SRI VENKATESWARA INTERNSHIP PROGRAM FOR RESEARCH IN ACADEMICS (SRI-VIPRA)

SRI-VIPRA

Project Report of 2024: SVP-2427

"Investigating the Neuro-Enzyme Targeting Abilities of Small Heterocyclic Compounds: A Novel Approach to Neurodegenerative Disease Treatment"

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SRIVIPRA PROJECT 2024

Title: "Investigating the Neuro-Enzyme Targeting Abilities of Small Heterocyclic Compounds: A Novel Approach to Neurodegenerative Disease Treatment"

Name of Mentor: Dr. POOJA **Name of Department: CHEMISTRY** **Designation: ASSISTANT PROFESSOR**	

List of students under the SRIVIPRA Project

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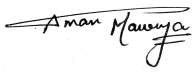
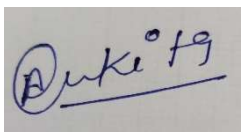
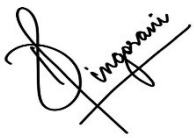
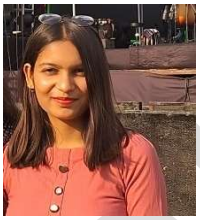
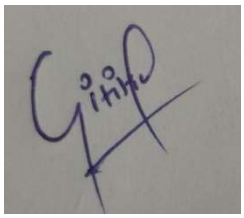
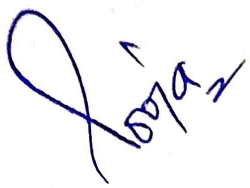
1		Aman Maurya	1522062	B.Sc.(H) Chemistry, SEM V	
2		Ankita	1522079	B.Sc.(H) Chemistry, SEM V	

3		Divya Hingorani	1122901	B.Sc.(P) Life Sciences, SEM V	
4		Nandini Pabreja	1522045	B.Sc.(H) Chemistry, SEM V	
5		Ashish Kumar Jha	1522069	B.Sc.(H) Chemistry, SEM V	
6		Gitika Nigam	1522024	B.Sc. (H) Chemistry, SEM V	



Signature of Mentor

Certificate of Originality

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP-2427 titled "Investigating the Neuro-Enzyme Targeting Abilities of Small Heterocyclic Compounds: A Novel Approach to Neurodegenerative Disease Treatment".

The participants have carried out the research project work under my guidance and supervision from 1st July, 2024 to 30th September 2024. The work carried out is original and carried out in an online/offline/hybrid mode.

Signature of Mentor

Acknowledgements

We want to start by expressing our deep gratitude to the Almighty for keeping us in good health throughout this journey. Our families have been our constant source of support and love, and we can't thank them enough.

A special mention goes to our mentor and guide, **Dr. Pooja**. Her guidance, suggestions, and unwavering encouragement have been invaluable. **Dr. Pooja's** effective communication and motivating words have sparked our curiosity and fueled our work. We are truly thankful for her generous time and expertise.

Working under her mentorship has been an incredible experience. Our project team deserves a round of applause too. They've been instrumental in spotting and fixing foundational errors, without which we wouldn't have met our project deadlines.

Lastly, our college deserves a heartfelt thank you for providing us with the resources, knowledge, and an unforgettable learning experience that has paved the way for the success of our project.

We owe them a great deal.

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INTRODUCTION

The number of deaths from neurological illnesses has been alarmingly rising since 1990. As of 2023, this number has risen to 40%, becoming neurological disorders the second biggest cause of death globally and the major cause of disability. By 2050, this increase is expected to almost double. Alzheimer's disease is a prevalent neurological condition that was ranked as the 7th biggest cause of mortality in the United States in 2022.

Acetylcholinesterase (AChE) is an enzyme that rapidly hydrolyzes acetylcholine (ACh) to terminate communication at the cholinergic synaptic cleft. AChE is the target of first-generation medicines used to treat Alzheimer disease (AD). Alzheimer's disease can also be caused by toxic clumps of amyloid-beta (Aβ), which disrupt synapses and eventually cause neurodegeneration and dementia. These aggregates can be amyloid plaques, intraneuronal Aβ, or soluble Aβ oligomers. A requirement for Aβ toxicity appears to be the existence of the microtubule-associated protein tau, whose hyperphosphorylated forms clump and deposit as neurofibrillary tangles in AD brains, blocking the passage of vital chemicals and nutrients.

Concepts including the neurotoxicity of excitatory neurotransmitters, altered insulin signaling, oxidative stress, inflammation, and therapeutic targets for the development of innovative medications for AD can help explain and provide a new theoretical basis for the etiology of dementia. Several studies have shown a link between the AChE activity of experimental animals and their memory and learning capacities.

The United States FDA database indicates that nitrogen heterocycles are present in 75% of approved novel small-molecule medicines, whereas oxygen-containing heterocycles rank second in terms of prevalence. In medicinal chemistry, coumarins are an advantageous scaffold and a flexible class of molecules. Their multitarget profile has previously been made public by several research institutions, especially in relation to neurodegenerative diseases. So, small heterocyclic compounds such as Nitrogen, Sulphur or Oxygen containing coumarins are ideal to show AChE inhibition.

Numerous pharmacological activities, such as anticoagulant, antibacterial, anticancer, antioxidant, anti-inflammatory, anti-tuberculosis, antiviral, and antidepressant properties, have been documented for coumarin derivatives containing one or more pharmacophores. Because of the strong biological significance of coumarin derivatives in pharmaceutical chemistry and drug discovery, it enhances efficacy and decreases toxicity of the synthesized hybrid molecules. This allows us to create a novel series of coumarin derivatives using the Perkin condensation, Knoevenagel condensation, and Pechmann reaction. Scientists have designed and synthesized new equivalents with improved AChE inhibitory activity and additional pharmacological effects, like inhibiting beta-secretase (BACE) to reduce Aβ deposition and

inhibiting monoamine oxidase (MAO), by identifying key structural features within the coumarin framework.

Furthermore, it has been found that coumarin derivatives protect neurons against Aβ-induced oxidative stress and free radical damage. It has been observed that in animal models, some coumarins produced from plants can mitigate memory loss caused by Aβ. As of right now, more than 1300 coumarins have been found in plants, microorganism and other natural sources. Coumarins have been discovered in 150 plant species from more than 30 families, including the Thymelaeaceae, Oleaceae, Apiaceae, Rutaceae, Astraceae, Fabaceae, Moraceae, Guttiferae, and Nyctaginaceae.K. Barot et al. Natural coumarins fall into eleven categories: simple coumarins, furanocoumarins (psoralene and angelicin types), pyranocoumarins (xanthyletin and seselin types), phenylcoumarins, and dicoumarins.

Because they target many pathways involved in the evolution and symptoms of Alzheimer's disease, coumarin-based drugs therefore offer a viable route for the development of new therapeutics for the condition. In order to cure Alzheimer's disease, researchers have created a variety of medicines that specifically target AChE in recent years. The comparison of several heterocyclic coumarin-based compounds as AChE inhibitors is the main topic of this article, which also compiles the experiments conducted *in vivo* and *in vitro*. This report compares various synthesis approaches for coumarin heterocyclic AChE inhibitors in an effort to identify the best chemical for treating AD.

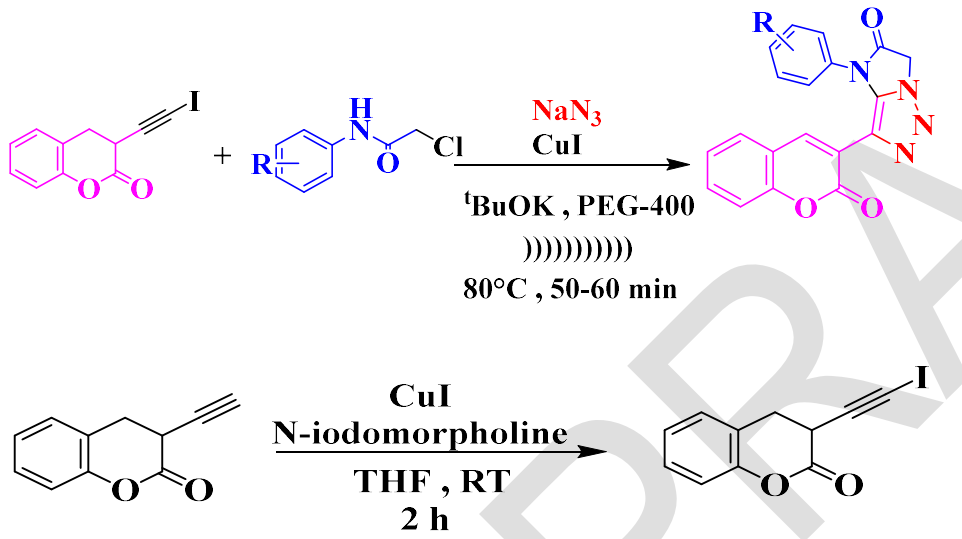
Different method for the synthesis of Coumarin derivatives.

Coumarin derivatives was synthesized by various type of methods that is opted by the several researchers in the different paper. Different types of coumarin derivative containing different type of moiety which possess different types of effects.

Synthesis of coumarin imidazole/triazole

- It was observed that Samala et al.2023 synthesized coumarin-imidazo[1,2-c] [1,2,3] triazoles via PEG-400 mediated one-pot reaction under ultrasonic irradiation. For one pot synthesis of coumarin imidazo derivatives, a starting compound that is 3-(iodoethynyl)-2H-chromen-2-one was synthesized. Further, CuI (1.2 mmol), N-iodomorpholine (0.045 mol), and 3-ethynyl-2H-chromen-2-one (0.03 mol) were dissolved in 30 mL of THF. At room temperature, a pale blood crimson precipitate was formed after one hour of stirring **(Scheme 1)**. The suspension was placed onto a 100

mL activated neutral alumina pad, and the filtrate was then collected. The recorded yield was 3.9 g (77%).

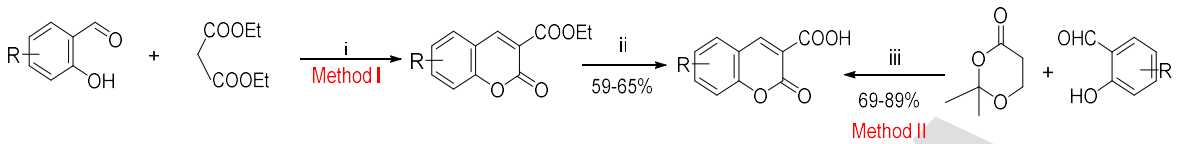


Scheme 1: PEG-400 mediated one-pot reaction

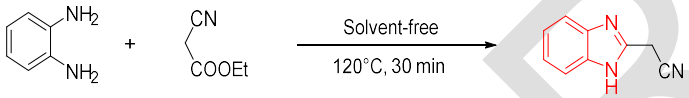
Synthesis of Coumarin-benzimidazole

- Paul.K.et.al.2013 synthesized various coumarin-benzimidazole derivatives and two crucial intermediates were involved in this synthesis. 1st intermediate, Coumarin-3-carboxylic acid was synthesized by two methods. (a) reaction between salicylaldehyde and dimethyl malonate followed by acidic hydrolysis under Knoevenagel condensation. (b) reaction between salicyaldehyde and Meldrum's acid, whereas 2nd intermediate, 2-(1H-benzo[d]imidazol-2-yl) acetonitrile was synthesized by reaction with *o*-phenylenediene (OPD) and ethyl cyanoacetate under solvent free conditions **(Scheme 4).** They synthesized 7-Bromo-coumarin-3-carboxylic acid (2) by following method (a) by treating Coumarin-3-carboxylic acid with OPD in polyphosphoric acid (PPA) (recorded yield-63%). A by-product:7-((2-aminophenyl) amino)-2-oxo-2H-chromene-3-carboxylic acid was obtained in 20 % yield. Also, 1a -d (recorded yield-70%) corresponding hybrids were synthesized when Coumarin-3-carboxylic acid was treated with OPD under solvent-free conditions at 100 °C. Following Vilsmeier–Haack formylation desired hybrids (4a-h) were synthesized when

corresponding hybrids 1 a-d were treated with methyl amine and ethyl amine in ethanol (EtOH) at room temperature (recorded yield was 50–59 %).

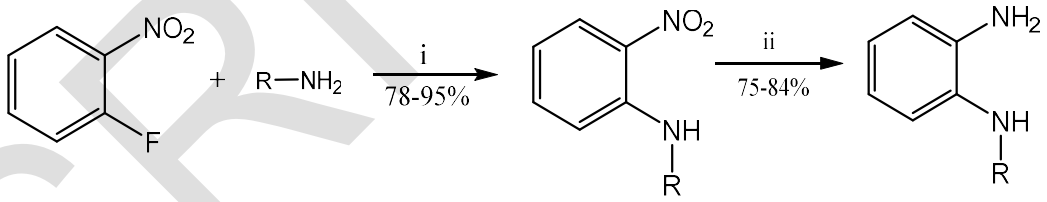


Reagent and conditions:-(i) Piperidine(cat.), EtOH, AcOH(cat.) reflux, 3-12h (ii) 0.5% NaOH, EtOH, reflux, 2h (or) 30% (w/w) aq. NaOH, EtOH, rt, 12h (iii) piperidinium acetate, EtOH, r.t. for 20 min then reflux for 2h.



Scheme 4. Synthesis of 1st and 2nd intermidiates

- 1,2-diaminobenzene derivatives were synthesized by using 2-fluoronitrobenzene with 4-substituted anilines in refluxing pyridine and reduction of the nitro group to amine in the presence of zinc-ammonium formate in MeOH through the SNAr reaction **(scheme 6).** Using 1,2-diaminobenzene derivatives Arya CG et al.2024 synthesized various direct-linked coumarin-benzimidazole hybrids highlighting aryl and n-butyl substituents at the N1-position of benzimidazole through Knoevenagel condensation reaction of these derivatives with coumarin-3-carboxylic acids in the presence of poly-phosphoric acid (PPA) at 154 °C **(scheme 7).**



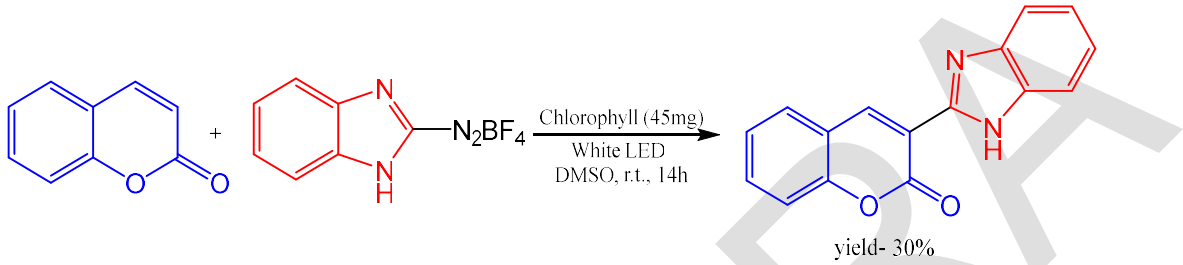
Reagents and conditions: (i)pyridine,110°C, 12h or DCM, r.t., 5h
(ii) Zn, ammonium formate, MeOH, r.t., 15 min.

Scheme 6. Synthesis of N-substituted o-phenylenediamine derivatives.

Synthesis of coumarin C3 benzimidazole from chlorophyll

- Moazzam A.etal.2022 developed a safe and metal-free method using aryldiazonium salt to directly intermolecularly arylate coumarin at position C3. Under visible light irradiation, chlorophyll was

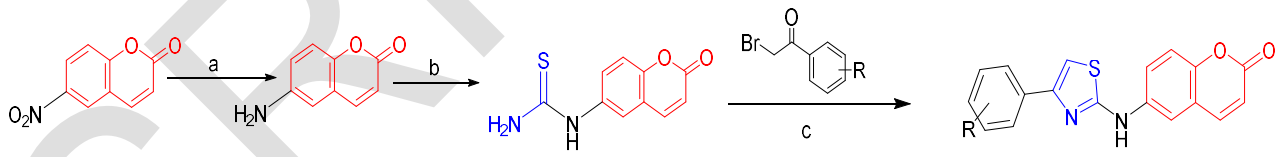
used as a biocatalyst (photosensitizer) (white LED light lamp). The advantages of this method include the utilization of visible light and the natural pigment chlorophyll as a photosensitizer as well as a simple and feasible synthetic procedure. **Scheme 8** shows the reaction between unsubstituted coumarin and the benzimidazole diazonium salt.



Scheme 8. Chlorophyll-catalyzed regioselective arylation of coumarin at C3 with diazonium salt of benzimidazole

Synthesis of Thiazole tethered coumarin

- Singh et al.2024 proposed a general procedure for the synthesis of phenyl substituted 6-(thiazol-2-ylamino)-2*H*-chromen-2-one. In this process, a solution of substituted 2-bromo-1-phenylethan-1-one (1 mmol) in EtOH (2 mL) was subjected to microwave radiation for 30 minutes at 80 °C, and then a 1-(2-oxo-2H-chromen-6-yl) thiourea (1 equiv.) was added. To yield the product, the precipitate was filtered, rinsed with water (5 mL), rinsed with cool ethanol (5 mL), and dried. The recorded yield was 61-93%.

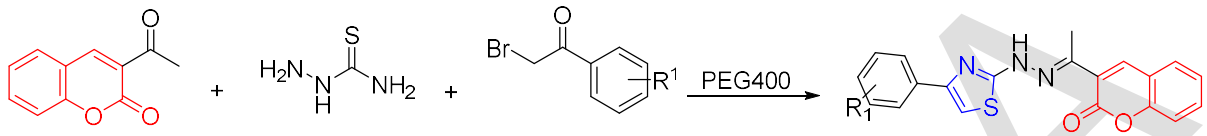


Reagents and conditions: a) Fe (8 equiv)/ AcOH, EtOH-H_2O, reflux, 1h; b) NH_4CN, aqueous HCl; 100°C, 87%; c) Substituted Phenacyl bromide, EtOH, microwave 6h, 61-93%

Scheme 11. Synthesis of Thiazole coumarin

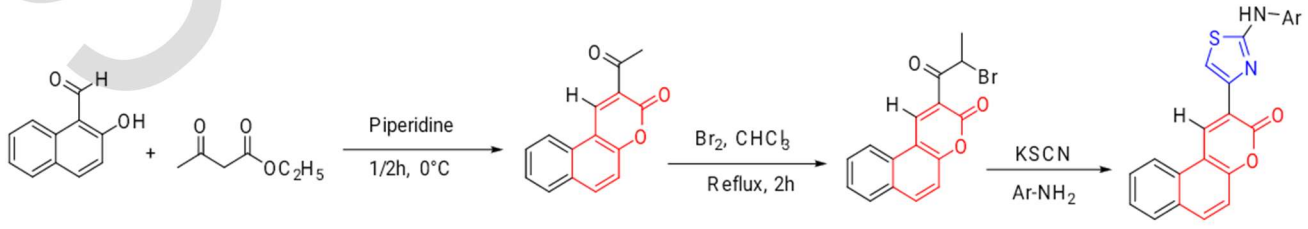
- Sadgir et al.2023 designed a general procedure for the synthesis of coumarin thiazole derivatives in which the 3-acetyl-coumarin 3 (0.01 mol) was added into a conical flask that contained 10 mL of PEG-400. Afterwards, at 70–80 °C, acetic acid was added and mixed for ten minutes. Following that, 0.01 mol of thiosemicarbazide was added, and the mixture was stirred for a further half an

hour. Substituted and unsubstituted phenacyl bromide (0.01 mol) was then added gradually throughout the course of the subsequent 30 minutes. The solid product was obtained. The reaction mixture was agitated for a further five minutes. To yield pure products, the substance was put into ice-cold water, filtered, and dried.



Scheme 12. Synthesis of coumarin appended thiazole hybrid heterocycles

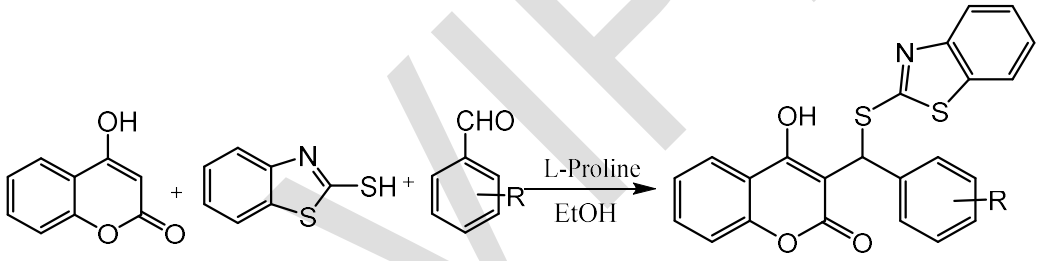
- Saeed, A.et al.2016 accomplished a general procedure for the synthesis of Thiazole Based Coumarinyl Scaffolds by addition of piperidine(1g) with constant shaking to a cold mixture of 2-hydroxy-1-naphthalaldehyde (0.1 mol) and ethylacetoacetate (0.1 mol, 12.63 ml). The 2-acetyl-3H-benzo[f]chromen-3-one was washed, filtered with ethanol and crystallized using pure water. Further, in a solution 2-acetyl-3H-benzo[f]chromen-3-one (0.01 mol, 2.38) in chloroform, 0.01 mol of bromine solution in chloroform was added with rapid shaking and this mixture was kep under heat and refux condition for 2-3 h. The solid was separated and crystallized using chloroform–ethanol (2:1) to yield pure 2-(2-bromoacetyl)-3H-benzo[f]chromen-3-one (0.001 m). In a round-bottom flask with a reflux condenser, the former was dissolved in 30 mL of ethanol, followed by addition of KSCN (0.001m). The resultant mixture was then agitated at 45–50 °C. After a one-hour period, substituted aniline (0.0012m) was added, the reaction mixture was refluxed, and TLC was used to track the reaction's progress. Cooling of the reaction mixture allowed for the extraction of the solid after 4-5 hours. The separated product was re-crystallized in ethanol for purification. A set of derivatives (from **1-10**), were created in this manner. The synthesis of coumarinyl derivatives was achieved with good yields and high purity using the method.



Synthesis of Thiazole Based Coumarinyl Scaffolds

Synthesis of coumarin benzothiazole

- Kadam et.al.2022 proposed a general procedure for the synthesis of 3-[(1,3-benzothiazol-2-ylsulfanyl) (phenyl)methyl]-2H-chromen-4-ol derivatives. In this method, L-proline (10 mol%) and substituted aldehyde (1 mmol) were dissolved in 10 mL ethanol in a 100 mL round-bottom flask and stirred for 10 minutes at room temperature. The addition of 4-hydroxy coumarin (1 mmol) and 2-mercapto benzothiazole (1.2 mmol) to this stirred mixture was followed. For about four to five hours, the resultant reaction mixture was stirred at room temperature. TLC monitored the reaction's progress. The product was filtered, cleaned with ethanol, and dried under vacuum when the reaction completed.



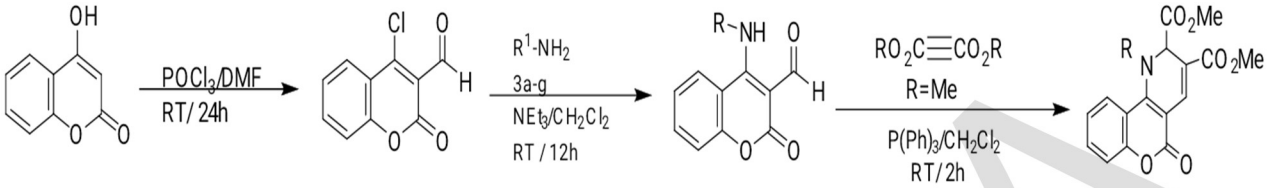
Scheme 18. Synthesis of 3-[(1,3- benzothiazol-2-ylsulfanyl) (phenyl)methyl]-2H-chromen-4- ol derivatives

Synthesis of coumarin pyridine

- In a solvent-free one-pot, four-component synthesis involving 3-acetylcoumarin, aromatic aldehydes, 1,3-indandione, and ammonium acetate, phthalimide-N-sulfonic acid (PISA) was used as a catalyst to produce 2H-chromen-indeno[1,2-b] pyridinone derivatives in good-to-excellent yields. In order to prepare novel indole and coumarin containing pyridine-3 carbonitrile derivatives in good-to-excellent yield, Krishnammagari et al. published an example of a one-pot, four-component condensation reaction of 3-acetylcoumarin, aldehydes, ammonium acetate, and 1H-indol-3-oxo-propanenitrile in acetic acid at a high temperature.

- Zahedi et al. synthesized coumarin dihydropyridine via a synthetic route in which 4–chloro-3-formylcoumarin was obtained from 4-hydroxycoumarin in the presence of phosphoryl chloride in

DMF and was further reacted with amine derivatives in the presence of NEt_3 in CH_2Cl_2 to obtain 4-amino-3-formylcoumarin derivatives, and final derivatives (a and b) were procured by reaction with dimethyl acetylene dicarboxylate or diethyl acetylene dicarboxylate.



- Zhou et al. synthesized 3-imidazolyl-[1,2-A] pyridinyl coumarin molecules; they refluxed Salicylaldehyde and ethyl acetoacetate were in ethanol with catalytic amounts of pyridine to produce 3-acetylcoumarin and 2-acetyl-3h-benzo(f)chromen-3-one through the Knoevenagel condensation reaction with yields of 71% and 86%, respectively. Further, 3-acetylcoumarin in solvent-free conditions was subjected to a [3+2] cycloaddition reaction with 2-aminopyridine where iodine was acting as a catalyst, and a series of coumarin derivatives were synthesized in the range of 10% to 43% yield.

Biological Studies

❖ **AChE Inhibition methods and materials for coumarin pyrazole hybrids**

Methods: AChE inhibition was measured using Ellman's method. The reaction mixture was incubated, and absorbance was measured at 412 nm.

Conclusion: The coumarin-pyrazole hybrids demonstrated moderate to high AChE inhibitory activity, indicating their potential as effective AChE inhibitors for treating Alzheimer's disease. Notable compounds include 7-(1H-pyrazol-1-yl)-4-methyl-2H-chromen-2-one. (Benazzouz-Touami, A.et al.2022)

❖ **AChE Inhibition methods and materials for coumarin benzimidazole derivatives**

Methods: Ellman's method was used to measure AChE inhibition. The reaction mixture was incubated, and absorbance was measured at 412 nm. (Sadgir, N. et al.2023)

Conclusion: The synthesized coumarin-benzimidazole derivatives exhibited significant AChE inhibitory activity. Compound 1, specifically 6-(Benzimidazol-2-yl)-coumarin, showed the highest binding affinity and inhibition activity, making it a promising candidate for further development as an AChE inhibitor.

❖ **AChE inhibition methods and materials for coumarin thiazole derivatives**

Methods: The AChE inhibition assay was done using the Ellman's method, which involved incubating the enzyme solution with the test compounds and initiating the reaction with acetylthiocholine iodide (AChI) and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). The decrease in absorbance at 412 nm, indicates enzymatic activity, which was measured to determine the inhibitory effects of the compounds.

Conclusion: The coumarin-thiazole hybrids, including (Methylamino)-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)acetamide (6a), N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-2-(propylamino)acetamide (6b), and 2-(Diethylamino)-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)acetamide (6c), exhibited a significant AChE inhibitory activity with IC50 values of 2.36 μM, 2.15 μM, and 0.043 μM, respectively. These results highlight their potential as effective inhibitors of AChE and suggest their utility in the development of therapies for Alzheimer's disease. (Sonmez F. et al.2017)

❖ **AChE inhibition methods and materials for coumarin-thiadiazole Hybrids**

Methods: AChE inhibition was measured using a modified Ellman's method. The reaction mixture, including the enzyme, test compounds, AChI, and DTNB, was incubated, and absorbance was measured at 412 nm.

Conclusion: Compounds such as 3-(2-Amino-1,3-thiazol-4-yl)coumarin exhibited significant AChE inhibitory activity, with the highest inhibition observed for this compound (IC50 = 43 nM). This indicates the potential of these coumarin-thiadiazole hybrids as effective AChE inhibitors (Karcz, D.et al.2022)

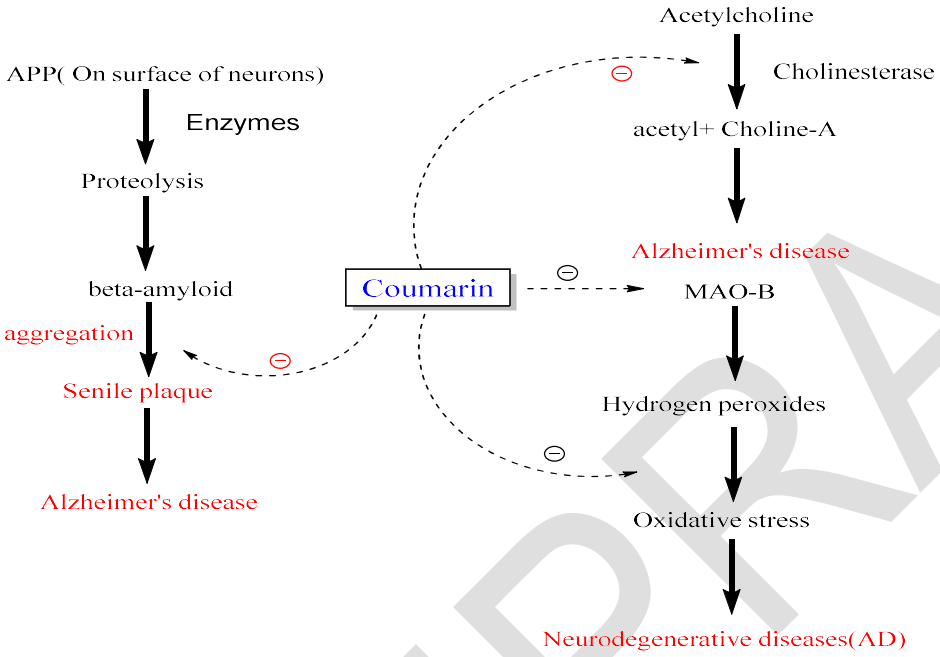


Figure 1. Mechanism of anti-alzheimer disease

❖ **In vitro methods and materials for coumarin pyrazole hybrids**

Methods: Cytotoxicity and antioxidant assays were performed to assess the compounds' safety and biological activity. Cell viability and antioxidant capacity were measured using standard assays.

Conclusion: The compounds, such as 7-(1H-pyrazol-1-yl)-4-methyl-2H-chromen-2-one, showed low cytotoxicity and significant antioxidant activity, suggesting their potential as safe and multifunctional therapeutic agents. (Benazzouz-Touami, A.et al.2022)

❖ **In silico methods and materials – Docking study for coumarin indole derivatives**

To comprehend the binding mechanisms of coumarin-indole derivatives with α-glucosidase, molecular docking was used. Using a model of α-glucosidase, the most powerful compounds (4a–d) were docked into the active site of the enzyme. Interactions were examined and binding energies were computed.

Docking Studies for In Silico Methods and Materials. Molecular docking was used to understand the binding processes between coumarin-indole derivatives and α-glucosidase. The most potent compounds

(4a-d) were docked into the enzyme's active site using an α-glucosidase model. Interactions were investigated and binding energies calculated. (Niri, D.et al.2022).

❖ **In Vivo methods and materials for coumarin benzimidazole derivatives**

Methods: Male Wistar rats were treated with the compounds. AChE activity was assessed in rat brain homogenates post-treatment.

Conclusion: The compounds showed significant AChE inhibition *in vivo*, supporting their potential as therapeutic agents for neurodegenerative diseases. (Sadgir, N. et al.2023)

❖ **In Vivo methods and materials for coumarin indole derivatives**

The yeast version of α-glucosidase was used to measure the in vitro anti-α-glucosidase activity. P-nitrophenyl glucopyranoside was used as the substrate to calculate the IC50 values.

The produced substances were put to the test against the enzyme and contrasted with the reference medication, acarbose. (Niri, D.et al.2022)

CONCLUSION

The studies on coumarin derivatives, including thiazole, thiadiazole, benzimidazole, and pyrazole hybrids, demonstrate their significant AChE inhibitory activity, low cytotoxicity, strong binding affinities in docking studies, and potential to enhance cognitive function in vivo. Compounds such as 6a, 6b, 6c, 3-(2-Amino-1,3-thiazol-4-yl)coumarin, 6-(Benzimidazol-2-yl)-coumarin, and 7-(1H-pyrazol-1-yl)-4-methyl-2H-chromen-2-one are highlighted for their potent activities. These findings highlight the future usage of these compounds as therapeutic agents for neurodegenerative diseases like Alzheimer's. In docking tests, compound 4d showed the lowest binding energy and competitive inhibition, making it a promising α-glucosidase inhibitor. This substance may provide a starting point for the creation of more effective α-glucosidase inhibitors. Compound 4d (3-phenoxy phenyl) had the lowest binding energy and competitive inhibition, making it a promising α-glucosidase inhibitor. This chemical may be use for developing more potent α-glucosidase inhibitors.

Abbreviation

Aβ- Amyloid-beta

AChE- Acetylcholinesterase

AD- Alzheimer's disease

BACE- Beta-secretase

BuChE- Butyrylcholinesterase

DCE- 1,2-Dichloroethane

DMSO- Dimethyl sulfoxide

DMF- Dimethylformamide

DNA- Deoxyribonucleic acid

DTNB- 5,5'-dithiobis-(2-nitrobenzoic acid)

hAChE- Human acetylcholinesterase

LED- Light Emitting Diode

MAO- Monoamine oxidase

MTT- 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide

NMP- N-Methylpyrrolidone

OPD- o-Phenylenediamine