Synthesis and Evaluation of Some Coumarin Based Schiff's Bases as Potential Antimicrobial Agents'

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Abstract: A series of the title compounds 3-(4-(4, 5-dihydro-5-(substituted phenyl)-1H-3-pyrazolyl) phenylimino) methyl)-4-chloro-2H-chromen-2-one **5(a-g)** have been synthesized. These compounds were characterized on the basis of their spectral (IR, ¹H NMR) data and evaluated for antimicrobial activity *in vitro* against gram positive bacteria, gram negative bacteria and fungi. The compound (**5b**) was found to be the most active with MIC of 20 µg/ml against all the tested organisms.

Keywords: Coumarin, Pyrazoline, Schiff's base, Antimicrobial activity.

Introduction

Development of resistance and adverse reactions towards existing antimicrobial therapy and a sharp rise in the population of immunocompromised patients has provided a thrust to the research in the area of antimicrobials. There are a number of reports that natural and synthetic coumarin derivatives possess antimicrobial activity. Novobiocin and chlorobiocin are established antimicrobials containing a coumarin skeleton. The biological effects of coumarins include antibacterial¹, anti-inflammatory², anti-AIDS³, antithrombotic⁴ and anti-tumorigenic⁴ effects. Similarly pyrazolines have been independently reported to possess antibacterial, antifungal⁵, anticancer⁶, antimalarial⁷ and anticonvulsant⁸ activity. In continuation of the work done in our lab on coumarin based antimicrobial agents⁹, we have designed and synthesized the series of compounds incorporating both, coumarin and pyrazoline moieties as potential antimicrobial agents against gram positive bacteria, gram negative bacteria and fungi.

Experimental

All the chemicals used in the synthesis were of laboratory grade. Melting points were determined in open capillary on Veego melting point electronic apparatus (VMP-D) and are uncorrected. The IR spectra of the synthesized compounds were recorded on Shimadzu

8400-S-FTIR spectrophotometer using potassium bromide. The ^1H NMR spectra were recorded in CDCl_3 using NMR Varian-Mercury 300 MHz spectrometer and chemical shifts are given in units as parts per million, downfield from tetramethylsilane (TMS) as an internal standard. To monitor the reactions as well as to establish the identity and purity of reactants and products, thin layer chromatography was performed on precoated aluminium sheets (silica gel 60 F₂₅₄, 6 x 2.5 cm) using toluene-methanol and chloroform-ethyl acetate as appropriate solvent systems. The spots were visualized under ultra-violet light or by exposure to iodine vapors.

The target compounds were synthesized as per the following scheme:

Scheme 1

General procedure for synthesis of 4-(4, 5-dihydro-5-(substituted phenyl)-1H-pyrazol-3-yl) benzenamine 4(a-g)

A mixture of 1-(4-aminophenyl)-3-(substituted phenyl)-prop-2-en-1-one **3(a-g)** (0.01 mol) and hydrazine hydrate (0.03 mol) in 20 ml absolute ethanol was taken in a 100 ml RBF. The reaction mixture was heated under reflux for 6-8 hrs then left overnight at 0°C. The crude

product which separated out was filtered, washed with water and recrystallized from ethanol. The physical and the spectral data are presented in **Table1**.

Table 1. Physical and spectral data of 4-(4, 5-dihydro-5-(substituted phenyl)-1*H*-pyrazol-3-yl) benzenamine **4(a-g)**.

Code No.	R	M. P.* (°C)	R _f * *	Percentage Yield (%)	IR (KBr,cm ⁻¹)
4a	H-	122-126	0.67	69	3446 (NH), 3330 & 3319 (NH ₂), 3087 (Ar-CH), 1523 (pyrazoline ring C=N), 1500(C=C).
4b	<i>p</i> -Cl-	135-139	0.71	60	3400(NH), 3383&3362(NH ₂), 3043(Ar-CH),1519 (pyrazoline ring C=N), 1489(C=C), 827 (C-Cl). ¹ H NMR (CDCl ₃ δ ppm):- 7.49-6.59 (8H, m, Ar- <i>H</i>), 5.81 (1H, s, <i>NH</i>), 4.87-4.84 (1H, t, <i>Hc</i>), 3.82 (2H, s, <i>NH</i> ₂), 3.46-3.43 (1H, dd, <i>Ha</i>), 2.98-2.95 (1H, dd, <i>Hb</i>).
4c	<i>m</i> -Cl-	137-140	0.68	65	3410(NH), 3334 & 3324 (NH ₂), 3035(Ar-CH), 1558 (pyrazoline ring C=N), 1489(C=C), 827(C-Cl).
4d	<i>o</i> -Cl-	133-136	0.66	68	3405(NH), 3390&3383(NH ₂), 2950(Ar-CH), 1510 (pyrazoline ring C=N), 1480(C=C), 829 (C-Cl).
4e	<i>p</i> -NO ₂ -	148-151	0.62	78	3409 (NH), 3345 & 3333 (NH ₂), 3036 (Ar-CH), 1600(pyrazoline ring C=N), 1500(NO ₂), 1450(C=C).
4f	<i>m</i> -NO ₂ -	145-150	0.71	80	3401(NH),3390&3383(NH ₂),3043(Ar-CH),1519(NO ₂), 1528(pyrazoline ring C=N), 1450 (C=C).
4g	<i>o</i> -OH-	125-128	0.65	74	3401(NH),3360&3334(NH ₂),3200(Ar-OH),3035(Ar-CH),1604(pyrazoline ringC=N), 1496 (C=C).

*All melting points are uncorrected, **Mobile phase- Benzene: Ethyl acetate = 9:1, Recrystalization solvent: Ethanol.

General procedure for synthesis of 3-(4-(4, 5-dihydro-5-(substituted phenyl)-1H-pyrazolyl) phenylimino) methyl)-4-chloro-2H-chromen-2-one (5a-g)

A mixture of 4-(4, 5-dihydro-5-(substituted phenyl)-1H-pyrazol-3-yl) benzenamine **4(a-g)** (0.01 mol) and 4-chloro-2-oxo-2H-chromene-3-carbaldehyde (**2**) (0.01mol) was taken in a 100 ml RBF containing 20ml of ethanol. Ethanolic NaOH (10%, 10ml) was added to the reaction mixture & it was heated under reflux for 6-8 hrs after which it was allowed to cool to room temperature. The solid obtained was filtered, washed with water, dried in air and recrystallized from ethanol. The physical and the spectral data are presented in **Table 2**.

Antimicrobial activity

Zone of inhibition (Cup-plate method)

All the target compounds **5(a-g)** were tested for their antimicrobial activity by agar cup plate method¹⁰. The organisms used for antibacterial activity were gram positive bacteria: *S. aureus* (ATCC 9144), *B. subtilis* (ATCC 6633) and *S. epidermis* (ATCC 12228), gram negative bacteria: *E. coli* (ATCC 25922), *S. typhi* and *P. aeruginosa* (ATCC 9027) and the media was nutrient agar broth. The antifungal activity was performed against *A. niger* (ATCC 10594) and *C. albicans* (ATCC 10231). The media being potato dextrose agar. Amoxycillin and fluconazole were used as standards for antibacterial and antifungal activity respectively. The observations are given in **Table 3**, **4** and **5** for gram positive bacteria, gram negative bacteria and fungi respectively.

Table 2. Physical and spectral data of 3-(4-(4, 5-dihydro-5-(substituted phenyl)-1H-pyrazolyl) phenylimino) methyl)-4-chloro-2H-chromen-2-one (**5a-g**).

Code No.	R	M.P. * (⁰ C)	** R _f	Percentage yield (%)	I.R. (KBr, cm ⁻¹)	¹ H NMR (CDCl ₃ , δppm)
5a	H-	208-210	0.68	71	3397(NH), 2922(Ar-CH), 1660(coumarin C=O), 1630(CH=N), 1602(pyrazoline ring C=N), 1463(C=C), 1190(coumarin C-O), 780(C-Cl).	9.21(1H,s,CH=N),8.48-8.02(12H,m,Ar-H), 6.18-6.16(1H, s, NH), 4.98-4.91(1H ,t, Hc), 3.66-3.69 (1H ,dd, Ha), 3.15-3.13 (1H, dd, Hb).

5b	<i>p</i> -Cl-	225-227	0.69	60	3403(NH), 3030 (Ar-CH), 1650(C=O), 1603(CH=N), 1538 (pyrazoline ring C=N), 1461(C=C), 1204(coumarin C-O), 780 (C-Cl)	9.12(1H,s,CH=N),8.79-7.24(12H, m, Ar-H), 6.22(1H, s, NH), 5.41-5.39 (1H ,t, Hc), 3.94-3.91 (1H ,dd, Ha), 3.22-3.19 (1H, dd, Hb).
5c	<i>m</i> -Cl-	228-230	0.72	62	3401(NH), 3064(Ar-CH), 1662(coumarin C=O),1605 (pyrazoline ring C=N), 1583(CH=N), 1461(C=C),1217 (coumarin C-O), 777 (C-Cl)	9.12(1H,s,CH=N),8.79-7.24 (12H, m, Ar-H), 6.22(1H, s, NH), 5.41-5.39(1H ,t, Hc), 3.94-3.91 (1H ,dd, Ha), 3.22-3.19 (1H, dd, Hb).
5d	<i>o</i> -Cl-	221-224	0.66	64	3401(NH), 3064 (Ar-CH), 1662(coumarin C=O),1605 (pyrazoline ring C=N), 1583(CH=N), 1461(C=C), 1217 (coumarin C-O), 777 (C-Cl)	9.12(1H,s,CH=N),8.79-7.24(12H, m, Ar-H), 6.22(1H, s, NH), 5.41-5.39(1H ,t, Hc), 3.94-3.91 (1H ,dd, Ha), 3.22-3.19 (1H, dd, Hb).
5e	<i>p</i> -NO ₂ -	230- 233	0.65	74	3390(NH),2925(Ar-CH), 1634(coumarin C=O), 1583(CH=N), 1558 (pyrazoline ring), 1500(NO ₂),1461(C=C), 1241(coumarin C-O), 780 (C-Cl),	9.29 1H,s,CH=N),8.87-7.21(12H, m, Ar-H), 6.23(1H, s, NH), 5.34-5.32(1H ,t, Hc), 3.41-3.39 (1H ,dd, Ha), 2.52-5.54 (1H, dd, Hb).
5f	<i>m</i> -NO ₂ -	229-232	0.67	76	3402(NH), 3076(Ar-CH), 1644(coumarin C=O), 1658(CH=N),1555 (pyrazoline ring C=N), 1489(C=C), 1265(coumarin C-O), 1510(NO ₂), 768 (C-Cl)	9.29 1H,s,CH=N),8.87-7.21 (12H, m, Ar-H), 6.23(1H, s, NH), 5.34-5.32(1H ,t, Hc), 3.41-3.39 (1H ,dd, Ha), 2.52-5.54 (1H, dd, Hb).
5g	<i>o</i> -OH-	229-241	0.70	68	3460(Ar-OH), 3319(NH), 2876(Ar-CH), 1624(coumarin C=O),1560(pyrazoline ring CH=N),1532(C=N), 1490(C=C), 1220(coumarin C-O), 790 (C-Cl)	9.20(1H,s,CH=N),8.83-7.21(12H,m,Ar-H), 6.69(1H,s,Ar-OH), 6.23(1H, s, NH), 5.34-5.32(1H ,t, Hc), 3.41-3.39 (1H ,dd, Ha), 2.52-2.54 (1H, dd, Hb).

*All melting points are uncorrected, **Mobile phase- Benzene: ethyl acetate = 9:1, Recrystalization solvent: Ethanol.

Table 3. Zone of inhibition against Gram positive bacteria:

Compound code	Concentration $\mu\text{g}/10\mu\text{l}$	Zone of inhibition in mm (SEM)		
		<i>S. aureus</i>	<i>S. epidermis</i> <i>epidermisepidermis</i>	<i>B. subtilis</i>
Amoxycillin	30	26(± 1.46)	27(± 1.39)	29(± 1.42)
5a	50	6(± 1.37)	7(± 1.42)	5(± 1.44)
	100	10(± 1.51)	13(± 1.53)	9(± 1.50)
	150	13 (± 1.36)	18 (± 1.41)	14 (± 1.62)
	5b	9(± 1.39)	7(± 1.40)	7(± 1.50)
5b	100	14(± 1.40)	13(± 1.38)	15(± 1.52)
	150	25 (± 1.52)	24 (± 1.54)	27 (± 1.61)
	5c	7(± 1.42)	9(± 1.36)	9(± 1.41)
	100	13(± 1.44)	12(± 1.37)	11(± 1.45)
5c	150	21 (± 1.63)	19 (± 1.55)	18 (± 1.47)
	5d	8(± 1.49)	5(± 1.49)	6(± 1.37)
	100	12(± 1.38)	10(± 1.52)	10(± 1.39)
	150	17 (± 1.52)	14 (± 1.44)	16 (± 1.64)
5d	5e	6(± 1.38)	6(± 1.41)	8(± 1.47)
	100	11(± 1.48)	11(± 1.45)	12(± 1.49)
	150	16 (± 1.38)	16 (± 1.42)	17 (± 1.43)
	5f	7(± 1.50)	4(± 1.55)	7(± 1.44)
5e	100	15(± 1.54)	9(± 1.41)	11(± 1.37)
	150	20 (± 1.60)	13 (± 1.51)	14 (± 1.66)
	5g	6(± 1.38)	5(± 1.36)	6(± 1.57)
	100	12(± 1.40)	8(± 1.46)	11(± 1.59)
5f	150	16 (± 1.65)	13 (± 1.46)	16 (± 1.54)

Table 4. Zone of inhibition against Gram negative bacteria:

Compound code	Concentration $\mu\text{g} / 10 \mu\text{l}$	Zone of inhibition in mm(SEM)		
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>
Amoxycillin	30	27 (± 1.37)	25 (± 1.35)	28 (± 1.30)
5a	50	9(± 1.40)	9(± 1.42)	8(± 1.53)
	100	14(± 1.37)	13(± 1.50)	15(± 1.60)
	150	15 (± 1.47)	15 (± 1.49)	17 (± 1.39)
5b	50	10(± 1.37)	10(± 1.38)	10(± 1.39)
	100	15(± 1.51)	14(± 1.44)	16(± 1.45)
	150	26 (± 1.45)	20 (± 1.48)	25 (± 1.50)
5c	50	10(± 1.44)	6(± 1.46)	10(± 1.42)
	100	12(± 1.54)	12(± 1.38)	13(± 1.53)
	150	19 (± 1.52)	19 (± 1.48)	20 (± 1.38)
5d	50	8(± 1.55)	9(± 1.49)	7(± 1.42)
	100	10(± 1.45)	12(± 1.51)	11(± 1.36)
	150	15 (± 1.44)	18 (± 1.37)	15 (± 1.51)
5e	50	5(± 1.44)	7(± 1.54)	9(± 1.39)
	100	11(± 1.59)	10(± 1.40)	10(± 1.41)
	150	17 (± 1.62)	13 (± 1.66)	14 (± 1.45)
5f	50	6(± 1.60)	9(± 1.39)	8(± 1.50)
	100	8(± 1.45)	12(± 1.44)	12(± 1.59)
	150	10 (± 1.41)	15 (± 1.45)	16 (± 1.39)
5g	50	4(± 1.52)	4(± 1.57)	7(± 1.63)
	100	9(± 1.46)	7(± 1.61)	10(± 1.58)
	150	12 (± 1.35)	9 (± 1.47)	14 (± 1.34)

Table 5. *Antifungal activity.*

Compound code	Concentration $\mu\text{g}/10\mu\text{l}$	Zone of inhibition in mm (SEM)	
		<i>A. niger</i>	<i>C. albicans</i>
Fluconazole	30	27 (± 1.39)	29 (± 1.42)
5a	50	6(± 1.42)	7(± 1.36)
	100	11(± 1.39)	13(± 1.40)
	150	14 (± 1.51)	16 (± 1.55)
5b	50	9(± 1.32)	10(± 1.34)
	100	15(± 1.39)	16(± 1.40)
	150	25 (± 1.46)	27 (± 1.36)
5c	50	8(± 1.44)	10(± 1.47)
	100	15(± 1.48)	17(± 1.43)
	150	21 (± 1.47)	23 (± 1.38)
5d	50	9(± 1.50)	11(± 1.52)
	100	13(± 1.42)	14(± 1.36)
	150	18 (± 1.62)	19 (± 1.51)
5e	50	7(± 1.37)	8(± 1.41)
	100	10(± 1.42)	11(± 1.43)
	150	16 (± 1.53)	17 (± 1.52)
5f	50	6(± 1.55)	7(± 1.51)
	100	11 (± 1.37)	12(± 1.38)
	150	14(± 1.46)	16 (± 1.44)
5g	50	6(± 1.42)	7(± 1.36)
	100	11(± 1.39)	13(± 1.40)
	150	14 (± 1.51)	16 (± 1.55)

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) against the above mentioned organisms was determined by the broth dilution method¹¹. The results are given in **Table 6**.

Table 6. Minimum inhibitory concentration for Gram positive & Gram negative bacteria:-

Compound Code	MIC ($\mu\text{g/ml}$)			MIC ($\mu\text{g/ml}$)		
	<i>B. subtilis</i>	<i>S. aureus</i> <i>B.subtilis</i>	<i>S. epidermis</i>	<i>E. coli</i>	<i>P. aeruginosa</i> <i>aaaeruginosa</i>	<i>S. typhi</i>
5a	25	25	30	25	30	30
5b	20	20	20	20	20	20
5c	20	25	35	30	35	25
5d	25	30	35	35	40	30
5e	30	35	30	35	30	25
5f	25	30	30	30	25	35
5g	30	25	30	35	30	35

Results and Discussion

4-Hydroxycoumarin (**1**)¹², 4-chloro-2-oxo-2*H*-chromene-3-carbaldehyde (**2**)¹³ and 1-(4-aminophenyl)-3-(substituted phenyl) prop-2-en-1-ones **3(a-g)**,^{14 and 15} were synthesized by using reported procedures. The physical and the spectral values were found to match with the reported values.

4-(4, 5-Dihydro-1-phenyl-5-(substituted phenyl)-1*H*-pyrazol-3-yl) benzene-amine **4 (a-g)** were synthesized by reaction of 1-(4-aminophenyl)-3-(substituted phenyl) prop-2-en-1-one **3(a-g)** with hydrazine hydrate using ethanol as solvent. These were then reacted with 4-chloro-2-oxo-2*H*-chromene-3-carbaldehyde (**2**) to yield the target compounds 3-(4-(4, 5-dihydro-5-(substituted phenyl)-1*H*-3-pyrazolyl) phenylimino) methyl)-4-chloro-2*H*-chromen-2-one (**5a-g**).

All the synthesized compounds **4(a-g)** were solids melting at around the range of 122-151⁰C (**Table 1**) and freely soluble in acetone and chloroform. The solid state IR (KBr) spectra of these compounds reveal a characteristic aromatic stretch between 3087-2950 cm⁻¹. The NH group present in the pyrazoline ring revealed peaks at around the range 3400-3446 cm⁻¹. The C=N group present in the pyrazoline ring peaks were observed at around the range 1510-1604 cm⁻¹. The stretching vibrations for free amino group are seen between 3319-3390 cm⁻¹ (**Table 1**). The ¹H NMR spectrum of the compound (**4b**) was recorded in CDCl₃. The compound (**4b**) revealed doublet at around 4.87-4.84 ppm for pyrazoline 5-*H* and singlet for NH at 5.81 ppm. The pyrazoline 4-CH₂ revealed a doublet of doublet at around 3.46-2.95 ppm. The singlet for NH₂ was observed at 3.82 ppm. The aromatic protons showed peaks at 7.30-7.49 & 6.68-6.5 ppm as multiplets (**Table 1**). All the target compounds **5(a-g)** were solids melting at around the range of 208-241⁰C and freely soluble in acetone and chloroform. The solid state IR (KBr, cm⁻¹) spectra of these compounds reveal a characteristic aromatic stretch between 2876-3076 cm⁻¹ and sharp carbonyl stretching vibration for coumarin lactone at 1624-1662 cm⁻¹ (**Table 2**). The -CH=N stretch of was observed at 1560-1658 cm⁻¹. No peak was observed for free amino group observed in the spectra of **4(a-g)** at around 3300-3200 cm⁻¹ indicating incorporation of this group into the Schiff's base.

The ¹H NMR spectra of the series of target compounds **5(a-g)** were recorded in CDCl₃. All compounds revealed a doublet of doublet for pyrazoline 5-*H* at around 4.98-4.91 ppm. The CH=N proton appeared at 9.29-9.12 ppm. (**Table 2**). The aromatic protons showed peaks at 8.87-7.29 ppm as multiplets. No peak was observed for free amino group observed in the

spectra of **4(a-g)** at 3.82 ppm confirms the formation of -CH=N group. This indicates the successful condensation of 4-(4, 5-dihydro-phenyl-5-(substituted phenyl)-1*H*-pyrazol-3-yl) benzenamine **4(a-g)** with 4-chloro-2-oxo-2*H*-chromene-3-carbaldehyde (**2**).

All the target compounds have shown good antimicrobial activity (**Table no. 3, 4, 5 and 6**) as compared to standard and compound **5b** exhibiting highest zone of inhibition and least MIC (20 µg/10µl) against all the organisms.

Conclusion

From the antimicrobial data of the synthesized compounds, we can conclude that the series of target compounds **5(a-g)**, 4-chloro-2-oxo-2*H*-chromene-3-carbaldehyde and 4-(4, 5-dihydro-phenyl-5-(substituted phenyl)-1*H*-pyrazol-3-yl) benzenamines hold promise as potential antimicrobial agents after further optimization.

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