Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of aryl isoxazole derivatives as metabotropic glutamate receptor 1 antagonists: A potential treatment for neuropathic pain

Gyeong hi Cho [a,b,†], TaeHun Kim [a,c,†], Woo Seung Son [a,b], Seon Hee Seo [a], Sun-Joon Min [a], Yong Seo Cho [a], Gyochang Keum [a], Kyu-Sung Jeong [b], Hun Yeong Koh [d], Jiyoun Lee [e,*], Ae Nim Pae [a,c,*]

[a] Center for Neuro-Medicine, Brain Science Institute, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea
[b] Department of Chemistry, Yonsei University, Seodaemun-gu, Seoul 120-749, Republic of Korea
[c] Biological Chemistry, Korea University of Science and Technology, Yuseong-Gu, Daejon 305-350, Republic of Korea
[d] Department of Chemistry, Inha University, Nam-gu, Incheon 402-751, Republic of Korea
[e] Department of Global Medical Science, Sungshin University, Seoul 142-732, Republic of Korea

ARTICLE INFO

Article history:
Received 14 October 2014
Revised 24 December 2014
Accepted 19 January 2015
Available online xxxx

Keywords:
mGluR1 antagonist
Metabotropic glutamate receptor
Neuropathic pain
Schizophrenia
Aryl isoxazole

ABSTRACT

Glutamate is the major excitatory neurotransmitter and known to activate the metabotropic and ionotropic glutamate receptors in the brain. Among these glutamate receptors, metabotropic glutamate receptor 1 (mGluR1) has been implicated in various brain disorders including anxiety, schizophrenia and chronic pain. Several studies demonstrated that the blockade of mGluR1 signaling reduced pain responses in animal models, suggesting that mGluR1 is a promising target for the treatment of neuropathic pain. In this study, we have developed mGluR1 antagonists with an aryl isoxazole scaffold, and identify several compounds that are orally active in vivo. We believe that these compounds can serve as a useful tool for the investigation of the role of mGluR1 and a promising lead for the potential treatment of neuropathic pain.

L-Glutamate is the most abundant neurotransmitter that plays a major role in neuronal excitability and neurotransmitter release in the mammalian central nervous system.[1] Glutamate activates two classes of receptors, G-protein coupled receptors called metabotropic glutamate receptors (mGluRs), and ligand gated ion channels called ionotropic glutamate receptors (iGluRs). Activation of the glutamate receptors is responsible for excitatory synaptic transmission and long-term potentiation and depression, which are thought to be the key process of cognitive function such as learning and memory.[2] In particular, it has been reported that mGluRs are involved in anxiety disorder, Parkinson's disease, autism, schizophrenia and chronic pain.[3] Due to their therapeutic implications in various disorders of the brain, great efforts have been made to identify small molecule modulators for the mGluRs.

The mGluRs are classified into group I (mGluR1 and mGluR5), group II (mGluR2 and mGluR3) and group III (mGluR6, mGluR7 and mGluR8) based on their structure, pharmacology and signal transduction.[4,5] Among these subtypes, group I mGluRs are highly expressed on the peripheral terminals of sensory neurons and involved in nociceptive processes.[6] More importantly, the mGluR1 is specifically distributed within the brain regions that are heavily involved in pain sensing, such as dorsal root ganglia, spinal cord, thalamus, and cerebral cortex.[6–9] In addition, several studies found that knockdown of mGluR1 expression significantly reduced nociceptive responses in animal models of pain.[10,11] Recently, highly selective allosteric antagonists of mGluR1 have been developed, and some of these small molecule modulators also demonstrated pain-suppressing activity in various chronic pain models in vivo.[12–18] Particularly, YM-230888 ((*R*)-*N*-cycloheptyl-6-({[(tetrahydro-2-furyl)methyl]amino}methyl)thieno[2,3-*d*]pyrimidin-4-ylamine), an orally active mGluR1 antagonist, has successfully demonstrated its antinociceptive activity in complete Freund's adjuvant-induced arthritic pain models.[12] Taken together, mGluR1 appears to be a promising target for the treatment of neuropathic pain.[12–14]

To develop selective modulators of mGluR1, much interest has focused on allosteric sites, due to the highly conserved nature of the glutamate binding sites of mGluRs. To date, selective mGluR1 antagonists with various scaffolds have been developed including

* Corresponding authors. Tel.: +82 2 920 7281; fax: +82 2 920 2027 (J.L.); tel.: +82 2 958 5185; fax: +82 2 958 5189 (A.N.P.).
E-mail addresses: jlee@sungshin.ac.kr (J. Lee), anpae@kist.re.kr (A.N. Pae).
† These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bmcl.2015.01.035
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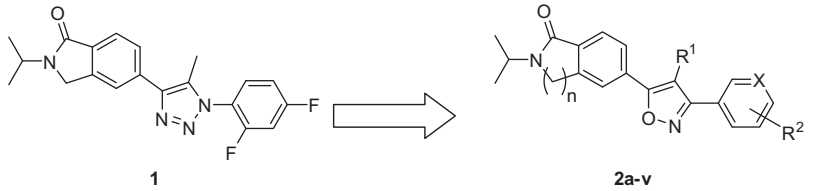
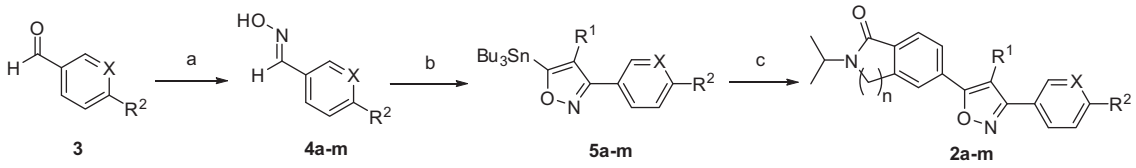


Figure 1. Design strategy for novel mGluR1 antagonists.



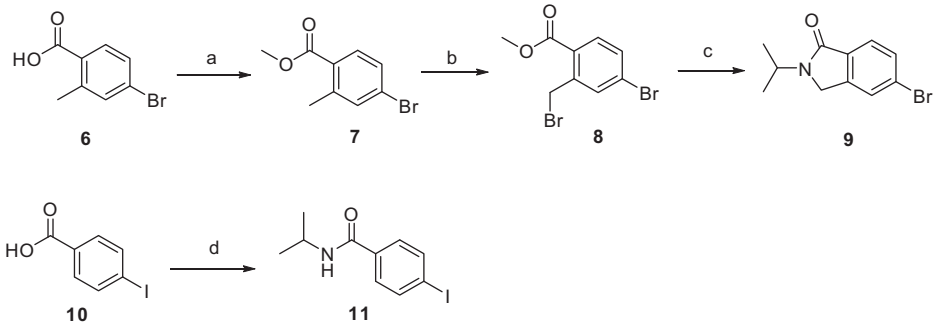
Scheme 1. Synthesis of Aryl isoxazole derivatives **2**. Reagents and conditions: (a) $HONH_2{\cdot}HCl$, Na_2CO_3, $EtOH/H_2O$, rt, 76–82%; (b) NCS, $KHCO_3$, 1-propinyl tributylstannane or ethynyltributylstannane, EtOAc, H_2O, rt, 43–57%; (c) aryl halide (**9**, **11** or methyl bromobenzoate), $Pd(PPh_3)_4$, PhMe, reflux, 44–63%.

triazafluorenone,[15–18] quinoline, isothiazole[19] and triazole[20,21] derivatives. Notably, Merck–Banyu laboratory developed a highly selective and potent antagonist, as depicted as compound **1** in Figure 1. Compound **1** demonstrated an excellent pharmacokinetic profile and an antipsychotic-like effect in an animal model without any noticeable toxicity, however, its analgesic activity was never tested in vivo.[20] On the basis of these findings, we decided to modify the triazole scaffold in compound **1** to an aryl isoxazole scaffold in hopes to develop selective mGluR1 antagonists with an antinociceptive activity. Herein, we report the synthesis and biological evaluation of novel aryl isoxazole derivatives as selective mGluR1 antagonists for the treatment of neuropathic pain.

The synthesis of aryl isoxazole derivatives **2** is described in Scheme 1. Substituted benzaldehyde or nicotinaldehyde compounds **3** were converted to oxime derivatives **4**, and then organostannanes were added via 1,3-dipolar addition to give compound **5**.[22] Subsequent Stille coupling reactions with isoindolinone fragment **9** or benzamide fragment **11** gave aryl isoxazole derivatives **2**.[23] The synthesis of isoindolinone fragment **9** started from 4-bromo-2-methylbenzoic acid (**6**) as shown in Scheme 2. Esterification of the starting material yielded compound **7**, which was further brominated by using NBS and a radical initiator, $[PhC(O)]_2O_2$ to produce compound **8**. Compound **8** was then subjected to a reflux with isopropyl amine to generate the isoindolinone fragment **9**.[20] The benzamide fragment **11** was synthesized from 4-iodobenzoic acid (**10**) via a peptide coupling reaction.

First, we tested compounds **2a**–**v** for antagonistic activity on human mGluR1. Additionally, we evaluated subtype selectivity on mGluR5, since mGluR1 and mGluR5 are structurally related and classified as group I. The antagonistic activity of compounds **2a**–**v** were determined by measuring changes in intracellular calcium concentration of human mGluR1 and mGluR5 expressing HEK cells[24] as shown in Table 1. We measured % inhibition of calcium release induced by glutamate at two fixed concentrations, 10 μM and 1 μM, respectively. All tested compounds showed moderate to high selectivity against mGluR1 over mGluR5, and compounds containing a halide-substituted phenyl group (**2a**–**c**, **2i**, **2q**, and **2t**) showed relatively good antagonistic activity. When the halide groups were replaced with more electron donating groups such as methoxy or methyl groups (**2g**, **2h**, **2s**, **2u**, and **2v**), their antagonistic activity appeared to be reduced significantly, suggesting that electron density in this region is important for antagonistic activity. Isoindolinone derivatives ($n = 1$; **2a**–**m**) seemed to show slightly better potency compared to benzamide derivatives ($n = 0$; **2n**–**v**). In addition, 4-methyl substituted isoxazole derivatives (R^1 = Me) demonstrated slightly better activity compared to their non-substituted counterparts (R^1 = H), indicating that conformational rigidity may affect antagonistic activity. We also attempted to modify the phenyl ring to a pyridine ring (X = N; **2d**–**e**, **2j**–**m**, and **2u**–**v**) and replaced the *N*-isopropyl group of the isoindolinone ring with a picolyl group to improve solubility. Modifications with more hydrophilic groups did improve overall solubility of the compounds, however, it did not enhance the antagonistic activity significantly.

Next, we selected several compounds with potent in vitro activity to assess metabolic stability and toxicity before we



Scheme 2. Synthesis of the fragments **9** and **11**. Reagents and conditions: (a) MeOH, H_2SO_4, reflux, 95%; (b) NBS, benzoyl peroxide, benzene, reflux, 100%; (c) isopropyl amine, Et_3N, PhMe, reflux, 80%; (d) isopropyl amine, EDCI, HOBt, *N*-methylmorpholine, CH_2Cl_2, rt, 70%.

Please cite this article in press as: Cho, G. h.; et al. *Bioorg. Med. Chem. Lett.* (2015), http://dx.doi.org/10.1016/j.bmcl.2015.01.035