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ORIGINAL ARTICLE

Synthesis of novel chalcone derivatives by conventional and microwave irradiation methods and their pharmacological activities

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Abstract Chalcones are abundant in edible plants and are considered to be the precursors of flavonoids and isoflavonoids. Chalcones belong to an important class of flavonoids, which may be prepared by Claisen–Schmidt condensation. They possess a wide range of biological activities and industrial applications. The 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. 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.

1. Introduction

Chalcones are abundantly present in nature from ferns to higher plants (Star and Marby, 1971). They are aromatic compounds with an unsaturated side chain and are often cytotoxic *in vitro* (Dhar, 1981). Chalcones have also been reported to be anti-inflammatory, analgesic and antipyretic (Satyanarayana and Rao, 1993). Some chalcones possess bactericidal, antifungal and insecticidal activity and some of their derivatives are reported to be antimutagenic (Torigoo et al., 1983). Chalcones are 1,3-diphenyl-2-propene-1-one (Nowakowska, 2007 and Maayan et al., 2005), 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 catalysed or acid catalysed reactions followed by dehydration. Chalcone is a common natural pigment and one of the

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important intermediates in the biosynthesis of flavonoids (Go et al., 2005). 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 (Kim et al., 2007), chemo preventive activity (Shen Jeu et al., 2005), cardiovascular disease (Liming et al., 2003), anticancer activity (Francesco et al., 2007), cytotoxic activity, antiprolifirative activity (Ducki et al., 1998), antimalarial activity (Chen et al., 1994), antiviral activity (Onyilagha et al., 1997) and anti-HIV activity (Xu et al., 2000). Therefore, in the present investigation it has been considered worthwhile to synthesize some new chalcone derivatives by conventional and microwave irradiation methods and to compare between two methods.

Microwave-induced organic reaction enhancement (MORE) chemistry 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 cytotoxic activity and antioxidant activities by standard methods.

2. Experimental

2.1. General procedure for the synthesis of chalcones by Claisen–Schmidt condensation

(Min et al., 2001; Dawey and Tivey, 1958; Kohler et al., 1967; Mehra, 1968):

*2.1.1. Synthesis of chalcones (**2a–2e**)*

(A) Conventional method: Equimolar quantities (0.001 mol) of 2-acetyl-5-methyl-furan 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 h, 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 the mobile phase.

(B) Microwave irradiation method: Equimolar quantities (0.001 mol) of 2-acetyl hetero cyclic derivatives 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 min at 180 watts.

2.1.2. The spectral data of the all chalcone derivatives is given below

*2.1.2.1. 1-(5-Methylfuran-2-yl)-3-phenylprop-2-en-1-one (**2a**).* Mol. formula: $C_{14}H_{12}O_2$, conventional method yield 61%, microwave irradiation yield 74%, the range of m.p. 124 ± 2 °C. IR (cm^{-1}): 3020 (C–H aromatic streching), 2924 (C–H methyl streching), 1660 (C═O), 1604 (HC═CH), 1214 (C–O–C). ^{1}H NMR (δ ppm): 2.45 (3H, s, C-5′–CH_3), 6.22 (1H, d, J = 4.2 Hz, C-4′-H), 7.25 (1H, d, J = 15.6 Hz, CO–CH═), 7.37–7.43 (5H, m, C-2″, 3″, 4″and 5″, 6″-H), 7.64 (1H, d, J = 4 Hz, C-3′-H), 7.96 (1H, d, J = 16.2 Hz, Ar–C–H═).

*2.1.2.2. 3-(4-Fluorophenyl)-1-(5-methylfuran-2-yl) prop-2-en-1-one (**2b**).* Mol. formula: $C_{14}H_{11}FO_2$, conventional method yield 65%, microwave irradiation yield 74%, the range of m.p. 92 ± 2 °C. IR (cm^{-1}): 1644 (C═O), 1591 (HC═CH), 1215 (C–O–C), 798 (C–F). ^{1}H NMR (δ ppm): 2.13 (3H, s, C-5′–CH_3), 7.45 (1H, d, J = 4 Hz, C-4′-H), 7.64 (1H, d, J = 16 Hz, CO–CH═), 7.70 (2H, d, J = 8.4 Hz, C-3″ and 5″-H), 7.75 (1H, d, J = 4.2 Hz, C-3′-H), 7.84 (2H, d, J = 8.4 Hz, C-2″ and 6″-H), 8.00 (1H, d, J = 16 Hz, Ar–C–H═).

*2.1.2.3. 3-(4-Chlorophenyl)-1-(5-methylfuran-2-yl) prop-2-en-1-one (**2c**).* Mol. formula: $C_{14}H_{11}ClO_2$, conventional method yield 78%, microwave irradiation yield 86%, the range of m.p. 126 ± 2 °C. IR (cm^{-1}): 3016 (C–H), 2924 (C–H), 1651 (C═O), 1602 (HC═CH), 1212 (C–O–C), 800 (C–Cl). ^{1}H NMR (δ ppm): 2.41 (3H, s, C-5′–CH_3), 6.25 (2H, d, J = 4 Hz, C-3′ and 4′-H), 7.26 (2H, d, J = 8.2 Hz, C-3″ and 5″-H), 7.40 (1H, d, J = 16 Hz, CO–CH═), 7.58 (2H, d, J = 9.4 Hz, C-2″ and 6″-H), 8.1 (1H, d, J = 16.4 Hz, Ar–C–H═).

*2.1.2.4. 3-(2,4-Dichlorophenyl)-1-(5-methylfuran-2-yl) prop-2-en-1-one (**2d**).* Mol. formula: $C_{14}H_{10}Cl_2O_2$, conventional method yield 64%, microwave irradiation yield 73%, the range of m.p. 120 ± 2 °C. IR (cm^{-1}): 3020 (C–H), 2924 (C–H), 1657 (C═O), 1601 (HC═CH), 1215 (C–O–C), 798 (C–Cl). ^{1}H NMR (δ ppm): 2.44 (3H, s, C-5′–CH_3), 6.23 (1H, d, J = 4 Hz, C-4′-H), 7.26–7.30 (2H, m, C-5″ and 6″-H), 7.35 (1H, d, J = 15.6 Hz, CO–CH═), 7.45 (1H, s, C-3″-H), 7.69 (1H, d, J = 8 Hz, C-3′-H), 8.16 (1H, d, J = 16 Hz, Ar–C–H═).

*2.1.2.5. 1-(5-methylfuran-2-yl)-3-(4-nitrophenyl) prop-2-en-1-one (**2e**).* Mol. Formula: $C_{14}H_{11}NO_4$, conventional method yield 53%, microwave irradiation yield 61%, the range of m.p. 174 ± 2 °C. IR (cm^{-1}): 1657 (C═O), 1603 (HC═CH), 1511 (Ar–NO_2), 1213 (C–O–C). ^{1}H NMR (δ ppm): 2.46 (3H, s, C-5′–CH_3), 6.25 (1H, d, J = 4 Hz, C-4′-H), 7.30 (1H, d, J = 4.2 Hz, C-3′-H), 7.44 (1H, d, J = 16 Hz, CO–CH═), 7.75 (2H, d, J = 9.2 Hz, C-2″ and 6″-H), 7.81 (2H, d, J = 9.4 Hz, C-3″ and 5″-H), 8.24 (1H, d, J = 16.4 Hz, Ar–C–H═).

2.2. Cytotoxicity test

2.2.1. Brine shrimp lethality bioassy (BSLT)

Brine shrimp lethality test has 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. 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.

2.2.2. 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 flask (1 L), filled with sterile artificial sea water under constant aeration for 38 h. After hatching, active nauplii free from egg shells were collected from the 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 light and the 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.

2.3. Statistical analysis

The percentage lethality was calculated from the mean survival larvae of compound 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 the Y-axis and aligned with the concentration on the X-axis, then the ED_{50} values were obtained.

2.4. Antioxidant activity

Free radicals are formed constantly in the 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 model 1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^{\cdot}$) scavenging activity (as it is a model for lipophilic radicals which initiate lipid peroxidation). The IC_{50} values of chalcones were tested for their antioxidant activity. Solvent used in both the tests for compounds was DMSO (dimethylsulfoxide).

2.4.1. 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 1.5×10^{-7} M methanolic solution of DPPH and various concentrations of the test substances and was kept in the dark for 50 min. 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 the X-axis and percentage inhibition on the Y-axis, at 50% of the percentage inhibition the line was drawn from Y-axis and aligned with the concentration on X-axis then the IC_{50} values were obtained. Tables 1–2.1, 2.2 and 3.

3. Results

Brine shrimp lethality test has been used as bioassay for a variety of toxic substances. All the chalcones (**2a–2e**) were tested for cytotoxic activity by the BSLT bioassay method. All the compounds were found to possess cytotoxic activity. Among them compounds **2a, 2c, 2b** showed a dose dependent cytotoxic

Table 1 Comparative reaction time and percentage yield of chalcone derivatives (**2a–2e**) by conventional and microwave irradiation methods.

S.No	Reaction time		Yeild (%)	
	Conventional (h)	MWI (min)	Conventional	MWI
2a	24	3	61	74
2b	24	4	65	74
2c	24	3.5	78	86
2d	24	3.5	64	73
2e	24	4	53	61

Table 2.1 Characterization of chalcone derivatives (**2a–2e**).

Compound no.	Elemental analysis	
	Calculated	Found
2a	C: 79.17 H: 5.65 O: 15.08	C: 79.2 H: 5.68 O: 15.1
2b	C: 72.98 H: 4.77 O: 13.90	C: 72.95 H: 4.80 O: 13.87
2c	C: 68.12 H: 4.46 O: 12.97	C: 68.09 H: 4.49 O:12.94
2d	C: 59.78 H: 3.55 O: 11.38	C: 59.81 H: 3.52 O: 11.35
2e	C: 54.18 H: 3.42 O: 24.88	C: 54.21 H: 3.45 O: 24.91

Table 2.2 Physical Properties.

S. no.	Molecular formula	M.P. (°C)	R_f Value
2a	$C_{14}H_{12}O_2$	124 ± 2	0.62
2b	$C_{14}H_{11}FO_2$	92 ± 2	0.64
2c	$C_{14}H_{11}ClO_2$	126 ± 2	0.66
2d	$C_{14}H_{10}Cl_2O_2$	120 ± 2	0.62
2e	$C_{14}H_{11}NO_4$	174 ± 2	0.56

Table 3 Cytotoxic activity of chalcones by using Brine shrimp lethality test (compounds **2a–2e**).

S.no.	Compounds	Solubility	ED_{50} μg/ml
2a	Phenyl	DMSO	24.27
2b	4″-Fluoro phenyl	—	39.26
2c	4″-Chloro phenyl	—	37.05
2d	2,4″-Dichloro phenyl	—	43.53
2e	4″-Nitro phenyl	—	45.38
Standard	(Podophyllotoxin)	—	3.88

Table 4 Antioxidant activity: Percentage inhibition of free radicals using DPPH method (compounds **2a–2e**).

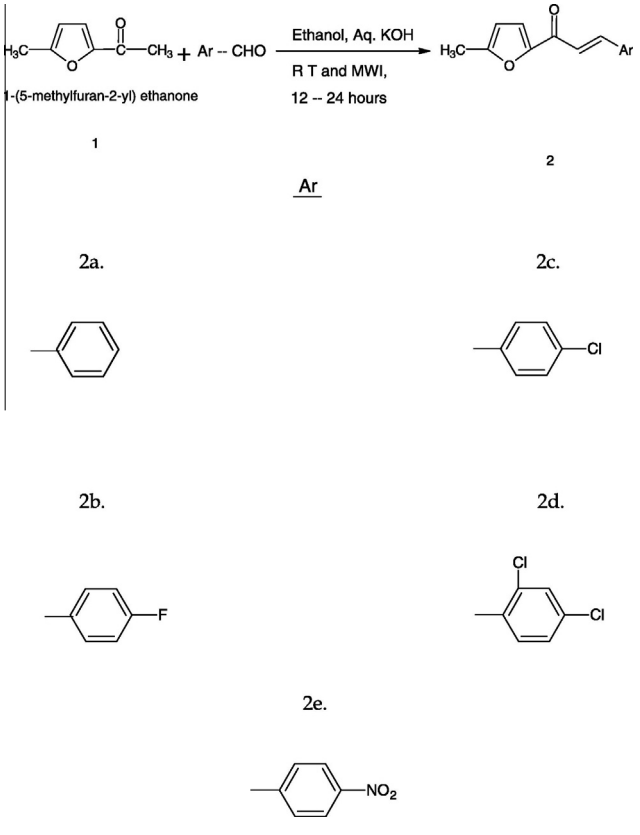
Compound	Quantity (μg/ml) percentage inhibition			
	25 μg/ml	50 μg/ml	100 μg/ml	IC_{50} μg/ml
2a	7.24	12.31	16.03	76.12
2b	4.05	7.12	11.04	65.04
2c	9.11	10.03	12.04	81.15
2d	8.35	9.24	10.47	75.20
2e	10.14	11.85	13.69	49.18
Ascorbic acid	16.13	38.11	62.34	0.61
	1 μg/ml	2.5 μg/ml	5 μg/ml	

activity at concentrations of (**2a**) 24.27 μg/ml, (2c) 37.05 μg/ml, (**2b**) 39.26 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 the BSLT assay method.

The *in vitro* antioxidant activity and scavenging effects of the (**2a–2e**) chalcones were evaluated by using different reactive species assay containing radical scavenging activity. The potency of the chalcone derivatives was estimated by IC_{50} values. The IC_{50} values of chalcone derivatives used in the present study were given in Table 5.

DPPH (1,1–diphenyl-2-picrylhydrazyl) radical scavenging activity was measured for all the chalcones (**2a–2e**). Among them, compounds **2a, 2b, 2c, 2d** and **2e** showed a dose dependent inhibition of radicals at concentrations of **25, 50** and **100** μg/ml.

Ascorbic acid, the well known antioxidant was used in the test for comparing the results, compound 5 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.



Scheme 1

4. Discussion

It is interesting to know that the diaryl chalcones, having electron releasing, electron withdrawing substituents like fluorine, chlorine as dichloro and nitro, pharmacophore especially at C-4 position of aromatic ring-B, showed excellent cytotoxic and antioxidant activities, when compared to that of other substituted chalcones.

5. Conclusion

All the synthesized (**2a–2e**) compounds were purified by recrystallization (solvent used was ethyl alcohol) or by column chromatography. The identification of compounds was established by single spot TLC, melting point and by spectral analysis involving IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. Since chalcones were widely reported to possess cytotoxic activity and antioxidant activities etc. All the chalcone derivatives were evaluated for the above mentioned activities and they have exhibited promising activity. Scheme 1.

From the cytotoxic activity and antioxidant activities it was proven that most of the chalcone derivatives are potent and possessing cytotoxic activity and antioxidant activities.

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