Tetrahydronaphthyridine and Dihydronaphthyridinone Ethers As Positive Allosteric Modulators of the Metabotropic Glutamate Receptor 5 (mGlu5)

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ABSTRACT: Positive allosteric modulators (PAMs) of metabotropic glutamate receptor 5 (mGlu5) represent a promising therapeutic strategy for the treatment of schizophrenia. Starting from an acetylene-based lead from high throughput screening, an evolved bicyclic dihydronaphthyridinone was identified. We describe further refinements leading to both dihydronaphthyridinone and tetrahydronaphthyridine mGlu5 PAMs containing an alkoxy-based linkage as an acetylene replacement. Exploration of several structural features including western pyridine ring isomers, positional amides, linker connectivity/position, and combinations thereof, reveal that these bicyclic modulators generally exhibit steep SAR and within specific subseries display a propensity for pharmacological mode switching at mGlu5, as well as antagonist activity at mGlu5. Structure—activity relationships within a dihydronaphthyridinone subseries uncovered 12c (VU0405372), a selective mGlu5 PAM with good in vitro potency, low glutamate fold-shift, acceptable DMPK properties, and in vivo efficacy in an amphetamine-based model of psychosis.

INTRODUCTION

Schizophrenia is a complex mental illness characterized by positive (hallucinations, paranoia, disorganized behavior) and negative symptoms (social withdrawal, anhedonia, flat affect) as well as cognitive dysfunction (deficits in attention, learning, and memory).1–4 Current treatments, including typical and second-generation atypical antipsychotics, are based largely upon the dopaminergic hypothesis of schizophrenia, which targets overactivation of subcortical dopamine D2 receptors.5 Both classes treat the positive symptoms; however, neither class of antipsychotics has made a substantial impact on the negative and cognitive symptoms.1–4 After several decades of clinical use, a significant understanding of the risks and side effect profiles that plague both classes of antipsychotics has been gained. These include extrapyramidal side effects, sexual dysfunction, weight gain (metabolic syndrome), agranulocytosis, increased cardiac risk, and poor patient compliance.3,6,7 The development of positive allosteric modulators (PAMs) of metabolotropic glutamate receptor 5 (mGlu5)8 as a novel approach to test the glutamate or N-methyl-D-aspartate (NMDA) receptor hypofunction hypothesis of schizophrenia9–14 has provided preclinical evidence for therapeutic potential in multiple psychosis and cognitive animal mod-
els,10,12–14 and, for more than a decade, industry and academic drug discovery groups have been in pursuit of brain penetrant small molecule mGlu5 potentiators.15,16 A testament to the success of the field has been the identification of over eight reported chemotypes15,16 with in vivo efficacy in preclinical models, beginning with 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB, 1),17 piperazinyl 1,2,4-oxadiazoles from Addex (ADX-47273, 2),18,19 and subsequently acetylenes N-methyl-5-(phenylethynyl)pyrimidin-2-amine (MPPA, 3).20 VU0360172 (4),21 LSN2463359 (8),22,23 piperazines VU0364289 (5)24 and 1-(4-(2-chloro-4-fluorophenyl)-piperazin-1-yl)-2-((pyridin-4-ylmethyl)ethanone (CPPZ, 6),25 ether VU0404251 (7),26 and triazole LSN2814617 from Lilly (9).23 However, recent findings from Merck-Addex and at the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD) have unveiled a target mediated CNS adverse-effect (AE) liability, driven by excessive glutamate fold potentiation27 or allosteric agonism,28,29 respectively; suggesting that PAMs with lower functional cooperativity with glutamate (e.g., glutamate fold-shift or potentiation) and devoid of allosteric agonism may be preferred for improved therapeutic index.30 More recently, we disclosed a phenoxy-based ether dihydrothiazolopyridone series31 with the representative in vivo tool compound VU0408899 (10) (Figure 1). Ether 10 represents, in part, efforts to constrain amides of type 7; however, due to inherent instability in acidic media (20% HP-β-CD in water, pH = 4) leading to the corresponding 2-hydroxycyclodextrinoid fragment, full exploration of the analogous system containing a benzoyl ether linkage was not possible. Limited in vitro structure–activity relationships (SAR) suggested a preference for the phenoxy-based linker within the dihydrothiazolopyridones (two examples tested);31 however, this trend was not found within monocyclic nicotineamides of type 7.26 Thus, it was unknown if alteration of the linker moiety within the context of a bicyclic system to more closely mimic 7 (e.g., dihydronaphthyridine core structures 12–17, Figure 2), would be productive for mGlu5 receptor potentiation. Prior studies within a dihydronaphthyridine class utilizing an acetylene-based linker, initially identified from an HTS screen, led to PAMs with submicromolar potency,32 including dihydronaphthyridinones 11, suggested that linkers employing either —CH2O— or —OCH3— might be successful as an acetylene replacement. Interestingly, evidence of subtle “molecular switches”15,33 within acetylenic dihydronaphthyridinones 11 via simple amide congeners led to fundamental changes in the mode of pharmacology (e.g., from PAMs to negative allosteric modulators or NAMs).15 The pursuit of ethers 12–20 were undertaken as part of a larger comprehensive study aimed to target four key structure–activity relationships: (1) endocyclic versus exocyclic amide, (2) phenoxy versus benzoxyl linker, (3) central pyridine ring isomers, and (4) site of the pendant group attachment to central heterocycle. It was unclear at the outset if ethers 12–20 would exhibit subtle changes in the mode of pharmacology similar to 1132 and other chemotypes30,34,35 or would maintain a higher fidelity for potentiation similar to CDPPB (1),17,36,37 N-[5-chloro-2-[(−1,3-dioxoisindolin-2-yl)methyl]phenyl]-2-hydroxy benzamide (CPPHA),38–40 piperazines (5–6),24,25,41 and glycine sulfonamide (ML332)42 based mGlu5 PAMs. Herein we describe the synthesis and pharmacological profile of a series of tetracyclodronaphthyridine and dihydronaphthyridinone ethers (12–20) as positive allosteric modulators of mGlu5.43–45 Low cooperativity PAM 12c (VU0405372) was ultimately identified and found to demonstrate a suitable balance of in vitro and in vivo properties for proof-of-concept studies in an amphetamine hyperlocomotor challenge model. In contrast to previously reported and related monocyclic ether series,26,31 these bicyclic modulator scaffolds were overall found to exhibit narrow SAR, low efficacy, and have a higher propensity for pharmacological “mode switching”15 upon chemical modification, demonstrating an SAR profile reminiscent of related acetylenic PAMs.32

![Figure 1](https://example.com/figure1.png)

**Figure 1.** mGlu5 receptor PAMs with reported efficacy in preclinical models of schizophrenia and cognition.17–26,31

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Evolution of tetracyclodronaphthyridine and dihydronaphthyridinone ether based mGlu5 allosteric modulators (12–20) from nicotinamide 720 and acetylenic modulator scaffold 11.32
RESULTS AND DISCUSSION

Chemistry. To prepare bicyclic analogues that most closely mimic the structural features found in 11, we initially prepared a series of dihydro-1,6-naphthyridin-5(6\textsubscript{H})-ones (12a−12t, Scheme 1). Starting from known compound 2-chloro-7,8-dihydro-1,6-naphthyridin-5(6\textsubscript{H})-one (21a, Supporting Information), substitution under basic conditions in the presence of various alcohols with heating afforded 12a−12h, 12q, and 12s in low to moderate yield. A subset was treated with alkylating agents under basic conditions, or with various aryl and heteroaryl halides using a copper iodide–diamine ligand protocol to effect a modified Ullman lactam cross-coupling to give the respective alkyl (12i−12n, 12s−12t) and N-aryl (12o−12p, 12r) lactam products.

Evaluation of the alternate ether linkage based upon an aryloxy methyl dihydronaphthyridinone, series 13, began synthetically from 21a as shown in Scheme 2. Protection with \textit{para}-methoxybenzyl chloride (PMBCl) afforded 21b in good yield. Suzuki−Miyaura coupling under Molander’s conditions using vinyl potassium trifluoroborate salt gave

Scheme 1. Synthesis of Alkoxy Dihydro-1,6-naphthyridinone Lactams 12a

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\text{Reagents and conditions: (a) 21a, R}^1\text{CH}_2\text{OH, KOT-Bu, DMF, 100 °C, 4−16 h, 23−63%; (b) lactam, R}^2\text{CH}_2\text{Br or R}^2\text{CH}_2\text{Cl, KOT-Bu, DMF, 60 °C, 2−4 h, 40−75%; (c) lactam, CuI (0.2 equiv), Ar/HetBr, N,N-dimethylethylene diamine, K}_2\text{CO}_3, \text{toluene, 120 °C, 18 h, 35−59%}.\n```

Scheme 2. Synthesis of Alkoxymethyl Dihydro-1,6-naphthyridinone Lactams 13a

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\text{Reagents and conditions: (a) 21a, PMBCl, KOT-Bu, DMF, 60 °C, 2 h, 66%; (b) vinyl potassium trifluoroborate, Cs}_2\text{CO}_3, \text{Pd(PPh}_3\text{)}_4, \text{EtOH, 80 °C, 2 h, 74%; (c) O}^\text{H}, \text{CH}_2\text{Cl}_2: \text{MeOH, −78 °C, 40 min, NaBH}_4, 0 °C; (d) R}^1\text{OH, DIAD, PPh}_3, \text{THF, 0 °C, 16 h, 35−40% (over two steps); (e) CAN, CH}_2\text{CN}: \text{H}_2\text{O, rt, 50 min, 60−65%; (f) 13a−b, R}^2\text{CH}_2\text{Br or R}^2\text{CH}_2\text{Cl, KOT-Bu, DMF, 60 °C, 2−4 h, 44−68%; (g) 13a−b, CuI (0.2 equiv), Ar/HetBr, N,N-dimethylethylene diamine, K}_2\text{CO}_3, \text{toluene, 120 °C, 18 h, 25−89%}.\n```

Scheme 3. Synthesis of Phenoxymethyl Dihydro-1,7-naphthyridinones Lactams 14a

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\text{Reagents and conditions: (a) 24, BH}_3, \text{THF, 0 °C, 16 h, 68%; (b) Zn(CN)}_2, \text{Cs}_2\text{CO}_3, \text{Pd(PPh}_3\text{)}_4, \text{DMF, 100 °C, 3 h, 56%; (c) DBAB, phenol, PPh}_3, \text{THF, 0−120 °C, 40 min, 80%; (d) NaOH, 115 °C, 3 h, quant; (e) CH}_2\text{I, K}_2\text{CO}_3, \text{DMF, rt, 16 h, 73%; (f) Bu}_3\text{Sn(allyl), Pd(PPh}_3\text{)}_4, \text{toluene, 125 °C, 16 h, 56%; (g) NaIO}_4, \text{OsO}_4, \text{THF, 0 °C−rt, 2 h, 74%; (h) RNH}_2, \text{THF-MeOH, NaBH(OAc)}_3, \text{rt, 16 h, 15−25%}.\n```

Chemistry. To prepare bicyclic analogues that most closely mimic the structural features found in 11, we initially prepared a series of dihydro-1,6-naphthyridin-5(6\textsubscript{H})-ones (12a−12t, Scheme 1). Starting from known compound 2-chloro-7,8-dihydro-1,6-naphthyridin-5(6\textsubscript{H})-one (21a, Supporting Information), substitution under basic conditions in the presence of various alcohols with heating afforded 12a−12h, 12q, and 12s in low to moderate yield. A subset was treated with alkylating agents under basic conditions, or with various aryl and heteroaryl halides using a copper iodide–diamine ligand protocol to effect a modified Ullman lactam cross-coupling to give the respective alkyl (12i−12n, 12s−12t) and N-aryl (12o−12p, 12r) lactam products.

Evaluation of the alternate ether linkage based upon an aryloxy methyl dihydronaphthyridinone, series 13, began synthetically from 21a as shown in Scheme 2. Protection with \textit{para}-methoxybenzyl chloride (PMBCl) afforded 21b in good yield. Suzuki−Miyaura coupling under Molander’s conditions using vinyl potassium trifluoroborate salt gave
vinyl dihydronaphthyridinone intermediate. Subsequent ozonolysis and reductive work-up gave intermediate alcohol. Mitsunobu alkylation in the presence of either phenol or 3-fluorophenol provided in 35–40% yield over two steps. Removal of the PMB protecting group using cerium ammonium nitrate proceeded smoothly to give 13b. Remaining analogues 13c–13i were prepared similarly as demonstrated for series 12, using alkylating conditions or copper-based arylation conditions to give alkyl and N-aryl lactam products 13c–13e and 13f–13i, respectively.

Within a third class of lactams 14 (Scheme 3), a benzyloxy linkage was retained and an alternate dihydro-1,7-naphthyridinone ring system was explored, placing the pyridine nitrogen at the position ortho to the lactam amide. Synthesis of series 14 began with borane reduction of 5,6-dichloronicotinic acid and subsequent cyanation using Pd(0) catalysis to give in moderate yield. Treatment of 25 under Mitsunobu conditions using phenol and di-tert-butyl azodicarboxylate (DBAB), followed by hydrolysis and ester formation, afforded chloroester 27. Allylation of 27 using Stille conditions with allyl tributyltin, followed by osmium tetroxide–sodium periodate mediated cleavage, provided key aldehyde intermediate 28 in good yield for the two steps. At this stage, installation of the lactam could be accomplished using a variety of amines in a one-pot two-step reductive alkylation, ring-cyclization reaction using sodium triacetoxborohydride in THF–MeOH to provide examples in low to moderate yield.

Synthesis of phenoxymethyl-dihydro-2,7-naphthyridinones 15 was accomplished using a seven-step sequence from methyl-6-methylnicotinate (29) as shown in Scheme 4 and represented the fourth class of dihydronaphthyridinones investigated. Chlorination followed by pyridine N-oxide formation using catalytic methyliodomethane(VII) in the presence of hydrogen peroxide gave intermediate 30. Polonovski rearrangement to give acetate 31 followed in low yield. Hydrolysis and subsequent Mitsunobu alkylation provided ether 32 in good yield. Installation of the vinyl moiety using Stille conditions provided key pyridine intermediate 33 in good yield. Vinylpyridine 33 smoothly underwent one-pot Michael addition and lactam cyclization using ammonia or several alkyl and aryl amines to give 15a–15d in moderate to excellent yield.

Alternative tetrahydronaphthyridines wherein the ether linkage is appended at the 3-position as a benzyloxy with an exocyclic amide were achieved according to Scheme 5. Starting bromide 34 was treated with HATU coupling conditions to give amides 35a–35c. Ullman-based etherification using CuI and 1,10-phenanthroline in toluene with heat afforded targets 20d–20g. The related aryloxymethyl targets retaining a similar substitution pattern and acyl-tetrahydronaphthyridine were prepared according to Scheme 6. Initial introduction of amide provided intermediate 35c in 96% yield. Introduction of 4-fluoro-benzamide provided intermediate 35c, followed by Stille cross-coupling to give vinyl 36. Subsequent ozonolysis and reductive work-up gave alcohol 37, which was elaborated via Mitsunobu alkylation to provide 20a–20c in low to moderate yields.

In Vitro Pharmacology. As reported previously, compounds were profiled in a rat mGlu5 low receptor expression cell line using a “triple add” protocol allowing for detection of agonism as well as positive and negative allosteric modulation simultaneously. In contrast, an allosteric modulator devoid of agonism in these low-expressing mGlu5, cell was found not to display a seizure liability in vivo. Thus, this recombinant mGlu5 cell line in conjunction with the “triple add” protocol provided an important initial in vitro assessment of both agonist and potentiation activity at mGlu5, which could be utilized to prioritize analogues for further progression.

Dihydronaphthyridinones. SAR are shown in Tables 1–4 (12–15, 20) and Figures 4–6 (16–19) and summarize in vitro potency optimization efforts from 10 distinct subseries. Initial efforts within Tables 1–2 were focused on further expanding our previously utilized pharmacophore noted within several chemotypes, including acetylene ethers 7 and 10, which retain a pyridyl nitrogen and an amide oxygen as...
Scheme 6. Synthesis of 3-Aryloxymethyl-tetrahydro-1,6-naphthyridine Amides 20a–20c

Reagents and conditions: (a) 34, 4-fluorobenzoic acid, HATU, DIPEA, DMF, rt, 16 h, 96%; (b) Bu3(vinyl)Sn, Pd(PPh3)4, toluene, 120 °C, 1 h, 82%; (c) O3, CH2Cl2:MeOH, −78 °C, 30 min, NaBH4, 0 °C; (d) R1OH, DIAD, polystyrene−PPh3, THF, rt, 16 h, 25–40% (over two steps).

potential hydrogen bond accepting groups in an optimal spatial arrangement as illustrated in Figure 3. As can be seen in Table 2, studies by AstraZeneca Pharmaceuticals48 and within the dihydrothiazolopyridone series10 have demonstrated successful utilization of the both benzyloxy- and phenoxymethyl-based spacers as replacements for the acetylene moiety, with varied potency depending on the nature of the heterocycle scaffold.26,31,48 Interestingly, in contrast to the unsubstituted benzyloxy lactams 12a−12b, alkoxymethyl-dihydronaphthyridinones 13a−13b were weak PAMs (a weak PAM results in a response that is greater than a 10% increase compared to the submaximal glutamate addition (EC20) but does not potentiate the overall glutamate EC50 response by at least 2-fold). Similarly, N-alkyl derivatives were not productive as PAMs, leading to compounds categorized as weak or inactive (13c−13e, 13i). Direct N-aryl derivatives were more beneficial, with the western 3-fluorophenyl congener 13g bearing an N-4-fluoro-phenyl proving to be potent (EC50 = 97 nM) and efficacious (Glu max = 72%). The 2-pyridyl derivative 13h was 5-fold less active (EC50 = 595 nM) and less efficacious. Fortunately, introduction of a 3-fluoro moiety on the distal phenyl consistently boosted potency (~2–3-fold) and proved transferrable among the subseries (e.g., 13f vs 13g). The potency for 13g−13h was overall similar to the benzoxyl direct comparators 12o−12p; however, phenoxy methyl-based PAMs 13g−13h have a 20−30% elevated Glu max response. Despite modification of the linker, PAM activity was retained, with no evidence of allosteric agonism.

Figure 3. Heterocycle, amide, linker pharmacophore relationships, and relative profile for “endocyclic” series 12−15 dihydronaphthyridinones and hypothetical benzyloxy lactam core regioisomers of 14−15.

1, in the case of 2-benzyloxy derivatives, half of the analogues were active as mGlu5 PAMs. Among active, the parent lactams (R = H, 12a−12h, 12q) showed a range of potency from 62 to 1171 nM with low to moderate maximal efficacy (glutamate max 43−68%). Notably, no evidence for allosteric agonism was observed for actives in the series. SAR on the R1 group demonstrated tolerance for meta-substituted fluorine and chlorine congeners 12c and 12e, respectively, representing two of the more interesting actives from this subseries on the basis of overall potency and efficacy (EC50 < 250 nM, Glu max >40%). n-Butyloxy derivatives 12q and 12r, reminiscent of a previously disclosed non-MPEP ether PAM, were not successful.47 The 2 and 3-pyridyl congeners 12g and 12h were also not tolerated at R1. Additionally, eastern alkyl derivatives 12i−12n containing linear, branched, cyclic, phenyl, and heterocyclic systems were either inactive (a <10% increase in the baseline EC20 glutamate fluorescence response in calcium assays at the highest concentration tested of 30 μM is defined as “inactive”) or had moderate potency and low efficacy (EC50 > 300 nM, PAM Glu max <40%, 12i−12n, 12s−12t). N-Aryl congeners 12o and 12p demonstrated comparable potency below 250 nM, with 12p slightly more efficacious (Glu max 50% vs 38%). Both the western fluoro and the eastern amide N-aryl SAR parallel the recently reported thiazolyl core structure system31; however, interestingly, N-alkyl derivatives were significantly better tolerated in the thiazolyl ring system.

We next turned to the alternate 2-phenoxymethyl derivatives shown in Table 2. Studies by AstraZeneca Pharmaceuticals48 and within the dihydrothiazolopyridone series10 have demonstrated successful utilization of the both benzyloxy- and phenoxymethyl-based spacers as replacements for the acetylene moiety, with varied potency depending on the nature of the heterocycle scaffold.26,31,48 Interestingly, in contrast to the unsubstituted benzyloxy lactams 12a−12b, alkoxymethyl-dihydronaphthyridinones 13a−13b were weak PAMs (a weak PAM results in a response that is greater than a 10% increase compared to the submaximal glutamate addition (EC50) but does not potentiate the overall glutamate EC50 response by at least 2-fold). Similarly, N-alkyl derivatives were not productive as PAMs, leading to compounds categorized as weak or inactive (13c−13e, 13i). Direct N-aryl derivatives were more beneficial, with the western 3-fluorophenyl congener 13g bearing an N-4-fluoro-phenyl proving to be potent (EC50 = 97 nM) and efficacious (Glu max = 72%). The 2-pyridyl derivative 13h was 5-fold less active (EC50 = 595 nM) and less efficacious. Fortunately, introduction of a 3-fluoro moiety on the distal phenyl consistently boosted potency (~2–3-fold) and proved transferrable among the subseries (e.g., 13f vs 13g). The potency for 13g−13h was overall similar to the benzoxyl direct comparators 12o−12p; however, phenoxy methyl-based PAMs 13g−13h have a 20−30% elevated Glu max response. Despite modification of the linker, PAM activity was retained, with no evidence of allosteric agonism.

In parallel, we evaluated isomeric dihydronaphthyridinones 14−15 (Table 3), which retain the linkage site for the distal phenyl para to the lactam amide; however, the pyridine nitrogen is adjacent to the lactam amide in a position reminiscent of a hydrogen bond accepting (HBA) pharmacophore proposed in a related ether series by Varnes and co-workers.49 Within both series 14 (Y = N) and series 15 (X = N) NH lactams (14a, 15a), N-methyl analogues (14b, 15b), and chiral (R)-3,3-dimethylbutan-2-yl substitute analogues, inspired by monocyclic ether 7, all proved to be inactive. The lack of activity in the calcium assay for parent lactams 14a and 15a versus the benzoxyl comparator 12a, in addition to western 3-fluoro congeners 13b (inactive) versus 12c (EC50 = 225 nM), suggests that for series 12−15 wherein the distal...
phenyl is maintained para to the lactam amide, the heteroatom arrangement between the linker oxygen and the pyridine nitrogen within series 12 is more preferred. However, N-aryl analogues have a unique SAR, suggesting that in this context the central tetrahydronaphthyridine scaffold is more critical for mGlu5 potentiation than the nature of the linker, as 12 and 13 are equally tolerated in the context of an N-aryl substituent.

4-Fluorophenyl PAMs 14d and 15d, which differ in the location of the central pyridine nitrogen, have comparable potency with dissimilar efficacy, with pyridine isomer 14d being one of the most efficacious PAMs reported across the tetrahydronaphthyridine subseries described. The related congener 13f was ∼2.5-fold more potent (EC50 = 198 nM) with 68% glutamate max, and 13g was 2-fold more potent than 13f. Relative to series 12, 12o is equipotent to 13g; however, 13g revealed a ∼2-fold higher maximal glutamate response (72% vs 38%). Although a more comprehensive survey was undertaken for series 12−13 relative to 14−15, on the basis of direct comparisons, general trends were noted between the series. A summary of the potency and efficacy profiles are found within Figure 3. Neither molecular switches nor allosteric agonism proved to be issues for 12−15 and SAR was generally steep. Active compounds within 12−13 have capacity for high potency (EC50 ≤ 200 nM) and moderate to high efficacy (%Glu max ≥ 50%), whereas actives within series 14−15, although more limited, exhibited moderate potency and variable efficacy (%Glu max 30% and 81%). Subtle changes in structure−efficacy/cooperativity relationships are best addressed via glutamate fold-shift experiments; however, glutamate max trends were consistent within series 12−15, and series 12 shows variable efficacies that encompass the full range reported for all subseries (Figure 3). In general, phenoxyl linkages show improved efficacy over benzyloxyl linkages for N-arylated analogues. The emerging pharmacophore for 12−13 suggested these dihydronaphthyridine scaffolds interact at the allosteric

Table 1. SAR of 2-Alkoxy-dihydronaphthyridinones 12

| Cmpd | R1 | R2 | mGlu5 pEC50 (±SEM) | mGlu6 EC50 (±SEM) | %Glu Max (±SEM) |
|------|----|----|-------------------|------------------|-----------------|
| 12a  | H  | H  | 5.98±0.04         | 1031             | 54±5.5          |
| 12b  | H  | H  | 5.93±0.08         | 1171             | 68±5.3          |
| 12c  | H  | H  | 6.65±0.12         | 225              | 50±3.2          |
| 12d  | H  | CCl | Inactive          |                  |                 |
| 12e  | H  | CCl | 7.21±0.18         | 62               | 43±5.6          |
| 12f  | H  | CCl | Inactive          |                  |                 |
| 12g  | H  | N  | Inactive          |                  |                 |
| 12h  | H  | N  | Inactive          |                  |                 |
| 12i  | H  | CH3 | Inactive          |                  |                 |
| 12j  | H  | i-Bu | Inactive         |                  |                 |
| 12k  | H  | i-Bu | Inactive         |                  |                 |
| 12l  | H  | CH3 | 6.35±0.09         | 444              | 36±3.4          |
| 12m  | H  | CH3 | 5.61±0.15         | 2473             | 39±5.1          |
| 12n  | H  | CH3 | 5.50±0.23         | 3173             | 36±5.6          |
| 12o  | H  | F  | 6.93±0.07         | 118              | 38±4.0          |
| 12p  | H  | F  | 6.67±0.07         | 212              | 50±4.8          |
| 12q  | H  | F  | Inactive          |                  |                 |
| 12r  | H  | F  | Inactive          |                  |                 |
| 12s  | H  | F  | 5.56±0.09         | 2765             | 23±1.4          |

Table 2. SAR of 2-Alkoxymethyl-dihydronaphthyridinones 13

| Cmpd | R1 | R2 | mGlu5 pEC50 (±SEM) | mGlu6 EC50 (±SEM) | %Glu Max (±SEM) |
|------|----|----|-------------------|------------------|-----------------|
| 13a  | H  | H  | >5.0               | >10,000          | 43±3.4          |
| 13b  | H  | H  | >5.0               | >10,000          | 34±4.2          |
| 13c  | H  | CH3 | Inactive          |                  |                 |
| 13d  | H  | i-Bu | 5.94±0.05         | 1146             | 38±2.9          |
| 13e  | H  | i-Bu | >5.0               | >10,000          | 33±2.0          |
| 13f  | H  | F  | 6.70±0.07         | 198              | 68±1.0          |
| 13g  | H  | F  | 7.01±0.07         | 97               | 72±3.9          |
| 13h  | H  | F  | 6.23±0.06         | 595              | 59±2.4          |
| 13i  | H  | F  | >5.0               | >10,000          | 36±0.8          |

*C*Calcium mobilization assay using HEK293 cells stably expressing rat mGlu5 receptors; values are the average of three or more independent determinations; Inactive, less than 10% change in calcium fluorescence compared to the EC20 glutamate response. *b*Expressed as amplitude of response using 30 μM test compound (percentage of maximal response versus 100 μM glutamate).
which were either inactive or weak PAMs (EC_{50} > 10 \mu M). Interestingly, a related more rigid, acetylene tetralone scaffold recently reported by Merz Pharmaceuticals GmbH\textsuperscript{51} displays a similar array of HBA moieties and was reported to have excellent PAM activity (EC_{50} < 100 nM) in a recombinant CHO cell line expressing human mGlu5.

**Acyl-tetrahydronaphthyridines.** Inspired in part by the Addex piperidine chemotype \textsuperscript{2,15,52} we postulated that perhaps a more extended pharmacophore placing the amide carbonyl moiety exocyclic of the tetrahydronaphthyridine scaffold as found within the Addex chemotype might prove viable (Figure 5). PAM 12c served as a precursor (Scheme 1) and allowed for facile access to representative benzyloxy targets 18a−d in two steps via borane reduction and subsequent amide coupling. Intriguingly, all proved to be weak to moderate antagonists of mGlu5 (Figure 5). At this juncture, in light of the unexpected switch in the mode of pharmacology for subseries 18, as well as the general challenges of multidimensional combinations of linker, core, attachment site, and lactam versus exocyclic amide (minimum of 24 formal combinations/scaffolds), we employed a ligand-based computational tool previously reported\textsuperscript{42} to prioritize and potentially streamline medicinal chemistry efforts and ligand design. Using this approach, we assessed ligand-based conformational ensembles of known highly efficacious PAMs, including Addex ligand \textsuperscript{2} and other lead molecules,\textsuperscript{53,54} with PAM activity below 150 nM and robust Glu max values above 70%. These low energy ensembles of PAMs served as flexible “hypotheses” or reference PAMs for a mutual flexible low energy shape-based alignment of proposed dihydronaphthyridone and tetrahydronaphthyridines using the Surflex-Sim algorithm as implemented in Sybyl.\textsuperscript{55,56} The molecular similarity and alignment optimization algorithms used in Surflex-Sim docking utilize a functional term to minimize the volume of molecular superpositions to construct an objective function for scoring superpositions of multiple molecules. The objective function can then be used as targets for virtual screening, or in this case, for scaffold hopping prioritization. The results from these studies using 2 are summarized in Figure 6 with a depicted overlay of the lowest energy structures for 2 versus 2-phenoxy methyl model amide 19 and 3-phenoxy methyl model amide 20. A Surflex-Sim score

![Figure 4. SAR summary of 3-benzyloxy and 3-phenoxymethyl-dihydronaphthyridinones 16−17.](image-url)

![Figure 5. Relationship between 2 and 18−20 and resulting first-generation exocyclic amides 18 utilizing 2-benzyloxy linkage.](image-url)

### Table 3. SAR of Isomeric Phenoxymethyl-dihydronaphthyridinones 14−15

| Compd | X   | Y   | R   | mGlu5 pEC_{50} (±SEM) | mGlu5 EC_{50} (nM) | %Glu Max (±SEM) |
|-------|-----|-----|-----|-----------------------|-------------------|-----------------|
| 14a   | CH  | N   | H   | Inactive              |                   |                 |
| 14b   | CH  | N   | CH3 | Inactive              |                   |                 |
| 14c   | CH  | N   |     | Inactive              |                   |                 |
| 14d   | CH  | N   |     | 6.27±0.09, 538        | 81±6.7            |                 |
| 15a   | N   | CH  | H   | Inactive              |                   |                 |
| 15b   | N   | CH  | CH3 | Inactive              |                   |                 |
| 15c   | N   | CH  |     | Inactive              |                   |                 |
| 15d   | N   | CH  |     | 6.39±0.35, 403        | 30±4.1            |                 |

\textsuperscript{4}Calcium mobilization assay using HEK293 cells stably expressing rat mGlu5 receptors; values are the average of three or more independent determinations; Inactive, less than 10% change in calcium fluorescence compared to the EC_{20} glutamate response. \textsuperscript{b}Expressed as amplitude of response using 30 \mu M test compound (percentage of maximal response versus 100 \mu M glutamate).
among certain mGlu5 modulator chemotypes.33 Within the phenoxy subseries analogues containing a 3-phenoxy methyl linker, and 3-benzyloxy fluoro congener 20c, success was found with 4-fluorobenzamide derivatives; in particular, the western phenyl and 3-fluoro phenyl derivatives 20a and 20b have statistically comparable potency and efficacy, with EC50 of 640 and 1090 nM, respectively, and a Glu max of 69%. In contrast, the 4-fluoro congener 20c was 2–3-fold less active. Three significant findings are evident among these lactam and exocyclic amide templates examined: (1) parent dihydronaphthyridinone 12c remains a confounding singleton relative to regioisomeric and positional congeners 13b, 14a, 15a, 16, and 17, (2) relative to earlier acetylenic dihyronaphthyridinones 11,32 no molecular switches were noted within dihydronaphthyridinone systems, however, steep SAR was evident, and (3) in contrast to dihydronaphthyridinones, exocyclic acyl-tetrahydronaphthyridines were highly sensitive to the linker attachment site, as 2-substituted based ethers 18 and 19, proved to be either weak antagonists or functionally inactive in the calcium assay. In addition, within the exocyclic acyl-tetrahydronaphthyridines, phenoxymethyl-based ethers were better tolerated and less susceptible to changes in the mode of pharmacology (e.g., 20a versus 20g); however, overall potency was generally diminished relative to series 12–13.

In light of the overall mGlu5, PAM potency (EC50 < 250 nM) and efficacy (Glu max >50%) observed for compounds 12c, 12p, 13f, and 13g, these PAMs were selected for further characterization (Table 5). In addition, the moderately potent PAM 14d (EC50 = 538 nM) was considered for further evaluation based upon its enhanced efficacy (Glu max = 81%).

Among the PAMs further examined, 12p, 13f, 13g, and 14d have similar cLogP and molecular weight (MW) by virtue of their common lactam N-aryl substituent, while the simple NH lactam 12c stands out as a low MW modulator (MW = 272) with excellent calculated ligand efficiency metrics (LipE and LELP, see Table 5).56,59 PAM 12c maintains the highest

(without additional information)
calculated LipE and lowest LELP among the set, suggesting a highly favorable enthalpy driven effect on cooperativity. Despite the less favorable physicochemical and LE properties for **12p**, **13f**, **13g**, and **14d** relative to **12c**, intrinsic clearance in human and rat microsomes suggested moderate predicted hepatic clearance and 1–10% fraction unbound. Subsequent selectivity profiling against mGlu1−4,6 using **12c**, **13g**, and **14d** revealed that N-aryl congeners **13g** and **14d** were also active as mGlu4, negative allosteric modulators (NAMs) with negative cooperativity fold-shift values between 0.2 and 0.3, while **12c** was fully selective for mGlu5 (mGlu1−4,6 EC50 > 10 μM, see Supporting Information).

**Additional Pharmacological Characterization of 12c.** In light of the unexpected lack of selectivity for **13g** and **14d** which maintain a similar N-aryl endocyclic amide pharmacophore found within **12p** and **13f**, we elected to continue to profile **12c** in more depth based on its overall potency, selectivity, and excellent physicochemical and ligand efficiency parameters (see Figure 7 for overall profile). Positive allosteric mGlu5 receptor revealed a small leftward shift in the glutamate concentration response curve (CRC) with increasing concentration of **12c** (55 nM to 30 μM), with no significant effect on the maximal response. A 2.2-fold leftward shift in the glutamate EC50 was observed in the presence of 30 μM **12c**, and a predicted allosteric modulator affinity of 2.17 μM and an efficacy cooperativity factor (log β) between glutamate and indicated allosteric modulator of 0.23 (cooperativity ~1.71) was defined as calculated using the operational model of allosterism.60 These results support a positive allosteric interaction between glutamate and **12c** and were confirmed in a cell line expressing the human mGlu5 receptor,60 where 10 μM **12c** produced a 3.3-fold leftward shift in the glutamate CRC and a PAM CRC potency of 370 nM (71% Glu max). In comparison to recently reported PAMs from our lab that have been profiled in glutamate progressive fold-shift studies up to 30 μM, including acetylenic picolinamides (e.g., ML254, maximum rat mGlu3 fold-shift 3.0)30 and dihydrothiazolopyridine 10 (maximum rat mGlu5 fold-shift 5.4),12c represents one of the weakest mGlu5 allosteric modulators reported in terms of measurable positive cooperativity.

**Extended DMPK Characterization and Ancillary Pharmacology of 12c.** Rat brain homogenate binding to assess fraction unbound in brain revealed a reasonable brain fm of 2.0% for **12c**. Inhibition of the major human cytochrome P450 (CYP) enzymes (2C9, 2D6, 3A4, 1A2) was measured in a cocktail assay using human liver microsomes and known substrates. PAM **12c** was found to display weak CYP inhibitory activity at 1A2 (IC50 = 25.7 μM), while no activity was observed against the other CYPs tested (IC50 > 30 μM). In kinetic aqueous solubility assays, **12c** displayed moderate to good solubility (pH 2, 4, 7.4: buffer: 38, 29, and 28 μM) and moderate solubility in fasted simulated intestinal fluid (Fassi, pH = 7.2) of 31 μg/mL (114 μM). In a nontoxic screen using 20% HP β-cyclodextrin, **12c** was a solution at 1 mg/mL and a microsusension at 3–5 mg/mL (pH 4.0). Rat pharmacokinetic studies (1 mg/kg IV, 10 mg/kg PO) revealed a high plasma clearance (CLpu) of 75 mL/min/kg and a short half-life (t1/2) of 0.3 h. After oral administration (10 mg/kg, 0.5% methyl cellulose, 1 mg/mL, noncrossover) to male Sprague–Dawley rats, **12c** reached an average maximal concentration (Cmax) of 3.36 μM with a corresponding time to reach Cmax (Tmax) of 0.5 h and an AUC0−24h of 62.6 μM-h, thus affording an estimated %F of 75. We performed a single dose in vivo screen to study the ability of **12c** to reverse amphetamine-induced hyperlocomotion in rats (PO, 56.6 mg/kg 20% HP β-cyclodextrin). Robust reversal of the hyperlocomotion response was noted at this dose (>30%, data not shown) and high brain exposure was observed at 1.5 h (Cbrain = 16.6 μM, 332 nM unbound), producing an average brain-to-plasma (B:P) ratio of 1.67 and unbound brain level above the rat in vitro EC50.

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**Table 5. Rat mGlu5 Potency Values and Data for Selected Key Compounds**

| Compd. | rmGlu5 EC50 (nM) | cLogP | LipE | LELP | CLint (h, r) (μL/min/kg) | CLHEP (h, r) (μL/min/kg) | PPB fm (h, r) (%) |
|--------|-----------------|-------|------|------|--------------------------|--------------------------|-------------------|
| **12c** | 225             | 2.62  | 4.03 | 5.76 | 25/175                   | 11/50                    | 0.047/0.034       |
| **12p** | 212             | 3.96  | 2.71 | 11.71| 18/138                   | 9.6/47                   | 0.010/0.025       |
| **13f** | 198             | 3.72  | 2.98 | 10.55| 18/108                   | 9.6/43                   | 0.021/0.036       |
| **13g** | 97              | 3.88  | 3.13 | 10.92| 8.8/121                  | 6.2/44                   | 0.014/0.029       |
| **14d** | 538             | 3.60  | 2.67 | 10.92| 19/33                    | 10/22                    | 0.096/0.110       |

For the mGlu5 pEC50 assay see Tables 1−4 and Experimental Section. cLogP was calculated using Adriana’s XLogP code. LipE = pEC50−cLogP; LELP = ligand efficiency/cLogP.46,59

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**Figure 7. Profile summary of **12c**.**

**Figure 8. Glutamate CRC in the presence of increasing concentrations of **12c**.**
Furthermore, in vivo B:P and in vitro $f_{\text{plasma}}/f_{\text{brain}}$ (1.7) measures were identical, with a calculated $K_{\text{pl}}$ of 1.1, suggesting passive CNS penetration for 12c in rat. Thus, 12c continued to demonstrate promising properties to warrant further evaluation in vivo. With respect to ancillary pharmacology, in a broad panel selectivity screen against 68 GPCRs, ion channels and transporters using 10 μM 12c, no significant off-target activity was noted (Eurofins Inc.).

In Vivo Pharmacological Characterization. Encouraged by its overall profile as a low fold-shift PAM, 12c was evaluated across a range of doses for its ability to reverse amphetamine-induced hyperlocomotion (AHL), an established model of antipsychotic activity (Figure 8). 12c dose-dependently decreased hyperlocomotion (AHL), an established model of antipsychotic activity (Figure 8). As seen in Figure 9, 12c dose-dependently affected open field activity in rats, as observed by a dose-dependent decrease in ambulation, with a maximal effect observed at a dose of 100 mg/kg PO, that produced an estimated brain unbound level 3-fold above its in vitro potency. Maximal efficacy of 12c was observed at a dose of 100 mg/kg with no significant motor impairment or overt neurological side effects. 12c provides another tool compound to complement the available mGlu5 PAMs with well characterized cooperativity profiles, 12c to better enable comparative studies with other chemotypes in native systems and in preclinical animal models.

CONCLUSIONS

Diverse mGlu5 modulator pharmacological profiles were attained within a series of tetrahydronaphthyridine and dihydrophosphorylmocon scaffolds through modifications of heteroatoms within the linker, core structure, and location of the pendant linkage moiety. N-Aryl dihydrophosphorylmocon congeners were nonselective and displayed negative cooperativity at mGlu5, and thus were not further progressed. Parent benzyloxy lactam 12c emerged as a suitable tool compound with good potency, excellent selectivity, and pharmacokinetic properties suitable for acute studies. Despite behaving in vitro as an ultralow cooperativity PAM in recombinant systems, 12c showed robust, dose-dependent effects in the reversal of amphetamine-induced hyperlocomotion, with a lowest active dose of 30 mg/kg PO, that produced an estimated brain unbound level 3-fold above its in vitro potency. Maximal efficacy of 12c was observed at a dose of 100 mg/kg with no significant motor impairment or overt neurological side effects. 12c provides another tool compound to complement the available mGlu5 PAMs with well characterized cooperativity profiles, 12c to better enable comparative studies with other chemotypes in native systems and in preclinical animal models.

EXPERIMENTAL SECTION

General. All reagents purchased from commercial suppliers were used without purification. Unless noted, all solvents used were anhydrous and all reactions were carried out under argon atmosphere. Microwave assisted reactions were performed in a single-mode reactor: Emrys Optimizer microwave reactor (Biotage). Analytical thin layer chromatography was performed on Analtech silica gel GF 250 μm plates and on silica gel 60 F254 plates (Merck) under standard techniques. Unless otherwise specified, preparative reverse-phase high performance liquid chromatography (RP-HPLC) purification was performed on a Gilson Inc. preparative UV-based system using a Phenomenex Luna C18 column (50 mm × 30 mm I.D., 5 μm), with an acetonitrile (unmodified)–0.1% trifluoroacetic acid in water gradient. Normal-phase silica gel preparative purification was performed using an automated CombiFlash RF from ISCO using either ISCO or Merck ready-to-connect cartridges. Analytical LC/MS was performed using the following instruments: (A) Agilent 1200 series with UV detection at 215 and 254 nm and ELSD detection (Polymer Laboratories PL-ELS 2100), utilizing an Accucore C18 2.6 μm, 2.1 mm × 30 mm column, a 1.1 min gradient, 7% [CH3CN/0.1% TFA]–95% [CH3CN/0.1% TFA] and a G6130 single quadrupole mass spectrometer or (B) ultra performance liquid chromatography (UPLC) measurement performed using an Acquity UPLC (Waters) system comprising a sampler organizer, a binary pump with degasser, a four column’s oven, a diode array detector (DAD), and a BEH-C18 column (1.7 μm, 2.1 mm × 50 mm) from Waters, with a flow rate of 1.0 mL/min at 50 °C without split to the MS detector. The gradient conditions consisted of 95% A (0.5 g/L ammonium acetate solution + 5% acetonitrile), 5% B (acetonitrile), to 40% A, 60% B in 3.8 min, to 5% A, 95% B in 4.6 min, kept until 5.0 min without split to the MS detector. The MS detector was configured with an ESCI dual ionization source (electrospray combined with atmospheric pressure chemical ionization). Nitrogen was used as the nebulizer gas. The source temperature was maintained at 140 °C. Low-resolution mass spectra (single quadrupole, SQD detector) were acquired by scanning from 100 to 1000 in 0.1 s using an interchannel delay of 0.08 s. The capillary needle voltage was 3 kV. The cone voltage was 25 V for positive ionization mode and 30 V for negative ionization mode.
acquisition was performed with MassLynx-Openlynx software. GC/MS measurement was performed using a 6890 series gas chromatograph (Agilent Technologies) system comprising a 7683 series injectors and autosampler, coupled to a 5973N MSD mass selective detector (single quadrupole, Agilent Technologies). The MS detector was configured with an electronic impact ionization source/chemical ionization source (EI/CI). EI low-resolution mass spectra were acquired by scanning from 50 to 550 at a rate of 14.29 scans/s. The source temperature was maintained at 230 °C. Helium was used as the nebulizer gas. Data acquisition was performed with Chemstation-Open Action software. GC/MS was carried out on a J&W HP-SMS column (20 m × 0.18 mm, 0.18 μm) from Agilent Technologies, with a flow rate of 0.7 mL/min. The temperature gradient applied was: initial temperature 50 °C, hold for 2.0 min, then a 50 °C/min ramp applied for 5.0 min until 300 °C and hold for 3.0 min in a 10 min run. Front inlet temperature was 250 °C. Split injection mode was used, 0.2 μL injection volume, with a 50:1 ratio into the GC/MS system. HRMS were obtained using a Micromass (Waters) Q-ToF API-US calibrated and verified with sodium iodide. The samples were diluted with a 50:50 0.1% formic acid (in Milli-Q)-acetone solution, directly infused using leucine–enkephalin (M + H = 556.2771) as a lock mass. Scan range was from 100 to 1000 Da, using a scan time of one second. The [M + H] or [M + Na] ion was observed. Purity of all final compounds was determined to be >98% by HPLC. Solvents for extraction, washing, and chromatography were HPLC grade. NMR spectra were recorded on either a Bruker DPX-400 or a Bruker AV-500 MHz spectrometer with standard pulse sequences. 1H Chemical shifts are reported as δ values in CDCl3 or DMSO-d6. Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, p = pentet, hext = hexet, sep = septet, dd = doublet of doubles, dt = doublet of triplets, dq = doublet of quartets, m = multiplet), coupling constant J reported in Hz. 13C chemical shifts are reported in δ values in CDCl3, as follows: chemical shift, C–F coupling constants (JC–F) reported in Hz. For a number of compounds, melting points (mp) were determined in open capillary tubes on a FP62 or on a FP81HTFF90 apparatus (Mettler). Melting points were measured with a temperature gradient of 10 °C/min (maximum temperature 300 °C), and the melting point was read from a digital display. Melting point values are peak values and were obtained with experimental uncertainties that are commonly associated with this analytical method. Optical rotation values were obtained on a JASCO P-2000 polarimeter.

Chemistry. Synthesis of presented compounds was performed according to Schemes 1–6. Experimental procedures, analytical data, HRMS, and NMR spectra can be found within Supporting Information. Synthesis and analytical data for representative members within series 12–19 are provided below.

1-Fluorobenzyl)-7,8-dihydro-1,6-naphthyridin-5(6H)-one (12a). Benzylic alcohol (85 μL, 0.82 mmol) was dissolved into DMF (1.6 mL) and treated with KOT-Bu (184 mg, 1.64 mmol) and stirred for 30 min. The mixture was treated with 2-chloro-7,8-dihydro-1,6-naphthyridin-5(6H)-one (20, 100 mg, 0.55 mmol, see Supporting Information) and heated to 100 °C for 4 h. The mixture was cooled to rt, acidified with 6 N HCl, and extracted with EtOAc (3×). The combined organic layers were washed with brine, dried over Na2SO4, and evaporated to dryness. The crude product mixture was purified by RP-HPLC (eluting with 40–90% MeCN/H2O with 0.1% TFA modifier) to afford the title compound (73 mg, 53%). 1H NMR (400 MHz, CDCl3) δ 8.20 (1H, d, J = 8.6 Hz), 7.76 (2H, m), 7.36 (3H, m), 6.75 (1H, d, J = 8.6 Hz), 5.85 (1H, bs), 5.43 (2H, s), 3.62 (2H, t, J = 6.8 Hz), 3.09 (2H, t, J = 6.8 Hz). 13C NMR (100 MHz, CDCl3) δ 166.4, 165.2, 158.0, 138.3, 136.7, 128.4, 128.0, 127.9, 118.3, 109.8, 68.1, 39.2, 30.7. HRMS (ES+, M + H) calculated for C12H12N2O2: 221.1290; found, 221.1289.

2-Butoxy-7,8-dihydro-1,6-naphthyridin-5(6H)-one (12b). Starting from 2-chloro-7,8-dihydro-1,6-naphthyridin-5(6H)-one (100 mg, 0.55 mmol) and following the general procedure outlined in 12a and 12c, RP-HPLC afforded the title compound as a powder (72 mg, 60%). 1H NMR (400 MHz, CDCl3) δ 8.17 (1H, d, J = 8.6 Hz), 6.67 (1H, d, J = 8.6 Hz), 5.84 (1H, bs), 4.33 (2H, t, J = 6.6 Hz), 3.61 (2H, t, J = 6.8 Hz), 3.06 (2H, t, J = 6.8 Hz). 13C NMR (100 MHz, CDCl3) δ 166.6, 165.1, 138.1, 117.9, 109.4, 66.3, 39.2, 30.9, 19.1, 13.7. HRMS (ES+, M + H) calculated for C12H13N2O2: 222.1341; found, 222.1340.
2-Phenoxymethyl)-7,8-dihydro-1,6-naphthyridin-5(6H)-one (13a). 2-Phenoxymethyl)-7,8-dihydro-1,6-naphthyridin-5(6H)-one (13a) (100 mg, 0.04 mmol) was dissolved in 2-mL DMF, and water (0.1 mL) was added. Cerium ammonium nitrate (42 mg, 0.04 mmol) was added, and after 15 min, the reaction was partitioned between EtOAc (5 mL) and brine (1 mL). The mixture was extracted with EtOAc (2x), and the organic layers concentrated, and purified by RP-HPLC to give title compound (2.6 mg, 28%). 3H NMR (400 MHz, CDCl3) δ 8.32 (1H, d, J = 8.0 Hz), 7.49 (1H, d, J = 8.0 Hz), 7.25 (2H, m), 6.76 (1H, m), 6.69 (2H, m), 6.94 (1H, bs), 5.10 (2H, s), 3.65 (2H, d, J = 6.6 Hz), 3.20 (2H, t, J = 6.7 Hz). LCMS tR = 0.732 min, > 98% at 215 and 254 nm, m/z = 255 [M + H]+, HRMS (ES+, M + H) calcd for C17H16N2O2, 291.1153; found, 291.1157.

2-(3-Fluoroxy)-phenoxymethyl)-7,8-dihydro-1,6-naphthyridin-5(6H)-one (13b). 2-(3-Fluoroxy)-phenoxymethyl)-7,8-dihydro-1,6-naphthyridin-5(6H)-one (13b) (100 mg, 0.04 mmol) was dissolved in CH2CN (5 mL) and water (2 mL) added. Cerium ammonium nitrate (419 mg, 0.77 mmol) was added, and after 15 min, the reaction was partitioned between EtOAc (10 mL) and brine (10 mL). The organic layers were concentrated and purified by RP-HPLC to give title compound as a solid (74 mg, 76%). 3H NMR (400 MHz, CDCl3) δ 8.36 (1H, d, J = 8.0 Hz), 7.53 (1H, d, J = 8.0 Hz), 7.20 (2H, m), 6.69 (1H, m), 6.67 (2H, m), 6.69 (2H, m), 6.94 (1H, bs), 5.20 (2H, s), 3.68 (2H, d, J = 6.6 Hz), 2.52 (2H, t, J = 6.7 Hz), 2.13 (2H, t, J = 6.7 Hz). 13C NMR (100 MHz, CDCl3) δ 165.3, 163.6 (d, Jc-F = 244.2 Hz), 159.9, 159.4 (d, Jc-F = 10.9 Hz), 158.3, 150.3, 134.0 (d, Jc-F = 9.8 Hz), 123.9, 119.9, 110.5 (d, Jc-F = 2.9 Hz), 108.3 (d, Jc-F = 21.1 Hz), 102.7 (d, Jc-F = 24.9 Hz), 70.5, 39.4, 30.9. HRMS (ES+, M + H) calcd for C17H15F3N2O2, 323.1039; found, 323.1037.

2-(3-Fluoroxy)-phenoxymethyl)-6-methyl-7,8-dihydro-1,6-naphthyridin-5(6H)-one (13c). 2-(3-Fluoroxy)-phenoxymethyl)-6-methyl-7,8-dihydro-1,6-naphthyridin-5(6H)-one (13c) (100 mg, 0.04 mmol) was dissolved in DMF (5 mL), and water (2 mL) added. Cerium ammonium nitrate (419 mg, 0.77 mmol) was added, and after 15 min, the reaction was partitioned between EtOAc (10 mL) and brine (10 mL). The organic layers were concentrated and purified by RP-HPLC to give title compound as a solid (74 mg, 76%). 3H NMR (400 MHz, CDCl3) δ 8.20 (1H, d, J = 8.0 Hz), 7.25 (1H, d, J = 8.0 Hz), 7.25 (2H, m), 6.67 (1H, m), 6.64 (2H, m), 5.10 (2H, s), 3.65 (2H, d, J = 6.6 Hz), 3.20 (2H, t, J = 6.7 Hz), 3.16 (2H, s). 13C NMR (100 MHz, CDCl3) δ 165.3, 159.0, 158.3, 134.0 (d, Jc-F = 9.8 Hz), 123.9, 119.9, 110.5 (d, Jc-F = 2.9 Hz), 108.3 (d, Jc-F = 21.1 Hz), 102.7 (d, Jc-F = 24.9 Hz), 70.5, 39.4, 30.9. HRMS (ES+, M + H) calcd for C17H16FN3O2, 323.1039; found, 323.1037.

2-((3-Fluoroxy)-phenoxymethyl)-6-(5-fluoropyridin-2-yl)-7,8-dihydro-1,6-naphthyridin-5(6H)-one (13d). 2-((3-Fluoroxy)-phenoxymethyl)-7,8-dihydro-1,6-naphthyridin-5(6H)-one (13d) (100 mg, 0.04 mmol) was dissolved in DMF (5 mL), and water (2 mL) added. Cerium ammonium nitrate (419 mg, 0.77 mmol) was added, and after 15 min, the reaction was partitioned between EtOAc (10 mL) and brine (10 mL). The organic layers were concentrated and purified by RP-HPLC to give title compound as a solid (74 mg, 76%). 3H NMR (400 MHz, CDCl3) δ 8.20 (1H, d, J = 8.0 Hz), 7.25 (1H, d, J = 8.0 Hz), 7.25 (2H, m), 6.67 (1H, m), 6.64 (2H, m), 5.10 (2H, s), 3.65 (2H, d, J = 6.6 Hz), 3.20 (2H, t, J = 6.7 Hz), 3.16 (2H, s). 13C NMR (100 MHz, CDCl3) δ 165.3, 159.0, 158.3, 134.0 (d, Jc-F = 9.8 Hz), 123.9, 119.9, 110.5 (d, Jc-F = 2.9 Hz), 108.3 (d, Jc-F = 21.1 Hz), 102.7 (d, Jc-F = 24.9 Hz), 70.5, 39.4, 30.9. HRMS (ES+, M + H) calcd for C17H16FN3O2, 323.1039; found, 323.1037.
mixture was diluted with CH₂Cl₂ and washed with a saturated solution of NaHCO₃ and brine. The organic layer was separated, dried (Na₂SO₄), filtered, and the solvents evaporated in vacuo. The crude product was purified by flash column chromatography (silica; 7N solution of ammonia in methanol in CH₂Cl₂ 0/100 to 4/96). The desired fractions were collected and the solvents evaporated in vacuo. The crude product was purified by flash column chromatography (silica; 7N solution of ammonia in methanol in CH₂Cl₂ 0/100 to 4/96). The desired fractions were collected and the solvents evaporated in vacuo to yield an impure fraction which was repurified by RP-HPLC on (C18 XBridge 19 mm × 100 mm, 5 μm). Mobile phase (gradient from 80% 0.1% NH₄CO₃H/NH₄OH pH 9 solution in water, 20% CH₃CN to 0% 0.1% NH₄CO₃H/NH₄OH pH 9 solution in water, 100% CH₃CN) to yield 14d as a white solid (15 mg, 15%). LCMS tᵣ = 2.00 min, > 98% at 215 and 254 nm, m/z = 349.1 [M + H].

1H NMR (500 MHz, CDCl₃) δ 7.07 (m, 2 H), 7.01 (t, J = 6.7 Hz, 2 H), 7.04–6.94 (m, 3 H), 5.26 (s, 2 H), 3.52 (td, J = 6.6, 2.9 Hz, 2 H), 3.06 (td, J = 6.6, 2.9 Hz, 2 H), 2.32 (t, J = 6.7 Hz, 2 H).

6-Phenoxyethyl-3,4-dihydro-2H-[2,7]naphthyridin-1-one (15a). A 7N solution of ammonia in methanol (5.5 mL) was added to 6-phenoxyethyl-4-vinyl-nicotinic acid methyl ester (33, 55 mg, 0.2 mmol). The mixture was stirred at 100 °C for 16 h. The solvents were evaporated in vacuo. The crude product was purified by flash column chromatography (silica; AcOEt in CH₂Cl₂ 0/100 to 100/0). The desired fractions were collected and the solvents evaporated in vacuo to yield an impure fraction which was triturated with Et₂O and repurified by RP-HPLC on (C18 XBridge 30 mm × 100 mm, 5 μm). Mobile phase (gradient from 80% 0.1% NH₄CO₃H/NH₄OH pH 9 solution in water, 20% CH₃CN to 0% 0.1% NH₄CO₃H/NH₄OH pH 9 solution in water, 100% CH₃CN) to yield 15a as a white solid (20 mg, 39%); mp 193 °C. LCMS tᵣ = 1.47 min, > 98% at 215 and 254 nm, m/z = 255.1 [M + H].

1H NMR (400 MHz, CDCl₃) δ 9.18 (s, 1 H), 7.43 (d, J = 0.5 Hz, 1 H), 7.36–7.28 (m, 2 H), 7.06–6.94 (m, 3 H), 6.26 (br s, 1 H), 5.24 (s, 2 H), 3.61 (td, J = 6.6, 2.9 Hz, 2 H), 3.03 (t, J = 6.7 Hz, 2 H).

2-Methyl-6-phenoxyethyl-3,4-dihydro-2H-[2,7]naphthyridin-1-one (15b). Starting from 6-phenoxyethyl-4-vinyl-nicotinic acid methyl ester (33, 55 mg, 0.2 mmol) and 2 M methanol in THF (5.5 mL) following the procedure described for compound 15a, compound 15b was obtained as a white solid (51 mg, 93%); mp 154 °C. LCMS tᵣ = 1.72 min, > 98% at 215 and 254 nm, m/z = 269.1 [M + H].

1H NMR (500 MHz, CDCl₃) δ 9.18 (s, 1 H), 7.38 (s, 1 H), 7.31 (s, 2 H), 7.04–6.95 (m, 3 H), 5.23 (s, 2 H), 3.59 (t, J = 6.6 Hz, 2 H), 3.16 (s, 3 H), 3.03 (t, J = 6.6 Hz, 2 H).

6-Phenoxyethyl-2(R)-(1,2,2-trimethyl-propyl)-3,4-dihydro-2H-[2,7]naphthyridin-1-one (15c). Acetic acid (0.006 mL, 0.11 mmol) was added to a solution of 6-phenoxyethyl-4-vinyl-nicotinic acid methyl ester (33, 29 mg, 0.11 mmol) and (R)-(+)-3,3-dimethyl-2-aminobutanoate (0.030 mL, 0.225 mmol) in MeOH (1 mL). The mixture was stirred at 100 °C for 16 h. Then (R)-(+)-3,3-dimethyl-2-aminobutanoate (0.030 mL, 0.225 mmol) and acetic acid (0.006 mL, 0.11 mmol) were added, and the mixture was heated at 100 °C for 2 days. The solvents were evaporated in vacuo and the residue diluted with CH₂Cl₂ and extracted with a saturated solution of NaHCO₃ and brine. The organic layer was separated, dried (Na₂SO₄), filtered, and the solvents evaporated in vacuo. The crude product was purified by flash column chromatography (silica; AcOEt in CH₂Cl₂ 0/100 to 30/70). The desired fractions were collected and the solvents evaporated in vacuo to yield compound 15c as a colorless oil which solidified upon standing at room temperature (29 mg, 79%). LCMS tᵣ = 3.10 min, > 98% at 215 and 254 nm, m/z = 339.1 [M + H].

1H NMR (500 MHz, CDCl₃) δ 9.19 (s, 1 H), 7.38 (s, 1 H), 7.31 (t, J = 8.1 Hz, 2 H), 7.03–6.92 (m, 3 H), 5.24 (s, 2 H), 4.83 (q, J = 7.2 Hz, 1 H), 3.59–3.43 (m, 3 H, 2 H), 3.06–2.93 (m, 1 H), 2.93–2.81 (m, 1 H), 1.21 (d, J = 6.9 Hz, 3 H), 0.99 (s, 9 H).

2-(4-Fluoro-phenyl)-6-phenoxyethyl-3,4-dihydro-2H-[2,7]naphthyridin-1-one (15d). Starting from 2-(4-Fluoro-phenyl)-4-vinyl-nicotinic acid methyl ester (33, 30 mg, 0.11 mmol) and 4-fluoroboronic acid (0.044 mL, 0.47 mmol) following the procedure described for compound 15c, title compound 15d was obtained as a white solid (24 mg, 62%); mp 198.8 °C. LCMS tᵣ = 2.66 min, > 98% at 215 and 254 nm, m/z = 349.1 [M + H].

1H NMR (500 MHz, CDCl₃) δ 9.24 (s, 1
Cyclopropyl[3-(3-fluorobenzoyl)oxy]-7,8-dihydro-1,6-naphthyridin-6(5H)-yl)methanone (20d). 3-[3-Bromo-7,8-dihydro-1,6-naphthyridin-6(5H)-yl](cyclopropyl)methanone (35a, 35 mg, 0.125 mmol), cesium carbonate (59 mg, 0.18 mmol), Cul (3 mg, 0.01 mmol), and 1,10-phenanthroline (3 mg, 0.02 mmol) were combined in a 2.0 mL microwave vial and placed under an argon atmosphere. Degassed toluene was added and the mixture heated at 110 °C for 4 h. The mixture was cooled to rt, filtered over Celite, and the filtrate concentrated to dryness. RP-HPLC purification afforded 20d as an off-white powder (15 mg, 33%). LCMS retention time (m/z) = 0.79 min, >98% at 215 and 254 nm, m/z = 328.1 [M + H]+. HRMS (ES+, M + H)+ calc for C18H18FN2O2: 327.1509; found, 327.1512.

(3-Benzylxoyl)-7,8-dihydro-1,6-naphthyridin-6(5H)-yl](4-fluorophenyl)methanone (20g). 3-Bromo-7,8-dihydro-1,6-naphthyridin-6(5H)-yl](4-fluorophenyl)methanone (35e, 28 mg, 0.09 mmol), benzyl alcohol (96 mg, 0.99 mmol), cesium carbonate (43 mg, 0.13 mmol), Cul (2 mg, 0.009 mmol), and 1,10-phenanthroline (3.2 mg, 0.018 mmol) were combined in a 2.0 mL microwave vial and placed under an argon atmosphere. Degassed toluene was added and the mixture heated at 110 °C for 4 h. The mixture was cooled to rt, filtered over Celite, and the filtrate concentrated to dryness. RP-HPLC purification afforded 20g as an off-white powder (6.9 mg, 22%). LCMS retention time (m/z) = 0.85 min, >98% at 215 and 254 nm, m/z = 363.1 [M + H]+. HRMS (ES+, M + H)+ calc for C22H20FN2O2: 362.1431; found, 362.1434.

Selectivity Screening. mGlus. To assess the effect of test compounds at mGlus, Ca2+ mobilization assays were performed as described previously. Briefly, HEK293 cells stably expressing rat mGlus were plated in black-walled, clear-bottomed, poly-n-lysine coated 384-well plates (Greiner Bio-One, Monroe, NC) in assay medium at a density of 20000 cells/well. Calcium flux was measured over time as an increase in fluorescence of the Ca2+ indicator dye, Fluor-4AM using a FDS 6000.Either vehicle or a fixed concentration of test compound (10 μM, final concentration) was added, followed 140 s later by a CRC of glutamate. Data were analyzed as described above.

Group II and Group III mGlus. The functional activity of the compounds was assessed at the rat group II and III mGlus receptors by measuring thallium flux through GIRK channels as previously described. Briefly, HEK293-GIRK cells expressing mGlu subtypes 2, 3, 4, 6, 7, or 8 were plated into 384-well, black-walled, clear-bottom poly-n-lysine coated plates at a density of 15000 cells/well in assay medium. A single concentration of test compound (10 μM) or vehicle was added, followed 140 s later by a CRC of glutamate (or L-AP4 for mGlu4) diluted in thallium buffer (125 mM NaHCO3, 1 mM MgSO4, 1.8 mM CaSO4, 5 mM glucose, 12 mM thallium sulfate, 10 mM HEPES), and fluorescence was measured using a FDS 6000. Data were analyzed as described previously.

In Vivo Pharmacology. Animal Husbandry. Animals were housed in the animal care facility certified by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) under a 12 h light/dark cycle (lights on, 7 a.m.; lights off, 7 p.m.) and had free access to food and water. The animals used in these experiments were food-deprived the evening before experimentation for oral administration of test compound. The experimental protocols performed during the light cycle were approved by the Institutional Animals Care and Use Committee of Vanderbilt University and conformed to the guidelines established by the National Research Council Guide for the Care and Use of Laboratory Animals.

Preparation of Test Article. 12c was formulated in volumes specific to the number of animals dosed each day. The solutions were formulated so that animals were injected with a maximal dosing volume of 10 mL/kg. The appropriate amount according to the dosage fixed concentration (55 nM to 30 μM) of mGlus compound was diluted in thallium buffer (125 mM NaHCO3, 1 mM MgSO4, 1.8 mM CaSO4, 5 mM glucose, 12 mM thallium sulfate, 10 mM HEPES) and fluorescence was measured using a FDS 6000. Data were analyzed as described previously.

Reversal of Amphetamine-Induced Hyperlocomotion. Studies were conducted using Small Open Field activity chambers (27 cm × 27 cm × 20 cm) (Kinder Scientific, Poway, CA) equipped with 16 horizontal (x- and y-axes) infrared photobeams. Changes in locomotor activity were measured as the number of photobeam breaks over time and were recorded with a Pentium 1 computer equipped with rat activity monitoring system software (Motor Monitor, Kinder Scientific, Poway, CA). Male Harlan Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 250–350 g were used. Animals were multiple fixed in the locomotor activity test chambers for 30 min. Animals were next pretreated for an additional 30 min with either vehicle or a dose of 12c po, followed by a subcutaneous injection of 1 mg/kg amphetamine or vehicle and monitored for an additional 60 min.
Treatments. Dose group 1, VAMP = 20% β-CD (12c vehicle), po + 1 mg/kg AMP, sc (n = 8). Dose group 2, 3AMP = 3 mg/kg 12c, po + 1 mg/kg AMP, sc (n = 6). Dose group 3, 10AMP = 10 mg/kg 12c, po + 1 mg/kg AMP, sc (n = 7). Dose group 4, 30AMP = 30 mg/kg 12c, po + 1 mg/kg AMP, sc (n = 8). Dose group 5, 56.6AMP = 56.6 mg/kg 12c, po + 1 mg/kg AMP, sc (n = 8). Dose group 6, 100AMP = 100 mg/kg 12c, po + 1 mg/kg AMP, sc (n = 8). Dose group 7, 100V = 100 mg/kg 12c, po + sterile water (AMP vehicle), sc (n = 8). Changes in locomotor activity were recorded for a total of 120 min. Data were expressed as changes in ambulation defined as the total number of photobeam breaks per 5 min interval. At the end of this behavioral study, each animal was euthanized, then decapitated, and the plasma and brain tissues were collected for the evaluation of exposure levels of 12c.

Data Analysis. Behavioral data were analyzed using a one-way ANOVA with main effects of treatment and time. For hsc analyses were performed using a Dunnett’s t-test with all treatment groups compared to the VAMP group using JMP 8.0 (SAS Institute, Cary, NC) statistical software. Data were graphed using SigmaPlot for Windows version 11.0 (Saugua, MA). A probability of p ≤ 0.05 was taken as the level of statistical significance. Percent effect and reversal calculations for the 3AMP, 10AMP, 30AMP, and 100AMP treatment groups were performed with the following formula relative to the VAMP treatment group: (1) total number of photobeam breaks in the time interval from t = 60 to t = 120 was calculated for each rat in each treatment group. (2) mean total number of photobeam breaks in the time interval from t = 60 to t = 120 was calculated for the VAMP group, (3) percent effect = ratio of the total number of photobeam breaks for each rat in each treatment group divided by the mean total number of photobeam breaks in the time interval from t = 60 to t = 120 of the VAMP group multiplied by 100, (4) percent reversal = 100 – the percent effect for each rat in each treatment group, (5) finally, the mean ± SE percent reversal for each treatment group was calculated from the individual percent reversal values. Percent effect and change calculations for the VAMP and 100 V treatment groups were given a score of 0, 1 or 2, with 0 = no effect, 1 = moderate effect, and 2 = robust full effect. All data were collected blinded to treatment for each animal. The following functional end points were scored. Autonomic nervous system functions: ptosis, exophthalmos, miosis, mydriasis, corneal reflex, pinna reflex, piloerection, respiratory rate, writhing, tail elevation, lacrimation, salivation, vasodilation, skin color, irritability, and rectal temperature. Somatomotor nervous system functions: motor activity, ataxia, arch/roll, tremors, leg weakness, rigid stance, spraddle, placing loss, grasping loss, righting loss, catalepsy, tail pinch reaction, escape loss, and physical appearance.

Data Analysis. Mean change values for each functional end point in the Irwin test battery of each treatment group were calculated using Microsoft Excel. Behavioral data were then analyzed using a one-way ANOVA with main effect of treatment. Post hoc analyses were performed using a Dunnett’s t-test with all treatment groups compared to the vehicle group using JMP 8.0 (SAS Institute, Cary, NC) statistical software. Data were graphed using SigmaPlot for Windows version 11.0 (Saugua, MA). A probability of p ≤ 0.05 was taken as the level of statistical significance.

Modified Irwin Neurological Test Battery. Male Harlan Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 250–300 g were used. Animals were given two training trials of 120 s on the rotarod with a 10 min interval between trials, followed by baseline assessment of performance with a 120 s trial, and any animals that did not reach a performance criteria of 85 s were excluded from the study. Animals were next pretreated for 30 min with vehicle (20% HP-β-CD) or a dose of 12c (n = 6 each group) and then tested on the rotarod using a 120 s trial. The amount of time in seconds that each animal remained on the rotarod was recorded; animals not falling off of the rotarod were given a maximal score of 120 s. Data were expressed as the mean latency to fall off the rotarod in seconds for each treatment group.

Data Analysis. Behavioral data were analyzed using a one-way ANOVA with main effects of treatment. Post hoc analyses were performed using a Dunnett’s t-test with all treatment groups compared to the vehicle group using JMP 8.0 (SAS Institute, Cary, NC) statistical software. Data were graphed using SigmaPlot for Windows version 11.0 (Saugua, MA). A probability of p ≤ 0.05 was taken as the level of statistical significance.

Modified Irwin Neurological Test Battery. Male Harlan Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 250–300 g were used. Animals were evaluated in the modified Irwin neurological test battery at t = 0 to provide a baseline measurement across each functional end point. Animals were next administered either vehicle (20% HP-β-CD) or a 100 mg/kg po dose of 12c (n = 6 each group) and then assessed after a 30 min, 1 h, and 4 h pretreatment interval in the modified Irwin neurological test battery. Changes in the different functional end points of the Irwin test battery were given a score of 0, 1 or 2, with 0 = no effect, 1 = moderate effect, and 2 = robust full effect. All data were collected blinded to treatment for each animal. The following functional end points were scored. Autonomic nervous system functions: ptosis, exophthalmos, miosis, mydriasis, corneal reflex, pinna reflex, piloerection, respiratory rate, writhing, tail elevation, lacrimation, salivation, vasodilation, skin color, irritability, and rectal temperature. Somatomotor nervous system functions: motor activity, ataxia, arch/roll, tremors, leg weakness, rigid stance, spraddle, placing loss, grasping loss, righting loss, catalepsy, tail pinch reaction, escape loss, and physical appearance.

Data Analysis. Mean change values for each functional end point in the Irwin test battery of each treatment group were calculated using Microsoft Excel. Behavioral data were then analyzed using a one-way ANOVA with main effect of treatment. Post hoc analyses were performed using a Dunnett’s t-test with all treatment groups compared to the vehicle group using JMP 8.0 (SAS Institute, Cary, NC) statistical software. Data were graphed using SigmaPlot for Windows version 11.0 (Saugua, MA). A probability of p ≤ 0.05 was taken as the level of statistical significance.

ASSOCIATED CONTENT

Supporting Information

mGlu selectivity for 12c, 13g, and 14d; operational model affinity and cooperativity calculations (12c), DMPK procedures and methods, rotarod, modified Irwin, Eurofins ancillary
pharmacology, compound characterization, NMR spectra for representative compounds, and subseries 19. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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**ABBREVIATIONS USED**

mGlu, metabotropic glutamate receptor; PAM, positive allosteric modulator; NAM, negative allosteric modulator; LTD, long-term depression; MPEP, 2-methyl-6-(phenylethynyl)pyridine; CPPHA, N-(4-chloro-2-((1,3-dioxo-5-yl)methyl)phenyl)-2-hydroxybenzamide; CDPPB, 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide; AHL, amphetamine-induced hyperlocomotion

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