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Analysis of the Potential Mechanism of Sanhua Decoction in Treating Ischemic Stroke Based on Network Pharmacology and Molecular Docking Technology

Wei Zhao[1] Dan Li[1] Min Yue[1] Cheng Yan[1] Feng Li[1] Yonghua Qi[1]

[1] School of Pharmacy, Xinxiang College, Xinxiang, Henan, China

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Address for correspondence Yonghua Qi, PhD, School of Pharmacy, Xinxiang College, No. 191, Jinsui Avenue East Section, Xinxiang University, Xinxiang, Henan, Henan 453003, China (e-mail: qyh@xxu.edu.cn).

Abstract

Objective The aim of this study was to explore the action mechanism of Sanhua decoction in treating ischemic stroke through network pharmacology and molecular docking technology.

Methods Active components and related targets of Sanhua decoction were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform. A "drug-active component-target" network was constructed, and core components were selected through topological analysis. Disease targets related to ischemic stroke were screened based on the Online Mendelian Inheritance in Man (OMIM), Therapeutic Target Database (TTD), GeneCards, DrugBank, and PharmGKB databases. The intersection of active component–related targets and ischemic stroke disease targets was identified to obtain potential targets of Sanhua decoction for treating ischemic stroke, represented using a Venn diagram. The STRING database was used to construct a protein–protein interaction (PPI) network of potential targets and filter for core targets. Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of core targets were performed using the DAVID database and Metascape platform. Molecular docking verification of core targets and core components was conducted using AutoDock.

Results A total of 52 active components and 142 related targets were screened from Sanhua decoction, with core active components including luteolin, nobiletin, β-sitosterol, eucalyptol, and aloe-emodin. There were 2,991 ischemic stroke–related targets, with 98 potential targets identified in the intersection with active component–related targets. An analysis of the PPI network analysis revealed 23 core targets, including serine/threonine-protein kinase 1 (AKT1), tumor protein p53 (TP53), and mitogen-activated protein kinase 3 (MAPK3). Enrichment analysis obtained 35 GO results and 41 signaling pathways. Molecular docking results indicated good binding between core components and core targets.

Conclusion Multiple components in the classic formula Sanhua decoction, such as luteolin and nobiletin, may play a role in treating ischemic stroke by regulating core targets like AKT1, TP53, and MAPK3, and participating in multiple signaling pathways.

Keywords
- Sanhua decoction
- ischemic stroke
- network pharmacology
- molecular docking technology

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Introduction

In recent years, the incidence of ischemic stroke has been rising annually, and due to its high mortality and disability rates, it severely impacts people's health and quality of life. A survey from 2019 indicated that stroke is the third leading cause of death and disability globally across all age groups, following neonatal diseases and ischemic heart disease.[1] At the same time, stroke is one of the major diseases leading to death in middle-aged and elderly individuals and significantly affects their physical health and quality of life. Acute ischemic stroke (AIS) is a severe medical emergency caused by the sudden cessation of blood supply to a part of the brain, potentially resulting in permanent damage.[2] Sanhua decoction is a classic traditional Chinese medicine (TCM) formula that has been used to treat ischemic stroke for thousands of years. This formula is derived from *Collection of Writings on the Mechanism of Disease, Suitability of Qi, and the Safeguarding of Life as Discussed in the 'Basic Questions' (Su Wen Bing Ji Qi Yi Bao Ming Ji)* and is now included in the national *Catalogue of Ancient Classic Famous Formulas (First Batch)* as the 55th entry.[3] It consists of four herbs: Dahuang (Rhei Radix et Rhizoma), Zhishi (Aurantii Fructus Immaturus), Houpo (Magnoliae Officinalis Cortex), and Qianghuo (Notopterygii Rhizoma et Radix). This formula is renowned for treating stroke, which functions to regulate qi, relieve stagnation, raise lucidness and lower turbidity, and open the pores. It can improve post-ischemic brain edema and blood–brain barrier permeability, demonstrating good clinical efficacy in treating AIS.[4,5] However, the mechanism by which Sanhua decoction exerts its effects on ischemic stroke remains unclear. This study employs network pharmacology and molecular docking technique to explore the active components and target sites of Sanhua decoction in treating ischemic stroke, aiming to preliminarily reveal and predict its potential action mechanism. The overall flowchart of the experimental plan is illustrated in ►**Fig. 1**.

Materials and Methods

Screening of Chemical Components and Relevant Targets of Sanhua Decoction

The chemical components of the herbs Dahuang (Rhei Radix et Rhizoma), Houpo (Magnoliae Officinalis Cortex), Qianghuo (Notopterygii Rhizoma et Radix), and Zhishi (Aurantii Fructus Immaturus) in Sanhua decoction were obtained through the traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP). Initial screening was conducted using thresholds of oral bioavailability (OB) $\geq$ 30% and drug likeness (DL) $\geq$ 0.18 to identify the active components.[6] The targets associated with the active components of Sanhua decoction were obtained from TCMSP and were unified for comparative analysis in the UniProt protein database (https://www.uniprot.org).

Construction of the "Drug-Active Component-Target" Network of Sanhua Decoction

The active components of Sanhua decoction and their associated targets were imported into Cytoscape 3.8.0 network analysis software to construct the "drug-active component-target" network diagram. In the diagram, nodes represent active components, and edges represent the interactions between active components and targets.[7,8] A topological analysis of the network was conducted to filter for core components.

Screening of Targets Related to Ischemic Stroke

Using "ischemic stroke" as a keyword, targets related to ischemic stroke were screened from the GeneCards, Online Mendelian Inheritance in Man (OMIM), Therapeutic Target Database (TTD), DrugBank, and PharmGKB databases.[9–12] After merging the targets obtained from the five databases and removing duplicates, disease-related targets were obtained.

Identification of Potential Targets of Sanhua Decoction for Treating Ischemic Stroke

The intersection of the targets related to the active components of Sanhua decoction and the ischemic stroke disease targets was taken to identify the potential targets of Sanhua decoction for treating ischemic stroke. A Venn diagram was created using an online diagram tool.

Construction of the Protein–Protein Interaction Network

The potential targets were imported into the STRING database, with the species limited to *Homo sapiens*. Isolated nodes were removed to obtain the protein–protein interaction (PPI) network diagram.[13] Topological analysis was performed using the CytoNCA plugin in Cytoscape 3.8.0 to filter out the core targets of Sanhua decoction for treating ischemic stroke.

Enrichment Analysis

Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were conducted on the potential core targets using the DAVID database and Metascape (http://metascape.org/gp/index.html) platform. For GO functional enrichment analysis, the species was set to "sapiens," and "Custom Analysis" was selected with a significance threshold of $p < 0.01$. For KEGG pathway enrichment analysis, a significance threshold of $p < 0.01$ was applied, and the results were visualized in bar charts and bubble charts.[14]

Molecular Docking Verification

The 2D structures of the top five core compounds ranked by degree were downloaded from the TCMSP database. The crystal structures of the top five targets with the highest degree values in the core target PPI network were downloaded from the PDB database (https://www.rcsb.org/). PyMoL software was used to remove unrelated ligands and nonprotein molecules (such as water molecules) from the target protein receptors.[15] The chemical structures of the ligands were downloaded from the PubChem database (http://zinc.docking.org/). AutoDock software was used for molecular docking of the above-mentioned target protein receptors and ligand molecules, setting the Grid Box centered on the ligand, and then the Autogrid module was used to obtain the docking active sites. The active components with the

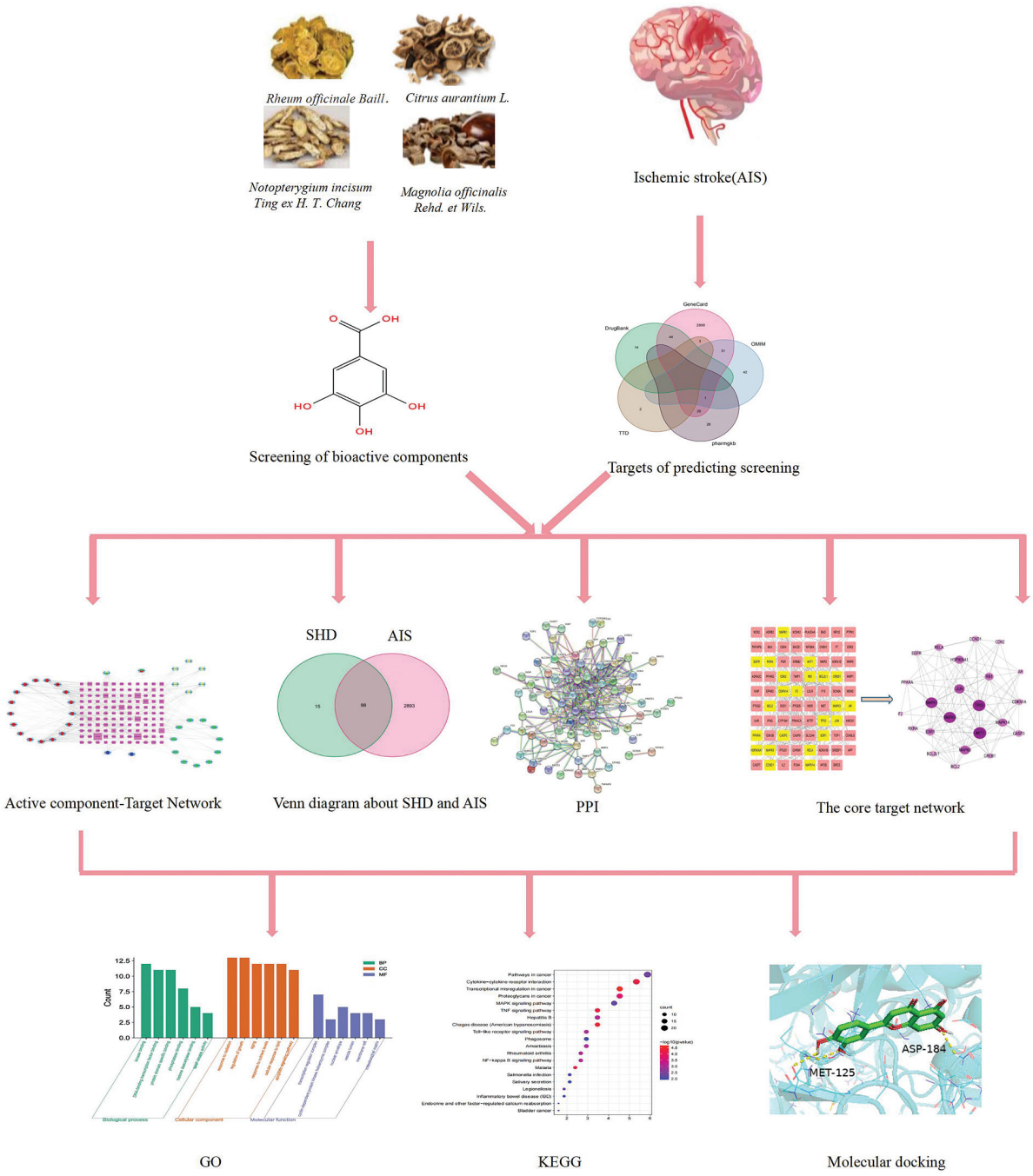

Fig. 1 Research plan flowchart.
Abbreviations: AIS, acute ischemic stroke; GO, Gene Ontology; PPI, protein–protein interaction; SHD, Sanhua Decoction.

highest binding energy were selected to the target protein, and visualize the docking results using PyMoL software.[16]

Results

Active Components and Related Targets of Sanhua Decoction

A total of 361 active compounds were obtained from the TCMSP for the formula of Sanhua decoction, including 92 from Dahuang (Rhei Radix et Rhizoma), 139 from Houpo (Magnoliae Officinalis Cortex), 65 from Qianghuo (Notopterygii Rhizoma et Radix), and 65 from Zhishi (Aurantii Fructus Immaturus). After preliminary screening with OB ≥30% and DL ≥0.18 as thresholds, 16 active components from Dahuang (Rhei Radix et Rhizoma), 2 from Houpo (Magnoliae Officinalis Cortex), 14 from Qianghuo (Notopterygii Rhizoma et Radix), and 22 from Qianghuo (Notopterygii Rhizoma et Radix) were identified. Among these, β-sitosterol is a common component of Dahuang (Rhei Radix et Rhizoma) and Qianghuo (Notopterygii Rhizoma et Radix), while imperatorin is a common component of Qianghuo (Notopterygii Rhizoma et Radix) and Zhishi (Aurantii Fructus Immaturus). After removing duplicates, a total of 52 active components were obtained, as shown in ►**Table 1**. A total of 511 active

Table 1 Information table of active components related to Sanhua decoction

No.	Molecule ID	Molecule name	OB/%	DL	Source
1	MOL002288	Emodin-1-O-β-D-glucopyranoside	44.81	0.80	Dahuang
2	MOL000358	β-sitosterol	36.91	0.75	Dahuang, Qianghuo
3	MOL002280	Torachrysone-8-O-β-D-(6'-oxayl)-glucoside	43.02	0.74	Dahuang
4	MOL002297	Daucosterol_qt	35.89	0.70	Dahuang
5	MOL000554	Gallic acid-3-O-(6'-O-galloyl)-glucoside	30.25	0.67	Dahuang
6	MOL002303	Palmidin A	32.45	0.65	Dahuang
7	MOL002259	Physciondiglucoside	41.65	0.63	Dahuang
8	MOL002251	Mutatochrome	48.64	0.61	Dahuang
9	MOL002293	Sennoside D_qt	61.06	0.61	Dahuang
10	MOL002276	Sennoside E_qt	50.69	0.61	Dahuang
11	MOL002235	EUPATIN	50.80	0.41	Dahuang
12	MOL002260	Procyanidin B-5,3′-O-gallate	31.99	0.32	Dahuang
13	MOL002268	Rhein	47.07	0.28	Dahuang
14	MOL000096	(-)-Catechin	49.68	0.24	Dahuang
15	MOL000471	Aloe-emodin	83.38	0.24	Dahuang
16	MOL002281	Toralactone	46.46	0.24	Dahuang
17	MOL005970	Eucalyptol	60.62	0.32	Houpo
18	MOL005980	Neohesperidin	57.44	0.27	Houpo
19	MOL011968	Coumarin,glycoside	33.07	0.78	Qianghuo
20	MOL000359	Sitosterol	36.91	0.75	Qianghuo
21	MOL004792	Nodakenin	57.12	0.69	Qianghuo
22	MOL011962	6'-Feruloylnodakenin	32.02	0.67	Qianghuo
23	MOL011963	8-geranoxy-5-methoxypsoralen	40.97	0.50	Qianghuo
24	MOL011975	Notoptol	62.97	0.48	Qianghuo
25	MOL001951	Bergaptin	41.73	0.42	Qianghuo
26	MOL011971	Diversoside_qt	67.57	0.31	Qianghuo
27	MOL001956	Cnidilin	32.69	0.28	Qianghuo
28	MOL002644	Phellopterin	40.19	0.28	Qianghuo
29	MOL002881	Diosmetin	31.14	0.27	Qianghuo
30	MOL001942	Isoimperatorin	45.46	0.23	Qianghuo
31	MOL001941	Ammidin	34.55	0.22	Qianghuo, Zhishi
32	MOL011969	Demethylfuropinnarin	41.31	0.21	Qianghuo
33	MOL013352	Obacunone	43.29	0.77	Zhishi
34	MOL013276	Poncirin	36.55	0.74	Zhishi
35	MOL013428	Isosakuranetin-7-rutinoside	41.24	0.72	Zhishi
36	MOL013440	Citrusin B	40.80	0.71	Zhishi
37	MOL005828	Nobiletin	61.67	0.52	Zhishi
38	MOL001803	Sinensetin	50.56	0.45	Zhishi
39	MOL013277	Isosinensetin	51.15	0.44	Zhishi

Table 1 (*Continued*)

No.	Molecule ID	Molecule name	OB/%	DL	Source
40	MOL009053	4-[(2S,3R)-5-[(E)-3-hydroxyprop-1-enyl]-7-methoxy-3-methylol-2,3-dihydrobenzofuran-2-yl]-2-methoxy-phenol	50.76	0.39	Zhishi
41	MOL007879	Tetramethoxyluteolin	43.68	0.37	Zhishi
42	MOL013435	Poncimarin	63.62	0.35	Zhishi
43	MOL013436	Isoponcimarin	63.28	0.31	Zhishi
44	MOL013437	6-Methoxy aurapten	31.24	0.30	Zhishi
45	MOL013279	5,7,4'-Trimethylapigenin	39.83	0.30	Zhishi
46	MOL013430	Prangenin	43.60	0.29	Zhishi
47	MOL013433	Prangenin hydrate	72.63	0.29	Zhishi
48	MOL005100	5,7-dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)chroman-4-one	47.74	0.27	Zhishi
49	MOL001798	Neohesperidin_qt	71.17	0.27	Zhishi
50	MOL000006	Luteolin	36.16	0.25	Zhishi
51	MOL002914	Eriodyctiol (flavanone)	41.35	0.24	Zhishi
52	MOL005849	Didymin	38.55	0.24	Zhishi

component–related targets were obtained from TCMSP, including 99 related to Dahuang (Rhei Radix et Rhizoma), 31 related to Houpo (Magnoliae Officinalis Cortex), 94 related to Qianghuo (Notopterygii Rhizoma et Radix), and 287 related to Zhishi (Aurantii Fructus Immaturus). After merging and removing duplicates, 142 active component–related targets for Sanhua Decoction were identified.

"Drug-Component-Target" Network of Sanhua Decoction

Using Cytoscape 3.8.0 software, the relationship network of active components and action targets of Sanhua decoction was drawn and analyzed, as shown in ►**Fig. 2**. This network consists of 159 nodes and 388 edges, where nodes represent active ingredients and related targets, and edges represent the interaction relationships between active components and related targets. The left side represents active ingredients, and the right side represents related targets. The *circular shapes on the blue rectangles* on the left represent different Chinese herbs, with *yellow* representing active components from Dahuang (Rhei Radix et Rhizoma), *blue* representing those from Houpo (Magnoliae Officinalis Cortex), *green* representing those from Qianghuo (Notopterygii Rhizoma et Radix), and *red* representing those from Zhishi (Aurantii Fructus Immaturus). The *green and red splicing* indicates common components of Qianghuo (Notopterygii

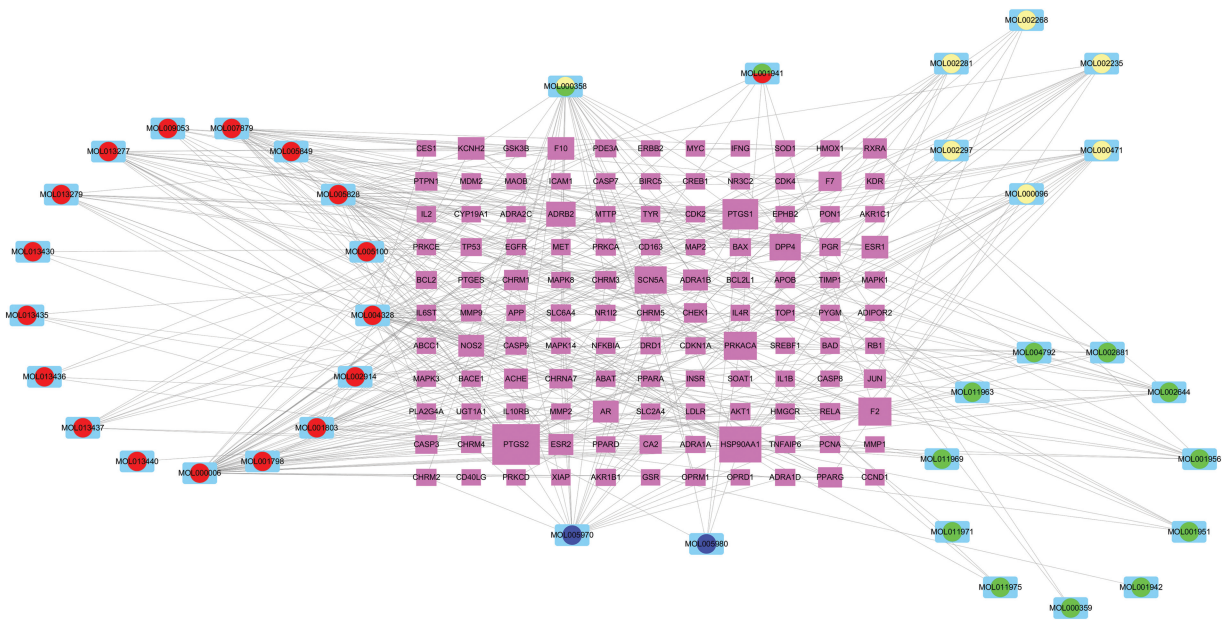

Fig. 2 "Drug-active component-target" network diagram of Sanhua decoction.

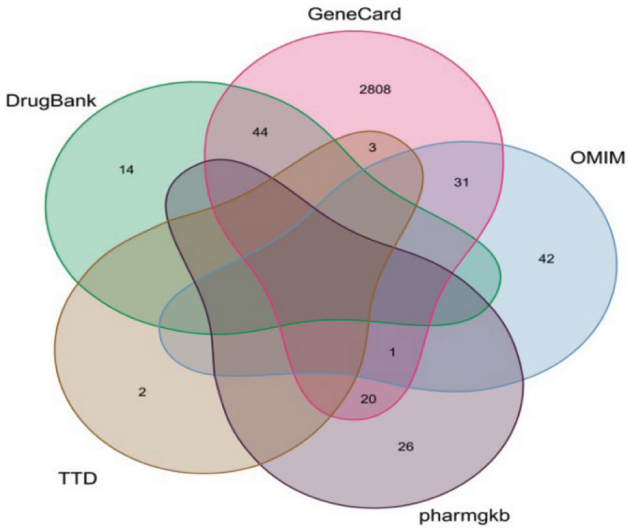

Fig. 3 Ischemic stroke disease targets.
Abbreviations: OMIM, Online Mendelian Inheritance in Man; TTD, Therapeutic Target Database.

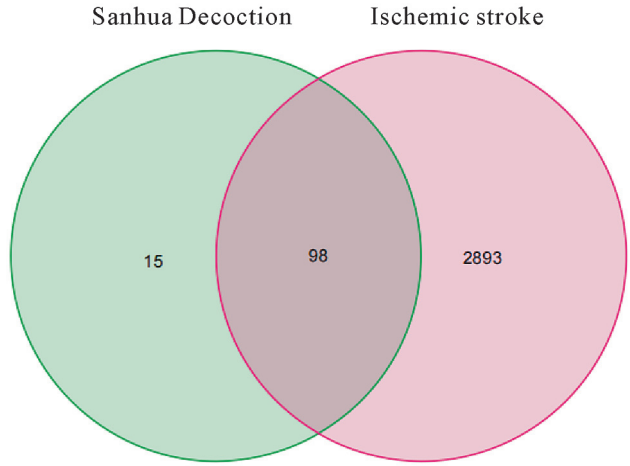

Fig. 4 Venn diagram of active component targets of Sanhua decoction and ischemic stroke–related targets.

Rhizoma et Radix) and Zhishi (Aurantii Fructus Immaturus), while the *yellow and green splicing* indicates common components of Dahuang (Rhei Radix et Rhizoma) and Qianghuo (Notopterygii Rhizoma et Radix). Topological analysis revealed that luteolin, hesperidin, and β-sitosterol are core components of Sanhua decoction.

Disease-Related Targets of Ischemic Stroke

Ischemic stroke–related targets were obtained from the GeneCards, OMIM, TTD, PharmGKB, and DrugBank databases. After merging and removing duplicates, a total of 2,991 ischemic stroke–related targets were identified, as shown in ►**Fig. 3**.

Acquisition of Potential Targets for Treating Ischemic Stroke with Sanhua Decoction

The intersection of targets related to the active components of Sanhua decoction and ischemic stroke disease targets yielded 98 potential targets for treating ischemic stroke with Sanhua decoction, which is illustrated in a Venn diagram, as shown in ►**Fig. 4**.

Protein–Protein Interaction Network

Potential targets were imported into STRING software, with the species set to *Homo sapiens*. After removing two free protein targets, the potential target PPI network was obtained, as shown in ►**Fig. 5**. This network consists of 79 nodes and 300 edges. Further screening using the CytoNCA plugin in Cytoscape software resulted in a core target PPI network containing 23 core targets and 117 edges, as shown in ►**Fig. 6**. Relevant information about the core targets is provided in ►**Table 2**.

Enrichment Analysis

Enrichment analysis of the 23 core targets resulted in 35 GO enrichment analysis results and 41 signaling pathways. The GO enrichment analysis includes 9 molecular function (MF) entries, 20 biological process (BP) entries, and 6 cellular component (CC) entries. MF primarily involves mitogen-activated protein kinase (MAPK) activity, tyrosine kinase binding proteins, and protein domain–specific binding; BP mainly concerns radiation response, growth regulation, cellular response to lipids, and apoptosis signaling pathways; CC includes transcription regulation complexes, cyclin-dependent protein kinase holoenzyme complexes, nuclear envelope, and vesicle lumen. Based on *p* values, the top six entries in each category were selected for bar chart visualization, as shown in ►**Fig. 7**, where lower *p* values indicate higher enrichment levels.

During the KEGG signaling pathway enrichment analysis, pathways were filtered with a threshold of $p < 0.01$, revealing that Sanhua decoction for treating ischemic stroke primarily involves cancer signaling pathway, MAPK signaling pathway, tumor necrosis factor (TNF) signaling pathway, toll-like receptor signaling pathway, NF-kappa B signaling pathway, and calcium reabsorption regulated by endocrine and other factors. The top 20 related pathways, sorted by *p* values, were visualized in a bubble chart, as shown in ►**Fig. 8**.

Molecular Docking Results

The top five compounds ranked by degree value in Sanhua decoction are luteolin, nobiletin, β-sitosterol, eucalyptol, and aloe-emodin, which were selected as ligands. The top five targets in the core PPI network, ranked by degree value, are serine/threonine-protein kinase 1 (AKT1), tumor protein p53 (TP53), MAPK3, MAPK1, and JUN, which were chosen as receptors. Molecular docking was conducted using AutoDock 4.2.6 software. The Grid Box settings were based on covering all active binding sites and essential residues, with specific parameters as follows: AKT1—box size (44 Å × 46 Å × 46 Å), centered at coordinates (21.72, 14.378, 9.831)Å; TP53—box size (74 Å × 56 Å × 60 Å), centered at coordinates (114.606, 87.11, −29.848)Å; MAPK3—box size (78 Å × 70 Å × 108 Å), centered at coordinates (44.329, 38.765, 73.25)Å; MAPK1—box size (60 Å × 64 Å × 64 Å), centered at coordinates (59.317, 26.146, 6.759)Å; and JUN—box size (50 Å × 68 Å

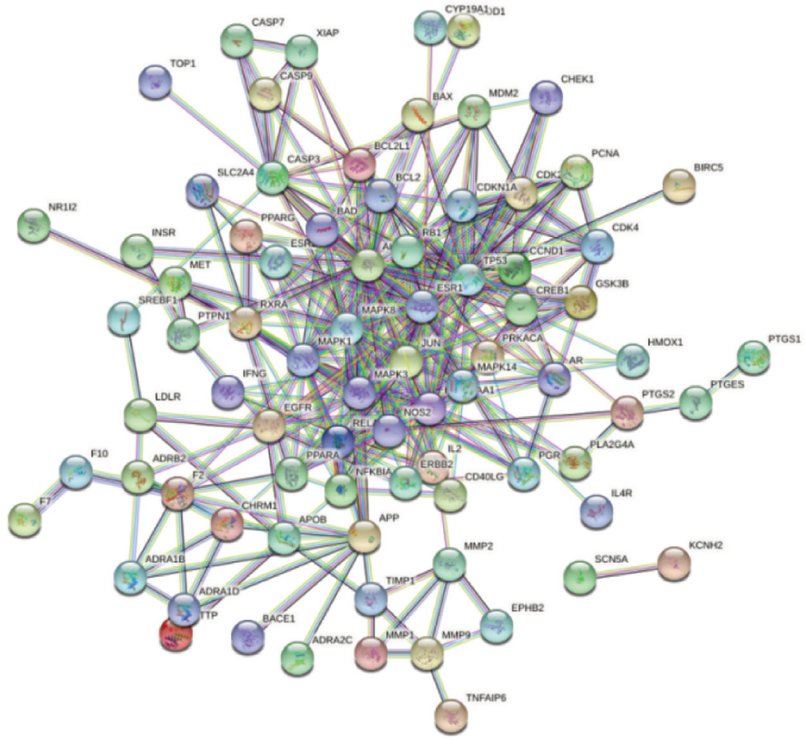

Fig. 5 Protein–protein interaction network.

× 52 Å), centered at coordinates (24.168, −16.901, −8.125)Å. It is generally accepted that the smaller the binding energy, the stronger the interaction between the active components and the protein. When the binding energy between the receptor and ligand is less than −4.25 kcal/mol, there is a certain level of binding activity; less than −5.0 kcal/mol indicates good binding activity; and less than −7.0 kcal/mol indicates strong binding activity. The analysis results showed that the binding energies of the five core compounds with the five core targets were all less than −5 kcal/mol, indicating a certain affinity between the core components and core targets. Specifically, the binding energy of AKT1 with aloe-emodin was the lowest at −7.1 kcal/mol; TP53 with luteolin had a binding energy of −8.0 kcal/mol; MAPK3 with aloe-emodin had a binding energy of −9.8 kcal/mol; MAPK1 with luteolin had a binding energy of −9.1 kcal/mol; and JUN with

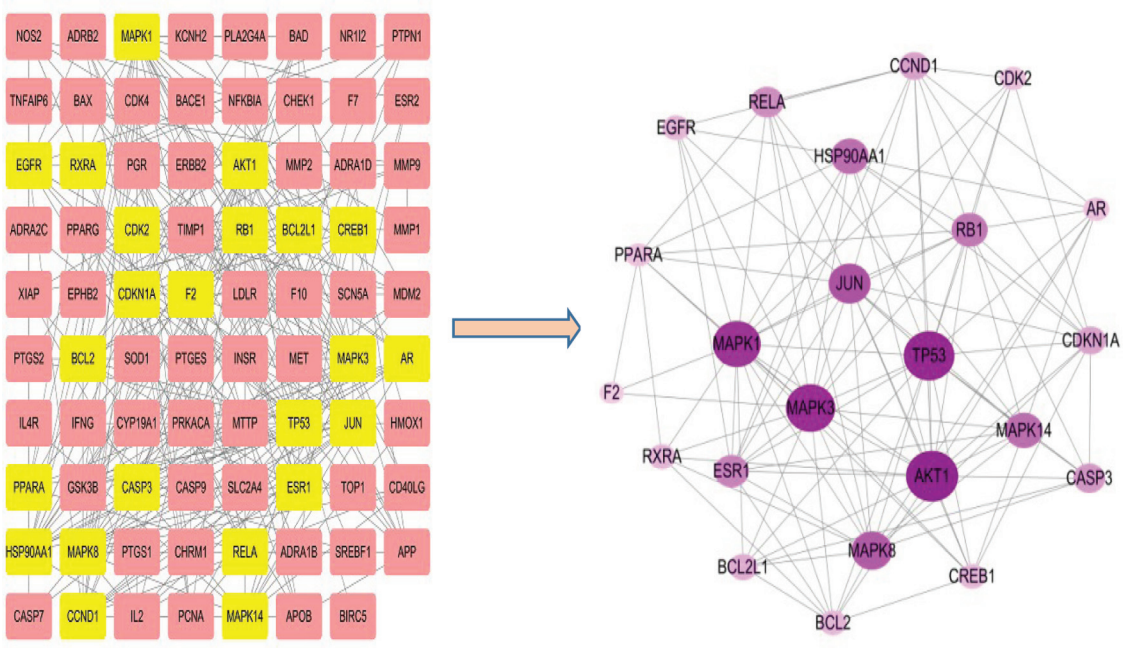

Fig. 6 Core target protein–protein interaction network.

Table 2 Core target relevant information for treating ischemic stroke with Sanhua decoction

No.	Targets	Betweenness	Closeness	Degree
1	AKT1	880.8163384	0.259136213	28
2	TP53	859.0440119	0.260869565	27
3	MAPK3	588.5295221	0.262626263	26
4	MAPK1	545.8901929	0.260869565	25
5	JUN	300.6738669	0.248407643	21
6	MAPK8	352.2462232	0.253246753	20
7	MAPK14	465.2555193	0.251612903	18
8	HSP90AA1	649.9711284	0.250803859	18
9	RB1	155.3833815	0.248407643	17
10	ESR1	240.0841519	0.247619048	16
11	RELA	166.4684357	0.241486068	15
12	CASP3	432.5606184	0.237804878	14
13	CCND1	60.40542785	0.237082067	13
14	CDKN1A	71.3202253	0.24	13
15	BCL2L1	91.60268614	0.235649547	12
16	EGFR	269.8408392	0.240740741	11
17	BCL2	25.58790286	0.234939759	11
18	CREB1	50.32150483	0.237804878	11
19	RXRA	195.4571818	0.230769231	11
20	AR	31.28356516	0.237804878	10
21	CDK2	46.19429769	0.226744186	10
22	PPARA	72.53105086	0.237082067	10
23	F2	392.1619381	0.225433526	9

both luteolin and aloe-emodin had a binding energy of −6.5 kcal/mol. The results are shown in ►**Table 3** and ►**Fig. 9**.

Discussion

According to the TCM theory, ischemic stroke falls under the category of "stroke (Zhong Feng)," with blood stasis being its fundamental pathological mechanism. This condition can often lead to sudden fainting and symptoms such as facial asymmetry, including the mouth, eyes, and lips. Currently, clinical treatment for ischemic stroke primarily focuses on inducing resuscitation and promoting blood circulation to resolve stasis, which has shown significant effectiveness.[17] Historically, Sanhua decoction has demonstrated notable efficacy in treating ischemic stroke in clinical practice. Systematic biological research indicates that multitarget interventions can enhance the effectiveness of drug treatments for complex systemic diseases. Network pharmacology is based on the "disease-target-drug" interaction network, systematically observing the interventions and effects of drugs on diseases, thereby revealing the mysteries of drug synergy within the body.[18] To further understand the potential mechanism of Sanhua decoction in treating ischemic stroke, we employed network pharmacology and molecular docking to explore the potential molecular action mechanism of its bioactive compounds.

In this experiment, we identified 52 active compounds from Sanhua decoction and 142 related targets. Through network topological analysis of "drug-active component-target," we found that the core compounds included luteolin, nobiletin, β-sitosterol, eucalyptol, and aloe-emodin. Previous studies have indicated that these compounds have therapeutic effects on cardiovascular diseases. Luteolin has been shown to effectively inhibit the proliferation, migration, and invasion of C918 cells, as well as the proliferation and migration of human umbilical vein endothelial cells (HUVECs). It can also inhibit the interaction between endothelial cells and C918 cells, possibly exerting its inhibitory effects through the phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB, also known as AKT) signaling pathway. Additionally, luteolin has been found to inhibit three modes of angiogenesis in uveal melanoma.[19] Nobiletin has been shown to reduce oxidative stress, exert anti-inflammatory effects, and increase blood flow, thereby improving the survival rate of random skin flaps.[20] These studies suggest the effectiveness and diversity of the active components in Sanhua decoction for treating ischemic stroke.

After mapping the active components of Sanhua decoction to related targets for ischemic stroke, 98 potential

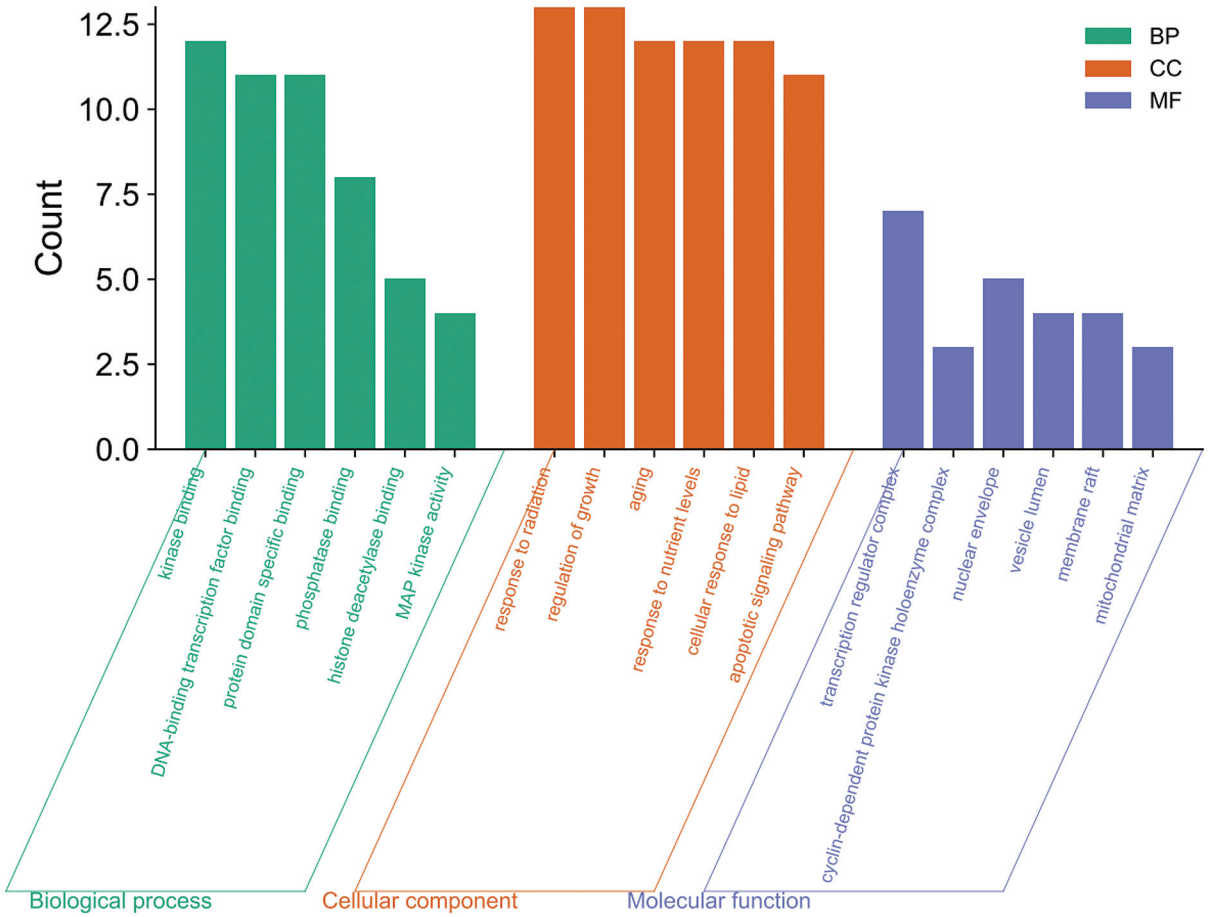

Fig. 7 Gene Ontology functional enrichment analysis of core targets.

targets for treating ischemic stroke with Sanhua decoction were identified. Analysis of the PPI network of these potential targets revealed that the core targets include AKT1, MAPK3, MAPK1, JUN, and TP53, all of which are involved in BPs such as transcription regulation, gene regulation, and apoptosis regulation. AKT is a serine/threonine protein kinase that exerts anti-apoptotic effects by phosphorylating downstream target proteins.[21–23] There are three isoforms of AKT: AKT1, AKT2, and AKT3, with AKT1 being widely expressed in body tissues.[24] AKT has neuroprotective effects;

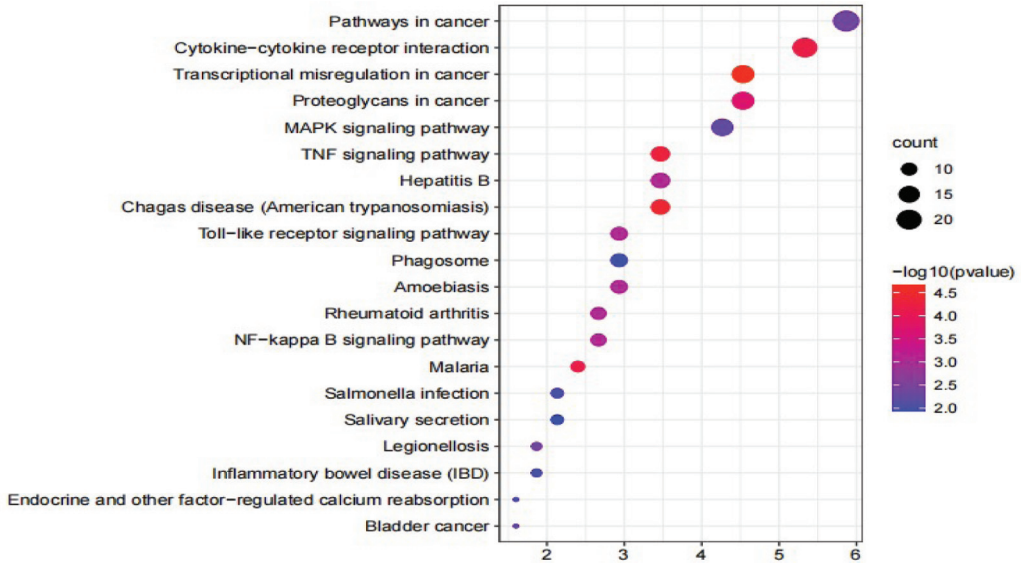

Fig. 8 KEGG signaling pathway enrichment analysis of core targets. Abbreviations: MAPK, mitogen-activated protein kinase; NF, nuclear factor; TNF, tumor necrosis factor.

Table 3 Molecular docking results of core components and core targets in Sanhua decoction

Compounds	Binding energy(kcal/mol)				
	AKT1	TP53	MAPK3	MAPK1	JUN
Luteolin	−6.3	−8.0	−9.4	−9.1	−6.5
Nobiletin	−5.9	−7.3	−8.5	−8.6	−5.9
β-sitosterol	−7.0	−6.7	−8.8	−7.0	−5.7
Eucalyptol	−5.3	−5.6	−5.5	−5.2	−4.8
Aloe-emodin	−7.1	−7.8	−9.8	−9.0	−6.5

studies have found that protein kinase D2 exerts neuroprotection by promoting the activation of AKT and CREB during ischemic stroke.[25] Early activation of AKT can reduce the infarct volume after cerebral ischemia-reperfusion injury and improve the oxygen supply/consumption balance in local brain tissue.[26] AKT also plays a significant role in angiogenesis; as an upstream signal of mammalian target of rapamycin (mTOR), it not only directly regulates the expression of vascular endothelial growth factor (VEGF) but also indirectly regulates the expression of various angiogenic factors.[27–32] MAPK is a key target in the treatment of ischemic stroke with Sanhua decoction playing a critical role in the regulation of apoptosis and inflammatory factor expression following ischemic stroke.[33] In the early stages of ischemia, p38 MAPK expression is elevated in neurons and glial cells[34]; activated p38 MAPK can promote the release of inflammatory cytokines, inducing the expression of adhesion molecules on vascular endothelial cells. These inflammatory cytokines, in turn, can promote the activation of p38 MAPK, creating a vicious cycle that exacerbates

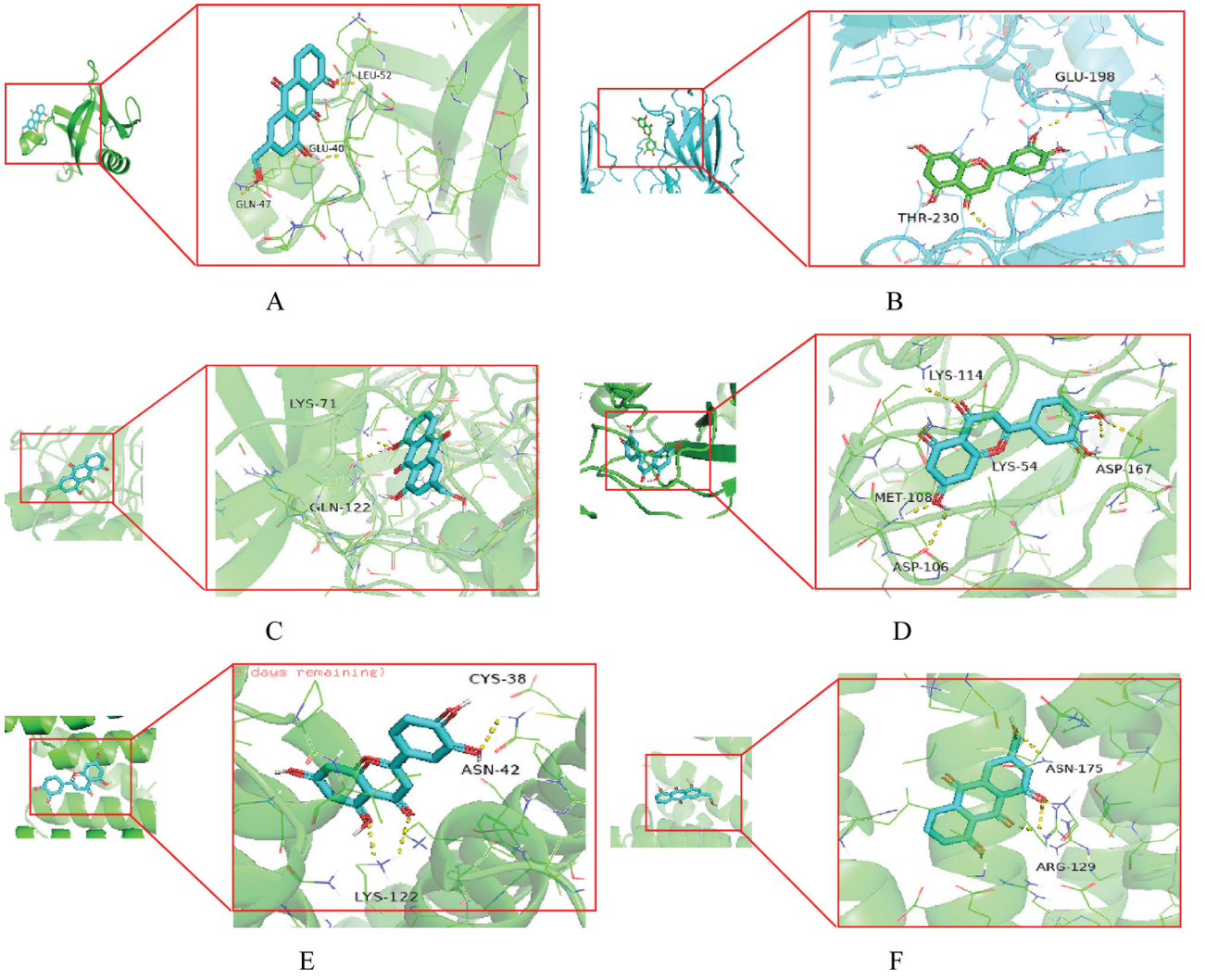

Fig. 9 Schematic diagram of molecular docking between core components and core targets.
Notes: (**A**) AKT1: Aloe-emodin. (**B**) TP53: Luteolin. (**C**) MAPK3: Aloe-emodin. (**D**) MAPK1: Luteolin. (**E**) JUN: Luteolin. (**F**) JUN: Aloe-emodin.

inflammation.[35] MAPK1 (ERK2), MAPK3 (ERK1), and p38 MAPK are highly homologous, and during ischemic stroke, they are involved in the regulation of proinflammatory cytokines such as interleukin-1β (IL-1β) and TNF-α by activating the MAPK cascade.[36] Therefore, inhibiting MAPK signal transduction has potential therapeutic prospects for improving inflammatory responses and blood–brain barrier disruption after ischemic stroke. TP53 is a tumor suppressor gene associated with the regulation of the cell cycle and apoptosis. During ischemic stroke, the brain is in a hypoxic environment, and cells are under stress, leading to disruption of DNA homeostasis.[37] Literature reports indicate that the TP53 Arg/Arg genotype controls the vulnerability of neuronal apoptosis and serves as a genetic marker for predicting adverse functional outcomes after stroke.[38] The findings of this study are consistent with previously published research.

Based on KEGG enrichment analysis, Sanhua decoction may treat ischemic stroke through multiple pathways. These signaling pathways include the cancer signaling pathway, MAPK signaling pathway, TNF signaling pathway, and toll-like receptor signaling pathway. The pathophysiology of ischemic stroke is very complex, with inflammation and immune responses being significant pathological changes in its progression, involving both the innate and adaptive immune systems. After a stroke, damaged nerve cells can induce glial cell activation, peripheral immune cell infiltration, and the release of inflammatory mediators, which further exacerbates blood–brain barrier damage and leads to the occurrence of cerebral edema, resulting in secondary brain injury.[39] TNF refers to a small protein secreted by macrophages, which plays an important role in the body, especially in immune and inflammatory responses. TNF is classified into two main types: TNF-α and TNF-β. Previous studies have reported that the expression level of TNF-α is significantly increased in patients with AIS, suggesting that TNF-α plays an important role in the pathogenesis of ischemic stroke.[40] TNF-α can also activate glial cells, mediate the expression of vascular endothelial cell adhesion molecules, and promote neutrophil infiltration.[41] Furthermore, research has shown that TNF-α can affect the permeability of the blood–brain barrier.

Conclusion

Based on network pharmacology and molecular docking, this study explores the mechanism of Sanhua decoction in treating ischemic stroke. It was found that active components in Sanhua decoction, such as luteolin, nobiletin, β-sitosterol, eucalyptol, and aloe-emodin, can participate in multiple BPs and pathways by regulating targets like AKT1, MAPK3, MAPK1, JUN, and TP53, thus exerting therapeutic effects on ischemic stroke. This research provides a theoretical reference for future studies on the mechanism of Sanhua decoction in treating ischemic stroke.

CRediT Authorship Contribution Statement

Wei Zhao: Methodology, formal analysis, investigation, writing -original draft. **Dan Li, Min Yue:** Project administration, resources, supervision, validation. **Feng Li, Cheng Yan:** Software, visualization, resources. **Yonghua Qi:** conceptualization, funding acquisition, resources.

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Conflict of Interest

The authors declare no conflict of interest.

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