

A network medicine and rank product-based framework for compound prioritization in Alzheimer's disease: application to

Pengfei Guo

The First Affiliated Hospital of Shenzhen University

Shengquan Hu

The First Affiliated Hospital of Shenzhen University

Mengmeng Jiang

The First Affiliated Hospital of Shenzhen University

Rui Hu

The First Affiliated Hospital of Shenzhen University

Yan Li

The First Affiliated Hospital of Shenzhen University

Yucui Ma

The First Affiliated Hospital of Shenzhen University

Yu Wei

Tianjin Tasly Digital Intelligence Chinese Medicine Technology Co., Ltd

Wenjia Wang

Tianjin Tasly Digital Intelligence Chinese Medicine Technology Co., Ltd

Zhengzhi Wu

szwzz001@email.szu.edu.cn

The First Affiliated Hospital of Shenzhen University

Research Article

Keywords: Alzheimer's disease, Tiantai No.1, Rank Product algorithm, network proximity, in vitro

Posted Date: September 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10781502/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

A network medicine and rank product-based framework for compound prioritization in Alzheimer's disease: application to Tiantai No.1

Pengfei Guo^{1,2}, Shengquan Hu^{1,2}, Mengmeng Jiang^{1,2}, Rui Hu^{1,2}, Yan Li^{1,2}, Yucui Ma¹, Yu Wei⁴, Wenjia Wang⁴, Zhengzhi Wu^{1,2,3*}

¹ The First Affiliated Hospital of Shenzhen University, Shenzhen Second People's Hospital Shenzhen, Shenzhen 518035, China

² Wu Zhengzhi Academician Workstation, NingBo College of Health Sciences, Ningbo, 315800, China

³ Hunan University of Traditional Chinese Medicine, Changsha, 410073, China

⁴ Tianjin Tasly Digital Intelligence Chinese Medicine Technology Co., Ltd., Tianjin 300410, China

Corresponding full address: No. 3002 Sungang West Road, Futian District, Shenzhen, Guangdong, 518035, China

Corresponding author E-mail: szwzz001@email.szu.edu.cn; **Tel.:** 0755-82530086

Abstract:

Background: Alzheimer's disease (AD) is a global public health challenge without curative therapies. Tiantai No.1, a botanical drug preparation comprising *Gastrodia elata*, *Panax ginseng*, and *Ginkgo biloba*, has shown clinical benefits in improving cognitive function in AD patients with mild cognitive impairment. However, its bioactive compounds and molecular mechanisms remain unclear.

Methods: Here, we employed an integrative approach combining network analysis, Rank Product algorithm, molecular docking, and *in vitro* assays to prioritize candidate compounds from Tiantai No.1 with potential anti-AD activity. We integrated multi-source AD-associated genes and compound-target data, constructed protein-protein interaction networks, and applied network proximity analysis to evaluate the association between Tiantai No.1 compounds and AD pathology. The Rank Product method was used to identify core targets. Molecular docking and 100-ns molecular dynamics simulations assessed binding stability between candidate compounds and prioritized targets. The protective effects of selected compounds were evaluated in glutamate (GLU)-induced HT22 neuronal cells.

Results: Network analysis revealed that Tiantai No.1 may modulate multiple pathways including calcium signaling, apoptosis, and PI3K-Akt signaling, with AKT1, IL6, SRC, and ALB as prioritized targets. *In vitro* assays showed that three candidate compounds, including genkwanin, diop, and formononetin, significantly enhancing cell viability in glutamate-injured HT22 cells. SPR analysis confirmed direct binding between diop and PTGS2 ($K_d = 3.07 \mu\text{M}$).

Conclusion: In conclusion, this study provides a hypothesis-generating framework for identifying candidate compounds from Tiantai No.1 with potential anti-AD activity, warranting further pharmacokinetic and pharmacodynamic validation.

Key words: Alzheimer's disease, Tiantai No.1, Rank Product algorithm, network proximity, *in vitro*

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia, accounting for approximately 60-70% of all dementia cases worldwide [1, 2]. It is characterized by the accumulation of amyloid-beta plaques and neurofibrillary tangles composed of hyperphosphorylated tau protein, leading to synaptic dysfunction, neuronal loss, and cognitive decline [3, 4]. AD is pathologically characterized by amyloid-beta accumulation and neurofibrillary tangles, while its clinical progression is driven by a multifactorial network involving oxidative stress, neuroinflammation, and neuronal injury, etc, collectively offering multiple therapeutic intervention points [5, 6]. Despite significant advances in understanding the molecular mechanisms underlying AD, effective disease-modifying therapies remain elusive, and current treatments primarily focus on symptomatic relief. The growing global burden of AD, driven by aging populations, underscores the urgent need for novel therapeutic strategies and biomarkers for early diagnosis and intervention.

Despite significant advances in understanding its pathogenesis, effective disease-modifying therapies remain limited. Traditional Chinese Medicine (TCM) has shown unique advantages in AD treatment, including multi-target effects, holistic regulation, and fewer side effects, making it a promising complementary approach to conventional therapies [7-9]. Min-Rui Ding et al. conducted a literature-based study and found that TCM can modulate multiple signaling pathways implicated in the pathological progression of AD, including the nuclear factor kappa-B, nuclear factor erythroid 2-related factor 2, and the Janus kinase/signal transducer and activator of transcription pathways. By regulating the crosstalk among these signaling networks, TCM helps maintain the balance of chronic inflammatory responses, attenuates oxidative stress damage, and modulates autophagic function, ultimately leading to the amelioration of cognitive impairment in AD patients [7]. Tiantai No.1, a herbal formulation primarily composed of *Rhizoma Gastrodiae*, *Radix Ginseng*, and *Folium Ginkgo*, has emerged as a potential therapeutic agent for AD and stress-related pathologies. A multicenter randomized double-blinded controlled clinical trial

demonstrated that Tiantai No.1 significantly improves cognitive impairment in patients with mild cognitive impairment and reduces the conversion rate from MCI to AD [10]. Preclinical studies utilizing diverse experimental models have elucidated its multi-target mechanisms. For example, in SAMP8 mice, Tiantai No.1 administration significantly reduced amyloid-beta deposition, enhanced cellular proliferation marker Ki67, suppressed glial fibrillary acidic protein-positive cells, and promoted LC3B-positive autophagosomes [11]. Further, in male SD rats and mesenchymal stem cells, Tiantai No.1 upregulated endoplasmic reticulum stress markers endoplasmic reticulum chaperone BiP mRNA and endoplasmic reticulum chaperone BiP mRNA and suppressed apoptosis Caspase-12 mRNA, highlighting its capacity to ameliorate ER stress-induced cellular damage [12]. These findings collectively underscore Tiantai No.1's pleiotropic mechanisms, spanning autophagy enhancement, anti-inflammatory signaling, and endoplasmic reticulum stress regulation, positioning it as a promising candidate for further translational investigation in neurodegenerative disorders. However, the material basis and mechanisms of Tiantai No.1 remain unclear.

TCM treats diseases through a "multi-component, multi-target" approach, which embodies the holistic philosophy of viewing the body as an integrated whole, and this aligns with the characteristic features of the multiple pathological mechanisms involved in AD [9, 13]. Network proximity algorithms offer a powerful systems-level framework for AD drug discovery by leveraging the topology of the human interactome. Operating under the "disease module" hypothesis, which posits that AD-associated genes cluster within specific network neighborhoods [14, 15], these methods quantify the topological distance between drug targets and disease modules, enabling the systematic prioritization of compounds acting on the broader pathological network rather than isolated targets [16-18]. Crucially, network proximity facilitates drug repurposing by identifying approved drugs whose targets lie close to AD modules, substantially accelerating discovery timelines. For example, identified 193 candidates via this pipeline, with 32.6% supported by prior literature [15]. Moreover, the rank product algorithm can be coupled with network-based approaches to enhance the reliability of target identification. By ranking genes or

proteins according to their differential expression or perturbation responses across multiple conditions, and then mapping these ranked lists onto protein-protein interaction (PPI) networks, researchers can prioritize disease-relevant modules with greater confidence. The computational efficiency and simplicity of the rank product method further facilitate its integration into high-throughput screening workflows, making it a practical tool for both target discovery and compound prioritization [19, 20]. Finally, the candidate compound and its target were subjected to molecular docking for binding mode prediction, molecular dynamics (MD) simulations for complex stability assessment, and surface plasmon resonance (SPR) for experimental binding affinity validation, collectively providing multi-level molecular evidence.

Combined with technologies such as network proximity, rank product, molecular docking, MD, SPR and *in vitro* experiment, this study comprehensively explored the potential molecular mechanisms of the main active compounds and key targets of Tiantai No.1 in the treatment of AD (**Fig. 1**). The findings provide a theoretical foundation for molecular drug design and optimization, as well as a reference for further targeted drug experiments and clinical applications.

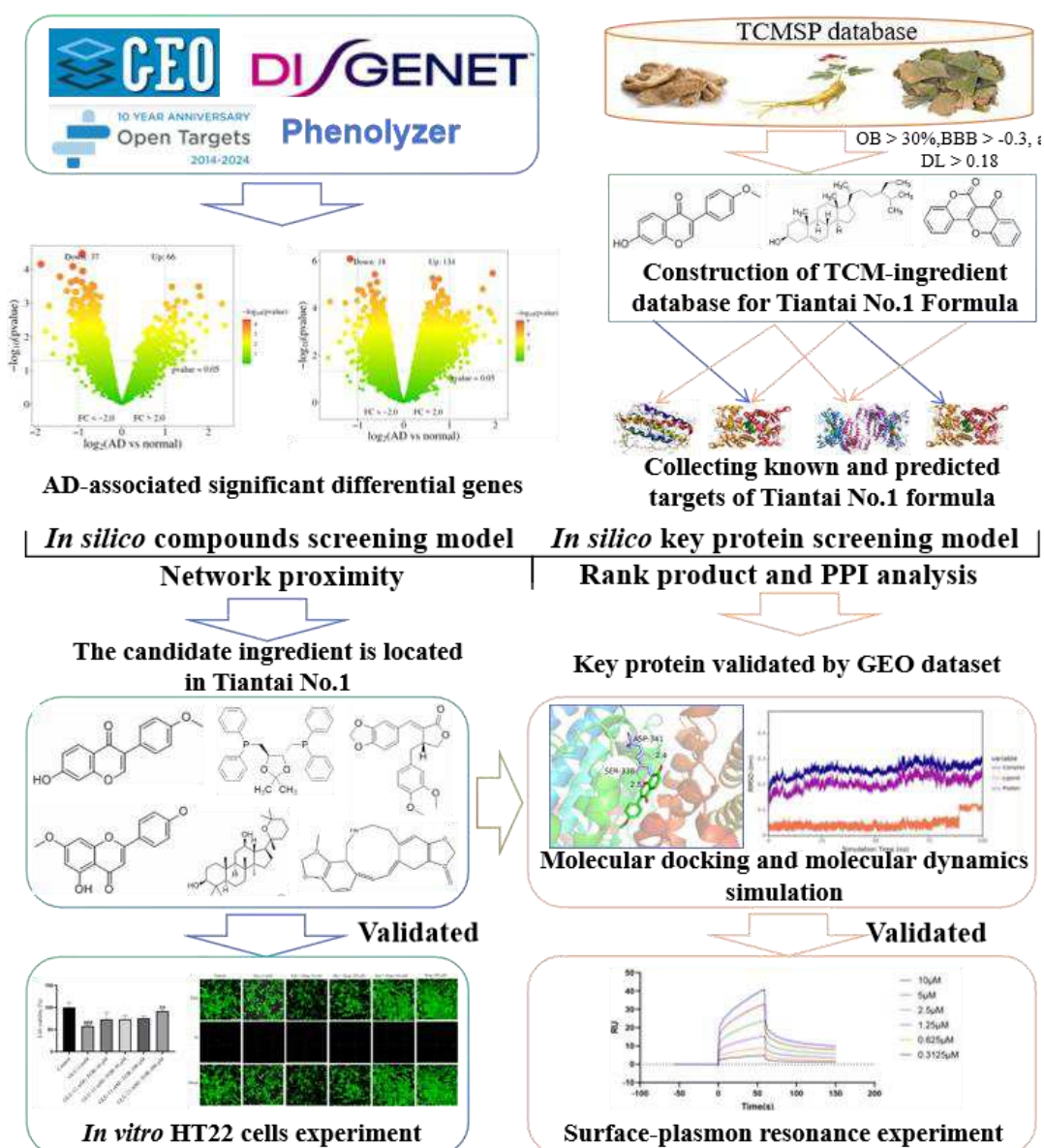


Fig. 1 Workflow illustrating the overall study design

Materials and methods

Botanical drug authentication and preparation

Tiantai No.1 was produced by Shenzhen General Factory of traditional Chinese medicine, batch No.: 030601. Tiantai No.1 is composed of three botanical drugs: *hizoma Gastrodiae*, *Radix Ginseng*, and *Folium Ginko*. All botanical drugs were authenticated by professor Zhengzhi Wu at Shenzhen Municipal Drug Inspection Institute based on morphological and microscopic characteristics according to the Chinese Pharmacopoeia (2020 edition). The dry extract was obtained through a sequential water-extraction and ethanol-precipitation procedure, lyophilized at -40°C, and preserved at -20°C in airtight containers. For experimental use, the lyophilized material was freshly reconstituted in sterile purified water to achieve the target concentrations.

Construction of TCM-compounds database for Tiantai No.1 Formula

Tiantai No.1 is composed of three TCM: *hizoma Gastrodiae*, *Radix Ginseng*, and *Folium Ginko*. This study collected the compounds of three botanical drugs from TCMSP database [21]. To prioritize compounds with favorable pharmacokinetic properties, we applied stringent selection criteria: oral bioavailability (OB) exceeding 30%, blood-brain barrier permeability (BBB) above -0.3, and drug-likeness (DL) surpassing 0.18. Candidates fulfilling these requirements were advanced to subsequent MOA explanation (**Appendix 1**).

Target prediction of TCM compounds

The putative pharmacological targets of compounds were systematically identified through 3 methods. 1) Collect known targets of compounds and predict new targets using the wSDTNBI algorithm (1) [22]. Molecular fingerprints were generated using RDKit's GetMorganFingerprint (radius = 2, useFeatures = True) to encode structural features. Algorithmic parameters were optimized as follows: $\alpha=0.4$ (similarity weight), $\beta = 0.2$ (network topology coefficient), $\gamma = -0.5$ (penalty factor), $\delta = 20$ (diffusion threshold), $\varepsilon = 4$ (iteration step size), with $k = 2$ resource diffusion cycles; 2) Predicting compounds targets using SysDT model in TCMSP database [21]; 3) Collecting literature evaluated compounds targets through HIT2.0 database [23]

(Appendix 2).

$$S_{wSDTNBI}=S_{norm}\circ S_{sim} \quad (1)$$

Mining of AD genes

AD gene mining came from three aspects: 1) Mining of AD phenotype candidate genes based on Phenolyzer database [24], including AD seed genes and predicted genes (predicted Score > 0.1); 2) Text mining-based AD gene collection from Diseases 2.0 database (Z-score > 5) [25]; 3) Optimization and mining of AD genes based on Open Targets Platform [26]. This study selected genes with a global score greater than 0.1; 4) DEGs mining from the GEO database (<https://www.ncbi.nlm.nih.gov/geo/>): Genome-wide expression profiles of the neocortex in AD patients versus controls (datasets GSE37263 and GSE37264). Subsequently, data normalization and batch effect correction were performed using the Clinical Bioinformatics Hub (<https://www.aclbi.com/static/index.html#/geo>). DEGs between the AD treatment group and the normal control group were then screened using thresholds of $|\log_2 \text{ fold change (FC)}| > 2$ and $P\text{-value} < 0.05$. Visualization of the DEGs was conducted through volcano plots and heatmaps (Appendix 3).

Construction of human protein-protein interaction network

The protein-protein interaction (PPI) data in this study were derived from the comprehensive work of Deisy Morselli Gysi et al [27], encompassing 5 principal interaction types: 1) binary PPIs (direct physical interactions), 2) affinity purification-mass spectrometry (AP-MS) identified complexes, 3) kinase-substrate relationships, 4) signaling pathway interactions, and 5) regulatory interactions, ultimately forming a curated interactome comprising 18,505 unique proteins and 327,924 experimentally validated interactions that represents one of the most complete human protein interaction networks currently available with particular strengths in signaling pathway coverage.

Screening of antiAD candidate compounds

In this study, an integrated statistical network model network proximity

algorithms was generated to prioritize compounds candidates based on the compound-target (T, S) interaction network and AD-associated genes. The principle of this algorithm is rooted in graph theory and network science, where the proximity or similarity between nodes is quantified by constructing and analyzing nodes and edges within a PPI network. By utilizing methods like network embedding or random walks, nodes in the network can be mapped to a low-dimensional vector space. The reference distance distribution was generated by calculating the proximity between compound-target groups and AD-associated genes with a procedure repeated 1,000 times. The mean $\mu_d(S,T)$ and s.d. $\sigma_d(S,T)$ of the reference distribution were used to convert an observed distance to a normalized distance, defining the proximity measure:

$$Z(S, T) = \frac{d(D, N) - \mu_d(D, N)}{\sigma_d(D, N)} \quad [28]$$

This analysis module references the methodology from the article by Morselli Gysi D et al. [27]. For each compound, network proximity algorithms was employed to calculate shortest distance between compound targets AD genes in PPI, with z-score < -4 defining significantly predicted compound-AD pairs [14].

Network construction

To further explore the multi-scale mechanisms of action (MOAs) of compounds in treating AD, gene sets were functionally annotated via the DAVID database (<https://david.ncifcrf.gov/summary.jsp>) to extract canonical pathways and GO terms significantly enriched in candidate compound-targeted AD-associated genes, respectively. Following enrichment analysis, , three network typologies were constructed: compound-target (C-T) network and target-pathway (T-P) network using Cytoscape 3.10.3. Illustrating associations between AD-related biological processes (BP), molecular functions (MF), cellular compounds (CC) by by <https://www.bioinformatics.com.cn> (last accessed on 10 Dec 2024), an online platform for data analysis and visualization.

Core gene screening based on Rank Product method

The Rank Product method is a non-parametric statistical approach first

introduced by Breitling et al. (2004) [29] for integrating prioritized rankings across multiple evaluation metrics in genomic studies, later adapted for computational compound discovery. This method operates through 3 key steps: 1) For each evaluation metric, an independent rank-ordered list is generated where n compounds are sorted by their performance on that specific metric. The rank position of the i -th compound ($i = 1, \dots, n$) across m different metrics is denoted as $r_{i1}, r_{i2}, \dots, r_{im}$ (2). 2) The composite Rank Product (RP) score for each gene is calculated as the geometric mean of its individual ranks. 3) Compounds are then re-ranked by their RP scores, where lower values indicate higher consensus priority across all metrics.

$$RP_i = (\prod_{k=1}^m r_{ik})^{1/m} \quad (2)$$

Analysis of key targets related to AD pathology

AlzData (<http://www.alzdata.org/>) [30] is an AD-specific database that aggregates high-throughput omics data. Using the gene symbols of compound-targeted targeting AD proteins as input, we performed correlation analyses with AD pathological markers (A β and tau) through AlzData. Additionally, normalized expression levels of these anti-AD targets in control and AD groups were compared using the “Differential expression” module based on datasets from GEO. All differential expression analyses were adjusted for the age and sex of the donors. The following datasets were utilized for each brain region: 1) entorhinal cortex: GSE26927, GSE26972, GSE48350, GSE5281; 2) hippocampus: GSE28146, GSE29378, GSE36980, GSE48350, GSE5281; 3) temporal cortex: GSE29652, GSE36980, GSE37263, GSE5281; 4) frontal cortex: GSE12685, GSE36980, GSE48350, GSE5281, GSE53890, GSE66333.

Molecular docking

Molecular docking analysis was performed using AutoDock Vina v1.2.0 to evaluate the binding affinities of candidate compounds to AD candidate genes. The 3D crystal structures of receptor proteins, including JUN (PDB ID: 1A02, resolution: 2.70 Å), SRC (PDB ID: 1A07, resolution: 2.20 Å) and ESR1 (PDB ID: 1A52, resolution: 2.80 Å) and the structures of 20 other proteins were retrieved from the RCSB PDB database (<https://www.rcsb.org/>). Protein structures were preprocessed

using MGL AutoDock Tools 1.5.7 to remove water molecules, add polar hydrogens, and assign Gasteiger charges. The docking grid box dimensions and center coordinates were predicted via Discovery studio 2016 based on “From Receptor Cavities” module. UniProt ID, PDB ID, and docking grid box dimensions and center coordinates for each protein are shown in **Appendix 4**. The 3D structures of 7 compounds were downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) and subjected to energy minimization using Chem3D 16.0 with the MMFF94 force field to optimize molecular geometries. The best binding poses were selected according to the lowest binding free energy (ΔG). compound-receptor complexes with $\Delta G \leq -5.0$ kcal/mol were considered stable conformations. PyMOL 2.5.4 was employed to visualize key binding interactions between compounds and active-site residues of AD candidate genes.

Molecular dynamics simulation

Molecular dynamics simulations were performed using GROMACS 2022.2 [31]. The protein was described by the Amber14SB force field, and the solvent was modeled using TIP3P water molecules. Small molecules were parameterized with AM1-BCC charges generated by antechamber and assigned GAFF2 atom types, which were then converted into GROMACS topologies using ACPYPE. Ion parameters were compatible with TIP3P (Joung-Cheatham). The complex was placed in a truncated dodecahedron box, with a minimum distance of ≥ 1.2 nm between the protein surface and the box boundary. TIP3P water molecules were added, and Na^+/Cl^- ions were included to neutralize the system and achieve a salt concentration of 0.15 M. The system was first energy-minimized using the Steepest Descent method (until $F_{\text{max}} < 1000 \text{ kJ mol}^{-1} \text{ nm}^{-1}$), followed by a 200 ps stepwise equilibration under NVT and NPT ensembles at 298 K. Production simulations were conducted for 100 ns under NPT conditions with a 2 fs time step and the Verlet cutoff scheme. Coulomb interactions were treated using the Particle Mesh Ewald (PME) method, while van der Waals and Coulomb interactions used a cutoff radius of 1.2 nm. All bonds involving hydrogen were constrained using the LINCS algorithm. Temperature and pressure were maintained at 298 K and 1 bar using the Nose-Hoover and Parrinello-Rahman

methods, respectively. Trajectories were saved every 10 ps. Post-simulation analyses, including root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), solvent accessible surface area (SASA), distance hydrogen bond number (Hbnum), residues contribution, binding energy, principal component analysis (PCA) and free energy landscape (FEL) analysis, were performed using GROMACS tools and VMD/PyMOL. When necessary, MM-PBSA energy evaluations were carried out using gmx_MMPBSA.

Chemicals and reagents

The HT-22 cell line was obtained from the Translational Medicine Research Institute of Shenzhen Second People's Hospital. Genkwanin (CAS ID: 437-64-9), diop (CAS ID: 32305-98-9), and formononetin (CAS ID: 485-72-3) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. A Cell Counting Kit-8 (CCK-8) was obtained from Bimake (Houston, TX, USA). The mouse monoclonal anti-COX2/Cyclooxygenase 2 antibody (clone 33/Cox-2, ab300668, Abcam, Cambridge, UK) was used in this study. The following reagents and materials of SPR were used in this study: CM5 Sensor Chip (BR-1005-30, Cytiva), Amine Coupling Kit (BR-100050, Cytiva), sodium acetate buffer solutions at pH 4.0, 4.5, and 5.0 (BR-100349, Cytiva), 10×PBS-P+(28995084, Cytiva), and dimethyl sulfoxide (DMSO, D8418, Sigma-Aldrich).

Cell culture

HT22 cells from the Translational Medicine Research Institute of the Second People's Hospital of Shenzhen, China. We were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, penicillin G (100 units/mL), and streptomycin (100 µg/mL) under 5% CO₂ in a humidified atmosphere at 37 °C. The adhered cells were grown to 70-80% confluence.

Cell viability assay

Cultured HT22 cells were added in 96-well plates at a density of 8×10^3 cells/well and grown for 24h. Compounds were dissolved and serially diluted with dimethyl sulfoxide (DMSO). Compounds with various concentrations or control (0.5% of dimethyl sulfoxide) were added to cells using liquid handling station equipped with

pintool system. After incubating for 2h, Glutamate (11 mM) was treated and further incubated for 24h. After incubation, the cells were added to each well with CCK8 solution at 37 °C in a humidified 5% CO₂ atmosphere for 1h. The absorbance of each well was measured at 450 nm using a microplate reader (Perkin Elmer, Waltham, MA, USA). The percentage of cell viability was expressed relative to the control cells.

Surface plasmon resonance analysis

The affinity between PTGS2 and Diop was analyzed using the Biacore T200 system. All SPR analyses were conducted on a BIAcore 1 K biosensor system (Cytiva, Marlborough, MA, USA) at 25 using a CMS sensor chip [32]. Protein immobilization was performed using a double-coupling method. The chip channels were activated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysulfosuccinimide at a flow rate of 10 µL/min. The PTGS2 (50 µg/mL in 1× PBS-P+buffer, pH=7.4) was immobilized on channel 4 at 10 µL/min, followed by ethanolamine blocking. Channel 3, treated similarly but without protein, served as a reference. For compound interaction studies, the running buffer was switched to 1 × PBS-P + containing 5% DMSO. Compounds were diluted to various concentrations in interaction buffer and injected over the immobilized protein surface at 30 µL/min with a contact time of 60 s. The chip was regenerated with 10 mM glycine-HCl (pH 2.0) for 5 min between cycles. Sensorgram data were processed using Biacore Insight Evaluation Software and fitted to a 1:1 Langmuir binding model to determine the association and dissociation constants.

FDA/PI double staining method

After exposing the cells to 11 mM glutamate for 2 hours, we treat them with varying concentrations of Diop (50, 100, and 200 µM). 24 hours post-treatment, cell viability is determined using the fluorescein diacetate (FDA)/propidium iodide (PI) double staining technique [33]. Following a 24 h co-incubation at 37 °C, the medium was discarded, and cells were stained with a pre-mixed FDA/PI solution (5 µg/mL, PI, 10 mg/mL FDA) for 5 min in the dark. Subsequently stained cells were observed and imaged under UV and phase-contrast microscopy. Five random fields were captured per well, Cell viability was calculated as the percentage of FDA-positive cells relative

to the total cell number (FDA-positive + PI-positive). This calculation reflects the proportion of metabolically active (viable) cells in the population.

Statistical analysis

Data are expressed as the mean $\pm$ SEM. Statistical analysis was performed in GraphPad Prism 5.01 software (GraphPad Software, Inc., CA, USA) using one-way analysis of variance followed by Dunnett's t test. $P < 0.05$ was considered statistically significant.

Results

Building a compound-target network

In this study, we identified 153 and 105 DEGs from the GSE37263 and GSE37264 datasets, respectively, as illustrated in **Fig. 2A-B**. Concurrently, 287, 527, and 158 disease-associated genes were retrieved from the Diseases 2.0, Open Targets, and Phenolyzer databases, with the 158 genes from Phenolyzer encompassing 103 seed genes **Fig. 2C**). Furthermore, compound-target interaction analyses using TCMSP, Netinfer, and HIT2.0 yielded 107, 497, and 46 potential therapeutic targets, respectively (**Fig. 2D**). Integrative data aggregation revealed a comprehensive dataset of 551 disease-related genes and 1,040 compound targets, as summarized in **Fig. 2E**.

Integration of 551 compound targets with 1,040 AD-associated genes revealed 137 potential AD-related candidates (**Appendix 5**) targeting by 23 active compounds, including 25 known genes and 114 predicted genes (**Fig. 2F**). The top 5 compounds ranked by degree value are beta-sitosterol (degree = 50), Girinimbina (degree = 47), Inermin (degree = 46), Fumarine (degree = 44), and formononetin (degree = 42). Meanwhile, the closeness centrality and betweenness centrality values of β -sitosterol are 0.45 and 1686.57 respectively, making it a key node in the network. Studies have shown that β -Sitosterol ameliorates memory and learning impairments in APP/PS1 mice by decreasing A β deposition[34]. The top 5 targets ranked by degree value are CASP9 (degree = 21), ESR2 (degree = 21), NOS3 (degree = 19), NR3C1 (degree = 19), and MAP2 (degree = 18), all of which are known targets. Based on a comprehensive analysis of the closeness centrality, betweenness centrality, and eigenvector centrality parameters, CASP9 are identified as key nodes in the network (**Table 1**). The topological properties of other network nodes are detailed in **Appendix 6**. Previous studies have shown that CASP9 activation in AD exacerbates neurodegeneration by cleaving tau into aggregation-prone fragments that form neurofibrillary tangles, creating a self-amplifying loop linking amyloid pathology to neuronal loss [35].

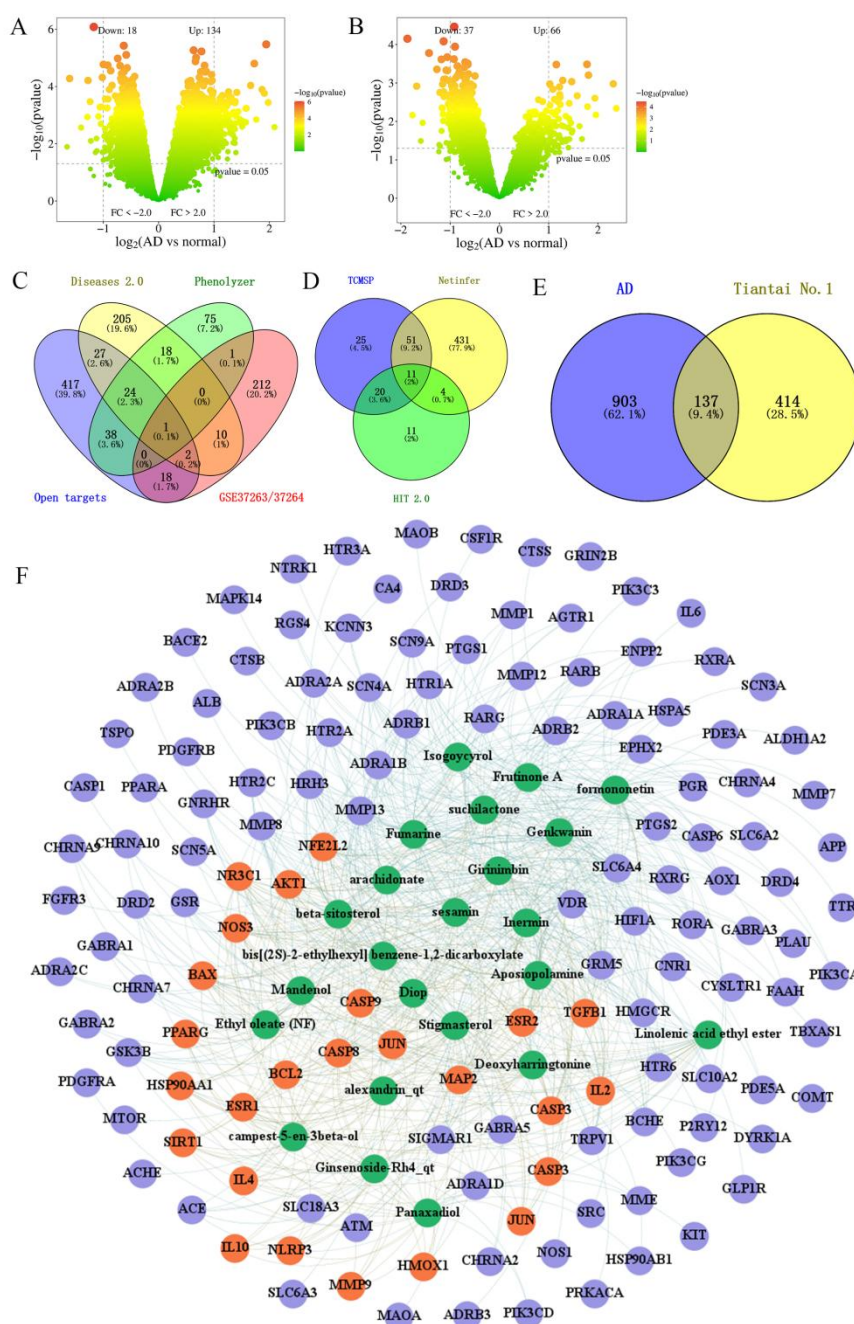


Fig. 2 Constructing Compound Target (C-T) network. (A, B) Volcanic maps of GSE37263 and GSE37264 DEGs. (C, D) The interrelationship between the number of AD and compound genes in different databases. (E) AD genes that can be targeted by compounds. (F) C-T network of compounds composed of known and predicted targets. In the Figure, the green nodes represent compounds, the orange nodes represent known targets of the compounds, and the blue nodes represent predicted targets of the compounds.

Table 1. Topology analysis of compounds-target (C-T) network

Compounds	Closeness centrality	Betweenness centrality	Eigenvector centrality	Degree	Target	Closeness centrality	Betweenness centrality	Eigenvector centrality	Degree
beta-sitosterol	0.45	1686.57	0.92	50	CASP9	0.51	433.41	0.77	21
Girinimbin	0.44	971.11	0.99	47	ESR2	0.50	353.47	0.81	21
Inermin	0.44	882.66	1.00	46	NOS3	0.51	406.94	0.68	19
Fumarine	0.43	532.12	1.00	44	NR3C1	0.47	262.34	0.73	19
formononetin	0.43	1755.14	0.71	42	MAP2	0.49	300.83	0.70	18
suchilactone	0.43	750.28	0.92	42	CASP3	0.48	281.41	0.70	18
Stigmasterol	0.43	519.56	0.89	41	CASP8	0.47	271.24	0.65	18
Frutinone A	0.42	923.37	0.78	40	JUN	0.45	255.1	0.65	18
sesamin	0.42	706.38	0.90	40	AKT1	0.44	189.87	0.69	18
Diop	0.42	783.97	0.83	39	TGFB1	0.47	255.19	0.66	17

GO enrichment analysis of the anti-AD targets of Tiantai No.1

As shown in **Fig. 3**, the top 10 enriched BP, MF, and CC terms were identified by DAVID database. The comprehensive details of GO analysis are provided in **Appendix 7**. The results demonstrated that the neuroprotective effects of Tiantai No.1 against AD were significantly associated with BP including adenylate cyclase-activating adrenergic receptor signaling pathway, response to xenobiotic stimulus and positive regulation of apoptotic process, etc (**Fig. 3A**). CC analysis identified critical localization patterns, with target proteins predominantly enriched in plasma membrane, synapse, neuron projection, postsynaptic membrane and dendrite (**Fig. 3B**). Moreover, MF profiling further revealed that Tiantai No.1 primarily interacts with identical protein binding, nuclear receptor activity and extracellular ligand-gated monoatomic ion channel activity, etc (**Fig. 3C**). However, these top-ranked GO terms (BP/MF/CC) exhibited limited capacity to delineate the direct therapeutic mechanisms of Tiantai No.1 in AD intervention. To comprehensively characterize its mode of action, we subsequently performed KEGG pathway enrichment analysis, which provided deeper insights into the systemic pharmacological network underlying Tiantai No.1's anti-AD properties.

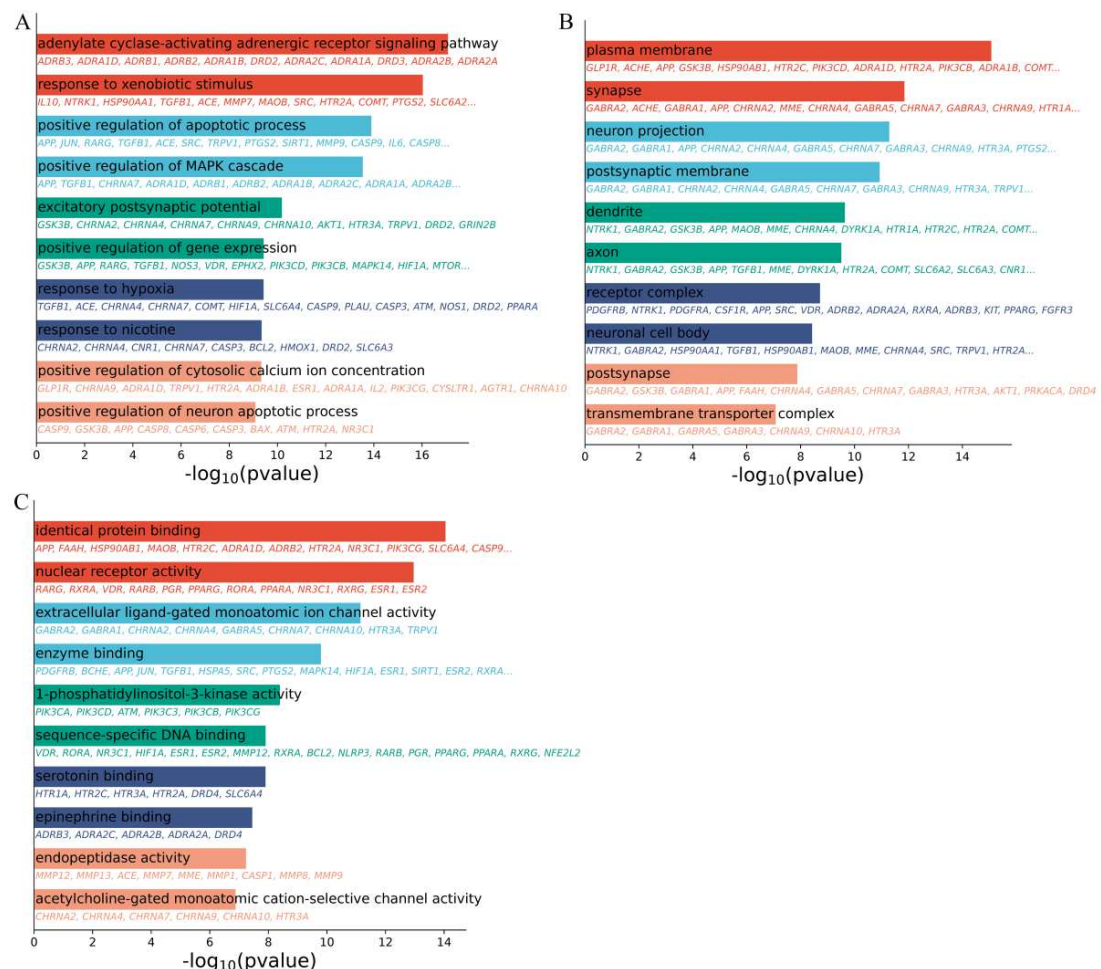


Fig. 3 GO enrichment analysis of Tiantai No 1 targeting AD genes. (A) 137 targets are associated with biological processes; (B) 137 targets are involved in cellular composition; (C) 137 targets perform molecular functions. The length of the bar chart represents the significance of the enriched entries

Literature-based KEGG pathway enrichment analysis of the anti-AD targets of Tiantai No. 1

To explore the key signaling pathways potentially mediating the anti-AD effects of TianTai No 1, we performed KEGG pathway enrichment analysis on the 137 candidate targets using the DAVID bioinformatics platform, with a statistical significance cutoff set at $p < 0.001$ (**Appendix 8**). A systematic literature search was conducted on PubMed using the query "([pathway name] AND Alzheimer's disease)" for each of the 97 analyzed pathways, which ultimately identified 33 pathways with experimentally validated associations to AD pathogenesis. The top 10 most significant

pathways with literature-validated associations to AD pathology were selected, including calcium, apoptosis, IL-17, estrogen, PI3K-Akt, Th17 cell differentiation, cGMP-PKG, Thyroid hormone, cAMP and JAK-STAT signaling pathway (**Table 2**). Meanwhile, we classified these pathways into four categories: environmental information processing, cellular processes, organismal systems, and human diseases (**Fig. 4A**).

In addition, we constructed a target-pathway network encompassing all evaluated and AD pathology-regulated pathways with their associated targets. As illustrated in **Fig. 4B**, the network comprises 127 nodes and 399 edges, including 33 literature-validated pathway nodes and 94 associated targets. Network analysis revealed that the top 5 pathways ranked by degree value were PI3K-Akt, Calcium, AD, neurodegeneration-multiple diseases, and apoptosis pathways. Notably, the Calcium pathway ($P\text{-Value} = 4.98\text{E-}10$) exhibited the highest statistical significance, followed by PI3K-Akt ($P\text{-Value} = 4.27\text{E-}08$) and apoptosis pathways ($P\text{-Value} = 8.01\text{E-}09$). This hierarchical significance pattern suggested differential regulatory importance among these critical pathways in AD pathogenesis. Microarray analysis of sprague-Dawley rat hippocampus revealed differential gene expression in nervous system development and apoptosis in the Tiantai No. 1 group compared to the model group [36]. Studies demonstrated that Tiantai No. 1 increased the expression of cAMP responsive element-binding protein in B104 neuronal cells [37]. In addition, the study revealed that genes can exert therapeutic effects against AD through multiple pathological mechanisms by participating in distinct pathways. For instance, CASP9 is involved in the apoptosis, PI3K-Akt signaling pathway, and thyroid hormone signaling pathway, modulating critical biological processes including signal transduction, neural cell proliferation, apoptosis, and metabolism [38, 39]. This suggested that Tiantai No. 1 potentially targets CASP9, representing an anti-apoptotic mechanism for treating AD.

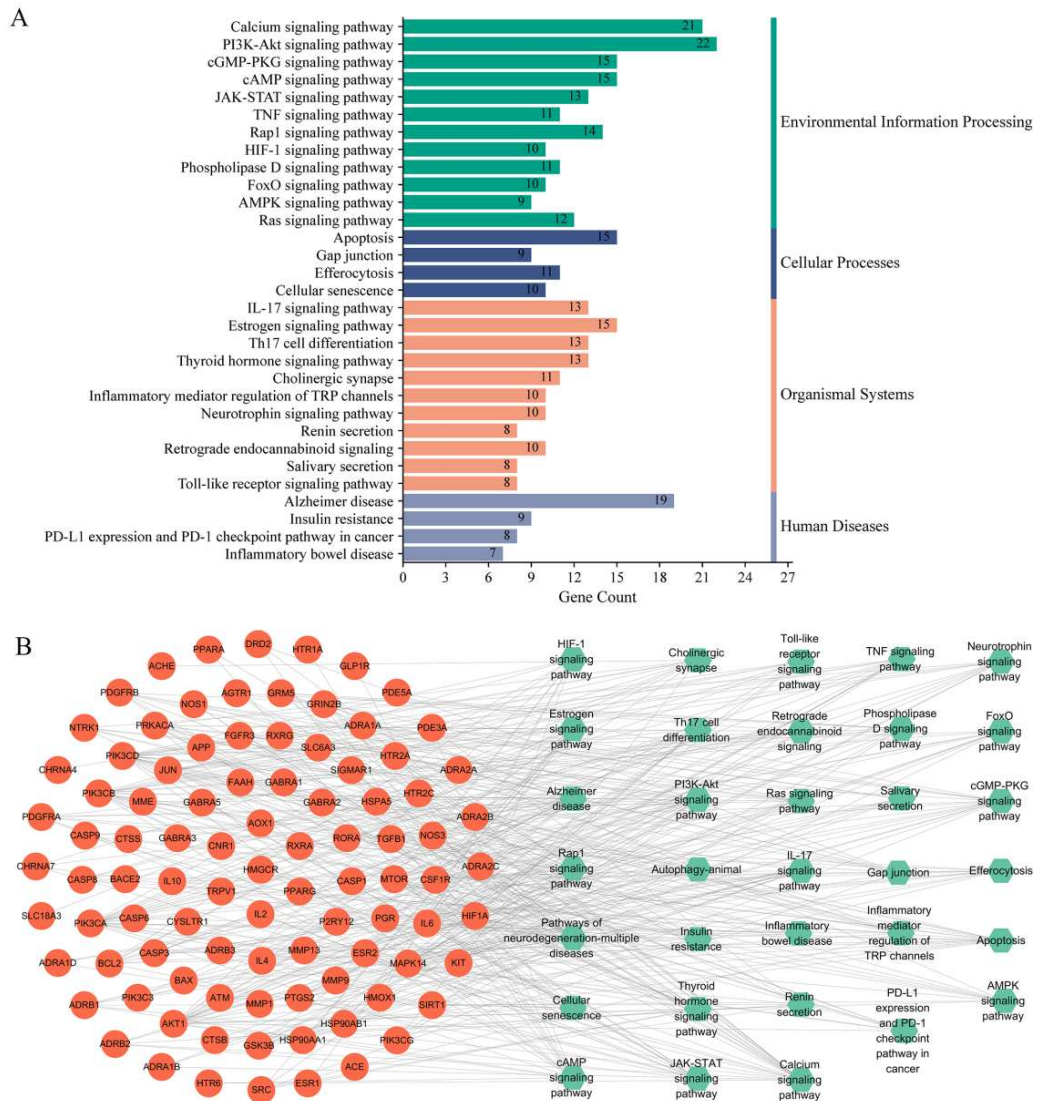


Fig. 4 KEGG enrichment analysis of Tiantai No 1 targeting AD genes. (A) Target-pathway network. The red nodes represent Tiantai No 1 targeting AD genes. The green nodes represent the pathways involved in literature validation to regulate AD pathology. **(B)** KEGG pathway classification. The horizontal axis of the bar chart represents the number of enriched pathways, while the vertical axis represents the enriched pathway entries and classifications.

Table 2. KEGG significantly enriched 10 pathways with literature verification

Term	Pathway	Count	P-Value	Genes	PMID
hsa04020	Calcium signaling pathway	21	4.98E-10	PDGFRB, NTRK1, PDGFRA, NOS3, CHRNA7, HTR2C, ADRA1D, ADRB1, ADRB2, HTR2A, ADRA1B, ADRA1A, GRIN2B, HTR6, GRM5, CYSLTR1, ADRB3, AGTR1, NOS1, PRKACA, FGFR3	33463465;36373976
hsa04210	Apoptosis	15	8.01E-09	NTRK1, JUN, PIK3CD, PIK3CB, CTSS, CASP9, CASP8, PIK3CA, CASP6, CASP3, BCL2, BAX, AKT1, ATM, CTSB	38472715;25322820
hsa04657	IL-17 signaling pathway	13	9.66E-09	GSK3B, JUN, HSP90AA1, HSP90AB1, MMP1, PTGS2, MAPK14, MMP9, IL4, IL6, MMP13, CASP8, CASP3	32582147;39748776
hsa04915	Estrogen signaling pathway	15	1.06E-08	JUN, HSP90AA1, HSP90AB1, NOS3, SRC, PIK3CD, PIK3CB, ESR1, MMP9, ESR2, PIK3CA, BCL2, AKT1, PGR, PRKACA	32297302;38726764
hsa04151	PI3K-Akt signaling pathway	22	4.27E-08	PDGFRB, NTRK1, PDGFRA, GSK3B, CSF1R, HSP90AA1, HSP90AB1, NOS3, PIK3CD, PIK3CB, IL2, MTOR, PIK3CG, CASP9, IL4, IL6, RXRA, PIK3CA, KIT, BCL2, AKT1, FGFR3	37186274;33935751
hsa04659	Th17 cell differentiation	13	4.67E-08	JUN, HSP90AA1, TGFB1, HSP90AB1, RORA, MAPK14, HIF1A, IL2, MTOR, IL4, IL6, RXRA, RXRG	36581132;34595688
hsa04022	cGMP-PKG signaling pathway	15	1.03E-07	NOS3, ADRA1D, ADRB1, ADRB2, ADRA1B, ADRA2C, ADRA1A, ADRA2B, PIK3CG, ADRA2A, ADRB3, PDE3A, AGTR1, AKT1, PDE5A	34093124;36400326

hsa04919	Thyroid hormone signaling pathway	13	1.65E-07	GSK3B, SRC, PIK3CD, PIK3CB, HIF1A, ESR1, MTOR, CASP9, RXRA, PIK3CA, AKT1, PRKACA, RXRG	39513900;29132997
hsa04024	cAMP signaling pathway	15	4.34E-06	GLP1R, JUN, HTR1A, PIK3CD, ADRB1, PIK3CB, ADRB2, GRIN2B, HTR6, PIK3CA, PDE3A, AKT1, DRD2, PPARA, PRKACA	36400326
hsa04630	JAK-STAT signaling pathway	13	5.12E-06	IL10, PDGFRB, PDGFRA, PIK3CD, PIK3CB, IL2, MTOR, IL4, IL6, PIK3CA, BCL2, AKT1, AOX1	36614305;36911202

Screening of antiAD candidate compounds by network proximity algorithms in Tiantai No.1

In this study, following preliminary was screened based on $OB \geq 30\%$, $DL \geq 0.18$ and BBB score ≥ -0.3 . Then, network proximity algorithms was employed to prioritize compounds in Tiantai No.1 with potential anti-AD activity based on target-disease association significance. The analysis revealed that all screened bioactive compounds exhibited statistically significant associations with AD pathogenesis ($z < -4$). Notably, preclinical evidence demonstrates that 11 out of 22 (50%) prioritized compounds significantly improved cognitive and memory performance, including β -sitosterol, formononetin, stigmasterol, girinimbine, and panaxadiol (**Table 3**). For example, Mechanistic studies demonstrated that formononetin alleviated $A\beta_{25-35}$ -induced cellular apoptosis through activation of the PI3K/Akt signaling pathway. Additionally, formononetin significantly enhanced learning and memory capabilities by suppressing $A\beta$ production and RAGE-dependent inflammatory signaling, augmenting α -secretase activity, and LRP1-dependent cerebral $A\beta$ clearance pathway [40, 41]. Among them, the PI3K/Akt signaling pathway is also one of the significant pathways identified by KEGG enrichment analysis for the anti AD effect of Tiantai No.1. Notably, stigmasterol and girinimbine have been confirmed as acetylcholinesterase inhibitors[42, 43]. Moreover, stigmasterol, panaxadiol, and sesamin can also treat AD through pathological mechanisms such as anti-oxidative stress, inhibiting synaptic dysfunction, and reducing amyloid- β toxicity [44-46]. Among the 11 candidate compounds, which deserve further preclinical validation, such as Frutinone A, Diop and suchilactone, etc. Therefore, the potential of Tiantai No.1 in the prevention and treatment of AD deserves to be further evaluated.

Table 3. Literature evidence on the anti-AD compounds in Tiantai No. 1.

Compounds	Mol ID	CAS ID	MW	OB (%)	BBB	DL	Proximity_distance	Proximity_Z-score	PMID
formononetin	MOL000392	485-72-3	268.28	69.67	0.02	0.21	0.640625	-10.52655085	39828895; 29191087; 35712702
inermine	MOL003648	2035-15-6	284.28	65.83	0.36	0.54	0.654545455	-10.32328622	35298568 29056913;
beta-sitosterol	MOL000358	83-46-5	414.79	36.91	0.99	0.75	0.77184466	-10.31748512	31908573; 21969169
bis[(2S)-2-ethylhexyl] benzene-1,2-dicarboxyl ate	MOL001490	CID: 7057920	390.62	43.59	0.68	0.35	0.680412371	-9.704899704	NA
frutinone A	MOL005321	38210-27-4	264.24	65.9	0.46	0.34	0.672727273	-9.43837499	NA
stigmasterol	MOL000449	83-48-7	412.77	43.83	1	0.76	0.73553719	-9.400153038	34055852; 38313373
diop	MOL002879	32305-98-9	390.62	43.59	0.26	0.39	0.69	-9.322251088	NA
linolenic acid ethyl ester	MOL007179	1191-41-9	306.54	46.1	1.09	0.2	0.744897959	-8.265668381	38123015

ethyl oleate (NF)	MOL002883	111-62-6	310.58	32.4	1.1	0.19	0.726315789	-8.135351422	NA
campest-5-en-3beta-ol	MOL005043	474-62-4	400.76	37.58	0.94	0.71	0.765306122	-8.014971504	28814827
suchilactone	MOL005384	50816-74-5	368.41	57.52	0.28	0.56	0.75	-7.968366747	NA
fumarine	MOL000787	130-86-9	353.4	59.26	-0.13	0.83	0.811965812	-7.734985929	34936968; 39525050
mandenol	MOL001494	544-35-4	308.56	42	1.14	0.19	0.818181818	-7.640588309	NA
panaxadiol	MOL005376	19666-76-3	460.82	33.09	0.23	0.79	0.8125	-7.311214968	30735733
deoxyharringtonine	MOL005317	36804-95-2	515.66	39.27	-0.25	0.81	0.773195876	-7.15579187	NA
girinimbine	MOL005356	23095-44-5	263.36	61.22	1.22	0.31	0.79047619	-7.136469936	39605111
isogoycyrol	MOL011594	#N/A	366.39	40.36	0	0.83	0.762376238	-6.930337079	NA
aposiopolamine	MOL005308	CID5319581	271.34	66.65	0.4	0.22	0.8	-6.601993865	NA
genkwanin	MOL005573	437-64-9	284.28	37.13	-0.24	0.24	0.871559633	-5.848751892	34315132
arachidonate	MOL005320	506-32-1	304.52	45.57	0.58	0.2	0.841584158	-5.745552689	20553961; 38866484
alexandrin_qt	MOL005399	#N/A	414.79	36.91	0.88	0.75	0.854166667	-5.546621383	NA
ginsenoside-Rh4_qt	MOL005348	#N/A	458.8	31.11	-0.18	0.78	0.878787879	-5.313922339	NA

Candidate genes screening by Rank Product

Based on degree values, the PPI network comprising 137 genes was constructed using Gephi_0.10.1 (**Fig. 5**). Then, PPI network imported into Cytoscape_v3.10.3 for network analysis, and finally, network topological parameters such as betweenness centrality, closeness centrality, and degree were obtained for 137 genes. This study conducted integrated scoring based on Rank Product. First, individual rank and rank score calculations were performed for betweenness centrality, closeness centrality, and degree. Subsequently, weighted rank, weighted rank-sorted, rank product, rank product-sorted, rank score product, and rank score product-sorted were computed. Through comprehensive evaluation, the final screening results of genes were determined by rank product-sorted in descending order (**Appendix 9**). As shown in **Table 4**, the top 10 genes ranked by the rank product were rAC-alpha serine/threonine-protein kinase (AKT1), interleukin-6 (IL6), proto-oncogene tyrosine-protein kinase Src (SRC), albumin (ALB), prostaglandin G/H synthase 2 (PTGS2), amyloid-beta precursor protein (APP), peroxisome proliferator-activated receptor gamma (PPARG), estrogen receptor (ESR1), caspase-3 (CASP3), and apoptosis regulator Bcl-2 (BCL2). Studies demonstrated that Tiantai No. 1 exhibits neuroprotective effects by reducing the accumulation of amyloid-beta and phosphorylated tau in SAMP8 mice [11]. Furthermore, Tiantai No. 1 protects neurons against amyloid-beta-induced neurotoxicity in B104 neuronal cells [37].

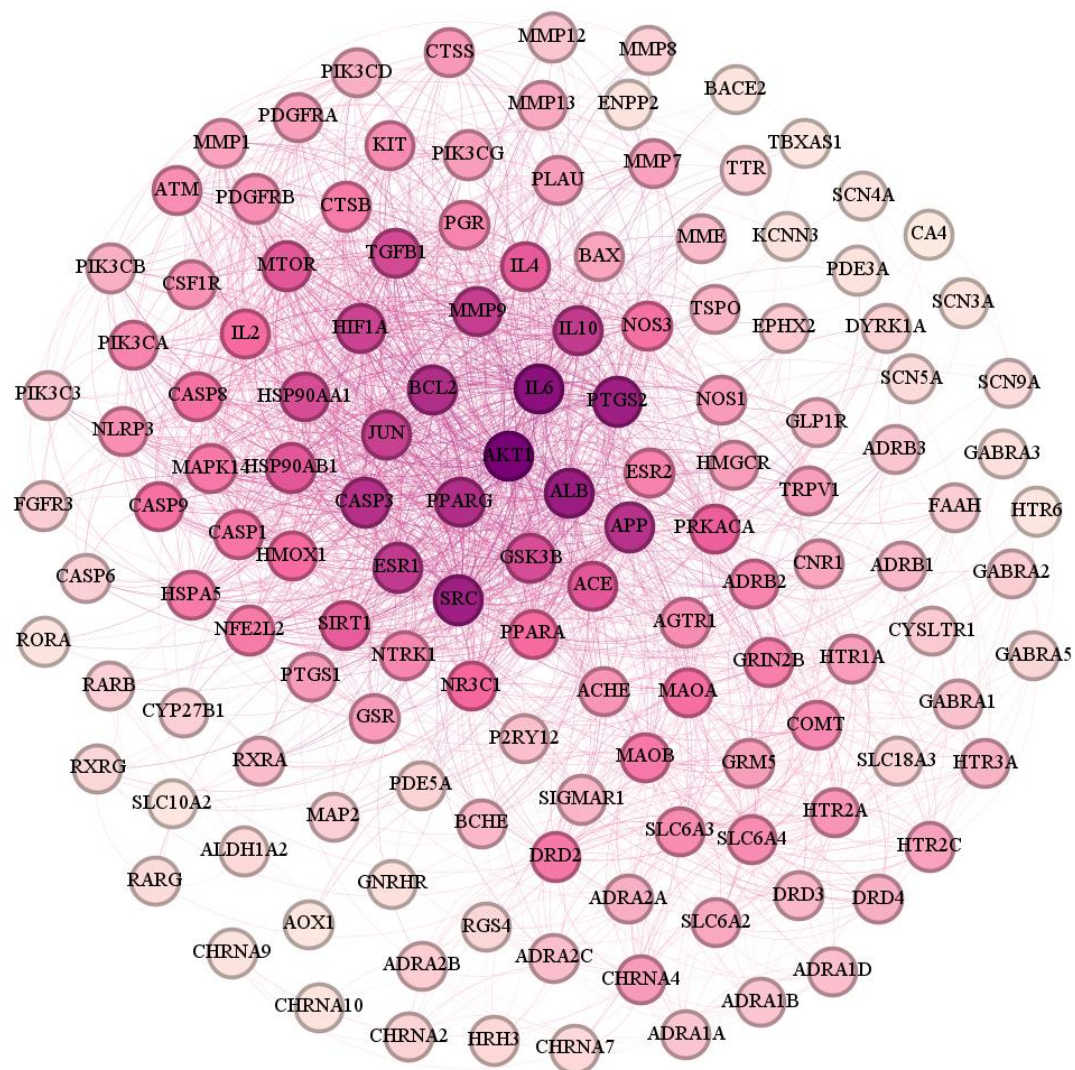


Fig. 5 Construction of PPI network with 137 genes. The color gradient from light to dark represents the magnitude of node degrees.

Table 4. Candidate genes screening of 137 genes by Rank Product

Name	Betweenness Centrality	Rank	Closeness Centrality	Rank	Degree	Rank	Rank product	Rank product sorting
AKT1	0.064045284	1	0.708333333	1	82	1	1	1
IL6	0.048297941	5	0.68	2	76	2	20	2
SRC	0.063694223	2	0.657004831	5	70	5	50	3
ALB	0.03499573	7	0.669950739	3	71	3	63	4
PTGS2	0.048429481	4	0.666666667	4	70	4	64	5
APP	0.053444141	3	0.644549763	6	62	9	162	6
PPARG	0.023223848	13	0.641509434	7	64	6	546	7
ESR1	0.035163957	6	0.62962963	10	59	10	600	8
CASP3	0.015877228	19	0.638497653	8	63	8	1216	9
BCL2	0.014825313	23	0.635514019	9	64	7	1449	10
IL10	0.012781795	25	0.621004566	11	59	11	3025	11
PRKACA	0.02430541	12	0.588744589	17	45	23	4692	12
JUN	0.010295186	32	0.604444444	12	57	13	4992	13
GSK3B	0.012547817	26	0.601769912	13	52	16	5408	14
ACE	0.017495509	17	0.588744589	18	48	18	5508	15
MMP9	0.00977744	34	0.601769912	14	58	12	5712	16

MAOA	0.025302372	11	0.578723404	21	40	28	6468	17
HIF1A	0.007633244	39	0.596491228	15	56	14	8190	18
DRD2	0.029041699	9	0.555102041	30	37	34	9180	19
HSP90AA1	0.007824824	38	0.591304348	16	52	17	10336	20

GEO dataset analysis of AD-associated key targets

The normalized expression values of the targets related to AD pathology in the healthy control and AD groups in the GEO dataset were analyzed with the “Differential expression” module of the AlzData database (**Fig. 6, Table 5**). Among these targets, AKT1, PTGS2, PPARG, APP, PPKACA, GSK3B, HSP90AA1, ALB and PTGS2 were significantly downregulated in AD patients compared to controls. Among them, the expression of AKT1 is in the entorhinal cortex, while the expression of other genes is in the temporal cortex (**Fig. 6A-G**). The expression of ALB was significantly downregulated in the temporal cortex in GSE5281 database (**Fig. 6D**). The expression of APP was significantly downregulated both in the temporal cortex and frontal cortex in AD patients compared to controls (**Fig. 6H**). The expression of BCL2, JUN, CASP3, HIF1A, IL6, MAOA and ACE was significantly upregulated in the in AD patients compared to controls (**Fig. 6J-P**). The expression of BCL2 and JUN is in the hippocampus (**Fig. 6J, K**), while the expression of HIF1A and MAOA is in the temporal cortex (**Fig. 6L, M**). The expression of CASP3 and IL6 is in the frontal cortex and entorhinal cortex, respectively (**Fig. 6N, O**). The expression of ACE was significantly upregulated in the temporal cortex in GSE37263 database (**Fig. 6P**). These results suggested that the potential pivotal role of key targets in AD pathogenesis.

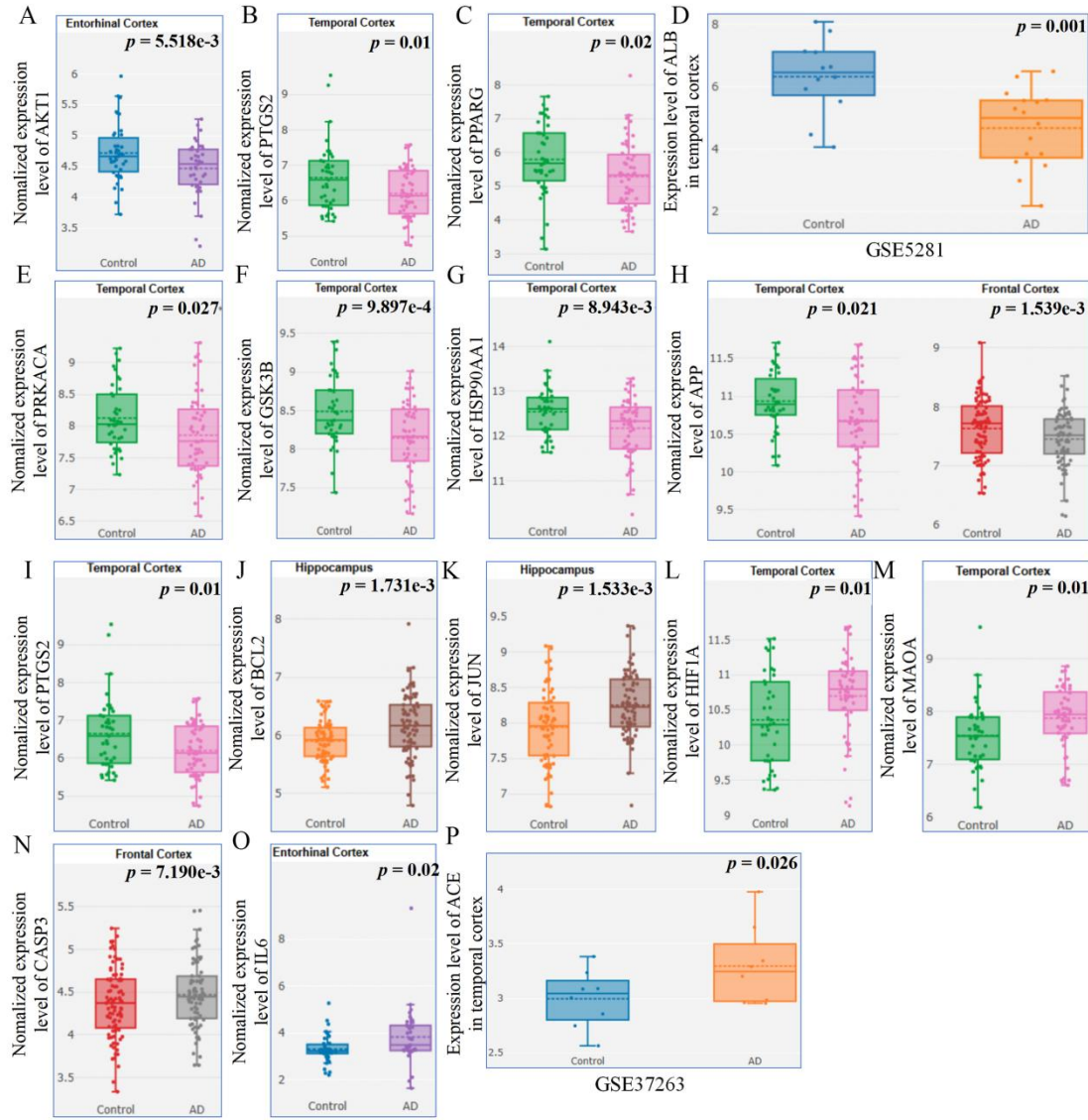


Fig. 6 Cross-platform normalized expression level of top 10 targets in PPI. (A–B) The normalized expression level of AKT1 and IL6 in entorhinal cortex. (C–D) The normalized expression level of PTGS2 and PPARG in temporal cortex. (E–F) The normalized expression level of CASP3 and BCL2 in frontal cortex and hippocampus, respectively. (G) The normalized expression level of APP in frontal cortex and temporal cortex. (H) The expression level of ALB and ACE in temporal cortex based on GSE5281 database ($n = 74$ in healthy control, $n = 87$ in the AD patients) and GSE37263 database ($n = 8$ in healthy control, $n = 8$ in the AD patients).

Table 5. Differentially expressed genes in AD across brain regions.

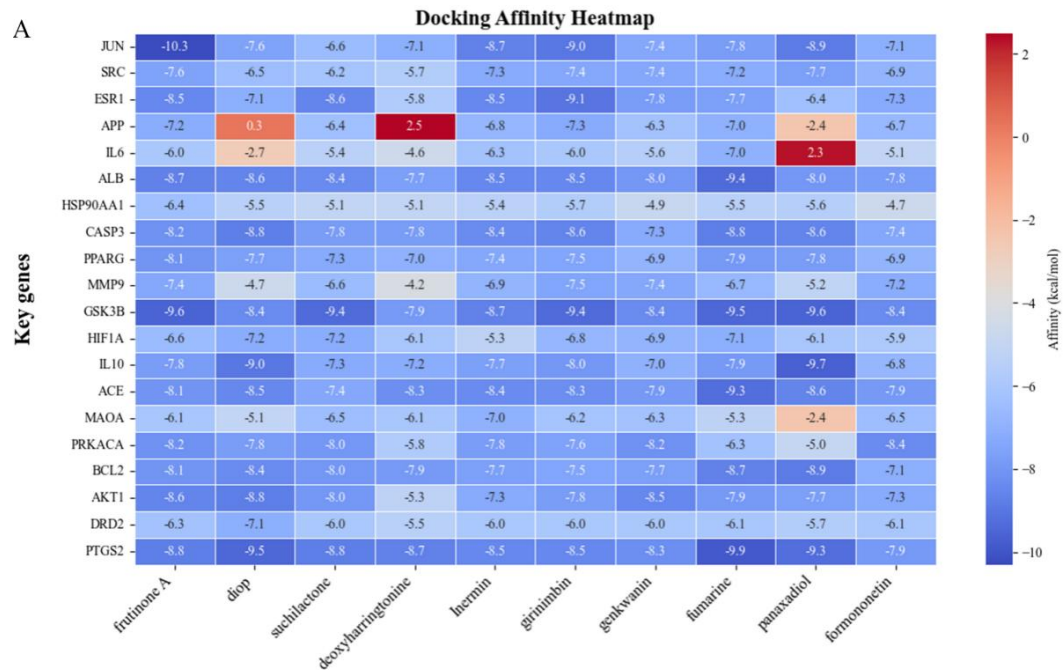
Genes	Brain region	log2 FoldChange	P-value	FDR
AKT1	Entorhinal cortex	-0.301	5.52E-03	0.042
IL6		0.515	0.02	0.09
APP	Frontal cortex	-0.234	1.54E-03	0.015
CASP3		0.138	7.19E-03	0.039
BCL2	Hippocampus	0.265	1.73E-03	0.028
JUN		0.275	1.53E-03	0.026
ALB		-1.65	0.001	0.013
APP		-0.261	0.021	0.085
PPARG		-0.517	0.02	0.082
PRKACA		-0.286	0.027	0.101
GSK3B		0.355	9.90E-04	0.009
MAOA	Temporal cortex	0.342	0.01	0.048
HIF1A		0.355	0.01	0.05
HSP90AA1		-0.36	8.94E-03	0.046
HIF1A		0.355	0.01	0.05
ACE		0.32	0.026	0.211
PTGS2		-0.459	0.01	0.05

Molecular docking results

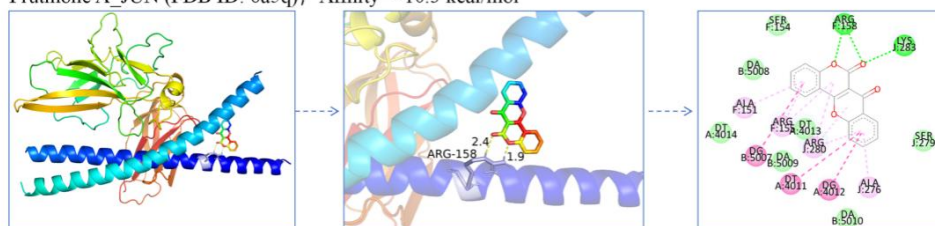
In this study, we selected both the top 20 ranked proteins (**Table 4**) and compounds that either lack literature validation or have been reported in fewer than 3 studies for molecular docking in Tiantai No. 1, respectively (**Fig. 7A**). Among them, frutinone A, suchilactone, inermin, girinimbin, and fumarine all had docking scores < -5 kcal/mol with the candidate genes. The complexes with the strongest binding energies were suchilactone-GSK3B (-9.4 kcal/mol), inermin-JUN/GSK3B (-8.7 kcal/mol), frutinone A-JUN (-10.3 kcal/mol), fumarine-PTGS2 (-9.9 kcal/mol), and girinimbin-GSK3B (-9.4 kcal/mol). For diop, deoxyharringtonine, genkwanin,

panaxadiol, and formononetin, the number of candidate genes with docking scores below -5 kcal/mol was 17, 17, 19, 17, and 19, respectively. Among these, the best binding energies were observed for diop-PTGS2 (-9.5 kcal/mol), deoxyharringtonine-PTGS2 (-8.7 kcal/mol), genkwanin-AKT1 (-8.5 kcal/mol), panaxadiol-GSK3B (-9.6 kcal/mol) formononetin-GSK3B and formononetin-PRKACA (-8.4 kcal/mol for both), and formononetin/panaxadiol-IL10 (-9.7 kcal/mol).

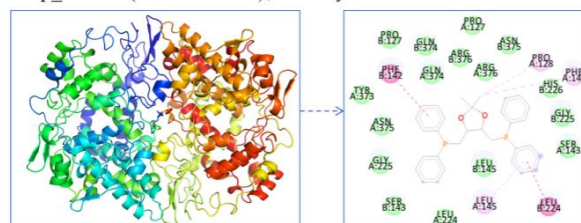
Docking analysis further confirmed that these bindings were mediated by hydrogen bond formation at the core proteins' active sites, with **Fig. 7** illustrating the 2D and 3D binding interactions and compound positioning within their respective protein pockets. Specifically, frutinone A effectively bound to the JUN active site, forming six hydrogen bonds (including two with ARG158 at 2.4 Å and 1.9 Å), along with van der waals forces, a Pi-Pi T-shaped interaction, a conventional hydrogen bond, and Pi-alkyl bonding (**Fig. 7B**). Similarly, diop interacted with PTGS2 through van der waals forces, alkyl bonding, a Pi-Pi T-shaped interaction, Pi-alkyl bonding, and amide-Pi stacked interaction (**Fig. 7C**). Suchilactone binding to GSK3B primarily involved hydrogen bonding (with ASN370 at 2.5 Å and GLU333 at 3.6 Å) and hydrophobic interactions, further supported by van der waals forces, carbon hydrogen bond, Pi-cation bonding, conventional hydrogen bond, and Pi-alkyl bonding (**Fig. 7D**). Please refer to **Fig. 7E-L** for detailed binding bond energies of other compounds and proteins. Please refer to **Table 6** for the binding methods of other complexes.



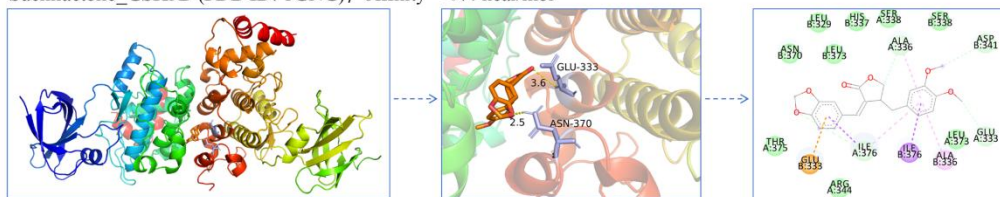
B Frutinone A_JUN (PDB ID: 6a5q), Affinity= -10.3 kcal/mol



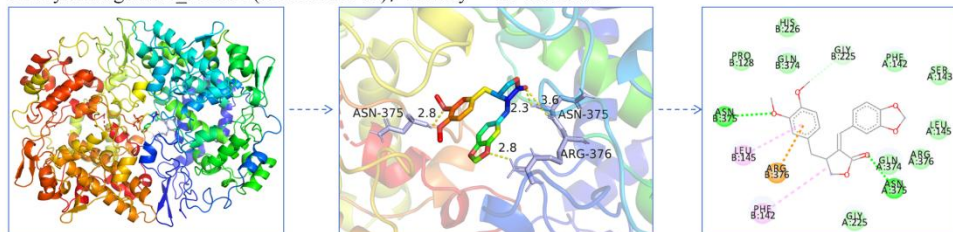
C Diop_PTGS2 (PDB ID: 5F19), Affinity= -9.5 kcal/mol



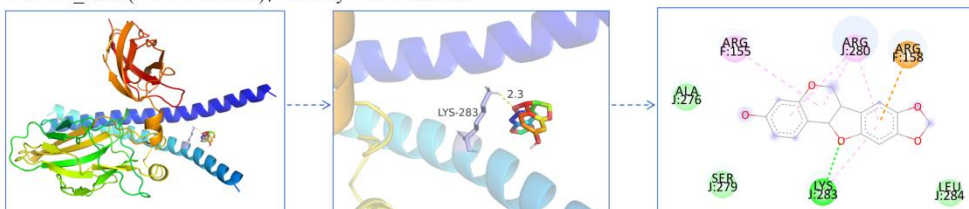
D Suchilactone_GSK3B (PDB ID: 1GNG), Affinity= -9.4 kcal/mol



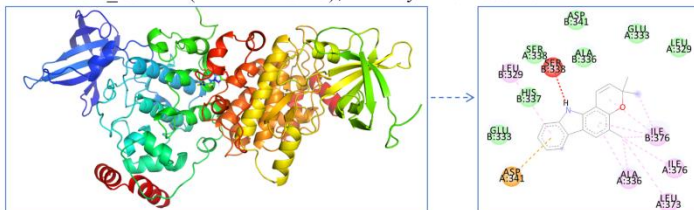
E Deoxyharringtonine_PTGS2 (PDB ID: 5F19), Affinity= -8.7 kcal/mol



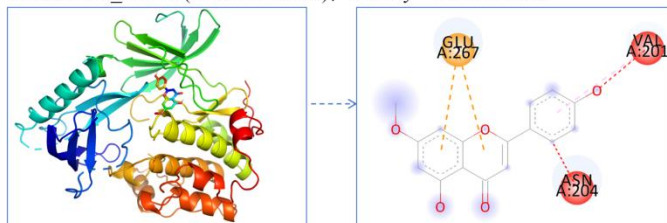
F Inermin_JUN (PDB ID: 1A02), Affinity= -8.7 kcal/mol



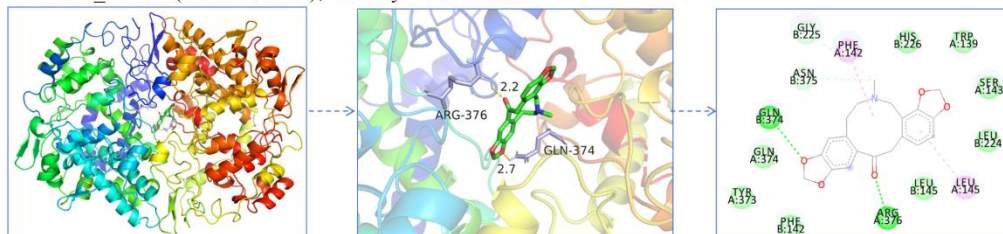
G Girinimbin_GSK3B (PDB ID: 1GNG), Affinity= -9.4 kcal/mol



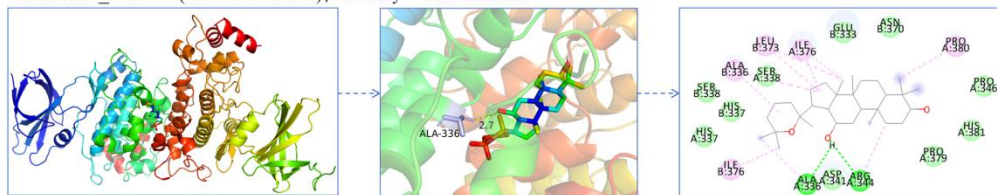
H Genkwanin_AKT1 (PDB ID: 3O96), Affinity= -8.5 kcal/mol



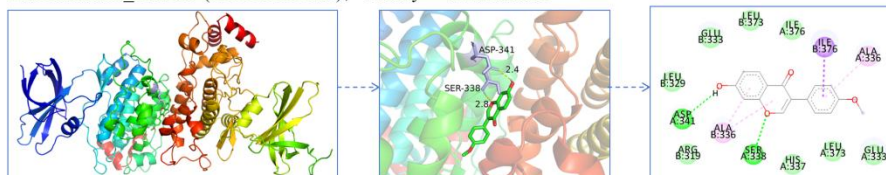
I Fumarine_PTGS2 (PDB ID: 5F19), Affinity= -9.9 kcal/mol



J Panaxadiol_GSK3B (PDB ID: 1GNNG), Affinity= -9.6 kcal/mol



K Formononetin_GSK3B (PDB ID: 1GNK), Affinity= -8.4 kcal/mol



- L
- | | | | |
|----------------------------|------------------|------------------|-----------|
| Van der Waals | Pi-Pi T-shaped | Alkyl | Pi-Anion |
| Conventional Hydrogen Bond | Pi-Alkyl | Pi-Sigma | Pi-Cation |
| Carbon Hydrogen Bond | Unfavorable Bump | Amide-Pi Stacked | |

Fig. 7 2D and 3D spaces of molecular docking of compounds with the active site of candidate proteins. The structure of protein-ligand drawn by PyMOL represents the binding site of compounds in the protein pocket at the left panels. The middle figure shows an enlarged view of the protein ligand interaction mode. The enlarged view of the types of binding bonds in the protein ligand interaction mode shown in the far right figure was drawn using Discovery Studio 2016.

Table 6. Docking score interaction of compounds with the candidate proteins.

Genes	Compound	Van der Waals	Conventional Hydrogen Bond	Carbon Hydrogen Bond	Pi-Pi T-shaped	Pi-Alky1	Unfavorable Bump	Alky1	Pi-Sigm a	Amide-Pi Stacked	Pi-Anio n	Pi-Cati on
AKT1	genkwanin	/	/	/	/	/	VAL A:201, ASN A:204	/	/	/	GLU A:267	/
	suchilactone	ASN B:370, LEU B:329, LEU A:373, LEU B:373, HIS B:337, SER A:338, SER B:338, THR A:375, ARG A:344	/	ILE A:376, ASP B:341, ALA A:336	/	ALA B:336	/	/	ILE B:376	/	GLU B:333	/
GSK3B	panaxadiol	SER A:338, HIS A:337, HIS B:337, ASP A:341, GLU B:333, ASN B:370, PRO A:346, PRO A:379, HIS A:381	ALA A:336, ARG A:344	/	/	/	/	ILE B:376, ALA B:336, LEU B:373, ILE A:376, PRO A:380	/	/	/	/
	formononeti n	LEU B:329, ARG B:319, GLU B:333, LEU B:373, ILE A:376, HIS A:337, LEU A:373, GLU A:333	/	ASP A:341, SER A:338	/	ALA B:336	/	/	ILE B:376	/	/	/

		SER F:154, DA B:5008, DT A:4014,			DG B:5007,	ALA F:151,						
	frutinone A	DT A:4013, DA B:5009, SER J:279,	/	ARG F:158,	DT A:4011,	ARG F:155,	/	/	/	/	/	/
		DA B:5010		LYS J:283	DG A:4012	ARG J:280, ALA						
						J:276						
	inermis	ALA J:276, SER J:279, LEU J:284	/	LYS J:283	/	ARG F:155,	/	ARG F:155,	/	/	/	ARG
JUN						ARG J:280		ARG J:280				F:158
						LEU B:329, ILE		LEU B:329,				
		GLU B:333, HIS B:337, SER A:338,				B:376, ILE		ILE B:376, ILE				
	girinimbine	ASP B:341, ALA B:336, GLU A:333,	/	/	/	A:376, ALA	/	A:376, ALA	/	/	ASP	/
		LEU A:329				A336,:LEU		A336,:LEU			A:341	
						A:373		A:373				
		TYR A:373, ASN A:375, GLY										
		A:225, SER B:143, LEU A:224, LEU				LEU A:145,		LEU A:145,				
PTGS2	diop	B:145, SER A:143, GLY B:225, HIS	/	/	PHE B:142,	PRO A:128,	/	PRO A:128,	/	/	/	/
		B:226, PRO B:127, GLN B:374,			LEU B:224	PHE A:142		PHE A:142				
		GLN A:374, ARG B:376, ARG										
		A:376, PRO A:127, ASN B:375										

	GLY A:225, GLN A:374, ARG									
deoxyharrin	A:376, LEU A:145, SER A:143, PHE	ASN A:375,			LEU B:145,					APG
gtonine	A:142, GLN B:374, PRO B:128, HIS	ASN B:375	GLY B:225	/	PHE B:142	/	/	/	/	B:376,
	B:228									
	GLN A:374, TYR A:373, PHE									
fumarine	B:142, LEU B:145, LEU B:224, SER	ASN B:375,	ARG A:376,		LEU A:145,					
		GLY B:225	GLN B:374	/	PHE A:142	/	/	/	/	/
	A:143, TRP A:143, HIS B:226									

Assessment of binding capacity by MD simulation

Molecular dynamics simulations revealed that all 3 complexes remained stable throughout the 100 ns simulation period, with backbone RMSD values fluctuating within the range of 2-3 Å. Among them, the PTGS2-Diop complex exhibited the smallest fluctuation amplitude, indicating its superior binding stability **Fig. 8a1, b1, c1**). The RMSF analysis results show that the RMSF values for most residues are below 0.15-0.20 nm (1.5-2.0 Å), indicating that the overall protein structure is relatively stable. RMSF values in a few residue regions exceed 0.25-0.30 nm (2.5-4.5 Å), demonstrating higher flexibility, which may be involved in conformational adjustments during ligand binding (**Fig. 8a2, b2, c2**). The radius of Rg for all 3 complexes remained within a relatively stable range throughout the simulation and exhibited only minor thermal fluctuations, indicating that the protein conformation remained compact and stable (**Fig. 8a3, b3, c3**). Similarly, SASA values remained largely stable overall during the simulation, with only minor fluctuations observed, suggesting that the solvent exposure of the system remained essentially constant (**Fig. 8a4, b4, c4**). The stable fluctuation of the distance during the simulation indicates that the relative spatial positions of the protein and ligand in all three complexes remain fixed, demonstrating the presence of strong and stable constraints (**Fig. 8a5, b5, c5**).

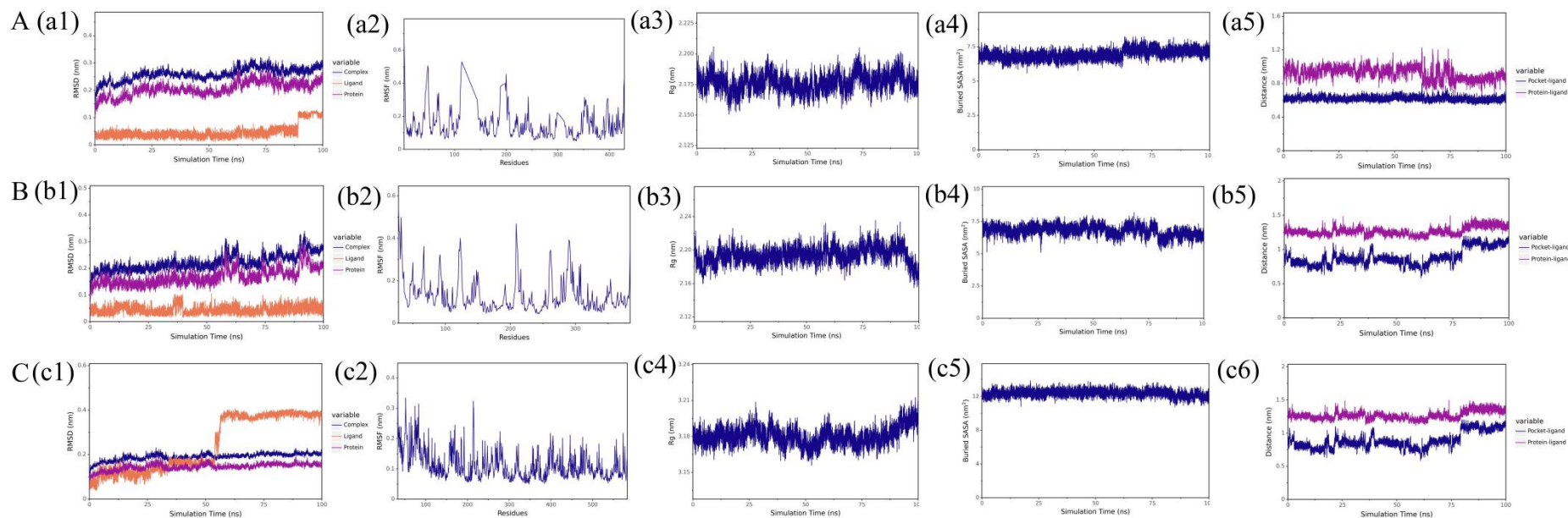


Fig. 8 Molecular dynamics simulation of AKT1-genkwanin, GSK3B-Formononetin and PTGS2-Diop complex. (A) RMSD, RMSF, Rg, SASA and Distance of AKT1-genkwanin; (B) RMSD, RMSF, Rg, SASA and Distance of GSK3B-Formononetin; (C) RMSD, RMSF, Rg, SASA and Distance of PTGS2-Diop complex.

Hydrogen bond analysis revealed that the complexes AKT1-genkwanin and GSK3B-Formononetin maintained a stable number of hydrogen bond interactions throughout the simulation, indicating favorable binding stability of the ligands within their respective binding pockets (**Fig. 9a1, 8b1**). In contrast, the PTGS2-Diop complex exhibited fewer hydrogen bonds, suggesting that other types of interactions (**Fig. 9c1**). The primary binding residues of the three complexes are shown in **Fig. 9a2, b2, c2**, among which AKT1-genkwanin exhibits the highest number of interacting residues. Additionally, the energy variations of all three complexes fluctuate within a limited range, demonstrating no significant changes (**Fig. 9a3, 8b3, 8c3**). PCA indicates that the conformational states of the ligands remained largely stable throughout the simulation, with the ligand in the AKT1-genkwanin complex exhibiting the highest conformational stability (**Fig. 9a4, 8b4, 8c4**). FEL analysis revealed that each system formed a distinct and concentrated low free energy basin with well-defined boundaries. The non-dispersed conformational distribution suggests the presence of a stable dominant conformation for each system (**Fig. 9a5, 8b5, 8c5**).

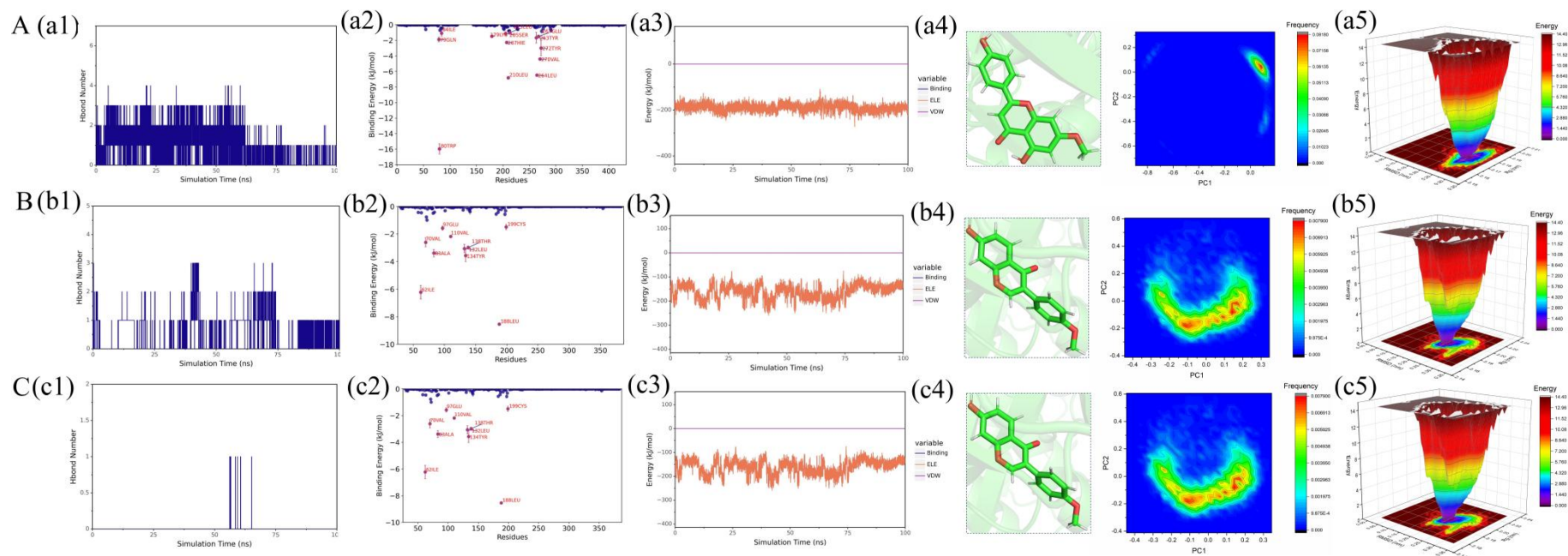


Fig. 9 Principal component analysis and free energy landscape analysis of complex. (A-C) Hbnum, residues contribution, binding energy, principal component analysis and free energy landscape analysis of AKT1-genkwanin (A), GSK3B-Formononetin (B) and PTGS2-Diop (C) complex, respectively.

Taking solvation energy into account, and comprehensively considering RMSD, Rg, distance, buried SASA, and interaction energy, the trajectory of the complex in its stable state was selected for binding energy calculation using the MM-PBSA method. The resulting energy terms related to binding are listed in the **Table 7**. ΔE_{MMPBSA} is the sum of ΔE_{ele} , ΔE_{vdw} , ΔE_{pol} , and ΔE_{nonpol} , while the Gibbs binding energy (ΔG_{bind}) is the sum of ΔE_{MMPBSA} and $-T\Delta S$. Analysis of the Table shows that in the complex, the ΔE_{vdw} is higher than ΔE_{nonpol} , and both are higher than ΔE_{ele} . Therefore, in the composition of the binding energy, van der Waals interactions play a major role, hydrophobic interactions play a secondary role, and electrostatic interactions serve as a supplementary contribution. The ΔE_{MMPBSA} for the small molecule and the protein are -103.939 ± 1.236 , -78.315 ± 1.472 and -169.08 ± 2.523 kJ/mol respectively, indicating a high binding energy and affinity between them. The small molecule binds stably to the protein, with van der Waals interactions being the dominant factor, hydrophobic interactions playing a secondary role, and electrostatic interactions providing a supplementary effect in the binding energy.

Table 7. Binding energy and its composition in stable state (Unit: kJ/mol)

Complex	ΔE_{vdw}	ΔE_{ele}	ΔE_{pol}	ΔE_{nonpol}	ΔE_{MMPBSA}	-T ΔS	ΔG_{bind}
AKT1-genkwanin	-175.066 ± 2.713	-12.754 ± 0.65	103.269 ± 2.228	-19.387 ± 0.083	-103.939 ± 1.236	7.638 ± 0.73	-96.3 ± 1.891
GSK3B-Formononetin	-132.856 ± 0.949	-5.782 ± 1.413	77.794 ± 3.824	-17.47 ± 0.026	-78.315 ± 1.472	9.041 ± 3.254	-69.273 ± 4.447
PTGS2-Diop	-233.397 ± 0.754	-4.914 ± 0.715	102.606 ± 3.593	-33.375 ± 0.258	-169.08 ± 2.523	-10.207 ± 2.481	-158.873 ± 4.11

Note: VDW: van der Waals interaction; ELE: electrostatic interaction; PB: polar solvation energy; SA: hydrophobic interaction; MM: sum of vdw and ele; MMPBSA: sum of mm, pb, and sa; -T ΔS : entropy contribution; ΔG : Gibbs binding free energy.

Tiantai No. 1 attenuated the cytotoxicity induced by the glutamate in HT-22 cells

HT22 cells are a mouse hippocampal neuron cell line. The hippocampus is one of the earliest and most severely affected brain regions in AD, closely related to learning and memory, therefore selecting hippocampal neurons has tissue relevance. An important characteristic of this cell line is its sensitivity to glutamate (GLU). GLU is the most important excitatory neurotransmitter in the central nervous system, but excessive amounts can cause excitotoxicity. This study discovered multiple potential anti AD compounds based on network proximity algorithm (**Table 3**). Therefore, this study used HT22 cells to examine the anti-AD effect of Tiantai No. 1. HT22 cells were treated with different concentrations of compounds for 12 h. As shown in **Fig. 10**, GLU at 11 mM significantly reduced the viability of HT-22 cells, which was blocked by diop (**Fig. 10A**), genkwnine (GEN) (**Fig. 10B**) and formononetin (FOR) (**Fig. 10C**). Specifically, the CCK-8 assay showed that HT22 cells treated with 100 μ M (84.46 ± 4.18 %) or 200 μ M (100.5 ± 4.18 %) of diop exhibited significant protection against GLU-induced toxicity. GEN and FOR exhibited significant protection against GLU-induced toxicity in HT22 cells treated with 200 μ M ($99.97 \pm 6.21\%$ and 91.77 ± 8.04 %, respectively). Subsequently, through SPR, the Diop evaluated for their binding ability to PTGS2. As depicted in **Fig. 10E**, the concentration-dependent binding of Diop with PTGS2 with Kd value is 3.07E-06. These results verified the direct strong binding between Diop and PTGS2.

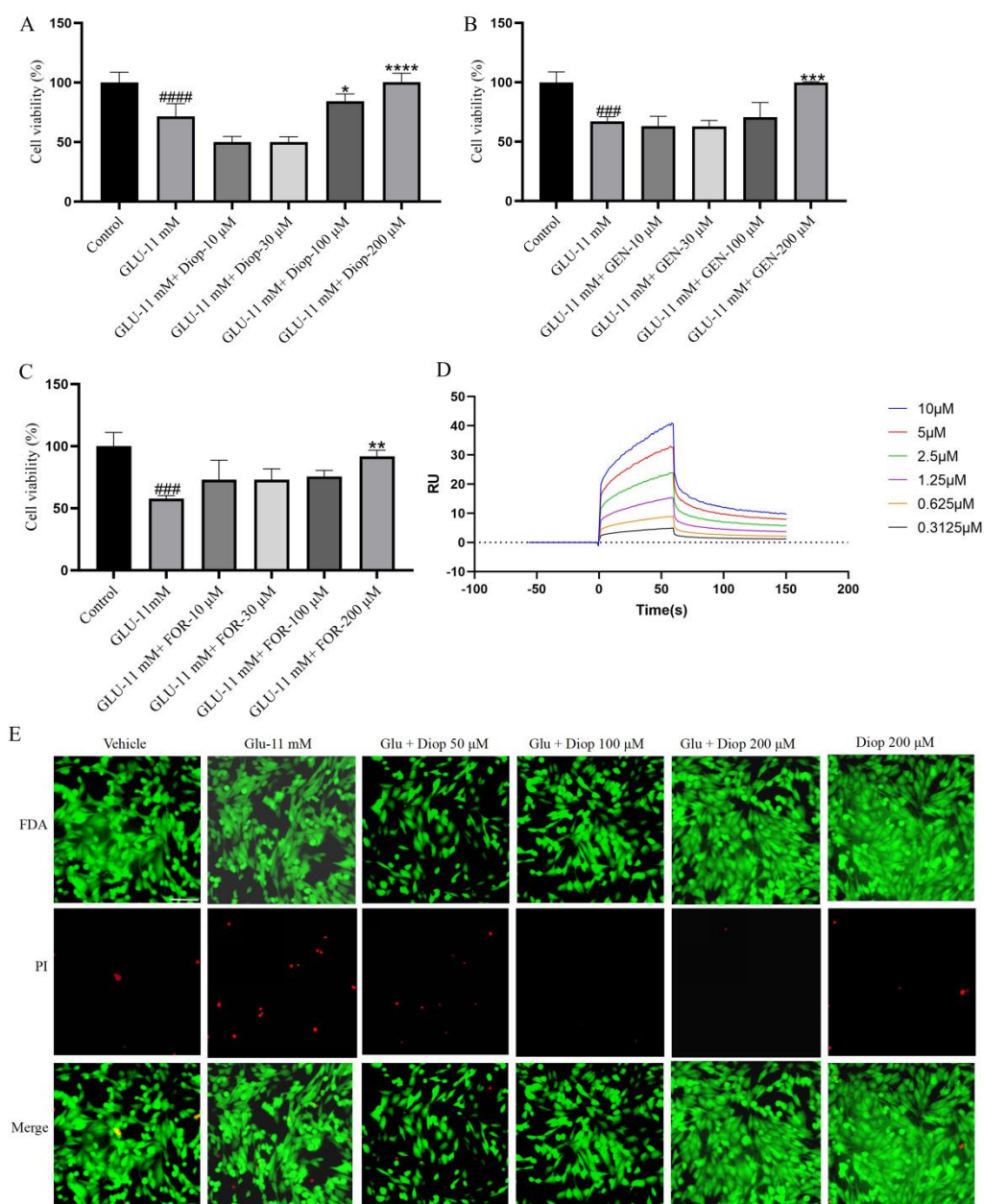


Fig. 9 Compounds of Tiantai No. 1 suppressed GLU-induced cell injury in HT22 cells. Cells were pre-treated for 2 h with diop (A), genkwanin (B) and formononetin (C) and then stimulated for 24 h with 11 mM GLU or not and the cell viability was detected by CCK8 assay. Data represent 3 independent experiments and are shown as mean $\pm$ SD. ### $p < 0.001$, #### $p < 0.0001$ compared with control, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared with GLU-induced group. (D) FDA/PI double staining. Scale bar: 20 μ m. Green fluorescence indicates the live cells while the red fluorescence represents the dead cells; (E) Surface plasmon resonance (SPR) analysis between Diop and PTGS2.

Discussion

AD is characterized by the accumulation of amyloid-beta plaques and neurofibrillary tangles in the brain, leading to progressive neuronal loss and cognitive decline, and currently has no cure [2, 3]. Additional contributing factors to AD include neuroinflammation, oxidative stress, synaptic impairment, among others [47]. As the most prevalent form of dementia, AD poses a major global health challenge, particularly amidst rising healthcare costs and an aging population. While available treatments can alleviate certain symptoms, they do not stop the underlying neurodegenerative process, underscoring the urgent need for innovative therapeutic strategies [17, 48]. Tiantai No.1 is a compound herbal formulation primarily composed of *Rhizoma Gastrodiae*, *Radix Ginseng*, and *Folium Ginkgo*. Previous *in vivo* and *in vitro* pharmacological studies have demonstrated that each of these medicinal constituents possesses anti-AD properties [49-53]. This study utilizes an integrated approach combining network proximity analysis, the RankProduct method, molecular docking, and *in vitro* experiments to investigate the therapeutic potential of Tiantai No. 1 against AD. The research aims to elucidate its active compounds, corresponding targets, associated pathways, and underlying biological mechanisms.

First, this study integrated the GEO datasets GSE37263 and GSE37264 with multiple public databases to collect AD-related genes. Using the SysDT model and the wSDTNBI algorithm, potential targets of the compounds were predicted, and a compound-target network was constructed. This revealed the multi-target anti-AD potential of Tiantai No.1 through such targets as CASP9, ESR2, and NOS3. Subsequently, GO and KEGG enrichment analyses were performed on the AD-related genes targeted by the compounds. The results indicated that Tiantai No.1 may exert its anti-AD effects by modulating multiple pathways, including the apoptotic process, PI3K-Akt signaling pathway, and cGMP-PKG signaling pathway. Next, in addition to meeting the screening criteria for compounds ($OB \geq 30\%$, $DL \geq 0.18$ and $BBB \geq -0.3$), we applied a network proximity algorithm to screen the compounds of Tiantai No.1 and evaluated the reliability of the screening results based on pharmacological literature. Compounds not yet reported in the literature were flagged

for further investigation. The screening identified 22 candidate compounds ($z < -4$), among which 11 (50%) have already been evaluated by *in vivo* or *in vitro* experiments, such as formononetin, inermin, and beta-sitosterol.

Formononetin (FOR), a phytoestrogen member of the flavonoid family derived from *Radix Puerariae* in Tiantai No.1, which has the pharmacological effects of antioxidative, anti-inflammatory, anti-apoptotic, and suppressing A β generation, etc [40, 54]. Study based on RNA-seq by Bo Liu et al. has demonstrated that FOR can alleviate cognitive impairment in mice by inhibiting neuroinflammation and alleviating the reductions in antioxidant capacity and SIRT-1 signaling [55]. A study by Miao Sun et al. demonstrated through *in vitro* experiments that FOR protects neurons from hypoxia-induced cytotoxicity by stimulating the ADAM10-sA β PP α pathway in hypoxic N2a-A β PP cells [56]. Furthermore, a study by Lizhi Chen et al. revealed that FOR attenuates A β ₂₅₋₃₅-induced cytotoxicity in HT22 cells by activating the PI3K/Akt signaling pathway and promoting the non-amyloidogenic cleavage of APP [41]. Molecular docking results indicated that FOR exhibits favorable binding affinity with APP and AKT1, with binding energies of -6.7 and -7.3 kcal/mol, respectively (**Table 5**). While molecular docking predicted favorable binding energies for several compound-protein pairs, these predictions require orthogonal experimental validation. We performed SPR analysis to confirm the binding between Diop and PTGS2 as a proof-of-concept, but binding affinities for other predicted pairs remain to be experimentally determined.

Fumarine (FMA), a natural alkaloid isolated from Chinese herbal medicine *Panax Ginseng C. A. Mey.*, which possesses a wide range of pharmacological activities such as anti-inflammatory, anticholinesterase, antioxidant, neuroprotective, and cytotoxic activities, etc [57]. Pharmacokinetic studies revealed that FMN effectively crossed the blood-brain barrier, reaching a concentration of 289.47 ng/g in both 3xTg-AD and P301S mice. Furthermore, FMN alleviated memory deficits and neuropathology by promoting tau ubiquitination and recruiting heat shock proteins HSP70 and HSC70 [58]. A study by Liying Lu et al. demonstrated that FMA exerts neuroprotective effects by reducing reactive oxygen species (ROS), upregulating the

p-AMPK/AMPK ratio and PGC1 α expression, as well as enhancing downstream mitochondrial biogenesis-related factors, including nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAM) [59].

Genkwanin (GKW), a methoxyflavone found in *Ginkgo Folium* in TCMSP database. *In vitro* and *in vivo* biological and pharmacological investigations showed that GKW exhibits remarkable antioxidant and anti-inflammatory activities, neuroprotective properties, antitumor capability, and antiviral effects [60]. A study by Peng Zeng et al. demonstrated that GKW exerts its therapeutic effects on AD by modulating core targets associated with tau pathology in N2A-APP cells, such as CDK5 and GSK3 β [61]. Our research indicates that the docking binding energy between GKW and GSK3B is -8.4 kcal/mol. Additionally, genkwanin exerts anti-inflammatory and anti-neurotoxic effects by suppressing MPP^{*}-induced activation of the TLR4/MyD88/NLRP3 inflammasome pathway in SH-SY5Y cells [61].

In this study, molecular docking and molecular dynamics simulations revealed favorable binding energies for the genkwanin-AKT1, formononetin-GSK3B, and Diop-PTGS2 complexes. Among these, the Diop-PTGS2 complex, which exhibited the most stable dynamics profile ($\Delta\text{EMMPBSA} = -169.08 \pm 2.523$ kcal/mol), was further evaluated by SPR experiments. The results suggest that PTGS2 could serve as a novel target of Diop for the treatment of AD. *In vitro* experiments demonstrated that diop, genkwanin, and formononetin at a concentration of 200 μM effectively protected HT22 cells against glutamate-induced cytotoxicity.

Therefore, several limitations should be acknowledged. First, the compound-target predictions relied on publicly available databases that may contain false positives and false negatives due to incomplete curation. Second, the network proximity algorithm predicts associations at a systems level but does not account for pharmacokinetic factors such as bioavailability, metabolism, or tissue distribution. Third, the identified compounds, particularly polyphenolic compounds, are known to exhibit non-specific binding in computational docking, and their concentrations in the final extract were not quantified. Therefore, the computational predictions presented

herein should be interpreted as hypothesis-generating rather than confirmatory, warranting further experimental validation including pharmacokinetic and pharmacodynamic studies.

Conclusion

In this study, the network proximity algorithm quantifies the association strength between compound targets and disease genes at the systemic level, thereby screening candidate compounds closely related to AD. Subsequently, the Rank Product method integrates and ranks potential targets based on multidimensional network topological parameters to identify core functional targets. By combining these two approaches, a complete logical chain from "compounds-target association" to "core target identification" is formed, systematically revealing the “multi-compounds, multi-target” synergistic mechanism of Tiantai No.1 in combating AD. The protective effect of compounds against AD pathogenesis was verified in HT22 cells. Our evidence indicated that the potential mechanism by which Tiantai No.1 ameliorates multiple pathological features of AD was direct synergy among effects on multiple ingredients, targets and pathways. Future research should focus on providing further *in vivo* experimental validation for the neuroprotective effects of Tiantai No.1 against AD. These results provide evidence supporting the clinical application of Tiantai No.1 in AD treatment.

Declarations

Ethics statement for the use of human and animal subjects

This study did not involve human or animal subjects. All data were obtained from publicly available sources or derived from existing literature reviews. No experiments involving human participants or animals were conducted as part of this research. Accordingly, ethical approval from an institutional review board and informed consent were not required.

Competing Interest: The authors declare no conflict of interest.

Author's Contribution

Pengfei Guo designed and executed the review, excavated data and performed the systematic analysis, and wrote the original draft; Mengmeng Jiang, Shengquan Hu, Hu Rui, Yan Li, Yucui Ma, Yu Wei and Wenjia Wang for formatting tables and figures and checking and calibrating; Zhengzhi Wu inspired this project, supervised the project, and revised the final manuscript.

Funding

The study was supported by The major new drug innovation project of the Ministry of Science and Technology of China (No.2017ZX09301001), National Natural Science Foundation of China (No.81574038), Shenzhen Science and Technology Program (JCYJ20220818101806014, JCYJ20250604180541051), Shenzhen Team-based Medical Science Research Program (2024YZZ11).

Availability of Data and Materials

All data in this study were obtained from unrestricted public sources or literature, with detailed references provided in the manuscript. All supporting materials are freely available for non-commercial use without confidentiality concerns.

Acknowledgement

We would like to extend our heartfelt gratitude to The Second People's Hospital of Shenzhen for their significant support and assistance in this study. The authors would like to thank Yucui Ma for his excellent technical support and Zhengzhi Wu for his critical review of the manuscript.

Reference

1. Soria Lopez, J. A.; González, H. M.; Léger, G. C., Alzheimer's disease. *Handb Clin Neurol* **2019**, 167, 231-255.
2. Scheltens, P.; De Strooper, B.; Kivipelto, M.; Holstege, H.; Chételat, G.; Teunissen, C. E.; Cummings, J.; van der Flier, W. M., Alzheimer's disease. *Lancet (London, England)* **2021**, 397, (10284), 1577-1590.
3. Rostagno, A. A., Pathogenesis of Alzheimer's Disease. *Int J Mol Sci* **2022**, 24, (1).
4. Lashley, T.; Schott, J. M.; Weston, P.; Murray, C. E.; Wellington, H.; Keshavan, A.; Foti, S. C.; Foiani, M.; Toombs, J.; Rohrer, J. D.; Heslegrave, A.; Zetterberg, H., Molecular biomarkers of Alzheimer's disease: progress and prospects. *Dis Model Mech* **2018**, 11, (5).
5. Andrade-Guerrero, J.; Santiago-Balmaseda, A.; Jeronimo-Aguilar, P.; Vargas-Rodríguez, I.; Cadena-Suárez, A. R.; Sánchez-Garibay, C.; Pozo-Molina, G.; Méndez-Catalá, C. F.; Cardenas-Aguayo, M. D.; Diaz-Cintra, S.; Pacheco-Herrero, M.; Luna-Muñoz, J.; Soto-Rojas, L. O., Alzheimer's Disease: An Updated Overview of Its Genetics. *Int J Mol Sci* **2023**, 24, (4).
6. Li, J.; Wang, L.; Zhang, X.; Shi, J.; Zhu, Y.; Wang, H.; Zhu, X.; Zhu, Q.; Luo, J. L., Translating Alzheimer's Disease Mechanisms into Therapeutic Opportunities. *Biomolecules* **2025**, 15, (9).
7. Ding, M. R.; Qu, Y. J.; Hu, B.; An, H. M., Signal pathways in the treatment of Alzheimer's disease with traditional Chinese medicine. *Biomed Pharmacother* **2022**, 152, 113208.
8. Fang, Z.; Tang, Y.; Ying, J.; Tang, C.; Wang, Q., Traditional Chinese medicine for anti-Alzheimer's disease: berberine and evodiamine from *Evodia rutaecarpa*. *Chin Med* **2020**, 15, 82.
9. Guo, P.; Zhang, B.; Zhao, J.; Wang, C.; Wang, Z.; Liu, A.; Du, G., Medicine-Food Herbs against Alzheimer's Disease: A Review of Their Traditional Functional Features, Substance Basis, Clinical Practices and Mechanisms of Action. *Molecules (Basel, Switzerland)* **2022**, 27, (3).
10. Wu, Z. Z.; Zhong, Q.; Sun, S. P., [A prospective multicenter randomized double-blinded controlled clinical trial on effects of tiantai no.1 in treating mild cognitive impairment]. *Zhongguo Zhong Xi Yi Jie He Za Zhi* **2010**, 30, (3), 255-8.
11. Li, Y. H.; Wang, X. S.; Chen, X. L.; Jin, Y.; Chen, H. B.; Jia, X. Q.; Zhang, Y. F.; Wu, Z. Z., Neuroprotective effect of the Chinese medicine Tiantai No. 1 and its molecular mechanism in the senescence-accelerated mouse prone 8. *Neural regeneration research* **2017**, 12, (2), 301-306.
12. Wu, Z. Z.; Li, Y. H.; Huang, A. C.; Li, M.; Zhang, X. L.; Wang, J. G.; Yang, M.; Chen, M. Y., Endoplasmic reticulum stress induced by tunicamycin and antagonistic effect of Tiantai No.1 (1) on mesenchymal stem cells. *Chin J Integr Med* **2010**, 16, (1), 41-9.
13. Chen, Z.; Wang, X.; Du, S.; Liu, Q.; Xu, Z.; Guo, Y.; Lin, X., A review on traditional Chinese medicine natural products and acupuncture intervention for Alzheimer's disease based on the neuroinflammatory. *Chin Med* **2024**, 19, (1), 35.
14. Cheng, F.; Desai, R. J.; Handy, D. E.; Wang, R.; Schneeweiss, S.; Barabási, A. L.; Loscalzo, J., Network-based approach to prediction and population-based validation of in silico drug repurposing. *Nature communications* **2018**, 9, (1), 2691.
15. Wu, Q.; Su, S.; Cai, C.; Xu, L.; Fan, X.; Ke, H.; Dai, Z.; Fang, S.; Zhuo, Y.; Wang, Q.; Pan, H.; Gu, Y.; Fang, J., Network Proximity-based computational pipeline identifies drug candidates for different pathological stages of Alzheimer's disease. *Computational and structural biotechnology journal* **2023**, 21, 1907-1920.

16. Zhao, J.; Guo, P.; Fang, J.; Wang, C.; Yan, C.; Bai, Y.; Wang, Z.; Du, G.; Liu, A., Neuroinflammation inhibition and neuroprotective effects of purpurin, a potential anti-AD compound, screened via network proximity and gene enrichment analyses. *Phytotherapy research : PTR* **2024**, 38, (11), 5474-5489.
17. Guo, P.; Zhao, J.; Zhang, B.; Wang, C.; Bai, Y.; Liu, A., Isorhapontigenin, a potential anti-AD natural product targeting multiple pathways, discovered via network proximity and gene enrichment analyses. *Phytomedicine Plus* **2025**, 5, (3), 100853.
18. Guo, P.; Zhao, J.; Wang, C.; Bai, Y.; Zhang, B.; Liu, A., Effect of DL0410 and tetrahydrocurcumin (LG0367) alone and in combination on learning and memory in vascular dementia. *European journal of pharmacology* **2026**, 178730.
19. Breitling, R.; Armengaud, P.; Amtmann, A.; Herzyk, P., Rank products: a simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. *FEBS letters* **2004**, 573, (1-3), 83-92.
20. Koziol, J. A., Comments on the rank product method for analyzing replicated experiments. *FEBS letters* **2010**, 584, (5), 941-4.
21. Ru, J.; Li, P.; Wang, J.; Zhou, W.; Li, B.; Huang, C.; Li, P.; Guo, Z.; Tao, W.; Yang, Y.; Xu, X.; Li, Y.; Wang, Y.; Yang, L., TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *Journal of cheminformatics* **2014**, 6, 13.
22. Wu, Z.; Ma, H.; Liu, Z.; Zheng, L.; Yu, Z.; Cao, S.; Fang, W.; Wu, L.; Li, W.; Liu, G.; Huang, J.; Tang, Y., wSDTNBI: a novel network-based inference method for virtual screening. *Chemical science* **2022**, 13, (4), 1060-1079.
23. Yan, D.; Zheng, G.; Wang, C.; Chen, Z.; Mao, T.; Gao, J.; Yan, Y.; Chen, X.; Ji, X.; Yu, J.; Mo, S.; Wen, H.; Han, W.; Zhou, M.; Wang, Y.; Wang, J.; Tang, K.; Cao, Z., HIT 2.0: an enhanced platform for Herbal Ingredients' Targets. *Nucleic acids research* **2022**, 50, (D1), D1238-d1243.
24. Yang, H.; Robinson, P. N.; Wang, K., Phenolyzer: phenotype-based prioritization of candidate genes for human diseases. *Nature methods* **2015**, 12, (9), 841-3.
25. Grissa, D.; Junge, A.; Oprea, T. I.; Jensen, L. J., Diseases 2.0: a weekly updated database of disease-gene associations from text mining and data integration. *Database (Oxford)* **2022**, 2022.
26. Ochoa, D.; Hercules, A.; Carmona, M.; Suveges, D.; Gonzalez-Urriarte, A.; Malangone, C.; Miranda, A.; Fumis, L.; Carvalho-Silva, D.; Spitzer, M.; Baker, J.; Ferrer, J.; Raies, A.; Razuvayevskaya, O.; Faulconbridge, A.; Petsalaki, E.; Mutowo, P.; Machlitt-Northen, S.; Peat, G.; McAuley, E.; Ong, C. K.; Mountjoy, E.; Ghousaini, M.; Pierleoni, A.; Papa, E.; Pignatelli, M.; Koscielny, G.; Karim, M.; Schwartzentruber, J.; Hulcoop, D. G.; Dunham, I.; McDonagh, E. M., Open Targets Platform: supporting systematic drug-target identification and prioritisation. *Nucleic acids research* **2021**, 49, (D1), D1302-d1310.
27. Morselli Gysi, D.; do Valle, Í.; Zitnik, M.; Ameli, A.; Gan, X.; Varol, O.; Ghiassian, S. D.; Patten, J. J.; Davey, R. A.; Loscalzo, J.; Barabási, A. L., Network medicine framework for identifying drug-repurposing opportunities for COVID-19. *Proceedings of the National Academy of Sciences of the United States of America* **2021**, 118, (19).
28. Guney, E.; Menche, J.; Vidal, M.; Barabási, A. L., Network-based in silico drug efficacy screening. *Nat Commun* **2016**, 7, 10331.
29. Koziol, J. A., A cautionary note on the rank product statistic. *FEBS letters* **2016**, 590, (11), 1586-91.

30. Xu, M.; Zhang, D. F.; Luo, R.; Wu, Y.; Zhou, H.; Kong, L. L.; Bi, R.; Yao, Y. G., A systematic integrated analysis of brain expression profiles reveals YAP1 and other prioritized hub genes as important upstream regulators in Alzheimer's disease. *Alzheimers Dement* **2018**, *14*, (2), 215-229.
31. Dutta, K.; Ravi, L., Molecular dynamic investigation for Roco4 kinase inhibitor as treatment options for parkinsonism. *J Mol Model* **2024**, *30*, (5), 133.
32. Ma, Y.; Hu, M.; Lai, J.; Li, J.; Wan, Q.; Sun, L.; Wu, J., Multi-omics and experimental validation reveal the mechanism of DanxiaTiaoban decoction in treating atherosclerosis. *Phytomedicine* **2025**, *147*, 157216.
33. Zhao, X.; Mou, C.; Xu, J.; Cui, W.; Shi, Y.; Wang, Y.; Luo, T.; Guo, W.; Ye, J.; Chen, W., Protection of Si Nanowires against A β Toxicity by the Inhibition of A β Aggregation. *Molecules* **2024**, *29*, (9).
34. Ye, J. Y.; Li, L.; Hao, Q. M.; Qin, Y.; Ma, C. S., β -Sitosterol treatment attenuates cognitive deficits and prevents amyloid plaque deposition in amyloid protein precursor/presenilin 1 mice. *Korean J Physiol Pharmacol* **2020**, *24*, (1), 39-46.
35. Avrutsky, M. I.; Troy, C. M., Caspase-9: A Multimodal Therapeutic Target With Diverse Cellular Expression in Human Disease. *Front Pharmacol* **2021**, *12*, 701301.
36. Li, Y. H.; Wu, Z. Z.; Cao, M. Q.; Li, M.; Sun, K. H.; Yang, M.; Chen, M. Y.; Huang, A. C., Effect of Tiantai No.1 on gene expression profiles in hippocampus of Alzheimer's disease rats by bioinformatic analysis. *Chin J Integr Med* **2015**, *21*, (2), 123-31.
37. Wu, Z. Z.; Huang, A. C.; de Vellis, J.; Li, Y. H., Effect of Tiantai No.1 on beta-amyloid-induced neurotoxicity and NF-kappa B and cAMP responsive element-binding protein. *Chinese journal of integrative medicine* **2008**, *14*, (4), 286-92.
38. Sepúlveda, P.; Ferreira, A. F. F.; Sandoval, C.; Bergoc, G.; Moreno, A. C. R.; Nunes, M. T.; Torrão, A. D. S., Thyroid Hormone Supplementation Restores Cognitive Deficit, Insulin Signaling, and Neuroinflammation in the Hippocampus of a Sporadic Alzheimer's-like Disease Rat Model. *Cells* **2024**, *13*, (21).
39. Wang, Y.; Lin, Y.; Wang, L.; Zhan, H.; Luo, X.; Zeng, Y.; Wu, W.; Zhang, X.; Wang, F., TREM2 ameliorates neuroinflammatory response and cognitive impairment via PI3K/AKT/FoxO3a signaling pathway in Alzheimer's disease mice. *Aging (Albany NY)* **2020**, *12*, (20), 20862-20879.
40. Fei, H. X.; Zhang, Y. B.; Liu, T.; Zhang, X. J.; Wu, S. L., Neuroprotective effect of formononetin in ameliorating learning and memory impairment in mouse model of Alzheimer's disease. *Biosci Biotechnol Biochem* **2018**, *82*, (1), 57-64.
41. Chen, L.; Ou, S.; Zhou, L.; Tang, H.; Xu, J.; Guo, K., Formononetin attenuates A β (25-35)-induced cytotoxicity in HT22 cells via PI3K/Akt signaling and non-amyloidogenic cleavage of APP. *Neurosci Lett* **2017**, *639*, 36-42.
42. Yesudas, R.; Thirumalairaj, V. K.; Durairaj, G.; Chacko, A.; Shanmugaasokan, L.; Gunasekaran, S., Stigmasterol- an Acetylcholinesterase Inhibitor from *Phormidium retzii* with relevance to Alzheimer's disease Therapy. *Int J Mol Cell Med* **2023**, *12*, (2), 100-107.
43. Taha, M. M. E.; Khalid, A.; Elfatih, F.; Mohan, S.; Sukumaran, S. D.; Ul-Haq, Z.; Aljahdali, I. A.; Oraibi, O.; Oraibi, B.; Alfaifi, H. A.; Alzahrani, A. H.; Farasani, A.; Jerah, A. A.; Babiker, Y. O. H.; Abdelwahab, S. I., Exploring the diverse acetylcholinesterase inhibitory potential of

- girinimbine: insights from in vitro assays, molecular docking, and simulation studies. *Cell Mol Biol (Noisy-le-grand)* **2024**, 70, (10), 154-160.
44. Pratiwi, R.; Nantasenamat, C.; Ruankham, W.; Suwanjang, W.; Prachayasittikul, V.; Prachayasittikul, S.; Phopin, K., Mechanisms and Neuroprotective Activities of Stigmasterol Against Oxidative Stress-Induced Neuronal Cell Death via Sirtuin Family. *Front Nutr* **2021**, 8, 648995.
 45. Ruankham, W.; Suwanjang, W.; Wongchitrat, P.; Prachayasittikul, V.; Prachayasittikul, S.; Phopin, K., Sesamin and sesamol attenuate H₂O₂-induced oxidative stress on human neuronal cells via the SIRT1-SIRT3-FOXO3a signaling pathway. *Nutr Neurosci* **2021**, 24, (2), 90-101.
 46. Liang, X.; Yao, Y.; Lin, Y.; Kong, L.; Xiao, H.; Shi, Y.; Yang, J., Panaxadiol inhibits synaptic dysfunction in Alzheimer's disease and targets the Fyn protein in APP/PS1 mice and APP-SH-SY5Y cells. *Life Sci* **2019**, 221, 35-46.
 47. Tönnies, E.; Trushina, E., Oxidative Stress, Synaptic Dysfunction, and Alzheimer's Disease. *J Alzheimers Dis* **2017**, 57, (4), 1105-1121.
 48. Varadharajan, A.; Davis, A. D.; Ghosh, A.; Jagtap, T.; Xavier, A.; Menon, A. J.; Roy, D.; Gandhi, S.; Gregor, T., Guidelines for pharmacotherapy in Alzheimer's disease - A primer on FDA-approved drugs. *J Neurosci Rural Pract* **2023**, 14, (4), 566-573.
 49. Zhao, W.; Wang, J.; Latta, M.; Wang, C.; Liu, Y.; Ma, W.; Zhou, Z.; Hu, S.; Chen, P.; Liu, Y., Rhizoma Gastrodiae Water Extract Modulates the Gut Microbiota and Pathological Changes of P-Tau(Thr231) to Protect Against Cognitive Impairment in Mice. *Front Pharmacol* **2022**, 13, 903659.
 50. Fu, Y.; Xu, Q.; Zhang, J.; Kang, C.; Yang, C.; Guo, L.; Zhang, C.; Zhou, T.; Xiao, C., Identifying the quality markers and optimizing the processing of Gastrodia rhizoma to treat brain diseases. *Front Pharmacol* **2024**, 15, 1396825.
 51. He, S.; Shi, J.; Ma, L.; Pei, H.; Zhang, P.; Shi, D.; Li, H., Total ginsenosides decrease A β production through activating PPAR γ . *Biomed Pharmacother* **2024**, 174, 116577.
 52. Zhu, C.; Liu, J.; Lin, J.; Xu, J.; Yu, E., Investigating the effects of Ginkgo biloba leaf extract on cognitive function in Alzheimer's disease. *CNS Neurosci Ther* **2024**, 30, (9), e14914.
 53. Nguyen, V. T. T.; Slotos, R. S.; Guilherme, M. D. S.; Nguyen, T. T.; Weisenburger, S.; Lehner, M. D.; Endres, K., Ginkgo biloba extract EGb 761[®] ameliorates cognitive impairment and alleviates TNF α response in 5xFAD Alzheimer's disease model mice. *Phytomedicine* **2025**, 136, 156327.
 54. Ma, X.; Wang, J., Formononetin: A Pathway to Protect Neurons. *Front Integr Neurosci* **2022**, 16, 908378.
 55. Liu, B.; Cui, D.; Liu, J.; Shi, J. S., Transcriptome analysis of the aged SAMP8 mouse model of Alzheimer's disease reveals novel molecular targets of formononetin protection. *Front Pharmacol* **2024**, 15, 1440515.
 56. Sun, M.; Zhou, T.; Zhou, L.; Chen, Q.; Yu, Y.; Yang, H.; Zhong, K.; Zhang, X.; Xu, F.; Cai, S.; Yu, A.; Zhang, H.; Xiao, R.; Xiao, D.; Chui, D., Formononetin protects neurons against hypoxia-induced cytotoxicity through upregulation of ADAM10 and sA β PP α . *J Alzheimers Dis* **2012**, 28, (4), 795-808.

57. Huang, W.; Kong, L.; Cao, Y.; Yan, L., Identification and Quantification, Metabolism and Pharmacokinetics, Pharmacological Activities, and Botanical Preparations of Protopine: A Review. *Molecules* **2021**, *27*, (1).
58. Sreenivasmurthy, S. G.; Iyaswamy, A.; Krishnamoorthi, S.; Senapati, S.; Malampati, S.; Zhu, Z.; Su, C. F.; Liu, J.; Guan, X. J.; Tong, B. C.; Cheung, K. H.; Tan, J. Q.; Lu, J. H.; Durairajan, S. S. K.; Song, J. X.; Li, M., Protopine promotes the proteasomal degradation of pathological tau in Alzheimer's disease models via HDAC6 inhibition. *Phytomedicine* **2022**, *96*, 153887.
59. Lu, L.; Pang, M.; Chen, T.; Hu, Y.; Chen, L.; Tao, X.; Chen, S.; Zhu, J.; Fang, M.; Guo, X.; Lin, Z., Protopine Exerts Neuroprotective Effects on Neonatal Hypoxic-Ischemic Brain Damage in Rats via Activation of the AMPK/PGC1 α Pathway. *Drug Des Devel Ther* **2024**, *18*, 4975-4992.
60. El Menyiy, N.; Aboulaghras, S.; Bakrim, S.; Moubachir, R.; Taha, D.; Khalid, A.; Abdalla, A. N.; Algarni, A. S.; Hermansyah, A.; Ming, L. C.; Rusu, M. E.; Bouyahya, A., Genkwanin: An emerging natural compound with multifaceted pharmacological effects. *Biomed Pharmacother* **2023**, *165*, 115159.
61. Zeng, P.; Fang, M.; Zhao, H.; Guo, J., A network pharmacology approach to uncover the key ingredients in Ginkgo Folium and their anti-Alzheimer's disease mechanisms. *Aging (Albany NY)* **2021**, *13*, (14), 18993-19012.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Tables.zip](#)
- [SupplementaryFiles.zip](#)