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Research article

Computational identification of Shenshao Ningxin Yin as an effective treatment for novel coronavirus infection (COVID-19) with myocarditis

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Abstract: *Background:* The newly identified betacoronavirus SARS-CoV-2 is the causative pathogen of the 2019 coronavirus disease (COVID-19), which has killed more than 4.5 million people. SARS-CoV-2 causes severe respiratory distress syndrome by targeting the lungs and also induces myocardial damage. Shenshao Ningxin Yin (SNY) has been used for more than 700 years to treat influenza. Previous randomized controlled trials (RCTs) have demonstrated that SNY can improve the clinical symptoms of viral myocarditis, reverse arrhythmia, and reduce the level of myocardial damage markers. *Methods:* This work uses a rational computational strategy to identify existing drug molecules that target host pathways for the treatment of COVID-19 with myocarditis. Disease and drug targets were input into the STRING database to construct protein–protein interaction networks. The Metascape database was used for GO and KEGG enrichment analysis. *Results:* SNY signaling modulated the pathways of coronavirus disease, including COVID-19, Ras signaling, viral myocarditis, and TNF signaling pathways. Tumor necrosis factor (TNF), cellular tumor antigen p53 (TP53), mitogen-activated protein kinase 1 (MAPK1), and the signal transducer and activator of transcription 3 (STAT3) were the pivotal targets of SNY. The components of SNY bound well with the pivotal targets, indicating there were potential biological activities. Conclusion: Our findings reveal the pharmacological role and molecular mechanism of SNY for the treatment of COVID-19 with myocarditis. We also, for the first time, demonstrate that SNY displays multi-component, multi-target, and multi-pathway characteristics with a complex mechanism of action.

Keywords: Shenshao Ningxin Yin (SNY); novel coronavirus infection (COVID-19); myocarditis; network pharmacology; molecular docking; SARS-CoV-2

1. Introduction

The 2019 novel coronavirus (COVID-19) pneumonia is an acute respiratory infectious disease caused by SARS-CoV-2, accompanied by fever, dry cough, and other clinical manifestations [1]. COVID-19 is closely related to the coronavirus that causes severe respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), as all are betacoronaviruses with the same ACE2 receptor [2]. SARS-CoV-2 causes severe respiratory distress by targeting the lungs and also induces myocardial damage [3,4]. Traditional Chinese Medicine (TCM) is uniquely advantageous for the treatment of viral diseases.

Shenshao Ningxin Yin (SNY) is composed of dwarf lilyturf tuber, Chinese angelica, ginseng, licorice root, white peony root, milkvetch root, Chinese Magnoliavine Fruit, Weeping Forsythia Capsulemagnoliavine fruit, weeping forsythia capsule, and honeysuckle flower (Table 1, Figure 1) and has been patented in China (Patent No. ZL 2020 1 0141213.1). This formula is derived from the Ren Shen Shao Yao decoction that stems from the spleen-stomach theory by Li Dongyuan. The original formula was used for more than 700 years to treat influenza. Dwarf lilyturf tuber has anti-ischemic and anti-inflammatory activities, and also improves microcirculation as a treatment of cardiovascular diseases [5]. The active components of milkvetch root can improve myocardial injury, activate macrophages, and regulate the human immune response by inducing the release of immune mediators [6–8]. SNY can improve the clinical symptoms of viral myocarditis, as well as reverse arrhythmia and reduce the levels of myocardial damage markers, such as CTNT, LDH, CK and CK-MB [9]. To investigate SNY as a potential treatment for COVID-19 with myocarditis, a mixture of valuable chemical probes would be required to understand the complex biological processes and identify potential therapeutic target genes or molecules. Analysis of its underlying mechanism can provide a reference basis for the clinical treatment of COVID-19 with myocarditis, and may even reduce mortality and improve patient prognosis. However, the specific molecular mechanisms have rarely been studied.

Table 1. Chinese herbal components contained in Shenshao Ningxin Yin (SNY).

No.	Chinese name	Chinese Pinyin name	Latin name	English name
1	麦冬	Mai dong	*Radix ophiopogonis*	Dwarf Lilyturf Tuber
2	当归	Dang gui	*Radix angelicae sinensis*	Chinese Angelica
3	人参	Ren shen	*Radix ginseng*	Ginseng
4	甘草	Gan cao	*Radix glycyrrhizae*	Liquorice Root
5	白芍	Bai shao	*Radix paeniae alba*	White Peony Root
6	黄芪	Huang qi	*Radix astragali*	Milkvetch Root
7	五味子	Wu weizi	*Fructus schisandrae chinensis*	Chinese Magnoliavine Fruit
8	连翘	Lian qiao	*Fructus forsythiae*	Weeping Forsythia Capsule
9	金银花	Jin yinhua	*Flos lonicerae*	Honeysuckle Flower

Computational methods for networks are not only utilized in structure-based drug design and pharmaceutical development, but also facilitate the characterization of molecular mechanisms resulting from complex ingredients, leading to the discovery of the therapeutic effect [10]. Molecule

docking uses chemometric methods to simulate the geometry and intermolecular forces of molecules and study the interactions among molecules. The aim is to find the best binding properties between small and large molecules (proteins) with known structures [11,12]. Experimentally screening all possible interactions among the ingredients of compounds and target proteins is impractical, so scientists use computational modelling and network approaches that are capable of accurately identifying potential drug-target interactions and serve as a basis for experimental studies [13,14]. This has been successfully used to illustrate the multitarget effects of TCM compounds for several diseases [15–17]. In this study, we simulated the mechanism of action of SNY on COVID-19 with myocarditis using a synthetic drug-ingredient-target-pathway network. Finally, molecular docking technology was used to verify the main compound-target pairs.

Figure 1. Images of the Chinese herbal components in Shenshao Ningxin Yin (SNY).

2. Materials and methods

2.1. Establishment of the chemical constituents and target sites database of SNY

Milkvetch root, Chinese magnoliavine fruit, weeping forsythia capsule, and honeysuckle flower were collected by searching the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://tcmspw.com/tcmspsearch.php) [18]. Oral bioavailability (OB) is the percentage of the pharmaceutical agent that is used and unchanged throughout systemic circulation [19,20]. Drug likeness (DL) is a concept of optimized pharmacokinetics and other properties, and is widely used during drug development to evaluate the potential of compounds [21]. To identify ingredients that may be absorbed orally and have curative effects, we selected the following threshold conditions for screening active SNY compounds: OB $\geq$ 30% and DL $\geq$ 0.18 [22]. By inputting the active compounds, the putative targets obtained from the DrugBank database were extracted from the TCMSP database.

The chemical constituents of dwarf lilyturf tuber were collected by searching the Integrative Pharmacology-based Research Platform of Traditional Chinese Medicine (TCMIP, http://www.tcmip.cn) [23]. All chemical constituents were screened by quantitative estimation of drug-likeness using a QED score, which was calculated according to the Pipeline Pilot ADMET collection model and included aqueous solubility, blood–brain barrier penetration, CYP450 2D6 inhibition, hepatotoxicity, human intestinal absorption, and plasma protein binding. The reported mean QED values for attractive and unattractive components in drug development were 0.67 and 0.49, respectively. The components of dwarf lilyturf tuber with moderate and good QED scores (QED $\geq$ 0.49) were retained. TCMIP uses a 2D structural similarity search of compounds to predict the target of the active ingredients using a Tanimoto coefficient similarity score with a drug marketed by the FDA [24]. The Tanimoto Score is in the range of 0–1, where 0 indicates completely different structures between ingredients and known drugs, and 1 indicates identical structures of two components. The target with a similarity score $\geq$ 0.8 is chosen as a predicted effective component of this Chinese medicine. Using the Uniprot database (https://www.uniprot.org/) [25], the targets collected from the TCMSP and TCMSP studies were identified as genes, which along with the database of chemical constituents, established the SNY targets.

2.2. Target prediction of COVID-19 with myocarditis

The GeneCards (https://www.genecards.org/) [26] and OMIM databases (https://www.omim.org/) [27] were searched using "novel coronavirus pneumonia", "COVID-19" and "myocarditis" search terms for targets associated with COVID-19 with myocarditis. Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/) was used to map the targets of the effective components of SNY with the target of COVID-19 with myocarditis to analyze the potential targets of SNY for treating this subgroup.

2.3. Network construction of the drug–compound target of SNY

An interaction network model was constructed using Cytoscape3.7.2 (https://cytoscape.org/) using the following nodes: Chinese herbs, compounds, and targets in SNY [28]. Nodes were connected to each other and each node was assigned important network topology parameters, including degree

of effect, which can be analyzed using NetworkAnalyzer in Cytoscape3.7.2. The larger the number of connected edges, the larger the corresponding degree, which indicates that the role of the target in the protein interaction network is more important.

2.4. Establishment of a protein–protein interaction (PPI) network

STRING 11.0 (https://STRING-db.org/) [29] contains known and predicted PPIs, where the credibility of the interactions is determined by the confidence level score (highest confidence, ≥ 0.9; high confidence or better, ≥ 0.7; medium confidence or better, ≥ 0.4; low confidence or better, ≥ 0.15). SNY targets were introduced into STRING 11.0 by defining the species as *Homo sapiens* with the confidence threshold set to 0.7; the free protein was removed, PPI was obtained, and the TSV format file was saved and imported into Cytoscape3.7.2. The output of the PPI network diagram of the potential targets of SNY was assessed in terms of degree value and core proteins were screened out.

2.5. Molecular docking of candidate components and targets

Molecular docking is a method used to predict the binding affinity and conformation of ligands by computing their physical and chemical parameters. Target 3D structure PDB format files were downloaded from RCSB PDB (https://www.rcsb.org/) [30], and the SDF format files of candidate compounds were obtained from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) [31].

Based on the analysis of the network topological properties of the SNY drug–compound target network, candidate compounds were selected. The core targets were obtained by analyzing the PPI network. ACE2 is a key target for COVID-19 with myocarditis [32,33], so candidate compounds were docked with the core targets and ACE2. Among the different structures of ACE2 in the RCSB PDB database, 1R4L was screened as it has an X-ray diffraction structure with a resolution of 3.00 Å without a protein break in the 3D conformation. It also has a potent inhibitor MLN-4760 (PubChem CID: 448281), which makes key binding interactions within the active site and offers insights on the action of specific residues involved in catalysis and substrate specificity [34]. The highly active ingredients reported previously for each target were retrieved from Pubchem and used as reference molecules for comparative study. PYMOL 2.3.0 software (https://pymol.org/) was used for dehydration/ligand/receptor analysis and Autodock 1.5.6 software (http://autodock.scripps.edu) was used to perform hydrogenation/charge calculation for proteins [35]. Parameters related to the receptor protein docking site were set to include the active pocket sites where small-molecule ligands may bind. The receptor protein and ligand small molecules were converted into pdbqt format and AutoDock Vina 1.1.2 was used to dock the receptor proteins with the small-molecule ligands.

2.6. Bioconcentration analysis of candidate targets

To illustrate how a target played a role in the pathway, the Metascape database (https://metascape.org/) for biological process (BP) enrichment analysis of gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotation enrichment analysis were used to analyze the biological processes in which core targets were involved [36]. Only items with a significance threshold of adjusted p-value < 0.05 were selected. Based on the literature, targets in 20 pathways highly associated with COVID-19 with myocarditis were sorted and imported as nodes in

Cytoscape3.7.2.

3. Results

3.1. Active constituents and targets of SNY

The chemical constituents and corresponding targets of Chinese angelica, ginseng, licorice root, white peony root, milkvetch root, Chinese magnoliavine fruit, weeping forsythia capsule, and honeysuckle flower were obtained from the TCPSP database, while the components of dwarf lilyturf tuber were retrieved from the TCMIP database. With values of OB > 30%, DL > 0.18, and QED ≥ 0.49, a total of 190 active ingredients were screened. Multiple types were included for dwarf lilyturf tuber (17), Chinese angelica (2), ginseng (17), licorice root (88), white peony root (8), milkvetch root (16), Chinese magnoliavine fruit (7), weeping forsythia capsule (18), and honeysuckle flower (17). After duplicates were removed, a total of 166 ingredients were stored for further study (Table S1). The 166 ingredients corresponded to 2133 active targets, broken down as follows: dwarf lilyturf tuber (196), Chinese angelica (40), ginseng (91), licorice root (1197), white peony root (71), milkvetch root (171), Chinese magnoliavine fruit (10), weeping forsythia capsule (183), and honeysuckle flower (174). After duplicates were removed, a total of 406 targets were stored for further study (Table S2).

3.2. Targets of COVID-19 with myocarditis

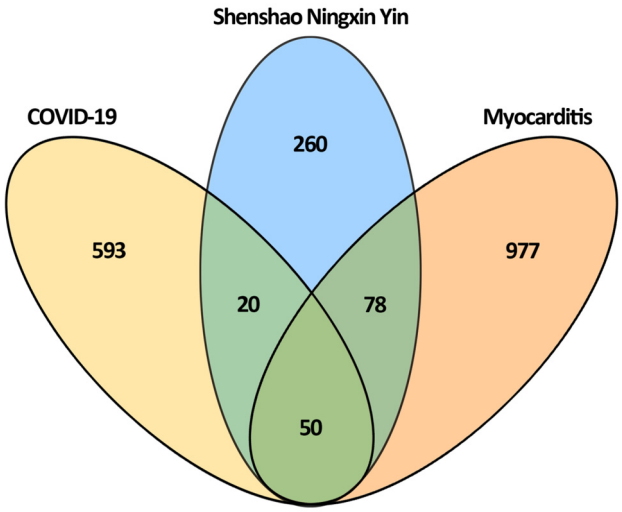

Figure 2. A total of 148 shared targets between Shenshao Ningxin Yin (SNY) and COVID-19 with myocarditis were identified.

The GeneCards and OMIM databases were used to retrieve COVID-19 and myocarditis disease targets, respectively. The results of the two databases were combined and de-weighted, resulting in

663 COVID-19-related genes and 1125 myocarditis-related genes. Using Venny 2.1.0, the targets of SNY were mapped to the target set of COVID-19 with myocarditis, which resulted in 148 potential targets of SNY (Figure 2, Table S3).

3.3. The drug-compound target network of SNY

Duplicates were deleted from the compound and target list and imported into Cytoscape3.7.2 to construct the drug-compound target network (Figure 3, Table S4). The network consisted of 581 nodes (166 compounds, 406 targets, 9 drugs) and 2999 edges. The V represents the Chinese herb, an ellipse represents the compound, and diamonds represent targets. The larger the node size, the larger the degree value of the node. High-degree nodes were located at key positions in the network. Cytoscape3.7.2 was used to analyze the topological properties of the network. The compounds in the network were arranged in descending order according to the degree value, and the compounds in the top 50 were selected as the candidate compounds of SNY (Table 2).

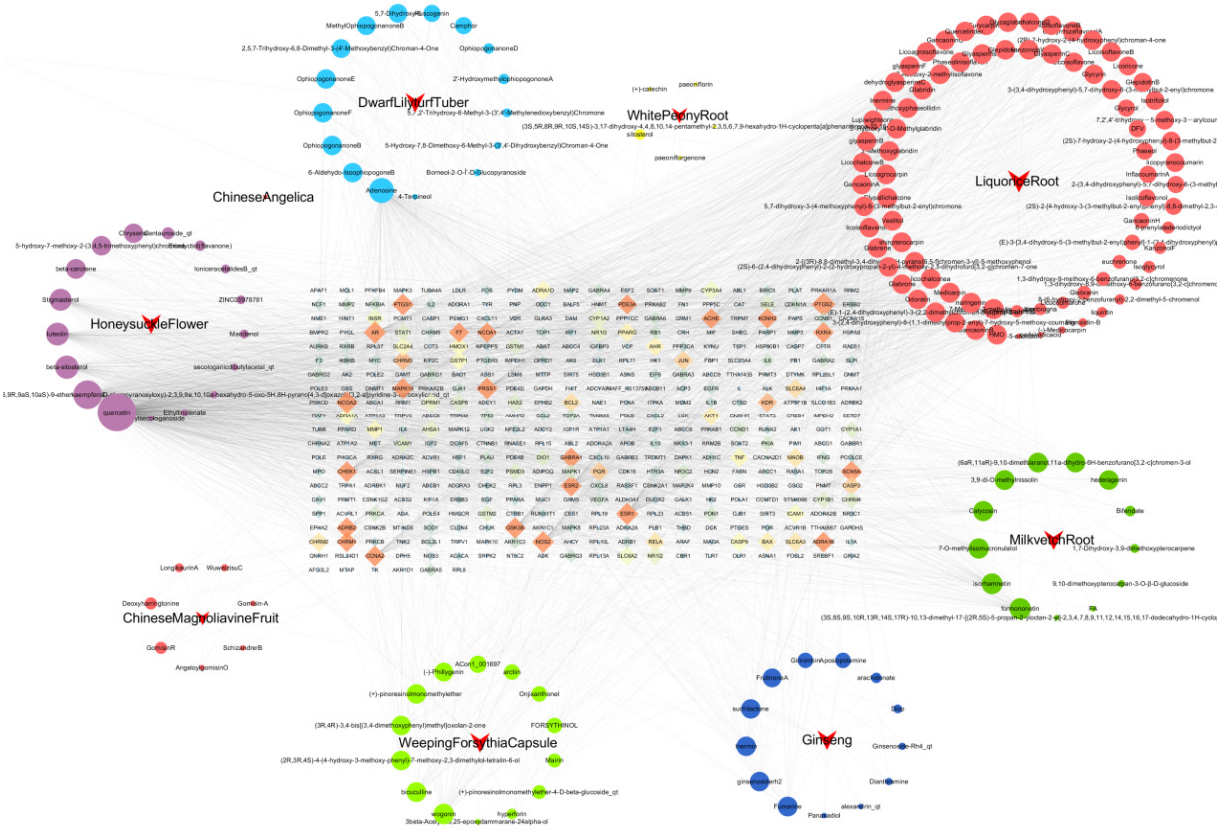

Figure 3. Drug-compound target network of Shenshao Ningxin Yin (SNY).

Table 2. Candidate compounds of Shenshao Ningxin Yin (SNY).

Sequence	Degree	Name	PubChem CID	Sequence	Degree	Name	PubChem CID
1	512	Quercetin	5280343	26	21	3'-Methoxyglabridin	5319439
2	251	Kaempferol	5280863	27	20	Kumatakenin	5318869
3	155	Adenosine	60961	28	20	Glabridin	124052
4	113	beta-Sitosterol	222284	29	20	3'-Hydroxy-4'-O-methylglabridin	15228662
5	100	Luteolin	5280445	30	20	1-Methoxyphaseollidin	480873
6	78	Stigmasterol	5280794	31	19	7-Acetoxy-2-methylisoflavone	268208
7	58	Formononetin	5280378	32	19	Astrapterocarpan	14077830
8	46	Isorhamnetin	5281654	33	18	Phaseolinisoflavan	4484952
9	38	Wogonin	5281703	34	17	Glyasperins M	None
10	33	7-O-Methylisomucronulatol	15689652	35	17	Corymbosin	10970376
11	32	Calycosin	5280448	36	16	Kanzonol W	15380912
12	32	7-Methoxy-2-methylisoflavone	354368	37	16	Hederagenin	73299
13	31	Naringenin	932	38	16	Glyasperin C	480859
14	25	Licochalcone a	5318998	39	16	Glepidotin A	5281619
15	25	Medicarpin	336327	40	15	Odoratin	13965473
16	24	Shinpterocarpin	10336244	41	15	Glabrone	5317652
17	24	4'-Methoxyglabridin	9927807	42	15	Gancaonin B	5317479
18	23	Vestitol	92503	43	15	Licoarylcoumarin	10090416
19	22	Licoagrocarpin	15840593	44	15	Kanzonol B	10881804
20	22	Glypallichalcone	5317768	45	14	Licoisoflavanone	392443
21	21	Bicuculline	10237	46	14	Glabrene	480774
22	21	beta-Carotene	5280489	47	14	5,7-Dihydroxy-3-[(4-methoxyphenyl)methyl]-8-methyl-4-oxochromene-6-carbaldehyde	5317212
23	21	Isoformononetin	3764	48	14	(2S)-6-(2,4-dihydroxyphenyl)-2-(2-hydroxypropan-2-yl)-4-methoxy-2,3-dihydrofuro[3,2-g]chromen-7-one	637112
24	21	Protopine	4970	49	14	Isolariciresinol	160521
25	21	3,9,10-Trimethoxypterocarpan	15689655	50	13	Glyasperin B	480784

3.4. Construction of the PPI network and screening of key targets

The common targets belonging to SNY and COVID-19 with myocarditis were introduced into the PPI network constructed using the STRING database, which resulted in 134 nodes and 2268 edges in the network (Figure 4A, Table S5). Each node in the network represents a different target. The number of interaction links between the targets is related to the role of the target in drug therapy. In the network, hub genes interact with each other through known (from curated databases and experimentally determined), predicted (gene neighborhood, fusions, and co-occurrence), and other (text-mining, coexpression, and protein homology) interactions. Four targets (TNF, TP53, AKT1 and STAT3) with the largest degree values (≥ 57) were selected as core targets (Figure 4B).

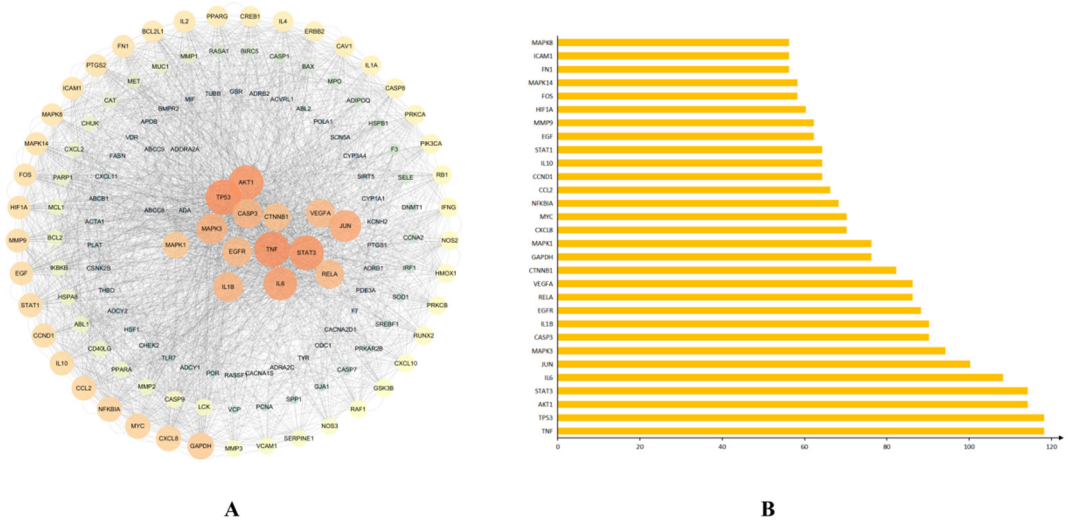

Figure 4. PPI network and core target degree ranking of Shenshao Ningxin Yin (SNY).

3.5. Verification of docking stimulation

Molecular docking experiments included 50 candidate compounds from SNY and core targets of the PPI network (STAT3 [PDB ID: 2AZ5], TP53 [PDB ID: 2H59], AKT1 [PDB ID: 1UNQ] and STAT3 [PDB ID: 5AX3]) and the key target of COVID-19 with myocarditis (ACE2 [PDB ID: 1R4L]) was selected. The candidate compounds from SNY were also compared to highly active ingredients reported previously, including TNF/SPD304 (PubChem CID: 5327044) [37], TP53/adenosine-5-diphosphoribose (PubChem CID: 447048) [38], AKT1/inositol 1,3,4,5-tetrakisphosphate (PubChem CID: 107758) [39], STAT3/5-iodotubercidin (PubChem CID: 97297) [40], and ACE2/MLN-476 [41]. The docking score indicates the binding ability of the compound to the target, and the smaller the number, the better the combination. Generally, a binding energy ≤ –5.0 kJ/mol is considered excellent [42–44]. Most of the 50 candidate compounds had lower binding energies and novel hydrogen bonding interactions with active residues of target receptors compared to the reference molecule (Figure 5, Table S6). The reference molecule scores included TNF/SPD304 (–9.1), TP53/adenosine-5-diphosphoribose (–9.4), AKT1/inositol 1,3,4,5-tetrakisphosphate (–4.7), STAT3/5-iodotubercidin (–7.0), and ACE2/MLN-476 (–9.0). Of the 50 candidate compounds, the

docking scores of TNF/glabrene (–9.3), TP53/kanzonol B (–10.5), AKT1/bicuculline (–5.8), STAT3/bicuculline (–9.4), and ACE2/glabrone (–10.4) were significantly better than the binding activity of the reference molecule.

The analysis of protein–ligand interactions can also help identify target sites. The stability of a protein–ligand complex is predominantly determined by the hydrogen bonds, which are the major stabilizers of the docked complexes. The binding mode between the small-molecule compound glabrene and the receptor protein TNF involves amino acid residues Tyr151 and Ser60, which form hydrogen bonds with the ligand. Residues Tyr59, Tyr151, Leu57, Leu120, Gly121, Tyr119, Ser60 and Gln61 form hydrophobic interactions with glabrene (Figure 6A). The binding mode between the small molecule kanzonol B and TP53 involves residues Gly188, Ser189, and Ala22, which form hydrogen bond interactions with kanzonol B. Amino acid residues Ser190, Phe33, Gly23, Arg34, Gly21, Tyr40, Lys11, Ala116, Phe48, Gln98, LLe159, LLe100 and Val160 form hydrophobic interactions with kanzonol B (Figure 6B). See Figure 6 for more details. These results suggest that the screened compounds may share a mechanism of action with the reference molecules.

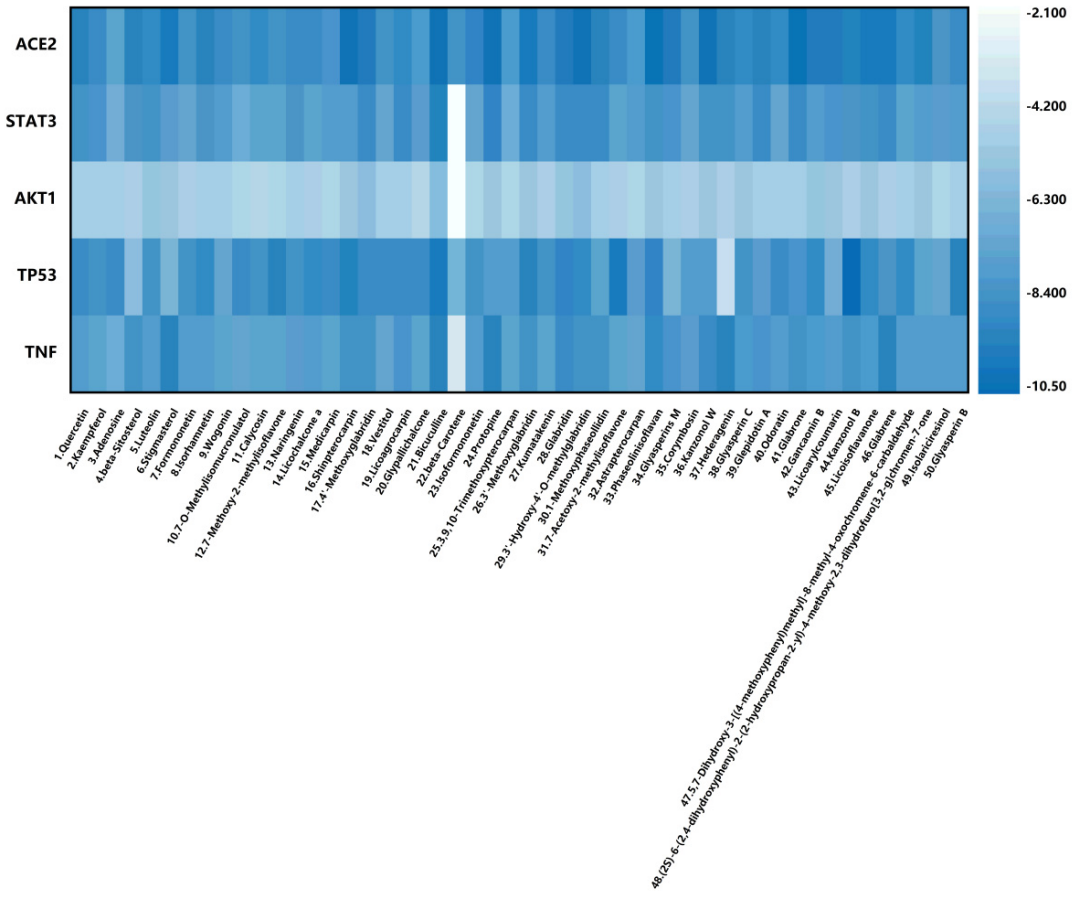

Figure 5. Heat map of docking scores for the core targets with candidate compounds from Shenshao Ningxin Yin (SNY).

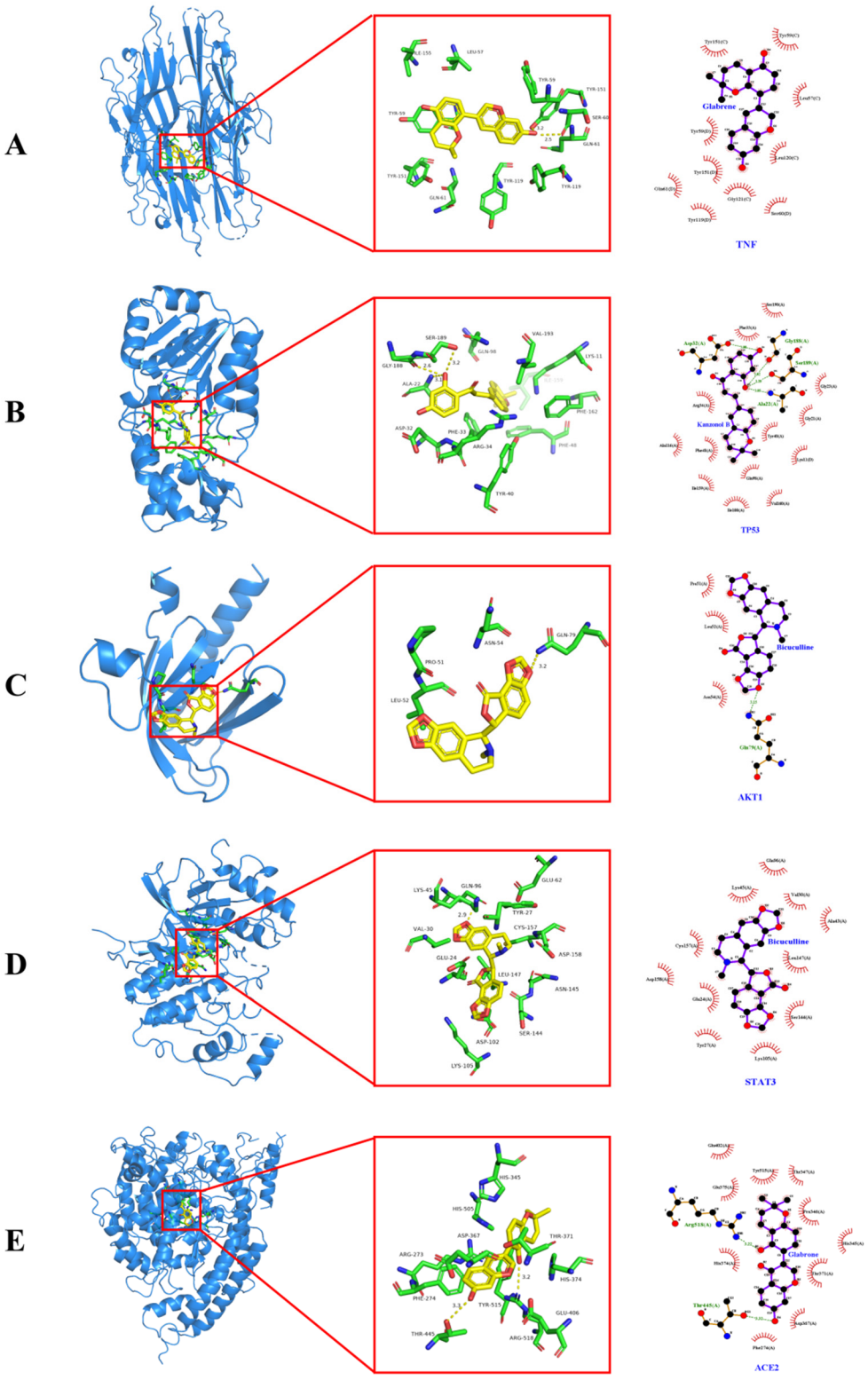

Figure 6. Molecular docking stimulation of the bioactive compound-core target. (A) TNF to glabrene; (B) TP53 to kanzonol B; (C) AKT1 to bicuculline; (D) STAT3 to bicuculline; (E) ACE2 to glabrone.

3.6. Enrichment findings for pivotal targets

To elucidate the mechanism of action of the effect of SNY on COVID-19 with myocarditis, enrichment analysis of GO biological processes was carried out using the Metascape database (Figure 7, Table S7). The targets of SNY were mainly involved in the inflammatory response from wounding (GO:0090594), heart morphogenesis (GO: 0003007), apoptotic heart process (GO: 0003015), response to tumor necrosis factor (GO: 0034612), and cytokine-mediated signaling pathway (GO: 0019221).

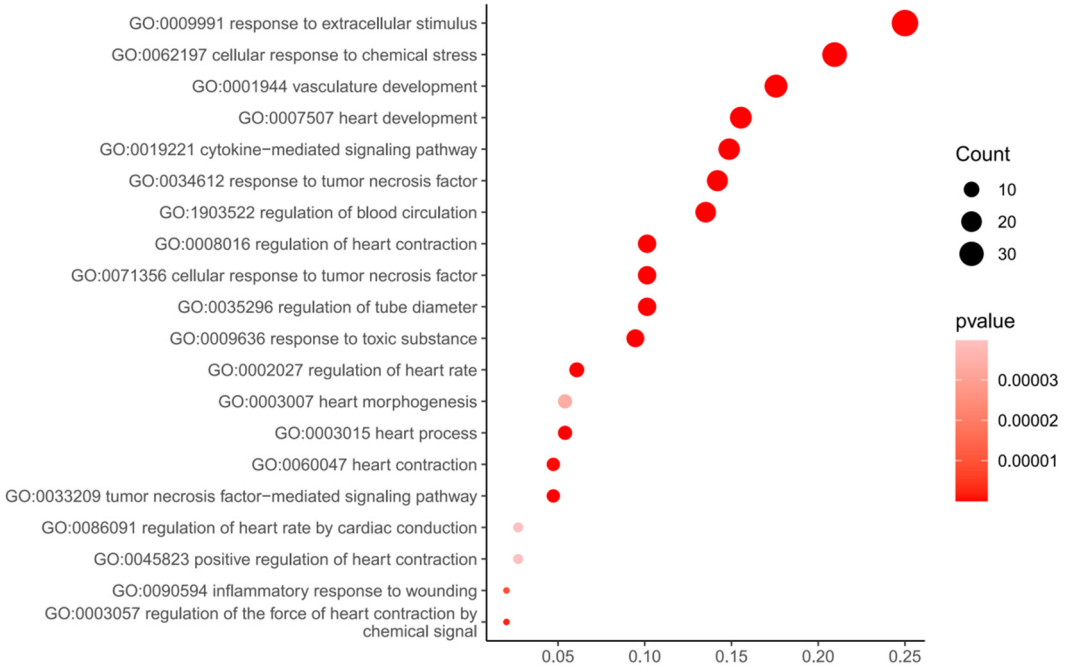

Figure 7. Gene ontology enrichment analysis.

According to the KEGG pathway enrichment analysis and the Metascape database, 20 signaling pathways significantly associated with COVID-19 with myocarditis infection were selected and visualized (Figure 8A, Table S8). The targets of SNY for COVID-19 infection were concentrated in the TNF signaling pathway (hsa04668), COVID-19 (hsa05171), Ras signaling pathway (hsa04014), renin secretion (hsa04924), and viral myocarditis (hsa05416). The results suggest that SNY plays an important role in the treatment of COVID-19 with myocarditis through multiple signaling pathways. The overall mechanism of action of SNY was further explored by analyzing the literature for signaling pathways reported to be associated with COVID-19 with myocarditis, which produced a potential target point map of key SNY regulation pathways (Figure 8B). Core signaling pathways were integrated and overlapped, and key targets were displayed more prominently in the signaling pathways. The drug, target, and related SNY signaling pathway were imported into Cytoscape3.7.2 to construct a network graph and visualize the relationships among components (Figure 9, Table S9). A total of 126 nodes and 505 edges were observed. The network diagram indicates that SNY is a multi-component, multi-target, and multi-pathway treatment.

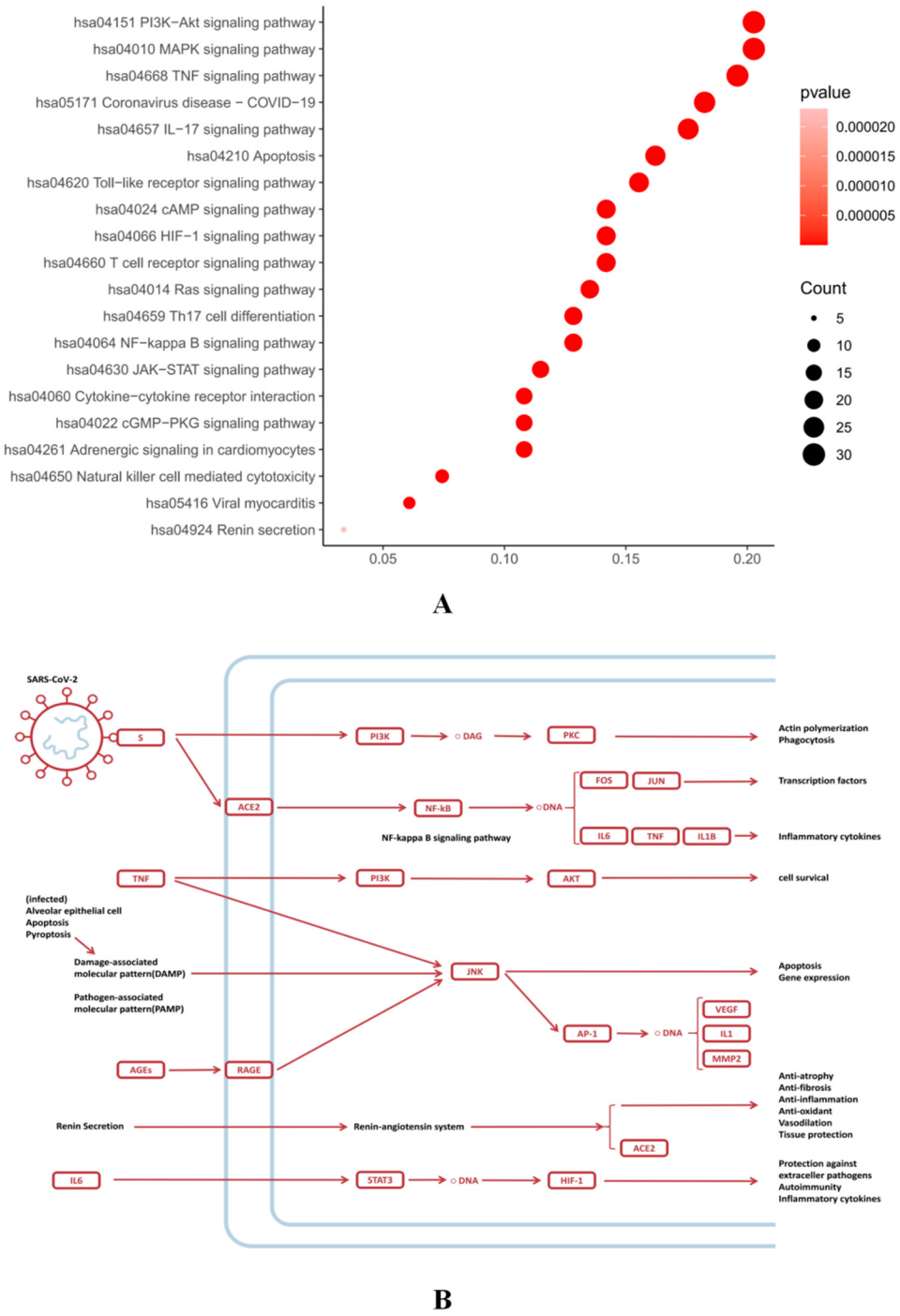

Figure 8. Enrichment findings of pivotal targets. (A) KEGG pathway enrichment analysis. (B) Key targets in the signaling pathways.

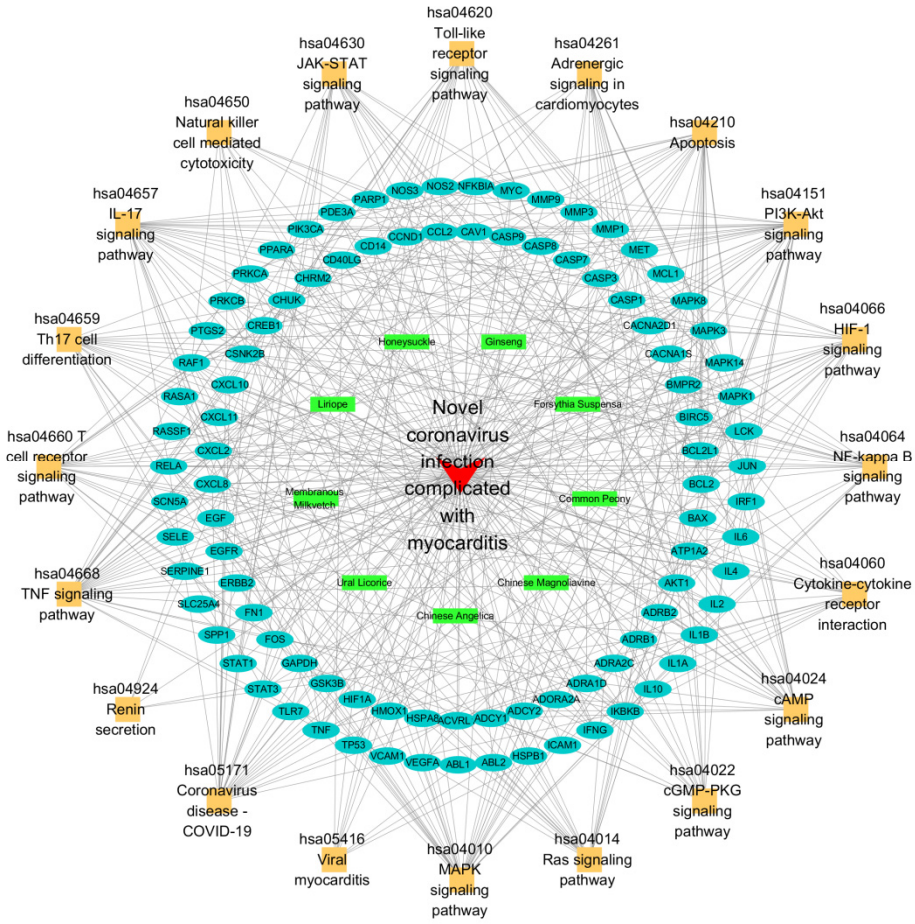

Figure 9. The drug–component–target–pathway network of Shenshao Ningxin Yin (SNY) for the treatment of COVID-19 with myocarditis.

4. Discussion

COVID-19 is a respiratory disease closely related to inflammation. The inflammatory storm caused by SARS-CoV-2 may spread to the cardiovascular system, causing cardiac immune activation and cardiac dysfunction. In addition, it can lead to target tissue fibrosis and microangiopathy, causing myocardial injury and subsequently leading to myocarditis. Damage to these extrapulmonary organs has become one of the factors associated with death. COVID-19 with myocarditis cannot be ignored, and therefore, an effective intervention for COVID-19 with myocarditis is an important way to reduce mortality and improve the prognosis of patients [45,46]. TCM has been used as an antiviral, to regulate immunity, and to promote body repair for thousands of years [47]. In TCM clinics, SNY is commonly prescribed for the treatment of viral myocarditis. SNY treatment of COVID-19 with myocarditis involves thousands of complex compounds that may act on various key targets and host cell cytokine signaling. A precise SNY therapy requires the identification of compounds that play an essential role in the treatment of COVID-19 with myocarditis. Due to the amount of time and effort needed to extract and purify natural products, this urgent need was addressed using computational methods for networks and molecular docking to facilitate drug design and screening for future experiments.

Network analysis demonstrated that of the 166 components of SNY, only quercetin, kaempferol,

luteolin, and formononetin had sizable interactions with the targets of COVID-19 infection with myocarditis. These components exhibit multiple biological activities, including anti-inflammatory, metabolism modulating, anti-oxidation, and anti-apoptosis. Quercetin and luteolin have cardioprotective effects against myocardial inflammation through an antioxidant anti-inflammatory mechanism [48,49]. Moreover, kaempferol can protect the heart from myocardial injury in isolated rat hearts through antioxidant activity and inhibition of glycogen synthase kinase-3beta [50]. In addition, formononetin has the potential to treat SARS-CoV-2 and improve myocardial injury. Indeed, a pathomorphological study of myocardial ischemia showed that medium- and high-dose treatment with formononetin significantly repaired myocardial injury [51]. Multiple components of SNY act together to treat COVID-19 with myocarditis. The target protein levels of TNF, IL6, NFKBIA, and STAT3 were excessively expressed in the PPI network. These results indicate a series of SNY targets for the treatment of COVID-19 with myocarditis.

The results of GO enrichment analysis indicated that targets were mainly involved in heart development, heart morphogenesis, response to tumor necrosis factor, and regulation of heart contraction force through chemical signaling. These results demonstrate that SNY can treat COVID-19 with myocarditis in multiple ways. IL6 is the central mediator of the cytokine storm, where it orchestrates the proinflammatory responses of immune cells, including the T lymphocytes [52]. This process results in T lymphocyte activation and the release of additional inflammatory cytokines, which in turn stimulates T lymphocytes, leading to a positive feedback loop of immune activation and myocardial damage [53]. Inhibition of IL6 can reduce the myocardial damage caused by COVID-19.

NFKBI is a family of transcription factors that regulates the expression of various pro-inflammatory cytokines, including IL6 and IL8, which are primarily responsible for the cytokine storm in COVID-19. Upon SARS-CoV-2 infection, the TNF signaling pathway is activated, which causes degradation of NFKBI inhibitory protein, resulting in the release and translocation of NFkB1 to the nucleus for transcription of various cytokine genes [54]. Candidate compounds bind well to TNF and block the TNF signaling pathways at critical steps, preventing the expression of various cytokine genes responsible for the COVID-19 cytokine influx. STAT3 participates in signal transmission for various cytokines and growth factor oncogenes. Its expression reduces inflammatory responses in mice with viral myocarditis [55]. Thus, the comprehensive regulation of TNF, IL6, NFKBIA, and STAT3 may explain the therapeutic effect of SNY on COVID-19 with myocarditis, although more detailed studies are needed.

Our pathway analyses indicated that cytokine–cytokine receptor interaction, Th17 cell differentiation, the IL-17 signaling pathway, and the TNF signaling pathway were mainly associated with COVID-19 with myocarditis. These results indicate that SNY may treat COVID-19 with myocarditis through multiple signaling pathways closely related to the disease's pathophysiological process. We infer that SNY may reduce the expression of inflammatory cytokines, inhibit the cascading reaction of an inflammatory outburst, and promote the absorption of inflammatory cells and tissue recovery. Levels of interferon, tumor necrosis factor, and interleukin tend to be elevated after SARS-CoV-2 infection, causing the release of large numbers of cytokine inflammatory mediators [56]. The cytokine storm and overactivation of immune cells in patients with COVID-19 results in severe acute myocarditis [57–59]. Inflammation can also cause endothelial cell dysfunction, impair the function of cardiac microcirculation, increase the procoagulant activity of blood, and accelerate heart injury in patients with COVID-19 infection [60,61].

In this study, the renin-angiotensin system (RAS) was found to be an important pathway. RAS

plays an important role in regulating blood pressure, electrolyte balance, and body fluid balance, and ACE2 is a key target in RAS [62]. Furthermore, we found that glabrone, an active ingredient in SNY, is highly efficient at binding to ACE2, with an absolute binding energy ≤ –5.0 kJ/mol. These findings suggest that the active ingredients in SNY may prevent viral entry into host cells and block binding to ACE2, thereby terminating viral RNA duplication. The RAS system has negative regulation points and ACE2 antagonizes ventricular hypertrophy, ventricular re-modeling, and oxidative stress. The S protein of SARS-CoV-2 has a high affinity for the ACE2 receptor, which is present on 7.5% of cardiomyocytes. The heart has its own susceptibility to SARS-CoV-2, which may be one of the mechanisms of direct myocardial injury. The upregulation of ACE2 expression is a compensatory response to myocardial injury and has a cardioprotective effect [63–65]. Therefore, SNY can function as a multidirectional intervention of COVID-19 infection with myocarditis. There may be other active components and targets in TCMs that have not been experimentally verified, suggesting that additional pharmacological mechanisms should be studied. Updating and optimizing artificial intelligence algorithms and fusing them with multi-modal data can provide new approaches for assessing the complex molecular mechanisms of action of TCMs with multiple components and targets.

5. Conclusions

We investigated the underlying mechanism of SNY for the treatment of COVID-19 with myocarditis by utilizing network computational methods. The results provide a reference point for the clinical treatment of COVID-19 with myocarditis and may reduce mortality and improve the prognosis of patients. SNY may treat this disease through mechanisms involving hub genes (TNF, TP53, AKT1, STAT3 and ACE2) and the regulation of the RAS, apoptosis, immune function, and inflammatory reactions. This research may facilitate the development of therapeutic methods that target COVID-19 with myocarditis. That said, extensive experimentation is necessary to unravel all of the possibilities, including *in vivo* and *in vitro* SNY efficacy experiments.

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Conflict of interest

The authors declare that there are no conflicts of interest related to this paper.

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