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RESEARCH ARTICLE

In Silico Insights into Marine Natural Products as AChE and BChE Inhibitors

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Abstract

Background: Alzheimer's Disease (AD), a progressive neurodegenerative disorder, remains a significant global health challenge. **Aim:** Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitors are crucial in managing the symptoms of AD, and exploring new, potent inhibitors is an ongoing area of research. **Methods:** This study investigates the therapeutic potential of marine-derived natural products as AChE and BChE inhibitors through a detailed *in silico* approach. A total of 675 compounds from the COCONUT Natural Product Database were screened, with compounds CNP0200466, CNP0325153, and CNP0017810 identified as top candidates for AChE inhibition, and compounds CNP0310051.1 and CNP0313638 identified for BChE inhibition. **Result:** The docking analysis revealed that these compounds exhibited significantly higher binding affinities compared to the standard drugs, Galantamine and Rivastigmine. For AChE (PDB: 4EY7), Galantamine showed a binding score of -9.6 kcal/mol, while compounds CNP0200466, CNP0325153, and CNP0017810 demonstrated stronger affinities of -12.6, -11.5, and -10.8 kcal/mol, respectively. Similarly, for BChE (PDB: 5DYW), Rivastigmine showed a binding score of -7.2 kcal/mol, while compounds CNP0310051.1 and CNP0313638 exhibited superior affinities of -11.0 and -10.9 kcal/mol, respectively. Pharmacokinetic evaluation using SwissADME confirmed that all compounds exhibited high gastrointestinal absorption, blood-brain barrier permeability, and no P-glycoprotein interaction, suggesting their potential for efficient CNS targeting. ADMET analysis revealed favorable renal clearance, non-hepatotoxic profiles, and no significant AMES toxicity. These results indicate that the marine-derived compounds identified in this study possess both high efficacy and favorable safety profiles. **Conclusion:** In conclusion, the promising docking results, coupled with favorable pharmacokinetic and toxicological properties, make these marine-derived compounds strong candidates for further preclinical and clinical studies.

Major Findings: The findings highlight the potential for these compounds to serve as novel therapeutic agents for Alzheimer's disease, paving the way for more effective treatments for this devastating disorder.

Keywords: AChE, ADMET, Alzheimer's, BChE, COCONUT Database, Molecular Docking

1. Introduction

As the most common type of dementia, Alzheimer's Disease (AD) affects millions of people worldwide, making it one of the biggest healthcare issues of the twenty-first century[1]. First identified by Alois Alzheimer in 1906, this progressive neurodegenerative disease is typified by a slow deterioration in cognitive function, especially memory and mental skills, which eventually results in significant impairment in day-to-day functioning[2]. Dementia affects over 50 million individuals globally, with AD accounting for 60–70% of cases. AD prevalence has increased to epidemic levels[3]. By 2050, this number is expected to triple, mostly as a result of aging populations and rising life expectancy[4]. People over 65 are primarily affected by the condition, and beyond age 65, the risk doubles every five years[5]. The financial burden is also significant; it is estimated that the yearly global expenses of direct medical care, informal care, and lost productivity total hundreds of billions of dollars[6]. The development of internal neurofibrillary tangles made of hyperphosphorylated tau protein

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and the buildup of extracellular amyloid-β (Aβ) plaques are the two main pathological hallmarks of AD, which is caused by complicated molecular pathways[7,8]. Brain shrinkage and gradual neuronal loss are caused by these degenerative alterations, especially in areas that are essential for memory and cognition[9]. Several risk factors have been found; however, the precise cause is still unknown. These include age as the main risk factor, genetic variables (especially the APOE ε4 allele), cardiovascular health, environmental factors, lifestyle choices, educational attainment, and cognitive reserve[10,11]. AD's clinical presentation usually progresses distinctively[12]. Early stages include modest memory loss, especially for recent events, planning and organizing difficulties, language and word-finding issues, and poor judgment[13]. Patients encounter worsening memory loss, trouble with everyday tasks, behavioral abnormalities, and confusion about time and location as the illness advances to the middle stages[14]. Severe cognitive decline, total reliance on carers, physical problems including trouble eating, and a lack of communication skills are characteristics of the late stages[15]. According to available data, several potentially changeable factors, such as consistent physical activity, social interaction, cognitive stimulation, following a Mediterranean diet, controlling cardiovascular risk factors, getting enough sleep, reducing stress, and quitting smoking, may lower the risk of AD[16,17]. Due to the intricacy of AD pathogenesis, numerous important treatment targets have been identified[18]. Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) have emerged as important targets, and cholinesterase inhibition is still the major therapeutic approach[19]. In numerous investigations, dual inhibition of AChE and BChE has produced encouraging findings[20]. Natural products have become a viable source of possible medication candidates in the search for new therapeutic agents[21]. With more than 400,000 natural items, the COCONUT (Collection of Open Natural Items) database has grown to be a vital tool for virtual screening campaigns in AD medication discovery[22]. This comprehensive database enables researchers to efficiently identify potential lead compounds targeting key proteins involved in AD pathogenesis[23]. Marine natural products, in particular, have gained significant attention due to their unique structural diversity and biological activities[24].

2. Materials and Methods

2.1 Protein Preparation

For this research project, two primary protein targets were selected based on their crucial roles in Alzheimer's disease pathology: Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE). The three-dimensional structures were obtained from the Protein Data Bank[25] using PDB IDs: 4EY7 for AChE and 5DYW for BChE. Following a thorough assessment of their resolution and structural quality factors, these particular structures were chosen. Since lower resolution values indicate more precise atomic position determination, which results in more accurate docking studies, both structures demonstrated resolutions better than 2.5Å (4EY7: 2.35Å, 5DYW: 2.50Å). This provides good structural reliability. The majority of residues were found to be in favoured regions, indicating the outstanding stereochemical quality of the protein backbone conformations, according to an analysis of the Ramachandran plots of these structures from the PROCHECK website depicted in **Figure 1.** UCSF Chimaera was used to prepare the protein structures[26]. Multiple chains were initially present for proteins 4ey7 and 5dyw; however, only chain A was kept because it contained all of the catalytic site information, making the study simpler and ensuring uniformity. By showing the entire backbone, the structural integrity was evaluated and any missing backbone information was found. Dock Prep, a Structure Editing tool in UCSF Chimaera, was used to optimize these gaps. Further, Hydrogen atoms were added, charges were assigned, water molecules and non-interacting ions were eliminated, and missing side chains were rebuilt as part of the preparation procedure. Each structure was then subjected to energy minimization to maximize conformations and remove any high-energy interactions that might have an impact on docking outcomes. The resulting optimized protein structures were saved in PDB format. Subsequently, AutoDock Tools 1.5.7 was employed for the grid box generation of each protein. The grid box dimensions and coordinates (x, y, z) were carefully recorded for the docking studies,

ensuring complete coverage of the active sites. Finally, the co-crystallized ligands were removed, and the proteins were converted and saved in PDBQT format, making them ready for molecular docking studies.

2.2 Dataset Preparation

In selecting compounds for this study, we utilized the COCONUT (Collection of Open Natural Products) database[22], specifically focusing on the Marine Natural Product collection. Initially, 5,264 marine-derived compounds were downloaded from the database. These marine natural products were chosen due to their unique structural diversity and potential biological activities against neurodegenerative targets. Although these compounds have been previously reported in the COCONUT database, they had not been specifically analyzed against the selected Alzheimer's disease targets (AChE, BChE), which introduces novelty to this research. To ensure drug-likeness and optimize the chances of good oral bioavailability, we employed Biovia Discovery Studio to filter the compounds based on Lipinski's rule of five and additional drug-like parameters. The following filters were applied: molecular weight (≤500 Da), number of hydrogen bond acceptors (≤10), number of hydrogen bond donors (≤5), polar surface area, and number of rotatable bonds. This filtering process resulted in a refined set of 675 compounds with favorable physicochemical properties for potential drug development. For the preparation of these compounds for docking studies, the 675 compounds were initially downloaded in CSV (Excel) format, which contained compound IDs and SMILES notations for each molecule. The SMILES were extracted and saved in a text file. Using Open Babel in Ubuntu-Linux OS, the text file format was converted to SMI file format. Taking the SMI file, a command was used in the Ubuntu terminal to convert the SMI file which contained 675 compounds into PDBQT format. During this process, an MMFF94 force field energy reduction gradient of 0.000001 kcal/mol/Å was applied, and 3D conformers were generated. This thorough preparation ensured that the compounds were optimized for subsequent docking studies. 2D structures of the top compounds were created using Chemdraw 22 software.

2.3 Docking Studies

AutoDock Vina[27] was utilized for conducting docking studies. The AutoDock Vina visual user interface (GUI) is powered by the MGL tools 1.5.7. Multiple ligand docking was being done using Perl script. The results of AutoDock Vina were then utilised to visualize interactions using Biovia Discovery Studio 2021.

2.4 Drug Likeness Studies

Although the initial selection from the COCONUT database applied Lipinski's Rule of Five to ensure basic drug-likeness, we conducted a more detailed pharmacokinetic evaluation using SwissADME and pKCSM to refine the selection further. While Lipinski's rules address essential aspects like molecular weight and lipophilicity, SwissADME[28] allowed us to assess a broader range of pharmacokinetic parameters, including solubility, Blood-Brain Barrier (BBB) permeability, P-glycoprotein (P-GP) substrate status, etc. pKCSM web server provided additional insights into pharmacokinetic properties such as Intestinal Absorption, metabolism insights, total clearance,

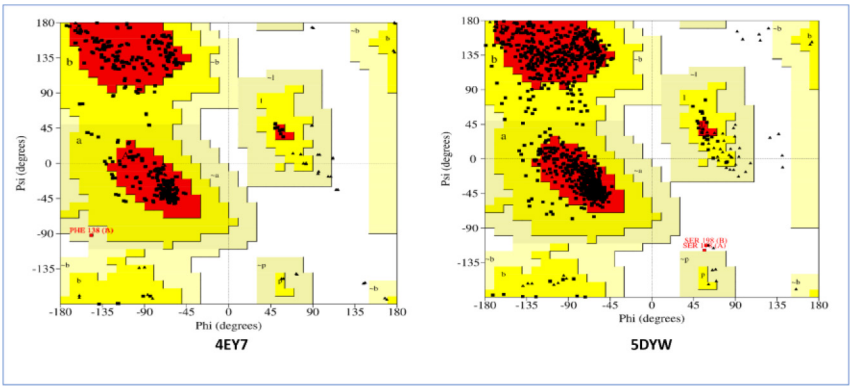


Figure 1. Ramachandran Plot of PDB ID: 4EY7 (AChE) and PDB ID: 5DYW (BChE).

AMES Toxicity which provides insights of Mutagenicity, and lastly skin sensitization property. After that one more online server tool Protox-III[29] was used for Toxicity assessment. By combining the analyses from SwissADME, pKCSM, and ProTox-III we ensured a rigorous, multi-dimensional evaluation that prioritized compounds with favorable ADME properties and low predicted toxicity.

3. Results and Discussion

3.1 Evaluation of Docking Protocol

Before the database was virtually screened, the AutoDock Vina docking process was extensively validated. To validate the co-crystallized ligands found in our protein of interest (PDB ID: 4ey7 and 5dyw) were removed and docked back into the same binding region After that the Root Mean Square Deviation (RMSD) was determined using Biovia Discovery studio. RMSD < 2.0 Å is generally considered successful validation. The co-crystallized ligand in 4ey7 showed an RMSD value of 0.4926 Å and the co-crystallized ligand in 5dyw showed an RMSD value of 1.77954 Å (Figure 2). Hence it verifies that our docking software and parameters can accurately reproduce the experimentally determined binding pose and can establish the reliability of our docking protocol[30].

3.2 Structure-based Virtual Screening

3.2.1 Screening and Docking Studies of Marine Natural Products

Following docking process validation, the marine natural product collection from the COCONUT Natural Product Database was screened. Lipinski's rule of five was applied using Biovia Discovery Studio 2021 software to filter the original dataset of 5264 compounds, resulting in a subset of 675 compounds. These compounds were then subjected to docking studies against AChE (PDB ID: 4EY7) and BChE (PDB ID: 5DYW). The standard reference inhibitors for AChE and BChE were galantamine and rivastigmine, respectively. Compounds were ranked using docking scores (in kcal/mol), and the top-performing ones were chosen for additional examination. Table 1 and Table 2 show the 2D structures and docking scores of the top compound against AChE and BChE respectively. For AChE (4EY7), the top compounds identified with the most favorable docking scores included CNP0200466, CNP0325153, and CNP0017810. Similarly, for BChE (5DYW), the best-performing compounds were CNP0310051.1 and CNP0313638. The docking scores of these compounds indicate their potential as effective inhibitors.

3.2.2 Key Interactions in AChE and BChE Active Sites

Based on literature[31-35] and molecular docking results, the following amino acid residues are known to play critical roles in the inhibition of AChE and BChE:

- AChE (4EY7):

1. Tyr A:337: Facilitates π-π stacking, stabilizing aromatic ligands.
2. Trp A:286: Involved in hydrophobic interactions, crucial for inhibitor anchoring.
3. Ser A:203: Commonly forms hydrogen bonds, enhancing binding stability.

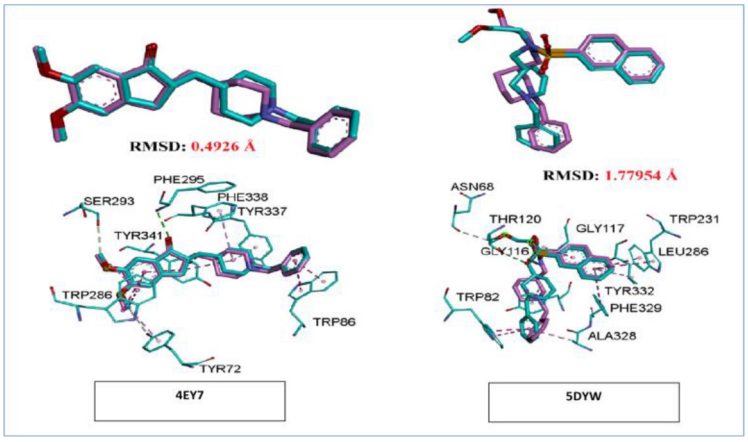


Figure 2. Co-crystallized ligands (Original in Blue and rocked in pink) superimposed for calculation of RMSD value.

Table 1. 2D structures of top compounds with Docking scores for AChE (4ey7)

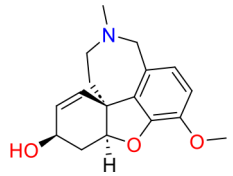
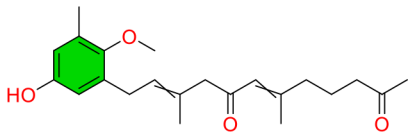
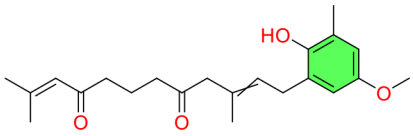
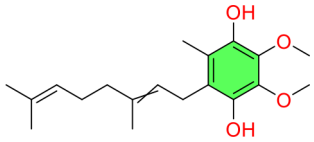
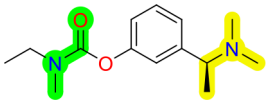
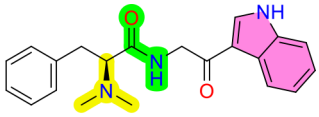
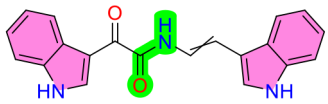
Sr. No.	Compound ID	2D Structure	Docking score (kcal/mol)
1	Galantamine		-9.6
2	CNP0200466		-12.6
3	CNP0325153		-11.5
4	CNP0017810		-10.8

Table 2. 2D structures of top compounds with docking scores for BChE(5dyw)

Sr. No.	Compound ID	2D Structure	Docking score (kcal/mol)
1	Rivastigmine		-7.2
2	CNP0310051.1		-11.0
3	CNP0313638		-10.9

4. Glu A:202: Contributes to electrostatic stabilization and ionic interactions.
5. Tyr A:124 and Phe A:295: Frequently interact through hydrogen bonding, aiding stability.

- BChE (5DYW):

1. Trp A:82: Critical for hydrophobic interactions, anchoring inhibitors in the active site.
2. Asp A:70: Forms hydrogen bonds, contributing to enhanced binding.
3. Ser A:198: A key catalytic residue often interacting with inhibitors.
4. Tyr A:332: Engages in π-π stacking interactions.
5. Gly A:116 and Ser A:287: Involved in stabilizing hydrogen bonds.

3.2.3 Analysis of Top Compounds with 2D Interaction Reference

The selected compounds demonstrated significant binding interactions with critical residues in the active sites of both AChE and BChE. These interactions are illustrated in 2D diagrams in Figures 3 and 4 for AChE and BChE respectively.

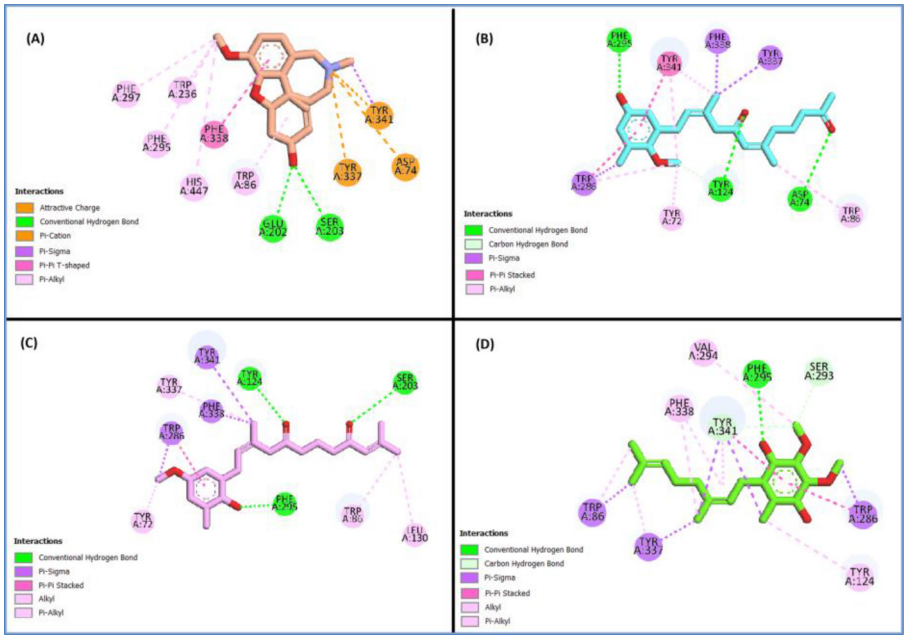


Figure 3. 2D Interactions of (a) Galantamine (b) CNP0200466 (c) CNP0325153 (d) CNP0017810 as AChE inhibitors.

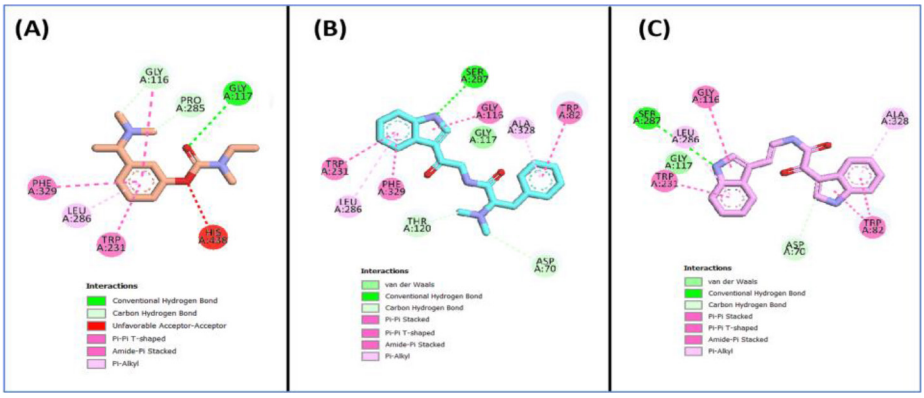


Figure 4. 2D Interactions of (a) Galantamine (b) CNP0310051.1 (c) CNP0313638 as BChE inhibitors.

The docking analysis revealed that the top-performing compounds for both AChE (4EY7) and BChE (5DYW) exhibited significant interactions with critical residues, matching and differentiating from the standard drugs Galantamine and Rivastigmine, respectively. For AChE, Galantamine formed key interactions with residues such as Tyr A:337 and Phe A:295, which stabilized the ligand through π-π stacking and hydrophobic interactions, and Ser A:203, which contributed to stability via hydrogen bonding. Similarly, compounds CNP0200466, CNP0325153, and CNP0017810 interacted with these critical residues, mirroring Galantamine's binding profile. However, CNP0200466 uniquely engaged with Tyr A:124 and Asp A:74 through hydrogen bonds, while CNP0017810 formed additional carbon-hydrogen bonds with Tyr A:341 and Ser A:293, providing enhanced stabilization. For BChE, Rivastigmine demonstrated significant interactions with residues like Trp A:231 (π-π stacking) and Gly A:117 (hydrogen bonding). The compounds CNP0310051.1 and CNP0313638 replicated these critical interactions, particularly with Trp A:231 and Ser A:287, which are essential for ligand binding. In addition to these shared interactions, CNP0310051.1 displayed unique hydrogen bonding with Thr A:120, while CNP0313638 engaged in van der Waals interactions with Gly A:117, offering improved binding stability compared to Rivastigmine. These

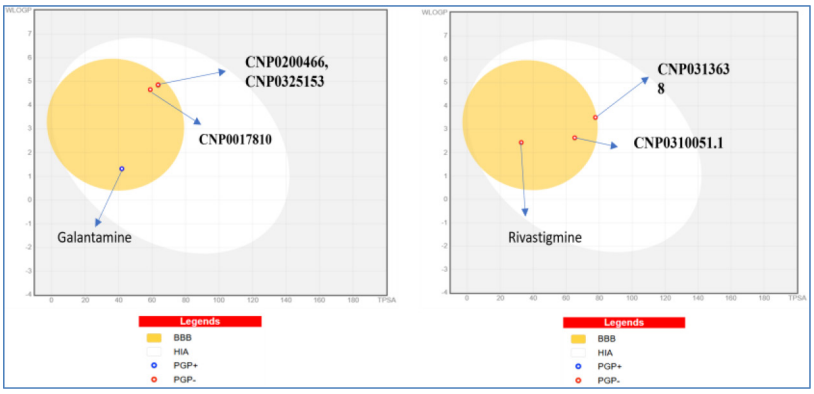


Figure 5. Boiled egg model.

differentiated interactions indicate the potential for enhanced specificity and binding affinity. The matched interactions with key residues underscore the ability of these compounds to mimic the inhibitory action of the standard drugs, while the unique binding features suggest their potential to provide improved therapeutic efficacy. These findings justify further evaluation of the top compounds as lead candidates for AChE and BChE inhibition, contributing to the development of novel therapeutic agents for Alzheimer's disease.

3.2.4 Future Directions

To comprehensively evaluate the drug potential of these compounds as AChE and BChE inhibitors, additional studies were conducted further:

- Drug-likeness Analysis: Utilizing SwissADME to ensure favorable pharmacokinetic properties.
- ADMET Analysis: Employing pKCSM to predict absorption, distribution, metabolism, excretion, and toxicity profiles.

These future studies, informed by the molecular insights gained here, will aid in identifying and optimizing promising candidates for Alzheimer's disease therapy.

3.3 Drug Likeness Study SwissADME

Though the COCONUT Natural Product Database applied Lipinski's Rule of Five to ensure basic drug-likeness, we further refined the selection of compounds through a more detailed pharmacokinetic evaluation using SwissADME. While Lipinski's rule provides an initial screening based on molecular properties, SwissADME allowed for a deeper analysis of crucial pharmacokinetic parameters, including Blood-Brain Barrier (BBB) permeability, P-glycoprotein (PGP) substrate status, and bioavailability scores. These additional evaluations were essential to confirm and expand upon the initial results from Lipinski's filter. For AChE, Galantamine, a standard drug, was identified as a PGP substrate with a log P value of 1.74, indicating moderate lipophilicity. In contrast, all marine-derived compounds (CNP0200466, CNP0325153, and CNP0017810) demonstrated no PGP substrate activity, suggesting they are less likely to be effluxed out of cells. This property enhances their therapeutic potential by promoting better cellular retention, ensuring more effective targeting in the central nervous system. The log P values for these compounds were slightly higher: CNP0200466 (3.03), CNP0325153 (3.03), and CNP0017810 (3.06) indicating greater lipophilicity, which is favorable for BBB penetration and targeting AChE (Table 3). For BChE, Rivastigmine, the standard drug, had a logP value of 2.34, while marine-derived compounds CNP0310051.1 (logP = 2.64) and CNP0313638 (logP = 3.51) showed higher logP values, suggesting enhanced BBB permeability (Table 4). Additionally, none of the selected compounds for both AChE and BChE were identified as PGP substrates, further supporting their potential for effective CNS targeting. To visually support these findings, a boiled egg diagram (Figure 5) was included, illustrating BBB permeability. The yellow section of the egg highlights compounds that are BBB-permeant, while Galantamine (represented in blue) is indicated as a PGP substrate (yes), and compounds in red are non-PGP substrates. This diagram helps emphasize the potential of these compounds for efficient drug delivery to the brain. The detailed SwissADME evaluation, beyond the initial

Table 3. SwissADME study of top compounds (AChE) for drug-likeness

Sr. No.	Compound No.	Molecular weight	BBB Permeant	P-GP Substrate	Bioavailability	Log P
1	Galantamine	287.35	Yes	Yes	0.55	1.74
2	CNP0200466	358.47	Yes	No	0.55	3.03
3	CNP0325153	358.47	Yes	No	0.55	3.03
4	CNP0017810	320.42	Yes	No	0.55	3.06

Table 4. SwissADME study of top compounds (BChE) for drug-likeness

Sr. No.	Compound No.	Molecular weight	BBB Permeant	P-GP Substrate	Bioavailability	Lipinski
1	Rivastigmine	250.34	Yes	Yes	0.55	2.34
2	CNP0310051.1	349.43	Yes	No	0.55	2.64
3	CNP0313638	329.35	Yes	No	0.55	3.51

Table 5. ADMET Prediction of Selected Compounds (AChE)

Sr No.	Compounds	Pharmacokinetics					
		Absorption	Metabolism		Excretion	Toxicity	
		Intestinal Absorption (HUMAN)	CYP		Total clearance	AMES Toxicity	SKIN SENSITIZATION
		Numeric (% absorbed)	2D6 SUB	3A4 INH	Numeric (log ml/min/kg)	Categorical (yes/no)	Categorical (yes/no)
1	Galantamine	93.796	No	Yes	0.979	No	No
2	CNP0200466	93.817	No	No	1.409	No	No
3	CNP0325153	96.097	No	No	1.479	No	No
4	CNP0017810	93.289	No	No	1.115	No	No

Table 6. ADMET Prediction of Selected Compounds (BChE)

Sr No.	Compounds	Pharmacokinetics					
		Absorption	Metabolism		Excretion	Toxicity	
		Intestinal Absorption (HUMAN)	CYP		Total clearance	AMES Toxicity	SKIN SENSITIZATION
		Numeric (% absorbed)	2D6 SUB	3A4 INH	Numeric (log ml/min/kg)	Categorical (yes/no)	Categorical (yes/no)
1	Rivastigmine	89.285	No	No	0.518	No	No
2	CNP0310051.1	93.009	Yes	Yes	1.101	No	No
3	CNP0313638	91.540	Yes	Yes	0.734	No	No

Lipinski filter, revealed promising pharmacokinetic properties for these compounds, making them strong candidates for further development as therapeutic agents for Alzheimer's disease.

3.4 pKCSM ADMET Analysis

In this comprehensive analysis, we evaluated the drug-like characteristics of the selected compounds using pKCSM, a platform that provides insights into ADME-Tox parameters and includes a "Theory" section to aid in interpreting the results. The compounds demonstrated excellent human intestinal absorption (>90%), with Rivastigmine being slightly lower (89.285%), indicating good oral bioavailability. All compounds showed favorable renal clearance and, more importantly, none exhibited AMES toxicity or skin sensitization,

Table 7. Toxicity prediction of standard and selected compounds (AChE)

Sr. No.	Compound	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Std	Galantamine	Inactive	Inactive	Active	Inactive	Active
1	CNP0200466	Inactive	Inactive	Inactive	Inactive	Inactive
2	CNP0325153	Inactive	Inactive	Inactive	Inactive	Inactive
3	CNP0017810	Inactive	Inactive	Inactive	Inactive	Inactive

A: Active; I: Inactive

Table 8. Toxicity prediction of standard and selected compounds (BChE)

Sr. no	Compound	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Std	Rivastigmine	Inactive	Inactive	Active	Inactive	Inactive
1	CNP0310051.1	Inactive	Inactive	Inactive	Inactive	Inactive
2	CNP0313638	Inactive	Inactive	Inactive	Inactive	Inactive

A: Active; I: Inactive

underscoring their safety profiles. From literature references[36-39], it has been established that interactions with cytochrome P450 enzymes, particularly CYP3A4 and CYP2D6, play a critical role in drug metabolism and the potential for drug-drug interactions. For AChE-targeted compounds (CNP0200466, CNP0325153, and CNP0017810), no inhibition of CYP3A4 or interaction with CYP2D6 was observed, suggesting a reduced likelihood of adverse interactions. In contrast, for BChE-targeted compounds (CNP0310051.1 and CNP0313638), although they acted as substrates for CYP2D6 and inhibited CYP3A4, this dual activity could prolong their half-life and potentially improve efficacy, provided careful dose adjustments are made. The *in silico* ADMET results indicate that all compounds possess favorable safety and pharmacokinetic profiles, making them strong candidates for further evaluation. These findings underscore the robust drug-like properties of the marine-derived natural products studied, paving the way for subsequent *in vitro* and *in vivo* validation. The detailed ADMET results for the selected compounds are summarized in Tables 5 and 6.

3.5 ProTox-III Toxicity Analysis

The toxicity of the standard drugs galantamine and rivastigmine, as well as the top filtered-out compounds, was predicted using ProTox-III. Cytotoxicity, Mutagenicity, Immunotoxicity, Carcinogenicity, and Hepatotoxicity were all examined. In contrast to conventional galantamine, which was active for immunotoxicity and cytotoxicity, compounds from the AChE investigation were inactive for all five toxicities. Although rivastigmine was active for immunotoxicity, substances from the BChE research were also inactive for all five toxicities. Tables 7 and 8 display the *in silico* toxicity results for the compounds chosen as AChE and BChE inhibitors, respectively.

4. Conclusion

This research explored the potential of marine-derived compounds as AChE and BChE inhibitors for Alzheimer's disease through a systematic *in-silico* approach, applying several predictive analyses to ensure an optimal balance between efficacy and safety. We began with a docking study using AutoDock Vina, where 675 compounds filtered from the COCONUT Natural Product Database were screened against AChE (PDB ID: 4EY7) and BChE (PDB ID: 5DYW). For AChE, compounds CNP0200466, CNP0325153, and CNP0017810 were identified as the top candidates. Similarly, for BChE, compounds CNP0310051.1 and CNP0313638 emerged as the most promising inhibitors. The docking results identified these compounds with superior binding affinities compared to the standard inhibitor galantamine for AChE and rivastigmine for BChE. Key interactions with residues critical for enzymatic inhibition were observed, which highlighted their potential for effective enzyme inhibition. SwissADME and pKCSM were then utilized to evaluate the drug-likeness and pharmacokinetic characteristics of the selected compounds. All compounds demonstrated high gastrointestinal absorption (>90%), blood-brain

barrier permeability, and non-P-glycoprotein substrate status, ensuring efficient central nervous system targeting and systemic efficacy. This analysis confirmed the compounds' ability to cross the BBB, a critical requirement for Alzheimer's disease therapeutics. Further refinement was achieved using pKCSM for ADMET analysis. All compounds showed excellent renal clearance, good CYP enzyme interaction profiles, and no indications of AMES toxicity and skin sensitization. The absence of AMES toxicity and skin sensitization confirmed their safety profiles, underscoring their suitability for further development. Finally, ProTox-III analysis revealed that all compounds were inactive for toxicity categories, including Hepatotoxicity, Carcinogenicity, immunotoxicity, Mutagenicity, and cytotoxicity, confirming their safety. This comprehensive evaluation ensured that the selected compounds possess optimal efficacy and safety characteristics. Overall, this systematic *in silico* study demonstrates the promising therapeutic potential of marine-derived natural products such as AChE and BChE inhibitors. The combined insights from docking, SwissADME, pKCSM, and ProTox-III studies justify their continued investigation in preclinical and clinical settings. The rigorous multi-parameter evaluation employed here ensures that these compounds not only exhibit potent enzyme inhibition but also possess favorable pharmacokinetic and toxicological profiles. These compounds, particularly CNP0200466, CNP0325153, and CNP0017810 for AChE, and CNP0310051.1 and CNP0313638 for BChE stand out as good candidates for further experimental and clinical studies, paving the way for their development as novel Alzheimer's therapeutics.

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6. Author Contibution

Prince Parmar: Conceptualization, Writing – original draft, preparation, Methodology, Formal analysis, Visualization, Data curation, Investigation, Validation. Pinkal Patel: Writing – review and editing, Supervision, Resources, Software. All authors have read and agreed to the published version of the manuscript.

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