

Docking-Guided Design and Biological Evaluation of a Lead Convolvine-Based Acetylcholinesterase Inhibitor for Alzheimer's Disease

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Docking-Guided Design and Biological Evaluation of a Lead Convolvine-Based Acetylcholinesterase Inhibitor for Alzheimer's Disease

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1. Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative condition marked by cognitive decline, memory loss, and cholinergic dysfunction [1]. Acetylcholinesterase (AChE) inhibition is still a crucial therapeutic approach, although existing medications like donepezil merely relieve symptoms and have negative side effects [2]. The traditional Ayurvedic Medhya Rasayana plant *Convolvulus pluricaulis* (Shankhpushpi) has been extensively documented for its neuroprotective and cognitive-enhancing qualities [3, 4]. Tropane alkaloids including convoline, convolidine, convolvine, and confoline are among its phytochemical constituents and provide a viable framework for logical drug design [3].

A targeted library of derivatives based on convolvine was created and assessed as possible AChE inhibitors in this work. Human AChE (PDB ID: 4PQE) was subjected to molecular docking using AutoDock Vina [5], and interactions with important catalytic residues were examined. FT-IR, NMR, and HRMS methods were used to characterise the top-ranked molecule, which was chosen for synthesis based on docking performance. Ellman's colorimetric technique was then used to determine its inhibitory activity [6].

Strong binding affinities were shown by the docking studies, and the chosen molecule outperformed the reference medication and showed advantageous interactions inside the catalytic region. Significant AChE inhibitory action with potency comparable to the standard was confirmed by in vitro assessment. Analysis of the structure–activity relationship revealed that substitution patterns are essential for controlling physicochemical characteristics and binding affinity.

These findings identify convolvine-based derivatives as promising scaffolds for the development of novel anti-Alzheimer's agents, warranting further pharmacological and in vivo validation.

2. Keywords

Alzheimer's disease; *Convolvulus pluricaulis*; convolvine-based derivatives; acetylcholinesterase inhibition; molecular docking; coumarin–nortropine ester; Ellman assay

3. Introduction

Alzheimer's disease (AD) is the most common cause of dementia, characterized by progressive neuronal loss and deficits in cholinergic neurotransmission [1]. Current acetylcholinesterase (AChE) inhibitors, such as donepezil, provide short-term symptomatic benefit but are limited by dose-related adverse effects and declining efficacy with prolonged use [2]. This therapeutic gap highlights the need for novel scaffolds with improved safety and sustained activity.

Convolvulus pluricaulis (Shankhpushpi), a traditional Medhya Rasayana herb in Ayurveda, is recognized for its cognition-enhancing and neuroprotective properties [3,4]. Its phytochemical profile includes tropane alkaloids such as convoline, convolidine, convolvine, and confoline. The rigid bicyclic framework of these alkaloids provides a rational basis for structural modification aimed at enhancing AChE inhibition.

In the present study, convolvine-based derivatives were designed and optimized through molecular docking against human AChE (PDB ID: 4PQE). Strategic substitutions were introduced to strengthen interactions with key catalytic residues and optimize physicochemical properties. The most promising candidate was synthesized, structurally characterized, and evaluated for AChE inhibitory activity using Ellman's colorimetric assay [6]. This integrated

approach establishes convolvine derivatives as promising scaffolds for anti-Alzheimer's drug development.

4. Compound Library

Convolvine, a tropane alkaloid isolated from *Convolvulus pluricaulis*, possesses a rigid bicyclic skeleton that provides a rational framework for acetylcholinesterase (AChE) inhibition. Structural modifications were introduced at key positions to generate a focused library of derivatives aimed at enhancing catalytic site interactions and optimizing physicochemical properties mention in **Figure 1**..

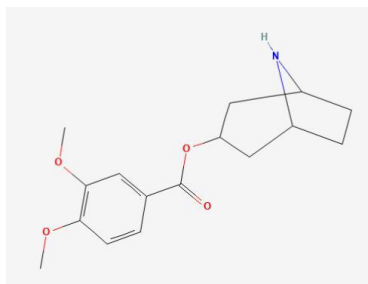


Figure 1. Chemical structure of convolvine (parent scaffold).

Common Structural Representation:-

The general structure of the convolvine scaffold and the position of structural modification are shown in Figure 2.

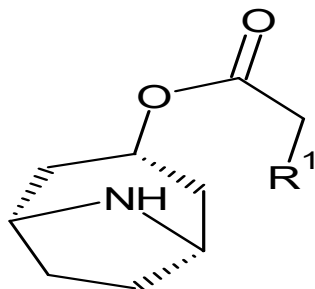


Figure 2: General structure of the convolvine scaffold showing the R-group position selected for structural modification.

The structure represents the parent convolvine scaffold used as the core framework for the design of the proposed derivatives. The general formula, Nortropane-O-C(=O)-R, indicates the ester linkage of the convolvine scaffold, with the R group representing the site at which different aromatic acid substituents can be introduced. In the present study, this R-group position was selected for structural diversification, while the core convolvine scaffold was retained. Different aromatic acid substituents were subsequently introduced at this position to generate the designed derivatives C1–C5. This modification strategy was used to investigate how changes in the aromatic substituent influence the molecular interactions and acetylcholinesterase inhibitory activity of the resulting compounds.

Compound (code)	Aromatic Acid Substituent (R group)
Convolvine	3,4-Dimethoxybenzoic acid
C1	Coumarin-3-carboxylic acid
C2	Naphthalene-1-carboxylic acid
C3	Coumarin-7-carboxylic acid
C4	Para-bromo benzoic acid
C5	Para-hydroxy benzoic acid

5. Designed Derivatives

The designed convolvine derivatives and their key structural modifications are summarized in Table 1.

Table 1. Designed convolvine– derivatives and key modifications

Code	IUPAC name	Formula	SMILES	Key modification
C1	8-azabicyclo[3.2.1]octan-3-yl 2-oxo-2 <i>H</i> -1-benzopyran-3-carboxylate	C ₁₇ H ₁₇ NO ₄	<chem>O=C(OC1CC2CCC(C1)N2)C1=CC2=CC=CC=C2OC1=O</chem>	Coumarin-3-carboxylic acid scaffold; reference compound for SAR analysis.[7,8,9]
C2	(1 <i>R</i> ,5 <i>S</i>)-8-azabicyclo[3.2.1]octan-3-yl naphthalene-1-carboxylate	C ₁₈ H ₁₉ NO ₂	<chem>O=C(OC1C[C@@H]2CC[C@H](C1)N2)C1=CC=CC2=CC=CC=C12</chem>	Naphthalene substitution lipophilicity [10] ↑
C3	(1 <i>R</i> ,5 <i>S</i>)-8-azabicyclo[3.2.1]octan-3-yl 2-oxo-2 <i>H</i> -1-benzopyran-7-carboxylate	C ₁₇ H ₁₇ NO ₄	<chem>O=C(OC1C[C@@H]2CC[C@H](C1)N2)C1=CC2=C(C=CC(=O)O2)C=C1</chem>	Coumarin-7-carboxylic acid [8]
C4	(1 <i>R</i> ,5 <i>S</i>)-8-azabicyclo[3.2.1]octan-3-yl 4-bromobenzoate	C ₁₄ H ₁₆ BrNO ₂	<chem>BrC1=CC=C(C=C1)C(=O)OC1C[C@@H]2CC[C@H](C1)N2</chem>	Para bromo benzoic acid
C5	(1 <i>R</i> ,5 <i>S</i>)-8-azabicyclo[3.2.1]octan-3-yl 4-hydroxybenzoate	C ₁₄ H ₁₇ NO ₃	<chem>OC1=CC=C(C=C1)C(=O)OC1C[C@@H]2CC[C@H](C1)N2</chem>	Hydroxy substitution ↑ H-bonding [11]

6.1 Materials and Methods:-

Docking Workflow

- **Ligand preparation:** convolvine structure was drawn in ChemDraw, converted to 3D, and energy-minimized using OpenBabel.
- **Protein preparation:** The three-dimensional structures of the target proteins were prepared using UCSF Chimera (version 1.16) [14], which included the removal of water molecules, addition of hydrogen atoms, and energy minimization. Binding sites were predicted using the CASTp server, following the workflow described by Saini et al. [15].
- **Docking:** Molecular docking was carried out using AutoDock Vina (version 1.1.2) to estimate binding affinities (kcal/mol) and analyze ligand–protein interactions. UCSF Chimera (version 1.16) [14] was used for protein preparation, grid box setup, and visualization of docking poses.
- **Validation:** Docking protocol was validated using the co-crystallized ligand present in the protein structure. The ligand was re-docked into the active site, and the predicted binding pose was compared with the original crystallographic conformation to assess accuracy.[15].
- **Visualization:** PyMOL and Discovery Studio Visualizer were used to analyze binding pockets and interaction residues. [16]

6.1.1. In Silico Drug-Likeness Evaluation

The drug-likeness of the designed convolvine derivatives (C1–C5) and the reference compound Donepezil was assessed using the **MolSoft** online server. The calculated molecular descriptors included molecular weight (MW), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), octanol/water partition coefficient (MolLogP), aqueous solubility (MolLogS), molecular polar surface area (MolPSA), blood–brain barrier (BBB) score, and drug-likeness score. In addition, each compound was evaluated for compliance with **Lipinski's Rule of Five** to determine its suitability as a potential orally active drug candidate.

6.1.2 In Silico Pharmacokinetic (ADME) Prediction

The pharmacokinetic properties of the designed convolvine derivatives (C1–C5) together with the reference drug Donepezil were predicted using the **pkCSM** web server. The computational analysis was performed to estimate the absorption, distribution, metabolism, and excretion (ADME) characteristics of the designed compounds, thereby providing an initial assessment of their pharmacokinetic behavior.

6.1.3 Absorption Prediction

The absorption profiles of the designed compounds were predicted using the pkCSM server. The evaluated parameters included aqueous solubility, Caco-2 cell permeability, human intestinal absorption, skin permeability (log K_p), and P-glycoprotein (P-gp) substrate and inhibitor predictions. These parameters were considered to estimate the oral absorption and membrane permeability of the designed derivatives.

6.1.4 Distribution Prediction

The distribution characteristics of the designed compounds were evaluated using the pkCSM server. The predicted parameters included the steady-state volume of distribution (VD_{ss}), fraction unbound in plasma (F_u), blood–brain barrier (BBB) permeability (log BB), and central nervous system (CNS) permeability (log PS). These descriptors were selected to estimate systemic distribution and the potential of the compounds to penetrate the central nervous system.

6.1.5 Metabolism and Excretion Prediction

The metabolism and excretion properties of the designed compounds were predicted using the pkCSM server. Metabolic assessment included substrate prediction for cytochrome P450 isoforms CYP2D6 and CYP3A4 together with inhibition prediction for CYP1A2, CYP2C19, CYP2C9, and CYP3A4. Excretion characteristics were evaluated by predicting total systemic clearance and renal organic cation transporter 2 (OCT2) substrate status.

6.1.6 In Silico Toxicity Prediction

The toxicity profiles of the designed convolvine derivatives (C1–C5) and Donepezil were predicted using the **pkCSM** and **ProTox-II** online platforms. Toxicity assessment included prediction of AMES mutagenicity, hERG I channel inhibition, hepatotoxicity, skin sensitization, acute oral toxicity (LD₅₀), and toxicity class. These computational analyses were performed to obtain a preliminary evaluation of the safety profile of the designed compounds prior to experimental investigation.

6.1.7 Reaction scheme:-

STEP:-I Synthesis of Coumarin-3-carboxylic acid (Knoevenagel Condensation)

Step I: Synthesis of Coumarin-3-carboxylic acid

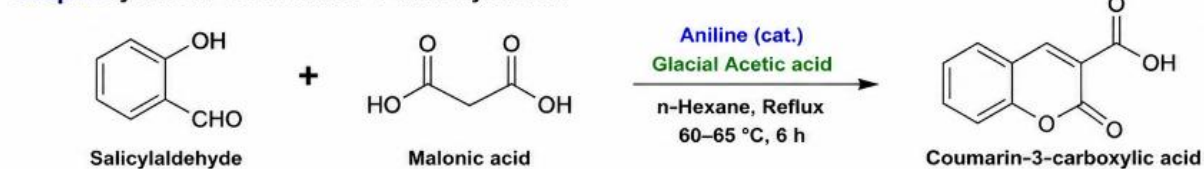


Figure:- 3 Synthesis of Coumarin-3-carboxylic acid.

STEP:II— Preparation of Coumarin-3-carbonyl chloride

Step II: Formation of Coumarin-3-carbonyl chloride

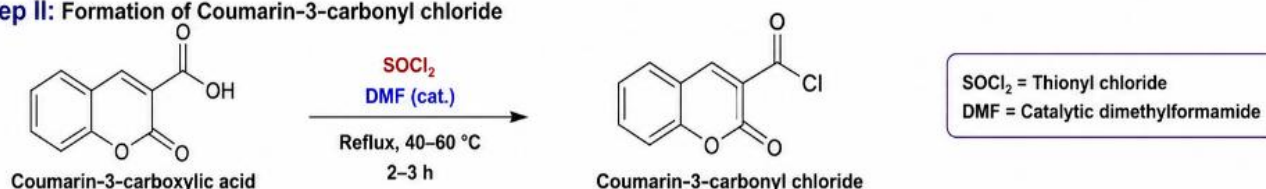


Figure:- 4 Preparation of Coumarin-3-carbonyl chloride

STEP:-III Protection of Nortropine as N-Boc-Nortropine

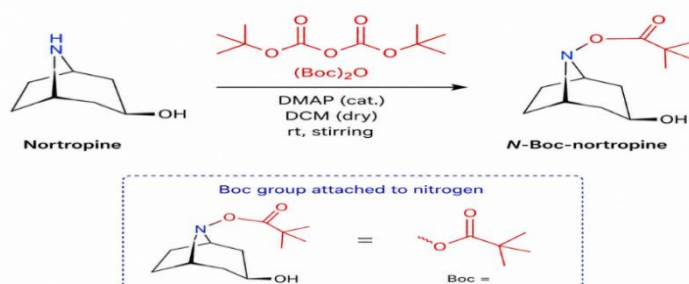


Figure:- 5 Protection of Nortropine as N-Boc-Nortropine

STEP IV. Synthesis of N-Boc-Protected Coumarin–Nortropine Ester

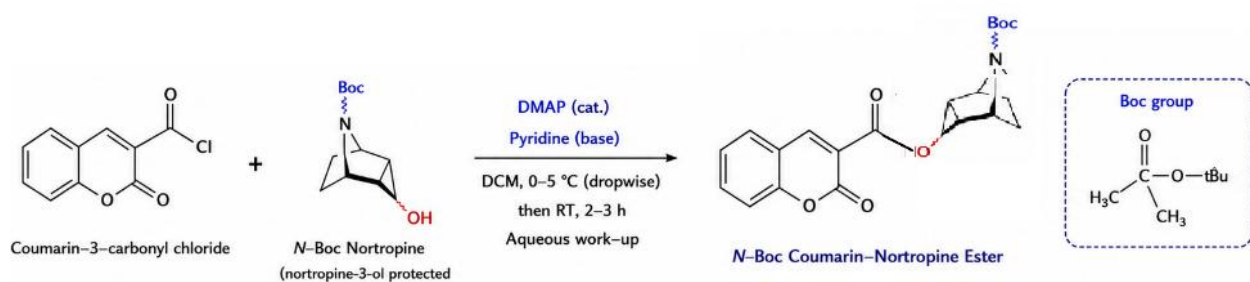


Figure:- 6 Synthesis of N-Boc-Protected Coumarin–Nortropine Ester

STEP :-V Boc Deprotection and Synthesis of Coumarin–Nortropine Ester (C1)

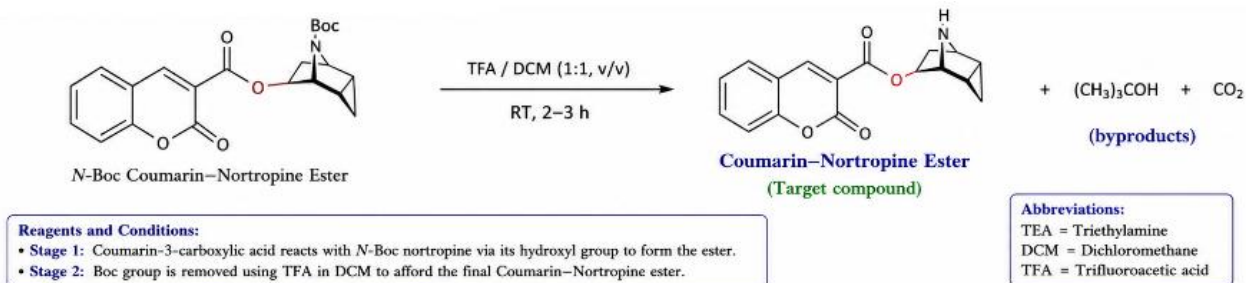


Figure:- 7 Boc Deprotection and Synthesis of Coumarin–Nortropine Ester (C1)

Step 1 – Synthesis of Coumarin-3-carboxylic acid (Knoevenagel Condensation)

Procedure: Coumarin-3-carboxylic acid was synthesized via a modified Knoevenagel condensation. Salicylaldehyde (31.5 mmol) and malonic acid (31.5 mmol) were dissolved in **n-hexane (8 mL)**, followed by the addition of glacial acetic acid (8.2 mmol) and catalytic aniline. The reaction mixture was refluxed at **60–65 °C for 12 h**, and the progress was monitored by TLC using **acetone:methanol:acetic acid (4:4:2, v/v/v)** as the mobile phase. After completion, the reaction mixture was cooled to room temperature, and the precipitated solid was collected by filtration, washed with cold **n-hexane**, and dried under reduced pressure to obtain **coumarin-3-carboxylic acid**. [18]. **Figure:- 3** Synthesis of Coumarin-3-carboxylic acid.

Step 2 – Preparation of Coumarin-3-carbonyl chloride

Procedure:- Coumarin-3-carboxylic acid (13.15 mmol) was suspended in excess thionyl chloride (SOCl₂) containing 2–3 drops of DMF as a catalyst. The reaction mixture was refluxed at 40–60 °C for 2–3 h until gas evolution ceased. Excess thionyl chloride was removed under reduced pressure to afford coumarin-3-carbonyl chloride, which was used directly in the subsequent esterification step without further purification. **Figure:- 4** Preparation of Coumarin-3-carbonyl chloride

Step 3 - Synthesis of N-Boc-Nortropine

Procedure:- Nortropine (17.70 mmol) was dissolved in dry **dichloromethane (DCM)**, followed by the addition of **di-tert-butyl dicarbonate (Boc₂O)** and catalytic **DMAP**. The reaction mixture was stirred at room temperature, and its progress was monitored by TLC. Upon completion, the reaction mixture was washed with water, and the organic layer was dried over anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure afforded **N-Boc-nortropine**, which was used directly in the next step. **Figure:- 5** Protection of Nortropine as N-Boc-Nortropine

Step 4:-Synthesis of Coumarin–Nortropine Ester (C1)

Procedure:- N-Boc-nortropine was dissolved in dry **DCM**, and **pyridine** was added as an acid scavenger. A solution of **coumarin-3-carbonyl chloride** in dry DCM was added dropwise at **0–5 °C** under continuous stirring. The reaction mixture was then allowed to reach room temperature and stirred until completion, as monitored by TLC. After aqueous work-up, the organic layer was dried over anhydrous sodium sulfate, and solvent removal under reduced pressure afforded the **N-Boc coumarin–nortropine ester**. **Figure:- 6** Synthesis of N-Boc-Protected Coumarin–Nortropine Ester

Step 5:- Boc Deprotection

Procedure:- The protected intermediate was treated with a **1:1 mixture of trifluoroacetic acid (TFA) and dichloromethane** at room temperature for **1–2 h**. The progress of the reaction was monitored by TLC. After completion, the solvent was removed under reduced pressure, and the residue was neutralized and washed with water to afford the target **Coumarin–Nortropine ester (C1)**. **Figure:- 7** Boc Deprotection and Synthesis of Coumarin–Nortropine Ester (C1)

TLC Conditions

Reaction progress was monitored using **silica gel 60 F254 TLC plates** and visualized under **UV light (254 nm)**. The mobile phase consisted of **acetone:methanol:acetic acid (4:4:2, v/v/v)**.

6.1.7 In Vitro Acetylcholinesterase (AChE) Inhibition Assay

The acetylcholinesterase (AChE) inhibitory activity of the synthesized lead compound (C1) was evaluated **in vitro** using **Ellman's colorimetric method** in a 96-well microplate format. The assay was performed at the **Infinite Biotech Institute of Research and Analytics, Sangli, Maharashtra, India**.

The reaction mixture consisted of phosphate buffer (pH 8.0), acetylcholinesterase enzyme, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and the test compound. The enzyme and test

compound were pre-incubated for **15 min** prior to initiation of the reaction. The enzymatic reaction was initiated by adding **acetylthiocholine iodide (ATChI)** as the substrate to obtain a final concentration of **1 mM**. Hydrolysis of ATChI by acetylcholinesterase generated thiocholine, which reacted with DTNB to produce the yellow-colored **5-thio-2-nitrobenzoate (TNB)** anion. The absorbance was recorded at **412 nm** using a microplate reader.

$$\text{AChE Inhibition (\%)} = \frac{\text{Abs 412}(\text{control}) - \text{Abs 412}(\text{extract})}{\text{Abs 412}(\text{control})} * 100$$

Donepezil was used as the positive control, whereas solvent-treated wells served as the negative control. The inhibitory activity of compound C1 was evaluated at concentrations of **20, 40, 60, 80, and 100 µg/mL**, and all measurements were performed in triplicate. The percentage inhibition of AChE activity was calculated using the following equation:

The half-maximal inhibitory concentration (**IC₅₀**) was determined from the concentration–response curve by plotting the percentage inhibition against the logarithm of the compound concentration.

7.1.0 Results and Discussion :-

7.1.1 Docking Results :-The docking interactions of convolvine, its derivatives, and the reference drug with AChE are summarized in Table 2.

Table no 2. Docking interactions of convolvine, derivatives, and reference drugs with AChE (PDB: 4PQE)

Compound	Docking Score (kcal/mol)	Hydrogen Bond Interactions	Hydrophobic / π - π Interactions
C1	−9.8	Ser203, His447	Trp86 (π-π), Tyr337
C2	−9.4	Tyr124	Phe338, Tyr337
C3	−8.4	Ser203, Glu334	Trp86, Tyr337
C4	−8.4	–	Tyr337, Phe295
C5	−8.4	Ser203, Tyr124	Trp86, Tyr337
Donepezil	−8.3	Ser203, His447	Trp86, Tyr337

The selected convolvine derivatives exhibited docking scores ranging from **−8.4 to −9.8 kcal/mol**, demonstrating binding affinities comparable to or better than **Donepezil (−8.3 kcal/mol)**. Among the designed compounds, **C1** showed the highest binding affinity (**−9.8 kcal/mol**) figure no: 8 and formed key interactions with **Ser203, His447, Trp86, and Tyr337**, similar to the reference drug. **C2** also exhibited strong binding (**−9.4 kcal/mol**) through interactions with **Tyr124, Phe338, and Tyr337**, whereas **C3–C5** maintained favorable interactions with catalytic and peripheral active-site residues. The enhanced binding affinities of the selected derivatives suggest that structural modification of the convolvine scaffold improved interactions within the AChE active site. Based on their docking performance, **C1–**

C5 were selected for subsequent pharmacokinetic and toxicity predictions, with **C1** identified as the lead compound for synthesis and biological evaluation. Lower docking score (more negative) indicates stronger binding affinity with AChE

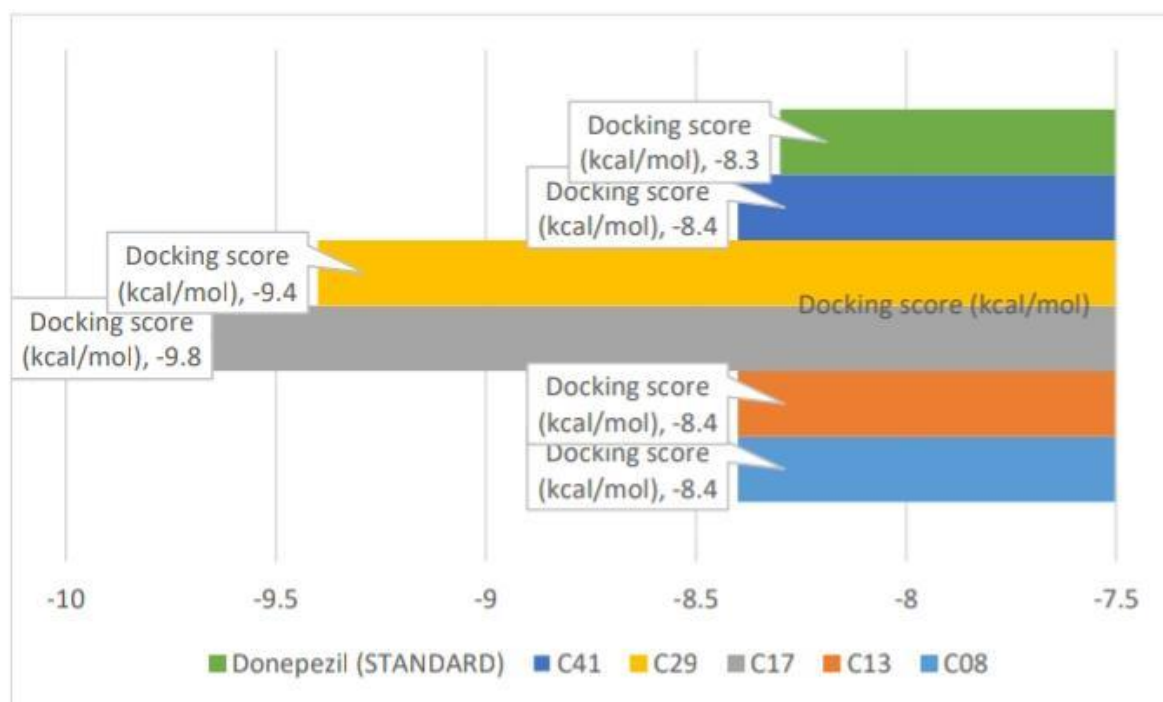


Figure:- 8 bar diagram docking score of c1 to c5

7.1.2 Results Docking Analysis

To confirm the accuracy of our docking protocol, the binding residues identified in this study were compared with those reported in recent literature. The Heliyon 2024 article on *Catunaregam spinosa* phytoconstituents (PDB ID: 4PQE) highlighted critical AChE residues including **Trp86, His447, Tyr337, Tyr341, Phe295, and Phe338** as pharmacophoric hotspots for ligand binding[17].

Our docking analysis of convolvine and its derivatives (C1–C5) consistently reproduced these interactions. Specifically, **Convolvine, C1, C3, C5, and Donepezil** engaged **Ser203, His447, Trp86, and Tyr337**, overlapping with the catalytic triad and aromatic gorge residues reported in the literature. **C2** showed hydrophobic contacts with **Tyr124 and Phe338**, while **C4** interacted with **Tyr337 and Phe295**, both validated binding residues. Notably, **C3** formed additional hydrogen bonding with **Glu334**,

This concordance between our computational findings and published data validates the docking workflow and confirms the pharmacological relevance of the designed derivatives. The overlap with literature-reported residues strengthens the conclusion that **C1 and C3 are optimized inhibitors**, engaging the same binding pockets as clinically established drugs.

C1 – Interaction with AChE (PDB: 4PQE)

C1 exhibited a docking score of **−9.8 kcal/mol**, confirming stronger binding affinity compared to convolvine. Docking was performed using a **20 × 20 × 20 Å grid box**, centered on the **SBD_Site_Sphere (ID:0)** with coordinates **XYZ −28.55, 23.09, −10.88 Å** and a binding radius of **7.38 Å**, ensuring accurate coverage of the active gorge.

The ligand formed **π – π stacking interactions with Trp86 and Trp286**, and **hydrophobic contacts with Tyr124, Tyr341**, stabilizing the compound within the aromatic gorge.

Validation against the Heliyon 2024 study (PDB ID: 4PQE) confirmed that **Trp86, His447, Tyr337, Tyr341, Phe295, and Phe338** are critical anchors for AChE inhibition. The overlap of C1 with **Trp86, Tyr341**, (**Figure:- 9 and Figure:- 10**) together with extended stabilization via **Trp286 and Tyr124**, demonstrates methodological accuracy and highlights the coumarin-3 substitution strategy as a rational design approach for enhanced AChE inhibition.

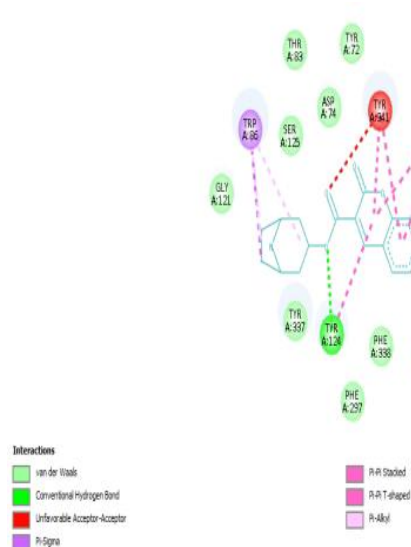


Figure:- 9 C1 – 2D interaction diagram with AChE (PDB: 4PQE)

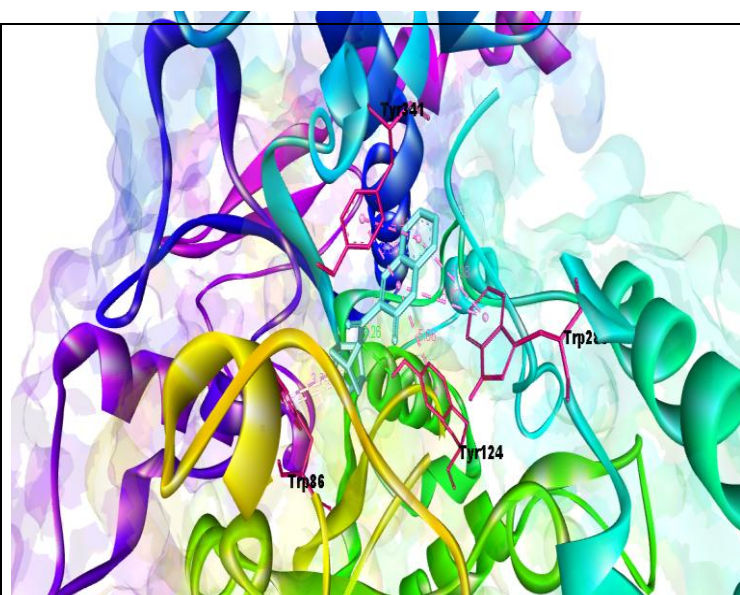
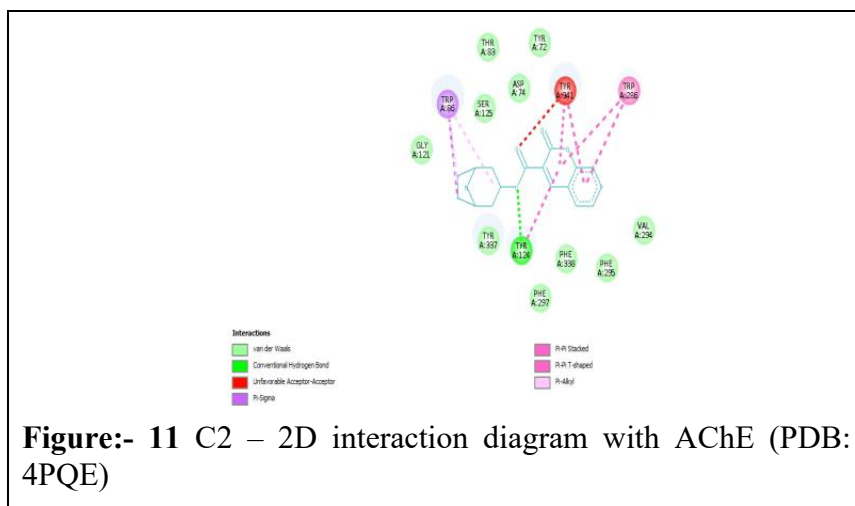
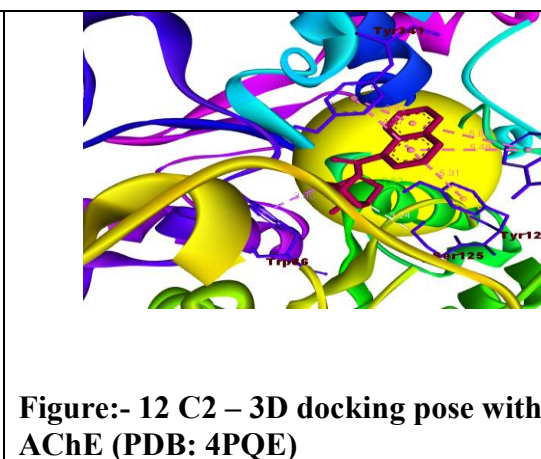


Figure:- 10 C1 – 3D docking pose with AChE (PDB: 4PQE)

C2 – Interaction with AChE (PDB: 4PQE)

C2 showed a docking score of **−9.4 kcal/mol**, indicating strong binding affinity within human acetylcholinesterase. Docking was performed in a **20 × 20 × 20 Å grid box**, centered on the **SBD_Site_Sphere (XYZ −28.10, 22.73 , −10.81 Å; radius 10.16 Å)** pose c2 **Figure:- 12**.

The ligand engaged in **π – π stacking with Trp86 and Trp286**, **hydrophobic contacts with Tyr124 and Tyr341**, and a **hydrogen bond with Ser125**, stabilizing its orientation in the aromatic gorge **Figure:- 11**.



Validation against the Heliyon 2024 study confirmed overlap with key residues (**Trp86, Tyr341**) and extended stabilization via **Trp286, Tyr124, and Ser125**, supporting the coumarin-derived scaffold as a rational design for AChE inhibition.

C3 – Interaction with AChE (PDB: 4PQE)

C3 exhibited a docking score of **−8.4 kcal/mol**, representing the strongest binding affinity among the tested compounds. Docking was performed using a **20 × 20 × 20 Å grid box**, centered on the **SBD_Site_Sphere (ID:0)** with coordinates **XYZ −28.55, 23.09, −10.89 Å** and a binding radius of **7.38 Å**, ensuring precise coverage of the active gorge **Figure:- 14**. The ligand formed **π–π stacking interactions with Trp86 and Trp286**, while **hydrophobic contacts were observed with Tyr337 and Tyr341**. A stabilizing hydrogen bond was also noted with **Ser293**, reinforcing ligand orientation within the aromatic gorge. Validation against the Heliyon 2024 study confirmed overlap with critical residues (**Trp86, Tyr337, Tyr341**) and highlighted additional stabilization via **Trp286 and Ser293**, supporting C3 as a promising coumarin-derived scaffold with enhanced affinity for AChE inhibition **Figure:- 13**.

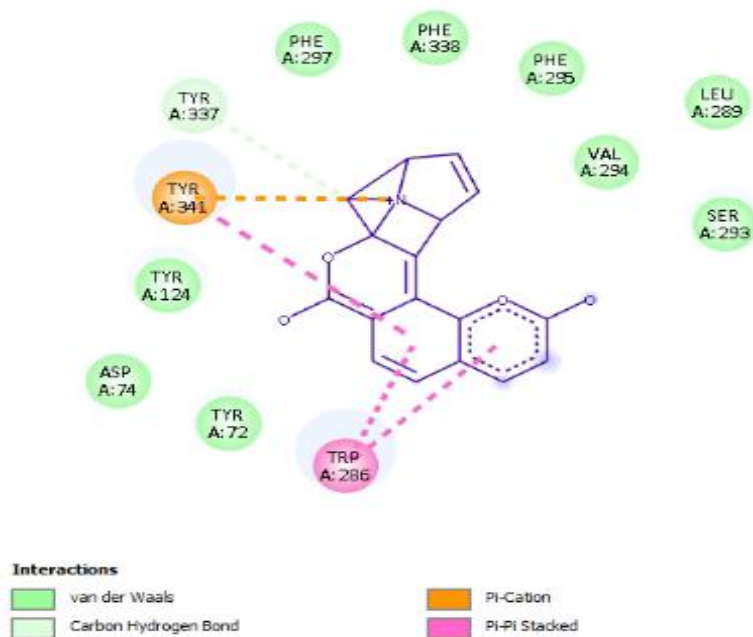


Figure:- 13 C3 – 2D interaction diagram with AChE (PDB: 4PQE)

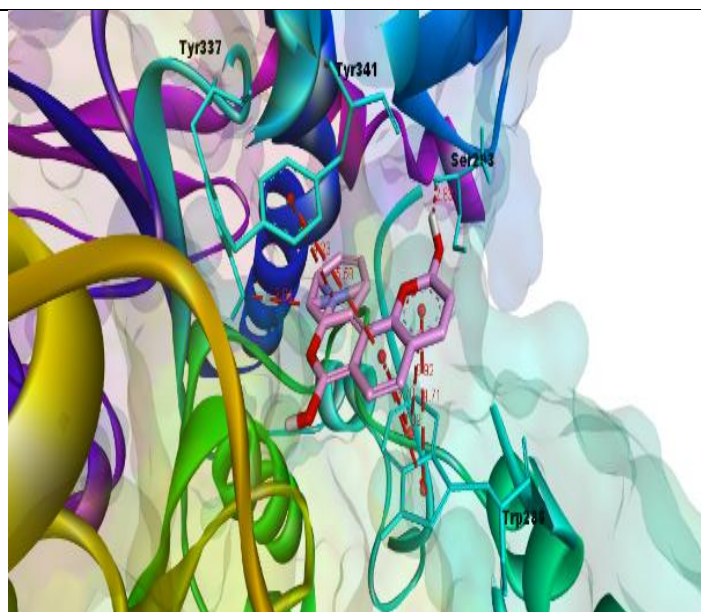


Figure:- 14
C3 – 3D docking pose with AChE (PDB: 4PQE)

C4 – Interaction with AChE (PDB: 4PQE)

C4 exhibited a docking score of **-8.3 kcal/mol**, reflecting weaker binding affinity compared to other derivatives. Docking was performed using a **20 × 20 × 20 Å grid box**, centered on the **SBD_Site_Sphere (ID:0)** with coordinates **XYZ -32.71, 22.60, -13.07 Å** and a binding radius of **7.38 Å**, ensuring accurate coverage of the active gorge **Figure:- 16**.

The ligand engaged in **hydrophobic contacts** with **Tyr337, Tyr341, Phe295, and Phe338**, along with additional stabilization via **Trp286, Tyr124, Val294, and Arg296**. **Figure:- 15** However, the absence of strong π - π stacking with Trp86 or hydrogen bonding with catalytic residues limited its overall affinity.

Validation against the Heliyon 2024 study confirmed partial overlap with reported anchors (**Tyr337, Phe295, Phe338**), but the weaker interaction profile suggests that the **para-bromo substitution** in C4 is less favorable for AChE inhibition.

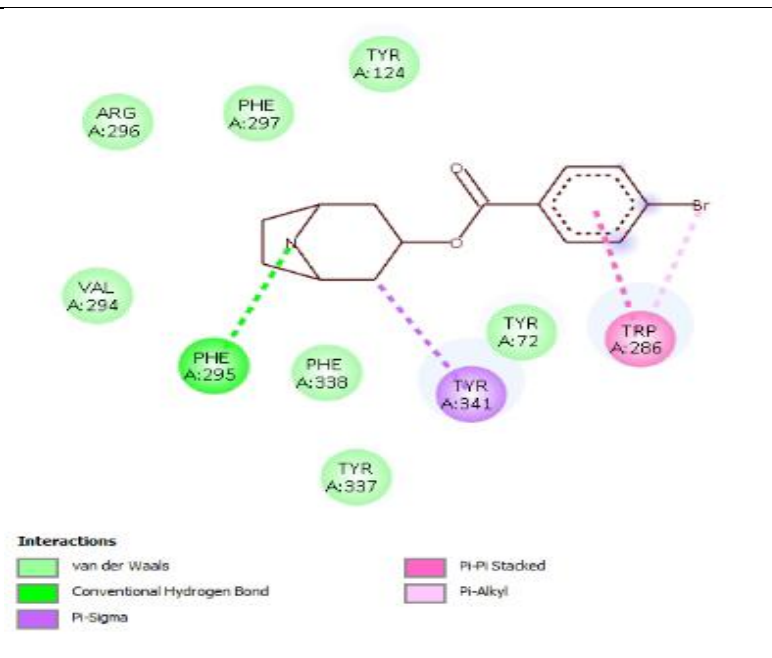


Figure:- 15 C4 – 2D interaction diagram with AChE (PDB: 4PQE)

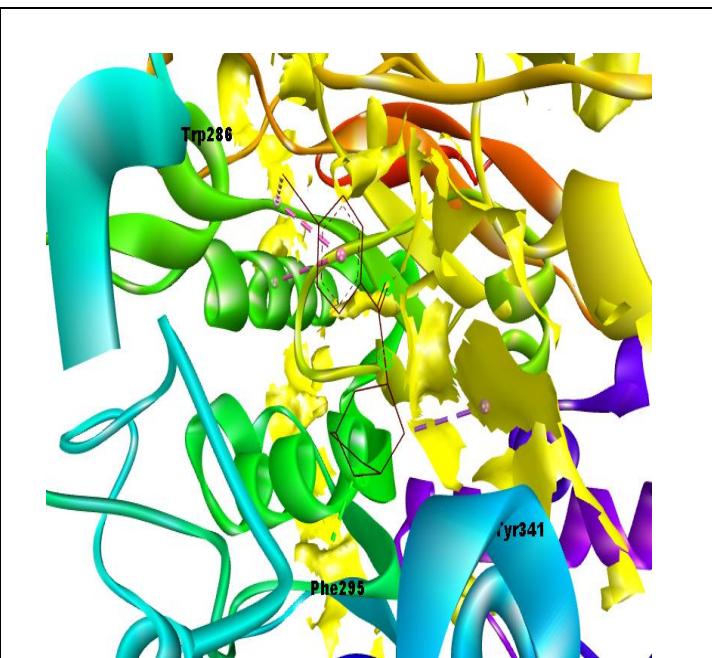


Figure:- 16 C4 – 3D docking pose with AChE (PDB: 4PQE)

C5 – Interaction with AChE (PDB: 4PQE)

C5 exhibited a docking score of **-8.4 kcal/mol**, reflecting moderate binding affinity within the active gorge of human acetylcholinesterase. Docking was performed using a **20 × 20 × 20 Å grid box**, centered on the **SBD_Site_Sphere (ID:0)** with coordinates **XYZ -24.88, 22.39, -8.76 Å** and a binding radius of **7.38 Å**, ensuring accurate placement within the catalytic site **Figure:- .16**

The ligand formed **hydrogen bonds with Ser203 and Tyr124**, while **π-π stacking interactions with Trp86 and Tyr341** contributed to stabilization within the aromatic gorge. Hydrophobic contacts with Gly121 and Ile451 further reinforced the binding orientation **Figure:-17**.

Validation against the Heliyon 2024 study confirmed overlap with critical residues (**Trp86, Tyr341, Tyr337**) and highlighted the stabilizing role of **Ser203 and Tyr124**, supporting C5 as a balanced scaffold with moderate affinity and improved solubility compared to other derivatives.

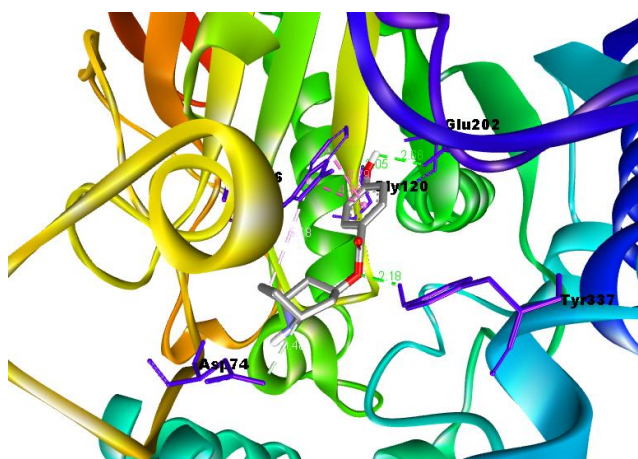


Figure:- 16 C5 – 2D interaction diagram with AChE (PDB: 4PQE)

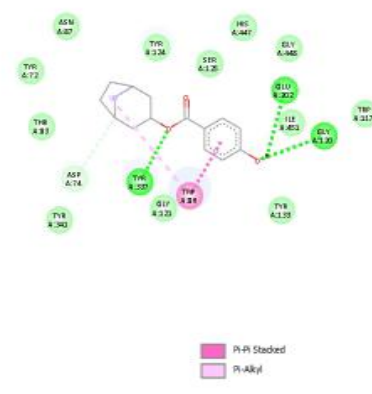


Figure:- 17
C5 – 3D docking pose with AChE (PDB: 4PQE)

Donepezil – Interaction with AChE (PDB: 4PQE)

Donepezil exhibited a docking score of **−8.2 kcal/mol**, consistent with its established role as a benchmark AChE inhibitor. Docking was performed using a **20 × 20 × 20 Å grid box**, centered on the **SBD_Site_Sphere (ID:0)** with coordinates **XYZ −28.68, 23.62, −11.18 Å** and a binding radius of **7.38 Å**, ensuring accurate placement within the catalytic gorge **Figure:-19**.

The ligand formed **π – π stacking** with **Trp86**, **hydrophobic contacts** with **Tyr337** and **Tyr341**, and **hydrogen bonding** with **His447** and **Ser203**, stabilizing its orientation relative to the catalytic triad. Validation against the Heliyon 2024 study confirmed overlap with critical residues (**Trp86**, **Tyr337**, **Tyr341**, **His447**), supporting Donepezil’s role as a reliable reference standard for comparative docking analysis **Figure:- 18**.

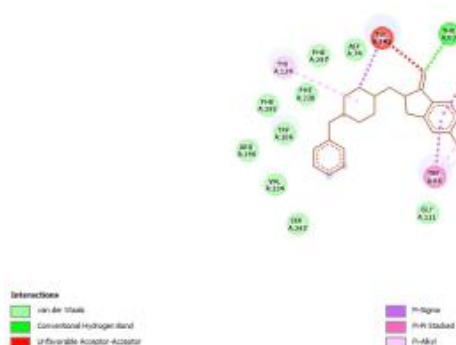


Figure:- 18 Donepezil – 2D interaction diagram with AChE (PDB: 4PQE)

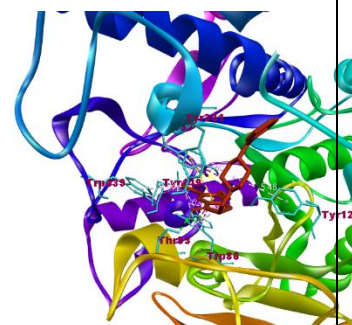


Figure:-19
Donepezil – 3D docking pose with AChE (PDB: 4PQE)

7.2. Absorption

The predicted absorption profiles of the designed convolvine derivatives are presented in **Table No:-3**. All compounds exhibited high predicted human intestinal absorption (>91%), indicating favorable oral absorption potential. C1, the synthesized lead compound, demonstrated excellent intestinal absorption (95.46%), moderate aqueous solubility ($-2.476 \log \text{ mol/L}$), acceptable Caco-2 permeability ($0.895 \log \text{ Papp} \times 10^{-6} \text{ cm/s}$), and was predicted to be a non-substrate of P-glycoprotein. These properties suggest efficient intestinal uptake with a lower likelihood of P-gp-mediated drug efflux. Among the remaining derivatives, C3 showed the highest intestinal absorption (96.06%), whereas C4 exhibited the highest Caco-2 permeability. Overall, the absorption profiles of the designed derivatives were comparable to or better than Donepezil, supporting their potential for oral administration.

Table no :- 3 The predicted absorption profiles of the designed convolvine derivatives

Compound	Water Solubility ($\log \text{ mol/L}$)	Caco-2 Permeability ($\log \text{ Papp} \times 10^{-6} \text{ cm/s}$)	Intestinal Absorption (%)	Skin Permeability ($\log \text{ Kp}$)	P-glycoprotein Substrate	P-glycoprotein I Inhibitor	P-glycoprotein II Inhibitor
C1	-2.476	0.895	95.462	-2.901	No	No	No
C2	-3.107	1.478	94.785	-2.519	Yes	No	No
C3	-2.521	0.952	96.059	-3.139	No	No	No
C4	-2.518	1.740	92.345	-2.700	No	No	No
C5	-1.333	1.231	91.188	-2.971	No	No	No
Donepezil	-4.648	1.273	93.707	-2.585	Yes	Yes	Yes

7.2 Distribution

The predicted distribution properties of the designed convolvine derivatives are summarized in **Table 4**. All compounds exhibited distribution characteristics consistent with potential central nervous system exposure. The synthesized lead compound, C1, showed moderate tissue distribution ($\text{VD}_{\text{ss}} = 0.577 \log \text{ L/kg}$), moderate blood–brain barrier permeability ($\log \text{ BB} = -0.230$), and CNS permeability ($\log \text{ PS} = -2.243$), indicating a balanced distribution profile. Among the remaining derivatives, C2 exhibited the highest predicted volume of distribution, C4 showed the greatest BBB permeability, C5 possessed the highest fraction unbound in plasma, whereas C3 displayed the lowest VD_{ss} and CNS permeability, suggesting comparatively lower tissue distribution. Overall, the predicted distribution profiles indicate that the designed convolvine derivatives possess physicochemical characteristics favorable for CNS drug development, with C1 demonstrating a distribution profile comparable to the reference drug Donepezil.

Table No:- 4 The predicted distribution properties of the designed convolvine derivatives

Compound	VD_{ss} ($\log \text{ L/kg}$)	Fraction Unbound (Fu)	BBB Permeability ($\log \text{ BB}$)	BBB Interpretation	CNS Permeability ($\log \text{ PS}$)
Donepezil	1.266	0.000	0.157	Moderate	-1.464
C1	0.577	0.274	-0.230	Moderate	-2.243
C2	1.151	0.137	0.277	Moderate	-1.696
C3	0.473	0.337	-0.197	Moderate	-2.881
C4	0.934	0.435	0.482	High	-2.731
C5	0.738	0.525	0.252	Moderate	-2.360

7.3 Metabolism and Excretion

The predicted metabolism and excretion profiles of the designed convolvine derivatives are presented in **Table 5**. All compounds were predicted to be **CYP3A4 substrates** and **non-substrates of CYP2D6**, while none showed inhibitory activity toward CYP1A2, CYP2C19, CYP2C9, or CYP3A4, indicating a low potential for CYP-mediated drug–drug interactions. The synthesized lead compound, **C1**, exhibited a predicted total clearance of **0.834 log mL/min/kg** and was identified as a **renal OCT2 substrate**, similar to Donepezil. Among the remaining derivatives, **C2** showed the highest predicted total clearance, whereas **C3** and **C5** were predicted to be non-substrates of renal OCT2. Overall, the designed derivatives displayed metabolism and excretion profiles comparable to Donepezil, supporting their favorable pharmacokinetic characteristics.

Table No:-5 The predicted metabolism and excretion profiles of the designed convolvine derivatives

Compound	CYP2D6 Substrate	CYP3A4 Substrate	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP3A4 Inhibitor	Total Clearance (log mL/min/kg)	Renal OCT2 Substrate
<i>Donepezil</i>	No	Yes	No	No	No	No	0.987	Yes
<i>C1</i>	No	Yes	No	No	No	No	0.834	Yes
<i>C2</i>	No	Yes	No	No	No	No	1.089	Yes
<i>C3</i>	No	Yes	No	No	No	No	0.990	No
<i>C4</i>	No	Yes	No	No	No	No	1.052	Yes
<i>C5</i>	No	Yes	No	No	No	No	1.069	No

7.4 Toxicity

The predicted toxicity profiles of the designed convolvine derivatives are summarized in **Table 6**. The synthesized lead compound, **C1**, was predicted to be non-mutagenic, non-hepatotoxic, non-skin sensitizing, and showed no hERG I inhibition, indicating a favorable preliminary safety profile. Its predicted **LD₅₀** value (**536 mg/kg**, Class IV) was comparable to that of the reference drug Donepezil. Among the remaining derivatives, **C3** and **C5** also exhibited acceptable toxicity profiles and belonged to **toxicity class IV**, with **C5** showing the highest predicted **LD₅₀** (**700 mg/kg**). In contrast, **C2** demonstrated positive **AMES mutagenicity** and, together with **C4**, was classified as **toxicity class III**. Overall, the toxicity predictions support **C1** as the most promising derivative, combining a favorable safety profile with the absence of major predicted toxicity liabilities[13] PROTOX SOFTWARE.

Table No:-6 The predicted toxicity profiles of the designed convolvine derivatives

Compound	AMES Toxicity	hERG I Inhibitor	Hepatotoxicity	Skin Sensitisation	LD ₅₀ (mg/kg)	Toxicity Class
<i>Donepezil</i>	No	No	Yes	No	505	IV
<i>C1</i>	No	No	No	No	536	IV
<i>C2</i>	Yes	No	No	No	116	III
<i>C3</i>	No	No	No	No	418	IV

C4	No	No	No	No	116	III
C5	No	No	No	No	700	IV

8.0 Characterization of Coumarin–Nortropine Ester (C1)

The target compound, **Coumarin–Nortropine ester (C1)**, was successfully synthesized as a **pale white solid** with an **isolated yield of 76%**. The compound exhibited an **R_f value of 0.46** and a **melting point of 328 °C**, indicating good purity and thermal stability. It was found to be soluble in **DMSO, ethanol, ethyl acetate, and chloroform**. The molecular formula and molecular weight were determined to be **C₁₇H₁₇NO₄** and **299.32 g/mol**, respectively. The chemical structure and identity of the synthesized compound were confirmed by **FTIR, ¹H NMR, LC–MS, and HPTLC** analyses.

Physicochemical characteristics of the synthesized Coumarin–Nortropine ester (C1).

Property	Observation
Compound	Coumarin–Nortropine ester (C1)
Molecular Formula	C ₁₇ H ₁₇ NO ₄
Molecular Weight (g/mol)	299.32
IUPAC Name	8-Azabicyclo[3.2.1]octan-3-yl 2-oxo-2H-1-benzopyran-3-carboxylate
Appearance	Pale white solid
Isolated Yield (%)	76
R _f Value	0.46
Melting Point (°C)	328
Solubility	DMSO, ethanol, ethyl acetate, and chloroform

8.1.FTIR Analysis C1 compound :-

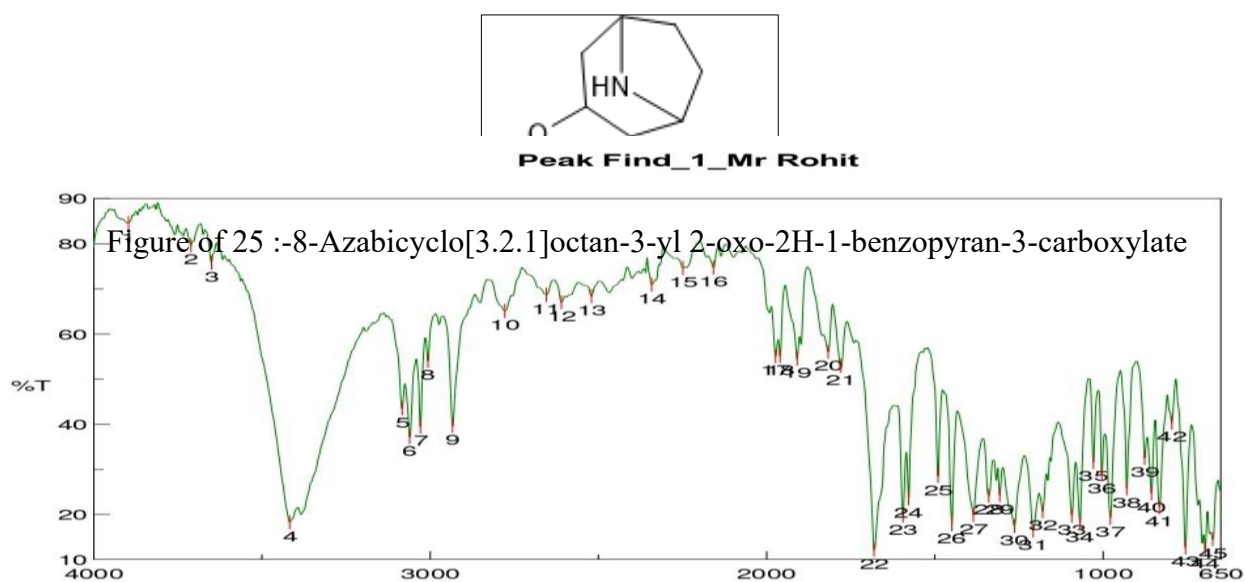


Figure:- 20 :- FTIR of synthesized Coumarin–Nortropine ester (C1

The FTIR spectrum of the synthesized **Coumarin–Nortropine ester (C1)** confirmed the successful formation of the target compound. A characteristic absorption band at **3418 cm⁻¹**

was assigned to **N–H stretching**, confirming the presence of the nortropine moiety. The absorption bands observed at **3083–3006 cm⁻¹** corresponded to **aromatic C–H stretching**, while the band at **2934 cm⁻¹** was attributed to **aliphatic C–H stretching**. A strong absorption at **1779 cm⁻¹** was assigned to the **ester carbonyl (C=O) stretching vibration**, confirming successful ester bond formation. The bands in the region of **1680–1578 cm⁻¹** were assigned to **aromatic C=C stretching** of the coumarin ring, whereas the absorptions at **1263–1179 cm⁻¹** corresponded to **ester C–O stretching** vibrations. Furthermore, the absence of the broad **carboxylic acid O–H stretching band (2500–3300 cm⁻¹)** indicated complete conversion of the carboxylic acid precursor into the corresponding ester. These spectral features are consistent with the proposed structure of **Coumarin–Nortropine ester (C1)** **Figure:- 20**.

The FTIR spectral data of the synthesized Coumarin–Nortropine ester (C1) are summarized in Table 7.

Table No 7 :- FTIR of synthesized **Coumarin–Nortropine ester (C1)**

Expected (cm ⁻¹)	Range	Observed (cm ⁻¹)	Peak	Assignment
3300–3500		3418		N–H Stretch; confirms presence of nortropine moiety
3000–3100		3083–3006		Aromatic C–H Stretch; confirms coumarin aromatic ring
2850–2950		2934		Aliphatic C–H Stretch; indicates nortropine backbone
1735–1790		1779		Ester C=O Stretch; confirms ester bond formation
1500–1700		1680–1578		Aromatic C=C Stretch; confirms conjugated coumarin system
1050–1300		1263–1179		Ester C–O Stretch; supports esterification
2500–3300		Absent		Carboxylic Acid O–H Stretch absent, indicating conversion of acid to ester

8.2 ¹H NMR Analysis:-

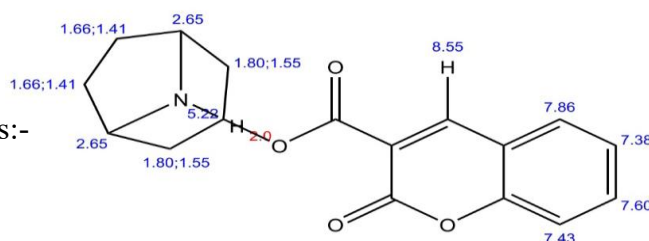


Figure :- 21 ¹H NMR interpretation c1 compound

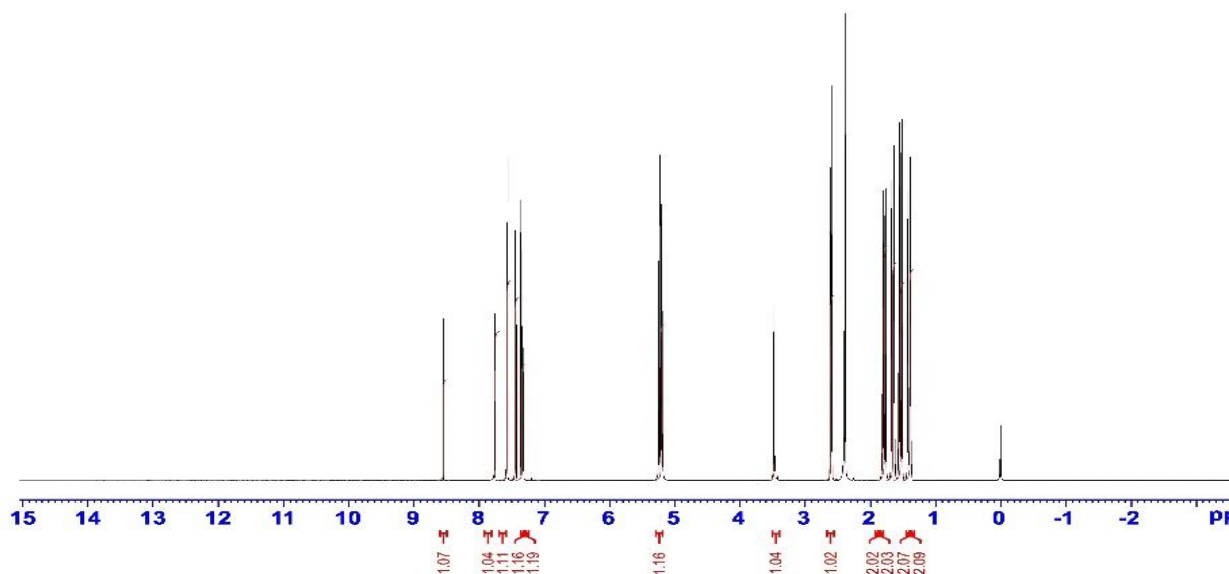


Figure :- 22 ¹H NMR interpretation c1 compound

The ¹H NMR spectrum of the synthesized **Coumarin–Nortropine ester (C1)** Figure :- 21 was consistent with the proposed molecular structure. The aliphatic protons of the **nortropine** moiety appeared as multiplets in the δ 1.41–3.52 ppm region, corresponding to the bridgehead and methylene protons of the bicyclic ring system. A doublet observed at δ 5.229 ppm (1H) was assigned to the proton adjacent to the ester linkage. The aromatic protons of the **coumarin** ring were observed between δ 7.389 and 8.551 ppm, with the singlet at δ 8.551 ppm corresponding to the characteristic proton of the coumarin system. The observed chemical shifts and splitting patterns were in good agreement with the proposed structure of **Coumarin–Nortropine ester (C1)**, confirming successful synthesis of the target compound

Figure :- 22. The ¹H NMR spectral data of the synthesized Coumarin–Nortropine ester (C1) are summarized in Table 8

Table no :- 8 ¹H NMR spectrum of the synthesized **Coumarin–Nortropine ester (C1)**

δ (ppm)	Splitting	Integration (rounded)	Assignment
1.413	m	2H	<u>Nortropine H</u>
1.555	m	2H	<u>Nortropine H</u>
1.661	m	2H	<u>Nortropine H</u>
1.807	m	2H	<u>Nortropine H</u>

2.652	d	1H	<u>Nortropine H</u>
3.520	m	1H	<u>Nortropine H</u>
5.229	d	1H	<u>Nortropine H</u>
7.389	m	1H	<u>Coumarin H</u>
7.438	m	1H	<u>Coumarin H</u>
7.603	m	1H	<u>Coumarin H</u>
7.862	d	1H	<u>Coumarin H</u>
8.551	s	1H	<u>Coumarin H</u>

Mass spectra of C-1:-

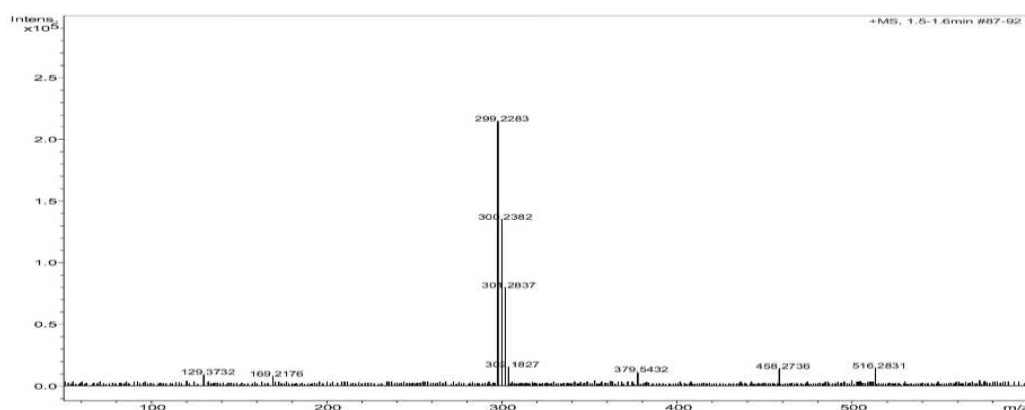


Figure :- 23 Mass spectra of C1 compound

The LC–MS spectrum of the synthesized **Coumarin–Nortropine ester (C1)** confirmed the molecular identity of the target compound. A prominent molecular ion peak was observed at **m/z 299.2283**, which is in good agreement with the calculated molecular weight (**299.32 g/mol**) of **C₁₇H₁₇NO₄**. The isotopic peaks observed at **m/z 300.2382** and **301.2837** further supported the molecular composition of the synthesized compound. The LC–MS data, together with the FTIR and ¹H NMR results, confirmed the successful synthesis of **Coumarin–Nortropine ester (C1)** Figure :- 23.

3.5.4. HPTLC Analysis

HPTLC analysis of the synthesized **Coumarin–Nortropine ester (C1)** was performed on **Merck silica gel 60 F₂₅₄ TLC plates** using **acetone:methanol:acetic acid (4:4:2, v/v/v)** as the mobile phase. The chromatogram exhibited a **single, sharp, and well-resolved peak** with an

Rf value of 0.46, indicating satisfactory chromatographic purity of the synthesized compound under the optimized experimental conditions Figure :- 24 HPTLC .

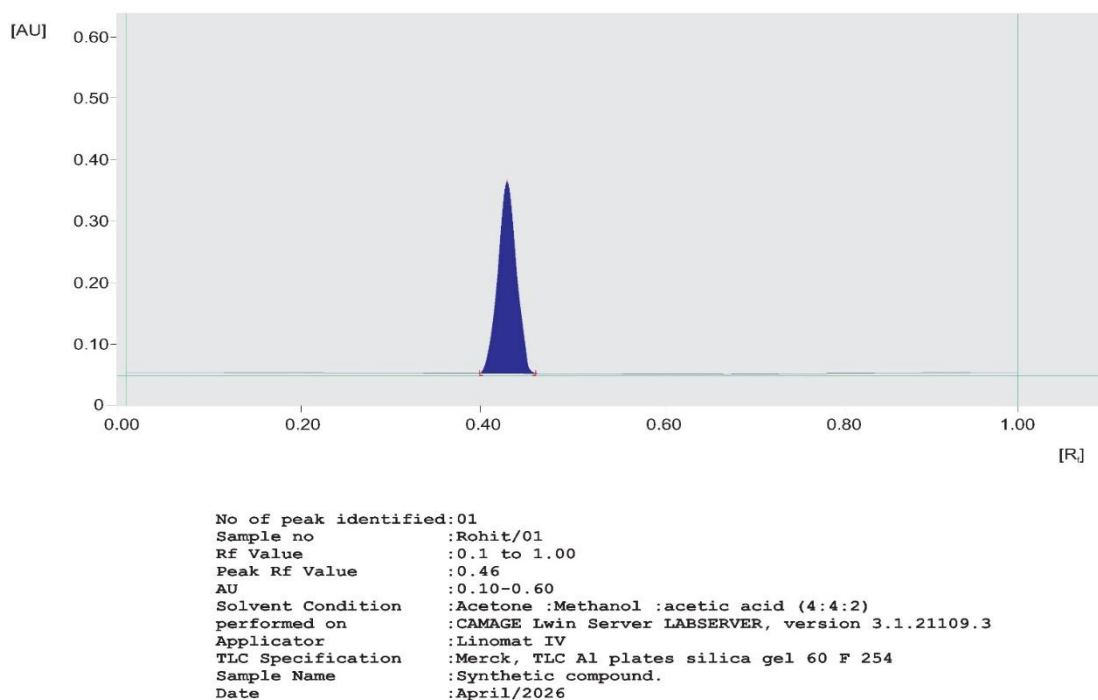


Figure :- 24 HPTLC chromatogram of the synthesized **Coumarin–Nortropine ester (C1)** showing a single chromatographic peak at **Rf = 0.46**.

In Vitro Acetylcholinesterase (AChE) Inhibitory Activity

The in vitro acetylcholinesterase (AChE) inhibitory activity of the synthesized **Coumarin–Nortropine ester (C1)** was evaluated using **Ellman's colorimetric assay**, with **Donepezil** used as the reference inhibitor. The assay was performed over a concentration range of **20–100 µg/mL**, and the inhibitory activity was determined by measuring the decrease in absorbance at **412 nm**. Compound **C1** exhibited concentration-dependent inhibition of AChE, showing **18.12%, 32.16%, 50.88%, 61.40%, and 69.01%** inhibition at **20, 40, 60, 80, and 100 µg/mL**, respectively. The calculated **IC₅₀** value of **C1** was **59.12 µg/mL**, whereas **Donepezil** showed inhibition values of **25.73%, 36.25%, 52.04%, 69.59%, and 80.70%** with an **IC₅₀** value of **57.96 µg/mL**. These results indicate that **C1** possesses significant acetylcholinesterase inhibitory activity, with potency comparable to the reference inhibitor

The in vitro acetylcholinesterase inhibitory activity of **C1** and **Donepezil** is presented in Table 9.

Table No :- 9 In vitro acetylcholinesterase inhibitory activity of Coumarin–Nortropine ester (C1) and Donepezil

Compound	20 µg/mL (%)	40 µg/mL (%)	60 µg/mL (%)	80 µg/mL (%)	100 µg/mL (%)	IC ₅₀ (µg/mL)
C1	18.12	32.16	50.88	61.40	69.01	59.12

Donepezil	25.73	36.25	52.04	69.59	80.70	57.96
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5. Discussion

The present study employed an integrated computational and experimental strategy to identify novel convolvine-derived acetylcholinesterase (AChE) inhibitors with potential therapeutic relevance for Alzheimer's disease. Structural modification of the convolvine scaffold generated a series of derivatives that were systematically evaluated through molecular docking, drug-likeness analysis, pharmacokinetic and toxicity prediction, followed by chemical synthesis and biological validation. Based on the overall computational performance, C1 was identified as the most promising candidate and was therefore selected for synthesis and subsequent experimental evaluation. The close agreement between the computational predictions and experimental observations demonstrates the effectiveness of the adopted lead optimization strategy.

The improved performance of C1 appears to arise from the rational incorporation of the coumarin moiety into the convolvine scaffold. Molecular docking suggested that this structural modification promotes favorable interactions within the catalytic gorge of AChE through a combination of hydrogen bonding and hydrophobic contacts. Such interactions are expected to improve ligand stabilization within the enzyme active site, thereby enhancing molecular recognition compared with the parent scaffold. These findings indicate that the selected structural modification successfully strengthened enzyme binding while preserving the fundamental pharmacophoric features of convolvine.

In addition to target affinity, the maintenance of appropriate physicochemical properties is essential during lead optimization. Drug-likeness analysis demonstrated that the designed derivatives retained molecular characteristics consistent with orally active drug candidates. Compliance with Lipinski's Rule of Five indicates that incorporation of the coumarin fragment did not adversely affect molecular size, lipophilicity, or hydrogen-bonding capacity. The balanced physicochemical profile of C1 therefore supports its suitability for continued optimization while maintaining favorable properties associated with drug development.

The predicted pharmacokinetic profile further supports the potential of C1 as a candidate for central nervous system drug discovery. Favorable intestinal absorption together with moderate blood–brain barrier permeability suggests that the compound may achieve adequate systemic exposure while retaining the ability to reach its therapeutic target within the brain. Furthermore, the predicted metabolic and excretion characteristics indicate an acceptable pharmacokinetic profile with a relatively low likelihood of undesirable metabolic interactions. Collectively, these observations suggest that optimization of the convolvine scaffold improved target affinity without compromising pharmacokinetic properties required for CNS-active molecules.

Early assessment of safety is equally important during the identification of lead compounds. Computational toxicity prediction indicated that C1 possesses a favorable preliminary safety profile, with no evidence of predicted mutagenicity, hepatotoxicity, skin sensitization, or hERG channel inhibition. Although these findings require confirmation through comprehensive experimental toxicological studies, the overall prediction suggests that the structural modifications introduced in the present work did not generate significant safety liabilities. This

favorable balance between efficacy-related properties and predicted safety further strengthens the suitability of C1 for continued investigation.

The computational findings were subsequently supported through experimental validation. The successful synthesis of C1 confirmed the feasibility of the proposed synthetic approach, while FTIR, ^1H NMR, and LC–MS characterization verified the formation and structural identity of the target molecule. Importantly, C1 exhibited measurable acetylcholinesterase inhibitory activity during in vitro evaluation, demonstrating that the favorable computational profile translated into biological activity. The consistency between molecular modeling and experimental observations highlights the reliability of the integrated workflow employed in this study and supports the value of combining computational screening with experimental validation during early-stage drug discovery.

The overall findings of this study are consistent with previous reports demonstrating that coumarin-based derivatives represent promising scaffolds for acetylcholinesterase inhibition owing to their ability to establish favorable interactions within the enzyme active site. The present work extends these observations by incorporating a coumarin pharmacophore into the natural alkaloid convolvine, thereby generating a hybrid scaffold that combines favorable molecular recognition with acceptable drug-like and pharmacokinetic characteristics. This integrated design strategy provides a useful framework for the development of structurally related AChE inhibitors with improved therapeutic potential.

In conclusion, the present investigation demonstrates that C1 possesses a balanced combination of favorable enzyme recognition, appropriate physicochemical characteristics, promising pharmacokinetic behavior, an encouraging predicted safety profile, and experimentally validated acetylcholinesterase inhibitory activity. Taken together, these findings identify C1 as a promising lead scaffold for further structural optimization and preclinical evaluation. More broadly, the integrated computational–experimental workflow described herein provides a rational and efficient strategy for the discovery of new acetylcholinesterase inhibitors for the treatment of Alzheimer's disease.

6. Conclusion

The present study successfully employed an integrated computational and experimental approach to develop novel convolvine–coumarin hybrid derivatives as potential acetylcholinesterase (AChE) inhibitors for the management of Alzheimer's disease. Rational structural modification of the parent convolvine scaffold generated a series of derivatives that were systematically evaluated using molecular docking, drug-likeness assessment, pharmacokinetic and toxicity prediction, followed by chemical synthesis, structural characterization, and in vitro biological evaluation. Among the designed molecules, C1 emerged as the most promising lead compound based on its balanced overall profile.

The computational findings indicated that C1 possessed favorable molecular recognition toward AChE together with desirable physicochemical characteristics, acceptable pharmacokinetic properties, and a promising preliminary safety profile. These predictions were further supported by the successful synthesis of C1, confirmation of its chemical structure through spectroscopic characterization, and demonstration of its acetylcholinesterase inhibitory activity in vitro. The consistency between the computational and experimental

findings supports the applicability of the integrated structure-based drug design strategy employed in the present study.

The results further demonstrate that incorporation of the coumarin pharmacophore into the convolvine scaffold represents a rational molecular design strategy capable of improving biological performance while maintaining drug-like characteristics and acceptable predicted pharmacokinetic and safety profiles. This balanced combination of favorable target affinity, physicochemical properties, and biological activity highlights the potential of the convolvine–coumarin hybrid framework for the development of new AChE inhibitors.

In conclusion, C1 represents a promising lead compound for the further development of acetylcholinesterase inhibitors targeting Alzheimer's disease. Future studies should focus on structural optimization together with comprehensive pharmacological, pharmacokinetic, toxicological, and in vivo investigations to further establish the therapeutic potential of this scaffold and facilitate its progression toward preclinical drug development

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Ethics, Consent to Participate, and Consent to Publish

Ethics, Consent to Participate, and Consent to Publish declarations: not applicable.

Reference:-

- [1] Lane, C.A., Hardy, J. and Schott, J.M. (2018), Alzheimer's disease. *Eur J Neurol*, 25: 59-70. <https://doi.org/10.1111/ene.13439>
- [2] Birks JS, Harvey RJ. Donepezil for dementia due to Alzheimer's disease. *Cochrane Database Syst Rev*. 2018;6:CD001190.**DOI:** 10.1002/14651858.CD001190.pub3

- [3] World Journal of Pharmaceutical Research. Formulation and Evaluation of Herbal Smart Pills for Jet Lag. *World J Pharm Res.* 2025;14(9) DOI: 10.20959/wjpr20259-36481
- [4] Hannan MA, Sultana A, Rahman MH, Sohag AAM, Dash R, Uddin MJ, Hossen MJ, Moon IS. Protective mechanisms of nootropic herb Shankhpashpi (*Convolvulus pluricaulis*) against dementia: Network pharmacology and computational approach. *Evid Based Complement Alternat Med.* 2022;2022:1015310. doi:10.1155/2022/1015310
- [5] Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem.* 2010;31(2):455–461. DOI: <https://doi.org/10.1002/jcc.21334>
- [6] Ellman GL, Courtney KD, Andres V Jr, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol.* 1961;7(2):88-95. doi:10.1016/0006-2952(61)90145-9
- [7] Saha, B., Das, A., Jangid, K., Kumar, A., Kumar, V., & Jaitak, V. (2024). Identification of coumarin derivatives targeting acetylcholinesterase for Alzheimer's disease by field-based 3D-QSAR, pharmacophore model-based virtual screening, molecular docking, MM/GBSA, ADME and MD Simulation study. *Current research in structural biology*, 7, 100124. <https://doi.org/10.1016/j.crstbi.2024.100124>
- [8] Kamel, N. N., Aly, H. F., Fouad, G. I., Abd El-Karim, S. S., Anwar, M. M., Syam, Y. M., Elseginy, S. A., Ahmed, K. A., Booles, H. F., Shalaby, M. B., Khalil, W. K. B., Sandhir, R., Deshwal, S., & Rizk, M. Z. (2023). Anti-Alzheimer activity of new coumarin-based derivatives targeting acetylcholinesterase inhibition. *RSC Advances*, 13(23), 14678–14692. <https://doi.org/10.1039/D3RA02344C>
- [9] Hasan, A. H., Abdulrahman, F. A., Obaidullah, A. J., Alotaibi, H. F., Alanazi, M. M., Noamaan, M. A., Murugesan, S., Amran, S. I., Bhat, A. R., & Jamalis, J. (2023). Discovery of Novel Coumarin-Schiff Base Hybrids as Potential Acetylcholinesterase Inhibitors: Design, Synthesis, Enzyme Inhibition, and Computational Studies. *Pharmaceuticals*, 16(7), 971. <https://doi.org/10.3390/ph16070971>
- [10] Aljohani, G., Al-Sheikh Ali, A., Alraqa, S. Y., Itri Amran, S., & Basar, N. (2021). Synthesis, molecular docking and biochemical analysis of aminoalkylated naphthalene-based chalcones as acetylcholinesterase inhibitors. *Journal of Taibah University for Science*, 15(1), 781–797. <https://doi.org/10.1080/16583655.2021.2005921>
- [11] Alotaibi, J. A. M., Sirwi, A., El-Halawany, A. M., Esmat, A., Mohamed, G. A., Ibrahim, S. R. M., Alzain, A. A., Halawa, T. F., Safo, M., & Abdallah, H. M. (2024). α -Glucosidase, butyrylcholinesterase and acetylcholinesterase inhibitory activities of phenolic compounds from *Carthamus tinctorius* L. flowers: *In silico* and *in vitro* studies. *Saudi pharmaceutical journal : SPJ : the official publication of the Saudi Pharmaceutical Society*, 32(7), 102106. <https://doi.org/10.1016/j.jsps.2024.102106>
- [12] Daina, A., Michielin, O. & Zoete, V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep* 7, 42717 (2017). <https://doi.org/10.1038/srep42717>

- [13] Priyanka Banerjee, Emanuel Kemmler, Mathias Dunkel, Robert Preissner, ProTox 3.0: a webserver for the prediction of toxicity of chemicals, *Nucleic Acids Research*, Volume 52, Issue W1, 5 July 2024, Pages W513–W520, <https://doi.org/10.1093/nar/gkae303>
- [14] Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. UCSF Chimera—A visualization system for exploratory research and analysis. *J Comput Chem*. 2004;25(13):1605–1612. doi:10.1002/jcc.20084
- [15] Nivatya HK, Singh A, Kumar N, Sharma L, Singh V, Mishra R, Gaur N, Mishra AK. Assessing molecular docking tools: understanding drug discovery and design. *Future J Pharm Sci*. 2025;11:111. doi: 10.1186/s43094-025-00611-1
- [16] BIOVIA, Dassault Systèmes. Discovery Studio Modeling Environment, Release 2019. San Diego: Dassault Systèmes; 2019.
- [17] Thandivel, S., Rajan, P., Gunasekar, T., Arjunan, A., Khute, S., Kareti, S. R., & Paranthaman, S. (2024). *In silico* molecular docking and dynamic simulation of anti-cholinesterase compounds from the extract of *Catunaregam spinosa* for possible treatment of Alzheimer's disease. *Heliyon*, 10(7), e27880. <https://doi.org/10.1016/j.heliyon.2024.e27880>
- [18] **Berhenke, L.F.; Monroe, E.; Britton, E.C.** *Preparation of coumarin-3-carboxylic acid*. U.S. Patent **2,338,569**, issued January 4, 1944. Application filed May 31, 1941, Serial No. 396,190. Assignee: The Dow Chemical Company, Midland, Michigan.