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Synthesis and biological evaluation of picolinamides and thiazole-2-carboxamides as mGluR5 (metabotropic glutamate receptor 5) antagonists

Hoang Nam Vu [a,b], Ji Young Kim [a,c], Ahmed H. E. Hassan [a,b], Kihang Choi [c], Jong-Hyun Park [a], Ki Duk Park [a,b], Jae Kyun Lee [a,b], Ae Nim Pae [a,b], Hyunah Choo [a,b], Sun-Joon Min [d,*], Yong Seo Cho [a,b,*]

[a] Center for Neuro-Medicine, Brain Science Institute, Korea Institute of Science and Technology (KIST), 5 Hwarangno 14-gil, Seongbuk-gu, Seoul 02792, Republic of Korea
[b] Department of Biological Chemistry, Korea University of Science and Technology (UST), 217 Gajungro, Yuseong-gu, Daejeon 34113, Republic of Korea
[c] Department of Chemistry, Korea University, Seoul 02841, Republic of Korea
[d] Department of Applied Chemistry, Hanyang University, Ansan, Gyeonggi-do 15588, Republic of Korea

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ABSTRACT

We described here the synthesis and biological evaluation of picolinamides and thiazole-2-carboxamides as potential mGluR5 antagonists. We found that a series of thiazole derivatives **6** showed better inhibitory activity against mGluR5. Compounds **6bc** and **6bj** have been identified as potent antagonists (IC_{50} = 274 and 159 nM) showing excellent in vitro stability profile. Molecular docking study using the crystal structure of mGluR5 revealed that our compounds **6bc** and **6bj** fit the allosteric binding site of mavoglurant well.

Glutamate, the principal excitatory neurotransmitter in the brain, regulates neuronal signal transmission through either inotropic or metabotropic glutamate receptors (iGluRs or mGluRs). The mGluRs belong to class C of the G-protein-coupled receptors (GPCRs), which are categorized into three groups (I, II and III) based on sequence homology, signal transduction mechanism and pharmacology. mGluR5, one of the group I receptors, is expressed postsynaptically and is mainly found in limbic brain areas including forebrain, striatal regions, and amygdala. Interaction of glutamate with mGluR5 results in activation of phospholipase C via G_q protein to release intracellular calcium ions, which leads to a variety of cellular responses.[1]

Regulation of mGluR5 has therapeutic potential in numerous preclinical models of diseases, including anxiety,[2] gastroesophageal reflux disease (GERD),[3] drug addiction,[4] and neuropathic pain.[5] Furthermore, recent study has demonstrated clinical evidence of the potential utility of mGluR5 antagonists. For example, basimglurant **3**, an mGluR5 negative allosteric modulator developed by Roche, is in phase II clinical trial for the treatment of depression and fragile X syndrome (Fig. 1).[6] Novartis researchers also developed mavoglurant **4** as a non-competitive mGluR5 inhibitor, which is currently in phase II clinical trial for Levodopa-induced dyskinesia.[7]

Up to date, a number of mGluR5 antagonists containing an alkyne subunit as a key structural motif have been reported and known to show high affinity to mGluR5 receptor.[8] Inspired by their therapeutic potentials, alkynylquinoline analogs have been investigated in our laboratory.[9] In fact, we have discovered 2-(pyridin-2-ylethynyl)quinoline, which has high inhibitory activity against mGluR5 showing excellent stability profile. Furthermore, this compound exhibited favorable in vivo activity in a behavior test of neuropathic pain mouse model. Despite the high potencies of acetylenic analogs, we have attempted to search for new nonalkynyl mGluR5 antagonists because an alkyne moiety is metabolically unstable to cause unfavorable side effects.[10] Recently, several groups reported that a series of arylcarboxamides or arylureas proved to be potent mGluR5 antagonists, which indicated that amide or urea functional group could be appropriate structural motif as a replacement of alkyne linkage.[11] On the basis of the arylamide structure, therefore, we designed a new class of mGluR5 antagonists as shown in Figure 2. Compared to the previously reported compounds, we envisioned that *meta*-substituted pyridine of **5** or *meta*-substituted thiazole of **6** would occupy the

* Corresponding authors.
E-mail addresses: sjmin@hanyang.ac.kr (S.-J. Min), ys4049@kist.re.kr (Y.S. Cho).

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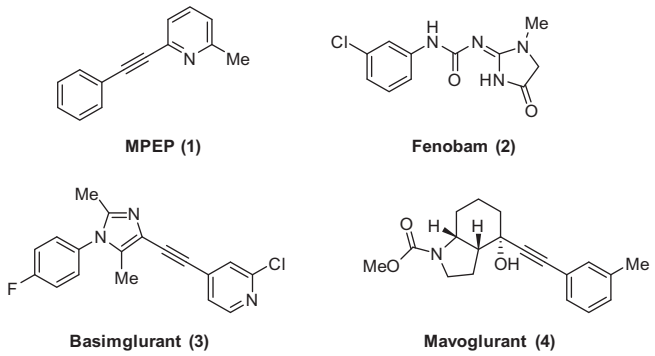

Figure 1. Representative mGluR5 antagonists.

binding pocket of terminal aryl pharmacophore of most mGluR5 antagonists. In addition, incorporation of substituted aromatic ring to the other side of the amide linker would provide molecular diversity in this series of compounds to explore structure–activity relationship (SAR) and stability profile.

Herein we report the synthesis and in vitro evaluation of picolinamides and thiazole-2-carboxamides as potent mGluR5 antagonists including structural insight resulting from computational docking study of our compounds in the crystal structure of mGluR5.

A series of picolinamide analogs **5** were easily synthesized through an amide coupling reaction using HBTU as a coupling reagent, as described in Scheme 1. In order to synthesize thiazole-2-carboxamides **6**, thiazole-2-carboxylic acids containing different substituents at the 2-position were required as substrates for amide coupling reactions. First, 4-methyl thiazole-2-carboxylic acid **9** was prepared by condensation of ethyl 2-amino-2-thioxoacetate **8** with chloroacetone followed by hydrolysis. Selective carbonylation of 2,4-dibromothiazole **10** via metal-halogen exchange produced the corresponding thiazole-2-carboxylate, which was also hydrolyzed to afford carboxylic acid **11**. Based on the Negishi type coupling reaction,[12] the synthesis of 4-cyano thiazole derivative **15** was achieved. Thus, thiazole triflate **13**, derived from condensation of thioglycolic acid **12** with ethyl cyanoformate followed by triflation, was treated with $Zn(CN)_2$ in the presence of palladium catalyst to produce ester **14**, which was converted to desired acid **15** by reduction and oxidation. Finally, a series of thiazole-2-carboxamides **6** were synthesized via either mixed anhydride or acid chloride intermediate.

In vitro antagonistic activities of picolinamides **5** against mGluR5 were evaluated using a fluorescence-based calcium mobilization assay.[13] The results of the in vitro assay of these compounds were shown in Table 1. Although plicolinamides **5** showed relatively low inhibitory activity against mGluR5 at the concentration of 10 μM and 1 μM, we found that the inhibition values of compounds **5a**, **5f**, **5l**, and **5m** were over 50% at 10 μM. Regarding the SAR of aromatic group on the left hand side, it seemed that the 2-pyridyl ring (**5a** and **5b**) was superior to the 3-pyridyl group (**5c**–**5i**). Most importantly, the *meta*-substituted phenyl ring was preferred when R^2 is methyl group (**5l** and **5m**).

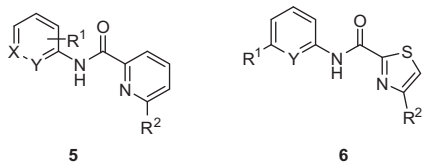

Figure 2. Structures of picolinamides **5** and thiazol-2-carboxamides **6**.

On the basis of this initial SAR, we decided to use 3-substituted phenyl and 6-substituted-2-pyridyl groups on the left hand side of thiazole derivatives for the next phase of SAR study.

Next, thiazole-2-carboxamide derivatives **6** were tested for their inhibitory activity against mGluR5 and the result is summarized in Table 2. At this time, the calcium-based functional assay was performed at the concentration of 1 μM because higher selection criteria were necessary due to searching for more potent lead compounds.[14] Among the tested compounds, eight compounds have more than 40% inhibitory activity against mGluR5. When R^2 is methyl group, compounds **6af**, **6ah**, and **6ai** bearing hydrogen, chloro and methyl groups on the pyridine ring showed potent inhibitory activity, comparable to the corresponding picolinamide **5a**. In case of 4-bromothiazole derivatives **6b**, substitution of either pyridyl ring or phenyl ring at the *meta*-position resulted in significant enhancement of potency. In particular, compounds **6bc** and **6bj** exhibited high antagonistic effect against mGluR5 at 1 μM. On the other hand, a set of 4-cyanothiazole derivatives **6c** showed a loss in potency. The overall SAR results indicated that the inhibitory activity of this series is highly sensitive to substitution at the 4-position of thiazole.

To further investigate pharmacological properties of the most potent compounds **6bc** and **6bj**, we examined their IC_{50} values, hERG inhibition, microsomal stability, and CYP inhibition.[15] The results are summarized in Table 3. IC_{50} values of compounds **6bc** and **6bj** were obtained by measuring inhibition values against mGluR5 at varied concentrations. In the hERG assay, depolarization potential inhibited by our compounds was measured using automated patch clamp device. The microsomal stability was determined by analysis of the remaining amount of compounds incubated in the human liver microsomes. For the CYP assay, the % remaining activity values of five human CYP450 isozymes after treatment with compounds **6bc** and **6cj** were obtained. The data confirmed that compounds **6bc** and **6bj** have excellent inhibitory activity against mGluR5 with IC_{50} values of 274 and 159 nM, which is comparable to that of mavoglurant **4** (IC_{50} = 110 nM in Ca^{2+} assay).[16] With regard to in vitro safety and stability, compound **6bc** had relatively higher % remaining activity of all the tested CYP isozymes compared to compound **6bj**, which suggested that **6bc** has better CYP stability than **6bj**. In addition, both compounds showed low blocking activity of hERG channel at three different concentrations. Compound **6bc** exhibited considerably good microsomal stability, whereas **6bj** was found to be rapidly metabolized in hepatic microsomes.

In order to rationalize the structure–activity relationship of thiazole derivatives, the binding modes of the most potent compounds **6bc** and **6bj** were evaluated using docking simulation with the crystal structure of the mGluR5 receptor recently reported by Heptares Therapeutics.[17] The energy-minimization of compounds **6bc** and **6bj** in the mGluR5 crystal structure confirmed that these compounds comparatively fit the allosteric binding site of mavoglurant **4**, as shown in Figure 3.[18] The 3-bromophenyl ring of compound **6bc** docks to the hydrophobic pocket formed by the 3-methylphenyl ring of **4** and the amide linker moiety sits well in a narrow channel surrounded by Pro655, Tyr659, Val806, and Ser809. The carbonyl group of **6bc** forms a hydrogen bond with Tyr659, which is likely to compensate for a loss of binding affinity in this region, where the hydrogen bonding between Ser809 and the hydroxyl group of **4** exists. Interestingly, the binding mode of compound **6bj** is opposite to that of compound **6bc**. Thus, the 4-bromothiazole segment of **6bj** sits in a cavity defined by several hydrophobic residues such as Ala813, Ala810, Ile625 and Pro655, which corresponds to the binding site of the 3-bromphenyl moiety of **6bc**. The hydrogen bonding between Tyr659 and the carbonyl oxygen of **6bj** was also observed. In addition, the methylpyridine tail of **6bj** favorably docks to the other side of hydrophobic binding