Letter

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

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VU6010608, a Novel $mGlu_7$ NAM from a Series of *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)benzamides

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ABSTRACT: Herein, we report the structure-activity relationships within a series of $mGlu_7$ NAMs based on a *N*-(2-(1*H*-1,2,4-triazol-1-yl)-5-(trifluoromethoxy)phenyl)benzamide core with excellent CNS penetration (K_ps 1.9 to 5.8 and $K_{p,uu}$s 0.4 to 1.4). Analogs in this series displayed steep SAR. Of these, VU6010608 (**11a**) emerged with robust efficacy in blocking high frequency stimulated long-term potentiation (LTP) in electrophysiology studies.

Of the eight subtypes of metabotropic glutamate receptors ($mGlu_{1-8}$), the physiology and therapeutic potential of $mGlu_7$ 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 $mGlu_7$ with anxiety, depression, epilepsy, schizophrenia, ADHD and autism.[3-11] Recent efforts from our labs, using non-selective Group III mGlu receptor positive allosteric modulators (PAMs) in combination with an $mGlu_7$ negative allosteric modulator (NAM), synaptic localization, and/or $mGlu_7$ knock-out mice to isolate effects of selective $mGlu_7$ activation, have identified a role for $mGlu_7$ in a mouse model of Rett syndrome and cognition.[12,13] Recent efforts to develop a first-in-class $mGlu_7$ PAM to further validate the Rett connection resulted in an $mGlu_7$-preferring PAM **1**, VU6005649, that demonstrated pro-cognitive effects on associative learning in wild type mice.[14] There have been several $mGlu_7$ NAM tools reported in the literature (**Figure 1**),[15-17] and the therapeutic potential of $mGlu_7$ has been reviewed.[18] In 2007, Tsukuba researchers reported on the first $mGlu_7$ 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 Therapeutics 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 $mGlu_2$.[20] Here, we describe the identification of a novel $mGlu_7$ NAM chemotype, devoid of electrophilic character, with potency comparable to **4** and high mGlu selectivity. Moreover,

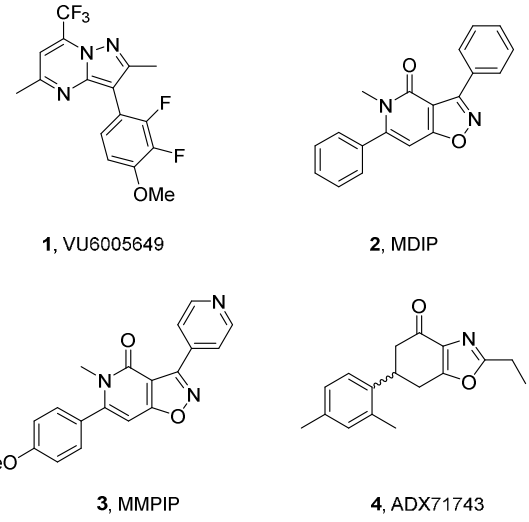


Figure 1. Structures of the recently reported $mGlu_7$-preferring PAM (**1**) and $mGlu_7$ NAMs (**2**-**4**), with a conserved 5,6-bicyclic chemotype.

this new series was brain penetrant and efficacious in electrophysiology studies.

As reported recently, we initiated a high-throughput screening (HTS) campaign on a 63,000 membered library, utilizing a triple-addition protocol, to simultaneously identify $mGlu_7$ agonists, PAMs, and NAMs.[14] This screen was very successful in identifying $mGlu_7$ PAM leads, which ultimately led to the development of **1**. This campaign also identified 156 $mGlu_7$ antagonists/NAMs; 75 of these compounds were validated after counter-screening against untransfected HEK cells and full concentration response curve (CRC) confirmation. Attractive chemotypes were evaluated against $mGlu_4$ and $mGlu_8$, and selective hits were then optimized via iterative parallel synthesis. Of these, HTS hit **5** (**Figure 2**), based on an *N*-(2-(1*H*-1,2,4-triazol-1-yl)phenyl)benzamide core, proved to be structurally distinct from the known $mGlu_7$ NAM chemotypes (**2-4**),[15-17] and was prioritized for optimization. Hit **5** was selective for $mGlu_7$ ($mGlu_7$ IC_{50} = 5.8 μM, pIC_{50} = 5.24±0.05, 19±3 L-AP4 Min (reduced an EC_{80} level of activation to an EC_{19}) and >30 μM versus $mGlu_4$ and $mGlu_8$), but, interestingly, appeared to be a partial antagonist as it did not completely block the agonist response. ADX71743 is more potent ($mGlu_7$ IC_{50} = 676 nM,

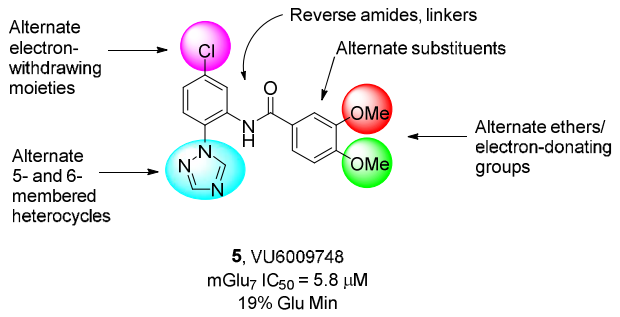
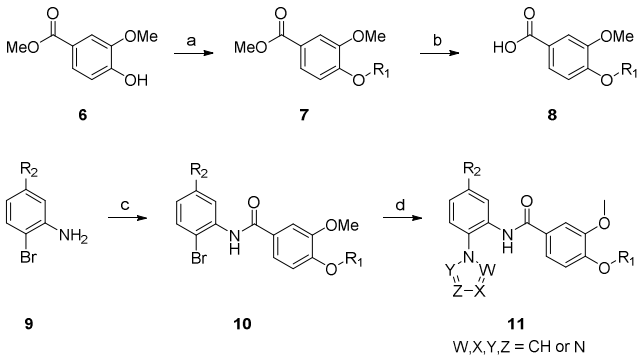


Figure 2. Structure of HTS hit **5** (VU6009748), and the multiple regions to be surveyed in the lead optimization campaign.

Scheme 1. Synthesis of Analogues **11**[a]



[a]Reagents and conditions: (a) R_1X, K_2CO_3, DMF, 100 °C, 3h, 94-97%; (b) LiOH, THF:H_2O (1:1), 60 °C, 2 h, quantitative; (c) PyClU, DIEA, CH_2Cl_2, 100 °C, mw, 20 min, 47-82%; azaheterocycle, *N,N*-dimethylcyclohexane-1,2-diamine, K_3PO_4, CuI, DMF, 100 °C, 16h, 51-68%.

pIC_{50} = 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 analogs **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 analogs **7** (diverse R_1), followed by ester hydrolysis to deliver benzoic acids **8**. A variety of 5-substituted (R_2), 2-bromoanilines **9** were coupled to acids **8** under PyClU conditions to give amides **10**. Finally, a copper-mediated Ullman coupling provided analogs **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, under standard conditions, where aryl and heteroaryl congeners were also surveyed.

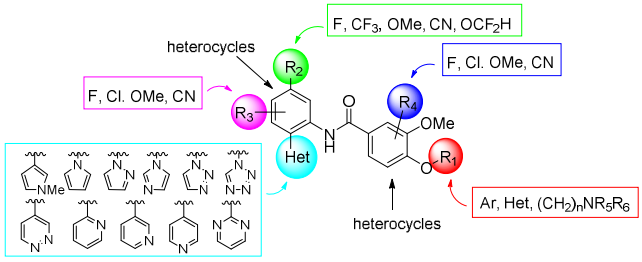
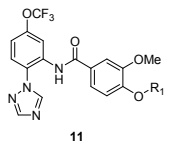


Figure 3. Substituents explored in analogs **11** that were inactive as $mGlu_7$ NAMs (IC_{50}s > 10 μM).

Evaluation of analogs **11** in our functional $mGlu_7$ assay[12-14,20] demonstrated steep SAR (akin to the SAR noted with the $mGlu_7$ PAM **1**), and compound **5** is included for comparison (R_2 = Cl).[14] Here, the majority of ~100 analogs prepared were inactive (IC_{50}s >10 μM) at $mGlu_7$ (**Figure 3**). The 1,2,4-triazole proved essential for $mGlu_7$ NAM activity, with other regioisomeric triazoles, pyrazoles, pyroles and imidazoles devoid of activity. Ring expansions to 6-membered azaheterocycles, accessed via Suzuki couplings, were also inactive. Similarly, the 5-position (R_2) was largely intolerant of change, with only an OCF_3 moiety proving superior to chlorine. While alternate ethers in the 4-position (R_1) were active, increasing steric bulk

Table 1. Structures and Activities of Compound **5** and Analogs **11**[a]



Compound	R_1	rmGlu$_7$ IC_{50} (μM)[a] [% L-AP4 Min ±SEM]	rmGlu$_7$ pIC_{50} (±SEM)	Rat K_p ($K_{p,uu}$)[b]
5	Me	5.8 [19±3]	5.24±0.05	ND
11a	Me	0.76 [15±3]	6.12±0.04	3.4 (0.48)
11b	Et	0.51 [11±2]	6.29±0.08	3.5 (0.83)
11c	^{n}Pr	0.78 [19±5]	6.11±0.05	1.9 (1.0)
11d	^{i}Pr	0.78 [14±4]	6.11±0.07	4.4 (0.89)
11e		0.71 [11±3]	6.15±0.11	5.8 (1.5)
11f		1.4 [10±3]	5.87±0.07	ND

[a]Calcium mobilization assays with rat mGlu$_7$/G$_{α15}$/HEK cells performed in the presence of an EC_{80} fixed concentration of L-AP4; values represent means from three (n=3) independent experiments performed in triplicate.[b] Total and calculated unbound brain:plasma partition coefficients determined at 0.25 h post-administration of an IV cassette dose (0.20–0.25 mg/kg) to male, SD rats (n = 1), in conjunction with *in vitro* rat plasma protein and brain homogenate binding assay data. ND = not determined.

led to a diminution in potency (e.g., **11f**, mGlu$_7$ IC_{50} = 1.4 μM). Importantly, the 3-methoxy moiety was also essential and intolerant to any modifications. Reverse amides, sulfonamides and α-trifluromethyl amines were not effective as linker replacements, and additional substituents to the ether aryl ring (even fluorine) led to a complete loss of mGlu$_7$ NAM activity, as did the incorporation of heterocyclic systems. However, the analogs **11a-f** with mGlu$_7$ NAM activity (**Table 1**) generally displayed favorable brain penetration (K_ps > 1, $K_{p,uu}$s >0.4) in our high throughput rat plasma:brain level (PBL) cassette paradigm,[21] and all proved to be partial antagonists (mGlu$_7$ Glu Min 10-19%).[21] The steep (all active analogs possessed IC_{50}s in the ~700 nM range) and flat (minor structural changes led to loss of mGlu$_7$ NAM activity) SAR profile of analogs **11** left five analogs (**11a-e**) for DMPK profiling to assess potential as *in vivo* tools.[14,20,21]

Table 2. *In vitro* DMPK Profiles of Analogs **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

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 towards a tool compound. In terms of cLogP (2.86), molecular weight (408), 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 amongst the new analogs **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 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 hour 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 both the 3- and 4-OCD_3 congeners as well as the 3,4-di-OCD_3 analog, 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 mGlu$_7$ 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* mGlu$_7$ IC_{50}. 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 μM) were two-fold above the *in vitro* mGlu$_7$ IC_{50}, 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* IC_{50}. 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]

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	1,550	59.0	2.00	0.43

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 μM) against $mGlu_{1,2,3,4,5,6,8}$, and was a highly selective $mGlu_7$ NAM (as opposed to **4**, which in our in-house mGlu selectivity panel, shows weak $mGlu_2$ activity).[19] Furthermore, in a broader ancillary pharmacology (a Eurofins Lead profiling panel of 68 GPCRs, ion channels and transporters), no significant activities were noted at 10 μM.[20]

$mGlu_7$ 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 $mGlu_7$ regulates long-term potentiation (LTP), and that antagonism of $mGlu_7$ 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 $mGlu_7$ 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]

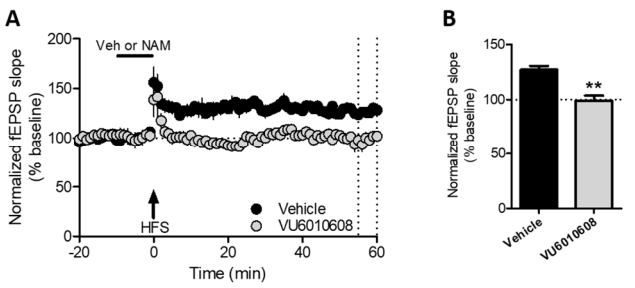


Figure 4. HFS-induced LTP is blocked by the $mGlu_7$ NAM VU6010608 (**11a**). (A) Time course showing the effects of vehicle or $mGlu_7$ NAM (VU6010608, 10 μM, grey symbols) followed by high-frequency stimulation (HFS; 2 x 100 Hz, 20 sec ISI) on fEPSP slope. (B) Comparison between drug treatment groups based on average slope from last 5 minutes of recording. (**p<0.01 compared to Vehicle; Student's t-test, n=4 per group). Data are expressed as mean ± SEM.

11a, a highly selective $mGlu_7$ 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 $mGlu_7$. 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 towards the goal of developing a robust *in vivo* $mGlu_7$ NAM probe, as well as the optimization of other $mGlu_7$ NAM hits from our HTS campaign, which will be reported in due course.

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Author Contributions

CWL, CMN, PJC, JMR, and BJS drafted/corrected the manuscript. CWR, KMM, PKS, HFR and DWE performed the chemical synthesis. CWL, PJC, CMN, JKR, and ALR oversaw the target selection and interpreted the biological data. EE, VBL, MTL, and ALR performed the *in vitro* molecular pharmacology studies and the HTS. ALB performed the *in vitro* and *in vivo* DMPK studies. JMR oversaw the *in vivo* experiments. DHR performed the *in vivo* studies. BJS performed the electrophysiology studies. All authors have given approval to the final version of the manuscript.

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ASSOCIATED CONTENT

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

ABBREVIATIONS

LTP, long-term potentiation; HFS, high frequency stimulation; metabotropic glutamate receptor (mGlu); PAM, positive allosteric modulator; NAM, negative allosteric modulator; HTS, high-throughput screen; PBL, plasma:brain level;

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20. See Supporting Information for full experimental details.
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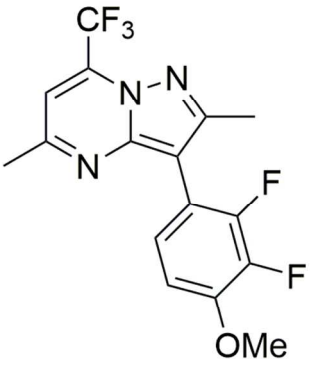

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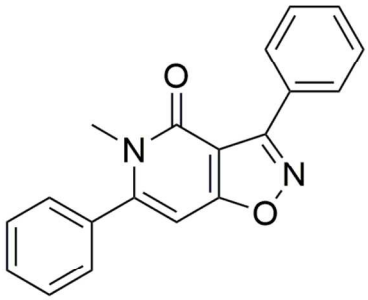
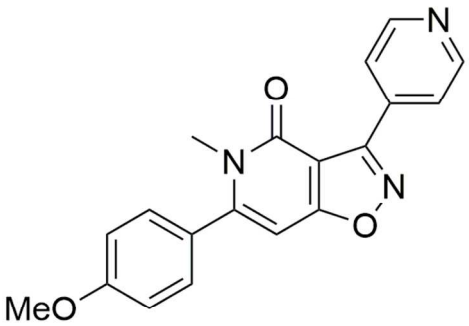


2, MDIP



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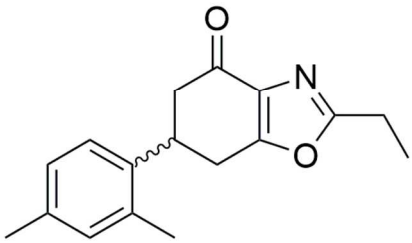

4, ADX71743

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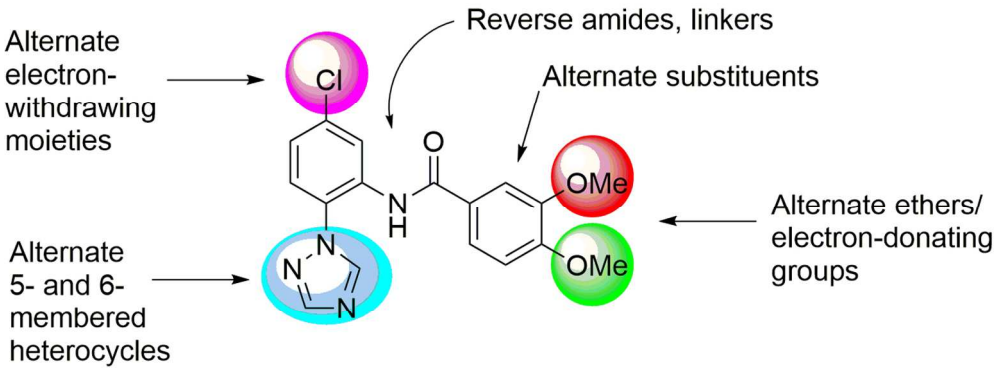

5, VU6009748
$mGlu_7$ IC_{50} = 5.8 μM
19% Glu Min

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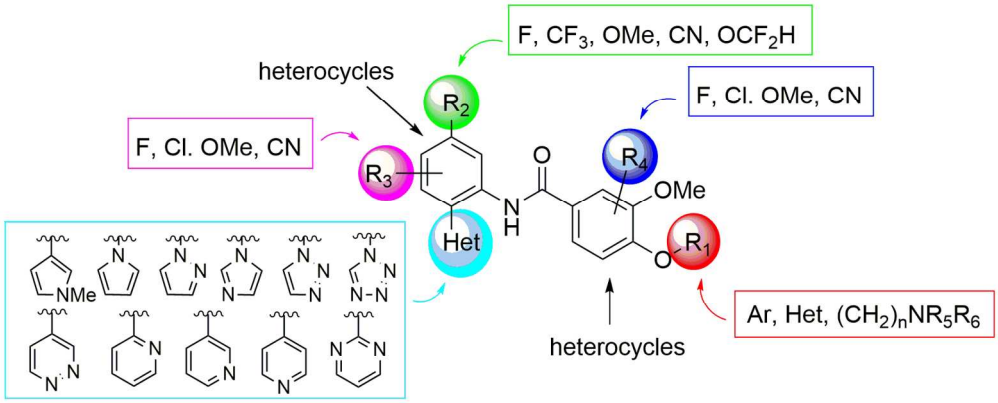

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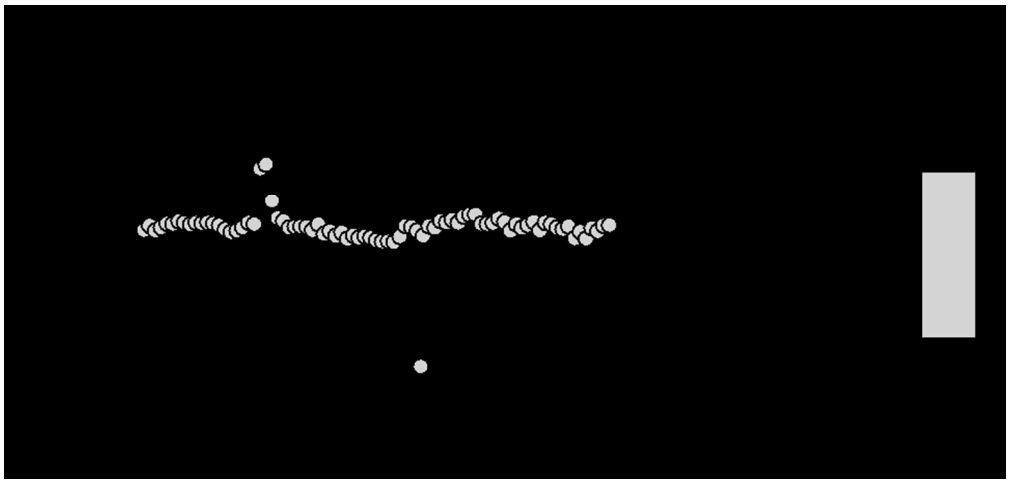

163x77mm (150 x 150 DPI)

VU6010608, a Novel mGlu$_7$ NAM from a Series of *N*-(2-(1*H*-1,2,4-trizol-1-yl)-5-(trifluoromethoxy)phenyl)benzamides

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