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## Discovery, Synthesis, and Structure Activity Relationship Development of a Series of *N*-(4-acetamido)phenylpicolinamides as Positive Allosteric Modulators of Metabotropic Glutamate Receptor 4 (mGlu<sub>4</sub>) with CNS Exposure in Rats

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### Abstract

Herein we report the discovery, synthesis and evaluation of a series of *N*-(4-acetamido)-phenylpicolinamides as positive allosteric modulators of mGlu<sub>4</sub>. Compounds from the series show submicromolar potency at both human and rat mGlu<sub>4</sub>. In addition, pharmacokinetic studies utilizing subcutaneous dosing demonstrated good brain exposure in rats.

### Introduction

G-Protein coupled receptors (GPCRs) comprise a large protein family of transmembrane receptors and have historically been the most studied class of drug targets. GPCRs are activated by orthosteric ligands binding which induce macromolecular protein changes and stimulation of signal transduction pathways and cellular responses inside the cell. The GPCR superfamily is grouped into three classes (class A (rhodopsin-like), B (secretin family) and C (metabotropic glutamate)).<sup>1</sup> The mGlu<sub>s</sub> belong to the Class C GPCR subfamily and are further subdivided into three classes.<sup>2</sup> Group I consists of mGlu<sub>1</sub> and mGlu<sub>5</sub>, Group II consists of mGlu<sub>2</sub> and mGlu<sub>3</sub>, and Group III consists of mGlu<sub>4</sub>, mGlu<sub>6</sub>,

<sup>‡</sup>Abbreviations: SAR: structure-activity relationship; mGluR: metabotropic glutamate receptor; PAM: positive allosteric modulator; HTS: high-throughput screening; Clint: intrinsic clearance; PPB: plasma protein binding; AUC: area under the curve; GPCR: G-protein coupled receptor; PHCCC: *N*-phenyl-7-(hydroxyimino)cyclopropa[*b*]chromen-1a-carboxamide; CNS: central nervous system; PD: Parkinson's disease; GIRK: G protein-coupled inwardly-rectifying potassium channel.

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**Supporting Information Available:** Experimental procedures, spectroscopic data, and NMR data for selected compounds and biological procedures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

mGlu<sub>7</sub> and mGlu<sub>8</sub>. Due to the lack of selectivity and physicochemical properties of orthosteric ligands of the mGlu<sub>s</sub>, significant effort has been initiated on identifying compounds that act via allosteric sites (i.e., sites different from the orthosteric site) on the receptors.<sup>3, 4</sup> This approach has been successful for the Group I and II glutamate receptors<sup>5</sup> (mGlu<sub>1</sub>,<sup>6, 7</sup> mGlu<sub>5</sub>,<sup>8, 9</sup> and mGlu<sub>2</sub><sup>8</sup>) with multiple tool compounds associated with each receptor; however, in contrast, the Group III family has been studied in much less detail due to the paucity of selective ligands – including allosteric modulators.

One subtype that has received much attention of late due to the discovery of selective allosteric ligands and its implication in a number of disease states, such as Parkinson's disease (PD), epilepsy, and anxiety, is mGlu<sub>4</sub>. The interest in targeting this receptor using allosteric modulation was initially sparked via studies with the compound *N*-phenyl-7-(hydroxyimino)cyclopropa[*b*]chromen-1*a*-carboxamide, PHCCC<sup>10, 11</sup>, **1**, a partially selective mGlu<sub>4</sub> positive allosteric modulator (PAM). With the exception of partial antagonist activity at mGlu<sub>1</sub>, **1** was shown to be selective versus the other mGlu<sub>s</sub>. Compound **1** has been an invaluable tool compound for the study of mGlu<sub>4</sub> and has been shown to be active in acute rodent models of Parkinson's disease (PD) when administered either via intracerebroventricular (icv) injection or systemically in a 50% DMSO vehicle. Although **1** has been a breakthrough compound for the study of mGlu<sub>4</sub>, it has substantial drawbacks due to its poor pharmacokinetic properties and relatively flat structure-activity relationship. Over the past three years, our laboratories and others have added to the list of selective and novel tool compounds for the study of mGlu<sub>4</sub> via positive allosteric modulation (Figure 1). Thus far, only two disclosed mGlu<sub>4</sub> PAMs (**3** (VU0361737)<sup>12</sup> and **4**<sup>13</sup>) have been shown to be CNS penetrant when dosed systemically in a nontoxic vehicle. In this communication, we herein disclose a second series of *N*-(4-acetamido)-phenylpicolinamides that act as mGlu<sub>4</sub> positive allosteric modulators and show that representative compounds are also CNS penetrant.

Starting from a functional high-throughput screening (HTS) initiated at Vanderbilt University, a number of *N*-(4-acetamido)-phenylcarboxamide compounds were discovered, as exemplified by compound **5** (Figure 2, Pubchem Compound ID (CID): 1314024; ChemBridge ID: 7637085). This compound represented a large class of hits that were identified by our HTS and signaled a starting point for a lead identification program in our laboratories.

## Results

The compounds were prepared via a common intermediate such that the phenyl core and the 2-chlorophenyl amide could be held constant while the furyl amide portion was evaluated (Scheme 1). To this end, the nitro aniline, **6**, was acylated with 2-chlorophenyl acid chloride to give **8**. Hydrogenation of the nitro group (Ra-Ni, H<sub>2</sub>) followed by amide formation led to compounds **9a–k** and **10a–d**. An alternative approach was employed for compounds **12a–n**. First, the aniline of **6** was di-Boc protected (Boc<sub>2</sub>O, DMAP), followed by nitro reduction (H<sub>2</sub> (1 atm), Pd/C) and picolinamide formation (picolinyl acid chloride, diisopropyl ethyl amine) leading to **12**. Finally, Boc deprotection (4 M HCl, dioxane) followed by amide formation yielded **12a–n**.

## Discussion

The initial SAR was centered around the furyl amide portion (**9a–k**) in order to replace the 5-bromo-2-furyl amide portion as this is a high molecular weight compound (MW = 449.7). Several heterocyclic replacements, as well as aryl and carbocycles, were evaluated. The two pharmacological assays we utilized to assess mGlu<sub>4</sub> PAM potency (1. Chinese Hamster

Ovary cells expressing human mGlu4 and the chimeric G protein Gq<sub>15</sub> to induce calcium mobilization and 2. HEK cells expressing rat mGlu4 in conjunction with G protein regulated Inwardly Rectifying Potassium (K) channels (GIRK) to induce thallium flux<sup>16</sup>) show some day-to-day variability in the maximal PAM response; for this reason, all data have been normalized to the % response of the control PAM, **1**, to compare relative efficacy.<sup>17, 18</sup> Initial removal of the bromine resulted in a more potent compound (**9a**, furyl, 977 nM), while the addition of a large phenyl group led to complete loss of activity (**9b**). Moving from the furyl compound to the saturated tetrahydrofuran (**9c**) also led to an inactive compound. Substituting the furan for sulfur containing heterocycles, such as thiophene and thiazole, resulted in compounds that were equipotent (**9d** and **9f**) or more potent (**9e**, 2-thiazole, 627 nM). Next, 6-membered heterocycles (**9g-i**) were evaluated with the 2-pyridyl compound, **9h**, exhibiting the best potency (99.5 nM). Modulation of the basicity of the pyridine nitrogen led to a 10-fold loss in potency (pyrazine, **9g**, 1780 nM; 4-pyrimidine, **9i**, 1330 nM). Replacement of the heterocyclic amides with carbocyclic amides also led to much reduced potency (cyclohexyl, **9k**, 1120 nM).

Having discovered a more potent replacement for the initial 5-bromo-2-furyl amide, we next turned our attention to the central core as this contains a 3-methoxy moiety which, when contained on the 2,4-dianiline species, could lead to bis-quinone formation. To this end, a number of substituted phenyl analogs were evaluated (Table 2). The 3-methoxy was replaced by both halogens (3-Cl, **10a**, 517 nM; 3-F, **10b**, 556 nM), trifluoromethyl (**10c**, 3750 nM) and ultimately removed (**10d**, 1650 nM). All of these substitutions led to a significant decrease in activity; however, we were encouraged to see that halogen substitution was tolerated and generated compounds with submicromolar potency.

Next, our attention turned to the amide portion that originally was the 2-chlorophenyl amide (Table 3). Noting that the fluoro- and chloro-phenyl compounds were equipotent, we utilized both of these cores when assessing the right-hand amide SAR and a series of aryl, cycloalkyl, alkyl and heteroaromatic amides were evaluated. A number of interesting observations regarding the SAR were gleaned from this study. The 2-chlorophenyl (**10a**, 517 nM) was superior to other 2-substituted compounds (2-fluorophenyl, **12f**, 2380 nM; 2-methylphenyl, **12a**, 958 nM; 2-methoxyphenyl, **12i**, inactive). Moving the halogen around the ring or adding a second halogen generally led to compounds with much reduced potency (**12g** and **12h**, inactive); however, the 2-chloro-4-fluoro compound (**12d**, 114 nM) was more active than the 2-chloro analog (**10a**, 517 nM). However, when the 2-chloro-4-fluoro was replaced with 2,4-difluoro (**12g**, inactive) there was a significant loss of potency (similar to the loss seen in **10a** vs. **12f**). Carbocycles were of equal potency (cyclohexyl, **12b**, 426 nM vs. phenyl, **12c**, 361 nM); however, the smaller isopropyl group lost significant activity (**12e**, 4270 nM). All of the heterocyclic substituents were much less active with the exception of 2-thiazole (**12k**, 403 nM).

Lastly, as this structural class represents a 2,4-dianiline, we were interested to determine if we could replace the internal core structure with a non-aniline compound (see Table 1, Supporting Information). We utilized an indole core structure and when the NH of either amide was replaced with an alkyl substituent, a significant loss of potency was seen. Alkylation of either amide also led to inactive compounds (data not shown).

With our best compounds evaluated for human and rat potency, we next turned our attention to evaluation of the compounds' ability to shift the glutamate response curve to the left, i.e. glutamate potentiation (fold shift, FS) as well as to assessment of the microsomal stability (intrinsic clearance) and the ability of the compounds to bind plasma proteins (see Table 4). For the most part, the GluMax (% **1**) was mirrored in the fold shift data, although a few exceptions are worth noting. First, the 2-thiazole (**9e**) and 2-pyridyl (**9g**) both had high

GluMax values (133.9% and 147.0%, respectively); however, this relatively small difference produced a significant difference in fold shift (32.2 vs. 72.0, respectively). Both of these values are the largest fold shifts reported to date for an mGlu<sub>4</sub> PAM. As observed for other allosteric modulators, the fold shift data, representative of compound efficacy, did not always track with the potency values (**10a**, 570 nM, 18.3 FS vs. **12d**, 169 nM, 6.7 FS).

Compounds were further evaluated by assessing their rate of intrinsic clearance in vitro as well as their ability to bind to plasma proteins (PPB) (see Table 2, Supporting Information). Two compounds possessed poor metabolic stability (**9e** and **15**). These compounds possess a labile methoxy group which could be the contributing factor their instability. Interestingly, **9g** also possesses a methoxy group but exhibits acceptable intrinsic clearance values (42.6 mL/min/kg). All of the other compounds tested exhibited acceptable intrinsic clearance (<50 mL/min/kg). All compounds evaluated were highly protein bound in rats (>99.5% bound). In addition to establishing in vitro pharmacology, metabolic stability and protein binding profiles, representative compounds (**9g**, **10a**, and **12a**) were tested for their inhibition of the major CYP450 enzymes (CYP1A2, 2C9, 3A4 and 2D6). These compounds showed no significant activity against CYP1A2 and CYP3A4 (>30 μM), but each of the compounds showed weak activity against CYP2C9 and CYP2D6 (>10 μM). Lastly, selectivity versus the other mGlu's was performed on **10a** and this compound was selective versus the Group I and Group II mGlu's; but showed weak PAM activity (as assessed by the ability of **10a** to left-shift the glutamate response curve at 30 μM) against mGlu<sub>7</sub> (5.3 FS) and mGlu<sub>8</sub> (3.8 FS) (see Table 2, Supporting Information). While compound is not completely selective for mGlu<sub>4</sub>, this compound seems to be group III mGlu preferring, which may allow for future rationally designed site directed mutagenesis studies to help confirm the binding location on the receptor.

Although there is a large range of fold shift values within compounds in this series, all other properties appear to be equivalent (intrinsic clearance, PPB, CYP inhibition profile); thus three representative compounds were chosen to further advance into in vivo PK studies (see Table 5). The hydrochloride salts of these compounds (**9g**, **10a**, **12a**) were synthesized and dosed subcutaneously (10 mg/kg) as an aqueous microsuspension containing 10% Tween-80, and the amount of compound present in brain and plasma was determined at 0.5, 1, 3, and 6 h after administration (Table 6). Uptake of **10a** (VU0366037) was substantial in both plasma and brain with a brain:plasma ratio of 1.04. Plasma exposure of VU0366038, **12a** and VU0415374, **9g** were comparable to **10a**; however, these compounds exhibited lower brain exposure with a brain:plasma ratio of 0.2 and 0.33, respectively.

## Conclusion

In summary, we have discovered and evaluated a novel series of *N*-(4-acetamido)-phenylpicolinamides as mGlu<sub>4</sub> positive allosteric modulators suitable as in vivo tool compounds. Compounds **9g**, **10a**, and **12a** exhibited submicromolar EC<sub>50</sub>'s against both human and rat mGlu<sub>4</sub>, and all three robustly left-shifted the glutamate response curve with **9g** and **10a** possessing fold shifts >15. In addition, compounds **10a** and **12a** showed acceptable in vitro PK parameters (intrinsic clearance) and were further evaluated for CNS exposure. **10a** showed good exposure in both plasma and brain, with a ratio of 1.04.

## Experimental Section

### General

The syntheses of selected compounds are described below. The general chemistry, experimental information, and syntheses of all other compounds are supplied in the

Supporting Information. Purity of all final compounds was determined by HPLC analysis is >95%.

#### ***N*-(4-(2-chlorobenzamido)-3-methoxyphenyl)picolinamide (9g)**

To a solution of **8g** (0.91 g, 2.98 mmol) in ethanol (EtOH):ethyl acetate (EtOAc) (200 mL, 1:1) was added Ra-Ni (~0.1 equivalents). An H<sub>2</sub> atmosphere (1 atm) was applied to the rxn mixture. After 16 h, the rxn was filtered through Celite and concentrated. The crude residue was deemed >85% pure by LCMS and carried through without further purification. To a solution of the aforementioned aniline in dimethylformamide (DMF) (25 mL) and diisopropyl ethylamine (DIEA) (5 mL, 28.7 mmol) was added 2-pyridinecarbonyl chloride (0.53 g, 2.98 mmol). After 12 h at rt, the rxn was added to EtOAc:H<sub>2</sub>O (150 mL, 1:1). The organic layer was washed with H<sub>2</sub>O (2 × 50 mL), Brine (50 mL) and dried (MgSO<sub>4</sub>). After filtration and concentration, the residue was purified by mass-guided preparative HPLC to afford amide **9g** (0.66 g, 58% yield over 2 steps). Analytical LCMS (Method 2): single peak (220 nm), R<sub>T</sub> = 1.167 min., *m/z* 382.0 [M + H]<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.64 (s, 1H), 9.58 (s, 1H), 8.74 (d, *J* = 4.4 Hz, 1H), 8.16 (d, *J* = 8.0 Hz, 1H), 8.07 (ddd, *J* = 8.0, 8.0, 1.6 Hz, 1H), 7.83 (d, *J* = 8.8 Hz, 1H), 7.75 (d, *J* = 2.0 Hz, 1H), 7.68 (dd, *J* = 6.4, 4.8 Hz, 1H), 7.58–7.46 (m, 5H), 3.82 (s, 3H); HRMS, calc'd for C<sub>20</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>Cl (M+H<sup>+</sup>), 382.0958; found 382.0958.

#### ***N*-(3-chloro-4-(2-chlorobenzamido)phenyl)picolinamide (10a)**

Following the general procedure above for **9g**, compound **10a** (0.62 g, 81%) was obtained as a tan solid: Analytical LCMS (Method 2): single peak (214 nm); R<sub>T</sub> = 1.496 min.; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.91 (s, 1H), 10.18 (s, 1H), 8.77 (d, *J* = 4.8 Hz, 1H), 8.23 (d, *J* = 2.0 Hz, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.10 (ddd, *J* = 8.0, 1.6, 1.6 Hz, 1H), 7.93 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.73–7.70 (m, 1H), 7.63–7.46 (m, 5H); HRMS, calc'd for C<sub>19</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>Cl<sub>2</sub> [M + H]<sup>+</sup>, 386.0463; found 386.0467.

#### ***N*-(3-chloro-4-(2-methylbenzamido)phenyl)picolinamide (12a)**

To a solution of **6** (1.67 g, 9.69 mmol) and dimethylaminopyridine (DMAP) (0.12 g, 0.97 mmol) in tetrahydrofuran (THF) (50 mL) at rt was added (Boc)<sub>2</sub>O (6.33 g, 29.0 mmol). After 16 h, the rxn was added to EtOAc:water (200 mL, 1:1) and the organic layer was separated and washed with Brine (50 mL), dried (MgSO<sub>4</sub>), filtered and concentrated. To a solution of the crude residue in EtOAc (200 mL) was added Rainey-Ni (~0.1 equivalent) and an H<sub>2</sub> atmosphere via balloon (1 atm) was applied. After 16 h, LCMS confirms loss of starting material, mixture was filtered through a Celite filter and concentrated and was carried on without purification. To a solution of the crude residue in dichloromethane (DCM) (20 mL) was added DIEA (3.4 mL, 24.2 mmol), followed by picolinoyl acid chloride hydrochloride (1.72 g, 9.69 mmol). After 16 h, the rxn was added to DCM:NaHCO<sub>3</sub> (aq) (200 mL, 1:1) and the organic layer was separated. The organic layer was washed with Brine (50 mL), dried (MgSO<sub>4</sub>), filtered and concentrated to afford amide **11** which was carried on without purification. To a solution of amide **11** in DCM (100 mL) at 0 °C was added 4M HCl in 1,4-dioxane (20 mL). After 15 min, the ice bath was removed. The violet red solution stirred for 12 h at rt and after TLC confirmed loss of starting material, the solvent was removed under vacuo. To a mixture of the crude residue in DMF (20 mL) at 0 °C was added DIEA (4.78 mL, 34.0 mmol) followed by *o*-toluoyl chloride (1.26 mL, 9.69 mmol). After 15 min, the ice bath was removed. After an additional 12 h, the rxn was added to EtOAc:H<sub>2</sub>O (200 mL, 1:1). The organic layer was washed with H<sub>2</sub>O (2 × 50 mL), Brine (50 mL) and dried (MgSO<sub>4</sub>). After filtration and concentration, the residue was purified by mass-guided preparative HPLC to afford amide **12a** (1.45 g, 41% overall yield). Analytical LCMS (Method 2): single peak (214 nm); R<sub>T</sub> = 1.482 min.; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.90 (s, 1H), 9.94 (s, 1H), 8.77 (d, *J* = 4.0 Hz, 1H), 8.22 (d, *J* = 2.0 Hz,



1H), 8.19 (d,  $J = 8.0$  Hz, 1H), 8.10 (ddd,  $J = 8.0, 2.0, 2.0$  Hz, 1H), 7.91 (d,  $J = 8.4$  Hz, 1H), 7.71 (ddd,  $J = 7.6, 4.8, 1.6$  Hz, 1H), 7.58 (d,  $J = 8.8$  Hz, 5H), 7.54 (d,  $J = 7.6$  Hz, 1H), 7.40 (dd,  $J = 7.2, 7.2$  Hz, 1H), 7.33–7.30 (m, 2H), 2.46 (s, 3H); HRMS, calc'd for  $C_{20}H_{17}N_3O_2Cl$   $[M + H]^+$ , 366.1009; found 366.1009.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

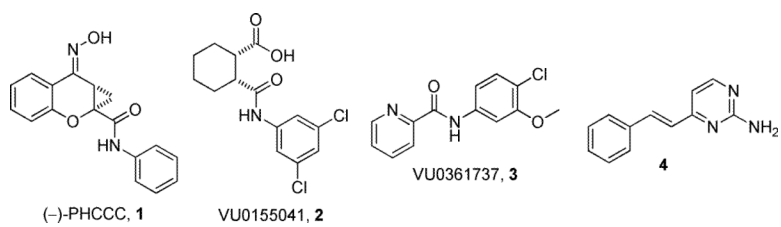
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## References and Notes

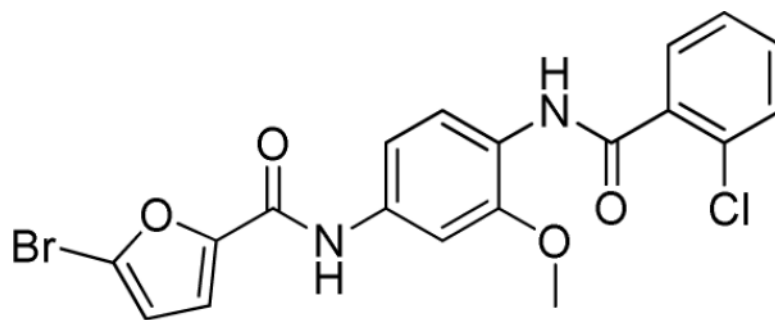
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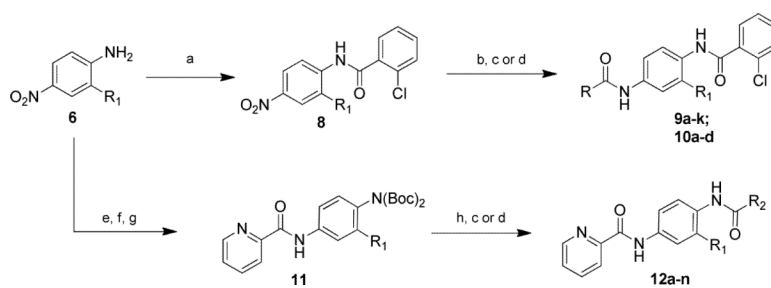
**Figure 1.** Structures of **1** and other recently reported mGlu<sub>4</sub> PAMs.





**5**  
**CID 1314024**

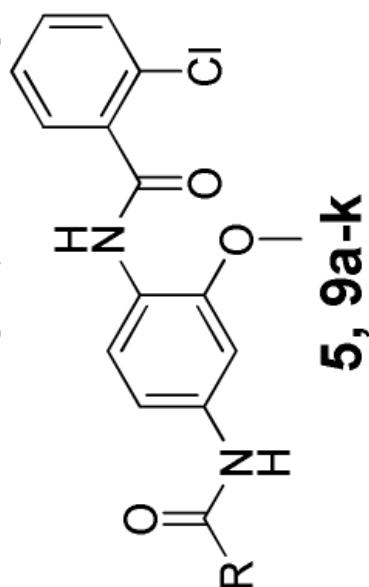
**Figure 2.**  
Structure of initial HTS hit.

**Scheme 1.**

Synthesis of N-(4-acetamido)-phenylcarboxamides, **9**, **10**, and **12**. All library compounds were purified by mass-directed prep LC where required.<sup>14, 15</sup>

Table 1

Human and Rat mGlu<sub>4</sub> potency and % GluMax response (as normalized to standard **1**) for selected furyl amide analogs.



Compd	R	hEC <sub>50</sub> (nM) <sup>a</sup>	GluMax (%Cmpd <b>1</b> ) <sup>b</sup>	rEC <sub>50</sub> (nM) <sup>d</sup>	GluMax (%Cmpd <b>1</b> ) <sup>b</sup>
<b>5</b>	5-bromo-2-furan	1440 ± 100	87.0 ± 1.2	1000 ± 90	125.4 ± 6.7
<b>9a</b>	2-furan	977 ± 99	62.7 ± 2.4	1010 ± 200	122.3 ± 2.9
<b>9b</b>	5-phenyl-2-furan	inactive <sup>c</sup>	16.3 ± 1.5	inactive <sup>c</sup>	16.3 ± 2.7
<b>9c</b>	2-tetrahydrofuran	inactive <sup>c</sup>	21.3 ± 2.9	>10,000	27.1 ± 3.0
<b>9d</b>	2-thiophene	1170 ± 150	63.4 ± 1.5	860 ± 90	96.7 ± 8.5
<b>9e</b>	2-thiazole	627 ± 80	77.6 ± 1.7	377 ± 101	133.9 ± 6.6
<b>9f</b>	4-thiazole	845 ± 39	86.8 ± 2.3	753 ± 164	132.4 ± 6.6
<b>9g</b>	2-pyridine	99.5 ± 9	79.4 ± 1.7	106 ± 28	147.0 ± 4.3
<b>9h</b>	pyrazine	1780 ± 140	74.3 ± 0.4	1120 ± 180	111.7 ± 6.6
<b>9i</b>	4-pyrimidine	1330 ± 90	77.9 ± 3.5	894 ± 203	133.1 ± 6.2
<b>9j</b>	phenyl	cannot fit <sup>d</sup>		cannot fit <sup>d</sup>	
<b>9k</b>	cyclohexyl	1120 ± 270	26.9 ± 5.3	1520 ± 630	43.2 ± 13.2
<b>1</b>		4740 ± 51		2820 ± 44	

<sup>a</sup>EC<sub>50</sub> and GluMax, are the average of at least three independent determinations performed in triplicate (Mean ± SEM shown in table).

<sup>b</sup>**1** is run as a control compound each day, and the maximal response generated in mGlu<sub>4</sub> CHO cells in the presence of mGlu<sub>4</sub> PAMs varies slightly in each experiment. Therefore, data were further normalized to the relative **1** response obtained in each day's run.

<sup>c</sup>Inactive compounds are defined as %GluMax did not surpass 2× the EC<sub>20</sub> value at the highest concentration (30 μM) for that day's run.

$p$  Curve could not be fit due to insolubility at higher concentrations.

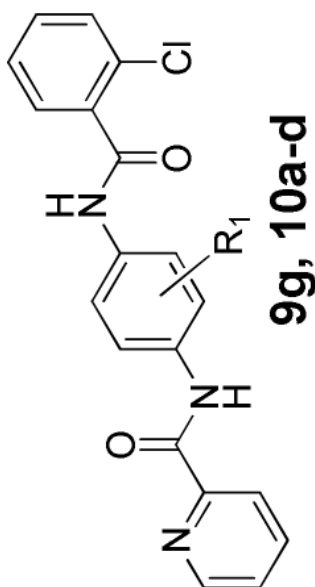
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Table 2

Human and Rat mGlu<sub>4</sub> potency and % GluMax response (as normalized to standard **1**) for selected central phenyl modification analogs.



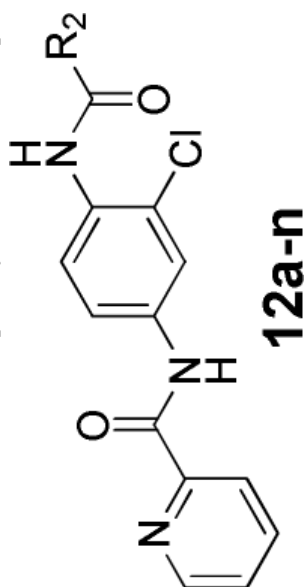
Compd	R <sub>1</sub>	hEC <sub>50</sub> (nM) <sup>a</sup>	GluMax (%Cmpd 1) <sup>b</sup>	rEC <sub>50</sub> (nM) <sup>a</sup>	GluMax (%Cmpd 1) <sup>b</sup>
<b>9g</b>	3-OMe	99.5 ± 9	79.4 ± 1.7	106 ± 28	147.0 ± 4.3
<b>10a</b>	3-Cl	517 ± 57	77.9 ± 1.6	570 ± 131	128.2 ± 5.6
<b>10b</b>	3-CF <sub>3</sub>	2090 ± 190	69.5 ± 1.5	2360 ± 230	101.9 ± 7.4
<b>10c</b>	H	1605 ± 200	33.6 ± 0.5	2260 ± 260	49.6 ± 5.4
<b>10d</b>	3-F	556 ± 76	59.7 ± 1.1	739 ± 92	91.4 ± 2.9

<sup>a</sup>EC<sub>50</sub> and GluMax, are the average of at least three independent determinations performed in triplicate (Mean ± SEM shown in table).

<sup>b</sup>Cmpd **1** is run as a control compound each day, and the maximal response generated in mGlu<sub>4</sub> CHO cells in the presence of mGlu<sub>4</sub> PAMs varies slightly in each experiment. Therefore, data were further normalized to the relative **1** response obtained in each day's run.

Table 3

Human and Rat mGlu<sub>4</sub> potency and % GluMax response (as normalized to standard **1**) for selected acetamido amide analogs.



Compd	R <sub>2</sub>	hEC <sub>50</sub> (nM) <sup>a</sup>	GluMax (%Cmpd <b>1</b> ) <sup>b</sup>	rEC <sub>50</sub> (nM) <sup>a</sup>	GluMax (%Cmpd <b>1</b> ) <sup>b</sup>
<b>12a</b>	2-methylphenyl	958 ± 48	72.5 ± 1.1	915 ± 161	123.1 ± 2.9
<b>12b</b>	cyclohexyl	426 ± 58	59.2 ± 1.0	592 ± 70	106.0 ± 0.7
<b>12c</b>	phenyl	361 ± 40	37.9 ± 1.3	518 ± 43	74.2 ± 8.7
<b>12d</b>	2-chloro-4-fluorophenyl	114 ± 26	61.1 ± 4.1	169 ± 28	118.8 ± 7.3
<b>12e</b>	isopropyl	4270 ± 540	60.0 ± 1.1	4820 ± 1160	109.0 ± 3.4
<b>12f</b>	2-fluorophenyl	2380 ± 170	34.5 ± 1.0	4390 ± 1140	71.6 ± 10.0
<b>12g</b>	2,4-difluorophenyl	inactive <sup>c</sup>	24.6 ± 1.5	2100 ± 780	43.5 ± 12.0
<b>12h</b>	3,5-dichlorophenyl	2150 ± 448	37.4 ± 3.2	2340 ± 817	61.0 ± 9.0
<b>12i</b>	2-methoxyphenyl	inactive <sup>c</sup>	16.4 ± 1.3	inactive <sup>c</sup>	19.0 ± 2.1
<b>12j</b>	5-thiazole	2180 ± 310	86.7 ± 2.0	1730 ± 170	124.8 ± 8.7
<b>12k</b>	2-thiazole	403 ± 28	61.5 ± 2.0	533 ± 145	105.5 ± 2.4
<b>12l</b>	2-thiophene	1040 ± 100	57.8 ± 1.8	1130 ± 140	93.8 ± 3.8
<b>12m</b>	N-methyl-2-indole	4720 ± 900	49.9 ± 4.1	2970 ± 290	87.1 ± 3.9
<b>12n</b>	5-phenyl-2-furyl	inactive <sup>c</sup>	24.9 ± 0.8	3350 ± 290	45.6 ± 10.8

<sup>a</sup>EC<sub>50</sub> and GluMax, are the average of at least three independent determinations performed in triplicate (Mean ± SEM shown in table).

<sup>b</sup>Cmpd **1** is run as a control compound each day, and the maximal response generated in mGlu<sub>4</sub> CHO cells in the presence of mGlu<sub>4</sub> PAMs varies slightly in each experiment. Therefore, data were further normalized to the relative **1** response obtained in each day's run.

<sup>c</sup>Inactive compounds are defined as %GluMax did not surpass 2× the EC<sub>20</sub> value at the highest concentration (30 μM) for that day's run.



Table 4

Fold shift, intrinsic clearance and plasma protein binding (PPB, rat) and CYP450 enzyme inhibition data for selected compounds.

Compd	rEC <sub>50</sub> (nM) <sup>a</sup>	GluMax (%Cmpd 1) <sup>b</sup>	rFS	Rat Clint (Cl <sub>HEP</sub> ), (mL/min/kg)	PPB (rat, %fu)	P450 (μM)				
						IA2	2C9	3A4	2D6	2D6
<b>9e</b>	377 ± 101	133.9 ± 6.6	32.2 ± 2.4	185.3 (50.8)	0.56	ND	ND	ND	ND	
<b>9g</b>	106 ± 28	147.0 ± 4.3	72.0 ± 4.5	42.6 (26.5)	0.33	>30	14.3	>30	12.8	
<b>10a</b>	570 ± 131	128.2 ± 5.6	18.3 ± 1.3	44.9 (27.3)	0.29	>30	11.8	>30	13.1	
<b>10d</b>	739 ± 92	91.4 ± 2.9	4.6 ± 0.1	31.2 (21.6)	0.58	ND	ND	ND	ND	
<b>12a</b>	915 ± 161	123.1 ± 2.9	9.6 ± 0.6	46.2 (27.8)	0.35	>10	14.1	>30	12.0	
<b>12d</b>	169 ± 28	118.8 ± 7.3	6.7 ± 2.0	54.0 (30.5)	ND	ND	ND	ND	ND	
<b>12k</b>	533 ± 145	105.5 ± 2.4	6.0 ± 0.6	ND	0.65	ND	ND	ND	ND	
<b>15</b>	1340 ± 200	107.1 ± 3.2	5.9 ± 1.1	605.2 (62.7)	0.16	ND	ND	ND	ND	

<sup>a</sup>EC<sub>50</sub> and GluMax, are the average of at least three independent determinations performed in triplicate (Mean ± SEM shown in table).

<sup>b</sup>Cmpd **1** is run as a control compound each day, and the maximal response generated in mGlu4 CHO cells in the presence of mGlu4 PAMs varies slightly in each experiment. Therefore, data were further normalized to the relative **1** response obtained in each day's run. ND = not determined.

**Table 5**

Rat Pharmacokinetic Data for Compounds VU0415374 (**9g**), VU0366037 (**10a**), and VU0366038 (**12a**) following SC dosing (10 mg/kg; 10% tween 80 microsuspension). AUC=area under the curve as assessed from 0–6 hours.

parameters	VU0415374, 9g	VU0366037, 10a	VU0366038, 12a
Plasma AUC (ng·hr/mL)	938	657.8	766.1
Plasma C <sub>max</sub> (ng/mL)	265	198.1	219.2
Brain AUC (ng·hr/g)	312	681.0	155.4
Brain C <sub>max</sub> (ng/g)	69.1	163.2	59.4
T <sub>max</sub> (h)	1	0.5	0.5
AUC <sub>0–6h, brain</sub> /AUC <sub>0–6h, plasma</sub>	0.33	1.04	0.2