Docking simulation of fragment library compounds to find new leads for specific WNK kinase inhibitors

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Abstract

Pseudohypoaldosteronism type II has been known as a rare autosomal dominant disorder caused by WNK1 [with no K (lysine) protein kinase-1] or WNK4. These serine/threonine kinases have unusual structures with a back pocket located just behind the ATP binding site. Moreover, a lysine residue (Lys233 in WNK1) in a glycine-rich loop plays a key role in their activity. In this work, we performed docking simulations of about 9,000 compounds from a fragment library with the back pocket of WNK1 in order to discover candidate lead compounds for development of specific inhibitors. Based on binding energy index, we selected β-tetralone (compound 5) as a lead structure that interacts with the back pocket, but not with the hinge region of WNK1.

Guided by the four predicted docking patterns of β-tetralone with the back pocket, we designed four derivatives A-D that were expected to form hydrogen bonds with Lys233. Docking studies indicated that these derivatives interact selectively with Lys233, but not with the hinge region. These compounds are considered potential lead compounds for developing selective WNK inhibitors.

Key Words: WNK1, WNK4, fragment library, docking simulation

Area of Interest: In silico drug discovery

1. Introduction

Among the WNK [with no K (lysine)] kinases, overexpression of WNK1 and mutations in WNK4 are associated with Pseudohypoaldosteronism type II [1]. The WNK kinases are structurally unusual in that a lysine residue present in the β3 strand of most serine/threonine kinases is replaced by cysteine, and their activity relies instead on a lysine residue in a glycine-rich loop [2]. In addition, X-ray analysis (PDB code: 3FPQ) of apo-WNK1 (Figure S1) revealed the presence of a
unique back pocket located just behind the ATP binding site. Therefore, we considered that compounds interacting with the back pocket and/or the essential lysine residue (Lys233 in the case of WNK1), but not interacting with the hinge region, would be good candidates for specific WNK kinase inhibitors. Conventional WNK1 and WNK4 kinase inhibitors do not interact with the key lysine residue [3-5]. Therefore, we performed docking simulations of WNK1 with compounds from a fragment library, with the aim of discovering new leads for developing specific inhibitors of WNK1 and WNK4. We focused on WNK1 in this work, because the kinase domains of WNK1 and WNK4 show high structural homology (87%).

2. Method

Among several crystal structures (PDB code: 3FPQ [6], 4PWN [7], 4Q2A [7], 5DRB [4], 5TF9 [5]) of WNK1 reported so far, we focused on 3FPQ-B (Figure S1b), which possesses a deep back pocket and contains glycerols in the hinge region and at the bottom of the back pocket. Docking simulation targeting a glycerol at the bottom of the back pocket was performed with the MOE-Dock program in the Molecular Operating Environment (MOE) [8]. The calculation allowed induced fit utilizing the rigid backbone for conformation of the template protein, with the Amber10:EHT force field, triangle matcher as placement, and GBVI/WSA dG (kcal/mol) as the binding affinity scoring function[9]. Simulations were run for a fragment library of about 9,000 compounds at the Drug Discovery Initiative, the University of Tokyo.

3. Results and Discussion

Docking simulation of fragment library compounds and WNK1 with glycerol in the hinge region was done using MOE-Dock program. From the docking simulations binding affinity of WNK1 with fragment compounds, GBVI/WSA dG, conduced to the top-ranked pose of each compound. As the result we obtained docking poses corresponding to 8,990 compounds with GBVI/WSA dG scores -7.55 to 8.62 kcal/mol. Examination of the top 20% ranking compounds suggested that most of them could be classified into three kinds of scaffolds (Types I-III). The most stable poses of representative compounds are shown in Table S1. Based on the GBVI/WSA dG values (-7.20 to -5.39 kcal/mol) and binding energy index (BEI) values (GBVI/WSA dG/MW*100), these compounds (1-12) were considered to interact with the bottom of the back pocket (Figure S2).

In particular, the BEI values of two small compounds (5 and 10) were good and five compounds (1-3, 5 and 6) contained 3,4-dihyronaphthalen-2(1H)-one as a partial structure. Therefore, we focused on β-tetralone (5) for further development.

Since small interacting compounds would not necessarily show inhibitory activity, we designed derivatives of β-tetralone (5) that would potentially interact with the critical lysine residue. Four docking poses (Poses A-D) were obtained by docking simulation of β-tetralone (5) without glycerol in the hinge region (Figure S3), suggesting that there are four possible binding modes of β-tetralone with the back pocket in WNK1. Therefore, we designed corresponding compounds A-D that might form a hydrogen bond with Lys233. In compounds A and B, the carbonyl group of β-tetralone structure can form the hydrogen bond with Lys233. On the other hand, there is hydrogen bonding between Lys233 and amido group or amidomethyl group which were introduced into the benzene ring of β-tetralone structure in compounds C and D. Furthermore, the benzylamino group in compound C and amino group in compound D were introduced into the benzene ring in order to
stabilize the interaction. Optimized poses of compounds A-D and their GBVI/WSA dG values are shown in Figure 1. Also, all of the compounds successfully interacted with Lys233 through hydrogen bond interactions that might push up specificity than ever before. Thus, compounds A-D are worth of potential lead candidate compounds for further development of specific inhibitors of WNK1 and WNK4. More detailed screening that is determined by reference to this work is currently underway.

**Table 1.** Scaffold types I-III and BEI values of docking compounds

| Type I | Type II | Type III |
|--------|---------|----------|
| ![Compounds](image1) | ![Compounds](image2) | ![Compounds](image3) |
| BEI: -3.30 | BEI: -3.66 | BEI: -3.53 |
| BEI: -3.74 | BEI: -3.53 | BEI: -3.29 |
| BEI: -3.76 | BEI: -3.29 | BEI: -4.18 |
| BEI: -2.92 | BEI: -4.18 | BEI: -3.53 |
| BEI: -3.69 | BEI: -3.53 | BEI: -3.72 |
| BEI: -3.32 | BEI: -3.72 | |

**Figure 1.** Designed lead compounds (A-D) corresponding to Pose A-D of β-tetralone (FigureS3).
4. Conclusion

In order to develop potent and specific WNK1 and WNK4 kinase inhibitors, we employed docking simulation to screen a fragment library for compounds that bind to the back pocket, which is a characteristic structure of WNK kinases. β-Tetralone was selected from among the top 20% ranked for binding affinity is regarded as a seed compound, and we designed a series of derivatives that were expected to interact with the key residue Lys233, based on four predicted docking patterns. Docking studies of the designed derivatives indicated that they all interacted with Lys233 in multiple binding modes. We think these derivatives will be useful as new leads for the development of potent and specific inhibitors of WNK1 and WNK4.

5. Supplements

Figures S1-S3 are available supplementary materials (1).
The atomic coordinates of following structures in PDB format are available supplemental data at: http://cbi-society.info/supplement/10.1273/cbij-17-30/
Compound A, Compound B, Compound C, and Compound D.

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