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Re-exploring the *N*-phenylpicolinamide derivatives to develop mGlu₄ ligands with improved affinity and in vitro microsomal stability



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ABSTRACT

In recent years, mGlu₄ has received great attention and research effort because of the potential benefits of mGlu₄ activation in treating numerous brain disorders, such as Parkinson's disease (PD). Many positive allosteric modulators of mGlu₄ have been developed. To better understand the role of mGlu₄ in healthy and disease conditions, we are interested in developing an mGlu₄ selective radioligand for in vivo studies. Thus, we had synthesized and studied [¹¹C]2 as a PET tracer for mGlu₄, which demonstrated some promising features as a PET radioligand as well as the limitation need to be improved. In order to develop an mGlu₄ 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 mGlu₄. The dideuteriumfluoromethoxy modified compound **24** is identified as a very promising mGlu₄ ligand, which has demonstrated enhanced affinity, improved in vitro microsomal stability, good selectivity and good permeability.

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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: mGlu₁ and mGlu₅, group II: mGlu₂ and mGlu₃, and group III: mGlu₄, mGlu₆, mGlu₇ and mGlu₈) 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, mGlu₄ has received great attention and research effort because of the potential benefits of mGlu₄ activation in treating numerous brain disorders, such as Parkinson's disease (PD).^{6,7} As a group III mGlu, mGlu₄ interacts with the G_{αi/o} subunit of

G-protein which negatively couples with adenylate cyclase to inhibit cAMP dependent signal pathways.^{8,9} The mGlu₄ 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 mGlu₄ receptor can be accomplished by two different mechanisms: orthosteric agonists (competing with l-glutamate) or noncompetitive positive allosteric modulators (PAMs). Most orthosteric ligands of mGlu₄ 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 mGlu₄ has spurred intense interest after (–)-PHCCC (**1**, *N*-phenyl-7-(hydroxyimino)-cyclopropa[b] chromen-1a-carboxamide), a partially selective mGlu₄ PAM, was discovered and demonstrated activity in models of neuroprotection and PD. Since then there has been substantial progress in identifying PAMs for mGlu₄.^{6,14,15} Figure 1 shows some representative mGlu₄ PAMs.^{6,14,16–20} Subsequent results with PAMs of mGlu₄ have further validated the antiparkinsonian activity in animal models of PD,^{11,17,21–24} in which this approach has opened a new avenue for

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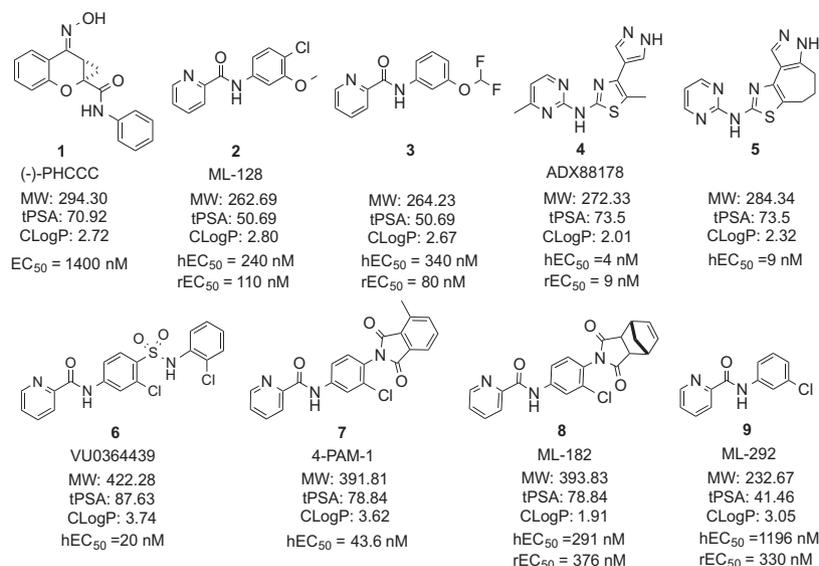


Figure 1. Some representative mGlu₄ PAMs.

developing nondopaminergic treatments for PD and for identifying a novel disease modifying therapeutics.

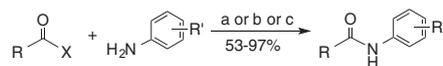
To better understand the role of mGlu₄ in healthy and disease conditions, we are interested in developing an mGlu₄ selective radioligand for in vivo study. As a noninvasive medical imaging technique and a powerful tool in neurological research, positron emission tomography (PET) offers a possibility 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 mGlu₁ and mGlu₅.¹⁵

Recently, we have reported a carbon-11 labeled PET ligand [¹¹C]**2** (*N*-(4-chloro-3-[¹¹C]methoxyphenyl)picolinamide),²⁵ which was based on a reported mGlu₄ PAM **2**.¹⁶ In 2009, two research groups at Addex Pharma²⁶ and Vanderbilt University¹⁶ have independently disclosed a series of small arylamide compounds as a new class of mGlu₄ PAMs. Engers et al. found from a high-throughput screening there were a number of small arylamide compounds having mGlu₄ 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 mGlu₄ 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_{0–8h,Brain}/AUC_{0–8h,Plasma}; (3) good in vitro potency and efficacy for both human and rat mGlu₄ compared to previous reported mGlu₄ PAM; (4) good selectivity over other mGlu subtypes; (5) compound **2** was the first mGlu₄ PAM to demonstrate efficacy in a preclinical rodent model of motor impairments associated with PD.⁶ Thus, we had synthesized and studied [¹¹C]**2** as a PET tracers for mGlu₄. This compound demonstrated some promising features as a PET radioligand such as the fast uptake into brain and the specific accumulation in mGlu₄-rich regions of the brain. However, in comparison to one of the best mGlu₅ PET tracer

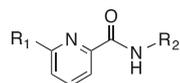
[¹⁸F]FPEB (3-[¹⁸F]fluoro-5-(2-pyridinylethynyl)benzonitrile),^{27,28} [¹¹C]**2** showed the decreased retention time in the brain, which may affect the quality of the imaging. The results indicate that the affinity and metabolic stability of this class of tracers need further optimization. We report here the synthesis and structure–affinity relationship study of new *N*-phenylpicolinamide derivatives to develop mGlu₄ 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 Schemes 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-pyridinyl) 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.



Scheme 1. Synthesis of the *N*-phenylpicolinamide derivatives. Reagents and conditions: (a) for carboxylic acids, EDC·HCl, HOBT·H₂O, 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, CH₂Cl₂, 4 h.

Table 1
SAR of *N*-phenylpicolinamide derivatives

Compd	R ₁	R ₂	MW	ClogP	tPSA	Affinity IC ₅₀ (nM)	Log(IC ₅₀ ± 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
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
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
25	H		248.25	2.39	50.69	3.7	-8.43 ± 0.03

Table 2
In vitro properties of the selected compounds

Compd	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 logconcentration versus time profile (T_{1/2} = Ln2/κ).

out on mGlu₁, mGlu₂, mGlu₅, mGlu₆ and mGlu₈. 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 mGlu₄ 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 mGlu₄. The dideuterium-fluoromethoxy modified compound **24** is identified as a very promising mGlu₄ 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 mGlu₄ PET tracer. Since a number of compounds have good affinity we are studying their PAM activity to mGlu₄ and potential therapeutic applications.

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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.2015.07.031>.

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