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Repurposing Studies of Berberine for Alzheimer's Disease: A Comparative Study with Galantamine Using Molecular Docking

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ABSTRACT :

The first module document describes the selection of target disease and protein structure.The goal of this module is to thoroughly study and understand the selection of disease target and protein structure processes.

The second module includes a deep study of literature review of galantamine and berberine.

The third module presents the molecular docking analysis of berberine. Molecular docking can be used to predict how a ligand will interact with its target receptor.

The fourth module its describes the interpretation result of standard and reference drug which we used in molecular docking study and conclusion of the molecular docking study .

INTRODUCTION

Drug discovery is a high-cost and time-consuming process with high failure rates. In recent years, new technologies, strategies, and approaches, such as drug repositioning, have been developed and applied to accelerate drug discovery. Drug repurposing (also called drug repositioning) is the process of identifying new therapeutic uses for existing drugs, including:

- Approved drugs
- Failed drugs (that passed safety but failed efficacy for the original indication)
- Shelved or investigational compounds

This new strategy has significant advantages over conventional drug discovery including,

- lower investment needs
- Shortened drug development timelines
- Potentially higher success rates[1]

IMPORTANCE OF DRUG REPURPOSING

Repurposing can identify new compounds based on phenotypic benefits without explicitly defining the mechanism of action. This can be directly tested in preclinical animal models, and
these results are more applicable to clinical applications and research. It may progress directly straight to Phase II clinical trials . There is a minimum risk of failure with repurposed drugs [2]

ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a progressive neurodegenerative disease. It is characterized by progressive cognitive deterioration together with declining activities of daily living and behavioral changes. Neurodegenerative disorders are characterized by the progressive loss of neurons that are critically involved in learning, thinking and remembering. It is characterized by unique pathological changes such as abnormal aggregation of amyloid β-peptide (Aβ), and hyperphosphorylated tau protein (p-tau) accumulation leading to neuroinflammation and oxidative stress[3].

- Early symptom**:** Short-term memory loss (difficulty remembering recent events)
- Progression**:** Language problems, disorientation, poor judgment, mood/personality changes
- Late stage**:** Severe cognitive impairment, loss of independence, difficulty swallowing, complete dependency

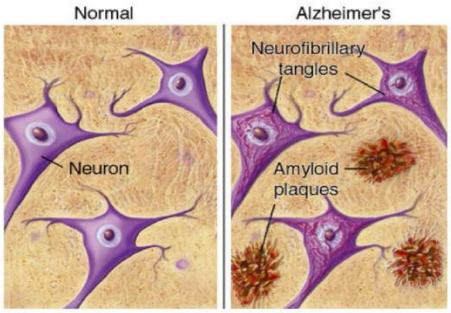


Fig1.1 Neurons of normal brain and alzheimer's one

Treatment

There is no definitive cure, but treatment focuses on symptom control and slowing progression

Pharmacological Treatment

A. Cholinesterase Inhibitors (Mild–Moderate AD)

Increase acetylcholine levels in the brain:

- **Donepezil**
- **Rivastigmine**
- **Galantamine**

B. NMDA Receptor Antagonist (Moderate–Severe AD)

- **Memantine**
  Reduces glutamate-mediated excitotoxicity.

C. Disease-Modifying Therapy (Early AD)

- **Anti-amyloid monoclonal antibodies** (e.g.,Lecanemab, Donanemab)
  These target amyloid plaques and may slow cognitive decline. Monitoring for ARIA (amyloid-related imaging abnormalities) is required.[4]

GALANTAMINE

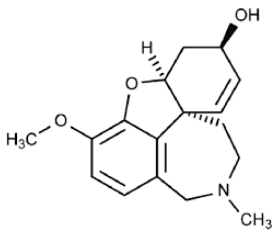


Fig1.2 The structure of galantamine

Galantamine is a tertiary alkaloid and reversible, competitive inhibitor of the acetylcholinesterase (AChE) enzyme, which is a widely studied therapeutic target used in the treatment of Alzheimer's disease.[5]

Following the discovery of its AChE-inhibiting properties, the cognitive effects of galantamine were studied in a wide variety of psychiatric disorders such as mild cognitive impairment, cognitive impairment in schizophrenia and bipolar disorder, and autism; however, redevelopment of the drug for Alzheimer's disease did not commence until the early 1990s due to difficulties in extraction and synthesis.

Galantamine blocks the breakdown of acetylcholine in the synaptic cleft, thereby increasing acetylcholine neurotransmission. Galantamine treats mild to moderate dementia caused by Alzheimer's disease. Galantamine increases acetylcholine (a brain chemical) levels in the central nervous system by blocking the action of the cholinesterase enzyme. This process helps transmit nerve signals that play a crucial role in memory.

Side Effects:

- Dizziness
- Drowsiness
- Weakness
- Loss of appetite
- Depression[6]

BERBERINE

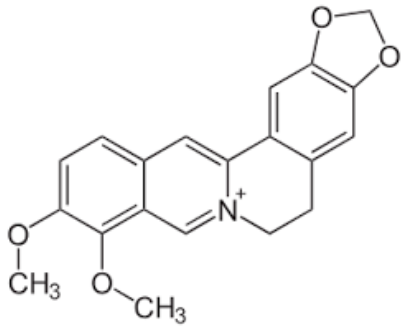


Fig1.3 The structure of berberine

Berberine is an organic compound classified as benzylisoquinoline alkaloid. Chemically, it is a quaternary ammonia compound.

name is derived from the genus of plants, *Berberis*. Berberine occurs in the roots, bark, stems, and leaves of *Berberis vulgaris* (barberry), *Berberis aristata* (tree turmeric), *Mahonia aquifolium* (Oregon grape) and *Hydrastis canadensis* (goldenseal).

Due to their yellow Its pigmentation, raw *Berberis* materials were once commonly used to dye wool, leather, and wood. Plants containing berberine have been used in traditional medicine, and berberine extracts are sold as dietary supplements. Other than in China as an over-the-counter drug, berberine is not approved as a prescription drug, regulated or proven safe in any country.

USES:

- **Canker sores:** Applying a gel containing berberine can reduce pain, redness, oozing, and the size of canker sores.
- **Diabetes:** Taking berberine by mouth seems to slightly reduce blood sugar levels in people with diabetes.
- **Hyperlipidemia:** Taking berberine by mouth, alone or with other ingredients, might help lower total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels in people with high cholesterol.
- **High blood pressure:** Taking 0.9 grams of berberine by mouth daily along with the blood pressure-lowering drug amlodipine reduces blood pressure better than taking amlodipine alone in people with high blood pressure[7]

PROTEIN STRUCTURE OF ACETYLCHOLINESTERASE

Acetylcholinesterase (AChE) is a crucial enzyme in cholinergic neurotransmission. It rapidly hydrolyzes the neurotransmitter acetylcholine into acetate and choline, thereby terminating synaptic transmission at cholinergic synapses. The three-dimensional (3D) structure of AChE has been extensively studied using X-ray crystallography, providing insight into its catalytic efficiency and role in drug design.

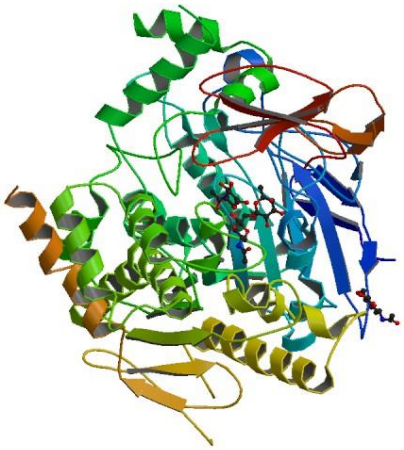


Fig 1.4 3D structure of acetylcholinesterase

Overall 3D Structure

The first high-resolution 3D structure of AChE was determined from *Torpedo californica* by Joel L. Sussman and colleagues in 1991.

AChE is:

- A globular protein
- Composed mainly of α-helices
- Approximately 537 amino acids in length (in Torpedo AChE)
- Organized into a compact ellipsoidal shape

The enzyme belongs to the α/β hydrolase fold family, a common structural motif found in many hydrolytic enzymes.

1.Deep and Narrow Active-Site Gorge

One of the most distinctive features of AChE is a ~20 Å deep aromatic gorge that penetrates halfway into the enzyme.

- Lined with aromatic amino acids (e.g., tryptophan, phenylalanine, tyrosine)
- Guides acetylcholine to the catalytic site

2. Catalytic Triad

At the base of the gorge lies the catalytic triad, typical of serine hydrolases:

- **Serine (Ser203 in human AChE)**
- **Histidine (His447)**
- **Glutamate (Glu334)**

These residues work together to hydrolyze acetylcholine through nucleophilic attack and formation of an acetyl-enzyme intermediate.

3. Peripheral Anionic Site (PAS)

Located at the entrance of the gorge:

- Regulates substrate entry
- Involved in amyloid-β aggregation in Alzheimer's disease
- Important target for dual-binding inhibitors[8]

The acetylcholinesterase (AChE) binding site is a deep narrow gorge present inside the enzyme where acetylcholine and inhibitory ligands bind.

It mainly consists of two regions: the catalytic active site (CAS), responsible for neurotransmitter hydrolysis, and the peripheral anionic site (PAS), involved in ligand recognition and stabilization.

LITERATURE REVIEW OF GALANTAMINE

1.Scott and Goa et al (2000), provided one of the earliest detailed reviews of galantamine's pharmacological profile. The authors described galantamine as a selective, reversible AChE inhibitor that enhances cholinergic transmission by increasing synaptic acetylcholine levels.[9]

2.Wilkinson and Murray et al (2001), examined galantamine treatment over 12 months. Patients receiving galantamine maintained cognitive performance longer than expected with natural disease progression. The study suggested that continuous treatment helps stabilize symptoms in mild to moderate AD.[10]

3.Samochocki *et al(2003)*,explained that galantamine works in two ways: it blocks acetylcholinesterase and also enhances nicotinic receptors. This dual action may improve communication between brain cells. The study helped explain why galantamine improves memory and attention. This mechanism makes it slightly different from some other cholinesterase inhibitors.[11]

LITERATURE REVIEW OF BERBERINE

4.Durairajan et al(2012), reported that berberine acts as a multi-target neuroprotective agent in Alzheimer's disease (AD). Their review highlighted berberine's ability to modulate amyloid-β (Aβ) production, inhibit tau hyperphosphorylation, reduce oxidative stress, and suppress neuroinflammatory pathways. The authors suggested that berberine's pleiotropic mechanisms make it a promising candidate for modifying AD pathology rather than providing only symptomatic relief.[12]

5.Asai et al(2007),demonstrated that berberine reduces amyloid-β levels by modulating amyloid precursor protein (APP) processing. The study showed that berberine decreased Aβ secretion in cultured neuronal cells by inhibiting β-secretase activity. These findings indicate that berberine may directly interfere with one of the core pathological features of AD.[13]

6.Zhu et al (2011),evaluated berberine in transgenic mouse models of AD and found significant improvements in spatial learning and memory performance. The study reported reduced amyloid plaque deposition and decreased neuronal loss in the hippocampus. These findings support berberine's potential in improving cognitive dysfunction associated with AD.[14]

7.Ji and Shen et al(2011), reported that berberine exhibits inhibitory activity against acetylcholinesterase (AChE), an important therapeutic target in Alzheimer's disease.

Their findings suggested that berberine enhances cholinergic neurotransmission by preventing acetylcholine breakdown, thereby potentially improving memory and cognitive function in AD models.[15]

MOLECULAR DOCKING

Molecular docking is a computational method widely used in pharmaceutical research, structural biology, and drug discovery to predict the interaction between a small molecule and a target macromolecule.
In most cases, the small molecule is referred to as a ligand, which may be a drug, phytochemical, peptide, or bioactive compound, while the macromolecule is usually a protein receptor such as an enzyme, receptor protein, or nucleic acid.
The primary objective of molecular docking is to determine the most stable binding orientation of the ligand within the active site of the receptor and to estimate the strength of the interaction between them.
Another important concept associated with docking is the "induced fit theory," which states that both the ligand and receptor may undergo conformational changes during binding to achieve maximum stability and biological activity.[16]

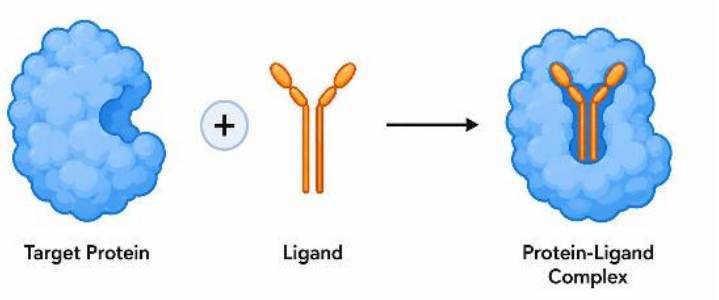


Fig 1.6 protein - ligand binding

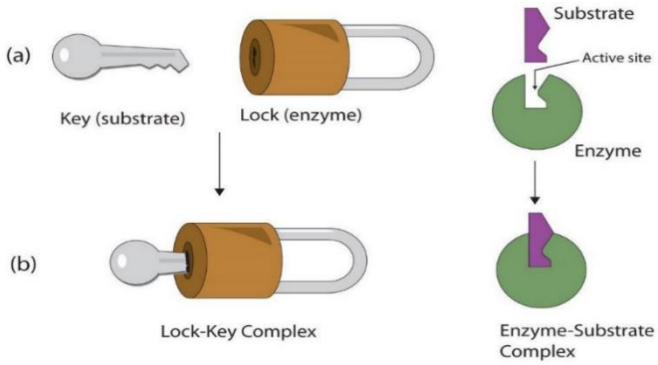


Fig 1.7 lock and key theory

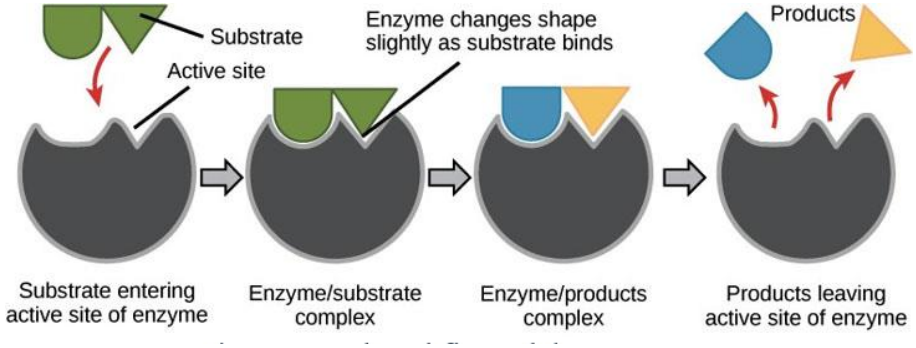


Fig 1.8 induced fit theory

TYPES OF MOLECULAR DOCKING

1. Rigid Docking

Rigid docking is the simplest and earliest form of molecular docking in which both the receptor and ligand are treated as rigid structures during the docking simulation. In this method, no conformational flexibility or bond rotation is allowed in either molecule. The ligand is docked into the active site of the receptor without changing the shape of the molecules.

It is based on the classical "lock and key" model proposed by Emil Fischer, where the receptor acts as a rigid lock and the ligand acts as a rigid key. Since molecular movement is restricted, the computational process becomes faster and requires less processing power.

2. Flexible Docking

Flexible docking is a more advanced and widely used docking approach in which the ligand is allowed to rotate, bend, and change its conformation during docking. It is based on the understanding that biomolecules are dynamic and adapt their structures during ligand binding.

Flexible docking is particularly important when studying ligands with multiple rotatable bonds or proteins with adaptable binding pockets.

3. Semi-Flexible Docking

Semi-flexible docking is an intermediate approach between rigid and flexible docking. In this method, the ligand is treated as flexible while the receptor remains rigid. The ligand can rotate around its bonds and adopt multiple conformations during docking, but the receptor structure remains unchanged.

Semi-flexible docking is one of the most commonly used docking strategies because it provides a balance between computational efficiency and prediction accuracy.[17]

Simulation-Based Approach

Simulation-based approaches in molecular docking involve the use of dynamic computational methods to mimic the physical movements and interactions of biomolecules over time. Unlike rigid or static docking methods, simulation approaches consider the flexibility and continuous motion of both ligands and receptors, making them more biologically realistic.

A major advantage of simulation-based docking is its high accuracy and realistic representation of molecular behavior. It is especially useful in studying flexible proteins, membrane proteins, enzyme mechanisms, and long-term stability of drug candidates.

Shape Complementarity Approach

The shape complementarity approach is one of the earliest and most widely used methods in molecular docking.

This approach is based on the principle that the ligand and receptor must possess complementary geometric shapes to fit together properly, similar to a "lock-and-key" mechanism.

The receptor binding pocket is represented as a surface or grid, and the ligand is rotated and translated systematically within the active site to identify the best fit.

One of the earliest and most popular docking programs using this approach is DOCK, which identifies potential ligand orientations by matching ligand atoms to spheres representing the receptor cavity.[18]

Major Steps Involved in Molecular Docking

The first step is *protein selection and preparation*, where the target protein structure is obtained from databases such as the Protein Data Bank (PDB). During preparation, water molecules, unwanted ligands, and ions are removed, while hydrogen atoms and charges are added to stabilize the protein structure.
The second step is *ligand preparation*. The ligand or drug molecule is selected from chemical databases like PubChem or DrugBank. The ligand structure is converted into a three-dimensional form, hydrogen atoms are added, and energy minimization is performed to obtain a stable conformation suitable for docking.
The next step is *active site identification*, where the binding pocket of the protein is identified using literature data or computational tools. Accurate identification of the binding site improves docking efficiency and reliability.
After identifying the active site, *grid generation* is performed. A grid box is created around the active site to define the area where the ligand can bind.
The core step is the *docking simulation*, where the ligand is placed into the binding pocket in different orientations and conformations. Docking software such as AutoDock Vina predicts the best binding pose based on interactions like hydrogen bonding, hydrophobic interactions, and electrostatic forces.
Next, *scoring function evaluation* is carried out to estimate the binding affinity between the ligand and protein. A more negative docking score usually indicates stronger and more stable binding interactions.
The generated docking poses are then analyzed in the *result analysis* step using visualization tools such as PyMOL and Discovery Studio.
Finally, *validation and post-docking studies* are performed to confirm the accuracy and stability of the docking results. Techniques such as RMSD calculation, molecular dynamics simulation, and ADMET analysis help evaluate the reliability and therapeutic potential of the ligand.

Berberine - AchE docking pose 3D

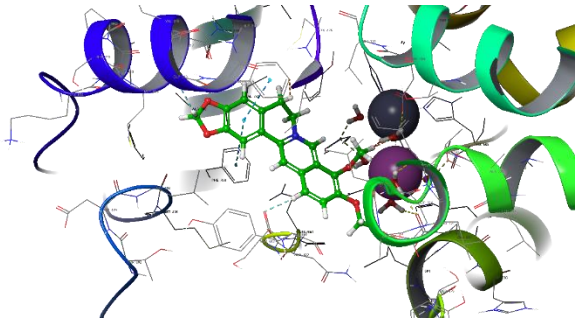


Fig 1.9

The image represents a molecular docking interaction between a ligand molecule and the active site of a target protein. The protein is shown in ribbon form with α-helices and loop regions surrounding the binding pocket, while the ligand is displayed in green stick representation at the center of the cavity.
The ligand interacts with nearby amino acid residues through hydrogen bonds and hydrophobic interactions, which help stabilize the ligand–protein complex. The dotted lines indicate hydrogen bonding between electronegative atoms of the ligand and amino acid residues of the protein.
Hydrophobic interactions between nonpolar regions of the ligand and the protein further enhance binding affinity and stability.

Galantamine – AchE docking pose 3D

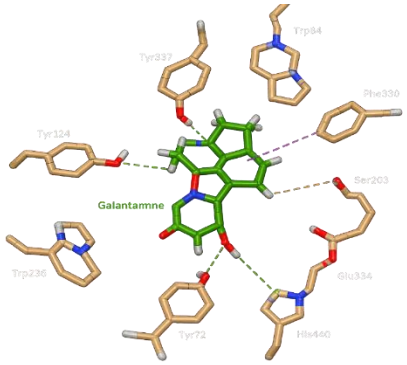


Fig 2.0

The 3D docking pose of galantamine with acetylcholinesterase (AChE) demonstrates that the ligand fits effectively within the active gorge of the enzyme through multiple stabilizing interactions. Galantamine is positioned near important amino acid residues such as Trp86, Tyr72, Phe295, Trp286, Ser203, His447, Glu202, and Phe338, which are involved in ligand recognition and catalytic activity.

Berberine – AchE docking pose 3D

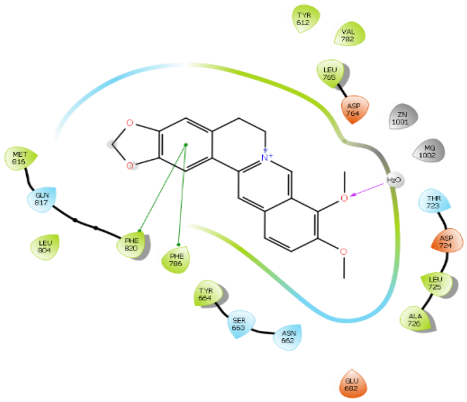


Fig 2.1

The docking analysis generally shows that berberine occupies the catalytic gorge of AChE and interacts with several important amino acid residues present in both the catalytic active site (CAS) and peripheral anionic site (PAS).

The positively charged quaternary ammonium structure of berberine facilitates strong π–π stacking and hydrophobic interactions with aromatic residues such as Trp84, Phe330, Tyr121, Tyr334, and Tyr337. These aromatic interactions are considered highly important because they stabilize the ligand inside the narrow active-site gorge of AChE.

Galantamine – AchE docking pose 2D

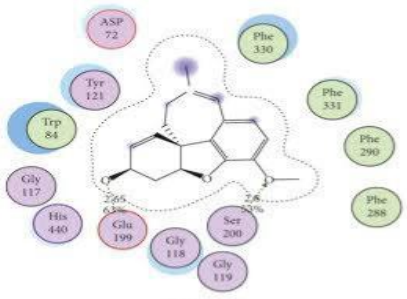


Fig 2.2

The 2D docking pose of galantamine with acetylcholinesterase (AChE) demonstrates how galantamine binds within the active site gorge of the enzyme through several important molecular interactions.

Galantamine mainly interacts with key amino acid residues such as Trp84, Phe330, His440, Ser200, and Glu199, which are involved in catalytic activity and substrate recognition. The ligand forms hydrogen bonds and hydrophobic interactions that stabilize the complex within the binding pocket.

Interaction Types Comparison of Galantamine and Berberine with Acetylcholinesterase (AChE)

Interaction Type	Galantamine	Berberine
Hydrogen Bonding	Forms multiple hydrogen bonds with catalytic site residues, helping proper orientation and stability	Forms fewer hydrogen bonds compared to galantamine
Hydrophobic Interactions	Moderate hydrophobic interactions within the active gorge	Strong hydrophobic interactions due to its planar aromatic structure
π–π Stacking Interactions	Limited π–π interactions	Strong π–π stacking with aromatic residues such as Trp84 and Phe330
Electrostatic Interactions	Mild electrostatic contribution	Significant electrostatic attraction because berberine is positively charged
Binding Site Preference	Mainly binds to the catalytic active site (CAS)	Can interact with both catalytic active site (CAS) and peripheral anionic site (PAS)
Binding Stability	Stable binding through hydrogen bonding network	Stable binding mainly through aromatic and hydrophobic interactions
Inhibitory Mechanism	Competitive inhibition of AChE	Mixed-type inhibition due to interaction with multiple binding regions
Overall Binding Nature	More polar interaction profile	More aromatic and hydrophobic interaction profile

Interpretation of Docking Result

The molecular docking study of galantamine with acetylcholinesterase (AChE) showed strong binding affinity within the active site of the enzyme. Galantamine was found to fit properly inside the catalytic gorge of AChE and formed stable ligand–protein interactions.

The docking analysis revealed the presence of hydrogen bonds, hydrophobic interactions, and van der Waals interactions with important amino acid residues such as Trp86, Tyr337, Ser203. These residues are essential for catalytic activity and stabilization of the ligand inside the active pocket.The molecular docking study of berberine with acetylcholinesterase demonstrated significant interaction within the active site of the enzyme. Berberine showed stable binding orientation inside the catalytic gorge and formed important hydrophobic interactions, π–π stacking, and van der Waals interactions with amino acid residues. These interactions contributed to the stabilization of the ligand–protein complex.

Compared with galantamine, berberine formed fewer hydrogen bonds, but its planar aromatic structure enhanced hydrophobic and π–π interactions within the active site. The docking score suggested good binding affinity toward AChE, indicating its potential inhibitory activity.

CONCLUSION

The docking study confirmed that galantamine possesses strong inhibitory potential against acetylcholinesterase through stable binding interactions and favorable docking affinity. The interaction with key catalytic residues validates its therapeutic effectiveness in Alzheimer's disease treatment.

The results indicate that galantamine acts as an efficient and reliable AChE inhibitor.

The docking analysis demonstrated that berberine exhibits favorable binding interactions with acetylcholinesterase and forms a stable ligand–protein complex. Strong hydrophobic and aromatic interactions support its inhibitory potential against AChE. Although its binding affinity is slightly lower than galantamine, it still shows significant inhibitory potential.

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