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Development of a novel, CNS-penetrant, metabotropic glutamate receptor 3 (mGlu₃) NAM probe (ML289) derived from a closely related mGlu₅ PAM

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Abstract

Herein we report the discovery and SAR of a novel metabotropic glutamate receptor 3 (mGlu₃) NAM probe (ML289) with 15-fold selectivity versus mGlu₂. The mGlu₃ NAM was discovered via a ‘molecular switch’ from a closely related, potent mGlu₅ positive allosteric modulator (PAM), VU0092273. This NAM (VU0463597, ML289) displays an IC₅₀ value of 0.66 μM and is inactive against mGlu₅. 2012

Keywords

metabotropic glutamate receptor 3; mGlu₃; molecular switch; NAM

The metabotropic glutamate receptors (mGlu_s) are members of the GPCR family C, characterized by a large extracellular amino-terminal agonist (venus fly-trap) binding domain.^{1,2} Eight mGlu_s have been cloned, sequenced and assigned to three groups (Group I: mGlu₁ and mGlu₅; Group II: mGlu₂ and mGlu₃; Group III: mGlu^{4,6,7,8}) based on their sequence homology, pharmacology, and coupling to effector mechanisms.^{1,2} Highly subtype-selective allosteric ligands (both PAMs, positive allosteric modulators, and/or NAMs, negative allosteric modulators) have been developed for mGlu₁, mGlu₂, mGlu₄,

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mGlu₅ and mGlu₇.³⁻¹¹ However, aside from mGlu₂ PAMs, most Group II ligands do not discriminate between mGlu₂ and mGlu₃; a necessary requirement as these two receptors have highly divergent expression and function.¹²⁻¹⁵ Thus, due to a lack of selective small molecule probes, it has been difficult to discern distinct pharmacological roles for mGlu₃, though numerous studies suggest mGlu₃ is involved in glial-neuronal communication and may have therapeutic potential for the treatment of schizophrenia, Alzheimer's disease, and depression.^{3-5,12,16-18}

To date, only two mGlu₃ NAMs have been reported (Fig. 1).^{19,20} The first, reported by Addex, is RO4491533 (**1**), a dual mGlu₂/mGlu₃ NAM (mGlu₂ IC₅₀ = 296 nM, mGlu₃ IC₅₀ = 270 nM) based on a benzodiazepinone nucleus that was efficacious in preclinical cognition and depression models.¹⁹ At about the same time, Lilly disclosed LY2389575 (**2**) as a selective mGlu₃ NAM;²⁰ however, when measuring native coupling of these receptors to G protein-coupled inwardly-rectifying potassium (GIRK) channels via thallium flux,²¹ we have observed that **2** is only ~4-fold selective for mGlu₃ over mGlu₂ (mGlu₂ IC₅₀ = 17 μM, mGlu₃ IC₅₀ = 4.2 μM)²². Thus, there is a critical need for potent and selective mGlu₃ ligands.

In the absence of an HTS campaign to identify novel mGlu₃ NAMs, we elected to take advantage of the propensity of certain mGlu₅ PAM chemotypes to easily modulate the mode of pharmacology or mGlu subtype selectivity with subtle structural alterations, ie. 'molecular switches'.²³⁻²⁷ One such chemotype that we^{26,27} and others²⁸ have reported on with a high propensity for displaying 'molecular switches' is represented by VU0092273 (**3**), a potent MPEP-site mGlu₅ PAM (Fig. 2).²⁷ Compound **3** also possessed weak mGlu₃ NAM activity (IC₅₀ ~ 10 μM, inhibits EC₈₀ by 72%, Fig. 2B), but otherwise showed no activity at the six other mGlu subtypes.

Thus, **3** became our lead compound from which to develop a potent and selective mGlu₃ NAM. As we have previously reported, due to the steep nature of allosteric modulator SAR (especially in series prone to 'molecular switches'), we pursued an iterative parallel synthesis approach for the chemical optimization of **3**.^{3,4} Previous work in this scaffold indicated that mGlu₅ PAM activity could be greatly diminished with substitution other than fluorine on the distal aryl ring, as well as with modifications to the amide moiety.²⁶ Therefore, our first generation library design (Fig. 3) initially held the 4-hydroxypiperidine amide constant, while surveying a diverse array of functionalized aryl/heteroaryl rings as well as other aliphatic groups. Once mGlu₃-preferring modifications were identified, these would be maintained while an amide scan would be performed to improve mGlu₃ NAM activity while eliminating mGlu₅ PAM activity.

Our first 48-member library was prepared as shown in Scheme 1, and purified, to >98% purity, by reverse phase chromatography.²⁹ Commercial 4-iodobenzoic acid **4** was coupled to 4-hydroxypiperidine, under standard EDC/HOBt conditions, to provide amide **5** in 95% yield. Once synthesized, **5** then underwent Sonogashira coupling reactions with a diverse array of 48 functionalized terminal acetylenes to provide analogs **6**.³⁰ True to allosteric modulator SAR, 47/48 of the analogs were either inactive on mGlu₃ (IC₅₀ >10 μM) or only afforded modest inhibition (5-50% Glu Min) of the glutamate EC₈₀. Only one compound, **7** (VU0457299), possessing a 4-methoxyphenyl moiety, displayed mGlu₃ NAM potency below 10 μM (mGlu₃ IC₅₀ = 3.8 μM, % Glu Min = 10.4±2.1). Interestingly, the regioisomeric 2-OMe and 3-OMe congeners were inactive.

Based on these data, the next round of library synthesis held the 4-methoxyphenyl moiety in **7** constant, and 48 amines³² were employed to survey alternative amides. This library, prepared according to Scheme 2, was far more productive, providing several analogs **10** with

mGlu₃ NAM potencies below 10 μM; however, SAR was still quite steep (Table 1). In general, polar (**10a-e**) and basic substituents (**10f** and **10g**) were the most efficacious. Of great interest was the enantioselective mGlu₃ inhibition displayed by the (*S*)-piperidine carboxylic acid **10c** (IC₅₀ = 5.7 μM) and the (*R*)-enantiomer **10d** (IC₅₀ >>10 μM, essentially inactive). This result led us to resolve racemic 3-hydroxymethyl analog **10e** (IC₅₀ = 2.1 μM), which afforded a full block of the EC₈₀. Following Scheme 2, both the (*R*)- and (*S*)-enantiomers of **10e**, **11** (VU0463597) and **12** (VU0463593) were prepared and assayed in the mGlu₃ GIRK assay (Fig. 4). Here, **11** (pIC₅₀ = 5.83±0.05, IC₅₀ = 1.5 μM) was 2-fold more potent than **12** (pIC₅₀ = 5.49±0.02, IC₅₀ = 3.3 μM), but both afforded full blockade (% Glu Mins of -4.4±1.9 and -1.7±1.3 respectively). Efforts now shifted towards more fully characterizing **11** (VU0463597).

We next evaluated the selectivity of **11**(VU0463597) between mGlu₂ and mGlu₅. Utilizing our mGlu₂ GIRK line, the IC₅₀ was much greater than 10 μM, with the CRC not reaching baseline at the highest concentration tested (30 μM) (Fig. 5A, triangles). Similarly, **11** was inactive for potentiating an EC₂₀ concentration of glutamate (Fig. 5B, triangles) or inhibiting an EC₈₀ concentration of glutamate (Fig. 5C, triangles) in our standard mGlu₅ calcium assay. As our calcium assays typically drive our mGlu drug discovery programs, we also evaluated **11** (VU0463597) in an mGlu₃ calcium assay in which mGlu₃ is co-expressed with the promiscuous G protein G_{α15} (Fig. 5B-C, squares). Here, we see slightly improved mGlu₃ NAM potency (pIC₅₀ = 6.18±0.03, IC₅₀ = 0.66 μM, % Glu Min = 2.1±0.3) compared to the mGlu₃/GIRK line. To verify that **11** antagonizes mGlu₃ via a non-competitive (allosteric) mechanism of action, we next performed a Schild analysis. In these studies, **11** dose-dependently induced a rightward shift and decreased the maximal efficacy of the orthosteric agonist glutamate (Fig. 6), consistent with a non-competitive (allosteric) mechanism of action. Thus, starting from a very potent mGlu₅ PAM (EC₅₀ = 0.27 μM), we were able to optimize and develop a potent and selective mGlu₃ NAM with high selectivity (~15-fold) versus mGlu₂ and complete specificity versus mGlu₅.

With this potent and selective mGlu₃ NAM in hand, we began profiling **11** in a battery of ancillary pharmacology and DMPK assays to assess the quality of this probe for potential *in vivo* studies. A Lead Profiling Screen at Ricerca³² (68 GPCRs, ion channels and transporters screened at 10 μM in radioligand binding assays) failed to identify any off target activities for **11** (no inhibition >25% @ 10 μM). In our tier 1 *in vitro* DMPK screen, compound **11** displayed no P450 inhibition in human liver microsomes (IC₅₀ >30 μM vs. 3A4, 2C9, 2D6 and 1A2), high plasma protein binding with fraction unbound (f_u) levels between 1 and 2% in both rat and human plasma, respectively; f_u determined in rat brain homogenate was 1%. Intrinsic clearance (CL_{int}) determined in rat and human liver microsomes indicated that compound **11** was rapidly cleared *in vitro* (rat, CL_{int} = 240 mL/min/kg; human, CL_{int} = 571.8 mL/min/kg). An *in vitro* to *in vivo* clearance correlation was established, as compound **11** was found to be a moderately cleared compound in rat (CL = 33 mL/min/kg) following intravenous administration (1 mg/kg); the low volume of distribution at steady state (V_{ss} 0.6 L/kg) and moderate clearance produced a relatively short t_{1/2} (16.8 min) *in vivo*. Metabolite ID studies in rat and human liver microsomes (Fig. 7) indicated that the principle biotransformation pathway was P450-mediated *O*-demethylation of **11** to generate the phenol **13**, a metabolite that was subsequently shown to be inactive at mGlu₃ and mGlu₅.

As our earlier SAR work indicated that the methyl ether was critical for mGlu₃ NAM activity, we performed an IP plasma:brain level (PBL) study to determine if we could achieve meaningful CNS exposure if first-pass metabolism was bypassed. Significantly, in a 10 mg/kg (10% Tween80 in 0.5% methylcellulose) IP plasma:brain level (PBL) study, we observed a brain (16.3 μM):plasma (9.7 μM) ratio of 1.67, indicating that **11** (VU0463597) was indeed centrally penetrant. Based on brain homogenate binding studies, this correlates

to ~163 nM free in rat brain at the 10 mg/kg dose, a value below the mGlu₃ IC₅₀ (0.66 μM); thus, in order to provide adequate target engagement, a 50 mg/kg dose may be required for *in vivo* efficacy with this first generation mGlu₃ NAM probe.

This project was an MLPCN Medicinal Chemistry FastTrack program, and based on the profile of **11**, it was declared an MLPCN probe and assigned the identifier ML289.³³ As such, ML289 is freely available upon request.³⁴

In summary, we have developed a potent, selective (>15-fold vs. mGlu₂) and centrally penetrant mGlu₃ NAM **11** (VU0463597 or ML289) with a good overall CYP profile. ML289 is also highly selective versus mGlu₅, which is notable as our lead was a 0.27 μM mGlu₅ PAM, and suggests ligand cross-talk between allosteric binding sites on mGlu₃ and mGlu₅. Once again, a subtle 'molecular switch', in the form of a *p*-OMe moiety, conferred selective mGlu₃ inhibition over mGlu₅ potentiation. Further chemical optimization efforts, as well as detailed molecular pharmacological characterization of ML289, are in progress and will be reported in due course.

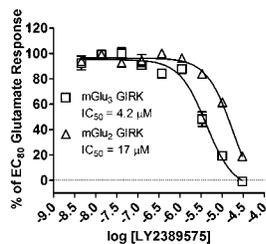
Acknowledgments

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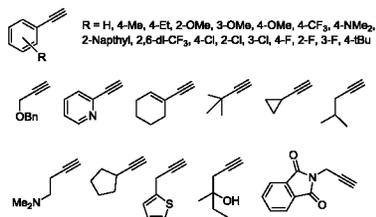
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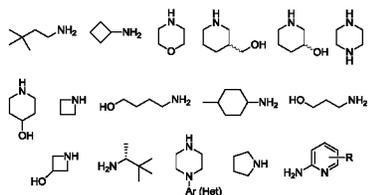
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30. Representative acetylenes employed in the 48-member library:



31. Representative amines employed in the 48-member library:



32. For full information on the targets in the Lead Profiling Screen at Ricerca, please see:

www.ricerca.com

33. For information on the MLPCN please see: <http://mli.nih.gov/mli/mlpcn/>

34. To request your free sample of ML289, please craig.lindsley@vanderbilt.edu

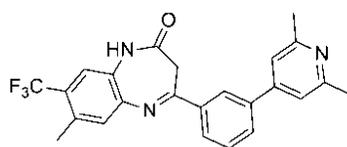
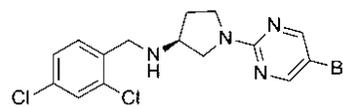
**1.** RO4491533mGlu₂ IC₅₀ = 296 nMmGlu₃ IC₅₀ = 270 nM**2.** LY2389575LY mGlu₂ IC₅₀ >12.5 μMLY mGlu₃ IC₅₀ = 190 nMVU mGlu₂ IC₅₀ = 17 μMVU mGlu₃ IC₅₀ = 4.2 μM

Figure 1. Structures of mGlu₃ NAMs RO4491533 (**1**) and LY2389575 (**2**), both dual mGlu₂/mGlu₃ NAMs.

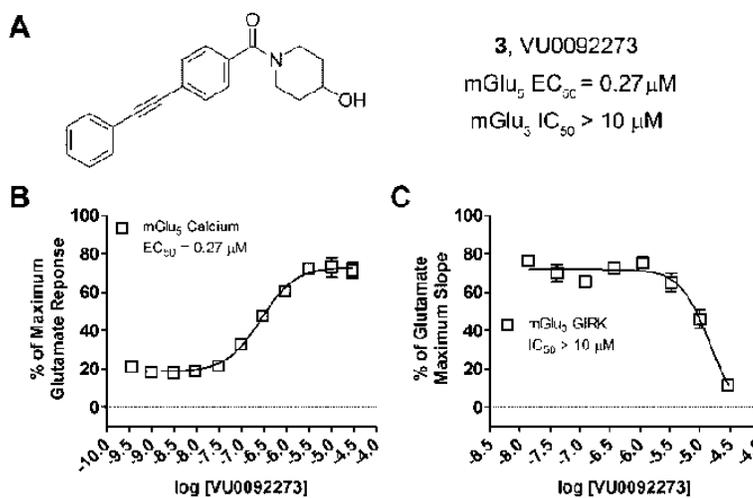


Figure 2.

A) Structure of VU0092273 (**3**), a potent mGlu₅ PAM (pEC₅₀ = 6.57±0.09, EC₅₀ = 0.27 μM). B) mGlu₅ PAM concentration-response curve (CRC) in presence of an EC₂₀ of glutamate. C) mGlu₃ antagonist CRC. **3** displayed weak NAM activity at mGlu₃ (IC₅₀ > 10 μM, inhibits EC₈₀ ~ 72%).

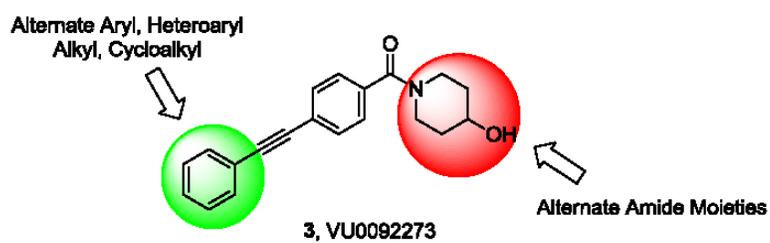


Figure 3. Library optimization strategy for VU0092273 (**3**) to improve mGlu₃ NAM activity while simultaneously eliminating mGlu₅ PAM activity.

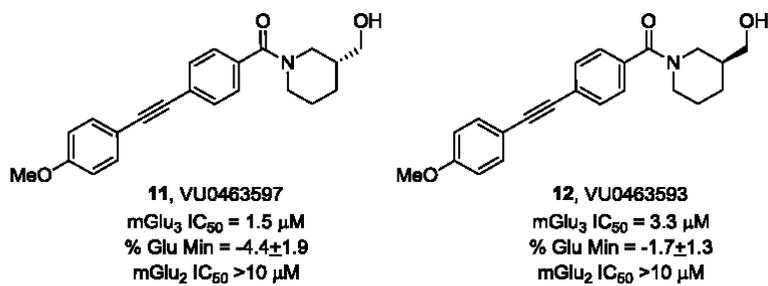


Figure 4.
Structures and activities of (*R*)-**11** and (*S*)-**12**, mGlu₃ NAMs.

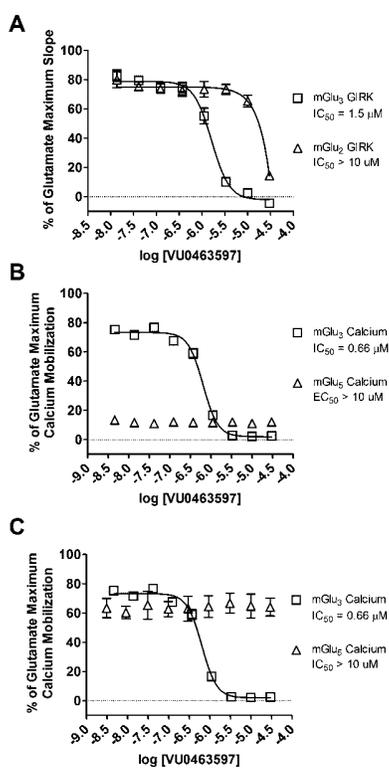


Figure 5. *In vitro* molecular pharmacology characterization of **11** (VU0463597). A) Concentration-response curves of mGlu₂ and mGlu₃ GIRK (antagonist mode). B) mGlu₃ calcium (antagonist mode) and mGlu₅ calcium (PAM mode). C) mGlu₃ calcium (antagonist mode) and mGlu₅ calcium (antagonist mode).

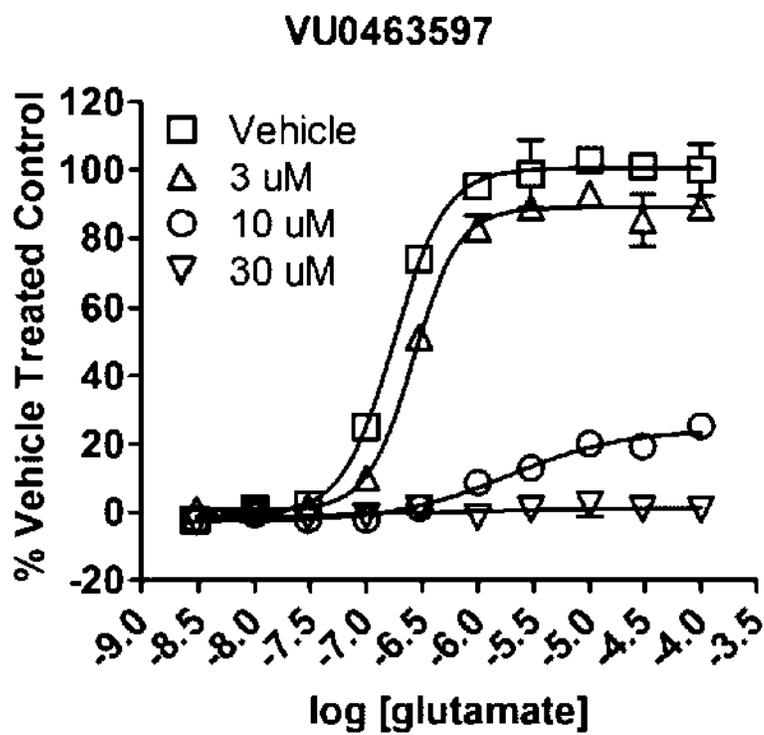


Figure 6. Schild Analysis of **11** (VU0463597). The concentration-response of glutamate for mGlu₃ GIRK is non-competitively inhibited by **11**.

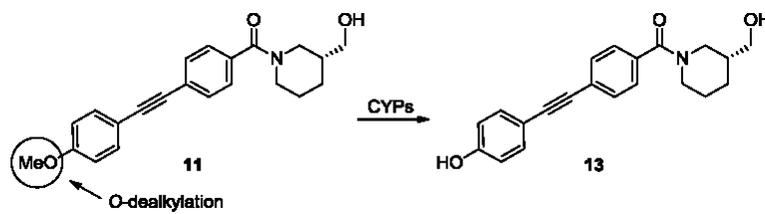
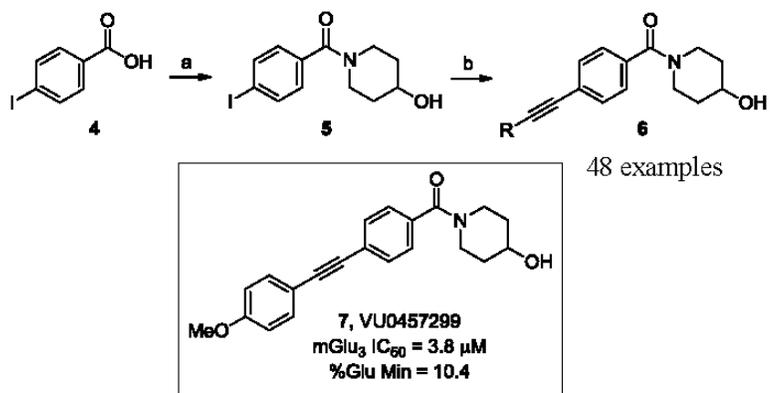
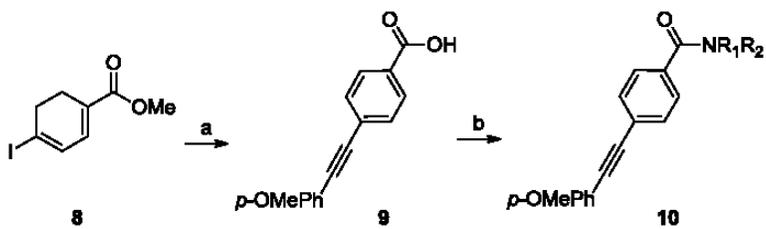


Figure 7.
Oxidative *O*-dealkylation of **11** in rat and human liver microsomes.

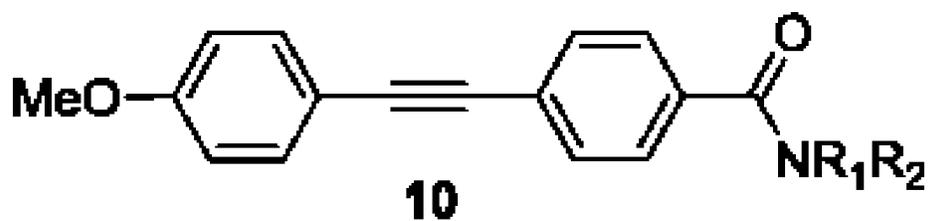
**Scheme 1.**

Reagents and conditions: (a) EDC, DMAP, DCM, DIPEA, 95%; (b) 20% CuI, 5% Pd(PPh₃)₄, 48 acetylenes (1.1 equiv.), DMF, DIEA, 60 °C, 1 h, 15-90%.

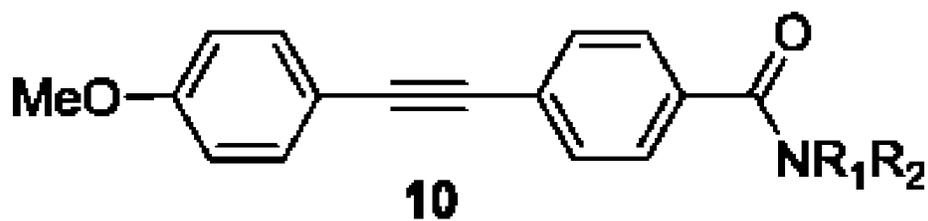
**Scheme 2.**

Reagents and conditions: (a) *i.* 20% CuI, 5% Pd(PPh₃)₄, 4-OMePh acetylene (1.1 equiv.), DMF, DIEA, 60 °C, 1 h, 82%, *ii*) KOH, aq. MeOH, 95%; (b) HNR₁R₂, EDC, DMAP, DCM, DIPEA, 40-96%.

Table 1

Structures and Activities of Analogs **10**.

Cmpd	NR ₁ R ₂	pIC ₅₀ ^a ±SEM	IC ₅₀ (μM) ^a	%Glu Min ^b ±SEM
7		5.42±0.04	3.8	10.4±2.1
10a		5.30±0.06	5.0	4.4±0.7
10b		5.61±0.07	2.5	-1.1±0.5
10c		5.24±0.01	5.7	2.2±1.1
10d			>10	16.4±2.5
10e		5.69±0.04	2.1	0.5±0.4
10f			>10	7.6±3.2



Cmpd	NR ₁ R ₂	pIC ₅₀ ^a ±SEM	IC ₅₀ (μM) ^a	%Glu Min ^b ±SEM
10g			>10	9.6±2.1

^aMeasured in an mGlu3 GIRK assay.

^b% Glu Min is the % inhibition of the compound on an EC80 concentration of glutamate. Values represent the mean ± standard error mean for three independent experiments performed in triplicate.