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# Mixed Fragment-based screening of WNK1 inhibitor

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Pseudohypoaldosteronism type II, known as a rare autosomal dominant disorder, is considered to be caused by overexpression of WNK [with no lysine(K)]1 or mutations in WNK4. Thus far, there have been only a few reports regarding the specific inhibitor for WNK1 or WNK4. We therefore explored lead compounds for WNK1 by mixed fragment-based drug discovery whereby competition experiment using surface plasmon resonance (SPR) enables us to select better lead compound in the early stages.

Mobility shift assay, by which inhibitory activity for phosphorylations of WNK1 was estimated, was examined in order to select hit compounds out of 9,000 fragments. Then we selected compounds having structural similarity to the hit compounds, and their binding abilities, including those of hit compounds, to WNK1 were tested using SPR. The competition assay by SPR was carried out using a single or a mixture of the candidate compounds.

Figure 1 shows competition assay about combination of candidate compounds 1 (1,3-isoquinolinediol) and 2 [N-(4-methyl-5-oxo-4,5-dihydro[1,2]dithiolo[4,3-b]pyrrol-6-yl)], and 3 (1,3-isoquinolinedione) and 4 (indirubin-3'-oxime). Compounds 1 and 2 were found to bind to the same site in WNK1 because the response of their mixture was almost the same as that of 2. In contrast, as shown in the right side of Figure 1, the response of the mixture of compounds 3 and 4 were the sum of those of the two. This clearly indicates compound 3 and 4 bind to the different sites in WNK1. The binding site of these compounds will be discussed using a docking study.

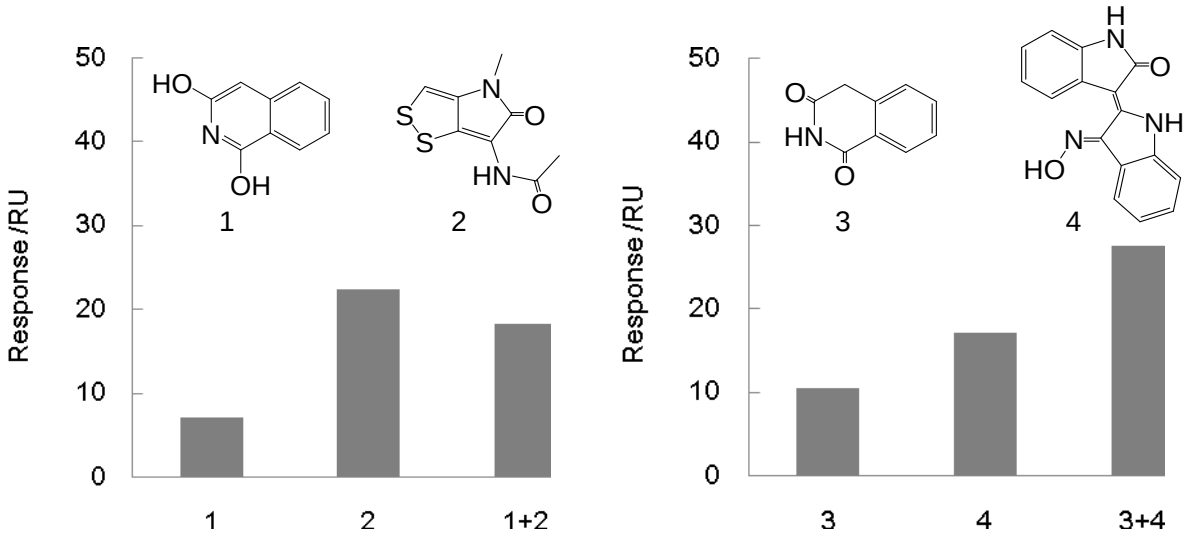


Fig. 1 Competition experiments using candidate compounds 1-4.