In Silico Analysis of Acetylcholine Modulation by Coumarin derivatives in Alzheimer's Disease

Dissertation

Submitted in the partial fulfillment of requirement for the award of the Degree of

Master of Science

In

Bioinformatics

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Candidate's Undertaking/Declaration

I, hereby declare that research work embodied in this dissertation entitled "***In Silico Analysis of Acetylcholine Modulation by Coumarin derivatives in Alzheimer's Disease***" has been prepared by me as a part of partial fulfilment of the **M.Sc. degree in Bioinformatics** under the supervision of Dr. Divya Kushwaha, Assistant Professor, Department of Chemistry, MMV, BHU. The matter embodied in this thesis is original and duly checked through a plagiarism detection tool approved by the HEI, as per UGC-INFLIBNET norms, and is Plagiarism free. The details of the plagiarism detection tool have been enclosed.

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Certificate

This is to certify that the research work embodied in this dissertation titled "***In Silico* Analysis of Acetylcholine Modulation by Coumarin derivatives in Alzheimer's Disease**" has been carried out by **Ms. Aditi Mishra**, as a partial fulfilment of the **M.Sc. degree in Bioinformatics,** under my supervision. Her work has been duly checked through a plagiarism detection tool approved by the HEI, as per UGC-INFLIBNET norms and is Plagiarism free.

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CERTIFICATE

This is to certify that **Ms. Aditi Mishra** (Enrolment No. **461294**), a student of M.Sc. IV semester, Department of Bioinformatics, Mahila Mahavidyalaya (MMV), Banaras Hindu University has satisfactorily completed her dissertation project ***"In Silico Analysis of Acetylcholine Modulation by Coumarin derivatives in Alzheimer's Disease"*** at MMV for partial fulfillment of the requirements for the award of degree of Master of Science in Bioinformatics (session 2022-24). This dissertation represents independent work carried out by the candidate.

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Acknowledgement

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Finally, I wish to express my gratitude to my parents for their love, encouragement and continuous support throughout the years of my education. This accomplishment would not be possible without them.

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Contents

1 Abstract:

Alzheimer's disease (AD) is a neurological illness that develops with time and causes memory loss and cognitive impairment. Acetylcholinesterase (AChE), an enzyme that hydrolyzes the neurotransmitter acetylcholine essential for cognitive processes is the target of one therapy strategy. Acetylcholine levels in the brain can be raised by inhibiting AChE, which will lessen Alzheimer's disease symptoms. At the moment, donepezil, an AChE inhibitor, is frequently used to treat Alzheimer's disease. Alternative AChE inhibitors, though, are required and may be more effective. This study aimed to investigate the possible AChE inhibitors in chemical and natural derivatives for the treatment of cognitive problems. Its aim is to point out the significance of coumarin derivatives in the creation of effective and secure Alzheimer's disease treatment medicines.

In-silico studies have been conducted to explore the potential of coumarin derivatives as AChE inhibitors, specifically warfarin (anticoagulant), esculetin (anticancer), daphnetin (enzyme inhibitor), osthole (antioxidant), and scopoletin (anti-inflammatory). The binding affinity of selected coumarin derivatives and AChE (PDB ID: 3I6M) was studied using molecular docking. Amongst these phytochemicals, hydroxycoumarin (Warfarin and osthole) gives the better docking result as compared to daphnetin, esculetin, and scopoletin. The binding potency of these coumarins in terms of their hydrophilic and hydrophobic interactions were investigated and compared with the known inhibitor donepezil. The results show that these coumarin derivatives have the potential to be new AChE inhibitors; some of the compounds have interactions that are more advantageous than donepezil in terms of binding affinities. Key structural characteristics and the interactions between structure and activity that contribute to the inhibitory activity were determined. The design and development of novel prospective AChE inhibitors generated from coumarin scaffolds for the treatment of Alzheimer's disease are greatly aided by the insights this study offers.

2 Introduction:

Alzheimer's disease (AD) is a degenerative neurologic ailment that worsens over time and impairs behaviour and memory. According to epidemiological research, the disease's prevalence may rise significantly during the next 20 years. Alzheimer's disease affects up to 5% of adults over 65 and 20% of those over 80. The cholinergic hypothesis, which suggested that a lack of cholinergic function in the brain is the cause of memory problems in people with this disease, has served as the foundation for the majority of therapeutic approaches.

As of right now, Alzheimer's disease has no known effective treatment, which could be partly attributed to the absence of a well-defined underlying mechanism. Several theories have been proposed to explain Alzheimer's disease based on the formation of extracellular amyloid-beta (Aβ) plaques and neurofibrillary tangles in the intracellular environment, gliosis, loss of synapses, and inflammation. These comprise the following:

1. the tau theory
2. the Aβ-amyloid oligomer hypothesis
3. the presenilin hypothesis
4. the Ca^{2+} dysregulation hypothesis
5. the lysosome hypothesis

The Aβ-amyloid hypotheses state that amyloid plaques cause synapto- and neurotoxicity as well as neurodegeneration because of an overproduction of the Aβ-amyloid peptide. The tau hypothesis states that there is an aberrant tau (tubulin-associated unit) phosphorylation, which causes aberrant neurofibrillary structure development. In healthy cells, tau proteins attach themselves to microtubules to encourage polymerization and stability. The calcium-sensing receptor (CaSR), a member of the G protein-coupled receptor (GPCR) family C that mediates calcium homeostasis and regulates intracellular signals, is the foundation of the Ca^{2+} dysregulation theory. CaSR deregulation has been linked to inflammation and neurodegenerative conditions like AD. Based on hereditary mutations in the genes encoding presenilin, the catalytic subunit of γ-secretase, the presenilin (PS) hypothesis postulates that presenilin cleaves the amyloid precursor protein (APP), increasing brain susceptibility and AD. Lastly, the lysosome theory is predicated on changes in the genes that control lysosomal pH resulting in impairment of the autophagy–lysosomal pathway.

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are the two types of enzymes linked to the illness. They have been shown to have the ability to quicken the assembly

of Aβ peptides into Alzheimer-type clumps, hence enhancing their neurotoxicity. Low acetylcholine, aberrant β-amyloid, T-protein aggregation, inflammation, and oxidative stress have all been reported in AD patients Acetylcholinesterase (AChE) inhibitors are the focus of Alzheimer's research, which is centred on the cholinergic system.

Alzheimer's disease patients have unique effects on cholinergic neurotransmission. Using acetylcholinesterase inhibitors to raise brain acetylcholine levels is one of the most promising ways to treat this illness.

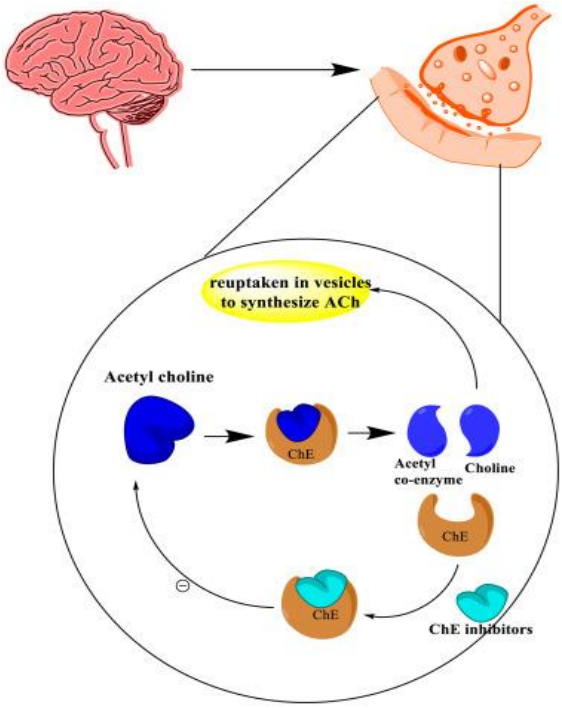


Figure 1: Mechanism of action of AChE inhibition in AD [adapted from reference 15]

AChE is a member of the carboxylesterase family of enzymes, type B. Carboxylesterase type B is a family of evolutionarily related proteins. It is a serine hydrolase that catalyses the breakdown of acetylcholine and other choline esters that function as neurotransmitters. Because of its rapid rate of catalysis, AChE is considered one of the most effective enzymes. The AChE active site is a 20 Å deep gorge, with three amino acids (Ser203, Glu334, and His447) forming the catalytic triad. A peripheral binding site is present nearby, beyond Tyr337. AChE inhibitors can interact with both of these sites. Kinetic studies have revealed that AChE has two distinct active sites, the ecstatic and the anionic, corresponding to the choline-binding pocket and the catalytic apparatus. The catalytic triad (Ser203, Glu334, and His447) is present in the esteratic site, which is the site of hydrolysis of acetylcholine into acetate and choline. The serine and histidine residues are present in other serine proteases, but the third amino acid is aspartate. In addition, the catalytic triad in AChE is of opposite chirality compared with other

proteases. Mechanistically, after the carboxylate is hydrolysed to free choline and acyl-enzyme, the latter undergoes a nucleophilic attack by a water molecule and is assisted by the histidine. The result of this reaction is the release of acetic acid and the free enzyme.

To stop the impulse-transmitted action of acetylcholine (AChE) through cholinergic synapses, the enzyme acetylcholinesterase (AChE) catalyses the hydrolysis of the ester-bound. While the exact cause of Alzheimer's disease (AD) is still unknown, presently, clinical trials investigating the therapy of Alzheimer's disease are utilizing reversible inhibitors of cholinesterase. By reducing the breakdown of acetylcholine at the synaptic location in the brain, anticholinesterase may interact with the central cholinergic system to improve memory and cognitive deficits in patients.

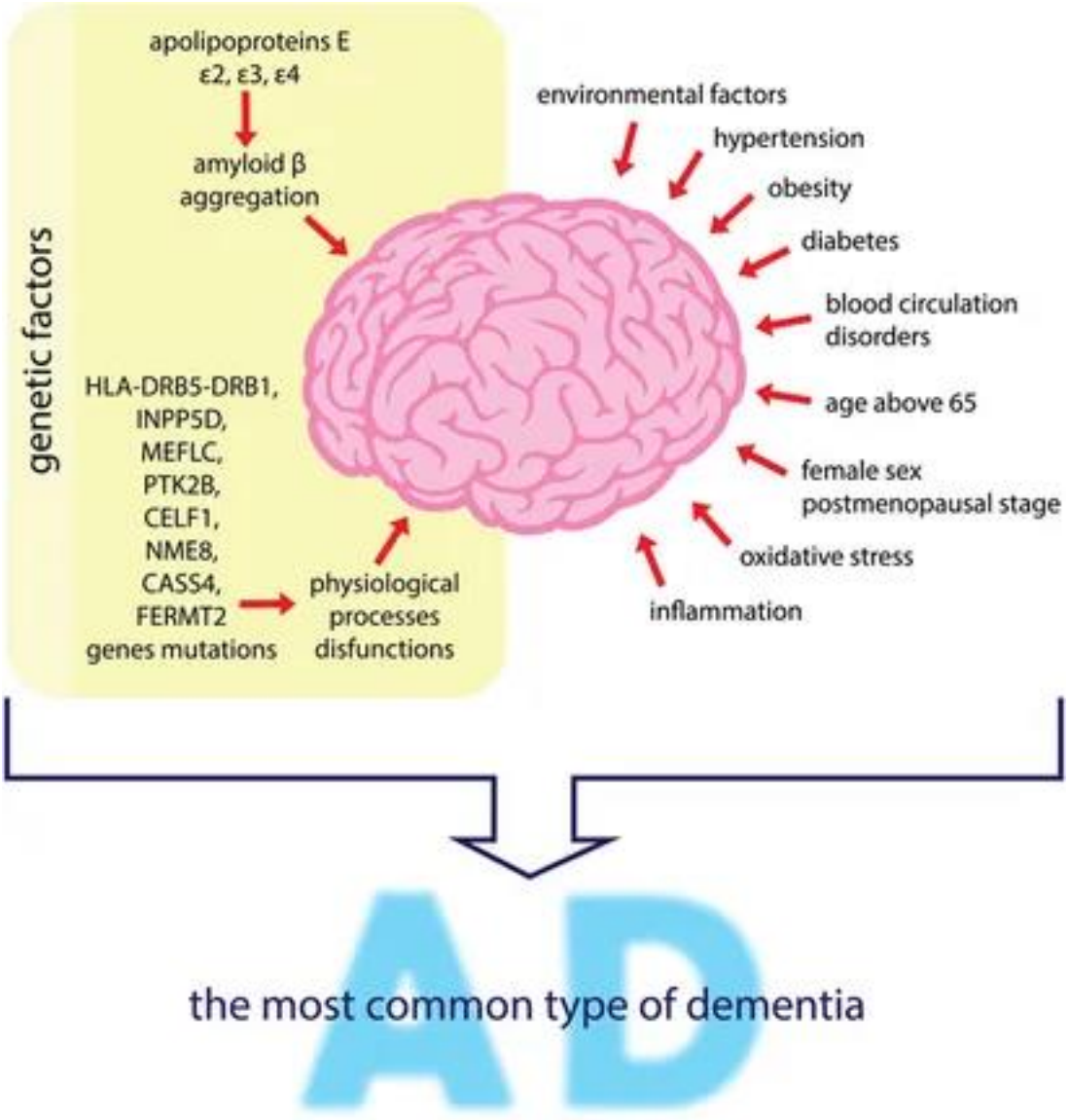
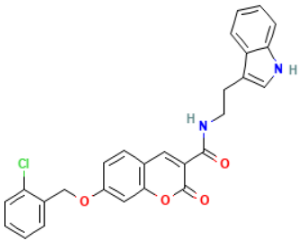


Figure 2. The most prevalent kind of dementia, AD, is influenced by certain factors. The two primary risk factors for developing AD are considered to be genetic and environmental.

Amyloid β aggregation is irregularly caused by apolipoprotein E. Genes like HLA-DRB5-DRB1, INPP5D, MEFLC, PTK2B, CELF1, NME8, CASS4, and FERMT2 might become mutated to cause dysfunctions in neural processes. The course of AD is further influenced by additional variables such oxidative stress, inflammation, blood circulation issues, diabetes, obesity, and hypertension. AD development is more likely in those over 65, particularly in postmenopausal women [adapted from reference16]

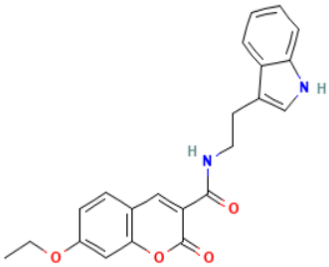
3 Literature Review

Saha et al. [1] studied a library of coumarin derivatives as acetylcholinesterase (AChE) inhibitors for the treatment of Alzheimer's disease. They performed computational modeling approaches such as 3D-QSAR, pharmacophore modeling, virtual screening, and molecular docking. They discovered a pharmacophore model that captured the key elements for AChE inhibition as well as a verified 3D-QSAR model. Ten HIT compounds were found using virtual screening and docking experiments. Of these, two leads (HIT 1 7-[(2chlorophenyl)methoxy]-N-[2-(1H-indol-3-yl)ethyl]-2-oxochromene-3-carboxamide (A) and HIT 2 7-ethoxy-N-[2-(1H-indol-3-yl)ethyl]-2-oxochromene-3-carboxamide (B) were given priority due to their favourable ADME features and thermodynamic stability in molecular dynamics simulations.



HIT 1

(A)



HIT 2

(B)

Baruah et al. [2] compared the standard medication donepezil to evaluate the inhibitory efficacy of two substituted coumarin derivatives on AChE activity in an aqueous buffer and in the presence of human serum albumin (HSA): chromenyl coumarate (CC) and 3,3'-methylene-bis(4-hydroxycoumarin) (MHC). According to Baruah et al., the experimental data demonstrated non-competitive inhibition, with CC demonstrating a greater inhibitory effect (IC_{50} = 48.49 ± 5.6 nM) than donepezil (IC_{50} = 74.13 ± 8.3 nM), underscoring its significance.

Prior structural-activity relationship (SAR) studies on coumarins were validated by the compound structure, which was crucial to the inhibitory efficiency. The inhibition potency of MHC (38% reduction) and CC (35% reduction) varied significantly in HSA, while the inhibition mechanism stayed unchanged. The peripheral anionic site was occupied by both substances (PAS) of AChE and moved the fluorescent indicator thioflavin T out of the binding pocket (Baruah et al.). The experimental findings were validated by MD simulations and molecular docking. The work lays the groundwork for additional research into these substances as possible AD medications and highlights how important it is to take drug delivery media into account when assessing inhibitory potencies.

Kamel et al. [3] synthesized new 2-oxo-chromene-7-oxymethylene acetohydrazide derivatives. The novel synthesised compounds were assessed in relation to donepezil and ascorbic acid, respectively, as antioxidant agents and acetylcholinesterase (AChE) inhibitors. One of the derivatives showed a DPPH scavenging activity of 57.14 ± 2.77% and an IC_{50} value of 0.802 μM. Moreover, it did not alter the blood profile, hepatic enzyme levels (AST, ALT, and ALP), or total urea in treated rats as compared to the control group, according to biochemical and haematological investigations. Furthermore, the examination of the hepatic, kidney, heart, and brain tissues in the treated rats' histological examinations showed no histopathological damage and normal architecture of the hepatic lobules and renal parenchyma. Derivative showed a positive safety profile and a good fit in the AChE enzyme's active site according to the molecular docking analysis. Comparing to the conventional medication donepezil, this derivative showed a promising anti-Alzheimer's disease efficacy.

K Nuthakki et al. [4] studied 8-acetyl-7-hydroxycoumarin as inhibitors of beta-site amyloid precursor protein cleaving enzyme 1 (BACE-1) and cholinesterase (ChE), two important targets in AD therapy. This compound demonstrated a moderate level of inhibitory action against BACE-1 and acetylcholinesterase (AChE), with IC_{50} values of 17.7 μM and 22.1 μM, respectively. Acknowledging coumarins as promising lead molecules, the scientists performed semisynthetic modifications to augment the inhibitory efficacy. An IC_{50} value of 3.3 μM for one of the semisynthetic derivatives, a trifluoromethyl-substituted coumarin chalcone, indicated a 5-fold increase in BACE-1 inhibition over the original drug. Interestingly, a combination of coumarin and donepezil, another semisynthetic derivative, showed dual suppression of IC_{50} values were found for both cholinesterase types (AChE and butyrylcholinesterase, BuChE), at 1.22 and 3.09 μM, respectively. The combination of coumarin and donepezil inhibits AChE in a non-competitive manner, as demonstrated by

research on enzyme kinetics and molecular modelling. The ability of the coumarin-donepezil hybrid to pass the blood-brain barrier, a critical prerequisite for possible AD therapies, was also demonstrated by the researchers. Furthermore, the hybrid molecule prevented amyloid-beta (Aβ) peptides from self-aggregating, which is a major pathogenic characteristic of AD. The study's findings demonstrate the promise of compounds derived from natural products, especially coumarins, as lead structures for the creation of multi-targeted Alzheimer's disease therapies. Given its dual inhibitory effect against cholinesterase and its capacity to prevent Aβ self-aggregation, the coumarin-donepezil hybrid is worthy of further study in preclinical models of Alzheimer's disease.

M Amin et al. [5] prepared twenty new 7-benzyloxy coumarin-based compounds containing different bioactive chemical fragments were synthesised by the researchers. The purpose of evaluating these compounds' AChE inhibitory activity was to find strong and specific inhibitors. Many synthesised compounds demonstrated impressive AChE inhibitory activity. Of these, five compounds showed particularly promising inhibitory potency, even surpassing that of the benchmark medication donepezil (IC_{50} = 0.711 μM). These compounds are (7-benzyloxy4{[(4phenylthiazol2(3H)ylidene)hydrazono]methyl}2Hchromen2one; IC_{50} 0.451μM),(7-benzyloxy-4-({[4-(4-methoxyphenyl)thiazol-2(3H)-ylidene]hydrazono}methyl) -2H-chromen-2-one; IC_{50} = 0.625 μM), (5-amino-1-[2-(7-benzyloxy-2-oxo-2H-chromen-4-yl)acetyl]-1H-pyrazole-4-carbonitrile; IC_{50} = 0.466 μM), (2-(7-benzyloxy-2-oxo-2H-chromen-4-yl)-N-(2-methylimino-4-phenylthiazol-3(2H)-yl)acetamide; IC_{50} = 0.500 μM), and (2-(7-benzyloxy-2-oxo-2H-chromen-4-yl)-N-[4-(4-methoxyphenyl)-2-methyliminothiazol-3(2H)-yl]acetamide; IC_{50} = 0.590 μM. The in-silico investigations, such as molecular docking simulations at the active site of recombinant human AChE (rhAChE) showed a link between the measured IC_{50} values of the tested compounds and the docking scores, indicating that the compounds had a binding pattern comparable to that of the co-crystallized ligand donepezil. The researchers also assessed the synthetic drugs' ADMET (absorption, distribution, metabolism, excretion, and toxicity) and other physicochemical characteristics, which provided insightful data for future development and optimisation. The results of this investigation demonstrated the potential of drugs based on 7-benzyloxycoumarin as effective AChE inhibitors and cognitive enhancers for the treatment of Alzheimer's disease. The common medication donepezil was shown to have lower inhibitory activity than several strong substances that the researchers were able to identify. A thorough grasp of the structure-activity

connections and binding processes of these compounds was made possible by the combination of in vitro, in vivo, and in silico investigations.

El-Sayed et al. [6] reported a new class of powerful phosphazine and phosphazide derivatives as selective β-amyloid aggregation inhibitors and acetylcholinesterase (AChE) inhibitors. Compared to donepezil (IC_{50} = 34.42 nM), 3,4-dimethoxy, cyclic acetal, and coumarin moiety containing derivatives displayed high AChE inhibitory activity (IC_{50} : 29.85 - 34.96 nM).

4 Tools/software:

4.1 PubMed/Google Scholar:

The National Library of Medicine (NLM) maintains PubMed, a comprehensive database of biological literature. More than 34 million citations from online books, life science journals, and MEDLINE are included in it. Users of PubMed can look up pertinent literature using keywords, authors, journals, or fields like abstract or title. It offers full-text article access, resources links, and citations in multiple forms.

A specialised search engine, **Google Scholar** indexes academic literature from publishers, professional associations, online repositories, and other websites. This includes articles, theses, books, abstracts, and court judgements. It is helpful for discovering highly referenced and significant works in a given field because it ranks search results according to their relevancy and the quantity of citations they have earned.

4.2 RCSB PDB:

A global database of 3D structural information for biological macromolecules, including proteins, nucleic acids, and complex assemblies, is the **Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB).** It offers tools for investigating, visualising, and analysing the more than 180,000 structures that are included. Scientists from all across the world have donated data to the RCSB PDB, which is crucial for comprehending the relationships, mechanisms, and functions of biological molecules.[7]

4.3 PubChem:

The National Centre for Biotechnology Information (NCBI) maintains PubChem, a database that offers details on the biological activity of small molecules, including chemical compounds and their characteristics. With information on chemical structures, calculated properties, interactions with biological targets, and reference to relevant literature, it has approximately 111 million compound records. For chemical information lookup, structure finding, and data analysis, PubChem is widely utilised in many domains, including as computational chemistry and drug development.[8]

4.4 Open Babel:

Designed to convert between several file formats used in computational chemistry and molecular modelling, Open Babel is a free, open-source chemical toolset. It offers a wide range of features, including data analysis, cheminformatics, and molecular mechanics, and supports more than 110 file formats. With its programming libraries, Open Babel can be integrated into other applications or utilised independently.[9]

4.5 AutoDock Vina:

AutoDock Vina is an open-source molecular docking and virtual screening programme that forecasts a ligand's (small molecule) binding mechanism and affinity with a protein target. It explores several binding modes using an iterative search method, assigning scores according to the expected affinities of each binding mode. For the purpose of finding possible drug candidates and comprehending how they interact with target proteins, AutoDock Vina is extensively utilised in computational chemistry and drug discovery.[10]

4.6 Discovery Studio:

Dassault Systèmes BIOVIA developed Discovery Studio, a comprehensive package of software tools for molecular modelling, simulation, and visualisation. It has modules for modelling proteins and small molecules, docking, modelling pharmacophores, QSAR analysis, and molecular dynamics simulations, among other activities. A graphical user interface and an extensive toolkit for drug design, structural biology, and computational chemistry research are offered by Discovery Studio.[11]

4.7 SCFBio:

The Computational Biology Unit at the University of Cape Town, South Africa, developed the SCFBio (Structural Computational Biology Group) web server. It offers a method for estimating proteins' active sites from their three-dimensional structures. The server ranks putative active site residues according to how likely they are to be found in the active site using a combination of sequence-based and structural methods. This instrument is helpful for researching protein-ligand interactions, developing inhibitors, and comprehending the catalytic processes of enzymes.[12]

4.8 SwissADMET:

The Swiss Institute of Bioinformatics created Swiss ADME, a web-based tool for assessing the absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics of small compounds. For a number of ADMET characteristics, including as water solubility, lipophilicity, blood-brain barrier permeability, P-glycoprotein binding, CYP inhibition, and AMES toxicity, it offers in silico predictions.[13]

4.9 PyMOL

A system for visualising molecules in three dimensions, called PyMOL, offers interactive tools for constructing, modifying, and animating molecules. PyMOL's huge collection of prebuilt molecular structures and intuitive interface makes it easy for users to visualise and explore complex molecules in three dimensions. Molecular data may be easily imported and exported because to its support for a wide range of file types. All things considered, PyMOL is a vital tool for anyone dealing with molecules and is very easy to use with little training.[14]

4.10 Molecular docking:

A computational method called "molecular docking" is used in drug design to forecast the orientation and affinity of a small molecule's (ligand's) binding to a target protein. It is essential for lead optimisation, virtual screening, and comprehending the molecular interactions that occur between ligands and their target receptors. In the process of docking, several ligand conformations and orientations are sampled inside the protein binding site, and each posture is scored using scoring methods that calculate binding affinity. AutoDock Vina and the docking modules in Discovery Studio are two well-known molecular docking programmes.

4.11 Drug-likeness:

The term "drug-likeness" refers to the combination of structural and physicochemical characteristics that a substance has to have in order to potentially be an oral active medication. Good absorption, distribution, metabolism, and excretion (ADME) characteristics are among the favourable pharmacokinetic and pharmacodynamic qualities that compounds with drug-like properties are more likely to display. A "Drug-Like" library of chemicals that meet specific drug-likeness requirements is made available by the ZINC database.

4.12 ADMET Properties:

The features of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) are important considerations in the development and discovery of new drugs. A compound's pharmacokinetic and pharmacodynamic behaviour in the body, as well as its bioavailability, distribution to target tissues, metabolic stability, and potential for toxicity, are determined by these qualities. It is possible to increase success rates and decrease late-stage failures in the drug discovery process by assessing and optimising ADMET characteristics early on.

4.13 Lipinski's Rule of Five:

A popular set of criteria for assessing a compound's drug-likeness based on its physicochemical characteristics is Lipinski's rule of five. According to the rule, if a molecule satisfies the following requirements, it is expected to have good oral bioavailability:

- Molecular weight ≤ 500 Daltons
- Calculated logP (partition coefficient) ≤ 5
- Number of hydrogen bond donors ≤ 5
- Number of hydrogen bond acceptors ≤ 10

Orally bioavailable compounds are less likely to break more than one of these guidelines. For lead optimisation in drug development, the ZINC database offers a "Lead-Like" library of compounds that adhere to Lipinski's rule of five.

5 Methodology:

5.1 Protein structure Retrieval:

The Protein Data Bank (PDB, www.rcsb.org) provided by the crystal structure of AChE (PDB ID: 3i6m in Figure 3) The PDB is an international database for biomolecular structure data that includes three-dimensional structural data for complex biomolecular assemblies, proteins, and nucleic acids. The crystal structure of acetylcholinesterase in association with the inhibitor donepezil, found at a resolution of 2.26 Å, is represented by the 3i6m entry. This resolution is considered quite good for X-ray crystallography and allows for detailed analysis of the atomic interactions within the protein. In this work, molecular docking using coumarin derivatives was performed using the 3i6m structure as the target receptor. This high-resolution crystal structure makes it easier to predict the binding interactions between ligands and AChE active site, which opens up new avenues for investigating possible AChE inhibitors made from coumarin scaffolds.

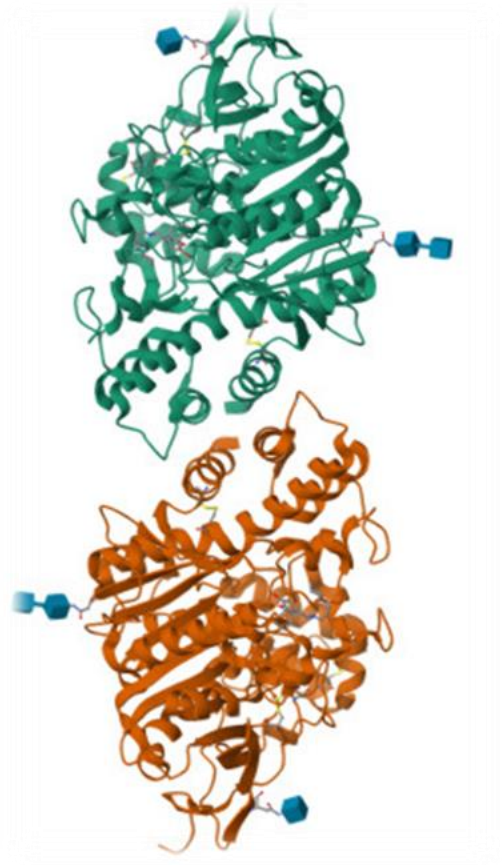


Figure 3: 3D Structure of Torpedo californica acetylcholinesterase complexed with N-piperidinopropyl-galanthamine taken from PDB (PDB id :3i6m) [7]

5.2 Ligand Preparation

The Drug Bank database provided the three-dimensional (3D) structures of the coumarin derivatives daphnetin, osthol, esculin, and warfarin. Several processes were taken to prepare these ligand structures for molecular docking and other computational research. Initially, the structures of the coumarin derivatives were retrieved in a suitable file format from the Drug Bank database. The Open Babel chemistry toolbox was then used to convert these recovered structural files into an appropriate format. The lowest energy conformations of the ligand structures were then achieved via energy minimization utilising the MMFF94 force field in Open Babel. Based on the target protein environment and physiological pH, appropriate protonation states and possible tautomeric forms of the ligands were produced and taken into consideration. For the ligand structures, partial atomic charges were computed using the method of Gasteiger charging. Lastly, the generated ligand structures were stored for use in later molecular docking investigations with AutoDock Vina in the PDBQT file format.

5.3 Molecular docking with Ligands:

The structure of the model P04058 (Figure 5) was used as a receptor for docking studies, and the docking process was carried out using the AutoDockTools (ADT) vina docking programme. Prior to docking, polar hydrogen was added, and Kollman charges were added to each atom. The structure was then saved in. pdbqt format. 3D structures of designed ligands were drawn using PubChem. Following this, all the structures were saved in.pdb format using OpenBabelGUI. In order to carry out the docking of the ligands, they were saved in. pdbqt format in ADT. A grid box with dimension 40×40×40 Å 3 with 0.375Å spacing and centred on 2.7737, 65.5108, and 66.0467 was created around the acetylcholinesterase ligand binding utilised, and 9 docked conformations were produced for every molecule. Genetic algorithms were used to calculate the energy. The binding energy and hydrogen bond interactions between the docked ligand conformations and the receptor acetylcholinesterase were examined. Biovia Discovery Studio was utilised to display the receptor with the ligand-binding site.

6 Results and Discussion:

6.1 Homology Modelling:

The AChE protein of the three-dimensional structure is obtained using SWISSMODEL. Because template 6g1u.1.A (Crystal structure of Torpedo Californica acetylcholinesterase in

complex 9-amino-6-choloro) in Figure 5 has 100% coverage and sequence identity, it is the one used for modelling. BLAST and hhBlits are used to search the SWISSMODEL library. The resulting structure has a 0.93/QMEAN as the score. QMEAN is a scoring function that uses a single model to generate absolute quality estimations on a local and global level. In relation to high-resolution X-ray structures, it assigns a Z score. A higher Z score indicates a better model.

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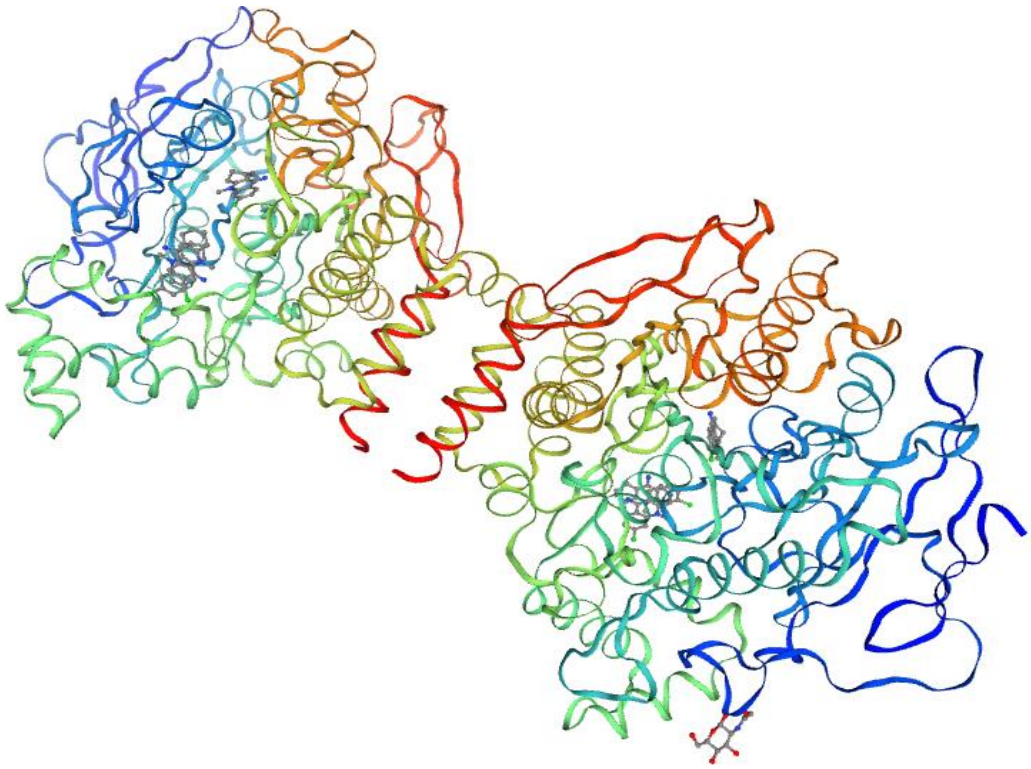


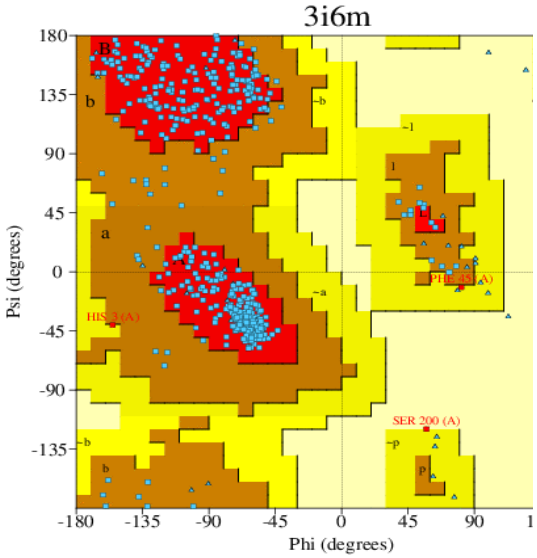
Figure 5: Model structure of P04058 of Torpedo Californica obtained from SWISS-MODEL

6.2 Ramachandran Plot:

It is a Plot made up of the torsional angles of the amino acid residues in a peptide chain, phi (φ) and psi (ψ). The dihedral angles are υ and ψ. The permitted area indicates the range of values for ϕ and ψ that are feasible for every amino acid. The torsion angles are N(i-1), C(i), Ca(i), and N(i), and ψ is C(i), Ca(i). The PROCHECK programme assessed the quality of the homology modeling-derived 3D model of P04058 (Figure 5). With a percentage of 89.0%, the Ramachandran plot revealed that the majority of the amino acid residues were in the most favoured region.

Remaining residues were distributed as follows: 10.3% were in additional allowed regions,

0.4% were in the generously allowed region, and only 0. 2% were in the forbidden region. Figure 6 displays the Ramachandran plot that PROCHECK produced.



PROCHECK statistics

1. Ramachandran Plot statistics

		No. of residues	%-tage
Most favoured regions	[A,B,L]	406	89.0%*
Additional allowed regions	[a,b,l,p]	47	10.3%
Generously allowed regions	[~a,~b,~l,~p]	2	0.4%
Disallowed regions	[XX]	1	0.2%*
Non-glycine and non-proline residues		456	100.0%
End-residues (excl. Gly and Pro)		2	
Glycine residues		44	
Proline residues		32	
Total number of residues		534	

Figure 6: Ramachandran Plot

6.3 AChE protein fragment structure analysis:

There are 534 amino acid residues in the AChE protein fragment's basic structure. 2 sheets, 3 beta-hairpin, 14 strands, 2 beta bulges, 29 helix-helix interactions, 26 helices, 44 beta turns, 3 disulphides, and 5 gamma turns make up the secondary structure, which was retrieved from the PDB sum database.

method of Gasteiger charging. Lastly, the generated ligand structures were stored for use in later molecular docking investigations with AutoDock Vina in the PDBQT file format.

6.4 Coumarin derivatives screened for AChE binding:

Coumarin (2H-1-benzopyran-2-one) are ubiquitous heterocycles which are known to possess a wide variety of biological activity. For current study we selected coumarin derivatives that are well established bioactive compounds (Figure 4).

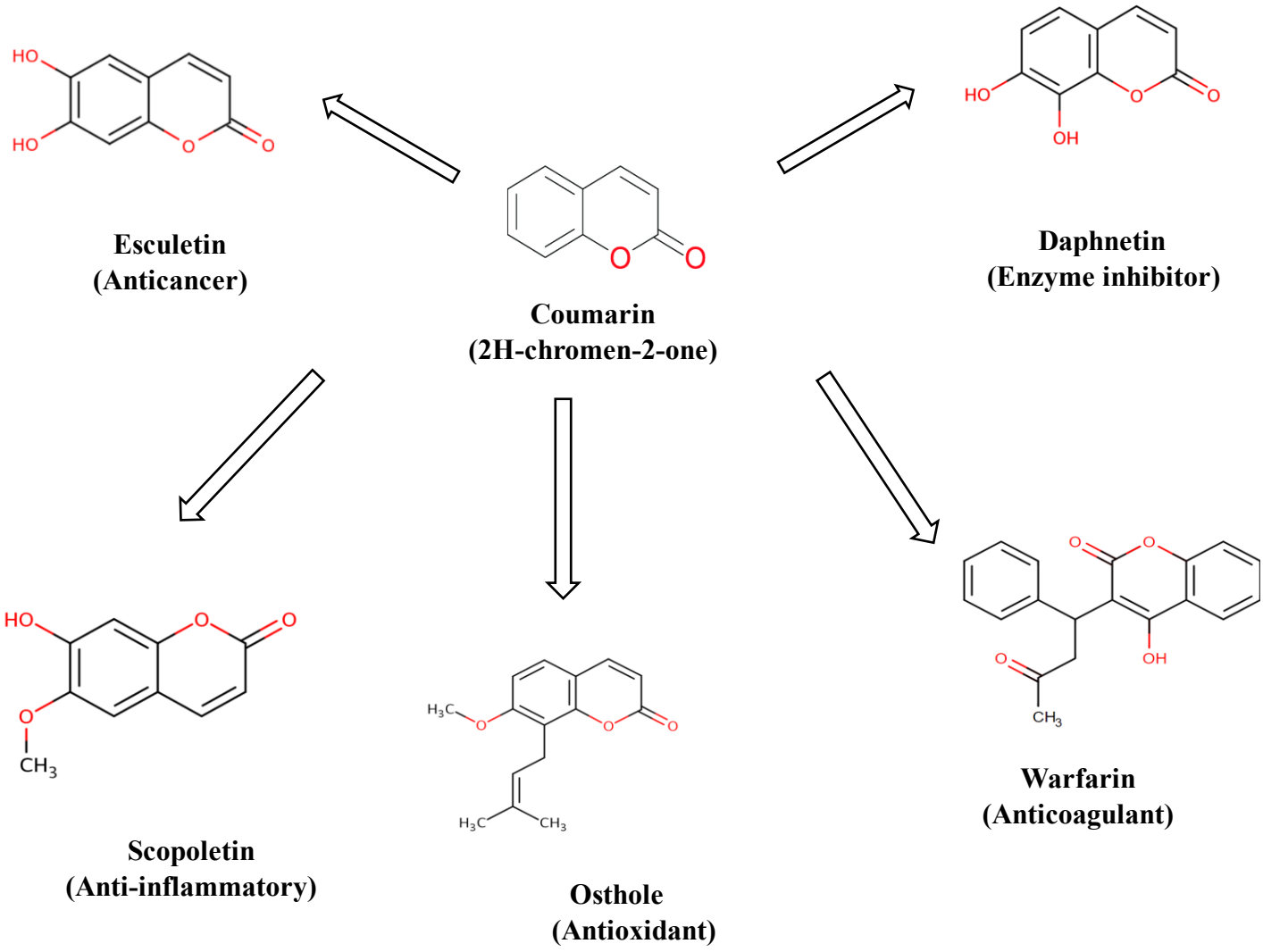


Figure 4: Structure of Coumarin derivatives

6.5 Summarized Docking Result:

Table 1 : Docking Result of Coumarin derivatives within binding pocket of AChE

S. No.	Ligand	Binding Affinity (kcal/mol)
1	Donepezil	-7.7
2	Coumarin	-10.3
3	Warfarin	-9.7
4	Esculetin	-7.4
5	Daphnetin	-7.8
6	Osthole	-8.9
7	Scopoletin	-7.5

- With a docking score of -10.3 kcal/mol, coumarin demonstrated the highest binding affinity and suggested a substantial interaction with the AChE protein.
- Osthole had a docking score of -8.9 kcal/mol, while Warfarin had a score of -9.7 kcal/mol, indicating significant binding as well.
- The binding affinity of donepezil**,** a recognised AChE inhibitor, was -7.7 KCal/mol.
- The binding affinities of esculetin**,** daphnetin**,** and scopoletin were comparatively weaker, with scores of -7.4, -7.8, and -7.5 kcal/mol, respectively.

6.6 Molecular docking of Ache Protein with Warfarin

Warfarin binds with the AChE receptor with the binding energy of -9.7 kcal/mol, on the surface of the AChE protein (Figure 7). Warfarin is stabilized in the interface by hydrophobic interaction with amino acid residues TRP 84, SER 122, PHE 331, and TYR 334. A conventional hydrogen bond is formed between the carbonyl (O) of warfarin and the hydroxyl hydrogen of SER122 and TYR121. Moreover, warfarin forms a pi-donor hydrogen bond with TRP 84 and showed π-π stacked interaction with PHE 331. It also establishes the π-π T-shape interaction with TYR 334 (Figure 8).

- In the 3D image interaction: Hydrophilic zones are indicated by blue patches.
- Hydrophobic regions are shown by brown spots.
- Different levels of hydrophilicity and hydrophobicity are represented by intermediate colours, which range from white to light blue/brown.

The hydrophobicity map makes the binding pocket's chemical environment easier to see. The specificity and affinity of binding of Warfarin depend on interactions with amino acid residues, such as hydrogen bonds with SER122 and TYR121.

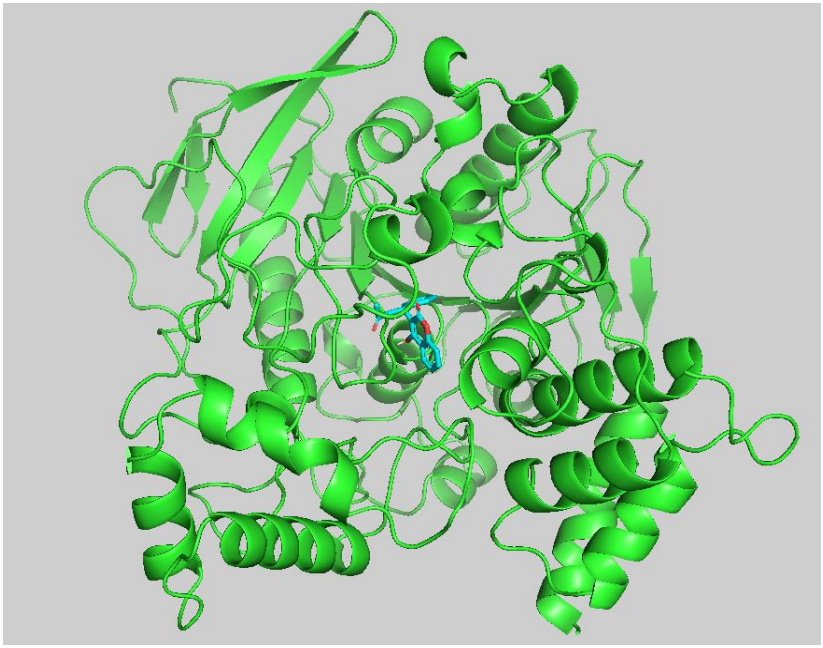
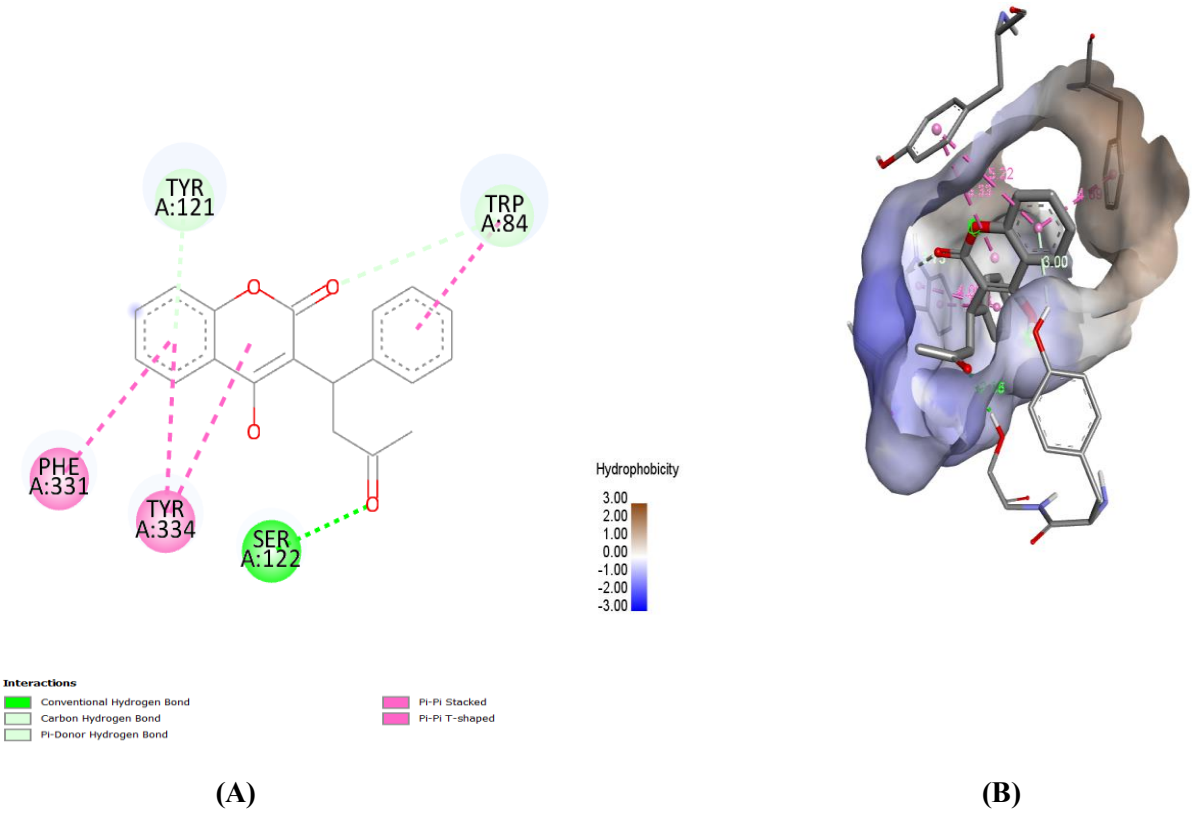


Figure 7: Binding of Warfarin with receptor AChE protein using PyMOL



(A) **(B)**

Figure 8: (A) 2D structure of AChE protein binding with Warfarin (B) 3D structure of AChE protein binding with Warfarin and all these interactions observed in Discovery Studio

6.7 Molecular docking of AChE Protein with Osthole:

Osthole interacted with AChE receptor with the binding energy of -8.9 kcal/mol in the pocket of AChE protein (Figure 9 & 10). Osthole is stabilized in the interface by H-bonds. The interacting amino acids in the case of Osthole are TYR121, PHE (331, 288, 233, 330), TRP 233, TYR 334, and HIS 440 residues. A conventional hydrogen bond is formed by the oxygen atom of lactone ring with the amino acid residue TYR 121 of the protein. The aromatic residues PHE 331, PHE 330, and TYR 334 were engaged in π-π T-shaped interactions with the molecule. Additionally, the pi-alkyl interactions were observed between the alkyl substituent and amino acids residues PHE 288, TRP233, HIS 440 and PHE 290.

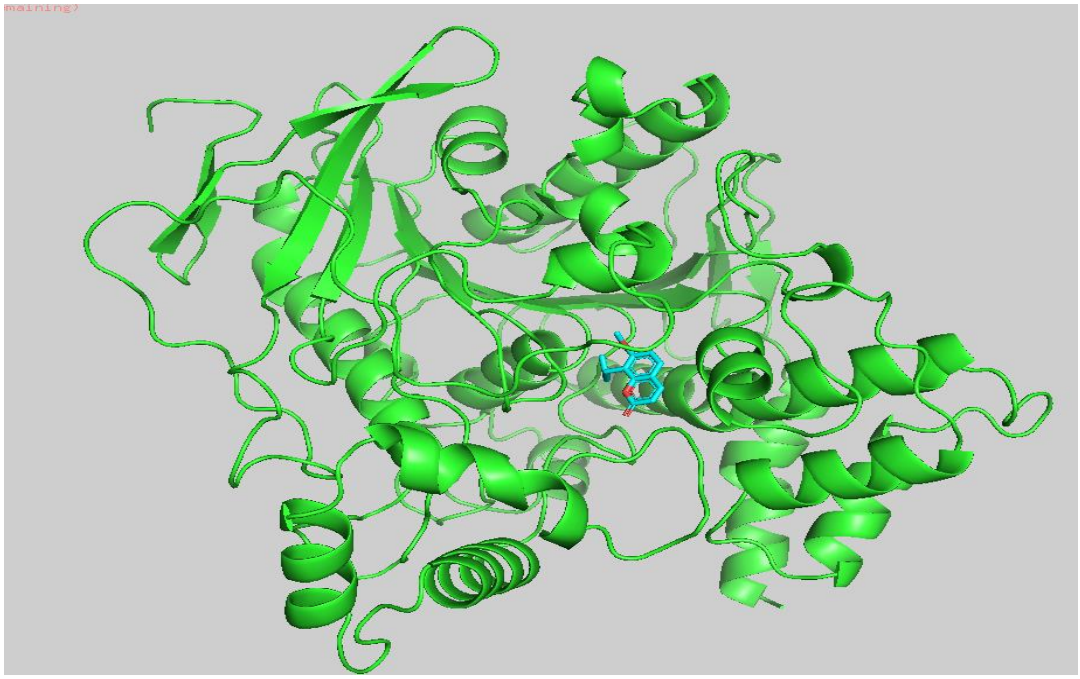
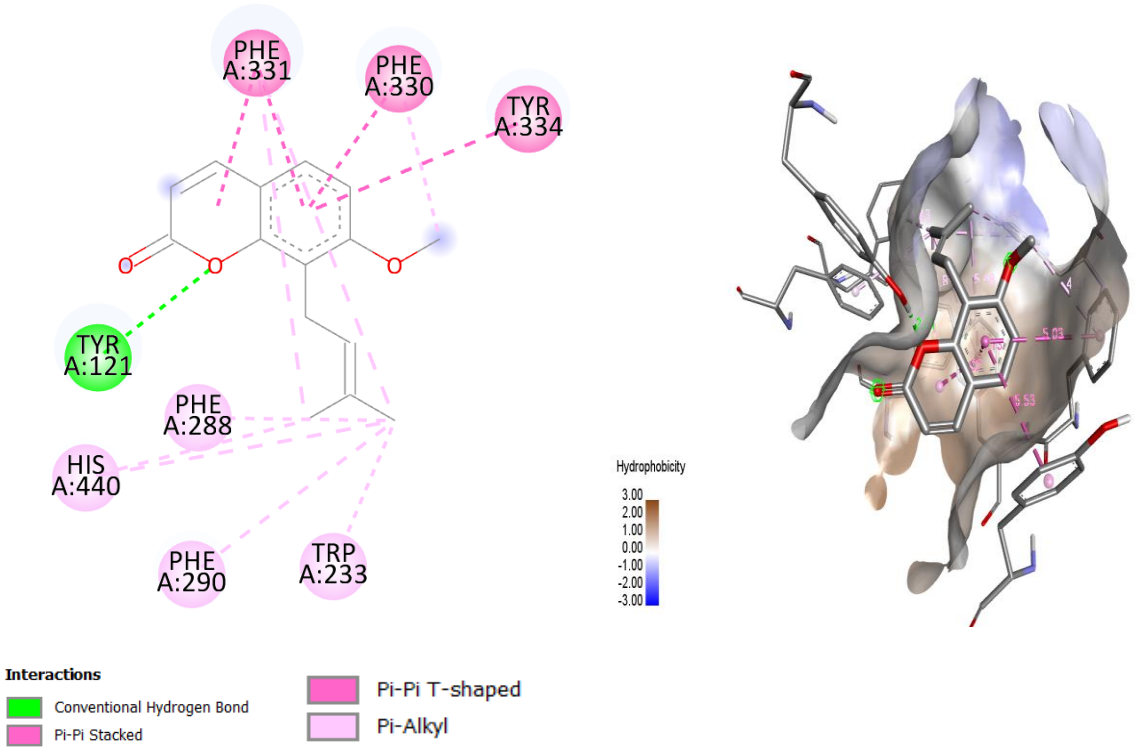


Figure 9*:* Binding of Osthole with receptor AChE protein using PyMOL



(A) **(B)**

Figure 10: (A) 2D structure of AChE protein binding with Osthole (B) 3D structure of AChE protein binding with Osthole and all these interactions observed in Discovery Studio

6.8 Molecular docking of AChE Protein with Daphnetin:

Daphnetin gave binding score of -7.8 kcal/mol with AChE protein (Figure 11 & 12). The ligand was showed hydrophobic interaction with amino acid residues TYR 130, GLY 117, TRP 84, and SER 122 residues. Moreover, two conventional hydrogen bonds were formed between the carbonyl oxygen and amino acid residues TYR 130 and GLY117, and one hydrogen bonds formed between hydroxyl group and SER122 residue. Furthermore, the molecule displays a π-π stacking contact with amino acid residue TRP 84.

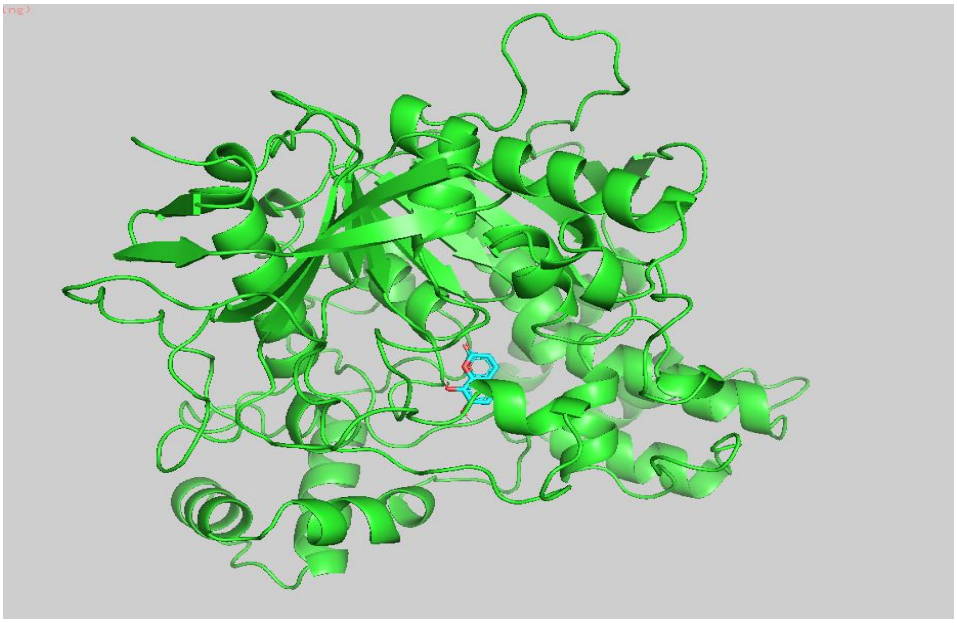
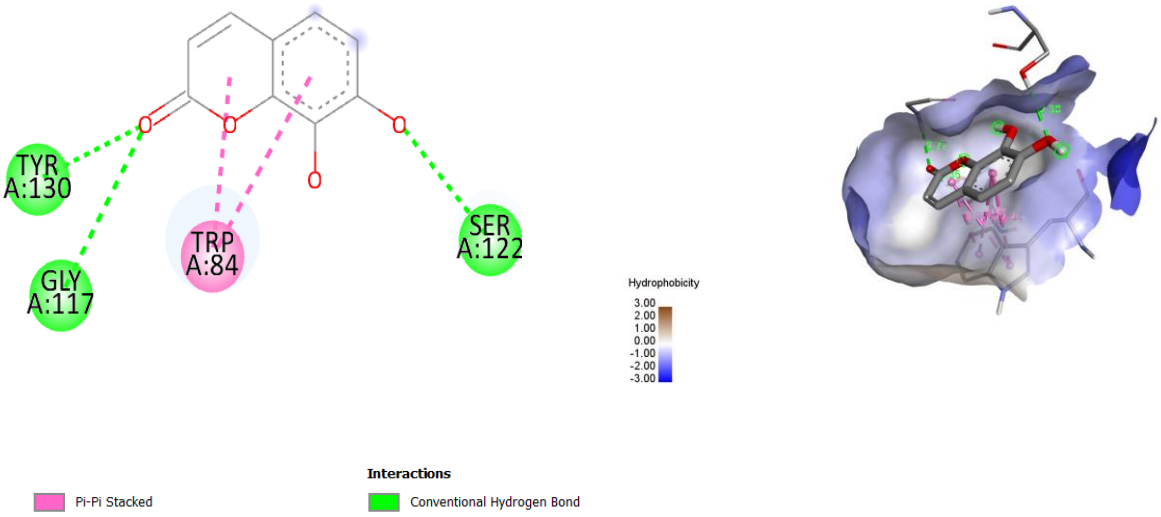


Figure 11: Binding of Daphnetin with receptor AChE protein using PyMOL



(A) **(B)**

Figure 12: (A) 2D structure of AChE protein binding with Daphnetin (B) 3D structure of AChE protein binding with Daphnetin and all these interactions observed in Discovery Studio

6.9 Molecular docking of AChE Protein with Esculetin

Esculetin binds with the AChE receptor with the binding energy of -7.4 kcal/mol (Figure 13 & 14). Esculetin is stabilized in the interface by H-bonds. The interacting amino acids in the case of Esculetin are TYR 130, GLY 441, and GLU 199 residues, of chain A. A conventional hydrogen bond is formed with the two amino acid residues TYR 130, and GLU 199 in chain A

of protein. In addition, the amino acid residue GLY 441 in the same chain A shows evidence of a carbon-hydrogen bond interaction in the molecule. Additionally, the molecule displays a pi-pi stacking contact with the identical chain A's amino acid residue TRP 84.

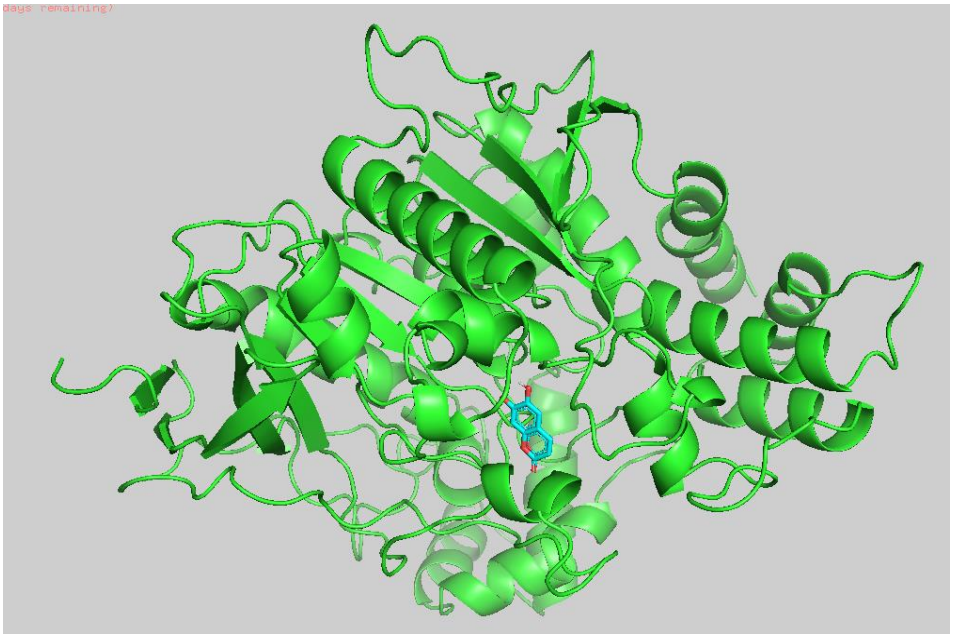


Figure 13: Binding of Esculetin with receptor AChE protein using PyMOL

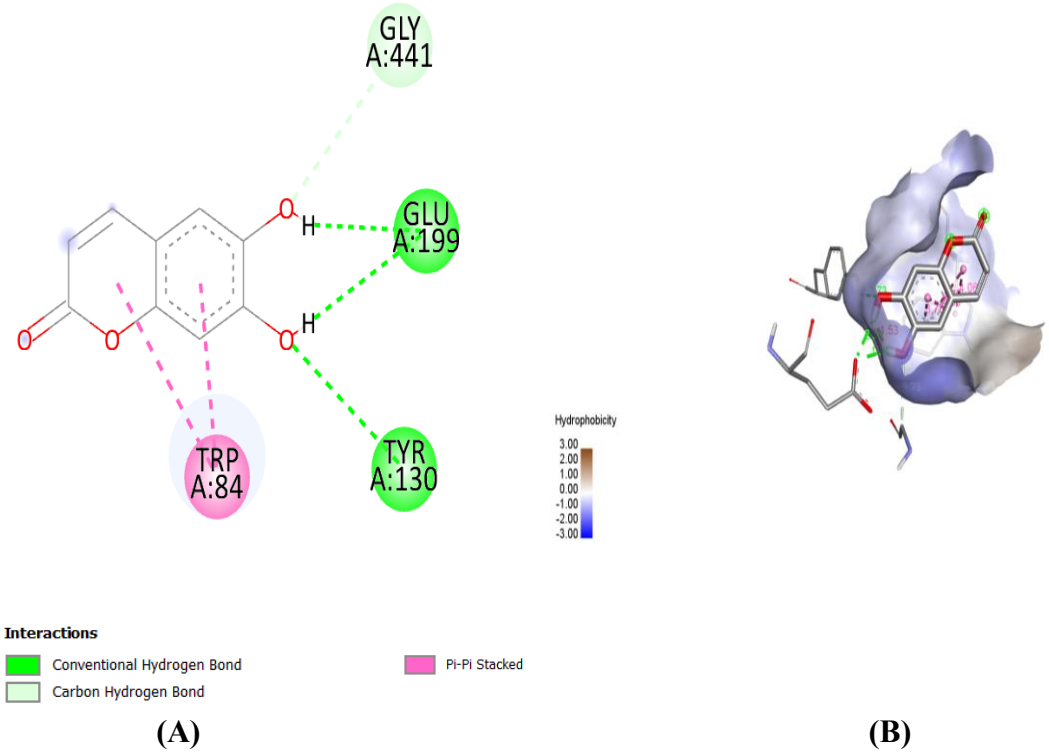


Figure 14: (A) 2D structure of AChE protein binding with Esculetin (B) 3D structure of AChE protein binding with Esculetin and all these interactions observed in Discovery Studio

6.10 Molecular docking of AChE Protein with Scopoletin:

Scopoletin bound to AChE receptor with the binding energy of -7.5 kcal/mol (Figure 15 &16). Scopoletin interacted with amino acid residues TYR 84, GLY441, ASN 85, and SER 122. Oxygen atom of methoxy group showed hydrogen bonding with amino acid residue SER 122. In addition, the amino acid residue GLY 441 and ASN 85 showed evident carbon-hydrogen bond interaction in the molecule. Furthermore, the molecule displays a pi-pi stacking contact with amino acid residue TRP 84.

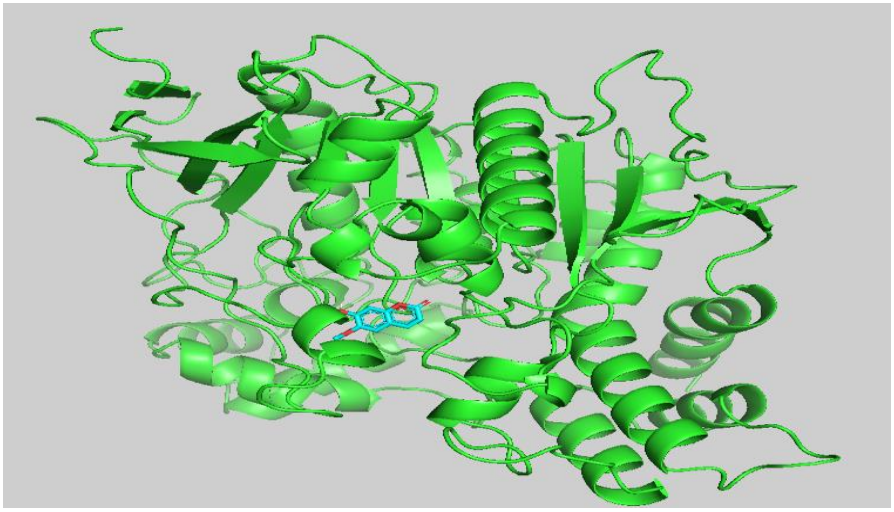


Figure 15: Binding of Scopoletin with receptor AChE protein using PyMOL

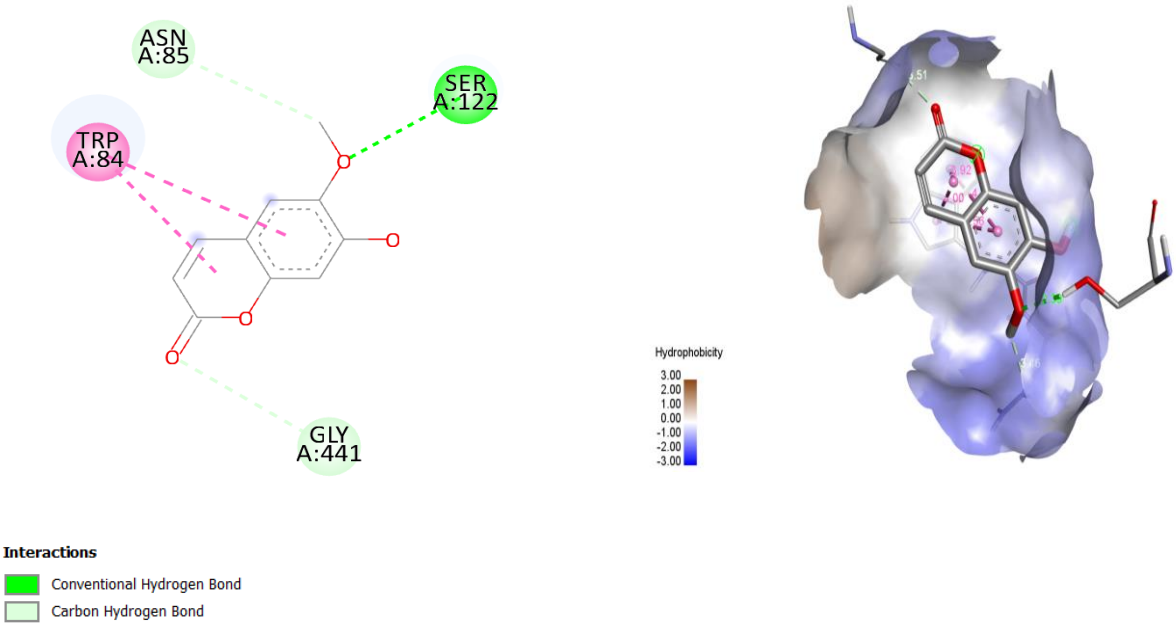


Figure 16: (A) 2D structure Scopoletin-AChE binding (B) 3D structure Scopoletin-AChE binding generated in Discovery Studio

6.11 Drug-likeness Screening of Coumarin derivatives

Table 2 : Drug-likeness Screening of Ligands

Parameter	Warfarin	Esculetin	Daphnetin	Osthole	Scopoletin	Donepezil
MW	310.35	178.14	178.14	244.29	206.2	379.50
A logP	3.40	1.20	1.20	3.31	1.81	4.36
H-bond acceptor	4	4	4	3	4	4
H-Bond donor	2	2	2	0	0	0
TPSA	70.67	70.67	70.67	39.44	48.67	38.77
Rotatable bond	4	0	0	3	2	6
Water solubility	-3.484	-2.847	-2.847	-4.372	-3.434	-2.425
Plasma protein binding	1.037	0.757	0.872	0.836	0.811	0.843
Blood-brain barrier	Yes	No	No	Yes	Yes	Yes
GI absorption	High	High	High	High	High	High
CYP1A2 inhibitor	No	Yes	Yes	Yes	Yes	No
CYP2C19 inhibitor	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No	Yes
CYP3A4 inhibitor	Yes	No	No	No	No	Yes
Carcinogenicity	No	No	No	No	No	No
Nephrotoxicity	No	No	No	No	Yes	No
Acute Oral Toxicity	Medium	High	Medium	High	Medium	Medium
Lipinski violations	0	0	0	0	0	0
Lipinski's rule of 5	Passed	Passed	Passed	Passed	Passed	Passed
Veber rule	Good	Good	Good	Good	Good	Good

In determining the drug-likeness of the ligands listed in the table several key parameters were considered, including Molecular weight (MW), lipophilicity (expressed as A logP), hydrogen bond characteristics (H-bond acceptor and donor counts), topological polar surface area (TPSA), rotatable bond count, water solubility, plasma protein binding, blood-brain barrier permeability, gastrointestinal (GI) absorption, and inhibition of important cytochrome P450 (CYP) enzymes.

6.11.1 Warfarin

Warfarin exhibits several advantageous drug-like characteristics, including a moderate molecular weight, ideal lipophilicity, and the capacity to pass through the blood-brain barrier a critical quality for medications that affect the central nervous system (CNS). Because it inhibits CYP3A4, there may be possible drug interactions. Therefore, while co-administering this medication with other medications that are metabolised by this enzyme system, caution must be exercised. Its distribution and pharmacokinetics may also be impacted by its medium plasma protein binding.

6.11.2 Esculetin:

Esculetin demonstrates favourable drug-like properties with strong gastrointestinal (GI) absorption and compliance with Lipinski's and Veber's criteria. Good membrane permeability, which is necessary for oral absorption, is suggested by its moderate lipophilicity. Esculetin, a CYP1A2 inhibitor, may alter the way concurrently administered medications are metabolised by this enzyme, which could result in drug interactions. Further investigation of its metabolic pathways and interactions is necessary.

6.11.3 Daphnetin:

Daphnetin exhibits characteristics of a medication, including high GI absorption and adherence to Veber's and Lipinski's criteria. Its lipophilicity and molecular weight are within permissible bounds for oral medications. Safety issues are brought up by the high acute oral toxicity and possible nephrotoxicity of daphnetin. In-depth preclinical research is required to evaluate its safety profile and identify any potential underlying toxicity pathways.

6.11.4 Osthole:

Osthole has promise for CNS-related applications due to its high GI absorption, strong water solubility, and ability to cross the blood-brain barrier. Its molecular characteristics fit the requirements for a medication nicely.

To determine Osthol safety profile and appropriateness for clinical use, extensive research is necessary due to its potential for nephrotoxicity and acute oral toxicity, much to Daphnetin.

6.11.5 Scopoletin

Lipinski and Veber's principles are followed by scopoletin, indicating strong water solubility and drug-like qualities. Its potential for CNS-related indications is increased by its ability to pass the blood-brain barrier.

Scopoletin is a CYP3A4 inhibitor, it may interact with medications that are metabolised by this enzyme system. This means that dose regimen modifications and close observation of possible drug interactions are required.

6.11.6 Donepezil:

Lipinski's and Veber's criteria are followed by donepezil, indicating that it has drug-like effects. Its molecular weight is another one of these attributes. For CNS-targeted therapeutics, its capacity to pass the blood-brain barrier is favourable.Donepezil's suppression of CYP3A4 and CYP2D6 can result in serious drug interactions, especially for people who take numerous drugs that are metabolised by these enzymes. In these situations, careful observation and dosage modifications can be required.

7 CONCLUSION:

This in-silico study explored the potential coumarin derivatives as acetylcholinesterase (AChE) inhibitors to treat Alzheimer's disease (AD). Several coumarin compounds showed higher binding affinities towards the AChE enzyme than donepezil, a medication that is utilised in clinical settings, according to molecular docking. When compared to donepezil (-7.7 kcal/mol), coumarin and its derivatives (-10.3 kcal/mol), osthol (-8.9 kcal/mol), and warfarin (-9.7 kcal/mol) displayed stronger interactions.

Compounds such as coumarin and its derivatives, warfarin, and osthole have favourable pharmacokinetic profiles similar to donepezil, including good gastrointestinal absorption and blood-brain barrier permeability, according to an evaluation of drug-likeness features. However, several coumarin derivatives were shown to have some safety issues, including nephrotoxicity, acute toxicity, and possible CYP enzyme inhibition. This emphasized the need for additional research and optimisation during lead compound development.

The results indicated that coumarin scaffolds could serve as a promising skeleton for the designing and development of new AChE inhibitors that specifically target Alzheimer's disease. These naturally occurring compounds present themselves as promising candidates with the potential to enhance the clinical performance of donepezil, the medicine currently in use, due to their favourable binding affinities and drug-like properties. To improve the potency, selectivity, and safety profiles of the lead compounds for the treatment of AD, future research should concentrate on their synthesis, experimental validation, and optimisation.

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