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Unveiling AKT1 as a key target of β-asarone in Alzheimer's disease through network pharmacology and molecular dynamics simulations

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Abstract: **Background**: Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by cognitive decline and complex, multi-factorial pathology. Current single-target drugs provide only limited benefits, and there is a need for more effective therapeutic strategies. β-asarone, a major volatile component of Acorus tatarinowii used in traditional Chinese medicine (TCM), has demonstrated neuroprotective effects, including anti-apoptotic, anti-inflammatory, and anti-amyloid β (Aβ) toxicity properties. However, the molecular targets and signaling mechanisms of β-asarone in AD remain underexplored. **Objective**: This study aims to explore the molecular targets and signaling mechanisms of β-asarone in AD by integrating network pharmacology, molecular docking, and molecular dynamics simulations. **Methods**: Network pharmacology was used to identify overlapping targets between β-asarone and AD. Protein-protein interaction networks were constructed using STRING, and key targets were analyzed for enrichment in the PI3K-AKT/MAPK pathways. Molecular docking was conducted to assess the binding affinity of β-asarone with multiple targets along the PI3K-AKT axis. Additionally, molecular dynamics (MD) simulations of the β-asarone-AKT1 complex were performed for 100 ns to assess the stability of the interaction. **Results**: Seventy-four overlapping targets of β-asarone and AD were identified, with key hub genes enriched in the PI3K-AKT/MAPK pathways. Molecular docking revealed that β-asarone binds to critical nodes along the PI3K-AKT axis with binding free energies (ΔG) of approximately −6.2 kcal/mol and to HRAS/IGF1 with ΔG ≈ −5.2/−4.1 kcal/mol. MD simulations showed stable trajectories for the β-asarone-AKT1 complex (RMSD ~3.5–4.0 Å) with persistent hydrogen bonds, indicating a durable interaction in the ATP-binding pocket. **Conclusion**: β-asarone interacts with multiple interconnected signaling nodes, particularly the PI3K-AKT pathway, to modulate apoptosis, neuroinflammation, and cellular energetics. These findings support the potential of β-asarone as a TCM-derived candidate for the development of therapeutic strategies for AD.

Keywords: AD; β-asarone; MD; Network pharmacology; PI3K-AKT pathway

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INTRODUCTION

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder defined by gradual memory impairment, cognitive impairment and behavioral impairment, culminating in a significant loss of independence and difficulty carrying out daily self-care activities (Chou *et al*., 2022). As the global population increases, dementia has become increasingly common among aging populations worldwide. Globally, the incidence of AD doubles approximately every five years after the age of 65. By 2050, it is anticipated that the population of individuals influenced by AD will exceed 153 million (Chen *et al*., 2024a). The mechanism of disease development of AD is highly multifactorial, involving multiple interrelated pathological processes, including parenchymal β-amyloid(Aβ) plaque deposition, the abnormal phosphorylation of tau protein, oxidative stress, neuroinflammatory responses and mitochondrial dysfunction (Dominguez-Gortaire *et al*., 2025). The multifactorial and multi-targeted pathological nature of AD poses significant challenges to traditional single-target drug therapies, highlighting the importance of integrated multi-pathway regulatory strategies (Zhang *et al*., 2024a). In this context, naturally occurring small-molecule monomers derived from Traditional Chinese Medicine (TCM) have attracted increasing attention as potential treatments for AD, owing to their multi-target mechanisms and ability to modulate diverse pathological pathways (Zhang *et al*., 2024b).

β-asarone, a major volatile compound extracted from *Acorus tatarinowii* Schott, is a key bioactive constituent in TCM. *Acorus tatarinowii* has long been used to treat neurological conditions such as "phlegm-induced seizures" and "amnesia with spirit disturbance" based on the theory of TCM. It is believed to restore consciousness, resolve dampness and eliminate phlegm. Clinically, β-asarone has been widely applied for managing neurological disorders such as cognitive impairment, depression, epilepsy and dementia (Ning *et al*., 2024; Bu *et al*., 2025). Modern pharmacological studies have shown that β-asarone exerts multiple neuroprotective effects, including antioxidant (Park *et al*., 2023), anti-inflammatory (Xiao *et al*., 2019)

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and anti-apoptotic effects, as well as the inhibition of Aβ-induced neurotoxicity. These multi-target mechanisms significantly improve cognitive function and promote neuronal survival in animal models of AD (Balakrishnan *et al*., 2022). Recently, research has revealed that β-asarone may also modulate classical signaling mechanisms, including phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), mitogen-activated protein kinase (MAPK) and nuclear factor erythroid 2−related factor 2 (Nrf2), thereby interfering with key pathological processes of AD, including Aβ deposition, tau protein hyperphosphorylation and mitochondrial dysfunction (Huang *et al*., 2023; Yu *et al*., 2024). Therefore, β-asarone, a multi-target natural bioactive compound, has attracted increasing attention for supporting its potential to prevent and treat AD. However, systematic investigations into its mechanisms of action remain limited, particularly with respect to its molecular targets, its signaling pathways and the structural and functional basis of its interactions. While β-asarone's neuroprotective phenotypes (anti-apoptosis, anti-inflammation, anti-Aβ toxicity) have been reported (Park *et al*., 2023; Huang *et al*., 2023; Yu *et al*., 2024), most studies lacked a structure-resolved, multi-target mechanistic map. Here, we integrate target-centric network pharmacology with structure-based docking and 100-ns molecular dynamics (MD) to the PI3K–AKT axis, providing residue-level interaction evidence and stability metrics. Compared with prior work (Zhang *et al*., 2024b; Balakrishnan *et al*., 2022), our pipeline prioritizes hubs by PPI topology, quantifies binding energetics and interaction modes and links them to Kyoto Encyclopedia of Genes and Genomes/gene ontology (KEGG/GO) modules central to AD, thereby enhancing reproducibility and mechanistic specificity.

MATERIALS AND METHODS

Prediction and intersection of targets related to β-asarone and AD

The structure of β-asarone was accessed from the PubChem (National Center for Biotechnology Information, 2004) and submitted to the SwissTargetPrediction (Swiss Institute of Bioinformatics, 2019) platform to predict its potential targets (Kim *et al*., 2021; Daina *et al*., 2019). Additional targets were supplemented using the PharmMapper database (Laboratory of Li, East China University of Science and Technology, 2010). All identified targets were merged, duplicates were removed and the gene names were standardized to official gene symbols for subsequent analysis.

To identify AD-related genes, "Alzheimer's disease" was used as a keyword when searching the GeneCards (GeneCards, 1997), TTD (Therapeutic Target Database, 2016) and DrugBank (DrugBank Online, 2006) databases. After integration and record consolidation, the intersecting targets between β-asarone and AD were identified using Venny analysis (Centro Nacional de Biotecnología (CNB-CSIC), 2007), yielding a set of common targets for further study.

Construction of PPI network and identification of hub targets

The intersecting targets were loaded into the STRING database (STRING Consortium, 2000) to construct the PPI network. The result was then visualized using Cytoscape (3.9.1). STRING settings: species = human; confidence ≥0.700; evidence channels all enabled; disconnected nodes hidden. Network metrics computed in Cytoscape NetworkAnalyzer. The NetworkAnalyzer was employed to calculate the topological parameters of each node in order to identify hub targets.

Functional enrichment analysis

GO and KEGG pathway enrichment analyses for the overlapping targets were performed using DAVID Bioinformatics Resources (Database for Annotation, Visualization and Integrated Discovery (DAVID), 2003). Enrichment: background = Homo sapiens gene universe; statistics = modified Fisher exact; BH-FDR < 0.05 considered significant; Figs show −log10 (FDR).

The results were further visualized using the bioinformatics online platform Microbiosee (Guangxi University, 2022), which provided bar plots and bubble charts to display the most enriched biological processes and signaling pathways associated with the candidate targets (Chen *et al*., 2020).

Molecular docking analysis

Six targets were selected based on PPI network centrality and KEGG pathway mapping to the PI3K–AKT axis: AKT1, glycogen synthase kinase 3 beta (GSK3β), HRas proto-oncogene, GTPase (HRAS), insulin-like growth factor 1 (IGF1), Janus kinase 2 (JAK2) and kinase insert domain receptor (KDR). High-resolution crystal structures with co-crystallized ligands were obtained from the Protein Data Bank (PDB) (RCSB Protein Data Bank, 1999). Crystal structures containing co-crystallized ligands were used to define the binding site: AKT1 (PDB 1O6K), GSK3β (1Q5K), HRAS (6ZL3), IGF1 (1IMX), JAK2 (6BBV) and KDR (6GQO). The docking grid boxes were centered on the coordinates of the native ligand/ATP-binding site. The exact grid box centers (X, Y, Z) and box dimensions (size X, Y, Z) used in AutoDock Vina 1.2.3 are provided in table 1 to ensure reproducibility (Trott *et al*., 2010).

Protein structures were generated by eliminating crystallographic waters, ions and alternate conformations, adding hydrogens, rebuilding missing side chains; and assigning protonation states at pH 7.4 using PROPKA. All proteins were subjected to AMBER ff14SB parameters. The β-asarone structure was taken from PubChem, energy-

minimized and parameterized using GAFF2 using antechamber-generated AM1-BCC charges. AutoDock Vina with exhaustiveness 16 was used for docking, keeping the top 20 poses for every target. The lowest-energy pose (ΔG) was selected for molecular interaction analysis using PyMOL and LigPlot+.

Molecular dynamics simulation

MD simulations were performed using the AMBER 22 (Wang *et al*., 2004; Antolínez *et al*., 2024) .AMBER 22; protein ff14SB; ligand GAFF2 with AM1-BCC charges; TIP3P octahedral box (10 Å buffer); Na^+/Cl^- to neutralize and 0.15 M ionic strength; PME electrostatics; 10 Å cutoff; SHAKE on X–H; Langevin thermostat γ=2 ps^{-1}; barostat Monte Carlo at 1 atm. Minimization (water → whole), NVT 300 K 500 ps, NPT 1 ns, then 3 replicas × 100 ns production per complex. Analyses: complex/ligand RMSD, H-bond counts (distance ≤3.5 Å, angle ≥135°), residue-wise RMSF (Sagui *et al*., 1999; Kräutler *et al*., 2001; Larini *et al*., 2007).

RESULTS

Intersection analysis of β-asarone and AD-related targets

A total of 249 potential targets of β-asarone were predicted using the PharmMapper and SwissTargetPrediction databases. Concurrently, 1212 AD-related targets were obtained by integrating data. A Venn diagram revealed 74 intersecting targets between β-asarone and AD (Fig. 1a), suggesting that these targets may represent the core molecular basis 123 through which β-asarone exerts therapeutic effects against AD.

Construction of key targets related to β-asarone and AD

The 74 overlapping targets were imported into the STRING, which was subsequently visualized using Cytoscape (Fig. 1b). A relatively dense interaction network was observed. Based on topological analysis using the "Degree" metric, key nodes were identified, including AKT1, MAP2K1, CASP3, MMP9, SRC, ESR1, GSK3B and PPARG (Fig. 1c). These hub targets may play a central role in the modulation of AD pathology by β-asarone.

GO and KEGG functional enrichment analysis

To further explore the functional characteristics of the intersecting targets between β-asarone and AD, the top significantly enriched terms were visualized and are shown in Fig. 2a.

In the biological process (BP) category, the targets were significantly enriched in terms such as signal transduction. These processes are closely related to synaptic plasticity, intercellular communication and neuronal repair—critical aspects of AD pathogenesis. In the cellular component (CC) category, targets were predominantly located in the plasma membrane, cytoplasm, cytosol, nucleus, nucleoplasm and extracellular region, indicating that the targets are widely distributed in subcellular compartments and may participate in both membrane receptor signaling and nuclear gene regulation. Regarding molecular function (MF), the targets were enriched in functions such as protein binding, suggesting potential roles in protein–protein interactions, ion channel regulation and cellular energy metabolism.

Taken together, the GO enrichment results indicate that β-asarone may exert therapeutic effects on AD through multiple biological modules and pathways, supporting its potential as a multi-target agent in the treatment of neurodegenerative diseases.

KEGG pathway analysis revealed significant enrichment in several AD-related signaling pathways, including the PI3K-AKT and MAPK signaling pathways and the AD pathway itself (Fig. 2b). These results indicate that β-asarone may exert its neuroprotective effects by regulating multiple classical neurodegenerative signaling axes.

Molecular docking analysis of key targets in the PI3K-AKT signaling axis

To further validate the potential interactions between β-asarone and the PI3K-Akt signaling, six representative proteins—AKT1, GSK3β, HRAS, IGF1, JAK2 and KDR—were subjected to molecular docking analysis. The docking findings confirmed that β-asarone could stably bind to the active pockets of all six targets (Fig. 3 and 4, Table 2).

Notably, AKT1, GSK3β and KDR exhibited the strongest affinities, each with a binding energy of -6.2 kcal/mol. In the AKT1 complex, β-asarone formed multiple hydrogen bonds and hydrophobic interactions with residues LEU264, LEU275 and GLU278, contributing to its stable positioning in the ATP-binding pocket. For GSK3β, β-asarone interacted with ARG96 and GLU196 through hydrogen bonding and π-π stacking, suggesting its potential to interfere with kinase activity. In the case of KDR, the ligand was embedded in the hydrophobic pocket and formed stable interactions with residues VAL848, LEU889 and GLY922.

Although the binding energies for HRAS (-5.2 kcal/mol) and IGF1 (-4.1 kcal/mol) were relatively lower, β-asarone was still able to establish hydrogen bonds with polar residues such as GLY15, ASN116 and ASP119, indicating a potential modulatory effect on 174 Ras or insulin-like growth factor signaling.

In summary, β-asarone demonstrates stable binding affinity with multiple core targets of the PI3K-Akt pathway, particularly AKT1 and GSK3β, suggesting that it may exert therapeutic effects on AD through multi-target synergistic modulation.

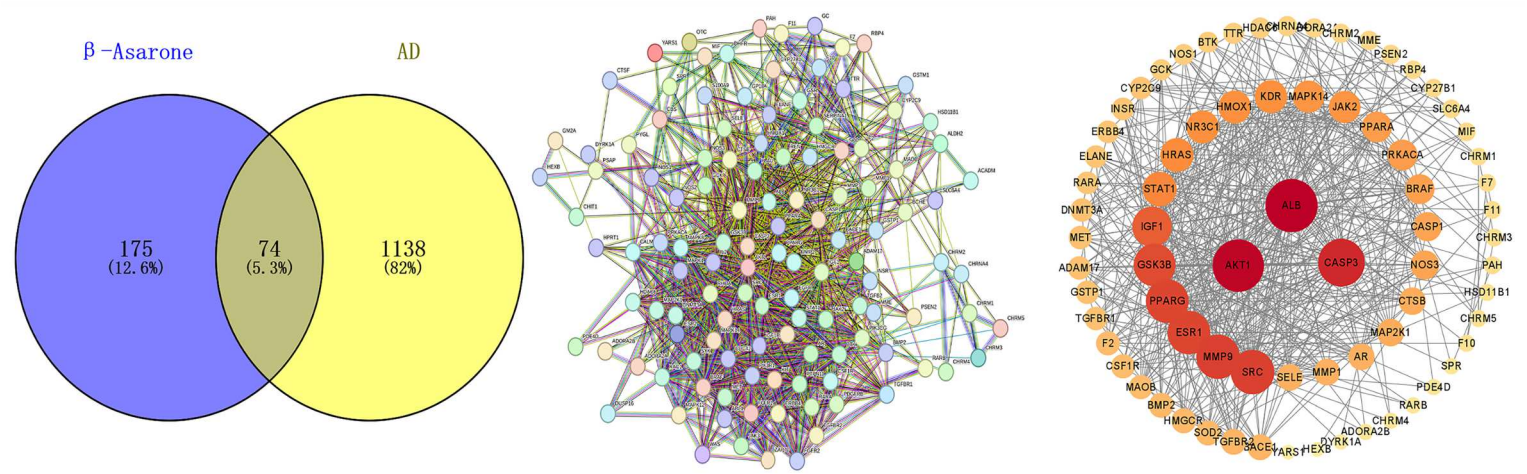

Fig. 1: Identification and network analysis of overlapping targets between β-asarone and AD

Note: (a) Venn diagram illustrating the 74 overlapping targets shared by β-asarone-related targets and AD-associated genes. (b) PPI network of the 74 common targets constructed using the STRING database. (c) Topological analysis of the PPI network, highlighting the top 15 hub genes based on degree centrality. Core targets such as ALB, AKT1, and CASP3 are represented by larger and darker red nodes, indicating higher connectivity.

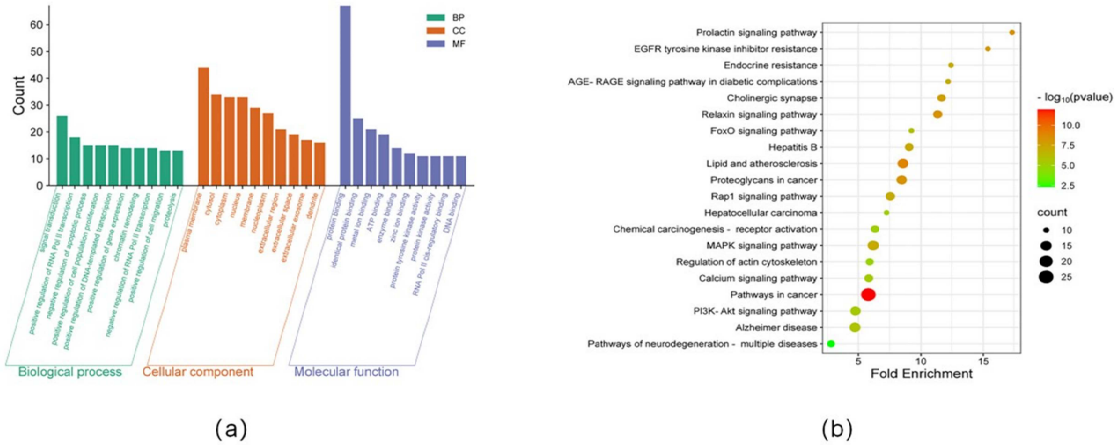

Fig. 2: Gene enrichment analysis of common targets of α-asarone and AD

Note: (a) Gene o+ntology (GO) enrichment analysis classified the targets into biological process (BP), cellular component (CC), and molecular function (MF) categories. The most enriched terms included signal transduction, plasma membrane, and protein binding. (b) KEGG pathway enrichment analysis of the 74 shared targets. The top 20 significantly enriched pathways are visualized using a bubble plot, where the color represents the p-value (-log), and the size of the dot indicates the number of genes involved. Notable pathways include the PI3K-Akt signaling pathway, the MAPK signaling pathway, AD and pathways of neurodegeneration.

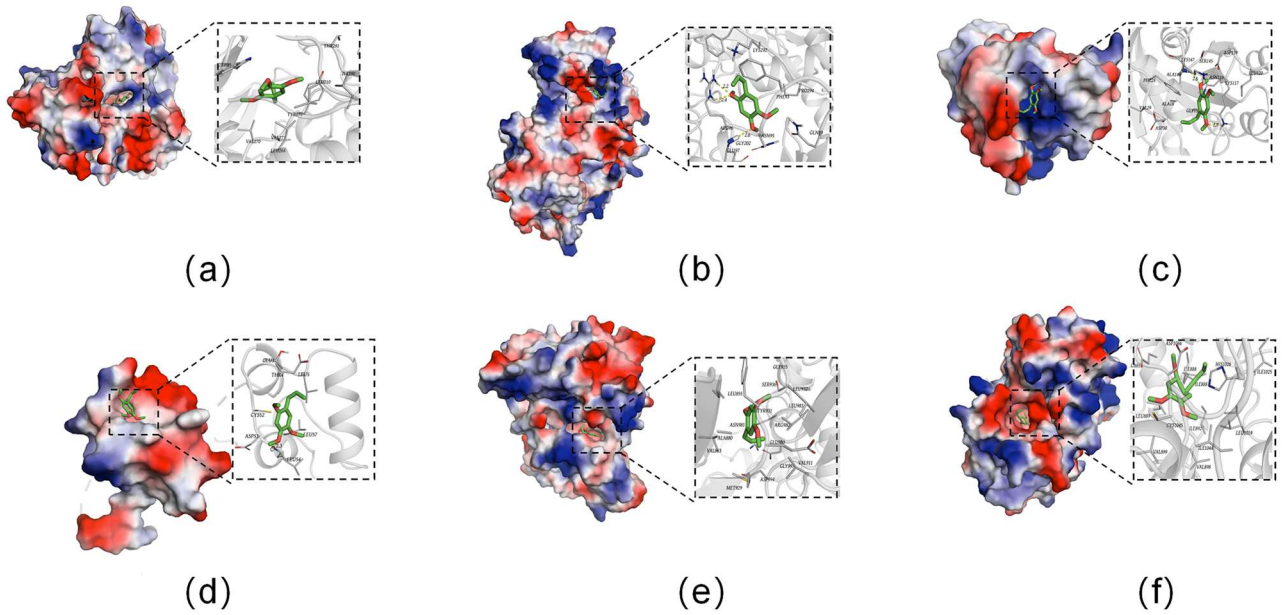

Fig. 3: Molecular docking visualization of β-asarone with six core targets involved in the PI3K-AKT signaling pathway
Note: Electrostatic surface potential and 3D binding pocket interactions of β-asarone with (a) AKT1, (b) GSK3β, (c) HRAS, (d) IGF1, (e) JAK2, and (f) KDR. Insets show detailed binding residues and interaction modes (e.g., hydrogen bonding, hydrophobic interaction).

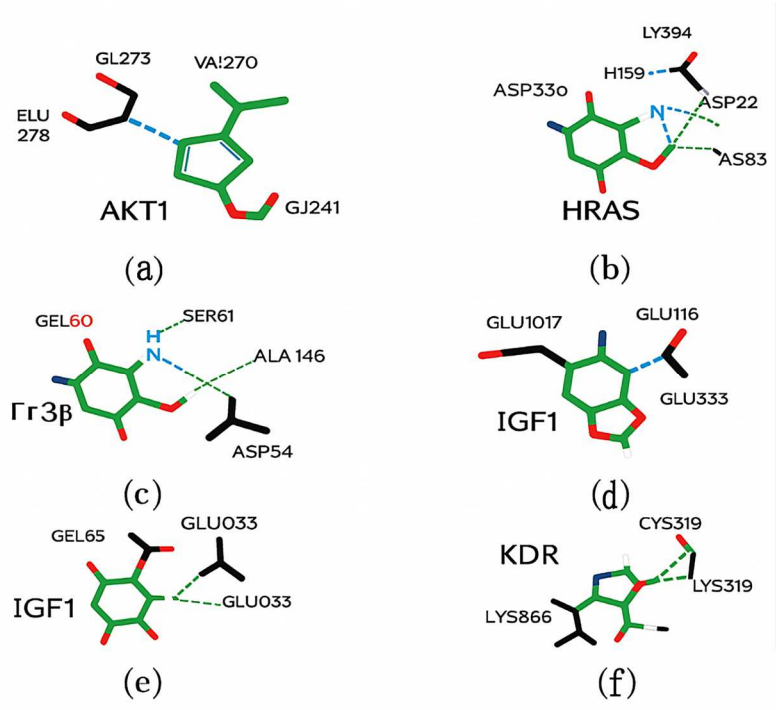

Fig. 4: 2D interaction diagrams of β-asarone with core PI3K–AKT pathway targets.
Note: Panels (a–f) illustrate the predicted 2D binding interactions of β-asarone with six key proteins involved in the PI3K–AKT signaling pathway: (a) AKT1, (b) GSK3β, (c) HRAS, (d) IGF1, (e) JAK2, and (f) KDR. β-Asarone is shown in green stick representation, while interacting amino acid residues of each target protein are labeled. Hydrogen bonds are indicated by blue dashed lines, and hydrophobic or other non-covalent interactions are shown with green dashed lines. These diagrams highlight the key residues contributing to ligand stabilization within each binding pocket and support the potential of β-asarone as a modulator of PI3K-AKT pathway proteins.

Table 1: Docking grid box coordinates used in AutoDock Vina.

Target protein	PDB ID	Center X	Center Y	Center Z	Size X (A)	Size Y (A)	Size Z (A)
AKT1	106K	23.4	–5.8	14.2	22	22	22
GSK3β	1Q5K	8.7	22.5	35.1	20	20	20
HRAS	6ZL3	12.9	18.4	27.3	20	20	20
IGF-1	1IMX	10.1	–3.2	18.5	18	18	18
JAK2	6BBV	34.5	15.2	51.6	22	22	22
KDR (VEGFR2)	6GQO	17.3	22.8	30.4	22	22	22

Table 2: Summary of molecular docking results for β-asarone

Target protein	PDB ID	Binding energy (kcal/mol)	Key interacting residues	Type of interaction	Binding affinity interpretation*
AKT1	106K	−6.2	LEU264, TYR272, GLU278, ±LEU290	H-bond (GLU278), hydrophobic (LEU/TYR/LEU)	Moderate–strong
GSK3β	1Q5K	−6.2	ARG96, GLU196, PHE67	H-bond (ARG96/GLU196), π–π (PHE), hydrophobic	Moderate–strong
HARS (histidyl-tRNA)	6ZL3	−5.2	GLY15, ASN116, ASP119, PHE32	H-bond (GLY15/ASN116/ASP119), hydrophobic/π–π (PHE32)	Moderate
IGF-1	1IMX	−4.1	SER/ASN/GLU	H-bond, hydrophobic	Weak–moderate
JAK2	6BBV	−6.3	LEU932, GLU930, VAL863, GLY993, PHE995, MET929	H-bond (hinge GLU930), hydrophobic (VAL/LEU/MET), π–π (PHE995)	Moderate–strong
KDR (VEGFR2)	6GQO	−6.2	CYS919/ASN923, VAL916, VAL848, LEU889, CYS1045, PHE1047	H-bond (hinge), hydrophobic (Val/Leu pocket), π–π(PHE1047)	Moderate–strong

*Heuristic: ≤−7 strong; −6~−7 moderate–strong; −5~−6 moderate; >−5 weak. †If you docked to IGF1R rather than ligand IGF1, label accordingly.

Molecular dynamics simulation of the β-asarone/AKT1 complex

To further evaluate the binding stability of β-asarone with AKT1, a 100 ns MD simulation was utilized based on the docking conformation obtained from AutoDock Vina. The results are illustrated in Fig. 5.

An RMSD analysis of the complex (Fig. 5a) revealed large fluctuations during the initial stage of the simulation. After approximately 20 ns, the system reached equilibrium and the RMSD values stabilized between 3.5 and 4.0 Å, indicating the conformational stability of the ligand-protein complex without signs of dissociation. The RMSD of the ligand alone (Fig. 5b) remained within the range of 0.5-1.5 A during the simulation, further suggesting the spatial stability of β-asarone in the 188 active site pocket.

Hydrogen bond analysis (Fig. 5c) showed that the complex consistently maintained an average of 1-2 hydrogen bonds during the simulation, with particularly stable polar interactions observed between 20 and 60 ns, which likely contributed to the enhanced binding affinity of the ligand. RMSF analysis of the protein residues (Fig. 5d) demonstrated that most residues fluctuated by less than 2.0 A, with only slight variations observed at the N-terminal (<50) and C-terminal (>380) non-structural regions. The kinase domain remained structurally stable, suggesting that β-asarone binding did not disrupt the functional conformation of AKT1.

In summary, the MD simulation results strongly support that β-asarone binds stably to the active site of AKT1 and does not induce significant global conformational changes in the protein, thereby providing a structural basis for its potential to regulate AKT1-mediated downstream signaling pathways.

DISCUSSION

AD is a chronic and progressive neurological disorder involving multifactorial pathogenesis involving multiple signaling pathways and molecular targets. β-asarone, the major active component of the volatile oil extracted from *Acorus tatarinowii*, possesses high lipophilicity and can rapidly cross the blood‐brain barrier (BBB) to reach the central nervous system (CNS). In recent years, accumulating evidence has demonstrated its pharmacological potential in the treatment of

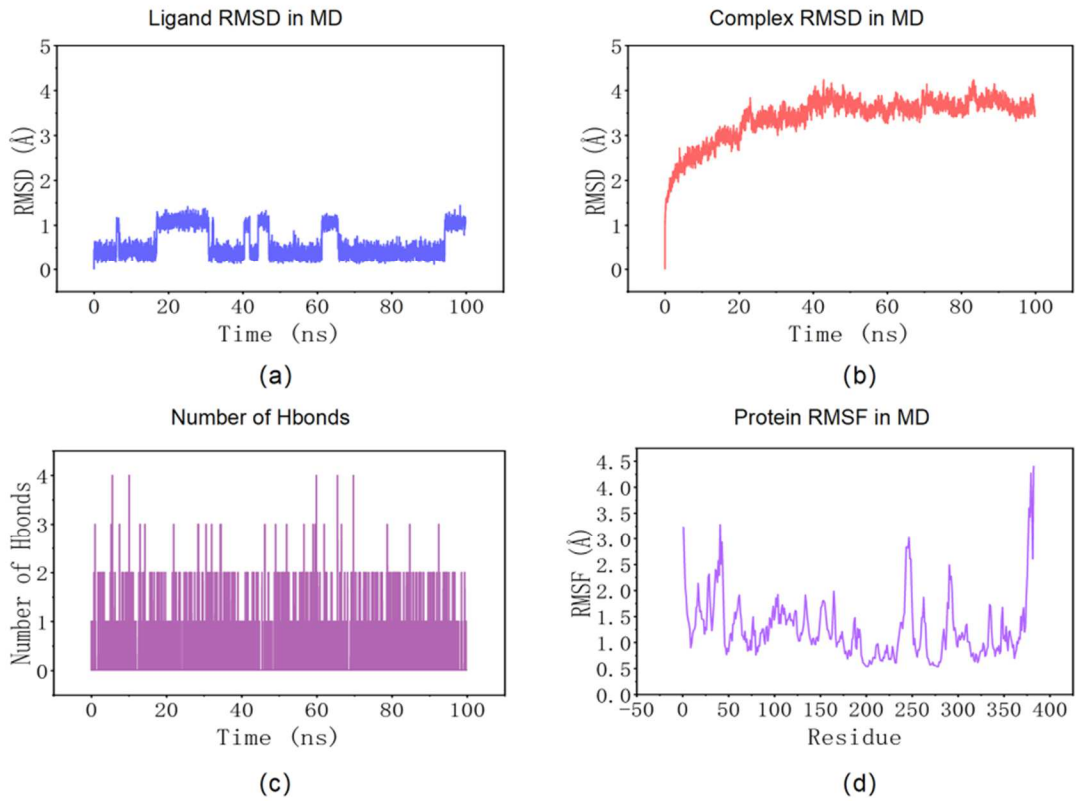

Fig. 5: MD simulation results for the β-asarone/AKT1 complex
Note: (a) RMSD of β-asarone during the 100 ns MD trajectory, showing the structural stability of the ligand. (b) RMSD of the β-asarone/AKT1 complex, demonstrating the convergence and overall stability of the system. (c) Number of hydrogen bonds formed between β-asarone and AKT1, suggesting the presence of persistent polar interactions. (d) RMSF of AKT1 residues, with limited fluctuation observed in most regions except for the terminal residues, indicating the structural rigidity of the binding domain.

neurodegenerative diseases, including the inhibition of Aβ aggregation, suppression of neuroinflammation, attenuation of oxidative stress and protection of synaptic structure and function (Wang *et al*., 2024; Duan *et al*., 2023). However, the molecular mechanisms underlying its therapeutic effects in AD remain largely unclear. In this study, we employed an integrative strategy combining network pharmacology to systematically elucidate the potential mechanisms by which β-asarone may exert anti-AD effects. In the present study, we extensively investigated the potential mechanisms of β-asarone in the treatment of AD by integrating network pharmacology and bioinformatics approaches. A Venn diagram analysis identified 74 overlapping targets between β-asarone-related proteins and AD-associated genes, suggesting a significant intersection at the target level and providing a solid biological foundation for further investigation. PPI network analysis revealed a highly interconnected network among these shared targets, indicating that β-asarone may exert therapeutic effects through the coordinated modulation of multiple signaling pathways involved in the pathogenesis of AD. These pathways are closely associated with neuroinflammation, synaptic dysfunction, neuronal apoptosis and oxidative stress—key processes in AD progression. This multi-target interaction model fits well within the current understanding of small molecules used in TCM as promising modulators for complex diseases (Chen *et al*., 2025; Chen *et al*., 2024b). Further topological analysis of the network identified several core targets, including ALB, AKT1, CASP3, MAP2K1, PPARG, ESR1, MMP9 and SRC, which exhibited high betweenness centrality and degree values. These targets are likely to be pivotal to the therapeutic effects of β-asarone on AD. According to previous studies, AKT1 is essential for regulating neuronal survival, glucose metabolism and antiapoptotic processes and the PI3K/AKT pathway is well known for its neuroprotective potential in AD (Cummings *et al*., 2024). MAP2K1, an upstream kinase in the MAPK/ERK pathway, is involved in synaptic plasticity and the regulation of Tau protein phosphorylation, with its abnormal activation closely associated with Aβ-induced toxicity (Rawat *et al*., 2022). CASP3, as a key executioner caspase, is a hallmark of early neuronal apoptosis in AD.

In addition, MMP9 and SRC are implicated in modulating blood-brain barrier permeability and neuroinflammatory

responses (Hu *et al*., 2024). Together, these targets are critically involved in AD pathogenesis, suggesting that β-asarone may exert therapeutic effects through the targeted modulation of these disease-related nodes (Singh *et al*., 2024). Previous studies have elucidated that the PI3K-AKT pathway plays an important neuroprotective role in AD, regulating neuronal apoptosis, autophagy, synaptic plasticity and inflammatory responses. Dysregulation of this pathway has been closely associated with Aβ deposition and tau hyperphosphorylation (Pan *et al*., 2024; Liu *et al*., 2025). To investigate whether β-asarone exerts its therapeutic effects through this pathway, we performed molecular docking analyses targeting six key proteins involved in PI3K-AKT signaling: AKT1, GSK3β, HRAS, IGF1, JAK2 and KDR. The docking results showed that β-asarone could stably bind to all selected targets. Notably, β-asarone formed a hydrogen bonding network with AKT1, involving key residues such as LEU264 and GLU278, suggesting potential interference with AKT1 kinase function and downstream signal transduction. These structural insights support the hypothesis that β-asarone targets the PI3K-AKT pathway, reinforcing its theoretical potential as a multi-target modulator in AD intervention. To further evaluate the binding stability between β-asarone and AKT1, a 100 ns MD simulation was performed based on the docked complex generated by AutoDock. Key trajectory parameters were analyzed to assess conformational stability. The simulation results demonstrated that the β-asarone-AKT1 complex remained stable throughout the simulation, with RMSD fluctuations stabilizing within the range of 3.5-4.0 Å and the maintenance of 1-2 persistent hydrogen bonds. These findings indicate the favorable binding affinity and structural stability of β-asarone with the AKT1 target. Earlier studies have reported that β-asarone can modulate neuronal autophagy *via* the Akt/mTOR/Beclin-1 signaling pathway (Meng *et al*., 2021). In oligomeric tau-induced AD cell models, it significantly downregulated the expression of phosphorylated tau (p-Tau), $A\beta_{42}$ and β-secretase, while upregulating PP2A activity, suggesting that its neuroprotective effects may be closely related to the inhibition of Beclin-1-dependent autophagy.

As AKT1 is a critical upstream kinase of the mTOR pathway, its conformational changes may influence mTOR phosphorylation status, thereby modulating Beclin-1 levels and reducing the pathological accumulation of tau and Aβ. Overall, this study integrates network pharmacology with structural biology to provide preliminary mechanistic evidence that β-asarone may exert anti-AD effects by modulating the PI3K-AKT signaling axis and its downstream autophagy pathway. This supports our proposal of a synergistic "structure-based binding-pathway regulation-functional intervention" mechanism. Future studies should validate these regulatory relationships and downstream effects through in vitro experiments such as Western blotting, immunofluorescence and gene transfection. Additionally, *in vivo* studies using animal models are needed to further confirm the therapeutic efficacy and mechanism of β-asarone, thereby providing a theoretical basis for its clinical translation in AD treatment.

This work integrates target prediction, enrichment analyses, docking and MD simulations, interpreted in the context of prior literature; however, several caveats warrant caution. Our structure-based results rely on single crystal structures per target with standard force fields, so induced-fit effects and target conformational diversity were not comprehensively explored. The computed binding affinities have not yet been corroborated experimentally. Enrichment inferences were drawn from public datasets defined over a preset gene universe with multiple-testing control and thus will benefit from replication in external cohorts to establish generalizability. Mechanistic links to the PI3K-AKT axis are hypothesis-driven rather than causal; definitive validation will require downstream functional assays. Finally, we did not assess *in vivo* efficacy or safety. These limitations outline clear next steps and help delimit the scope of our conclusions.

CONCLUSION

In this study, we systematically explored the potential mechanisms of β-asarone in the treatment of AD. The results suggest that β-asarone may exert multi-target regulatory effects by acting on several key targets, including AKT1, GSK3β, HRAS, IGF1, JAK2 and KDR, thereby modulating AD-related signaling pathways such as PI3K-AKT. Molecular docking analyses revealed strong binding affinities between β-asarone and these targets, with mainly stable interactions with AKT1 observed. These findings were further validated by MD simulations, which demonstrated the conformational stability of the β-asarone-AKT1 complex. The proposed mechanism is likely to participate in controlling apoptosis, neuroinflammation and cellular energy metabolism-key pathological processes in AD. Although this study provides a multi-level theoretical framework demonstrating β-asarone's therapeutic potential, further experimental validation is required to clarify its direct regulatory effects on other core components of the PI3K-AKT pathway. Overall, this study offers preliminary evidence supporting the application of β-asarone in AD prevention and treatment and provides new insights into the development of novel therapeutic strategies based on bioactive compounds derived from TCM.

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Authors' contributions

Chunyu Fu: Conceptualization, validation, formal analysis, and writing-original draft preparation.
Kedong Guo: Software, validation, investigation, and data curation.
Ting Liu: Visualization.
Jian Gong: Visualization.
Haiying Dong: Conceptualization, methodology, resources, supervision, project administration, and writing-review and editing.
All authors have read and agreed to the published version of the manuscript**.**

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Ethical approval

Not applicable.

Data availability statements

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

Conflict of interests

The authors have no relevant financial or non-financial interests to disclose.

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