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Discovery of 6-(pyrimidin-5-ylmethyl)quinoline-8-carboxamide negative allosteric modulators of metabotropic glutamate receptor subtype 5

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Abstract

Based on previous work that established fused heterocycles as viable alternatives for the picolinamide core of our lead series of mGlu₅ negative allosteric modulators (NAMs), we designed a novel series of 6-(pyrimidin-5-ylmethyl)quinoline-8-carboxamide mGlu₅ NAMs. These new quinoline derivatives also contained carbon linkers as replacements for the diaryl ether oxygen atom common to our previously published chemotypes. Compounds were evaluated in a cell-based functional mGlu₅ assay, and an exemplar analog **27** was more than 60-fold selective versus the other seven mGlu receptors. Selected compounds were also studied in metabolic stability assays in rat and human S9 hepatic fractions and exhibited a mixture of P450- and non-P450-mediated metabolism.

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); Quinoline

Glutamate is the major excitatory transmitter in the mammalian central nervous system and activates both ionotropic (iGlu) and metabotropic glutamate (mGlu) receptors. While the iGlu receptors are ligand-gated ion-channels, the mGlu receptors are a family of eight G protein-coupled receptors (GPCRs). Based on their structure, function, and effects on downstream signaling pathways, the mGlu receptors have been classified into three groups. Group I receptors (mGlu₁ and mGlu₅), which are found in postsynaptic locations, are coupled via G_q to the activation of phospholipase C. On the other hand, group II receptors (mGlu₂₋₃), which are found both pre and postsynaptically, and group III receptors (mGlu₄, mGlu₆₋₈), which are found primarily in presynaptic locations, are coupled via G_{i/o} to the inhibition of adenylyl cyclase activity. The orthosteric glutamate binding site of the mGlu receptors is contained in the extracellular domain; however, the allosteric binding sites that have been discovered to date are found in the transmembrane domain.^{1,2}

The design of drug-like orthosteric ligands that are highly selective for a single mGlu receptor versus the other seven mGlu family members can be quite challenging. One method for overcoming this hurdle that has proven successful for a variety of mGlu receptors has been the use of approaches focused on compounds that interact with an allosteric site.³ Among these efforts, the design and optimization of negative allosteric modulators (NAMs) of mGlu₅ has been pursued extensively.⁴ Likewise, preclinical and clinical studies have indicated many potentially interesting therapeutic applications for mGlu₅ NAMs.¹⁻⁴ In fact, 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} Regrettably, in most instances, the primary efficacy endpoints were not met;⁵⁻⁹ however, some secondary efficacy endpoints have been noted in both MDD⁷ and PD-LID.¹⁰

Small molecule mGlu₅ NAM research has been a major focus of our group and recently culminated in the identification of a highly optimized mGlu₅ NAM candidate for clinical evaluation, VU0424238 (**1**) (Fig. 1),¹¹ a member of a series of picolinamide mGlu₅ NAMs. We also recently reported on the discovery of an additional backup series of heterocyclic mGlu₅ NAMs exemplified by [1,2,4]triazolo[1,5-*a*]pyridine-8-carboxamide **2**.¹² The discovery of **2** was based on a hypothesis that an internal hydrogen bond between the amide NH and the 1-nitrogen of the heteroaryl core of **2** would help orient the molecule similarly to the postulated conformation of VU0424238 (**1**), where an analogous hydrogen bond between the amide NH and the picolinamide nitrogen was assumed likely. The discovery of **2** and its analogs opened the door to investigate other heterocyclic cores such as quinoline-8-carboxamides. In fact, we synthesized compound **3**, a quinoline analog of **2**, and verified that it was similarly potent versus mGlu₅. Still, to further enhance the novelty of such new compounds and explore new SAR, we also sought to modify additional portions of the scaffold (Fig. 1). Specifically, we chose to replace the oxygen linker that had been constant

across the chemotypes found in both **1** and **2** as well as other related series^{13,14} by exchanging it with several carbon linkers. This Letter describes our efforts toward that end.

Synthetic work on the aforementioned ether analogs had employed nucleophilic aromatic substitution (S_NAr) as the means for joining the northern heteroaryl ring to the core of the scaffold.^{11–14} Our plan to move to a carbon linker would thus necessitate a substantially different synthetic approach (Scheme 1).¹⁵ Our first targets were a small set of ketone-linker containing analogs **11–14**. 6-Bromo-8-chloroquinoline **4** was converted to vinyl analog **5** via a Suzuki cross-coupling reaction with potassium vinyltrifluoroborate.¹⁶ Ozonolysis of **5** followed by treatment with dimethyl sulfide afforded aldehyde **6**. Metal-halogen exchange of 5-bromopyrimidine was carried out at low temperature, and the resultant organolithium intermediate was treated with **6** *in situ* to yield secondary alcohol **7**. Oxidation of **7** was accomplished with manganese oxide to provide ketone **8**. A palladium-catalyzed reaction with zinc cyanide gave nitrile **9**, and subsequent acidic hydrolysis with heating gave the penultimate acid **10**. Formation of amide analogs **11–14** was accomplished with phosphorous oxychloride in pyridine, conditions that we have employed successfully in the past.^{11–14}

Monofluoromethylene linker analogs **20–21** and difluoromethylene linker analogs **26–36** were prepared from intermediate **8** (Scheme 2). Suzuki cross-coupling reaction with potassium vinyltrifluoroborate as before afforded vinyl intermediate **15**. Reduction of the ketone was accomplished with sodium borohydride to provide alcohol **16**, which was treated with diethylaminosulfur trifluoride (DAST) to yield **17**. Ozonolysis of **17** followed by treatment with dimethyl sulfide afforded aldehyde **18**, which was subsequently oxidized to penultimate acid **19** with sodium chlorite. Conversion of **19** to amides **20–21** was carried out as previously described. Preparation of difluoromethylene linker analogs **26–36** was analogous; however, the route sequence was modified such that conversion of ketone **8** to difluoromethylene intermediate **22** was the initial step in the synthesis.

Synthesis of analogs with methoxy (**47–48**) and methyl (**51–52**) substituents at the methylene linker employed an alternative method for joining the northern heteroaryl ring to the quinoline core (Scheme 3). Here, a Suzuki cross-coupling reaction between potassium vinyltrifluoroborate and 5-bromopyrimidine **37** was used to access vinyl intermediate **38**. Bromination of the olefin, followed by treatment with 1,8-diazabicycloundec-7-ene (DBU), gave vinyl bromide **39**. Meanwhile, a palladium-catalyzed coupling of methyl 6-bromoquinoline-8-carboxylate **40** with bis(pinacolato)diboron provided boronic ester **41**.¹⁷ A Suzuki coupling between **39** and **41** was used to join the northern pyrimidine ring to the quinoline core and gave intermediate **42**. Ozonolysis of **42** followed by treatment with dimethyl sulfide afforded ketone **43**, which was reduced to alcohol **44** with sodium borohydride. Alcohol **44** was methylated with potassium bis(trimethylsilyl) amide and methyl iodide to yield **45**. Treatment of **45** with aqueous lithium hydroxide gave penultimate acid **46**, which was converted to analogs **47** and **48** as previously described. Intermediate **42** was also subjected to a palladium catalyzed hydrogenation to afford reduced intermediate **49**, which was subsequently converted to analogs **51** and **52** utilizing previously outlined chemistry.

Ketone linker analogs **11–14** were the first compounds from this work to be evaluated in our functional assay that measures the inhibition of Ca^{2+} mobilization induced by an EC_{80} concentration of glutamate in HEK293A cells expressing rat mGlu₅ (Table 1).¹⁸ The heteroaryl amides were chosen as these had proven effective for engendering mGlu₅ potency in other similar chemotypes.^{11,12,14} Interestingly, in this series, substitution of the pyridin-2-yl amide proved critical for potency; both **12** and **13** were more than 10-fold more active than **11**. The most potent analog in this set was 4-methylthiazol-2-yl amide **14**.

With the knowledge that ketones can be metabolically unstable, we decided to study that possibility with **14**. Incubation of **14** in rat whole blood at 37 °C for one hour showed near complete conversion to a metabolite thought to be the result of ketone reduction (Fig. 2). To verify this result, the secondary alcohol **53** was prepared through sodium borohydride reduction of **14**. Indeed, alcohol **53** was confirmed as the major metabolite from the rat whole blood stability study. To determine if alcohol **53** might be an active metabolite, it was also tested in the mGlu₅ functional assay; however, it was only a weak antagonist ($\text{IC}_{50} > 10 \mu\text{M}$). Finally, to verify if this metabolic event occurred *in vivo*, a rat pharmacokinetics (PK) study was conducted with **14** using intravenous (IV) dosing (Table 2). Not surprisingly, compound **14** was consumed quite rapidly with a corresponding appearance of metabolite **53** in the plasma. With such data in hand, we were keen to evaluate non-ketone carbon linkers.

Table 3 presents the SAR for four new carbon linkers in the context of the 5-fluoropyridin-2-yl and 4-methylthiazol-2-yl amides, groups that had demonstrated activity in this series and other favorable properties in related historical series.^{11,12,14} Both the monofluoromethylene analogs **20–21** and difluoromethylene analogs **26–27** demonstrated potency on par with ketone comparators **13** and **14** (Table 1). On the other hand, larger substituents on the methylene carbon in the form of methoxy (**47–48**) and methyl (**51–52**) negatively impacted mGlu₅ activity. Analogs **20–21** and **47–52** were tested as racemates. With the most potent compound (**27**) bearing the difluoromethylene linker, we prepared and tested several additional amide derivatives with that linker (Table 4). SAR here showed little tolerance for further substitution as all new pyridin-2-yl analogs (**28–35**) were less potent than 5-fluoropyridin-2-yl derivative **26**. Likewise, increasing the size of the 4-substituent on the thiazol-2-yl amide from methyl (**27**) to cyclopropyl (**36**) led to a 3.5-fold drop in mGlu₅ potency.

We have previously demonstrated that an unsubstituted pyrimidin-5-yl ether moiety is a potential site for aldehyde oxidase (AO) and/or xanthine oxidase (XO)-mediated metabolism.^{11,20–22} Since predicting human pharmacokinetics for compounds that are largely metabolized by AO and/or XO can be challenging due to their variable expression across preclinical species,^{20,23} we were interested in profiling some of these new compounds to understand what fraction of their metabolism was mediated by P450 versus non-P450 mechanisms. Thus, we evaluated **21** and **27** in metabolic stability assays in rat and human liver S9 hepatic fractions both with and without the addition of NADPH (Table 5).²⁴ Since NADPH is required for cytochrome P450-mediated oxidations, experiments that lack NADPH provide an assessment of non-P450-mediated metabolism. Whereas VU0424238 (**1**) was metabolized almost exclusively by AO/XO,²⁰ **21** and **27** demonstrated a mixture of P450- and non-P450-mediated metabolism. Interestingly, while **21** exhibited a higher

percentage of P450-mediated metabolism in rat S9 than in human S9 hepatic fractions, the profile for **27** was similar across species. Such results highlight how subtle the SAR can be for P450 versus non-P450-mediated metabolism. Of note, while these experiments do not conclusively identify AO/XO as the specific mechanism for the non-P450 mediated component of metabolism, our prior studies with related unsubstituted pyrimidin-5-yl ethers^{20–22} suggest AO/XO involvement.

Since analog **27** was the most potent analog in this series, we chose to profile that compound further (Table 6). Thus, selectivity was assessed versus the other seven mGlu receptors utilizing fold-shift experiments that assess the effect of 10 μ M **27** on the concentration response curves (CRC) of an orthosteric agonist in cell lines expressing each individual mGlu receptor.²⁵ Compound **27** was inactive versus the other seven mGlu receptor subtypes, indicating a selectivity for mGlu₅ of at least 60-fold. An analogous fold-shift experiment was conducted in HEK293A cells expressing human mGlu₅, and not surprisingly, 10 μ M **27** fully blocked the glutamate CRC in that cell line. This result was consistent with prior studies on compounds from related chemotypes where the difference between rat and human mGlu₅ activity was negligible.^{11,14} The extent to which **27** was bound to proteins was also assessed, and the compound was highly bound in both rat (f_u = 0.015) and human (f_u = 0.014) plasma.²⁶ Finally, a P450 inhibition profile was determined for **27**, and the compound was a moderate inhibitor of CYP3A4 and CYP2C9 and a potent inhibitor of CYP1A2.²⁶

When compared to clinical candidate VU0424238 (**1**) or backup analog **2**, compound **27** does not offer as attractive of an overall profile (Table 6), and significant room for optimization within this series remains. Nonetheless, the work described herein demonstrates that a variety of single carbon linkers, including both sp² and sp³ hybridized carbons, can be accessed from common intermediates and function as competent isosteric replacements for diaryl ethers. In addition, the utility of the quinoline core is consistent with our previously published work utilizing nitrogen-containing fused heterocycles¹² as replacements for the picolinamide core of our lead series. Finally, it is worth noting that such trends may be somewhat general with the potential to translate to other chemotypes designed to interact with a variety of targets.

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15. For detailed synthetic procedures see Conn PJ, Lindsley CW, Emmitte KA, Felts AS. US Patent. 9,533,982. Jan 3.2017. Synthesis of compound **27** is presented here as an exemplar compound from this series: 8-Chloro-6-vinylquinoline (**5**): 6-Bromo-8-chloroquinoline **4** (1.49 g, 6.14 mmol), PdCl₂(dppf) (100 mg, 0.122 mmol), potassium vinyltrifluoroborate (823 mg, 6.14 mmol) and triethylamine (857 μ L, 6.15 mmol) were dissolved in ethanol (61 mL) in a round-bottom flask and heated to 80 °C until the reaction was judged complete by LCMS (~2 h). The reaction was cooled, filtered through celite, and washed with 1:1 hexanes:ethyl acetate. The solvents were removed *in vacuo*, and the crude material was purified by flash chromatography on silica gel to afford 1.17 g (100%) of the title compound as an off-white solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.96 (dd, *J* = 4.2, 1.6 Hz, 1H), 8.40 (dd, *J* = 8.3, 1.6 Hz, 1H), 8.19 (d, *J* = 1.8 Hz, 1H), 7.96 (d, *J* = 1.6 Hz, 1H), 7.63 (dd, *J* = 8.3, 4.2 Hz, 1H), 6.89 (dd, *J* = 17.7, 10.9 Hz, 1H), 6.10 (d, *J* = 17.6 Hz, 1H), 5.46 (d, *J* = 11.0 Hz, 1H); ES-MS [M+H]⁺: 190.0. 8-Chloroquinoline-6-carbaldehyde (**6**): Intermediate **5** (4.08 g, 21.5 mmol) was dissolved in CH₂Cl₂ (108 mL) and methanol (108 mL) and cooled to -78 °C. Ozone was bubbled through the reaction until disappearance of **5** was observed by TLC. Air was bubbled through the reaction to remove excess ozone and dimethyl sulfide (47.7 mL, 645 mmol) was added. The reaction was allowed to warm to room temperature and stirred for 2 h. Water was added, and the reaction was extracted with CH₂Cl₂ (3x). The combined organics were dried over MgSO₄, filtered, and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 3.19 g (77%) of the title compound as a white solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.15 (s, 1H), 9.17 (dd, *J* = 4.12, 1.7 Hz, 1H), 8.70 (dd, *J* = 8.3, 1.6 Hz, 1H), 8.66 (d, *J* = 1.6 Hz, 1H), 8.26 (d, *J* = 1.7 Hz, 1H), 7.79 (dd, *J* = 8.3, 4.2 Hz, 1H); ES-MS [M+H]⁺: 192.1. (8-Chloroquinolin-6-yl)(pyrimidin-5-yl)methanol (**7**): 5-Bromopyrimidine (2.65 g, 16.7 mmol) was dissolved in THF (34 mL) and ether (34 mL) in an oven-dried round bottom flask and cooled to -100 °C. *n*-Butyllithium (1.6M in hexanes) (10.4 mL, 16.6 mmol) was added dropwise while ensuring the internal reaction temperature remained below -90 °C. After addition the reaction was stirred for 15 minutes at -100 °C and intermediate **6** (3.19 g, 16.7 mmol) was added dropwise as a solution in THF (34 mL) while ensuring the internal reaction temperature remained below -90 °C. The reaction was then allowed to gradually warm to room temperature. The reaction was quenched with water, and the mixture was extracted with ethyl acetate (3x). The combined organics were dried over MgSO₄, filtered, and concentrated *in vacuo*. Purification by

flash chromatography on silica gel afforded 3.67 g (81%) of the title compound as a white solid: ^1H NMR (400 MHz, DMSO- d_6) δ 9.09 (s, 1H), 8.99 (dd, J = 4.2, 1.6 Hz, 1H), 8.86 (s, 2H), 8.46 (dd, J = 8.4, 1.6 Hz, 1H), 8.06 (d, J = 1.4 Hz, 1H), 7.98 (d, J = 1.7 Hz, 1H), 7.64 (dd, J = 8.3, 4.2 Hz, 1H), 6.58 (d, J = 4.2 Hz, 1H), 6.06 (d, J = 4.2 Hz, 1H); ES-MS $[\text{M}+\text{H}]^+$: 272.1. 8-Chloroquinolin-6-yl(pyrimidin-5-yl)methanone (**8**): Intermediate **7** (3.67 g, 13.5 mmol) was dissolved in CH_2Cl_2 (113 mL), and MnO_2 (8.22g, 94.5 mmol) was added. The reaction was stirred overnight, filtered, and concentrated *in vacuo* to afford 3.63 g (100%) of the title compound as a white solid: ^1H NMR (400 MHz, DMSO- d_6) δ 9.48 (s, 1H), 9.21 (s, 2H), 9.17 (dd, J = 4.2, 1.7 Hz, 1H), 8.68 (dd, J = 8.4, 1.7 Hz, 1H), 8.54 (d, J = 1.8 Hz, 1H), 8.29 (d, J = 1.8 Hz, 1H), 7.78 (dd, J = 8.3, 4.2 Hz, 1H); ES-MS $[\text{M}+\text{H}]^+$: 270.1. 8-Chloro-6-(difluoro(pyrimidin-5-yl)methyl)quinoline (**22**): DAST (1.7 mL, 13 mmol) was added to intermediate **7** (500 mg, 1.85 mmol) dissolved in CH_2Cl_2 (12.5 mL) in a sealed vessel, and the reaction was heated to 40 °C overnight. An additional portion of DAST (500 μL , 6.5 mmol) was added, and the reaction was heated at 40 °C for an additional 24 hours. The reaction was cooled and quenched by slow addition to ice. The layers were separated, and the aqueous was extracted with CH_2Cl_2 . The combined organics were dried over MgSO_4 , filtered, and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 152 mg (28%) of the title compound as a yellow solid: ^1H NMR (400 MHz, DMSO- d_6) δ 9.39 (s, 1H), 9.19 (dd J = 4.3, 1.7 Hz, 1H), 9.17 (s, 2H), 8.75-8.69 (m, 2H), 8.67 (dd, J = 8.4, 1.6 Hz, 1H), 7.83 (dd, J = 8.4, 4.3 Hz, 1H); ES-MS $[\text{M}+\text{H}]^+$: 291.9. 6-(Difluoro(pyrimidin-5-yl)methyl)-8-vinylquinoline (**23**): Intermediate **22** (152 mg, 0.521 mmol), $\text{PdCl}_2(\text{dppf})$ (17 mg, 0.021 mmol), potassium vinyltrifluoroborate (84 mg, 0.63 mmol), and triethylamine (73 μL , 0.52 mmol) were dissolved in isopropanol (3.5 mL) in a round-bottom flask and heated to 100 °C until the reaction was judged complete by LCMS (~2 h). The reaction was cooled, filtered through celite, and washed with 5% methanol in CH_2Cl_2 . The solvents were removed *in vacuo*, and the crude material was purified by flash chromatography on silica gel to afford 71 mg (48%) of the title compound as a pale-yellow solid: ^1H NMR (400 MHz, DMSO- d_6) δ 9.38 (s, 1H), 9.18 (s, 2H), 9.04 (dd, J = 4.2, 1.7 Hz, 1H), 8.50 (dd, J = 8.3, 1.6 Hz, 1H), 8.27-8.17 (m, 2H), 7.93 (dd, J = 18.0, 11.3 Hz, 1H), 7.66 (dd, J = 8.3, 4.2 Hz, 1H), 6.21 (d, J = 18.0 Hz, 1H), 5.56 (d, J = 11.3 Hz, 1H); ES-MS $[\text{M}+\text{H}]^+$: 284.0. 6-(Difluoro(pyrimidin-5-yl)methyl)quinoline-8-carbaldehyde (**24**): Intermediate **23** (71 mg, 0.25 mmol) was dissolved in CH_2Cl_2 (2.5 mL) and cooled to -78 °C. Ozone was bubbled through the reaction until disappearance of **23** was observed by TLC. Air was bubbled through the reaction to remove excess ozone and dimethyl sulfide (556 μL , 7.52 mmol) was added. The reaction was allowed to warm to room temperature and stirred for 2 h. The reaction was concentrated *in vacuo*, and purification by flash chromatography on silica gel afforded 21 mg (29%) of the title compound as a yellow solid: ^1H NMR (400 MHz, CDCl_3) δ 9.52 (s, 1H), 7.63 (s, 1H), 7.48 (d, J = 3.6 Hz, 1H), 7.26 (s, 2H), 6.71 (d, J = 8.2 Hz, 1H), 6.66-6.60 (m, 2H), 5.98 (dd, J = 8.4, 4.3 Hz, 1H); ES-MS $[\text{M}+\text{H}]^+$: 286.0. 6-(Difluoro(pyrimidin-5-yl)methyl)quinoline-8-carboxylic acid (**25**): Intermediate **24** (21 mg, 0.074 mmol) was dissolved in dioxane (1.5 mL) and a solution of sodium acetate (21 mg, 0.26 mmol) in AcOH (484 μL) and H_2O (484 μL) was added followed by a solution of sulfamic acid (14 mg, 0.15 mmol) in H_2O (1.1 mL) and a solution of sodium chlorite (17 mg, 0.18 mmol) in H_2O (1.4 mL). The reaction was stirred for 1.5 hours and concentrated to approximately one-third its total volume. The resulting solution was extracted with 3:1 CHCl_3/IPA (3x), and the combined organics were dried over MgSO_4 , filtered, and concentrated *in vacuo* to afford 22 mg (99%) of the title compound as an off-white solid: ^1H NMR (400 MHz, DMSO- d_6) δ 9.40 (s, 1H), 9.22-9.14 (m, 3H), 8.82 (d, J = 7.9 Hz, 1H), 8.71 (s, 1H), 8.59 (s, 1H), 7.95-7.86 (m, 1H); ES-MS $[\text{M}+\text{H}]^+$: 302.2. 6-(Difluoro(pyrimidin-5-yl)methyl)-*N*-(4-methylthiazol-2-yl)quinoline-8-carboxamide (**27**): Intermediate **25** (7.0 mg, 0.023 mmol) and 4-methylthiazol-2-amine (2.9 mg, 0.025 mmol) were dissolved in pyridine (0.75 mL) in a flame-dried round-bottom flask. The reaction was cooled to -15 °C and phosphorus oxychloride (2.4 μL , 0.026 mmol) was added while ensuring the temperature remained at -15 °C. After stirring for 30 min at -15 °C, and the reaction was quenched with ice-water and neutralized with 10% aqueous K_2CO_3 . The mixture was extracted with EtOAc (3x), and the combined organics were dried over MgSO_4 , filtered, and concentrated *in vacuo*. Purification by reverse-phase chromatography afforded 5.8 mg (63%) of the title compound as a white solid. ^1H NMR (400 MHz, DMSO- d_6) δ 9.41 (s, 1H), 9.28 (d, J = 2.8 Hz, 1H), 9.19 (s, 2H), 8.77 (d, J = 8.0 Hz, 1H), 8.74-8.64 (m, 2H), 7.87 (dd, J = 4.2, 8.2 Hz, 1H), 6.86 (s, 1H), 2.31 (s, 3H); ES-MS $[\text{M}+\text{H}]^+$: 398.3; HRMS, calc'd for $\text{C}_{17}\text{H}_{10}\text{F}_2\text{N}_6\text{O}_2$ [M], 397.0811; found 397.0809.

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25. For a detailed description of the mGlu fold-shift assays employed to assess selectivity, see reference 11.
26. For a detailed description of the equilibrium dialysis plasma protein binding assays and cytochrome P450 cocktail inhibition assay in pooled human liver microsomes, see reference 11.

- New negative allosteric modulators of metabotropic glutamate receptor subtype 5
- Quinoline-8-carboxamides are functional as a picolinamide replacement
- Certain carbon-based linkers are alternatives to an ether linker

Figure 1. Picolinamide mGlu₅ NAM candidate VU0424238 (**1**), [1,2,4]triazolo[1,5-*a*]pyridine-8-carboxamide mGlu₅ NAM **2**, quinoline-8-carboxamide **3**, and our plan for development of a new quinoline-8-carboxamide mGlu₅ NAM scaffold.

Figure 2.
Major metabolic pathway of compound **14** in rat whole blood at 37 °C (1 h).

Scheme 1.

Reagents and conditions: (a) $\text{H}_2\text{C}=\text{CHBF}_3\text{K}$, $\text{PdCl}_2(\text{dppf})$, NEt_3 , EtOH , 80°C , 100%; (b) O_3 , CH_2Cl_2 , MeOH , -78°C , then Me_2S , -78°C to rt, 77%; (c) 5-bromopyrimidine, $n\text{-BuLi}$, THF , ether, -100°C , then **6**, -100°C to rt, 81%; (d) MnO_2 , CH_2Cl_2 , 100%; (e) $\text{Zn}(\text{CN})_2$, $\text{Pd}(\text{PPh}_3)_4$, DMF , microwave, 140°C , 20 min, 70%; (f) H_2SO_4 , AcOH , sealed tube, 120°C , 64%; (g) H_2NR^2 , POCl_3 , pyridine, -15°C , 14% (**10**→**11**), 21% (**10**→**12**), 42% (**10**→**13**), 14% (**10**→**14**).

Scheme 2.

Reagents and conditions: (a) $\text{H}_2\text{C}=\text{CHBF}_3\text{K}$, $\text{PdCl}_2(\text{dppf})$, NEt_3 , *i*-PrOH, 100 °C, 68% (**8**→**15**), 48% (**22**→**23**); (b) NaBH_4 , MeOH, THF, 0 °C to rt, 99%; (c) Et_2NSF_3 , CH_2Cl_2 , 40 °C, 37% (**16**→**17**), 28% (**8**→**22**); (d) O_3 , CH_2Cl_2 , MeOH, -78 °C, then Me_2S , -78 °C to rt, 24% (**17**→**18**) 29% (**23**→**24**); (e) NaClO_2 , NaOAc , AcOH , H_2O , $\text{H}_2\text{NSO}_3\text{H}$, dioxane, 94% (**18**→**19**), 99% (**24**→**25**); (f) H_2NR^2 , POCl_3 , pyridine, -15 °C, 10% (**19**→**20**), 24% (**19**→**21**), 11–63% (**25**→**26–36**).

Scheme 3.

Reagents and conditions: (a) $\text{H}_2\text{C}=\text{CHBF}_3\text{K}$, $\text{PdCl}_2(\text{dppf})$, NEt_3 , $n\text{-PrOH}$, sealed tube, $100\text{ }^\circ\text{C}$, 53%; (b) Br_2 , CHCl_3 , $0\text{ }^\circ\text{C}$, then DBU, 65%; (c) bis(pinacolato)diboron, $\text{PdCl}_2(\text{dppf})$, KOAc, DMSO, microwave, $120\text{ }^\circ\text{C}$, 10 min, 99%; (d) $\text{PdCl}_2(\text{dppf})$, 1M Na_2CO_3 (aq), microwave, $100\text{ }^\circ\text{C}$, 10 min, 61%; (e) O_3 , CH_2Cl_2 , MeOH, $-78\text{ }^\circ\text{C}$, then Me_2S , $-78\text{ }^\circ\text{C}$ to rt, 39%; (f) NaBH_4 , MeOH, THF, $0\text{ }^\circ\text{C}$ to rt, 52%; (g) $\text{KN}(\text{SiMe}_3)_2$, MeI, DMF, PhMe, $0\text{ }^\circ\text{C}$; 26%; (h) 1M LiOH (aq), dioxane, 99% (45→46), 99% (49→50); (i) H_2NR^2 , POCl_3 , pyridine, $-15\text{ }^\circ\text{C}$, 26% (46→47), 27% (46→48), 11% (50→51), 8% (50→52); (j) H_2 (1 atm), 10% Pd/C, MeOH, 96%.

Table 1mGlu₅ activity of 6-ketoquinoline analogs

11 R = 6-F 12 R = 6-Me 13 R = 5-F 14			
No.	mGlu ₅ pIC ₅₀ (±SEM) ^a	mGlu ₅ IC ₅₀ (nM) ^a	% Glu max (±SEM) ^{a,b}
11	< 5.0	> 10,000	12.5 ± 8.4 ^c
12	6.38 ± 0.22	417	1.5 ± 0.1
13	6.07 ± 0.45	842	2.7 ± 1.0
14	6.77 ± 0.19	172	1.3 ± 0.1

^a Calcium mobilization rat mGlu₅ assay; values are avg. of *n* = 3^b Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); avg. of *n* = 3^c Concentration response curve does not plateau

Table 2 Rat pharmacokinetics of **14** and metabolite **53**^{a,b}

Compound 14		Metabolite 53	
half-life (min)	30	half-life (min)	171
MRT ^c (min)	19	MRT ^c (min)	107
Cl _p (mL/min/kg)	3430	Cl _p (mL/min/kg)	94
V _{ss} (L/kg)	50	V _{ss} (L/kg)	10

^a n=2 male Sprague-Dawley rats; dose = 1.0 mg/kg^b IV formulation: 10% ethanol, 90% PEG 400^c MRT = mean residence time

Table 3

mGlu₅ activity of quinoline linker analogs

		Series A		Series B	
No.	Series	X	mGlu ₅ pIC ₅₀ (±SEM) ^a	mGlu ₅ IC ₅₀ (nM) ^a	% Glu max (±SEM) ^{a,b}
20	A	CHF	6.66 ± 0.23	217	1.5 ± 0.3
21	B	CHF	6.42 ± 0.25	378	1.7 ± 0.1
26	A	CF ₂	6.41 ± 0.28	392	4.5 ± 2.2
27	B	CF ₂	6.79 ± 0.31	161	1.4 ± 0.3
47	A	CHOMe	5.46 ± 0.30	3480	2.9 ± 0.1
48	B	CHOMe	5.76 ± 0.25	1750	1.8 ± 0.3
51	A	CHMe	5.82 ± 0.23	1520	1.5 ± 0.2
52	B	CHMe	6.23 ± 0.18	589	1.4 ± 0.0

^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 4

mGlu₅ activity of quinoline difluoromethylene linker analogs

No.	Series	R	mGlu ₅ pIC ₅₀ (±SEM) ^a	mGlu ₅ IC ₅₀ (nM) ^a	% Glu max (±SEM) ^{a,b}
26	A	5-F	6.41 ± 0.28	392	4.5 ± 2.2
27	B	Me	6.79 ± 0.31	161	1.4 ± 0.3
28	A	6-Me	5.96 ± 0.23	1100	2.7 ± 0.5
29	A	6-Et	5.44 ± 0.21	3600	7.8 ± 6.2
30	A	6-OMe	5.79 ± 0.24	1610	7.1 ± 4.7
31	A	5-Me	6.17 ± 0.26	676	3.0 ± 0.6
32	A	5-CN	< 4.5	> 30,000	—
33	A	4-Me	< 5.0	> 10,000	44 ± 12 ^c
34	A	4-OMe	< 4.5	> 30,000	—
35	A	4-CN	< 4.5	> 30,000	—
36	B	cyc-Pr	6.25 ± 0.27	558	3.0 ± 0.5

^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

^cConcentration response curve does not plateau

Table 5

Metabolic stability in rat and human S9 hepatic fractions

		human S9			
No.		+NADPH		-NADPH	
		CL _{INT} ^a	CL _{HEP} ^b	CL _{INT} ^a	CL _{HEP} ^b
21		98.2	17.3	31.3	12.6
27		110	17.6	19.6	10.1
No.		+NADPH		-NADPH	
		CL _{INT} ^a	CL _{HEP} ^b	CL _{INT} ^a	CL _{HEP} ^b
21		199	51.8	34.7	23.2
27		163	49.0	38.3	24.8

^aCL_{INT} = intrinsic clearance in units of mL/min/kg^bCL_{HEP} = predicted hepatic clearance in units of mL/min/kg calculated according to the formula: CL_{HEP} = (QH × CL_{INT})/(QH + CL_{INT}) where QH = hepatic flow of 21 (human) or 70 (rat)¹⁹

Table 6 Comparison of **27** to VU0424238 (**1**) and **2**

	1	2	27
pharmacology			
mGlu ₅ IC ₅₀ (nM)	11	20	161
selectivity vs. other mGlu ₅	>900	>500	>60
plasma protein binding ^a			
rat plasma f_u	0.106	0.066	0.015
human plasma f_u	0.120	0.071	0.014
P450 inhibition (IC ₅₀ in μ M) ^b			
CYP3A4	>30	>30	5.9
CYP2D6	>30	>30	26.5
CYP2C9	12.1	>30	5.3
CYP1A2	1.0	15.1	0.3

^aFraction unbound (f_u) as measured by an equilibrium dialysis assay using rat or human plasma^b Assayed in pooled human liver microsomes in the presence of NADPH with CYP-specific probe substrates