Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2011, 3(5):710-717

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Antioxidant and antibacterial activities of some novel chalcone derivatives and their synthesis by conventional and microwave irradiation methods

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ABSTRACT

In an effort to develop antioxidant and antibacterial agents, a series of chalcones were prepared by Claisen-Schmidt condensation of appropriate aldehydes and ketones by base catalyzed or acid catalyzed followed by dehydration , in the presence of aqueous solution of potassium hydroxide and ethanol at room temperature. The synthesized compounds were characterized by means of their IR, ^{1}H NMR spectral data and elemental analysis. All the compounds were tested for their anti oxidant and antibacterial activities by the cup plate method. 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.

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

INTRODUCTION

Chalcones having α, β-unsaturated carbonyl system is one of the most useful Michael acceptor and undergo Michael type nucleophillic addition followed by intra molecular cyclization and aromatization resulting a large number of heterocyclic and cyclic potentially useful system. The

chalcones are considered to be precursors of flavonoids and isoflavonoids when found as naturally- occurring compounds, but it could be considered that their true importance is extended in two branches. The biological activity associated with them, including anti-inflammatory, [1-3] antimitotic, [4] anti-leishmanial, [5]anti-invasive, [6,7] anti-tuberculoid, [8] anti-fungal, [9] anti-malarial, [10,11] anti-tumor, and anti-oxidant properties [12] as well as their recognized synthetic utility in the preparation of pharmacologically-interesting heterocyclic systems like pyrazolines, which have also been largely studied owing to their pharmacological activities, which includes anti-tumor, [13] anti-inflammatory, [14] anti-parasitary, [15] anti-depressive, anticonvulsant, [16] antimicrobial, [17] anti nociceptives [18] and nitric oxide synthase inhibitors, associated with diseases such as Alzheimer, Huntington, and inflammatory arthritis [19].

The structures of the various synthesized compounds were confirmed on the basis of their elemental and spectral (IR, ^{1}H NMR and MASS) data. Therefore, in the present investigation it has been considered worthwhile to synthesize some new chalcone derivatives by conventional and microwave irradiation methods and comparison between two methods

EXPERIMENTAL SECTION

General procedure for the synthesis of chalcones by Claisen-Schmidt condensation [20-25]**: Synthesis of chalcones (1-5):-**

(a) (Conventional). Equimolar quantities (0.001mol) of 2-acetyl-5-chloro–thiophene and respective aldehydes (0.001mol), were mixed and dissolved in minimum amount (3ml) of alcohol, to this aqueous potassium hydroxide solution (0.003mol) was added slowly and mixed occasionally for 24 hrs, at room temperature. Completion of the reaction was identified by observing on precoated TLC plates. After completion of the reaction, the reaction mixture was poured into crushed ice, if necessary 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), using ethylacetate and hexane mixture as mobile phase.

(b) (MWI). Equimolar quantities (0.001mol) of acetyl hetero cyclic compound and respective aldehydes (0.001mol) were mixed and dissolved in minimum amount (3ml) of alcohol; to this aqueous potassium hydroxide solution (0.003mol) was added slowly and mixed. The entire reaction mixture was microwave irradiated for about 2-6 minutes at 180 watts.

All the five compounds have been analyzed by using IR, ^{1}H NMR and MASS spectral data was given below.

Spectral data of the following synthesized compounds:

1-(5-chlorothiophen-2-yl)-3-(4-nitrophenyl) prop-2-en-1-one (1): Mol. Formula: $C_{13}H_8Cl_2NO_3S$, Conventional method Yield 62, Microwave Irradiation 79 %, m.p. 186 ± 2^{o}C. IR (cm^{-1}) : 1654 (C=O), 1608 (HC=CH), 3036 (C-H), 802 (C-Cl), 1513 (Ar-NO_2), 764 (C-S). ^{1}H NMR (δ ppm): 7.02 (1H, d, J=4 Hz, C-4'-H), 7.40 (1H, d, J=16 Hz, CO-CH=), 7.68 (1H, d, J=4 Hz, C-3'-H), 7.78 (2H, d, J=10.2 Hz, C- 2" and 6"-H), 7.82 (1H, d, J=15.6 Hz, Ar-C-H=), 8.28 (2H, d, J=8.6 Hz, C-3" and 5"-H).

3-(4-chloro-3-nitrophenyl)-1-(5-chlorothiophen-2-yl) prop-2-en-1-one (2): Mol. Formula: $C_{13}H_7$ Cl_2N O_3S, Conventional method Yield 68 % , Microwave Irradiation 79 %, m.p. 153 ± 2^oC. IR (cm^{-1}) : 1647 (C=O), 1596 (HC=CH), 3079 (C-H) , 1519 (Ar-NO_2), 824(C-Cl), 764 (C-S). ^1H NMR (δ ppm) : 5.31 (2H, d, J=4 Hz, C-3' and 4'-H), 5.55 (1H, d, J=15.2 Hz, CO-CH=), 6.12 (1H, d, J=8 Hz, C- 5"-H), 6.29 (1H, d, J=8.2 Hz, C- 6"-H), 6.40 (1H, s, C- 2" -H), 6.80 (1H, d, J=15.4 Hz, Ar-C-H=).

3-(3-bromo-4-methoxyphenyl)-1-(5-chlorothiophen-2-yl) prop-2-en-1-one (3): Mol. Formula: $C_{14}H_{10}Br$ Cl O_2S, Conventional method Yield 62 % , Microwave Irradiation 78 %, m.p. 111 ± 2^oC. IR (cm^{-1}) : 1649 (C=O), 1578 (HC=CH), 3079 (C-H) , 1217 (C-O-C), 799 (C-Cl), 730 (C-S). ^1H NMR (δ ppm) : 3.8 (3H, m, C-4"- OCH_3), 6.93 (1H, d, J=8.4 Hz, C-5"-H), 7.01 (1H, d, J=4 Hz, C-4'-H), 7.21 (1H, d, J=16 Hz, CO-CH=), 7.26 (1H, s, C-2"-H), 7.52 (1H, d, J=8.6 Hz, C-6"-H), 7.64 (1H, d, J=4 Hz, C-3'-H), 7.74 (1H, d, J=16 Hz, Ar-C-H=).

1-(5-chlorothiophen-2-yl)-3-(4-methylphenyl) prop-2-en-1-one (4): Mol. Formula: $C_{14}H_{11}$ Cl O S, Conventional method Yield 65 % , Microwave Irradiation 74 %, m.p. 122 ± 2^oC. IR (cm^{-1}) : 1642 (C=O), 1609 (HC=CH), 3031(C-H), 795 (C-Cl), 730 (C-S) ,^1H NMR (δ ppm) : 2.39 (3H, s, C-4"- CH_3), 6.97(1H, d, J=4 Hz, C-4'-H) ,7.20 (2H, d, J=8 Hz, C-3" and 5"-H), 7.27 (1H, d, J=16 Hz, CO-CH=), 7.50 (2H, d, J=8.2 Hz, C-2" and 6"-H), 7.62 (1H, d, J=4 Hz, C-3'-H), 7.85 (1H, d, J=15.4 Hz, C- Ar-C-H=). ^{13}C NMR : 181.07(C-1), 144.53(C-2'), 144.45(C-5'), 141.37(C-3'), 139.43(C-1"), 131.83(C-4'), 131.04 (C-4"), 129.75 (C-3" and 5"), 128.57 (C-2" and 6"), 127.69 (C-2), 119.38 (C-3), 21.53 (C-4"-CH_3). MS (*m/z*, %) : 263.3 $[M+H]^+$.

1-(5-chlorothiophen-2-yl)-3-(2,5-dimethylphenyl) prop-2-en-1-one (5): Mol. Formula: $C_{14}H_{13}$ Cl O S, Conventional method Yield 62 % , Microwave Irradiation 74 %, m.p. 80 ± 2^oC .IR (cm^{-1}) : 1641 (C=O), 1585 (HC=CH), 3031(C-H), 772 (C-S). ^1H NMR (δ ppm) : 2.36 (3H, s, C-5"-CH_3), 2.42 (3H, s, C-2"-CH_3), 7.01 (1H, d, J=4 Hz, C-4'-H), 7.12 (2H, s, C-3" and 4"-H),7.26 (1H, d, J=10 Hz, CO-CH=), 7.47 (1H, s, C-6"-H), 7.66 (1H, d, J=4 Hz, C- 3'-H), 8.15 (1H, d, J=15.4 Hz, Ar-C-H=).

Pharmacological Activities:-

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, two *in vitro* antioxidant models 1,1-Diphenyl-2-picrylhydrazyl radical ($DPPH^•$) scavenging activity (as it is a model for lipophilic radicals which initiate lipid peroxidation) . The IC_{50} values of chalcones tested for their antioxidant activity. Solvent used in both the tests for compounds was DMSO (Dimethylsulphoxide).

ii). DPPH free-radical scavenging activity:-

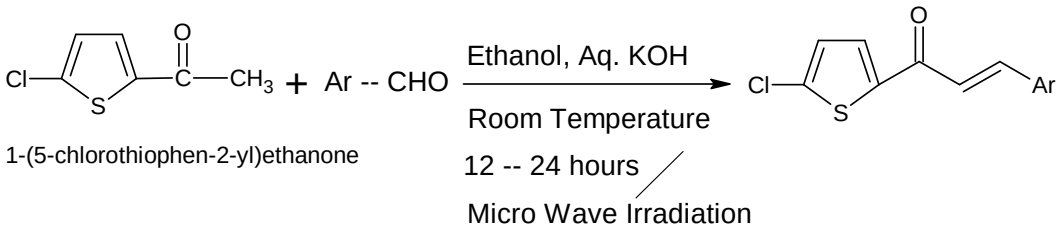
DPPH (1,1–diphenyl-2-picrylhydrazyl) radical scavenging activity was measured by the method of Lamaison *et al.* The reaction mixture contained $1.5X10^{-7}$ M methanolic solution of DPPH and

various concentrations of the test substances and were kept in dark for 50 minutes. Optical density (OD) of samples was measured at 517 nm against a blank, and IC_{50} values were calculated (using linear regression analysis) by plotting a graph, taking concentration on X-axis and percentage inhibition on Y-axis, at 50% of the percentage inhibition the line was drawn from Y-axis and aligned with the concentration on X-axis then got the IC_{50} values.

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.

SCHEME

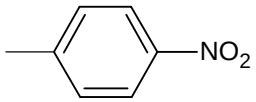


1-(5-chlorothiophen-2-yl)ethanone

(1-5)

Ar

1.



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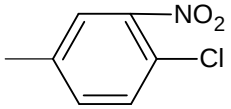

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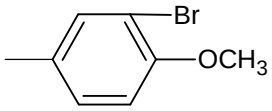

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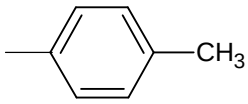

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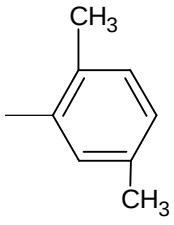

Each test compound (5mg) was dissolved in dimethyl sulfoxide (5 ml, Analytical R grade) at a concentration of 1000 µg/ml. Ciprofloxacin solution was also prepared at a concentration of 1000 µg/ml in a sterile distilled water. All the compounds were tested at a concentration of

0.025ml (25µg), 0.05ml (50µg), 0.2ml (200µg) and 05ml (500µg) level and DMSO used as a control. The solutions of each test compound, standard solution of (500 g) 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± 1^0C 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 tests of components were carried out in triplicate.

Table-1: Comparative reaction time and percentage yield of chalcone derivatives by conventional and microwave irradiation methods

S.No	Reaction time		Yeild (%)	
	Conventional (hr)	MWI (min)	Conventional	MWI
1	24	3.5	64	79
2	24	3.5	68	79
3	24	3.0	62	78
4	24	3.0	65	74
5	24	3.0	62	74

Table -2: Characterization of chalcone derivatives

Compound No	Rf value	M.P	Elemental analysis	
			Calculated	Found
1	0.51	186± 2°C	C: 53.11 H: 2.72 S: 10.89	C: 53.07 H: 2.69 S: 10.86
2	0.49	153± 2°C	C: 47.56 H: 2.13 S: 9.75	C: 47.58 H: 2.16 S: 9.78
3	0.67	111± 2°C	C: 46.98 H: 2.79 S: 8.94	C: 46.97 H: 2.82 S : 8.97
4	0.57	122± 2°C	C: 63.9 H: 4.18 S: 12.00	C: 63.87 H: 4.15 S: 12.03
5	0.54	80± 2°C	C: 65.1 H: 4.69 S: 11.5	C: 65.7 H: 4.72 S: 11.48

Table.3.1, Antioxidant activity of chalcone derivatives (1-5): Percentage inhibition of free radicals using DPPH method (Compounds 1-5)

Compounds	**Quantity (µg/ml) Percentage inhibition**			
	25 µg/ml	**50 µg/ml**	**100 µg/ml**	**IC_{50} µg/ml**
1	**10.32**	**12**	**19.26**	**64.83**
2	9.57	13.42	16.28	>100
3	**7.14**	**9.62**	**20.06**	**49.56**
4	2.13	7.7	8.55	>100
5	9.99	12.16	19.82	>100
Ascorbic acid	16.13 1 µg/ml	38.11 2.5 µg/ml	62.34 5 µg/ml	3.81

Table-3.2: Antibacterial activity of chalcone derivatives (1-5)

C0MPOUNDS	(Gram +Ve), Zone of inhibition (in 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	-	2	8	16	-	3	7	15
2	-	3	10	18	-	3	8	14
3	-	3	9	17	-	3	8	15
4	-	4	12	21	-	4	11	20
5	-	5	13	22	-	5	12	21
Ciprofloxacin			-	24	-	-	-	23
Control			-	-	-	-	-	-

(-) no zone of inhibition

Table-3.2: Antibacterial activity of chalcone derivatives (1-5)

C0MPOUNDS	(Gram -Ve), Zone of inhibition (in 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	11	17	-	4	12	18
2	-	4	12	19	-	5	13	19
3	-	4	11	17	-	5	12	18
4	-	7	15	20	-	7	14	20
5	-	8	16	22	-	8	16	21
Ciprofloxacin			-	26	-	-	-	24
Control			-	-	-	-	-	-

RESULTS AND DISCUSSION

In the present study, we first performed the synthesis of derivatives by conventional and microwave irradiation method in **Scheme** but to reduce the reaction time, it was decided to synthesize the compounds with microwave irradiation, which can be more effective, faster, and energy efficient in addition; we compared those with others that were obtained via conventional heating methods and results were mentioned in Table no 1 and table no 2**.**

The *in vitro* antioxidant activity and scavenging effects of the 5 chalcones were evaluated by using different reactive species assay containing DPPH radical scavenging activity. The potency of the chalcone derivatives was estimated by IC_{50} values. The IC_{50} values of chalcone derivatives synthesized in the present study were given in **Tables 3.1.** The free radical scavenging activity of all the chalcones (**1-5**) were evaluated through their ability to quench the $DPPH^{\bullet}$ using ascorbic acid as reference. Among them compounds **1, 3** showed a dose dependent inhibition of 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 present in **Table 3.1**.Ascorbic acid, the well known antioxidant was used in test for comparing the results, at

concentrations of **1, 2.5** and **5** µg/ml; compound **3** appears to be the best among all the tested compounds. Few of the chalcone derivatives showed good percentage inhibition but their IC_{50} values were more. Hence they were less potent among the tested compounds with respect to IC_{50} values.

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 500µg/ml compared with the standard drugs. In particular, compounds **4, 5** possessed maximum activity on all the bacterial strains which may be due to the presence of methyl at C-4; dimethyl at C-2 and C-5 position , respectively on aromatic ring-B of chalcone . Other compounds also showed mild to moderate activity at both the concentration levels on all organisms. The results and complete data of test were presented in **Table 3.2**.

Acknowledgement
Authors are thankful to Dept. of pharmaceutical chemistry, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam (A.P.) India for providing the facilities to carry out research work.

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