Computational Study of Novel Natural Inhibitors Targeting 3-Phosphoinositi-Dependent Protein Kinase 1(PDK1)

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Abstract

Objective To screen ideal lead compounds with potential inhibition of 3-phosphoinositi-dependent protein kinase 1 (PDK1) from ZINC15 database, which is beneficial to drug design and improvement.

Methods The Discovery Studio 4.5 computer-aided virtual screening technique was used to screen potential inhibitors of PDK1. Libdock was used for virtual screening and scoring of candidate compounds, ADME module was used for physical and chemical properties and toxicity analysis, and CDOCKER module was used for molecular docking analysis. The binding affinity of ligand-PDK1 was studied through molecular docking, and the stability of ligand-PDK1 in the natural environment was analyzed through molecular dynamics simulation.

Results Two natural compounds ZINC00000157721 and ZINC000034189841 were screened from ZINC15 database. These two compounds have no CYP2D6 inhibition, easy to pass the blood-brain barrier, no hepatotoxicity, high binding affinity with PDK1, higher stability in the natural environment than positive drug BX-795, and stable existence.

Conclusions The results show that ZINC00000157721 and ZINC000034189841 are ideal and safe lead compounds and have a potential inhibitory effect on PDK1. These compounds are safe candidates and may provide the basis and premise for the design and optimization of specific PDK1 inhibitors.

Introduction

Glioma is the most common primary malignant craniocerebral tumor in the central nervous system [1]. It can be seen in both adults and children and is caused by glial cell carcinoma of the brain and spinal cord [2]. At present, the main treatments for glioma are surgery, radiotherapy, chemotherapy, targeted therapy, etc., but glioma is prone to relapse and has a poor prognosis. Chemotherapy is the main treatment method, but the applicability of chemotherapy is greatly limited due to the increasing resistance of tumor cells to chemotherapeutic drugs and multiple side effects. One reason is that certain genetic changes cause the expression of certain enzymes in the body to become abnormal, preventing cancer drugs from acting on tumor cells[3].

3-phosphoinositide-dependent protein kinase 1 (PDK1), a member of the AGC (cAMP-dependent, cGMP-dependent and protein kinase C) group, is encoded by the PDPK1 gene located at 16p13.3. It has been shown that the amplification of PDK1 can lead to a poor prognosis of glioma [4]. PDK1 has three ligand-binding sites: substrate binding site, catalytic ATP binding site and PDK1 interaction fragment (PIF) binding site [5]. PIF pocket recruitment has the downstream substrate kinase of hydrophobic motif (HM), which stimulates the intrinsic activity of PDK1. Catalytic ATP binding sites exist in a variety of protein kinases, and the development of ATP non-competitive inhibitors has the great potential [6]. PDK1 is an important protease that mediates the PI3K-AKT-mTOR signaling pathway, and its activity is regulated by the phosphorylation/dephosphorylation cycle. Overexpression of PDK1 is a major factor causing cancer [7]. When PDK1 is overexpressed, PDK1 hyper-phosphorylates AKT on the T308 residue, causing abnormal activation of the PI3K-AKT-mTOR signaling pathway, leading to malignant transformation of cells, which is

related to migration, adhesion, tumor angiogenesis and extracellular matrix degradation of tumor cells, as well as inhibiting apoptosis [8].

In general, preventing overexpression of the PI3K-AKT-mTOR signaling pathway is an effective anti-tumor pathway, but PDK1 can abnormally activate this pathway, which is one of the reasons for the failure of chemotherapy for malignant tumors. Therefore, how to select effective lead compounds to inhibit PDK1 is an important step in drug development and cancer treatment. At present, there are more studies on PDK1 inhibitors including gsk-470 (GSK2334470), staurosporine, bx-795, etc. [9–13], among which bx-795 has the most in-depth research. The aminopyridine skeleton of bx-795 binds to the catalytic ATP binding site of PDK1, competitively inhibiting the binding of ATP, thereby preventing the overexpression of PDK1 and its over-activation of the downstream pathway, thereby inhibiting cell cancerization. Bx-795 also inhibits the phosphorylation of AKT at Thr308, thereby inhibiting its activity [14]. Therefore, PDK1 inhibitor is an effective drug for anti-tumor therapy. This study aimed to identify more effective compound inhibitors than bx-795 from the natural drug library for the treatment of cancer.

Natural products and derivatives, with potential biological functions and special molecular structures, play an important role in today's drug market and have made important contributions to the design and improvement of drugs [15–17]. In this study, a series of structural biological and chemical methods, such as molecular docking and molecular dynamics simulation, were used to screen and identify compounds with potential inhibitory functions related to PDK1. What’s more, we extracted the candidate drugs and their pharmacological characteristics from ZINC15 database, and detected the toxicity, metabolism, gastrointestinal absorption and distribution of the candidate compounds. The significance of this study lies in the search for the lead compounds of PDK1 inhibitors, to provide a basis for the development and improvement of tumor therapy drugs.

Methods And Materials

Discovery Studio Software and Ligand Library

Discovery Studio 4.5 software (BIOVIA, San Diego, California, USA) is a new generation of molecular modeling in the field of life science and environmental simulation software, a protein characterization, homology modeling, molecular mechanics calculation and molecular dynamics simulation, based on the structure of the drug design and drug design based on small molecule, combinatorial library design and analysis, and other functions, is designed to provide protein modeling and the design and improvement of the related drugs. At present, this method has been used to select a large number of lead compounds and optimize and improve their drug candidates. For Discovery Studio, LIBDOCK module can be used for simulated screening of lead compounds, ADME module for physical and chemical properties and toxicity analysis, and CDOCKER module for molecular docking analysis. The ZINC15 database is a virtual screening compound library, a collection of merchant compound libraries, supported and maintained by the Irwin and Shoichet laboratories in the department of pharmaceutical chemistry at the university of California, San Francisco (UCSF). After screening a large number of molecules through ZINC database, the screened compounds that may be active can be directly purchased from suppliers through the links provided by ZINC, to facilitate the determination of drug activity in vitro. The ZINC15 database is a free database of

commercially available compounds provided by the Irwin and Shoichet laboratories in the department of pharmaceutical chemistry at the UCSF.

Structure-Based Virtual Screening Using LibDock

The catalytic ATP binding sites of PDK1 were selected to screen compounds that might inhibit PDK1. LibDock is a rigid body-based docking module, and the LibDock module of Discovery Studio 4.5 [18] is used for virtual filtering. It uses a grid placed at the binding site as well as polar and non-polar probes to calculate the hotspots of the protein. The hotspots then align the ligands to form favorable interactions. Ligand minimization using the Smart Minimiser algorithm and the CHARMM force field (Cambridge, Massachusetts, USA). After ligand minimization, all ligand positions were ranked according to ligand scores. Download the 2.0-A crystal structure of PDK1(protein database identifier: 3nax) from the protein database and import them into the LibDock working environment. The chemical structure of PDK1 is shown in Fig. 1. The protein is prepared by removing the crystalline water and surrounding heteroatoms, then adding hydrogen, protonizing, ionizing, and minimizing energy. Energy minimization is carried out by CHARMM force field and Smart minimization algorithm [19]. Perform 2,000 steps with a root mean square gradient tolerance of 12.277, and the final root mean square gradient is 0.690. The binding site is determined using the prepared protein. The BX-795 binding site was used to determine the active site of docking. Through LibDock, all the prepared ligands were docked at the specified active sites for virtual analysis. According to LibDock score, rank and group all docking positions.

Absorption, Distribution, Metabolism, and Excretion and Toxicity Prediction

The ADME module of Discovery Studio 4.5 was used to calculate the absorption, distribution, metabolism and excretion (ADME) of the candidate compounds, including water-solubility, ability to penetrate the blood-brain barrier, human gastrointestinal tract absorption, hepatotoxicity, plasma protein binding level, and cytochrome P450 2D6(CYP2D6) inhibition. Pharmacological properties such as toxicity of all possible compounds were calculated using the TOPKAT (toxicity prediction by Komputer assistive technology) module. These properties should be fully considered when selecting suitable candidate drugs for PDK1.

Molecule Docking

The CDOCKER module in Discovery Studio 4.5 is used for molecular docking research. CDOCKER is a molecular docking method based on CHARMM force field, which can produce high-precision docking results. The receptor remains rigid and the ligand is allowed to bend during the docking process. Each complex pose requires a calculation of the CHARMM energy (interaction energy plus ligand strain) and interaction energy of the ligand-binding affinity. The crystal structure of PDK1 was obtained from the protein database. Normally, crystalline water molecules are removed in the process of rigid and semi-flexible docking [20, 21], excluding the effect of fixed water molecules on the conformation of the receptor-ligand complex. And then you add hydrogen to the protein. In order to prove the reliability of the combined pattern, the initial inhibiting compound bx-795 was extracted from the binding site and compared with the crystal structure connected to PDK1. PDK1 binding site is defined as the region within the radius of 5-a of the geometric center of mass of the ligand bx-795. During the docking, the ligand is combined with the group residue of the binding site. The

identified structure is buttoned in the combined pocket of PDK1. CDOCKER process was performed, and different positions of each ligand-PDK1 complex were analyzed.

Molecular Dynamics Simulation

In the prediction of the molecular docking program, the optimal binding conformation of ligand-pdk1 complex should be selected and prepared for molecular dynamics simulation. The receptor-ligand complex was placed in a rhombic box and an explicit periodic boundary solvated water model was used. To simulate the ecological environment, sodium chloride was added to the system and the ionic strength was 0.145. Then, the system is placed in the CHARMM force field, and the system is relaxed by minimizing the energy (the fastest descent is 500 steps, and the conjugate gradient is 500 steps), so that the RMS gradient is 0.227. The system was slowly driven from the initial temperature of 296K to the target temperature of 302K for a total of 2ps, and the equilibrium simulation of 5ps was carried out. The molecular dynamics simulation (production) lasted for 30ps and the time step was 1fs. The simulation was carried out at a constant temperature and atmospheric pressure close to 300K. Then, the Ewald algorithm was used to calculate the remote static electricity, and the linear constraint algorithm was used to fix all the hydrogen bonds involved. The trajectory of potential energy, root-mean-square deviation and structural characteristics is determined through the trajectory protocol of Discovery Studio 4.5 with the initial complex Settings as the reference.

Result

Virtual Screening of Natural Products Database Against PDK1

The catalytic ATP binding site is an important regulatory site of PDK1. By blocking the binding site to ATP, the abnormal activation of the downstream PI3K-AKT-mTOR signaling pathway can be blocked to prevent the occurrence of tumors. Therefore, this site is selected as the reference site. The molecules of 17,931 listed biological genes were extracted from the ZINC15 database. The pharmacological properties of these compounds were compared and analyzed using PDK1 as a receptor protein. Bx-795 was selected as the positive drug for control. After a series of screenings, 6374 compounds could bind PDK1 stably. Table 1 lists the top 20 compounds.

Table 1
Top 20 Ranked Compounds with Higher LibDock Scores

Number	Compounds	Libdock Score
1	ZINC000013374322	175.288
2	ZINC000035271475	155.276
3	ZINC000002528510	152.963
4	ZINC000001577210	151.351
5	ZINC000002525131	147.97
6	ZINC000002528486	147.583
7	ZINC000002528509	147.23
8	ZINC000031156069	144.352
9	ZINC000019340795	144.089
10	ZINC000100064387	143.721
11	ZINC000003831331	143.241
12	ZINC000003819461	142.374
13	ZINC000004098820	142.209
14	ZINC000002526388	141.936
15	ZINC000005999135	141.04
16	ZINC000004073899	140.138
17	ZINC000004654841	140.039
18	ZINC000034189841	138.985
19	ZINC000014592909	138.77
20	ZINC000031167746	138.488

ADME and Toxicity Prediction

Using the ADME module of Discovery Studio 4.5, the pharmacological properties of the bx-795 and 20 candidate compounds were predicted, including aqueous-solubility, ability to cross the blood-brain barrier, CYP2D6 inhibition, hepatotoxicity, human-intestinal absorption level, and ability to bind to plasma proteins (Table 2). According to the predicted results, all the compounds except ZINC000005999135 were soluble in water. CYP2D6 is an essential enzyme for drug metabolism, and five compounds, ZINC000004654841, ZINC000004098820, ZINC00064387, ZINC000002528486 and ZINC00000000167746, are CYP2D6 inhibitors. In terms of hepatotoxicity, 8 compounds were similar to bx-795 with toxicity, and the remaining 12 were nontoxicity. In terms of intestinal absorption, ZINC000005999135, ZINC000003831331 and

ZINC000002525131 could hardly be absorbed. ZINC000004073899, ZINC000031156069, ZINC000013374322 and other six compounds have weak binding to plasma proteins.

Table 2
Adsorption, Distribution, Metabolism, and Excretion Properties of Compounds

number	Compounds	Solubility Level	BBB Level	CYP2D6	Hepatotoxicity	Absorption Level	PPB Level
1	ZINC000001577210	2	2	0	0	0	0
2	ZINC000003819461	3	3	0	0	0	1
3	ZINC000004654841	2	4	1	1	0	1
4	ZINC000034189841	2	1	0	0	0	1
5	ZINC000035271475	3	4	0	0	1	1
6	ZINC000004098820	2	2	1	1	0	1
7	ZINC000100064387	2	4	1	1	0	1
8	ZINC000004073899	4	4	0	1	2	0
9	ZINC000031156069	2	2	0	0	0	0
10	ZINC000013374322	4	4	0	0	2	0
11	ZINC000005999135	0	4	0	0	3	1
12	ZINC000002528509	3	3	0	0	0	0
13	ZINC000002526388	3	3	0	0	0	1
14	ZINC000002528486	2	4	1	1	0	1
15	ZINC000002528510	3	3	0	0	0	1
16	ZINC000019340795	4	4	0	1	1	0
17	ZINC000003831331	0	4	0	0	3	1
18	ZINC000014592909	3	3	0	1	0	0
19	ZINC000002525131	1	4	0	0	3	1
20	ZINC000031167746	2	4	1	1	2	1

BBB, blood-brain barrier; CYP2D6, cytochrome P-450 2D6; PPB, plasma protein binding

Aqueous-solubility level: 0, extremely low; 1, very low, but possible; 2, low; 3, good.

BBB level: 0, very high penetrant; 1, high; 2, medium; 3, low; 4, undefined.

CYP2D6 level: 0, noninhibitor; 1, inhibitor.

Hepatotoxicity: 0, nontoxic; 1, toxic.

Human-intestinal absorption level: 0, good; 1, moderate; 2, poor; 3, very poor.

PPB: 0, absorbent weak; 1, absorbent strong.

number	Compounds	Solubility Level	BBB Level	CYP2D6	Hepatotoxicity	Absorption Level	PPB Level
21	bx-795	2	4	0	1	0	1
BBB, blood-brain barrier; CYP2D6, cytochrome P-450 2D6; PPB, plasma protein binding							
Aqueous-solubility level: 0, extremely low; 1, very low, but possible; 2, low; 3, good.							
BBB level: 0, very high penetrant; 1, high; 2, medium; 3, low; 4, undefined.							
CYP2D6 level: 0, noninhibitor; 1, inhibitor.							
Hepatotoxicity: 0, nontoxic; 1, toxic.							
Human-intestinal absorption level: 0, good; 1, moderate; 2, poor; 3, very poor.							
PPB: 0, absorbent weak; 1, absorbent strong.							

In addition to pharmacological properties, safety should also be fully considered in this study. Using the TOPKAT module of Discovery Studio 4.5, toxicity indicators for 20 compounds and the bx-795 were determined, such as rodent carcinogenicity (based on the NTP dataset), developmental toxicity potential properties, and Ames mutagenesis (Table 3). According to the results, 13 compounds had no mutagenesis and 3 compounds had no toxicity. In both rats and mice, the bx-795 was not predicted to be rodent carcinogenic. In conclusion, ZINC000001577210 and ZINC000034189841 are ideal lead compounds without CYP2D6 inhibition. Compared with other compounds, ZINC000001577210 and ZINC000034189841 were easy to cross the blood-brain barrier, free of hepatotoxicity, and easy to be absorbed through the intestinal tract. Especially, ZINC000001577210 had very low Ames mutagenicity and no developmental toxicity. Therefore, ZINC000001577210 and ZINC000034189841, which are relatively safe candidates, can be used for follow-up studies. (Fig. 2)

Table 3
Toxicities of Compounds

		Mouse NTP		Rat NTP			
Number	Compounds	Female	Male	Female	Male	Ames	DTP
1	ZINC000013374322	0.002	0	1	0.015	0	0.095
2	ZINC000035271475	0.993	0	1	0.989	0.739	1
3	ZINC000002528510	0.999	0.036	0	0.999	0.999	0.769
4	ZINC000001577210	0	0.173	0	0.952	0	0.04
5	ZINC000002525131	0	1	1	1	0	1
6	ZINC000002528486	0.603	0.001	0	0.535	0.996	0.019
7	ZINC000002528509	0.999	0.041	0	0.999	0.999	0.745
8	ZINC000031156069	0.198	1	0	0.022	0.081	1
9	ZINC000019340795	0.782	0.205	1	0.659	0	0.998
10	ZINC000100064387	1	0	0.997	0.001	0	0.999
11	ZINC000003831331	1	1	0	0.993	0	1
12	ZINC000003819461	0.997	0	0	0.966	0.003	1
13	ZINC000004098820	0.989	0	1	0.265	0.977	1
14	ZINC000002526388	0.999	0.041	0	0.999	0.999	0.745
15	ZINC000005999135	0	0.001	0.073	0	0	1
16	ZINC000004073899	0.615	1	1	0.015	0.09	0.938
17	ZINC000004654841	1	1	0	0.994	0.019	1
18	ZINC000034189841	0.999	0	1	0.268	0.97	1
19	ZINC000014592909	1	0.999	1	0.999	0	1
20	ZINC000031167746	0.001	1	1	1	0	1
21	bx-795	0	0.007	0	0.064	0	1
NTP, U.S. National Toxicology Program; DTP, developmental toxicity potential.							
NTP < 0.3(noncarcinogen);>0.8(carcinogen).							
Ames < 0.3(nonmutagen);>0.8(mutagen).							
DTP < 0.3(nontoxic);>0.8(toxic).							

Analysis of Ligand Binding

Using the CDOCKER module of Discovery Studio 4.5, ZINC000001577210 and ZINC000034189841 were docked in the molecular structure of PDK1 to study the ligand-binding mechanism of these two lead compounds and bx-795. Calculate the CDOCKER potential energy, as can be seen from Table 4, ZINC000001577210 and ZINC000034189841 have similar potential energy to the drug bx-795 (59.694 kcal/mol), indicating that the binding affinity of these two compounds with PDK1 resembles that of bx-795. By computing research also has carried on the hydrogen bond structure and π-related interactions (Figure 3,Figure 4). According to the hydrogen bond analysis results (Table 5), ZINC000001577210 formed 3 pairs of hydrogen bonds with PDK1, by the O3 of the compound with ASP223:HN of PDK1, H31 of the compound with ASP223:HN of PDK1 and H35 of the compound with ASP223:HN. 6 pairs of hydrogen bonds were formed between ZINC000034189841 and PDK1, by the O9 of the compound with LYS111:HZ2 of PDK1, H38 of the compound with GLY91:O of PDK1, H39 of the compound with GLY91:O of PDK1, etc. For the positive drug bx-795, it formed 11 pairs of hydrogen bonds with PDK1. According to the analysis and calculation, the hydrogen bonds formed between bx-795 and PDK1 are more than these two candidate compounds, but the bonds are almost longer, so the stability of these two compounds still needs further study and analysis. In addition to the hydrogen bonding π-related interactions of compounds and PDK1 were formed (Table 6). LYS111 residue of ZINC000034189841 only formed one pair of π-related interactions with PDK1, respectively ZINC000001577210 formed 4 pairs of π-related interactions with PDK1, and positive drug bx-795 formed 14 pairs of π-related interactions with PDK1.

Table 4
CDOCKER Potential Energy of Compounds with PDK1

Compounds	-CDOCKER Potential Energy (kcal/mol)
ZINC000001577210	64.9018
ZINC000034189841	50.2122
PDK1	59.694

Table 5
Hydrogen Bond Interaction Parameters for Each Compound with PDK1 Residues

Receptor	Compound	Donor Atom	Receptor Atom	Distances (Å)
3nax	ZINC000001577210	ASP223:HN	ZINC000001577210:O3	2.83
		ZINC000001577210:H31	ASP223:O	2.95
		ZINC000001577210:H35	ASP223:O	2.16
	ZINC000034189841	LYS111:HZ2	ZINC000034189841:O9	1.66
		ZINC000034189841:H38	GLY91:O	1.91
		ZINC000034189841:H39	GLY91:O	2.51
		ZINC000034189841:H44	ASP223:O	1.94
		TYR126:HH	ZINC000034189841	2.80
		SER92:HB1	ZINC000034189841:O15	2.51
	PDK1	Molecule:H42	VAL143:O	1.97
		Molecule:H37	MET134:O	2.25
		Molecule:H40	VAL143:O	2.47
		Molecule:H40	Molecule:I29	2.89
		ASP223:HN	Molecule:O1	2.44
		Molecule:H35	VAL143:O	2.28
		Molecule:H47	ASP223:O	1.96
		THR222:HA	Molecule:O1	2.21
		Molecule:H37	VAL143:O	2.16
		Molecule:H41	VAL143:O	0.89
		Molecule:H49	ASP223:O	2.24
		Molecule:H54	PHE224:O	2.68
		Molecule:H55	GLY91:O	2.88

Table 6
π-Related Interaction Parameters for Each Compound with PDK1

Receptor	Compound	Donor Atom	Receptor Atom	Distances (Å)
3nax	ZINC000001577210	VAL96:HG11	ZINC000001577210	2.78
		ZINC000001577210	ALA109	4.21
		ZINC000001577210	LYS111	4.88
		ZINC000001577210	VAL143	5.29
	ZINC000034189841	ZINC000034189841	LYS111	4.47
	PDK1	TYR126	Molecule	4.19
		A:PHE224	Molecule	5.09
		Molecule	PHE142	4.91
		A:LEU88	Molecule	5.31
		VAL96	Molecule	4.75
		ALA109	Molecule	3.81
		VAL143	Molecule	5.1
		Molecule:I29	LEU145	4.5
		Molecule	LEU159	4.57
		Molecule	VAL96	5.48
		Molecule	LYS111	4.61
		Molecule	LEU159	5.44
		Molecule	ALA227	5.04
		Molecule	LEU196	5.08

Molecular Dynamics Simulation

In order to evaluate the stability of the ligand-PDK1 complex in a natural environment, a molecular dynamics simulation module was carried out. CDOCKER module was used to obtain the original conformation from the molecular docking experiment. The potential energy diagram and RMSD curve of each compound are shown in Fig. 5. After 15ps, the trajectories of each complex reached equilibrium, and the RMSD curve and potential energy of these complexes were stable with time. The potential energy of ZINC000001577210-PDK1 complex is lower than that of ZINC000001577210-PDK1 complex, indicating that ZINC000001577210 is more stable. Molecular dynamics simulation results show that these compounds and PDK1 through hydrogen bonding and π-related interactions combination, contribute to the stability of these complexes. In conclusion, both of the two compounds can interact with PDK1, and the complexes can stably exist in the natural environment and have a regulatory effect on PDK1.

Discussion

Glioma is the most aggressive and malignant type of brain tumor and accounts for the majority of brain cancer deaths. Therefore, research on its prevention and treatment is extremely important[22]. PDK1, a member of the AGC protein kinase family, activates the PI3K-AKT-mTOR signaling pathway. Overexpression of PDK1 activates this pathway, which can lead to the development and progression of cancer. At present, there are many studies on the inhibitors of PDK1 catalytic ATP binding site. Although great progress has been made in the design and development of PDK1 inhibitor, only bx-795 was selected as a positive drug in this study. Currently, the bx-795 has been further studied, but due to the limitations of this drug, more PDK1 inhibitors need to be screened for clinical treatment.

Inhibiting excessive activation of PDK1 has the potential to treat tumors, but the currently studied inhibitor bx-795 has obvious limitations. First, bx-795 is not a specific inhibitor of PDK1. Compared with PDK1, bx-795 has a more significant inhibiting effect on TBK1 and IKKϵ. Bx-795 is the most effective inhibitor of these two protein kinases. When using bx-795 to inhibit PDK1, the physiological effect of TBK1 and IKKϵ may be affected severely [23, 24]. Secondly, according to the experimental results of this study, the bx-795 has poor water-solubility and hepatotoxicity, which indicates that the high dose of bx-795 has a small inhibitory effect on PDK1, but produces more serious liver diseases. Therefore, it is extremely urgent to screen more safe and efficient PDK1 inhibitors.

In this study, 17931 compounds for sale were extracted from the ZINC database for virtual screening, and then LIBDOCK, ADME, TOPKAT, CDOCKER and molecular dynamics simulation were conducted. The LibDock score showed the degree of energy optimization and conformational stability. Compounds with a high LibDock score had better energy optimization and conformational stability[25, 26]. Through the calculation of LibDock module, it was found that 6374 compounds could combine with PDK1 stably. According to the LibDock score, the top 20 compounds were selected for research and calculation.

ADME and toxicity analyses were performed on 20 compounds and positive drugs to assess their pharmacological properties. The results showed that ZINC000001577210 and ZINC000034189841 were ideal lead compounds. Compared with other compounds, these two compounds have no CYP2D6 inhibition, easy to pass the blood-brain barrier, and no hepatotoxicity. They are high-quality drug candidates with great potential in the clinical application of cancer treatment. Although the remaining 18 compounds have toxicity or other risk factors, they also have the potential for drug development and clinical application by regulating certain groups or atoms to reduce negative effects. In conclusion, ZINC000001577210 and ZINC000034189841 are ideal lead compounds for further analysis.

CDOCKER module was used to study the chemical bonds and energy of lead compounds. CDOCKER module calculation shows that the potential energy of ZINC000034189841 and ZINC000001577210 is similar to that of BX-795, indicating that the binding affinity of these two compounds with PDK1 resembles that of bx-795.

Finally, molecular dynamics simulation was carried out to evaluate the stability of the two compounds in the natural environment. According to the results of RMSD and potential energy of these ligand-PDK1 complexes, the trajectories of these complexes reached equilibrium after 15ps, and the RMSD curve and potential energy were stable over time, thus proving that these two complexes could stably exist in the natural environment. Modification of these compounds may result in a more stable structure of complexes. In conclusion, the two compounds found in this study have appropriate pharmacological properties, high affinity with PDK1, and good stability in the natural environment, and have great potential for PDK1-related drug development.

Nowadays, the design and development of oncology drugs have attracted much attention, but progress is slow. This study shows that the most important thing in drug design is to screen ideal lead compounds. In this study, five modules of LibDock, ADME, TOPKAT, CDOCKER and molecular dynamics simulation of Discovery Studio 4.5 were used to analyze and screen candidate compounds. Through the analysis of the pharmacological properties, binding affinity and stability of these two lead compounds, it is found that they are indeed due to the positive drug BX-795. Through a series of simulations and calculations, ZINC000001577210 and ZINC000034189841 are the most promising drugs for the treatment of glioma. However, it should be noted that a lot of improvement, optimization and experimental verification are needed before these drugs can be marketed. In addition, the research methods of this study play a guiding role in the screening of lead compounds for cancer treatment.

Although accurate simulation analysis and calculation are carried out in this study, there are still some limitations. The screened lead compounds still need to prove their effects in further studies such as animal experiments, and more indicators, such as IC50, need to be evaluated to further improve and optimize the drugs.

Conclusion

In this study, a series of computer-aided structures and chemical techniques such as virtual screening, ADME, molecular docking, toxicity prediction, and molecular dynamics simulation were used to screen out the lead compounds inhibiting PDK1 function. Through analysis and calculation, ZINC000001577210 and ZINC000034189841 are safe drug candidates with high potential. In addition, some candidate compounds with obvious pharmacological properties were selected, which provided a necessary premise for drug design and optimization of PDK1 or other proteins.

Declarations

Conflict of interest

The authors declare no conflict of interest.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding on reasonable request.

Author contributions

Sheng Zhong and Zhen Guo designed experiments; Gaojing Dou, Xiaye Lv and Xinhui Wang wrote the manuscript; Bo Wu, Hongyu Wang, Yuting Jiang and Xiaoxia Liu carried out experiments; Shuya Hou, Luodan Liang, Zhenghe Chen, Yonggao Mou and analyzed experiments results.Zhenghe Chen, Yonggao Mou and Gaojing Dou revised the manuscript, figures and tables.

References

1. Chen R, Smith-Cohn M, Cohen AL, Colman H. Glioma Subclassifications and Their Clinical Significance. Neurotherapeutics. 2017;14(2):284–97. doi:10.1007/s13311-017-0519-x.
2. Braunstein S, Raleigh D, Bindra R, Mueller S, Haas-Kogan D. Pediatric high-grade glioma: current molecular landscape and therapeutic approaches. J Neurooncol. 2017;134(3):541–9. doi:10.1007/s11060-017-2393-0.
3. Ceylan H. Identification of hub genes associated with obesity-induced hepatocellular carcinoma risk based on integrated bioinformatics analysis. Med Oncol. 2021;38(6):63. doi:10.1007/s12032-021-01510-0.
4. Emmanouilidi A, Falasca M. Targeting PDK1 for Chemosensitization of Cancer Cells. Cancers (Basel). 2017;9(10). doi:10.3390/cancers9100140.
5. Biondi RM. Phosphoinositide-dependent protein kinase 1, a sensor of protein conformation. Trends Biochem Sci. 2004;29(3):136–42. doi:10.1016/j.tibs.2004.01.005.
6. Xu X, Chen Y, Fu Q, Ni D, Zhang J, Li X, et al. The chemical diversity and structure-based discovery of allosteric modulators for the PIF-pocket of protein kinase PDK1. J Enzyme Inhib Med Chem. 2019;34(1):361–74. doi:10.1080/14756366.2018.1553167.
7. Velpula KK, Guda MR, Sahu K, Tuszynski J, Asuthkar S, Bach SE, et al. Metabolic targeting of EGFRvIII/PDK1 axis in temozolomide resistant glioblastoma. Oncotarget. 2017;8(22):35639–55. doi:10.18632/oncotarget.16767.
8. Karar J, Maity A. PI3K/AKT/mTOR Pathway in Angiogenesis. Front Mol Neurosci. 2011;4:51. doi:10.3389/fnmol.2011.00051.
9. Yang C, Huang X, Liu H, Xiao F, Wei J, You L, et al. PDK1 inhibitor GSK2334470 exerts antitumor activity in multiple myeloma and forms a novel multitargeted combination with dual mTORC1/C2 inhibitor PP242. Oncotarget. 2017;8(24):39185–97. doi:10.18632/oncotarget.16642.
10. Yagi M, Kantarci A, Iwata T, Omori K, Ayilavarapu S, Ito K, et al. PDK1 regulates chemotaxis in human neutrophils. J Dent Res. 2009;88(12):1119–24. doi:10.1177/0022034509349402.

11. Signore M, Pelacchi F, di Martino S, Runci D, Biffoni M, Giannetti S, et al. Combined PDK1 and CHK1 inhibition is required to kill glioblastoma stem-like cells in vitro and in vivo. Cell Death Dis. 2014;5(5):e1223. doi:10.1038/cddis.2014.188.
12. Barile E, De SK, Pellecchia M. PDK1 inhibitors. Pharm Pat Anal. 2012;1(2):145–63. doi:10.4155/ppa.12.17.
13. Feldman RI, Wu JM, Polokoff MA, Kochanny MJ, Dinter H, Zhu D, et al. Novel small molecule inhibitors of 3-phosphoinositide-dependent kinase-1. J Biol Chem. 2005;280(20):19867–74. doi:10.1074/jbc.M501367200.
14. Dangelmaier C, Manne BK, Liverani E, Jin J, Bray P, Kunapuli SP. PDK1 selectively phosphorylates Thr(308) on Akt and contributes to human platelet functional responses. Thromb Haemost. 2014;111(3):508–17. doi:10.1160/th13-06-0484.
15. Li J, Zhang K, Yi N. A Bayesian hierarchical model for detecting haplotype-haplotype and haplotype-environment interactions in genetic association studies. Hum Hered. 2011;71(3):148–60. doi:10.1159/000324841.
16. Newman DJ. Developing natural product drugs: Supply problems and how they have been overcome. Pharmacol Ther. 2016;162:1–9. doi:10.1016/j.pharmthera.2015.12.002.
17. Ceylan H, Budak H, Kocpinar EF, Baltaci NG, Erdogan O. Examining the link between dose-dependent dietary iron intake and Alzheimer's disease through oxidative stress in the rat cortex. J Trace Elem Med Biol. 2019;56:198–206. doi:10.1016/j.jtemb.2019.09.002.
18. Rao SN, Head MS, Kulkarni A, LaLonde JM. Validation studies of the site-directed docking program LibDock. J Chem Inf Model. 2007;47(6):2159–71. doi:10.1021/ci6004299.
19. Brooks BR, Brooks CL 3rd, Mackerell AD Jr, Nilsson L, Petrella RJ, Roux B, et al. CHARMM: the biomolecular simulation program. J Comput Chem. 2009;30(10):1545–614. doi:10.1002/jcc.21287.
20. 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. doi:10.1371/journal.pone.0074761.
21. 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. J Comput Aided Mol Des. 2015;29(1):89–100. doi:10.1007/s10822-014-9807-2.
22. Ludwig K, Kornblum HI. Molecular markers in glioma. J Neurooncol. 2017;134(3):505–12. doi:10.1007/s11060-017-2379-y.
23. Bain J, Plater L, Elliott M, Shpiro N, Hastie CJ, McLauchlan H, et al. The selectivity of protein kinase inhibitors: a further update. Biochem J. 2007;408(3):297–315. doi:10.1042/bj20070797.
24. McIver EG, Bryans J, Birchall K, Chugh J, Drake T, Lewis SJ, et al. Synthesis and structure-activity relationships of a novel series of pyrimidines as potent inhibitors of TBK1/IKKε kinases. Bioorg Med Chem Lett. 2012;22(23):7169–73. doi:10.1016/j.bmcl.2012.09.063.
25. Ceylan H. A bioinformatics approach for identifying potential molecular mechanisms and key genes involved in COVID-19 associated cardiac remodeling. Gene Rep. 2021;24:101246.

doi:10.1016/j.genrep.2021.101246.

26. Işık M, Demir Y, Durgun M, Türkeş C, Necip A, Beydemir Ş. Molecular docking and investigation of 4-(benzylideneamino)- and 4-(benzylamino)-benzenesulfonamide derivatives as potent AChE inhibitors. Chem Pap. 2020;74(5):1395–405. doi:10.1007/s11696-019-00988-3.

Figures

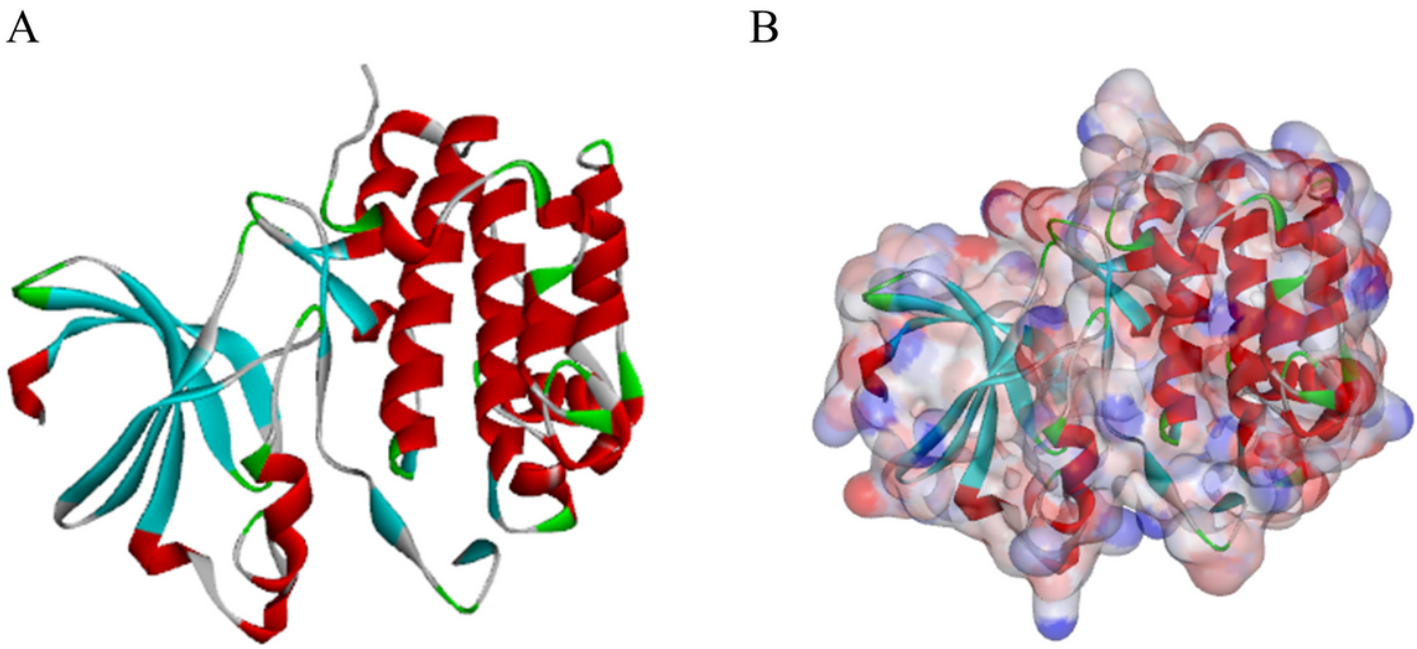

Figure 1

Molecular structure of 3-phosphoinositide-dependent protein kinase 1(PDK1). (A) Initial molecular structure. (B) Surface of the binding area added. Blue represents a positive charge, and red represents a negative charge.

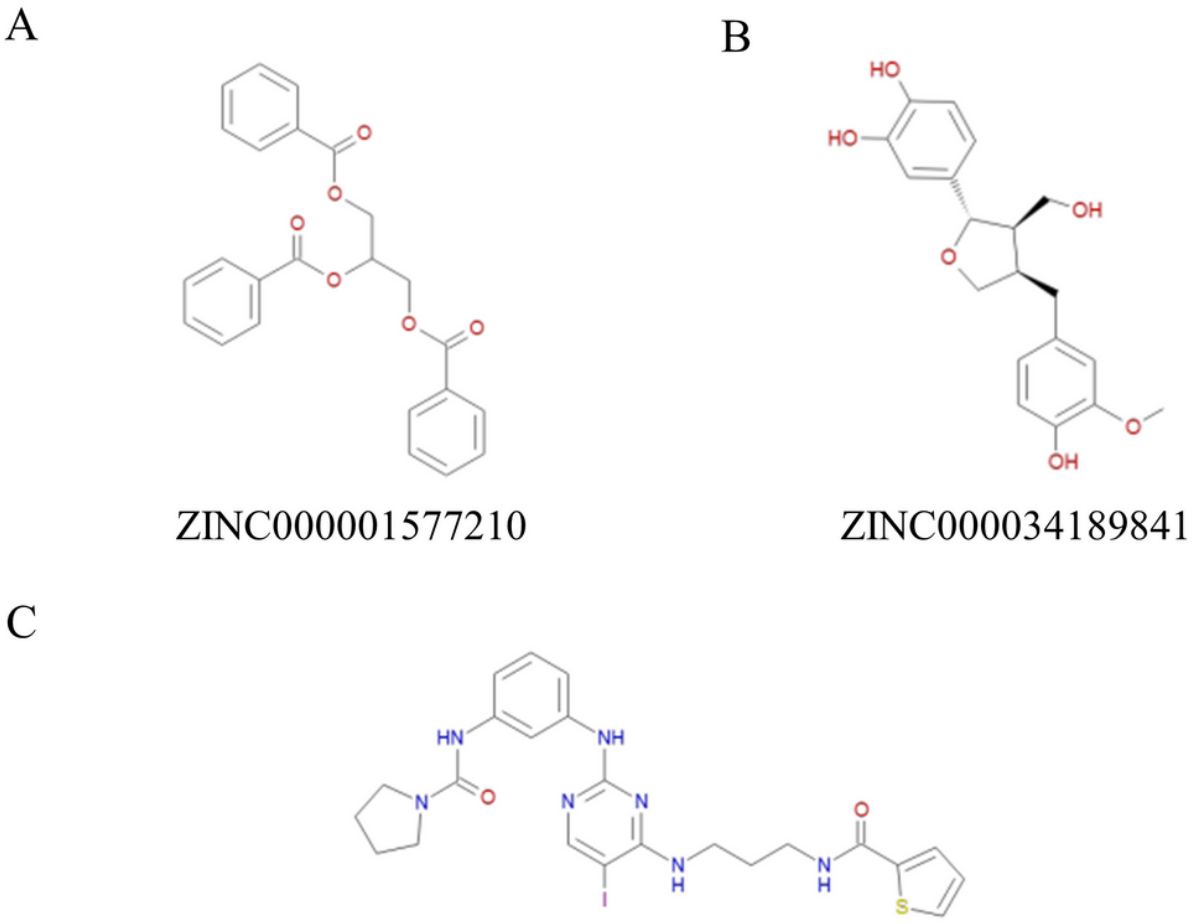

Figure 2

Structures of novel compounds selected from virtual screening and bx-795.

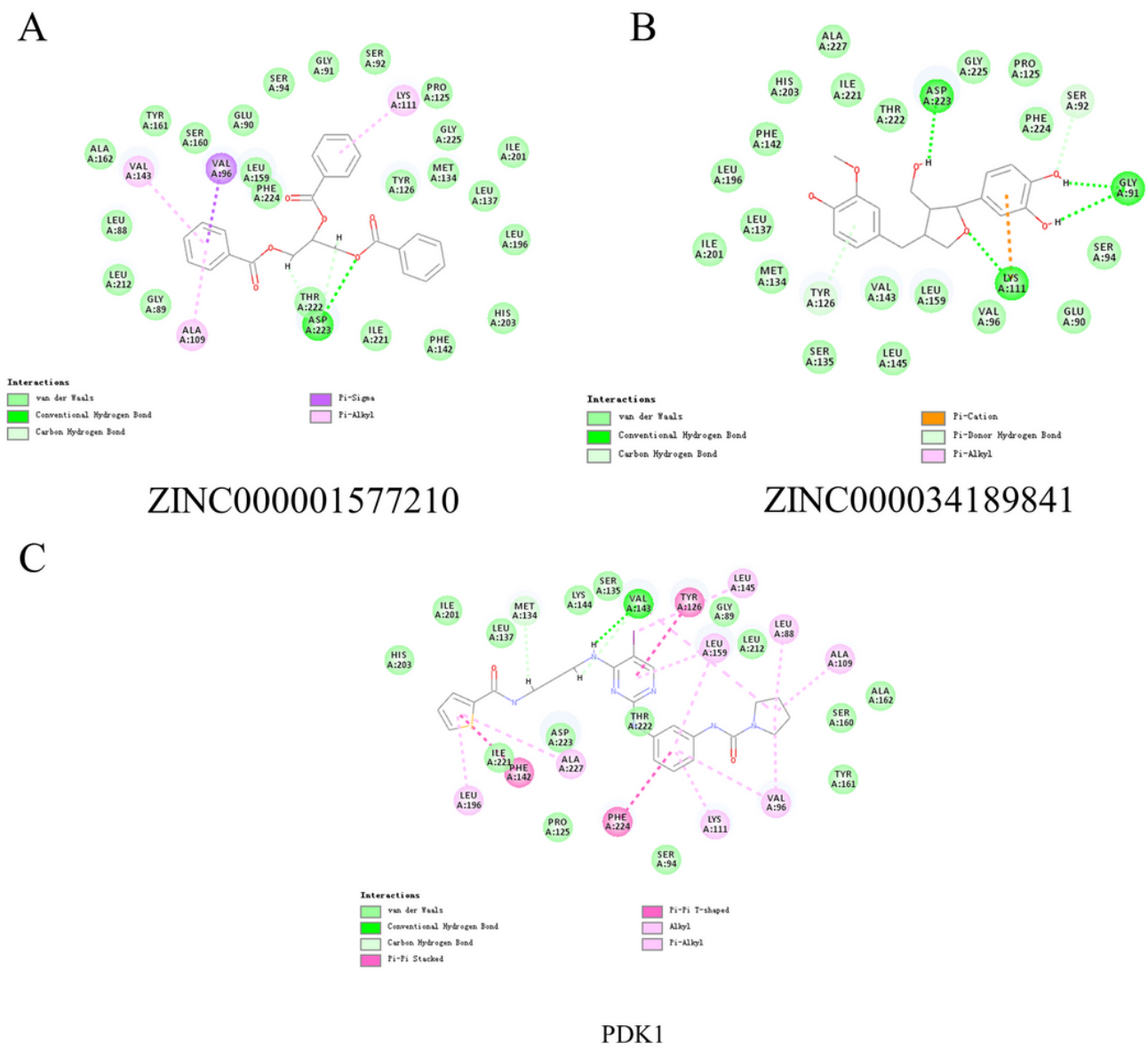

Figure 3

Schematic drawing of interactions between ligands and PDK1. The surface of the binding areas was added. Blue represents positive charge; red represents negative charge; and ligands are shown in sticks, with the structure around the ligand-receptor junction shown in thinner sticks. (A) ZINC000001577210-PDK1 complex. (B) ZINC000034189841-PDK1 complex.

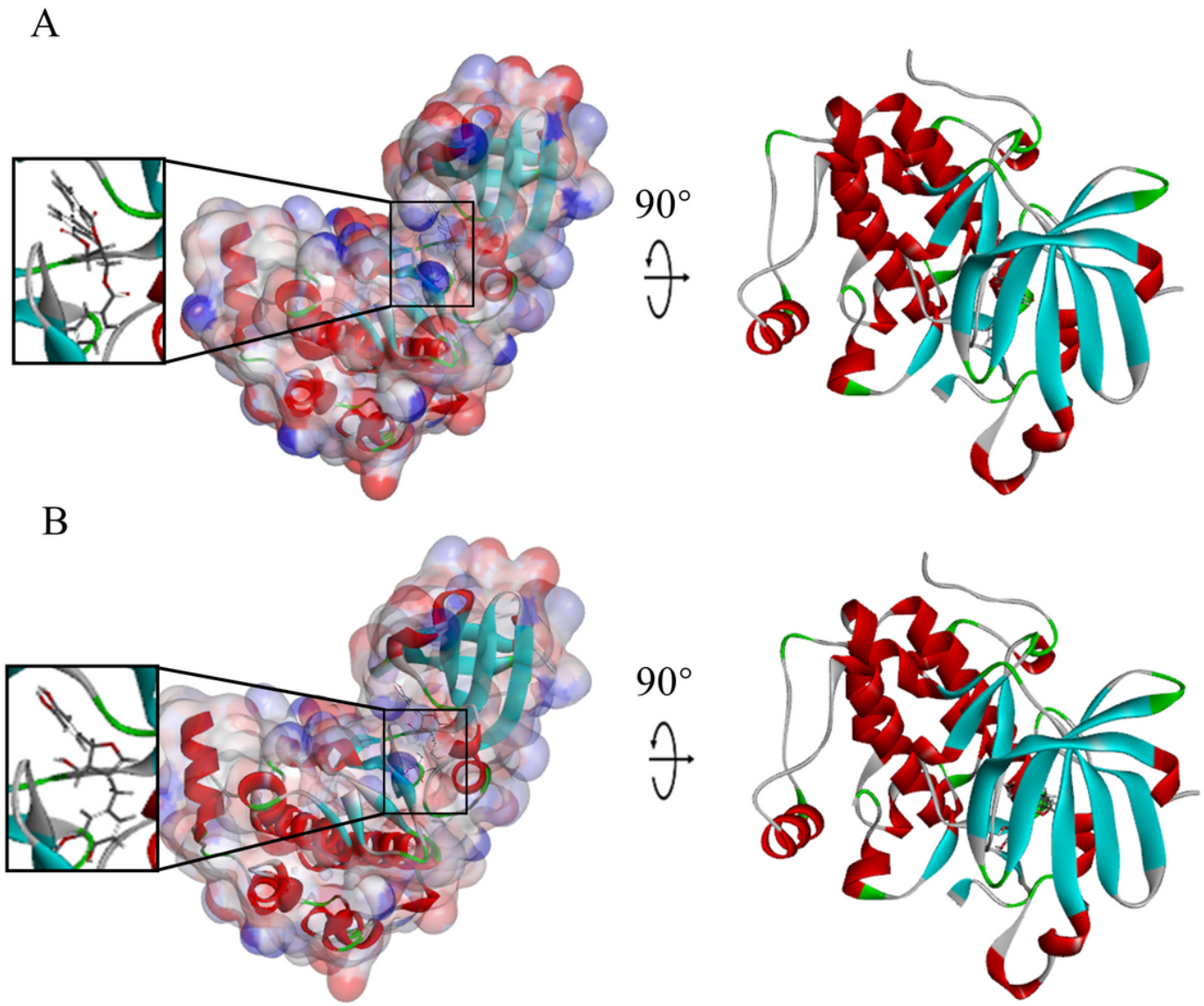

Figure 4

Schematic of intermolecular interaction of the predicted binding modes of (A) ZINC000001577210 with PDK1, (B) ZINC000034189841 with PDK1, and (C) bx-795 with PDK1.

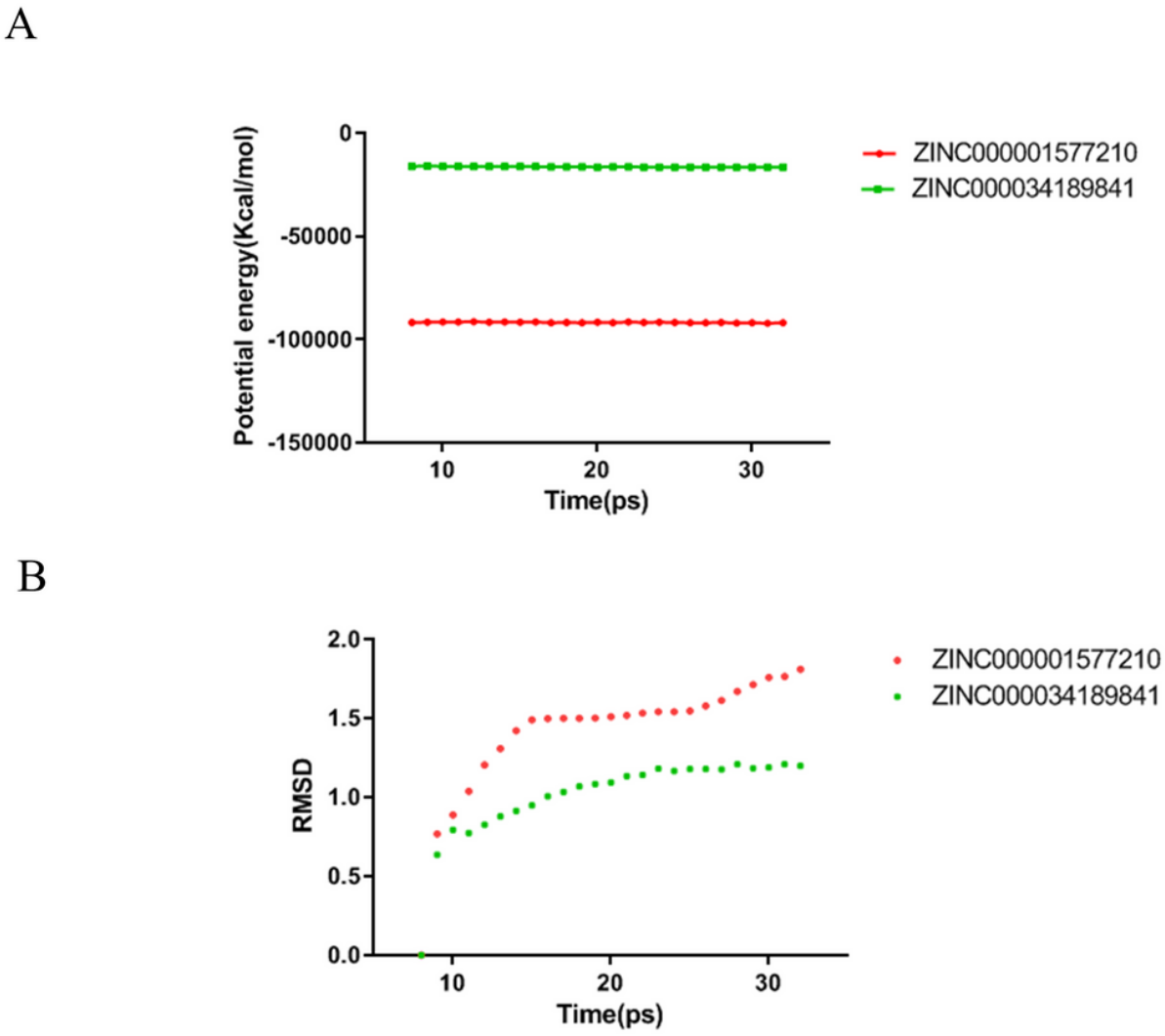

Figure 5

Results of molecular dynamics simulation of the compounds ZINC000001577210 and ZINC000034189841. (A) Potential energy, RMSD, root-mean-square deviation. (B) Average backbone root-mean-square deviation.