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Discovery of imidazo[1,2-*a*]-, [1,2,4]triazolo[4,3-*a*]-, and [1,2,4]triazolo[1,5-*a*]pyridine-8-carboxamide negative allosteric modulators of metabotropic glutamate receptor subtype 5

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Abstract

Based on a hypothesis that an intramolecular hydrogen bond was present in our lead series of picolinamide mGlu₅ NAMs, we reasoned that an inactive nicotinamide series could be modified through introduction of a fused heterocyclic core to generate potent mGlu₅ NAMs. In this Letter, we describe the synthesis and evaluation of compounds that demonstrate the viability of that approach. Selected analogs were profiled in a variety of *in vitro* assays, and two compounds were evaluated in rat pharmacokinetic studies and a mouse model of obsessive-compulsive disorder. Ancillary pharmacology screening revealed that members of this series exhibited moderate inhibition of the dopamine transporter (DAT), and SAR was developed that expanded the selectivity for mGlu₅ versus DAT.

Graphical abstract

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Keywords

Metabotropic glutamate receptor subtype 5 (mGlu₅); Negative allosteric modulator (NAM); Central nervous system (CNS); G-protein coupled receptor (GPCR); Heterocycle

The major excitatory transmitter in the mammalian central nervous system (CNS), glutamate or L-glutamic acid, activates both ionotropic and metabotropic glutamate (mGlu) receptors. The mGlu receptors are a family of eight G-protein-coupled receptors (GPCRs) that have been further classified into three groups. The group I receptors (mGlu₁ and mGlu₅) are found in postsynaptic locations and are coupled via G_q to the activation of phospholipase C. The group II (mGlu_{2,3}) receptors are found both pre and postsynaptically, while the group III (mGlu₄, mGlu_{6,8}) receptors are primarily presynaptic in localization; both group II and III receptors are coupled via G_{i/o} to the inhibition of adenylyl cyclase activity and regulation of ion channels. Whereas the glutamate binding site is located in the extracellular domain of the mGlu receptors, allosteric binding sites that have been identified at present are found in the transmembrane domain.^{1,2}

The design of orthosteric ligands that are sufficiently selective for an individual mGlu versus the other members of the mGlu family has proven difficult. On the other hand, highly selective allosteric modulators have been developed for several of the mGlu,³ with negative allosteric modulators (NAMs) of mGlu₅ representing one of the most advanced areas in this growing field.⁴ Potential therapeutic applications for mGlu₅ NAMs are extensive and include many different types of psychiatric and neurodegenerative disorders.¹⁻⁴ Phase II clinical trials have been conducted with small molecule mGlu₅ NAMs in fragile X syndrome (FXS),^{5,6} major depressive disorder (MDD),⁷ obsessive-compulsive disorder (OCD),⁸ and Parkinson's disease levodopa-induced dyskinesia (PD-LID).^{9,10} Unfortunately, most of these studies have failed to meet primary efficacy endpoints.⁵⁻⁹ Still, certain secondary efficacy endpoints have been noted in MDD⁷ and PD-LID.¹⁰

We have been engaged in a quest for a highly optimized mGlu₅ NAM for some time and recently disclosed our candidate for clinical evaluation, VU0424238 (**1**) (Fig. 1).¹¹ A related analog that differed only in the nature of the northern heteroaryl ether, VU0424222 (**2**), was also extensively profiled. Both compounds were potent mGlu₅ antagonists in our functional assay that measures the inhibition of Ca²⁺ mobilization induced by an EC₈₀ concentration of glutamate in HEK293A cells expressing rat mGlu₅.¹² During an early phase of the optimization process that identified the picolinamide core of our lead series and led to the discovery of **1** and **2**, we noted that nicotinamide analogs **3** and **4** (Fig. 1) were devoid of mGlu₅ activity up to the top concentration tested (30 μM) in our assay.

We hypothesized that the picolinamide of our lead series may form an intramolecular hydrogen bond that is not found in nicotinamide congeners (Fig. 2).¹³ Furthermore, we reasoned that it was also possible that such an interaction favored a conformation suitable for binding to the target. Thus, we postulated that modification of the nicotinamide core to a fused heterocycle such as an imidazo- or triazolopyridine would introduce a nitrogen atom capable of forming a similar intramolecular hydrogen bond and leading to a favorable disposition of the amide and core for interacting with mGlu₅. In a search for additional novel

scaffolds capable of delivering highly optimized mGlu₅ NAMs, we set out to evaluate this hypothesis. This Letter details our efforts toward that end.

A variety of synthetic methods were employed to access our desired targets, but all routes began with 3-bromo-5-fluoropyridin-2-amine **5** (Scheme 1).¹⁴ Oxidation of the amine with potassium persulfate in sulfuric acid afforded 2-nitropyridine **6**. Nucleophilic aromatic substitution with 3-fluoro-5-hydroxypyridine was facile and proceeded at room temperature to provide ether **7**. Reduction of the nitro group was accomplished via catalytic hydrogenation to yield amine **8**. Cyclization with chloroacetaldehyde under basic conditions generated imidazo[1,2-*a*]pyridine **9**. The aryl bromide was converted to nitrile **10** via a palladium-catalyzed reaction with zinc cyanide, and basic hydrolysis of **10** with heating gave the penultimate acid **11**. Generation of the amide targets **12–16** was accomplished through a simple coupling with the desired 2-aminopyridine monomers.

Synthesis of [1,2,4]triazolo[4,3-*a*]pyridine analogs was initiated from intermediate **8** or **17** (Scheme 2).¹⁵ Compound **17** was prepared akin to **8** utilizing 5-hydroxypyrimidine instead of 3-fluoro-5-hydroxypyridine. A Sandmeyer reaction with sodium nitrite and hydrogen fluoride-pyridine was employed to generate 2-fluoropyridines **18** and **19**.¹⁶ Nucleophilic aromatic substitution with hydrazine under microwave conditions gave intermediates **20** and **21**. Cyclization was accomplished via microwave heating in either triethyl orthoformate or triethyl orthoacetate to provide [1,2,4]triazolo[4,3-*a*]pyridines **22–24**. As before, a palladium-catalyzed cyanation/basic hydrolysis sequence was used to access the penultimate acids **28–30**. Conversion to the amide targets **31–33** proceeded as previously described as well.

Synthesis of [1,2,4]triazolo[1,5-*a*]pyridine analogs began with intermediates **8**, **17**, and **34** (Scheme 3).¹⁷ Compound **34** was prepared akin to **8** utilizing 3-chloro-5-hydroxypyridine instead of 3-fluoro-5-hydroxypyridine. In this case, **8**, **17**, and **34** were treated with either dimethylformamide dimethyl acetal or dimethylacetamide dimethyl acetal followed by hydroxylamine to afford intermediates **35–38**. Cyclization was effected through treatment with trifluoroacetic anhydride to provide the [1,2,4]triazolo[1,5-*a*]pyridines **39–42**. The remaining steps followed the previously outlined sequence to afford the final amide analogs **51–54**. The synthesis of certain analogs (**64–66**) with more sensitive functional groups in the northern pyridyl ether followed an alternative synthesis (Scheme 4). In this case, a palladium-catalyzed carbonylation reaction was used to convert bromides **55–57** to their corresponding methyl esters **58–60**, which could be hydrolyzed at room temperature to yield acids **61–63**. Amide bond formation as before afforded targets **64–66**.

Finally, synthesis of 5-cyanopyridin-3-yl ether (**73**) and 5-(difluoromethoxy)pyridine-3-yl ether (**76**) analogs required a modified approach (Scheme 5). 2-Methoxyethoxymethyl (MEM) ether intermediate **67** was converted to ester **68** via the previously described carbonylation reaction. Cleavage of the MEM protecting group was accomplished with methanesulfonic acid to afford **69**. Reaction with *N*-phenyl triflimide and base was used to access aryl triflate **70**, which was converted to nitrile **71** using a palladium catalyzed cyanation. Alcohol **69** was also alkylated with sodium chlorodifluoroacetate to generate

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difluoromethyl ether **74**. Intermediates **71** and **74** were converted to analogs **73** and **76**, respectively, utilizing the methods previously described.

We have established the pyrimidin-5-yl ether moiety found in compounds such as **1** (Fig. 1) as a site for aldehyde oxidase (AO)-mediated metabolism.^{11,18,19} Accurately predicting human pharmacokinetics with compounds that are substantially metabolized by AO can be more challenging due in part to its variable expression across preclinical species.²⁰ While compound **2** was not metabolized by AO, compound **1** ultimately had other characteristics that cumulatively yielded a superior profile compared to **2**.¹¹ Still, we remained interested in identifying a compound that was not subject to AO-mediated metabolism as a potential backup to **1**. As such, we returned to the 5-fluoropyridin-3-yl ether found in **2** for much of the work described herein.

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Evaluation of imidazo[1,2-*a*]pyridines **12–16** (Table 1) revealed SAR around the amide portion of the scaffold that was consistent with those observed in the picolinamide series.¹¹ Although, 5-fluoropyridine **14** was the most potent analog in this set, it was 6.5-fold less active than picolinamide **2** (Fig. 1). Still, we decided to profile these analogs in a variety of *in vitro* assays to more completely evaluate their potential (Table 2).²¹ While certain analogs (**12–14**) were marginally less bound to plasma proteins than **2**, and all were 2 to 5-fold less potent inhibitors of CYP1A2 than **2**, these were viewed as small gains in light of the reduced mGlu₅ activity for these compounds relative to **2**. In addition, all of the imidazo[1,2-*a*]pyridine analogs (**12–16**) were more rapidly metabolized by both rat and human liver microsomes than picolinamide **2**. Given the superior potency of the 5-fluoropyridin-2-yl carboxamide **14**, much of the remaining SAR work was carried out in the context of that particular amide group.

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Results from screening [1,2,4]triazolo[4,3-*a*]pyridines **31–33** (Series A) and [1,2,4]triazolo[1,5-*a*]pyridines **51–53** (Series B) revealed clear SAR as well (Table 3). First, the introduction of the additional nitrogen atom relative to the imidazo[1,2-*a*]pyridine core had no substantial impact on mGlu₅ activity as **31** and **51** were comparable in potency to **14**. Second, compounds **32** and **52** showed that substitution of the triazole ring with a methyl group was unfavorable for mGlu₅ activity, resulting in 14 and 70-fold drops in potency, respectively. Finally, while we expected that the pyrimidin-5-yl ethers **33** and **53** would be prone to AO-mediated metabolism,^{18,19} these compounds were also examined for the sake of comparison. As was the case with picolinamides **1** and **2**, both **33** and **53** were less potent than their respective 5-fluoropyridin-3-yl ether counterparts **31** and **51**; however, the impact was more pronounced in the case of analog **33**.

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Selected analogs (**31**, **51**, and **53**) were advanced to additional *in vitro* assays for further profiling (Table 4).²¹ Gratifyingly, several areas of improvement relative to prior compounds were noted. First moving from the imidazo[1,2-*a*]pyridine core (**14**) to either a [1,2,4]triazolo[4,3-*a*]pyridine core (**31**) or [1,2,4]triazolo[1,5-*a*]pyridine core (**51**) increased fraction unbound, reduced P450 inhibition, and increased metabolic stability. Importantly, both compounds had improved P450 inhibition profiles and increased fraction unbound relative to picolinamide **2** as well. Whereas **51** was predicted to have low clearance ($<1/3$ hepatic blood flow)²² in both rats and humans, analog **31** was predicted to have moderate

clearance ($\frac{1}{3}$ to $\frac{2}{3}$ hepatic blood flow)²² in both species. Pyrimidin-5-yl ether **53** has a similar P450 inhibition profile to **51**; however, **53** is less protein bound than either **31** or **51**. Clearance in rats and humans was predicted to be low for **53** but may be underestimated if AO plays a substantial role in its *in vivo* metabolism, which cannot be measured in a hepatic microsomal stability assay.

Pleased with the improvements seen with **31** and **51**, we continued profiling those compounds to better assess their potential utility. First, selectivity versus the other mGlu receptors was measured in fold-shift experiments that assess the effect of 10 μ M test compound on the concentration response curves of an orthosteric agonist in cell lines expressing the mGlu receptor of interest.²³ Both **31** and **51** were inactive versus all other mGlu receptors, indicating that their selectivity for mGlu₅ is at least 370 and 500-fold, respectively. Permeability and efflux potential was evaluated in Madin-Darby canine kidney (MDCK) cells transfected with the human MDR1 gene.²⁴ While there was no evidence of P-glycoprotein (P-gp) mediated efflux with **51**, the ER for **31** was 2.4, which indicates a low potential for efflux with that compound. Extent of binding to rat brain homogenates was assessed for each compound. Compound **31** was similarly bound to rat plasma and brain homogenates while analog **51** was 2.4-fold more highly bound to rat brain homogenates than rat plasma.

Although the polar surface area (PSA) of **31** and **51** was somewhat higher than desirable (PSA = 91),²⁵ other calculated properties such as molecular weight (368) and cLogP (**31** clogP = 1.4; **51** clogP = 2.0)²⁵ for **31** and **51** were consistent with those associated with orally available CNS drugs.²⁶ As such, having a better understanding of the *in vitro* profiles for **31** and **51**, we next moved those compounds into *in vivo* assays (Table 6). Rat pharmacokinetics studies showed **31** and **51** to have similar profiles. Both compounds exhibited low plasma clearance. While the liver microsomal stability assay almost perfectly predicted the *in vivo* clearance of **51**, the *in vivo* clearance of **31** was 2.5-fold lower than predicted. Unfortunately, oral bioavailability for both compounds was lower than desirable and at the low end of acceptable for progression in our estimation. Fortunately, CNS penetration was good with both compounds. The unbound brain to unbound plasma ratio ($K_{p,uu}$) for **51** was near unity, suggesting distribution equilibrium between the two compartments.²⁷ The $K_{p,uu}$ for **31** was slightly more than 2.0, which raises the possibility of active influx; however, several additional studies would be required to determine this conclusively.

Naïve mice are known to bury foreign objects such as glass marbles in deep bedding, and inhibition of this behavior has been utilized as an assay for measuring the efficacy of certain anxiolytics such as benzodiazepines.²⁸ This assay models the repetitive and perseverative behavior associated with obsessive-compulsive disorder (OCD) as opposed to novelty-induced anxiety,²⁹ and we have employed this assay as a convenient first tier behavioral assay for measuring the *in vivo* efficacy of our novel mGlu₅ NAMs.^{11,30,31} Thus, we advanced **31** and **51** into the marble burying assay using an optimized dose (3.0 mg/kg) of our clinical candidate **1** as a positive control (Fig. 3). Again, the results were similar with both compounds as each had a minimum efficacious dose of 3.0 mg/kg and produced a similar effect to the optimized dose of **1** at 10 mg/kg.

As noted to this point, **31** and **51** have similar profiles across several different assays; however, we favored **51** by a small margin due to a superior P450 inhibition profile, marginally better rat pharmacokinetics, and reduced potential for active transport. Thus, we selected **51** for progression to a commercial radioligand binding assay panel of 68 diverse targets³² to assess potential ancillary pharmacology at a concentration of 10 μ M. Overall, the profile for **51** was quite clean with only two significant responses.³³ Inhibition of binding at the platelet activating factor (PAF) was weak (55%); however, strong inhibition (89%) of binding to the dopamine transporter (DAT) was observed. Interaction with DAT was not entirely surprising as **2** (86% inhibition of binding at 10 μ M) had a similar profile in the same assay. Since inhibition of DAT is associated with abuse potential,³⁴ the lack of significant DAT inhibition with **1** was one, though by no means the only, factor that led to its selection as our clinical candidate.¹¹ To evaluate the functional potency of **51** at DAT, it was examined in a cell-based assay that measured inhibition of dopamine uptake.³⁵ As predicted by the radioligand binding assay, **51** was a moderately potent ($IC_{50} = 730$ nM) inhibitor of dopamine uptake, which amounts to approximately 35-fold selectivity for mGlu₅ versus DAT. It is worth noting that certain atypical DAT inhibitors have been posited as potential therapeutics for stimulant abuse.³⁶ Likewise, several preclinical studies with mGlu₅ NAMs have pointed toward a potential role for mGlu₅ inhibition in the treatment of addiction.³⁷ Thus, a dual mGlu₅/DAT inhibitor might represent an interesting profile for the treatment of stimulant addiction. Inhibition of DAT might also be advantageous in certain therapeutic areas that overlap with those for an mGlu₅ NAM such as depression³⁸ and Parkinson's disease.³⁹ Still, in this particular case, we were interested in determining if activity at DAT could be modulated while maintaining potent inhibition of mGlu₅.

Knowing that pyrimidin-5-yl ether **1** lacked significant affinity for DAT, we hypothesized that modifications to the 5-fluoropyridin-3-yl ether of **51** may be a viable approach for reducing DAT activity. Thus, we prepared and tested a variety of new derivatives in this region of the scaffold (Table 7). Alternative substituents at the 5-position reduced potency at mGlu₅; however, the impact with the 5-cyano group (**73**) was only 2-fold. Likewise, addition of a second fluoro group to the pyridine ring (**64–66**) was deleterious to mGlu₅ activity, but **64** and **65** were within 2- and 3-fold of **51**, respectively. Screening analogs **64**, **65**, and **73** in our panel of *in vitro* assays as before (Table 8) showed that each compared favorably to **51** in some areas, though there were some notable differences. 4,5-Difluoropyridine **64** was predicted to have higher clearance in both rats and humans relative to **51** and was a more potent inhibitor of CYP1A2. 5,6-Difluoropyridine **65** was more highly protein bound and was predicted to have higher clearance in rats than **51**. Finally, 5-cyanopyridine **73** was a more potent inhibitor of CYP3A4 and CYP2C9 than **51**; however, the P450 inhibition profile of **73** was still considered acceptable.

Interested to determine the impact of these structural modifications on DAT activity relative to **51**, we began that process with these same three analogs as well as its isomer (**31**) and pyrimidin-5-yl ether **53** (Table 9). Initially, affinity for the DAT receptor was measured in the radioligand binding assay.⁴⁰ Not surprisingly, analog **31** showed similar results to its isomer (**51**). In contrast, DAT binding was reduced with several compounds including pyrimidin-5-yl ether **53**, 5,6-difluoropyridine **65**, and 5-cyanopyridine **73**. As such, these three analogs

were next evaluated in the dopamine uptake inhibition functional assay.³⁵ All three analogs were less potent than **51** in this assay, and had wider margins of selectivity for mGlu₅ versus DAT.

In conclusion, we have established that certain fused heterocycles are able to serve as competent isosteres for the pyridine core found in our picolinamide lead series. While imidazo[1,2-*a*]pyridine analogs were rapidly metabolized, highly protein bound, and had undesirable P450-inhibition profiles, compounds with more attractive profiles were obtained with [1,2,4]triazolo[1,5-*a*]pyridine and [1,2,4]triazolo[4,3-*a*]pyridine cores. Two such analogs were low clearance compounds in rats with similar efficacy in a mouse model of OCD. Finally, inhibition of DAT could be modulated through modification of the northern heteroaryl ether portion of the chemotype, and three analogs (**53**, **65**, and **73**) with >50-fold selectivity for mGlu₅ versus DAT were identified.

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- The synthesis of *N*-(5-Fluoropyridin-2-yl)-6-((5-fluoropyridin-3-yl)oxy)imidazo[1,2-*a*]pyridine-8-carboxamide (**14**) is representative of analogs with the imidazo[1,2-*a*]pyridine core. (a) 3-Bromo-5-fluoro-2-nitropyridine (**6**). Potassium persulfate (20.2 g, 74.7 mmol) was added to

sulfuric acid (21.4 mL) and the mixture was stirred at rt for 5 min. The mixture was cooled to 0 °C and **5** (3.57 g, 18.7 mmol) was added. The reaction was allowed to warm to rt, and the viscous mixture was stirred for 1 h to ensure that all of **5** dissolved in the reaction mixture. After 1 h, the reaction was quenched with ice water and neutralized with aqueous ammonium hydroxide. The mixture was extracted with ethyl acetate (3x), dried over MgSO₄, filtered, and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 2.20 g (54%) of the title compound as a pale yellow solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.70 (dd, *J* = 7.7, 2.5 Hz, 1H), 8.67 (d, *J* = 2.5 Hz, 1H). (b) 3-Bromo-5-((5-fluoropyridin-3-yl)oxy)-2-nitropyridine (**7**). Compound **6** (1.16 g, 5.35 mmol), 3-fluoro-5-hydroxypyridine (712 mg, 6.30 mmol), potassium carbonate (1.45 g, 10.5 mmol) and DMF (13 mL) were added to a round-bottom flask and stirred at rt for 4 h. The reaction was filtered and washed with ethyl acetate. The filtrate was washed with water (2x), and the aqueous layer was extracted with ethyl acetate. The combined organics were dried over MgSO₄, filtered, and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 1.50 g (91%) of the title compound as a light brown solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.54 (d, *J* = 2.4 Hz, 1H), 8.50-8.48 (m, 2H), 8.35 (d, *J* = 8.3 Hz, 1H), 7.88 (dt, *J* = 9.9, 2.3 Hz, 1H); ES-MS [M+H]⁺: 314.0. (c) 3-Bromo-5-((5-fluoropyridin-3-yl)oxy)pyridin-2-amine (**8**). Compound **7** (2.24 g, 7.13 mmol) was dissolved in MeOH (48 mL) and catalytic “Raney” nickel was added. The reaction was evacuated and purged with hydrogen and stirred vigorously overnight under a hydrogen atmosphere. The reaction was filtered through celite and washed with 3:1 chloroform/IPA. The organics were concentrated *in vacuo*, and the crude product was filtered through a short pad of silica gel using 89:10:1 DCM/MeOH/NH₄OH. The organics were concentrated *in vacuo* to give 1.92 g (95%) of the title compound as a brown solid that was used without further purification: ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.31 (d, *J* = 2.4 Hz, 1H), 8.24-8.22 (m, 1H), 7.93 (d, *J* = 2.6 Hz, 1H), 7.79 (d, *J* = 2.6 Hz, 1H), 7.39 (dt, *J* = 10.4, 2.4 Hz, 1H), 6.27 (s, 2H); ES-MS [M+H]⁺: 284.0. (d) 8-Bromo-6-((5-fluoropyridin-3-yl)oxy)imidazo[1,2-*a*]pyridine (**9**). Compound **8** (500 mg, 1.76 mmol), chloroacetaldehyde solution (5.23 mL, ~50% in water), potassium carbonate (487 mg, 3.52 mmol) and IPA (4.4 mL) were added to a microwave vial and heated in a microwave reactor at 170 °C for 20 min. The reaction was filtered and washed with 5% MeOH in DCM. The filtrate was concentrated *in vacuo* and purified by flash chromatography on silica gel to afford 352 mg (65%) of the title compound as a brown solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.73 (d, *J* = 2.0 Hz, 1H), 8.41 (d, *J* = 2.2 Hz, 1H), 8.40-8.39 (m, 1H), 8.07 (s, 1H), 7.72-7.64 (m, 3); ES-MS [M+H]⁺: 307.8. (e) 6-((5-Fluoropyridin-3-yl)oxy)imidazo[1,2-*a*]pyridine-8-carbonitrile (**10**). Compound **9** (352 mg, 1.14 mmol), tetrakis(triphenylphosphine)palladium(0) (132 mg, 0.114 mmol), zinc cyanide (161 mg, 1.37 mmol, 1.20 eq) and DMF (5 mL) were added to a microwave vial and heated in a microwave reactor at 140 °C for 15 minutes. The reaction was diluted with ethyl acetate and washed with water (2x). The combined aqueous layers were extracted with ethyl acetate, and the combined organics were dried over MgSO₄, filtered, and concentrated *in vacuo*. Purification by reverse-phase chromatography afforded 235 mg (81%) of the title compound as a yellow solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.00 (d, *J* = 2.2 Hz, 1H), 8.42-8.39 (m, 2H), 8.12 (d, *J* = 2.2 Hz, 1H), 8.09 (d, *J* = 1.0 Hz, 1H), 7.77 (d, *J* = 1.0 Hz, 1H), 7.68 (dt, *J* = 2.4, 10.4 Hz, 1H); ES-MS [M+H]⁺: 255.2. (f) 6-((5-Fluoropyridin-3-yl)oxy)imidazo[1,2-*a*]pyridine-8-carboxylic acid (**11**). Compound **10** (235 mg, 0.924 mmol) was dissolved in dioxane (4.6 mL), and 2N aqueous NaOH (2.3 mL) was added. The mixture was heated in a sealed tube at 100 °C for 18 h and after cooling, the reaction was neutralized to pH 4–5 with 2N aqueous HCl. The water and solvent were removed *in vacuo*, and the crude reaction was dissolved in 10% MeOH/DCM. The undissolved salts were filtered off, and the filtrate was concentrated *in vacuo* to afford 250 mg (99%) of the title compound as a white solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.11 (d, *J* = 2.2 Hz, 1H), 8.46-8.39 (m, 2H), 8.27 (d, *J* = 1.6 Hz, 1H), 8.05 (d, *J* = 2.2 Hz, 1H), 7.96 (d, *J* = 1.6 Hz, 1H), 7.70 (dt, *J* = 2.4, 10.3 Hz, 1H); ES-MS [M+H]⁺: 274.0. (g) *N*-(5-Fluoropyridin-2-yl)-6-((5-fluoropyridin-3-yl)oxy)imidazo[1,2-*a*]pyridine-8-carboxamide (**14**). Compound **11** (25 mg, 0.092 mmol) and 5-fluoro-2-aminopyridine (21 mg, 0.18 mmol) were dissolved in pyridine (1 mL) in a flame-dried round-bottom flask. The reaction was cooled to –15 °C, and phosphorus oxychloride (17 µL, 0.18 mmol) was added dropwise while keeping the temperature below –15 °C. After stirring for 30 min at –15 °C, the reaction was quenched with ice-water and neutralized with 10% aqueous K₂CO₃. The mixture was extracted with ethyl acetate (3x), and the combined organics were dried over MgSO₄, filtered, and concentrated *in vacuo*. Purification by reverse-phase chromatography

afforded 12 mg (36%) of the title compound as a white solid: ^1H NMR (400 MHz, DMSO- d_6) δ 8.99 (d, J = 2.3 Hz, 1H), 8.45-8.40 (m, 3H), 8.36 (dd, J = 4.1, 9.2 Hz, 1H), 8.15 (d, J = 1.0 Hz, 1H), 7.97 (d, J = 2.3 Hz, 1H), 7.88-7.81 (m, 2H), 7.70 (dt, J = 2.3, 10.3 Hz, 1H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 160.03, 159.31 (d, $J(\text{C},\text{F})$ = 257.3 Hz), 156.05 (d, $J(\text{C},\text{F})$ = 248.9 Hz), 154.69 (d, $J(\text{C},\text{F})$ = 5.3 Hz), 147.85 (d, $J(\text{C},\text{F})$ = 2.2 Hz), 142.98, 140.56, 136.45 (d, $J(\text{C},\text{F})$ = 3.5 Hz), 135.96 (d, $J(\text{C},\text{F})$ = 25.3 Hz), 133.29, 132.67 (d, $J(\text{C},\text{F})$ = 22.8 Hz), 125.71 (d, $J(\text{C},\text{F})$ = 19.6 Hz), 122.99, 122.82, 120.11, 115.62, 114.72 (d, $J(\text{C},\text{F})$ = 4.4 Hz), 112.74 (d, $J(\text{C},\text{F})$ = 21.5 Hz); HRMS: calculated for $\text{C}_{18}\text{H}_{11}\text{F}_2\text{N}_5\text{O}_2$ [M], 367.0881; found 367.0884.

15. The synthesis of *N*-(5-Fluoropyridin-2-yl)-6-((5-fluoropyridin-3-yl)oxy)-[1,2,4]triazolo[4,3-*a*]pyridine-8-carboxamide (**31**) is representative of analogs with the [1,2,4]triazolo[4,3-*a*]pyridine core. (a) 3-Bromo-2-fluoro-5-((5-fluoropyridin-3-yl)oxy)pyridine (**18**). Compound **8** (2.65 g, 9.33 mmol) was dissolved in HF-pyridine (23 mL) in a HDPE vessel. The reaction was cooled to 0 °C, and sodium nitrite (965 mg, 14.0 mmol) was added in portions. After 5 min at 0 °C, the reaction was poured onto ice and made basic with saturated aqueous NaHCO_3 . The mixture was extracted with ethyl acetate (2x), dried over MgSO_4 , filtered, and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 2.44 g (91%) of the title compound as a light yellow solid: ^1H NMR (400 MHz, DMSO- d_6) δ 8.43 (d, J = 2.4 Hz, 1H), 8.37 (d, J = 1.89 Hz, 1H), 8.32 (dd, J = 7.3, 2.7 Hz, 1H), 8.19-8.17 (m, 1H), 7.66 (dt, J = 10.2, 2.4 Hz, 1H); ES-MS $[\text{M}+\text{H}]^+$: 287.0. (b) 3-Bromo-5-((5-fluoropyridin-3-yl)oxy)-2-hydrazineylpyridine (**20**). Compound **18** (2.44 g, 8.50 mmol), hydrazine (2.67 mL, 85 mmol) and EtOH (42 mL) were added to a microwave vial and heated in a microwave at 120 °C for 15 min. The reaction was concentrated *in vacuo*, dissolved in ethyl acetate, and washed with water. The organic layer was dried over MgSO_4 , filtered, and concentrated *in vacuo* to afford 2.52 g (99%) of the title compound as a white solid: ^1H NMR (400 MHz, DMSO- d_6) δ 8.32 (d, J = 2.4 Hz, 1H), 8.24-8.22 (m, 1H), 8.09 (d, J = 2.4 Hz, 1H), 7.84 (d, J = 2.4 Hz, 1H), 7.50 (s, 1H), 7.39 (dt, J = 10.3, 2.3 Hz, 1H), 4.27 (s, 2H); ES-MS $[\text{M}+\text{H}]^+$: 299.0. (c) 8-Bromo-6-((5-fluoropyridin-3-yl)oxy)-[1,2,4]triazolo[4,3-*a*]pyridine (**22**). Compound **20** (2.56 g, 8.56 mmol) and triethylorthoformate (22 mL) were added to a microwave vial and heated in a microwave at 180 °C for 20 min. The reaction was concentrated *in vacuo* and purified by flash chromatography on silica gel to afford 2.50 g (94%) of the title compound as a white solid: ^1H NMR (400 MHz, DMSO- d_6) δ 9.30 (s, 1H), 8.66 (d, J = 1.9 Hz, 1H), 8.43-8.41 (m, 2H), 7.88 (d, J = 1.9 Hz, 1H), 7.71 (dt, J = 10.3, 2.3 Hz, 1H); ES-MS $[\text{M}+\text{H}]^+$: 309.0. (d) 6-((5-Fluoropyridin-3-yl)oxy)-[1,2,4]triazolo[4,3-*a*]pyridine-8-carbonitrile (**25**). Compound **22** (2.50 g, 8.09 mmol), tetrakis(triphenylphosphine)palladium(0) (935 mg, 0.809 mmol), zinc cyanide (1.14 g, 9.71 mmol) and DMF (40 mL) were added to a microwave vial and heated in a microwave reactor at 140 °C for 15 minutes. The reaction was diluted with ethyl acetate and washed with water (2x). The combined aqueous layers were extracted with ethyl acetate, and the combined organics were dried over MgSO_4 , filtered, and concentrated *in vacuo*. Purification by reverse-phase chromatography afforded 1.86 g (90%) of the title compound as a yellow solid: ^1H NMR (400 MHz, DMSO- d_6) δ 9.36 (s, 1H), 8.96 (d, J = 2.0 Hz, 1H), 8.46-8.41 (m, 2H), 8.37 (d, J = 2.0 Hz, 1H), 7.72 (dt, J = 2.3, 10.4 Hz, 1H); ES-MS $[\text{M}+\text{H}]^+$: 256.2. (e) 6-((5-Fluoropyridin-3-yl)oxy)-[1,2,4]triazolo[4,3-*a*]pyridine-8-carboxylic acid (**28**). Compound **25** (1.86 g, 7.29 mmol) was dissolved in dioxane (37 mL) and 2N aqueous NaOH (18.5 mL) was added. The mixture was heated in a sealed tube at 100 °C for 18 h, and after cooling, the reaction was neutralized to pH 4-5 with 2N aqueous HCl. The water and solvent were removed *in vacuo*, and the crude reaction was dissolved in 10% MeOH/DCM. The undissolved salts were filtered off, and the filtrate was concentrated *in vacuo* to afford 740 mg (37%) of the title compound as a white solid: ^1H NMR (400 MHz, DMSO- d_6) δ 9.10 (d, J = 2.4 Hz, 1H), 8.43 (s, 1H), 8.42-8.38 (m, 3H), 7.11 (dt, J = 2.4, 11.2, Hz, 1H); ES-MS $[\text{M}+\text{H}]^+$: 275.0. (f) *N*-(5-Fluoropyridin-2-yl)-6-((5-fluoropyridin-3-yl)oxy)-[1,2,4]triazolo[4,3-*a*]pyridine-8-carboxamide (**31**). Compound **28** (740 mg, 2.70 mmol) and 5-fluoro-2-aminopyridine (605 mg, 5.40 mmol) were dissolved in pyridine (27 mL) in a flame-dried round-bottom flask. The reaction was cooled to -15 °C, and phosphorus oxychloride (302 μL , 3.24 mmol) was added dropwise while keeping the temperature below -15 °C. After stirring for 30 min at -15 °C, the reaction was quenched with ice-water and neutralized with 10% aqueous K_2CO_3 . The mixture was extracted with ethyl acetate (3x), and the combined organics were dried over MgSO_4 , filtered, and concentrated *in vacuo*. Purification by reverse-phase chromatography afforded 484 mg (49%) of the title compound as a white solid: ^1H NMR (400 MHz, DMSO- d_6) δ 9.54 (d, J = 2.3 Hz, 1H), 8.85 (s, 1H), 8.45 (d, J = 1.9 Hz, 1H),

8.43-8.41 (m, 2H), 8.34 (dd, $J = 4.1, 9.2$ Hz, 1H), 8.30 (d, $J = 2.2$ Hz, 1H), 7.87 (td, $J = 3.0, 8.6$ Hz, 1H), 7.69 (dt, $J = 2.4, 10.4$ Hz, 1H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 159.29 (d, $J(\text{C},\text{F}) = 257.1$ Hz), 158.98, 156.19 (d, $J(\text{C},\text{F}) = 249.6$ Hz), 154.89 (d, $J(\text{C},\text{F}) = 5.5$ Hz), 153.88, 147.45 (d, $J(\text{C},\text{F}) = 2.1$ Hz), 146.28, 144.32, 136.27 (d, $J(\text{C},\text{F}) = 3.4$ Hz), 136.07 (d, $J(\text{C},\text{F}) = 25.5$ Hz), 132.66 (d, $J(\text{C},\text{F}) = 22.75$ Hz), 127.83, 126.08, 125.85 (d, $J(\text{C},\text{F}) = 19.6$ Hz), 120.23, 114.72 (d, $J(\text{C},\text{F}) = 4.5$ Hz), 112.51 (d, $J(\text{C},\text{F}) = 21.9$ Hz); HRMS: calculated for $\text{C}_{17}\text{H}_{10}\text{F}_2\text{N}_6\text{O}_2$ [M], 368.0833; found 368.0838.

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17. The synthesis of *N*-(5-fluoropyridin-2-yl)-6-((5-fluoropyridin-3-yl)oxy)-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxamide (**51**) is representative of analogs with the [1,2,4]triazolo[1,5-*a*]pyridine core. (a) *N*-(3-Bromo-5-((5-fluoropyridin-3-yl)oxy)pyridin-2-yl)-*N'*-hydroxyformimidamide (**35**). Compound **8** (1.92 g, 6.76 mmol) was dissolved in IPA (24 mL) and DMF-DMA (1.18 mL, 8.79 mmol) was added. The reaction was heated to reflux for 3 h and subsequently cooled to 50 °C. Hydroxylamine hydrochloride (611 mg, 8.79 mmol) was added, and stirring continued for an additional 2 hours at 50 °C. The reaction was cooled and concentrated *in vacuo* to afford the title compound as a yellow solid that was used without further purification. ^1H NMR (400 MHz, DMSO- d_6) δ 10.73 (s, 1H), 8.39 (d, $J = 2.3$ Hz, 1H), 8.33-8.31 (m, 1H), 8.22 (d, $J = 2.6$ Hz, 1H), 8.17 (d, $J = 9.7$ Hz, 1H), 8.13 (d, $J = 2.6$ Hz, 1H), 7.90 (d, $J = 9.6$ Hz, 1H), 7.55 (dt, $J = 10.3, 2.4$ Hz, 1H); ES-MS $[\text{M}+\text{H}]^+$: 327.0. (b) 3-Bromo-5-((5-fluoropyridin-3-yl)oxy)-2-hydrazineylpyridine (**39**). Crude compound **35** was dissolved in THF (47 mL) and cooled to 0 °C. Trifluoroacetic anhydride (1.49 mL, 10.5 mmol) was added dropwise, and the reaction was stirred at rt for 16 h. The reaction was quenched with saturated aqueous NaHCO_3 . The mixture was extracted with ethyl acetate (3x), and the combined organics were dried over MgSO_4 , filtered, and concentrated *in vacuo* to afford 2.07 g (99%) of the title compound as a light brown solid that was used without further purification: ^1H NMR (400 MHz, DMSO- d_6) δ 9.23 (d, $J = 2.0$ Hz, 1H), 8.59 (s, 1H), 8.42-8.40 (m, 1H), 8.39 (d, $J = 2.4$ Hz, 1H), 8.14 (d, $J = 2.0$ Hz, 1H), 7.68 (dt, $J = 10.4, 2.4$ Hz, 1H); ES-MS $[\text{M}+\text{H}]^+$: 309.0. (c) 6-((5-Fluoropyridin-3-yl)oxy)-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonitrile (**43**). Compound **39** (967 mg, 3.13 mmol), tetrakis(triphenylphosphine)palladium (0) (361 mg, 0.313 mmol), zinc cyanide (441 mg, 3.75 mmol) and DMF (15.6 mL) were added to a microwave vial and heated in a microwave at 140 °C for 15 min. The reaction was concentrated *in vacuo* and purified by flash chromatography on silica gel to afford 650 mg (81%) of the title compound as a light brown solid: ^1H NMR (400 MHz, DMSO- d_6) δ 9.59 (d, $J = 2.2$ Hz, 1H), 8.73 (s, 1H), 8.58 (d, $J = J = 2.2$ Hz, 1H), 8.44 (d, $J = 1.9$ Hz, 1H), 8.41 (d, $J = 2.3$ Hz, 1H), 7.69 (dt, $J = 10.5, 2.5$ Hz, 1H); ES-MS $[\text{M}+\text{H}]^+$: 256.1. (d) 6-((5-Fluoropyridin-3-yl)oxy)-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxylic acid (**47**). Compound **43** (650 mg, 2.55 mmol) was dissolved in dioxane (12.7 mL) and 2N aqueous NaOH (6.4 mL) was added. The mixture was heated in a sealed tube at 100 °C for 18 h and, after cooling, the reaction was neutralized to pH 4–5 with 2N aqueous HCl . The water and solvent were removed *in vacuo*, and the crude reaction was dissolved in 10% MeOH/DCM. The undissolved salts were filtered off, and the filtrate was concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 318 mg (46%) of the title compound as a brown solid. ^1H NMR (400 MHz, DMSO- d_6) δ 9.09 (d, $J = 2.2$ Hz, 1H), 8.45 (s, 1H), 8.40 (s, $J = 2.4$ Hz, 1H), 7.97 (d, $J = 2.4$ Hz, 1H), 7.79 (d, $J = 2.4$ Hz, 1H), 7.65 (dt, $J = 10.4, 2.4$ Hz, 1H); ES-MS $[\text{M}+\text{H}]^+$: 274.8. (e) *N*-(5-fluoropyridin-2-yl)-6-((5-fluoropyridin-3-yl)oxy)-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxamide (**51**). Compound **47** (60 mg, 0.22 mmol) and 5-fluoro-2-aminopyridine (49 mg, 0.44 mmol) were dissolved in pyridine (2.2 mL) in a flame-dried round-bottom flask. The reaction was cooled to –15 °C and phosphorus oxychloride (25 μL , 0.26 mmol) was added dropwise while keeping the temperature below –15 °C. After stirring for 30 min at –15 °C, the reaction was quenched with ice-water and neutralized with 10% aqueous K_2CO_3 . The mixture was extracted with ethyl acetate (3x), and the combined organics were dried over MgSO_4 , filtered, and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 30 mg (37%) of the title compound as a white solid: ^1H NMR (400 MHz, DMSO- d_6) δ 9.55 (d, $J = 2.3$ Hz, 1H), 8.86 (s, 1H), 8.45 (d, $J = 2.0$ Hz, 1H), 8.44-8.41 (m, 2H), 8.35 (dd, $J = 4.1, 9.1$ Hz, 1H), 8.36 (d, $J = 4.1$ Hz, 1H), 7.87 (td, $J = 3.1, 8.6$ Hz, 1H), 7.70 (dt, $J = 2.4, 10.4$ Hz, 1H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 159.30 (d, $J(\text{C},\text{F}) = 257.0$ Hz), 159.00, 156.19 (d, $J(\text{C},\text{F}) = 249.4$ Hz), 154.90 (d, $J(\text{C},\text{F}) = 5.4$ Hz), 153.88, 147.47 (d, $J(\text{C},\text{F}) = 2.1$ Hz), 146.30, 144.32, 136.27 (d, $J(\text{C},\text{F}) = 3.4$ Hz), 136.08 (d, $J(\text{C},\text{F}) = 25.5$ Hz), 132.66 (d, $J(\text{C},\text{F}) = 22.8$ Hz), 127.85, 126.10, 125.87 (d, $J(\text{C},\text{F}) = 19.6$ Hz), 120.25, 114.73

(d, $J(\text{C},\text{F}) = 4.5 \text{ Hz}$), 112.51 (d, $J(\text{C},\text{F}) = 21.8 \text{ Hz}$); HRMS: calculated for $\text{C}_{17}\text{H}_{10}\text{F}_2\text{N}_6\text{O}_2$ [M], 368.0833; found 368.0838.

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Figure 1.
Picolinamide mGlu₅ NAM clinical candidate VU0424238 (**1**) and related analog VU0424222 (**2**) and inactive nicotinamide analogs **3** and **4**

Figure 2.
The fused heterocycle approach to a new series of mGlu₅ NAMs

Figure 3. Marble burying assay results with **31** (A) and **51** (B); all compounds were delivered via intraperitoneal (IP) dosing and formulated in 10% polysorbate 80 in sterile water; male Harlan CD-1 mice; $n = 6$ per treatment group; 15 min pre-treatment; 30 min burying time; *, $p < 0.05$ versus vehicle control group, Dunnett's test; bars denote marbles buried.

Scheme 1.

Reagents and conditions: (a) K₂S₂O₈, H₂SO₄, 0 °C to r.t., 54%; (b) 3-fluoro-5-hydroxypyridine, K₂CO₃, DMF, 91%; (c) H₂ (1 atm.), Raney Ni, MeOH, 95%; (d) ClCH₂CHO, K₂CO₃, ⁱPrOH, microwave, 170 °C, 20 min., 65%; (e) Zn(CN)₂, Pd(PPh₃)₄, DMF, microwave, 140 °C, 15 min., 81%; (f) 2N NaOH (aq.), dioxane, sealed tube, 100 °C, 18 h., 99%; (g) substituted 2-aminopyridine, POCl₃, pyridine, -15 °C, 61% (R = 6-F), 42% (R = 6-Me), 36% (R = 5-F), 55% (R = 5-Me), 60% (R = 5-OMe).

Scheme 2.

Reagents and conditions: (a) HF·pyridine, NaNO₃, 0 °C, 91% (**8**→**18**), 84% (**17**→**19**); (b) H₂NNH₂, EtOH, microwave, 120 °C, 15 min., 99% (**18**→**20**) 97% (**19**→**21**); (c) RC(OEt)₃, microwave, 180 °C, 20 min., 94% (**20**→**22**), 46% (**20**→**23**), 76% (**21**→**24**); (d) Zn(CN)₂, Pd(PPh₃)₄, DMF, microwave, 140 °C, 15 min., 90% (**22**→**25**), 92% (**23**→**26**), 21% (**24**→**27**); (e) 2N NaOH (aq.), dioxane, sealed tube, 100 °C, 18 h., 37% (**25**→**28**), 92% (**26**→**29**), 99% (**27**→**30**); (f) 5-fluoro-2-aminopyridine, POCl₃, pyridine, -15 °C, 49% (**28**→**31**), 45% (**29**→**32**), 83% (**30**→**33**).

Scheme 3.

Reagents and conditions: (a) Me₂NCR(OMe)₂, ⁱPrOH, reflux, then NH₂OH·HCl, 50 °C; (b) (F₃CCO)₂O, THF, 0 °C to r.t., 99% (**8**→**39**), 45% (**8**→**40**), 95% (**17**→**41**), 99% (**34**→**42**); (c) Zn(CN)₂, Pd(PPh₃)₄, DMF, microwave, 140 °C, 15 min., 81% (**39**→**43**), 78% (**40**→**44**), 42% (**41**→**45**), 50% (**42**→**46**); (d) 2N NaOH (aq.), dioxane, sealed tube, 100 °C, 18 h., 46% (**43**→**47**), 89% (**44**→**48**), 99% (**45**→**49**), 99% (**46**→**50**); (e) 5-fluoro-2-aminopyridine, POCl₃, pyridine, -15 °C, 37% (**47**→**51**), 62% (**48**→**52**), 59% (**49**→**53**), 15% (**50**→**54**).

Scheme 4.

Reagents and conditions: (a) Pd(OAc)₂, 1,3-bis(diphenylphosphino) propane, NEt₃, MeOH, CO (60 psi), 60 °C, 48% (**55**→**58**), 69% (**56**→**59**), 83% (**57**→**60**); (b) 1N NaOH (aq.), dioxane, 99% (**58**→**61**), 97% (**59**→**62**), 99% (**60**→**63**); (c) 5-fluoro-2-aminopyridine, POCl₃, pyridine, -15 °C, 50% (**61**→**64**), 51% (**62**→**65**), 94% (**63**→**66**).

Scheme 5.

Reagents and conditions: (a) $\text{Pd}(\text{OAc})_2$, 1,3-bis(diphenylphosphino) propane, NEt_3 , MeOH, CO (60 psi), 60 °C, 98%; (b) MeSO_3H , CHCl_3 , 63%; (c) $\text{PhN}(\text{SO}_2\text{CF}_3)_2$, NEt_3 , DMF, CH_2Cl_2 , 0 °C, 99%; (d) $\text{Zn}(\text{CN})_2$, $\text{Pd}(\text{PPh}_3)_4$, DMF, microwave, 140 °C, 15 min., 59%; (e) 1N NaOH (aq.), dioxane, 99% (**71**→**72**), 99% (**74**→**75**); (f) 5-fluoro-2-aminopyridine, POCl_3 , pyridine, -15 °C, 46% (**72**→**73**), 50% (**75**→**76**); (g) $\text{ClCF}_2\text{CO}_2\text{Na}$, K_2CO_3 , DMF, 95 °C, 18 h, 16%.

Table 1mGlu₅ activity of imidazo[1,2-*a*]pyridine core analogs

No.	R	mGlu ₅ pIC ₅₀ (±SEM) ^a	mGlu ₅ IC ₅₀ (nM) ^a	% Glu max (±SEM) ^{a,b}
12	6-F	7.42 ± 0.02	38	1.5 ± 0.2
13	6-Me	7.18 ± 0.01	66	1.5 ± 0.2
14	5-F	7.69 ± 0.15	20	1.6 ± 0.1
15	5-Me	7.13 ± 0.10	74	1.4 ± 0.2
16	5-OMe	7.26 ± 0.08	55	1.6 ± 0.2

^aCalcium mobilization rat mGlu₅ assay; values are avg. of *n* = 3^bAmplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); avg. of *n* = 3

Table 2Further *in vitro* profiling of imidazo[1,2-*a*]pyridines

No.	f_u^a		CL _{hep} (mL/min/kg) ^b		P450 Inhibition IC ₅₀ (μM) ^c				
	rat	hum	rat	hum	3A4	2D6	2C9	1A2	
2	0.015	0.011	34.8	7.3	4.3	>30	5.4	0.2	
12	0.023	0.026	56.1	17.9	2.3	24.8	1.6	0.6	
13	0.021	0.024	61.8	19.7	2.1	>30	1.0	0.5	
14	0.028	0.020	54.5	15.1	3.9	>30	5.7	0.8	
15	0.005	0.016	53.7	19.3	1.9	>30	6.9	0.8	
16	0.014	0.028	43.7	17.3	2.0	>30	5.7	1.1	

^aFraction unbound (f_u) as measured by an equilibrium dialysis assay using rat or human plasma^bPredicted hepatic clearance (CL_{hep}) based on the measured intrinsic clearance in rat or human liver microsomes^cAssayed in pooled human liver microsomes in the presence of NADPH with CYP-specific probe substrates

Table 3

mGlu₅ activity of triazolopyridine core analogs

		Series A		Series B	
No.	A	R	mGlu ₅ pIC ₅₀ (±SEM) ^a	mGlu ₅ IC ₅₀ (nM) ^a	% Glu max (±SEM) ^{a,b}
31	CF	H	7.57 ± 0.11	27	2.3 ± 0.1
32	CF	Me	6.43 ± 0.01	372	2.7 ± 0.9
33	N	H	6.49 ± 0.15	324	1.6 ± 0.2
51	CF	H	7.69 ± 0.05	20	1.5 ± 0.2
52	CF	Me	5.85 ± 0.33	1410	3.3 ± 1.6
53	N	H	7.13 ± 0.05	74	1.5 ± 0.2

^a Calcium mobilization rat mGlu5 assay; values are avg. of *n* 3

^b Amplitude of response in the presence of 30 μ M test compound as a percentage of maximal response (100 μ M glutamate); avg. of $n = 3$

Table 4

Further *in vitro* profiling of triazolopyridines

No.	f_u^a		CL _{hep} (mL/min/kg) ^b		P450 Inhibition IC ₅₀ (μM) ^c				
	rat	hum	rat	hum	3A4	2D6	2C9	1A2	
2	0.015	0.011	34.8	7.3	4.3	>30	5.4	0.2	
14	0.028	0.020	54.5	15.1	3.9	>30	5.7	0.8	
31	0.051	0.100	28.5	11.6	17.8	>30	>30	4.3	
51	0.066	0.071	12.8	5.8	>30	>30	>30	15.1	
53	0.168	0.313	5.3	6.2	>30	>30	>30	9.9	

^a Fraction unbound (f_u) as measured by an equilibrium dialysis assay using rat or human plasma

^b Predicted hepatic clearance (CL_{hep}) based on the measured intrinsic clearance in rat or human liver microsomes

^c Assayed in pooled human liver microsomes in the presence of NADPH with CYP-specific probe substrates

Table 5

Permeability, efflux potential, and brain homogenate binding

No.	MDCK-MDR1 Permeability				f_u^c	
	P_{app} (A-B) ^a	P_{app} (B-A) ^a	ER ^b	rat plasma	human plasma	rat brain
31	4.7	11.1	2.4	0.051	0.100	0.065
51	10.0	13.1	1.3	0.066	0.071	0.028

^a Apparent permeability coefficient (P_{app}) expressed as 10^{-6} cm/s

^b Efflux ratio (ER) is the ratio of B-A P_{app} to A-B P_{app}

^c Fraction unbound (f_u) as measured by an equilibrium dialysis assay using rat plasma, human plasma, or rat brain homogenates

Table 6

Rat pharmacokinetics

IV PK (1 mg/kg) ^a				
No.	$t_{1/2}$ (min) ^b	MRT (min) ^b	CL _p (mL/min/kg) ^b	V _{SS} (L/kg) ^b
31	79	118	14.0	1.6
51	81	143	11.0	1.6

PO PK (10 mg/kg) ^c				
No.	T _{max} (h) ^d	C _{max} (nM) ^d	AUC (nM·h) ^d	% F ^d
31	3	573	5670	17
51	3	673	8300	20

Tissue Distribution Study (3 mg/kg PO) ^e				
No.	plasma (nM)	brain (nM)	K _p ^f	K _{p,uu} ^f
31	269	445	1.7	2.1
51	361	508	1.4	0.6

^a $n = 2$; vehicle = 10% ethanol, 70% PEG 400, 20% saline

^b $t_{1/2}$ = plasma half-life; MRT = mean residence time; CL_p = plasma clearance; V_{SS} = volume of distribution at steady-state

^c $n = 2$; vehicle = 0.1% polysorbate 80 in 0.5% methyl cellulose

^d T_{max} = time at which maximum concentration occurs; C_{max} = maximum concentration; AUC = area under the curve; F = bioavailability

^e $n = 1$; vehicle = 0.1% polysorbate 80 in 0.5% methyl cellulose

^f K_p = total brain to total plasma ratio; K_{p,uu} = unbound brain (brain f_u · total brain) to unbound plasma (plasma f_u · total plasma) ratio

Table 7Analogues of **51**

No.	R	mGlu ₅ pIC ₅₀ (±SEM) ^a	mGlu ₅ IC ₅₀ (nM) ^a	% Glu max (±SEM) ^{a,b}
51	5-F	7.69 ± 0.05	20	1.5 ± 0.2
54	5-Cl	7.05 ± 0.18	89	1.1 ± 0.2
64	4,5-di-F	7.39 ± 0.02	41	2.0 ± 0.1
65	5,6-di-F	7.25 ± 0.08	56	1.6 ± 0.3
66	2,5-di-F	6.93 ± 0.07	117	2.2 ± 0.6
73	5-CN	7.36 ± 0.14	44	2.3 ± 0.4
76	5-OCHF ₂	6.89 ± 0.02	129	1.7 ± 0.2

^aCalcium mobilization rat mGlu₅ assay; values are avg. of $n = 3$ ^bAmplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); avg. of $n = 3$

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Table 8

Further *in vitro* profiling of analogs of **51**

No.	f_u^a		CL _{hep} (mL/min/kg) ^b		P450 Inhibition IC ₅₀ (μM) ^c				
	rat	hum	rat	hum	3A4	2D6	2C9	1A2	
51	0.066	0.071	12.8	5.8	>30	>30	>30	>30	15.1
64	0.066	0.067	46.7	13.6	>30	>30	>30	>30	4.1
65	0.023	0.031	25.8	5.2	23.0	>30	>30	>30	>30
73	0.065	0.135	13.9	7.0	10.6	>30	20.5	14.6	

^a Fraction unbound (f_u) as measured by an equilibrium dialysis assay using rat or human plasma

^b Predicted hepatic clearance (CL_{hep}) based on the measured intrinsic clearance in rat or human liver microsomes

^c Assayed in pooled human liver microsomes in the presence of NADPH with CYP-specific probe substrates

Table 9

DAT inhibition profiles

No.	DAT Binding ^a % Inhibition at 10 μ M	DAT Functional ^b IC ₅₀ (μ M)	DAT IC ₅₀ /mGlu ₅ IC ₅₀
51	89	0.730	36.5
31	91	ND ^d	ND ^d
53	60	>10 ^c	>135
64	87	ND ^d	ND ^d
65	67	2.87	51.3
73	79	3.29	74.8

^a n = 2; Displacement of [¹²⁵I]RTI-55 in CHO-K1 cells expressing human DAT; positive control = GBR-12909, DAT binding IC₅₀ = 0.71 nM in this study; see reference 40

^b n = 2; Inhibition of [³H]dopamine uptake in CHO-K1 cells expressing human DAT; positive control = nomifensine, DAT IC₅₀ = 15 nM in this study; see reference 35

^c 42% inhibition at top concentration of 10 μ M

^d ND = not determined