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Research Article

SYNTHESIS OF CHALCONE AND THEIR DERIVATIVES AS ANTIMICROBIAL AGENTS

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ABSTRACT

In a wide search program towards new and efficient antimicrobial agents, substituted chalcones have been synthesized by condensing benzaldehyde derivatives with acetophenone derivatives in dilute ethanolic sodium hydroxide solution at room temperature according to Claisen – Schmidt condensation. The structures of these compounds have been investigated by infra red spectroscopy, nuclear magnetic resonance spectroscopy and mass spectrometry. The antimicrobial activity of the novel products was evaluated by Filter Paper Disc diffusion Method. The compound 1b showed excellent activity against *S. aureus* at both concentration i.e. 500µg/ml and1000 µg/ml.

Keywords: Chalcone derivates, Antimicrobial agents.

INTRODUCTION

There is growing interest in the pharmacological potential of natural products is chalcones constitute an important group of natural products. Chemically, they consist of open chain flavanoids in which the two aromatic rings are joined by a three carbon α .β unsaturated carbonyl system The presence of a reactive α, β unsaturated keto function in chalcones is found to be responsible for their antimicrobial activity[1] In recent years a variety of chalcones have been reviewed for their cytotoxic, anticancer chemoprevenive and mutagenic as well as antiviral, insecticidal and enzyme inhibitory properties[2,3].

A number of chalcones having hydroxy, alkoxy groups in different position have been reported to possess anti-bacterial[4], antiulcer[5], antifungal[6], antioxidant[7], vasodilatory[8], antimitotic[9], antimalarial [10], antileshmanial[11] and inhibition of chemical mediators release, inhibition of leukotriene B_4[12], inhibition of tyrosinase[13,14] and inhibition of aldose reductase[15] activities. Appreciation of these findings motivated us to synthesize chalcones as a potential template for antimicrobial agents. It must be noted that this scaffold provides substitution pattern on benzylidenacetophenones nucleus.

EXPERIMENTAL.

The melting points were recorded in open sulphuric acid or oil bath using thermometer and were uncorrected. IR spectra were recorded using Perkin-Elmer FTIR-RX_1 spectrophotometer. A ^{1}HNMR spectrum was recorded using $CDCl_3$ on Bruker Avance (400 MHz) and their chemical shifts are recorded in δ (parts per million) units with respect to tetramethyl silane (TMS) as internal standard. Mass spectra were recorded on a Waters Q-T of micro MS. All the reagents and solvents used were of analytical grade and were used as supplied unless otherwise stated. Progress of the reactions was monitored using TLC, performed on aluminium plates precoated with silica gel-G, using chloroform: methanol (92:8) as the solvent systems and the spots were visualized by exposure to iodine vapors.

General Procedure

Chalcones were synthesized by base catalyzed Claisen-Schmidt condensation reaction of appropriately substituted acetophenones and aldehydes by known literature method[16].

A mixture of benzaldehyde derivatives (0.01 mol) and acetophenone derivatives (0.01 mol) was dissolved in 10 ml rectified spirit in a 250 ml round-bottomed flask equipped with a magnetic stirrer. Then 10 ml NaOH solution (1g in 10ml H_2O) was added drop wise to the reaction mixture on vigorous stirring for 30 minutes when solution became turbid. The reaction temperature was maintained between 20-25˚ C using a cold water bath on the magnetic stirrer. After vigorous stirring for 4-5 hours the reaction mixture was neutralized by 0.1-0.2N HCl whereby the precipitation occurred. On filtering off, the crude chalcones were dried in air and recrystallized by rectified spirit. The residue was purified on column chromatography (silica gel with 10% ethyl acetate in hexane) to afford pure chalcones (Scheme 1). The chemical profile of the compounds is as shown in Table 1.

3-(4-methoxy phenyl)-1-(4-bromophenyl)-2-propen-1-one (1a)

To a stirred mixture of p-bromo acetophenone (10mmol) and p-methoxy benzaldehyde (10mmol) in rectified spirit (10mL), sodium hydroxide (10.0 mL) was added drop wise and treated as in the general procedure to give 1a. Recrystalization from rectified spirit.. IR (nujol) cm^{-1}: 1658 (>C=O in conjugation with C=C), 1596,1540 (>C=C< in conjugation with C=O), 722 (-Br);^{1}HNMR ($CDCl_3$),δ (ppm): 7.85(d, 2H, Ar 3', 5'H), 7.87 (d, 2H, Ar 2',6'H), 7.58(d,1H_a, J = 16 Hz, =CH), 7.61(d, 1H_b, J =16Hz, =CH), 6.92 (d, 2H, Ar 2",6"-H), 6.94(d,2H, Ar 3", 5"- H), 3.84(s,3H, Ar 4"-OCH_3); Mass spectrum (EI,*m/z*): 318 ($M^{+\cdot}$+1) Exact mass of molecular ion *m/z* = 317.175, calculated for $C_{16}H_{13}BrO_2$: 317.177.

3-(4-methoxy phenyl)-1-(4-iodophenyl)-2-propen-1-one (1b)

To a stirred mixture of p-iodo acetophenone (10mmol) and p-methoxy benzaldehyde (10mmol) in rectified spirit (10mL), sodium hydroxide (10.0 mL) was added drop wise and treated as in the general procedure to give 1b. Recrystalization from rectified spirit.. IR (nujol) cm^{-1}: 1656 (>C=O in conjugation with C=C), 1597,1541 (>C=C< in conjugation with C=O), 660(-I);^{1}HNMR ($CDCl_3$),δ (ppm): 7.72(d, 2H, Ar 3', 5'H), 7.83 (d, 2H, Ar 2',6'H), 7.58(d,1H_a, J = 16 Hz, =CH), 7.60(d, 1H_b, J =16Hz, =CH), 6.92 (d, 2H, Ar 2",6"-H), 6.94(d,2H, Ar 3", 5"- H), 3.85(s,3H, Ar 4"-OCH_3);Mass spectrum (EI,*m/z*): 365 ($M^{+\cdot}$+1) Exact mass of molecular ion *m/z* = 364.175, calculated for $C_{16}H_{13}IO_2$: 364.178.

3-(4-methoxy phenyl)-1-(4-methoxyphenyl)-2-propen-1-one (1c)

To a stirred mixture of p-methoxy acetophenone (10mmol) and p-methoxy benzaldehyde (10mmol) in rectified spirit (10mL), sodium hydroxide (10.0 mL) was added drop wise and treated as in the general procedure to give 1c. Recrystalization from rectified spirit.. IR (nujol) cm^{-1}: 1655 (>C=O in conjugation with C=C), 1599,1528 (>C=C< in conjugation with C=O), 1017(-OCH_3);^{1}HNMR ($CDCl_3$),δ (ppm): 6.98(d, 2H, Ar 3', 5'H), 8.02 (d, 2H, Ar 2',6'H), 7.41(d,1H_a, J = 16 Hz, =CH), 7.76(d, 1H_b, J =16Hz, =CH), 7.61 (d, 2H, Ar 2",6"-H), 6.92(d,2H, Ar 3", 5"- H), 3.89(s,3H, Ar 4"-OCH_3); Mass spectrum (EI,*m/z*): 270 ($M^{+\cdot}$+2) Exact mass of molecular ion *m/z* = 268.303, calculated for $C_{17}H_{18}O_3$: 268.304.

3-(4-methoxy phenyl)-1-(4-ethoxyphenyl)-2-propen-1-one (1d)

To a stirred mixture of p-ethoxy acetophenone (10mmol) and p-methoxy benzaldehyde (10mmol) in rectified spirit (10mL), sodium hydroxide (10.0 mL) was added drop wise and treated as in the general procedure to give 1d. Recrystalization from rectified spirit.. IR (nujol) cm^{-1}: 1654 (>C=O in conjugation with C=C), 1599,1526 (>C=C< in conjugation with C=O), 1048(-OC_2H_5), 1026(-

OCH_3);^{1}HNMR ($CDCl_3$),δ (ppm): 1.40-1.42(t, 3H, -CH_3), 4.02-4.06(q, 2H, -CH_2), 6.95 (d, 2H, Ar 3', 5'H), 8.02 (d, 2H, Ar 2',6'H), 7.50(d,1H_a, J = 16 Hz, =CH), 7.76(d, 1H_b, J =16Hz, =CH), 7.61 (d, 2H, Ar 2",6"-H), 6.91 (d,2H, Ar 3", 5"- H), 3.83(s,3H,Ar"-OCH_3). Mass spectrum (EI,*m/z*): 283 ($M^{+\cdot}$+1) Exact mass of molecular ion *m/z* = 282.329, calculated for $C_{18}H_{18}O_3$: 282.330.

3-(4-hydroxy phenyl)-1-(4-methoxyphenyl)-2-propen-1-one (1e)

To a stirred mixture of p-methoxy acetophenone (10mmol) and p-hydroxy benzaldehyde (10mmol) in rectified spirit (10mL), sodium hydroxide (10.0 mL) was added drop wise and treated as in the general procedure to give 1e. Recrystalization from rectified spirit.. IR (nujol) cm^{-1}: 1640 (>C=O in conjugation with C=C), 1598,1528 (>C=C< in conjugation with C=O), 1020(-OCH_3), 3659(-OH); ^{1}HNMR ($CDCl_3$),δ (ppm): 3.87(s,3H,Ar-4'-OCH_3), 6.89(d, 2H, Ar 3', 5'H), 7.94 (d, 2H, Ar 2',6'H), 7.51(d,1H_a, J = 16 Hz, =CH), 7.71(d, 1H_b, J =16Hz, =CH), 7.45 (d, 2H, Ar 2",6"-H), 6.96(d,2H, Ar 3", 5"- H), 8.03(s,1H, Ar 4"- OH); Mass spectrum (EI,*m/z*): 255 ($M^{+\cdot}$+1) Exact mass of molecular ion *m/z* =254.278, calculated for $C_{16}H_{14}O_3$: 254.280.

3-(4-hydroxyphenyl)-1-(4-chlorophenyl)-2-propen-1-one (1f)

To a stirred mixture of p-chloro acetophenone (10mmol) and p-hydroxy benzaldehyde (10mmol) in rectified spirit (10mL), sodium hydroxide (10.0 mL) was added drop wise and treated as in the general procedure to give 1f. Recrystalization from rectified spirit; IR (nujol) cm^{-1}: 1652 (>C=O in conjugation with C=C), 1592,1556 (>C=C< in conjugation with C=O), 754(-Cl),1390(-OH); ^{1}HNMR ($CDCl_3$),δ (ppm): 8.95(s,1H,-OH), 7.45(d, 2H, Ar 3', 5'H), 7.82 (d, 2H, Ar 2',6'H), 7.20(d,1H_a, J = 16 Hz, =CH), 7.86(d, 1H_b, J =16Hz, =CH), 7.52 (d, 2H, Ar 2",6"-H), 6.61(d,2H, Ar 3", 5"- H); Mass spectrum (EI,*m/z*): 259 ($M^{+\cdot}$+1) Exact mass of molecular ion *m/z* = 258.0445, calculated for $C_{15}H_{11}ClO_2$: 258.0447.

3-(4-Hydroxyphenyl)-1-(4-bromophenyl)-2-propen-1-one (1g)

To a stirred mixture of p-bromo acetophenone (10mmol) and p-hydroxy benzaldehyde (10mmol) in rectified spirit (10mL), sodium hydroxide (10.0 mL) was added drop wise and treated as in the general procedure to give 1g. Recrystalization from rectified spirit; IR ((nujol) cm^{-1}: 1650 (>C=O in conjugation with C=C), 1590,1548 (>C=C< in conjugation with C=O), 654(-Br),1365(-OH); ^{1}HNMR ($CDCl_3$),δ (ppm): 8.92(s,1H,-OH), 7.55 (d, 2H, Ar 3', 5'H), 7.72 (d, 2H, Ar 2',6'H), 7.12(d,1H_a, J = 16 Hz, =CH), 7.76(d, 1H_b, J =16Hz, =CH), 7.42 (d, 2H, Ar 2",6"-H), 6.56(d,2H, Ar 3", 5"- H); Mass spectrum (EI,*m/z*): 303 ($M^{+\cdot}$+2) Exact mass of molecular ion *m/z* =301.9940, calculated for $C_{15}H_{11}BrlO_2$: 301.9942.

3-phenyl-1-(4-bromophenyl)-2-propen-1-one (1h)

To a stirred mixture of p-bromo acetophenone (10mmol) and benzaldehyde (10mmol) in rectified spirit (10mL), sodium hydroxide (10.0 mL) was added drop wise and treated as in the general procedure to give 1h. Recrystalization from rectified spirit.

IR (nujol) cm^{-1}: 1652 (>C=O in conjugation with C=C), 1568,1517 (>C=C< in conjugation with C=O), 678(-Br);^{1}HNMR ($CDCl_3$),δ (ppm): 7.44(d, 2H, Ar 3', 5'H), 7.89 (d, 2H, Ar 2',6'H), 7.44(d,1H_a, J = 16 Hz, =CH), 7.78(d, 1H_b, J =16Hz, =CH), 7.61 (d, 2H, Ar 2",6"-H), 7.40(d,2H, Ar 3", 5"- H), 7.22(s,1H, Ar 4"- H); Mass spectrum (EI,*m/z*): 286 ($M^{+\cdot}$+1) Exact mass of molecular ion *m/z* = 285.997, calculated for $C_{15}H_{11}BrO$: 285.999

3-phenyl-1-(4-iodophenyl)-2-propen-1-one (1i)

To a stirred mixture of p-iodo acetophenone (10mmol) and benzaldehyde (10mmol) in rectified spirit (10mL), sodium hydroxide (10.0 mL) was added drop wise and treated as in the general procedure to give 1i. Recrystalization from rectified spirit.

IR (nujol) cm^{-1}: 1646 (>C=O in conjugation with C=C), 1552,1498 (>C=C< in conjugation with C=O), 565(-I); ^{1}HNMR ($CDCl_3$),δ (ppm): 7.40(d, 2H, Ar 3', 5'H), 7.82 (d, 2H, Ar 2',6'H), 7.38(d,1H_a, J = 16 Hz, =CH), 7.74(d, 1H_b, J =16Hz, =CH), 7.53 (d, 2H, Ar 2",6"-H), 7.35(d,2H, Ar 3", 5"- H), 7.18(s,1H, Ar 4"- H); Mass spectrum (EI,*m/z*): 334 ($M^{+\cdot}$+1) Exact mass of molecular ion *m/z* = 333.983, calculated for $C_{15}H_{11}ClO$: 333.985.

3-phenyl-1-(4-ethoxyphenyl)-2-propen-1-one (1j)

To a stirred mixture of p-ethoxy acetophenone (10mmol) and benzaldehyde (10mmol) in rectified spirit (10mL), sodium hydroxide (10.0 mL) was added drop wise and treated as in the general procedure to give 1j. Recrystalization from rectified spirit.

IR (nujol) cm^{-1}: 1648(>C=O in conjugation with C=C), 1576,1534(>C=C< in conjugation with C=O), 1048 (-OC_2H_5); ^{1}HNMR ($CDCl_3$),δ (ppm): 1.39-1.43(t, 3H, -CH_3), 4.03-4.08(q, 2H, -CH_2), 6.95 (d, 2H, Ar 3', 5'H), 8.02 (d, 2H, Ar 2',6'H), 7.51(d,1H_a, J = 16 Hz, =CH), 7.76(d, 1H_b, J =16Hz, =CH), 7.61 (d, 2H, Ar 2",6"-H), 7.36-7.39 (m,3H, Ar 3",4", 5"- H); Mass spectrum (EI,*m/z*): 253($M^{+\cdot}$+1) Exact mass of molecular ion *m/z* = 252.1150, calculated for $C_{17}H_{16}O_2$:252.1151.

Antibacterial activity

Antimicrobial activity of all synthesized compounds were determined by disc diffusion method[17]. All human pathogenic bacteria viz *Staphylococcus aureus* (737), *Pseudomonas aeruginosa* (1688) were procured from Institute of Microbial Technology, Chandigarh. 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. Stock solutions of synthesized compounds diluted in dimethyl sulphoxide (1% DMSO) to give final concentration of 500µg/ml and 1000 µg/ml. A reference standard for both gram positive and gram negative bacteria was made by dissolving accurately weighed quantity of chloramphenicol (500 and 1000 µg/mL, respectively) in sterile distilled water, separately. The incubation was carried out at 37ºC 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-2.

RESULTS AND DISCUSSION

The structures of synthesized compounds were confirmed by IR, ^{1}HNMR and mass spectral analysis.

The titled compounds were confirmed by IR spectral data showing sharp bands in the range between 1630-1660 cm^{-1} indicated the presence of C=O group. Compounds (1a-1h) were also confirmed by ^{1}HNMR spectral analysis. Inspection of the ^{1}HNMR spectra suggested that the chalcones were geometrically pure and configured trans ($J_{Ha\text{-}Hb}$ = 16 Hz. The results revealed that majority of the synthesized compounds showed varying degrees of inhibition against Gram positive bacteria shown in Table 2. The 1b showed excellent activity against *S. aureus* at both concentration i.e. 500µg/ml and1000 µg/ml. The compounds1b, 1i , 1d, 1h & 1g ,1f have shown good to moderate activity against *S aureus* at both concentration i.e. 500µg/ml and 1000 µg/ml. Three of the chalcones with antistaphylococcal activity (1c,1e &1j) gave no inhibitory zones probably due to their low diffusion potential into agar media.

Finally, no activity was observed for compounds against *P.aeruginosa,* a Gram negative organism. It is widely known that Gram positive and negative organisms have significantly different membrane compositions and architecture[18] which would explain the selective activity of the present compounds against Gram positive *S. aureus.*

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Table 1: Physical constants of the synthesized compounds

Comp code	R1	R2	Molecular formula	M. Wt	Yield %	MP(°C)
1a	O CH_3	Br	$C_{16}H_{13}BrO_2$	317.17	88	197-198
1bb	OCH_3	I	$C_{16}H_{13}IO_2$	364.18	80	199-201
1c	OCH_3	OCH_3	$C_{17}H_{18}O_3$	268.30	68	175-177
1d	OC_2H_5	OCH_3	$C_{18}H_{18}O_3$	282.33	65	186-188
1e	OH	OCH_3	$C_{16}H_{14}O_3$	254.28	48	238-240
1f	OH	Cl	$C_{15}H_{11}ClO_2$	258.04	32	235-237
1g	OH	Br	$C_{15}H_{11}BrO_2$	301.99	30	265-268
1h	H	Br	$C_{15}H_{11}$ BrO	285.99	88	155-157
1i	H	I	$C_{15}H_{11}$ IO	333.98	88	154-156
1j	H	-OC_2H_5	$C_{17}H_{16}O_2$	252.11	84	140-143

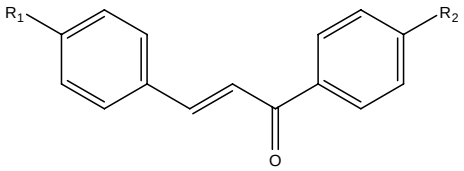
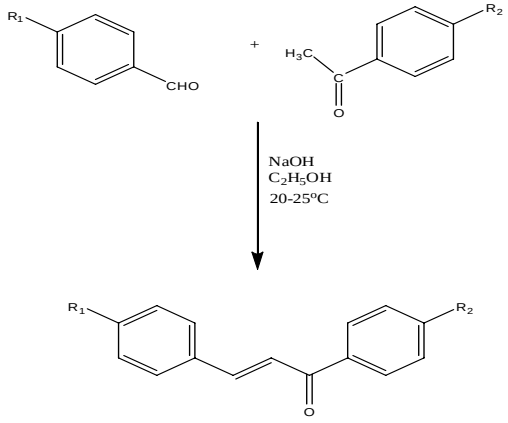


Table 2: Antimicrobial activity of the synthesized compounds

Compounds	Antibacterial activity (%inhibition)			
	Staphylococcus aureus (737)		*Pseudomonas aeruginosa* (1688)	
	500 µg/mL	1000 µg/mL	500 µg/mL	1000 µg/mL
1a	25.0	34.8	-	-
1b	29.8	40.3	-	-
1c	-	-	-	-
1d	23.8	33.6	-	-
1e	-	-	-	-
1f	21.4	32.3	-	-
1g	22.6	32.8	-	-
1h	23.0	33.3	-	-
1i	24.5	34.7	-	-
1j	-	-	-	-
Chloramphenicol	42.3	55.2	63.7	78.9
DMSO	1.4		1.2	

Scheme 1: Scheme of synthesis of compounds



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