Technology and Health Care 29 (2021) S239–S256
DOI 10.3233/THC-218023
IOS Press

Molecular mechanism underlying the hypolipidemic effect of *Shanmei* Capsule based on network pharmacology and molecular docking

Qian Wang[a,1], Lijing Du[b,1], Jiana Hong[a], Zhenlin Chen[a], Huijian Liu[c], Shasha Li[d], Xue Xiao[a,*] and Shikai Yan[a,b,*]
[a]*Institute of Traditional Chinese Medicine, Guangdong Pharmaceutical University, Guangzhou, Guangdong 510006, China*
[b]*School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, China*
[c]*Shanxi Taihang Pharmaceutical Co., Ltd, Changzhi, Shanxi 046000, China*
[d]*The Second Clinical College of Guangzhou University of Chinese Medicine, Guangzhou, Guangdong 510006, China*

Abstract.
BACKGROUND: *Shanmei* Capsule is a famous preparation in China. However, the related mechanism of *Shanmei* Capsule against hyperlipidemia has yet to be revealed.
OBJECTIVE: To elucidate underlying mechanism of *Shanmei* Capsule against hyperlipidemia through network pharmacology approach and molecular docking.
METHODS: Active ingredients, targets of *Shanmei* Capsule as well as targets for hyperlipidemia were screened based on database. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were performed via Database for Annotation, Visualization, and Integrated Discovery (DAVID) 6.8 database. Ingredient-target-disease-pathway network was visualized utilizing Cytoscape software and molecular docking was performed by Autodock Vina.
RESULTS: Seventeen active ingredients in *Shanmei* Capsule were screened out with a closely connection with 34 hyperlipidemia-related targets. GO analysis revealed 40 biological processes, 5 cellular components and 29 molecular functions. A total of 15 signal pathways were enriched by KEGG pathway enrichment analysis. The docking results indicated that the binding activities of key ingredients for PPAR-α are equivalent to that of the positive drug lifibrate.
CONCLUSIONS: The possible molecular mechanism mainly involved PPAR signaling pathway, Bile secretion and TNF signaling pathway via acting on MAPK8, PPARγ, MMP9, PPARα, FABP4 and NOS2 targets.

Keywords: *Shanmei* Capsule, hyperlipidemia, network pharmacology, molecular docking

1. Introduction

Hyperlipidemia is one of the most prevalent global chronic metabolic diseases, which is closely related

[1]These authors contributed equally to this work.
[*]Corresponding authors: Xue Xiao, Institute of Traditional Chinese Medicine, Guangdong Pharmaceutical University, Guangzhou, Guangdong 510006, China. E-mail: erxiaohappy@163.com. Shikai Yan, School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, China. E-mail: shkyan@sjtu.edu.cn.

to many high-incidence cardiovascular diseases, such as hypertension, diabetes, coronary heart disease and atherosclerosis [1–3]. It is characterized as elevated contents of total cholesterol (TC), triglyceride (TG), low-density-lipoprotein cholesterol (LDL-C), as well as decreased high-density-lipoprotein cholesterol (HDL-C) levels [4]. With the elevating prevalence and morbidity, hyperlipidemia has become a critical global issue for public health. Regulating lipid metabolism disorders may be a potential approach to decelerating or preventing the progression of hyperlipidemia. At present, statins, such as atorvastatin, lovastatin and simvastatin, are among the most commonly used lipid-lowering drugs to reduce plasma lipids. Statins are generally regarded as safe and tolerable drugs, however, certain controversies remain. A survey analyzed all case reports from the FDA AE Reporting System database linking muscle-related adverse events to statin use. The results show that dose-dependent side effects occur across the statin class [5–8]. Thus, it is necessary to develop and identify an alternative drug that may be valuable in regulating lipid metabolism. Compound formulae of traditional Chinese medicine (TCM) emphasizes emphasizes the synergism among active ingredients, with proper herbs and relevant dosage to synergize the desirable effects and minimize side effects integrally. Compared with Western medicine, TCM are considered relatively safe and produces fewer adverse reactions [9,10].

TCM has been used for thousands of years for the treatment of hyperlipidemia in China, with advantages of multitarget mechanisms, remarkable curative effect and safety. They are featured by global regulation of glucose and lipid metabolism, as well as energy homeostasis via active ingredients in a prescription [11]. *Shanmei* Capsule, a famous preparation in China, is made of a combination of *Crataegi Folium* (called Shanzhaye in Chinese, the leaves of *Grataegus pinnatifida* Bge. *var. major N.E. Br.* or *Grataegus pinnatifida* Bge.) and Dahurian rose fruit (called Cimeiguo in Chinese, the fruit of *Rosa davurica Pall. var. davurica*) ethanol extract in proportion [12]. *Crataegi Folium* and Dahurian rose fruit both are well-known traditional medicinal plants, which have been confirmed to possess various biological and pharmacological activities, such as anti-inflammatory, antioxidant, hypocholesterolaemic and hypolipidaemic effects [13–15]. In clinical application, they are included in remedies on their own or in combination with other herbs to regulate lipid metabolism disorders induced by cardiovascular diseases. The above evidence suggests that *Shanmei* Capsule may be an alternative approach for treating hyperlipidemia. However, the hypolipidemic effect and related activity mechanisms of *Shanmei* Capsule against hyperlipidemia have yet to be revealed. Due to the complexity of active ingredients and the unknown synergistic effects of multiple components, it remains a laborious task to clarify the molecular mechanism of the *Shanmei* Capsule.

With the booming development of systems biology and bioinformatics, network pharmacology approach has been used successfully for the discovery of the key targets and their potential molecular mechanisms [16,17]. Contrary to "one drug, one target, one disease" principle of traditional drug design, network pharmacology based on the "drug-disease-target" interaction network, which comprehensively and systematically evaluates the intervention or effect of drugs on diseases. Network pharmacology combines the multi-level information of "ingredient-disease-target-pathway", which corresponded with the characteristics of TCM. Molecular docking is an important computer-aided drug discovery method, which can be used to predict the interactions between drug ingredients and target proteins, and the possible binding sites of drugs [18,19]. Interactions between drug molecules and target proteins provide important information on drug discovery, which will help towards the development of drugs to treat hyperlipidemia.

In this study, network pharmacology approach was applied to investigate the relationships between active ingredients, core targets and pathways. Based on the results of network analysis and molecular docking, multi-ingredient, multi-target, and multi-pathway potential mechanisms related to *Shanmei* Capsule against hyperlipidemia were predicted.

2. Methods

2.1. Collection of active ingredients for Shanmei Capsule

Crataegi Folium and Dahurian rose fruit were used as keywords to screen the candidate ingredients of *Shanmei* Capsule. All the chemical ingredients were queried based on the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (http://tcmspw.com/tcmsp.php) and literatures. The structural information regarding biological activities of small molecules, including CAS numbers and Canonical SMILES strings, were obtained from the Scifinder database (https://scifinder.cas.org/scifinder/) and PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [20].

2.2. Target acquisition for drug and disease-related

Target prediction of *Shanmei* Capsule was performed using Swiss Target Prediction (http://www.swisstargetprediction.ch/) [21]. The Canonical SMILES strings obtained in 2.1 was introduced into the Swiss Target Prediction to obtain human-related targets for active ingredients. Using hyperlipidemia as a keyword, the candidate target of hyperlipidemia was searched by the GeneCard database (version 4.14, https://www.genecards.org/). After removing the duplicate targets, the overlapping targets related to active ingredients and hyperlipidemia were selected as the candidate targets, and then imported into the STRING database (http://string-db.org/) for gene interaction analysis.

2.3. GO and KEGG pathway enrichment analysis

Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analysis were carried out based on the Database for Annotation, Visualization and Integrated Discovery (DAVID, version 6.8, http://www.david.niaid.nih.gov). Results with values of $P < 0.01$ were selected, and Human was defined as the species. DAVID is a powerful gene functional database, which can provide comprehensive functional annotations of pathways, including biological processes, cellular component and molecular functions. After getting the results, these pathways were visualized as a bubble chart by imageGP (http://www.ehbio.com/ImageGP/), a free online tool for data analysis.

2.4. Network construction and analysis

Network construction and analysis were performed using Cytoscape 3.3.0 (download from https://cytoscape.org/). The nodes in different shapes and colors of the network represented active ingredients, targets, pathways, and the edges indicated the interaction of them. In this study, degree refers to the number of directly connected nodes with one another. It is considered that the greater the number of degree is, the greater the influence is. The topological parameters of network, such as degree, were calculated by Network Analyzer (Cytoscape plugin).

2.5. Molecular docking

The candidate ingredients of *Shanmei* Capsule and lifibrate (positive control drug) were chosen for molecular docking, and the results were visualized using PyMOL software (https://www.pymol.org/). The detailed steps were as follows. Firstly, molecular structure of active ingredients and the protein crystal structure of the hyperlipidemia-related target were downloaded from the ZINC 15 (http://zinc.docking.

org/) and RCSB PDB database (https://www.rcsb.org/), respectively. Prior to performing the docking process, the target protein structure was pretreated by AutoTools, including removal of water molecules, addition with hydrogens and so on. Secondly, the active pocket on protein structure was predicted by GetBox Plugin (PyMOL plugin) [22,23]. The values of the utilized parameters in autodock software were presented in Table S1. Finally, molecular docking simulation was performed using the Autodock Vina software. According to the scoring method, the docking models with the lowest score and binding-energy value were selected and visualized for subsequent analysis.

2.6. ADME and toxicology prediction

The 2D structures (in .sdf format) and simplified molecular input line entry specification (SMILES) codes of 17 key active ingredients were downloaded from the PubChem database (http://pubchem.ncbi.nlm.nih.gov). Then, a SwissADME web tool (http://www.swissadme.ch/index.php) was employed to predict ADME (Absorption, Distribution, Metabolism and Excretion) parameters, including bioavailability score, gastrointestinal (GIT) absorption and blood-brain barrier (BBB). Toxicity analysis was done by Toxtree (version 3.1.0.1851), a generic open source application, which is able to estimate toxic hazard by making predictions for a number of toxicological endpoints by different modules, such as carcinogenicity prediction (genotoxic and non-genotoxic) and Ames carcinogenicity tests.

3. Results

3.1. Screening of Shanmei Capsule active ingredients

A total of 59 candidate active ingredients were screened from the TCMSP, including 25 from *Crataegi Folium*, 29 from Dahurian rose fruit, and 5 shared ingredients. Details are shown in Table 1. The obtained candidate ingredients and all of the potential targets were applied to construct a network of ingredient-target interactions, including 330 nodes (59 ingredients and 271 targets) and 928 edges, as shown in Fig. 1. The degree of targets was adopted as a characteristic topological parameter to define the significance of candidate ingredients. The interactions indicated that one ingredient could regulate numerous targets, such as quercetin, kaempferol, β-sitosterol, isorhamnetin, ursolic acid, chlorogenic acid and so on, suggesting that *Shanmei* Capsule has multi-ingredients and multi-target characteristics for the treatment of hyperlipidemia.

3.2. Prediction of candidate targets of hyperlipidemia

A total of 1018 potential targets to have correlations with hyperlipidemia were found via the GeneCards database. After merging hyperlipidemia-related targets and active ingredient targets, 34 overlapping targets were considered as candidate targets (Table 2, Fig. 2). Further linking of target ingredients with candidate targets based on Cytoscap 3.3.0 resulted in a network of disease-ingredient-target, with 51 nodes and 301 edges (Fig. 3). Among them, 17 red dovetail nodes represent active ingredients of *Shanmei* Capsule, and 34 purple ellipse nodes represent candidate targets of hyperlipidemia. After analyzing the topological characteristics of each node in the interaction network, it shows that the node degrees of eight targets, HMGCR, MMP9, MAPK8, PPARα, PPARγ, FABP4, ALOX5 and NOS2 are more than 13, which implied that these targets might be key targets for hyperlipidemia. Previous study found that these targets were primarily involved in inflammatory reactions, lipid metabolism, etc.

Table 1
A list of the selected compounds in *Shanmei* Capsule for network analysis

Source	Compound	Molecular formula	CAS number
Crataegi Folium	Tyramine	$C_8H_{11}NO$	51-67-2
Crataegi Folium	Vitamin c	$C_6H_8O_6$	50-81-7
Crataegi Folium	Cyanidol	$C_{15}H_{11}ClO_6$	528-58-5
Crataegi Folium	Heriguard	$C_{16}H_{18}O_9$	327-97-9
Crataegi Folium	Caffeic acid	$C_9H_8O_4$	331-39-5
Crataegi Folium	Vitamin-G	$C_6H_8O_6$	83-88-5
Crataegi Folium	Isorhamnetin	$C_{16}H_{12}O_7$	480-19-3
Crataegi Folium	Vitexin	$C_{21}H_{20}O_{10}$	3681-93-4
Crataegi Folium	Caffeate	$C_9H_8O_4$	3843-74-1
Crataegi Folium	Nicotinic acid	$C_6H_5NO_2$	59-67-6
Crataegi Folium	Hyperin	$C_{21}H_{20}O_{12}$	482-36-0
Crataegi Folium	Stigmasterol	$C_{29}H_{50}O$	68555-08-8
Crataegi Folium	Orientin	$C_{21}H_{20}O_{11}$	28608-75-5
Crataegi Folium	Maslinic acid	$C_{30}H_{48}O_4$	4373-41-5
Crataegi Folium	Octacosanol	$C_{28}H_{58}O$	557-61-9
Crataegi Folium	Thiamine	$C_{12}H_{18}Cl_2N_4OS$	70-16-6
Crataegi Folium	Orbitol	$C_6H_{14}O_6$	50-70-4
Crataegi Folium	Ent-Epicatechin	$C_{15}H_{14}O_6$	35323-91-2
Crataegi Folium	p-coumaric acid	$C_9H_8O_3$	501-98-4
Crataegi Folium	Isoorientin	$C_{21}H_{20}O_{11}$	4261-42-1
Crataegi Folium	2-methoxyphenethylamine	$C_9H_{13}NO$	2045-79-6
Crataegi Folium	(1S)-1-phenylethanamine	$C_8H_{11}N$	2627-86-3
Crataegi Folium	Vitexin-2-O-rhamnoside	$C_{27}H_{30}O_{14}$	64820-99-1
Crataegi Folium	Diethylamine	$C_4H_{11}N$	109-89-7
Crataegi Folium	Valamine	$C_4H_{11}N$	78-81-9
Crataegi Folium/Dahurian rose fruit	Sitosterol	$C_{29}H_{50}O$	83-46-5
Crataegi Folium/Dahurian rose fruit	Rutin	$C_{27}H_{30}O_{16}$	153-18-4
Crataegi Folium/Dahurian rose fruit	Kaempferol	$C_{15}H_{10}O_6$	520-18-3
Crataegi Folium/Dahurian rose fruit	Ursolic acid	$C_{30}H_{48}O_3$	77-52-1
Crataegi Folium/Dahurian rose fruit	Quercetin	$C_{15}H_{10}O_7$	117-39-5
Dahurian rose fruit	luteolin-7-O-rutinoside	$C_{27}H_{30}O_{15}$	25694-72-8
Dahurian rose fruit	Hyperoside	$C_{21}H_{20}O_{12}$	482-36-0
Dahurian rose fruit	Isoliquiritigenin	$C_{15}H_{12}O_4$	961-29-5
Dahurian rose fruit	β-sitosterol	$C_{29}H_{50}O$	64997-52-0
Dahurian rose fruit	Tiliroside	$C_{30}H_{26}O_{13}$	20316-62-5
Dahurian rose fruit	Hesperidin	$C_{28}H_{34}O_{15}$	520-26-3
Dahurian rose fruit	Oleanolic acid	$C_{30}H_{48}O_3$	508-02-1
Dahurian rose fruit	Stearic acid	$C_{18}H_{36}O_2$	57-11-4
Dahurian rose fruit	Palmitic acid	$C_{18}H_{36}O_2$	57-10-3
Dahurian rose fruit	Oleic acid	$C_{18}H_{34}O_2$	112-80-1
Dahurian rose fruit	Linoleic acid	$C_{18}H_{32}O_2$	60-33-3
Dahurian rose fruit	Linolenic acid	$C_{18}H_{30}O_2$	463-40-1
Dahurian rose fruit	Eicosadienoic acid	$C_{20}H_{36}O_2$	2091-39-6
Dahurian rose fruit	Strictinin	$C_{27}H_{22}O_{18}$	517-46-4
Dahurian rose fruit	Heptacosane	$C_{27}H_{56}$	593-49-7
Dahurian rose fruit	Cerotic acid	$C_{26}H_{52}O_2$	506-46-7
Dahurian rose fruit	Daucosterol	$C_{35}H_{60}O_6$	474-58-8
Dahurian rose fruit	Betulinic acid	$C_{30}H_{48}O_3$	472-15-1
Dahurian rose fruit	Gallic acid	$C_7H_6O_5$	149-91-7
Dahurian rose fruit	Furfuryl acetate	$C_7H_8O_3$	623-17-6
Dahurian rose fruit	n-hexyl furan	$C_{10}H_{16}O$	3777-70-6
Dahurian rose fruit	2-furan-2-yl-pentanal	$C_9H_{12}O_2$	31681-26-2
Dahurian rose fruit	β-sitosterol acetate	$C_{31}H_{52}O_2$	915-05-9

Table 1, continued

Source	Compound	Molecular formula	CAS number
Dahurian rose fruit	2-amino-propionic acid ethyl ester	$C_5H_{11}NO_2$	17344-99-9
Dahurian rose fruit	Isobutyl formate	$C_5H_{10}O_2$	542-55-2
Dahurian rose fruit	Isoamyl acetate	$C_7H_{14}O_2$	123-92-2
Dahurian rose fruit	Ethyl hexanoate	$C_8H_{16}O_2$	123-66-0
Dahurian rose fruit	Ethyl 1-acetylcyclopropane-1-carboxylate	$C_8H_{12}O_3$	32933-03-2
Dahurian rose fruit	Ethyl palmitoleate	$C_{18}H_{34}O_2$	56219-10-4

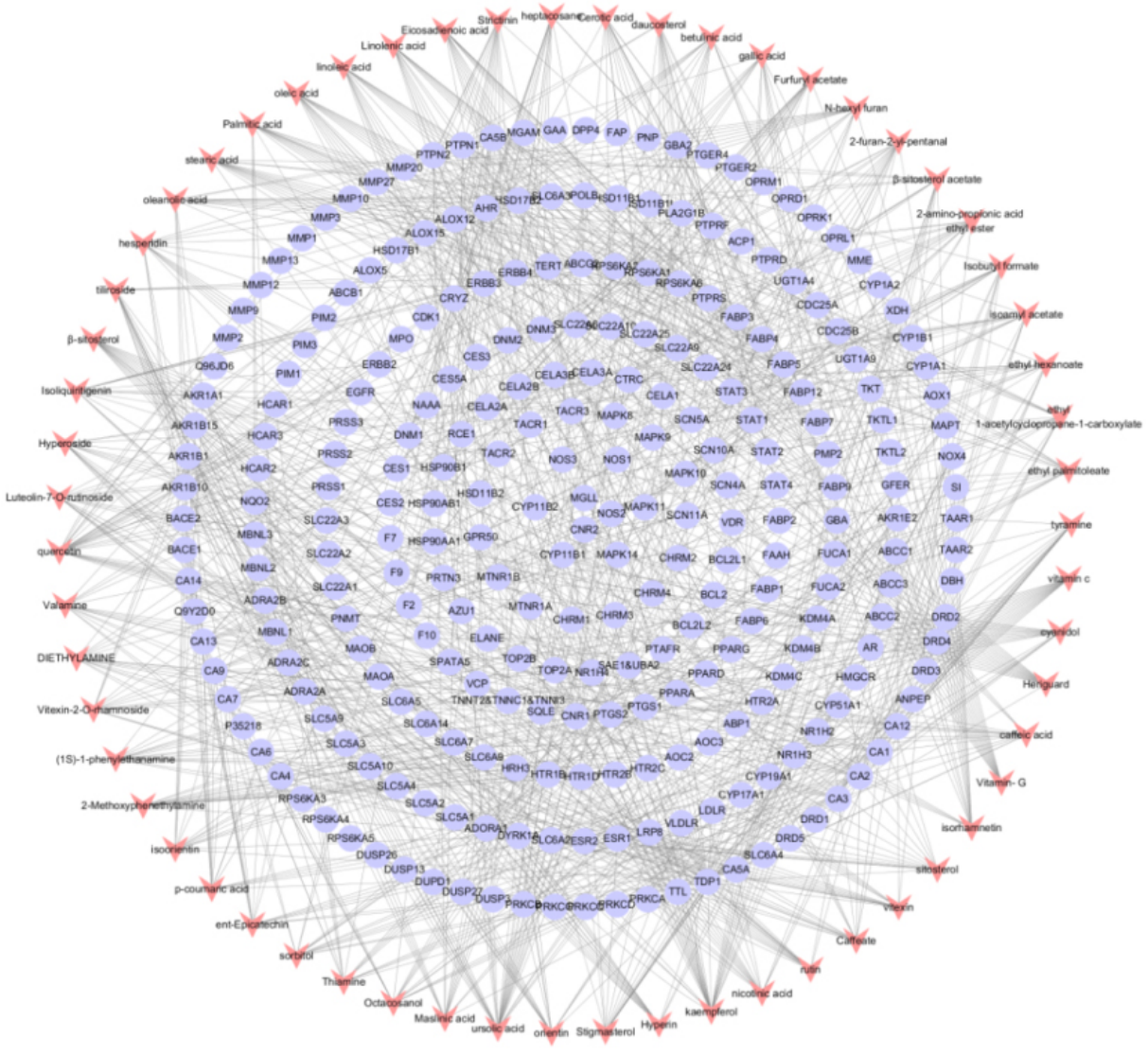

Fig. 1. Establishment of component-target network. Components are shown as red dovetails nodes and target are marked as purple circular nodes.

Table 2
Related parameters of potential targets of *Shanmei* Capsule

No.	Uniprot ID	Target gene	Target protein
1	P15121	AKR1B1	Aldo-keto reductase family 1 member B1
2	O60218	AKR1B10	Aldo-keto reductase family 1 member B10
3	P08253	MMP2	72 kDa type IV collagenase
4	P14780	MMP9	Matrix metalloproteinase-9
5	P03956	MMP1	Interstitial collagenase
6	P08254	MMP3	Stromelysin-1
7	P10636	MAPT	Microtubule-associated protein tau
8	P10275	AR	Androgen receptor
9	P04035	HMGCR	3-hydroxy-3-methylglutaryl-coenzyme A reductase
10	P11511	CYP19A1	Aromatase
11	P01130	LDLR	Low-density lipoprotein receptor
12	P03372	ESR1	Estrogen receptor
13	Q92731	ESR2	Estrogen receptor beta
14	P09917	ALOX5	Arachidonate 5-lipoxygenase
15	P08183	ABCB1	ATP-dependent translocase ABCB1
16	P35869	AHR	Aryl hydrocarbon receptor
17	P04626	ERBB2	Receptor tyrosine-protein kinase erbB-2
18	P00533	EGFR	Epidermal growth factor receptor
19	P05164	MPO	Myeloperoxidase
20	Q9UNQ0	ABCG2	ATP-binding cassette sub-family G member 2
21	P15090	FABP4	Fatty acid-binding protein
22	P07148	FABP1	Fatty acid-binding protein
23	P37231	PPARγ	Peroxisome proliferator-activated receptor gamma
24	Q07869	PPARα	Peroxisome proliferator-activated receptor alpha
25	P21554	CNR1	Cannabinoid receptor 1
26	P35354	PTGS2	Prostaglandin G/H synthase 2
27	P00734	F2	Prothrombin
28	Q96RI1	NR1H4	Bile acid receptor
29	P08246	ELANE	Neutrophil elastase
30	P07900	HSP90AA1	Heat shock protein HSP 90-alpha
31	P45983	MAPK8	Mitogen-activated protein kinase 8
32	Q16539	MAPK14	Mitogen-activated protein kinase 14
33	P29474	NOS3	Nitric oxide synthase
34	P35228	NOS2	Nitric oxide synthase

3.3. GO and KEGG pathway enrichment analysis

To further clarify relevant functions and pathways, GO functional analyses and KEGG pathway enrichment were performed for above 34 core targets using DAVID database. GO enrichment analysis was annotated in the following three categories, including biological process, cellular component and molecular function, with $P < 0.01$ serving as the threshold. There were 40 biological processes involved, including oxidation-reduction processes, intracellular receptor signaling pathway, regulation of inflammatory response, positive regulation of MAP kinase activity, etc. (Fig. 4A). In the cellular component group, GO terms were mainly included nucleus, nucleoplasm, extracellular space, perinuclear region of cytoplasm and basolateral plasma membrane (Fig. 4B). Figure 4C shows that the enriched molecular functions of targets are mainly associated with RNA polymerase II transcription factor activity, enzyme binding, steroid hormone receptor activity, etc. These results indicated that the potential mechanism of *Shanmei* Capsule against hyperlipidemia was related to oxidation-reduction processes, intracellular receptor signaling, and inflammatory response. A total of 15 signal pathways were enriched by KEGG pathway enrichment analysis (Table 3), including PPAR signaling pathway, Bile secretion and TNF signaling pathway, et al. Among them, Pathway in cancer is a cancer-related signaling pathway. Recent

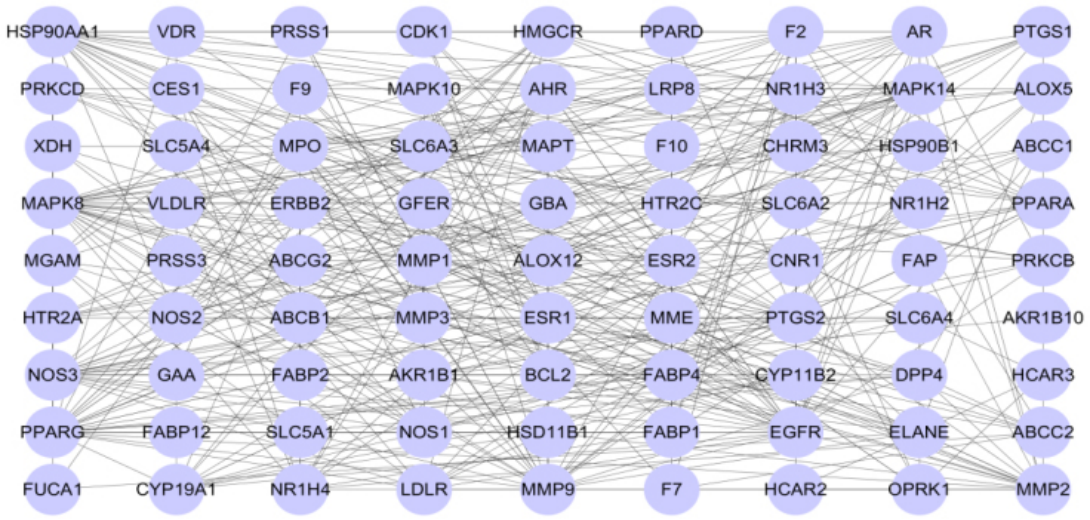

Fig. 2. Target-target interaction network between active ingredients of *Shanmei* Capsule against hyperlipidemia.

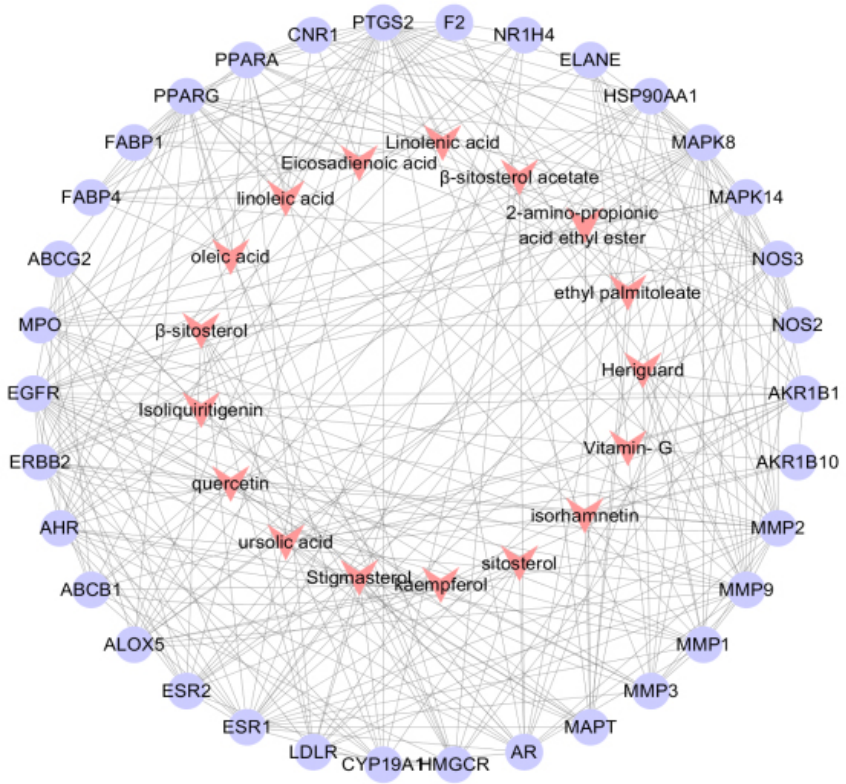

Fig. 3. Component-target-diseas interactive network of *Shanmei* Capsule. Red dovetails nodes are the main active ingredients of *Shanmei* Capsule, and purple circular nodes are the potential targets for treating hyperlipidemia.

Table 3
Pathway enrichment of potential targets based on KEGG pathway analysis

No.	Pathway description	P value	Nr. Genes	Ratio
1	Pathways in cancer	0.00001	11	32.4
2	Estrogen signaling pathway	0.00001	7	20.6
3	Bladder cancer	0.00004	5	14.7
4	PPAR signaling pathway	0.00024	5	14.7
5	Bile secretion	0.00027	5	14.7
6	TNF signaling pathway	0.00140	5	14.7
7	Ovarian steroidogenesis	0.00150	4	11.8
8	Toxoplasmosis	0.00160	5	14.7
9	Proteoglycans in cancer	0.00210	6	17.6
10	Hepatitis C	0.00320	5	14.7
11	Prolactin signaling pathway	0.00420	4	11.8
12	Transcriptional misregulation in cancer	0.00710	5	14.7
13	Prostate cancer	0.00770	4	11.8
14	GnRH signaling pathway	0.00840	4	11.8
15	HIF-1 signaling pathway	0.00970	4	11.8

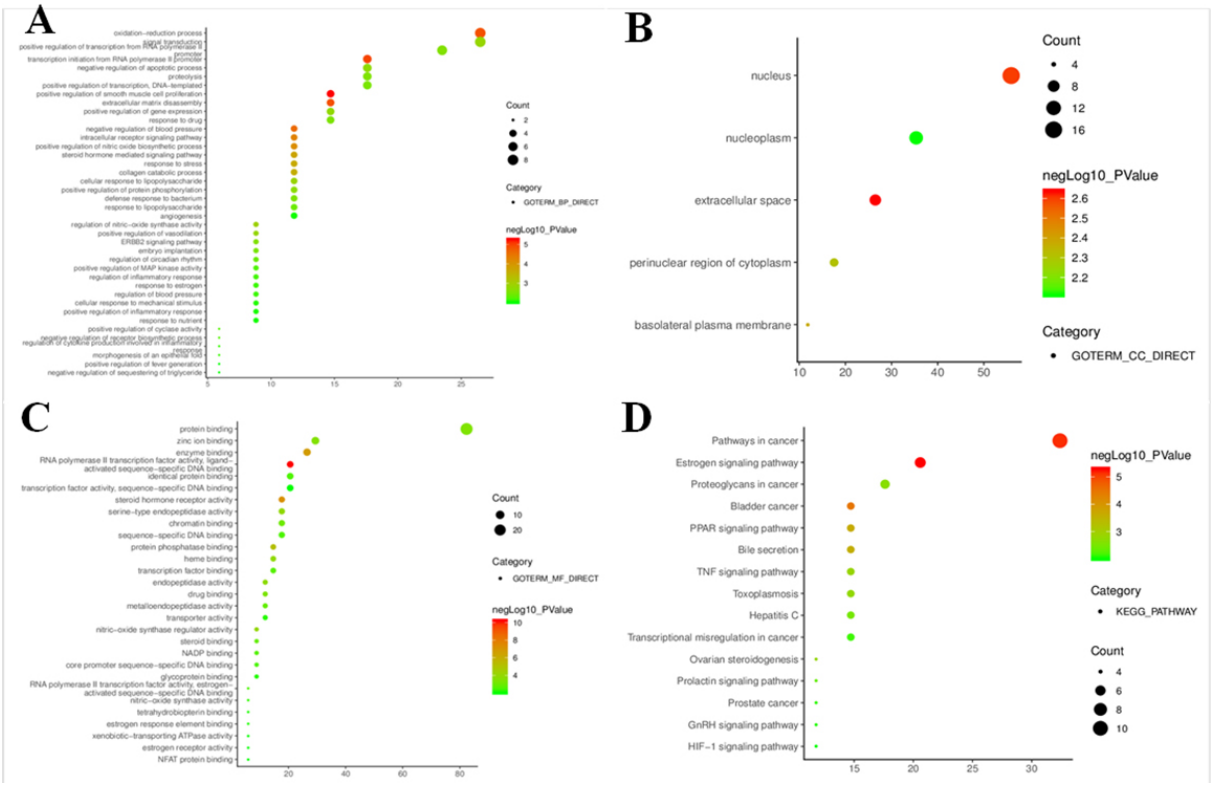

Fig. 4. Go and KEGG pathway enrichment bubble diagrams for candidate targets ($P \leqslant 0.01$). (A) GO analysis of Biological processes, (B) GO analysis of cellular components, (C) GO analysis of molecular functions, (D) KEGG analysis.

studies have reported that a carcinogenic role of the metabolic syndrome in variety of cancers [24]. Based on these results, *Shanmei* Capsule may attenuate hyperlipidemia partially by regulating inflammation or apoptosis, lipid metabolism and decrease oxidative stress during hyperlipidemia progression.

At last, in order to elucidate the connection between the core targets and corresponding pathways, a

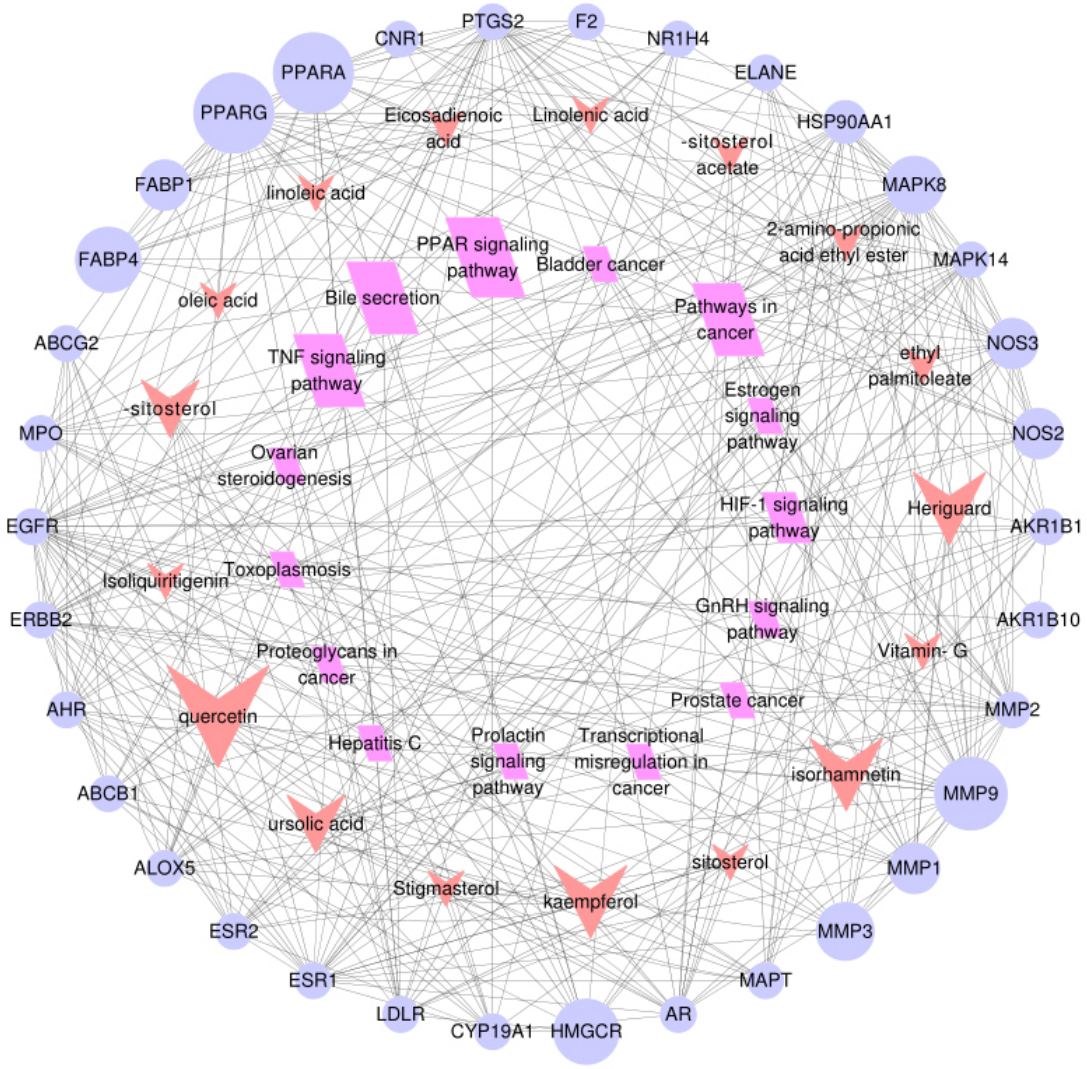

Fig. 5. The component-target-pathway network for *Shanmei* Capsule. Purple circular nodes represent the candidate targets, red dovetails nodes represent the active ingredients and rose red parallelogram nodes represent the KEGG pathways. The size of nodes was proportional with the degree and larger sizes represent greater associations with hyperlipidemia treatment.

component-target-pathway interaction network map was generated by Cytoscape 3.3.0 (Fig. 5). The 17 red dovetails nodes represent the active pharmaceutical ingredient, the 34 purple circular nodes denote the potential targets, and the 15 rose red parallelogram nodes represent the pathways. And the node sizes depend on degree value. The larger the node is, the larger the degree value is. There were 66 nodes and 340 edges were involved in the association between these targets, which reflected the biological process of the key targets. These results were revealed that *Shanmei* Capsule take effect in hyperlipidemia treatment by multi-components, multi-targets and multi-pathways.

3.4. Docking results analysis

Molecular docking was performed among 17 ingredients and 34 potential targets using the Autodock

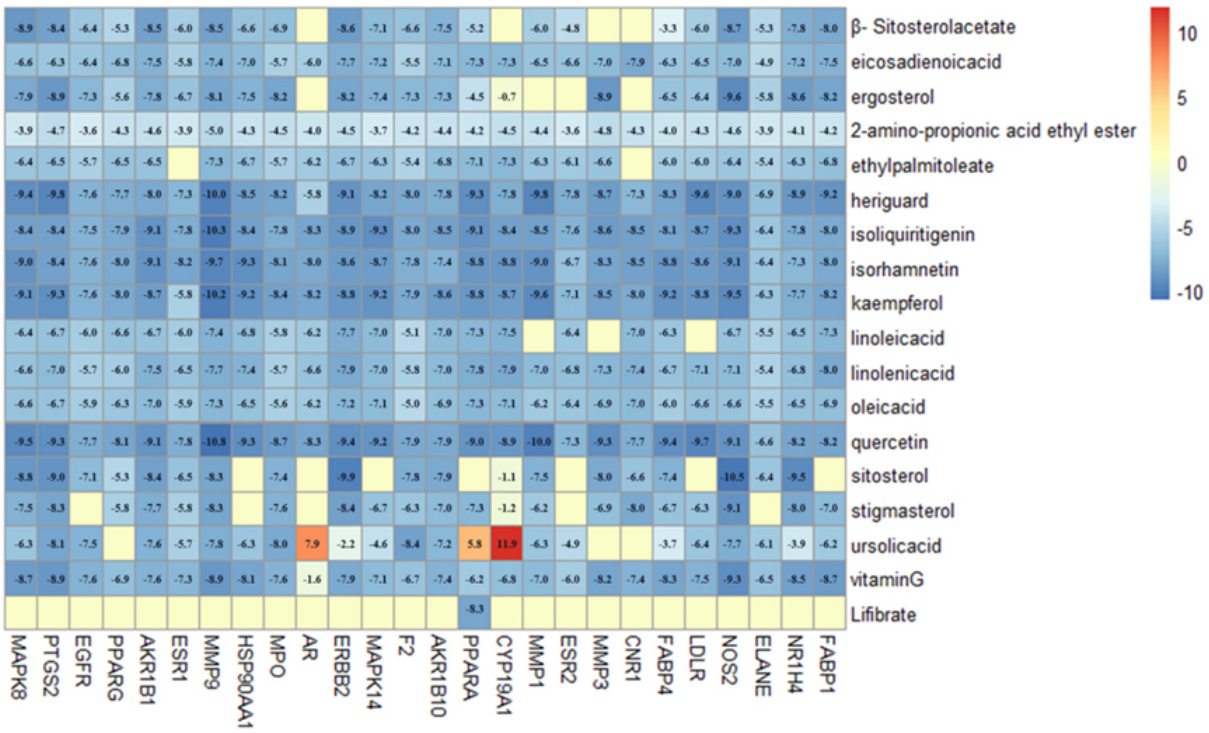

Fig. 6. The heat map of molecule docking scores.

Vina software, and detailed results are presented in Fig. 6. Affinity was the binding score for the molecular docking, and when the docking score value was smaller, the binding affinity to target protein was stronger. The docking score < -7 indicated a high binding activity [25]. The molecular docking results revealed that the docking scores of 47% active ingredients were lower than -7, and the ingredients with the lowest scores among these targets were quercetin and chlorogenic acid (heriguard) (Fig. 6). PPAR-α is a key target of lifibrate (positive drug), which belongs to the nuclear hormone receptor family, which is linked to dyslipidemia and diabetes. The docking results showed that the binding activity of quercetin, kaempferol, isorhamsin and chlorogenic acid for PPAR-α are equivalent to, and even better than that of the positive drug lifibrate. The molecular docking models are illustrated in Fig. 7. These findings indirectly verified that *Shanmei* Capsule had a regulatory effect on hyperlipidemia targets. At the same time, the above molecular docking results were consistent with those of previous network screening, which verified the reliability of network pharmacology.

3.5. Results of drug ADME and toxicity estimations

The ADME properties of 17 active ingredients were calculated by SwissADME web tool. As is can be observed in Table 4, all the ingredients, besides heriguard, have bioavailability score above the threshold of 0.5, which might suggest good oral bioavailability. The majority of the tested ingredients have no ability to penetrate the blood-brain barrier, thus presenting a low risk for central nervous system side effects. Based on toxicity tests, it appears that all the ingredients have no nongenetic carcinogen properties, with only vitaminG was predicted to be genetic carcinogen. The results of SwissADME calculations and toxicity tests provided useful information about the selected ingredients and found to be free from high risks of undesired effects.

Table 4
ADME and toxicity prediction results of 17 key ingredients in *Shanmei* Capsule

No.	Compound	GI absorption	BBB permeant	Bioavailability score	Genetic carcinogen	Nongenetic carcinogen	Ames
1	β-Sitosterolacetate	Low	No	0.55	−	−	−
2	Eicosadienoicacid	High	No	0.56	−	−	−
3	Ergosterol	Low	No	0.55	−	−	−
4	2-amino-propionic acid ethyl ester	High	No	0.55	−	−	−
5	Ethylpalmitoleate	High	Yes	0.55	−	−	−
6	Heriguard	Low	No	0.11	−	−	−
7	Isoliquiritigenin	High	Yes	0.55	−	−	+
8	Isorhamnetin	High	No	0.55	−	−	+
9	Kaempferol	High	No	0.55	−	−	−
10	Linoleicacid	High	Yes	0.56	−	−	−
11	Linolenicacid	High	Yes	0.56	−	−	−
12	Oleicacid	High	No	0.56	−	−	−
13	Quercetin	High	No	0.55	−	−	+
14	Sitosterol	Low	No	0.55	−	−	−
15	Stigmasterol	Low	No	0.55	−	−	−
16	Ursolicacid	Low	No	0.56	−	−	−
17	VitaminG	Low	No	0.55	+	−	+

Notes: (−) negative; (+) positive.

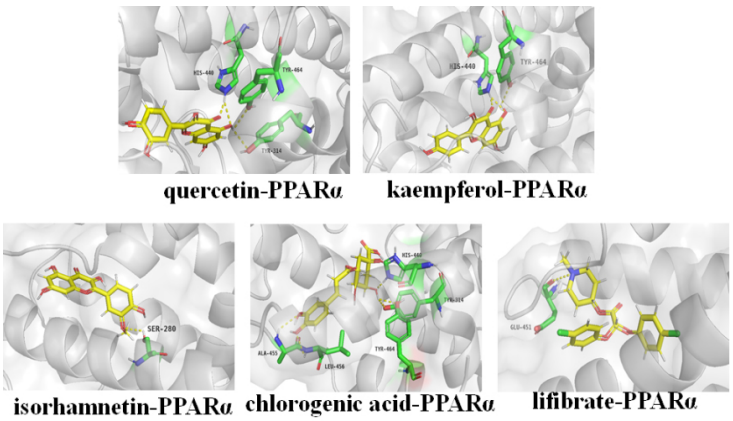

Fig. 7. Molecular models of bioactive ingredients and positive drug binding to the predicted PPARα target. (A) Quercetin, (B) Heriguard, (C) Lifibrate.

4. Discussion

Hyperlipidemia is one of the most prevalent global chronic metabolic diseases, which usually involved in a disorder of lipid metabolism and accompanied by elevated contents of TC, TG, LDL-C, as well as decreased HDL-C levels. During this process, blood lipid level, oxidative stress, inflammation, and other factors might be the key link of the development of dyslipidemia-induced hyperlipidemia. *Shanmei* Capsule, a famous preparation in China, is made of a combination of *Crataegi Folium* and Dahurian rose fruit ethanol extract in proportion. *Crataegi Folium* and Dahurian rose fruit both are well-known traditional medicinal plants, which have been confirmed to possess various biological and pharmacological activities, such as anti-inflammatory, antioxidant and hypolipidaemic effects. Therefore, in this study, network pharmacology approach and molecular docking were applied to investigate the

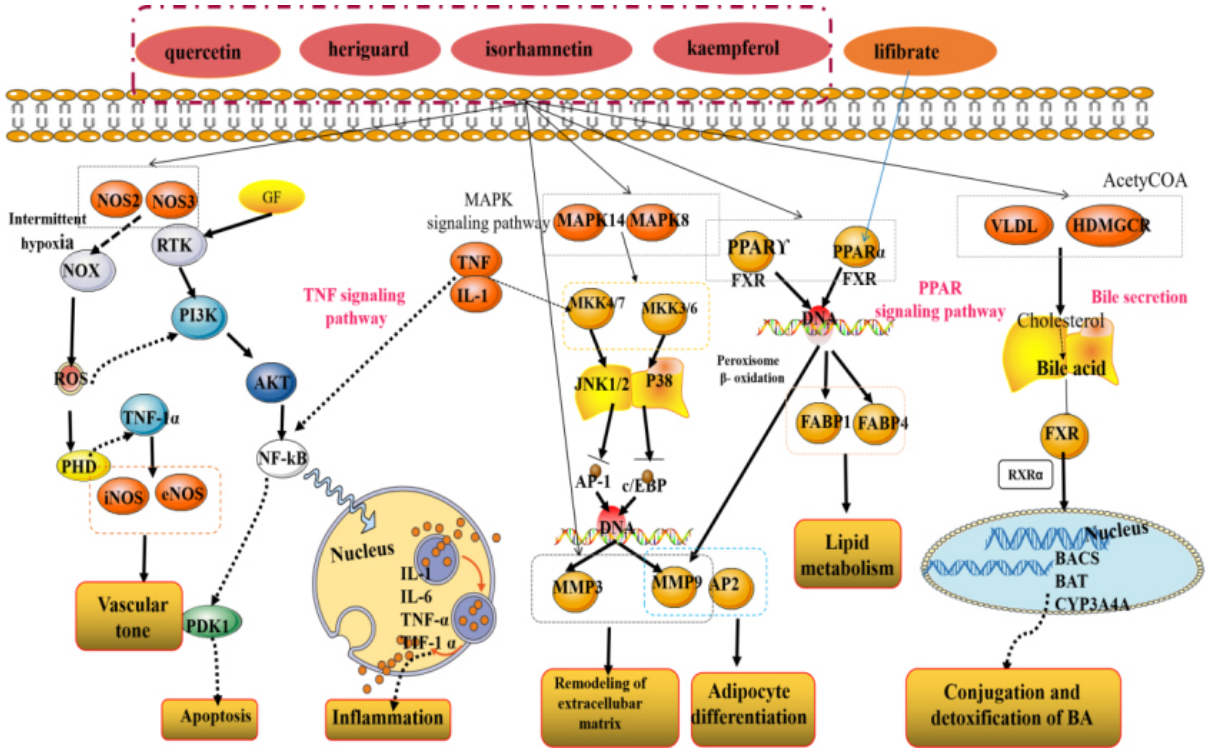

Fig. 8. Detailed signaling pathway of *Shanmei* Capsule in the treatment of hyperlipidemia.

potential mechanism of multi-ingredient, multi-targets, and multi-pathway on *Shanmei* Capsule against hyperlipidemia (Fig. 8) [26–30].

Via network pharmacology analysis, a total of 17 key active ingredients were regarded to be effective on hyperlipidemia, including quercetin, kaempferol, isorhamnetin, chlorogenic acid, ursolic acid and β-sitosterol, etc. Quercetin, one of antioxidant substances, has been reported that it could upregulate the expression of PPARα. Quercetin and its metabolites induced eNOS activity by AMPK phosphorylation, which resulted in an improvement in vessel function. They were also inhibited the formation of foam cell through activating PPARγ-ABCA1 pathway, and thereby improving lipid levels [31,32]. Foam cell accumulation in atherosclerotic lesions is a critical early stage process of atherosclerosis. Treatment with kaempferol markedly suppresses oxidized low-density lipoprotein-induced macrophage foam cell formation, which promotes an increase in cholesterol efflux and a decrease in lipid accumulation in foam cells [33]. Zhang et al. [34] indicated that isorhamnetin is a dietary source of PPARγ antagonist. Isorhamnetin treatment inhibited the adipocyte differentiation induced by the PPARγ agonist rosiglitazone, and ameliorated insulin resistance, which may be beneficial to prevent obesity development and hepatic steatosis. Several researchers reported that anti-lipidemic and Anti-diabetic effects of chlorogenic acid are mediated through the activation of AMPK (AMP-activated protein kinase). Chronic administration of chlorogenic acid inhibited hepatic glucose-6-phosphatase expression and activity, improved lipid homeostasis and skeletal muscle glucose uptake, attenuated hepatic steatosis, which in turn improved glucose tolerance, fasting glucose level, dyslipidemia and insulin sensitivity in Leprdb/db mice [35,36]. Ursolic acid is a triterpenoid compound commonly present in fruits, vegetables and TCM, and that have a wide range of biological functions, such as anti-inflammatory, antihyperlipidemic, hypoglycemic

and antioxidant properties [37]. He et al. [38] showed that ursolic acid inhibited 3T3-L1 preadipocyte differentiation and adipogenesis via the LKB1/AMPK pathway, so as to suppress 3T3-L1 preadipocyte differentiation and lipid accumulation by regulating the transcriptional factors and their downstream lipogenic targets. β-sitosterol is a plant sterol. Experimental and clinical studies have shown that β-sitosterol has hypolipidemic, anti-diabetic, hepatoprotective, anti-cancer, and anti-arthritic role [39]. In summary, the main active ingredients (e.g., quercetin, kaempferol, isorhamnetin, chlorogenic acid, ursolic acid and β-sitosterol) of *Shanmei* Capsule in the treatment of hyperlipidemia could regulate multiple cellular signaling pathways, thereby involving in the regulation of lipid synthesis and metabolism, lowering cholesterol levels, and preventing or delaying the development of dyslipidemia.

Thirty-four potential targets consist of HMGCR, MMP9, MAPK, PPARα, PPARγ, FABP4, NOS2, etc., were screened as effective targets of *Shanmei* Capsule against hyperlipidemia. HMGCR (HMG-CoA reductase), is a rate-limiting enzyme of cholesterol biosynthesis. HMGCR inhibitors (e.g., statins) are currently the mainstay of treatment for dyslipidemia, which can reduce the levels of cholesterol via blocking mevalonate synthesis [40]. MMP9, belongs to matrix metalloproteinase (MMP) molecular family, has been considered as a key target for treating metabolic diseases. Studies have shown that MMP inhibitor was closely associated with calcific aortic valve disease (CAVD) among high fat induced mice, and that these enzymes might be important targets for preventing CAVD development [41]. Intracellular mitogen-activated protein kinases (MAPK) are an important class of proline-dependent protein kinases, which involved in the regulation of various biological functions, such as inflammation, cell proliferation and differentiation. Aouadi et al. [42] demonstrated that p38MAPK increased adipogenesis via inhibition of C/EBPβ and PPARγ activations. PPAR, a member of the nuclear receptor transcription factor family, has 3 isoforms: PPARα, PPARβ and PPARγ. It is known that lipid metabolism could be regulated by PPAR, which were shown to decrease lipoprotein secretion, accelerate triglyceride catabolism, and influences serum total- and LDL-cholesterol levels. On the other hand, PPARγ stimulates the fatty acids storage in adipocytes and regulates glucose metabolism, thereby improving insulin sensitivity [43]. FABP4, also known as Adipocyte fatty acid–binding protein (A-FABP), is a major cytoplasmic protein in adipocytes. It is shown that FABP4 is expressed at high levels in adipose tissues, especially in adipocyte differentiation [44–46]. NO is generated from L-arginine catalyzed by a class of nitric oxide synthases (NOS), including neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). All three NOS isoforms were expressed in atherosclerotic plaque. Once the enzyme is expressed, iNOS is regulated by the transcription fact and catalyzes the high expression of NO. On the contrary, eNOS and nNOS produce a small number of NO in a highly regulated way [47]. The above results indicated that *Shanmei* Capsule treatment may play its role of hypolipidemic via multi-targets.

The KEGG enrichment analysis indicated that the main pathways involved in the hyperlipidemia treatment process are PPAR signaling pathway, Bile secretion and TNF signaling pathway. PPAR signaling pathway participate in many physiological processes implicated in cardiovascular diseases associated with hyperlipidemia and diabetes, including lipid metabolisms, energy metabolisms, and inflammatory reactions, etc. PPARα is an essential transcription factor involved in lipid metabolism and inflammation, and there is predominantly expressed in β-oxidation activities and tissues with high mitochondrial (e.g., liver) [48]. While PPARγ is mainly expressed in immune system and adipose tissue, where it modulates genes involved in cell differentiation, cell cycle regulation, insulin sensitivity and glucose uptake [49]. Bile acids are famous for their effects on lipid digestion and cholesterol homeostasis. The conversion of cholesterol to bile acids (i.e., bile acid synthesis) in the liver is the major pathway of cholesterol removal from the body [50], which itself is endogenous ligands of the farnesoid X receptor (FXR). FXR, together with small heterodimer partner (SHP), plays an important role in linking bile acids

regulation with lipid, lipoprotein, and glucose metabolism [51,52]. As for TNF signaling pathway, it has direct effect on inflammation, whereas many cytokines such as TNF-α are able to increase lipolysis, which in turn contributes to a chronic state of insulin resistance and increases circulating free fatty acids. It has been reported that expanding adipose tissue results in chronic low-grade inflammation that leads to the development of metabolic disorders, including insulin resistance, dyslipidemia, and type 2 diabetes [53,54].

The potential binding sites and intermolecular interaction of active ingredients for target proteins were further elaborated by docking analysis. PPARα is commonly considered as a key target of lifibrate, and at the same time it is also an important target screened from the network pharmacology of *Shanmei* Capsule. The melocule docking results confirmed the potential hypolipidemic mechanism of *Shanmei* Capsule, showing excellent affinity towards PPARα target protein. The above findings also support the network pharmacology analysis results of *Shanmei* Capsule. Therefore, such compounds could provide a molecular foundation for the subsequent development of novel pharmacological inhibitors against hyperlipidemia.

5. Conclusion

In the present study, a network pharmacology approach in combination with molecular docking was proposed to explore the underlying mechanism of action of *Shanmei* Capsule against hyperlipidemia. The active ingredients of *Shanmei* Capsule in hyperlipidemia treatment are composed of 59 ingredients. Among them, quercetin, kaempferol, isorhamsin and chlorogenic acid are main active ingredients. The possible molecular mechanism mainly involved PPAR signaling pathway, Bile secretion and TNF signaling pathway via acting on MAPK8, PPARγ, MMP9, PPARα, FABP4 and NOS2 targets. In conclusion, *Shanmei* Capsule, as a traditional Chinese medicine preparation, has multiple constituents, multiple targets and multiple pathways properties, which could serve as a promising therapeutic drug for hyperlipidemia.

Acknowledgments

This work was supported by the Key Research and Development Program of Shanxi Province (201903D321205).

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

References

[1] Nelson RH. Hyperlipidemia as a risk factor for cardiovascular disease. Primary Care. 2013; 40: 195-211.
[2] Navar-Boggan AM, Peterson ED, D'Agostino RBSr, Neely B, Sniderman AD, Pencina MJ. Hyperlipidemia in early adulthood increases long-term risk of coronary heart disease. Circulation. 2015; 131(5): 451-458.
[3] Genest J, McPherson R, Frohlich J, Anderson T, Campbell N, Carpentier A, et al. 2009 canadian cardiovascular society/canadian guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult-2009 recommendations. Canadian Journal of Cardiology. 2009; 25(10): 567-579.

[4] Zeng W, Huang KE, Luo Y, Li DX, Chen W, Yu XQ, et al. Nontargeted urine metabolomics analysis of the protective and therapeutic effects of citri reticulatae chachiensis pericarpium on high-fat feed-induced hyperlipidemia in rats. Biomedical Chromatography. 2020; 34: 4795.
[5] Mancini GB, Baker S, Bergeron J, Fitchett D, Frohlich J, Genest J, et al. Diagnosis, prevention, and management of statin adverse effects and intolerance: Canadian consensus working group update (2016). Canadian Journal of Cardiology. 2016; 32: S35-65.
[6] Hippisley-Cox J, Coupland C. Unintended effects of statins in men and women in England and Wales: population based cohort study using the QResearch database. BMJ. 2010; 340: 2197.
[7] Du Souich P, Roederer G, Dufour R. Myotoxicity of statins: mechanism of action. Pharmacology & Therapeutics. 2017; 175: 1-16.
[8] Hoffman KB, Kraus C, Dimbil M, Golomb BA. A survey of the FDA's AERS database regarding muscle and tendon adverse events linked to the statin drug class. PloS One. 2012; 7(8): 42866.
[9] Chu SM, Shih WT, Yang YH, Chen PC, Chu YH. Use of traditional Chinese medicine in patients with hyperlipidemia: a population-based study in Taiwan. Journal of Ethnopharmacology. 2015; 168: 129-135.
[10] El-Tantawy WH, Temraz A. Natural products for controlling hyperlipidemia: review. Archives of Physiology and Biochemistry. 2019; 125(2): 128-135.
[11] Fang PH, Yu M, Shi MY, Bo P, Gu XW, Zhang ZW. Baicalin and its aglycone: a novel approach for treatment of metabolic disorders. Pharmacological Reports. 2020; 72(1): 13-23.
[12] Editorial Committee of Chinese Pharmacopoeia. Chinese Pharmacopoeia. Medical Science and Technology Press 2020.
[13] Yin JJ, Qu JG, Zhang WJ, Lu DR, Gao YC, Ying XX, et al. Tissue distribution comparison between healthy and fatty liver rats after oral administration of hawthorn leaf extract. Biomedical Chromatography. 2014; 28: 637-647.
[14] Fu JH, Zheng YQ, Li P, Li XZ, Shang XH, Liu JX. Hawthorn leaves flavonoids decreases inflammation related to acute myocardial ischemia/reperfusion in anesthetized dogs. Chinese Journal of Integrative Medicine. 2013; 19(8): 582-588.
[15] Jung HJ, Sa JH, Song YS, Shim TH, Park EH, Lim CJ. Anti-Inflammatory, anti-angiogenic, and anti-nociceptive activities of the chloroform fraction of a methanol extract from Rosa Davurica Pall. leaves in experimental animal models. Immunopharmacology and Immunotoxicology. 2011; 33: 186-192.
[16] He D, Huang JH, Zhang ZY, Du Q, Peng WJ, Yu R, et al. A network pharmacology-based strategy for predicting active ingredients and potential targets of Liuwei Dihuang Pill in treating type 2 diabetes mellitus. Drug Design Development and Therapy. 2019; 13: 3989-4005.
[17] Song XQ, Zhang Y, Dai E, Wang L, Du HT. Prediction of triptolide targets in rheumatoid arthritis using network pharmacology and molecular docking. International Immunopharmacology. 2020; 80: 106179.
[18] Elokely KM, Doerksen RJ. Docking challenge: protein sampling and molecular docking performance. Journal of Chemical Information and Modeling. 2013; 53(8): 1934-1945.
[19] Comeau SR, Gatchell DW, Vajda S, Camacho CJ. C lusPro: an automated docking and discrimination method for the prediction of protein complexes. Bioinformatics. 2004; 20(1): 45-50.
[20] Wang Y, Cheng T, Bryant SH. PubChem BioAssay: a decade's development toward open high-throughput screening data sharing. SLAS Discovery. 2017; 22(6): 655-666.
[21] Gfeller D, Michielin O, Zoete V. Shaping the interaction landscape of bioactive molecules. Bioinformatics. 2013; 29(23): 3073-3079.
[22] Parikesit AA, Nurdiansyah R. The predicted structure for the anti-sense siRNA of the RNA polymerase enzyme (RdRp) gene of the SARS-CoV-2. Berita Biologi. 2020; 19: 97-108.
[23] Tambunan USF, Bramantya N, Parikesit AA. In silico modification of suberoylanilide hydroxamic acid (SAHA) as potential inhibitor for class II histone deacetylase (HDAC). BMC Bioinformatics. 2011; 12(Suppl 13): S23.
[24] Uzunlulu M, Telci Caklili O, Oguz A. Association between metabolic syndrome and cancer. Annals of Nutrition and Metabolism. 2016; 68(3): 173-179.
[25] Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry. 2010; 31(2): 455-461.
[26] Chinetti G, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. Inflammation Research. 2000; 49(10): 497-505.
[27] Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, Deeb S, et al. PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. Embo Journal. 1996; 15(19): 5336-5348.
[28] Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-Mediated Inhibition of Insulin Receptor Tyrosine Kinase Activity in TNF-α- and Obesity-Induced Insulin Resistance. Science. 1996; 271(5249): 665-668.
[29] Enslen H, Raingeaud J, Davis RJ. Selective activation of p38 mitogen-activated protein (MAP) kinase isoforms by the MAP kinase kinases MKK3 and MKK6. Journal of Biological Chemistry. 1998; 273(3): 1741-1748.
[30] Pineda Torra I, Claudel T, Duval C, Kosykh V, Fruchart JC, Staels B. Bile acids induce the expression of the human

peroxisome proliferator-activated receptor alpha gene via activation of the farnesoid X receptor. Molecular Endocrinology. 2003; 17(2): 259-272.
[31] Wang LL, Zhang ZC, Hassan W, Li Y, Liu J, Shang J. Amelioration of free fatty acid-induced fatty liver by quercetin-3-O-β-D-glucuronide through modulation of peroxisome proliferator-activated receptor-alpha/sterol regulatory element-binding protein-1c signaling. Hepatology Research. 2016; 46(2): 225-238.
[32] Sun LQ, Li E, Wang F, Wang T, Qin ZP, Niu SH, et al. Quercetin increases macrophage cholesterol efflux to inhibit foam cell formation through activating PPARγ-ABCA1 pathway. International Journal of Clinical and Experimental Pathology. 2015; 8(9): 10854-10860.
[33] Li XY, Kong LX, Li J, Zhou YD. Kaempferol suppresses lipid accumulation in macrophages through the downregulation of cluster of differentiation 36 and the upregulation of scavenger receptor class B type I and ATP-binding cassette transporters A1 and G1. International Journal of Molecular Medicine. 2013; 31(2): 331-338.
[34] Zhang Y, Gu M, Cai WJ, Yu LJ, Feng L, Zhang L, et al. Dietary component isorhamnetin is a PPARγ antagonist and ameliorates metabolic disorders induced by diet or leptin deficiency. Surface Science Reports. 2016; 18(6): 19288.
[35] Ong KW, Hsu A, Tan BKH. Anti-diabetic and anti-lipidemic effects of chlorogenic acid are mediated by ampk activation. Biochemical Pharmacology. 2013; 85: 1341-1351.
[36] McCarty MF. A chlorogenic acid-induced increase in GLP-1 production may mediate the impact of heavy coffee consumption on diabetes risk. Medical Hypotheses. 2005; 64: 848-853.
[37] Rao VS, De Melo CL, Queiroz MGR, Lemos TLG, Menezes DB, Melo TS, et al. Ursolic acid, a pentacyclic triterpene from sambucus australis, prevents abdominal adiposity in mice fed a high-fat diet. Journal of Medicinal Food. 2011; 14(11): 1375-1382.
[38] He YH, Li Y, Zhao TT, Wang YW, Sun CH. Ursolic acid inhibits adipogenesis in 3T3-L1 adipocytes through LKB1/AMPK pathway. PLoS One. 2013; 8(7): 70135.
[39] Ponnulakshmi R, Shyamaladevi B, Vijayalakshmi P, Selvaraj J. In silico and *in vivo* analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. Toxicology Mechanisms and Methods. 2019; 29(4): 276-290.
[40] Osea L, Budinski D, Hounslow N, Arnesonc V. Long-term treatment with pitavastatin is effective and well tolerated by patients with primary hypercholesterolemia or combined dyslipidemia. Atherosclerosis. 2010; 210(1): 202-208.
[41] Jung JJ, Razavian M, Kim HY, Ye YP, Golestani R, Toczek J, et al. Matrix metalloproteinase inhibitor, doxycycline and progression of calcific aortic valve disease in hyperlipidemic mice. Scientific Reports; 2016; 194(10): 225-231.
[42] Aouadi M, Laurent K, Prot M, Marchand-Brustel YL, Binétruy B, Bost F. Inhibition of p38MAPK increases adipogenesis from embryonic to adult stages. Diabetes. 2006; 55(2): 281-289.
[43] Yilmaz-Aydogan H, Kurnaz O, Kucukhuseyin O, Akadam-Teker B, Kurt O, Eronat AP, et al. Different effects of PPARA, PPARG and ApoE SNPs on serum lipids in patients with coronary heart disease based on the presence of diabetes. Gene. 2013; 523(1): 20-26.
[44] Xu AM, Tso AWK, Cheung BMY, Wang Y, Wat NMS, Fong CHY, et al. Circulating adipocyte-fatty acid binding protein levels predict the development of the metabolic syndrome: a 5-year prospective study. Circulation. 2007; 115(12): 1537-1543.
[45] Tso AWK, Xu AM, Sham PC, Wat NMS, Wang Y, Fong CHY, et al. Serum adipocyte fatty acid binding protein as a new biomarker predicting the development of type 2 diabetes: a 10-year prospective study in a Chinese cohort. Diabetes Care. 2007; 30(10): 2667-2672.
[46] Coll B, Cabre A, Alonso-Villaverde C, Lazaro I, Aragonés G, Parra S, et al. The fatty acid binding protein-4 (FABP4) is a strong biomarker of metabolic syndrome and lipodystrophy in HIV-infected patients. Atherosclerosis. 2008; 199(1): 147-153.
[47] Wilcox JN, Subramanian RR, Sundell CL, Tracey WR, Pollock JS, Harrison DG, et al. Expression of multiple isoforms of nitric oxide synthase in normal and atherosclerotic vessels. Arteriosclerosis Thrombosis and Vascular Biology. 1997; 17: 2479-2488.
[48] Schoonjans K, Staels B, Auwerx J. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. Journal of Lipid Research. 1996; 37(5): 907-925.
[49] Guan Y, Breyer MD. Peroxisome proliferator-activated receptors (PPARs): novel therapeutic targets in renal disease. Kidney International. 2001; 60: 14-30.
[50] Asgharpour A, Kumar D, Sanyal A. Bile acids: emerging role in management of liver diseases. Hepatology International. 2015; 9(4): 527-533.
[51] Zheng Z, Zhao Z, Li S, Lu X, Jiang M, Lin J, et al. Altenusin, a non-steroidal microbial metabolite, attenuates non-alcoholic fatty liver disease by activating the farnesoid X receptor. Molecular Pharmacology. 2017; 92(4): 425-436.
[52] Kim KH, Choi S, Zhou Y, Kim EY, Lee JM, Saha PK, et al. Hepatic FXR/SHP axis modulates systemic glucose and fatty acid homeostasis in aged mice. Hepatology. 2017; 66(2): 498-509.
[53] Croft M, Benedict CA, Ware CF. Clinical targeting of the TNF and TNFR superfamilies. Nature Reviews Drug Discovery. 2013; 12(2): 147-168.

[54] Oliveira MC, Menezes-garcia Z, Henriques MCC, Soriani FM, Pinho V, Faria AMC, et al. Acute and sustained inflammation and metabolic dysfunction induced by high refined carbohydrate-containing diet in mice. Obesity. 2013; 21(9): 396-406.

Supplementary material

Table S1
Grid parameters of the protein-ligand interaction for the key targets

Protein	PDB ID	Grid parameters of the protein-ligand interaction					
		Center x	Center y	Center z	Size x	Size y	Size z
AKR1B10	1ZUA	−24.9	23.9	15.0	60.0	60.0	60.0
AR	2AX6	26.9	3.5	4.4	17.8	18.5	12.5
CNR1	5TGZ	32.3	19.0	292.2	39.8	73.1	70.4
CYP19A1	3EQM	85.2	51.3	42.8	21.9	24.7	21.4
EGFR	5U8L	13.4	−4.7	−15.9	21.7	29.1	47.6
ELANE	1H1B	10.5	8.9	15.9	39.2	52.8	31.0
ERBB2	3PP0	16.4	17.4	26.2	17.4	23.9	17.2
ESR1	3UUC	−10.1	−16.2	−3.9	18.1	26.0	25.5
ESR2	3OLL	−10.6	45.0	10.2	20.4	32.2	16.6
F2	3K65	22.9	0.9	2.4	16.9	22.7	14.6
FABP4	5Y0F	5.6	−7.5	−19.2	17.4	16.1	18.6
LDLR	1IJQ	−3.1	39.3	47.6	50.0	48.0	52.0
MAPK8	4L7F	−5.1	53.8	4.3	19.1	17.3	27.5
MAPK14	6SFI	53.4	69.4	17.4	19.7	23.8	21.7
MMP1	966C	8.8	−10.9	38.9	20.4	18.4	18.8
MMP3	1D7X	−7.4	19.8	29.6	40.0	40.0	40.0
MMP9	4H1Q	29.2	6.0	18.9	37.5	22.8	25.2
MPO	5MFA	−27.5	4.9	0.3	64.4	65.2	84.7
NOS2	4NOS	3.1	95.2	17.2	26.8	19.1	30.0
NR1H4	6HL1	10.9	−14.7	12.3	17.9	23.7	14.5
PPARA	5HYK	8.7	33.9	19.3	14.5	18.0	19.8
PPARG	6T9C	14.8	17.3	17.7	21.9	17.8	18.8
PTGS2	5F19	28.1	41.0	56.6	54.1	75.2	45.6
AKR1B1	5OUK	4.5	2.7	4.8	27.0	24.7	20.5
FABP1	3STK	−0.9	−8.8	19.4	24.2	18.4	28.0
HAP90AA1	4LWE	−0.7	−36.0	−25.3	17.1	17.9	17.3