International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.3, pp 1462-1469, July-Sept 2011

Synthesis and Cytotoxic, Anti oxidant activity of 1, 3- Diphenyl -2- Propene -1-one derivatives

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Abstract: Chalcones belong to an important class of flavonoids, which may be prepared by Claisen reaction. They possess a wide range of biological activities and industrial applications. Kostanecki was the first to give the term chalcone and who did pioneering work in the synthesis of naturally coloring compounds. 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. Chemoprotection by chalcones may be a consequence of their antioxidant properties, mediated via inhibition or induction of metabolic enzymes, by an anti-invasive effect or a reduction in nitric oxide production. 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 cytotoxic activity and antioxidant activity.
Keywords: Claisen-Schmidt condensation, Microwave irradiation, Cytotoxic activity, Antioxidant activity.

INTRODUCTION

Chalcones are abundantly present in nature from ferns to higher plants [1]. They are aromatic compounds with an unsaturated side chain and are often cytotoxic *in vitro* [2]. Chalcones have also been reported to be anti-inflammatory, analgesic and antipyretie[3]. Some chalcones possess bactericidal, antifungal and insecticidal activity and some of their derivatives are reported to be antimutagenic [4]. Chalcones are 1,3-diphenyl-2-propene-1-one[5,6], 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 followed by dehydration. Chalcone is a common natural pigment and one of the important intermediate in the biosynthesis of flavonoids [7]. Synthetic and naturally occurring chalcones have been extensively studied and developed as one of the

pharmaceutically important molecules. chalcone derivatives are screened for their anti-inflammatory activity[8], chemopreventive activity[9],cardiovascular disease[10], anticancer activity[11],cytotoxic activity[12],atiprolifirative activity[13],antimalarial activity[14],antiviral actiivity[15], anti–HIV activity[16]. 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.

Microwave-induced organic reaction enhancement (MORE) chemistry10 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 1H NMR and IR analysis. The compounds were tested for their cytotoxic activity and antioxidant activities by standard methods.

EXPERIMENTAL

General procedure for the synthesis of chalcones by Claisen-Schmidt condensation [17-22]:

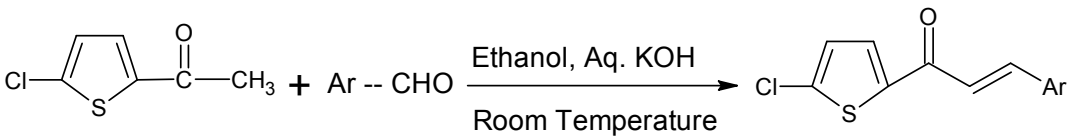
Synthesis of chalcones (1-5):-

(a) Conventional method: 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) Microwave irradiation method: Equimolar quantities (0.001mol) of acetyl hetrocyclic compounds 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-phenylprop-2-en-1-one (1) (Table (1a),(1b)), 3-(3-bromophenyl)-1-(5-chlorothiophen-2-yl) prop-2-en-1-one (2) (Table(2a),(2b)), 1-(5-chlorothiophen-2-yl)-3-(4-fluoro phenyl) prop-2-en-1-one (3) s(Table (3a),(3b)), 3-(4-chlorophenyl)-1-(5-chlorothiophen-2-yl) prop-2-en-1-one (4) (Table (4a),(4b)), 1-(5-chlorothiophen-2-yl)-3-(2,4-dichlorophenyl) prop-2-en-1-one (5) (Table (5a),(5b)).

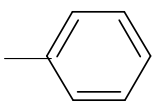
SCHEME .1



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

Ar

1.



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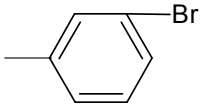

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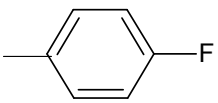

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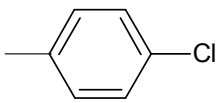

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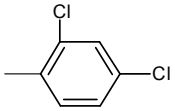

Table 1. 1-(5-chlorothiophen-2-yl)-3-phenylprop-2-en-1-one (1):
1(a) Physical data:

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

1(b) Spectral data :

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

^1H 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=).

Table 2. 3-(3-bromophenyl)-1-(5-chlorothiophen-2-yl) prop-2-en-1-one (2):
2(a) 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	C 47.7
							H 2.44	H 2.46
10 % Ethyl acetate / Hexane TLC - R_f : 0.67							S 9.76	S 9.73

2 (b) Spectral data :

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

Table 3. 1-(5-chlorothiophen-2-yl)-3-(4-fluorophenyl) 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		
$C_{13}H_8ClFOS$	266.7	114± 2°C	24 hrs	76	2.5 min	87	C 58.49	C 58.47
							H 2.99	H 2.96
10 % Ethyl acetate / Hexane TLC - R_f : 0.58							S 11.99	S 12.02

3 (b) Spectral data :

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

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

Molecular formula	MWt	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	C 55.13
							H 2.82	H 2.80
10 % Ethyl acetate / Hexane TLC - R_f : 0.66							S 11.30	S 11.27

4 (b) Spectral data:

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

Table 5. 1-(5-chlorothiophen-2-yl)-3-(2,4-dichlorophenyl) 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		
$C_{13}H_7Cl_3OS$	317.6	140 ± 2°C	24 hrs	88	1.5 Min	98	C 49.1	C 49.07
							H 2.20	H 2.23
10 % Ethyl acetate / Hexane TLC - R_f : 0.52							S 10.07	S 10.09

5 (b) 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=).

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. 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.

Table-6.1
Cytotoxic activity of chalcones by using Brine shrimp lethality test (compounds 1-5)

S.NO	COMPOUNDS	Solubility	ED_{50} µg/ml
1	Phenyl	DMSO	19.04
2	3''- bromo phenyl	-	41.24
3	4''- fluoro phenyl	-	39.04
4	4''- chloro phenyl	-	40.28
5	2,4''- dichloro phenyl	-	43.02
Standard	(Podophyllotoxin)	-	3.88

Table.6.2
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	14.26	27.4	30.11	56.13
2	11.27	13.06	23.45	72.25
3	9.82	12.43	25.03	64.18
4	17.55	23.1	43.23	Less active
5	19.87	27.43	47.13	89.04
Ascorbic acid	16.13 1 µg/ml	38.11 2.5 µg/ml	62.34 5 µg/ml	3.81

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.

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 ($DPPH^{\bullet}$) 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).

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.

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 (**1-5**) were tested for cytotoxic activity by the BSLT bioassay method. All the compounds were found to possess cytotoxic activity. Among them compounds 1 compound showed a dose dependent cytotoxic activity at concentrations of (1) 19.04 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**.**

ANTIOXIDANT ACTIVITY:-

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 6.2.

DPPH-radical scavenging activity:

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 6.2.

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.

ACKNOWLEDGEMENT

I am thankful to my guide Dr.V.Girija Sastry, Dept. of pharmaceutical chemistry, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam - 530003 (A.P.) India. For providing the facilities to carry out research work. To my beloved parents (Father: - Md. Rafeeq Ahmed, Mother: - Khyrunnisa begum). For their moral support.

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