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Synthesis and Antibacterial Activity of Coumarin and Its Derivatives

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Coumarin and its different derivatives were prepared by treating ethyl acetoacetate with phenol and its different derivatives in acidic media. Product formation was confirmed by spectroscopic techniques *i.e.* IR, NMR *etc*. The antimicrobial activities of synthesized compounds were checked against different gram positive and gram negative bacteria by agar ditch method.

Keywords: Coumarin, Ethyl acetoacetate, Phenol derivatives.

INTRODUCTION

Conventional antibacterial treatment is going towards a disaster due to the fast development of resistance to existing agents[1]. However, the plant kingdom constitutes a source of new chemicals, which may be important for their potential use in medicine[2]. Coumarins are plant secondary metabolites compounds that show different biological activity according to their substitution patterns.

Coumarin (1,2-benzopyrone, 2*H*-1-benzopyran-2-one) is a fragrant organic chemical compound in the benzopyrone chemical class. Coumarin was first isolated in 1822 from tonka bean[3]. Pleasant odour of the seeds is due to coumarin and therefore used in the perfume industry. It is bitter in taste, however in large infused doses, it may cause haemorrhage and liver damage as well as it can paralyze the heart. It is therefore controlled as a food additive in many countries.

Coumarin and its derivatives were used in the treatment of cancer[4], oedemas[5] and they also shown promising biological activity. They are found to be antibacterial[6], anti-thrombotic[7], vasodilatory[8], anti-mutagenic[9] and anti-tumourigenic[10]. In this study pure coumarin and its different derivatives were prepared, characterized by ^{1}H NMR and ^{13}C NMR. Their antibacterial activity was evaluated against various Gram-positive and Gram-negative bacteria and they proved to be good antibacterial agent.

EXPERIMENTAL

Acetic acid, sodium acetate, salicylaldehyde, conc. H_2SO_4, phenol, ethyl acetoacetate, α-naphthol, resorcinol, *p*-toluenesulphonic acid and other solvents of analytical grade were purchased from sigma Aldrich. All chemicals were pure enough and used directly without further purification.

Synthesis of 2*H*-chromene-2-one: (1.72 g, 14 mmol) 2 mL of salicylaldehyde, (2.50 g, 30.5 mmol) fused sodium acetate and (4.76 g, 79.0 mmol) 5 mL acetic acid was transferred to a 250 mL Erlenmeyer flask duly installed with an air reflux condenser; the top end of which was provided with $CaCl_2$ guard tube and heated the mixture in an oil-bath for a duration of 6 h between 180-190 °C. Then cooled the contents of flask and ground them finely with the help of mortar and piston. Weighed out the crude product and dissolved the crude product in petroleum ether to get pure coumarin crystals of white colour, (1.2 g, 58 %) separated after the evaporation of ether.

Synthesis of 4-methyl-2*H*-chromene-2-one: According to the procedure by Reddy *et al.*[11], catalyst H_2SO_4/silica gel (100 mg) was dispersed in a mixture of phenol (0.94 g, 10 mmol) and β-keto esters (1.52 mL, 12 mmol) in a 25 mL round bottom flask equipped with a distillation condenser. The reaction mixture was stirred vigorously at 120 °C. The progress of the reaction was monitored by TLC. At the end reaction mixture was dissolved in CH_3OH and filtered to recover the catalyst. The solvent was evaporated by heating to obtain the crude product. Thus product was washed with water, filtered and dried at 100 °C. Product purification was done by dissolving it in 20 mL 1 M NaOH and then reproduced with 10 mL 2 M H_2SO_4 solution. The pure product (0.83 g, 52 %) was dried in a high vacuum.

Synthesis of 4,6-dimethyl-2*H*-chromene-2-one: To get desired product *m*-cresol (1.1 g, 10 mmol) and β-keto esters (1.52 mL, 12 mmol) were mixed with 100 mg H_2SO_4 /silica

gel catalyst in a round bottom flask which was attached with a distillation condenser. The content was stirred vigorously at 120 °C. The reaction progress was checked by TLC. On completion of the reaction, the reaction mixture was treated with CH_3OH and filtered to separate the catalyst. The filtrate was evaporated to get the crude product. Thus obtained product was washed with water, filtered and dried at 100 °C. The product was purified by dissolving in 20 mL of 1 M NaOH and then regenerated with 10 mL of 2 M H_2SO_4 solution. The pure product of yellowish colour (0.76 g, 41 %) was dried in a high vacuum.

Synthesis of 4-methyl-2*H*-benzo[*h*]chromene-2-one: α-naphthol (1.45 g, 10 mmol) and β-keto esters (1.52 mL, 12 mmol) were mixed in a 25 mL round bottom flask equipped with a distillation condenser in the presence of 100 mg H_2SO_4/silica gel catalyst. The reaction mixture was stirred forcefully at 120 °C. The completion of the reaction was checked by TLC. Finally the reaction mixture was treated with CH_3OH and filtered to recover the catalyst. The solvent was evaporated under reduced pressure to obtain the crude product. Thus obtained product was washed with water, filtered and dried at 100 °C. The product was purified by dissolving in 20 mL 1 M NaOH and then regenerated with 10 mL 2 M H_2SO_4 solution. The pure product of light yellow colour (0.98 g, 46 %) was dried in a high vacuum.

Antibacterial study: In order to study the bacterial activity of above synthesized compounds different bacterial strains *i.e. E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis* and Methicillin-resistant *Staphylococcus aureus* were collected from the Department of Microbiology, University of Punjab Lahore, Pakistan.

Preparation of test compound: 2 mg of each compound was dissolved in 1 mL of DMSO and diluted to 3 different concentrations *i.e* 0.2, 0.02 and 0.002 mg/0.1 mL for microbiological assays.

Preparation of L.B broth and agar plates: L.B broth was prepared in five test tubes for 5 bacterial strains. Agar plate diffusion technique was applied to develop L.B nutrient medium.

The composition of the medium was (g/L) tryptone (1 g), yeast extract (0.5 g), sodium chloride (0.5 g); agar (1.5-2.0 g) and water (100 mL). Every synthesized compound was tested according to pre mentioned concentration by dissolving in DMSO, while DMSO itself was used as control for comparison.

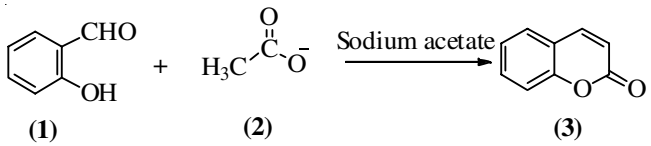
N-Agar media was autoclaved and 25-30 mL of the media was added into the 9 cm diameter Petri-dish, allowed to solidify and then 1 mL bacterial suspension was transferred to the plate at 27 °C for 24 h. The wells were made in the plates with the help of autoclaved pasture pipette and then it was filled with the synthetic compound dissolved in DMSO. The 100 µg/mL concentration was used and the activity of compound was determined by measuring the inhibition zone.

Perkin-Elmer FT-IR 783 spectrophotometer was used to record IR spectras. Jeol ECS 300 NMR spectrometer was used for ^1H NMR and ^{13}C NMR measurements. For protons, the chemical shifts were measured relative to tetramethylsilane (TMS) at δ = 0 ppm.

RESULTS AND DISCUSSION

Coumarin has been used as an aroma accompaniment in pipe tobaccos and different alcoholic drinks, although it is banned as a food additive, due to its hepatotoxicity in animals. Coumarin itself does not has any anticoagulant property, but it can be converted into 4-hydroxycoumarin, then further it is converted into actual anticoagulant discoumarol, a fermentation product and mycotoxin. Because of their varied biological activities, the preparation of coumarin and its derivatives has attracted the attention of organic chemists. Following coumarin derivatives were prepared and characterized.

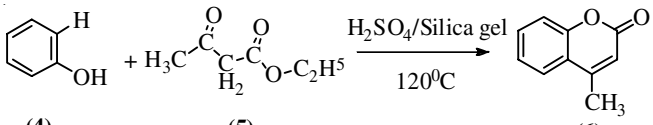
2*H*-Chromene-2-one: IR (film, ν_{max}, cm^{-1}): 3268.61 (Ar CH str), 2972.19 (CH str), 1680.22 (C=O lactone), 1391.34 (ester C-O str), 1100 (C-O-C cyclic str), δ_H (300 MHz; $CDCl_3$) 7.72 (1H, d, *J* = 9.6, -C*H*=CHCOO-), 7.58-7.45 (2H, m, ArH), 7.37-7.24 (2H, m, ArH), 6.42 (1H, d, *J* = 9.5, -CH=C*H*COO-); δ_C (75 MHz; $CDCl_3$) 160.7, 154.1, 143.4, 131.8, 127.8, 124.4, 118.8, 116.9, 116.7. R_f 0.34 (EtOAC-pet. ether, 50:50) (Fig. 1).



(1) **(2)** **(3)**

Fig. 1. Synthesis of coumarin

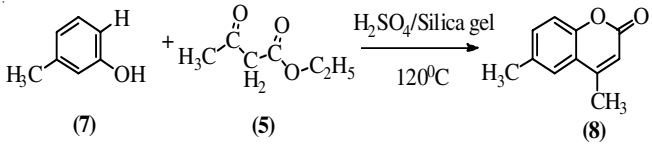
4-Methyl-2*H*-chromene-2-one: IR (film, ν_{max}, cm^{-1}): 3077.22 (Ar CH str), 2972.19 (CH str), 1590 (C=O lactone), 1391.34 (ester C-O str), 1122.95 (C-O-C cyclic str). δ_H (300 MHz; $CDCl_3$) 7.61 (1H, d, *J* = 7.9, ArH), 7.53 (1H, m, 1H, ArH), 7.35- 7.26 (2H,m, ArH), 6.28 (1H, s, -C(CH_3)=C*H*COO-), 2.44 (3H, s, CH_3); δ_C (75 MHz; $CDCl_3$) 160.7 (0), 153.4 (0), 152.3 (0), 131. 7 (1), 124.5 (1), 124.1 (1), 119.8 (0), 116.9 (1), 115.0 (1), 18.5 (3); R_f 0.41 (EtOAC-pet. ether, 50:50) (Fig. 2).



(4) **(5)** **(6)**

Fig. 2. Synthesis of 4-methyl-2*H*-chromene-2-one

4,6-Dimethyl-2*H*-chromene-2-one: IR (film, ν_{max}, cm^{-1}): 2924.12 (CH str), 1709 (C=O lactone), 1610.5 (C=C), 1571 (ester C-O str) δ_H (300 MHz; $CDCl_3$) 7.38 (1H, s, -C*H*=C(CH_3)), 7.33 (1H, dd, *J* = 8.5, 1.8 -C(CH_3)-C*H*=CH), 7.22 (1H, d, *J* = 8.5, -C(CH_3)-CH=C*H*), 6.27 (1H, m, -CO-C*H*=C(CH_3)), 2.42 (3H, s, -CH_3), 2.42 (3H, s, -CH_3); δ_C (75 MHz; $CDCl_3$) 161.0, 152.3, 151.6, 133.8, 132.6, 124.4, 119.6, 116.7, 115.0, 20.9, 18.6. R_f 0.29 (EtOAC-pet. ether, 50:50) (Fig. 3).

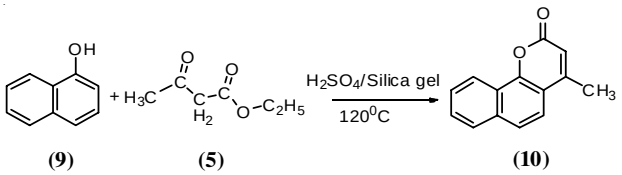


(7) **(5)** **(8)**

Fig. 3. Synthesis of 4,6-dimethyl-2*H*-chromene-2-one

4-Methyl-2*H*-benzo[*h*] chromene-2-one: IR (film, ν_{max}, cm^{-1}): 3079.49 (C-H sp^2), 2982.35 (C-H sp^3), 1643.22 (C=O lactone), 1610.3 and 1454.42 (C=C aromatic), 1060.22 (C-O). δ_H (300 MHz; $CDCl_3$) 8.42 (1H, m, -C*H*=C(CH_3)), 8.18 (1H,

d, *J* = 8.4, -C*H*=CH), 7.94 (1H, d, *J* = 9.0, -C*H*=CH), 7.88 (1H, d, *J* = 8.0, -C*H*=CH), 7.67 (1H, ddd, *J* = 8.4, 7.0, 1.4, -C*H*=CH), 7.55 (1H, ddd, *J* = 8.1, 7.1, 1.1, -C*H*=CH), 7.41 (1H, d, *J* = 9.0, -C*H*=CH), 2.43 (3H, s, -CH_3). δ_C (75 MHz; $CDCl_3$) 160.8, 153.8, 139.0, 133.0, 130.2, 128.9, 128.9, 128.2, 126.0, 121.3, 116.9, 115.5, 112.9. R_f 0.49 (EtOAC-pet. ether, 50:50) (Fig. 4).



(9) **(5)** **(10)**

Fig. 4. Synthesis of 4-methyl-2*H*-benzo[*h*]chromene-2-one

Four different coumarin and its derivatives were prepared in acidic media with good yield (Table-1). Four different phenol derivatives were utilized to synthesize above said compounds. In synthesis of coumarin and its derivatives -OH of phenol derivatives behaved as a nucleophile, carbon atom of  group attached to ethoxy group (-OC_2H_5) behaved as an electrophile and -OC_2H_5 group removed as a leaving group. In the 2nd step "O" atom of -$COCH_3$ group helped to bring the intermediate into cyclic form. Finally the product was regenerated in acidic media.

In order to check the antibacterial activities of the synthetic compounds, different Gram-positive and Gram-negative bacteria were used *i.e. Staphylococcus aureus*, *Bacillus subtilis*, Methicillin-resistant *Staphylococcus aureus* and *E. coli*, *Pseudomonas aeruginosa*, *S. typhi*.

The response of synthesized compounds against different pathogens and measurement of their inhibition zones are given in the Table-2.

It is obvious from Table-2 that compounds labelled as Z1-HZ1 showed varying degree of inhibition zone against any Gram-positive and Gram-negative bacteria. The minimum inhibition concentrations (MIC) of above four synthesized compounds against each bacterial strain are given in Table-3.

TABLE-1
PRODUCT WITH THEIR YIELDS

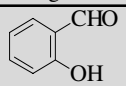
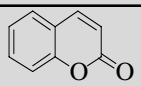
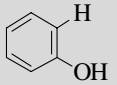
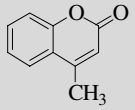
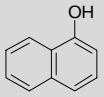
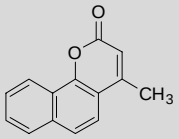
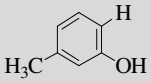
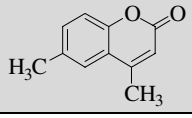
Entry number	Sample description	Starting material	Product	Yield (%)
1	Z1			58
2	Z2			52
3	Z3			46
4	HZ1			41

TABLE-2
ANTIBACTERIAL ACTIVITY DATA OF COUMARIN AND DERIVATIVES

Synthesized compounds (50 µg)	Diameter of zone of Inhibition (mm)					
	Gram Positive			Gram Negative		
	MRSA	*Bacillus subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*	*S. typhi*
Z1	-	-	22	35	50	-
Z2	15	-	-	32	55	30
Z3	10	10	-	25	44	-
HZ1	22	-	19	28	60	-

Note: Mean inhibition zones are measured in mm

TABLE-3
MIC OF COUMARIN AND DERIVATIVES

Synthesized compounds	Gram positive			Gram negative		
	MRSA (µg)	*Bacillus subtilis*	*S. aureus*	*P. aeruginosa* (µg)	*E. coli* (µg)	*S. typhi*
Z1	-	-	15	20	30	-
Z2	10	-	-	20	30	20
Z3	10	10 µg	-	20	20	-
HZ1	20	-	10	10	40	-

Note: MIC values are µg/mL of the compound

It can be seen in Table-3 that all the compounds are not equally active against all bacterial strains but all are active against *E. coli* and *P. aeruginosa*. The growth of *Bacillus subtilis* and *S. typhi* were inhibited only by Z2 and Z3, respectively and rest of the compounds were found to be inactive against these two bacterial strains. Z1 and HZ1 found to be least active against *E. coli* but they are very active against *S. aureus* and *P. aeruginosa*.

Conclusion

Coumarin and its derivatives were synthesized by using different phenol derivatives. The synthesized compounds were then tested against different Gram-positive and Gram-negative bacteria. All the synthesized compounds showed activity against any one of the bacterial strain by inhibiting their growth.

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