Bioorganic & Medicinal Chemistry Letters 26 (2016) 5669–5673

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Identification of lead small molecule inhibitors of glycogen synthase kinase-3 beta using a fragment-linking strategy

Jinhee Kim [a], Yonghoon Moon [a,b], Sungwoo Hong [a,b,*]

[a] Center for Catalytic Hydrocarbon Functionalization, Institute for Basic Science (IBS), Daejeon 305-701, Republic of Korea
[b] Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 305-701, Republic of Korea

ARTICLE INFO

Article history:
Received 29 September 2016
Revised 19 October 2016
Accepted 21 October 2016
Available online 22 October 2016

Keywords:
Glycogen synthase kinase-3 beta
Inhibitor
Fragment-linking strategy
7-Azaindole
Kinase

ABSTRACT

Glycogen synthase kinase-3 beta (GSK3β) kinase serves as a promising therapeutic target for the treatment of various human diseases, such as diabetes, obesity, and Alzheimer's disease. In this study, we report lead GSK3β inhibitors identified using a fragment-linking strategy. Through the systematic exploration, a six-atom chain unit bearing the rigid double bond was found to be a suitable linker connecting two fragments, which enables favorable contacts with backbone groups of residues in the pockets. As a consequence, potent GSK3β inhibitor **9i** was found with IC_{50} values of 19 nM. The binding mode analysis indicates that the activities of the inhibitors appear to be achieved by the establishment of multiple hydrogen bonds and hydrophobic interactions in the ATP-binding site of GSK3β. The good biochemical potencies and structural uniqueness of the inhibitors support consideration in the further study to optimize the biological activity.

Glycogen synthase kinase-3 (GSK3) protein is a serine/threonine kinase, which was originally identified as a regulator of glycogen metabolism [1]. This kinase subfamily includes two homologous isoforms GSK3α and GSK3β which are 95% identical at the amino acid level [2]. GSK3 kinase plays an important role in a variety of physiological processes, including cell cycle progression, microtubule stability, apoptosis, signaling, and transcription [3]. Among two different isoforms, GSK3β potentiates to modulate blood glucose level by impairing the catalytic activity of glycogen synthase through phosphorylation [4]. Besides its role in the conversion of glucose to glycogen, GSK3β is a key kinase to obtain abnormal hyperphosphorylation on tau, which causes destabilization of microtubules, subsequent dissociation, and aggregation of tau to form neurofibrillary tangles (NFTs) [5]. Taken together, regulation of GSK3β activity would be a promising therapeutic strategy for the treatment of various human diseases, such as diabetes, obesity, and Alzheimer's disease.

A variety of GSK3β inhibitors, including lithium, natural products, and synthetic molecules, have been developed in recent decades through intensive drug discovery programs [5]. Despite the achievements in both high potency and chemical structure diversity, GSK3β targeted therapeutic approaches by small molecule inhibitors have often been unsuccessful because of the selectivity issues against other related kinases [6a,b,7]. Off-target kinase inhibition can cause toxicity due to cross-interaction of multiple pathways.

Fragment-based drug discovery (FBDD) approach presents an appealing alternative to the current drug discovery process by either growing or linking two fragments [8]. Fragment-linking approach is conceptually most fascinating in fragment-based drug discovery approaches and can lead to a rapid buildup of fragments using an appropriate linker moiety to generate new high-affinity ligands [9]. Once two or more fragments are identified in high affinity regions to determine favorable spots, they are chemically linked in an optimal fashion to benefit from a superadditivity effect [10]. In this strategy, even non-selective initial core could be developed into a highly selective inhibitor by linking fragments in two neighboring sites. Fragment-linking strategy is, however, considerably more challenging than growing strategy because the linker should not disrupt any important functionality of the fragments and even subtle change of the conformation can cause the loss of key interactions between the initial fragment and biological target. Therefore, the optimally linked molecules should completely retain the key interactions of the individual fragments in an additive manner and maintain the optimum binding geometries of the original fragment hits. In this study, we have designed and identified promising lead GSK3β inhibitors using a fragment-linking strategy in which two fragments were connected with an optimum linker-length.

* Corresponding author at: Center for Catalytic Hydrocarbon Functionalization, Institute for Basic Science (IBS), Daejeon 305-701, Republic of Korea, Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 305-701, Republic of Korea.
E-mail address: hongorg@kaist.ac.kr (S. Hong).

http://dx.doi.org/10.1016/j.bmcl.2016.10.060
0960-894X/

The availability of the substantial 3D-structural information of GSK3β has enhanced opportunities for the identification of potent and selective inhibitors utilizing structure-based drug design [5]. Through our concurrent program supporting the development of GSK3β inhibitors, we recently identified hit compound **1** (IC_{50} = 1.45 μM) by a systematic fragment-based *de novo* design (FBDD) procedure. Compound **1** is comprised of three parts in order to maximize the interactions of an inhibitor with three binding regions in the active site of GSK3β. Fig. 1 presents the schematic binding mode of **1** in the ATP binding site of GSK3β. The 7-azaindole and indole fragments were linked together by an amide linkage to investigate the main interactions in Region 2 and 3. The aprotic nitrogen on the central 7-azaindole moiety and the neighboring pyrrolic nitrogen accept and donate a hydrogen bond in the bidentate form with the back bone of Asp133 and Val135 in the hinge region. According to proposed binding mode, the phenyl group in 7-azaindole is directed toward the polar binding pocket near the salt bridge comprised of Lys85 and Glu97 and Asp200 in the DFG motif, which has been known as a pocket for potency and selectivity [5]. Based on these premises, we turned to our attention to linker design and modifications to increase inhibitory activity. As the starting point for generating derivatives, the lowest-energy conformation of **1** was analyzed to estimate the energy penalty resulting from the different geometry of the bioactive conformation as show in Fig. 2. The systematic evaluation of the conformational features indicates that the geometry of 7-azaindole and indole fragments in compound **1** likely to be fixed in a folded conformation, probably caused by intramolecular pi-stacking interaction. This spatial restriction may prevent the indole moiety of **1** from accessing Region 3 by distorting the linker. With the binding mode set in this way, we speculated that more rigid linkers can possibly increase the interactions with the residue by forcing the indole moiety to extend into Region 3 and make favorable contacts with residues.

With a goal to develop a new structural class of potent GSK3β inhibitors, our initial round of analogues was focused around the incorporation of suitable linkers while fixing 7-azaindole and indole fragments (Table 1). The general synthetic route for the preparation of the target compounds is shown in Scheme 1. The synthesis commenced with DCC/HOBt mediated coupling of Cbz protected glycine (**2**) and indole-3-ethylamine (**3**), followed by Cbz deprotection under catalytic hydrogenation conditions to afford the amine **4**. The resulting amine **4** was coupled with azaindoles **5** [11] to furnish the target compounds **1**, **6a**, **6b**, **6c**, and **6d**. To build compounds bearing acrylamide as a linking group, target compounds **9** were prepared by a two step sequence, shown in Scheme 1b. Thus, the methyl acrylate group was installed by a palladium-catalyzed cross-coupling with 5-bromo-7-azaindole derivatives **7** [12] to furnish the ester compounds, which were hydrolyzed under either acidic or basic conditions. Finally, amides **9** were produced from the resulting carboxylic acids **8** and appropriate amines in the presence an amide coupling reagent (HBTU). For the synthesized derivatives, IC_{50} (50% inhibitory

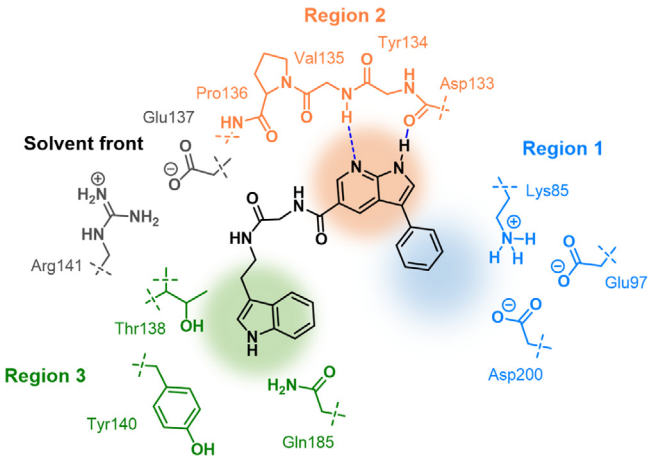


Fig. 1. Structure and schematic binding mode of **1**.

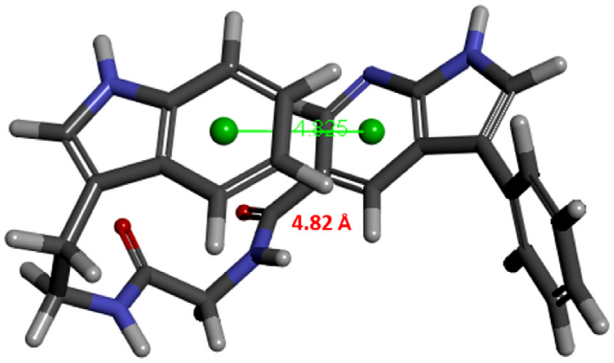
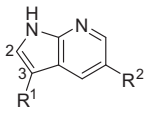
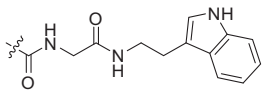
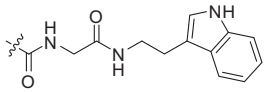
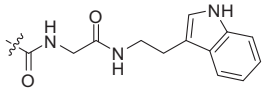
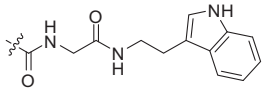
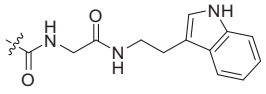
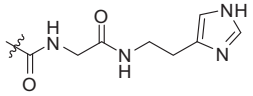
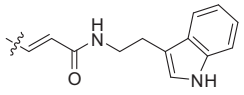
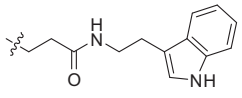


Fig. 2. Calculated conformation of **1**.

Table 1
Exploration of the groups and linker



Entry	R^1	R^2	GSK3β IC_{50} (μM)
1	–Ph		1.45
6a	–H		>50
6b[a]	–Ph		>50
6c	–COPh		15.7
6d[b]	–Ph		>50
6e	–Ph		2.61
9a	–Ph		0.356
10	–Ph		1.03

[a] 2-Phenyl group instead of 3-phenyl group.
[b] Indole instead of central 7-azaindole core.