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Design, Synthesis, Characterization and Evaluation of Anti-Inflammatory Activity of Amino Thiazole Coumarin Derivatives

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Abstract

This research aims to design, synthesize, and assess the in-vitro anti-inflammatory activity of aminothiazole coumarin derivatives by reacting 3-bromoacetyl coumarin with thiourea in ethanol to give 5 substituted coumarin derivatives. The synthesized compounds were designed & evaluated to investigate their binding interactions with the protein target 3PGH. All the newly synthesized aminothiazole coumarin derivatives are characterised by IR, 1H NMR and mass spectroscopy. Compounds were evaluated for in-vitro anti-inflammatory activity by using membrane stabilization assay & BSA denaturation assay, measuring their ability to modulate key markers of inflammation. Compounds **5b**, **5c** and **5e** exhibited significant activity and Compound **5d** showed moderate activity by membrane stabilization assay. Compound 5c exhibited significant activity & Compound **5c** showed moderate activity by BSA denaturation assay. Compounds **5b** and **5c** are found to exert significant anti-inflammatory activity by membrane stabilization assay as well as BSA denaturation assay and can serve as potential compounds as anti-inflammatory agents to treat inflammation in future

Keywords: Anti-inflammatory; Coumarin; diclofenac; Cell-based assay; Molecular Docking

1. Introduction

Inflammation is a complex and dynamic response of the body's immune [1] system to harmful stimuli such as pathogens, damaged cells, toxins, or irritants. It is a protective mechanism designed to eliminate the initial cause of cell injury, clear out damaged cells, and initiate tissue repair. However, prolonged or chronic inflammation [2] can contribute to the development of various disorders, including cardiovascular [3] diseases, arthritis [4] and cancer [5]. Therefore, regulating inflammation [6] remains a significant focus in pharmaceutical research, with non-steroidal anti-inflammatory drugs (NSAIDs) being the primary treatment option. NSAIDs exert their anti-inflammatory [7], analgesic [8] and antipyretic [9] effects by inhibiting cyclooxygenase (COX) enzymes [10], specifically COX-1 and COX-2, which are responsible for the conversion of arachidonic acid to prostaglandins (PGs). Prostaglandins play a pivotal role in the inflammatory process [11] by promoting vasodilation, increasing vascular permeability, and recruiting immune cells to the site of injury or infection. While COX-1 is constitutively expressed in most tissues and is involved in maintaining normal physiological functions [12], such as gastric mucosa protection and platelet aggregation, COX-2 is induced during inflammation and is primarily responsible for the production of pro-inflammatory prostaglandins. Traditional NSAIDs non-selectively inhibit both COX-1 and COX-2, leading to effective relief of inflammation but also causing undesirable side effects [13], particularly in the GI tract and cardiovascular system. These side effects, including peptic ulcers [14], GI bleeding, renal dysfunction, and increased cardiovascular risk, have driven the search for safer, more selective anti-inflammatory agents. The limitations of traditional NSAIDs have sparked interest in the discovery and development of novel molecules with selective COX-2 inhibition or alternative anti-inflammatory mechanisms. Among the various classes of organic compounds explored for this purpose, heterocyclic compounds have shown tremendous potential due to their diverse chemical structures and biological activities. Coumarins, a class of heterocyclic compounds characterised by a benzopyrone structure, have gained particular attention for their broad spectrum [15,16] of pharmacological properties, including anticoagulant, anticancer [17,18], antimicrobial [19], antiviral [20,21] and anti-inflammatory [22-24] activities. Coumarins are naturally occurring compounds found in many plants, and their synthetic derivatives have been extensively studied for various therapeutic applications [25-29]. The anti-inflammatory activity of coumarins is of great interest because they have been shown to inhibit the synthesis of prostaglandins, similar to NSAIDs, without the severe side effects associated with traditional NSAID use. Additionally, coumarins possess antioxidant properties, which allow them to scavenge reactive oxygen species (ROS) and protect cells from oxidative stress-induced damage, a key factor in chronic inflammation. One of the primary areas of research on coumarins involves modifying their

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chemical structure to enhance their biological activity. The C-3 and C-4 positions of the coumarin core are particularly important for functionalization with various pharmacophoric groups, which can significantly impact their therapeutic potential. For instance, 3-substituted coumarins have been found to exhibit potent anti-inflammatory effects, making them attractive candidates for drug development. Previous studies have explored a range of modifications, including the introduction of carboxamides, sulfonamides, and halogenated groups, to improve the anti-inflammatory activity of coumarins. In this study, we focus on the goal of developing novel anti-inflammatory agents. Specifically, we aim to synthesise coumarin derivatives with substitutions at the 3-position to investigate their potential as selective anti-inflammatory and their ability to mitigate the adverse effects typically associated with conventional NSAIDs. The rationale behind this approach is based on the well-established structure-activity relationship (SAR) of coumarins, where substitutions at the 3-position have been shown to enhance anti-inflammatory activity while minimising toxicity. Once synthesized and characterized, the biological evaluation of these coumarin derivatives involves assessing their anti-inflammatory activity using in vitro assay. Additionally, molecular docking studies are performed to evaluate the binding affinity of the synthesized derivatives to 3PGH, providing insight into their potential as selective inhibitors. Our study aims to contribute to the growing body of research on coumarins by providing new insights into the design and development of substituted 3-coumarin derivatives as anti-inflammatory agents. By focusing on the modification of the coumarin structure at the 3-position, we hope to identify compounds with improved selectivity for anti-inflammatory activity, reduced side effects, and enhanced therapeutic potential. Flurbiprofen, a well-known NSAID, is used as the reference drug in the docking study to benchmark the anti-inflammatory activity of the synthesized coumarin derivatives. The synthesis and evaluation of novel coumarin derivatives represent a promising avenue for addressing this challenge. By leveraging the unique chemical properties of coumarins and their ability as an anti-inflammatory, this research seeks to develop new anti-inflammatory agents that offer the therapeutic benefits of traditional NSAIDs without the associated risk.

2. Experimental:

2.1. Material procurement:

Chemicals were procured from Sigma-Aldrich Merck. The identification and characterization of synthesized compounds were done on the basis of physical along with chemical and spectral analysis data such as Melting point (M.P.), Thin layer chromatography (TLC), Infrared Spectroscopy (IR), Nuclear Magnetic Resonance Spectroscopy (1H NMR), Mass Spectroscopy. The melting point was determined on Vego melting point apparatus (VMP PM, 32/1105) and was uncorrected. Thin Layer Chromatography done by using (G-60 mesh) silica gel. Reactions monitored by recoated TLC, it visualized either by iodine vapours chamber or by UV cabinet. Rf value calculation done for synthesized compounds using n-Hexane: ethyl acetate (8:2). The IR spectra of intermediate as well as final derivatives were recorded on Fourier Transform Infrared Spectrophotometer by using KBr as a standard (JASCO FTIR). Bruker spectrometer with CDCl3 as solvent used for 1H-NMR spectra. Tetramethylsilan (TMS) as an internal standard to indicate Chemical shifts in parts per million values. GC-MS were carried out by using BRUKER Compass Data Analysis 4.2.

2.2. Synthesised Compounds of Substituted 3-Coumarin Derivatives

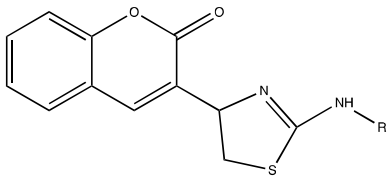
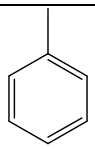
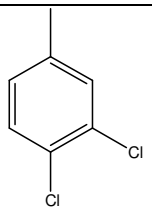
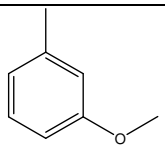
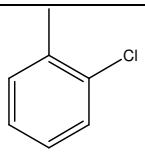


Figure 1: General structure of 3-Substitued Coumarin Derivative.

The synthesis scheme of five novel 3-substituted coumarin derivatives is shown in scheme 1. Figure 1 represents the structure of coumarin and its derivatives are listed in Table 1.

Table 1: Substitution table of synthesised Coumarin derivatives.

Sr. No	Compound Code	R
1	5a	-H
2	5b	
3	5c	
4	5d	
5	5e	

2.3. *in vitro* anti-inflammatory assays:

To perform the anti-inflammatory activity of synthesized substituted 3-coumarin derivatives, following two in-vitro anti-inflammatory assays were performed.

2.3.1. Membrane stabilization assay

The RBC membrane stabilization assay is a laboratory test used to assess a substance's or compound's capacity to safeguard and stabilize cell membranes. The experiment is carried out to evaluate a substance's cytoprotective or anti-inflammatory capabilities. In this test, Lower haemolysis or decreased haemoglobin release indicates a stronger membrane stabilization effect of the tested substance Assesses compounds' protective effects on cell membranes and their ability to reduce membrane damage caused by inflammation, oxidative stress, or other stressors. This method provides information on the therapeutic potential of drugs for diseases related to inflammation or membrane rupture

Procedure

The RBC membrane stabilization assay was performed to evaluate the anti-inflammatory activity of test samples. Whole blood was centrifuged at 2500 rpm for 5 minutes, the supernatant was discarded, and the packed cell volume was measured after washing and clearing the supernatant three times. A 40% suspension of the packed cells was reconstituted with phosphate-buffered saline. Different concentrations of test samples were prepared along with vehicle control and unheated control. RBC suspension was incubated with test samples at 54°C for 20 minutes, except for the unheated control. After cooling and centrifugation, the absorbance was measured at 560 nm, and the percentage of haemolysis and membrane stabilization were calculated. Diclofenac was used as the standard reference. The of haemolysis was using the following equation:

$$Precent\ inhibition\ = \{\left[1 - \frac{(Abs.of\ TSH\ -\ Abs.of\ TSUH\ )}{(Abs.of\ CSH\ -\ Abs.of\ TSUH)}\right]\} \times 100$$

Equation 1. The formula for percent inhibition for the membrane stabilization assay

Where;
Abs = Absorbance
TSH = Test sample heated
TSUH = Test sample unheated
CSH = Control sample heated

2.3.2. BSA Denaturation Assay

A laboratory test called the BSA (Bovine Serum Albumin) denaturation assay is used to evaluate a substance's or compound's capacity to stop the denaturation or unfolding of BSA protein under particular circumstances. It is frequently used to assess a compound's anti-inflammatory or protein-stabilizing effects. The BSA denaturation assay evaluates a substance's capacity to prevent the unfolding of BSA protein under denaturing conditions. It measures the degree of denaturation by observing changes in absorbance or fluorescence properties, indicating the substance's potential for anti-inflammatory or protein-stabilizing effects.

Procedure

The protocol was performed as per previously reported references. Different concentrations of the Test Samples were prepared along with on vehicle control. 200 µl of 1% w/v BSA in PBS (pH 6.4) was added to 1800 µl of test Samples. All tubes were incubated for 20 minutes at 37°C and then for 5 minutes at 70°C. Tubes were allowed to cool at RT. The turbidity was measured at 660 nm by using Multiskan SkyHigh Plate Reader. The Control represented 100% protein denaturation. Diclofenac was used as a reference standard. The of BSA was calculated using the following equation;

$$Precent\ Inhibition\ = \frac{(Abs.of\ control\ -\ Abs.of\ Test)}{Abs.of\ control} \times 100$$

Equation 2. Formula for BSA denaturation assay

2.4. Prediction of Drug Likeness and ADMET Properties:

The compounds were examined by application of Lipinski's Rule of Five, which declares of the molecule should not exceed more than 500 Daltons, that it can comprise a maximum of hydrogen bonds (05), hydrogen bond acceptors (10), rotatable bonds (10) and should be the number should not exceed more than above numbers. The tPSA characteristic was associated to the transportation of molecules passively across the blood brain barrier and membranes. All the compounds had tPSA less than 140, all studied compounds meet the Gastrointestinal absorption criteria. All the molecules had WLOGP values less than 5 (which indicates whether a chemical is harmful or not) (table 3). These chemicals were moderately easy to synthesize (less than 5 on the scale). This indicates that these molecules will be easy to produce in the laboratory and will be active, drug-like, and have oral bioavailability. The compounds showed excellent intestine absorption percent and it was above 90%. The BBB permeability (logBB) values were more than -2.5. Moreover, the Central Nervous System permeability (Log PS) values of all compounds were less than -3 which indicates that all the compounds can penetrate the CNS. It was noted that all the compounds were not toxic (table 4).

2.5. Selection of Target

The protein target 3PGH was specifically chosen for this study due to its established role in inflammatory processes, particularly as it relates to the cyclooxygenase pathway. While other proteins and pathways could potentially contribute to anti-inflammatory activity, targeting 3PGH aligns directly with the study's objective of evaluating the anti-inflammatory potential of coumarin-thiazole derivatives. Additionally, the availability of structural data for 3PGH facilitates precise computational docking studies, allowing for a detailed evaluation of ligand-protein interactions. Exploring other targets would require significant diversification of the study focus, which could dilute the depth of analysis on the synthesized compounds' interactions with this well-characterized protein. Future studies can expand on this work by considering alternative pathways and targets to provide a broader understanding of the anti-inflammatory mechanisms.

2.6. Selection of a Standard Drug

In the docking studies, flurbiprofen was selected as the reference compound due to its well-documented role as a non-steroidal anti-inflammatory drug (NSAID) with potent cyclooxygenase (COX) inhibitory activity. This property makes it an ideal comparator for evaluating the binding interactions of the synthesized coumarin derivatives with the target protein, 3PGH. Its established binding parameters provide a benchmark for assessing the relative binding affinities and potential of the novel compounds as COX inhibitors, aligning with the study's objective of identifying effective anti-inflammatory agents. For the in vitro biological assays, diclofenac was employed as the standard reference due to its recognized efficacy in membrane stabilisation and protein denaturation assays. These assays are widely utilized to evaluate anti-inflammatory activity, and diclofenac's dual COX inhibition and established performance in such experimental protocols make it a reliable control.

Using diclofenac ensures the results of the synthesized compounds can be directly compared to a clinically relevant NSAID, providing meaningful insights into their therapeutic potential. The rationale for employing different standards lies in their specific attributes relevant to the study phases. Flurbiprofen's molecular docking data ensure robust computational validation, while diclofenac's experimental performance supports practical biological activity assessment. This dual-standard approach offers a comprehensive evaluation of the synthesised derivatives' anti-inflammatory properties, enhancing the findings' reliability and translational potential

2.7. Docking Studies:

To investigate the binding interactions of the target compounds within the active site several tools were utilized, including Autodock 4.2.6, UCSF Chimera 1.15, Biovia Discovery Studio Visualizer, ACD/ChemSketch, and Open Babel GUI 3.1.1 for molecular docking and chemical analysis. Additionally, pkCSM, a web-based tool, was employed for ADMET predictions.

2.8. Receptor Preparation

A three-dimensional X-ray crystal structure of the target protein, 3PGH, was obtained from the PDB database. The protein structure was prepared by removing ions, water molecules, and the bound ligand. Polar hydrogens and Kollman charges were subsequently added to the receptor. The protein file was processed and converted into the appropriate format after applying charges using AutoDock Tool 4 (ADT). Grid parameter files (.gpf) were generated by setting the grid box dimensions to 40 × 40 × 40 with a grid spacing of 0.375 Å. The grid was centred on the receptor with coordinates X = 22.013690, Y = 0.252828, and Z = 52.794034. The grid log file (.glg) was then created, followed by the generation of a docking log file (.dlg) using input parameter files (.dpf). Docking simulations were performed using the Lamarckian genetic algorithm, enabling a thorough exploration of the conformational space. Each docking experiment consisted of 10 runs, with the maximum number of evaluations and generations set to 2,500,000 and 27,000 respectively. Following docking, the resulting receptor-ligand complexes were analysed using the AutoDock Tool to identify optimal binding poses. Discovery Studio was used for visualization and ranking of the docked interactions, with binding energy serving as the primary criterion for evaluating receptor-ligand affinity.

3. Results and Discussion

3.1. Docking Studies

The docking analyses of the synthesized amino coumarin-thiazole derivatives were conducted to elucidate their molecular interactions with the target protein, 3PGH, obtained from the Protein Data Bank (PDB). The primary objective was to evaluate the binding affinities and interaction profiles of these derivatives, thereby identifying potential candidates for the development of advanced anti-inflammatory agents. The docking results unequivocally demonstrated that all the synthesized derivatives exhibited significant binding affinities, with compounds 5b and 5c exhibiting superior interactions compared to the reference NSAID, flurbiprofen (Figures 2, 3, and 4). Compound 5c, characterized by dichloro substituents at the third and fourth positions of the aromatic ring, demonstrated the highest binding affinity among the synthesized compounds. This exceptional affinity is attributed to its ability to establish stabilizing hydrogen bonds with pivotal residues, such as arginine and tyrosine, within the active site of 3PGH. These interactions not only anchor the compound firmly within the binding pocket but also enhance its spatial orientation, optimizing the inhibitory potential. Similarly, compound 5e, containing a chloro substituent at the second position of the aromatic ring, displayed robust binding affinity, facilitated by the formation of two hydrogen bonds with key residues in the active site. Furthermore, compound 5b, which incorporates a phenyl substituent at the second position of the thiazole ring, exhibited substantial binding affinity. The phenyl group likely augments hydrophobic interactions and aligns spatially to form stabilizing hydrogen bonds with arginine and tyrosine residues. The structural modifications at the 3-position of the coumarin scaffold emerged as a crucial determinant of enhanced biological activity and target specificity. Extensive structure-activity relationship (SAR) studies have validated that strategic substitutions at this position substantially augment ligand-target interactions. Halogen substituents, exemplified by the dichloro groups in compound 5c, intensify hydrophobic interactions within the protein's active site, contributing to the stabilization of the ligand-protein complex. Moreover, electron-withdrawing groups at the 3-position exert an electronic modulation on the coumarin core, enhancing its propensity for hydrogen bonding and electrostatic interactions with critical residues. Similarly, bulky substituents, such as phenyl groups as seen in compound 5b, effectively occupy hydrophobic pockets, thereby amplifying binding affinity and reducing nonspecific interactions. The coumarin-thiazole moiety, a shared pharmacophoric feature of the synthesized derivatives, was found to play an integral role in their molecular interactions. This structural motif facilitates multiple stabilizing interactions with the target protein, including hydrogen bonds and π-π stacking with aromatic residues. These interactions are pivotal for achieving high binding affinity and structural stability within the active site, underscoring the significance of the coumarin-thiazole framework in drug design. Comparative docking analysis further highlighted that the binding affinities of the synthesized derivatives were either comparable to or exceeded that of flurbiprofen. This finding underscores the successful enhancement of the coumarin framework's interaction profile through the incorporation of functional groups at the 3-position. These modifications not only improved the binding dynamics but also enhanced the drug-

like properties of the derivatives, contributing to their potential as viable therapeutic candidates. The results of this study emphasize the promise of amino coumarin-thiazole derivatives as prospective leads in the design of next-generation anti-inflammatory drugs. By leveraging SAR principles, the rational placement of substituents at the 3-position offers a strategic avenue to fine-tune biological activity, target selectivity, and pharmacokinetic properties. Future research should focus on the exploration of additional substituents and advanced computational modelling to refine these derivatives further for preclinical and clinical evaluation. Such efforts have the potential to culminate in the development of anti-inflammatory therapeutics with superior efficacy, minimized adverse effects, and enhanced target specificity.

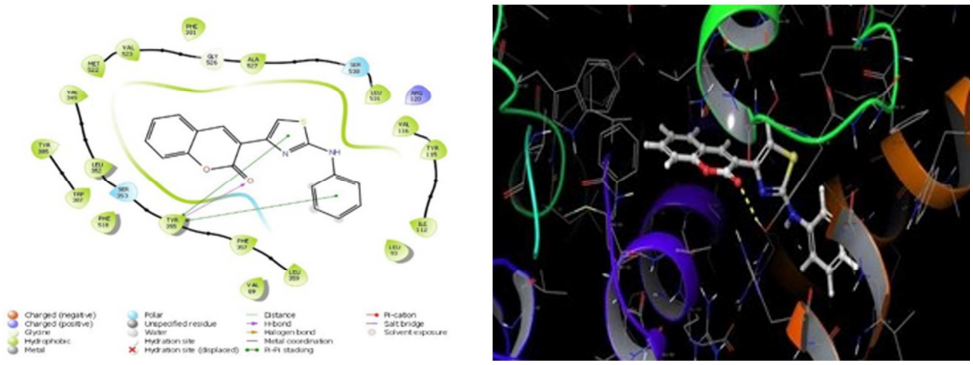


Figure 2: Docking images of compound 5b

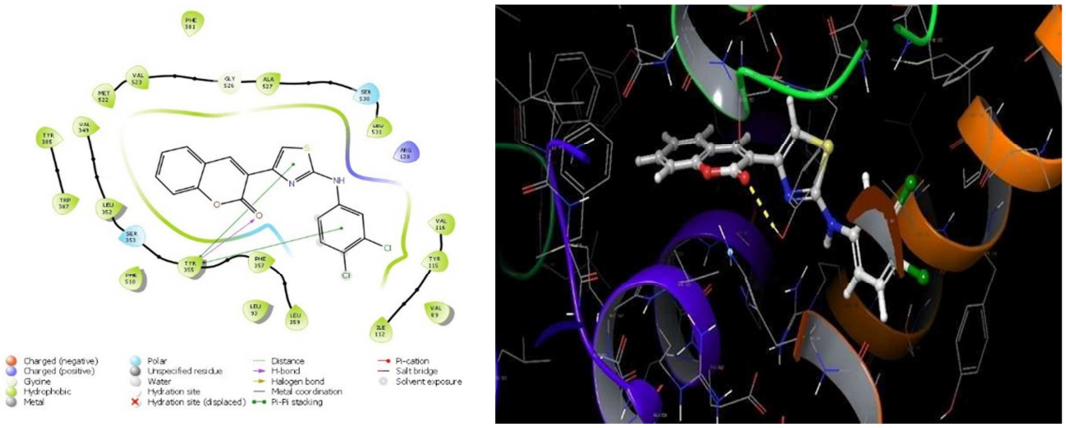


Figure 3: Docking study of compound 5c

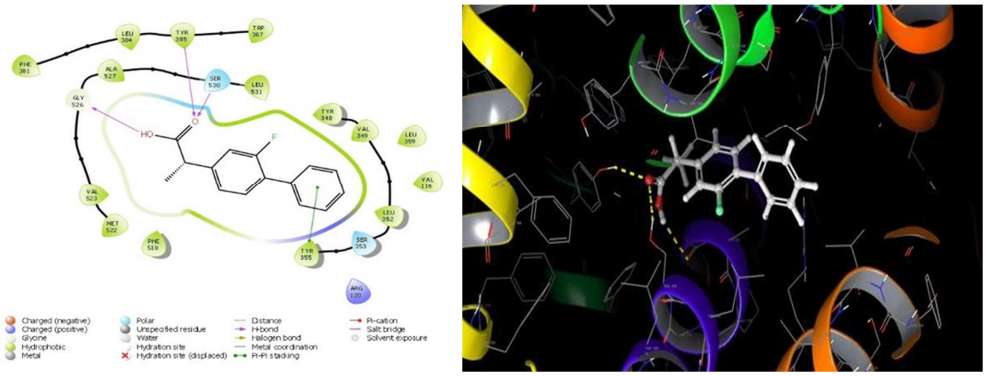


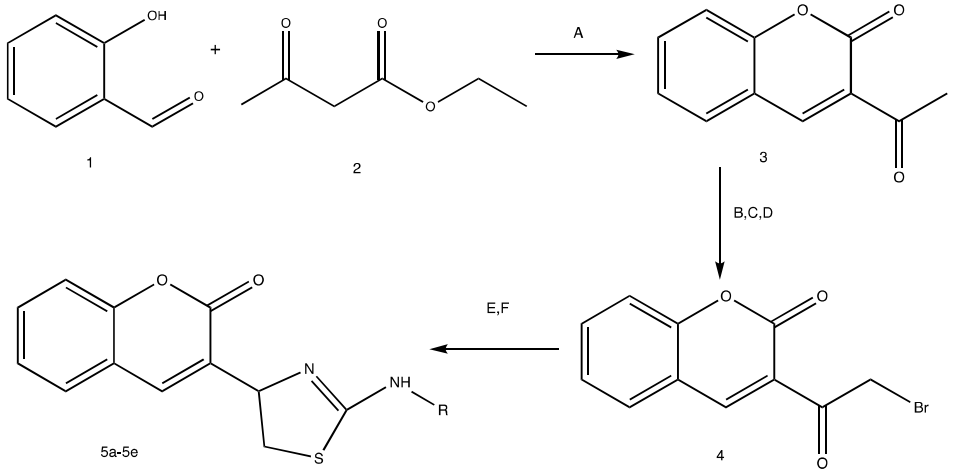
Figure 4: Docking study of flurbiprofen

Table 2: Binding energy of synthesised compounds.

Sr. No	Compound Code	Coumarin Derivatives	Binding Energy
1	5a	3-(2-amino-1,3-thiazol-4-yl)-2H-1-chromen-2-one	-8.345
2	5b	3-(2-anilino-1,3-thiazol-4-yl)-2H-1-chromen-2-one	-10.055
3	5c	3-[2-(3,4-dichloroanilino)-1,3-thiazol-4-yl)-2H-1-chromen-2-one	-10.465
4	5d	3-[2-(3-methoxyanilino)-1,3-thiazol-4-yl)-2H-1-chromen-2-one	-9.906
5	5e	3[2-(2-chloroanilino)-1,3-thiazol-4-yl)-2H-1-chromen-2-one	-10.14
6	-	Flurbiprofen	-8.786

3.2. Chemistry:

Active compounds (5a–5e) were synthesised through a three-step synthetic process outlined in Scheme 1. In the first step, the starting materials 2-hydroxybenzaldehyde (1) and ethyl acetoacetate (2), each at a concentration of 1 mmol, were treated with piperidine (A) as a catalyst at room temperature with continuous stirring for 1 hr, resulting in the formation of 3-acetyl-2H-1-benzopyran-2-one (3). In the second step, compound 3 was reacted with 1-bromopyrrolidine-2,5-dione (B) in the presence of acetonitrile (C) and chloroform (D) under reflux conditions at temperature of 40ºC, yielding 3-(bromoacetyl)-2H-1-chromen-2-one (4). Finally, the active compounds (5a–5e) were synthesised by refluxing compound 4 with thiourea (E) in ethanol (F) for 1 hour, producing the desired products.



Reagents: A: Piperidine, B: 1-bromopyrolidine-2,5-dione, C: acetonitrile, D: Chloroform, E: thiourea, F: ethanol.

Scheme 1: Synthesis Scheme of Coumarin Derivatives.

3.3. Spectroscopy results:

3-(2-amino-1,3-thiazol-4-yl)-2H-1-chromen-2-one: $C_{12}H_8N_2O_2S$, Yield: 89.23%, R_f: 0.56, m.p.: 229-231^{0}C, FTIR(ν KBr, cm-1): 3013.5(Aromatic C-H),1483.4(Aromatic C=C), 3244.3(N-H Stre), 1625.4(C=N stre), 1718.2(C=O stre),1262.8(C-N stre) (Aromatic), 1H NMR-δ 7.05 (1H, s), 7.29 (1H, m), 7.37-7.61 [2H, m], 7.79 (1H, ddd, 8.5 (1H,S), HR-MS-(M+Na^+) 245.03 found 267.02

3-(2-anilino-1,3-thiazol-4-yl)-2H-1-chromen-2-one: $C_{18}H_{12}N_2O_2S$, Yeild: 79.23%, R_f:0.84, m.p.:232-235^{0}C, FTIR(ν KBr,cm-1):3110.29(Aromatic C-H),1535.95 (Aromatic C=C),3284.2095(N-H),1693.08(C=N), 1718.19(C=O stre),1270.94 C-N (Aromatic), 1H-NMR-δ 6.83-7.04 [2H, 6.89 (tt), 6.99 (s)], 7.17-7.36 [5H, 7.23 (tdd), 7.30 (m), 7.30 (m)], 7.38-7.61 [2H, m], 7.80 (1H, m), 7.98 (1H, s), 8.4-8.5 [2H, (dd)], LC-MS-(M+) 321 found 321.

3-[2-(3,4-dichloroanilino)-1,3-thiazol-4-yl]-2H-1-chromen-2-one: $C_{18}H_9NO_2SCl_2$, Yield: 91.50%, R_f: 0.89, m.p.: 238-240^{0}C, FTIR(ν KBr, cm-1):3013.54 (Aromatic C-H),1483.44 (Aromatic C=C),3244.27 (N-H),1625.43 (C=N) ,1718.19 (C=O),1262.82 C-N (Aromatic) ,760.46 (C-Cl stre) , 1H-NMR-δ 7.00 (1H, s), 7.25-7.66 [6H, 7.30 (m), 7.38 (dd), 7.42 (dd), 7.45 (m), 7.54 (m), 7.59 (dd)],7.74-7.95 [2H, m] LC-MS-(M+) at 388.9.,

3-[2-(3-methoxyanilino)-1,3-thiazol-4-yl]-2H-1-chromen-2-one: $C_{19}H_{14}N_2O_3S$, Yield: 89.26%, R_f: 0.82, m.p.: 234-237°C, FT-IR(ν KBr, cm-1): 3024.38(Aromatic C-H),1448.46(Aromatic C=C),3362.98 (N-H stre),1683.21 (C=N stre),1727.21 (C=O stre),1246.37 C-N (Aromatic) ,1302.00 (C-O stre), 1H-NMR-δ 3.76 (3H, s), 6.67 (1H, ddd), 7.00 (1H, s), 7.16-7.37 [4H, 7.22 (td), 7.30 (m), 7.31 (m), 7.31(td)], 7.38-7.61 [2H, m], 7.80 (1H, m), 7.98 (1H, s), LC-MS-(M+) 350.9.

3[2-(2-chloroanilino)-1,3-thiazol-4-yl)-2H-1-chromen-2-one: $C_{18}H_{11}N_2O_2SCl$, Yield: 79.43%, R_f: 0.77, m.p.: 239-242^{0}C; 1H-MNR = δ 4.00 (1H, s, NH); δ 8.56 (1H, s, CH); δ 7.42 (1H, s, CH); δ 7.84 (1H, s, CH); δ 7.40 (1H, s, CH); δ 8.20 (1H, s, CH); δ 6.75 (1H, s); δ 7.42 (1H, s, CH); δ 7.65 (1H, s); δ 7.42 (1H, s); δ 8.05 (1H, s)

3.4. Drug-likeness properties of newly designed derivatives

Table 3: Drug-likeness properties of newly synthesised coumarin derivatives

Compound Code	Molecular weight (g/mol)	WLOGP	HBD	HBA	RO5	RB	tPSA(Å)	SA
5a	244.27	2.51	1	3	0	1	97.36	2.88
5b	320.37	4.66	1	3	0	3	83.37	3.20
5c	389.26	5.97	1	3	0	3	83.37	3.20
5d	350.39	4.67	1	4	0	4	92.60	3.25
5e	354.81	5.31	1	3	0	3	83.37	3.20

(MW: Molecular weight, HBD: Hydrogen bond donor, HBA: Hydrogen bond acceptor, RO5: Rule of five, RB: Rotatable bonds, tPSA: Total polar surface area, SA: Synthetic Accessibility)

3.5. ADMET properties of newly synthesised derivatives.

Table 4: ADMET properties of newly synthesised coumarin derivatives.

Compound Code	ABS int (%)	Dist Log BB	Dist Log PS	2D6	3A4	1A2	2C19	2C9	2D6	3A4	Excreation TC	AMES Toxicity
5a	95.039	-0.255	-1.99	N	Y	Y	N	N	N	N	0.148	N
5b	92.753	0.49	-1.505	N	Y	Y	Y	N	N	Y	0.099	N
5c	90.921	0.375	-1.281	N	Y	Y	Y	N	N	N	0.15	N
5d	93.525	0.404	-1.696	N	Y	Y	Y	N	N	Y	0.254	N
5e	91.026	0.459	-1.388	N	Y	Y	Y	Y	N	Y	0.235	N

3.6. In-vitro anti-inflammatory assay of the synthesized derivatives:

Anti-inflammatory activity of synthesized amino thiazole coumarin derivatives was checked by preforming *in-vitro* anti-inflammatory assay of the synthesised derivatives has been carried out using two experimental procedures.

3.6.1. Membrane stabilisation assay

Table 5: Membrane stabilisation: Diclofenac

Conc (µg/ml)	Log Conc	Abs 1	Abs 2	Abs 3	Avg. Abs	% Inhibition	% Inhibition	% Inhibition	Avg. Inhibition
Unheated	-	0.065	0.054	0.057	0.059	-	-	-	-
Control	-	0.827	0.805	0.856	0.830	-	-	-	-
12.5	1.097	0.322	0.312	0.323	0.743	13.415	10.405	9.951	40.049
25	1.398	0.290	0.295	0.288	0.424	52.595	54.735	50.597	46.496
50	1.699	0.254	0.254	0.242	0.257	72.730	74.273	75.856	55.895
75	1.875	0.217	0.299	0.214	0.156	85.703	87.247	89.219	57.467
100	2	0.186	0.169	0.174	0.111	93.150	94.837	91.982	72.784
150	2.176	0.105	0.103	0.096	0.088	96.653	95.485	96.783	90.149

Table 6: Membrane stabilisation assay of compound 5c

Conc (µg/ml)	Log Conc	Abs 1	Abs 2	Abs 3	Avg. Abs	% Inhibition	% Inhibition	% Inhibition	Avg. Inhibition
Unheated	-	0.065	0.054	0.057	0.059	-	-	-	-
Control	-	0.827	0.805	0.856	0.830	-	-	-	-
19.35	1.291	0.812	0.790	0.768	0.790	2.302	5.161	8.003	5.156
39.063	1.592	0.797	0.788	0.767	0.784	4.264	5.421	8.200	5.962
78.125	1.893	0.780	0.769	0.735	0.762	6.465	7.885	12.269	8.873
156.25	2.194	0.642	0.624	0.673	0.646	24.453	26.689	20.385	23.842
312.50	2.495	0.565	0.606	0.613	0.595	34.341	29.067	28.138	30.515
625	2.796	0.451	0.472	0.416	0.446	49.183	46.456	53.677	49.772
1250	3.097	0.203	0.255	0.207	0.222	81.344	74.598	80.825	78.922
2500	3.398	0.102	0.155	0.107	0.121	94.447	87.571	93.799	91.939

The absorbance value at 560 nm for compound 5c gradually decrease with concentration, which could indicate dose-dependent protection of the RBC membrane. This is consistent with what you would expect if the test compound has a membrane-stabilizing effect. Diclofenac, a known anti-inflammatory drug, shows absorbance values for the reference standard drug. The percent inhibition values show an increasing trend with increasing concentration (Table 5). This trend is indicative of greater membrane stabilization at higher concentrations. The values range from 5.156% at 19.35 µg/ml to 91.939% at 2500 µg/ml, which is reasonable and expected for a compound with good membrane-stabilizing properties for test sample (compound 5c). Diclofenac shows a % inhibition of 90.149 % at 150 µg/ml, which is in line with its known pharmacological effects (Table 6).

3.6.2. BSA Denaturation Assay

Table 7: BSA Denaturation assay of Diclofenac

Conc (µg/ml)	Log Conc	Abs 1	Abs 2	Abs 3	Avg. Abs	% Inhibition	% Inhibition	% Inhibition	Avg. Inhibition
Control	-	1.179	1.141	1.115	1.145	-	-	-	-
12.5	1.097	1.035	1.063	0.981	1.026	9.575	7.183	14.333	10.364
25	1.398	0.505	0.528	0.524	0.519	55.895	53.886	54.236	54.672
50	1.699	0.384	0.365	0.319	0.356	66.473	68.124	72.104	68.901
75	1.875	0.241	0.223	0.185	0.217	78.918	80.503	83.817	81.08
100	2	0.121	0.101	0.120	0.114	89.432	91.179	89.520	90.044
150	2.176	0.075	0.069	0.059	0.068	93.417	93.959	94.826	94.067

Table 8: BSA denaturation assay of Compound 5c.

Conc (µg/ml)	Log Conc	Abs 1	Abs 2	Abs 3	Avg. Abs	% Inhibition	% Inhibition	% Inhibition	Avg. Inhibition
Control	-	1.179	1.141	1.115	1.145	-	-	-	-
19.35	1.291	0.803	0.833	0.904	0.847	29.869	27.249	21.048	26.055
39.063	1.592	0.771	0.772	0.801	0.781	32.646	32.576	30.044	31.755
78.125	1.893	0.725	0.735	0.740	0.733	36.690	35.808	35.371	35.956
156.25	2.194	0.709	0.698	0.685	0.697	38.044	39.039	40.175	39.086
312.50	2.495	0.548	0.525	0.506	0.526	52.114	54.148	55.808	54.023
625	2.796	0.360	0.350	0.329	0.346	68.603	69.432	71.266	69.767
1250	3.097	0.195	0.199	0.165	0.186	82.943	82.620	85.590	83.718
2500	3.398	0.071	0.085	0.061	0.072	93.764	92.576	94.672	93.671

The absorbance decreases with increasing concentration of the test compound, which suggests that the compound is preventing protein denaturation. This trend is consistent with expected results for a substance with anti-inflammatory or protein-stabilizing properties. The percent (%) inhibition values calculated from the absorbance data reflect a dose-dependent inhibition of BSA denaturation (Table 7 and 8). The values increase progressively as the concentration of the test compound increases, which aligns with what would be expected for a compound that is effective at preventing protein denaturation. IC_{50} Values of Compound 5c and Standard drug were calculated based on the in-vitro assays performed and the IC_{50} values are represented in Table 9.

Table 9: IC_{50} values based on the in-vitro assay

Test Item	Membrane Stabilization		BSA Denaturation	
	Log IC50 Value	IC50 Value (µg/ml)	Log IC50 Value	IC50 Value (µg/ml)
Standard	1.432	27.051	1.455	28.51
3-[2-(3,4-dichloroanilino)-1,3-thiazol-4-yl)-2H-1-chromen-2-one	3.059	114.4	3.020	104.8

4. Conclusion

In summary, this research successfully designed, synthesized, and characterized a range of amino thiazole coumarin derivatives, assessing their anti-inflammatory properties through in-vitro experiments. Among the synthesized compounds, 5b, 5c, and 5e exhibited the strongest binding interactions with the target protein 3PGH, outperforming the reference drugs flurbiprofen in molecular docking studies and diclofenac biological assays. The findings suggest that these derivatives possess significant potential as anti-inflammatory agents, with compound 5c demonstrating the best activity in terms of membrane stabilization and protein denaturation inhibition. Although their anti-inflammatory efficacy was moderate compared to diclofenac, the results highlight promising opportunities for further optimization of these compounds to enhance their therapeutic potential and minimize adverse effects, offering a promising foundation for the development of new anti-inflammatory drugs based on coumarin scaffolds.

5. Conflict of interest

The authors have no conflict of interest

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