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Synthesis of novel coumarin derivatives and its biological evaluations

Swayam Sourav Sahoo[1], Smita Shukla[2], Subhangankar Nandy[3*] and Himanshu Bhusan Sahoo[3]

[1]Department of Pharma. Chemistry, Roland Institute of Pharma. Sciences, Berhampur, Orissa, India
[2]Department of Chemistry, Vedica College of Pharmacy, Bhopal, MP, India
[3]Department of Pharmacology, Vedica College of Pharmacy, Bhopal, MP, India

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ABSTRACT

Coumarins possess a number of biological activities like anticoagulant, antimicrobial, anti-inflammatory, analgesic, antioxidant, anticancer, antiviral, antimalarial etc. Coumarin belongs to a group as benzopyrones, which consists of a benzene ring joined to a pyrone nucleus. In the present study, the thirteen new coumarin derivatives are synthesized and characterized by IR and ^{1}H NMR spectra. These newly formed Coumarin derivatives were screened for anti-inflammatory activity by carrageenan induced rat paw edema model and antibacterial activity against Stophylococcus aureus as well as Escherichia coli by cup plate method. The synthesized coumarin derivatives were administered orally in the dose of 10 mg/kg. Ibuprofen and amoxicillin were taken as standard for anti-inflammatory and antibacterial activity respectively. The result of present investigation showed that the compounds 7, 8 & 12 showed significantly ($P < 0.001$) inhibition against Carrageenan induced rat paw edema, but all new compounds(1-13) significantly ($P<0.001$) zone of inhibition against bacterial activity as compared to control group.

***Key words*:** Synthesis of Coumarin derivatives, Anti-inflammatory activity, Carrageenan induced rat paw edema, Zone of inhibition, Antibacterial activity.

INTRODUCTION

Inflammatory diseases are becoming common in aging society throughout the world. Recent studies indicate that the mediators and cellular effectors of inflammation are important constituents of the local environment of tumors [1]. Inflammation in the body's response to noxious or injurious stimuli, characterized by warmth, redness of the skin, pain, swelling and loss of function. It is a part of host defense mechanism. There are several tissue factors that are known to be involved in the inflammatory reactions such as release of histamines, bradykinin and prostaglandins [2].

Coumarins (2H-1-benzopyran-2-ones) are important oxygen containing fused heterocycles used in drugs and dyes [3]. Coumarins be bound their class name to 'coumarou' the vernacular name of the Tonka bean (*Dipteryx odorata willd, Fabaceae)*, from which coumarin itself was isolated in 1820[4]. They are the family of lactones containing benzopyrone skeletal framework that have enjoyed isolation from plant as well as total synthesis in the laboratory

[5]. The incorporation group as a fused component into parent coumarin alters the property of parent coumarin and converts it into a more useful product [6]. Coumarin is plant flavonoids widely distributed in nature. Natural coumarins are known to have antidiabetic activity [7], anabolic antioxidant and hepato protective activities [8]. Substituted coumarins derivatives have been reported to have variety of biological activities. The potent antibiotics like Novobiocin, Coumaromycin and Chartesium are coumarin derivatives. Recently, the interest on these compounds has been revived owing to their use as fluorescent markers in the biochemical determination of enzymes. Coumarin derivatives can be synthesized by one of such methods as the Claisen rearrangement [9], Perkin reaction [10], Pechmann reaction [11], Witting reaction [12], as well as the Knoevnagel condensation [13]. Derivatives of coumarins usually occur naturally as secondary metabolite present in seed, roots and leaves of many plant species [14]. Microwave irradiation has since been proven to be extremely useful for promoting and simplifying many condensation reactions which can be carried out both in solvent and under solvent free condition. The essence of this work was synthesis of coumarin derivatives using microwave irradiation in comparison with conventional methods. These investigations have revealed their potentials as versatile biodynamic agent for example-3-heteroaryl substituted coumarin and benzocoumarins of potential interest as pharmaceuticals and photochromic dyes [15]. Similarly various coumarin chalcones in the solvent free media exhibit high potency as antibacterial agent [3].Introduction of fluoro and sulfonamide moieties into coumarin side chain hoping for an improvement of biological activity because incorporation of fluorine to various heterocycles is known to influence the biological activity [16] and the sulfonamide moiety itself possesses important antibacterial [17] and antitumor activity [18]. Specifically 1, 5 substituted benzothiazepine [19] are well known compounds for diverse therapeutically properties like antimicrobial [20], antihypertensive [21], calcium channel blocker [22], blood platelet aggregation inhibitory [23] and coronary vasodilatory effects [24]. In the present study, we have evaluated the anti-inflammatory and antibacterial activity of some newly synthesized coumarin derivatives.

MATERIALS AND METHODS

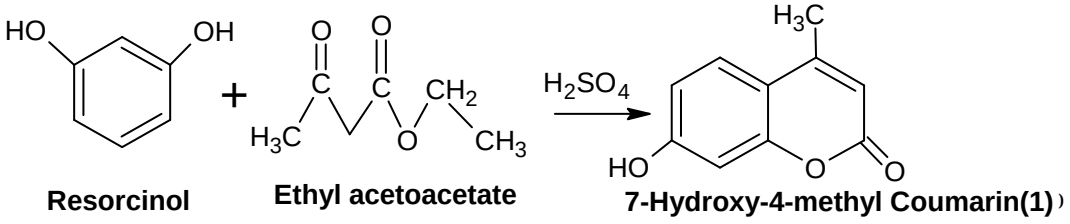
Chemicals and instruments required

The chemicals are used as of analytical grade i.e. Resorcinol, Ethylacetoacetate, Conc. H_2SO_4, 7-hydroxy-4-methyl Coumarin, Conc. HNO_3, benzene, Iron powder, Ethanol, conc. HCl, Pyridine, NaOH, dil. HCl, acetic/ maleic/ succinic/ pthalic anhydride, Glacial acetic acid, benzaldehyde, p-nitrobenzaldehyde, 4-bromo benzaldehyde, 3,4-dichloro benzaldehyde, dichloroacetyl chloride, anhydrous potassium carbonate, chloroacetyl chloride, anhydrous potassium carbonate and acetone.

All the melting points were determined in open capillaries, using Boitus melting point apparatus, expressed in ^{0}C. The IR spectra of the compounds were recorded on Shimadzu IR Affinity FTIR spectrophotometer using KBr discs and the values are expressed in cm^{-1}. The ^{1}H NMR spectra of compounds were recorded on Bruker Avance Ii 400 MHz NMR spectrophotometer using DMSO as an internal standard and the values are expressed in δ ppm. The elemental analyses of the compounds were recorded on Elemental Vario El Iii, Carlo Erba 1108 Elemental analyzer. The structures of synthesized compounds were confirmed by spectral and elemental analysis.

Synthesis of 7-Hydroxy-4-Methyl Coumarin

Procedure: About 150 ml. of conc. H_2SO_4 in a 500 ml beaker was stirred with external ice water cooling until the temperature of acid become about 5 ^{0}C – 10^{0}C. 37 gm of powdered resorcinol was added to 45 ml. of ethyl acetoacetate until a complete solution was obtained. Then this solution was added slowly to H_2SO_4. In such a way that the temperature does not rise above 10^{0}C and the stirring was continued for ½ an hour. The mixture is poured into the ice/cold water & the solid product is separated, filtered out and dried. Then the crude product was recrystallized from ethanol. The resultant Yields: 85%, Melting point: 192 ^{0}C, Molecular Formula: $C_{10}H_8O_3$, Molecular Mass: 176.17 and Solubility: Methanol, Ethanol, Pyridine, H_2SO_4 etc.

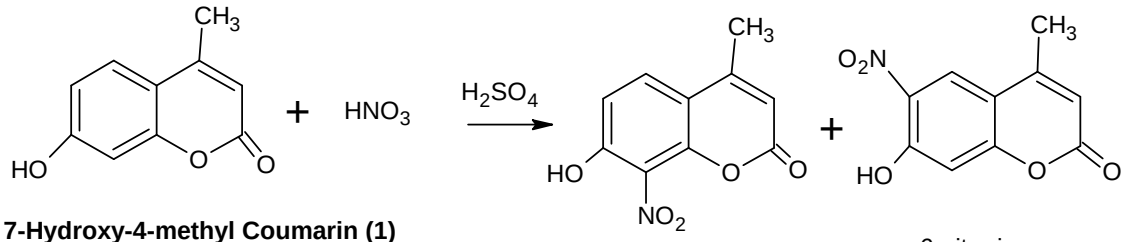


Resorcinol **Ethyl acetoacetate** **7-Hydroxy-4-methyl Coumarin(1)**

Spectral characterization: The IR spectra (in KBr) 7-hydroxy-4-mrthyl Coumarin showed characteristic bands at 1678 cm^{-1} describes the lactone group (C=O), and at 3437 cm^{-1} describes the hydroxyl group (-OH).

Synthesis of 8-nitro-7-Hydroxy-4-Methyl Coumarin

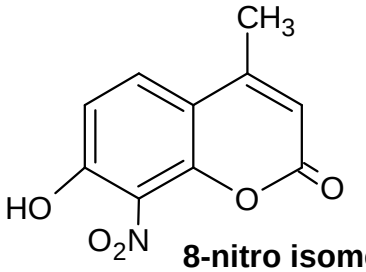
Procedure: The nitration of 7-hydroxy-4-methyl Coumarin using concentrated nitric acid and sulphuric acid at 5^oC gave two nitro isomers i.e. 7-hydroxy-4-methyl-8-nitro Coumarin & 7-hydroxy-4-methyl-6-nitro Coumarin. In a conical flask 7-hydroxy-4-methyl Coumarin (12 gm) was dissolved in conc. H_2SO_4 acid (100 ml.) and then keep the flask in an ice bath. When the temperature inside the flask is below 1^0C, 20 ml of nitrating mixture (5ml of concentrated nitric acid and 15 ml of concentrated sulphuric acid) taking care that the temperature does not rise above 10 ^0C. After the addition was completed, removed the flask from the ice bath and keep it at room temperature for an hour. The flask shaked occasionally during this period and then poured with stirring in a beaker containing crushed ice. The crude product filtered which is a mixture of 6 and 8 nitro derivatives and washed with cold water. Transfer the crude mixture in a conical flask containing ethanol and boiled. The residue is 6-nitro-4-methyl-7-hydroxy coumarin, m.p 260-262 ^0C. Concentrated the filtrate, and cooled in an ice bath, 8-nitro derivative soon crystallized out. Recrystallized from ethanol and collect 8-nitro-4-methyl-7-hydroxy coumarin, m.p 255-256 ^0C. Yields: 60%, Melting point: 255-256 ^0C, Molecular Formula: $C_{10}H_7NO_5$, Molecular Mass: 221.17.



7-Hydroxy-4-methyl Coumarin (1)

8-nitro isomer(2)

6-nitro isomer

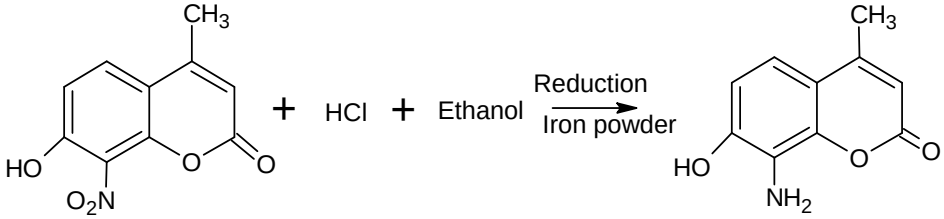


8-nitro isomer (2)

Spectral characterization: The IR spectrum (KBr) of 8-nitro isomer showed characteristic bands at 1726 cm^{-1} (lactone C=O), and at 3284 cm^{-1} (OH).

Synthesis of 8-Amino-7-hydroxy-4-methyl Coumarin

Procedure: Iron powder (8 gm) was added portion wise with stirring to a hot mixture of 8-nitro-7-hydroxy-4-methyl Coumarin (4.4 gm, 0.02 moles) in ethyl alcohol (20 ml) and concentrated hydrochloric acid (30 ml) at reflux temperature. After completion of the addition, the refluxing was continued for 6 hours. Upon cooling a white precipitate formed, which was filtered off, washed with water, dried and recrystallized. Yields: 50%, Melting point: 280 ^0C, Molecular Formula: $C_{10}H_9NO_3$, Molecular Mass: 191.2, Solubility: Pyridine, 10% aq NaOH, DMF, DMSO etc.



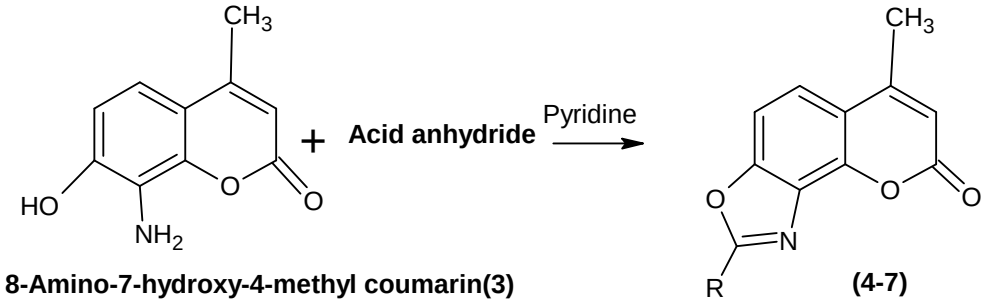
8-Nitro-7-hydroxy-4-methyl Coumarin(2)

8-Amino-7-hydroxy-4-methyl coumarin (3

Spectral characterization: The IR spectra showed bands at 3084 cm^{-1} (amine -NH_2), broad band at 3287 cm^{-1} (hydroxyl -OH) and bands 1710 cm^{-1} (lactone -C=O). ^{1}H-NMR of **3** in DMSO showed signals of 2.45 ppm (3H, s, CH_3), bands at 7.0 - 7.6 ppm (3H, dds, aromatic protons) and bands at 11.8 (1H, broad OH).

Synthesis of 6-methyl-2-substituted-8H-pyrano [2, 3-e] benzoxazol-8-ones

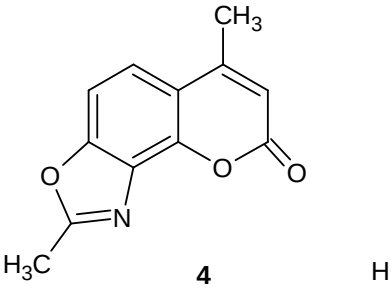
Procedure: To a solution of 3rd synthetic compound (0.42 gm, 0.002 mole) in pyridine (10 ml), an appropriate acid anhydride (0.002 mole), namely, acetic anhydride, maleic anhydride, succinic anhydride and pthalic anhydride was added. The reaction mixture was refluxed for 10 hours, the pyridine was distilled under reduced pressure, and the residue was washed with water and dissolved in sodium hydroxide solution (5%, 10 ml). The reaction mixture was filtered off and neutralized by dilute hydrochloric acid. The precipitate formed was filtered off, washed with water, dried and recrystallized. (4-7). The compound of **4, 5, 6, 7** having yields of 52%**,** 67%**,** 58%**,** 82% respectively; Melting point of > 300 ^{0}C**,** 283 ^{0}C**,** 260 ^{0}C**,** >300^{0}C respectively; Molecular Formulas were $C_{12}H_9NO_3$, $C_{14}H_7NO_5$, $C_{14}H_{11}NO_5$, $C_{18}H_{11}NO_5$ respectively; Molecular Mass were 215.2, 271.22, 273.24, 320.0 respectively and Solubility in DMF, DMSO.



8-Amino-7-hydroxy-4-methyl coumarin(3) **(4-7)**

R=
-CH_3 (Acetic anhydride)
-CH=CH-COOH (Maleic anhydride)
-CH_2-CH_2-COOH (Succinic abhydride)
-C_6H_4-COOH (Pthalic anhydride)

4: 6-methyl-2-methyl-8H-pyrano [2, 3-e] benzoxazol-8-ones
5: 6-methyl-2-propeonate-8H-pyrano [2, 3-e] benzoxazol-8-ones
6: 6-methyl-2-propanoate-8H-pyrano [2, 3-e] benzoxazol-8-ones
7: 6-methyl-2-[p-benzoate]-8H-pyrano [2, 3-e] benzoxazol-8-ones



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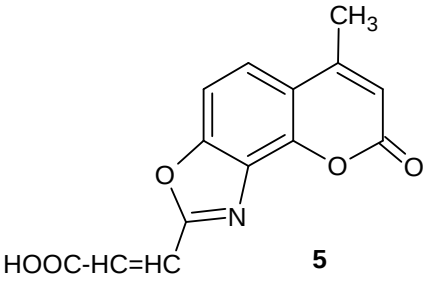

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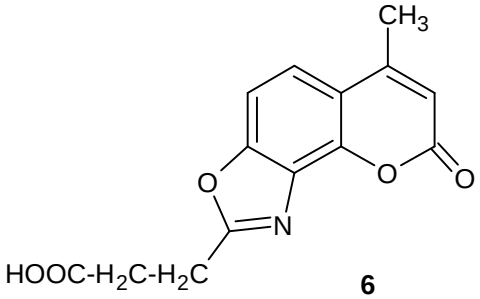

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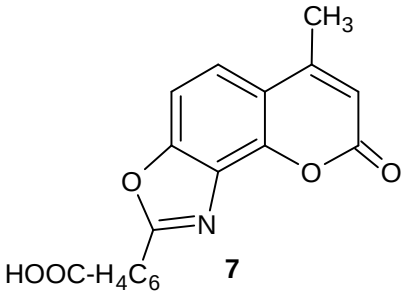

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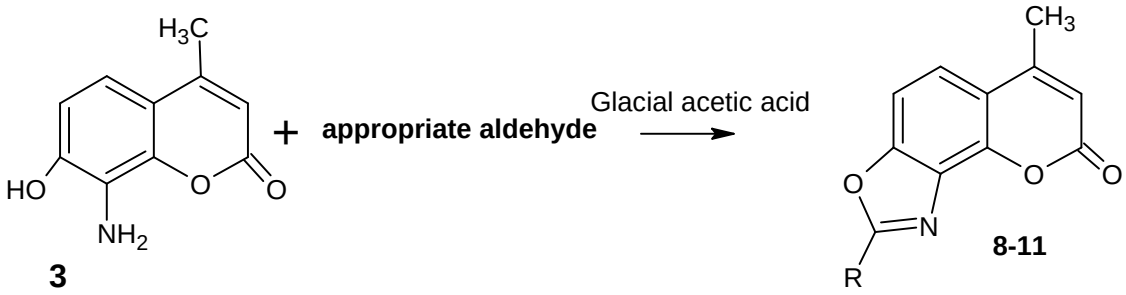
Spectral characterization: The IR spectrum of **(7)** showed characteristic bands at 1624 cm^{-1} (C=N), 1693 cm^{-1} (lactone C=O) and 3284 cm^{-1} (acid OH). The ^{1}H-NMR spectrum in DMSO, **(7)** showed signals at 2.42 ppm (3H, s, CH_3), 6.14, 7.02 and 7.04 ppm (3H, sdd, Coumarin protons), 7.5-7.8 ppm (4 H, m, aromatic protons) and at 11.6 ppm (1H, broad OH).

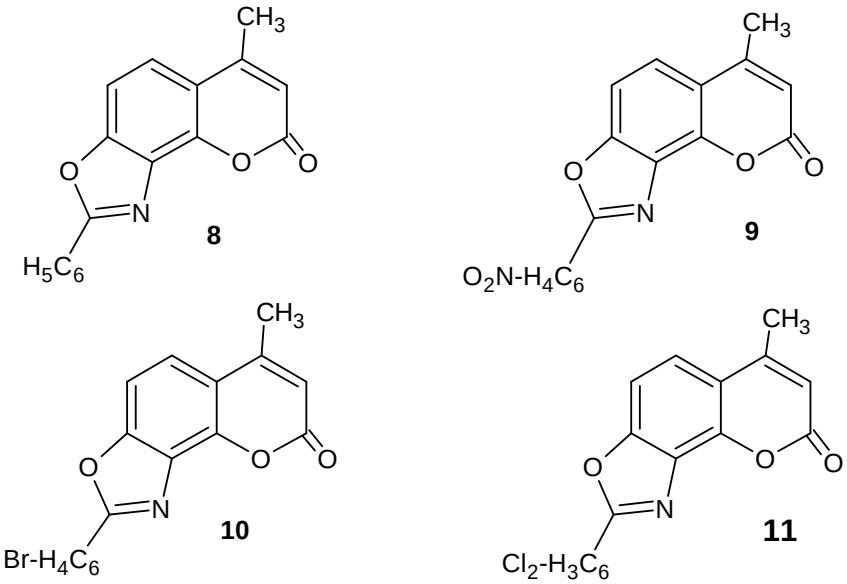
Synthesis of 6-methyl-2-substituted-8H-pyrano [2, 3-e] benzoxazol-8-one

Procedure: To a solution of **3** (0.42 gm, 0.002 mole) in glacial acetic acid (20 ml), and the appropriate aldehyde namely, benzaldehyde, p-nitrobenzaldehyde, 4-bromo benzaldehyde, 3,4-dichloro benzaldehyde (0.002mole) was refluxed for 15 hours, cooled, poured into ice/cold water. The precipitate formed was filtered off and recrystallized. **(8-11)**



R=
- C_6H_5 (Benzaldehyde)
- C_6H_4-NO_2 (p-nitro benzaldehyde)
- C_6H_4-Br (4-bromo benzaldehyde)
-C_6H_4-Cl_2 (3,4-dichloro benzal dehyde)

8: 6-methyl-2-benzayl-8H-pyrano [2, 3-e] benzoxazol-8-ones
9: 6-methyl-2-[p-nitro benzayl]-8H-pyrano [2, 3-e] benzoxazol-8-ones
10: 6-methyl-2-[p-bromo benzyl]-8H-pyrano [2, 3-e] benzoxazol-8-ones
11: 6-methyl-2-[3', 4'-dichloro benzayl]-8H-pyrano [2, 3-e] benzoxazol-8-ones

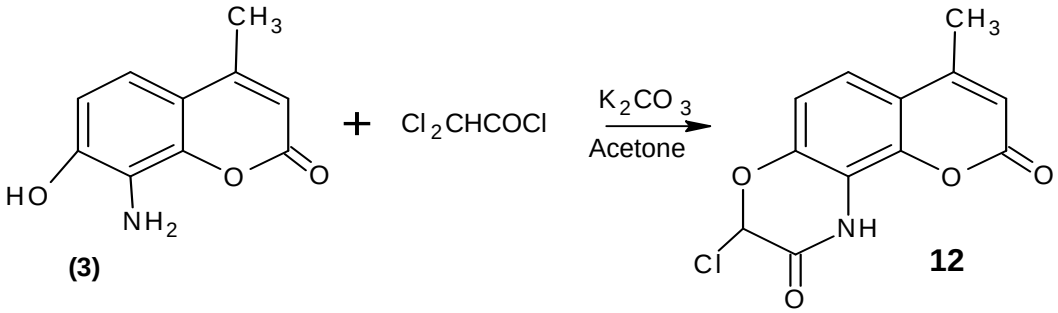


The compounds of **8, 9, 10, 11** having Yields 56%**,** 82%**,** 45% **,** 65% respectively; Melting point having 232 ^{0}C**,** 245 ^{0}C**,** 253 ^{0}C**,** 220 ^{0}C respectively; Molecular Formula having $C_{17}H_{11}NO_3$, $C_{17}H_{10}N_20_5$, $C_{17}H_{10}BrNO_3$, $C_{17}H_9C_{l2}NO_3$ respectively; Molecular Formula having 277.24, 322.28, 356.17, 346.16 respectively and Solubility in DMF, DMSO.

Spectral characterization: The IR spectrum (KBr) showed characteristic bands at 1625 cm^{-1} (C=N) and at 1720 cm^{-1} (lactone C=O). ^{1}H-NMR spectrum (DMSO) showed signal at 2.42 ppm (3H, s, CH3), at 6.13, 7.0 and 7.5 ppm (3H, dds, coumarin protons) and at 11.5 ppm (1H, broad OH).

Synthesis of 3-chloro-7-methyl-9H-pyrano [2, 3-e] benzo-1, 4-oxazine-2, 9-Dione

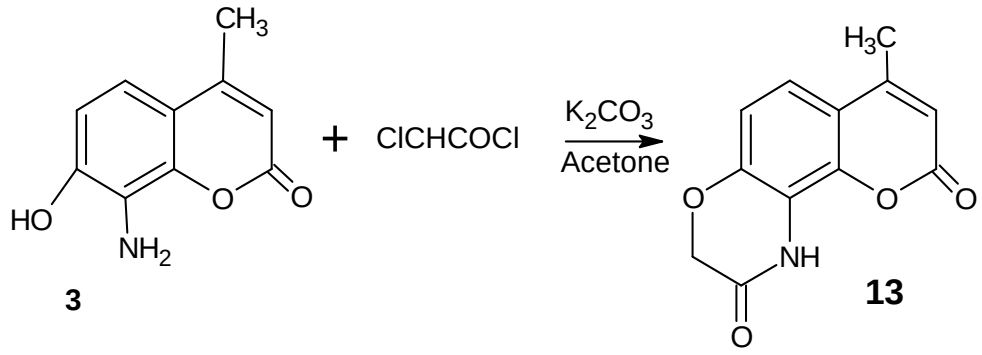
A mixture of **3** (0.44 gm, 0.002 moles), dichloroacetyl chloride (0.2 ml, 0.002 moles) and anhydrous potassium carbonate (0.5 gm) in acetone (20 ml) was refluxed for 10 hours. The reaction mixture was cooled and poured into ice/cold water. The precipitate formed was filtered off and recrystallized. Yields: 95%, Melting point: 262 ^{0}C, Molecular Formula: $C_{12}H_8ClNO_4$, Molecular Mass: 264.52, Solubility: Ethanol, $CDCl_3$, DMSO etc.



Spectral characterization: The IR spectrum of **(12)** showed characteristic bands at 3284 cm^{-1} (NH), 1720 cm^{-1} (lactone C=O) and 1624 cm^{-1} (NHCO).

Synthesis of 7-methyl-9H-pyrano [2, 3-e] benzo-1, 4-oxazine-2, 9-Dione

Procedure: A mixture of **3** (0.44 gm, 0.002 mole), chloroacetyl chloride (0.17 ml, 0.002 mole) and anhydrous potassium carbonate (0.5 gm) in acetone (20 ml) was refluxed for 3 hours, cooled then the reaction mixture was cooled and poured into ice/cold water. The precipitate formed was filtered off and recrystallized. Yields: 85%, Melting point: 285 ^{0}C; Molecular Formula: $C_{12}H_9NO_4$, Molecular Mass: 230.1, Solubility: Ethanol, $CDCl_3$, DMSO etc.



Spectral characterization: The IR spectrum of (13) showed characteristic bands at 3284 cm^{-1} (NH), 1726 cm^{-1} (lactone C=O) and 1620 cm^{-1} (NHCO). The ^{1}H-NMR spectrum in DMSO showed signal 2.42 ppm (3H, s, CH_3), 6.15, 7.0 and 7.5 ppm (3H, sdd, Coumarin protons) and at 11.7 ppm (1H, broad, NH).

Biological Evaluation

In view of varied biological importance of different series of coumarin derivatives, it is felt worthwhile in evaluate them for possible activities. The newly formed synthesized compounds were screened for anti-inflammatory and antibacterial activity.

Animals and Treatment

Healthy male rats (Wistar albino) of 4-8 weeks old were selected after physical and behavioural veterinary examination from Institutional Animal House of Roland Institute of Ph. Sciences, Berhampur, Orissa. All experiments involving animals complies with the ethical standards of animal handling and approved by Institutional Animal ethics committee (IAEC Regd. No: 926/ab/06/CPSCSEA). The weight range was fall within ± 20% of the

mean body for each sex at the time of initiation of treatment. The animals were kept in polypropylene cages (six rats per cage) and maintained on a standard laboratory diet and water ad libitum. They were housed in an air-conditioned room with 12:12 h light and dark cycle at least 7 day prior to experiment. The room temperature (about 23ºC) and humidity (about 60%) were controlled automatically.

Anti-inflammatory activity

The compounds were tested for anti-inflammatory activity by Carrageenan induced rat paw oedema model [25]. Inflammation was induced by injecting 0.05 ml of 1% carageenan suspension subcutaneously into the sub plantar region of the left hind paw and 0.05 ml of saline was injected into the sub plantar region of the left hind paw for all groups. Albino rats of either sex were divided into 15 groups of six animals each. One hr. prior to carageenan injection, the groups III to XV treated with new Coumarin derivative (1 to 13) administered 10 mg/kg, DMSO was given to group-I used as carageenan treated control and standard drug Ibuprofen (5mg/kg) was administered to group-II. All the doses were administered orally. Anti-inflammatory activity was evaluated by measuring carageenan induced rat paw oedem before carageenan injected and after carageenan injection of time intervals 1^{st}, 2^{nd} and 3^{rd} hour using Plethysmometer. The percent increase of paw oedema volume [26, 27] was determined at 1^{st}, 2^{nd} and 3^{rd} hrs after induction of inflammation.

The percent inhibition of paw oedema volume is calculated using the formula,

$$\text{Percent inhibition} = 1 - \frac{Y_t}{Y_c} \times 100$$

Where, Y_t= Average increase in paw volume in groups tested with test compounds.
Y_c= Average increase in paw volume in control

The results and statistical analysis of anti-inflammatory activity of Ibuprofen and the compounds tested are shown in tables [28, 29].

Antibacterial activity

The antibacterial activity of newly synthesized Coumarins was conducted against Gram positive bacteria i.e. *Stophylococcus aureus* and Gram negative bacteria i.e. *Escherichia coli* by using cup plate method. [30, 31, 32] Amoxicillin was employed as reference standard to compare the results. Nutrient broth was used for the preparation of inoculation of the bacteria and nutrient agar was used for the screening methods.

Each test compound (5 mg) was dissolved in dimethyl sulphoxude (DMSO) (5ml) at a concentration of 1000 µg/ml. amoxicillin solution were also prepared at a concentration of 1000 µg/ml in sterilized distilled water. All the compounds were tested at a concentration of 0.05 ml (50 µg) and 0.1 ml (100 µg) level and DMSO used as a control. The solutions of each test compound, control and references standards (0.05 and 0.1 ml) were added separately in the cups and the plates were kept undistributed for at least 2 hours in refrigerator to allow diffusion of the solution properly into nutrient agar medium. Petridish were subsequently incubated at 37 ± 1^0C for 24 hours. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader. All the experiments were carried out in triplicates and compared to control.

Statistical analysis

Results were expressed as the Mean ± standard error means (S.E.M.). The comparison of data within groups was performed by the analysis of variance using ANOVA test. Significant difference between control and experimental groups was assessed by Dunnett's test. A probability level of less than 1 % ($P < 0.01$) was considered significant. The statistical analysis was made by using Systat 7.0.

RESULTS AND DISCUSSION

Table 1: The Melting Point, % of yield, molecular formula, molecular mass of new synthesized compounds.

Comp. No.	Melting Point	Yield (%)	Molecular formula	Molecular mass
1	192	85	$C_{10}H_8O_3$	176.17
2	255	60	$C_{10}H_7NO_5$	221.17
3	280	50	$C_{10}H_9NO_3$	191.2
4	>300	52	$C_{12}H_9NO_3$	215.2
5	283	67	$C_{14}H_7NO_5$	271.22
6	260	58	$C_{14}H_{11}NO_5$	273.24
7	>300	82	$C_{18}H_{11}NO_5$	320.0
8	232	56	$C_{17}H_{11}NO_3$	277.24
9	245	82	$C_{17}H_{10}N_20_5$	322.28
10	253	45	$C_{17}H_{10}BrNO_3$	356.17
11	253	65	$C_{17}H_9C_{l2}NO_3$	346.16
12	262	95	$C_{12}H_8ClNO_4$	264.52
13	285	85	$C_{12}H_9NO_4$	230.1

Table 2: The Elemental analysis of new synthesized compounds.

Compound No.	Elemental Analysis % (Calculated/found)		
	C	H	N
1	68.18 / 66.52	4.54/4.81	0.00/0.14
2	51.29/48.83	3.21/3.39	6.3/6.8
3	62.8/62.21	4.7/3.47	6.32/6.11
4	66.9/64.12	3.97/3.41	6.52/6.3
5	61.9/53.73	2.71/3.61	5.2/6.0
6	61.00/54.5	4.0/3.4	5.1/6.2
7	57.2/54.7	3.58/3.44	4.38/6.31
8	73.6/72.65	3.97/3.82	5.95/6.16
9	63.35/63.29	3.10/3.71	8.8/9.05
10	57.3/50.29	2.81/3.61	3.93/4.17
11	58.9/52.1	2.6/3.58	4.04/5.92
12	54.52/53.21	3.23/3.5	5.3/6.3
13	62.6/45.31	3.9/3.8	6.0/5.4

Table 3: Effect of coumarin derivatives (1-13) on % of inhibition after 1st, 2nd and 3rd hour in 1% Carrageenan-induced rat paw oedema

Compound	% of inhibition		
	After 1 hr	After 2 hr	After 3 hr
1	2.54 ± 0.066	5.46 ± 1.765	13.22 ± 0.870
2	1.37 ± 0.0987	4.65 ± 1.263	8.75 ± 1.381
3	1.25 ± 0.165	6.3 ± 1.793	27.88 ± 1.499*
4	3.67 ± 0.023	8.41 ± 2.42	11.97 ± 1.527
5	-	3.97 ± 0.254	7.21± 1.583
6	-	-	2.87 ± 1.618
7	6.25 ± 0.89	11.05 ± 1.92	39.7 ± 1.758**
8	8.75 ± 0.543*	14.73 ± 0.764*	42.8 ±2.213**
9	-	-	3.1 ± 2.425
10	-	-	1.7± 1.983
11	2.1 ± 0.879	8.65 ± 0.893	13.82 ± 1.487
12.	11.25 ± 0.781**	22.63 ± 0.223**	49.33 ± 1.543**
13	3.84 ± 0.553	7.12 ± 0.0	12.21 ± 1.251
Ibuprofen	20.8 ± 0.342	35.7 ± 1.994	57.14 ± 1.769
Control	-	-	-

*All values were represented as Mean ±SEM, Where N= 6; Dunnett´s test were used for testing the significance difference in variable that passed. The threshold of Statistical significance was set at * P < 0.01 and ** P < 0.001 versus control.*

The anti-inflammatory activity of the some newly synthesized Coumarins has been evaluated by using Carrageenan-induced rat paw oedema method [33, 34]. These three compounds (7, 8 and 12) showing significantly inhibition (P < 0.001) after three hrs of carrageenan induction. The results of the evaluation have been viewed by taking Ibuprofen as the standard drug and were represented in **Table 3.** The compound 12 showed maximum antiiflammatory effect on time dependant study and this may due to the presence of chlorine at 3 positions, methyl at 7 positions on the

aromatic ring of the coumarin respectively. It was also observed that the compound 7 & 8 carrying methyl group at 6 positions respectively, showed moderate activity. The initial phase is due to the release of histamine, serotonin and kinin in the first hour after the administration of carrageenan, a more pronounced second phase is attributed to release of bradykinin, prostaglandin and lysosome. The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents [35-44].

Table 4: Antibacterial activity of Coumarin Derivatives

Compounds	Zone of inhibition (in mm)	
	S. aureus	***E. coli***
1	16 ± 1.29 **	18 ± 1.91**
2	17 ±1.93 **	20 ± 1.43 **
3	17 ± 1.74 **	18 ± 1.21 **
4	18 ± 1.87 **	16 ± 1.54 **
5	16 ± 1.06 **	17 ± 1.3 **
6	19 ± 1.21**	16 ± 1.76 **
7	19 ± 1.39 **	17 ± 1.09 **
8	17 ± 1.21**	18 ± 1.23 **
9	18 ± 1.65 **	19 ± 1.98 **
10	21 ± 1.26 **	21 ± 1.65 **
11	23 ± 1.43 *	24 ± 1.43 **
12	22 ± 1.6*	27 ± 1.54 **
13	17 ± 1.04**	17 ± 1.61 **
Amoxicillin	29 ± 1.32	36 ± 1.43

*All values were represented as Mean ±SEM, Where N= 3; Dunnett´s test were used for testing the significance difference in variable that passed. The threshold of Statistical significance was set at * P < 0.01 and ** P < 0.001 versus control (Amoxicillin).*

The derivatives were merely active against bacteria which support the role of Coumarins as defensive compounds. All the compounds **(1-13, 45, 46 and 47)** have been evaluated for their antibacterial activity against ***Staphylococcus aureus*** (Gram positive) and ***Escherichia coli*** (Gram negative), using agar cup-plate method. The results Showing Significant zone of inhibition ($P < 0.001$) as compared with Amoxicillin (standard). The antibacterial activity results were presented in **Table 4.** In particular, compounds **11 & 12** possessed maximum activity which may due to presence of chlorine on aromatic ring of Coumarins. Other compounds also showed mild to moderate activity at 0.1 ml concentration level on all organisms.

CONCLUSION

The result of present study indicates that Compound 12 ($C_{12}H_8ClNO_4$) possess maximum anti inflammatory as well as antibacterial activity among all new synthesized products of coumarin. However further studies are needed to establish molecular mechanisms, which are responsible for these biological activity.

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