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Synthesis, characterization of some novel coumarin derivatives and evaluation of their pharmacological activities

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ABSTRACT

A series of new 3-[1-(6-Chloropyrimidin-4-yl)-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one(5a-j) were synthesized by the reaction between 4-aryl substituted-3-(4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-ones (4a-j) and 4,6-Dichloro pyrimidine in the presence of pyridine. The former was obtained by cyclisation of coumarin chalcone derivatives with hydrazine hydrate. The structures of the synthesized compounds have been established by IR and ^{1}H NMR dada. Further they have been screened for anti-inflammatory and antibacterial activities.

Key words: Coumarin, pyrimidine, pyrazole, anti-inflammatory, antibacterial.

INTRODUCTION

Coumarin (or) 1, 2- Benzopyrone (or) Benzo-alpha-pyrones are a very large and important family of compounds. Their structure consists of fused pyrone and benzene rings at position 2. These are present in significant amounts in plants and more than 1300 coumarins were identified from natural sources. The synthesis of coumarins and their derivatives has attracted considerable attention from organic and medicinal chemists for many years as a large number of natural products contain this heterocyclic nucleus [1-3].

They are widely used as additives in food, perfumes, cosmetics, pharmaceuticals and optical brighteners [4], dispersed fluorescent and laser dyes [5]. Furthermore, the pharmacological and biochemical properties as well as therapeutic applications of coumarines depend upon the pattern of substitution. In view of this, coumarins have attracted intense interest in recent years because of their diverse pharmacological properties. Hence, coumarine derivatives have been reported to possess, antibacterial [6-7], anticoagulant [8], antitubercular [9], antimicrobial [10], anti-inflammatory [11], anticancer [12], analgesic [13-14], antimalerial [15], antifungal [16] etc. Thus the synthesis of this heterocyclic nucleus is of much interest. Coumarins have been synthesized by several routes including pechmann, Perkin, Knoevenagel, Reformatsky and Wittig reactions.

Pyrazoline is five-membered heterocyclic having two adjacent nitrogen atoms with in the ring. It has only one endocyclic double bond and is basic in nature. Among its various derivatives, 2-pyrazolines seem to be the most frequently studied pyrazoline type compounds. 2-Pyrazolines can be considered as a cyclic hydrazine moiety [17-19]. Pyrazoline derivatives also posses wide pharmacological activities like antimicrobial [20], anti-inflammatory [21] etc.

Pyrimidines are the heterocyclic aromatic compounds similar to benzene and pyridine containing two nitrogen atoms at positions 1 and 3 of the six membered rings [22-23]. The literature survey indicated that a wide range of pharmacological activities are exhibited by the compounds encompassing pyrimidine nucleus. In addition to this, various analogs of Pyrimidines have been found to posses antibacterial [24], antifungal [25], anti-inflammatory [26], analgesic [27], antihypertensive [28], antiviral [29], antidiabetic [30], anticonvulsant [31], anticancer activity [32] and many of pyrimidines derivatives are reported to possess potential central nervous system (CNS) depressant properties [33] and also act as calcium channel blockers [34] etc.

From the thorough literature survey it was found that coumarins and pyrazoline posses broad spectrum of activities. When one biologically active molecule is linked to another, the resultant molecule generally has increased potency. Hence in the present work we attempted to fuse Coumarin, Pyrazoline and Pyrimidine moieties. It is contemplated that this combination is expected to result in a significant increase in anti-inflammatory and antibacterial activities

Most of the coumarin derivatives were synthesized starting from commercial salicylaldehydes according to the pathway reported in scheme. The required coumarin chalcones were prepared by the condensation of 3-acetylcoumarin with substituted aromatic aldehydes in the presence of piperidine in chloroform. The resulting coumarin chalcones upon cyclisation with hydrazine in the presence of pyridine afford 4-aryl substituted-3-(4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-ones (4a-j).

Finally treatment of 4-aryl substituted-3-(4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-ones with 4,6-dichloro pyrimidine in the presence of pyridine and ethanol gave 3-[1-(6-Chloropyrimidin-4-yl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl]-2*H*-chromen-2-one(5a-j) in 54-68% yield (table 1).

MATERIALS AND METHODS

All the chemicals and solvents used for this work were procured from Vijaya chemicals; Hyd. Melting points were determined on a capillary melting point apparatus and are uncorrected.

The progress of the reaction was monitored by thin layer chromatography (TLC) on silica gel plates. The IR spectra (in KBr pellets) were recorded on a Shimadzu FTIR 157 spectrophotometer. ^{1}H NMR spectra were recorded on a bruker WM- 250 at 250MHz using TMS as internal standard (Chemical shifts in values).

General procedures:

Synthesis of 3-acetyl coumarin: A mixture of salisaldehyde (0.1M), ethylacetoacetate was stirred and cooled. To this 3-5 drops of piperidine was added with shaking. The mixture was maintained at freezing temperature for 2-3hrs. This resulted in separation of a yellow coloured solid mass. The crude product was then recrystallized in ethanol.

Synthesis of 3-Cinnamoyl coumarins: A mixture of 3-acetyl coumarin (1eq) and substituted aromatic aldehydes (1.2eq) in chloroform was stirred with catalytic amount of piperidine under reflux for 1.5-2hrs. Mixture was cooled and chloroform was removed under vaccum, the resulting residue was dried and recrystallized with appropriate solvent.

Synthesis of 4-aryl substituted-3-(4, 5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-ones (4a-j): 2.5g of pyridine was added to the mixture of coumarin chalcone (0.05M) and hydrazine hydrate (0.2M). The reaction mixture was refluxed for 5-8hrs., cooled and neutralized by 2N HCl. Then reaction mixture was poured into crushed ice. The ppt obtained was filtered and recrystallized with appropriate solvent.

Synthesis of 3-[1-(6-Chloropyrimidin-4-yl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl]-2*H*-chromen-2-one(5a-j) : Pyridine (0.01 M) was dissolved in 25 ml of alcohol and then added (0.01 M) of 4a in small portions with continuous stirring. To the above mixture 0.01mole of 4,6-Dichloro pyrimidine was added and the contents of the flask were stirred well for 15 min, refluxed for 2hrs at refluxing temperature. The reaction mixture was cooled and poured on to 200g of crushed ice with constant stirring. The reaction mixture was neutralized to litmus by adding dilute Acetic acid to get the solid product and was collected by filtration and recrystallized with appropriate solvent.

3-[1-(6-Chloropyrimidin-4-yl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl]-2*H*-chromen-2-one (5a):
(KBr, γmax, cm-1): 1649 (C=O), 1544(C=N), 3099 (Ar-H), C-Cl (766); 1H-NMR (400MHz, $CDCl_3$, δ ppm):8.42(s,1H, pyrimidine), 7.52 (s,1H, coumarin), 7.12-7.34 (m,9H, Ar-H), 7.02 (s,1H, pyrimidine), 4.52 (t,1H, pyrazoline), 3.42(d,1H CH_2), 3.33(d,1H, CH_2).

3-(5-(4-Chlorophenyl)-1-(6-Chloropyrimidin-4-yl)-4,5-dihydro-1*H*-pyrazol-3-yl]-2*H*-chromen-2-one (5b):
(KBr, γmax, cm-1): 1646 (C=O), 1547(C=N), 3058 (Ar-H), C-Cl (770), 1192(OCH_3); 1H-NMR (400MHz, $CDCl_3$, δ ppm):8.47(s, 1H, pyrimidine), 7.5 (s, 1H, coumarin), 7.18-7.22(m, 8H, Ar-H), 7.11(s, 1H, pyrimidine), 4.52(t, 1H, pyrazoline), 3.43(d, 1H CH_2), 3.33(d, 1H, CH_2).

3-(5-(2-Chlorophenyl)-1-(6-Chloropyrimidin-4-yl)-4,5-dihydro-1*H*-pyrazol-3-yl]-2*H*-chromen-2-one(5c):
(KBr, γmax, cm-1): 1648 (C=O), 1544(C=N), 3097 (Ar-H), C-Cl (766); 1H-NMR (400MHz, $CDCl_3$, δ ppm):8.46(s, 1H, pyrimidine), 7.6 (s, 1H, coumarin), 7.12-7.27(m,8H, Ar-H), 6.98(s, 1H, pyrimidine), 4.49(t, 1H, pyrazoline), 3.42(d,1H CH_2), 3.36(d,1H, CH_2).

3-[1-(6-Chloropyrimidin-4-yl)-5-(4-nitrophenyl)-4, 5-dihydro-1*H*-pyrazol-3-yl]-2*H*-chromen-2-one (5d):
(KBr, γmax, cm-1): 1647 (C=O), 1566(C=N), 3084 (Ar-H), C-Cl (780); 1H-NMR (400MHz, $CDCl_3$, δ ppm):8.48(s,1H, pyrimidine), 8.15-8.21(m,2H, Ar-H) 7.51 (s,1H, coumarin), 7.38-7.42(m,2H, Ar-H), 7.12-7.26(m,4H, Ar-H), 6.92(s,1H, pyrimidine), 4.46(t,1H, pyrazoline), 3.45(d,1H CH_2), 3.36 (d,1H, CH_2).

3-[1-(6-Chloropyrimidin-4-yl)-5-(2-nitrophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-2*H*-chromen-2-one (5e):
(KBr, γmax, cm-1): 1647 (C=O), 1566(C=N), 3084 (Ar-H), C-Cl (780); 1H-NMR (400MHz, $CDCl_3$, δ ppm):8.44(s,1H, pyrimidine), 8.23(d,1H, Ar-H) 7.52 (s,1H, coumarin), 7.36-7.45(m,3H, Ar-H), 7.14-7.26(m,4H, Ar-H), 6.95(s,1H, pyrimidine), 4.26(t,1H, pyrazoline), 3.37(d,1H CH_2), 3.25 (d,1H, CH_2).

3-[1-(6-Chloropyrimidin-4-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-2*H*-chromen-2-one (5f):
(KBr, γmax, cm-1): 1648 (C=O), 1551(C=N), 3069 (Ar-H), C-Cl(768), 1190(OCH_3); 1H-NMR (400MHz, $CDCl_3$, δ ppm):8.43(s,1H, pyrimidine), 7.42 (s,1H, coumarin), 7.04-7.32 (m,6H, Ar-H), 7.13 (s,1H, pyrimidine), 6.45(m,2H Ar-H), 4.26 (t,1H, pyrazoline), 3.26(s,3H,-CH_3), 3.22(d,1H CH_2), 3.13(d,1H, CH_2).

3-[1-(6-Chloropyrimidin-4-yl)-5-(4-(-dimethylamino)-phenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-2*H*-chromen-2-one (5g):
(KBr, γmax, cm-1): 1649 (C=O), 1551(C=N), 3063 (Ar-H), C-Cl(762), 1196(OCH_3); 1H-NMR (400MHz, $CDCl_3$, δ ppm):8.62(s,1H, pyrimidine), 7.75-7.96(m,4H,Ar-H), 7.56 (s,1H, coumarin), 7.42-7.55(m,4H, Ar-H), 6.65(s, 1H, pyrimidine), 4.92(t,1H, pyrazoline), 3.46(d,1H CH_2), 3.85(d,1H, CH_2), 2.85(s,6H,N,N- Dimethylamine).

3-[1-(6-Chloropyrimidin-4-yl)-5-(3-hydroxy-4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-2*H*-chromen-2-one (5h):
(KBr, γmax, cm-1): 1649 (C=O), 1554(C=N), 3086 (Ar-H), C-Cl (776); 1H-NMR (400MHz, $CDCl_3$, δ ppm):8.48(s, 1H, pyrimidine), 7.4 (s, 1H, coumarin), 7.14-7.26(m, 4H, Ar-H), 7.01(s, 1H, pyrimidine), 6.75(d,2H,Ar-H), 6.58-6.67(m,2H,Ar-H), 5.15(s,1H,-OH), 4.53(t,1H, pyrazoline), 3.41(d,1H CH_2), 3.36(d,1H, CH_2).

3-[1-(6-Chloropyrimidin-4-yl)-5-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-2*H*-chromen-2-one (5i):
(KBr, γmax, cm-1): 1646 (C=O), 1559(C=N), 3086 (Ar-H), C-Cl (776); 1H-NMR (400MHz, $CDCl_3$, δ ppm):8.45(s,1H, pyrimidine), 7.31 (s,1H, coumarin), 7.13-7.24(m,4H, Ar-H), 6.96(s,1H, pyrimidine), 6.72-6.85(m,4H, Ar-H), 5.15(s,1H,-OH), 4.35(t,1H, pyrazoline), 3.36(d,1H CH_2), 3.27 (d,1H, CH_2).

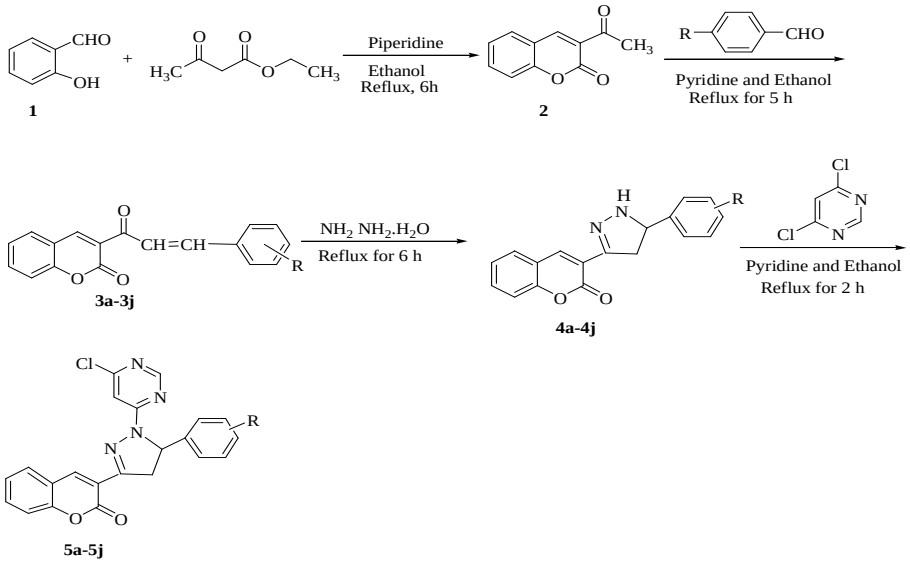
3-[1-(6-Chloropyrimidin-4-yl)-5-(2-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]-2*H*-chromen-2-one (5j):
(KBr, γmax, cm-1): 1647 (C=O), 1561(C=N), 3086 (Ar-H), C-Cl (776); 1H-NMR (400MHz, $CDCl_3$, δ ppm):8.46(s,1H, pyrimidine), 7.41 (s,1H, coumarin), 7.12-7.24(m,4H, Ar-H), 7.02(s,1H, pyrimidine), 6.65-6.82(m,4H, Ar-H), 5.12(s,1H,-OH), 4.29(t,1H, pyrazoline), 3.36(d,1H CH_2), 3.27 (d,1H, CH_2).

RESULTS AND DISCUSSION

The reaction sequence leading to the formation of desired heterocyclic compounds are outlined in scheme.

Treatment of o-hydroxy benzaldehyde (1) with ethylacetoacetate in the presence of piperidine gave 3-acetyl coumarin (2). Later on condensation with substituted aromatic aldehydes gave chalcones (3a-j). Cyclisation of chalcones with hydrazine hydrate gave 4-aryl substituted-3-(4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-ones (4a-j). Finally treatment of 4-aryl substituted-3-(4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-ones with 4,6-dichloro pyrimidine in the presence of pyridine and ethanol gave 3-[1-(6-Chloropyrimidin-4-yl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl]-2*H*-chromen-2-one(5a-j). The structures of the synthesized compounds have been established on the basis of their spectral (IR, ^{1}HNMR Spectroscopy) studies. Amongst the compound tested for anti-inflammatory and antibacterial activity some compound exhibited promising activity and some exhibited significant activity. Physical data of synthesized compounds are presented in Table No.1

Scheme



Physical data of 3-[1-(6-Chloropyrimidin-4-yl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl]-2*H*-chromen-2-one(5a-j).

Table No. 1

S.No	Product code	R	Mol. Formula(Mol. Weight)	M.P (Yield %)
1	5a	H	$C_{22}H_{15}ClN_2O_2$(402.8)	256 (56)
2	5b	p-Cl	$C_{22}H_{14}Cl_2N_4O_2$(437.2)	276(58)
3	5c	o-Cl	$C_{22}H_{14}Cl_2N_4O_2$(437.2)	279(59)
4	5d	p-NO_2	$C_{22}H_{14}Cl_2N_5O_4$(447.8)	281(54)
5	5e	o-NO_2	$C_{22}H_{14}Cl_2N_5O_4$447.8)	284(62)
6	5f	p-OCH_3	$C_{23}H_{17}ClN_4O_3$(432.8)	272(59)
7	5g	$N(CH3)_2$	$C_{24}H_{20}ClN_5O_2$(445.9)	285(65)
8	5h	p-OCH_3, m-OH	$C_{23}H_{17}ClN_4O_4$(448.8)	287(62)
9	5i	p-OH	$C_{22}H_{15}ClN_4O_3$(418.8)	265(68)
10	5j	o-OH	$C_{22}H_{15}Cl_2N_4O_3$(418.8)	267(65)

BIOLOGICAL EVALUATION

Anti-inflammatory activity

Synthesized compounds were screened for anti-inflammatory activity (In vivo) by carrageen induced rat paw oedema model. Diclofenac sodium at dose of 20mg/kg body weight served as standard. Statistical analysis was carried out to determine the percentage reduction in rat paw oedema volume and the results are presented in Table No 2.

Data showing anti-inflammatory activity of Coumarin derivatives in Carrageenan induced acute rat paw oedema model

Table No. 2

Group	Treatment	Dose Mg/kg	Paw oedema volume							
			After 1st hr		After 2 nd hr		After 3rd hr		After 4th hr	
			Mean	% ROV	Mean	% ROV	Mean	% ROV	Mean	% ROV
1	Control	0.5ml	0.76	-	0.85	-	0.98	-	1.07	-
2	Standard	20	0.45	40.78	0.43	49.41	0.39	60.20	0.32	70.09
3	5a	200	0.51	32.89	0.56	34.11	0.60	38.77	0.62	42.06
4	5b	200	0.56	26.31	0.60	29.41	0.65	33.67	0.70	54.57
5	5c	200	0.55	27.63	0.61	28.23	0.66	32.67	0.71	54.64
6	5d	200	0.49	35.52	0.45	45.88	0.50	48.97	0.51	52.33
7	5e	200	0.50	34.21	0.52	38.82	0.55	43.87	0.58	53.79
8	5f	200	0.48	36.84	0.51	40.00	0.56	42.85	0.60	43.92
9	5g	200	0.58	23.68	0.62	27.05	0.68	30.61	0.74	30.84
10	5h	200	0.50	34.21	0.52	38.82	0.55	43.87	0.57	46.72
11	5i	200	0.47	33.23	0.51	37.01	0.54	42.12	0.56	46.11
12	5j	200	0.49	35.52	0.53	39.25	0.56	44.54	0.58	48.20

Antibacterial activity:

The resulted 10 compounds were also screened for antibacterial activity at a concentration of 50µg/ml and 100µg/ml using DMF as a control against *Staphylococcus aureus, Bacillus pumilus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa* by disk-diffusion method on nutrient agar media. Ampicillin and Gentamycin were used as standard drugs for the comparison at the concentration 50 µg/ml and 100 µg/ml against Gram positive and Gram negative bacteria used for the study and the results are presented in Table No 3.

Data showing anti-bacterial activity of Coumarin derivatives:

Table No.3

Sample Code	*Inhibition zone diameter in mm									
	S.aureus		*B.subtilis*		*B.pumilis*		*E.coli*		*P.aureginosa*	
	50µg	100µg	50µg	100µg	50µg	100µg	50µg	100µg	50µg	100µg
5a	3	7	4	8	5	9	4	9	6	10
5b	7	11	8	14	6	13	12	14	8	12
5c	5	7	4	9	4	8	7	10	5	8
5d	7	16	7	17	7	15	11	17	7	15
5e	3	10	5	12	3	9	6	12	6	11
5f	8	19	8	18	9	18	12	20	8	18
5g	7	18	8	19	8	18	8	19	11	22
5h	8	17	7	17	8	16	8	17	9	18
5i	6	15	6	16	7	13	10	16	10	21
5j	2	8	5	11	4	9	-	8	9	20
Gentamycin	13	19	12	17	15	20	13	24	15	25
Ampicillin	15	23	14	24	13	23	14	22	14	23
DMF	-	-	-	-	-	-	-	-	-	-

**Average of triplicate ± Standard deviation; Note: '-'denotes no activity, 8-12 mm poor activity, 13-17 mm moderate activity, 18-20 above good.*

CONCLUSION

Ten new compounds of 3-[1-(6-Chloropyrimidin-4-yl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl]-2*H*-chromen-2-one(5a-j) were synthesized. All the synthesized compounds were characterized by IR, ^{1}HNMR spectral studies. The synthesized compounds were screened for anti-inflammatory and antibacterial activities. The results presented on above tables reveals that compounds 5b,5c,5d and 5e exhibited maximum inhibition of 54.57%, 54.64%,52.33% and 53.79% respectively where as compounds 5i and 5j exhibited a moderate inhibition of 46.1% and48.20% respectively in carrageen induced rat hind paw oedema model. While compounds 5a, 5b, 5c, 5 d, 5 e, and 5f, were found to exhibit moderate antibacterial activities. Among these various compounds 5g, 5i and 5j, were showed good activities in particular against gram-negative bacteria.

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