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Jesaconitine from Aconitum Species as a Potential Acetylcholinesterase Inhibitor: An *in Silico* Docking Study for Alzheimer's Disease Management

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Abstract

Background & Objectives: One of the established pharmacological strategies for slowing the progression of Alzheimer's disease (AD) involves the inhibition of the acetylcholinesterase (AChE) enzyme. Current research has increasingly focused on the identification of novel compounds, particularly naturally derived metabolites, that exhibit potent modulatory activity alongside favorable toxicological profiles. In this context, diterpenoid alkaloids represent a promising therapeutic class for modulating AD pathology through AChE inhibition.

Material & Methods: In this *in silico* study, molecular docking analyses were performed to screen and characterize diterpenoid alkaloids with the potential to attenuate AD progression.

Results: Jesaconitine demonstrated a binding affinity of −6.72 kcal/mol, surpassing that of the reference inhibitor Tacrine (−6.21 kcal/mol). Docking simulations revealed critical interactions within the active site of AChE, including conventional hydrogen-bonding networks involving the residues Ser125, Asn87, and Tyr337.

Conclusion: The findings of the present study identify Jesaconitine as a promising lead compound for the management of AD through AChE inhibition, based on *in silico* docking predictions. Moreover, these results provide a theoretical framework for the development of novel AChE inhibitors and indicate that Jesaconitine warrants further experimental and pharmacological investigation.

Keywords: Acetylcholinesterase, Jesaconitine, Diterpene alkaloid, Molecular docking, Alzheimer's disease.

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the gradual deterioration of memory, cognitive function, and overall mental performance (1, 2). Global epidemiological reports indicate that approximately 36 million individuals were living with AD in 2010, and this number is projected to increase dramatically to nearly 115 million by 2050 (3, 4). These projections underscore the substantial and escalating burden of AD on global public health and highlight the urgent need for the development of effective therapeutic strategies to mitigate its devastating consequences. Cholinesterases constitute a family of enzymes responsible for catalyzing the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, an essential biochemical process required for maintaining normal cholinergic signaling and neuronal communication (5). Among these enzymes, acetylcholinesterase (AChE; EC 3.1.1.7) is predominantly localized in neuronal tissues, skeletal muscles, and erythrocyte membranes (6-8).

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Although cholinesterases are encoded by distinct genes located on human chromosomes, these enzymes share highly comparable catalytic domains and active-site architectures. From a pharmacological perspective, inhibition of cholinesterase activity has been widely pursued as an effective therapeutic strategy to enhance synaptic acetylcholine concentrations by preventing its enzymatic degradation (9, 10). Despite extensive efforts directed toward the treatment of AD, only a limited number of cholinesterase inhibitors, including Donepezil, Rivastigmine, and Tacrine (Figure 1), have received regulatory approval for clinical application (11-13). Consequently, the continued identification and characterization of novel cholinesterase inhibitors, particularly those derived from natural or plant-based sources, remain critically important for the advancement of therapeutic interventions against AD and related neurodegenerative disorders.

Diterpene alkaloid derivatives constitute a structurally diverse class of naturally occurring secondary metabolites predominantly identified in plant genera such as *Aconitum*, *Delphinium*, and *Spiraea*. These compounds exhibit a broad spectrum of pharmacological and biochemical activities, including analgesic, anti-inflammatory, antiarrhythmic, and neuroactive effects (14, 15). Their intricate molecular architecture, which combines a diterpenoid backbone with nitrogen-containing functional groups, contributes to their high affinity for a variety of biological targets, including ion channels, neurotransmitter receptors, and key metabolic enzymes. Recent pharmacological investigations have suggested that certain diterpene alkaloid derivatives may possess neuromodulatory and neuroprotective properties, thereby highlighting their potential utility in the management of neurodegenerative disorders (16, 17). Although previous studies have primarily focused on their cardiovascular and neuronal toxicity profiles, increasing attention has recently been directed toward their interactions with cholinergic enzymes, particularly AChE. Nevertheless, current evidence regarding AChE inhibition by diterpene alkaloid derivatives remains limited and largely preliminary, while the underlying molecular mechanisms have yet to be comprehensively elucidated. Accordingly, further biochemical and molecular investigations are warranted to clarify their potential role as AChE modulators and to evaluate their suitability as lead compounds for anti-Alzheimer drug discovery. Molecular docking has emerged as a cornerstone technique in structure-

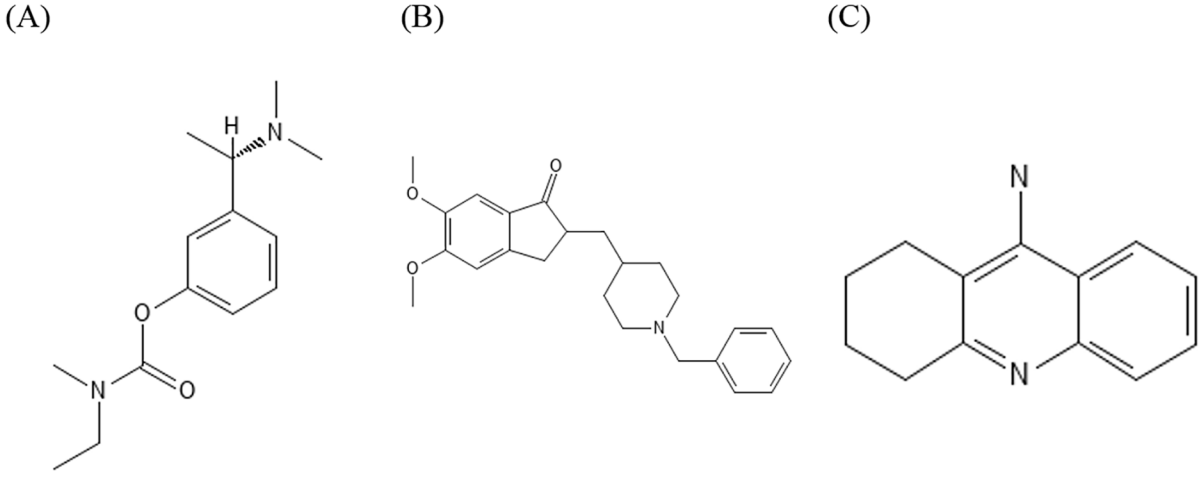

Figure 1. Chemical structure of inhibitors for AChEs: (A) Rivastigmine, (B) Donepezil, and (C) Tacrine.

based drug discovery, offering a sophisticated computational framework for predicting and optimizing molecular interactions with remarkable accuracy and reliability (18, 19). Through the application of advanced algorithms and simulation protocols, molecular docking facilitates the virtual screening and modeling of ligand–receptor complexes within biologically relevant environments (20, 21). Through the application of advanced algorithms and simulation protocols, molecular docking facilitates the virtual screening and modeling of ligand–receptor complexes within biologically relevant environments (22, 23). This approach enables the evaluation of molecular conformations, binding affinities, and energetic parameters, thereby assisting in the identification of promising bioactive compounds and the elucidation of their potential mechanisms of action (22,23). Despite the widespread application of computational screening approaches in drug discovery, studies specifically investigating diterpene alkaloids and their molecular interactions with AChE remain scarce. Therefore, the present study aimed to evaluate the binding affinity of Jesaconitine toward AChE and to compare its interaction profile with that of the standard drug Tacrine in order to assess its therapeutic potential for AD management. The findings derived from this investigation may contribute to the rational design of novel therapeutic agents for AD and related neurodegenerative disorders, thereby advancing the search for potent and selective cholinergic modulators.

Materials and Methods

Enzymes structure preparation for docking

The crystallographic structure of AChEs from *Torpedo californica* (PDB ID: 4PQE) was retrieved from the Protein Data Bank (PDB) (https://www.rcsb.org/). Prior to docking simulations, the protein structure was refined and prepared using UCSF Chimera version 1.7. Structural preprocessing included the assignment of appropriate force-field parameters, the addition of hydrogen atoms, and the calculation of partial atomic charges. Missing side chains and unresolved residues were also reconstructed to ensure the structural completeness and stability of the enzyme model. These preparatory procedures were conducted to generate an energetically favorable and geometrically reliable receptor conformation suitable for subsequent molecular docking analyses.

Enzyme active site delineation

The identification of residues constituting the enzyme's active site pocket was executed using the Computed Atlas of Surface Topography of proteins (CASTp) server (http://sts.bioe.uic.edu/castp/index.html?2pk9) (24, 25). The CASTp computational framework is based on advanced developments in computational geometry and surface-area analysis, providing a robust platform for the identification of solvent-accessible cavities and binding pockets. One of the principal strengths of the CASTp methodology lies in its ability to accurately define the interface between buried pocket volumes and the surrounding solvent environment. Furthermore, the platform employs rigorous analytical procedures for the comprehensive characterization of pockets and cavities. Importantly, this approach overcomes the limitations commonly associated with grid-based methodologies and surface discretization techniques, thereby providing rotationally invariant and geometrically robust descriptors of binding sites. Collectively, these procedures ensured that the identified active site accurately represented the biologically relevant binding region required for subsequent molecular docking evaluations.

Ligands preparation and docking validation

The initial three-dimensional (3D) structures of the diterpene alkaloid candidates were retrieved from the PubChem Compound Database (https://pubchem.ncbi.nlm.nih.gov/)(Figure 2 and Table 1).

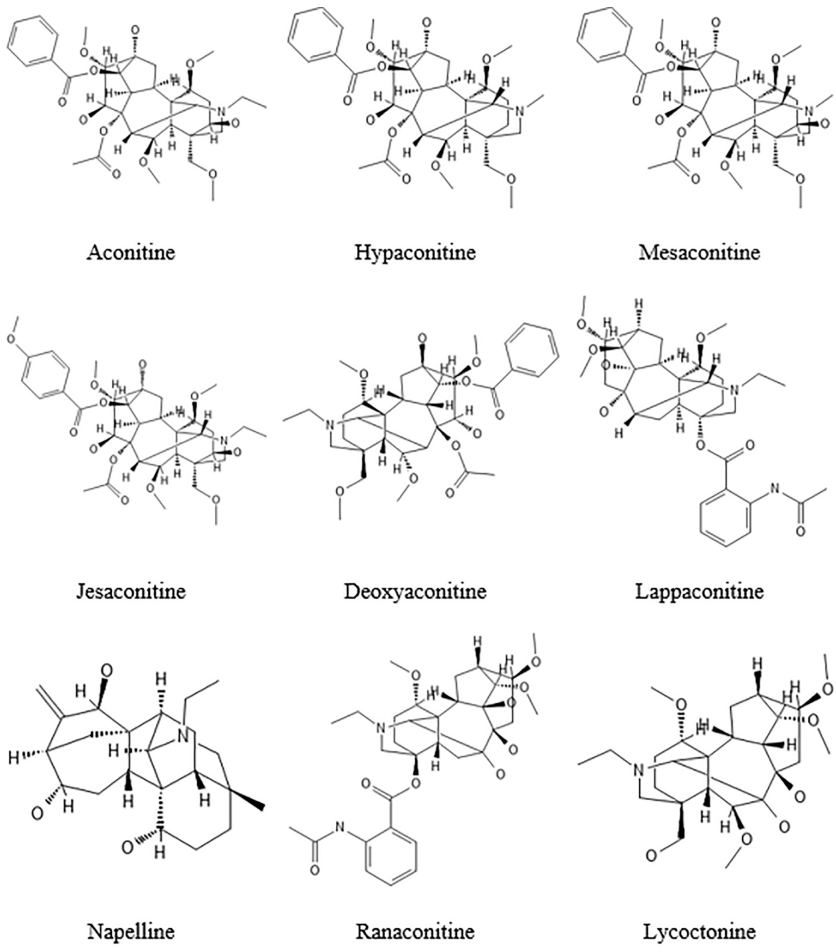

Figure 2. The molecular structures of the diterpene alkaloid against AChEs.

Table 1. The chemical data of selected diterpene alkaloid.

No.	Compound	Sources	M_w (g/mol)	Molecular Formula	PubChem ID
1	Aconitine	*Aconitum napellus*	645.7	$C_{34}H_{47}NO_{11}$	245005
2	Hypaconitine	*Aconitum carmichaeli*	615.7	$C_{33}H_{45}NO_{10}$	91973803
3	Mesaconitine	*Aconitum japonicum*	631.7	$C_{33}H_{45}NO_{11}$	16401314
4	Jesaconitine	Aconitum *japonicum*	675.8	$C_{35}H_{49}NO_{12}$	76963334
5	Deoxyaconitine	*Aconitum carmichaeli*	629.7	$C_{34}H_{47}NO_{10}$	44445634
6	Lappaconitine	*Aconitum sinomontanum*	584.7	$C_{32}H_{44}N_2O_8$	90479327
7	Napelline	*Aconitum napellus*	359.5	$C_{22}H_{33}NO_3$	17749282
8	Ranaconitine	*Aconitum ranunculifolium*	600.7	$C_{32}H_{44}N_2O_9$	78358494
9	Lycoctonine	*Aconitum lycoctonum*	467.6	$C_{25}H_{41}NO_7$	76956004

Each structure was subsequently converted into Protein Data Bank (PDB) format using the BIOVIA Discovery Studio (DS) suite (26). The resulting preprocessed PDB files were then utilized as input ligands for the molecular docking simulations. To ensure the reliability and robustness of the computational protocol, a validation procedure was conducted prior to the primary docking analyses. Nine diterpene alkaloids derived from *Aconitum* species were selected to investigate their potential as a novel class of AChE inhibitors. These compounds were not selected arbitrarily; rather, they were chosen to represent the structural diversity characteristic of this chemical class. The selected compounds included Aconitine, Hypaconitine, Mesaconitine, Jesaconitine, Deoxyaconitine, Lappaconitine, Napelline, Ranaconitine, and Lycoctonine. The calculated docking scores (kcal/mol), obtained from the docking simulations, demonstrated a strong and favorable correlation, thereby supporting the validity of the employed computational approach. Following successful validation, the selected diterpene alkaloids were docked into the prepared binding pocket of the 4PQE receptor structure. Docking calculations were performed using the AutoDock module integrated within DS version 2.5. This software employs a shape-based search algorithm to explore ligand conformational space and predict the most favorable orientation (pose) within the enzyme active site. The resulting docked conformations were subsequently evaluated on the basis of their predicted binding free energies, with particular emphasis placed on the lowest-energy configurations as indicators of potential binding affinity.

Molecular docking scoring

Molecular docking simulations were carried out to characterize the putative binding modes of the diterpene alkaloids within the active site of 4PQE. All docking calculations were performed using the AutoDock 4.2.6 software package. To ensure physiological relevance, the protonation states of ionizable amino acid residues were assigned at pH 7.4.

Prior to the docking simulations, comprehensive preparatory procedures were applied to both the receptor and ligand structures. These procedures included the systematic removal of non-essential crystallographic water molecules, the assignment of appropriate partial atomic charges using established methodologies such as the Gasteiger-Kollman formalism, and the addition of polar hydrogen atoms to facilitate chemically realistic intermolecular interactions.

A computational grid map was subsequently generated to define the search space for ligand placement within the active site. The grid dimensions were set to x = 60, y = 60, and z = 60 points, with a grid spacing of 0.375 Å. These parameters provided adequate spatial sampling coverage around the selected active-site residues. Conformational sampling and optimization within the defined search space were conducted using the Lamarckian Genetic Algorithm (LGA), which enables efficient exploration of the configurational landscape and identification of energetically favorable ligand conformations.

For each ligand, 15 independent docking runs were performed to generate multiple binding poses. Among the generated conformations, the pose exhibiting the most favorable, that is, the lowest, binding energy was selected as the optimal binding configuration. Finally, detailed two-dimensional interaction profiles between the selected ligand poses and the key residues of the 4PQE active site were visualized and analyzed using DS visualization tools, thereby facilitating the identification of hydrogen bonds, hydrophobic contacts, and π–π stacking interactions.

Results

High-throughput docking for lead compound prioritization against AChEs

The identification of potent and selective enzyme inhibitors, which constitutes a critical

step in the development of novel therapeutic agents, has been substantially accelerated through the application of *in silico* screening methodologies. By computationally filtering extensive chemical libraries, virtual screening (VS) circumvents the labor-intensive and time-consuming nature of conventional high-throughput experimental screening, thereby enabling the rapid prioritization of candidate molecules for subsequent empirical validation (27-29). This strategy is particularly advantageous when applied to structurally complex natural-product scaffolds such as diterpene alkaloids, as it facilitates a rational and targeted approach to drug discovery against neurological targets, including AChE.

To systematically evaluate the inhibitory potential of the screened diterpene alkaloid library against AChE, molecular docking simulations were performed. The resulting binding affinities, expressed as estimated free energies of binding, are presented in Table 2. These quantitative parameters serve as important indicators of ligand-enzyme complex stability and provide valuable insights into the active-site residues involved in mediating ligand binding.

Table 2. Docking score by the diterpene alkaloid during docking with AChEs, Van der waals (VDW), Hydrogen-bond (H-bond).

Diterpene alkaloid	Docking energy (Kcal/mol)	Van der waals	Hydrogen-bond	Electrostatics	Residual interaction
Aconitine	-6.19	-3.57	-2.41	-0.11	Trp236 (Pi-Pi-Stacked), Tyr337 (Pi-Pi-Stacked),Phe338 (Pi-Pi-Stacked), Tyr341 (VDW), Ser125 (H-Bond)
Hypaconitine	-6.11	-3.52	-2.22	-0.31	Tyr341 (VDW), Ser125 (H-Bond), Ser229 (C H-Bond), Trp86 (Pi-Pi-Stacked), Tyr72 (VDW)
Mesaconitine	-5.32	-3.62	-1.44	-0.11	Trp236 (Pi-Pi-Stacked), Tyr337 (Pi-Pi-Stacked), Phe338 (Pi-Pi-Stacked), Tyr341 (VDW), Ser125 (H-Bond)
Jesaconitine	-6.72	-4.23	-1.25	-0.67	Trp236 (Pi-Pi-Stacked), Tyr337 (H-Bond and Pi-Pi-Stacked),Phe338 (Pi-Pi-Stacked), Tyr341 (VDW), Ser125 (H-Bond), Asn87 (H-Bond), Ser229 (C H-Bond), Trp86 (Pi-Pi-Stacked), Tyr72 (VDW)
Deoxyaconitine	-5.48	-3.44	-1.31	-0.29	Tyr341 (VDW), Ser125 (H-Bond), Ser229 (C H-Bond), Trp86 (Pi-Pi-Stacked), Tyr72 (VDW)
Lappaconitine	-6.16	-3.72	-1.16	-0.21	Ser125 (H-Bond), Ser229 (C H-Bond), Trp86 (Pi-Pi-Stacked)
Napelline	-5.97	-2.88	-1.52	-0.18	Ser125 (H-Bond), Ser229 (C H-Bond), Trp86 (Pi-Pi-Stacked)
Ranaconitine	-6.14	-4.35	-1.42	-0.14	Trp236 (Pi-Pi-Stacked), Tyr337 (Pi-Pi-Stacked),Phe338 (Pi-Pi-Stacked), Tyr341 (VDW), Ser125 (H-Bond)
Lycoctonine	-5.28	-2.66	-1.41	-0.72	Tyr341 (VDW), Ser125 (H-Bond), Ser229 (C H-Bond), Trp86 (Pi-Pi-Stacked), Tyr72 (VDW)
Tacrine (Ref.)	-6.21	-4.19	-1.31	-0.82	Trp236 (Pi-Pi-Stacked), Tyr337 (Pi-Pi-Stacked),Phe338 (Pi-Pi-Stacked), Tyr341 (VDW), Ser125 (H-Bond), Ser229 (C H-Bond), Trp86 (Pi-Pi-Stacked), Tyr72 (VDW)

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The docking analysis demonstrated that all investigated diterpene alkaloids exhibited thermodynamically favorable interactions within the AChE binding gorge, as evidenced by their negative docking energy values. For comparative benchmarking, the commercially established AChE inhibitor Tacrine yielded a binding energy of −6.21 kcal/mol under the present simulation conditions. The nine diterpene alkaloids selected from PubChem on the basis of structural similarity displayed varying binding affinities toward the target enzyme. Among them, Jesaconitine exhibited the most favorable docking score (−6.72 kcal/mol), thereby emerging as the strongest predicted binder within the investigated compound set. This binding-energy profile strongly suggests an enhanced intrinsic binding affinity and the formation of energetically favorable molecular interactions within the AChE active site relative to the reference inhibitor.

Although Jesaconitine has previously been investigated for several pharmacological properties, the present study is, to the best of our knowledge, the first to report its binding affinity toward AChE. While the improvement in docking energy relative to Tacrine is moderate, the observed interaction profile indicates considerable potential for further structural optimization and lead development.

The molecular docking simulations provided important mechanistic insights into the probable binding behavior of the investigated diterpene alkaloids in comparison with the reference inhibitor Tacrine within the catalytic gorge of AChE. Particular emphasis was placed on characterizing the non-covalent interactions governing ligand binding and stabilization.

As illustrated in Figures 3A-D, Jesaconitine exhibited a complex and multifaceted interaction network with residues located within the AChE active site. The compound established substantial hydrophobic interactions, including π–π stacking and T-shaped interactions

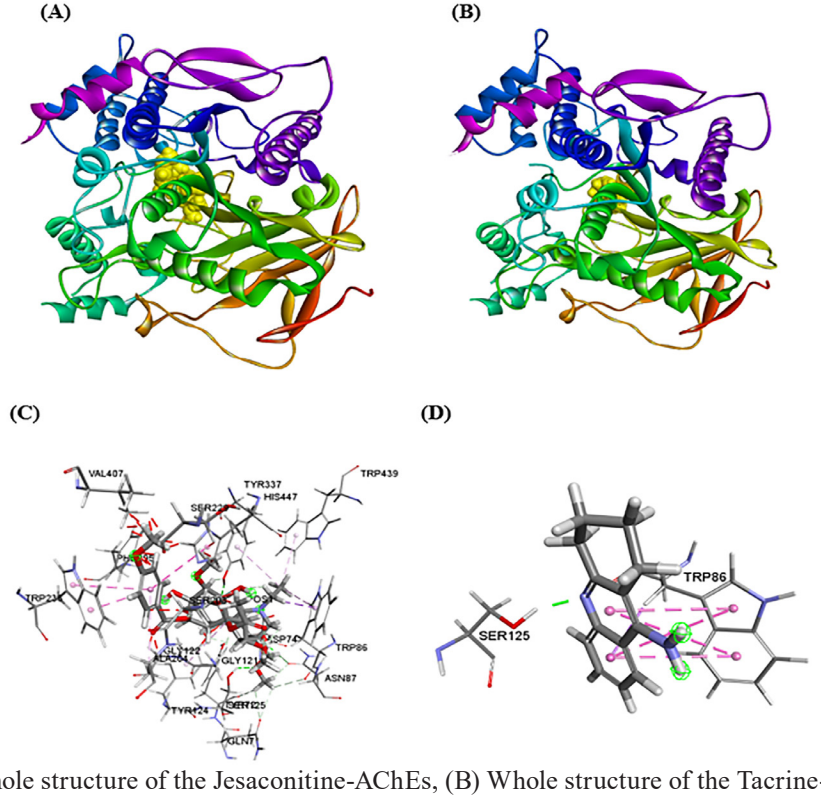

Figure 3. (A) Whole structure of the Jesaconitine-AChEs, (B) Whole structure of the Tacrine-AChEs, (C) 3D interaction of Jesaconitine-AChEs, (D) 3D interaction of Tacrine-AChEs.

represented by pink dashed lines, with critical aromatic residues (Fig. 4A). Specifically, interactions were observed with Trp236 and Tyr337. These hydrophobic contacts appear to play an essential role in anchoring the nonpolar regions of the diterpene scaffold within the hydrophobic pocket of the enzyme, analogous to the binding behavior reported for established AChE inhibitors.

In addition to hydrophobic interactions, Jesaconitine formed several stabilizing hydrogen bonds, indicated by green dashed lines in the interaction diagrams. Notably, hydrogen-bonding interactions were identified with the backbone or side-chain atoms of Ser125, Asn87, and Tyr337. The involvement of characteristic catalytic and peripheral-site residues, including Trp86 and Tyr337, suggests that Jesaconitine occupies a binding region structurally comparable to that targeted by classical AChE inhibitors.

Figures 3A-D additionally illustrate the binding conformation adopted by the reference compound Tacrine within the AChE active site. The interaction profile of Tacrine was found to be primarily mediated through its quaternary nitrogen center, which formed a strong electrostatic and hydrogen-bonding interaction with the side chain of Ser125. This interaction has been extensively documented in previous AChE inhibition studies. Furthermore, the planar aromatic scaffold of Tacrine engaged in pronounced π–π stacking interactions, particularly with Trp86, thereby stabilizing the inhibitor within the active-site gorge (Figure 4B).

A direct comparison of the two binding modes revealed that, although the diterpene alkaloid exhibited a more intricate interaction

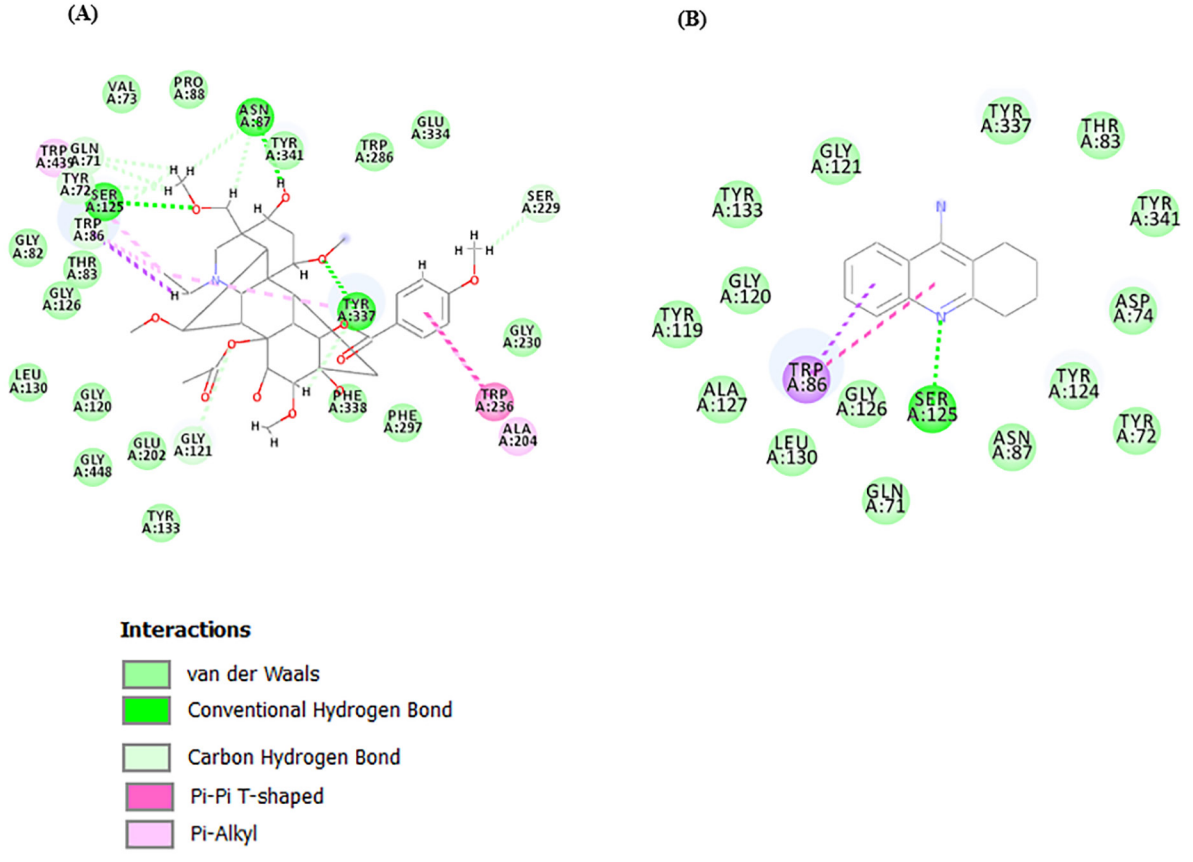

Figure 4. (A) 2D interaction of Jesaconitine-AChEs, (B) 2D interaction of Tacrine-AChEs. Hydrogen bonds are shown as green dashed lines. Pi-Pi stacking interactions are indicated in orange.

network owing to its larger and more highly functionalized structure, it nevertheless shared several critical anchoring interactions with the reference inhibitor Tacrine. Importantly, Jesaconitine successfully reproduced key molecular interactions required for potent inhibition, including strong hydrophobic association with residues such as Trp86 and interactions with residues located in close proximity to the catalytic region, including Ser125.

The greater number and diversity of interactions observed for Jesaconitine, particularly those involving peripheral-site residues such as Tyr337 and Trp236, suggest that this compound may possess enhanced binding affinity or potentially exert allosteric modulatory effects beyond the predominantly orthosteric binding mode exhibited by the smaller Tacrine molecule. Moreover, the presence of extensive polar interactions within the diterpene alkaloid complex implies favorable desolvation energetics upon binding, which may contribute to the lower and more favorable predicted binding-energy score.

The superior binding affinity predicted for Jesaconitine relative to Tacrine may be attributed to the presence of additional hydroxyl groups capable of forming supplementary hydrogen bonds with residues such as Ser125, Asn87, and Tyr337, as well as to the 4-methoxybenzoate moiety, which participates in favorable hydrophobic interactions within the acyl-binding pocket.

Nevertheless, several limitations of the present study should be acknowledged. Owing to restricted access to external databases during the study period, the comparative analysis was confined to the standard inhibitor Tacrine. Future investigations incorporating broader database accessibility should evaluate the binding performance of Jesaconitine against additional natural AChE inhibitors, including Galantamine and Huperzine A.

Discussion

The findings of the present study suggest that diterpene alkaloids may represent promising candidates for targeting AChE. Molecular docking analysis demonstrated that Jesaconitine exhibits a favorable binding orientation within the enzyme active site and interacts with several key residues implicated in inhibitor recognition and stabilization. These observations indicate that the molecular architecture of Jesaconitine possesses structural features capable of supporting effective binding to AChE.

One particularly noteworthy aspect of Jesaconitine is its structurally complex natural-product scaffold. Owing to its highly functionalized architecture, the compound is capable of establishing multiple types of intermolecular interactions simultaneously within the enzyme binding pocket. Such interactions may enhance the overall stability of the ligand-enzyme complex. In contrast to smaller inhibitors, larger and more functionally diverse molecules often achieve strong binding through the cumulative effect of numerous weak interactions, including hydrogen bonding, hydrophobic contacts, and π-related interactions, which collectively contribute to improved binding stability and affinity.

The present findings further demonstrate that Jesaconitine does not necessarily require an identical binding mode to that of Tacrine in order to exhibit favorable interaction characteristics. Although Tacrine is a well-established AChE inhibitor, naturally derived compounds may adopt distinct binding conformations while still retaining substantial inhibitory potential. This observation is particularly important because it suggests that diterpene alkaloids could serve as a novel source of structurally unique AChE inhibitors with alternative interaction mechanisms.

In addition, Jesaconitine may serve as a valuable lead compound for future structure-based drug design. Its molecular scaffold

provides multiple opportunities for structural modification aimed at enhancing binding affinity, selectivity, and biological activity. Consequently, Jesaconitine may constitute an attractive starting point for the rational development of novel natural product-derived inhibitors targeting cholinergic dysfunction.

Nevertheless, the findings of the present study should be interpreted with appropriate caution. Although molecular docking provides valuable insights into potential ligand-binding modes and interaction patterns, it cannot independently confirm actual inhibitory activity under biological conditions. Therefore, additional investigations, including molecular dynamics simulations, pharmacokinetic analyses, and experimental enzyme inhibition assays, are required to validate and extend the present findings.

Collectively, the results of this study identify Jesaconitine as a promising natural compound for further investigation as a potential AChE inhibitor. Moreover, the findings support the broader hypothesis that diterpene alkaloids may possess considerable therapeutic potential in the development of novel treatment strategies for cholinergic-related neurodegenerative disorders.

Limitations of the Study

Several limitations of the present study should be acknowledged. First, the docking analyses were performed using *T. californica* AChE, which, although highly homologous to the human enzyme, may not fully reproduce the structural and functional characteristics of human AChE. Second, molecular dynamics (MD) simulations and ADMET (absorption, distribution, metabolism, excretion, and toxicity) predictions were not conducted, thereby limiting the assessment of ligand stability, pharmacokinetic behavior, and toxicity profiles under physiological conditions. Third, the absence of experimental validation represents an important limitation; consequently, *in vitro* enzymatic assays and additional biological studies are necessary to confirm the predicted inhibitory activity of Jesaconitine. Finally, although Jesaconitine exhibited a more favorable binding affinity than Tacrine, the observed difference was relatively modest, suggesting that the compound should presently be regarded as a lead structure for further optimization rather than as a definitive drug candidate.

Conclusion

The present study demonstrated the significant potential of diterpene alkaloids as a promising source of novel AChE inhibitors, as supported by *in silico* molecular docking analyses. Detailed docking investigations revealed that a panel of nine alkaloids, including Lycoctonine, Ranaconitine, Napelline, Lappaconitine, Deoxyaconitine, Jesaconitine, Mesaconitine, Hypaconitine, and Aconitine, exhibited favorable binding affinities toward the active site of AChE, as reflected by their calculated binding-energy values.

Among the investigated compounds, Jesaconitine displayed the most favorable interaction profile and the lowest predicted binding energy, thereby emerging as the most promising lead candidate for further pharmaceutical development targeting AChE dysfunction in AD. The superior binding characteristics observed for Jesaconitine suggest that this compound may serve as a valuable structural template for the future design and optimization of novel cholinergic modulators.

Overall, this *in silico* investigation successfully identified Jesaconitine as a potential AChE inhibitor and further highlighted the therapeutic promise of diterpene alkaloids in the development of new treatment strategies for AD and related neurodegenerative disorders.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding

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Ethical Considerations

This study received ethical approval from the Iran National Science Foundation (INSF) under Project No. 40405653.

Code of Ethics

No40405653

Author's Contributions

Akbar Nasri: Conceptualization, Writing original draft. Morteza Sadeghi: Formal analysis, Supervision, Writing original draft, Methodology, Data curation.

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