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Computational Analysis of Potential Inhibitors Selected Based On Structural Similarity for the Src SH2 Domain

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***Abstract*—**The inhibition of SH2 domain regulated protein-protein interactions is an attractive target for developing an effective chemotherapeutic approach in the treatment of disease. Molecular simulation is a useful tool for developing new drugs and for studying molecular recognition. In this study, we searched potential drug compounds for the inhibition of SH2 domain by performing structural similarity search in PubChem Compound Database. A total of 37 compounds were screened from the database, and then we used the LibDock docking program to evaluate the inhibition effect. The best three compounds (AP22408, CID 71463546 and CID 9917321) were chosen for MD simulations after the LibDock docking. Our results show that the compound CID 9917321 can produce a more stable protein-ligand complex compared to other two currently known inhibitors of Src SH2 domain. The compound CID 9917321 may be useful for the inhibition of SH2 domain based on these computational results. Subsequently experiments are needed to verify the effect of compound CID 9917321 on the SH2 domain in the future studies.

***Keywords*—**Nonpeptide inhibitor, Src SH2 domain, LibDock, molecular dynamics simulation.

I. Introduction

THE Srchomology 2 (SH2) domains were identified through sequence similarities in the non-catalytic regions of Src related protein tyrosine kinase [1]. SH2 domains have vital roles in intracellular signaling process, and this domain is consisted of ~100 amino acids. Concerning about the structure of SH2, it consists of 2 parallel α-helices and a central anti-parallel β-strands. SH2 domains are found in a larger number of proteins which can bind to phosphorylated tyrosine residues, and these proteins are kinases, transcription factors and adaptor proteins. Besides, these domains had been identified that they regulate kinase activity to influence many cell responses, including proliferation, apoptosis, immune response, gene transcription and growth [2]. Abnormal protein-protein interactions in these domains can cause many disease states. For therapeutic intervention, the Src SH2 domain had been taken as a possible target in several diseases like colon carcinoma, breast adenocarcinoma, and osteoporosis by developing specific inhibitors. The inhibition of a protein's function by prohibiting its SH2 domain is a possible way to modulate normal SH2-domain function.

SH2 domains were found to bind phosphopeptides or peptidomimetics that contained phosphotyrosine or phospho-tyrosine-like motifs [3], [4]. Recently, Morlacchi et al. had reported a novel peptidomimetic inhibitor targeting the Src SH2 domain of signal transducer and activator of transcription 6 (STAT6) [5]. Duan et al. proposed potential selective inhibitors for treating cancer by targeting the Src SH2 domain-containing phosphatase 2 (Shp2) [6]. There are two binding sites in the SH2 domain: one interacts with phosphotyrosine groups on ligands and another one takes up an aliphatic side chain. Therefore, many Src SH2 inhibitors are developed by synthesizing peptides containing phosphorylated tyrosine residues (pTyr). For example, the Src SH2 domain had been found that it could bind with pTyr-Glu-Glu-Lle (pYEEI) peptide with high affinity [7]. Except for the phosphopeptide ligands, some researches dedicated in the development of nonpeptide SH2 inhibitors [8], [9]. Shakespeare et al. developed a nonpeptide SH2 inhibitor called as AP22408, which contained 3'-4'-diphosonophenylalanine (Dpp) as a phosphotyrosine mimic [9]. The Dpp moiety of this drug exhibited bone-targeting properties, and furthermore this drug demonstrated *in vivo* antiresorptive activity in a parathyroid hormone-induced rat model.

However, the efficient inhibition of specific SH2 domain through small-molecule inhibitors for treating diseases remains a difficult issue. The purpose of this paper is to focus on the interactions between the nonpeptide SH2 inhibitors and the SH2 domain from the Src kinase. We try to use molecular simulation methods to compare the inhibition effects of several nonpeptide inhibitors on the Src SH2 domain. This study may provide a useful reference to research more effective inhibitors of the SH2 domain.

II. Materials and Methods

A. Protein Model

The protein model used in this computational study was downloaded from Protein Data Bank (PDB; http://www.rcsb.org/pdb/). The SH2 domain-inhibitor (AP22408) complex has been solved by NMR and it is present in PDB with the code 1FBZ. The conformation of this protein-ligand complex was generated from the report by Shakespeare et al. [9] and the resolution of this complex is 2.4 Å. In the structure of SH2 domain, there are two binding pockets that involve in the ligand recognition. One of two pockets is a positively charged region, which corresponds to the phosphotrysine binding site. The charge of another pocket is neutral and this pocket is linked by hydrophobic residues. These two pockets were included in the defined bind site in the molecular simulation processes.

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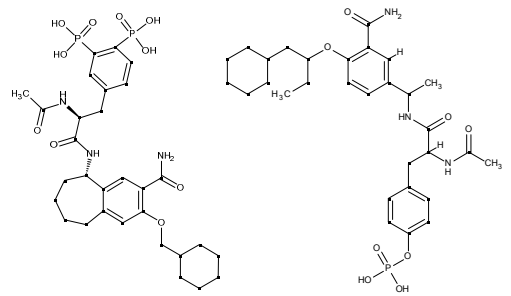
B. Drug Compounds

All drug compounds used in this study were basically collected from a chemical structure database called PubChem (https://pubchem.ncbi.nlm.nih.gov/) except for AP22408. The structure file of AP22408 was obtained directly from the PDB file (PDB code: 1FBZ). Other chemical compounds were obtained by using the three selection criteria: (1) We used the keywords "Src SH2 inhibitor" to search the compounds in PubChem Compound Database; (2) The structural similarity between AP22408 and the selected compound had to be over 95% by performing structure search in PubChem Compound Database; (3) Three compounds of SH2 inhibitors (CID 67698995, 11798263, and 46936211) were selected from literatures [8], [10]. By doing this, 31 compounds were collected and ready for using in the subsequent simulation steps. Fig. 1 shows the chemical structures of selected compounds.



(a) (b)

Fig. 1 Chemical structures of (a) AP22408 and (b) CID 67698995

C. Docking Simulation

The software for molecular simulation used in this study was Discovery Studio 3.5 (DS 3.5; Accerlrys Inc., San Diego, CA). The docking between ligand and protein was evaluated by using LibDock docking program. LibDock is a high-throughput algorithm for docking ligands into an active binding site on the receptor, which is also a site-features docking algorithm. The 31 screened compounds were docked with the binding site of SH2 domain altogether by using LibDock Program. The ligand and protein atoms were applied with CHARMM force field before running LibDock. Ligand conformations were aligned to receptor interaction sites and the best poses were reported in the end of docking simulations. Each pose was evaluated according to the LibDock score, and three ligands with top LibDock scores were selected for further examinations.

D. Molecular Dynamics Simulation

Molecular dynamics (MD) simulations were performed using DS 3.5 Standard Dynamics Cascade and Dynamics package. Samples of ligand-receptor complexes were applied with the CHARMM polar hydrogen force filed and solvated by applying explicit periodic boundary in a solvation model before running MD simulations. MD simulations were conducted under the setting parameters, which were listed as follows: steepest descent of energy minimization was 500, steps of conjugate gradient minimization were 500, the system was heated from 50K to 300K within 2 ps, and steps of equilibration were 1000. Besides, the parameters of electrostatics and Apply SHAKE Constraint were chosen as Particle Mesh Ewald (PME) and true, respectively. The simulations were performed with a total production time of 200 ps. For other parameters, we adopted default setting values. We used the functions of Analyze Trajectory to analyze root mean square deviations (RMSDs) of protein-ligand complexes and ligands, total energy of protein-ligand complex, and hydrogen bond (H-bond) after MD simulation. The simulations were carried out on a dual-processor workstation (Intel Xeon, eight-core, 2.0 GHz) with a memory size of 20 GB.

III. Results and Discussion

A. Docking Simulation

According to the results obtained from LibDock simulation, all ligands were ranked by the LibDock scores. TABLE I shows the top three ligands that have good binding affinities with the defined active site on SH2 domain. The LibDock scores are ranging from 135.2 ~ 136.9. We found that, there are two compounds having a higher LibDock scores than AP22408 (shown in Table I). Concerning about the binding stability, each compound could produce 4 or 5 intermolecular hydrogen bonds (H-bonds) with the amino acid residues on SH2 domain in the best docking pose. We selected these three compounds in the following MD simulations in order to further investigate dynamic behaviors of protein-ligand complexes and evaluate the stability of ligand binding in the light of the variations of intermolecular H-Bonds at different time points.

TABLE I
The Ligands with Top Three Highest LibDock Score

Molecule name	LibDock score	Number of H-bond in the best pose
CID 71463546	136.9	5
CID 9917321	136.0	5
AP22408	135.2	4

B. Energy and RMSD Values in MD Simulations

Binding stability of three selected compounds can be validated according to the results obtained from MD simulations. We sampled 80 data points by setting a regular interval from the 200 ps simulation trajectory. RMSDs of protein-ligand complexes are shown in Fig. 2, and Fig. 3 shows that the RMSD values for individual ligand. The average value of RMSD for each ligand was calculated over the simulation trajectory. The average RMSD value of protein-ligand complex with CID 71463546, CID 9917321, or AP22408 was 1.51±0.2 Å, 1.62±0.3 Å and 1.55±0.29 Å, respectively. Regarding to the ligands, the average RMSD values of CID 71463546, CID 9917321, and AP22408 were 2.13±0.28 Å, 1.86±0.28 Å and 1.86±0.36 Å for the 200 ps simulation, respectively. The lower RMSD values of ligands suggest that ligands can have more stable interactions with the receptor. From this viewpoint, CID 9917321 and AP22408 could produce nearly equivalent stability with the receptor. Total energies for three kinds of protein-ligand complexes were ranging from –39,560

to –39,366 kcal/mol (shown in Fig. 4). The total energies of ligand-protein complexes were approximately identical to each other for the 200 ps simulation. Among them, CID 71463546 has the lowest total energy with the receptor.

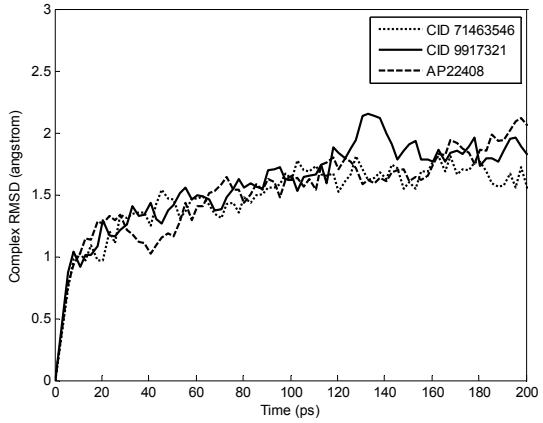


Fig. 2 The RMSD values of protein-ligand complexes during MD simulation

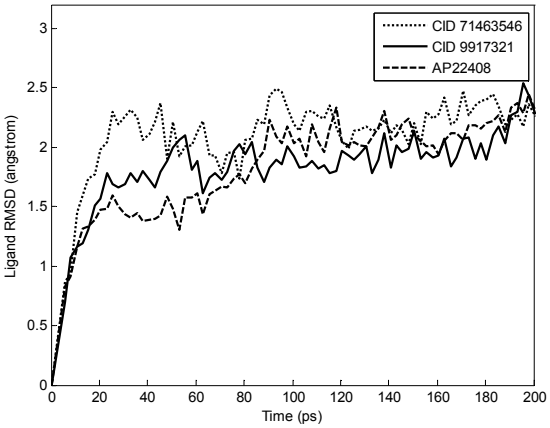


Fig. 3 The RMSD values of ligands at the binding site of SH2 domain during MD simulation

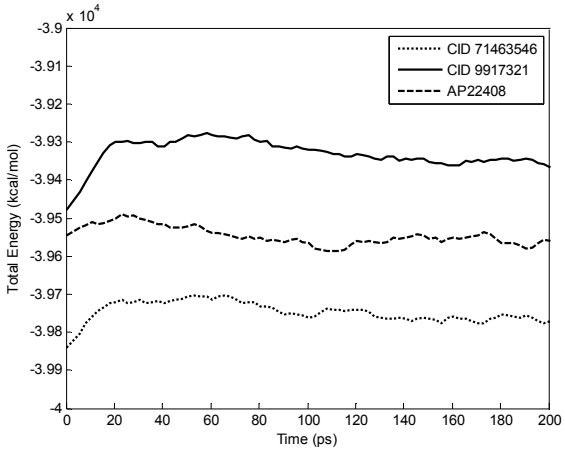


Fig. 4 Total energies of protein-ligand complexes during MD simulation

C. Comparisons between Binding Abilities of Ligands through MD Simulations

From the analysis of MD simulation trajectory, we found that H-bonds could be formed or disrupted with protein side chains at different time points over the simulation trajectory. Table II lists the number of intermolecular H bonds for each ligand at different time points. The results of MD simulations showed that CID 71463546 could produce at least 4 intermolecular H-bonds with the SH2 domain in the simulation trajectory. However, some H-bonds were not stable and disrupted during the simulation trajectory. Among these H-bonds, only three stable H-bonds were formed with CID 71463546 by amino acid residues Arg232, Lys260 and Ser272. ILE271 was also an important residue for contributing one of the four H-bonds in the end of 200 ps simulation. But, the H-bond formed between residue ILE271 and CID 71463546 was absent at 100 ps in the simulation trajectory. For CID 9917321, the compound produced at most 6 intermolecular H-bonds with the SH2 domain in the 200 ps simulation. Based on the number of H-bonds, we suggest that CID 991732 is capable of forming a more stable ligand-protein complex compared to other two compounds. The number of H-bonds presented here is different from the results obtained from LibDock simulation, which is mainly caused by using different algorithms in the simulations.

TABLE II
THE NUMBER OF INTERMOLECULAR H BONDS FOR EACH LIGAND AT DIFFERENT TIME POINTS

Molecule	Time point(ps)			
	50	100	150	200
CID 71463546	4	4	6	4
CID 9917321	6	6	7	6
AP22408	5	3	3	4

In the final 200 ps of analysis, amino acid residues Arg 232, Ser234, Cys242 and Lys 260 were key residues for forming six H-bonds with CID 991732 (shown in Fig. 5). The compound CID 991732 bound to the SH2 domain through two H-bonds at Arg232, two single H-bonds at Cys242 and Ser234, and two H-bonds at Lys260. These four amino residues are involved in the phosphotrysine binding site on the SH2 domain. The data obtained from PubChem Compound Database reveal that CID 77463546 and AP22408 are currently known inhibitors for Src SH2 domain. The compound CID 991732 is not recorded as a SH2 domain-inhibitor in PubChem Compound Database. In accordance with the biological data, AP22408 inhibited Src-ligand binding with an IC50 of 0.3 μM [9], [11]. In a parathyroid hormone-induced rat model, the drug demonstrates that it has *in vivo* antiresorptive activity while the animals were given with a dose of 50 mg/kg twice daily. For another compound CID 11798263, this ligand has an IC50 of 6.5 μM according to the data in PubChem Compound Database. This compound just got a LibDock score of 129.98 in the docking simulation in this study. Basically, the findings from computational simulations are consistent with the bioactivity data. However, the inhibition effect of CID 991732 on the SH2 domain is still needed to validate by performing further experimental studies. In summary, this study proposes a

potential compound for using as a SH2 domain-inhibitor.

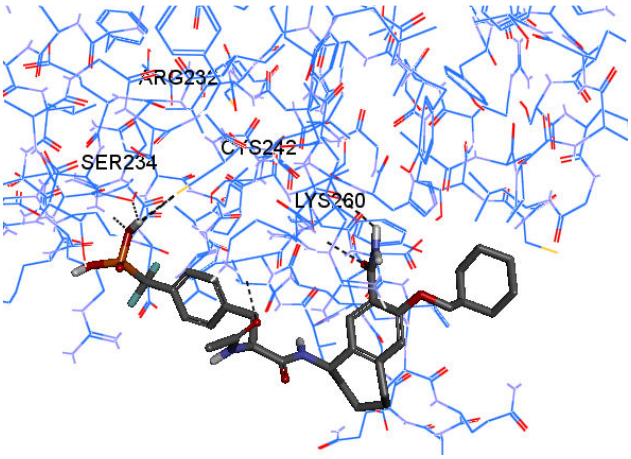


Fig. 5 Intermolecular interactions between protein side chains and docked ligand (CID 9917321). H-bonds are denoted with dotted lines

IV. Conclusions

In this computational study, we had identified the compound CID 9917321 might have a possibility to become an effective inhibitor for the Src SH2 domain. Besides, our results of MD simulations indicated that CID 991732 could have a more stable binding reaction with the SH2 domain than the other two compounds. In order to verify the effect of CID 9917321 on the inhibition of SH2 domain, computational analysis like quantitative structure–activity relationship (QASR) and biological experiments are necessary in the future study. These findings in this study may provide a useful reference to design a better, more effective inhibitor of the Src SH2 domain.

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