

Material Basis and Mechanism of Chansu Injection for COVID-19 Treatment Based on Network Pharmacology and Molecular Docking Technology

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Material Basis and Mechanism of Chansu Injection for COVID-19 Treatment Based on Network Pharmacology and Molecular Docking Technology

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Abstract

Background: Coronavirus disease 2019 (COVID-19) is an emerging and rapidly evolving disease with no effective drug treatment. Traditional Chinese medicines have been widely used to treat COVID-19 in China. Chansu and its major active constituent, bufalin, exert broad-spectrum antiviral effects. Although the clinical efficacy of Chansu injection for COVID-19 treatment has been confirmed, its mechanism of action remains unclear.

Objectives: In this study, we used network pharmacology and molecular docking technology to explore the potential material basis and mechanism of action of Chansu injection for COVID-19 treatment.

Methods: The main components of Chansu injection were determined using high-performance liquid chromatography (HPLC). The PharmMapper, SwissTargetPrediction, SEA, and TCMID databases were used to screen for the active ingredients and therapeutic targets of Chansu injection while the OMIM and GeneCards Suite databases were used to search for COVID-19-related targets. The STRING database was used for protein–protein interaction (PPI) network construction and topological analysis while DAVID was used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of the core targets. Molecular docking of the three Chansu injection components (i.e., cinobufagin, resibufogenin, and bufalin) to angiotensin-converting enzyme II, spike (S) protein, 3CL protease, and RNA-dependent RNA polymerase was also carried out.

Results: The three Chansu injection compounds were identified using HPLC. A total of 236 drug-related targets and 16,611 disease-related targets were identified, and 77 common targets were determined through mapping. The PPI mapping results revealed 16 core targets obtained through topological analysis and screening. Further, GO and KEGG pathway enrichment analyses revealed that the PI3K and JAK–STAT signaling

pathways are the major pathways regulated by Chansu injection in COVID-19 treatment. The molecular docking results suggest that the three Chansu injection components have high binding energies to the S protein.

Conclusions: This study revealed that the potential mechanism of Chansu injection for COVID-19 treatment involves multiple targets and pathways, thereby providing a scientific basis for its clinical application and further research.

Keywords: Chansu Injection; COVID-19; network pharmacology; molecular docking technology

Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged suddenly in China in November 2019 [1]. This disease has become a global pandemic with severe health issues around the world [2,3]. This is the third pandemic that has occurred during the last 20 years of the 21st century, the first of which was the SARS pandemic in 2003 and the second, Middle East respiratory syndrome (MERS) pandemic in 2015 [4–6]. Worldometers real-time statistics show that as of February 1, 2021, SARS-CoV-2 has caused more than 100 million infections and more than 2.22 million deaths. As a result, it is considered a public health emergency of international concern [7–9].

SARS-CoV-2 is the seventh coronavirus found to infect humans. Similar to SARS-CoV and MERS-CoV, SARS-CoV-2 belongs to the β -subtype coronavirus [10]. Its genetic similarity to that of the bat coronavirus is 96% and it shares the same cell receptor, angiotensin-converting enzyme II (ACE2), found in SARS-CoV. SARS-CoV-2 is a single-stranded RNA with the largest RNA virus [11,12]. It can trigger an antiviral immune response upon entry into organisms, causing slight or moderate symptoms that are difficult to notice in most patients. Severe cases display excessive inflammatory responses, leading to severe progressive pneumonia and even acute respiratory distress syndrome, septic shock, coagulation disorders, and multi-organ failure [13,14]. Owing to its severity and rapid progression, there is an urgent need to develop novel medicines for the emerging coronavirus [15].

To date, no medicines specific for coronavirus treatment are available. Before the COVID-19 pandemic in China, the coverage and effectiveness of Chinese medicines were greater than 90% [16]. Results of clinical observation revealed that Chinese medicine could effectively alleviate symptoms, reduce the development of slight-to-moderate symptoms into severe cases, increase the recovery rate, decrease the death rate, and promote rehabilitation [17]. The full participation of Chinese medicine has become a highlight of the anti-COVID-19 campaign in China and has contributed to the pandemic's global campaign. As a result, Chinese medicines have been increasingly acknowledged.

Chansu is obtained from the excreta of the skin and postauricular glands of *Bufo bufo gargarizans*. It fights inflammation and infection, and regulates immunity [18,19]. Chansu injection is now being widely administered to inhibit bacteria, fight viruses, suppress pain, reduce inflammation, inhibit the proliferation of tumor cells, increase patient's albumin level after radiochemical therapy, and induce tumor cell apoptosis. Favorable clinical therapeutic efficiency and safety assessments have also been acquired in the ancillary treatment of cancer and chronic hepatitis. The main active ingredients in Chansu, such as bufalin, resibufogenin, and cinobufagin, have very strong antiviral effects [20,21]. In a clinical trial, Hue Fen et al. found that Chansu injection significantly improved the respiratory function of patients with severe

COVID-19, suggesting a marked clinical effect and no side effects [22].

Network pharmacology is an emerging discipline that integrates the technology and content of polypharmacology, computational biology, and network analysis. Because the integrality and systematicity of network pharmacology are consistent with the Chinese medicine theory, it has been widely used in the prediction and characterization of potential targets and active ingredients of Chinese medicine [23]. Molecular docking technology is a theoretical simulation approach to predict the binding pattern and affinity of medicines using computers to study the receptors' characteristics as well as the interaction between receptors and medicinal molecules [24]. In this study, we attempted to identify the effective ingredients and targets of Chansu injection for COVID-19 treatment using a network pharmacological method. Molecular docking was also used to investigate the binding capacity of protein receptors to small medicine molecules based on geometric mapping and energy mapping, a key-and-lock principle. The material basis and molecular mechanism of Chansu injection for COVID-19 treatment was explored. A flowchart of the research methodology is presented in Figure 1.

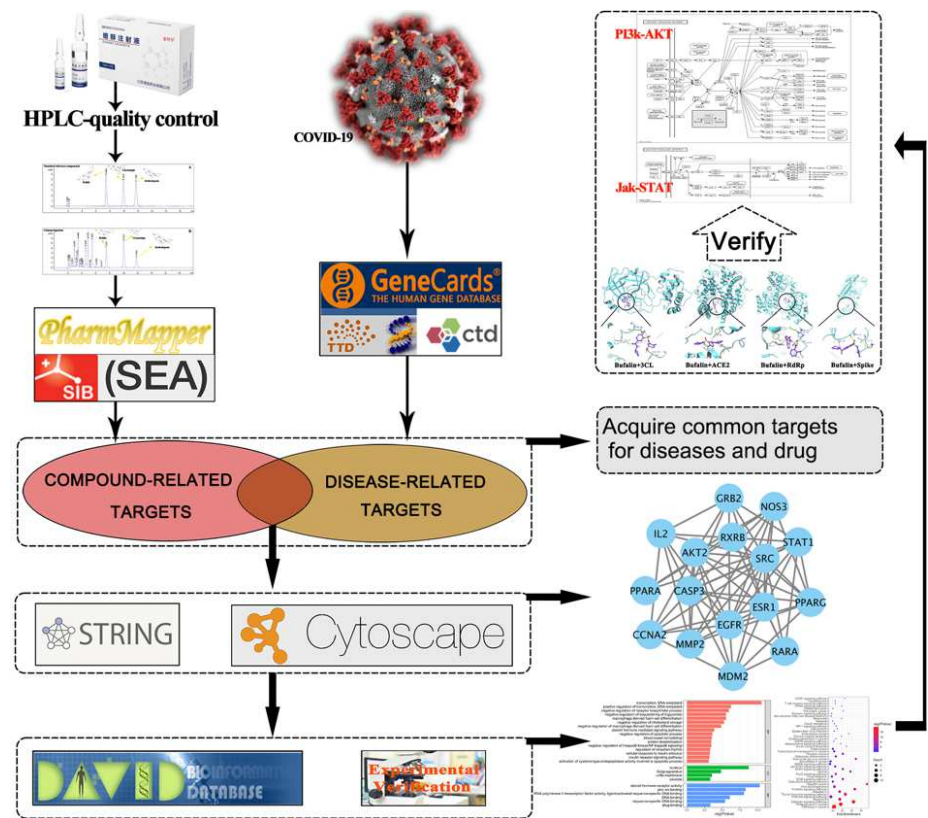


Figure 1. Flowchart of the study

Materials and methods

Database

PharmMapper (<http://lilab-ecust.cn/pharmmapper/>), Universal Protein (UniProt,

<http://www.uniprot.org/>) database, similarity ensemble approach (SEA, <http://sea.bkslab.org>) database, Swiss database (SwissTargetPrediction, <https://www.sib.swiss>), STRING (<https://string-db.org>), the Database for Annotation, Visualization, and Integrated Discovery (DAVID, <https://david.ncifcrf.gov>), GeneCards database platform (<http://www.genecards.org>), Comparative Toxicogenomics Database (CTD, <http://ctd.mdibl.org>), and Therapeutic Target Database (TTD, <http://bidd.nus.edu.sg/group/ttd/ttd.asp>) were used.

Ingredient analysis

The Chansu injection sample was supplied by the Pujin Pharmaceutical Cooperation (Jiangsu, China). The chromatographic conditions and system compatibility tests included the Agilent Zorbax SB-C18 chromatographic column (column length 250 mm, inner diameter 4.6 mm, particle size 5 μ m) and acetonitrile/0.1% ammonium acetate solution (50:50) for the mobile phase. The detection wavelength was 296 nm. Theoretical plate counts were calculated according to the bufalin level peak, which was not less than 3,000. To prepare the control solution, an appropriate amount of bufalin, resibufogenin, and cinobufagin was calibrated precisely, and 1.6 μ g each of bufalin, resibufogenin, and cinobufagin was added to 1 mL of 50% acetonitrile. The control solution (20–50 μ L) and 50 μ L of the test samples were loaded into a high-performance liquid chromatography (HPLC) equipment and the calculations were derived with an external method. Every 1 mL of sample should contain 1.2–4.8 μ g of bufalin (C₂₄H₃₄O₄), cinobufagin (C₂₆H₃₄O₆), and resibufogenin (C₂₄H₃₂O₄).

Acquisition of potential targets

Potential targets of the active ingredients of Chansu injection were searched online on PharmMapper, SEA, and Swiss, using the index words bufalin, resibufogenin, and cinobufagin. The combined results were considered potential targets.

Acquisition of targets for COVID-19

The index words coronavirus disease, coronavirus infections, and severe acute respiratory syndrome were searched in CTD, TTD, and GeneCards Suite, respectively. The index word search was conducted on January 20, 2021. The combined results of the three databases were used, and duplications were removed to obtain the corresponding targets for COVID-19.

Acquisition of the crosstalk targets of the disease and medicine

The corresponding targets of Chansu injection related to COVID-19 were mapped using the R language data package (version 3.6.1). The crosstalk targets were considered potential targets for COVID-19 treatment with Chansu injection.

Network construction

A medicine–target disease network diagram was prepared by introducing the acquired information of crosstalk targets and active ingredients into the Cytoscape software. Thereafter, a topological analysis was performed.

Acquisition of the PPI diagram

The acquired crosstalk targets were recorded in the STRING database, using the genus, *Homo sapiens*. The free targets were deleted, and the resultant file, string_interactions.tsv, was introduced into the Cytoscape software for topological analysis. Core targets were determined according to the degree and centrality of the intermediate value.

GO and KEGG enrichment analysis

The function of the core targets was described and annotated to investigate its pathway. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analyses were conducted using the DAVID database. For GO enrichment analysis, molecular function (MF), biological process (BP), and cellular component (CC) were used to confine and describe genes. The potential signaling pathway for COVID-19 treatment using Chansu injection was determined through KEGG enrichment analysis.

Molecular docking

The molecular structure of the active ingredients of Chansu injection was downloaded from PubChem while those of M^{pro}, ACE2, 3CL protease, and spike protein were downloaded from the PDB database. The data were processed using MGLTools 1.5.6 and saved as a pdbqt file. The active sites and grid box coordinates of M^{pro}, ACE2, 3CL protease, and spike protein were defined according to their built-in ligands. The size of each box was 30 × 30 × 30 grids. Small-molecule protein docking was conducted using AutoDock Vina 1.1.2, and the conformation with the highest score was mapped and analyzed using PyMOL and Maestro 11.9.

Results

Active ingredients of Chansu injection

The major components of Chansu injection were analyzed using HPLC. On comparison with standard reference compounds, three compounds were identified: bufalin, resibufogenin, and cinobufagin. Representative chromatograms of the standard reference compounds (A) and Chansu injection components (B) are shown in Figure 2.

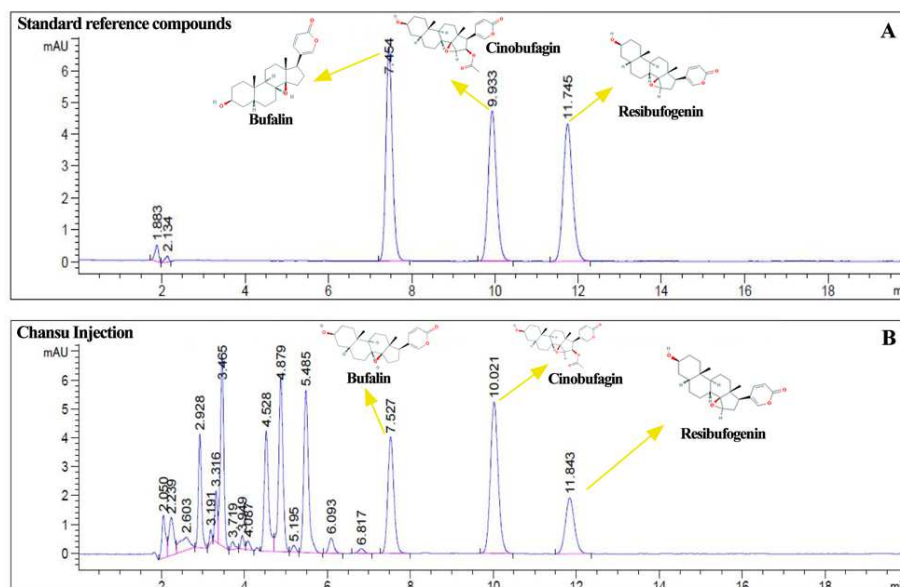


Figure 2. HPLC chromatograms of the components of Chansu injection

Potential targets of Chansu injection

The active ingredients of Bufalin, Resibufogenin, and Cinobufagin were searched online in PharmMapper, SEA, and Swiss databases, and the results were combined and duplicates were eliminated to obtain a total of 236 potential targets.

Crosstalk targets of Chansu injection and COVID-19

By searching GeneCards, CTD, and TTD, a total of 16,611 disease-related targets were obtained (7,927 from GeneCards, 14,774 from CTD, and 2 from TTD). When the potential targets of Chansu injection were mapped to disease-related targets, a total of 77 crosstalk targets were identified. In particular, COVID-19 was found to have 96, 99, and 92 crosstalk targets with bufalin, cinobufagin, and resibufogenin, respectively (Figure 3).

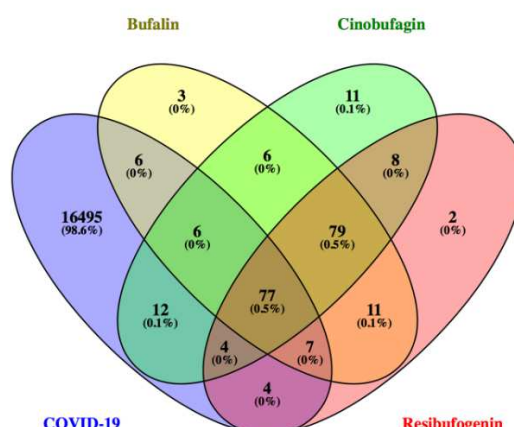


Figure 3. Venn diagram

Medicine–target disease network diagram

In the medicine–target disease network diagram (Figure 4), the outer light blue nodes represent targets, the inner dark blue nodes represent the active ingredients of Chansu injection, the red nodes represent medicines, and the green nodes represent diseases. Topological analysis of the network diagram showed that there were 82 nodes and 137 connecting lines. Further, the network centralization, heterogeneity, and density were 0.199, 1.096, and 0.032, respectively.

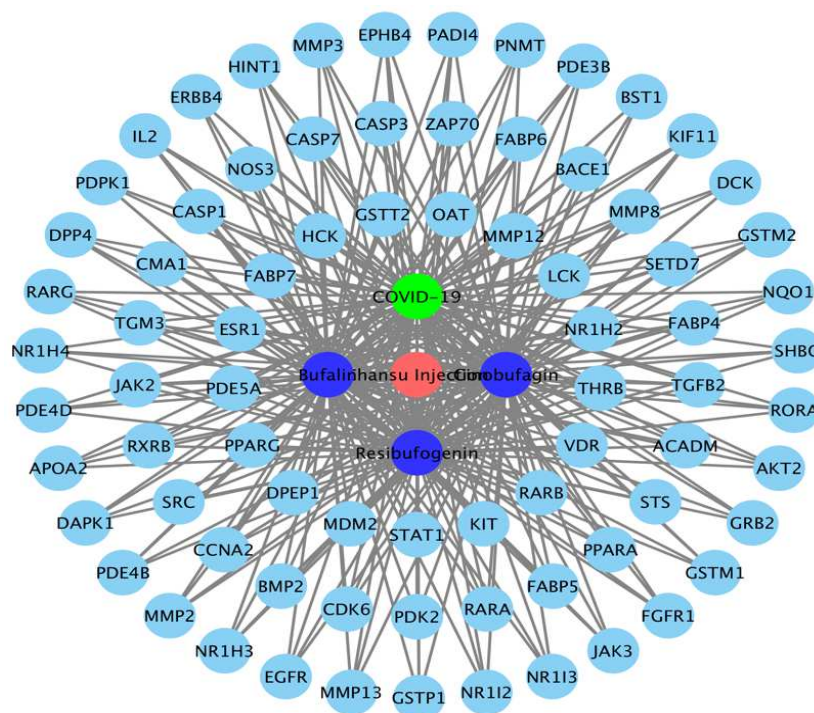


Figure 4. Network diagram of the drug–target disease

PPI diagram and core target filtration

Information concerning the crosstalk targets of the disease and drug was introduced into the STRING database for analysis using the genus, *Homo sapiens*. Four free targets were deleted, and the resultant file “string_interactions.tsv” was introduced into the Cytoscape software to draw the protein–protein interaction (PPI) diagram. Thereafter, a topological analysis was performed. The nodes with retention value and the centrality of intermediate values above the mean values are the core targets (Figure 5).

As shown in Table 1, core targets were obtained, namely AKT2, GRB2, EGFR, CCNA2, RXRB, RARA, STAT1, PPARG, ESR1, SRC, NOS3, IL2, PPARA, MMP2, MDM2, and CASP3.

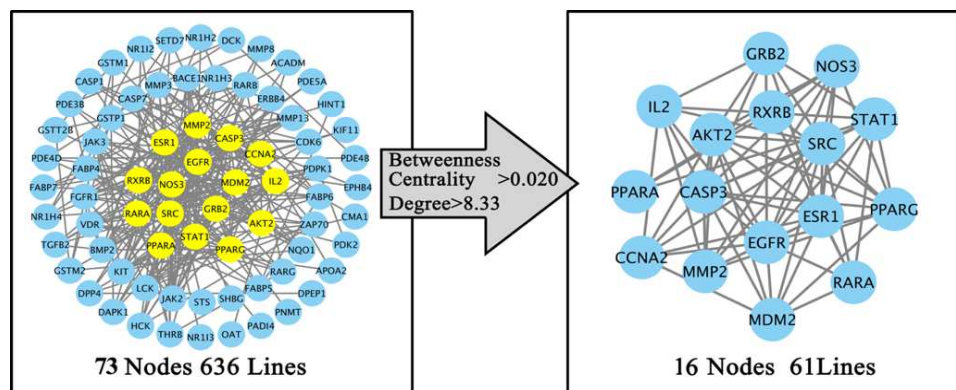


Figure 5. PPI diagram and filtration of the core targets

Table 1. Core targets of Chansu injection

Gene name	Protein name	Uniprot ID
AKT2	RAC-beta serine/threonine-protein kinase	P31751
GRB2	Growth factor receptor-bound protein 2	P62993
EGFR	Epidermal growth factor receptor	P00533
CCNA2	Cyclin-A2	P20248
RXRβ	Retinoic acid receptor RXR-beta	P28702
RARα	Retinoic acid receptor alpha	P10276
STAT1	Signal transducer and activator of transcription 1- alpha/beta	P42224
PPARγ	Peroxisome proliferator-activated receptor gamma	P37231
ESR1	Estrogen receptor	P03372
SRC	Proto-oncogene tyrosine-protein kinase Src	P12931
NOS3	Nitric oxide synthase	P29474
IL2	Interleukin-2	P60568
PPARα	Peroxisome proliferator-activated receptor alpha	Q07869
MMP2	72 kDa type IV collagenase	P08253
MDM2	E3 ubiquitin-protein ligase Mdm2	Q00987
CASP3	Caspase-3	P42574

GO and KEGG enrichment analysis

Based on the GO enrichment analysis, a total of 26 entries had a P value less than 0.05, including 16 BP, 4 CC, and 6 MF (Figure 6). These entries are involved in cell proliferation, modulation of apoptosis and programmed death, cascading response of intracellular signals, immune responses, nucleic acid metabolism processes, transcription, and translation. Based on the KEGG enrichment analysis, the phosphoinositide 3-kinase (PI3K–Akt) and JAK–STAT signaling pathways differed significantly and were closely related to viral infection (Figure 7).

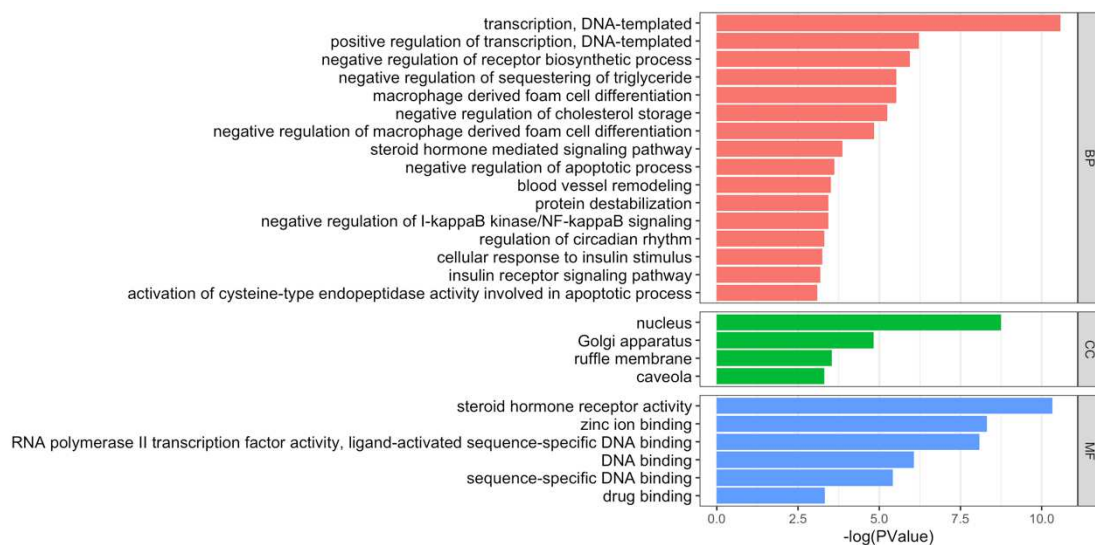


Figure 6. The results of GO enrichment analysis

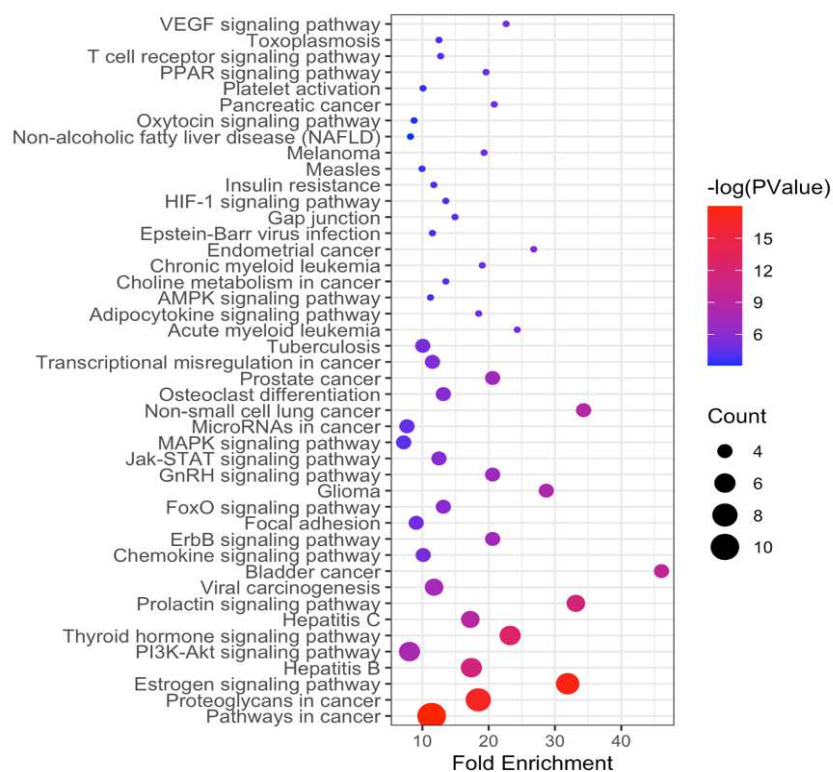


Figure 7. The results of KEGG enrichment analysis

Molecular docking

As shown in Figure 8, bufalin had the highest binding efficiency to 3CL protease, ACE2, RNA-dependent RNA polymerase (RdRp), and spike protein, with an absolute binding efficiency above 7, indirectly suggesting that the Chansu injection has a good therapeutic effect on SARS-CoV-2. The molecular docking diagram of bufalin and virus proteins is shown in Figure 9.

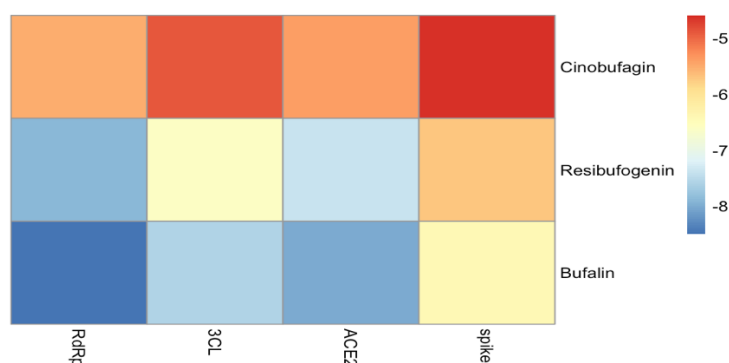


Figure 8. Diagram of the molecular docking efficiency

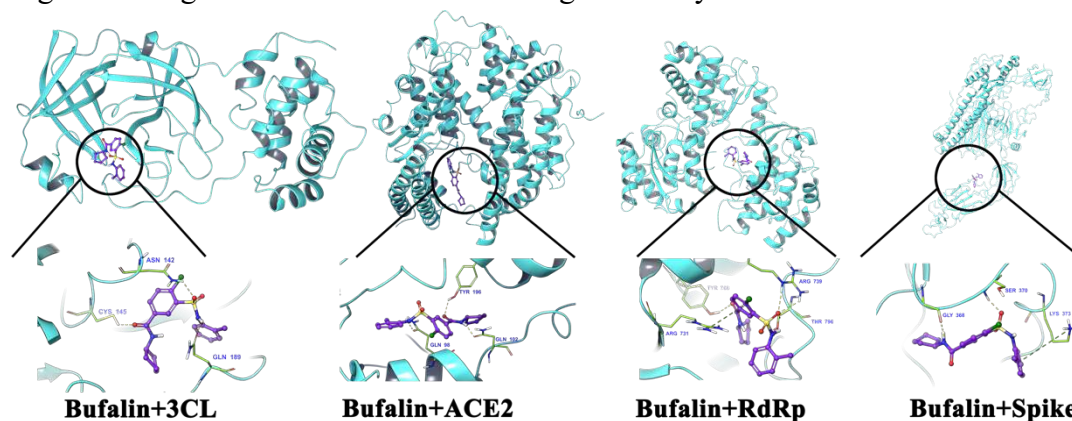


Figure 9. Molecular docking of Chansu injection to 3CL protease, AEC2, RdRp, and spike protein

Discussion

COVID-19 is a global pandemic in the 21st century that has greatly threatened human health and safety [25]. Chinese medicines can enhance the self-immunity and reconstruction capacity of the human body when used to prevent infectious diseases, thereby maintaining homeostasis [26]. They function to balance immunity, eliminate inflammation, and avoid or delay inflammation outburst by regulating immunity, inflammation, endocrine function, signal transduction, and other BPs, and inhibiting the production of bacterial endotoxins through multiple ingredients and targets (Huang et al., 2020). Chinese medicines have a unique advantage and provide effective control strategies in severe conditions, such as global pandemics, which lack effective treatments.

Previously, bufalin, the main active ingredient of Chansu, was found to have a broad-spectrum antiviral activity that can inhibit mouse hepatitis virus, feline infectious peritonitis virus, MERS-CoV, and vesicular stomatitis virus that infect host cells via the ATP1A1-mediated Src signaling pathway [28,29]. In addition, ATP1A1 plays a crucial role in the infection of host cells by Ebola and Respiratory Syncytial Virus (RSV) [30,31].

A 50-sample clinical [22] study showed that Chansu injection could significantly improve the respiratory function of patients with severe COVID-19. In fact, the PaO₂/FiO₂ and ROX of 95.2% patients in the treatment group were improved, while

those in the control group improved by 68.4% and 73.7%, respectively. The respiration-supported recovery time of the treatment group was one day shorter than that of the control group. Meanwhile, the results of the safety index analysis showed that Chansu injection did not have evident toxic side effects at the tested dosage.

As Chansu injection can effectively treat COVID-19, network pharmacology was used to study the molecular mechanism of Chansu injection, which might be involved in the regulation of cell proliferation, cell apoptosis, programmed cell death, intracellular signaling cascade response, immune response, nucleic acid metabolism, transcription, translation, and other BPs, in COVID-19 treatment. Based on the KEGG enrichment analysis results, the PI3K–Akt and JAK–STAT signaling pathways may play a critical role. Further, the molecular docking results suggested that bufalin, one of the active ingredients in Chansu injection, has a very high binding efficiency to 3CL protease, ACE2, RdRp, and spike protein, with an absolute binding efficiency above 7, suggesting that the active ingredients in Chansu injection may have stopped viral entry into host cells and blocked its binding to ACE2 and the binding of the SARS-CoV-2 protein to the hydrolase of SARS-CoV-2 3CL protease, thereby terminating viral RNA duplication. This study has laid the foundation for future confirmation of the molecular mechanisms of Chansu injection in COVID-19 treatment.

At present, the role of the PI3K–Akt signaling pathway in various diseases has been confirmed. In humans, the PI3K–Akt pathway regulates multiple cellular and biological processes, such as tumor immunity, virus infection, and inflammatory recovery. PI3K is an intracellular phosphatidylinositol kinase (PIK) that also has Ser/Thr kinase and PIK activities, which participate in the regulation of various cellular functions, such as proliferation, differentiation, and glucose transportation [32,33]. The PI3K–Akt signaling pathway was found to be involved in the infection processes of different types of viruses, including RNA and DNA viruses, which mainly regulate viral activity in the early infection stage, recognize host cell infection, and mediate cell transformation [34–37].

Iranian scholars found that [38] viruses depend on two types of receptors to enter cells: ACE2 and CD147. Other molecular factors on the cell surface that facilitate viral genome entry into host cells include TMPRSS2 and furin protease. CD147 and furin protease both help to induce the PI3K/Akt signal transduction pathway. Further, the endocytosis of SARS-CoV-2 is mediated by clathrin, and this pathway is regulated by PI3K/Akt signal transduction. Once the virus binds to ACE2, the cell membrane sinks and viral endocytosis occurs. A reduction of ACE2 on the cell surface helps increase the level of serum angiotensin II (Ang II), which has been proven in COVID-19 patients. However, the binding of Ang II to its type I receptor (AT1R) activates this signaling pathway, thereby regulating the SARS-CoV-2 infection to host cells and virus duplication in infected cells. Therefore, the PI3K/Akt signaling pathway could regulate the activation of activator protein-1 (AP-1) and nucleic factor- κ B (NF- κ B), reduce the expression of inflammatory cytokines such as Interleukin-6 (IL-6) and Tumor necrosis factor alpha (TNF- α), inhibit the cascading reaction of an inflammatory outburst, and promote the absorption of inflammatory cells and tissue recovery.

The JAK/STAT signaling pathway plays a crucial role in the regulation of cell growth,

survival, and pathogen resistance [39–42]. Professor Cuijun found that [43] type I interferon pretreatment could significantly interfere with the duplication of SARS-CoV-2, suggesting a certain sensitivity of SARS-CoV-2 to type I interferon cells. The induced interferon was released to regulate the JAK–STAT-mediated expression of multiple downstream antiviral cytokines and inhibit virus duplication and amplification. The M^{pro} in SARS-CoV-2 could promote autophagic degradation of the transcriptomic factor, STAT1, to weaken the entrance of STAT1 into the nucleus. This process inhibits the activity of type I interferon cells, reduces the expression of the downstream active ingredient of the JAK–STAT pathway, and helps COVID-19 patients acquire a natural immune escape.

A study [44] by Carl H June’s research group at the University of Pennsylvania (USA) found that the cytokine release syndrome (CRS) is the primary lethal cause for SARS and MERS patients, as well as COVID-19 patients. When a coronavirus enters the body, monocytes, macrophages, and dendritic cells secrete IL-6 and other inflammatory factors. The downstream activity of IL-6 mainly includes the classical cis and trans pathways. In the cis pathway, IL-6 forms a complex with the IL-6 membrane and gp130 and activates the JAK and STAT3 pathways. IL-6 is mainly expressed in immune cells; therefore, this pathway can activate immune cells to trigger CRS. In the trans pathway, the highly concentrated IL-6 binds to the soluble IL-6R and forms a dipolymer with gp130. As gp130 is widely expressed on the surface of all cells, the downstream JAK–STAT3 pathway can activate cells that do not express IL-6R, such as epithelial cells. Further, it may induce increased vascular endothelial growth factor (VEGF), monocyte chemoattractant protein-1 (MCP-1), and Interleukin-8 (IL-8) levels, additional release of IL-6, and reduction of E-cadherin, eventually leading to acute respiratory distress syndrome (ARDS).

Conclusion

In summary, in this study, we determined the binding efficiency of Chansu injection to the main protein of the novel coronavirus and explored the functioning mechanism of this Chinese medicine for COVID-19 treatment using network pharmacology and molecular docking based on highly effective in clinical studies. The results showed that Chansu injection could regulate virus duplication, activate immune cells, and alleviate inflammatory responses through the PI3K–Akt and JAK–STAT signaling pathways. The Chansu injection treats patients with COVID-19 by acting on SARS-CoV-2 and ACE2. The results of this study provide theoretical and experimental evidence for COVID-19 treatment using Chansu injection.

Abbreviations

COVID-19: Coronavirus disease 2019; TCMSP: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; DL: Drug-like; OB: Oral bioavailability; PPI: Protein-protein interaction; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; BP: Biological process; CC: Cellular component; MF: Molecular function.

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Availability of data and materials

The data and materials used to support the finding of this study are available from the corresponding author upon request.

Authors' contributions

XZ and QW designed the study. YX, WP, DH, WZ, and FF performed the experiments. YX and WP analyzed the data. YX, WP and DH wrote the main manuscript. XZ and QW were responsible for quality control of the study. All authors reviewed the manuscript.

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Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Footnotes

Yong Xu and Wenpan Peng contributed equally to this work.

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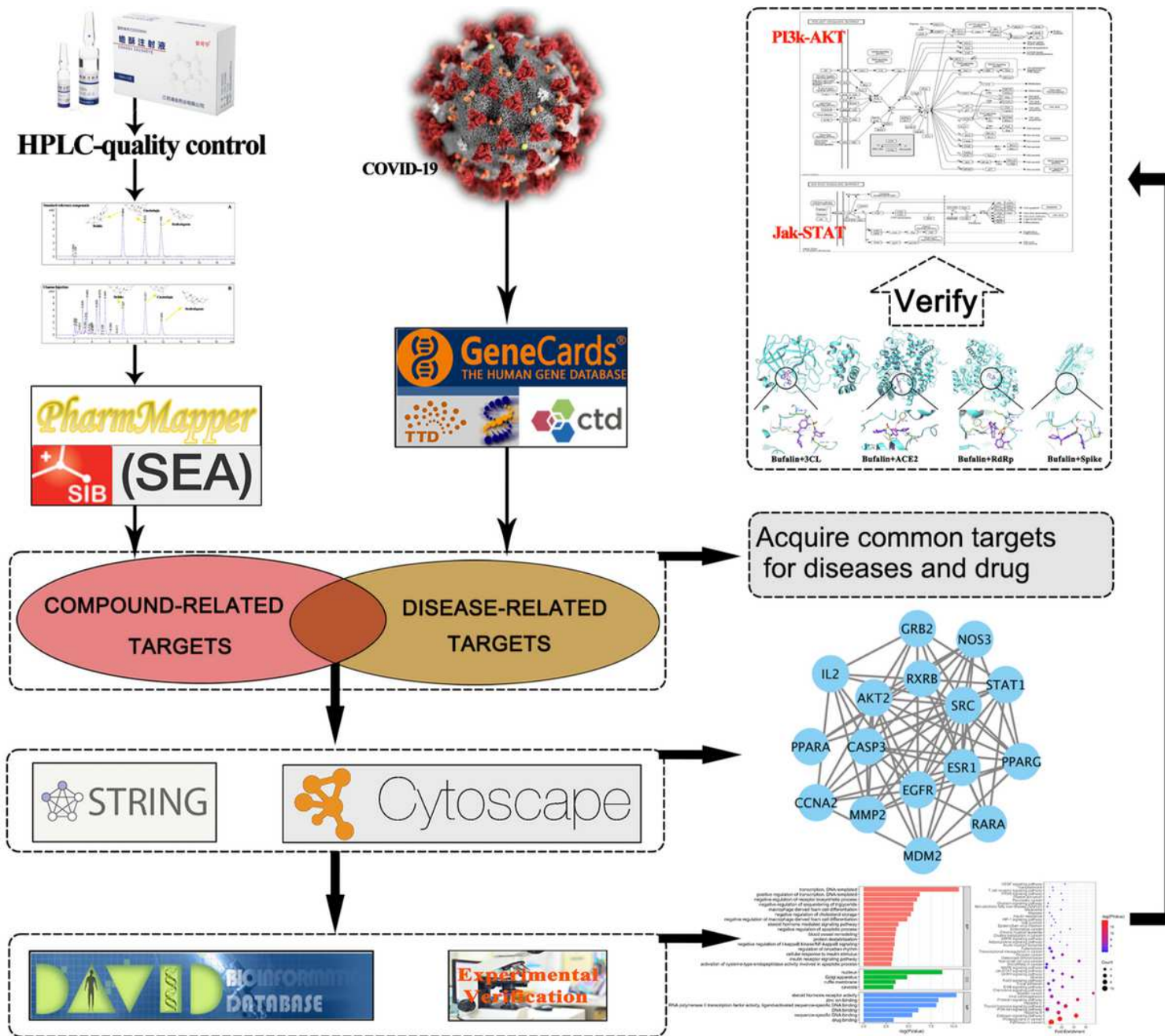
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Flowchart of the study



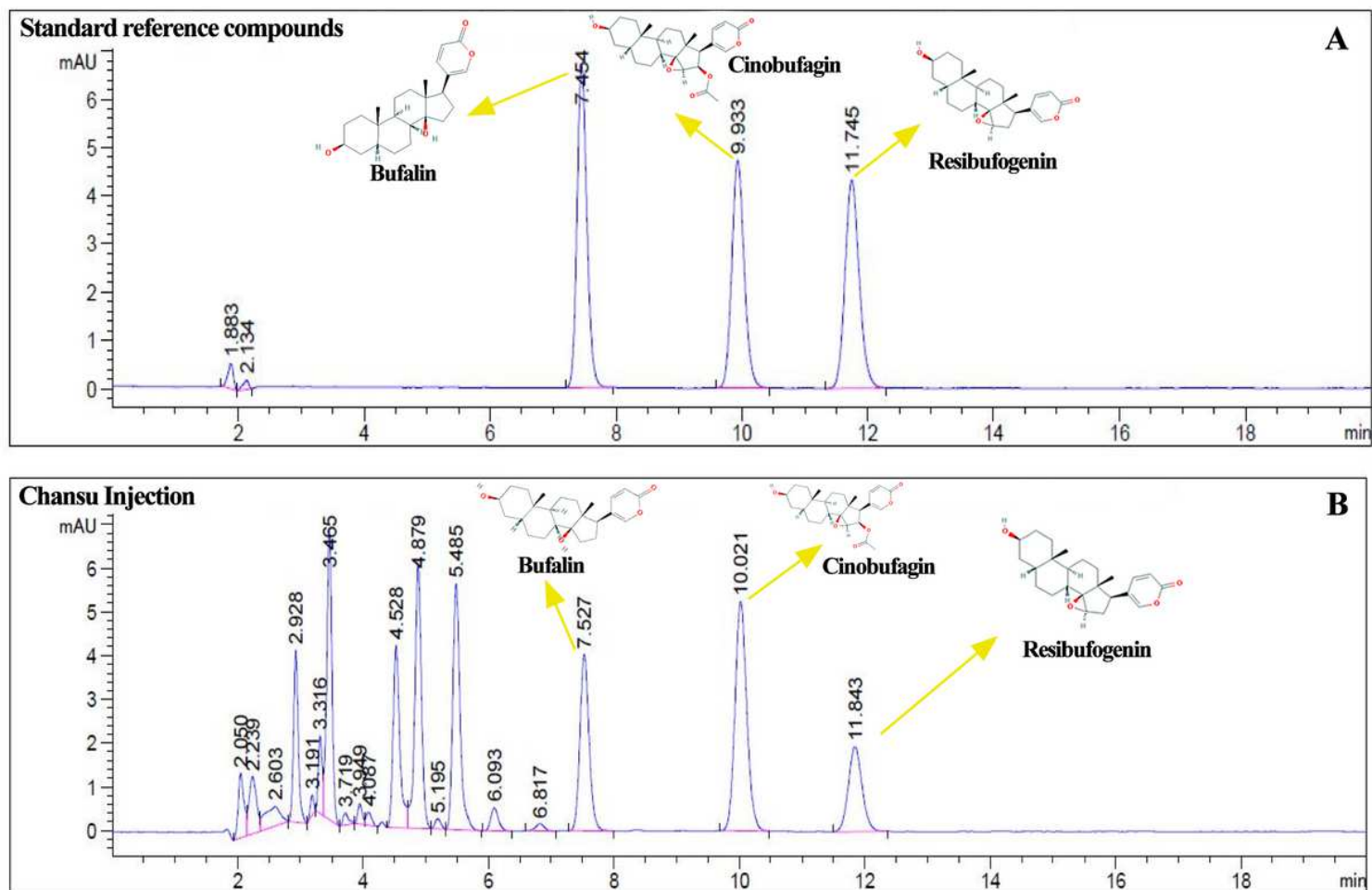


Figure 2

PLC chromatograms of the components of Chansu injection

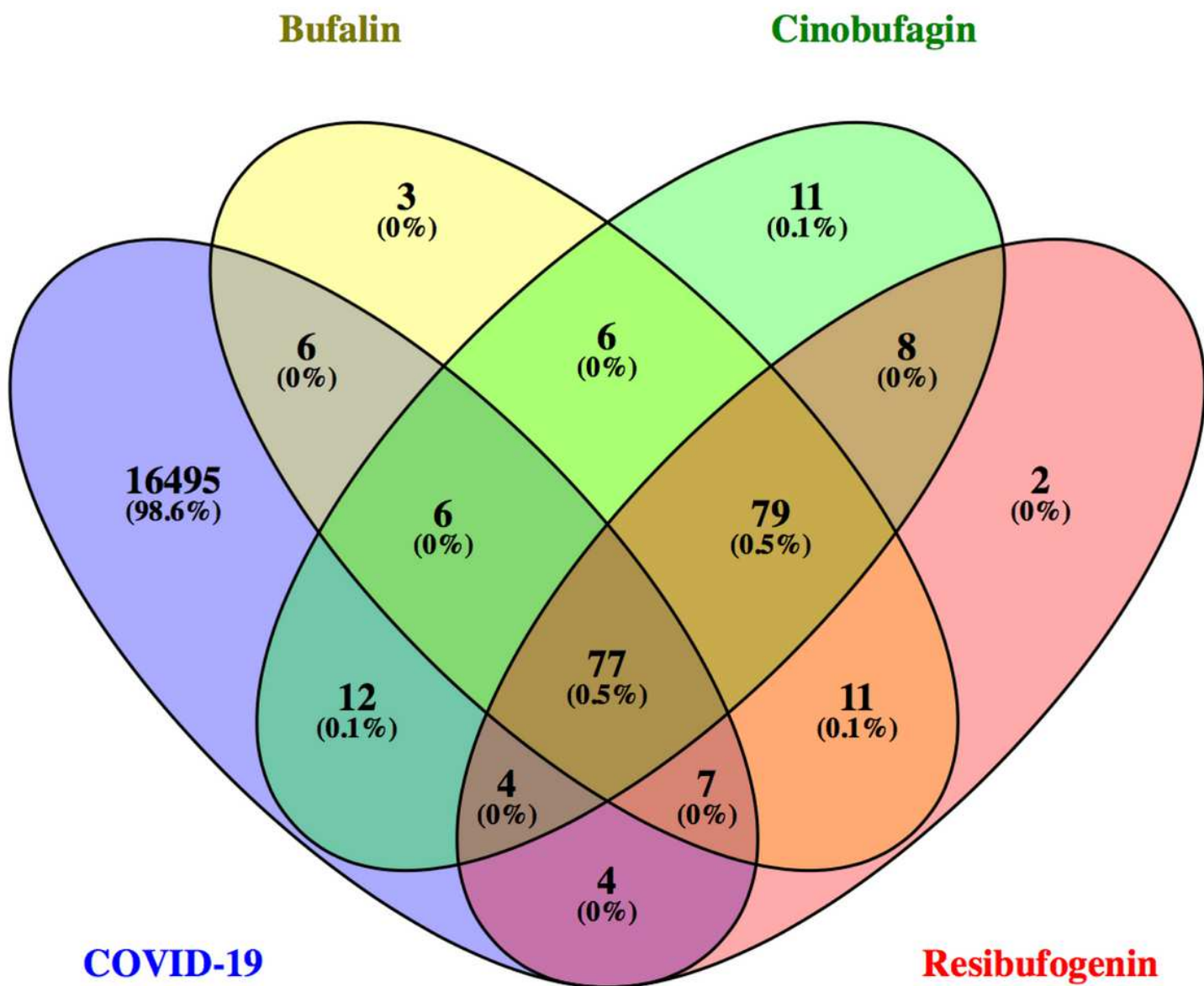


Figure 3

Venn diagram

Network diagram of the drug–target disease

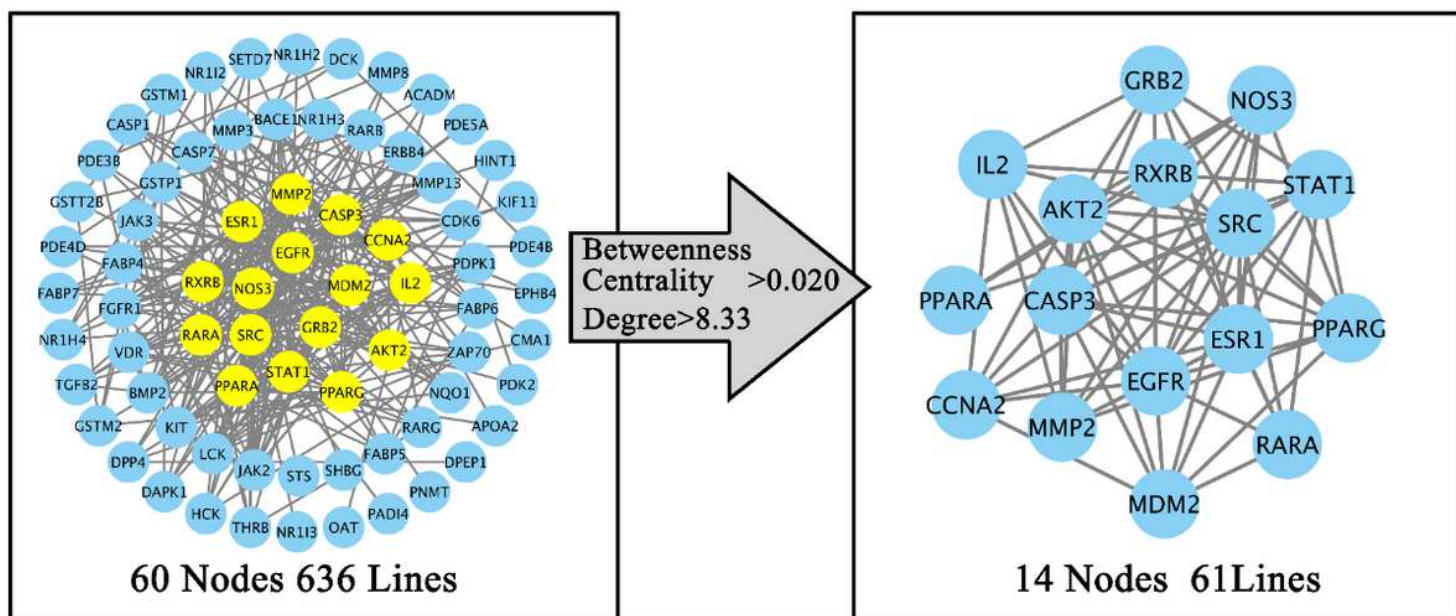


Figure 5

PPI diagram and filtration of the core targets

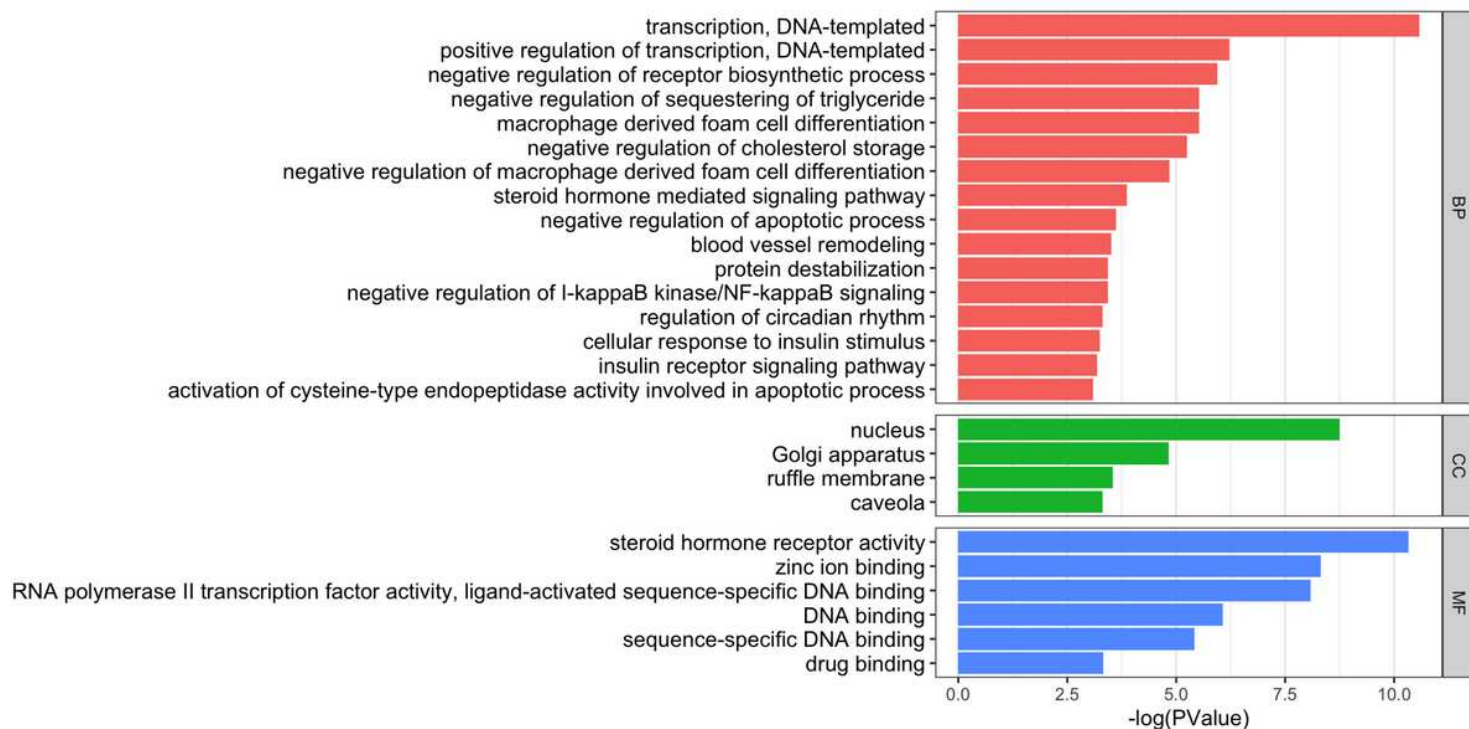


Figure 6

The results of GO enrichment analysis

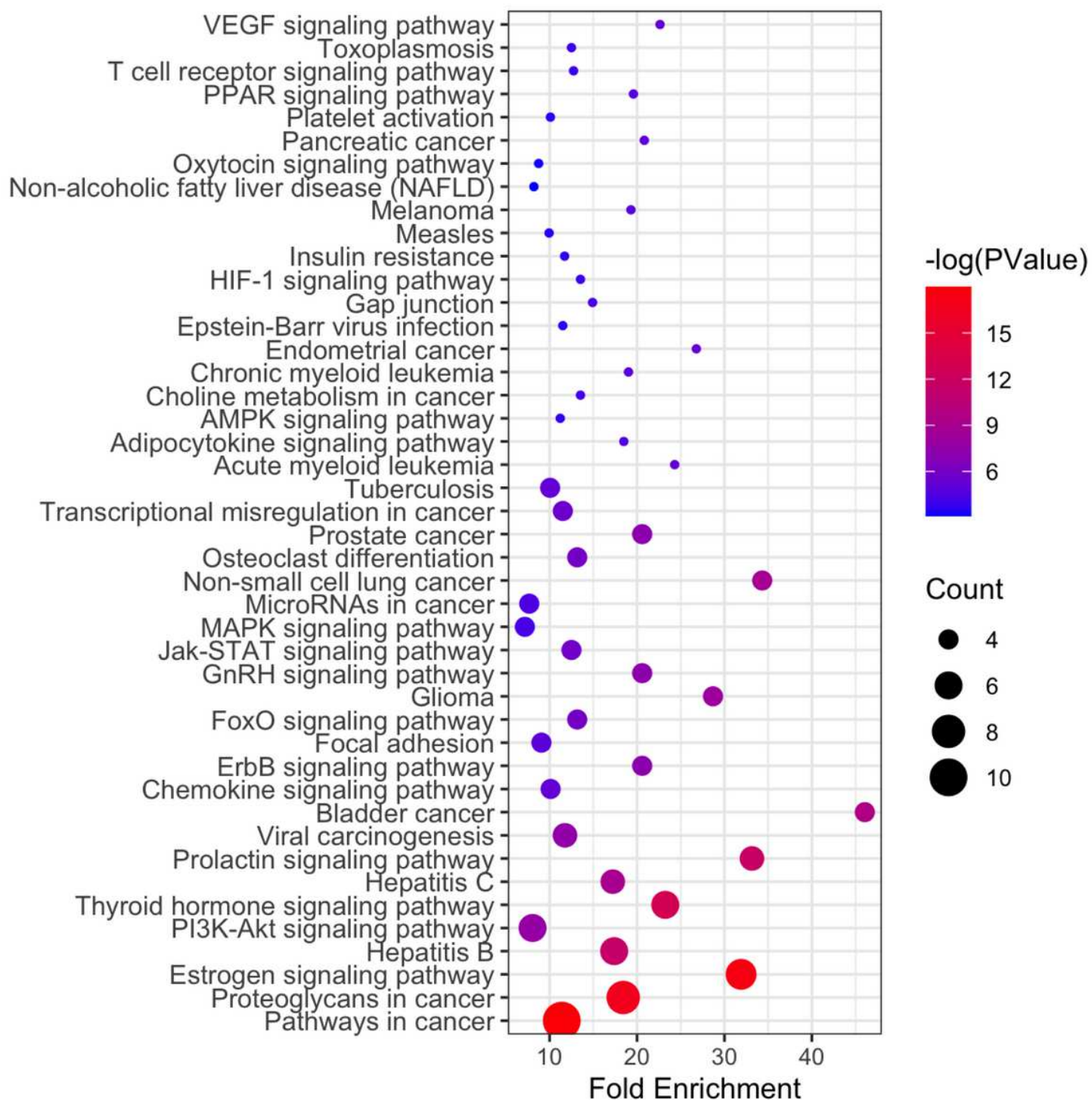


Figure 7

The results of KEGG enrichment analysis

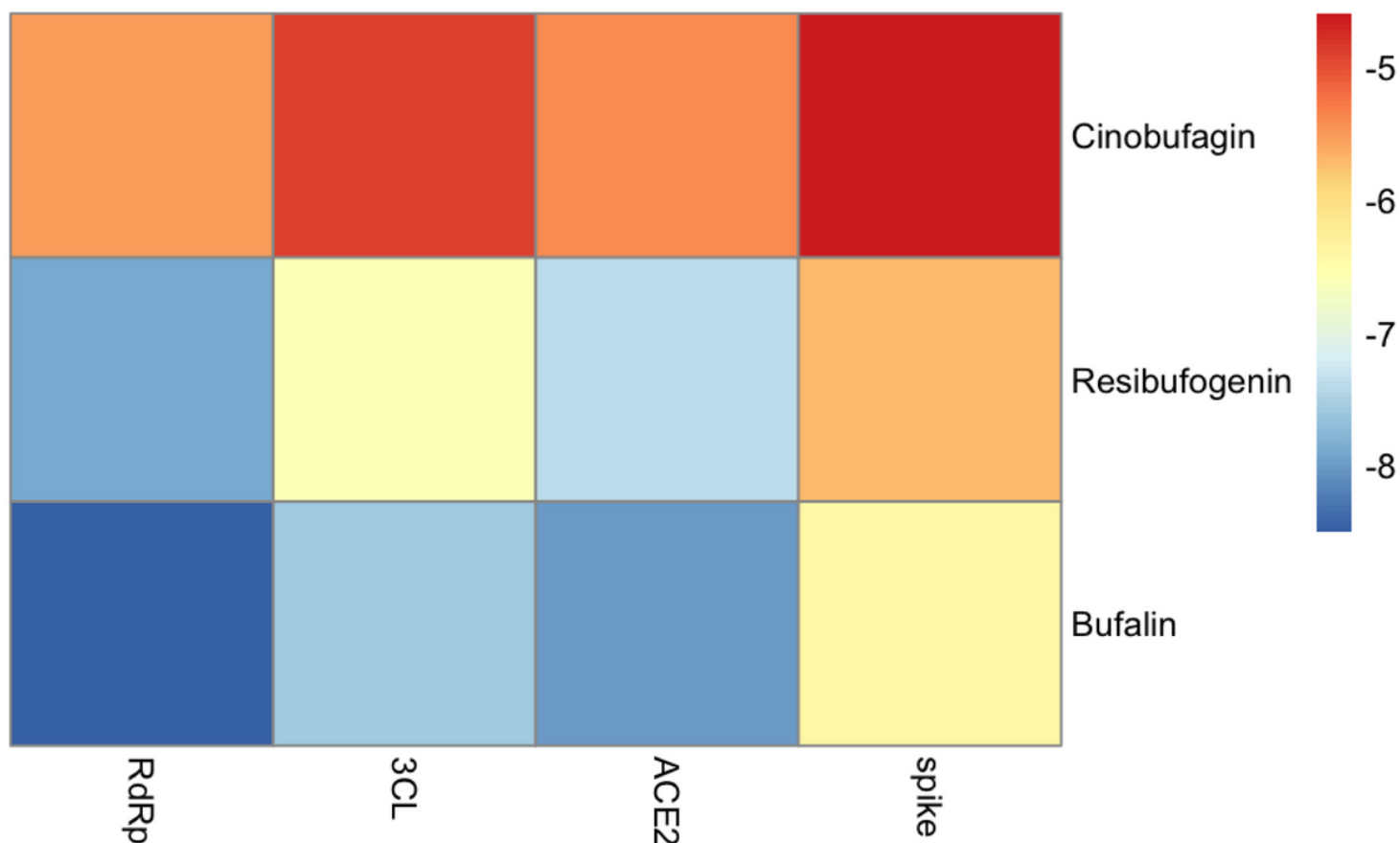


Figure 8

Diagram of the molecular docking efficiency

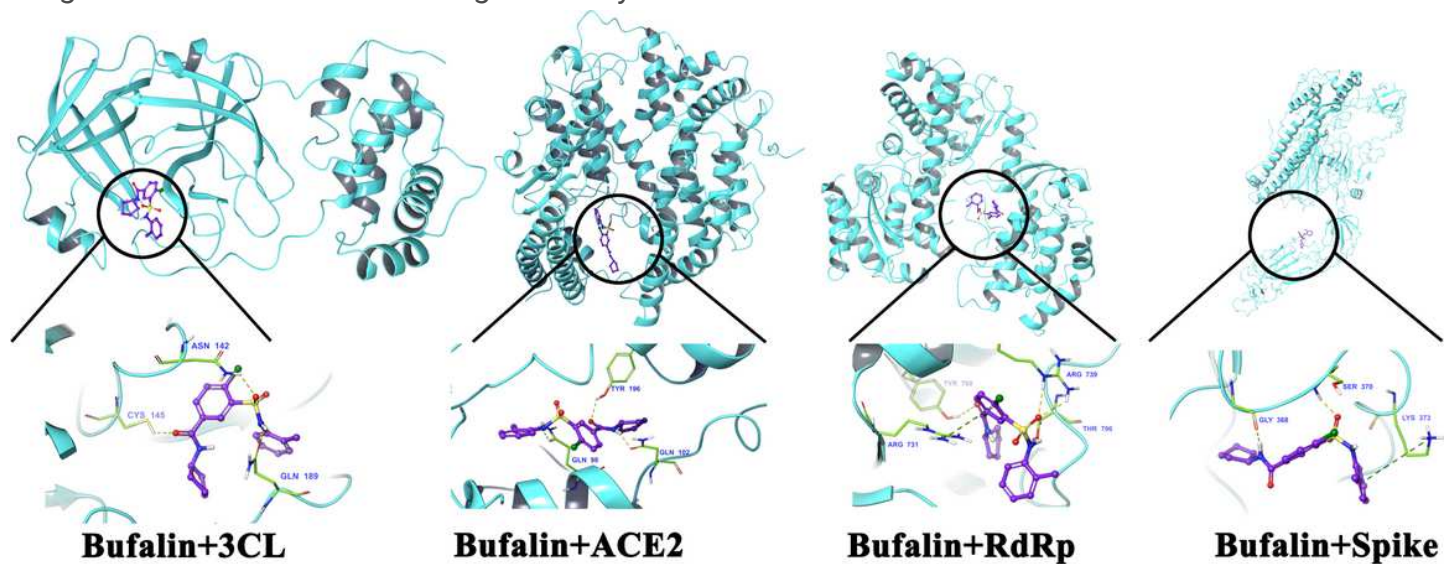


Figure 9

Molecular docking of Chansu injection to 3CL protease, AEC2, RdRp, and spike protein