Reveal the Potential Molecular Mechanisms of Simiao Pills in the Treatment of Hyperuricemic Nephropathy Based on Network Pharmacology with Molecular Docking

Yin-Hong Geng
The First Affiliated Hospital of Zhengzhou University
Jia-Hui Yan
Huazhong University of Science and Technology
Liang Han
Huazhong University of Science and Technology
Zhe Chen
Huazhong University of Science and Technology
Sheng-Hao Tu
Huazhong University of Science and Technology
Lin-Qi Zhang
The First Affiliated Hospital of Henan University of traditional Chinese Medicine
Chun-Dong Song
The First Affiliated Hospital of Henan University of traditional Chinese Medicine
Feng-Yang Duan
The First Affiliated Hospital of Henan University of traditional Chinese Medicine
Ya-Fei Liu (yafeiliutjh@gmail.com)
The First Affiliated Hospital of Zhengzhou University

Research Article

Keywords: Simiao Pills, Hyperuricemic nephropathy, Compounds, Targets, Network pharmacology, Molecular docking

Posted Date: April 20th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1558866/v1

Abstract

Objective. A network pharmacology integrated molecular docking strategy was used to predict the underlying molecular mechanism of Simiao pills (SMP) in the treatment of hyperuricemic nephropathy (HN).

Methods. The active compounds and targets of SMP were screened by TCMSP database. Then, the disease databases such as CTD, DisGeNET, DrugBank, GeneCards, OMIM, TTD and PharmGKB were searched to determine the targets related to HN. STRING and Cytoscape software were applied to analysis and construct PPI network. R software was used to screen common targets, find the core targets of SMP in the treatment of HN, and perform Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis on the core targets, to clarify the core targets and mechanism of action of SMP in the treatment of HN. Finally, active compounds and core targets were selected for molecular docking verification by AutoDock Vina and Pymol software.

Results. In this study, 65 related active compounds of SMP, 203 drug-related targets and 91 common targets of SMP and HN were screened. The network suggests that baicalein, kaempferol, quercetin, wogonin, beta-sitosterol and rutaecarpine may be the effective compounds of SMP in the treatment of HN. GO and KEGG pathway enrichment analyses identified the mechanism. Molecular docking revealed that the active compounds in SMP showed strong affinity towards the core protein receptors.

Conclusions. SMP has a synergistic mechanism of multi-molecules, multi-targets and multi-pathways in the treatment of HN, which provides a theoretical basis for further exploring the molecular mechanism of SMP in the treatment of HN.

1. Introduction

Hyperuricemic nephropathy (HN) is characterized by the increase of uric acid (UA) relating to the purine metabolic disorder or the decrease of renal excretion, which makes UA and its crystals deposit in the kidney, and finally causes renal damage. It was previously thought that HN was rare in China. Due to improved living standards and altered dietary habits, such as increasing in intake of protein and purine-rich foods, the incidence of HN increased significantly in recent decades [1]. Kidney is the most important organ to excrete UA. Overproduction or underexcretion of UA lead to UA accumulate in the kidneys and cause kidney damage. The possible mechanisms of uric acid-induced kidney injury include the following aspects. For one thing, UA can be directly via obstruction of renal tubules by urate crystal deposition, causes tubular apoptosis, tubular necrosis, and interstitial inflammation. For another, UA is able to cause vascular endothelial injury, activate the renin-angiotensin-aldosterone system, and stimulate the inflammatory cascade, indirectly aggravating kidney injury. Both mechanisms are likely to reduce the glomerular filtration rate and eventually lead to renal fibrosis and renal failure, while kidney injury further induces elevated blood UA. The two mechanisms promote each other, forming a vicious circle. Therefore,

it is particularly important to improve renal UA excretion function and repair renal inflammatory damage in the treatment of HN.

At present, the commonly used clinical drugs for the treatment of HN are mainly through mechanisms such as inhibition of UA synthesis and enhancement of urate excretion, including allopurinol [2], benzbromarone and febuxostat. However, with the decline of renal function of patients, especially non-dialysis patients with chronic kidney disease (CKD) stage 3–4 caused by HN, the conventional medications are limited. Moreover, they have no beneficial effects on renal inflammation and injury, and could also bring about serious adverse reactions such as allergy and cardiovascular events [3]. Nowadays, there is no obvious breakthrough in the treatment of HN in western medicine. In recent years, studies have shown that Traditional Chinese medicine can significantly lower blood UA level and protect renal tubulointerstitial injury indexes of HN patients [4]. Simiao pill (SMP), derived from Chengfang Biandu, comprised of *Rhizoma Atractyodis* (Cang Zhu), *Cortex Phellodendri* (Huang Bo), *Radix Vladimiriae* (Niu Xi), and *Semen Coicis* (Yi Yi Ren), is able to clear away heat, dryness and dampness, strengthen tendons and bones, and treat dampness-heat syndrome. Basic and clinical studies have indicated that it was used for the treatment of hyperuricemia and gout. Studies have manifested that SMP could alleviate high fructose-induced glomerular renal injury in rats by up-regulating Sirt1 and inhibiting the activation of nuclear factor -κB (NF-κB) / NLRP3 inflammasome [5]. In addition, domestic scholars analyzed the therapeutic effect of SMP on HN from the perspective of "state target syndrome differentiation", which organically combined “macro syndrome differentiation” and “micro target practice” [6]. However, the mechanisms and potential targets of SMP in the treatment of HN remain unclear, which is a major limitation for its widespread clinical application. Based on network pharmacology, this study explored the main effective compounds, action targets and action pathways of SMP in the treatment of HN, aiming to provide a source of ideas for future research, and to provide a guide for clinical application. The detailed flowchart is shown in Fig. 1.

2. Methods

2.1. Search and screening of gene targets and components of SMP

The active ingredients and gene targets of SMP which including *Rhizoma Atractyodis*, *Cortex Phellodendri*, *Radix Vladimiriae* and *Semen Coicis* were searched from Traditional Chinese Medicine Systems Pharmacology (TCMSP) Database and analysis platform (http://tcmspw.com/ tcmsp.php). We screened out bioactive ingredients by evaluating oral bioavailability (OB) and drug-likeness (DL), with DL ≥ 0.18 and OB ≥ 30% as the thresholds. The human gene abbreviation of each target was determined by Perl software and UniProt database to obtain the possible target proteins of SMP.

2.2. Search and screening the related genes of HN.

Using “Gouty nephropathy” or “Uric acid nephropathy” or “Hyperuricemic nephropathy” as the search term, the Human Gene Database (GeneCards, https://www.genecards.org/), Online Mendelian Inheritance in Man (OMIM, https:// www.omim.org/), the Pharmacogenomics Knowledge Base database (PharmGKB, https://www.pharmgkb.org/), DrugBank database (https://www.drugbank.ca/), DisGeNET database (https://www.disgenet.org/), Terapeutic Target Database (TTD, http://db.idrblab.net/ttd/), and Comparative Toxicogenomics Database (CTD, http://ctdbase.org/) were used to query and screen gene targets. The pooled results from each database were combined and duplicates removed.

2.3. Determination of the drug-disease common targets.

Venn diagrams of the SMP-HN-related targets were generated using ‘Venn Diagram’ packages in R 4.1.1 software and the drug and disease gene targets acquired from the above two steps were intersected to obtain the common targets of SMP and HN.

2.4. Construction of the compound-target network

The candidate compounds and potential targets were used to construct the compound-target network by the Cytoscape 3.8.0 software.

2.5. Construction of protein–protein interaction (PPI) networks

The protein–protein interaction (PPI) network of HN-related targets of SMP was constructed by String (https://string-db.org/cgi/input.pl) and Cytoscape online tools. The network topology analyses, including degree centrality (DC), betweenness centrality (BC), closeness centrality (CC), eigenvector centrality (EC), local average connectivity-based method (LAC) and network centrality (NC) were performed by the CytoNCA. Then, the key compounds and main disease targets were screened according to their topological parameters.

2.6. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

Through R software and related packages, the enrichment analysis of GO and KEGG were carried out under the conditions of fisher test $p < 0.05$ and $q < 0.05$ (q is the corrected p value). The smaller p value is, the higher enrichment degree is. GO enrichment analyses were performed for each of the three GO categories: biological process (BP), cellular component (CC), and molecular function (MF) and screened out the first 10 functional categories to construct a histogram. The top 30 entries with the highest enrichment degree in KEGG are drawn into the histogram with R software and related packages.

2.7. Molecular Docking.

The 3D structure of the core target proteins was retrieved and downloaded from the (PDB) database (http://www.rcsb.org). The small molecule ligands and non-protein molecules such as water molecules in the target protein were removed by using PyMOL software and saved as PDB files. PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used to download PDB lattice documents of main active

partitioned 2D structure. For small molecules, the ChemBio3D 2014 software was used to minimize their energy. The 3D structure is 2D file structure generated by Open babel and saved as Mol2 files. Finally, they were imported into AutoDock Vina for molecular docking verification. Molecular docking aims to calculate binding affinities between target protein and a ligand. More negative binding energy is taken as evidence in which the receptor and ligand possesses a better affinity.

3. Results

3.1. Target Screening of SMP and HN.

With OB ≥ 30% and DL ≥ 0.18 as filtering conditions, 75 active compounds were screened out. After the removal of duplicates, 65 compounds were selected (Table 1).

Table 1
OB and DL of 65 active compounds in SMP

MOL ID	Compound	OB/%	DL	Source
MOL000085	beta-daucosterol_qt	36.91	0.75	CZ□NX
MOL000088	beta-sitosterol 3-O-glucoside_qt	36.91	0.75	CZ
MOL000092	daucosterin_qt	36.91	0.76	CZ
MOL000094	daucosterol_qt	36.91	0.76	CZ
MOL000098	quercetin	46.43	0.28	HB□NX
MOL000173	wogonin	30.68	0.23	CZ□NX
MOL000179	2-Hydroxyisoxypropyl-3-hydroxy-7-isopentene-2,3-dihydrobenzofuran-5-carboxylic	45.2	0.2	CZ
MOL000184	NSC63551	39.25	0.76	CZ
MOL000186	Stigmasterol 3-O-beta-D-glucopyranoside_qt	43.83	0.76	CZ
MOL000188	3β-acetoxyatractylone	40.57	0.22	CZ
MOL000358	beta-sitosterol	36.91	0.75	HB□NX
MOL000359	sitosterol	36.91	0.75	YYR
MOL000422	kaempferol	41.88	0.24	NX
MOL000449	Stigmasterol	43.83	0.76	HB□ NX□ YYR
MOL000622	Magnograndiolide	63.71	0.19	HB
MOL000762	Palmidin A	35.36	0.65	HB
MOL000785	palmatine	64.6	0.65	HB□NX
MOL000787	Fumarine	59.26	0.83	HB
MOL000790	Isocorypalmine	35.77	0.59	HB
MOL000953	CLR	37.87	0.68	YYR
MOL001006	poriferasta-7,22E-dien-3beta-ol	42.98	0.76	NX
MOL001131	phellamurin_qt	56.6	0.39	HB
MOL001323	Sitosterol alpha1	43.28	0.78	YYR
MOL001454	berberine	36.86	0.78	HB□NX
MOL001455	(S)-Canadine	53.83	0.77	HB

MOL ID	Compound	OB/%	DL	Source
MOL001458	coptisine	30.67	0.86	HB□NX
MOL001494	Mandenol	42	0.19	YYR
MOL001771	poriferast-5-en-3beta-ol	36.91	0.75	HB
MOL002372	(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	33.55	0.42	YYR
MOL002636	Kihadalactone A	34.21	0.82	HB
MOL002641	Phellavin_qt	35.86	0.44	HB
MOL002643	delta 7-stigmastenol	37.42	0.75	HB□NX
MOL002644	Phellopterin	40.19	0.28	HB
MOL002651	Dehydrotanshinone II A	43.76	0.4	HB
MOL002652	delta7-Dehydrosophoramine	54.45	0.25	HB
MOL002656	dihydroniloticin	36.43	0.81	HB
MOL002659	kihadanin A	31.6	0.7	HB
MOL002660	niloticin	41.41	0.82	HB
MOL002662	rutaecarpine	40.3	0.6	HB
MOL002663	Skimmianin	40.14	0.2	HB
MOL002666	Chelerythrine	34.18	0.78	HB
MOL002668	Worenine	45.83	0.87	HB
MOL002670	Cavidine	35.64	0.81	HB
MOL002671	Candletoxin A	31.81	0.69	HB
MOL002672	Hericenone H	39	0.63	HB
MOL002673	Hispidone	36.18	0.83	HB
MOL002714	baicalein	33.52	0.21	NX
MOL002776	Baicalin	40.12	0.75	NX
MOL002882	[(2R)-2,3-dihydroxypropyl] (Z)-octadec-9-enoate	34.13	0.3	YYR
MOL002894	berberrubine	35.74	0.73	HB
MOL002897	epiberberine	43.09	0.78	NX
MOL003847	Inophyllum E	38.81	0.85	NX

MOL ID	Compound	OB/%	DL	Source
MOL004355	Spinasterol	42.98	0.76	NX
MOL005438	campesterol	37.58	0.71	HB
MOL006392	dihydroniloticin	36.43	0.82	HB
MOL006401	melianone	40.53	0.78	HB
MOL006413	phellochin	35.41	0.82	HB
MOL006422	thalifendine	44.41	0.73	HB
MOL008118	Coixenolide	32.4	0.43	YYR
MOL008121	2-Monoolein	34.23	0.29	YYR
MOL012461	28-norolean-17-en-3-ol	35.93	0.78	NX
MOL012505	bidentatoside,ii_qt	31.76	0.59	NX
MOL012537	Spinoside A	41.75	0.4	NX
MOL012542	β-ecdysterone	44.23	0.82	NX
MOL013352	Obacunone	43.29	0.77	HB

A total of 2901 target genes of SMP were attained from TCMSP database. Duplicate targets were deleted, revealing 203 targets. Samely, 1098 HN-related target genes were collected from seven existing resources, namely GeneCards, OMIM, PharmGKB, DrugBank, TTD, DisGeNET, and CTD (Fig. 2a).

3.2. Targets and Compounds of SMP in the Treatment of HN.

By taking the intersection of drug targets and disease targets, we got 91 intersection targets (Fig. 2b).

3.3. Construction of Network.

Cytoscape software was used to visualize the network of SMP in the treatment of HN (Fig. 3). The network is made up of 124 nodes and 273 edges.

3.4. Core Targets in PPI network

The 91 potential targets were imported into the String database to build the PPI network. It revealed that 1475 edges and 91 nodes in the PPI network (Fig. 4a). The degree of each node is calculated with CytoNCA. The hub proteins in the PPI network which included tumor protein p53 (TP53), fructooligosaccharide (FOS), matrix metallopeptidase 9 (MMP9), hypoxia-inducible factor 1-alpha (HIF1α), caspase-3 (CASP3), vascular endothelial growth factor A (VEGFA), C-C motif chemokine 2

(CCL2), JUN, epidermal growth factor (EGF), matrix metallopeptidase 2 (MMP2), fibronectin 1 (FN1), prostaglandin-endoperoxide synthase 2 (PTGS2), interleukin-8 (CXCL8), and interleukin-1 beta (IL-1β) were screened out (Fig. 4d) after two topological analysis (Fig. 4b 4c). It contained 14 nodes and 91 lines.

3.5. Functional enrichment analysis of GO and KEGG.

We conducted GO analyses including BP, CC and MF and KEGG pathway enrichment. The results showed that 1879 BP, 24 CC and 124 MF were enriched by GO analysis, and 148 KEGG pathways were enriched by pathway enrichment. The top 10 BP, CC, and MF results were selected to draw the GO function histogram (Fig. 5a). The top 30 pathways with the highest degree of KEGG enrichment were selected to establish the histogram (Fig. 5b).

3.6. Molecular Docking.

Docking was implemented with AutoDock Vina. As shown in Fig. 6, almost all active ingredients had better binding activity with core targets. Partial Outcomes of molecular docking analysis are presented in Fig. 7.

4. Discussion

HN could eventually lead to renal failure (RF), seriously threatening the life and health of patients [7–8]. Currently, the drugs of clinical treatment in HN are mainly divided into inhibition of UA synthesis, increase of UA excretion, promote UA decomposition. As mentioned above, these drugs are limited when the glomerular filtration rate decreases and probably cause a range of adverse reactions. With the development of TCM, a new therapeutic direction has been provided. Studies have shown that SMP can also enhance renal UA excretion ability, reduce UA level and improve renal insufficiency of hyperuricemia mice by regulating renal organic anion transporter in hyperuricemia mice [9]. Due to fewer adverse reactions and lower cost, SMP has attracted increasing interest in recent years. This research is to unravel the mechanism and molecular docking of SMP in the treatment of HN.

According to Fig. 4 and Table 1, it could be concluded that quercetin, baicalein, kaempferol and others were the main active components of SMP. Quercetin, a typical flavonoid, was the main component of *Cortex Phellodendri* and *Radix Vladimiriae*. Studies have suggested that quercetin can decrease UA and protect kidney by regulating the expression levels of renal tissue transporter and urinary regulatory protein [10]. Quercetin has also been reported to relieve HN by regulating NLRP3 inflammasome and TLRs signaling pathway [11]. Baicalein and kaempferol were the active compound of *Radix Vladimiriae*. Baicalein inhibits inflammation by down-regulating NF-κB, mitogen-activated protein kinase (MAPK), PI3K-Akt and other pathways [12]. Kaempferol belongs to the flavonol group. It has been reported that kaempferol significantly increase antioxidant resistance and suppress xanthine oxidase (XOD) in hyperuricemia rats [13]. In addition, quercetin [14], stigmasterol [15], baicalein [16] can reduce UA level by inhibiting the activity of XOD in hyperuricemia mice. SMP [17] can down-regulate URAT1 and up-regulate OAT1 renal UA transporter, inhibit XOD activity, and enhance antioxidant enzyme activity, so as to reduce

UA level and protect kidney. It could be concluded that SMP and its various active ingredients can effectively reduce UA level and improve body injury by inhibiting XOD activity, regulating UA transporter, and anti-inflammation and anti-oxidation through multiple channels and multiple targets.

Related core target proteins of SMP active ingredients were obtained in the PPI network, such as VEGFA, PTGS2, IL-1β, CCL2, TP53, FN1, HIF1α, FOS, JUN, MMP9, CASP3, EGF, MMP2 and CXCL8. VEGFA plays crucial role in angiogenesis and maintenance of vascular stability. PTGS2 catalyzes the conversion of arachidonic acid to prostaglandins, which plays a major role in the biological environment, including homeostasis, gastric mucosal integrity, renal function, and inflammatory response [18]. IL-1β is a pro-inflammatory gene and its expression increases gradually with the occurrence of inflammation. CCL2 can not only induce the aggregation of inflammatory cells such as neutrophils, monocytes and lymphocytes to the lesion site, but also induce the synthesis of other cytokines such as IL-2, IL-6 or cell adhesion molecules [19]. TP53 is a well-known tumor suppressor gene. It can also indirectly regulate cell cycle regulation and DNA damage repair [20]. Inhibition of p53 significantly promotes IL-1β induced chondrocyte senescence [21]. The potential role of TP53 in inflammatory regulation is related to inhibit the proinflammatory cytokine IL-6 [22]. It has been shown that overexpression of FN1 can result in activation of TGF-Akt/PI3K/Akt pathway promoting the survival of cells [23]. HIF1α is considered as the master transcriptional regulator of cellular and developmental response to hypoxia, specifically in vascularization and angiogenesis, energy metabolism, cell survival, and tumor invasion. C-Jun N-terminal kinase (JNK) is an important member of the MAPK family. Former studies pointed out that the JNK signaling pathway could possess a significant role in almost all physiological and pathological processes such as differentiation, proliferation and apoptosis of all cellular types [24]. Studies have shown that in adenine nephropathy rats, the activation of JNK signaling pathway has an important role in in renal tubular epithelial cell apoptosis and renal injury [25]. C-FOS is a highly conserved gene widely expressed at low levels in most cells, and is an important member of the immediate early gene family. C-FOS gene is highly expressed in renal tissue, and affects the occurrence and development of nephropathy by participating in the regulation of cell proliferation, differentiation, apoptosis, production of inflammatory factors and tissue fibrosis. Experimental studies [9] demonstrated that c-FOS expression was increased in renal tissues of rats with chronic glomerulonephritis, and the mechanism of c-FOS expression mediated by syK/Ras/C-FOS signaling pathway might be completed. The SYK/Ras/C-FOS pathway is activated by extracellular signaling molecules. Activate the downstream factor and eventually enhance c-fos expression. Activated FOS protein binds to c-Jun family proteins to form transcription factor AP-1, which increases the expression of tumor necrosis factor (TNF)-α, IL-8, IL-6 and other inflammatory factors, and participates in the inflammatory response in the course of chronic glomerulonephritis. MMP2 is a member of the MMP family. MMP2 is involved in the decomposition of glomerular ECM in the pathological process of various glomerular diseases [26, 27]. Epidermal growth factor receptor (EGFR) is widely expressed in the kidney of mammals with different cell types and regulates cell proliferation, differentiation and apoptosis by activating MAP kinase, JAK/STAT, SRC kinase and PI3K intracellular pathways [28–30]. CXCL8 is known chemokines for neutrophils [31]. CXCL8 reduces pathological kidney damage and inflammatory cell infiltration in the kidney by inhibiting the

inflammatory response induced by neutrophils [32]. Caspase (CASP) 3 is a typical apoptotic effect CASP, which plays an extremely critical role in body development, apoptosis and inflammation [33]. Overall, it is speculated that SMP acts as treating HN through anti-inflammation, apoptosis, cell cycle regulation and anti-oxidative stress, etc.

According to the results of enrichment analysis, many pathways containing NF-κB, IL-17, TNF, p53 and HIF-1 were found relating to the mechanism of SMP treating HN. IL-17 is a characteristic cytokine secreted by TH17 cells and participates in the inflammatory response in vivo. Studies have found that [34] regulatory T cells and TH17 cells play a vital role in the disease development of acute gout arthritis rats. However, IL-17 neutralizing antibody can reduce renal inflammatory infiltration in HN [35] and improve the symptoms of HN. It has been shown that increased UA level can induce apoptosis of renal tubular cells by disrupting the balance between anti-apoptotic and pro-apoptotic proteins [36]. In addition, renal tubular epithelial cell apoptosis has also been observed in renal biopsies of patients with familial gout nephropathy [37]. TNF signaling pathway is strongly correlated with the secretion of inflammatory factors in the body, and induced the activation of NF-κB, P38 α and their downstream effector kinase MK2 by binding to TNFR1 receptor, thereby promoting the transcription of target genes, mRNA stability and translation [38]. It concluded that the bioactive components of SMP act on multiple targets to treat HN through multiple pathways.

There are still many deficiencies, such as the most active ingredient-target proteins were not confirmed by clinical experiment, the limitations of various platform databases and related software algorithms.

5. Conclusion

Network pharmacology and molecular docking were carried out to investigate the possible mechanisms of SMP in the treatment of HN. It could be concluded that SMP plays the role of anti-inflammatory, apoptosis, cell cycle regulation and antioxidant stress through multiple metabolic pathways and multiple targets in the treatment of HN. In future, the mechanisms of SMP for HN revealed by this study are needed to be further investigated in vitro experiments. However, this study still provides some underlying recommendations for the follow-up researches.

Declarations

Declaration of competing interest

All authors state that there is no conflict of interest.

Funding

YFL received a National Natural Science Foundation of China (81701601). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability statement

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

Ethical approval

All analyses were based on database thus no ethical approval and patient consent are required.

Author contributions

Data Curation: YJH and HL. Formal analysis: GYH. Investigation: CZ. Supervision: TSH, DFY. Writing—original draft: GYH. Writing—review and editing: ZLQ, SCD, and Y-FL

References

1. He L, Zou S, Nie Y. Effect of reducing uric acid prescription on renal tubulointerstitial lesions in uric acid nephropathy animals [J] Chinese Journal of International Medical Journal, 2003, (3):213–216
2. Wu W, Zhang R, Ma F. Effect of allopurinol on serum creatinine and urea nitrogen levels in patients with gout nephropathy [J]. Chinese journal of biochemical medicine, 2017, 37 (9): 170–172.
3. Yang X, Xue Y, Zou H. Clinical application and Adverse reactions of Febuxostat [J]. Journal of adverse drug reactions, 2014, 16 (3): 168–170.
4. Zhang J, Zhao J, Liu L. Effect of Spleen-tonifying kidney-relieving decoction combined with Western Medicine on immune function and microinflammation in patients with chronic renal failure [J]. Journal of Modern Integrated Chinese and Western Medicine, 2017, 26(35):3906–3908.
5. Ma C, Kang L, Ren H, Zhang D, Kong L. Simiao pill ameliorates renal glomerular injury via increasing Sirt1 expression and suppressing NF-κB/NLRP3 inflammasome activation in high fructose-fed rats. Journal of Ethnopharmacology. 2015, 172:108–117.
6. Deng C, Liu Q, Ouyang M, Hu M, Zhou K. Analysis of the effect of Simiao powder on hyperuricemia nephropathy based on "state target syndrome differentiation". China Medical Herald. 2020, 17(34):138–141.
7. Xing R, Meng D, Ren W. Exploration on the establishment of animal models for gouty nephropathy complicated with chronic renal failure [J]. Chin J IntegrTrad West Med, 2011,31(10):1409–1413
8. Pschowski R, Briegel S, Von Haehling S, Doehner W, Bender TO, Pape UF, *et al*. Effects of dialysis modality on blood loss, bleeding complications and transfusion requirements in critically ill patients with dialysis-dependent acute renal failure[J]. AnaesthIntensive Care, 2015, 43(6):764–770.
9. Hu QH, Jiao RQ, Wang X, Lv YZ, Kong LD. Simiao pill ameliorates urate underexcretion and renal dysfunction in hyperuricemic mice [J]. J Ethnopharmacol, 2010, 128(3):685–692.
10. Hu QH, Zhang X, Wang X, Jiao RQ, Kong LD. Quercetin regulates organic ion transporter and uromodulin expression and improves renal function in hyperuricemic mice[J]. Eur J Nutr, 2012,51(5):

593–606.

11. Hu Q, Miao M, Lu G, Ji H. Effects of quercetin on the expression of NLRP3 and TLRs in uric acid nephropathy rats [J]. Chinese herbal medicine, 2013, 44 (24):3496–3502.
12. Feng T, Zhou L, Gai S, Zhai Y, Gou N, Wang X, *et al*. Acacia catechu (L.f.)Willd and Scutellaria baicalensis Georgi extracts suppress LPS-inducedpro-inflammatory responses through NF-κB, MAPK, and PI3K-Akt signaling pathways in alveolar epithelial type II cells[J]. Phy-tother Res,2019,33(12):3251–3260.
13. F. Haidari, "Effects of parsley (Petroselinum crispum) and its flavonol constituents, kaempferol and quercetin, on serum uric acid levels, biomarkers of oxidative stress and liver xanthine oxidoreductase aactivity in Oxonate-induced hyperuricemic rats," Iranian Journal of Pharmaceutical Research: IJPR, 2011, 10(4):811 – 819
14. Zhang C, Wang R, Zhang G, Gong D. Mechanistic insights into the inhibition of quercetin on xanthine oxidase. Int J Biol Macromol. 2018; 112:405–412.
15. Ferraz-Filha ZS, Michel Araújo MC, Ferrari FC, Dutra IP, Saúde-Guimarães DA. Tabebuia roseoalba: In Vivo Hypouricemic and Anti-inflammatory Effects of Its Ethanolic Extract and Constituents. Planta Med. 2016;82(16):1395–1402.
16. Shieh DE, Liu LT, Lin CC. Antioxidant and free radical scavenging effects of baicalein, baicalin and wogonin[J]. Anticancer Res, 2000, 20(5a):2861–5.
17. Hua J, Huang P, Zhu CM, Yuan X, Yu CH. Anti-hyperuricemic and nephroprotective effects of Modified Simiao Decoction in hyperuricemic mice. J Ethnopharmacol. 2012;142(1):248–252.
18. HAWKEY C J. COX-2 inhibitors [J]. Lancet,1999,353(9149):307–314.
19. Watson AES, Goodkey K, Footz T, Voronova A. Regulation of CNS precursor function by neuronal chemokines [J]. Neurosci Lett, 2020, 715:134533.
20. E. M. Pinto and G. P. Zambetti. "What 20 years of research has taught us about the TP53 p.R337H mutation," Cancer, 2020, 126(21):4678–4686.
21. Lu H, Wang D, Li H, Zhong J, Lin Y, Xu X, *et al*, "GPR39 agonist TC-G 1008 ameliorates IL-1β-induced chondrocyte senescence," Artificial Cells, Nanomedicine, and Biotechnology, 2019, 47(1):2612–2617
22. Zhang T. "p53 predominantly regulates IL-6 production and suppresses synovial inflammation in fibroblast-like synoviocytes and adjuvant-induced arthritis," Arthritis Research & Terapy, 2016, 18(1):271
23. Zhang H, Chen X, Xue P, Ma X, and Li J, "FN1 promotes chondrocyte differentiation and collagen production via TGFβ/PI3K/Akt pathway in mice with femoral fracture," Gene, 2020, 769:145–253
24. Oya M, Mikami S, Mizuno R, Marumo, K, Mukai M, Murai M. C-Jun activation in acquired cystic kidney disease and renal cell carcinoma[J]. J Urol, 2005,174(2):726.
25. Tu Y, Sun W, Wan Y. Dahuang Fuzi Decoction ameliorates tubular epithelial apoptosis and renal damage via inhibiting TGF-β1-JNK signaling pathway activation in vivo: J Ethnopharmacol, 2014; 156:115–124

26. Sekiuchi M, Kudo A, Nakabayashi K, Kanai-Azuma M, Akimoto Y, Kawakami H, *et al* Expression of matrix metalloproteinases 2 and 9 and tissue inhibitors of matrix metalloproteinases 2 and 1 in the glomeruli of human glomerular diseases: the results of studies using immunofluorescence, in situ hybridization, and immunoelectron microscopy. Clin Exp Nephrol 2012, 16:863–874.
27. Kluger MA, Zahner G, Paust HJ, Schaper M, Magnus T, Panzer U, *et al*. Leukocyte-derived MMP9 is crucial for the recruitment of proinflammatory macrophages in experimental glomerulonephritis. Kidney Int 2013, 83: 865–877.
28. Schlessinger J. Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. Cell. 2002; 110: 669–672
29. Hynes NE, Lane HA. ERBB receptors and cancer: The complexity of targeted inhibitors. Nat Rev Cancer. 2005; 5: 341–354
30. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol, 2001; 2: 127–137
31. Yan W, Li F, Qin Y, Ren Y, Zheng L, Dai X, *et al*. Evaluation of recombinant CXCL8(3–73) K11R/G31P in muscle fibrosis and Trichinella larvae encapsulation in a murine model of trichinellosis. Int Immunopharmacol. 2016; 35:323.
32. Li F, Zhang X, Mizzi C, Gordon JR. CXCL8 (3–73) K11R/G31P antagonizes the neutrophil chemoattractants present in pasteurellosis and mastitis lesions and abrogates neutrophil influx into intradermal endotoxin challenge sites in vivo. Vet Immunol Immunopathol. 2002;90(1–2):65.
33. Bergmann A, Steller H. Apoptosis, stem cells, and tissue regeneration. Sci Signal 2010;3(145):re8.
34. Dai XJ, Tao JH, Fang X, Xia Y, Li XM, Wang YP, *et al*. Changes of treg/Th17 ratio in spleen of acute gouty arthritis rat induced by MSU crystals [J]. Inflammation, 2018, 41(5):1955–1964.
35. Raucci F, Iqbal AJ, Saviano A, Minosi P, Piccolo M, Irace C, *et al*. IL-17A neutralizing antibody regulates monosodium urate crystal-induced gouty inflammation[J]. Pharmacol Res, 2019,147:104351.
36. Quan H, Peng X, Liu S, Bo F, Yang L, Huang Z, *et al*. Differentially expressed protein profile of renal tubule cell stimulated by elevated uric acid using SILAC coupled to LC-MS [J]. Cell Physiol Biochem, 2011,27(1):91–98.
37. Lhotta K, Gruber J, Sgonc R, Fend F, König P. Apoptosis of tubular epithelial cells in familial juvenile gouty nephropathy [J]. Nephron, 1998, 79 (3):340–344.
38. So T, Ishii N. The TNF – TNFR family of co-signal mole-cules [J]. Adv Exp Med Biol, 2019, 1189:53–84.

Figures

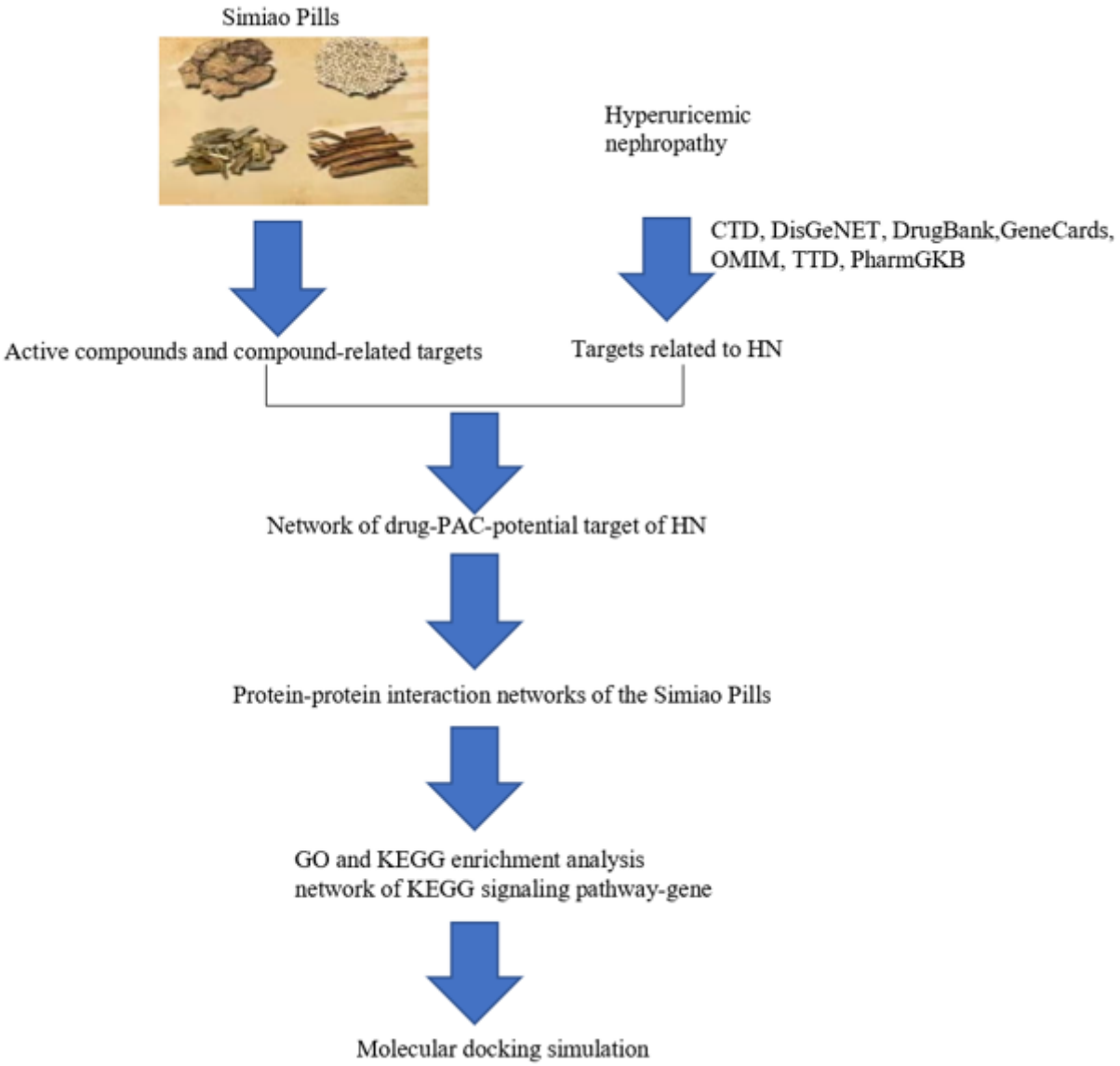

Figure 1

Flow diagram of the study.

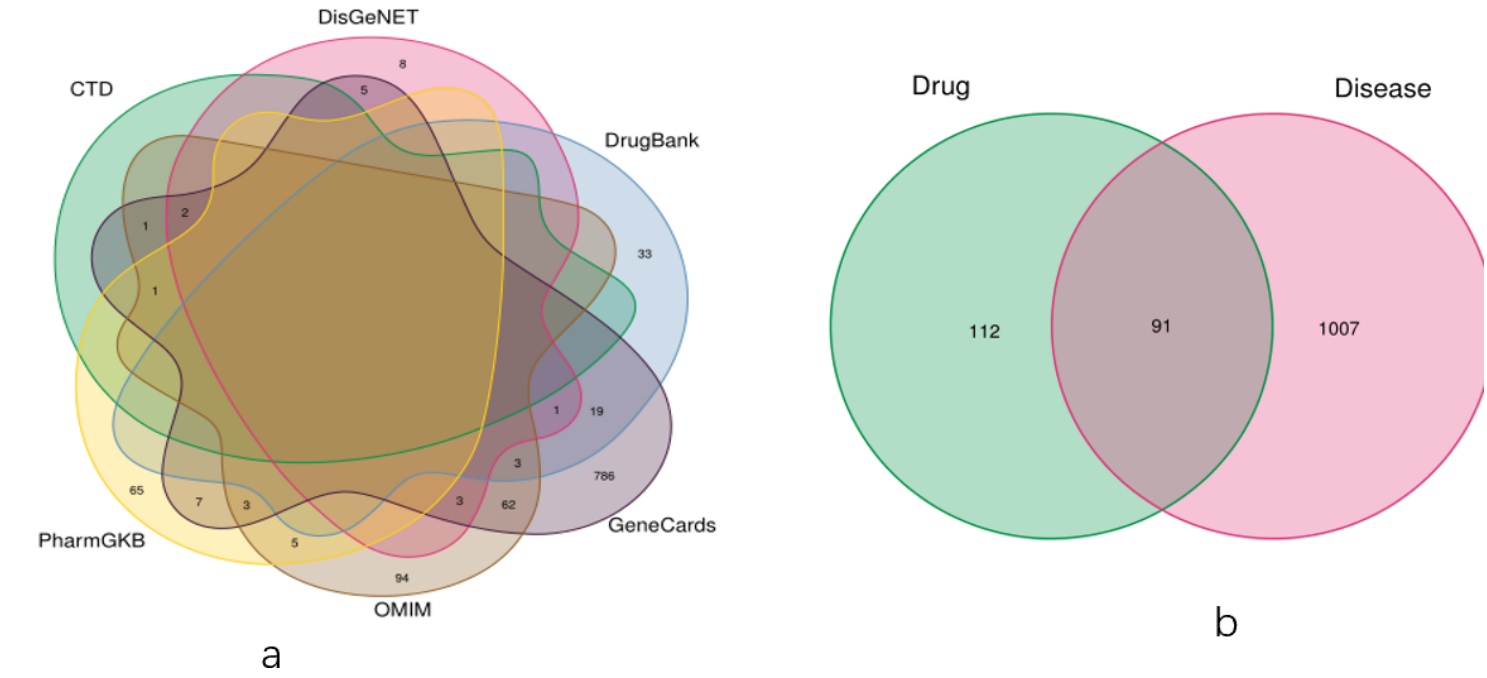

Figure 2

a: Venn diagram of disease targets. b: Venn diagram of SMP targets and HN.

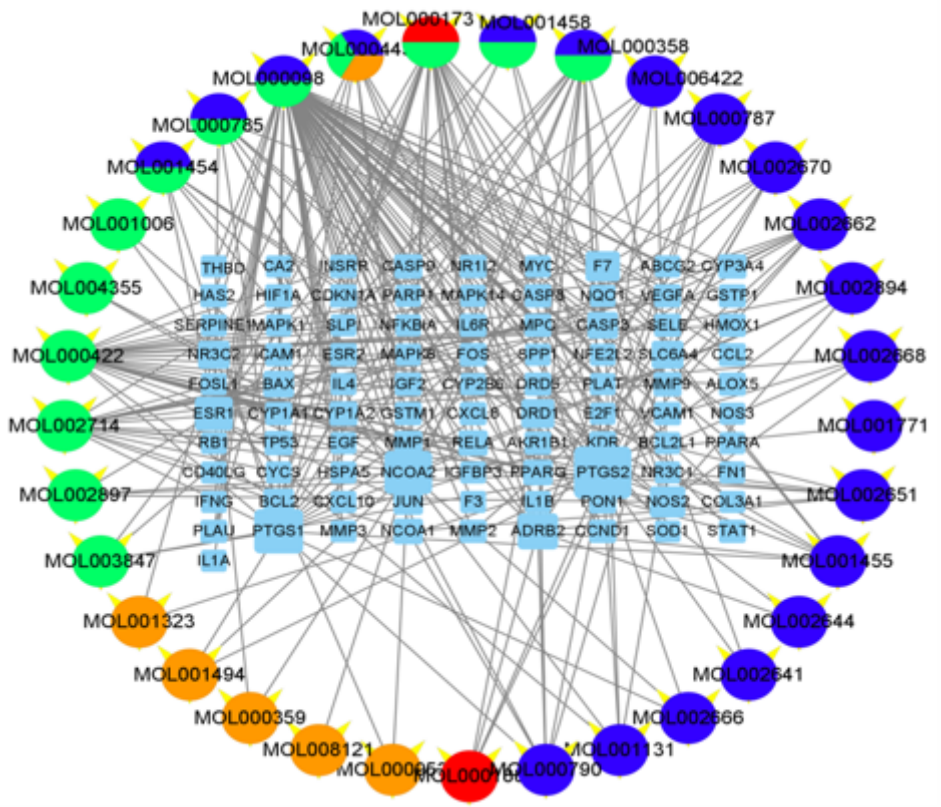

Figure 3

A network of the drug-PAC-potential target of HN. The circle represents the active compounds. Different colors distinguish drug sources of each compound; blue represents the active compound from Cortex

Phellodendri (Huang Bo), red represents the active compound from Rhizoma Atracylodis (Cang Zhu), orange represents the active compound from Semen Coicis (Yi Yi Ren), green shows the active compound from Radix Vladimiriae (Niu Xi), and purple indicates the common compound of various drugs. The square node on the middle denotes the target.

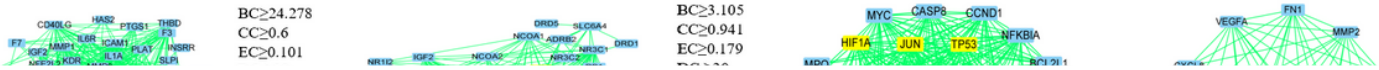

Figure 4

PPI network of the SMP.

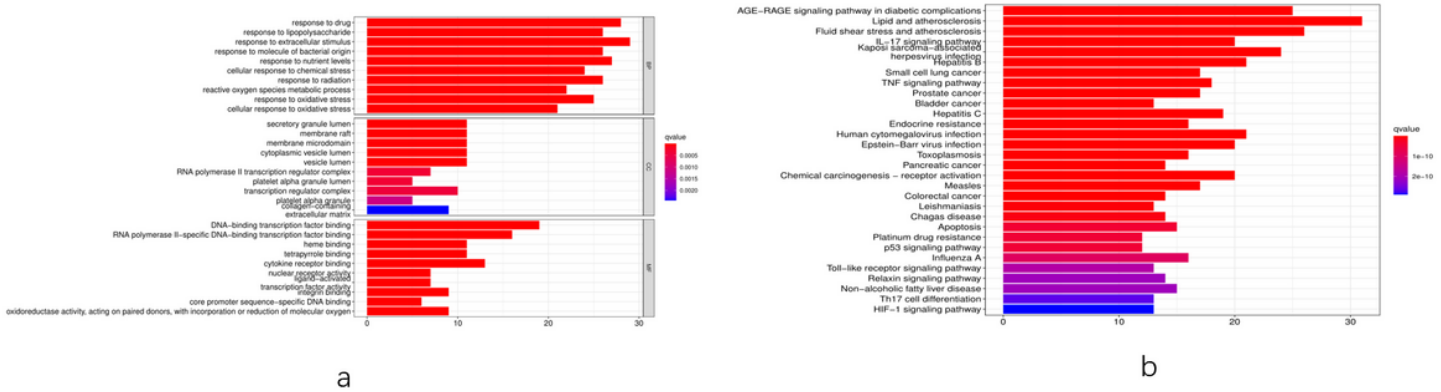

Figure 5

a: GO functional enrichment analysis. GO analysis of putative targets. The y-axis demonstrates the top 10 significantly enriched BP, CC, and MF categories, whereas the x-axis displays the number of enrichment genes of these terms (P<0.05). The color denotes the different P value range, and the redder it is, the more significant enrichment. Noted: BP: biological process, CC: cellular compound, MF: molecular function

b KEGG pathway enrichment analysis. Note. KEGG enrichment analysis of putative targets. The y-axis outlines the top 30 significantly enriched KEGG pathways, whereas the x-axis displays the number of enrichment genes of these terms (P<0.05). The color denotes the different P value range; the redder it is, the more significant enrichment.

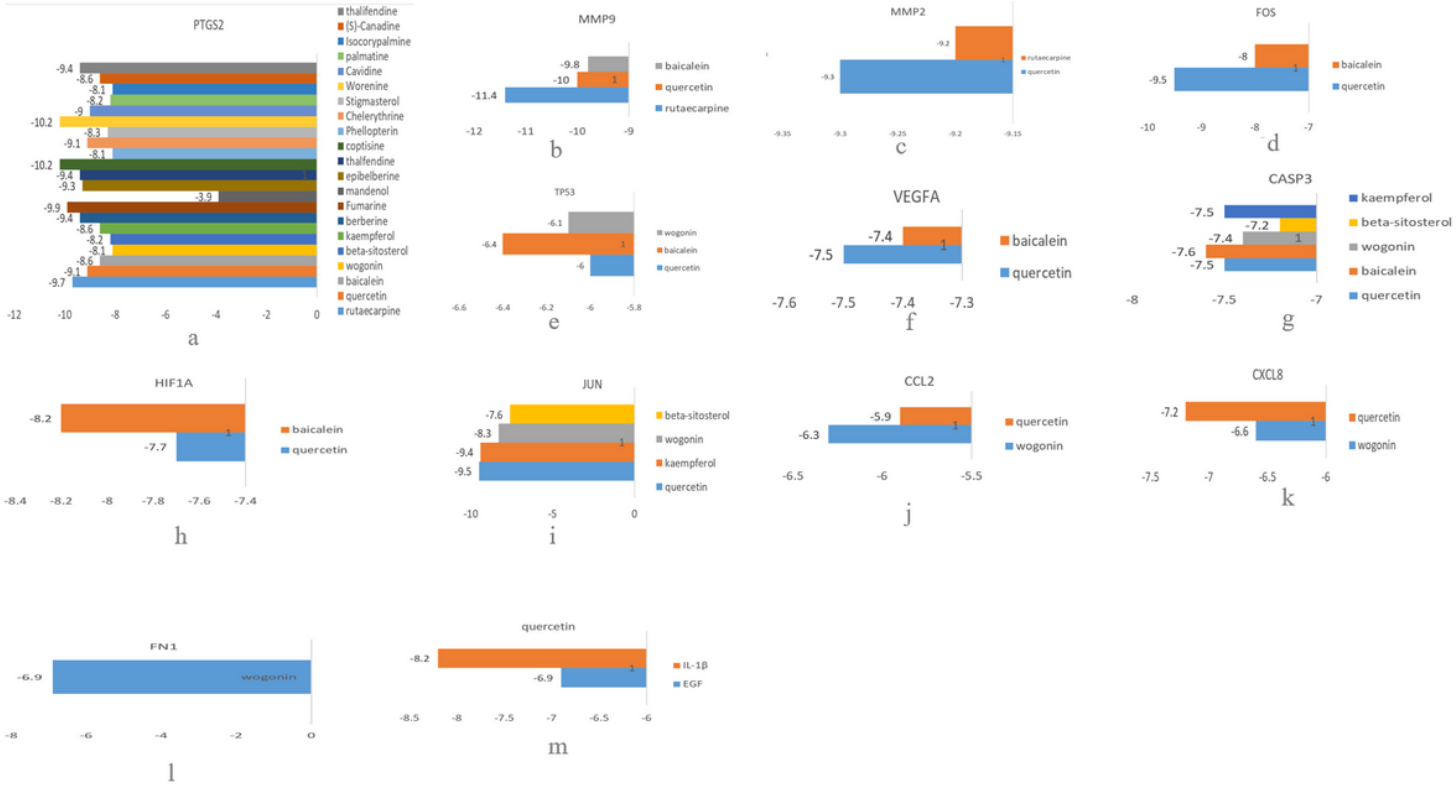

Figure 6

The affinity of the putative compounds with core targets. Note：The x-axis displays affinity in units (kcal/mol) and the y-axis represents different compound compounds (a, b, c, d, e, f, g, h, i, j, k, l) or core targets (m)

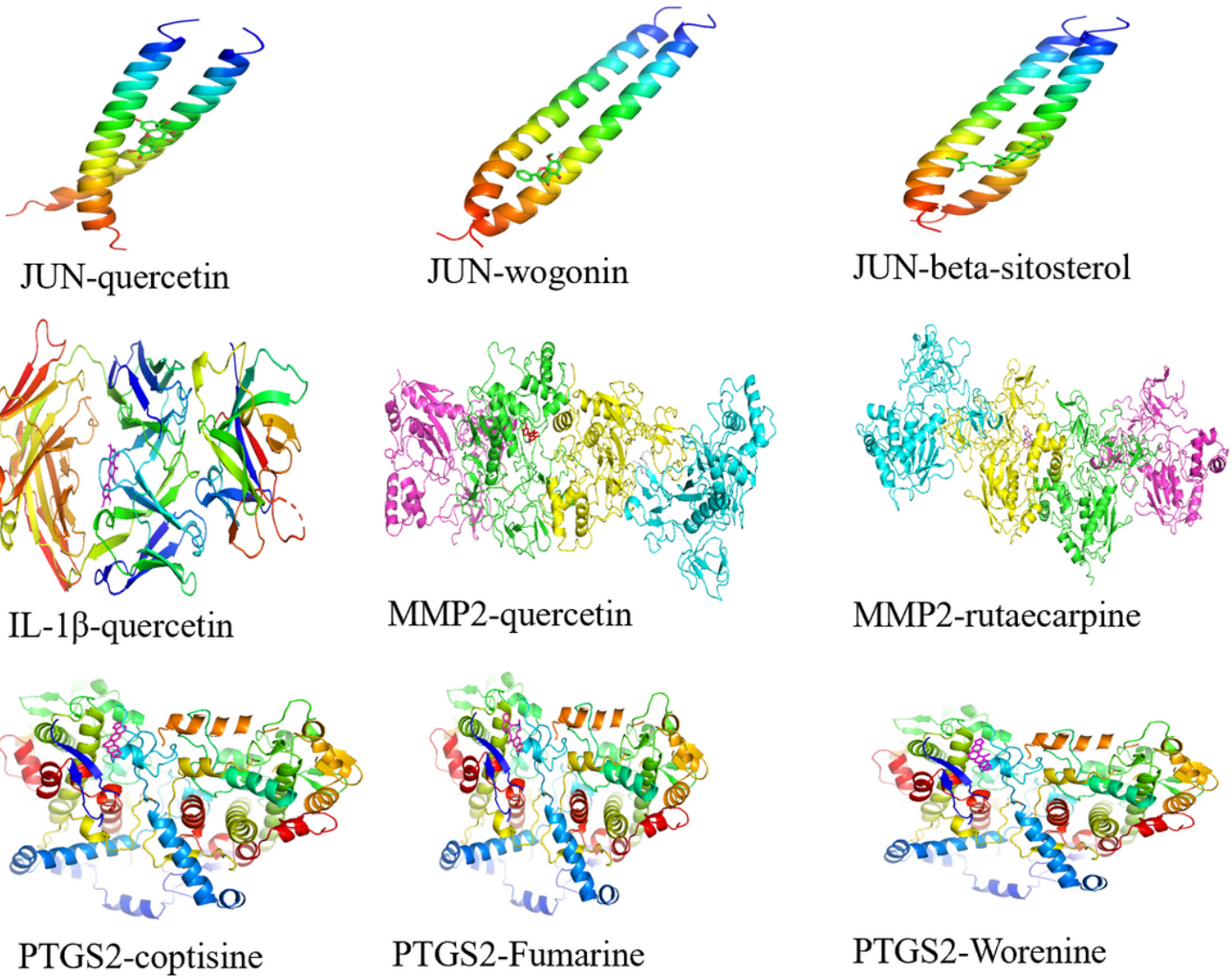

Figure 7

Molecular docking.