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Structure-based design of benzo[e]isoindole-1,3-dione derivatives as selective GSK-3β inhibitors to activate Wnt/β-catenin pathway

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ABSTRACT

Deregulation of Wnt/β-catenin pathway is closely related to the pathogenesis of neurodegenerative diseases such as Alzheimer's disease (AD), and glycogen synthase kinase 3β (GSK-3β), the central negative regulator of Wnt pathway, is regarded as an important target for these diseases. Here, we report a series of benzo[e]isoindole-1,3-dione derivatives as selective GSK-3β inhibitors by rational-design and synthesis, which show high selectivity against GSK-3β over Cyclin-dependent kinase 2 (CDK2), and significantly activate the cellular Wnt/β-catenin pathway. The structure–activity relationship of these GSK-3β inhibitors was also explored by *in silico* molecular docking.

1. Introduction

Wnt/β-catenin signaling pathway plays essential roles in embryonic development and tissue homeostasis [1–5]. Deregulation of Wnt/β-catenin pathway is closely associated with neurodegenerative diseases such as Alzheimer's disease [6–8], Parkinson disease [9,10] and Schizophrenia [11,12]. Glycogen synthase kinase 3β (GSK-3β) acts as the key negative regulator of Wnt/β-catenin signaling pathway, and is therefore regarded as an important target for these diseases [13–18].

GSK-3β is a constitutively active serine/threonine protein kinase belonging to the CMGC protein kinase family that include cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAP kinases), glycogen synthase kinases (GSK-3) and CDK-like kinases. The structure of GSK-3β is highly homologous to that of other CMGC members such as cyclin-dependent kinases (CDKs) [19–21]. In consistent with this homology, many GSK-3β inhibitors are also potent inhibitors of CDKs [22–25]. Selectivity is thus considered as a major issue to develop potent GSK-3β inhibitors as probes for biological functions of GSK-3β and drugs for the treatment of various diseases [18,26–31].

During our development of a chemical genetic approach to analyzing biological systems by using zebrafish assay, we identified a potent GSK-3β inhibitor (Fig. 1, compound **1**) bearing a benzo[e]isoindole-1,3-dione core structure from a public library, which inhibits the eye and forebrain formations of zebrafish embryos, resembling a typical Wnt overexpression phenotype [32]. A series of synthetically more feasible derivatives (Fig. 1, compounds **1a** and **1b**) are also developed to inhibit GSK-3β and activate Wnt pathway, but the selectivity of these inhibitors over CDK2 is relatively low [33], which is similar to that of compound **1**. Kinase selectivity profiling has shown that compound **1** is highly selective against GSK-3β over other kinases except for CDK2 [32]. In this study, we optimized these inhibitors to be highly selective to GSK-3β over CDK2 by structure-based rational design.

2. Results and discussion

2.1. Rational design of selective GSK-3β inhibitors

The homology between the ATP sites of GSK-3β and CDK2 is over 86%, so many GSK-3β inhibitors also potently inhibit CDK2. For example, compound **1b** inhibits GSK-3β and CDK2 with a comparable potency. To improve the selectivity, we explored the regions outside the ATP site based on the crystal structures of GSK-3β and CDK2, and searched for the potential residues that can be used to design selective GSK-3β inhibitors. Detailed structural analysis revealed that the residues located at the helix D immediately following the hinge region are significantly different between GSK-3β and CDK2 (Fig. 2). GSK-3β is characterized by positively charged residues (Arg141 and Arg144) in this region, while CDK2 is characterized by negatively charged residues (Asp86 and Asp92). The difference of the electrostatic potential in this region makes it possible to design selective inhibitors against GSK-3β but not CDK2. We used compound **1b** as the starting point, and introduced sulfonyl group to interact with the positively charged

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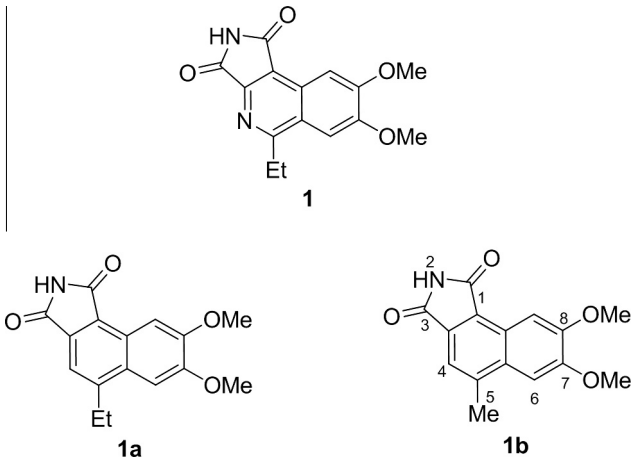


Fig. 1. Chemical structures of GSK-3β inhibitors (**1**, **1a** and **1b**) with benzo[e]isoindole-1,3-dione core structure.

residues Arg141 and Arg144 at the helix D of GSK-3β. The sulfonyl group could be linked to the methyl group at the 5-position of the benzo[e]isoindole-1,3-dione scaffold by 4-amino piperidine group with the consideration of size and solubility of the linker. Such modification of compound **1b** would improve the binding affinity with GSK-3β by the favorable electrostatic interactions, while which might impair the binding affinity with CDK2 due to the repulsion between the partially negatively charged sulfonyl group and the negatively charged residues of CDK2.

2.2. Chemistry

The desired benzo[e]isoindole-1,3-dione derivatives **8a–i** were synthesized in modest to good yields as shown in Scheme 1. The sulfonyl piperidine amine substrates (**5a–i**) were synthesized by the condensation of 4-Boc-aminopiperidine and sulfonyl chloride followed by removing the Boc protecting group. The precursor **6** that has been reported previously [33] served as the starting material. After bromination with NBS and AIBN, the compound **7**

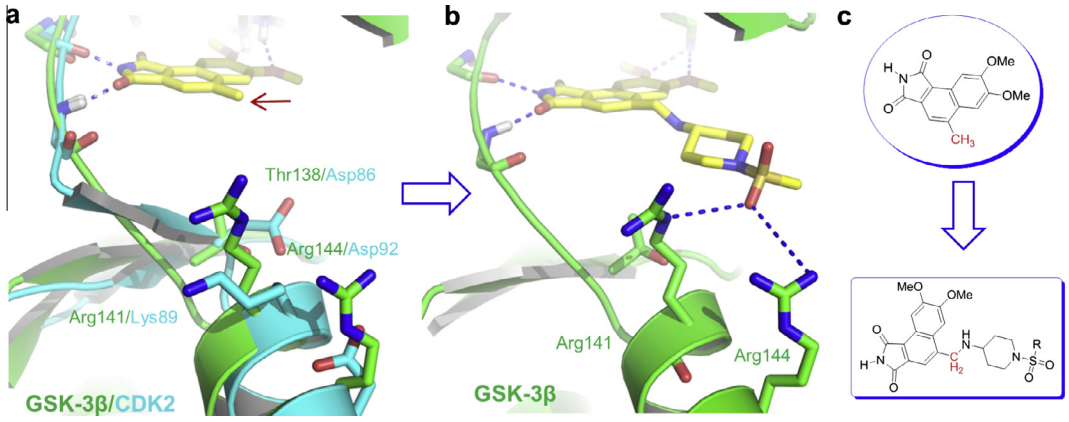
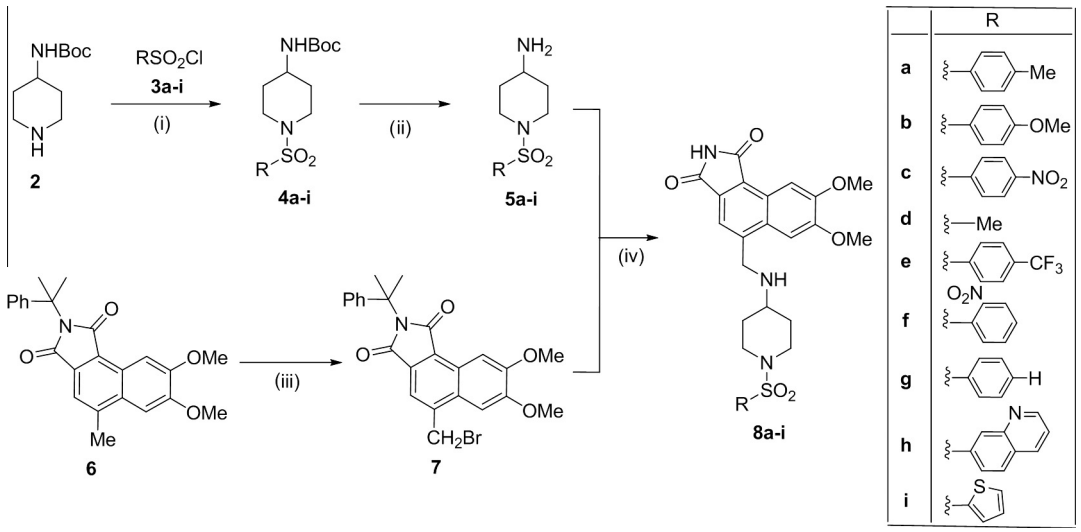


Fig. 2. Designed strategy of selective GSK-3β inhibitors. (a) Structural comparison between GSK-3β (green) and CDK2 (cyan), different residues at the helix D were shown in stick representation. (b) Interaction between the designed inhibitor and GSK-3β, the hydrogen bonds were shown by blue dash lines. (c) Chemical modifications of the parent compound **1b** to selective GSK-3β inhibitors.



Scheme 1. Synthesis of **8a–i**. (i) Boc_2O, DCM, Et_3N, stirring, rt, 4 h; (ii) TFA, stirring, rt, 10 h; (iii) NBS, AIBN, DCM, stirring, 40 °C; (iv) K_2CO_3, THF, 0 °C to room temperature, stirring overnight; and then TFA, stirring, 50 °C, 10 h.