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Discovery of 3-aminopicolinamides as metabotropic glutamate receptor subtype 4 (mGlu4) positive allosteric modulator warheads engendering CNS exposure and in vivo efficacy

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ABSTRACT

This letter describes the further chemical optimization of the picolinamide-derived family of $mGlu_4$ PAMs wherein we identified a 3-amino substituent to the picolinamide warhead that engendered potency, CNS penetration and in vivo efficacy. From this optimization campaign, VU0477886 emerged as a potent (EC_{50} = 95 nM, 89% Glu Max) $mGlu_4$ PAM with an attractive DMPK profile (brain:plasma K_p = 1.3), rat CL_p = 4.0 mL/min/kg, $t_{1/2}$ = 3.7 h) and robust efficacy in our standard preclinical Parkinson's disease model, haloperidol-induced catalepsy (HIC).

Metabotropic glutamate receptor subtype 4 ($mGlu_4$) has garnered a great deal of interest as a novel, non-dopaminergic target for the potential treatment of Parkinson's disease (PD) by virtue of selective activation via positive allosteric modulators (PAMs)[1–6] and normalization of the overactive indirect pathway within the basal ganglia circuitry.[7,8] Beyond symptomatic treatment of motor disturbances, emerging data suggests $mGlu_4$ PAMs may also be neuroprotective, and hence disease-modifying.[1–6] As discussed in the field, SAR can be steep for certain allosteric chemotypes, and adequate CNS penetration, as well as overall DMPK properties, have been an ongoing challenge in the development of $mGlu_4$ PAMs.[6,9,10] An optimization program in our lab based on an HTS hit has afforded a diverse array of picolinamide-based $mGlu_4$ PAMs **1**–**5** (Fig. 1),[11–15] and by surveying bioisosteres, the identification of our first preclinical candidate, VU0418506 (**6**).[16] Our back-up program sought to distance from the pyrazolo[4,3-*b*]pyridine head group of **6**, and one aspect focused on functionalized picolinamides, leveraged en route to **7**.[16] In this Letter, we will detail the discovery, SAR, DMPK profiles and in vivo efficacy of the newly identified 3-aminopicolinamide $mGlu_4$ PAM warheads.

In route to the pyrazolo[4,3-*b*]pyridine head group of **6** (EC_{50} = 67 nM, pEC_{50} = 7.17 ± 0.07, 109.3 ± 3.8% Glu Max), we had synthesized and evaluated a single 3-aminopicolinamide, **7** (EC_{50} = 587 nM, pEC_{50} = 6.24 ± 0.03, 88.1 ± 2.0% Glu Max) analog (Fig. 2).[16] While ~7-fold less potent than **6**, **7** represented a structurally distinct and unexplored chemotype. Thus, we quickly prepared a diverse set of either 3- or 4-functionalized picolinamides **8**–**15**; however, all proved to be inactive as $mGlu_4$ PAMs, suggesting that the 3-amino moiety, and the presence of a hydrogen bond donor, in **7** was a required pharmacophore.

We then elected to survey the 3-aminopicolinamide warhead in the context of a broader array of Eastern moieties (e.g., imides, aryl/heteroaryl, sulfonamides, ethers) previously shown to engender $mGlu_4$ PAM activity, maintaining a key *meta*-chloro moiety. The chemistry to assemble analogs **17** was straight forward, as the Eastern moieties (R = phthalimides, imides, lactams and ethers)

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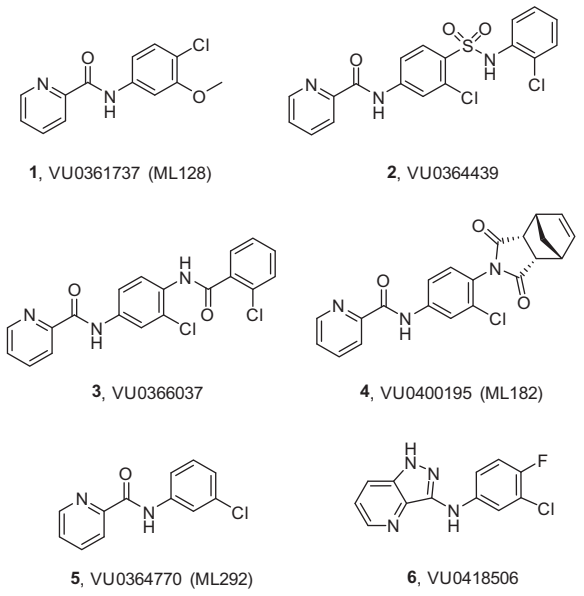


Figure 1. Structures of picolinamide-based $mGlu_4$ PAMs (**1**–**5**), and the first preclinical candidate, VU0418506 (**6**).

have all previously been described[11–15] and were available as functionalized anilines **16a**–**d** (Scheme 1).

The SAR for analogs **17** (Table 1) represented a departure from simple picolinamides **1**–**5** or the pyrazolo[4,3-*b*]pyridine head group of **6**, displaying a steep phenotype. Phthalimides, such as **17a** and **17d,** displayed good PAM activity, as did certain isoindolinones, such as **17e** and **17h**. However, electronics played a key role in activity, as an electron-rich isoindolinone, **17h,** possessed an EC_{50} of 319 nM, whereas an electron-deficient congener, **17g**, was devoid of PAM activity (EC_{50} > 10 μM). This proved to be a general trend (F and CN also devoid of PAM activity). Lactams **17j**–**s** also displayed a range of $mGlu_4$ PAM activity, from inactive to 758 nM, driven mainly by lipophilicity and steric bulk. Here, only two analogs, **17a**, a phthalimide congener, and **17t**, an ether derivative, displayed potency (EC_{50}s below 100 nM) comparable to the clinical candidate, **6**. The unsubstituted phthalimide **17a** was potent at both human and rat $mGlu_4$ (EC_{50}s of 94.5 nM and 128 nM, respectively), and possessed attractive physicochemical properties (MW = 392, *c*Log*P* = 2.52 and TPSA = 105 Å^2). Similarly, the chemically distinct, pyrimidinyl ether **17t** was even more potent (human and rat $mGlu_4$ EC_{50}s of 64.6 nM and 46.6 nM, respectively) and also possessed favorable physicochemical properties (MW = 396, *c*Log*P* = 2.27 and TPSA = 103). However, before locking into these two novel $mGlu_4$ PAMs for more detailed characterization, we wanted to fully evaluate other SAR within this series of $mGlu_4$ PAMs.

As the 3-aminopicolinamide moiety afforded potent $mGlu_4$ PAMs, we then surveyed the activity of the corresponding 3-hydroxypicolinamide analog of **17a**, **18** (Fig. 3). Compound **18** ($mGlu_4$ EC_{50} = 125 nM, 115.4% Glu Max) proved to be of comparable potency to **17a**, but offered no advantage in terms of physiochemical properties. Then, in the context of **17t**, we surveyed alternative heteroaromatic amino amides, and found that the isonicotinamide derivative, **19**, was devoid of $mGlu_4$ PAM activity (EC_{50} > 10 μM), while a pyrazine analog, **20**, was active (EC_{50} = 296 nM, 103.9% Glu Max), but lost ~5-fold relative to **17t**. Therefore, we advanced both **17a** and **17t** in a battery of in vitro and in vivo DMPK assays.[11–16]

For in vivo tool compounds, the in vitro and in vivo DMPK profiles of both **17a** (VU0477886) and **17t** (VU0483872) were attractive. PAM **17a** displayed moderate intrinsic clearance in both rat and human microsomes (CL_{HEP} 51 mL/min/kg and 5.8 mL/min/kg, respectively), limited free fraction (F_u (*h*, *r*) of 0.007 and 0.003) and a mixed cytochrome P_{450} profile (3A4, 2D6 and 1A2 IC_{50}s > 30 μM, 2C9 IC_{50} = 7.2 μM). In Sprague Dawley (SD) rats, **17a** was a low clearance compound (CL_P = 3.98 mL/min/kg) with a low volume (V_{ss} = 0.85 L/kg) and a 3.7 h half-life. In a rat plasma:brain level (PBL) cassette study (0.25 mg/kg, 0.25 h, IV), **17a** effectively partitioned into the CNS (K_p = 1.31) and was not a human P-gp substrate (efflux ratio = 1.2). Similarly, PAM **17t** displayed moderate to high intrinsic clearance in both rat and human microsomes (CL_{HEP} 46.1 mL/min/kg and 17.71 mL/min/kg, respectively), limited free fraction (F_u (*h*, *r*) of 0.004 and 0.004) and a mixed cytochrome P_{450} profile (2C9 2D6 IC_{50}s > 30 μM, 1A2 IC_{50} = 2.6 μM and 3A4 IC_{50} = 21.1 μM). In SD rats, **17t** was a low clearance compound (CL_P = 13.7 mL/min/kg) with a uniform volume (V_{ss} = 0.86 L/kg) and a 1.5 h half-life. In a rat PBL cassette study (0.25 mg/kg, 0.25 h, IV) **17t** displayed moderate partitioning into the CNS (K_p = 0.36) and was not a human P-gp substrate (efflux ratio = 1.9). Both **17a** and **17t** displayed excellent selectivity versus the other mGlu receptors. Thus, both new PAMs possessed acceptable profiles to advance into our standard rodent pharmacodynamic model for Parkinson's disease (PD), reversal of haloperidol-induced catalepsy (HIC).[11–16]

PAM **17a** (VU0477886) was evaluated first via an oral route of administration in the HIC model (Fig. 4), and compared side-by-side with the preclinical candidate, **6** (VU0418506). In this study, a 1 mg/kg p.o. dose of **17a**, proved to be as efficacious as **6** in reversing a robust 1.5 mg/kg dose of haloperidol. As our previous PBL study was IV, we also collected plasma and brain samples from

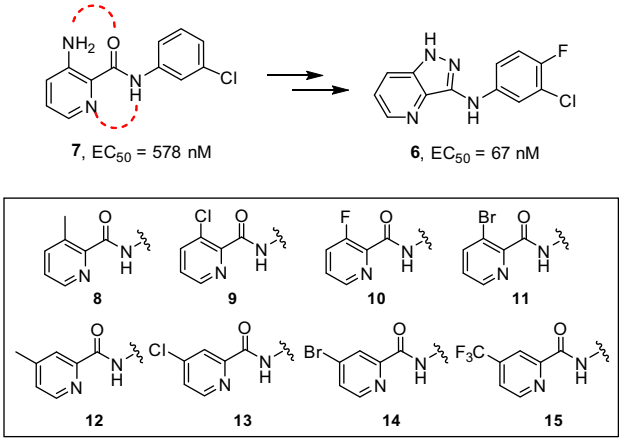
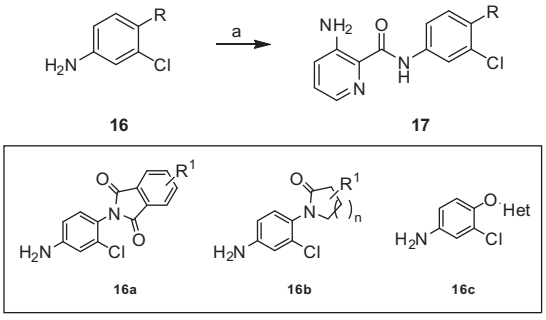
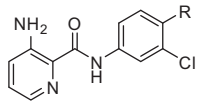


Figure 2. Structures of the lone 3-aminopicolinamide $mGlu_4$ PAM (**7**), and a diverse array of inactive functionalized picolinamide head groups **8**–**15** (inset).

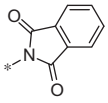
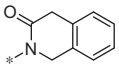
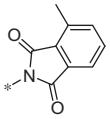
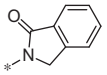
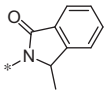
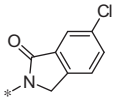
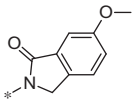
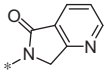
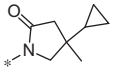
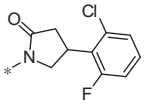
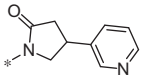
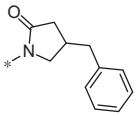
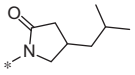


Scheme 1. Preparation of analogs **17**. *Reagents and conditions:* (a) 3-aminopicolinic acid or 3-hydroxypicolinic acid, HATU, DIEA, DCM:DMF, rt, 24 h, 65–90%.[17]

Table 1
Structures and $mGlu_4$ PAM activity of analogs **17**

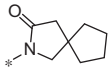
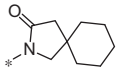
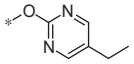
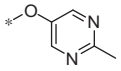


17

Compd	R	$mGlu_4$ EC_{50}(μM)[a]	pEC_{50} ± SEM[a]	% Glu Max[b]
17a		0.095	7.02 ± 0.50	89.1 ± 3.9
17b		2.19	5.66 ± 0.07	63.2 ± 1.8
17c		0.921	6.04 ± 0.09	60.7 ± 2.2
17d		0.689	6.16 ± 0.14	122.5 ± 6.2
17e		0.236	6.63 ± 0.21	43.9 ± 6.2
17f		3.96	5.40 ± 0.01	76.8 ± 0.6
17g		>10	>5	42.3 ± 2.6
17h		0.319	6.50 ± 0.07	71.7 ± 11.1
17i		1.25	5.90 ± 0.05	68.9 ± 0.8
17j		>10	>5	39.6 ± 4.7
17k		>10	>5	12.7 ± 3.0
17l		2.80	5.55 ± 0.07	66.2 ± 1.4
17m		1.86	5.73 ± 0.05	75.9 ± 0.9
17n		2.52	5.60 ± 0.13	92.8 ± 2.4
17o		0.758	6.12 ± 0.05	45.3 ± 4.1
17p		1.61	5.79 ± 0.04	74.5 ± 2.7

(*continued on next page*)

Table 1 (*continued*)

Compd	R	$mGlu_4$ EC_{50}(μM)[a]	pEC_{50} ± SEM[a]	% Glu Max[b]
17q		>10	>5	13.8 ± 3.3
17r		1.28	5.89 ± 0.08	52.0 ± 3.5
17s		>10	>5	47.7 ± 2.7
17t		0.065	7.19 ± 0.11	97.9 ± 4.0
17u		1.35	5.87 ± 0.15	89.8 ± 2.0

[a] Calcium mobilization $mGlu_4$ assay; values are average of $n = 3$.
[b] Amplitude of response in the presence of 30 μM test compound, normalized to a standard compound, PHCCC (100% Glu Max).[11–16]

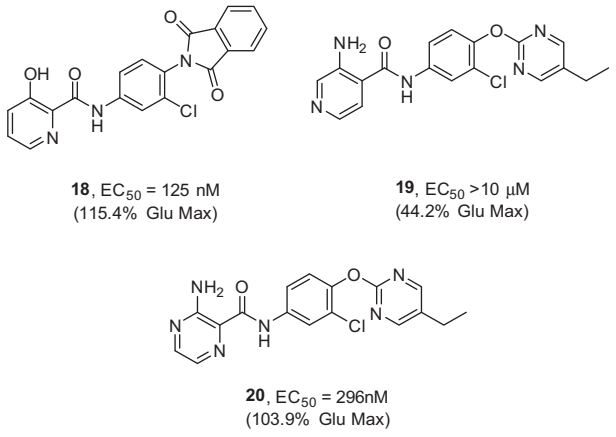


Figure 3. Structures and $mGlu_4$ PAM activities of close analogs of **17a** and **17t**, highlighting the unique pharmacology of the 3-aminopicolinamide.

the oral HIC study to assess PK/PD. In this case, total brain levels reached 334 nM ($K_p = 0.3$), a value greater than 2-fold above the rat $mGlu_4$ PAM EC_{50} (128 nM).

PAM **17t** (VU0483872), in the same paradigm, proved to be more efficacious than **6** (Fig. 5), displaying a minimum effective dose (MED) of 1 mg/kg p.o. and robust reversal at 3 mg/kg p.o. comparable to candidate **6** at 10 mg/kg p.o. Once again, as our previous PBL study was IV, we also collected plasma and brain samples from the oral HIC study to assess PK/PD. In this case, total brain levels reached 172 nM ($K_p = 0.2$), a value greater than 3.5-fold above the rat $mGlu_4$ PAM EC_{50} (46.6 nM). Thus, both 3-aminopicolinamide derivatives proved to be potent $mGlu_4$ PAMs in vitro and in vivo.

In summary, we report the first detailed SAR of a novel series of $mGlu_4$ PAMs based on a 3-aminopicolinamide warhead. New $mGlu_4$ PAMs **17a** (VU0477886) and **17t** (VU0483872) proved to be potent PAMs, possessed favorable physicochemical properties and DMPK properties, displayed excellent CNS penetration and demonstrated robust in vivo efficacy in the HIC preclinical model of PD. Moreover, **17t** performed as well, if not better than our first $mGlu_4$ PAM preclinical candidate **6** (VU0418506). While attractive in many aspects, neither **17a** nor **17t** advanced as back-ups to **6** due to low F_u, suboptimal multispecies PK and poor projected

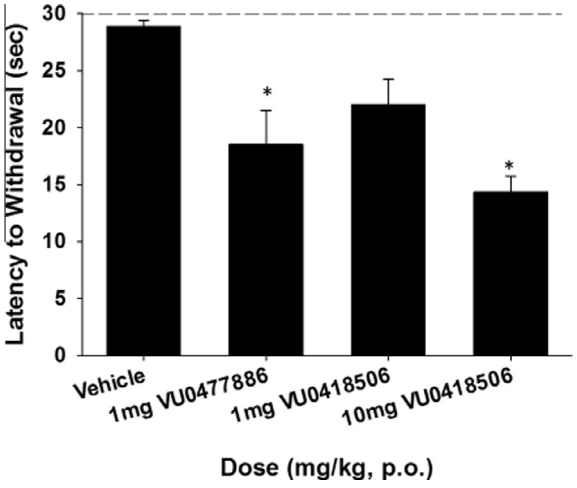


Figure 4. Reversal of haloperidol-induced catalepsy (HIC) with either **17a** (VU0477886) or preclinical candidate **6** (VU0418506). Dosed orally as a microsuspension in 0.1% Tween80/0.5% methylcellulose bead-milled 30 min after haloperidol (1.5 mg/kg). $n = 9$–10 rats/group, $^*p < 0.05$ in comparison to vehicle.

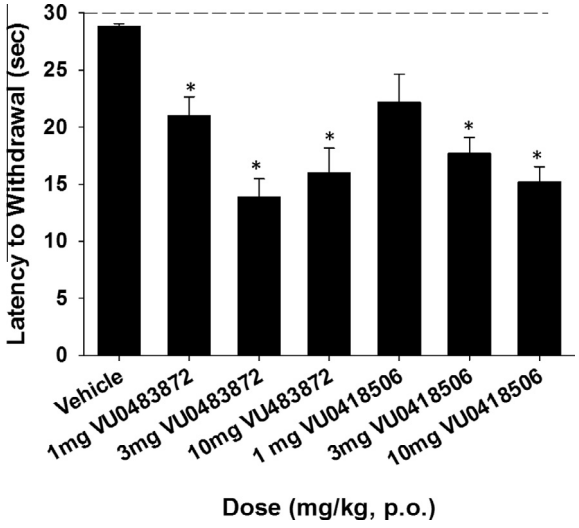


Figure 5. Reversal of haloperidol-induced catalepsy (HIC) with either **17t** (VU0483872) or preclinical candidate **6** (VU0418506). Dosed orally as a microsuspension in 0.1% Tween80/0.5% methylcellulose bead-milled 30 min after haloperidol (1.5 mg/kg). $n = 9$–10 rats/group, $^*p < 0.05$ in comparison to vehicle.

human PK. However, both represent new in vivo tool compounds to study $mGlu_4$ potentiation, and demonstrate solid PK/PD relationships in HIC. Efforts towards suitable back-up compounds continue and will be reported in due course.

Acknowledgments

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17. Representative synthesis of **17a** (VU0477886). *N*-(3-chloro-4-(1,3-dioxoisoindolin-2-yl)phenyl)-3-aminopicolinamide. In a vial, 300 mg (0.993 mmol, 1.0 equiv) of 2-(2-chloro-4-nitrophenyl)isoindoline-1,3-dione were resuspended in dioxane. The suspension was cold in an ice bath and purged with argon. A previously prepared solution of tin(II) chloride (4.5 equiv) in concentrated hydrochloric acid (5 M concentration of $SnCl_2$) was added dropwise to the suspension. After 2 h of stirring at room temperature, the reaction was neutralized carefully with aqueous potassium carbonate 20%, filtered and extracted with diethyl ether. The organic phase was dried with magnesium sulphate, filtered and the volatiles eliminated in vacuo to yield a yellow solid (196 mg, 72%). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ (ppm): 7.97 (2H, m), 7.90 (2H, m), 7.14 (1H, d, J = 8.5 Hz), 6.76 (1H, d, J = 2.4 Hz), 6.60 (1H, dd, J = 8.6 Hz, J = 2.4 Hz), 5.74 (2H, s, –NH_2).^{13}C NMR (100.6 MHz, DMSO-d_6) δ (ppm): 167.5, 151.5, 135.3, 132.7, 131.78, 131.76, 123.9, 116.5, 113.6, 113.0. In a vial, 0.088 mmol (1.2 equiv) of the 3-aminopicolinic acid were added and dissolved in 0.5 mL mixture of DCM:DIEA (9:1), then 41 mg (0.110 mmol, 1.5 equiv) of HATU were added. The mixture was stirred for 10 min, and 20 mg (0.073 mmol, 1.0 equiv) *N*-(4-aminophenyl)phthalimide dissolved in 0.5 mL of DCM:DIEA (9:1), followed by 3 drops of DMF. The reaction was stirred for 24 h at room temperature. After this time, the reaction was quenched with the addition of water, and was worked up by extraction with DCM (2 mL, thrice). The organic phased was filtered through a phase separator, volatiles were evaporated, the crude was dissolved in DMSO and purified by preparative HPLC. Cream powder. ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 10.33 (1H, s), 8.16 (1H, d, J = 2.3 Hz), 8.01 (2H, m), 7.95 (1H, dd, J = 4.2 Hz, J = 1.1 Hz), 7.83 (2H, m), 7.74 (1H, dd, J = 8.6 Hz, J = 2.3 Hz), 7.35 (1H, d, J = 8.6 Hz), 7.25 (1H, dd, J = 8.4 Hz, J = 4.3 Hz), 7.09 (1H, dd, J = 8.4 Hz, J = 1.2 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 166.8, 165.6, 146.3, 139.9, 136.6, 134.4, 133.6, 131.9, 130.7, 128.6, 127.9, 125.4, 124.4, 120.6, 118.3. HRMS (TOF, ES+) $C_{20}H_{14}ClN_4O_3$ [M+H]+ calcd mass 393.0754, found 394.0753.