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Letter

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Design and synthesis of *N*-aryl phenoxyethoxy pyridinones as highly selective and CNS penetrant $mGlu_3$ NAMs

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KEYWORDS: Negative allosteric modulator (NAM), metabotropic glutamate receptor 3 ($mGlu_3$), depression, VU6010572, physiochemical properties

ABSTRACT: Herein, we detail the optimization of the $mGlu_3$ NAM, VU0650786, via a reductionist approach to afford a novel, simplified $mGlu_3$ NAM scaffold that engenders potent and selective $mGlu_3$ inhibition ($mGlu_3$ IC_{50} = 245 nM, $mGlu_2$ IC_{50} >30 μM) with excellent CNS penetration (rat brain:plasma K_p = 1.2, $K_{p,uu}$ = 0.40). Moreover, this new chemotype, exemplified by VU6010572, requires only four synthetic steps, displays improved physiochemical properties and *in vivo* efficacy in a mouse tail suspension test (MED = 3 mg/kg i.p.).

GRM3, the gene that encodes metabotropic glutamate receptor subtype 3 ($mGlu_3$), represents a significant locus associated with schizophrenia, substance abuse disorders and bipolar disorder; moreover, single-nucleotide polymorphisms (SNPs) within *GRM3* are linked to cognitive deficits.[1-6] Dual $mGlu_{2/3}$ negative allosteric modulators (NAMs) **1-5** have demonstrated therapeutic potential in Alzheimer's disease, anxiety, obsessive-compulsive disorder, autism spectrum disorders and cognition (**Figure 1**).[6-11] Moreover, the $mGlu_{2/3}$ NAM decoglurant **5** advanced into human Phase II clinical trials for depression.[12-14] Despite the therapeutic relevance and clinical interest, few highly selective $mGlu_3$ NAMs exist to define the contribution of $mGlu_3$ inhibition.[15-19] Early $mGlu_3$ NAM tool compounds **6** and **7** (**Figure 2**), derived from 'molecular switches' within $mGlu_5$ positive allosteric modulator (PAM) ligands,[20,21] enabled study of selective $mGlu_3$ inhibition and highlighted a key role for $mGlu_3$ in the regulation of synaptic plasticity in medial prefrontal cortex (mPFC) as well as antidepressant and anxiolytic activity.[22] In particular, VU0650786, **8**, ($mGlu_2$ IC_{50} >30 μM, $mGlu_3$ IC_{50} = 392 nM, rat brain:plasma K_p = 1.7; $K_{p,uu}$ = 0.78) has emerged as a highly valuable $mGlu_3$ NAM *in vivo* probe; however, it requires a nine step synthesis.[23] Thus, we hoped to simplify the VU0650786 chemotype and also improve upon physicochemical properties in a next generation $mGlu_3$ NAM *in vivo* probe with a strong intellectual property (IP) positon.

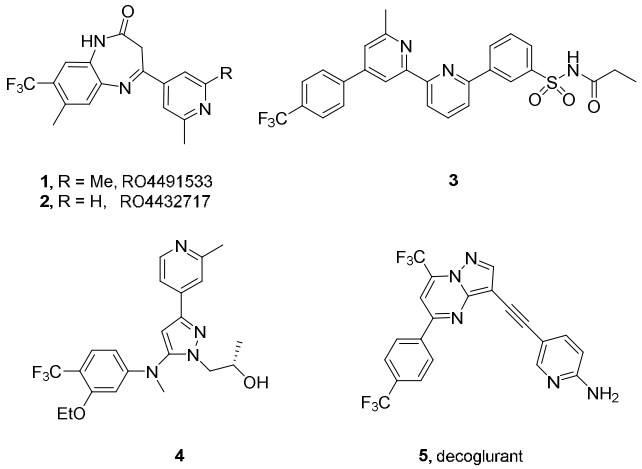


Figure 1. Structures of reported dual $mGlu_{2/3}$ NAMs **1**-**5** that have provided target validation for Group II mGlu inhibition in multiple CNS disorders.

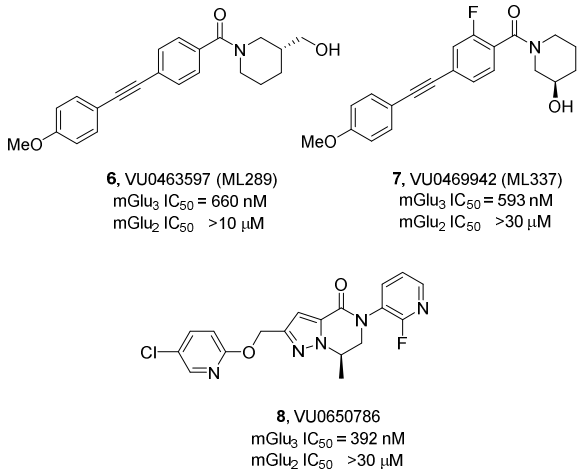


Figure 2. Structures and *in vitro* $mGlu_2$ / $mGlu_3$ potencies of reported $mGlu_3$ NAMs **6-8**, all derived from $mGlu_5$ PAM scaffolds via 'molecular switches'.

Using **8** as a lead, our goal was to reduce molecular complexity and enhance physicochemical properties in a next generation $mGlu_3$ NAM. We elected to deconstruct the heterobicyclic dihydropyrazolo[1,5-*a*]pyrazine-4(5*H*)-one core of **8**, and replace it with an ethereal, aliphatic linker and either an *N*-aryl pyrimidine or *N*-aryl pyridine head-piece (**Figure 3**) to provide greater conformational flexibility and rapid synthesis.

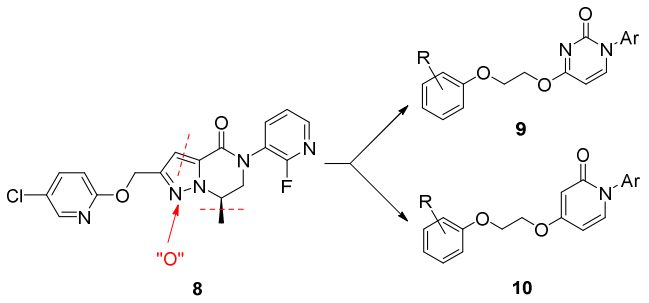
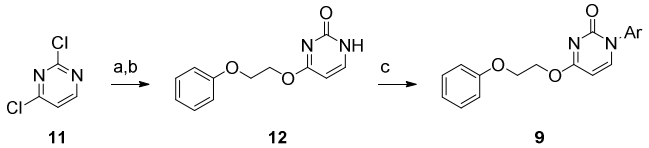


Figure 3. Optimization plan to deconstruct **8** into more flexible cores **9** and **10** with improved predicted physicochemical properties.

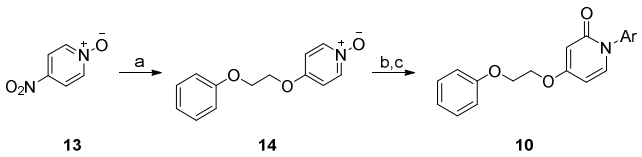
The chemistry to access scaffolds **9** and **10** proved straightforward.[24] For scaffold **9** (**Scheme 1**), commercially available 2,4-dichloropyrimidine **11** underwent an S_NAr reaction with 2-phenoxyethanol, followed by a second S_NAr with water to afford pyrimidinone **12**. Finally, a copper-mediated *N*-arylation step delivered analogs **9** in good yields in only three steps.[24] Similarly, pyridine analogs **10** were all prepared in a three step fashion (**Scheme 2**). Here, commercial 4-nitropyridine-1-oxide **13** undergoes an S_NAr reaction with 2-phenoxyethanol to provide **14**. *N*-oxide migration provides the pyridine core, which is then *N*-alkylated under copper catalysis with aryl boronic acids to provide analogs **10**.[24] Variations on this scheme were used to generate analogs **10** where the unsubstituted phenyl moiety was replaced with functionalized aryl and heteroaryl moieties.

Scheme 1. Synthesis of Analogues **9**[a]



[a]Reagents and conditions: (a) 2-phenoxyethanol, NaH, DMF, 0 °C to rt, 46%; (b) K_2CO_3, DABCO, H_2O, 1,4-Dioxane, 70 °C, 83%; (c) 8-hydroxyquinoline, CuI, K_2CO_3, DMSO, microwave, 160 °C, 30 min, 16-45%.

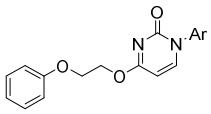
Scheme 2. Synthesis of Analogues **10**[a]



[a]Reagents and conditions: (a) 2-phenoxyethanol, NaH, DMF, 0 °C to 100 °C, 81%; (b) Ac_2O, microwave 140 °C, 60 min, then 1N LiOH, 50 °C, 57%; (c) $ArB(OH)_2$, $Cu(OAc)_2$, pyridine, 4Å MS, DCM, air, 44-65%.

A limited number of pyrimidine analogs **9** were prepared, as SAR was steep and selectivity versus $mGlu_5$ eroded, but Table 1 highlights key analogs in this series. While not productive en route to a new $mGlu_3$ *in vivo* probe, **9a** was a potent $mGlu_3$ NAM (IC_{50} = 295 nM) with no discernable activity at $mGlu_2$ (IC_{50} >30 μM). Relative to **8**, potency was enhanced, molecular weight reduced and fraction unbound in plasma doubled (rat $f_{u,plasma}$ = 0.16), all of which validated the reductionist approach.

Table 1. Structures and activities of analogs **9**.[a]



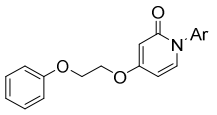
Entry	Ar	$mGlu_3$ IC_{50} (μM)[a] [Glu Min ±SEM]	$mGlu_3$ pIC_{50} (±SEM)	$mGlu_5$ EC_{50} (μM)[a] (pEC_{50}, %Glu Max)
9a		0.29 [3.94±1.1]	6.53±0.08	1.2 (5.9, 97)
9b		>10 [15.5±5.4]	<5	3.5 (5.5, 77)
9c		1.02 [3.77±0.76]	5.99±0.25	0.43 6.4, 83)

[a]Calcium mobilization assays with $mGlu_3/G_{qi5}$-CHO cells performed in the presence of an EC_{80} fixed concentration of glutamate; values represent means from three (n=3) independent experiments performed in triplicate.

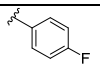
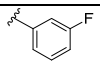
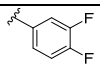
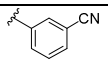
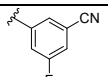
However, as alluded to above, **9a**, while selective versus $mGlu_{1,2,4,6,7,8}$, was an $mGlu_5$ PAM (EC_{50} = 1.2 μM, 97% Glu Max). Interestingly, **9c** was a more potent $mGlu_5$ PAM (EC_{50} = 427 nM, 83% Glu Max) than $mGlu_3$ NAM (IC_{50} = 1.02 μM). These finding were not entirely unexpected, as eliminating $mGlu_5$ PAM activity was a major facet of the optimization effort that delivered **8**.[23] Would deletion of a single nitrogen atom in **9** to yield analogs **10** eliminate the $mGlu_5$ PAM activity while maintain all of the other favorable properties?

Table 2 highlights SAR for the pyridinone analogs **10** which proved more robust than NAMs **9**, with direct analogs of **9b** and **9c** significantly more potent (**10b** and **10c**) $mGlu_3$ NAMs. Relative to **8**, potency was enhanced, molecular weight reduced and fraction unbound in plasma doubled (rat $f_{u,plasma}$ = 0.12 to 0.16), all of which further validated the reductionist approach. Moreover, all were highly selective versus $mGlu_2$ (IC_{50}s > 30 μM), and $mGlu_5$ PAM activity diminished ($mGlu_5$ EC_{50}s in the 400 nM to 6 μM ranges). However, analogs such as **10a** and **10c** emerged with unique, dual $mGlu_3$ NAM/$mGlu_5$ PAM pharmacological profiles; in contrast, **10d** displayed ~18-fold selectivity as an $mGlu_3$ NAM versus $mGlu_5$ PAM activity.

Table 2. Structures and activities of analogs **10**.[a]



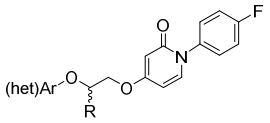
10

Entry	Ar	$mGlu_3$ IC_{50} (μM)[a] [Glu Min ±SEM]	$mGlu_3$ pIC_{50} (±SEM)	$mGlu_5$ EC_{50} (μM)[a] (pEC_{50}, % Glu Max)
10a		0.93 [2.69±0.7]	6.03±0.12	0.56 (6.2, 97)
10b		0.39 [3.45±1.1]	6.41±0.09	1.9 (5.7, 88)
10c		0.18 [3.53±1.1]	6.74±0.09	0.34 (6.5, 91)
10d		0.34 [3.63±1.2]	6.47±0.08	6.0 (5.2, 87)
10e		0.27 [3.39±1.0]	6.57±0.11	1.3 (5.9, 27)

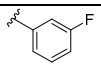
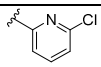
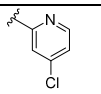
[a]Calcium mobilization assays with $mGlu_3/G_{qi5}$-CHO cells performed in the presence of an EC_{80} fixed concentration of glutamate; values represent means from three (n=3) independent experiments performed in triplicate.

In an effort to eliminate $mGlu_5$ PAM activity, we held the 4-fluorophenyl moiety of the pyridinone constant and surveyed replacements for the western phenyl moiety as well as a methyl substituent on the ether chain, generating analogs **15** (**Table 3**).[24] While $mGlu_3$ activity and selectivity versus $mGlu_2$ was maintained ($mGlu_2$ IC_{50}s > 30 μM), $mGlu_5$ PAM activity persisted in **15a-c** ($mGlu_5$ EC_{50}s in the 1.7 μM to 9 μM ranges), but greatly diminished relative to analogs **10**, with the pyridine ether moieties. However, the racemic methyl congener **15d** proved exceptional. **15d** was a moderately potent $mGlu_3$ NAM (IC_{50} = 1.2 μM), but proved to be selective versus both $mGlu_2$ (IC_{50}s > 30 μM) and $mGlu_5$ (EC_{50}s > 30 μM). In addition, **15d** had a clean CYP_{450} inhibition profile (IC_{50} > 30 μM versus 3A4, 2D6, 2C9 and 1A2), good fraction unbound ($f_{u,plasma}$ = 0.04 (rat),

Table 3. Structures and activities of analogs **15**.[a]

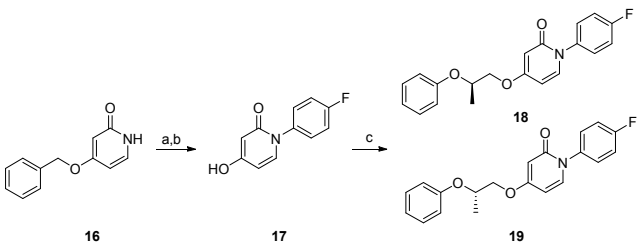


15

Entry	Ar (het)	R	$mGlu_3$ IC_{50} (μM)[a] [Glu Min ±SEM]	$mGlu_3$ pIC_{50} (±SEM)	$mGlu_5$ EC_{50} (μM)[a] (pEC_{50}, % Glu Max)
15a		H	0.35 [3.56±1.1]	6.46±0.06	1.5 (5.8, 93)
15b		H	0.45 [3.62±1.2]	6.35±0.04	7.8 (5.1, 94)
15c		H	1.45 [3.77±0.76]	5.84±0.12	8.8 (5.1, 82)
15d		Me	1.23 [3.57±0.84]	5.91±0.14	>30 (<4.5)

[a]Calcium mobilization assays with $mGlu_3/G_{qi5}$-CHO cells performed in the presence of an EC_{80} fixed concentration of glutamate; values represent means from three (n=3) independent experiments performed in triplicate.

Scheme 3. Synthesis of enantiomers **18** and **19**[a]



16 **17** **19**

[a]Reagents and conditions: (a) 4-FPhB(OH)$_2$, Cu(OAc)$_2$, pyridine, 4Å MS, DCM, air, 72%; (b) 10% Pd/C, H_2 (1 atm), MeOH, 18 h, 99%; (*R*)-2-phenoxypropan-1-ol or (*S*)-2-phenoxypropan-1-ol, PPh_3, D^{t}BAD, THF, rt,18h, 72-75%.

0.10 (human) and $f_{u,brain}$ = 0.05 (rat), was highly CNS penetrant in rat (brain plasma K_p = 1.7, $K_{p,uu}$ = 1.3) and showed moderate predicted hepatic clearance (rat CL_{hep}= 43.2 mL/min/kg, human CL_{hep}=10.7 mL/min/kg; based on microsomal intrinsic clearance data).[24,25] Thus, efforts focused on synthesizing and evaluating the discrete enantiomers of **15d**.

The synthesis of the (*R*) and (*S*) enantiomers of **15d** is shown in Scheme 3.[24] Commercially available pyridine **16** is subjected to the standard copper catalyzed arylation with 4-fluorophenyl boronic acid, followed by 10% Pd/C hydrogenation to provide **17**. A Mitsunobu reaction with either (*R*)-2-phenoxypropan-1-ol or (*S*)-2-phenoxypropan-1-ol, both known compounds,[24] delivers **18** and **19**, respectively in good overall yields and enantiopurity. When assessed in our assays, the (*R*)-enantiomer **18** was devoid of activity at both $mGlu_3$ and $mGlu_2$ (IC_{50}s > 30 μM), demonstrating significant enantiopreference. In contrast (**Figure 4**), the (*S*)-enantiomer **19** proved to be a potent $mGlu_3$ NAM (IC_{50} = 245 nM, pIC_{50} = 6.61±0.12, 3.33±0.31) with high selectivity versus not only $mGlu_2$ (IC_{50} > 30 μM) and $mGlu_5$

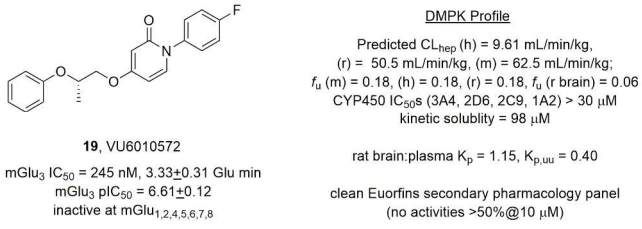


Figure 4. Structure, molecular pharmacology and DMPK profile of **19**.

(EC_{50} > 30 μM), but all mGlu receptors (inactive at $mGlu_{1,4,6,7,8}$). In terms of its DMPK profile, **19** displayed an attractive profile with no CYP_{450} inhibition liabilities (IC_{50}s > 30 μM), good fraction unbound in plasma ($f_{u,plasma}$s ~0.18 for human, rat and mouse), moderate predicted hepatic clearance across species, and, relative to **8**, kinetic solubility (PBS buffer at pH 7.4) improved 4-fold (98 μM). In rat, **19** was highly CNS penetrant (brain:plasma K_p = 1.15, $K_{p,uu}$ = 0.40) and mouse (K_p = 1.17, $K_{p,uu}$ = 0.26). Before any *in vivo* behavior was performed, we also explored broader ancillary pharmacology beyond the mGlus in a Eurofins radioligand binding panel of 68 GPCRs, ion channels, transporters and nuclear hormone receptors.[24,26] Gratifyingly, no significant activities were noted (no inhibition >50% @ 10 μM).

With interest in Group II NAMs for depression-related behaviors, and as potentially novel antidepressants, we evaluated **19** in a mouse tail suspension study[27,28] side-by-side with a new CNS penetrant $mGlu_2$ NAM (**20**, VU6001966)[24,29-32] to dissect the role of the individual Group II mGluRs in this paradigm. The two NAMs were administered i.p. and compared relative to a 30 mg/kg i.p. dose of ketamine (**Figure 5**). The $mGlu_3$ NAM **19** (VU6010572) showed robust efficacy in this model at 3 mg/kg (roughly comparable to the effect elicited by ketamine at 30 mg/kg), while the $mGlu_2$ NAM **20** (VU6001966) was inactive up to 30 mg/kg i.p. Exposure was measured from these studies, and **19** achieved total brain levels of ~1.2 μM (K_p = 1.2, $K_{p,uu}$ = 0.27 for this mouse PK/PD study) at the 3 mg/kg dose (~5-fold above the $mGlu_3$ IC_{50}), while **20** achieved total brain levels of ~14 μM (~180-fold above the $mGlu_2$ IC_{50}) at the highest dose tested (30 mg/kg i.p.). For the majority of our allosteric modulator programs, total brain exposure, and not free brain levels, is the best correlate of *in vivo* efficacy.[22,23,33-35] These early data support a greater contribution of $mGlu_3$ inhibition for the antidepressant effects of dual $mGlu_{2/3}$ NAMs (and in agreement with previous studies with **8**),[23] but studies are underway in other antidepressant behavioral paradigms and in both mice and rats to strengthen these preliminary findings.

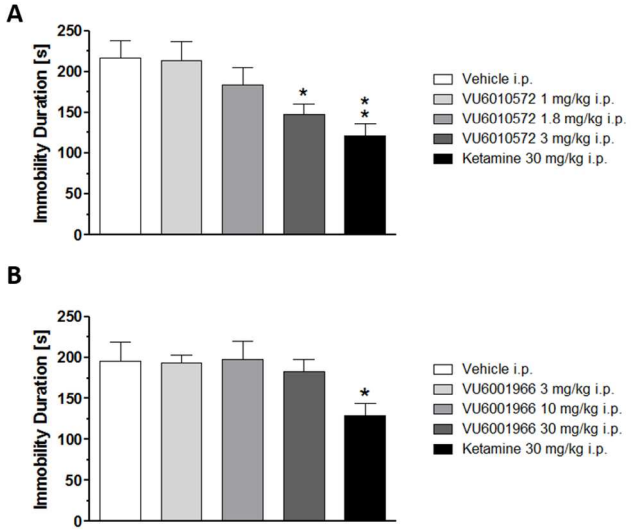


Figure 5. Mouse tail suspension test in CD-1 mice with A) $mGlu_3$ **19** and B) $mGlu_2$ NAM **20**. The MED for $mGlu_3$ **19** is 3 mg/kg i.p., while the $mGlu_2$ NAM **20** is without effect up to 30 mg/kg in this paradigm. Ketamine (the positive control) displays efficacy at 30 mg/kg in this paradigm. Vehicle: 10% Tween 80 in H_2O (10 mL/kg), *n* = 10-14 mice per dose group. *p <0.05 vs. vehicle, **p < 0.01 versus vehicle.[24]

In summary, we have discovered the next generation of highly selective and CNS penetrant $mGlu_3$ NAMs by a reductionist strategy that lowered molecular weight, improved physicochemical and DMPK properties, while also reducing the synthetic route by 50%, relative to **8**. Moreover, a head-to-

head comparison of highly selective and CNS penetrant $mGlu_2$ and $mGlu_3$ NAMs in a mouse tail suspensions test, to assess potential antidepressant phenotype, indicated the $mGlu_3$ inhibition is the dominant mGlu subtype responsible for efficacy. Further anti-depressant paradigms in both mice and rats are underway, and results will be reported in due course.

AUTHOR INFORMATION

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Author Contributions

CWL wrote the manuscript and oversaw the medicinal chemistry. KAE designed compounds and JLE, KAB, MFL, MBM and SRB performed chemical synthesis. PJC, ALR and CMN performed and analyzed molecular pharmacology data. SC, RDMM, TMB and ALB oversaw and analyzed in vitro and in vivo DMPK data. CKJ and MB performed and analyzed the mouse tail suspension assay/data.

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

Metabotropic glutamate receptor (mGlu); PAM, positive allosteric modulator; NAM, negative allosteric modulator; $mGlu_3$, metabotropic glutamate receptor subtype 3; PBL, plasma:brain level; K_p, plasma:brain partitioning coefficient; $K_{p,uu}$, unbound plasma:unbound brain partitioning coefficient; DCM, dichloromethane; DABCO, 1,4-diazabicylco[2.2.2]octane; MED, minimum effective dose; CYP; cytochrome P450; PK/PD, pharmacokinetic/pharmacodynamics; DMPK, drug metabolism and pharmacokinetics; DtBAD, di-*tert*-butyl azodicarboxylate.

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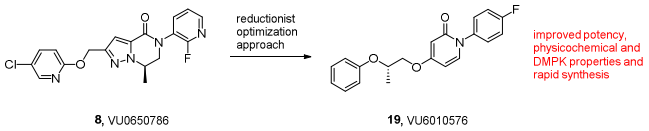
Table of Contents Graphic



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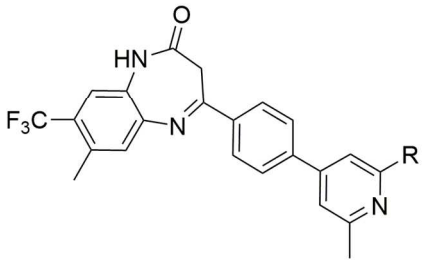

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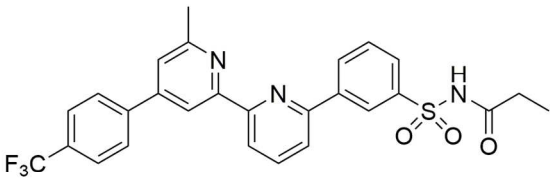

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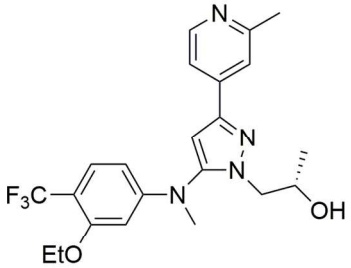

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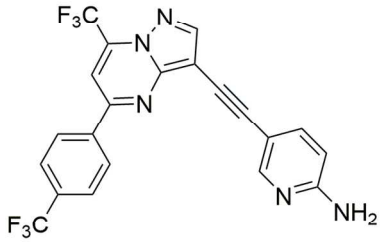

5, decoglurant

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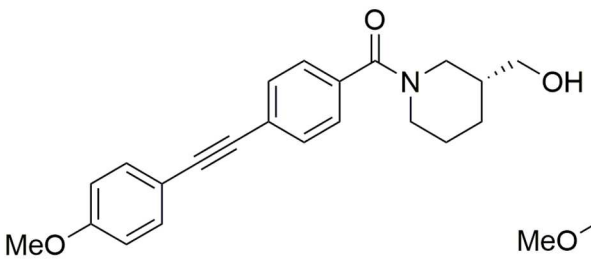
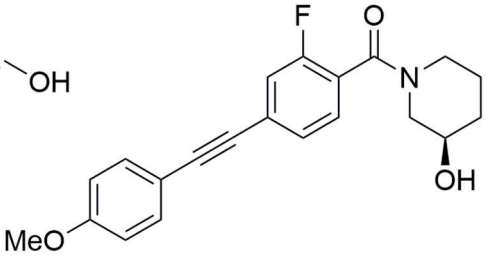
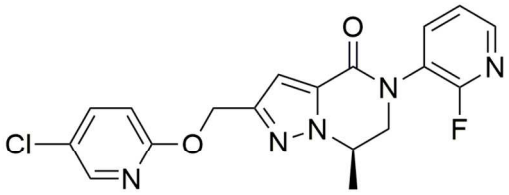


6, VU0463597 (ML289)
$mGlu_3$ IC_{50} = 660 nM
$mGlu_2$ IC_{50} >10 μM



7, VU0469942 (ML337)
$mGlu_3$ IC_{50} = 593 nM
$mGlu_2$ IC_{50} >30 μM



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$mGlu_2$ IC_{50} >30 μM

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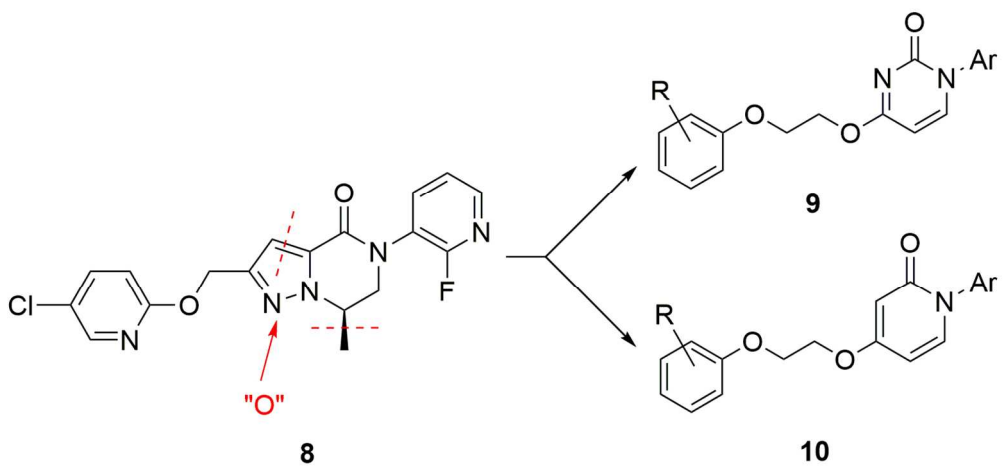

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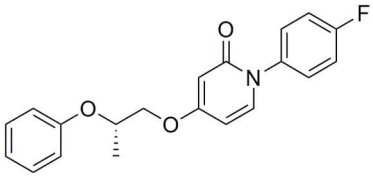

19, VU6010572

$mGlu_3$ IC_{50} = 245 nM, 3.33±0.31 Glu min
$mGlu_3$ pIC_{50} = 6.61±0.12
inactive at $mGlu_{1,2,4,5,6,7,8}$

DMPK Profile

Predicted CL_{hep} (h) = 9.61 mL/min/kg,
(r) = 50.5 mL/min/kg, (m) = 62.5 mL/min/kg;
f_u (m) = 0.18, (h) = 0.18, (r) = 0.18, f_u (r brain) = 0.06
CYP450 IC_{50}s (3A4, 2D6, 2C9, 1A2) > 30 μM
kinetic solublity = 98 μM

rat brain:plasma K_p = 1.15, $K_{p,uu}$ = 0.40

clean Euorfins secondary pharmacology panel
(no activities >50%@10 μM)

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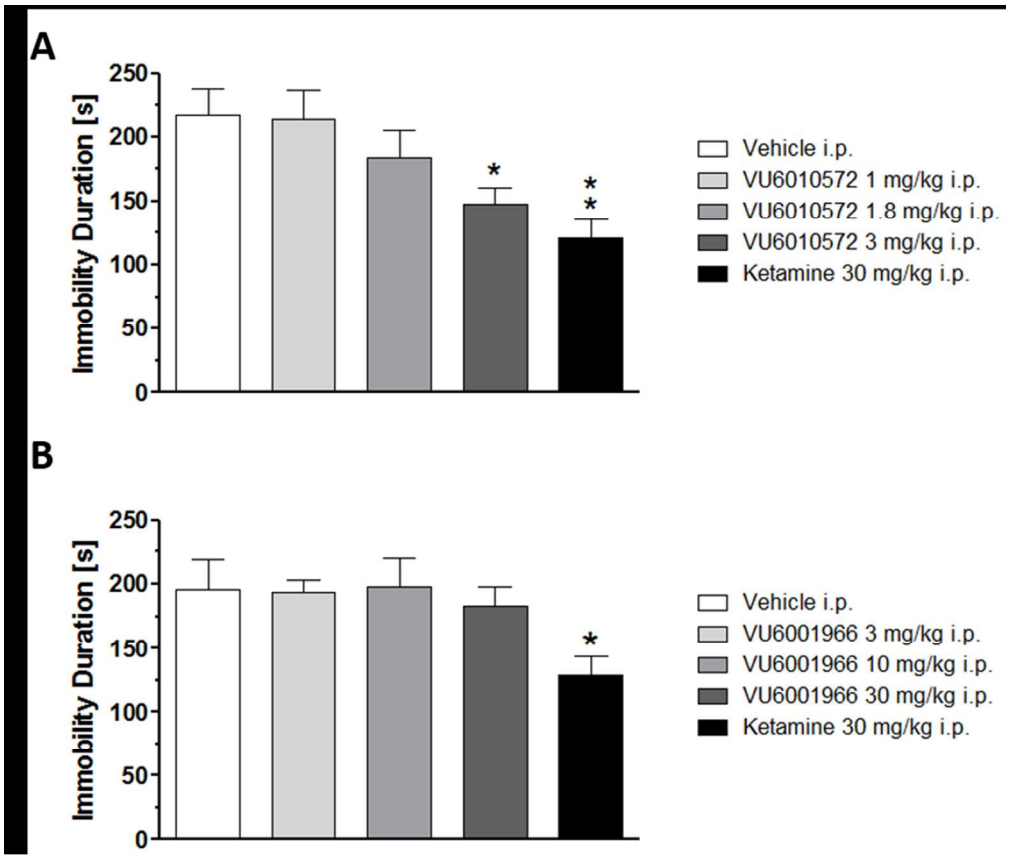

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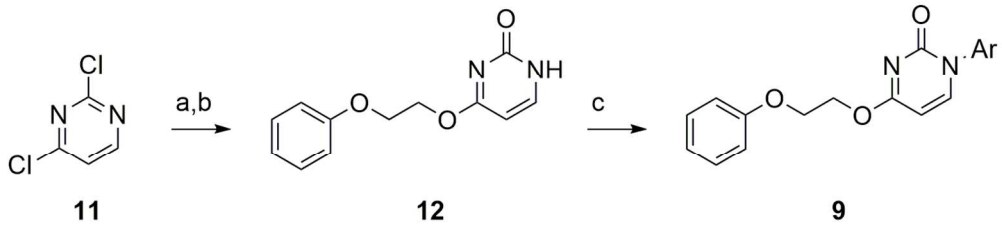

11 **12** **9**

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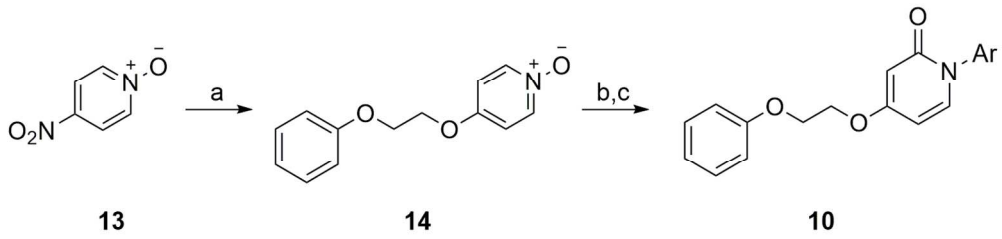

13 **14** **10**

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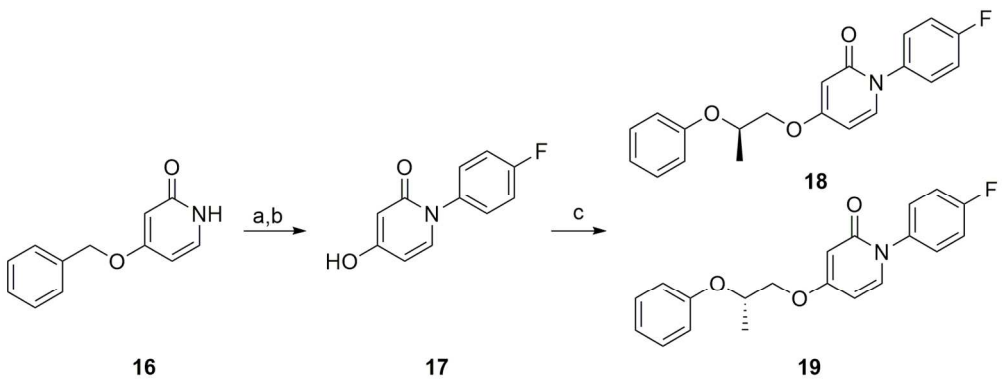

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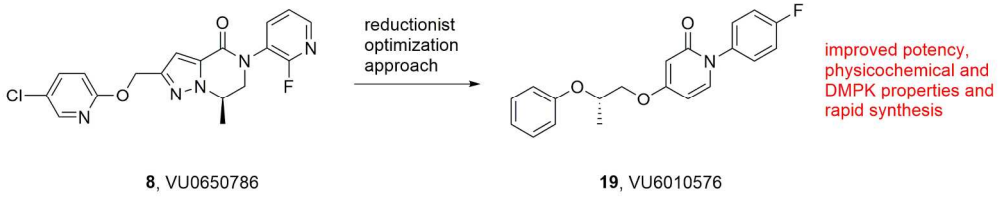

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