Synthesis and Antimicrobial Activity of Some Chalcone Derivatives

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Abstract: In an effort to develop antimicrobial agents, a series of chalcones were prepared by Claisen-Schmidt condensation of appropriate acetophenones with appropriate aromatic aldehydes in the presence of aqueous solution of potassium hydroxide and ethanol at room temperature. The synthesized compounds were characterized by means of their IR, ¹H-NMR spectral data and elemental analysis. All the compounds were tested for their antibacterial and antifungal activities by the cup plate method.

Keywords: Chalcone, Synthesis, Antibacterial activity, Antifungal activity

Introduction

Chalcones are well known intermediates for synthesizing various heterocyclic compounds. The compounds with the backbone of chalcones have been reported to possess various biological activities such as antimicrobial¹, anti-inflammatory², analgesic³, antiplatelet⁴, antiulcerative⁵, antimalarial⁶, anticancer⁷, antiviral⁸, antileishmanial⁹, antioxidant¹⁰, antitubercular¹¹, antihyperglycemic¹², immunomodulatory¹³, inhibition of chemical mediators release¹⁴, inhibition of leukotriene B₄¹⁵, inhibition of tyrosinase¹⁶ and inhibition of aldose reductase¹⁷ activities. The presence of a reactive α,β -unsaturated keto function in chalcones is found to be responsible for their antimicrobial activity. In the present communication we report the reaction of various acetophenone derivatives with different aromatic aldehyde derivatives to form chalcones (3a-1). The structures of the various synthesized compounds were assigned on the basis of IR, ¹H-NMR spectral data and elemental analysis. These compounds were also screened for their antimicrobial activity.

Experimental

Melting points were determined in open capillary tubes and are uncorrected. The IR spectra were recorded in KBr on Perkin-Elmer BX Spectrophotometer. The ¹H-NMR were recorded

in CDCl₃ on Bruker Spectrospin AV 400 MHz Spectrometer using TMS as an internal standard. The elemental analyses were performed on Carlo Erba 1108 elemental analyzer. The purity of the compounds was checked by TLC-using Silicagel-G (Merck). Column chromatography was performed on Silica gel (Merck, 60-120 mesh).

General procedure for the preparation of 1-(substitutedphenyl)-3-(substituted-phenyl)-2-propen-1-ones (3_{a-l})

A mixture of substituted acetophenones (0.01 mole) and aryl aldehydes (0.01 mole) was stirred in ethanol (30 mL) and then an aqueous solution of potassium hydroxide (15 mL) was added to it. The mixture was kept over night at room temperature and then it was poured into crushed ice and acidified with dilute hydrochloric acid. The chalcone derivative precipitates out as solid. Then it was filtered and crystallized from ethanol (Scheme 1).

Scheme 1

3_a : R ₁ = R ₃ = R ₄ = R ₆ = H, R ₂ = Br, R ₅ = Cl	3_g : R ₁ = OH, R ₂ = R ₄ = R ₆ = H, R ₃ = R ₅ = Cl
3_b : R ₁ = R ₄ = R ₆ = H, R ₂ = Br, R ₃ = R ₅ = Cl	3_h : R ₁ = OH, R ₂ = R ₃ = R ₄ = R ₆ = H, R ₅ = F
3_c : R ₁ = R ₃ = R ₄ = R ₆ = H, R ₂ = Br, R ₅ = F	3_i : R ₁ = OH, R ₂ = R ₃ = H, R ₄ = R ₅ = R ₆ = OCH ₃
3_d : R ₁ = R ₃ = H, R ₂ = Br, R ₄ = R ₅ = R ₆ = OCH ₃	3_j : R ₁ = R ₂ = OH, R ₃ = R ₄ = R ₆ = H, R ₅ = NO ₂
3_e : R ₁ = R ₃ = R ₄ = R ₅ = R ₆ = H, R ₂ = Br	3_k : R ₁ = R ₂ = OH, R ₃ = R ₄ = R ₅ = R ₆ = H
3_f : R ₁ = OH, R ₂ = R ₃ = R ₄ = R ₆ = H, R ₅ = Cl	3_l : R ₁ = OH, R ₂ = R ₃ = R ₄ = R ₆ = H, R ₅ = Cl

1-(4'-Bromophenyl)-3-(4-chlorophenyl)-2-propen-1-one (3_a)

Yield 88%; m.p. 191-193°C; IR (KBr, cm⁻¹): 1732 (C=O), 1637 (CH=CH), 852 (C-Cl), 836 (C-Br); ¹H-NMR (CDCl₃, δ ppm): 7.81 (1H, d, J=16 Hz, =CH-Ar), 7.34 (1H, d, J=16 Hz, -CO-CH=), 7.30-7.72 (8H, m, Ar-H). Anal. Calcd. for C₁₅H₁₀OClBr: C, 55.91; H, 3.11; O, 4.97. Found: C, 55.85; H, 3.22; O, 4.79.

1-(4'-Bromophenyl)-3-(2,4-dichlorophenyl)-2-propen-1-one (3_b)

Yield 91%; m.p. 148-150°C; IR (KBr, cm⁻¹): 1736 (C=O), 1632 (CH=CH), 865 (C-Cl), 835 (C-Br); ¹H-NMR (CDCl₃, δ ppm): 7.80 (1H, d, J=16 Hz, =CH-Ar), 7.40 (1H, d, J=16 Hz, -CO-CH=), 7.35 (1H, s, Ar-H), 8.03 (2H, d, Ar-H), 7.55-7.63 (4H, m, Ar-H). Anal. Calcd. for C₁₅H₉OCl₂Br: C, 50.59; H, 2.52; O, 4.49. Found: C, 50.46; H, 2.49; O, 4.56.

1-(4'-Bromophenyl)-3-(4-fluorophenyl)-2-propen-1-one (3_c)

Yield 86%; m.p. 175-177°C; IR (KBr, cm⁻¹): 1720 (C=O), 1628 (CH=CH), 838 (C-Br), 815 (C-F); ¹H-NMR (CDCl₃, δ ppm): 7.81 (1H, d, J=16 Hz, =CH-Ar), 7.06 (1H, d, J=16 Hz, -CO-CH=), 7.30-7.74 (8H, m, Ar-H). Anal. Calcd. for C₁₅H₁₀OBrF: C, 59.03; H, 3.27; O, 5.24. Found: C, 59.10; H, 3.31; O, 5.15.

1-(4'-Bromophenyl)-3-(3,4,5-trimethoxyphenyl)-2-propen-1-one (3_d)

Yield 88%; m.p. 138-139°C; IR (KBr, cm⁻¹): 1728 (C=O), 1636 (CH=CH), 1148 (C-OCH₃), 842 (C-Br); ¹H-NMR (CDCl₃, δ ppm): 7.82 (1H, d, J=16 Hz, =CH-Ar), 7.27 (1H, d, J=16 Hz, -CO-CH=), 3.90 (9H, s, 3 x OCH₃), 6.79 (2H, s, Ar-H), 7.58 (2H, d, Ar-H), 7.65 (2H, d, Ar-H). Anal. Calcd. for C₁₈H₁₇O₄Br: C, 57.30; H, 4.51; O, 16.98. Found: C, 57.25; H, 4.54; O, 16.85.

1-(4'-Bromophenyl)-3-phenyl-2-propen-1-one (3_e)

Yield 91%; m.p. 114-116⁰C; IR (KBr, cm⁻¹): 1756 (C=O), 1622 (CH=CH), 848 (C-Br); ¹H-NMR (CDCl₃, δ ppm): 7.82 (1H, d, J=16 Hz, =CH-Ar), 7.40 (1H, d, -CO-CH=), 7.30-7.78 (9H, m, Ar-H). Anal. Calcd. for C₁₅H₁₁OBr: C, 62.73; H, 3.83; O, 5.57. Found: C, 62.75; H, 3.94; O, 5.61.

1-(2'-Hydroxyphenyl)-3-(4-chlorophenyl)-2-propen-1-one (3_f)

Yield 82%; m.p. 154-156⁰C; IR (KBr, cm⁻¹): 3435 (OH), 1647 (C=O), 1582 (CH=CH), 810 (C-Cl); ¹H-NMR (CDCl₃, δ ppm): 7.94 (1H, d, J=16 Hz, =CH-Ar), 6.70 (1H, d, J=16 Hz, -CO-CH=), 12.80 (1H, s, C-2'-OH), 7.25-7.80 (8H, m, Ar-H). Anal. Calcd. for C₁₅H₁₁O₂Cl: C, 69.94; H, 4.25; O, 12.38. Found: C, 69.98; H, 4.29; O, 12.40.

1-(2'-Hydroxyphenyl)-3-(2,4-dichlorophenyl)-2-propen-1-one (3_g)

Yield 92%; m.p. 178-180⁰C; IR (KBr, cm⁻¹): 3434 (OH), 1639 (C=O), 1574 (CH=CH), 862 (C-Cl); ¹H-NMR (CDCl₃, δ ppm): 7.82 (1H, d, J=16 Hz, =CH-Ar), 6.98 (1H, d, J=16 Hz, -CO-CH=), 12.50 (1H, s, C-2'-OH), 7.19 (1H, s, Ar-H), 6.87-8.80 (6H, m, Ar-H). Anal. Calcd. for C₁₅H₁₀O₂Cl₂: C, 61.45; H, 3.41; O, 10.92. Found: C, 61.39; H, 3.60; O, 10.80.

1-(2'-Hydroxyphenyl)-3-(4-fluorophenyl)-2-propen-1-one (3_h)

Yield 87%; m.p. 189-191⁰C; IR (KBr, cm⁻¹): 3432 (OH), 1687 (C=O), 1638 (CH=CH), 830 (C-F); ¹H-NMR (CDCl₃, δ ppm): 7.81 (1H, d, J=16 Hz, =CH-Ar), 6.89 (1H, d, -CO-CH=), 12.70 (1H, s, C-2'-OH), 6.90-7.85 (8H, m, Ar-H). Anal. Calcd. for C₁₅H₁₁O₂F: C, 74.38; H, 4.54; O, 13.22. Found: C, 74.40; H, 4.52; O, 13.28.

1-(2'-Hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)-2-propen-1-one (3_i)

Yield 86%; m.p. 180-182⁰C; IR (KBr, cm⁻¹): 3433 (OH), 1636 (C=O), 1570 (CH=CH), 1127 (OCH₃); ¹H-NMR (CDCl₃, δ ppm): 7.87 (1H, d, J=16 Hz, =CH-Ar), 7.05 (1H, d, J=16 Hz, -CO-CH=), 12.90 (1H, s, C-2'-OH), 3.95 (9H, s, 3 x OCH₃), 6.91 (2H, s, Ar-H), 6.95-7.98 (4H, m, Ar-H). Anal. Calcd. for C₁₅H₁₈O₅: C, 68.78; H, 5.73; O, 25.47. Found: C, 68.72; H, 5.65; O, 25.39.

1-(2', 4'-Dihydroxyphenyl)-3-(4-nitrophenyl)-2-propen-1-one (3_j)

Yield 91%; m.p. 138-140⁰C; IR (KBr, cm⁻¹): 3414 (C-NO₂), 1688 (C=O), 1640 (CH=CH), 1324 (C-NO₂); ¹H-NMR (CDCl₃, δ ppm): 7.87 (1H, d, J=16 Hz, =CH-Ar), 6.65 (1H, d, J=16 Hz, -CO-CH=), 5.32 (1H, s, C-4'-OH), 7.18-7.67 (7H, m, Ar-H). Anal. Calcd. for C₁₅H₁₁O₅N: C, 63.15; H, 3.85; O, 28.07; N, 4.91. Found: C, 63.10; H, 3.91; O, 28.35; N, 4.89.

1-(2',4'-Dihydroxyphenyl)-3-phenyl-2-propen-1-one (3_k)

Yield 65%; m.p. 158-160⁰C; IR (KBr, cm⁻¹): 3100 (OH), 1720 (C=O), 1640 (CH=CH); ¹H-NMR (CDCl₃, δ ppm): 7.93 (1H, d, J=16 Hz, =CH-Ar), 6.81 (1H, d, J=16 Hz, -CO-CH=), 5.40 (1H, s, C-4'-OH), 7.10-7.92 (8H, m, Ar-H). Anal. Calcd. for C₁₅H₁₂O₃: C, 75.00; H, 5.00; O, 20.00. Found: C, 75.12; H, 5.09; O, 19.98.

1-(2'-Hydroxyphenyl)-3-(4-chlorophenyl)-2-propen-1-one (3_l)

Yield 91%; m.p. 151-153⁰C; IR (KBr, cm⁻¹): 3433 (OH), 1640 (C=O), 1564 (CH=CH), 824 (C-Cl); ¹H-NMR (CDCl₃, δ ppm): 7.84 (1H, d, J=16 Hz, =CH-Ar), 6.94 (1H, d, J=16 Hz, -CO-CH=), 12.65 (1H, s, C-2'-OH), 6.95-7.85 (8H, m, Ar-H). Anal. Calcd. for C₁₅H₁₁O₂Cl: C, 69.94; H, 4.25; O, 12.38. Found: C, 69.59; H, 4.32; O, 12.45.

Results and Discussion

Antimicrobial activity

The newly synthesized compounds (3_{a-i}) were screened for their antibacterial activity against two gram positive bacteria viz., *Bacillus pumilis*, *Bacillus subtilis* and two gram negative bacteria viz., *Escherichia coli*, *Proteus vulgaris* by using cup plate method^{18,19}. The agar medium was purchased from HI media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. Discs measuring 6.25 mm in diameter were punched from Whatman No.1 filter paper. The test compounds were prepared in different concentrations using dimethylsulfoxide. Solutions of the test compounds were prepared by dissolving 5 mg each in 5 mL of dimethylsulfoxide at a concentration of 1000 µg/mL. Volumes of 0.05 mL and 0.1 mL of each compound were used for testing. The cups each of 9 mm diameter were made by scooping out medium with a sterilized cork borer in a petri dish which was streaked with the organisms.

The solutions of each test compound (0.05 and 0.1 mL) were added separately in the cups and petri dishes were subsequently incubated. A reference standard for both gram positive and gram negative bacteria was made by dissolving accurately weighed quantity of chloramphenicol (200 and 1000 µg/mL, respectively) in sterile distilled water, separately. The incubation was carried out at 37°C for 24h. All the experiments were carried out in triplicate. Simultaneously, controls were maintained by employing 0.1 mL of dimethylsulfoxide which did not reveal any inhibition. Zones of inhibition produced by each compound was measured in mm. The results of antibacterial studies are given in Table 1.

Table 1. Antibacterial activity of chalcone derivatives

Compound	Zone of inhibition (in mm)							
	<i>B.pumilis</i>		<i>B.subtilis</i>		<i>E.coli</i>		<i>P.vulgaris</i>	
	0.05 mL	0.1 mL	0.05 mL	0.1 mL	0.05 mL	0.1 mL	0.05 mL	0.1 mL
3 _a	6	8	8	10	7	9	-	-
3 _b	7	9	9	11	8	10	8	10
3 _c	8	10	7	9	9	11	-	-
3 _d	12	14	11	13	13	15	-	-
3 _e	8	10	7	9	9	12	8	11
3 _f	7	9	8	10	9	11	-	-
3 _g	8	10	9	11	7	9	-	-
3 _h	7	9	8	10	7	9	-	-
3 _i	9	12	8	13	11	14	-	-
3 _j	10	12	11	13	8	11	9	12
3 _k	7	9	8	11	8	10	-	-
3 _l	7	9	8	10	7	9	-	-
Chloramphenicol	-	-	16	-*	14	-*	10	-*

(-) indicates no zone of inhibition; (-*) indicates inhibition not done.

All those compounds screened for antibacterial activity were also tested for their antifungal activity using potato-dextrose-agar (PDA) medium by same cup plate method against *Aspergillus niger*, *Rhizopus oryzae* and *Aspergillus flavus*. The PDA medium was purchased from HI media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium and PDA medium was done as per the standard procedure. The solutions of test compounds were prepared by a similar procedure described under the antibacterial activity. Each test compound (5 mg) was dissolved in 5 mL of dimethylsulphoxide (1000 µg/mL). Volumes of 0.05 and 0.1 mL of each compound were

used for testing. A reference standard drug fluconazole (200 and 1000 µg/mL respectively) and dimethylsulphoxide as a control which did not reveal any inhibition. The experiments were performed in triplicate in order to minimize the errors. Zone of inhibition produced by each compound was measured in mm. The results of antifungal studies are given in Table 2.

Table 2. Antifungal activity of chalcone derivatives

Compound	Zone of inhibition (in mm)					
	<i>A.niger</i>		<i>R.oryzae</i>		<i>A.flavus</i>	
	0.05 mL	0.1 mL	0.05 mL	0.1 mL	0.05 mL	0.1 mL
3 _a	10	14	9	13	8	11
3 _b	6	8	7	10	8	10
3 _c	6	8	7	9	6	8
3 _d	7	9	8	11	-	-
3 _e	6	8	7	9	-	-
3 _f	6	8	7	11	8	9
3 _g	7	9	8	9	6	8
3 _h	7	8	9	10	7	8
3 _i	6	8	7	9	8	9
3 _j	6	8	7	9	-	-
3 _k	6	8	7	9	6	7
3 _l	8	9	8	10	6	9
Fluconazole	25	-*	-	-	-	-

(-) indicates no zone of inhibition; (-*) indicates inhibition not done.

The screening results revealed that the compounds 3_{a-l} showed significant antimicrobial activity. In particular compounds 3_b, 3_e and 3_j only showed mild inhibitory action on *P.vulgaris*. Compounds 3_b, 3_d and 3_i have shown significant activity on *B.pumilis*, *B.subtilis* and *E.coli*. Compound 3_a (R = 4-chlorophenyl) have shown high potency especially against *A.niger* and *R.oryzae*. Compounds 3_d, 3_e and 3_j have not shown any inhibition against *A.flavus*. All the organisms employed at a concentration of 1000 µg/mL (0.01 mL dose level) showed considerable antibacterial and antifungal activities and are comparable to that of standard drugs chloramphenicol and fluconazole, respectively.

Conclusion

Compounds with electron releasing groups such as methoxy and hydroxyl showed better antibacterial activity than the others not having such groups. Compounds having pharmacophores such as, chloro, dichloro and fluoro groups have exhibited more antifungal activity on all the three fungi than the others. These results suggest that the chalcone derivatives have excellent scope for further development as commercial antimicrobial agents. Further experiments were needed to elucidate their mechanism of action.

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