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NETWORK PHARMACOLOGY ANALYSIS OF YINAOAN CAPSULES HOSPITAL PREPARATION FOR TREATING EPILEPSY BASED ON MULTIPLE PATHWAY INFORMATION

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ABSTRACT

Based on network pharmacology and various route information, investigate prospective substances and mechanisms of hospital preparation of Yinaoan capsule (YNA) in the treatment of epilepsy. The compounds of YNA were collected using the Traditional Chinese Medicine System Pharmacology Database (TCMSP), Traditional Chinese Medicines Integrated Database (TCMID), Encyclopedia of Traditional Chinese Medicine (ETCM), and related literature, and the pkCSM platform was used to predict the pharmacokinetic parameters. The targets of compounds were predicted by SwissTargetPrediction; Collect epilepsy targets and the information of Anti-epileptic drugs from databases such as Genecards and TTD, then obtain intersection targets of YNA compounds and epilepsy. KEGG pathway enrichment analysis was performed through the DAVID database. Gephi was used to construct the network. Finally, the compounds were confirmed by Autodock Vina. 27 key pathways of YNA (Calcium signaling pathway, cAMP signaling pathway, etc.) can be obtained by KEGG pathway analysis. 25 core targets (MAPK3, PRKCA, etc.) and 20 key compounds (GC195, ZNX069, etc.) were obtained by network analysis. The major molecules and the core targets have a strong binding interaction, as demonstrated by molecular docking. By working on MAPK3, PRKCA, and other core targets through important chemicals like GC195 and ZNX069 to activate the Calcium signaling pathway, the cAMP signaling system, and other pathways, YNA may have anti-epileptic effects.

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Introduction

A seizure is a brief episode of symptoms brought on by abnormally high or synchronized neural activity in the brain [1]. Epilepsy is a brain illness defined by a lasting propensity to have epileptic seizures [2, 3]. Epilepsy affects people of all ages, races, social classes, and geographic locations [4, 5]. According to the 2016 Global Burden of Disease Collaborators, epilepsy accounts for a relevant fraction of the worldwide disease burden, affecting approximately 46 million people [6, 7]. Although the etiology agent can be identified, it remains an unknown cause in about half of cases [8]. Currently, epilepsy is a treatable disorder, with up to 80% going into prolonged seizure remission and up to 50% continuing to be seizure-free after stopping treatment [4, 9]. Anti-epileptic drugs are the primary treatment for seizures, and they have effective responses in 70% of patients, mainly using monotherapy [10]. However, 20-30% of all patients are not responsive to treatment with pharmaceuticals, which is described as “drug-resistant epilepsy” [11]. In addition, the use of anti-epileptic drugs will bring many adverse effects [12]. The first generation of anti-epileptic drugs mainly caused neurological effects. When the patient is

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pregnant, it is necessary to choose anti-epileptic drugs carefully to avoid teratogenic effects on the fetus. The patients treated with anti-epileptic drugs have a two-fold to three-fold increased risk of bone fractures [13].

In China, epilepsy was first recorded in the "Huangdi Neijing", which is considered to be related to congenital loss [14]. "Danxi Xinfa" believes that epilepsy is related to phlegm [15]. "Zhangshi Yitong" states that the treatment of epilepsy should nourish the kidney as the foundation and eliminate phlegm as the appearance [16]. Traditional Chinese medicine (TCM) has a long history in the treatment of epilepsy, which can be summarized into the following four aspects: ① nourishing qi and cultivating vitality, nourishing the kidney, invigorating the spleen, and strengthening the foundation; ② eliminating phlegm and removing blood stasis; ③ detoxifying; ④ activating spirit to resuscitate brain [17]. TCM treatment of epilepsy is characterized by various methods, significant curative effects, and few side effects, which can alleviate seizures of different types and improve the quality of life of patients [18].

The Yinaoan capsules (YNA) in this study were developed by Prof. Liu Mao-cai of the Guangzhou University of Traditional Chinese Medicine. It combines pharmacology and his years of experience in treating epilepsy based on the experienced formula for epilepsy "ChuXian powder" of Mr. Lin Xia-quan, a famous veteran Chinese medicine practitioner in Guangdong Province. The main composition of YNA is *Ziziphus jujuba* (Suan Zao Ren-SZR), *Paeonia lactiflora* (Bai Shao-BS), *Angelica sinensis* (Dang Gui-DG), *Gastrodia elata* (Tian Ma-TM), *Arisaema erubescens* (Zhi Nan Xing-ZNX), *Buthus martensii* (Quan Xie-QX), *Scolopendra subspinipes mutilans* (Wu Gong-WG), *Glycyrrhiza uralensis* (Gan Cao-GC). Animal experiments have shown that YNA can prolong the incubation period, lower the amplitude of convulsive discharge, and shorten the lasting time of convulsive seizures, and these effects were similar to those of phenytoin sodium [19]. In addition, the combined clinical application of YNA and anti-epileptic drugs has better overall efficacy than that of anti-epileptic drugs alone [20].

Although studies have shown that YNA exerts anti-epileptic effects, the active compounds and mechanisms remain to be elucidated. Therefore, network pharmacology can be used for preliminary exploration [21]. Currently, in the network pharmacology studies, existing studies analyze the potential effects of herbal medicines by exploring more drug information, such as principal component analysis of herbal compounds with molecular descriptors of FDA-approved anti-infective and anti-inflammatory drugs based on structure-effect relationship, which in turn leads to predict the anti-infective or anti-inflammatory efficacy of herbs [22]. In addition, some researchers perform hierarchical clustering of herbal component target profiles with FDA-approved drug target profiles to analyze the potential mechanism of herbal medicines [23]. However, there is still a lot of room for the utilization of disease and disease drug pathway information in network pharmacology research. In this study, a network pharmacological method integrating disease and disease drug pathway information was proposed to provide a reference for the study of the mechanism of YNA.

Materials and Methods

YNA Compounds and Target Collection

The compounds of 8 herbal medicines in YNA were collected by Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), Traditional Chinese Medicine Integrated Database (TCMID), The Encyclopedia of Traditional Chinese Medicine (ETCM), and related literature. Among them, considering that glycosylated compounds may be deglycosylated *in vivo* through glycosidase hydrolysis, glycoside ligands of these compounds are also included in the TCMSP database. pkCSM (http://biosig.unimelb.edu.au/pkcsm/) was used to predict the pharmacokinetic parameters of compounds. In this article, two parameters related to the blood-brain barrier, BBB permeability (blood-to-brain drug partition measurements at steady state (log BB)) and CNS permeability (blood-brain permeability-surface area product (logPS)) were selected to screen active compounds.

After screening, SwissTargetPrediction was used to predict the target of the potential active compounds. The SwissTargetPrediction algorithm predicts the most likely macromolecular targets by combining 2D and 3D similarities of small molecules.

YNA-Epilepsy Intersection Targets

To find disease targets associated with epilepsy, the following resources were used: OMIM database (https://omim.org/), Genecards (version 5.13, https://www.genecards.org/), TTD (http://db.idrblab.net/web/), DisGenet (version v7.0, https://www.disgenet.org/), and Drugbank (version 5.1.9, https://go.drugbank.com/). The search was conducted using the keyword "epilepsy". Subsequently, the compound's targets and disease targets are intersected to obtain potential targets of YNA for the treatment of epilepsy.

Anti-Epileptic Drugs and Target Collection

Drugbank and TTD were used to collect anti-epileptic drugs and their targets. Then the US Food and Drug Administration (FDA), the National Medical Products Administration (NMPA), the Pharmaceuticals and Medical Devices Agency (PMDA), and the European Medicines Agency (EMA), were used to confirm whether drugs are still circulating in the four major markets.

Analysis of KEGG Pathway Enrichment

The collected Gene symbols of YNA-epilepsy targets, epilepsy targets, and anti-epileptic drug targets were imported into DAVID (version 2021, https://david.ncifcrf.gov/). Identifier, Background, and Pathways menus are respectively selected as

OFFICIAL_GENE_SYMBOL, Homo sapiens, and KEGG_PATHWAY to obtain YNA-epilepsy, epilepsy, and anti-epilepsy drugs related KEGG pathway enrichment analysis results, respectively.

Network Construction

According to the results of the KEGG pathway enrichment analysis, the Gephi (Version 0.9.6) software was used to construct a target-key pathway network and a compound-core target network respectively, to analyze the core targets and key compounds in YNA.

Molecular Docking

The 3D structure of the compounds was collected through the TCMSP and Pubchem. Chemdraw (Version 16.0, Cambridge Soft, USA) would be used to draw the compounds for which there was no structural information in the two databases. Lastly, batch conversion of SDF format files into mol2 format files was done using Open Babel GUI (Version 2.4.1), and molecular docking was carried out using Autodock Vina (Version 1.1.2, Scripps Research, USA). The 3D structure of the target protein can be downloaded through the PDB database (http://www.rcsb.org/) and saved in the pdbqt format before docking. The target protein's eutectic ligand is where the docking box is positioned. Target proteins without eutectic ligands are wrapped as much as feasible by the docking box. The configuration file's (config file) parameter values are exhaustiveness=8, energy_range=3, and num_modes=9. The better the binding capability after docking, the lower the docking threshold.

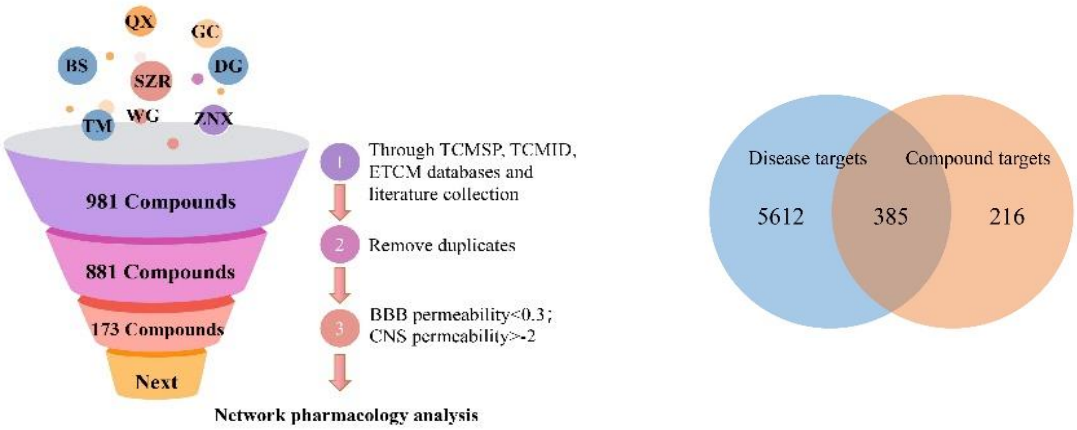
Results and Discussion

YNA Compounds and Target Collection Results

Nine hundred eighty-one constituents of the herbal remedies found in YNA were gathered from relevant literature and databases. There are 881 compounds left among them, with 100 compounds identified in different herbal treatments more than once. Then, 173 compounds were screened based on log BB> 0.3 and logPS> -2. These compounds are more likely to pass through the blood-brain barrier and penetrate the central nervous system [24], thereby exerting anti-epileptic effects. After screening, 173 compounds were obtained. The compound screening process is shown in **Figure 1a**. These compounds were predicted by SwissTargetPrediction and resulted in 601 targets.

YNA-Epilepsy Targets Collection Results

From Genecards OMIM, DisGenet, TTD, and Drugbank databases, 5997 targets altogether were obtained from the keyword "Epilepsy". Subsequently, the compound targets and disease targets were intersected to obtain 385 targets, which are potential targets for YNA to treat epilepsy, as shown in **Figure 1b**.



a) b)

Figure 1. a) The compounds screening process. b) Venn map of YNA target for treating epilepsy.

Anti-Epileptic Drugs and Their Target Collection Results

A total of 64 anti-epileptic drugs were collected from the Drugbank and TTD. 45 anti-epileptic drugs have corresponding targets. They are still circulating in the four major markets, which have been confirmed by the NMPA, FDA, EMA, and PMDA. The remaining 11 drugs had been discontinued or not approved in four regions, and the other 8 drugs have no clear targets. A total of 108 targets were obtained from 45 drugs. They are effective targets that were able to be acted by drugs to exert anti-epileptic effects.

KEGG Pathway Enrichment Analysis Results

Import the collected 385 YNA-epilepsy intersection targets, 5997 epilepsy disease targets, and 108 anti-epileptic drug targets

into DAVID, respectively, to obtain 92, 117, and 21 pathways (P<0.05). P value is used to sort every path. Three approaches were taken to obtain the main pathways of YNA-epilepsy: Map the top 10% of illness pathways into pathways of YNA-epilepsy intersection targets, acquire the top 10% of YNA-epilepsy intersection target pathways, and map all anti-epileptic medication routes into pathways of YNA-epilepsy intersection target pathways. Through the first step, 9 key pathways can be obtained, including the Calcium signaling pathway, cAMP signaling pathway, and Serotonergic synapse, etc. 8 supplementary key pathways can be obtained through the second step, including the Retrograde endocannabinoid signaling pathway, FoxO signaling pathway, and Neutrophil extracellular trap formation, etc. It is worth noting that although Lysosome and Thermogenesis rank 8th and 9th respectively in the disease pathway, they do not exist in the YNA-epilepsy pathway, which means that they are probably not the key pathway for the treatment of epilepsy by YNA, so they are not selected. Through the third step, 10 supplementary key pathways are obtained, including GABAergic synapse, Glutamatergic synapse Taste transduction, etc. Among them, Cardiac muscle contraction, Cortisol synthesis and secretion, Aldosterone synthesis and secretion, and Renin secretion rank 11th, 18th, 19th, and 20th in the drug pathway, respectively, but it does not exist in the YNA-Epilepsy pathway, and likewise is not selected as the key pathways. Finally, 27 key pathways were obtained. the details of the relationship between these pathways and epilepsy are shown in **Table 1**.

It can be seen from **Table 1** that the top 10% of the YNA-epilepsy pathway, that is, the first 9 pathways all exist in the disease pathway, and the cAMP signaling pathway and Apoptosis rank high in the disease pathway. In addition, the 10 key pathways selected from the top 10% of disease pathways all exist in the YNA-epilepsy pathway. These provide data to support the effect of YNA in the treatment of epilepsy. Among the first 9 YNA-epilepsy pathways, 4 are not involved in the current anti-epileptic drugs pathway, but 17 of the 21 anti-epileptic drug pathways exist in the YNA-epileptic pathway. It not only shows that there are multiple mechanisms of YNA in the treatment of epilepsy but also indicates the unique advantages of TCM compared with chemical drugs. New epilepsy mechanisms are constantly being proposed, such as the importance of neuroinflammation to the occurrence and development of epilepsy, and the treatment of neuroinflammation has also become an important therapeutic approach [25]. According to the pathway information, such as the PI3K-Akt signaling pathway, YNA may also be related to the treatment of neuroinflammation.

Table 1. Information on key pathways.

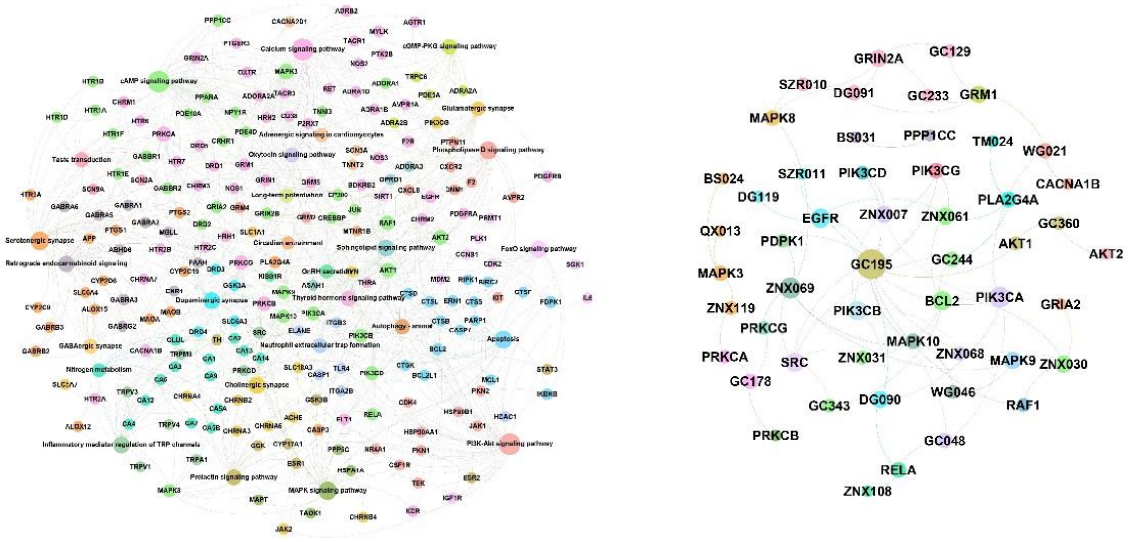
No.	Pathway	Function	Ranking in YNA-Epilepsy pathway	Rank in the disease pathway	Rank in the Anti-epileptic drug pathway
1	Calcium signaling pathway	It is an important pathway for epilepsy [26].	1	35	4
2	cAMP signaling pathway	It is related to the occurrence and treatment of epilepsy [27].	2	4	9
3	Serotonergic synapse	It is related to the treatment of epilepsy [28].	3	29	6
4	Nitrogen metabolism	It plays an important role in maintaining ammonia homeostasis and preventing epilepsy [29].	4	40	-
5	Apoptosis	Epilepsy leads to neuronal apoptosis [30].	5	5	-
6	Cholinergic synapse	It is crucially involved in the modulation of epilepsy [31].	6	28	10
7	Inflammatory mediator regulation of TRP channels	It could be an emerging target for seizure disorders [32].	7	69	14
8	Phospholipase D signaling pathway	It plays a unique pathophysiological function in epileptic seizures [33].	8	77	-
9	Prolactin signaling pathway	Prolactin has a neuroprotective effect [34].	9	19	-
10	Retrograde endocannabinoid signaling	It controls GABA release [35].	21	1	2
11	FoxO signaling pathway	Neuronal death induced by seizures is affected by this pathway [36].	10	2	-
12	Neutrophil extracellular trap formation	The neutrophil is a mediator of neuronal hyperexcitability [37].	69	3	-
13	PI3K-Akt signaling pathway	It is related to the occurrence and treatment of epilepsy [38].	11	6	-
14	GnRH secretion	Epilepsy can lead to dysregulation of this pathway [39].	35	7	17
15	Sphingolipid signaling pathway	It is involved in various neurological disorders [40].	13	10	-
16	Autophagy-animal	It is involved in epileptogenesis [41].	47	11	-
17	Thyroid hormone signaling pathway	It is involved in a form of juvenile myoclonic epilepsy [42].	28	12	-
18	GABAergic synapse	It is the pathway of action of many anti-epileptic drugs [43].	37	14	1

19	Glutamatergic synapse	It is a therapeutic pathway for epilepsy [44].	58	43	3
20	Taste transduction	The altered influx of calcium and other ions leads to impaired taste transduction [45].	24	-	5
21	Circadian entrainment	Sleep disturbance is a common complication of epilepsy [46].	82	59	7
22	Adrenergic signaling in cardiom yocytes	In chronic epilepsy, this pathway is activated and damages the myocardium [47].	71	49	8
23	Long-term potentiation	It can be used to treat epilepsy [48].	36	56	12
24	MAPK signaling pathway	It is related to the occurrence and treatment of epilepsy [27].	41	32	13
25	Dopaminergic synapse	The main function of GABA neurons and receptors is to regulate this pathway [49].	15	21	15
26	Oxytocin signaling pathway	Oxytocin has anticonvulsant and neuroprotective effects [50].	63	46	16
27	cGMP-PKG signaling pathway	It affects synaptic transmission and membrane excitability [51].	51	22	21

-: Not exist in the corresponding pathways.

KEGG Pathway Enrichment Analysis Results

In this study, 27 key pathways and corresponding targets were used to construct the key pathway-target network to obtain the core targets of YNA in the treatment of epilepsy. As shown in **Figure 2a**, the network includes 242 nodes (27 pathways, 215 targets) and 632 edges. In this network, the 25 core targets whose degree value is greater than 5 are shown in **Table 2**. This not only shows the importance of these targets for the treatment of epilepsy but also reflects that there may be multiple mechanisms by which YNA can treat epilepsy. The core target-compound network was further constructed from the core targets and their corresponding compounds to explore the key compounds, as shown in **Figure 2b**. **Table 3** lists the 20 key compounds with a degree value greater than 1 in the network. They are potential active compounds in YNA for treating epilepsy. Among them, Anethole has been shown to have anti-epileptic effects [52]. Cis-isoeugenol [53] and Methyleugenol [54] have reported antioxidant effects. Antioxidants are thought to exert neuroprotective effects in the treatment of epilepsy [55]. Methyleugenol has also been shown to have anti-epileptic and neuroprotective effects [56]. It is worth further studying their anti-epileptic effects.



a) b)

Figure 2. Network analysis. a) The key pathway-target network. b) The core target-compound network.

Table 2. The core targets retrieved from the key pathway-target network.

No.	Gene Nane	Degree
1	MAPK3	21
2	PRKCA	19
3	PRKCG	16
4	PRKCB	16
5	AKT2	16
6	AKT1	16

7	RAF1	16
8	PIK3CD	13
9	PIK3CB	13
10	PIK3CA	13
11	MAPK9	10
12	MAPK8	10
13	MAPK10	10
14	CACNA1B	7
15	GRM1	7
16	RELA	7
17	PPP1CC	7
18	EGFR	6
19	GRIN2A	6
20	GRIA2	6
21	PLA2G4A	6
22	PDPK1	6
23	BCL2	6
24	PIK3CG	6
25	SRC	6

Table 3. The core targets attained from the key pathway-target network.

No.	Mol ID	Degree	Compound
1	GC195	11	2-Tetradecanone
2	ZNX069	6	1-Acetyl-beta-carboline
3	ZNX030	4	7,10-Octadecadienoic acid, methyl ester
4	ZNX061	4	Methyl pentadecanoate
5	DG090	4	cis-Isoeugenol
6	ZNX007	4	Trioxsalen
7	GC244	3	Methyl 12-methyltetradecanoate
8	WG021	3	2-Decanone
9	DG091	3	2-Methyl-5-decanone
10	GC048	3	Anethole
11	GC178	3	1-Pentadecanol
12	GC360	2	Tetrahydroharmine
13	GC343	2	Methyl linoleate
14	ZNX031	2	8,11,14-Docosatrienoic acid methyl ester
15	GC129	2	5,6,7,8-Tetrahydro-2,4-dimethylquinoline
16	GC233	2	5,6,7,8-Tetrahydro-4-methylquinoline
17	SZR010	2	O-Nornuciferine
18	WG046	2	4-Methylbenzoic acid anhydride
19	ZNX119	2	Methyl cis-11-eicosenoate
20	ZNX068	2	Methyleugenol

The 20 compounds were docked with the 25 core targets screened respectively. Most of the scores are less than -6 kcal/mol, which shows that the predicted binding of key compounds and core targets is good, and it is worth further digging. **Figure 3** illustrates the possible binding mechanisms of the two compounds, 2-tetradecanone, and 1-acetyl-beta-carboline, to their

respective top-scoring targets. These compounds have degree values greater than 4. 2-tetradecanone formed a hydrogen bond with the amino acids Cys424 of RAF1, Gly219 of GRIA2, and Gly250 of GRIN2A. 1. A hydrogen bond was formed between acetyl-beta-carboline and the Gly250 and Ser242 amino acids of GRIN2A and GRIA2. Hydrophobic contacts make up the remaining interactions. It is evident that the binding of the active chemicals to the core target is mostly mediated by hydrophobic interactions. The docking data is crucial for future further verification in addition to providing more evidence that the major compounds have significant relationships with the primary targets.

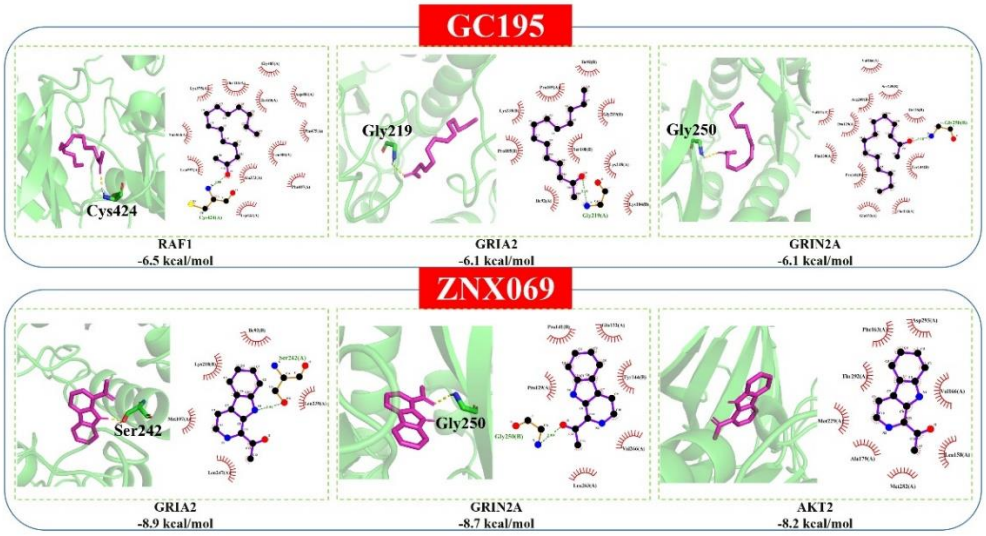


Figure 3. The predicted binding mode of GC195 and ZNX069 to their respective top three-scoring targets.

The potential mechanisms of YNA to exert anti-epileptic effects can be obtained by the KEGG pathway and network analysis. **Figure 4** shows some of the key mechanisms. YNA may act on core targets such as MAPK3 and PRKCA through 2-Tetradecanone and other compounds, thereby activating key pathways such as the Calcium signaling pathway, cAMP signaling pathway, Serotonergic synapse, and GABAergic synapse. According to the information provided by the KEGG pathway database [57], subsequent effects include Regulation of synaptic transmission and neuronal excitability; Neuroprotection; Hyperpolarization decreased excitability, etc. This study employed network pharmacology, which has been used extensively in the study of TCM [58], to predict the active ingredients and YNA's mechanisms of action for the management of epilepsy. However, since the conclusions obtained are all based on *in silico* data, further experimental verification is still needed, which is also one of the limitations of this study. We also hope to carry out related pharmacological experiments in the future to further analyze the anti-epileptic mechanisms of YNA and achieve more accurate clinical application for it.

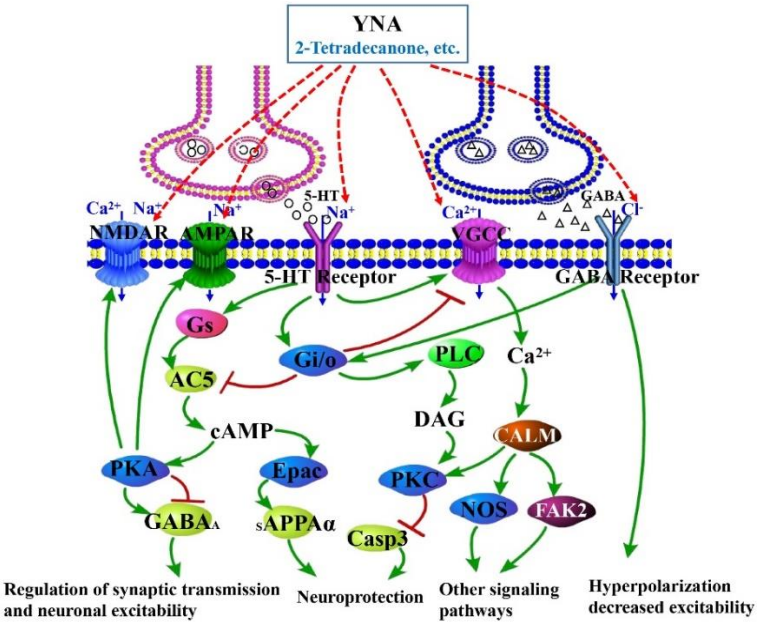


Figure 4. The potential mechanism of YNA in treating epilepsy.

Conclusion

In this study, network pharmacology integrated with data on illness pathways and anti-epileptic medication pathways was used to investigate the possible substances and processes of YNA in the treatment of epilepsy. The key pathways, targets, and compounds of YNA have been preliminarily obtained, and the preliminary confirmation has been carried out by molecular docking. According to the findings, YNA may have anti-epileptic effects via activating the calcium signaling system, the cAMP signaling pathway, and other pathways by acting on MAPK3, PRKCA, and other core targets through important chemicals like GC195 and ZNX069. This work can serve as a foundation for future clinical research in addition to providing some evidence for the therapeutic effects of YNA. In addition, the strategy of combining multiple pathway information can provide new ideas for the study of network pharmacology.

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Conflict of interest: None

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Ethics statement: None

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