Research Article

Synthesis and Evaluation of Novel 7-hydroxy-4-methyl Coumarin Derivatives for their Anti-Microbial and Anti-Oxidant activities

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ABSTRACT

Coumarins are a class of compounds with benzopyrone ring system. The esters of Coumarin ring serves a good anti-bacterial and anti-oxidant activity. The purpose of this investigation was to develop new Coumarin derivatives with increased biological activities. In the present work 7-hydroxy-4-methyl Coumarin was synthesized by the reaction of Resorcinol with Ethyl acetoacetate in the presence of conc, H2SO4. The 7-hydroxy-4-methyl Coumarin so formed was fused with substituted Benzaldehyde in the presence of piperdine as a catalyst for 2 hrs. (**2a-2g**). the compound formed was refluxed for an hour with substituted benzoic acid in the presence of conc.H_2SO_4 which followed esterification reaction to give compounds (**3a-3g**). The structures of the final newly synthesized compounds were confirmed from IR and ^{1}HNMR and Mass spectra. The newly synthesized compounds were screened for their anti-bacterial and anti-oxidant activities. Among the synthesized compounds **3b, 3c, 3d,3e, 3f and 3g** possess good anti-bacterial and compounds **3a, 3b, 3e** possess good anti-oxidant activities.

Keywords: 7-hydroxy-4-methyl Coumarin, esters, anti-bacterial activity, anti-oxidant activity.

INTRODUCTION

Coumarins are becoming an attractive target of extensive research due to its inherent diverse properties. The most widely reported activities are anti-inflammatory, antibacterial, anti-oxidant, anti-cancer, anti-coagulant, etc. Coumarins scavenge reactive oxygen species and causes suppression of inflammation. Substitution of various groups at 4 and 7 positions f Coumarin ring resulted in good biological activities. Synthesis of Coumarin derivatives with substitution at 3, 4 and 7 positions resulted in increase in anti-inflammatory, anti-microbial and anti-oxidant activities.

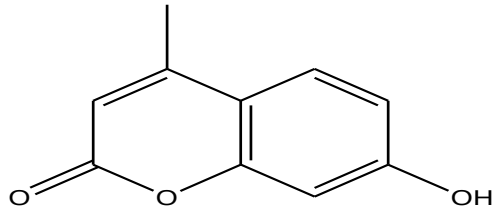


Fig.1 7-hydroxy-4-methyl Coumarin

It was observed that ester linked with Coumarin ring at 3 and 7position was found to possess good anti-inflammatory, anti-microbial activities, anti-oxidant activities, etc. Hence it was decided to modify the structural nucleus of Coumarin at 3rd and 7th positions by substituting with different groups linked by ester with a view to increase their biological activity. On consideration of the above observations, it was worthwhile to synthesize some Coumarin Derivatives.

Experimental

Materials and Methods

All reagents were used as received from commercially sources without purification. Resorcinol, Ethyl acetoacetate, conc. Sulphuric acid, Piperidine (catalytic grade), Benzaldehyde, Benzoic acid derivatives were obtained from PDVVPF's College of Pharmacy. All chemicals used were of L.R. grade.

Chemistry

Progress of reaction was monitored by Thin Layer Chromatography (TLC) using glass plates pre-coated with Silica Gel-G (Loba layer thickness 0.25mm). Melting points of the newly synthesized compounds were with a Veego electronic melting point apparatus (model VMP-D) and were reported uncorrected.1HNMR 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. Molecular mass of the compound was determined by mass to charge (m/z) ratio. The mass spectra were obtained on WATERS Q-TOF MICROMASS (LC-MS). Elemental analyses were obtained using an elemental analyzer.

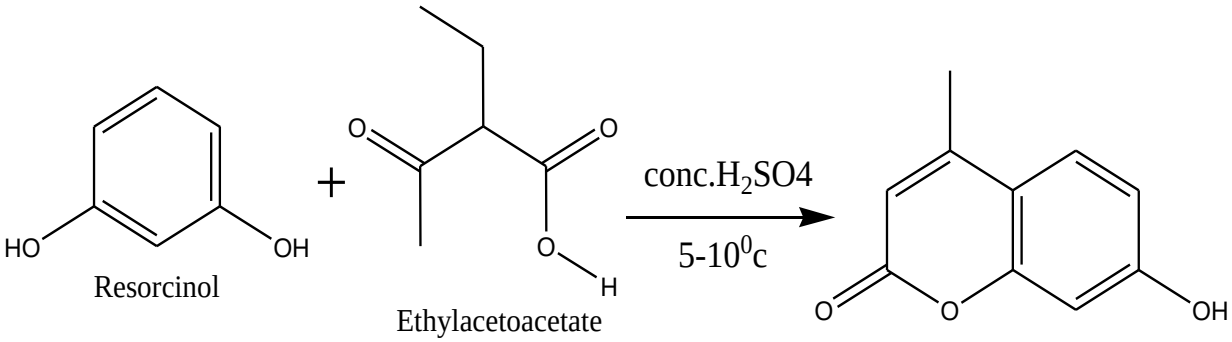
Synthesis

Scheme-1

General procedure for the preparation of 7-hydroxy-4-methyl Coumarin (1)

This compound was synthesized according to the procedure: About 81 ml. 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 ^{0}C – 10^{0}C. 20 gm. of powdered resorcinol was added to 25 ml. of ethyl acetoacetate until a complete solution was obtained. Then this solution was added slowly to H_2SO_4. 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, dried and recrystallized from ethanol. m. p. 192-194^{0}c (87% yields) Molecular Formula: $C_{10}H_8O_3$. Molecular Mass: 176.17. Solubility: Ethanol.

Step-1



Resorcinol

Ethylacetoacetate

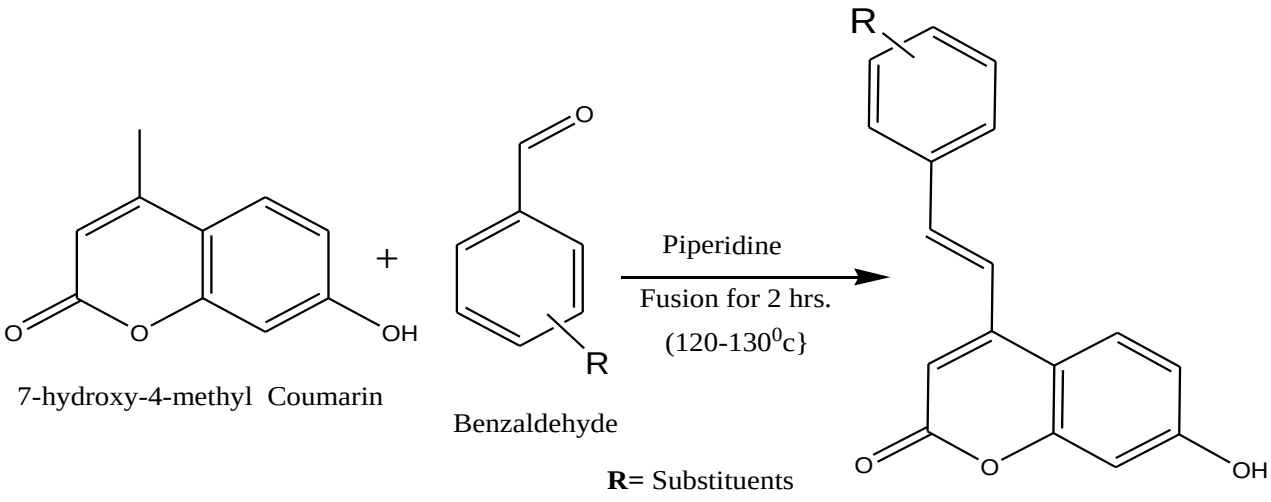
7-hydroxy-4-methyl Coumarin

1

Fig-2

Step-2 General procedure for the preparation of compounds (2a-2g)

The 7-hydroxy-4-methylcoumarin (0.01 mol.) **1** was fused with aromatic aldehydes (0.01 mol.) at (120-130°C) in the presence of piperdine as catalyst condensation takes place to give Coumarin derivatives (**2a-2g**).



7-hydroxy-4-methyl Coumarin

Benzaldehyde

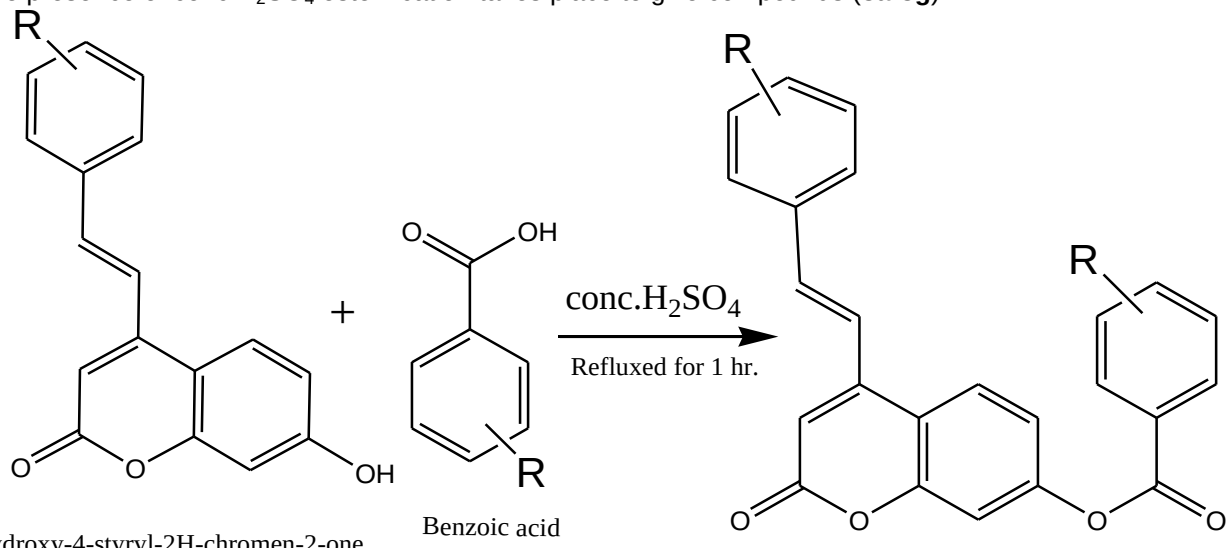
R= Substituents

Fig-3

7-hydroxy- 4-styryl-2H-chromen-2-one derivatives (**2a-2g**)

Step-3 General procedure for the preparation of compounds (3a-3g)

The compounds (**2a-2g**) (0.01mol.) were refluxed for an hour with benzoic acid derivatives (0.01mol.) in the presence of conc.H_2SO_4 esterification takes place to give compounds (**3a-3g**).



7-hydroxy-4-styryl-2H-chromen-2-one

Benzoic acid

(2-oxo-4-styryl-2H-chromen-7yl) benzoate derivatives **(3a-3g)**

Fig.-4 **R**= Substituents

Chemistry

2-oxo-(4-styryl)-2H-chromen-7-yl benzoate (3a)

Mol. form. $C_{24}H_{16}O_4$, Mol. Wt.: 368.38, yield:78%, mp:309^{0}C, C=78.25%(78.23%); H=4.38%(4.37%); O=17.37%(17.35%), IR (KBr in cm^{-1}) 1724(C=O, ester), 1601(C=C ,aromatic),^{1}HNMR (DMSO)(8.14 C=O),(7.21 C=C).

[2-oxo-4-(4-hydroxy)-styryl-2H-chromen-7yl]-4-hydroxybenzoate(3b)

Mol.form.$C_{24}H_{16}O_6$, Mol. Wt.: 400.38, yield:67%, mp:333^{0}C, m/e: 400.09 (100.0%), 401.10 (27.2%), 402.10 (4.7%), C=72.00%(72%); H=4.03%(4.0%); O=23.98%(23.95%), IR (KBr in cm^{-1}),3300(OH),1708(C=O), 1606(C=C), ^{1}HNMR 7.97(C=O), 5.0(OH)

[2-oxo-4-(2-chloro)-styryl-2H-chromen-7yl] benzoate (3c)

Mol.form.$C_{24}H_{15}ClO_4$ Mol. Wt.: 402.83, yield:55%, MP:352^{0}C, m/e: 402.07 (100.0%), 404.06 (32.0%), 403.07 (27.1%), 405.07 (8.9%), 404.07 (4.3%), 406.07 (1.4%), C=71.56%(71.55%); H=3.75%(3.73%); Cl=8.80%(8.75%); O=15.89%(15.88%) IR (KBr in cm^{-1}),800(Cl), 1708(C=O), 1606(C=C), ^{1}HNMR CH 8.14(C=O),CH 7.09(Cl), CH 6.14(C=C)

[2-oxo-4-(4-nitro)-styryl-2H-chromen-7yl] 4-methyl benzoate (3d)

Mol.form.$C_{25}H_{17}NO_6$ Mol. Wt.: 427.41, yield:46%, MP:354^{0}C m/e: 427.11 (100.0%), 428.11 (28.3%), 429.11 (5.1%) , C=70.25%(70.23%); H=4.01%(4.00%); N=3.28%(3.25%); O=22.46%(22.45%) IR (KBr incm^{-1}),1560(NO2), 1372(-NH), 1708(C=O), 1606(C=C),^{1}HNMR CH 7.70(OC), CH 8.14(NO_2).

[2-oxo-4-(4-hydroxy)-styryl-2H-chromen-7yl]-4-methoxy benzoate (3e)

Mol. form. $C_{25}H_{18}O_6$

Mol. Wt.: 414.41, yield:67%, MP:354^{0}C, m/e: 414.11 (100.0%), 415.11 (28.0%), 416.12 (3.9%), 416.11 (1.2%), C=72.46%(72.45%); H=4.38%(4.35%); O=23.16%(23.15%), IR (KBr incm^{-1}),2860(OCH_3), 1708(C=O), 1606(C=C),^{1}HNMR CH 8.3 (CO), CH_3 3.73(CH_3).

[2-oxo-4-styryl-2h-chromen-7yl]-4-chloro benzoate (3f)

Mol. Form. $C_{24}H_{15}ClO_4$ Mol. Wt.: 402.83, yield:66% MP:353^{0}C, m/e: 402.07 (100.0%), 404.06 (32.0%), 403.07 (27.1%), 405.07 (8.9%), 404.07 (4.3%), 406.07 (1.4%) C=71.56%(71.55%); H=3.75%(3.74%); Cl=8.80%(8.78%); O=15.89%(15.88%), IR (KBr incm^{-1}),800(Cl), 1708(C=O), 1606(C=C), ^{1}HNMR CH 8.8(CO).

[2-oxo-4-(4-aminostyryl)-2h-chromen-7yl] 3-amino benzoate (3g)

Mol. Form. $C_{24}H_{18}N_2O_4$ Mol. Wt.: 398.41, yield:57%, MP:301^{0}C, m/e: 398.13 (100.0%), 399.13 (27.1%), 400.13 (4.5%), C=72.35%(72.33%); H=4.55%(4.54%); N=7.03%(7.01%); O=16.06%(16.05%), IR

(KBr incm^{-1}),3371(NH), 1708(C=O), 1606(C=C), ^{1}HNMR CH 6.41(NH), NH2 4.0(NH aromatic).

^{1}HNMR spectra of selected compounds

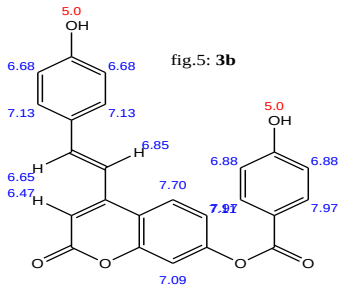


fig.5: **3b**

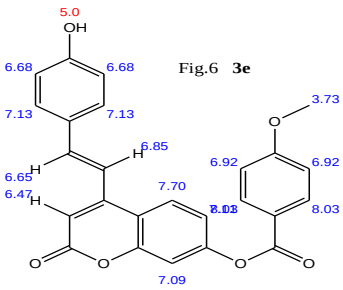


Fig.6 **3e**

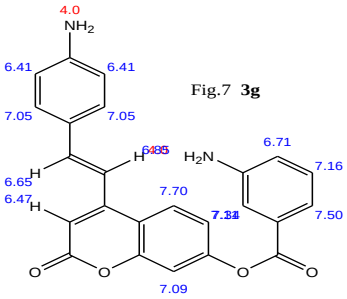


Fig.7 **3g**

Biological Screening

Antibacterial activity

Cup Plate method

The microbiological activity of the synthesized compounds was tested by Agar diffusion method using Cup Plate method against Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*S.aureus*). *Streptomycin* was used as standard drug. 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* solution was also prepared at a conc. 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. The solutions 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^{0}C 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. Solvent DMSO as control was included in every experiment of determining zone of inhibition as a control to ensure that it has no effect on the bacterial growth. Each experiment was done in a duplicate.

Anti-oxidant activity

Hydrogen Peroxide Scavenging Activity

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations ((250, 500, 750 and 1000 µg/mL)) of synthesized compounds were added to a hydrogen peroxide solution (0.6 mL, 40 mM). Ascorbic acid was used as a reference standard. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min. against a blank solution containing phosphate buffer without hydrogen peroxide. Hydrogen peroxide percentage scavenging activity was then calculated using Equation

$$\text{Scavenging effect (\%)} = \frac{A_0 - A_t}{A_t} * 100$$

Where A_0 was the absorbance of control and A_t was the absorbance of test compounds or standard.

RESULTS AND DISCUSSION

The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. In general the compound [2-oxo-4-(4-hydroxy)-styryl-2H-chromen-7yl]-4-hydroxybenzoate(**3b**),[2-oxo-4-(2-chloro)-styryl-2H-chromen-7yl] benzoate**(3c**), [2-oxo-4-(4-nitro)-styryl-2H-chromen-7yl] 4-methyl benzoate(**3d**), [2-oxo-4-(4-hydroxy)-styryl-2H-chromen-7yl] 4-methoxy benzoate(**3e**), [2-oxo-4-styryl-2h-chromen-7yl] 4-chloro benzoate (**3f**), [2-oxo-4-(4-aminostyryl)-2h-chromen-7yl] 3-amino benzoate(**3g**) showed good activity against both Gram-negative and Gram-positive bacteria as compared to 2-oxo-(4-styryl)-2H-chromen-7-yl benzoate (**3a**).This may to presence of chloro, nitro, amino, hydroxy and methyl groups present on aromatic ring.

In addition to above activity, the newly synthesized Coumarin derivatives showed significant anti-oxidant activity. The compounds to 2-oxo-(4-styryl)-2H-chromen-7-yl benzoate (**3a**), [2-oxo-4-(4-hydroxy)-styryl-2H-chromen-7yl]-4-hydroxybenzoate(**3b**) and [2-oxo-4-(4-hydroxy)-styryl-2H-chromen-7yl] 4-methoxy benzoate (**3e**) showed good anti-oxidant activity. This may be due to the presence of styryl ring substituted with hydroxy groups.

CONCLUSION

In general, all Coumarin derivatives shows antimicrobial activity at Conc. 100 µg/ml. The chloro, hydroxy and nitro group containing Coumarin compounds showed better activity than their parent compounds against the bacterial strain *E. coli* and *S. aureus* .The chloro, nitro, amino and hydroxy groups are essential for increased activity. Similarly the Coumarin derivatives shows good anti-oxidant activity at conc.1000 µg/ml. The hydroxy group is essential for good anti-oxidant activity.

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Table 1: Anti-bacterial activity of Coumarin derivatives

S.no.	Compounds Conc. (100µg/ml)	Zone of Inhibition(in mm)	
		E.coli	*S.aureus*
1	3a	12	11
2	3b	15	13
3	3c	16	12
4	3d	14	10
5	3e	13	12
6	3f	17	14
7	3g	16	15
8	*Streptomycin*	23	20

Table 2: Anti-oxidant activity of Coumarin derivatives

S.no.	Compounds Conc. (1000µg/ml)	% Inhibition
1	3a	55
2	3b	62
3	3c	43
4	3d	41
5	3e	56
6	3f	39
7	3g	34
8	Ascorbic acid	70

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