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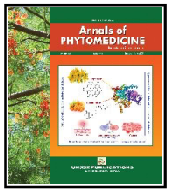

Original Article : Open Access

Design and synthesis of isoxazole-based chromene-2-one derivative active against diabetic inflammation

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Abstract

A series of coumarin-based isoxazole derivatives, 5(a-e), were synthesized and characterized through spectral analysis. Structural confirmation was achieved *via* IR and NMR spectroscopy, where key spectral features validated the successful formation of the target compounds. Physiochemical property analysis using the ProTox online tool revealed that all synthesized compounds possess both hydrophobic and hydrophilic components, adhere to Lipinski's rule of five, and exhibit log P values below 5, suggesting favorable drug-likeness and pharmacological potential. Molecular docking studies were conducted using PyRx software to evaluate the interaction of compounds 5(a-h) with the PPAR-α receptor (PDB: 2P54). The docking scores ranged between –8.0 and –9.3 kcal/mol, indicating strong binding affinities. Among these, compound 5d exhibited the most favorable interaction, forming stabilizing electrostatic interactions within the active site. The binding pattern was compared with the standard drug lobeglitazone. The synthesized derivatives were further assessed for their biological activity. Anti-inflammatory potential was evaluated against the COX-2 enzyme, where all compounds exhibited inhibitory activity at 10 and 50 µg/ml. Notably, compound 5e demonstrated the highest inhibition (86.6%) at 50 µg/ml. Additionally, anti-diabetic activity was examined against α-amylase and α-glucosidase enzymes. All compounds displayed significant inhibition, with compound 5d showing the most potent activity. These findings suggest that the synthesized coumarin-based isoxazole derivatives possess promising anti-inflammatory and antidiabetic potential, warranting further pharmacological investigation.

1. Introduction

Diabetes mellitus is a common condition caused by an abnormal glucose metabolism. This condition disrupts the systems that govern the synthesis and efficiency of insulin, the most critical hormone for controlling glucose metabolism. Diabetes affects around 830 million individuals worldwide as of 2024 (Alemany, 2024). This number has risen substantially from 200 million in 1990. The prevalence is notably increasing in low and middle-income countries. Type 2 diabetes accounts for over 90% of all cases and is influenced by factors such as high body mass index, poor diet, and low physical activity . Diabetic inflammation is a key feature of diabetes and its complications (Oguntibeju, 2019; desai *et al.,* 2024). Several factors contribute to the increase in inflammation in diabetes, including hyperglycemia (high blood sugar), advanced glycation end products (AGE), oxidative stress, insulin resistance (insulin resistance, a hallmark of type 2 diabetes, causes an imbalance in lipid metabolism, leading to the accumulation of free fatty acids and pro-inflammatory mediators in tissues), adipose tissue dysfunction, chronic activation of the immune system, gut dysbiosis, oxidative and endoplasmic reticulum (ER) stress, lipotoxicity, mitochondrial dysfunction, genetic and epigenetic factors, coexisting conditions. While traditional anti-inflammatory drugs have limitations, newer therapies targeting specific inflammatory pathways, advanced metabolic drugs, and lifestyle interventions are transforming the management of diabetic inflammation (Nowotny *et al.*, 2015; Khanal *et al.*, 2024; Patil *et al.*, 2024). Ongoing clinical trials and emerging technologies promise further breakthroughs. Advanced treatments for diabetic inflammation focus on addressing chronic low-grade inflammation, a key driver of insulin resistance, beta-cell dysfunction, and complications such as cardiovascular and renal diseases (Rajesh *et al.*, 2023; Khanal *et al.*, 2024).

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Heterocyclic compounds are organic compounds that include at least one element other than carbon (a heteroatom) within their ring structure. The most prevalent heteroatoms are nitrogen (N), oxygen (O), and sulfur (S), but additional elements like phosphorus and selenium can also be found. Heterocyclic chemistry is a broad area having applications in organic synthesis, pharmaceutical chemistry, and industrial processes. Lobeglitazone is an aromatic ether that belongs to the thiazolidinedione class of drugs. It is synthesized from the rosiglitazone backbone by substituting the pyrimidine moiety and adding the para-methoxyphenol functional group at the fourth position. Similarly, isoxazole is a five-membered aromatic heterocyclic compound consisting of three carbon atoms, one nitrogen atom, and one oxygen atom in its ring structure. Its chemical formula is C_2H_2 NO, and the arrangement features an oxygen and a nitrogen atom adjacent to each other in the ring. Due to their diverse biological activities, isoxazole and its derivatives are widely studied and used in medicinal chemistry. It has been reported as antimicrobial activity, anti-inflammatory agents, anticancer activity, central nervous system (CNS) drugs, and antioxidant, antitubercular, and Antidiabetic activity (Khanal and Patil, 2022; Pinho, 2005). Its derivatives often have improved pharmacokinetics and reduced side effects compared to other heterocycles.

Peroxisome proliferator activated receptor α (PPAR-α) is a nuclear receptor and transcription factor that plays a crucial role in regulating lipid metabolism, energy homeostasis, and inflammation. It controls the expression of genes involved in fatty acid oxidation, lipid transport, and ketogenesis. It facilitates the breakdown of fatty acids for energy, particularly during fasting. Upon activation by ligands, PPAR-α forms a heterodimer with the retinoid x receptor. This complex binds to specific DNA sequences known as PPREs (PPAR response elements) in the promoter regions of target genes to regulate transcription. It is implicated in metabolic disorders such as diabetes, obesity, and non-alcoholic fatty liver disease. Due to their involvement, it has become an attractive target for therapeutic interventions to manage metabolic and inflammatory diseases. Other receptors responsible for diabetes mellitus include GPCRS (G-protein coupled receptor) and GPL-1 receptor. Metformin, sulfonylurea, and other commonly used diabetic medications. The most frequent adverse effects reported were tiredness, nausea, stomach discomfort, diarrhea, heart disease, difficulty breathing, allergic response, back pain, xerostomia, headache, an increased risk of fractures, renal illness, lack of appetite, eye problems, and weight gain (Janani and Kumari, 2015; Rajesh *et al.,* 2024; Rajesh *et al.,* 2024; Dheeraj *et al.,* 2024; Dwivedi and Shastry, 2023).

In the current study, *in silico* and *in vitro* studies have been conducted to elucidate the antidiabetic ability of synthesized compounds the clinically prescribed antidiabetic drugs were used for comparing the relative efficacy during experiment.

2. Materials and Methods

2.1 Molecular docking

The 2D structures of all designed ligands were created using ChemDraw 20.0, and their canonical SMILES were generated. These were then converted into PDB format using Discovery Studio Visualizer 2019 (BIOVIA Discovery Studio Visualizer; https://discover.3ds.com/discovery-studio-visualizer-download). Ligand energy minimization was carried out using the MMFF97 force field, and the structures were subsequently converted into PDBQT format for docking. Target protein structures were retrieved from the UniProt database (https://www.uniprot.org/) to identify available structures in the Protein Data Bank (RCSB; https://www.rcsb.org/), specifically selecting the crystal structure of PPAR alpha (PDB ID: 2P54). The receptor 2P54, with a resolution of 1.79 Å, contained a single chain (Chain A) with a sequence of 267 amino acid residues and no mutations. Molecular docking studies were conducted using the PyRx virtual screening tool (Ashtekar *et al.,* 2023; Dwivedi and Shastry, 2024; Bharat *et al.*, 2023).

2.2 Prediction of ADME properties

Assessing the ADME properties of molecules provides preliminary insights into their drug-like potential. This process predicts key physicochemical characteristics and evaluates lipinski's rule of five parameters, including hydrogen bond donors, hydrogen bond acceptors, and log P values, which are crucial for biological activity. In this study, the ADME properties of the selected docked ligands were analyzed using the ProTox 3.0 web portal (tox.charite.de/protox3/) (Ismail and Uzairu, 2019; Khanal *et al.*, 2024; Banerjee *et al.*, 2018).

2.3 Synthetic method

Step-I: Synthesis of 7-hydroxy-4-methyl coumarine

Take 37 ml of concentrated sulfuric acid in a large beaker and keep the temperature between 4 and 50°c. Transfer 9.2 g of powdered resorcinol to 10.96 ml of ethyl acetoacetate, stirring constantly, until a full solution is achieved. Add the solution gently to the concentrated sulphuric acid in the preceding solution, stirring for 30 min. Pour the reaction mixture into 300 ml of cold water. Recrystallize methanol. 7-Hydroxy-4-methycoumarin is purified in sodium hydroxide pallets (10 g) and dissolved in water (100 ml). Now, add diluted hydrochloric acid until a precipitate form and the solution turns acidic. The filtrate is next filtered and collected on filter paper, where it is decolorized with methanol and charcoal (Palaniappan and Shekhar, 2004).

Step-II: Procedure for the synthesis of (E)-7-hydroxy-4-2H-chromen-2-one

2(a-e)

0.030 mole of 7-hydroxy-4-methycoumarin and 0.030 mole of sub aromatic aldehyde were dissolved in 30 ml of dry chloroform. The catalytic quantity of piperidine is added to the reaction mixture and refluxed for 1.5 h. The chloroform is distilled off and the residue is washed with methanol (Palaniappan and Shekhar, 2004).

Step-III: Procedure for the preparation of(E)-7-(4 acetylphenyl)-4-2H-chromen-2-one 3(a-e)

A round-bottom flask was filled with a mixture of compounds (3.7 g, 0.014 mole), anhydrous potassium carbonate (1.93 g, 0.014 mole), and a small amount of KI as a catalyst. The mixture was stirred for 20 minutes in dry acetone (25 ml), after which 4-chloroacetophenone (1.7 ml, 0.014 mol) was added. The reaction was stirred for 48 h at 80°C, with progress monitored by TLC. After cooling to room temperature, the mixture was filtered through a Celite pad and washed with 60 ml of acetone. The solvent was removed, and the crude product was recrystallized from diethyl ether to obtain the desired compound (Palaniappan and Shekhar, 2004).

Step-IV: General procedure for the synthesis of 7-(4-(3-phenylacryloly phenyl)-4-(E)-2H-chromen-2-one 4(a-e)

A total of 4.0 g (0.014 mole) of compound 3 was dissolved in 50 ml of DMF and stirred continuously at room temperature. Subsequently, a 40% KOH solution was introduced to the reaction mixture, which was kept stirring for 24 h at room temperature. After the reaction was complete, the mixture was poured over crushed ice and neutralized with HCl. The product that formed was collected by filtration, rinsed with water, dried, and recrystallized from ethanol to yield the final compound. In the same manner, compounds 4(a-e) were also synthesized (Palaniappan and Shekhar, 2004).

Step-V: Procedure for the synthesis of (E)-7-(4-(3-phenylisoxazole-5-yl)phenyl)-4-2H-chromen-2one 5(a-e)

After dissolving compound 4 (4.0 g, 0.010 mole) in 25 ml of ethanol, hydroxylamine hydrochloride (0.69 g, 0.010 mole) was added to the mixture. After that, the reaction mixture was mixed with a 40% KOH solution and refluxed for 10 hrs. TLC was used to track the reaction's development. Following the reaction's conclusion, the mixture was cooled, added to crushed ice, and neutralized with HCL. The alcohol to compound product was separated, filtered, cleaned with water, dried, and recrystallized. Likewise, other compounds 5(a-e) were produced (Jung *et al.*, 2024; Al Abdeen and Mustafa, 2022).

2.4 *In vitro* anti-inflammatory activity

2.4.1 Cyclooxygenase (COX-2) inhibition

A colorimetric human COX-2 inhibitor screening test kit (Sigma-Aldrich) was used to detect COX-2 inhibition. In accordance with the manufacturer's procedure, the identified compounds were employed in inhibition experiments. A Varioskan Flash microtitre plate reader with SkanIt software 2.4.3 RE was used to measure the absorbance at 415 nm (Ambati *et al.*, 2017).

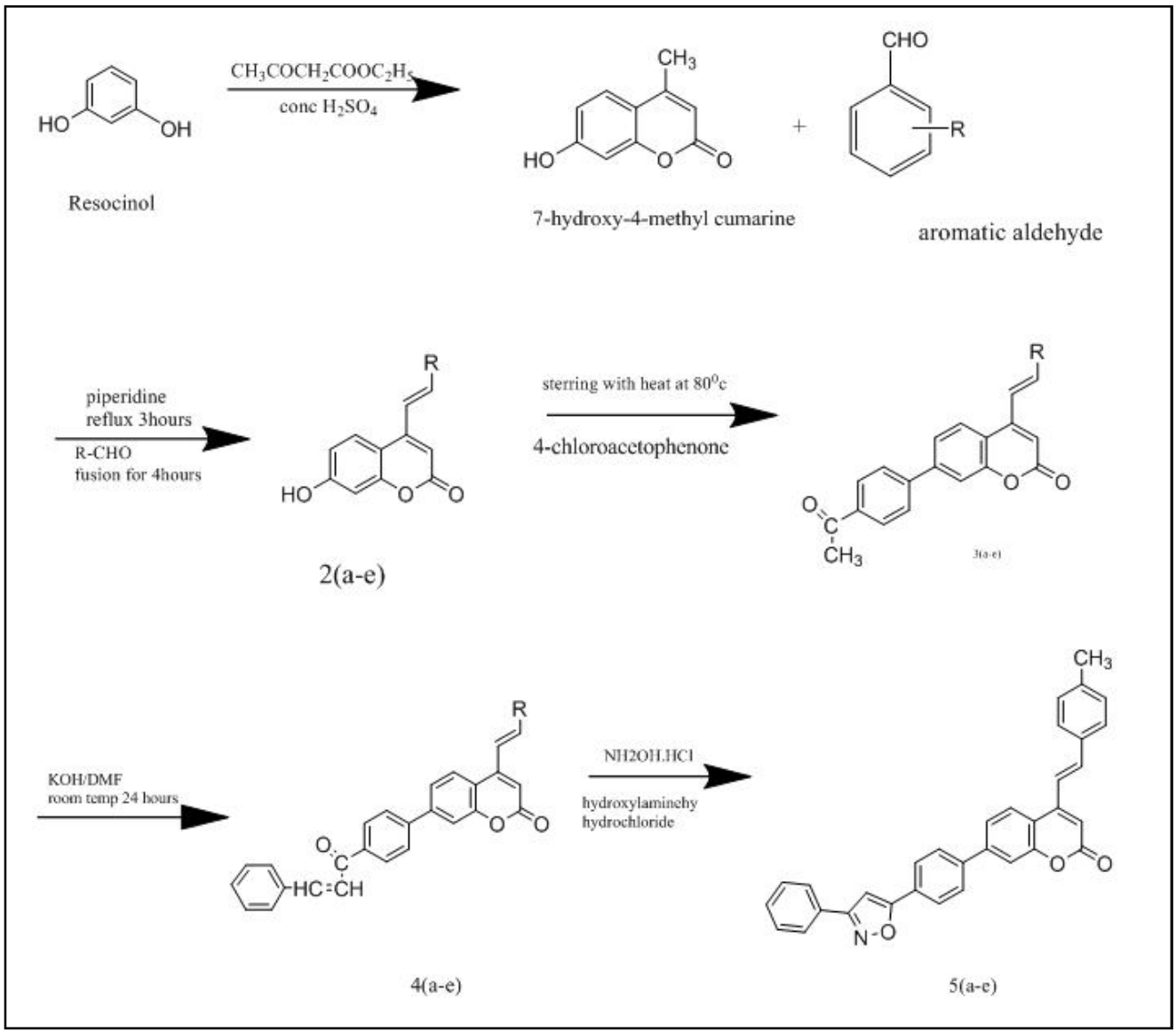

Figure 1: Scheme for synthesis of novel isoxazole based chromen-2-one derivatives.

2.5 *In vitro* antidiabitic activity

2.5.1 Alpha Amylase inhibition assay

α-Amylase solution of concentration 1mg/ml was prepared. The DNSA reagent was prepared by the addition of 80 ml 0.5 m NaOH, 2.1 g 3,5-Dinitrosalicylic acid, and 30 g Sodium potassium tartrate, which was heated at 75°C. 200 µl sodium phosphate buffer (pH 6.9), 20 µl of the enzyme, and 500 µl test compounds of various concentrations (10-50 µg/ml) were mixed jointly in test tubes and incubated at 25°C for 10 min. 200 µl of 1% starch solution and 400 µl of DNSA reagent were added to this solution, and the mixture was again incubated at 25°C for 10 min. The test tubes were cooled to room temperature. 5 ml of distilled water was added to all the test tubes. The absorbance of the solution was quantified at 540 nm (Agrawal and Gupta, 2016; Bindu *et al.*, 2024).

2.5.2 Alpha-glucosidase inhibition assay

50 µl of phosphate buffer (100 mm, pH=6.8), 10 µl of α-glucosidase solution (1 U/ml), and 20 µl of different concentrations of test compounds (10-50 µg/ml) were taken in a 96 well plate and incubated at 37°C for 15 min. 50 µl p-nitrophenyl α- glucopyranoside solution (5 mm) was transferred to the above solution and incubated further at 37°C for 20 min. Sodium carbonate (0.1 M) was added to stop the reaction. The absorbance was then observed at 405 nm. The percentage inhibition was calculated and reported (Nair *et al.*, 2013).

3. Results

3.1 Molecular docking

The designed compounds have been docked against PPAR alpha receptor the binding affinities have been noticed in the range of – 8.0 to – 9.3 as shown in Table 1. The interaction with amino acid has shown in Figure 2.

Table 1: Molecular docking scores of designed compounds with PPAR alpha receptor

Comound	Binding affinity
5a	– 9.2
5b	– 8.8
5c	– 8.9
5d	– 9.3
5e	– 9
Lobeglitazone	– 8

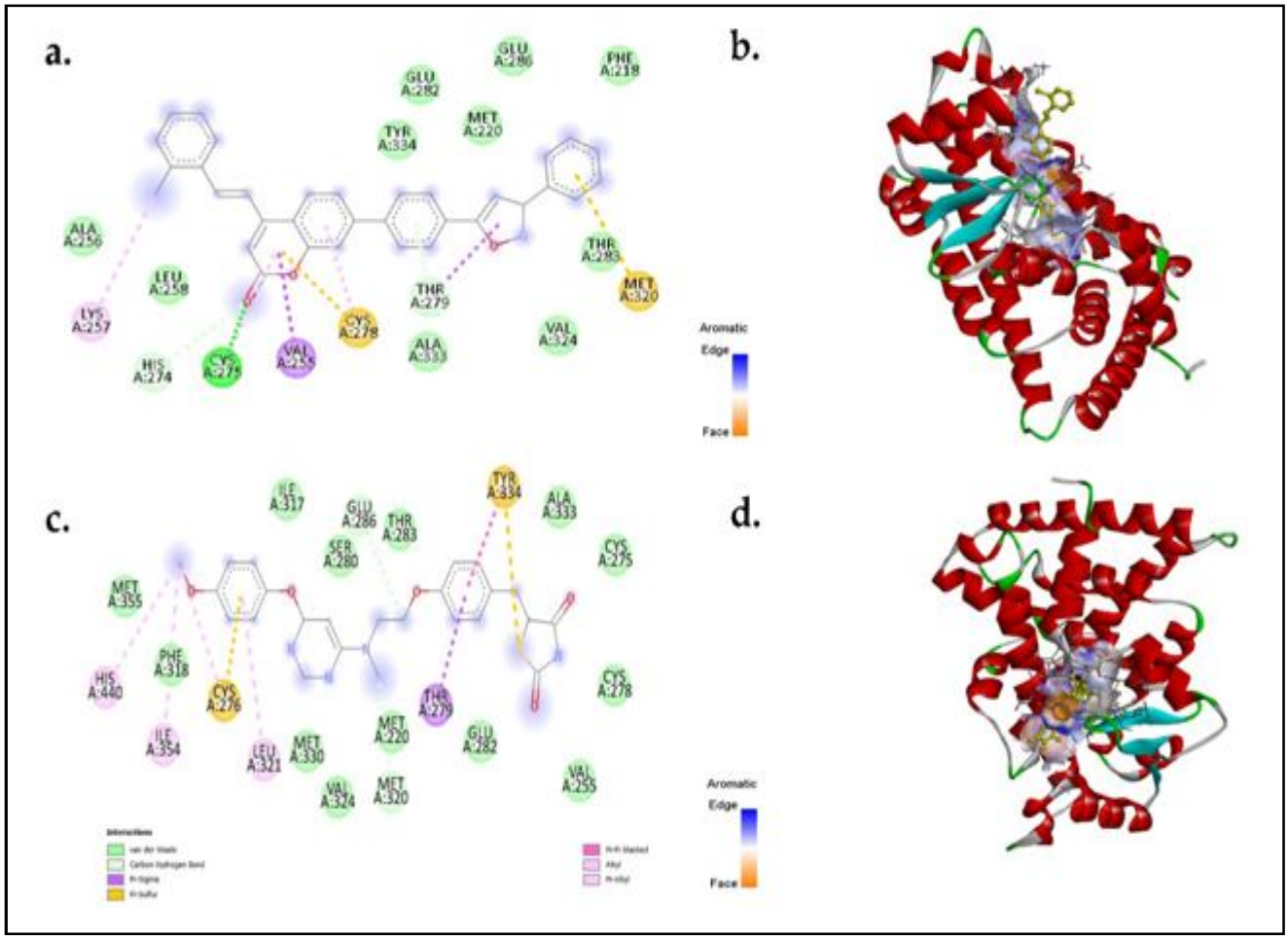

Figure 2: 2D and 3D interaction of (a), (b) 5d (c), (d) Lobeglitazone with 2P54.

3.2 ADME properties

The designed and synthesized compounds physicochemical and druggable parameters predicted using ProTox online tool, where the molecular weight under the range of 480-513, the number of H-bond donors, number of H-bond acceptors, and partition coefficient (QPlogP (o/w)) under the prescribe range which over all states the violation of lipinski's rule of five and its depicted in Table 2.

Table 2: Predicted ADME profile of compounds

Comp. code	Molecular weight	Donor H	Acceptor H	QPlogP (o/w)	Rule of five
5a	481.54	0	2	8.26	0
5b	497.54	0	3	7.96	0
5c	495.52	0	3	7.76	0
5d	501.96	0	2	8.61	1
5e	512.51	0	3	8.38	1
Lobeglitazone	480.54	1	10	4.02	0

3.3 Synthesis

The synthesized compounds using scheme as depicted in Figure 1. All the physicochemical parameters have been showed in Table 3.

Compound(E)-4-(4-methylstyryl)-7-(4-(3-phenylisoxazol-5-yl)phenyl)-2H-chromen-2-one (5a)

White crystalline powder (Ethanol), IR (KBr, cm^{-1}): 2961.00, 2913.12(C-H; aromatic), 1726.70 (C=O), 1514.23 (C=C), 1200.26 (C-O-C); ^{1}H NMR (DMSO-d_6): 5λ 2.27 (3H, s), 6.06 (1H, s), 7.14-7.78 (13H, 7.21 , 7.23 (ddd,7.31 (s), 7.38 (dd, 1.8 Hz), 7.43 (d), 7.55 (ddd, 7.56 (tdd), 7.62 (dddd), 7.67 (dd), 7.72 (dd)), 7.93 (2H, ddd), 8.09-8.24 (4H, 8.15 (dtd,), 8.18 (ddd). m/z: 481.17.

Compound (E)-4-(4-methoxylstyryl)-7-(4-(3-phenylisoxazol-5-yl) phenyl)-2H-chromen-2-one (5b)

White crystalline powder (Ethanol), IR (KBr, cm^{-1}): 2958.67, 2915.13 (C-H; aromatic), 1726.76 (C=O), 1512.05 (C=C), 1201.92 (C-O-C) ^{1}H NMR (DMSO-d_6): λ 3.79 (3H, s), 5.88 (1H, s), 7.04-7.21 (3H, 7.11 (d), 7.15 (ddd)), 7.26-7.77 (10H, 7.31 (s), 7.37 (dd), 7.41 (d), 7.46 (ddd), 7.56 (tdd), 7.62 (dddd), 7.66 (dd), 7.71 (dd), 7.92 (2H, ddd), 8.09-8.24 (4H, 8.15 (dtd), 8.17 (ddd). m/z: 497.16.

Compound (E)-7-(4-(3-phenylisoxazol-5-yl) phenyl)-4styryl-2H-chromen-2-one (5c)

White crystalline powder (Ethanol), IR (KBr, cm^{-1}): 2919.20, 2856.59 (C-H; aromatic), 1731.57 (C=O), 1509.80 (C=C), 1204.18 (C-O-C). ^{1}H NMR (DMSO-d_6): λ 6.20 (1H, s), 7.18-7.81 (11H, 7.25 (d), 7.38 (dd), 7.40 (s), 7.43 (d), 7.57 (tdd), 7.63 (dddd), 7.67 (dd), 7.73 (dd), 7.75 (ddd)), 7.87-8.24 (8H, 7.93 (ddd), 8.04 (ddd), 8.15 (dtd), 8.18 (ddd), 10.00 (1H, s).m/z: 495.15.

Compound (E)-4-(2-chlorolstyryl)-7-(4-(3-phenylisoxazol-5-yl) phenyl)-2H-chromen-2-one (5d)

White crystalline powder (Ethanol), IR (KBr, cm^{-1}): 2960.92, 2920.10 (C-H; aromatic), 1704.45 (C=O), 1506.63 (C=C), 1154.82 (C-O-c), 761.30 (Cl). ^{1}H NMR (DMSO-d_6): λ 6.20 (1H, s), 7.18-7.81 (11H, 7.25 (d), 7.38 (dd), 7.40 (s), 7.43 (d), 7.57 (tdd), 7.63 (dddd), 7.67 (dd), 7.73 (dd), 7.75 (ddd), 7.87-8.24 (8H, 7.93 (ddd), 8.04 (ddd), 8.15 (dtd), 8.18 (ddd), 10.00 (1H, s). m/z: 501.11.

Compound (E)-4-(2-nitrostyryl)-7-(4-(3-phenylisoxazol-5-yl) phenyl)-2H-chromen-2-one (5e)

White crystalline powder (Ethanol), IR (KBr, cm^{-1}): 2924.43, 2855.26 (C-H;aromatic), 1774.92 (C=O), 1603.77 (C=C), 1267.74 (C-O-C), 1331.21 (-NO_2). ^{1}H NMR (DMSO-d_6) : λ 6.31 (1H, s), 7.30-7.45 (3H, 7.37 (d), 7.38 (dd), 7.40 (s)), 7.50-8.00 (11H, 7.57 (tdd), 7.59 (ddd), 7.63 (dddd), 7.68 (ddd), 7.68 (d), 7.68 (dd), 7.75 (dd), 7.84 (ddd), 7.94 (ddd), 8.09-8.28 (5H, 8.15 (dtd), 8.18 (ddd), 8.22 (ddd). m/z: 512.14.

Table 3: Physicochemical data of coumarin based isoxazole derivatives 5(a-e)

Comp.Code	Ar	Molecular weight	Colour	Yield (%)	M.P (°C)	Rf value
5a	4-CH_3C-H_5	481.55	White crystalline powder	68.0	182-185	0.59
5b	4-$OCH_3C_6H_5$	495.53	White crystalline powder	64.0	184-187	0.58
5c	4-$CHOC_6H_5$	495.53	White crystalline powder	67.5	183-186	0.62
5d	2-ClC_6H_5	501.97	Yellow crystalline powder	62.0	181-185	0.60
5e	2-$NO_2C_6H_5$	512.52	Yellow crystalline powder	63.9	182-186	0.63

3.4 *In vitro* anti-inflammatory and antidiabetic activity

Compound 5(a-e) were assessed for its *in vitro* activity where COX-2 inhibtion results showen that the standard drug (indomethacin) have highest inhibion followed by 5e, 5c, 5b as it has showed in Figure 3. Similarly, antidiabetic activity has been tested and the results state that the compound 5c showed prominent inhibion against alpha amylase enzyme compare to standard drug (lobeglitazone) as depicted in Figure 4. The alpha glusidase assay result indicates that the compound 5d shows prominent inhibition as compare to the standard drug (lobeglitazone) the results depicted in Figure 5.

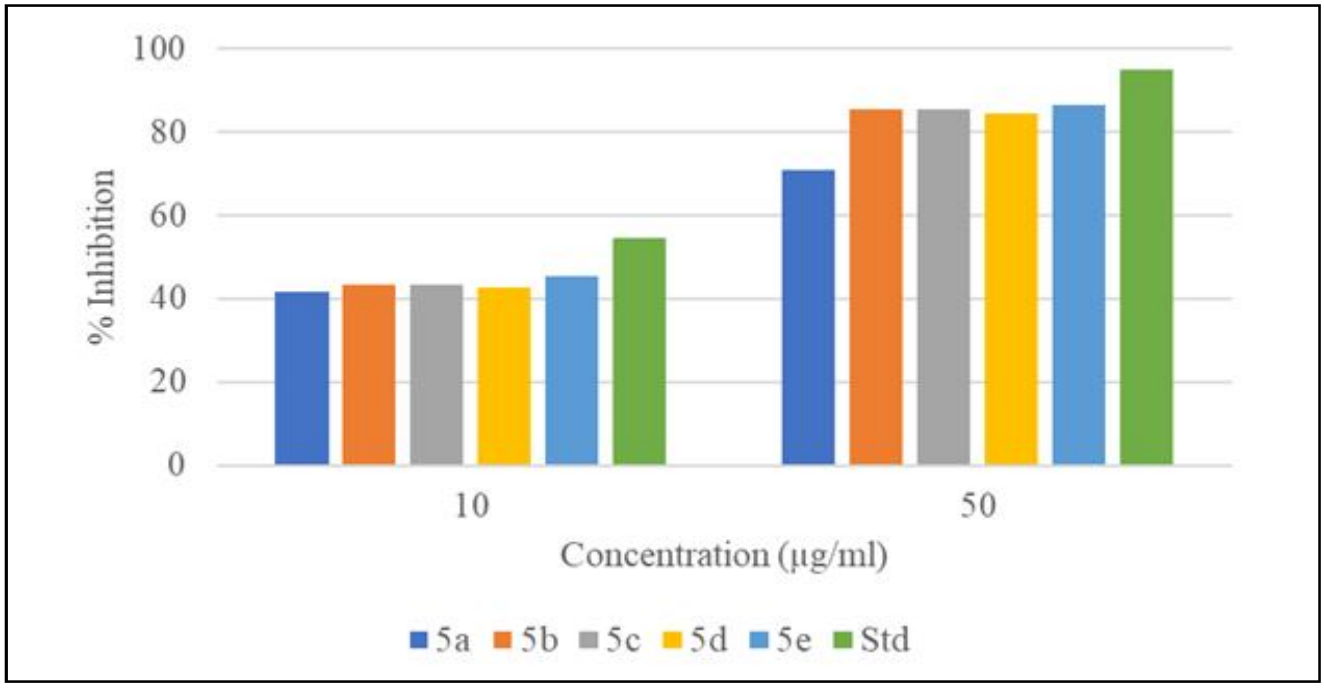

Figure 3: Percentage inhibition of cyclooxygenase (COX-2) enzyme by synthesized compounds 5(a-e).

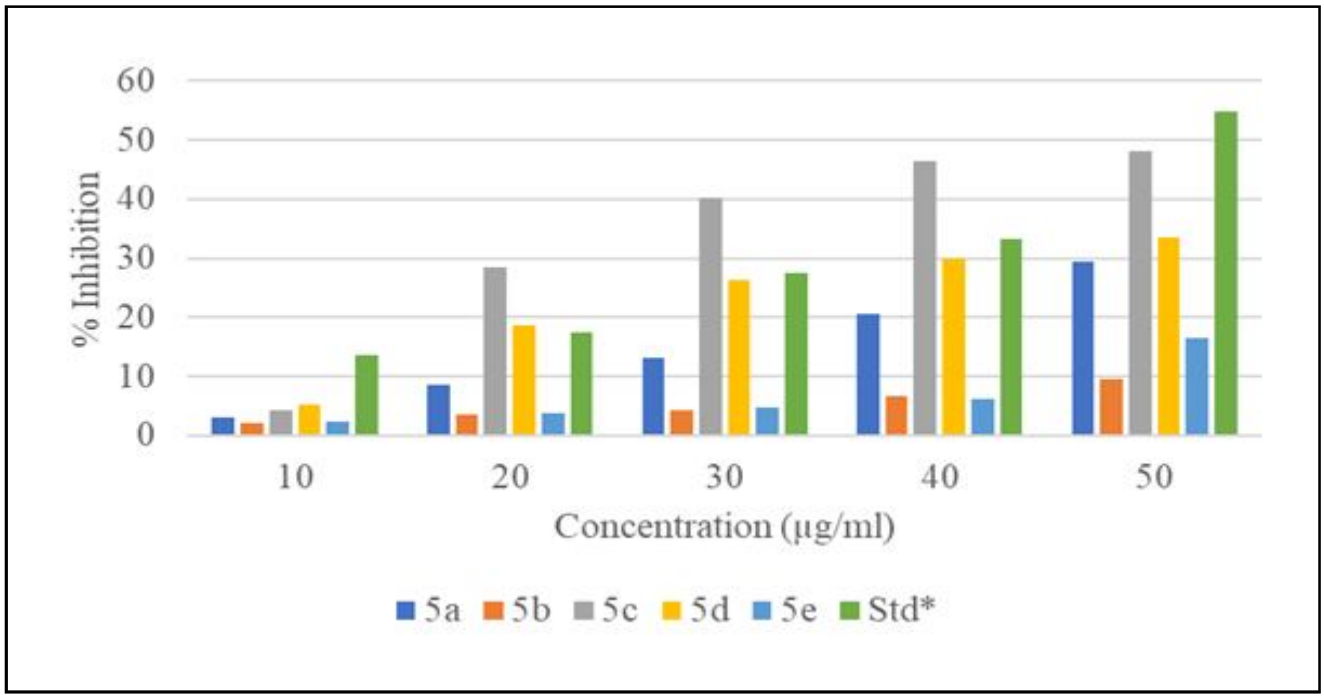

Figure 4: Antidiabetic activity of coumarin based isoxazole derivatives 5(a-e) by alpha- amylase inhibition method.

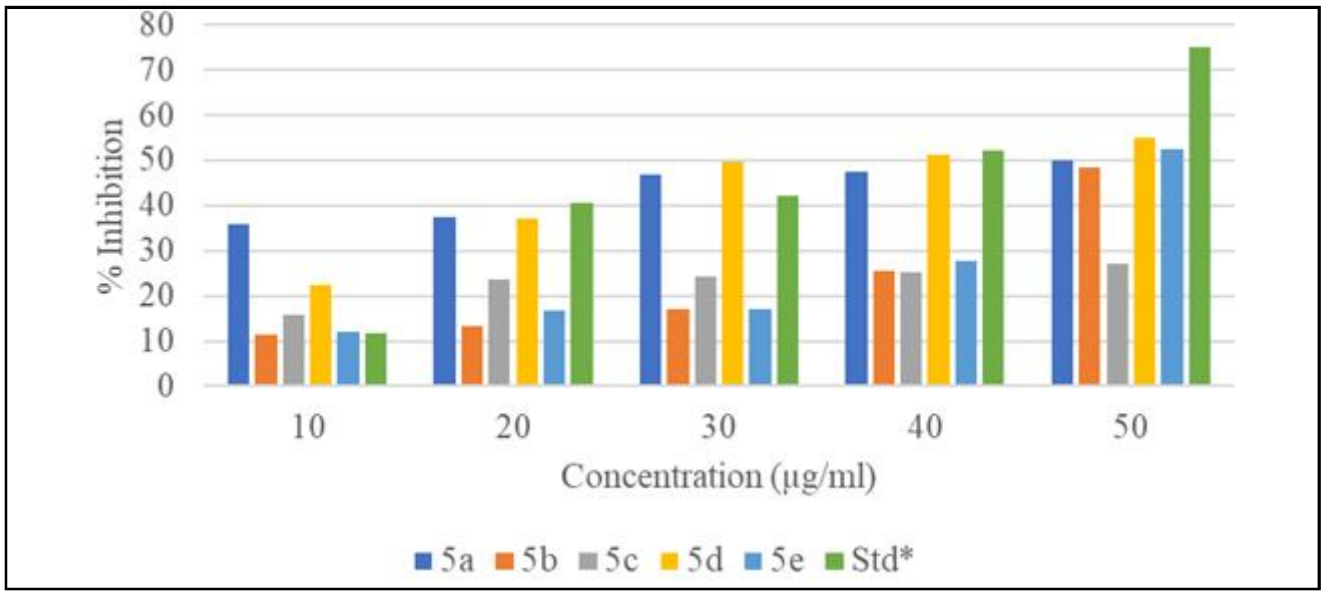

Figure 5: Antidiabetic activity of coumarin-based isoxazole derivatives 5(a-e) by alpha-glucosidase inhibition method.

4. Discussion

The final compounds 5(a-e) were subjected to physiochemical property analysis using the ProTox online tool. The results indicated that all compounds possess both hydrophobic and hydrophilic components. Furthermore, these compounds adhere to lipinski's rule of five, exhibiting a molecular weight of less than 500, a hydrogen bond donor count of less than 5, and a hydrogen bond acceptor count of less than 10. The log P values for these compounds were found to be less than 5, suggesting favorable drug-likeness. These physiochemical properties indicate that the compounds have the potential for pharmacological efficacy and may be considered for further clinical evaluation. The physical properties of compounds 5(a-e) and the ADME properties of compounds 5(a-h) are summarized in Table 2.

To gain deeper insights into the interaction patterns of the synthesized compounds 5(a-h) with the receptor "PPAR alpha bound with SRC1 peptide and GW735" (PDB Code: 2P54), molecular docking studies were conducted using PyRx software. The docking scores ranged between – 8 and – 9.3 kcal/mol, suggesting a strong binding affinity. Among these, compound 5d demonstrated the best fit within the active site, stabilized by various electrostatic interactions. The docking scores are detailed in Table 1, while the binding pattern and amino acid interactions are depicted in Figure 2(a, b). For comparison, the binding pattern of the standard drug lobeglitazone is illustrated in Figure 2(c, d).

The synthesis of coumarin-based isoxazole derivatives followed a designed scheme, as shown in Figure 1. The synthetic pathway involved five distinct steps to yield the final compounds. Structural confirmation of the intermediates and final compounds 5(a-e) was achieved through spectral analysis. Specifically, the IR spectrum of 5(a-e) displayed a peak at 1726.70 cm^{-1} (Ar C=O) (Rajesh *et al.*, 2024), confirming the presence of the carbonyl functional group. Additionally, the chemical shift at 7.38 ppm (1H, s) in the 1H NMR spectrum validated the formation of compound 5a. These spectral values were consistent with the designed structures, affirming successful synthesis.

The synthesized compounds 5(a-e) were evaluated for their anti-inflammatory activity against the COX-2 inhibitor (Rajita *et al.*, 2024; Singh *et al.*, 2024). The percentage inhibition was calculated and compared with the standard drug, lobeglitazone. All compounds exhibited inhibitory activity at concentrations of 10 and 50 µg/ml, with compound 5e demonstrating the highest inhibition (86.6%) at 50 µg/ml. These results, as presented in Figure 3, facilitated the establishment of a structure activity relationship for these compounds.

Additionally, the antidiabetic activity of the synthesized compounds was assessed against two key enzymes, alpha-amylase and alpha-glucosidase. The percentage inhibition was determined and compared with lobeglitazone. All compounds displayed inhibitory effects at concentrations of 10-50 µg/ml. Among them, compound 5d exhibited the most potent activity, as illustrated in Figure 4,5. These findings further highlight the therapeutic potential of these synthesized compounds.

5. Conclusion

The comprehensive analysis of the synthesized compounds 5(a-e) demonstrated promising physiochemical properties, including adherence to lipinski's rule of five and favorable drug-likeness, as determined using the ProTox online tool. Their balanced hydrophobic and hydrophilic components suggest their potential for pharmacological applications. Additionally, docking studies with the PPAR alpha receptor revealed strong binding affinities, with compound 5d exhibiting the most stable interactions. The successful synthesis of coumarin-based isoxazole derivatives through a well-defined synthetic pathway was confirmed using spectral data. Furthermore, biological evaluations established that compound 5e displayed the highest anti-inflammatory activity against the COX-2 inhibitor, while compound 5d exhibited potent antidiabetic effects against alpha-amylase and alpha-glucosidase. Overall, the study

highlights the therapeutic potential of these synthesized compounds for anti-inflammatory and antidiabetic applications. Future studies, including *in vivo* evaluations and clinical assessments, will be necessary further to validate their efficacy and safety for potential pharmaceutical use.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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