Original Article

Exploring the mechanism of Shenqisherong pill against cervical spondylotic myelopathy by network pharmacology and molecular docking

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Background: Shenqisherong pill (SQSRP) has been used clinically to treat cervical spondylotic myelopathy (CSM) with satisfactory results; however, its active ingredients and mechanisms are unclear. The present study aimed to explore the active ingredients and molecular mechanisms of SQSRP against CSM using network pharmacology and molecular docking.

Methods: The compounds in SQSRP were obtained from public databases and related literature, and oral bioavailability (≥30%) and drug-likeness (≥0.18) were screened using absorption, distribution, metabolism, and excretion (ADME) criteria. Compounds-related and CSM-related target genes were identified using public databases, and the overlapping genes between compounds and CSM target genes were identified using a Venn diagram. Cytoscape and STRING were used to construct, visualize, and analyze the interaction network between these overlapping targets. Gene Ontology (GO) and KEGG pathway enrichment analysis of overlapping targets used Omicshare tools and constructed a compound-overlapping targets network, target-pathway network, and compound-target-pathway network using Cytoscape. Finally, molecular docking software was used to verify the targets.

Results: A total of 447 compounds in SQSRP were identified, and ADME screening identified 96 compounds as potentially active ingredients. A total of 249 compound-related genes and 280 CSM-related genes were identified using public databases, and 53 overlapping genes were identified. The results of compound targets and protein-protein interaction network analysis showed that the pharmacological effects of SQSRP against CSM involved 56 compounds and 53 genes. The results of GO and KEGG pathway enrichment analysis suggested that the therapeutic effects of SQSRP against CSM were exerted by reducing inflammation, inhibiting apoptosis, and protecting neurons. The molecular mechanisms may be strongly associated with PI3K-Akt, MAPK, IL-17, and TNF, which might be pivotal signaling pathways.

Conclusions: The active ingredients and mechanisms of SQSRP against CSM were investigated using network pharmacology. The findings proved that the pill could treat CSM through multi-component, multi-target, and multi-pathway synergy and provide a theoretical basis for the subsequent extraction of active ingredients from SQSRP.

Keywords: Shenqisherong pill (SQSRP); cervical spondylotic myelopathy; network pharmacology; active ingredient; mechanism

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Introduction

Cervical spondylotic myelopathy (CSM) is a common degenerative disease that causes spinal cord injury (1). It is triggered by the degeneration changes of the cervical intervertebral junction structures, such as disc herniation, posterior vertebral spur, ossification of the posterior longitudinal ligament, yellow ligament hypertrophy, or calcification that causes the cervical spinal cord to suffer from chronic progressive compression (1,2). CSM is the most common cause of spinal cord dysfunction in adults (3). It often causes pain and decreases the quality of life of patients. In severe cases, it could lead to disability (4). Also, it brings about a great economic burden on the family and society. Previous studies have been reported that the incidence and prevalence of CSM are at least 4.1 and 60.5 per 100,000, respectively, and will gradually increase as the global population ages, making it a growing public health concern (4,5).

Presently, the treatment of CSM is mainly divided into surgical and conservative approaches. A large number of studies have shown that the decompression of cervical spine surgery can prevent further development of symptoms, improve patients' dysfunction, relieve pain, and improve the quality of life of patients (6-8). However, some studies have shown that there is no evidence that surgical treatment is better for mild to moderate myelopathy than conservative treatment. Thus, it is recommended that patients with mild symptoms be first treated conservatively (9,10). Furthermore, due to the risk of surgical treatment and complications post-surgery, a single surgical method cannot meet the clinical needs. Hence, a conservative treatment that can effectively control the condition and promote the recovery of patients is an urgent requisite.

Shenqisherong pill (SQSRP) was formulated based on the clinical experience of Professor Qi Shi, a well-known traditional Chinese medicine (TCM) practitioner in China and Shanghai Longhua Hospital. SQSRP is a representative TCM formula used clinically in Longhua Hospital to treat CSM for more than 20 years. It has provided a favorable therapeutic effect for patients with mild to moderate CSM. SQSRP is composed of *Radix astragali* (RA), *Radix salviae Miltiorrhizae* (RSM), *Saline cistanche* (SC), and musk. In addition, accumulating evidence has indicated that numerous active ingredients from the herbs of SQSRP had significant anti-inflammatory and neuroprotective effects (11-15). Our previous studies have shown that SQSRP reduces local inflammation, improves spinal cord ischemia or blood stasis, relieves continued spinal cord injury, and thus improves patients' clinical symptoms and promotes recovery (16,17). It also exerts an adequate clinical effect in the treatment of patients with CSM in the early and middle stages and has been approved by the National Medical Products Administration for clinical trials (clinical trial acceptance number: CXZL1900016). However, the molecular mechanisms underlying SQSRP against CSM are yet unclear and need further investigation.

SQSRP, like other TCM formulas, has a complex composition with multi-component, multi-target, and multi-pathway synergy characteristics. Thus, these features and the mechanism of action are difficult to analyze using conventional experimental methods (18,19). TCM emphasizes dialectical treatment. Many diseases are often caused by multiple factors, and the symptoms can change dynamically with disease progression (20,21). Therefore, it is difficult to achieve curative effects by relying on a single drug component and a single target (22). Over the years, research on the mechanism of the efficacy of TCM has always been the focus and difficulty of TCM research. Network pharmacology is an emerging discipline based on systems biology theory and network analysis of biological systems (23). It is employed to explore the intervention and influence of drugs on disease networks (22,24). Intriguingly, network pharmacology emphasizes multi-pathway regulation of the signaling pathway, which is in accordance with the characteristics of TCM-based syndrome differentiation, multiple components, and multiple targets (25). Network pharmacology provides a novel method for the study of the efficacy of TCM and has been increasingly used in the research of the mechanism of TCM in treating various diseases; for example, the mechanism of *Zhichan* powder in the treatment of Parkinson's disease and the multi-component synergy mechanism of *Banxia xiexin* decoction on irritable bowel syndrome (26,27).

In this study, we used a network pharmacological and molecular docking approach to explore the molecular

Table 1 Databases and software used in this study

Description of methods	Databases and software
Ingredients database building	TCMSP (http://www.tcmspw.com/tcmsp.php), TCMID (http://www.megabionet.org/tcmid/), Shanghai Institute of Organic Chemistry of CAS. Chemistry Database (http://www.organchem.csdb.cn)
Screening for bioactive compounds	TCMSP (http://www.tcmspw.com/tcmsp.php)
Screening of potential targets of bioactive compounds and diseases in database	TCMSP (http://www.tcmspw.com/tcmsp.php), UniProt (https://www.uniprot.org/), OMIM (https://omim.org/), GeneCards (https://www.genecards.org/), Bioinformatics and Evolutionary Genomics (http://bioinformatics.psb.ugent.be/webtools/Venn/)
Construction of PPI network	STRING ver 11.0 (https://string-db.org/), Cytoscape (ver 3.7.2)
GO and KEGG pathway enrichment analysis	Omnicshare (https://www.omicshare.com/tools/)
Network construction	Cytoscape (ver 3.7.2)
Molecular docking	PubChem (https://pubchem.ncbi.nlm.nih.gov/), Open Babel (ver 2.3.2), Protein Data Bank (http://www.rcsb.org/pdb), PyMOL (ver 2.3.4), AutoDockTools (ver 1.5.6), AutoDock Vina (ver 1.1.2)

TCMSP, Traditional Chinese Medicine Systems Pharmacology; TCMID, Traditional Chinese Medicines Integrated Database; OMIM, Online Mendelian Inheritance in Man; PPI, protein-protein interaction; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

mechanism of SQSRP in the treatment of CSM. Firstly, we screened and collected the related targets in CSM and the active ingredients of SQSRP through network pharmacology databases. Then, the interactions among the overlapping targets were deduced via STRING. Next, the overlapping targets were uploaded to the Omicshare analysis platform for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. Subsequently, the pharmacological data were integrated into the target-pathway (T-P) network and compound target-pathway (C-T-P) network to identify the key compounds and targets of SQSRP in the treatment of CSM. Finally, the molecular docking method was used to verify the binding effect of the compounds to the targets. The database or software used in this study is shown in *Table 1*, and the workflow is illustrated in *Figure 1*.

We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/apm-21-408).

Methods

Ingredients database building

The chemical ingredients of the four herbal medicines in SQSRP were collected from Traditional Chinese Medicines Systems Pharmacology (TCMSP) database (http://www.tcmspw.com/tcmsp.php) (28), Traditional Chinese Medicine Integrated Database (TCMID) (http://www.megabionet.org/tcmid) (29), Shanghai Institute of Organic Chemistry of CAS. Chemistry Database (http://www.organchem.csdb.cn), and related literature (30-32).

Screening for bioactive compounds

The components of TCM formulas are complex and diverse and may not be absorbed by the human body to achieve the desired treatment of the disease. Therefore, in order to screen out compounds with high activity in SQSRP, the collected components were imported into the TCMSP database to find the absorption, distribution, metabolism, and excretion (ADME) information. Oral bioavailability (OB) indicates the percentage of a drug being absorbed into the circulatory system and generates pharmacological effects after ingesting into the human body (33). The OB was calculated using the OBioavail 1.1 system and was set at a benchmark of ≥30% as OB less than 30% is regarded as low orally bioavailable drugs (34-36). The drug-likeness (DL) indicates the impression of a molecule's pharmacodynamics in the body and determines molecules with similar characteristics and targets. The DL was calculated using the Tanimoto coefficient and was set at a benchmark of ≥0.18 (34,35). The compounds without ADME information were

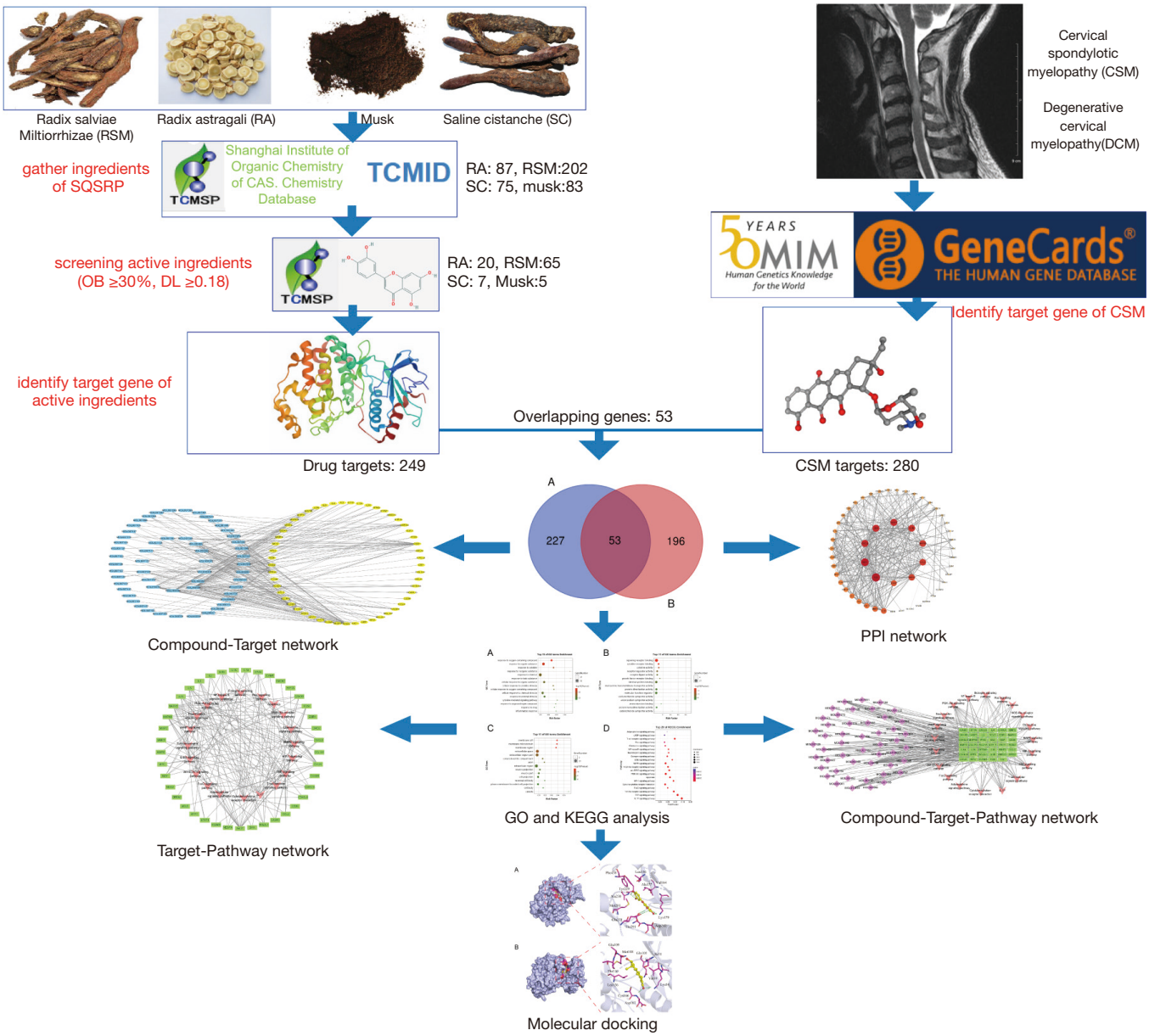

Figure 1 The workflow for network pharmacology analysis of SQSRP in treating CSM. CSM, cervical spondylotic myelopathy; SQSRP, Shenqisherong pill; DCM, degenerative cervical myelopathy; RSM, Radix Salviae Miltiorrhiza; RA, Radix Astragali; SC, Saline Cistanche; OB, oral bioavailability; DL, drug-likeness; TCMSP, Traditional Chinese Medicine Systems Pharmacology; TCMID, Traditional Chinese Medicines Integrated Database; OMIM, Online Mendelian Inheritance in Man; PPI, protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

excluded, and the final list was reserved only for those components that met both OB ≥30% and DL ≥0.18. In addition, some compounds that did not meet the screening criteria but had crucial pharmacological effects were also retained for further analysis.

Screening of potential targets of bioactive compounds and diseases in databases

The protein target information of each bioactive compound was obtained from the TCMSP database. Compounds without target information were excluded. Subsequently,

these protein targets were introduced into the UniProt database (https://www.uniprot.org/) to retrieve the corresponding gene symbol (37). The protein targets that were not identified in the database were removed.

CSM-related target genes were collected from the Online Mendelian Inheritance in Man (OMIM) (https://omim.org/) and GeneCards (https://www.genecards.org/) databases (38,39). The search keywords were as follows: cervical spondylotic myelopathy or degenerative cervical myelopathy. To date, all SQSRP-associated and CSM-associated target genes were collected, and the duplicate genes were deleted. Then, an online Venn mapping software (http://bioinformatics.psb.ugent.be/webtools/Venn/) was used to identify and visualize the overlapping genes between the compound and the CSM target genes. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Construction of protein-protein interaction (PPI) network for overlapping genes

In order to explore the interaction between the protein targets of anti-CSM and identify the key proteins, the overlapping genes were imported into STRING 11.0 database (https://string-db.org/) (40), and species was set to "*Homo sapiens*" after "Multiple Proteins" was selected; the other parameters were default. The obtained protein interaction results were saved in TSV format. The node 1, node 2, and combined score information in the files were retained and imported into Cytoscape software to draw the interaction network (41). The Network Analyzer tool in Cytoscape software was used to analyze the network. The node size and color were set to reflect the size of the degree value, and the thickness of the edge was set to reflect the size of the combination score to obtain the final PPI network. To further identify the key proteins in the network, node 1 and node 2 in the export file were analyzed to obtain the number of adjacent nodes for each protein. Then, the bar graph of the top 30 proteins was visualized using the bar plot function in the R language.

GO and KEGG pathway enrichment analysis of overlapping genes

To illustrate the role of anti-CSM target proteins in gene function and understand the potential mechanism of SQSRP against CSM, we performed GO function and KEGG pathway enrichment analysis of overlapping genes using Omicshare tools, a free online analysis platform (https://www.omicshare.com/tools/) (42).

Network construction

In order to explore the pharmacological mechanisms of SQSRP in the treatment of CSM, we constructed the regulatory network of SQSRP. Firstly, the interactions between compounds and overlapping targets were collected from the TCMSP database and the research results of the gene symbol to construct the interaction network of overlapping genes and compounds using Cytoscape 3.7.2 software. Then, combined with the data of 20 interested pathways from KEGG analysis, a T-P network and a C-T-P network were constructed using Cytoscape 3.7.2 software. Finally, the Network Analyzer tool was used to assess the topology of the network and identify the key target genes or compounds in the network.

Molecular docking

The compounds in Spatial Data File (SDF) file format were searched via the PubChem website (https://pubchem.ncbi.nlm.nih.gov/) and converted into Protein Data Bank (PDB) file using the Open Babel 2.3.2 software (43,44). The crystal structures of the receptor proteins were retrieved from the PDB database (http://www.rcsb.org/pdb). Then, water removal and ligand removal were performed on the receptor protein using the PyMOL 2.3.4 software, and the receptor protein was modified by hydrogenation and charge balance using AutoDockTools software (45,46). Subsequently, the receptor protein and small ligand molecules were converted into PDBQT format. The molecular docking of the receptor protein and small ligand molecules was carried out using the AutoDock Vina 1.1.2 software (Spacing (angstrom) =1, size_x =50, size_y =50, size_z =50 in each target) (47). The docking process was evaluated by the genetic algorithm. All docking run options were default values. Finally, the docking results with the lowest score were visualized using the PyMOL 2.3.4 software.

Results

Compounds of SQSRP

SQSRP consists of four herbal medicines, namely RA, RSM, SC, and musk. After removing the duplicate ingredients, a total of 447 compounds were identified in

SQSRP, including 87 in RA, 202 in RSM, 75 in SC, and 83 in musk (Table S1).

Identification of SQSRP bioactive compounds

A total of 96 bioactive components (OB ≥30%, DL ≥0.18) were identified in four herbal medicines in SQSRP by ADME screening, including 20 in RA, 65 in RSM, 7 in SC, and 5 in musk. Additionally, two ingredients that did not meet the screening criteria were retained on the active ingredients list because of their specific therapeutic effects. For example, our previous studies have shown that echinacoside reduces the inflammatory response in a rat model of CSM via inhibition of excessive mitochondrial fission (48). Chen *et al.* showed that glycine has a neuroprotective role in neuron damage caused by oxygen-glucose deprivation by regulating the level of microRNA-301a (49). Some studies exhibited that testosterone had a neuroprotective effect and the ability to induce myelin regeneration in multiple sclerosis (50). The detailed information of the 96 active ingredients is shown in *Table 2*.

Target gene identification of bioactive compounds and disease

After screening by the TCMSP database and the UniProt database, a total of 249 target genes corresponding to 84 compounds were collected (Table S2). As listed in Table S3, a total of 280 CSM-related target genes were identified from the OMIM and GeneCards databases. The Venn diagram (*Figure 2*) showed 53 overlapping genes between 280 CSM-related and 249 compounds-related genes. These overlapping molecules represent potential target genes for SQSRP in the treatment of CSM.

Analysis of PPI network for overlapping genes

In order to explore the correlation among the 53 anti-CSM-related targets, a PPI network was constructed. The nodes in the PPI network represent proteins, and edges represent interactions between the proteins. The size of the node and the shade of the color represent the degree value. The higher the degree value of a node, the more critical its role in the network. The detailed information on 53 target genes in the PPI network is shown in Table S4.

As shown in *Figure 3*, the network contains a large number of interlaced lines, indicating a strong correlation among these targets. This phenomenon indicates that SQSRP has a synergistic effect on multiple closely related targets in the treatment of CSM. The visualization results of the degree values of the targets in the network are shown in *Figure 4*. The top ten targets are interleukin 6 (IL-6), AKT serine/threonine kinase 1 (Akt1), C-X-C motif chemokine ligand 8 (CXCL8), transcription factor AP-1 (JUN), vascular endothelial growth factor A (VEGFA), interleukin-1 beta (IL-1B), C-C motif chemokine 2 (CCL2), mitogen-activated protein kinase 8 (MAPK8), mitogen-activated protein kinase 1 (MAPK1), and intercellular adhesion molecule 1 (ICAM1); these occupy a critical position in the entire network and are speculated as the key targets for SQSRP efficacy in the treatment of CSM.

GO and KEGG pathway enrichment analysis of overlapping genes

The results of GO enrichment analysis include three categories: biological process (BP), molecular function (MF), and cellular component (CC). The data of BP, MF, and CC enrichment analysis of the 53 anti-CSM target genes were collected from Omicshare, and the top 15 significantly enriched terms in the three categories ($P<0.05$) were visualized using the Omicshare tools (*Figure 5A-5C*). RichFactor refers to the ratio of the number of genes located in the term entry among the differentially expressed genes to the total number of genes located in the term entry among all genes. The larger the RichFactor, the higher the degree of enrichment. The size of the bubble indicates the number of genes. The larger the bubble, the greater the number of genes enriched to the term. The shade of the bubble color indicates the level of significance; the redder the color, the greater the concentration of the term. Our results indicated that SQSRP might be responsive to oxygen-containing compound, cytokine stimulus, toxic substances, and inflammatory reaction via signaling receptor binding, cytokine receptor binding, cytokine activity, and growth factor receptor binding in the membrane raft, membrane region, extracellular region, axon, and neuron part to exert an anti-CSM potential.

KEGG pathway enrichment analysis of overlapping genes was performed using the Omicshare online analysis platform at $P<0.05$ for screening; consequently, a total of 132 signaling pathways were obtained. Literature screening retrieved 20 KEGG pathways visualized using the Omicshare tools (*Figure 5D*), and the relevant information of those pathways are listed in *Table 3*. The current results indicated that the molecular mechanisms of SQSRP against

Table 2 Information about the active ingredients of SQSRP after ADME screening

Mol ID	Compound	OB (%)	DL	MW	AlogP	Hdon	Hacc	Caco-2	BBB	FASA-	HL	Herb
MOL001601	1,2,5,6-tetrahydrotanshinone	38.75	0.36	280.34	2.98	0	3	0.96	0.39	0.33	18.05	RSM
MOL001659	Poriferasterol	43.83	0.76	412.77	7.64	1	1	1.44	1.03	0.22	5.34	RSM
MOL001771	Poriferast-5-en-3beta-ol	36.91	0.75	414.79	8.08	1	1	1.45	1.14	0	5.07	RSM
MOL001942	Isoimperatorin	45.46	0.23	270.3	3.65	0	4	0.97	0.66	0.27	–1.44	RSM
MOL002222	Sugiol	36.11	0.28	300.48	4.99	1	2	1.14	0.7	0.27	14.62	RSM
MOL002651	Dehydrotanshinone II A	43.76	0.4	292.35	4.22	0	3	1.02	0.52	0.33	23.71	RSM
MOL002776	Baicalin	40.12	0.75	446.39	0.64	6	11	–0.85	–1.74	0.36	17.36	RSM
MOL000569	Digallate	61.85	0.26	322.24	1.53	6	9	–0.76	–1.52	0.43	5.29	RSM
MOL000006	Luteolin	36.16	0.25	286.25	2.07	4	6	0.19	–0.84	0.39	15.94	RSM
MOL006824	α-amyrin	39.51	0.76	426.8	7.35	1	1	1.37	1.2	0.23	3.06	RSM
MOL007036	5,6-dihydroxy-7-isopropyl-1, 1-dimethyl-2, 3-dihydrophenanthren-4-one	33.77	0.29	298.41	4.38	2	3	1.19	0.8	0.29	14.91	RSM
MOL007041	2-isopropyl-8-methylphenanthrene-3,4-dione	40.86	0.23	264.34	4.16	0	2	1.23	0.81	0.43	14.89	RSM
MOL007045	3α-hydroxytanshinoneIIa	44.93	0.44	310.37	3.56	1	4	0.53	0.22	0.3	23.78	RSM
MOL007048	(E)-3-[2-(3,4-dihydroxyphenyl)-7-hydroxy-benzofuran-4-yl] acrylic acid	48.24	0.31	312.29	3.21	4	6	0.18	–0.89	0.4	8.87	RSM
MOL007049	4-methylenemiltirone	34.35	0.23	266.36	4.33	0	2	1.25	0.87	0.38	14.6	RSM
MOL007050	2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde	62.78	0.4	356.4	3.58	2	6	0.35	–0.73	0.24	7.89	RSM
MOL007051	6-o-syringyl-8-o-acetyl shanzhiside methyl ester	46.69	0.71	628.64	–1.13	5	16	–1.73	–2.08	0.22	9.94	RSM
MOL007058	Formyltanshinone	73.44	0.42	290.28	3.36	0	4	0.54	–0.28	0.41	24.12	RSM
MOL007059	3-beta-Hydroxymethyllenetanshiquinone	32.16	0.41	294.32	3.16	1	4	0.38	–0.48	0.36	22.51	RSM
MOL007061	Methylenetanshinquinone	37.07	0.36	278.32	4.26	0	3	1.03	0.46	0.36	24.33	RSM
MOL007063	Przewalskin a	37.11	0.65	398.49	2.25	1	6	–0.26	–0.69	0.38	1.63	RSM
MOL007064	Przewalskin b	110.32	0.44	330.46	3.18	1	4	0.34	0.22	0.32	2.17	RSM
MOL007068	Przewaquinone B	62.24	0.41	292.3	2.99	1	4	0.39	–0.45	0.38	24.94	RSM
MOL007069	Przewaquinone c	55.74	0.4	296.34	3.31	1	4	0.42	–0.3	0.32	23.7	RSM
MOL007070	(6S,7R)-6,7-dihydroxy-1, 6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10, 11-dione	41.31	0.45	312.34	2.34	2	5	–0.06	–0.68	0.32	22.54	RSM
MOL007071	Przewaquinone f	40.31	0.46	312.34	2.07	2	5	–0.09	–0.9	0.29	22.45	RSM
MOL007077	Sclareol	43.67	0.21	308.56	4.27	2	2	0.84	0.51	0.27	4.71	RSM

Table 2 (*continued*)

Table 2 (*continued*)

Mol ID	Compound	OB (%)	DL	MW	AlogP	Hdon	Hacc	Caco-2	BBB	FASA-	HL	Herb
MOL007079	Tanshinaldehyde	52.47	0.45	308.35	3.83	0	4	0.57	–0.07	0.32	23.49	RSM
MOL007081	Danshenol B	57.95	0.56	354.48	2.59	1	4	0.53	0.11	0.3	4.28	RSM
MOL007082	Danshenol A	56.97	0.52	336.41	2.01	1	4	0.33	–0.01	0.34	5.15	RSM
MOL007085	Salvilenone	30.38	0.38	292.4	4.26	0	2	1.46	1.07	0.35	20.81	RSM
MOL007088	cryptotanshinone	52.34	0.4	296.39	3.44	0	3	0.95	0.51	0.29	17.3	RSM
MOL007093	dan-shexinkum d	38.88	0.55	336.41	2.83	1	4	0.67	–0.15	0.35	30	RSM
MOL007094	Danshenspiroketallactone	50.43	0.31	282.36	3.24	0	3	0.88	0.51	0.34	15.19	RSM
MOL007098	Deoxyneocryptotanshinone	49.4	0.29	298.41	4.32	1	3	0.85	0.24	0.3	27.17	RSM
MOL007100	Dihydrotanshinlactone	38.68	0.32	266.31	2.77	0	3	1.26	0.81	0.38	5.42	RSM
MOL007101	Dihydrotanshinonel	45.04	0.36	278.32	2.86	0	3	0.95	0.43	0.4	18.32	RSM
MOL007105	Epidanshenspiroketallactone	68.27	0.31	284.38	2.37	0	3	0.9	0.61	0.33	1.77	RSM
MOL007107	C09092	36.07	0.25	286.5	5.98	1	1	1.63	1.54	0.25	–0.16	RSM
MOL007108	Isocryptotanshi-none	54.98	0.39	296.39	3.59	0	3	0.93	0.34	0.3	31.92	RSM
MOL007111	Isotanshinone II	49.92	0.4	294.37	4.66	0	3	1.03	0.45	0.3	24.73	RSM
MOL007115	Manool	45.04	0.2	304.57	5.5	1	1	1.28	1.16	0.28	5.81	RSM
MOL007118	Microstegiol	39.61	0.28	298.46	4.75	1	2	1.05	0.99	0.33	4.52	RSM
MOL007119	Miltionone I	49.68	0.32	312.39	3.33	1	4	0.35	–0.11	0.35	41.49	RSM
MOL007120	Miltionone II	71.03	0.44	312.39	2.14	1	4	0.62	0.03	0.28	2.91	RSM
MOL007121	Miltipolone	36.56	0.37	300.43	2.74	1	3	0.5	0.17	0.3	1.7	RSM
MOL007122	Miltirone	38.76	0.25	282.41	4.73	0	2	1.23	0.87	0.32	14.82	RSM
MOL007123	Miltirone II	44.95	0.24	272.32	0.77	1	4	0.04	–0.25	0.35	2.24	RSM
MOL007124	Neocryptotanshinone ii	39.46	0.23	270.35	3.61	1	3	0.76	0.16	0.32	26.98	RSM
MOL007125	Neocryptotanshinone	52.49	0.32	314.41	3.01	2	4	0.35	–0.13	0.28	14.46	RSM
MOL007127	1-methyl-8,9-dihydro-7H-naphtho[5,6-g]benzofuran-6,10,11-trione	34.72	0.37	280.29	3.21	0	4	0.5	–0.27	0.33	37.89	RSM
MOL007130	Prolithospermic acid	64.37	0.31	314.31	2.77	4	6	0.1	–0.75	0.42	8.82	RSM
MOL007132	(2R)-3-(3,4-dihydroxyphenyl)-2-[(Z)-3-(3,4-dihydroxyphenyl) acryloyl]oxy-propionic acid	109.38	0.35	360.34	2.69	5	8	–0.33	–1.02	0.41	2.01	RSM
MOL007140	(Z)-3-[2-[(E)-2-(3,4-dihydroxyphenyl)vinyl]-3,4-dihydroxy-phenyl]acrylic acid	88.54	0.26	314.31	2.82	5	6	–0.09	–0.77	0.43	4.31	RSM
MOL007141	Salvianolic acid g	45.56	0.61	340.3	2.2	4	7	–0.14	–0.97	0.45	2.4	RSM
MOL007142	Salvianolic acid j	43.38	0.72	538.49	3.78	6	12	–0.82	–2.14	0.44	5.77	RSM
MOL007143	Salvilenone I	32.43	0.23	270.4	2.88	1	2	1.13	0.77	0.3	1	RSM
MOL007145	Salviolone	31.72	0.24	268.38	4.05	1	2	1.04	0.72	0.36	0.33	RSM

Table 2 (*continued*)

Table 2 (*continued*)

Mol ID	Compound	OB (%)	DL	MW	AlogP	Hdon	Hacc	Caco-2	BBB	FASA-	HL	Herb
MOL007149	NSC 122421	34.49	0.28	300.48	4.99	1	2	1.08	0.63	0.29	14.56	RSM
MOL007150	(6S)-6-hydroxy-1-methyl-6-methylol-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-quinone	75.39	0.46	312.34	2.42	2	5	0.03	−0.74	0.29	23.45	RSM
MOL007151	Tanshindiol B	42.67	0.45	312.34	2.34	2	5	0.05	−0.63	0.33	22.25	RSM
MOL007152	Przewaquinone E	42.85	0.45	312.34	2.34	2	5	−0.04	−0.65	0.32	22.44	RSM
MOL007154	Tanshinone iia	49.89	0.4	294.37	4.66	0	3	1.05	0.7	0.31	23.56	RSM
MOL007155	(6S)-6-(hydroxymethyl)-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-dione	65.26	0.45	310.37	3.57	1	4	0.44	−0.31	0.29	23.48	RSM
MOL007156	Tanshinone VI	45.64	0.3	296.34	2.44	2	4	0.48	−0.28	0.38	15.21	RSM
MOL000211	Mairin	55.38	0.78	456.78	6.52	2	3	0.73	0.22	0.26	8.87	RA
MOL000239	Jaranol	50.83	0.29	314.31	2.09	2	6	0.61	−0.22	0.29	15.5	RA
MOL000296	Hederagenin	36.91	0.75	414.79	8.08	1	1	1.32	0.96	0	5.35	RA
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	0.78	428.82	8.54	1	1	1.45	1.09	0	5.22	RA
MOL000354	Isorhamnetin	49.6	0.31	316.28	1.76	4	7	0.31	−0.54	0.32	14.34	RA
MOL000371	3,9-di-O-methylnissolin	53.74	0.48	314.36	2.89	0	5	1.18	0.63	0	9	RA
MOL000374	5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside	41.72	0.69	642.67	−0.95	9	16	−2.47	−3.62	0	2.52	RA
MOL000378	7-O-methylisomucronulatol	74.69	0.3	316.38	3.38	1	5	1.08	0.84	0	2.98	RA
MOL000379	9,10-dimethoxypterocarpan-3-O-β-D-glucoside	36.74	0.92	462.49	0.74	4	10	−0.63	−1.5	0	13.06	RA
MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	64.26	0.42	300.33	2.64	1	5	0.93	0.55	0	8.49	RA
MOL000387	Bifendate	31.1	0.67	418.38	2.56	0	10	0.15	−0.06	0	17.96	RA
MOL000392	Formononetin	69.67	0.21	268.28	2.58	1	4	0.78	0.02	0	17.04	RA
MOL000398	Isoflavanone	109.99	0.3	316.33	2.42	2	6	0.53	0.17	0	15.51	RA
MOL000417	Calycosin	47.75	0.24	284.28	2.32	2	5	0.52	−0.43	0	17.1	RA
MOL000422	Kaempferol	41.88	0.24	286.25	1.77	4	6	0.26	−0.55	0	14.74	RA
MOL000433	FA	68.96	0.71	441.45	0.01	7	13	−1.5	−2.59	0	24.81	RA
MOL000438	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	67.67	0.26	302.35	3.13	2	5	0.96	0.34	0	2.9	RA

Table 2 (*continued*)

Table 2 (*continued*)

Mol ID	Compound	OB (%)	DL	MW	AlogP	Hdon	Hacc	Caco-2	BBB	FASA-	HL	Herb
MOL000439	Isomucronulatol-7,2'-di-O-glucosiole	49.28	0.62	626.67	−0.68	8	15	−2.22	−3.36	0	0.93	RA
MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.05	0.48	314.31	3.11	2	6	0.89	−0.04	0	7.95	RA
MOL000098	Quercetin	46.43	0.28	302.25	1.5	5	7	0.05	−0.77	0.38	14.4	RA & SC
MOL000358	Beta-sitosterol	36.91	0.75	414.79	8.08	1	1	1.32	0.99	0.23	5.36	SC
MOL005320	Arachidonate	45.57	0.2	304.52	6.41	1	2	1.27	0.58	0.26	7.56	SC
MOL005384	Suchilactone	57.52	0.56	368.41	3.73	0	6	0.82	0.28	0.28	9.03	SC
MOL007563	Yangambin	57.53	0.81	446.54	2.6	0	8	0.67	0.01	0.14	3.61	SC
MOL008871	Marckine	37.05	0.69	475.69	5.3	3	5	0.86	−0.1	0.17	13.56	SC
MOL008875	Echinacoside	3.14	0.38	786.81	−1.36	12	20	−3.11	−4.2	0.32		SC
MOL000953	Cholesterol	37.87	0.68	386.73	7.38	1	1	1.43	1.13	0.2	4.52	Musk
MOL000737	Morin	46.23	0.27	302.25	1.5	5	7	0	−0.77	0.41	15.51	Musk
MOL010919	17-beta-estradiol	12.41	0.32	272.42	3.81	2	2	0.95	0.46	0.27		Musk
MOL001232	TES	12.93	0.35	288.47	3.33	1	2	0.68	0.29	0.26		Musk
MOL000050	GLY	48.74	0	75.08	−0.98	3	3	−0.56	−1.03	0	11.95	Musk

SQSRP, Shenqisherong Pill; ADME, absorption, distribution, metabolism, and excretion; OB, oral bioavailability; DL, drug-likeness; MW, molecular weight; AlogP, Atomic log P; Hdon, hydrogen-bond donors; Hacc, hydrogen-bond acceptors; Caco-2, caco-2 permeability; BBB, blood-brain barrier; FASA-, fractional negative surface area; HL, half-life; RSM, Radix Salviae Miltiorrhiza; RA, Radix Astragali; SC, Saline Cistanche.

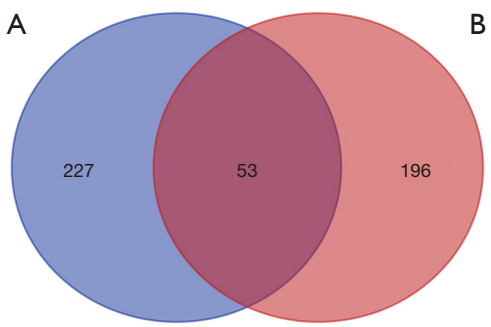

Figure 2 Overlapping genes between 280 CSM-related genes (A) and 249 compounds-related genes (B). CSM, cervical spondylotic myelopathy.

CSM were closely related to these 20 signaling pathways.

Results of network construction-based analysis

According to the TCMSP database and the research results of the gene symbol, 56 compounds that interacted with the 53 overlapping genes were identified. The interaction network of 53 overlapping genes and 56 compounds, containing 109 nodes and 476 edges, was constructed using Cytoscape 3.7.2 (*Figure 6*). To screen out the crucial active components and key targets of SQSRP against CSM, T-P and C-T-P networks were also constructed using Cytoscape 3.7.2 software. The nodes in the networks represent interacting protein target genes, compounds, or pathways. The edges represent the interactions between the nodes. The degree value of the nodes represents the number of interacting genes, compounds, or pathways. The higher the degree value of the node, the more critical the corresponding genes, compounds, or pathways are for the treatment of CSM.

The degree value of the compounds or genes was specifically set, and the topology of the network was analyzed to screen out the crucial active components and key targets of SQSRP against CSM. The degree values of

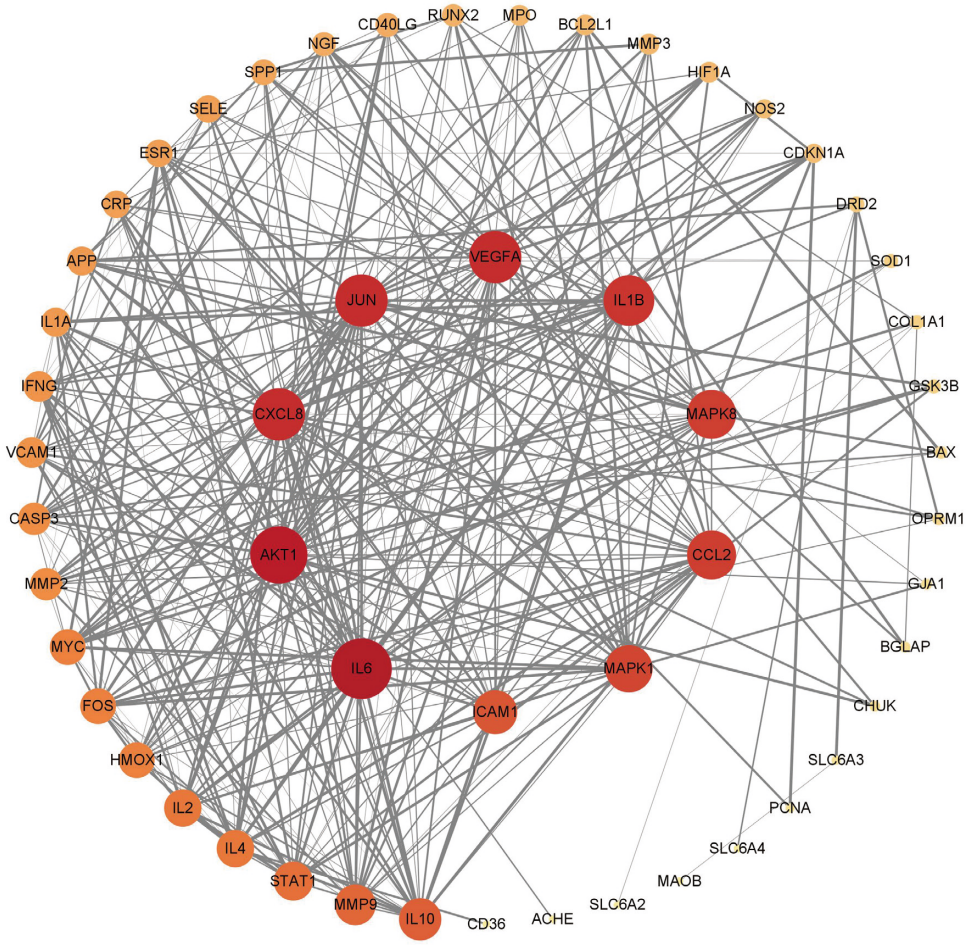

Figure 3 PPI network for overlapping genes (target genes of SQSRP treating CSM). PPI, protein-protein interaction; SQSRP, Shenqisherong pill; CSM, cervical spondylotic myelopathy.

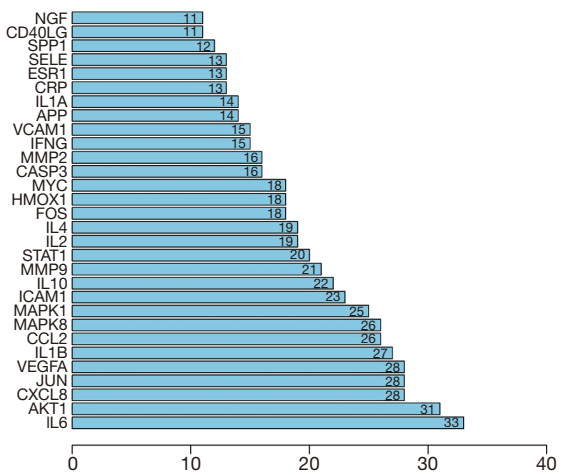

Figure 4 Barplot chart of 30 targets of maximum degree value in the PPI network. PPI, protein-protein interaction.

the nodes in the network are shown in *Table 4*. As shown in *Figure 6* and *Figure 7*, four active ingredients (quercetin, luteolin, 17-beta-estradiol, and kaempferol) have high degree values. In the T-P network, we found that Akt1 and MAPK1 have high degree values, 16 and 15, respectively (*Figure 8*). In addition, IL-17, TNF, PI3K-Akt, and MAPK signaling pathways have high degree values, 15, 15, 14, and 12, respectively (*Figures 7*,*8*). Thus, it can be speculated that these compounds, targets, and pathways have a pharmacological effect in the treatment of CSM by SQSRP. Further analysis showed that 41/56 compounds are connected to at least two targets, and 36/53 targets are connected to at least two compounds (*Figure 3*), i.e., the same active ingredient may act on different targets, and different active ingredients may act on the same target. These data indicated that SQSRP exerts its medicinal effect

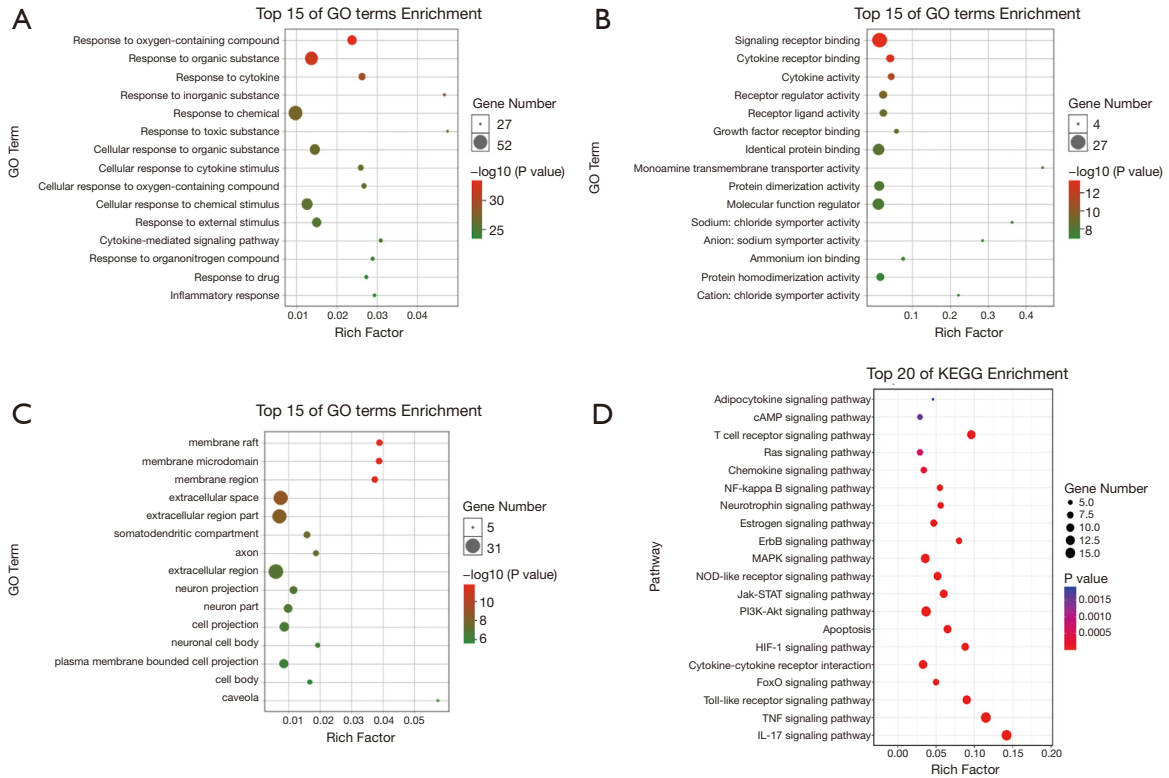

Figure 5 GO and KEGG pathway enrichment analyses of anti-CSM targets of SQSRP (P<0.05). Biological process (A), molecular function (B), cellular component (C), and KEGG (D). GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; CSM, cervical spondylotic myelopathy; SQSRP, Shenqisherong pill.

in CSM treatment through a multi-component, multi-target, and multi-pathway synergistic effect.

Molecular docking

We used molecular docking studies to investigate the putative interaction between active ingredients in SQSRP and key anti-CSM targets. The binding energy and docking parameters are shown in *Table 5*. The lower the energy value, the better the combination effects. The two docking results had the lowest scores (*Figure 9*). *Figure 9A* shows the binding mode between receptor protein Akt1 and Luteolin ligand small molecule. Ala230, Lys179, Thr291, and Glu278 form hydrogen bonds with small ligand molecules. The amino acid residues Tyr229, Phe438, Leu156, ALA177, Val164, Asp292, and Met281 form hydrophobic interactions with the small ligand molecules. *Figure 9B* shows the binding mode between the receptor protein MAPK1 and the small ligand molecule 17-beta-estradiol. The amino acid residues Asp167 and Lys54 interact with the small ligand molecule via hydrogen bonds. The amino acid residues, Val39, Ile31, Gln105, Met108, Glu109, Thr110, Leu156, and Cys166, interact with small ligand hydrophobic molecules. These docking results showed that the active compounds had a high affinity with key targets, especially luteolin and 17-beta-estradiol.

Discussion

Network pharmacology can comprehensively and systematically analyze the role of drug molecules in diseases and has been increasingly used in the research of mechanisms underlying TCM formulas (51). In this study, the active ingredients of SQSRP were analyzed using the network pharmacological method—the C-T network was constructed, and the interaction between the compounds and the targets was analyzed. The C-T network showed that the therapeutic effect of SQSRP on CSM was closely related to 56 active ingredients and 53 target genes. The analysis of the degree value of compounds in the network found that the key active

Table 3 Signaling pathways/target genes related to CSM regulated by SQSRP

Pathway ID	Pathway	Number	Target genes
ko04657	IL-17 signaling pathway	15	*GSK3B, MAPK1, MMP9, MAPK8, CCL2, IFNG, IL4, IL1B, IL6, MMP3, CASP3, CXCL8, FOS, JUN, CHUK*
ko04668	TNF signaling pathway	15	*SELE, ICAM1, MAPK1, MMP9, MAPK8, CCL2, IL1B, IL6, AKT1, MMP3, VCAM1, CASP3, FOS, JUN, CHUK*
ko04151	PI3K-Akt signaling pathway	14	*GSK3B, MAPK1, COL1A1, IL2, VEGFA, IL4, SPP1, CDKN1A, NGF, IL6, MYC, AKT1, BCL2L1, CHUK*
ko04010	MAPK signaling pathway	12	*MAPK1, MAPK8, VEGFA, IL1A, IL1B, NGF, MYC, AKT1, CASP3, FOS, JUN, CHUK*
ko04620	Toll-like receptor signaling pathway	11	*MAPK1, MAPK8, STAT1, SPP1, IL1B, IL6, AKT1, CXCL8, FOS, JUN, CHUK*
ko04060	Cytokine-cytokine receptor interaction	11	*IL1B, IL2, IFNG, IL1A, IL6, CCL2, CD40LG, IL10, NGF, CXCL8, IL4*
ko04660	T cell receptor signaling pathway	11	*MAPK1, IL2, IFNG, AKT1, GSK3B, JUN, FOS, CD40LG, IL10, CHUN, IL4*
ko04210	Apoptosis	10	*BAX, MAPK1, MAPK8, NGF, AKT1, CASP3, FOS, BCL2L1, JUN, CHUK*
ko04630	Jak-STAT signaling pathway	10	*IL2, IFNG, IL4, STAT1, CDKN1A, IL6, IL10, MYC, AKT1, BCL2L1*
ko04621	NOD-like receptor signaling pathway	10	*MAPK1, MAPK8, CCL2, STAT1, IL1B, IL6, CXCL8, BCL2L1, JUN, CHUK*
ko04066	HIF-1 signaling pathway	9	*NOS2, MAPK1, HMOX1, HIF1A, IFNG, VEGFA, CDKN1A, IL6, AKT1*
ko04915	Estrogen signaling pathway	8	*MMP2, ESR1, MAPK1, MMP9, OPRM1, AKT1, FOS, JUN*
ko04068	FoxO signaling pathway	7	*MAPK8, MAPK1, IL10, AKT1, CHUN, IL6, CDKN1A*
ko04012	ErbB signaling pathway	7	*GSK3B, MAPK1, MAPK8, CDKN1A, MYC, AKT1, JUN*
ko04722	Neurotrophin signaling pathway	7	*GSK3B, BAX, MAPK1, MAPK8, NGF, AKT1, JUN*
ko04064	NF-kappa B signaling pathway	7	*ICAM1, CD40LG, IL1B, VCAM1, CXCL8, BCL2L1, CHUK*
ko04062	Chemokine signaling pathway	7	*GSK3B, MAPK1, CCL2, STAT1, AKT1, CXCL8, CHUK*
ko04014	Ras signaling pathway	7	*MAPK1, MAPK8, VEGFA, NGF, AKT1, BCL2L1, CHUK*
ko04024	cAMP signaling pathway	6	*MAPK1, MAPK8, AKT1, DRD2, FOS, JUN*
ko04920	Adipocytokine signaling pathway	4	*MAPK8, CD36, AKT1, CHUK*

CSM, cervical spondylotic myelopathy; SQSRP, Shenqisherong Pill.

ingredients were quercetin, luteolin, 17-beta-estradiol, and kaempferol. Quercetin is a natural antioxidant that has been used as an anti-inflammatory drug (52,53). Several animal experiments have shown that quercetin reduces neuroinflammation and apoptosis after spinal cord injury and protects the neurons (21,52,54,55). Luteolin is a natural flavonoid compound with various pharmacological activities, such as anti-inflammatory, anti-allergic, and anti-tumor (56). Reportedly, luteolin protects the nerves by reducing oxidative stress and inhibiting inflammatory response and apoptosis after spinal cord injury (57). In addition, it exerts an analgesic effect on both acute and inflammatory pain as well as neuropathic pain (58). 17-beta-estradiol is the active ingredient in musk. Studies have shown that 17-beta-estradiol reduces secondary damage after spinal cord injury in rats (59,60). This neuroprotective effect could be attributed to increased Kir4.1 and glutamate transporter one expression in astrocytes by 17-beta-estradiol, thereby improving the

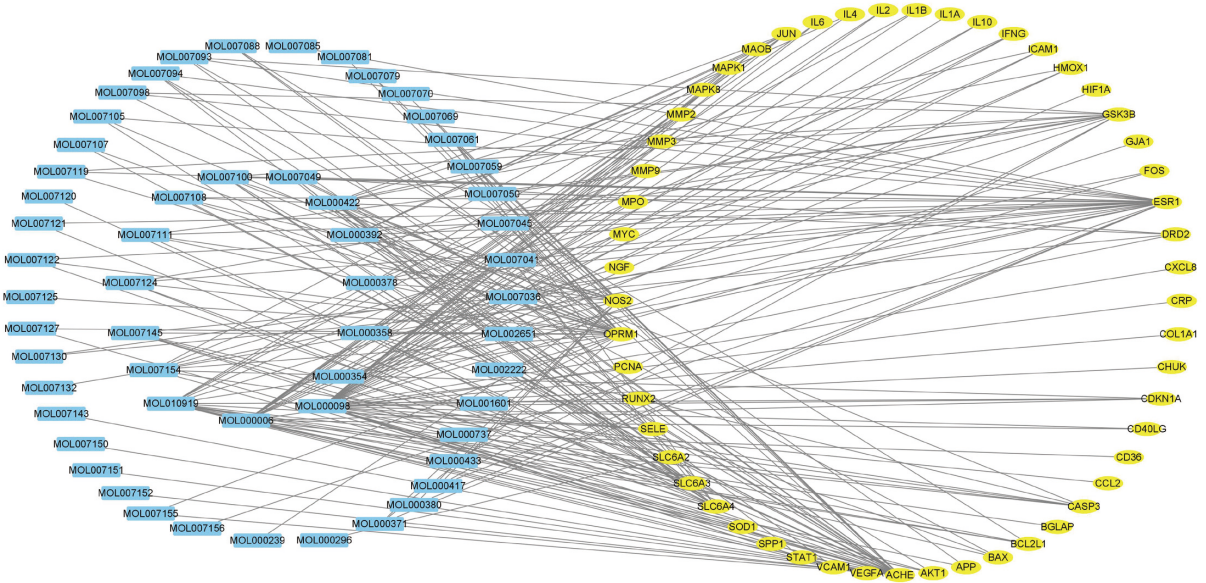

Figure 6 The C-T network of 56 compounds related to CSM. Compounds are denoted by blue rectangles and targets by yellow ovals. C-T, compound-target; CSM, cervical spondylotic myelopathy.

Table 4 Degree value of 56 compounds and 53 target genes in C-T network

Compound	Value	Gene	Value
MOL000098	38	*ACHE*	32
MOL000006	19	*OPRM1*	31
MOL010919	17	*ESR1*	25
MOL000422	13	*NOS2*	14
MOL000392	9	*SLC6A4*	13
MOL007154	8	*GSK3B*	12
MOL007100	7	*SLC6A3*	9
MOL007145	6	*CASP3*	6
MOL000358	5	*JUN*	6
MOL000378	5	*CDKN1A*	4
MOL000354	5	*BCL2L1*	4
MOL007124	5	*AKT1*	4
MOL007111	5	*DRD2*	4
MOL007108	5	*VCAM1*	3
MOL007049	5	*SELE*	3
MOL000371	4	*BAX*	3
MOL007094	4	*MAOB*	3

Table 4 (*continued*)

Table 4 (*continued*)

Compound	Value	Gene	Value
MOL007093	4	*SLC6A2*	3
MOL007041	4	*IFNG*	3
MOL000417	3	*ICAM1*	3
MOL000380	3	*HMOX1*	3
MOL007122	3	*MAPK1*	3
MOL007119	3	*MMP9*	3
MOL007105	3	*MMP2*	3
MOL007098	3	*IL1B*	2
MOL007088	3	*SOD1*	2
MOL007061	3	*STAT1*	2
MOL007050	3	*MYC*	2
MOL002651	3	*FOS*	2
MOL002222	3	*CD40LG*	2
MOL007155	2	*IL4*	2
MOL007130	2	*IL2*	2
MOL007127	2	*APP*	2
MOL007121	2	*IL6*	2

Table 4 (*continued*)

Table 4 (*continued*)

Compound	Value	Gene	Value
MOL007107	2	*IL10*	2
MOL007079	2	*VEGFA*	2
MOL007069	2	*BGLAP*	1
MOL007059	2	*NGF*	1
MOL007045	2	*CD36*	1
MOL007036	2	*RUNX2*	1
MOL001601	2	*SPP1*	1
MOL000737	1	*CHUK*	1
MOL000433	1	*CRP*	1
MOL000296	1	*MPO*	1
MOL000239	1	*IL1A*	1
MOL007156	1	*COL1A1*	1
MOL007152	1	*CXCL8*	1
MOL007151	1	*CCL2*	1
MOL007150	1	*GJA1*	1
MOL007143	1	*HIF1A*	1
MOL007132	1	*MMP3*	1
MOL007125	1	*MAPK8*	1
MOL007120	1	*PCNA*	1
MOL007085	1		
MOL007081	1		
MOL007070	1		

C-T, compound-target.

homeostasis of potassium and glutamate (61). Kaempferol is an active flavonoid substance with anti-inflammatory, antioxidant, and anti-viral effects that can alleviate LPS-induced neuroinflammation and BBB dysfunction in mice by inhibiting HMGB1 release and downregulating TLR4/MyD88 pathway (62). In addition, many compounds, such as formononetin in RA, tanshinone IIA in RSM, have anti-inflammatory and neuroprotective effects in the C-T network (63,64). Thus, these findings suggested that the main active ingredients in SQSRP have therapeutic effects on CSM.

In order to explore the correlation among 56 CSM-related target genes, the PPI network was constructed (*Figure 3*). Further analysis found that target genes, such as IL-6, Akt1, CXCL8, JUN, VEGFA, IL-1b, CCL2, MAPK8, MAPK1, and ICAM1 occupied an important position in the network, indicating that these target genes were vital to the action of SQSRP against CSM. To illustrate the role of 53 target genes in gene function and signaling pathways, we performed GO function enrichment and KEGG pathway enrichment analyses. The current study demonstrated that SQSRP was mainly involved in the regulation of nitrogen compounds, reactive oxygen species, cytokines, lipids, and inflammation. The results of KEGG enrichment analysis and T-P network (*Figures 5D* and *8*) showed that these 20 signaling pathways were closely related to the therapeutic effects of SQSRP against CSM and PI3K-Akt, MAPK, IL-17, and TNF were the pivotal signaling pathways. In the pathological process of CSM, progressive compression can cause chronic hypoxic-ischemic damage to the spinal cord tissue, triggering a series of inflammatory reactions and leading to neuron and oligodendrocyte apoptosis. Several studies have shown that the PI3K-Akt signaling pathway after spinal cord injury significantly reduces apoptosis and protects the nerves (65,66). Thus, the MAPK pathway is critical for the pathogenesis of neuropathic pain and spinal cord apoptosis, which is closely related to the occurrence and development of CSM (67,68). Furthermore, chronic persistent neuropathic pain could be alleviated by suppressing the expression of IL-17A in the IL-17 signaling pathway (69). The pro-inflammatory cytokine TNF-α mediates the recruitment of inflammatory cells into injured spinal cord tissues, and by inhibiting the expression of TNF-α, the inflammation in CSM is reduced (70). The current research suggested that the signaling pathways mentioned above may play crucial roles in the inflammatory response, apoptosis, and neuroprotection and exert the therapeutic effects of SQSRP against CSM.

The results of the C-T-P network analysis indicated that 17-beta-estradiol, kaempferol, luteolin, and quercetin are the pivotal active ingredients and Akt1 and MAPK1 are the critical targets of SQSRP against CSM. Akt1, Akt2, and Akt3 and isoforms of the Akt kinase (71). The Akt kinase plays a vital role in the body's physiological and pathological signaling mechanism as it is involved in cellular functions, such as cell survival, proliferation, migration, and gene expression (72). Some studies have shown that the upregulation of Akt1 activation and activation of the PI3K/Akt pathway contributes to the neuroprotective effect during spinal cord injury (73-76). The MAPK1, also known as ERK2, is a member of the MAP kinase signal transduction pathway. The activation of MAPK1 provides neuroprotection during spinal cord injury (77-79). Molecular docking showed that luteolin and 17-beta-

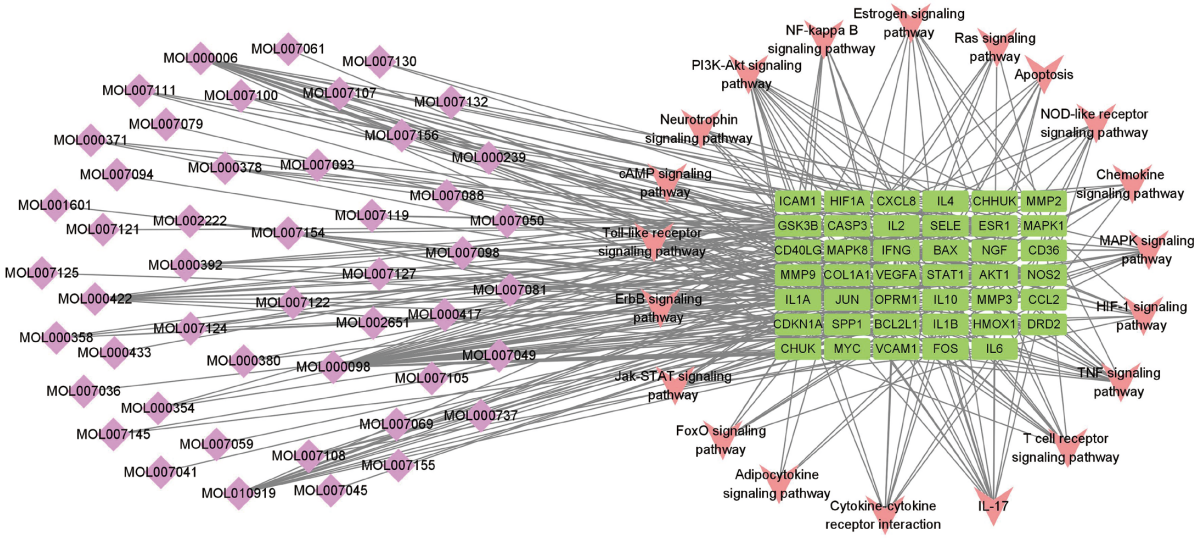

Figure 7 Compound-target-pathway network. Violet diamonds represent active ingredients in SQSRP. Green rectangles represent overlapping genes of SQSRP and CSM. Purple circles represent proteins that directly or indirectly interacted with common targets. Red arrows represent enriched pathways. SQSRP, Shenqisherong pill; CSM, cervical spondylotic myelopathy.

estradiol tightly bind to Akt1 and MAPK1, respectively, suggesting that the critical components of SQSRP could reduce nerve injury by regulating inflammation, reducing stress response, and reducing neuronal apoptosis.

Nevertheless, the present study has several limitations. First, network pharmacology is an approach to discover the network of drug actions through a combination of drug-target networks and biological networks (22). However, this qualitative analysis of the interaction of "drug-component-target-disease" is limited in explaining the overall compatibility mechanism of the TCM compounds as the databases might show discrepancies due to numerous sources of information and theoretical and experimental data (80).

Second, the TCM formula contains a variety of herbs and could be prescribed in different ratios upon the rationale of the physician and the perceived syndrome of the patients. The different ratios of the herbs and the solvent used as a vehicle of the prescription may alter the concentrations of the compounds in the body after consumption, and uncertainty to the concentrations absorbed in the body may occur due to the pharmacokinetics of absorption, distribution, metabolism, excretion, and toxic effects (ADMET) parameters in the body (81). Nonetheless, the dose-efficacy correlation between the compounds and disease is critical, and it is difficult to quantify the effect of the compounds with the current network pharmacology (82). Therefore, further validation experiments on drug concentration are required to accurately reflect the effective components of SQSRP in the treatment of CSM.

Third, the TCM formula is composed of various herbs that play different roles during the treatment according to the principles of TCM theories and therapies (83). However, the results showed that some of the compounds are relatively low in the original herbs, thereby implying that they only exert theoretical significance and do not contribute to the clinical therapeutic value of TCM towards this disease.

Fourth, several active ingredients such as quercetin, kaempferol, and β-sitosterol were found in many herbs and exhibited pharmacological actions on the target disease. Owing to this phenomenon, the specificity of the medicinal activity of these herbs is yet controversial. However, natural products usually act through the modulation of multiple targets rather than a single, specific target. This synergistic action of natural products could address multiple targets of diseases and contribute to the holistic approach of TCM (84). Nevertheless, bioinformatics analysis and further *in vivo* and *in vitro* pharmacological experimental validations are imperative to support this hypothesis (85).

In summary, the results of network pharmacological analysis indicated that the anti-CSM effect of SQSRP

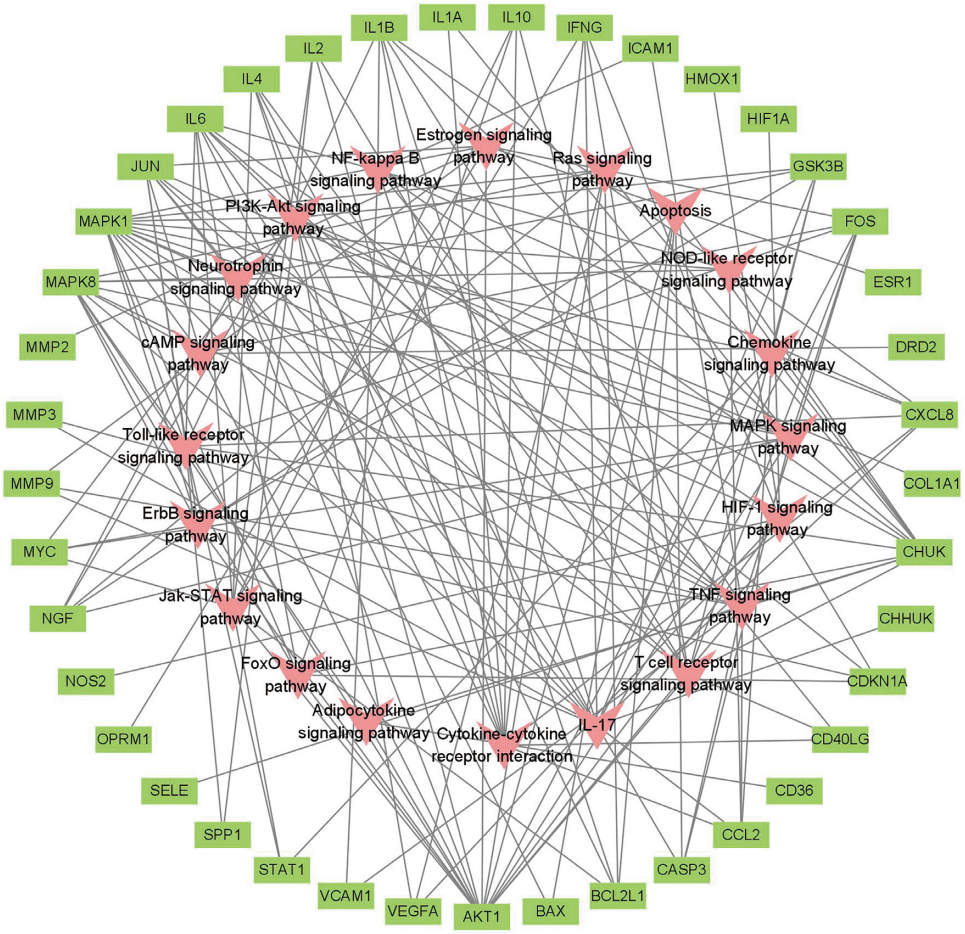

Figure 8 Target-pathway network. Enriched pathways are denoted by red arrows and targets by green rectangles.

was achieved through multi-compound, multi-target, and multi-pathway synergy. The C-T-P network and KEGG enrichment analysis identified 20 signaling pathways closely related to the anti-CSM effect of SQSRP and four key signaling pathways in the network. Next, we verified the interactions between these compounds and targets, as well as targets and targets. The results of network pharmacology provided a theoretical basis for the subsequent extraction of active ingredients from SQSRP to treat CSM.

Conclusions

In this study, for the first time, the potential mechanism of SQSRP against CSM was investigated using network pharmacology. We identified the therapeutic effects of SQSRP against CSM that were effectuated by reducing inflammation, inhibiting apoptosis, and protecting neurons (*Figure 10*). The molecular mechanisms were closely related to 20 pathways, and PI3K-Akt, MAPK, IL-17, and TNF might be the critical signaling pathways (*Figure 8*). Four key compounds and two key targets were identified by comprehensive analysis. These targets were further verified by molecular docking. To elucidate the mechanism of action of SQSRP in the treatment of CSM, we conducted an *in vivo* pharmacokinetic study, determined the pharmacokinetic characteristics of the active compounds by liquid mass spectrometry, and observed the anti-inflammatory and inhibition of apoptosis effects of the active ingredients at different concentrations through *in vivo* and *in vitro* models.

Table 5 Molecular docking fraction

Protein	Grid_size	Docking score (kcal/mol)			
		17-beta-estradiol	kaempferol	luteolin	quercetin
AKT1	50×50×50	−8.7	−8.0	−8.7	−8.7
MAPK1	50×50×50	−8.9	−8.4	−8.3	−8.0

AKT1, AKT serine/threonine kinase 1; MAPK1, mitogen-activated protein kinase 1.

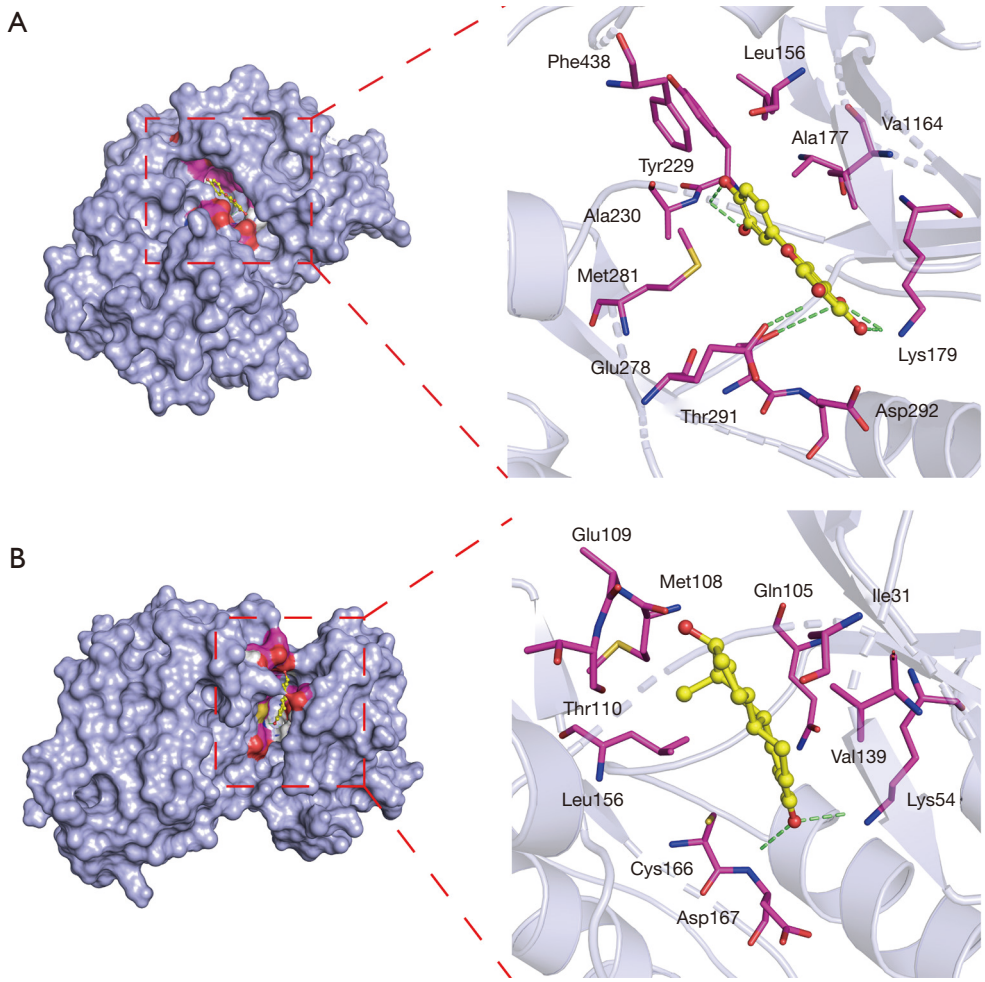

Figure 9 Interaction of active ingredients with key targets. AKT1 protein-luteolin (A), MAPK1 protein-17-beta-estradiol (B). AKT1, AKT serine/threonine kinase 1; MAPK1, mitogen-activated protein kinase 1.

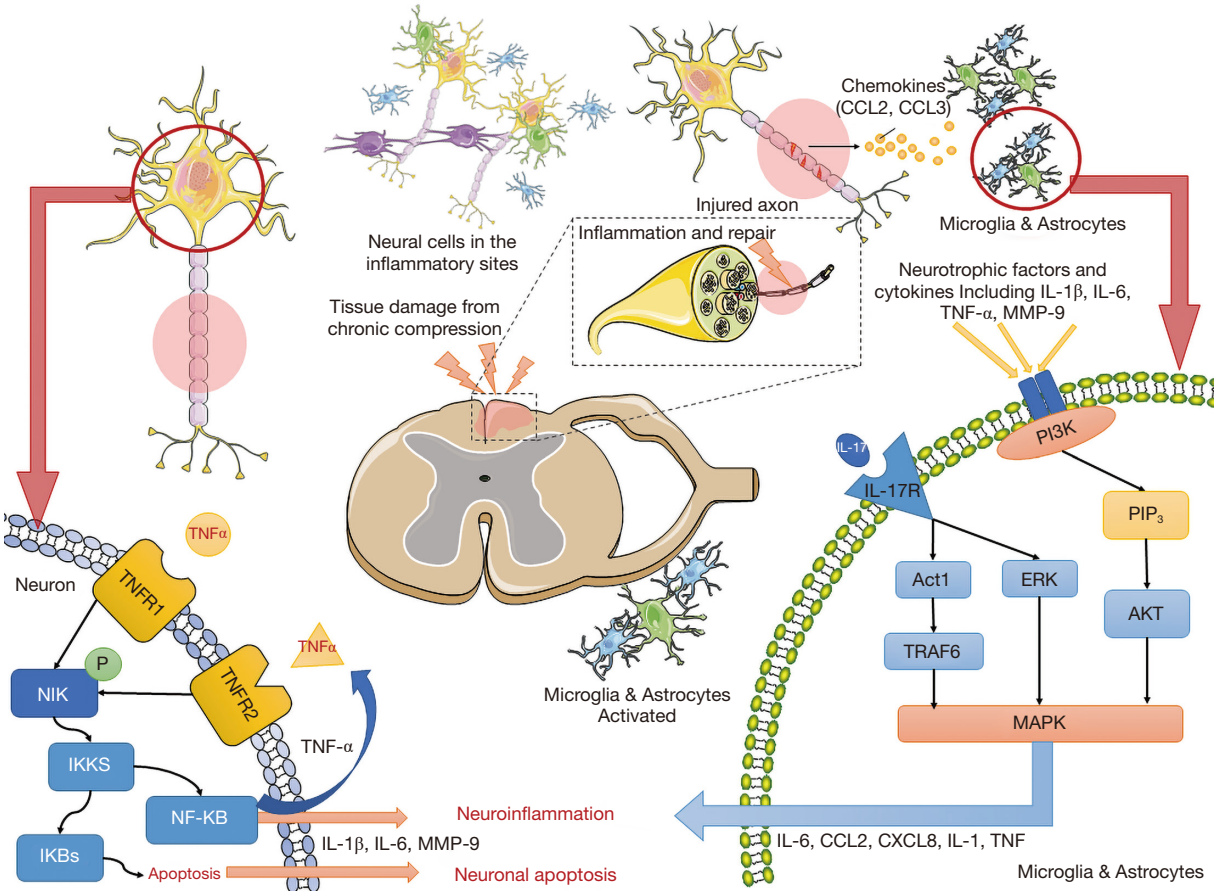

Figure 10 The mechanism of effect of SQSRP in treating CSM. The molecular docking analysis reveals that luteolin can modulate the PI3K/AKT signaling pathway by binding with the AKT1, while the 17-beta-estradiol can modulate the MAPK signaling pathway by binding with the MAPK1. Both actions could contribute in reducing neuroinflammation. On top of that, the SQSRP also exhibit functions of reducing neuronal apoptosis. Therefore, it can be deduced that SQSRP can provide neuroprotection with the functions listed above. SQSRP, Shenqisherong Pill; CSM, cervical spondylotic myelopathy; PI3K, phosphatidylinositide 3-kinases; AKT1, AKT serine/threonine kinase 1; MAPK1, mitogen-activated protein kinase 1.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as

revised in 2013).

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Supplementary

Table S1 A total of 447 compounds in SQSRP

Compound	Herb	Compound	Herb	Compound	Herb
protocatechuic acid	RSM	methyltanshinonate	RSM	Pulegone	SC
5-isopropyl-2-methylbicyclo[3.1.0] hex-2-ene	RSM	microstegiol	RSM	alpha-humulene	SC
[(3R)-3,7-dimethylocta-1,6-dien-3-yl] acetate	RSM	miltionone I	RSM	eugenol	SC
Cymol	RSM	miltionone II	RSM	Tyrosol	SC
Satol	RSM	miltipolone	RSM	MTL	SC
NERYLACETATE	RSM	Miltirone	RSM	8-epi-Loganic acid	SC
(1R,2R,4S)-2,4-diisopropenyl-1-methyl-1-vinylcyclohexane	RSM	miltirone II	RSM	8-Epilpganic acid_qt	SC
EIC	RSM	neocryptotanshinone ii	RSM	Pinoresinol	SC
Oktadekan	RSM	neocryptotanshinone	RSM	Hentriacontan	SC
protocatechualdehyde	RSM	przewalskin	RSM	salidroside	SC
1,2,5,6-tetrahydrotanshinone	RSM	1-methyl-8,9-dihydro-7H-naphtho[5,6-g]benzofuran-6,10,11-trione	RSM	succinic acid	SC
beta-Chamigrene	RSM	paramiltioic acid	RSM	Sitogluside	SC
Poriferasterol	RSM	potassium salvianolate d	RSM	beta-sitosterol	SC
poriferast-5-en-3beta-ol	RSM	prolithospermic acid	RSM	TGL	SC
D-Camphene	RSM	(2S,3S)-2-(3,4-dihydroxyphenyl)-7-hydroxy-4-[(E)-3-hydroxy-3-oxoprop-1-enyl]-2,3-dihydrobenzofuran-3-carboxylic acid	RSM	genistein	SC
isoimperatorin	RSM	(2R)-3-(3,4-dihydroxyphenyl)-2-[(Z)-3-(3,4-dihydroxyphenyl)acryloyl] oxy-propionic acid	RSM	n-Heptadecanol	SC
(R)-linalool	RSM	salviacoccin	RSM	arachidonate	SC
cyanidol	RSM	danshensu	RSM	suchilactone	SC
Oleanolic acid deriv.	RSM	salvianic acid c	RSM	Acteoside_qt	SC
SPBio_002209	RSM	salvianolic acid a	RSM	WLN: VHR	SC
Moslene	RSM	salvianolic acid c	RSM	Hyacinthin	SC
1H-Cycloprop(e)azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, (1aR-(1aalpha,4aalpha,7beta,7abeta,7balpha))-	RSM	salvianolic acid d	RSM	MENTHOL	SC
sugiol	RSM	salvianolic acid e	RSM	Yangambin	SC
HEPTACOSANE	RSM	(Z)-3-[2-[(E)-2-(3,4-dihydroxyphenyl)vinyl]-3,4-dihydroxy-phenyl]acrylic acid	RSM	Propyl methyl trisulfide	SC

Table S1 (*continued*)

https://dx.doi.org/10.21037/apm-21-408

Table S1 (*continued*)

Compound	Herb	Compound	Herb	Compound	Herb
caffeic acid	RSM	salvianolic acid g	RSM	cistanoside D	SC
PENTACOSANE	RSM	salvianolic acid j	RSM	Liriodendrin	SC
(-)-beta-Phellandrene	RSM	salvilenone I	RSM	liriodendrin_qt	SC
oleanolic acid	RSM	salviol	RSM	BUA	SC
Dehydrotanshinone II A	RSM	salviolone	RSM	4-Phenylbicyclo[2,2,2] octan-1-ol	SC
VIV	RSM	salvipisone	RSM	Henicosanoic acid	SC
Baicalin	RSM	methyl (1S,4aS,5R,7S,7aS)-5,7-dihydroxy-7-methyl-1-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy-4a,5,6,7a-tetrahydro-1H-cyclopenta[d]pyran-4-carboxylate	RSM	MSM	SC
δ-cadinol	RSM	shanzhiside methyl ester_qt	RSM	(+)-pinoresinol-O-β-D-glucopyranoside	SC
[(1S)-endo]-(-)-Borneol	RSM	NSC 122421	RSM	m-Anisidine	SC
succinic acid	RSM	(6S)-6-hydroxy-1-methyl-6-methylol-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-quinone	RSM	cyanidol	SC
beta-caryophyllene	RSM	Tanshindiol B	RSM	(1R,4aR,4bR,10aS)-7-isopropyl-1,4a-dimethyl-2,3,4,4b,5,6,10,10a-octahydrophenanthrene-1-carboxylic acid	SC
L-Serin	RSM	Przewaquinone E	RSM	(1S,4S,7R)-7-bicyclo[2.2.2]oct-2-enol	SC
Threonin	RSM	Tanshilactone	RSM	quercetin	SC
PHA	RSM	tanshinone iia	RSM	Cistanin	SC
NSC733507	RSM	(6S)-6-(hydroxymethyl)-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g] benzofuran-10,11-dione	RSM	Cistanoside A	SC
LPG	RSM	tanshinone VI	RSM	Cistanoside A_qt	SC
(2S)-2-amino-3-[(2R)-2-amino-3-hydroxy-3-oxopropyl]disulfanylpropanoic acid	RSM	tanshinone i	RSM	.beta.-D-Glucopyranoside, 2-(4-hydroxy-3-methoxyphenyl) ethyl 3-O-(6-deoxy-.alpha.-L-mannopyranosyl)-4-O-[(2E)-3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]-	SC
tigogenin	RSM	Spirostan-3-ol, (3beta,5alpha,25S)-	RSM	Cistanoside E	SC

Table S1 (*continued*)

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Table S1 (*continued*)

Compound	Herb	Compound	Herb	Compound	Herb
Monomethyl lithospermate	RSM	uvaol	RSM	Cistanoside E_qt	SC
Physcion	RSM	β-cadinol	RSM	Cistanoside F	SC
Stenol	RSM	apigenin	RSM	Cistanoside H	SC
TMH	RSM	stearic acid	RSM	1-Methyl-4-isoallyl-cyclohexane	SC
GLY	RSM	hexadecane	RSM	[(7S,8R)-7-hydroxy-5,6,7,8-tetrahydro-3H-pyrrolizin-1-yl]methyl (2R)-2-hydroxy-2-(1-hydroxyethyl)-3-methylbutanoate	SC
ursolic acid	RSM	Henicosane	RSM	Epimedin A	SC
Gulutamine	RSM	luteolin-7-o-glucoside	RSM	16-Triacontanol	SC
L-	RSM	(R)-p-Menth-1-en-4-ol	RSM	Leonuride(ajugol)	SC
(-)-Epicedrol	RSM	Germacrene D	RSM	Leonuride(ajugol)_qt	SC
Leucinum	RSM	alpha-Farnesene	RSM	methyl 2-dimethylaminoacetate	SC
h-Met-h	RSM	(1R,4S,4aR,8aR)-4-isopropyl-1,6-dimethyl-3,4,4a,7,8,8a-hexahydro-2H-naphthalen-1-ol	RSM	[(2R,3R,4S,5R,6R)-5-acetyloxy-6-[2-(3,4-dihydroxyphenyl)ethoxy]-2-(hydroxymethyl)-4-[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxyoxan-3-yl] (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate	SC
L-Lysin	RSM	vanillic acid	RA	Marckine	SC
Glucosol	RSM	EIC	RA	cistanoside B	SC
DTY	RSM	Mairin	RA	cistanoside B_qt	SC
digallate	RSM	Heriguard	RA	Ethyl disulfide	SC
isoferulic acid	RSM	Jaranol	RA	echinacoside	SC
luteolin	RSM	Rhamnocitrin	RA	echinacoside_qt	SC
Prolinum	RSM	alexandrin	RA	2,3-Dimethylheptane	SC
8-isopropylidene-1,5-dimethylcyclodeca-1,5-diene	RSM	hederagenin	RA	n-Decyl glucoside	SC
1,5-Dihydroxy-3-methylanthraquinone	RSM	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	RA	SBU	SC
ASI	RSM	isorhamnetin	RA	2,6-diisobutyl-4-methylphenol	SC
L-Valin	RSM	lupeol	RA	3,4,5,5-Tetramethyl-2-cyclopenten-1-one	SC

Table S1 (*continued*)

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Table S1 (*continued*)

Compound	Herb	Compound	Herb	Compound	Herb
oleic acid	RSM	3,9-di-O-methylnissolin	RA	3,4-Dioxymethulene-5methoxy-1-(1-oxopropyl) benzene	SC
L-Ile	RSM	3-Hydroxy-2-picoline	RA	n-Triacontanol	SC
α-amyrin	RSM	(2S)-4-methoxy-7-methyl-2-[1-methyl-1-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-methylol-tetrahydropyran-2-yl]oxy-ethyl]-2,3-dihydrofuro[3,2-g]chromen-5-one	RA	17-beta-estradiol	Musk
palmitic acid	RSM	5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside	RA	1beta,2beta,5alpha,11-tetraacetoxy-8alpha-benzoyl-4alpha-hydroxy-7beta-nicotinoyl-dihydroagarofuran	Musk
(E)-3-(3-hydroxy-4,5-dimethoxy-phenyl) acrylic acid	RSM	5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside_qt	RA	2,6-decamethylene pyridine	Musk
5,6-dihydroxy-7-isopropyl-1,1-dimethyl-2,3-dihydrophenanthren-4-one	RSM	7,2'-dihydroxy-3',4'-dimethoxyisoflavone-7-O-β-D-glucoside	RA	2,6-nonamethylene pyridine	Musk
1,2-DT-Quinone	RSM	7-hydroxy-3-(2-hydroxy-3,4-dimethoxy-phenyl)chromone	RA	22-cyclopentyloxil-22-deisopentyl-3beta-hydroxyl-guranstanol	Musk
Dehydromiltirone	RSM	7-O-methylisomucronulatol	RA	3'(s)-hydroxy-4'(r)angeloyloxy-3',4'-dihydroxanthyletin	Musk
Henicosyl formate	RSM	9,10-dimethoxypterocarpan-3-O-β-D-glucoside	RA	3,5-dihydroxybenzoic acid	Musk
1-ketoisocryptotanshinone	RSM	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	RA	3-methylcyclotridecan-1-one	Musk
2-isopropyl-8-methylphenanthrene-3,4-dione	RSM	13-hydroxy-9,11-octadecadienoic acid	RA	3alpha,17-dihydroxy-5beta-androstane	Musk
3-epicorosolic,acid	RSM	Arabinose,d	RA	3alpha-hydroxy-5alpha-androstan-17-one	Musk
1-(3,4-dihydroxyphenyl)-2-hydroxyethanone	RSM	D-Galacturonic acid, homopolymer	RA	3alpha-hydroxy-androst-4-ene-17-one	Musk
3,7-dimethylocta-2,6-dien-1-yl formate	RSM	DL-Glucuronic acid	RA	3beta,17alpha-dihydroxy-5alpha-androstane	Musk
3α-hydroxytanshinoneIIa	RSM	isoferulic acid	RA	3beta-hydroxy-5alpha-androstan-17-one	Musk
3beta-Hydroxytanshinone IIA	RSM	Fucopyranose, L-	RA	3beta-hydroxy-androst-5-ene-17-one	Musk
(1R,4R,5S)-1-isopropyl-4-methyl-4-bicyclo[3.1.0]hexanol	RSM	Bifendate	RA	3α-hydroxy-5β-androstan-17-one	Musk
(E)-3-[2-(3,4-dihydroxyphenyl)-7-hydroxy-benzofuran-4-yl]acrylic acid	RSM	gamma-aminobutyric acid	RA	3α-hydroxy-androst-4-ene-17-one	Musk

Table S1 (*continued*)

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Table S1 (*continued*)

Compound	Herb	Compound	Herb	Compound	Herb
4-methylenemiltirone	RSM	FERULIC ACID (CIS)	RA	3α-ureido-androst-4-en-17-one	Musk
2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde	RSM	daidzein	RA	3α-ureido-androst-4-en-17β-ol	Musk
6-o-syringyl-8-o-acetyl shanzhiside methyl ester	RSM	Ononin	RA	3β-hydroxy-5α-androstan-17-one	Musk
6-o-syringyl-8-o-acetyl shanzhiside methyl ester_qt	RSM	formononetin	RA	3β-hydroxy-androst-5-ene-17-one	Musk
7-oxoroyleanone2	RSM	Soyasaponin I	RA	5 alpha-androstan-3,17-dione	Musk
(4bS,8aS,10S)-10-hydroxy-2-isopropyl-4b,8,8-trimethyl-5,6,7,8a,9,10-hexahydrophenanthrene-3,4-dione	RSM	choline	RA	5 beta-androstan-3 alpha, 17 alpha-diol	Musk
9-methyl lithospermate b	RSM	GGB	RA	5 beta-androstan-3 alpha, 17 beta-diol	Musk
TNP00297	RSM	(+)-Syringaresinol	RA	5 beta-androstan-3,17-dione	Musk
Heriguard	RSM	cis-p-Coumarate	RA	5-cis-cyclopentadecen-1-one	Musk
formyltanshinone	RSM	isoflavanone	RA	5-cis-cyclotetradecen-1-one	Musk
3-beta-Hydroxymethyllenetanshiquinone	RSM	Docosanoate	RA	5α-androstan-3,17-dione	Musk
Lithospermic acid B	RSM	Flavaxin	RA	5α-androstane-3β,17α-diol	Musk
Methylenetanshinquinone	RSM	astragalosidel	RA	5β-androstan-3,17-dione	Musk
neo-przewaquinone a	RSM	astragalosidel_qt	RA	5β-androstan-3α,17α-diol	Musk
przewalskin a	RSM	astragalosideII	RA	5β-androstan-3α,17β-diol	Musk
przewalskin b	RSM	astragalosideII_qt	RA	6-hydroxy-musizin-8-o-beta-d-glucoside	Musk
przewalskin c	RSM	astragalosideIII	RA	allantoin	Musk
przewalskin d	RSM	astragalosideIII_qt	RA	alpha-estradiol	Musk
Przewaquinone A	RSM	astragalosideIV	RA	androst-4,6-diene-3,17-dione	Musk
Przewaquinone B	RSM	astragalosideIV_qt	RA	androst-4-ene-3,17-dione	Musk
przewaquinone c	RSM	AstragalosideIV	RA	androsterone	Musk
(6S,7R)-6,7-dihydroxy-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-dione	RSM	AstragalosideIV_qt	RA	cholest-4-ene-3-one	Musk
przewaquinone f	RSM	Astraisoflavanin	RA	cholesterol	Musk
(2S)-3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid	RSM	Mucronulatol	RA	cholesteryl ferulate	Musk
saloilenone	RSM	astrachrysoside A	RA	cyclotetradecan-1-one	Musk
salvianolic acid b	RSM	Caffeate	RA	cyclovirobuxine	Musk
salvianolic acid n	RSM	rutin	RA	decamine	Musk

Table S1 (*continued*)

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Table S1 (*continued*)

Compound	Herb	Compound	Herb	Compound	Herb
Saprothoquinone	RSM	Lariciresinol	RA	estragole	Musk
sclareol	RSM	Calycosin	RA	hydroxymuscopyridine a	Musk
Tannin	RSM	3'-Hydroxy-4'-methoxyisoflavone-7-O-beta-D-glucoside	RA	hydroxymuscopyridine b	Musk
tanshinaldehyde	RSM	astrasieversianin XV	RA	morin	Musk
Tanshinol A	RSM	XLS	RA	musclide a1	Musk
Danshenol B	RSM	nicotinic acid	RA	muscol	Musk
Danshenol A	RSM	kaempferol	RA	muscone	Musk
Z-8-Hexadecen-1-ol acetate	RSM	rhamnocitrin-3-O-glucoside	RA	muscopyridine	Musk
Aethiopinone	RSM	RAM	RA	musennin	Musk
Salvilenone	RSM	asernestioside A	RA	n-nonane	Musk
carnosol	RSM	asernestioside A_qt	RA	n-nornuciferine	Musk
cryptotanshinone	RSM	asernestioside B	RA	normuscone	Musk
Cyclotetradecane	RSM	asernestioside B_qt	RA	s-methyl cysteine	Musk
dan-shexinkum a	RSM	Crystal VI	RA	testosterone	Musk
dan-shexinkum b	RSM	betaine	RA	α-estradiol	Musk
dan-shexinkum c	RSM	coumarin	RA	β-estradiol	Musk
dan-shexinkum d	RSM	linolenic acid	RA	methyl palmitate	Musk
danshenspiroketallactone	RSM	FA	RA	triolein	Musk
danshenspiroketallactoneii	RSM	acetylastragaloside I	RA	aspartate asparagic acid asparaginic acid aspartic acid	Musk
daucosterol	RSM	acetylastragaloside I_qt	RA	glycine	Musk
dehydrouvaol	RSM	(Z)-1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one	RA	serine	Musk
deoxyneocryptotanshinone	RSM	Hirsutrin	RA	glutamic acid	Musk
dihydroisotanshinoneI	RSM	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	RA	urea	Musk
Istidina	RSM	isomucronulatol-7,2'-di-O-glucosiole	RA	methyl oleate methyl-9-octadecenoate	Musk
dihydrotanshinlactone	RSM	isomucronulatol-7,2'-di-O-glucosiole_qt	RA	Δ4-cholestenone-3	Musk
dihydrotanshinoneI	RSM	LUPENONE	RA	cholestanol	Musk
diisopro-penyl methyl vinyl cyclohexane2	RSM	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	RA	cholic acid	Musk
dimetbyl lithosper-mate b	RSM	L-	RA	3α-hydroxyandrostan-4-en-17β-one	Musk

Table S1 (*continued*)

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Table S1 (*continued*)

Compound	Herb	Compound	Herb	Compound	Herb
dimethyllithospermate	RSM	Prolinum	RA	androst-4-en-3,17-dione	Musk
epidanshenspiroketallactone	RSM	palmitic acid	RA	5α-androstane-3,17-dione	Musk
ethyl lithospermate	RSM	quercetin	RA	5β-androstane-3,17-dione	Musk
C09092	RSM	Neral	SC	5β-androstane-3α,17β-diol	Musk
isocryptotanshi-none	RSM	WLN: Q1R	SC	5β-androstane-3α',17β-diol	Musk
isosalvianolic acid c	RSM	(+)-Ledol	SC	valine	Musk
isotanshinone iib	RSM	Leonurine	SC	3β- hydroxy-5β-androstan-17-one	Musk
Isotanshinone II	RSM	acteoside	SC	3β- hydroxy-androst-5-en-17-one	Musk
Isotanshinone I	RSM	decaffeoylacteoside	SC	5α-androstane-3,17-diol	Musk
lithospermic acid	RSM	Geniposidic acid	SC	5β-androstane-3α,17α-diol	Musk
manool	RSM	geniposidie acid_qt	SC	3β-hydroxyandrost-5-en-17-one	Musk
methylrosmarinate	RSM	Dauricine (8CI)	SC	androst-4-one-3,17-dione	Musk

SQSRP, Shenqisherong Pill; RSM, Radix Salviae Miltiorrhiza; RA, Radix Astragali; SC, Saline Cistanche.

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Table S2 After screening the TCMSP and the Uniprot databases, a total of 249 target genes corresponding to 84 compounds were identified

Mol ID	Compound	OB (%)	DL	Herb	Gene Symbol
MOL001601	1,2,5,6-tetrahydrotanshinone	38.75	0.36	RSM	*PTGS1/CHRM3/CHRM1/SCN5A/CHRM5/PTGS2/HTR3A/CHRM4/RXRA/OPRD1/ADRA1A/CHRM2/ADRA1B/SLC6A3/ADRB2/ADRA1D/OPRM1/GABRA1/NCOA2/NCOA1/SLC6A4*
MOL001659	Poriferasterol	43.83	0.76	RSM	*PGR/NR3C2*
MOL001771	poriferast-5-en-3beta-ol	36.91	0.75	RSM	*PGR/NCOA2*
MOL001942	isoimperatorin	45.46	0.23	RSM	*PTGS2*
MOL002222	sugiol	36.11	0.28	RSM	*CHRM3/CHRM1/SCN5A/CHRM5/PTGS2/CHRM4/OPRD1/ACHE/ADRA1A/CHRM2/ADRA1B/ADRB2/ADRA1D/DRD2/OPRM1*
MOL002651	Dehydrotanshinone II A	43.76	0.4	RSM	*CHRM3/CHRM1/ESR1/AR/SCN5A/PPARG/CHRM5/PTGS2/CHRM4/OPRD1/ACHE/ADRA1A/ADRB2/OPRM1/GABRA1/NCOA1*
MOL002776	Baicalin	40.12	0.75	RSM	
MOL000569	digallate	61.85	0.26	RSM	*PTGS2/AKR1B1*
MOL000006	luteolin	36.16	0.25	RSM	*PTGS1/AR/PTGS2/PRSS1/NCOA2/RELA/EGFR/AKT1/VEGFA/CCND1/BCL2L1/CDKN1A/CASP9/MMP2/MMP9/MAPK1/IL10/RB1/TNFSF15/JUN/IL6/CASP3/TP63/NFKBIA/TOP1/MDM2/APP/MMP1/PCNA/ERBB2/PPARG/HMOX1/CASP7/ICAM1/MCL1/BIRC5/IL2/CCNB1/TYR/IFNG/IL4/TOP2A/GSTP1/SLC2A4/INSR/CD40LG/PTGES/NUF2/ADCY2/MET*
MOL006824	α-amyrin	39.51	0.76	RSM	
MOL007036	5,6-dihydroxy-7-isopropyl-1,1-dimethyl-2,3-dihydrophenanthren-4-one	33.77	0.29	RSM	*PTGS1/CHRM3/CHRM1/SCN5A/PTGS2/RXRA/ACHE/ADRA1A/ADRA1B/ADRB2/OPRM1/NCOA2/NCOA1*
MOL007041	2-isopropyl-8-methylphenanthrene-3,4-dione	40.86	0.23	RSM	*PTGS1/CHRM3/CHRM1/ESR1/AR/SCN5A/PPARG/CHRM5/PTGS2/HTR3A/CHRM4/RXRA/ADRA1A/CHRM2/ADRA1B/SLC6A3/ADRB2/ADRA1D/SLC6A4/OPRM1/GABRA1/CCNA2/NCOA2*
MOL007045	3α-hydroxytanshinoneIIa	44.93	0.44	RSM	*CHRM1/SCN5A/CHRM5/PTGS2/OPRD1/ACHE/ADRB2/OPRM1/PRSS1/NCOA1*
MOL007048	(E)-3-[2-(3,4-dihydroxyphenyl)-7-hydroxy-benzofuran-4-yl]acrylic acid	48.24	0.31	RSM	*PTGS2*
MOL007049	4-methylenemiltirone	34.35	0.23	RSM	*PTGS1/CHRM3/CHRM1/ESR1/AR/SCN5A/PPARG/CHRM5/PTGS2/ADRA2A/ADRA2C/CHRM4/RXRA/OPRD1/ADRA1A/CHRM2/ADRA1B/SLC6A3/ADRB2/ADRA1D/SLC6A4/DRD2/OPRM1/GABRA1/NCOA2/NCOA1*
MOL007050	2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde	62.78	0.4	RSM	*NOS2/ESR1/AR/PPARG/ESR2/MAPK14/GSK3B/CCNA2*

Table S2 (*continued*)

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Table S2 (*continued*)

Mol ID	Compound	OB (%)	DL	Herb	Gene Symbol
MOL007051	6-o-syringyl-8-o-acetyl shanzhiside methyl ester	46.69	0.71	RSM	
MOL007058	formyltanshinone	73.44	0.42	RSM	*AR/PTGS2/RXRA/NCOA1*
MOL007059	3-beta-Hydroxymethyllenetanshiquinone	32.16	0.41	RSM	*CHRM1/PTGS2/RXRA/OPRD1/ACHE/ADRA1A/ADRB2/OPRM1/PRSS1/NCOA1*
MOL007061	Methylenetanshinquinone	37.07	0.36	RSM	*CHRM3/CHRM1/SCN5A/CHRM5/PTGS2/RXRA/OPRD1/ACHE/ADRA1A/CHRM2/ADRB2/SLC6A4/OPRM1/GABRA1/PRSS1/NCOA1*
MOL007063	przewalskin a	37.11	0.65	RSM	*NR3C2/NR3C1*
MOL007064	przewalskin b	110.32	0.44	RSM	*PTGS2/PGR/NR3C2/NR3C1/NCOA2/NCOA1*
MOL007068	Przewaquinone B	62.24	0.41	RSM	*PTGS2/RXRA/PRSS1/NCOA1*
MOL007069	przewaquinone c	55.74	0.4	RSM	*PTGS1/CHRM3/CHRM1/SCN5A/CHRM5/PTGS2/CHRM4/OPRD1/ACHE/ADRA1A/CHRM2/ADRB2/OPRM1/GABRA1/NCOA1*
MOL007070	(6S,7R)-6,7-dihydroxy-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g] benzofuran-10,11-dione	41.31	0.45	RSM	*PTGS2/ACHE/PRSS1/NCOA1*
MOL007071	przewaquinone f	40.31	0.46	RSM	*PTGS2/PRSS1/NCOA1*
MOL007077	sclareol	43.67	0.21	RSM	*PTGS2*
MOL007079	tanshinaldehyde	52.47	0.45	RSM	*CHRM1/PTGS2/OPRD1/ACHE/ADRB2/OPRM1/PRSS1/NCOA1*
MOL007081	Danshenol B	57.95	0.56	RSM	*PTGS2/PGR/OPRM1/NR3C1/NCOA1*
MOL007082	Danshenol A	56.97	0.52	RSM	*PTGS1/KCNH2/SCN5A/PTGS2/RXRA/NCOA1*
MOL007085	Salvilenone	30.38	0.38	RSM	*PTGS1/ESR1/AR/CHRM5/PTGS2/HTR3A/ESR2*
MOL007088	cryptotanshinone	52.34	0.4	RSM	*PTGS1/CHRM3/CHRM1/SCN5A/CHRM5/PTGS2/CHRM4/OPRD1/ADRA1A/CHRM2/ADRA1B/ADRB2/ADRA1D/OPRM1/NCOA2/NCOA1/PGR/GABRA1/RELA/STAT3/CCND1/BCL2L1/TNF/SF15/APP/EDN3/BIRC5*
MOL007093	dan-shexinkum d	38.88	0.55	RSM	*NOS2/PTGS1/KCNH2/CHRM1/ESR1/AR/SCN5A/PPARG/PTGS2/RXRA/ACHE/ADRA1B/ADRB2/ESR2/GSK3B/CHEK1/PRSS1/CCNA2/NCOA2/NCOA1*
MOL007094	danshenspiroketallactone	50.43	0.31	RSM	*PTGS1/CHRM3/CHRM1/ESR1/SCN5A/CHRM5/PTGS2/CHRM4/RXRA/ACHE/ADRA1A/CHRM2/ADRA1B/ADRB2/ADRA1D/CHRNA2/SLC6A4/OPRM1/GABRA1*
MOL007098	deoxyneocryptotanshinone	49.4	0.29	RSM	*PTGS1/CHRM3/CHRM1/ESR1/AR/SCN5A/CHRM5/PTGS2/CHRM4/RXRA/OPRD1/ADRA1A/CHRM2/ADRA1B/ADRB2/ADRA1D/OPRM1/GSK3B/NCOA2/NCOA1*

Table S2 (*continued*)

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Table S2 (*continued*)

Mol ID	Compound	OB (%)	DL	Herb	Gene Symbol
MOL007100	dihydrotanshinlactone	38.68	0.32	RSM	*NOS2/PTGS1/CHRM3/CHRM1/ESR1/AR/SCN5A/PPARG/CHRM5/PTGS2/HTR3A/RXRA/ACHE/ADRA1A/ADRA1B/SLC6A3/ADRB2/ADRA1D/SLC6A4/OPRM1/GABRA1/GSK3B/PRSS1/CCNA2*
MOL007101	dihydrotanshinoneI	45.04	0.36	RSM	*PTGS1/SCN5A/PTGS2/HTR3A/RXRA/ADRA1A/ADRA1B/ADRB2/GABRA1/NCOA2/NCOA1*
MOL007105	epidanshenspiroketallactone	68.27	0.31	RSM	*PTGS1/CHRM3/CHRM1/ESR1/SCN5A/CHRM5/PTGS2/CHRM4/RXRA/OPRD1/ADRA1A/CHRM2/ADRA1B/ADRB2/ADRA1D/SLC6A4/OPRM1/GABRA1*
MOL007107	C09092	36.07	0.25	RSM	*CHRM3/CHRM1/SCN5A/ACHE/ADRA1A/CHRM2/ADRA1B/ADRB2/ADRA1D/OPRM1*
MOL007108	isocryptotanshi-none	54.98	0.39	RSM	*NOS2/PTGS1/CHRM3/CHRM1/ESR1/AR/SCN5A/CHRM5/PTGS2/CHRM4/RXRA/OPRD1/ACHE/ADRA1A/CHRM2/ADRA1B/ADRB2/ADRA1D/DRD2/OPRM1/GABRA1/PRSS1/NCOA2/NCOA1*
MOL007111	Isotanshinone II	49.92	0.4	RSM	*NOS2/CHRM3/CHRM1/ESR1/AR/SCN5A/CHRM5/PTGS2/RXRA/OPRD1/ACHE/ADRA1A/CHRM2/ADRB2/OPRM1/ESR2/GABRA1/GSK3B/CHEK1/CCNA2*
MOL007115	manool	45.04	0.2	RSM	*NCOA2*
MOL007118	microstegiol	39.61	0.28	RSM	
MOL007119	miltionone I	49.68	0.32	RSM	*PTGS1/CHRM3/CHRM1/ESR1/AR/SCN5A/PTGS2/RXRA/OPRD1/ADRA1A/CHRM2/ADRA1B/ADRB2/OPRM1/NR3C1/GSK3B/CCNA2/NCOA2/NCOA1*
MOL007120	miltionone II	71.03	0.44	RSM	*PTGS2/ACHE/PGR/NR3C1/NCOA2/NCOA1*
MOL007121	miltipolone	36.56	0.37	RSM	*ESR1/ACHE*
MOL007122	Miltirone	38.76	0.25	RSM	*PTGS1/CHRM3/CHRM1/ESR1/AR/DRD5/SCN5A/CHRM5/PTGS2/ADRA2C/CHRM4/RXRA/OPRD1/ADRA1A/CHRM2/ADRA1B/SLC6A3/ADRB2/ADRA1D/OPRM1/NCOA2*
MOL007123	miltirone II	44.95	0.24	RSM	
MOL007124	neocryptotanshinone ii	39.46	0.23	RSM	*PTGS1/CHRM3/CHRM1/ESR1/AR/SCN5A/PTGS2/CHRM4/RXRA/OPRD1/ADRA1A/CHRM2/ADRA1B/SLC6A3/ADRB2/ADRA1D/SLC6A4/OPRM1/GABRA1/GSK3B/CCNA2*
MOL007125	neocryptotanshinone	52.49	0.32	RSM	*PTGS1/CHRM3/CHRM1/SCN5A/PPARG/PTGS2/ADRA1B/ADRB2/ADRA1D/OPRM1/NCOA2/NCOA1*

Table S2 (*continued*)

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Table S2 (*continued*)

Mol ID	Compound	OB (%)	DL	Herb	Gene Symbol
MOL007127	1-methyl-8,9-dihydro-7H-naphtho[5,6-g]benzofuran-6,10,11-trione	34.72	0.37	RSM	*PTGS1/CHRM3/SCN5A/CHRM5/PTGS2/RXRA/ACHE/ADRA1A/ADRB2/OPRM1/GABRA1/NCOA1*
MOL007130	prolithospermic acid	64.37	0.31	RSM	*NOS2/PTGS1/ESR1/AR/PTGS2/PRSS1*
MOL007132	(2R)-3-(3,4-dihydroxyphenyl)-2-[(Z)-3-(3,4-dihydroxyphenyl)acryloyl]oxy-propionic acid	109.38	0.35	RSM	*ESR1/AR/PPARG/PTGS2/PRSS1/CCNA2*
MOL007140	(Z)-3-[2-[(E)-2-(3,4-dihydroxyphenyl)vinyl]-3,4-dihydroxy-phenyl]acrylic acid	88.54	0.26	RSM	
MOL007141	salvianolic acid g	45.56	0.61	RSM	*PTGS2*
MOL007142	salvianolic acid j	43.38	0.72	RSM	*F7/PRSS1*
MOL007143	salvilenone I	32.43	0.23	RSM	*PTGS2/RXRA/ACHE/PGR/NR3C1/NCOA2/NCOA1*
MOL007145	salviolone	31.72	0.24	RSM	*PTGS1/CHRM3/CHRM1/DRD5/SCN5A/CHRM5/PTGS2/ADRA2A/HTR3A/CHRM4/OPRD1/ACHE/SLC6A2/ADRA1A/CHRM2/ADRA2B/ADRA1B/SLC6A3/ADRB2/CHRNA2/SLC6A4/DRD2/OPRM1/GABRA1/GABRG3/GABRE*
MOL007149	NSC 122421	34.49	0.28	RSM	
MOL007150	(6S)-6-hydroxy-1-methyl-6-methylol-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-quinone	75.39	0.46	RSM	*PTGS2/ACHE/PRSS1/NCOA1*
MOL007151	Tanshindiol B	42.67	0.45	RSM	*PTGS2/ACHE/NCOA1*
MOL007152	Przewaquinone E	42.85	0.45	RSM	*PTGS2/ACHE/NCOA1*
MOL007154	tanshinone iia	49.89	0.4	RSM	*CHRM3/CHRM1/SCN5A/CHRM5/PTGS2/CHRM4/OPRD1/ACHE/ADRA1A/CHRM2/ADRB2/OPRM1/NCOA1/RXRA/RELA/BCL2/FOS/CDKN1A/MMP9/JUN/AHSA1/CASP3/TP63/NFKBIA/FASN/EDNRA/EDN3/CYP3A4/CYP1A2/MYC/CYP1A1/NR1I2/NPM1/ECE1/PARP4/CALCR/ITGB3*
MOL007155	(6S)-6-(hydroxymethyl)-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-dione	65.26	0.45	RSM	*CHRM1/SCN5A/PTGS2/OPRD1/ACHE/ADRA1A/ADRB2/OPRM1/PRSS1/NCOA1*
MOL007156	tanshinone VI	45.64	0.3	RSM	*PTGS1/ESR1/AR/SCN5A/PPARG/PTGS2/NCOA2/NCOA1*
MOL000211	Mairin	55.38	0.78	RA	*PGR*
MOL000239	Jaranol	50.83	0.29	RA	*NOS2/PTGS1/AR/SCN5A/PTGS2/ESR2/CHEK1/PRSS1/NCOA2*
MOL000296	hederagenin	36.91	0.75	RA	*PGR/NCOA2/CHRM3/CHRM1/CHRM2/ADRA1B/GABRA1/GRIA2/ADH1B/ADH1C/LYZ/PTGS1/SCN5A/PTGS2/RXRA/SLC6A2*

Table S2 (*continued*)

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Table S2 (*continued*)

Mol ID	Compound	OB (%)	DL	Herb	Gene Symbol
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	0.78	RA	*PGR*
MOL000354	isorhamnetin	49.6	0.31	RA	*NOS2/PTGS1/ESR1/AR/PPARG/PTGS2/ESR2/MAPK14/GSK3B/PRSS1/CCNA2/NCOA2/PYGM/CHEK1/AKR1B1/NCOA1/F7/ACHE/GABRA1/MAOB/GRIA2/RELA/NCF1/OLR1*
MOL000371	3,9-di-O-methylnissolin	53.74	0.48	RA	*NOS2/PTGS1/CHRM3/CHRM1/ESR1/ADRB1/SCN5A/PTGS2/HTR3A/ADRA2C/RXRA/ACHE/ADRA1B/ADRB2/ADRA1D/OPRM1/GABRA1/PRSS1/NCOA2*
MOL000374	5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside	41.72	0.69	RA	
MOL000378	7-O-methylisomucronulatol	74.69	0.3	RA	*NOS2/PTGS1/CHRM3/KCNH2/CHRM1/ESR1/AR/ADRB1/SCN5A/PPARG/CHRM5/PTGS2/ADRA2C/CHRM4/RXRA/OPRD1/ADRA1A/CHRM2/ADRA1B/SLC6A3/ADRB2/ADRA1D/SLC6A4/ESR2/GABRA1/MAPK14/GSK3B/CHEK1/RXRB/PRSS1/CCNA2/NCOA2*
MOL000379	9,10-dimethoxypterocarpan-3-O-β-D-glucoside	36.74	0.92	RA	*PTGS2/NCOA2*
MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	64.26	0.42	RA	*NOS2/PTGS1/CHRM3/CHRM1/ESR1/SCN5A/PTGS2/HTR3A/RXRA/ACHE/ADRA1B/ADRB2/ADRA1D/GABRA1/PRSS1/NCOA2/NCOA1/CHRM4*
MOL000387	Bifendate	31.1	0.67	RA	*PTGS2/KDR/MET/PTGS1*
MOL000392	formononetin	69.67	0.21	RA	*NOS2/PTGS1/CHRM1/ESR1/AR/PPARG/PTGS2/RXRA/ADRA1A/SLC6A3/ADRB2/SLC6A4/ESR2/MAPK14/GSK3B/MAOB/CHEK1/PRSS1/CCNA2/PKIA/ACHE/JUN/PPARG/IL4/ATP5F1B/ND6/HSD3B2/HSD3B1*
MOL000398	isoflavanone	109.99	0.3	RA	
MOL000417	Calycosin	47.75	0.24	RA	*NOS2/PTGS1/ESR1/AR/PPARG/PTGS2/RXRA/ESR2/MAPK14/GSK3B/CHEK1/PRSS1/CCNA2/NCOA2/ADRB2*

Table S2 (*continued*)

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Table S2 (*continued*)

Mol ID	Compound	OB (%)	DL	Herb	Gene Symbol
MOL000422	kaempferol	41.88	0.24	RA	*NOS2/PTGS1/AR/PPARG/PTGS2/NCOA2/PRSS1/PGR/CHRM1/ACHE/SLC6A2/CHRM2/ADRA1B/GABRA1/F7/RELA/IKBKB/AKT1/BCL2/BAX/TNFSF15/JUN/AHSA1/CASP3/MAPK8/MMP1/STAT1/PPARG/HMOX1/CYP3A4/CYP1A2/CYP1A1/ICAM1/SELE/VCAM1/NR1I2/CYP1B1/ALOX5/HAS2/GSTP1/AHR/PSMD3/SLC2A4/NR1I3/INSR/DIO1/PPP3CA/GSTM1/GSTM2/AKR1C3/SLPI*
MOL000433	FA	68.96	0.71	RA	*GSK3B*
MOL000438	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	67.67	0.26	RA	
MOL000439	isomucronulatol-7,2'-di-O-glucosiole	49.28	0.62	RA	
MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.05	0.48	RA	*PTGS2/RXRA/PRSS1*
MOL000098	quercetin	46.43	0.28	RA & SC	*PTGS1/AR/PPARG/PTGS2/NCOA2/AKR1B1/PRSS1/KCNH2/SCN5A/ADRB2/MMP3/F7/RXRA/ACHE/GABRA1/MAOB/RELA/EGFR/AKT1/VEGFA/CCND1/BCL2/BCL2L1/FOS/CDKN1A/EIF6/BAX/CASP9/PLAU/MMP2/MMP9/MAPK1/IL10/EGF/RB1/TNFSF15/JUN/IL6/AHSA1/CASP3/TP63/ELK1/NFKBIA/POR/ODC1/CASP8/TOP1/RAF1/SOD1/PRKCA/MMP1/HIF1A/STAT1/RUNX1T1/ERBB2/ACACA/HMOX1/CYP3A4/CYP1A2/CAV1/MYC/F3/GJA1/CYP1A1/ICAM1/IL1B/CCL2/SELE/VCAM1/PTGER3/CXCL8/PRKCB/BIRC5/DUOX2/NOS3/HSPB1/SULT1E1/MGAM/IL2/NR1I2/CYP1B1/CCNB1/PLAT/THBD/SERPINE1/COL1A1/IFNG/ALOX5/IL1A/MPO/TOP2A/NCF1/ABCG2/HAS2/GSTP1/NFE2L2/NQO1/PARP1/AHR/PSMD3/SLC2A4/COL3A1/CXCL11/CXCL2/DCAF5/NR1I3/CHEK2/INSR/CLDN4/PPARA/PPARD/HSF1/CRP/CXCL10/CHUK/SPP1/RUNX2/RASSF1/E2F1/E2F2/ACPP/CTSD/IGFBP3/IGF2/CD40LG/IRF1/ERBB3/PON1/DIO1/PCOLCE/NPEPPS/HK2/RASA1/GSTM1/GSTM2*
MOL000358	beta-sitosterol	36.91	0.75	SC	*PGR/NCOA2/PTGS1/PTGS2/KCNH2/CHRM3/CHRM1/SCN5A/CHRM4/ADRA1A/CHRM2/ADRA1B/ADRB2/CHRNA2/SLC6A4/OPRM1/GABRA1/BCL2/BAX/CASP9/JUN/CASP3/CASP8/PRKCA/PON1/MAP2*
MOL005320	arachidonate	45.57	0.2	SC	*PTGS1/PTGS2/RXRG/NCOA2*
MOL005384	suchilactone	57.52	0.56	SC	*KCNH2/SCN5A/PTGS2/F7/ADRB2/NCOA1/PTGS1/RXRA/ADRA1D*
MOL007563	Yangambin	57.53	0.81	SC	*KCNH2/SCN5A/PTGS2/CACNA1S/NCOA2*

Table S2 (*continued*)

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Table S2 (*continued*)

Mol ID	Compound	OB (%)	DL	Herb	Gene Symbol
MOL008871	Marckine	37.05	0.69	SC	*PTGS2*
MOL000953	Cholesterol	37.87	0.68	Musk	*PGR/NR3C2/NCOA2*
MOL000737	morin	46.23	0.27	Musk	*PTGS1/AR/PPARG/PTGS2/TOP1/EDN3/ABCB1/ALOX5/CD36/DIO1/BATF3*
MOL010919	17-beta-estradiol	12.41	0.32	Musk	*CHRM3/CHRM1/CHRM5/CHRM4/RXRA/ACHE/ADRA1A/CHRM2/SLC6A3/ADRB2/ADRA1D/SLC6A4/OPRM1/AKT1/BCL2/BCL2L1/CDKN1A/MMP2/MAPK1/FGF13/TNFSF15/CASP3/CASP8/SOD1/CAT/PRKCA/TEP1/MMP1/MMP13/EDNRA/CYP3A4/CAV1/CASP7/CYP1A1/IL1B/NGF/SELE/VCAM1/PRKCD/FN1/LITAF/GABBR1/TYR/IFNG/ABCG2/GSTP1/SMAD2/HSF1/PCOLCE/PGR/CXCL12/CXCR4/PTGES/AKR1B1/KLK3/CDC37/APOA1/SMAD3/BGLAP/LEP/ITGAM/GPER1/TSHB/CSF3R/TGFBR2/ELN/FBN1/NOTCH1/JAG1/HSPA2/NCOA1/OCLN/DUSP1/ACP5/CASP6/PCDHGB3/KLK10*
MOL001232	TES	12.93	0.35	Musk	*RXRA/PGR/AKR1C3*
MOL000050	GLY	48.74	0	Musk	*PTGS1/PTGS2/F7/KYNU/AKR1B1/GABRA1/SLC6A9/CTSD*

TCMSP, Traditional Chinese Medicine Systems Pharmacology; OB, oral bioavailability; DL, drug-likeness; RSM, Radix Salviae Miltiorrhiza; RA, Radix Astragali; SC, Saline Cistanche.

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Table S3 A total of 280 CSM-related target genes

Gene Symbol			
TP53	Tumor Protein P53	IFNG	Interferon Gamma
FGFR3	Fibroblast Growth Factor Receptor 3	IDUA	Alpha-L-Iduronidase
COL2A1	Collagen Type II Alpha 1 Chain	ACAN	Aggrecan
MTHFR	Methylenetetrahydrofolate Reductase	CXCL8	C-X-C Motif Chemokine Ligand 8
PRKN	Parkin RBR E3 Ubiquitin Protein Ligase	DKK1	Dickkopf WNT Signaling Pathway Inhibitor 1
BAX	BCL2 Associated X, Apoptosis Regulator	MAPT	Microtubule Associated Protein Tau
HLA-DQB1	Major Histocompatibility Complex, Class II, DQ Beta 1	TRPV4	Transient Receptor Potential Cation Channel Subfamily V Member 4
IL6	Interleukin 6	TGFB3	Transforming Growth Factor Beta 3
HLA-DRB1	Major Histocompatibility Complex, Class II, DR Beta 1	HLA-A	Major Histocompatibility Complex, Class I, A
APOE	Apolipoprotein E	CRP	C-Reactive Protein
VEGFA	Vascular Endothelial Growth Factor A	MTOR	Mechanistic Target Of Rapamycin Kinase
MMP2	Matrix Metallopeptidase 2	CYCS	Cytochrome C, Somatic
TNF	Tumor Necrosis Factor	SLC26A2	Solute Carrier Family 26 Member 2
MMP3	Matrix Metallopeptidase 3	BMP4	Bone Morphogenetic Protein 4
IL1B	Interleukin 1 Beta	TIMP3	TIMP Metallopeptidase Inhibitor 3
IL10	Interleukin 10	IL2	Interleukin 2
COL1A1	Collagen Type I Alpha 1 Chain	IL1RN	Interleukin 1 Receptor Antagonist
COL11A2	Collagen Type XI Alpha 2 Chain	TIMP1	TIMP Metallopeptidase Inhibitor 1
COMP	Cartilage Oligomeric Matrix Protein	TIMP2	TIMP Metallopeptidase Inhibitor 2
MMP9	Matrix Metallopeptidase 9	EXT2	Exostosin Glycosyltransferase 2
GALNS	Galactosamine (N-Acetyl)-6-Sulfatase	H19	H19 Imprinted Maternally Expressed Transcript
HIF1A	Hypoxia Inducible Factor 1 Subunit Alpha	TNFRSF1B	TNF Receptor Superfamily Member 1B
ARSB	Arylsulfatase B	MPO	Myeloperoxidase
HLA-B	Major Histocompatibility Complex, Class I, B	TNFRSF1A	TNF Receptor Superfamily Member 1A
COL9A2	Collagen Type IX Alpha 2 Chain	IDS	Iduronate 2-Sulfatase
VDR	Vitamin D Receptor	FAS	Fas Cell Surface Death Receptor
COL9A3	Collagen Type IX Alpha 3 Chain	FXN	Frataxin
MYC	MYC Proto-Oncogene, BHLH Transcription Factor	MTR	5-Methyltetrahydrofolate-Homocysteine Methyltransferase
BMP2	Bone Morphogenetic Protein 2	BDNF	Brain Derived Neurotrophic Factor
ESR1	Estrogen Receptor 1	MBP	Myelin Basic Protein
CREBBP	CREB Binding Protein	IL17A	Interleukin 17A
AKT1	AKT Serine/Threonine Kinase 1	ALB	Albumin
MAPK1	Mitogen-Activated Protein Kinase 1	H2AC18	H2A Clustered Histone 18

Table S3 (*continued*)

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Table S3 (*continued*)

Gene Symbol			
APP	Amyloid Beta Precursor Protein	PRNP	Prion Protein
GUSB	Glucuronidase Beta	CD4	CD4 Molecule
CALCA	Calcitonin Related Polypeptide Alpha	RUNX2	RUNX Family Transcription Factor 2
COL9A1	Collagen Type IX Alpha 1 Chain	IL4	Interleukin 4
PSEN1	Presenilin 1	SOD1	Superoxide Dismutase 1
ENO2	Enolase 2	NFKB1	Nuclear Factor Kappa B Subunit 1
FOXP3	Forkhead Box P3	SPP1	Secreted Phosphoprotein 1
NOS2	Nitric Oxide Synthase 2	SERPINH1	Serpin Family H Member 1
DCN	Decorin	CCR6	C-C Motif Chemokine Receptor 6
DPYSL5	Dihydropyrimidinase Like 5	GFAP	Glial Fibrillary Acidic Protein
IL1A	Interleukin 1 Alpha	COL1A2	Collagen Type I Alpha 2 Chain
EXT1	Exostosin Glycosyltransferase 1	NGF	Nerve Growth Factor
S100B	S100 Calcium Binding Protein B	B2M	Beta-2-Microglobulin
RMRP	RNA Component Of Mitochondrial RNA Processing Endoribonuclease	JUN	Jun Proto-Oncogene, AP-1 Transcription Factor Subunit
CCL2	C-C Motif Chemokine Ligand 2	TTPA	Alpha Tocopherol Transfer Protein
FGF2	Fibroblast Growth Factor 2	EXOC1	Exocyst Complex Component 1
ABCD1	ATP Binding Cassette Subfamily D Member 1	STAT1	Signal Transducer And Activator Of Transcription 1
BTD	Biotinidase	HTT	Huntingtin
SNCA	Synuclein Alpha	CD8A	CD8a Molecule
SERPINA3	Serpin Family A Member 3	CDC42	Cell Division Cycle 42
CD40	CD40 Molecule	CD36	CD36 Molecule
AQP4	Aquaporin 4	HMOX1	Heme Oxygenase 1
IL2RA	Interleukin 2 Receptor Subunit Alpha	BCL2L1	BCL2 Like 1
SLC2A1	Solute Carrier Family 2 Member 1	TSC1	TSC Complex Subunit 1
IL18	Interleukin 18	CDKN1A	Cyclin Dependent Kinase Inhibitor 1A
MOG	Myelin Oligodendrocyte Glycoprotein	HMGCR	3-Hydroxy-3-Methylglutaryl-CoA Reductase
CCL5	C-C Motif Chemokine Ligand 5	CTSB	Cathepsin B
MAPK8	Mitogen-Activated Protein Kinase 8	LMNB1	Lamin B1
COL6A1	Collagen Type VI Alpha 1 Chain	CRYAB	Crystallin Alpha B
ENPP1	Ectonucleotide Pyrophosphatase/ Phosphodiesterase 1	SMARCA4	SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A, Member 4
PTH	Parathyroid Hormone	HTRA1	HtrA Serine Peptidase 1
BGLAP	Bone Gamma-Carboxyglutamate Protein	NTRK1	Neurotrophic Receptor Tyrosine Kinase 1

Table S3 (*continued*)

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Table S3 (*continued*)

Gene Symbol			
MATN3	Matrilin 3	IL15	Interleukin 15
HLA-G	Major Histocompatibility Complex, Class I, G	ANKH	ANKH Inorganic Pyrophosphate Transport Regulator
RPS27A	Ribosomal Protein S27a	IL5	Interleukin 5
BMP7	Bone Morphogenetic Protein 7	CHAT	Choline O-Acetyltransferase
FUCA1	Alpha-L-Fucosidase 1	CSF2	Colony Stimulating Factor 2
CD274	CD274 Molecule	GC	GC Vitamin D Binding Protein
KL	Klotho	ARSH	Arylsulfatase Family Member H
CDK5	Cyclin Dependent Kinase 5	SERPINC1	Serpin Family C Member 1
PCNA	Proliferating Cell Nuclear Antigen	F2	Coagulation Factor II, Thrombin
IFNA1	Interferon Alpha 1	RHOA	Ras Homolog Family Member A
CNTF	Ciliary Neurotrophic Factor	CD28	CD28 Molecule
RAD51	RAD51 Recombinase	F5	Coagulation Factor V
ICAM1	Intercellular Adhesion Molecule 1	HTR2A	5-Hydroxytryptamine Receptor 2A
LTA	Lymphotoxin Alpha	IL13	Interleukin 13
PLP1	Proteolipid Protein 1	CD40LG	CD40 Ligand
CCL3	C-C Motif Chemokine Ligand 3	GZMB	Granzyme B
XPO1	Exportin 1	GSN	Gelsolin
TTR	Transthyretin	ITGA4	Integrin Subunit Alpha 4
PTPRC	Protein Tyrosine Phosphatase Receptor Type C	AIFM1	Apoptosis Inducing Factor Mitochondria Associated 1
ACTA2	Actin Alpha 2, Smooth Muscle	SLC6A3	Solute Carrier Family 6 Member 3
GSK3B	Glycogen Synthase Kinase 3 Beta	TGIF1	TGFB Induced Factor Homeobox 1
CTLA4	Cytotoxic T-Lymphocyte Associated Protein 4	CNP	2',3'-Cyclic Nucleotide 3' Phosphodiesterase
NBAS	NBAS Subunit Of NRZ Tethering Complex	CX3CR1	C-X3-C Motif Chemokine Receptor 1
ARSA	Arylsulfatase A	WRN	WRN RecQ Like Helicase
HNRNPA1	Heterogeneous Nuclear Ribonucleoprotein A1	MICB	MHC Class I Polypeptide-Related Sequence B
TBP	TATA-Box Binding Protein	GJA1	Gap Junction Protein Alpha 1
ATP7A	ATPase Copper Transporting Alpha	CCL4	C-C Motif Chemokine Ligand 4
TARDBP	TAR DNA Binding Protein	ANGPT2	Angiopoietin 2
SELE	Selectin E	NEU1	Neuraminidase 1
CCR5	C-C Motif Chemokine Receptor 5	ALPP	Alkaline Phosphatase, Placental
ANXA5	Annexin A5	PRF1	Perforin 1
VCAM1	Vascular Cell Adhesion Molecule 1	TKT	Transketolase

Table S3 (*continued*)

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Table S3 (*continued*)

Gene Symbol			
TIA1	TIA1 Cytotoxic Granule Associated RNA Binding Protein	HSP90AA1	Heat Shock Protein 90 Alpha Family Class A Member 1
PDCD1	Programmed Cell Death 1	GLUD1	Glutamate Dehydrogenase 1
CXCR3	C-X-C Motif Chemokine Receptor 3	OPRM1	Opioid Receptor Mu 1
GDNF	Glial Cell Derived Neurotrophic Factor	HNRNPA2B1	Heterogeneous Nuclear Ribonucleoprotein A2/B1
COL10A1	Collagen Type X Alpha 1 Chain	CNR1	Cannabinoid Receptor 1
ADAMTSL1	ADAMTS Like 1	NEFH	Neurofilament Heavy Chain
TIMP4	TIMP Metallopeptidase Inhibitor 4	SLC1A3	Solute Carrier Family 1 Member 3
IHH	Indian Hedgehog Signaling Molecule	SLC6A4	Solute Carrier Family 6 Member 4
TF	Transferrin	MIR199B	MicroRNA 199b
EPRS1	Glutamyl-Prolyl-TRNA Synthetase 1	SOX2-OT	SOX2 Overlapping Transcript
FMOD	Fibromodulin	TBK1	TANK Binding Kinase 1
AFP	Alpha Fetoprotein	GARS1	Glycyl-TRNA Synthetase 1
NTF3	Neurotrophin 3	HAVCR2	Hepatitis A Virus Cellular Receptor 2
TSC2	TSC Complex Subunit 2	PTH1R	Parathyroid Hormone 1 Receptor
AGER	Advanced Glycosylation End-Product Specific Receptor	PIGA	Phosphatidylinositol Glycan Anchor Biosynthesis Class A
BMP1	Bone Morphogenetic Protein 1	ITGAL	Integrin Subunit Alpha L
SUMF1	Sulfatase Modifying Factor 1	CALR	Calreticulin
HMGB1	High Mobility Group Box 1	SLC1A2	Solute Carrier Family 1 Member 2
BMPR1A	Bone Morphogenetic Protein Receptor Type 1A	STAT5A	Signal Transducer And Activator Of Transcription 5A
IFNB1	Interferon Beta 1	CCL11	C-C Motif Chemokine Ligand 11
CREB1	CAMP Responsive Element Binding Protein 1	ITIH4	Inter-Alpha-Trypsin Inhibitor Heavy Chain 4
MAOB	Monoamine Oxidase B	SLC18A2	Solute Carrier Family 18 Member A2
TAC1	Tachykinin Precursor 1	PTHLH	Parathyroid Hormone Like Hormone
BACE1	Beta-Secretase 1	ITGB2	Integrin Subunit Beta 2
CYP2D6	Cytochrome P450 Family 2 Subfamily D Member 6	ZBTB16	Zinc Finger And BTB Domain Containing 16
HCRT	Hypocretin Neuropeptide Precursor	DNAH8	Dynein Axonemal Heavy Chain 8
DDX41	DEAD-Box Helicase 41	GRM1	Glutamate Metabotropic Receptor 1
CASP3	Caspase 3	APOH	Apolipoprotein H
CHST3	Carbohydrate Sulfotransferase 3	SLC6A2	Solute Carrier Family 6 Member 2
ANXA6	Annexin A6	CAPN2	Calpain 2
MDK	Midkine	DNTT	DNA Nucleotidylexotransferase
RHOD	Ras Homolog Family Member D	PRDX1	Peroxiredoxin 1

Table S3 (*continued*)

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Table S3 (*continued*)

Gene Symbol			
ST3	Suppression Of Tumorigenicity 3	PROM1	Prominin 1
TGFB1	Transforming Growth Factor Beta 1	CCR1	C-C Motif Chemokine Receptor 1
CHUK	Component Of Inhibitor Of Nuclear Factor Kappa B Kinase Complex	CREB3	CAMP Responsive Element Binding Protein 3
DRD2	Dopamine Receptor D2	ELAVL4	ELAV Like RNA Binding Protein 4
AVPR2	Arginine Vasopressin Receptor 2	REG1A	Regenerating Family Member 1 Alpha
XDH	Xanthine Dehydrogenase	FOS	Fos Proto-Oncogene, AP-1 Transcription Factor Subunit
CACNA1A	Calcium Voltage-Gated Channel Subunit Alpha1 A	PTPN13	Protein Tyrosine Phosphatase Non-Receptor Type 13
COL6A6	Collagen Type VI Alpha 6 Chain	HCCS	Holocytochrome C Synthase
IL17RC	Interleukin 17 Receptor C	SEMA6A	Semaphorin 6A
ATXN3	Ataxin 3	FASLG	Fas Ligand
TH	Tyrosine Hydroxylase	ACHE	Acetylcholinesterase

CSM, cervical spondylotic myelopathy.

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Table S4 Detailed information on 53 target genes in the PPI network

Gene symbol (Target)	Degree	Betweenness Centrality	Closeness Centrality	UniProt ID	Target name
IL6	33	0.070776979	0.684210526	P05231	Interleukin-6
AKT1	31	0.098266869	0.65	P31749	AKT serine/threonine kinase 1
JUN	28	0.047069746	0.634146341	P05412	Transcription factor AP-1
CXCL8	28	0.154890009	0.658227848	P10145	C-X-C motif chemokine ligand 8
VEGFA	28	0.054655425	0.641975309	P15692	Vascular endothelial growth factor A
IL1B	27	0.024322703	0.619047619	P01584	Interleukin-1 beta
MAPK8	26	0.047016417	0.619047619	P45983	Mitogen-activated protein kinase 8
CCL2	26	0.016985801	0.611764706	P13500	C-C motif chemokine 2
MAPK1	25	0.034682244	0.611764706	P28482	Mitogen-activated protein kinase 1
ICAM1	23	0.012918565	0.590909091	P05362	Intercellular adhesion molecule 1
IL10	22	0.008381957	0.577777778	P22301	Interleukin-10
MMP9	21	0.018285701	0.571428571	P14780	Matrix metalloproteinase-9
STAT1	20	0.017051734	0.547368421	P42224	Signal transducer and activator of transcription 1
IL4	19	0.042728768	0.553191489	P05112	Interleukin-4
IL2	19	0.005335698	0.559139785	P60568	Interleukin-2
FOS	18	0.016126953	0.559139785	P01100	Proto-oncogene c-Fos
MYC	18	0.016227643	0.559139785	P01106	Myc proto-oncogene protein
HMOX1	18	0.010557918	0.553191489	P09601	Heme oxygenase 1
CASP3	16	0.012374631	0.547368421	P42574	Caspase-3
MMP2	16	0.010989907	0.541666667	P08253	Matrix metalloproteinase-2
IFNG	15	0.004030982	0.52	P01579	Interferon gamma
VCAM1	15	0.003172345	0.536082474	P29533	Vascular cell adhesion molecule 1
IL1A	14	0.009791488	0.509803922	P01583	Interleukin-1 alpha
APP	14	0.097708698	0.559139785	P05067	Amyloid-beta precursor protein
ESR1	13	0.029961754	0.504854369	P03372	Estrogen receptor 1
CRP	13	0.001191280	0.5	P02741	C-reactive protein
SELE	13	0.001387722	0.509803922	P16581	E-selectin
SPP1	12	0.024751413	0.525252525	P10451	Secreted phosphoprotein 1
NGF	11	0.007358082	0.509803922	P01138	Nerve growth factor
CD40LG	11	0.001137256	0.485981308	P29965	CD40 ligand
RUNX2	10	0.016702605	0.481481481	Q13950	Runt-related transcription factor 2
BCL2L1	9	0.002900018	0.5	P07817	Bcl-2-like protein 1
HIF1A	9	0.001365322	0.47706422	Q16665	Hypoxia-inducible factor 1-alpha
MMP3	9	0.000301015	0.485981308	P08254	Matrix metalloproteinase-3

Table S4 (*continued*)

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Table S4 (*continued*)

Gene symbol (Target)	Degree	Betweenness Centrality	Closeness Centrality	UniProt ID	Target name
MPO	9	0.000075415	0.47706422	P05164	Myeloperoxidase
CDKN1A	8	0.011154689	0.456140351	P38936	Cyclin-dependent kinase inhibitor 1
NOS2	8	0.000637683	0.468468468	P35228	Nitric oxide synthase
DRD2	6	0.148567119	0.460176991	P14416	D(2) dopamine receptor
GSK3B	5	0.000772650	0.468468468	P49841	Glycogen synthase kinase-3 beta
COL1A1	5	0.003499825	0.433333333	P02452	Collagen alpha-1
SOD1	5	0.000349841	0.464285714	P00441	Superoxide dismutase 1
BAX	4	0.000031423	0.419354839	Q07812	Apoptosis regulator BAX
OPRM1	4	0.002713683	0.460176991	P35372	Mu-type opioid receptor 1
CHUK	3	0.000350509	0.433333333	015111	Conserved helix-loop-helix ubiquitous kinase
BGLAP	3	0.000150830	0.366197183	P02818	Bone Gla protein
GJA1	3	0.000034279	0.433333333	P17302	Gap junction alpha-1
PCNA	2	0	0.342105263	P12004	Proliferating cell nuclear antigen
SLC6A3	2	0.038461538	0.320987654	Q01959	Solute carrier family 6 member 3
CD36	1	0	0.35862069	Q13965	Thrombospondin receptor
SLC6A4	1	0	0.317073171	P31645	Solute carrier family 6 member 4
ACHE	1	0	0.361111111	P22303	Acetylcholinesterase
SLC6A2	1	0	0.317073171	P23975	Solute carrier family 6 member 2
MAOB	1	0	0.244131455	P27338	Monoamine oxidase type B

PPI, protein-protein interaction.

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