Elucidation of the active compounds and molecular mechanisms of Smilacis Chinae Rhizoma for treatment of pelvic inflammatory disease based on network pharmacology and MMGBSA-docking

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Research

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Abstract

Background Smilacis Chinea Rhizoma (SCR) is widely used in the treatment of pelvic inflammatory disease (PID). However, its active ingredients and the mechanisms against PID remain elusive. This study aimed to clarify the active ingredients and explore their molecular mechanisms on PID.

Method Network pharmacology and MMGBSA-docking exploited the active compounds and mechanisms against PID, as well as validating the binding mode of candidate targets.

Results Network pharmacology revealed 32 active compounds and 718 compound-related targets mapped to 91 pathways which were clustered 7 genres (e.g., immunoregulation). C-T-P network and PPI analysis illustrated 17 PID-related targets, indicating that SCR may decrease inflammation, ameliorate fibrosis, and inhibit microorganisms via bidirectionally regulating IL-17 signaling pathway. Furthermore, active compounds were uncovered that bound to prostaglandin-endoperoxide synthase 2, matrix metalloprotein-9, lipocalin, signal transducer and activator of transcription 3, myeloperoxidase, and tumor necrosis factor. 19 active compounds (e.g., rutin (-66.43 kcal/mol), moracin M (-37.01 kcal/mol) and oxylesveratrol (-38.84 kcal/mol)) were found to show excellent binding free energy, demonstrating that H-bond, Pi electron cloud and electrostatic potential as the main binding ability to these targets.

Conclusion Approach of network pharmacology and MMGBSA-docking revealed the active ingredients, such as rutin, moracin M, and oxylesveratrol, in SCR and dissected it exhibits the therapeutic effects (e.g., decrease inflammation, ameliorate fibrosis, and inhibit microorganisms) of PID by the bidirectional regulation of IL-17 signaling pathway.

Background

Pelvic inflammatory disease (PID), the infection and inflammation of the female upper genital tract, is a common cause of infertility, chronic pain, and ectopic pregnancy [1]. Diagnosis and management are challenging, largely due to a polymicrobial etiology that is not fully delineated [2]. Reportedly, it is estimated that 2.5 million American women aged between 18 to 44 have received a PID diagnosis in their lifetime [3], and one in eight women with a history of PID encountered difficulties in getting pregnant [4]. PID treatment is mainly based on broad-spectrum antibiotic regimens and surgical treatment [2, 5]. Antibiotics are effective in lessening short-term morbidity but have no effects on long-term complications, due to the disease’s complex mechanism and long-term process [6]. Although the incidence of PID has decreased because of screening for gonorrhea and chlamydia and the early intervention of broad-spectrum antibiotics, damage to the reproductive system caused by infection has not ameliorated [7]. Therefore, the therapeutic goal for the treatment of PID ought to include both short-term microbiological effects and long-term prevention of sequelae [6]. In addition, the use of antibiotics is limited by the emergence of antibiotic resistance and PID without an identified pathogen. In order to inhibit the progress, alleviate the long-term sequelae of PID and avoid antibiotic resistance, it is often used in conjunction with traditional Chinese medicine (TCM) [8, 9, 10].

TCM are designed to maintain the balance of body’s functions utilizing a lot of intricate compounds in herbs. Because multiple constituents may produce synergistic regulation on different targets, elucidating the mechanisms of TCM always takes lots of time and resources. There is no doubt that network pharmacology recently emerged as a new field including physiology, genetics, biochemistry and molecular simulation via integrating various research methods to investigating complicated mechanism of multiple compounds. Nowadays, network pharmacology has been applied for revealing the pharmacological mechanism of TCM from perspective of entirety. For instance, most of the ingredients from well-researched herbs are carried out molecular simulation, such as, pharmacophore matching and inverse-docking to clarify the candidate targets which is available for researchers to further illustrate the integral mechanism of TCM. Otherwise, the particle interaction, a role of fundament of integral regulation, is the same concerned, MMGBSA docking provides a view of molecular binding and a calculation of binding free energy to ensure compounds bound targets possess enough energy to engender reaction of biochemistry. Integrating network pharmacology and MMGBSA-docking makes it possible to systematically decode the active compounds and mechanism of TCM in the network.

Smilacis Chinea Rhizoma (SCR) is commonly known as ‘Baqia’ (or ‘Jin Gang Teng’) and it has been widely used in TCM for the treatment of PID and has been formulated into granules, syrup, and capsules, demonstrating a good curative effect [10–13]. Based on the previous studies, steroid saponin, flavonoids, glycoside, stilbene and organic acids were the principal chemical compounds in SCR, and demonstrated anti-cancer, anti-inflammatory, anti-oxidation and hypolipidemic effects [14]. The flavonoid derivatives such as engeletin, lsoharmatin and quercetin are the mainly constituents for anti-inflammatory and anti-oxidation by inhibiting extracellular regulatory protein kinase and Smad2/3 protein phosphorylation, thereby relieving the degree of fibrosis in the uterus [15], and reducing iron ions and scavenging free radicals respectively [16, 17]. Therefore, these ingredients group, which can be divided into four categories, are the focus of this study. However, what regulated roles these active compounds played in SCR remains unclear and relevant mechanism against PID remains to be determined.
Hence, we evaluated the whole candidate targets of active compounds and provided a perspective of the integral mechanism via enriching the functions of targets and dissected the molecular mechanism in PID using the combined approach of network pharmacology and MMGBSA-docking.

**Methods And Materials**

**Screening of active compounds**

SCR compounds were systematically listed as ligands from published paper mining [18,19] and TCMSP database (http://tcmspw.com/tcmsp.php) [20]. All compound structures from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) [21] were filtered utilizing the “Lipinski rules” of the Molinspiration database (https://www.molinspiration.com/cgi-bin/properties) [22]. In the field of drug discovery, the Lipinski rules were used to screen the compound database in order to eliminate molecules which were unsuitable for drug use, including $n·OHNH \leq 5$, $n·ON \leq 10$, $MW \leq 500$, $miLogP \leq 5$. Compounds that meet the Lipinski rules were used in network pharmacology and MMGBSA-docking [23,24]. The 2D structures (.sdf format) of all compounds were generated by ChemBioOffice2014 [25].

**Candidate targets of active compounds and pathways enrichment**

Compounds that satisfied Lipinski rules were uploaded to SwissTargetPrediction (http://www.swisstargetprediction.ch/) [26], PharmMapper (http://www.lilab-ecust.cn/pharmmapper/) [27] and SEA (http://sea.bkslab.org/) [28] to obtain candidate targets. All the genes should be from “*Homo sapiens*” in order to clarify the function of critical targets, and were proofread by Uniprot database (https://www.uniprot.org/) [29]. Finally, the whole targets were used for enrichment of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways via David database (https://david.ncifcrf.gov/) [30]. To reduce the redundancy, the newly developed Functional Annotation Clustering in David database was applied to report groups/displays similar annotations together which makes the biology clearer and more focused to be read. The grouping algorithm is based on the hypothesis that similar annotations should have similar gene members. In this sense, the more common genes annotations share, the higher chance they will be grouped together.

**Network construction**

Recently, network pharmacology has been gradually used in the field of Chinese medicine to investigate the pharmacologic mechanisms [31-33]. In this study, network pharmacology was applied to analyze the interaction between SCR and PID and the selection of critical targets. To identify the intersection of targets between PID and SCR, title of “pelvic inflammatory disease” was placed in GeneCards (https://www.genecards.org/) [34], DisGeNET (https://www.disgenet.org/) [35] and Drugbank (https://www.drugbank.ca/) [36] to obtain gene names of PID targets, which manually confirmed that each target had a clinical study in PID. Additionally, the intersection genes (compound-related and disease-related targets) were used to perform annotation analysis of the obtained crossover genes by using Gene Ontology (GO) and the KEGG pathway analysis functions in the STRING platform (https://string-db.org/) [37], with the intersection genes directly mapped to the pathway. Cytoscape was used [38] to visualize a network of “Compound-target-pathway”, as well as networks of “Compound-candidate targets” and “Functional Annotation Clustering of KEGG Pathway” in “Candidate targets of active compounds and pathways enrichment”. In these networks, each compound, target and pathway were indicated by node, and the interactions between each node were described by edges. These networks were established to project an overview of the interactions among compounds, targets and pathways.

**Protein–protein interaction (PPI) analysis**

In order to reveal the direct and indirect roles active compounds of SCR played in the pelvic inflammatory targets. The intersection targets were introduced STRING platform and generated a graphical network of PPI. In the network, each node represents all the proteins produced by a single, protein-coding gene locus, and edges represent protein-protein associations which are meant to be specific and meaningful, i.e. proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding each other. Ultimately, to clarify interfering mechanism compounds broke into PPI network, targets with high degree and relating to critical pathway in the PPI network were selected to operate MMGBSA-docking.

**Docking between PID crucial targets and active compounds**

Due to the algorithm defect of the database on target prediction, a thorough docking was performed to improve the credibility of network pharmacology. The crystal structures of selected targets were obtained from RCSB (http://www.rcsb.org/) [39], and the crucial targets were docked with active compounds filtered by Lipinski rules.

Schrödinger Glide was used to pretreat the 3D protein structure for docking, including adjusting the bond orders to ensure the stability of the chemical bonds between the atoms, adding the missing hydrogen atoms and amino acid residues, optimizing the orientation of amino acids.
and hydrogen atoms, and optimizing the distribution of H-bonds, removing water molecules and heterogeneous molecules. Finally, energy minimization with a force field OPLS-2005 was supplied.

At the end of pretreatment, Sitemap attached to Maestro was used to identify top-ranked potential receptor binding sites, following which the Receptor Grid Generation module was employed to select the active cavity and generate a grid in protein. Next, the active compounds were introduced to Maestro and optimized by the liquid simulation of OPLS-2005 all-atoms force field in the LigPrep module, as well as combined into a ligand package. The docking accuracy was standard (SP), as well as flexible docking. At this point, the docking preparation was completed, and the scaling factor and partial charge cutoff of van der Waals radius scaling 1.0 and 0.25 were used to generate the grids on active sites and ligand package were selected to perform molecular docking in the Ligand Docking module. Next, the generated Glide Gscore was used to assess the affinity between compounds and proteins. Moreover, crucial targets were docked with their self-ligands to set positive contrast, and their Glide Gscores were used to measure whether compounds possessed a good affinity to the protein and standardized the score of compounds to be visualized as a heat map by MeV [40].

**Binding free energy calculation (MM-GBSA) based on SP docking**

Good poses and good score are obvious by SP docking but what was the binding free energy of the docked complex was another problem. Docking results showed the active compounds did bind to the active site of protein but could this association last long enough to elicit any potential biological response, as biological response largely depends upon the binding free energy of the association. Therefore, the docked complexes in SP mode were subjected to binding free energy calculation (MM-GBSA) using prime module of Maestro [41]. A total of 19 active compounds were selected for this analysis.

**Results**

**Network of active compound-candidate targets**

After filtering was operated for Lipinski rules, thirty-two in sixty-eight compounds were screened as active compounds in SCR. and listed in Supplementary Table 1. Chemical formats of smile and sdf were generated to predict the potential targets. Then 718 potential targets (some were duplicates) associated with compounds were screened from the SwissTargetPrediction, PharmMapper, and SEA server, respectively. Fig. 1 elucidated the relationship between compounds and candidate targets. Three main clustering huddles consisted of 32 active compounds indicated that different compounds were apt to interact different targets. Interestingly, the candidate targets of organic acids, such as vanillic acid, syringic acid, oleanolic acid and protocatechuic acid, and avonoids both had apparently characteristic targets and common targets between each other. This kind of phenomenon was based on a lot of complicated compounds in traditional herbs such as SCR.

**Enriched analysis of candidate targets**

To further explore the crucial mechanism of compound-related sophisticated targets, 718 targets were uploaded David database to obtain KEGG pathways to clarify the potential functions of compounds. Due to the defect of database, only 715 targets were identified and associated with 91 effective KEGG pathways (Supplementary Table 2). For sake of better interpreting the principal function of pathways, Functional Annotation Clustering was carried out. As Fig. 2 shown, seven annotation clustering huddles comprised of 91 KEGG pathways respectively were related to metabolism, nervous system, nervous and humoral regulation, hormone production and release, infection, immunoregulation, acid and lipid metabolism and comprehensive regulation. The result manifested effect of the multiple and potential regulations of SCR. All above were corresponded to the existing researches [42-45].

**Compound-target-pathway network and analysis of GO and KEGG**

In the above results, we especially concerned the immunoregulation and the inflammatory pathway in comprehensive regulation, therefore, we further researched the essential mechanisms on PID. Firstly, eighty-six PID-related targets were retrieved from databases. Then, a total of 17 Intersection targets were obtained and uploaded to STRING platform to enrich functions. Next, the top five results of GO enrichment (A) and the top 10 KEGG pathways (B) were selected as shown in Fig. 3. The biological processes, including response to chemical, cellular response to chemical stimulus, response to toxic substance, female pregnancy, etc., indicated that the predicted proteins commonly respond to exogenous substances and participate in female pregnancy. Furthermore, the molecular function and cellular component indicated that compounds may affect the cytokine receptor, antioxidant activity, and peroxidase activity by binding to the proteins. To clarify the relationship between herb and disease, a network of the compounds, targets, and pathways were visualized by Cytoscape. We found that a total of 28 compounds could act on 17 key targets and associate with 20 effective pathways (Fig. 4), a bigger node implying the degree of multiple regulations.

**PPI network analysis**
17 intersection targets were analyzed using the PPI network in STRING platform (Fig. 6). Relevant parameters of network were as follows, 1. number of nodes and edges: 17 and 76, 2. average node degree and expected number of edges: 8.94 and 19, 3. PPI enrichment p-value: <1.0e-16. Otherwise, Red nodes (MMP9, TNF, IL6, PTGS2, LCN2) and purple nodes (IL2, STAT3, IL6) were used to highlight the IL-17 signaling pathway and Th17 cell differentiation, respectively. MPO mentioned in “3.3” and all highlighted targets except inflammatory factor (IL2 and IL6) were selected as core targets to illuminate the SCR interfering mechanism against PID utilizing MMGBSA-docking.

**Molecular docking with binding free energy based MMGBSA**

The heat map was employed to stick out the features of 32 active compounds as shown in Fig. 7 (Original data is presented in Supplementary Table 1). According to the distance metric of Average Dot Product in Mev, compounds that had a high activity clustered together excellently, with a high affinity to PTGS2, LCN2, TNF, MPO, STAT3, and MMP-9. Rutin (10; -10.758), moracin M (44; -9.326) and oxyresveratrol (30; -8.098) were found to occupy the top score, which exceeded or neared the original ligands in verified docking (Supplementary Table 3). Furthermore, compounds demonstrated a binding affinity to one or several targets. According to the cutoff of 0.5 (Fig.7), 19 capital protein-ligand molecular interactions were analyzed and are illustrated in Table 1.

Further analysis demonstrated that rutin possessed a strong binding ability to LCN2, TNF (Fig.8 D, D1: rutin and LCN2; Fig.8 E, E1: rutin and TNF), especially with PTGS2. As shown in Fig.8 A, A1, based on the geometric matching, it was observed that rutin and the active cavity of PTGS2 protein matched well, completely wrapping in the loop region formed by amino acids. In terms of interaction, the electrostatic potential ([purplish-blue: positive region, such as -OH]) of rutin adapted to surface of the protein (A), and the two glycosides on rutin formed three hydrogen bonds (B) with GLN 434, HIE 214, and TYR 385, indicating that the compound was stable in the pocket of PTGS2. As shown in Fig.8 B, B1, oxyresveratrol (-38.84 kcal/mol) bound to MPO by forming six H-bonds with ARG C 504, NAG D 641, TRP A 32, ARG A 31, ARG B 31, TRP B 32. The aglycon with a big volume only demonstrated two -OH, with a lower electrostatic potential compared with glycosyl, indicating that compound was situated at a stable region. Alternatively, Fig.8 C, C1 demonstrated that moracin M (-37.01 kcal/mol) separately formed one interaction of pi-cation and three H-bonds with LYS A 45, GLU A 62, GLN A 96, GLU B 360, affecting the spatial configuration of STAT3. Finally, Fig. F, F1 indicated that oxyresveratrol formed two Pi-pi stacking with TYR 179 and PHE 192, three H-bonds with ALA 191, HIS 210, and GLY 233, and the distribution of electrostatic potential focus on the benzene ring on both sides, thus approaching the surface of MMP-9, which illustrated the formation process of a stable configuration. Based on the above results, the H-bond and electrostatic potential were the main factors regulating the function of targets.

**Discussion**

Traditionally, in modern medicine, drugs were designed to target specific proteins relevant to the disease. In TCM, herbs can possess even hundreds of compounds, and each compound could have multiple targets. Network pharmacology provided holistic perspective (e.g., Compound-targets; targets-pathway; Compound-target-pathway-disease; PPI analysis) to dig out the potential mechanisms for illustrating the integral regulation-based active compounds of TCM and conducting the consequent experiment.

According to C-T-P network, we proposed a simple inference as shown in Fig. 5 (Details are presented in Supplementary Fig. 1), with the IL-17 signaling pathways (the highest value of -log10(FDR)) considered potentially efficacious by means of in-depth excavation of the above network. IL-17 signaling pathway reportedly had dual regulatory roles in pro-inflammatory and host defense processes [46, 47]. On the one hand, excessive secretion of IL-17A and IL-17F from Th17 cells can induce massive inflammatory factors including IL-6, IL-1β and TNF-α. IL-17 signaling pathway can also synthesize prostaglandin E2 (PGE2) by inducing prostaglandin-endoperoxide synthase 2 (PTGS2), and the vasodilator effect of PGE2 also promoted inflammatory cells to enter the site of inflammation, so the activation of IL-17 signaling pathway had a strong pro-inflammatory effect [48]. In previous study, SCR down-regulated the expression of IL-6, IL-1β, TNF-α, IL-2 and PTGS2 in PID model rats, but did not elaborate the down-regulation mechanism [15, 19]. Therefore, combined with the results of this study, this may be the SCR inhibition of IL-17 signaling pathway.

On the other hand, in the process of immunoregulation, the regulation of IL-17 signaling pathway can recruit neutrophil to the inflammatory region to release MPO [49] and induce gene expression of neutrophil gelatinase-associated lipocalin (LCN2) and matrix metalloproteinases (MMPs) [46, 50]. These proteins made important impacts in host defense. For instance, matrix metalloprotein 9 (MMP-9) was activated to promote embryo formation, wound healing, transfer of inflammatory cells [51, 52]. Interestingly, it was reported that SCR up-regulated the expression of MMP-2 and MMP-9 in PID rat models and down-regulated the MMPs inhibitor TIMP-1, thereby restoring the balance between MMPs and TIMP-1 and reducing tissue fibrosis during PID [15]. Collectively, these results demonstrated the multi-target regulation of compounds in SCR.

Although the above network provided a clear view of integral regulation of SCR, it was still a barrier to validate the facticity of each research data and was difficult to give consideration to the critical effect of each result. Hence, how to select a crucial result (targets or pathways) to
concern and validate was a issue worth pondering carefully for researchers. Therefore, PPI analysis played an essential role which help us find the key targets and pathways and explore that a single target can cause an radioactive effect in the network. In this study, IL-17 signaling pathway and Th17 cell differentiation-related targets were considered focuses in the PPI. As previously reported, prostaglandin-endoperoxide synthase 2 (PTGS2) is inducible and usually produces inflammatory prostaglandins, which mediate responses to physiological stress (infection, inflammation), stimulate chronic inflammation, and are a target for non-steroidal anti-inflammatory drugs (NSAIDs) [53]. As shown in Table 1 and Fig. 8, the binding capacity of rutin to PTGS2 (-66.43 kcal/mol) was greater than that demonstrated by its own ligand, suggesting that rutin could be a potential novel inhibitor, and down-regulate the activity of this enzyme. Additionally, all the active compounds (Fig. 7) mostly belonged to flavonoids and stilbenes. Based on existing pharmacodynamic investigations, these flavonoids and stilbenes have achieved obvious anti-inflammatory effects and ameliorated fibrosis in PID animal models by inhibiting the synthesis or release of histamine, 5-HT, and PGE2, as well as enhanced the production of MMP-9 in uteri [15, 56]. Particularly, engeletin (1), polydatin (4) and resveratrol (5) have inhibited the release of IL-6 and TNF-α [57]. Rutin (10) inhibited the release of IL-2 and TNF-α[19]. These results indicated that active ingredients inhibit PTGS2 to decrease the synthesis of PGE2 [42], including rutin (10), polydatin (4), oxyresveratrol (30) and piceatannol (21), demonstrating a good binding ability. Oxyresveratrol indicated a high affinity to MMP-9 and may be the cause of up-regulation. MPO and LCN2 which inhibited or eliminated the microorganisms [54, 55] were regulated by polydatin and rutin. So far, a part of results in network pharmacology has been validated and discussed via MMGBSA-docking and paper-mining, which can yet be regarded as effective method to elucidate the active compound of SCR and relevant mechanisms against PID.

Conclusions And Further Prospect

Network pharmacology and MMGBSA-docking methods were applied to explore the active ingredients of SCR and provided an integral view of mechanism against PID. The principal regulating function of 32 potent ingredients for treatment of PID were uncovered, we found that 718 candidate targets map to 91 effective pathways which were mainly clustered seven genres (e.g., infection, immunoregulation and comprehensive regulation). Furthermore, in the PPI and C-T-P network analysis, 17 PID-related targets mapped to IL17-signaling pathway and Th17 cell differentiation. After MMGBSA-docking, a total of 19 active compounds, including rutin (-66.43 kcal/mol), moracin M (-37.01 kcal/mol) and oxyresveratrol (-38.84 kcal/mol) showed greater binding force to the therapeutic targets.

Overall, active ingredients of SCR exhibited a strong affinity to therapeutic targets of PID, thereby contributing to decreasing inflammation, ameliorating fibrosis, and inhibiting or eliminating microorganisms via bidirectional regulation of IL-17 signaling pathway. However, it was a time-consuming and risky process to draw this kind of conclusion, analysis of the network had to be up against a problem on how to select the principal results to focus, which is a big challenge but a core in network pharmacology. Therefore, valid validation of results (e.g., MMGBSA-docking, animals and biochemistry) is as same important. Through the analysis of this study, IL-17 pathway was found to probably play a critical role in the development and treatment of PID, but relevant research was lacking and incomplete. Hence, we are devoted to in-depth investigation of the therapeutic mechanisms on PID.

Abbreviations

TCM: Traditional Chinese Medicine; SCR: Smilacis Chinea Rhizoma; PID: Pelvic Inflammatory Disease; TCMSP: Traditional Chinese Medicine System and Pharmacology; SEA: Similarity ensemble approach; PPI: Protein-protein interaction; MMGBSA: Molecular Mechanics Generalized Born Surface Area; OPLS-2005: Optimum-polarized ligand simulation-2005; SP: Standard precision; KEGG: Kyoto Encyclopedia of Genes and Genomes; GO: Gene Ontology; NSAIDs: non-steroidal anti-inflammatory drugs; C-T-P: Compound-target-pathway.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agree to publish this paper.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interest
The authors declare that they have no competing interests.

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Author's contributions

Yi Zhang, Gang Fan, Yunsen Zhang designed this study; Zikuang Zhao, Huimin Chen colleted the relevant data, Yunsen Zhang drafted the manuscript; Wenxiang Wang, Qi Li, Xuanhao Li Yi Zhang, Gang Fan proofread the manuscript.

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### Tables

| Table 1 Docking table with bonding characterization and binding energies in kcal/mol with 19 active compounds involved in six therapeutic targets. Bold font indicates two same bonds, MMGBSA dG Bind: The binding energy of the receptor and ligand as calculated by the Prime Energy, a Molecular Mechanics + Implicit Solvent Energy. Function (kcals/mol) = PrimeEnergy(Optimized Complex) - PrimeEnergy(Optimized Free Ligand) - PrimeEnergy(Optimized Free Receptor) |
|---|

| No. | Name         | Structure | Glide G score | MMGBSA dG Bind (kcal/mol) | Bonding Interaction | Bond Length(Å) | Bond Type | Binding Protein |
|-----|--------------|-----------|---------------|---------------------------|---------------------|---------------|-----------|-----------------|
| 1   | Engeletin    | ![Structure](image1) | -8.342        | -28.9                     | ASP A 158, ASP A 97, SER A 144, TYR A 27, LYS A 45 | 1.76, 1.53, 1.72, 1.69, 2.70 | H-acc, H-don | STAT3 (PDB ID: 5ax3) |
| 2   | Isoengeletin | ![Structure](image2) | -7.195        | -42.43                    | APS A 158, ASP A 97, ASP A 102, TYR A 27, MET A 99 | 1.86, 1.84, 2.01 | H-acc, H-don | STAT3 |
| 3   | Astilbin     | ![Structure](image3) | -7.839        | -44.37                    | ASP A 102, ASP A 97, ASP A 158, ASN A 145, SER A 144, MET A 99 | 2.43, 2.36 | H-acc, H-don | STAT3 |
| 4   | Polydatin    | ![Structure](image4) | -8.846        | -44.64                    | ASP A 158, GLU A 62, GLU B 360, ASN A 145, SER A 144, TYR A 27, GLN A 45, LYS A 45 | 1.67, 1.97, 1.75, 2.09, 1.94, 2.50, 2.06 | H-acc, H-don | STAT3 |
| 5   | Resveratrol  | ![Structure](image5) | -7.252        | -36.38                    | ASP D 321, TRP B 32, LYS C 505 | 1.76, 1.62 | H-acc, Pi-Pi stacking | MPO (PDB ID: 5f19) |
| 10  | Rutin        | ![Structure](image6) | -10.758       | -66.43                    | TYR A 385 | 1.96 | H-acc, H-don | PTGS2 |
| 11  | Kaempferol   | ![Structure](image7) | -7.734        | -47.73                    | ALA B 191, GLY B 233, HIS B 230, PHE B 110 | 2.16, 2.28 | H-acc, H-don, Pi-Pi stacking | MMP9 (PDB ID: 5ue4) |
| 12  | Dihydrokaempferol | ![Structure](image8) | -8.167        | -32.62                    | GLU A 62, ASP A 158, GLU B 360, MET A 99, GLN A 96, LYS A 45 | 1.68, 2.44, 2.24, 2.33 | H-acc, H-don | STAT3 |
| 17  | Quercetin    | ![Structure](image9) | -8.367        | -38.06                    | ASP A 158, GLU B 360, GLU A 62, MET A 99, GLN A 96, LYS A 45 | 1.71, 1.57, 1.60, 2.29 | H-acc, H-don | STAT3 |
| 18  | Isorhamnetin | ![Structure](image10) | -6.347        | -45.64                    | ARG A 81, LYS A 125, TYR A 106 | 1.84, 1.84 | H-acc, H-don, Pi-Pi stacking | LCN2 (PDB ID: 1x89) |
| 21  | Piceatannol  | ![Structure](image11) | -9.223        | -53.00                    | PHE A 210, HIS A 386, THR A 212 | 1.67, 2.02, 1.67, 2.02 | H-acc, H-don | PTGS2 |
| 30  | Oxyresveratrol| ![Structure](image12) | -8.098        | -38.84                    | ASP D 321, TRP B 32, ARG A 31 | 1.75, 1.62, 1.70 | H-acc, H-don, Pi-Pi stacking | MPO |
| 43  | Maackoline   | ![Structure](image13) | -8.503        | -52.32                    | ALA A 199, TYR A 385, GLN A 454, HIS A 386 | 1.97, 2.00, 2.51 | H-acc, H-don, Pi-Pi stacking | PTGS2 |
| 44  | Moracin M    | ![Structure](image14) | -9.326        | -37.01                    | GLU A 62, GLU B 360, GLN A 96, LYS A 45 | 1.58, 1.62, 2.14 | H-acc, H-don, Pi-Pi stacking | STAT3 |
| 47  | Gentisic acid| ![Structure](image15) | -5.852        | -23.11                    | TYR A 106, LYS A 125, LYS A 125 | 1.87, 2.05 | H-acc, H-don, Pi-Pi stacking | LCN2 |
| 48  | Dihydroquercetin| ![Structure](image16) | -6.874        | -42.01                    | TYR D 151, TYR D 59 | 1.76 | H-acc, Pi-Pi stacking | TNP (PDB ID: 2ax5) |
| 58  | Kaempferide  | ![Structure](image17) | -7.962        | -47.23                    | GLY B 233, ALA B 191, HIS B 230, PHE B 10 | 2.37, 2.13, 2.62 | H-acc, H-don, Pi-Pi stacking | MMP9 |
| 60  | Gramine      | ![Structure](image18) | -7.419        | -31.00                    | GLY C 121 | 2.15 | H-acc, H-don, Pi-Pi stacking | TNF |
| 62  | Dihydrokaempferide | ![Structure](image19) | -8.275        | -34.39                    | TRP A 387, THR A 206 | 2.27, 1.89 | H-acc, H-don, Pi-Pi stacking | PTGS2 |
Figure 1

Network of 32 candidate compounds predicted to have 718 candidate protein targets. The network consists of active compounds and candidate targets, including 749 nodes and 2681 edges. The candidate targets of organic acids, such as vanillic acid, syringic acid, oleanolic acid and protocatechuic acid, and flavonoids both have apparently characteristic targets and common targets between each other.

Figure 2

Functional Annotation Clustering of KEGG Pathway enriched by 715 candidate targets. The network consists of KEGG pathways and annotation clustering huddles, including 99 nodes and 93 edges. 7 annotation clustering huddles comprised of 91 KEGG pathways respectively were related to metabolism, nervous system, nervous and humoral regulation, hormone production and release, infection, immunoregulation, acid and lipid metabolism and comprehensive regulation.
Figure 3
GO and KEGG pathway enrichment of intersection targets. A indicates GO analysis; B indicates KEGG pathways. Color of word: red denotes Biologic Process; blue denotes Molecular Function; green denotes Cellular Component; gray denotes KEGG Pathway.

Figure 4

Compound-target-pathway (C-T-P) network. The network consists of compounds, targets, and pathways, including 64 nodes and 146 edges. 28 components interact with 17 target proteins and are associated with PID through 20 pathways. Blue indicates KEEG pathways, pink indicates compounds, green indicates targets, and purple indicates IL-17 signaling pathway and mapped targets.

Figure 5

Regulation mechanism in PID rat model. Orange implies targets of PID; brown implies signaling factors; Gray implies the potential mediator; “↑” implies up-regulation; “↓” implies down-regulation; “?” implies uncertainty.
Figure 6

Protein-protein interaction analysis of 17 PID-related targets. Red node (MMP9, TNF, IL6, PTGS2, LCN2) and purple node (IL2, STAT3, IL6) were used to highlight the IL-17 signaling pathway and Th17 cell differentiation, respectively.
Figure 7

Clustering heat map between compounds and PID targets. Due to restraint of software, & indicates ",". Dimethoxy and glucopyranosylcinnamic abbreviate "Di" and "glu" respectively. 19 active compounds were operated MMGBSA-docking in the cutoff of 0.5.
Figure 8

Binding sites between compounds and proteins. A, A1: rutin and PTGS2; B, B1: oxyresveratrol and MPO; C, C1: moracin M and STAT3; D, D1: rutin and LCN2; E, E1: rutin and TNF; F, F1: oxyresveratrol and MMP9. A, B, C, D, E, F imply 2D binding models; A1, B1, C1, D1, E1, F1 imply interaction of electrostatic potential.

Supplementary Files

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