Published in final edited form as:

ACS Chem Neurosci. 2016 September 21; 7(9): 1192–1200. doi:10.1021/acscchemneuro.6b00035.

Discovery, Synthesis, and Preclinical Characterization of *N*-(3-Chloro-4-fluorophenyl)-1*H*-pyrazolo[4,3-*b*]pyridin-3-amine (VU0418506), a Novel Positive Allosteric Modulator of the Metabotropic Glutamate Receptor 4 (mGlu₄)

Darren W. Engers^{†,§}, Anna L. Blobaum^{†,§}, Rocco D. Gogliotti^{†,§}, Yiu-Yin Cheung^{†,§}, James M. Salovich^{†,§}, Pedro M. Garcia-Barrantes[§], J. Scott Daniels^{†,§}, Ryan Morrison^{†,§}, Carrie K. Jones^{†,§}, Matthew G. Soars[⊥], Xiaoliang Zhuo[⊥], Jeremy Hurley[⊥], John E. Macor[⊥], Joanne J. Bronson[⊥], P. Jeffrey Conn^{†,§,||}, Craig W. Lindsley^{†,‡,§}, Colleen M. Niswender^{†,§,||}, and Corey R. Hopkins^{†,‡,§,*}

[†]Department of Pharmacology, Vanderbilt University, Nashville, Tennessee 37232, United States

[‡]Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37232, United States

[§]Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University, Nashville, Tennessee 37232, United States

^{||}Vanderbilt Kennedy Center, Vanderbilt University, Nashville, Tennessee 37232, United States

[⊥]Bristol-Myers Squibb Co., Research and Development, 5 Research Parkway, Wallingford, Connecticut 06492, United States

Abstract

The efficacy of positive allosteric modulators (PAMs) of the metabotropic glutamate receptor 4 (mGlu₄) in preclinical rodent models of Parkinson's disease has been established by a number of groups. Here, we report an advanced preclinically characterized mGlu₄ PAM, *N*-(3-chloro-4-fluorophenyl)-1*H*-pyrazolo[4,3-*b*]pyridin-3-amine (VU0418506). We detail the discovery of VU0418506 starting from a common picolinamide core scaffold and evaluation of a number of amide bioisosteres leading to the novel pyrazolo[4,3-*b*]pyridine head group. VU0418506 has been characterized as a potent and selective mGlu₄ PAM with suitable in vivo pharmacokinetic properties in three preclinical safety species.

*Corresponding Author. Phone: 615-936-6892. Fax: 615-778-1414. corey.r.hopkins@vanderbilt.edu.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscchemneuro.6b00035.

Chemical synthesis of all analogs, in vitro Pharmacology procedures, in vitro PK methods, in vivo PK methods, Selectivity and initial toxicology evaluation of **26**, 4-day in vivo PK study of **26**, human hepatocyte induction methods and in vitro biotransformation protocols (PDF)

Author Contributions

C.R.H. and C.W.L. oversaw and designed the chemistry. D.W.E., R.D.G., Y.Y.C., J.M.S., and P.M.B. performed the synthetic chemistry work. A.L.B., J.S.D., and R.M. designed the pharmacokinetic experiments. P.J.C. and C.M.N. designed and analyzed the in vitro pharmacology experiments. C.R.H. and C.M.N. wrote the manuscript with input from all authors.

Graphical Abstract

26**VU0418506**hmGlu₄ EC₅₀ = 68 nMrmGlu₄ EC₅₀ = 46 nM

B:P = 2.1

Keywords

Metabotropic glutamate receptor 4; mGlu₄; CYP induction; Parkinson's disease; pyrazolo[4,3-b]pyridine

Parkinson's disease (PD) is a chronic movement disorder resulting from the loss of dopaminergic neurons in the basal ganglia (BG).¹ The four core motor symptoms of PD are tremor, rigidity, bradykinesia, and postural instability, all of which can impact simple task completion as the disease progresses. At present there is no cure for PD, and there are only medications that provide symptomatic relief. Generally, afflicted individuals are given dopamine receptor agonists or levodopa (L-DOPA) to combat motor symptoms, the latter in combination with carbidopa, which allows L-DOPA to reach the brain before being converted to dopamine.² Unfortunately, not all symptoms are responsive to drug treatment (with rigidity and bradykinesia responding the best), leaving a significant portion of the disease population poorly treated; additionally, drug responsiveness wanes over disease progression.³ Other therapeutics can aid in disease management (e.g., anticholinergics); however, a small, but significant, portion of PD patients remain unresponsive to current treatment options. In certain individuals, deep brain stimulation (DBS), a surgical procedure wherein electrodes are implanted within nuclei of the BG, has been shown to have dramatic results.⁴ However, careful patient selection is an important step for DBS success and only a small number of patients qualify for this surgery. Thus, the treatment options for the symptomatic and disease-modifying intervention for PD remain limited, particularly as the disease progresses.

Motor function is controlled by the BG through two distinct pathways from the striatum: the direct and indirect pathways.^{5,6} These pathways have opposite effects on motor activity:

activation of the direct pathway promotes movement, and the indirect pathway inhibits it. In PD patients, the balance between these two pathways is lost, leading to reduced inhibitory input to the internal globus pallidus and substantia nigra pars reticulata via the direct pathway and an overall increase in the excitatory flow to these output nuclei via the polysynaptic indirect pathway.^{5,6} Over the past several years, the metabotropic glutamate receptor 4 (mGlu₄) has gained attention as a potential target for pharmacological intervention to normalize the BG circuitry in PD patients.^{7,8} This receptor is expressed presynaptically on terminals projecting from the striatum to the globus pallidus external segment (GPe), the first synapse of the BG indirect pathway (the “striato-pallidal” synapse).⁹ Activation of mGlu₄ at these terminals has been shown to reduce inappropriate GABA release onto the GPe, normalizing motor output.^{5,6,9} Additionally, expression of mGlu₄ in the striatum and other BG structures has been postulated to be involved in positive responses on motor output induced by activation or potentiation of mGlu₄.

Over the past several years, our laboratories and others have disclosed a series of picolinamide-based mGlu₄ positive allosteric modulators (PAMs) (Figure 1).¹⁰ This scaffold has proven to deliver potent and selective mGlu₄ PAMs and several of these compounds have been used in preclinical anti-Parkinsonian animal models.^{11–14} Compounds **3**^{15,16} and **7**^{16,17} were shown to reverse haloperidol-induced catalepsy after systemic administration (administered either subcutaneously (SC) or via an oral (PO) route). Within the picolinamide series of mGlu₄ PAMs, we have extensively characterized compound **7** and have shown that this ligand is effective when administered alone in reversing haloperidol-induced catalepsy, forelimb asymmetry-induced by unilateral 6-hydroxydopamine (6-OHDA) lesions in the median forebrain bundle, and deficits in attention induced by bilateral 6-OHDA nigrostriatal lesions in rats.¹⁷ In addition to activity when administered alone, **7** was shown to enhance the activity of preladenant (an A_{2A} antagonist previously in clinical trials for PD therapy) in the reversal of haloperidol-induced catalepsy.¹⁷ Lastly, when administered with an inactive dose of L-DOPA, **7** potentiated the effects of L-DOPA in reversing forelimb asymmetry, suggesting that mGlu₄ PAMs may also provide LDOPA sparing activity.¹⁷ Unfortunately, the picolinamide scaffold suffered from poor stability *in vivo* due to some extent, the instability of the amide bond. Thus, we initiated a medicinal chemistry effort to find a suitable replacement for the picolinamide head group with the goal of identifying a potent and selective mGlu₄ PAM with improved pharmacokinetic properties.

RESULTS AND DISCUSSION

We have previously explored the structure–activity relationship (SAR) around the right-hand portion of the molecule and have identified several groups that are tolerated (amides, sulfonamides, imides).^{12–14} Thus, our initial SAR optimization in this report started with the evaluation of known amide bioisosteres¹⁸ while keeping the 2-pyridine moiety intact, as our previous work has shown that this group is optimal for activity (Table 1). We have previously evaluated the reduction of the carbonyl group to the benzyl amine, the reversal of the amide, and the introduction of a cyclopropyl group as a carbonyl replacement.¹⁴ However, each of these substitutions led to significant loss of activity. Replacing the picolinamide moiety with the 2-pyridyl sulfonamide, **8**, led to a complete loss of activity against mGlu₄. To test the hypothesis that the planarity around this group needed to be

maintained, we next evaluated two cyclic amide bioisosteres.¹⁸ First, the 1,2,4-oxadiazole group was introduced, **9**, but this replacement led to an inactive compound. Next, we introduced the triazine moiety, **10**, to keep the hydrogen bond donor group available as in the secondary amide; however, this change led to an inactive compound as well. To introduce steric bulk to the amide bond, we alkylated the secondary amide, **11**; that, too, led to an inactive compound. Lastly, an amino group was introduced to generate an internal hydrogen bond with the carbonyl group, and this was a productive change (**12**, 578 nM). Taking into account our previous attempts,^{14,16} as well as this current endeavor, it appears that very little change is tolerated around the picolinamide portion of this scaffold.

Having established that the aminopicolinamide head group imparted significant mGlu₄ PAM activity, we next examined other cyclized head groups to systematically explore the SAR around this portion of the molecule (Table 2). We first looked to cyclize the amide NH portion with the pyridine moiety as this region was thought to participate in an internal hydrogen bond. However, cyclizing this portion of the molecule, **13**, led to an inactive compound, confirming the previous results observed with alkylated compounds. Efforts were then directed at cyclization of compounds while keeping the NH bond intact. The azabenzimidazole compound, **14**, was inactive; however, the 2-pyridyl benzimidazole, **15**, was active, although 5-fold less potent than **7**. A productive change was introduction of the benzoxazole head group, **16**, which was equipotent with **7**, but 4-fold less active than **3**. The two quinazolin-4-one analogues were not well tolerated (**17** and **18**), even though both compounds possessed the carbonyl and NH components of the amide bond. Other 5- and 6-membered cyclized moieties that either kept the nitrogen of the pyridine constant (**20** and **22**) or the heteroatom of the carbonyl (**19** and **21**), or both, **23**, were also not tolerated. However, when we contracted the 6-membered ring of **23** and introduced an additional heteroatom in the form of an isoxazolo[4,5-*b*]pyridine group,¹⁹ we were pleased to see a significant improvement in mGlu₄ PAM activity (**24**, EC₅₀ = 339 nM). This compound has the elements of the pyridine and carbonyl; however, it does not contain the additional hydrogen bond found in the aminopyridine. Synthesis of a compound that retains an NH in the form of the indazole group led to a potent PAM (**25**, EC₅₀ = 67 nM). Interestingly, this compound does not contain the nitrogen of the putative pyridine group as present in the previous active compounds. Introduction of the pyrazolo[4,3-*b*]pyridine group²⁰ did not significantly increase activity (**26**, EC₅₀ = 68 nM). In addition, inclusion of a 4-fluoro atom in order to block oxidative metabolism in this position was also well tolerated. Overall, these compounds represent a novel chemical scaffold for mGlu₄ PAMs.

Having identified replacement head groups for the picolinamide that were potent mGlu₄ PAMs, we next determined if these compounds displayed improved pharmacokinetic properties compared to the picolinamides **3** and **7** in a battery of in vitro assays (Table 3). Compounds **3** and **7**, unsubstituted picolinamides, were unstable in liver microsomes with high intrinsic clearance in rat that approached the rat hepatic blood flow rate.^{21,22} However, both compounds displayed moderate free fraction in both rat and human plasma.^{22,23} Compounds were also evaluated for their inhibition of the cytochrome P450 (CYP450) enzymes using a cocktail approach in human liver microsomes as a first-tier screen for potential drug–drug interaction liability. Each of these compounds was a potent inhibitor of

CYP1A2 (**3**, 130 nM; **7**, 550 nM), but showed no significant activity against CYP2C9, CYP2D6, or CYP3A4 (>30 μ M). The aminopicolinamide compound, **12**, showed a very similar profile to both **3** and **7**, with high predicted clearance in liver microsomes, moderate free fraction in rat and human plasma, and potent inhibition of CYP1A2, but with inactivity against the other CYPs. Neither the isoxazolo[4,5-*b*]pyridine, **24**, nor the indazole, **25**, provided any improvement in the in vitro PK properties; in fact, while each of these was more highly protein bound in rat and human, other portions of their overall profile were comparable to the previous compounds profiled. Significant improvement, however, was seen with the pyrazolo[4,3-*b*]pyridine compound, **26**. Compound **26** had significantly lower intrinsic clearance in both rat ($CL_{\text{HEP}} = 12.1$ mL/min/kg) and human ($CL_{\text{HEP}} = 10.1$ mL/min/kg) liver microsomes. In addition, **26** displayed moderate free fraction in both rat ($F_u = 0.028$) and human ($F_u = 0.017$) plasma. **26** was a potent inhibitor of CYP1A2 but was much less active against CYP2C9 ($IC_{50} = 8.9$ μ M) and CYP2D6 and CYP3A4 (>30 μ M).

To establish an in vitro/in vivo correlation (IV/IVC), we next evaluated both **24** and **26** in in vivo PK experiments to assess clearance (Table 3). Both compounds demonstrated an excellent correlation in rats between in vitro and in vivo parameters, with **24** showing suprahepatic clearance ($CL_p = 117$ mL/min/min) and a short half-life ($t_{1/2} = 31$ min), while **26** showed moderate clearance ($CL_p = 29$ mL/min/kg) and a moderate half-life ($t_{1/2} = 91$ min) in rats (Tables 3 and 4).²⁴ To better understand the metabolic instability difference between **24** and **26**, we performed a metabolic soft-spot analysis (Figure 2). Analysis of the two compounds revealed that the major metabolite of **24** was N–O bond cleavage of the isoxazolo[4,5-*b*]pyridine moiety, whereas the major metabolite of **26** was oxodefluorination, albeit in low abundance. Based on these in vitro and in vivo PK parameters, compound **26**, VU0418506, was chosen for advancement.

To further profile **26**, we prepared > 10 g as outlined in Scheme 1. 3-Fluoro-2-formylpyridine, **27**, was heated in the presence of hydrazine which led to the pyrazolo[4,3-*b*]pyridine which was then brominated (2 M NaOH, Br₂), yielding 3-bromo-1*H*-pyrazolo[4,3-*b*]pyridine, **28**, in 29% yield over the two steps. Next, the nitrogen was protected as the Boc amide (Boc₂O, DMAP, Et₃N) in good yield (88%). Finally, the target compound **26** was completed via a Buchwald-Hartwig amination of **29** and **30** (Pd₂(dba)₃, X-Phos, Cs₂CO₃) followed by Boc deprotection (TFA, CH₂Cl₂) in good overall yield (70% for two steps).^{25,26}

We next evaluated **26** in a functional assay using the rat mGlu₄ protein and demonstrated that its activity was similar to that observed for the human receptor ($pEC_{50} = 7.34 \pm 0.04$, 46 nM). To assess the selectivity of **26** for mGlu₄ versus the other mGlu receptors, we performed selectivity assays in fold-shift format for the eight mGlu receptors.¹⁴ **26** was found to be selective versus the Group I and Group II mGlu receptors (Figure 3A–C, E) and against mGlu₇ and mGlu₈ (Figure 3G and H), two of the Group III receptors. The only other mGlu receptor that appears to functionally interact (with equipotency) with **26** is mGlu₆ (Figure 3F), a receptor primarily expressed in the retina. In addition to mGlu selectivity, **26** was tested against a wider panel of GPCRs, enzymes, ion channels, nuclear hormone receptors and transporters (Supporting Information Table 1) and was found to be inactive

against all of the targets, except monoamine oxidase-B (MAO-B, 330 nM). A scan against a panel of 96 kinase targets (DiscoverX, KINOMEScan, www.discoverx.com) revealed no significant activity (<30%). To further derisk **26** for potential advancement into preclinical toxicological studies, we progressed it into a cardiac channel panel IC₅₀ screen (ChanTest Cardiac Channel Panel, www.chantest.com), and tested for activity versus nine cardiac ion channels (including hERG); **26** was inactive up to 100 μ M against all nine channels (Supporting Information Table 2). Lastly, **26** was negative in a mini-AMES test (EuroFins, formerly Ricerca; www.eurofins.com) against TA98 and TA100 with and without S9.

Further in vivo PK profiling of **26** revealed that the compound had excellent oral bioavailability in both rats (F = 95%) and dogs (F = 36%) (Table 4). In addition, evaluation of **26** in a nonhuman primate (using a rhesus IV cassette) revealed a low clearance molecule (CL_p = 13 mL/min/kg) with a moderate half-life ($t_{1/2}$ = 3 h) (Table 4). Lastly, a plasma/brain PO snapshot study in rats showed substantial uptake of **26** in both the plasma and brain with a K_p (brain/plasma ratio (0–6 h)) of 2.10 (Table 4). A more detailed discussion of the CSF levels and in vivo efficacy is detailed in the companion paper, Niswender et al., submitted.

Before moving Compound **26** into extensive in vivo testing and chronic dosing paradigms, we investigated the potential of the potent CYP1A2 inhibitory activity observed with compound **26** to affect dosing strategies. Activity against CYP1A2 is not surprising for these compounds as it is well-known that small poly aromatic groups inhibit this enzyme.²⁷ However, as CYP1A2 is also the primary enzyme responsible for the metabolism of **26**, we evaluated the potential of **26** to autoinduce CYP1A2 expression or activity in cryopreserved hepatocytes.²⁸ **26** was shown to be a potent inducer of CYP1A2 activity (EC_{50} = 9.8 μ M and E_{max} = 104 fold-induction) and this manifested in a multiday pharmacokinetic study in rodents where, upon day 4 of dosing, significantly reduced plasma levels of **26** were detected in both male and female Sprague–Dawley (SD) rats (15 mg/kg PO, 1 $\times$ daily) (Supporting Information Figure 1). Total reductions in plasma AUC in male SD rats were from 1148 μ M·hr (day 1) to 200 μ M·hr (day 4); similar results were observed in female SD rats at the same dose. Therefore, due to this CYP1A2 autoinduction profile in rodents, **26** is not suitable for chronic dosing, but still remains a valuable tool compound for acute studies as highlighted in our companion paper, Niswender et al., submitted.

CONCLUSION

In summary, we report the discovery and characterization of a novel chemical scaffold as an advanced preclinical positive allosteric modulator of mGlu₄, VU0418506 (**26**), the overall profile of which is shown in Table 5. We have shown that VU0418506 is potent against both the human (EC_{50} = 68 nM) and rat (EC_{50} = 46 nM) receptors and is selective against the other mGlu receptors, except retinally restricted mGlu₆. While VU0418506 autoinduced CYP1A2 activity which limits its use in chronic dosing assays, it is anticipated that it will remain a valuable research tool compound for acute dosing. Extensive in vivo PK studies show that VU0418506 displays good bioavailability in two preclinical species (rat and dog) and is not a substrate for P-glycoprotein (Pgp, Table 5). Further selectivity profiling of VU0418506 revealed that it is selective against a number of kinases and other target families (GPCRs, ion channels, etc.). Initial examination of potential toxicological end points showed

that VU0418506 is clean against a panel of ion channels associated with cardiac toxicity and it negative in a mini-AMES assay with and without S9. Further in vivo examination of VU0418506 in a number of preclinical animal models of Parkinson's disease and other CNS disease models is reported in Niswender et al., submitted.

METHODS

General

All NMR spectra were recorded on a 400 MHz AMX Bruker NMR spectrometer. ^1H and ^{13}C chemical shifts are reported in δ values in ppm downfield with the deuterated solvent as the internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, b = broad, m = multiplet), integration, coupling constant (Hz). Low resolution mass spectra were obtained on an Agilent 6120 or 6150 instrument with ESI source. Method A: MS parameters were as follows: fragmentor, 70; capillary voltage, 3000 V; nebulizer pressure, 30 psig; drying gas flow, 13 L/min; drying gas temperature, 350 °C. Samples were introduced via an Agilent 1290 UHPLC comprised of a G4220A binary pump, G4226A ALS, G1316C TCC, and G4212A DAD with ULD flow cell. UV absorption was generally observed at 215 and 254 nm with a 4 nm bandwidth. Column: Waters Acquity BEH C18, 1.0×50 mm, 1.7 μm . Gradient conditions: 5% to 95% CH_3CN in H_2O (0.1% TFA) over 1.4 min, hold at 95% CH_3CN for 0.1 min, 0.5 mL/min, 55 °C. Method B: MS parameters were as follows: fragmentor, 100; capillary voltage, 3000 V; nebulizer pressure, 40 psig; drying gas flow, 11 L/min; drying gas temperature, 350 °C. Samples were introduced via an Agilent 1200 HPLC comprised of a degasser, G1312A binary pump, G1367B HPALS, G1316A TCC, G1315D DAD, and a Varian 380 ELSD (if applicable). UV absorption was generally observed at 215 and 254 nm with a 4 nm bandwidth. Column: Thermo Accucore C18, 2.1×30 mm, 2.6 μm . Gradient conditions: 7% to 95% CH_3CN in H_2O (0.1% TFA) over 1.6 min, hold at 95% CH_3CN for 0.35 min, 1.5 mL/min, 45 °C. High resolution mass spectra were obtained on an Agilent 6540 UHD Q-TOF with ESI source. MS parameters were as follows: fragmentor, 150; capillary voltage, 3500 V; nebulizer pressure, 60 psig; drying gas flow, 13 L/min; drying gas temperature, 275 °C. Samples were introduced via an Agilent 1200 UHPLC comprised of a G4220A binary pump, G4226A ALS, G1316C TCC, and G4212A DAD with ULD flow cell. UV absorption was observed at 215 and 254 nm with a 4 nm bandwidth. Column: Agilent Zorbax Extend C18, 1.8 μm , 2.1×50 mm. Gradient conditions: 5% to 95% CH_3CN in H_2O (0.1% formic acid) over 1 min, hold at 95% CH_3CN for 0.1 min, 0.5 mL/min, 40 °C. For compounds that were purified on a Gilson preparative reversed-phase HPLC, the system comprised of 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. UV wavelength for fraction collection was user-defined, with absorbance at 254 nm always monitored. Method: Phenomenex Axia-packed Luna C18, 30×50 mm, 5 μm column. Mobile phase: CH_3CN in H_2O (0.1% TFA). Gradient conditions: 0.75 min equilibration, followed by user defined gradient (starting organic percentage, ending organic percentage, duration), hold at 95% CH_3CN in H_2O (0.1% TFA) for 1 min, 50 mL/min, 23 °C. Solvents for extraction, washing and chromatography were HPLC grade. All reagents were purchased from Aldrich Chemical Co. and were used without purification.

3-Bromo-1H-pyrazolo[4,3-b]pyridine (28)

A mixture of 3-fluoro-2-formylpyridine, **27** (1.0 equiv), and anhydrous hydrazine (8.0 equiv) was heated to 110 °C. After 16 h, the reaction was cooled to rt and slowly poured onto ice water. After extracting with EtOAc (3×), the collected organic layers were washed with brine (100 mL), dried (MgSO₄), filtered, and concentrated to provide a dark brown oil. LCMS (method A): single peak (214 and 254 nm), $R_T = 0.102$ min, MS (ESI⁺) $m/z = 120.2$ [M + H]⁺. The crude residue was dissolved in 2 M NaOH (0.5 M), and a solution of Br₂ (1.0 equiv) in 2 M NaOH (1.0 M) was added dropwise. After 3 h at rt, NaHSO₃ (aqueous) (1 mL) was added followed by 4 N HCl (~1.0 M). A solid precipitated formed and was collected by filtration and air-dried affording **28** as an off-white solid (29% yield over two steps). LCMS (method A): single peak (214 and 254 nm), $R_T = 0.270$ min, MS (ESI⁺) $m/z = 200.0$ [M + H]⁺.

tert-Butyl 3-Bromo-1H-pyrazolo[4,3-b]pyridine-1-carboxylate (29)

To a solution of **28** (1.0 equiv), DMAP (10 mg), and Et₃N (1.15 equiv) in dry DMF (0.25 M) was added Boc₂O (1.1 equiv) at rt. After 16 h at rt, the reaction was added to EtOAc/H₂O (1:1). The separated organic layer was washed with water (2×) and brine, dried (MgSO₄), filtered, and concentrated to provide desired product **29** (88% yield). Material was taken through without further purification. LCMS (method A): single peak (214 and 254 nm), $R_T = 0.793$ min, MS (ESI⁺) $m/z = 298.2$ [M + H]⁺.

N-(3-Chloro-4-fluorophenyl)-1H-pyrazolo[4,3-b]pyridin-3-amine (25)

A mixture of **29** (1.0 equiv), Cs₂CO₃ (2.0 equiv), Pd₂(dba)₃ (5 mol %), and 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (XPhos) (10 mol %) in a round-bottomed flask were subjected to evacuation and purging with nitrogen (3×). A solution of 3-chloro-4-fluoroaniline, **30** (1.1 equiv), in 1,4-dioxane (0.1 M) was added. The reaction mixture was heated to 100 °C. After 12 h, the reaction was added to EtOAc/H₂O (1:1) and the organic layer was separated. The aqueous layer was reextracted with EtOAc (2×). The collected organic layers were washed with brine, dried (MgSO₄), filtered, and concentrated to afford a crude solid. The material was taken through to the next step. The crude residue was dissolved in DCM (0.1 M) at 0 °C, and TFA (1.0 M) was added. The ice bath was removed. After an additional 30 min, LCMS confirmed the loss of starting material. The solvent was removed under reduced pressure and the residue was purified by reverse phase liquid column chromatography (35–65% acetonitrile/water with 0.1% trifluoroacetic acid) to yield **26** as an orange solid (70% over two steps). LCMS (method B): single peak (214 and 254 nm), $R_T = 0.753$ min, MS (ESI⁺) $m/z = 263.1$ [M + H]⁺. ¹H NMR (400 MHz, *d*₆-DMSO): δ 9.55 (br s, 1H), 8.49 (s, 1H), 8.11–8.10 (m, 1H), 7.99 (d, *J* = 8.0 Hz, 1H), 7.66 (m, 1H), 7.46 (m, 1H), 7.30 (dd, *J* = 9.2, 8.8 Hz, 1H). HRMS calcd for C₁₂H₉N₄FCl [M + H]⁺, 263.0500; found, 263.0499.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

The authors would like to thank the Michael J. Fox Foundation for Parkinson's Research and Bristol-Myers Squibb for their support of the program to develop mGlu4 PAMs.

The authors declare the following competing financial interest(s): C.M.N., C.K.J., A.L.B., D.W.E., J.S.D., C.W.L., C.R.H., and P.J.C. have received funding and royalties from Bristol-Myers Squibb.

REFERENCES

1. Schapira AHV. Neurobiology and treatment of Parkinson's disease. *Trends Pharmacol. Sci.* 2009; 30:41–47. [PubMed: 19042040]
2. Smith Y, Wichmann T, Factor SA, DeLong MR. Parkinson's disease therapeutics: new developments and challenges since the introduction of Levodopa. *Neuropsychopharmacology.* 2012; 37:213–246. [PubMed: 21956442]
3. Carlsson A. Treatment of Parkinson's with L-DOPA. The early discovery phase, and a comment of current problems. *J. Neural Transm.* 2002; 109:777–787. [PubMed: 12111467]
4. Breit S, Schulz JB, Benabid A-L. Deep brain stimulation. *Cell Tissue Res.* 2004; 318:275–288. [PubMed: 15322914]
5. DeLong MR, Wichmann T. Circuits and circuit disorders of the basal ganglia. *Arch. Neurol.* 2007; 64:20–24. [PubMed: 17210805]
6. Wichmann T, DeLong MR. Functional neuroanatomy of the basal ganglia in Parkinson's disease. *Adv. Neurol.* 2003; 91:9–18. [PubMed: 12442660]
7. Marino MJ, Williams DL Jr, O'Brien JA, Valenti O, McDonald TP, Clements MK, Wang R, DiLella AG, Kinney GG, Conn PJ. Allosteric modulation of group III metabotropic glutamate receptor 4: a potential approach to Parkinson's disease treatment. *Proc. Natl. Acad. Sci. U. S. A.* 2003; 100:13668–13673. [PubMed: 14593202]
8. Marino MJ, Conn PJ. Glutamate-based therapeutic approaches: allosteric modulators of metabotropic glutamate receptors. *Curr. Opin. Pharmacol.* 2006; 6:98–102. [PubMed: 16368268]
9. Valenti O, Marino MJ, Wittmann M, Lis E, DiLella AG, Kinney GG, Conn PJ. Group III metabotropic glutamate receptor-mediated modulation of the striatopallidal synapse. *J. Neurosci.* 2003; 23:7218–7226. [PubMed: 12904482]
10. (a) Hopkins CR, Lindsley CW, Niswender CM. mGluR4-positive allosteric modulation as potential treatment for Parkinson's disease. *Future Med. Chem.* 2009; 1:501–513. [PubMed: 20161443] (b) Lindsley CW, Niswender CM, Engers DW, Hopkins CR. Recent progress in the development of mGluR4 positive allosteric modulators for the treatment of Parkinson's disease. *Curr. Top Med. Chem.* 2009; 9:949–963. [PubMed: 19754407] (c) Robichaud AJ, Engers DW, Lindsley CW, Hopkins CR. Recent progress on the identification of metabotropic glutamate 4 receptor ligands and their potential utility as CNS therapeutics. *ACS Chem. Neurosci.* 2011; 2:433–449. [PubMed: 22860170]
11. Reynolds, IJ. Metabotropic glutamate receptors as therapeutic targets in Parkinson's disease. 6th International Meeting on Metabotropic Glutamate Receptors Taormina; 14 September to 19 September 2008; Sicily, Italy. 2008.
12. Engers DW, Gentry PR, Williams R, Bolinger JD, Weaver CD, Menon UN, Conn PJ, Lindsley CW, Niswender CM, Hopkins CR. Synthesis and SAR of novel, 4-(phenylsulfamoyl)phenylacetamide mGlu4 positive allosteric modulators (PAMs) identified by functional high-throughput screening (HTS). *Bioorg. Med. Chem. Lett.* 2010; 20:5175–5178. [PubMed: 20667732]
13. Engers DW, Field JR, Le U, Zhou Y, Bolinger JD, Zamorano R, Blobaum AL, Jones CK, Jadhav S, Weaver CD, Conn PJ, Lindsley CW, Niswender CM, Hopkins CR. Discovery, synthesis, and structure-activity relationship development of a series of N-(4-acetamido)phenylpicolinamides as positive allosteric modulators of metabotropic glutamate receptor 4 (mGlu4) with CNS exposure in rats. *J. Med. Chem.* 2011; 54:1106–1110. [PubMed: 21247167]

14. Jones CK, Engers DW, Thompson AD, Field JR, Blobaum AL, Lindsley SR, Zhou Y, Gogliotti RD, Jadhav S, Zamorano R, Bogenpohl J, Smith Y, Morrison R, Daniels JS, Weaver CD, Conn PJ, Lindsley CW, Niswender CM, Hopkins CR. Discovery, synthesis, and structure-activity relationship development of a series of N-4-(2,5-dioxopyrrolidin-1-yl)phenylpicolinamides (VU0400195, ML182): characterization of a novel positive allosteric modulator of the metabotropic glutamate receptor 4 (mGlu₄) with oral efficacy in an antiparkinsonian animal model. *J. Med. Chem.* 2011; 54:7639–7647. [PubMed: 21966889]
15. Hopkins CR, Niswender CM, Lewis LM, Weaver CD, Lindsley CW. Discovery of a potent, selective and in vivo active mGluR4 positive allosteric modulator. 2010 Bookshelf ID: NBK47352.
16. Engers DW, Niswender CM, Weaver CD, Jadhav S, Menon UN, Zamorano R, Conn PJ, Lindsley CW, Hopkins CR. Synthesis and evaluation of a series of heterobiaryl amides that are centrally penetrant metabotropic glutamate receptor 4 (mGluR4) positive allosteric modulators (PAMs). *J. Med. Chem.* 2009; 52:4115–4118. [PubMed: 19469556]
17. Jones CK, Bubser M, Thompson AD, Dickerson JW, Turle-Lorenzo N, Amalric M, Blobaum AL, Bridges TM, Morrison RD, Jadhav S, Engers DW, Italiano K, Bode J, Daniels JS, Lindsley CW, Hopkins CR, Conn PJ, Niswender CM. The mGlu4 positive allosteric modulator VU0364770 produces efficacy alone and in combination with L-DOPA or an adenosine A2A antagonist in preclinical rodent models of Parkinson's disease. *J. Pharmacol. Exp. Ther.* 2012; 340:404–421. [PubMed: 22088953]
18. Meanwell NA. Synopsis of some recent tactical application of bioisosteres in drug design. *J. Med. Chem.* 2011; 54:2529–2591. [PubMed: 21413808]
19. Conn, PJ.; Lindsley, CW.; Hopkins, CR.; Weaver, CD.; Niswender, CM.; Engers, DW. Benzisoxazoles and azabenzisoxazoles as mglur4 allosteric potentiators, compositions, and methods for treating dysfunction. Patent WO2011/100614. 2011. p. 112
20. Conn, PJ.; Lindsley, CW.; Hopkins, CR.; Niswender, CM.; Gogliotti, RD.; Salovich, JM.; Engers, DW.; Cheung, YY. Pyrazolopyridine, pyrazolopyrazine, pyrazolopyrimidine, pyrazolothiophene and pyrazolothiazole compounds as mGluR4 allosteric potentiators and for treating neurological dysfunctions. Patent WO2011/100607. 2011. p. 1-123.
21. Obach RS. Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes. *Drug Metab. Dispos.* 1999; 27:1350–1359. [PubMed: 10534321]
22. Chang G, Steyn SJ, Umland JP, Scott DO. Strategic Use of Plasma and Microsome Binding To Exploit in Vitro Clearance in Early Drug Discovery. *ACS Med. Chem. Lett.* 2010; 1:50–63. [PubMed: 24900175]
23. Kalvass JC, Maurer TS. Influence of nonspecific brain and plasma binding on CNS exposure: implications for rational drug discovery. *Biopharm. Drug Dispos.* 2002; 23:327–338. [PubMed: 12415573]
24. Bridges TM, Morrison RD, Byers FW, Luo S, Daniels JS. Use of a novel rapid and resource-efficient cassette dosing approach to determine the pharmacokinetics and CNS distribution of small molecule 7-transmembrane receptor allosteric modulators in rat. *Pharmacol. Res. Perspect.* 2014; 2:e00077. [PubMed: 25505618]
25. Guram AS, Rennels RA, Buchwald SL. A simple catalytic method for the conversion of aryl bromides to arylamines. *Angew. Chem., Int. Ed. Engl.* 1995; 34:1348–1350.
26. Driver MS, Hartwig JF. Carbon-nitrogen-bond-forming reductive elimination of arylamines from palladium(II) phosphine complexes. *J. Am. Chem. Soc.* 1997; 119:8232–8245.
27. Wang B, Zhou SF. Synthetic and natural compounds that interact with human cytochrome P450 1A2 and implications in drug development. *Curr. Med. Chem.* 2009; 16:4066–4218. [PubMed: 19754423]
28. Lin JH. CYP induction-mediated drug interactions: in vitro assessment and clinical implications. *Pharm. Res.* 2006; 23:1089–1116. [PubMed: 16718615]

ACS Chem Neurosci. Author manuscript; available in PMC 2017 September 21.

Figure 2.
Met ID in rat liver microsomes.

Figure 3.

Compound **26** potentiates the glutamate response in an mGlu₄ expressing cell line (D) and is inactive against other mGlu receptors, except mGlu₆ (F).

Scheme 1.

Synthesis of 26 (VU0418506)a

^aReagents and conditions: (a) NH₂NH₂, 100 °C; (b) 2 M NaOH, Br₂, 29% over two steps;(c) Boc₂O, DMAP, Et₃N, DMF, 88%; (d) Cs₂CO₃, X-Phos, Pd₂(dba)₃, 1,4-dioxane, 100 °C;(e) TFA, CH₂Cl₂, rt, 70% over two steps.

Table 1.

Amide Replacements

Cmpd		mGlu ₄ pEC ₅₀ (± SEM) ^a	mGlu ₄ EC ₅₀ (nM) ^a	%GluMax (± SEM) ^{a,b}
3		6.62 ^c	240 ^c	
7		5.96 ^c	1,100 ^c	
8			Inactive ^d	46 ^d
9		<4.5	>30,000	15.3 ± 2.7
10		<4.5	>30,000	25.1 ± 2.7
11		<4.5	>30,000	16.1 ± 1.9
12		6.24 ± 0.03	578	88.1 ± 2.0

^aCalcium mobilization mGlu₄ assay; values are average of $n = 3$.

^bAmplitude of response in the presence of 30 μ M test compound, normalized to a standard compound, PHCCC (100% GluMax).

^cData taken from ref 16.

^d $n = 1$.

Table 2

Aminopicolinamide Replacements

Cmpd		mGlu ₄ pEC ₅₀ (± SEM) ^a	mGlu ₄ EC ₅₀ (nM) ^a	%GluMax (± SEM) ^{a,b}
13		<4.5	>30,000	14.4 ± 2.2
14		<4.5	>30,000	34.8 ± 7.3
15		5.25 ± 0.07	5645	113.6 ± 7.6
16		6.04 ± 0.04	915	35.7 ± 1.8
17		<5	>10,000	25.9 ± 2.6
18		<4.5	>30,000	17.1 ± 2.4
19		<4.5	>30,000	20.5 ± 0.7
20		<4.5	>30,000	16.5 ± 2.7
21		<4.5	>30,000	19.6 ± 2.0

Cmpd		mGlu ₄ pEC ₅₀ (± SEM) ^a	mGlu ₄ EC ₅₀ (nM) ^a	%GluMax (± SEM) ^{a,b}
22		<4.5	>30,000	19.2 ± 3.5
23		<5	>10,000	23.5 ± 2.6
24		6.47 ± 0.01	339	106.5 ± 1.9
25		7.18 ± 0.03	67	113.7 ± 3.8
26		7.17 ± 0.07	68	109.3 ± 0.8

^a Calcium mobilization mGlu₄ assay; values are average of $n = 3$.

^b Amplitude of response in the presence of 30 μM test compound, normalized to a standard compound, PHCCC (100% GluMax).

Table 3
In Vitro and in Vivo PK Properties of Selected Compounds (Predicted Hepatic Clearance, PPB, CYP Inhibition)

compd	CL _{HEP} (mL/min/kg) ^a		plasma protein binding (F _u) ^b		CYP450 IC ₅₀ (μM) ^c				rat IV (1 mg/kg)	
	rat	human	rat	human	1A2	2C9	2D6	3A4	CL (mL/min/kg)	t _{1/2} (min)
3	60.4	17.2	0.024	0.023	0.13	>30	>30	>30		
7	58.7	18.2	0.019	0.021	0.55	>30	>30	>30		
12	64.1	20.3	0.024	0.026	0.2	>30	>30	>30		
24	44	ND	0.004	0.002	<0.1	>30	>30	>30	117	30.6
25	48.6	16.7	0.009	0.009	<0.1	3.6	>30	13.2	71.7	144
26	12.1	10.1	0.028	0.017	<0.1	8.9	>30	>30	29	91

^aPredicted hepatic clearance based on intrinsic clearance in rat and human liver microsomes.

^bF_u = fraction unbound.

^cIC₅₀ determinations of major CYP enzymes in human liver microsomes using specific probe substrate-metabolite pairs for each CYP.

ND = not determined.

Table 4

In Vivo PK for Lead Compounds

	26		
PK Parameters (IV)	Rat, 1 mg/kg	Dog, 1 mg/kg	Rhesus, Cassette
CL _p (mL/min/kg)	29	36	13
V _{ss} (L/kg)	4.0	2.4	2.5
t _{1/2} (min)	91	221	173
MRT (min)	132	66	184
AUC (hr·ng/mL)	608	515	
PK Parameters (PO)	Rat	Dog, 3 mg/kg	
	3 mg/kg	10 mg/kg	
C _{max} (ng/mL)	429	875	375
T _{max} (hr)	1	2	0.75
AUC PO (hr·ng/mL)	1895	7418	556
%F	100	95	36
Plasma:Brain (PO, 0 – 6 h, 10 mg/kg)			
AUC _{plasma} ₀₋₆	8771		
AUC _{brain} ₀₋₆	18415		
B:P	2.1		

Table 5

Summary of 26 (VU0418506)

26, VU0418506**26, VU0418506**

human mGlu ₄ EC ₅₀ (nM)	68 nM
rat mGlu ₄ EC ₅₀ (nM)	46 nM
rat fold shift (@30 μM)	15.5
MW	262.7
cLogP	3.96
TPSA	53.6
intrinsic clearance (mL/min/kg)	
human CL _{HEP}	10.1
rat CL _{HEP}	12.1
dog CL _{HEP}	18.9
mouse CL _{HEP}	50.1
rhesus CL _{HEP}	11.5
plasma protein binding (% F _u)	
human	1.7
rat	2.8
dog	0.9
mouse	1.9
rhesus	1.4
CYP450 inhibition (μM)	
1A2	<0.1
2C9	8.9
2D6	>30
3A4	>30
2C19	6.1
in vivo PK parameters (rat IV, 1 mg/kg)	
CL (mL/min/kg)	29
V _{ss} (L/kg)	4.0
AUC (ng·h/mL)	608
MRT (min)	132
t _{1/2} (min)	91
in vivo PK parameters (rat PO, 10 mg/kg)	
C _{max} (ng/mL)	875
T _{max} (h)	2
AUC PO (ng·h/mL)	7418

26, VU0418506**26, VU0418506**

%F	95%
rat CNS PO PBL (0–6 h)	
plasma (ng·h/mL)	8771
brain (ng·h/mL)	18 415
B:P	2.1
P_{app} , A–B (10^{-6})	24
P_{app} , B–A (10^{-6})	14
efflux ratio	0.6