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Exploring the Inhibitory Potential of Podolactone B against Human Acetylcholinesterase: A Docking Study

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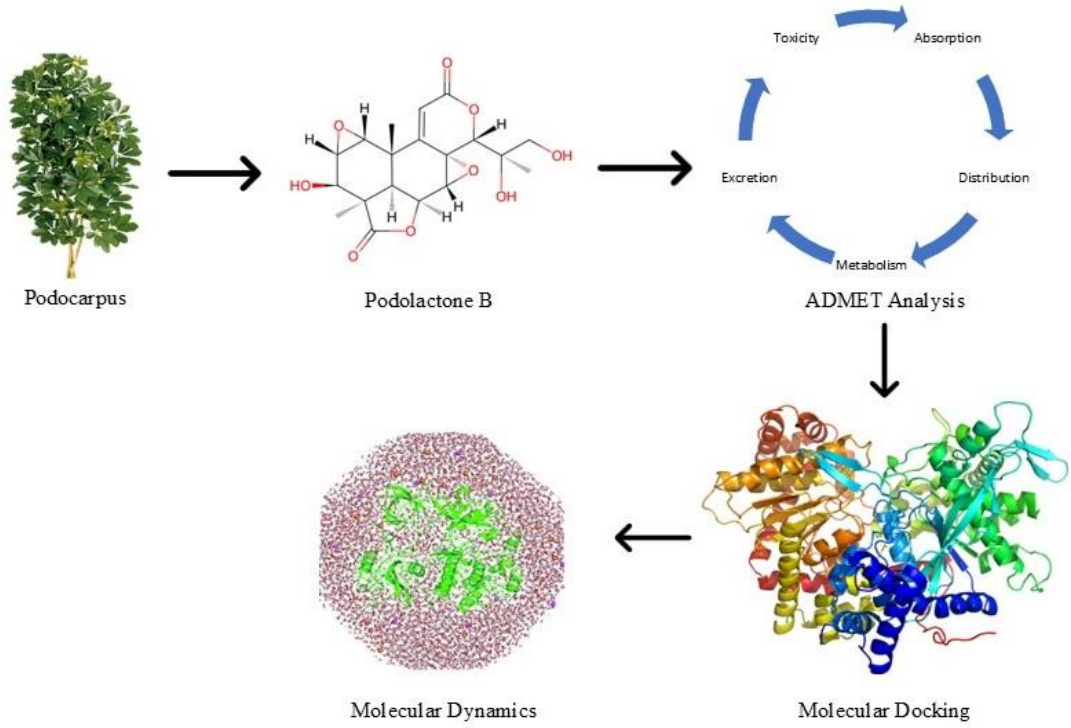
Graphic abstract:



Abstract: In this work, a novel therapeutic drug for treating Alzheimer's disease is molecularly simulated. The cholinergic hypothesis is the treatment approach used in this investigation. With a substance derived from the natural podocarpus derivative Podolactone B. The goal was to alter cholinergic activity by inhibiting Acetylcholinesterase. The study was performed In-silico Molecular docking in AutoDock Vina, performed on Galantamine and Podolactone B against the Crystal Structure of Human Acetylcholinesterase and PyMOL software was used to investigate the binding mode and interaction of the ligand with the

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receptor. Molecular dynamics in Gromacs software, the trajectory of stimulation was examined using a variety of tools, including the radius of gyration (RG), solvent accessible surface area (SASA), hydrogen bonding, protein root mean square deviation (RSMD), and root mean square fluctuation (RMSF), to study structural and dynamic properties of the simulated system, such as its overall shape flexibility and interaction with surrounding solvent. MMPBSA simulations were performed on the complex of target. To ascertain the binding affinity and the contributions of various energy terms to the total binding energy for inhibition, the resulting energy components were examined. This study shows that Podolactone B has a good binding affinity might operate as an acetylcholinesterase inhibitor.

Keywords: Podolactone B, acetylcholinesterase, Alzheimer's disease, podocarpus, docking studies, molecular dynamics

1. Introduction

The prevalence of Alzheimer's disease (AD) is on the rise due to the aging population, yet no effective treatments have been developed to delay or prevent the neurodegeneration associated with AD [1]. Existing evidence suggests that AD is influenced by a combination of environmental, genetic, epigenetic, and metabolic factors [2]. The most prevalent type of dementia, AD causes deficits in language and visuospatial abilities, and behavioral issues like aggression, apathy, and depression. Genetic factors contribute to around 70% of the risk for developing AD [3]. By 2050, there will be three times as many persons with dementia worldwide as there are now—roughly 47 million people. Effective prevention of AD requires timely diagnosis and multidisciplinary management [4]. The pathogenesis of AD involves the formation of β-amyloid (Aβ) plaque aggregates in the brain's cortical and limbic regions, along with the development of intracellular neurofibrillary tangles caused by hyper phosphorylation of τ-protein [4–6]. AD also leads to increased activity of acetylcholinesterase, which play a crucial role in the functioning of the cholinergic system. The cholinergic system is severely affected in AD-associated dementia, resulting in significant impairments and complications [7]. The cholinergic hypothesis of AD proposes that the loss of cholinergic neurons and reduced acetylcholine (ACh) levels contribute to cognitive decline. ACh plays a crucial role in memory, learning, and attention, and its deficiency impairs neurotransmission, leading to AD symptoms like memory loss. Inhibiting acetylcholinesterase (AChE), the enzyme that breaks down ACh, increases its availability and improves cholinergic function, alleviating cognitive deficits [8,9].

Due to the constant and early cholinergic impairment seen in AD, acetylcholinesterase has emerged as a possible therapeutic target for easing symptoms. Myasthenia gravis (MG) was successfully treated by inhibiting peripheral AChE, demonstrating the viability of targeting AChE for medical treatment. Inhibition of the central nervous system (CNS) is selective, but currently, galantamine, rivastigmine, donepezil, and memantine are the four medications that can be used to treat AD [10]. The first three of them are AChE inhibitors, although memantine works in a different way. AChE and butyrylcholinesterase (BChE) are the two different forms of cholinesterase enzymes. While BChE is mostly located in the liver, AChE is mainly present in the blood and brain synapses. The two enzymes' ability to hydrolyze their respective substrates—ACh in the case of AChE and butyrylcholine in the case of BChE—is the major difference between them. A synthetic substance called BCh is utilized to distinguish between AChE and BChE receptors. AChE and BChE are the targets of many AD medications, albeit some show varied degrees of selectivity [11].

In this case, the phytoconstituent derived from podocarpus, specifically podolactone B, possess antioxidant properties so it may inhibit acetylcholinesterase. Antioxidants aid in decreasing the buildup of free radicals and subsequently mitigating oxidative stress. These antioxidants can be administered as drugs or as hydrogels or nanofibers made of them by combining them with other biomaterials. The medication can be directly administered via intraperitoneal injection, intragastric method, or oral intake. Different compounds used for direct

administration have been extensively documented [12].

The biological activities of plants belonging to the Podocarpus genus have been extensively studied, revealing a diverse range of effects including plant-growth. Regulation [13,14] antibacterial activity [15] and anti-proliferative properties [16–19]. These activities are mainly attributed to specific chemical compounds known as podolactones or nagilactones [20,21] which, according to the conjugated arrangement between the B and C rings, are divided into three classes (types A-C). Podolactones of type A have the structure [8(14), 9(11)-dienolide], whereas those of types B and C have the groups [7α,8α-epoxy-9(11)-enolide] and [7(8), 9(11)-dienolide], respectively [17,20,22]. In the case of Podocarpus neriifolius, several podolactones have been identified, including the cytotoxic nagilactone C [23] and sulfur-containing derivatives such as podolactones C and D [24–26]. Furthermore, new findings from the leaves of this plant have revealed the presence of a lignan known as neriilignan and a novel cyclopeptide known as neriitide A [27]. With the aim of discovering potential lead anticancer agents from natural sources, a research effort, supported by a multidisciplinary program project grant, focused on investigating a root sample of P. neriifolius. As a result, one new B-type podolactone (2) and three known B-type podolactones (1, 3, and 4), along with three known totarane-type diterpenes (5–7), were isolated and purified. The resulting compounds' antiproliferative qualities were assessed against a panel of human cancer cell lines, including ovarian, breast, colon, and melanoma, after their structures were determined [28].

The Podocarpus derivatives have shown their potential in various areas, such as antioxidant, anti-inflammatory, antimicrobial, anticancer, and neuroprotective activities. They have also been explored for their potential in traditional medicine and as a source of natural products for drug discovery and development. The study is to assess the effectiveness of podolactone, a specific phytoconstituent, as an inhibitor of acetylcholinesterase (AChE). This evaluation will be carried out using molecular docking techniques. In the field of drug discovery, virtual screening has proven to be a successful strategy. In a study conducted by Iman et al. published in CNS & Neurological Disorders - Drug Targets (2018, Vol. 17, No. 1), *in silico* approaches have been instrumental in addressing various biological challenges [29–34] and identifying new inhibitors for neurological disorders [35]. This process enables the search for compounds possessing specific key features that could serve as potential leads. By applying Lipinski's rule of five [36], the pharmacological activity of drugs can be assessed. Molecular docking techniques are employed to determine the most favorable orientations and stable ligand-protein complexes. Building upon these principles, the objective of the current study was to employ virtual screening to identify potential inhibitors of acetylcholinesterase. Following an initial screening by Lipinski's rule of five, compounds from the protein database underwent further filtration according to pharmacokinetic parameters. The substances' capacity to cross the blood-brain barrier (BBB), Caco2 cell permeability, human intestinal absorption (HIA), and plasma protein binding were also assessed. Promising leads with potential pharmacological activity were subjected to absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis through molecular docking [37]. The results indicated that four screened compounds exhibited efficacy as AChE inhibitors, suggesting their potential utility in the treatment of Alzheimer's disease (AD). Subsequently, compounds demonstrating the most favorable drug-like properties can be synthesized for further investigation.

In this study Galantamine is used as a standard (reference) drug due to its established role as a clinically approved acetylcholinesterase inhibitor for AD. Its well-documented pharmacological profile provides a reliable benchmark to evaluate Podolactone B’s binding efficacy and therapeutic potential [38]. This ensures relevance, clinical applicability, and comparability in the study. The primary aim of this research is to investigate whether Podolactone B has the capability to act as an inhibitor of acetylcholinesterase (AChE), which is a target enzyme associated with Alzheimer's disease through molecular docking and dynamics technique.

2. Computational Method

2.1. Docking simulation software

Using the AutoDock Vina-1.2.0 in silico screening tool, molecular docking simulations were performed to evaluate the potential binding affinity between the lead molecules under investigation and the enzyme target AChE (crystal structure of human acetylcholinesterase) with protein data bank (PDB) code 4PQE.

2.2. Molecular docking

For this study, molecular docking was performed on galantamine and podolactone B against the acetylcholinesterase PDB ID 4PQE. First, the 3D structures of the Galantamine and Podolactone B were obtained from the Pubchem website and prepared for docking using the Open Babel software. The receptor, Crystal Structure of Human Acetylcholinesterase PDB ID 4PQE was also prepared for docking. The search space and grid box dimensions were configured to 70 x 78 x 60 with centre x = -27.328, centre y = 24.390, and centre z = -0.467 in AutoDock Vina. The docking runs were then started for the docking protocol. The binding affinities of the resultant docking positions were examined, and the best poses were chosen for additional examination. PyMOL software was used to analyse the ligand-receptor interactions and binding mode [39–41].

2.3. In silico physicochemical properties

The physicochemical parameters were evaluated using the SwissADME website [42,43]. Podolactone B and Galantamine in this study had their physicochemical properties, includes the amount of rotatable bonds, H-bond acceptors, H-bond donors, lipophilicity, and topological polar surface area (TPSA), all of which were confirmed. The drug-likeness profile was verified using both Lipinski and Veber's standards.

2.4. In silico ADMET evaluation

The pharmacokinetics of small compounds is assessed using the pKCSM accessible web server by examining ADMET (absorption, distribution, metabolism, excretion, and toxicity) parameters [43,44]. Intestinal absorption is a factor in the absorption rate. The blood-brain barrier's (logBB) permeability is used to determine the brain's distribution. Metabolic potential is predicted by the CYP inhibition model (CYP2C9 and CYP3A4). Excretion is anticipated based on the renal OCT2 substrate and total clearance. Medication toxicity can be predicted by hepatotoxicity.

2.5. Molecular dynamics simulation

A computational method called molecular dynamics (MD) simulation makes use of Newton's equations of motion to examine how atoms move within molecules. In this case, the simulation was performed using the Gromacs software package, which is widely used and well-established MD simulation software [45,46]. The protein-ligand complex was minimised in vacuum as the initial stage of the simulation procedure. In order to reduce the system's potential energy, the atomic coordinates of the complex are iteratively adjusted using the steepest descent algorithm. Using the SPC water model, the compound was solvated in a periodic box of water following minimisation. The SPC water model is a basic model that uses a single point charge to represent water molecules. It is frequently used as a foundation for more intricate water models. Additionally, the complex was kept at a salt concentration of 0.15 M by appropriately doping it with sodium and chloride ions. After undergoing an NPT (constant pressure, constant temperature) equilibration phase, the resultant complex was run through a production run in the NPT ensemble for 100 ns (nanoseconds). Systems under constant temperature and pressure, which are frequently seen in biological systems, are simulated using the NPT ensemble. The GROMOS 54a7 force field was utilized in the simulation. This force field was chosen for its reliability and widespread application in simulating protein-ligand interactions. Lastly, the Gromacs software package's tools—which include the protein root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (RG), solvent accessible surface area (SASA), and hydrogen bonding (H-Bond) were used to analyse the simulation's trajectory. These analyses allow researchers to study various structural and dynamic properties of the simulated system, such as its overall shape, flexibility, and interactions with the surrounding solvent.

2.6. Binding free energy calculation (MM/PBSA) approach

For this study, Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) calculations

were performed on the complex of the target. The computation was done using the final 50 ns of each complex's Gromacs trajectory. Initially, using the Gromacs program, the complicated structures were ready for computation. This included creating topology files and adding an explicit solvent. The g_MMPBSA program was used to set up the MMPBSA calculation, and during the final 50 ns of the trajectory, the energy decomposition was carried out on each complex. To ascertain the binding affinity and the contributions of various energy terms to the total binding energy, the resulting energy components were examined. [47].

Table 1. Docking score and type of interaction of galantamine and podolactone B against Human Acetylcholinesterase.

Compound Name	Binding Affinity kcal/mol	Hydrogen-forming interaction
Galantamine	-8.0	A:TYR337, A:TRP86, A:TRP439, A:TYR124, A:ASP74, A:THR83, A:ASN87
Podolactone B	-6.5	A:THR83, A:GLY121, A:TYR124, A:SER125, A:TYR337, A:HIS447, A:TRP86

3. Results and discussion

3.1. Molecular docking analysis

In this study, galantamine and podolactone B were evaluated for their ability to bind to the crystal structure of human acetylcholinesterase PDB ID 4PQE. Molecular docking, a computer method, was used to analyse the binding patterns of these chemicals. AutoDock Vina software was used as the docking program. The protein complex was previously characterized and its structure was recorded in the Protein Data Bank (PDB) with the identification number 4PQE. Docking scores of the galantamine and podolactone B compounds against the test proteins are tabulated in Tables 1.

3.2. In silico physiochemical properties

According to reports, 95% of potential drug molecules fail during the development stages of the drug discovery process, and 50% of these failures are attributable to unfavorable physicochemical and ADMET qualities. Test compounds should be sorted based on their drug-likeness characteristics to prevent this failure. The concept of drug-likeness is helpful in virtual screening to choose the right chemical candidates and to prevent drug development failure. Thus, to estimate the physicochemical properties for this investigation, we used the SwissADME website. The outcomes are shown in Table 2.

Galantamine and Podolactone B both demonstrated no deviation from Lipinski's rules. Galantamine and Podolactone B both had LogP values of 1.91 and -0.45, respectively. It is deemed acceptable for the molecular weights, number of hydrogen bond donors, and number of hydrogen bond acceptors to all stay within the range of fewer than 500, 10, and 5, respectively [48]. Additionally, with numbers of rotatable bonds and total polar surface area (TPSA) values within the range for oral availability [49]. As a result, Podolactone B demonstrated positive drug-likeness, indicating that it may make for a promising medication candidate.

3.3. In silico ADMET properties

pkCSM was used to evaluate the ADMET criteria [44] and are shown in Table 3. Galantamine and podolactone B, both showed human intestinal absorption at rates of 94.99% and 59.77%, respectively, according to in silico study of ADMET.

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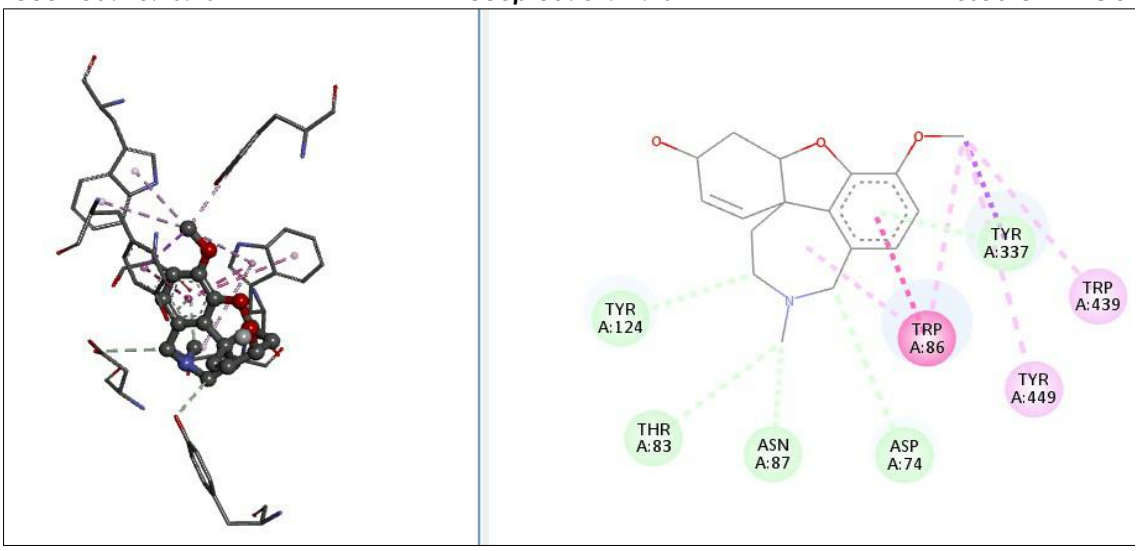

Figure 1. Molecular docking simulation of galantamine.

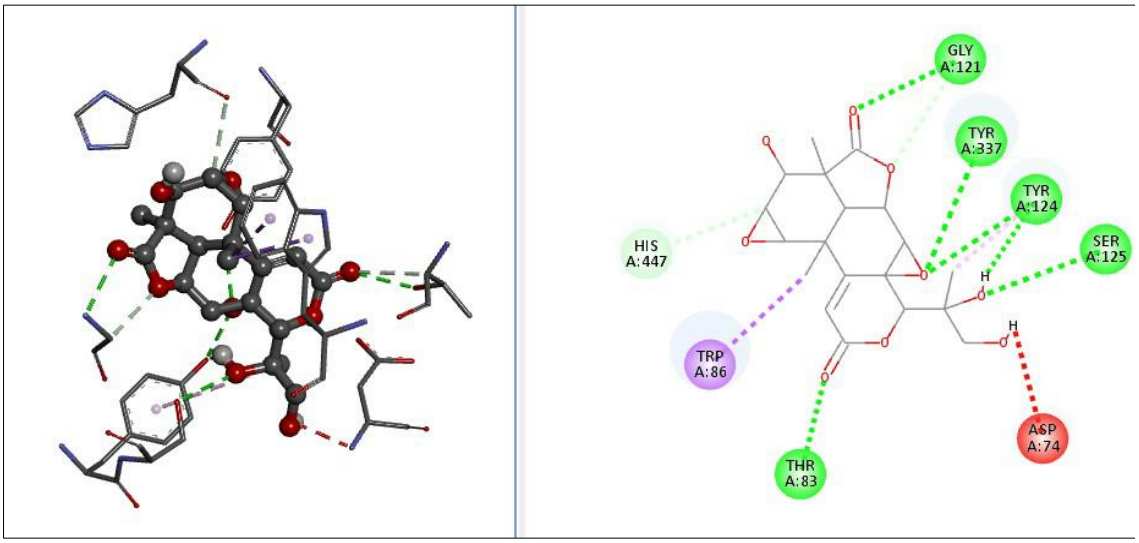

Figure 2. Molecular docking simulation of podolactone B.

Table 2. Physicochemical properties predicted by *in silico* analysis (SwissADME program).

Compound Name	Physiochemical Properties							
	MW (g/mol)	TPSA [a] (Å²)	nRB	nHBA	nHBD	Log Po/w	GI absorption	Rule of Five (violations)
Galantamine	287.35	41.93	1	4	1	1.91	High	0
Podolactone B	394.37	138.35	2	9	3	-0.45	Low	0

Table 3. ADMET parameters predicted by in silico analysis (pKCSM program).

	Absorption	Distribution	Metabolism	Excretion	Toxicity

Compound Name	Intestinal Absorption (Human) (% Absorbed)	BBB Permeability (Log BB)	CYP2C9	CYP3A4	Total Clearance (Log ml/min/kg)	Renal OCT2 Substrate	Hepatotoxicity
Galantamine	94.99	-0.081	No	No	0.991	Yes	Yes
Podolactone B	59.77	-0.922	No	No	0.566	No	No

Lower absorption of podolactone B was observed, which may be a direct result of elevated TPSA because elevated TPSA reduces membrane permeability [50]. Blood brain barrier (BBB) permeability (logBB) was used to determine how both substances were distributed throughout the brain. Polar chemicals cannot enter the brain because of the BBB, which shields it from damage. It is interesting to note that the tested podolactone B can cross the BBB because the log BB value is greater than -1. This could be explained by the fact that podolactone abides by the TPSA value Veber rule of less than 140.

The metabolism, excretion and toxicity parameters of galantamine and podolactone B were also examined. The body contains a superfamily of crucial detoxifying enzymes called cytochrome P450, which is mainly found in the liver and small intestine. They participate in the oxidation of xenobiotics and their removal. P450 3A4 and P450 2C9 are the two P450 drug metabolizing enzymes that are most frequently found in the liver and small intestine [51]. Galantamine and podolactone B did not inhibit the CYP2C9 and CYP3A4 isoforms of cytochrome P450 as shown in Table 3. Thus, podolactone B might not have an impact on the CYP450 enzyme's activity, which means it is unlikely to have an impact on the CYP2C9 and CYP3A4 drug substrates' metabolism and clearance.

The kidney is crucial for the removal of drugs. A main renal uptake transporter known as the organic cation transporter 2 (OCT2) is involved in the clearance and disposal of organic cation medications. Since Podolactone B did not exhibit any drug-drug interactions that would have decreased the renal clearance of an OCT2 substrate, it is unlikely that it is an OCT2 substrate. The results of the calculation of the renal total clearance are shown in Table 3. Hepatotoxicity was studied with regard to toxicity. It's interesting to note that podolactone B demonstrated no liver toxicity. In conclusion, the best ADMET profiles were seen with podolactone B. However, in vitro and in vivo investigations are required to corroborate this.

3.4. Molecular Dynamics (MD)

The simulation was used to gain insights into how the inhibitor binds to the target protein and how it affects the activity, as well as to predict potential binding sites and inhibitor mechanisms. Vera–bound system was utilised to study the dynamic behavior of the targeted protein. (i) Protein alone [4PQE-APO], (ii) Protein bound to GAL [4PQE-GAL], (iii) Protein bound to POB [4PQE-POB].

The RMSDs of the 4PQE-APO, 4PQE-GAL and 4PQE-POB protein complexes, measured over a 100 ns period, were found to be 0.25+/-0.015 nm, 0.29+/-0.019 nm and 0.26+/-0.116 nm respectively. These values indicate that there was little structural change in the protein complexes during the simulation, and the RMSDs did not deviate significantly from that of the unbound protein (Figure 3). Throughout the simulation, the relative stability of complex molecules is represented by these RMSD data. Overall, the RMSD findings show that during the simulation, each of the four protein complexes under consideration remained largely stable. The complexes' differences were negligible, indicating that their stability is nearly identical. This is likely due to the fact that they all contain similar components in their structures.

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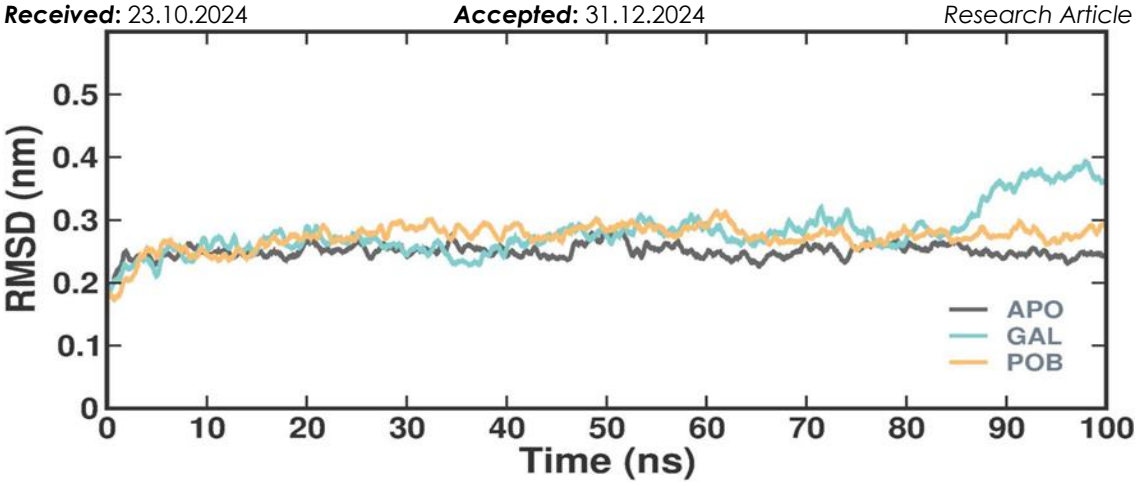

Figure 3. Root means square deviation of backbone atoms of 4PQE-APO, 4PQE-GAL and 4PQE-POB.

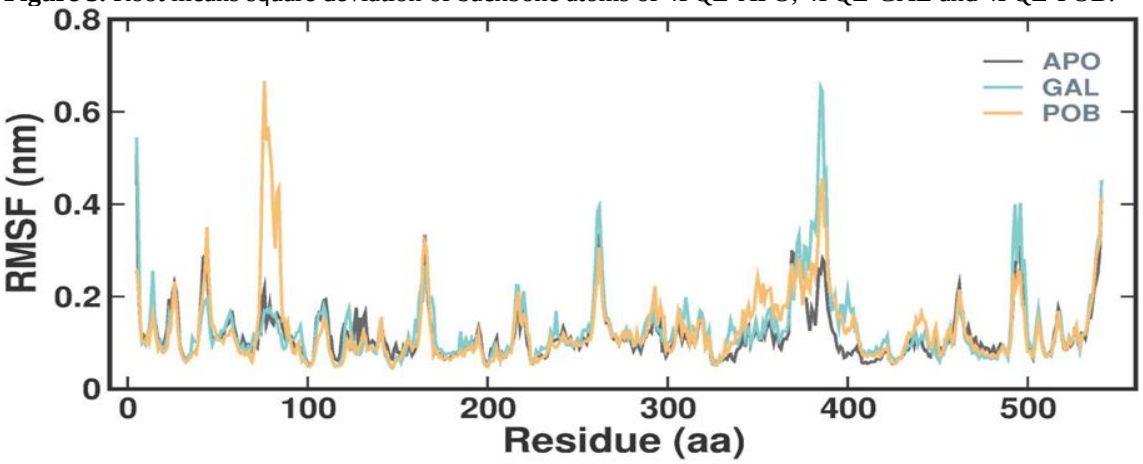

Figure 4. Root means square fluctuation of c-alpha atoms of 4PQE-APO, 4PQE-GAL and 4PQE-POB.

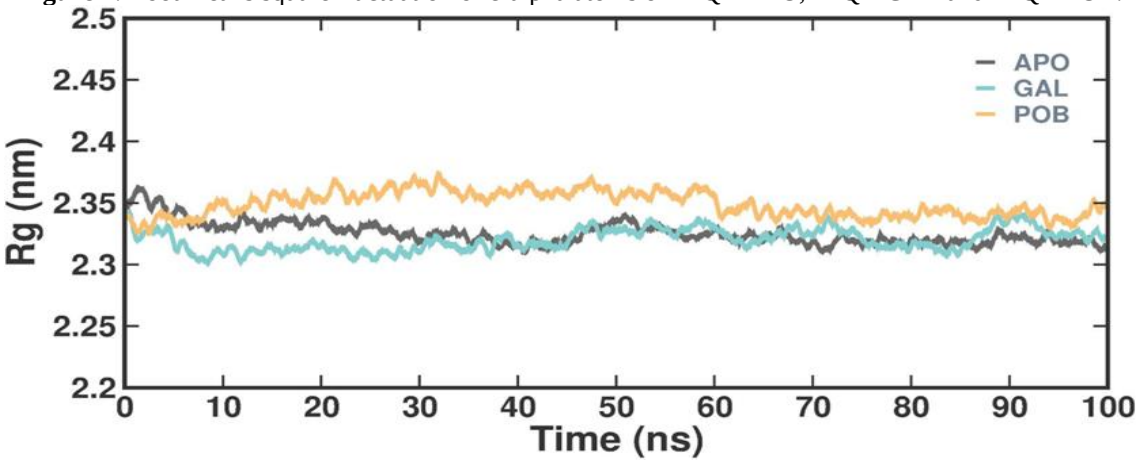

Figure 5. RG of backbone atoms of with 4PQE-APO, 4PQE-GAL and 4PQE-POB.

RMSF (root mean square fluctuation) analysis is used to identify which amino acids of a protein experience the most movement during a molecular dynamics simulation. This information can be used to understand how the presence or absence of a ligand affects the stability of the protein. In this case, the RMSF values were determined over a 100 ns simulation timescale, giving an idea of how the

protein's vibrations change over time. The RMSF results for 4PQE-APO, 4PQE-GAL and 4PQE-POB complexes as depicted in Figure 4. The outcome indicated that no appreciable structural alterations occurred over the 100 ns simulation.

The radius of gyration (Rg) is a measure of the compactness of a protein's structure, which can be calculated as the mass-weighted average distance of atoms from the protein's center of mass. The Rg plot can be used to visualize how the overall shape and folding of the protein changes over time during a MD simulation. The Rg plot in Figure 5 illustrates the changes in the protein's structure at different time points during the simulation trajectory. The Rg value pattern of the 4PQE-APO, 4PQE-GAL, and 4PQE-POB complexes was consistent throughout the experiment. The average RG value from 0 to 100 ns for 4PQE-APO, 4PQE-GAL and 4PQE-POB protein complex proteins were 2.31+/-0.014 nm, 2.32+/-0.019 nm and 2.35+/-0.021 nm respectively. The radius of gyration is a useful metric for measuring the structural flexibility of the protein complex. According to the protein complex's Rg values, the complex's morphologies folding at various trajectories do not significantly differ from one another. This implies that the protein complex has maintained its structural stability during the simulation. Further stable Rg values observed in the simulations suggest that Podolactone B binding does not disrupt the protein's structural integrity, maintaining its functional conformation.

The change in SASA was analysed to quantify the hydrophobic core's compactness. SASA reflects changes in protein surface exposure to solvent. Stable SASA values indicate that ligand binding does not expose the hydrophobic core, preserving protein stability and functionality. Figure 6 illustrates how the SASA of the 4PQE-APO, 4PQE-GAL, and 4PQE-POB proteins changes over time. The average SASA value from 0 to 100 ns for 4PQE-APO, 4PQE-GAL and 4PQE-POB protein complex proteins were 218.01+/-4.15 nm, 217.46+/-1.11 nm and 218.16+/-1.51 nm respectively. This suggests that the protein at the structural level has not changed during the simulation.

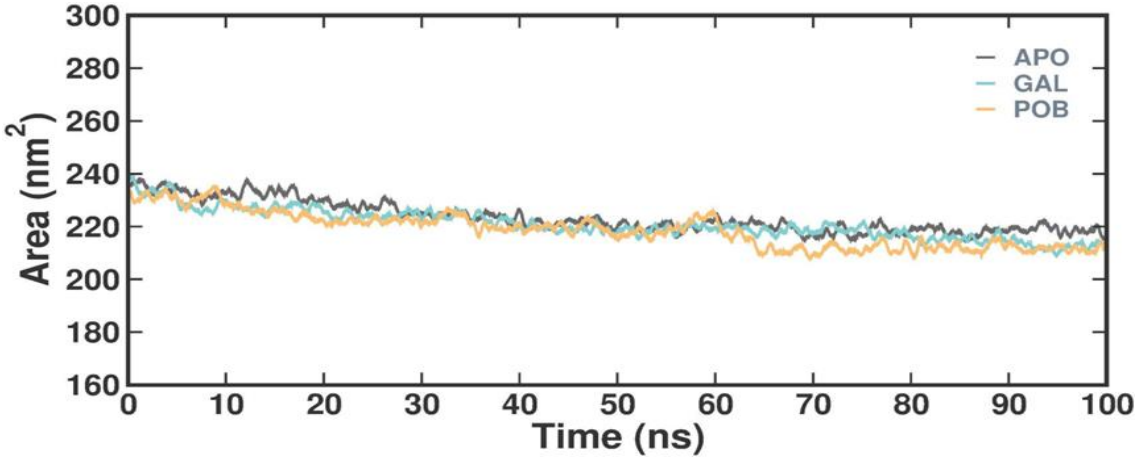

Figure 6. SASA of backbone atoms of with 4PQE-APO, 4PQE-GAL and 4PQE-POB

3.5. Hydrogen Bond (H-bond)

The stability of protein-ligand complexes can be attributed to the formation of hydrogen bonds between the protein and the ligand. In this research, the hydrogen bonds that were identified through molecular docking analysis were further confirmed through simulation analysis. Consistent hydrogen bonding during simulations highlights the strength and specificity of Podolactone B's interaction with the active site, supporting its potential as an effective inhibitor. The results of the hydrogen bond analysis for the complexes with 4PQE-GAL and 4PQE-POB are shown in Figure 7.

3.6. Principal component analysis

A statistical method for reducing the dimensionality of data and extracting significant characteristics or patterns is principal component analysis, or PCA. In structural biology, it is a widely used method for analysing conformational changes in proteins or protein-ligand complexes. The PCA analysis was performed on the 4PQE-APO, 4PQE-GAL and

4PQE-POB to examine the structural differences between the various protein samples (Figure 8).

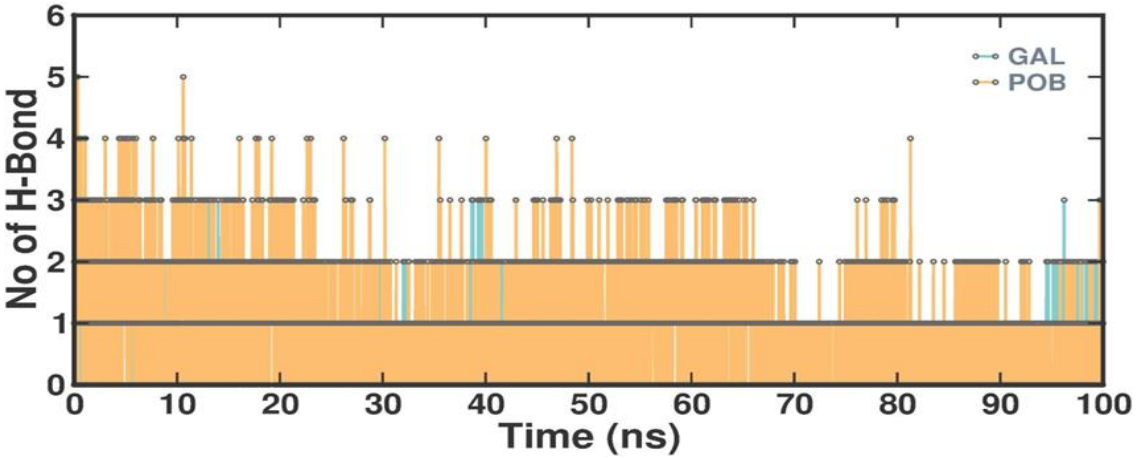

Figure 7. H-Bond of 4PQE-GAL and 4PQE-POB.

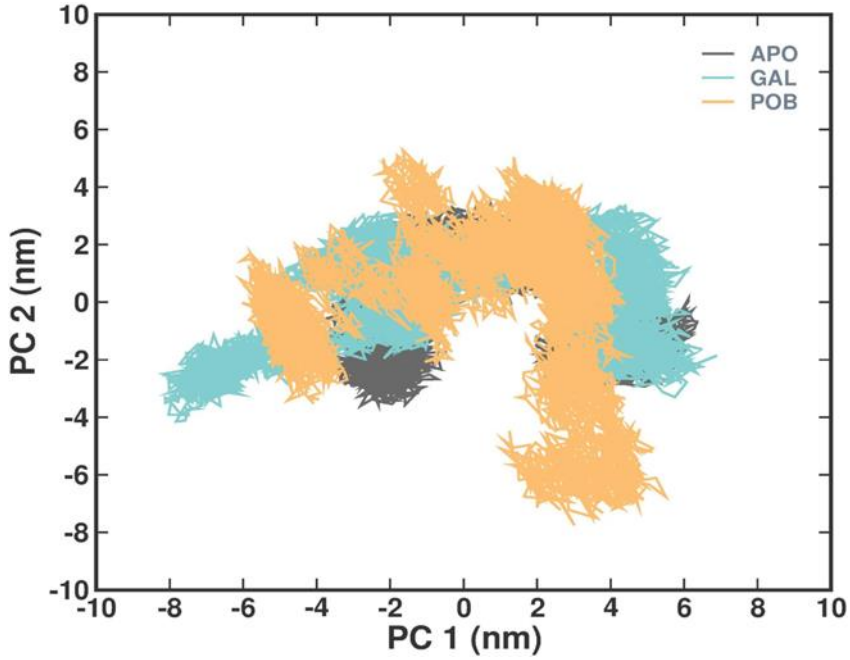

Figure 8. Principal component analysis.

3.7. MM – PBSA

In Table 4, the binding free energy calculations of galantamine (GAL) and podolactone B (POB) with the AChE enzyme (4PQE) were performed using the MM-PBSA method, to assess their potential as AChE inhibitors. The calculated binding energies provide insight into the stability and affinity of the compounds for the enzyme's active site, which is crucial for AChE inhibition.

Galantamine, a well-known AChE inhibitor, exhibited a binding energy of -137.027 ± 9.907 kJ/mol. This value reflects the balance between favorable van der Waals and electrostatic interactions, as well as the solvation effects. Specifically, the van der Waals energy for galantamine was -188.506 ± 12.680 kJ/mol, indicating strong non-covalent interactions between the compound and the enzyme. The electrostatic energy of galantamine was -25.165 ± 13.050 kJ/mol, which supports the compound's favorable interaction with the enzyme's charged residues. However, the polar solvation energy of 94.358 ± 19.019 kJ/mol suggests that solvation is less favorable, contributing to the total binding energy.

In comparison, podolactone B (POB) demonstrated a slightly stronger binding energy of -166.479 ± 13.332 kJ/mol. Notably, the van der Waals energy for POB was -189.790 ± 12.663 kJ/mol, which is comparable to that of galantamine, indicating similarly strong molecular interactions with the enzyme. However, POB exhibited a less favorable electrostatic energy of -16.794 ± 8.176 kJ/mol, which could suggest a slightly weaker interaction with charged residues on the enzyme. Furthermore, the polar solvation energy for POB was 57.465 ± 12.973 kJ/mol, which is significantly lower than that for galantamine. This suggests that POB may experience a more favorable solvation environment, contributing to its higher binding affinity.

The binding free energy calculations suggest that podolactone B has comparable, if not stronger, potential as an AChE inhibitor relative to galantamine. While POB's electrostatic interactions appear slightly weaker than those of galantamine, its favorable van der Waals and solvation energies may compensate for this, leading to a potentially more stable and effective binding at the AChE active site. These findings support the hypothesis that podolactone B could be a promising lead compound for further AChE inhibition studies and the development of therapeutic agents for diseases such as Alzheimer's.

Table 4. Binding free energy calculations of galantamine and podolactone B with 4PQE using MM-PBSA method.

System	van der Waal energy	Electrostatic energy	Polar solvation energy	Binding energy
GAL	-188.506 +/- 12.680 kJ/mol	-25.165 +/- 13.050 kJ/mol	94.358 +/- 19.019 kJ/mol	-137.027 +/- 9.907 kJ/mol
POB	-189.790 +/- 12.663 kJ/mol	-16.794 +/- 8.176 kJ/mol	57.465 +/- 12.973 kJ/mol	-166.479 +/- 13.332 kJ/mol

4. Conclusions

Molecular docking studies demonstrated that Podolactone B has a strong binding affinity for its receptor, indicating its potential as an effective natural acetylcholinesterase inhibitor. Molecular dynamics simulations further confirmed the compound's stability and provided insights into the contribution of various energy terms to its overall binding energy, supporting its therapeutic potential. This in silico analysis positions Podolactone B as a promising lead compound, paving the way for future in vitro and in vivo studies to evaluate its efficacy, safety, and mechanism of action. These findings also lay the groundwork for exploring structural modifications to enhance its pharmacological properties. By shedding light on the molecular interactions between Podolactone B and AChE, a key enzyme in Alzheimer's disease pathology, this study offers valuable insights into its role as an AChE inhibitor. The results can inform the design of more effective therapeutic agents, guide structure-activity relationship (SAR) studies, and support the development of combination therapies.

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Authors' contributions

The author AB involved in Investigation, Conceptualization, Resources, Data curation, Writing – original draft, Visualization and Project administration. The author NVR involved in Software, Validation, and Formal analysis. The author CA involved in Conceptualization, Validation, Investigation, Writing – review and editing, and Visualization. SJ involved in Conceptualization, Methodology/Study design and Supervision. The author LB involved in Software, Validation, Resources, Data curation. The author

SD and MS involved in Writing –review and editing and Visualization. The author LSW involved in Resources, Data curation and Validation. VS involved in Conceptualization, Methodology/Study design and Supervision. All authors read and approved the final version of the manuscript.

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