Computational Study on Novel Natural Inhibitors Targeting Janus Kinase 3

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Abstract

Objective: The aim of this study is to screen and identify novel leading compounds which can inhibit protein Janus Kinase 3 (JAK3) from a drug library (ZINC database) to provide precise target therapy for lung cancer.

Methods: A set of computation-aided structural biology methods and chemical virtual screening techniques were carried out to screen novel inhibitor compounds. Libdock scores for potential inhibitors of JAK3 were calculated using fast docking method-virtual screening. Next, ADMET properties (absorption, distribution, metabolism, excretion, and toxicity) were conducted to predict their pharmacological characteristics. The binding affinity as well as the interactions between the candidate compounds and JAK3 were calculated and visualized by precise molecular docking algorithm. Ultimately, molecular dynamics simulation (MD) was performed to estimate the stability of the ligand-JAK3 complex under natural environment.

Results: After screening, two novel natural compounds, ZINC000014952116 and ZINC00000393864, were finally selected as leading compounds from the ZINC15 database, which possessed less Ames mutagenicity, rodent carcinogenicity and developmental toxicity potential than other candidate compounds. Additionally, they didn't inhibit the activity of CYP2D6. Molecular dynamics simulation analysis showed that ZINC000014952116 and ZINC00000393864 could interact with JAK3 steadily, and their ligand-JAK3 complexes could keep stable under natural situation, and act as regulatory role to JAK3.

Conclusion: This study analyzed that ZINC000014952116 and ZINC00000393864 were ideal natural inhibitors targeting JAK3 from the ZINC15 database, which could also provide more options and resources for other cancer chemotherapy.

Introduction

Lung cancer is the most common cancer around the world, the incidence rate accounts for 13% of newly diagnosed cancer cases and has a high mortality rate [1] The most common type of lung cancer is non-small cell lung carcinomas (NSCLC), which accounts for about 85% of all lung cancer cases[2]. The subgroup of non-small cell lung carcinomas are mainly squamous cell carcinoma, adenocarcinoma and large cell carcinoma[3]. Although diagnostic and treatment strategies of non-small cell lung carcinomas have made great progress in recent years, the five-year survival rate of lung cancer is less than 15%, and still had a poor prognosis[4]. Therefore, in order to treat more effectively and improve the survival rate and life quality of patients with lung cancer, not only the molecular mechanism of the occurrence and development of lung cancer need to be realized, but also more potential drugs which had precise targeted

therapy of JAK3 need to be screened and excavated.

Janus kinases (JAKs), a family of non-receptor protein-tyrosine kinases, consist of JAK1, JAK2, JAK3, and TYK2 (tyrosine kinase-2), which play an important role in cytokine signaling and are closely linked to both cancer and inflammatory diseases[5]. The Janus kinase family is regulated by numerous cytokines including interleukins, interferons, and hormones such as erythropoietin, thrombopoietin, and growth hormone[6]. Followed by the binding of a cytokine to its receptor, JAKs could auto-phosphorylate and trans-phosphorylate other proteins. JAKs phosphorylate sites on the cytokine receptor cytoplasmic tails, which create docking sites for signaling effectors, principally the signal transducers and activators of transcription (STATs). The STATs are then phosphorylated, resulting in nuclear translocation and regulating the expression of thousands of proteins[7]. Also, it has been shown that the activation of STAT3 can drive tumor metastasis, and JAK1 and JAK3 function at upstream of STAT3, which could be an necessary cascade reaction for activation of STAT3[8]. Meanwhile, studies have indicated that when the activity of JAK3 protein is inhibited, the occurrence of tumor metastases is reduced more than tenfold and JAKs knockdown reduced the clonal-growth and migratory ability of lung cancer cells[9]. So maybe it can be concluded that JAK3 plays an important role in tumor metastasis. Except the pathogenic effect of lung cancer, JAK3 mutation is also reported to be the hub gene in the development of acute lymphoblastic leukemia [10]. For example, the expression of JAK 3 pathway gene was up-regulated with a strong activity in the B-lineage acute lymphoblastic leukemia (ALL) patients[11].

In conclusion, the Jaks family is closely related to the occurrence, development and metastasis of a variety of tumors, among which JAK3 is the most important hub gene, and its mediated signaling pathway keep highly active in the growth of tumor cells. As a result, taking JAK3 as a target for tumor therapy and seeking a strategy to effectively inhibit JAK3 lead compounds have become a hot spot. Currently, JAK3 was found to be specific to cysteine residues in front of solvent exposure in the ATP binding bag, while it was replaced by serine in three other subtypes of the kinase family. According to this characteristic, FM-381, a covalently reversible JAK3 inhibitor, was previously discovered with excellent selectivity in the JAK family and the entire kinome. However, the bioavailability has not been fully demonstrated yet [12]. Therefore, this study aimed to find more potential compounds comprehensively which was obtained from natural product library that can effectively inhibit the function of JAK3 protein. Because natural products and their derivatives have potential biological functions and unique molecular structure as advantages, and the feasibility and effectiveness of natural compounds have been proved in some researches [13-15], this study screened small molecules from the ZINC15 database by a set of high-precision techniques, such as virtual screening, molecular docking, toxicity prediction and other structural and chemical methods to achieve the goal.

Results

Virtual screening of natural products database against JAK3

Arginine residues, as the crucial regulatory sites of JAK3, played an essential role in regulating the JAK3 protein, in that small molecules binding to this region may change the posture of the protein and so that inactivate the function of JAK3. Thus, this pocket region was selected as the active reference binding site for ligands to dock at. Molecule structure of JAK3 was obtained from protein data bank (PDB ID: 6GLB) and selected as the receptor protein. FM-381, a covalent-reversible JAK3 inhibitor, was elected as the reference compound. Firstly, after fast screening method Libdock, a total of 13653 small molecules from the ZINC15 database had docked successfully with the protein JAK3, among which, 1028 natural products had higher Libdock scores than the reference compound (Libdock score: 127.568). The highest 20 compounds according to Libdock scores were selected as candidate compounds for the following study, as shown in **Table 1**.

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

The ADME prediction module of Discovery Studio 4.5 was used to predict pharmacologic properties of all the 20 selected ligands as well as FM-381，which included aqueous solubility level, blood-brain barrier level (BBB), CYP2D6 inhibition, hepatotoxicity, human intestinal absorption level, and plasma protein binding properties (PPB) (**Table 2**). Results indicated that the aqueous solubility (defined in water at 25°C) of these selected ligands varied a wide range, the same as the blood-brain barrier level: totally 8 ligands had a high solubility level (scores $\geqslant$ 3), 6 ligands had a moderate solubility level (score = 2), and the rest ligands were low-soluble in water. All compounds were predicted to be non-inhibitors of cytochrome P450 2D6 (CYP2D6) except ZINC000028968101. Almost half of the compounds were toxic to liver. For human intestinal absorption level, 16 ligands had a good absorption level (scores $\geqslant$ 3). In terms of plasma protein binding properties, ZINC000150338786, ZINC000004096878, ZINC000028968101, ZINC000008220036, and ZINC000004096059 could bind with plasma protein well. As for the reference ligand property, it was predicted to be non-inhibition to CYP2D6 and had a high binding force with plasma protein, what's more, it was calculated to be toxic to liver.

Safety and toxicity need be considered fully as well in screening candidate drugs. To identify the safety of the top 20 compounds, this study made full use of computational method of TOPKAT module from Discovery Studio 4.5, to predict and analyze different kinds of toxicity indicators of these compounds, such as developmental toxicity potential (DTP), ames mutagenicity (AMES) and rodent carcinogenicity (based on the U.S. National Toxicology Program dataset). As shown in **Table 3**, results showed that only two compounds, ZINC000003938642 and ZINC000008220036, had no developmental toxicity potential, meanwhile 12 compounds had non ames mutagenicity. In the aspect of reference ligand, it was predicted to be carcinogenicity to mouse and rat, no developmental potential. Finally, considering all the characteristics mentioned above, ZINC000014952116 and ZINC00000393864 were identified as ideal lead compounds, owing to their less ames mutagenicity, rodent carcinogenicity and developmental toxicity potential than other compounds, which were not CYP2D6 inhibitors, not toxic to liver, high soluble in water,

high intestinal absorption as well. Consequently, ZINC000014952116 and ZINC00000393864 were chosen for the subsequent research (**Figure 1**).

Analysis of ligand binding and ligand pharmacophore

To figure out how these compounds combined with JAK3, this study utilized CDOCKER module to dock ZINC000014952116 and ZINC00000393864 into the binding pocket of JAK3, and CDOCKER potential energy was computed and listed in **Table 4**. RMSD between the docked site and the crystal structure of the FM-381-JAK3 complex was 0.7413 Å, suggesting that the CDOCKER module which was carried out in this study were credible. After CDOCKER, results showed that the binding affinity of ZINC000014952116 (-54.1495kcal/mol) and ZINC00000393864 (-51.5194kcal/mol) with JAK3 was lower than FM-381-JAK3 complex (-49.2387kcal/mol). Structural computation study was used to perform hydrogen bonds and π-related interactions (**Figures 2 and 3**). Results showed that ZINC000003938642 formed 13 pairs of hydrogen bonds with JAK3, by the O35 of the compound with LYS855:NZ of JAK3, the O15 of the compound with ARG953:NH2 of JAK3, the H57 of the compound with ASP967:OD2 of JAK3, the H74 of the compound with LEU828:O of JAK3, the H76 of the compound with LYS830:O of JAK3, the H98 of the compound with GLN864:O of JAK3, the O11 of the compound with ASN954:CA of JAK3, the O35 of the compound with GLY969:CA of JAK3, the H58 of the compound with ASP967:OD2 of JAK3, the H61 of the compound with ASN954:OD1 of JAK3, the H73 of the compound with ARG953:O of JAK3, the H77 of the compound with LYS830:O of JAK3, the H84 of the compound with LYS830:O of JAK3.Meanwhile,it also formed one pair of alkyl with JAK3,which is from C3 of the compound to VAL836 of JAK3.There were a pi-pi interaction observed between ZINC000003938642 and LYS855:NZ of JAK3 and a pi-alkyl interaction between ZINC000003938642 and LEU857 of JAK3. ZINC000014952116 formed 4 pairs of hydrogen bonds with JAK3, by the O27 of the compound with LYS855:NZ of JAK3, the O40 of the compound with CYS909:N of JAK3, the H51 of the compound with LEU828:O of JAK3, the H69 of the compound with GLY834:O of JAK3.Also,it formed two pi-sigma interactions, one is between LEU828:CD1 of JAK3 and the compound, the other is between LEU956:CD1 of JAK3 with the compound. Meantime, two pi-alkyl interactions formed between JAK3 and the compound, including ALA853 of JAK3 with the compound and LEU857 of JAK3 with the compound. As to the reference compound FM-381 (23), it formed 7 pairs of hydrogen bonds with JAK3, by the N19 of the compound with LEU905:HN of JAK3, the N16 of the compound with ARG911:HH12 of JAK3, the N16 of the compound with ARG911:HH22 of JAK3, the H48 of the compound with GLU903:O of JAK3, the N19 of the compound with TYR904:HA of JAK3, the N16 of the compound with ARG953:HD1 of JAK3, the H28 of the compound with LEU905:O of JAK3.Also,8 π-related interactions were formed in the complex, including LEU828:HB2, MET902:SD, ALA853, LEU956, LEU828, VAL836, VAL884, and CYS909 of JAK3 with the compound. Moreover, it formed 2 alkyl interactions, by ALA966 and VAL836 of JAK3 with the compound. The detailed information of these chemical bonds between ligands and JAK3 protein was shown in **Table 5**.

Molecular dynamics simulation

After analyzing the ligand binding mechanism and ligand pharmacophore, molecular dynamics simulation module test was then carried out to further evaluate the stabilities of the ligand-JAK3 complexes under natural environment conditions. RMSD curves and potential energy chart of each complex were displayed in **Figure 4** on the basis of the primitive conformations from the molecular docking experiment through CDOCKER module. From **Figure 4**, it could be seen that the trajectories of two complexes attained equilibrium after 130 ps; Potential energy and RMSD of these complexes began to level off as time went on. The molecular dynamics simulation result indicated that the stability of these complexes could attribute to these hydrogen bonds and π-related interactions between compounds and JAK3. Furthermore, these results demonstrated that ZINC000014952116 and ZINC00000393864 could interact with JAK3, and their complexes with JAK3 could be stable in a natural environment, and acted as regulatory roles to JAK3 the same as the reference FM-381 did.

Discussion

Lung cancer is one of the main causes of cancer death worldwide. The existing treatment strategies for non-small cell lung carcinoma (NSCLC) are resection and adjuvant chemotherapy, but the deficiency also remains obvious like a high risk of recurrence and death [16]. Recent studies have shown that adjuvant chemotherapy can improve survival in patients with early stage of non-small cell carcinoma [17]. What's more, one of the causes of NSCLC is epidermal growth factor receptor (EGFR) mutations, which initially respond to tyrosine kinase inhibitors (TKIs)[18] However, EGFR-TKIS is less effective when the gatekeeper T790M is mutated, so EGFR T790M inhibitors are needed to suppress the activity of EGFR T790M. It has been shown that JAK3 inhibitorⅥ could be regarded as a kind of selective EGFR T790M inhibitor and there has similarities between JAKs and EGFR T790M [19]. Consequently，looking for novel natural JAK3 inhibitor is pivotal in the treatment of cancer. At present, a relatively mature inhibitor of JAK3 is FM-381, which is also the reference drug ligand selected in this study. However, it still has some limitations that its bioavailability has not been proven, and indicators like pharmacological properties has not been tested.

In this study, firstly after virtual screening, a total of 13,653 small molecules in ZINC15 database were successfully docked at the binding region of JAK3 protein .These compounds were ranked from highest to lowest by their Libdock scores. Compounds with higher Libdock scores suggested more stable conformation and better energy optimization than those with lower Libdock scores, which also meant that the compounds could bind better to the protein. Subsequently, the top 20 compounds on the basis of Libdock scores were selected for further study. ADME and toxicity prediction were performed on the selected compounds to understand their absorption, distribution, metabolism, excretion and toxicity properties in vivo. The results showed that ZINC000014952116 and ZINC00000393864 had no hepatotoxicity, which indicated that they were relatively safe when used for a long time and would cause little damage

to the liver, suggesting that these two compounds were safer when metabolized by the liver than the reference ligand (predicted with toxic to liver). Moreover, compared with other compounds, they could bind to plasma proteins, facilitating absorption and distribution in the body, which had the same properties as the reference ligand. In addition, the non-inhibition of CYP2D6 for these two compounds and reference ligand indicated their importance for liver drug enzyme. Subsequently, toxicity predictions showed ZINC000014952116 and ZINC00000393864 had less Ames mutagenicity, rodent carcinogenicity and developmental toxicity potential, which indicated that they were potentially less harmful to humans. In a word, a series of advantages of ZINC000014952116 and ZINC00000393864 made them be selected as ideal candidates and be carried out for further analysis.

The following research adopted the CDOCKER module by docking ZINC000014952116 and ZINC00000393864 with JAK3 protein to display how these compounds combine with JAK3 and the type of the interacting chemical bonds. It turned out that CDOCKER interaction energy of ZINC000014952116 and ZINC00000393864 were both lower than the reference ligand FM-381, which suggested that the two compounds could bind to JAK3 more stable than the reference ligand. It was also supported by the evidence that the two potential compounds formed more chemical bonds with JAK3 than the reference ligand FM-381, elucidating the stability of the ligand-JAK3 complexes.

At last, molecular dynamics simulation was used to describe the movement of molecules and reflect their stability in the natural environment. The results indicated that the trajectories of two complexes attained equilibrium after 130 ps; Potential energy and RMSD of these complexes begins to level off as time goes on. Therefore, it can be deemed that they were able to exist stably in the natural environment. Furthermore, based on these results, we can improve the drug to reduce its adverse effects and improve its stability.

In conclusion, in this study, JAK3 inhibitors with stronger and more stable ligand binding were obtained by using virtual screening, providing more options for cancer chemotherapy. At the same time, the technological methods used in this study can be extended to the screening of drugs acting on molecular targets of other diseases. A batch of drugs with potential research value can be preliminarily screened only by take advantage of computer software, which greatly saves research costs and improves research efficiency. It's worth noting that there are some limitations to this approach.It cannot completely simulate and determine the stability and effect of drugs in the living environment, which requires more detailed clinical studies for the preliminary screened drugs.

Conclusions

In this study, a series of computer-aided structural and chemical analysis techniques (including virtual screening, molecular docking, ADME, toxicity prediction, etc.) were used to screen and identify desirable lead compounds with the function of inhibiting JAK3.Finally, ZINC000014952116 and ZINC00000393864 with a series of advantages were selected as ideal compounds from the ZINC15 database. At the same time, this

study determined the chemical properties of the candidate drugs, which provided a reference for the further selection of drugs for clinical use, and was helpful for the improvement of drugs.

Materials and Methods

Discovery studio software and ligand library

Discovery Studio 4.5 software (BIOVIA, San Diego, California, USA) is a comprehensive molecular modeling and environmental simulation software,which is applied in the study of protein structure and function, as well as in drug discovery. With high-quality graphics, proven technology, and an integrated environment, DS integrates the preservation and management of experimental data with professional-level modeling and simulation tools. At present, the main functions of DS include characterization of proteins (including protein-protein interaction), homology modeling, molecular mechanics calculation and molecular dynamics simulation, based on the structure of the drug design tools (including ligand-protein interaction, new drug design and molecular docking), based on small molecule drug design tools (including quantitative structure-activity relationship, pharmacophore, database selection, ADMET) and combinatorial library design and analysis, etc. Libdock module of Discovery Studio was applied to screen drug Molecules; CDOCKER module was carried out for docking study;and ADME module was used for pharmacologic properties. The ZINC15 database was selected to screen JAK3 inhibitors in this study. The ZINC15 database is a free database of commercially-available compounds for virtual screening. The ZINC15 database is a free database of commercially available compounds provided by the Irwin and Shoichet Laboratories, Department of Pharmaceutical Chemistry, University of California, San Francisco (San Francisco, California, USA).

Structure-based virtual screening using Libdock

Ligand-binding pocket region of JAK3 was selected as the binding site to screen potential compounds to inhibit JAK3. The Libdock module of Discovery Studio 4.5 was used for virtual screening. Libdock is a strictly based docking module. It calculates protein hotspots using a grid placed at the binding sites and both polar and non-polar probes.These hot spots are further used to arrange ligands for favorable interactions.Smart Minimiser algorithm and CHARMM force field (Harvard University, Cambridge, Massachusetts, USA) were used to minimize ligands, and all ligand positions were ranked according to ligand scores. The 1.9-A crystal structure of human JAK3 was extracted from the protein database (protein bank identification: 6GLB), and the inhibitor FM-381 (23) was downloaded from the Zinc15 database and imported into the working environment of Libdock. The chemical structure of JAK3 is shown in Figure 5. The protein was prepared by removing crystal water and other heteroatoms around it, followed by addition of hydrogen, protonation, ionization, and energy minimization. The CHARMm force field and the Smart Minimiser algorithm were applied for energy minimization. The minimization performed 2000 steps and the final

RMS gradient was 0.7413. The prepared protein was prepared to define the binding site. Using the ligands (FM-381 (23)) binding site, the active site for docking was generated. Virtual screening was used by docking all the prepared ligands at the defined active site using Libdock. According to the Libdock score, all the docked poses were ranked and grouped.

ADME (Absorption, Distribution, Metabolism, Excretion) and toxicity prediction

The ADME module of Discovery Studio 4.5 was employed to calculate the absorption, distribution,metabolism, excretion of selected compounds,such as their aqueous solubility, blood-brain barrier (BBB) penetration, cytochrome P450 2D6 (CYP2D6) inhibition, hepatotoxicity, human intestinal absorption, plasma protein binding (PPB) level.TOPKAT (Toxicity Prediction by Komputer Assisted Technology) modules of DS4.5 was also employed to calculate the toxicity and other properties of all the potential compounds, including rodent carcinogenicity, ames mutagenicity and developmental toxicity potential. These pharmacological properties were comprehensively considered in the screening of suitable JAK3 candidates.

Molecule docking and pharmacophore prediction

CDOCKER module of Discovery Studio 4.5 was used for molecular docking study. CDOCKER is a molecular docking method based on CHARMM force field, which can produce high-precision docking results. The CHARMM force field was employed for both receptors and ligands. Receptor is held rigid, while ligands are allowed to flex when docking. The CDOCKER module calculate the CHARMM energy and the interaction energy for each complex pose,which indicated ligand binding affinity. Crystal structure of JAK3 was obtained from the protein data bank. The crystal water molecules were generally removed in a rigid and semi-flexible docking process, causing the fixed water molecules to possibly affect the conformation of the receptor-ligand complex. The water molecules were removed, and hydrogen atoms were added to the protein. In order to prove the reliability of the analysis results, the initial inhibitor compound FM-381 (23) was extracted from the ZINC15 database, the same to the Natural Products screened, and then FM-381 (23) was docked into the crystal structure of JAK3 to compare the root-mean- square deviation (RMSD) with these 2 conformations. During the docking process, the ligands were allowed to bind with the residues of protein groups within the binding site sphere. The structures of identified hits were prepared and docked into the binding site of JAK3. Different poses of each ligand-JAK3 complex were generated and analyzed based on CDOCKER interaction energy.

Molecular dynamics simulation

The best binding conformations of the ligand-JAK3 complexes among the poses predicted by the molecule docking program were selected and prepared for molecular dynamics simulation. The ligand-receptor complex was put into an ortho-rhombic box and solvated with an explicit periodic boundary solvation water model. In order to simulate the physiological environment, solidum chloride were added to the system

with the ionic strength of 0.145. Then the system was subjected to the CHARMM force field and relaxed by energy minimization (500 steps of steepest descent and 500 steps of conjugated gradient), with the final root mean square gradient of 0.7413. The system was slowly driven from an initial temperature of 296 K to the target temperature of 302 K for 2 ps, and equilibration simulations were run for 5 ps. Molecular dynamics simulation (production module) was performed for 25 ps with time step of 1 fs. The simulation was performed with the normal pressure and temperature system at a constant temperature of nearly 300 K during the process.The particle mesh Ewald algorithm was used to calculate long-range electrostatics, and the linear constraint solver algorithm was adapted to fix all bonds involving hydrogen. With initial complex setting as a reference, a trajectory was determined for RMSD, potential energy, and structural characteristics through the Discovery Studio 4.5 analysis trajectory protocol.

Reference

1.FRENKEL M, SLATER R, SAPIRE K, et al. 2018. Complementary and Integrative Medicine in Lung Cancer: Questions and Challenges. J Altern Complement Med [J], 24: 862-871.

2.SHI L, XU Z, YANG Q, et al. 2019. IL-7-Mediated IL-7R-JAK3/STAT5 signalling pathway contributes to chemotherapeutic sensitivity in non-small-cell lung cancer. Cell Prolif [J], 52: e12699.

3.YANG Y, DING L, HU Q, et al. 2017. MicroRNA-218 functions as a tumor suppressor in lung cancer by targeting IL-6/STAT3 and negatively correlates with poor prognosis. Mol Cancer [J], 16: 141.

4.SIEGEL R L, MILLER K D, JEMAL A 2018. Cancer statistics, 2018. CA Cancer J Clin [J], 68: 7-30.

5.KETTLE J G, ÅSTRAND A, CATLEY M, et al. 2017. Inhibitors of JAK-family kinases: an update on the patent literature 2013-2015, part 1. Expert Opin Ther Pat [J], 27: 127-143.

6.ROSKOSKI R, JR. 2016. Janus kinase (JAK) inhibitors in the treatment of inflammatory and neoplastic diseases. Pharmacol Res [J], 111: 784-803.

7.ROBINSON M F, DAMJANOV N, STAMENKOVIC B, et al. 2020. Efficacy and Safety of PF-06651600 (Ritlecitinib), a Novel JAK3/TEC Inhibitor, in Patients With Moderate-to-Severe Rheumatoid Arthritis and an Inadequate Response to Methotrexate. Arthritis Rheumatol [J], 72: 1621-1631.

8.WANG P, YU B, WANG C, et al. 2020. C-terminal of E1A binding protein 2 promotes the malignancy of osteosarcoma cells via JAK1/Stat3 signaling. J Cell Commun Signal [J], 14: 67-76.

9.CHUANG C H, GREENSIDE P G, ROGERS Z N, et al. 2017. Molecular definition of a metastatic lung cancer state reveals a targetable CD109-Janus kinase-Stat axis. Nat Med [J], 23: 291-300.

10.BROUX M, PRIETO C, DEMEYER S, et al. 2019. Suz12 inactivation cooperates with JAK3 mutant signaling in the development of T-cell acute lymphoblastic leukemia. Blood [J], 134: 1323-1336.

11.UCKUN F M, PITT J, QAZI S 2011. JAK3 pathway is constitutively active in B-lineage acute lymphoblastic leukemia. Expert Rev Anticancer Ther [J], 11: 37-48.

12.FORSTER M, CHAIKUAD A, DIMITROV T, et al. 2018. Development, Optimization, and

Structure-Activity Relationships of Covalent-Reversible JAK3 Inhibitors Based on a Tricyclic Imidazo[5,4- d]pyrrolo[2,3- b]pyridine Scaffold. J Med Chem [J], 61: 5350-5366.
13.LI W, YUAN B, ZHAO Y, et al. 2021. Transcriptome profiling reveals target in primary myelofibrosis together with structural biology study on novel natural inhibitors regarding JAK2. Aging [J], 13.
14.YANG L, LI W, ZHAO Y, et al. 2019. Computational Study of Novel Natural Inhibitors Targeting O-Methylguanine-DNA Methyltransferase. World neurosurgery [J], 130: e294-e306.
15.ZHONG S, WU B, YANG W, et al. 2021. Effective natural inhibitors targeting poly ADP-ribose polymerase by computational study. Aging [J], 13: 1898-1912.
16.UPRETY D, MANDREKAR S J, WIGLE D, et al. 2020. Neoadjuvant Immunotherapy for NSCLC: Current Concepts and Future Approaches. J Thorac Oncol [J], 15: 1281-1297.
17.NAGASAKA M, GADGEEL S M 2018. Role of chemotherapy and targeted therapy in early-stage non-small cell lung cancer. Expert Rev Anticancer Ther [J], 18: 63-70.
18.DA CUNHA SANTOS G, SHEPHERD F A, TSAO M S 2011. EGFR mutations and lung cancer. Annu Rev Pathol [J], 6: 49-69.
19.NISHIYA N, SAKAMOTO Y, OKU Y, et al. 2015. JAK3 inhibitor VI is a mutant specific inhibitor for epidermal growth factor receptor with the gatekeeper mutation T790M. World J Biol Chem [J], 6: 409-418.

Figures

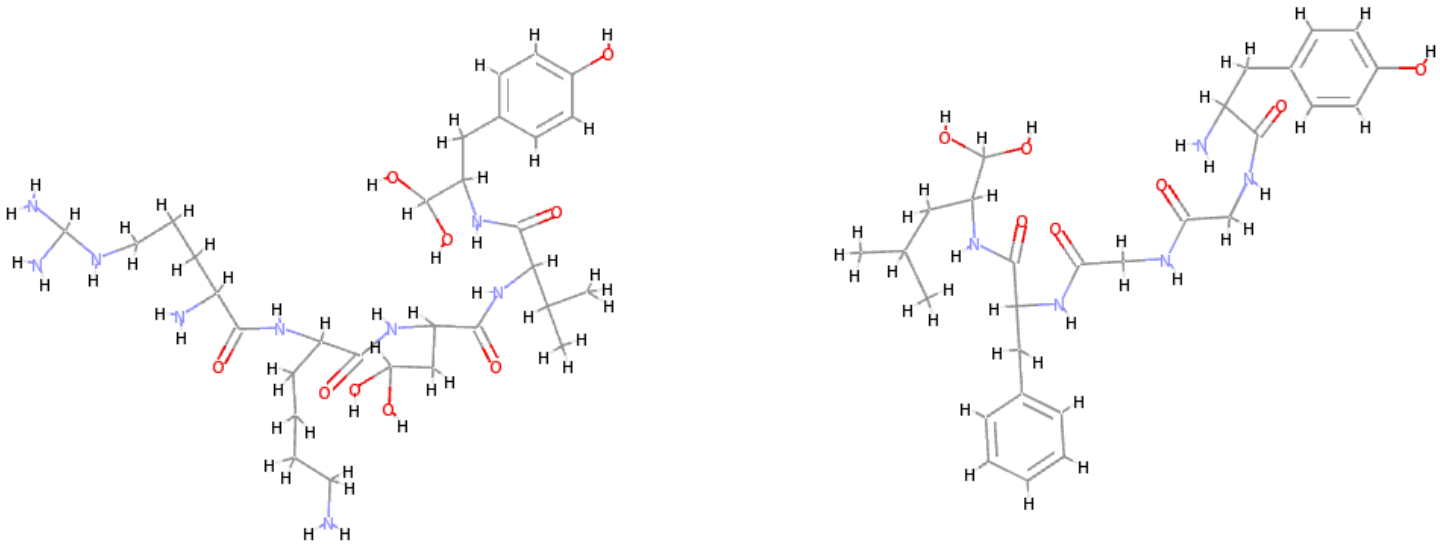

Figure 1

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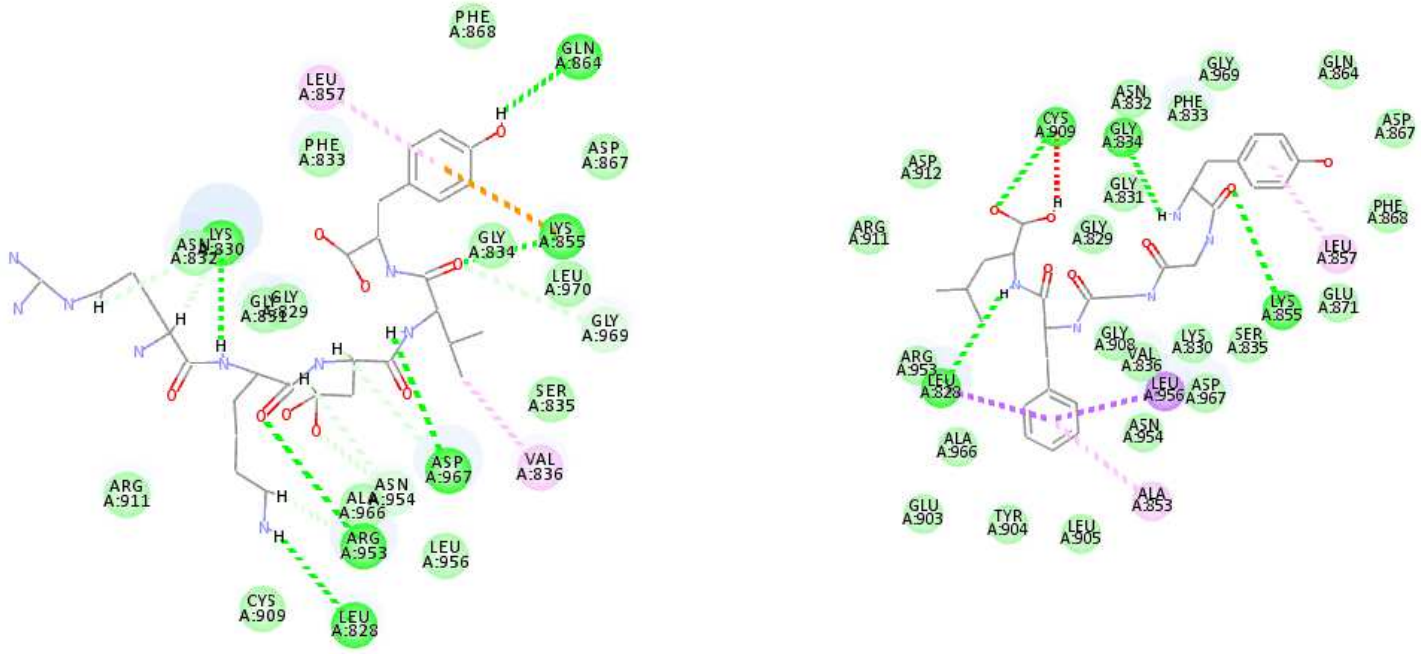

Figure 2

RMSD between the docked site and the crystal structure of the FM-381-JAK3 135 complex was 0.7413 Å, suggesting that the CDOCKER module which was carried out 136 in this study were credible. After CDOCKER, results showed that the binding affinity 137 of ZINC000014952116 (-54.1495kcal/mol) and ZINC00000393864 (-51.5194kcal/mol) 138 with JAK3 was lower than FM-381-JAK3 complex (-49.2387kcal/mol). Structural 139 computation study was used to perform hydrogen bonds and π-related interactions

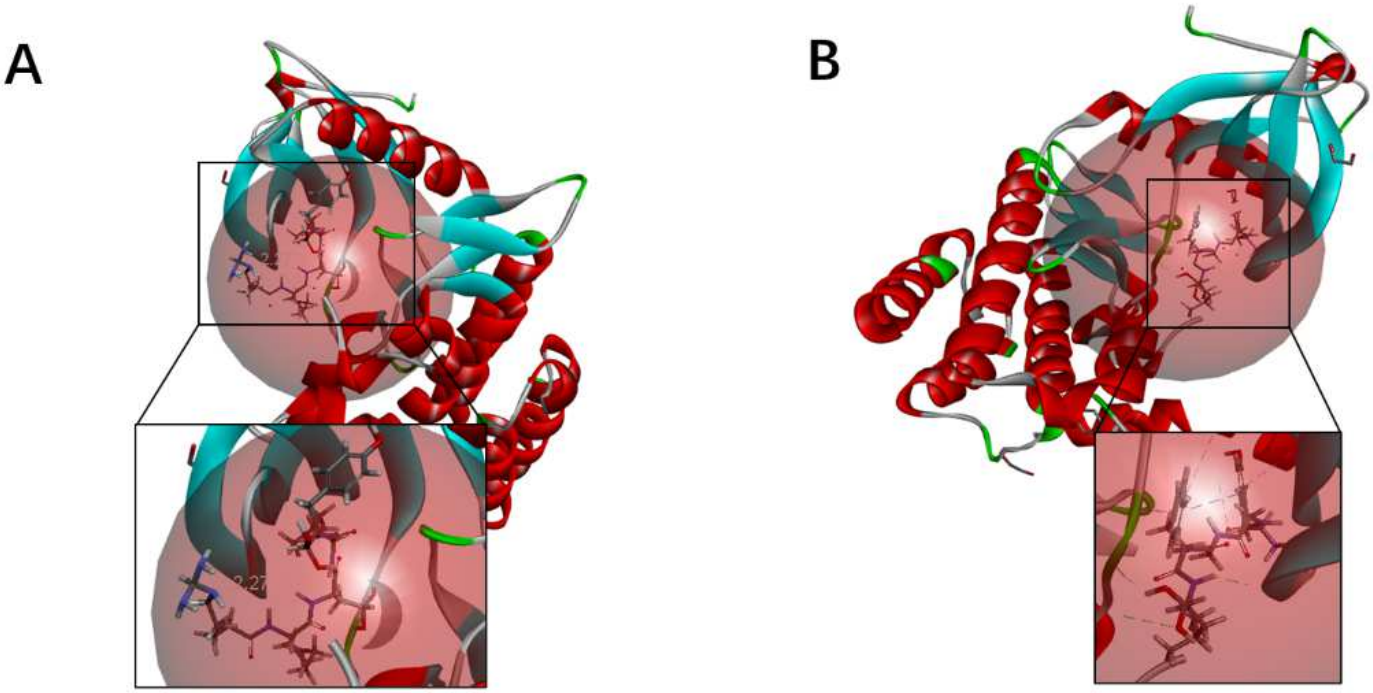

Figure 3

Results showed that ZINC000003938642 formed 13 pairs of hydrogen bonds with JAK3, by the O35 of the compound with LYS855:NZ of JAK3, the O15 of the compound with ARG953:NH2 of JAK3, the H57 of the compound with ASP967:OD2 of JAK3, the H74 of the compound with LEU828:O of JAK3, the H76 of the compound with LYS830:O of JAK3, the H98 of the compound with GLN864:O of JAK3, the O11 of the compound with ASN954:CA of JAK3, the O35 of the compound with GLY969:CA of JAK3, the H58 of the compound with ASP967:OD2 of JAK3, the H61 of the compound with ASN954:OD1 of JAK3, the H73 of the compound with ARG953:O of JAK3, the H77 of the compound with LYS830:O of JAK3, the H84 of the compound with LYS830:O of JAK3.

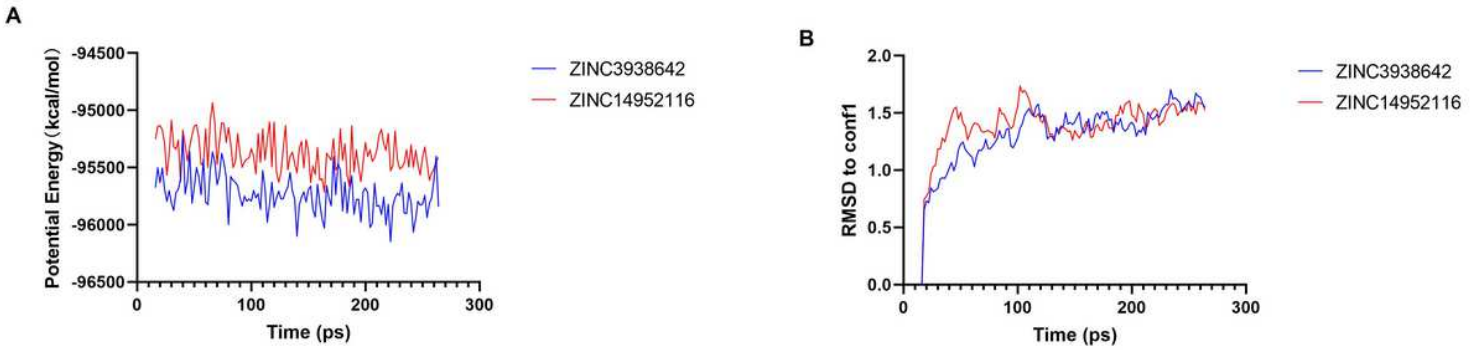

Figure 4

RMSD 176 curves and potential energy chart of each complex were displayed on the 177 basis of the primitive conformations from the molecular docking experiment through 178 CDOCKER module. It could be seen that the trajectories of two 179 complexes attained equilibrium after 130 ps; Potential energy and RMSD of these 180 complexes began to level off as time went on.

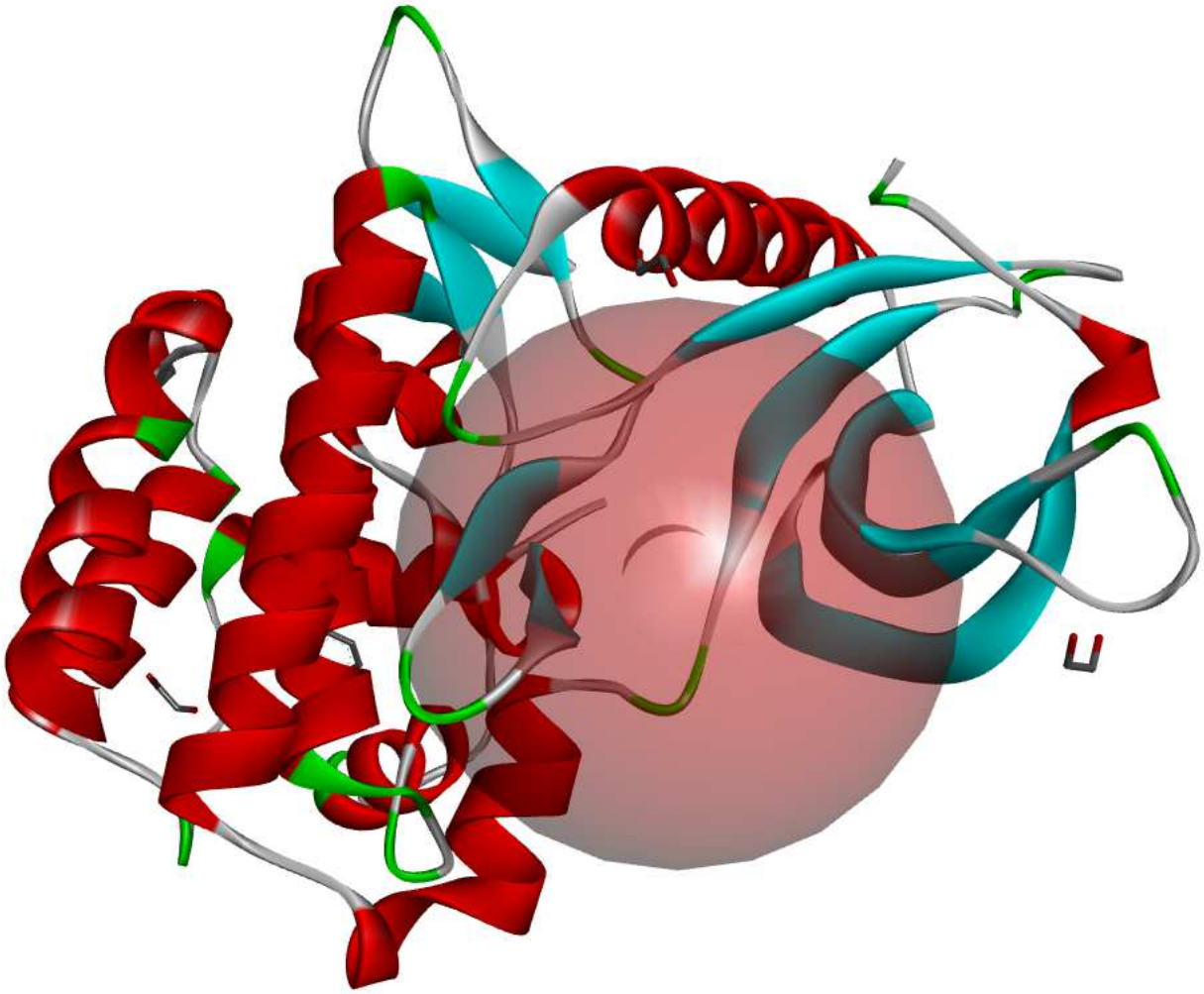

Figure 5

The 1.9-A crystal structure of human 294 JAK3 was extracted from the protein database (protein bank identification: 6GLB), and 295 the inhibitor FM-381 (23) was downloaded from the Zinc15 database and imported into 296 the working environment of Libdock. The chemical structure of JAK3 is shown in fig.