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CONVENTIONAL AND MICROWAVE ASSISTED SYNTHESIS OF CHALCONES AND THEIR BIOLOGICAL EVALUATION

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ABSTRACT

Chalcones are synthesized by Claisen-Schmidt condensation, which involves cross-aldol condensation of appropriate aldehydes and ketones by base catalyzed or acid catalyzed reaction followed by dehydration. Chalcone is a common natural pigment and one of the important intermediate in the biosynthesis of flavonoids. Chalcones are synthesized by conventional and microwave assisted synthesis methods. By microwave assisted synthesis, a considerable increase in the reaction rate has been observed and that too, with better yields. The compounds have been screened for antibacterial activity and antioxidant activity.

Key words: Claisen-Schmidt condensation, Microwave irradiation, Antibacterial, Antioxidant activity.

INTRODUCTION

Chalcones are 1,3-diphenyl-2-propene-1-one[1,2], in which two aromatic rings are linked by a three carbon α, β-unsaturated carbonyl system. These are abundant in edible plants and are considered to be the precursors of flavonoids and isoflavonoids. Chalcones are synthesized by Claisen-Schmidt condensation, which involves cross-aldol condensation of appropriate aldehydes and ketones by base catalyzed or acid catalyzed reaction followed by dehydration. Chalcone is a common natural pigment and one of the important intermediate in the biosynthesis of flavonoids[3]. Synthetic and naturally occurring chalcones

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have been extensively studied and developed as one of the pharmaceutically important molecules. Chalcone derivatives are screened for their anti-inflammatory activity[4], chemopreventive activity[5], cardiovascular disease[6], anticancer activity[7], cytotoxic activity[8], antiprolifirative activity[9], antimalarial activity[10], antiviral actiivity[11] and anti–HIV activity[12]. Therefore, in the present investigation, it has been considered worthwhile to synthesize some new chalcone derivatives by conventional and microwave irradiation methods and a comparison has been made between two methods.

Microwave-induced organic reaction enhancement (MORE) chemistry[10] is gaining popularity as a non-conventional technique for rapid organic synthesis. Important features of this technique are easy access to very high temperature, good control over energy input in a reaction, higher yields and rapid synthesis of organic compounds.

The synthesized compounds were purified by recrystallization and chromatography. The compounds were characterized by ^{1}H NMR and IR analysis. The compounds were tested for their antibacterial and antioxidant activities by standard methods.

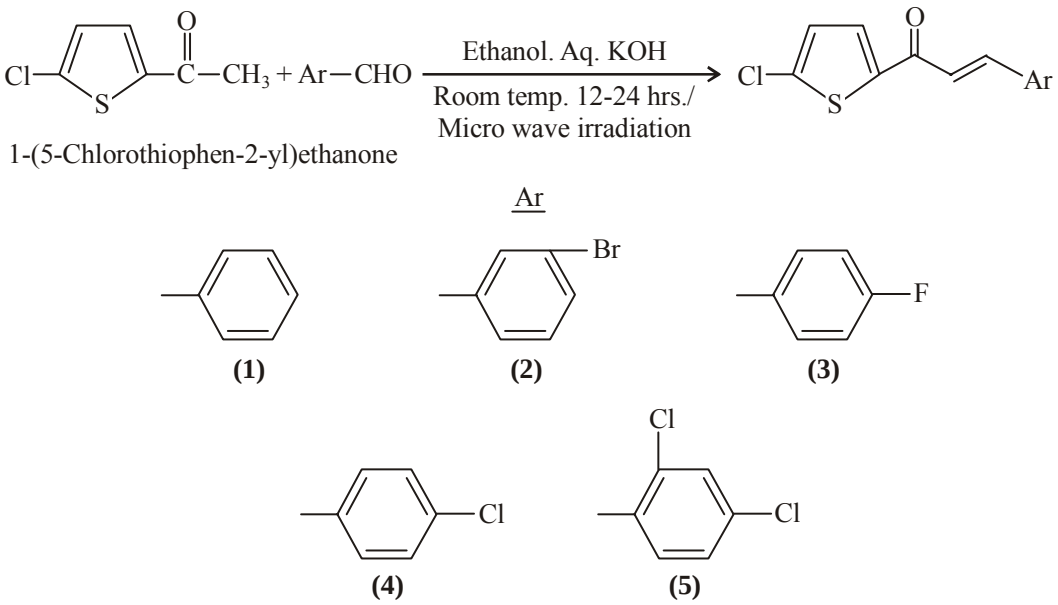
EXPERIMENTAL

General procedure for the synthesis of chalcones by Claisen-Schmidt condensation[13-18]

Synthesis of chalcones (1-5)

(a) Conventional method: Equimolar quantities (0.001 mol) of 2-acetyl-5-chloro–thiophene and respective aldehydes (0.001 mol), were mixed and dissolved in minimum amount (3 mL) of alcohol. To this, aqueous potassium hydroxide solution (0.003 mol) was added slowly and mixed occasionally for 24 hrs, at room temperature. Completion of the reaction was identified by observing on precoated TLC plates of Merck. After completion of the reaction, the reaction mixture was poured into crushed ice, if necessary, it is acidified with dil HCl. The solid separated was filtered and dried. It was purified by recrystallization or by column chromatography performed on silica gel (100-200 Mesh, Merck), using ethyl acetate and hexane mixture as mobile phase.

(b) Microwave irradiation method: Equimolar quantities (0.001 mol) of acetyl hetrocyclic compounds and respective aldehydes (0.001 mol) were mixed and dissolved in minimum amount (3 mL) of alcohol. To this, aqueous potassium hydroxide solution (0.003 mol) was added slowly and mixed. The entire reaction mixture was microwave irradiated for about 2-6 minutes at 180 watts.



Scheme 1

(1). 1-(5-Chlorothiophen-2-yl)-3-phenylprop-2-en-1-one (1)

Physical data

Molecular formula	M. Wt.	M.P.	Time and % Yield				Elemental analysis (%)	
			Conventional		Microwave irradiation		Calculated	Found
			T	% Y	T	% Y		
$C_{13}H_9ClOS$	248.7	106 ± 2°C	24 hrs	85	1.5 min	94	C 62.7 H 3.60	C 62.9 H 3.57
10% Ethyl acetate/Hexane ; TLC - R_f : 0.60							S 12.86	S 12.88

Spectral data

IR (cm^{-1}): 643 (C=O), 1587 (HC = CH), 3093 (C-H aromatic ring streching), 802 (C-Cl), 756 (C-S)

^{1}H NMR (δ ppm): 6.99 (1H, d, J = 8.4 Hz, C-4'-H), 7.26 (1H, d, J = 15.6 Hz, CO-CH=), 7.3-7.5 (5H, m, Ph-H), 7.68 (1H, d, J = 9.6 Hz, C-3'-H), 7.82 (1H, d, J = 16 Hz, Ar-C-H=).

(2). 3-(3-Bromophenyl)-1-(5-chlorothiophen-2-yl) prop-2-en-1-one (2)

Physical data

Molecular formula	M. Wt.	M.P.	Time and % Yield				Elemental analysis (%)	
			Conventional		Microwave irradiation		Calculated	Found
			T	% Y	T	% Y		
$C_{13}H_8BrClOS$	327.6	102 ± 2°C	24 hrs	80	2.0 min	91	C 47.68 H 2.44	C 47.7 H 2.46
10 % Ethyl acetate/Hexane ; TLC - R_f : 0.67							S 9.76	S 9.73

Spectral data

IR (cm^{-1}): 1645 (C=O), 1588 (HC=CH), 3078, 3062 (C-H), 807 (C-Br), 785 (C-S).

^{1}H NMR (δ ppm): 7.01 (1H, d, J = 4 Hz, C-4'-H), 7.26 (1H, d, J = 16 Hz, CO-CH=), 7.30 (1H, d, J = 4 Hz, C-6"-H), 7.53 (1H, t, J = 8 Hz, C-5"-H), 7.64 (1H, d, J = 4 Hz, C-3'-H), 7.69 (1H, s, C-2"-H), 7.75 (1H, d, J = 16 Hz, Ar-C-H=).

(3). 1-(5-Chlorothiophen-2-yl)-3-(4-fluorophenyl) prop-2-en-1-one (3)

Physical data

Molecular formula	M . Wt.	M.P.	Time and % Yield				Elemental analysis (%)	
			Conventional		Microwave irradiation		Calculated	Found
			T	% Y	T	% Y		
$C_{13}H_8ClFOS$	266.7	114 ± 2°C	24 hrs	76	2.5 min	87	C 58.49 H 2.99	C 58.47 H 2.96
10 % Ethyl acetate/Hexane ; TLC - R_f : 0.58							S 11.99	S 12.02

IR (cm^{-1}): 1646 (C=O), 1586.9 (HC=CH), 3093 (C-H), 803 (C-F), 724 (C-S).

^{1}H NMR (δ ppm): 7.0 (1H, d, J = 4 Hz, C-4'-H), 7.12 (2H, d, J = 8.6 Hz, C-3" and 5"-H), 7.21 (1H, d, J = 16 Hz, CO-CH=), 7.61 (2H, d, J = 8.4 Hz, C-2" and 6"-H), 7.63 (1H, d, J = 4 Hz, C-3'-H), 7.81 (1H, d, J = 16 Hz, Ar-C-H=).

(4). 3-(4-Chlorophenyl)-1-(5-chlorothiophen-2-yl) prop-2-en-1-one (4)

Physical data

Molecular formula	M. Wt.	M.P.	Time and % Yield				Elemental analysis (%)	
			Conventional		Microwave irradiation		Calculated	Found
			T	%Y	T	% Y		
$C_{13}H_8Cl_2OS$	283	144 ± 2°C	24 hrs	87	1.5 min	96	C 55.1 H 2.82	C 55.13 H 2.80
10 % Ethyl acetate/Hexane ; TLC - R_f : 0.66							S 11.30	S 11.27

Spectral data

IR (cm^{-1}): 1647 (C=O), 1592 (HC=CH), 797 (C-Cl), 768 (C-S).

^{1}H NMR (δ ppm): 7.03 (1H, d, J = 4 Hz, C-4'-H), 7.31 (1H, d, J = 15.6 Hz, CO-CH=), 7.42 (2H, d, J = 8.4 Hz, C-3" and 5"-H), 7.59 (2H, d, J = 8.6 Hz, C-2" and 6"-H), 7.71 (1H, d, J = 3.8 Hz, C-3'-H), 7.85 (1H, d, J = 16 Hz, Ar-C-H=).

(5). 1-(5-Chlorothiophen-2-yl)-3-(2,4-dichlorophenyl) prop-2-en-1-one (5)

Physical data

Molecular formula	M. Wt.	M.P.	Time and % Yield				Elemental analysis (%)	
			Conventional		Microwave irradiation		Calculated	Found
			T	% Y	T	% Y		
$C_{13}H_7Cl_3OS$	317.6	140 ± 2°C	24	88	1.5	98	C 49.1 H 2.20	C 49.07 H 2.23
10 % Ethyl acetate/Hexane ; TLC - R_f : 0.52							S 10.07	S 10.09

Spectral data

IR (cm^{-1}): 1643 (C=O), 1588 (HC=CH), 792 (C-Cl), 771 (C-S).

^{1}H NMR (δ ppm): 7.02 (1H, d, J = 4 Hz, C-4'-H), 7.30 (1H, s, C-3"-H), 7.32 (1H, d, J = 16 Hz, CO-CH=), 7.46 (1H, d, Hz, C-5"-H), 7.61 (1H, d, J = 8.4 Hz, C-6"-H), 7.67 (1H, d, J = 4.2 Hz, C-3'-H), 8.08 (1H, d, 15.8 Hz, Ar-C-H=).

Antioxidant activity

Free radicals are formed constantly in human system either as accidental products during metabolism or deliberately during the process of phagocytosis or due to environmental pollutants, ionizing radiations, ozone, heavy metal poisoning, cigarette smoking and chronic alcohol intake. Free radicals being highly reactive can oxidize biomolecules leading to tissue injury and cell death.

In the present study, *in vitro* antioxidant activity has been determined using nitro blue tetrazolium (NBT). The IC_{50} values of chalcones were obtained. Solvent used in the test for compounds was DMSO (Dimethylsulphoxide).

Procedure for superoxide free-radical scavenging activity

NBT-riboflavin photoreduction method

Superoxide scavenging activity of the compounds was determined by Mc Cord and Fridovich method, which depends on light induced superoxide generation by riboflavin and corresponding reduction of nitro blue tetrazolium (NBT). The assay mixture contained EDTA (Ethylenediamine tetraacetic acid) solution (6.6 mM) containing NaCN (3 μg), riboflavin (2 μM), NBT (50 μM), test substances and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 mL. The absorbance at 560 nm were measured before and 15 minutes after illumination. All tests were run in triplicate and mean values were used to calculate percentage scavenging ability and IC_{50} values were calculated (using linear regression analysis). The inhibitory effects of samples on the generation of superoxide anions were estimated by the equation -

$$\text{Percentage Inhibition} = [(A_0 - A_1) \times 100] / A_0 \quad \ldots(1)$$

Where A_0 is the absorbance with no addition of sample and A_1 is the absorbance with addition of sample.

Antibacterial activity

The anti bacterial activity of synthesized chalcones were conducted against two gram positive bacteria *viz., Bacillus subtilis* and *Staphylococcus aureus* and two gram negative bacteria *viz., Escherichia coli, Salmonella abony* by using cup plate method. Ciproflaxacin was employed as reference standard to compare the results.

Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5 mL, Analytical R grade) at a concentration of 1000 μg/mL. Ciprofloxacin solution was also prepared at a

concentration of 1000 µg/mL in a sterile distilled water. All the compounds were tested at a concentration of 0.025 mL (25 µg), 0.05 mL (50 µg), 0.2 mL (200 µg) and 0.5 mL (500 µg) level and DMSO used as a control. The solutions of each test compound, standard solution (500 µg) was added separately in the cups and the plates were kept undisturbed for at least 2 hours in refrigerator to allow diffusion of the solution properly into nutrient agar medium. Petri dishes were subsequently incubated at 37 ± 1°C for 24 hrs. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader. All the observations were carried out in triplicate.

RESULTS AND DISCUSSION

Antioxidant activity

The *in vitro* antioxidant activity and scavenging effects of the 5 chalcones were evaluated by using different reactive species assay containing NBT-superoxide free-radical scavenging activity. The potency of the chalcone derivatives was estimated by IC_{50} values**.**

NBT-superoxide radical scavenging activity

All the chalcones **(1-5)** were found to scavenge the superoxides generated by photo-reduction of riboflavin. Among them, compounds **(2)**, **(3)** and **(5)** showed a dose dependent inhibition of superoxide radicals at concentrations of 25, 50 and 100 µg/mL. The remaining compounds exhibited less activity when compared to the above compounds at similar concentration levels and are presented in Table 1.

Table 1: Percentage inhibition of superoxide radical using NBT-riboflavin photo-reduction method (Compounds 1-5)

Compounds	Quantity (µg/mL) percentage inhibition			
	25 µg/mL	**50 µg/mL**	**100 µg/mL**	**IC_{50} µg/mL**
1	13.42	16.26	25.75	87.24
2	9.04	7.89	5.89	57.13
3	7.53	6.01	5.17	68.03
4	5.36	2.21	0	Less active
5	4.13	3.53	0	44.48
Gallic acid	31.21 0.25 µg/mL	40 0.5 µg/mL	59.83 0.75 µg/mL	0.61 µg/mL

Gallic acid, the known antioxidant was employed in the study for comparing the results, at concentrations of 0.25, 0.5 and 0.75 µg/mL. Compound **(3)** appeared to be the best among all the tested compounds. Few of the chalcone derivatives showed good percentage of inhibition but their IC_{50} values were more. Hence, they were less potent among the tested compounds with respect to IC_{50} values.

Antibacterial activity

The antibacterial activity of all the synthesized chalcone derivatives **(1-5)** was evaluated against two gram positive bacteria *viz., Bacillus subtilis* and *Staphylococcus aureus* and two gram negative bacteria *viz., Escherichia coli* and *Salmonella abony*, by using cup plate method. Ciproflaxacin was employed as reference standard to compare the results.

Compounds **(1-5)** exhibited significant antibacterial activity at both the concentrations like 200 and 500 µg/mL compared with the standard drugs. In particular, compounds **(3)**, **(4)** and **(5)** possessed maximum activity on all the bacterial strains which may be due to the presence of dichloro at C-2,4; bromine at C-3; fluorine at C-4; respectively on aromatic ring-B of chalcone. Other compounds also showed mild to moderate activity at both the concentration levels on all organisms. Compounds **(3)** and **(5)** appear to be the best among all the tested compounds. The results and complete data of test are presented in Table 2.

Table 2: Antibacterial activity of chalcone derivatives (1-5)

Compds.	(Gram +ve), Zone of inhibition (mm)							
	Bacillus subtilis				***Staphylococcus aureus***			
	25 µg/mL	**50 µg/mL**	**200 µg/mL**	**500 µg/mL**	**25 µg/mL**	**50 µg/mL**	**200 µg/mL**	**500 µg/mL**
1	-	-	7	14	-	2	6	11
2	-	4	12	18	-	3	8	17
3	-	5	13	21	-	4	9	20
4	-	4	13	21	-	5	9	21
5	-	4	12	20	-	5	10	19
Ciprofloxacin			-	24	-	-	-	23
Control			-	-	-	-	-	-

(-) No zone of inhibition

Table 2: Antibacterial activity of chalcone derivatives (1-5)

Compds.	(Gram -ve), Zone of inhibition (mm)							
	Escherichia coli				***Salmonella abony***			
	25 µg/mL	**50 µg/mL**	**200 µg/mL**	**500 µg/mL**	**25 µg/mL**	**50 µg/mL**	**200 µg/mL**	**500 µg/mL**
1	-	3	7	13	-	3	9	13
2	-	4	11	19	-	5	12	18
3	-	6	14	21	-	6	15	22
4	-	7	15	21	-	6	14	21
5	-	7	16	22	-	7	14	21
Ciprofloxacin			-	26	-	-	-	24
Control			-	-	-	-	-	-

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