Evaluation of Synthetic Cytochrome P₄₅₀-Mimetic Metalloporphyrins To Facilitate “Biomimetic” Biotransformation of a Series of mGlu₅ Allosteric Ligands

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ABSTRACT: Allosteric ligands within a given chemotype can have the propensity to display a wide range of pharmacology, as well as unexpected changes in GPCR subtype selectivity, typically mediated by single-atom modifications to the ligand. Due to the unexpected nature of these “molecular switches”, chemotypes with this property are typically abandoned in lead optimization. Recently, we have found that in vivo oxidative metabolism by CYP₄₅₀ can also engender molecular switches within allosteric ligands, changing the mode of pharmacology and leading to unwanted toxicity. We required a higher-throughput approach to assess in vivo metabolic molecular switches, and we turned to a “synthetic liver”, a 96 well kit of biomimetic catalysts (e.g., metalloporphyrins) to rapidly survey a broad panel of synthetic CYP₄₅₀’s ability to oxidize/“metabolize” an mGlu₅ PAM (VU0403602) known to undergo an in vivo CYP₄₅₀-mediated molecular switch. While the synthetic CYP₄₅₀ did generate a number of oxidative “metabolites” at known “hot spots”, several of which proved to be pure mGlu₅ PAMs comparable in potency to the parent, the known CYP₄₅₀-mediated in vivo ago-PAM metabolite, namely, VU0453103, was not formed. Thus, this technology platform has potential to identify hot spots for oxidative metabolism and produce active metabolites of small-molecule ligands in a high-throughput, scalable manner.

INTRODUCTION

From steep structure–activity relationships to family subtype selectivity issues to a potential lack of conservation of allosteric sites (e.g., functional activity) across species, allosteric GPCR drug discovery is fraught with challenges and development caveats.1−13 However, no challenge is more daunting than that of “molecular switches”, subtle structural changes to an allosteric ligand, often single-atom (e.g., introduction of a methyl, fluoro, amino, or hydroxyl moiety), that can reverse the mode of pharmacology (e.g., from a positive to a negative allosteric modulator, PAM to NAM, or vice versa) or change family subtype selectivity.1−11 Fortunately, molecular switches are not present across all allosteric ligands but occur rather randomly within certain highly planar chemotypes, suggesting shallow induced fit pockets. While molecular switches have oftentimes been proven beneficial to gain entry to activity at a given receptor subtype (e.g., conversion of an mGlu₅ PAM to a selective mGlu₅ PAM12 or an mGlu₅ PAM to a selective mGlu₅, NAM13), they can also convert a nontoxic “pure” mGlu₅ PAM (VU0403602, 1) to neurotoxic mGlu₅ ago-PAM (VU0453103, 2) in vivo via an unexpected CYP₄₅₀-mediated oxidation (Figure 1).14 The latter is challenging to de-risk, without multiple in vivo studies, performed with and without CYP₄₅₀ inhibitors, to ascertain parent and metabolite drug levels as well as neurotoxicity in rodent seizure models, and ultimately preparative CYP₄₅₀ incubations to identify the structure of the neurotoxic metabolite.14 Within a fast-moving lead optimization effort, employing an in vivo de-risking strategy is not practical in terms of time, cost, and the likelihood of success. Based on these realities, we were intrigued by newly available biomimetic oxidation (BMO) kits, advertised as a “synthetic liver”, mimicking the oxidative metabolism of multiple CYP₄₅₀ enzymes (e.g., synthetic metalloporphyrins).15 Importantly, 96 individual synthetic livers are in each well of a 96 well plate,
allowing an expansive survey of synthetic biotransformation of a ligand, coupled with production kits to readily scale the chemistry from any distinct well (e.g., synthetic CYP450), enabling isolation and characterization of the “metabolite(s).” If successful and productive, this could revolutionize our current, laborious approach to identifying allosteric ligand chemotypes susceptible to in vivo molecular switches and more readily focus optimization efforts on chemical series devoid of molecular switches.

## RESULTS AND DISCUSSION

Knowing the in vitro and in vivo metabolism of 1,13 as well as the relevant CYP450-mediated oxidative metabolites, we elected to assess the ability of a commercial BMO kit15 to metabolize mGlu5 PAM 1 and determine if any of the 96 synthetic livers could generate 2 or other known metabolites. Incubation of 1 with the screening BMO kit led to the production of only four metabolite-like compounds M3–M6 (Figure 2).16 The metabolite of greatest interest, the mGlu5 ago-PAM 2, produces a parent ion adduct of [M + H]+ with m/z 311 and MS/MS produced the minor fragment of m/z 293 resulting from the loss of water (−18 Da; −H₂O), while the water loss fragment from 1 (m/z 295 [M + H]+) results in a m/z of 277. The next major fragments of 2 result from, first, the loss of the cyclobutanol moiety producing an [M − 71 + 2H]+ ion at m/z 241 and, second, fragmentation of the carbon nitrogen bond producing the benzoyl ion at m/z 223. For 1, the fragments associated with the water-assisted hydrolysis of the amide bond and the loss of the cyclobutyl group are also observed in the MS/MS and MS/MS/MS (MS³) at m/z 242 and m/z 241, respectively. The last major fragment observed in both 1 and 2 is the decarboxylation producing a fragment with a m/z of 196. Since a standard of 2 was available, we were able to determine via retention time analysis that no detectable amount of 2, representing hydroxylation of the 3-position on the cyclobutyl moiety of 1, was produced by any of the 96 individual synthetic metalloporphyrins in the BMO kit.16

The BMO screening kit did produce four other oxidative species, and analysis of the unknowns produced from 1 (M3–M6) were first analyzed by UPLC MS/MS/MS (UPLCMS).16 The parent ion of M3 was observed as the [M + H]+ adduct at m/z 311, and the first notable fragment from the MS/MS was the loss of water corresponding to the m/z at 293. The major fragment at m/z 257 resulted from the loss of the cyclobutyl moiety, which is an increase of 16 Da from the removal of the cyclobutyl moiety observed at m/z 241 in 1 and 2. The next series of fragments at m/z 242 and 241 were the same fragments observed in 1 and 2, however, a new fragment at m/z 240 represented the benzoyl ion fragment. This fragment at m/z 240 is also observed as the major fragment in the MS³ of m/z 257. The remaining two fragments are decarboxylation and oxygen loss fragmentation at m/z 196 and an interesting fragment at m/z 214. This m/z 214 fragment corresponded to the loss of −43 Da from m/z 257, which would correspond to the loss of oxygen and hydrogen cyanide. These fragments at m/z 257 and 240 indicate that the oxygen was not attached to the cyclobutyl moiety like in 2, and thus, the fragment at m/z 214 indicates that there was a high likelihood that M3 is the N-oxide of 1, a metabolite detected in small amounts from traditional metabolite identification (MetID) studies. A simple comparison of the retention time, fragmentation patterns, and coinjection (average of the coinjection retention time analysis t_R = 0.923 ± 0.000 min) with an available standard, synthesized to support the previous MetID studies, confirmed the identity of M3 as 4 (VU0409496), the N-oxide of 1 (Figure 3).16

The biomimetic kit afforded 3.3 mg of M4. UPLCMS³ of M4 found a parent ion of [M + H]+ at m/z 241, which corresponded to the primary amide of 1. MS/MS of the parent ion gave two fragments, the water-assisted hydrolysis at m/z 242 and the major fragment at m/z 224, the benzoyl ion. The MS³ of m/z 224 gave the decarboxylation fragment at m/z 196. All listed fragments indicate the identity of M4 as the primary amide of 1. The ¹H NMR of M4 displayed the same seven aromatic proton signals observed in 1 and lacked signals corresponding to the cyclobutyl moiety.16 The only extra signal observed is the result of an increased barrier of rotation due to the intramolecular hydrogen bond observed in picolinamides

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**Figure 1.** Major biotransformation of pure mGlu5 PAM, namely, VU0403602 (1), to M1, a potent, neurotoxic mGlu5 ago-PAM (VU0453103, 2), and M2, the inactive product of amide hydrolysis 3 observed in male rat hepatic S9 fractions. Other minor metabolites were the corresponding N-oxide or result of oxidation of the acetylene moiety.

**Figure 2.** Observed key fragments of M3–M6. Key HMBC correlations in blue.

**Figure 3.** Confirmed structure of M3 as the N-oxide 4 (VU0409496).
resulting in the primary amide having two unique signals at δH 7.80 (1H, br s, NH) and δH 5.69 (1H, br s, NH). The biomimetic kit also afforded 1.8 mg of M5. UPLCMS of M5 found a parent ion of [M + H]+ at m/z 327, which corresponds to the addition of two oxygens to 1 (+32 Da; +O × 2). The first fragment resulted from the loss of water with m/z 309. The familiar loss of the cyclobutyl moiety and the water-assisted hydrolysis fragments were present at m/z 273 and 274; however, both fragments were still +32 Da heavier than 1 (m/z 241 and 242) when these same losses occurred. MS of the major fragment m/z 273 indicated the loss of ammonia (−17 Da; −NH3) to afford the benzoyl ion at m/z 256 followed by the loss of hydrogen cyanide (−27 Da; −HCN) at m/z 246 and decarboxylation at m/z 228, all of which are still +32 Da heavier than their counterpart’s fragments from 1. These observations indicated that the two oxygens must be attached to the phenylethynylpyridine moiety and not as the N-oxide since HCN was lost and both oxygens still remained.

The 1H NMR spectrum of M5 was obtained to hopefully glean more structural information. The chemical shifts as expected for the cyclobutyl moiety remain constant. The proton resonances from the picolinamide and the 2-fluorobenzyl moiety were all deshielded up to δH 9.11 (1H, br dd, J = 2.1, 0.8 Hz), 8.42 (1H, dd, J = 8.1, 2.1 Hz), 8.34 (1H, dd, J = 8.2, 0.8 Hz), 7.78 (1H, dt, J = 7.8, 1.3 Hz), 7.74 (1H, ddd, J = 8.8, 2.7, 1.6 Hz), 7.53 (1H, td, J = 8.4, 5.2 Hz), and 7.41−7.32 (2H, m), 7.25 (1H, dd, J = 9.7, 2.8 Hz), and 7.15−7.06 (1H, m) observed in 1. The only exchangeable signal observed was the amide NH proton at δH 8.17 (1H, br d, J = 7.9 Hz). The 1H NMR revealed that no carbon−hydrogen bonds were replaced with carbon−oxygen bonds, indicating the ethynyl moiety as the most likely position for the addition of the two oxygens. In order to determine if oxidation occurred at the triple bond, an HMBC spectrum of M5 was obtained. Careful examination of the HMBC correlations from two of the picolinamide protons at δH 9.11 and δH 8.42 shows that they both display correlations to a ketone at δC 191.1, while two protons from the 2-fluorobenzyl moiety at δH 7.78 and δH 7.74 both display correlations to a ketone at δC 191.0. These observations support the transformation of the triple bond into a di-ketone assigning the potential structure as M5.

The biomimetic kit provided only 0.5 mg of M6, which was found to have a parent ion of [M + H]+ m/z 299. This mass was rather interesting since it was only 4 Da higher in mass than 1, which indicated that functionalities were lost and added. Notable fragments observed in the MS/MS were the loss of water at m/z 288 (−18 Da; −H2O) and the loss of the cyclobutyl moiety [M − 55 + 2H]2+ to afford the major fragment at m/z 245. Further fragmentation (MS3) of m/z 245

Scheme 1. Synthesis of Putative Synthetic Metabolites M4−M6

<table>
<thead>
<tr>
<th>Step</th>
<th>Reaction</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
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<tbody>
<tr>
<td>i</td>
<td>6</td>
<td>HATU, DIPEA, DMF, rt, 30 min; 85%.</td>
<td></td>
</tr>
<tr>
<td>ii</td>
<td>7</td>
<td>CuI, DEA, DMF, 60 °C, 2 h, microwave; 80%.</td>
<td></td>
</tr>
<tr>
<td>iii</td>
<td>8</td>
<td>Pd(PPh3)4, CuI, DIPEA, DMF, 60 °C, microwave, 45 min; 56%.</td>
<td></td>
</tr>
<tr>
<td>iv</td>
<td>9</td>
<td>HATU, DIPEA, DMF, rt, 1 h; 76%.</td>
<td></td>
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<tr>
<td>v</td>
<td>10</td>
<td>EDAC, DIPEA, hydroxybenzotriazole, (NH4)2CO3, THF, rt, 2 h; 32%.</td>
<td></td>
</tr>
<tr>
<td>vi</td>
<td>11</td>
<td>MgSO4, KMnO4, NaHCO3, acetone/H2O, rt, 1 h; 21%.</td>
<td></td>
</tr>
<tr>
<td>vii</td>
<td>12</td>
<td>(NH4)2CO3, THF/H2O, 65 °C, 16 h; 8%.</td>
<td></td>
</tr>
<tr>
<td>viii</td>
<td>13</td>
<td>NaOH, DMF/H2O, 60 °C, 2 h.</td>
<td></td>
</tr>
<tr>
<td>ix</td>
<td>14</td>
<td>HATU, DIPEA, DMF, rt, 1 h; 16% over two steps.</td>
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resulted in the formation of the water-assisted amide hydrolysis product at $m/z$ 246, the loss of ammonia to form the benzoyl ion ($-17$ Da; $-\text{NH}_3$) at $m/z$ 228, loss of hydrogen cyanide ($-27$ Da; $-\text{HCN}$) at $m/z$ 218, and decarboxylation at $m/z$ 200. Assuming that a carbon was lost and adding 12 Da to $m/z$ 299 gives a $m/z$ of 311 indicate that there is a high likelihood that a carbon was removed. Since all lost fragments have been identified as fragments lost from M3–M5 and since the added oxygen was still present after decarboxylation, the lost carbon and the added oxygen must be distinct from the phenyl-ethynylpyridine moiety. Examination of the $^1$H NMR shows that the cyclobutyl moiety and amide proton remain unchanged and all seven protons from the picolinamide and 2-fluorobenzyl were still present at $\delta_H$ 8.92 (1H, br d, $J = 1.6$ Hz), 8.33 (1H, d, $J = 8.1$ Hz), 8.22 (1H, dd, $J = 8.1$, 2.1 Hz), 7.60–7.47 (3H, m), and 7.36 (1H, tdd, $J = 8.1$, 2.6, 1.3 Hz). Since the cyclobutyl moiety, picolinamide, and the 2-fluorobenzyl moiety were not modified, it left the ethynyl linkage as the only possible place for modification. If the ethynyl linkage was replaced with a ketone, then the loss of a carbon and the addition of an oxygen would be accounted for. It has also been shown to be possible to convert internal alkynes into shorter ketones with the use of porphyrins. This assumption would assign the structure as M6.

In order to unambiguously confirm the structures and identities of M4–M6, we synthesized de novo these putative synthetic metabolites and compared spectral data and retention times (Scheme 1). Starting from commercial acid
5, a HATU-mediated amide coupling with cyclobutyl amine 6 afforded amide 7 in 85% yield. A Sonogashira coupling between 7 and 3-fluorophenyl acetylene 8 provided 1 in 56% yield. Alternatively, 1 was also prepared via a different route. Direct Sonogashira coupling of acid 5 with 8 delivered acid 3, which could then be employed to access 1 via a standard amide coupling. Acid 3 can also be employed to give the primary carboxamide M4 (9) via an amide coupling sequence with (NH4)2CO3, albeit in only 32% yield. Following a known literature protocol, the acetylene moiety in 1 was smoothly converted to the diketone M5 (10) by treatment with K2MnO4. The last putative metabolite M6 (14) was accessed in two steps. A palladium-catalyzed acylation between boronic acid 11 and acid chloride 12 gave ketone 13 in low yield. Hydrolysis of the acid and another HATU-mediated amide coupling with 6 delivered M6 (14) in low yield over two steps (16%).

To confirm the structure of M4 as the primary amide of 1, it was synthesized (Scheme 1) and compared via retention time, fragmentation patterns, coinjection, and 1H NMR comparison (see the Supporting Information). The comparison analysis confirmed M4 as 9 (average of the coinjection retention time analysis $t_R = 1.047 \pm 0.001$ min), the primary amide of 1 (Figure 4). Similarly, the proposed structure of M5, diketone 10, was synthesized on scale (Scheme 1) to aid in the confirmation of the structure. The analysis of the retention time (average of the coinjection retention time analysis $t_R = 0.957 \pm 0.001$ min), fragmentation patterns, coinjection, and 1H NMR comparison confirmed the identity of M5 as the diketone 10. Once again, to confirm the proposed structure of M6, 14 was synthesized on scale (Scheme 1). Analysis of the retention time (average of the coinjection retention time analysis $t_R = 0.969 \pm 0.001$ min), mass fragmentation patterns, coinjection, and 1H NMR confirmed structure M4 for M6. With M3–M6 in hand, they were evaluated in our standard mGlu5 calcium mobilization functional assay, and M5 and M6 were inactive in both PAM and NAM modes; however, M3 and M4 proved more interesting.

The N-oxide, M3 (4, VU0409496) proved to be a potent, pure PAM in our standard screening assays (human EC50 = 38.7 nM, pEC50 = 7.41 ± 0.06, 71.5 ± 2.6% Glu Max; rat EC50 = 28 nM, pEC50 = 7.55 ± 0.06, 88.5 ± 3.6% Glu Max), roughly equipotent to the parent 1 and similarly devoid of mGlu5 agonism (human EC50 > 30 μM). This active metabolite was produced in very small quantities in native systems, but the BMO kit produced M3 in a significant amount to warrant characterization. Further evaluation of M3 found it to be non-CNS penetrant, with modest fraction unbound ($f_u (h, r) = 0.012, 0.033$) and a metabolic activator of 3A4; therefore, M3 was not viable as a lead. The primary carboxamide M4 (VU6025968), a new synthetic oxidative metabolite not observed in native systems, was also an active mGlu5 PAM. As we have sufficient quantity from the BMO production kit, we screened both the kit-produced M4 and synthetic M4 in the human mGlu5 assay. The kit-produced M4 afforded a pure PAM profile, with an EC50 of 510 nM (75% Glu Max), and the synthetic-derived M4 was equivalent (EC50 = 315 nM, 77%), and no agonism noted up to 30 μM. Importantly, it was significant to identify active oxidative metabolites from the BMO kit that retained the desired mode of pharmacology (pure PAM versus ago-PAM) that were either produced in minor quantities or not observed in classical native system preparations.

### CONCLUSIONS

In summary, the BMO kit proved to afford a highly expedient synthetic liver producing a number of known metabolites of 1, as well as novel synthetic metabolites in quantities sufficient for full characterization and biological assessment. MetID studies of the synthetic analogs were confirmed by independent synthesis, and unexpectedly based on the structures of M3–M6, two were potent pure mGlu5 PAM, that is, active metabolites. We hoped that the BMO kit would produce the potent, neurotoxic mGlu5 ago-PAM (VU0453103, 2) of pure PAM 1, the result of an oxidative-metabolism-induced molecular switch; however, this was not observed in any of the 96 individual synthetic livers. Still, the technology platform has utility in producing on scale synthetic oxidative metabolites, but the biomimetic catalysts (e.g., metalloporphyrins) may not fully encompass all of the oxidative chemistry of endogenous CYPs. Further work in this arena is in progress, and additional studies with other allosteric ligands will be reported in due course.

### EXPERIMENTAL SECTION

#### General Methods

All reactions were carried out employing standard chemical techniques under air unless noted. Solvents used for extraction, washing, and chromatography were HPLC-grade. All reagents were purchased from commercial sources and were used without further purification. All microwave reactions were carried out in sealed tubes in a Biotage Initiator microwave synthesis reactor. Analytical HPLC was performed on an Agilent 1200 LCMS with a gradient of 5–95% MeCN in 0.1% TFA water over 1.5 min and UV detection at 215 and 254 nm along with ELS detection and electrospray ionization, with all final compounds showing >95% purity. High-resolution mass spectra were obtained on an Agilent 6540 UHD Q-TOF with the ESI source. Automated flash column chromatography was performed on a Teledyne ISCO Combi-Flash system. Reversed-phase HPLC was performed on a Gilson preparative reversed-phase HPLC system comprising a 333 aqueous pump with solvent-selection valve, 334 organic pump, GX-271 or GX-281 liquid handler, two column switching valves, and a 155 UV detector. The UV wavelength for fraction collection was user-defined, with absorbance at 254 nm always monitored. The column used was Phenomenex Axia-packed Gemini C18 (30 × 50 mm, 5 μm). For the acidic method, the mobile phase was CH3CN in H2O (0.1% TFA). Gradient conditions were 0.75 min equilibration followed by a user-defined gradient (starting organic percentage, ending organic percentage, duration), held at 95% CH3CN in H2O (0.1% TFA) for 1 min, 50 mL/min, 23 °C. For the basic method, the mobile phase was CH3CN in H2O (0.05% v/v NH4OH). Gradient conditions were 0.75 min equilibration followed by a user-defined gradient (starting organic percentage, ending organic percentage, duration), held at 95% CH3CN. LCMS® experiments were carried out on an Agilent 1290 UPLC system coupled to a Thermo Scientific LTQ XL Linear Ion trap mass spectrometer using CH3CN and 10 mM ammonium formate with pH adjusted to 4.1 as the mobile phases with a Phenomenex Kinetex C18 (2.6 μ, 2.1 × 50 mm, 100 Å column) held at 50 °C with a CERA Temperature Controller at 1 mL/min. All NMR spectra were recorded on a 400 MHz Bruker AV-400 instrument. 1H chemical shifts are reported as δ values in ppm relative to the residual solvent peak (CDCl3 = 7.28).
7.26, DMSO-d₆ = 2.50, CD₂OD = 4.78). Data are reported as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, ddq = doublet of doublets of quartets, sext = sextet, m = multiplet), coupling constant (Hz), and integration. ¹³C chemical shifts are reported as δ values in ppm relative to the residual solvent peak (CDCl₃ = 77.2, DMSO-d₆ = 39.5, CD₂OD = 49.15).

**Compound Information.** 5-Bromo-N-cyclobutylpicolinamide (7). To a vial were added S-bromopicromionic acid (S) (1.24 mmol), HATU (1.49 mmol), DMF (5 mL), DIPEA (4.95 mmol), and cyclamolyamine (6) (1.49 mmol). The mixture was allowed to stir for 30 min at rt after which time LCMS indicated product formation. The sample was filtered and purified on normal-phase chromatography using 0–20% EtOAc in hexanes to give S-bromo-N-cyclobutylpicolinamide (7) as a white solid (85% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.58 (1H, dd, J = 2.2, 0.6 Hz), 8.06 (1H, dd, J = 8.3, 0.6 Hz), 8.01 (1H, br s), 7.95 (1H, dd, J = 8.3, 2.2 Hz), 4.62–4.51 (1H, m), 2.47–2.37 (2H, m), 2.09–1.97 (2H, m), 1.83–1.73 (2H, m); ¹³C NMR (100 MHz, CDCl₃) δ 162.6, 149.3, 148.6, 140.1, 124.0, 123.8, 44.8, 31.3, 15.4; LCMS: m/z = 885 mm (98%); m/z = 256.3 [M + H]⁺; HRMS (ESI) m/z [M + H]⁺ calc for C₁₀H₉BrN₂O⁺ 255.0128, found 255.0126.

**Route 2:** S-(3-Fluorophenylethynyl)picolinic acid (3) (0.331 mmol) was dissolved in DMF (0.580 mL). DIPEA (0.660 mmol) was added, and the reaction solution was stirred for 30 min. HATU (0.400 mmol) was added, and the reaction solution was stirred for 30 min. Cyclobutylamine (6) (0.400 mmol) was added, and the reaction solution was stirred for 1 h at rt. At completion, the reaction solution was filtered and purified by reversed-phase chromatography to give N-cyclobutyl-S-(3-fluorophenylethynyl)picolinamide (1) as an off-white solid (76% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.68 (1H, s), 8.18 (1H, d, J = 8.1 Hz), 8.10 (1H, br d, J = 7.5 Hz), 7.94 (1H, dd, J = 2.0, 8.1 Hz), 7.39–7.31 (2H, m), 7.28–7.22 (1H, m), 7.16–7.09 (1H, m), 4.62 (1H, sext, J = 8.2 Hz), 2.51–2.41 (2H, m), 2.15–2.02 (2H, m), 1.88–1.76 (2H, m); ¹³C NMR (100 MHz, CDCl₃) δ 162.7, 162.6 (d, J_C–F = 247.5 Hz), 150.5, 148.8, 140.0, 130.3 (d, J_C–F = 8.6 Hz), 127.8 (d, J_C–F = 3.0 Hz), 124.1 (d, J_C–F = 9.6 Hz), 122.6, 121.8, 118.7 (d, J_C–F = 23.0 Hz), 116.7 (d, J_C–F = 21.1 Hz), 93.4 (d, J_C–F = 3.6 Hz), 86.5, 44.8, 31.3 (double intensity), 15.4; [M + H⁺]; HRMS (ESI) m/z [M + H⁺] calc for C₁₀H₉F₂NO⁺ 295.0714, found 295.0714.

**N-Cyclobutyl-S-(3-fluorophenylethynyl)picolinamide N-Oxide (M₃, 4).** The compound was available as a 10 mM DMSO stock solution from previous work conducted by VCNDD. Synthesis and chiral resolution were previously reported.

**N-Cyclobutyl-S-(3-fluorophenylethynyl)picolinamide N-Oxide (M₃, 4).** The compound was available as a 10 mM DMSO stock solution from previously patented work at VCNDD. ¹H NMR (400 MHz, CDCl₃) δ 8.70 (1H, br d, J = 7.0 Hz), 8.21 (1H, dd, J = 0.6, 8.1 Hz), 7.97 (1H, dd, J = 2.0, 8.1 Hz), 8.71 (1H, br s), 7.40–7.32 (2H, m), 7.29–7.23 (1H, m), 7.16–7.06 (1H, m), 5.70 (1H, br), ¹³C NMR (100 MHz, CDCl₃) δ 166.1, 162.5 (d, J_C–F = 247.5 Hz), 150.8, 148.2, 140.0, 130.5 (d, J_C–F = 8.7 Hz), 127.9 (d, J_C–F = 3.2 Hz), 124.0 (d, J_C–F = 9.4 Hz), 123.4, 122.1, 118.7 (d, J_C–F = 23.2 Hz), 116.8 (d, J_C–F = 21.2 Hz), 93.7 (d, J_C–F = 3.5 Hz), 86.4; LCMS: m/z = 0.824 min (98%); m/z = 241.0 [M + H⁺]; HRMS (ESI) m/z [M + H⁺] calc for C₁₀H₉F₂NO⁺ 241.0772, found 241.0774.

**N-Cyclobutyl-S-(2-(3-fluorophenyl)-2-oxooctacyl)picolinamide (M₅, 10).** N-Cyclobutyl-S-(3-fluorophenylethynyl)picolinamide (1) (0.025 mmol) was dissolved in acetone (2 mL). A suspension of sodium bicarbonate (0.130 mmol) and magnesium sulfate (43 mg, 0.350 mmol) in water (0.74 mL) was added followed by potassium permanganate (87 mg, 0.550 mmol). The reaction solution was stirred for 1 h at rt. At completion, hexanes were added (5 mL), and the
solution was decanted. The remaining residue was purified by reversed-phase chromatography to give N-cyclobutyl-5-(3-fluorobenzoyl)-2-oxoacetamidinamide (M5, 10) as a yellow solid (21% yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 9.11 (1H, dd, J = 0.8, 2.1 Hz), 8.42 (1H, dd, J = 2.1, 8.2 Hz), 8.33 (1H, dd, J = 0.8, 8.2 Hz), 8.17 (1H, br d, J = 7.9 Hz), 7.78 (1H, d, J = 1.4, 7.8 Hz), 7.74 (1H, ddd, J = 1.6, 2.7, 8.8 Hz), 4.60 (1H, sext, J = 8.1 Hz), 2.49−2.39 (2H, m), 2.12−1.99 (2H, m), 1.86−1.74 (2H, m); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 191.1, 191.0 (d, $^{13}$C−F = 2.3 Hz), 163.0 (d, $^{13}$C−F = 250.0 Hz), 161.9, 154.1, 149.8, 138.9, 134.4 (d, $^{13}$C−F = 6.6 Hz), 131.1 (d, $^{13}$C−F = 7.7 Hz), 130.1, 126.4 (d, $^{13}$C−F = 3.1 Hz), 122.7 (d, $^{13}$C−F = 21.4 Hz), 122.5, 116.6 (d, $^{13}$C−F = 22.8 Hz), 44.9, 31.2 (double intensity), 15.4; LCMS: $f_k$ = 1.028 min (>98%), m/z = 327.4 [M + H]⁺; HRMS (ESI) m/z [M + H]⁺ calcd for C$_{19}$H$_{16}$FN$_2$O$_3$ 327.1139, found 327.1143.

*Methyl 5-(3-Fluorobenzoyl)picolinate (13).* Methyl picolinate-5-boronic acid (11) (0.570 mmol), 3-fluorobenzoyl chloride (12) (0.470 mmol), potassium carbonate (1.420 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.0500 mmol) were placed in a vial that was purged with N$_2$. THF (2.1 mL) and water (0.30 mL) were added, and the reaction solution was heated overnight at 65 °C. The reaction solution was filtered through Celite and washed with EtOAc, and the solvent was concentrated via vacuum. The resulting residue was used immediately in the next step, being dissolved in 0.1 N HCl (pH = 4−5) with metabotropic glutamate receptors. *Chem. Rev.* 2016, 116, 6707−6741.


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(15) For information, see https://www.hepatochem.com/chemistry-screening-kits/bmo-kit/.

(16) See the Supporting Information for full details.


