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Conventional and Microwave assisted Synthesis of 1, 3- Diphenyl -2- Propenone derivatives and Cytotoxic, Anti bacterial activites

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Abstract: In an effort to develop cytotoixc 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, 1H-NMR spectral data and elemental analysis. Cytotoxicity against tumour cell lines may be the result of disruption of the cell cycle, inhibition of angiogenesis, interference with p53-MDM2 interaction, mitochondrial uncoupling or induction of apoptosis. Structural requirements for cytotoxic activity vary according to the mechanisms of action. All the compounds were tested for their cytotoxic activity (by BSLT method) 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: Claisen-Schmidt condensation, Microwave irradiation, Cytotoxic activity, Antibacterial 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 hetero - cyclic systems like pyrazolines and pyrimidines, 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, 1H 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. The compounds were tested for their cytotoxic activity and antibacterial activities by standard methods.

EXPERIMENTAL

General procedure for the synthesis of chalcones by Claisen-Schmidt condensation [20-25]:

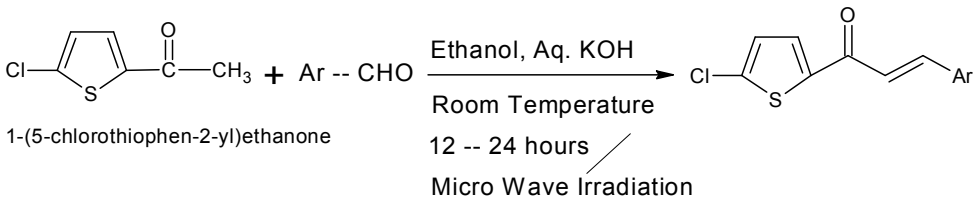
Synthesis of chalcones (1-7):-

(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 of Merck. 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, Merck), 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.

Physical data and spectral data of each compound is mentioned 1-(5-chlorothiophen-2-yl)-3-[4-(dimethyl amino) phenyl] prop-2-en-1-one (1) (Table (1a),(1b)), 1-(5-chlorothiophen-2-yl)-3-(4-methoxyphenyl) prop-2-en-1-one (2) (Table(2a),(2b)), 11-(5-chlorothiophen-2-yl)-3-(3,4-dimethoxyphenyl) prop-2-en-1-one(3) (Table (3a),(3b)), 1-(5-chlorothiophen-2-yl)-3-(3,4,5-trimethoxyphenyl) prop-2-en-1-one (4) (Table (4a),(4b)), 1-(5-chlorothiophen-2-yl)-3-(4-ethoxy -phenyl) prop-2-en-1-one (5) (Table (5a),(5b)). 1-(5-chlorothiophen-2-yl)-3-(1*H*-pyrrol-2-yl) prop-2-en-1-one (6) (Table (6a),(6b)). 1-(5-chlorothiophen-2-yl)-3-(1*H*-indol-3-yl) prop-2-en-1-one (7) (Table (7a),(7b)).

SCHEME .1



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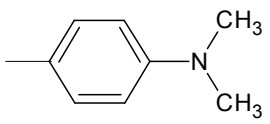

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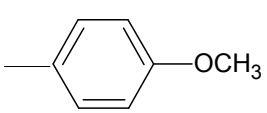

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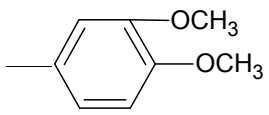

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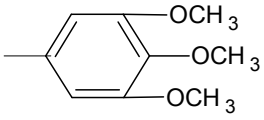

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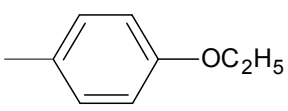

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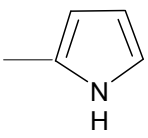

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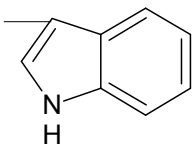

1. 1-(5-chlorothiophen-2-yl)-3-[4-(dimethyl amino) phenyl] prop-2-en-1-one (1):
1 (a) Physical data:

Molecular formula	M .Wt	M.P	Time and (%) Yield				Elemental analysis (%)	
			Conventional		Micro wave Irradiation		Calculated	Found
			T	% Y	T	% Y		
$C_{15}H_{14}ClNOS$	291.7	128 ± 2°C	24 hrs	54	4.5 min	62	C 61.73	C 61.75
							H 4.62	H 4.64
15 % Ethyl acetate / Hexane TLC - R_f : 0.58							S 10.92	S 10.89

1 (b) Spectral data:

IR (cm^{-1})	:	1625 (C=O), 1551 (HC=CH), 1317 (C-N-C), 772 (C-S).
^1H NMR (δ ppm)	:	3.05 (6H, s, C-4''- $N(CH_3)_2$), 6.77 (2H, d, J=9.6 Hz, C-3'' and 5''-H), 6.98 (1H, d, J=4 Hz, C-4'-H), 7.16 (1H, d, J=16.2 Hz, CO-CH=), 7.52 (2H, d, J=9.2 Hz, C-2'' and 6''-H), 7.61 (1H, d, J=4 Hz, C-3'-H), 7.84 (1H, d, J=16 Hz, Ar-C-H=).

2. 1-(5-chlorothiophen-2-yl)-3-(4-methoxyphenyl) prop-2-en-1-one (2):
2 (a) Physical data:

Molecular formula	M .Wt	M.P	Time and (%) Yield				Elemental analysis (%)	
			Conventional		Micro wave Irradiation		Calculated	Found
			T	% Y	T	% Y		
$C_{14}H_{11}ClO_2S$	278.7	118 ± 2°C	24 hrs	54	2.5 min	66	C 60.24	C 60.21
							H 3.91	H 3.93
10 % Ethyl acetate / Hexane TLC - R_f : 0.64							S 11.48	S 11.45

2 (b) Spectral data:

IR (cm^{-1})	:	1643 (C=O), 1587 (HC=CH), 1228 (C-O-C), 800 (C-Cl), 722 (C-S).
^1H NMR (δ ppm)	:	3.86 (3H, s, C-4''- OCH_3), 6.88 (2H, d, J=8.4 Hz, C-3'' and 5''-H), 7.01 (1H, d, J=4 Hz, C-4'-H), 7.18 (1H, d, J=15.6 Hz, CO-CH=), 7.60 (2H, d, J=8.4 Hz, C-2'' and 6''-H), 7.62 (1H, d, J=4.4 Hz, C-3'-H), 7.83 (1H, d, J=15.2 Hz, Ar-C-H=).

3. 1-(5-chlorothiophen-2-yl)-3-(3,4-dimethoxyphenyl) prop-2-en-1-one (3):
3 (a) Physical data:

Molecular formula	**M.Wt**	**M.P**	**Time and (%) Yield**				**Elemental analysis (%)**	
			Conventional		**Microwave Irradiation**		**Calculated**	**Found**
			T	**%Y**	**T**	**% Y**		
$C_{15}H_{13}ClO_3S$	308.7	130 ± 2°C	24 Hrs	52	2.5 min	61	C 58.36	C 58.38
							H 4.07	H 4.1
25 % Ethyl acetate / Hexane TLC - R_f : 0.57							S 10.3	S 10.27

3 (b) Spectral data:

IR (cm^{-1})	:	3084 (C-H), 1644 (C=O), 1586 (HC=CH), 1259 (C-O-C), 795 (C-Cl), 714(C-S).
^1H NMR (δ ppm)	:	3.93 (3H, s, C-3''- OCH_3), 3.95 (3H, s, C-4''- OCH_3), 6.89 (1H, d, J=8.2 Hz, C-5''-H), 6.99 (1H, d, J=4 Hz, C-4'-H), 7.16 (1H, d, J=I5.2 Hz, CO-CH=), 7.22-7.26 (2H, d, J=9.6 Hz, C-2''and 6''-H) 7.64 (1H, d, J=3.6 Hz, C- 3'-H), 7.81 (1H, d, J=16.2 Hz, Ar-C-H=).

4. 1-(5-chlorothiophen-2-yl)-3-(3,4,5-trimethoxyphenyl) prop-2-en-1-one (4):
4 (a) Physical data:

Molecular formula	M .Wt	M.P	Time and (%) Yield				Elemental analysis (%)	
			Conventional		Microwave Irradiation		Calculated	Found
			T	%Y	T	%Y		
$C_{16}H_{15}ClO_4S$	338.8	110 ± 2°C	24 hrs	55	2.5 Min	67	C 56.8	C 56.78
							H 4.3	H 4.33
25 % Ethyl acetate / Hexane TLC - R_f : 0.52							S 9.4	S 9.43

4 (b) Spectral data:

IR (cm^{-1}) : 3095 (C-H), 1646 (C=O), 1585 (HC=CH), 1217 (C-O-C), 819 (C-Cl), 769(C-S).

^{1}H NMR (δ ppm) : 1.57 (3H, s, C-4"- OCH_3),
3.91 (6H, d, J=8 Hz, C-3" and 5"- OCH_3)
6.84 (2H, s, C-2" and 6"-H),
7.01 (1H, d, J=4.2 Hz, C- 4'-H),
7.22 (1H, d, J=10.6 Hz, CO-CH=),
7.64 (1H, d, J=4 Hz, C- 3'-H),
7.79 (1H, d, J=9.4 Hz, Ar-C-H=).

5. 1-(5-chlorothiophen-2-yl)-3-(4-ethoxyphenyl) prop-2-en-1-one (5)
5 (a) Physical data :

Molecular formula	M.Wt	M.P	Time and (%) Yield				Elemental analysis (%)	
			Conventional		Microwave Irradiation		Calculated	Found
			T	% Y	T	%Y		
$C_{15}H_{13}ClO_2S$	292.7	134 ± 2°C	24 Hrs	58	3.0 min	67	C 61.4	C 61.37
							H 4.28	H 4.3
15 % Ethyl acetate / Hexane TLC - R_f : 0.61							S 10.9	S 10.87

5 (b) Spectral data:

IR (cm^{-1}) : 1643 (C=O), 1585 (HC=CH), 1220 (C-O-C), 772 (C-S).

^{1}H NMR (δ ppm) : 1.40 (3H, t, C-4"-OCH_3),
4.10 (2H, dd, C-4"-OCH_2),
6.93 (2H, d, J=9.6 Hz, C-3" and 5"-H)
6.98 (1H, d, J=3.8 Hz, C-4'-H),
7.21 (1H, d, J=15.8 Hz, CO-CH=),
7.56 (2H, d, J=10 Hz, C- 2" and 6"-H)
7.62 (1H, d, J=4 Hz, C-3'-H),
7.82 (1H, d, J=16 Hz, Ar-C-H=).

6. 1-(5-chlorothiophen-2-yl)-3-(1*H*-pyrrol-2-yl) prop-2-en-1-one (6):
6 (a) Physical data:

Molecular formula	M.Wt	M.P	Time and (%) Yield				Elemental analysis (%)	
			Conventional		Microwave Irradiation		Calculated	Found
			T	% Y	T	%Y		
$C_{11}H_8ClNOS$	237.7	162 ± 2°C	24 hrs	48	3.5 min	62	C 55.53	C 55.51
							H 3.18	H 3.21
20 % Ethyl acetate / Hexane TLC - R_f : 0.54							N 5.86	S 5.84

6 (b) Spectral data:

IR (cm^{-1})	:	3242 (N-H), 1635(C=O), 1545(HC=CH) 1284 (C-N-C), 771 (C-S), 731 (C-Cl).
^{1}H NMR (δ ppm)	:	4.10 (1H, m, N-H), 6.20 (2H, d, J=8 Hz, C-3" and 5"-H), 6.91 (1H, d, J=8 Hz, C-4"-H), 7.21 (1H, d, J=4 Hz, C-4'-H), 7.32 (1H, d, J=15.6 Hz, CO-CH=), 7.61 (1H, d, J=4.2 Hz, C- 3'-H), 7.84 (1H, d, J=16 Hz, Ar-C-H=).

7. 1-(5-chlorothiophen-2-yl)-3-(1*H*-indol-3-yl) prop-2-en-1-one (7):
7 (a) Physical data:

Molecular formula	M .Wt	M.P	Time and (%) Yield				Elemental analysis (%)	
			Conventional		Microwave Irradiation		Calculated	Found
			T	% Y	T	% Y		
$C_{15}H_{10}ClNOS$	287.7	158 ± 2°C	24 hrs	51	2.5 min	60	C 62.56	C 62.53
							H 3.41	H 3.38
35 % Ethyl acetate / Hexane TLC - R_f : 0.46							N 4.86	N 4.89

7 (b) Spectral data:

IR (cm^{-1})	:	3212 (N-H), 1633(C=O), 1519 (HC=CH) 1220 (C-N-C), 770 (C-S).
^{1}H NMR (δ ppm)	:	7.20 (1H, d, J=4 Hz, C-4'-H), 7.22-7.27 (4H, m, C-4", 5", 6"and 7"-H), 7.53 (1H, d, J=15.2 Hz, CO-CH=), 8.15 (1H, d, J=4.2 Hz, C-3'-H), 8.28 (1H, d, J=16 Hz, Ar-C-H=). 9.95 (1H, s, C- 3"-H), 12.13 (1H, s, N-H).

Table-6.1
Cytotoxic activity of chalcones by using Brine shrimp lethality test (compounds 1-7)

S.NO	COMPOUNDS	Solubility	ED_{50} µg/ml
1	4''- (dimethyl amino) phenyl	-	1.26
2	4''- methoxy phenyl	-	4.02
3	3'',4''- dimethoxy phenyl	-	39.64
4	3'',4'',5''- trimethoxy phenyl	-	44.46
5	4''- ethoxy phenyl	-	1.64
6	1*H*- pyrrol-2-yl	-	44.46
7	1*H*- indol-3-yl	-	41.75
Standard	(Podophyllotoxin)	-	3.88

Table-6.2
Antibacterial activity of chalcone derivatives (1-7)

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	-	9	16	23	-	9	17	23
2	-	5	12	18	-	5	13	18
3	-	6	13	20	-	6	13	19
4	-	8	14	22	-	7	14	21
5	-	6	11	20	-	5	14	19
6	-	7	13	21	-	8	15	21
7	-	8	16	22	-	9	16	23
Ciprofloxacin			-	26	-	-	-	24
Control			-	-	-	-	-	-

CYTOTOXICITY TEST :

Brine shrimp lethality bioassy (BSLT):-

Brine shrimp lethality test have been used as bioassay for a variety of toxic substances. This method has also been applied to plant extracts in order to facilitate the isolation of biologically active compounds[19, 20, 21]. A general bioassay that appears capable of detecting a broad spectrum of bioactivity, present in crude extracts and in synthetic compounds is the brine shrimp lethality bioassay, rather than more tedious and expensive *in vitro* and *in vivo* antitumor assays. Furthermore, it does not require animal serum as is needed for cytotoxicities.

Procedure:

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of medicinal plants[160,161]. Brine shrimps (Artemia salina) were hatched using brine shrimp eggs in a conical shaped vessel (1L), filled with sterile artificial sea water under constant aeration for 38 h. After hatching, active nauplii free from egg shells were collected from brighter portion of the chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing 5 ml of brine solution. In each experiment, test substances whose activities are to be checked were added to the vial according to their concentrations and maintained at room temperature for 24 h under the light and surviving larvae were counted. Experiments were conducted along with control (vehicle treated), different concentrations (1-5000 µg / ml) of the test substances in a set of three tubes per dose. Replicas should be maintained to get accurate results.

Statistical analysis

The percentage lethality was calculated from the mean survival larvae of compounds treated tubes and control. ED_{50} values were obtained by (best-fit line method) 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 ED_{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.

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 0.5ml (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 components were carried out in triplicate.

RESULTS AND DISCUSSION

CYTOTOXIC ACTIVITY:-

Brine shrimp lethality test (BSLT):

Brine shrimp lethality test has been used as bioassay for variety of toxic substances. All the chalcones (**1-7**) were tested for cytotoxic activity by the BSLT bioassay method. All the compounds were found to possess cytotoxic activity. Among them compounds 11,12,15 compounds showed a dose dependent cytotoxic activity at concentrations of (11) 1.26 µg/ml, , (12) 4.02 µg/ml, (15) 1.64 µg/ml respectively. The remaining compounds exhibited less activity when compared to the above mentioned compounds at various concentration levels. The degree of lethality is directly proportional to the concentration of the synthesized compounds. Podophyllotoxin was used as a standard drug for BSLT assay method. The results and complete data of test presented in Table 6.1.

ANTIBACTERIAL ACTIVITY:-

The antibacterial activity of all the synthesized chalcone derivatives (**1-7**) 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 (**1-7**) exhibited significant antibacterial activity at both the concentrations like 200 and 500µg/ml compared with the standard drugs. In particular, compounds **1, 4, 6 and 7** possessed maximum activity on all the bacterial strains which may be due to the presence of methyl at C-4; dimethyl amino at C-4 position, trimethoxy at C-3,4,5 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 6.2.

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