RESEARCH HIGHLIGHT

Identification of a series of 3-(benzo[d]oxazol-2-yl)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl) pyridin-2-amines, as a new class of G-protein-coupled receptor kinase 2 and 5 inhibitor

Cheon Ho Park[1], Mi Young Lee[1], Jeong Hyun Lee[1], Byung Ho Lee[1], Kwang-seok Oh[1,2]

[1]*Research Center for Drug Discovery Technology, Korea Research Institute of Chemical Technology, Daejeon, 305-343, Republic of Korea*
[2]*Department of Medicinal and Pharmaceutical Chemistry, University of Science and Technology, Daejeon, 305-350, Republic of Korea*

Correspondence: Kwang-Seok Oh
E-mail: ksoh@krict.re.kr
Received: February 27, 2014
Published online: May 6, 2014

The arising critical implications of G-protein-coupled receptor kinase 2 and 5 (GRK2 and 5) in cardiovascular disease including myocardial infarction and heart failure, have been attracting attention and inhibitors of GRK2 and 5 were considered as a novel therapeutic strategy to prevent and treat cardiovascular disease. Despite this large therapeutic potential, to date few GRK2 and 5 inhibitors have been reported under development. As a part of drug research program to identify novel and potent GRK2 and 5 inhibitor, we found that a series of 3-(benzo[d]oxazol-2-yl)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl) pyridin-2-amines was a novel scaffold for GRK2 and 5 inhibitor. Moreover, the N-substituted benzoxazole derivative (1o), bearing a pyrrolidine at R3 position, exhibited the most potent inhibitory activity to GRK5 and moderate to GRK2. This research highlight discusses the processing and findings of the recent study.

Keywords: G-protein coupled receptor kinase-2 (GRK2); GRK5; Benzoxazole; Heart failure

To cite this article: Park C, et al. Identification of a series of 3-(benzo[d]oxazol-2-yl)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl) pyridin-2-amines, as a new class of G-protein-coupled receptor kinase 2 and 5 inhibitor. Receptor Clin Invest 2014; 1: e100. doi: 10.14800/rci.100.

Heart failure (HF), leading cause of death and illness worldwide, often occurs as an end-phase of pathological myocardial hypertrophy in the elderly [1]. Initially, hypertrophy is regarded as an accommodative cardiac response that contributes to keep cardiac output from a variety of stress such as systemic hypertension and myocardial infarction. However, hypertrophy accompanied by chronically elevated stresses represents a shift from adaptive to maladaptive response that leads to cardiac remodeling and dysfunction, eventually resulting in HF [2, 3].

Multiple molecular pathways regulate cardiac hypertrophy via modulation of G protein-coupled

receptors (GPCRs) to a variety of ligands, such as hormones, neurotransmitters, and environmental stimuli [4]. Several mechanisms have been studied extensively but remain unclear. Among these pathways, GPCR kinases (GRKs)-mediated regulation of GPCR through direct phosphorylation of receptors have proposed as a general mechanism [5]. When an agonist binds to GPCR, it activates a cellular signal transduction cascade through G proteins, but also induces GRKs/β-arrestin-mediated events to prevent excess stimulation. An interaction between GRKs and agonist-bound GPCRs lead to binding of β-arrestin, where it can phosphorylate the activated receptor, resulting in inhibition of further G protein activation which are deeply implicated with HF [6]. One of the defining characteristics of HF is pathologic impairment of β-adrenergic receptor (β-AR) systems. In the failing heart, the decrease of cardiac output induces to increase circulating catecholamines concentration, which subsequently results in severe uncoupling of β-ARs and a loss of inotropic reserve, and it appears to closely correlate with GRK2 and 5 up-regulation [7, 8].

GRK2 is known as β-AR kinase that is expressed highly in the heart. After the relationship between cardiac remodeling and overexpression of GRK2 had been found, GRK2 has been considered as a pharmaceutical target for the treatment of HF [9]. Indeed, GRK2 inhibition, such as a cardiac specific overexpression of GRK2 inhibitory peptide, βARKct, or by cardiac-specific gene deletion in animals, effectively ameliorates ventricular dysfunction in animal models of HF [10]. In contrast to GRK2, GRK5, the other major cardiac GRK, has been defined not only by its β-ARs desensitization property but also by nuclear accumulation in failing heart and nuclear histone deacetylase (HDAC) kinase activity [11]. Noteworthy, nuclear HDAC kinase activity of GRK5 enhances fetal gene expression relating to cardiac hypertrophy [12], and thus reducing activity of GRK5 in the nucleus represents a viable drug target to treat HF.

As such, GRK2 and 5 are emerging as an attractive therapeutic target. Despite this large therapeutic potential, to date few GRK2 inhibitors have been identified and GRK5 inhibitors in public have been unknown. Recently, some pharmaceutical companies such as Takeda Pharmaceuticals, Inc., Sanofi, and Aerie pharmaceuticals have developed potent GRK2 inhibitors which possess the binding activity in the active site of GRK2, however these have not advanced to clinical trials (http://integrity.thomson-pharma.com/integrity/ reports).

In our recent study entitled "Design and synthesis of novel 3-(benzo[d]oxazol-2-yl)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)pyridin-2-amine derivatives as selective GRK2 and 5 kinase inhibitors [13] ", we performed a pilot of high throughput screening (HTS) campaign, chemical synthesis, biochemical evaluation, and structure-activity relationships (SAR) based on novel scaffolds. The pilot HTS campaign for the search of GRK2 and 5 novel scaffolds was carried out using the library of the most diverse set consisting of 15,040 compounds and 284 focus-library set. These chemical libraries used in this study were supported by the Korea Chemical Bank (KCB), which is operated by Korea Research Institute of Chemical Technology with participation of nation-wide industries, academia and research institution involved in drug discovery programs. The chemical library of KCB was classified into 200 major classes and 1,200 subclasses with skeletal and functional group diversity and maintained under the "PharmaCore" system to analyze the chemical diversity. From a pilot HTS campaign, we have identified a novel series of benzoxazole derivatives, containing the amine group, as hit compounds. The SAR investigation focused on the exploring to the effects of substituents on amine group of benzoxazole derivatives at R2 and R3 position. Of amine substituents, piperidinyl and pyrrolidinyl substituent on R2 position of benzoxazole has moderate inhibitory activity toward GRK5. Moreover, the N-substitution of pyrrolidine at R3 position of benzoxazole exhibits the most potent inhibitory activity to GRK5 and moderate to GRK2, while the other introduction of pyrazole, aromatic-oxy, heteroaromaticoxy, amide, sufonyl amide, and urea functionalities at R3 position of benzoxazole was not observed acceptable GRK2 and 5 inhibitions. Among derivatives, the 3-(benzo[d]oxazol-2-yl)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl) pyridin-2-amine (**1o**) was found to exhibit the most potent inhibitory activity to GRK2 and 5 with IC_{50} values of 0.46, 0.059 μM, respectively.

In the molecular docking study using the crystal structure of GRK2, the residue D272 and M274 in the hinge region of GRK2 was particulary defined as interaction site, which hydrogen bonds were similar with those of GRK1 and GRK6 with ATPs. In addition, the π-cation interation with K220 and the hydrophobic interation with V205 and L324 of GRK2 enhances the activity of the compound **1o.** Moreover, the pyrrolidine moiety of compound **1o** has a hydrophobic core to interact with F202, L222, L235, and G337 of GRK2. These results indicate that the N-substitution of pyrrolidine at R3 position of benzoxazole, which serves as hydrophobic core donor, might be a critical role for inhibition to GRK2.

In the present, we have begun to explore cell-based and animal model-based pharmacological evaluation of compound **1o.** Particularly, we expect that benzoxazlole derivatives may represent a new class of potent GRK2 and 5 inhibitors. Our findings provide important information for the design of novel GRK2 or 5 inhibitors.

Acknowledgments

This work was financially supported by the Korea Healthcare technology R&D Project (A100096) of MHW, Republic of Korea. We thank KCB of Korea Research Institute of Chemical Technology for providing chemical library.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Dorn GW, 2nd, Force T. Protein kinase cascades in the regulation of cardiac hypertrophy. J Clin Invest 2005; 115: 527-537.
2. Heineke J, Molkentin JD. Regulation of cardiac hypertrophy by intracellular signaling pathways. Nat Rev Mol Cell Biol 2006; 7: 589-600.
3. Mckinsey TA, Olson EN. Toward transcriptional therapies for the failing heart: Chemical screens to modulate genes. J Clin Invest 2005; 115: 538-546.
4. Pierce K, Premont RT, Lefkowitz RJ. Seven-transmembrane receptors. Nat Rev Mol Cell Biol 2002; 3: 639-650.
5. Reiter E, Lefkowitz RJ. GRKs and β-arrestins. Roles in receptor silencing, trafficking, and signaling. Trends Endocrinol Metab 2006; 17: 159-165.
6. Nakaya M, Chikura S, Watari K, Mizuno N, Mochinaga K, Mangmool S, et al. Induction of Cardiac fibrosis by β-blocker in G protein-independent and G-protein-coupled receptor kinase 5/β-arrestin2-dependent signaling pathways. J Biol Chem 2012; 287: 35669-35677.
7. Iaccarino G, Tomhave ED, Lefkowitz RJ, Koch WJ. Reciprocal in vivo regulation of myocardial G protein-coupled receptor kinase expression by β-adrenergic receptor stimulation and blockade. Circulation 1998; 98: 1783-1789.
8. Ping P, Anzai T, Gao M, Hammond HK. Adenylyl cyclase and G protein receptor kinase expression during development of heart failure. Am J Physiol 1997; 273: H707-717.
9. Ungerer M, Bohm M, Elce JS, Erdmann E, Lohse MJ. Altered expression of beta-adrenergic receptor kinase and beta1-adrenergic receptors in the failing human heart. Circulation 1993; 87: 454-463.
10. Koch WJ, Rockman HA, Samama P, Hamilton RA, Bond RA, Milano CA, et al. Cardiac function in mice overexpressing the beta-adrenergic receptor kinase or a beta ARK inhibitor. Science 1995; 268: 1350-1353.
11. Pitcher JA, Freedman NJ, Lefkowitz RJ. G-protein-coupled receptor kinases. Annu Rev Biochem 1998; 67: 653-692.
12. Martini JS, Raake P, Vinge LE, DeGeorge BR, Jr., Chuprun JK, Harris DM, Gao E, Eckhart AD, Pitcher JA, Koch WJ. Uncovering a protein-coupled receptor kinase-5 as a histone deacetylases kinase in the nucleus of cardiomyocytes. Proc Natl Acad Sci U S A 2008; 105: 12457-12462.
13. Cho SY, Lee BH, Jung H, Yun CS, Ha JD, Kim HR, et al. Design and synthesis of novel 3-(benzo[d]oxazol-2-yl)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)pyridin-2-amine derivatives as selective G-protein-coupled receptor kinase-2 and -5 inhibitors. Bioorg Med Chem Lett 2013; 23: 6711-6716.