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Synthesis and Screening of Anti-inflammatory activity of Coumarins bearing oxy acetic acid moiety

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A B S T R A C T

Coumarins and its derivatives are considered as the most active classes of heterocycles, which possess a broad spectrum of biological activity. They have been proven to be active as antibacterial, antifungal, anti-inflammatory, anti-depressant, anti-HIV and antitumor agents. Moreover, Coumarin and its related derivatives have been used as inhibitors of lipoxygenase (LOX) and cyclooxygenase (COX) pathways of arachidonic acid metabolism. In the present study, we have evaluated the anti-inflammatory and antimicrobial activity of some newly synthesized Coumarin derivatives. In search for new anti-inflammatory agents with improved safety profiles, some new 7-hydroxycoumarin derivatives bearing functionalized aryl oxy acetic acid moieties were synthesized. This prompted us to synthesize a series of novel Coumarin derivatives with oxy acetic acid moiety and evaluate their anti-inflammatory activity. The structures of the final newly synthesized compounds were confirmed from IR, ^{1}HNMR and Mass spectra. Among the newly synthesized compounds,3e, 3f, 3g, 3h, 3i and 3j showed maximum anti-inflammatory activity in comparison to standard drug Ibuprofen.

Keywords: 7-hydroxy-4-methyl Coumarin, Oxy acetic acid moiety, Anti-inflammatory activity, Rat paw edema model, IR, NMR.

A R T I C L E I N F O

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1. Introduction

The literature survey reveals that derivatives of Coumarins and aryl oxy-acetic acidpossess versatile biological activities such as antibacterial, antifungal, anti-inflammatory, anticancer, antiviral, antioxidant. Coumarins and its related derivatives have been used as inhibitors of lipoxygenase (LOX) and cyclooxygenase (COX) pathways of arachidonic acid metabolism. In the Present work we aim to design novel Coumarin derivatives linked with oxy acetic acid moiety. To establish the scheme for synthesis of selected designed molecules. To achieve the synthesis of series of 7-hydroxy,4-methyl Coumarin derivatives. To characterize the synthesized compound by melting point, thin layer chromatography and spectral analysis like nuclear magnetic resonance spectroscopy, infrared spectroscopy and mass spectroscopy. To perform biological screening of the synthesized compounds for anti-inflammatory activity.[1,2,3]

2. Experimental

All reagents were used as received from commercial sources without purification. Resorcinol, Ethyl acetoacetate, conc. Sulphuric acid, Piperidine (catalytic grade), Benzaldehyde and its substituted derivatives were obtained from PDVVPF's College of Pharmacy. All chemicals used were of L.R. grade. The experimental protocol for anti-inflammatory activity using rat paw edema model was approved by Institutional Animal Ethics Committee (IAEC) with Ref. PDVVPF'S COP/IAEC/ PG08/2016 and conducted according to guidelines for the use and care of animals according to CPCSEA guidelines.

Chemistry:

Melting points of the newly synthesized compounds were with a Veego electronic melting point apparatus (model VMP-D).^{1}HNMR spectra were recorded with the aid of BRUKER AVANCEV II 400 NMR spectrometer in DMSO as solvent and TMS as internal standard, chemical shifts are given in ppm. The IR spectra of compounds were recorded on a SHIMADZU Happ-Ganzel spectrometer at 4cm^{-1} frequency. The mass spectra was obtained on WATERS Q-TOF MICROMASS (LC-MS). Progress of reaction was monitored by Thin Layer Chromatography (TLC) using glass plates pre-coated with Silica Gel-G.

Procedure for Synthesis

Step-I:

General procedure for the Synthesis of 7-hydroxy-4-methyl Coumarin: About 134ml. of conc. H_2SO_4 in a 250 ml beaker was stirred with external ice water cooling until the temperature of acid become about 5^0C-10^0C. 33 gm. (0.01mol.) of powdered resorcinol was added to 35 ml.(0.01 mol.) 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 10^0C 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: 192^0C, Molecular Formula: $C_{10}H_8O_3$, Molecular Mass: 176.17 and Solubility: Methanol, Ethanol, Pyridine, H_2SO_4, etc. [5]

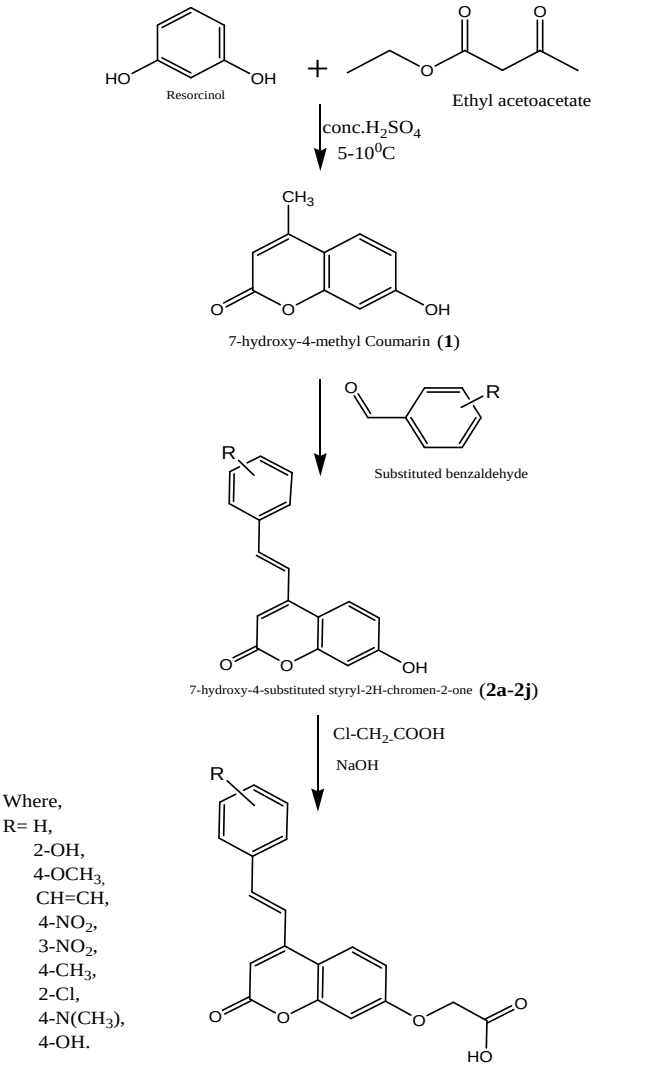


Figure 1: Scheme of synthesis

Step-II:

General Procedure for the Synthesis of Substituted 7-hydroxy-4-styryl-2H-chromen-2-one derivatives:

Equimolars from 7-hydroxy-4-methyl Coumarin (0.01mol.) and aromatic aldehydes (Substituted benzaldehyde) (0.01mol.) were fused together in the presence of catalytic amount of piperdine for about 2 hrs. (120-130°C). After the reaction has finished, the mixture was cooled, treated with ethanol and poured onto ice/water. The formed precipitate was filtered out and recrystallized from ethanol. Melting points of the synthesized compounds were determined and were recorded. [6]

Step-III:

General Procedure for the Synthesis of Substituted 2-[2-oxo-4-styry l-2H-chromen-7yl] oxy acetic acid

derivatives: To A mixture of 1 gm. Of compound and 3.5ml of 33% NaOH solution a test tube, 2.5 ml of chloro acetic acid solution was added and heated gently on boiling water bath for an hour. After cooling the mixture was cooled and diluted with 10 ml of water and acidified to Congo red with dil. HCl. It was extracted with 10 ml of ether. The ethereal extract was washed with 2.5 ml of sodium carbonate solution and acidified with dil. HCl to Congo red. The aryl oxy acetic acid derivatives which separate out were collected and were recrystallized from aq. Ethanol. Melting points of newly synthesized compounds were determined and were recorded uncorrected. [7]

IR spectrum interpretation data:

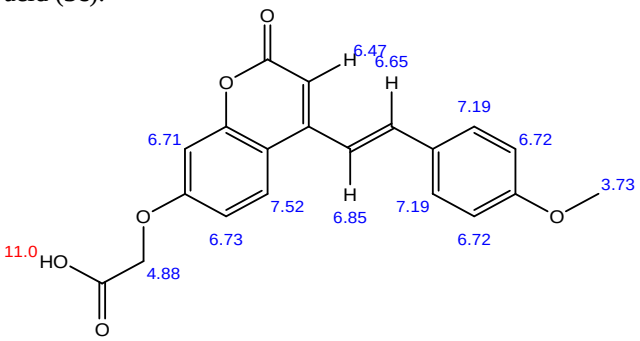
- 2-[2-oxo-4(4-methoxystyryl)-2H-chromen-7-yl] oxy acetic acid (**3c**), 3317cm^{-1}(CH=CH), 2993cm^{-1}(C-H), 1672 cm^{-1} (C=O), 1511cm^{-1}(C=C), 3025cm^{-1}(OCH3).
- 2- [2- oxo -4(4-phenyl buta-1,3 dienyl)-2H-chromen-7-yl]oxy acetic acid (**3d**),3317cm^{-1}(CH2), 2989cm^{-1}(C-H), 1672cm^{-1}(C=O),309cm^{-1}(C-H)(stretch), 1176cm^{-1}(CH3).
- 2-[2-oxo-4(-4-nitro styryl)-2H-chromen-7-yl] oxy acetic acid (**3e**).1062cm^{-1}(NO_2), 1384cm^{-1} (C-NO_2), 3051cm^{-1}(C-H), 2922cm^{-1}(C-H), 2358cm^{-1}(C-H)(stretch).
- 2-[2-oxo-4(4-methyl styryl)-2H-chromen-7-yl] oxy acetic acid (**3g**). 2358cm-1(C-H) (stretch), 2970cm^{-1}(CH), 33340 cm^{-1} (O-H), 1705cm^{-1}(CO), 1514cm^{-1}(C-H).
- 2-[2-oxo-4-(2-chloro styryl)-2H-chromen-7-yl]oxy acetic acid (**3h**). 800cm^{-1}(C-Cl), 3319cm^{-1} (CH=CH), 3097cm^{-1}(C-H)(stretch), 746cm^{-1}(C-Cl), 1674cm^{-1}(C=O).

^{1}H NMR Spectrum interpretation:

^{1}H NMR Spectrum interpretationof2-[2-oxo-4(4-methoxy styryl)-2H-chromen-7-yl] oxy acetic acid (**3c**). 6.5-7.5δ ppm [m, 1H, C-H (Ar-H)], 3.7δ ppm [s,3H, C-H (methyl)], 6.8δ ppm [d,2H, C-H(ethylene)], 8.7δ ppm [s,1H, O-H(carboxylic)], 4.7δ ppm [d, 2H, C-H(methylene)].

^{1}HNMR spectra of selected compound:

2-[2-oxo-4(4-methoxy styryl)-2H-chromen-7-yl] oxy acetic acid (**3c**).



Mass Spectra:

Mass Spectra of 2-[2-oxo-4-styryl-2H-chromen-7-yl] oxy acetic acid (**3a**). 328(100%) M^{+1}Peak, Molecular weight was found to be 328 which is almost equal to calculated molecular weight 322.

Procedure:

Anti-inflammatory activity:

The experimental protocol was approved by Institutional Animal Ethical Committee with Ref. PDVVPF'S COP/ IAEC/PG08/2016 and conducted according to guidelines for the use and care of animals. All the synthesized compounds were subjected to Anti-inflammatory activity using carrageenan induced rat paw edema model.

1. Adult, healthy Wister rats of either sex of weight 200-250 gm. were randomly assigned to three groups (n=6), weighed and marked.

a. GROUP-I (Control):0.05ml of 1% carrageenan suspension was given s/c in the sub planter region of left hind paw.

b. GROUP-II (Standard): 0.05ml of 1% carrageenan suspensionwas given s/c in the sub planter region of left hind paw + Ibuprofen at a dose of 5mg/kg was given.

c. GROUP-III (Test): 0.05ml of 1% carrageenan suspensionwas given s/c in the sub planter region of left hind paw + 10mg/kg test compound was given.

2. Paw edema was induced by sub plantar injection of 0.05 ml of carrageenan solution into the left hind paw of rats of all groups.
3. All the other doses were administered orally. Anti-inflammatory activity was evaluated by measuringCarrageenan induced rat paw edema before carrageenan injected and after carrageenan injection of time interval of 3rd hour using Plethysmometer.[14]
4. The percent increase of paw edema volume was determined at 3rd hr. after induction of inflammation.
5. The percent inhibition of paw edema volume is calculated using the formula,
   Percent inhibition =V_c- V_t /V_c*100
   Where, V_t= Average increase in paw volume in groups tested with test compounds.
   V_c= Average increase in paw volume in control
6. The results and statistical analysis of anti-inflammatory activity of Ibuprofen and the compounds are expressed as mean ± SEM.[4-17]

Antimicrobial Activity

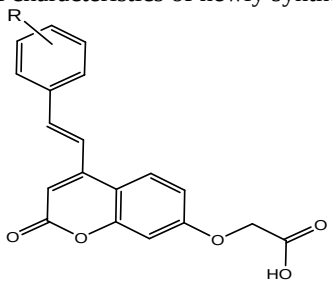
The antimicrobial activity of newly synthesized Coumarins was conducted against Gram positive bacteria i.e. Staphylococcus aureus and Gram negative bacteria i.e. Escherichia coli by using cup plate method. Streptomycin and Benzyl penicillin were employed as reference standard to compare the results. 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 sulphoxide (DMSO) (5ml) at a concentration of 1000 µg/ml. Streptomycin and Benzyl penicillin 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

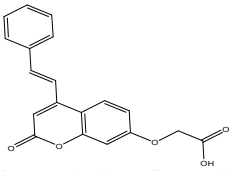
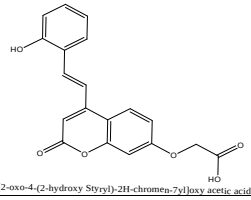
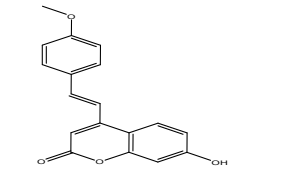
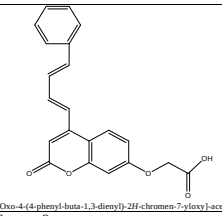
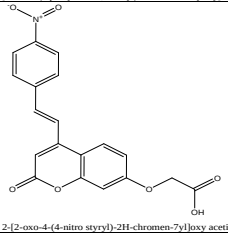
nutrient agar medium. Petridishes were subsequently incubated at 37±10C for 24 hours. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader. All the experiments were carried out in duplicates and compared to standard. [4-7,10,14-18]

3. Results and Discussion

Drugs that can suppress the effect of inflammation in rat paw can be considered as significant. The results of anti-inflammatory activity of the synthesized compounds are represented below:

Table-1: Physicochemical characteristics of newly synthesized compounds (3a-3j):



Code	Structures	Molecular Formula	Molecular weight	Melting point (^{0}C)	Yield (%)	R_f value
3a	 2-[2-oxo-4- Styryl-2H-chromen-7yl]oxy acetic acid	$C_{20}H_{16}O_5$	322.31	>350	70.20	0.4
3b	 2-[2-oxo-4-(2-hydroxy Styryl)-2H-chromen-7yl]oxy acetic acid	$C_{19}H_{14}O_6$	338.31	>350	68.25	0.45
3c	 7-hydroxy-4-(4methoxy-styryl)-2H-chromen-2-one	$C_{20}H_{16}O_6$	352.34	>350	55.90	0.44
3d	 [2-Oxo-4-(4-phenyl-buta-1,3-dienyl)-2*H*-chromen-7-yloxy]-acetic acid	$C_{21}H_{16}O_5$	348.34	198-200	65.75	0.41
3e	 2-[2-oxo-4-(4-nitro styryl)-2H-chromen-7yl]oxy acetic acid	$C_{19}H_{13}NO_7$	367.31	256-258	70.13	0.47
3f		$C_{19}H_{13}NO_7$	367.31	260-261	72.17	0.43

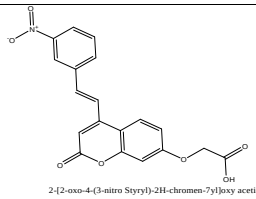
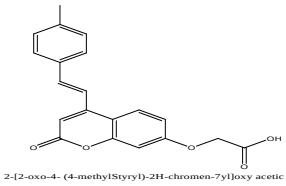
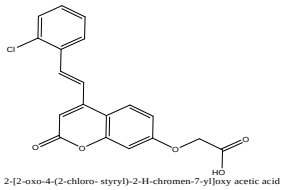
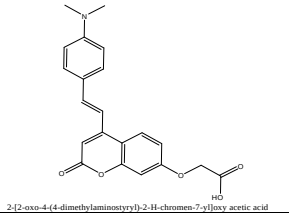
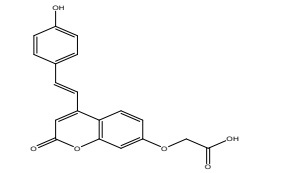
	 2-[2-oxo-4-(3-nitro Styryl)-2H-chromen-7yl]oxy acetic acid					
3g	 2-[2-oxo-4- (4-methylStyryl)-2H-chromen-7yl]oxy acetic	$C_{20}H_{16}O_5$	336.34	>350	68.14	0.49
3h	 2-[2-oxo-4-(2-chloro- styryl)-2-H-chromen-7-yl]oxy acetic acid	$C_{19}H_{13}ClO_5$	356.29	294-295	75.80	0.4
3i	 2-[2-oxo-4-(4-dimethylaminostyryl)-2-H-chromen-7-yl]oxy acetic acid	$C_{21}H_{19}NO_5$	365.38	>350	68.23	0.43
3j	 2-[2-oxo-4-(4-hydroxy styryl)-2-H-chromen-7-yl]oxy acetic acid	$C_{19}H_{14}O_6$	338.31	>350	45.65	0.44

Table-2: Anti-inflammatory activity of synthesized compounds (**3a-3j**)

Group	**Drug**	**Dose (mg./kg.)**	**Mean increase in Paw edema vol.(ml)± SEM After 3 hrs.**	**Inhibition of Paw edema (%)**
1.	Control	-	0.85±0.02ns	-
2.	Standard	5mg./kg.	0.11±0.02***	**73**
3.	3a	10mg./kg.	0.42±0.01	43
4.	3b	10mg./kg.	0.45±0.02ns	40
5.	3c	10mg./kg.	0.43±0.02	42
6.	3d	10mg./kg.	0.41±0.02	44
7.	3e	10mg./kg.	0.27±0.02*	58
8.	3f	10mg./kg.	0.19±0.02***	66
9.	3g	10mg./kg.	0.28±0.01*	57
10.	3h	10mg./kg.	0.20±0.02**	65
11.	3i	10mg./kg.	0.25±0.01*	60
12.	3j	10mg./kg.	0.23±0.02*	62

N=6; One way ANOVA followed by multiple Tukey's comparison test.* P<0.05; *P<0.01; ***P<0.001 when compared with control, ns-non significant. Inhibition of Paw edema (%) $=V_c-V_t / V_c*100$

Where, V_c= Mean increase in Paw volume in the control group of rats.

V_t= Mean increase in Paw volume in rats treated with test compound group of rats.

All the compounds **(3a-3j)** have been evaluated for their antibacterial activity against *Staphylococcus aureus* (Gram positive-ATCC2079) and *Escherichia coli* (Gram negative-ATCC2089), using agar cup-plate method. The results Showing Significant zone of inhibition as compared with standard drugs-*Streptomycin* (for Gram negative bacteria) and *Benzyl penicillin* (for Gram positive bacteria).[11,12] The antibacterial activity results were presented in Table-3.

Table 3: Antimicrobial activity of synthesized compounds (**3a-3j**)

Compounds	Zone of Inhibition (in mm)			
	E. coli(Gram negative)		*S.aureus(Gram positive)*	
	50 µg/ml	**100µg/ml**	**50µg/ml**	**100µg/ml**
Control	-	-	-	-
Standard	**20**	**23**	**18**	**20**
3a	16	19	15	17
3b	10	06	09	07
3c	14	16	13	15
3d	08	09	10	06
3e	13	18	12	14
3f	14	16	15	17
3g	09	07	05	06
3h	13	15	17	12
3i	05	11	08	09
3j	06	07	05	09

Discussion:

Anti-inflammatory of newly synthesized compound shows that, Compounds**3e, 3f, 3g, 3h, 3i** and**3j**showed maximum anti-inflammatory activity in comparison to standard drug Ibuprofen. Antimicrobial activity of the synthesized compounds was performed by Cup Plate method in the drug concentration range 50 µg. /ml (0.05ml) and 100 µg. /ml (0.1ml) against *S. aureus (*ATCC2079*)* and *E. coli* (ATCC2089). The results of anti-microbial activity are shown as zone of inhibition in mm and are quoted in Table-3.In particular, compounds **3a, 3c, 3e, 3f** and **3h**possessed maximum activity. Other compounds also showed mild to moderate activity at 50 µg/ml and 100 µg/ml concentration levels on all micro-organisms. All the results are compared with standard drugs. [11,12]

4. Conclusion

Compounds 2-[2-oxo-4(4-nitro styryl)-2H-chromen-7yl]oxy acetic acid (**3e**), 2-[2-oxo-4(3-nitro styryl)-2H-chromen-7yl]oxy acetic acid (**3f**), 2-[2-oxo-4(4-methyl styryl)-2H-chromen-7yl]oxy acetic acid (**3g**), 2-[2-oxo-4(2-chloro styryl)-2H-chromen-7yl]oxy acetic acid (**3h**), 2-[2-oxo-4(4-dimethylamino styryl)-2H-chromen-7yl]oxy acetic acid (**3i**), 2-[2-oxo-4(4-hydroxy styryl)-2H-chromen-7yl]oxy acetic acid (**3j**) showed maximum anti-inflammatory activity.

Compounds 2-[2-oxo-4- styryl-2H-chromen-7yl]oxy acetic acid (**3a**), 2-[2-oxo-4(4-methoxy styryl)-2H-chromen-7yl]oxy acetic acid (**3c**), 2-[2-oxo-4(4-nitrostyryl)-2H-chromen-7yl]oxy acetic acid (**3e**), 2-[2-oxo-4(3-nitro styryl)-2H-chromen-7yl]oxy acetic acid (**3f**), 2-[2-oxo-4(2-chloro styryl)-2H-chromen-7yl]oxy acetic acid(**3h**) showed good anti-microbial activity against both Gram positive and Gram negative microbes.

5. Acknowledgement

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