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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

Andrew S. Felts [a,b], Alice L. Rodriguez [a,b], Ryan D. Morrison [a,b], Daryl F. Venable [a,b], Anna L. Blobaum [a,b], Frank W. Byers [a,b], J. Scott Daniels [a,b], Colleen M. Niswender [a,b], Carrie K. Jones [a,b], P. Jeffrey Conn [a,b], Craig W. Lindsley [a,b,c], Kyle A. Emmitte [a,b,c,*]

[a] *Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University Medical Center, Nashville, TN 37232, USA*
[b] *Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, USA*
[c] *Department of Chemistry, Vanderbilt University, Nashville, TN 37232, USA*

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ABSTRACT

Selective negative allosteric modulators (NAMs) of each of the group I metabotropic glutamate receptors ($mGlu_1$ and $mGlu_5$) 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 mGlus. One compound, VU0467558 (**29**), demonstrated near equipotent activity at both receptors, selectivity versus other mGlus, a favorable ancillary pharmacology profile, and CNS exposure in rodents.

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 (mGlus). The mGlus 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 mGlus ($mGlu_1$ and $mGlu_5$) 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 (Ca^{2+}) and activation of protein kinase C (PKC). Conversely, both the group II ($mGlu_2$ and $mGlu_3$) and group III ($mGlu_4$, $mGlu_6$, $mGlu_7$, and $mGlu_8$) mGlus 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 mGlus.[4–8] Among the most advanced areas within this field are small molecule negative allosteric modulators (NAMs) of $mGlu_1$[9,10] and $mGlu_5$.[11–15] In fact, at least one $mGlu_1$ NAM, a 1,4-diaryl-5-methyl-1,2,3-triazole developed by researchers at Merck-Banyu, reached clinical candidate status.[16,17] The $mGlu_5$ 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 $mGlu_1$ and $mGlu_5$ 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 mGlus. Numerous preclinical studies with

* Corresponding author at present address: Dept. of Pharmaceutical Sciences, UNT System College of Pharmacy, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76017, USA. Tel.: +1 817 735 0241; fax: +1 817 735 2603.
E-mail address: kyle.emmitte@unthsc.edu (K.A. Emmitte).

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selective $mGlu_1$ and $mGlu_5$ NAM tools demonstrate overlap in potential therapeutic applications. For example, accounts of efficacy in preclinical models of anxiety,[23–28] addiction,[29–45] and pain[46–50] 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 $mGlu_1$ NAMs,[9] and concerns regarding the psychotomimetic effects of certain $mGlu_5$ 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 $mGlu_5$ 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 $mGlu_1$ and $mGlu_5$ while demonstrating selectivity versus other members of the mGlu family. The $mGlu_1$ NAM co-activity in **1** was not entirely unexpected as Lilly had previously identified and characterized a selective $mGlu_1$ NAM tool, LY456236 (**2**) from the same chemotype.[53] Furthermore, more highly optimized $mGlu_1$ NAMs from quinazoline-like scaffolds had also been previously reported, including compound **3** from Pfizer[48] 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 $mGlu_5$ NAMs[52] led us to believe that secondary amines would be required for activity at that target. Evaluation of the Pfizer series of $mGlu_1$ NAMs[48] indicated that saturated lipophilic groups would be favorable for $mGlu_1$ activity. We were keen to incorporate saturated groups at this position as increased sp^3 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.

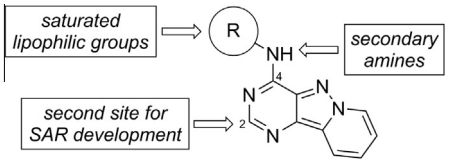


Figure 2. Proposal for development of novel $mGlu_{1/5}$ NAMs from a pyrido[1′,2′:1,5]pyrazolo[4,3-d]pyrimidin-4-amine scaffold.

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** (R^1 = H), **7** was heated via microwave irradiation in formamide. Synthesis of intermediate **9** (R^1 = CH_3) 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

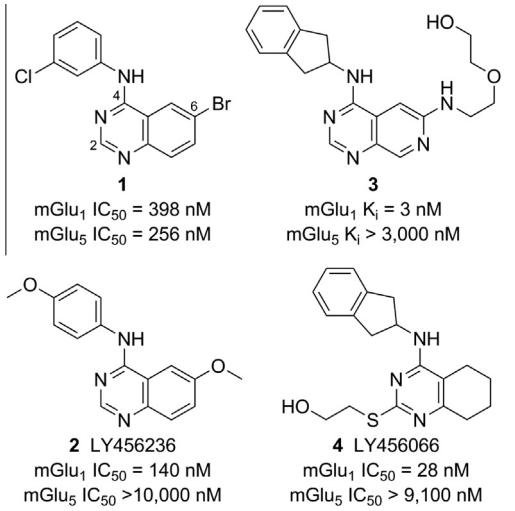
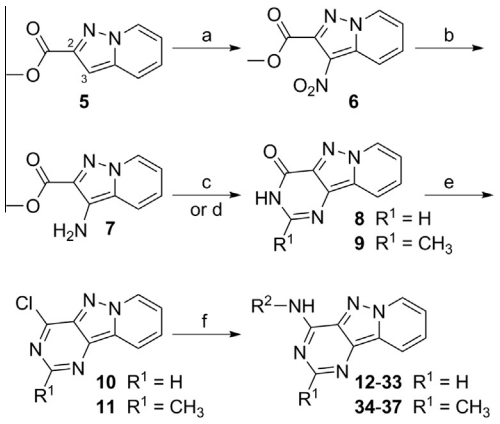


Figure 1. Quinazoline and quinazoline-like group I mGlu NAMs.



Scheme 1. Reagents and conditions: (a) KNO_3, H_2SO_4, 0 °C to rt, 79%; (b) $SnCl_2$, concd 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%.