Synthesis, Characterization and Primary Antimicrobial, Antifungal Activity Evaluation of Schiff bases of 4-Chloro-(3-substituted- phenylimino) - methyl-[2H] - chromene-2-one

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Abstract: Coumarins show promising antimicrobial and antifungal activity, this prompted to us to synthesize new derivatives of coumarins. 4-Chloro-2-oxo-2H-chromene-3-carbaldehyde was reacted with substituted aniline and rectified spirit to obtain a new series of Schiff bases of 4-chloro-(3-substituted-phenylimino) -methyl-[2H]-chromene-2-one (**3a-i**). Their structures were confirmed by IR, ¹H NMR spectral data. The synthesized compounds were investigated for antimicrobial and antifungal activities. The data reported showed that the effect of variation in chemical structure was rather unpredictable. Seldom did a particular structural modification lead to uniform alteration in activity in all tests. The substitution which appeared to be most important for high order of activity in the number of test was the 3-chloro phenylimino group. The observed antimicrobial and antifungal activities are attributed to the substitution at 3rd position of phenylimino moiety.

Keywords: Schiff base, Antimicrobial Activity, Antifungal Activity, 4-Chloro-3-coumarinaldehyde.

Introduction

Coumarins have a long history of having number of pharmacological activities such as anticoagulant, antithrombotic, antimutagenic, vasodilator, LOX and CLOX inhibitors and it can also used in treatment of edema¹⁻³. The recent success of coumarins as antimicrobial and antifungal has further highlighted the importance of this class in medicinal chemistry⁴⁻¹⁰. A systematic investigation of this class of compound revealed that coumarin derivatives containing pharmacophore agent plays an important role in medicinal chemistry. It has been reported that naturally occurring coumarin derivatives such as 2H-1-benzopyran-2-one, 4-hydroxycoumarins shows different pharmacological activities which are clinically important. As literature survey shows that, Schiff bases too have antimicrobial and antifungal activity this promoted to us to synthesize new derivatives of coumarins^{11, 12}. In view of these above, an

attempt has been undertaken for the synthesis of new Schiff bases of 4-chloro-(3-substituted-phenylimino)-methyl-[2H]-chromene-2-one, possessing moderate biological activities. The synthesized compounds were tested for their possible antimicrobial and antifungal activities.

Experimental

Melting points were estimated with Veego (VMP-D) melting point apparatus in open capillaries and are uncorrected. Purity of compounds was checked by TLC. IR spectra (KBr, cm^{-1}) were recorded on a Shimadzu 8400-SFT-IR spectrometer. ^1H NMR (CDCl_3) on a Varian-Mercury-300 MHz spectrometer using TMS as an internal reference (chemical shift in δppm).

Preparation of 4-hydroxycoumarin (1)

To a mixture of *o*-hydroxyacetophenone (10.0 mol), sodium (25.0 mol) and diethylcarbonate (30.0 mol), mixed well and heated at 160°C with constant stirring. The mixture was diluted with xylene (30 mL) and further heated at 160°C for 1 h and then the mixture was poured into cold water (400 mL). NaOH (2 N) was used to make the mixture alkaline and mixture was stirred with diethyl ether. After which the aqueous phase was collected and then acidified with HCl (6 N). Product was filtered, washed with ice cold water and dried in vacuum desiccator.

Preparation of 4-chloro-2-oxo-2H-chromene-3-carbaldehyde (2)

To a stirred mixture of 4-hydroxycoumarin (0.06 mol) in anhydrous DMF (0.6 mol) was added to which POCl_3 (0.18 mol) was added drop wise at -10° to -5°C . Mixture was then stirred for 1 h at room temperature and heated and stirred for 2 h at 60°C . After the reaction was completed, the mixture was kept overnight at 0°C . The separated pale yellow solid was collected by filtration and washed successively with Na_2CO_3 solution (5%) and water and was dried in air.

Preparation of Schiff base of 4-chloro-3-coumarinaldehyde (3)

4-Chloro-3-Coumarinaldehyde, (0.005 mol) of aniline and rectified spirit, was reflux for 20 min. Water was then added. The oil that separated was induced to crystallize by rubbing with glass rod and the solid was collected by filtration. After washing well with cold ethanol (88%), the crude Schiff base was dried and recrystallised from aq. methanol. Compounds (**3a-i**) were prepared similarly by using different arylaldehydes. Their melting points, % yields and molecular formula are given in Table 1.

Table 1. Characterization data of compounds (**3a-i**).

Compd No.	R	Molecular formula	Molecular weight	Melting point, $^\circ\text{C}$	R _f Value	
					Percentage yield, %	
3a	2-NO ₂	C ₁₆ H ₉ ClN ₂ O ₄	328.71	295-296	0.52	76.68
3b	4-NO ₂	C ₁₆ H ₉ ClN ₂ O ₄	328.71	162-164	0.63	67.48
3c	4-Cl	C ₁₆ H ₉ Cl ₂ NO ₂	318.15	167-168	0.62	75.94
3d	3-OCH ₃	C ₁₇ H ₁₂ ClNO ₃	313.74	167-168	0.65	73.71
3e	4-OCH ₃	C ₁₇ H ₁₂ ClNO ₃	313.74	126-127	0.58	60.89
3f	2-OCH ₃	C ₁₇ H ₁₂ ClNO ₃	313.74	119-121	0.64	70.94
3g	3-CH ₃	C ₁₇ H ₁₂ ClNO ₂	297.74	165-170	0.58	64.18
3h	4-CH ₃	C ₁₇ H ₁₂ ClNO ₂	297.74	203-205	0.61	67.56
3i	H	C ₁₆ H ₁₀ ClNO ₂	283.71	179-180	0.68	78.01

1: m.p. 213-215 °C, yield: 93.4%, IR (KBr in cm^{-1}): 3421(O-H str.), 2885, 3003, 3073 (C-H str.), 1647(C=O str.); R_f : 0. 8. **2:** m.p. 120-122°C, yield: 72%, IR (KBr in cm^{-1}): 3061.13, 2918.40 (C-H str.) 1741.78 (C=O str.); 758.05(C-Cl str). ^1H NMR (δ ppm) (CDCl_3): 7.3-8.13(4H,s,Ar-H), 10.34(s,1H,CHO). R_f : 0. 83

3a: (R= 2- NO_2) m.p. 295-296 °C, yield: 76.68%, IR (KBr in cm^{-1}): 3078.49 (C-H str.), 1739.85(C=O str.), 1602.90(C=N str.), 796.12(C-Cl str.). ^1H NMR (δ ppm) (CDCl_3): 8.758(s,1H,-CH=N-), 7.601(t,2H,Ar-H), 7.828-7.990(d,1H,Ar-H), 8.192(d,1H,Ar-H), 7.455-7.579(m,2H,Ar-H).

3b: (R= -4- NO_2): m.p. 162-164 °C, yield: 67.48%, IR (KBr in cm^{-1}): 3082.35, 3051.49 (C-H str.), 1716.70(C=O str.), 1519.96(C=N str.), 758.05(C-Cl str.): ^1H NMR (δ ppm) (CDCl_3): 8.758(s,1H,-CH=N-), 7.601(t,2H,Ar-H), 7.828-7.990(d,1H,Ar-H), 8.192(d,1H, Ar-H), 7.455-7.579(m,2H, Ar-H).

3c: (R= 4-Cl): m.p. 167-168 °C, yield: 75.94%, IR (KBr in cm^{-1}): 3117, 3080, 3063, (C-H str.), 1693(C=O str.), 1541.18(C=N str.), 717.54(C-Cl str.). ^1H NMR (δ ppm) (CDCl_3): 8.758(s,1H,-CH=N-), 7.601(t,2H,Ar-H), 7.828-7.990(d,1H,Ar-H), 8.192(d,1H,Ar-H), 7.455-7.579(m,2H,Ar-H).

3d: (R=3- OCH_3): m.p. 200-201 °C, yield: 73.71%, IR (KBr in cm^{-1}): 3066.92, 3016.77 (C-H), 1739(C=O), 1473.66(C=N), 759.98(C-Cl): ^1H NMR (δ ppm) (CDCl_3): 8.758(s,1H,-CH=N-), 7.601(t,2H,Ar-H), 7.828-7.990(d,1H,Ar-H), 8.192(d,1H,Ar-H), 7.455-7.579(m,2H,Ar-H), 3.431(s, 1H, OCH_3).

3e: (R=4 - OCH_3): m.p. 126-127 °C, yield: 60.89%, IR (KBr in cm^{-1}): 3078, 2924(C-H str.), 1714(C=O str.), 1518.03(C=N str.), 754.19(C-Cl str.): ^1H NMR (δ ppm) (CDCl_3): 8.758 (s,1H,-CH=N-), 7.601(t,2H,Ar-H), 7.828-7.990(d,1H,Ar-H), 8.192(d,1H,Ar-H), 7.455-7.579(m,2H,Ar-H), 3.431(s,1H, OCH_3).

3f: (R=2- OCH): m.p. 119-121 °C, IR (KBr in cm^{-1}): 3061.13(C-H), 1739.85(C=O), 1602.50(C=N), 769.98(C-Cl): ^1H NMR (δ ppm) (CDCl_3): 8.758(s,1H,-CH=N-), 7.601(t,2H,Ar-H), 7.828-7.990(d,1H,Ar-H), 8.192(d,1H,Ar-H), 7.455-7.579(m,2H,Ar-H).

3g: (R=3 CH_3): m.p. 165-170 °C, yield: 64.18%, IR (KBr in cm^{-1}): 3340.82, 3061.13(C-H), 1730.99(C=O), 1504.53(C=N), 754.19(C-Cl): ^1H NMR (δ ppm) (CDCl_3): 8.758(s,1H,-CH=N), 7.601(t,2H,Ar-H), 7.828-7.990(d,1H,Ar-H), 8.192(d,1H,Ar-H), 7.455-7.579(m,2H,Ar-H), 2.441(s,3H, CH_3).

3h: (R=4 CH_3): m.p. 179-180 °C, yield: 78.01%, IR (KBr in cm^{-1}): 3061.13, 2677.29(C-H), 1741(C=O), 1500.67(C=N), 780.30(C-Cl): ^1H NMR (δ ppm) (CDCl_3): 8.758(s,1H,-CH=N-), 7.601(t,2H,Ar-H), 7.828-7.990(d,1H,Ar-H), 8.192(d,1H,Ar-H), 7.455-7.579(m,2H,Ar-H).

3i: (R=H): m.p. 203-205 °C, yield: 65.17%, IR (KBr in cm^{-1}): 3117.07, 3063 (C-H), 1693.56(C=O), 1541.18(C=N), 717.54(C-Cl): ^1H NMR (δ ppm) (CDCl_3): 8.758(s,1H,-CH=N), 7.601(t,2H,Ar-H), 7.828-7.990(d,1H,Ar-H), 8.192(d,1H,Ar-H), 7.455-7.579(m,2H, Ar-H), 2.441(s,3H, CH_3).

Statistical analysis

Data were analyzed by one -way ANOVA followed by Dunnett's *t*-test using computerized Graph Pad Instat version 3.05.

Antibacterial activity

In vitro antimicrobial study was carried on Muller hinton agar (Hi-media) plates (37 °C, 24 h) by agar diffusion cup plate method¹³. All the compounds were screened for antimicrobial activity at 50,100, 150, 200 $\mu\text{g/mL}$ concentration against the following bacterial strains

Staphylococcus aureus, *Bacillus subtilis*, *Escherichia coli*. Minimum inhibitory concentration (MIC) of the test solution was determined by using broth dilution method. Antifungal activity was tested on Muller hinton agar (Hi-media) plates (37 °C, 24 h) by agar diffusion cup plate method¹³, against *Candida albicans* and *Aspergillus niger* at conc. Level at 50, 100, 150, 200 µg/mL. Amoxycillin and Fluconazole were used as standard for antimicrobial and antifungal activity under similar conditions. DMSO was used as solvent control for both antimicrobial and antifungal activities. The results are presented in Table 2.

Table 2. Antimicrobial and antifungal activity of compounds 3(a-i).

S. No.	Compound name	Concentration µg/10µL	Zone of inhibition in mm				
			Bacteria			Fungi	
			<i>E.Coli</i>	<i>B.Subtilis</i>	<i>S.Aureus</i>	<i>A.Niger</i>	<i>C.Albicans</i>
1.	3a	50	-	-	-	-	-
		100	13	11	12	9	7
		150	14	16	18	14	11
		200	25	22	24	19	15
2.	3b	50	-	-	-	-	-
		100	12	11	10	8	8
		150	18	17	15	12	12
		200	24	23	21	17	16
3.	3c	50	8	7	7	-	-
		100	16	14	15	10	10
		150	24	21	22	16	15
		200	32	28	30	22	21
4.	3d	50	-	-	-	-	-
		100	10	11	10	7	7
		150	15	17	15	11	10
		200	21	23	20	15	14
5.	3e	50	-	-	-	-	-
		100	8	10	9	7	10
		150	12	15	14	10	15
		200	17	21	19	14	20
6.	3f	50	-	-	-	-	-
		100	9	10	8	7	9
		150	13	15	12	11	14
		200	18	20	17	15	19
7.	3g	50	-	-	-	-	-
		100	11	12	13	9	8
		150	16	18	19	13	12
		200	22	24	26	18	17
8.	3h	50	-	-	-	-	-
		100	10	11	12	9	8
		150	15	16	18	14	12
		200	21	22	24	19	16
9.	3i	50	-	-	-	-	-
		100	9	7	8	7	-
		150	13	10	12	9	8
		200	18	14	17	13	11

Results and Discussion

Spectral characterization (IR, ^1H NMR) of final compound is provided in this section. The ^1H NMR spectrum of all the Schiff base compound is in between 7-8.758 (Aromatic H) and 2.65-3.82 (S of CH_3). The characterization data of synthesized compounds is provided in Table 1. Biological results are reported in Table 2, which also included results of standard drugs. There was moderate antimicrobial and antifungal activity associated with synthesized compounds. The significant changes in potency resulted from minor change in chemical structure is shown in Table 2.

Synthetic scheme

Scheme 1.

Conclusion

A total of 9 compounds were synthesized and screened for their antimicrobial activity against *S. aureus*, *B. subtilis* and *E. coli* and antifungal activity against *A. niger* and *C. albicans*. The MIC of all compounds was determined. The compounds were found to have moderate antimicrobial activity.

The observed antimicrobial and antifungal activities are attributed to the substitution of 3-chloro, 3-methoxy group at 3 position of phenylimino ring of synthesized compounds. Obviously, the comparative evaluation of active compounds will require further studies; the data reported in this article may be helpful guide for the medicinal chemist who is working in this area.

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