Novel Natural Inhibitors Targeting AKT1 by Computational Study

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Research Article

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Abstract

Object: This study was designed to screen ideal lead compounds and drug candidates with an inhibitory effect on AKT1 from the drug library (ZINC database).

Methods: A battery of computer-aided virtual techniques were used to identify potential inhibitors of AKT1. LibDock is used for structure-based screening followed by ADME (absorption distribution, metabolic excretion) and toxicity prediction. Molecular dynamics simulations were used to evaluate the stability of ligand-receptor complexes.

Results: Two new natural compounds ZINC000049872065 and ZINC000021992902 were found to bind to AKT1 in the ZINC database, showing a higher binding affinity. Also, they were predicted to have lower rodent carcinogenicity, Ames mutagenicity, developmental toxicity potential, and high tolerance to cytochrome P4502D6. Molecular dynamics simulation shows that ZINC000049872065 and ZINC000021992902 have more favorable potential energies with AKT1, which can exist stably in the natural environment.

Conclusion: This study suggests that ZINC000049872065 and ZINC000021992902 are ideal potential inhibitors of AKT1 targeting. These compounds are safe drug candidates and have important implications for the design and improvement of C-MET target drugs.

Introduction

Many cancers (including ovarian, lung, and pancreatic cancers) have been reported associated with abnormal overexpression or activation of AKT, which is concerned with increased cancer cell proliferation and survival[1]. AKT, also known as protein kinase B (PKB), is a serine/threonine kinase that belongs to the AGC (AGC kinases) family of kinases. Besides, AKT is a central signaling node of the PAM(PI3K/AKT/mTOR) pathway which is a well-known pathway in the regulation of tumorigenesis [2]. Among all three AKT isoforms (AKT1, AKT2 and AKT3), AKT1 is critical in various cell physiological activities. These constantly activated activities in multifarious tumors include transcription, apoptosis, glucose metabolism, cell proliferation, and cell migration [3]. AKT1 involved in the local tumor growth and AKT2 involved in the distant tumor dissemination, while AKT3 has a poorer prognostic value [4]. In the breast, the role of AKT3 has been less studied so far, but it seems to have a more preponderant role in triple negative tumors [5].

Dysregulation of the PI3K/AKT pathway cooperates with many human malignancies. For example, mutation of AKT-activated is contributed to increase the risk of colon cancer. Activated AKT signaling promotes mitotic arrest, polyploidy, and hepatocellular carcinoma; hyperactivation of AKT depletes hematopoietic stem cells and induces leukemia in mice. AKT, as the major regulatory factor in PI3K/AKT signaling pathway, once activated, phosphorylates its myriad substrates via its kinase activity [6]. In summary, the AKT family members influence many functions of tumor cell including transcription, apoptosis, glucose metabolism, cell proliferation, and cell migration, especially AKT1. It plays an important role in invasion and proliferation of tumor cell. Therefore, the selection of effective AKT1 inhibitors will plays an important role in drug development and cancer treatment.

Ipatasertib is a highly selective small-molecule inhibitor of AKT. It is in development as a single agent and also in combination with other therapies for the treatment of cancers. Double-blind phase II study of ipatasertib in combination with abiraterone and prednisone/prednisolone showed trends towards improved radiographic progression-free survival (PFS) and overall survival (OS) compared with placebo in patients with mCRPC (metastatic castration-resistant prostate cancer) who had a PTEN loss [7]. Ipatasertib is a great inhibitor of PI3K/AKT1/PTEN-altered tumors, however, AKT2 and AKT3 have a poorer prognostic value compared with AKT1[4,5,8](1). Therefore, AKT1 inhibitors are potential for anti-tumor treatments. This study aimed to screen natural compounds from natural drugs that are more effective than Ipatasertib in treating cancer.

Natural products, as lead compounds, can be transformed into new drugs through appropriate structural modification, which is an important source of new drug research in pharmaceutical industry [9,10]. In recent years, several targeted drugs have been reported to inhibit AKT1[11]. In order to screen and verify lead compounds which can potentially regulate AKT1, a range of structural biological and chemical methods were used in this study. Some properties of these compounds such as absorption, distribution, metabolism, excretion and toxicity were also predicted in our study. Providing the research object for the development of AKT1 inhibitors, we list a series of drug candidates, besides, we have also provided their pharmacological properties.

Methods

Discovery studio software and ligand libraries

Discovery Studio is a suite of software to simulate small and large molecule systems, which is designed to screen, design and modify potential drugs through structural chemistry and structural biology calculations, thereby identifying and refining a wide range of lead compounds and candidate drug approaches [12]. The LibDock and ADME (absorption, distribution, metabolism, excretion) modules of Discovery Studio 4.5 software (DS4.5, Accelrys, Inc.) are applied in virtual screening. CDOCKER is used for docking research. Natural Products (NP) database in the ZINC database was used to screen AKT1 inhibitors as a selection. The Irwin and Shoichet laboratories, which is in the department of pharmaceutical chemistry at the University of California, San Francisco (UCSF) providing the ZINC database as a free commercial compound database.

Use LibDock for structure-based virtual filtering

The ligand-binding pocket region of AKT1 was selected to identify new compounds that might inhibit AKT1 as the binding site. Virtual filtering is performed using the LibDock module of Discovery Studio 4.5[13]. LibDock is a rigid docking program. It uses grids placed at binding sites and polar and non-polar probes to calculate protein hotspots. To form favorable interactions the hotspots are furtherly used to align ligands, as well as the Smart Minimiser algorithm and CHARMM force field (Cambridge, Massachusetts, USA) for ligand minimization. All ligand positions were ranked by ligand scores after minimization. The 2.45Å crystal structure of AKT1 (Protein Data Bank identifier: 3MV5) and Ipatasertib was downloaded from the Protein data bank (PDB) and imported into LibDock's work environment. The chemical structure of AKT1 is shown in **figure 1**. Proteins are made by removing crystalline water and other heteroatoms and then adding hydrogen,

protonation, ionization, and energy minimization. The CHARMM force field and Smart Minimiser algorithm were used to energy minimization [14]. With a root mean square (RMS) gradient tolerance of 12.277, 2000 steps were performed in the minimization with an, which resulted in an RMS gradient of 0.09778. To define binding sites the prepared proteins were used, the Ipatasertib binding site was selected as the active site for docking. By using LibDock, all prepared ligands were docked at defined active sites for virtual screening. According to the LibDock score, all docking positions are sorted and grouped by compound name.

ADME (Absorption, Distribution, Metabolism, and Excretion) and Toxicity Prediction

The ADME module of Discovery Studio 4.5 is used to calculate the absorption, distribution, metabolism, and excretion of selected compounds, also used the DS4.5 TOPKAT (toxicity prediction by Computer assistive technology) module to calculate all potential compounds toxicity and other properties, including its water-soluble, blood-brain barrier (BBB) permeability, cytochrome P4502D6 (CYP2D6), liver toxicity, human intestinal absorption, plasma protein (PPB) levels, rodent carcinogenicity, ames respectively and developmental toxicity potential. These pharmacological properties should be taken into full consideration when selecting AKT1 drug candidates.

Molecular Dynamics Simulation

The best binding conformations of each compounds-3MV5 complex were chosen for molecular dynamics simulation. an orthorhombic box was built for the ligand-receptor complex was put into an orthorhombic box and solvated with an explicit periodic boundary solvation water model. Solidum (ionic strength of 0.145) chloride was poured into the system for the sake of simulating the physiological environment. Then the CHARMM force field and energy minimization were prepared for the system (500 steps of steepest descent and 500 steps of conjugated gradient), with a result showing that the final root means square gradient of 0.227. The system was slowly driven from an initial temperature (296K) to the target temperature(320K) in 2 ps, and equilibration simulations were performed for 5 ps. Molecular dynamics simulation (production module) was run for 25 ps and the time step was 1 fs. The simulation was run with the normal pressure and temperature system (300K) during the process. Long-range electrostatics were calculated by the particle mesh Ewald algorithm, and all bonds involving hydrogen were fixed by the linear constraint solver algorithm. Select initial complex setting as a reference, Discovery Studio 4.5 analysis trajectory protocol was used for a trajectory determined for RMSD, potential energy, and structural characteristics.

Results

Virtual Screening of Natural Products Database Against AKT1

The ligand-binding pocket played a major role in the regulatory sites of AKT1. As a result, we select this pocket region as the reference site. Amount to 17931 named for-sale biogenic molecules were screened from the ZINC15 database. We select AKT1 as the receptor protein in order to compare the pharmacologic properties with other compounds. There are 7764 compounds could dock with AKT1 and the top 20 compounds with higher scores were listed in Table 1.

Table 1
Top 20 Ranked Compounds with LibDock Scores

Number	Compounds	Libdock score
1	ZINC000014951634	168.745
2	ZINC000014951658	166.943
3	ZINC000028968101	145.408
4	ZINC000049872065	141.722
5	ZINC000002509755	140.459
6	ZINC000015122022	140.179
7	ZINC000002528486	139.207
8	ZINC000001531664	138.246
9	ZINC000013328774	137.404
10	ZINC000021992902	135.637
11	ZINC000030725991	134.058
12	ZINC000002526388	133.986
13	ZINC000008844372	133.522
14	ZINC000013378636	133.393
15	ZINC000073280937	132.993
16	ZINC000030730842	132.136
17	ZINC000006528354	132.056
18	ZINC000044281738	131.796
19	ZINC000028882432	131.625
20	ZINC000008214697	131.398

ADME and Toxicity Prediction

ADME module of Discovery Studio 4.5 was used for predicting selected ligands' pharmacologic properties with Ipatasertib (Table 2). From aqueous solubility prediction (defined in water at 25 C), most of the compounds had a good solubility in water. Four compounds had a medium blood-brain barrier level while others were undefined. 70% of the compounds were predicted to be noninhibitors with CYP2D6, which had a great impact on drug metabolism. As for hepatotoxicity, 8 compounds were found to be nontoxic, which was similar to Ipatasertib. Four compounds were found had good human intestinal absorption as Ipatasertib had. Plasma protein binding properties showed 10 compounds had weak absorption.

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

Number	Compounds	Solubility Level	BBB Level	CYP2D6	Hepatotoxicity	Absorption Level	PPB Level
1	ZINC000014951634	3	4	0	0	3	0
2	ZINC000014951658	3	4	0	0	3	0
3	ZINC000028968101	1	4	1	1	3	1
4	ZINC000049872065	3	4	0	0	2	0
5	ZINC000002509755	2	2	1	1	0	1
6	ZINC000015122022	2	4	1	0	2	1
7	ZINC000002528486	2	2	1	1	0	1
8	ZINC000001531664	2	4	0	1	3	0
9	ZINC000013328774	3	4	0	1	3	0
10	ZINC000021992902	3	4	0	0	1	0
11	ZINC000030725991	0	4	0	1	2	0
12	ZINC000002526388	2	4	1	1	0	1
13	ZINC000008844372	3	4	0	1	1	0
14	ZINC000013378636	2	4	1	0	2	1
15	ZINC000073280937	2	4	0	1	2	1
16	ZINC000030730842	4	4	0	1	3	0
17	ZINC000006528354	3	2	0	1	0	1
18	ZINC000044281738	0	4	0	1	3	1
19	ZINC000028882432	3	4	0	0	3	0
20	ZINC000008214697	2	4	0	0	3	1
21	Ipatasertib	2	2	0	0	0	0

Safety had to be greatly considered in this study. In order to make sure the safety of the 20 compounds, a series of toxicity indexes of the compounds and Ipatasertib were detected by using a computational method (Table 3). Results indicated that 12 compounds were found to be non-mutagenic, and 7 compounds were found with no developmental toxicity potential. Ipatasertib was found to have high rodent carcinogenicity in both female mouse and female rat. In consideration of all the above results, we select ZINC000049872065 and ZINC000021992902, because these two were non-CYP2D6 inhibitors, non-hepatotoxic and less

carcinogenic lead compounds in comparison with other compounds. In general, ZINC000049872065 and ZINC000021992902 were chosen as safe drugs and for the following study (Fig. 2).

Table 3
Toxicities of Compounds

Number	Compounds	Mouse NTP		Rat NTP		Ames	DTP
		Female	Male	Female	Male		
1	ZINC000014951634	0.089	0	1	0	0	1
2	ZINC000014951658	1	0	1	0	0	1
3	ZINC000028968101	1	0.021	0.06	0.997	1	1
4	ZINC000049872065	0.353	0	0.752	0.006	0	0
5	ZINC000002509755	0.603	0.001	0	0.535	0.996	0.019
6	ZINC000015122022	0	1	1	0	1	1
7	ZINC000002528486	0.603	0.001	0	0.535	0.996	0.019
8	ZINC000001531664	0.999	1	0	1	0	1
9	ZINC000013328774	0.865	1	1	1	0.674	1
10	ZINC000021992902	0.198	0	0.033	0.251	0	0
11	ZINC000030725991	0	0	0.163	1	0	1
12	ZINC000002526388	0.999	0.041	0	0.999	0.999	0.745
13	ZINC000008844372	1	1	1	1	0	1
14	ZINC000013378636	0	1	1	0	1	1
15	ZINC000073280937	0.312	1	0	0.185	0	1
16	ZINC000030730842	1	1	0.001	0	1	0
17	ZINC000006528354	0.151	0	1	0	0.002	1
18	ZINC000044281738	0	0	1	0	0.007	0
19	ZINC000028882432	0.081	1	0	0.987	0	1
20	ZINC000008214697	0	0.003	0	1	0	0
21	Ipatasertib	1	0	0.985	0	0	1

Analysis of Ligand Binding

By being docked into the molecule structure of AKT1 by CDOCKER module, the two selected compounds and Ipatasertib’s ligand binding mechanisms with AKT1 were studied. In comparison with ZINC00004987206 and

ZINC000021992902, the CDOCKER potential energy of the reference ligand Ipatasertib were significantly higher. These indicated that the binding affinity of AKT1 with ZINC000049872065 and ZINC000021992902 was higher than it with Ipatasertib (Table 4). Through a structural computation study, we also performed Hydrogen bonds and π -related interactions. (Figs. 3 and 4). Results illustrated that 12 pairs of hydrogen bonds of ZINC000049872065 with AKT1 were formed. Also, the complex formed 1 pair of π -related interactions, by the compound itself with PHE161 of AKT1. ZINC000021992902 didn't form any π -related interactions with AKT1, but it formed 10 hydrogen bonds with AKT1, by O34 of the compound with GLY294:HN of AKT1, O18 of the compound with GLY157:HA1 of AKT1, O18 of the compound with GLY157:HA2 of AKT1, et al. As for reference Ipatasertib, it formed 5 hydrogen bonds with AKT1 and 1 π -related interactions with AKT1, by VAL164 of Ipatasertib with C20 with AKT1. (Tables 5 and 6)

Table 4
CDOCKER Potential Energy of Compounds with 3MV5

Compounds	-CDOCKER Potential Energy (kcal/mol)
ZINC000049872065	58.7801
ZINC000021992902	56.4843
Ipatasertib	49.9388

Table 5
Hydrogen Bond Interaction Parameters for Each Compound with 3MV5

Receptor	Compound	Donor Atom	Receptor Atom	Distances (Å)
3MV5	ZINC000049872065	GLY159:HN	ZINC000049872065:O22	2.64
		LYS179:HZ1	ZINC000049872065:O35	1.88
		GLY294:HN	ZINC000049872065:O35	2.85
		ZINC000049872065:H52	THR291:OG1	2.53
		ZINC000049872065:H54	GLU234:OE1	1.97
		ZINC000049872065:H56	LEU156:O	2.36
		GLY157:HA1	ZINC000049872065:O18	2.31
		GLY157:HA2	ZINC000049872065:O18	2.45
		ZINC000049872065:H49	GLU234:OE1	2.41
		ZINC000049872065:H51	ASP292:OD2	2.48
		ZINC000049872065:H55	GLU234:OE1	2.23
		ZINC000049872065:H75	GLU191:OE1	2.63
	ZINC000021992902	GLY294:HN	ZINC000021992902:O34	2.74
		ZINC000021992902:H51	ASP292:OD2	2.24
		ZINC000021992902:H53	GLU234:OE1	1.96
		ZINC000021992902:H55	LEU156:O	2.52
		GLY157:HA1	ZINC000021992902:O18	2.32
		GLY157:HA2	ZINC000021992902:O18	2.36
		ZINC000021992902:H48	GLU234:OE1	2.38
		ZINC000021992902:H49	ASP292:OD2	2.72
		ZINC000021992902:H54	GLU234:OE1	2.29
		ZINC000021992902:H74	GLU191:OE1	3.08
	Ipatasertib	LYS276:HZ1	Molecule:O8	1.69
		Molecule:H56	GLU234:OE1	2.08
		GLY157:HA1	Molecule:N16	2.91
		Molecule:H44	ASN279:OD1	2.83
		Molecule:H46	ASP292:OD2	2.47

Table 6
Pi-Related Interaction Parameters for Each Compound with 3MV5

Receptor	Compound	Donor Atom	Receptor Atom	Distances (Å)
3MV5	ZINC000049872065	A:PHE161	ZINC000049872065	4.48
	ZINC000021992902	/	/	/
	lpatasertib	Molecule:C20	A:VAL164	4.45

Molecular Dynamics Simulation

A molecular dynamics simulation module was built to assess the stability of the ligand-AKT1 complexes under natural environmental circumstances. The original conformations were got from the CDOCKER module through the molecular docking experiment. RMSD curves and potential energy chart of each complex were shown in Fig. 5. The time when the trajectories of each complex reached equilibrium is 15ps. With time going, RMSD and potential energy of these complexes reached stable state. In summary, ZINC000049872065 and ZINC000021992902 could interact with AKT1, and the complexes existed stably in the natural environment.

Discussion

Many cancers are associated with abnormal overexpression or activation of AKT, which is concerned with increased cancer cell proliferation and survival [1]. In comparison with normal esophageal mucosa (27.7% or 23/83 cases), high expression levels of the phosphorylated AKT protein were found more (90.4% or 75/83 cases) in patients with esophageal squamous cell carcinoma (ESCC). AKT1 is critical in various physiological activities and these constantly activated activities in multifarious tumors include transcription, apoptosis, glucose metabolism, cell proliferation, and cell migration[3]. Therefore, the key to inhibit tumor is to find an inhibitor of AKT1.

Recently, the PI3K/AKT signaling pathway is considered to be a potential target in treating cancer [15]. AKT is a central signaling node of the PI3K pathway and could be an ideal target for improving pathway inhibition. At present, a large number of PI3K/AKT pathway inhibitors have been extensively studied. However, the application and clinical significance of those drugs have been largely restricted by such potential adverse effects as pneumonitis and hepatotoxicity [15, 16]. MK-2206, one of allosteric AKT inhibitors, was used for monotherapy treatment. Some study showed the monotherapy treatment of MK-2206 had a good tolerance, however, its impact on patient benefit has been minimal. GSK690693, a potent ATP-competitive AKT inhibitor, has modest kinase selectivity, but its oral is poor [16]. Hence, it is urgent to find more effective inhibitors for clinical applications. In this study, lpatasertib was chosen as a reference drug.

To do virtual screening and analysis, Discovery Studio's numerous sections were used in this study. Results indicated that 7764 compounds were eligible to bind stably with AKT1. Based on the LibDock score, further study of the top 20 compounds was pooled.

In order to evaluate the pharmacologic properties of these compounds, ADME and toxicity predictions of the selected compounds were used in this study. Outcomes indicated that ZINC000049872065 and ZINC000021992902 had a good solubility and a good absorption level. Additionally, they were non-inhibitors of CYP2D6 and non-hepatotoxic. Besides, in comparison with other compounds, these two compounds were also found to have less developmental toxicity potential, rodent carcinogenicity and mutagenicity. Hence, ZINC000049872065 and ZINC000021992902 were selected as safe drug candidates and further analysis was performed. For another, the remaining drugs still had a possible function in drug development despite their possessed toxicities or negative effects.

The bonding mechanism and chemical bonds of the selected candidate compounds were also researched. CDOCKER module computation illustrated that ZINC000049872065 and ZINC000021992902 has a lower CDOCKER interaction energy compared with the reference ligand Ipatasertib. The results indicated that Ipatasertib had a lower binding affinity with AKT1 than these two compounds.

In the end, molecular dynamics simulation was used to investigate their stability in the natural environment. Calculation of RMSD and potential energy of these ligand-AKT1 complexes demonstrated the time when the trajectories of each complex reached equilibrium is 15ps. As time goes, RMSD and potential energy of these complexes reached stable state, which showed ZINC000049872065 and ZINC000021992902 could interact with AKT1 and the complexes were stable in the natural environment. On account of the results, these 2 compounds could be used for drug development and refinement.

Screening ideal lead compounds was the most critical step of current drug designation. In this study, a battery of computer-aided virtual techniques was used to identify possible inhibitors of AKT1. LibDock was applied for structure-based screening followed by ADME and toxicity prediction. To confirm the binding affinity mechanism between the ligand and AKT1, molecular docking was conducted. To assess the stability of ligand- AKT1 complexes, molecular dynamics simulations were used. The results indicated that these 2 compounds might potentially influence cancers. But it is all known that without refining and improving some drug thousands of times. Therefore, the refinement and improvement of them are significant in the following research.

Although this study was well-designed and precise measurements have been conducted, some shortcomings still exist. To confirm our results, such further experiments as animal testing will be performed. Besides, more indicators which should be assessed in the future include half-maximal inhibitory concentration and half-maximal effective concentration.

Conclusion

In order to screen and identify the ideal lead compounds as the inhibitors of AKT1, a battery of computer-aided structural was conducted. Two compounds which were chosen as the safe drug candidates were ZINC000049872065 and ZINC000021992902. These two compounds were critical in AKT1 inhibitor development. Besides, pharmacologic properties of drug candidates which could make a great contribution to AKT1 or other proteins in medication design and improvement was provided.

Declarations

Conflict of interest

There is no conflict of interests.

Author contributions

Bo Wu, Sheng Zhong and Wenzhuo Yang designed experiments; Zhiyun Zhang wrote manuscript; Xiaye Lv, Gaojing Dou, Xinhui Wang and Junliang Ge carried out experiments; Xuefeng Pan and Hongyu Wang analyzed experiments results.

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Figures

Figure 1

Molecular structure of 3MV5. (A) Initial molecular structure. (B) Surface of binding area added. Blue represents positive charge, and red represents negative charge.

A

ZINC000049872065

B

ZINC000021992902

C

Ipatasertib

Figure 2

Structures of Ipatasertib and novel compounds selected from virtual screening.

Figure 3

Schematic of intermolecular interaction of the predicted binding modes of (A) ZINC000049872065 with 3MV5, (B) ZINC000021992902 with 3MV5, and (C) Ipatasertib with 3MV5.

Figure 4

Schematic drawing of interactions between ligands and 3MV5. The surface of 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) ZINC000049872065-3MV5 complex. (B) ZINC000021992902-3MV5 complex.

A

B

Figure 5

Results of molecular dynamics simulation of the compounds ZINC000049872065 and ZINC000021992902. (A) Potential energy, Average backbone root-mean-square deviation. (B) RMSD, root-mean-square deviation.