RECORDSOF CHEMICAL SCIENCES

ADME-TOX and Ligand-Protein Interaction of *P*-Substituted (E)-benzylidenchroman-4-ones Derivatives for the Treatment of Dementia

[1,2] *Maryam Ismail Usman*

https://doi.org/10.33003/frscs_2024_0304/02

Accepted: 15th Nov, 2024
Published: 12th Dec, 2024

1. Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu-Bello University, Zaria
2. Department of Chemistry, Al-Qalam University, Katsina, Katsina State.

**Corresponding Author:*
Maryam Ismail Usman
greatmaryam85@gmail.com

FRsCS Vol.3 No. 4 (2024)
Official Journal of Dept. of Chemistry, Federal University of Dutsin-Ma, Katsina State.
http://rudmafudma.com

ISSN (Online): 2705-2362
ISSN (Print): 2705-2354

Abstract

Dementia remains a significant health challenge affecting elderly individual worldwide. The development of novel Acetylcholinesterase inhibitors (AChEI) is crucial in the fight against dementia. This study aims to evaluate the ADMET and ligand-protein interactions of Benzylidenechroman-4-one derivatives as potential AChE inhibitors against Dementia through in silico methods. The SwissADME platform was utilized for drug-likeness and toxicity predictions, assessing ADME parameters and pharmacokinetic properties. ProTox 3.0 web tool was employed for toxicity assessment. Molecular docking analysis was conducted using AutoDock Vina with the crystal structures of target proteins AChE. The interactions were analyzed using BIOVIA Discovery Studio. The compounds demonstrated strong binding affinities to the active sites of the target proteins, suggesting effective inhibition capabilities. Theoretical oral bioavailability was promising based on Lipinski's rule of five and GI absorption. Toxicity predictions indicated low toxicity for the ligands. The molecular docking study suggests that the Benzylidenechroman-4-one derivatives are promising candidates as AChE inhibitors against Dementia. The compounds exhibit strong binding affinities and specific interactions with key residues, indicating potential as AChE drugs. Experimental validation is required to confirm these in silico predictions.

Keywords: Dementia, Acetylcholinesterase Inhibitory activity, Benzylidenechroman-4-ones, Molecular docking, Pharmacokinetic and Interaction studies

Introduction

Dementia is commonly known as aging disease characterized by progressive decline in cognitive function and behavioral disorders among elderly people especially 65 years and above are affected by this neurodegenerative disorder (Piacentini, et al 2014; Zheng et al 2016; Peauger et al., 2017). It is presumed that, in the nearing 2 decades, the rate of dementia patients will be highly elevated (Dhingra and Kumar, 2012). The cause of AD is doubtful however, it is evident from literature, that low level of neurotransmitters, especially acetylcholine, amyloid-beta (Aβ) aggregates, oxidative stress, and the concentrations of metals interdependently play key roles in the neurodegeneration process (Akrami, et al., 2014). The cholinergic system, beta amyloid (Aβ) protein, tau protein, oxidative stress, inflammation, and toxic metal ions are the main hypotheses that explain the causes and progression of AD. In line with these hypotheses, increasing cholinergic activity with cholinesterase inhibitory compounds, inhibiting Aβ aggregate formation, and reducing the hyperphosphorylation of tau protein are the main treatment approaches for the disease (Begüm, 2020). The primary target of dementia is acetylcholinesterase, which is the enzyme that catalyse the hydrolyses

and ester. Other targets include Butylcholinesterase and Monoamine oxidase (MAO-B). Colovic et al., 2013 reported that increasing levels of acetylcholine in the brain of dementia patients increases inhibition activity. Acetylcholinesterase is responsible for cerebral blood flow modulation, tau protein phosphorylation, fibrillary tangle, and beta-amyloid aggregation. Acetylcholine (ACh) is the naturally occurring neurotransmitter found in the nervous, muscles, and central nervous system, and its hydrolysis into choline and acetic acid is catalyzed by acetylcholinesterase (AChE). The cholinergic hypothesis is based on the presumption that the inhibition of AChE would prevent the hydrolysis of ACh so that the level of ACh in cholinergic synapses is increased. Both cholinesterases belong to a large protein family containing the α/β hydrolase fold. (Marek, et al., 2013). Benzylidenechroman-4-one is a promising scaffold used for the design of new remedies for the treatment of this disease. Literature evidence reveals that both synthetic and natural homoisoflavonoids exhibit many biological activities including anti-microbial, anti-cancer, anti-inflammatory, anti-oxidant, antifungal, anti-inflammatory, antiproliferative, antihistamic, antiallergic, antiviral, phosphodiesterase isoenzyme-inhibiting, antimutagenic, and protein tyrosine kinase (PTK) inhibitor activities ((Roy, et al., 2013; Namdar et al. 2013). A few drugs such as donepezil, rivastigmine, galanthamine, and tacrine, were developed for the treatment of dementia and another form of Alzheimer's disease. Unfortunately known of these remedies were found to alleviate the disease completely. However, they increase the availability of ACh in the synaptic cleft, which is capable of reversing the scopolamine-induced cognitive deficit and impairment of learning and memory (Santanu, et al 2020). However, tacrine is associated with hepatotoxicity thus it is rarely used. On the other hand, donepezil and rivastigmine which are commonly used in the early-to-moderate stages of AD often present adverse effects and are not completely effective (Colovic et al., 2013). However, clinical trial studies revealed that galantamine shows a promising pharmacological profile and clinically relevant neuroprotective effects in AD (Akrami et al., 2014). This research aimed to evaluate the Binding Affinity, predict ADMETox, and study ligand interaction with existing amino acid residues of protein (4MOE) for the treatment of dementia. The 3D crystal structure of Protein (Recombinant Human Acetylcholinesterase in Complex with 9 derivatives of Benzylidenechroman-4-one) was used to perform docking. The molecular docking was performed using Autodock Vina and BIOVIA Discovery Studio.

Materials and Methods

Softwares

Autodock Tools (Trott and Olson, 2011), UCSF Chimera (Petersen et al. 2004), Swiss ADME (webserver), Protox-II (webserver), Discovery Studio 2021, Spartan 14 ChemDraw Ultra 12.0, and Cygwin64 Terminal, and Pubchem.

Protein Preparation

The 3D structure of the protein (Acetylcholinesterase – 4MOE) was retrieved from the Protein Data Bank in the RCSB (Research Collaboratory For Structural Bioinformatics) site in PDB Format. Before molecular docking, a protein was prepared using the BIOVIA Discovery Studio software package and UCSF Chimera. During preparation polar hydrogens, proper bonds, bond orders, hybridization, and charges were added and water molecules and heteroatoms were removed from the protein crystal structure for the prevention of unwanted interaction while docking was done using AutoDock Vina. A structure-based in silico

procedure was applied to discover the binding mode of the ligands using Grid parameters (_x = 26, y = 14, and z = 18). Autodock tools were used to add hydrogen and partial charges for protein and ligands using Gasteiger charges (Sefa et al., 2022: Karthika et al., 2020).

Ligand Preparation

The library of bioactive molecules with drug-likeness properties was retrieved using PubChem and ChEMBL Database and filtered based on activity values and criteria like, Target protein, assays, molecular properties (e.g., molecular weight, LogP), and IC50. The compounds were exported in SMILES format (SDF or CSV). The SMILES were converted to structures Using ChemDraw. Optimization and energy minimization calculations of the 3D structure were done using Spartan 14 and the minimized structures were converted to PDBQT using Autodock vina (Sefa et al., 2022; Karthika et al., 2020).

Virtual Screening (Using SwissADME and Protox II webservers)

ADME-Tox prediction was predicted by the methods adopted by Priyanka et al., 2018 and Karthika et al., 2020. These online tools predict the (Absorption, Distribution, Metabolism Excretion, and Toxicity) properties of the chemical structure of the ligands (Karthika et al., 2020).

Molecular docking and Visualization

The ligands were docked against the target protein(4MOE) using AutoDock Vina Tool and Cygwin. Cygwin is a virtual screening software that tests the binding affinity (docking Score) of multiple ligands against a target protein. AutoDock Vina tool was used for the docking of the retrieved compounds against 4MOE protein. The entire protein was covered under a grid box and docked. The scoring function (binding affinity) of the virtual screening tool predicted the degree of successful interaction between ligands against the amino acid residues using BIOVIA Discovery Studio software. The Discovery Studio visualization tool was used to visualize the docked compounds (Janson et al., 2020: Karthika et al., 2020)

Results and Discussion

Table 1: Grid-box parameters for the enzyme (4MOE)

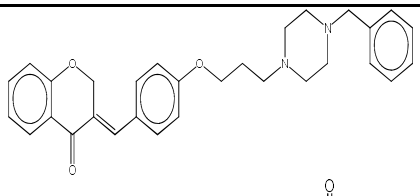
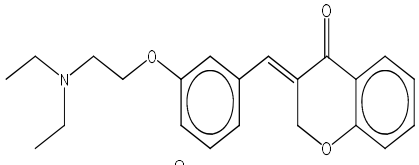
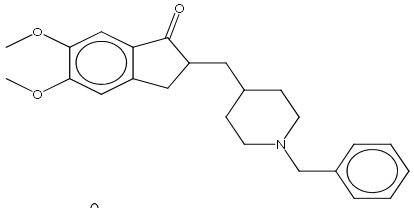
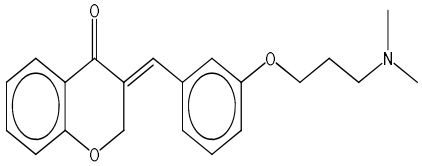
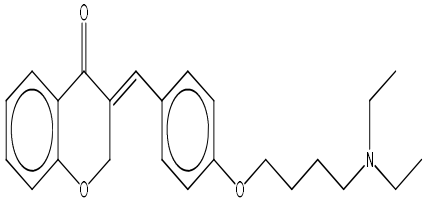
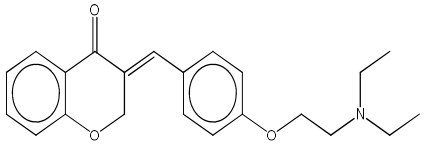
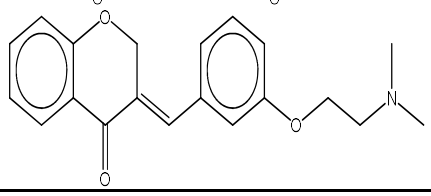
	Grid-box Size			**Center**		
	X	**Y**	**Z**	**X**	**Y**	**Z**
4MOE	26	14	18	-17.171	-42.504	25.612

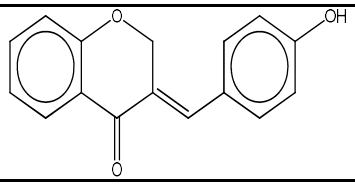
Table 2: SwissADME for Prediction ADME

Ligand	**MW**	**HBA**	**HBD**	**MLogP**	**GI absorption**	**BBB permeant**
1	468.59	5	0	3.13	High	Yes
2	351.44	4	0	2.56	High	Yes
3	379.49	4	0	3.06	High	Yes
4	337.41	4	0	2.34	High	Yes
5	379.49	4	0	2.98	High	Yes
6	351.43	4	0	2.56	High	Yes
7	323.39	4	0	2.11	High	Yes
8	252.26	3	1	2.02	High	Yes

All the ligands are within the acceptable range(<500 Da), however, ligand 1 (468.59 Da) is on y the higher side, which may reduce absorption. Satisfy Lipinski's rule of five, indicating good drug-like properties. Their high gastrointestinal absorption (GI) potential suggests efficient oral bioavailability and their moderate lipophilicity (MLogP ranges from 2.02-3.13) suggests good solubility. Ligands 1-7 have no HBD, which might reduce solubility, ligand 8 has 1 HBD, potentially improving interactions with the target, while their HBA may enhance their binding Interaction. All ligands permeate the BBB, making them suitable for CNS targets.

Table 3: Chemical Description of Ligands

Ligand ID	Molecular Formula	2D-Representation	IUPAC Name
1	**$C_{36}H_{39}N_2O_3$**		(E)-3-(4-(3-(4-benzylpiperazin-1-yl)propoxy)benzylidene)chroman-4-one
2	**$C_{22}H_{25}NO_3$**		(E)-3-(3-(2-(diethylamino)ethoxy)benzylidene)chroman-4-one
3	**$C_{23}H_{31}NO_3$**		2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one
4	**$C_{21}H_{25}NO_3$**		(E)-3-(3-(3-(dimethylamino)propoxy)benzylidene)chroman-4-one
5	**$C_{24}H_{32}NO_3$**		(E)-3-(4-(4-(diethylamino)butoxy)benzylidene)chroman-4-one
6	**$C_{22}H_{27}NO_3$**		(E)-3-(4-(2-(diethylamino)ethoxy)benzylidene)chroman-4-one
7	**$C_{22}H_{27}NO_3$**		(E)-3-(3-(2-(diethylamino)ethoxy)benzylidene)chroman-4-one

8	**$C_{16}H_{14}O_3$**		(E)-3-(4-hydroxybenzylidene)chroman-4-one

Virtual Screening

Table 4: Protox 3.0 Prediction of Toxicity

Ligand	Hepatotoxicity	Neurotoxicity	Respiratory toxicity	Cardiotoxicity	immunotoxicity	AChE	LD50(mg/kg)
1	Nil	Active	Active	Nil	Active	Active	2500
2	Nil	Active	Active	Nil	Active	Nil	2500
3	Nil	Active	Active	Nil	Active	Active	505
4	Nil	Active	Active	Nil	Active	Nil	388
5	Nil	Active	Active	Nil	Active	Active	2500
6	Nil	Active	Active	Nil	Active	Nil	2500
7	Nil	Active	Active	Nil	Active	Active	2500
8	Nil	Active	Active	Nil	Active	Active	2500

All ligands are active to Immunotoxicity and respiratory toxicity, suggesting potential risks to immune-related adverse effects and risk to respiratory function, They show a neurotoxic effect, which aligns with their intended function as AChE inhibitors for CNS targets. However, all the ligands are inactive to hepatoxicity and cardiotoxicity indicating a lack of liver and heart toxicity, which is favourable for drug safety. Ligands 1,3,5,7 and 8 show AChE toxicity, which may indicate off-target effects that could compromise the therapeutic profile or lead to adverse reactions, ligands 2, 4, and 6 are inactive for AChE toxicity, making them more favorable candidates for further development. Ligands 1, 2, 5, 6, 7, and 8 show relatively low toxicity, as their LD50 values above 2000 mg/kg are typically considered non-toxic, these ligands could be safer for further exploration as drug candidates. Ligand 3 has moderate toxicity, an LD50 value between 300 and 1000 mg/kg is often classified as moderately toxic. Ligand 4 is the most toxic among the group, with LD_{50} values below 500 mg/kg.

Docking Analysis

Table 5: Binding Affinity and IC_{50}

Ligand	Scoring function (Kcal/mol)	IC_{50} (nM)

1	-10.0	4720
2	-9.50	30300
3	-9.40	24.70
4	-9.30	25720
5	-9.70	14160
6	-9.30	3290
7	-9.30	1410
8	-8.60	99900

The most favourable binding affinity is observed with ligand 1, indicating a strong interaction with the target protein. Other ligands show moderate binding affinities ranging from -8.60 to 9.50 Kcal/mol ligand 3. Ligand 3 is the most potent ligand requiring only 24.70 nM to inhibit 50 % of the target activity. However, its LD50 value suggests moderate toxicity, which needs improvement before it can be considered safe for further development followed by ligands 7,6 with their high LD50 (2500 mg/kg). Ligands 1, 5, and 4 display lower potency with IC50 values that are increasingly less competitive for drug development. Ligand 2 is a weak inhibitor, requiring a very high concentration for 50 % inhibition. Ligand 8 is the weakest ligand, with IC50 99900 nM, indicating very poor potency. Its development as a drug candidate is not recommended unless its structure is significantly modified.

Post-docking Analysis

Table 4 describes the binding interactions of the Native ligand Donepezil with human AChE (AChE) in the crystal structure PDB ID (4MOE). These interactions include various types of non-covalent bonds, such as pi-pi stacking, Van der Waals forces, hydrogen bonds, and alkyl interactions, contributing to Donepezil's strong binding affinity for AChE. Trp 286 participates in pi-pi stacking and Van der Waals interactions, anchoring the compound's aromatic moieties. Tyr 341 and Phe 338 provide additional pi-pi and alkyl interactions stabilizing the compounds in the gorge of the gorge of AChE. His 447 forms hydrogen bonds and Van der Waals contacts, critical for the catalytic center. Tyr 124 stabilizes the compounds through hydrophobic interaction.

Table 4: Binding Energies of Benzylidenechroman-4-one derivatives and respective cocrystallized ligands

PDB ID	LIGAND	Conventional H-Bond	C-H Bond	Pi-Pi Stacking	Pi-Alkyl	Pi-Sigma Bond	Vanderwaals	Unfavorable donor-donor
4MOE	0	PHE A295,TYR A124	Val A294, TYR A337	TYR A341	PHE A338, PHE A 297	TRP A286	Nil	Nil
	1	PHE A295,TYR A124	SER A293	TYR A341, TRP A86	Nil	Nil	Nil	Nil
	2	Nil	Nil	PHE A297, TRP A286	TYP A337	Nil	Nil	Nil
	3	Nil	TYR A341, SER A293	TYR A124, TRP A286	LEU A289, TRP A86, PHE A295, PHE A338, PHE A297, HIS A447	Nil	Nil	Nil
	4	SER A203	Nil	TRY A124, TRP A86	VAL A294, TRP A286	Nil	Nil	Nil
	5	Nil	TYR A124	TRP A286, TYR A341	HIS A447, TYR A337, PHE A338	TRP A86	Nil	Nil
	6	Nil	TYR A337	TYR A341, TRP A286, PHE A297	PHE A338, PHE A295, HIS A447	Nil	Nil	Nil
	7	Nil	Nil	TRP A286	Nil	Nil	Nil	Nil
	8	TYR A124	Nil	TRP A86, TYR A337, TYR A341	Nil	Nil	Nil	PHE A295

Post-docking Analysis

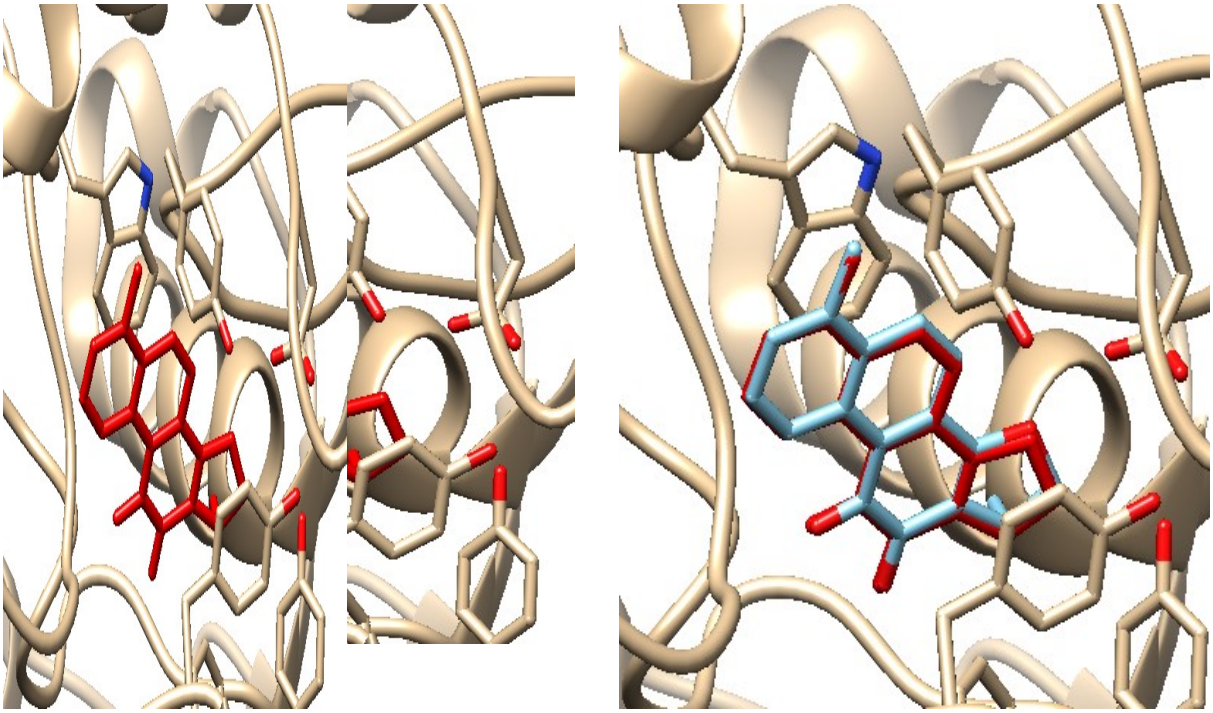


Figure 1: Receptor and Native ligand before and after docking

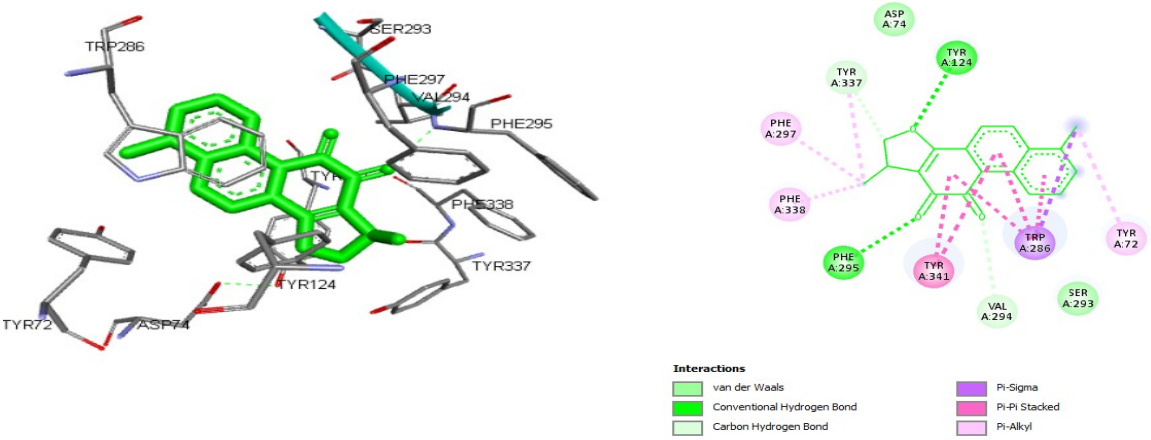


Figure 2: 3D and 2D binding interaction of Lig 0 at active site of Acetylcholinesterase (4MOE)

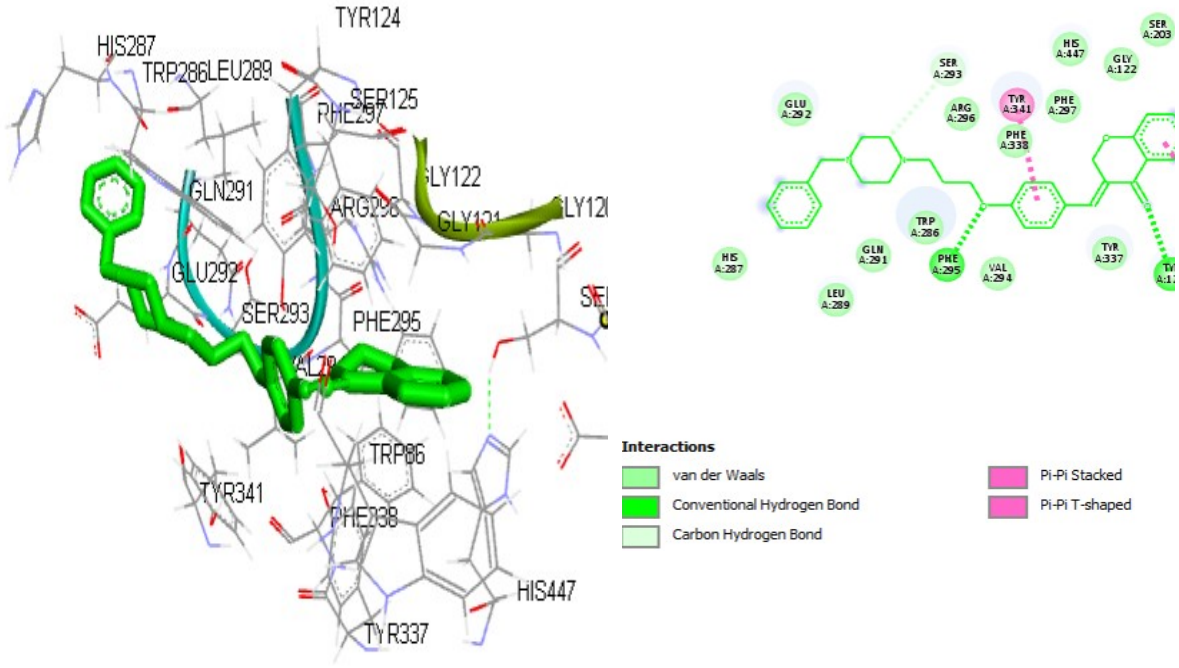


Figure 3: 3D and 2D binding interaction of Lig 1 at active site of Acetylcholinesterase (4MOE)

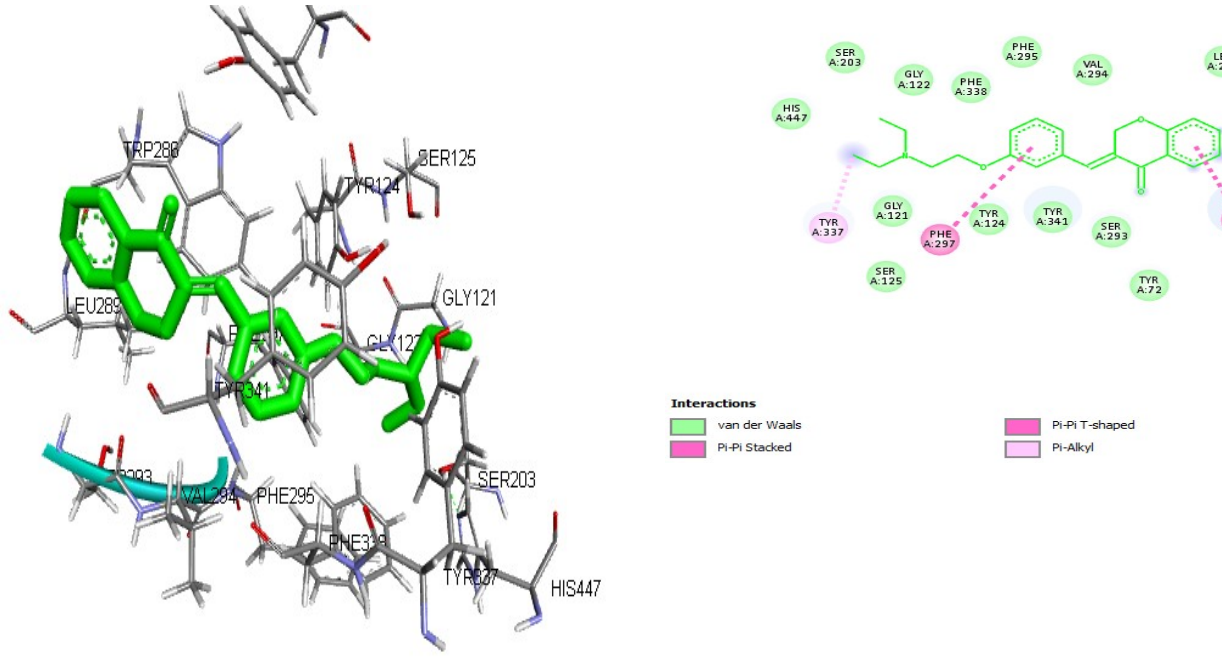


Figure 4: 3D and 2D binding interaction of Lig 2 at the active site of Acetylcholinesterase (4MOE)

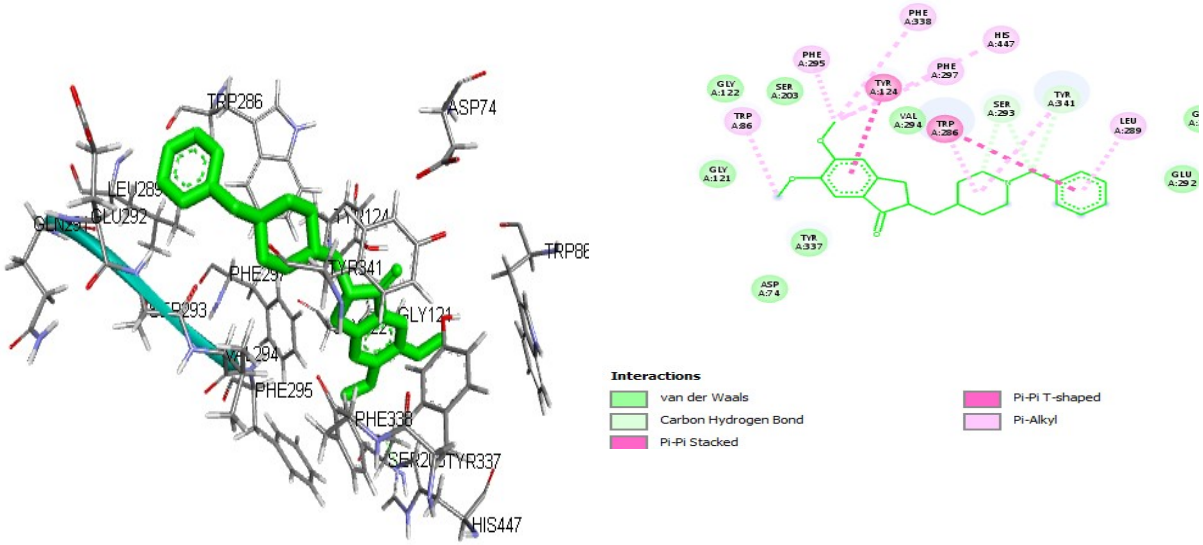


Figure 5: 3D and 2D binding interaction of Lig 3 at active site of Acetylcholinesterase (4MOE)

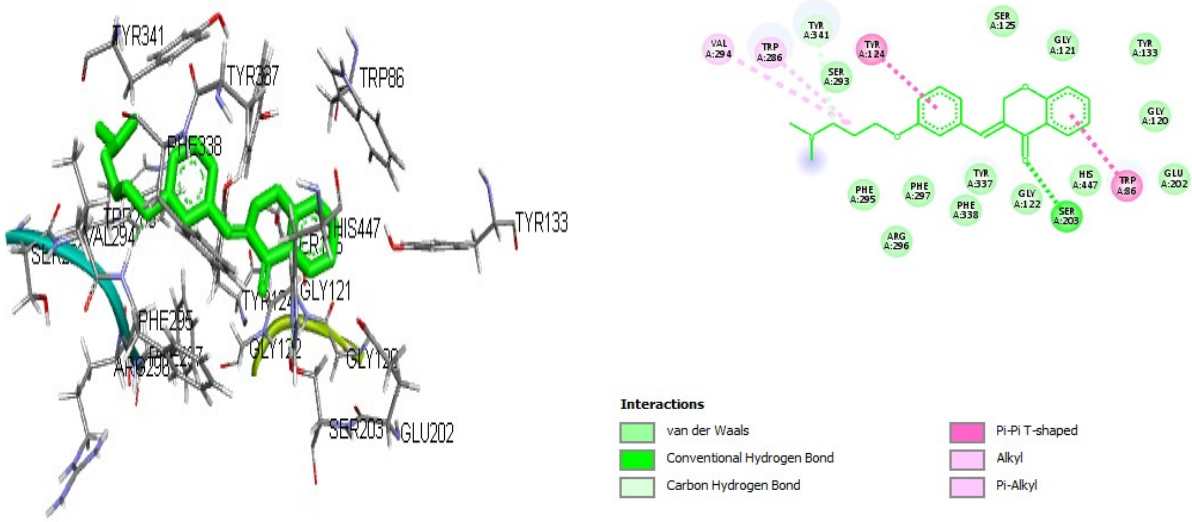


Figure 6: 3D and 2D binding interaction of lig4 at active site of Acetylcholinesterase (4MOE)

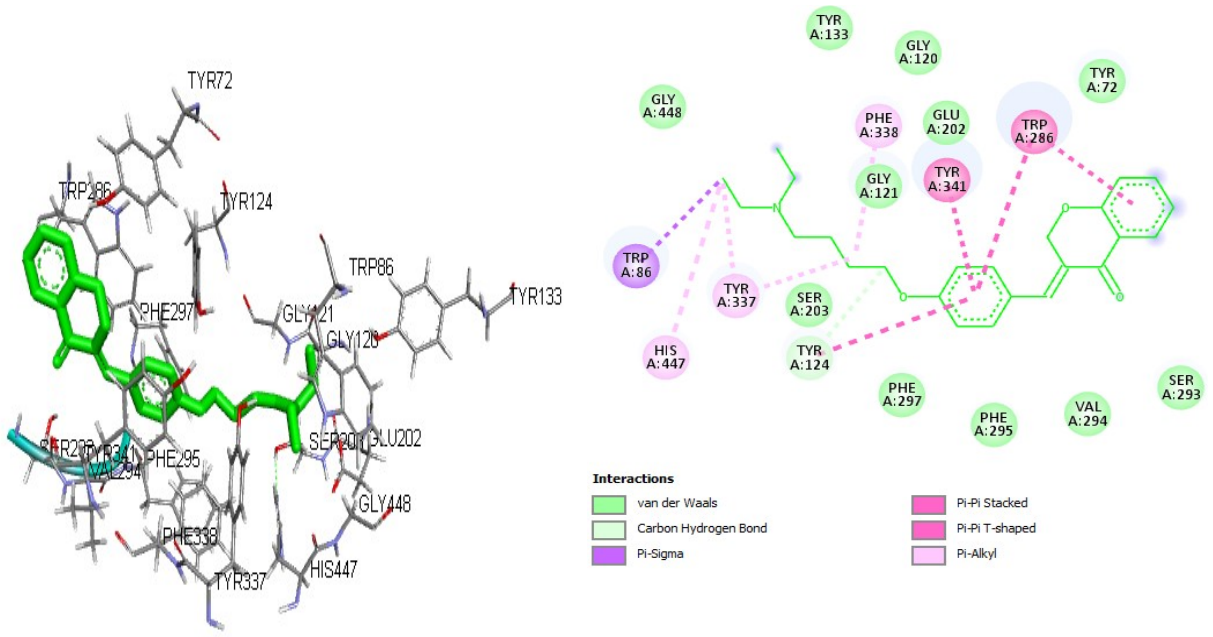


Figure 7: 3D and 2D binding interaction of lig5 at active site of Acetylcholinesterase (4MOE)

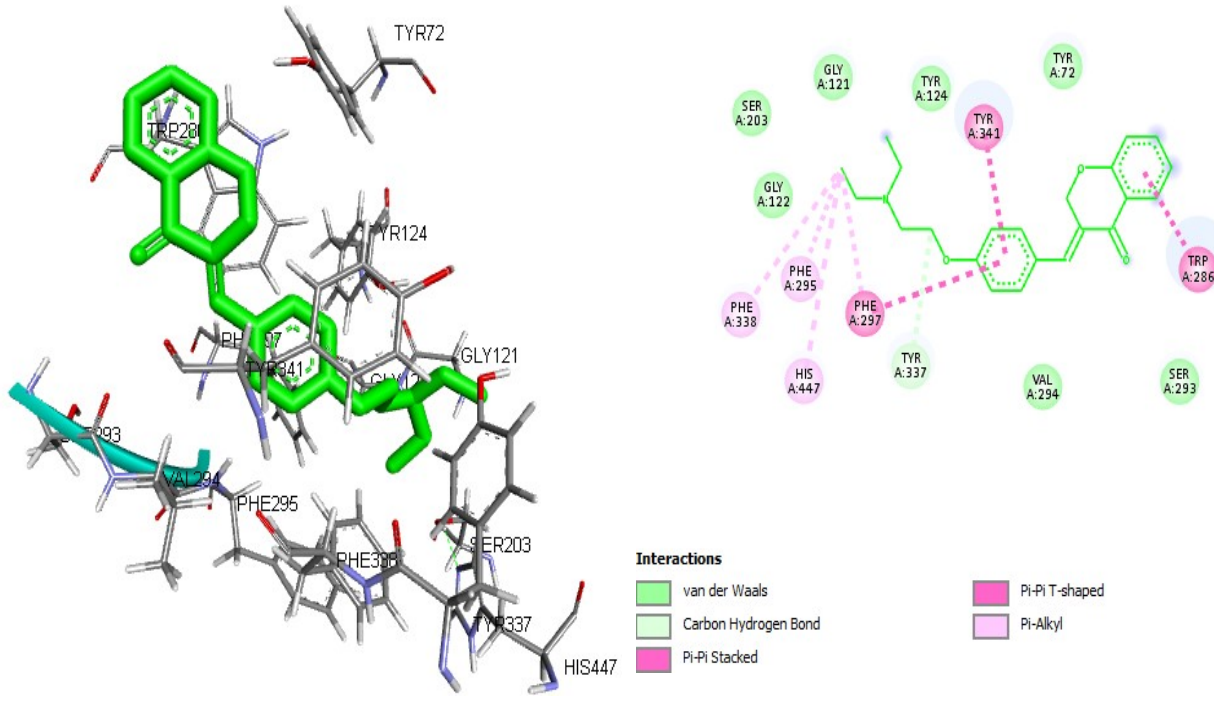


Figure 8: 3D and 2D binding interaction of lig6 at active site of Acetylcholinesterase (4MOE)

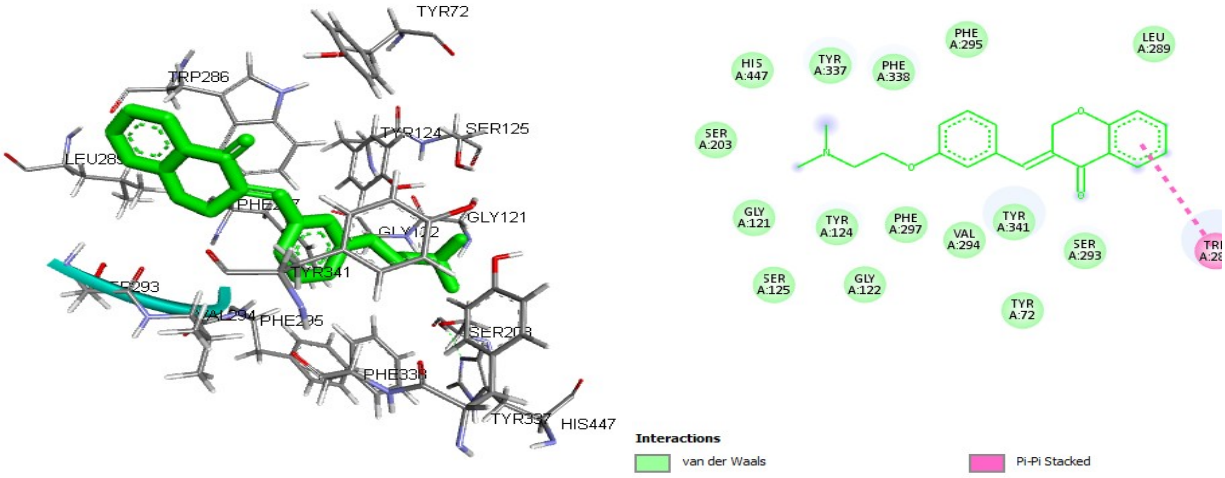


Figure 9: 3D and 2D binding interaction of lig7 at active site of Acetylcholinesterase (4MOE)

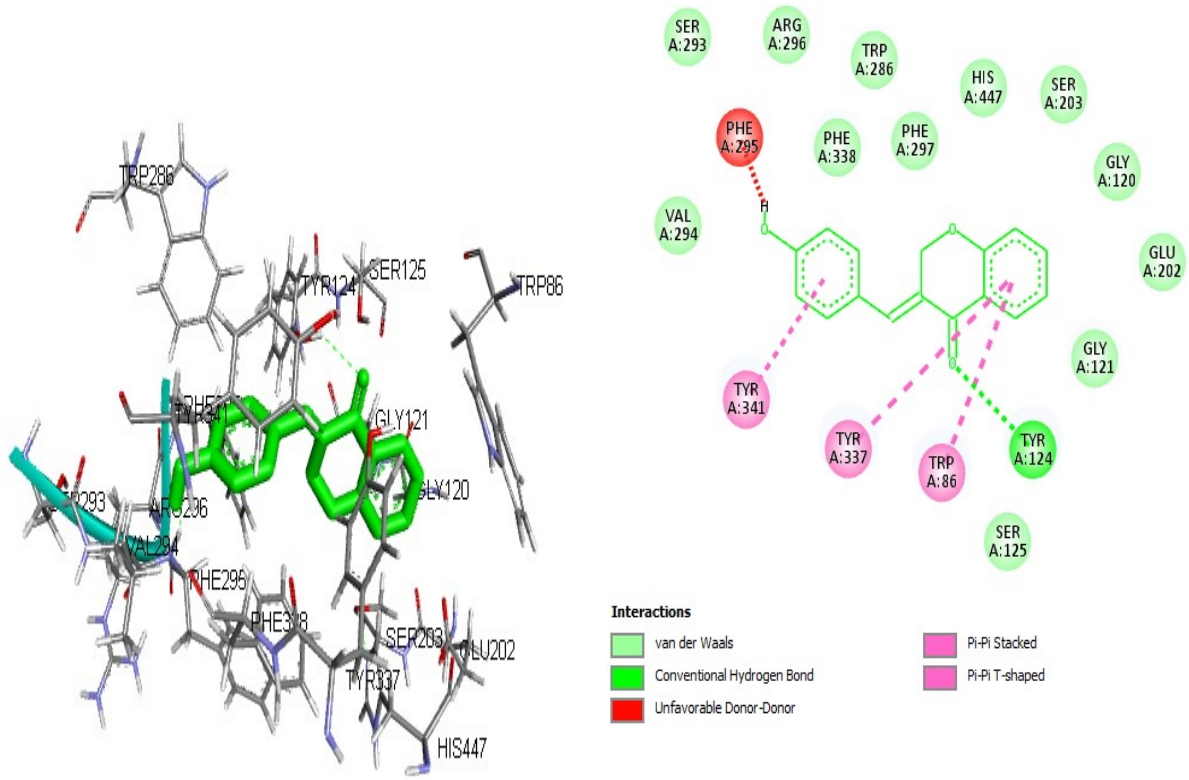


Figure 10: 3D and 2D binding interaction of lig8 at active site of Acetylcholinesterase (4MOE)

Conclusion

The comprehensive evaluation of ligands 1 to 8 based on their ADMET properties and docking interactions with human AChE reveals critical insights into their potential as drug candidates. Ligands 1 and 6 emerged as the most promising candidates due to high docking scores, diverse and stable interactions, and favourable ADMET profile. They exhibited strong interactions with critical residues such as Trp 286, Tyr 341, His 447 and Phe 338 via pi-pi stacking, hydrophobic stabilization, Van der Waal forces, and hydrogen bonding. Ligand 8 experiences unfavourable stacking or steric hindrance, reducing its binding efficiency despite favourable ADMET properties.

Recommendation

These findings support further optimization and experimental validation of ligands 1 and 6 as potential AChE inhibitors for Alzheimer's disease treatment.

References

Akrami HBF, Mirjalili M, Khoobi H, Nadri A, Moradi A, Sakhteman S, Emami A, Foroumadi AS (2014). Indolinone-based acetylcholinesterase inhibitors: synthesis, biological activity and molecular modeling, European Journal of Medicinal Chemistry (2014), doi:10.1016/j.ejmech.2014.01.017.

Begüm NS, Derya O, Ulviye AC, Serkan L, Betül KC, Yusuf Ö, Zafer AK (2020). Design, Synthesis, and Structure–Activity Relationships of Thiazole Analogs as Anticholinesterase Agents for Alzheimer's Disease. 25, 4312; doi:10.3390/molecules25184312.

Colovic MB, Krstic, DZ, Lazarevic-Pasti, TD, Bondzic AM, Vasic VM (2013). Acetylcholinesterase Inhibitors: Pharmacology and Toxicology. Current Neuropharmacology, 11(3), 315–335. doi:10.2174/1570159x1131103000 6.

Jason TA, Noorul SM, Hemalatha S (2022); In Silico Molecular Docking Approach Against Enzymes Causing Alzheimer's Disease Using *Borassus flabellifer* Linn. Appl Biochem Biotechnol. 194(4): 1804–1813. doi: 10.1007/s12010-021-03779-3

Karthika PB, Arunagiri A, Ruckmani K, and Shanmugarathinam A (2020). Insilico Method for Prediction of Maximum Binding Affinity and Ligand-Protein Interaction Studies on Alzheimer's Disease International Journal of Research -Granthaalayah, 8(11), 362-370. doi.org/10.29121/granthaalayah.v8.i11.2020.2472.

Marek B, Anna W, Michalina H, Natalia G, Christoph A, Sotriffer BM (2013). Structure-Based Search for New Inhibitors of Cholinesterases Int. J. Mol. Sci., 14, 5608-5632; doi:10.3390/ijms14035608.

Peauger L, Azzouz R, Gembus V, Ţînţaş ML, Sopková-de OS, Bohn P, Levacher V (2017). Donepezil-Based Central Acetylcholinesterase Inhibitors employing a "Bio-Oxidizable" Prodrug Strategy: Design, Synthesis, and in Vitro Biological Evaluation. Journal of Medicinal Chemistry, 60(13), 5909–5926. doi:10.1021/acs.jmedchem.7b007 02.

Piacentini R, De Chiara G, Li Puma DD, Ripoli C, Marcocci ME, Garaci E, Palamara AT, Grassi C. (2014). HSV-1 and Alzheimer's disease: More than a hypothesis. Front. Pharmacol. 5.

Roy SK, Kumari N, Gupta S (2013). 7-Hydroxy-(E)-3-phenylmethylene-chroman-4-one analogues as efflux pump inhibitors against Mycobacterium

smegmatis mc2 155. Eur J Med Chem 66:499–507.

Sefa CA, Demet D, Ali OC, Aysen EO, Sevim A (2022). Computational Investigation of the Interaction Mechanism of Some antiAlzheimer Drugs with the Acetylcholinesterase Enzyme. Open Journal of Nano ISSN: 2147-0081 (2023) 8–1, DOI: 10.56171/ojn.1110326..

Zheng C, Xia Y, Pan Y, Chen J. (2016). Automated identification of dementia using medical imaging: A survey from a pattern classification perspective. Brain Inform., 3, 17–27.