Brief Article

Discovery of an orally bioavailable and Central Nervous System (CNS) penetrant mGlu7 Negative Allosteric Modulator (NAM) in vivo tool compound: N-(2-(1H-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)-4-(cyclopropylmethoxy)-3-methoxybenzamide (VU6012962)

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Discovery of an orally bioavailable and Central Nervous System (CNS) penetrant $mGlu_7$ 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)

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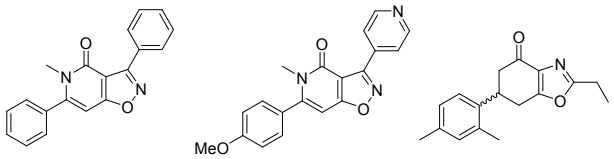
Supporting Information Placeholder

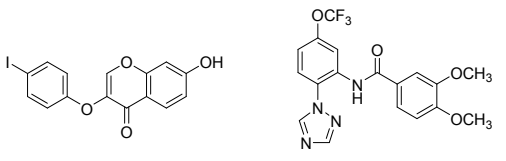
ABSTRACT: Herein, we report the discovery of a new, orally bioavailable and CNS-penetrant metabotropic glutamate receptor 7 ($mGlu_7$) negative allosteric modulator (NAM) that achieves exposure in cerebral spinal fluid (CSF) 2.5x above the *in vitro* IC_{50} at minimum effective doses (MEDs) of 3 mg/kg in preclinical anxiety models.

INTRODUCTION

Metabotropic glutamate receptor subtype 7 ($mGlu_7$) is one of the family of eight mGlu receptors ($mGlu_{1-8}$). Human genetic and mouse data have associated $mGlu_7$ with anxiety, autism, ADHD, schizophrenia, epilepsy, depression and Rett syndrome.[1-15] Early studies with first generation $mGlu_7$ negative allosteric modulators (NAMs), such as **1**[16,17], **2**[16,17], **3**[18] and **4**[19], 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 $mGlu_7$ 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 $mGlu_7$ 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* IC_{50}, the estimated unbound brain levels based on a brain homogenate binding assay indicated that **5** would not achieve levels above the *in vitro* $mGlu_7$ IC_{50} in terms of free brain concentrations. Despite this limitation, like **3**, $mGlu_7$ NAM **5** demonstrated robust efficacy in native tissues



1, MDIP **2**, MMPIP **3**, ADX71743



4, XAP044 **5**, VU6010608

Figure 1. Structures of reported $mGlu_7$ NAMs **1**-**5**, yet the field still requires a robust *in vivo* tool compound for target validation studies.

in blocking high frequency-stimulated long term potentiation (LTP) in the hippocampus.[21] Here, we disclose efforts aimed at the continued optimization of $mGlu_7$ NAM **5**, and the development of a new, highly selective $mGlu_7$ NAM *in vivo* tool compound that achieves predicted unbound CNS levels in excess of the *in vitro* IC_{50} 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 $mGlu_7$ 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 $mGlu_7$ NAM potency and PK, and 2) explore a larger collection of diverse 4-ether analogs **7**.

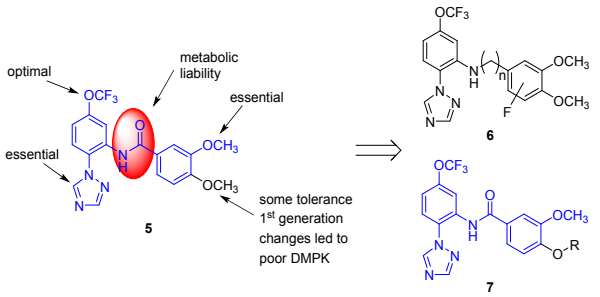
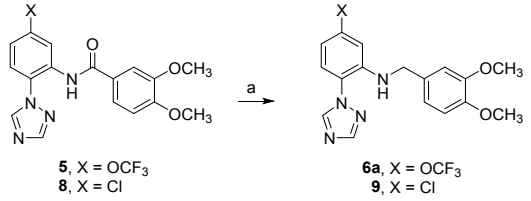


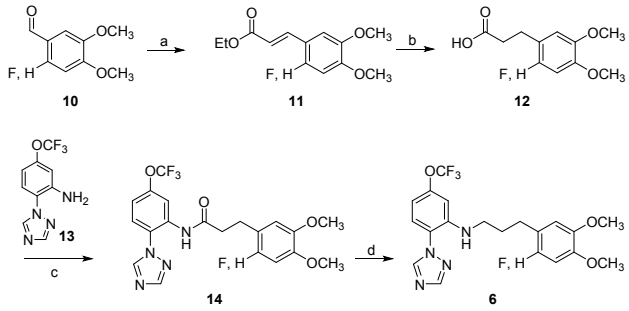
Figure 2. Overview of $mGlu_7$ NAM **5** SAR and metabolic liabilities, suggesting a path forward towards a new, structurally distinct, *in vivo* rodent tool compound. The minimum/essential pharmacophore of **5** is in blue.

Scheme 1. Synthesis of amine-linked congeners **6a** and **9**.[a]



[a]Reagents and Conditions: (a) $LiAlH_4$ (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]

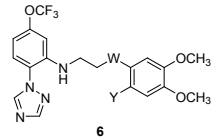


[a]Reagents and Conditions: (a) (carbethoxymethylene)triphenylphosphorane, CH_2Cl_2, rt, 16h, 90-94%; (b) i. $NaBH_4$, AcOH, Pd/C, toluene, rt, 1h, ii. LiOH, THF:H_2O (1:1), rt, 3h, 86-90%; (c) **13**, PyClU, DIEA, CH_2Cl_2, mw 100 °C, 30 min, 38-53%; (d) $LiAlH_4$ (3.5 equiv., 1.0 M in THF), THF, 0 °C, 2 h, 45-60%.

With large quantities of $mGlu_7$ 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 $LiAlH_4$ (**Scheme 1**) to afford **6a** and **9**, respectively.[22] While **5** was a moderately potent $mGlu_7$ NAM (IC_{50} = 760 nM, pIC_{50} = 6.12±0.04, 15±3% L-AP4 min), both **6a** (IC_{50} = 5.9 μM, pIC_{50} = 5.23±0.12, 17±3% L-AP4 min) and **9** (IC_{50} = 7.9 μM, pIC_{50} = 5.1±0.10, 21±3% L-AP4 min) lost considerable $mGlu_7$ 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 PyClU-mediated amide coupling with known **13** provided **14**, and reduction of the amide carbonyl with $LiAlH_4$ 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 $mGlu_7$ 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_us 0.01 to 0.03) or rat brain homogenate binding (f_u<0.003). Related analogs of **6b-d**, wherein the OCF_3 moiety was replaced with a chlorine atom, fared worse in terms of both potency and disposition, and numerous analogs proved inactive (IC_{50}s > 10 μM). Thus, the pursuit of an *in vivo* tool compound refocused on analogs **7**.

Table 1. Structures and rat $mGlu_7$ activities of analogs **6**[a]

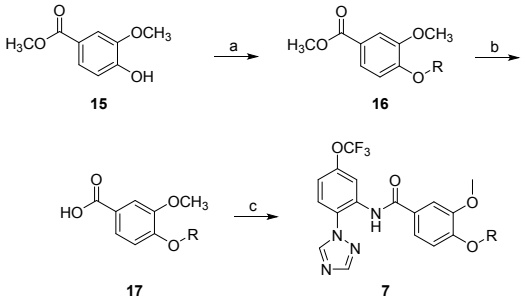


6

Entry	W	Y	$mGlu_7$ IC_{50} (μM)[a] (pIC_{50}±SEM)	% L-AP_4 Min±SEM	Rat CL_{hep} (mL/min/kg)
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

[a]Calcium mobilization assay with rat $mGlu_7/G_{\alpha 15}$/HEK cells performed in the presence of an EC_{80} fixed concentration of L-AP4 (a more potent agonist at $mGlu_7$ compared to glutamate); values represent means from three (n = 3) independent experiments in triplicate. ND = not determined. CL_{hep}, predicted hepatic clearance.

Scheme 3. Synthesis of 4-alkoxy congeners **7**.[a]

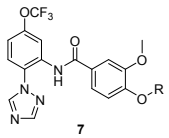


15 16 17 7

[a]Reagents and Conditions: (a) R-Br, K_2CO_3, MeCN, mw, 170 °C, 1h, 79-96%; (b) LiOH, THF:H_2O (1:1), rt, 3h, 88-97%; (c) **13**, PyClU, DIEA, CH_2Cl_2, mw 100 °C, 30 min, 38-53%.

To explore a broader range of 4-alkoxy analogs **7**, commercial ester **15** was alkylated with various alkyl bromides under microwave conditions to affords analogs **16** in yields ranging from 79-96%.[22] Ester hydrolysis smoothly afforded acid **17**, which was subsequently coupled to aniline **13** under PyClU 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,

Table 2. Structures and rat $mGlu_7$ activities of analogs **7**[a]



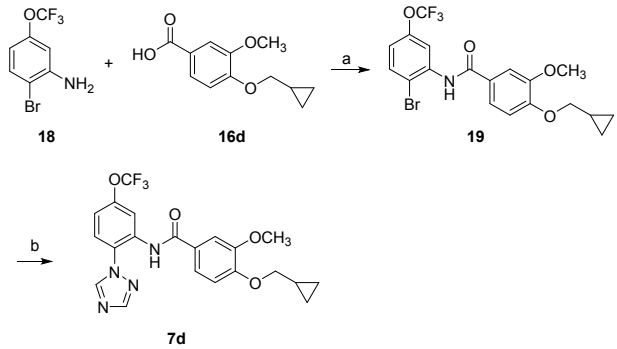
7

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

[a]Calcium mobilization assay with rat $mGlu_7/G_{\alpha 15}$/HEK cells performed in the presence of an EC_{80} fixed concentration of L-AP4 (a more potent agonist at $mGlu_7$ compared to glutamate); values represent means from three (n = 3) independent experiments in triplicate. ND = not determined. CL_{hep}, predicted hepatic clearance.

but isopropylmethyl (**7c**) restored $mGlu_7$ NAM activity, and cyclopropylmethyl (**7d**) proved optimal (IC_{50} = 350 nM, pIC_{50} = 6.46±0.10, 12.6±1.5% L-AP_4 min), and the most potent within this chemotype to date. Larger moieties, such as **7e-7g,** lost activity. Beyond an enhancement in $mGlu_7$ NAM potency, **7d** also showed a significant improvement in predicted hepatic clearance (rat CL_{hep} = 15.9 mL/min/kg), 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 PyClU-mediated coupling to provide **19**. An Ullmann coupling reaction installed the requisite 1,2,4-triazole to deliver **7d** in ~500 mg scale.[22]

Scheme 4. Scale-up route to access **7d**.[a]



18 16d 19 7d

[a]Reagents and Conditions: (a) PyClU, DIEA, CH_2Cl_2, mw 100 °C, 30 min, 53%; (b) *H*-1,2,4-triazole, trans-*N,N'*-dimethylcyclohexane-1,2-diamine, K_3PO_4, CuI, DMF, 100 °C, 16h, 56%.

NAM **7d** possessed an acceptable molecular weight (448 g/mol), cLogP (3.8) and polar surface area (84.7 Å^2). In addition to low predicted hepatic clearance in rat (CL_{hep} = 15.9

mL/min/kg), **7d** also showed moderate predicted hepatic clearance in mouse (CL_{hep} = 44.0 mL/min/kg) 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 (PBL) IV cassette study,[22,23] NAM **7d** displayed high brain penetration (rat K_p = 2.35 ([brain$_{tot}$] = 375 nM), $K_{p,uu}$ = 0.75 ([brain$_{unbound}$] = 3.4 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 (CL_p = 31.5 mL/min/kg), moderate volume (V_{ss} = 1.9 L/kg) and a short half-life ($t_{1/2}$ = 40 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 mGlu$_7$ versus the other seven mGlu receptors (>10 μM versus mGlu$_{1,2,3,4,5,6,8}$) as well as largely devoid of ancillary pharmacology (compound activity at only one target, 5-HT$_{2B}$ receptor, that was greater than 50% at 10 μM) in a Eurofins Lead Profiling panel of 68 GPCRs, ion channels and transporters.[22] Based on 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 (i.p.) 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 ([plasma]$_{tot}$ = 598 nM: [brain]$_{tot}$ = 745 nM) and a $K_{p,uu}$ of 0.38 ([plasma]$_{unbound}$ = 16.7 nM: [brain]$_{unbound}$ = 6.4 nM); however, the CSF:plasma K_p was 2.15, with levels of **7d** in CSF of 1.3 μM, or ~3.8-fold above the *in vitro* IC_{50}. As with recent mGlu$_4$ PAMs, assessing CSF exposure was critical, as opposed to simply estimating free brain levels (i.e., $K_{p,uu}$).[24,25]

Based on the potency, efficacy, disposition and high concentrations in CSF of **7d**, we progressed NAM **7d** as an *in vivo* rodent tool molecule. First generation mGlu$_7$ 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 7B**).

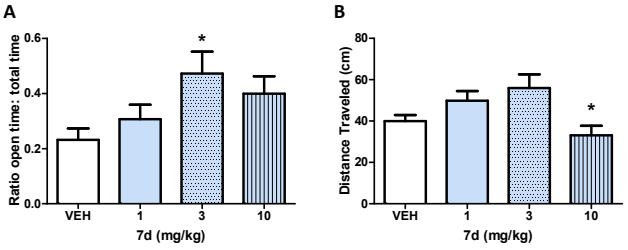
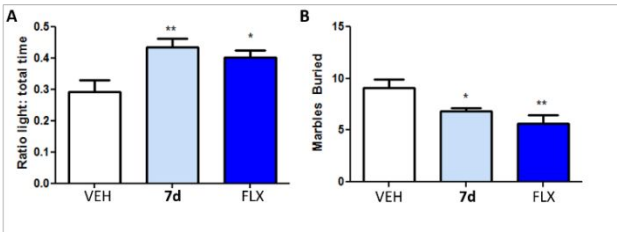


Figure 3. NAM **7d** decreases anxiety in the elevated zero maze (EZM) assay in mice. A) Intraperitoneal administration of **7d** increases time spent in the open arms (MED = 3 mg/kg); One-way ANOVA; (F[3,39]=3.112, p = 0.0382), Bonferroni's post hoc test; VEH vs. 3 mg/kg, p<0.05. B). The 10 mg/kg dose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

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 i.p.) or fluoxetine (15 mg/kg i.p.) 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 hour time point used for treatment in mice. These studies revealed values of [plasma]$_{tot}$ = 303 nM and [CSF] = 883 nM; this CSF level is 2.5x higher than the *in vitro* IC_{50} of 350 nM. Taken together, mGlu$_7$ 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.

Figure 4. NAM **7d** is efficacious at 3 mg/kg in both the light/dark box and marble burying assays and is comparable to fluoxetine. A) Administration of both 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 s. FLX, p<0.01. All



data points were run with Grubb's outlier test).

CONCLUSION

In summary, we have reported on the discovery of a new, structurally distinct mGlu$_7$ 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 brain levels (CSF concentrations) ~4-fold above the *in vitro* IC_{50}. Moreover, NAM **7d** is highly selective for mGlu$_7$ versus the other seven mGlu receptor subtypes and across large ancillary pharmacology panels. Like first generation mGlu$_7$ 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) as well as ^{1}H and ^{13}C NMR and Hi-Res 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 hours, 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. NH_4Cl x 2, brine, dried over $MgSO_4$, 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). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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+) calc'd for $C_{21}H_{19}F_3N_4O_4$, 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 CH_2Cl_2 (15 mL) in a Biotage microwave vial was added **17d** (1.48 g, 6.64 mmol), *N,N*-diisopropylethylamine (3.47 mL, 19.9mmol), and chlorodipyrrolidinocarbenium hexafluorophosphate (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 NH_4Cl. The layers were separated, and the aqueous layer was washed with DCM x 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). ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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+) calc'd for $C_{19}H_{17}BrF_3NO_4$, 459.0293; found 459.0296.

ASSOCIATED CONTENT

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ABBREVIATIONS USED

$mGlu_7$, metabotropic glutamate receptor subtype 7; CRC, concentration-response-curve; NAM, negative allosteric modulator; PBL, plasma:brain level; SAR, structure-activity-relationships; CSF, cerebral spinal fluid; EZM, elevated Z maze.

SUPPORTING INFORMATION AVAILABLE

The general chemistry, experimental information, and syntheses of all other compounds are supplied in the Supporting Information, as well as *in vitro* and *in vivo* pharmacology and DMPK methods as well as the availability of Molecular Formula Strings and Supplemental Figures. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

(1) Niswender, C.M. and Conn, P.J. Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Ann. Rev. Pharmacol. Toxicol.* **2010**, *50*, 295−322.

(2) Lindsley, C.W.; Emmitte, K.A.; Hopkins, C.R.; Bridges, T.M.; Gregory, K.J.; Niswender, C.M.; Conn, P.J. Practical strategies and concepts in GPCR allosteric modulator discovery: Recent advances with metabotropic glutamate receptors. *Chem. Rev.* **2016**, *116*, 6707-6741.

(3) Fisher, N.M.; Seto, M.; Lindsley, C.W.; Niswender, C.M. Metabotropic Glutamate Receptor 7: A new therapeutic target in neurodevelopmental disorders. *Front. Mol. Neurosci.* **2018**, *11*, article 387.

(4) Sansig, G.; Bushell, T. J.; Clarke, V. R.; Rozov, A.; Burnashev, N.; Portet, C.; Gasparini, F.; Schmutz, M.; Klebs, K.; Shigemoto, R.; Flor, P.J.; Kuhn, R.; Knoepfel,T.; Schroeder, M.; Hampson, D. R.; Collett,V. J.; Zhang, C.; Duvoisin, R.M.; Collingridge, G.L.; van Der Putten, H. Increased seizure susceptibility in mice lacking metabotropic glutamate receptor 7. *J. Neurosci*. **2001**, *21*, 8734−8745.

(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.

(6) Palucha, A.; Klak, K.; Branski, P.; van der Putten, H.; Flor, P.J.; Pilc, A. Activation of the mGlu7 receptor elicits antidepressant-like effects in mice. *Psychopharmacology* **2007**, *194*, 555-562.

(7) Callaerts-Vegh, Z.; Beckers, T.; Ball, S.M.; Baeyens, F.; Callaerts, P.F.; Cryan, J.F.; 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.

(8) Mitsukawa, K.; Mombereau, C.; Lötscher, E.; Uzunov, D.P.; van der Putten, H.; Flor, P.J.; Cryan, J.F. 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.

(9) Hölscher, C.; Schmid, S.; Pilz, P.K.; Sansig, G.; van der Putten, H.; Plappert, C.F. Lack of the metabotropic glutamate receptor subtype 7 selectively modulates Theta rhythm and working memory. *Learn. Mem* **2005**, *12*, 450-455.

(10) Hölscher, C.; Schmid, S.; Pilz, P.K.; Sansig, G.; van der Putten, H.; Plappert, C.F. 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.

(11) Bushell, T.J.; Sansig, G.; Collett, V.J.; van der Putten, H.; Collingridge, G.L. Altered short-term synaptic plasticity in mice lacking the metabotropic glutamate receptor mGlu7. *ScientificWorldJournal* **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.

(13) Gogliotti R.G.; Senter R.K.; Fisher N;, Adams J.; Zamorano R.; Walker A.G.; Blobaum A.L.; Engers D.W.; Hopkins C.R.; Daniels J.S.; Jones, C.K.; Lindsley C.W.; Xiang Z.; Conn P.J.; and Niswender C.M. $mGlu_7$ potentiation rescues cognitive, social, and respiratory phenotypes in a mouse model of Rett syndrome. *Sci. Trans. Med.* **2017**, 9, eaai7459.

(14) Jalan-Sakrikar, N.; Field, J.R.; Klar, R.; Mattmann, M.E.; Gregory, K.J.; Zamorano, R.; Engers, D.W.; Bollinger, S.R.; Weaver, C.D.; Days, E.L.; Lewis, L.M.; Utley, T.J.; Hurtado, M.; Rigault, D.; Acher, F.; Walker, A.G.; Melancon, B.J.; Wood, M.R.; Lindsley, C.W.; Conn, P.J.; Xiang, Z.; Hopkins, C.R.; Niswender, C.M. 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.

(15) Abe, M.; Seto, M.; Gogliotti, R.G.; Loch, M.T.; Bollinger, K.A.; Chang, S.; Engelberg, E.M.; Luscombe, V.B.; Harp, J.M.; Bubser, M.; Engers, D.W.; Jones, C.K.; Rodriguez, A.L.; Blobaum, A.L.; Conn, P.J.; Niswender, C.M.; Lindsley, C.W. 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.

(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.

(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.

(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, S.A.; Salt, T.E.; 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.

(19) Gee, C.E.; Peterlik, D.; Neuhäuser, C.; Bouhelal, R.; Kaupmann, K.; Laue, G.; Uschold-Schmidt, N.; Feuerbach, D.; Zimmermann, K.; Ofner, S.; Cryan, J.F.; van der Putten, H.; Fendt, M.; Vranesic, I.; Glatthar, R.; Flor, P.J. 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.

(20) Niswender, C.M.; Johnson, K.A.; Miller, N.R.; Ayala, J.E.; Luo, Q.; Williams, R.; Saleh, S.; Orton, D.; Weaver, C.D.; Conn, P.J. Context-dependent pharmacology exhibited by negative allosteric modulators of metabotropic glutamate receptor 7. *Mol. Pharmacol*. **2010**, *77*, 459–468.

(21) Reed, C.W.; McGowan, K.M.; Spearing, P.K.; Stansley, B.J.; Roenfanz, H.F.; Engers, D.W.; Rodriguez, A.L.; Engelberg, E.M.; Luscombe, V.B.; Loch, M.T.; Remke D.H.; Rook, J.M.; Blobaum, A.L.; Conn, P.J.; Niswender, C.M.; Lindsley, C.W. VU6010608, a novel $mGlu_7$ NAM from a series of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)benzamides. *ACS Med. Chem. Lett.* **2017**, *8*, 1326-1330.

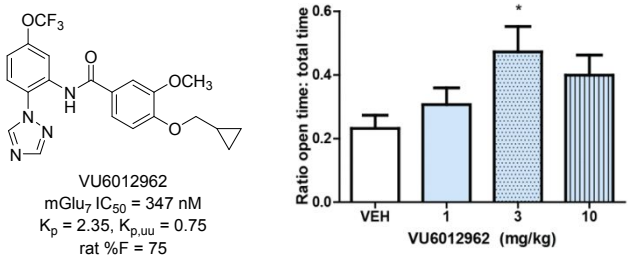
(22) See Supporting Information for full experimental details.

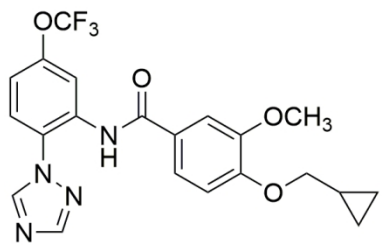
(23) Bubser, M.; Bridges, T.M.; Dencker, D.; Gould, R.W.; Grannan, M.; Noetzel, M.J.; Lamsal, A.; Niswender, C.M.; Daniels, J.S.; Poslusney, M.S.; Melancon, B.J.; Tarr, J.C.; Byers, F.W.; Wess, J.; Duggan, M.E.; Dunlop, J.; Wood, M.W.; Brandon, N.J.; Wood, M.R.; Lindsley, C.W.; Conn, P.J.; Jones C.K. Selective activation of M_4 muscarinic acetylcholine receptors reverses MK-801-induced behavioral impairments and enhances associative learning in rodents. *ACS Chem. Neurosci.* **2014**, *5*, 920-942.

(24) Engers, D.W.; Blobaum, A.L.; Gogliotti, R.D.; Cheung, Y-Y.; Salovich, J.M.; Garcia-Barrantes, P.M.; Daniels, J.S.; Morrison, R.; Jones, C.K.; Soars, M.G.; Zhuo, X.; Hurley, J.; Macor, J.E.; Bronson, J.J.; Conn, P.J.; Lindsley, C.W.; Niswender, C.M.; Hopkins, C.R. Discovery, synthesis and preclinical characterization of *N*-(3-chloro-4-fluorophenyl)-1*H*-pyrazolo[4,3-*b*]pyridin-3-amine (VU0418506), a novel positive allosteric modulator of the metabotropic glutamate receptor 4 ($mGlu_4$). *ACS Chem. Neurosci.* **2016**, *7*, 1192-1200.

(25) Panarese, J.D.; Engers, D.W.; Wu, Y-J.; Bronson, J.J.; Macor, J.E.; Chun, A.; Rodriguez, A.L.; Felts, A.S.; Engers, J.L.; Emmitte, K.A.; Castelhano, A.L.; Kates, M.J.; Nader, M.A.; Jones, C.K.; Blobaum, A.L.; Conn, P.J.; Niswender, C.M.; Hopkins, C.R.; Lindsley, C.W. Discovery of VU2957 (Valiglurax): An $mGlu_4$ positive allosteric modulator evaluated as a preclinical candidate for the treatment of Parkinson's Disease. *ACS Med. Chem. Lett., web asap*.

TABLE OF CONTENTS GRAPHIC





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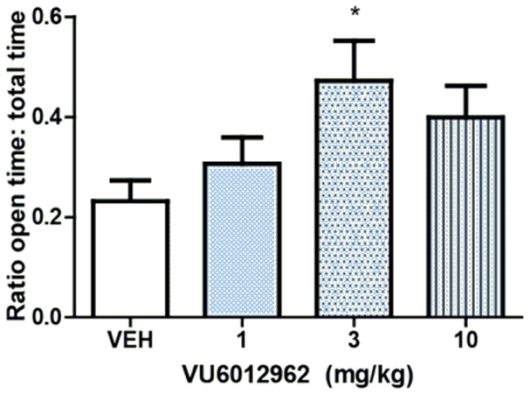
rat %F = 75



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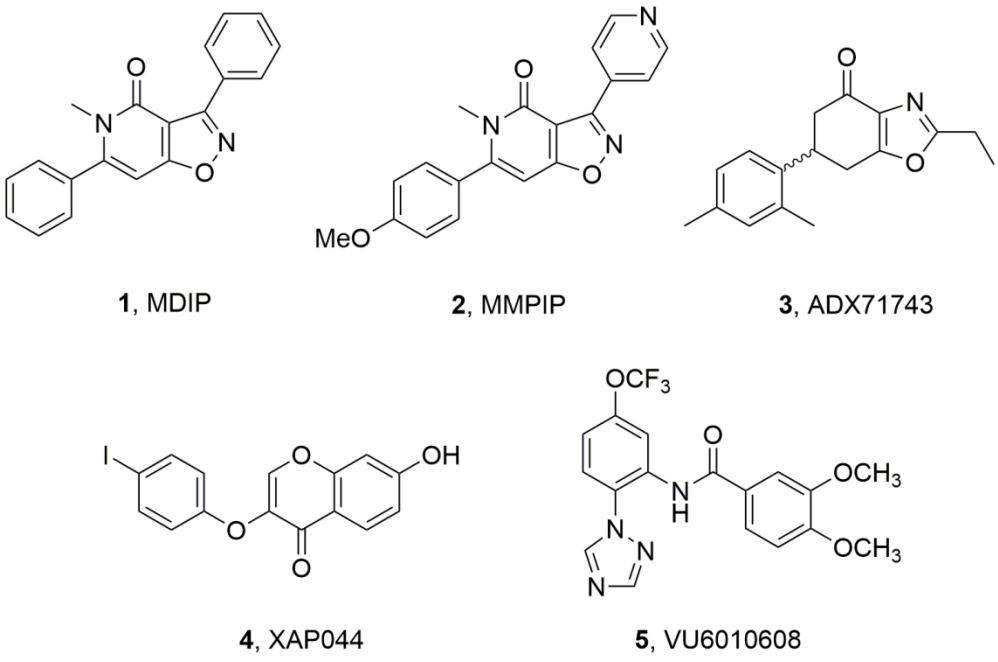

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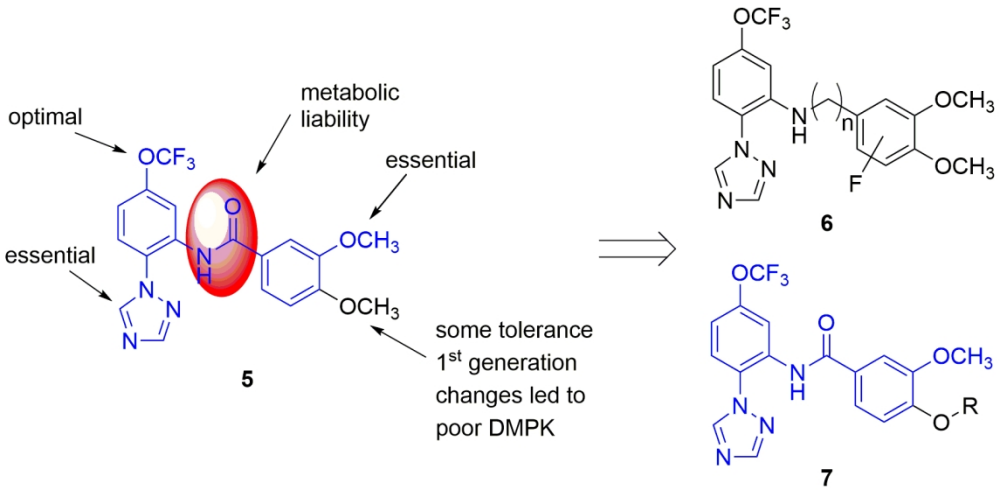

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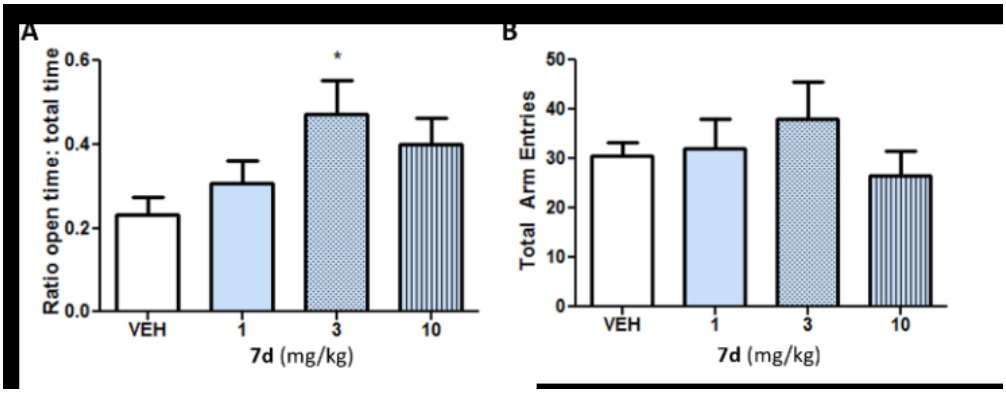

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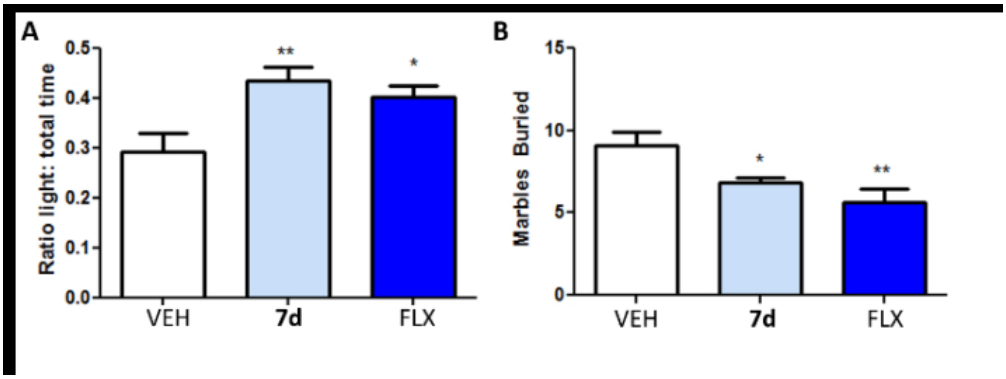

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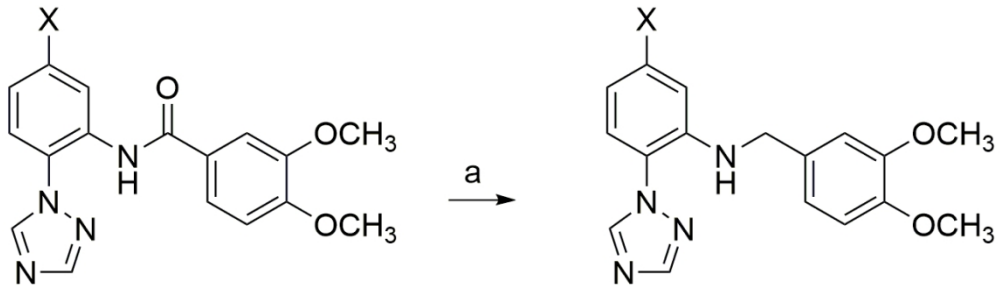

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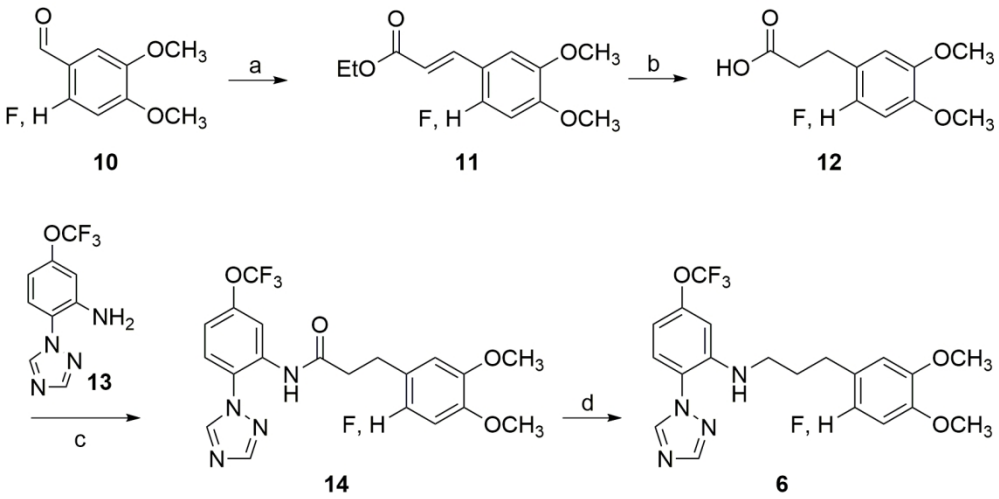

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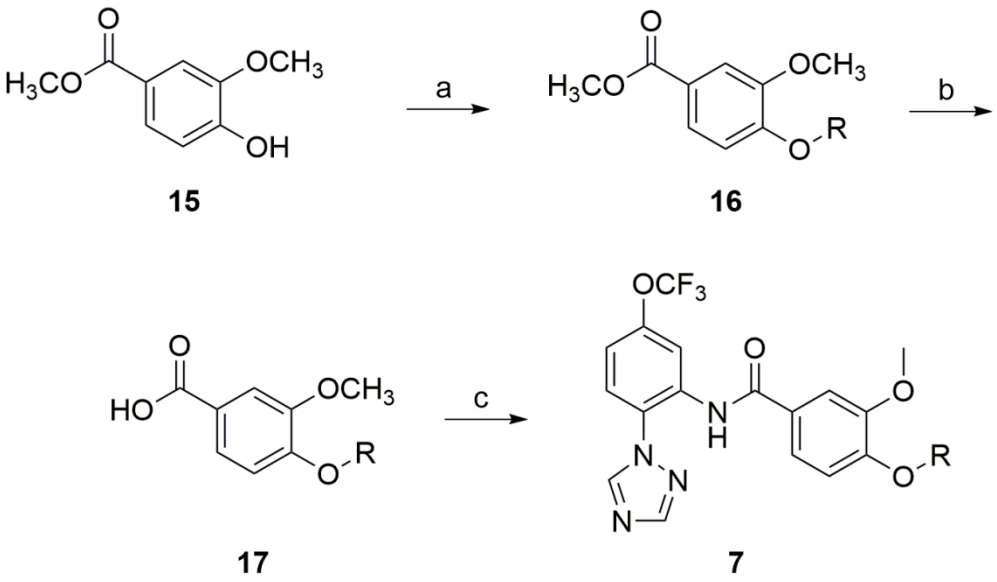

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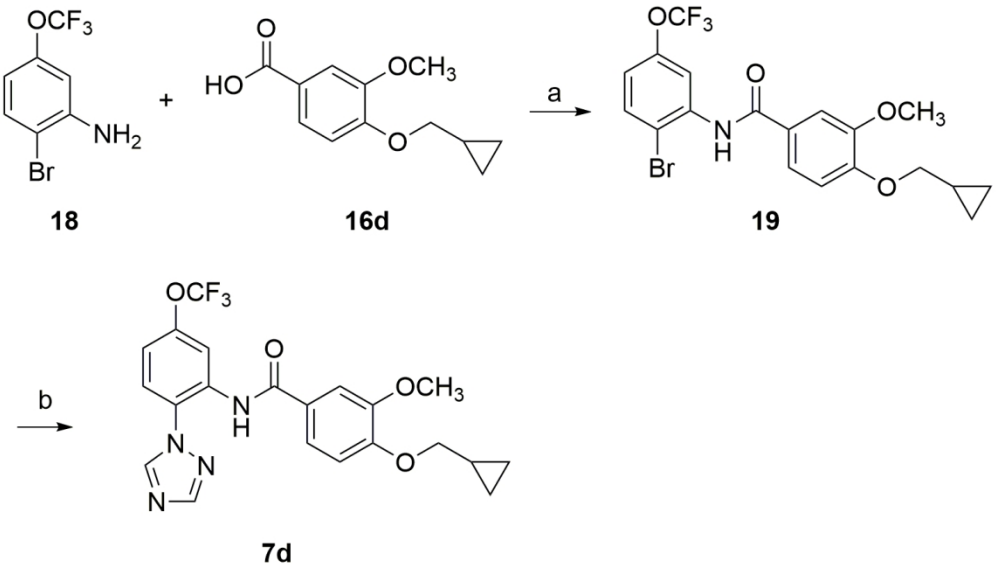

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