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Research Article

SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITIES OF NEWLY SYNTHESIZED COUMARIN DERIVATIVES

JV. Chavhan[1*] and SS. Patil[2]

[1]P. D. Polytechnic Amravati, Maharashtra, India.
[2]Director and Head Lifelong learning and extension,
Department Sant Gadgebaba Amravati University, Amravati, Maharashtra, India.

ABSTRACT

In this work, substituted coumarin based flavones have been synthesized via chalcone. The study was conducted with two primary objectives. The first objective was to synthesize coumarin based flavones and second objective was to study antimicrobial activity of synthesized compounds. The structures of the compounds were elucidated by elemental and spectral (IR, ^{1}HNMR, and MS) analysis. The reactions are easy to conduct, under mild conditions, and form coumarine substituted flavones in moderate to excellent yields.

Keywords: Chalcone, Coumarine, Flavones and antimicrobial activity.

1. INTRODUCTION

Coumarins are a class of naturally occurring compounds, found in variable levels throughout the plant kingdom. Today coumarins are very important in the pharmaceutical field due to their wide occurrence, and versatile pharmacological activity associated with low toxicity profile such as antimicrobial, anticoagulant, antioxidant, and anticancer activities[1-4]. Applications of coumarins range from additives in food, perfumes, and cosmetics, to the preparation of insecticides, optical brighteners, and tunable laser dyes[3]. Coumarin itself was reported to have an immunostimulatory activity on macrophages and other cells of the immune system. This results in the use of coumarin in chronic infections such as chronic brucellosis, mycoplasmosis, toxoplasmosis, and Q fever[1].

Flavonoids are a class of natural compounds possessing a wide range of pharmacological activities[5]. Flavonoids are widely present in the plant kingdom exhibiting a broad range of biological activities, including antibacterial, antifungal, antiviral, anti-allergic, anti-inflammatory, and anti-proliferative and antioxidant activities[6-9]. It has been reported that the intensity of the antimicrobial activity of a flavonoid strongly depends on its chemical structure, which is particularly influenced by the number and position of various functional groups. At present the flavonoids are the subject of medical research and there are several reports of their oestrogenic activity, antitumour activity[10]. Historically preparations containing flavonoids as the principal active constituent have been used to treat various human disorders[6]. The Old Testament refers to the healing property of Propolis. This antimicrobial property has been attributed to the presence of high proportions of flavonoids[11, 12]. The activity of flavonoids is probably due to their ability to form complexes with extracellular and soluble proteins and with bacterial cell walls.

Thus it was worthwhile to synthesize flavonoids and to evaluate them for antimicrobial activity.

2. MATERIAL AND METHODS

Melting points were determined on a Veego Melting Point Apparatus Mod. VMP-DS ± 0.5˚C accuracy and are uncorrected. The ^{1}H NMR spectra were recorded on a BRUKER Spectrometer (400 MHz). Chemical shifts were reported in parts per million using tetramethylsilane as an internal standard and were given in δ units. The solvent for NMR spectra was DMSO. Infrared spectra were taken on SHIMADZU-FTIR-8400 Spectrophotometer instrument in the frequency range of 4000-400 cm^{-1} by KBr powder method. The Mass spectra were recorded by MS-SHIMADZU-QP2010. All reactions were monitored by thin layer

chromatography, carried out on 0.2 mm silica gel 60F254 (Merk) plates using UV light for detection. Common reagent grade chemicals are either commercially available and were used without further purification.

Preparation of 6-chloro-4-methyl-7-hydroxycoumarin (1)

1 gm of 4-chlororesorcinol was added to 1.18 ml ethyl acetoacetate and this mixture was cooled. 7 ml conc. H_2SO_4 was added with const. stirring for 12hr. reaction mixture was poured in crushed ice. The solid product obtained was dissolve in 5% NaOH and then acidified to obtained brownish solid of 6-chloro-4-methyl-7-hydroxycoumarin.

It is brownish crystalline solid; Yield 59%; mp. 220°C; IR spectrum (KBr), υ (cm^{-1}): 1480.70 cm^{-1} (C=C str.); 1680.90 cm^{-1} (C=O str.); 3450.15 cm^{-1} (O-H str.); ^{1}HNMR spectrum (δ ppm): 2.52 (3H, d, CH_3); 4.82 (1H, s, OH); 6.24 (1H, d, CH); 7.1-7.69 (Ar-H).

Preparation of 6-chloro-4-methyl-2-oxo-2H chromen-7-yl acetate (2)

The mixture of 6-chloro-4-methyl-7-hydroxycoumarin 0.1M and 0.1M of acetyl chloride stirred for 1 hr. The reaction mixture was poured in crushed ice, to get the 4-methyl-2-oxo-2H-chromen-7yl acetate. It is crystallized from ethanol.

It is brownish crystalline solid; yield 63%; mp.198°C; IR spectrum (KBr), υ (cm^{-1}): 1480.70 cm^{-1} (C=C str.); 1680.15 cm^{-1} (C=O str.); ^{1}HNMR spectrum (δ ppm): 2.92 (3H, s, CH_3); 3.05 (3H, d, CH_3); 6.23 (1H, d, CH); 6.8-7.4 (Ar-H).

Preparation of 8-acetyl-6-chloro-7-hydroxy-4-methyl-2H-chromen-2-one (3)

0.235gm of acetylated compound and 5gm of $AlCl_3$ was heated at 145-150˚C in oil bath for an hour, with $CaCl_2$ tube to prevent from moisture. HCl gas was continuously evolved. The reaction mixture was treated with ice and concentrate HCl. Thus the solid product obtained was collected washed with water and crystallized from ethanol.

It is brownish crystalline solid; yield 67%; mp. 195°C; IR spectrum (KBr), υ (cm^{-1}): 1445.78 cm^{-1} (C=C str.); 1671.91 cm^{-1} (C=O ketone str.); 3082.68 cm^{-1}(O-H str.); ^{1}HNMR spectrum (δ ppm): 2.92 (3H, s, CH_3); 3.05 (3H, d, CH_3); 6.23 (1H, d, CH); 6.8-7.4 (Ar-H).

Preparation of 6-chloro-8-[(2E)-3-(4-methoxyphenyl)prop-2-enoyl]-7-hydroxy-4-methyl-2H-chromen-2-one (4a)

Compound (3) and 4-methoxybenzaldehyde was taken in 1:1 proportion, to this 50 ml of ethanol as a solvent, and 9ml of 40 % KOH was added, the reaction mixture was refluxed for 2 hrs to get the compound (4a). The product obtained was washed with water and crystallised from ethanol.

Similarly (4b) was synthesized by reaction of compound (3) with 4-nitrobenzaldehyde.

6-chloro-8-[(2E)-3-(4-methoxyphenyl)prop-2-enoyl]-7-hydroxy-4-methyl-2H-chromen-2-one (4a)

It is brownish crystalline solid; yield 67%; mp. 183°C; IR spectrum (KBr), υ (cm^{-1}) : 1450.29 cm^{-1} (Ar C=C str.); 1248.40 cm^{-1} (C-O-C str.); 1684.45 cm^{-1} (C=O ketone str.); 3370 cm^{-1} (O-H str.); 740.42 cm^{-1} (C-Cl str.); ^{1}HNMR spectrum (δ ppm): 2.23 (3H, s, CH_3); 2.75 (3H, s, O-CH_3); 10.52 (1H, s, OH); 5.96 (1H, d, CH); 6.83 (1H, d, CH); 7.52 (1H, d, CH); 7.28-7.39 (Ar-H).

6-chloro-8-[(2E)-3-(4-nitrophenyl)prop-2-enoyl]-7-hydroxy-4-methyl-2H-chromen-2-one (4b)

It is brownish crystalline solid; yield 65%; mp. 190°C; IR spectrum (KBr),υ (cm^{-1}): 1449.14 cm^{-1} (Ar C=C str.); 1248.43 cm^{-1} (C-O-C str.); 1345.24/1588 cm^{-1} (NO_2 str.); 1683.48 cm^{-1} (C=O ketone str.); 3367.15 cm^{-1}(O-H str.); 740.39 cm^{-1} (C-Cl str.); ^{1}HNMR spectrum (δ ppm): 2.34 (3H, s, CH_3); 9.32 (1H, s, OH); 6.09 (1H, d, CH); 6.96 (1H, d, CH); 7.50 (1H, d, CH); 7.28-7.29 (Ar-H).

Synthesis of 6-chloro-8-(4-methoxyphenyl)-4-methyl-2H,10H-pyrano[2,3-f]chromene-2,10-dione (5a)

Few iodine crystals were added in the mixture of compound (4a) and DMSO. The reaction mixture was refluxed for 1 hr. to get the final product 5-chloro-8-(4-methoxyphenyl)-4-methyl-2H,10H-pyrano[2,3-f]chromene-2,10-dione (5a).

Similarly compound (5b) was prepared.

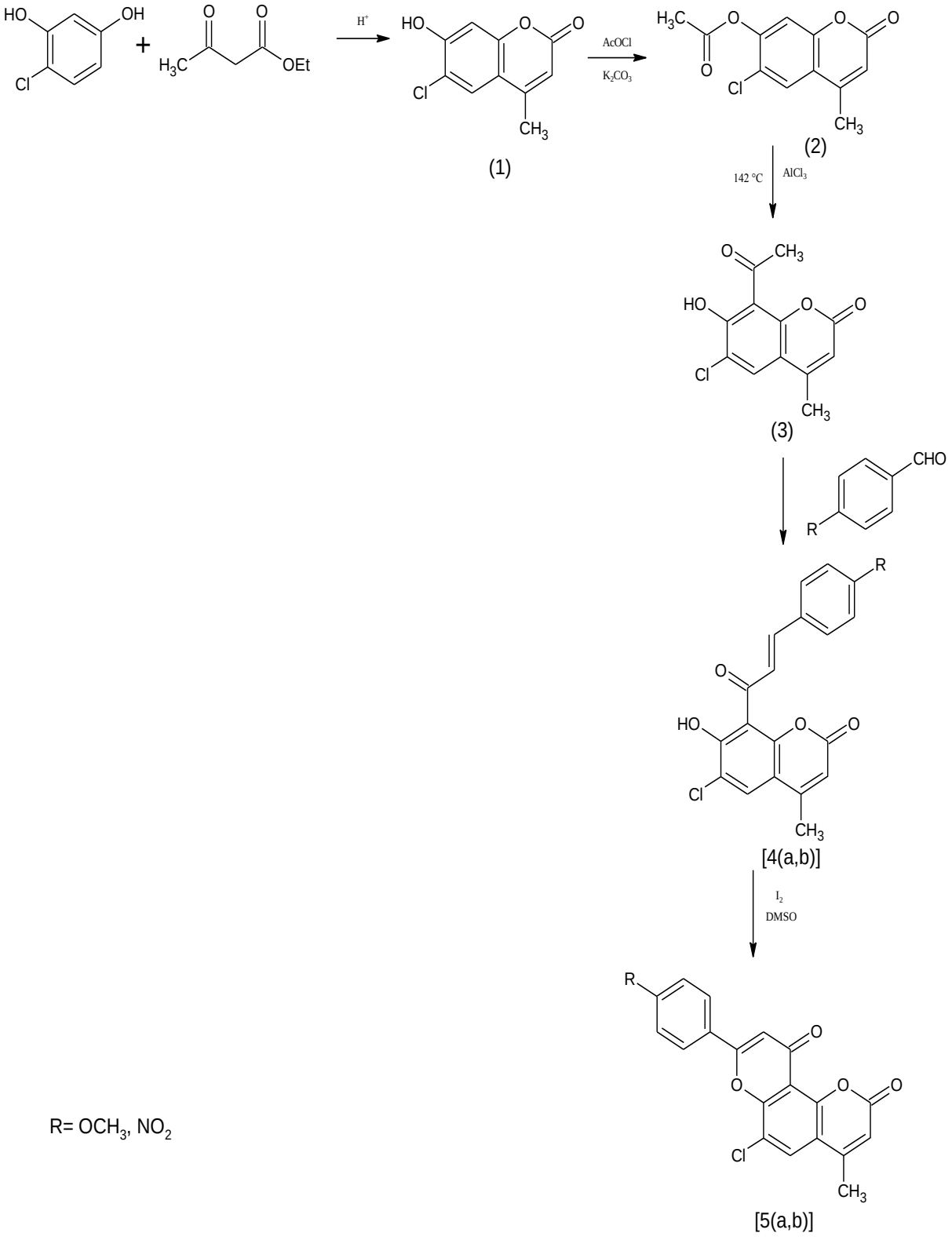
6-chloro-8-(4-methoxyphenyl)-4-methyl-2H,10H-pyrano [2,3-f]chromene-2,10-dione (5a)

It is brownish crystalline solid; yield 69%; mp.186°C; IR spectrum (KBr), υ (cm^{-1}): 1473.52 cm^{-1} (Ar C=C str.); cm^{-1} 1700.39 cm^{-1} (C=O ketone str.); 1263.93 cm^{-1}(C-O-C str.); 748.93 cm^{-1} (C-Cl str.); ^{1}HNMR spectrum (δ ppm): 2. 23 (3H, s, CH_3); 2.55 (3H, s, CH_3); 6.01 (1H, d, CH); 6.8 (1H, d, CH); 7.4-7.5 (Ar-H).

6-chloro-8-(4-nitrophenyl)-4-methyl-2H,10H-pyrano[2,3-f]chromene-2,10-dione (5b)

It is brownish crystalline solid; yield 68%; mp. 167°C; IR spectrum (KBr), υ (cm^{-1}): 1474.23 cm^{-1} (Ar C=C str.); 1700.97 cm^{-1} (C=O ketone str.); 1267.08 cm^{-1}(C-O-C str.); 748.77 cm^{-1} (C-Cl str.); 1344.21/1600.43 cm^{-1} (NO_2 str.); ^{1}HNMR spectrum (δ ppm): 2. 67 (3H, s, CH_3); 7.02 (1H, d, CH); 7.28 (1H, d, CH); 7.58-7.72 (Ar-H).

REACTION SCHEME



3. RESULTS AND DISCUSSION

The structures of compounds (4a,b) and (5a,b) were confirmed on the basis of spectral analysis. The IR spectrum of chalcones (4a,b) showed a characteristic band due to C-O-C str. (1248.40-1248.43 cm^{-1}); C=C str. (1449.14-1450.29 cm^{-1}); C=O str. (1683.48-1684.45 cm^{-1}); O-H str. (3367.15-3370 cm^{-1}); vibration band indicates formation of chalcone. ^{1}H-NMR (DMSO) spectrum of chalcone showed a signal at δ 5.96-6.09 (*d*, 1H, CH); 6.83-6.96 (*d*, 1H, CH); 7.50-7.52 (*d*, 1H, CH) and δ 9.32-10.52 (*s*, 1H, OH) confirms presence of chalcone.

The IR spectrum of (5a,b) exhibited a band due to C=O str. (1697–1700 cm^{-1}); C=C str. (1473–1474 cm^{-1}); and C-O-C ring str. (1263-1267 cm^{-1}) and C-Cl str. (748.77-748.93 cm^{-1}) stretching vibration band which indicates the presence of the flavone ring. Further, in their ^{1}H-NMR (DMSO) spectrum, the appearance of a signal at δ 6.01-7.02 (*d*, 1H, CH flavone) and δ 6.8-7.28 (*d*, 1H, CH, flavone) confirms the presence of flavone ring.

3.1 Antibacterial activity

The target molecules were tested for antibacterial activity against the variety of test organisms *Escherichia coli*, *Pseudomonas* (gram-negative bacteria) and *Staphylococcus aureus, Salmonella typhi* (gram-positive bacteria) by disc diffusion method with concentration 0.1 mg/ml. The screening results indicate that compound **5b** shows high activity against *Pseudomonas* and both compound shows good activity against *S. typhi*, *S. aureus*, *E. coli* and **5a** shows low activity against *Pseudomonas.*

Table 1: Antibacterial activity of the compounds 5a and 5b

Compound	Antibacterial activity Diameter of zone of inhibition (in mm)			
	S. aureus	*S. paratyphi*	*E. coli*	*Pseudomonas*
5a	7	8	4	7
5b	9	9	5	10
Oxacillin (standard)	15	14	12	16

The zones of inhibition of the reference compound oxacillin are also given in Table 1. The result indicates that the presence of chloro, methoxy, and nitro groups enhanced the antibacterial activity. However, no specific structure–activity relationship could be established.

4. CONCLUSION

The successful synthesis of chalcone and flavone compounds follows a mild, efficient route with a good to moderate yield. In present work we synthesized flavones by reacting chalcones with iodine crystals in DMSO medium. The synthesized compounds exhibited good antimicrobial activity.

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