Library Progress International *Print version ISSN 0970 1052*
Vol.44 No. 3, Jul-Dec 2024: P.13052-13069 *Online version ISSN 2320 317X*

Original Article *Available online at www.bpasjournals.com*

Synthetic Approaches and Biological Evaluation of Novel Coumarin Based Compounds

Nitesh Kumar Singh[1],Vikrant Verma[2], Manish Pathak[2] , Sokindra Kumar[3]

[1]Research Scholar, Department of Pharmaceutical chemistry, Faculty of Pharmacy, Kharvel Subharti College of Pharmacy, Swami Vivekanand Subharti University, Meerut(U.P) INDIA -250005, vijeetsingh84@rediffmail.com
[2]Department of Pharmaceutical chemistry,Faculty of Pharmacy, Kharvel Subharti College of Pharmacy, Swami Vivekanand Subharti University, Meerut(U.P) INDIA -250005
[3]Department of Pharmacology, Faculty of Pharmacy, Swami Vivekanand Subharti University, Meerut -250005, (U.P), INDIA

How to cite this article: Nitesh Kumar Singh,Vikrant Verma, Manish Pathak, Sokindra Kumar (2024). Synthetic Approaches and Biological Evaluation of Novel Coumarin Based Compounds. *Library Progress International*, 44(3), 13052-13069.

Abstract
Coumarins, which are 2H-1-benzopiran-2-one compounds originating from some plants, have been proven to be anticancer, antibacterial, and anticancer agents. Around of these structures are currently accepted for the treatment of cardiovascular disease (warfarin), antibiotics (novobiocin or chlorobiocin), and anticancer medications (geiparvarin). We created 38 coumarin derivatives (2–50), 22 of which were novel, in this project by replacing the eighth carbon of the benzopiran ring with nearly aromatic and aliphatically substituted piperidine and piperazines. This was done because of the structure's great potential and the dearth of research on molecules resulting from the benzopiranone heterocycle. The produced compounds' cytotoxicity, analgesic, and anti-inflammatory qualities were evaluated. The targeted molecules were made in two steps. Using the Pechmann reaction, the 7-hydroxy-4-methyl-chromen-2-one coumarin scaffold was created in the first step. Another phase was the derivatization of the 7-hydroxy-4-methyl-chromen-2-one coumarin scaffold (compound 1) by addition of piperidine and aromatic and aliphatic substituted piperazine groups.

In vitro tests were used to evaluate the cytotoxicity, analgesic, and anti-inflammatory properties. MTT assay was employed to assess the cytotoxicity of MCF-7 breast cancer cells and RAW264.7 macrophages. The nitrite inhibition test was employed to evaluate anti-inflammatory activity using RAW264.7 macrophage cells. The reference drugs for the cytotoxicity and anti-inflammatory tests were L-NAME and indomethacin (IND). PGE2 production for the painkilling effect was noted. The outcomes indicated that compounds 11, 23, and 31 have promising anti-inflammatory activity. Compound 11 produced better results than the reference drugs and were three times more active than IND. Moreover, compound 11 confirmed little cytotoxicity and a moderate analgesic effect.

Keywords: Coumarins, anti-inflammatory activity, cytotoxicity, analgesic activity, piperazine, piperidine.

INTRODUCTION

Coumarins (2H-1-benzopyran-2-one) are compounds also found in a variety of plants (Fig. 1). Since their discovery in the seeds of Coumarouna odorata Aube (Diptryx odorata) [1], these chemicals have been referred to as "coumarin". Coumarin derivatives have been linked to a wide variety of biological activities in the literature, including anticoagulant [2, 3], antibacterial [4], anti-inflammatory [5], anticancer [6], and antidepressant [7]. Nowadays, the vast range of functionalities makes this building block the preferred choice for developing novel medications. The relevance of the coumarin ring in pharmaceutical research has sped up the advance of new synthesis methods. Coumarin scaffold can be produced via the Pechmann, Perkin, Wittig, Kostanecki-Robinson,Knovenagel, and Reformatsky reactions [8].

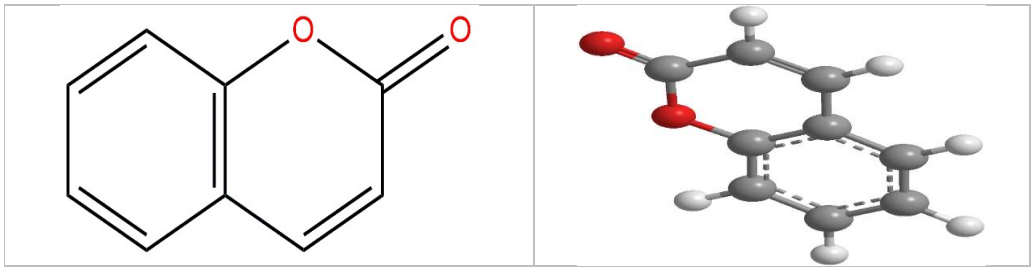


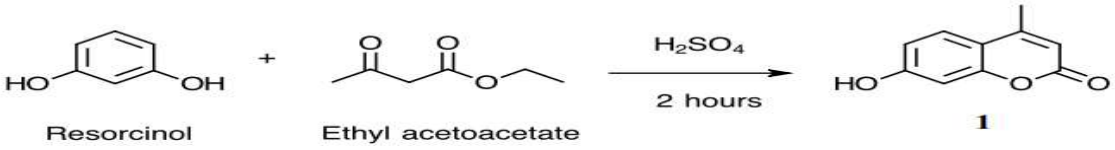
Fig. 1 Structure of Coumarin

Many of the drugs that are now on the market have the coumarin molecule. For instance, illnesses affecting the cardiovascular system commonly employ warfarin as an anticoagulant [8]. Another important coumarin derivative is used as an antimicrobial. The most well-known medications made from coumarins are the antibiotics Novobiocin and Chlorobiocin. They are very active against Gram-positive bacteria because they stop the DNA gyrase enzyme from functioning [9, 10]. It has also been discovered that plants with the coumarin structure contain geiparvarin [6].

METHODS OF SYNTHESIS

1.1. General Procedure A: Synthesis of Coumarin Scaffold

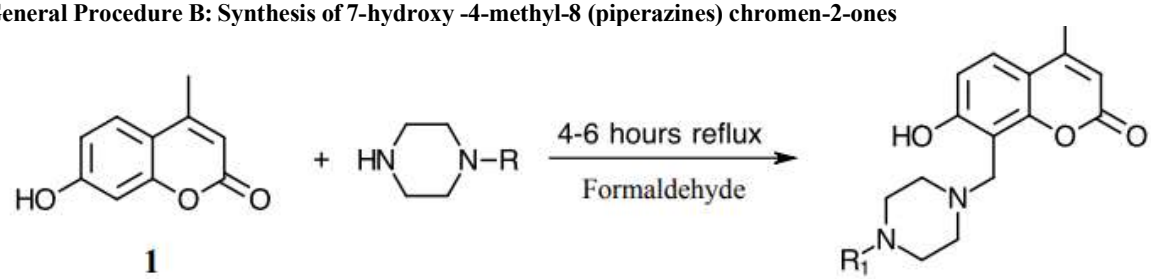
1.1.



Scheme 1

1.22 eq of resorcinol was liquified in 20 mL of saturated H2SO4. Subsequently, 1.0 equivalent of ethyl acetoacetate was gradually added, and the mixture was left to stir at 0–5 degrees Celsius for two hours. The reaction mixture was set on ice water for two hours. After a water wash, the solid was recrystallized from EtOH. Ultimately, 7-Hydroxy-4-methyl-chromen-2-one, a pure white solid, was produced [11].

1.1. General Procedure B: Synthesis of 7-hydroxy -4-methyl-8 (piperazines) chromen-2-ones

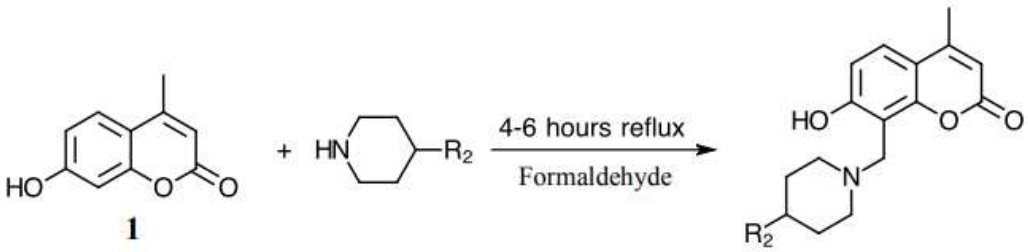


1.1.

Scheme 2

Following the dissolution of Compound 1 (7-Hydroxy-4-methyl-chromen-2-one; 1.0eq) in 5 mL of 95% EtOH, the reaction media was supplemented with formaldehyde (0.2 mL) and piperazine derivative (s)(R1) (1.0eq). Reflux occurred in the reaction mixture for four to six hours. After the four to six hours, the reaction mixture was cooled, and the solvent was then dissolved under vacuum. Once light-yellow oils were obtained, a small amount of cooled acetone was added. The whitish solids were then crystallized using acetone [12].

Synthesis of 7-hydroxy-4-methyl-8- (piperidine) chromen-2-one



Scheme 3

1.1. Compound 1 (7-Hydroxy-4-methyl-chromen-2-one) was liquefied in 95% EtOH, and the reaction medium was then supplemented with formaldehyde and piperazine derivative(s) (R2). Reflux occurred in the reaction mixture for four to six hours. The solvent was vacuum-evaporated after the reaction medium had cooled for four to six hours. Once light-yellow oils were obtained, a tiny amount of cooled acetone was added. The white crystals were then crystallized using acetone [12].

1.1. **Analytical Methods**

1.1. **Melting Point Determination**

1.1. Using a Mettler Toledo FP62 capillary melting apparatus, the melting points of synthesized compounds were ascertained.

1.1. **Analysis of Thin Layer Chromatography TLC Plates:**

1.1. TLC 20x20 aluminum sheets 60 F254 (Merck) Silica gel Systems of Solvents: 50:50 ethyl acetate:n-hexane Pulling Procedure: The solvent system was poured into the TLC chamber, and it was left for an hour to achieve a suitable saturation. Spot determination: Some piperazine and piperidine derivatives were visualized using ninhydrine dye, and spots of the synthesized compounds and their starting materials were identified using UV light (254/365 nm).

1.1. **Spectrometric Methods**

1.1. **Fourier-Transform Infrared Spectroscopy (FT-IR)**

1.1. The Fourier Transform Potassium bromide pellets were utilized along with a PerkinElmer Spectrum One series FT-IR instrument to conduct infrared spectroscopy analyses. The expression for spectrums was cm-1.

1.1. **H-NMR**

1.1. Tetramethylsilane (TMS) was used as the reference, and dimethylsulfoxide (DMSO-d6) and deuterated chloroform (CDCl3) were used as the solvents. The 1H-NMR spectra were recorded using a Bruker Spectrospin Avance DPX-400 (400 MHz). Parts per million (ppm) was the unit of measurement for the chemical shifts.

1.1. **C-NMR Spectra**

1.1. Tetramethylsilane (TMS) was utilized as the reference in the 13C-NMR spectra, which were recorded at 400 MHz. Dimethylsulfoxide (DMSO-d6) and deuterated chloroform (CDCl3) were employed as the solvents. Parts per million were used to identify the chemical shifts (ppm).

1.1. **LC-MS Analyses**

- The Waters UPLC was used to record the LC-MS spectra. Utilized was a photo diode detector (UPLC LG 500 nm). The column was the BEH C8 X Bridge column. Phase of mobility:

- 0-1 min: ACN: water: 1% formic acid = 9: 90: 1
- 1-2 min: ACN: water: 1% formic acid = 24: 75: 1
- 2-3 min: ACN: water: 1% formic acid = 0: 99: 1
- 3-12 min: ACN: water: 1% formic acid = 9: 90: 1

1.1. Biological Activity Tests

1.1. Cell culture and viability

The cells' viability was assessed using the MTT (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. Compounds liquified in dimethyl sulfoxide (DMSO) were added to plated RAW264.7 cells (2x105 cells per millilitre) at a concentration of 100µM (the highest concentration that could be used). Each well received 0.5 mg/mL of MTT after a 24-hour incubation period, and the mixture was then raised for an additional two hours at 37°C. Following the removal of the plate medium, 100 µl of isopropanol was added to each well. A UV-spectrophotometric plate reader was used to measure the transmission density of the MTT formazan at 540 nm. The absorbance ratio, expressed as a percentage, between the chemically exposed cells and the cells preserved with 0.5% DMSO (as a control) was utilized to ascertain theviability of the system. Every measurement was carried out three times [13].

1.1. MCF-7 %Cell Viability Protocol

The cells' feasibility was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Following 70–80% confluency, the human breast cancer cell line MCF-7 was refined on 48-well plates and exposed to 10 µM dimethyl sulfoxide (DMSO)-derived compounds. After incubating for 24 hours, 0.5 mg/mL of the generated MTT was further, and it was incubated at 37 °C for 2 hours. Following the incubation time, 100 µL of isopropanol was added to each well, and the optical density of MTT formazan at 540 nm was measured using a UV spectrophotometric plate reader. The transmission density of the cells unprotected to the chemicals was defined as the number of live cells among the cells treated with a 0.5% DMSO control. Every measurement was made three times [14].

1.1. Evaluation of Anti-inflammatory Activity by Nitrite Assay

The anti-inflammatory efficacy of the compounds was assessed by measuring the stable nitric oxide (NO) metabolite, nitrite, using the Griess test (Kiemer and Vollmar, 1997). To summarize, RAW-264.7 cells were plated in 48-well plates at a density of 2x105 cells per millilitre. After that, the plates were incubated for a full day at 37°C with 5% CO2. After a two-hour pre-treatment with 100µM (the maximum dose used), plated cells were stimulated with 1µg/mL lipopolysaccharide (LPS) for twenty-two more hours. The culture supernatant was mixed with 50 µL of Griess reagent (1% sulphanilamide, 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride in 5% phosphoric acid) and allowed to stand at room temperature for half an hour. The mixture's absorbance at 570 nm was measured with a microplate reader. **The amount of nitrite in the test samples was calculated [15].**

Utilize the sodium nitrite standard bow. The positive controls were L-NAME (Nω-Nitro-L-arginine methyl ester hydrochloride) prepared in DMEM and 100 µM of Indomethacin dissolved in DMSO. The data are shown as a ratio of [NO] treated cells to [NO]untreated cells, with percentages used to indicate any differences. Everybody was measured three times.

1.1. Analgesic Activity

As directed by the manufacturer, the prostaglandin E2 ELISA Kit (Abcam, UK) was used to quantify the analgesic activity using cell supernatants preserved with LPS and compounds.

RESULTS AND DISCUSSION:

In this work, novel equivalents of 7-hydroxy-4-methylchrome-2-one, piperazine and piperidine, were synthesized and it's invitro cytotoxicity, analgesic, and anti-inflammatory characteristics were evaluated.

Chemistry

The analysis of the retrosynthetic pathway of the selected mixes is displayed under (Fig. 2). The final compounds are made up of two key parts: a coumarin scaffold and piperazine/piperidine groups. When H2SO4

was present, the coumarin fraction was created via the Pechmann reaction. The piperazine and piperidine groups were then added to the coumarin ring's ninth position using formaldehyde and ethanol.

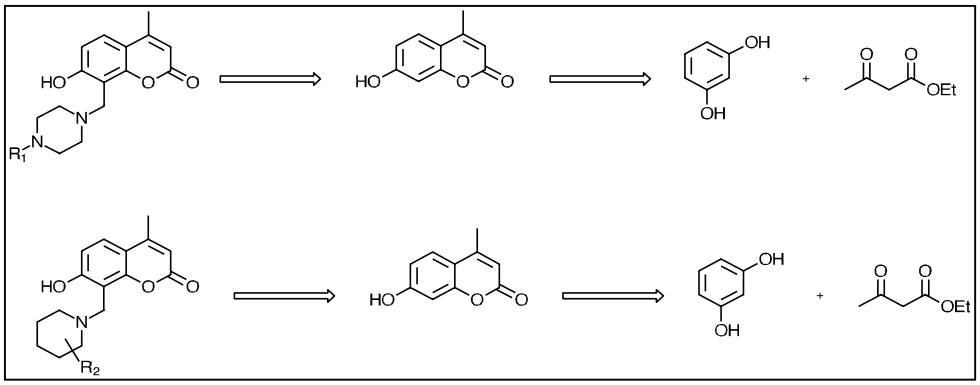


Fig. 2 Retro synthesis of piperazine/ piperidine analogues

Step1: Synthesis of 7-hydroxy-4-methylchromen-2-one (Compound 1)

In order to synthesize Compound 1, (7-hydroxy-4-methylchromen-2-one), the Pechmann reaction was utilized as a guide. Resorcinol and ethyl acetoacetate were the starting chemicals, and an acidic medium was used to conduct the reaction. The action and the situation mechanism are explained below (Fig. 5.2 and Sch. 1)

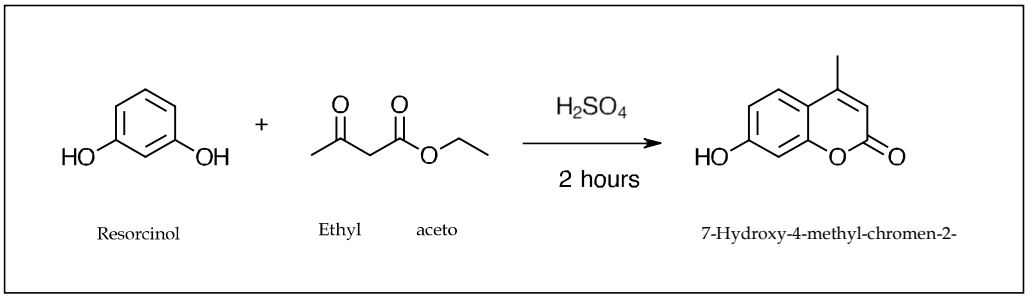
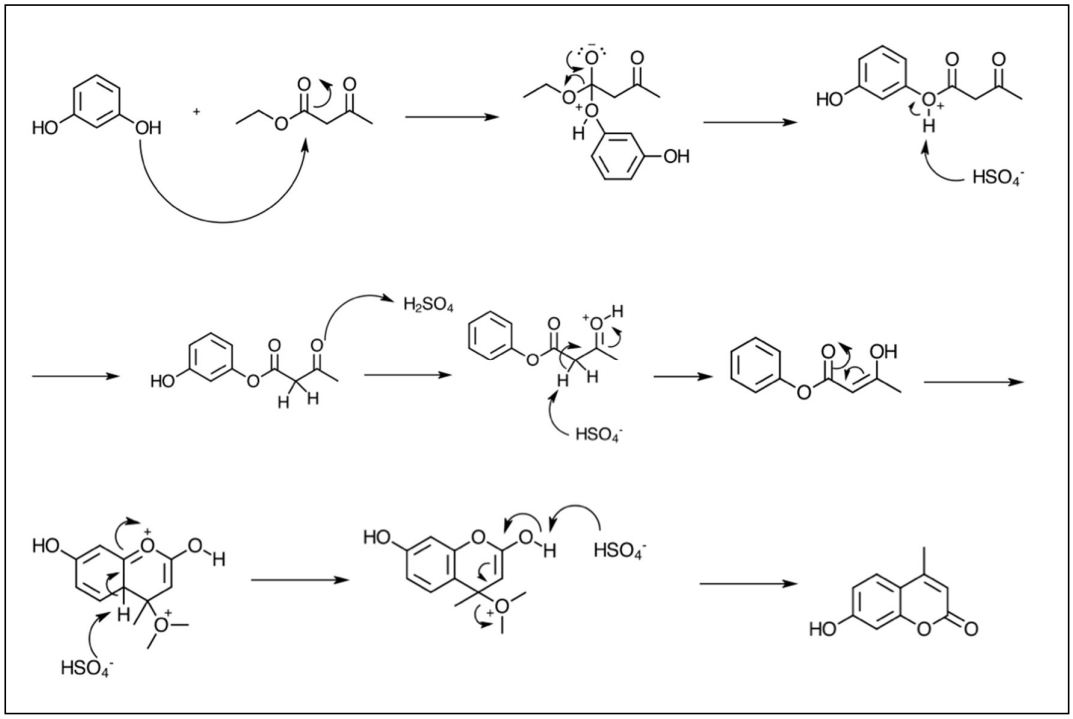


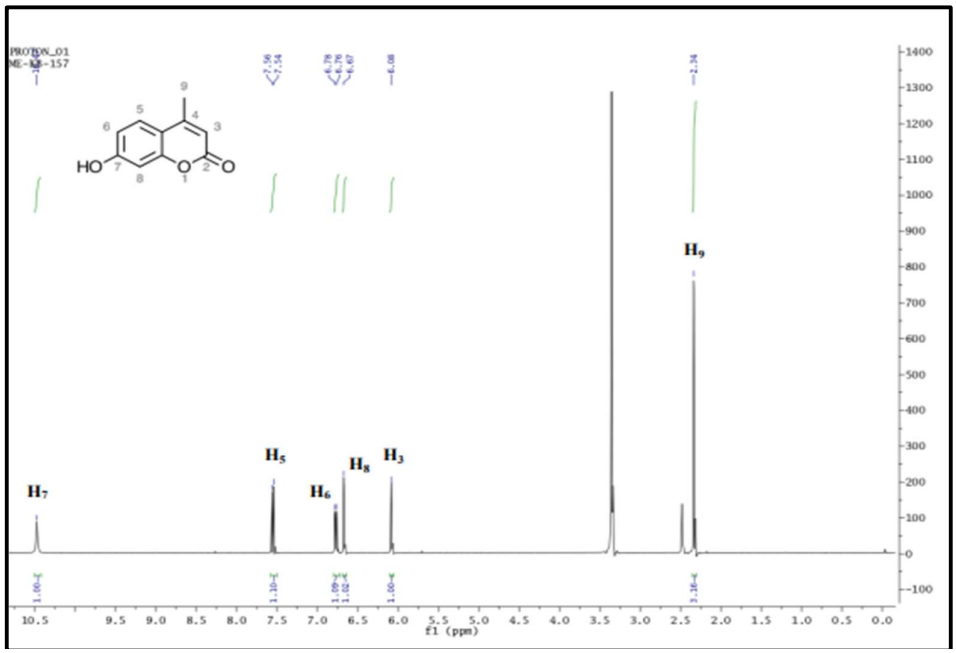
Fig. 3 Synthesis scheme of 7-hydroxy-4-methylchromen-2-one

1.2 MECHANISM:



Scheme 4. Mechanism of 7-hydroxy-4-methyl-chromen-2-one

Compound 1 was characterized using 1 H-NMR, 13 C-NMR, LCMS, and FTIR following its synthesis (Fig. 4-7). The 1 H-NMR analysis indicates that the aromatic H5 is detected at 7.57 ppm (J=8.7Hz) as a doublet. The proton that is adjacent to it, H6, produces a doublet at 6.78 ppm (J1=8.7Hz, J2=2.4Hz) due to a meta coupling with H8, in addition to the ortho coupling with H5. The doublet for the H8 proton obtained at 6.68 ppm with the same J=2.4 Hz coupling constant confirms this finding. The H3 proton, which exhibits a typical cis-allylic coupling with the methyl moiety bond to the cyclic double bond, is represented by the quartet at 6.10 ppm (J=1.2Hz). Lastly, the doublet obtained confirms the presence of the methyl (4).



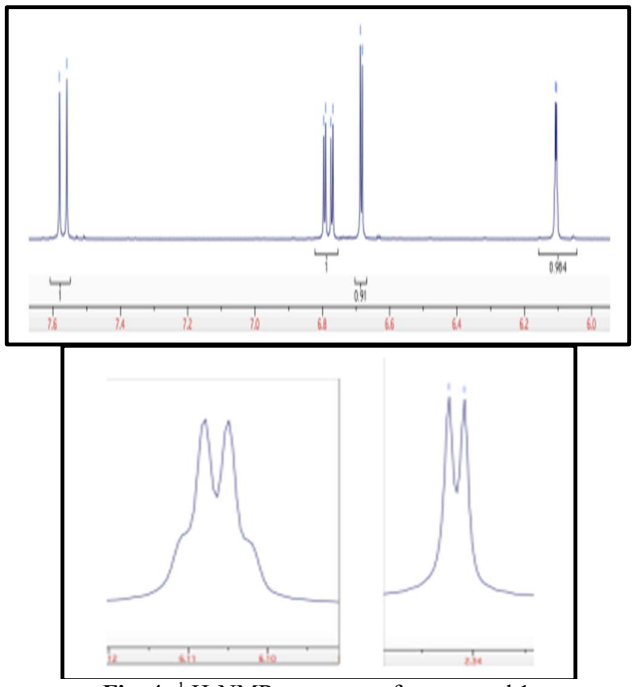


Fig. 4: [1] H-NMR spectrum of compound 1

Since the spectrum showed a typical carbonyl signal at 161 ppm, a peak at 18 ppm for the methyl C9 carbon, and 8 signals that are typical of sp2 hybridized carbons for the C3, C4, and C5-C8 (aromatic carbons of coumarin), the acquisition of the coumarin scaffold was also validated by 13C-NMR (Fig. 5).

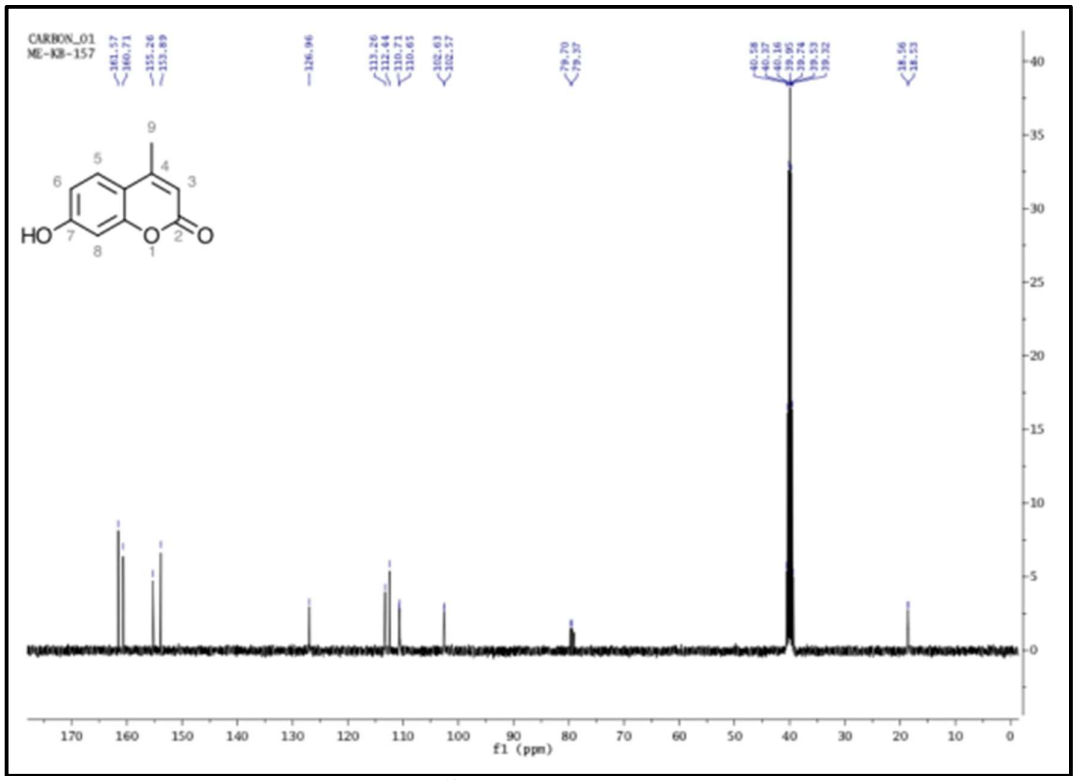


Fig.5: [13]C-NMR of compound 1

The mass spectrum, which showed a M+H peak at 176.8 (m/z) in addition to the molecule's isotopic peaks at

178.1 (m/z) and 179.1 (m/z), further supported the cyclization (Fig. 6). Ultimately, the UV-Vis spectrum, which showed an inflection at 220 nm, a minimum at 260 nm, and a maximum at 320 nm, was consistent with the literature(Fig. 7).

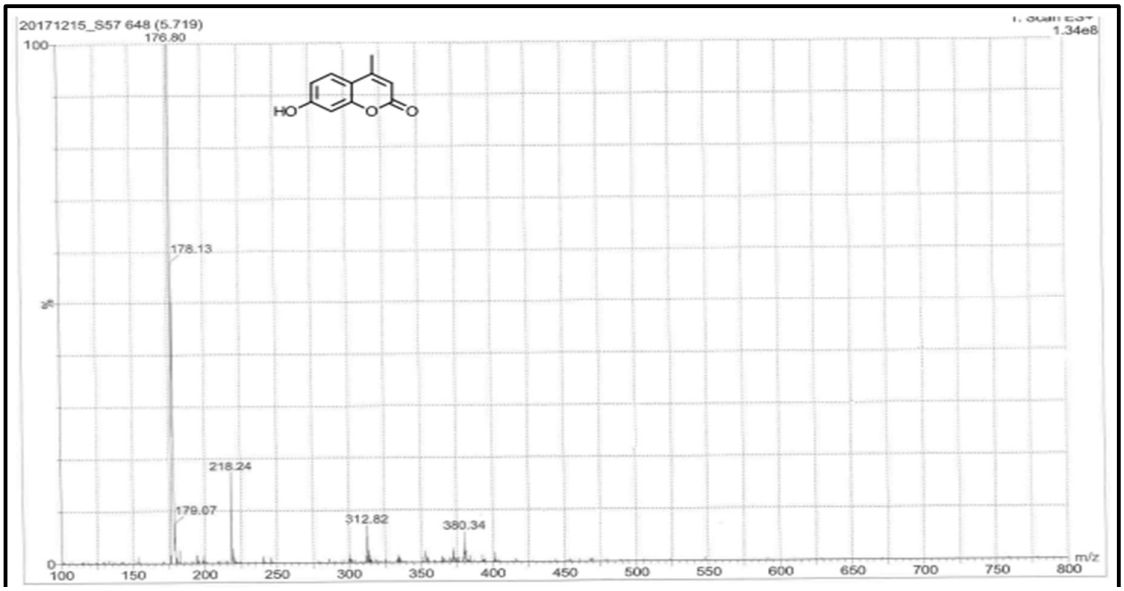


Fig. 6: LC-MS spectrum of compound 1

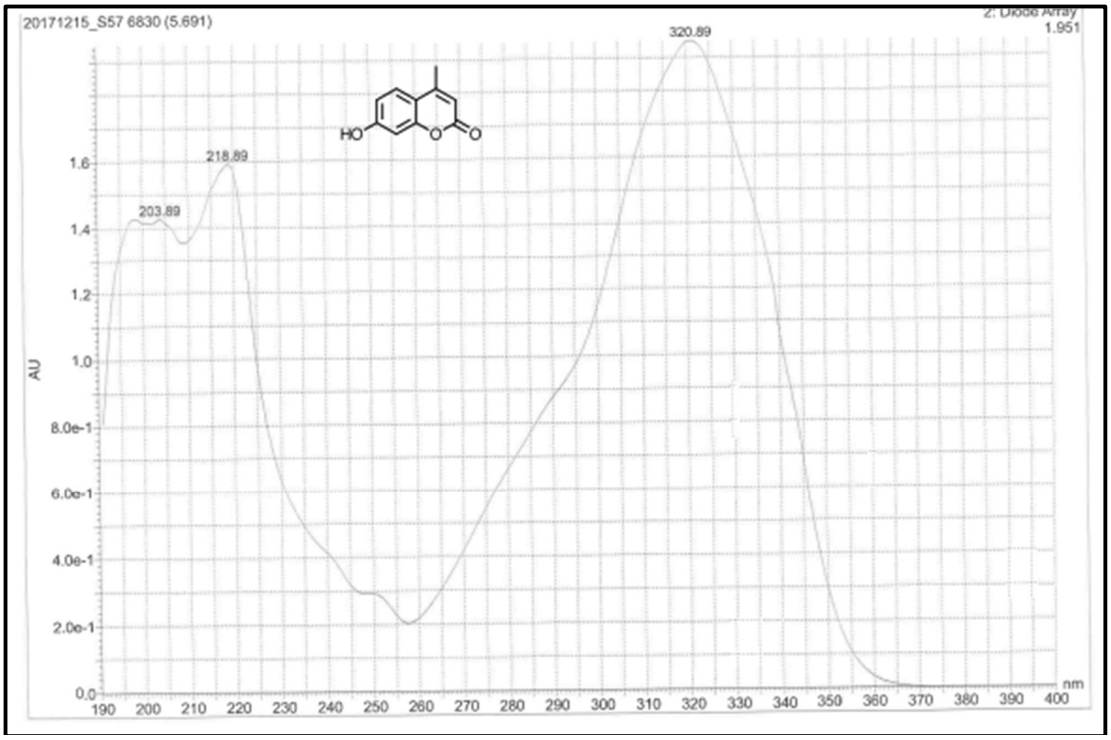


Fig. 7: UV- Vis spectrum of compound

Step 2: Synthesis of Piperazine and PiperidineAnalogues

In another stage, the generated chemical experienced the sequence of derivatizations involving piperazine and piperidine molecules (Fig. 8). To create the required molecules, formaldehyde was rummage-sale to attach the heterocyclic moieties at the ninth position in compound 1. It is thought that this mechanism is at work. In this procedure, formaldehyde is first protonated, and a nucleophile then targets the nitrogen of either the piperazine or the piperidine's secondary amine function. The production of an iminium ion after proton exchanges allows

compound 1 to attack the electrophilic carbon, which in turn allows the piperazine or piperidine heterocycle to be bound by a methylene linker at the eighth situation of coumarin ring (Sch. 2).

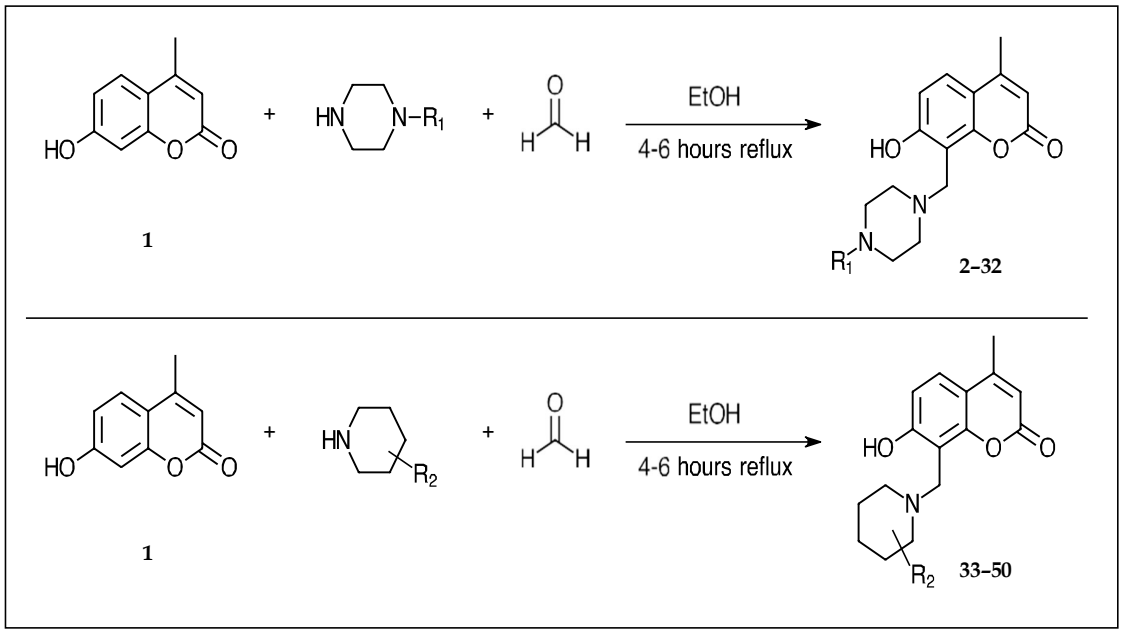
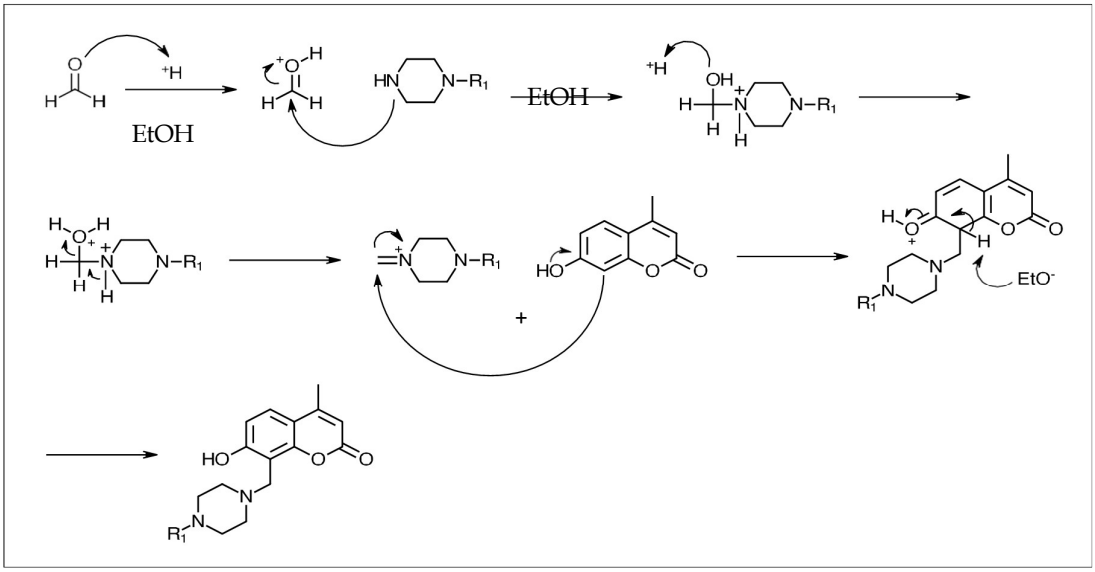


Fig. 8: Synthesis of targeted compounds 2-50

1.3 MECHANISM:



Scheme 5. General reaction mechanism of the compounds 2-50

1.3. The goal was to synthesize fifty coumarin derivatives, of which eighteen (containing seven new structures) and thirty-two (containing fifteen new structures) were piperazine derivatives.

1.3. Piperazine derivatives (2-32)

The N4 atom of the heterocycle in 32 different piperazines was substituted with several aliphatic and aromatic medieties to form this family of derivatives. **Table 1** displays the yields of 26 products, which varied from

acceptable to moderate. As mentioned before, the structure was verified using spectroscopic methods [16].
Table 1. Piperazine derivatives yields

Compound	% Yield	Compound	% Yield
2	63	18	50
3	32	19	60
4	47	20	47
5	26	21	28
6	50	22	62
7	78	23	38
8	35	24	54
9	-	25	68
10	-	26	36
11	25	27	-
12	31	28	76
13	-	29	15
14	-	30	60
15	53	31	38
16	64	32	51
17	67		

Piperidine derivatives (33-50)

The second class of compounds was created using eighteen different mono- or disubstituted piperidine molecules. Twelve of the target compounds were successfully synthesised, with normally good yields. Spectroscopic methods were used once more for structure confirmation. The yields attained for the eighteen piperidine derivatives are displayed in Table 2 [16].

Table 2. Piperidine derivatives yields

Compound	%Yield	Compound	% Yield
33	50	42	40
34	66	43	-
35	48	44	-
36	20	45	75
37	52	46	68
38	-	47	64
39	-	48	56
40	-	49	24
41	52	50	-

Biology
Cytotoxicity

First, the generated compound's cytotoxicity was evaluated. Using RAW264.7 macrophage cells—which are non-carcinogenic—the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was employed to perform the test. The ratio of treated cell absorbance to treated cell absorbance with 0.5% DMSO, the solvent used to melt the chemicals, was utilized to determine the viability of the experiment. 100 µM of the generated compounds were applied to the cells. The percentages were used to express the numbers in Table 3. The data analysis revealed that the synthesized structures are typically not dangerous, with the exception of the p-chlorobenzyl substituted piperazine derivative 23, which had a viability of 36% at 100 µM.

As cells were treated at extremely high doses and agreed that the practicality did not drop below 10%, this chemical may still be considered moderately cytotoxic.

Following outcomes with healthy cells, some of the assemblies were tested for anticancer activity on MCF-7 breast cancer cells. The compounds were confirmed at a dosage of 10 µM for their negligible cytotoxicity on macrophages. Once again, no cytotoxicity was discovered for virtually all of the structures under analysis, with the exception of molecules **17** and **23**, whose viability decreased to 37 and 23%, respectively. These values, while inadequate to designate a structure as an antiproliferative molecule, are significant and can be used to develop novel, highly potent coumarin derivatives, such as molecules **17** and **23.**

Table 3. Cytotoxicity results on RAW264.7 macrophage and MCF-7 breast cancer cell lines

	Compound	R	RAW 264.7	MCF-7
Piperazines	**1**	-	78,9±9,9	**109± 1,2**
	2	-	ND	**ND**
	3	CH_3	76,11±11	**102± 4,9**
	4	CH_2CH_3	76± 11,2	**101± 3,6**
	5	CH_2CH_2OH	66± 6,6	**106± 1,5**
	6	$CH_2{=}CHCH_2$	70± 8,1	**96± 6,9**
	7	CH_2CH_2Ph	ND	**ND**
	8	CH_2CH_2CN	65± 8,9	**ND**
	11	$COOCH_2Ph$	64± 3,4	**ND**
	12	$COOCH_2CH_3$	66± 6	**ND**
Piperidines	**15**	Ph	66± 4,3	**116± 4,0**
	16	*p*-Cl-Ph	87± 1,9	**90± 4,4**
	17	*p*-F-Ph	67± 13,6	**37± 3,8**
	18	*p*-Br-Ph	64± 9,5	**ND**
	19	*p*-CH_3-Ph	60± 8,3	**85± 1,7**
	20	*p*-NO_2-Ph	59± 2,3	**102± 2,1**
	21	*o*-CN-Ph	55± 6,9	**93± 2,2**
	22	*p*-OMe-Ph	64± 7,8	**85± 1,7**
	23	*p*-Cl-Bn	36± 5,1	**23± 1,9**
	24	*p*-OH-Ph	65± 7,7	**ND**
	25	3,4-$(Cl)_2$-Ph	77± 3,7	**95± 3,9**
	26	*p*-CF_3-Ph	ND	**ND**
	28	*o*-F-Ph	77± 11,8	**ND**
	30	Benzo[1.3]dioxo l-5yl	65± 12,7	**ND**
	31	Naphthylmethyl	68± 5,2	**ND**
	32	Pyridin-2-yl	64± 11	**ND**
	33	-	61±12,5	**ND**

34	4-CH_3	67±5,4	**ND**
35	2-CH_3	79± 10,7	**ND**
36	3,5-$(CH_3)_2$	77± 6,4	**ND**
37	2-CH_2CH_3	69± 13,6	**ND**
41	3-CH_2OH	77± 6,1	**ND**
42	2-$COOCH_2CH_3$	81± 2,9	**ND**
45	4-COOH	57±3,1	**ND**
46	3-COOH	ND	**ND**
47	4-Ph	ND	**ND**
48	4-(*p*-OH-Ph)	61±5,2	**ND**
49	**4-Bn**	**59± 10,7**	**ND**

ND: Not determined

Anti-inflammatoryActivity

The NitriteAssay

The anti-inflammatory qualities of the produced compounds were assessed using the nitrite inhibition test. Nitrite levels are crucial markers of inflammation since they are known to be markedly raised in inflammatory tissues. After inducing an inflammatory state in RAW264.7 macrophage cells with LPS, the resultant compounds were added to the growth media at a concentration of 100 µM. Since compounds 2, 7, 26, 47, and 48 were insoluble in DMSO, they were excluded from the investigation. As reference medications, N-nitro-L-arginine methyl ester hydrochloride (L-NAME) and indomethacin (IND) (100 µM) were used. Measured and compared to levels obtained for LPS-induced cells preserved with the solvent used to melt the molecules (0.5%DMSO+LPS), the nitrite levels in the culture supernatant were determined. Results are given as the ratio [NO] treated cells/[NO]untreated cells and expressed as percentages in the following table (Table 4).

The findings indicate that compound 1, the unsubstituted coumarin molecule, does not show any anti-inflammatory properties. Data from piperazine compounds with aliphatic moieties substituted (2–12) show that small alkyl groups, such as those that are methyl, ethyl, allyl, or substituted with hydroxy or cyano, are inert. Substances having an acylated nitrogen have a greater activity. As a matter of fact, the ethyl carbamate derivative 12 decreased by 11%. In addition, nitrite levels were reduced by 90% when compound 11 was substituted with benzyl carbamate in the cells. This compound had the greatest activity of all those examined, having three times the potency of the standard indomethacin molecule.

When aromatic rings were used in place of piperazines, positive outcomes were also seen. The activity of the molecules was much enhanced by substituting the phenyl-substituted piperazine derivative, despite the fact that it only caused a 14% drop in NO levels. Even while the inhibition levels for p-Cl (16), p-F (17), p-Br (18), p-OCH3 (22), and p-OH (24) were still rather low (30, 23, 23, 20, 17, and 47%, respectively), it was found that substituting the phenyl ring at its paraposition enhanced the compounds' bioactivity. Compound 20 (59%), p-NO2, showed the greatest activity level. Only the p-CH3(19) structure defied this pattern; no inhibitory ability was found for it.

Table 4. Anti-inflammatory activities of the tested compounds.

Compound	R	% Nitrite Reduction
1	-	**-2,0± 10,4**
2	-	**NR**
3	CH_3	**-2,8 ± 8,3**
4	CH_2CH_3	**7,1±5,0**
5	CH_2CH_2OH	**9,0±4,2**
6	CH_2=$CHCH_2$	**-1,6 ± 2,3**
7	CH_2CH_2Ph	**NR**
8	CH_2CH_2CN	**1,1±1,0**
11	$COOCH_2Ph$	**90,1±8,7**
12	$COOCH_2CH_3$	**11,5±4,9**
15	Ph	**13,9±3,7**

16	*p*-Cl-Ph	**21,6±7,4**
17	*p*-F-Ph	**30,0±6,6**
18	*p*-Br-Ph	**23,2±11,2**
19	*p*-CH_3-Ph	**1,6±6,8**
20	*p*-NO_2-Ph	**58,8±8,4**
21	*o*-CN-Ph	**20,2±7,2**
22	*p*-OMe-Ph	**17,3±4,8**
23	*p*-Cl-Bn	**83,2±8,2**
24	*p*-OH-Ph	**47,2±2,8**
25	3,4-$(Cl)_2$-Ph	**42,5±4,2**
26	*p*-CF_3-Ph	**NR**
28	*o*-F-Ph	**2,1±3,5**
30	Benzo [1.3] dioxol-5yl	**26,0±2,0**
31	Naphthylmethyl	**73,0±6,1**
32	Pyridin-2-yl	**3,7±2,2**
33	-	**-6,0 ± 3,4**
34	4-CH_3	**-3,1 ± 7,4**
35	2-CH_3	**17,1±3,9**
36	3,5-$(CH_3)_2$	**-4,1 ± 3,0**
37	2-CH_2CH_3	**-0,9 ± 3,4**
41	3-CH_2OH	**-0,7 ± 5,2**
42	2-$COOCH_2CH_3$	**-22,6± 10,1**
45	4-COOH	**5,7±0,9**
46	3-COOH	**-2,6 ± 6,9**
47	4-Ph	**NR**
48	4-(*p*-OH-Ph)	**NR**
49	4-Bn	**38,5±5,2**
	IND+LPS	**31,5±6,8**
	L-NAME +LPS	**44,4±6,2**
	NR: No result	

Substitution at the ortho-position showed little activity, with compounds 21, 28, and 29 inhibiting by 20, 2, and 24%, respectively. It appears that the electron-withdrawing or electron-attracting properties of the substituted groups had no effect on bioactivity, since comparable activities were obtained for the p-NO2 and p-OH compounds 20 and 24, the first of which was an extremely effective electron-withdrawing group and the second an excellent electron-donating group. Finally, with 83 and 73% inhibition, respectively, the derivatives of p-chlorobenzyl (23) and naphthylmethyl (31) displayed the greatest findings for this class of compounds, indicating that a methylene spacer could be required for the bioactivity to reach significant levels.NO levels were seen to stay steady, if not rise, in response to treatment with 100µM of the piperidine compounds (33–49). This implies that there are no anti-inflammatory qualities present in these structures. For several of the compounds (11, 17, 19, 20, 21, 22, 23, 24, 28, 29, 31, 31), a range of doses from 6.25 to 100µM was investigated to see whether or not the bioactivities discovered were dose-dependent. The findings are shown in the following table, which groups the compounds according to their activities: the most active compounds (11, 23, 31,), the compounds with inhibition percentages of about 50% (20, 24), the compounds with inhibition percentages of less than 30% (17, 21, 29), and the inactive compounds (19, 22, 28) (Table 5).

Table 5. Reduction % of nitrite concentration for different compound concentrations

NO inhibition from70 to 90%

	11	23	31
100µM	90,1±8,7	83,2±8,2	**73,0±6,1**
50µM	65,5±6,4	26,7±6,8	**66,5±4,5**
25µM	30,2±10,0	-14,2 ± 2,7	**50,3±5,5**
12.5µM	16,9±9,2		**11,4±7,8**
6.25µM	**-16,1 ± 5,2**		**-15,1 ± 5,2**

NO inhibition from 50 to70%

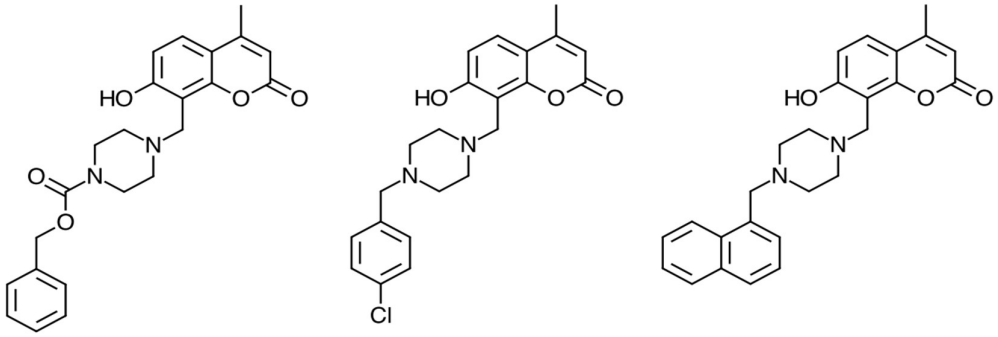
	20	24
100µM	58,8±8,4	**47,2±2,8**
50µM	49,4±3,1	**45,5±2,0**
25µM	**53,3±7,5**	

No Inhibition from 20 -50 %

	17		21
100µM	30,0±6,6		**20,2±7,2**
50µM	23,0±4,9		**2,1±4,0**
25µM	8,5±8,6		
	Inactive Compounds		
	19	**22**	**28**
100µM	1,6±6,8	17,3±4,8	**2,1±3,5**
50µM	**11,9±6,4**	**18,6±4,0**	**0,3±4,1**

The most active compound 11, reduced its inhibitory ability from 90.1% to 65.5% when given at a 50 M dosage. When compound11's concentration fell to 6.25 M, it ceased to prevent inflammation, indicating that the compound's anti-inflammatory properties are dose-dependent. Compound 23's activity was found to drastically decrease, but other than that, it matched compound 11's pattern. At 100 M of this chemical, the reduction in nitrite levels was 83.2%; however, at 25 M, it fell to -14.2%. As for the relationship between the concentration and activity of compound 31, it was found that the nitrite decreases levels did not change significantly up to 25 M, indicating that compound 31 had an activity profile that was slightly distinct from the others. Compound 31 below 25M, however, was discontinued**.**

The results demonstrated that the inhibitory activity of compounds 20 and 24 was unaffected by the concentration used, since the nitrite levels remained rather consistent across all tested concentrations. Compound 29 was completely inert at 50 µM. The discreetly active compounds 17, 21, and 29 caused the nitrite levels in the cells to decrease as expected.
The activity of compounds 22 and 28 will unchanged with concentration as expected. It's interesting to note, nevertheless, that compound 19's anti-inflammatory efficacy increased at 50µM. More studies on this chemical at lower levels might be carried out to evaluate if this unexpected shift in the activity is still visible. As a result of our research, we discovered that substituting a piperidine moiety or alkyl-substituted piperazines into the coumarin scaffold does not result in anti-inflammatory activity, whereas aryl-substituted piperazine heterocycles appear to be appropriate for the development of novel coumarin substituted anti-inflammatory drugs (Fig. 9).



11 (90%) **23** (83%) **31** (73%)

Fig. 9: Structures of the most active compounds: 11, 23, and 31.

Monitoring of PGE_2 Production

The bulk of anti-inflammatory drugs work via the arachidonic acid route. This theory states that inhibition of prostaglandin H2 synthesis stops the making of PGE2, which is responsible for pain perception. As a result, following compounds 11, 20, 24, 25, 31, and 49 shown capable anti-inflammatory actions, their analgesic potential was evaluated. The analgesic impact was evaluated using the prostaglandin E2 Elisa kit, which quantifies prostaglandin E2 (PGE2) synthesis. Compound 23 was eliminated from our investigation because of its cytotoxicity, despite the fact that it also had a strong anti-inflammatory activity. Actually, since their cytotoxicity will restrict the amount of PGE2 that may be created, substances whose cytotoxicity results in availability of less than 70% are unable to offer significant data. The found data are gathered in Table 6.

Table 6. Analgesic activity test results

COMPOUND	PGE_2 PRODUCTION (PG/ML)
11	**2768,6**
20	**3418,9**
24	**2663,6**
25	**2830,7**
31	**3269,7**
49	**3215,8**
%0.5DMSO+ LPS	**3325,84**
IND+LPS	**265,38**
L-NAME+LPS	**3380,54**

Compounds **11, 24**, and **25** did not significantly outperform the reference drug indomethacin in terms of PGE2 production reduction (t-test, $p<0.1$). Compound **11** (PGE2 production 2768.6 pg/mL) showed a minor analgesic effect in addition to its highest anti-inflammatory impact.

1.4 CONCLUSION

1.5 Our objective for this study was to create forty-nine coumarin derivatives per piperazine and piperidine groups substituted at the eighth position. We successfully synthesized thirty-eight, with twenty-two being unique. The toxicity and anti-inflammatory effects of the synthetic substances were investigated. The results indicated that the chemicals synthesized had no anticancer properties. As a result, this study showed no correlation between coumarin derivatives' anti-inflammatory and anticancer characteristics. Piperazine compounds were substantially more active than piperidines in terms of anti-inflammatory activity, as evidenced by the NO inhibition assay.
Compounds 11, 23, and 31 had the greatest anti-inflammatory effect, being 2 to 3 times more potent than the reference indomethacin medicine. Furthermore, the activities of these medications (11, 23, 31) were shown to be dosage dependent. Compound 11 too resulted in a slight decrease in PGE2 generation and modest cytotoxicity. Replacing the eighth position of the coumarin molecule with aryl-substituted piperazine moieties has been effective in developing novel anti-inflammatory drugs.

Conflict of Interest
None declared.

Financial support and sponsorship
Nil

REFERENCES

1. Sarker SD, Nahar L. Progress in the Chemistry of Naturally Occurring Coumarins. In: Kinghorn AD, Falk H, Gibbons S, Kobayashi Ji, editors. Progress in the Chemistry of Organic Natural Products 106. Springer International; 2017: 241-304
2. Liu J-H, Xu S-X, Yao X-S, Kobayashi H. Angelol-type coumarins from Angelica pubescence f. biserrata and their inhibitory effect on platelet aggregation. Phytochemistry 1995; 39(5):1099-101.
3. Bouckaert C, Zhu S, Govers-Riemslag JWP, Depoorter M, Diamond SL, Pochet L. Discovery and assessment of water soluble coumarins as inhibitors of the coagulation contact pathway. Thromb Res. 2017; 157:126-33.
4. Ostrov DA, Hernández Prada JA, Corsino PE, Finton KA, Le N, Rowe TC. Discovery of Novel DNA Gyrase Inhibitors by High-Throughput Virtual Screening. Antimicrob Agents Chemother. 2007; 51(10):3688-98.
5. Kontogiorgis CA, Hadjipavlou-Litina DJ. Synthesis and Antiinflammatory Activity of Coumarin Derivatives. J Med Chem 2005; 48(20):6400-8.
6. Borges F, Roleira F, Milhazes N, Santana L, Uriarte E. Simple Coumarins and Analogues in Medicinal Chemistry: Occurrence, Synthesis and Biological Activity. Curr Med Chem. 2005; 12(8):887-916.
7. González-Gómez JC, Santana L, Uriarte E, Brea J, Villazón M, Loza MI, et al. New arylpiperazine derivatives with high affinity for α1A, D2 and 5-HT2A receptors. Bioorg Med Chem Lett. 2003; 13(2):175-8.
8. Xin-Mei P, Guri LVD, Cheng- He Z. Current Developments of Coumarin Compounds in Medicinal Chemistry. Curr Pharm Des. 2013; 19(21):3884-930.
9. Galkin A, Fallarero A, Vuorela PM. Coumarins permeability in Caco-2 cell model. J Pharm Pharmacol. 2009; 61(2):177-84.
10. Sairam KV, Gurupadayya BM, Chandan RS, Dattatri KN, Vishwanathan B. A Review on Chemical Profile of Coumarins and their Therapeutic Role in the Treatment of Cancer. Curr Drug Deliv. 2016; 13(2):186-201.
11. Chen Y, Wang S, Xu X, Liu X, Yu M, Zhao S, et al. Synthesis and Biological Investigation of Coumarin Piperazine (Piperidine) Derivatives as Potential Multireceptor Atypical Antipsychotics. J Med Chem. 2013; 56(11):4671-90.
12. Mazzei M, Nieddu E, Miele M, Balbi A, Ferrone M, Fermeglia M, et al. Activity of Mannich bases of 7-hydroxycoumarin against Flaviviridae. Bioorg Med Chem. 2008; 16(5):2591-605.
13. Liao JC, Deng JS, Chiu CS, Huang SS, Hou WC, Lin WC, Huang GJ. Chemical compositions, anti-inflammatory, antiproliferative and radical-scavenging activities of Actinidia callosa var. ephippioides. The American journal of Chinese medicine. 2012;40(05):1047-62.
14. Jarrett JM. Synthesis and In-Vitro Cell Viability/Cytotoxicity Studies of Novel Pyrrolobenzodiazepine Derivatives.

15. Kiemer AK, Vollmar AM. Effects of different natriuretic peptides on nitric oxide synthesis in macrophages. Endocrinology. 1997 Oct 1;138(10):4282-90.

16. Wyrzykiewicz E, Wendzonka M, Kędzia B. Synthesis and antimicrobial activity of new (E)-4-[piperidino (4'-methylpiperidino-, morpholino-) N-alkoxy]stilbenes. Eur J Med Chem. 2006; 41(4):519-25.