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Synthesis and biological activity studies of some novel pyrazoline derivatives

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ABSTRACT

*The title compounds were synthesized by refluxing different substituted chalcones **(4a-j)** with 2-(3-chloro-4-methyl-2-oxo-2H-chromen-7-yloxy)acetohydrazide **(3)** in presence of glacial acetic acid. Compound **(3)** was prepared by the reaction of 3-chloro-7-hydroxy-4-methyl-2H-chromen-2-one **(1)** with ethyl chloroacetate and subsequent treatment with hydrazine hydrate. The newly synthesized compounds were characterized by ^{1}H NMR, IR, and Mass spectral data. Further all the compounds were screened for antimicrobial activity.*

Keywords: Chalcones, 2-(3-chloro-4-methyl-2-oxo-2*H*-chromen-7-yloxy)acetohydrazide, 3-chloro-7-hydroxy-4-methyl-2*H*-chromen-2-one, antimicrobial activity.

INTRODUCTION

Heterocyclic compounds are acquiring more importance in recent years because of their immense biological and pharmacological potency. Various biologically active synthetic compounds have five membered nitrogen containing heterocyclic ring in their structures. Many compounds bearing pyrazoles and their reduced forms pyrazolines constitute an interesting class of heterocycles due to their synthetic versatility and effective biological activities such as antimicrobial [1,2], antiviral [3], anti-inflammatory [4,5], antidepressant [6], antitubercular [7], antiamoebic [8], analgesic [9] activities. Literature survey reveals several synthetic protocols for the synthesis of these compounds and the presence of this core in any molecule plays a key role in enhancing the activity. On the other hand, coumarin and its derivatives represent one of the important class of heterocyclic compounds possessing a wide range of biological activities. These include antibacterial [10], antifungal [11,12], antitumor [13,14], herbicidal, anti-inflammatory [15] activities. Coumarins are oxygen containing heterocycles widely distributed in nature. They are also used as additives in food, perfumes, agrochemicals, pharmaceuticals, and in the preparation of insecticides, optical brighteners, dispersed fluorescent and dye lasers.

Chalcones are 1,3-diaryl-2-propen-1-ones are natural or synthetic compounds prepared by claisen-schmidt condensation of aromatic aldehydes with acetophenones in presence of base and alcohol as solvent medium [16,17]. These compounds found application in the synthesis of various heterocyclic compounds.

Keeping in view of the above interesting pharmacological features, we hereby report the synthesis and antimicrobial activity of a series of new pyrazoline derivatives.

MATERIALS AND METHODS

All the solvents and reagents were obtained from commercial sources and were used without further purification. Melting points were determined in open capillaries and were uncorrected. TLC was used to monitor the progress of all reactions and to check the purity of compounds. The IR spectra (KBr pellets) were recorded on a JASCO FT/IR-5300 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer with TMS as an internal standard. Mass spectra were recorded on LCMS-2010A, SHIMADZU spectrometer.

Procedure for the synthesis of ethyl 2-(3-chloro-4-methyl-2-oxo-2*H*-chromen-7-yloxy)acetate (2):
A mixture of 3-chloro-7-hydroxy-4-methyl-2*H*-chromen-2-one **1** (0.05 mol), ethyl chloroacetate (0.05 mol) and potassium carbonate in dry acetone was refluxed for 30 hrs. The progress of the reaction was monitored by TLC up to completion. The reaction mixture was filtered hot and the solvent was distilled off from the filtrate. The solid thus obtained was purified by recrystallization from ethanol [18]. Yield: 92%; m.p: 110-112°C.

Procedure for the synthesis of 2-(3-chloro-4-methyl-2-oxo-2*H*-chromen-7-yloxy)acetohydrazide (3):
A mixture of compound **2** (0.05 mol) and hydrazine hydrate (0.05 mol) in ethanol was refluxed for 4-5 hrs. The reaction mixture was poured into ice cold water. The solid product was collected by filtration, washed with water, dried and finally recrystallized from ethanol [19].

Yield: 92%; m.p: 185-187°C; ^{1}H NMR (400 MHz, DMSO): δ 2.60 (s, 3H, CH_3), 4.41 (s, 2H, NH_2), 4.69 (s, 2H, OCH_2), 7.09-7.87 (m, 3H, Ar-H), 9.46 (s, 1H, NH); LCMS (m/z): 283 $(M+H)^+$.

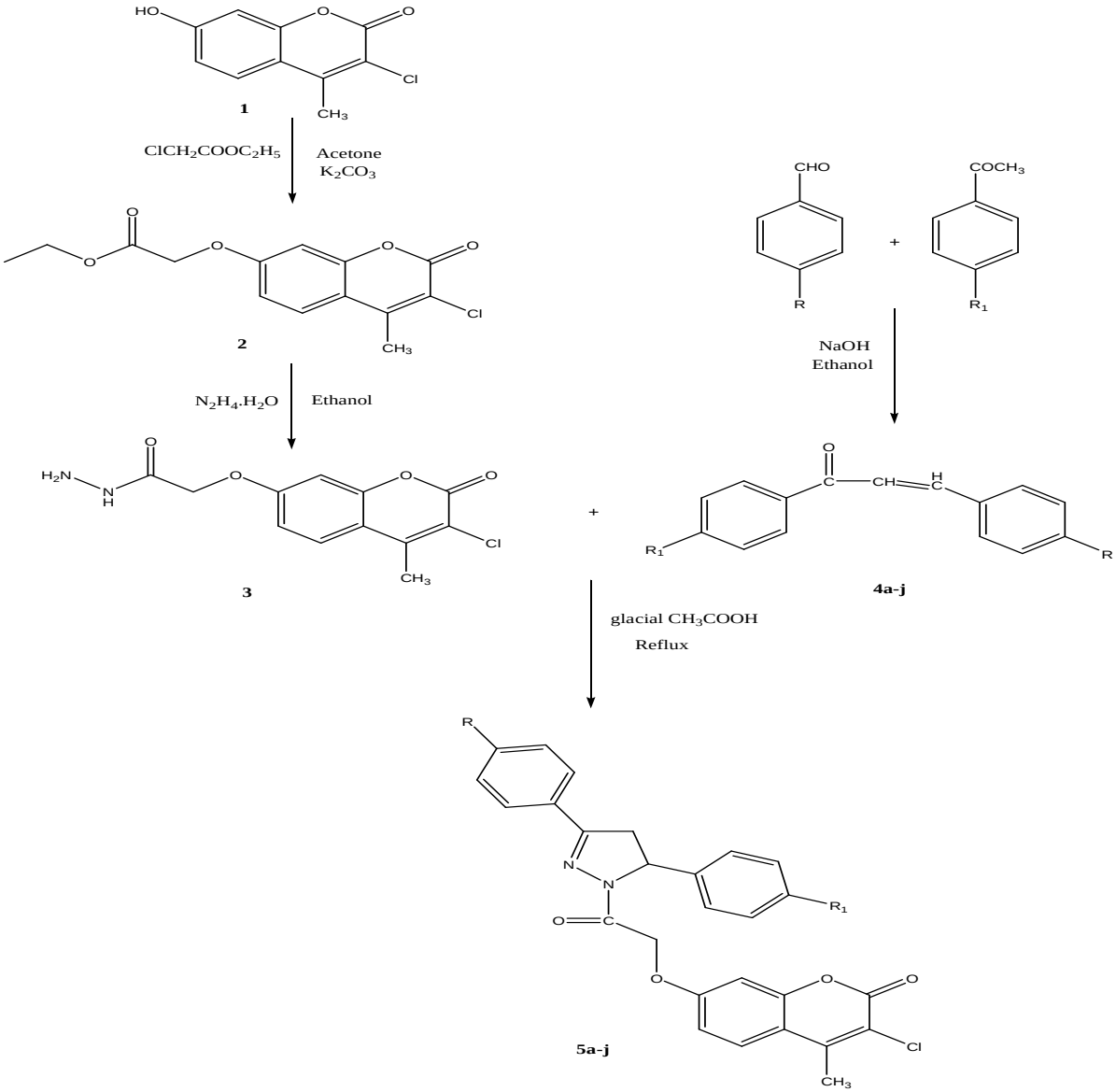
General procedure for the preparation of chalcones (4a-j):
To a solution of substituted aldehydes (0.01 mol) and substituted aryl acetophenones (0.01 mol) in ethanol, a solution of NaOH (6 ml, 40%) was added and the mixture was stirred for 24 hrs at room temperature [20]. The resultant reaction mixture was poured onto crushed ice, acidified with dil.HCl. The solid obtained was filtered, dried and recrystallized from ethanol.

General procedure for the synthesis of pyrazoline derivatives (5a-j):
To a solution of chalcones **4a-j** (0.01 mol) in 25 ml of glacial acetic acid, 2-(3-chloro-4-methyl-2-oxo-2*H*-chromen-7-yloxy)acetohydrazide **3** (0.01 mol) was added. The reaction mixture was refluxed for 8 hrs and left overnight. Excess of solvent was removed under reduced pressure and the resultant residue was treated with ice cold water. The solid mass obtained was filtered, washed with water and recrystallized from a suitable solvent.

Spectral data of synthesized compounds
Synthesis of 7-(2-(3,5-bis(4-chlorophenyl)-4,5-dihydropyrazol-1-yl)-2-oxoethoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (5a):
Yield: 60%; m.p: 205-207°C; IR (KBr): 3059 (Ar C-H), 1679 (C=O), 1585 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 2.5 (s, 3H, CH_3), 3.12-3.39 (m, 2H, CH_2), 4.58 (s, 2H, OCH_2), 5.28 (m, 1H, CH), 7.60-8.18 (m, 11H, Ar-H); Anal. Calcd for $C_{27}H_{19}Cl_3N_2O_4$: C, 59.85; H, 3.53; N, 5.17; Found: C, 59.87; H, 3.56; N, 5.19.

Synthesis of 7-(2-(3-(4-chlorophenyl)-4,5-dihydro-5-(4-hydroxyphenyl)pyrazol-1-yl)-2-oxoethoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (5b):
Yield: 66%; m.p: 210-212°C; IR (KBr): 3066 (Ar C-H), 1681 (C=O), 1580 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO): δ 2.4 (s, 3H, CH_3), 3.08-3.36 (m, 2H, CH_2), 4.62 (s, 2H, OCH_2), 5.48 (m, 1H, CH), 7.30-7.90 (m, 11H, Ar-H), 9.72 (s, 1H, OH); Anal. Calcd for $C_{27}H_{20}Cl_2N_2O_5$: C, 61.96; H, 3.85; N, 5.35; Found: C, 61.99; H, 3.88; N, 5.39.

Synthesis of 7-(2-(5-(4-aminophenyl)-4,5-dihydro-3-(4-nitrophenyl)pyrazol-1-yl)-2-oxoethoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (5c):
Yield: 62%; m.p: 220-222°C; ^{1}H NMR (400 MHz, DMSO): δ 2.1 (s, 3H, CH_3), 2.80-3.11 (m, 2H, CH_2), 4.0 (s, 2H, NH_2), 4.66 (s, 2H, OCH_2), 5.30 (m, 1H, CH), 7.77-8.30 (m, 11H, Ar-H); LCMS (m/z): 533 $(M+H)^+$; Anal. Calcd for $C_{27}H_{21}ClN_4O_6$: C, 60.85; H, 3.97; N, 10.51; Found: C, 60.81; H, 3.99; N, 10.54.



Scheme-1: Synthetic route for the compounds (5a-j)

Compound	R	R_1
5a	4-Cl	4-Cl
5b	4-Cl	4-OH
5c	4-NO_2	4-NH_2
5d	4-NO_2	4-Cl
5e	4-NO_2	4-NO_2
5f	4-NO_2	4-OH
5g	4-Cl	4-NH_2
5h	4-Cl	4-NO_2
5i	4-Cl	H
5j	4-OH	H

Synthesis of 7-(2-(5-(4-chlorophenyl)-4,5-dihydro-3-(4-nitrophenyl)pyrazol-1-yl)-2-oxoethoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (5d):
Yield: 65%; m.p: 209-211°C; IR (KBr): 3051 (Ar C-H), 1682 (C=O), 1574 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 2.1 (s, 3H, CH_3), 2.93-3.23 (m, 2H, CH_2), 4.62 (s, 2H, OCH_2), 5.42 (m, 1H, CH), 7.13-7.71 (m, 11H, Ar-H); Anal. Calcd for $C_{27}H_{19}Cl_2N_3O_6$: C, 58.71; H, 3.47; N, 7.61; Found: C, 58.67; H, 3.45; N, 7.67.

Synthesis of 7-(2-(4,5-dihydro-3,5-bis(4-nitrophenyl)pyrazol-1-yl)-2-oxoethoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (5e):
Yield: 58%; m.p: 224-226°C; IR (KBr): 3074 (Ar C-H), 1669 (C=O), 1585 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 2.4 (s, 3H, CH_3), 3.10-3.39 (m, 2H, CH_2), 4.73 (s, 2H, OCH_2), 5.30 (m, 1H, CH), 7.25-7.78 (m, 11H, Ar-H); LCMS (m/z): 563 $(M+H)^+$; Anal. Calcd for $C_{27}H_{19}ClN_4O_8$: C, 57.61; H, 3.40; N, 9.95; Found: C, 57.65; H, 3.43; N, 9.91.

Synthesis of 7-(2-(4,5-dihydro-5-(4-hydroxyphenyl)-3-(4-nitrophenyl)pyrazol-1-yl)-2-oxoethoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (5f):
Yield: 62%; m.p: 216-218°C; ^1H NMR (400 MHz, DMSO): δ 1.98 (s, 3H, CH_3), 2.97-3.27 (m, 2H, CH_2), 4.46 (s, 2H, OCH_2), 5.72 (m, 1H, CH), 7.77-8.30 (m, 11H, Ar-H), 10.20 (s, 1H, OH); LCMS (m/z): 534 $(M+H)^+$; Anal. Calcd for $C_{27}H_{20}ClN_3O_7$: C, 60.74; H, 3.78; N, 7.87; Found: C, 60.77; H, 3.82; N, 7.82.

Synthesis of 7-(2-(5-(4-aminophenyl)-3-(4-chlorophenyl)-4,5-dihydropyrazol-1-yl)-2-oxoethoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (5g):
Yield: 65%; m.p: 197-199°C; IR (KBr): 3063 (Ar C-H), 1682 (C=O), 1576 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 2.5 (s, 3H, CH_3), 2.93-3.19 (m, 2H, CH_2), 3.75 (s, 2H, NH_2), 4.52 (s, 2H, OCH_2), 5.16 (m, 1H, CH), 7.25-7.78 (m, 11H, Ar-H); Anal. Calcd for $C_{27}H_{21}Cl_2N_3O_4$: C, 62.08; H, 4.05; N, 8.04; Found: C, 62.11; H, 4.08; N, 7.99.

Synthesis of 7-(2-(3-(4-chlorophenyl)-4,5-dihydro-5-(4-nitrophenyl)pyrazol-1-yl)-2-oxoethoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (5h):
Yield: 64%; m.p: 232-234°C; IR (KBr): 3078 (Ar C-H), 1668 (C=O), 1580 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 1.9 (s, 3H, CH_3), 2.91-3.19 (m, 2H, CH_2), 4.68 (s, 2H, OCH_2), 5.72 (m, 1H, CH), 7.18-7.79 (m, 11H, Ar-H).

Synthesis of 7-(2-(3-(4-chlorophenyl)-4,5-dihydro-5-phenylpyrazol-1-yl)-2-oxoethoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (5i):
Yield: 70%; m.p: 215-217°C; IR (KBr): 3071 (Ar C-H), 1665 (C=O), 1562 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 2.5 (s, 3H, CH_3), 3.10-3.39 (m, 2H, CH_2), 4.65 (s, 2H, OCH_2), 5.34 (m, 1H, CH), 7.72-8.31 (m, 12H, Ar-H); Anal. Calcd for $C_{27}H_{20}Cl_2N_2O_4$: C, 63.92; H, 3.97; N, 5.52; Found: C, 63.95; H, 4.01; N, 5.54.

Synthesis of 7-(2-(4,5-dihydro-3-(4-hydroxyphenyl)-5-phenylpyrazol-1-yl)-2-oxoethoxy)-3-chloro-4-methyl-2*H*-chromen-2-one (5j):
Yield: 70%; m.p: 227-229°C; IR (KBr): 3063 (Ar C-H), 1676 (C=O), 1589 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO): δ 2.2 (s, 3H, CH_3), 2.84-3.14 (m, 2H, CH_2), 4.68 (s, 2H, OCH_2), 5.38 (m, 1H, CH), 7.45-8.05 (m, 12H, Ar-H), 9.28 (s, 1H, OH); LCMS (m/z): 489 $(M+H)^+$; Anal. Calcd for $C_{27}H_{21}ClN_2O_5$: C, 66.33; H, 4.33; N, 5.73; Found: C, 66.28; H, 4.35; N, 5.77.

Biological Evaluation
Antimicrobial activity
The antimicrobial activity of newly synthesized compounds was determined using agar well diffusion method [21]. All the compounds were tested invitro for their antibacterial activity against *Bacillus substilis* (Gram positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* (Gram negative bacteria) using nutrient agar medium **(Table 1)**. Antifungal activity was carried out against *Aspergillus niger* and *Aspergillus flavus* using potato dextrose agar medium **(Table 2)**. Streptomycin and Fluconazole were used as standard drugs for antibacterial and antifungal activity respectively. DMSO was used as solvent control. The compounds were tested at a concentration of 100 µg/ml against both bacterial and fungal strains.

Preparation of Nutrient agar medium

To prepare 1 lit of nutrient agar medium 3 g of beef extract, 3 g of peptone, 15 g of agar was used. The ingredients were accurately weighed and dissolved in a liter of distilled water before the addition of agar. The P^H of the medium was adjusted to 7.0 by adding few drops of 0.1 N NaOH/HCl. Later this medium was transferred to conical flasks and plugged with nonabsorbent cotton. Medium was then sterilized by autoclaving at 15lbs pressure for 15 mins, cooled and used for the study.

Preparation of Potato dextrose agar medium

200 g of potato slices were boiled with distilled water. Dextrose and agar were weighed separately. 20 g of dextrose was mixed with potato infusion. 20 g of agar was added as a solidifying agent. These constituents were mixed thoroughly and later this medium was transferred to conical flasks and plugged with nonabsorbent cotton. Medium was then sterilized by autoclaving at 15lbs pressure for 15 mins, cooled and used for the study.

Method of testing

The sterilized media was poured onto the sterilized petri dishes (20-25 ml, each petri dish) and allowed to solidify. Wells of 6 mm diameter was made in the solidified media with the help of sterile borer and solutions of the test compounds were added with the help of micropipette. A sterile swab was used to evenly distribute microbial suspension over the surface of solidified media. The plates were incubated at 37°C for 24 hrs in case of antibacterial activity and 72 hrs at 25°C for antifungal activity. The zone of inhibition was measured in mm scale.

Table 1. Antibacterial activity of synthesized compounds (5a-j)

Compound	Zone of inhibition (mm) at 100 µg/ml concentration		
	B.subtilis	***E.coli***	***P.aeruginosa***
5a	11	10	08
5b	14	13	13
5c	16	14	14
5d	17	16	15
5e	10	09	-
5f	12	11	11
5g	18	16	17
5h	17	14	15
5i	06	05	-
5j	10	12	09
Streptomycin	24	22	22
Control (DMSO)	-	-	-

Table 2. Antifungal activity of synthesized compounds (5a-j)

Compound	Zone of inhibition(mm) at 100 µg/ml concentration	
	Aspergillus niger	***Aspergillus flavus***
5a	14	14
5b	10	11
5c	12	10
5d	14	16
5e	15	16
5f	08	06
5g	-	-
5h	13	15
5i	09	07
5j	-	-
Fluconazole	25	25
Control (DMSO)	-	-

RESULTS AND DISCUSSION

Chemistry

The procedure adopted for the synthesis of title compounds **(5a-j)** are shown in scheme-1. The ester compound **(2)** was obtained by reacting compound **(1)** with ethyl chloroacetate and potassium carbonate in dry acetone. Compound

(2) on treatment with hydrazine hydrate in presence of ethanol yielded hydrazide compound **(3)** in good yields. The compound **(3)** on reaction with chalcones **(4a-j)** in presence of glacial acetic acid at reflux temperature gave pyrazoline derivatives **(5a-j)**. The synthesized compounds were purified by recrystallization using appropriate solvents and some purified by column chromatography. The structures of the title compounds were established by ^{1}H NMR, IR, mass spectral data. The IR spectrum of **5a** exhibited absorption bands for C=O, C=N at 1679, 1585 cm^{-1} respectively. The ^{1}H NMR spectrum of **5a** showed a singlet at δ 2.5 due to CH_3 protons. A multiplet at δ 3.12-3.39 corresponds to CH_2 protons of pyrazoline. Another singlet at δ 4.58 corresponds to OCH_2 protons. A multiplet at δ 5.28 is due to CH proton of pyrazoline. Aromatic protons appeared as multiplet at δ 7.60-8.18, confirmed the structure of the compound.

Biological activity

Antimicrobial studies

All the synthesized compounds were screened for antimicrobial activity by agar well diffusion method. The results showed that among the tested compounds **5b, 5c, 5d, 5g, 5h** exhibited good activity against both Gram positive and Gram negative bacteria. Compound **5i** showed least activity against *Bacillus subtilis* and *Escherichia coli*. Compound **5e** and **5i** showed no activity against *Pseudomonas aeruginosa*. The compounds **5a, 5d, 5e, 5h** exhibited good activity against *Aspergillus niger* and *Aspergillus flavus*. Compounds **5g, 5j** do not exhibited antifungal activity towards both the organisms. Compounds **5f, 5i** showed least activity towards *Aspergillus niger* and *Aspergillus flavus* and remaining compounds showed moderate activity.

CONCLUSION

In this study a series of novel pyrazoline derivatives containing coumarin moiety were synthesized and evaluated for antimicrobial activity. For antibacterial activity various bacteria, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* were used and results were compared with the standard drug streptomycin. Antifungal activity was performed against *Aspergillus niger* and *Aspergillus flavus* using fluconazole as standard. The screening results revealed that most of the compounds were found to exhibit significant antimicrobial activity.

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