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Synthesis and Antimicrobial Activity of Some New Substituted Coumarin

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ABSTRACT

Coumarins were prepared by heating of 2-hydroxy substituted acetophenone and resorcinol with acetoacetic ester in presence of catalytic amount of 2-methylpiperidine.Characterisation and structural elucidation were done on the basis of chemical, analytical and spectral analysis. The antimicrobial activities of these coumarins were assayed against the test organism E.coli, S.typhi, S.paratyphi, P.vulgaris, S.aureus. All bacterial species used in present investigation are human pathogens. The coumarins were tested against pathogenic bacteria for their antimicrobial activity by using cup plate diffusion method and for determination of minimum inhibitory concentration (MIC) values by serial dilution method.

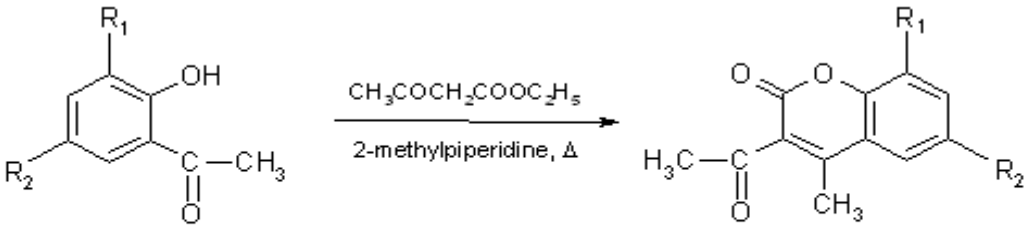
Keywords: Synthesis, Antimicrobial, Coumarin.

INTRODUCTION

During the last twenty years, the study of the biological activities of coumarin derivatives has been the aim of many researchers. Coumarins possess a number of biological activities like anticoagulant, antimicrobial, anti-inflammatory, analgesic, anticancer, etc [1-4]. Literature survey reveals that numbers of derivatives of coumarin have been reported for their pharmacological activities. Asma A.Al-Rifai et al [5] have been reported synthesis, characterization and antimicrobial activity of some new coumarin derivatives. Gadaginamath et al [6] have synthesized and studied the antimicrobial activity of 3- substituted coumarin. Boregowada et al [7] have been reported synthesis and biological evaluation of 4-(3-hydroxy-benzofuran-2-yl) coumarins. A green process for synthesis of coumarin derivatives like 6-hydroxycoumarin, 7-hydroxycoumarin, 6-amino-4-methylcoumarin, etc. have been given by Chaudhary and Datta [8]. Avin et al [9] have been reported synthesis and tumor inhibitory activity of novel coumarin analogs targeting angiogenesis and apoptosis. Synthesis, pharmacological study and docking calculations of new benzo[f] coumarin derivatives as dual inhibitors of enzymatic systems involved in neuro degenerative diseases have been studied by Matos et al [10]. From the literature, it appears that much work has been done on several derivatives of coumarin for their antimicrobial activity.

MATERIALS AND METHODS

Synthesis of 3-acetyl-4,6-dimethylcoumarin (L_1): Reaction mixture of 2-hydroxy-5-methylacetophenone (0.01 M, 1.5 g), acetoacetic ester (0.01 M, 1.26 mL) and 2-methylpiperidine (5 drops) was stirred. This solution was added dropwise in conc. H_2SO_4 (10 mL). The mixture was stirred, and the temperature was kept below 10°C by means of ice and salt. After all the solution has been added, the reaction mixture was stirred continuously for 30 min. The reaction mixture was poured in ice-cold water with vigorous stirring. The precipitate was collected on filter and washed with cold water and recrystalized from ethanol, a dark green shiny crystals of (L1) were obtained, m.p.- 70°C, Yield - 75%.



(L1)

Where, R_1 = -H

R_2 = -CH_3

Properties and Constitution of the Compound (L1)

1. It is greenish crystalline solid, m.p. 70°C.
2. The compound was found to be soluble in CCl_4, benzene, acetone, acetic acid and was insoluble in water.
3. It gives yellow coloration after addition of aqueous ferric chloride solution which clearly indicates that the phenolic hydroxy group is absent and involved into cyclization.
4. The Rf value was found to be 0.72 in benzene solvent on silica gel-G with layer thickness of 0.3 mm.
5. Elemental analysis

Elements	% Found	% Calculated
Carbon	72.03	72.22
Hydrogen	5.15	5.55

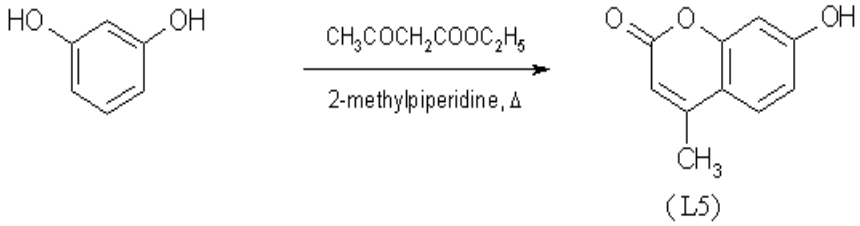
From the analytical data, molecular formula of the compound (L1) was found to be $C_{13}H_{12}O_3$.

6. The IR spectrum of the compound (L1) was carried out in KBr pellets. The important absorption can be correlated as ,2923 (-C-H (alkyl) stretching), 1916(>C=O stretching), 1745(lactone C=O stretching), 1491(>C=C< stretching), 1247(C-O-C stretching).
   IR Spectrum indicates the cyclization by:
   a) Absence of band in the range of 3600-3200 cm^{-1} for phenolic OH group.
   b) The appearance of band at 1247 cm^{-1} for C-O-C stretching.
7. The NMR spectrum of compound (L1) was recorded in DMSO. The chemical shift can be correlated as,7.61-6.81(s, 3H, Ar-H), 2.61(s, 3H,-$COCH_3$), 2.28(s, 3H,-CH_3).

Table-1: Synthesis, mp, Rf value of Coumarins

Compd No.	R_1	R_2	Mole. formula	m.p.(^{0}C)	Rf values
L_1	-H	$-CH_3$	$C_{13}H_{12}O_3$	70	0.72
L_2	$-NO_2$	-Cl	$C_{12}H_8O_5NCl$	75	0.87
L_3	-Br	-Cl	$C_{12}H_8O_3BrCl$	55	0.81
L_4	-H	-Cl	$C_{12}H_9O_3Cl$	80	0.76

Synthesis of 4-methyl-7-hydroxycoumarin (L_5): Interaction of resorcinol (0.01 M, 1.1 g) with acetoacetic ester (0.01 M, 1.26 mL) in presence of catalytic amount of 2-methylpiperidine (5 drops). This reaction mixture was added dropwise in conc. H_2SO_4 (10 mL). The mixture was stirred, and the temperature was kept below 10°C. After all the solution has been added, stirred the mixture continuously for 30 min. Poured the reaction mixture in ice-cold water with vigorous stirring. The precipitate was collected on filter and washed with cold water and recrystallized from ethanol to obtained colourless crystals of (L5), m.p.- 189°C, Yield - 85%



(L5)

Properties and Constitution of the Compound (L5)

1. It is colourless crystalline solid, m.p. 189°C.
2. The compound was found to be soluble in acetone, acetic acid, benzene and was insoluble in water.
3. It gives purple colouration with aqueous ferric chloride indicating the presence of phenolic OH group.
4. The Rf value was found to be 0.74 in benzene solvent on silica gel-G with layer thickness of 0.3 mm.
5. Elemental analysis

Elements	% Found	% Calculated
Carbon	67.77	68.18
Hydrogen	4.39	4.54

From the analytical data, molecular formula of the compound (L5) was found to be $C_{10}H_8O_3$.

6. The IR spectrum of the compound (L5) was carried out in KBr pellets. The important absorption can be correlated as, 3498(Ar-OH stretching), 2902(-C-H (alkyl) stretching), 1671(lactone C=O stretching), 1605(>C=C< stretching), 1248(C-O-C stretching).
   IR Spectrum indicates the cyclization by:
   a. The appearance of band at 1248 cm^{-1} for C-O-C stretching.
7. The NMR spectrum of compound (L5) was recorded in DMSO. The chemical shift can be correlated as, 10.46(s, 1H, Ar-OH), 7.97-6.03, (s, 4H, Ar-H), 2.57-2.38(d, 3H,-CH_3).

Following coumarins are prepared:

1) 3-Acetyl-4,6-dimethylcoumarin (L1)
2) 3-Acetyl-4-methyl-6-chloro-8-nitrocoumarin (L2)
3) 3-Acetyl-4-methyl-6-chloro-8-bromocoumarin (L3)
4) 3-Acetyl-4-methyl-6-chlorocoumarin (L4)
5) 4-Methyl-7-hydroxycoumarin (L5)

Antimicrobial Activity: Any chemical substance inhibiting the growth or causing the death of a microorganism is known as 'antimicrobial agent'. A survey of literature reveals that extensive work has been done on many heterocyclic compounds for their antimicrobial activities including both gram positive and gram negative pathogens [11-17]. Total five synthesized compounds were studied for their antimicrobial activities. All the pathogens tested during analysis are human pathogens. For testing the antimicrobial activity the compounds were assayed against *E. coli, S. typhi, S. paratyphi, P. vulgaris* and *S. aureus*.

The activities of compounds were tested against all the pathogens by serial dilution method [18,19] for determining the MIC value, all these compounds were dissolved in CCl_4. The MIC value less than 1000 µg mL^{-1}, such compounds are highly active. It was found that all the compounds are highly and moderately active against bacteria. Generally, less is the concentration more is the active compound. The comparative study of MIC values of the compounds L_1, L_2, L_3, L_4 and L_5 are given in table 2.

Table 2. Minimum inhibitory concentration (MIC) values of Compounds L_1-L_5 in µg mL^{-1}

Sr. No.	Compds.	*E. coli*	*S. typhi*	*S. paratyphi*	*P. vulgaris*	*S. aureus*
1.	L_1	1100	800	920	860	1000
2.	L_2	900	740	800	780	900
3.	L_3	1400	1150	1000	1060	1200
4.	L_4	1200	1280	1360	1440	1600
5.	L_5	1600	700	860	920	1060

RESULTS AND DISCUSSION

Activity against *E. coli*: The antimicrobial activity of the synthesized compounds against *E. coli* is highly remarkable; L_2 is highly active because of -Cl and $-NO_2$ groups. L_1, L_3 and L_4 are moderately active because of presence of -Cl and -Br groups, while L_5 is weakly active.

Activity against *S. typhi*: The antimicrobial activity of the synthesized compounds again remarkable and considerable. Three out of five compounds show higher activity (L_1 is 800 µg mL^{-1}, L_2 is 740 µg mL^{-1} and L_5 is 700 µg mL^{-1}) whereas remaining two compounds L_3 (1150 µg m L^{-1}) and L_4 (1280 µg m L^{-1}) are moderately active. It can also be noted that when $-NO_2$ group and -Cl group are present in the molecule the reactivity enhances, which increases the potency of the drug. This probably may be due to higher resonance stability provided by these groups and hence prolonged activity of the compounds.

Activity against *S. paratyphi*: Out of five compounds for which activity was measured against *S. paratyphi* a total of three compounds found to be highly active i.e. L_1 (920 µg m L^{-1}), L_2 (800 µg m L^{-1}) and L_5 (860 µg m L^{-1}). Whereas two compounds are found to be moderately active i.e., L_3 (1000 µg m L^{-1}) and L_4 (1360 µg m L^{-1}).

Activity against *P. vulgaris*: All the five compounds were tested against *P. vulgaris*. L_1, L_2 and L_5 are found to be highly active while all others are moderately active.

Activity against *S. aureus*: It is observed from table 2 that, five compounds are tested against *S. aureus* pathogens from which compound L_2 is highly active (900 µg m L^{-1}). L_1 (1000 µg m L^{-1}), L_3 (1200 µg m L^{-1}) and L_5 (1060 µg m L^{-1}) are moderately active while L_4 (1600 µg m L^{-1}) is weakly active. So active compounds can be used for treatment of wound infection after biological, pharmaceutical, medical study and if these do not have any toxic side effects.

APPLICATIONS

From table 2, it is observed that MIC values for *E. coli* ranges from 900-1600 µg m L^{-1}. MIC values for *S. typhi* ranges from 700-1280 µg m L^{-1}. MIC values for *S. paratyphi, P. vulgaris* and *S. aureus* are found to be ranging from 800-1360 µg m L^{-1}, 780-1440 µg m L^{-1} and 900-1600 µg m L^{-1} respectively. It was observed that all the above compounds are having good antimicrobial activity and no one is inactive.
It is seen that compound L_2 is highly active against all microbes because of the presence of -NO_2, -Cl and -CH_3 groups. So from the result it has been observed that the presence of nitro group and chloro group increases the activity and increase in activity is also related to the presence of heterocyclic nucleus in addition to benzenoid nucleus. So these synthesized drugs can be used as the best alternative drugs for the treatment of diseases caused by *E. coli*, *S. typhi, S. paratyphi, P. vulgaris* and *S. aureus* only after the pharmaceutical, biochemical and medicinal significance, if these drugs do not have toxic and other side effects.

CONCLUSIONS

In conclusion, the present paper describes the synthesis and antimicrobial activity of five new coumarin derivatives and was screened against five bacterial strains such *E. coli*, *S. typhi, S. paratyphi, P. vulgaris* and *S. aureus*. It is observed that compound 3-acetyl-4-methyl-6-chloro-8-nitrocoumarin is highly active against all microbes because of the presence of -NO_2, -Cl and -CH_3 groups.

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