

Exploring the Quantum Chemical and Inhibitory Footprint of Coumarin Derivatives Against α -Amylase: A Unified *in Silico* and ADMET Profiling Study

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Abstract

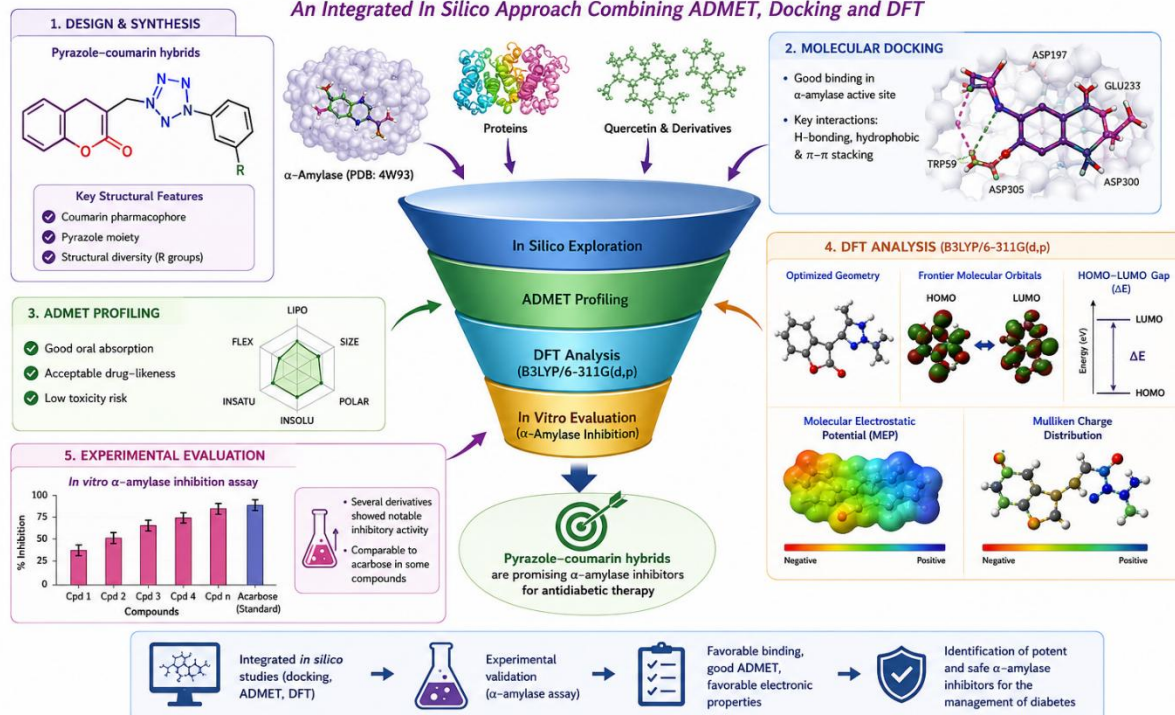
Diabetes mellitus is a prevalent metabolic disease associated with persistent elevation of blood glucose and represents a significant challenge in the search for effective and well-tolerated therapeutic agents. Targeting α -amylase, a key enzyme involved in the hydrolysis of dietary carbohydrates, offers a promising approach for controlling postprandial glucose excursions. In this study, a novel series of pyrazole-linked coumarin derivatives was rationally designed, synthesized, and investigated for their potential as α -amylase inhibitors through a combination of computational and experimental methodologies. Molecular docking analysis was employed to elucidate the binding modes of the synthesized compounds within the α -amylase active site and to identify important ligand–protein interactions contributing to their inhibitory activity. In silico ADMET assessment was subsequently performed to examine their physicochemical characteristics, drug-likeness, pharmacokinetic profiles, and potential toxicity, with most compounds displaying favorable predicted properties. DFT calculations further provided insights into the structural and electronic characteristics of the derivatives by analyzing optimized geometries, frontier molecular orbitals, HOMO–LUMO energy gaps, and molecular electrostatic potential distributions. The synthesized compounds were structurally characterized using appropriate analytical methods and subjected to in vitro α -amylase inhibition assays, with acarbose serving as the standard inhibitor. The experimental results, supported by the computational investigations, identified several derivatives with notable inhibitory activity and favorable molecular properties. Overall, the findings suggest that pyrazole–coumarin hybrids represent a promising chemical framework for further optimization and development of potential α -amylase-targeting antidiabetic agents.

Keywords: Pyrazole–coumarin hybrids, α -Amylase inhibition, Molecular docking, Frontier Molecular Orbital, Molecular Electrostatic Potential.

Graphical abstract

Pyrazole-Coumarin Hybrids as α -Amylase Inhibitors

An Integrated In Silico Approach Combining ADMET, Docking and DFT



1. Introduction

Diabetes mellitus (DM) is a prevalent chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, defective insulin action, or both. It is commonly associated with symptoms such as excessive urination, thirst, increased hunger, fatigue, and weight loss, while postprandial hyperglycemia is an important feature of disease progression. Prolonged hyperglycemia can induce vascular dysfunction and oxidative stress, contributing to severe microvascular and macrovascular complications, including retinopathy, nephropathy, cardiovascular disease, stroke, and peripheral tissue damage. [1-3]

α -Amylase is an important carbohydrate-digesting enzyme responsible for the breakdown of dietary starch into smaller oligosaccharides and glucose-producing intermediates. It catalyzes the cleavage of α -1,4-glycosidic bonds in complex carbohydrates, facilitating their subsequent digestion and absorption in the gastrointestinal tract. Excessive α -amylase activity can accelerate carbohydrate hydrolysis and contribute to a rapid elevation of blood glucose following meals. Therefore, suppression of α -amylase activity represents a valuable therapeutic approach for delaying carbohydrate digestion and limiting postprandial glucose elevation, which may help improve overall glycemic control in diabetes mellitus. [4,5]

Coumarin (2H-chromen-2-one) is a versatile medicinal chemistry scaffold with diverse biological activities. Molecular hybridization of coumarin with bioactive heterocycles such as pyrazole offers a promising strategy for developing novel α -amylase inhibitors. Incorporation of these pharmacophoric units may enhance interactions with the α -amylase active site and improve the biological potential of the resulting derivatives. [6,7]

The integration of computational techniques has greatly enhanced modern drug discovery by enabling the rational design and evaluation of potential therapeutic compounds. Molecular docking is a valuable *in silico* approach for predicting the binding modes and interactions of ligands within the active site of target proteins. In the present study, pyrazole-based coumarin derivatives were designed and computationally evaluated to investigate their structure–activity relationships and interaction patterns with α -amylase, providing a theoretical basis for the development of novel antidiabetic agents for Type 2 diabetes mellitus. [8,9]

Density Functional Theory (DFT) is a widely used quantum mechanical approach for investigating the electronic structure and physicochemical properties of molecules. It provides valuable insights into molecular stability, chemical reactivity, and electronic behavior by calculating parameters such as total energy, frontier molecular orbitals, and energy gaps. DFT calculations complement molecular docking by offering a deeper understanding of the electronic characteristics that influence molecular interactions with biological targets. Consequently, DFT has become an important computational tool in drug discovery, aiding in the rational design and optimization of potential therapeutic candidates. [10]

2. Materials and methods

2.1. Computational Evaluation

2.1.1. Therapeutic Target Identification

The crystal structure of human pancreatic α -amylase (PDB ID: 1B2Y) was obtained from the Protein Data Bank (PDB) and selected as the target protein for structure-based computational analysis. The experimentally determined three-dimensional structure was considered suitable for investigating potential inhibitor-enzyme interactions. Prior to docking,

the protein structure was prepared and optimized using the Protein Preparation Wizard in Schrödinger Maestro 12.5, including appropriate structural refinement and optimization steps to generate a reliable receptor model for subsequent molecular docking studies. ^[11]

2.1.2. Lead identification and Optimization

A structure-based virtual screening approach was used to identify potential inhibitors of human pancreatic α -amylase. Fifty drug-like compounds were retrieved from the ZINC database and screened using iGEMDOCK v2.1. The compounds were ranked based on their predicted binding energies and interactions with key active-site residues. Compounds showing favorable binding scores and relevant interactions were shortlisted as potential lead candidates for further molecular docking and computational evaluation. ^[12]

2.1.3. Quantum Chemical Analysis

Density functional theory (DFT) calculations were performed to investigate the optimized molecular structures and electronic properties of the selected scaffold coumarin. The molecular geometries were optimized at the B3LYP/6-311G(d,p) level of theory in the gas phase employing the Gaussian 09 W program. Frequency calculations were subsequently performed to confirm the optimized structures as stable minima by ensuring the absence of imaginary frequencies. Frontier molecular orbital analysis was carried out by calculating the energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), along with the HOMO–LUMO energy gap (ΔE), to assess the electronic stability and chemical reactivity of the compounds. Molecular electrostatic potential (MEP) analysis was performed to identify electron-rich and electron-deficient regions that may contribute to intermolecular interactions. Global reactivity descriptors, including chemical hardness, softness, electronegativity, chemical potential, and electrophilicity index, were also calculated from the frontier orbital energies. ^[13]

2.1.4. Pharmacokinetic Profiling

Following virtual screening and preliminary literature assessment, the prioritized compounds were subjected to lead identification and optimization based on their drug-like and pharmacokinetic characteristics. molinspiration was employed to assess key physicochemical descriptors and drug-likeness parameters, including molecular weight, logP, hydrogen-bond donors (HBD), hydrogen-bond acceptors (HBA), and compliance with Lipinski's Rule of Five. Subsequently, pharmacokinetic profiling was performed using pkCSM to predict key ADME properties and identify potential pharmacokinetic liabilities. Compounds exhibiting favorable drug-likeness and predicted pharmacokinetic profiles were prioritized as potential lead candidates for further molecular docking and biological evaluation. ^[14]

2.1.5. Molecular Interaction Analysis

Structure-based molecular docking was carried out using the Glide module of Schrödinger Maestro to investigate the binding affinity and interaction patterns of the synthesized compounds toward human pancreatic α -amylase. The prepared ligands were docked into the enzyme's predefined active-site region, and the resulting poses were evaluated based on Glide docking scores, binding orientation, and ligand–protein interaction profiles. The docked complexes were analyzed to characterize key hydrogen-bonding, hydrophobic, π – π stacking, π –alkyl, alkyl, and van der Waals interactions contributing to ligand recognition and binding stabilization. Compounds exhibiting favorable docking scores and complementary interactions with catalytically or functionally relevant active-site residues were considered potential α -

amylase inhibitors and prioritized for subsequent in-silico validation and experimental biological evaluation.^[15]

2.2. Synthetic Investigation

Step 1: Synthesis of 3-acetyl coumarin

A mixture of salicylaldehyde (0.204 mol) and ethylacetoacetate (0.255 mol) was cooled and maintained at 0-5°C, piperidine was added dropwise to the mixture while stirring (Doebner modification). The reaction mixture was left overnight, resulting in the formation of a yellow coloured solid. The solid thus obtained was filtered and recrystallized with ethanol to give 3-acetyl coumarin as fine yellow needles.

Step 2: Synthesis of chalcone

Equimolar quantities of substituted aromatic aldehydes and 3-acetyl coumarin were taken in a 250 ml beaker, which was dissolved in 10 ml of rectified spirit (ethanol). This mixture was stirred using a mechanical stirrer at 20-25°C. While stirring NaOH (30%) was added dropwise to this mixture for 30 mins, the solution becomes turbid, and continued stirring for 4-5 hours while maintaining the temperature. After stirring has completed, the reaction mixture was neutralised by using 0.2 N HCl, whereby the precipitate occurs. It was filtered, and the crude chalcone was dried in air and recrystallised with ethanol.

Step 3: Synthesis of Pyrazoles

A mixture of chalcones (3 mmole) and thiosemicarbazide (14 mmole) was refluxed in ethanol (50ml) containing 0.5 ml of concentrated hydrochloric acid for 8 hours and poured on 100 g of crushed ice. The residue thus obtained was filtered and recrystallised with methanol.

Scheme

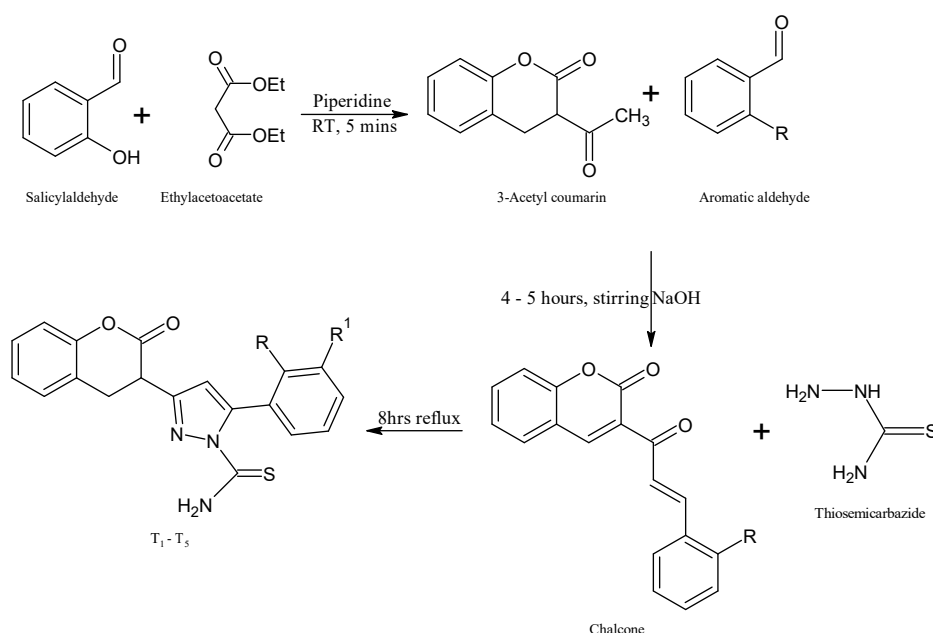


Figure 1 Scheme for synthesis of pyrazole-coumarin derivatives

Table 1: Chemical substituents of T₁₋₅ derivatives

Compound code	R	R ¹
T ₁	H	H
T ₂	Cl	H
T ₃	NO ₂	H
T ₄	OCH ₃	OCH ₃
T ₅	OH	OCH ₃

2.3. Spectral Characterization

Physicochemical Parameters such as Solubility, Melting Point analysis by Visible Range melting point apparatus (LABINDIA), Thin Layer Chromatography were performed for the synthesized compounds. Characterization of the synthesized compounds was performed by UV spectroscopy, Infrared spectroscopy, MASS spectroscopy on JASCO V-530, Department of Pharmaceutical Analysis, SRIPMS, COP, Coimbatore. PMR spectra were recorded on BRUKER ULTRA SHIELDED NMR-400 MHz at VIT University, Chennai. MASS spectra were recorded on GC-MS at VIT University, Chennai.

2.4. Biological Evaluation

In vitro anti-diabetic potential of the synthesized coumarin derivatives were studied by performing the enzyme inhibition assay using carbohydrate digestive enzyme α -amylase. The study was carried out with porcine pancreatic α -amylase with starch as substrate. acarbose was selected as the standard drug for comparison of the results.

It is based on the principle that α -amylase digests the starch in reaction mixture to yield maltose. The maltose produced would reduce the 3,5-dinitrosalicylic acid in the colouring agent to 3-amino 5-nitrosalicylic acid. The reaction mixture produced a colour change from orange to red. The intensity of red colour will be directly proportional to the amount of maltose produced. When an enzyme inhibitor is present in the reaction mixture digestion of starch, production of maltose and the intensity of red colour produced will be less. ^[16]

3. Results and discussion

3.1. Computational Evaluation

3.1.1. Therapeutic Target Identification

The crystal structure of **human pancreatic α -amylase (PDB ID: 1B2Y)** was retrieved from the RCSB Protein Data Bank and selected for molecular docking studies. As a key enzyme involved in **starch hydrolysis and carbohydrate digestion**, α -amylase represents a relevant therapeutic target for controlling postprandial glucose levels. The structure provided a suitable basis for evaluating the binding interactions and inhibitory potential of the synthesized compounds.

3.1.2. Lead identification and Optimization

The docking results of α -amylase (PDB ID: 1B2Y) with the 50 similar structural ligands are reported in Table 2. The best docked structures should have lower binding energies.

Table 2: Virtual screening results of selected HITS using iGEMDOCK

S.NO	COMPOUND CODE	Kcal/mol	S.NO	COMPOUND CODE	Kcal/mol
1	Zinc_18249417	-90.53	26	Zinc_2063189	-66.85
2	Zinc_20031600	-83.49	27	Zinc_18141403	-79.99
3	Zinc_9365179	-75.5	28	Zinc_634140	-68.29
4	Zinc_19166762	-84.51	29	Zinc_8442294	-67.43
5	Zinc_1700294	-94.06	30	Zinc_8442293	-59.04
6	Zinc_841803	-71.13	31	Zinc_8442288	-55.47
7	Zinc_3901268	-71.31	32	Zinc_651582	-62.16
8	Zinc_19799526	-82.5	33	Zinc_8442281	-55.46
9	Zinc_982962	-102.76	34	Zinc_730699	-83.88
10	Zinc_19989886	-82.58	35	Zinc_8442279	-69.79
11	Zinc_2258599	-96.1	36	Zinc_8442278	-77.97
12	Zinc_19990070	-79.21	37	Zinc_8442277	-71.89
13	Zinc_5556455	-95.45	38	Zinc_8442276	-62.55
14	Zinc_19990034	-95.57	39	Zinc_8442275	-78.22
15	Zinc_1240782	-94.08	40	Zinc_8442273	-60.38
16	Zinc_19794473	-76.95	41	Zinc_8442272	-86.63
17	Zinc_5286115	-95.45	42	Zinc_8442271	-73.91
18	Zinc_984053	-95.57	43	Zinc_8442270	-88.19
19	Zinc_5519407	-94.08	44	Zinc_8442268	-60.29
20	Zinc_2758246	-76.95	45	Zinc_8442295	-93.49
21	Zinc_8575396	-104.43	46	Zinc_634138	-83.9
22	Zinc_12378847	-108.44	47	Zinc_1019824	-78.77
23	Zinc_6182368	-90.93	48	Zinc_8442266	-88.9
24	Zinc_6219168	-94.9	49	Zinc_8442267	-81.93
25	Zinc_19318821	-93.13	50	Zinc_8442265	-76.15

3.1.3. Quantum Chemical Analysis

3.1.3.1. Frontier molecular orbital analysis

Frontier molecular orbital analysis was performed to evaluate the electronic structure and chemical reactivity of the investigated scaffolds. The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) were calculated using DFT at the B3LYP/6-311G(d,p) level. The HOMO represents the electron-donating region of a molecule, whereas the LUMO indicates its electron-accepting capability. The spatial distribution and energy of these orbitals provide insight into charge-transfer characteristics, molecular reactivity, and potential interaction with the target protein. The calculated HOMO and LUMO energies were further used to derive relevant quantum chemical reactivity descriptors,

providing additional information on the electronic properties and stability of the studied scaffold.

Frontier molecular orbital (FMO) analysis was performed to investigate the electronic properties and reactivity of the coumarin and pyrazole–coumarin scaffolds. The calculated HOMO and LUMO energies of the coumarin scaffold were -6.84 and -2.27 eV, respectively, resulting in a HOMO-LUMO energy gap (ΔE) of 4.57 eV. In comparison, incorporation of the pyrazole moiety into the coumarin framework resulted in HOMO and LUMO energies of -6.24 and -2.21 eV, respectively, with a reduced energy gap of 4.03 eV. Thus, the pyrazole–coumarin scaffold exhibited a decrease of approximately 0.54 eV in the HOMO-LUMO gap compared with the parent coumarin scaffold.

The reduction in the HOMO-LUMO energy gap following incorporation of the pyrazole moiety indicates a modification of the electronic structure of the parent coumarin framework. The relatively higher HOMO energy of the pyrazole-coumarin scaffold suggests an increased electron-donating tendency, while its slightly lower LUMO stabilization indicates that the scaffold retains favorable electron-accepting characteristics. Consequently, the reduced ΔE of the pyrazole-coumarin scaffold suggests comparatively greater electronic softness, polarizability, and ease of electronic excitation than the parent coumarin scaffold. In contrast, the larger energy gap observed for coumarin is indicative of comparatively greater electronic stability and lower susceptibility to electronic excitation.

Overall, the FMO analysis demonstrates that incorporation of the pyrazole moiety substantially influences the electronic characteristics of the coumarin scaffold. The decrease in the HOMO-LUMO gap from 4.57 eV for coumarin to 4.03 eV for pyrazole–coumarin indicates enhanced electronic reactivity and charge-transfer propensity of the modified scaffold. These findings suggest that pyrazole incorporation can modulate the electronic properties of the coumarin framework and may contribute to favorable molecular interactions with biological targets.

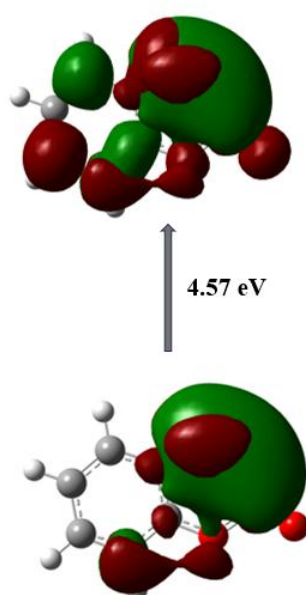


Figure 2 HOMO-LUMO plot for coumarin

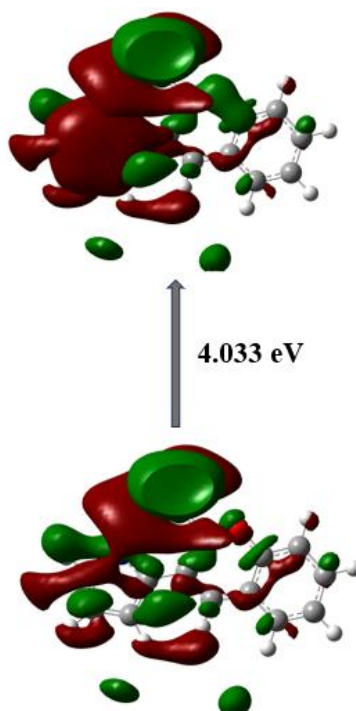


Figure 3 HOMO-LUMO plot of pyrazole-coumarin

3.1.3.2. Molecular Electrostatic Potential (MEP) Analysis

The molecular electrostatic potential (MEP) analysis was performed to investigate the electronic distribution and reactive regions of the selected scaffolds. The MEP maps provide a visual representation of the electrostatic potential, enabling identification of electron-rich and electron-deficient regions that may participate in intermolecular interactions.

The MEP maps showed distinct negative, positive, and neutral electrostatic potential regions, reflecting differences in the electronic environments of the scaffolds. The most negative regions, represented by red colour, are mainly associated with electron-rich atoms and are favourable for electrophilic attack and hydrogen-bond donation. In contrast, the blue regions indicate positive electrostatic potential and electron-deficient sites that may favour nucleophilic interactions or act as hydrogen-bond acceptor regions.

The distribution of electrostatic potential varied among the compounds due to differences in their substituents, heteroatoms, and electronic structures. Oxygen- and nitrogen-containing functional groups contributed significantly to the electron-rich regions, which may facilitate interactions with complementary residues in the target enzyme active site. These electrostatic features may also influence the orientation and binding of the compounds within the receptor.

Overall, the MEP analysis identified distinct reactive regions and supported the potential of the investigated scaffolds to participate in hydrogen bonding, electrostatic, and other non-covalent interactions. The differences in the size, shape, and distribution of electrostatic potential may contribute to variations in binding affinity and inhibitory activity, complementing the molecular docking findings.

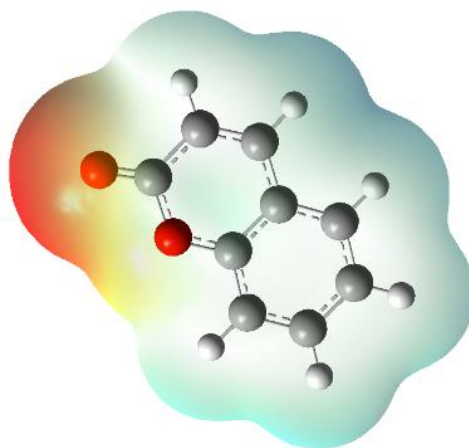


Figure 4 Molecular Electrostatic Potential (MEP) of coumarin

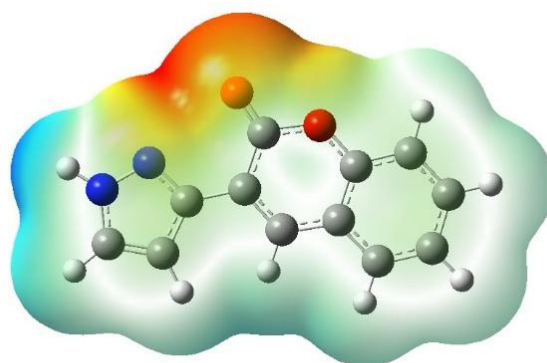


Figure 5 Molecular Electrostatic Potential (MEP) of pyrazole-coumarin

3.1.3.3. Mulliken atomic charges

Mulliken population analysis was performed at the B3LYP/6-311G(d,p) level to evaluate the electronic charge distribution of the coumarin and pyrazole-coumarin scaffolds. The calculated Mulliken charges were used to identify the relative electron-rich and electron-deficient regions and to assess the changes in electronic distribution following incorporation of the pyrazole moiety.

For coumarin, the two oxygen atoms exhibited negative Mulliken charges of -0.393 and -0.455 e, indicating electron-rich regions. Significant charge polarization was also observed in the carbon framework, with the most positive and negative carbon centers showing charges of +1.727 and -1.830 e, respectively. This heterogeneous distribution reflects the polarized electronic character of the conjugated coumarin system and indicates potential sites for electrostatic and non-covalent interactions.

In pyrazole-coumarin, the oxygen atoms retained negative charges of -0.371 and -0.426 e. Importantly, incorporation of the pyrazole ring introduced two electronically differentiated nitrogen atoms, with charges of -0.314 and +0.031 e. The negatively charged nitrogen represents an additional electron-rich site that may participate in hydrogen-bonding and

electrostatic interactions. The most positive and negative carbon centers showed charges of +0.807 and -1.888 e, respectively.

Table 3: Mulliken atomic charge distribution of coumarin and pyrazole-coumarin

Parameter	Coumarin	Pyrazole-coumarin
O atom 1	-0.393 e	-0.371 e
O atom 2	-0.455 e	-0.426 e
Pyrazole N 1	-	-0.314 e
Pyrazole N 2	-	+0.031 e
Most positive C	+1.727 e	+0.807 e
Most negative C	-1.830 e	-1.888 e

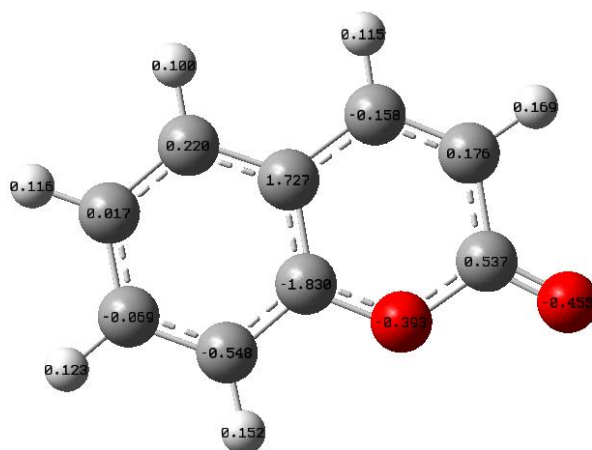


Figure 6 Mulliken atomic charge distribution of coumarin

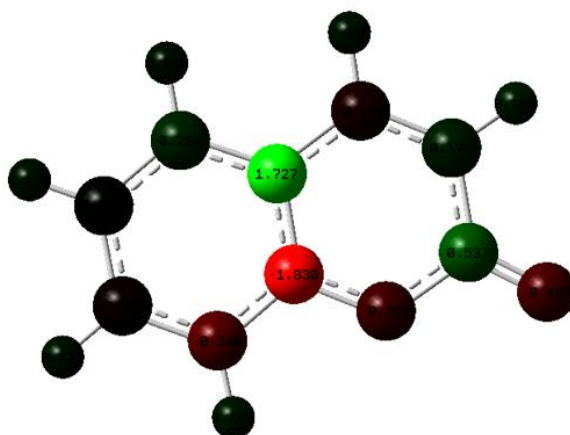


Figure 7 Colour-coded electrostatic charge distribution of coumarin

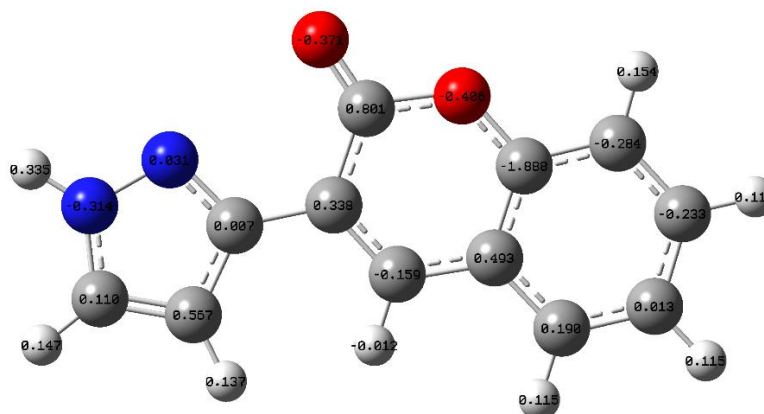


Figure 8 Mulliken atomic charge distribution of pyrazole-coumarin

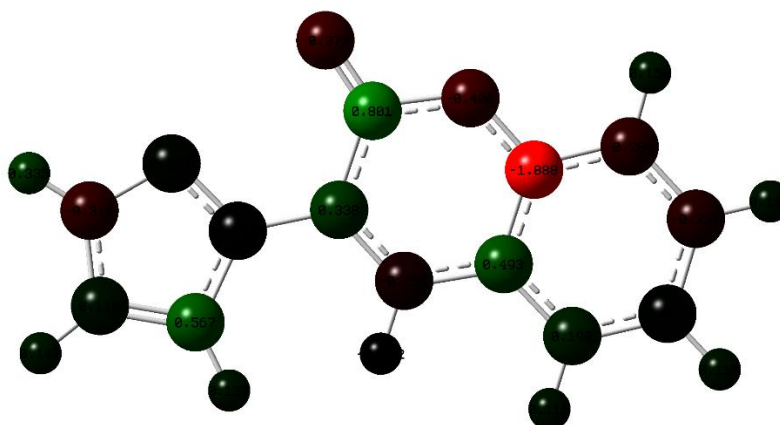


Figure 9 Colour-coded electrostatic charge distribution of pyrazole-coumarin

Overall, the B3LYP/6-311G(d,p) Mulliken analysis indicates that pyrazole incorporation substantially redistributes the electronic charge of the parent coumarin scaffold. The introduction of the negatively polarized pyrazole nitrogen creates an additional electron-rich region, while the reduction in the maximum positive carbon charge from +1.727 to +0.807e indicates redistribution of electron density and reduced positive polarization at that site. The most negative carbon center remained strongly polarized (−1.830 vs. −1.888 e). Thus, the pyrazole–coumarin scaffold exhibits a more diversified distribution of electronically favorable regions, which may influence molecular recognition and non-covalent interactions with biological targets.

The colour-coded charge distribution further supported the numerical Mulliken charge analysis. Electron-rich regions were predominantly associated with the negatively charged oxygen atoms, while incorporation of the pyrazole ring introduced an additional electron-rich nitrogen centre. The pyrazole–coumarin scaffold exhibited a more differentiated colour distribution than the parent coumarin, reflecting redistribution of electron density following

structural modification. The presence of distinct electron-rich and electron-deficient regions may facilitate hydrogen-bonding and electrostatic interactions with complementary sites in biological targets.

3.1.4. Pharmacokinetic Profiling

Based on the virtual screening outcomes, five coumarin-based molecules were selected as promising lead candidates and further optimized through drug-likeness and ADMET profiling. Their predicted physicochemical properties, including molecular weight, lipophilicity (logP), hydrogen-bonding capacity, and aqueous solubility, were assessed using the molinspiration server. The compounds were evaluated according to Lipinski's Rule of Five to estimate their oral drug-likeness and potential for favorable gastrointestinal absorption. Overall, this analysis provided an initial assessment of the suitability of the selected coumarin derivatives for further pharmacokinetic evaluation and biological investigation.

Table 4: Drug likeness screening of ligands T₁₋₅ by molinspiration

S.NO	COMPOUND CODE	M LOG P	MOLECULAR WEIGHT	NO. OF H ACCEPTORS	NO. OF H DONORS	NO. OF VIOLATIONS
1	T ₁	3.21	341.42	5	3	0
2	T ₂	3.84	383.86	5	3	0
3	T ₃	3.29	394.41	8	2	0
4	T ₄	2.86	409.47	7	3	0
5	T ₅	2.55	395.44	7	4	0

All five compounds (T₁–T₅) complied with Lipinski's Rule of Five, suggesting favorable drug-likeness and potential for oral administration. Pharmacokinetic and toxicity profiling using the pkCSM platform further indicated generally favorable predicted ADME characteristics, including acceptable aqueous solubility and absorption and distribution profiles. The toxicity predictions revealed no significant liabilities related to mutagenicity, carcinogenicity, or skin sensitization among the evaluated compounds. Collectively, these findings support the favorable drug-like, pharmacokinetic, and predicted safety profiles of T₁–T₅ and justify their further evaluation as potential therapeutic candidates.

Table 5: Drug likeness screening of T₁₋₅ by pkCSM

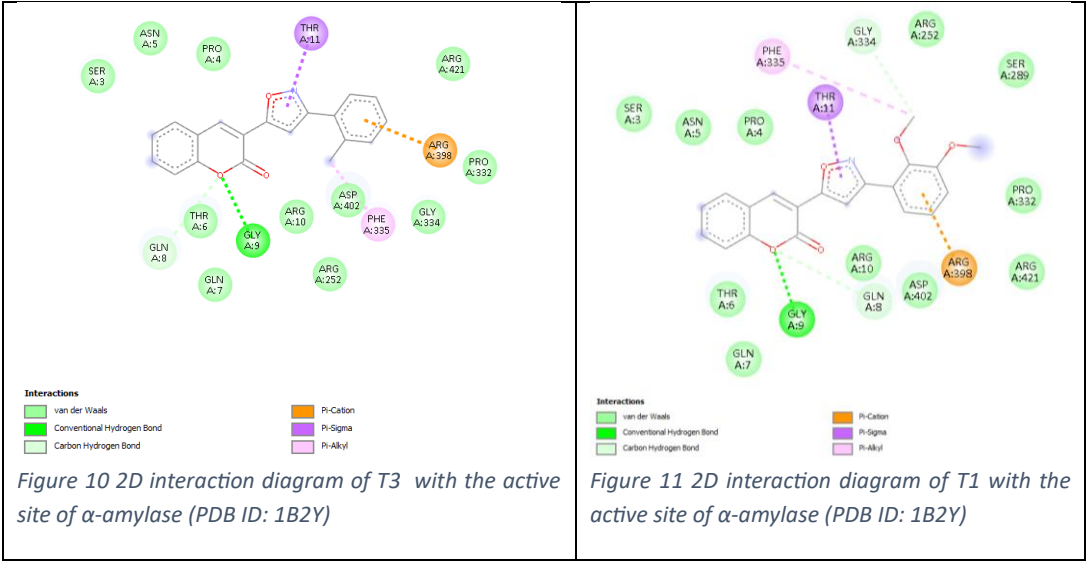
Compound code	CaCO ₂ permeability	Intestinal absorption	Skin permeability	P-glycoprotein substrate	P-glycoprotein inhibitor	BBB permeability	Volume of distribution (VDss)	CYP3A4 substrate	CYP3A4 inhibitor	CYP2D6 inhibitor	CYP2C9 inhibitor	CYP2C19 inhibitor	Total clearance	Renal OCT2 substrate	AMES toxicity	hERG inhibition	Hepatotoxicity	LD ₅₀	Skin sensitization
T ₁	0.92	96.6	-2.7	Yes	No	-0.5	0.04	Yes	No	No	Yes	Yes	-0.0	Yes	No	No	Yes	2.10	No
T ₂	0.63	94.9	-2.7	Yes	Yes	-0.7	0.08	Yes	Yes	No	Yes	yes	-0.07	Yes	Yes	No	Yes	2.20	No
T ₃	0.78	99.5	-2.7	Yes	Yes	-1.0	-0.1	Yes	Yes	No	Yes	Yes	0.03	No	Yes	No	No	2.55	No
T ₄	0.87	100	-2.7	Yes	Yes	-0.9	0.08	Yes	Yes	No	Yes	Yes	0.16	Yes	No	No	Yes	2.54	No
T ₅	0.31	100	-2.7	Yes	Yes	-0.7	0.24	Yes	Yes	No	Yes	Yes	0.01	No	No	No	Yes	2.67	No

3.1.5. Molecular Interaction Analysis

Molecular docking studies were performed using the Glide module of the Schrödinger Maestro suite to investigate the binding affinity and interaction patterns of the designed ligands with the selected protein targets. The docking results demonstrated that many compounds shown binding energies lower than those of the reference compounds. For the α -amylase enzyme, the reference compound acarbose showed a docking score of -7.85 kcal/mol. Among the designed compounds, docking scores revealed that T₃ exhibited strong binding affinities towards α -amylase, with a docking score of -8.3 kcal/mol. These compounds interacted with key residues such as Arg252, Pro4, Asp402, Gly334, and Thr11. The docking results indicated that compound T₃ exhibited the highest binding affinity, suggesting strong interactions with active site residues.

Table 6: Molecular docking scores of T₁₋₅ derivatives against α -amylase

S.No	Compound name	Docking score (Kcal/mol)	Interactions
1	T ₁	-7.94	Arg398, Phe335, Gly334, Thr11, Gly9, Pro4
2	T ₂	-7.88	Asp402, Arg398, Phe335, Thr11, Pro4, Gly9
3	T ₃	-8.3	Arg252, Pro4, Asp402, Gly334, Thr11
4	T ₄	-7.74	Arg10, Phe335, Gly334, Thr11, Asp402, Arg252
5	T ₅	-7.85	Gly9, Asp402, Thr11, Gly334, Phe335
6	Acarbose	-7.85	Asp197, Glu233, Asp356



3.2. Synthetic Investigation

3.2.1. Physical characterization

All the 5 new compounds T₁₋₅ obtained through the scheme were synthesized with good yield and evaluated by their physical characterization (melting point and TLC).

Table 7: Physical characterization of T₁₋₅ derivatives

S.NO	Compound code	R	R ¹	Molecular formula	Molecular weight	% yield	Melting Point C	Rf value
1	T ₁	H	H	C ₁₉ H ₁₅ N ₃ O ₂ S	341.4	67%	177°C	0.56
2	T ₂	Cl	H	C ₁₉ H ₁₄ ClN ₃ O ₂ S	383.8	76%	172°C	0.42
3	T ₃	NO ₂	H	C ₁₉ H ₁₄ N ₄ O ₄ S	394.4	87%	188°C	0.66
4	T ₄	OCH ₃	OCH ₃	C ₂₁ H ₁₉ N ₃ O ₄ S	409.4	46%	166°C	0.43
5	T ₅	OH	OCH ₃	C ₂₀ H ₁₇ N ₃ O ₄ S	354.3	57%	169°C	0.77

3.3. Spectral Characterization

The structures of synthesized compounds during the present investigation were established on the basis of chemical data, IR, UV, PMR and MASS spectra data.

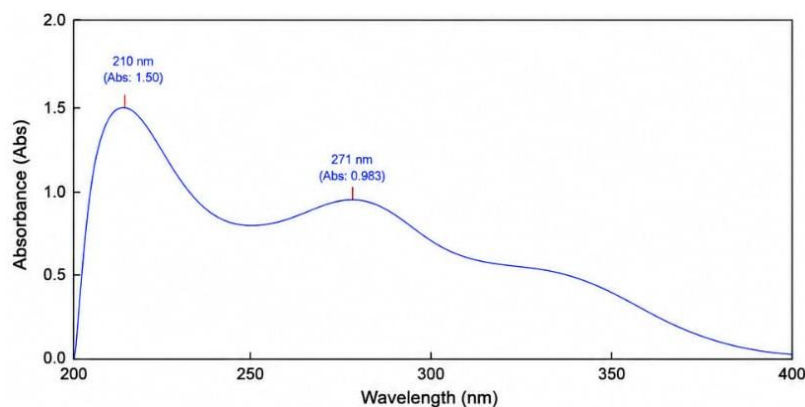


Figure 12 UV spectra of T3

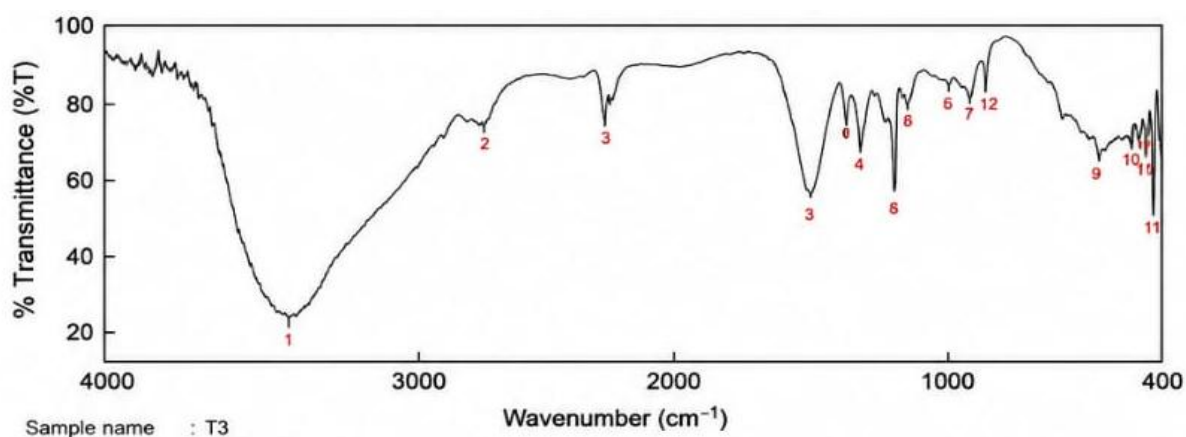


Figure 13 IR spectra of T3

3.4. Biological Evaluation

Procedure:

From 1 mg/ml stock solution, different concentrations (5-500 $\mu\text{g/ml}$) of coumarin derivatives were prepared by adding a few drops of dimethylsulfoxide and the volume made up with water. About 500 μl of α -amylase (0.5 $\mu\text{g/ml}$) was added and was incubated for 10 minutes at room temperature. Then, 500 μl of 1 % starch solution was added and incubated for another 10 minutes. After that, 1 ml of the coloring agent was added to the reaction mixture and heated in a boiling water bath for 5 minutes. After cooling, 10 ml of distilled water was added for dilution. To measure the absorbance of the colored extracts, a blank was prepared for each set of concentrations of test sample by replacing the enzyme solution with buffer. Control incubations representing 100% enzyme activity were prepared by replacing the test drug with water. The absorbance was then measured at 540 nm. The α -amylase inhibition was expressed as percentage of inhibition, and the IC_{50} values were determined by linear regression plots with varying concentrations of synthesized coumarin against percentage inhibition. ^[17]

All newly synthesized coumarin-based pyrazole (T₁–T₅) derivatives were evaluated for *in vitro* α -amylase inhibitory activity at concentrations ranging from 5–500 $\mu\text{g/mL}$, using acarbose as the reference drug. All compounds exhibited a concentration-dependent increase in inhibition, and IC₅₀ values were determined from inhibition–concentration plots. Among the pyrazole derivatives, T₁ and T₃ demonstrated the most potent α -amylase inhibitory activity (IC₅₀ = 24 and 25 $\mu\text{g/mL}$, respectively), comparable to acarbose, while T₂ exhibited moderate activity (IC₅₀ = 35 $\mu\text{g/mL}$) and T₄ and T₅ showed the least inhibition (IC₅₀ = 75 and 150 $\mu\text{g/mL}$). Overall, compounds T₁ and T₃ emerged as the most promising α -amylase inhibitors in the synthesized series.

Table 7: *In vitro* assay values of T₁₋₅ derivatives against α -amylase enzyme

Compound code	5 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$	25 $\mu\text{g/ml}$	50 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$	400 $\mu\text{g/ml}$	500 $\mu\text{g/ml}$	IC ₅₀ $\mu\text{g/ml}$
T ₁	34.00	42.50	55.30	61.00	64.00	71.00	79.00	82.03	24
T ₂	24.54	35.00	46.81	49.09	65.00	75.00	85.00	88.00	35
T ₃	33.75	41.25	54.50	60.41	68.75	77.50	80.16	85.40	25
T ₄	19.58	32.91	37.30	45.33	54.66	65.50	76.25	79.50	75
T ₅	21.96	27.84	30.98	40.39	46.60	52.54	56.47	66.80	150
Standard (Acarbose)	27.28	46.17	52.05	63.34	73.11	79.24	82.21	94.01	12

4. Conclusion

The present study established pyrazole-coumarin hybrids as promising α -amylase-targeting antidiabetic scaffolds through an integrated computational, synthetic, and biological approach. Five derivatives (T₁–T₅) were successfully synthesized through a three-step strategy and characterized using physicochemical, TLC, UV, IR, PMR, and mass spectrometric analyses.

Lead identification involved virtual screening of 50 ZINC compounds against human pancreatic α -amylase (PDB ID: 1B2Y), followed by molecular docking of the synthesized derivatives, which demonstrated favorable interactions within the enzyme active site. DFT analysis at the B3LYP/6-311G(d,p) level showed a reduced HOMO–LUMO gap from 4.57 eV for coumarin to 4.03 eV for pyrazole–coumarin, indicating enhanced electronic softness and charge-transfer potential. MEP and Mulliken charge analyses further demonstrated significant redistribution of electron density following pyrazole incorporation.

Drug-likeness and ADMET profiling indicated that all derivatives complied with Lipinski's Rule of Five and exhibited generally acceptable predicted pharmacokinetic and toxicity profiles. *In vitro* α -amylase inhibition studies using porcine pancreatic α -amylase and acarbose as the reference inhibitor demonstrated concentration-dependent activity, with T₁ and T₃ showing the highest inhibitory potential.

Overall, the combined findings from virtual screening, docking, DFT analysis, ADMET prediction, synthesis, characterization, and *in vitro* evaluation support the pyrazole–coumarin framework as a promising lead for further SAR studies, optimization, mechanistic investigations, and *in vivo* evaluation toward the development of potential agents for controlling postprandial hyperglycemia.

References

1. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract.* 2018;138:271-281.
2. Baena-Díez JM, Peñafiel J, Subirana I, Ramos R, Elosua R, Marín-Ibañez A, et al. Risk of cause-specific death in individuals with diabetes: a competing risks analysis. *Diabetes Care.* 2016;39(11):1987-1995.
3. Kitabchi AE, Umpierrez GE, Miles JM, Fisher JN. Hyperglycemic crises in adult patients with diabetes. *Diabetes Care.* 2009;32(7):1335-1343.
4. Kumar S, et al. Synthesis and biological evaluation of isoxazole-coumarin hybrids as potential antidiabetic agents. *Bioorg Chem.* 2018;77:451-460.
5. Karrouchi K, et al. Pyrazole-based derivatives as potential therapeutic agents: a review. *Molecules.* 2018;23(1):134.
6. Venugopala KN, et al. Synthesis and biological activity of coumarin derivatives. *Biomed Rep.* 2013;1(2):189-196.
7. Salehi B, et al. The therapeutic potential of coumarins as antidiabetic agents. *Front Pharmacol.* 2019;10:1147.
8. Chikhale RV, et al. Identification of mycobacterial thymidylate kinase inhibitors: a comprehensive pharmacophore, machine learning, molecular docking, and molecular dynamics simulation studies. *Mol Divers.* 2024;28:1947-1964.
9. Alp M, et al. Drug screening of α -amylase inhibitors as candidates for treating diabetes. *J Cell Mol Med.* 2023;27:2249-2260.
10. Mustapha B, et al. Computational approach: 3D-QSAR, molecular docking, molecular dynamics simulation investigations, drug-likeness, and DFT score evaluation of a potential novel and retrosynthesis of some TR-H derivatives as *Streptococcus pneumoniae* drug. *Karbala Int J Mod Sci.* 2025;11:328-339.
11. Mateev E, et al. Repurposing of FDA-approved drugs as dual-acting MAO-B and AChE inhibitors against Alzheimer's disease: an *in silico* and *in vitro* study. *J Mol Graph Model.* 2023;122:108471.
12. Abbasi SA, et al. Synthesis of modified 1,3,4-thiadiazole incorporating substituted thiosemicarbazide derivatives: elucidating the *in vitro* and *in silico* studies to develop promising anti-diabetic agent. *Results Chem.* 2024;8:101556.
13. Panigrahi D, Sahu SK. Computational approaches: atom-based 3D-QSAR, molecular docking, ADME-Tox, MD simulation and DFT to find novel multi-targeted antitubercular agents. *BMC Chem.* 2025;19:39.
14. Chalkha M, Akhazzane M, Moussaid FZ, Daoui O, Nakkabi A, Bakhouch M, et al. Design, synthesis, characterization, *in vitro* screening, molecular docking, 3D-QSAR, and ADME-Tox investigations of novel pyrazole derivatives as antimicrobial agents. *New J Chem.* 2022;46:2747-2760.
15. Adegboye AA, Khan KM, Salar U, Aboaba SA, Chigurupati S, Fatima I, et al. 2-Aryl benzimidazoles: synthesis, *in vitro* α -amylase inhibitory activity, and molecular docking study. *Eur J Med Chem.* 2018;150:248-260.

16. Makarand A, Amisha V, Yusuf AV. Synthesis and evaluation of chalcone derivatives for its alpha amylase inhibitory activity. Org Chem Indian J. 2014;10:192-204.
17. Thalapaneni NR, Chidambaram KA, Ellappan T, Sabapathi ML, Mandal SC. Inhibition of carbohydrate digestive enzymes by *Talinum portulacifolium* (Forssk) leaf extract. J Complement Integr Med. 2008;5(1).