Published in final edited form as:
Bioorg Med Chem Lett. 2015 September 15; 25(18): 3956–3960. doi:10.1016/j.bmcl.2015.07.031.

Re-exploring the *N*-phenylpicolinamide derivatives to develop mGlu$_4$ ligands with improved affinity and *in vitro* microsomal stability

Zhaoda Zhang[a], **Kun-Eek Kil**[a], **Pekka Poutiainen**[a], **Ji-Kyung Choi**[a], **Hye-Jin Kang**[b], **Xi-Ping Huang**[b], **Bryan L Roth**[b], and **Anna-Liisa Brownell**[a,*]

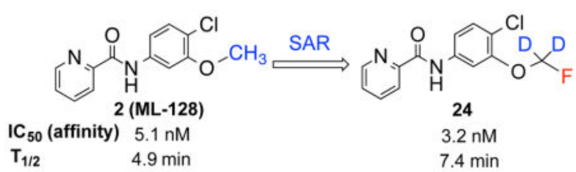
[a]Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Charlestown, Massachusetts 02129, United States

[b]Department of Pharmacology, University of North Carolina Chapel Hill School of Medicine, Chapel Hill, North Carolina 27514, United States

Abstract

In recent years, mGlu$_4$ has received great attention and research effort because of the potential benefits of mGlu$_4$ activation in treating numerous brain disorders, such as Parkinson's disease (PD). Many positive allosteric modulators of mGlu$_4$ have been developed. To better understand the role of mGlu$_4$ in healthy and disease conditions, we are interested in developing an mGlu$_4$ selective radioligand for *in vivo* studies. Thus, we had synthesized and studied [^{11}C]**2** as a PET tracer for mGlu$_4$, which demonstrated some promising features as a PET radioligand as well as the limitation need to be improved. In order to develop an mGlu$_4$ ligand with enhanced affinity and improved metabolic stability, we have modified, synthesized and evaluated a series of new *N*-phenylpicolinamide derivatives. The SAR study has discovered a number of compounds with low nM affinity to mGlu$_4$. The dideuteriumfluoromethoxy modified compound **24** is identified as a very promising mGlu$_4$ ligand, which has demonstrated enhanced affinity, improved *in vitro* microsomal stability, good selectivity and good permeability.

Graphical Abstract



*For correspondence or reprints contact: Anna-Liisa Brownell Athinoula A. Martinos Center for Biomedical Imaging 149, 13th Street Suite 2301 Charlestown, MA 02129 United States, abrownell@partners.org, Phone: 617-726-3807.

Supplementary data
Supplementary data (experimental procedures and spectroscopic characterization of all new compounds) associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.bmcl.

Keywords

metabotropic glutamate receptor subtype 4 ($mGlu_4$); positive allosteric modulator (PAM); positron emission tomography (PET); affinity; metabolic stability; structure-affinity relationship (SAR)

L-Glutamate is the most abundant excitatory neurotransmitter in the CNS (Central nerve system) of vertebrates and probably mediates more than 50% of all synapses.[1,2] Two major classes of receptors, mGlu and iGlu, are involved in glutamate signal transfer. The mGlu belong to Class C of the GPCR (G protein-coupled receptor) super family, which are thought to exist as dimers and have a distinct large extracellular N-terminus. This extracellular N-terminal domain contains two hinged globular domains referred as the Venus Flytrap Domain (VFD), which is the orthosteric binding site for the endogenous ligand, L-glutamate.[3] The mGlu can be further divided into three subgroups including eight known receptor subtypes (group I: $mGlu_1$ and $mGlu_5$, group II: $mGlu_2$ and $mGlu_3$, and group III: $mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$) based on their structural similarity, ligand specificity, and preferred coupling mechanisms.[4] The mGlu are involved in glutamate signaling in almost every excitatory synapse in CNS, and they have distinctive biodistribution in CNS depending on subtypes and subgroups.[5] In recent years, $mGlu_4$ has received great attention and research effort because of the potential benefits of $mGlu_4$ activation in treating numerous brain disorders, such as Parkinson's disease (PD).[6,7] As a group III mGlu, $mGlu_4$ interacts with the $G_{\alpha i/o}$ subunit of G-protein which negatively couples with adenylate cyclase to inhibit cAMP dependent signal pathways.[8,9] The $mGlu_4$ is expressed at multiple synapses throughout the basal ganglia, mainly localized presynaptically and expressed in the striatum, hippocampus, thalamus, and cerebellum.[4,10,11] Its activation reduces neurotransmitter release, a mechanism implicated in the pathophysiology of PD. The activation of the $mGlu_4$ receptor can be accomplished by two different mechanisms: orthosteric agonists (competing with L-glutamate) or noncompetitive positive allosteric modulators (PAMs). Most orthosteric ligands of $mGlu_4$ made in the past lack clear subtype selectivity and BBB (Blood-brain barrier) penetration, but notable examples exist of selective and brain penetrant orthosteric agonists, such as LSP4-2022.[12,13] Much recent effort has been focused on the development of allosteric modulators, which target the seven-transmembrane spanning domain. In particular, the allosteric modulation of $mGlu_4$ has spurred intense interest after (−)-PHCCC (**1**, N-phenyl-7-(hydroxyimino)-cyclopropa[*b*]chromen-1*a*-carboxamide), a partially selective $mGlu_4$ PAM, was discovered and demonstrated activity in models of neuroprotection and PD. Since then there has been substantial progress in identifying PAMs for $mGlu_4$.[6,14,15] Figure 1 shows some representative $mGlu_4$ PAMs.[6,14,16,17,18,19,20] Subsequent results with PAMs of $mGlu_4$ have further validated the antiparkinsonian activity in animal models of PD,[11,17,21,22,23,24] in which this approach has opened a new avenue for developing nondopaminergic treatments for PD and for identifying a novel disease modifying therapeutics.

To better understand the role of $mGlu_4$ in healthy and disease conditions, we are interested in developing an $mGlu_4$ selective radioligand for *in vivo* study. As a noninvasive medical imaging technique and a powerful tool in neurological research, positron emission tomography (PET) offers a possibility to visualize and analyze the target receptor expression under physiological and pathophysiological conditions. PET is being applied more often to detect disease-related biochemical changes before the disease-associated anatomical changes could be found by standard medical imaging modalities. Moreover, PET tracers serve as invaluable biomarkers during the development of potential therapeutic drugs. Thus, extensive research efforts have been directed toward the development of PET radioligands suitable for probing mGlu such as $mGlu_1$ and $mGlu_5$.[15]

Recently, we have reported a carbon-11 labeled PET ligand [^{11}C]**2** (*N*-(4-Chloro-3-[^{11}C]methoxyphenyl)picolinamide)[25], which was based on a reported $mGlu_4$ PAM **2**[16]. In 2009, two research groups at Addex Pharma[26] and Vanderbilt University[16] have independently disclosed a series of small arylamide compounds as a new class of $mGlu_4$ PAMs. Engers et al. found from a high-throughput screening there were a number of small arylamide compounds having $mGlu_4$ PAM activity. They reported the SAR study, *in vitro* pharmacokinetic (PK) parameters and *in vivo* rat PK, which included the SAR results for sixteen *N*-phenylpicolinamide derivatives.[16] Compounds **2** and **3** were the most potent $mGlu_4$ PAMs in this series and showed some potentially suitable properties for PET tracer development, which include: 1) Rapid penetration into rat brain following intraperitoneal injection (T_{max} for brain: 0.5 h); 2) High brain:plasma (B/P) partition coefficients for both compounds (B/P=4.1 for **2** and 9.9 for **3**), in which B/P was determined by $AUC_{0\text{–}8h,\ Brain}/AUC_{0\text{–}8h,\ Plasma}$; 3) Good *in vitro* potency and efficacy for both human and rat $mGlu_4$ compared to previous reported $mGlu_4$ PAM; 4) Good selectivity over other mGlu subtypes; 5) Compound **2** was the first $mGlu_4$ PAM to demonstrate efficacy in a preclinical rodent model of motor impairments associated with PD.[6] Thus, we had synthesized and studied [^{11}C]**2** as a PET tracers for $mGlu_4$. This compound demonstrated some promising features as a PET radioligand such as the fast uptake into brain and the specific accumulation in $mGlu_4$-rich regions of the brain. However, in comparison to one of the best $mGlu_5$ PET tracer [^{18}F]FPEB (3-[^{18}F]fluoro-5-(2-pyridinylethynyl)benzonitrile)[27,28], [^{11}C]**2** showed the decreased retention time in the brain, which may affect the quality of the imaging. The results indicate that the affinity and metabolic stability of this class of tracers need further optimization. We report here the synthesis and structure-affinity relationship study of new *N*-phenylpicolinamide derivatives to develop $mGlu_4$ ligands with improved affinity and metabolic stability.

We have modified and synthesized a series of new *N*-phenylpicolinamide derivatives for SAR study, in which the syntheses are shown in Scheme 1 – 3 (see Supplementary data). Three most active known compounds in this series (**2**, **3**, and **10**) were also synthesized and evaluated as the reference compounds for optimization. On the basis of previous SAR results[16], we modified compound **2** at three positions (3- or 4-phenyl, 6-pyrindyl) as illustrated in Figure 2. It is known that the SAR of this series was tight,[6,16] so we started with minor modifications based on the reported data. As shown in Table 1, the modifications include the isosteric replacement of hydrogen by fluorine or deuterium, oxygen by sulfur, methoxy by cyano group and change for different halogen atoms. The radiolabeling strategy was also considered in lead optimization design to generate the facile labeling positions for either C-11 or F-18 tracer.

Since poor BBB permeability and high nonspecific binding (NSB) are among the most frequent causes for failure in CNS PET ligand development, it is necessary to consider some important physicochemical parameter such as MW, ClogP and tPSA at the design stage. It has been recently proposed that more desirable ranges for CNS drugs are ClogP <3, MW < 360 and 40 < tPSA < 90.[29] As shown in Table 1, all compounds except **12** and **22** possess the favorable physicochemical parameters, making them ideal candidates for CNS ligand development.

The lead compounds **2** and **3** were identified as $mGlu_4$ PAMs by using functional assays (calcium mobilization assays for human $mGlu_4$ and thallium flux assays for rat $mGlu_4$) and characterized with EC_{50}, the maximum response and the fold shift values.[16] It is known that the EC_{50} value may not always correlated closely to the affinity value for PAM.[30] It is very important to study the binding affinity for developing PET ligands. Thus, we prepared the tritium-labeled compound **2** ([^{3}H]**2**, *N*-(4-chloro-3-(methoxy-t_3)phenyl)picolinamide) for competitive binding assay.[31] The synthesized compounds were characterized with competitive binding studies using $mGlu_4$ transfected CHO cells by increasing the concentration of test materials from 0.01 nM to 10 μM in presence of 2 nM of [^{3}H]**2**, in which the binding affinities to $mGlu_4$ were described as IC_{50} values (Table 1).[32]

In structure-affinity study, we first evaluated the substitutions at the 4-phenyl position by keeping the 3-methoxy group constant. The 4-phenyl position of *N*-phenylpicolinamide was tolerated with some substitutions as demonstrated in known compounds **6–8**, in which compounds **6** and **7** were reported very potent but poor brain penetration.[20] Thus we limited the 4-phenyl substitutions for different halogens. The results show that the 4-chloro substitution give the best affinity, in which the affinity values of **2** and **10–13** are in the following order: Cl < H < F < I < Br. Larger halogen substitutions such as iodine and bromine led to substantial loss in affinity. It was then found that the 3-methylthio group was superior to the 3-methoxy group by comparing compounds **13** and **14**, showing a 2.8 fold enhancement in affinity.

On the other hand, compounds **15–17** had been incorporated a fluorine atom at 6-pyrindyl position of *N*-phenylpicolinamide, which can have a relatively facile fluorine-18 labeling. Compared to **2** and **3,** the affinity of **15** and **16** was not significantly reduced.

Next we turned our attention to the 3-phenyl position. It is considered that the metabolic stability was one of major issues for ML-128 (**2**), in which the 3-methoxy group was identified as the soft group. The 3-phenyl position was also very sensitive with substitutions. It was reported a simple change of 3-difluoromethoxy in compound **3** to 3-trifluoromethoxy group imparted a more than 10 fold loss of activity.[16] Our initial effort was directed at 3-cyano substitution, in which ^{11}C-cyanation may be carried out through a palladium-mediated cyanation or the Rousenmund-von Braun reaction.[33] Five 3-cyanophenyl compounds (**18**–**22**) with different 4-phenyl substitutions were evaluated. The results show that the 3-cyano-4-chloro-analog **20** give a similar affinity compared to the 3-methoxy-4-chloro-analog **2**. The affinity values of **18**–**22** are depending on 4-phenyl substitution and in the following order: Cl < F < H < Br < I, which shows different substitution effect compared to 3-methoxy analogs **2** and **10–13**. We then replaced 3-methoxy with 3-fluoromethoxy for two reasons: first, since both 3-methoxy- and 3-difluoromethoxy-analogs exhibited the activity, fluoromethoxy should be also active; second, it generates a position for fluorine-18 labeling. Fluorine-18 is often the radionuclide of choice for both its physical and nuclear characteristics. Its half-life is long enough to carry out relatively extended imaging protocols when compared to what is possible with carbon-11. This facilitates kinetic studies and high-quality metabolic and plasma analysis. However, fluorine-18 labeling is normally limited to chemical structures already containing a fluorine atom and the possible labeling strategies are limited for the preparation of radiotracers of high specific radioactivity. The result shows that 3-fluoromethoxy compound **23** has an improved affinity (3.2 nM) compared to that (5.1 nM) of **2**, which improves 1.6 fold. However, 3-fluoromethoxy group may not be metabolically stable, since the 3-methoxy and 3-difluoromethoxy groups were metabolically unstable in compounds **2** and **3**. On the other hand, 3-trifluoromethoxy analog of **3** was significantly more stable but lack activity.[16] Hence, we had applied a 3-dideuteriumfluoromethoxy group to replace 3-fluoromethoxy group as shown in compounds **24** and **25**. Deuterium isotope effects have been used to reduce *in vivo* metabolic rates. For example, Zhang et al. reported that a deuterium-substituted analog (with $^{18}FD_2CO$) as a radioligand for peripheral benzodiazepine receptor (PBR) had remarkably prolonged the half-life ($T_{1/2}$) in mice brain.[34] The deuterium substitution may reduce the rate of defluorination initiated by cleavage of the C–H bond without altering the binding affinity to $mGlu_4$. The result shows that the 3-dideuteriumfluoromethoxy modified compounds **24** and **25** have excellent affinity.

On the basis of the affinity of these picolinamide derivatives, we subsequently determined the *in vitro* microsomal stability of the selected compounds that include **2**–**4** and **23**–**24** (Table 2). Compound **4** (ADX88178) is one of a most potent $mGlu_4$ PAM to date and was shown to be orally active in a number of preclinical *in vivo* PD models.[14,22] As Table 2 shows, the dideuteriumfluoromethoxy-compound **24** ($T_{1/2}$ = 7.4 min) is more stable than the corresponding fluoromethoxy-analog **23** ($T_{1/2}$ = 5.8 min) and the methoxy-analog **2**($T_{1/2}$ = 4.9 min). It was reported that the cleaving rate of the C–H bond wasabout 6.7 times faster than that of C–D bond at 25 °C.[34] On the other hand, the half time and the difference of the metabolic rates of the dideuteriumfluoromethoxy analog and the fluoromethoxy analog depended on the level of the enzyme. In developing the PET ligand for PBR, Zhang et al. found that the half time ($T_{1/2}$) in the plasma was 2.575 min for the deuterium-substituted analog (with $^{18}FD_2CO$) and 2.367 min for the non-deuterated analog. However, the half time ($T_{1/2}$) of the deuterium-substituted analog in the brain was >60 min, whereas that of for the non-deuterated analog was only 2.227 min.[34] We anticipate that the difference of the half times in the brain between compounds **24** and **23** as well as **2** could be more significant. Compared to **4**, compound **24** has the same affinity and a similar *in vitro* microsomal stability. It is clear that compound **24** has both enhanced affinity and improved *in vitro* microsomal stability compared to **2**.

The selectivity of compound **24** was also determined among the various mGlu subtypes, in which the functional assays were carried out on $mGlu_1$, $mGlu_2$, $mGlu_5$, $mGlu_6$ and $mGlu_8$. Compound **24** showed little activity against these mGlu (Supporting Information).

In addition, the permeability values of **2–4** and **23** were measured using BBB PAMPA model at pH 7.4, which characterized the rate across the BBB due to passive diffusion. The determined effective permeability (P_e) values are summarized in Table 2, in which the P_e results for internal highly and low permeable standards are 160 for propranolol and <2.8 for atenolol, respectively. This result indicates that compounds **23** and **24** have good BBB permeability. Although high BBB passive permeability does not necessary translate to sufficient unbound drug concentration in the brain because of potential intrinsic clearance and efflux transport, it is beneficial for CNS drug candidates.

In summary, we have modified, synthesized and evaluated a series of new *N*-phenylpicolinamide derivatives. Our research further demonstrated that *N*-phenylpicolinamide is a good template to develop $mGlu_4$ ligands, which has offered extensive SAR results by us and other labs.[16,26] The SAR study has discovered a number of compounds with good affinities (<10 nM) to $mGlu_4$. The dideuteriumfluoromethoxy modified compound **24** is identified as a very promising $mGlu_4$ ligand, which has demonstrated enhanced affinity, improved *in vitro* microsomal stability, good selectivity and good permeability. Compound **24** is considered as an attractive candidate for future labeling with fluorine-18 as an $mGlu_4$ PET tracer. Since a number of compounds have good affinity we are studying their PAM activity to $mGlu_4$ and potential therapeutic applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding was provided by NIBIB-R01EB012864 and NIMH-R01MH91684 to A.-L. B. Authors would like to acknowledge supporting grants for the instrumentation 1S10RR029495-01, 1S10RR026666-01, and 1S10RR023452-01. The mGlu functional data was generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2013-00017-C (NIMH PDSP). The NIMH PDSP is Directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA. Financial support for PP from The Orion Farmos Research Foundation, Kuopio University Foundation, and Sigrid Juselius Foundation is gratefully acknowledged.

References

1. Conn PJ. Physiological roles and therapeutic potential of metabotropic glutamate receptors. Annals of the New York Academy Sciences. 2003; 1003:12–21.
2. Watkins JC, Jane DE. The glutamate story. British Journal of Pharmacology. 2006; 147(S1):S100–S108. [PubMed: 16402093]
3. Hampson, DR.; Rose, EM.; Antflick, JE. The structure of metabotropic glutamate receptors. In: Gereau, RWSGT., editor. The glutamate receptors. Human Press; Totowa: 2008. p. 363-386.
4. Conn PJ, Pin JP. Pharmacology and functions of metabotropic glutamate receptors. Annual Review of Pharmacology and Toxicology. 1997; 37:205–237.
5. Riedel G, Platt B, Micheau J. Glutamate receptor function in learning and memory. Behavioral Brain Research. 2003; 140:1–47.
6. 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 Chemical Neuroscience. 2011; 17:433–449. [PubMed: 22860170]

7. Amalric M, Lopez S, Goudet C, Fisone G, Battaglia G, Nicoletti F, Pin JP, Acher FC. Group III and subtype 4 metabotropic glutamate receptor agonists: Discovery and pathophysiological applications in Parkinson's disease. Neuropharmacology. 2013; 66:53–64. [PubMed: 22664304]

8. Fettagutti F, Balani-Guerra B, Corsi M, Nakanishi S, Corti C. Activation of the extracellular signal regulated kinase 2 by metabotropic glutamate receptors. European Journal of Neuroscience. 1999; 11:2073–2082. [PubMed: 10336676]

9. Niswender CM, Conn PJ. Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annual Review of Pharmacology and Toxicology. 2010; 50:295–322.

10. Corti C, Aldegheri L, Somogyi P, Ferraguti F. Distribution and synaptic localization of the metabotropic glutamate receptor 4 (mGluR4) in the rodent CNS. Neuroscience. 2002; 110:403–420. [PubMed: 11906782]

11. Valenti O, Mannaioni G, Seabrook GR, Conn PJ, Marino MJ. Group III metabotropic glutamate-receptor-mediated modulation of excitatory transmission in rodent substantia nigra pars compacta dopamine neurons. Journal of Pharmcology and Experimental Therapeutics. 2005; 313:1296–1304.

12. Goudet C, Vilar B, Courtiol T, Deltheil T, Bessiron T, Brabet I, Oueslati N, Rigault D, Bertrand HO, McLean H, Daniel H, Amalric M, Acher F, Pin JP. A novel selective metabotropic glutamate receptor 4 agonist reveals new possibilities for developing subtype selective ligands with therapeutic potential. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2012; 26(4):1682–93. [PubMed: 22223752]

13. Cajina M, Nattini M, Song D, Smagin G, Joergensen EB, Chandrasena G, Bundgaard C, Toft DB, Huang X, Acher F, Doller D. Qualification of LSP1-2111 as a Brain Penetrant Group III Metabotropic Glutamate Receptor Orthosteric Agonist. ACS Medicinal Chemistry Letters. 2014; 5(2):119–123. [PubMed: 24900783]

14. Lindsley CW, Hopkins CR. Metabotropic glutamate receptor 4 ($mGlu_4$)-positive allosteric modulators for the treatment of Parkinson's disease: historical perspective and review of the patent literature. Expert Opinion on Therapeutic Patents. 2012; 22(5):461–481. [PubMed: 22506633]

15. Zhang, Z.; Brownell, A-L. Imaging of Metabotropic Glutamate Receptors (mGluR)s. In: Bright, P., editor. Neuroimaging - Clinical applications. InTech - Open Access Publisher; Rijeka, Croatia: 2012. p. 499-532.

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). Journal of Medicinal Chemistry. 2009; 52:4115–4118. [PubMed: 19469556]

17. 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 ($mGlu_4$) with Oral Efficacy in an Antiparkinsonian Animal Model. Journal of Medicinal Chemistry. 2011; 54(21):7639–7647. [PubMed: 21966889]

18. Kalinichev M, Le Poul E, Bolea C, Girard F, Campo B, Fonsi M, Royer-Urios I, Browne SE, Uslaner JM, Davis MJ, Raber J, Duvoisin R, Bate ST, Reynolds IJ, Poli S, Celanire S. Characterization of the novel positive allosteric modulator of the metabotropic glutamate receptor 4 ADX88178 in rodent models of neuropsychiatric disorders. Journal of Pharmacology and Experimental Therapeutics. 2014; 350(3):495–505. 11. [PubMed: 24947466]

19. Hong SP, Liu KG, Ma G, Sabio M, Uberti MA, Bacolod MD, Peterson J, Zou ZZ, Robichaud AJ, Doller D. Tricyclic Thiazolopyrazole Derivatives as Metabotropic Glutamate Receptor 4 Positive Allosteric Modulators. Journal of Medicinal Chemistry. 2011; 54(14):5070–5081. [PubMed: 21688779]

20. 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. Current Topics in Medicinal Chemistry (Sharjah, United Arab Emirates). 2009; 9(10):949–963.

21. 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. Journal of Neuroscience. 2003; 23:7218–7226. [PubMed: 12904482]
22. Le Poul E, Bolea C, Girard F, Poli S, Charvin D, Campo B, Bortoli J, Bessif A, Luo B, Koser AJ, Hodge LM, Smith KM, DiLella AG, Liverton N, Hess F, Browne SE, Reynolds IJ. A potent and selective metabotropic glutamate receptor 4 positive allosteric modulator improves movement in rodent models of Parkinson's disease. Journal of Pharmacology and Experimental Therapeutics. 2012; 343(1):167–177. [PubMed: 22787118]
23. Bennouar KE, Uberti MA, Melon C, Bacolod MD, Jimenez HN, Cajina M, Kerkerian-Le Goff L, Doller D, Gubellini P. Synergy between L-DOPA and a novel positive allosteric modulator of metabotropic glutamate receptor 4: Implications for Parkinson's disease treatment and dyskinesia. Neuropharmacology. 2013; 66:158–169. [PubMed: 22491024]
24. Iderberg H, Maslava N, Thompson AD, Bubser M, Niswender CM, Hopkins CR, Lindsley CW, Conn PJ, Jones CK, Cenci MA. Pharmacological stimulation of metabotropic glutamate receptor type 4 in a rat model of Parkinson's disease and L-DOPA-induced dyskinesia: Comparison between a positive allosteric modulator and an orthosteric agonist. Neuropharmacology. 2015 Ahead of Print.
25. Kil KE, Zhang Z, Jokivarsi K, Gong C, Choi JK, Kura S, Brownell AL. Radiosynthesis of *N*-(4-chloro-3-[^{11}C]methoxyphenyl)-2-picolinamide ([^{11}C]ML128) as a PET radiotracer for metabotropic glutamate receptor subtype 4 (mGlu$_4$). Bioorganic and Medicinal Chemistry. 2013; 21:5955–5962. [PubMed: 23978356]
26. Bolea C. Preparation of amido derivatives and their use as positive allosteric modulators of metabotropic glutamate receptors. 2009 2008-EP59043,2009010454, 20080710.
27. Patel S, Hamill T, Connolly B, Jagoda E, Li W, Gibson R. Species differences in mGluR5 binding sites in mammalian central nervous system determined using in vitro binding with [^{18}F]F-PEB. Nuclear Medicine and Biology. 2007; 34:1009–17. [PubMed: 17998106]
28. Wang J, Tueckmantel W, Zhu A. Pellegrino D, Brownell A-L, Synthesis and preliminary biological evaluation of 3-[(18)F]fluoro-5-(2-pyridinylethynyl)benzonitrile as a PET radiotracer for imaging metabotropic glutamate receptor subtype 5. Synapse. 2007; 61(12):951–61. [PubMed: 17787003]
29. Zhang L, Villalobos A, Beck EM, Bocan T, Chappie TA, Chen L, Grimwood S, Heck SD, Helal CJ, Hou X, Humphrey JM, Lu J, Skaddan MB, McCarthy TJ, Verhoest PR, Wager TT, Zasadny K. Design and Selection Parameters to Accelerate the Discovery of Novel Central Nervous System Positron Emission Tomography (PET) Ligands and Their Application in the Development of a Novel Phosphodiesterase 2A PET Ligand. Journal of Medicinal Chemistry. 2013; 56(11):4568–4579. [PubMed: 23651455]
30. Kenakin T, Onaran O. The ligand paradox between affinity and efficacy: can you be there and not make a difference? Trends Pharmacol Sci. 2002; 23(6):275–280. [PubMed: 12084633]
31. Kil KE, Poutiainen P, Zhang Z, Zhu A, Choi JK, Jokivarsi K, Brownell AL. Radiosynthesis and Evaluation of an 1^{8}F-Labeled Positron Emission Tomography (PET) Radioligand for Metabotropic Glutamate Receptor Subtype 4 (mGlu$_4$). Journal of Medicinal Chemistry. 2014; 57(21):9130–9138. [PubMed: 25330258]
32. Poutiainen P, Kil K-E, Zhang Z, Kuruppu D, Tannous B, Brownell A-L. Co-operative binding assay for the characterization of mGlu$_4$ allosteric modulators. Neuropharmacology. 2015
33. Miller PW, Long NJ, Vilar R, Gee AD. Synthesis of ^{11}C, ^{18}F, ^{15}O, and ^{13}N radiolabels for positron emission tomography. Angew Chem, Int Ed. 2008; 47(47):8998–9033.
34. Zhang MR, Maeda J, Ito T, Okauchi T, Ogawa M, Noguchi J, Suhara T, Halldin C, Suzuki K. Synthesis and evaluation of *N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-[^{18}F]fluoromethoxy-d_2-5-methoxybenzyl)acetamide: a deuterium-substituted radioligand for peripheral benzodiazepine receptor. Bioorg Med Chem. 2005; 13(5):1811–1818. [PubMed: 15698799]

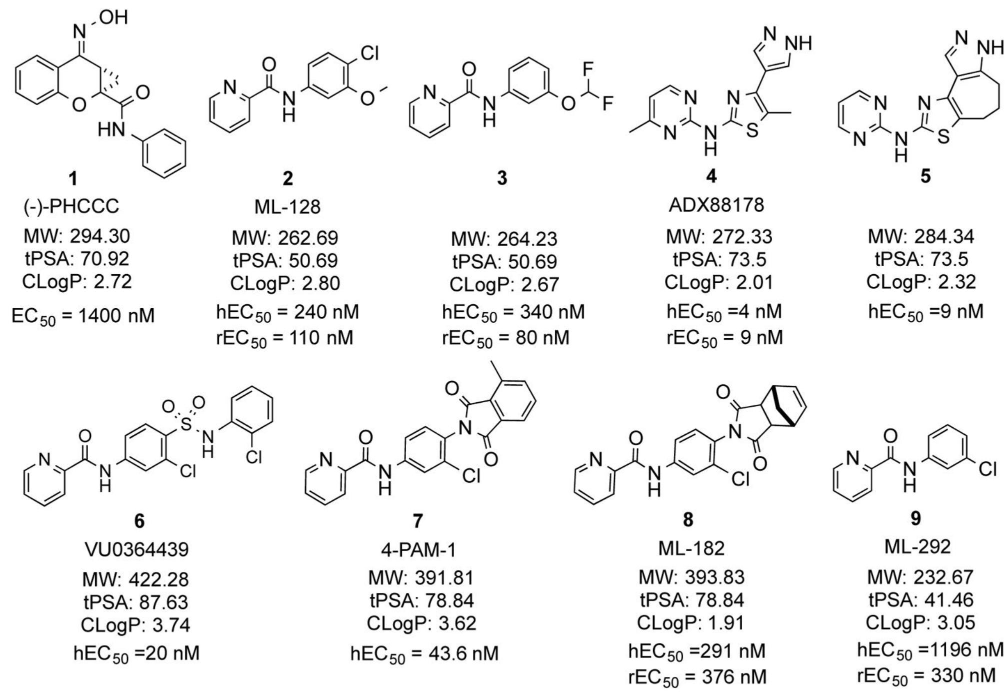
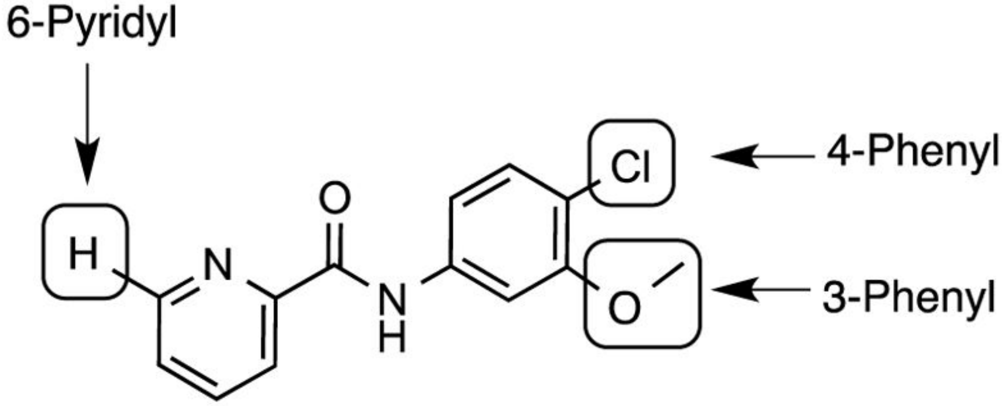


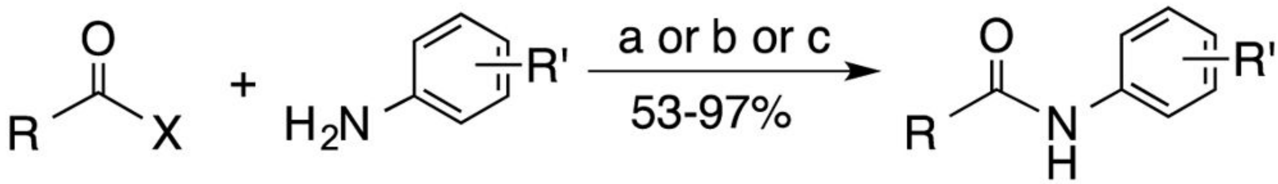
Figure 1.

Some representative $mGlu_4$ PAMs.



2

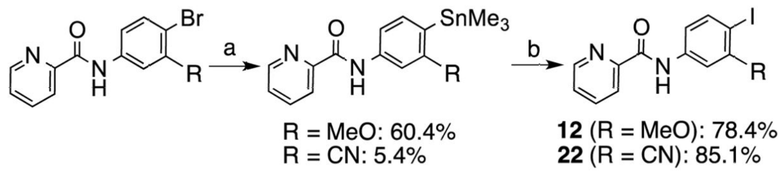
Figure 2.
Modificatons on compound **2**



Scheme 1.

Synthesis of the *N*-phenylpicolinamide derivatives.

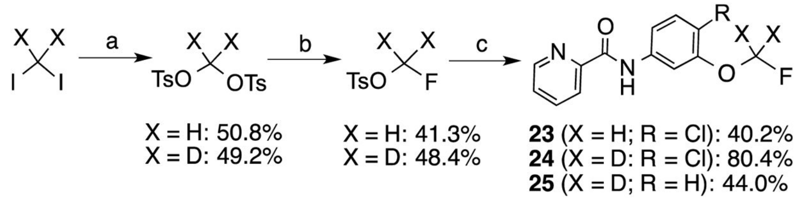
Reagents and conditions: (a) for carboxylic acids, EDC.HCl, HOBt.H_2O, DIPEA, dioxane; (b) for carboxylic acids, 1. thionyl chloride, benzene, reflux for 2 h; 2. TEA, THF, 40 °C, 1 h; (c) for acid chloride, DIPEA, CH_2Cl_2, 4h.



Scheme 2.

Synthesis of the *N*-phenylpicolinamides **12** and **22.**

Reagents and conditions: (a) $(SnMe_3)_2$, $Pd(PPh_3)_4$, Toluene, reflux, 8.5 h; (b) I_2, CH_2Cl_2, 2 h.



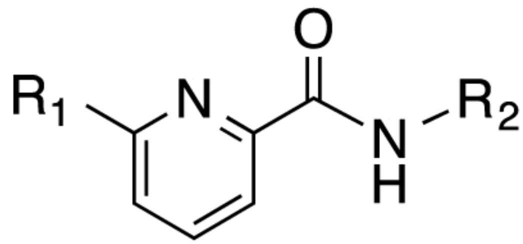
Scheme 3.

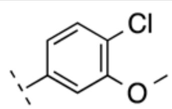
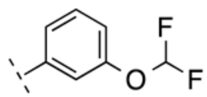
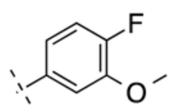
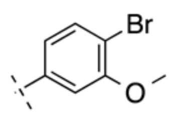
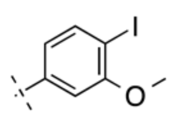
Synthesis of the *N*-(3-fluoromethoxyphenyl)picolinamides **23 – 25.**

Reagents and conditions: (a) Ag(OTs), MeCN, reflux, overnight; (b) CsF, $HO(CH_2O)_6H$, reflux, 3.5 h; (c) *N*-(4-R-3-hydroxyphenyl)picolinamide, K_2CO_3, 40–50 °C, 3 days.

Table 1

SAR of *N*-phenylpicolinamide derivatives.

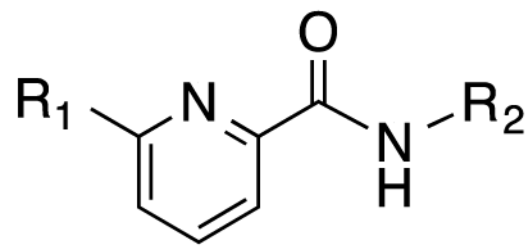


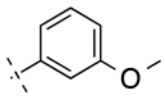
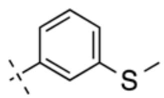
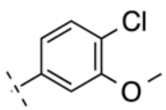
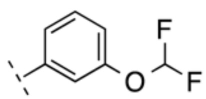
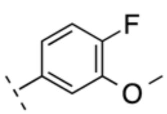
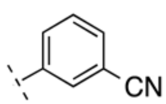
Compd	R_1	R_2	MW	CLogP	tPSA	Affinity IC_{50} (nM)	$Log(IC_{50} \pm SE)$
2	H		262.69	2.80	50.69	5.1	−8.29 ± 0.09
3	H		264.23	2.67	50.69	4.6	−8.34 ± 0.08
10	H		246.24	2.26	50.69	31.6	−7.50 ± 0.09
11	H		307.15	2.95	50.69	322	−6.49 ± 0.08
12	H		354.15	3.16	50.69	146	−6.84 ± 0.10

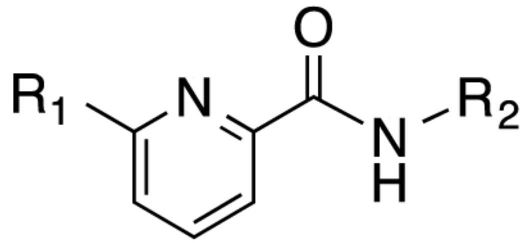
Author Manuscript

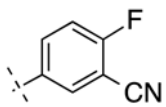
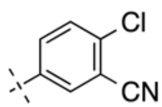
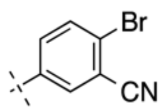
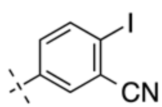
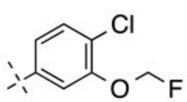
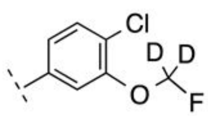
Author Manuscript

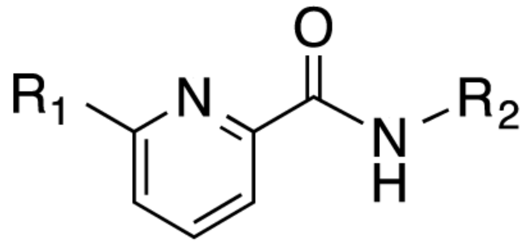
Author Manuscript



Compd	R_1	R_2	MW	CLogP	tPSA	Affinity IC_{50} (nM)	Log(IC_{50} ± SE)
13	H		228.25	2.22	50.69	13.7	−7.86 ± 0.10
14	H		244.31	2.81	41.46	4.9	−8.31 ± 0.07
15	F		280.68	2.96	50.69	7.3	−8.13 ± 0.11
16	F		282.22	2.85	50.69	6.7	−8.18 ± 0.10
17	F		264.23	2.42	50.69	89.2	−7.05 ± 0.10
18	H		223.23	1.89	65.25	10.4	−7.98 ± 0.05



Compd	R_1	R_2	MW	CLogP	tPSA	Affinity IC_{50} (nM)	$Log(IC_{50} \pm SE)$
19	H		241.23	2.06	65.25	7.4	−8.13 ± 0.04
20	H		257.68	2.50	65.25	5.3	−8.28 ± 0.04
21	H		302.13	2.78	65.25	47	−7.33 ± 0.08
22	H		349.13	3.04	65.25	172	−6.77 ± 0.08
23	H		280.68	2.97	50.69	3.2	−8.49 ± 0.07
24	H		282.70	2.97	50.69	3.2	−8.49 ± 0.11



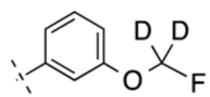
Compd	**R$_1$**	**R$_2$**	**MW**	**CLogP**	**tPSA**	**Affinity IC$_{50}$ (nM)**	**Log(IC$_{50}$ ± SE)**
25	H		248.25	2.39	50.69	3.7	−8.43 ± 0.03

Table 2

In Vitro properties of the selected compounds.

Compound	Affinity IC_{50} (nM) (n=3)	κ^{a}	SEM(κ) (n=2)	$T_{1/2}$ (min)	Avg. P_e (10^{-6} cm/s)
2	5.1	0.141	0.010	4.9	256
3	4.6	0.132	0.008	5.2	214
4	3.2	0.099	0.005	7.0	257
23	3.2	0.120	0.010	5.8	272
24	3.2	0.093	0.005	7.4	

[a]The decay constant that is slope of log concentration vs time profile ($T_{1/2}$=Ln2/κ).