Discovery of Novel Inhibitors of Dual-Specificity Phosphatase Pyst2 with Structure-Based Virtual Screening

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Protein tyrosine phosphatases (PTPs) are a family of the regulatory enzymes that are responsible for the dephosphorylation of phosphotyrosine residues in the protein substrates. A series of experimental evidence has been reported so far to support the correlation between malfunctions in PTP activity and various diseases including cancer, neurological disorders, and diabetes.† This has made PTPs be a promising target for drug discovery. Of the various PTPs, Pyst2 (also known as MKP-X and DUSP7) is a member of the Pyst subfamily of dual-specificity phosphatases (DUSPs) and known to have substrate selectivity for the classical p42 (ERK2) isoform of the mitogen-activated protein kinase (MAPK).‡,§

Pyst2 was known to be overexpressed in leukemia and other malignant cells,¶,†¶ which indicates that Pyst2 can be a target for the development of cancer drugs.¶ Despite such a pharmaceutical importance, no small-molecule Pyst2 inhibitors has been reported so far. In this study, we identify two novel inhibitors of Pyst2 based on the structure-based drug design protocol involving the virtual screening with docking simulations and in vitro enzyme assay. It will be shown that the docking simulation with the improved scoring function can be a useful technique for elucidating the activities of the identified inhibitors, as well as for enriching the chemical library with molecules that are likely to have inhibitory activities.¶

The docking library for Pyst2 comprising about 85,000 compounds was constructed from the chemical database distributed by Interbioscreen (http://www.ibscreen.com) containing approximately 30,000 natural and 320,000 synthetic compounds. The selection was based on the drug-like filters that adopt only the compounds with physicochemical properties of potential drug candidates¶ and without reactive functional group(s). All of the compounds included in the docking library were then subjected to the Corina program to generate their three-dimensional atomic coordinates, followed by the assignment of Gasteiger-Marsilli atomic charges.¶ We used the AutoDock program† in the virtual screening of Pyst2 inhibitors because the outperformance of its scoring function over those of the others had been shown in several target proteins.¶,¶¶ To increase the accuracy of scoring function, we implemented the solvation energy term proposed by Kang et al.†¶ Three dimensional structure of Pyst2 was obtained from homology modeling with Pyst3 as the structural template that reveals the sequence identity of 81% with Pyst2.†¶

Of the 85,000 compounds subject to the virtual screening with docking simulations, 100 top-scored compounds were selected as virtual hits. 92 of them were available from the compound supplier and were tested for inhibitory activity against Pyst2 by in vitro enzyme assay. As a result, we identified 2 compounds that inhibited the catalytic activity of Pyst2 by more than 50% at the concentration of 50 µM. The chemical structures and the inhibitory activities of the newly identified inhibitors are shown in Figure 1 and Table 1, respectively. We note that compounds 1 and 2 possess a benzoic acid group at the end of the molecular structure. The calculated binding free energies of these two potent inhibitors are also similar: 26.8 and 27.2 kcal/mol for 1 and 2, respectively. To the best of our knowledge, neither of these compounds has been reported as Pyst2 inhibitors so far. Judging from the moderate potency and the structural difference, both of the newly identified inhibitors can serve as a new inhibitor scaffold for further development by structure-activity relationship (SAR) methods to optimize the inhibitory activities.

The calculated binding mode of 1 in the active site of Pyst2 is shown in Figure 2. It is seen that the carboxylate group of the inhibitor points toward the catalytic residue Cys280 at a distance within 4 Å. We also note that the carboxylate group of 1 forms two hydrogen bonds with the side-chain guanidium group of Arg286 in a bidentate

Figure 1. Chemical structures of the newly identified Pyst2 inhibitors.
manner, which seems to be the most significant binding force for stabilizing 1 in the active site. A stable hydrogen bond is also established between the inhibitor carboxylate group and the backbone amide group of Leu281 in the active site. Because the carboxylate group of 1 resides in a close proximity to Cys280 and is stabilized by the formation of multiple hydrogen bonds at the active site, the benzoate moiety seems to be an effective surrogate for the substrate phosphate group that can bind tightly in the active site. The inhibitor 1 can be further stabilized by the hydrophobic interactions of its aromatic groups with the side chains of Pro228, His250, Ala282, Ile284, and Phe323. Judging from the structural features of the calculated Pyst2-1 complex, the inhibitory activity of 1 seems to stem from the establishments of multiple hydrogen bonds and hydrophobic interactions in the active site in a simultaneous fashion.

Figure 3 shows the most stable binding mode of 2 in the active site of Pyst2 obtained from docking simulations. The binding mode of 2 is similar to that of 1 in that the inhibitor benzoate group points toward the catalytic residue Cys280, and is stabilized by the bidentate hydrogen bond with the side chain of Arg286 and the backbone amide groups in the active site. However, one of the hydrogen bonds between the carboxylate group and the side-chain guanidium ion of Arg286 appears to be established in a weaker form in Pyst2-2 than in Pyst2-1 complex, which is reflected in the lengthening of hydrogen bond distance from 1.70 Å in the latter to 2.14 Å in the former. This seems to have an effect of lowering the inhibitory activity of 2. As a consequence of such a weakening in the hydrogen bond, however, a new hydrogen bond is formed with the backbone amide group of Ala282 at the bottom of the active site. As in the Pyst2-1 complex, the hydrophobic interactions seem also to be a significant binding force for stabilizing 2 in the active site of Pyst2 because its hydrophobic groups form van der Waals contacts with the side chains of His250, Leu281, Ala282, Ile284, Pro319, and Phe323. Because the hydrophobic interactions are established in a similar fashion in the two enzyme-inhibitor complexes, the weakening of a hydrogen bond between the inhibitor carboxylate group and the side chain of Arg286 should be responsible for the lower inhibitory activity of 2 than 1.

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References