

Prediction of the Active Ingredients, Potential Targets, and Signaling Pathways in ZaoRenDiHuang Capsules for Treatment of Insomnia Based on Network Analysis and Molecular Docking

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Research

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Abstract

Background Insomnia is a major global public health issue with a high incidence, which presents a significant economic burden. Importantly, insomnia is often accompanied by a myriad of symptoms during the daytime, the most common being insomnia dizziness, headache, malaise, fatigue, anxiety, and even contribute to several diseases. However, the action mode of multi-component and multi-target for Chinese medicine could be a promising therapy for insomnia. According to the previous research, the ZaoRenDiHuang (ZRDH) Capsules showed the noteworthy anti-insomnia effect. Up to now, active ingredients, potential targets, and signaling pathways and mechanism of action are not yet clear. In this study, network pharmacology was employed to elucidate the potential anti-insomnia mechanism of ZRDH.

Methods In this study, an integrated pharmacology approach was implemented, which involved evaluation of absorption, distribution, metabolism and excretion of ZRDH, data mining of the insomnia targets, protein-protein interaction (PPI) network analysis, enrichment analysis, and molecular docking simulation, to predict the bioactive components, potential targets, and molecular mechanism of ZRDH for insomnia.

Results In this work, 44 anti-insomnia components of ZRDH and 65 anti-insomnia targets of insomnia were filtrated through database mining. The Drug-Disease network was constructed and

five key components Jujuboside A, Schizandrin A, Schizandrin C, Schizandrin B, and Spinosin, were further obtained. Sixty-five key targets were identified by topological analysis. Sequential studies turned out, NMURI, CAICR, GABA, TAER2, ORDS, CYS1TR2, HTR1B, TLR4 were the common key targets. Docking studies indicated that the bioactive compounds could stably bind the pockets of target proteins. The findings of Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotation suggested that the Neuroactive ligand-receptor interaction, Serotonergic synapse CAMP signaling pathway, HIF-1 α signaling pathway, Toll-like receptor signaling pathway, anti-insomnia through data mining and network analysis.

Conclusion: In summary, potential mechanisms involved in ZRDH treatment for insomnia involves multiple components and multiple target points as well as multiple pathways. These findings may offer a profile for further investigations of the anti-fibrotic mechanism of ZRDH.

Background

Insomnia is a major global public health issue with a high incidence, which presents a significant economic burden [1]. Globally, approximately 25% of people are not satisfied with their sleep, 10–15% report symptoms of sleepless related to daytime consequences [2–4]. For the health-care system, the economic burden of Insomnia is US\$ 5,010 per person per year, while individuals with good sleep well are US\$ 421 per year [5]. Importantly, insomnia is often accompanied by a myriad of symptoms during the daytime, the most common being insomnia dizziness, headache, malaise, fatigue, anxiety, and even

contribute to several diseases [6, 7]. The strong association are between chronic insomnia and medical disorders [8]. A meta-analysis of 13 prospective studies demonstrated that insomnia is related to an increased risk of developing cardiovascular events [9]. In the US National Health Interview Survey and the US National Comorbidity Survey, findings consistently showed that insomnia was likely to present with anxiety, mood, impulse-control, and substance misuse disorders [4–10]. Therefore, it is of utmost urgency to implement effective medical management for insomnia.

Currently, treatment for insomnia includes psychobehavioural intervention and pharmacological treatment. Although psychobehavioural therapy is generally well accepted by patients, it is not always feasible due to the scarce availability of clinicians with psychobehavioural skills [11–13].

Thus, pharmacological therapy is the first treatment choice for sleepless patients including antihistamines, melatonin, and herbal preparations, BzRAs, chronobiotic agents, and low-dose doxepin hydrochloride, antidepressants, antipsychotics, and anticonvulsants [1]. However, barriers to implementation include several side-effects such as drowsiness, dizziness, daytime sedation, cognitive impairment, motor incoordination, ataxia, gastrointestinal upset, weight gain, and hypomania [14–19]. Furthermore, these drugs are addictive and can produce physical and psychological dependence after use. It is an urgent need to develop alternative therapeutics.

In China, most of patients turn to Chinese medicine (CM) for insomnia. Two recent meta-analyses demonstrated that Chinese medicine could correct the imbalance and effectively improve sleep [20, 21]. ZaoRenDiHuang Capsule (ZRDH) was approved by the China Food and Drug Administration for treatment of insomnia. ZRDH comprises five different kinds of medicines as illustrated in Table 1. The previous study reported that ZRDH could improve the quality of sleep and relieve anxiety and depression. Yet, the underlying mechanisms and the targets of ZRDH remain unclear.

Table 1
Five different kinds of herbal medicines in ZaoRenDiHuang Capsule (ZRDH).

Latin name	English name	Chinese name	Abbreviation
Fructus Schisandrae Chinensis	Chinese magnoliavine fruit	Wu Wei Zi	WWZ
Semen Ziziphi Spinosae	Spine date seed	Suan Zao Ren	SZR
Rhizoma Coptidis	Golden thread	Huang Lian	HL
Radix Scutellariae	Baical skullcap root	Huang Qin	HQ
Radix Rehmanniae Recens	Fresh rehmannia root	Di Huang	DH

Given the characteristics of a ZRDH compound, the composition is complex. To reveal the main pharmacological action mechanisms of ZRDH, we employed a network pharmacology strategy. Network pharmacology is based on high-throughput omics data analysis, network database retrieval, and it combines systems biology with multidirectional pharmacology, which could provide a novel strategy to research the mechanism of action of complex CM formulae [22–24]. In this work, we performed a

network pharmacology approach to predict the active ingredients, potential targets, and signaling pathways in ZRDH for treatment of insomnia and implemented the molecular docking validation. The flowchart of the whole study design is illustrated in Fig. 1.

Materials And Methods

Screening the active compounds and putative targets of ZRDH

In this work, we identified the chemical components of ZRDH from the traditional Chinese medicine system pharmacology database and analysis platform (TCMSP, <https://tcmospw.com/tcmosp.php>. 2020-11-14) [25]. The parameters are set as following: the oral bioavailability (OB) was set to $\geq 30\%$ [26], the drug-likeness (DL) was set to ≥ 0.18 [27], and the drug half-life (HL) was set to ≥ 4 hours [28]. Based on the components identified, corresponding putative targets were predicted by the spatial conformation of the collected compounds from Swiss Target Prediction Tools in Swiss Institute of Bioinformatics (SwissTargetPrediction, <http://www.swisstargetprediction.ch/>. 2020-11-14) [29, 30].

Collection Of Target Proteins For Related Insomnia

We used the 'Insomnia' and 'Sleepless' as keywords in the therapeutic target database (TTD, <https://db.idrblab.org/ttd/>.2020-11-14) [31], the Drugbank database (Drugbank, <https://www.drugbank.ca/>.2020-11-14) [32], OMIM database (OMIM, <https://omim.org>.2020-11-14) and DisGeNET database (DisGeNET, <http://www.disgenet.org/>. 2020-11-14) [33, 34], to search for insomnia-related targets, convert the target protein name to gene name through the UniProt database (<https://www.UniProt.org/>)[35]. Subsequently, we acquired all insomnia targets after removing repetitive targets.

Constructing A Protein-protein Interaction (ppi) Network And Enrichment Analysis

We constructed the Venn diagram to show the numbers of targets obtained from ZRDH and Insomnia. The shared targets between ZRDH and Insomnia might be potential targets for ZRDH in treating insomnia. Then, the overlapping targets were processed by the STRING database to construct the protein-protein interaction (PPI) network. PPIs with high confidence ranges (high > 0.7) for data scores were included in this work [36, 37]. Subsequently, a potential key target network was constructed by Cytoscape 3.7.1 (<https://www.cytoscape.org/>.2020-11-14), and the network parameters were systematically analyzed [38][39].

To illustrate the potential mechanism of ZRDH and its effects on insomnia, the functional information was calculated and analyzed using the database for annotation, visualization and integrated discovery (DAVID) tools (DAVID version 6.8, <https://david.ncifcrf.gov/>, 2020-11-14), and P-value < 0.01 and FDR < 0.05 were set as the parameter for differential screening [40]. The results of the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were visualized by R software 3.4.1.

Screening for hub genes via topological analysis and cluster analysis

PPI network was analyzed according to degree centrality (DC), Betweenness centrality (BC), and close centrality (CC). The filter parameters were set to greater than the median. In order to further elaborate the classification of hub genes, we implemented a cluster analysis via MCODE algorithm. MCODE finds clusters (highly interconnected regions) in a network. Clusters mean different things in different types of networks. For instance, clusters in a protein-protein interaction network are often protein complexes and parts of pathways, while clusters in a protein similarity network represent protein families [41].

Constructing the interaction network of ZRDH treating Insomnia and Molecular docking validation

The targets from the filtered PPI network were mapped to drug ingredients. Accordingly, we constructed the interaction network between ZRDH and Insomnia. Subsequently, the above-mentioned interaction network analysis generated potential hub genes active in ZRDH treatment. Binding activity between the active drug components and the hub genes were evaluated by flexible molecular docking using Surflex-Dock software [42]. Surflex-Dock uses an idealized active site ligand called a prototype molecule as a target for generating a hypothetical conformation of a molecule or a molecular fragment [42]. These hypothetical conformations are all scored by the Hammerhead scoring function, which also serves as the objective function for the local optimization of the conformation [43]. Through the crossover process, a large number of conformations are assembled from the complete molecule to achieve flexible docking. The crystal structure of the target proteins was obtained from the Protein Data Bank (PDB, <https://www.rcsb.org/>)[44]. The structural formula (MOL2 format) of the compounds were available at the TCMSP database and PubChem. The protein targets were processed by removing water, adding hydrogen, and extracting the ligand structure accordingly and finally, Surflex-Dock v.2.1 was run to perform molecular docking.

Results

Screening the active compounds and putative targets of ZRDH

A total of 61 compounds were screened. Of these compounds, 7 were from Wuweizi, 8 from Suanzaoren, 6 from Baishao, 23 from Huangqin, 7 from Baishao, 2 from Dihuang and 8 from Huanglian. Accordingly, 723 targets were identified via Swiss Target Prediction Tools. The potential compounds of the ZRDH

formulation and the respective ADME parameters are shown in detail in **Supplementary Material 1**. And the potential targets of ZRDH in detail are shown in **Supplementary Material 2**.

Constructing A Protein–protein Interaction (ppi) Network And Enrichment Analysis

The intersection (222 common targets) between drug targets and disease targets, is shown in Fig. 2 and the details of the shared targets are shown in **Supplementary Material 3**. Using the String database, a protein–protein interaction (PPI) network was constructed. 207 common targets were identified with network attributes (207 Nodes, 1167 Edges) (Fig. 3A). And then over the top of that, enrichment analysis was performed via DAVID. GO enrichment analysis yielded GO entries ($p < 0.01$, $FDR < 0.05$) comprising 320 biological processes (BP), 34 cellular components (CC), and 37 molecular functions (MF). The top 10 entries were selected from BP, CC, and MF, respectively, in order of $-\lg P$ value (Fig. 4).

In total, 96 terms ($p < 0.01$, $FDR < 0.05$) were obtained from the KEGG pathway enrichment analysis using DAVID data. The top 20 entries were selected according to the $-\lg P$ value to draw a bubble diagram (Fig. 5). The main pathways included the Neuroactive ligand – receptor interaction Calcium signaling pathway, Serotonergic synapse CAMP signaling pathway, HIF – 1a signaling pathway, Proteoglycans in cancer, Central carbon metabolism in cancer, Prostate cancer, Thyroid hormone signaling pathway, Pathways in cancer, Prolactin signaling pathway, Type II diabetes mellitus, Estrogen signaling pathway, Hepatitis B, Neurotrophin signaling pathway, Toll – like receptor signaling pathway, Influenza A, Morphine addiction, Endometrial cancer, Bladder cancer.

Screening for hub genes via topological analysis and cluster analysis

To illustrate the hub targets of ZRDH and its effects on Insomnia, PPI network was analyzed via topological analysis. As shown in Fig. 3B, 65 nodes and 379 edges were identified in the PPI network (Network Properties: Degree > 20 , Betweenness > 0.014 , Closeness > 0.404). Subsequently, an MCODE analysis of the PPI network was implemented. The identified targets were clustered as four groups (**Cluster 1**: CNR1, DRD3, ADCY1, HTR1A, DRD4, MTNR1B, DRD2, ADRA2A, GPER1, ADORA1, ADRA2B, ADRA2C, FPR2; **Cluster 2**: F2, EDNRA, PIK3R1, HTR2A, HTR2C, TACR1, GRM5, ADRA1B; **Cluster 3**: AKT1, IL2, CCND1, GAPDH, MMP9, PPARG, RELA, STAT1, ESR1, VEGFA; **Cluster 4**: MMP2, CREBBP, FGF2, PRKCA, ICAM1, SERPINE1, CASP3, MTOR, ERBB2, HRAS, TLR4, AR, TNF, HIF1A), which represented potential key targets for the therapeutic effects of ZRDH, as shown in Fig. 3C.

Molecular Docking Validation

We mapped the components of ZRDH based on these 65 targets to construct the drug (components)–disease (insomnia targets) network shown in Fig. 6A. The binding ability and of herbal components to core protein targets were validated by molecular simulations. Using molecular docking by Surflex-Dock

modeling, a docking score greater than 3 was considered as a stable compound binding to the protein. In this respect, the top 20 nodes were selected from the drug-disease network (Fig. 6B) according to the degree value. Jujuboside A, Schizandrin A, Schizandrin C, and Schizandrin B, Spinosin, the five uppermost anti-insomnia components were selected, as shown in Fig. 7.

Discussion

In this work, 65 potential targets related to the treatment of insomnia were identified. Several studies reported the anti-insomnia activity of jujuboside A [45, 46]. The hydrolysate of jujuboside A can form hydrogen bonds through the residues β 2-Thr2667 and β 2-Thr229 at the GABAA-R binding site to act as hypnotic sedation [47]. Spinosin, as a representative component of flavonoids in jujube seeds, can significantly increase the hypnotic effect of pentobarbital-induced reduction in sleep latency and prolong sleep time [48]. Its mechanism of action may be through 5-HT Realized by the receptor system [49]. Schizandrin A, Schizandrin C, and Schizandrin B can enhance the sleep effect of pentobarbital sodium at subthreshold sleep dose in mice [50]. There have also been numerous studies showing a strong relationship between sleep deprivation and changes in vascular endothelial growth factor [51, 52]. Hypoxia is the main stimulatory factor of the increase of VEGF level, which is related to the occurrence of cardiovascular diseases. Elevated circulating VEGF levels in patients with sleep apnea are due to subclinical symptoms associated with cardiovascular disease or arteriosclerosis, rather than to respiratory disease and Hypoxia itself, therefore, reducing the concentration of VEGF has clinical significance for improving sleep quality [53].

From the perspective of targets and pathways, the neuroactive ligand receptor interaction pathway is a collection of all ligand receptors on the plasma membrane that are related to intracellular and extracellular pathways. The receptors include neuromodulin receptor subtype 1 (NMURI), Calcitonin receptor (CAICR), GABA A receptor B2 (GABRB2), Tachykinin receptor subtype 2 (TAER2), Dopamine receptor subtype 5 (ORDS), Cysteinyl white three Related targets such as ene receptor subtype 2 (CYS1TR2) and 5-HT receptor 1B (HTR1B). In this study, this pathway involves the most potential targets and is most closely related to insomnia. Among them, Schizandrin A, Schizandrin C, and Schizandrin B, Spinosin are involved in the regulation of this pathway. Jujuboside A may regulate this pathway by acting on GABRA1, GABRA2, and GABRA5, thereby exerting an anti-insomnia effect.

The 5-HT energy system and the dopamine (DA) energy system are related to the metabolism of the monoamine neurotransmitter 5-HT and its metabolites. Liu Jie et al performed a behavioural study of rats showed that the effect of sauerkraut-schisandrae could reduce the content of DA, 5-HT and its metabolite 5-indole acetic acid (5-HIAA) in the brain tissue of anxious mice [54]. The anti-anxiety mechanism may be related to the 5-HT energy system and the DA energy system. As the precursor of 5-HT, tryptophan is an amphipathic essential amino acid, which plays an important role in protein synthesis, protein-protein interaction, important metabolic functions, and signal transduction. Studies have shown that the improvement of sleep-in rats may be related to the increase of 5-HT levels in the hypothalamus and brainstem [47]. The increase in the number of night awakenings s related to the decrease in newborn

sleep latency [47, 48]. In this study, we discovered key targets that apigenin, salvianolic acid A, caffeic acid, and protocatechuic acid in ZRDH may act on 5-HT through CYP1A2, DDC, MAOA, and MAOB targets, respectively. The energy system and the DA energy system participate in the regulation of tryptophan metabolism to play a role in the treatment of insomnia. In addition, the sedative molecular mechanism of spinosin is very closely related to the regulation of GABA and 5-HT receptors [55].

KEGG pathway analysis results show that more gene targets are enriched in inflammation-related diseases and signaling pathways, which indicates that ZRDH may act on these inflammatory factor targets to regulate inflammation signaling pathways, inhibit inflammation. The role of insomnia. Among them, Tol-like receptor signaling pathway, HIF-1a, cAMP signaling pathway indicated that insomnia is related to inflammation. Studies have shown that sleep deprivation can damage hippocampal cAMP signal transduction, and the imbalance of cAMP conduction and sleep loss have an important role [56]. The instantaneous increase in cAMP levels in hippocampal excitatory neurons during sleep deprivation is sufficient to prevent insufficient memory consolidation due to sleep loss. In addition, sleep deprivation can reduce the phosphorylation of cAMP response element binding protein (CREB) in the hippocampus under basal conditions and prevent CREB phosphorylation [57].

Current studies have shown that Tol-like receptor 4 (TLR4 signaling pathway) in the Tol-like receptor signaling pathway activates the inflammatory signaling cascade in response to endogenous and pathogen-related ligands known to be associated with sleep loss. Therefore, TLR4 may be a mediator of some inflammation-related sleep loss effects, and growing evidence has provided convincing reasons for evaluating the role of TLR4 as a pro-inflammatory mediator of sleep deprivation cells [58]. Tol-like receptors have a regulatory effect on the type of adaptive immune response, and TLR4 has the effect of activation and induction of immune cell synthesis and pro-inflammatory cytokine release [59, 60].

Conclusion

This study systematically explored the active ingredients, potential targets, and signaling pathways in ZaoRenDiHuang capsules for the treatment of insomnia. The network interaction diagram of key 45 components of the network was constructed, corresponding to 65 key anti-insomnia action targets. Furthermore, Jujuboside A, Schizandrin A, Schizandrin C, Schizandrin B, and Spinosin, the five uppermost anti-insomnia components were selected. In addition, the molecular docking verified the good activity of the active compounds against insomnia targets. Finally, it was found that four functional pathways were involved in Neuroactive ligand – receptor interaction, Serotonergic synapse CAMP signaling pathway, HIF – 1a signaling pathway, Toll – like receptor signaling pathway, anti-insomnia through data mining and network analysis. Overall, this study provides a reference and scientific basis for further research on the anti-insomnia effect of ZRDH.

Declarations

Availability of data and materials

The data sets used in this study may be obtained from the corresponding authors by email by request.

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Contributions

FML, XLT and ZZ proposed and designed this work. DJ, JHZ and YQZ contributed to writing the manuscript. XDA, LYD, and SHZ investigated the study analysis. LYD and YHZ revised the manuscript. All authors read and approved the final manuscript.

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Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

These authors declare that there are no conflicts of interest regarding this work.

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61. Legends.

Figures

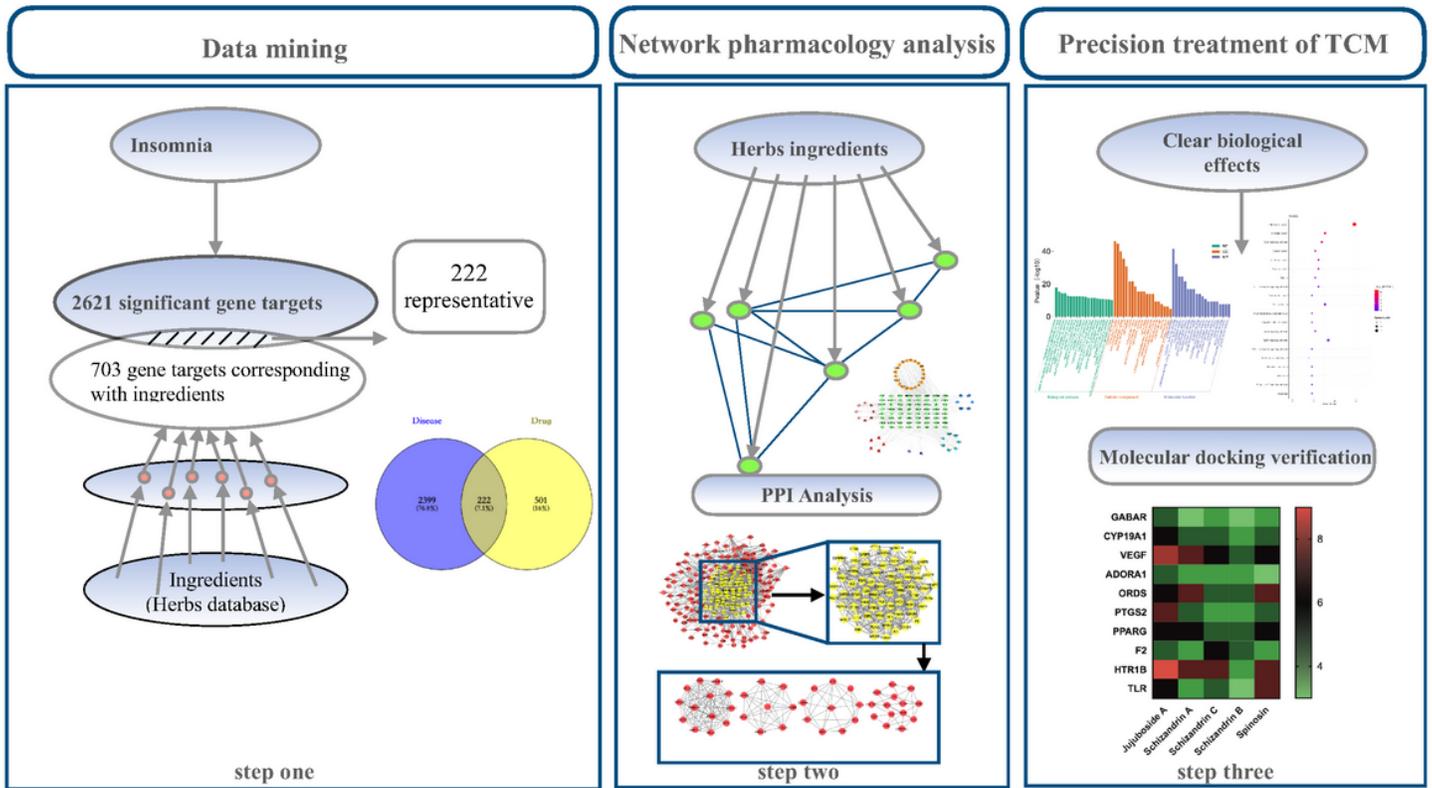


Figure 1

Schematic diagram of the integrated pharmacology strategy approach that combines quantitative analysis of components, network analysis, and molecular docking to investigate the mechanisms of ZRDH treatment against insomnia.

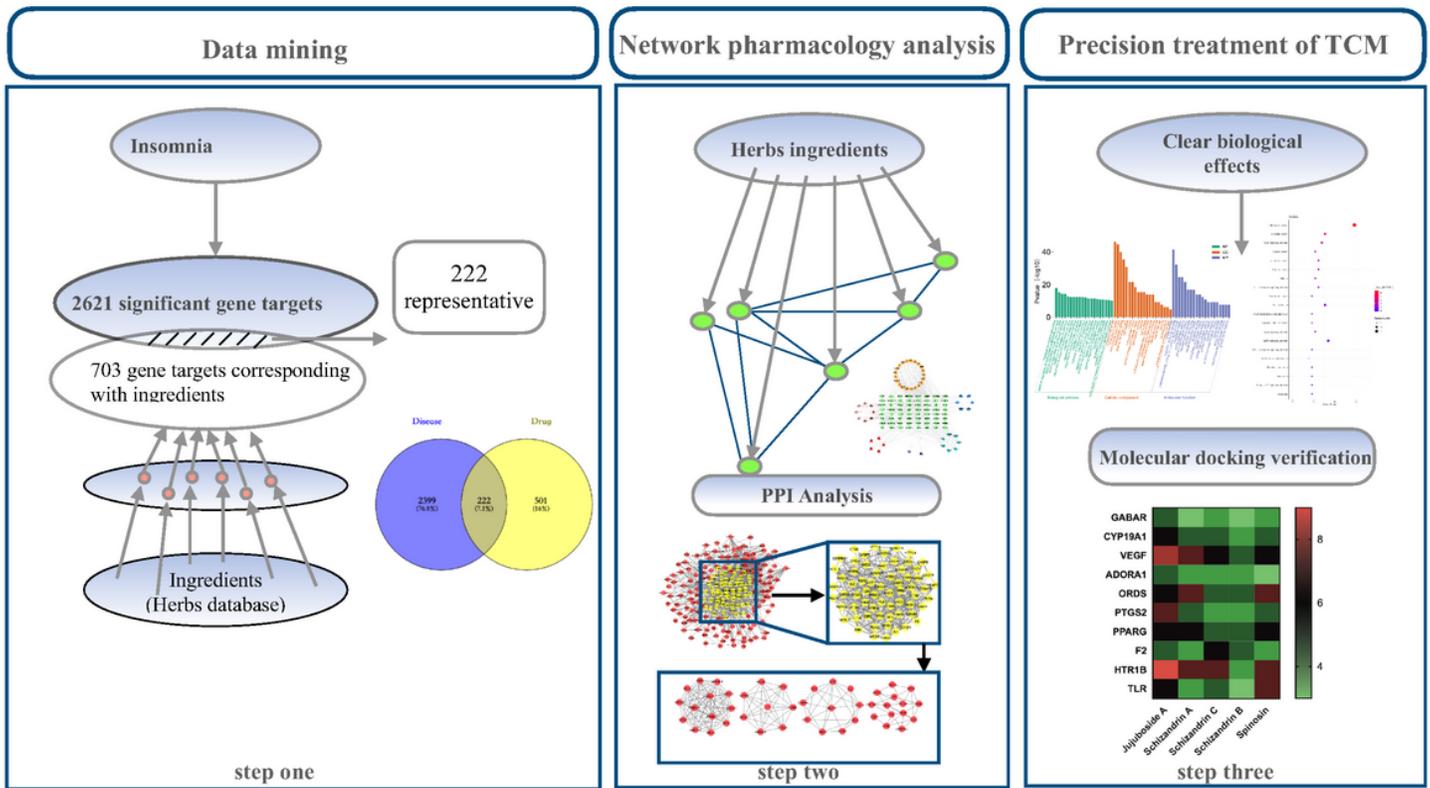


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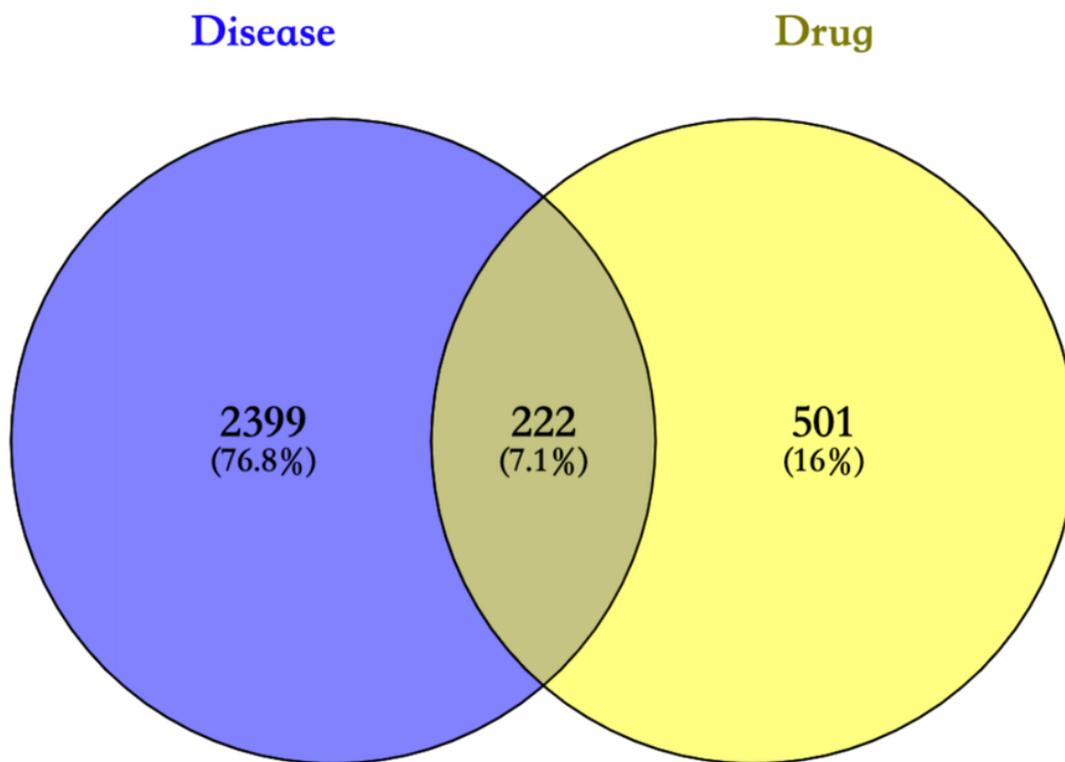


Figure 2

Wayne diagram of commo targets of ZRDH therapy and insomnia.

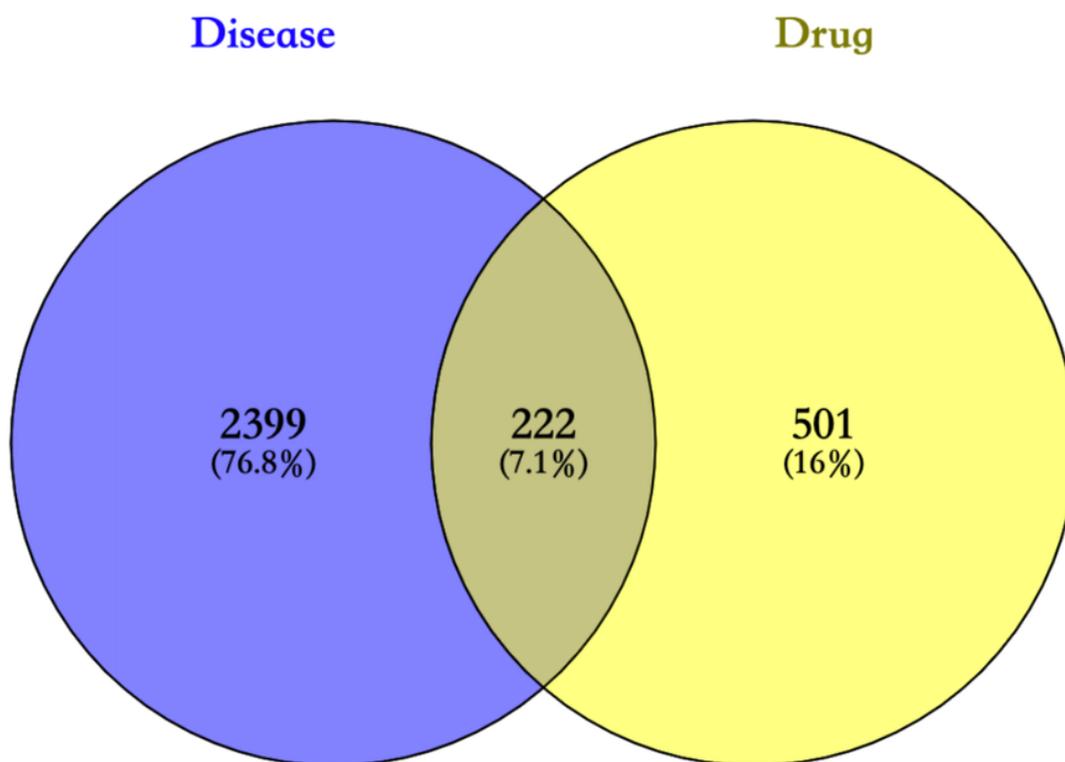


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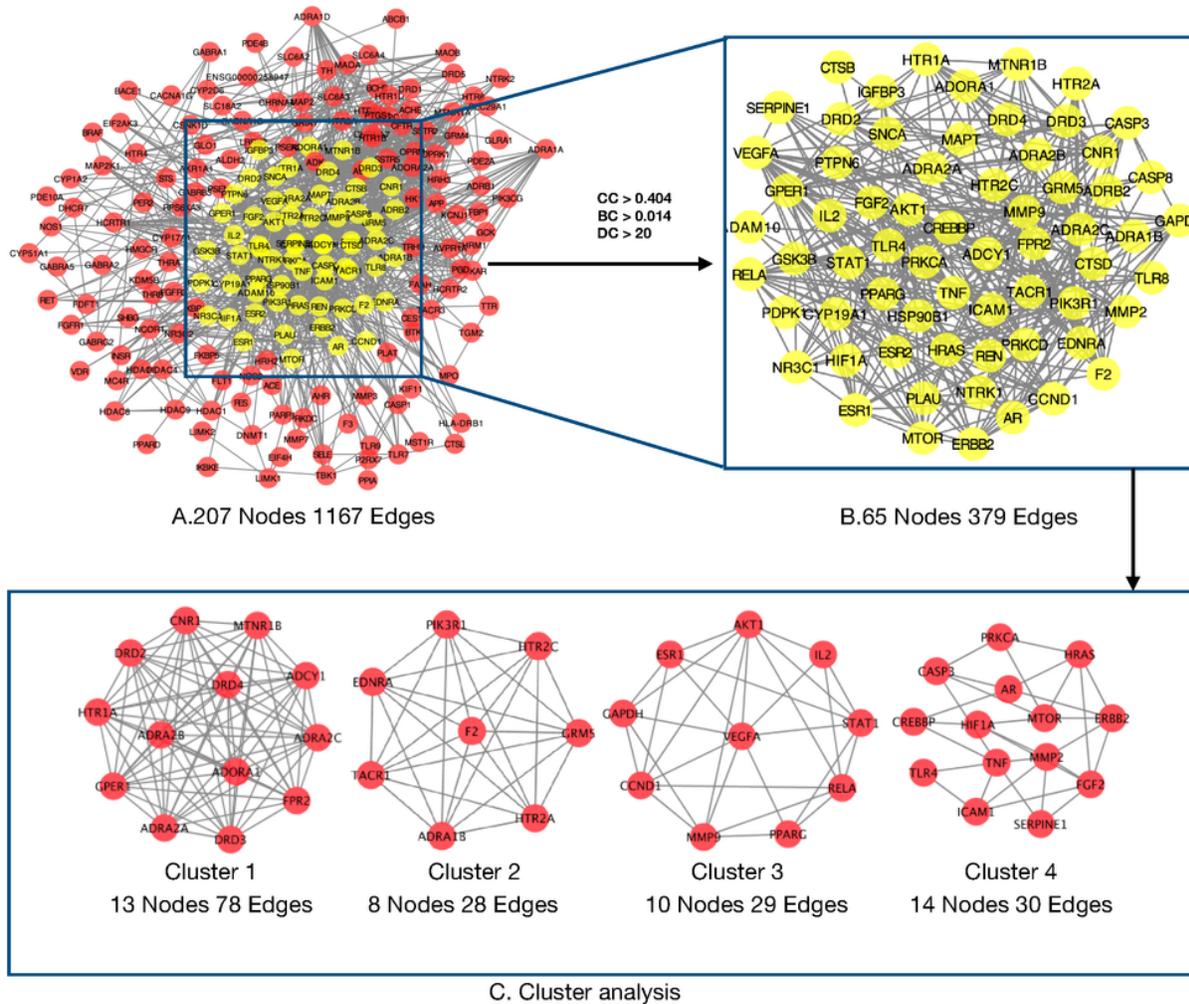


Figure 3

Identification of candidate targets for ZRDH against Insomnia via Protein-protein interaction Analysis. A. Protein-protein interaction (PPI) networks of shared targets between ZRDH and Insomnia analyzed by STRING 11.0. B. The most significant module identified by the topology selection. C. The core 65 targets (hub targets) in the PPI network was cluster analysis using the MCODE plug-in.

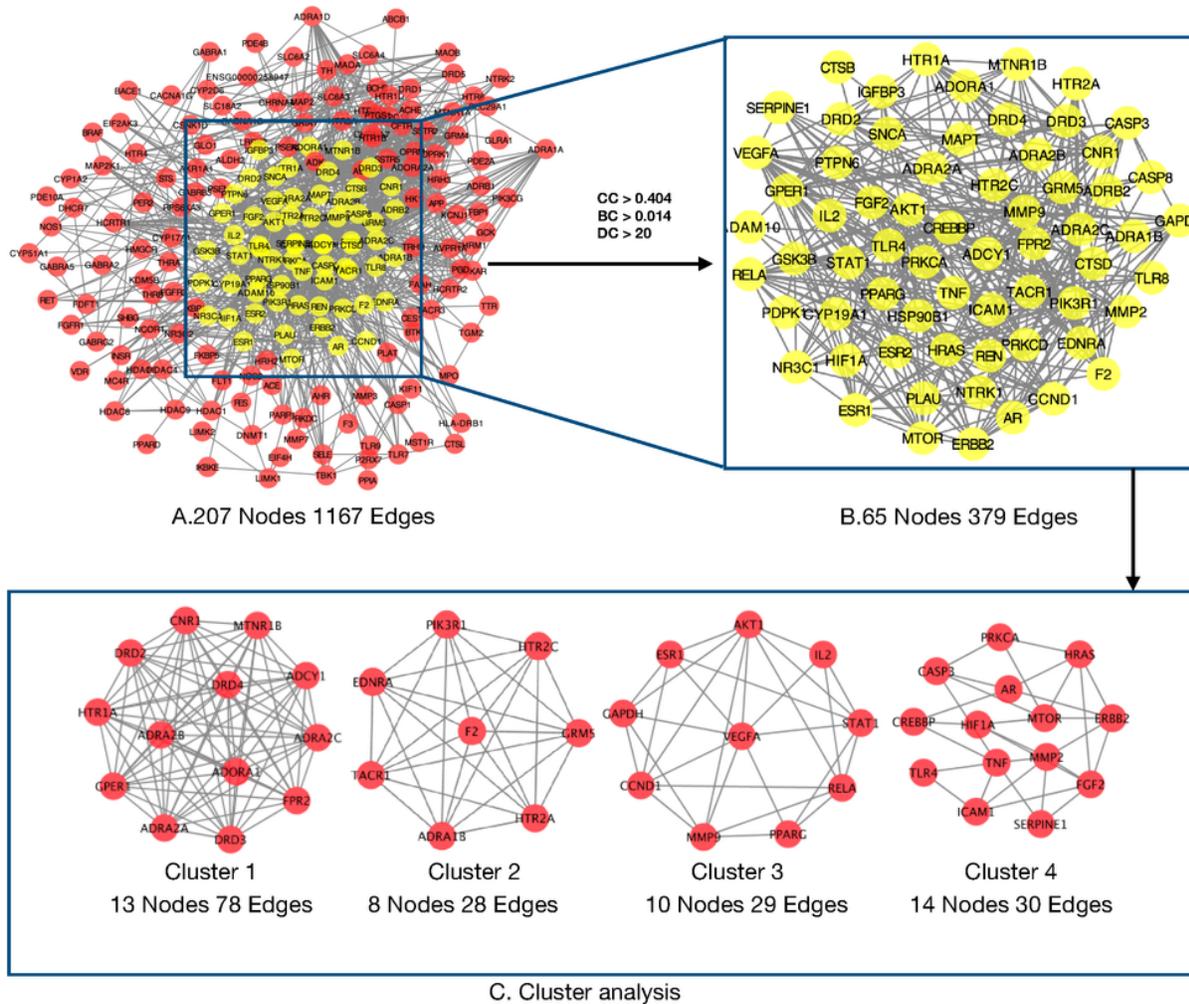


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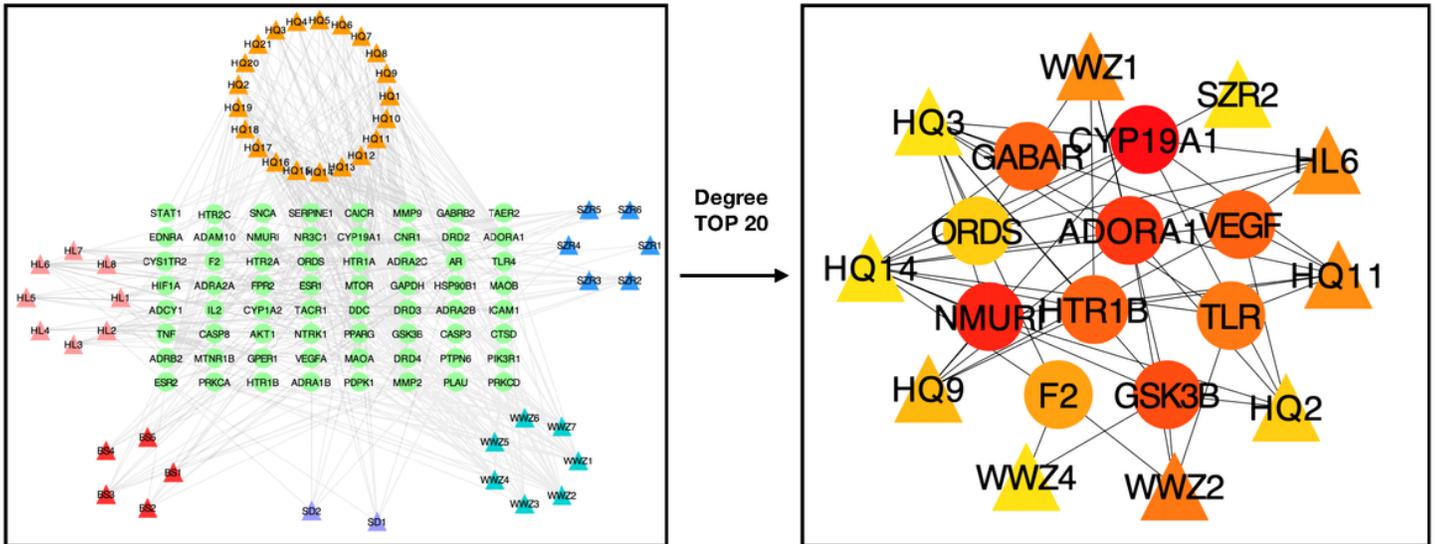


Figure 4

Enrichment analysis of the potential targets of ZRDH against Insomnia by R software 3.4.2 for the Gene Ontology database. Top 10 biological process (BP) terms, cellular component (CC) terms, and molecular function (MF) terms are shown as green bars, orange bars, and purple bars, respectively according to “P-value < 0.01 and FDR < 0.05”.

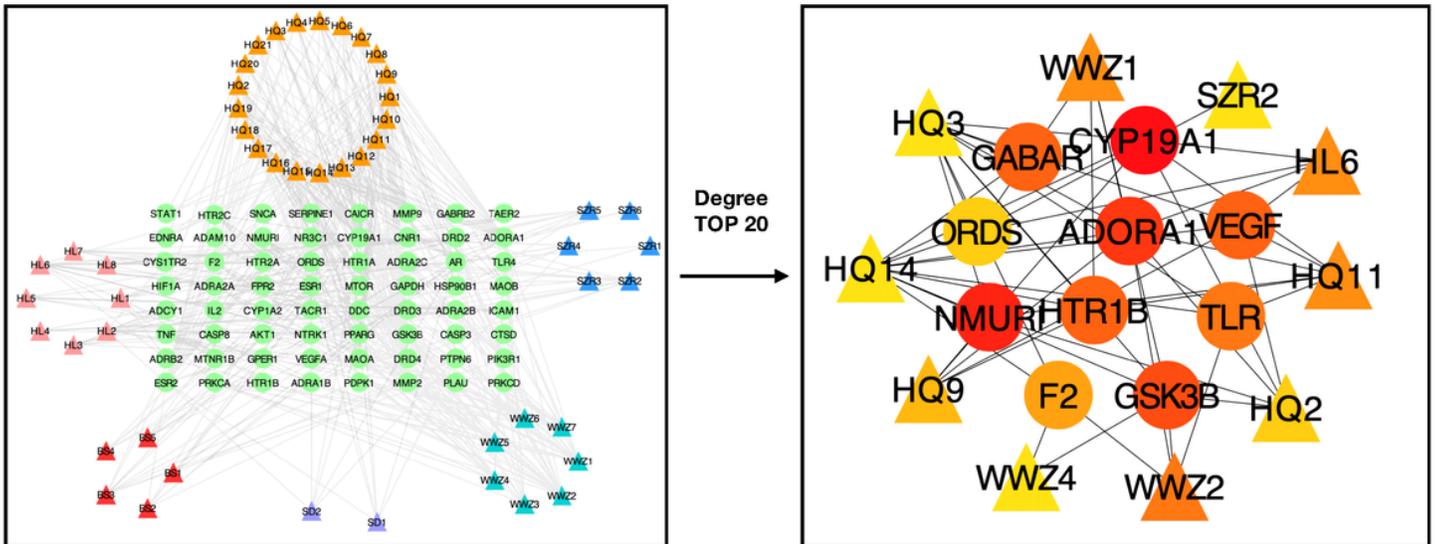


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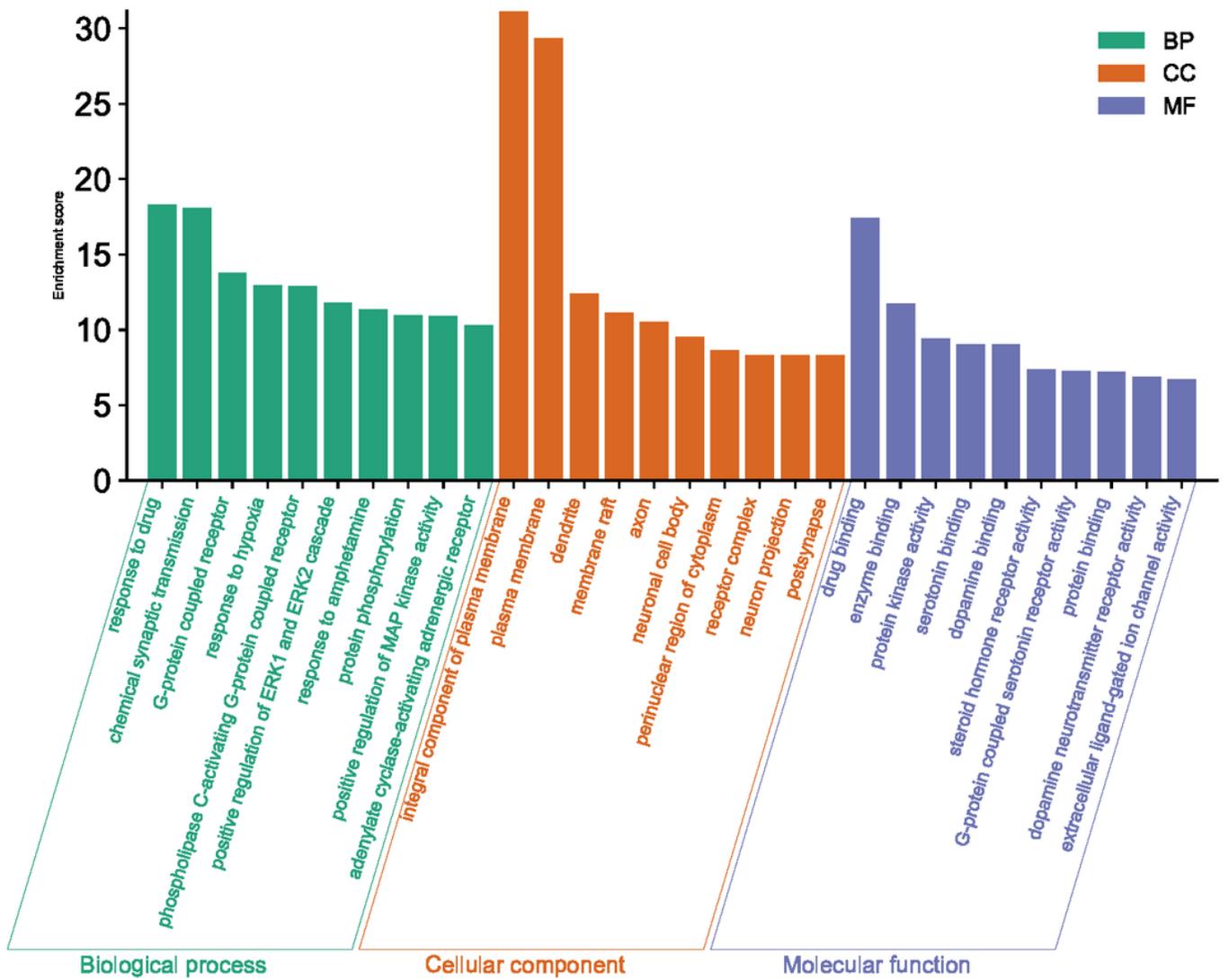


Figure 5

Enrichment analysis for KEGG. Top 20 KEGG pathways listed by bubble chart according to the “P-value < 0.01 and FDR < 0.05”.

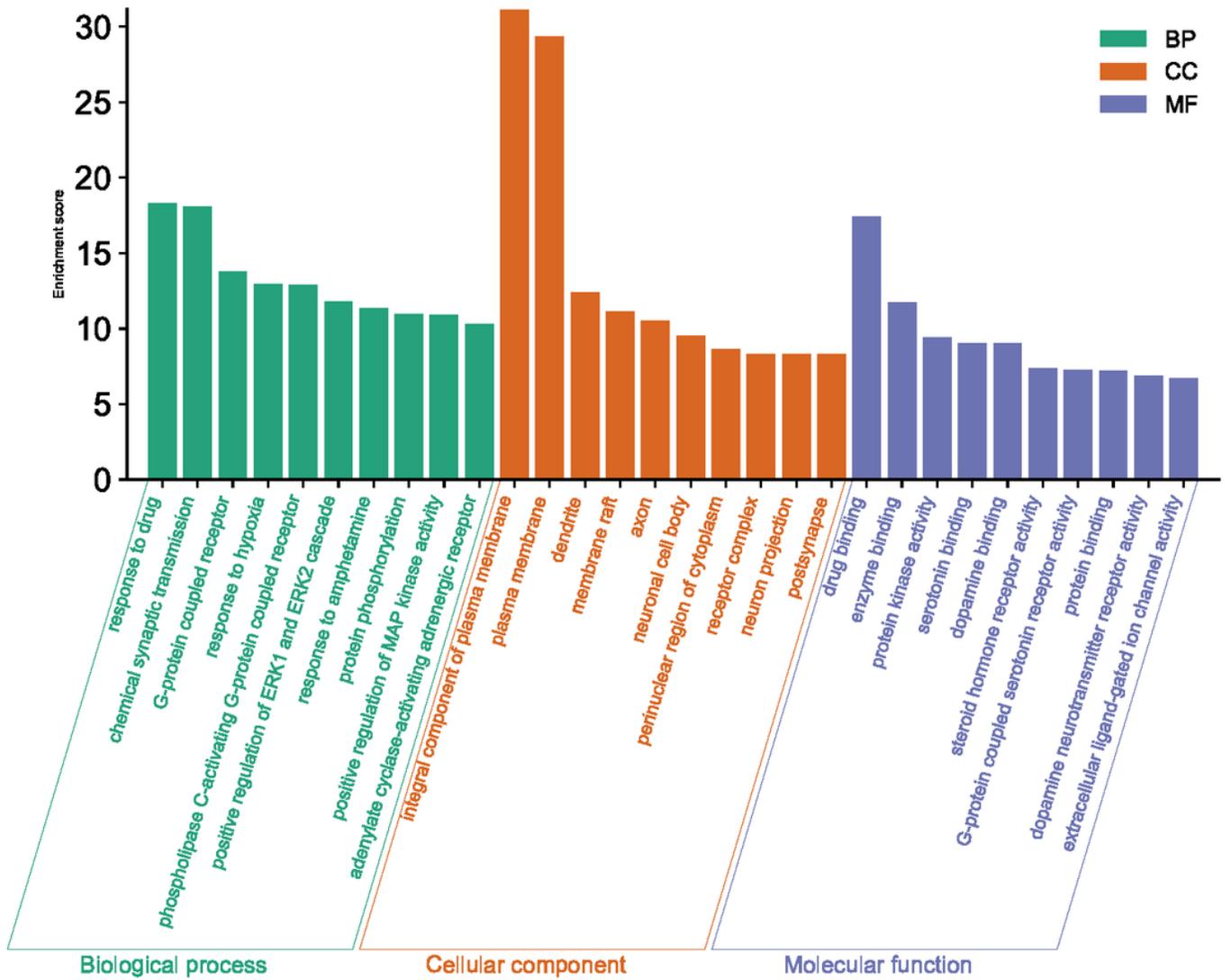


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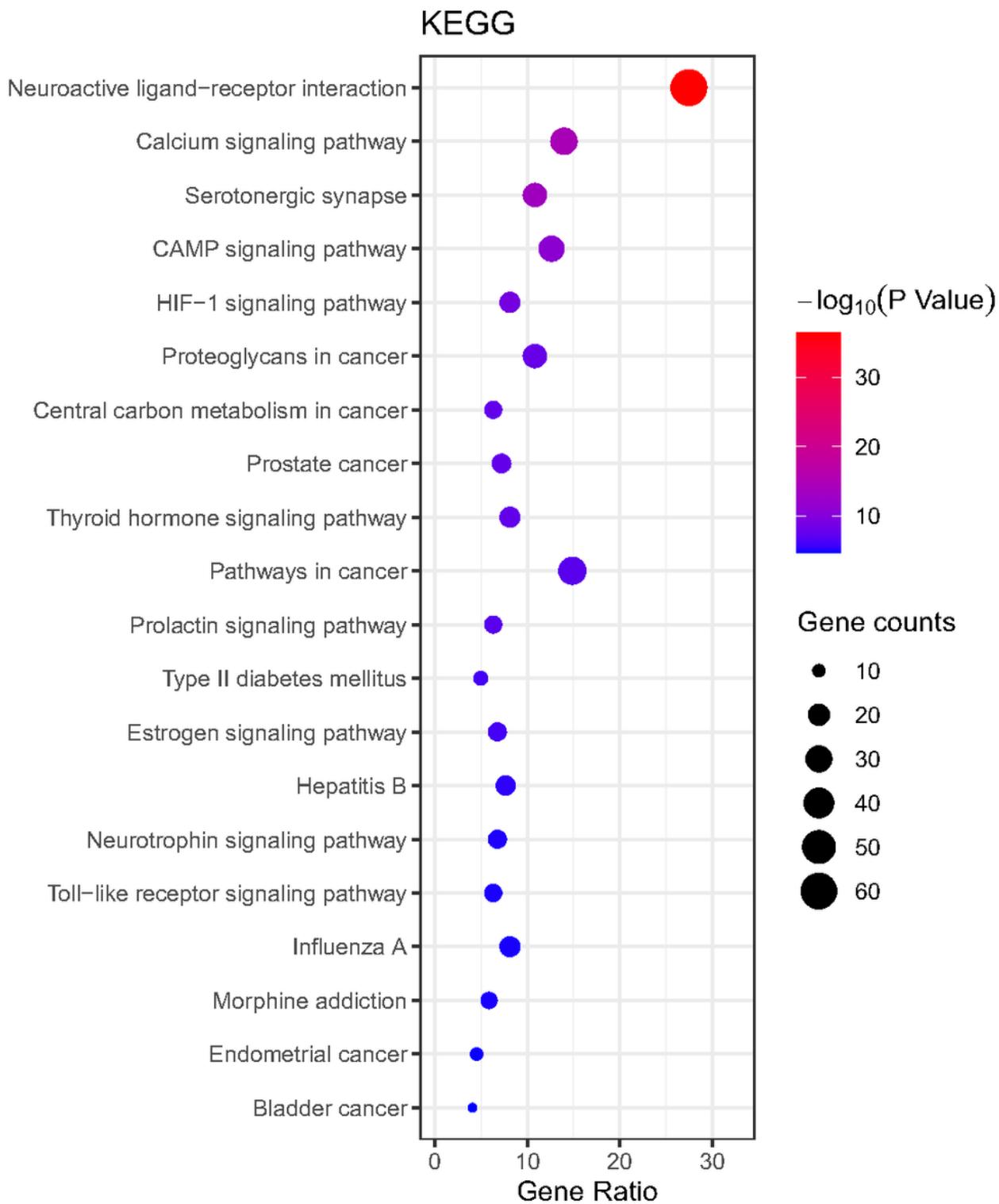


Figure 6

Construction of the drug (herbal ingredients)–disease (Insomnia) network. The nodes representing drug candidate compounds are shown as triangles, and the targets are indicated by circles.

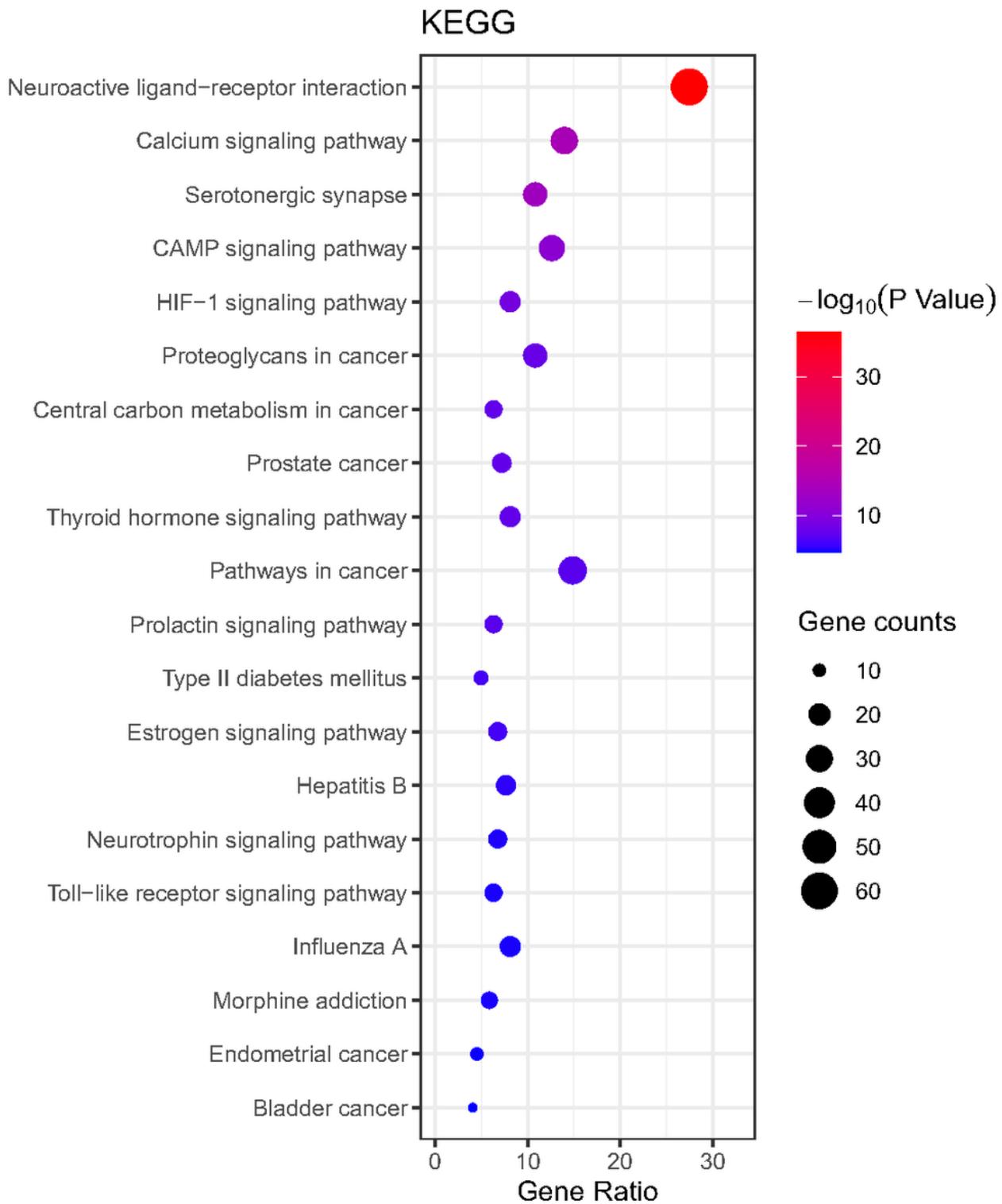


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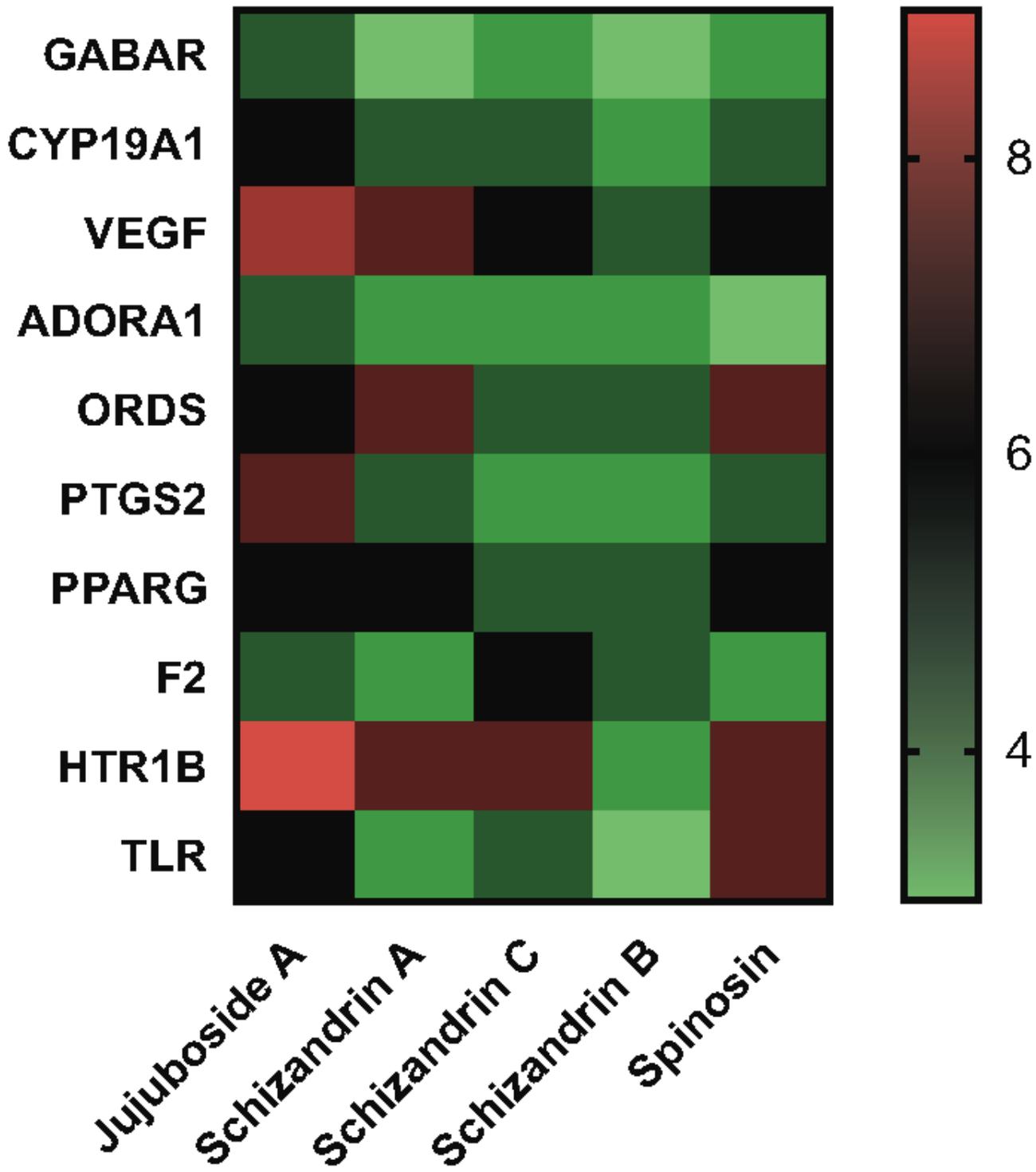


Figure 7

Heat map of Molecular docking.

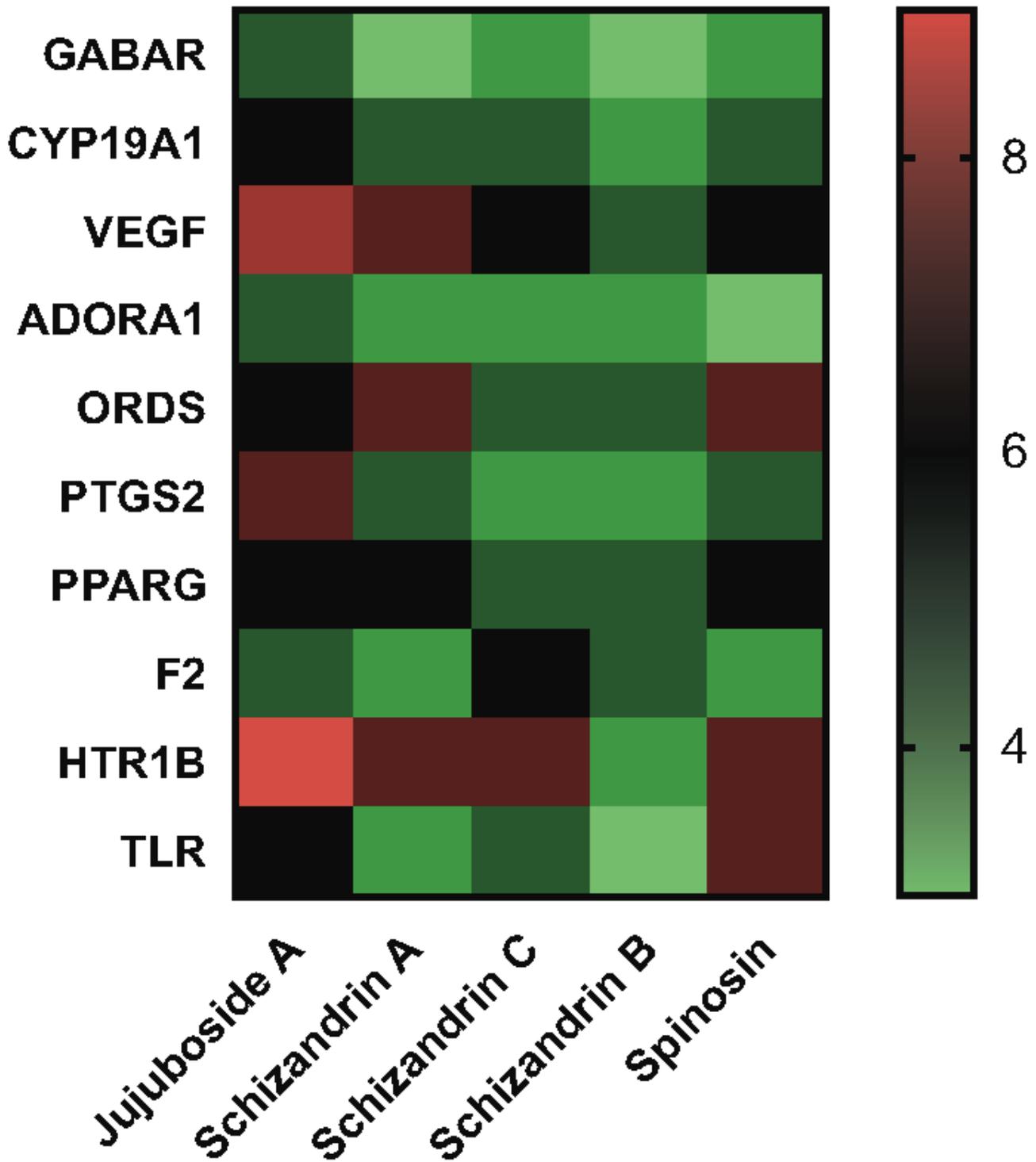


Figure 7

Heat map of Molecular docking.

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