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Molecular Docking Analysis of Novel Acetylcholinesterase Inhibitors for Alzheimer's Disease Treatment

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ABSTRACT

***Introduction*:**
Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by memory loss and cognitive decline. One therapeutic strategy involves inhibiting acetylcholinesterase (AChE), the enzyme responsible for acetylcholine degradation in synaptic clefts. AChE inhibitors enhance cholinergic neurotransmission, thereby alleviating cognitive symptoms associated with AD. This study aimed to identify and evaluate novel AChE inhibitors structurally related to rivastigmine using computational techniques, including virtual screening and molecular docking, to discover potential lead compounds for AD therapy.

Materials and Methods:
The crystal structure of AChE (PDB ID: 6M0E) was obtained from the Protein Data Bank. Ligands with over 95% structural similarity to rivastigmine, based on the Tanimoto coefficient, were retrieved from PubChem. The ligands were energy-minimized and screened virtually using PyRx. Molecular docking was performed with AutoDock 4.2, and docking results were analyzed in terms of binding energy, inhibition constant (Ki), and interaction profiles to assess inhibitory potential.

Results:
Among the screened compounds, Ligand 13 exhibited the most favorable binding affinity, with a binding energy of −5.07 kcal/mol and an inhibition constant of 192.84 μM. Interaction analysis revealed that Ligand 13 formed three hydrogen bonds with key residues Ser215 and Arg177 in the AChE active site, suggesting stronger binding than rivastigmine.

Conclusion:
Ligand 13 emerged as a promising AChE inhibitor candidate for AD treatment. Further studies involving pharmacokinetic, toxicity, and experimental validation are necessary to confirm its therapeutic potential.

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Introduction

Alzheimer's disease (AD) is the most prevalent form of dementia and represents a progressive neurodegenerative disorder characterized by memory impairment, cognitive decline, and loss of functional independence. This condition profoundly affects the quality of life of patients and their caregivers, and its increasing global prevalence imposes a substantial healthcare and socioeconomic burden. As there is currently no definitive cure for AD, existing therapeutic strategies are primarily symptomatic, aiming to alleviate cognitive deficits and slow disease progression (1,2).

One of the principal therapeutic approaches involves the inhibition of acetylcholinesterase (AChE), the key enzyme responsible for hydrolyzing acetylcholine at neuronal synapses. The depletion of acetylcholine is a well-recognized hallmark of AD pathophysiology, and restoring its levels through AChE inhibition can enhance cholinergic transmission and cognitive performance. Clinically approved AChE inhibitors (AChEIs) such as donepezil, galantamine, and rivastigmine are commonly prescribed for mild to moderate AD. Among them, rivastigmine exhibits a distinctive dual inhibitory mechanism targeting both acetylcholinesterase and butyrylcholinesterase, providing broader cholinergic support. However, despite its therapeutic benefits, rivastigmine is associated with several limitations, including gastrointestinal side effects, modest efficacy, and frequent dosing requirements, which collectively restrict its clinical potential (2,3,4).

Structural modification of rivastigmine offers a rational strategy to develop novel derivatives with improved pharmacological profiles. By introducing specific functional groups or chemical substitutions, it is possible to enhance inhibitory potency, increase blood–brain barrier permeability, improve solubility, and strengthen metabolic stability. Furthermore, certain derivatives may exhibit additional neuroprotective activities by mitigating oxidative stress and neuroinflammation—two key pathological processes contributing to AD progression (1,2,3,4).

Computational methods such as virtual screening and molecular docking have emerged as efficient and cost-effective approaches for the rational design and evaluation of potential drug candidates. Molecular docking enables the prediction of binding modes, interaction patterns, and binding affinities between ligands and target enzymes, thereby facilitating the identification of compounds with high inhibitory potential (4–7). The accuracy of such in silico methods is further strengthened by the availability of high-resolution crystal structures of AChE and the detailed understanding of its active site topology. Given these considerations, employing computational techniques to identify rivastigmine-like compounds with enhanced pharmacokinetic and pharmacodynamic properties presents a promising avenue for drug discovery. By systematically analyzing the molecular interactions, binding energies, and inhibition constants of structurally similar derivatives, it becomes possible to pinpoint lead molecules with superior efficacy and safety profiles (3,4,6,7,8).

This research is significant because it addresses the urgent need for more effective and safer therapeutic options for Alzheimer's disease—a condition with immense global health implications and limited current treatment efficacy. By integrating structure-based virtual screening and molecular docking analyses, this study aims to contribute to the rational design of next-generation AChE inhibitors. The identification of novel rivastigmine analogs with improved pharmacological performance could not only enhance treatment outcomes but also offer valuable insights into the structural determinants of enzyme inhibition, ultimately aiding the development of innovative therapeutics for Alzheimer's disease (3,4,8,9).

Objective

The primary objective of this study was to identify and evaluate novel AChE inhibitors with structural similarity to rivastigmine using comprehensive computational approaches. Specifically, through virtual screening, molecular docking, and detailed interaction analyses, the research aimed to

predict the binding affinity, inhibition constant, and inhibitory potential of selected compounds against AChE. By systematically comparing their binding energies and molecular interaction profiles with those of rivastigmine, this study sought to discover promising lead compounds that may serve as potential candidates for the development of more potent and safer therapeutic agents for Alzheimer's disease.

Materials and Methods

Protein Preparation: The crystal structure of acetylcholinesterase (PDB ID: 6M0E) was obtained from the Protein Data Bank (http://www.rcsb.org). Initially, the enzyme structure was loaded into UCSF Chimera software (https://www.cgl.ucsf.edu/chimera/) for molecular docking preparation. The PDB structure's Chain A, which has 542 amino acid residues, was chosen. Gasteiger charges and hydrogen atoms were introduced after the ligands were eliminated. The constructed enzyme was then saved for docking investigations in PDB format (10).

Virtual Screening and Molecular Docking: Several accessible chemical compound databases have been established, storing millions of chemical molecules. In this study, the PubChem chemical library (https://pubchem.ncbi.nlm.nih.gov/) was used to identify and screen chemical molecules similar to rivastigmine as a reference structure for shape-based screening. The number of chemical compounds that were similar to rivastigmine was lowered to 19 following screening with a Tanimoto coefficient of 95%. Notably, a great place to find intriguing chemicals with possible action against biological targets is the PubChem database. Following the use of the B3LYP/6-31G base set with HyperChem 7.5 software to minimize the structural energy of the molecules, Autodock Vina (PyRx) was used to carry out the second stage of virtual screening. PyRx is a computational drug design tool that uses a sophisticated docking approach for virtual screening (10,11). The ligands with the best binding energy from this phase were selected for the third screening phase, which was conducted through docking of each selected ligand from the previous step using AutoDock4.2 software. It has been shown that AutoDock4.2 performs better than AutoDock Vina in ligand docking analysis (12). Using docking methods, the ability to estimate performance, scoring, and evaluating the protein-ligand interactions can be used to predict binding affinity. The crystal structure 4M0E was used to define the acetylcholinesterase binding site. The binding site was defined after ligand removal from the enzyme's crystal structure and using the central points of the grid box with coordinates: X: -11.56, Y: 1.199, and Z: -6.555, and the grid spacing was set to 0.375 Å. The number of grid points in X, Y, and Z dimensions were chosen to be 44, 46, and 46, respectively, such that the grid box volume was appropriate for each ligand and covered the entire active site. During docking, the protein was considered rigid, while the ligands were flexible. The interactions between the ligand and protein were analyzed using the Genetic Algorithm (GA), a stochastic search algorithm inspired by natural selection and genetics for computational optimization. The number of GA runs for each independent run was fixed at 100. AutoDock's other settings were left at their default settings. For all reference and target ligands, the same grid box size and comparable characteristics were employed. The optimal conformations were found by molecular docking using the Lamarckian genetic method. The outcomes were stored for further docking interaction analysis. LigPlot software was also used to examine the binding interactions between the ligands and the enzyme (10-12).

Results:

Structure-Based Virtual Screening and Molecular Docking: Based on similarity searches against rivastigmine, the reference molecule for therapeutic development, a total of 19 compounds were retrieved from three databases. The PyRx program was utilized for the second stage of structure-based virtual screening. Four ligands with the highest binding energies were chosen as response ligands in this phase, as shown in (Figure 1).

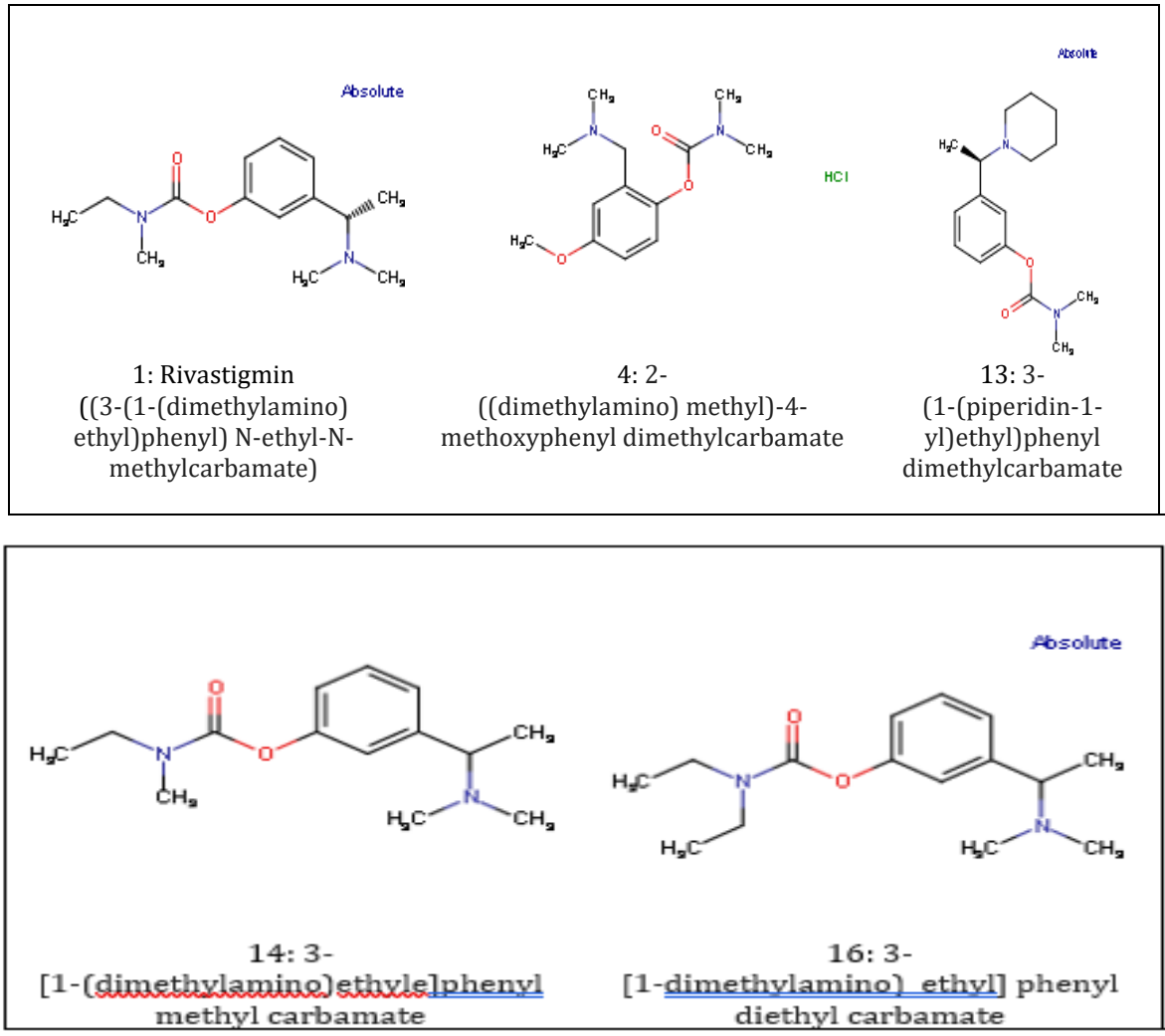


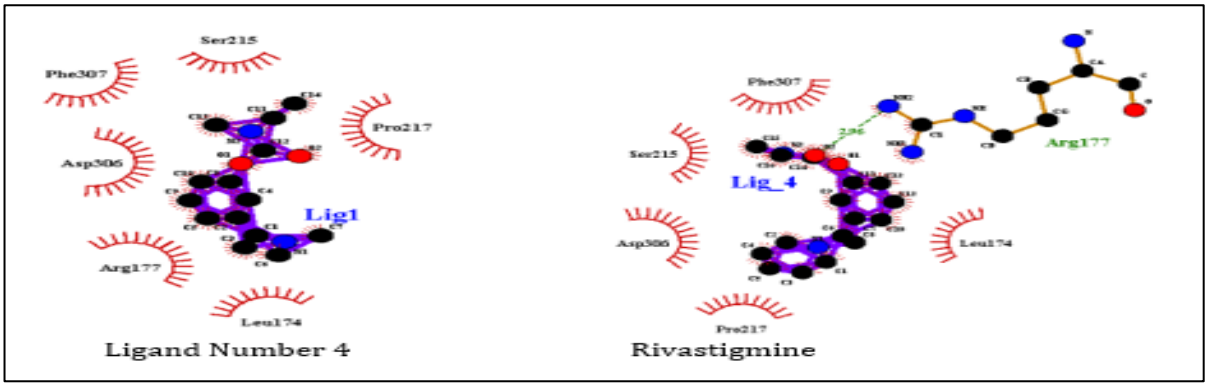
Fig1: Two-dimensional structure of rivastigmine (as the reference ligand), and the top 4 compounds identified through structure-based virtual screening.

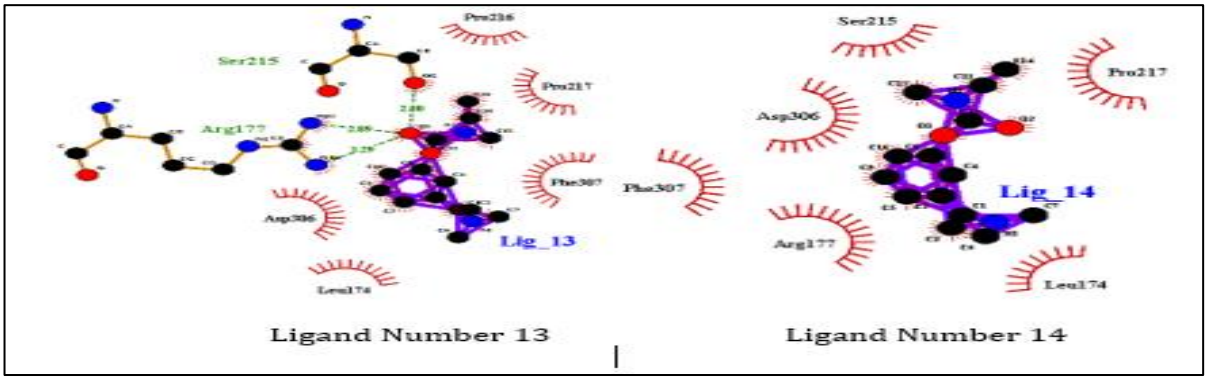
Molecular Docking and Binding Affinity: Next, AutoDock4.2 was used to independently dock each of the four produced ligands and rivastigmine to the binding site of the target protein, acetylcholinesterase. Protein-ligand interactions were ranked according to their strength using the binding energy score, which is a numerical number. Better binding between the ligand and the protein is indicated by higher negative binding energy values. Using the relevant clustering diagram and physical data like the inhibition constant and binding energy, the optimal conformer for docking between each ligand was chosen. In particular, the amount of conformers in each cluster and the binding energy from the clustering diagram for each ligand were used to determine which cluster best matched the ligand's conformers. A conformer would be chosen as the final docking conformer for acetylcholinesterase protein-ligand interactions if it had the lowest values of any physical parameters (binding energy and inhibition constant) in a cluster. Table 1 displays the inhibition constant and binding energy for the compounds in the best cluster.

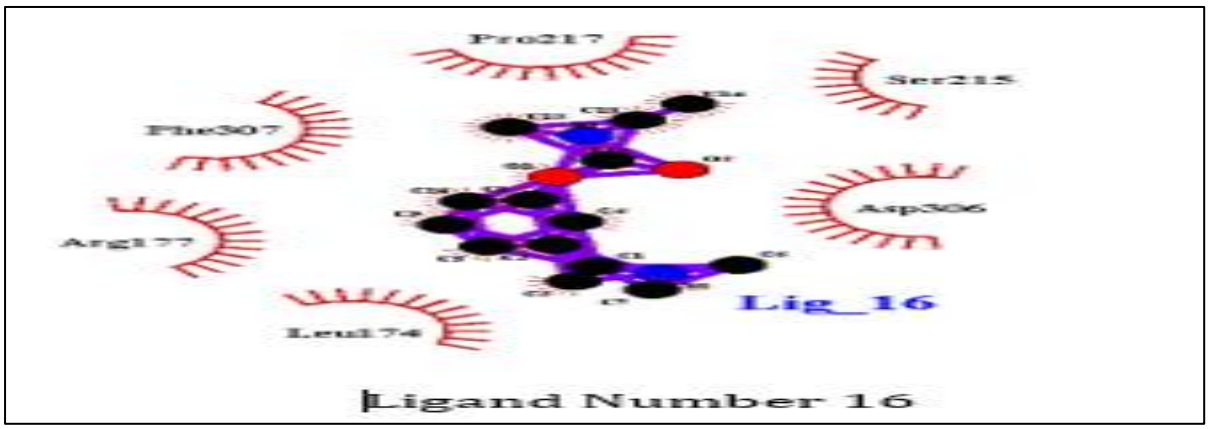
Table 1: Binding Energy and Inhibition Constant of Compounds Against Acetylcholinesterase Enzyme

Ligand Number 16	Ligand Number 14	Ligand Number 13	Ligand Number 4	Rivastigmine	Name Ligand
-4.32	-4.43	-5.07	-4.99	-4.35	Binding Energy(kcal/mol)
240.28	233.37	192.84	221.47	242.92	Inhibition Constant(μm)

As shown in Table 1, Ligand 13 exhibits a binding energy of -5.07 kcal/mol and an inhibition constant of 192.84 µM. These results indicate that Ligand 13 performs better compared to rivastigmine, which has a binding energy of -4.35 kcal/mol and an inhibition constant of 242.92 µM. Therefore, Ligand 13 not only has higher inhibitory potency but also a lower inhibition constant compared to rivastigmine. These differences highlight the high potential of Ligand 13 as a promising alternative for Alzheimer's treatment, particularly in inhibiting acetylcholinesterase enzyme. Interaction Analysis with Acetylcholinesterase: To further investigate the interactions of the ligands with acetylcholinesterase, the molecular interactions were analyzed using Lig Plus software. The results are shown in Figure 2, where the two-dimensional interaction images of each ligand with the enzyme's active site are displayed. Ligand 13 demonstrated the highest binding affinity due to its ability to form three hydrogen bonds with the amino acids Ser215 and Arg177, in addition to hydrophobic interactions. Other ligands, such as Ligand 4, formed only one hydrogen bond, while rivastigmine, Ligand 14, and Ligand 16 mainly formed hydrophobic interactions.







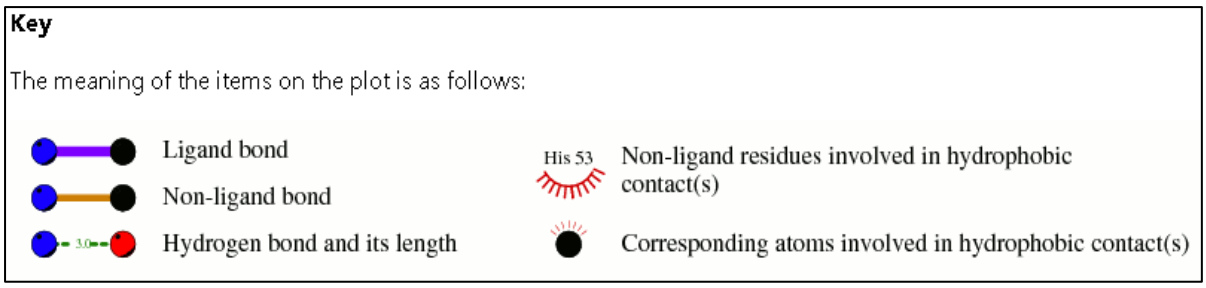


Fig 2: Two-dimensional interaction image of each ligand with the active site of acetylcholinesterase.

Comparative Analysis of Hydrogen Bonding and Hydrophobic Interactions: The number of hydrogen bonds formed by each ligand was crucial in determining their binding affinity. Ligand number 13 formed the most hydrogen bonds, contributing significantly to its higher binding affinity. Figure 2 clearly illustrates the hydrogen bond formations and hydrophobic interactions of each ligand with the enzyme.

Discussion

Alzheimer's Disease and the Need for Effective Treatments: Alzheimer's disease, as one of the most common neurodegenerative disorders globally, requires innovative and effective treatments. The search for compounds with high inhibitory properties against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) has gained significant attention. These enzymes are essential for the normal function of the nervous system, and inhibiting them may help reduce the symptoms and progression of Alzheimer's disease (4,8,12-14).

Significance of Molecular Docking and Virtual Screening: In this study, molecular docking and virtual screening methods were used to identify new inhibitors of AChE. These computational tools are highly efficient for drug design due to their low cost and high speed compared to traditional experimental methods (12,13). The results showed that compound number 13 exhibited superior binding energy and inhibition constant compared to rivastigmine, the standard Alzheimer's treatment (8,12,14).

Role of Hydrogen Bonds in Inhibitory Potency: The contribution of hydrogen bonding to increasing inhibitory efficacy was one of the study's main conclusions. Ligand number 13 had a greater binding affinity and stronger inhibitory potential than other ligands because it was able to make three hydrogen bonds with amino acids Ser215 and Arg177 in the enzyme's active site. In line with other research, this findings highlights the significance of hydrogen bonding in maintaining the stability of the protein-ligand complex (12-16).

Hydrophobic Interactions and Their Role in Binding Affinity: The stability of the enzyme-ligand complex is aided by hydrophobic contacts as well as hydrogen bonds. Together with the extra hydrogen bonds, ligand number 13's hydrophobic interactions remained comparable to those of rivastigmine, greatly enhancing the binding to the enzyme's active site. This implies that maximizing inhibitory efficacy requires a mix of hydrogen bonds and hydrophobic interactions (1,10,15-17).

Comparison with Previous Studies: The results of this study align with those of similar research. For example, Uba and Yelekçi in Turkey in 2018 investigated the structural modifications in rivastigmine through molecular docking and found that adding electron-donating groups to the structure could enhance interactions with the enzyme's active site, thereby increasing binding energy and decreasing the inhibition constant.

This is consistent with the improvements observed in Ligand number 13 (12). Additionally, this study shares similarities with the research conducted by Asma Khalaf Alshamari et al. in Saudi Arabia in 2024, who explored thiouracil derivatives as dual inhibitors of AChE and BChE for Alzheimer's treatment. Both studies observed high inhibitory potential and favorable pharmacokinetic properties for the tested compounds (18).

Limitations and Future Directions: While the results are promising, there are several limitations to this study. The findings from molecular docking are based on computational predictions and must be

validated through in vitro and in vivo studies. Furthermore, the pharmacokinetic and pharmacodynamic properties of Ligand number 13, particularly its permeability across the blood-brain barrier, solubility, and metabolic stability, need to be thoroughly evaluated to assess its clinical feasibility (12,15-18).

Conclusion

AD remains one of the most challenging and impactful neurodegenerative disorders in global healthcare, with no definitive cure currently available. Existing therapeutic agents, including AChE inhibitors such as rivastigmine, primarily provide symptomatic relief by enhancing cholinergic transmission. However, their clinical utility is limited due to adverse effects, modest efficacy, and the need for frequent administration. These challenges emphasize the urgent necessity for the discovery and rational design of novel AChE inhibitors with improved potency, selectivity, and safety profiles. In this context, computational methodologies—particularly virtual screening and molecular docking—offer efficient and cost-effective strategies for drug discovery.

These in silico approaches enable the systematic evaluation of molecular interactions between ligands and target enzymes, allowing accurate predictions of binding affinity, complex stability, and inhibitory potential prior to experimental validation.

In the present study, the crystal structure of acetylcholinesterase was utilized along with advanced computational tools, including PyRx and AutoDock 4.2, to identify and analyze rivastigmine-like compounds. Nineteen candidate molecules were screened based on structural similarity and docking performance. Among these, Ligand 13 exhibited the most favorable docking parameters, characterized by a more negative binding energy and a lower inhibition constant (Ki) compared to rivastigmine, suggesting a higher inhibitory potential toward AChE. Detailed molecular interaction analysis revealed that Ligand 13 formed three hydrogen bonds with active-site residues in addition to hydrophobic interactions, which may account for its enhanced binding affinity and inhibitory potency. In contrast, rivastigmine and most other ligands primarily engaged in hydrophobic contacts or formed fewer hydrogen bonds. These findings highlight the potential of structural modification of rivastigmine to enhance its pharmacological and physicochemical properties. Moreover, the outcomes of molecular docking provide valuable insights that can guide subsequent experimental and in vitro validation studies, expediting the early stages of drug development. Overall, this study demonstrates the effectiveness of computational approaches in identifying novel AChE inhibitors with promising therapeutic potential for Alzheimer's disease. By integrating virtual screening, molecular docking, and interaction analysis, this research contributes to a rational framework for designing next-generation cholinesterase inhibitors. Ultimately, such strategies may help reduce the global burden of Alzheimer's disease and improve patients' quality of life through the development of safer and more effective therapeutic agents.

References

1. Kamatham PT, Shukla R, Khatri DK, Vora LK. Pathogenesis, diagnostics, and therapeutics for Alzheimer's disease: Breaking the memory barrier. Ageing Research Reviews. 2024; 101:102481.
2. Knopman DS, Amieva H, Petersen RC, Chételat G, Holtzman DM, Hyman BT, Nixon RA, Jones DT. Alzheimer disease. Nature reviews Disease primers. 2021;7(1):33.
3. Castellani RJ, Rolston RK, Smith MA. Alzheimer disease. Disease-a-month: DM. 2010; 56(9):484.
4. Peitzika SC, Pontiki E. A review on recent approaches on molecular docking studies of novel compounds targeting acetylcholinesterase in Alzheimer disease. Molecules. 2023 Jan 21; 28(3):1084.
5. Dighe SN, De la Mora E, Chan S, Kantham S, McColl G, Miles JA, Veliyath SK, Sreenivas BY, Nassar ZD, Silman I, Sussman JL. Rivastigmine and metabolite analogues with putative Alzheimer's disease-modifying properties in a Caenorhabditis elegans model. Communications Chemistry. 2019;2(1):35.
6. Gottwald MD, Rozanski RI. Rivastigmine, a brain-region selective acetylcholinesterase inhibitor for treating Alzheimer's disease: review

and current status. Expert opinion on investigational drugs. 1999;8(10):1673-82.
7. Zhang J, Zhang Y, Wang J, Xia Y, Zhang J, Chen L. Recent advances in Alzheimer's disease: Mechanisms, clinical trials and new drug development strategies. Signal transduction and targeted therapy. 2024;9(1):211.
8. Thai QM, Pham TN, Hiep DM, Pham MQ, Tran PT, Nguyen TH, Ngo ST. Searching for AChE inhibitors from natural compounds by using machine learning and atomistic simulations. Journal of Molecular Graphics and Modelling. 2022; 115:108230.
9. Jang C, Yadav DK, Subedi L, Venkatesan R, Venkanna A, Afzal S, Lee E, Yoo J, Ji E, Kim SY, Kim MH. Identification of novel acetylcholinesterase inhibitors designed by pharmacophore-based virtual screening, molecular docking and bioassay. Scientific reports. 2018;8(1):14921.
10. Mahmood Janlou MA, Sahebjamee H, Yazdani M, Fozouni L. Structure-based virtual screening and molecular dynamics approaches to identify new inhibitors of Staphylococcus aureus sortase A. Journal of Biomolecular Structure and Dynamics. 2024;42(3):1157-69.
11. Allouche AR. Gabedit—A graphical user interface for computational chemistry softwares. Journal of computational chemistry. 2011; 32(1): 174-82.
12. Uba AI, Yelekçi K. Carboxylic acid derivatives display potential selectivity for human histone deacetylase 6: Structure-based virtual screening, molecular docking and dynamics simulation studies. Computational Biology and Chemistry. 2018; 75:131-42.
13. Walczak-Nowicka ŁJ, Herbet M. Acetylcholinesterase inhibitors in the treatment of neurodegenerative diseases and the role of acetylcholinesterase in their pathogenesis. International journal of molecular sciences. 2021; 22(17):9290.
14. Thawabteh AM, Ghanem AW, AbuMadi S, Thaher D, Jaghama W, Karaman D, Karaman R. Recent Advances in Therapeutics for the Treatment of Alzheimer's Disease. Molecules. 2024; 29(21):5131.
15. Li Q, Yang H, Chen Y, Sun H. Recent progress in the identification of selective butyrylcholinesterase inhibitors for Alzheimer's disease. European journal of medicinal chemistry. 2017; 132:294-309.
16. Thandivel S, Rajan P, Gunasekar T, Arjunan A, Khute S, Kareti SR, Paranthaman S. In silico molecular docking and dynamic simulation of anti-cholinesterase compounds from the extract of Catunaregam spinosa for possible treatment of Alzheimer's disease. Heliyon. 2024;10(7).
17. Martins MM, Branco PS, Ferreira LM. Enhancing the therapeutic effect in Alzheimer's disease drugs: The role of Polypharmacology and Cholinesterase inhibitors. ChemistrySelect. 2023;8(10):e202300461.
18. Alshamari AK, Hassan NA, Alshammari OA, Basiony EA, Alshammari MZ, Hassan AA. Synthesis, Biological Evaluation, Molecular Docking, Molecular Dynamics, and ADME Studies of Novel Thiouracil Derivatives as Dual Inhibitors of Butyrylcholinesterase and Acetylcholinesterase Enzymes. Journal of Molecular Structure. 2024:141154.