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In-vitro anti-inflammatory and antimicrobial activity of synthesized some novel pyrazole derivatives from coumarin chalcones

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ABSTRACT

Some novel series of Heterocyclic Pyrazole derivatives were synthesized by reacting the various substituted Coumarin chalcones (which are prepared by claisen-schmidt condensation *method*) *with phenyl hydrazine in catalytic GAA and ethanol media were refluxed, the obtained series of compounds are characterized by Physical and spectroscopic data. And all the synthesized compounds were evaluated for anti-inflammatory (in-vitro) & antimicrobial activity.*

Key words: Coumarin chalcones, Pyrazole, anti-inflammatory and antimicrobial activity.

INTRODUCTION

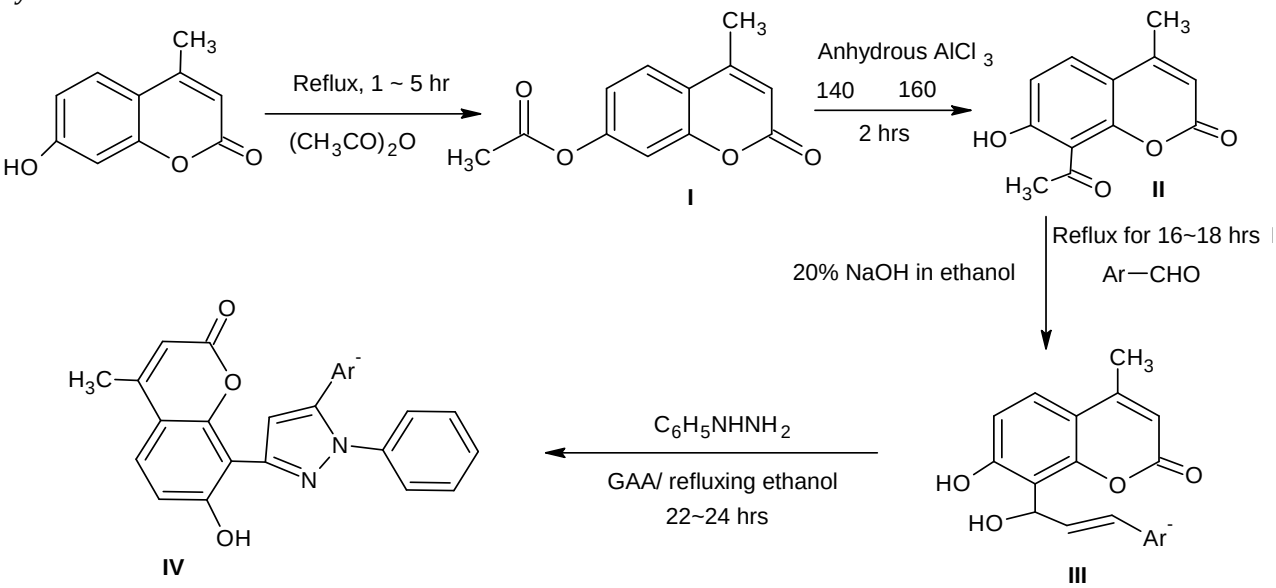
There are a number of reports that natural and synthetic Coumarin derivatives posses antimicrobial activity [1,2]. Novobiocin and chlorobiocin are established antimicrobials containing a Coumarin skeleton. So in the present work we synthesized some 8 novel pyrazole heterocyclic derivatives from substituted 8-[(1E)-1-phenylprop-1-en-2-yl]-2H-chromen-2-one (i.e Coumarin chalcones by **claisen-schmidt condensation)**[3,4,5] with phenyl hydrazine. The aim is done because synthetic Coumarin chalcones itself posse's versatile pharmacological and biological activities like antimicrobial, anti-inflammatory, anti-HIV, anticoagulant, anticancer, antihypertensive, hypoglycemic and antileshmanial activities so on. And the Pyrazole structure containing compound shows predominant role on antimicrobial, anti-inflammatory, antidepressant and anti-tubercular activities. So the above mentioned 8 novel substituted 8-(1,5-diphenyl-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (IV-a-h) heterocyclic compounds were synthesized [6,7], the structure was characterized by physical and spectroscopic analysis like M.P, TLC, IR, ^{1}H-NMR & FAB-Mass [8,9]. And all the synthesized compounds were subjected to study their *In-vitro* anti-inflammatory activity were performed by using inhibition of albumin denaturation technique which was studied according to Muzushima and Kabayashi with slight modification [10,11]. Antimicrobial activity like anti-bacterial activity over G+ve ***Staphylococcus aureus and*** G-ve ***Escherichia coli*** and antifungal activity over ***Candida albicans*** and ***Aspergillus Niger*** by the disc diffusion method by measuring diameter of zone of inhibition in mm [12,13].

MATERIALS AND METHODS

Experimental

Melting point were determined by open capillary method which are uncorrected, the synthesized compounds are characterized and identified by elemental analysis, FT-IR by KBr method using Shimadzu 300 MHz FT-IR Spectrophotometer. Some selected compounds were subjected to ^{1}H-NMR spectra data were recorded on Bruker 400 MHZ in $CDCl_3$ using TMS as an internal standard and FAB-Mass for structural confirmation, all the compounds are screened for *In-vitro* anti-inflammatory and antimicrobial activity.

Synthetic scheme*:*



Ar = a) C_6H_5, b) p $(CH_3)_2N$-C_6H_4 , c) p OCH_3-C_6H_4 , d) o OH-C_6H_4, e) Furan , f) p Cl- C_6H_4 , g) m NO_2- C_6H_4 , h) p OH- C_6H_4

Synthesis of 8-(1, 5-diphenyl-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (IV a – h)

I. Synthesis of 7-acetoxy-4-methyl Coumarin (I) :

A mixture of 7-hydroxy-4-methyl Coumarin (0.16 mol, 28.2 gm) and acetic anhydride (0.56 mol, 52.87 ml) was refluxed for 1-5hr under anhydrous conditions. While the solution was hot, it was poured into crushed ice and the product was separated out which was filtered and washed with cold water. The obtained product was recrystallized from ethanol. **M.P.- 160±2^0 C**

II. Synthesis of 8-acetyl-7-hydroxy-4-methyl Coumarin (II):

The above obtained 7-acetoxy-4-methyl Coumarin (0.01 mol) and anhydrous $AlCl_3$ (0.03 mol) was heated under anhydrous conditions in an oil bath at 125^{0}C and the temperature was raised and maintained for 2 hr at to 145-160^{0}C. To this reaction mixture the crushed ice was added and acidified with dilute HCl stirring the mixture and was left for 2-3 hr in order to decompose the complex. The separated product was filtered, washed with water and recrystallized from ethanol. **M.P - 182±2^0 C**

III. Synthesis of 8-[(1*E*)-1-phenylprop-1-en-2-yl]-2*H*-chromen-2-one (III) :

A mixture of 8-acetyl-7-hydroxy-4-methyl Coumarin [0.01M] and substitute aromatic aldehyde [0.01M] was stirred in 30 ml of ethanol at the room temperature in the presence of 15ml of 20% NaOH was added to the mixture. This mixture was stirred for 16-18 hrs at room temperature and this reaction mixture was poured into crushed ice and acidifies with dilute HCl to neutral. The chalcones derivative is precipitates out as solid then it was filtered, dried and recrystallized from ethanol.

IV. Synthesis of 8-(1,5-diphenyl-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (IV-a – h)

A mixture of 0.01M Coumarin chalcones and 0.01M of phenyl hydrazine were refluxed in 30ml ethanol and after half an hour add catalytic amount of glacial acetic acid and relaxation was continued for 22-24 hrs. The reacting mixture was cooled and poured into crushed ice, the solid product was precipitated out, and this was washed twice with cold water and recrystallized from ethanol. The reaction is monitored by TLC. All the compounds are characterized by physical and spectral data as shown below.

Table 1 Physical data for the synthesized compounds BP (1-8)

S.No	Comp code	Mol. Formula	M.Wt (grm)	% YIELD	M.P ^{0}C	Calculated % of Elements			
						C	H	N	O
1	BP-1	$C_{25}H_{18}N_2O_3$	394.4	65	182	76.13	4.60	7.10	12.17
2	BP-2	$C_{27}H_{23}N_3O_3$	437.5	58	212	74.12	5.30	9.60	10.97
3	BP-3	$C_{23}H_{16}N_2O_4$	384.4	45	196	73.57	4.75	6.60	15.08
4	BP-4	$C_{25}H_{18}N_2O_4$	410.4	51	205	73.16	4.42	6.83	15.59
5	BP-5	$C_{26}H_{20}N_2O_4$	424.4	58	181	71.87	4.20	7.29	16.65
6	BP-6	$C_{25}H_{17}ClN_2O_3$	428.9	62	211	70.01	4.00	6.53	11.19
7	BP-7	$C_{25}H_{17}N_3O_5$	439.4	56	206	68.33	3.90	9.56	18.21
8	BP-8	$C_{25}H_{18}N_2O_4$	410.4	61	215	72.80	4.89	6.79	15.52

Spectral data for the synthesized compounds BP (1-8)

BP-1: 8-(1,5-diphenyl-1H-pyrazol-3-yl)-7-hydroxy-4-methyl-2H-chromen-2-one.
IR (KBr) cm-1 : 1575 (C=C), 1690 (C=O), 1260 (C-O-C) 1595 (C=N), 1380 (C-N). ^{1}H-NMR (CDCl3 δ in ppm) 2.30 (s, 3H, CH_3), 12.02(s, 1H, OH), 6.70 - 8.10(m, 12H, Ar-H).

BP-2: 7-hydroxy-8-[5-(4-dimethylaminophenyl)-1-phenyl-1H-pyrazol-3-yl]-4-methyl-2H-chromen-2-one.
IR (KBr) cm-1 : 1580 (C=C), 1675 (C=O), 1285 (C-O-C), 1565 (C=N), 1375 (C-N). ^{1}H-NMR (CDCl3 δ in ppm) 2.44 (s, 3H, CH_3), 12.28(s, 1H, OH), 6.40-7.50(m, 12H, Ar-H), 3.25(s,6H,$N(CH_3)_2$), Mass M/z=438.

BP-3: 7-hydroxy-8-[5-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-3-yl]-4-methyl-2H-chromen-2-one.
IR (KBr) cm-1 : 1565 (C=C), 1685 (C=O), 1255 (C-O-C), 1580 (C=N), 1390 (C-N).

BP-4: 7-hydroxy-8-[5-(2-hydroxyphenyl)-1-phenyl-1H-pyrazol-3-yl]-4-methyl-2H-chromen-2-one.
IR (KBr) cm-1 : 1610 (C=C), 1665 (C=O), 1275 (C-O-C), 1570 (C=N), 1385 (C-N).

BP-5: 8-[5-(furan-3-yl)-1-phenyl-1H-pyrazol-3-yl]-7-hydroxy-4-methyl-2H-chromen-2-one.
IR (KBr) cm-1 : 1585 (C=C), 1685 (C=O), 1265 (C-O-C), 1590 (C=N), 1365 (C-N) ^{1}H-NMR (CDCl3 δ in ppm) 2.32 (s, 3H, CH_3), 12.10 (s, 1H, OH), 6.80 - 7.60(m, 12H, Ar-H), 3.87(s, 3H, OCH_3). Mass M/z = 423.

BP-6: 8-[5-(4-chlorophenyl)-1-phenyl-1H-pyrazol-3-yl]-7-hydroxy-4-methyl-2H-chromen-2-one.
IR (KBr) cm-1 : 1580 (C=C), 1670 (C=O), 1250 (C-O-C), 1565 (C=N), 1385 (C-N).

BP-7: 7-hydroxy-4-methyl-8-[5-(3-nitrophenyl)-1-phenyl-1H-pyrazol-3-yl]-2H-chromen-2-one.
IR (KBr) cm-1 : 1590 (C=C), 1655 (C=O), 1280 (C-O-C), 1575 (C=N), 1375 (C-N).

BP-8: 7-hydroxy-8-[5-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-3-yl]-4-methyl-2H-chromen-2-one.
IR (KBr) cm-1 : 1565 (C=C), 1665 (C=O), 1285 (C-O-C), 1560 (C=N), 1380 (C-N)^{1}H-NMR (CDCl3 δ in ppm) 2.26 (s, 3H, CH_3), 12.20(s, 1H, OH), 6.70 - 7.80(m, 12H, Ar-H), 11.18(s, 1H, Ar-OH, Mass M/z = 410.

Biological activity:

Anti-inflammatory activity (*in-vitro model*) [10,11]

The synthesized compounds are screened for anti-inflammatory activity by using inhibition of albumin denaturation technique, which was studied according to Muzushima and Kabayashi with slight modification.

The standard drug and test compounds were dissolved in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions was less than 2.0%. Test solution (1 ml) containing different conc. of drugs was mixed with 1 ml of 1% mM albumin solution in phosphate

buffer and incubated at 27±1^0 C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60^0 ± 1^{0}C in water bath for 10 min. After cooling, the turbidity was measured at 660 nm (UV-Visible Spectrophotometer SL-159, Elico India Ltd.). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken. The ibuprofen was used as standard drug. Results are tabulated in table no 2.

$$\% \text{ of inhibition} = \left(\frac{Vt}{Vc} - 1 \right) \times 100$$

Where, Vt = mean absorbance value of test group.
Vc = mean absorbance value of control group.

Table 2

S.No	Sample	Mean absorbance value ± SEM	% Inhibition of denaturation
1.	Control	0.0800	-
2.	Ibuprofen	0.155 ± 0.0080	93.75
3.	BP1	0.125 ± 0.0070	56.25
4.	BP2	0.128 ± 0.0065	60.01
5.	BP3	0.124 ± 0.0035	55.03
6.	BP4	0.142 ± 0.0050	78.25
7.	BP5	0.132 ± 0.0073	72.12
8.	BP6	0.129 ± 0.0080	61.25
9.	BP7	0.133 ± 0.0072	73.25
10.	BP8	0.138 ± 0.0070	76.50

Antimicrobial activity [12, 13] :

All synthesized compounds were screened for antibacterial and antifungal activity by cup plate method from the standard procedure; the two concentrations are taken i.e. 50 & 100 µg/ml over a different bacterial strains and fungal strains as shown in table. The values obtained are compared with the values produced from the standard drugs like Amoxicillin for bacterial and Fluconazole for fungal and the dimethyl formamide (DMF) was used as control for both the strains. Some of the compounds show significant property compared with the standard and other shows moderate. This will be shown in the table no 3.

Table 3 Mean zone of inhibition in (mm)

S.No	Compound Code.	Staphylococcus aureus (G $^{+ve}$)		E.coli (G $^{-ve}$)		Candida albicans		Aspergillus Niger	
		50 µg	100µg	50µg	100µg	50µg	100µg	50µg	100µg
1	Amoxicillin	22	24	25	28	-	-	-	-
2	Fluconazole	-	-	-	-	18	22	20	24
2	BP1	17 (0.77)	20 (0.83)	21 (0.84)	23 (0.82)	14 (0.78)	18 (0.82)	17 (0.85)	21 (0.87)
3	BP2	18 (0.82)	21 (0.87)	22 (0.88)	24 (0.86)	16 (0.89)	19 (0.86)	18 (0.90)	20 (0.83)
4	BP3	18 (0.82)	21 (0.87)	23 (0.92)	24 (0.86)	17 (0.94)	19 (0.86)	18 (0.90)	21 (0.87)
5	BP4	17 (0.77)	20 (0.83)	21 (0.84)	23 (0.82)	15 (0.83)	17 (0.77)	16 (0.80)	19 (0.79)
6	BP5	19 (0.86)	22 (0.92)	21 (0.84)	25 (0.89)	16 (0.89)	19 (0.86)	18 (0.90)	22 (0.92)
7	BP6	17 (0.77)	19 (0.79)	19 (0.76)	21 (0.75)	16 (0.89)	18 (0.82)	17 (0.85)	19 (0.79)
8	BP7	19 (0.86)	22 (0.92)	23 (0.92)	24 (0.86)	17 (0.94)	19 (0.86)	18 (0.90)	21 (0.87)
9	BP8	19 (0.86)	19 (0.79)	20 (0.80)	21 (0.75)	15 (0.83)	17 (0.77)	18 (0.90)	22 (0.92)
10	Control (DMF)	-	-	-	-	-	-	-	-

RESULTS AND DISCUSSION

The Coumarin chalcones was prepared by claisen-schmidt condensation method by reacting acetoxy Coumarin with different substituted aldehyde(III a-h) and continuing to prepare 8-(1,5-diphenyl-1*H*-pyrazol-3-yl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (IV a-h) by reacting Coumarin chalcones with phenyl hydrazine in ethanol media. The reaction was monitored by TLC using silica gel 60 and final compounds melting point was determined by open capillary method and structure was determined by FT-IR by KBr method, ^{1}H-NMR and FAB-Mass spectroscopy. All the compounds are subjected to anti-inflammatory activity in-vitro model were the results are show in the table and the compound BP-4>8>7>5>6 posses good inhibitory of protein denaturation remaining compounds showed moderate activity. In the same way antimicrobial activity were performed to all compounds in which compound BP-2, 3, 5 and 7 posses significant antibacterial and antifungal activity and rest of the compounds showed moderate activity.

CONCLUSION

The synthesized compounds are identified by spectral data and compounds shows significant to moderate activity for *in-vitro* anti-inflammatory, some compounds shows prominent antimicrobial(anti-bacterial & anti-fungal), based upon this the further studies will be done in future.

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