

Article

## Exploration of Allosteric Agonism Structure-Activity Relationships within an Acetylene Series of Metabotropic Glutamate Receptor 5 (mGlu5) Positive Allosteric Modulators (PAMs): discovery of 5-((3-fluorophenyl)ethynyl)-N-(3-methyloxetan-3-yl)picolinamide (ML254)

Mark Turlington, Meredith J. Noetzel, Aspen Celeste Chun, Ya Zhou, Rocco D. Gogliotti, Elizabeth D. Nguyen, Karen J. Gregory, Paige N. Vinson, Jerri M. Rook, Kiran K. Gogi, Zixiu Xiang, Thomas M. Bridges, J. Scott Daniels, Carrie K. Jones, Colleen M Niswender, Jens Meiler, P. Jeffrey Conn, Craig W Lindsley, and Shaun R. Stauffer

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2 **Exploration of Allosteric Agonism Structure-Activity Relationships within an Acetylene Series of Metabotropic**  
3 **Glutamate Receptor 5 (mGlu<sub>5</sub>) Positive Allosteric Modulators (PAMs): discovery of 5-((3-fluorophenyl)ethynyl)-**  
4 ***N*-(3-methyloxetan-3-yl)picolinamide (ML254)**  
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6 Mark Turlington<sup>a,b,c</sup>, Meredith J. Noetzel<sup>a,b</sup>, Aspen Chun<sup>a,b,c</sup>, Ya Zhou<sup>a,b,c</sup>, Rocco D. Gogliotti<sup>a,b,c</sup>, Elizabeth D. Nguyen<sup>f</sup>,  
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8 Scott Daniels<sup>a,b,c</sup>, Carrie Jones<sup>a,b,c</sup>, Colleen M. Niswender<sup>a,b,c</sup>, Jens Meiler<sup>a,d,f,g</sup>, P. Jeffrey Conn<sup>a,b,c</sup>, Craig W. Lindsley<sup>a,b,c,d</sup>,  
9 Shaun R. Stauffer<sup>a,b,c,d\*</sup>  
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13 <sup>a</sup>*Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, USA*

14 <sup>b</sup>*Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University Medical Center, Nashville, TN 37232, USA*

15 <sup>c</sup>*Vanderbilt Specialized Chemistry Center for Probe Development (MLPCN), Nashville, TN 37232, USA*

16 <sup>d</sup>*Department of Chemistry, Vanderbilt University, Nashville, TN 37232, USA*

17 <sup>e</sup>*Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC, 3052*

18 <sup>f</sup>*Center for Structural Biology, Vanderbilt University Medical Center, Nashville, TN 37232, USA*

19 <sup>g</sup>*Vanderbilt Institute for Chemical Biology, Vanderbilt University Medical Center, Nashville, TN 37232, USA*

20 *\*To whom correspondence should be addressed: shaun.stauffer@vanderbilt.edu*  
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29 **ABSTRACT:** Positive allosteric modulators (PAMs) of metabotropic glutamate receptor 5 (mGlu<sub>5</sub>) represent a  
30 promising therapeutic strategy for the treatment of schizophrenia. Both allosteric agonism and high glutamate fold-shift  
31 have been implicated in the neurotoxic profile of some mGlu<sub>5</sub> PAMs; however, these hypotheses remain to be adequately  
32 addressed. To develop tool compounds to probe these hypotheses, the structure-activity relationship of allosteric agonism  
33 was examined within an acetylenic series of mGlu<sub>5</sub> PAMs exhibiting allosteric agonism in addition to positive allosteric  
34 modulation (ago-PAMs). PAM **38t**, a low glutamate fold-shift allosteric ligand (maximum fold-shift ~3.0), was selected  
35 as a potent PAM with no agonism in the *in vitro* system used for compound characterization and in two native  
36 electrophysiological systems using rat hippocampal slices. PAM **38t** (ML254) will be useful to probe the relative  
37 contribution of cooperativity and allosteric agonism to the adverse effect liability and neurotoxicity associated with this  
38 class of mGlu<sub>5</sub> PAMs.  
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## 53 INTRODUCTION

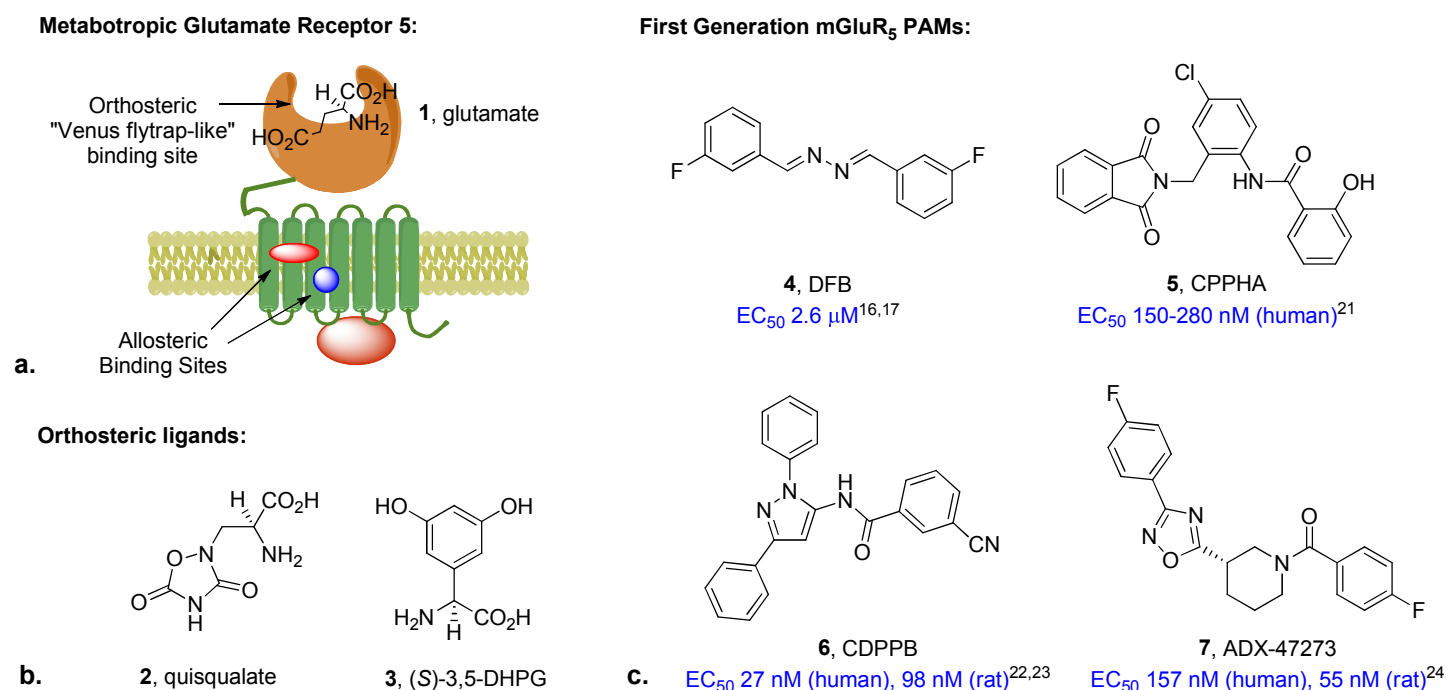
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55 A largely under-met medical need affecting approximately 1% of the world's population, schizophrenia is a  
56 complex mental illness characterized by three symptom clusters including positive symptoms (hallucinations, paranoia,  
57 disorganized behavior), negative symptoms (social withdrawal, anhedonia, flat affect), and cognitive dysfunction (deficits  
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1 in attention, learning, and memory).<sup>1-4</sup> Current treatments for schizophrenia were developed based on the dopaminergic  
2 hypothesis of schizophrenia which points to over-activation of subcortical dopamine D<sub>2</sub> receptors as a causative factor for  
3 the positive symptoms of the disease.<sup>5,6</sup> Accordingly, first-generation typical antipsychotics (e.g. haloperidol) act as D<sub>2</sub>  
4 antagonists and second-generation atypical antipsychotics (e.g. clozapine, risperidone) act as mixed D<sub>2</sub>/5-HT<sub>2A</sub>  
5 antagonists as well as having activity at other receptors.<sup>5,6</sup> Both classes are routinely used to treat the positive symptoms  
6 of schizophrenia and several statistical analyses have revealed that there is little evidence for improved efficacy of  
7 atypical over typical antipsychotics except in severe cases of schizophrenia.<sup>7,8</sup> However, the two classes are different in  
8 their side-effect profiles. While typical antipsychotics are plagued by extra-pyramidal side effects (movement disorders)  
9 atypical antipsychotics often offer improved side-effect profiles but are associated with significant weight gain.<sup>8</sup> In  
10 addition to the considerable adverse effect profiles, neither class of antipsychotics has a substantial impact on the negative  
11 and cognitive symptoms of the disorder, and 20% of patients are non-responsive to treatment.<sup>1</sup> These severe limitations  
12 highlight the need to develop new treatments for schizophrenia.  
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26 In addition to the dopaminergic pathways, disruptions in many neuronal circuits including the glutamatergic,  
27 GABAergic, and cholinergic pathways are observed in schizophrenic patients.<sup>3</sup> Importantly, abnormalities in  
28 glutamatergic circuits have been linked with all three symptom clusters of schizophrenia, fueling the development of the  
29 glutamate hypothesis of schizophrenia as a means to address all symptom clusters. Clinical observations have revealed  
30 that phenylcyclidine (PCP) and ketamine, antagonists of the ionotropic N-Methyl-D-aspartate (NMDA) glutamate  
31 receptor (NMDAR), produce schizophrenic-like symptoms.<sup>1-4, 9-13</sup> Furthermore, administration of high doses of the  
32 NMDA co-agonists glycine, *D*-serine, and *D*-cycloserine improves positive, negative, and cognitive symptoms in  
33 schizophrenic patients,<sup>1-2, 14-15</sup> generating excitement that enhancement of glutamatergic neurotransmission could be a  
34 novel strategy for the treatment of schizophrenic symptoms. While direct activation of NMDAR results in toxicity  
35 associated with ion channel overactivation,<sup>1-2</sup> the metabotropic glutamate receptor 5 (mGlu<sub>5</sub>) is closely associated with  
36 NMDAR function and may provide an indirect means to rescue NMDAR hypofunction with a lower propensity for  
37 toxicity. Numerous studies indicate that mGlu<sub>5</sub> plays a critical role in modulating neurotransmission in forebrain circuits  
38 implicated in NMDAR antagonist mediated psychotomimetic effects, and the ability to more subtly modulate NMDAR  
39 activity has been proposed to result in a potentially lower propensity for toxicity.<sup>1-2,4,13,16-17</sup>  
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56 mGlu<sub>5</sub> is a member of the Family C G protein-coupled receptors (GPCRs) that bear a large extracellular “Venus  
57 flytrap-like” domain that serves as the binding site for glutamate. The high sequence homology of the glutamate binding  
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sites among the eight known metabotropic glutamate receptor (mGlu) subtypes has made the development of subtype selective mGlu<sub>5</sub> orthosteric agonists difficult.<sup>2,4</sup> In contrast, spatially distinct allosteric binding sites that reside in the transmembrane domain of the receptor (Figure 1) possess less sequence conservation across the mGlu subtypes, making possible the discovery of mGlu<sub>5</sub> selective ligands. Allosteric ligands can activate the receptor directly (allosteric agonists) or can modulate the activity of the receptor in the presence of the endogenous ligand glutamate, either enhancing (positive allosteric modulators, PAMs) or diminishing (negative allosteric modulators, NAMs) receptor activity.<sup>18-20</sup> Allosteric modulators with no agonist activity may provide a more subtle and physiologically-relevant approach to restoring target function in comparison to orthosteric/allosteric agonists as receptor response only occurs in the presence of the endogenous agonist.<sup>18-20</sup> In addition, allosteric modulators often offer improvements in chemical tractability and improved properties for central nervous system (CNS) exposure over traditional orthosteric glutamate analogs, such as quisqualate and (*S*)-3,5-DHPG which have difficulty passing the blood-brain barrier.<sup>16,18-20</sup>

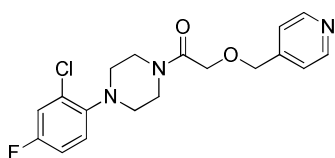


**Figure 1.** a. Representative mGlu<sub>5</sub> receptor (protomer of dimer) with orthosteric and allosteric binding sites. b. Orthosteric mGlu<sub>5</sub> ligands. c. 1<sup>st</sup> generation mGlu<sub>5</sub> PAMs.

The advantages of allosteric modulators have fueled the development of highly subtype selective and CNS penetrant mGlu<sub>5</sub> PAMs.<sup>16-17,21</sup> The earliest mGlu<sub>5</sub> PAMs are represented by the four chemotypes shown in Figure 1. 3,3'-difluorobenzaldazine (DFB), developed by Merck & Co. in 2003, represented the first subtype selective mGlu<sub>5</sub> PAM, and the ensuing discovery of *N*-(4-chloro-2-((1,3-dioxoisindolin-2-yl)methyl)phenyl)-2-hydroxybenzamide (CPPHA) in

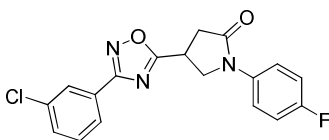
2004 provided a compound with improved potency and properties suitable for use in native preparations.<sup>21</sup> Shortly thereafter, the first highly useful *in vivo* tool compound, 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide (CDPPB) was discovered by Merck scientists,<sup>22,23</sup> and researchers at Addex Pharmaceuticals reported a chemically distinct mGlu<sub>5</sub> PAM, ADX-47273 (**7**), with suitable properties for *in vivo* studies.<sup>24</sup> Utilizing these compounds, studies in animal models have added evidence to the promise of mGlu<sub>5</sub> allosteric activation as a novel therapeutic strategy for the treatment of schizophrenia. PAM **6** has been shown to possess efficacy in animal models predictive of positive symptoms (amphetamine-induced hyperlocomotion (AHL), prepulse inhibition of acoustic startle reflex), cognitive deficits (behavioral and cognitive flexibility, Morris water maze (MWM)), and negative symptoms (sucrose preference).<sup>16,22-23,36</sup> Studies with **6** have revealed similar efficacy in reversal of positive symptoms (conditioned avoidance responding, apomorphine-induced climbing, AHL) and improvements in cognition (novel object recognition, five-choice serial reaction time test, MWM).<sup>16,24,36</sup> In the wake of these studies, numerous novel mGlu<sub>5</sub> PAM chemotypes have been discovered and optimized, leading to improvements in potency and physiochemical properties.<sup>16,17</sup> Representatives of major chemotypes are shown in Figure 2 and many of these compounds have demonstrated efficacy in antipsychotic and cognition models.<sup>27-36</sup> In addition, Lilly recently revealed mGlu<sub>5</sub> PAMs LSN2814617 (**10**) and LSN2463359 (**14**).<sup>28</sup> Both **10** and **14** are reported to shift a concentration-response curve for the group I orthosteric agonist, DHPG, in rat cortical neurons with relatively weak efficacies compared to historical mGlu<sub>5</sub> PAMs (**10**, FS = 2.8 at 3 μM; **14**, FS = 2.0 at 1 μM). *In vivo*, these compounds have been reported to exert significant effects in multiple models including: methylazoxymethanol (MAM) neurodevelopmental model of schizophrenia, wake-promoting properties in acute sleep-wake electroencephalogram (EEG), and reversal of NMDAR antagonist SDZ 220,581-induced disruptions in delayed-matching-to-position (DMTP).<sup>28</sup> Interestingly, these low efficacy PAMs do not substantially impact baseline performance on their own in DMTP, nor do they have significant effects in reversal of hyperlocomotion in the AHL model, which is in contrast to previously reported mGlu<sub>5</sub> PAMs that maintain higher efficacies (orthosteric agonist concentration-response curve EC<sub>50</sub> fold-shift greater than 3.0).<sup>17,23-25,30,33,36</sup>

## N-Aryl Piperazines



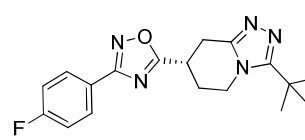
**8, CPPZ**  
EC<sub>50</sub> 550 nM (human)<sup>25</sup>

## N-Aryl Pyrrolidinones



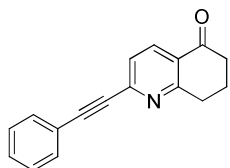
**9**  
EC<sub>50</sub> 11 nM (human)<sup>26,27</sup>

## Tetrahydrotriazolopyridines

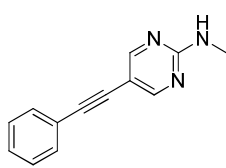


**10, LSN2814617**  
EC<sub>50</sub> 42 nM (rat), 52 nM (human)<sup>28</sup>

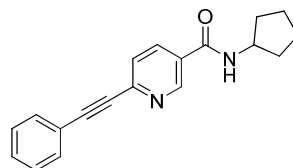
## Acetylenes



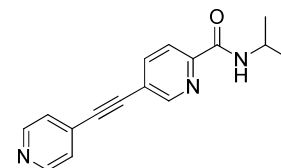
**11**  
EC<sub>50</sub> 34 nM (rat), 172 nM (human)<sup>29</sup>



**12, MPPA**  
EC<sub>50</sub> 14 nM (rat)<sup>30</sup>

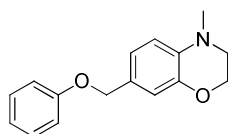


**13**  
EC<sub>50</sub> 30 nM (human)<sup>31</sup>

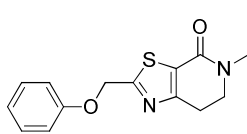


**14 LSN2463359**  
EC<sub>50</sub> 24 nM (rat), 33 nm (human)<sup>28</sup>

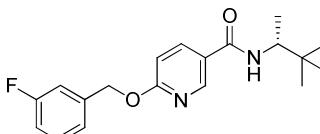
## Ethers



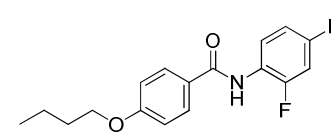
**15**  
EC<sub>50</sub> 50 nM (human)<sup>32</sup>



**16**  
EC<sub>50</sub> 1300 nM (rat)<sup>33</sup>



**17**  
EC<sub>50</sub> 2.2 nM (rat)<sup>34</sup>

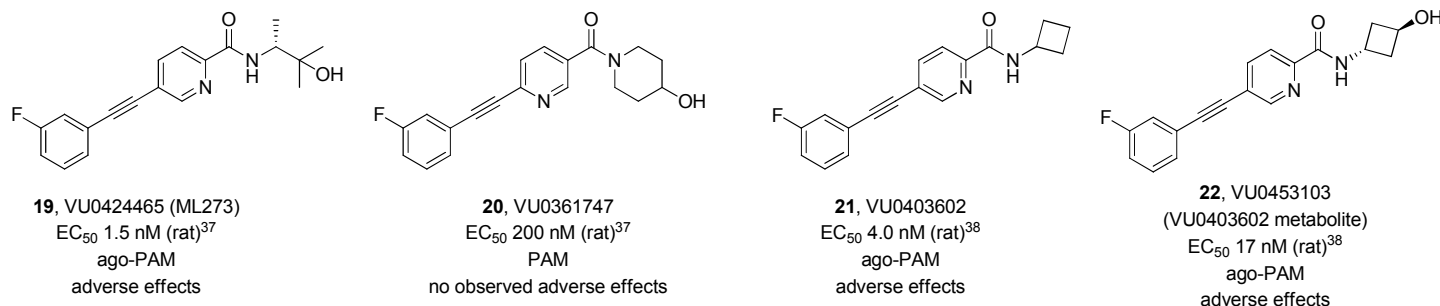


**18, VU0357121**  
EC<sub>50</sub> 33 nM (rat)<sup>35</sup>

**Figure 2.** mGlu<sub>5</sub> PAM chemotypes and reported PAM potencies.

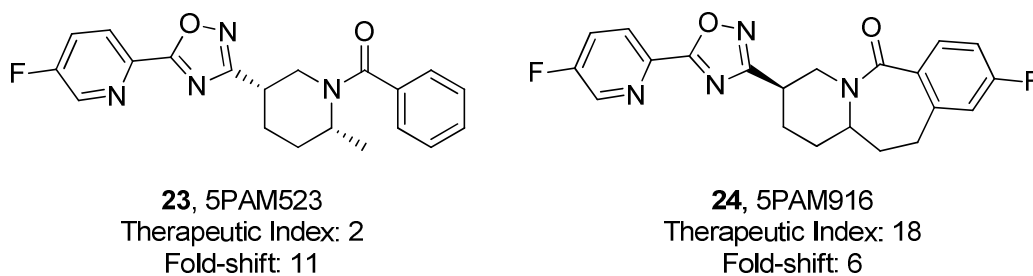
Despite the therapeutic promise of mGlu<sub>5</sub> PAMs, recent findings have begun to reveal a CNS adverse-effect (AE) liability for certain classes of mGlu<sub>5</sub> PAMs.<sup>37-39</sup> The seizure-inducing effects of group I mGlu *orthosteric* agonists is well known<sup>40,41</sup>; however, the potential for mechanism-related AEs associated with *allosteric* mGlu<sub>5</sub> receptor activation was not appreciated until recently. Studies in our laboratories have demonstrated that allosteric modulator **19** (VU0424465, ML273, Figure 3) causes seizure activity in rodents.<sup>37</sup> This effect can be blocked by treatment with 2-methyl-6-(2-phenylethynyl) pyridine (MPEP), an allosteric mGlu<sub>5</sub> NAM, pointing to the role of mGlu<sub>5</sub> in the observed neurotoxicity. Our recombinant mGlu<sub>5</sub> cell line with low receptor density is predicted to have a better correlation with the functional response observed in native systems and thus serves as a more definitive assay system to detect allosteric agonism.<sup>43</sup> Allosteric modulator, **19** displays both allosteric agonism and PAM activity (ago-PAM activity) in a mGlu<sub>5</sub> cell line with low receptor density, as well as in native systems such as astrocytes.<sup>37</sup> In contrast, nicotinamide acetylene allosteric modulator **20** (VU0361747, Figure 3) was devoid of agonism in these low-expressing mGlu<sub>5</sub> cells and did not display a seizure liability *in vivo*.<sup>37</sup> Thus, allosteric agonist activation of mGlu<sub>5</sub>, similar to orthosteric activation, can contribute to an AE liability *in vivo* and epileptogenesis in native systems. Further evidence in support of this hypothesis surfaced from profiling of **21** (VU0403602, Figure 3). Similarly to ago-PAM **19**, picolinamide acetylene **21** demonstrates agonist activity in a mGlu<sub>5</sub> low-expressing cell line and native systems and possesses a seizure liability in animal models.<sup>38</sup>

Further complicating the pharmacological effects of **21**, its principle *in vivo* metabolite **22** (VU0453103) also displays significant ago-PAM activity, suggesting that formation of active metabolites is possible and may contribute to an adverse effect profile *in vivo*. These findings highlight the need to avoid an ago-PAM pharmacological profile in both the parent compound and its major metabolites in the quest to develop allosteric ligands for mGlu<sub>5</sub> receptor activation.



**Figure 3.** Acetylene mGlu<sub>5</sub> PAMs used to assess seizure activity in animal models.<sup>37,38</sup>

Concurrent with these findings, a collaborative effort between Merck and Addex Pharmaceuticals revealed that mGlu<sub>5</sub> PAMs within their piperidine and caprolactam chemotypes induced significant convulsions and neuronal cell death in mice (Figure 4).<sup>39</sup> In contrast mGlu<sub>5</sub> knock-out mice that were administered the compounds did not display these adverse effects. These compounds did not possess ago-PAM activity in astrocytes suggesting allosteric agonism is not necessarily required to induce adverse effects; although the pharmacological profile of the major metabolites was not reported. While the most toxic compound reported 5PAM523 (**23**, Figure 4) had a therapeutic index of just 2-fold ( $C_{\max}$  minimum active dose amphetamine-induced hyperlocomotion versus  $C_{\max}$  minimum adverse effect dose), higher therapeutic indices were observed for the additional compounds reported. Of these, 5PAM916 (**24**) was found to have the highest therapeutic index (18-fold) and the lowest glutamate EC<sub>50</sub> fold-shift (6-fold at 10  $\mu$ M). These observations suggest that the efficacy to shift a glutamate concentration-response curve, or more precisely PAM cooperativity, may play an important role in neurotoxicity, such that improved safety margins may be realized by designing compounds possessing lower cooperativity.<sup>39</sup>



**Figure 4.** Merck-Addex compounds found to induce neurotoxicity.<sup>39</sup>

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These reports, along with the adverse effect liability of allosteric agonism observed in our laboratories, suggest that PAMs devoid of agonism in situations where mGlu<sub>5</sub> expression is low and possessing moderate to low efficacy may be preferable for developing compounds with optimal safety margins. To further investigate the safety implications of ago-PAM pharmacology we initiated medicinal chemistry efforts to explore the structural determinants of ago-PAM activity in the **19** scaffold.<sup>37</sup> We targeted the development of highly potent ( $\leq 10$  nM) tool compounds structurally similar to **19** that did not exhibit allosteric agonism in our *in vitro* assay systems. In addition, the glutamate fold-shift profile of promising pure PAMs was also investigated as an indicator of cooperativity. Herein we report a detailed structure-activity relationship (SAR) of allosteric agonism within this class of acetylene based mGlu<sub>5</sub> PAMs. This study led to the optimization and characterization of low cooperativity PAM acetylenes that possess *in vitro* pharmacological and pharmacokinetic profiles that will enable further studies to address ongoing questions concerning the therapeutic index of mGlu<sub>5</sub>.

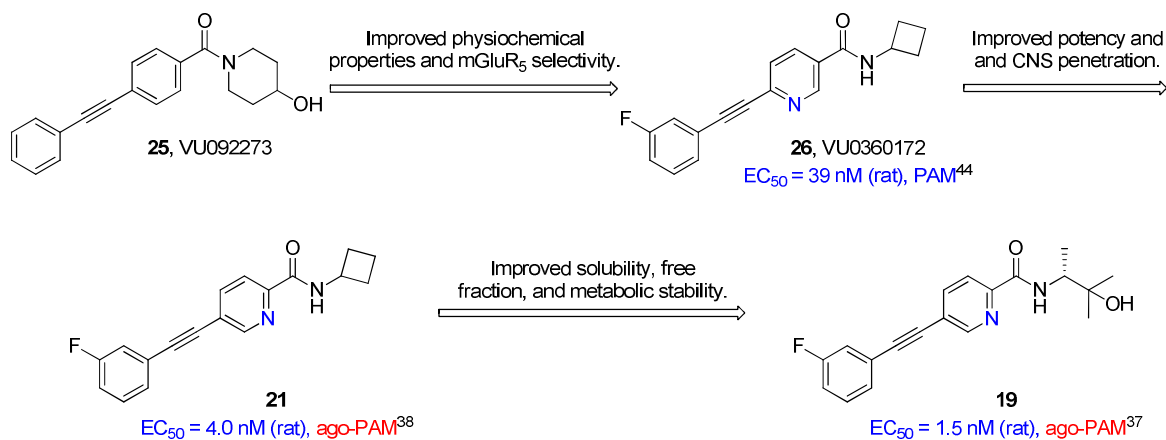
## 28 RESULTS AND DISCUSSION

### 31 Structure-Activity Relationship (SAR) Design

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Our development of acetylenic mGlu<sub>5</sub> PAMs originated with a high throughput screen (HTS) lead VU0092273 (**25**, Figure 5) that was quickly optimized to the nicotinamide analog, VU0360172 (**26**, Figure 5).<sup>42</sup> The introduction of the central pyridyl ring in **26** enabled salt formation leading to improved water solubility and the first orally active acetylene mGlu<sub>5</sub> PAM, despite a high degree of plasma protein binding (98.9%) and low CNS exposure (brain to plasma ratio = 0.13). Subsequent efforts to improve physiochemical properties within this class led to the discovery that picolinamide analogs, as represented by **21** (Figure 5),<sup>38</sup> which show a general improvement in potency, still possess a high degree of plasma protein binding (>99% bound in human and rat). Both **21** and **26** were found to undergo substantial oxidative metabolism of the eastern cyclobutane ring (see Figure 3).<sup>38</sup> With these findings we turned our efforts toward reducing lipophilicity and addressing metabolism of the eastern alkyl group through the introduction of functional groups bearing additional hydrogen bond donors and/or acceptors in this portion of the molecule. This effort led to the discovery of **19** bearing a tertiary carbinol which contributed to improved solubility, reduced metabolism, and reduced plasma protein binding (2.8% bound in rat, 2.7% bound in human).





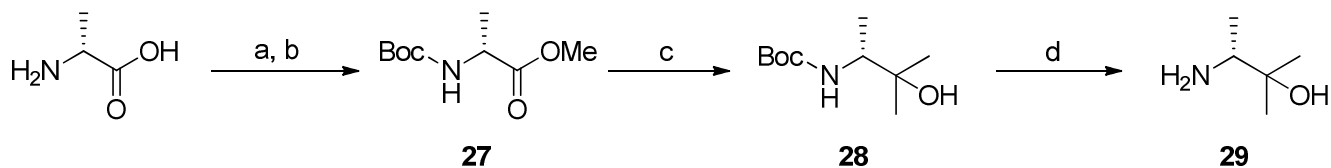
**Figure 5.** SAR development of Vanderbilt acetylene mGlu<sub>5</sub> PAMs. (Potency values refer to the low density rat mGlu<sub>5</sub> receptor cell line.<sup>43</sup>)

As **19** represented one of the first compounds within the acetylene class to possess ago-PAM activity in the low-expressing receptor cell line,<sup>37</sup> we initiated exploration of SAR around this analog to probe the structural determinants of allosteric agonism. Analysis of **19** yielded three important structural features to explore: the western aryl ring, the heteroaryl core, and the eastern amide region. The western aryl ring was hypothesized to be a structural region that might impact *in vitro* PAM to NAM profile mode switching as observed in related acetylenes.<sup>17</sup> The importance of the core region was suggested by the fact that nicotinamide containing acetylenes **26** and **20** were active as PAMs but devoid of agonist activity (Figures 3 and 5). Lastly, the hydroxyl motif in the eastern amide region of **19** and metabolite **22**<sup>38</sup> was identified as a common structural motif among acetylenes possessing adverse effect profiles; therefore, alternate functional groups designed to maintain good pharmacokinetic and physicochemical properties were targeted.

## Chemistry

To explore western aryl and core analogs (*R*)-3-amino-2-methylbutan-2-ol, **29**, was first prepared as shown in Scheme 1.<sup>35</sup> Starting from (D)-alanine, the amino acid was transformed into the methyl ester upon treatment with SOCl<sub>2</sub> in MeOH. Protection of the amine as the *tert*-butyl carbamate yielded intermediate **27** in 88% yield over the 2 steps. Reaction with MeMgBr (3.0 equiv.) yielded tertiary alcohol **28** in 63% yield, and deprotection in the presence of TFA/CH<sub>2</sub>Cl<sub>2</sub> afforded (*R*)-3-amino-2-methylbutan-2-ol, **29**. While the amino alcohol could be stored as the TFA salt and used over a period of several weeks, best results were obtained by removing the *tert*-butyl carbamate immediately prior to use.

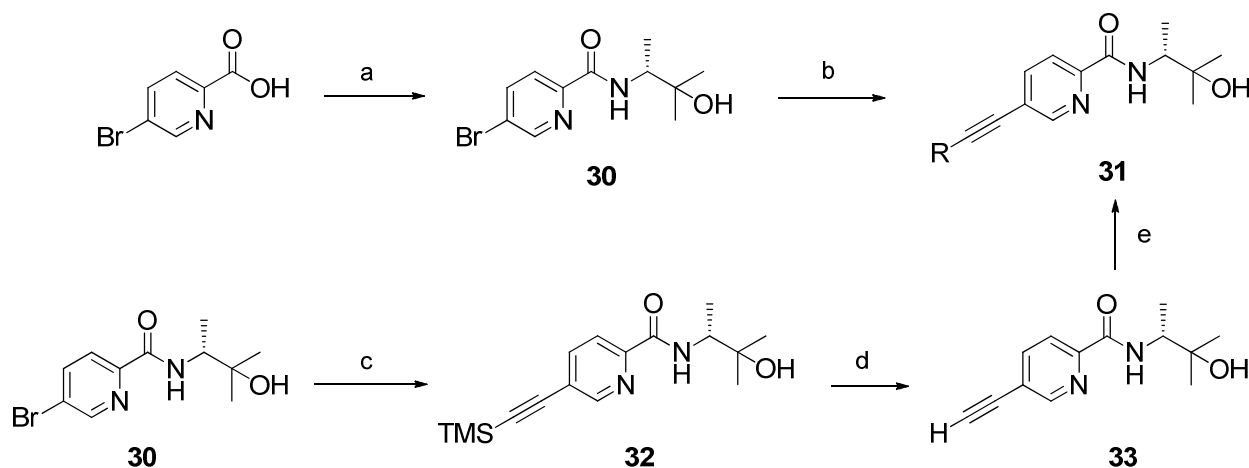
### Scheme 1. Synthesis of (*R*)-3-amino-2-methylbutan-2-ol<sup>a</sup>



13 "Reagents and conditions: (a)  $\text{SOCl}_2$ , MeOH, 0 °C to rt; (b)  $\text{Boc}_2\text{O}$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , 0 °C to rt, 88% over 2 steps; (c)  
14 MeMgBr (3.0 equiv), THF, 0 °C to rt, 63%; (d) TFA/ $\text{CH}_2\text{Cl}_2$  (1:2), 0 °C, 97%.

15 The desired analogs to explore the structural properties of compounds possessing allosteric agonism were  
16 accessed in the parallel synthetic sequences shown in Schemes 2-4. The western aryl region was rapidly diversified  
17 utilizing key intermediates **30** and **33**. 5-Bromo-picolinamide **30** was accessed via a HATU mediated amide coupling of  
18 5-bromopicolinic acid with **29** in 81% yield. Palladium catalyzed Sonogoshira coupling of **30** with aryl and cyclic alkyl  
19 acetylenes provided the desired western analogs **31** in 50-90% yield. When the desired acetylene coupling partners were  
20 unavailable, analogs were synthesized from terminal acetylene **33**. Sonogoshira coupling of 5-bromo-picolinamide **30**  
21 with trimethylsilylacetylene to yield **32**, followed by removal of the silyl protecting group with  $\text{K}_2\text{CO}_3$  in MeOH/THF  
22 provided terminal acetylene **33** in 73% yield over 2 steps. Sonogoshira coupling of the resulting terminal acetylene with  
23 aryl and heteroaryl bromides provided the desired analogs in 43-62% yield.

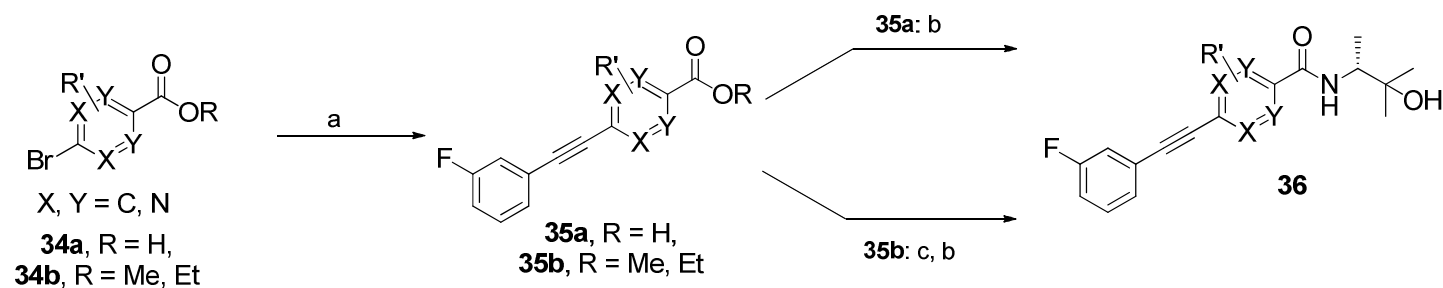
### 33 Scheme 2. Synthesis of western aryl analogs<sup>a</sup>



53 "Reagents and conditions: (a) **29**, HATU, DIPEA, DMF, rt, 81% (b) terminal acetylene, CuI,  $\text{PdCl}_2(\text{PPh}_3)_2$ ,  
54  $\text{Et}_2\text{NH}$ , DMF, 90 °C, 50-90%; (c) trimethylsilylacetylene, CuI,  $\text{PdCl}_2(\text{PPh}_3)_2$ ,  $\text{Et}_2\text{NH}$ , DMF, 90 °C, 77%; (d)  
55  $\text{K}_2\text{CO}_3$ , MeOH/THF, rt, 95%; (e) aryl bromide, CuI,  $\text{PdCl}_2(\text{PPh}_3)_2$ ,  $\text{Et}_2\text{NH}$ , DMF, 90 °C, 43-62%.

Synthesis of heteroaryl core analogs was achieved as displayed in Scheme 3. Sonogoshira coupling of 4-bromo-heteroaryl carboxylic acids **34a** and 1-ethynyl-3-fluorobenzene yielded biaryl acetylenes **35a** in 30-97 % yield, which were then subjected to a HATU mediated amide bond coupling with **29** to access core analogs **36**. Where the desired carboxylic acids were not available the corresponding esters **34b** were used to access intermediate **34b** via a Sonogoshira coupling with 1-ethynyl-3-fluorobenzene. Saponification of ester **35b** with LiOH in THF/H<sub>2</sub>O and amide coupling with **29** yielded the desired analogs.

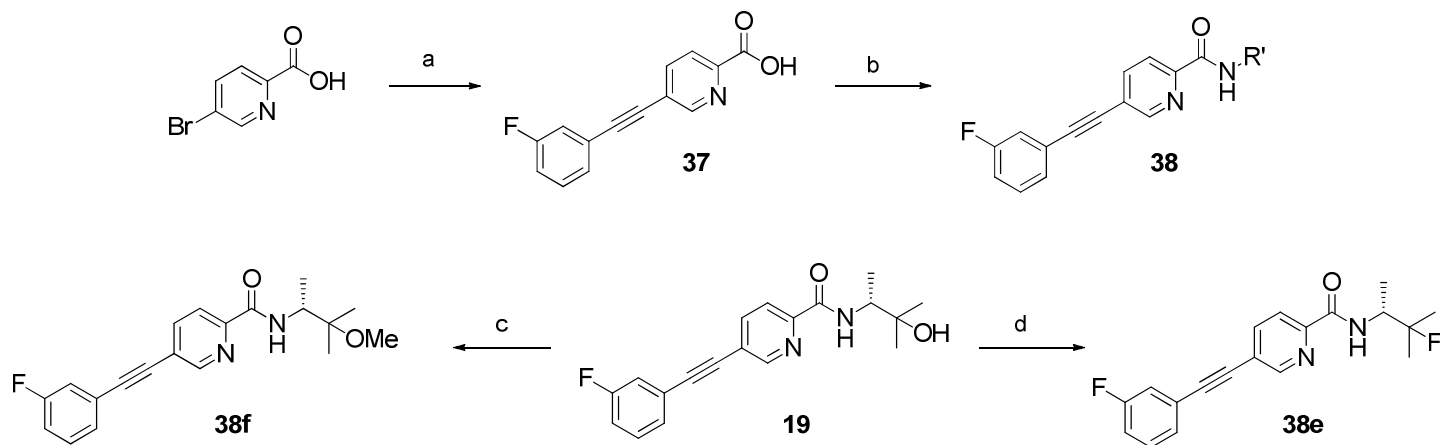
### Scheme 3. Synthesis of core analogs<sup>a</sup>



<sup>a</sup>Reagents and conditions: (a) 1-ethynyl-3-fluorobenzene, CuI, PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>, Et<sub>2</sub>NH, DMF, 90 °C, 30-97%; (b) **29**, HATU, DIPEA, DMF, rt, (c) LiOH, THF/H<sub>2</sub>O, rt.

The eastern amide region was explored utilizing ethynyl-picolinic acid **37** as shown in Scheme 4. Following coupling of 5-bromopicolinic acid with 1-ethynyl-3-fluorobenzene to yield **37**, a variety of amines were installed through HATU mediated amide bond formation. To understand the effect of the chiral center, (*S*)-3-amino-2-methylbutan-2-ol was prepared from L-alanine in a sequence analogous to that shown in Scheme 1, and a variety of commercially available chiral amines bearing the (*R*)- and (*S*)-configuration at the chiral center were investigated. In addition, to systematically explore the effects of the eastern hydroxyl on ago-PAM activity several additional analogs were prepared directly from **19**. Methylation of the tertiary hydroxyl by treatment with NaH in THF followed by reaction with MeI afforded methyl ether **38f**. The hydroxyl group was also transformed into the corresponding fluoro-analog via reaction of the alcohol with DAST (CH<sub>2</sub>Cl<sub>2</sub>, -78 °C) to access **38e**.

### Scheme 4. Synthesis of eastern amide analogs<sup>a</sup>

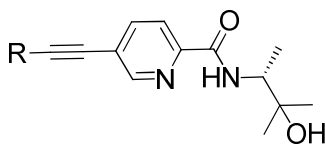


<sup>a</sup>Reagents and conditions: (a) 1-ethynyl-3-fluorobenzene, CuI, PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>, Et<sub>2</sub>NH, DMF, 90 °C, 65%; (b) amine, HATU, DIPEA, DMF, rt; (c) NaH, MeI, THF, 0 °C to rt, 58%; (d) DAST, CH<sub>2</sub>Cl<sub>2</sub>, -78 °C to rt, 30%.

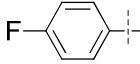
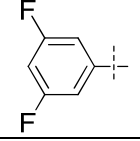
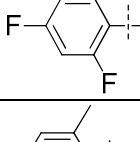
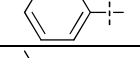
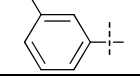
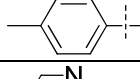
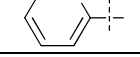
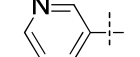
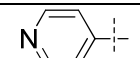
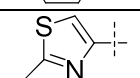
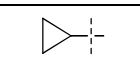
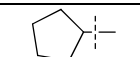
### *In Vitro* Pharmacology

Compounds were profiled in our rat mGlu<sub>5</sub> low receptor expression cell line using a “triple add” protocol in accord with previously published procedures.<sup>42,43</sup> Activity observed using the low receptor expressing cell line correlates with the functional response observed in native systems and allows for detection of allosteric agonism. The “triple add” protocol allows compounds to be evaluated for agonism as well as positive and negative allosteric modulation simultaneously.<sup>43</sup>

**Table 1. Rat mGlu<sub>5</sub> Potency and % GluMax Response for Western Aryl Analogs**



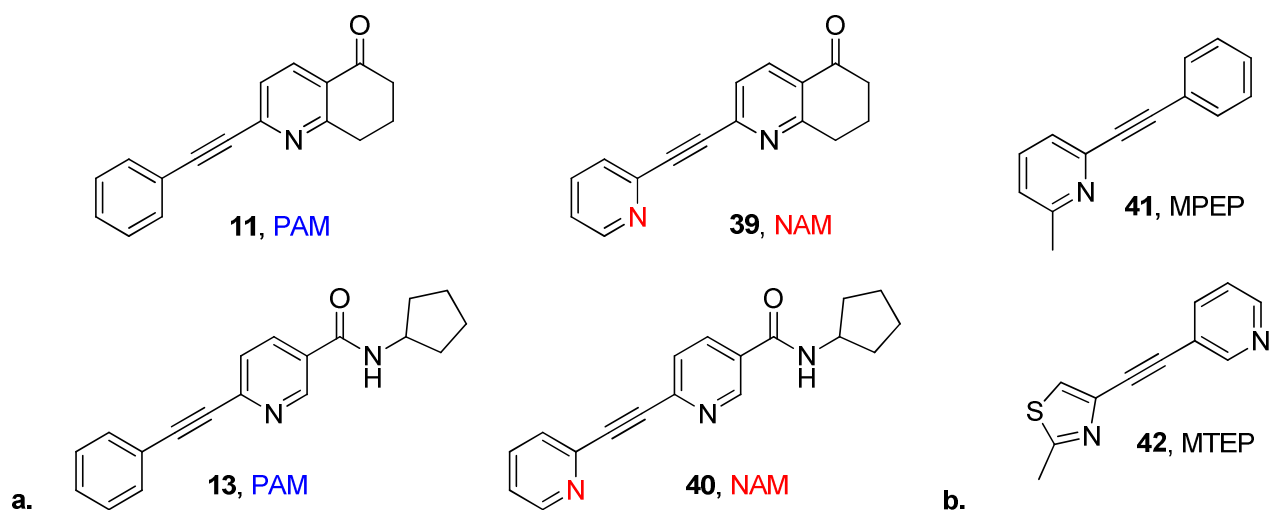
Compound	R	Potentiator (Rat mGlu <sub>5</sub> )			Agonist (Rat mGlu <sub>5</sub> )			Category
		pEC <sub>50</sub> (± SEM) <sup>a</sup>	EC <sub>50</sub> (nM) <sup>a</sup>	GluMax (± SEM) <sup>a</sup>	pEC <sub>50</sub> (± SEM) <sup>a</sup>	EC <sub>50</sub> (nM) <sup>a</sup>	GluMax (± SEM) <sup>a</sup>	
31a		8.38 ± 0.14	4.1	60.9 ± 5.1	6.06 ± 0.14	874	43.2 ± 5.2	ago-PAM
31b		8.10 ± 0.07	8.0	60.0 ± 2.9	5.94 ± 0.10	1,160	29.6 ± 4.0	ago-PAM
19		8.31 ± 0.02	4.9	63.5 ± 2.7	6.04 ± 0.10	904	37.1 ± 5.7	ago-PAM

1	<b>31c</b>		7.91 ± 0.07	12.3	66.2 ± 2.1	5.82 ± 0.12	1,520	38.4 ± 5.7	ago-PAM
2									
3	<b>31d</b>		7.24 ± 0.10	58.2	61.6 ± 1.4	5.44 ± 0.01	3,670	37.9 ± 4.3	ago-PAM
4									
5									
6									
7	<b>31e</b>		7.58 ± 0.09	26.6	61.2 ± 1.4	5.43 ± 0.18	3,690	38.2 ± 8.9	ago-PAM
8									
9									
10									
11	<b>31f</b>		6.66 ± 0.04	217	59.9 ± 1.8	4.91 ± 0.02	12,400	24.1 ± 5.3	ago-PAM
12									
13									
14	<b>31g</b>		8.19 ± 0.16	6.5	68.6 ± 3.3	5.87 ± 0.03	1,360	20.8 ± 2.9	ago-PAM
15									
16									
17	<b>31h</b>		6.97 ± 0.02	107	63.1 ± 2.0	--	--	--	PAM
18									
19									
20	<b>31i</b>		7.38 ± 0.19	41.3	57.5 ± 6.14	--	--	--	PAM
21									
22	<b>31j</b>		6.46 ± 0.03	344	71.5 ± 4.4	5.03 ± 0.05	9,300	25.9 ± 8.3	ago-PAM
23									
24	<b>31k</b>		7.37 ± 0.13	43.0	66.6 ± 2.6	5.33 ± 0.06	4,660	22.4 ± 6.6	ago-PAM
25									
26									
27	<b>31l</b>		7.23 ± 0.50	58.4	66.6 ± 3.0	--	--	--	PAM
28									
29	<b>31m</b>		5.89 ± 0.00	1289	63.4 ± 6.0	--	--	--	PAM
30									
31	<b>31n</b>		7.03 ± 0.28	93.3	63.7 ± 3.8	5.01 ± 0.06	9,750	14.8 ± 5.7	ago-PAM
32									

<sup>a</sup>pEC<sub>50</sub>, EC<sub>50</sub>, and % GluMax response are the average of at least three independent measurements performed in duplicate or triplicate.

The structure-activity relationship (SAR) are shown in Tables 1-3. SAR around the western aryl region reveals that most manipulations to this portion of the molecule do not eliminate ago-PAM activity. As seen in compounds **19** and **31b-31c**, positioning of the fluorine atom only modestly affected potency and efficacy with des-fluoro compound **31a** and 3-fluoro congener **19** preferred for both agonist and PAM potency. Incorporation of the sterically larger methyl group led to a concomitant decrease of PAM and agonist activity, except in the case of *meta*-substituted analog **31g**. While agonism was retained for *ortho*- and *meta*-substituted derivatives **31f** and **31g**, methyl substitution at the *para*-position led to PAM **31h**. Introduction of a western pyridyl also proved to impact agonist activity, leading to the discovery of PAM **31i**, bearing a western 2-pyridine. Structurally, **31i** is intriguing as PAM to NAM mode switching has been observed upon the introduction of the 2-pyridyl functionality within the tetralone acetylene series reported by Merz Pharmaceuticals<sup>29</sup> and in the acetylene scaffold by reported by Ritzén and coworkers (Figure 6).<sup>31,44</sup> Thus, it appears that, within the acetylene scaffold, the 2-pyridyl functionality can engender inhibitory effects in some cases. Moreover, when functionalities are present in other regions of the molecule that lead to receptor activation (e.g. agonism or ago-PAM) the 2-pyridyl group

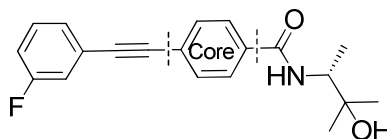
appears to moderate these strong agonist effects. The positioning of the pyridyl nitrogen also appears to be critical for attenuation of the agonism, as the 3- and 4-substituted pyridines (**31j**, **31k**) maintain ago-PAM activity. Given that the 2-pyridyl motif is structurally reminiscent of mGlu<sub>5</sub> antagonist MPEP, we also investigated the methyl thiazole motif found in the mGlu<sub>5</sub> antagonist MTEP to give **31i**, which also elicited a PAM profile with no observed agonism. Finally, cyclic alkyl groups were explored, with cyclopropyl derivative **31m** found to be a PAM, although with greatly reduced potency. Ago-PAM cyclopentyl derivative **31n** exhibited a smaller degree of allosteric agonism relative to aryl analogs.



**Figure 6.** a. PAM to NAM mode switching with incorporation of the 2-pyridyl motif.<sup>29,44</sup> b. Acetylene based allosteric antagonists.

In parallel, explorations of the core region of the molecule revealed interesting structural features of allosteric agonism. Deletion of the nitrogen in benzamide **36a** yielded a highly potent ago-PAM. Interestingly, although nicotinamide analog **36b** displayed reduced potency in comparison with picolinamide **19** it still displayed significant allosteric agonism. As nicotinamide acetylene analogs exemplified by **20** (Figure 3) and **26** (Figure 5) are generally PAMs devoid of agonism, these results suggest that the eastern amide portion of the molecule is partially responsible for the observed allosteric agonism. Core modifications were able to eliminate allosteric agonism in some instances, as 3- and 4-substituted methyl analogs **36d** and **36e** as well as pyrimidine **36f** were found to lack apparent agonist activity. Regioisomeric pyrimidine **36g**, however, displayed ago-PAM activity, as did pyridazine, pyrazine, and thiazole core analogs (**36h-36j**).

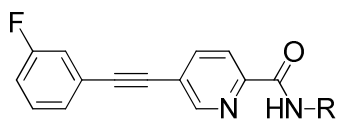
**Table 2. Rat mGlu<sub>5</sub> Potency and % GluMax Response for Core Analogs**



Compound	R	Potentiator (Rat mGlu <sub>5</sub> )			Agonist (Rat mGlu <sub>5</sub> )			Category
		pEC <sub>50</sub> (± SEM) <sup>a</sup>	EC <sub>50</sub> (nM) <sup>a</sup>	GluMax (± SEM) <sup>a</sup>	pEC <sub>50</sub> (± SEM) <sup>a</sup>	EC <sub>50</sub> (nM) <sup>a</sup>	GluMax (± SEM) <sup>a</sup>	
36a		8.12 ± 0.06	7.5	53.9 ± 4.3	5.98 ± 0.03	1,040	43.6 ± 4.3	ago-PAM
19		8.31 ± 0.02	4.9	63.5 ± 2.7	6.04 ± 0.10	904	37.1 ± 5.7	ago-PAM
36b		7.10 ± 0.04	80.0	64.9 ± 1.5	5.17 ± 0.08	6,800	26.6 ± 5.1	ago-PAM
36c		7.77 ± 0.05	17.1	64.9 ± 2.1	5.96 ± 0.01	1,090	9.2 ± 1.7	ago-PAM
36d		6.98 ± 0.04	105.4	66.4 ± 3.2	--	--	--	PAM
36e		6.62 ± 0.04	242.1	66.6 ± 1.2	--	--	--	PAM
36f		6.91 ± 0.13	121.8	68.7 ± 3.1	--	--	--	PAM
36g		7.32 ± 0.18	47.6	64.1 ± 3.0	5.38 ± 0.22	4,130	19.2 ± 5.5	ago-PAM
36h		7.66 ± 0.07	21.9	70.2 ± 3.4	5.67 ± 0.05	2,120	16.5 ± 3.9	ago-PAM
36i		7.90 ± 0.03	12.5	64.4 ± 3.1	5.52 ± 0.02	3,040	17.2 ± 3.7	ago-PAM
36j		7.48 ± 0.05	33.5	62.1 ± 3.8	5.32 ± 0.03	4,780	26.1 ± 2.9	ago-PAM

<sup>a</sup>pEC<sub>50</sub>, EC<sub>50</sub>, and % GluMax response are the average of at least three independent measurements performed in duplicate or triplicate.

We next turned our attention to exploring the eastern amide region within the picolinamide core as this structural motif appeared to have a significant bias toward ago-PAM activity. Maintaining the propyl backbone found in **19**, we prepared a number of analogs, systematically varying substituents to explore the SAR profile of allosteric agonism. Based on our initial hypothesis that the tertiary carbinol contributes to allosteric agonism and adverse effects, our first round of SAR involved deletion and modification of the tertiary hydroxyl. Surprisingly, *tert*-butyl analogs **38a** and **38b** and *iso*-propyl analogs **38c** and **38d** displayed potent ago-PAM activity and replacement of the hydroxyl with fluorine (**38e**) resulted in a potent ago-PAM.

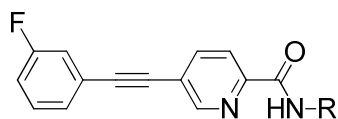
Table 3. Rat mGlu<sub>5</sub> Potency and % GluMax Response for Eastern Amide Analogs Lacking Polar Functionalities

Compound	R	Potentiator (Rat mGlu <sub>5</sub> )			Agonist (Rat mGlu <sub>5</sub> )			Category
		pEC <sub>50</sub> (± SEM) <sup>a</sup>	EC <sub>50</sub> (nM) <sup>a</sup>	GluMax (± SEM) <sup>a</sup>	pEC <sub>50</sub> (± SEM) <sup>a</sup>	EC <sub>50</sub> (nM) <sup>a</sup>	GluMax (± SEM) <sup>a</sup>	
38a		7.75 ± 0.19	17.8	66.0 ± 2.7	5.56 ± 0.07	2,751.8	31.2 ± 2.7	ago-PAM
38b		7.28 ± 0.12	51.9	70.8 ± 3.6	5.27 ± 0.17	5,403.4	20.0 ± 2.8	ago-PAM
38c		8.35 ± 0.09	4.5	69.3 ± 0.8	5.83 ± 0.25	1,469.1	22.5 ± 1.4	ago-PAM
38d		7.69 ± 0.11	20.4	66.7 ± 2.7	5.65 ± 0.04	2,224.1	13.1 ± 1.4	ago-PAM
38e		8.28 ± 0.04	5.3	68.6 ± 3.0	5.69 ± 0.08	2,064.5	42.8 ± 2.5	ago-PAM
38f		--	--	--	--	--	--	Inactive
38g		7.46 ± 0.04	34.4	65.9 ± 1.3	5.52 ± 0.03	3,009.9	8.3 ± 2.2	ago-PAM
38h		7.54 ± 0.03	28.9	58.2 ± 2.7	--	--	--	PAM
38i		7.78 ± 0.13	16.6	63.9 ± 1.0	5.59 ± 0.25	2,573.1	9.5 ± 2.1	ago-PAM
38j		7.43 ± 0.10	36.7	61.4 ± 1.6	--	--	--	PAM
38k		7.08 ± 0.12	84.0	68.1 ± 3.44	< 5.0	> 10,000.0	4.7 ± 1.3	ago-PAM

<sup>a</sup>pEC<sub>50</sub>, EC<sub>50</sub>, and % GluMax response are the average of at least three independent measurements performed in duplicate or triplicate.

For optically active compounds **38a-38d** a slight preference was observed for the (*R*)-enantiomer. Interestingly, capping the tertiary carbinol as the methyl ether (**38f**) resulted in an inactive compound. As changes to the tertiary carbinol were not successful in preventing ago-PAM activity, we deleted the methyl group adjacent to the amide while maintaining the eastern alkyl chain carbon backbone length found in **19**. This exploration proved fruitful with *iso*-butyl analog **38h** and methyl-cyclopropyl analog **38j** found to be potent PAMs; however, the effect was subtle and the trend was not entirely clear as the propyl analog **38g**, *tert*-butyl analog **38i**, and the methyl-cyclobutyl analog **38k** displayed weak agonism.



Table 4. Rat mGlu<sub>5</sub> Potency and % GluMax Response for Eastern Amide Analogs Bearing Polar Functionalities

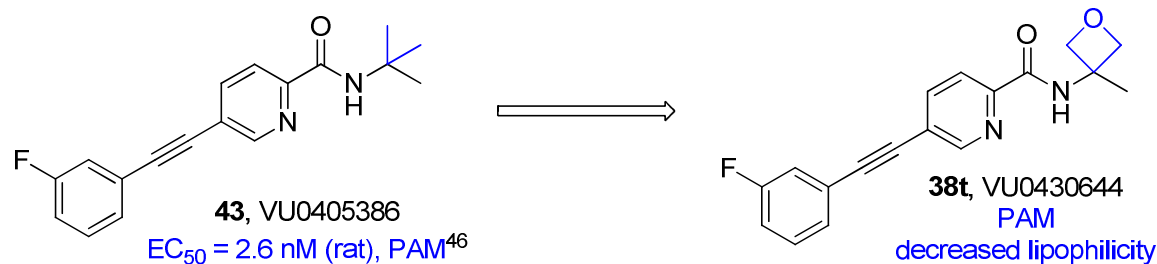
Compound	R	Potentiator (Rat mGlu <sub>5</sub> )			Agonist (Rat mGlu <sub>5</sub> )			Category
		pEC <sub>50</sub> (± SEM) <sup>a</sup>	EC <sub>50</sub> (nM) <sup>a</sup>	GluMax (± SEM) <sup>a</sup>	pEC <sub>50</sub> (± SEM) <sup>a</sup>	EC <sub>50</sub> (nM) <sup>a</sup>	GluMax (± SEM) <sup>a</sup>	
19		8.31 ± 0.02	4.9	63.5 ± 2.7	6.04 ± 0.10	904	37.1 ± 5.7	ago-PAM
38l		--	--	--	--	--	--	Inactive
38m		8.10 ± 0.11	7.9	60.9 ± 4.6	6.14 ± 0.10	729	9.3 ± 3.3	ago-PAM
38n		7.46 ± 0.02	34.6	61.5 ± 2.5	5.40 ± 0.08	4,020	20.0 ± 6.0	ago-PAM
38o		--	--	--	--	--	--	Inactive
38p		6.87 ± 0.04	136	61.9 ± 2.8	--	--	--	PAM
38q		6.65 ± 0.09	224	67.0 ± 1.7	4.97 ± 0.22	10,700	5.8 ± 1.1	ago-PAM
38r		7.68 ± 0.11	20.8	52.1 ± 7.1	--	--	--	PAM
38s		7.66 ± 0.03	21.9	60.8 ± 4.7	5.86 ± 0.01	1,390	21.4 ± 2.2	ago-PAM
38t		8.03 ± 0.06	9.3	45.5 ± 2.4	--	--	--	PAM
38u		7.97 ± 0.23	10.7	60.4 ± 3.4	--	--	--	PAM
38v		7.33 ± 0.11	47.3	65.7 ± 5.0	5.55 ± 0.03	2,840	16.3 ± 2.5	ago-PAM

<sup>a</sup>pEC<sub>50</sub>, EC<sub>50</sub>, and % GluMax response are the average of at least three independent measurements performed in duplicate or triplicate.

Since deletion of the tertiary carbinol did not yield a general strategy to prevent allosteric agonism, we next explored analogs bearing the eastern alcohol in order to gain an understanding of the SAR of allosteric agonism when a hydrogen bond donor was maintained. The effect of the configuration of the chiral center was first explored and, surprisingly, the opposite enantiomer of **19**, (*S*)-**38l**, was inactive, suggesting that the position of the tertiary hydroxyl group is critical in receptor activation at the allosteric binding site. In contrast, both enantiomers of optically active derivatives **38a** to **38d** were active ago-PAMs, with only a slight preference for the (*R*)-enantiomer. Removal of steric

1 bulk surrounding the alcohol (**38m**) and adjacent to the amide (**38n**) did not remove allosteric agonism, while alcohol **38o**  
2 possessing no steric bulk was inactive. Interestingly, extension of the carbon backbone in analog **38p** resulted in a loss of  
3 agonist activity, suggesting that allosteric agonism within this series is sensitive to the location of the tertiary alcohol.  
4

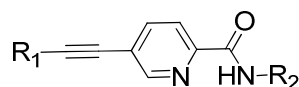
5  
6 We next pursued alternatives to the tertiary alcohol, investigating functional groups that conceptually could serve  
7 as a “molecular lock”, such that the functionality possesses desirable physiochemical and DMPK properties, which  
8 intentionally deter formation of hydroxylated metabolites specifically within the eastern amide moiety that may engender  
9 undesirable pharmacology on their own.<sup>39</sup> Fluorinated alkyl amides were explored, and trifluoroethyl derivative **38q** was  
10 found to be an ago-PAM with moderate potency. Trifluoroalkyl derivatives possessing a chiral center afforded increased  
11 activity (**38r-s**). Interestingly, these analogs yielded enantiospecific activity, with (*R*)-**38r** representing a highly potent  
12 PAM and (*S*)-**38s** displaying ago-PAM activity. We also explored oxetanes since this motif has been utilized as a  
13 surrogate for geminal dimethyl groups and represent a polar alternative for the introduction of steric bulk as well as  
14 possessing hydrogen bond accepting properties.<sup>45</sup> In particular, the oxetane was appealing as a means to address the high  
15 lipophilicity and plasma protein binding associated with our recently reported PAM VU0405386 (**43**),<sup>46</sup> thus, we  
16 prepared the 3-methyl substituted oxetane as a *tert*-butyl replacement (**38t**, Figure 7). The oxetane can also be envisioned  
17 as an isosteric replacement for the cyclobutyl moiety found in **21** (Figure 3), which was found to generate the 3-hydroxy  
18 metabolite **22** (Figure 3) that contributed in part to an adverse effect profile. Incorporation of the oxetane moiety proved  
19 successful and **38t** represents the most potent PAM ( $EC_{50} = 9.3$  nM) discovered thus far within this series. Maintaining  
20 the oxetane and extending the carbon chain length one carbon led to equipotent PAM **38u**. Further extension to the two  
21 carbon variant **39v** maintained potentiation activity; however, weak allosteric agonism returned ( $EC_{50} = 2.8$   $\mu$ M, Glu Max  
22 16.3%). Collectively, the eastern amide SAR strongly suggests that the general structural elements of the 2-methylbutan-  
23 2-ol motif (**29**) introduced to increase solubility are strongly biased toward allosteric agonism and that other eastern amide  
24 groups can be identified with favorable physiochemical properties that result in PAMs lacking agonist activity.  
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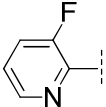
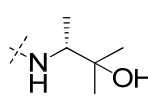
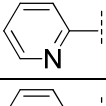
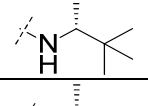
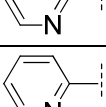
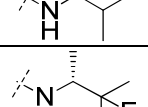
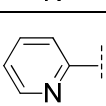
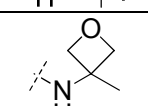
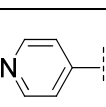
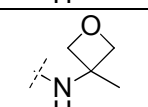
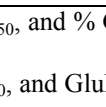
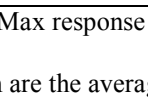
58 **Figure 7.** Oxetane surrogate for steric bulk with decreased lipophilicity.  
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In light of these results, a final round of SAR was pursued to probe the effects of the basicity of the pyridyl nitrogen in pure PAM **31i** and to investigate combination of the western pyridyl with a small subset of eastern alkyl groups such as the oxetane motif. We first studied the effects of the basicity of the pyridyl nitrogen through the introduction of electron withdrawing fluorine atoms around the pyridine ring. If a basic nitrogen at the 2-position of the pyridine ring helps to stabilize a conformation of the receptor not disposed toward allosteric agonism, we hypothesized that decreased basicity of the pyridyl nitrogen at this position might weaken this interaction and restore allosteric agonism. As shown in Table 5, introduction of fluorine reveals that the basicity of the pyridyl nitrogen is in fact important, as fluoro-substituted pyridyls **31o** – **31r** display allosteric agonism. We subsequently designed several analogs to explore whether the 2-pyridyl functionality could serve as a “molecular lock” to prevent allosteric agonism in compounds bearing eastern amide alkyl groups found to possess agonism when combined with the 3-fluorophenyl western aryl ring. This study revealed that the 2-pyridyl motif prevented allosteric agonism in some but not all cases. Modulator **44b** and **44c** exhibited PAM activity; however, *tert*-butyl analog **44a** displayed ago-PAM activity. We then pursued a hybrid picolinamide acetylene containing the western 2-pyridyl and eastern oxetane amide motifs since these substructures were discovered to be two of the most preferred structural elements to maintain PAM activity without apparent agonism (**44d**). Unexpectedly, this combination led to mode switching, yielding an antagonist. Moving the nitrogen to the 4-position and maintaining the oxetane (**45**) restored some potentiation, resulting in a weak PAM.

**Table 5. Rat mGlu<sub>5</sub> Potency and % GluMax Response for Pyridyl Analogs**



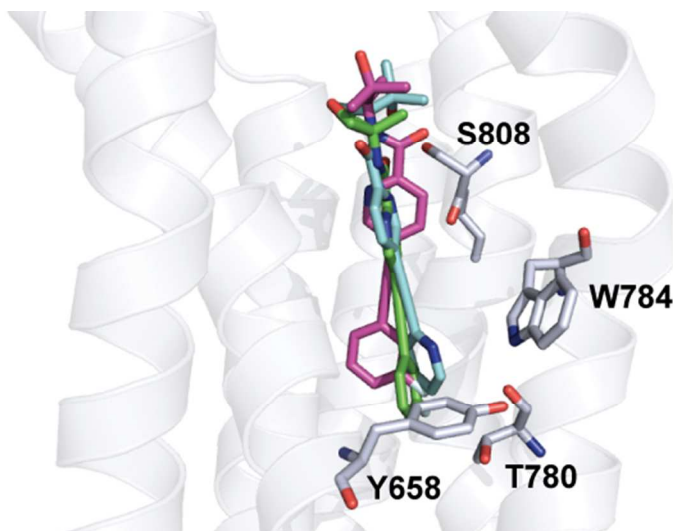
Compd	R <sub>1</sub>	R <sub>1</sub>	Potentiator (Rat mGlu <sub>5</sub> )			Agonist (Rat mGlu <sub>5</sub> )			Category
			pEC <sub>50</sub> (± SEM) <sup>a</sup>	EC <sub>50</sub> (nM) <sup>a</sup>	GluMax (± SEM) <sup>a</sup>	pEC <sub>50</sub> (± SEM) <sup>a</sup>	EC <sub>50</sub> (nM) <sup>a</sup>	GluMax (± SEM) <sup>a</sup>	
<b>31i</b>			7.03 ± 0.14	92.9	69.7 ± 4.3	--	--	--	PAM
<b>31o</b>			7.87 ± 0.24	13.6	63.0 ± 1.7	5.89 ± 0.15	1,300	8.3 ± 2.4	ago-PAM
<b>31p</b>			7.55 ± 0.07	28.2	64.6 ± 3.0	5.77 ± 0.04	1,700	13.5 ± 3.1	ago-PAM
<b>31q</b>			7.44 ± 0.36	36.0	66.2 ± 2.8	5.40 ± 0.22	3,980	33.0 ± 7.8	ago-PAM

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
<b>31r</b>			$7.47 \pm 0.04$	33.8	$60.6 \pm 4.3$	$5.43 \pm 0.01$	3,700	$29.2 \pm 3.6$	ago-PAM									
<b>44a</b>			$7.20 \pm 0.06$	63.3	$63.0 \pm 4.9$	$5.21 \pm 0.06$	6,110	$24.2 \pm 3.7$	ago-PAM									
<b>44b</b>			$7.10 \pm 0.07$	79.0	$58.5 \pm 3.0$	--	--	--	<b>PAM</b>									
<b>44c</b>			$7.65 \pm 0.10$	22.2	$54.9 \pm 3.3$	--	--	--	<b>PAM</b>									
<b>44d</b>			$6.14 \pm 0.20^b$	731 <sup>b</sup>	$16.7 \pm 4.5^b$	--	--	--	antagonist									
<b>45</b>			$6.16 \pm 0.10$	691	$36.0 \pm 6.9$	--	--	--	Weak PAM									

<sup>a</sup>pEC<sub>50</sub>, EC<sub>50</sub>, and % GluMax response are the average of at least three independent measurements performed in duplicate or triplicate.

<sup>b</sup>pIC<sub>50</sub>, IC<sub>50</sub>, and GluMin are the average of three independent measurements performed in triplicate.

In an effort to gain further insights into the interaction of ago-PAM versus PAM preferring functional groups within the mGlu<sub>5</sub> binding pocket PAMs **31i** and **38t** along with ago-PAM **19** were docked into our recently generated comparative model of the transmembrane region of mGlu<sub>5</sub> (Figure 8).<sup>47</sup> Interestingly, in comparison with previously docked picolinamide PAMs (e.g. **43** and **21**),<sup>47</sup> all three compounds sit higher in the binding pocket; this may be attributable to the inclusion of the additional hydrogen bond acceptor on the eastern amide. Similar to what we observed for other acetylenic PAMs, computationally it was difficult to differentiate whether the eastern amide is buried deep within the pocket, or points towards the extracellular space, likely a reflection of the highly linear structure for the class. However, models wherein the eastern amide points towards the extracellular space placed the compounds in proximity of residues previously found to be critical for the function of acetylene PAMs and MPEP (Figure 8).<sup>47</sup> The hydroxyl and amide carbonyl of the modulators were within 3 Å of S808 and the 2-pyridyl western aryl ring was within 3 to 5 Å from Y658, T780 and W784. This pose was chosen as the most likely binding conformation due to its consistency with existing data, and the second possible pose is shown in the Supplementary Material (see section VI).



**Figure 8.** mGlu<sub>5</sub> binding pose for pure-PAMs **31i** (cyan color) and **38t** (green color) and ago-PAM **19** (magenta).

In an attempt to probe these putative poses and elucidate the molecular determinants of agonism further, we examined the impact of four key point mutations (Y658V, T780A, W784A and S808A) on modulator affinity, cooperativity and agonism by applying an operational model of allosterism to glutamate concentration-response curves in the absence and presence of varying concentrations of each PAM, i.e. a progressive fold-shift experiment (Supplementary Material, see sections II-V). All three compounds were sensitive to alanine substitution of T780A; however, the observed reductions in affinity (10-30 fold) were not as substantial as those reported for previous picolinamides (e.g. **43** and **21**).<sup>47</sup> The overall profile of **38t** across all four point mutations was comparable to the prior picolinamides,<sup>47</sup> and in particular both Y658V and S808A engendered a NAM switch identical to previously reported (5-((3-fluorophenyl)ethynyl)pyridin-2-yl)(3-hydroxyazetid-1-yl)methanone (VU0405398).<sup>47</sup> The most striking differences are observed for **31i**, as Y658V had no effect on affinity or cooperativity, despite causing marked reductions in affinity, including abolishment of PAM activity, for all other picolinamide PAMs tested to date. Furthermore, **31i** showed a gain in allosteric agonist activity at W784A, a mutation known to reduce the negative cooperativity of MPEP and increase positive cooperativity of other PAMs.<sup>47</sup> Based upon the pose depicted in Figure 8 and the 2-pyridyl “molecular switch” trends discussed previously (*vide supra*, see Figure 6), it may be hypothesized that the 2-pyridyl western aryl within acetylenic mGlu<sub>5</sub> modulators has a key interaction with W784, favoring less active receptor states. In the W784A mutant where this interaction is absent, modulator **31i** more readily facilitates active receptor conformations.

Having gained insights into the allosteric agonism SAR and potential models for receptor/residue-ligand interaction, we turned our attention to PAM glutamate cooperativity due to its impact on therapeutic index. We selected

PAMs lacking agonist activity from this investigation that display potency values  $\leq 200$  nM and evaluated their ability to left shift the glutamate concentration-response curve at a concentration of 10  $\mu$ M (Table 6). This analysis revealed a range of glutamate fold-shift values from 1.3 to 5.3-fold. This distribution of fold-shift values provides useful tool compounds to probe the impact of cooperativity on therapeutic index in PAMs within the biaryl acetylene chemotype. Due to its excellent potency and low cooperativity, PAM **38t** was selected for further characterization and declared an MLPCN probe.

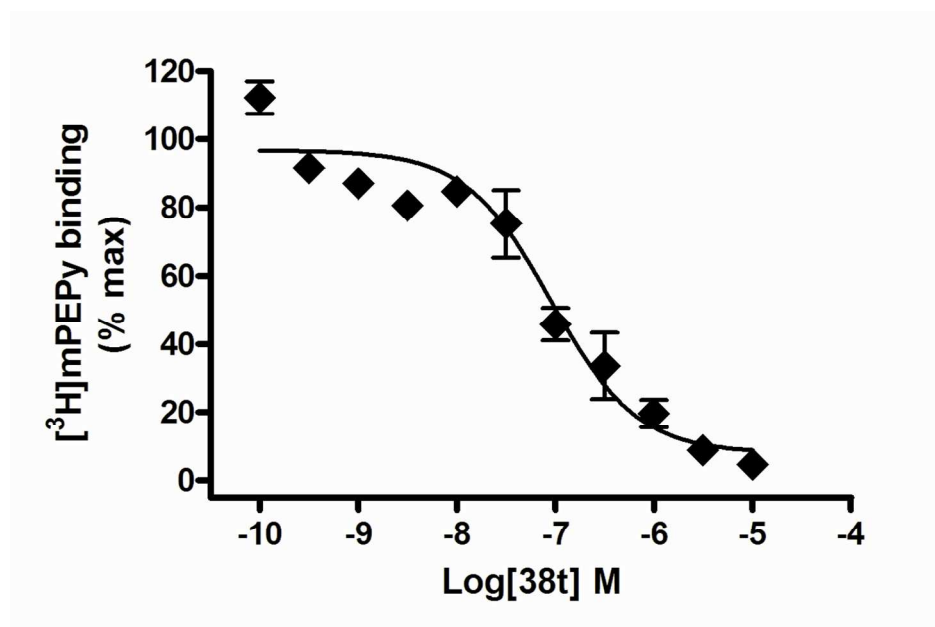
**Table 6. Rat mGlu<sub>5</sub> Cooperativity (Fold-Shift) of Selected PAMs Lacking Apparent Allosteric Agonist Activity.**

Compound	Structure	Glutamate Fold-Shift	Compound	Structure	Glutamate Fold-Shift
<b>31i</b>		1.7	<b>38p</b>		5.3
<b>31l</b>		1.6	<b>38r</b>		1.5
<b>36d</b>		2.3	<b>38t</b>		2.8
<b>36f</b>		4.0	<b>38u</b>		3.3
<b>38h</b>		1.9	<b>44b</b>		1.3
<b>38j</b>		1.6	<b>44c</b>		1.5

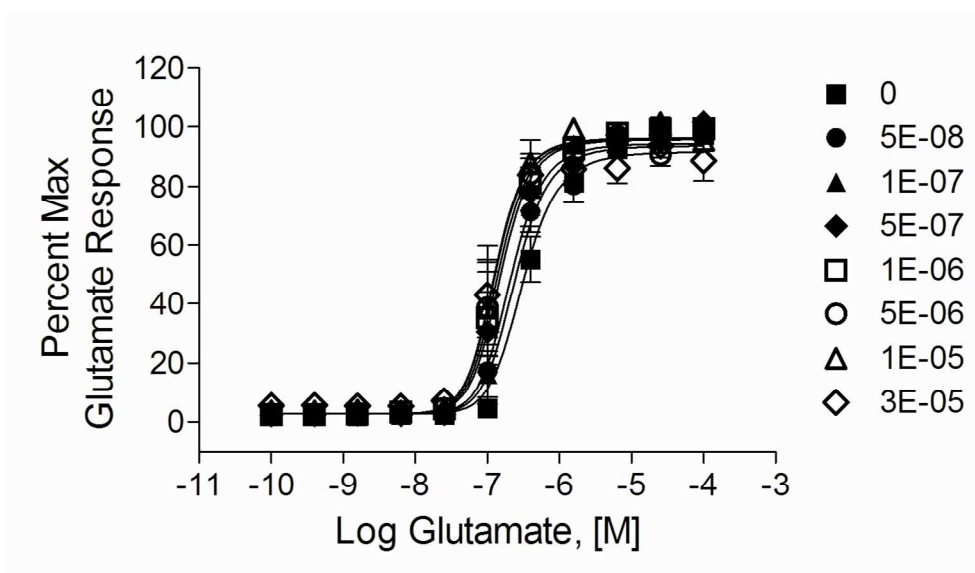
<sup>a</sup>PAM concentration tested was 10  $\mu$ M, values represent the average of three independent measurements performed in triplicate.

To validate that **38t** interacts with mGlu<sub>5</sub> at the MPEP binding site radioligand binding studies were performed with [<sup>3</sup>H]methoxyPEPy. Increasing concentrations of **38t** resulted in complete inhibition of [<sup>3</sup>H]methoxyPEPy binding supporting a competitive interaction between the two ligands (Figure 9). **38t** exhibited a K<sub>i</sub> of 90 nM, representing a ~10-

1 fold higher functional activity ( $EC_{50} = 9.3 \text{ nM}$ ) compared to binding. To utilize compounds in *in vivo* assays it is  
2 important to determine if they are selective for mGlu<sub>5</sub> compared with other mGlu subtypes. A 10  $\mu\text{M}$  concentration of  
3 PAM **38t** did not shift the glutamate (or L-AP4) concentration response curve when evaluated using cells expressing any  
4 of the other mGlu subtypes (mGlu<sub>1-4, 6-8</sub>, see Supplementary Material section I) demonstrating high selectivity for mGlu<sub>5</sub>.  
5 In addition, screening of 10  $\mu\text{M}$  **38t** against a panel of 68 GPCRs, ion channels and transporters revealed no significant off  
6 target activity (Eurofins Inc.). Finally, oxetane **38t** was evaluated in progressive fold-shift experiments (50 nM to 30  
7  $\mu\text{M}$ ). The shift in the glutamate concentration response curve in the presence of increasing concentrations of modulator is  
8 shown in Figure 10. Increasing concentration of modulator resulted in a fold-shift that reached a maximum of  
9 approximately 3.0-fold at 5  $\mu\text{M}$  with a predicted affinity of -6.81 (154 nM) and an efficacy cooperativity factor ( $\log\beta$ )  
10 between glutamate and indicated allosteric modulator of 0.34 (cooperativity  $\sim 2.2$ ).  
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Figure 9. **38t** fully displaces [<sup>3</sup>H]methoxyPEPy binding.



**Figure 10.** **38t** shifts the glutamate concentration response curve leftward with increasing concentrations of modulator: 1.7 at 50 nM; 1.7 at 100 nM; 2.2 at 500 nM; 2.7 at 1  $\mu$ M; 3.0 at 5  $\mu$ M; 2.8 at 10  $\mu$ M and 3.2 at 30  $\mu$ M

The *in vitro* drug metabolism and pharmacokinetic (DMPK) profile of **38t** was next determined, with the hope that the oxetane motif would help to mitigate previously observed metabolism and improve physiochemical properties within this chemotype. Gratifyingly, oxetane **38t** displayed low *in vitro* metabolism with a predicted hepatic clearance ( $CL_{\text{HEP}}$ ) of 1.6 mL/min/kg in rat and 0.2 mL/min/kg in human, a significant improvement in comparison to similar compounds within this series (**19**: predicted  $CL_{\text{HEP}}$  of 34.5 mL/min/kg in rat and 3.5 mL/min/kg in human; **21**: predicted  $CL_{\text{HEP}}$  of 55.6 mL/min/kg in rat<sup>38</sup>). PAM **38t** also possesses improved fraction unbound ( $f_u$ ) as measured by plasma protein binding assay using equilibrium dialysis, with oxetane **38t** 3.5% unbound in human plasma and 3.6% unbound in rat plasma. In comparison, more lipophilic **21** displays human and rat  $f_u$  plasma values < 1%.<sup>38</sup> Rat brain homogenate binding was used to determine fraction unbound in brain for **38t**; these studies revealed  $f_u$  brain values of 1.6%. To assess drug-drug interactions, inhibition of the major human cytochrome P450 (CYP) enzymes (2C9, 2D6, 3A4, 1A2) was measured in human liver microsomes and **38t** was found to display inhibitory activity at 1A2 ( $IC_{50}$  = 5.30  $\mu$ M) while no activity was observed against the other CYPs tested ( $IC_{50}$  > 30  $\mu$ M). Solubility of **38t** was found to be modest with a Fassif (fasted simulated intestinal fluid) solubility of 10-23  $\mu$ g/mL.

To verify its PAM pharmacological profile in native systems **38t** was examined for induction of long-term depression (LTD) at the Schaffer collateral – CA1 (SC-CA1) synapse in the hippocampal formation. LTD at this synapse is known to be modulated by mGlu<sub>5</sub> activation, and orthosteric mGlu<sub>5</sub> agonists such as (*S*)-3,5-DHPG have been shown to



1 elicit LTD.<sup>48</sup> Similarly ago-PAM **19** induces LTD,<sup>38</sup> however, **38t** does not induce LTD on its own (Figure 11;  $100.3 \pm$   
2  $3.7$  % baseline 55min after compound washout). This provides further evidence that **38t** does not elicit a response on its  
3 own in native systems. In addition, prior studies involving **19** showed the induction of epileptiform activity in CA3  
4 pyramidal neurons in hippocampal preparations. We performed similar studies with **38t** to assess agonist activity in this  
5 native CNS preparation. PAM **38t** had no significant effect on either the inter-event interval ( $127.9 \pm 7.7$  % of baseline)  
6 or amplitude ( $101.2 \pm 5.0$  % of baseline) of spontaneous firing supporting an agonism-free profile for **38t** (data not  
7 shown). These data demonstrate that **38t** acts as a pure PAM in two hippocampal native systems.

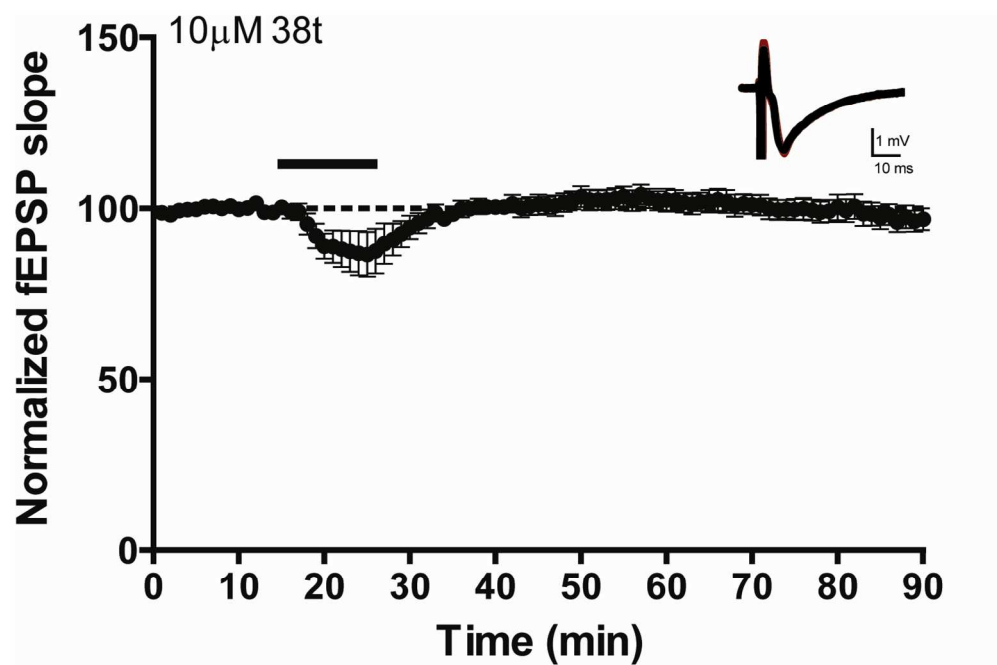


Figure 11. **38t** has no effect on long-term depression at the SC-CA1 synapse in rat hippocampus.

## CONCLUSION

Although mGlu<sub>5</sub> PAMs represent a promising therapeutic strategy for the treatment of schizophrenia, recent reports have raised concerns over a seizure liability and neurotoxicity associated with some chemotypes. Allosteric agonism within the **19** acetylenene chemotype and a high cooperativity (glutamate fold-shift) within the Merck-Addex piperidine and caprolactam series are pharmacological profiles that have been associated with an adverse effect liability. We have extensively explored the SAR of allosteric agonism within the acetylene amide chemotype providing insight into the structural elements contributing to allosteric agonism. In general, the structural elements of the eastern amide were found to have the greatest impact on the presence or absence of allosteric agonism. Replacement of the western 3-  
ACS Paragon Plus Environment

1 fluorophenyl with the 2-pyridyl motif was found to eliminate allosteric agonism in many but not all cases. The  
2 computational docking and mutagenesis data highlight the subtleties of interactions within the common allosteric site,  
3 wherein key residues, in particular W784 and S808, are hypothesized to be engaged in key interactions important for  
4 receptor-modulator interaction and function. Future studies to understand if the functional consequences of these mutants  
5 are indicative of a direct residue side-chain-modulator interaction, or an indirect allosteric interaction will be important to  
6 pursue.  
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13 Due to the potential impact of cooperativity on adverse effects the glutamate fold-shift profile of potent PAMs  
14 was examined revealing PAMs possessing low to moderate efficacy as assessed by glutamate fold-shift. This distribution  
15 of cooperativity profiles will enable studies to test the impact of glutamate fold-shift on neurotoxicity within this  
16 chemotype. Based on its efficacy profile and lack of apparent agonism *in vitro*, highly potent oxetane **38t** was further  
17 characterized and found to possess significantly improved DMPK properties compared with other acetylenes within this  
18 series. **38t** was also profiled in native systems and exhibited no allosteric agonism for the induction of LTD at the  
19 Schaffer collateral – CA1 (SC-CA1) synapse or epileptiform activity in CA3 pyramidal neurons. Preliminary experiments  
20 using a high dose of **38t** in an *in vivo* model of psychosis demonstrate robust reversal of amphetamine induced  
21 hyperlocomotion (data not shown) with no overt behavioral disturbances; however, definitive PK-PD studies in this and  
22 other models, including fluorojade neurotoxicity studies, are needed in order to fully ascertain the anticipated *in vivo*  
23 properties of PAM **38t**. PAM **38t** represents a highly potent tool compound with acceptable pharmacokinetic properties  
24 that will enable further studies to probe the therapeutic index of low fold-shift PAMs. Such studies involving **38t** and  
25 other structurally diverse PAMs are underway and will be reported in due course. mGlu<sub>5</sub> PAM **38t** (ML254) is an  
26 MLPCN probe and is freely available upon request.<sup>60</sup>  
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## 46 EXPERIMENTAL SECTION.

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49 **General.** All reagents purchased from commercial suppliers were used without purification. Unless noted all solvents  
50 used were anhydrous and all reactions were carried out under argon atmosphere. Analytical thin layer chromatography  
51 was performed on Analtech silica gel GF 250 micron plates. Preparative RP-HPLC purification was performed on a  
52 Gilson Inc. preparative UV-based system using a Phenomenex Luna C18 column (50 x 30 mm I.D., 5 μm) with an  
53 acetonitrile (unmodified)-0.1% trifluoroacetic acid in water gradient. Normal-phase silica gel preparative purification was  
54 performed using an automated Combi-flash Rf from ISCO. Analytical LC/MS was performed on an Agilent 1200 Series  
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with UV detection at 215 and 254 nm and ELSD detection (Polymer Laboratories PL-ELS 2100), utilizing an Accucore C18 2.6 $\mu$ , 2.1 x 30 mm column, a 1.1 min gradient, 7% [CH<sub>3</sub>CN/ 0.1%TFA] – 95 [CH<sub>3</sub>CN/ 0.1%TFA] and a G6130 single quadrupole mass spectrometer. Purity of all final compounds was determined to be >98% by analytical HPLC. Solvents for extraction, washing and chromatography were HPLC grade. NMR spectra were recorded on a Bruker 400 MHz spectrometer. <sup>1</sup>H chemical shifts are reported as  $\delta$  values in CDCl<sub>3</sub> or CDOD<sub>3</sub>. Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, p = pentet, hex = hextet, sep = septet, dd = doublet of doublets, dq = doublet of quartets, m = multiplet), coupling constant reported in Hz. <sup>13</sup>C chemical shifts are reported in  $\delta$  values in CDCl<sub>3</sub> or CDOD<sub>3</sub> as follows: chemical shift, C-F coupling constants ( $J_{C-F}$ ) reported in Hz. Low resolution mass spectra were obtained on an Agilent 1200 series 6130 mass spectrometer. HRMS were obtained using a Micromass (Waters) Q-ToF API-US calibrated and verified with sodium iodide. The samples were diluted with a 50:50 0.1% Formic Acid (in Milli-Q):Acetonitrile solution, directly infused using Leucine-Enkephalin (M+H=556.2771) as a lockmass. Scan range was from 100-1000 Da, using a scan time of one second. The [M+H] or [M+Na] ion was observed. Optical rotation values were obtained on a JASCO P-2000 polarimeter.

## Chemistry.

**(R)-*t*-Butyl (3-hydroxy-3-methylbutan-2-yl)carbamate (28).** (D)-Alanine (15 g, 0.168 mol, 1 equiv) was dissolved in MeOH (75 mL, 2.2 M) and cooled to 0 °C. SOCl<sub>2</sub> (20.8 mL, 0.286 mol, 1.7 equiv) was *slowly* added taking care to control the exotherm. The reaction mixture was stirred overnight warming to room temperature. After the reaction was determined to be complete by TLC, the MeOH and excess SOCl<sub>2</sub> were carefully removed by vacuum distillation. The resulting oil was redissolved in MeOH (50 mL) and the solvent was removed by rotary evaporation. The resulting methyl ester was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (150 mL, 1.1 M) and cooled to 0 °C. Et<sub>3</sub>N (70.2 mL, 0.504 mol, 3 equiv) and Boc<sub>2</sub>O (44 g, 0.202 mol, 1.2 equiv) were added and the reaction mixture was allowed to warm to room temperature and stirred overnight. After the reaction was determined to be complete by TLC, the precipitates formed during the reaction were removed via filtration through celite rinsing with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was washed with citric acid (sat'd aq, 1 x 50 mL), dried with Na<sub>2</sub>SO<sub>4</sub>, and concentrated via rotary evaporation yielding (R)-methyl 2-((tert-butoxycarbonyl)amino)propanoate as a yellow oil (30 g, 88% yield).  $[\alpha]_D^{20} = 3.6$  (c = 1.3, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  5.07 (1H, bs), 4.28 (1H, m), 3.71 (3H, s), 1.41 (9H, s), 1.35 (3H, d,  $J = 7.2$  Hz); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  173.8, 155.0, 79.7, 52.2, 49.1, 28.2, 18.5; HRMS (ES+, M+H) calcd for C<sub>9</sub>H<sub>18</sub>NO<sub>4</sub>: 204.1236, found: 204.1236.

(*R*)-methyl 2-((*tert*-butoxycarbonyl)amino)propanoate (5g, 24.6 mmol, 1 equiv) was dissolved in THF (125 mL, 0.2 M) and cooled to 0 °C. MeMgBr (32.8 mL, 98.4 mmol, 5 equiv, 3.0 M soln in Et<sub>2</sub>O) was added slowly and the reaction was allowed to warm to room temperature and stirred overnight. The reaction was then quenched *carefully* with NH<sub>4</sub>Cl (sat'd aq) and extracted with EtOAc (3 x 75 mL). The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated. The crude oil was purified by silica gel chromatography eluting with Hex/EtOAc (0-75% EtOAc) with the desired product eluting between 30-50% EtOAc. (*R*)-*tert*-butyl (3-hydroxy-3-methylbutan-2-yl)carbamate was isolated as a clear oil (3.15 g, 63% yield).  $[\alpha]_D^{20} = 1.9$  (c = 1.4, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ ; 4.82 (1H, d, *J* = 7.2 Hz), 3.54 (1H, bs), 2.58 (1H, bs), 1.41 (9H, s), 1.19 (3H, s), 1.14 (3H, s), 1.09 (3H, d, *J* = 6.8 Hz); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 156.3, 79.3, 72.8, 54.5, 28.3, 27.3, 25.6, 16.1; HRMS (ES+, M+H) calcd for C<sub>10</sub>H<sub>22</sub>NO<sub>3</sub>: 204.1600, found: 204.1598.

**(*R*)-3-Amino-2-methylbutan-2-ol (29).** In a scintillation vial (*R*)-*tert*-butyl (3-hydroxy-3-methylbutan-2-yl)carbamate (1 equiv) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (0.1 M) and cooled to 0 °C. Trifluoroacetic acid (0.2M) was added and the reaction mixture was stirred for 1 h. The starting material was determined to be consumed by TLC and the reaction mixture was concentrated resulting in a brown oil. The resulting (*R*)-3-amino-2-methylbutan-2-ol TFA salt was dissolved in DMF (0.2 M) and used without purification.

**(*R*)-5-Bromo-N-(3-hydroxy-3-methylbutan-2-yl)picolinamide (30).** In a scintillation vial (*R*)-*tert*-butyl (3-hydroxy-3-methylbutan-2-yl)carbamate (1.5 g, 7.4 mmol, 1 equiv) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (6 mL, 0.12 M) and cooled to 0 °C. Trifluoroacetic acid (3 mL, 0.25 M) was added and the reaction mixture was stirred for 1 h. The starting material was determined to be consumed by TLC, the reaction mixture was concentrated via rotary evaporation resulting in a brown oil. The resulting (*R*)-3-amino-2-methylbutan-2-ol TFA salt was dissolved in DMF (5 mL) and used without purification. In a 100 mL round bottom flask 5-bromopicolinic acid (1.49 g, 7.4 mmol, 1.0 equiv) and HATU (3.1 g, 8.14 mmol, 1.1 equiv) were combined in DMF (25 mL, 0.3 M). *N,N*-Diisopropylethylamine (3.2 mL, 18.25 mmol, 5 equiv) was then added and the reaction was stirred for 15 min. The (*R*)-3-amino-2-methylbutan-2-ol TFA salt was added as a solution in DMF and the reaction was stirred 18 h after which the reaction was determined to be complete by LC/MS. The reaction mixture was partitioned between EtOAc (75 mL) and H<sub>2</sub>O (25 mL). After separating the aqueous layer the organic layer was washed again with H<sub>2</sub>O (2 x 15 mL). The organic layer was then dried with Na<sub>2</sub>SO<sub>4</sub>, concentrated, and purified via silica gel chromatography eluting with Hex/EtOAc (0-75% EtOAc) with the aryl bromide eluting at 40% EtOAc. The product was isolated as a brown oil (1.72 g, 81% yield).  $[\alpha]_D^{20} = -13.7$  (c = 0.81, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 8.51 (1H, bs), 8.09 (1H, d, *J* = 9.2 Hz), 8.01 (1H, m), 7.90 (1H, m), 4.04 (1H, m), 3.03 (1H, bs), 1.22 (9 H,bs); <sup>13</sup>C NMR (100

MHz, CDCl<sub>3</sub>) δ 163.4, 149.1, 148.2, 139.8, 123.7, 123.6, 72.6, 53.5, 27.4, 25.8, 15.7; HRMS (ES<sup>+</sup>, M+Na) calcd for C<sub>11</sub>H<sub>15</sub>N<sub>2</sub>O<sub>2</sub>BrNa: 309.0215, found: 309.0212.

### General Methods for Series 31.

**Method A, 31a-n.** In a scintillation vial, (*R*)-5-bromo-*N*-(3-hydroxy-3-methylbutan-2-yl)picolinamide, **31**, (1 equiv) was placed under argon atmosphere, and dissolved in DMF (0.25M). PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (0.05 equiv) CuI (0.1 equiv) were added, followed by an alkyne (1.25 equiv) and Et<sub>2</sub>NH (6 equiv). The reaction mixture was heated to 90 °C for 45 min after which the reaction was determined to be complete by LC/MS. The reaction mixture was filtered through a Fisherbrand Nylon 0.45μm syringe filter and purified directly by preparative RP-HPLC eluting with 0.1% TFA in H<sub>2</sub>O/ MeCN (10 – 90% MeCN).

**Method B, 31o-r.** In a scintillation vial, (*R*)-5-ethynyl-*N*-(3-hydroxy-3-methylbutan-2-yl)picolinamide, **33**, (1 equiv) was placed under argon atmosphere, and dissolved in DMF (0.25M). An aryl halide (1 equiv), Pd(PPh<sub>3</sub>)<sub>4</sub> (0.05 equiv) and CuI (0.1 equiv) added, followed by Et<sub>3</sub>N (17 equiv). The reaction mixture heated to 60 °C until the reaction was determined to be complete by LC/MS (1 to 2 h). The reaction mixture was filtered through a Fisherbrand Nylon 0.45μm syringe filter and purified directly by preparative RP-HPLC eluting with 0.1% TFA in H<sub>2</sub>O/ MeCN (10 – 90% MeCN).

**(*R*)-5-((3-Fluorophenyl)ethynyl)-*N*-(3-hydroxy-3-methylbutan-2-yl)-3-methylpicolinamide (31d).** LCMS: t<sub>R</sub> = 0.798 min, >98% at 215 and 254 nm, *m/z* = 341.2 [M + H]<sup>+</sup>. [α]<sub>D</sub><sup>20</sup> = -14.2 (c = 0.74, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.48 (1H, d, *J* = 1.6 Hz), 8.24 (1H, d, *J* = 8.8 Hz), 7.70 (1H, d, *J* = 1.2 Hz), 7.32 (2H, m), 7.24 (1H, m), 7.10 (1H, m), 4.10 (1H, dq, *J* = 8.9, 6.9 Hz), 2.73 (3H, s), 2.68 (1H, bs), 1.27 (9H, m); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 165.6, 162.3 (d, *J*<sub>C-F</sub> = 245.6 Hz), 147.6, 146.0, 143.0, 135.0, 130.1 (d, *J*<sub>C-F</sub> = 8.6 Hz), 127.6 (d, *J*<sub>C-F</sub> = 3.0 Hz), 124.0 (d, *J*<sub>C-F</sub> = 9.4 Hz), 121.8, 118.5 (d, *J*<sub>C-F</sub> = 22.8 Hz), 116.4 (d, *J*<sub>C-F</sub> = 21.1 Hz), 92.8, 86.2, 73.1, 53.4, 27.6, 25.5, 20.3, 16.0; HRMS (ES<sup>+</sup>, M+H) calcd for C<sub>20</sub>H<sub>22</sub>FN<sub>2</sub>O<sub>2</sub>: 341.1665, found: 341.1663.

**(*R*)-*N*-(3-Hydroxy-3-methylbutan-2-yl)-5-(pyridin-2-ylethynyl)picolinamide (31i).** LCMS: t<sub>R</sub> = 0.533 min, >98% at 215 and 254 nm, *m/z* = 310.2 [M + H]<sup>+</sup>. [α]<sub>D</sub><sup>20</sup> = -21.1 (c = 0.53, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 8.71 (1H, bs), 8.64 (1H, d, *J* = 4.6 Hz), 8.17 (2H, d, *J* = 8.1 Hz), 7.99 (1H, dd, *J* = 8.1, 1.7 Hz), 7.71 (1H, dt, *J* = 7.8, 1.5 Hz), 7.55 (1H, d, *J* = 7.9 Hz), 7.28 (1H, m), 4.11 (1H, dq, *J* = 8.9, 6.7 Hz), 2.70 (1H, bs), 1.27 (3H, d, *J* = 6.8 Hz), 1.26 (6H, d, *J* = 8.1 Hz); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 163.7, 150.7, 150.2, 148.8, 142.4, 140.1, 136.3, 127.4, 123.4, 121.9, 121.7, 93.4, 85.1, 72.9, 53.7, 27.6, 25.8, 15.9; HRMS (ES<sup>+</sup>, M+H) calcd for C<sub>18</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub>: 310.1556, found: 310.1554.

**(R)-N-(3-Hydroxy-3-methylbutan-2-yl)-5-(pyridin-4-ylethynyl)picolinamide (31k).** LCMS:  $t_R = 0.461$  min, >98% at 215 and 254 nm,  $m/z = 310.2$   $[M + H]^+$ .  $[\alpha]_D^{20} = -27.8$  ( $c = 0.48$ ,  $CHCl_3$ );  $^1H$  NMR (400MHz,  $CDCl_3$ )  $\delta$  8.67 (1H, dd,  $J = 1.6$  Hz), 8.65 (1H, bs), 8.20 (1H, m), 8.17 (1H, bs), 7.97 (1H, dd,  $J = 8.2, 2.0$  Hz), 7.41 (2H, d,  $J = 4.9$  Hz), 4.12 (1H, dq,  $J = 9.5, 7.0$  Hz), 2.59 (1H, bs), 1.29 (3H, d,  $J = 6.9$  Hz), 1.28 (6H, d,  $J = 5.6$  Hz);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  163.6, 150.6, 149.8, 149.0, 140.1, 130.3, 125.6, 121.8, 121.7, 91.5, 89.8, 72.9, 53.7, 27.6, 25.8, 15.9; HRMS (ES+, M+H) calcd for  $C_{18}H_{20}N_3O_2$ : 310.1556, found: 310.1554.

**(R)-5-Ethynyl-N-(3-hydroxy-3-methylbutan-2-yl)picolinamide (33).** In a 100 mL round bottom flask (R)-5-bromo-N-(3-hydroxy-3-methylbutan-2-yl)picolinamide (1.5 g, 5.2 mmol, 1 equiv),  $PdCl_2(PPh_3)_2$  (187 mg, 0.26 mmol, 0.05 equiv), and CuI (99 mg, 0.52 mmol, 0.1 equiv) were combined, placed under argon atmosphere, and dissolved in DMF (15 mL, 0.35M). Trimethylsilylacetylene (1.1 mL, 7.8 mmol, 1.5 equiv) was added, followed by  $Et_2NH$  (3.2 mL, 31.2 mmol, 6 equiv). The reaction mixture heated to 90 °C for 45 min after which the reaction was determined to be complete by LC/MS. The reaction mixture was diluted with EtOAc (45 mL) and washed with  $H_2O$  (3 x 15 mL). The organic layer was dried with  $Na_2SO_4$ , concentrated and purified via silica gel chromatography eluting with Hex/EtOAc (0 to 75 % EtOAc) with the trimethylsilyl protected acetylene eluting at 40% EtOAc as a pale yellow oil (1.22 g, 77% yield).  $^1H$  NMR (400MHz,  $CDCl_3$ )  $\delta$  8.55 (1H, bs), 8.10 (1H, d,  $J = 9.1$  Hz), 8.14 (1H, dd,  $J = 8.1, 1.8$  Hz), 7.85 (1H, dd,  $J = 8.1, 2.0$  Hz), 4.10 (1H, m), 2.65 (1H, bs), 1.27 (3H, d,  $J = 7.7$  Hz), 1.26 (6H, d,  $J = 5.8$  Hz), 0.26 (9H, s).

The trimethylsilyl protected acetylene (1.22 g, 4.0 mmol, 1 equiv) was dissolved in MeOH/THF (1:1, 16 mL, 0.25 M) and  $K_2CO_3$  (1.1 g, 8.0 mmol, 2 equiv) was added. The reaction was stirred at room temperature for 1h after which it was determined to be complete by LC/MS. The reaction mixture was diluted with  $H_2O$  (5 mL) and extracted with EtOAc (3 x 20 mL). The organic layer was dried with  $Na_2SO_4$ , concentrated and purified via silica gel chromatography eluting with Hex/EtOAc (0 to 100 % EtOAc) with the acetylene eluting at 60 – 80% EtOAc as a pale yellow oil (883 mg, 95% yield).  $[\alpha]_D^{20} = -4.9$  ( $c = 0.73$ ,  $CHCl_3$ );  $^1H$  NMR (400MHz,  $CDCl_3$ )  $\delta$  8.61 (1H, d,  $J = 1.2$  Hz), 8.17 (1H, bs), 8.14 (1H, dd,  $J = 8.0, 0.3$  Hz), 7.90 (1H, dd,  $J = 8.1, 2.0$  Hz), 4.10 (1H, dq,  $J = 9.1, 6.9$  Hz), 3.34 (1H, s), 2.58 (1H, s), 1.27 (3H, d,  $J = 6.9$  Hz), 1.26 (6H, d,  $J = 5.8$  Hz);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  163.7, 151.0, 148.8, 140.4, 121.8, 121.7, 82.7, 79.9, 73.0, 53.7, 27.6, 25.7, 15.9; HRMS (ES+, M+Na) calcd for  $C_{13}H_{16}N_2O_2Na$ : 255.1109, found: 255.1107.

### General Methods for Series 36.

**Method A, 36a-b, d-e, h-j.** In a scintillation vial, an aryl halide carboxylic acid, **34a**, (1 equiv),  $PdCl_2(PPh_3)_2$  (0.05 equiv), and CuI (0.1 equiv) were combined, placed under argon atmosphere, and dissolved in DMF (0.25M). 3-

1 fluorophenylacetylene (1.25 equiv) was added, followed by Et<sub>2</sub>NH (6 equiv). The reaction mixture heated to 90 °C for 45  
2 min after which the reaction was determined to be complete by LC/MS. The reaction mixture was filtered through a  
3 Fisherbrand Nylon 0.45µm syringe filter and purified directly by preparative RP-HPLC eluting with 0.1% TFA in H<sub>2</sub>O/  
4 MeCN (10 – 90% MeCN) to yield the desired acetylene carboxylic acid **35a**.  
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8 In a scintillation vial acetylene carboxylic acid **35a** (1.0 equiv) and HATU (1.1 equiv) were combined in DMF (0.25 M).  
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10 *N,N*-Diisopropylethylamine (DIPEA, 5 equiv) was then added. After the reaction mixture was stirred for 10 min a  
11 solution of freshly prepared (*R*)-3-amino-2-methylbutan-2-ol TFA salt in DMF (1.1 equiv) was added and the reaction  
12 was stirred until determined to be complete by LC/MS (2 to 20 h). The reaction mixture was filtered through a  
13 Fisherbrand Nylon 0.45µm syringe filter and purified directly by preparative RP-HPLC eluting with 0.1% TFA in H<sub>2</sub>O/  
14 MeCN (10 – 90% MeCN).  
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22 **Method B, 36c, f, g.** In a scintillation vial an aryl halide carboxylic ester, **34b**, (1 equiv), Pd(PPh<sub>3</sub>)<sub>4</sub> (0.05 equiv) and CuI  
23 (0.1 equiv) were combined, placed under an argon atmosphere, and dissolved in DMF (0.25 M). 3-fluorophenylacetylene  
24 (1.25 equiv) was added, followed by Et<sub>3</sub>N (17 equiv). The reaction mixture heated to 60 °C until the reaction was  
25 determined to be complete by LC/MS (1 to 2 h). The reaction mixture was filtered through a Fisherbrand Nylon 0.45µm  
26 syringe filter and purified directly by preparative RP-HPLC eluting with 0.1% TFA in H<sub>2</sub>O/ MeCN (10 – 90% MeCN) to  
27 yield the desired acetylene carboxylic ester **35b**.  
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35 Methyl or ethyl ester **35b** (1 equiv) was dissolved in THF:H<sub>2</sub>O (4:1, 0.1 M), and LiOH (3 equiv) was added. The reaction  
36 was vigorously stirred until the starting material was observed to be consumed by LC/MS (30 min to 16h). The reaction  
37 was quenched with 2 M HCl and extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, dried with  
38 Na<sub>2</sub>SO<sub>4</sub>, concentrated, and used without further purification. In a scintillation vial the resulting acetylene carboxylic acid  
39 (1.0 equiv) and HATU (1.1 equiv) were combined in DMF (0.25 M). *N,N*-Diisopropylethylamine (DIPEA, 5 equiv) was  
40 then added. After the reaction mixture was stirred for 10 min a solution of freshly prepared (*R*)-3-amino-2-methylbutan-  
41 2-ol TFA salt in DMF (1.1 equiv) was added and the reaction was stirred until determined to be complete by LC/MS (2 to  
42 20 h). The reaction mixture was filtered through a Fisherbrand Nylon 0.45µm syringe filter and purified directly by  
43 preparative RP-HPLC eluting with 0.1% TFA in H<sub>2</sub>O/ MeCN (10 – 90% MeCN).  
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53 **(*R*)-6-((3-Fluorophenyl)ethynyl)-*N*-(3-hydroxy-3-methylbutan-2-yl)nicotinamide (36b).** LCMS: t<sub>R</sub> = 0.765 min,  
54 >98% at 215 and 254 nm, *m/z* = 326. 9 [M + H]<sup>+</sup>. [α]<sub>D</sub><sup>20</sup> = -7.5 (c = 0.57, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 8.98  
55 (1H, d, *J* = 1.8 Hz), 8.10 (1H, dd, *J* = 8.1, 2.3 Hz), 7.55 (1H, d, *J* = Hz), 7.32 (3H, m), 7.08 (1H, m), 6.76 (1H, d, *J* = 8.8  
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Hz), 4.13 (1H, dq,  $J = 8.8, 6.8$  Hz), 2.62 (1H, bs), 1.29 (6H, d,  $J = 4.4$  Hz), 1.27 (3H, d,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  164.8, 162.3 (d,  $J_{\text{C-F}} = 245$  Hz), 148.3, 145.3, 135.3, 130.1 (d,  $J_{\text{C-F}} = 8.5$  Hz), 129.1, 128.0 (d,  $J_{\text{C-F}} = 7.0$  Hz), 126.9, 123.5 (d,  $J_{\text{C-F}} = 9.4$  Hz), 118.8 (d,  $J_{\text{C-F}} = 23.0$ ), 116.8 (d,  $J_{\text{C-F}} = 21.0$  Hz), 90.0 (d,  $J_{\text{C-F}} = 3.4$  Hz), 88.7, 72.6, 53.7, 28.0, 26.3, 15.7; HRMS (ES+, M+H) calcd for  $\text{C}_{19}\text{H}_{20}\text{FN}_2\text{O}_2$ : 327.1509, found: 327.1510.

**(R)-6-((3-Fluorophenyl)ethynyl)-N-(3-hydroxy-3-methylbutan-2-yl)-5-methylnicotinamide (36e).** LCMS:  $t_{\text{R}} = 0.816$  min, >98% at 215 and 254 nm,  $m/z = 341.2$   $[\text{M} + \text{H}]^+$ .  $[\alpha]_{\text{D}}^{20} = -17.9$  ( $c = 0.43$ ,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.57 (1H, s), 8.17 (1H, d,  $J = 7.2$  Hz), 8.07 (1H, s), 7.36 (2H, s), 7.24 (1H, m), 7.09 (1H, m), 4.12 (1H, dq,  $J = 8.9, 6.9$  Hz), 2.55 (3H, s), 1.71 (1H, bs), 1.29 (3H, d,  $J = 6.8$  Hz), 1.28 (6H, d,  $J = 9.0$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  164.2, 162.4 (d,  $J_{\text{C-F}} = 245.7$  Hz), 150.6, 150.5, 148.2, 130.1 (d,  $J_{\text{C-F}} = 8.6$  Hz), 127.6 (d,  $J_{\text{C-F}} = 3.0$  Hz), 124.2 (d,  $J_{\text{C-F}} = 9.2$  Hz), 122.9, 122.6, 118.6 (d,  $J_{\text{C-F}} = 22.7$  Hz), 116.4 (d,  $J_{\text{C-F}} = 21.1$  Hz), 96.8, 85.4, 73.1, 53.8, 27.6, 25.6, 20.4, 15.9; HRMS (ES+, M+H) calcd for  $\text{C}_{20}\text{H}_{22}\text{FN}_2\text{O}_2$ : 341.1665, found: 341.1664.

**(R)-2-((3-Fluorophenyl)ethynyl)-N-(3-hydroxy-3-methylbutan-2-yl)pyrimidine-5-carboxamide (36f).** LCMS:  $t_{\text{R}} = 0.708$  min, >98% at 215 and 254 nm,  $m/z = 327.9$   $[\text{M} + \text{H}]^+$ .  $[\alpha]_{\text{D}}^{20} = -8.9$  ( $c = 0.67$ ,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.12 (2H, s), 7.43 (1H, d,  $J = 7.8$  Hz), 7.34 (2H, m), 7.14 (1H, td,  $J = 9.2, 2.4$  Hz), 6.75 (1H, d,  $J = 8.8$  Hz), 4.14 (1H, dq,  $J = 8.8, 6.9$  Hz), 2.31 (1H, bs), 1.30 (6H, s), 1.28 (3H, d,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.8, 162.2 (d,  $J_{\text{C-F}} = 246.1$  Hz), 156.1, 154.3, 130.2 (d,  $J_{\text{C-F}} = 8.4$  Hz), 128.6 (d,  $J_{\text{C-F}} = 3.1$  Hz), 125.9, 122.6 (d,  $J_{\text{C-F}} = 9.4$  Hz), 119.4 (d,  $J_{\text{C-F}} = 23.1$  Hz), 117.5 (d,  $J_{\text{C-F}} = 21.2$  Hz), 88.6 (d,  $J_{\text{C-F}} = 3.5$  Hz), 88.2, 72.5, 53.7, 28.0, 26.4, 15.7; HRMS (ES+, M+H) calcd for  $\text{C}_{18}\text{H}_{19}\text{FN}_3\text{O}_2$ : 328.1461, found: 328.1459.

**5-((3-Fluorophenyl)ethynyl)-N-(3-hydroxy-3-methylbutyl)picolinamide (38p).** LCMS:  $t_{\text{R}} = 0.870$  min, >98% at 215 and 254 nm,  $m/z = 326.9$   $[\text{M} + \text{H}]^+$ .  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.61 (1H, bs), 8.42 (1H, m), 8.15 (1H, d,  $J = 8.1$  Hz), 7.90 (1H, dd,  $J = 8.1, 2.0$  Hz), 7.31 (2H, m), 7.22 (1H, m), 7.07 (1H, m), 3.63 (2H, q,  $J = 6.1$  Hz), 2.44 (1H, bs), 1.82 (2H, t,  $J = 7.0$  Hz), 1.30 (6H, s);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  163.8, 162.3 (d,  $J_{\text{C-F}} = 245.7$  Hz), 150.4, 148.7, 139.7, 130.1 (d,  $J_{\text{C-F}} = 8.6$  Hz), 127.6 (d,  $J_{\text{C-F}} = 3.1$  Hz), 123.9 (d,  $J_{\text{C-F}} = 9.4$  Hz), 122.7, 121.5, 118.5 (d,  $J_{\text{C-F}} = 22.8$  Hz), 116.4 (d,  $J_{\text{C-F}} = 21.1$  Hz), 93.1 (d,  $J_{\text{C-F}} = 3.5$  Hz), 86.3, 70.5, 42.0, 35.8, 29.6; HRMS (ES+, M+H) calcd for  $\text{C}_{19}\text{H}_{20}\text{FN}_2\text{O}_2$ : 327.1509, found: 327.1508.

**(R)-5-((3-Fluorophenyl)ethynyl)-N-(1,1,1-trifluoropropan-2-yl)picolinamide (38r).** LCMS:  $t_{\text{R}} = 1.030$  min, >98% at 215 and 254 nm,  $m/z = 336.9$   $[\text{M} + \text{H}]^+$ .  $[\alpha]_{\text{D}}^{20} = 10.5$  ( $c = 0.59$ ,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.68 (1H, d,  $J = 1.4$  Hz), 8.20 (1H, d,  $J = 8.1$  Hz), 8.11 (1H, d,  $J = 9.8$  Hz), 7.97 (1H, dd,  $J = 7.8, 1.8$  Hz), 7.35 (2H, m), 7.27 (1H, m),



7.10 (1H, m), 4.88 (1H, m), 1.46 (3H, d,  $J = 7.0$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  163.3, 162.4 (d,  $J_{\text{C-F}} = 245.7$  Hz), 150.5, 147.4, 139.9, 130.2 (d,  $J_{\text{C-F}} = 8.6$  Hz), 127.7 (d,  $J_{\text{C-F}} = 3.1$  Hz), 125.3 (q,  $J_{\text{C-F}} = 279.3$  Hz), 123.8 (d,  $J_{\text{C-F}} = 9.4$  Hz), 123.2, 122.0, 118.6 (d,  $J_{\text{C-F}} = 22.9$  Hz), 116.6 (d,  $J_{\text{C-F}} = 21.0$  Hz), 93.7, 86.1, 46.5 (q,  $J_{\text{C-F}} = 31.6$  Hz), 14.4 (d,  $J_{\text{C-F}} = 1.5$  Hz); HRMS (ES+, M+H) calcd for  $\text{C}_{17}\text{H}_{13}\text{F}_4\text{N}_2\text{O}$ : 337.0964, found: 337.0966.

**(S)-5-((3-Fluorophenyl)ethynyl)-N-(1,1,1-trifluoropropan-2-yl)picolinamide (38s).** LCMS:  $t_{\text{R}} = 1.030$  min, >98% at 215 and 254 nm,  $m/z = 336.9$   $[\text{M} + \text{H}]^+$ .  $[\alpha]_{\text{D}}^{20} = -10.0$  ( $c = 0.82$ ,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$  8.68 (1H, d,  $J = 1.4$  Hz), 8.20 (1H, d,  $J = 8.1$  Hz), 8.11 (1H, d,  $J = 9.8$  Hz), 7.97 (1H, dd,  $J = 7.8, 1.8$  Hz), 7.35 (2H, m), 7.27 (1H, m), 7.10 (1H, m), 4.88 (1H, m), 1.46 (3H, d,  $J = 7.0$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  163.3, 162.4 (d,  $J_{\text{C-F}} = 245.7$  Hz), 150.5, 147.4, 139.9, 130.2 (d,  $J_{\text{C-F}} = 8.6$  Hz), 127.7 (d,  $J_{\text{C-F}} = 3.1$  Hz), 125.3 (q,  $J_{\text{C-F}} = 279.3$  Hz), 123.8 (d,  $J_{\text{C-F}} = 9.4$  Hz), 123.2, 122.0, 118.6 (d,  $J_{\text{C-F}} = 22.9$  Hz), 116.6 (d,  $J_{\text{C-F}} = 21.0$  Hz), 93.7, 86.1, 46.5 (q,  $J_{\text{C-F}} = 31.6$  Hz), 14.4 (d,  $J_{\text{C-F}} = 1.5$  Hz); HRMS (ES+, M+H) calcd for  $\text{C}_{17}\text{H}_{13}\text{F}_4\text{N}_2\text{O}$ : 337.0964, found: 337.0963.

**5-((3-Fluorophenyl)ethynyl)-N-(3-methyloxetan-3-yl)picolinamide (38t).** LCMS:  $t_{\text{R}} = 0.879$  min, >98% at 215 and 254 nm,  $m/z = 310.9$   $[\text{M} + \text{H}]^+$ .  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$  ;  $\delta$  8.64 (1H, d,  $J = 1.4$  Hz), 8.29 (1H, bs), 8.13 (1H, d,  $J = 7.8$  Hz), 7.93 (1H, dd,  $J = 8.1, 2.0$  Hz), 7.33 (2H, m), 7.24 (1H, m), 7.09 (1H, m), 4.94 (2H, d,  $J = 6.4$  Hz), 4.57 (2H, d,  $J = 6.5$  Hz), 1.77 (3H, s);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.9, 162.3 (d,  $J_{\text{C-F}} = 245.9$  Hz), 150.4, 148.2, 139.8, 130.1 (d,  $J_{\text{C-F}} = 8.6$  Hz), 127.6 (d,  $J_{\text{C-F}} = 3.0$  Hz), 123.8 (d,  $J_{\text{C-F}} = 9.3$  Hz), 122.7, 121.3, 118.5 (d,  $J_{\text{C-F}} = 22.9$  Hz), 116.5 (d,  $J_{\text{C-F}} = 21.0$  Hz), 93.5 (d,  $J_{\text{C-F}} = 3.5$  Hz), 86.2, 81.8, 53.6, 23.6; HRMS (ES+, M+H) calcd for  $\text{C}_{18}\text{H}_{16}\text{FN}_2\text{O}_2$ : 311.1196, found: 311.1197.

**5-((3-Fluorophenyl)ethynyl)-N-((3-methyloxetan-3-yl)methyl)picolinamide (38u).** LCMS:  $t_{\text{R}} = 0.797$  min, >98% at 215 and 254 nm,  $m/z = 325.1$   $[\text{M} + \text{H}]^+$ .  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$  8.67 (1H, d,  $J = 1.3$  Hz), 8.29 (1H, bs), 8.20 (1H, d,  $J = 7.6$  Hz), 7.97 (1H, dd,  $J = 6.0, 2.0$  Hz), 7.35 (2H, m), 7.26 (1H, m), 7.10 (1H, m), 4.59 (2H, d,  $J = 6.0$  Hz), 4.44 (2H, d,  $J = 6.0$  Hz), 3.68 (2H, d,  $J = 6.5$  Hz), 1.40 (3H, s);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  164.2, 162.4 (d,  $J_{\text{C-F}} = 245.9$  Hz), 150.5, 148.2, 139.9, 130.2 (d,  $J_{\text{C-F}} = 8.7$  Hz), 127.7 (d,  $J_{\text{C-F}} = 3.0$  Hz), 123.9 (d,  $J_{\text{C-F}} = 9.4$  Hz), 122.7, 121.8, 118.6 (d,  $J_{\text{C-F}} = 22.8$  Hz), 116.6 (d,  $J_{\text{C-F}} = 21.1$  Hz), 93.4 (d,  $J_{\text{C-F}} = 3.7$  Hz), 86.3, 80.3, 45.9, 40.2, 22.0; HRMS (ES+, M+H) calcd for  $\text{C}_{19}\text{H}_{18}\text{FN}_2\text{O}_2$ : 325.1352, found: 325.1353.

**5-((3-Fluorophenyl)ethynyl)picolinic acid (37).** In a 100 mL round bottom flask 5-bromopicolinic acid (3g, 14.8 mmol, 1 equiv),  $\text{PdCl}_2(\text{PPh}_3)_2$  (519 mg, 0.74 mmol, 0.05 equiv), and CuI (282 mg, 1.48 mmol, 0.1 equiv) were combined, placed under an argon atmosphere and dissolved in DMF (50 mL, 0.3M). 3-Fluorophenylacetylene (2.05 mL, 17.76 mmol, 1.2

equiv) was added, followed by Et<sub>2</sub>NH (9.2 mL, 88.8 mmol, 6 equiv). The reaction mixture heated to 90 °C for 45 min after which the reaction was determined to be complete by LC/MS. The crude reaction mixture was diluted with EtOAc (50 mL) and H<sub>2</sub>O (50 mL). After separating the organic layer, the aqueous layer was washed with EtOAc (2 x 25 mL). The aqueous layer was then acidified to ~ pH = 2 with 2M HCl, and extracted with EtOAc (3 x 50 mL). The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub>, concentrated, and used without further purification. The product was isolated as a white solid (2.32 g, 65%). <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD) δ 8.81 (1H, bs), 8.16 (2H, m), 7.44 (2H, m), 7.34 (1H, d, *J* = 9.7 Hz), 7.21 (1H, m); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ 167.0, 163.9 (d, *J*<sub>C-F</sub> = 244.4 Hz), 152.5, 148.0, 141.3, 131.7 (d, *J*<sub>C-F</sub> = 8.6 Hz), 129.0, 125.8, 125.2 (d, *J*<sub>C-F</sub> = 9.3 Hz), 124.8, 119.4 (d, *J*<sub>C-F</sub> = 23.4 Hz), 117.7 (d, *J*<sub>C-F</sub> = 21.4 Hz), 94.8, 86.8; HRMS (ES<sup>+</sup>, M+H) calcd for C<sub>14</sub>H<sub>9</sub>FNO<sub>2</sub>: 242.0617, found: 242.0618.

**Amide Analogs 19, 38a-d, g-v.** In a scintillation vial 5-((3-fluorophenyl)ethynyl)picolinic acid, **37**, (1.0 equiv) and HATU (1.1 equiv) were combined in DMF (0.25 M). *N,N*-Diisopropylethylamine (DIPEA, 3 equiv) was then added. The reaction mixture was stirred for 10 min after which an amine (1.1 equiv) was added. The reaction was stirred until determined to be complete by LC/MS (2 to 20 h). The reaction mixture was filtered through a Fisherbrand Nylon 0.45μm syringe filter and purified directly by preparative RP-HPLC eluting with 0.1% TFA in H<sub>2</sub>O/ MeCN (10 – 90% MeCN).

**(*R*)-5-((3-Fluorophenyl)ethynyl)-*N*-(3-hydroxy-3-methylbutan-2-yl)picolinamide (19).** LCMS: *t*<sub>R</sub> = 0.731 min, >98% at 215 and 254 nm, *m/z* = 327.1 [M + H]<sup>+</sup>. [α]<sub>D</sub><sup>20</sup> = -22.2 (c = 0.43, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 8.65 (1H, dd, *J* = 1.3, 0.7 Hz), 8.19 (2H, dd, *J* = 8.1, 0.7 Hz), 7.95 (1H, dd, *J* = 8.1, 2.0 Hz), 7.34 (2H, m), 7.24 (1H, m), 7.09 (1H, m), 4.13 (1H, dq, *J* = 9.1, 6.8 Hz), 2.50 (1H, bs), 1.29 (3H, d, *J* = 6.8 Hz), 1.28 (6H, d, *J* = 8.0 Hz); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 163.8 162.3 (d, *J*<sub>C-F</sub> = 245.7 Hz), 150.4, 148.4, 139.8, 130.1 (d, *J*<sub>C-F</sub> = 8.6 Hz), 127.7 (d, *J*<sub>C-F</sub> = 3.0 Hz), 123.9 (d, *J*<sub>C-F</sub> = 9.3 Hz), 122.5, 121.8, 118.5 (d, *J*<sub>C-F</sub> = 22.9 Hz), 116.5 (d, *J*<sub>C-F</sub> = 21.0 Hz), 93.3, 86.3, 73.1, 53.8, 27.6, 25.7, 15.9; HRMS (ES<sup>+</sup>, M+H) calcd for C<sub>19</sub>H<sub>20</sub>FN<sub>2</sub>O<sub>2</sub>: 327.1509, found: 327.1507.

**(*R*)-*N*-(3-Fluoro-3-methylbutan-2-yl)-5-((3-fluorophenyl)ethynyl)picolinamide (38e).** (*R*)-5-((3-fluorophenyl)ethynyl)-*N*-(3-hydroxy-3-methylbutan-2-yl)picolinamide, **19** (40 mg, 0.12 mmol, 1.0 equiv) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (1.2 mL, 0.1 M) and cooled to -78 °C. DAST (19μL, 0.12 mmol, 1.0 equiv) was added dropwise and the reaction was allowed to slowly warm to rt over 4 h. The reaction was determined to be complete by LC/MS and was quenched carefully by the addition of H<sub>2</sub>O (0.5 mL). The aqueous layer was extracted with EtOAc (3 x 5 mL), dried Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by preparative RP-HPLC eluting with 0.1% TFA in H<sub>2</sub>O/ MeCN (10 – 90% MeCN) to afford the title compound in 30% yield. LCMS: *t*<sub>R</sub> = 0.926 min, >98% at 215 and 254 nm, *m/z* = 329.1 [M + H]<sup>+</sup>. [α]<sub>D</sub><sup>20</sup> =

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-5.9 (c = 1.16, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 8.68 (1H, d, *J* = 1.3 Hz), 8.19 (1H, dd, *J* = 12.0, 0.4 Hz), 8.14 (1H,  
d, *J* = 9.8 Hz), 7.96 (1H, dd, *J* = 8.1, 2.0 Hz), 7.35 (2H, m), 7.27 (1H, m), 7.10 (1H, m), 4.29 (1H, m), 1.57 (1H, bs), 1.46  
(3H, d, *J* = 16.7 Hz), 1.40 (3H, d, *J* = 16.7 Hz), 1.33 (3H, d, *J* = 6.9 Hz); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 163.2, 162.4 (d,  
*J*<sub>C-F</sub> = 22.9 Hz), 150.5, 148.4, 139.8, 130.1 (d, *J*<sub>C-F</sub> = 8.7 Hz), 127.7 (d, *J*<sub>C-F</sub> = 3.2 Hz), 124.0 (d, *J*<sub>C-F</sub> = 9.4 Hz), 122.6,  
121.8, 118.6 (d, *J*<sub>C-F</sub> = 21.8 Hz), 116.5, (d, *J*<sub>C-F</sub> = 21.0 Hz), 96.6 (d, *J*<sub>C-F</sub> = 170.7 Hz), 93.3 (d, *J*<sub>C-F</sub> = 3.4 Hz), 86.4, 51.8 (d,  
*J*<sub>C-F</sub> = 22.2 Hz), 24.6 (d, *J*<sub>C-F</sub> = 7.8 Hz), 24.3 (d, *J*<sub>C-F</sub> = 7.4 Hz), 15.6 (d, *J*<sub>C-F</sub> = 3.7 Hz); HRMS (ES+, M+H) calcd for  
C<sub>19</sub>H<sub>18</sub>F<sub>2</sub>N<sub>2</sub>ONa: 351.1285, found: 351.1282.

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**(R)-5-((3-Fluorophenyl)ethynyl)-N-(3-methoxy-3-methylbutan-2-yl)picolinamide (38f).** (R)-5-((3-  
fluorophenyl)ethynyl)-N-(3-hydroxy-3-methylbutan-2-yl)picolinamide, **19** (68 mg, 0.21 mmol, 1.0 equiv) was dissolved  
in THF (2 mL, 0.1 M) and cooled to 0 °C. NaH (11.1 mg, 0.46 mmol, 2.2 equiv) was added. The reaction mixture was  
warmed to rt and stirred for 10 min. Methyl iodide (15 μL, 0.23 mmol, 1.1 equiv) was added and the reaction mixture  
was stirred at room temperature overnight. Analysis of the reaction mixture by LC/MS revealed ~70% of the desired  
product, ~20% starting material, and ~10% dimethylated product. The reaction was quenched with NH<sub>4</sub>Cl (sat'd, aq) and  
extracted with EtOAc (3 x 5 mL). The organic layers were dried with Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by preparative  
RP-HPLC eluting with 0.1% TFA in H<sub>2</sub>O/ MeCN (10 – 90% MeCN) to afford the product in 58% yield. LCMS: t<sub>R</sub> =  
0.824 min, >98% at 215 and 254 nm, *m/z* = 341.2 [M + H]<sup>+</sup>. [α]<sub>D</sub><sup>20</sup> = -31.7 (c = 0.65, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400MHz,  
CDCl<sub>3</sub>) δ 8.66 (1H, d, *J* = 1.6 Hz), 8.21 (1H, d, *J* = 9.6 Hz), 8.17 (1H, d, *J* = 8.1 Hz), 7.92 (1H, dd, *J* = 8.1, 1.9 Hz), 7.32  
(2H, m), 7.23, (1H, m), 7.07 (1H, m), 4.18 (dq, 1H, *J* = 9.5, 6.7 Hz), 3.25 (3H, s), 1.23 (3H, d, *J* = 6.9 Hz), 1.20 (6H, d, *J*  
= 11.6 Hz), ; <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 163.0, 162.3 (d, *J*<sub>C-F</sub> = 245.6 Hz), 150.4, 148.8, 139.6, 130.1 (d, *J*<sub>C-F</sub> = 8.6  
Hz), 127.6 (d, *J*<sub>C-F</sub> = 3.0 Hz), 124.0 (d, *J*<sub>C-F</sub> = 9.3 Hz), 122.2, 121.6, 118.5 (d, *J*<sub>C-F</sub> = 22.9 Hz), 116.4 (d, *J*<sub>C-F</sub> = 21.1 Hz),  
93.0, 86.4, 75.9, 52.3, 49.4, 22.0, 21.9, 15.5; HRMS (ES+, M+H) calcd for C<sub>20</sub>H<sub>22</sub>FN<sub>2</sub>O<sub>2</sub>: 341.1665, found: 341.1664.

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**(S)-5-((3-Fluorophenyl)ethynyl)-N-(3-hydroxy-3-methylbutan-2-yl)picolinamide (38l).** LCMS: t<sub>R</sub> = 0.738 min, >98%  
at 215 and 254 nm, *m/z* = 327.1 [M + H]<sup>+</sup>. [α]<sub>D</sub><sup>20</sup> = 24.3 (c = 0.48, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 8.64 (1H, dd, *J*  
= 1.3, 0.7 Hz), 8.19 (1H, bs), 8.18 (1H, dd, *J* = 8.1, 0.7 Hz), 7.94 (1H, dd, *J* = 8.1, 2.0 Hz), 7.34 (2H, m), 7.24 (1H, m),  
7.09 (1H, m), 4.13 (1H, dq, *J* = 9.1, 6.8 Hz), 2.66 (1H, bs), 1.29 (3H, d, *J* = 6.8 Hz), 1.28 (6H, d, *J* = 8.0 Hz); <sup>13</sup>C NMR  
(100 MHz, CDCl<sub>3</sub>) δ 163.8 162.3 (d, *J*<sub>C-F</sub> = 245.7 Hz), 150.4, 148.4, 139.8, 130.1 (d, *J*<sub>C-F</sub> = 8.6 Hz), 127.6 (d, *J*<sub>C-F</sub> = 3.0  
Hz), 123.9 (d, *J*<sub>C-F</sub> = 9.3 Hz), 122.5, 121.8, 118.5 (d, *J*<sub>C-F</sub> = 22.9 Hz), 116.5 (d, *J*<sub>C-F</sub> = 21.0 Hz), 93.3, 86.3, 73.1, 53.7,  
27.6, 25.7, 15.9; HRMS (ES+, M+H) calcd for C<sub>19</sub>H<sub>20</sub>FN<sub>2</sub>O<sub>2</sub>: 327.1509, found: 327.1507.

**General Amide Coupling and Sonogashira Two-step Procedure (44a,b,d, 45).**

In a scintillation vial 5-bromopicolinic acid (1.0 equiv) and HATU (1.1 equiv) were combined in DMF (0.25 M). *N,N*-Diisopropylethylamine (DIPEA, 5 equiv) was then added. After the reaction mixture was stirred for 10 min the desired amine was added and the reaction was stirred until determined to be complete by LC/MS (2 to 20 h). The reaction mixture was filtered through a Fisherbrand Nylon 0.45 $\mu$ m syringe filter and purified directly by preparative RP-HPLC eluting with 0.1% TFA in H<sub>2</sub>O/ MeCN (10 – 90% MeCN).

In a scintillation vial, the aryl bromide (1 equiv), PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (0.05 equiv), and CuI (0.1 equiv) were combined, placed under argon atmosphere, and dissolved in DMF (0.25M). The desired ethynylpyridine (2- or 4-ethynylpyridine) (1.25 equiv) was added, followed by Et<sub>2</sub>NH (6 equiv). The reaction mixture heated to 90 °C for 45 min after which the reaction was determined to be complete by LC/MS. The reaction mixture was filtered through a Fisherbrand Nylon 0.45 $\mu$ m syringe filter and purified directly by preparative RP-HPLC eluting with 0.1% TFA in H<sub>2</sub>O/ MeCN (10 – 90% MeCN) to yield products **44a-d** and **45**.

**(R)-N-(3-Methylbutan-2-yl)-5-(pyridin-2-ylethynyl)picolinamide (44b).** LCMS: t<sub>R</sub> = 0.727 min, >98% at 215 and 254 nm, *m/z* = 294.2 [M + H]<sup>+</sup>. [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -50.0 (c = 0.78, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  8.72 (1H, d, *J* = 1.7 Hz), 8.65 (1H, d, *J* = 4.7 Hz), 8.20 (1H, d, *J* = 8.1 Hz), 8.00 (1H, dd, *J* = 6.1, 2.0 Hz), 7.89 (1H, d, *J* = 9.0 Hz), 7.73 (1H, dt, *J* = 7.7, 1.6 Hz), 7.57 (1H, d, *J* = 7.8 Hz), 7.30 (1H, m), 4.05 (1H, m), 1.84 (1H, sep, *J* = 6.5 Hz), 1.21 (3H, d, *J* = 6.7 Hz), 0.97 (6H, dd, *J* = 6.8, 4.0 Hz); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  162.8, 150.7, 150.2, 149.1, 142.4, 140.1, 136.3, 127.4, 123.4, 121.7, 121.6, 93.3, 85.1, 50.2, 33.1, 18.6, 18.5, 17.6; HRMS (ES<sup>+</sup>, M+H) calcd for C<sub>18</sub>H<sub>20</sub>N<sub>3</sub>O: 294.1606, found: 294.1607.

**(R)-N-(3-Fluoro-3-methylbutan-2-yl)-5-(pyridin-2-ylethynyl)picolinamide (44c).** (*R*)-N-(3-hydroxy-3-methylbutan-2-yl)-5-(pyridin-2-ylethynyl)picolinamide, **31i** (39 mg, 0.12 mmol, 1.0 equiv) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (1.2 mL, 0.1 M) and cooled to -78 °C. DAST (19 $\mu$ L, 0.12 mmol, 1.0 equiv) was added dropwise and the reaction was allowed to slowly warm to rt over 4 h. The reaction was determined to be complete by LC/MS and was quenched carefully by the addition of H<sub>2</sub>O (0.5 mL). The aqueous layer was extracted with EtOAc (3 x 5 mL), dried Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by preparative RP-HPLC eluting with 0.1% TFA in H<sub>2</sub>O/ MeCN (10 – 90% MeCN) to afford the title compound in 81% yield. LCMS: t<sub>R</sub> = 0.671 min, >98% at 215 and 254 nm, *m/z* = 312.2 [M + H]<sup>+</sup>. [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -14.0 (c = 0.46, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  8.75 (1H, dd, *J* = 1.9, 0.6 Hz), 8.67 (1H, d, *J* = 4.0 Hz), 8.20 (1H, dd, *J* = 8.1, 0.6 Hz), 8.15 (1H, d, *J* = 9.8 Hz), 8.02 (1H, dd, *J* = 8.1, 2.0 Hz), 7.74 (1H, dt, *J* = 7.8, 1.8 Hz), 7.58 (1H, d, *J* = 7.8 Hz), 7.31 (1H, m),

4.28 (1H, m), 1.45 (3H, d,  $J = 16.2$  Hz), 1.45 (3H, d,  $J = 16.4$  Hz), 1.45 (3H, d,  $J = 6.9$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  163.0, 150.8, 150.3, 148.7, 142.4, 140.1, 136.3, 127.4, 123.5, 122.0, 121.7, 96.5 (d,  $J_{\text{C-F}} = 170.8$  Hz), 93.5, 84.8, 51.4 (d,  $J_{\text{C-F}} = 22.3$  Hz), 24.5 (d,  $J_{\text{C-F}} = 5.8$  Hz), 24.2 (d,  $J_{\text{C-F}} = 5.3$  Hz), 15.5 (d,  $J_{\text{C-F}} = 3.8$  Hz); HRMS (ES+, M+H) calcd for  $\text{C}_{18}\text{H}_{19}\text{FN}_3\text{O}$ : 312.1512, found: 312.1511.

***N*-(3-Methyloxetan-3-yl)-5-(pyridin-2-ylethynyl)picolinamide (44d)**. LCMS:  $t_{\text{R}} = 0.453$  min, >98% at 215 and 254 nm,  $m/z = 294.2$  [M + H] $^+$ .  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$  8.72 (1H, d,  $J = 1.2$  Hz), 8.66 (1H, d,  $J = 4.4$  Hz), 8.28 (1H, bs), 8.15 (1H, d,  $J = 8.1$  Hz), 8.02 (1H, dd,  $J = 9.2, 3.0$  Hz), 7.73 (1H, td,  $J = 7.8, 1.6$  Hz), 7.58 (1H, d,  $J = 7.8$  Hz), 7.31 (1H, m), 4.94 (2H, d,  $J = 6.3$  Hz), 4.58 (2H, d,  $J = 6.5$  Hz), 1.77 (3H, s);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.9, 150.8, 150.3, 148.6, 142.4, 140.3, 136.4, 127.4, 123.6, 122.4, 121.4, 93.7, 84.9, 81.9, 53.7, 23.7; HRMS (ES+, M+H) calcd for  $\text{C}_{17}\text{H}_{16}\text{N}_3\text{O}_2$ : 294.1243, found: 294.1240.

***N*-(3-Methyloxetan-3-yl)-5-(pyridin-4-ylethynyl)picolinamide (45)**. LCMS:  $t_{\text{R}} = 0.354$  min, >98% at 215 and 254 nm,  $m/z = 294.2$  [M + H] $^+$ .  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$  8.67 (1H, d,  $J = 1.2$  Hz), 8.65 (2H, m), 8.29 (1H, bs), 8.16 (1H, d,  $J = 8.1$  Hz), 7.98 (1H, dd,  $J = 8.1, 1.9$  Hz), 7.41 (2H, d,  $J = 5.6$  Hz), 4.93 (2H, d,  $J = 6.4$  Hz), 4.58 (2H, d,  $J = 6.4$  Hz), 1.77 (3H, s);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.9, 150.5, 149.9, 148.8, 140.1, 130.2, 125.5, 122.0, 121.4, 91.7, 89.5, 81.8, 53.7, 23.6; HRMS (ES+, M+H) calcd for  $\text{C}_{17}\text{H}_{16}\text{N}_3\text{O}_2$ : 294.1243, found: 294.1242.

### Fluorescence-Based Calcium Flux Assay (Concentration-response curve (potency) and glutamate fold shift (efficacy)).

For measurement of compound-evoked increases in intracellular calcium, HEK293 cells stably expressing rat mGlu<sub>5</sub> were plated in 384-well,<sup>44</sup> poly-D-lysine coated, black-walled, clear-bottomed plates in 20  $\mu\text{L}$  of assay medium (DMEM supplemented with 10% dialyzed fetal bovine serum, 20 mM HEPES and 1 mM sodium pyruvate) at a density of 15,000 cells/well. Cells were grown overnight at 37°C/5% CO<sub>2</sub>. The next day, medium was removed from the cells and they were incubated with 20  $\mu\text{l}$ /well of 1  $\mu\text{M}$  Fluo-4AM (Invitrogen, Carlsbad, California) prepared as a 2.3 mM stock in dimethyl sulfoxide (DMSO) and mixed in a 1:1 ratio with 10% (w/v) pluronic acid F-127 and diluted in calcium assay buffer (Hank's Balanced Salt Solution (HBSS; Invitrogen, Carlsbad, CA) supplemented with 20 mM HEPES and 2.5 mM probenecid, pH 7.4) for 50 min at 37°C. Dye loading solution was removed and replaced with 20  $\mu\text{l}$ /well of assay buffer. For PAM potency curves, mGlu<sub>5</sub> compounds were diluted in calcium assay buffer and added to the cells followed by the addition of an EC<sub>20</sub> concentration of glutamate 140 sec later, and then an EC<sub>80</sub> concentration of glutamate 60 sec later. For

1 fold-shift experiments either a single concentration (10  $\mu\text{M}$ ) or multiple fixed concentrations (50 nM - 30  $\mu\text{M}$ ) of mGlu<sub>5</sub>  
2 compound or vehicle were added followed by the addition of a concentration-response curve (CRC) of glutamate 140  
3 seconds later. Calcium flux was measured over time as an increase in fluorescence using a Functional Drug Screening  
4 System 6000 (FDSS 6000, Hamamatsu, Japan). The change in relative fluorescence over basal was calculated before  
5 normalization to the maximal response to glutamate.  
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### 10 11 **Selectivity Screening.**

12 mGlu<sub>1</sub>. To assess the effect of test compounds at mGlu<sub>1</sub>, Ca<sup>2+</sup> mobilization assays were performed as described  
13 previously (Hammond et al., 2010; Noetzel et al., 2012). Briefly HEK293 cells stably expressing rat mGlu<sub>1</sub> were plated in  
14 black-walled, clear-bottomed, poly-D-lysine coated 384-well plates (Greiner Bio-One, Monroe, NC) in assay medium at a  
15 density of 20,000 cells/well. Calcium flux was measured over time as an increase in fluorescence of the Ca<sup>2+</sup> indicator  
16 dye, Fluo-4AM using a FDSS 6000. Either vehicle or a fixed concentration of test compound (10  $\mu\text{M}$ , final  
17 concentration) was added followed 140 sec later by a CRC of glutamate. Data were analyzed as described above.  
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28 Group II and Group III mGlu<sub>s</sub>. The functional activity of the compounds of interest was assessed at the rat group II and III  
29 mGlu receptors by measuring thallium flux through GIRK channels as previously described (Niswender et al., 2008).  
30 Briefly, HEK293-GIRK cells expressing mGlu subtypes 2, 3, 4, 6, 7 or 8 were plated into 384-well, black-walled, clear-  
31 bottom poly-D-lysine coated plates at a density of 15,000 cells/well in assay medium. A single concentration of test  
32 compound (10  $\mu\text{M}$ ) or vehicle was added followed 140 sec later by a CRC of glutamate (or L-AP4 for mGlu<sub>7</sub>) diluted in  
33 thallium buffer (125 mM NaHCO<sub>3</sub>, 1 mM MgSO<sub>4</sub>, 1.8 mM CaSO<sub>4</sub>, 5 mM glucose, 12 mM thallium sulfate, 10 mM  
34 HEPES) and fluorescence was measured using a FDSS 6000. Data were analyzed as described previously (Niswender et  
35 al., 2008).  
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### 47 **Radioligand binding.**

48 Membranes were prepared from HEK293A cells expressing rat mGlu<sub>5</sub>. Cells were harvested and pelleted by  
49 centrifugation and re-suspended in ice-cold homogenization buffer (50 mM Tris-HCl, 10 mM EDTA, 0.9% NaCl, pH7.4),  
50 and homogenized by 3 x 10 sec bursts. Cell fractions were separated by centrifugation and the resulting pellet re-  
51 suspended in ice-cold assay buffer (50 mM Tris-HCl, 0.9% NaCl, pH7.4). For inhibition binding experiments, membranes  
52 (50  $\mu\text{g}$ /well) were incubated with 7 nM [<sup>3</sup>H]methoxyPEPy and a range of concentrations of test ligand for 1 h at room  
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1 temperature with shaking in assay buffer. 10  $\mu$ M MPEP was used to determine non-specific binding. Assays were  
2 terminated by rapid filtration using a Brandel 96-well plate Harvester, and washed three times with ice-cold assay buffer.  
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4 The next day MicroScint20 was added and radioactivity was counted.  
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### 7 **Electrophysiology (LTD and epileptiform studies).**

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10 All animals used in these studies were cared for in accordance with the NIH Guide for the Care and Use of Laboratory  
11 Animals. 30-40 (LTD experiments) or 24-30 (epileptiform experiments) day old male Sprague–Dawley rats were used.  
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13 The brains were quickly removed and submerged into ice-cold cutting solution (in mM: 110 sucrose, 60 NaCl, 3 KCl,  
14 1.25  $\text{NaH}_2\text{PO}_4$ , 28  $\text{NaHCO}_3$ , 5 glucose, 0.6 (+)-sodium-L-ascorbate, 0.5  $\text{CaCl}_2$ , 7  $\text{MgCl}_2$ ). All solutions were  
15 continuously bubbled with 95%  $\text{O}_2$ /5%  $\text{CO}_2$ . Transverse slices (400  $\mu$ m) were made using a vibratome (Leica VT100S).  
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17 For LTD experiments, individual hippocampi were microdissected out and transferred to a room temperature mixture  
18 containing equal volumes of cutting solution and artificial cerebrospinal fluid (ACSF; in mM: 125 NaCl, 2.5 KCl, 1.25  
19  $\text{NaH}_2\text{PO}_4$ , 25  $\text{NaHCO}_3$ , 25 glucose, 2  $\text{CaCl}_2$ , 1  $\text{MgCl}_2$ ) and equilibrated for 30 min, followed by room temperature ACSF  
20 for 1 h. For epileptiform experiments, individual hippocampi were transferred directly into room temperature ACSF (in  
21 mM: 124 NaCl, 5 KCl, 1.25  $\text{NaH}_2\text{PO}_4$ , 26  $\text{NaHCO}_3$ , 10 glucose, 2  $\text{CaCl}_2$ , 1.2  $\text{MgSO}_4$ ) and equilibrated for 1 h. Slices were  
22 transferred to a submersion recording chamber and equilibrated for 5-10 min at 30-32°C. A bipolar-stimulating electrode  
23 was placed in the stratum radiatum near the CA3-CA1 border in order to stimulate the Schaffer collaterals. Recording  
24 electrodes were filled with ACSF and placed in the stratum radiatum of area CA1 (LTD experiments) or in the pyramidal  
25 cell body layer of CA3 (epileptiform experiments). Field potential recordings were acquired using a Multiclamp 700B  
26 (Warner Instruments) amplifier and pClamp 9.2 software. For stimulation based experiments an intensity that produced  
27 50–60% of the maximum was used as the baseline stimulation.  $\text{mGlu}_5$  compounds were diluted to the appropriate  
28 concentrations in DMSO and applied to the bath using a perfusion system. Sampled data was analyzed by averaging three  
29 sequential field excitatory postsynaptic potentials (fEPSPs) slopes, followed by normalizing to the average slope  
30 calculated during the predrug period (percent of baseline). For epileptiform experiments, spontaneous events were  
31 measured using MiniAnalysis (Synaptosoft Inc, NJ) and inter-event interval (IEI) was normalized to the baseline  
32 response.  
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### 55 **Comparative modeling of receptor.**

1 The comparative model of mGlu<sub>5</sub> was constructed as described previously (Gregory et al., 2013). In brief, the X-ray  
2 crystal structure for human  $\beta$ 2-adrenergic receptor (PDB ID: 2RH1)<sup>50</sup> was chosen as a template based on its high sequence  
3 similarity to mGlu<sub>5</sub>. A profile to profile sequence alignment of TM regions between Class C hepta-helical transmembrane  
4 regions and Class A crystal structure templates was directly adopted from Muhlemann et al., 2006,<sup>50</sup> with the exception of  
5 TM2, TM4 and TM7, which were based on the alignment of CaSR with Class A hepta-helical regions.<sup>51</sup> The sequence  
6 alignment was used to thread the amino acid sequence of the mGlu<sub>5</sub> transmembrane helical region onto the backbone  
7 coordinates of the  $\beta$ 2-adrenergic receptor. The protein structure prediction software package Rosetta 3.4<sup>51</sup> was used to  
8 rebuild the loop regions between the helices using Monte Carlo Metropolis (MCM) fragment replacement combined with  
9 cyclic coordinate descent loop closure (CCD).<sup>53,54</sup> The resulting full sequence models were then subjected to eight  
10 iterative cycles of side chain repacking and gradient minimization of  $\phi$ ,  $\psi$  and  $\chi$  angles in Rosetta Membrane.<sup>56</sup> Over  
11 5,000 comparative models of mGlu<sub>5</sub> were generated and clustered for structural similarity using bcl::Cluster.<sup>57</sup> The lowest  
12 energy model from the largest cluster was used for further ligand docking studies.

### 27 **Computational docking of ligands.**

30 Ligands **19**, **38t** and **31i** were computationally docked into the comparative model of mGlu<sub>5</sub> using Rosetta Ligand.<sup>57-59</sup>  
31 Each modulator was allowed to sample docking poses in a 5 Å radius centered at the putative binding site for allosteric  
32 modulation, determined by the residues known to affect MPEP affinity.<sup>47</sup> Once a binding mode had been determined by  
33 the docking procedure, 10 low energy conformations of the ligand created by MOE (Molecular Operating Environment,  
34 Chemical Computing Group, Ontario, Canada) were tested within the site. Side-chain rotamers around the ligand were  
35 optimized simultaneously in a Monte-Carlo minimization algorithm. The energy function used during the docking  
36 procedure contains terms for van der Waals attractive and repulsive forces, hydrogen bonding, electrostatic interactions  
37 between pairs of amino acids, solvation, and a statistical term derived from the probability of observing a side-chain  
38 conformation from the Protein Data Bank. For each modulator, over 5,000 docked complexes were generated and  
39 clustered for structural similarity using bcl::Cluster.<sup>57</sup> The lowest energy binding mode from the two largest clusters for  
40 each modulator, encompassing ligand positions for which the eastern amide was pointing either towards or away from the  
41 extracellular surface, were used for further analysis.



**SUPPORTING INFORMATION AVAILABLE.** mGlu selectivity for **38t**, progressive glutamate fold-shift analysis,  $pK_B$  and  $\log\beta$  calculations, DMPK procedures, compound characterization, and NMR data for compounds. This information is available free of charge via the Internet at <http://pubs.acs.org>.

**CORRESPONDING AUTHOR:** Tel 615-936-8407, email [shaun.stauffer@vanderbilt.edu](mailto:shaun.stauffer@vanderbilt.edu)

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#### ABBREVIATIONS USED.

mGlu, metabotropic glutamate receptor, PAM, positive allosteric modulator, NAM, negative allosteric modulator, SAM, silent allosteric modulator, PCP, phenylcyclidine, NMDAR, ionotropic N-Methyl-D-aspartate glutamate receptor, DHPG, dihydroxyphenylglycine, LTD, long-term depression, MTEP, 3-((2-methyl-4-thiazolyl)ethynyl)pyridine, MPEP, 2-methyl-6-(phenylethynyl)pyridine, DFB, 3,3'-difluorobenzaldazine, CPPHA, *N*-(4-chloro-2-((1,3-dioxoisindolin-2-yl)methyl)phenyl)-2-hydroxybenzamide, CDPPB, 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide, AHL, amphetamine-induced hyperlocomotion, MWM, Morris water maze, MAM, methylazoxymethanol, EEG, electroencephalogram, DMTP, delayed-matching-to-position, MLPCN, molecular libraries probe production centers network.

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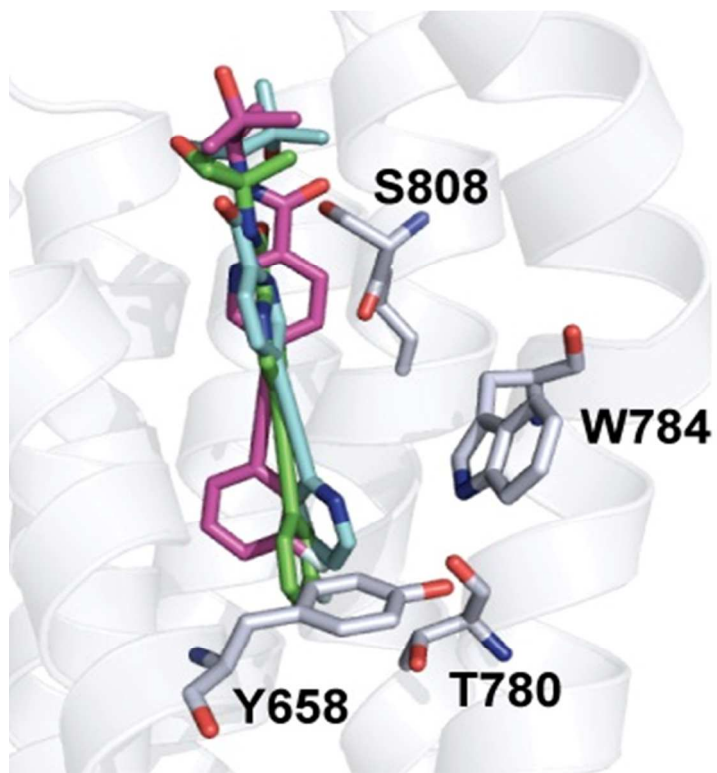
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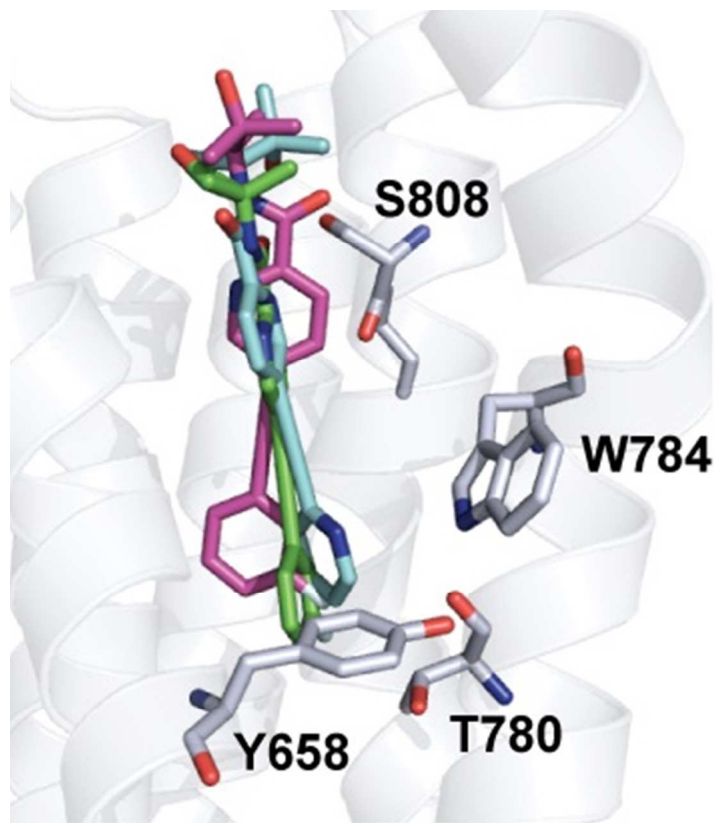
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## TABLE OF CONTENTS GRAPHIC.





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