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Synthesis, *In Silico*, and *In Vitro* Evaluation of a Coumarin-Based Schiff Base as a Potential Anti-Alzheimer's Agent

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Abstract

This study investigates the anti-Alzheimer's potential of coumarin-based Schiff bases. Among the series of synthesized compounds, the phenyl hydrazine derivative of 3-Acetyl coumarin (PHC) was selected based on its high docking score. Given the limited treatment options for Alzheimer's disease (AD), this research aims to explore novel therapeutic approaches using coumarin derivatives. In silico studies demonstrated strong binding interactions of PHC with acetylcholinesterase (AChE), with a binding affinity of -10.7 kcal/mol, supporting its role as a multi-target directed ligand. Pharmacokinetic evaluations using Swiss ADME software indicated favourable drug-like properties, including good gastrointestinal absorption, blood-brain barrier permeability, and compliance with Lipinski's rule of five. In vitro assays showed that PHC effectively inhibits AChE, with an IC_{50} value of 52.08 µg/mL. Notably, it interacts with both the catalytic active site and the peripheral anionic site of AChE, enhancing its potential as a multi-target ligand. Additionally, PHC exhibited moderate antioxidant activity, which may help mitigate oxidative stress, a key factor in AD pathology. These findings indicate that PHC holds significant anti-Alzheimer's potential through its multifunctional properties, highlighting its promise as a therapeutic candidate for AD.

Keywords

Acetylcholinesterase (AChE) inhibition, Alzheimer's disease, Coumarin, multi-target ligand, Schiff base.

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INTRODUCTION

Dementia is a term used to describe a marked decline in cognitive function that disrupts an individual's ability to carry out daily tasks. Among the various forms of dementia, Alzheimer's disease (AD) is the most common, responsible for over two-thirds of cases in people aged 65 and older. AD is a progressive neurodegenerative condition that starts with subtle symptoms and gradually leads to a deterioration of cognitive and behavioural capacities. These capacities include memory, understanding, language skills, attention, reasoning, and decision-making. While Alzheimer's disease is not a direct cause of death, it greatly elevates the risk of complications that can ultimately prove fatal.[1] Individuals diagnosed with Alzheimer's disease (AD) present

with abnormal beta-amyloid peptide deposits, a reduction in neurotransmitter levels, and elevated oxidative stress.[2]

The current treatment landscape for Alzheimer's disease (AD) predominantly revolves around symptomatic relief, with drugs such as acetylcholinesterase inhibitors (AChEIs) like donepezil, rivastigmine, and galantamine providing only modest improvements in cognitive function. These therapies primarily target the cholinergic deficit in AD but fail to alter the underlying pathophysiological processes of the disease.[3] The progressive nature of AD necessitates the development of disease-modifying agents (DMAs) that can simultaneously address multiple pathological hallmarks, such as amyloid-beta (Aβ) accumulation, tau hyperphosphorylation, oxidative stress, and neuroinflammation. There is an increasing demand for novel, safe, and effective AChEIs that not only enhance cholinergic transmission but also exhibit multifunctional properties, such as neuroprotective effects, anti-amyloidogenic potential, and antioxidant activity.[4] Recent studies highlight the importance of discovering multifunctional compounds to potentially halt or slow the progression of AD, rather than merely managing its symptoms.

To develop more effective and selective AChE inhibitors with fewer side effects, it is crucial to target specific structural features of the enzyme, which can be beneficial for treating Alzheimer's disease (AD).[5] The crystallographic analysis of AChE reveals that the enzyme has a deep, narrow gorge about 20 Å long. Within this gorge, two primary binding sites are found: the catalytic active site (CAS) at the bottom, and the peripheral anionic site (PAS) near the entrance.[6,7] Studies indicate that successful AChE inhibitors should consist of three key components: a core ring system that interacts with PAS, a basic center that binds to CAS, and a linker connecting the two to meet the enzyme's structural needs.[8,9]

Despite the limited number of approved therapies, heterocyclic compounds have surfaced as potential candidates in the research of Alzheimer's disease (AD). Their capacity to engage with critical biological targets has led to extensive exploration of heterocyclic scaffolds for their therapeutic potential in managing AD. [10]

Coumarin is a naturally occurring organic compound belonging to the benzopyrone family, characterized by a fused benzene and α-pyrone ring.[11] The coumarin ring, known for its structural simplicity and electron-rich nature, serves as an appealing molecular framework. It can be readily modified with various functional groups, allowing it to be linked with other bioactive fragments or compounds to produce more potent compounds with favorable bioactivity and physicochemical characteristics.[12] Coumarin derivatives have demonstrated both anti-inflammatory effects and impacts on the central nervous system.[13] Coumarins are promising compounds for developing acetylcholinesterase inhibitors (AChEIs) due to their ability to undergo chemical modifications at various positions within their core structure.[14] In addition to AChE inhibition, the coumarin scaffold exhibits multiple biological activities relevant to Alzheimer's disease. These include the inhibition of β-secretase-1 (BACE-1), antagonism of cyclooxygenase (COX) and lipoxygenase (LOX), modulation of the cannabinoid receptor 2 (CB2), activation of the gamma-aminobutyric acid (GABA) receptor, antagonism of the NMDA receptor, and inhibition of monoamine oxidase (MAO).[15]

Research has shown that coumarin was able to bind to the PAS of AChE via aromatic π-π stacking interactions and therefore could serve as one part of the dual binding mode of action.[16] To discover new dual-acting acetylcholinesterase (AChE) inhibitors, the coumarin moiety was fused with primary amines. The coumarin moiety, known for its aromatic properties, was selected for its potential to inhibit cholinesterase (ChE) by binding to the peripheral anionic site (PAS) of AChE, while a primary amine-containing moiety was incorporated to facilitate interactions with the catalytic active site (CAS) of AChE. This strategic design aims to enhance the compound's binding affinity and inhibitory efficacy, ultimately contributing to its therapeutic potential against Alzheimer's disease.

MATERIALS AND METHODS

A number of Schiff bases of 3- Acetyl coumarin were selected. All the chemicals and reagents used in this work were of synthetic grade.

Molecular docking

Molecular docking of the 3-Acetyl coumarin derivatives was conducted using PyRx software with the AutoDock Vina algorithm to assess binding affinity and interactions with Alzheimer's-related targets, including acetylcholinesterase (AChE). This analysis provided insights into the structural features contributing to the compounds' inhibitory potential, forming a basis for further experimental validation.[17]

Methodology of docking

Target identification and retrieval

Crystallographic structures of the targets of interest (receptors/ enzymes) were obtained from PDB

(Protein Data Bank) and saved in standard 3D coordinate format.

Table 1 Protein target and its PDB ID

TARGET	PDB ID
AChE	4PQE

Molecular visualization with BIOVIA Discovery Studio

Molecular visualization plays an important role in the analysis and presentation of modelling studies. BIOVIA Discovery Studio 2021 v21.1 is a software designed for this purpose, utilizing techniques like molecular modelling, structure-based drug design, virtual screening, and cheminformatics. It allows for both 2D and 3D visualization of interactions between a drug and its target protein, highlighting key amino acids involved and the types of bonds formed during the interaction.

Swiss ADME: Predicting pharmacokinetic properties

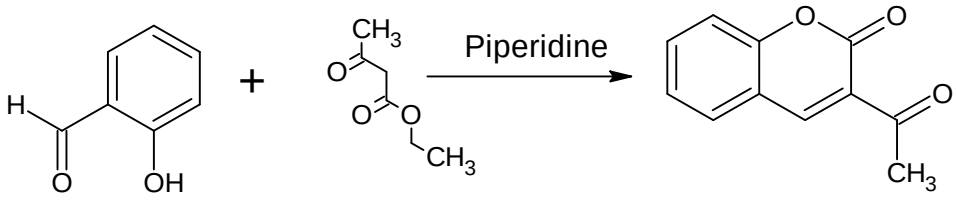
The Swiss ADME tool, developed by the Swiss Institute of Bioinformatics, was used to predict the ADME (Absorption, Distribution, Metabolism, and Excretion) properties of the proposed compounds. This software generated predictive models, including the Boiled Egg model, iLOGP for lipophilicity, and the Bioavailability Radar, providing insights into the drug-likeness and pharmacokinetic behaviour of the compounds. [18]

Synthesis

Synthesis of 3- acetyl coumarin

Step 1

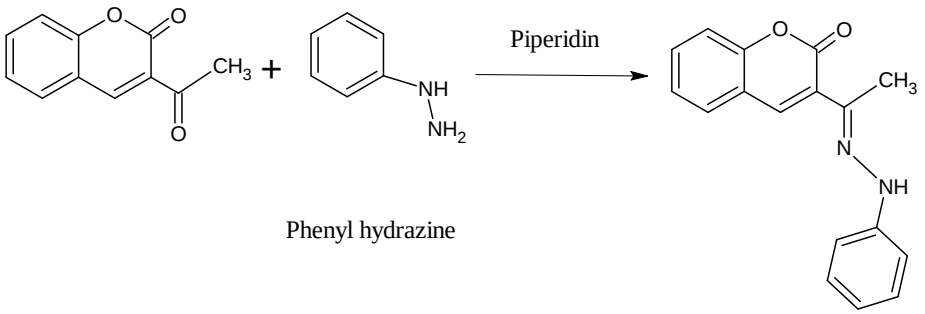
The synthesis of the 3-Acetyl coumarin was carried out by reacting salicylaldehyde with ethyl acetoacetate, using piperidine as a catalyst. The reaction mixture was stirred for 20 minutes. The reaction was monitored using thin-layer chromatography (TLC) using a solvent system consisting of ethyl acetate and n-hexane in a 2:3 ratio. The separated, yellow-coloured solid product was recrystallized using ethanol. [19-21]



Synthesis of 3-[(1*E*)-1-(2-phenylhydrazinylidene) ethyl]-2*H*-1-benzopyran-2-one

Step 2

A mixture of 1 (5 mmol), phenylhydrazine (5 mmol), and acetic acid (10 mL) was stirred at room temperature for 10 minutes. The reaction mixture was then poured into 30 mL of ice-cold water, causing the solid to separate. The solid was filtered, washed twice with an aqueous acetic acid solution (1:1, 5 mL each), and dried. The reaction was monitored using a solvent system of n-hexane: ethyl acetate in a ratio of 3.5:1.5. The crude product was purified by recrystallization from ethanol solvent to obtain PHC. [22]



3-[(1*E*)-1-(2-phenylhydrazinylidene)ethyl]-2*H*-1-benzopyran-2-one

PHC

Physical characterization

Melting point determination:

The melting points of the synthesized compounds were determined in open capillaries using a melting point apparatus.

Spectral characterization

The synthesized compounds were characterized using Fourier-transform infrared (FTIR) spectroscopy, UV-visible spectroscopy, and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. FTIR spectra were obtained on a JASCO FTIR model in the 4000–400 cm^{-1} range using KBr pellets at the College of Pharmaceutical Sciences, Government Medical College Trivandrum. UV-visible spectra were recorded on a HITACHI UV-visible spectrophotometer 5300 at the same college. The ^{1}H NMR spectra were acquired using a JEOL (JNM-ECZ400S) instrument operating at 400 MHz with chloroform-D as the solvent at the Government College for Women, Vazhuthacaud.

***In-vitro* assays**

Determination of acetylcholinesterase inhibitory activity using microplate assay [23-25]

The experiment utilized a 50 mM Tris-HCl buffer at pH 8.0. Acetylcholinesterase (AChE) from *Electrophorus electricus* was prepared in a stock solution (222 U/mL) stored at -80°C and diluted with 0.1% BSA in buffer. DTNB was prepared in a buffer with 0.1 M NaCl and 0.02 M $MgCl_2$, while acetylthiocholine iodide (ATCI) was dissolved in deionized water. In each well of a 96-well plate, we added 100 µL of 3 mM DTNB, 20 µL of 0.26 U/mL AChE, 40 µL of buffer, and 20 µL of different extract concentrations (25, 50, and 100 µg/mL). The plate was incubated for 15 minutes at 25˚C, and absorbance was measured at 412 nm. The reaction was initiated by adding 20 µL of ATCI, with absorbance recorded at 5 and 20 minutes. The percentage inhibition was calculated using the formula:

% inhibition = {(E - ΔOD) / E} × 100, where E represents the blank value.

DPPH radical scavenging activity (Hydrogen donating activity) [26-28]

A 0.1 mM DPPH solution was prepared by dissolving 3.94 mg of DPPH in 100 ml of methanol and stored in darkness to prevent light-induced degradation. To each 1 ml sample of PHC and standard ascorbic acid solution at varying concentrations (25, 50, 100, and 250 µg/ml for compounds; 25, 50, and 100 µg/ml for ascorbic acid), 3 ml of DPPH solution was added. The mixtures were shaken and incubated in darkness at room temperature for 30 minutes. Absorbance was measured at 517 nm, with ascorbic acid as the standard, DPPH solution with methanol as the control, and methanol as the blank. All experiments were performed in triplicate, and the average values were calculated. The percentage of DPPH radical scavenging activity was determined using the formula:

% inhibition = [(OD of control - OD of test) / OD of control] × 100

where OD is the optical density.

RESULTS AND DISCUSSION

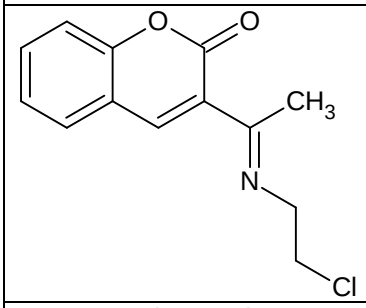
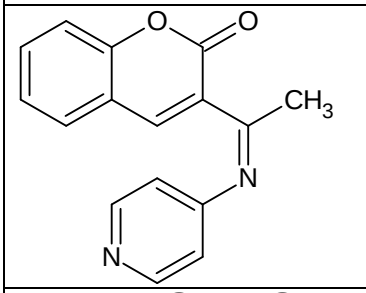
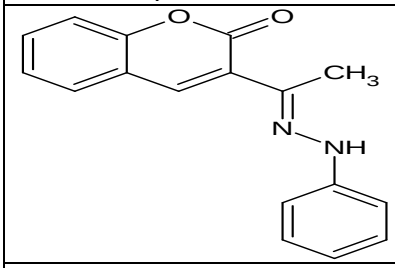
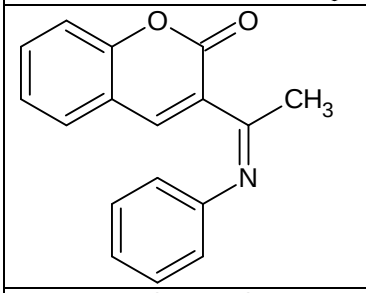
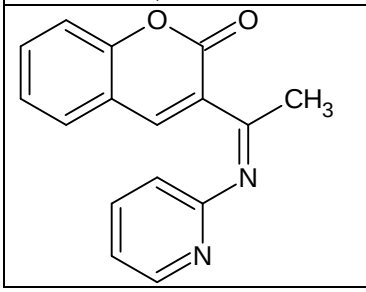
***In-silico* studies**

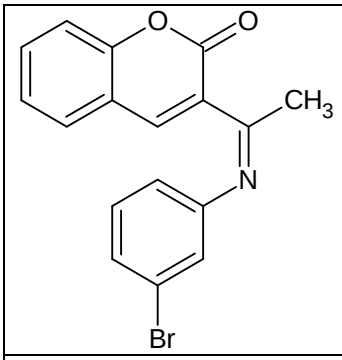
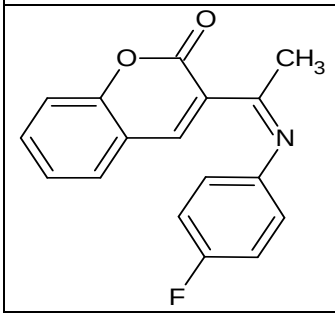
In-silico studies were successfully carried out with the aid of commercially available as well as freely available software and online tools. A new series of coumarin derivatives was analysed using software products.The structures of the synthesized compounds were designed using ACD ChemSketch 12.0.

Docking studies

Molecular docking studies were performed utilizing PyRx and BIOVIA Discovery Studio 2021 to evaluate the binding affinities of the compounds with the enzyme acetylcholinesterase. (PDB ID: 4PQE).

Table 1 Docking score of the selected series of coumarin derivatives

COMPUONDS	DOCKING SCORE – 4PQE (Kcal/mol)
	High: -7.7 Low: -6.7
	High: -7.8 Low: -6.8
	High: -10.7 Low: -8.5
	High: - 7 Low: - 6.4
	High: - 10.2 Low - 7.2

	High: - 8.1 Low: - 7.6
	High: - 8.1 Low: - 7.6

Among the series of coumarin derivatives, the phenyl hydrazine derivative (PHC - marked in the circle) with high docking scores was selected for further studies.

2-D Interaction of PHC on receptors

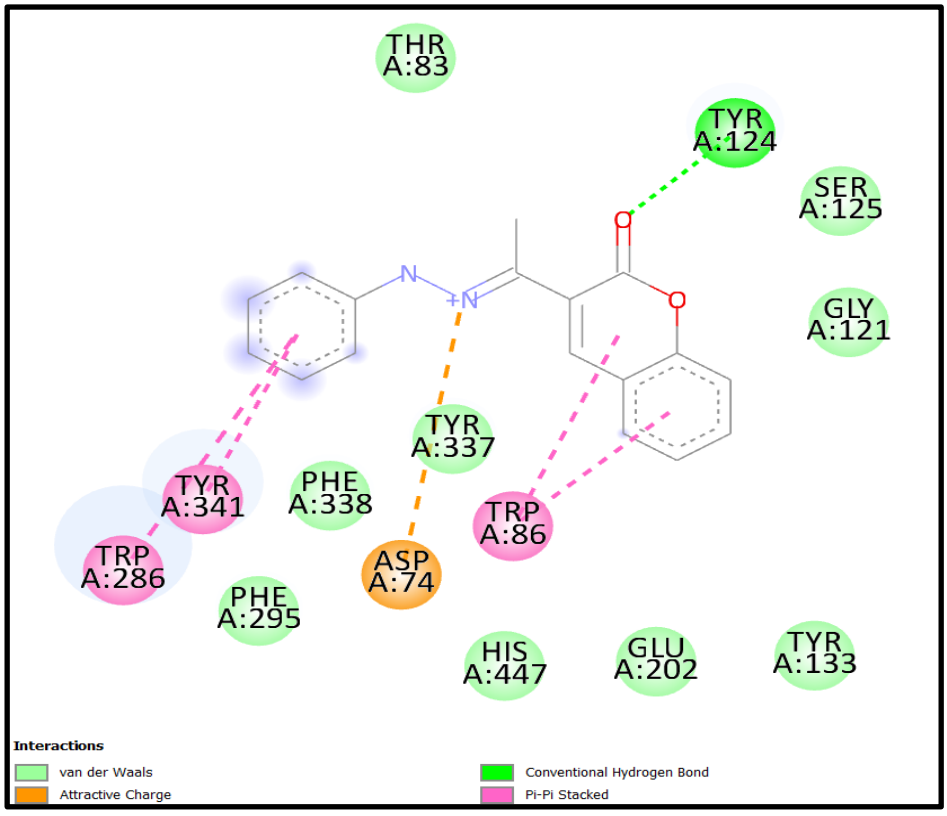


Figure 1: 2-D interaction of PHC with 4PQE

The binding interactions of PHC with resemblance to those of donepezil, suggesting acetylcholinesterase (AChE) show a strong comparable inhibitory potential against Alzheimer's-

related targets. Molecular docking analysis revealed that the benzyl piperidine moiety of donepezil interacts with TRP A:86 via π–π stacking [29] —a key interaction that is similarly observed with the coumarin ring of PHC, enhancing its stability in the active site. Additionally, both donepezil and the PHC demonstrate interactions with TRP A:124, further strengthening their binding affinity within the enzyme's active site. Notably, the nitrogen linker of donepezil interacts with ASP A:74 in the peripheral anionic site (PAS) [30], an interaction pattern that is also mirrored by PHC. Several other residues, including TYR A:337 and PHE A:338, engage in van der Waals interactions with the PHC, contributing to its stable positioning within the AChE active site. Based on these docking studies, it is evident that the PHC fits well within the AChE active site, mimicking the dual-site binding pattern of donepezil. This similarity in binding interactions highlights the compound's potential as a lead candidate for further optimization as an AChE inhibitor and as a promising anti-Alzheimer's agent.

Pharmacokinetic analysis using SWISS ADME software

The *in-silico* pharmacokinetic analysis of the PHC was conducted using the Swiss ADME web tool, providing insights into its potential as drug candidate for Alzheimer's disease (AD). One of the key outcomes of this study was the favourable prediction for blood-brain barrier (BBB) permeability, a critical factor for CNS-targeted drugs. The compound exhibited characteristics such as appropriate lipophilicity and small molecular size, which suggest passive diffusion across the BBB, a prerequisite for exerting pharmacological effects in the brain. In addition to BBB permeability, the compound adhered to Lipinski's Rule of Five, which assesses drug-likeness based on molecular weight, logP, and hydrogen bonding potential. The Schiff base's compliance with these criteria, including a molecular weight below 500 Da, logP values less than 5, and acceptable hydrogen bond donors and acceptors, indicates good oral bioavailability and the potential for systemic absorption. These pharmacokinetic properties highlight the suitability of the Schiff base compounds for oral administration, a critical consideration for long-term AD therapy.

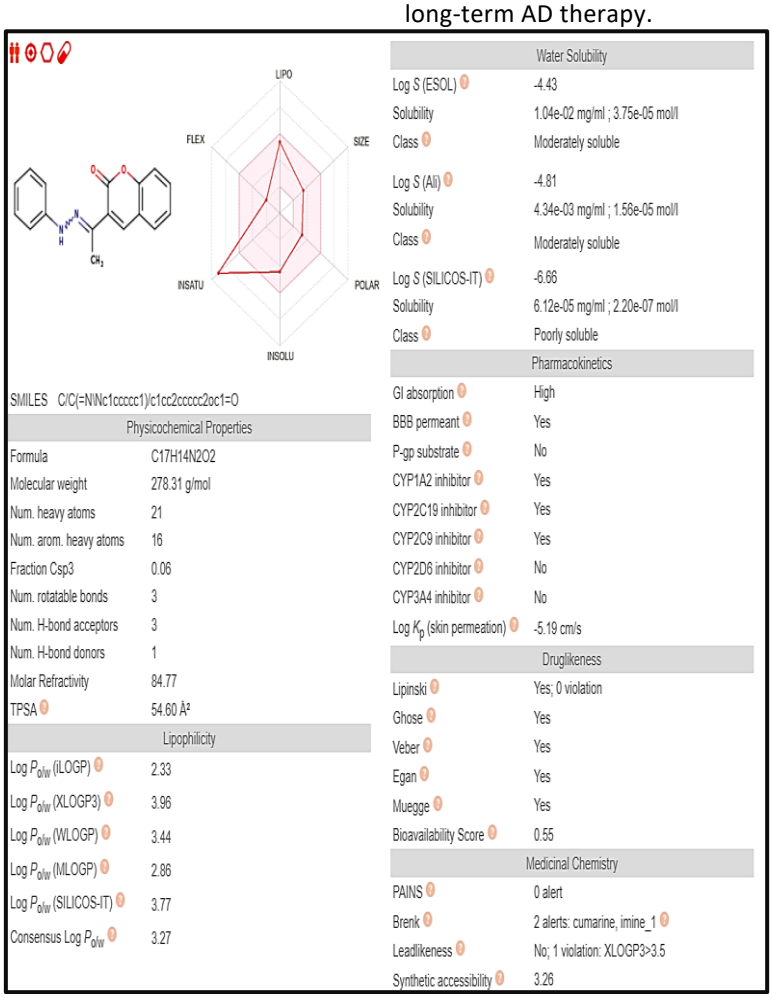


Figure 2: *In-silico* pharmacokinetic analysis of PHC

The PHC exhibits favourable gastrointestinal (GI) permeability, indicating efficient absorption and bioavailability when administered orally. Additionally, it demonstrates the ability to cross the blood-brain barrier (BBB), a critical feature for the treatment of Alzheimer's disease, as effective therapeutics must reach the brain to exert their therapeutic effects. Furthermore, both PHC adhere to Lipinski's Rule of Five, a set of guidelines used to evaluate drug-likeness. This rule suggests that an orally active drug typically has no more than five hydrogen bond donors, no more than ten hydrogen bond acceptors, a molecular weight under 500 Daltons, and a partition coefficient (log P) not exceeding 5. By following these criteria, the PHC shows promising pharmacokinetic profiles suitable for further development as Alzheimer's therapeutics.

Synthesis of Schiff base of 3-acetyl coumarin

The synthetic strategy involving the formation of 3-Acetyl coumarin and its derivatives were carried out as outlined. The percentage yield of the PHC was found to be 87%.

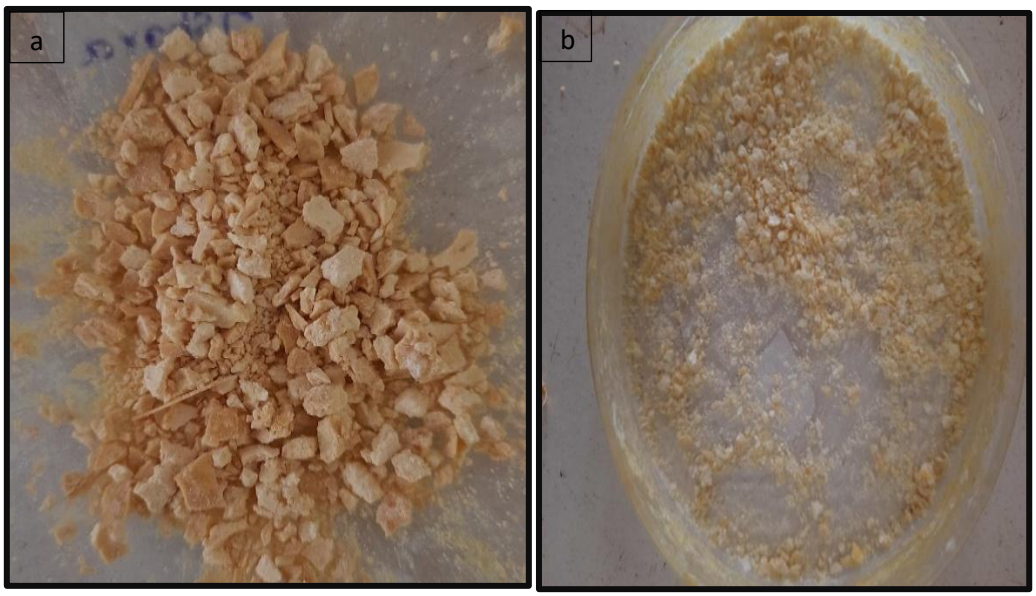


Figure 3: Photographs of synthesized intermediate Compound, 3-Acetyl coumarin. a) Separated solid mass b) Recrystallised product

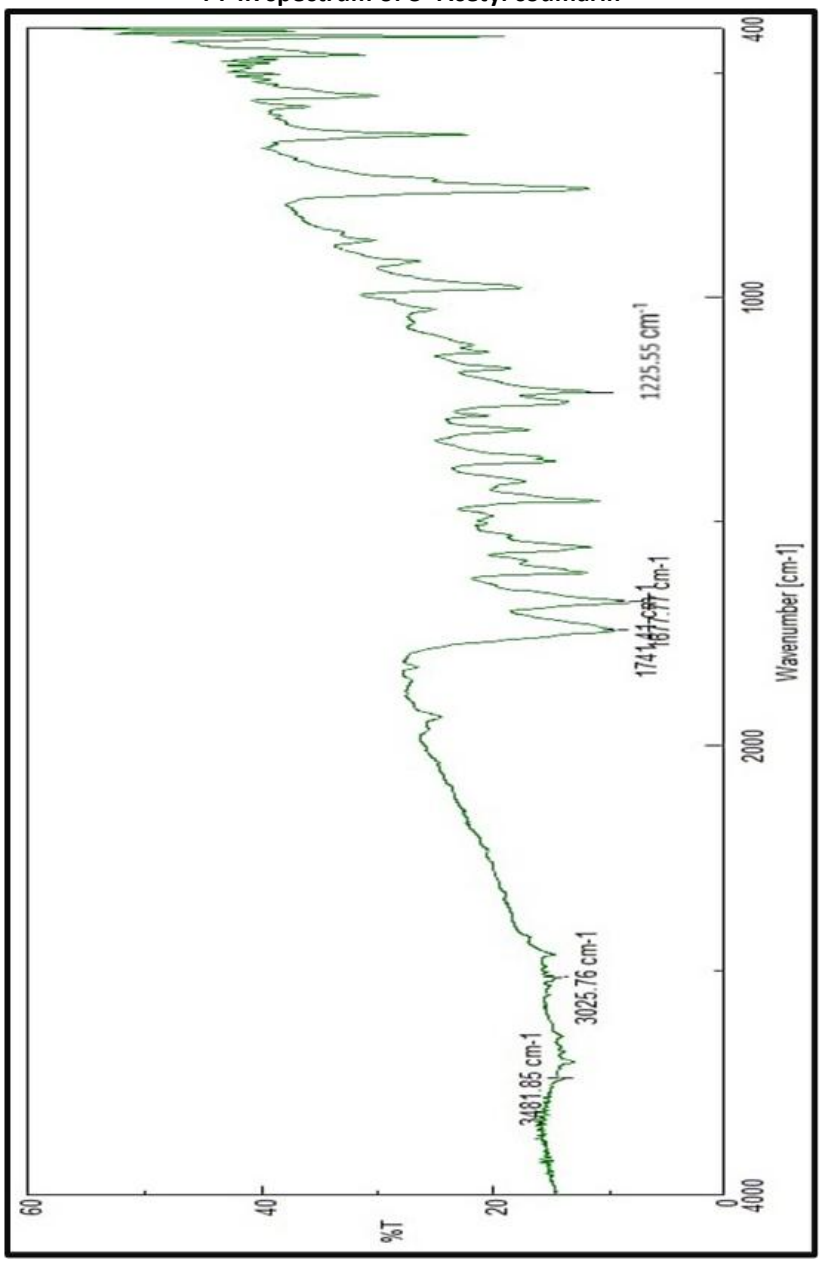
Figure 4: Photographs of synthesized PHC

Table 2 Physicochemical data of synthesized compounds

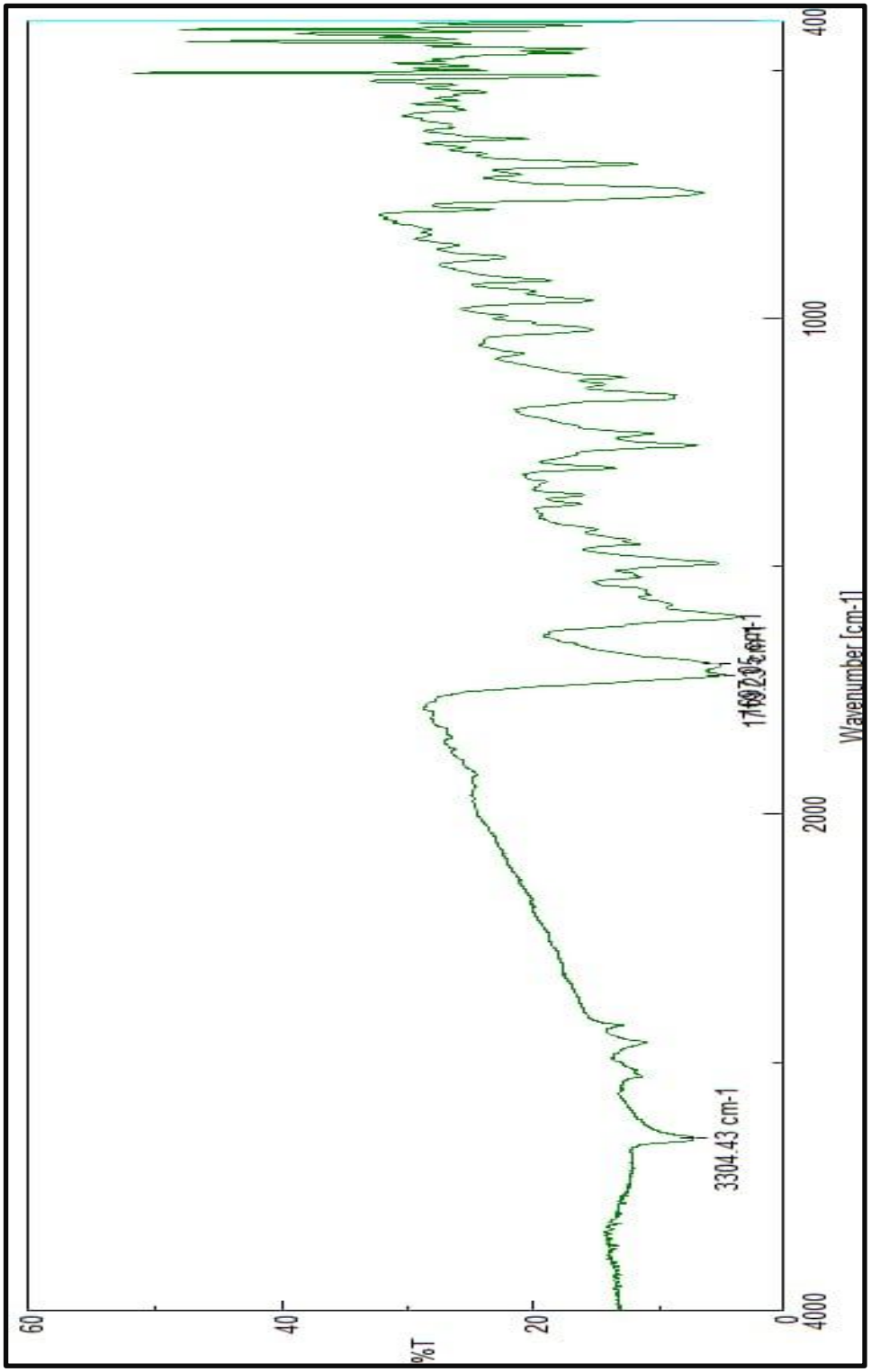
DATA	3-AC (INTERMEDIATE)	PHC
Molecular formula	$C_{17}H_{14}N_2O_2$	$C_{17}H_{14}N_2O_2$
Calculated molecular weight	188.18 G/Mol	278.31 g/mol
Melting point	115-119 °C	170-177 °C
Percentage yield	91.5%	87.0%
Color	Pale Yellow	Orange
Odor	Characteristic	Characteristic
Solubility in water	Insoluble	Insoluble
Solubility in organic solvents (Ethanol, Methanol & DMSO)	Soluble	Soluble

The FTIR spectral analysis of the compounds was carried out and the characteristic absorption peaks confirmed that conversions had taken place. FTIR spectra were recorded on JASCO FTIR model 4100.

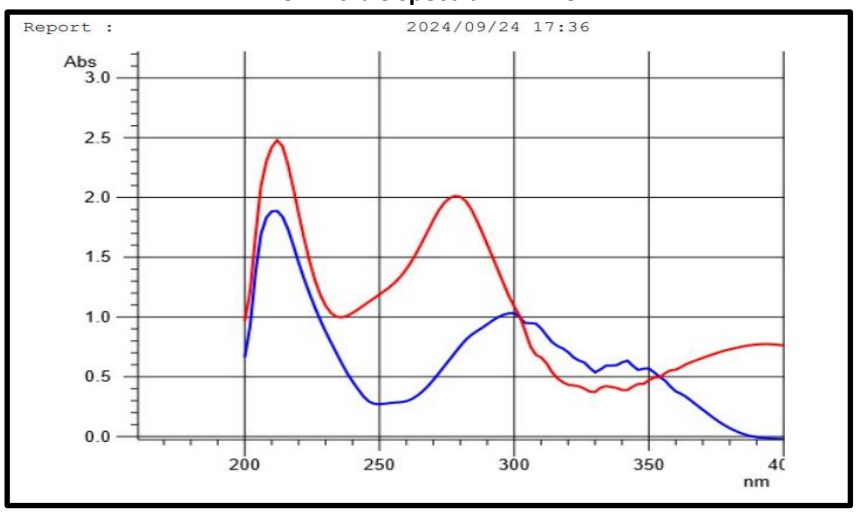
FT-IR spectrum of 3- Acetyl coumarin



FT-IR spectrum of PHC



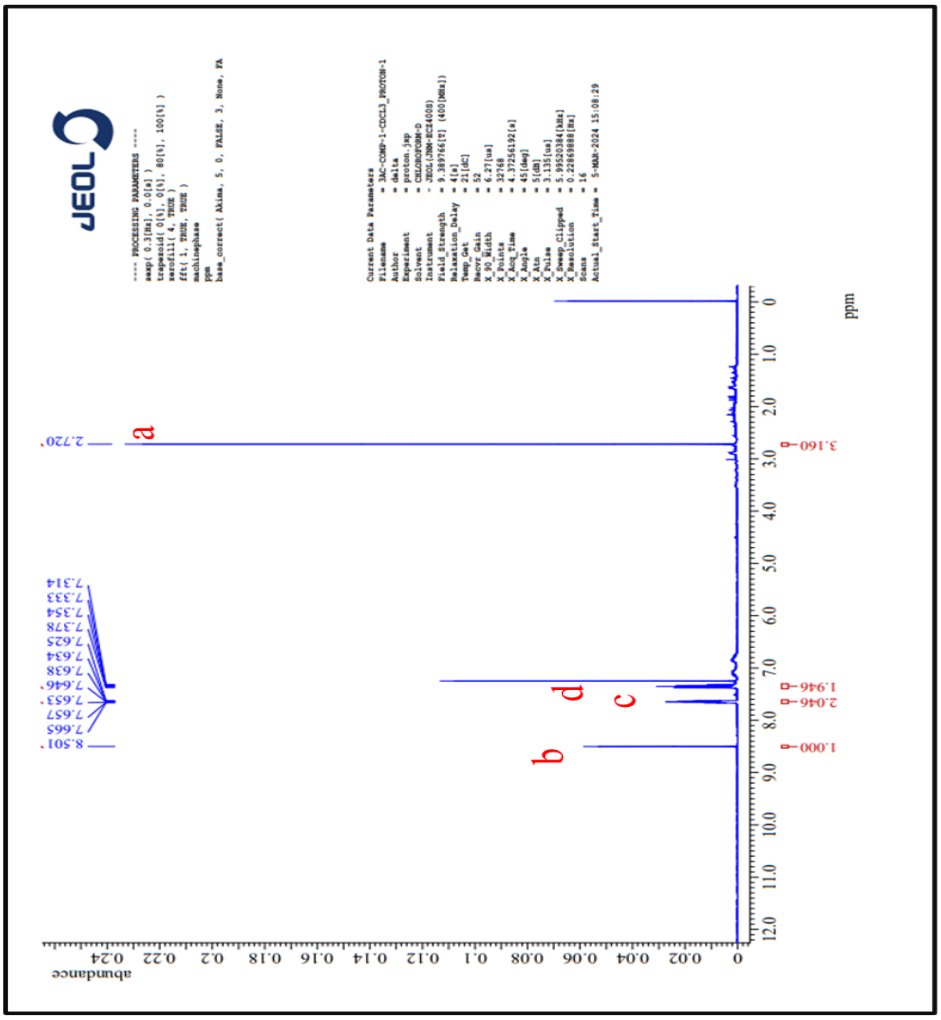
UV visible spectrum – PHC



3 AC :– 300 nm

PHC :- 278 nm

^{1}H NMR Spectrum of 3-Acetyl coumarin



^{1}H NMR Spectrum of PHC

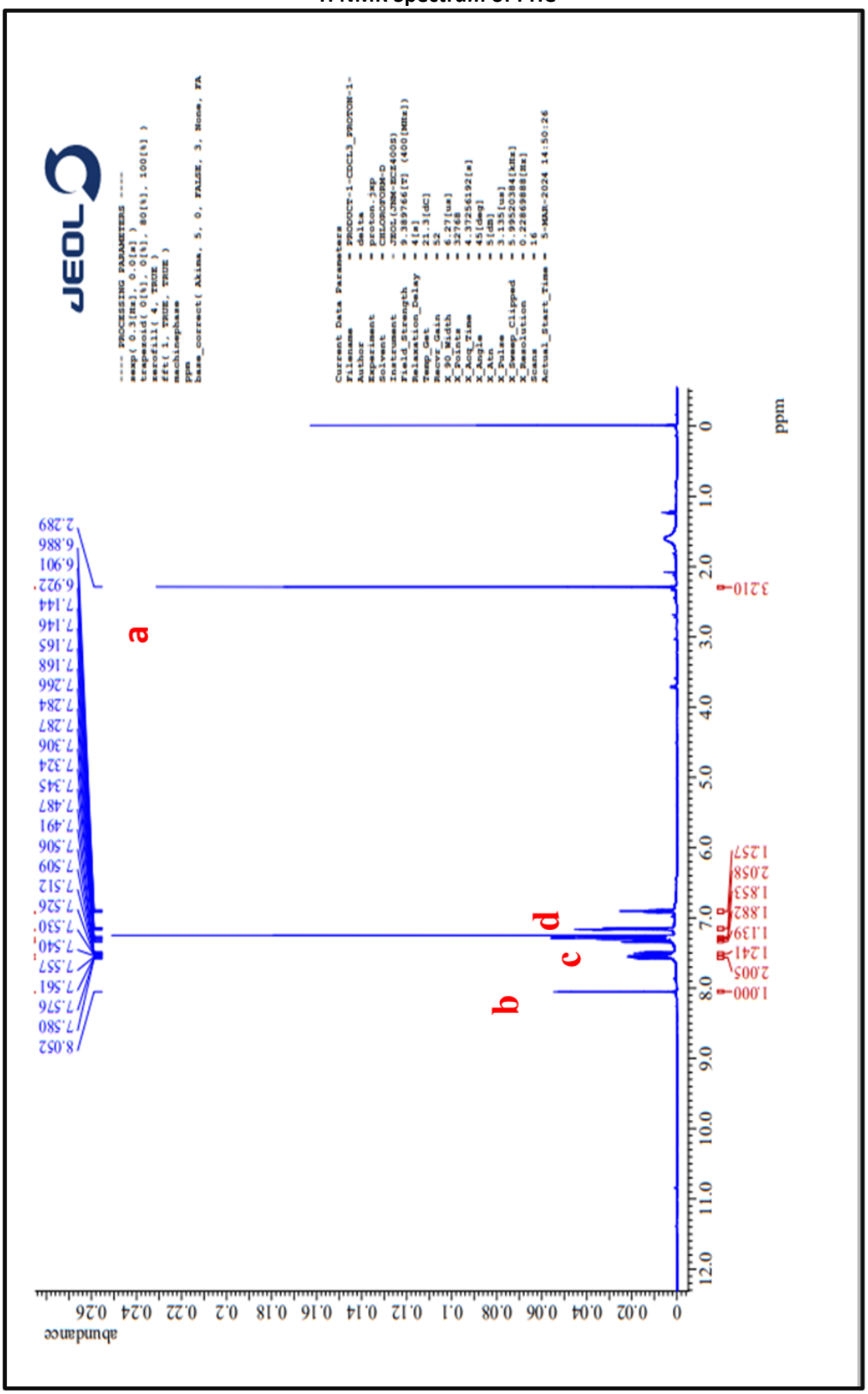


Table 3 FTIR spectral data of the synthesized compounds

CODE	IR DATA (cm^{-1})
3-AC (INTERMEDIATE)	C=O Stretching (1741.41), C=C Stretching (1677.77), C-H Stretching (3025.76), C-O Stretching (1225.55)
PHC	N-H Stretching (3304.43), OC=O Stretching (1719.23), C=N Stretching (1697.05)

UV visible spectral data

Table 4 UV-visible spectral data of synthesized compounds

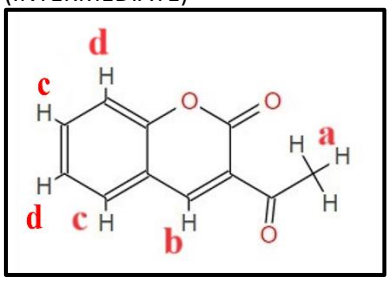
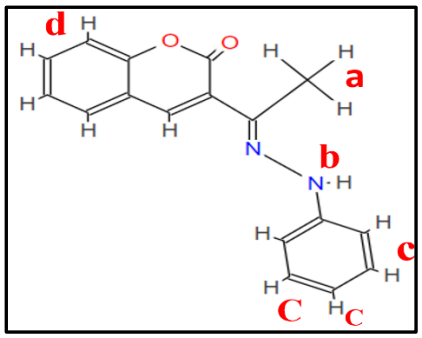
CODE	UV Absorption Maxima (λ_{max}) (nm)
3-AC (INTERMEDIATE)	300 nm
PHC	278 nm

The UV-visible spectrum of the intermediate compound, 3-Acetyl coumarin, displayed a characteristic absorption peak at 300 nm. In contrast, the synthesized final compound exhibited a peak at 278 nm, indicating a shift in absorption wavelength. This change in the UV-visible absorption pattern reflects structural modifications associated with the synthesis process. Thus, the observed spectral differences between the intermediate and final compounds provide evidence of product formation and confirm the completion of the synthetic reaction.

^{1}H NMR Spectral data

Proton NMR spectra were recorded in chloroform-d. Chemical shifts are reported in delta (ppm)

Table 5 ^{1}H NMR spectral data of the synthesized compounds

STRUCTURE	PROTON TYPE	CHEMICAL SHIFT (ppm)
3 -A C (INTERMEDIATE) 	a – (s,3H)	2.720
	b – (m, 1H)	8.501
	c – (m,2H)	7.665-7.634
	d– (m,2H)	7.378-7.314
PHC 	a – (s,3H)	2.289
	b – (m, 1H)	8.052
	c – (m,3H)	7.580-7.487
	d – (m,7H)	6.886-7.345

***In vitro* pharmacological studies**

Determination of acetylcholinesterase (AChE) inhibitory activity using microplate assay

The acetylcholinesterase (AChE) inhibitory activity of the synthesized PHC was evaluated using a microplate assay, a common method for assessing enzyme inhibition by potential therapeutic compounds. PHC demonstrated significant inhibitory activity, with an IC_{50} value of 52.08 µg/mL, calculated using ED50 PLUS V 1.0 software. This IC_{50} value represents the concentration at which PHC inhibits 50% of AChE's enzymatic activity, highlighting its potential in blocking AChE. This is particularly relevant for Alzheimer's disease, as AChE is responsible for the breakdown of acetylcholine, a neurotransmitter essential for cognitive function.

Table 6: Percentage inhibition of acetylcholinesterase by PHC

Concentration (µg/mL)	PHC	
	ΔOD	Percentage of inhibition
E	0.126	0
25	0.0884	29.84
50	0.0536	57.46
100	0.0344	72.70

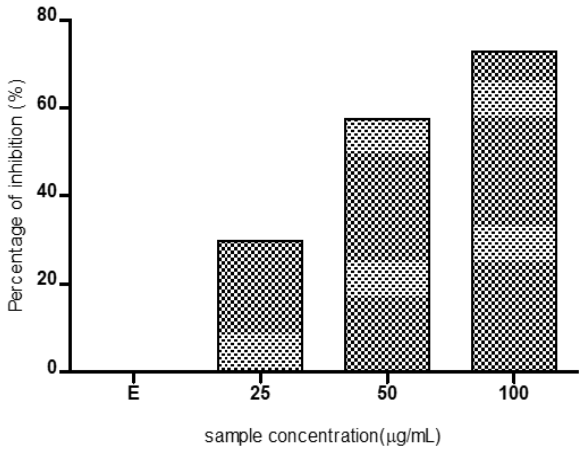


Figure 5: Graphical representation of acetylcholinesterase inhibitory activity of PHC.

When compared to donepezil, a clinically approved and effective AChE inhibitor, PHC demonstrated a comparable IC_{50} value, suggesting it may have similar potency in inhibiting AChE. Donepezil's effectiveness in clinical settings is attributed to its ability to bind efficiently within the active site of AChE, preventing the breakdown of acetylcholine and enhancing cholinergic transmission, which is essential for improving cognitive symptoms in Alzheimer's patients. The similar IC_{50} value of PHC suggests that it may interact with AChE's active site in a way akin to donepezil, effectively reducing enzyme activity and potentially enhancing acetylcholine levels.

This comparable inhibitory potency positions PHC as a strong candidate for further investigation and optimization. With additional structural modifications, it may be possible to improve PHC's selectivity, potency, and pharmacokinetic properties, making it a promising lead compound for developing new, effective anti-Alzheimer's drugs. These findings underline PHC's potential to act as a therapeutic alternative or adjunct to existing AChE inhibitors, potentially contributing to more effective treatment options for Alzheimer's disease. [31]

DPPH radical scavenging activity (Hydrogen donating activity)

DPPH radical scavenging assay, the most widely used method for screening antioxidant activity was employed during the study. This assay is based on the ability of antioxidants to donate hydrogen atoms or electrons to neutralize the DPPH radical, leading to a decrease in absorbance. [32,33] Since oxidative stress is a major contributing factor in neurodegenerative diseases like Alzheimer's [34,35], evaluating the antioxidant capacity of the synthesized coumarin Schiff bases is crucial for understanding their potential therapeutic value.

DPPH radical scavenging activity was recorded in terms of percentage inhibition. The percentage inhibition of DPPH radical by different concentrations of PHC was compared with that of ascorbic acid.

Table 7: Percentage inhibition of DPPH radical scavenging activity shown by Ascorbic acid & PHC

Group	Sample	Concentration (µg/ml)	Optical density 517 nm	Inhibition of DPPH radical (%)	IC_{50} (µg/mL)
CONTROL			1.147 ± 0.021		
STANDARD	Ascorbic acid	25	0.569 ± 0.041	35.3095 ± 2.334	12.38
		50	0.483 ± 0.030	69.8343 ± 1.758	
		100	0.035 ± 0.0050	85.6146 ± 0.127	
		200	0.279 ± 0.0034	94.5945 ± 0.460	
TEST	PHC	25	0.107 ± 0.0028	8.1081 ± 0.714	129.63
		50	0.864 ± 0.029	24.6730 ± 0.848	
		100	0.702 ± 0.041	38.7968 ± 2.575	
		200	0.281 ± 0.013	75.5013 ± 1.837	

As depicted in the table, PHC showed percentage inhibitions of 75.501% at a concentration of 200 µg/mL. However, it's activity was lower than that of the standard (ascorbic acid), which exhibited a percentage inhibition of 93.465%. The IC_{50} values were determined by regression analysis using Microsoft Excel. The IC_{50} values for compound was significantly higher (129.63 µg/mL) compared to the standard drug, ascorbic acid (12.38 µg/mL), indicating that the synthesized compound possesses lower free radical scavenging activity than ascorbic acid. This suggests that the synthesized coumarin Schiff bases possess moderate antioxidant activity to the highly potent ascorbic acid.

Previous studies on coumarin derivatives have reported moderate antioxidant activity, with IC_{50} values generally higher than those of standard antioxidants like ascorbic acid.[36] The results of this study are consistent with such findings, suggesting that while coumarin Schiff bases can exhibit some level of free radical scavenging, their antioxidant potential is typically lower compared to more conventional antioxidants. Despite this, moderate antioxidant activity can still contribute to neuroprotection by reducing oxidative stress in Alzheimer's disease models.

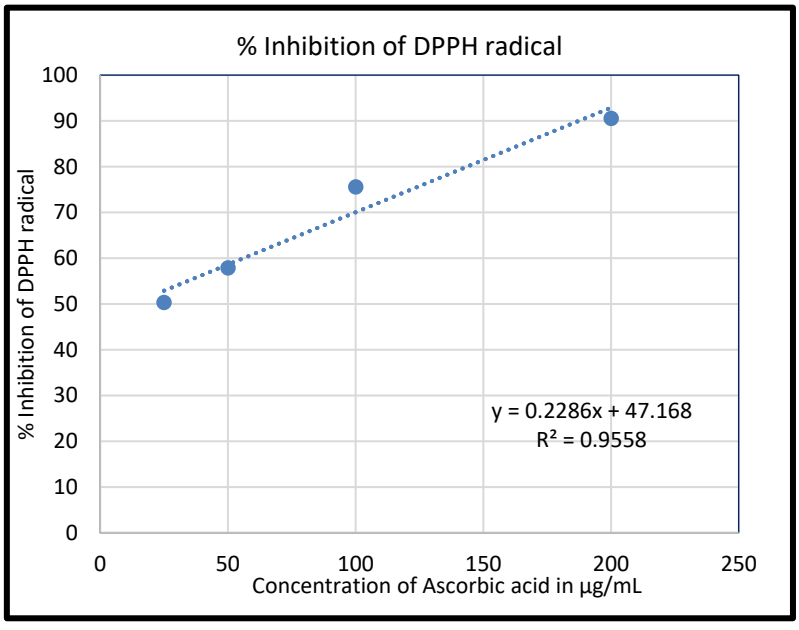


Figure 6: Standard curve of Ascorbic acid

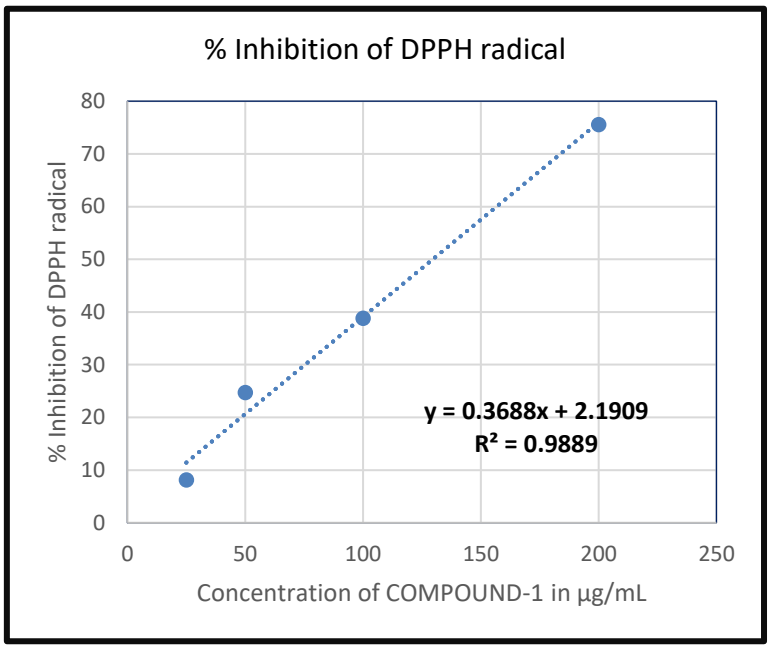


Figure 7: Standard curve of PHC

CONCLUSION

The study successfully synthesized and characterized a coumarin-based Schiff base, the phenyl hydrazine derivative (PHC), as a potential anti-Alzheimer's agent. *In-silico* molecular docking studies demonstrated that the compound fits well in the acetylcholinesterase (AChE) active site. Pharmacokinetic analysis using SwissADME indicated favorable drug-like properties, including blood-brain barrier permeability, compliance with Lipinski's Rule of Five, and good oral bioavailability, supporting its potential as a CNS-active therapeutic. *In vitro* studies demonstrated the compound's efficacy in inhibiting AChE activity, a crucial therapeutic target in Alzheimer's disease, with an IC_{50} value of 52.08 µg/mL. Furthermore, antioxidant assays revealed moderate DPPH radical scavenging activity, which may provide neuroprotective benefits by mitigating oxidative stress in Alzheimer's disease. Though less potent than ascorbic acid, the compound's antioxidant activity could still contribute to its overall therapeutic efficacy. Together, these findings highlight the compound as a promising lead for further development as an anti-Alzheimer's drug, offering potential advantages as both an AChE inhibitor and an antioxidant.

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