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Ligand-based Virtual Screening. Identification of Potential Acetylcholinesterase Inhibitors

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Abstract: *The primary purpose of this paper was to find new possible acetylcholinesterase inhibitors by utilizing molecular docking to assess the binding affinity of 30 distinct ligands to the AChE protein with the PDB ID: 6f25. The ligands were identified using SwissSimilarty and starting from the chemical structure of donepezil. The Discovery Studio package was used to evaluate ligand-protein interactions, while AutoDock Vina was employed for molecular docking. The reference ligand had an affinity of -7.8 Kcal/mol for the protein. Out of the 30 ligands, ZINC000032101422 had the highest affinity towards the protein (-10.4 Kcal/mol), followed by ZINC000049037726 with an affinity of -10.1 Kcal/mol and ZINC000034964947 with an affinity of -9.9. These findings suggest that these chemicals have the potential to be effective acetylcholinesterase inhibitors. In-silico studies provide a foundation for identifying potential compounds, but it is essential to validate these findings through further studies.*

Keywords*: acetylcholinesterase, donepezil, molecular docking, ligand-based virtual screening*

1. Introduction

Alzheimer's disease (AD) is a chronic illness progressively affecting multiple brain areas. AD is associated with the accumulation of amyloid-β (Aβ) peptide and protein tau aggregation as neurofibrillary tangles in neurons [1]. Modifications in neurons, microglia, and astroglia contribute to the disease's progression long before cognitive decline appears [2]. There have been significant developments in the understanding of the molecular basis of the disease in recent years, which has led to the identification of potential biomarkers for diagnosis, risk assessment, treatment trials, therapeutic targets, and novel drug targets. Despite advances in Alzheimer's research, clinical evaluation of cognitive ability remains the primary method of identifying the condition [3].

Acetylcholine (Ach) is necessary for optimal brain function. People with low ACh show a gradual cognitive decline (thinking, memory, and reasoning). Augmenting cholinergic neurotransmission remains the primary strategy for addressing the cognitive and behavioural symptoms that are associated with mild and moderate AD [4]. The drugs that improve cholinergic neurotransmission are called acetylcholinesterase inhibitors (AChEIs). The primary mechanism behind these drugs consists of inhibiting the breakdown of acetylcholine. The first drug of this class, tacrine, was approved by the FDA in 1993, with studies beginning as early as 1984. In 2013 the drug was withdrawn from use, and currently, the representative drugs of this class are donepezil, rivastigmine, and galantamine. These drugs act by slowing the cognitive decline associated with AD. In the search for better AChEIs, there were multiple attempts to modify the structure of tacrine [5].

Molecular docking is a popular computational tool in drug discovery and development. Molecular docking studies give us an image of the interactions between proteins and small molecules. This approach illuminates the behaviour of small molecules in the binding sites of target proteins. Molecular docking involves two stages: first, predicting the ligand's location and orientation within the binding site of the protein, and second, evaluating the binding affinity of the ligand for the target protein. It can aid researchers in identifying novel drug candidates, optimizing their potency and selectivity, and understanding the mechanism of action of current medications. Molecular docking is also used in the process of screening large libraries of small compounds against many targets, molecular docking offers

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a cost-effective and time-efficient alternative to experimental approaches [6,7]. Ligand-based virtual screening (LBVS) prioritizes candidate molecule selection based on similarity to an active ligand. By comparing the similarity of prospective ligands to known active chemicals, LBVS can be used to find novel ligands [8].

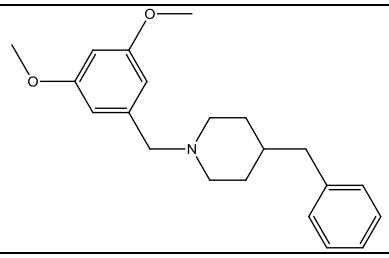
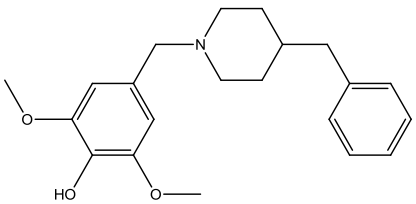
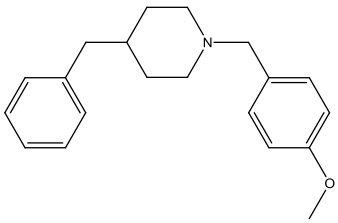
This study examines donepezil, an FDA-approved drug for treating mild to severe (AD). Donepezil is associated with side effects such as gastrointestinal issues and muscle weakness. Several molecular docking studies have focused on derivatives of donepezil to identify potential therapeutic agents for AD. For example, one study explored the replacement of donepezil's piperidine ring with different rings found in natural compounds. Another study aimed to discover new AChE inhibitors derived from donepezil, and it identified two compounds, IP-13 and IP-15, that have better inhibitory potential than donepezil [9, 10].

As effective treatments for AD are lacking, the search for potential therapeutic agents remains of utmost importance. This study aimed to identify potential AChEIs by evaluating the binding affinity of 30 ligands with chemical structures similar to donepezil. The ligands were identified using the SwissSimilarity tool [11, 12]. The molecular docking studies were conducted using AutoDock Vina 1.5.7 software [13]. The ligand-protein interactions were explored using the Discovery Studio suite [14].

2. Materials and methods

2.1. Ligand selection and ligand preparation

Starting from the chemical structure of donepezil and using the SwissSimilarity suite, which searches for molecules that are structurally similar to a reference molecule, a ligand-based virtual screening was performed, identifying 30 chemical compounds from the ZINC Drug-like database [15]. The database was searched using the extended connectivity circular molecular fingerprint screening method. The ligands were downloaded from the ZINC database and converted to a compatible file format using the Babel suite [16]. For reference, the chemical structure of donepezil was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in the .sdf format and then translated to a compatible format using Babel. Next, the ligands underwent a process of adding rotatable bonds using AutoDock Vina version 1.5.7. The chemical structures of the identified compounds are presented in Figure 1, providing a visual representation of the identified ligands and their structural similarity to donepezil.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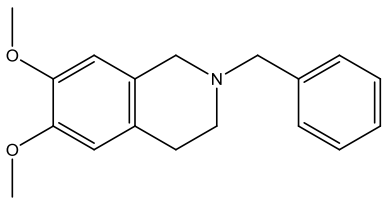	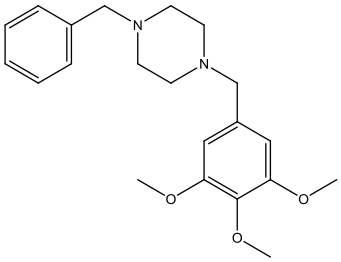	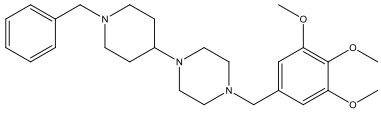
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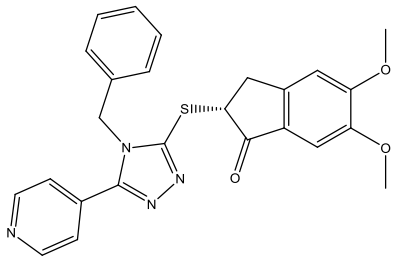	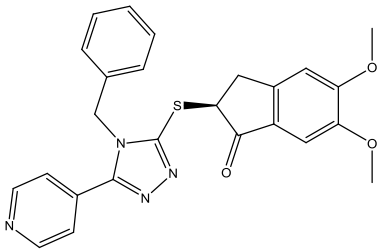	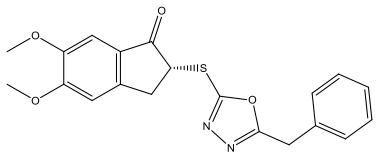
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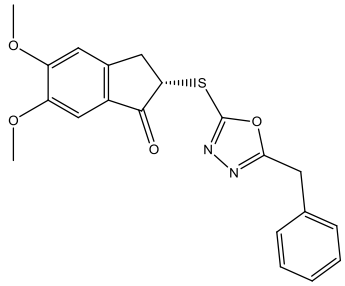	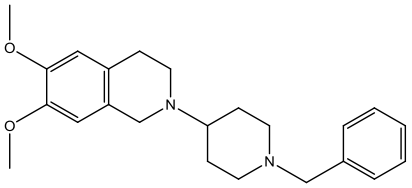	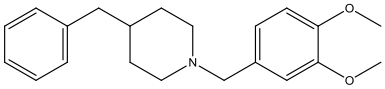
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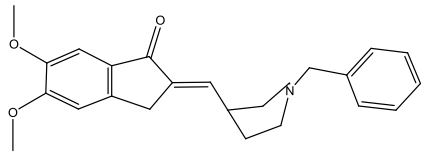	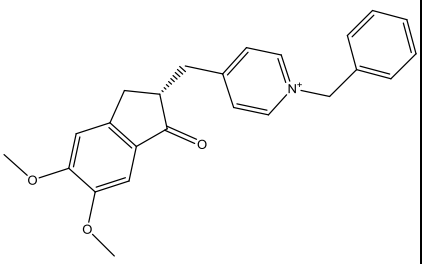	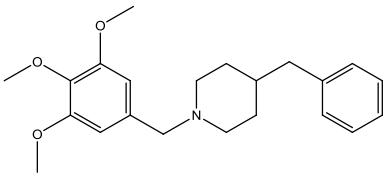
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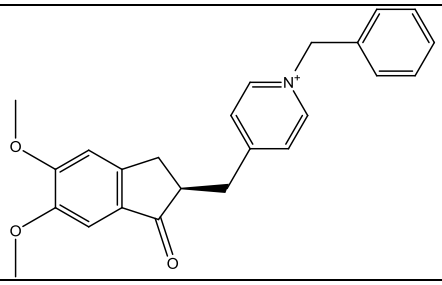	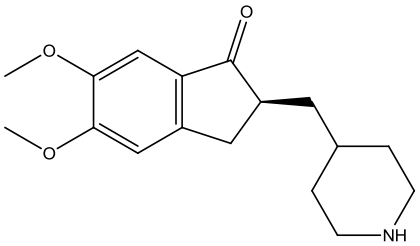	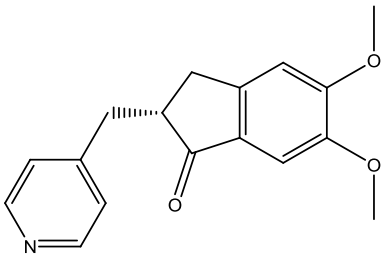
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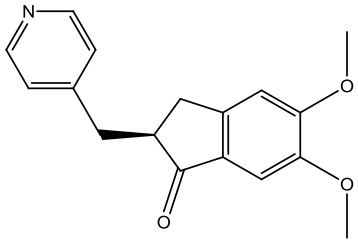	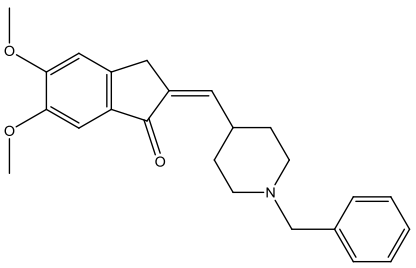	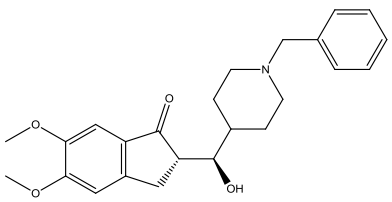
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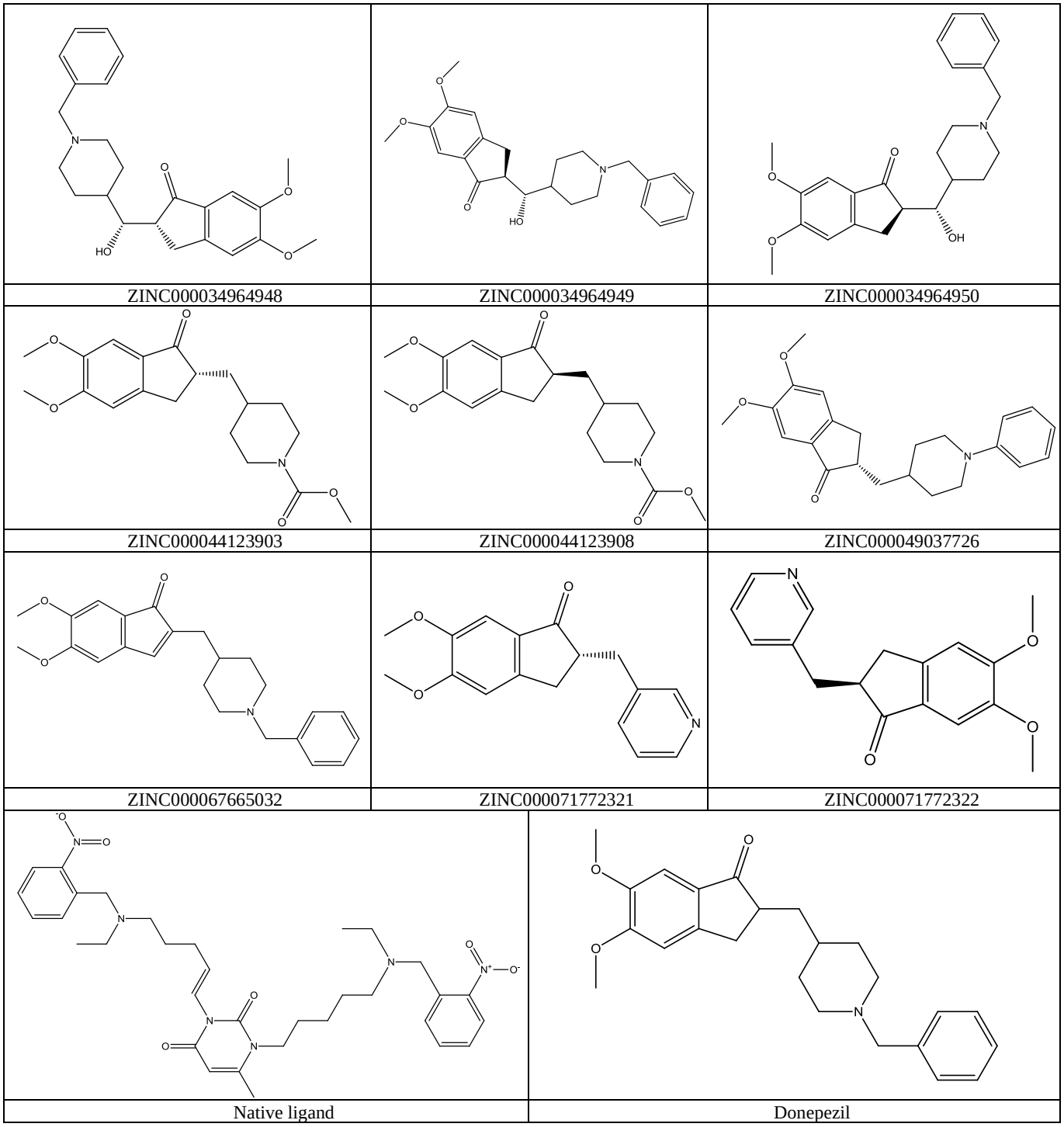


Figure 1. The chemical structure of the investigated compounds, the native ligand, and the reference ligand, donepezil

2.2. Protein selection and preparation

The protein of interest (AChE) was downloaded as a .pdb file from the Protein Data Bank (a large database of protein structures). Before performing molecular docking, all co-crystalized ligands and water molecules were removed using the Molegro Molecular Viewer 2.5 software (http://molexus.io/). These molecules must be removed to avoid undesired interactions with the bound ligands. After this process, the protein was imported into AutoDockVina, further optimizing the structure for the molecular docking process. The optimization procedure often involves the addition of missing hydrogen atoms, assigning correct charges to protein atoms, and optimizing the protein structure by reducing energy

utilizing force fields. After this process, the protein may be saved as a PDBQT file, which is the file format required by AutoDock Vina for docking simulations. This file contains the protein atoms' 3D coordinates and information on the atom types, partial charges, and other parameters necessary for the molecular docking simulation. Figure 2 presents the 3D representation of the target protein.

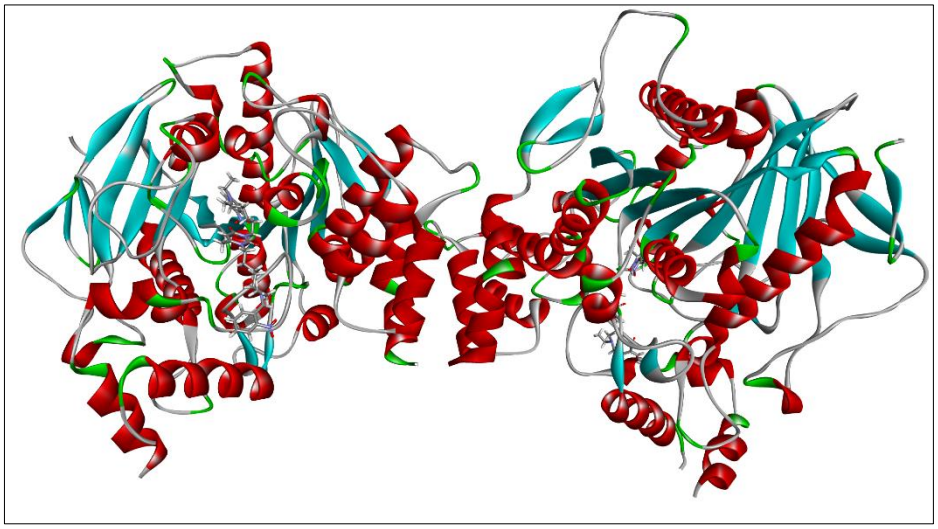


Figure 2. 3D representation of AChE

2.3. Molecular docking

The molecular docking method includes predicting a ligand's configuration when it binds to a target protein. The ligands and target protein were prepared and inputted into AutoDock Vina. The docking grid was determined based on the target protein's active site. After the docking simulation, the ligand-protein interactions were analyzed using the Discovery Studio suite, which allows for the visualization of the binding modes of the ligands within the active site of the protein. [10]. The docking procedure's accuracy was determined by determining the root-mean-square (RNSD) deviation between the native ligand, and the re-docked native ligand, a lower RMSD value indicates a more accurate docking result. The binding affinities of the investigated compound were compared to the reference ligand (donepezil), investigating the potential of these compounds as AChEIs.

3. Results and discussions

3.1. Molecular docking validation

The AChE protein selected for this molecular docking study has the PDB ID 6f25. The grid box was set to X=192.15, Y=89.74, and Z=6.98, with a size of 60x60x60. The native ligand of 6f25, known as C35, was used for validation. The native ligand of 6f25, C35, was used as a reference molecule for validation purposes. The RMSD value between the native ligand and the re-docked ligand was calculated to be 1.53 Å, which falls within the acceptable range reported in the literature [17]. The binding affinities of the investigated ligands were obtained and are presented in Table 1, providing valuable insights into their potential as AChEIs.

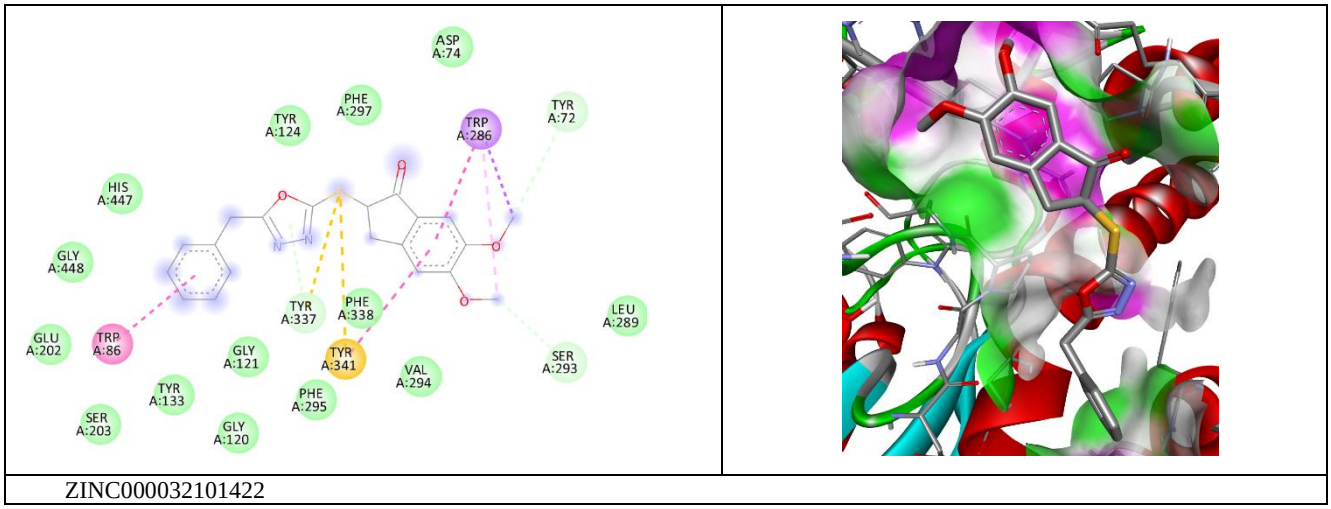
Table 1. Table type styles (Table caption is indispensable).

Compound	Binding affinity Kcal/mol	Compound	Binding affinity Kcal/mol
C35	-6.4	ZINC000004689710	-7.9
ZINC000000210773	-6.4	ZINC000026657992	-8.0
ZINC000044123903	-6.6	ZINC000032100613	-8.0
ZINC000021989894	-6.7	ZINC000071772322	-8.6

ZINC000022055510	-6.7	ZINC000034964949	-8.7
ZINC000032101417	-6.8	ZINC000013744635	-8.8
ZINC000000240265	-7	ZINC000000597028	-8.9
ZINC000000603084	-7.0	ZINC000021989891	-8.9
ZINC000019831630	-7.2	ZINC000000819183	-9.0
ZINC000071772321	-7.5	ZINC000019537660	-9.0
ZINC000034964950	-7.6	ZINC000003964923	-9.5
ZINC000032100616	-7.7	ZINC000044123908	-9.6
ZINC000034964948	-7.7	ZINC000000240780	-9.8
Donepezil	-7.8	ZINC000034964947	-9.9
ZINC000004990243	-7.8	ZINC000049037726	-10.1
ZINC000057523201	-7.8	ZINC000032101422	-10.4

The virtual screening experiment revealed the binding affinity (in kcal/mol) of the selected ligands to the target protein, with donepezil serving as a control molecule. The binding affinities values represent the intensity of the interaction between the ligands and the target protein, with lower values suggesting greater binding.

Of the 30 investigated ligands, 16 showed a higher affinity towards the protein than the reference ligand, ZINC000032101422 with a binding affinity of -10.4 kcal/mol, ZINC000049037726 with a binding affinity of -10.1 kcal/mol, and ZINC000034964947 with a binding affinity of -9.9 kcal/mol had the highest binding affinity. On the other hand, 13 compounds showed weaker binding affinity than donepezil, such as C35 (the native ligand), with a binding affinity of -6.4 kcal/mol. Two of the investigated ligands (ZINC000004990243, ZINC000057523201) had the same binding affinity for the protein as the reference ligand. Figure 3 presents the 2D and 3D diagrams of the compounds with a high affinity towards the protein, the native ligand, and donepezil.



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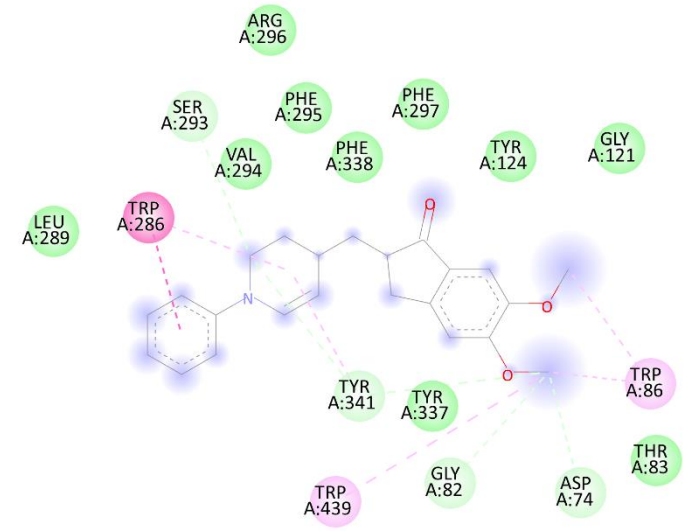

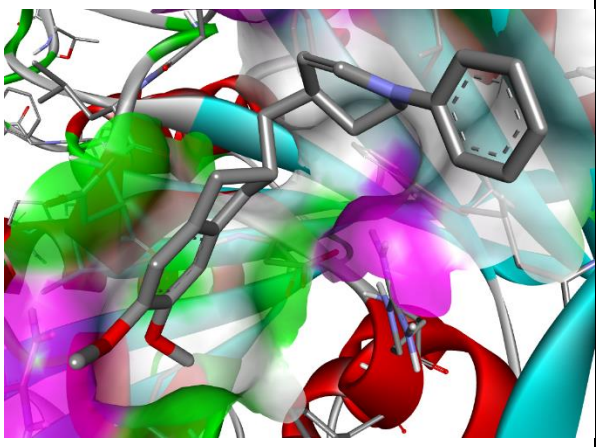

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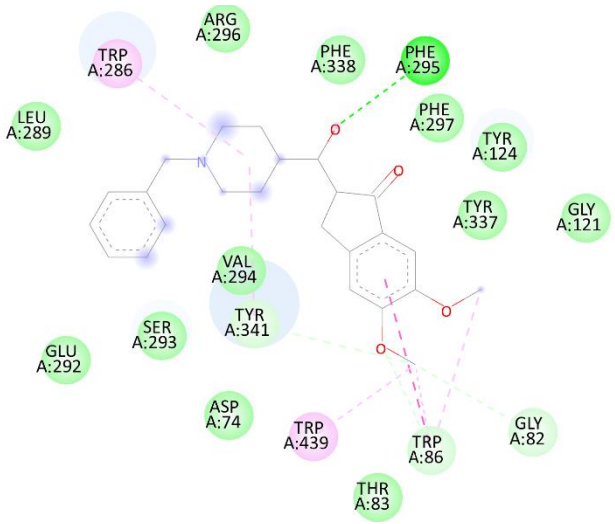

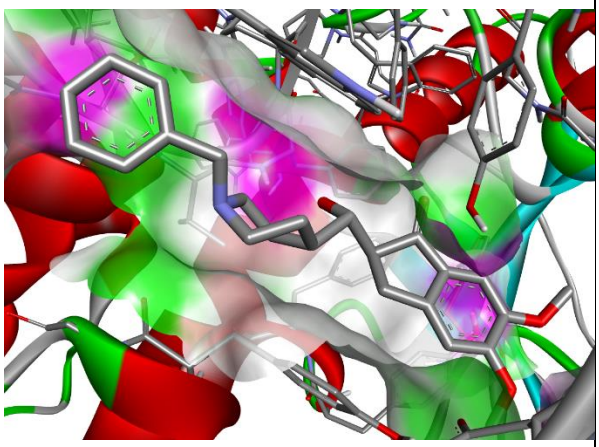

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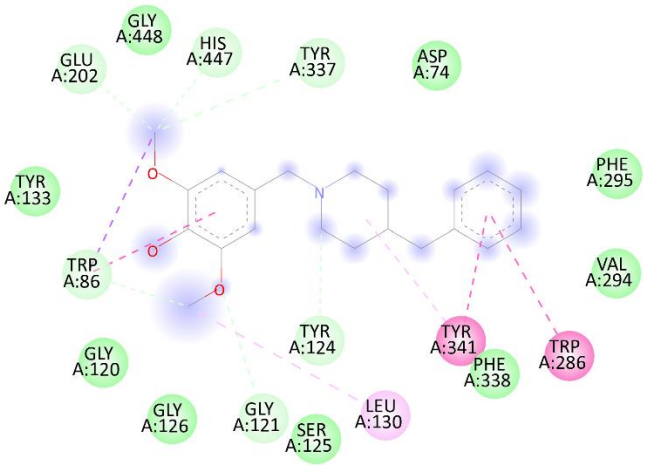

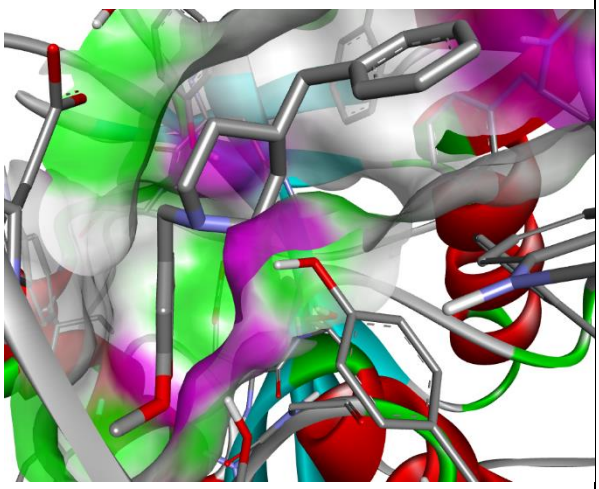

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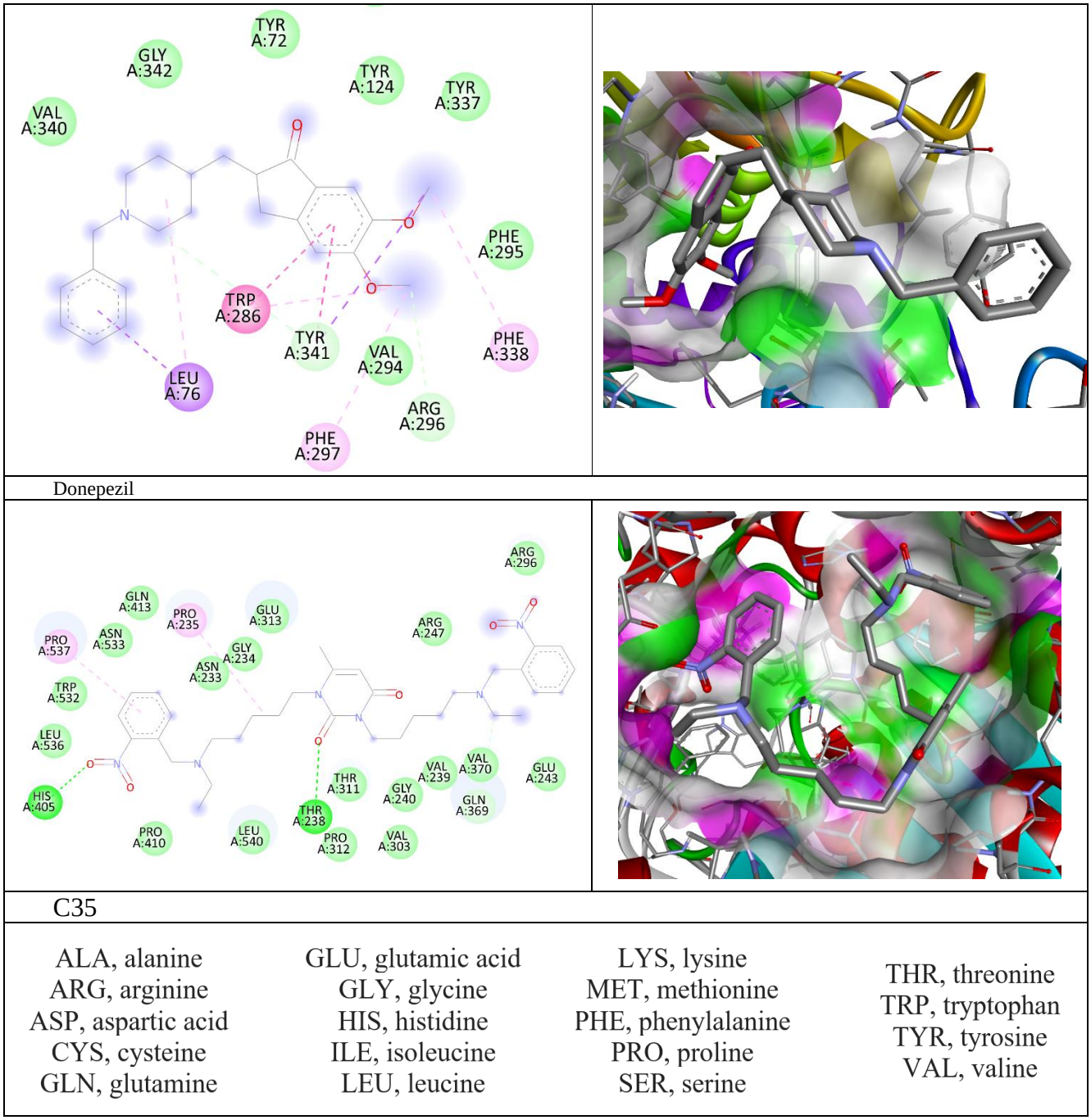

Figure 3. The compounds that had the highest affinity towards 3CLpro. 2D-left 3D-right

The highest affinity compound binds to the following amino acids: TRP286, TYR72, SER293, TYR341, TYR337, and TRP86. ZINC000049037726 binds to TRP86, ASP74, GLY82, TRP439, TYR341, TRP286, and SER293. ZINC000034964947 binds to TRP286, PHE295, GLY82, TRP86, TRP439, and TYR341. ZINC000000240780 binds to TRP286, TYR341, LEU130, TYR124, GLY121, TRP86, GLU202, HIS447, and TYR337. Donepezil, a reversible acetylcholinesterase inhibitor, binds to the following amino acids: PHE338, ARG296, PHE297, TYR341, TRP286, and LEU76. The native ligand of the protein binds to PRO235, PRO537, HIS405, and THR238.

4. Conclusions

Because of the absence of viable therapies, AD remains a big issue in the field of medicine. This paper highlights the utility of in-silico techniques in the drug development process, notably ligand-based

virtual screening and molecular docking investigations. This study identified 16 chemicals with greater binding affinity to AChE than donepezil, a routinely used AChEI in the treatment of AD. These findings imply that these compounds have potential in the treatment of AD. Nonetheless, 13 molecules, including the native ligand, had lower binding affinity than donepezil. The use of ligand-based virtual screening, combined with molecular docking studies, provides a powerful approach for identifying potential drug candidates and designing new molecules with improved pharmacological properties. Although there are drawbacks to in-silico studies, such as a lack of trust in the scoring function and the difficulties in simulating human physiological conditions, the addition of artificial intelligence technologies is expected to enhance these approaches in the near future dramatically. As a result, in-silico studies remain a valuable tool for drug development, with the potential to create and find new therapeutic agents for AD and other disorders.

References

1. MASTERS, C., BATEMAN, R., BLENNOW, K. et al., (2015), Alzheimer's disease. Nat Rev Dis Primers, 1, 15056. https://doi.org/10.1038/nrdp.2015.56
2. SCHELTENS, P., DE STROOPER, B., KIVIPELTO, M., HOLSTEGE, H., CHÉTELAT, G., TEUNISSEN, C. E., CUMMINGS, J., VAN DER FLIER, W. M., (2021), Alzheimer's disease. The Lancet, 397(10284), 1577-1590. https://doi.org/10.1016/S0140-6736(20)32205-4
3. WOLFE, M. S., (2018), The molecular and cellular basis of neurodegenerative diseases: underlying mechanisms. Academic Press.
4. MARUCCI, G., BUCCIONI, M., DAL BEN, D., LAMBERTUCCI, C., VOLPINI, R., AMENTA, F., (2021), Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease. Neuropharmacology, 190, 108352. https://doi.org/10.1016/j.neuropharm.2020.108352
5. CUI, X., GUO, Y., FANG, J., et al., (2019), Donepezil, a drug for Alzheimer's disease, promotes oligodendrocyte generation and remyelination. Acta Pharmacol Sin, 40, 1386-1393. https://doi.org/10.1038/s41401-018-0206-4
6. MENG XY, ZHANG HX, MEZEI M, CUI M., Molecular docking: a powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des. 2011 Jun;7(2):146-57. doi: 10.2174/157340911795677602. PMID: 21534921; PMCID: PMC3151162.
7. MORRIS GM, LIM-WILBY M., Molecular docking. Methods Mol Biol. 2008;443:365-82. doi: 10.1007/978-1-59745-177-2_19. PMID: 18446297.
8. HAMZA A, WEI NN, ZHAN CG., Ligand-based virtual screening approach using a new scoring function. J Chem Inf Model. 2012 Apr 23;52(4):963-74. doi: 10.1021/ci200617d.
9. SAHOO, R. K., (2022), Acetylcholinesterase (AchE) receptor-based in-silico drug discovery for Alzheimer's Disease. ChemRxiv. https://doi.org/10.26434/chemrxiv.16642406.v1
10. MISHRA, C. B., KUMARI, S., MANRAL, A., PRAKASH, A., SAINI, V., LYNN, A. M., TIWARI, M., (2017), Design, synthesis, in-silico and biological evaluation of novel donepezil derivatives as multi-target-directed ligands for the treatment of Alzheimer's disease. Eur J Med Chem, 125, 736-750. https://doi.org/10.1016/j.ejmech.2016.09.057
11. BRAGINA, ME., DAINA, A., PEREZ, MAS., MICHIELIN, O. ZOETE, V., SwissSimilarity 2021 Web Tool: Novel Chemical Libraries and Additional Methods for an Enhanced Ligand-Based Virtual Screening Experience., Int. J. Mol. Sci, 2022, 23(2), 811.
12. ZOETE, V., DAINA, A., BOVIGNY, C., MICHIELIN, O. SwissSimilarity: A Web Tool for Low to Ultra High Throughput Ligand-Based Virtual Screening., J. Chem. Inf. Model., 2016, 56(8), 1399.
13. TROTT, O., OLSON, A. J., (2010), AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem, 31(2), 455-461. https://doi.org/10.1002/jcc.21334
14. BIOVIA, Dassault Systèmes, (2023), Discovery Studio. San Diego: Dassault Systèmes.
15. IRWIN JJ, SHOICHET BK. ZINC--a free database of commercially available compounds for virtual screening. J Chem Inf Model. 2005 Jan-Feb;45(1):177-82. doi: 10.1021/ci049714+.

16. BOYLE, N. M., BANCK, M., JAMES, C. A., et al. (2011), Open Babel: An open chemical toolbox. J Cheminform, 3, 33. https://doi.org/10.1186/1758-2946-3-33
17. RAMÍREZ, D., CABALLERO, J., (2018), Is it reliable to take the molecular docking top scoring position as the best solution without considering available structural data? Molecules, 23(5), 1038. https://doi.org/10.3390

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