New Natural Compound Inhibitors of Checkpoint Kinase-1(CHK1) Based on Computational Study

Hui Li
The First Hospital of Jilin University
jianxin Xi
Jilin University
Zhenhua Wang
Jilin University
Han Lu
Jilin University
Zhishan Du
Jilin University
Bo Wu
The First Hospital of Jilin University
Sheng Zhong
Cancer Hospital of Sun Yat sen University
Yida Peng
Jilin University
Yonggao Mou (mouyg@sysucc.org.cn)
Cancer Hospital of Sun Yat sen University

Research Article

Keywords: Discovery Studio 4.5, Inhibitor, CHK1, Prexasertib, Virtual screening

Posted Date: May 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-509168/v1

Abstract

As a malignant tumor of the ovary, the general treatment principle of ovarian cancer is surgical treatment, supplemented by chemotherapy, and some patients can use targeted drugs. Its treatment effect is relatively poor, so the prognosis is poor, the mortality rate is high. To contribute to drug design and refinement, ideal lead compounds with potential inhibitory effects on ATP-competitive CHK1 (Checkpoint kinase-1) inhibitors were downloaded from the drug library (ZINC15 database) and screened afterwards. The ATP-competitive CHK1 inhibitors were identified by using computer-aided virtual screening technology. We first calculated the LibDock score through the docking of proteins and molecules, and then analyzed the pharmacological and toxicological properties. Then, we performed precise docking of the small molecules selected in the above steps with CHK1 protein to analyze their docking mechanism and affinity. Next, we used molecular dynamics simulation to make a assessment if the ligand-CHK1 complex were stable in natural environment. As the result shown, ZINC000008214547 and ZINC000072103632 were proved to bind with CHK1 with a higher binding affinity and stability. Additionally, their toxicological analysis shows that they are less toxic and will not inhibit the activity of cytochrome P-450 2D6. In the simulation of molecular dynamics, we also found that ZINC000008214547-CHK1 and ZINC000072103632-CHK1 complexes' potential energy were more favorable compared with reference ligand, Prexasertib. Not only that, the two complexes also showed better stability in the natural environment. So, all results elucidated that ZINC000008214547 and ZINC000072103632 were favorable lead inhibitors of CHK1 protein. ZINC000008214547 and ZINC000072103632 were safe and had the potential to inhibit CHK1 protein. They may contribute a solid foundation for the development of CHK1 target drug.

Introduction

Ovarian cancer is a malignant tumor, one of the most general tumor associated with the female reproductive organs. And cutaneous cancer is the most common of ovarian cancer (1). The general treatment principle is surgical treatment, supplemented by chemotherapy, and some patients can use targeted drugs. Treatment effect is relatively poor, and mortality is high. As an important adjuvant therapy for ovarian cancer, chemotherapy count a lot in the initial therapy and recurrence treatment of ovarian cancer. Although there is an initial response for ovarian cancer to some chemotherapy, there is a high recurrence rate. And ovarian cancer is easy to metastasize and relapse(2). With the more and more common use of chemotherapy drugs, tumors have acquired resistance to chemotherapy drugs, hindering the development of chemotherapy(3). Surgical treatment, initial cell abatement surgery and platinum-based chemotherapy are effective against this type of cancer, but it is prone to relapse and eventually develops platinum resistance. Therefore, it is of great significance to research and develop more targeted drugs for not only the treatment of ovarian cancer but also development of targeted therapy.

Studies have shown that Prexasertib has significant anti-tumor activity in ovarian cancer's therapy(4). Prexasertib is ATP-competitive CHK1 (Checkpoint kinase-1) inhibitor and Its effectiveness and selectivity have been proved in many studies. As a Ser/Thr kinase, CHK1 protein kinase regulates cell survival, cell cycle as well as embryogenesis (5)(6). DDR(DNA damage Response) is the main regulatory mechanism in tumor formation, and CHK1 is the most common core protein in DDR to coordinate, detect and repair DNA damage processes (7, 8). CHK1 blocks the cell cycle of tumor cells by phosphorylating downstream molecules of cell cycle's signaling pathway. Once the cell cycle is blocked, DNA damage can be repaired, which will lead to tumor cells' reduction of

sensitivity to radiotherapy and increase of resistance to chemotherapeutic drug. Moreover, the cells dominated by CHK1 protein kinase can grow and become the main body in the tumor, which will promote the production of more malignant tumor cells and lead to the production of drug-resistant clones and tumor recurrence in clinical practice(9). This limits the effectiveness of traditional anticancer drugs in treating cancer by directly damaging DNA.

When normal cells are damaged in DNA, CHK1 can be phosphorylated and activated by ATR and ATM. And after being activated, CHK1 can phosphorylate itself, thus activating the phosphatase cell division cycle 25(Cdc25) family. In addition, the phosphorylated Cdc25C can make the substrate CDK2(Cyclin dependent kinase 2) highly phosphorylated, thus inactivating the cyclin B-CDK2 complex and finally causing the G2 phase stagnation cell cycle conversion delay(10). During this period, tumor suppressor proteins P53 and CHK1 repair damaged cells at cell cycle checkpoint, such as G1, S, S and G2, respectively, so that damaged DNA can be repaired in a timely manner(11). However, most tumor cells are inactivated with p53 gene and lack of G1 checkpoint. So they can only rely on CHK1 to repair damaged DNA molecules(12). When CHK1 inhibitor is used in combination with some anti-tumor drugs that cause DNA damage, tumor cells without the p53 gene lack G1 checkpoint. And CHK1's inhibition leads to lack of S and G2 checkpoint. So damaged tumor cells eventually induce premature death through mitotic mutations or apoptosis pathways. But for normal cells, they contain p53 gene, and lacking of G2 checkpoint has little influence. So damaged DNA can be repaired at G1 checkpoint(13). Therefore, when combined with DNA damage reagents, CHK1 inhibitors can improve the sensitivity and selectivity of DNA damage reagents to cancer cells and reduce adverse reactions. This makes it possible to treat cancer with CHK1 inhibitors.

Generally speaking, DNA damage reagents are anti-tumor drugs, but CHK1 can repair DNA-damaged tumor cells. It leads to the reduction or failure of chemotherapy or radiotherapy for malignant tumors and it is conducive to the growth of tumors. Therefore, CHK1 protein kinase is a research hot spot in recent years. And CHK1 inhibitor is a potential adjuvant for anti-tumor therapy. Furthermore, the selection of lead compounds that can effectively inhibit CHK1 is essential for the development of drugs and the therapy of cancer.

Currently, the most studied CHK1 inhibitors include AZD7762(14),SCH900776(15)Prexasertib(LY2606368)(16) and so on. Moreover, Prexasertib as an CHK1 inhibitor, can promote DNA damage process by increasing the replication stress. And preclinical studies have shown that Prexasertib induces DNA damage in the presence of Cdc25A and CDK2(17, 18). Prexasertib can kill tumor cells by docking with CHK1, preventing CHK1 from repairing damaged DNA. Whether administrated alone or in combination with other drugs, it can effectively inhibit tumor growth and exert anti-tumor activity. The researchers published Prexasertib for platinum resistance and relapse/refractory ovarian cancer patients with excellent results(4). Therefore, Prexasertib was selected as the reference drug for the experiment.

However, the current inhibitor Prexasertib still has significant therapeutic limitations. Studies have shown that Prexasertib can cause adverse reactions during treatment, with neutropenia being the most common side effect (19). In addition, Prexasertib is hepatotoxic and hematological toxicity (17). What's more, although Prexasertib promotes the DNA damage process, chemotherapeutic drug resistance is inevitable. Because of these limitations of Prexasertib, screening more inhibitors of CHK1 is essential for the therapy of cancer. Therefore, in this study, we aimed to select inhibitors more effective than Prexasertib from the natural drug library.

Due to their unique molecular structure and potential biological functions, natural products and their derivatives occupy an important position in today's pharmacological market and make great contributions to drug design and improvement (20, 21). Therefore, Discovery Studio 4.5's several modules, like LibDock, CDOCKER, ADME (absorption, distribution, metabolism, excretion), Toxicity Prediction, were applied for screening and identifying ideal inhibitors of CHK1. In addition, we also assessed the ADME properties and Toxicological properties of selected candidate molecules. In this study, a series of favorable inhibitors of CHK1 as well as their pharmacological and toxicological properties were found by comparing to Prexasertib, which lays a solid foundation for the development of CHK1 inhibitors and drug development.

Material And Methods

Software and ZINC15 database

Discovery Studio 4.5(DS 4.5) is a comprehensive software and widely used in life science, which can give us the ability to provide molecular modeling and environmental simulation.And it just based on Windows/Linux systems and personal computers launched by biovia. It has the ability to provide chemical/biological data display, simulation/analysis, construction Three-dimensional molecules, display dynamic changes, three-dimensional mapping and many other functions. DS can be applied to the following research fields of life sciences: new drug discovery, bioinformatics, structural biology, enzymology, immunology, virology, genetics and developmental biology, tumor research. With DS 4.5, we found a large number of potential inhibitors of CHK1. Firstly, we used LibDock module of DS 4.5 to screen the small molecules that can be docked with CHK1. Then we use CDOCKER for more precise protein and molecule docking. And ADME and TOPKAT module were also used to analyze pharmacological and toxicological properties. Small molecules were downloaded from ZINC15 database, a free database form the Irwin and Shoichet Laboratories, Department of Pharmaceutical Chemistry, University of California, San Francisco.

Virtual Screening Based on LibDock

Virtual screening was performed by DS 4.5's LibDock module that is rigidity - based(22). The first step was to calculate Hotspots that characterize the receptor site. Docking was performed after the formation of multiple conformations of the ligand. Finally, the optimization and scoring of the docking were carried out. Libdock module docked these conformations into the binding pocket of the receptor based on the principle of matching the conformation of small molecules with the hotspot of the receptor interaction. Libdock module's biggest advantage is that it is fast, can be operated in parallel, and is suitable for large-scale virtual screening. All positions of molecules were ranked according to ligand score. We choose the binding pocket region where Prexasertib bind with CHK1 as the binding region for screening. We downloaded the 2.5°crystal structure of human CHK1 and inhibitor Prexasertib from PDB (the protein database). Figure 1 shows CHK1' chemical structure. The crystalline water and other surrounding heteroatoms were removed before the proteins were prepared, and then hydrogenating, protonating, ionizing, and minimizing the energy. After determining the binding site with the prepared protein,we used the ligand Prexasertib to bind the position to generate the docking active site. Then virtual filtering is performed by LibDock to dock molecules at the defined region. In the next step, rank and group all docking positions founded on the LibDock score.

ADME and Toxicity calculation

The DS 4.5's ADME module was applied to assess molecules' CYP2D6 (cytoplasmic p-450 2D6)inhibition, plasma protein binding levels, aqueous solubility, human intestinal absorption, blood-brain barrier penetration, and hepatotoxicity. The DS 4.5's TOPKAT module was used to calculate molecules' toxicological properties, including U.S. National Toxicology Program rodent carcinogenicity, developmental toxicity potential, Ames mutagenicity, chronic oral LOAEL (lowest observed adverse effect level) and LD50 (rat oral median lethal dose). All of the above calculations were taken into consideration when potential inhibitors of CHK1.

CDOCKER analysis and Pharmacophore assessment

DS 4.5's CDOCKER module was carried out for high-precision docking on the basis of CHARMM force field. In this research, both the receptor and ligand use CHARMM force field. CHK1 remains rigid in the process of docking, whereas the ligands were allowed to bend. The interaction energy is an indicator of the affinity between the ligand and the protein. We calculated the interaction energy of each complex posture of the ligand with the protein. We downloaded CHK1's crystal structure from PDB. In order to avoid affecting the structure of the receptor-ligand complex, we decided to remove the crystal water molecule during the preparation of the protein(23, 24). Then we added hydrogen atoms to the protein. Next, in order to prove the reliability of the binding mode, we deleted Prexasertib from the binding site, and then re-docked CHK1 with it again. Regarding the scope of the binding site of CHK1, we selected a region with a radius of 5 A° from the geometric center of the ligand Prexasertib. During docking, the ligand interacted and combined with CHK1 in the defined region. According to the CDOCKER interaction energy, the different ligand -CHK1 complexes' stances were calculated. The pharmacophore formation module of 3D-QSAR was used to display the pharmacophore of a compound. Each molecule contained up to 255 conformations, but only those conformations within the energy threshold of 10kcal/mol could be retained.

Molecular Dynamics Simulation

In the above analysis, the best ligand conformation was selected for molecular dynamics simulation analysis. Firstly, in an orthogonal box,we put the ligand-receptor complex and established a clear periodic boundary solvated water model. Next,in this system, we simulate the physiological environment by adding sodium chloride with an ionic strength of 0.145. For the rest of the process, CHARMM force field had an effect on the system. And the system was relaxed by the energy minimization method(the conjugate gradient and the steepest descent were 500 steps and 500 steps, respectively). The final root mean square gradient was 0.227. The system's temperature rose slowly from 296K to 302K for 2ps. And the equilibrium simulation of 5Ps was carried out. The production module of molecular dynamics simulation was carried out at 25ps. And the time step of the production module was 1fs. In order to evaluate the long-range electrostatic, the particle mesh Ewald algorithm has also been applied. Also, the constant temperature was set at 300K in this procedure. What's more, all hydrogen bonds were fixed through the linear constraint solver algorithm. By trajectory protocol of DS 4.5, structural characteristics, potential energy and RMSD's trajectory were drawn according to the initial complex setup.

Results

Screening inhibitors of CHK1 virtually

The binding pocket of ligand is CHK1's essential region for regulation, because small molecules binding to the active site can compete with ATP protein kinase and prevent CHK1 phosphorylation, thus preventing DNA repair of damaged cells. Therefore, this pocket area is chosen as the reference point. We downloaded 17,799 molecules in total from the ZINC15 database. CHK1 was selected as the receptor protein and downloaded from PDB. The inhibitor Prexasertib was selected as the reference ligand. Result showed that 17,799 compounds met the conditions of stable binding with CHK1, among which 3394 compounds' score of LibDock was higher than Prexasertib's (116.085). The top 20 were shown below (Table 1).

Table 1
Top 20 ranked compounds with higher libdock scores than Prexasertib.

Number	Compounds	Libdock score	Number	Compounds	Libdock score
1	ZINC000014712793	178.116	11	ZINC000014811803	140.08
2	ZINC000014951634	163.03	12	ZINC000008214547	139.899
3	ZINC000095620725	157.444	13	ZINC000002526388	139.775
4	ZINC000004097774	155.724	14	ZINC000001557162	139.666
5	ZINC000045337516	151.8	15	ZINC000070455322	139.15
6	ZINC000100634117	142.081	16	ZINC000100634116	139.035
7	ZINC000005766341	141.807	17	ZINC000072103632	138.676
8	ZINC000014780940	141.215	18	ZINC000006036395	138.343
9	ZINC000006036380	140.983	19	ZINC000017596232	137.96
10	ZINC000014780926	140.3	20	ZINC000005640039	137.616

Pharmacological and Toxicological Prediction

Firstly, we used the DS 4.5's ADME module to calculate the pharmacological properties of not only Prexasertib but also all ligands selected, such as PPB (plasma protein binding properties), human intestinal absorption, BBB (brain/blood barrier), aqueous solubility, hepatotoxicity and CYP2D6 (cytochrome P450 2D6) binding (Table 2). In addition to ZINC000095620725, all compounds were showed basically soluble in water in the result of aqueous solubility prediction which was defined at a water temperature of 25˚C. CYP2D6 ,as an important enzyme in drug metabolism ,most compounds were proved to be no inhibiting effect on it except ZINC000014780940 in the predictions, ZINC000014780926 and ZINC000002526388. What's more, after we finished the hepatotoxicity prediction, we found 15 compounds to be nontoxic and 5 compounds to be similar to the toxicity of Prexasertib. For the next module, 3 compounds□ZINC000008214547, ZINC000002526388 and ZINC000072103632 □indicated a higher absorption level than Prexasertib in the prediction of human intestinal absorption. Last but not least, 11 compounds were showed to have a high bounding force with plasma protein but the others didn't not.

Table 2
ADME (Adsorption, Distribution, Metabolism, Excretion) properties of compounds.

Number	Compounds	Solubility Level[a]	BBB level[b]	CYP2D6[c]	Hepatotoxicity[d]	Absorption Level[e]	PPB Level[f]
1	ZINC000014712793	4	4	0	0	3	0
2	ZINC000014951634	3	4	0	0	3	0
3	ZINC000095620725	0	4	0	0	3	1
4	ZINC000004097774	2	4	0	0	3	0
5	ZINC000045337516	1	4	0	0	3	1
6	ZINC000100634117	3	4	0	0	2	0
7	ZINC000005766341	1	4	0	0	3	1
8	ZINC000014780940	2	4	1	1	2	1
9	ZINC000006036380	2	1	0	0	1	1
10	ZINC000014780926	2	4	1	1	2	1
11	ZINC000014811803	3	4	0	0	3	0
12	ZINC000008214547	2	4	0	0	0	0
13	ZINC000002526388	2	4	1	1	0	1
14	ZINC000001557162	3	4	0	1	1	0
15	ZINC000070455322	1	4	0	1	3	0
16	ZINC000100634116	3	4	0	0	2	0
17	ZINC000072103632	3	4	0	0	0	0
18	ZINC000006036395	2	1	0	0	1	1
19	ZINC000017596232	1	4	0	0	3	0
20	ZINC000005640039	2	4	0	0	1	1
21	Prexasertib	2	4	0	1	1	0
a Aqueous-solubility level: 0 (extremely low); 1 (very low, but possible); 2 (low); 3 (good)							
b Blood Brain Barrier level: 0 (Very high penetrant); 1 (High); 2 (Medium); 3 (Low); 4 (Undefined)							
c Cytochrome P450 2D6 level: 0 (Non-inhibitor); 1 (Inhibitor)							
d Hepatotoxicity: 0 (Nontoxic); 1 (Toxic)							
e Human-intestinal absorption level: 0 (good); 1 (moderate); 2 (poor); 3 (very poor)							
f Plasma Protein Binding: 0 (Absorbent weak); 1 (Absorbent strong)							

The safety of drugs is of paramount importance, so this study has also done a full study on the safety of these compounds. Therefore, the of Discovery Studio 4.5'TOPKAT module was applied to predict different toxicity indicators of the compound and Prexasertib based on computational methods to demonstrate the safety of the candidate compounds. In this module, four indicators including rodent carcinogenicity (based on the U.S. National Toxicology Program data set), developmental toxicity potential properties, and Ames mutagenicity as well as rat oral LD50 and chronic oral LOAEL, were predicted (Table 3) From the results, we can learned that 19 molecules were to have non-mutagenic effect and 14 molecules were to have no developmental toxicity potential properties. About the Rodent Carcinogenicity, It can be seen that only ZINC000006036380 was toxic to both male and female mouse, and ZINC000006036395 was toxic to female mouse only. In addition, ZINC000004097774 was mildly toxic to male rat. Put all of the above results together, two compounds, ZINC000008214547(compound 1) and ZINC000072103632(compound 2) were determined to potential ideal lead compounds, because hepatotoxicity, CYP2D6 inhibitors, Ames mutagenicity, rodent carcinogenicity, and developmental toxicity potential were all not found compared with other compounds. In summary, ZINC000008214547 and ZINC000072103632 proved to be safe drug candidates and could be used as candidate drugs for subsequent studies. (Fig. 2)

Table 3
Toxicities of compounds.

Number	Compounds	Mouse NTP[a]		Rat NTP[a]		AMES[b]	DTP[c]
		Female	Male	Female	Male		
1	ZINC000014712793	0.671	0.614	0.395	0.553	0.344	0.698
2	ZINC000014951634	0.428	0.614	0.436	0.542	0.392	0.613
3	ZINC000095620725	0.351	0.455	0.386	0.611	0.523	0.812
4	ZINC000004097774	0.577	0.625	0.365	0.729	0.023	0.744
5	ZINC000045337516	0.458	0.597	0.312	0.620	0.000	0.576
6	ZINC000100634117	0.453	0.586	0.447	0.572	0.426	0.598
7	ZINC000005766341	0.459	0.665	0.470	0.552	0.109	0.676
8	ZINC000014780940	0.534	0.518	0.142	0.297	0.000	0.524
9	ZINC000006036380	0.987	0.999	0.262	0.348	0.000	0.902
10	ZINC000014780926	0.299	0.439	0.422	0.483	0.000	0.618
11	ZINC000014811803	0.105	0.525	0.082	0.078	0.421	0.691
12	ZINC000008214547	0.267	0.456	0.299	0.531	0.196	0.681
13	ZINC000002526388	0.393	0.588	0.519	0.517	0.045	0.635
14	ZINC000001557162	0.534	0.518	0.142	0.297	0.000	0.524
15	ZINC000070455322	0.136	0.016	0.228	0.482	0.001	0.462
16	ZINC000100634116	0.047	0.350	0.040	0.060	0.160	0.722
17	ZINC000072103632	0.265	0.025	0.241	0.232	0.000	0.397
18	ZINC000006036395	0.999	0.000	0.401	0.067	0.000	0.979
19	ZINC000017596232	0.489	0.594	0.364	0.475	0.739	0.775
20	ZINC000005640039	0.542	0.613	0.248	0.249	0.010	0.584
21	Prexasertib	0.345	0.465	0.467	0.534	0.667	0.486
a < 0.3 (Non-Carcinogen); >0.7 (Carcinogen)							
b < 0.3 (Non-Mutagen); >0.7 (Mutagen)							
c < 0.3 (Non-Toxic); >0.7 (Toxic)							

Ligand Binding and Ligand Pharmacophore analysis

Through the CDOCKER module, we studied the mechanism of ligand binding of these compounds to CHK1 by docking these compounds(Prexasertib, ZINC000008214547 and ZINC000072103632) with the molecular

structure of CHK1. After calculating and displaying, the CDOCKER potential energy were shown in Table 4. The value of RMSD between the Prexasertib and CHK1 complex was 0.75 Å about the docked pose and the crystal structure. The result indicated that the CDOCKER module employed in this study was extremely repeatable and dependable in terms of repeatability. As Table 5 shows, relative to the reference ligand Prexasertib (-45.4587 kcal/mol), both ZINC000008214547 and ZINC000072103632 had a lower CDOCKER potential energy, which suggested that these two molecules could integrate with CHK1 better than that of Prexasertib. Next, for the purpose of analyzing the situation of combination about the hydrogen bonds and Alkyl interaction, Pi-Alkyl interaction and Pi-Sigma interaction of ligands-CHK1 complex(Figure 3 and Fig. 4) ligand and CHK1 bonds, we adopted the measure of structural computation. The results are described below, 1 pairs of hydrogen bonds was formed between ZINC000072103632 and CHK1, by the O3 of the compound with TYR20:OH of CHK1. Also, 9 Alkyl interactions were presented in the complex. For ZINC000008214547, there were 5 pairs of Alkyl interaction with CHK1, by 3 pairs of LEU15 of CHK1 with the ligand, LEU137 of CHK1 with the ligand and C28 of the ligand with LEU137 of CHK1. There were also 4 hydrogen bonds in the complex. About the existence of the bonds of the reference compound Prexasertib, it formed 4 hydrogen bonds with CHK1, by the N23 of the ligand with LYS38:HZ3 of CHK1, H43 of the ligand with GLU85:O, N26 of the ligand with CYS87:HN, and H46 of the ligand with CYS87:O. Nine Pi-Alkyl interaction and 1 Pi-Sigma interaction were also formed with CHK1.(Table 5 and Table 6) In regard to pharmacophore of these 2 compounds, 46 feature pharmacophores in ZINC000008214547 and 16 feature pharmacophores in ZINC000072103632 were showed up in the results. Otherwise, hydrogen bond acceptor,hydrogen bond donor and hydrophobic center was displayed in ZINC000008214547 and ZINC000072103632 displayed hydrogen acceptor and hydrophobic center. (Fig. 5)

Table 4
CDOCKER interaction energy, relative energy and asolute energy of compounds with CHK1.

Compounds	CDOCKER Interaction energy (Kcal/mol)
ZINC000008214547	-46.9068
ZINC000072103632	-56.5031
Prexasertib	-45.4587

Table 5
Hydrogen bond interaction parameters for each compound and CHK1 residues.

Receptor	Compound	Donor atom	Receptor Atom	Distances (Å)
h-CHK1	ZINC000008214547	A:GLU91:OE2	ZINC000008214547:H66	2.78
		A:GLU134:O	ZINC000008214547:H66	2.1
		A:TYR20:OH	ZINC000008214547:H68	2.55
		A:ASP148:OD1	ZINC000008214547:H64	1.91
	ZINC000072103632	A:TYR20:OH	ZINC000072103632:O3	2.77
	Prexasertib	A:LYS38:HZ3	Molecule: N23	2.14
		A:GLU85:O	Molecule: H43	1.97
		A:CYS87:HN	Molecule: N26	2.12
		A:CYS87:O	Molecule: H46	2.74

Table 6
Alkyl interaction, Pi-Alkyl interaction and Pi-Sigma interaction parameters for each compound and CHK1 residues.

Interaction parameters	Receptor	Compound	Donor atom	Receptor Atom	Distances (Å)
Alkyl interaction	h-CHK1	ZINC000008214547	A:LEU15	ZINC000008214547	4.49
			A:LEU15	ZINC000008214547	5.13
			A:LEU15	ZINC000008214547	4.84
			A:LEU137	ZINC000008214547	4.87
			A:LEU137	ZINC000008214547:C28	4.41
		ZINC000072103632	A:VAL68	ZINC000072103632:C26	3.92
			A:LEU84	ZINC000072103632:C26	3.70
			A:LEU84	ZINC000072103632:C27	4.46
			A:ALA36	ZINC000072103632:C27	4.15
			A:ALA23	ZINC000072103632:C27	3.98
			A:ALA23	ZINC000072103632	5.13
			A:LEU15	ZINC000072103632	4.32
			A:LEU15	ZINC000072103632	5.17
			A:LEU37	ZINC000072103632	4.86
Pi-Alkyl interaction		Prexasertib	A:LEU137	Molecule	5.33
			A:VAL68	Molecule	5.04
			A:LEU84	Molecule	4.86
			A:VAL23	Molecule	5.05
			A:ALA36	Molecule	4.39
			A:ALA36	Molecule	5.06
			A:LEU137	Molecule	4.63
			A:CYS87	Molecule	4.94
			A:LEU15	Molecule	4.98
Pi-Sigma interaction		Prexasertib	A:LEU15:HD12	Molecule	2.84

Molecular Dynamics Simulation

On account of the importance of evaluating the stability of ligand-CHK1 complex in natural environment, a new module, molecular dynamics simulation module was designed. We previously completed molecular docking

experiments through CDOCKER module, and the original conformation was obtained. Two important results, RMSD curves and potential energy chart of each complex was showed in Fig. 6. After 85 ps, the trend of the curve about these two compounds reached equilibrium, and RMSD and potential energy of these complexes always stayed stable over time. The results of molecular dynamics simulation indicated that the formation of these p-related interactions and hydrogen bonds formed by molecules and CHK1 take effect on the stabilization of these complexes. All of the above results validated that these 2 compounds could interact with CHK1, and their complexes could be stable when existed in a natural environment and had the same adjustable effects on CHK1 as the reference Prexasertib did.

Discussion

Ovarian cancer, as a malignant tumor of the ovaries, can occur at any age. The histological types are diverse and mainly epithelial(25). And because ovarian cancer is not easy to be found in the early stage, the treatment effect is relatively poor in the late stage. The recurrence rate after treatment is high, and the mortality rate is also very high. Prexasertib, as a typical CHK1 inhibitor, has been shown that there is a reliable effect in the treatment of ovarian cancer, especially high-grade serous carcinoma(4).But there are some side effects. In addition,Initial cell abatement surgery and platinum-based chemotherapy are effective against this type of cancer, but it is prone to relapse and eventually develops platinum resistance. For these patients with poor prognosis, new treatment strategies are needed,and other CHK1 inhibitors need to be sought for treatment(26).

DNA damage Response (DDR) is the main regulatory mechanism in tumor formation(7). As a serine-threonine protein kinase, CHK1 could regulate the cellular activities. CHK1 is an important component of DRR pathway and a major regulatory molecule of arresting the cell cycle which is DNA damage-dependent (27). CHK1 contribute to DNA damage repair as a strategic molecule,which is an important cause of malignant tumor growth and a hindrance to treatment. The mechanism which CHK1 inhibitors kill cancer cells is to prevent the phosphorylation of CHK1 itself, resulting in the failure of phosphorylation of its substrate and the failure of cells to stay at the G2-M check point in the process of cell cycle. Thus, the collapse occurs, causing the killing of tumor cells(28, 29). Therefore, the search for a suitable CHK1 inhibitor is crucial to the resistance to tumor growth and treatment to tumor. For the past few years,novel clinical CHK1 inhibitors have been developing and they are given in combination with other anticancer drugs to improve the therapeutic effect of tumor are always of great value and meaningful(4, 16).

Over time, the design and development of CHK1 inhibitors has made great progress(19). As Prexasertib is part of a relatively mature CHK1 inhibitor, we take it for granted as the reference drug in our research. The potential of CHK1 inhibitors in tumor therapy is unquestionable. The detailed structural characteristics of CHK1 and the basis for identifying Prexasertib in this study could help improve the structural design of CHK1 inhibitors and could ultimately be used to improve enzymes for cancer treatment.

In this study, a big part of the main content is to select Discovery Studio 4.5’6 modules (LibDock, ADME, TOPKAT, CDOCKER, 3D-QSAR and molecular dynamics simulation) to screen and analyze the biochemical structure properties of novel potential inhibitors. Apart from these, we also studied and investigated the molecular conformation, pharmacological properties, binding affinity, and stability of the selected compounds to provide stronger evidence for their high performance over the reference inhibitor Prexasertib.

Firstly, there were 17799 named product compounds in the ZINC15 database for us to conduct virtual screening. What's more, all of these named product molecules were purchasable and natural. The LibDock score served as an indicator of evaluating the effect of energy optimization and conformational stability.Therefore,we can draw the conclusion that the higher the LidDock score is, the better energy optimization and comformational stability are. After scoring these compound by using Discovery Studio 4.5's libdock module, we first selected 3,394 compounds that were considered to have high affinity with CHK1. At the same time, another 105 compounds were selected on account of having a higher scores in the Libdock module than the reference inhibitor Prexasertib (Lib-dock score: 116.085, ranking: 106) among these compounds. To put it another way, in terms of stable conformation and better energy optimization,these 105 compounds were superior to Prexasertib. Finally, the top 20 natural compounds scoring by LibDock module are selected and concentrated for subsequent research.

Secondly, ADME(absorption, distribution, metabolism, excretion) and toxicity properties are two important indicators to evaluate pharmacological properties of drugs, so we used the ADME module and TOPKAT module to predict these two indicators. AS a result, two compounds(ZINC000008214547 and ZINC000072103632) are considered to be ideal lead compounds. Because they have a good level of dissolution in water, which implies that they can be absorbed well in the body and they show non-inhibition to cytochrome P4502D6(CYP2D6) which means they are no hepatotoxicity. In addition, three toxicity indexes, the Ames mutagenicity, rodent carcinogenicity, and developmental toxicity potential of these two compounds are lower than those of other compounds, which also indicate their potential applications in drug development. Moreover, specific groups and atoms can be designed to alter pharmacological properties. Therefore, We can't categorically assume that the other compounds on the list that are less desirable. Perhaps, with some design, these compounds can also work in unexpected ways and shows their potential value in drug development. In summary, compared with the current research, ZINC000008214547 and ZINC000072103632 are the most valuable high quality drug candidates and most likely to play the role we expect.

Thirdly, CDOCKER module played an important role in the study of chemical bonding and bonding mechanisms in this study. We obtained CDOCKER interaction energy of ZINC000008214547 and ZINC000072103632 after calculating by CDOCKER module. The values were lower than the reference ligand Prexasertib(-45.4587 kcal/mol)apparently. This leads us to the conclusion that these two molecules can better bind with CHK1 with a high affinity in comparison with Prexasertib. Nevertheless, the chemical structure of these two molecules should also be analyzed, which we do with 3D-QSAR module. The two compounds combined with CHK1 had similar type and number of chemical bonds to Prexasertib, so these two compounds could also stably bind to CHK1 through active site Cys145, which possibly help to competitively inhibit the activity of CHK1 and thus playing a role in increasing tumor lethality.

Furthermore, in the same way, feature pharmacophores of these two compounds is analysed by 3D-QSAR. Computation results illustrated 46 feature pharmacophores in ZINC000008214547 and 16 feature pharmacophores in ZINC000072103632.Apart from these, the result also showed us ZINC000008214547 can act as hydrogen bond donor, hydrogen bond acceptor and hydrophobic center and ZINC000072103632 can act as hydrogen acceptor and hydrophobic center. After predicting the pharmacophore of these two molecules by computational methods. The results mean that these 2 molecules probably have a more suitable and valid pharmacophore than Prexasertib. In future research, diverse specific groups could be added to the two

compounds to elaborate the design and refinement of the drugs, thus increasing their significance in drug therapy and drug improvement.

Finally, the stability of these two compounds was appraised by performing molecular dynamics simulation module. The data relating to RMSD and potential energy of these ligand-CHK1 complexes were obtained in this step. And the results displayed that the time of the trajectories of complexes to reach equilibrium is about 85 ps. Both RMSD and potential energy of the complexes don't change over time and stay stable all the time. So that proved that these 2 complexes have the ability of stable existence in the natural environment. Taking all the above results into consideration, they provides the possibility to develop and design drugs, such as modification and purification, to bind ligands and receptors more firmly. It is also worth noting that a similar skeleton in their chemical structures can represent as agonists or inhibitors, and the inverse effect is caused by the addition or removal of different groups or atoms. From what has been discussed above, the natural compounds identified in this study have the advantages of strong pharmacological action, strong binding affinity with CHK1 and good stabilization, which can provide valuable resources for the development of CHK1-related drugs.

At present, the design and development of oncology drugs has always been a topic of interest to scientists around the world, although the road to research and development of oncology drugs is tortuous and difficult. This study about screening of ideal lead compounds is of great significance and is a crucial step in drug design. It lays a firm foundation for future drug designation and development. Through a sequence of advanced technical calculations, We speculate that these two promising molecules probably possess of bright prospects and significant significance in the future treatment of ovarian cancer. In addition, this study provides effective guidance and techniques for screening lead compounds with some potential therapeutic value. This advanced approach could help identify more lead compounds in the future and contribute to drug development.

Although this study has been carefully designed and accurately measured, we admit that there are still some limitations in this study. In fact, it takes thousands of refinements and refinements to bring a drug to market successfully. Therefore, subsequent research can focus directly on refining and improving the lead compounds selected in this study.

Conclusion

The study based on the structural aided by the computer, chemical techniques and a series of studies were conducted consisting of virtual screening, molecular docking, ADME, and toxicity prediction to screen out and confirm desirable lead compounds which will have potential inhibitory effects on CHK1. Two compounds (ZINC000008214547 and ZINC000072103632) were selected as safety ideal lead compound, which will make great contribution to the subsequent development of CHK1 inhibitors. Moreover, out of consideration of uncertainty, this research offers a table of drug candidates and their properties in the terms of pharmacological for everyone's review, which may also help to pave the way for later drug design and improvement of CHK1 or other proteins.

Declarations

Acknowledgements

We thanked the foundation of open innovation experiment of College of Basic Medical Sciences, Jilin University (2018)

Author Contribution statement

This study was completed with a team work. Every author has made substantial contributions to the study. Sheng Zhong has come up with the conception. Addtionally, Hui Li has done the design of the work. The experiment and data collection part was completed by jianxin Xi, Zhenhua Wang. Furthermore, analysis of the data was done by Han Lu. As for interpretation of the data, Zhishan Du has contributed a lot to this part. What's more, Weihang Li was responsible for the creation of new software used in the work. Bo Wu, Shanshan Jiang and Yida Peng have drafted the work. And Yonggao Mou has substantively revised it.

Competing Interest Statement

All authors declare no conflicts of interest related to this manuscript, and all authors have approved the publication of this work.

References

1. Ramalingam P. Morphologic, Immunophenotypic, and Molecular Features of Epithelial Ovarian Cancer. Oncology (Williston Park, NY). 2016;30(2):166-76.
2. Bowtell DD, Böhm S, Ahmed AA, Aspuria PJ, Bast RC, Jr., Beral V, et al. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. Nature reviews Cancer. 2015;15(11):668-79.
3. Chamberlain MC. Anticancer therapies and CNS relapse: overcoming blood-brain and blood-cerebrospinal fluid barrier impermeability. Expert review of neurotherapeutics. 2010;10(4):547-61.
4. Parmar K, Kochupurakkal BS, Lazaro JB, Wang ZC, Palakurthi S, Kirschmeier PT, et al. The CHK1 Inhibitor Prexasertib Exhibits Monotherapy Activity in High-Grade Serous Ovarian Cancer Models and Sensitizes to PARP Inhibition. Clinical cancer research : an official journal of the American Association for Cancer Research. 2019;25(20):6127-40.
5. Walworth N, Davey S, Beach D. Fission yeast chk1 protein kinase links the rad checkpoint pathway to cdc2. Nature. 1993;363(6427):368-71.
6. Chen CH, Chu PC, Lee L, Lien HW, Lin TL, Fan CC, et al. Disruption of murine mp29/Syf2/Ntc31 gene results in embryonic lethality with aberrant checkpoint response. PloS one. 2012;7(3):e33538.
7. Zhang Y, Hunter T. Roles of Chk1 in cell biology and cancer therapy. International journal of cancer. 2014;134(5):1013-23.
8. Walker M, Black EJ, Oehler V, Gillespie DA, Scott MT. Chk1 C-terminal regulatory phosphorylation mediates checkpoint activation by de-repression of Chk1 catalytic activity. Oncogene. 2009;28(24):2314-23.
9. Rundle S, Bradbury A, Drew Y, Curtin NJ. Targeting the ATR-CHK1 Axis in Cancer Therapy. Cancers. 2017;9(5).
10. Graves PR, Lovly CM, Uy GL, Piwnica-Worms H. Localization of human Cdc25C is regulated both by nuclear export and 14-3-3 protein binding. Oncogene. 2001;20(15):1839-51.

11. Zhou BB, Bartek J. Targeting the checkpoint kinases: chemosensitization versus chemoprotection. Nature reviews Cancer. 2004;4(3):216-25.
12. Kawabe T. G2 checkpoint abrogators as anticancer drugs. Molecular cancer therapeutics. 2004;3(4):513-9.
13. Powell SN, DeFrank JS, Connell P, Eogan M, Preffer F, Dombkowski D, et al. Differential sensitivity of p53(-) and p53(+) cells to caffeine-induced radiosensitization and override of G2 delay. Cancer research. 1995;55(8):1643-8.
14. Itamochi H, Nishimura M, Oumi N, Kato M, Oishi T, Shimada M, et al. Checkpoint kinase inhibitor AZD7762 overcomes cisplatin resistance in clear cell carcinoma of the ovary. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2014;24(1):61-9.
15. Herůdková J, Paruch K, Khirsariya P, Souček K, Krkoška M, Vondálová Blanářová O, et al. Chk1 Inhibitor SCH900776 Effectively Potentiates the Cytotoxic Effects of Platinum-Based Chemotherapeutic Drugs in Human Colon Cancer Cells. Neoplasia (New York, NY). 2017;19(10):830-41.
16. Sen T, Tong P, Stewart CA, Cristea S, Valliani A, Shames DS, et al. CHK1 Inhibition in Small-Cell Lung Cancer Produces Single-Agent Activity in Biomarker-Defined Disease Subsets and Combination Activity with Cisplatin or Olaparib. Cancer research. 2017;77(14):3870-84.
17. Hong D, Infante J, Janku F, Jones S, Nguyen LM, Burris H, et al. Phase I Study of LY2606368, a Checkpoint Kinase 1 Inhibitor, in Patients With Advanced Cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2016;34(15):1764-71.
18. King C, Diaz HB, McNeely S, Barnard D, Dempsey J, Blosser W, et al. LY2606368 Causes Replication Catastrophe and Antitumor Effects through CHK1-Dependent Mechanisms. Molecular cancer therapeutics. 2015;14(9):2004-13.
19. Babiker HM, McBride A, Cooke LS, Mahadevan D. Therapeutic potential of investigational CHK-1 inhibitors for the treatment of solid tumors. Expert opinion on investigational drugs. 2017;26(9):1063-72.
20. Newman DJ. Developing natural product drugs: Supply problems and how they have been overcome. Pharmacology & therapeutics. 2016;162:1-9.
21. Li JW, Vederas JC. [Drug discovery and natural products: end of era or an endless frontier?]. Biomeditsinskaia khimiia. 2011;57(2):148-60.
22. Rao SN, Head MS, Kulkarni A, LaLonde JM. Validation studies of the site-directed docking program LibDock. Journal of chemical information and modeling. 2007;47(6):2159-71.
23. Kalani K, Agarwal J, Alam S, Khan F, Pal A, Srivastava SK. In silico and in vivo anti-malarial studies of 18β glycyrrhetinic acid from Glycyrrhiza glabra. PloS one. 2013;8(9):e74761.
24. Sarvagalla S, Singh VK, Ke YY, Shiao HY, Lin WH, Hsieh HP, et al. Identification of ligand efficient, fragment-like hits from an HTS library: structure-based virtual screening and docking investigations of 2H- and 3H-pyrazolo tautomers for Aurora kinase A selectivity. Journal of computer-aided molecular design. 2015;29(1):89-100.
25. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA: a cancer journal for clinicians. 2017;67(1):7-30.
26. Naumann RW, Coleman RL. Management strategies for recurrent platinum-resistant ovarian cancer. Drugs. 2011;71(11):1397-412.
27. Dai Y, Grant S. New insights into checkpoint kinase 1 in the DNA damage response signaling network. Clinical cancer research : an official journal of the American Association for Cancer Research.

2010;16(2):376-83.
28. Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. Nature. 2004;432(7015):316-23.
29. Patil M, Pabla N, Dong Z. Checkpoint kinase 1 in DNA damage response and cell cycle regulation. Cellular and molecular life sciences : CMLS. 2013;70(21):4009-21.

Figures

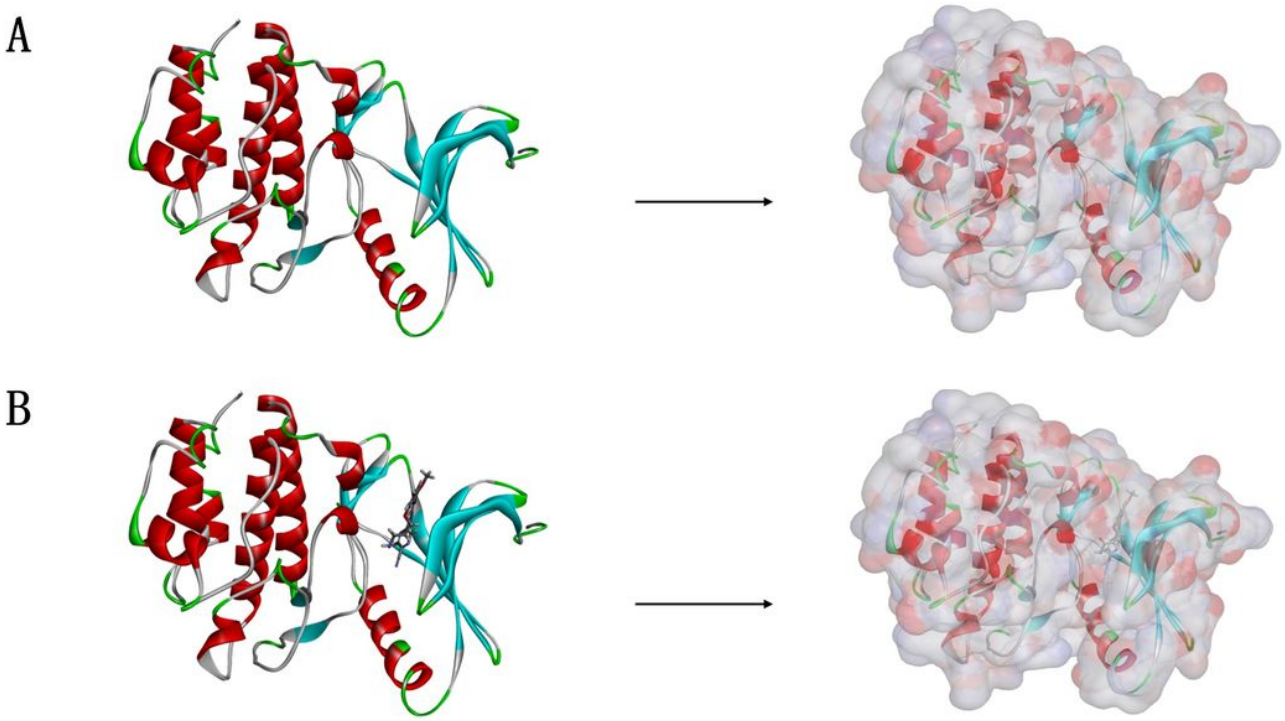

Figure 1

(A) The molecular structure of CHK1. Initial molecular structure was shown, and the surface of the molecule was added. (B) The complex structure of CHK1 with Prexasertib. Initial complex structure was shown, and the surface of the complex was added. Blue represented positive charge, red represented negative charge.

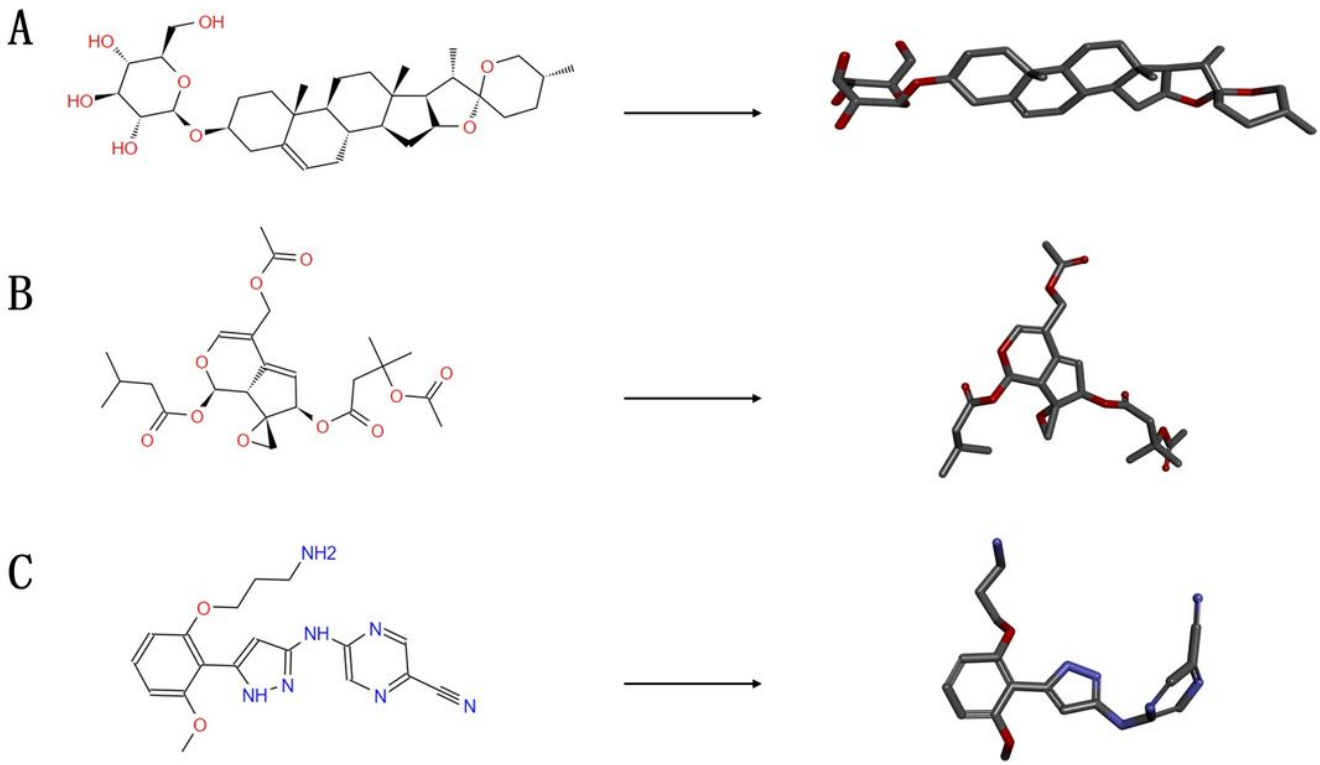

Figure 2

The 2D structures of CHK1 and novel compounds selected from virtual screening by Chemdraw. And 3D structures of CHK1 and novel compounds selected from virtual screening by DS 4.5. (A) ZINC000008214547; (B) ZINC000072103632; (C) Prexasertib.

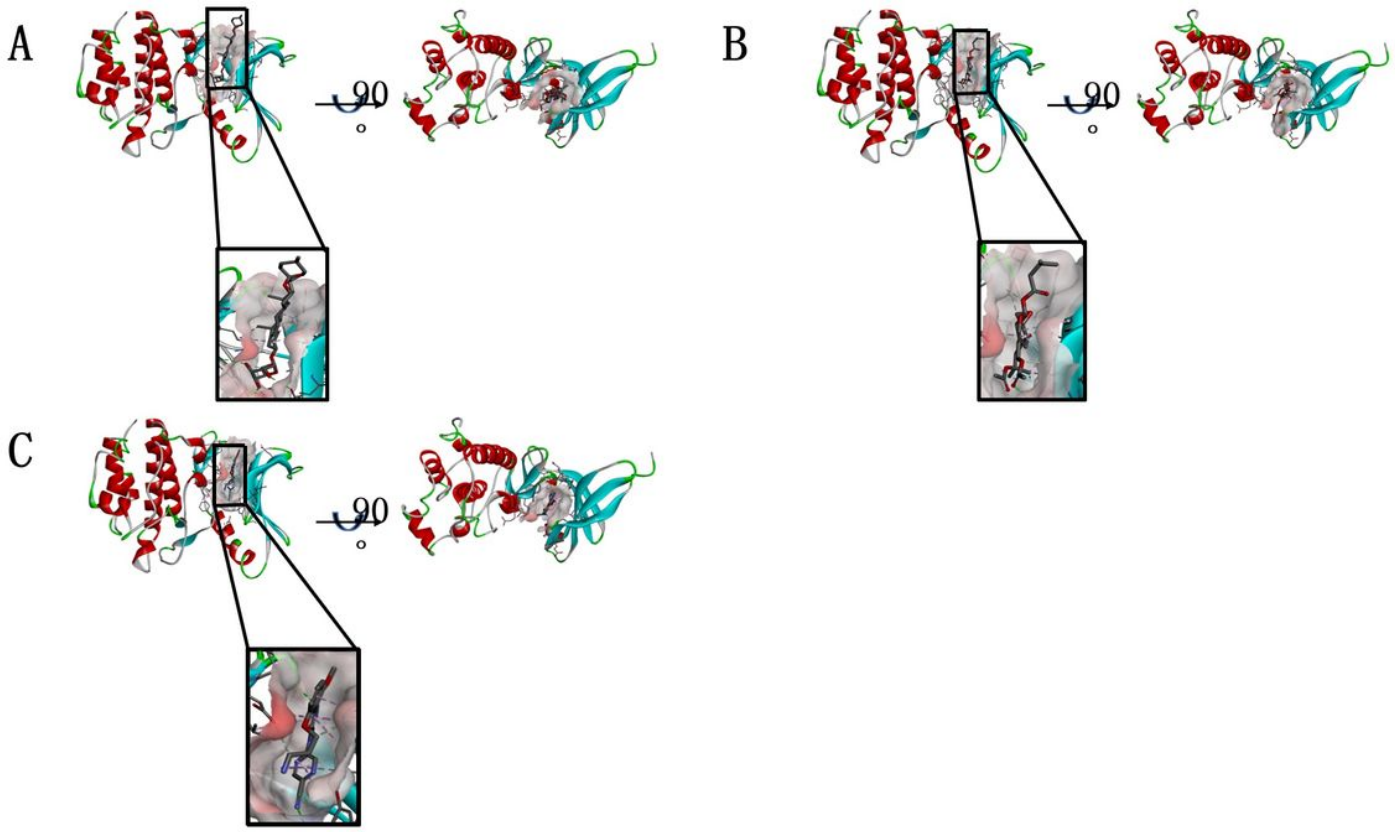

Figure 3

(A) ZINC000008214547-CHK1 complex. Schematic drawing of interactions between ligands and CHK1, and the Charge surface of the junction pocket were added, Blue represented positive charge, red represented negative charge, and ligands were shown in sticks, the structure around the ligand-receptor junction were shown in thinner sticks. (B) ZINC000072103632-CHK1 complex. Schematic drawing of interactions between ligands and CHK1, and the Charge surface of the junction pocket were added, Blue represented positive charge, red represented negative charge, and ligands were shown in sticks, the structure around the ligand-receptor junction were shown in thinner sticks. (C) Prexasertib-CHK1 complex. Schematic drawing of interactions between ligands and CHK1, and the Charge surface of the junction pocket were added. Blue represented positive charge, red represented negative charge, and ligands were shown in sticks, the structure around the ligand-receptor junction were shown in thinner sticks.

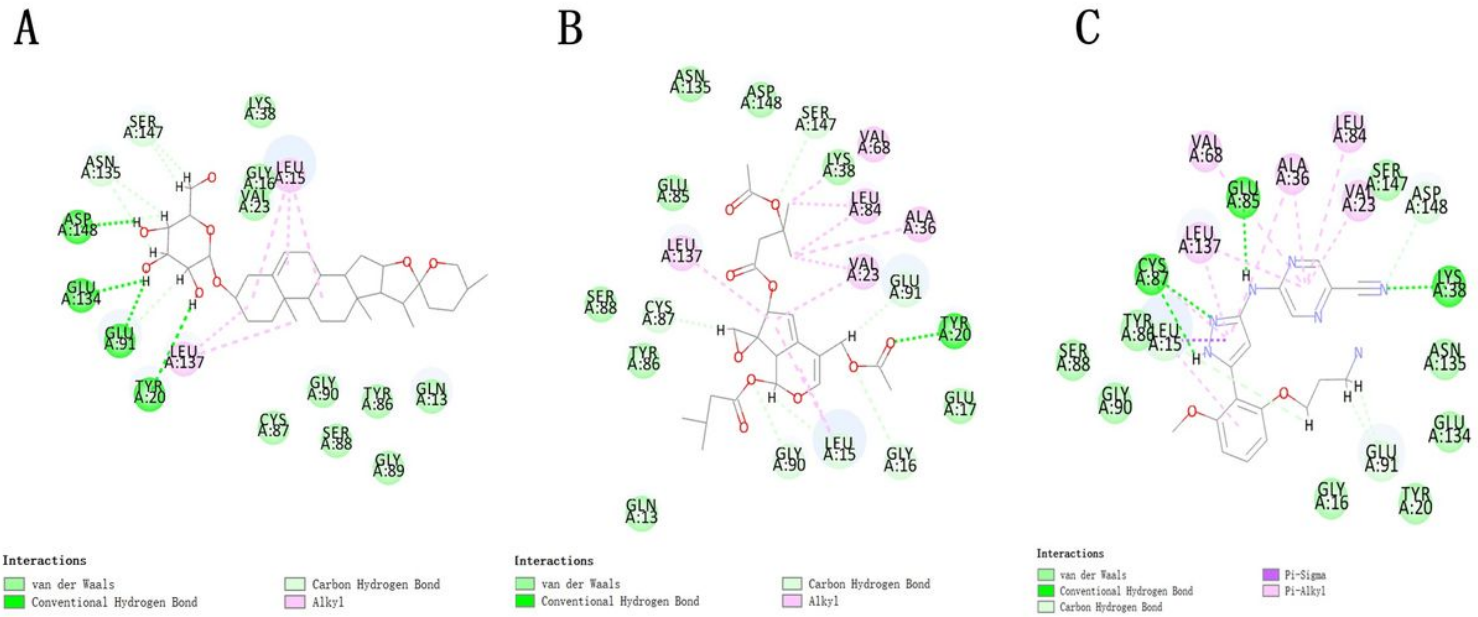

Figure 4

The inter-molecular interaction of the predicted binding modes of (A) ZINC000008214547 to CHK1; (B) ZINC000072103632 to CHK1, (C) Prexasertib to CHK1.

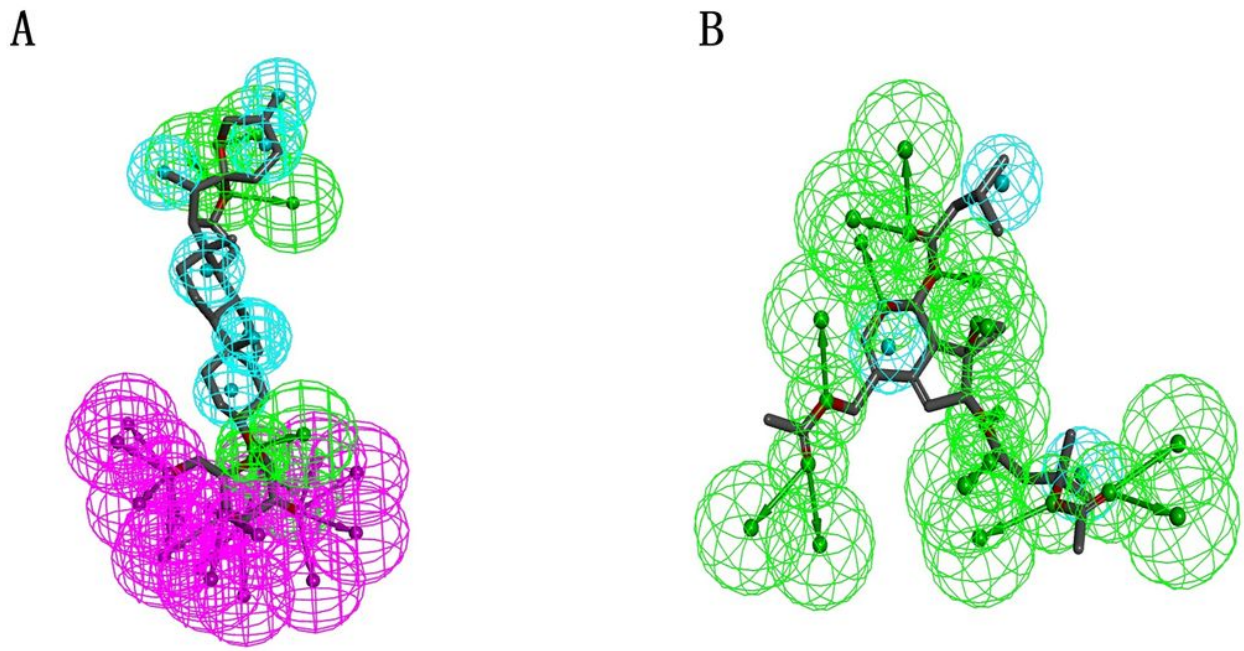

Figure 5

Pharmacophore predictions using 3D-QSAR. (A) ZINC000008214547: Green represents hydrogen acceptor, blue represents hydrophobic center, purple represents hydrogen donor. (B) ZINC000072103632: Green represents hydrogen acceptor, blue represents hydrophobic center.

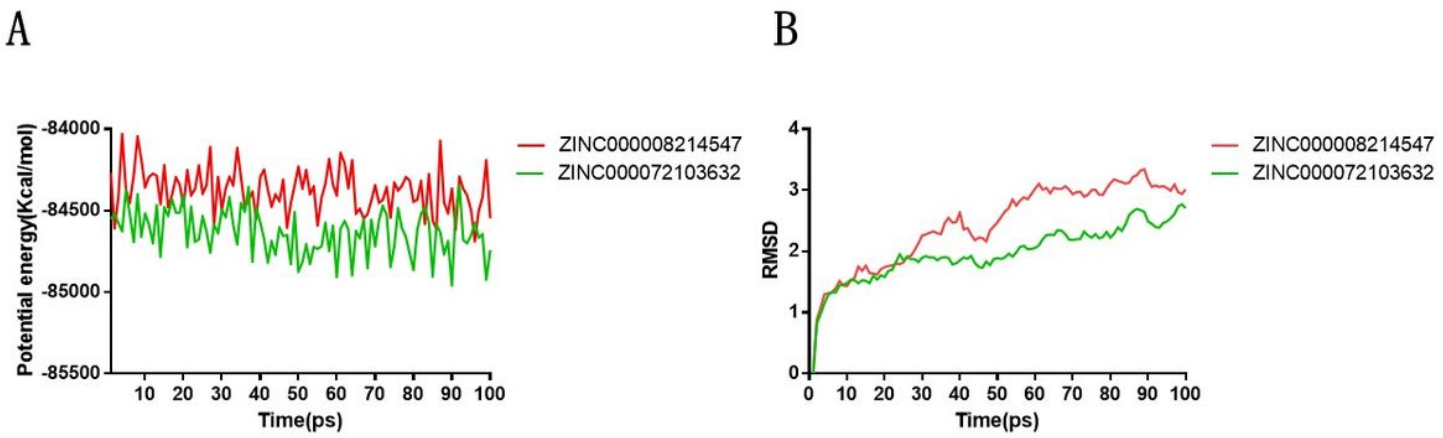

Figure 6

Results of molecular dynamics simulation of three complexes. (A) Potential Energy of ZINC000008214547-CHK1 complex; (B) Potential Energy of ZINC000072103632-CHK1 complex; (C) Average backbone RMSD of ZINC000008214547-CHK1 complex; (C)Average backbone RMSD of ZINC000072103632-CHK1 complex.