ARTICLE
Traditional Medicine Research 2023;8(10):57. https://doi.org/10.53388/TMR20230507001

Molecular mechanisms of Tibetan medicine Sug-Mel-sum-thang in treating insomnia disorder: network pharmacology, molecular docking, and surface plasmon resonance

Jin-Peng Ma[1, 2], Rang-Ji Cai[1], Ji Zang[1], Cairang Nanjia[1*]

[1]College of Pharmacy, Qinghai University for Nationalities, Xining 810000, China. [2]College of Pharmacy, Xiamen University, Xiamen 361000, China.

[*]**Corresponding to:** Cairang Nanjia, College of Pharmacy, Qinghai University for Nationalities, No. 3 Bayi Middle Road, Chengdong District, Xining 810000, China. E-mail: cnanjia@qhmu.edu.cn.

Author contributions

Ma JP organized data and wrote the first draft, Cairang NJ revised the draft, acquired funding, and supervised, Cai RJ wrote a first draft, and Zang J analyzed data. All the authors participated in the final approval of the manuscript.

Competing interests

The authors declare no conflicts of interest.

Acknowledgments

This study was supported by grants from the Projects of the National Social Science Foundation of China (20XMZ026) and the Qinghai Provincial Natural Science Foundation Applied Basic Research Project (2019-ZJ-7088).

Peer review information

Traditional Medicine Research thanks Hong Li and other anonymous reviewers for their contribution to the peer review of this paper.

Abbreviations

SMST, Sug-Mel-sum-thang; ID, insomnia disorder; PPI, protein-protein interaction; SPR, surface plasmon resonance; BBB, blood-brain barrier.

Citation

Ma JP, Cai RJ, Zang J, Cairang NJ. Molecular mechanisms of Tibetan medicine Sug-Mel-sum-thang in treating insomnia disorder: network pharmacology, molecular docking, and surface plasmon resonance. *Tradit Med Res*. 2023;8(10):57. doi: 10.53388/TMR20230507001.

Executive editor: Xin-Yi Yang.

Received: 07 May 2023; **Accepted:** 20 June 2023; **Available online:** 03 July 2023.

Abstract

Background: The Tibetan medicine Sug-Mel-sum-thang (SMST) is often used to treat insomnia disorder (ID); however, the underlying mechanism remains unclear. In this study, network pharmacology prediction was conducted to analyze the molecular mechanisms of SMST in treating ID. **Methods:** The active ingredients of the three herbs used in SMST were obtained from the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences. Chemistry Database [DB/OL]. The chemical formulae of the active ingredients were downloaded from PubChem in canonical SMILES format. These were then imported into the SwissTargetPrediction platform to identify drug targets. ID targets were obtained from databases such as DrugBank, GeneCards, and DisGeNET. Potential targets of SMST for the treatment of ID were identified using the online Venny mapping platform. The potential target protein-protein interaction was constructed using the STRING platform, and the obtained protein-protein interaction information was imported into Cytoscape 3.7.1 for image optimization and core gene extraction and ClueGO in Cytoscape 3.7.1 was used for the enrichment analysis of potential targets. Cytoscape 3.7.1 was also used to construct the regulatory network of the Tibetan medicine compound target pathway and conduct a topological analysis. **Results:** The mechanism of action of SMST in the treatment of ID involved 72 compounds, including nerolidol, apigenin, luteolin, and piperine. The key targets identified were COMT, CNR1, AKT1, SLC6A4, TNF, CTNNB1, and CHRNA4. The enrichment analysis obtained 69 KEGG pathways, mainly related to Alzheimer's disease, cAMP signaling pathway, serotonergic synapse, and other pathways. The core active ingredient molecule docked with the target to a higher degree. The results showed that the active ingredient exhibited good binding activity with the related targets. Some parts with good docking fractions were selected for surface plasmon resonance analysis. **Conclusion:** Various active components in SMST play a role in the treatment of ID by acting on key targets, such as COMT and CNR1, to regulate multiple signaling pathways.

Keywords: Sug-Mel-sum-thang; drug target; insomnia disorder; Tibetan medicine

Traditional Medicine Research 2023;8(10):57. https://doi.org/10.53388/TMR20230507001

Highlights

For the first time, network pharmacology was used to explore the mechanism of Sug-Mel-sum-thang in the treatment of insomnia disorders, and to provide theoretical support for the clinical application of Sug-Mel-sum-thang.

Medical history of objective

Sug-Mel-sum-thang was first recorded in the *Four Medical Tantras*, one of the most important collections of Tibetan medical texts with a history of 1,400 years of clinical application.

Sug-Mel-sum-thang is used in Tibetan medicine to treat insomnia disorders.

Background

Insomnia disorder (ID) is a common clinical condition. The prevalence of ID is approximately 10–20%, of which approximately 50% of cases have a chronic course [1]. ID is mainly characterized by dissatisfaction with sleep duration or quality, difficulty in initiating or maintaining sleep, severe anxiety, and daytime dysfunction. Moreover, it frequently co-occurs with other medical or psychiatric conditions, such as pain and depression. Persistent insomnia impairs physical health, leading to reduced quality of life and physical or mental illness. Despite its high incidence, little is known about the etiology, pathology, or physiology of ID. The classification and diagnosis of ID has undergone significant changes over the last decade, and the perception of ID has shifted from being a symptom to a disease [2, 3].

The most widely prescribed drugs for ID treatment are benzodiazepines and benzodiazepine receptor agonists, which bind to the γ-aminobutyric acid site of the benzodiazepine receptor and increase the intrinsic activity of the inhibitory neurotransmitter γ-aminobutyric acid to exert a therapeutic effect. However, approximately 40% of patients with chronic ID fail to achieve sustained remission with these treatments, and their safety and efficacy are limited by the tolerance and dependence that develop with long-term use [4, 5]. The Tibetan medicine Sug-Mel-sum-thang (SMST) has been used to treat ID for more than 1,400 years, and is considered a classic formula based on the theory of Tibetan medicine. It comprises three herbs commonly used in Tibetan medicine: *Amomum kravanh Pierre* ex Gagnep. (Bai dou kou), *Cuminum cyminum* L. (Zi ran), and *Piper longum* L. (Bi ba). SMST was first recorded in the Four Medical Tantras, one of the most important collections of Tibetan medical texts with a history of 1,400 years of clinical application [6]. Because Mongolian medicine and Tibetan medicine are similar, SMST is used in the clinic as “Sugmuler-3 flavor soup”, and its formula differs slightly from that of the Tibetan medicine SMST [7]. Although SMST is frequently used in clinical practice and has a significant curative effect [7, 8], the mechanism whereby it ameliorates ID is unclear, thus restricting its development and application.

With the rapid development of bioinformatics, molecular biology, medicinal chemistry, cell biology, and other disciplines, various biological databases have emerged for querying active ingredients in single and compound medicines and predict the targets of drugs and diseases. Predicting the action targets of drugs and diseases and analyzing the mechanism of drug action aid in the rapid development of traditional Chinese medicine [9–11]. Bioinformatics can also be used to conduct modern research on Tibetan medicine and analyze its mechanism of action of Tibetan medicine. In this study, the mechanism of action of SMST in the treatment of ID was predicted using network pharmacology and molecular docking technology and a scientific basis for the treatment of ID by SMST was proposed. The development of SMST provides novel insights and can serve as reference for future studies.

Materials and methods

The study methodology is illustrated in Figure 1.

Ingredients and drug targets of SMST

The components and potential targets of SMST were obtained from the Shanghai Institute of Organic Chemistry of the Chinese Academy of Sciences. Chemistry Database [DB/OL]. Collection of *A. kravanh*, *P. longum*, and *C. cyminum* in SMST A total of 72 compounds were obtained from Tibetan medicines. The chemical formula of canonical SMILES was obtained from PubChem (https://pubchem. ncbi. nlm. nih. gov/). The canonical SMILES file was imported into the SwissTargetPrediction platform (http://www.swisstargetprediction.ch/), and its attribute was set to “homosapiens” to predict the targets of the chemical components.

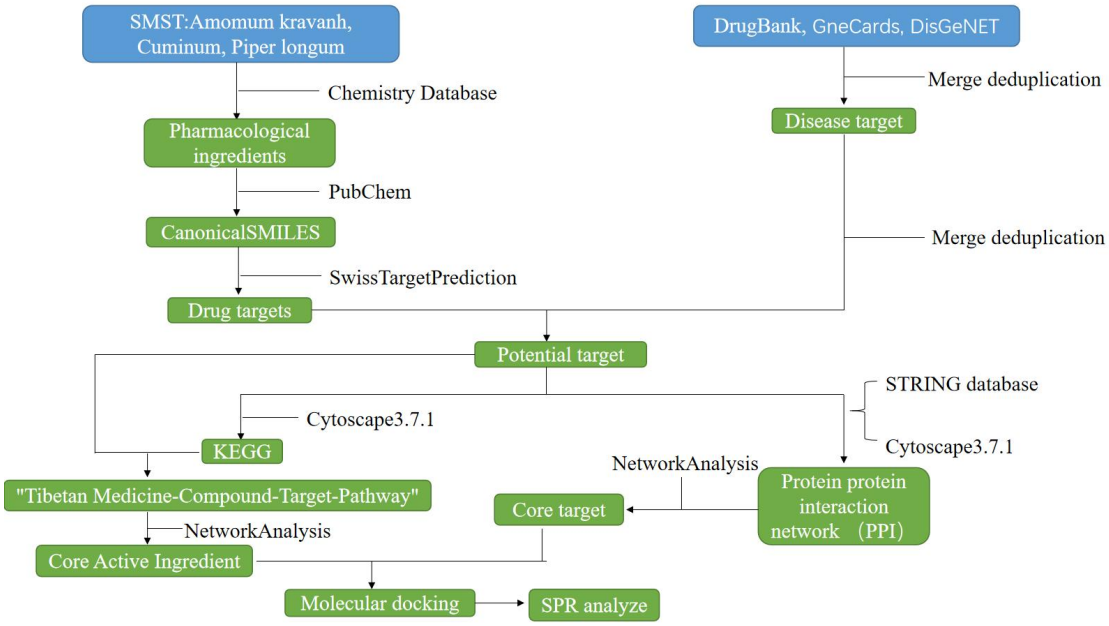

Figure 1 Illustration of the study strategy. PPI, protein-protein interaction; SPR, surface plasmon resonance; SMST, Sug-Mel-sum-thang; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Acquisition of disease target and construction of Venn diagram
Disease targets of insomnia were obtained from DrugBank (https://go. drugbank. com/), GeneCards (https://genecards. weizmann. ac. il/v3/), and DisGeNET (http://www. disgenet. org/). Disease targets from the three databases were combined to remove duplicates. The intersection of the drug and disease targets was determined and the potential target was represented as a Venn diagram.

Protein-protein interaction (PPI) network construction
The potential targets of item "2.2" were imported into the STRING database (https://cn.string-db.org/), and the biological species was set to "Homosapiens". The protein interaction network map was obtained and saved in tab separated values format. The PPI data file was imported into Cytoscape 3.7.1 for image optimization, and network analysis was performed to analyze the network topology parameters of the effective components.

Enrichment analysis
The potential targets of item "2.2" were imported into the plug-in ClueGO in Cytoscape 3.7.1, "Selectspecies" was set to "Homosapiens", and a P value of < 0.05 was set for KEGG pathway enrichment analysis.

Construction of "Tibetan medicine-compound-target-pathway" regulatory network
The potential targets of item "2.2" and their corresponding compounds were combined with the KEGG pathway enrichment analysis results of item "2.4" to construct a "Tibetan medicine-compound-target-pathway" network using Cytoscape 3.7.1 software. Network analysis was performed to analyze the network topology parameters of the active ingredients and determine the core active ingredients.

Molecular docking
The core active ingredients in item "2.5" was compared with the 14 targets of AKT1, SLC6A3, SLC6A4, COMT, TNF, CHRNA4, CTNNB1, GRM5, DRD2, ESR1, CNR1, CYP3A4, GRIN2A, and HTR2A in item "2.3" molecular docking was done. The 3D molecular structure of the active ingredient was downloaded from the PubChem database and the target protein structure was downloaded from the protein data bank database (https://www. rcsb. org/) based on human origin, resolution, protein length, and related reports. The target protein was imported into Maestro software for hydrogen bonding optimization, dehydration, and energy minimization. Subsequently, the processed proteins and small molecules were imported into Maestro software for molecular docking.

Surface plasmon resonance (SPR) analysis
A phosphate-buffered saline solution containing 1% dimethyl sulfoxide as the driving buffer was prepared. The CNR1 and COMT proteins (Proteintech) were coupled to a CM5 chip (Cytiva) using a biomolecular interaction detector. Apigenin and piperine were dissolved in dimethyl sulfoxide and diluted to 8 concentrations (16, 8, 4, 2, 1, 0.5, 0.25, and 0.125 mM). Each solution was diluted 100 times with phosphate-buffered saline to obtain the test solutions. The ligand dissociation was recorded using a biomolecular interaction detector. Affinity and kinetic fitting analyses of the ligands and small molecules were performed using the software provided with the instrument.

Results

Active ingredients and targets of SMST
A total of 72 active compounds from *A. kravanh*, *P. longum*, and *C. cyminum* were obtained from the Chemical Professional Database of the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences. Among the 72 active ingredients, 25 were from *A. kravanh*, 14 were from *P. longum*, 33 were from *C. cyminum*, and seven were common components of *C. cyminum* and *A. kravanh*, as shown in Table 1. Drug targets were obtained using the SwissTargetPrediction platform. A total of 207 targets were obtained for *A. kravanh*, 445 for *P. longum*, and 257 for *C. cyminum*. Target 623 was obtained by combining the above targets and removing duplicates.

Table 1 Information on active ingredients of medicines retained in the database

Compound	Molecular formula	Source
Dihydropiperlonguminine	$C_{16}H_{21}NO_3$	Piper longum
Guineesine/guineensine	$C_{24}H_{33}NO_3$	Piper longum
Sylvatine	$C_{24}H_{33}NO_3$	Piper longum
Diaeudesmin	$C_{22}H_{26}O_6$	Piper longum
Tetrahydropiperic acid	$C_{12}H_{14}O_4$	Piper longum
Sesamin	$C_{20}H_{18}O_6$	Piper longum
Piperundecalidine	$C_{23}H_{29}NO_3$	Piper longum
Pipernonaline	$C_{21}H_{27}NO_3$	Piper longum
Dehydropipernonaline	$C_{21}H_{25}NO_3$	Piper longum
Piperlonguminine	$C_{16}H_{19}NO_3$	Piper longum
Pipercide	$C_{22}H_{29}NO_3$	Piper longum
Piperine	$C_{17}H_{19}NO_3$	Piper longum
Piperidine	$C_5H_{11}N$	Piper longum
N-isobutyldeca-trans-2e,4e-dienamide	$C_{14}H_{25}NO$	Piper longum
Syringol	$C_8H_{10}O_3$	Cuminum
5,6-octadecadienoic acid	$C_{18}H_{32}O_2$	Cuminum
Apigenin-7-o-glucoside	$C_{21}H_{20}O_{10}$	Cuminum

Table 1 Information on active ingredients of medicines retained in the database (*continued*)

Compound	Molecular formula	Source
Luteolin	$C_{15}H_{10}O_6$	Cuminum
α-phellandrene	$C_{10}H_{16}$	Cuminum
β-phellandrene	$C_{10}H_{16}$	Cuminum
Phellandral	$C_{10}H_{16}O$	Cuminum
1,4-p-menthadien-7-al	$C_{10}H_{14}O$	Cuminum
P-menth-3-en-7-al	$C_{10}H_{16}O$	Cuminum
Apigenin	$C_{15}H_{10}O_5$	Cuminum
Apigetrin	$C_{21}H_{20}O_{10}$	Cuminum
α-terpinene	$C_{10}H_{16}$	Cuminum
γ-terpinene	$C_{10}H_{16}$	Cuminum
Chrysoeriol	$C_{16}H_{12}O_6$	Cuminum
β-farnesene	$C_{15}H_{24}$	Cuminum
Cuminalcohol	$C_{10}H_{14}O$	Cuminum
Cuminaldehyde	$C_{10}H_{12}O$	Cuminum
Choline	$C_5H_{14}NO^+$	Cuminum
Cephalin	$C_9H_{18}NO_8P$	Cuminum
Geranial	$C_{10}H_{16}O$	Cuminum
β-bisabolene	$C_{15}H_{24}$	Cuminum
Perillaldehyde	$C_{10}H_{14}O$	Cuminum
α-Terpineol	$C_{10}H_{18}O$	Cuminum
Cynaroside	$C_{21}H_{20}O_{11}$	Cuminum
1,3-p-menthadien-7-al	$C_{10}H_{14}O$	Cuminum
Phosphatidylcholine	$C_{42}H_{80}NO_8P$	Cuminum
Bornyl acetate	$C_{12}H_{20}O_2$	Amomum kravanh
γ-patchoulene	$C_{15}H_{24}$	Amomum kravanh
Sabinene hydrate	$C_{10}H_{18}O$	Amomum kravanh
Myrcenol	$C_{10}H_{18}O$	Amomum kravanh
Borneol	$C_{10}H_{18}O$	Amomum kravanh
Nerolidol	$C_{15}H_{26}O$	Amomum kravanh
Farnesol	$C_{15}H_{26}O$	Amomum kravanh
Sabinene	$C_{10}H_{16}$	Amomum kravanh
Aromadendrene	$C_{15}H_{24}$	Amomum kravanh
1,4-cineole	$C_{10}H_{18}O$	Amomum kravanh
3-carene	$C_{10}H_{16}$	Amomum kravanh
Carvone	$C_{10}H_{14}O$	Amomum kravanh
Camphor	$C_{10}H_{16}O$	Amomum kravanh
Camphene	$C_{10}H_{16}$	Amomum kravanh
(cis-trans) farnesol	$C_{15}H_{26}O$	Amomum kravanh
(trans-trans) farnesol	$C_{16}H_{28}O$	Amomum kravanh
4-terpineol	$C_{10}H_{18}O$	Amomum kravanh
Bisabolene	$C_{15}H_{24}$	Amomum kravanh
Limonene	$C_{10}H_{16}$	Amomum kravanh, Cuminum
β-caryophyllene	$C_{15}H_{24}$	Amomum kravanh, Cuminum
Myrcene	$C_{10}H_{16}$	Amomum kravanh, Cuminum
P-cymol	$C_{10}H_{14}$	Amomum kravanh, Cuminum
Cineole	$C_{10}H_{18}O$	Amomum kravanh, Cuminum
α-pinene	$C_{10}H_{16}$	Amomum kravanh, Cuminum
β-pinene	$C_{10}H_{16}$	Amomum kravanh, Cuminum

Potential targets of SMST in the treatment of ID

The ID targets were screened using the DrugBank, Gencards, and DisGeNET databases. After de-duplication, 813 target genes were identified. Using the Venny mapping platform, the active ingredients of SMST were intersected with ID-related targets, and 107 potential targets were obtained, as shown in Figure 2.

Construction of PPI network of potential targets of SMST in the treatment of ID

The potential targets of SMST treatment ID were uploaded to the STRING database to obtain the PPI network and related information, and the PPI information was imported into Cytoscape 3.7.1 to obtain the PPI network diagram (Figure 3). Network analysis was performed to analyze the network topology parameters of the active ingredients. We found that the AKT1, SLC6A3, SLC6A4, COMT, TNF, CHRNA4, CTNNB1, GRM5, DRD2, ESR1, CNR1, CYP3A4, GRIN2A, and HTR2A nodes had high degree values. These targets are important in the network and are core targets for SMST in ID therapy.

Enrichment analysis

KEGG pathway enrichment analysis was performed on the potential targets of SMST in the treatment of ID using the ClueGO plug-in in Cytoscape. The results showed that 69 pathways may be associated with ID, including Alzheimer's disease, cAMP signaling pathway, serotonergic synapse, calcium signaling pathway, and dopaminergic synapse, as shown in Figure 4. Cytoscape 3.7.1 was used to construct a

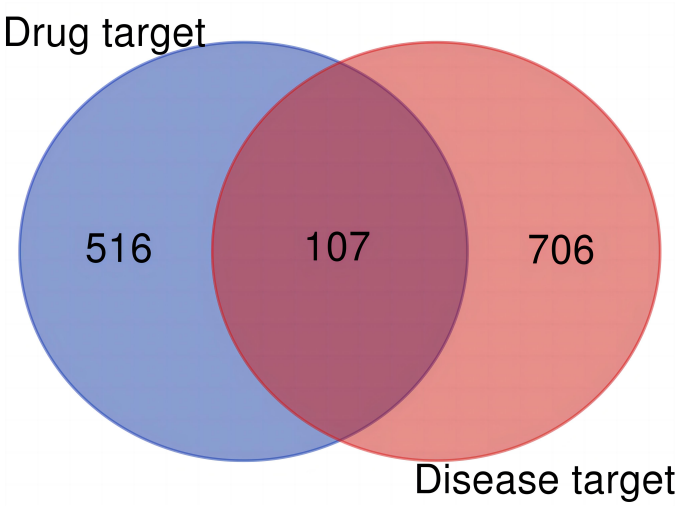

Figure 2 Venn diagram of the common targets between the prediction of SMST drug targets and ID targets. SMST, Sug-Mel-sum-thang; ID, insomnia disorder.

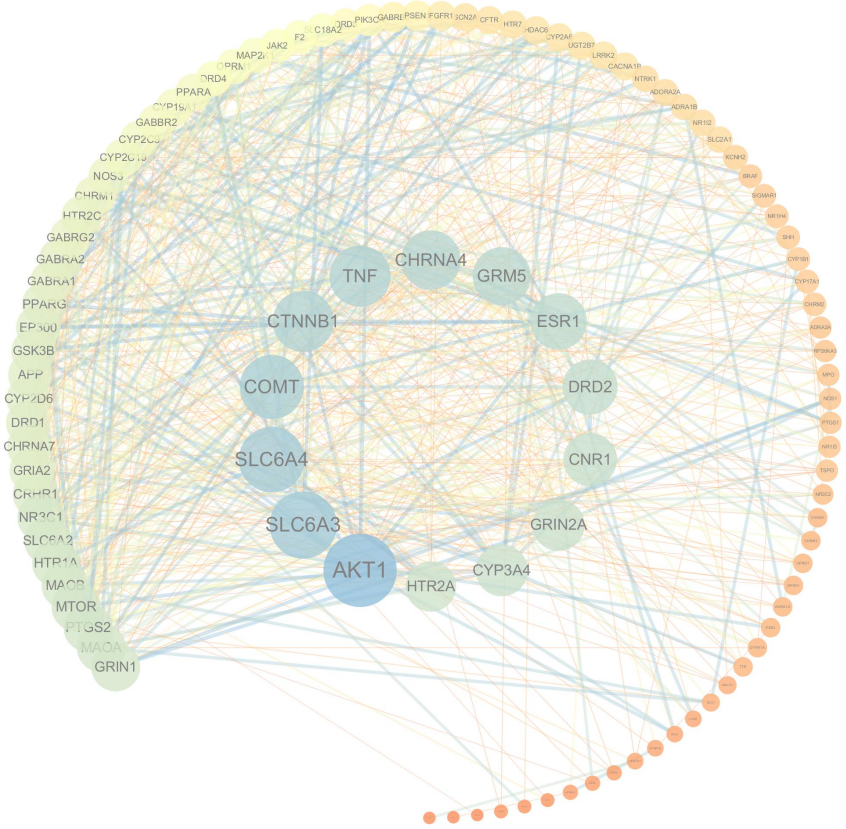

Figure 3 Interaction network between SMST and ID common targets. SMST, Sug-Mel-sum-thang; ID, insomnia disorder.

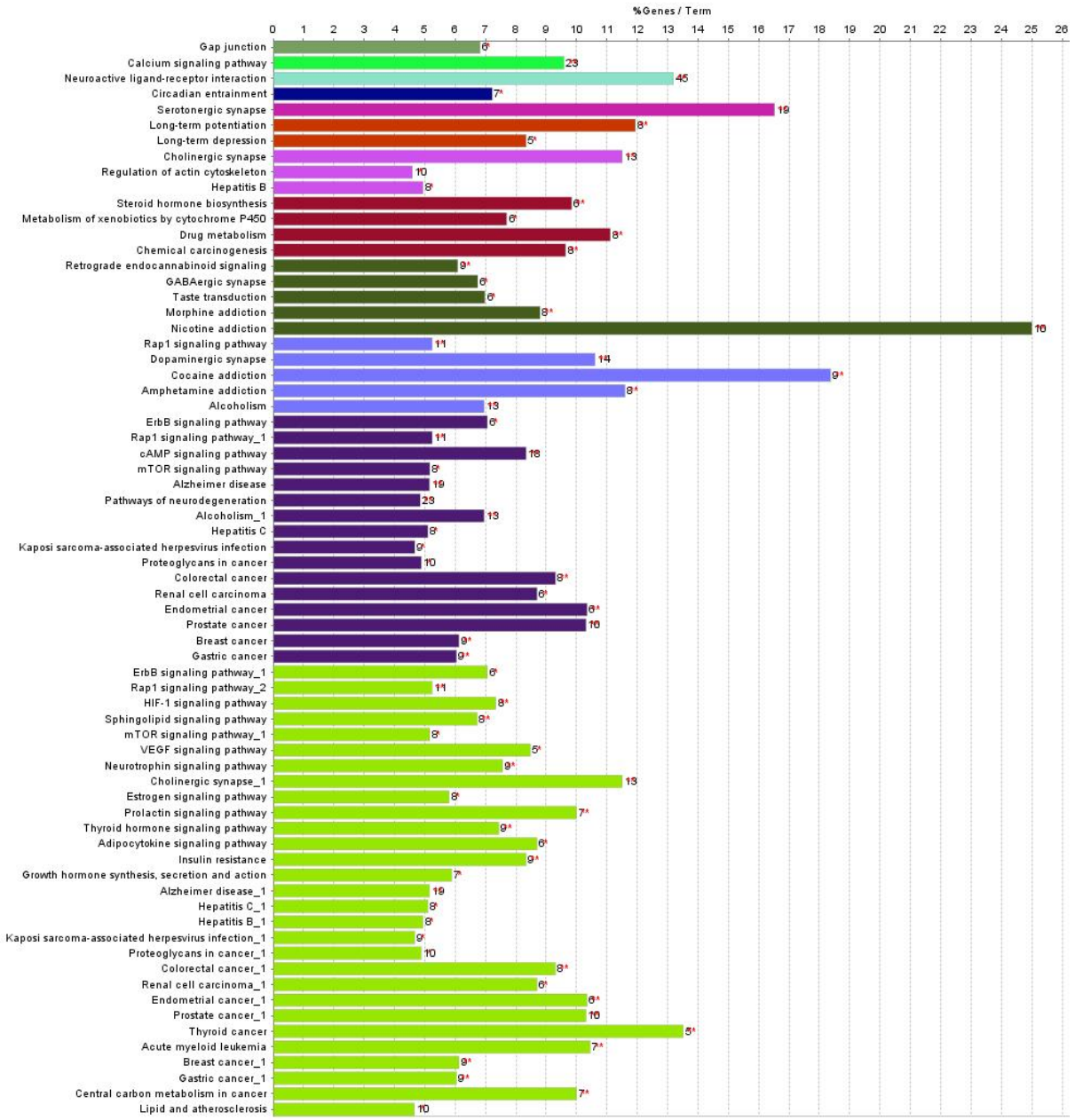

Figure 4 Analysis of KEGG function enrichment of Sug-Mel-sum-thang (SMST). SMST, Sug-Mel-sum-thang; KEGG, Kyoto Encyclopedia of Genes and Genomes.

network diagram of the "Tibetan medicine-compound-target-pathway". The graph nodes represent active ingredients, squares represent targets, inverted triangles represent compounds, quadrilaterals represent pathways, and hexagons are traditional Chinese medicines. The area and color transparency of the node represents the degree values. The larger the area and darker the color, the more important the node, as shown in Figure 5.

Molecular docking

Molecular docking was conducted to explore the binding activity between the target protein and its corresponding components. The active ingredients with node degrees greater than twice the median in the "Tibetan medicine-compound-target-pathway" network diagram constructed herein were screened as the core ingredients of SMST for the treatment of ID (Table 2). Node degree values greater than twice the median in the PPI network were selected as the core targets, which could be considered the core targets of ID treatment. These 13 genes were selected as receptors, as shown in Table 3.

It is generally believed that the smaller the docking score, the lower the binding energy to the receptor and the stronger the potential activity of the ligand. The results obtained by molecular docking showed that Van der Waals forces, Pi-alkyl, H-bond, Pi-Pi stacking, and other forces were present, indicating that the core active components and the target have good binding activity. The molecular docking results are shown in Figure 6, and part of the molecular docking is shown in Figure 7.

SPR results

The results of the SPR affinity test revealed the KD values of piperine, apigenin, and CNR1 to be 0.000977 μmol/L and 0.008527 μmol/L, respectively, as shown in Figures 8 and Figures 9. The KD value of piperine and COMT was 38.3 μmol/L (Figure 10). The SPR results confirmed that apigenin, piperine, CNR1, piperine, and COMT exhibited good binding.

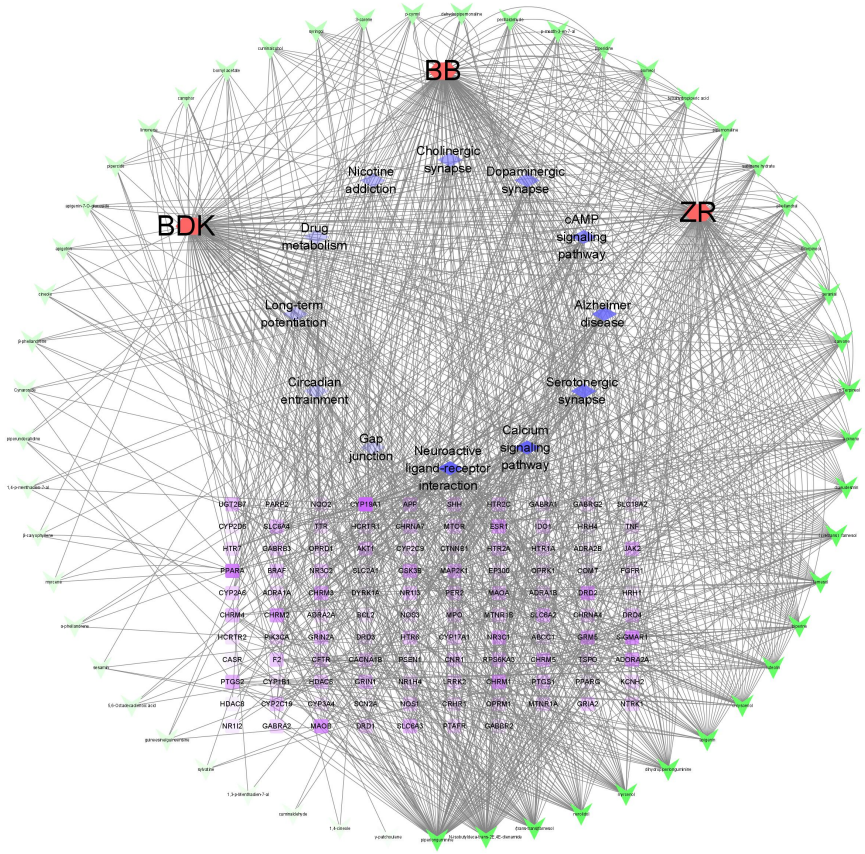

Figure 5 "Tibet medicine-compound-target-pathway" network diagram of SMST. SMST, Sug-Mel-sum-thang.BB, *Piper longum*; BDK, *Amomum kravanh*; ZR, *Cuminum cyminum.*

Table 2 Information on core components of SMST for ID treatment

CanonicalSMILES	Compound	Degree	source
CC(C)CNC(=O)C=CC=CC1=CC2=C(C=C1)OCO2	Piperlonguminine	77	Piper longum
CC(=CCCC(=CCCC(=CCO[Si](C)(C)C)C)C)C	(trans-trans)farnesol	41	Amomum kravanh
CC(=CCCC(=CCCC(C)(C=C)O)C)C	Nerolidol	34	Amomum kravanh
CC(C)(CCCC(=C)C=C)O	Myrcenol	33	Amomum kravanh
C(=O)(/C=C/CCc1cc2OCOc2cc1)NCC(C)C	Dihydropiperlonguminine	32	Piper longum
c1(cc(=O)c2c(o1)cc(cc2O)O)c1ccc(cc1)O	Apigenin	31	Cuminum
c1(cc(=O)c2c(o1)cc(cc2O)O)c1cc(c(cc1)O)OC	Chrysoeriol	31	Cuminum
c1(cc(=O)c2c(o1)cc(cc2O)O)c1cc(c(cc1)O)O	Luteolin	29	Cuminum
N1(CCCCC1)C(=O)/C=C/C=C/c1cc2OCOc2cc1	Piperine	27	Piper longum

SMST, Sug-Mel-sum-thang; ID, insomnia disorder.

Table 3 Molecular docking target protein structure information

Target	PDB ID
AKT1	1UNQ
SLC6A4	5I6X
COMT	6I3C
TNF	6U66
CTNNB1	5IVN
CHRNA4	6CNJ
GRM5	6FFI
ESR1	7BAA
DRD2	7JVR
CNR1	5U09
CYP3A4	5HDZ
GRIN2A	5H8F
HTR2A	6WH4

PDB, protein data bank database.

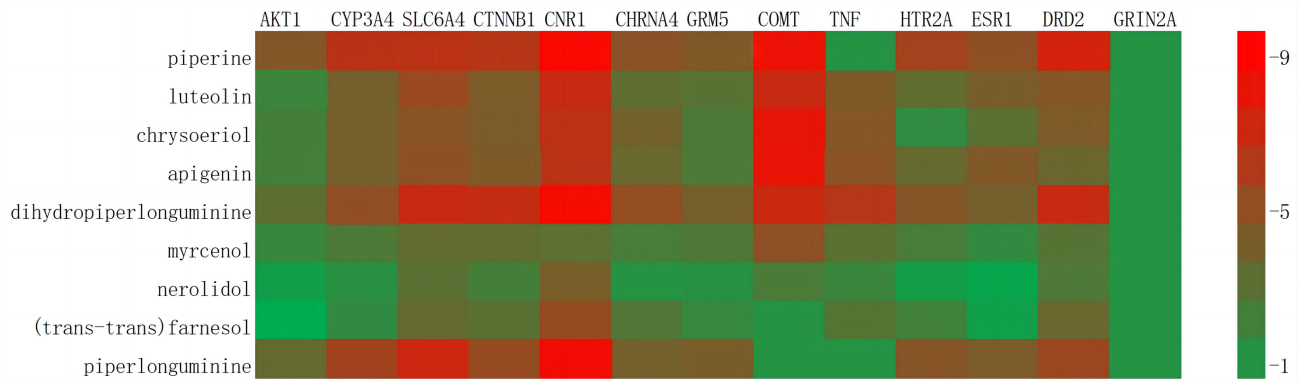

Figure 6 Molecular docking scoring heat map of the core active ingredients and key targets in SMST. SMST, Sug-Mel-sum-thang.

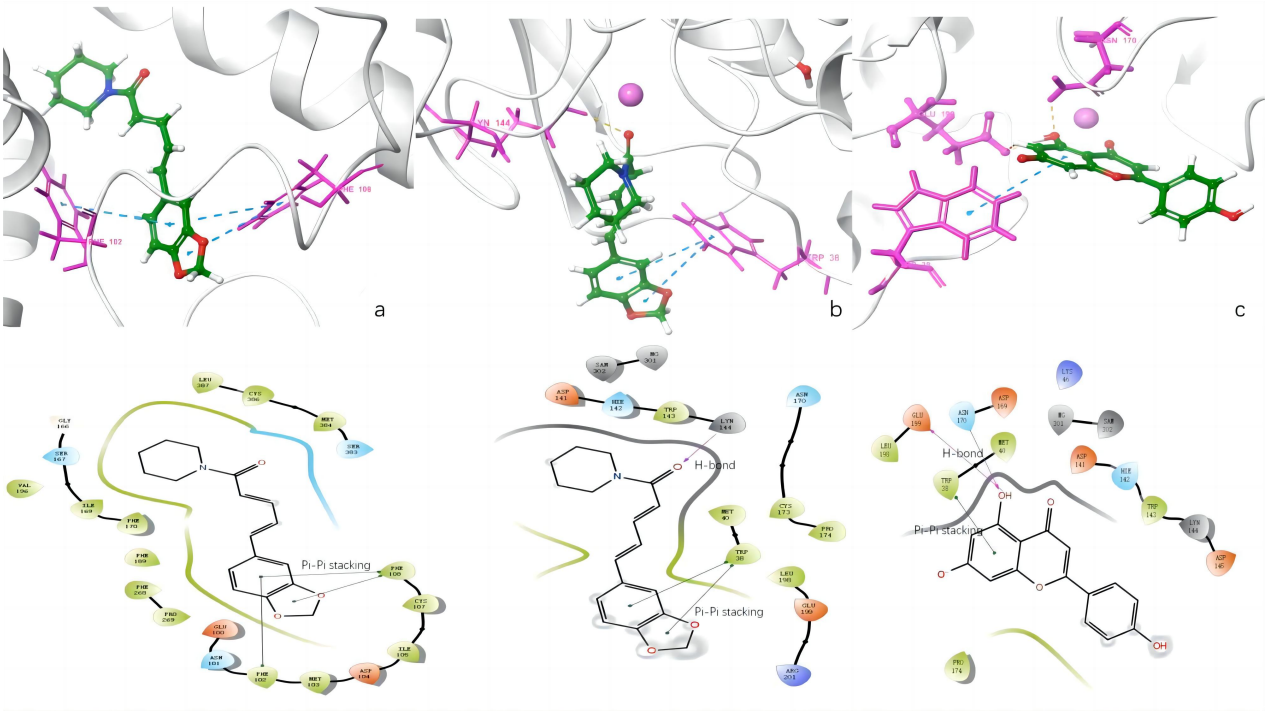

Figure 7 Part of the molecular docking diagram of the active ingredients in SMST with key targets (top: 3D, bottom: 2D). a: CNR1-piperine; b: COMT–piperine; c: COMT–apigenin. SMST, Sug-Mel-sum-thang.

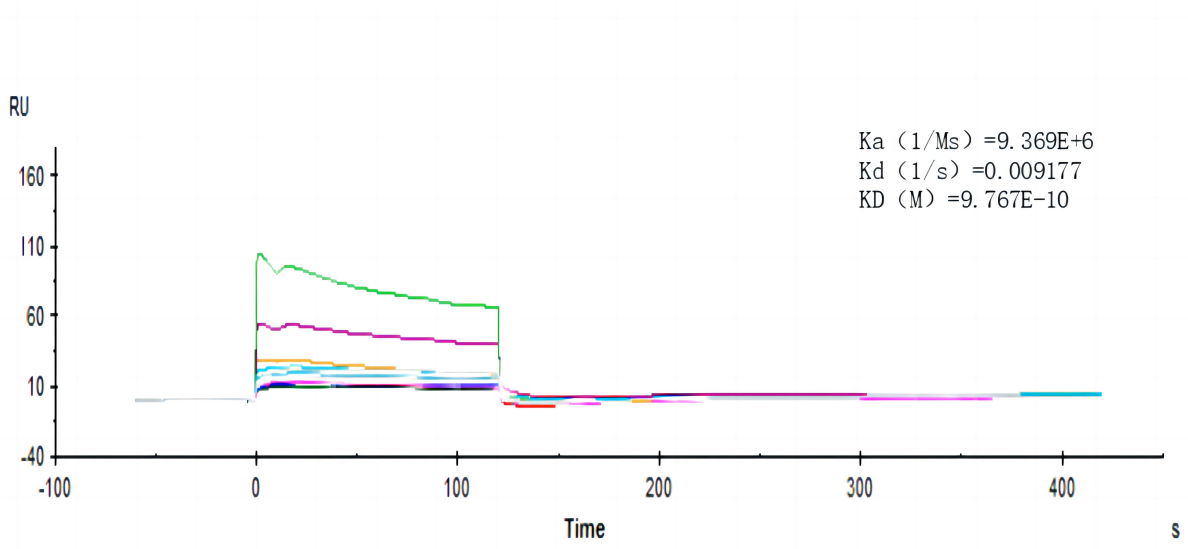

Figure 8 CNR1-piperine

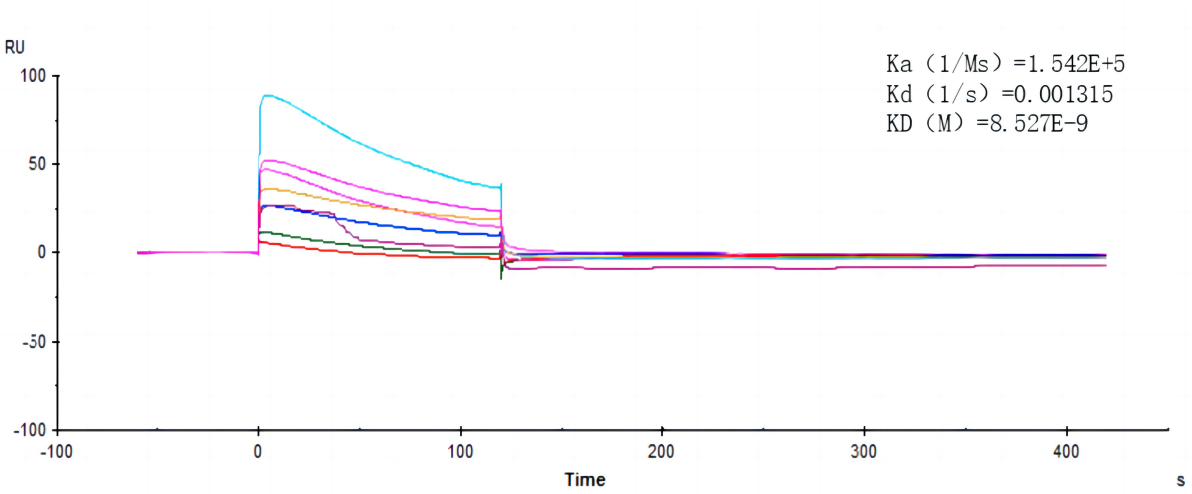

Figure 9 CNR1-apigenin

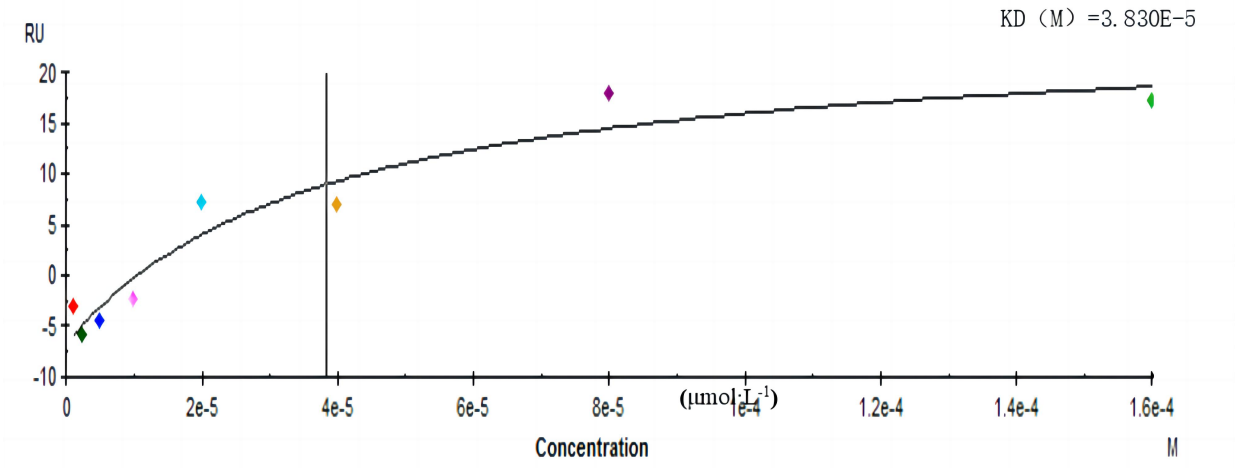

Figure 10 COMT-piperine. (From right to left, the concentration is 160, 80, 40, 20, 10, 5, 2.5, 1.25 μM)

Discussion

Sleep is an essential physiological process in humans, as it is necessary for individual survival and has several functions, such as improving memory, promoting growth and development, delaying aging, and enhancing immunity. Research shows that approximately one-third of adults have ID, which is currently treated with benzodiazepines and other sleep-helping drugs (including anti-anxiety drugs, antidepressants, melatonin steroids, and hormone drugs). However, these drugs generally have disadvantages such as strong drug dependence, easy addiction, and easy development of drug resistance [12].

Traditional Tibetan medicine has unique sleep-improving properties and has accumulated a wealth of clinical prescriptions. SMST is the first choice of Tibetan medicine in ID treatment. In Tibetan medicine, ID is considered a disorder of "rLung" in the "three humors", and the combination of sweet and pungent herbs and "hot" and "greasy" effects will have a good therapeutic effect. SMST is a classic Tibetan medicinal formula composed of three herbs: *A. kravanh*, *P. longum*, and *C. cyminum*. *A. kravanh* has an acrid taste, whereas *P. longum* and *C. cyminum* are sweet and acrid. These three herbs have the effect of being "hot" and "greasy", and can thus inhibit the "rLung" disease and soothe the nerves, thus aiding in sleep.

Nine core components in SMST were obtained by combining the PPI network and the "Tibetan medicine-compound-target-pathway" network constructed in this study and screening the active ingredients with a node degree greater than twice the median. Apigenin has been used to improve the symptoms of chronic ID and has shown preliminary efficacy [13, 14]. Both insomnia and depression are central nervous system diseases. The blood-brain barrier (BBB) facilitates the supply of nutrients to the brain, regulates the brain microenvironment, and influences the biological activities of various compounds [15]. The BBB also protects the central nervous system from compounds that negatively affect its function. Studies have shown that flavonoids can easily penetrate the BBB in the following order: genistein > isoliquiritigenin > apigenin > puerarin > kaempferol > hesperidin > rutin > quercetin [16, 17]. Therefore, apigenin may cross the BBB and play a role in ID treatment [13].

The KEGG pathway enrichment results showed that the treatment of ID by SMST mainly involved the Alzheimer's disease, cAMP signaling, serotonergic synapse, and calcium signaling pathways. Alzheimer's disease, the most common type of dementia, is a neurodegenerative disorder characterized by memory deficits, particularly forgetting recent information, impaired recall, loss of time tracking, problem-solving, language, and difficulty in identification. Research suggests that ID is involved in the pathogenesis of Alzheimer's disease

and may affect its symptoms and development [18–24].

Molecular docking results showed that the core ingredients, piperine, apigenin, and piperlonguminine, had good docking activity with the target proteins COMT, CNR1, and SLC6A4. BIAcore technology can monitor the entire reaction process of bonded substances on the surface of metal films by tracking and detecting the SPR angle of incident light in the medium in real-time. For example, the entire process of the binding and dissociation of biomolecules can be detected in real-time. Through the BIAcore experiment, it was verified that CNR1 combined well with piperine and apigenin, and COMT combined well with piperine [25–28]. Therefore, it can be concluded that the active ingredients of SMST combine well with the target protein and can play a role in ID treatment. No animal experiments were performed in this study. The sedative and hypnotic effects of the predicted core compound on mice were observed, and core compounds with good effects were selected to further explore the mechanism of action of ID therapeutics and provide support for clinical application.

Conclusion

In this study, a comprehensive analytical method combining active ingredient screening, target prediction, molecular docking, and SPR technology was used for the first time to identify the main active ingredients in SMST. Additionally, the possible molecular mechanisms underlying ID treatment were elucidated. This study provides theoretical and experimental bases for the development and application of SMST in ID therapy.

References

1. Buysse DJ. Insomnia. *JAMA* 2013;309(7):706–716. Available at: http://doi.org/10.1001/jama.2013.193
2. Morin CM, Drake CL, Harvey AG, et al. Insomnia disorder. *Nat Rev Dis Primers* 2015;1(1). Available at: http://doi.org/10.1038/nrdp.2015.26
3. Sutton EL. Insomnia. *Ann Intern Med* 2021;174(3):ITC33–ITC48. Available at: http://doi.org/10.7326/AITC202103160
4. Riemann D, Nissen C, Palagini L, Otte A, Perlis ML, Spiegelhalder K. The neurobiology, investigation, and treatment of chronic insomnia. *Lancet Neurol* 2015;14(5):547–558. Available at: http://doi.org/10.1016/S1474-4422(15)00021-6
5. Morin CM, Vallières A, Guay B, et al. Cognitive Behavioral Therapy, Singly and Combined With Medication, for Persistent Insomnia. *JAMA* 2009;301(19):2005. Available at: http://doi.org/10.1001/jama.2009.682
6. Li MJ, Zhang JK, Li HL, et al. Study on the Sedative and Hypnotic Effects of Tibetan Medicine Sugmel Sumtang. *J Liaoning Univ* 2020;47(01):65–70. Chinese. Available at: https://wenku.baidu.com/view/f73d347832b765ce0508763231126edb6f1a767f?fr=xueshu&_wkts_=1687319142596
7. Zhang JK, Cai RNJ, Nan JDZ, et al. An Overview and Perspective of Tibetan Medicine Sug sMel sum thang in Treating Insomnia. *Chin J Ethnomed Ethnopharm* 2018;27(23):85–87. Chinese. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C44YLTlOAiTRKibYlV5Vjs7iLik5jEcCI09uHa3oBxtWoB7jdU8N8rVidBEjXiUrr2QrjN2hifcKLel1gb74smG0&uniplatform=NZKPT
8. Suo NZM, Dang CJ. Tibetan medicine Sug sMel sum thang treated 60 cases of insomnia. *Chin J Ethnomed Ethnopharm* 2018;24(07):45+80. Chinese. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C44YLTlOAiTRKibYlV5Vjs7i0-kJR0HYBJ80QN9L51zrP7YszauUrZ4eyuN98TjJf3TOB-NMnhjHMhAw-0Q6xkVB&uniplatform=NZKPT
9. Han L, Han Y. Network Pharmacology-Based Study on the Active Component and Mechanism of the Anti-Gastric-Cancer Effect of Herba Sarcandrae. J Healthc Eng 2021;2021:1-12. Available at: http://doi.org/10.1155/2021/3001131
10. Liu B, Wang A, Cao Z, Li J, Zheng M, Xu Y. Mechanism of Pilose Antler in Treatment of Osteoporosis Based on Network Pharmacology. *J Healthc Eng* 2022;2022:1–7. Available at: http://doi.org/10.1155/2022/5298892
11. Xu R, Yang X, Tao Y, et al. Analysis of the Molecular Mechanism of Evodia rutaecarpa Fruit in the Treatment of Nasopharyngeal Carcinoma Using Network Pharmacology and Molecular Docking. *J Healthc Eng* 2022;2022:6277139. Available at: https://doi.org/10.1155/2022/6277139
12. Kay Stacey M, Attarian H. Advances in the management of chronic insomnia. *BMJ* 2016:i2123. Available at: http://doi.org/10.1136/bmj.i2123
13. Salehi B, Venditti A, Sharifi-Rad M, et al. The Therapeutic Potential of Apigenin. *Int J Mol Sci* 2019;20(6):1305. Available at: http://doi.org/10.3390/ijms20061305
14. Zick SM, Wright BD, Sen A, Arnedt JT. Preliminary examination of the efficacy and safety of a standardized chamomile extract for chronic primary insomnia: A randomized placebo-controlled pilot study. *BMC Complement Altern Med* 2011;11(1). Available at: http://doi.org/10.1186/1472-6882-11-78
15. Campos Bedolla P, Walter FR, Veszelka S, Deli MA. Role of the Blood–Brain Barrier in the Nutrition of the Central Nervous System. *Arch Med Res* 2014;45(8):610–638. Available at: http://doi.org/10.1016/j.arcmed.2014.11.018
16. Végh K, Riethmüller E, Hosszú L, et al. Three newly identified lipophilic flavonoids in Tanacetum parthenium supercritical fluid extract penetrating the Blood-Brain Barrier. *J Pharm Biomed Anal* 2018;149:488–493. Available at: http://doi.org/10.1016/j.jpba.2017.11.029
17. Yang Y, Bai L, Li X, et al. Transport of active flavonoids, based on cytotoxicity and lipophilicity: An evaluation using the blood–brain barrier cell and Caco-2 cell models. *Toxicol In Vitro* 2014;28(3):388–396. Available at: http://doi.org/10.1016/j.tiv.2013.12.002
18. Sadeghmousavi S, Eskian M, Rahmani F, Rezaei N. The effect of insomnia on development of Alzheimer's disease. *J Neuroinflammation* 2020;17(1):289. Available at: http://doi.org/10.1186/s12974-020-01960-9
19. Bubu OM, Brannick M, Mortimer J, et al. Sleep, Cognitive impairment, and Alzheimer's disease: A Systematic Review and Meta-Analysis. *Sleep* 2016;40(1). Available at: http://doi.org/10.1093/sleep/zsw032
20. Almondes KM de, Costa MV, Malloy-Diniz LF, Diniz BS. Insomnia and risk of dementia in older adults: Systematic review and meta-analysis. *J Psychiatr Res* 2016;77:109–115. Available at: http://doi.org/10.1016/j.jpsychires.2016.02.021
21. Chen PL, Lee WJ, Sun WZ, Oyang YJ, Fuh JL. Risk of Dementia in Patients with Insomnia and Long-term Use of Hypnotics: A Population-based Retrospective Cohort Study. *PLoS One* 2012;7(11):e49113. Available at: http://doi.org/10.1371/journal.pone.0049113
22. Yaffe K, Nettiksimmons J, Yesavage J, Byers A. Sleep Quality and Risk of Dementia Among Older Male Veterans. *Am J Geriatr Psychiatry* 2015;23(6):651–654. Available at: http://doi.org/10.1016/j.jagp.2015.02.008
23. Tsapanou A, Gu Y, Manly J, et al. Daytime Sleepiness and Sleep Inadequacy as Risk Factors for Dementia. *Dement Geriatr Cogn Disord Extra* 2015;5(2):286–95. Available at: http://doi.org/10.1159/000431311
24. Hung CM, Li YC, Chen HJ, et al. Risk of dementia in patients with primary insomnia: a nationwide population-based case-control study. *BMC Psychiatry* 2018;18(1). Available at: http://doi.org/10.1186/s12888-018-1623-0

25. Zimmermann P, Egea Jimenez AL. Study of PDZ–Peptide and PDZ–Lipid Interactions by Surface Plasmon Resonance/BIAcore. *Methods Mol Biol* 2021:75–87. Available at: http://doi.org/10.1007/978-1-0716-1166-1_5
26. Drake AW, Tang ML, Papalia GA, Landes G, Haak-Frendscho M, Klakamp SL. Biacore surface matrix effects on the binding kinetics and affinity of an antigen/antibody complex. *Anal Biochem* 2012;429(1):58–69. Available at: http://doi.org/10.1016/j.ab.2012.06.024
27. Myszka DG. Analysis of small-molecule interactions using Biacore S51 technology. *Anal Biochem* 2004;329(2):316–323. Available at: http://doi.org/10.1016/j.ab.2004.03.028
28. Chen X, Wu Y, Chen C, et al. Identifying potential anti-COVID-19 pharmacological components of traditional Chinese medicine Lianhuaqingwen capsule based on human exposure and ACE2 biochromatography screening. *Acta Pharm Sin B* 2021;11(1):222–36. Available at: http://doi.org/10.1016/j.apsb.2020.10.002