Synthesis and Evaluation of Some Coumarin Containing Potential Antimicrobial Agents

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Abstract: A series of the Schiff's bases incorporating coumarin and chalcone moieties, 3-(4-(4-(substituted phenyl)prop-1-ene-3-one) phenylimino) methyl)-4-chloro-2h-chromen-2-one 4(a-g) were synthesized as potential antimicrobial agents. These compounds were characterized on the basis of their spectral (IR, ¹H NMR) data and evaluated for antimicrobial activity in vitro against gram positive and gram negative bacteria and fungi. Compound 4b was found to be most active with an MIC of 20 µg/mL against all the tested organisms.

Keywords: Coumarins, Chalcone, Schiff's base, Antibacterial, Antifungal activity.

Introduction

There are a number of reports that natural and synthetic coumarin derivatives possess antimicrobial activity¹⁻². Novobiocin and chlorobiocin are established antimicrobials containing a coumarin skeleton. A number of the amino substituted coumarin derivatives have been reported as the inhibitors of DNA Gyrase as potential antibacterials³. Elgamel *et al*⁴ have reported synthesis and antimicrobial properties of hydrazino derivatives of 5-chloro-6-formylfuranocoumarins. Similarly chalcones have also been shown to display antimicrobial activity⁵. Development of resistance towards existing antimicrobial therapy and a sharp rise in the population of immunocompromised patients have provided a thrust to the research in the area of antimicrobials. In continuation of the work done in our laboratory on coumarin based antimicrobial agents⁵, we have designed a series of compounds incorporating coumarin and chalcone moieties in the structure in hope of achieving a better antimicrobial activity. The compounds have been synthesized via simple steps given in Scheme 1.

Experimental

All the chemicals used in the synthesis were of laboratory grade. Melting points were determined in open capillary on Veego (model: VMP-D) electronic apparatus and are

uncorrected. The IR spectra of the synthesized compounds were recorded on shimadzu 8400-S FT-IR 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 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 f_{254} , 5 x 2.5 cm) using toluene-methanol and chloroform-ethyl acetate as appropriate solvent systems.

Scheme 1

General procedure for Synthesis of 3-(4-(4-substituted phenyl) prop- 1-ene-3-one) phenylimino) methyl)-4-chloro-2H-chromen-2-one 4(a-g)

4-chloro-3-coumarinaldehyde (0.01mol) was dissolved in 20ml of rectified spirit. To this solution the chalcone **3(a-g)** (0.01 mol) was added and refluxed for 7-8 hrs. On completion of the reaction, water was added to this solution until cloudiness persisted. It was then kept aside to cool when the product separated out as precipitates. The solid obtained was filtered, washed with water, dried in air and recrystallized from methanol. The physical and spectral data is provided in **Table I**.

Table I. Physical and spectral data of synthesized compounds.

Code No.	R	* Melting Point(°C)	* * R _f	% Yield	I.R (KBr, cm ⁻¹)	¹ H NMR (CDCl ₃ , δ ppm)
4a	H	272 -274	0.45	92	2922, 2851(Ar stretch), 1737 (coumarin C=O), 1642 (Aliphatic C=C)1638 (C=O), 1558(C=N),	9.33(1H, s,-CH=N), 8.8(1H,d,-CH), 8.4-7.8(1H,d,-CH), 8.4-7.4 (13H,m,-Ar-H)
4b	<i>p</i> -Cl	265 -267	0.55	90	2922,2851(Ar stretch), 1750 (coumarinC=O)& 1634(C=O),1655 (AliphaticC=C)1534 (C=N), 846(C-Cl)	9.3(1H,s,-CH=N), 8.8(1H,d,-H), 8.6(1H,d,-H),8.5-7.5 (12H,m,Ar-H)
4c	<i>m</i> -Cl	260 -262	0.57	91	2922, 2850(Ar stretch), 1760 (coumarin C=O), 1639 (C=O), 1568(C=N), 1657 (Aliphatic C=C), 836(C-Cl)	9.3(1H,s,-CH=N), 8.4(1H,d,-CH), 8.5-7.4(12H,m,Ar-H), 8.8(1H, d,-CH)
4d	<i>p</i> -NO ₂	220 -222	0.47	89	2959,2880(Ar stretch),1745 (coumarinc=O),1644(C=O), 1558(C=N), 1644 (aliphatic C=C) 1361(Ar-NO ₂)	9.3(1H,s,-CH=N), 8.4(1H,d,-CH), 8.5-7.4(12H,m,Ar-H), 8.8(1H, d,-CH)
4e	<i>m</i> -NO ₂	275 -278	0.50	91	2848, 2770 (Ar stretch),1755(coumarin C=O),1632 (C=O), 1568(C=N), 1643 (Aliphatic C=C), 1452(NO ₂)	9.3(1H,s,-CH=N), 8.8(1H,d,-CH), 8.6(1H,d,H), 8.4-7.4 (12H,m,Ar-H),
4f	<i>o</i> -OH	214 -217	0.56	93	3319, 3228(OH Ar stretch), 2876(Ar stretch),1734(coumarin C=O),1639 (C=O), 1523(C=N stretch), 1344 (C=C stretch)	9.4(1H,s,-CH=N), 8.1(2H, d,- H), 8.0(2H, d,-H),7.7-7.4(11H,m,Ar-H), 5.3(1H,s,OH)
4g	<i>o</i> -Cl	254 -258	0.54	90	2922, 2851(Ar stretch), 1750 (coumarin C=O), 1645 (C=O), 1534 (C=N), 1659 (aliphatic C=C)	9.2(1H,s,-CH=N), 8.8(2H, d,- H), 8.5-7.4 (12H,m,Ar-H)

*All melting points are uncorrected.
Recrystallization Solvent: Methanol.

**Mobile Phase- Toulene: Methanol 9:1.

Antimicrobial activity

Zone of inhibition

All the target compounds **4(a-g)** were tested for their antimicrobial activity by agar cup plate method⁷ by using nutrient agar broth media. The organisms used 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). The antifungal activity was evaluated against *A. Niger* (ATCC 10594) and *C. Albicans* (ATCC 10231) by using potato dextrose agar as media. Amoxycillin and fluconazole were used as standards for antibacterial and antifungal activity respectively. The observations are given in **Table II** and **III** for antibacterial activity and in **Table IV** for antifungal activity.

Table II. Zone of inhibition of synthesized compounds against gram positive bacteria.

Compound Code	Concentration $\mu\text{gm}/10\mu\text{l}$	Zone of inhibition in mm (SEM)		
		<i>B.subtilis</i>	<i>S.aureus</i>	<i>S.epidemis</i>
Amoxycillin (30)		31 (± 1.56)	33 (± 1.55)	30 (± 1.64)
4a	100	11 (± 1.06)	14 (± 1.16)	10 (± 1.26)
	120	16 (± 1.36)	18 (± 1.48)	12 (± 1.16)
	150	23 (± 1.46)	26 (± 1.59)	19 (± 1.39)
4b	100	13 (± 1.11)	12 (± 1.21)	11 (± 1.33)
	120	16 (± 1.44)	16 (± 1.56)	15 (± 1.36)
	150	26 (± 1.53)	30 (± 1.67)	25 (± 1.52)
4c	100	9 (± 1.06)	11 (± 1.10)	7 (± 1.21)
	120	14 (± 1.32)	17 (± 1.43)	9 (± 1.46)
	150	18 (± 1.33)	24 (± 1.49)	11 (± 1.22)
4d	100	15 (± 1.18)	16 (± 1.29)	10 (± 1.28)
	120	21 (± 1.26)	20 (± 1.22)	12 (± 1.16)
	150	24 (± 1.42)	25 (± 1.53)	23 (± 1.43)
4e	100	10 (± 1.16)	11 (± 1.16)	10 (± 1.15)
	120	15 (± 1.12)	15 (± 1.11)	12 (± 1.10)
	150	20 (± 1.40)	21 (± 1.41)	22 (± 1.42)
4f	100	11 (± 1.40)	10 (± 1.32)	9 (± 1.33)
	120	13 (± 1.31)	16 (± 1.38)	11 (± 1.36)
	150	23 (± 1.42)	22 (± 1.41)	18 (± 1.32)
4g	100	7 (± 1.23)	8 (± 1.25)	12 (± 1.26)
	120	13 (± 1.28)	12 (± 1.26)	15 (± 1.22)
	150	19 (± 1.37)	18 (± 1.32)	21 (± 1.40)

Table III: Zone of inhibition of synthesized compounds against gram negative bacteria.

Compound Code	Concentration $\mu\text{gm}/10\mu\text{l}$	Zone of inhibition in mm (SEM)		
		<i>B. subtilis</i>	<i>S. aureus</i>	<i>S. epidemis</i>
Amoxycillin (30)		31 (± 1.56)	33 (± 1.55)	30 (± 1.64)
4a	100	11 (± 1.06)	14 (± 1.16)	10 (± 1.26)
	120	16 (± 1.36)	18 (± 1.48)	12 (± 1.16)
	150	23 (± 1.46)	26 (± 1.59)	19 (± 1.39)
4b	100	13 (± 1.11)	12 (± 1.21)	11 (± 1.33)
	120	16 (± 1.44)	16 (± 1.56)	15 (± 1.36)
	150	26 (± 1.53)	30 (± 1.67)	25 (± 1.52)
4c	100	9 (± 1.06)	11 (± 1.10)	7 (± 1.21)
	120	14 (± 1.32)	17 (± 1.43)	9 (± 1.46)
	150	18 (± 1.33)	24 (± 1.49)	11 (± 1.22)
4d	100	15 (± 1.18)	16 (± 1.29)	10 (± 1.28)
	120	21 (± 1.26)	20 (± 1.22)	12 (± 1.16)
	150	24 (± 1.42)	25 (± 1.53)	23 (± 1.43)
4e	100	10 (± 1.16)	11 (± 1.16)	10 (± 1.15)
	120	15 (± 1.12)	15 (± 1.11)	12 (± 1.10)
	150	20 (± 1.40)	21 (± 1.41)	22 (± 1.42)
4f	100	11 (± 1.40)	10 (± 1.32)	9 (± 1.33)
	120	13 (± 1.31)	16 (± 1.38)	11 (± 1.36)
	150	23 (± 1.42)	22 (± 1.41)	18 (± 1.32)
4g	100	7 (± 1.23)	8 (± 1.25)	12 (± 1.26)
	120	13 (± 1.28)	12 (± 1.26)	15 (± 1.22)
	150	19 (± 1.37)	18 (± 1.32)	21 (± 1.40)

Table IV: Zone of inhibition of synthesized compounds against fungi.

Compound Code	Concentration $\mu\text{g/ml}$	Zone of inhibition in mm (SEM)	
		<i>A. niger</i>	<i>C.albicans</i>
Fluconazole (35)		26 (± 1.32)	28 (± 1.37)
4a	100	10 (± 1.40)	12 (± 1.41)
	120	11 (± 1.42)	13 (± 1.44)
	150	15 (± 1.45)	14 (± 1.42)
4b	100	16 (± 1.31)	15 (± 1.35)
	120	18 (± 1.30)	17 (± 1.38)
	150	24 (± 1.32)	25 (± 1.35)
4c	100	12 (± 1.42)	9 (± 1.44)
	120	13 (± 1.45)	14 (± 1.41)
	150	20 (± 1.50)	17 (± 1.49)
4d	100	13 (± 1.48)	11 (± 1.46)
	120	16 (± 1.44)	10 (± 1.41)
	150	18 (± 1.47)	13 (± 1.40)
4e	100	13 (± 1.40)	12 (± 1.41)
	120	14 (± 1.39)	13 (± 1.43)
	150	17 (± 1.49)	15 (± 1.48)
4f	100	9 (± 1.21)	12 (± 1.22)
	120	11 (± 1.24)	14 (± 1.25)
	150	18 (± 1.20)	15 (± 1.27)
4g	100	12 (± 1.70)	11 (± 1.71)
	120	13 (± 1.72)	13 (± 1.73)
	150	16 (± 1.72)	17 (± 1.65)

Minimum inhibitory concentration

The Minimum inhibitory concentration (MIC) against the above organisms was determined by the method of broth dilutions⁸. The results are given in **Table V**.

Table V: Minimum inhibitory concentration for the synthesized compounds ($\mu\text{g/ml}$).

Compound Code	Gram Positive Bacteria			Gram Negative Bacteria		
	<i>B.subtilis</i>	<i>S.aureus</i>	<i>S. epidermis</i>	<i>E.coli</i>	<i>S.typhi</i>	<i>P.aeruginosa</i>
4a	20	30	30	30	25	30
4b	20	20	20	20	20	20
4c	25	25	35	20	30	30
4d	30	30	35	35	30	25
4e	25	25	30	25	35	30
4f	25	30	30	35	30	30
4g	30	35	35	25	25	25

Results and Discussion

4-Hydroxycoumarin⁹ (**1**), 4-chloro-2-oxo-2h-chromene-3-carbaldehyde¹⁰ (**2**) and 1-(4-aminophenyl)-3-(substituted phenyl) prop-2-en-1-ones¹¹ **3(a-g)**, were synthesized by using reported procedures. The physical and the spectral values were found to match with the reported values. The targeted Schiff's bases, 3-(4-(4-(substituted phenyl) prop-1-ene-3- one) phenylimino methyl) 4chloro-2H-chromen-2-one **4(a-g)** were synthesized by reacting equimolar quantities of 4-chloro-2-oxo-2h-chromene-3-carbaldehyde (**2**) and 1-(4-aminoaryl)-3-(substituted phenyl) prop-2-en-1-one **3(a-g)** in rectified spirit. The compounds **4(a-g)** obtained are solids melting at around the range of 214- 274°C. The solid state IR (KBr) spectra (**Table I**) of these compounds reveal a characteristic aromatic stretch between 2900-3100cm⁻¹. Sharp α , β -unsaturated carbonyl group stretch at around 1620-1741 cm⁻¹ and sharp carbonyl stretching vibration for coumarin lactone at around 1750 cm⁻¹ were also seen. The stretching vibrations for C=N group are seen at around 1534-1602 cm⁻¹. The ¹H NMR spectra (**Table I**) of were recorded in CDCl₃. The aromatic protons showed peaks at 7.4-8.6 ppm as multiplet. The olefinic protons displayed characteristic doublets at around 8.8-8.5 and 8.4-8.3. Absence of peak by aldehydic proton confirms formation of schiff's base. Absence of singlet by amino protons confirms that the intermediates the chalcone **3(a-g)** have been condensed successfully with 4-chloro-2-oxo-2H-chromene-3-carbaldehyde (**2**). This is further confirmed by appearance of imine peak proton at around 9.4 ppm.

All the target compounds **4(a-g)** have shown good in-vitro antimicrobial activity (**Table III** and **IV**) against all the tested gram +ve and gram -ve bacteria and fungi. Compound **4b** exhibited highest zone of inhibition and least MIC (20 $\mu\text{g/mL}$) against all organisms.

Conclusions

From the antimicrobial data of the synthesized compounds, we can conclude that the series of targeted compound **4(a-g)** 3-(4-(4-(Substituted phenyl)prop-1-ene-3-one)phenylimino)methyl)-4-chloro-2*H*-chromen-2-ones hold promise as potential antimicrobial agents after further optimisation.

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