Non-Quinone Sulfone Analog as a Cdc25 Inhibitor: Biological Evaluation and Structural Determinants of Cdc25A and Cdc25B Selectivity

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Cdc25 phosphatases, as activators of the Cdk/cyclins, are important in cell cycle control. Because Cdc25A and Cdc25B have oncogenic properties and are overexpressed in various human tumors, they are attractive drug targets for anticancer therapies.[1] To date, many of the most potent Cdc25 inhibitors are quinone-containing compounds.[2] Among them, Cpd 5 (1) has provided useful information in future design of potent Cdc25 inhibitors for both experimental and therapeutic purposes.[3] However, the redox properties of the naphthoquinones can generate toxic reactive oxygen species.[4] Non-quinone Cpd 5 analog 2 with the sulfone was therefore synthesized and exhibited Cdc25A inhibition with the reasonable activity (IC_{50} = 7.5 μM).[5]

Cpd 5 (1)   **2**   **3**
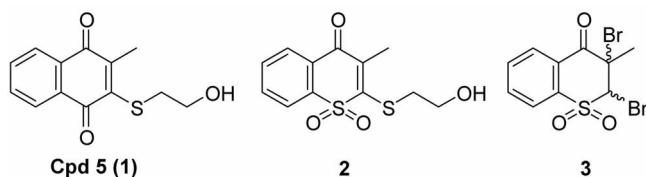
Interestingly, we also observed the unexpected result that the precursor 3 of compound 2 had the strong inhibition activity (IC_{50} = 2.1 μM) against Cdc25A. Consistent with enzyme inhibition, it showed the inhibitory activity of Hep3B cell growth and did not generate reactive oxygen species when compared to quinone-containing Cdc25 inhibitors.[6] Moreover, this compound has interesting properties because it is more selective for hepatoma cells compared to normal cells and is 25-fold more potent for Cdc25A than Cdc25B or Cdc25C. Therefore, we investigated the detail mechanisms of inhibitory actions and binding modes with 3 against Cdc25 in this study.

Since kinetic analysis and dilution experiment[7] of compound 3 and Cdc25 phosphatases revealed reversible competitive inhibition (*data not shown*), we examined its binding mode in the catalytic site with docking simulations. The AutoDock program[8] was used in this docking simulation because the outperformance of its binding energy function over those of the others had been shown in several target proteins.[9] In order to enhance the accuracy of the binding energy function, an improved solvation energy term for a ligand developed by Kang *et al.*[10] was introduced in the original binding energy function. This modification of the solvation free energy term is expected to increase the accuracy in docking simulation, because the underestimation

of ligand solvation often leads to the overestimation of the binding affinity of a ligand with many polar atoms.[11] Furthermore, cubic equation approach was applied to obtain the dielectric constant required in computing the interatomic electrostatic interactions between Cdc25 phosphatases in **3**.[12]

With respect to the determination of protonation states of the ionizable residues, we used the atomic distance data in the X-ray structures of Cdc25A[13] and Cdc25B.[14] For example, the side chains of Asp and Glu residues were assumed to be neutral if their carboxylate oxygens of OD or OE atoms were located within 3.5 Å from a hydrogen-bond accepting group including the backbone aminocarbonyl oxygen. Similarly, the side chains of lysine and histidine were assumed to be protonated unless their respective nitrogen atom was in proximity of a hydrogen-bond donating group. In this way the catalytic residues in the active site (Cys430 and Glu431 in Cdc25A, and Cys473 and Glu474 in Cdc25B) were assumed to be ionized while ND1 and NE2 atoms of the histidine side chain (His429 for Cdc25A, and His472 for Cdc25B) appeared to be protonated and deprotonated, respectively.

Prior to the actual docking simulations, the original X-ray crystal structures of Cdc25A and Cdc25B was equilibrated in aqueous solution through 1 nanosecond molecular dynamics simulation with the AMBER program, which had been successful in modeling the structures of proteins[15] and nucleic acids[16] in solution. The equilibration procedure started with the addition of sodium ions as the counterions to neutralize the total charge of all-atom model of Cdc25 phosphatases. The system was then immersed in a rectangular solvent box containing about 7000 TIP3P water molecules. After 1000 cycles of energy minimization to remove bad van der Waals contacts, we equilibrated the system beginning with 20 ps equilibration dynamics of the solvent molecules at 300 K. The next step involved equilibration of the solute with a fixed configuration of the solvent molecules for 10 ps at 10, 50, 100, 150, 200, 250, and 300 K. Then, the equilibration dynamics of the entire system was performed at 300 K for 500 ps using the periodic boundary condition. The SHAKE algorithm[17] was applied to fix all bond lengths involving hydrogen atom. We used a time step of 1.5 fs and a nonbonding-interaction cutoff radius of 12 Å.

Compared views in Figure 1 are the calculated binding free energies and binding modes of 3 in the catalytic sites of

(a)

E389
Y386
M488   R385
E431
R436
C430
E435
S433   F432
S434
ΔG$_{bind}$ = -16.6 kcal/mol

(b)

Y428
M531
E431
R479
R427
E474
C473
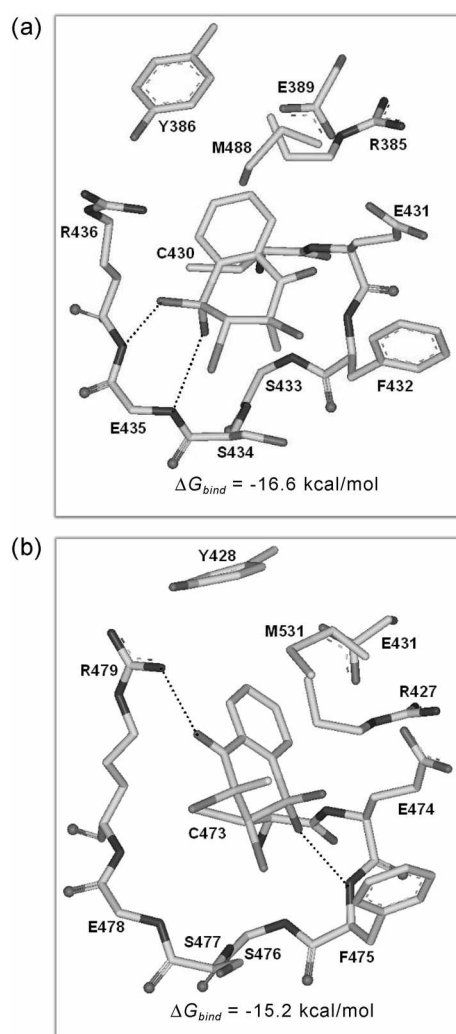E478
S477
S476   F475
ΔG$_{bind}$ = -15.2 kcal/mol

Figure 1. Comparative view of the binding free energies and binding modes of **3** in the catalytic sites of (a) Cdc25A and (b) Cdc25B. Carbon atoms of Cdc25 phosphatases and **3** are shown in green and cyan, respectively. Each dotted line indicates a hydrogen bond.

Cdc25A and Cdc25B. We note that consistent with in vitro enzyme assay data, **3** is predicted to be more stabilized in the catalytic site of Cdc25A than in that of Cdc25B. The difference in binding free energy amounts to 1.3 kcal/mol, which corresponds to 10-fold decrease in inhibition constant (K$_i$). It is also noteworthy that **3** exhibits different hydrogen bonding patterns in the catalytic sites of the two Cdc25 phosphatases. As can be seen Figure 1a, the two oxygens of the sulfoxide groups of **3** are hydrogen bonded to backbone amidic nitrogen of Glu435 and Arg436 in Cdc25A-**3** complex. On the other hand, only one oxygen atom of **3** forms a hydrogen bond with the backbone amide group of Phe475 in the active site of Cdc25B. In this complex, another hydrogen bond is established between the inhibitor carbonyl group and the side chain of Arg479. Judging from the same number of the hydrogen bonds, the extent of hydrogen-bond stabili-

zation of **3** seems to be similar in the active sites of the two Cdc25A and B.

The major structural difference around the active sites of the two Cdc25 phosphatases is in the position of the Met residue located at the top of active site. Met488 points toward the cavity of the active site in Cdc25A, whereas the corresponding Met531 is directed outward to bulk solvent in Cdc25B. Apparently, such a difference in active site geometry can serve as a clue for designing the selective Cdc25 phosphatase inhibitors. As shown in Figure 1, indeed, the hydrophobic interaction between the phenyl ring of the inhibitor and the active site Met residue is established in a stronger form in the Cdc25A-**3** complex than that Cdc25B-**3** complex. This difference in the strengths of hydrophobic interactions can thus be invoked to explain the stronger binding of **3** in the active site of Cdc25A than in that of Cdc25B.

In summary, compound **3** has been known to possess the protein phosphatase Cdc25A and B inhibitory activity and selectivity. We now show that compound **3** caused reversible inhibition of Cdc25 phosphatases and displayed competitive inhibitor kinetics. Based on its kinetic profile, docking simulations were also used to provide structural insights into the Cdc25A and Cdc25B inhibition selectivity.

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