Molecular Docking, Molecular Dynamics Simulations, and Free Energy Calculation Insights into the Binding Mechanism between VS-4718 and Focal Adhesion Kinase

Mingsong Shi, Tao Chen, Siping Wei, Chenyu Zhao, Xinyu Zhang, Xinghui Li, Xinyi Tang, Yan Liu, Zhuang Yang,* and Lijuan Chen*

Cite This: ACS Omega 2022, 7, 32442−32456
Read Online

ABSTRACT: Focal adhesion kinase (FAK) is a 125 kDa nonreceptor tyrosine kinase that plays an important role in many carcinomas. Thus, the targeting of FAK by small molecules is considered to be promising for cancer therapy. Some FAK inhibitors have been reported as potential anticancer drugs and have entered into clinical development; for example, VS-4718 is currently undergoing clinical trials. However, the lack of crystal structural data for the binding of VS-4718 with FAK has hindered the optimization of this anticancer agent. In this work, the VS-4718/FAK interaction model was obtained by molecular docking and molecular dynamics simulations. The binding free energies of VS-4718/FAK were also calculated using the molecular mechanics generalized Born surface area method. It was found that the aminopyrimidine group formed hydrogen bonds with the C502 residue of the hinge loop, while the D564 residue of the T-loop interacted with the amide group. In addition, I428, A452, V484, M499, G505, and L553 residues formed hydrophobic interactions with VS-4718. The obtained results therefore provide an improved understanding of the interaction between human FAK and VS-4718. Based on the obtained binding mechanism, 47 novel compounds were designed to target the adenosine 5′-triphosphate-binding pocket of human FAK, and ensemble docking was performed to assess the effects of these modifications on the inhibitor binding affinity. This work is also expected to provide additional insights into potential future target design strategies based on VS-4718.

INTRODUCTION

Focal adhesion kinase (FAK, also known as protein tyrosine kinase 2, PTK2) is a nonreceptor tyrosine kinase and is one of the FAK family members. In a biological context, FAK plays a key role in the adhesion, motility, invasion, metastasis, and survival of cancer cells. FAK has been described as a protein that possesses increased tyrosine phosphorylation, which is of particular importance in many carcinomas, including papillary thyroid carcinoma,1 neck cancer,2-5 malignant melanomas,6,7 bladder cancer,8 intrahepatic cholangiocarcinoma,9 ovarian cancer,10 esophageal cancer,11 breast cancer,12 and pancreatic ductal adenocarcinoma.13,14 Additionally, high levels of FAK in cancer patients are generally associated with poor prognosis. Thus, FAK has been considered a promising potential target for cancer therapy.

The FAK (1052 residues) consists of three domains (Figure 1), namely, the N-terminal 4.1 protein, ezrin, radixin, moesin (FERM) homology domain (residues 35-355), the middle protein kinase domain (residues 422-680), and the C-terminal focal adhesion target (FAT) domain (residues 707-1052).15 The kinase domain of FAK (also known as the catalytic domain) possesses a highly conserved amino acid sequence and structure that is formed from an N-terminal
Gly) motif is highly conserved and follows the T-loop, which is referred to as a hinge loop. It should be noted here that the DFG (Asp-Phe-Gly) activation loop or A-loop), or (iii) rotation of the α-C-helix in the N-lobe. Generally, such rearrangements are based on one of the following processes: (i) movement of the P-loop (i.e., the glycine-rich loop), (ii) movement of the T-loop (namely, the activation loop or A-loop), or (iii) rotation of the α-C-helix in the N-lobe. It should be noted here that the DFG (Asp-Phe-Gly) motif is highly conserved and follows the T-loop, which serves as an important regulator of the various kinase processes: (i) movement of the P-loop (i.e., the glycine-rich loop), (ii) movement of the T-loop (namely, the activation loop or A-loop), or (iii) rotation of the α-C-helix in the N-lobe. It should be noted here that the DFG (Asp-Phe-Gly) motif is highly conserved and follows the T-loop, which serves as an important regulator of the various kinase activities. However, partial rearrangement of the T-loop can be induced by different kinase inhibitors for open and/or closed conformations. For example, pyrazolobenzothiazine can bind with the open conformation of the T-loop of FAK (PDB ID: 4I4F) (Figure S1). In addition, pyrrolo[2,3-d]imidazole can bind with the DFG-in (the side chain of D564 pointing into the active pocket of human FAK) motif of FAK (PDB ID: 3PXK), while pyrazolobenzothiazine can bind with the DFG-out (the side chain of D564 pointing out the active pocket of human FAK) motif (PDB ID: 4I4F) (Figure S2). Thus, the conformational rearrangement of the active pocket of the kinase domain plays an important role in the design of novel kinase inhibitors. However, the conformation that occurs in the binding of VS-4718 with FAK remains unclear. Therefore, for this study, four models of FAK were selected as the initial receptor structures for constructing VS-4718/FAK complexes, namely, FAK-I (DFG-in and T-loop open), FAK-II (DFG-in and T-loop closed), FAK-III (DFG-out and T-loop open), and FAK-IV (DFG-out and T-loop closed).

Till date, several small-molecule inhibitors of FAK have been reported, and some are currently under clinical development, including defactinib (VS-6063, PF-04554878), GSK2256098, VS-6062 (PF-00562271, PF-5662271), CEP-37440, BI-853520 (IN-10018), and VS-4718 (PND-1186) (Figure S3). Thus, the conformational rearrangement of the active pocket of the kinase domain plays an important role in the design of novel kinase inhibitors. However, the conformation that occurs in the binding of VS-4718 with FAK remains unclear. Therefore, for this study, four models of FAK were selected as the initial receptor structures for constructing VS-4718/FAK complexes, namely, FAK-I (DFG-in and T-loop open), FAK-II (DFG-in and T-loop closed), FAK-III (DFG-out and T-loop open), and FAK-IV (DFG-out and T-loop closed).

Till date, several small-molecule inhibitors of FAK have been reported, and some are currently under clinical development, including defactinib (VS-6063, PF-04554878), GSK2256098, VS-6062 (PF-00562271, PF-5662271), CEP-37440, BI-853520 (IN-10018), and VS-4718 (PND-1186) (Figure S3). Thus, the conformational rearrangement of the active pocket of the kinase domain plays an important role in the design of novel kinase inhibitors. However, the conformation that occurs in the binding of VS-4718 with FAK remains unclear. Therefore, for this study, four models of FAK were selected as the initial receptor structures for constructing VS-4718/FAK complexes, namely, FAK-I (DFG-in and T-loop open), FAK-II (DFG-in and T-loop closed), FAK-III (DFG-out and T-loop open), and FAK-IV (DFG-out and T-loop closed).

As the compound of interest in the current study, VS-4718 is a reversible and selective inhibitor that exhibits an IC_{50} (50% inhibitory concentration) value of 1.5 nM in an in vitro kinase assay. The VS-4718 has also demonstrated a cellular IC_{50} of ~100 nM in malignant pleural mesothelioma cell lines and breast carcinoma. Furthermore, it has a median relative cellular IC_{50} of 1.22 μM against the pediatric preclinical testing program cell line. These results indicate that VS-4718 possesses on-target and off-target concentrations of <100 nM and >1.0 μM, respectively, in these cell lines. In terms of its clinical development, VS-4718 has been demonstrated to act as a potential inhibitor for triple-negative breast cancer stem cells. It is currently being evaluated in advanced cancer (NCT02651727), metastatic nonhematologic malignancies (NCT01849744), and acute myeloid or B-cell acute lymphoblastic leukaemia (NCT02215629) (Table S1). Additionally, five off-targets for VS-4718 have been reported with >65% inhibition at 1 μM, thereby indicating that optimization of VS-4718 is necessary to obtain selective FAK inhibitors based on VS-4718. In this context, the binding mechanism of VS-4718 could be used to provide reference for the development of new antitumor drugs to target FAK.

It is known that receptor–ligand interactions play an important role in the elucidation of drug-target binding mechanisms; hence, a reliable receptor–ligand structure is essential to permit structure- or knowledge-based drug development. Because molecular docking can create a static image of the drug–target complex, it has been employed in the area of drug design. Furthermore, molecular dynamics (MD) simulations have also been employed to help elucidate the interactions present during drug binding. Till date, no crystal structural data are currently available for the binding of VS-4718 with FAK, which hinders the development of superior FAK inhibitors. We herein report a VS-4718/FAK binding model obtained through the use of molecular docking and all-atom MD simulations. More specifically, the binding free energies are also calculated using the molecular mechanics generalized Born surface area.
In addition, the hot residues present in the VS-4718/FAK binding models, which are likely to alter the binding affinity of VS-4718 with FAK, are identified by analyzing the energy decomposition for each residue. Ultimately, our aim is to carry out simulations that will provide the binding mechanism for VS-4718 with FAK, in addition to useful information that will facilitate the development of innovative FAK inhibitors.

**MATERIALS AND METHODS**

**Molecular Docking.** To date, more than 35 crystal structures have been published for human FAK (UniProt ID: Q05397) in the PDB, with 24 being known to form complex structures between the FAK kinase domain and either an inhibitor or ADP (Table S2). When bound to an inhibitor, the T-loop (i.e., the active, or A-loop) of FAK can form an open or closed conformation; for example, 4-((1R,2R)-2-(dimethylamino)cyclopentyl)amino)-5-(trifluoromethyl)pyrimidin-2-yl)amino)-N-methylbenzenesulfonamide binds with the open conformation of the T-loop (PDB ID: 6YY6), while N-methyl-N-3-(2-(2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)methyl)pyridin-2-yl)-methanesulfonamide binds with the closed conformation (PDB ID: 6YQ8) (Figure S4). In addition, the DFG domain, which is also an important loop for determining the kinase activity, can form the in or out conformation, such as in the case of DFG-in upon binding with N-(3-(((5-cyano-2-phenyl-1H-pyrrolo[2,3-b]pyridin-4-yl)methyl)pyridin-2-yl)-N-methylmethanesulfonamide (PDB ID: 4GU6) or DFG-out upon binding with 1-(4-(6-amino-9H-purin-9-yl)-phenyl)-3-(3-(tert-butyl)-1-(p-tolyl)-1H-pyrrozol-5-yl)urea (PDB ID: 4KY9) (Figure S5). However, little information is currently available regarding the conformation of the FAK active pocket with VS-4718. Therefore, we employed four representative conformations of the active groove to construct the VS-4718/FAK complex in our docking study. More specifically, these conformational models are as follows: (i) with an open T-loop and DFG-in, FAK-I (PDB ID: 6YY6); (ii) with a closed T-loop and DFG-in, FAK-II (PDB ID: 6IBZ); (iii) with an open T-loop and DFG-out, FAK-III (PDB ID: 4KY9); and (iv) with a closed T-loop and DFG-out, FAK-IV (PDB ID: 4EBV) (Figure S6).

The three-dimensional crystal structure of human FAK (UniProt ID: Q05397) coocrystallized with BI-4464 (PDB ID: 6IBZ) was obtained from the PDB, as were those of 6YY6, 4KY9, and 4EBV. These four crystal structures were selected as models for molecular docking. PMYOL 2.1 was used to prepare the coocrystallized structures of FAK by removing the coocrystallized ligand molecules. The crystallographic water molecules were removed to obtain the final protein structures and maintain chain A within the molecular docking.

Two-dimensional structures of VS-4718 and the other compounds were sketched using InDraw software and were converted into three-dimensional structures using OpenBabel 3.1. These structures were then minimized with the semiempirical PM3 method using MOPAC2016 (Stewart Computational Chemistry, Colorado Springs, CO; http://OpenMOPAC.net). The structures of the protein, VS-4718, and all other compounds were pretreated using AutoDockTools 1.5.6 with hydrogen atoms included, followed by Gasteiger charging and unreasonable atomic overlap adjustment. A 40 × 40 × 40 grid map with a 0.375 Å grid spacing was generated using AutoGrid v.4.2, this grid map was based on the center of the ATP-binding groove for FAK. Two hundred conformation per system were generated using the Lamarckian genetic algorithm in AutoDock v.4.2. Finally, the optimal conformation for each docking model was selected based on the docking experiment that gave the best rational orientation in the active pocket, as referenced to previously reported crystal complex structures for inhibitor/FAK complexes.

**MD Simulations.** The MD simulations have become increasingly important in the context of understanding the interactions between receptors (e.g., proteins, enzymes, or cyclodextrins) and ligands (e.g., inhibitors, stabilizers, and supermolecules). Therefore, MD simulations were carried out to explore the binding between VS-4718 and the FAK protein. The initial VS-4718/FAK complex structures obtained from the docking model were used as the initial complex conformations for subsequent simulations. Because the standard force field of a small molecule cannot be obtained, the general Amber force field generation procedure (version 2; GAFF2) was used to generate the force fields of the various ligand molecules examined in this work, including that of VS-4718. For this purpose, the ligand structure was drawn using InDraw and translated into three dimensions using OpenBabel v3.1 prior to structural optimization at the B3LYP/6-31G* level of theory using Gaussian09 software. Then, electrostatic potential was also calculated using the B3LYP/6-31G* method. Subsequently, the restrained electrostatic potential protocol was used to fit the partial atomic charges of the small molecule. Meanwhile, the standard Amber ff19SB force field was employed to create the topology parameters for the FAK protein. The standard residue protonation approach was employed based on the residue obtained in the ff19SB force field (pH = 7.0). Subsequently, the ligand/FAK complex systems were solvated with a cuboid box of TIP3P water at a relative distance of 15 Å from all protein atoms, and a single chloride ion was added to ensure that the entire system was in an electrically neutral state. The final system included the FAK protein, the ligand molecule, and the solvent water molecules.

To avoid the unfavorable interactions produced by additional solvents and ions, the system was minimized by initially restraining the atoms of the protein and ligand molecules to optimize the coordinates of the water molecules and counterions, wherein the weight for the positional restraints was set at 2.0 kcal/mol/Å². Subsequently, minimization of the overall system was carried out without any constraints. Langevin dynamics with a 2.0 ps−1 collision frequency was used to increase the overall system temperature from 0 to 300 K in an NVT ensemble, while the isotropic position scaling method was applied to maintain the system pressure at 1 bar. Subsequently, the NPT ensemble was applied to equalize the system at 300 K and 1 bar. Simulations were then performed for heating, the application of a constant pressure, and the process of equilibrium maintenance for 200 ps in each case. Finally, the two systems with different conformations of FAK were subjected to a 500 ns MD simulation under the above conditions to collect the necessary data required for analysis. In these simulations, periodic boundary conditions were used to avoid unphysical edge effects, while the SHAKE algorithm was employed to constrain the covalent bonds of the protein and ligand hydrogen atoms. To mitigate long-range electrostatic forces, the particle mesh Ewald algorithm was used.
applied during the simulation, wherein a cutoff distance of 12 Å was employed to simplify the short-range electrostatic forces. This cutoff was also used to simplify the van der Waals interactions. The seed for the pseudo-random number generator was based on the current date and time. System preparation and analysis of the simulation results were conducted using AmberTools21,24. However, it should be noted that the simulations were performed using the CUDA version of PMEMD in AMBER201,25 to decrease the required simulation time through the use of Nvidia GPUs. The coordinates of the simulation were saved every 10 ps to obtain the trajectories for the complex systems. The CPTRAJ19,20 model was employed to analyze the data obtained from the MD trajectories.

Binding Free Energy Calculations. Qualitative and quantitative analyses are both important when determining the binding free energies of inhibitor–protein binding interactions. Currently, several methods are available for estimating the absolute binding free energy between a protein and an inhibitor, such as the linear interaction energy,79–80 the linear response approximation,108 the solvated interaction energy,104 the free energy pathway,103 and the molecular mechanics Poisson Boltzmann (or generalized Born) surface area (MM-PB/GBSA)103,104 approach. However, the MM/GBSA approach is considered the most efficient means for evaluating ligand and enzyme systems,25 106 and its framework can be represented by the following equations

\[
\Delta G_{\text{binding}} = G_{\text{complex}} - G_{\text{protein}} - G_{\text{ligand}}
\]

\[
G = E_{\text{gas}} + E_{\text{sol}} - TS
\]

\[
E_{\text{gas}} = E_{\text{int}} + E_{\text{vdW}} + E_{\text{ele}}
\]

\[
E_{\text{solv}} = E_{\text{GB}} + E_{\text{surf}}
\]

where \(G_{\text{complex}}\), \(G_{\text{protein}}\), and \(G_{\text{ligand}}\) are the free energies of binding for VS-4718 + FAK, FAK, and VS-4718, respectively. The value of \(\Delta G_{\text{binding}}\) can be summarized based on the enthalpic term (\(\Delta H = \Delta E_{\text{gas}} + \Delta E_{\text{int}}\)) and the entropic term (\(\Delta S\)), while the molecular mechanical energy (\(E_{\text{gas}}\)) can be divided into van der Waals forces (\(E_{\text{vdW}}\)), electrostatic forces (\(E_{\text{ele}}\)), and the intramolecular energy (\(E_{\text{int}}\)), which can themselves be obtained from a statistical average based on the ff19SB force field. In addition, the solvation free energy (\(E_{\text{sol}}\)) can be decomposed into electrostatic (\(E_{\text{GB}}\)) and nonelectrostatic (\(E_{\text{surf}}\)) components. More specifically, the \(E_{\text{surf}}\) component is the combined effect of the unfavorable cost of surface formation and the favorable van der Waals interactions between the solute and the solvent. In this term, \(E_{\text{surf}}\) can be evaluated using \(\gamma \cdot \Sigma A + b\), where \(\gamma = 0.0072 \text{ kcal/Å}^2\) and \(b = 0.0 \text{ kcal/mol}\) and where the LCPO method106 is used to estimate the solvent accessible surface area (SA). Furthermore, the GB equation107,108 was applied to calculate the \(E_{\text{GB}}\) contribution. For the purpose of this work, the dielectric constants for the solute and the exterior were set to 1 and 80, respectively. One thousand snapshots were extracted from the final 200 ns of the MD trajectory to give a statistical average for the MM/GBSA method, and an entropic term was added to improve the accuracy for the binding of VS-4718 with FAK. Normal model analysis coupled with the quasi-harmonic model was applied to estimate the entropic contribution, which was based on 100 snapshots from the final 200 ns of the MD trajectory.109

To determine the essential residues involved in the binding interactions between VS-4718 and FAK, the contribution of each residue was evaluated at the atomic level using the energy decomposition method to obtain the binding free energy. The electrostatic contribution to the solvation energy was determined from the GB model, as in the case of the binding free energy calculation, while the single trajectory simulation was used to calculate the binding free energy; the internal energy calculation was excluded. Moreover, based on the corresponding SA, the nonpolar solvation energy per atom was obtained, and the entropic contribution of each residue was included for the energy decomposition calculations. The contribution of each given residue was estimated at the atomistic level by summing the contribution of the overall atoms present in the residue. Similarly, the contributions of the backbone and the side chain were determined by summing the relevant atoms in each case. One thousand snapshots extracted from the 200 ns simulation were also used to estimate the energy decomposition. Finally, all energies involved in the binding of VS-4718 with FAK were calculated using the AMBER MMPBA.py program.10 All methods employed during this study are outlined in Figure S40.

RESULTS AND DISCUSSION

Initial Models. Prior to carrying out the docking experiment, a redocking strategy was employed to evaluate the docking power of the docking procedure. The docking power is defined as the root-mean-square deviation (rmsd) between the conformation from molecular docking and the crystal conformation of the ligand molecule. More specifically, the selected crystal complex structures for 6YYV (PDB ID, FAK-I), 6I8Z (FAK-II), 4K9Y (FAK-III), and 4EBV (FAK-VI) were redocked, and the rmsd between the crystal structure conformation of the ligand and the conformation with the lowest energy (i.e., −8.73, −11.90, −13.25, and −10.03 kcal/mol for FAK-I, FAK-II, FAK-III, and FAK-IV, respectively) for docking was found to be <1.0 Å in each case (Figure S7). It should be noted here that the ATP binding site of FAK was also considered in the FAK-VI model because the original ligand was bound to the allosteric site (Figure S8). Thus, as an example of one redocking experiment, the lowest binding free energy conformation of redocking for the FAK-II model was determined to be −13.25 kcal/mol, and the conformation number of this cluster was 1960, which gives an occupancy of 98% based on the 2000 docking conformations (Figure S9). These results indicate that the docking strategy is suitable for docking the ligand molecule at the ATP binding site. In addition, it was found that VS-4718 was bound to the ATP binding site of FAK, and therefore the same docking strategy was used to construct the VS-4718/FAK-I, VS-4718/FAK-II, VS-4718/FAK-III, and VS-4718/FAK-IV complexes.

The initial conformations for VS-4718 binding with FAK were obtained from docking experiments, wherein the lowest binding free energies were −8.74, −10.11, and −8.61 kcal/mol for the FAK-I, FAK-II, and FAK-III systems, respectively (Figure S10). Although the binding models based on FAK-I and FAK-II (i.e., with DFG-in) were similar (Figure S11), the binding models with DFG-out (i.e., FAK-III and FAK-IV) were not considered in the context of the defined standards for competitive ATP inhibitors.65,66,111,112 More specifically, there was a large spatial similarity between the ligands and a competitive ATP inhibitor, and hydrogen bonds were not present between the ligands and the hinge loop of FAK. These
results suggest that the DFG-in conformation is the most plausible in the binding of VS-4718 with FAK. Furthermore, the greater binding affinity between VS-4718 and FAK-II (cf., FAK-I, based on the docking score) indicates that the closed conformation of the T-loop plays an important role in enhancing the binding of VS-4718 with FAK. Thus, the model of VS-4718 binding with FAK-II was selected for further experiments.

To obtain a more accurate conformation of VS-48718 binding with FAK-II, the subsequent docking experiment incorporated 2000 different conformations that could be divided into >5 clusters (Figure S12), wherein the lowest free energy conformation within each cluster was selected as its representative conformation. The binding models of cluster 1 (defined as FAK-II-1) and 4 (defined as FAK-II-4) were considered to meet the above-described standards for competitive ATP inhibitors. Thus, two binding conformations (i.e., the conformation with the lowest binding score in cluster 1 or cluster 4) were selected for further examining the binding of VS-4718 with human FAK (Figure 2), wherein the two nitrogen atoms (N3 and N5) of the pyrimidine—amine moiety form two hydrogen bonds with the nitrogen and oxygen atoms in the C502 residue (Figure S13). In addition, an unfavorable acceptor–acceptor interaction was found between the C502 residue and the methoxy oxygen atom of VS-4718. Furthermore, in the case of FAK-II-1, the O1 atom of the ligand forms a hydrogen bond with the D564 residue in the DFG domain of FAK. Together, these data suggest that the pyrimidine–amine moiety of the inhibitor plays an important role in the binding characteristics. Moreover, the pyrimidine side chains, such as the 2-methoxy-4-morpholinophenyl group, are responsible for orienting the conformation of the inhibitor when bound with FAK. Similarly, the trifluoromethyl group occupies the hydrophobic cavity, while the N-methylbenzamide group points toward the T-loop region.

System Stability. Because the docking experiments did not consider any additional interactions between VS-4718 and FAK or rearrangement of the residues present in the active site of FAK, MD simulations were carried out for the two VS-4718/FAK complex systems to obtain additional information relating to the binding mechanism. More specifically, MD simulations were performed over 500 ns for the various protein–ligand complexes, at which point the rmsd values of the heavy atoms in the protein backbone and in the ligands had reached a plateau (Figure S14). Only small fluctuations of the rmsd values were observed for the protein kinase domain, with values of 1.77 ± 0.25 Å and 1.77 ± 0.19 Å being determined for FAK-II-1 and FAK-II-4, respectively, thereby indicating that the FAK structures were stable. In contrast, the fluctuations for the ligands were 1.51 ± 0.26 and 2.00 ± 0.24 Å for FAK-II-1 and FAK-II-4, respectively, which indicates that VS-4718 was reoriented in the active pocket for the FAK-II-4 system. This result agrees with the radius of gyration (Figure S15) and the surface area (Figure S16) determined for the VS-4718/FAK complex system, and therefore our observations indicate that these MD simulations are suitable for analyzing the interactions between VS-4718 and FAK.

The root-mean-square fluctuation (RMSF), which is based on the fluctuation of residues, was then used to quantify the stabilities of specific residues during the MD simulations. For this purpose, the RMSF values were analyzed based on the 500 ns MD trajectory for each VS-4718/FAK complex system (Figure S17), and it was found that for both FAK-II-1 and FAK-II-4, the amino acid residues at FAK positions 564–592 (i.e., the T-loop) exhibited a greater degree of fluctuation than those present in other regions (Figure 3A). This observation, which is consistent with the rmsd results, indicates that the T-loop of FAK is unstable during these simulations. In addition, the A-loop conformations (564–592 for human FAK) were in good agreement with those of other kinases, such as salt-inducible kinase 2, microtubule affinity-regulating kinase 2, and microtubule affinity-regulating kinase 4. In our simulations, the initial conformation of FAK-II was closed, which promoted a greater degree of VS-4718 binding. However, as previously mentioned, the T-loops can form open or closed conformations, which are able to recognize various inhibitors; other protein kinases also have a similar recognition mechanism.

Based on the obtained docking scores, the closed conformation of the FAK T-loop was identified as the optimal conformation for VS-4718 binding with FAK, as indicated by the snapshots obtained at 100, 200, 300, 400, and 500 ns from the MD trajectory. These snapshots were aligned and are shown for the FAK-II-1 and FAK-II-4 system in Figures S18 and S19, respectively. The initial and final structures of the two complexes were also extracted to check the possible structural changes during the MD simulation (Figure 3B,C), and it was found that the structure of the FAK A-loop (i.e., the T-loop) adopted an induced-fit conformation in the presence of an inhibitor. However, in the DFG domain, the T-loop maintained a closed conformation, thereby indicating that overall, VS-4718 is bound to the ATP binding site of FAK via a closed conformation of the T-loop.

Analysis of the Hydrogen-Bonding Network. The initial docking models identified a hydrogen bonding network between VS-4718 and FAK. However, only a few hydrogen bonds were formed between VS-4718 and the FAK protein (Figure 4). Some potential residues for hydrogen bonds were identified in the active site, but less than two hydrogen bonds were found in the majority of simulation times, with an average number of 2.43 and 1.88 hydrogen bonds being determined for FAK-II-1 and FAK-II-4, respectively (Figure S20). The occupancy of the hydrogen bonds was also assessed in the
500 ns simulation for the VS-4718/FAK complexes, wherein the N3 atom of VS-4718 acted as a donor atom to form a hydrogen bond with the C502 residue of FAK (82.98 and 61.75% occupancies for FAK-II-1 and FAK-II-4, respectively). The oxygen atom of the C502 residue also took part in hydrogen bonding with the N5 atom of VS-4718, acting as an acceptor, with occupancies of 96.53 and 66.64% being determined for the FAK-II-1 and FAK-II-4 systems, respectively. Furthermore, it was determined that two of the hydrogen bonds formed between FAK and VS-4718 were located within the diamino-pyridine ring; these interactions represent the universal hydrogen bonds that are commonly found for FAK inhibitors and other protein kinase inhibitors. Moreover, the 7H-pyrrolo[2,3-d]pyrimidine, diamino-pyrimidine, and thieno[3,2-d]pyrimidine rings also contribute to the binding model in a manner similar to the indazole ring, thereby indicating that the indazole ring can be replaced with a pyrazole or pyridine ring.

In addition, the D564 residue present in the DFG region formed hydrogen bonds with VS-4718, although these were weaker than those formed at the C502 residue. Interestingly, in the FAK-II-1 system, the hydrogen bond between this residue and the O1 atom of VS-4718 (46.11% occupancy) was not

Figure 3. Fluctuation in the FAK conformation during binding with VS-4718. (A) RMSF for the FAK backbone residues during the 500 ns simulation. Frames of the VS-4718/FAK complexes at 0 nm (green) and 500 ns (cyan) for the (B) FAK-II-1 and (C) FAK-II-4 systems. The FAK protein is represented in its cartoon form, while the VS-4718 ligand is shown in the stick format. The T-loop of FAK is highlighted.

Figure 4. Hydrogen bond analysis for the VS-4718/FAK systems. (A) Distribution of the number of hydrogen bonds for total 50 000 frames during the 500 ns simulation. (B) Occupancy of each hydrogen bond as a percentage of the investigated period (500 ns), during which specific hydrogen bonds were formed. A hydrogen bond was defined when the distance between the acceptor and donor atoms was <3.5 Å and the internal acceptor···H-donor angle was >120°. Also shown are schematic diagrams of hydrogen bonding in the (C) FAK-II-1 and (D) FAK-II-4 systems.
observed in the FAK-II-4 system. However, greater hydrogen bonding occupancy was found between the D564 residue and the trifluoromethyl group of FAK-II-4 compared to that of the FAK-II-1 system, which was attributed to translation of the N-methylbenzamide group conformation to orientate the coordination of a Mg$^{2+}$ ion to stabilize the Mg—ATP interactions at the catalytic subunit of the cyclic adenosine monophosphate-dependent protein kinase.\cite{126} As a result, repositioning of the D564 side chain would prevent ATP binding at the binding site.

Subsequently, MD simulation trajectories were employed to calculate the distances between the donor and acceptor atoms, in addition to the angles between the donor, hydrogen, and acceptor atoms. More specifically, the hydrogen bond between the C502 residue of FAK and the N3 atom of VS-4718 in the FAK-II-1 system (distance $= 3.15 \pm 0.24$ Å) was stronger than the FAK-II-4 system (distance $= 3.42 \pm 0.40$ Å) (Figure S21), and a similar trend was also observed for the hydrogen bond between the C502 residue and the N5 atom (distances $= 3.15 \pm 0.17$ and $3.44 \pm 0.33$ Å for the FAK-II-1 and FAK-II-4 systems, respectively) (Figure S22). In addition, similar hydrogen bonds were found between the N3 atom and the C502 residue, indicating that these two hydrogen bonds play the same key role in the binding of VS-4718 with FAK, as confirmed experimentally for the interactions between FAK and BI-4464 (distances $= 3.05$ and $3.06$ Å, PDB ID: 6I8Z\cite{125}) (Figure S23). Furthermore, hydrogen bonding between the D564 residue and both the trifluoromethyl group and the O1 atom of VS-4718 were examined (Figures S24 and S25). It was found that replacing the O1 atom in the FAK-II-1 system with a trifluoromethyl group in the FAK-II-4 system reduced the binding affinity, as confirmed by measurement of the corresponding angles (Figures S26 and S27). Overall, these results suggest that the FAK-II-1 system is a superior model for investigating the binding of VS-4718 with FAK, wherein hydrogen bonding plays an important role in orienting VS-4718 into the ATP binding site. Our results also suggest that the methyl group of the N-methylbenzamide moiety can also be substituted for ethyl, cyclopropyl, and propyl chains to retain a similar binding affinity.

**Analyses of the Interaction Fingerprints.** To quantitatively characterize the interactions between VS-4718 and the human FAK ATP binding pocket, the interaction fingerprints of the FAK-II-1 and FAK-II-4 complexes were calculated using IChem\cite{127,130} during the final 200 ns of the MD simulations (Figure 5). It was found that the I428, V436, A452, V484, M499, L501, V509, S509, N551, L553, L567, and S568 residues of FAK form hydrophobic interactions with the inhibitor in both systems, wherein the A452, V484, M499, and L567 residues provide the greatest stabilization with a proportion of $\sim 1.00$ (defined as the proportion of frames exhibiting interactions divided by the total (10 000) frames obtained in the final 200 ns of the simulation). In addition, the L553 residue can form hydrophobic interactions with the pyridine moiety of VS-4718, while the I428 residue forms hydrophobic interactions with the benzene ring of the N-methylbenzamide group. It was also found that the S568 and N551 residues contributed toward hydrophobic interactions in the FAK-II-4 system but not in the FAK-II-1 system, and therefore the hydrogen bond attributed to the D564 residue in the FAK-II-1 system (see Figure 4B) can be considered to compensate for these missing interactions. As mentioned above, fingerprint analysis confirmed that the D564 residue forms a hydrogen bond with VS-4718 in the FAK-II-1 system (proportion $= 0.64$) but not in the FAK-II-4 system (proportion $= 0.02$). However, the hydrophobic segment of D564 contributes similarly to binding in both systems (proportions $= 0.97$ and 1.00 for the FAK-II-1 and FAK-II-4 systems, respectively). Moreover, C502 was found to form hydrogen bonds in both systems through interaction with the dianisopyridine group of the inhibitor (proportion $\sim 1.00$), and this result is consistent with the hydrogen bond analyses section in this work. The above results therefore indicate that hydrogen bonding between the inhibitor and the C502 and D564 residues is key to stabilizing the orientation of the VS-4718 molecule within the binding pocket.

**Binding Free Energies.** As described above, the interactions between VS-4718 and the FAK can be depicted by docking experiments and MD simulations. However, these procedures did not allow the determination of binding affinities of the inhibitor to the two model systems. Thus, the MM/GBSA method was employed to calculate the
absolute VS-4718/FAK binding free energies for both systems (Tables 1, S3, and S4). It was found that all entropy values \(\Delta S_{\text{total}}\) and enthalpies \(E_{\text{gas}} + G_{\text{gas}}\) were negative (i.e., less than \(-23.10\) and \(-55.80\) kcal/mol, respectively), which indicates that the formation of a binding complex is an enthalpy-driven process. For FAK-II-1, the calculated binding free energy \(\Delta G_{\text{bind}}\) was approximately \(-33.13\) kcal/mol, whereas for FAK-II-4, the \(\Delta G_{\text{bind}}\) value was \(-32.70\) kcal/mol, thereby confirming that both the FAK-II-1 and FAK-II-4 systems constituted the preferred models. This conformation of FAK-II-1 was also previously observed in the crystal structure of a methanesulfonylamine diaminopyrimidine inhibitor bound with FAK (PDB ID: 3BZ3) (Figure S28).

Overall, the perfect binding model for VS-4718/FAK involves the side chain (N-methylbenzamide group) of the ligand pointing toward the DFG region, which permits stable binding at the ATP binding site of FAK. The obtained binding free energy therefore indicates that VS-4718 can bind strongly with FAK, as confirmed by the experimentally obtained IC\(_{50}\) value of 1.5 nM.\(^{20}\)

The binding energy can usually be decomposed into its polar \(E_{\text{ele}} + E_{\text{GB}}\) and nonpolar \(E_{\text{vdW}} + E_{\text{solv}}\) terms. More specifically, for the FAK-II-1 and FAK-II-4 systems examined herein, the polar terms were determined to be 10.90 and 12.42 kcal/mol, respectively, using the MM/GBSA method. It should be noted here that a positive value for the polar contribution indicates that the polar interactions between VS-4718 and FAK are antagonistic to this binding. In contrast, van der Waals \(E_{\text{vdW}}\) interactions \((-61.39\) and \(-60.89\) kcal/mol for FAK-II-1 and FAK-II-4, respectively) acted as the main nonpolar contribution and were conducive to binding. Furthermore, the nonpolar terms were determined to be \(-68.91\) and \(-68.22\) kcal/mol for FAK-II-1 and FAK-II-4, respectively. These similar values for the two systems support our previous observation that they exhibited a similar binding affinity toward the inhibitor. Overall, these results indicate that hydrophobic (i.e., nonpolar) interactions are predominantly responsible for the binding of VS-4718 with human FAK.

**Free Energy Decomposition.** The calculated binding free energies presented in Table 1 show that the nonpolar (i.e., hydrophobic) term plays the most important role in complex formation. Because the per-residue free energy decomposition strategy is known to enable analysis of the inhibitor--protein interactions,\(^{55,131-134}\) the interaction energies between the various residues of FAK and VS-4718 were computed using the MM/GBSA decomposition protocol (Tables S5 and S6). As indicated by the obtained results, several hydrophobic residues possessed substantial subtotal binding free energies. In the FAK-II-1 system, the L501 and C502 residues present in the hinge loop made a favorable contribution to binding (i.e., \(-1.85\) kcal/mol, Figure 6) because the C502 residue can form two hydrogen bonds with VS-4718. It should be noted that the residues of the hinge loop that form hydrogen bonds with the ATP-competitive inhibitor are known to be conserved for FAK\(^{28,31,52}\) and for other protein kinases.\(^{135-146}\) In addition, the hydrophobic interaction between the side chain of L501 and the methoxy group of VS-4718 was the main contributor for this residue, giving a value of \(-1.75\) kcal/mol. However, the methoxy group of the inhibitor generated an electrostatic repulsion with C502 due to the proximity of this group to the carbonyl oxygen atom of C502 (Figure S29). Variation in the position of this methoxy group has therefore been used to increase the inhibitor selectivity toward different protein kinases, such as in the case of dasatinib.\(^{141}\) Therefore, the presence of a substituent at this position is of particular importance. Ideally, the methoxy group could be substituted by a halogen atom or a small alky chain to increase the binding affinity and prevent electrostatic repulsion with C502.

In addition, the L553 residue also provided a contribution of more than \(-2.90\) kcal/mol due to a stable hydrophobic interaction with the pyridine ring of VS-4718 (Figure S30). Based on previous studies, it would be expected that substitution of the pyridine moiety with pyrimidine would reduce the distance between the ligand and the residue to increase the binding affinity.\(^{58}\) (Figure S31). Indeed, the pyrimidine ring has been incorporated into other FAK inhibitors, such as VS-6063 and VS-6062,\(^{65}\) suggesting that the hydrophobic interaction between the VS-4718 inhibitor and the L553 residue of FAK could be vital for ligand binding. Furthermore, the I428 residue on the P-loop is of particular importance due to its ability to form hydrophobic interactions with both the pyridine ring and the methoxybenzene ring of VS-4718. In such cases, the side chain of I428 is responsible for the interaction with the methoxybenzene ring, as observed from the distance and angle of the interacting structure (Figure S32).

### Table 1. Binding Free Energy for the VS-4718/FAK Complex and Decomposition into Electrostatic Interactions, van der Waals Interactions, Solvation Free Energies, and Entropy Values

| Energy (kcal/mol)          | FAK-II-1   | FAK-II-4   |
|----------------------------|------------|------------|
| \(\Delta E_{\text{vdW}}\)  | -61.39 (2.79)* | -60.89 (2.58) |
| \(\Delta E_{\text{ele}}\)  | -14.44 (3.21) | -11.86 (3.42) |
| \(\Delta E_{\text{GB}}\)   | 25.34 (2.57)  | 24.28 (2.83)   |
| \(\Delta E_{\text{solv}}\)  | -7.52 (0.27)  | -7.33 (0.27)   |
| \(\Delta E_{\text{gas}}\)  | -75.83 (4.05) | -72.75 (4.44) |
| \(\Delta G_{\text{bind}}\) | 17.81 (2.57)  | 16.95 (2.81)   |
| \(\Delta G_{\text{gas}}\)  | -58.02 (3.19) | -55.80 (3.04) |
| \(\Delta S_{\text{total}}\) | -24.89 (5.41) | -23.10 (5.48) |
| \(\Delta G_{\text{sol}}\)  | -33.13 (6.28) | -32.70 (6.27) |

\(*\Delta E_{\text{vdW}}\): contribution of the van der Waals energy to the free energy of binding; \(\Delta E_{\text{ele}}\): contribution of the electrostatic energy to the free energy of binding; \(\Delta E_{\text{GB}}\): contribution of the polar solvation energies to the free energy of binding; \(\Delta E_{\text{solv}}\): contribution of the nonpolar solvation energies to the free energy of binding; \(\Delta E_{\text{gas}}\): contribution of \(\Delta E_{\text{vdW}} + \Delta E_{\text{ele}}\) to the free energy of binding; \(\Delta E_{\text{gb}}\): contribution of \(\Delta E_{\text{GB}} + \Delta E_{\text{solv}}\) to the free energy of binding; \(\Delta G_{\text{bind}}\): the final estimated binding free energy from \(\Delta E_{\text{gas}} + \Delta E_{\text{vdW}} - \Delta S_{\text{total}}\). *The uncertainties (shown in parentheses) were calculated as the root-mean-square error for each frame extracted during the MM/GBSA process.
However, due to the nonaromatic nature of the resulting seven-membered ring, the affinity of BJG-03-025 was reduced to 20 nM, thereby confirming that the presence of an aromatic ring is essential at this site. Moreover, the tricyclic benzopyrimidodiazepinone core has been shown to act as a privileged scaffold for the generation of potent and selective kinase inhibitors. Therefore, it can be inferred that other tricyclic cores may be used to increase the selectivity of FAK inhibitors. Additionally, the methoxybenzene ring also formed hydrophobic interactions with the G505 residue (−1.60 kcal/mol), and it has been shown that the G505 and I428 residues can form a “clip” to bind the methoxybenzene ring (Figure S34), thereby suggesting that this moiety cannot be substituted by other nonaromatic rings.

It should also be noted here that the DFG motif serves as an important regulator of kinase activities. In addition, it is highly conserved and follows the T-loop and therefore can be used in the design of novel inhibitors. In this motif, the D564 residue forms a hydrogen bond with the O1 atom of VS-4718, which contributed an energy of −1.95 kcal/mol. Furthermore, the L567 residue on the T-loop was found to contribute an energy of −2.39 kcal/mol to the binding of VS-4718 with FAK, and this interaction can be attributed to hydrophobic binding with the benzene ring of the N-methylbenzamide moiety (Figure S35). Importantly, if the conformation of the T-loop is open, L567 will point toward the solvent environment and destroy this key hydrophobic contribution, and therefore the aromatic N-methylbenzamide ring must be retained in the structure. Furthermore, we found that the DFG-motif forms an α-helical structure in VS-4718/FAK (Figure S36), which enables multiple interactions, such as those between the D564/L567 residues and VS-4718. Indeed, this α-helical conformation has been demonstrated to provide selectivity between FAK and PYK2 (61% sequence identity with FAK in the kinase domain). More specifically, the benzene ring of VS-4718

Figure 6. Key residues involved in VS-4718 binding with human FAK. The energy was decomposed into its backbone and side-chain components for each residue in the FAK-II-1 (A) and FAK-II-4 (B) systems. The energy also decomposed into its nonpolar solvation, polar solvation, electrostatic, and van der Waals components for each residue in the FAK-II-1 (C) and FAK-II-4 (D) systems. The binding energy was decomposed using the MM/GBSA method.
provides a favorable hydrophobic interaction with L567 to
decrease the off-rate, which can be used to increase the
selectivity between FAK and PYK2. These results therefore
suggest that a DFG-in conformation with a closed T-loop is
necessary in the FAK structure to obtain selective FAK
inhibitors.

With the exception of the D564 residue, we found that the
key residues involved in VS-4718 binding with human FAK
in the FAK-II-4 system were similar to those in the FAK-II-1
system. More specifically, D564 is unable to form a hydrogen
bond with the O1 atom of VS-4718 in the FAK-II-4 system,
which ultimately changes the backbone contribution from
−1.17 kcal/mol in FAK-II-1 to −0.31 kcal/mol in FAK-II-4.
Overall, the I428, A452, L501, C502, G505, L553, D564,
and L567 residues appear to be the key residues for VS-4718
binding with human FAK.

**Design Strategies.** Based on the central aminopyrimidine
hinge, VS-4718 was modified at four different regions. More
specifically, the interactions with the solvent-exposed pocket
and the interactions with the nonconserved upper lobe
residues were probed (R1), and the FAK back-pocket
interface containing an induced helix was modified (R2). In
addition, the DFG helix induced in FAK after ligand binding
was investigated (R3), while the influence of the central
aminopyrimidine group was evaluated (R4) (Figure 7). Thus,
based on the interaction model between VS-4718 and human
FAK, 47 novel compounds were designed to target the ATP-
binding pocket of human FAK (Figure S37), and ensemble
docking was performed to assess the effects of these
modifications on the inhibitor binding affinity.

As shown in Figures S38 and S39, the representative
conformations of the various clusters of the FAK-II-1 and
FAK-II-4 systems were analyzed using the rmsd values
obtained for the Cα of the protein structure, and similar
conformations were obtained for two complex systems in each
case. In addition, based on the cluster analysis, the first cluster
of the MD simulation was determined to be the representative
conformation for this MD simulation because its occupancy
was >38.2% for FAK-II-1 and >70.7% for FAK-II-4. The
centroid frames in each cluster were therefore selected as
representative conformations for ensemble docking.

Overall, 10 conformations of the complex systems were
employed in the ensemble docking calculations, and the
docking scores for the novel compounds were selected from
the lowest binding free energy of each docking (Table S7).
As indicated, modification of the R1 region had little influence on
the binding affinity, although it was necessary to retain this
region to prevent loss of the inhibitor activity. In addition,
certain modifications of the R2 region increased the binding
affinity (e.g., in the cases of R2a, R2d, and R2g–R2k), while in
the R3 region, an improved binding affinity was obtained when
the N-methylbenzamide group was present (R3a–R3j). In
contrast, aromatic heterocycle group replaced with the benzene
ring of benzamide group (R3k–R3p) decreased the binding
affinity. Finally, modification of the R4 segment appears key for
optimizing the main hydrogen bonding network, and it was
found that the R4d and R4e structures constituted novel
frameworks to improve binding with the C502 residue.

## CONCLUSIONS

The inhibition of FAK has shown potential as a therapeutic
treatment of various carcinomas, such as breast, ovarian, and
neck cancers. Previously, VS-4718 was reported as a selective,
reversible inhibitor of FAK, with an IC50 value of 1.5 nM. Such
inhibition can be attributed to a conformational rearrangement of
the kinase domain of human FAK upon inhibitor binding.
Therefore, we carried out a molecular modeling study of the
binding mechanism between VS-4718 and FAK to probe the
key interactions responsible for this action. More specifically,
molecular docking studies, MD simulations, binding free
energy calculations, and energy decomposition studies
provided critical information regarding the molecular inter-
actions and binding affinities within the VS-4718/FAK
complexes, and a reasonable interaction model between the
inhibitor and the protein was established. Overall, the obtained
results indicated that VS-4718 can be modified to enhance its
binding affinity with FAK based on the following strategies: (i)
maintenance of the N-methylbenzamide moiety for binding
with the Asp-Phe-Gly (DFG) motif of FAK, (ii) enhancement of
the interactions with the back pocket of FAK, and (iii)
obtimation of the hydrogen-bonding interactions from the
diaminopyrimidine group. Overall, the present study not only
facilitates a better understanding of the binding mechanism of
human FAK with VS-4718 but also provides additional insights
into potential future design strategies for this inhibitor. The
synthesis and biological evaluation of novel inhibitors targeting
FAK will be considered in the near future, and the results will
be reported in due course.

## ASSOCIATED CONTENT

* Supporting Information

The Supporting Information is available free of charge at
https://pubs.acs.org/doi/10.1021/acsomega.2c03951.

Figure 7. Representation of the key interactions between VS-4718 and FAK, and the four modification regions. (A) Interactions between VS-4718 and the key residues of human FAK determined herein. (B) Modification of VS-4718 at four different regions (R1, R2, R3, and R4).
Conformations of FAK, inhibitor structures, binding models, docking results, rmsd plots, gyration radius plots, RMSF plots, snapshots, distance plots, angle plots, cluster results, hydrogen bond analysis results, clinical trials of VS-4718, crystal structures of human FAK, binding free energy, free energy decompositions, and docking score of designing inhibitors (PDF).

Author Information

Corresponding Authors
Zhuang Yang — State Key Laboratory of Biotherapy, West China Hospital of Sichuan University, Chengdu 610041, China; orcid.org/0000-0002-8915-9944; Phone: +86-28-85164063; Email: young9008@126.com
Liju Chen — State Key Laboratory of Biotherapy, West China Hospital of Sichuan University, Chengdu 610041, China; Phone: +86-28-85164063; Email: chenlj125@163.com

Authors
Mingsong Shi — State Key Laboratory of Biotherapy, West China Hospital of Sichuan University, Chengdu 610041, China
Tao Chen — State Key Laboratory of Biotherapy, West China Hospital of Sichuan University, Chengdu 610041, China
Siping Wei — Key Laboratory for Chemistry and Molecular Engineering of Medicinal Resources, Guangxi Normal University, Guilin 541004, China; Department of Medicinal Chemistry, School of Pharmacy, Southwest Medical University, Luzhou 646000, China
Chenyu Zhao — West China School of Basic Medical Sciences and Forensic Medicine, Sichuan University, Chengdu 610041, China
Xinyu Zhang — West China School of Pharmacy, Sichuan University, Chengdu 610041, China
Xinghui Li — West China School of Pharmacy, Sichuan University, Chengdu 610041, China
Xinyi Tang — West China School of Pharmacy, Sichuan University, Chengdu 610041, China
Yan Liu — State Key Laboratory of Biotherapy, West China Hospital of Sichuan University, Chengdu 610041, China

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c03951

Author Contributions
Conceptualization, M.S. and T.C.; methodology, M.S.; software, M.S.; validation, M.S., S.W., C.Z., and X.Z.; formal analysis, M.S. and X.L.; investigation, M.S. and X.T.; resources, M.S. and Y.L.; data curation, M.S.; writing—original draft preparation, M.S.; writing—review and editing, M.S., Z.Y., and L.C.; visualization, M.S.; supervision, M.S. and Z.Y.; project administration, M.S. and L.C.; funding acquisition, M.S., S.W., and Z.Y. All authors have read and agreed to the published version of the manuscript.

Funding
This research was sponsored by the National Natural Science Foundation of China (82073569), the 1.3.5 project for disciplines of excellence, West China Hospital, Sichuan University (ZYGD20001), Post-Doctor Research Project, West China Hospital, Sichuan University (2021HXBH017), and Open Project of State Key Laboratory of Chemistry and Molecular Engineering of Medicinal Resources (CMEMR2021-B10).

Notes
The authors declare no competing financial interest.

Acknowledgments
Certain data were obtained from the National Supercomputing Center of Guangzhou and the Chengdu Supercomputing Center. The authors thank Editage (www.editage.cn) for English language editing.

References
(1) Ignjatović, V. B.; Miljuš, J. R. J.; Rončević, J. V.; Tatić, S. B.; Denić, T. M. I.; Dorić, I. D.; Selemetjev, S. A. Focal adhesion kinase splicing and protein activation in papillary thyroid carcinoma progression. Histochim. Cell Biol. 2022, 157, 183–194.
(2) Pang, X.-J.; Liu, X.-J.; Liu, Y.; Liu, W.-B.; Li, Y.-R.; Yu, G.-X.; Tian, X.-Y.; Zhang, Y.-B.; Song, J.; Jin, C.-Y.; et al. Drug discovery targeting focal adhesion kinase (FAK) as a promising cancer therapy. Molecules 2021, 26, 4250.
(3) Dawson, J. C.; Serrels, A.; Stupack, D. G.; Schlaepfer, D. D.; Frame, M. C. Targeting FAK in anticancer combination therapies. Nat. Rev. Cancer 2021, 21, 313–324.
(4) Chauhan, A.; Khan, T. Focal adhesion kinase—an emerging viable target in cancer and development of focal adhesion kinase inhibitors. Chem. Biol. Drug Des. 2021, 97, 774–794.
(5) Zhang, Y. X.; Sun, X. C. Role of focal adhesion kinase in head and neck squamous cell carcinoma and its therapeutic prospect. Oncotargets Ther. 2020, 13, 10207–10220.
(6) Del Mistro, G.; Riemann, S.; Schindler, S.; Beissert, S.; Kontermann, R. E.; Ginolhac, A.; Halder, R.; Presta, L.; Sinkkonen, L.; Sauter, T.; et al. Focal adhesion kinase plays a dual role in trail resistance and metastatic outgrowth of malignant melanoma. Cell Death Dis. 2022, 13, 54.
(7) Mousson, A.; Legrand, M.; Steffan, T.; Vauchelles, R.; Carli, P.; Gies, J. P.; Lehmann, M.; Zubér, G.; De Mey, J.; Dujardin, D.; et al. Inhibiting FAK-paxillin interaction reduces migration and invadopodia-mediated matrix degradation in metastatic melanoma cells. Cancers 2021, 13, 1871.
(8) Quispe, P. A.; Lavecchia, M. J.; León, I. E. Focal adhesion kinase inhibitors in the treatment of solid tumors: Preclinical and clinical evidence. Drug Discovery Today 2022, 27, 664–674.
(9) Song, X. H.; Xu, H. W.; Wang, P.; Wang, J. X.; Aifo, S.; Wang, H. C.; Xu, M.; Liang, B. Y.; Che, L.; Qiu, W.; et al. Focal adhesion kinase (FAK) promotes cholangiocarcinoma development and progression via yap activation. J. Hepatol. 2021, 75, 888–899.
(10) Li, H.; Gao, Y.; Ren, C. Focal adhesion kinase inhibitor BI 853520 inhibits cell proliferation, migration and emt process through P38/AKT/MTOR signaling pathway in ovarian cancer. Discover Oncol. 2021, 12, 29.
(11) Zhang, Y. M.; Liu, S.; Zhou, S.; Yu, D. D.; Gu, J. J.; Qin, Q.; Cheng, Y.; Sun, X. C. Focal adhesion kinase: Insight into its roles and therapeutic potential in oesophageal cancer. Cancer Lett. 2021, 496, 93–103.
(12) Rigiacciolo, D. C.; Cirillo, F.; Tala, M.; Muglia, L.; Gutkind, J. S.; Maggiolini, M.; Lappano, R. Focal adhesion kinase fine tunes multifaceted signals toward breast cancer progression. Cancers 2021, 13, 645.
(13) Osipov, A.; Blair, A. B.; Liberto, J.; Wang, J. X.; Li, K. Y.; Herbst, B.; Xu, S. Q.; Li, N.; Niu, R.; Rashid, D.; et al. Inhibition of focal adhesion kinase enhances antitumor response of radiation therapy in pancreatic cancer through CD8+ T cells. Cancer Biol. Med. 2021, 18, 206.
(14) Le Large, T. Y. S.; Bijlsma, M. F.; El Hassouni, B.; Mantini, G.; Lagerweij, T.; Henneman, A. A.; Funel, N.; Kok, B.; Pham, T. V.; de Haas, R.; et al. Focal adhesion kinase inhibition synergizes with NAB-
paclitaxel to target pancreatic ductal adenocarcinoma. J. Exp. Clin. Cancer Res. 2021, 40, 91.

(15) Nowakowski, J.; Cronin, C. N.; McRee, D. E.; Knuth, M. W.; Nelson, C. G.; Pavletic, N. P.; Rogers, J.; Sang, B. C.; Scheibe, D. N.; Swanson, R. V.; et al. Structures of the cancer-related Aurora-A, FAK, and EphA2 protein kinases from nanovolume crystallography. Structure 2002, 10, 1659–1667.

(16) Thomas, T.; Roux, B. Tyrosine kinases: Complex molecular systems challenging computational methodologies. Eur. Phys. J. B 2021, 94, 203.

(17) Olivieri, C.; Wang, Y. J.; Li, G. C.; Manu, V. S.; Kim, J.; Stultz, B. R.; Neibergall, M.; Perctelli, F.; Muretta, J. M.; Thomas, D. D.; et al. Multi-state recognition pathway of the intrinsically disordered protein kinase inhibitor by protein kinase A. Life 2020, 9, No. e55607.

(18) Schünke, S.; Stoldt, M.; Lecher, J.; Kaupp, U. B.; Willbold, D. Structural insights into conformational changes of a cyclic nucleotide-binding domain in solution from mesorhizobium loti K1 channel. Proc. Natl. Acad. Sci. U.S.A. 2011, 108, 6121–6126.

(19) Wilmann, M.; Gautel, M.; Mayans, O. Activation of calcium/calmodulin regulated kinases. Cell. Mol. Biol. 2000, 46, 883–894.

(20) Xie, Y.; Lord, C. L.; Clarke, B. P.; Ivey, A. L.; Hill, P. S.; McDonald, W. H.; Wente, S. R.; Ren, Y. Structure and activation mechanism of the yeast RNA pol II CTD kinase CTDK-1 complex. Proc. Natl. Acad. Sci. U.S.A. 2021, 118, e2019163118.

(21) Tavernier, N.; Thomas, Y.; Vagneron, S.; Maisonneuve, P.; Orlicky, S.; Mader, P.; Regmi, S. G.; Van Hove, L.; Levinson, N. M.; Gasmi-Seabrook, G.; et al. Bora phosphorylation substitutes in trans Orlicky, S.; Mader, P.; Regmi, S. G.; Van Hove, L.; Levinson, N. M.; Gasmi-Seabrook, G.; et al. Bora phosphorylation substitutes in trans.
epithelial to mesenchymal transition and suppresses migratory-associated integrins in lung cancer cells. Pharmacutics 2021, 13, 554.
(49) Genheden, S.; Ryde, U. The MM/GBSA and MM/PBSA methods to estimate ligand-binding affinities. Expert Opin. Drug Discovery 2015, 10, 449–461.
(50) King, E.; Aitchison, E.; Li, H.; Luo, R. Recent developments in free energy calculations for drug discovery. Front. Mol. Biosci. 2021, 8, 712085.
(51) Huang, K. F.; Luo, S.; Cong, Y. L.; Zhong, S. S.; Zhang, J. Z.; Duan, L. L. An accurate free energy estimator: Based on MM/PBSA combined with interaction entropy for protein-ligand binding affinity. Nanoscale 2020, 12, 10737–10750.
(52) Greene, D.; Qj, R. X.; Nguyen, R.; Qia, T. Y.; Luo, R. Heterogeneous dielectric implicit membrane model for the calculation of MMPBSA binding free energies. J. Chem. Inf. Model. 2019, 59, 3041–3056.
(53) Wang, C.; Greene, D.; Xiao, L.; Qi, R.; Luo, R. Recent developments and applications of the MMPBSA method. Front. Mol. Biosci. 2018, 4, 87.
(54) Shi, M.; Zhang, C.; Xie, Y.; Xu, D. Stereoselective inclusion mechanism of ketoprofen into beta-cyclodextrin: Insights from molecular dynamics simulations and free energy calculations. Theor. Chem. Acc. 2014, 133, 1556.
(55) Shi, M.; He, J.; Weng, T.; Shi, N.; Qj, W.; Guo, Y.; Chen, T.; Chen, L.; Xu, D. The binding mechanism of NWHD-870 to bromodomain-containing protein 4 based on molecular dynamics simulations and free energy calculation. Phys. Chem. Chem. Phys. 2022, 24, 5125–5137.
(56) Liu, K.; Li, D.; Zheng, W.; Shi, M.; Chen, Y.; Tang, M.; Yang, T.; Zhao, M.; Deng, D.; Zhang, C.; et al. Discovery, optimization, and evaluation of quinazolinone derivatives with novel linkers as orally efficacious phosphoinositide-3-kinase delta inhibitors for treatment of inflammatory diseases. J. Med. Chem. 2021, 64, 8951–8970.
(57) Law, R. P.; Nunes, J.; Chung, C. W.; Bantscheff, M.; Buda, K.; Dai, H.; Evans, J. P.; Flinders, A.; Klimaszew ska, D.; Lewis, A. J.; et al. Discovery and characterisation of highly cooperative FAK-degrading PROTACS. Angew. Chem., Int. Ed. 2021, 60, 23327–23334.
(58) Berger, B. T.; Amaral, M.; Kolh, D. B.; Nunes-Alves, A.; Musil, D.; Heinrich, T.; Schroeder, M.; Neil, R.; Wang, J.; Navratilova, I.; et al. Structure-kinetic relationship reveals the mechanism of selectivity of FAK inhibitors over PYK2. Cell Chem. Biol. 2021, 28, 686–698.
(59) Thilfault, D. G.; Fromm, P.; Martin-Garcia, J. M. Hydrocarbon-stapled paxillin peptide bound to the focal adhesion targeting (FAT) domain of the focal adhesion kinase (FAK), 2020; 10.2210/pdb6PW8/pdb.
(60) Momin, A. A.; Hameed, U. F. S.; Arold, S. T. Passenger sequences can promote interlaced dimers in a common variant of the maltose-binding protein. Sci. Rep. 2019, 9, 20396.
(61) Kadaré, G.; Gervasi, N.; Brami-Cherrier, K.; Blockus, H.; El Messari, S.; Arold, S. T.; Girault, J. A. Conformational dynamics of the maltose-binding protein. Structure 2021, 29, 4722–4738.
(62) Sanner, M. F. Python: A programming language for software exploration 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences. Nucleic Acids Res. 2021, 49, D437–D451.
(63) The PyMOL Molecular Graphics System, version 2 Schrödinger, LLC.
(64) O’Boyle, N. M.; Banck, M.; James, C. A.; Morley, C.; Vandermeersch, T.; Hutchison, G. R. Open babel: An open chemical toolbox. J. Cheminf. 2011, 3, 33.
(65) Stewart, J. P. Optimization of parameters for semi-empirical methods I-method. J. Comput. Chem. 1989, 10, 209–220.
(66) Sanner, M. F. Python: A programming language for software integration and development. J. Mol. Graphics 1999, 17, 57–61.
(67) Gasteiger, J.; Marsili, M. Iterative partial equalization of orbital electronegativity—a rapid access to atomic charges. Tetrahedron 1980, 36, 3219–3228.
(68) Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. Autodock4 and autodocktools4: Automated docking with selective receptor flexibility. J. Comput. Chem. 2009, 30, 2785–2791.
(69) Fuhrmann, J.; Rurainski, A.; Lenhof, H. P.; Neumann, D. A new Lamarckian genetic algorithm for flexible ligand-receptor docking. J. Comput. Chem. 2010, 31, 1911–1918.
(70) Shi, M.; Zhao, M.; Wang, L.; Liu, K.; Li, P.; Liu, J.; Cai, X.; Chen, L.; Xu, D. Exploring the stability of inhibitor binding to SIK2 using molecular dynamics simulation and binding free energy calculation. Phys. Chem. Chem. Phys. 2021, 23, 13216–13227.
(71) Shi, M.; Xu, D. Molecular dynamics investigations suggest a non-specific recognition strategy of 14-3-3-igma protein by tweezer: Implication for the inhibition mechanism. Front. Chem. 2019, 7, 237.
(72) Chen, J. Z.; Wang, X. Y.; Zhang, J. Z. H.; Zhu, T. Effect of substituents in different positions of aminothiazole hinge-binding...
scaffolds on inhibitor-CDK2 association probed by interaction entropy. ACS Omega 2018, 3, 18052–18064.

(86) Chen, J. Z.; Zhang, S. L.; Wang, W.; Pang, L. X.; Zhang, Q. G.; Liu, X. G. Mutation-induced impacts on the switch transformations of the GDP- and GTP-bound K-RAS: Insights from multiple replica quantum mechanics energy surfaces in solution. J. Chem. Inf. Model. 2016, 61, 1954–1969.

(87) Wang, J. M.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. Development and testing of a general AMBER force field. J. Comput. Chem. 2004, 25, 1157–1174.

(88) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; et al. Gaussian 09; Gaussian, Inc., Wallingford CT, 2009.

(89) Bayly, C. I.; Cieplak, P.; Cornell, W. D.; Kollman, P. A. A well-behaved electrostatic potential based method using charge restraints for deriving atomic charges: The RESP model. J. Phys. Chem. 1993, 97, 10269–10280.

(90) Tian, C.; Kasavajhala, K.; Belfon, K. A. A.; Raguelle, L.; Huang, H.; Miques, A. N.; Bickel, J.; Wang, Y. Z.; Pincay, J.; Wu, Q.; et al. Ff9sb: Amino-acid-specific protein backbone parameters trained against quantum mechanics energy surfaces in solution. J. Chem. Theory Comput. 2020, 16, 528–552.

(91) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. 1983, 79, 926–935.

(92) Ryckaert, J.-P.; Ciccotti, G.; Berendsen, H. J. C. Numerical integration of the cartesian equations of motion of a system with constraints: Molecular dynamics of n-alkanes. J. Comput. Phys. 1977, 23, 327–341.

(93) Darden, T.; York, D.; Pedersen, L. Particle mesh ewald: An nlog(n) method for ewald sums in large systems. J. Chem. Phys. 1993, 98, 10089–10092.

(94) Case, D. A.; Aplikula, H. M.; Belfon, K.; Ben-Shalom, I. Y.; Brozell, S. R.; Cerrutti, D. S.; Cheatham, T. E.; Cisneros, G. A.; Cruevo, V. W. D.; Darden, T. A.; et al. Amber 2012; University of California: San Francisco, 2012.

(95) Roe, D. R.; Cheatham, T. E. Parallelization of cpyptraj enables large scale analysis of molecular dynamics trajectory data. J. Comput. Chem. 2018, 39, 2110–2117.

(96) Roe, D. R.; Cheatham, T. E. Ptraj and cpyptraj: Software for processing and analysis of molecular dynamics trajectory data. J. Chem. Theory Comput. 2013, 9, 3084–3095.

(97) Perdih, A.; Baren, U.; Solmajer, T. Binding free energy calculations of n-sulphonyl-glutamic acid inhibitors of murine ligase. J. Mol. Biol. 2009, 39, 983–996.

(98) Baren, U.; Martinek, V.; Florián, J. Free energy simulations of un-catalyzed DNA replication fidelity: Structure and stability of T center dot G and dTTP center dot G terminal DNA mismatches flanked by a single dangling nucleotide. J. Phys. Chem. B 2006, 110, 10557–10566.

(99) Agüst, J.; Medina, C.; Samuelsson, J. E. A new method for predicting binding affinity in computer-aided drug design. Protein Eng. 1994, 7, 385–391.

(100) Lee, F. S.; Chu, Z. T.; Bolger, M. B.; Warshel, A. Calculations of antibody-antigen interactions: Microscopic and semi-microscopic evaluation of the free energies of binding of phosphorylcholine analogs to MCPC603. Protein Eng. 1992, 5, 215–228.

(101) Naim, M.; Bhat, S.; Banik, K. N.; Dennis, S.; Chowdhury, S. F.; Siddiqi, I.; Drabik, P.; Sulea, T.; Bayly, C. I.; Jakalian, A.; et al. Solvated interaction energy (SIE) for scoring protein-ligand binding affinities. I. Exploring the parameter space. J. Chem. Inf. Model. 2007, 47, 122–133.

(102) Gilson, M. K.; Zhou, H. X. Calculation of protein-ligand binding affinities. Annu. Rev. Biophys. Biomol. Struct. 2007, 36, 21–42.

(103) Srinivasan, J.; Cheatham, T. E.; Cieplak, P.; Kollman, P. A.; Case, D. A. Continuum solvent studies of the stability of DNA, RNA, and phosphomimidate DNA helices. J. Am. Chem. Soc. 1998, 120, 9401–9409.

(104) Lee, M. S.; Salisbury, F. R.; Olson, M. A. An efficient hybrid explicit/implicit solvent method for biomolecular simulations. J. Comput. Chem. 2004, 25, 1967–1978.

(105) Shi, M.; Wang, L.; Li, P.; Liu, J.; Chen, L.; Xu, D. Dasatinib-SIK2 binding elucidated by homology modeling, molecular docking, and dynamics simulations. ACS Omega 2021, 6, 11025–11038.

(106) Weiser, J.; Shenkin, P. S.; Still, W. C. Approximate atomic surfaces from linear combinations of pairwise overlaps (LCPO). J. Comput. Chem. 1999, 20, 217–230.

(107) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. Semianalytical treatment of solvation for molecular mechanics and dynamics. J. Am. Chem. Soc. 1990, 112, 6127–6129.

(108) Srinivasan, J.; Trevathan, M. W.; Beroza, P.; Case, D. A. Application of a pairwise generalized born model to proteins and nucleic acids: Inclusion of salt effects. Theor. Chem. Acc. 1999, 101, 426–434.

(109) Gao, P. C.; Li, Z. L. Computation of the boltzmann entropy of a landscape: A review and a generalization. Lands. Ecol. 2019, 34, 2183–2196.

(110) Miller, B. R.; McGee, T. D.; Swails, J. M.; Homeyer, N.; Gohlke, H.; Roitberg, A. E. MMPBSA.py: An efficient program for end-state free energy calculations. J. Chem. Theory Comput. 2012, 8, 3314–3321.

(111) Yen-Pon, E.; Li, B.; Abeçorrón-García-de-Eulate, M.; Tomkiewicz-Raulet, C.; Dawson, J.; Lietha, D.; Frame, M. C.; Coumoul, X.; Garbay, C.; Etche-Quelquejeu, M.; et al. Structure-based design, synthesis, and characterization of the first inhibitor of focal adhesion kinase. ACS Chem. Biol. 2018, 13, 2067–2073.

(112) Zhou, J.; Bronowska, A.; Le Coq, J.; Lietha, D.; Gräfer, F. Allosteric regulation of focal adhesion kinase by PitP and ATP. Biophys. J. 2015, 108, 698–705.

(113) Ahari, S.; Mogharrab, N.; Navapour, L. Interconversion of inactive to active conformation of MARK2: Insights from molecular modeling and molecular dynamics simulation. Arch. Biochem. Biophys. 2017, 630, 66–80.

(114) Ahari, S.; Mogharrab, N.; Navapour, L. Structure and dynamics of inactive and active MARK4: Conformational switching through the activation process. J. Biomol. Struct. Dyn. 2020, 38, 2468–2481.

(115) Tokarski, J. S.; Newitt, J. A.; Chang, C. Y. J.; Cheng, J. D.; Witekkind, M.; Kieber, S. E.; Kish, K.; Lee, F. Y. F.; Borrillier, R.; Longardo, L.; et al. The structure of dasatinib (BMS-354825) bound to activated ABL kinase domain elucidates its inhibitory activity against imatinib-resistant abl mutants. Cancer Res. 2006, 66, 5790–5797.

(116) Cowan-Jacob, S. W.; Fendrich, G.; Floersheimer, A.; Furet, P.; Liebetanz, J.; Rummel, G.; Rheinberger, P.; Centeleghe, M.; Fabbro, D.; Manley, P. Structural biology contributions to the discovery of drugs to treat chronic myelogenous leukaemia. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2007, 63, 80–93.

(117) Shah, N. R.; Tancioni, I.; Ward, K. K.; Lawson, C.; Chen, X. L.; Jean, C.; Sulzmaier, F. J.; Uryu, S.; Millar, N. L. G.; Connolly, D. M.; et al. Analyses of merlin/NF2 connection to FAK inhibitor responsiveness in serious ovarian cancer. Gynecol. Oncol. 2014, 134, 104–111.

(118) Sun, Z. C.; Jiang, Q. W.; Li, J.; Guo, P. J. The potent role of salt-inducible kinases (SIKs) in metabolic homeostasis and tumorigenesis. Signal Transduction Targeted Ther. 2020, 5, 150.

(119) Saha, B. C.; Kumari, R.; Kushumesh, R.; Ambasta, A.; Sinha, B. P. Status of Rho kinase inhibitors in glaucoma therapeutics-an overview. Int. Ophthalmol. 2022, 42, 14, 42.
adhesion kinase inhibitors. Part I. Bioorg. Med. Chem. Lett. 2006, 16, 2173–2176.

(122) Chen, T.; Liu, Y.; Shi, M.; Tang, M.; Si, W.; Yuan, X.; Wen, Y.; Chen, L. Design, synthesis, and biological evaluation of novel covalent inhibitors targeting focal adhesion kinase. Bioorg. Med. Chem. Lett. 2021, 31, 128433.

(123) Qi, Y. H.; Li, Y.; Fang, Y.; Gao, H.; Qiang, B. C.; Wang, S. X.; Zhang, H. B. Design, synthesis, biological evaluation, and molecular docking of 2,4-diaminopyrimidine derivatives targeting focal adhesion kinase as tumor radio-tracers. Mol. Pharm. 2021, 18, 1634–1642.

(124) Wang, S.; Zhang, R.-H.; Zhang, H.; Wang, Y.-C.; Yang, D.; Zhao, Y.-L.; Yan, G.-Y.; Xu, G.-B.; Guan, H.-Y.; Zhou, Y.-H.; et al. Design, synthesis, and biological evaluation of 2,4-diamino pyrimidine derivatives as potent fak inhibitors with anti-cancer and anti-angiogenesis activities. Eur. J. Med. Chem. 2021, 222, 113573.

(125) Cho, H.; Shin, I.; Yoon, H.; Jeon, E.; Lee, J.; Kim, Y.; Ryu, S. S.; Song, C.; Kwon, N. H.; Moon, Y.; et al. Identification of thieno 3,2-d pyrimidine derivatives as dual inhibitors of focal adhesion kinase and FMS-like tyrosine kinase 3. J. Med. Chem. 2021, 64, 11934–11957.

(126) Knighton, D. R.; Zheng, J. H.; Ten Eyck, L. F.; Ashford, V. A.; Xuong, N. H.; Taylor, S. S.; Sowadski, J. M. Crystal structure of the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase. Science 1991, 253, 407–414.

(127) Southan, C. Caveat usor: Assessing differences between major chemistry databases. ChemMedChem 2018, 13, 470–481.

(128) Da Silva, F.; Desaphy, J.; Rognan, D. IChem: A Versatile Toolkit for Detecting, Comparing, and Predicting Protein-Ligand Interactions. ChemMedChem 2018, 13, 507–510.

(129) Da Silva, F.; Desaphy, J.; Bret, G.; Rognan, D. IChemPIC: A random forest classifier of biological and crystallographic protein-protein interfaces. J. Chem. Inf. Model. 2015, 55, 2005–2014.

(130) Marcou, G.; Rognan, D. Optimizing fragment and scaffold docking by use of molecular interaction fingerprints. J. Chem. Inf. Model. 2007, 47, 195–207.

(131) Ahmad, F.; Albultti, A.; Tariq, M. H.; Din, G.; ul Qamar, M. T.; Ahmad, S. Discovery of potential antiviral compounds against hendra virus by targeting its receptor-binding protein (G) using computational approaches. Molecules 2022, 27, 554.

(132) Zubair, M. S.; Maulana, S.; Widodo, A.; Pitopang, R.; Arba, M.; Hariono, M. GC-MS, LC-MS/MS docking and molecular dynamics approaches to identify potential sars-cov-2 3-chromotrypti-

sin-like protease inhibitors from zingiber officinale roscoe. Molecules 2021, 26, 5230.

(133) Musheen, Z. T.; Hameed, A. R.; Al-Hasani, H. M. H.; Ahmad, S.; Li, G. L. Computational determination of potential multiprotein targeting natural compounds for rational drug design against sars-cov-2. Molecules 2021, 26, 17.

(134) Corts-Hernandez, P.; Nuñez, R. V.; Dominguez-Ramirez, L. Docking and molecular dynamics predictions of pesticide binding to the calyx of bovine beta-lactoglobulin. Int. J. Mol. Sci. 2020, 21, 1988.

(135) Yang, T.; Hu, M.; Chen, Y.; Xiang, M.; Tang, M.; Qi, W.; Shi, M.; He, J.; Yuan, X.; Zhang, C.; et al. N-(pyrimidin-2-yl)-1,2,3,4-tetrahydrosquinoxalin-6-amine derivatives as selective janus kinase 2 inhibitors for the treatment of myeloproliferative neoplasms. J. Med. Chem. 2020, 63, 14921–14936.

(136) Wang, L.; Zheng, Y.; Li, D.; Yang, L.; Lei, L.; Yan, W.; Zheng, W.; Tang, M.; Shi, M.; Zhang, R.; et al. Design, synthesis, and bioactivity evaluation of dual-target inhibitors of tubulin and src kinase guided by crystal structure. J. Med. Chem. 2021, 64, 8127–8141.

(137) Liedha, D.; Eck, M. J. Crystal structures of the FAK kinase in complex with TAE226 and related bis-anilino pyrimidine inhibitors reveal a helical DFG conformation. PLoS One 2008, 3, No. e3800.

(138) Haile, P. A.; Casillas, L. N.; Votta, B. J.; Wang, G. Z.; Charnley, A. K.; Dong, X. Y.; Bury, M. J.; Romano, J. J.; Mehllmann, J. F.; King, B. W.; et al. Discovery of a first-in-class receptor interacting protein 2 (RIP2) kinase specific clinical candidate, 2-(4-(benzo[d]thiazol-5-y)lamo)-6-(tert-butylsulfonyl)quinazolin-7-yl) oxy)ethyl di-

hydrogen phosphate, for the treatment of inflammatory diseases. J. Med. Chem. 2019, 62, 6482–6494.

(139) Chen, Y.; Yuan, X.; Tang, M.; Shi, M.; Yang, T.; Liu, K.; Deng, D.; Chen, L. Degrading FLT3-ITD protein by proteolysis targeting chimeras (PROTAC). Bioorg. Chem. 2022, 119, 105508.

(140) Yang, T.; Cui, X.; Tang, M.; Qi, W.; Zhu, Z.; Shi, M.; Yang, L.; Pei, H.; Zhang, W.; xie, L.; et al. Identification of a novel 2,8-diazaspiro 4.5 decan-1-yl derivative as a potent and selective dual TYK2/JAK1 inhibitor for the treatment of inflammatory bowel disease. J. Med. Chem. 2022, 65, 3511–3517.

(141) Lombardo, L. J.; Lee, F. Y.; Chen, P.; Norris, D.; Barrish, J. C.; Behnia, K.; Castaneda, S.; Cornelius, L. A. M.; Das, J.; Doweyko, A. M.; et al. Discovery of n-(2-chloro-6-methylphenyl)-2-(6-((2-hydroxyethyl)-piperazin-1-yl)-2-m ethylpyrimidin-4-ylamo)thiazole-5-carboxamide (BMS-354825), a dual SRC/ABL kinase inhibitor with potent antitumor activity in preclinical assays. J. Med. Chem. 2004, 47, 6685–6661.

(142) Groendyke, B. J.; Nabet, B.; Mohrardt, M. L.; Zhang, H. S.; Peng, K.; Koide, E.; Coffey, C. R.; Che, J. W.; Scott, D. A.; Bass, A. J.; et al. Discovery of a pyrimidothiazolodiazepine as a potent and selective focal adhesion kinase (FAK) inhibitor. ACS Med. Chem. Lett. 2021, 12, 30–38.

(143) Amaro, R. E.; Baudry, J.; Chodera, J.; Demir, O.; McCammon, J. A.; Miao, Y. L.; Smith, J. C. Ensemble docking in drug discovery. Biophys. J. 2018, 114, 2271–2278.

(144) Vilar, S.; Costanzi, S. Application of monte carlo-based receptor ensemble docking to virtual screening for GPCR ligands. In G protein coupled receptors: Modeling, activation, interactions and virtual screening; Conn, P. M., Ed.; Methods in enzymology; Elsevier Academic Press Inc, 2013; Vol. 522, pp 263–278.