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Exploring the active components and mechanism of modified bazhen decoction in treatment of chronic cerebral circulation insufficiency based on network pharmacology and molecular docking

Zhongbo Xu, MS[a], Manyang Shen, BS[b], Lin Li, MD[a,*]

Abstract

Modified bazhen decoction (MBZD) is a classical Chinese medicine formula with potential efficacy in the treatment of chronic cerebral circulation insufficiency (CCCI), and its main components and potential mechanisms are still unclear. The study aimed to investigate the active ingredients and mechanism of action of MBZD in treating CCCI through network pharmacology combined with molecular docking. The chemical composition and targets of 11 Chinese herbs in MBZD were retrieved utilizing the traditional Chinese medicine systems pharmacology database and analysis platform platform, and the targets for CCCI were screened by Genecards, online mendelian inheritance in man, therapeutic target database, and comparative toxicogenomics database databases. The targets were genetically annotated with the Uniprot database. We created a compound-target network employing Cytoscape software and screened the core targets for the treatment of CCCI by CytoNCA clustering analysis; the AutoDock Vina program performed molecular docking study of crucial targets. One thousand one hundred ninety-one active compounds were obtained, 2210 corresponding targets were predicted, 4971 CCCI-related targets were obtained, and 136 intersecting genes were identified between them. The central core targets were IL6, MAPK14, signal transducer and activator of transcription 3, RELA, VEGFA, CCND1, CASP3, AR, FOS, JUN, EGFR, MAPK1, AKT1, MYC, and ESR1; gene ontology functional enrichment analysis yielded 911 gene ontology items ($P < .01$), while Kyoto Encyclopedia of Genes and Genomes pathway enrichment yielded 138 signal pathways ($P < .01$), primarily including oxidative reactions, vascular regulation, apoptosis, and PI3K-Akt signaling pathway. The molecular docking results showed that the core active component of MBZD had good binding with the main target.

This research initially uncovered the mechanism of action of MBZD via multi-component-multi-target-multi-pathway for the treatment of CCCI, providing the theoretical basis for the clinical application of MBZD.

Abbreviations: BP = biological process, CC = cellular component, CCCI = chronic cerebral circulation insufficiency, LAC = local mean connectivity-based method, MBZD = modified bazhen decoction, STAT3 = signal transducer and activator of transcription 3, TCM = traditional Chinese medicine.

Keywords: chronic cerebral circulation insufficiency, modified bazhen decoction, molecular docking, network pharmacology, traditional Chinese medicine

1. Introduction

Chronic cerebral circulation insufficiency (CCCI) is a common chronic cerebrovascular disease with dizziness, headache, sleeplessness, and memory loss as its principal clinical signs. It is mostly middle-aged and elderly patients and seriously harms the lives of middle-aged and elderly patients. Modern medicine believes that chronic cerebral insufficiency is the pre-stage of cerebral infarction and is closely related to various diseases such as vascular dementia and Alzheimer.[1] According to statistics, about 80% of the elderly over 75 will have varying degrees of chronic cerebral insufficiency, and those over 60 will generally appear.[2]

Currently, modern medicine's treatment of CCCI patients mainly focuses on controlling the underlying disease, expanding blood vessels, improving cerebral circulation, nourishing the brain nerves, and failing to achieve satisfactory therapeutic effects.[3] Traditional Chinese medicine (TCM) has, in comparison, shown clear benefits in the treatment of CCCI. In the theory of Chinese medicine, most patients with CCCI suffer from deficiency of vital energy, blood stasis and turbid phlegm.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are publicly available.

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How to cite this article: Xu Z, Shen M, Li L. Exploring the active components and mechanism of modified bazhen decoction in treatment of chronic cerebral circulation insufficiency based on network pharmacology and molecular docking. Medicine 2023;102:29(e34341).

Received: 4 April 2023 / Received in final form: 22 June 2023 / Accepted: 23 June 2023

http://dx.doi.org/10.1097/MD.0000000000034341

Therefore, supplementing the vital energy, removing blood stasis, and dissipating phlegm are the fundamental TCM therapeutic approaches for CCCI. Modified bazhen tang (MBZD) is a commonly used herbal prescription for the therapies of CCCI at our hospital. It consists of 11 Chinese herbs, including Huangqi(*Hedysarum Multijugum Maxim*), Danshen (*Radix Salviae*), Gegen (*Radix Puerariae*), Dangshen (*Codonopsis Radix*), Chishao (*Radix Paeoniae Rubra*), Fuling (*Poria cocos*), Baizhu (*Atractylodes Macrocephala Koidz*), Chuanxiong (*Chuanxiong Rhizoma*), Danggui (*Angelicae Sinensis Radix*), Shichangpu (*Acoritataninowii Rhizoma*) and Tiannanxing (*Arisaematis Rhizoma*). MBZD can alleviate CCCI patients symptoms significantly in the clinic, improving the function of cerebrovascular reserve and cerebral blood flow.[3] In addition, it can alleviate dizziness, headache, heavy head, and other symptoms, but its mechanism of action is yet unknown.

When the Chinese medicine formula treats diseases, there is a complicated many-to-many network relationship between chemical components, predictive targets, and disease targets. How to clarify the mechanism of action of traditional Chinese medicine and its compound preparations is a significant issue. The development of network pharmacology depends on the fast growth of systems biology, network biology, chemical biology, and computer technology.[4] Network pharmacology can analyze the biological behavior of various drug molecules operating on diverse targets, cells, and organs from the genetic and molecular levels, which can predict and disclose the effects and mechanisms of the different drug molecules.[5] According to the interaction network of "drug-chemical component-target-disease," through network topology analysis, systematically and comprehensively observe and study the intervention and influence of drugs on diseases, revealing the mystery of the multi-molecular properties of drugs that act synergistically on the human body. Network pharmacology has the characteristics of holistic and systemic nature, which coincides with the holistic view of Chinese medicine, and is currently one of the important methods for the discovery of the material basis of Chinese medicine efficacy.[6] Therefore, from the perspective of network pharmacology, a "multi-component-multi-target" network of traditional Chinese medicine compounds is established to predict, reveal and clarify the molecular mechanism of MBZD in treating CCCI. The specific research process is shown in Figure 1.

2. Materials and methods

2.1. Screening of potential pharmacodynamic compounds and related targets in MBZD

The traditional Chinese medicine systems pharmacology database and analysis platform (https://old.tcmsp-e.com/tcmsp.php)[7] was used to collect the effective active ingredients and targets of 11 kinds of traditional Chinese medicines in the MBZD, including Huangqi, Danshen, Gegen, Dangshen, Chishao, Fuling, Baizhu, Chuanxiong, Danggui, Shichangpu, and Tiannanxing. Active chemical compounds are selected depending on the pharmacokinetics absorption, distribution, metabolism, and excretion characteristics of each component, using oral bioavailability > 30% and drug-likeness > 0.18 as screening requirements[8]; import the active ingredients of the compound into the DrugBank database (https://go.drugbank.com/)[9] for matching, deleting duplicate, nonhuman, and irregular targets, and finally, screen out potential targets. Then, with the Uniprot database(https://www.uniprot.org/),[10] the full name of targets was converted to official gene symbols, and the target without the corresponding gene name was deleted.

2.2. Screening of related targets in CCCI

Using Genecards database(https://www.genecards.org/),[11] online mendelian inheritance in man data (http://www.omim.org/),[12] comparative toxicogenomics database database (http://ctdbase.org/)[13] and therapeutic target database database (http://db.idrblab.net/ttd/),[14] search with "Chronic cerebral circulation insufficiency" as a keyword to get CCCI-related targets. Finally, the TCM compound drug target was intersected with CCCI-related targets, and the document "drug-disease" was obtained for the intersection target of TCM and CCCI.

2.3. Gene ontology and pathway enrichment analysis

ClusterProfiler,[15] a program inside the R 4.04 software (https://www.r-project.org/), did a functional enrichment analysis on drug-disease targets. A cutoff P value and Q value < 0.05 were adopted to signify statistical significance. GO, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment annotation results were visualized using the GGplot2 R software package to draw a bubble diagram.

2.4. Network construction

Network construction was performed as follows: The intersection of CCCI disease targets and the active ingredient of MBZD compound was input into Cytoscape software (https://cytoscape.org/) to build the compound-target and target-pathways network. Additionally, using the "Network Analyzer" function to obtain its topology parameters, the core compounds are screened according to the "Degree" value; The target genes of the drug-disease intersection were imported into the database STRING (https://cn.string-db.org/)[16] with the settings of "Homo sapiens" and a confidence level ≥ 0.7 to construct a protein-protein interaction (PPI) network.[17] After that, the PPI network outcomes were visualized using Cytoscape software.

2.5. Network cluster analysis and core target selection

The CytoNCA plug-in[18] in Cytoscape software was used for the cluster analysis to calculate computing degree centrality, intermediate centrality, tight centrality cellular component (CC), eigenvector centrality, local mean connectivity-based method (LAC) and network centrality in PPI. These parameters are the primary method for identifying critical nodes in network analysis. The higher the index, the more influential the role of nodes in the network. Nodes and networks higher than the median of the above 6 topological parameters are extracted to screen out the core target.

2.6. Validation of molecular docking

The main TCM components were selected to conduct molecular docking with the core targets. The 2-dimensional structure diagram of the compound was downloaded from the PubChem database[19] (https://pubchem.ncbi.nlm.nih.gov/) and imported into Chem3D software to draw the 3-dimensional structure diagram. After optimization, the structure diagram was saved in the format of MOL2. AutoDock Tools1.5.6 prepares receptors, including adding hydrogen and setting docking parameters.[20] Every ligand and receptor data is stored in the PDBQT format. AutoDock Vina was utilized to analyze and validate the binding affinity and predicted network pharmacology outcomes of the compound-target interaction. Pymol 2.3.0 software (https://pymol.org/2/) was used to depict the crucial model.

3. Results

3.1. Potential pharmacodynamic components

Using the traditional Chinese medicine systems pharmacology database and analysis platform database, 1191 active

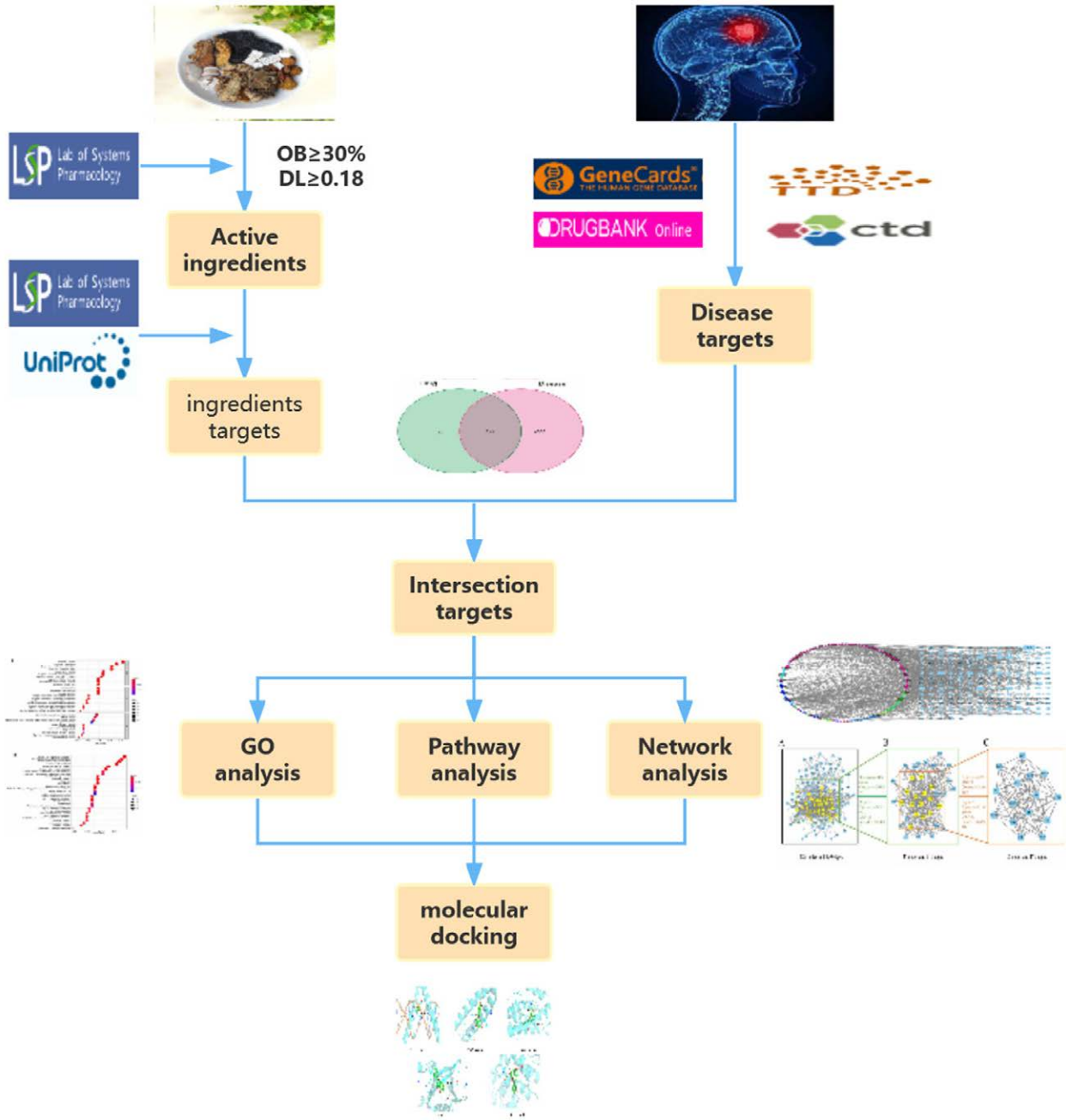


Figure 1. The flowchart of the whole study's methodology.

components were retrieved from the eleven herbal medications comprising MBZD. A total of 181 active ingredients were screened, including 20 active ingredients of Huangqi, 65 active ingredients of Danshen, 4 active ingredients of Gegen, 21 active ingredients of Dangshen, 29 active ingredients of Chishao, 15 active ingredients of Fuling, 7 active ingredients of Baizhu, 7 active ingredients of Chuanxiong, 2 active ingredients of Danggui, 4 active ingredients of Shichangpu, and 7 active ingredients of Tiannanxing.

3.2. Targets of MBZD and CCCI

The DRUGBANK database predicted the component targets of MBZD. After gene annotation of the Uniprot database, 2210 annotated genes were obtained. In addition, 4971 genes related to CCCI were retrieved from DRUGBANK, online mendelian inheritance in man, comparative toxicogenomics database, and therapeutic target database databases. Using R software to draw a VENN diagram (Fig. 2), a total of 136 intersection targets were obtained from the MBZD and diseases, namely the action targets of MBZD in the treatment of CCCI.

3.3. Functional enrichment analysis

GO enrichment analysis of drug-disease targets shows that in terms of biological processes (BP), the targets were mainly enriched in the "response to drug," "cellular response to chemical stress," and "response to metal ion," Regarding CC, the targets were mainly enriched in the "membrane raft," "membrane microdomain," and "membrane region." In terms of molecular functions, the targets were mainly enriched in "DNA – binding transcription factor binding," "amide binding," and "RNA

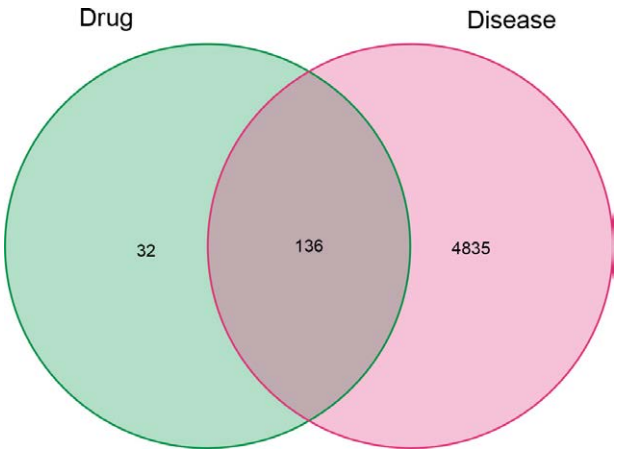


Figure 2. Venn diagram showing shared targets of CCCI and MBZD. CCCI = chronic cerebral circulation insufficiency, MBZD = modified bazhen decoction.

polymerase II – specific DNA – binding transcription factor binding." The top 10 functionally enriched associated with MBZD against CCCI were plotted as bubble maps (Fig. 3A).

KEGG enrichment analysis revealed the total involvement of 165 signaling pathways, of which 138 were $P < .01$. The MBZD against CCCI mainly involved the PI3K-Akt signaling pathway, AGE-RAGE signaling pathway, HIF-1 signaling pathway, IL-17 signaling pathway, p53 signaling pathway, Cellular senescence, Apoptosis, etc, and was associated with Human cytomegalovirus infection, Kaposi sarcoma-associated, herpesvirus infection, Hepatitis, Virus infection, Cancer and other diseases are closely related (Fig. 3B). Meanwhile, according to the results of the KEGG enrichment analysis, the PI3K-Akt signaling pathway was the main pathway of MBZD against CCCI. The KEGG Mapper platform was used to map the pathway mechanism of the PI3K-Akt signaling pathway (Fig. 4).

3.4. Network construction

The "component-targets" network diagram of MBZD in the treatment of CCCI was drawn by Cytoscape 3.7.2 software (Fig. 5A). There were 316 nodes in the network, including 201 nodes of target genes and 115 nodes of active ingredients. Among them, the left circle represents the active ingredients of compound MBZD, different colors represent different traditional Chinese medicine ingredients, and the right grid represents the target genes of active ingredients, represented by a blue rectangle. The larger the node, the more linked components represent the target gene. Through network analysis, it was found that the top 5 active ingredients of Degree value in MBZD were quercetin, luteolin, kaempferol, tanshinone IIA, and 7-O-Methylisomucronulatol (Table 1). To display the correlation between top pathways and target proteins based on KEGG enrichment analysis, we constructed a target-pathway network and visualized the relationship between the hub anti-CCCI targets, and their corresponding pathways. The network contains 61 nodes and 109 edges, connecting top 5 pathways, 81 targets (Fig. 5B).

3.5. Network cluster analysis and core target selection

One hundred thirty-6 intersection targets TSV text were imported into Cytoscape software to draw a PPI network diagram (Fig. 6A) which consists of 129 nodes and 820 edges. The Cytonca plug-in in Cytoscape software was used twice to screen candidate targets for PPI network. In the first round of screening, according to the topological analysis parameters: Betweenness = 54.54066866, Closeness = 0.223367698, Degree = 9, Eigenvector = 0.034260273, LAC = 4.4, Network = 5.771645022, the new network was screened out to contain 37 nodes and 316 edges, namely the yellow nodes (Fig. 6B). The parameters of the second round are Betweenness = 256.0598225, Closeness = 0.243902439, Degree = 22, Eigenvector = 0.130245864, LAC = 10.3, Network = 13.84654836. Finally, 15 core targets (IL6, MAPK14, signal transducer and activator of transcription 3 (STAT3), RELA, VEGFA, CCND1, CASP3, AR, FOS, JUN, EGFR, MAPK1, AKT1, MYC, and ESR1) were screened, including 15 nodes and 176 edges (Fig. 6C) (Table 2). These genes may be the core targets of MBZD against CCCI.

3.6. Molecular docking verification

The top 5 active compounds in this study performed molecular docking with the top 5 core targets, and the molecular docking binding energy score and docking parameters are shown in Table 3. Generally, the binding energy < – 5.0 kJ/mol is the standard for stable ligand and receptor binding in molecular docking. The lower the binding energy, the higher the molecular matching stability. The present results revealed that all docking binding free energies were less than –5.0 kJ/mol. Some molecular docking binding patterns are shown in Figure 7, where gray is protein receptor, green is small molecular ligand, and yellow dotted line is hydrogen bond formed. Tanshinone IIA docked with AKT1 through DG-308, DA-308 and degree centrality-310; with MYC through ARG-23; with IL6 through LEU-65, MET-68 and ARG-169; and with STAT3 through GLU-91 and HIS-13. JUN docked with kaempferol is hydrogen bonded to a single amino acid residue via SER-540 and THR-526.

4. Discussion

The application of network pharmacology to investigate the mechanism of herbal compounding for treating diseases has become a hot research topic. The network pharmacology technique elucidates the mechanism of the effect of TCM from a systematic viewpoint and presents a multifaceted research strategy for varied, complicated TCM compounding. In this research, we used a network pharmacology approach to uncover the possible molecular mechanisms of MBZD against CCCI. We discovered that 115 active MBZD components may function on 136 CCCI-associated targets. Further functional enrichment analysis revealed that MBZD might act on multiple BP of CCCI and interfere with CCCI through the PI3K-Akt signaling pathway, HIF-1 signaling pathway, and AGE-RAGE signaling pathway, thus confirming the multi-component, multi-channel, and multi-target characteristics of MBZD.

The top 5 active ingredients in the PPI network were: quercetin (Astragalus), luteolin (Radix Salviae), kaempferol (Astragalus, Calamus), tanshinone IIA (Radix Salviae), and 7-O-methylisomaltol (Astragalus). These herbs are the core drugs of MBZD, indicating that the results of the network pharmacological study coincide with the clinical application of the herbal formulas. Quercetin is a natural flavonoid with various biological activities, including antitumor, anti-inflammatory, antioxidant and anti-apoptotic. It was found that quercetin inhibited the accumulation of oxidants in cells and the inactivation of phosphorylated phospho-p53 in the nucleus, thereby reducing endothelial cell apoptosis induced by oxidative effects.[21] Lin et al[22] found that quercetin ameliorates atherosclerosis by inhibiting dendritic cell activation, thereby reducing endothelial cell dysfunction. Additionally, some scholars have experimentally found that quercetin may contribute to the neuroprotective effect of focal cerebral ischemia by regulating the expression of thioredoxin.[23] Luteolin, one of the natural compounds, has physiological functions such as mitigation of oxidative damage and resistance to inflammatory response, reduction of tumor multiplicity, and antibacterial, cardiovascular, cerebrovascular,

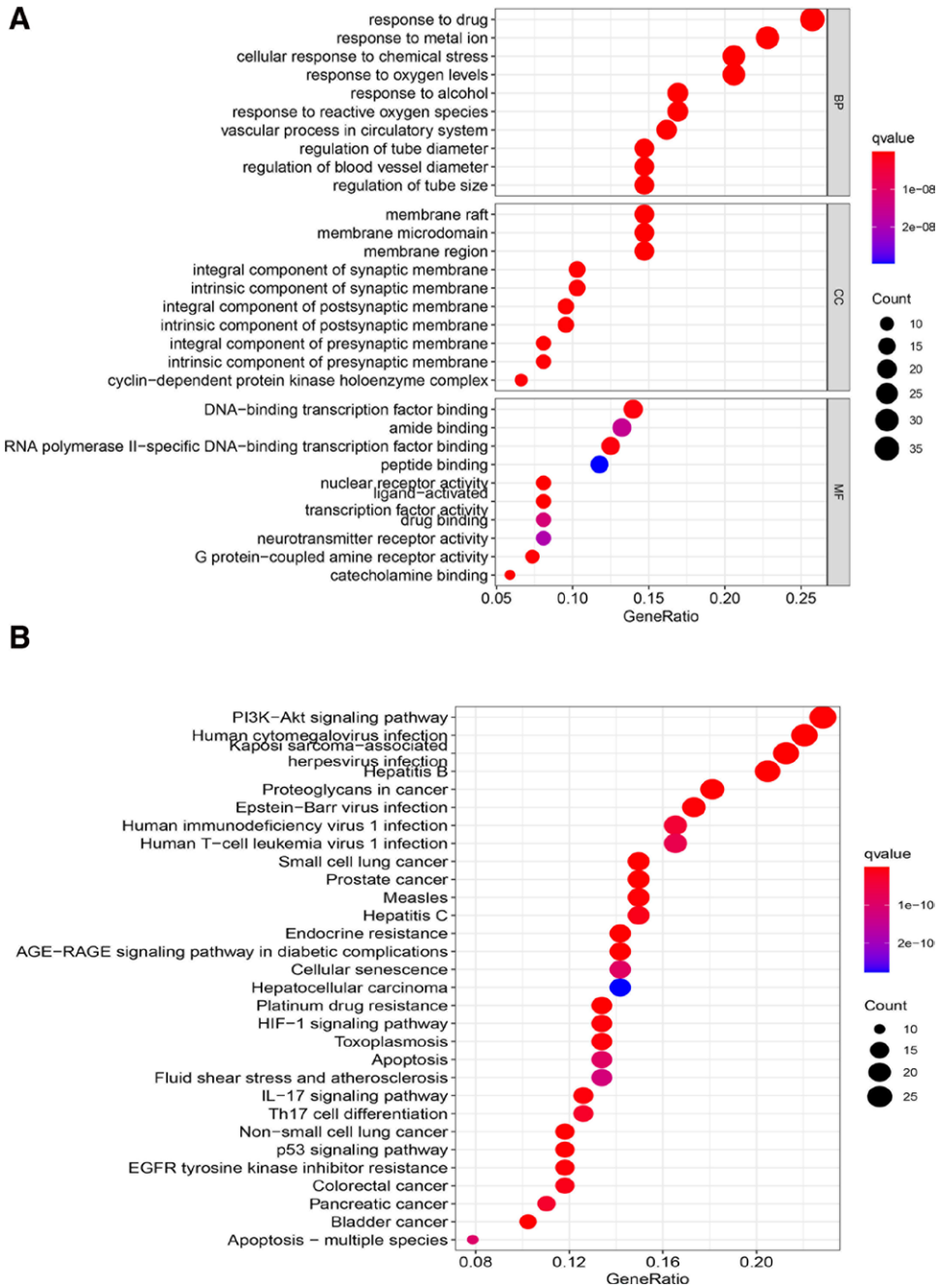


Figure 3. Functional enrichment analysis. (A). Bubble chart of GO enrichment (B). Bubble chart of KEGG pathways (The color of the bubbles represents the *P* value, while the size denotes the number of genes).

and respiratory protective effects.[24–26] Kaempferol is a flavonoid, mainly from the rhizome of kaempferia, a plant of the ginger family, and is widely found in various traditional Chinese medicines. Yu L et al[27] found that kaempferol glycoxib inhibited the expression of OX-42, glial fibrillary acidic protein, and phosphorylated STAT3 and achieved neuroprotective effects in rat brain tissues damaged by ischemia-reperfusion of brain tissue. Similarly, experimental animal studies have shown that Kaempferol can significantly improve the volume of cerebral infarction, improve pathological tissue changes, reduce apoptosis and expression of apoptotic factors, and play a protective role in brain tissue.[28] Radix Salviae is a critical traditional Chinese medicine, taken from the dried root and rhizome of the plant Salvia miltiorrhiza, family Labiatae. It is widely used in China to treat cardiovascular and cerebrovascular diseases.[29] Radix Salviae contains many components, among which tanshinone IIA is the main active ingredient in Radix Salviae, which has various pharmacological effects, such as antitumor, antioxidant, anti-inflammatory, and antibacterial effects.[30] Some studies have shown that tanshinone IIA can significantly improve brain tissue edema and infarct volume after 24 hours of focal cerebral ischemia, and similarly enhances superoxide dismutase activity, reduces malondialdehyde levels, and decreases nitric oxide and inducible nitric oxide synthase expression.[31,32]

In addition, fifteen potential targets consisting of AKT1, MYC, IL6, STAT3, JUN, etc, were screened as core targets of MBZD against CCCI. AKT1 is a serine/threonine protein kinase that regulates many processes, including angiogenesis, metabolism, proliferation, cell survival, and growth.[33] AKT1 gene deletion induces vascular endothelial cell dysfunction, cellular vascular

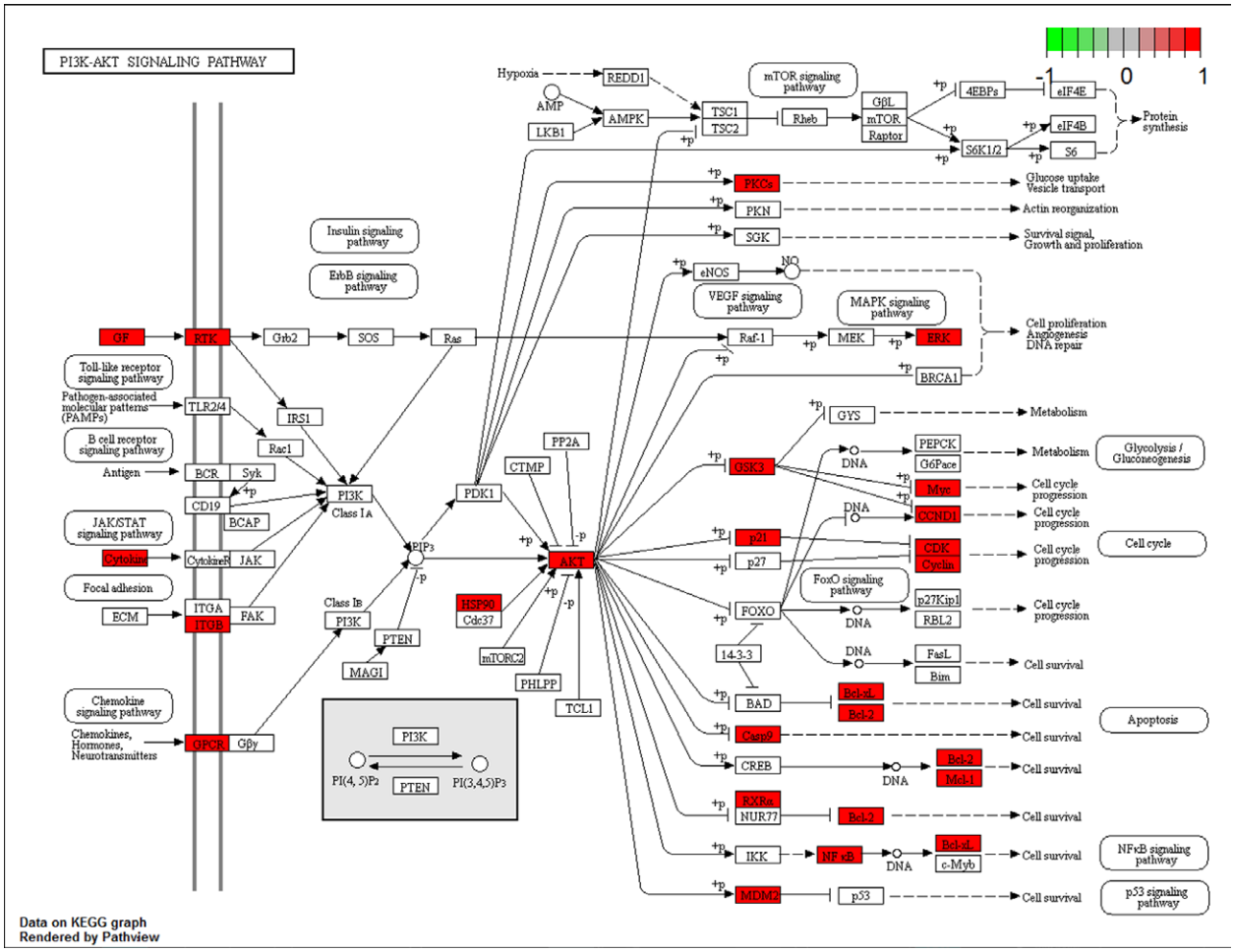


Figure 4. PI3K-Akt signaling Pathway map of CCCI against MBZD. (The red rectangles represent the crucial targets). CCCI = chronic cerebral circulation insufficiency, MBZD = modified bazhen decoction.

flat altered migration and survival of smooth muscle, promoting atherosclerosis, which is the most important causative agent of cerebral arterial insufficiency.[34,35] IL6 is an effector in regulating metabolic processes and immune function of the body and is the most important mediator of inflammatory processes in the body.[36] Studies have shown that IL6 is directly involved in the inflammatory response of the vascular endothelium, leading to thickening of the intima of arteries and also promotes the expression of intracellular adhesion molecules, which are involved in the formation of atherosclerosis.[37,38] In a state of cerebral ischemia, damaged brain tissue generates an immune response, and inflammatory cells are activated to secrete IL6 as an essential inflammatory mediator involved in secondary brain injury.[39] STAT3 is present in neurons and endothelial cells of blood vessels, astrocytes, and microglia. During the acute phase of cerebral ischemia, STAT3 exerts neuroprotective effects by inhibiting neuronal apoptosis and oxidative stress, thereby reducing the size of cerebral infarction. Studies have shown that STAT3 protein phosphorylation levels increase after cerebral ischemia, causing activation of the JAK2/STAT3 signaling pathway, which in turn mediates the postischemic inflammatory response and neuronal apoptosis.

Through the enrichment of core targets to understand their BP, molecular information, CC and related pathways, the mechanism of action of the herbal formula was revealed at a deeper level. The results of GO enrichment analysis in this study showed that the BP of the core targets were mainly involved in oxidative reactions and vascular regulation, suggesting that MBZD mainly exerted its effects on the prevention and treatment of CCCI through regulating vascular vascular diameter and antioxidant reactions. Enrichment analysis at the level of molecular functional information of the core targets showed that the relevant components of MBZD could act through DNA transcription factors and RNA transcription factors. Enrichment analysis at the CC level showed that the active ingredients of MBZD exerted their pharmacological effects by acting on various important functional sites such as cell membranes.

In this study, the KEGG pathway enrichment analysis revealed that the PI3K-Akt signaling pathway had the highest enrichment fold value, indicating that this pathway is the most important pathway for the anti-CCCI effect of MBZD, suggesting that MBZD may improve the disease by regulating PI3K-Akt signaling pathway activation. At the same time, the KEGG analysis also involved apoptosis and programmed cell death of senescent cells. PI3K/Akt pathway, as one of the vital cascade signaling pathways, is involved in the process of cerebral ischemogenesis and development, and is central in crosstalk among various mechanisms such as apoptosis, autophagy, oxidative stress and inflammatory response.[40] Studies have confirmed that the PI3K/Akt pathway is progressively inhibited during the pathological injury of cerebral ischemia-reperfusion, and by intervening to trigger and activate this pathway, the trend of excessive apoptosis can be reversed, thus reducing the extent of cerebral ischemia-reperfusion injury.[41] In order to verify the speculation of network pharmacology, this study selected the essential potent substance of MBZD and the core target for molecular docking verification, and the results showed that the critical potent

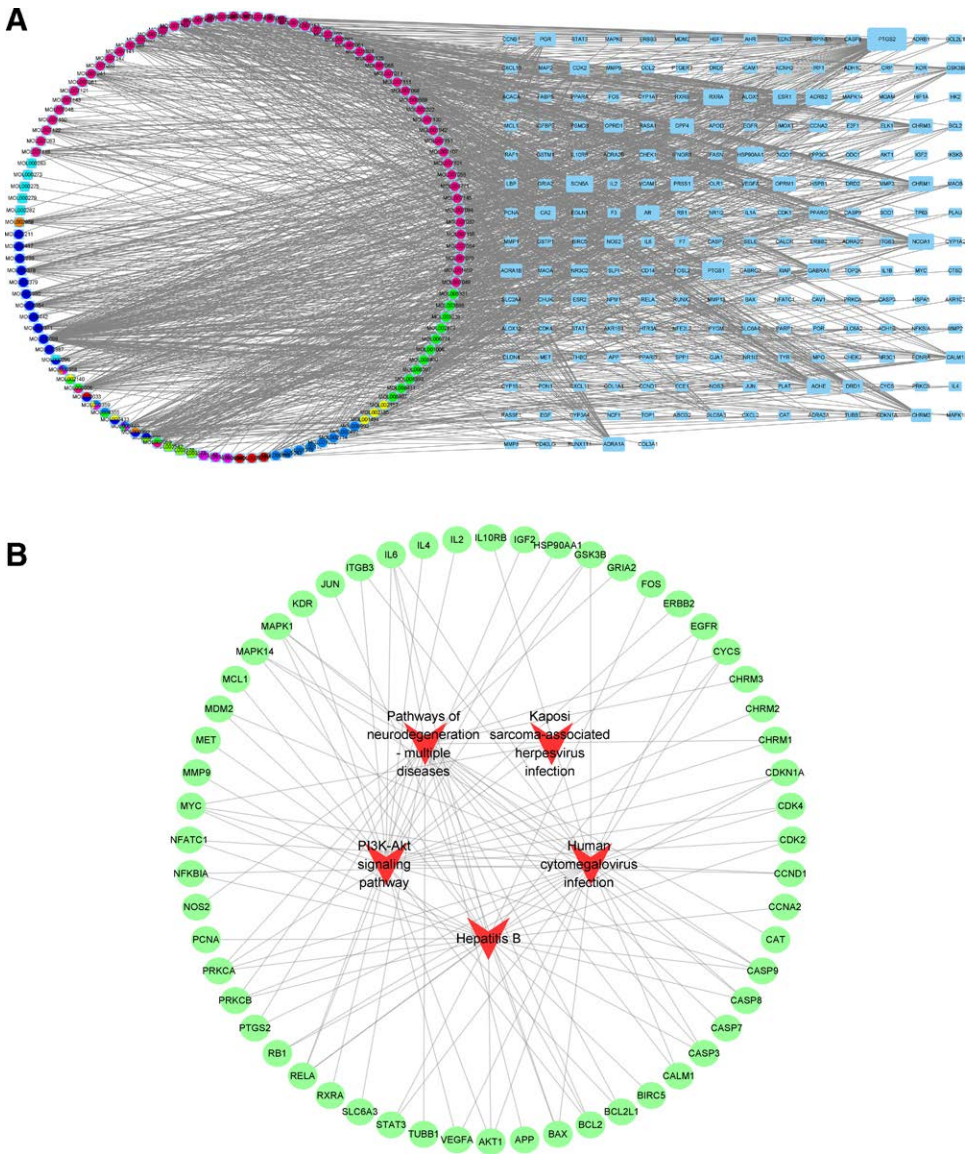
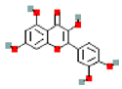
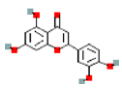
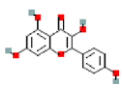
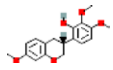


Figure 5. Network diagram of MBZD. (A). The component-target network. (B). The target-pathways network, MBZD = modified bazhen decoction.

Table 1

Top 5 compounds information.

Compound	Molecule structure	Degree	OB	DL	Herb
Quercetin		123	46.43	0.28	Huangqi
Luteolin		48	36.16	0.25	Dangshen Danshen
Kaempferol		47	41.88	0.24	Huangqi Shichangpu
Tanshinone IIA		35	49.89	0.40	Danshen
7-O-methylisomucronulatol		33	74.69	0.30	Huangqi

OB = oral bioavailability.

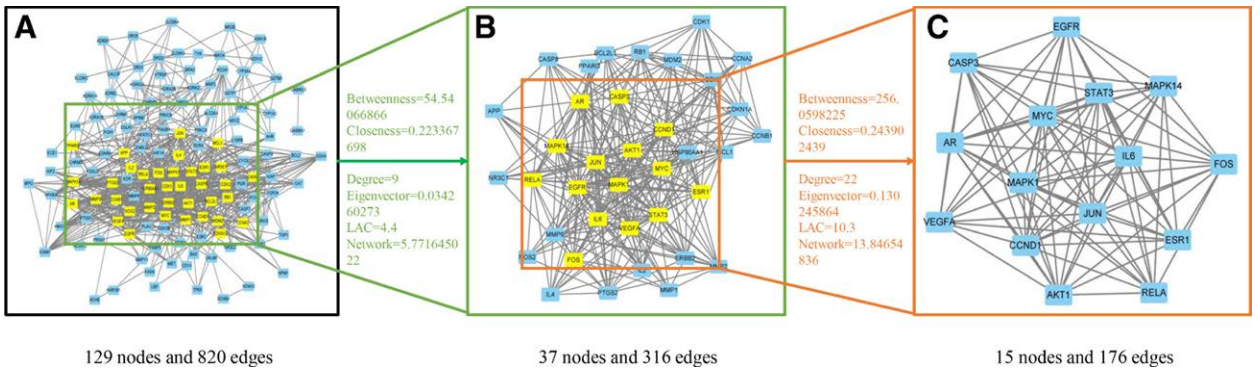


Figure 6. PPI network and cluster analysis of candidate targets of MBZD against CCCI. (A). The interactive PPI network of MBZD putative targets and CCCI -related targets. (B). PPI network of significant proteins extracted from A. (C). PPI network of candidate MBZD targets for CCCI treatment extracted from B. CCCI = chronic cerebral circulation insufficiency, MBZD = modified bazhen decoction.

Table 2

Specific information of the 15 key target genes.

Uniprot ID	Name	Description	Degree	Betweenness	Closeness
P31749	AKT1	RAC-alpha serine/threonine protein kinase	29	70.12873	0.837209
P01106	MYC	Myc proto-oncogene protein	29	66.13998	0.837209
P05231	IL6	Interleukin-6	27	45.75236	0.8
P40763	STAT3	Signal transducer and activator of transcription 3	27	44.61759	0.8
P05412	JUN	Transcription factor Jun	27	62.17815	0.8
P24385	CCND1	G1/S-specific cyclin-D1	26	43.15975	0.782609
P28482	MAPK1	Mitogen activated protein kinase 1	26	48.95696	0.782609
P00533	EGFR	Epidermal growth factor receptor	23	37.7368	0.734694
P15692	VEGFA	Vascular endothelial growth factor A	22	32.89219	0.72
P42574	CASP3	Caspase-3	21	25.40147	0.705882
P10275	AR	Androgen receptor	21	21.90491	0.705882
Q16539	MAPK14	Mitogen activated protein kinase 14	20	16.57684	0.692308
P9WHG9	RELA	Bifunctional (p) ppGpp synthase/hydrolase RelA	19	24.47301	0.679245
P03372	ESR1	Estrogen receptor	19	15.32111	0.679245
P01100	FOS	Protein c-Fos	17	12.07774	0.654545

STAT3 = signal transducer and activator of transcription 3.

Table 3

The binding energy of compound and core targets.

	Binding free energy (kcal·mol – 1)				
Active compound	**AKT1**	**MYC**	**IL6**	**STAT3**	**JUN**
7_O_Methylisomucronulatol	–7	–6.8	–6	–6.9	–6.6
Kaempferol	–7.6	–7.9	–7.1	–6.6	–7.1
Luteolin	–7.9	–7.9	–7.3	–7.7	–7.5
Quercetin	–7.3	–8	–7.2	–6.5	–7.2
Tanshinone IIA	–8.8	–8.9	–7.5	–7.8	–8.3

STAT3 = signal transducer and activator of transcription 3.

substance of MBZD and the core target protein could bind stably, which verified the prediction of network pharmacology about the mechanism of action of MBZD. However, there are certain limitations to the present study. Firstly, the current sample size of these datasets was small. Therefore, further studies using high-throughput sequencing experiments with larger clinical samples would be valuable. Secondly, some key genes and pathways were not found to be associated with IPF in previous studies. However, there are limitations in this study, that is, the mechanism of action of MBZD in the treatment of CCCI is only explored at the theoretical level, and the results of the experimental validation analysis still need to be improved.

5. Conclusion

In summary, MBZD may regulate the expression of key genes such as AKT1, MYC, IL6, STAT3, JUN, etc, and then act on multiple signaling pathways such as PI3K/Akt through the active ingredients such as quercetin, luteolin, kaempferol, tanshinone IIA and 7-O-Methylisomucronulatol, which together exert the effect of treating CCCI from multiple aspects such as regulating vascular vessel diameter and antioxidant response. This study reflects the multi-component, multi-target, and multi-pathway characteristics of MBZD for the treatment of CCCI, which will establish the theoretical foundation for further research on MBZD for the treatment of CCCI.

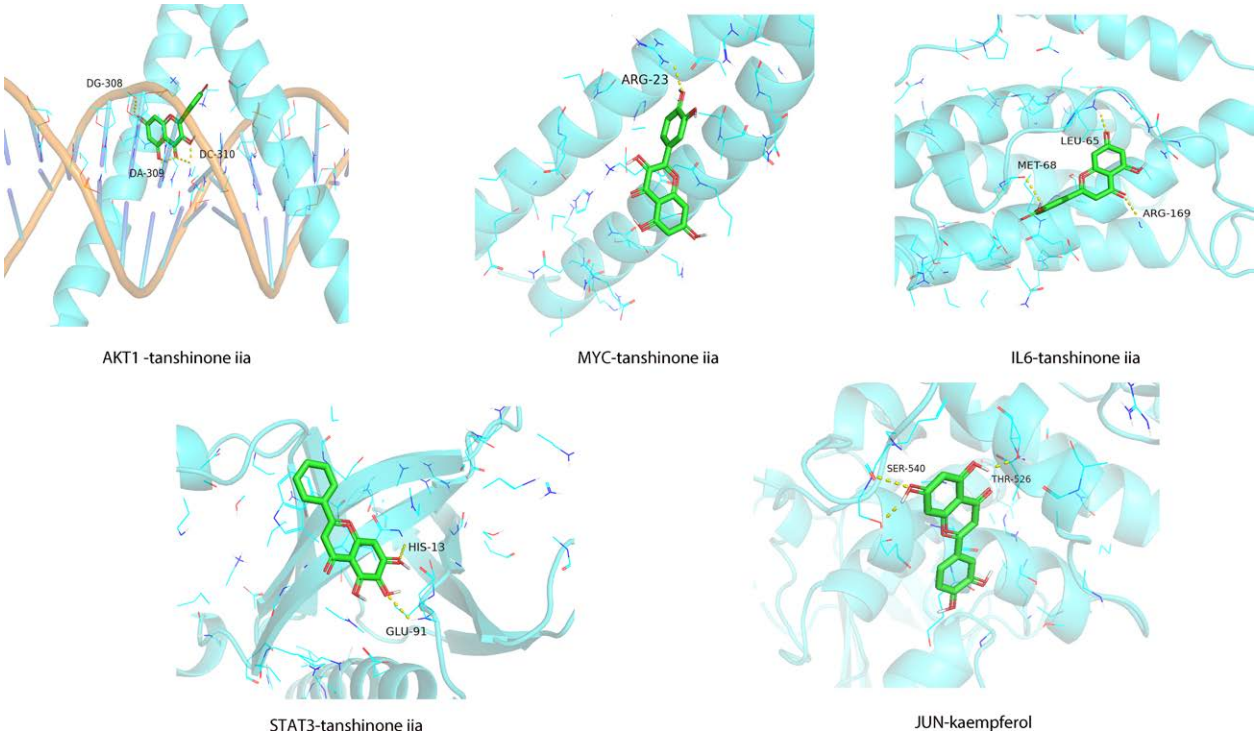


Figure 7. Diagram of molecular docking.

Acknowledgements

This research was sponsored by the Jiangxi Provincial TCM Science and Technology Plan Project (2021A248) and Jiangxi Provincial Natural Science Foundation (20202BABL206141).

Author contributions

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