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Development of Novel, CNS Penetrant Positive Allosteric Modulators for the Metabotropic Glutamate Receptor Subtype 1 ($mGlu_1$), Based on an *N*-(3-Chloro-4-(1,3-dioxoisoindolin-2-yl)phenyl)-3-methylfuran-2-carboxamide Scaffold, That Potentiate Wild Type and Mutant $mGlu_1$ Receptors Found in Schizophrenics

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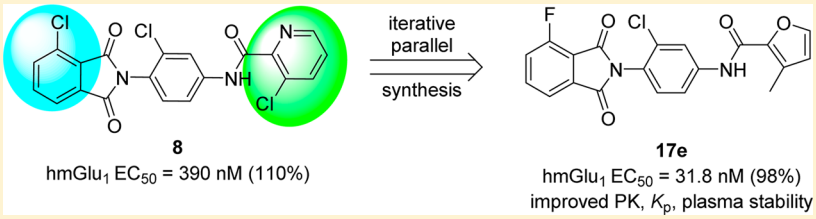
Supporting Information



ABSTRACT: The therapeutic potential of selective $mGlu_1$ activation is vastly unexplored relative to the other group I mGlu receptor, $mGlu_5$; therefore, our lab has focused considerable effort toward developing $mGlu_1$ positive allosteric modulators (PAMs) suitable as in vivo proof of concept tool compounds. Optimization of a series of $mGlu_1$ PAMs based on an *N*-(3-chloro-4-(1,3-dioxoisoindolin-2-yl)phenyl)-3-methylfuran-2-carboxamide scaffold provided **17e**, a potent ($mGlu_1$ EC_{50} = 31.8 nM) and highly CNS penetrant (brain to plasma ratio (K_p) of 1.02) $mGlu_1$ PAM tool compound, that potentiated not only wild-type human $mGlu_1$ but also mutant $mGlu_1$ receptors derived from deleterious *GRM1* mutations found in schizophrenic patients. Moreover, both electrophysiological and in vivo studies indicate the $mGlu_1$ ago-PAMs/PAMs do not possess the same epileptiform adverse effect liability as $mGlu_5$ ago-PAMs/PAMs and maintain temporal activity suggesting a broader therapeutic window.

INTRODUCTION

Since the discovery in the 1980s that glutamate (Glu) activated metabotropic glutamate (mGlu) receptors, in addition to α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors, these class C GPCRs have been the focus of intense research and drug discovery efforts.[1,2] There are eight subtypes of mGlu receptors, $mGlu_{1-8}$, that are classified into three groups (group I, $mGlu_{1,5}$; group II, $mGlu_{2,3}$; group III, $mGlu_{4,6,7,8}$) based on sequence homology, pharmacology, and signal transduction mechanism.[1] While early efforts to understand the physiological role of group I mGlu receptors relied on orthosteric, "glutamate-derived" agonists, excitotoxicity and seizure liability limited their utility.[1−9] Later efforts employed allosteric ligands that afforded subtype selectivity as well as mitigation of the adverse pharmacology by mimicking physiological conditions.[1−9] Of the group I mGlus, $mGlu_5$ is by the far the most understood, due in large part to a diverse array of allosteric ligands, both positive allosteric modulators (PAMs) and negative allosteric modulators (NAMs), for which clinical candidates have been developed (and clinical proof-of-concept has been shown in the case of $mGlu_5$ NAMs).[5−12] In contrast, a diverse array of $mGlu_1$ NAMs exists,[2] but very few $mGlu_1$ PAMs have been reported, despite being the first PAMs for any mGlu receptor.[3] The first generation $mGlu_1$ PAMs **1**−**3** and **5** were developed by Roche (Figure 1) in the early 2000s and were potent ($mGlu_1$ EC_{50} values of 56−200 nM), but the drug metabolism and pharmacokinetic (DMPK) profiles were poor and the majority displayed significant species differences (only **2** and **5** proved to potentiate both human and rat $mGlu_1$).[13−16] While **5** has been used in the literature to validate the role of $mGlu_1$ in various CNS disorders,[17−20] it is highly protein bound (F_u = 0.01), CNS penetration is modest (K_p = 0.29), and it possesses a short half-life ($t_{1/2}$ = 54 min) in rat, rendering **5**, as well as the later introduced **4**, less than ideal as in vivo tool compounds by

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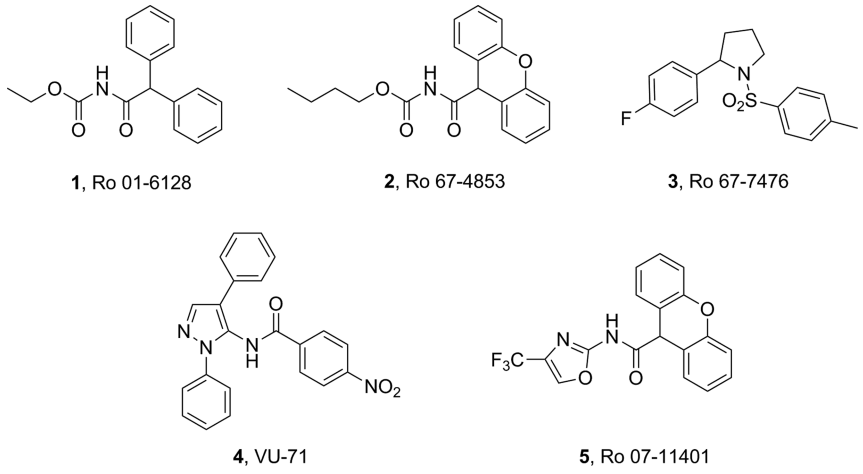

Figure 1. Structure of the known $mGlu_1$ PAMs **1**−**5**, upon which target validation and therapeutic potential have been based. $mGlu_1$ PAMs **1**−**3** were the first described, but only **2** and **5** are active at both rat and human $mGlu_1$. In addition to pronounced species differences, all possessed poor physicochemical and DMPK properties, which limited their utility as in vivo proof of concept tool compounds.

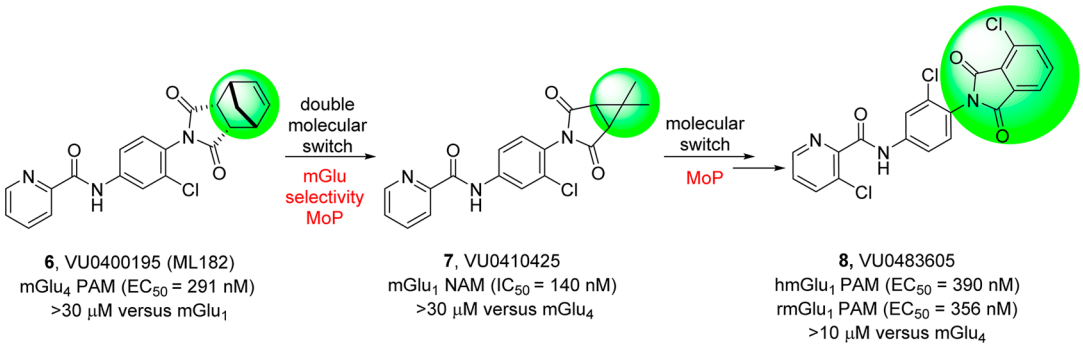

Figure 2. Second generation $mGlu_1$ PAM **8**, derived from a series of "molecular switches", converting a highly selective $mGlu_4$ PAM **6** into an $mGlu_1$ NAM **7** and then, with limited optimization, conversion into a selective $mGlu_1$ PAM with comparable activity at human, rat, and mutant $mGlu_1$ receptors.

today's standards. However, as the only mGu_1 PAM tool compound with CNS exposure, the field had no other option than to employ the lone existing tool to validate and understand the receptor's role in numerous CNS disorders.[17−20] Thus, to provide an $mGlu_1$ PAM in vivo tool compound with a more desired profile (F_u > 3%, K_p > 0.8, clean CYP profiles) to ensure robust target engagement and validation, as well as a distinct chemotype to avoid chemotype-related pharmacology, our lab has recently focused on the development of next generation $mGlu_1$ PAMs and ago-PAMs.

While our interest has historically resided in the development of $mGlu_5$ PAMs and NAMs for various CNS disorders,[5−12] two recent, independent studies identified 12 rare, deleterious nonsynonymous single nucleotide polymorphisms (nsSNPS) in the *GRM1* gene, which encodes $mGlu_1$, in schizophrenic patients.[21,22] These findings led us to explore these loss of function mutant receptors and assess if signaling could be restored with an $mGlu_1$ PAM.[23,24] In lieu of an HTS campaign to provide the desired $mGlu_1$ tool compounds, we relied on the propensity of certain allosteric ligands to possess "molecular switches".[25,26] In this case, an iterative parallel synthesis optimization effort focused on the $mGlu_4$ PAM **6** (based on an *N*-(3-chloro-4-((3a*R*,4*S*,7*R*,7a*S*)-1,3-dioxo-1,3,3a,4,7,7a-hexahydro-2*H*-4,7-methanoisoindol-2-yl)phenyl)picolinamide scaffold) provided the highly selective $mGlu_1$ NAM **7** (Figure 2), which underwent a "double molecular switch" (e.g., a switch for mGlu receptor selectivity from $mGlu_4$ to $mGlu_1$ as well as a concomitant switch in the mode of pharmacology from PAM to NAM). Further optimization resulted in an additional mode of pharmacology (MoP) "molecular switch" to afford the highly selective $mGlu_1$ PAM **8** ($mGlu_1$ EC_{50} = 390 nM) and a fundamentally new $mGlu_1$ PAM chemotype.[23] Both Roche's **5** and our **8** were able to potentiate (i.e., render glutamate a more potent agonist) all of the mutant $mGlu_1$ receptors (as well human WT $mGlu_1$) and to restore efficacy (Glu Max) in a limited number of the mutant receptors. There are also double and triple mutants that correlate with even worse disease prognosis, and those are currently being generated for evaluation with $mGlu_1$ PAMs.[17,18] Across all of our diverse family A, B, and C GPCR PAM programs, the pharmacological parameters that predict in vivo efficacy vary greatly.[5−8,27,28] In some case, we observe no effect on E_{max}, yet see robust in vivo efficacy, even with a low fold-shift, while for other GPCRs, increases in either E_{max} or large fold-shifts are required.[5−8,27−37] Thus, the ideal pharmacological profile for an $mGlu_1$ PAM against these mutant receptors is not clear, and we have yet to characterize PAMs against the double and triple $mGlu_1$ mutants.[21,22] On the basis of the susceptibility of $mGlu_1$ to change its response to allosteric modulators due to small changes in its sequence (i.e., the species disconnect observed between human and rat form of the receptor due to a L757V change), we were pleased to see that the mutant $mGlu_1$

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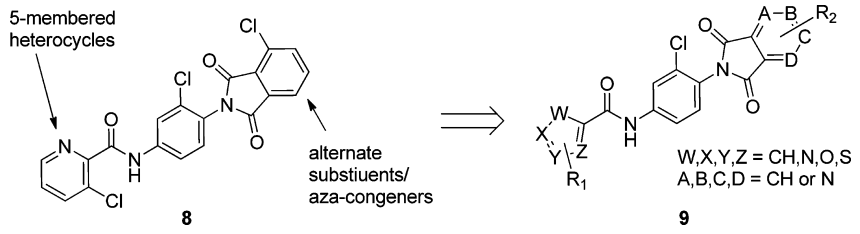
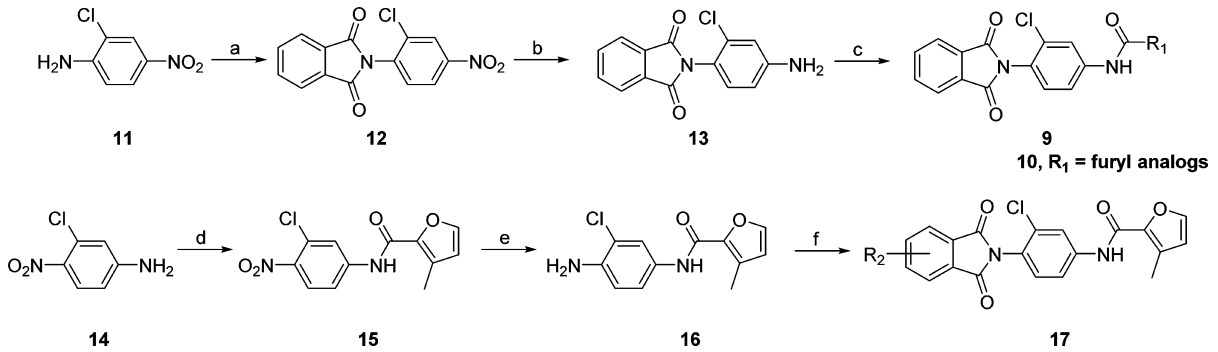


Figure 3. Optimization plans for $mGlu_1$ PAM **8** to enhance potency, stability, and CNS penetration via analogs **9**.

Scheme 1. General Synthetic Route to Access $mGlu_1$ PAMs 9, 10, and 17[a]



[a]Reagents and conditions: (a) phthalic anhydride, AcOH, reflux, 88%; (b) $SnCl_2$, HCl, dioxane, rt, 72%; (c) RCO_2H or substituted furylcarboxylic acids, HATU, DIEA, DCM, rt, 43−96%; (d) 3-methyl-2-furanyl chloride, DIEA, DCE, microwave 120 °C, 30 min, 97%; (e) $SnCl_2$, HCl, dioxane, rt, 96%; (f) phthalic anhydrides, AcOH, reflux, 34−92%.

receptors explored could still be modulated with small molecule PAMs.[24] Thus, these preliminary data fueled our interest in $mGlu_1$ as a potential new schizophrenia target for patients harboring these mutations (as well as to revisit other indications for $mGlu_1$ PAMs); however, the DMPK profile of **8**, while improved over **5** in terms of K_p (0.84 vs 0.29),[23] could still be further optimized in order to obtain a more suitable tool for in vivo studies (e.g., improve potency, PK, F_u). This is also essential for target validation with a PAM.[5−8,27,28] Our studies have shown that for some mGlu receptors, such as $mGlu_4$, free brain levels of the PAM correlate with efficacy, while for others, such as $mGlu_5$, total brain levels of the PAM afford the most robust correlate.[5−8,27−37] Thus, to truly validate $mGlu_1$ PAMs/ago-PAMs across a broad range of CNS disorders, a toolkit of chemical probes with diverse structures, pharmacology, and DMPK profiles are required.

RESULTS AND DISCUSSION

Chemistry. Focused optimization around **8** to enhance $mGlu_1$ PAM potency and improve the DMPK profile was plagued by crossover activity at $mGlu_4$, driven in large part by the picolinamide moiety engendering $mGlu_4$ PAM activity when subtle electronic and/or steric perturbations were performed.[23,37,38] On the basis of the structure−activity relationships (SAR) we have previously developed around this chemotype for $mGlu_4$ PAM activity, our optimization plan for **8** was modified to focus on a diverse array of five-membered heterocyclic amides to replace the picolinamide moiety, as well as key substituents on the phthalimide group, providing analogs **9** (Figure 3).[23,37,38] Both structural modifications were designed to diminish activity at $mGlu_4$, but the impact on $mGlu_1$ PAM activity, while hopeful, was less certain.

As shown in Scheme 1, analogs **9** were readily prepared in three steps from commercial materials and in good overall yields.[39] Starting from aniline **11**, condensation with phthalic anhydride in refluxing acetic acid provided **12** in 88% yield. Reduction of the nitro moiety with tin(II) chloride afforded **13** in 72% yield, and a HATU-mediated amide coupling gives analogs **9** in yields ranging from 43% to 96%, depending on the heterocyclic carboxylic acid employed. The same sequence of steps provides the furylamide based analogs **10**. Finally, analogs **17** were also prepared in three steps from aniline **14**. Here, microwave-assisted acylation of **14** with 3-methyl-2-furanyl chloride affords furylamide **15** in 97% yield. Once again, a tin(II) chloride mediated reduction of the nitro group provided aniline **16** (96%), which was condensed with phthalic anhydride in refluxing acetic acid to yield analogs **17** (34−92%).[39]

SAR for the initial diversity library of analogs **9** was strikingly "flat" (Table 1), with only 2 of 14 analogs possessing any measurable $mGlu_1$ PAM activity. The 2-furylamide congener **9a** was the most active ($mGlu_1$ EC_{50} = 550 nM, pEC_{50} = 6.26 ± 0.10, 76 ± 6% Glu Max), while related derivatives, such as the 2-thienyl **9b** and 2-pyrrolyl **9c** were devoid of $mGlu_1$ activity ($mGlu_1$ EC_{50} values of >10 μM). Only the 4-thiazolyl analog **9h** showed modest activity ($mGlu_1$ EC_{50} = 4.5 μM, pEC_{50} = 5.35 ± 0.10, 107 ± 7% Glu Max), while the regioisomeric thiazole **9i** was inactive, as were oxazoles (**9d** and **9e**), other aza-congeners, as well as a tetrahydrofuran **9n**. It is worth noting that the inactive compounds described did not decrease the glutamate response during the screening, which excludes a switch of mode of pharmacology to NAMs as it has been observed in other cases; however, the possibility that these modifications could generate silent allosteric modulator (SAMs) or a "molecular switch" that changes the subtype selectivity toward other mGlu receptor has not been explored or ruled out.

On the basis of these data, we prepared a library of diverse furylamides **10**, surveying a wide range of substituents (Table 2). Like analogs **9**, SAR for this series was similarly "flat";

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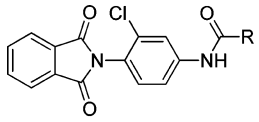
Table 1. Structures and Activities for Selected Five-Membered Heterocyclic Amides 9

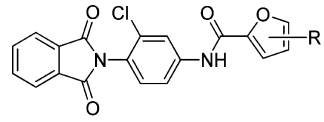


9

Compd.	R	hmGlu$_1$ EC$_{50}$ (μM)[a]	pEC$_{50}$ (±SEM)[a]	% Glu Max (±SEM)[a]
9a		0.55	6.26±0.10	76±6
9b		>10.0	>5.00	-
9c		>10.0	>5.00	-
9d		>10.0	>5.00	-
9e		>10.0	>5.00	-
9f		>10.0	>5.00	-
9g		>10.0	>5.00	-
9h		4.47	5.35±0.10	107±7
9i		>10.0	>5.00	-
9j		>10.0	>5.00	-
9k		>10.0	>5.00	-
9l		>10.0	>5.00	-
9m		>10.0	>5.00	39±4
9n		>10.0	>5.00	-

[a]Calcium mobilization mGlu$_1$ assays. Values are average of three (n = 3) independent experiments performed in triplicate.

Table 2. Structures and Activities for Selected Furylamides 10



10

Compd.	Furyl	hmGlu$_1$ EC$_{50}$ (μM)[a]	pEC$_{50}$ (±SEM)[a]	% Glu Max (±SEM)[a]
10a		>10.0	>5.00	60±7
10b		>10.0	>5.00	30±4
10c		>10.0	>5.00	-
10d		>10.0	>5.00	55±3
10e		>10.0	>5.00	45±4
10f		>10.0	>5.00	43±6
10g		>10.0	>5.00	-
10h		>10.0	>5.00	35±3
10i		>10.0	>5.00	30±4
10j		0.097	7.01±0.15	118±3
10k		>10.0	>5.00	-
10l		>10.0	>5.00	-
10m		>10.0	>5.00	-
10n		>10.0	>5.00	-

[a]Calcium mobilization mGlu$_1$ assays. Values are average of three (n = 3) independent experiments performed in triplicate.

however, many of these analogs showed weak PAM activity at ~10 μM (% Glu Max 30–60%). Again, a single derivative **10j**, a 3-methylfuryl congener, emerged with promising activity (mGlu$_1$ EC$_{50}$ = 97 nM, pEC$_{50}$ = 7.01 ± 0.15, 118 ± 3% Glu Max). Interestingly, substituents in the 5-position (**10a–i**) were uniformly inactive, as were fused benzofurans **10m** and **10n**. Addition of a methyl group to the 5-position of **10j**, providing the 3,5-dimethyl analog **10l**, also resulted in a loss of mGlu$_1$ PAM activity.

The next round of optimization held the 3-methylfurylamide of **10j** constant and surveyed alternative substituents on the phthalimide moiety delivering analogs **17** (Table 3), providing, for the first time, robust SAR and a collection of potent and highly efficacious mGlu$_1$ PAMs (EC$_{50}$ values of 24.3–98.8 nM). In this instance, small groups in either the 3- or 4-position of the phthalimide moiety afforded potent mGlu$_1$ PAMs (**17a–f**), and the 4-aza derivative was also tolerated (**17g**). Efficacy (% Glu Max) was tightly maintained (86–98%) across all analogs **17**. Surprisingly, analogs **17** all retained some residual mGlu$_4$ PAM activity, with selectivity versus mGlu$_4$ ranging from 5.9- to >100-fold.

Molecular Pharmacology. From this set of analogs **17** (Table 3), three compounds were selected for further characterization: **17d**, the 4-Cl analog (mGlu$_1$ EC$_{50}$ = 80.9 nM, pEC$_{50}$ = 7.09 ± 0.10, 94 ± 9% Glu Max, 27.7-fold versus mGlu$_4$); **17e**, the 3-F analog (mGlu$_1$ EC$_{50}$ = 31.8 nM, pEC$_{50}$ = 7.49 ± 0.15, 98 ± 6% Glu Max, 34.9-fold versus mGlu$_4$); and **17g** the 4-aza analog (mGlu$_1$ EC$_{50}$ = 98.8 nM, pEC$_{50}$ = 7.01 ± 0.08, 94 ± 4% Glu Max, >100-fold versus mGlu$_4$).[39] These three analogs displayed comparable potency at rat mGlu$_1$ and enhanced efficacy and represent a 3- to >10-fold improvement in mGlu$_1$ PAM potency over our initial lead mGlu$_1$ PAM **8** (Figure 4). As shown in Figure 5, all three mGlu$_1$ PAMs induced a parallel leftward shift of the glutamate concentration–response curve (CRC). **17d** induces a maximum 8.3-fold shift at 10 μM, with modest agonism (<20%) noted at the highest concentration. **17e** induces a maximum 11.4-fold shift at 10 μM, with higher levels of agonism (~35%) noted at the highest concentration, and **17f** induces a maximum 10.9-fold shift at 10 μM, with modest agonism (<20%) noted at the highest concentration in cells expressing human mGlu$_1$. On the basis of these profiles, these modulators are best described as mGlu$_1$ ago-PAMs in this calcium mobilization assay, though the degree of allosteric agonism varies.[23,24,39] As glutamatergic tone may vary across brain regions, possessing tool compounds with a range of pure PAM to ago-PAM activity is very attractive and will be useful in in vivo proof of concept studies and translation.[5–8,27,28] We also assessed selectivity of **17d**, **17e**, and **17g** versus the other remaining six mGlu receptors in our

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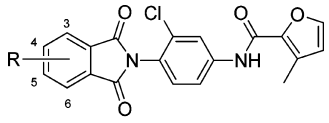
Table 3. Structures and Activities for Selected 3-Methyl-2-furylcarboxamide Analogs 17



17

compd	R	$hmGlu_1$ EC_{50} (nM)[a]	pEC_{50} (±SEM)[a]	% Glu Max (±SEM)[a]	$hmGlu_4$ EC_{50} (nM)[a]	pEC_{50} (±SEM)[a]	% Glu Max[b] (±SEM)[a]	fold selective vs $mGlu_4$
17a	3-Me	24.3	7.61 ± 0.16	86 ± 3	620	6.21 ± 0.18	153 ± 11	25.5
17b	4-Me	54.4	7.26 ± 0.21	86 ± 5	323	6.49 ± 0.26	69 ± 6	5.9
17c	3-Cl	33.0	7.48 ± 0.32	91 ± 6	1140	5.94 ± 0.22	163 ± 22	34.5
17d	4-Cl	80.9	7.09 ± 0.10	94 ± 9	2250	5.64 ± 0.11	89 ± 5	27.8
17e	3-F	31.8	7.49 ± 0.15	98 ± 6	1110	5.95 ± 0.16	122 ± 14	34.9
17f	4-F	24.1	7.62 ± 0.07	88 ± 3	712	614 ± 0.30	97 ± 7	29.7
17g	4-Aza	98.8	7.01 ± 0.08	94 ± 4	>10 000	>5.00	18 ± 4	>100

[a]Calcium mobilization $mGlu_1$ assays. Values are average of three (n = 3) independent experiments performed in triplicate. [b]Glu Max is expressed as % of PHCCC response.

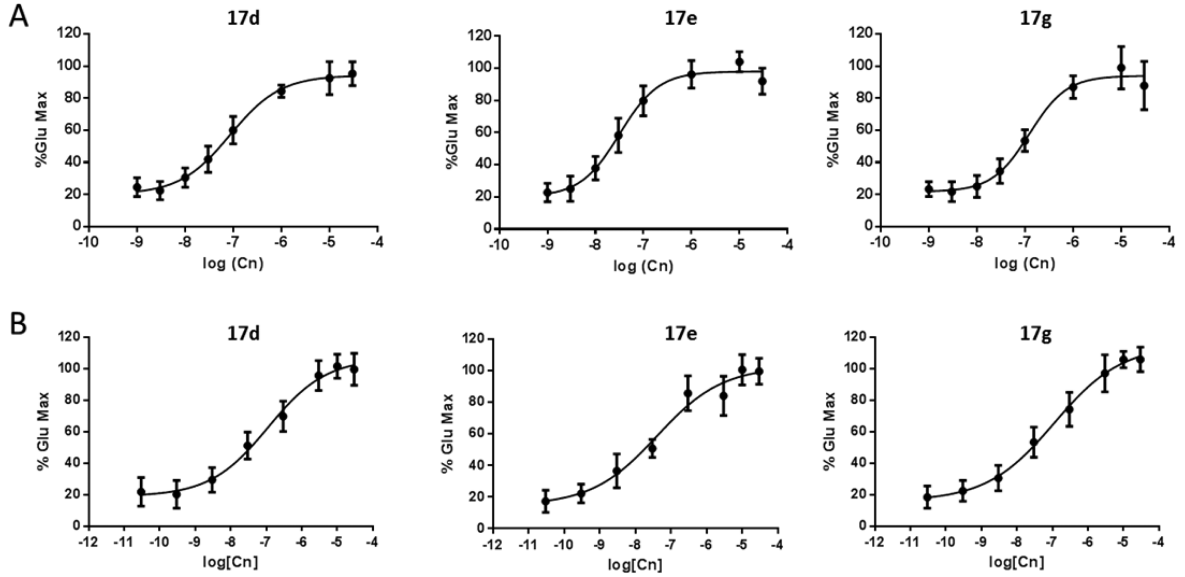


Figure 4. All three $mGlu_1$ PAMs (**17d**, **17e**, and **17g**) are equipotent and equiefficacious on human and rat $mGlu_1$ WT receptors. (A) Concentration−response curves (CRCs) on human $mGlu_1$ WT receptors: **17d**, $mGlu_1$ EC_{50} = 80.9 nM, pEC_{50} = 7.09 ± 0.10, 94 ± 9% Glu Max; **17e**, $mGlu_1$ EC_{50} = 31.8 nM, pEC_{50} = 7.49 ± 0.15, 98 ± 6% Glu Max; and **17g** $mGlu_1$ EC_{50} = 98.8 nM, pEC_{50} = 7.01 ± 0.08, 94 ± 4% Glu Max. (B) Concentration−response curves (CRCs) on rat $mGlu_1$ WT receptors: **17d**, $mGlu_1$ EC_{50} = 113 nM, pEC_{50} = 6.95 ± 0.13, 107 ± 4% Glu Max; **17e**, $mGlu_1$ EC_{50} = 40.7 nM, pEC_{50} = 7.39 ± 0.18, 103 ± 5% Glu Max; and **17g** $mGlu_1$ EC_{50} = 113 nM, pEC_{50} = 6.95 ± 0.15, 115 ± 5% Glu Max.

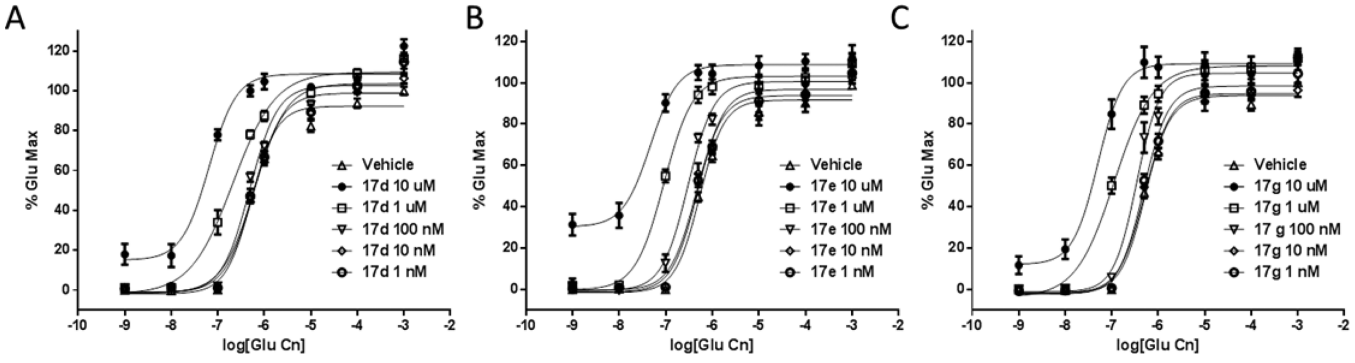


Figure 5. All three $mGlu_1$ PAMs induce a concentration-dependent, parallel leftward shift of the glutamate concentration−response curve (CRC) in cells expressing the human $mGlu_1$ WT receptor. (A) **17d** induces a ∼8.3-fold shift, with modest (<20%) agonism noted at the 10 μM concentration. (B) **17e** induces a ∼11.4-fold shift, with noted (∼35%) agonism at the 10 μM concentration, and (C) **17g** induces a ∼10.9-fold shift, with modest (<20%) agonism noted at the 10 μM concentration. Thus, all three are more accurately described as $mGlu_1$ ago-PAMs in this assay.

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Table 4. DMPK Characterization of $mGlu_1$ PAMs 5, 8, 17d, 17e, and 17g[a]

parameter	5	8	17d	17e	17g
human CL_{INT} (mL min^{-1} kg^{-1})	262	17.2	2.90	3.10	167
human CL_{HEP} (mL min^{-1} kg^{-1})	19.4	9.46	2.54	2.70	18.6
rat CL_{INT} (mL min^{-1} kg^{-1})	661	39.9	22.8	207	405
rat CL_{HEP} (mL min^{-1} kg^{-1})	63.3	9.46	17.2	52.3	59.7
human f_u plasma	0.014	unstable	unstable	0.051	unstable
rat f_u plasma	0.003	unstable	unstable	0.030	0.030
CYP450 IC_{50} (μM)					
1A2	13	>30	>30	25.2	>30
2C9	0.6	>30	>30	6.45	>30
2D6	>30	>30	>30	>30	>30
3A4	>30	>30	>30	>30	>30
rat iv PK (0.2 mg/kg)					
$t_{1/2}$ (min)	54	ND	91.1	53.6	5.38
MRT (min)	50	ND	53.5	64.1	7.85
CL_p (mL min^{-1} kg^{-1})	35	ND	33.0	13.3	382
V_{ss} (L/kg)	1.34	ND	1.76	0.85	3.00
rat iv PBL (0.25 h, 0.2 mg/kg)					
C_n plasma (ng/mL)	276	336	173	200	12.5
C_n brain (ng/mL)	81	284	108	204	35.2
K_p	0.29	0.84	0.63	1.02	2.81

[a]ND, not determined.

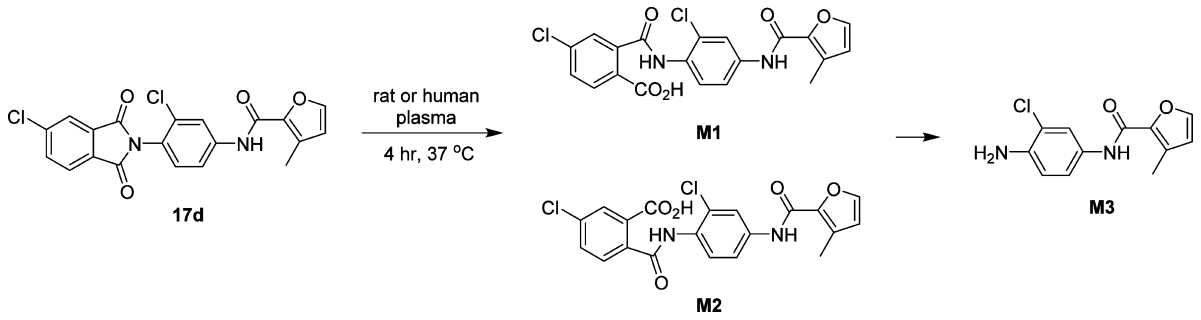

Figure 6. Ex vivo metabolism of **17d** in rat and human plasma. Upon incubation in either rat or human plasma at 37 °C, parent **17d** is exclusive species at $t = 0$, but by 4 h, no parent remains. Hydrolysis of the phthalimide moiety is complete in both rat and human plasma by 4 h, providing the regioisomeric phthalamic acids **M1** and **M2** as major metabolites, as well as the minor **M3**, the product of amide hydrolysis of **M1** and **M2**.

standard fold-shift assay paradigm at a 10 μM concentration.[30,31,33,36] **17d** and **17g** were selective against $mGlu_{2,3,5,6,7,8}$, inducing less than a ~2-fold shift of the glutamate CRC at 10 μM. **17e** was selective versus $mGlu_{2,3,8}$ (less than ~2-fold shift of glutamate CRC at 10 μM, a modest 2.7-fold shift at $mGlu_5$, and larger shifts at $mGlu_6$ (6.7) and $mGlu_7$ (7.0). Thus, the $mGlu_1$ PAMs are suitable for in vivo studies.

Metabolism and Disposition. A major goal of the optimization effort was to improve not only $mGlu_1$ PAM potency but also the DMPK profile relative to the lead **8** (and **5**) to provide an in vivo tool compound. A hallmark of the earlier $mGlu_1$ PAMs was moderate to high clearance in rodent species, mixed cytochrome P450 (CYP) profiles, poor CNS penetration (in the case of **5**, K_p < 0.3), and in the case of **8**, instability in human and rat plasma, making it impossible to assess free fraction (F_u) and thus free drug levels.[23] Therefore, we profiled **5**, **8**, **17d**, **17e**, and **17g** in a battery of in vitro and in vivo DMPK assays (Table 4) to determine if this second generation series of $mGlu_1$ PAMs addressed key issues.[39] As shown in Table 4, all three analogs addressed different aspects of the major limitations (low K_p, high plasma protein binding and plasma instability). **17d** displayed low hepatic clearance in both human (CL_{HEP} = 2.54 mL min^{-1} kg^{-1}) and rat (CL_{HEP} = 17.2 mL min^{-1} kg^{-1}), a clean CYP profile (IC_{50} > 30 μM versus CYP 1A2, 2C9, 2D6, and 3A4), moderate in vivo clearance (CL_p = 33 mL min^{-1} kg^{-1}), reasonable half-life ($t_{1/2}$ = 91 min), and good CNS penetration (brain/plasma ratio K_p of 0.63). However, **17d**, like **8**, was unstable in both human and rat plasma, though acceptable in vivo. In contrast, **17g**, the 4-aza congener, displayed high hepatic clearance in both human (CL_{HEP} = 18.6 mL min^{-1} kg^{-1}) and rat (CL_{HEP} = 59.7 mL min^{-1} kg^{-1}), a clean CYP profile (IC_{50} > 30 μM versus CYP 1A2, 2C9, 2D6, and 3A4), superhepatic in vivo clearance (CL_p = 283 mL min^{-1} kg^{-1}), exceedingly short half-life ($t_{1/2}$ = 5.4 min), yet high CNS penetration (brain/plasma ratio K_p of 2.81). Interestingly, **17g** was unstable in human plasma but stable in rat plasma (f_u = 0.03). **17e** represents a middle ground between these two extremes of disposition and emerged as the $mGlu_1$ ago-PAM with the most balanced profile for in vivo studies. For the first time in this series, **17e** was stable in both human and rat plasma, enabling determination of plasma free fraction (human f_u = 0.05, rat f_u = 0.03). **17e** displayed low hepatic clearance in human (CL_{HEP} = 2.70 mL min^{-1} kg^{-1}) and high in rat (CL_{HEP} = 59.7 mL min^{-1} kg^{-1}) and a mixed but acceptable CYP profile (IC_{50} > 30 μM versus CYP 2D6 and 3A4, IC_{50} = 25.2 μM at 1A2, IC_{50} = 6.45 μM at 2C9).

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Interestingly, there was not a good in vitro/in vivo (IVIVC) correlation, as **17e** possessed low in vivo clearance (CL_p = 13.3 mL min^{-1} kg^{-1}), a comparable half-life ($t_{1/2}$ = 53.6 min) to **5**, and improved CNS penetration (brain/plasma ratio K_p of 1.02).

The plasma instability of **8**, **17d**, and **17g** was not entirely unexpected based on literature precedent with reported hydrolysis of phthalimide-containing drugs in plasma.[40,41] To assess this as the source of plasma instability, we evaluated **17d** in rat and human plasma at a 10 μM incubation concentration at 0 and 4 h time points at 37 °C (Figure 6) and analyzed it by mass spectrometry to identify metabolites.[39] In both rat and human plasma, the parent compound is clearly present at t = 0, but by 4 h, no parent **17d** remains, but three metabolites are formed: M1 (major), M2 (major), and M3 (minor). As predicted from the literature, M1 and M2 correspond to hydrolysis of the phthalimide to afford the two regioisomeric phthalamic acids, M1 and M2, and the aniline M3, resulting from amide hydrolysis of M1 and M2. However, the presence of the electronegative fluorine atom in **17e**, presumably from inductive effects, stabilizes the phthalimide moiety to enable stability in plasma such that F_u can be determined.

Activity at *GRM1* Mutants. Though selective $mGlu_1$ activation has been suggested as a mechanism for therapeutic intervention in a variety of CNS disorders, our interest primarily resides in schizophrenia and the presence of mutant $mGlu_1$ receptors derived from deleterious *GRM1* mutations found in schizophrenic patients (Figure 7).[21,22] Recently we reported that we developed stable HEK-293 cell lines expressing 9 of the 12 $mGlu_1$ mutant receptors, confirmed that they were loss-of-function mutations and not due to loss of receptor cell surface expression and that $mGlu_1$ PAMs **5** and **8** could potentiate calcium signaling induced by activation of these mutant receptors with glutamate or *S*-3,5-dihydroxyphenylglycine (DHPG), restoring functionality in cells expressing mGlu1 possessing K563N and P1015A mutations (i.e., those which had only a small negative impact on glutamate signaling), while in other mutants, enhancements in glutamate potency, but not efficacy, were observed.[23] Thus, $mGlu_1$ PAMs may be a viable therapeutic intervention in patients harboring these mutations. We selected four of the nine single point mutant receptors (Figure 7), three in the transmembrane domain (P729T, A863E, Y632H) and one in the intracellular C-terminal domain (P1014S), and evaluated the ability of the three PAMs to restore the reduction in glutamate-mediated calcium signaling utilizing a fold-shift experiment as shown previously for human WT $mGlu_1$ (Figure 5). It was pleasing to see that the SAR driven on human $mGlu_1$ to deliver the second generation $mGlu_1$ PAMs is retained across WT and the mutant $mGlu_1$ receptors. Across the four mutants, $mGlu_1$ PAMs **17d**, **17e**, and **17g** induce a parallel-leftward shift of the glutamate CRC, providing maximum shifts of ~10-fold at 10 μM compound concentration and, in some cases, modest increases in glutamate efficacy (Figure 8). Similar to human WT mGlu1, **17e** displayed robust ago-PAM activity across all four mutants (up to ~40%), and this was noted at concentrations of compound above 1 μM, while ago-PAM activity was greatly diminished for **17d** and **17g** at these mutants compared to the WT receptor. As the major deficit in these mutant receptors is the decrease in the efficacy of the receptor signal, the possibility of these probes having a therapeutic benefit in schizophrenic patients carrying these mutations is still an open question. The increase in sensitivity toward glutamate might be beneficial; however, in vivo studies in mutant mice harboring these mutations should be carried out in order to assess if this feature is sufficient to observe an improvement in the phenotype.

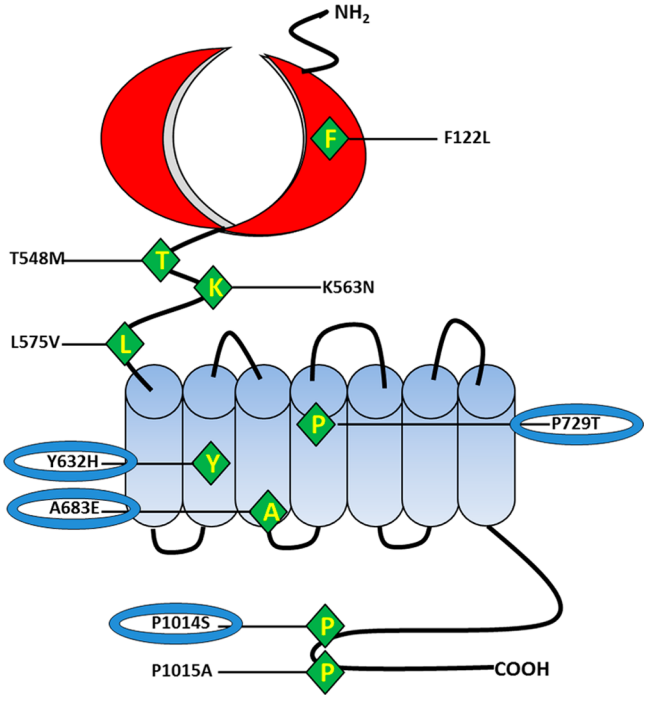

Figure 7. Cartoon representation of monomeric of human $mGlu_1$, indicating nine of the nonsynonymous single nucleotide polymorphisms (nsSNPs) in the $mGlu_1$ receptor found in schizophrenic patients. Circled in blue are the four single point points in this study, three in the transmembrane domain (P729T, A863E, Y632H), and one in the intracellular, C-terminal domain (P1014S).

Adverse Effect Liability. While early efforts to understand the physiological role of group I mGlu receptors relied on orthosteric, "glutamate-derived" agonists, excitotoxicity, and seizure liability limited their utility.[1−9] For example, the group I dual $mGlu_{1/5}$ agonist DHPG induces severe epileptiform activity,[42,43] and this liability weighed heavily on early $mGlu_5$ PAM programs,[30,31,42−47] as it was not clear if this was due to $mGlu_1$, $mGlu_5$, or both; moreover, it was not clear if a pure $mGlu_5$ PAM would avoid this liability.[42−47] Since this field has matured, multiple $mGlu_5$ PAMs, across diverse chemotypes that possessed even modest ago-PAM or pure PAMs that in vivo generated ago-PAM metabolites activity, have been shown to induce epilepitform activity in rat hippocampal CA3 region in vitro as well as racine scale (4+) seizures in rodents.[30,31,42−47] This severe adverse effect liability has hindered and slowed the development of this promising antipsychotic target. Thus, with the development of **17e**, we were poised to assess the adverse effect liability of an $mGlu_1$ ago-PAM/PAM with excellent CNS penetration and free drug levels. To determine if a selective $mGlu_1$ ago-PAM/PAM has any effect in inducing the epileptiform activity, we performed experiments analogous to those described in the previous studies and recorded spontaneous extracellular spike activity from CA3 pyramidal cell body layer in a mouse hippocampal slice preparation.[34,43] Bath application of the $mGlu_1$ PAM **17e** (10 μM, >300-fold above the $mGlu_1$ EC_{50}) did not induce epileptiform activity, reflected by no change in frequency of spontaneous spike activity following application of the $mGlu_1$ PAM (Figure 9).

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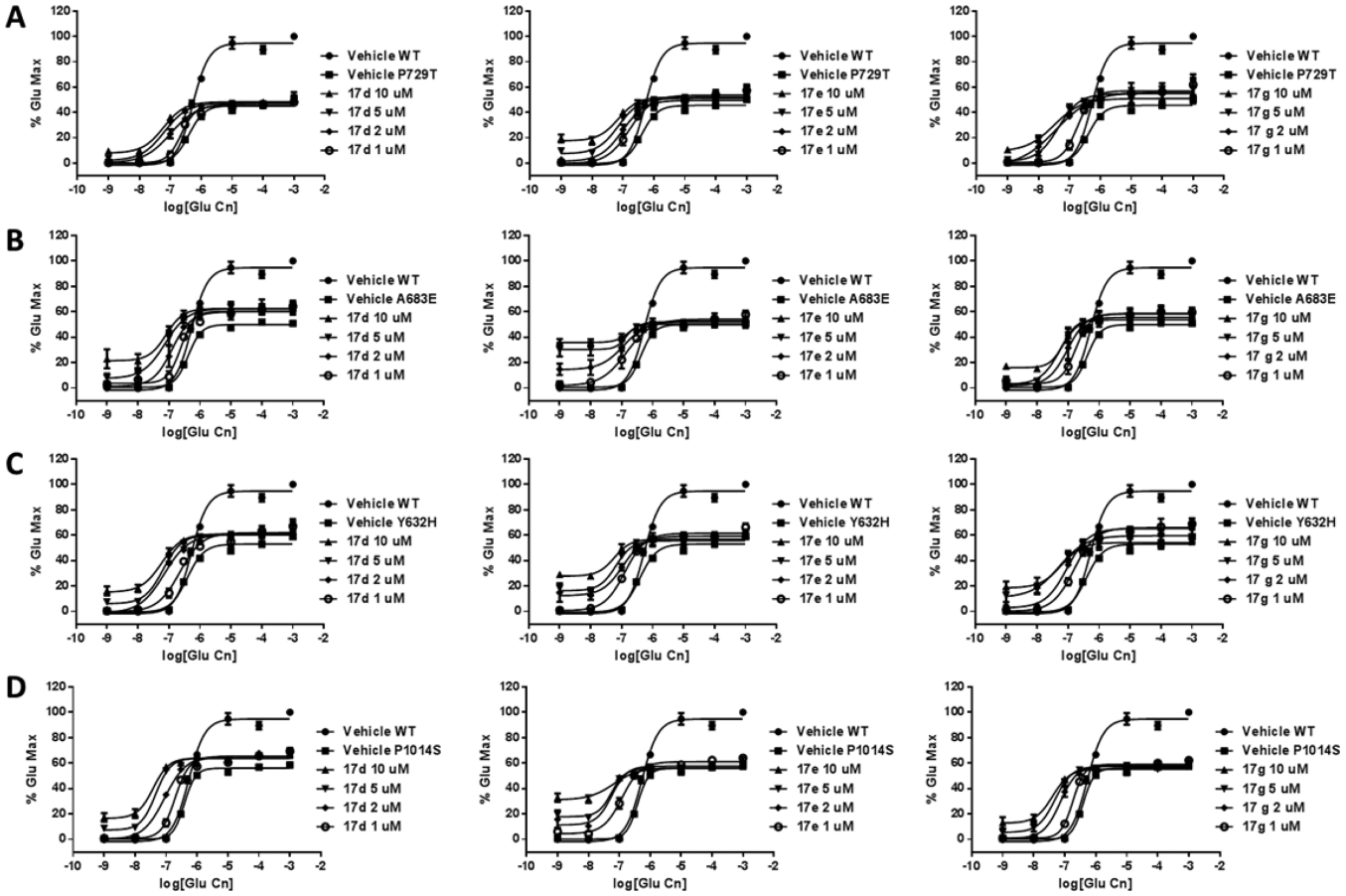

Figure 8. All three $mGlu_1$ PAMs **17d**, **17e**, and **17g** induce a parallel leftward shift of the glutamate concentration−response-curve (CRC) in cells stably expressing human $mGlu_1$ mutant receptors (P729T (A), A683E (B), Y632H (C), P1014S(D)) found in schizophrenics. As in WT human $mGlu_1$ receptors, ago-PAM activity is noted, with **17e** showing robust ago-PAM activity across the four schizophrenia mutants evaluated.

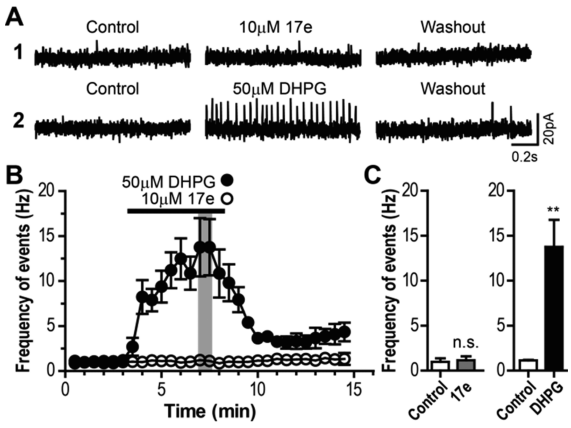

Figure 9. mGlu1 ago-PAM **17e** does not induce epileptiform activity in CA3 region of the hippocampus. (A) Sample traces of extracellular recordings from CA3 pyramidal cell body layer in hippocampal slices in control, during application and after washout of 10 μM **17e** (A1) and 50 μM DHPG (A2). (B) Average time courses of frequency of spontaneous activity before, during, and after application of **17e** (n = 6) and DHPG (n = 6), respectively. The gray bar indicates the time point at which the data were taken to make statistical comparison to the control levels. (C) Bar graphs summarizing the effects of 10 μM **17e** (left) and 50 μM DHPG (right) on the frequency of spontaneous activity in CA3 region of the hippocampus: (n.s.) p = 0.36; (**) p = 0.0084; paired t test.

The frequency of spike activity after application of 10 μM **17e** was 1.16 ± 0.44 Hz, which is not significantly different from the baseline control (0.99 ± 0.38 Hz; p = 0.38, n = 6, paired t test), despite possessing ∼35% agonism of $mGlu_1$ at this concentration. As a positive control, we applied the group I mGlu agonist DHPG, which induced marked epileptiform activity, as evidenced by a robust increase in frequency of spontaneous activity following application of 50 μM DHPG (13.8 ± 3.0 Hz, compared to 0.94 ± 0.06 Hz in baseline control; p = 0.0084, n = 6, paired t test).[39]

Previous reports have demonstrated that intracerebroventricular injections of the group I mGlu agonist DHPG can induce behavioral convulsions in rats.[39,42,43] DHPG activates both $mGlu_1$ and $mGlu_5$ receptors. Our group has previously shown that $mGlu_5$ ago-PAM (*R*)-5-((3-fluorophenyl)ethynyl)-*N*-(3-hydroxy-3-methylbutan-2-yl)picolinamide (VU0424465, **18**) causes seizure activity and behavioral convulsions in rats.[44] Therefore, to assess the potential contributions of $mGlu_1$ activation in the induction of seizure activity, we evaluated the behavioral effects of selective $mGlu_1$ PAMs, **17d**, **17e**, and **17g** alongside the selective $mGlu_5$ ago-PAM **18** in mice.[39] Male C57Bl/6 mice (25−30g) were administered $mGlu_5$ ago-PAM **18** (1 or 3 mg/kg ip) or $mGlu_1$ ago-PAMs **17d**, **17e**, or **17g** (100 mg/kg ip), and behavioral responses were monitored continuously for 3 h. Behavioral convulsions were measured using a modified Racine score (0−5). Systemic administration of **18** induced severe, dose-dependent behavioral manifestations of seizure activity with peak responses observed approximately

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5 min after administration (Figure 10) in accord with previous data in rats.[44] In contrast, administration of a much higher dose

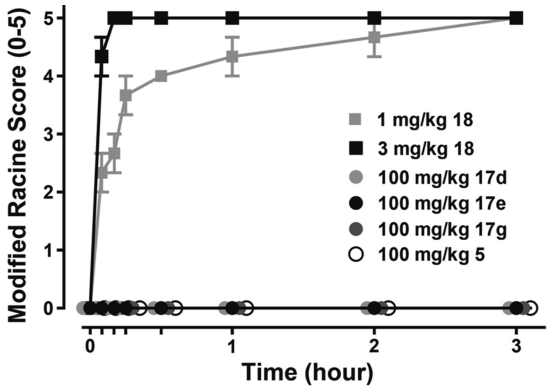

Figure 10. Metabotropic glutamate receptor subtype 1 positive allosteric modulators do not induce behavioral convulsions in mice. Severe, dose-dependent behavioral convulsions were observed in mice administered the $mGlu_5$ ago-PAM **18** (1 and 3 mg/kg, 10% Tween 80, 10 mL/kg) beginning 5 min following intraperitoneal dosing as measured by the modified Racine scale. In contrast, $mGlu_1$ ago-PAMs/PAMs **5**, **17d**, **17e**, and **17g** induce no adverse effects in mice at doses that reach brain concentration significantly higher than the compound's EC_{50} at $mGlu_1$ (100 mg/kg, 10% Tween 80, 10 mL/kg). As two distinct $mGlu_1$ PAM chemotypes afford the same effect, confidence is high that $mGlu_1$ differentiates from $mGlu_5$ adverse effect liability. Data represent the mean ± SEM, n = 3.

of the $mGlu_1$ ago-PAM **17d**, **17e**, or **17g** induced no measurable behavioral disturbances at any time point (Figure 10).[39] Of note, **17e** achieved total brain exposure of 9.2 μM, ~288-fold above the $mGlu_1$ PAM EC_{50} (31.8 nM) and >10-fold when corrected for free drug levels (rBHB F_u = 0.04). Similarly, **17d** and **17g** achieved total brain levels of 1.4 and 2.52 μM, respectively, both an order of magnitude above their respective $mGlu_1$ EC_{50} values. Therefore, selective $mGlu_1$ activation did not induce observable seizure activity or behavioral convulsions despite achieving high brain concentrations that are significantly higher than those required for maximum potentiation of $mGlu_1$ activation, and these in vivo data are supported by the electrophysiology data (Figure 9). To ensure this was not a chemotype driven effect, we also assessed Roche's **5** in this model at 100 mg/kg ip. For the first time, these data suggest that the epileptiform activity induced by the group I dual $mGlu_{1/5}$ agonist is solely mediated by agonism at $mGlu_5$, and both in vitro electrophysiology data and in vivo data support this conclusion; moreover, these findings suggest that $mGlu_1$ ago-PAMs/PAMs may have a larger therapeutic window than $mGlu_5$ ago-PAMs/PAMs and avoid this key adverse effect liability.

CONCLUSION

In summary, we optimized a series of $mGlu_1$ PAMs based on an *N*-(3-chloro-4-(1,3-dioxoisoindolin-2-yl)phenyl)-3-methylfuran-2-carboxamide scaffold, driven by multidimensional, iterative parallel synthesis to provide a second generation of potent and CNS penetrant $mGlu_1$ PAMs (**17d**, **17e**, and **17g**) with DMPK profiles to enable in vivo proof of concept studies. All three PAMs potentiated not only wild-type human $mGlu_1$ but also mutant $mGlu_1$ receptors derived from deleterious *GRM1* mutations found in schizophrenic patients, which will enable translational studies. From this effort, **17e**, a potent ($mGlu_1$ EC_{50} = 31.8 nM) and highly CNS penetrant (brain to plasma ratio (K_p) of 1.02) $mGlu_1$ PAM emerged as a new in vivo proof-of-concept tool compound, along with **17d** and **17g**, with a balanced DMPK profile and overcoming key liabilities of its predecessors **5** and **8**. Moreover, we showed for the first time that $mGlu_1$ ago-PAMs do not induce epileptiform activity in the CA3 region of the hippocampus and do not induce seizures in vivo at drug concentrations far above the $mGlu_1$ PAM EC_{50}, suggesting that the adverse effect liability of group I agonists, such as DHPG, is solely mediated by agonism at $mGlu_5$. Further in vivo studies and continuing efforts around this scaffold are in progress and will be reported in due course.

EXPERIMENTAL SECTION

Chemistry. All reactions were carried out employing standard chemical techniques under inert atmosphere. Solvents used for extraction, washing, and chromatography were HPLC grade. Unless otherwise noted, all reagents were purchased from Aldrich Chemical Co. and were used without further purification. Analytical thin layer chromatography was performed on 250 μm silica gel plates from Sorbent Technologies. Analytical HPLC was performed on an Agilent 1200 LCMS with UV detection at 215 and 254 nm along with ELSD detection and electrospray ionization, with all final compounds showing >95% purity and a parent mass ion consistent with the desired structure. All NMR spectra were recorded on a 400 MHz Brüker AV-400 instrument. ^{1}H chemical shifts are reported as δ values in ppm relative to the residual solvent peak (DMSO-d_6 = 2.50, $CDCl_3$ = 7.26). Data are reported as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet), coupling constant (Hz), and integration. ^{13}C chemical shifts are reported as δ values in ppm relative to the residual solvent peak (DMSO-d_6 = 39.52, $CDCl_3$ = 77.16). Low resolution mass spectra were obtained on an Agilent 1200 LCMS with electrospray ionization. High resolution mass spectra were recorded on a Waters QToF-API-US plus Acquity system with electrospray ionization. Automated flash column chromatography was performed on a Teledyne ISCO Combiflash Rf system. Preparative purification of library compounds was performed on a Gilson 215 preparative LC system. Purity for all final compounds was >95%, and each showed a parent mass ion consistent with the desired structure (LCMS).

2-(2-Chloro-4-nitrophenyl)isoindoline-1,3-dione (12). In a flask, 200 mg (1.622 mmol, 1.2 equiv) of 2-chloro-4-nitroaniline and 280 mg (1.350 mmol, 1.0 equiv) of phthalic anhydride were added and dissolved in 5 mL of acetic acid. The mixture was refluxed for 24 h. After cooling the solution a precipitate is formed. The solid was filtered and carefully triturated with cold methanol to obtain a light yellow powder (359 mg, 88%). ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 8.45 (1H, d, J = 2.4 Hz), 8.28 (1H, dd, J = 8.7 Hz, J = 2.5 Hz), 8.00 (2H, m), 7.86 (2H, m), 7.58 (1H, d, J = 8.6 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 165.6, 148.2, 135.6, 134.9, 134.6, 131.6, 131.4, 125.7, 124.2, 122.6.

2-(4-Amino-2-chlorophenyl)isoindoline-1,3-dione (13). In a vial, 300 mg (0.993 mmol, 1.0 equiv) of **12** were resuspended in dioxane. The suspension was cold in an ice bath and purged with argon. A previously prepared solution of tin(II) chloride (847 mg, 4.469 mmol, 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 sulfate, filtered and the volatiles were eliminated in vacuo to yield a yellow powder (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.

General Synthesis of *N*-(3-Chloro-4-(1,3-dioxoisoindolin-2-yl)phenyl)amides (9 and 10). In a vial, 0.088 mmol (1.2 equiv) of

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the carboxylic acid was added and dissolved in 0.5 mL mixture of DCM/DIEA (9:1), then an amount of 41 mg (0.110 mmol, 1.5 equiv) of HATU was added. The mixture was stirred for 10 min, and an amount of 20 mg (0.073 mmol, 1.0 equiv) of **13** dissolved in 0.5 mL of DCM/DIEA (9:1) was added, 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.

***N*-(3-Chloro-4-(1,3-dioxoisoindolin-2-yl)phenyl)-3-methylfuran-2-carboxamide (10j).** Cream powder. ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 8.14 (1H, s), 8.06 (1H, d, J = 2.4 Hz), 7.97 (2H, m), 7.81 (2H, m), 7.64 (1H, dd, J = 8.6 Hz, J = 2.4 Hz), 7.40 (1H, d, J = 1.5 Hz), 7.32 (1H, d, J = 8.6 Hz), 6.43 (1H, d, J = 1.5 Hz), 2.47 (3H, s). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 166.7, 157.1, 142.8, 141.4, 139.6, 134.4, 133.7, 131.8, 130.8, 129.9, 124.8, 123.9, 120.8, 118.3, 116.0, 11.2. HRMS (TOF, ES+) $C_{20}H_{14}ClN_2O_4$ $[M + H]^+$ calcd mass 381.0642, found 381.0644.

***N*-(2-Chloro-4-nitrophenyl)-3-methylfuran-2-carboxamide (15).** In a microwave vial, an amount of 912 mg (5.28 mmol, 1.2 equiv) of 2-chloro-4-nitroaniline was added and dissolved in 20 mL of DCE/DIEA (9:1), followed by addition of 500 μL (4.40 mmol, 1.0 equiv) of the 3-methylfurancarbonyl chloride. The reaction was heated in the microwave at 120 °C for 30 min. The reaction was cooled to room temperature and water was added, causing the precipitation of the product. The crude was filtrated in vacuo and titrated with methanol to give a cream solid (1195 mg, 97% yield). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ (ppm): 10.71 (1H, s), 8.27 (1H, d, J = 2.2 Hz), 8.14 (1H, d, J = 9.1 Hz), 7.99 (1H, dd, J = 9.1 Hz, J = 2.2 Hz), 7.88 (1H, d, J = 1.5 Hz), 6.66 (1H, d, J = 1.5 Hz), 2.37 (3H, s). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ (ppm): 158.1, 144.9, 144.3, 141.9, 141.4, 130.3, 127.7, 126.9, 121.7, 119.0, 116.4, 11.5. HRMS (TOF, ES+) $C_{12}H_9ClN_2O_4$ $[M^+]$ calcd mass 280.0251, found 280.0254.

***N*-(4-Amino-2-chlorophenyl)-3-methylfuran-2-carboxamide (16).** In a flask, an amount of 480 mg (1.71 mmol, 1.0 equiv) of **15** was suspended in dioxane. The suspension was cold in an ice bath and purged with argon. A previously prepared solution of tin(II) chloride (1.45 g, 7.70 mmol, 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 sulfate, filtered and the volatiles were eliminated in vacuo to yield a cream solid (410 mg, 96% yield). ^{1}H NMR (400.1 MHz, DMSO-d_6) δ (ppm): 9.87 (1H, s), 7.76 (1H, d, J = 1.6 Hz), 7.74 (1H, d, J = 2.3 Hz), 7.41 (1H, dd, J = 8.7 Hz, J = 2.3 Hz), 6.82 (1H, d, J = 8.7 Hz), 6.58 (1H, d, J = 1.6 Hz), 5.51 (2H, br), 2.32 (3H, s). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ (ppm): 157.5, 143.8, 142.3, 139.8, 129.9, 127.7, 121.8, 121.2, 117.8, 116.4, 116.0, 11.4. HRMS (TOF, ES+) $C_{12}H_{11}ClN_2O_2$ $[M^+]$ calcd mass 250.0509, found 250.0513.

General Synthesis of Substituted *N*-Acyl-*N*-(4-aminophenyl)-3-methylfuran-2-carboxamides (17). In a vial, 30 mg (0.12 mmol, 1.0 equiv) of the aniline and 0.18 mmol (1.5 equiv) of the phthalic anhydride were added and dissolved in 1 mL of acetic acid. The mixture was heated at 110 °C while stirring for 4 h. After this time, volatiles were evaporated, the crude was dissolved in DMSO and resolved by preparative HPLC.

***N*-(3-Chloro-4-(4-methyl-1,3-dioxoisoindolin-2-yl)phenyl)-3-methylfuran-2-carboxamide (17a).** Cream powder. ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 8.15 (1H, s), 8.05 (1H, d, J = 2.4 Hz), 7.79 (1H, d, J = 7.4 Hz), 7.65 (2H,m), 7.55 (1H, d, J = 7.7 Hz), 7.39 (1H, d, J = 1.5 Hz), 7.29 (1H, d, J = 8.6 Hz), 6.43 (1H, d, J = 1.6 Hz), 2.47 (3H, s), 2.46 (3H, s). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 167.5, 166.8, 157.1, 142.8, 141.4, 139.5, 138.6, 136.7, 133.9, 133.8, 132.3, 130.8, 129.9, 128.6, 125.0, 121.5, 120.8, 118.2, 116.0, 17.7, 11.2. HRMS (TOF, ES+) $C_{21}H_{16}ClN_2O_4$ $[M + H]^+$ calcd mass 395.0799, found 395.0798.

***N*-(3-Chloro-4-(5-methyl-1,3-dioxoisoindolin-2-yl)phenyl)-3-methylfuran-2-carboxamide (17b).** Cream powder. ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 8.15 (1H, s), 8.05 (1H, d, J = 2.3 Hz), 7.84 (1H, d, J = 7.7 Hz), 7.77 (1H, d, J = 7.4 Hz), 7.62 (1H, dd, J = 8.6 Hz, J = 2.4 Hz), 7.59 (1H, d, J = 7.7 Hz), 7.38 (1H, d, J = 1.5 Hz), 7.30 (1H, d, J = 8.6 Hz), 6.42 (1H, d, J = 1.5 Hz), 2.56 (3H, s), 2.46 (3H, s). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 166.9, 166.8, 157.1, 145.8, 142.8, 141.4, 139.6, 135.0, 133.8, 132.2, 130.8, 129.9, 129.2, 124.9, 124.4, 123.8, 120.8, 118.3, 116.0, 22.0, 11.2. HRMS (TOF, ES+) $C_{21}H_{16}ClN_2O_4$ $[M + H]^+$ calcd mass 395.0799, found 395.0798.

***N*-(3-Chloro-4-(4-chloro-1,3-dioxoisoindolin-2-yl)phenyl)-3-methylfuran-2-carboxamide (17c).** Cream powder ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 8.16 (1H, s), 8.06 (1H, d, J = 2.3 Hz), 7.88 (1H, m) 7.74 (2H, m), 7.63 (1H, dd, J = 8.6 Hz, J = 2.3 Hz), 7.39 (1H, d, J = 1.2 Hz), 7.29 (1H, d, J = 8.6 Hz), 6.42 (1H, d, J = 1.2 Hz), 2.46 (3H, s). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 165.2, 164.3, 157.2, 142.9, 141.4, 139.8, 136.1, 135.2, 133.9, 133.7, 132.0, 130.7, 130.0, 127.6, 124.4, 122.3, 120.8, 118.3, 116.1, 11.2. HRMS (TOF, ES+) $C_{20}H_{13}Cl_2N_2O_4$ $[M + H]^+$ calcd mass 415.0252, found 415.0254.

***N*-(3-Chloro-4-(5-chloro-1,3-dioxoisoindolin-2-yl)phenyl)-3-methylfuran-2-carboxamide (17d).** Cream powder. ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 8.16 (1H, s), 8.06 (1H, d, J = 2.3 Hz), 7.94 (1H, d, J = 1.7 Hz), 7.90 (1H, d, J = 8.0 Hz), 7.77 (1H, dd, J = 8.0 Hz, J = 1.7 Hz), 7.63 (1H, dd, J = 8.6 Hz, J = 2.3 Hz), 7.40 (1H, d, J = 1.4 Hz), 7.30 (1H, d, J = 8.6 Hz), 6.43 (1H, d, J = 1.4 Hz), 2.46 (3H, s). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 165.8, 165.4, 157.1, 142.9, 141.4, 141.2, 139.8, 134.5, 133.6, 133.5, 130.7, 130.0, 129.8, 125.1, 124.4, 124.3, 120.8, 118.3, 116.1, 11.2. HRMS (TOF, ES+) $C_{20}H_{13}Cl_2N_2O_4$ $[M + H]^+$ calcd mass 415.0252, found 415.0251.

***N*-(3-Chloro-4-(4-fluoro-1,3-dioxoisoindolin-2-yl)phenyl)-3-methylfuran-2-carboxamide (17e).** Cream powder. ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 8.16 (1H, s), 8.06 (1H, d, J = 2.3 Hz), 7.80 (2H, m), 7.65 (1H, dd, J = 8.6 Hz, J = 2.4 Hz), 7.49 (1H, m), 7.39 (1H, d, J = 1.4 Hz), 7.30 (1H, d, J = 8.6 Hz), 6.42 (1H, d, J = 1.4 Hz), 2.46 (3H, s). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 165.5, 163.3, 157.9 ($^1J_{CF}$, d, J = 266 Hz), 157.2, 142.9, 142.7, 141.4, 139.8, 137.0 ($^3J_{CF}$, d, J = 7.6 Hz), 133.8 (($^2J_{CF}$, d, J = 22.6 Hz), 130.8, 130.0, 124.3, 122.7 ($^2J_{CF}$, d, J = 19.6 Hz), 120.8, 120.1 ($^4J_{CF}$, d, J = 3.2 Hz), 118.7, 118.3,116.1, 11.2. HRMS (TOF, ES+) $C_{20}H_{13}ClFN_2O_4$ $[M + H]^+$ calcd mass 399.0548, found 399.0547.

***N*-(3-Chloro-4-(5-fluoro-1,3-dioxoisoindolin-2-yl)phenyl)-3-methylfuran-2-carboxamide (17f).** Cream powder. ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 8.16 (1H, s), 8.06 (1H, d, J = 2.3 Hz), 7.97 (1H, dd, J = 8.2 Hz, J = 4.5 Hz), 7.64 (2H, m), 7.47 (1H, td, J = 8.5 Hz, J = 2.2 Hz), 7.40 (1H, d, J = 1.5 Hz), 7.30 (1H, d, J = 8.6 Hz), 6.43 (1H, d, J = 1.5 Hz), 2.47 (3H, s). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 165.6, 165.3, 157.1, 142.9, 141.4, 139.8, 134.6 ($^3J_{CF}$, d, J = 9.7 Hz), 133.7, 130.7, 130.0, 127.6 ($^4J_{CF}$, d, J = 2.9 Hz), 126.3 ($^3J_{CF}$, d, J = 9.1 Hz), 124.5, 121.5 ($^2J_{CF}$, d, J = 22.9 Hz), 120.8, 118.3, 116.1, 111.6 ($^2J_{CF}$, d, J = 25.0 Hz), 11.2. HRMS (TOF, ES+) $C_{20}H_{13}ClFN_2O_4$ $[M + H]^+$ calcd mass 399.0548, found 399.0546.

***N*-(3-Chloro-4-(1,3-dioxo-1,3-dihydro-2*H*-pyrrolo[3,4-*c*]-pyridin-2-yl)phenyl)-3-methylfuran-2-carboxamide (17g).** Cream powder. ^{1}H NMR (400.1 MHz, DMSO-d_6) δ (ppm): 10.47 (1H, s), 9.31 (1H, s), 9.21 (1H, d, J = 4.8 Hz), 8.21 (1H, d, J = 2.2 Hz), 8.06 (1H, d, J = 4.8 Hz), 7.92 (1H, dd, J = 8.7 Hz, J = 2.2 Hz), 7.86 (1H, d, J = 1.4 Hz), 7.56 (1H, d, J = 8.7 Hz), 6.65 (1H, d, J = 1.4 Hz), 2.37 (1H, s). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ (ppm): 166.2, 165.9, 158.0, 156.8, 145.1, 144.5, 141.7, 141.4, 139.3, 132.3, 131.5, 129.4, 125.9, 124.1, 121.1, 120.0, 117.8, 116.3, 11.5. HRMS (TOF, ES+) $C_{19}H_{12}ClN_3O_4$ $[M]^+$ calcd mass 381.0516, found 381.0519.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00727.

Detailed pharmacology, DMPK, electrophysiology, and in vivo behavioral methods (PDF)

Molecular formula strings (CSV)

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Author Contributions

P.M.G.-B. performed chemical synthesis and molecular pharmacology. H.P.C. performed molecular pharmacology and mutagenesis. C.M.N., P.J.C., and C.W.L. oversaw the molecular pharmacology and mutagenesis. F.W.B. did all animal dosing and drawing. C.W.L. and A.L.B. performed and analyzed all DMPK studies. Z.X. performed the electrophysiology, and J.M.R. performed the in vivo behavioral studies. C.W.L. oversaw all aspects of the work (medicinal chemistry, pharmacology, DMPK) and wrote the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

$mGlu_1$, metabotropic glutamate receptor subtype 1; nsSNP, nonsynonymous single nucleotide polymorphism; DMPK, drug metabolism and pharmacokinetics; PAM, positive allosteric modulator; NAM, negative allosteric modulator; Glu, glutamate

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