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Design and synthesis of 7-hydroxy-1*H*-benzoimidazole derivatives as novel inhibitors of glycogen synthase kinase-3β

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Abstract—A hydroxy functional group was introduced as the hydrogen bond donor and acceptor at the hinge region of protein kinase in order to develop novel ATP-competitive inhibitors. Several derivatives of 7-hydroxyl-1*H*-benzoimidazole were designed as inhibitors of glycogen synthase kinase-3β with the help of ab initio calculations and a docking study. Enzymatic assay and an X-ray complex study showed that these designed compounds were highly potent ATP-competitive inhibitors.

Glycogen synthase kinase-3β (GSK-3β) is a serine/threonine protein kinase that inhibits the activity of glycogen synthase (GS), tau, and numerous other substrates by means of phosphorylation.[1] On the basis of such biochemical actions, there is much evidence that it may be a good target for diabetes and several central nervous system diseases, such as dementia and bipolar disease. For example, in the fatty tissue of mice suffering from fatty diabetes, the GSK-3β activity has been observed to be twice that of normal mice.[2] Transgenic mice programmed to express GSK-3β in the brain have abnormal neurons caused by the hyperphosphorylating tau of the neurofibrillary tangle, which plays an important role in dementia.[3] Moreover, patients with type II diabetes[4] and dementia[5] show a higher level of GSK-3β expression than normal controls. Bipolar disorder has been treated with lithium and valproic acid, well-known GSK-3β inhibitors.[6] Thus, we have designed novel GSK-3β inhibitors using proprietary structure-based technologies.

As was done for other known kinase inhibitors,[7,8] we targeted the adenosine triphosphate (ATP) binding site,

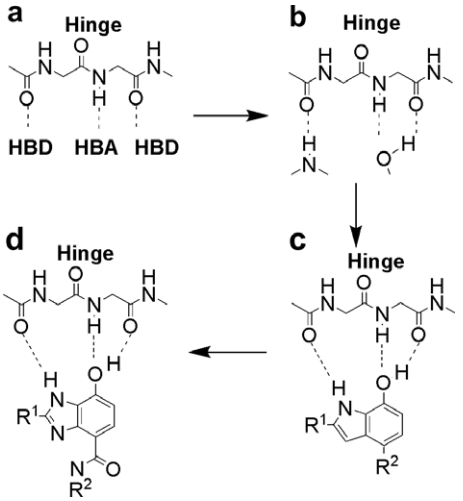

Figure 1. Scheme for designing new kinase inhibitors. (a) Pharmacophore representation of inhibitor at the hinge region in protein kinase, HBD represents hydrogen bond donor and HBA represents hydrogen bond acceptor. (b) A secondary amine group is adopted as the HBD and an hydroxy group is adopted as the HBD and HBA. (c) 7-Hydroxy indole is designed as a skeleton. (d) For synthetic feasibility, 7-hydroxy benzoimidazole is selected as a scaffold.

Keywords: Kinase inhibitor; Drug design; Glycogen synthase kinase; GSK; 7-Hydroxy-1*H*-benzoimidazole.
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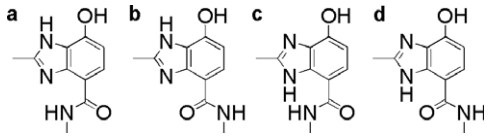


Figure 2. Four possible conformers **a**, **b**, **c**, and **d** of designed scaffold.

specifically the hydrogen bond network at the hinge region of the kinase. To bind to this region, most known kinase inhibitors include a hydrogen bond acceptor flanked by two hydrogen bond donors on the basis of pharmacophoric analysis (Fig. 1).[9] In our design, a hydroxy functional group (—OH) is expected to act as both a hydrogen bond donor and an acceptor at the same time. A secondary amine (—NH—) is considered as another hydrogen bond donor. Initially, we adopted 7-hydroxyl indole as a skeleton, but changed to 7-hydroxy benzimidazole because of synthetic difficulties. Moreover, to facilitate the derivatization, we decided to introduce an amide into the *para*-position of the phenyl ring of the skeleton.

Considering the internal hydrogen bond of the designed scaffold, only four conformers are possible as summarized in Figure 2. Structural optimization was performed for each conformer using the DFT method with B3LYP and 6-31G* basis sets in the Gaussian 03 package[10] on the Linux system. The results are summarized in Table 1. However, conformers **c** and **d** cannot make a multiple hydrogen bond network properly at the hinge region of the kinase. Thus, conformer **a** is the lowest-energy conformer for a new scaffold, considering the hydrogen bond network.

Table 1. Ab initio optimization results of conformers **a**, **b**, **c** and **d**

Conformer	Energy difference (Kcal/mol)	Torsion angle (degree)
d	0.0	176
a	4.1	0
c	6.0	25
b	13.9	140

A docking study was performed with the published structure (PDB code: 1I09)[11] using the Affinity module of InsightII[12] employing the cvff force field. All solvent molecules were removed and explicit hydrogen atoms were generated at pH 7.4. The binding site atoms were defined as the residue atoms within 5 Å from the initial position of the ligand. During the docking process, the binding site atoms and the ligand atoms were flexible. The Monte Carlo and the Simulated Annealing methods were applied to generate and minimize the possible poses. A representative result is depicted in Figure 3 using the structure of compound **6a** (Scheme 1 and Table 2). The compound fits well in the GSK-3β protein structure with a reasonable conformation like conformer **a**. It binds to the hinge region to make hydrogen bonds. As expected, the hydrogen bond distance was measured to be 2.88 Å between the Val135 C=O and imidazole NH of the compound, 2.83 Å between the Val135 NH and the oxygen of the hydroxy group and 2.54 Å between the Asp133 C=O and the oxygen of OH. Additionally, the corresponding hydrogen bond angles are 172°, 148° and 137°, respectively. These results indicate that the designed scaffold can bind to the ATP binding site like most other kinase inhibitors.

The designed inhibitors were synthesized using the methods in Scheme 1. 3-Amino-4-methoxybenzoic acid (**1**) was reacted with MeOH under acidic conditions to give

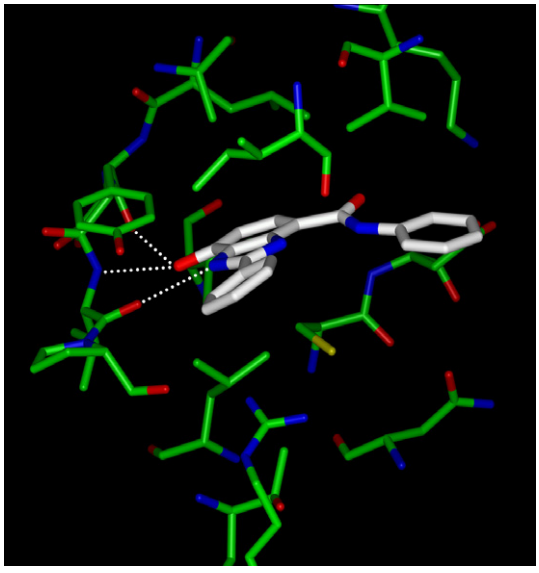
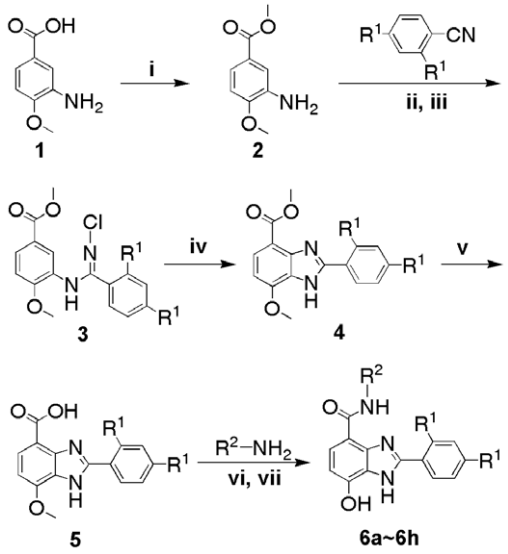
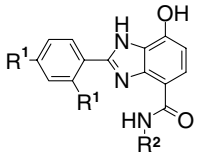


Figure 3. Docking result of compound **6a**. The key interaction between GSK-3β and compound **6a** is the hydrogen bond network in the hinge region depicted by a white dotted line. The carbon atom in the ligand is colored white, the carbon atom in the protein is green, oxygen is red, and nitrogen is blue.



Scheme 1. Reagents and conditions: (i) SOC_{12}/MeOH, reflux, 2 h (yield: 98%); (ii) *p*-TSA, 180 °C; (iii) NaOCl, 50% aq MeOH, 0.5 h (yield: 30–70%); (iv) Na_2CO_3 solution, 60 °C, 1 h (yield: 70–95%); (v) $LiOH{\cdot}H_2O$, THF/MeOH/H_2O (3:1:1), reflux, 4 h (yield: 90–98%); (vi) EDC, HOBt, DMAP, DMF, rt, 8 h (yield: 30–60%); (vii) $AlCl_3$, toluene, reflux, 5 h (yield: 20–60%).

Table 2. GSK-3β inhibition results for synthesized compounds



Compound	R^1	R^2	% inhibition at 5 μM (%)	IC_{50} (nM)
Ref.			99	70
6a	H		86	
6b	H		93	870
6c	H		91	580
6d	H		100	25
6e	Cl		64	
6f	Cl		64	
6g	Cl		87	1000
6h	Cl		99	15

methyl 3-amino-4-methoxybenzoate (**2**). Amidine intermediates (**3**) were prepared by treating *p*-TSA and NaOCl at 180 °C and rt, respectively. These amidines were cyclized with sodium carbonate solution to give methyl 7-methoxy-2-*o*-, *p*-substituted phenyl-1*H*-benzimidazole-4-carboxylate (**4**) and hydroxylated by $LiOH{\cdot}H_2O$ to give a benzimidazole-4-carboxylic acid (**5**) in good yield. Various final compounds (**6**) were prepared by treating R^2-amine with EDC coupling reagents. We incorporated phenyl, benzyl, phenethyl and *p*-methanesulfoneamide-phenethyl amine as summarized in Table 2.

The inhibitory activities of the synthesized compounds were determined using the published method.[13] We used 3-(1-methylindol-3-yl)-4-[3-(2-aminoethoxy)phenyl]-1*H*-pyrrole-2,5-dione as a reference compound, of which IC_{50} value was reported as 20 nM in the patent[14] and was determined as 70 nM in this system. Compound **6a**, the first synthesized basic structure, was evaluated as giving 86% inhibition at 5 μM concentration. To increase the steric attraction, two chlorides were introduced at R^1 but had no effect on the inhibitory activity. As the size of R^2 increased, the potency of the inhibitor was improved in compounds **6b**–**6h**. In particular, compound **6h** was evaluated as having an IC_{50} value of 15 nM, probably due to the hydrophilic interaction between two oxygens of the methanesulfoneamide and three NHs in the side chains of Arg141, Gln185 and Arg220.

To confirm the calculated binding mode of the designed scaffold, the X-ray structure of GSK-3β bound with compound **6h** was determined at 3.2 Å resolution.[15] This showed that the designed hydrogen bond network in the hinge region is realized (Fig. 4a). The hydrogen bond distances are measured to be 3.31 Å between the Val135 C=O and imidazole NH of compound **6h**, 3.36 Å between the Val135 NH and oxygen of the hydroxy group and 2.55 Å between the Asp133 C=O and oxygen of OH. Additionally, the corresponding hydrogen bond angles between GSK-3β and compound **6h** are 130°, 129° and 132°, respectively. Moreover, the torsion angle between the benzimidazole ring and amide is in concordance with our expectation based on ab initio calculations. When the two structures from X-ray studies and calculations are superimposed along the backbone of the kinase hinge region, the benzimidazole rings almost overlap (Fig. 4b).

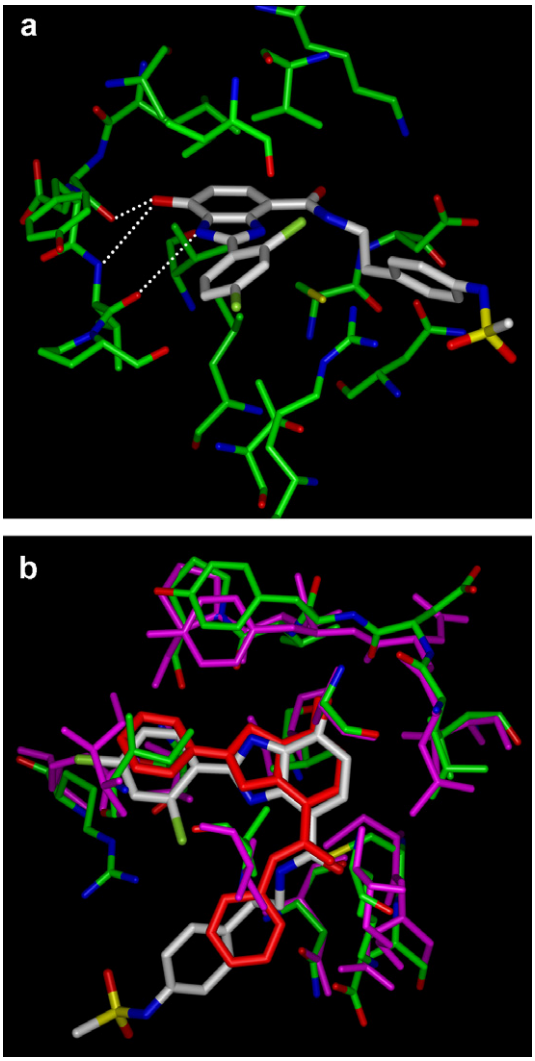


Figure 4. (a) X-ray complex structure of GSK-3β and compound 6 h. The hydrogen bond network in the hinge region is depicted by a white dotted line. The carbon atom in the ligand is colored white, the carbon atom in the protein is green, oxygen is red, and nitrogen is blue. (b) Comparison between X-ray and docking structures. The docking structure with **6a** is colored red, the corresponding protein is purple, the X-ray complex structure 6 h is white and the corresponding protein is cyan.

In summary, we have designed new kinase inhibitors by considering the hydrogen bond network between the kinase protein and ligands. The binding conformation and positioning of the designed inhibitors were predicted using ab initio calculation and a molecular docking study and confirmed through enzymatic assay and X-ray crystallography.

Acknowledgments

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