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Development of a novel, CNS-penetrant, metabotropic glutamate receptor 3 ($mGlu_3$) NAM probe (ML289) derived from a closely related $mGlu_5$ PAM

Douglas J. Sheffler [a,b,c,†], Cody J. Wenthur [a,b,†], Joshua A. Bruner [b,†], Sheridan J.S. Carrington [a,b], Paige N. Vinson [a,b], Kiran K. Gogi [a,b], Anna L. Blobaum [a,b,c], Ryan D. Morrison [a,b,c], Mitchell Vamos [e], Nicholas D.P. Cosford [e], Shaun R. Stauffer [a,b,d], J. Scott Daniels [a,b,c], Colleen M. Niswender [a,b], P. Jeffrey Conn [a,b,c], Craig W. Lindsley [a,b,c,d,*]

[a] *Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, USA*
[b] *Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University Medical Center, Nashville, TN 37232, USA*
[c] *Vanderbilt Specialized Chemistry Center for Probe Development (MLPCN), Nashville, TN 37232, USA*
[d] *Department of Chemistry, Vanderbilt University, Nashville, TN 37232, USA*
[e] *Apoptosis and Cell Death Research Program and Conrad Prebys Center for Chemical Genomics, Sanford-Burnham Medical Research Institute, 10901 N. Torrey Pines Rd., La Jolla, CA 92037, USA*

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ABSTRACT

Herein we report the discovery and SAR of a novel metabotropic glutamate receptor 3 ($mGlu_3$) NAM probe (ML289) with 15-fold selectivity versus $mGlu_2$. The $mGlu_3$ NAM was discovered via a 'molecular switch' from a closely related, potent $mGlu_5$ positive allosteric modulator (PAM), VU0092273. This NAM (VU0463597, ML289) displays an IC_{50} value of 0.66 μM and is inactive against $mGlu_5$.

The metabotropic glutamate receptors (mGlus) are members of the GPCR family C, characterized by a large extracellular amino-terminal agonist (venus fly-trap) binding domain.[1,2] Eight mGlus have been cloned, sequenced, and assigned to three groups (Group I: $mGlu_1$ and $mGlu_5$; Group II: $mGlu_2$ and $mGlu_3$; 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 $mGlu_1$, $mGlu_2$, $mGlu_4$, $mGlu_5$, and $mGlu_7$.[3–11] However, aside from $mGlu_2$ PAMs, most Group II ligands do not discriminate between $mGlu_2$ and $mGlu_3$; 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 $mGlu_3$, though numerous studies suggest $mGlu_3$ 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 $mGlu_3$ NAMs have been reported (Fig. 1).[19,20] The first, reported by Addex, is RO4491533 (**1**), a dual $mGlu_2$/$mGlu_3$ NAM ($mGlu_2$ IC_{50} = 296 nM, $mGlu_3$ IC_{50} = 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 $mGlu_3$ NAM;[20] however, when measuring native coupling of these receptors to G protein-coupled inwardly-rectifying potassium (GIRK) channels via

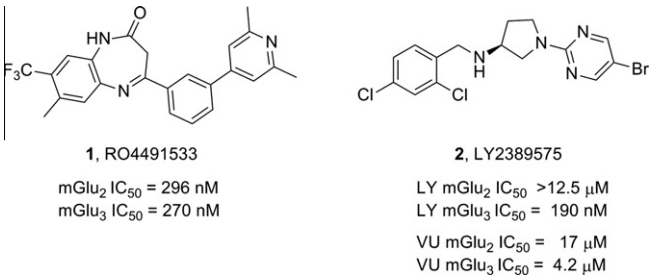

Figure 1. Structures of $mGlu_3$ NAMs RO4491533 (**1**) and LY2389575 (**2**), both dual $mGlu_2$/$mGlu_3$ NAMs.

* Corresponding author.
E-mail address: craig.lindsley@vanderbilt.edu (C.W. Lindsley).
† These authors contributed equally to this work.

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thallium flux,[21] we have observed that **2** is only ~4-fold selective for $mGlu_3$ over $mGlu_2$ ($mGlu_2$ IC_{50} = 17 μM, $mGlu_3$ IC_{50} = 4.2 μM).[22] Thus, there is a critical need for potent and selective $mGlu_3$ ligands.

In the absence of an HTS campaign to identify novel $mGlu_3$ NAMs, we elected to take advantage of the propensity of certain $mGlu_5$ PAM chemotypes to easily modulate the mode of pharmacology or mGlu subtype selectivity with subtle structural alterations, that is 'molecular switches'.[23–27] One such chemotype that we[26,27] and others[28] have reported on with a high propensity for displaying 'molecular switches' is represented by VU0092273 (**3**), a potent MPEP-site $mGlu_5$ PAM (Fig. 2).[27] Compound **3** also possessed weak $mGlu_3$ NAM activity (IC_{50} ~10 μM, inhibits EC_{80} by 72%, Fig. 2**B**), 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 $mGlu_3$ 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 $mGlu_5$ 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 $mGlu_3$-preferring modifications were identified, these would be maintained while an amide scan would be performed to improve $mGlu_3$ NAM activity while eliminating $mGlu_5$ 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-hydroxypiperdine, 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 $mGlu_3$ (IC_{50} >10 μM) or only afforded modest inhibition (5–50% Glu Min) of the glutamate EC_{80}. Only one compound, **7** (VU0457299), possessing a 4-methoxyphenyl moiety, displayed $mGlu_3$ NAM potency below 10 μM ($mGlu_3$ IC_{50} = 3.8 μM, % Glu Min = 10.4 ± 2.1).

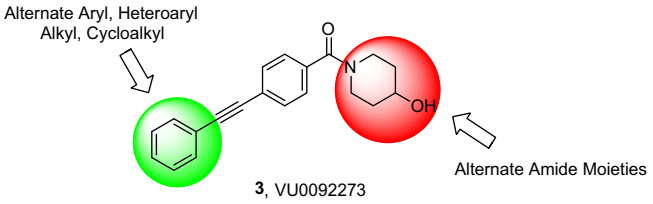
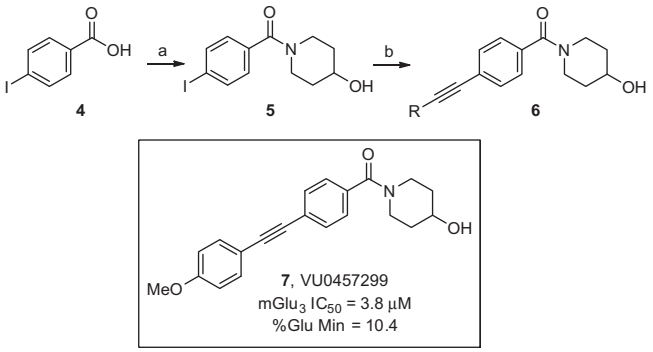


Figure 3. Library optimization strategy for VU0092273 (**3**) to improve $mGlu_3$ NAM activity while simultaneously eliminating $mGlu_5$ PAM activity.



Scheme 1. Reagents and conditions: (a) EDC, DMAP, DCM, DIPEA, 95%; (b) 20% CuI, 5% $Pd(PPh_3)_4$, 48 acetylenes (1.1 equiv), DMF, DIEA, 60 °C, 1 h, 15–90%.

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 amines[31] were employed to survey alternative amides. This library, prepared according to Scheme 2, was far more productive, providing several analogs **10** with $mGlu_3$ 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 $mGlu_3$ inhibition displayed by the (*S*)-piperidine carboxylic acid **10c** (IC_{50} = 5.7 μM) and the (*R*)-enantiomer **10d** (IC_{50} >>10 μM, essentially inactive). This result led us to resolve racemic 3-hydroxymethyl analog **10e**

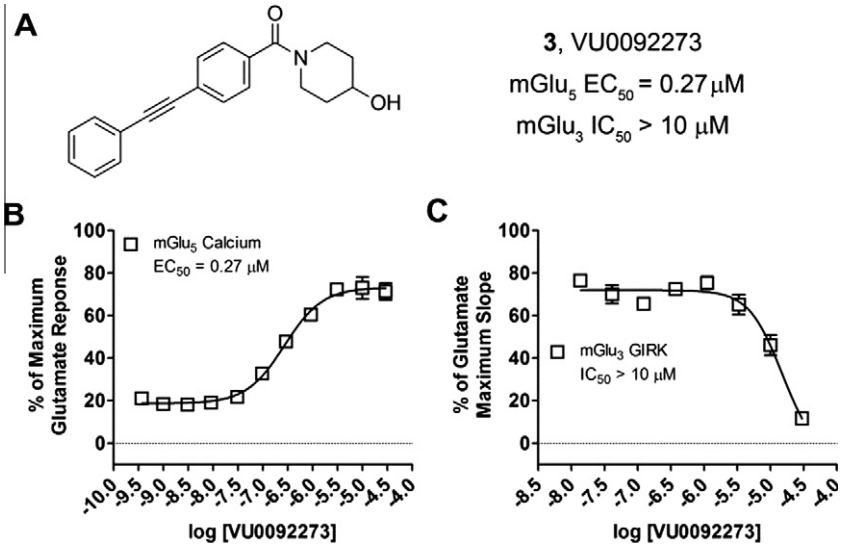
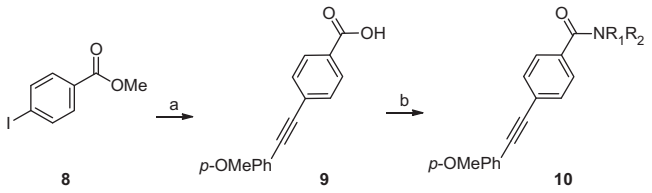
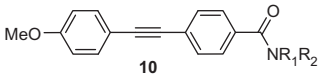


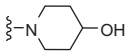
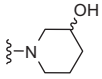
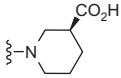
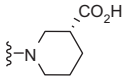
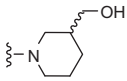
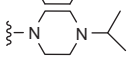
Figure 2. (A) Structure of VU0092273 (**3**), a potent $mGlu_5$ PAM (pEC_{50} = 6.57 ± 0.09, EC_{50} = 0.27 μM). (B) $mGlu_5$ PAM concentration–response curve (CRC) in presence of an EC_{20} of glutamate. (C) $mGlu_3$ antagonist CRC **3** displayed weak NAM activity at $mGlu_3$ (IC_{50} >10 μM, inhibits EC_{80} ~72%).



Scheme 2. Reagents and conditions: (a) (i) 20% CuI, 5% $Pd(PPh_3)_4$, 4-OMePh acetylene (1.1 equiv), DMF, DIEA, 60 °C, 1 h, 82%;(ii) KOH, aq MeOH, 95%; (b) HNR_1R_2, EDC, DMAP, DCM, DIPEA, 40–96%.

Table 1
Structures and activities of analogs **10**



Compd	NR_1R_2	pIC_{50} ± SEM	IC_{50}[a] (μM)	%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
10g			>10	9.6 ± 2.1

[a] Measured in an $mGlu_3$ GIRK assay.
[b] % Glu Min is the % inhibition of the compound on an EC_{80} concentration of glutamate. Values represent the mean ± standard error mean for three independent experiments performed in triplicate.

(IC_{50} = 2.1 μM), which afforded a full block of the EC_{80}. Following Scheme 2, both the (*R*)- and (*S*)-enantiomers of **10e**, **11** (VU0463597) and **12** (VU0463593) were prepared and assayed in the $mGlu_3$ GIRK assay (Fig. 4). Here, **11** (pIC_{50} = 5.83 ± 0.05, IC_{50} = 1.5 μM) was twofold more potent than **12** (pIC_{50} = 5.49 ± 0.02, IC_{50} = 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).

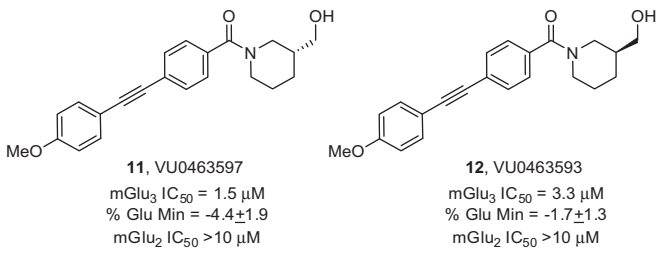


Figure 4. Structures and activities of (*R*)-**11** and (*S*)-**12**, $mGlu_3$ NAMs.

We next evaluated the selectivity of **11**(VU0463597) between $mGlu_2$ and $mGlu_5$. Utilizing our $mGlu_2$ GIRK line, the IC_{50} 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 EC_{20} concentration of glutamate (Fig. 5**B**, triangles) or inhibiting an EC_{80} concentration of glutamate (Fig. 5**C**, triangles) in our standard $mGlu_5$ calcium assay. As our calcium assays typically drive our mGlu drug discovery programs, we also evaluated **11** (VU0463597) in an $mGlu_3$ calcium assay in which $mGlu_3$ is co-expressed with the promiscuous G protein $G_{\alpha 15}$ (Fig. 5B and C, squares). Here, we see slightly improved $mGlu_3$ NAM potency (pIC_{50} = 6.18 ± 0.03, IC_{50} = 0.66 μM, % Glu Min = 2.1 ± 0.3) compared to the $mGlu_3$/GIRK line. To verify that **11** antagonizes $mGlu_3$ via a non-competitive (allosteric) mechanism of action, we next performed a Schild analysis. In these

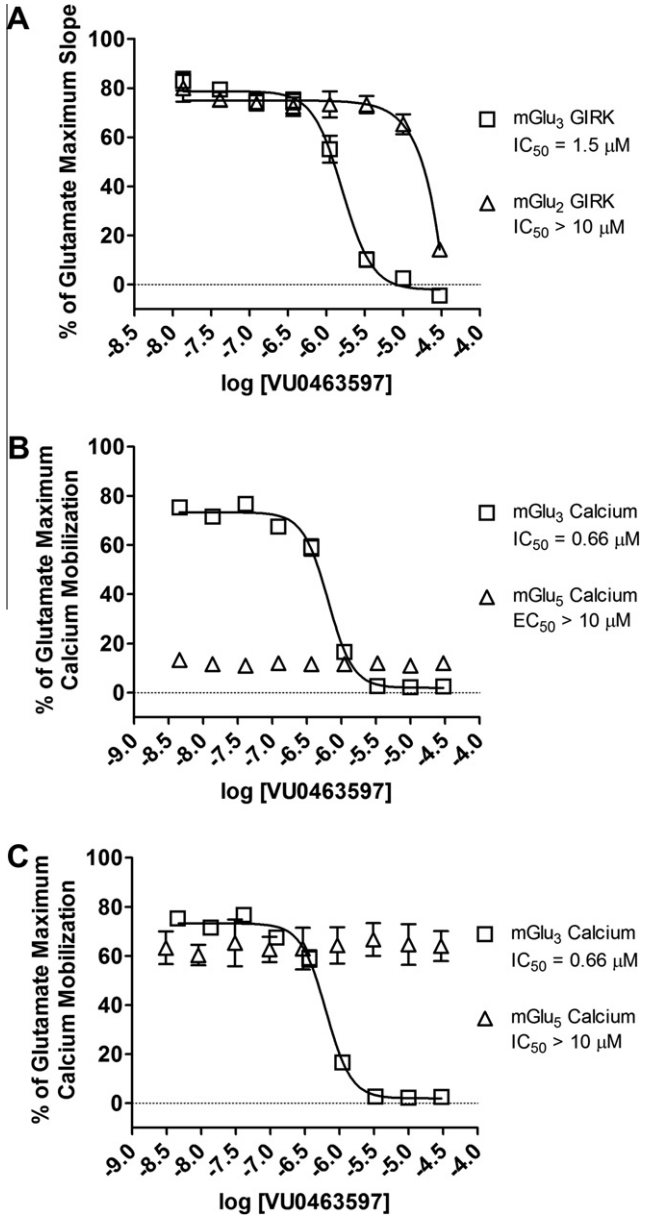


Figure 5. In vitro molecular pharmacology characterization of **11** (VU0463597). (A) Concentration–response curves of $mGlu_2$ and $mGlu_3$ GIRK (antagonist mode). (B) $mGlu_3$ calcium (antagonist mode) and $mGlu_5$ calcium (PAM mode). (C) $mGlu_3$ calcium (antagonist mode) and $mGlu_5$ calcium (antagonist mode).

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 $mGlu_5$ PAM (EC_{50} = 0.27 μM), we were able to optimize and develop a potent and selective $mGlu_3$ NAM with high selectivity (~15-fold) versus $mGlu_2$ and complete specificity versus $mGlu_5$.

With this potent and selective $mGlu_3$ 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 Ricerca[32] (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% at 10 μM). In our tier 1 in vitro DMPK screen, compound **11** displayed no P450 inhibition in human liver microsomes (IC_{50} >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 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 $mGlu_3$ and $mGlu_5$.

As our earlier SAR work indicated that the methyl ether was critical for $mGlu_3$ 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 $mGlu_3$ IC_{50} (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 $mGlu_3$ NAM probe.

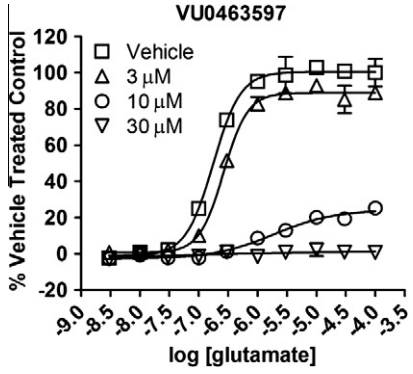


Figure 6. Schild analysis of **11** (VU0463597). The concentration–response of glutamate for $mGlu_3$ GIRK is non-competitively inhibited by **11**.

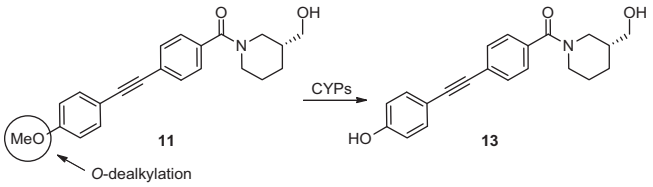


Figure 7. Oxidative O-dealkylation of **11** in rat and human liver microsomes.

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 $mGlu_2$) and centrally penetrant $mGlu_3$ NAM **11** (VU0463597 or ML289) with a good overall CYP profile. ML289 is also highly selective versus $mGlu_5$, which is notable as our lead was a 0.27 μM $mGlu_5$ PAM, and suggests ligand cross-talk between allosteric binding sites on $mGlu_3$ and $mGlu_5$. Once again, a subtle 'molecular switch', in the form of a *p*-OMe moiety, conferred selective $mGlu_3$ inhibition over $mGlu_5$ 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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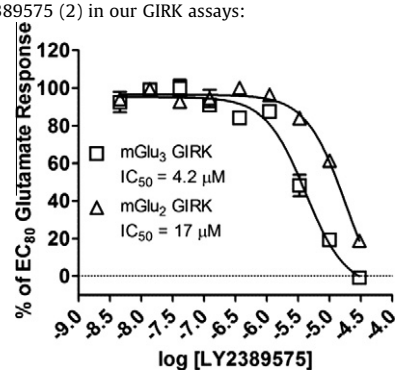
22. CRCs for LY2389575 (2) in our GIRK assays:



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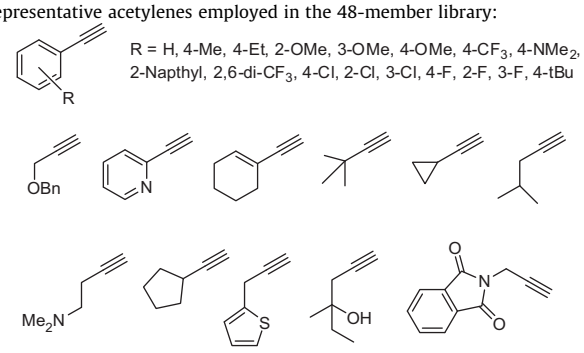
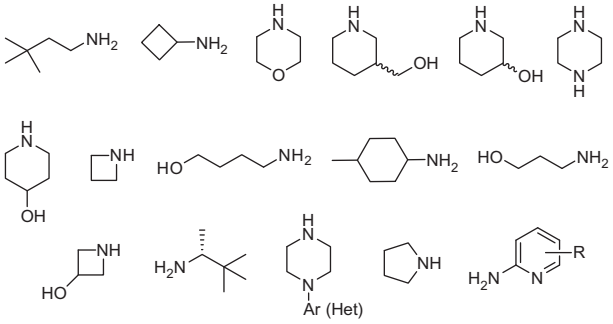
30. Representative acetylenes employed in the 48-member library:



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 email: craig.lindsley@vanderbilt.edu.