Competitive fragment assay for the selective inhibitor of WNKs kinase

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Abstract

Pseudohypoaldosteronism type II is a rare, familial, autosomal-dominant hypertensive disease that is caused by mutations of WNK (with no lysine [K]) protein kinases 1 and 4. WNKs lack a lysine residue in the β3 strand that is generally conserved in protein kinases. WNK 1 and WNK4 share 87% homology, and possess an unusual back pocket just behind the catalytic lysine residue (Lys233 in WNK1). Therefore, compounds interacting with both the back pocket and catalytic lysine residue could be selective inhibitors. Here, we screened a fragment library for inhibitors of WNK1-mediated phosphorylation by means of mobility shift assay and surface plasmon resonance (SPR)-based binding assay. Among the identified inhibitors, some interacted with the back pocket rather than the hinge region of WNK1, as determined by SPR competitive binding assay. The results of kinase profiling suggest these compounds are promising leads for development of selective inhibitors of WNK 1 and 4.

Key Words: WNK1, WNK4, Fragment compounds, SPR, competition assay

Area of Interest: In silico drug discovery

1. Introduction

Hyperfunctions of WNK (with no lysine [K]) protein kinase 1 (WNK1) and WNK4 are associated with pseudohypoaldosteronism type II, a rare, autosomal-dominant, hypertensive disorder [1]. These WNK kinases show a high degree of homology, and differ from other protein kinases in that a lysine residue in the β3 strand is replaced with cysteine [2]. Further, an X-ray crystal structure of apo-WNK1 (PDB code: 3FPQ) revealed the presence of a back pocket just behind the ATP-binding site. Interestingly, the protein kinase inhibitor staurosporine does not show high inhibitory activity towards WNK1 [3]. This could be explained by a computational docking study [4], which indicated that Lys233 restricts staurosporine’s interaction with the hinge region of
WNK 1 (Figure S1). Accordingly, we hypothesized that compounds interacting with the back pocket and Lys233 in WNK1, but not with the hinge region, would be promising candidates for specific inhibitors. Among reported WNK1 and WNK4 kinase inhibitors, only one is so far known to interact with the back pocket [3, 5-6].

Fragment-based drug discovery (FBDD) is a useful tool for finding compounds that bind to a unique target [7, 8]. Therefore, in this work we screened a large fragment library by means of mobility shift assay and surface plasmon resonance-based competitive binding assay in order to discover compounds that interact with both the back pocket and catalytic lysine residue, as new leads for developing specific inhibitors of WNK1 and WNK4. Screening was focused on WNK1 activity, taking account of the high homology (87%) of the two kinases.

2. Methods

2.1 Mobility Shift Assay

The WNK1-inhibitory activity of about 9000 compounds in a fragment library at the Drug Discovery Initiative, the University of Tokyo, was screened using mobility shift assay. A mixture containing 1.5 μM fluorescein amidite (FAM)-labeled OSR1 peptide substrate (Toray Research Center, Tokyo, Japan), 20 μM ATP, 10 ng/μL WNK1 (Carna Biosciences, Kobe, Japan), 50 mM Hepes (pH 7.4), 5 mM MgCl₂, 3 mM MnCl₂, 1 mM DTT, 1% Protease Inhibitor Cocktail Set V (Merck Darmstat, Germany), 1% Phosphatase Inhibitor Cocktail Set III (Merck), 0.01% Brij-35 and a test compound (5% DMSO) was incubated at room temperature for 6 h. The reaction was terminated by addition of 4 mM EDTA. Phosphorylated and unphosphorylated peptides were separated and detected by a Lab-Chip EZ Reader II (PerkinElmer).

2.2 Surface Plasmon Resonance (SPR) Measurements

WNK1 was immobilized onto a CM5 Sensor Chip using an amine coupling kit (GE Healthcare). A sample in buffer solution containing 10 mM HEPES (pH 7.4), 150 mM NaCl, 50 μM EDTA, 0.1% Tween 20, and 5% DMSO was injected at a constant rate (30 μL/min) for the first 1 min for association. We then monitored dissociation for 3 min using a Biacore T200 (GE Healthcare). The binding response and the equilibrium dissociation constant (K_d) with respect to WNK1 were determined using the instrument software.

3. Results and Discussion

Screening of the fragment library for inhibitory activity towards WNK1-mediated phosphorylation activity by mobility shift assay afforded 81 compounds with IC₅₀ values of less than 200 μM. Elimination of non-specific binders based on SPR results left 50 candidates. Among them, 3 compounds showed potent inhibition of WNK1-mediated phosphorylation; the IC₅₀ values of compounds 1-3 were 8.9 μM, 10.3 μM, and 5.8 μM, and their K_d values were >40 μM, 3 μM, 1 μM, respectively (Table 1).
Next, competitive-binding assay was performed by SPR to investigate the binding sites of these compounds. The principle is that when binding of one compound becomes saturated, another compound is added, and then if the binding sites are different, the binding amount will be equal to the sum of binding at the two sites [9]. Firstly, compound 3 was mixed with staurosporine, which is known to bind in the hinge region. The observed binding amount was the sum of those of each compound, and therefore compound 3 and staurosporine have different binding sites (Figure 1a). This result suggests that compound 3 binds to the back pocket rather than the hinge region. Compounds 1 and 3 also bound to different sites (Figure 1b). However, 2 and 3 bound to the same site (Figure 1c). This result suggests the compound 2 also binds mainly to the back pocket. Next, kinase profiling of compound 2 was performed to examine the selectivity. Among 50 kinases examined, 8 were inhibited by at least 50% at 10 μM (Figure 2). These results suggest that it may be possible to develop specific WNK inhibitors by structural development of compounds 2 and 3.

![Figure 1](image.png)

**Figure 1.** Competitive binding assay of staurosporine(STS) and three hit compounds
(a) Compound 3 with STS, (b) Compound 3 with compound 1 (c) Compound 3 with compound 2.

![Figure 2](image.png)

**Figure 2.** Kinase profiling of the compound 2 using mobility shift assay
Carried out at Carna Biosciences. ATP concentration was approximately equal to the Km value of each kinase.

Docking simulations using MOE software indicate that compounds 1 and 3 bind to WNK1 at the hinge region and the back pocket, respectively (Figure S4). Compound 3 also binds to the key Lys233 residue through a hydrogen bond, and there appears to be enough space around the binding...
site for derivatization. And then GRID analysis [10] shows that the hydrophobic favorable site (gray mesh) spreads around Leu369 and the hydrogen-bond donor site (blue mesh) exists around carbonyl group of Leu369 (Figure S5). Therefore, as the compound that reflected GRID analysis 4,7-dimethyl-5-aminomethyl-1,3-isoquinolininedione derivative compound 4 of compound 3 was designed (Figure S6). GBVI/WSA dG scores [11] of WNK1 with compound 3 and 4, were -5.88 and -5.95 kcal/mol, respectively. Thus, we consider that compounds 3 are promising lead compounds for developing specific inhibitors of WNK1 and WNK4.

4. Supplementary Information

Figures S1-S6 are in the supplementary materials (1) available at: http://doi.org/10.1273/cbij.17.34

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