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N-Alkylpyrido[1',2':1,5]pyrazolo-[4,3-d]pyrimidin-4-amines: A new series of negative allosteric modulators of mGlu_{1/5} with CNS exposure in rodents

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Abstract

Selective negative allosteric modulators (NAMs) of each of the group I metabotropic glutamate receptors (mGlu₁ and mGlu₅) have been well characterized in the literature and offer potential as therapeutics in several disorders of the central nervous system (CNS). Still, compounds that are potent mGlu_{1/5} NAMs with selectivity versus the other six members of the mGlu family as well as the balance of properties required for use *in vivo* are lacking. A medicinal chemistry effort centered on the identification of a lead series with the potential of delivering such compounds is described in this Letter. Specifically, a new class of pyrido[1',2':1,5]pyrazolo[4,3-d]pyrimidin-4-amines was designed as a novel isosteric replacement for 4-aminoquinazolines, and compounds from within this chemotype exhibited dual NAM activity at both group I mGlu. One compound, VU0467558 (**29**), demonstrated near equipotent activity at both receptors, selectivity versus other mGlu, a favorable ancillary pharmacology profile, and CNS exposure in rodents.

Graphical Abstract

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29 VU0467558

mGlu₁ IC₅₀ = 69 nM
 mGlu₅ IC₅₀ = 129 nM
 other mGlu > 10 μM

Keywords

Metabotropic glutamate receptor subtype 1 (mGlu₁); Metabotropic glutamate receptor subtype 5 (mGlu₅); Negative allosteric modulator (NAM); Central nervous system (CNS); G-protein coupled receptor (GPCR)

Glutamate (L-glutamic acid), the major excitatory transmitter in the mammalian central nervous system (CNS), produces its effects through binding to both ionotropic and metabotropic glutamate receptors (mGlu). The mGlu comprise a family of eight G-protein-coupled receptors (GPCRs) that are further divided according to their structure, preferred signal transduction mechanisms, and pharmacology. The group I mGlu (mGlu₁ and mGlu₅) are located post-synaptically and are coupled via G_q to the activation of phospholipase C, an event that leads to the elevation of intracellular calcium (Ca²⁺) and activation of protein kinase C (PKC). Conversely, both the group II (mGlu₂ and mGlu₃) and group III (mGlu₄, mGlu₆, mGlu₇, and mGlu₈) mGlu are found predominantly pre-synaptically and are coupled via G_{i/o} to the inhibition of adenylyl cyclase activity. Orthosteric binding sites are located in the extracellular N-terminal domain within the mGlu family. In contrast, the majority of allosteric binding sites that have been discovered to date are contained in the transmembrane domain.¹⁻³

Given the high homology across orthosteric binding sites within the mGlu family, the design of highly selective orthosteric ligands has been quite challenging. One approach to circumvent this challenge that has proven successful in many instances has been the development of selective allosteric modulators of the individual mGlu.⁴⁻⁸ Among the most advanced areas within this field are small molecule negative allosteric modulators (NAMs) of mGlu₁^{9,10} and mGlu₅.¹¹⁻¹⁵ In fact, at least one mGlu₁ NAM, a 1,4-diaryl-5-methyl-1,2,3-triazole developed by researchers at Merck-Banyu, reached clinical candidate status.^{16,17} The mGlu₅ NAM field is still more advanced, with multiple compounds advancing to the clinic, including phase II studies with basimglurant (Roche),^{18,19} dipraglurant (Addex),²⁰ and mavoglurant (Novartis).^{21,22}

Highly selective mGlu₁ and mGlu₅ NAMs are both clearly interesting, and many high quality small molecule probes from multiple chemotypes exist for each. Still, we were surprised to find no reports of compounds suitable for rodent studies that are potent NAMs of both group I receptors while maintaining selectivity against the other mGlu. Numerous preclinical studies with selective mGlu₁ and mGlu₅ NAM tools demonstrate overlap in potential therapeutic applications. For example, accounts of efficacy in preclinical models of anxiety,²³⁻²⁸ addiction,²⁹⁻⁴⁵ and pain⁴⁶⁻⁵⁰ point toward a potential role for antagonism of

each group I receptor in these disorders. Still, the literature has documented concerns regarding the potential for motor and cognitive side effects with mGlu₁ NAMs,⁹ and concerns regarding the psychotomimetic effects of certain mGlu₅ NAMs have been documented as well.^{11,51} Thus, it is reasonable to question whether there will be a negative impact in combining such activities; however, one should not assume such will necessarily be the case. It may even be possible that a dual mGlu_{1/5} NAM could offer an improved safety profile by reducing the occupancy at each receptor required for efficacy relative to agents selective for only one of these receptors. Such studies suggest the possibility of a potential benefit with dual mGlu_{1/5} inhibition and warrant efforts to identify a lead series capable of delivering that profile.

During the course of our own efforts to identify and optimize selective mGlu₅ NAMs, we identified a series of 6-substituted-4-anilinoquinazolines represented by screening hit **1** (Fig. 1).⁵² Interestingly, compound **1** and additional analogs within this series were essentially equipotent against mGlu₁ and mGlu₅ while demonstrating selectivity versus other members of the mGlu family. The mGlu₁ NAM co-activity in **1** was not entirely unexpected as Lilly had previously identified and characterized a selective mGlu₁ NAM tool, LY456236 (**2**) from the same chemotype.⁵³ Furthermore, more highly optimized mGlu₁ NAMs from quinazoline-like scaffolds had also been previously reported, including compound **3** from Pfizer⁴⁸ and LY456066 (**4**) from Lilly.⁵⁴

Based on these observations, we rationalized that it might be possible to design a selective mGlu_{1/5} NAM with properties suitable for use in rodent behavioral assays. Quinazolines are among the most studied scaffolds in medicinal chemistry and are extensively described in the primary and patent literature;⁵⁵ thus, we sought to develop a novel isostere for this moiety. Literature searching revealed that one chemotype that had yet to be prepared and studied to any degree biologically was a series of pyrido[1',2':1,5]pyrazolo[4,3-*d*]pyrimidin-4-amines (Fig. 2). Given such novelty and a high probability that such compounds would function as quinazoline isosteres, we were quite interested in pursuing these targets. Our own aforementioned work related to mGlu₅ NAMs⁵² led us to believe that secondary amines would be required for activity at that target. Evaluation of the Pfizer series of mGlu₁ NAMs⁴⁸ indicated that saturated lipophilic groups would be favorable for mGlu₁ activity. We were keen to incorporate saturated groups at this position as increased sp³ character has been linked to improved properties and drug-likeness.⁵⁶ Finally, given the substitution pattern in LY456066 (**4**, Fig. 1), we identified the 2-position worthy of SAR development as well.

It was envisioned that analogs could be accessed through known key intermediate amine **7** (Scheme 1). The synthesis of **7** from commercially available methyl ester **5** was previously described in the literature.⁵⁷ We employed a modified version of the published synthesis of **7**. Specifically, nitration of **5** with potassium nitrate in sulfuric acid afforded 3-nitro intermediate **6** in good yield, and a tin(II) chloride reduction of the nitro group gave amine **7** in moderate yield. For synthesis of intermediate **8** (R¹ = H), **7** was heated via microwave irradiation in formamide. Synthesis of intermediate **9** (R¹ = CH₃) was accomplished through microwave irradiation in triethyl orthoacetate under mildly acidic conditions; however, this transformation was much more sluggish than was observed for the preparation of **8**.

Conversion of **8** and **9** to heteroaryl chlorides **10** and **11**, respectively, was carried out in moderate yield by microwave irradiation in phosphorous oxychloride. Installation of 4-position amines was accomplished through a nucleophilic aromatic substitution reaction with the requisite primary amine (R^2NH_2).

Synthesis of analogs with amines at the 2-position of the scaffold employed an alternative route that also began with key intermediate **7** (Scheme 2). Heating of **7** with ethyl chloroformate in dioxane, followed by treatment with potassium *tert*-butoxide under microwave irradiation, afforded intermediate **38** in near quantitative yield. Conversion of **38** to dichloro intermediate **39** was accomplished in high yield through microwave heating in phosphorous oxychloride. Room temperature nucleophilic aromatic substitution of trans-methylcyclohexylamine proceeded selectively at the 4-position of the template to provide penultimate intermediate **40**. The selectivity for the 4-position mirrors results obtained in similar transformations involving 2,4-dichloroquinazolines.^{58,59} Final stage diversification was carried out as described above through heating **40** with the desired amine (R^3NH_2) via microwave irradiation.

Evaluation of all new compounds for their activity at mGlu₁ and mGlu₅ was conducted using functional cell-based assays. These fluorescence-based assays employ cell lines stably expressing either rat mGlu₁ or rat mGlu₅ and measure the ability of the compound to block the mobilization of calcium induced by an EC₈₀ concentration of glutamate.^{60,61} The format for these assays employs a “triple-add” protocol that allows for the detection of agonists, positive allosteric modulators (PAMs), and NAMs.⁶² A number of 4-position amine analogs (**12–19**) were prepared that were considered somewhat flexible, meaning that the sp³ carbon to which the amine was attached was not part of a cyclic ring system (Table 1). Several analogs were prepared and tested as pairs of enantiomers with a chiral methyl group (**12–17**). In each case, the (*R*)-enantiomer analogs (**13**, **15**, and **17**) were preferred relative to their (*S*)-enantiomer counterparts (**12**, **14**, and **16**) with regards to mGlu₁ NAM activity. The mGlu₁ NAM activity was also improved by moving from increasingly lipophilic cyclopropyl (**12**, **13**) to *tert*-butyl (**14**, **15**) to cyclohexyl (**16**, **17**) analogs. The 3,3-dimethylbutan-1-amine analog **18** proved the most potent mGlu₁ NAM in this set. On the other hand, 2-methylbutan-2-amine analog **19** was only a weak mGlu₁ NAM, inhibiting the glutamate response only at the top concentration tested (30 μ M). Finally, mGlu₅ NAM activity with these compounds was generally weak or inactive (> 30 μ M); however, **18** demonstrated modest mGlu₅ NAM potency that was approximately ten-fold less than its mGlu₁ NAM activity. Such a result confirmed the possibility of accessing dual mGlu_{1/5} NAMs from within this scaffold.

Results obtained with cycloalkyl amine analogs **20–26** provided further encouragement (Table 2). Potency at both mGlu₁ and mGlu₅ was generally enhanced by increasing size and lipophilicity; however, a preference for mGlu₁ versus mGlu₅ was noted with all analogs. For example, a clear potency enhancement at both receptors was observed in moving from cyclohexyl analog **20** to cycloheptyl analog **21** to cyclooctyl analog **22**. Additionally, while potency was good with 2,3-dihydro-1*H*-inden-2-amine **23**, adamantyl derivatives **24** and **25** were among the most potent compounds in this set. Notably, installation of a tertiary hydroxyl group on the adamantine ring (**26**), a modification that reduced lipophilicity

substantially relative to direct comparator **25**, also reduced potency at both receptors. Finally, while mGlu₁ NAM potency was excellent in the case of a few analogs in this set, mGlu₅ NAM potency remained modest in those same analogs.

Having demonstrated that increased lipophilicity and/or steric bulk on the 4-position increased potency, we decided to prepare substituted analogs of cyclohexyl amine **20**. New analogs (Table 3) contained functional groups designed to further probe this trend while continuing to search for increased mGlu₅ NAM activity. Difluorocyclohexyl amine **27** reduced mGlu₁ NAM activity relative to **20** (Table 2) and failed to enhance mGlu₅ NAM activity. Fortunately, other analogs were more promising. For instance, both *trans*-4-methylcyclohexyl amine **28** and 4,4-dimethylcyclohexyl amine **29** demonstrated excellent mGlu₁ NAM potency, and **29** proved a breakthrough compound with excellent activity at mGlu₅ as well. Additional compounds prepared from various commercially available chiral amines (**30–33**) offered good potency as mGlu₁ NAMs (**30–32**); however, mGlu₅ potency failed to approach that observed with **29**. Such results are yet another example of the narrow SAR that is often discovered in the design of allosteric modulators of GPCRs and a reminder of the importance of preparing and testing multiple analogs even when there are only subtle structural differences across compounds.

Prior to the discovery of **29** and in parallel to its preparation, we evaluated substitution of the 2-position of the scaffold with methyl (**34–37**) and amine (**41–45**) groups (Table 4). The patent application that covers LY456066 (**4**) (Fig. 1) indicates that alkyl and *N*-alkyl groups can function as alternatives to the *S*-alkyl groups of that compound,⁶³ and we viewed these as more drug-like alternatives. For these analogs, we employed four of the 4-position amines that offered good to excellent mGlu₁ NAM activity and modest mGlu₅ NAM potency. Unfortunately, modest reduction in mGlu₁ NAM potency was observed with a 5-methyl substituent relative to hydrogen, and no mGlu₅ activity was noted with these compounds. The 5-amino analogs were even more disappointing, with only primary amine **41** offering weak mGlu₁ NAM activity and other compounds inactive at both receptors. Given the intolerance of substitution at this position, we decided to move forward with further profiling of **29** as it was the only compound that was essentially equipotent at both receptors. It should be noted that it has yet to be established that a compound that is equipotent at both group I mGlu will ultimately prove optimal. If a potent mGlu₁ NAM with moderate mGlu₅ NAM co-activity was of interest, this chemotype appears highly tractable for that profile. Still, compound **29** (VU0467558) represented a novel and potent dual mGlu_{1/5} NAM with reduced lipophilicity (3.57 vs. 5.09) and enhanced sp³ character relative to hit **1** (Fig.1). Thus, **29** was a logical choice for further profiling from within this new lead series.⁶⁴

First, selectivity was assessed versus the remaining six members of the mGlu family. The effect of 10 μM **29** on the orthosteric agonist concentration-response curve (CRC) was assessed in fold-shift experiments,^{65,66} and no significant effects were observed. Hence, **29** exhibited excellent selectivity (>75-fold) for the group I mGlu receptors. To evaluate the ancillary pharmacology profile (Table 5), **29** was screened in a commercially available radioligand binding assay panel of 68 clinically relevant targets.⁶⁷ For 62 of the 68 targets no significant responses were noted; significant responses were defined as greater than or equal to 50% inhibition of binding. Four of the six remaining targets showed only modest

inhibition of radioligand binding while substantial inhibition was found at the closely related receptors, adenosine A₁ and A_{2A}. Additionally, since 4-aminoquinazolines are well-known scaffolds for kinase inhibitors,⁵⁵ we profiled **29** in an extensive kinase panel of 369 kinases.⁶⁸ In this large panel, 10 μ M **29** only inhibited more than 50% of the activity of eight kinases, and half of these were members of a single family, the casein kinase 1 family. Overall, the selectivity of **29** was considered encouraging, and evaluation of the compound in assays designed to assess its potential for use *in vivo* was deemed worthwhile.

Since unbound fraction (f_u) can be important to consider alongside potency when correlating *in vivo* efficacy to exposure, equilibrium dialysis protein binding assays were conducted in both plasma and brain homogenates (Table 6).^{69,70} Compound **29** was highly protein bound, and the fraction unbound was higher in plasma than brain for both rat and mouse. Metabolic stability was gauged by measuring the intrinsic clearance of **29** in liver microsomes and using that value to calculate the predicted hepatic clearance (CL_{HEP}).^{70,71} Unfortunately, the predicted hepatic clearance for **29** was near hepatic blood flow for both species. Finally, the potential for drug–drug interactions with **29** was assessed in a human liver microsomes (HLM) cocktail assay with probe substrates for four common P450s. Compound **29** was judged quite clean according to this assay, with little P450 inhibition observed. With an *in vitro* DMPK profile in hand, we moved forward with *in vivo* studies in rodents.⁷³

The clearance in rats and the CNS penetration for **29** was assessed simultaneously using intravenous (IV) dosing (Table 7).^{70,74} The *in vitro* microsomal stability assay proved predictive as the compound exhibited high-clearance and a short half-life. Such a result may point toward microsomal stability as a useful assay for further optimization within this series. Fortunately, the CNS penetration for **29** was excellent with a brain to plasma ratio (K_p) of 5.3 in rats. Moreover, the unbound brain to unbound plasma ratio was approximately one, indicating distribution equilibrium between the compartments and a low probability of the compounds being a substrate for transporters.⁷⁵ Given the high clearance observed in rats, intraperitoneal (IP) dosing was chosen as a route of administration that might provide therapeutically relevant plasma drug levels. Thus, IP PK studies in rats and mice at 10 and 30 mg/kg, respectively, were carried out.⁷⁰ Tissue distribution studies in mice were conducted in parallel using the same conditions. CNS penetration in mice was quite similar to that observed in rats, with no evidence of efflux. Additionally, exposure using this route of administration and these doses was quite good in both species. For example, using the aforementioned data, it is estimated that **29** achieved an unbound brain concentration of approximately 100 nM in both rats and mice, which is near the functional IC₅₀ value at both mGlu₁ and mGlu₅.

In conclusion, we have designed a new lead series of pyrido[1',2':1,5]pyrazolo[4,3-*d*]pyrimidin-4-amines for the development of mGlu_{1/5} NAMs with selectivity versus other members of the mGlu family. One member of this series, **29** (VU0467558), is a potent antagonist of both group I receptors with essentially equal activity and can be dosed IP in rats and mice to reach therapeutically relevant levels of unbound compound in the CNS. As such, **29** represents an attractive lead for further optimization. Future work to explore the SAR around modification of the tricyclic core, the identification and elimination of

metabolic hotspots, and refinement of the ancillary pharmacology in this novel series is planned.

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64. Synthesis of **29** (VU0467558): (a) methyl 3-nitropyrzolo[1,5-*a*]pyridine-2-carboxylate (**6**). Compound **5** (4.75 g, 27.0 mmol, 1.0 eq) was dissolved in sulfuric acid (90 mL) and cooled to 0 °C. Potassium nitrate (3.41 g, 33.7 mmol, 1.25 eq) was added and the reaction was allowed to warm to room temperature. Stirring was continued for 3.5 hours at which point the reaction was poured into ice water. The mixture was extracted with 3:1 CHCl₃/IPA (3×), and the combined organics were dried (MgSO₄), filtered and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 4.69 g (79%) of the title compound as yellow solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.04 (d, *J* = 6.9 Hz, 1H), 8.27 (d, *J* = 8.9 Hz, 1H), 7.97 (t, *J* = 7.9 Hz, 1H), 7.48 (td, *J* = 7.0, 1.1 Hz, 1H), 3.98 (s, 3H); ES-MS [*M*+1]⁺: 222.1. (b) methyl 3-aminopyrzo[1,5-*a*]pyridine-2-carboxylate (**7**). Tin chloride (20.1 g, 106 mmol, 5.0 eq) was dissolved in concentrated hydrochloric acid (35.3 mL) and added dropwise to a solution of compound **6** (4.69 g, 21.2 mmol, 1.0 eq) in dioxane (106 mL). The reaction was stirred for 30

minutes and neutralized with saturated sodium bicarbonate. The reaction was filtered and washed with 3:1 CHCl₃/IPA. The layers were separated and the aqueous was extracted with CHCl₃/IPA (2×). The combined organics were dried (MgSO₄), filtered and concentrated *in vacuo* to afford 2.62 g (65%) of the title compound as a yellow solid that was used without further purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.37 (d, *J* = 6.9 Hz, 1H), 7.75 (d, *J* = 8.7 Hz, 1H), 6.92–6.84 (m, 2H), 5.29 (s, 2H), 3.83 (s, 3H); ES-MS [M+1]⁺: 192.1. (c) pyrido[1',2':1,5]pyrazolo[4,3-*d*]pyrimidin-4(3*H*)-one (**8**). Compound **7** (661 mg, 0.346 mmol, 1.0 eq) was suspended in formamide (7 mL) and heated in a microwave reactor at 200 °C for 60 minutes. The solid was filtered and dried to afford 630 mg (98%) of the title compound as a brown solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.91 (d, *J* = 7.0 Hz, 1H), 8.12 (d, *J* = 8.82 Hz, 1H), 7.94 (s, 1H), 7.46 (t, *J* = 7.4 Hz, 1H), 7.3 (t, *J* = 6.9 Hz, 1H); ES-MS [M+1]⁺: 187.1. (d) 4-chloropyrido[1',2':1,5]pyrazolo[4,3-*d*]pyrimidine (**10**). Compound **8** (1.22 g, 6.55 mmol, 1.0 eq) was suspended in phosphorus oxychloride (16.4 mL) and heated in a microwave reactor at 140 °C for 20 minutes. The reaction was concentrated *in vacuo* and the residue was dissolved in methylene chloride and stirred with potassium carbonate for 30 minutes. The mixture was filtered and concentrated *in vacuo*. Purification by flash chromatography on silica gel afforded 787 mg (59%) of the title compound as a yellow solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.29 (d, *J* = 6.8 Hz, 1H), 8.91 (s, 1H), 8.58 (d, *J* = 8.6 Hz, 1H), 7.81 (t, *J* = 7.4 Hz, 1H), 7.74 (t, *J* = 6.8 Hz, 1H); ES-MS [M+1]⁺: 205.2. (e) N-(4,4-dimethylcyclohexyl)pyrido[1',2':1,5]pyrazolo[4,3-*d*]pyrimidin-4-amine (VU0467558, **29**). Compound **10** (787 mg, 3.85 mmol, 1.0 eq), 4,4-dimethylcyclohexan-1-amine hydrochloride (1.18 g, 7.21 mmol, 1.9 eq) and *N,N*-Diisopropylethylamine (3.35 mL, 19.2 mmol, 5.0 eq) were dissolved in DMF (10 mL) and heated in a microwave reactor at 150 °C for 10 minutes. The reaction was concentrated *in vacuo* and purified by flash chromatography on silica gel to afford 670 mg (59%) of the title compound as a white solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.91 (d, *J* = 6.9 Hz, 1H), 8.29 (s, 1H), 8.20 (d, *J* = 8.7 Hz, 1H), 7.95 (d, *J* = 8.2 Hz, 1H), 7.48 (t, *J* = 7.1 Hz, 1H), 7.37 (td, *J* = 7.0, 1.4 Hz, 1H), 4.20–4.08 (m, 1H), 1.76–1.63 (m, 4H), 1.44–1.37 (m, 2H), 1.35–1.25 (m, 2H), 0.94 (d, *J* = 10.8 Hz, 6H); ES-MS [M+1]⁺: 296.2.

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All rodent PK experiments were conducted in accordance with the National Institute of Health regulations of animal care covered in Principles of Laboratory Animal Care (National Institutes of

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Figure 1.
Quinazoline and quinazoline-like group I mGlu NAMs

Figure 2.
Proposal for development of novel mGlu_{1/5} NAMs from a pyrido[1',2':1,5]pyrazolo[4,3-d]pyrimidin-4-amine scaffold

Scheme 1.

Reagents and conditions: (a) Reagents and conditions: (a) KNO_3 , H_2SO_4 , 0 °C to r.t., 79%; (b) $SnCl_2$, con. HCl , dioxane, 65%; (c) For $R^1 = H$, $HCONH_2$, microwave, 200 °C, 60 min., 98%; (d) For $R^1 = CH_3$, $CH_3C(OEt)_3$, formic acid, microwave, 180 °C, 60 min., 24%; (e) $POCl_3$, microwave, 140 °C, 20 min., 59% ($R^1 = H$), 54% ($R^1 = CH_3$); (f) R^2NH_2 , DIEA, DMF, microwave, 150 °C, 15 min., 43-84%.

Scheme 2.

Reagents and conditions: (a) (i) ClCO_2Et , dioxane 100°C , 16 h, (ii) KO^tBu , isopropanol, microwave, 160°C , 15 min., 99%; (b) POCl_3 , microwave, 140°C , 20 min., 84%; (c) *trans*-4-methylcyclohexylamine, DIEA, DMF, rt, 16h., 76%; (d) $R^3\text{NH}_2$, DIEA, DMF, microwave, 150°C , 10 min., 48-77%.

Table 1
4-Position SAR for flexible alkyl amines

The chemical structure shows a 4-substituted indazole where the substituent R is attached to the nitrogen at the 4-position. The R groups are defined as follows: I is a cyclopropyl group, II is a 1-methylcyclopropyl group, III is a 3,3-dimethylbutyl group, and IV is a 2,2,3-trimethylbutyl group.

Cpd	R	A	cLogP ^a	mGlu ₁ pIC ₅₀ (± SEM) ^b	mGlu ₁ IC ₅₀ (nM) ^b	% Glu Max (± SEM) ^{b,c}	mGlu ₅ pIC ₅₀ (± SEM) ^b	mGlu ₅ IC ₅₀ (nM) ^b	% Glu Max (± SEM) ^{b,c}
12	I	cyclopropyl	2.26	< 4.5	> 30,000	—	< 4.5	> 30,000	—
13	II	cyclopropyl	2.26	< 5.0 ^{de}	> 10,000 ^{de}	71.6 ^e	< 4.5	> 30,000	—
14	I	<i>tert</i> -butyl	3.30	5.18 ± 0.05	6,540	−0.2 ± 3.0	< 4.5	> 30,000	—
15	II	<i>tert</i> -butyl	3.30	6.05 ± 0.07	883	2.2 ± 0.1	< 4.5	> 30,000	—
16	I	cyclohexyl	3.52	5.65 ± 0.03	2,240	0.9 ± 0.2	< 5.0 ^d	> 10,000 ^d	61.8 ± 4.3
17	II	cyclohexyl	3.52	6.33 ± 0.10	462	2.4 ± 0.3	< 5.0 ^d	> 10,000 ^d	18.4 ± 6.9
18	III	—	3.26	6.47 ± 0.07	341	1.5 ± 0.3	5.44 ^e	3,600 ^e	1.3 ^e
19	IV	—	2.58	< 5.0 ^d	> 10,000 ^d	12.6 ± 4.3	< 4.5	> 30,000	—

^aCalculated using ChemDraw Professional, version 15.0 (PerkinElmer Informatics, Inc.)

^bCalcium mobilization assay; values are average of n = 3

^cAmplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of n = 3

^dConcentration-response curve (CRC) does not plateau

^eAverage of n = 2

Table 2
4-Position SAR for cycloalkyl amines

Cpd	R	n	cLogP ^a	mGlu ₁ pIC ₅₀ (± SEM) ^b	mGlu ₁ IC ₅₀ (nM) ^b	% Glu Max (± SEM) ^{b,c}	mGlu ₅ pIC ₅₀ (± SEM) ^b	mGlu ₅ IC ₅₀ (nM) ^b	% Glu Max (± SEM) ^{b,c}
20	I	1	2.77	5.57 ± 0.13	2,720	-0.5 ± 0.8	< 4.5	> 30,000	—
21	I	2	3.19	6.65 ± 0.07	225	2.0 ± 0.3	< 5.0 ^d	> 10,000 ^d	20.0 ± 14.1
22	I	3	3.60	7.03 ± 0.09	94	1.9 ± 0.1	5.48 ^e	3,290 ^e	1.3 ^e
23	II	—	3.19	6.61 ± 0.05	247	2.1 ± 0.2	5.57 ± 0.13	2,700	17.2 ± 10.6
24	III	—	3.23	7.08 ± 0.05	84	2.9 ± 0.6	5.76 ± 0.41	1,750	3.4 ± 1.3
25	IV	—	3.05	6.83 ± 0.04	148	2.6 ± 0.5	5.63 ± 0.25	2,360	4.8 ± 5.3
26	V	—	1.74	6.32 ± 0.04	484	2.4 ± 0.3	< 4.5	> 30,000	—

^aCalculated using ChemDraw Professional, version 15.0 (PerkinElmer Informatics, Inc.)

^bCalcium mobilization assay; values are average of n = 3

^cAmplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of n = 3

^dCRC does not plateau

^eAverage of n = 2

Table 3
4-Position SAR for substituted cyclohexyl amines

The figure shows a general chemical template for a 4-position substituted cyclohexyl amine. The template consists of a bicyclic system (a benzene ring fused to a pyridine ring) with an amine group (-NH-) at the 4-position. The amine group is connected to a cyclohexyl ring. The cyclohexyl ring is substituted with various R groups, labeled I through VII. The R groups are defined as follows: I is a cyclohexyl ring with a fluorine atom at the 1-position; II is a cyclohexyl ring with a methyl group at the 1-position; III is a cyclohexyl ring with a methyl group at the 1-position and a methyl group at the 2-position; IV is a cyclohexyl ring with a methyl group at the 1-position and a methyl group at the 2-position, with a different stereochemistry than III; V is a cyclohexyl ring with a methyl group at the 1-position and a methyl group at the 2-position, with a different stereochemistry than IV; VI is a cyclohexyl ring with a methyl group at the 1-position and a methyl group at the 2-position, with a different stereochemistry than V; VII is a cyclohexyl ring with a methyl group at the 1-position and a methyl group at the 2-position, with a different stereochemistry than VI.

Cpd	R	cLogP ^a	mGlu ₁ pIC ₅₀ (± SEM) ^b	mGlu ₁ IC ₅₀ (nM) ^b	% Glu Max (± SEM) ^{b,c}	mGlu ₅ pIC ₅₀ (± SEM) ^b	mGlu ₅ IC ₅₀ (nM) ^b	% Glu Max (± SEM) ^{b,c}
27	I	2.33	< 5.0 ^d	> 10,000 ^d	39.0 ± 7.7	< 4.5	> 30,000	—
28	II	3.10	7.03 ± 0.08	93	2.0 ± 0.2	5.64 ± 0.22	2,270	2.0 ± 0.5
29	III	3.57	7.16 ± 0.08	69	2.9 ± 0.2	6.89 ± 0.29	129	1.6 ± 0.5
30	IV	2.58	6.63 ± 0.05	232	2.5 ± 0.6	< 5.0 ^d	> 10,000 ^d	9.9 ± 9.2
31	V	3.71	6.82 ± 0.03	152	2.7 ± 0.2	< 5.0 ^d	> 10,000 ^d	9.0 ± 8.3
32	VI	3.71	6.63 ± 0.12	233	3.1 ± 0.4	5.68 ^e	2,090 ^e	2.3 ^e
33	VII	3.92	5.79 ± 0.04	1,610	7.1 ± 2.9	< 4.5	> 30,000	—

^aCalculated using ChemDraw Professional, version 15.0 (PerkinElmer Informatics, Inc.)

^bCalcium mobilization assay; values are average of n = 3

^cAmplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of n = 3

^dCRC does not plateau

^eAverage of n = 2

Table 4
2-Position SAR

Cpd	Series	R	cLogP ^a	mGlu ₁ pIC ₅₀ (± SEM) ^b	mGlu ₁ IC ₅₀ (nM) ^b	% Glu Max (± SEM) ^{b,c}	mGlu ₅ pIC ₅₀ (± SEM) ^b	mGlu ₅ IC ₅₀ (nM) ^b	% Glu Max (± SEM) ^{b,c}
34	I	CH ₃	4.01	5.75 ± 0.09	1,800	2.6 ± 0.7	< 4.5	> 30,000	—
35	II	CH ₃	4.62	6.12 ± 0.04	759	2.4 ± 0.7	< 4.5	> 30,000	—
36	III	CH ₃	4.83	5.79 ± 0.04	1,610	7.1 ± 2.9	< 4.5	> 30,000	—
37	IV	CH ₃	4.14	5.67 ± 0.07	2,120	0.6 ± 0.8	< 4.5	> 30,000	—
41	I	NH ₂	2.81	5.25 ^e	5,600 ^e	0.58 ^e	< 4.5	> 30,000	—
42	I	NHCH ₃	3.11	< 4.5	> 30,000	—	< 4.5	> 30,000	—
43	I	N(CH ₃) ₂	3.90	< 4.5	> 30,000	—	< 4.5	> 30,000	—
44	I	NHCH(CH ₃) ₂	3.76	< 4.5	> 30,000	—	< 4.5	> 30,000	—
45	I	NHCH ₂ CH ₂ OCH ₃	2.95	< 4.5	> 30,000	—	< 4.5	> 30,000	—

^aCalculated using ChemDraw Professional, version 15.0 (PerkinElmer Informatics, Inc.)

^bCalcium mobilization assay; values are average of n = 3

^cAmplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of n = 3

^dCRC does not plateau

^eAverage of n = 2

Table 5Ancillary Pharmacology Profile for 10 μ M **29** (VU0467558)

Radioligand Binding Panel Significant Results ^a	
Target	Inhibition of Binding
adenosine A ₁	92%
adenosine A _{2A}	94%
adenosine A ₃	63%
serotonin 5-HT _{2B}	65%
norepinephrine transporter (NET)	65%
sodium channel, site 2	55%
Kinase Inhibition Profile Significant Results ^b	
Kinase	Inhibition of Kinase Activity
casein kinase 1 α 1 (CK1 α 1)	65.3%
casein kinase 1 δ (CK1 δ)	85.1%
casein kinase 1 ϵ (CK1 ϵ)	75.9%
casein kinase 1 γ 2 (CK1 γ 2)	50.4%
DAP kinase-related apoptosis-inducing protein kinase 1 (DRAK1)	52.9%
Serine-arginine protein kinase 1 (SRPK1)	79.4%
Serine-arginine protein kinase 2 (SRPK2)	75.2%
TRAF2 And NCK Interacting Kinase (TNIK)	58.8%

^a greater than or equal to 50% inhibition of binding at 10 μ M **29**^b greater than or equal to 50% inhibition of kinase activity at 10 μ M **29**

Table 6*In vitro* DMPK Profile for **29** (VU0467558)

Protein Binding (f_u) ^a		Predicted Hepatic Clearance (CL _{HEP}) ^b	
	plasma	brain	
rat	0.037	0.008	rat 63.6 mL/min/kg
mouse	0.025	0.010	mouse 84.5 mL/min/kg

P450 Inhibition IC ₅₀ (μM) ^c			
3A4	2D6	2C9	1A2
>30	>30	>30	9.6

^a f_u = fraction unbound; equilibrium dialysis assay^b calculated from measured intrinsic clearance in liver microsomes^c Assayed in pooled HLM in the presence of NADPH

Table 7Rodent PK Results for **29** (VU0467558)

Rat IV PK ^{a,b}		Tissue Distribution Studies		
half-life	34 min		rat ^b	mouse ^{c,d}
CL _p	98 mL/min.kg	time point	15 min	30 min
V _{ss}	3.1 mL/min/kg	K _p ^e	5.3	3.3
		K _{p,uu} ^f	1.1	1.3

Rodent IP PK Time Course Studies		
	rat ^{a,g}	mouse ^{c,d}
dose	10 mg/kg	30 mg/kg
plasma T _{max}	15 min	20 min
plasma C _{max}	2.30 μM	3.00 μM
plasma AUC	3.23 μM·h	2.86 μM·h

^a male Sprague-Dawley rats (n = 2 per time point)^b 0.2 mg/kg; vehicle = 10% ethanol, 40% PEG 400, 50% DMSO^c male CD-1 mice (n = 3 per time point)^d 30 mg/kg; vehicle = 10% Tween 80 in water^e K_p = total brain to total plasma ratio^f K_{p,uu} = unbound brain (brain *f*_u · total brain) to unbound plasma (plasma *f*_u · total plasma) ratio^g 10 mg/kg; vehicle = 10% Tween 80 in water