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Research Article

# In-silico Studies of Potential Anti-Alzheimer Compounds from *Spondias dulcis*

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**Abstract**
Alzheimer's is a chronic neurodegenerative disease characterized by low levels of acetylcholine and the accumulation of abnormal neuritic plaques, leading to rapid memory decline and cognitive impairment. Compounds found in the *kedondong* plant (*Spondias dulcis*) have been reported to exhibit in vitro activity as acetylcholinesterase inhibitors. This study examines the potential of active compounds in Spondias dulcis in their interaction with acetylcholinesterase, an enzyme implicated in the pathogenesis of Alzheimer's disease. The enzyme was obtained from the Protein Data Bank (PDB ID: 4EY7). The test ligands were screened based on Lipinski's rule and docked with the receptor. The results of molecular docking which yielded the five best affinity energy values were followed by ADMET testing (absorption, distribution, metabolism, excretion, and toxicity). The test ligand ellagic acid deoxyhexoside showed binding energy at -11.213 kcal/mol. Molecular dynamics simulations were performed using YASARA with AMBER14 force fields for 50 ns. The test ligand ellagic acid eoxyhexoside showed an MM-PBSA value of -51.277 kcal/mol and exhibited good complex stability with an average total RMSD value of 2 Å and low inter-residue fluctuation values. These findings are consistent with the results obtained from the comparator ligand, donepezil. Therefore, compounds in *Spondias dulcis* have the potential to act as acetylcholinesterase inhibitors and can be considered for the development of therapies for Alzheimer's disease.

**Keywords**: Alzheimer, molecular docking, molecular dynamic, *Spondias dulcis*

## 1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder that causes a progressive decline in cognitive abilities, including memory, understanding, language, concentration, and reasoning. Generally, AD affects people over the age of 65[1]. According to the WHO in 2017, there are 47 million people worldwide who have AD, and 10 million new cases are found every year. The cholinergic hypothesis suggests that the cholinergic system in individuals with Alzheimer's disease (AD) is impaired. Cholinergic activity plays a crucial role in sustaining attention and memory. tudies have shown a significant loss of cholinergic neurons and a reduction in acetylcholine release in the brains of AD patients[2]. In addition, the presence of free radicals can trigger the formation of abnormal structures, namely amyloid fibrils, which are also found in the brains of Alzheimer's patients[3].

Traditional medicine is often employed in local healing practices for disease treatment due to its natural properties and minimal side effects. Using natural products for treatment is often favoured due to their ability to target multiple factors involved in disease progression through a multitarget mechanism. *Spondias dulcis*, also known as *kedondong*, is a fruit-bearing plant classified as an angiosperm belonging to the mango family or Anacardiaceae. It is widely distributed in tropical and subtropical regions, including Indonesia. The plant is known for its beneficial fruit, leaves, and bark properties[2]. According to previous studies, the ethanol extract of

the *Spondias dulcis* plant demonstrated antioxidant activity, with IC50 values of 13.687 µg/mL for the leaves, 17.609 µg/mL for the bark, and 19.109 µg/mL for the fruit pulp[4]. Additionally, *Spondias dulcis* has shown vigorous inhibitory activity against the enzyme acetylcholinesterase in vitro, with inhibition values of 9.95 ± 0.53 mg GALAE/g from the water extract, 10.33 ± 1.09 mg GALAE/g from the ethyl acetate extract, and 7.81 ± 0.62 mg GALAE/g from the methanol extract[5]. Therefore, exploring bioactive compounds from *Spondias dulcis* as potential inhibitors of acetylcholinesterase is a promising strategy in the search for alternative therapies based on natural products.

The present study aimed to employ computational approaches to identify potential inhibitors of the acetylcholinesterase enzyme, specifically focusing on the molecular interactions between ligands and receptors through molecular docking. Molecular dynamics simulations validated these interactions to observe the stability of interactions within the complex structure and the fluctuations of amino acid residues in acetylcholinesterase.

## 2. RESEARCH METHODS

### Materials

This research was conducted using a computer with a Windows 11 Professional 64-bit operating system. The software utilized includes BIOVIA Discovery Studio, Chimera, Marvin JSketch, AutoDock Tools 4, and Yet Another Scientific Artificial Reality Application (YASARA) Dynamic with license code 349861572. All test ligand materials use bioactive compounds from the leaf and stem bark of Spondias dulcis that were identified in the previous study [5–8], and acetylcholinesterase as the receptor

### Preparation of Receptor Structure

The acetylcholinesterase enzyme is the receptor of choice in this study. The 3D structure of acetylcholinesterase with PDB ID 4EY7 was downloaded from the RSCB PDB (https://www.rcsb.org/) in .pdb[9] format. Then the structure from chain A complex was prepared using Chimera by removing water molecules, adding polar hydrogen, and separating it from the cocrystallized ligand. Subsequently, charges were assigned to the structure and it was saved in .pdbqt format.

### Preparation of Ligands

The ligand's 2D structure was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in *.sdf* format. All ligand tests were prepared before screening by protonation and energy minimization using Marvin Sketch, and then the ligands were saved in *.pdb* format were used for further analysis.

### Lipinski's Rule of Five

To be considered a potential drug, ligands must adhere to Lipinski's rule of five, which assesses whether a test ligand can function as an active oral drug. This evaluation uses the SwissADME webserver (http://www.swissadme.ch/) by inputting the canonical smile of the ligand and examining parameters including molecular weight < 500 g/mol, hydrogen acceptors < 10, hydrogen donors < 5, Log P value < 5, and molecular refractivity within the range of 41-131. A ligand passes Lipinski's rule if it meets these parameters with no more than two exceptions[10]. Those that pass this test are then subjected to docking studies

### Validation of Docking Method

The docking method was validated using PyRx 0.8 with the AutoDock Vina system. The conformations obtained from redocking were overlaid with the original ligand conformations from crystallography, measured by the Root Mean Square Deviation (RMSD) values, which are acceptable if < 2 Å[11]. The grid box settings were used to determine the space for ligand binding to the receptor during docking, which was determined based on the position of the cocrystallized ligand already bound to the protein macromolecule when the complex was downloaded.

### Molecular Docking and Visualization

In this stage, each prepared ligand was docked with the acetylcholinesterase protein using AutoDock Tools and the Webinar web server, which employs the same algorithm as AutoDock Vina. The simulation results provided ligand conformations ranked based on their binding energy values, from lowest to highest. The top five ligands with the lowest binding energy values were then visualized using BIOVIA Discovery Studio to examine the interactions between the ligands and the receptor.

### Pharmacokinetics Propeties

The test ligand with the lowest binding energy value was further evaluated by predicting its pharmacokinetic properties, including absorption, distribution, metabolism, excretion, and toxicity (ADMET). This was achieved by inputting the canonical SMILES nto the admeSAR web server (http://lmmd.ecust.edu.cn/admetsar2/)[12]. The data will show positive and negative signs indicating whether or not it can occur, as well as decimal values from 0 to 1 indicating the percentage likelihood or unlikelihood of occurrence.

### Molecular Dynamic

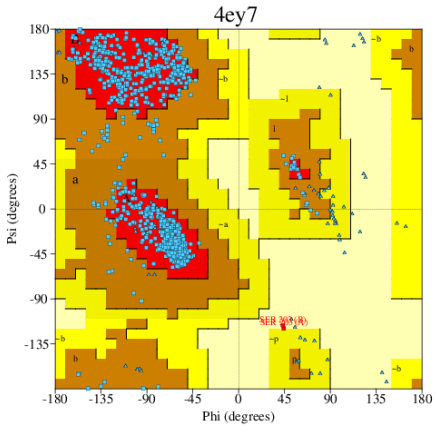
The compound identified as a potential AChE inhibitor was subjected to molecular dynamics (MD) simulations using YASARA Dynamics. The potential ligand with the best pose was complexed with the AChE enzyme using Chimera software, and donepezil was used as a comparison ligand during the simulation. he YASARA program consolidates the preparation, minimization, equilibration, and production processes into a single parameter file, known as md_runfast.mcr, to define parameters such as the AMBER14 force field, a temperature of 310K, a pH of 7.4, a NaCl concentration of 0.9%, and a pressure of 1 atm. In this research, the length of the MD run was 50,000 ps (50 ns), and snapshots were taken every 25 ps. After production, the analysis step was run using three-parameter files: md_analyze to obtain root mean square deviation (RMSD) analysis, md_analyzeres to obtain root mean square fluctuation (RMSF) analysis, and md_analyzebindingenergy to obtain molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) analysis.

## 3. RESULTS AND DISCUSSION

### Selection of Structure and Active Site

The receptor used in this study is the three-dimensional structure of the acetylcholinesterase enzyme protein code 4EY7)[9]. This structure represents the tertiary structure of the human acetylcholinesterase enzyme complexed with donepezil as a co-crystallized ligand. Based on the Ramachandran plot analysis shown in **Figure 1**, the receptor has a resolution of 2.35 Å and a percentage of non-glycine residues in the most favoured regions of 90.8%, which indicates that the protein structure is of good quality[13].

Before docking the ligand to the receptor, the molecular docking protocol was validated by redocking the co-crystallized ligand, donepezil, into the active site of AchE. It was found that the redocked conformation of the ligand perfectly overlapped with the co-crystallized ligand shown in **Figure 2**, with an MSD of 0.197 Å; the results indicate that the PyMol program accurately repositions the donepezil ligand within the active site of AChE. A docking protocol is acceptable when the RMSD value between the docking pose and the crystallographic ligand pose is less than 2.0 Å[14].




**Ramachandran Plot Statistic**

| | | No. of residues | %-tage |
|---|---|---|---|
| Most favoured regions | [A,B,L] | 786 | 90.8% |
| Additional allowed regions | [a,b,l,p] | 78 | 9.0% |
| Generously allowed regions | [~a,~b,~l,~p] | 0 | 0.0% |
| Disallowed regions | [XX] | 2 | 0.2%* |
| Non-glycine and non-proline residues | | 866 | 100.0% |
| End-residues (excl. Gly and Pro) | | 7 | |
| Glycine residues | | 100 | |
| Proline residues | | 92 | |
| Total number of residues | | 1065 | |

**Figure 1.** Ramachandran Plot

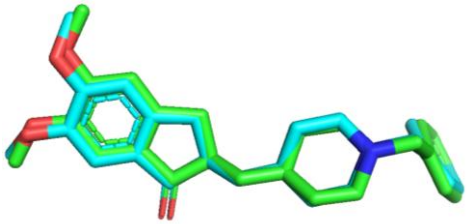


**Figure 2.** Validation of molecular docking protocols using PyMol program fot the crystal structure of acetylcholinestrease (AChE) in complex with donepezil inhibitor. Green is the co-crystal ligand, meanwhile blue is the redocking pose

### Lipinski's Rule of Five

To determine the potential of a chemical compound as an active oral drug, it must have good absorption properties to be effectively taken up by the body. Evaluating the physicochemical properties is a crucial step in predicting the drug's absorption rate, which generally occurs in the gastrointestinal tract through passive diffusion[15]. This process requires the drug to pass through the separating membrane, which can be traversed only by molecules with suitable parameters, as predicted by Lipinski's Rule of Five.

Lipinski's Rule of Five is a widely used guideline for drug discovery that predicts the biological activity of orally administered molecules based on specific chemical and physical properties. Important physicochemical properties to analyze include a molecular weight of less than 500 Da, a logP value between -0.4 and 5, fewer than 5 hydrogen bond donors, fewer than 10 hydrogen bond acceptors, and a molar refractivity of less than 130. The compound is considered capable of penetrating the gastrointestinal and cell membranes and reaching the target enzyme effectively if it meets these criteria with no more than two exception[10].

The molecular weight parameter determines the diffusion ability of a compound; compounds with smaller molecular weights more easily penetrate cell membranes[16]. The logP value indicates the lipophilicity of a compound; a high logP value shows that the compound is easily soluble in organic solvents and hydrophobic, while a low logP value shows that the compound is hydrophilic and difficult to penetrate cell membranes[17]. The number of hydrogen bond donors and acceptors affects the energy required for absorption; more hydrogen bonds increase the energy needed[18]. Molar refractivity (RM) indicates the steric properties of a compound that influence drug interactions with receptors[19]. In this study, we found that geraniin and rutin did not pass Lipinski's rule of 5 because they did not meet more than 2 of the 5 criteria; thus, they were not considered for further analysis.

**Table 1. Result of Lipinski's rule of five test**

| No | Compound | MW (gram/mol) <500 | H Donor <5 | H acceptor <10 | log P <5 | RM 4-131 | *Lipinski Rule of five* |
|---|---|---|---|---|---|---|---|
| 1 | *Galic acid* | 424.45 | 1 | 5 | 4.88 | 119,15 | Yes |
| 2 | *Galic acid hexoside* | 332.26 | 7 | 10 | -1,41 | 71,44 | Yes |
| 3 | *Methylgallate* | 184.15 | 3 | 5 | 0,57 | 43,79 | Yes |
| 4 | *brevifolin carboxylic acid* | 292.20 | 4 | 7 | 0.04 | 68,18 | Yes |
| 5 | *Geraniin* | 952.65 | 14 | 27 | -1,70 | 208,10 | No |
| 6 | *Valoneic acid-dilactone* | 470.30 | 7 | 12 | 1,06 | 112,83 | Yes |
| 7 | *Brevifolin* | 196.20 | 1 | 4 | 1,45 | 51,64 | Yes |
| 8 | *Methyl brevifolin carboxylate* | 306,23 | 3 | 8 | 0,43 | 72,50 | Yes |
| 9 | *Ellagic acid deoxyhexoside* | 448.34 | 6 | 12 | -0,19 | 106,27 | Yes |
| 10 | *Ellagic acid* | 302.19 | 4 | 8 | 1,00 | 75,31 | Yes |
| 11 | *trimethylellagic acid* | 344.28 | 1 | 8 | 2,16 | 88,72 | Yes |
| 12 | *syringic acid* | 198.17 | 2 | 4 | 0,99 | 48,41 | Yes |
| 13 | *salicylic acid*[1] | 138.12 | 2 | 2 | 1,24 | 35,42 | Yes |
| 14 | *Rutin* | 610.52 | 10 | 16 | -1,29 | 141,38 | No |
| 15 | *Quercetin* | 304,24 | 5 | 7 | 1,23 | 78,03 | Yes |
| 16 | *kaempferol 3-O-glucoside* | 448,38 | 7 | 11 | -0,25 | 108,13 | Yes |
| 17 | *quercitrin (quercetin 3-O-rhamnoside)* | 448,38 | 7 | 11 | 0,16 | 109 | Yes |
| 18 | *Myricetin* | 318,24 | 6 | 8 | 0,79 | 80,06 | Yes |
| 19 | *[3-hydroxy-3-methylbut-1-enyl]-5,7-dimethoxy-2-phenyl-2,3-dihidrokromen-4-on* | 368,43 | 1 | 5 | 4,51 | 107,54 | Yes |
| 20 | *Scopoletin* | 192.17 | 1 | 4 | 1,52 | 51 | Yes |
| 21 | *catechin* | 290,27 | 5 | 6 | 0,85 | 74,33 | Yes |
| 22 | *Luteolin* | 286,24 | 4 | 6 | 1,73 | 111,13 | Yes |
| 23 | *naringenin 7-O-glucoside (prunin)* [*1] | 434,40 | 6 | 10 | 0,23 | 103,69 | Yes |
| 24 | *(epi)catechin-gallate* | 442,38 | 7 | 10 | 1,25 | 110,04 | Yes |
| 25 | *Naringenenin* | 272,26 | 3 | 5 | 1,84 | 71,57 | Yes |
| 26 | *Pinoresinol* | 353,39 | 2 | 6 | 2,26 | 94,90 | Yes |
| 27 | *Salsolinol* | 179,22 | 3 | 3 | 1,03 | 54,64 | Yes |
| 28 | *Morphine* | 285,34 | 2 | 4 | 1,47 | 82,27 | Yes |
| 29 | *Ajmalin* | 326,44 | 2 | 4 | 1,53 | 99,87 | Yes |
| 30 | *Scoparon* | 206,20 | 0 | 4 | 2,42 | 55,47 | Yes |
| 31 | *3-methyl-6-[[2-(2-methylbut-3-en-2-yl)-1H-indole-3-yl] methylidene] piperazin-2,5-dion* | 351,49 | 3 | 2 | 4,81 | 111,77 | Yes |
| 32 | *4-hydroxy-3-(3-methylbut-2-enyl)phenyl]ethanone* | 204,27 | 1 | 3 | 2,92 | 62,38 | Yes |

**Table 2.** Binding energy values for the docked complexes, as obtained from AutoDock Vina.

| Compound | Binding Affinity (kcal mol$^{-1}$) | Compound | Binding Affinity (kcal mol$^{-1}$) |
|---|---|---|---|
| Donepezil (control ligand) | -12,451 | trimethylellagic acid | -9.452 |
| Epicatechin gallate | -12,041 | [3-hydroxy-3-methylbut-1-enyl]-5,7-dimethoxy-2-phenyl-2,3-dihydrochromen-4-on | -8,876 |
| Ellagic acid deoxyhexoside | -11,213 | 4-hydroxy-3-(3-methylbut-2-enyl)phenyl]ethanone) | -8,692 |
| Valoneic acid dilactone | -10,489 | quercitrin (quercetin 3-O-rhamnoside) | -8,545 |
| Luteolin | -10,337 | Gallic acid hexoside | -8,527 |
| Naringenin | -10,324 | Scoparon | -7,989 |
| Catechin | -10,312 | Scopoletin | -7,603 |
| Morphine | -10,271 | Salsolinol | -7,436 |
| 3-methyl-6-[[2-(2-methylbut-3-en-2-yl)-1H-indole-3-yl] methylidene] piperazin-2,5-dion | -10,269 | Ellagic acid | -7,324 |
| Methyl brevifolin carboxylate | -10,205 | Brefivolin | -6,916 |
| Kaempferol 3-O glucoside | -10,177 | Methylgallate | -6,789 |
| Myricertin | -10,024 | Salysilic acid | -6,737 |
| Brevifolin carboxylic acid | -9,908 | Syringic acid | -6,61 |
| Pinoresinol | -9,876 | Gallic acid | -6,53 |
| Ajmalin | -9,862 | Prunin | -6,093 |
| *Quercetin* | -9.573 | | |

**Molecular Docking and Visual Analysis**

Docking was initially performed using Webina AutoDock Vina, which was run on the Webina webserver. Donepezil, a co-crystallographic ligand bound in AchE structure, was regarded as the control ligand, so that the test ligands with stronger binding than or close to the binding of donepezil can be considered as potential ligands for development. The thirty compounds were docked using the grid box parameters obtained during the docking validation stage. The grid box, with dimensions of 20 Å x 20 Å x 20 Å and spacing of 0.375 Å, is centered on the enzyme's active site (coordinates: x = -13.88; y = -43.906; z = 27.108). The binding energy values for the ligands are tabulated in **Table 2**, showing varied results; the control ligand donepezil, has a value of -12.44 kcal/mol.

We observed that none of the test ligands exhibited stronger binding energy than donepezil, the control ligand. However, the top five test ligands approached the binding energy value of the reference ligand, with epicatechin gallate at -12.041 kcal/mol, ellagic acid deoxyhexoside at -11.213 kcal/mol, valoneic acid dilactone at -10.489 kcal/mol, luteolin at -10.337 kcal/mol, and naringenin at -10.324 kcal/mol. The stronger the binding energy of a ligand to the receptor, the more negative the value obtained[20]. This variation in binding energy is predicted to be due to differences in ligand binding to amino acids in the acetylcholinesterase receptor, which can determine interactions and the most stable molecular geometry. Additionally, the AutoDock-Vina scoring function is empirical, consisting of Gaussian interactions, hydrogen bonding interactions, hydrophobic interactions, and covalent bonds during the measurement[21].

We continued the molecular docking process with visualization of the top five ligands with the highest binding affinity to observe interactions with the receptor in BIOVIA Discovery Studio. The interaction results showed that the control ligand, donepezil, was stabilized by hydrophobic contacts with several amino acid residues, including Tyr337, Phe338, Trp86, Trp286, and Tyr341. Hydrophobic contact occurs when nonpolar molecules associate, often through Van der Waals interactions. Additionally, donepezil forms a hydrogen bond with the ketone group of the indanone ring positioned at Phe295, as shown in **Figure 3.**

Acetylcholine, as a native substrate, interacts with several amino acids, including Ser203, His447, and Glu334. Therefore, donepezil works reversibly by binding to the enzyme's active site, preventing the enzyme from interacting with cetylcholine. Based on **Figure 3b**, the compound epicatechin gallate has the most hydrogen bonds compared to the other test ligands. Hydrogen bonds are formed between the hydroxyl (-OH) groups of the ligand and the hydroxyl

(-OH) groups of the residues Ser203, Tyr124, Tyr341, and Ser124, as well as with the carbonyl (-CO) group of the residue Trp86. These hydrogen bonds are more numerous than those of other test ligands. Additionally, the ligand undergoes pi stacking with the aromatic groups of the amino acid residues Phe338 and Trp286, forming a van der Waals interaction; this occurs because between molecules with charge and discharge are in close proximity[22]. Additionally, there are less favourable hydrogen acceptor-acceptor and donor-donor interactions with the amino acid residues Trp86 and Trp122, which can disrupt the stability of the complex.

In the compound ellagic acid deoxyhexoside, hydrogen bonds form between the ligand's carbonyl (-CO) group and the amino group of the Phe295 amino acid residue. This interaction is also present in the control ligand, donepezil. Additionally, the Tyr124 residue forms two strong and stable hydrogen bonds with the ligand's ether (-O-) and hydroxyl (-OH) groups, with a radius of less than 2.5 Å. Meanwhile, the aromatic group of the Trp86 residue forms van der Waals forces with the ligand's alkyl group, and the same aromatic group of the Tyr341 residue interacts with several aromatic groups on the ligand, resulting in pi stacking that creates van der Waals forces and organizes hydrophobic contacts, as shown in **Figure 3c**. In the ligand valoneic acid dilactone Figure **3d**, two hydrogen bond interactions form between the ligand's carbonyl (-CO) groups and the amino groups of the Phe295 amino acid residue, similar to the interaction seen in donepezil. Additionally, hydrogen bonds form between the carbonyl (-CO) groups of the Trp86 amino acid residue and the alcohol (-OH) groups of the ligand at the catalytic site.

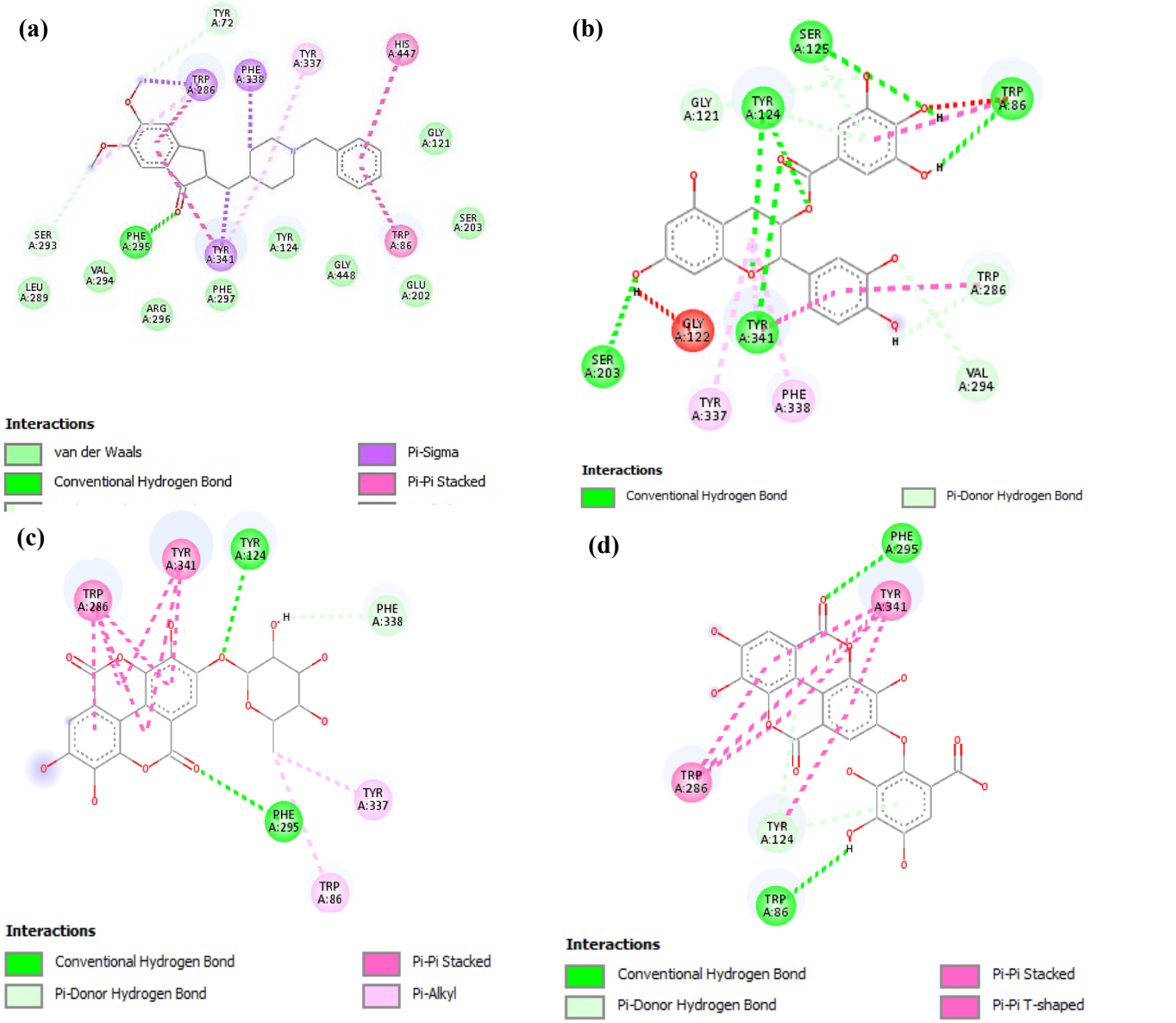



**Figure 3.** Visualization of 2D interactions between AChE with **(a)** donepezil, **(b)** epicatechin gallate, **(c)** ellagic acid deoxyhexoside **(d)** valoneic acid dilactone

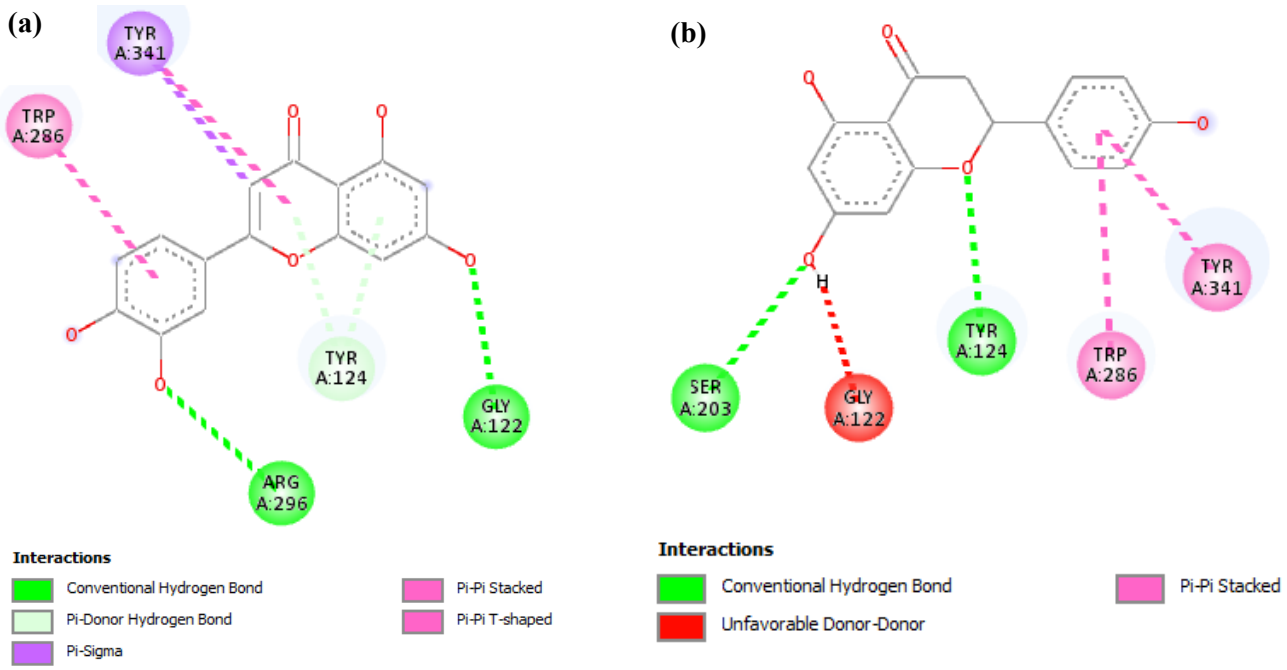



**Figure 4.** Visualization of 2D interactions between AChE with **(a)** luteolin, **(b)** naringenin

In the luteolin compound **Figure 4.a**, a hydrogen bond forms between the ligand's hydroxyl (-OH) group and the amino group of the Arg296 amino acid residue. Additionally, the naringenin compound forms hydrogen bonds between the ligand's hydroxyl (-OH) and ether (-$OCH_2$) groups and the hydroxyl groups of Ser203 and Tyr124, both of which are located in the enzyme's catalytic site **Figure 4b**. Moreover, there are donor-donor hydrogen bonds with the Gly122 amino acid residue, which are considered less favorable interactions as they can disrupt the stability of the ligand-receptor complex[23].

**Pharmacokinetic Properties**

In drug development, predicting pharmacokinetic properties is a crucial step to avoid failures and unnecessary side effects. This pharmacokinetic prediction uses the ADMET test, which consists of absorption, distribution, metabolism, excretion, and toxicity. We used the admetSAR program (http://lmmd.ecust.edu.cn/admetsar2/) to predict the pharmacokinetics of the five ligands with the strongest binding energies. The absorption prediction was tested using Human Intestinal Absorption (HIA) values, the distribution test measured the Blood-Brain Barrier (BBB), the metabolic profile was assessed for CYP inhibition, and the toxicity profile included carcinogenicity, hepatotoxicity, and LD50[12]. Predicting drug absorption in the small intestine is essential for identifying potential oral drug candidates that can enter the systemic circulation. Human Intestinal Absorption (HIA) values indicate active substance absorption in the human intestine[24,25]. Based on **Table 3**, the control ligand donepezil and the test ligands epicatechin gallate, luteolin, and naringenin show good absorption levels.

The Blood-Brain Barrier (BBB) parameter indicates a drug's ability to penetrate the brain's protective barrier, which is crucial for Alzheimer's drugs targeting the central nervous system (CNS)[25]. **Table 3** shows that all test ligands have a low potential to cross the BBB. Drugs undergo biotransformation in the liver, involving the enzyme Cytochrome P-450, especially the CYP3A4 subtype, which is found in hepatocytes and intestinal mucosal cells. Some drugs can inhibit CYP3A4, posing a risk when taken with other medications, as decreased enzyme activity prevents drug conversion into excretable forms, leading to higher body circulation and potential toxicity[26]. **Table 3** indicates that luteolin and naringenin may inhibit CYP3A4.

Toxicity predictions are crucial for drug candidate design. This study predicts hepatotoxicity, carcinogenicity, and acute oral toxicity. According to **Table 3**, donepezil, epicatechin gallate, and valenoic acid dilactone may cause liver damage, but none of the five test ligands show carcinogenic potential. Acute toxicity, indicated by LD50 (the dose lethal to 50% of test animals)[27], places the test ligands in category III, meaning they are slightly toxic. Ellagic acid deoxyhexoside was selected for molecular dynamics simulations due to its favorable absorption profile and lower toxicity compared to other test ligands.

**Table 3**. Properties of pharmacokinetics AChE inhibitor compounds

| Compound | HIA | BBB | Inhibition CYP3A4 | Carcinogenic | Hepatotoxicity | $LD_{50}$ (mg/kg) |
|---|---|---|---|---|---|---|
| Donepezil | (+) 0,9838 | (+) 0,9250 | (-) 0,7411 | (-) 0,9600 | (+) 0,8677 | 505 |
| epicatechin gallate | (+) 0.8422 | (-) 0.800 | (-) 0,7662 | (-) 1.000 | (+) 0.6250 | 1000 |
| ellagic acid deoxyhexoside | (+) 0.6842 | (-) 0.8774 | (-) 0,8636 | (-) 0.9900 | (-) 0.9900 | 5000 |
| valoneic acid-dilactone | (+) 0.6378 | (-) 0.8000 | (-) 0,7876 | (-) 1.000 | (+) 0.6125 | 1000 |
| Luteolin | (+) 0.9071 | (-) 0.7750 | (+) 0,6951 | (-) 1.000 | (-) 0.5125 | 3919 |
| Naringenin | (+) 0.9450 | (-) 0.7750 | (+) 0,8988 | (-) 0.9600 | (-) 0.7500 | 2000 |

**Molecular Dynamic Simulation of Acetylcholinestrease Complex Structure**

The Molecular Dynamic Dynamics Simulation (MD) method can validate the stability of the complex structure's interaction stability by demonstrating the structural conformation's stability, the flexibility of the amino acid residues affected by docking, and estimating the free energy changes associated with the molecular system. The molecules subjected to molecular dynamics include the enzyme complex with the control ligand, acetylcholinesterase-donepezil (AChED), and the enzyme with the test ligand, acetylcholinesterase-ellagic acid deoxyhexoside (AChEE).

The interaction between natural compound structures and acetylcholinesterase can alter the enzyme's mechanism, impacting its catalytic activity. High binding affinity restricts the movement of the bound atom and stabilizes the catalytic site of the acetylcholinesterase macromolecule [28]. RMSD (Root Mean Square Deviation) values can analyze this effect quantitatively. If the RMSD values are ≤ 2Å for 5 ns within the initial 10 ns of the production run, the complex is considered stable[29]. Dynamic stability can be observed when the complex is in equilibrium, where no significant fluctuations occur, and a simulation time of 50 ns is sufficient to observe the convergence of both complexes. Based on the RMSD trajectory in **Figure 5** for the AChED complex, the RMSD value is around 1.8 Å, with the highest deviation value being 2.326 Å at 8 ns of simulation time. Meanwhile, the RMSD value for the AChEE complex is approximately 2 Å, with the highest deviation value of 2.39 Å occurring at 38 ns of simulation time. The stabilization of the RMSD value indicates that the maximum conformation of the protein bound to the ligand has been achieved, allowing the protein to maintain its position[30].

When a ligand binds to a receptor, it induces conformational changes and alters the thermal stability of the protein, which is necessary to produce biological [31]. In **Figure 6a**, the ligand donepezil binds to the enzyme's catalytic site, where the ligand does not significantly alter the enzyme's conformation, and no unfolding events occur that could reduce the therapeutic effect produced by the ligand. Instead, only stable dynamic movements occur due to the positioning effect of donepezil. These changes also occur in the AChEE complex, as shown in **Figure 6b**.

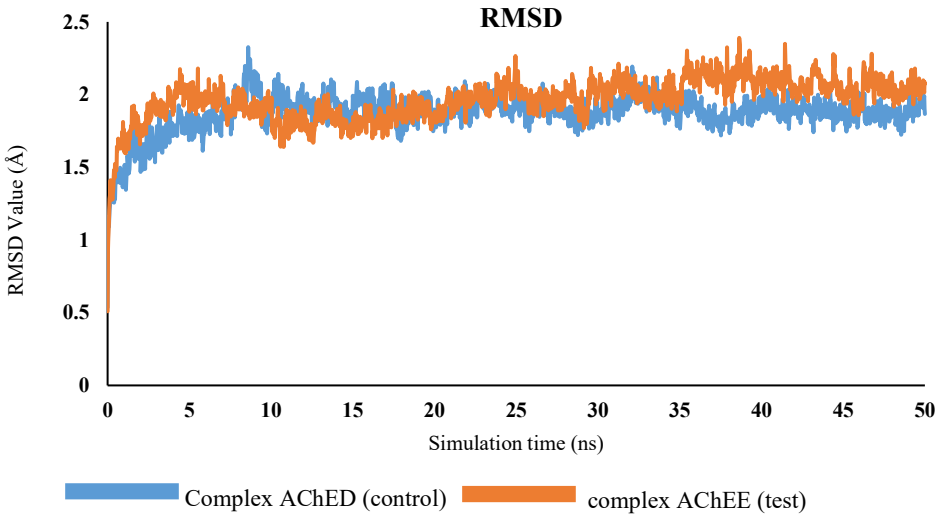



**Figure 5.** RMSD values of AChED and AChED complexes

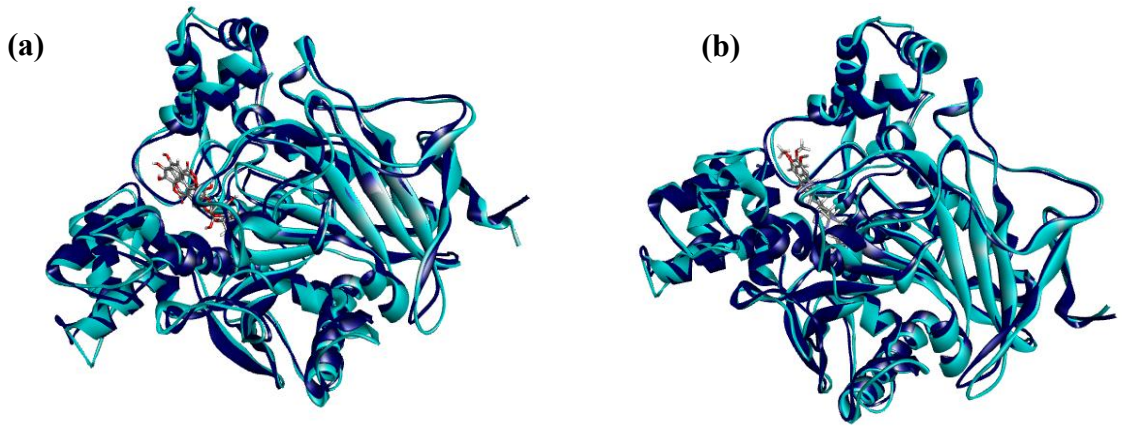



**Figure 6**. Superimpose (a) AChED complex, (b) AChEE complex before (dark blue) and after (light blue).

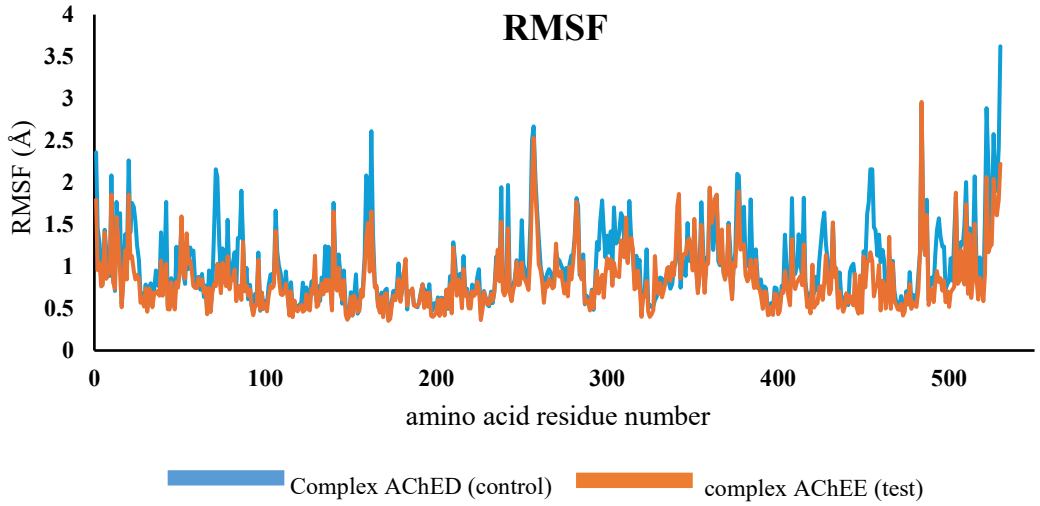



**Figure 7.** RMSF values of AChED and AChED complexes

Root Mean Square Fluctuation (RMSF) measures the deviation between a particle's position and its initial position. Unlike RMSD, RMSF is calculated for each residue that makes up a protein by measuring the movement of the residues during the simulation. A low RMSF value indicates low flexibility of a residue and high stability. The RMSF values for the AChEE complex in **Figure 7** show a similar pattern to the reference complex AChED because the ellagic acid deoxy hexoside ligand interacts with identical residues as the control ligand donepezil.

The RMSF values in **Figure 7** depict the fluctuations of amino acid residues in the complex structure during the simulation. Amino acid residues in the protein's active site and those that form bonds with the ligand exhibit lower RMSF values compared to amino acid residues that do not interact with the ligand.

**Figure 8** shows that residues Trp286, Tyr341, Tyr337, and Trp86, which form hydrophobic contacts with the ligand ellagic acid deoxy hexoside, exhibit lower RMSF values, indicating their stability. Notably, Tyr337 has a higher RMSF value of 1.12 Å in the active site area. Similarly, residues Tyr124 and Phe295, which form hydrogen bonds with the ligand, also display lower RMSF values, underscoring their role in maintaining the structural integrity of the protein-ligand complex. It is observed that the RMSF values for the AChED and AChEE complexes show a slight difference and do not exhibit significant fluctuations, ranging from 0.39 to 0.85 Å. However, for the Tyr337 residue, the RMSF value reaches 1.1 Å. Nonetheless, this fluctuation is not very significant as it is still below 2Å, indicating that the increase in RMSF values only suggests bond stretching

The MM-PBSA (Molecular Mechanics/Poisson-Boltzmann Surface Area) analysis is a computational approach used to calculate the free energy of molecules involved in

Van der Waals and electrostatic interactions, along with solvation effects on the stability of a complex[33]. MM-PBSA calculations are also necessary to validate the accuracy of docking scores obtained from Autodock Vina. The MM-PBSA energy values in **Table 4** indicate that the control complex, AChED, has a value of -51.408 kJ/mol. In contrast, the test complex, AChEE, has an MM-PBSA value of -51.277 kJ/mol. These values show nearly identical results with slight differences in the potential and solvation energy components. In the context of YASARA Dynamics, a binding energy value closer to a positive direction suggests a stronger binding[34]. Therefore, AChEE exhibits slightly better binding than AChED.

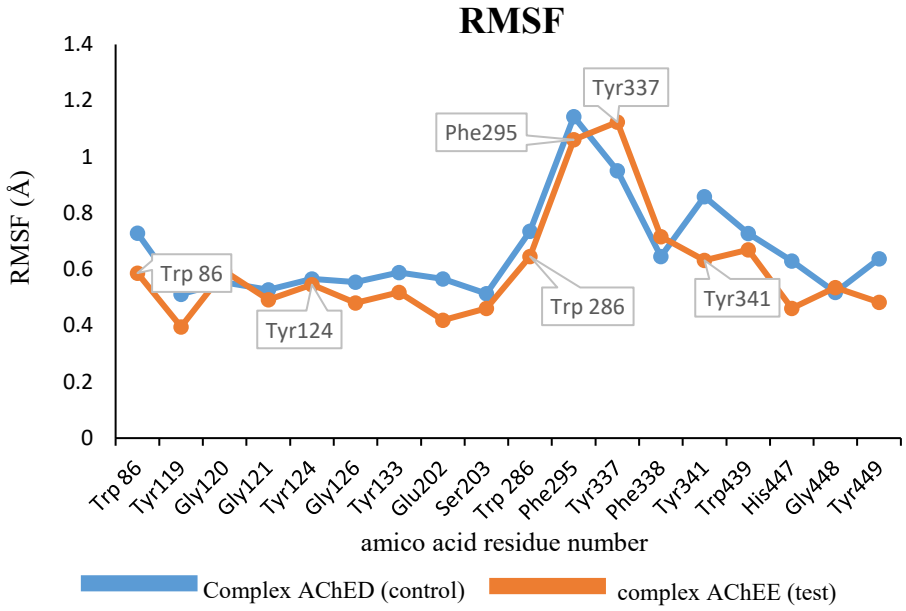



**Figure 8.** RMSF values of AChED and AChED complexes in enzym's active site

**Table 4.** Binding Energy of MMPBSA Complex AChE for 50 ns

| Energy Components (Kj/mol) | System | |
|---|---|---|
| | Acetylcholinesterase–donepezil (AChED) | Acetylcholinesterase–ellagic acid deoxy hexoside (AChEE) |
| Binding Energy | -51.408 | -51.277 |
| Receptor Potential Energy (EpotRecept) | -26.19 | -26.4 |
| Receptor Solvation Energy (EsolvRecept) | -24.93 | -24.7 |
| Ligand Potential Energy (EpotLigand) | -26.29 | -26.7 |
| Ligand Solvation Energy (EsolvLigand) | -25.13 | -24.6 |
| Complex Potential Energy (EpotComplex) | -26.19 | -26.4 |
| Complex Solvation Energy (EsolvComplex) | -24.94 | -24.7 |

## 4. CONCLUSIONS

We have demonstrated that ellagic acid deoxy hexoside from Spondias dulcis may effectively inhibit acetylcholinesterase by forming a binding energy of -11.213 kcal/mol at the enzyme's active site. Molecular dynamics simulation analysis indicates that both the MM-PBSA energy, RMSD of the complex, and RMSF of the amino acid residues suggest that the AChEE (acetylcholinesterase-ellagic acid deoxy hexoside) complex exhibits good dynamic stability. In conclusion, based on the collective findings of this study, ellagic acid deoxyhexoside holds promise as a candidate for developing effective acetylcholinesterase inhibitors. Further in vitro and in vivo experiments are necessary to validate and advance these results.

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