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Microwave Assisted Synthesis Of Novel A,B – Unsaturated Carbonyl Derivatives And Their Activity.

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Abstract: A series of novel chalcone derivatives have been synthesized and tested for their Cytotoxic and antioxidant activity. Chalcones were synthesized by Microwave Irradiation technique (MWI). The main advantages of MWI are higher yields and rapid synthesis. Hence, it prompted us to synthesize chalcones by MWI. Cytotoxic acitivity was carried out by Brine Shrimp Lethality Assay (BSLT) and antioxidant activity by NBT-riboflavin photo reduction method. The synthesized derivatives have shown good cytotoxic and antioxidant potential.
Key words: Chalcones; Microwave irradiation; Cytotoxic activity; 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[3]. Several biological activities of chalcones can be attributed due to the presence of unique template molecule. 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[4], chemopreventive activity[5], cardiovascular disease[6], anticancer activity[7], cytotoxic activity[8], antiprolifeirative activity[9], antimalarial activity[10], antiviral activity[11], anti–HIV activity[12]. This prompted us to synthesize some new chalcone derivatives. Chalcones are synthesized by Claisen-Schmidt condensation, which involves base catalyzed or acid catalyzed cross aldol condensation of appropriate aldehydes and ketones, followed by dehydration.

Microwave-induced organic reaction enhancement (MORE) chemistry[13,14] is gaining popularity as a non-conventional technique because of higher yields under milder reaction conditions, higher product purities, easy access to very high temperature, good control over energy input in a reaction, and rapid synthesis of organic compounds. Spectacular accelerations produced in many chemical reactions as a consequence of heating rate can be seen in microwave-assisted

organic synthesis, which cannot be achieved in conventional technique. Some reactions which do not occur by conventional heating can be achieved by microwave synthesis. The effect of microwave radiation in chemical reactions is due to the combination of thermal and non-thermal effects. When compared to conventional technique, the energetic coupling takes place at molecular level in microwave irradiation technique. Microwave heating is dependent on the properties of the material.

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.

EXPERIMENTAL

CHEMISTRY

All the melting points of the derivatives were determined by digital melting point apparatus (SMP 10). The ^{1}H NMR spectra were recorded on a Bruker AV 400 MHZ in DMSO, using TMS as an internal standard. Shifts reported are relative to the signal of the solvent used in each case and coupling constants are reported in Hz (s: singlet, bs: broad singlet, d: doublet, t: triplet, dd: double doublet, dt: double triplet, m: multiplet). IR spectra were recorded using a ALPHA FT-IR spectrometer (Bruker). High resolution mass spectra were obtained on Agilent 6100 Series Single Quadrupole LC/MS. Microwave-assisted reactions were carried out in a Catalyst Scientific Microwave Oven System (Model: CATA 2R, Make: Catalyst System, Pune, India). Precoated TLC plates from Merck were used. Commercial compounds were purchased from Aldrich Chemical Co.

GENERAL PROCEDURE FOR THE SYNTHESIS OF CHALCONES (3A-3E) BY CLAISEN-SCHMIDT CONDENSATION MICROWAVE IRRADIATION METHOD:

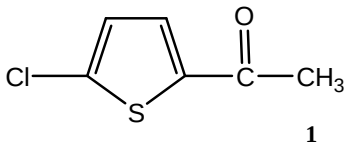
Equimolar quantities (0.001mol) of 2-acetyl-5-chloro–thiophene and respective aldehydes (0.001mol) were mixed and dissolved in minimum amount (3ml) of alcohol; to this aqueous potassium hydroxide solution (0.003mol) was added slowly and mixed. The entire reaction mixture was microwave irradiated for about 2-6 minutes at 180 watts[15,16,17]. 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. The elemental analysis, Melting points of all the derivatives were calculated and presented in Table 1.

Table 1: Physical Properties (R_f, M.P & Elemental analysis) chalcone derivatives,

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

SCHEME

Synthesis of Chalcones by claisen-Schmidt Condensation



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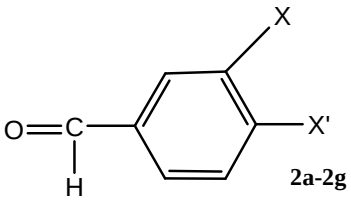


2a-2g

2a, X=H, X'=NO_2,

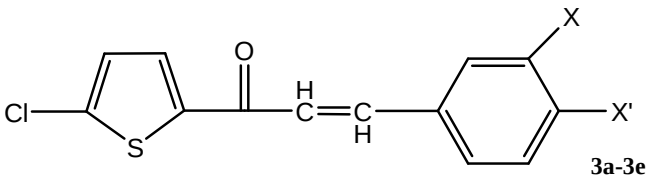
2b, X=NO_2, X'=Cl,

2c, X=Br, X'=OCH_3

2d, X=H, X'=CH_3,

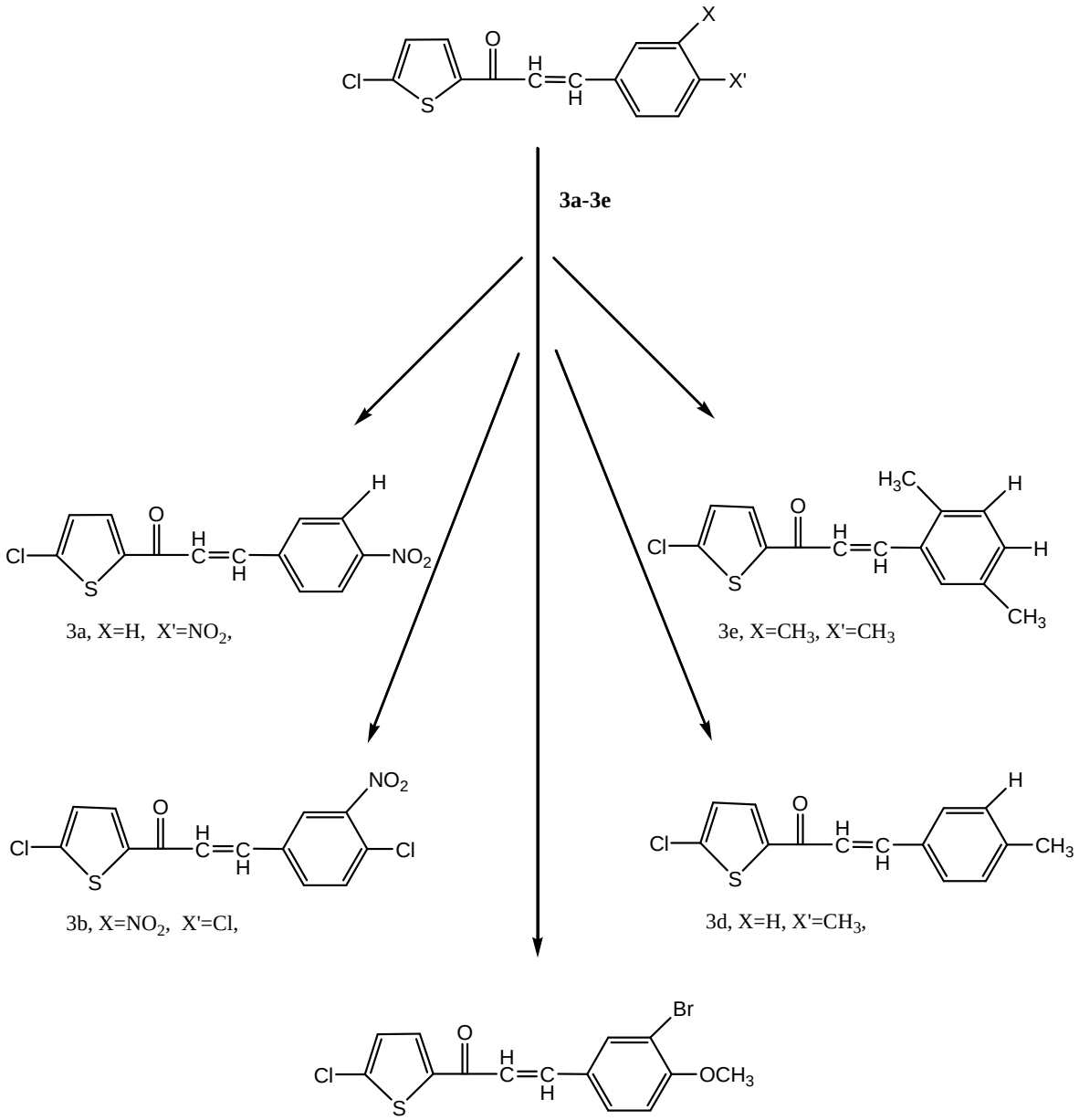
2e, X=CH_3, X'=CH_3

Ethanol, aq.KOH
Conventional,
RT, 12-24 hrs/
MWI. 1-12, Min.



3a-3e

Structures of synthesized chalcone derivatives



SPECTRAL DATA OF THE FOLLOWING SYNTHESIZED COMPOUNDS:

***1-(5-chlorothiophen-2-yl)-3-(4-nitrophenyl) prop-2-en-1-one (1)*:** Mol. Formula: $C_{13}H_8$ Cl_2 N O_3S, Microwave Irradiation Yield 79 %, m.p. 186 ± 2 ^oC. IR (cm^{-1}) : 1654 (C=O), 1608 (HC=CH), 3036 (C-H), 802 (C-Cl), 1513 (Ar-NO_2), 764 (C-S). ^1H 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, Microwave Irradiation Yield 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, Microwave Irradiation

Yield 78 %, m.p. 111 ± 2 ^{o}C. IR (cm^{-1}) : 1649 (C=O), 1578 (HC=CH), 3079 (C-H) , 1217 (C-O-C), 799 (C-Cl), 730 (C-S). ^{1}H 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, Microwave Irradiation Yield 74 %, m.p. 122 ± 2 ^{o}C. IR (cm^{-1}) : 1642 (C=O), 1609 (HC=CH), 3031(C-H), 795 (C-Cl), 730 (C-S) ,^{1}H 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). Mass: 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, Microwave Irradiation Yield 74 %, m.p. 80 ± 2 ^{o}C .IR (cm^{-1}) : 1641 (C=O), 1585 (HC=CH), 3031(C-H), 772 (C-S). ^{1}H 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 ACTIVITY

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[18, 19, 20]. 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[21,22,23,24,25,26], 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.

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 and obtained 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 *in vitro* antioxidant activity nitro blue tetrazolium (NBT). The IC_{50} values of chalcones tested for their antioxidant activity. Solvent used in the test for compounds was DMSO (dimethylsulphoxide).

NBT-RIBOFLAVIN PHOTO REDUCTION 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)[27,28,29]. The assay mixture contained EDTA (Ethylenediamine tetra-acetic acid) solution (6.6 mM) containing NaCN (3µg), riboflavin (2µM), NBT (50µM), test substances and phosphate buffer (67mM, P^{H} 7.8) in a final volume of 3 mL. The absorbance at 560nm 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:
Percentage Inhibition = $[(A_0 - A_1) \times 100] / A_0$

Where A_0 is the absorbance with no addition of sample

A_1 is the absorbance with addition of sample.

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 (**3a-3e**) were tested for cytotoxic activity by the BSLT bioassay method. All the compounds were found to possess cytotoxic activity. Among them compounds **3c, 3d, 3e** showed a dose dependent cytotoxic activity at concentrations of (**3c**) 27.14µg/ml, (**3d**) 3.73µg/ml, (**3e**) 4.51µg/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 2.**

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 (**3a-3e**) were found to scavenge the superoxides generated by photo-reduction of riboflavin. Among them, compounds **3b, 3d, 3e** 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 were present in **Table 3, Figure 1.**

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 **3c** appeared 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.

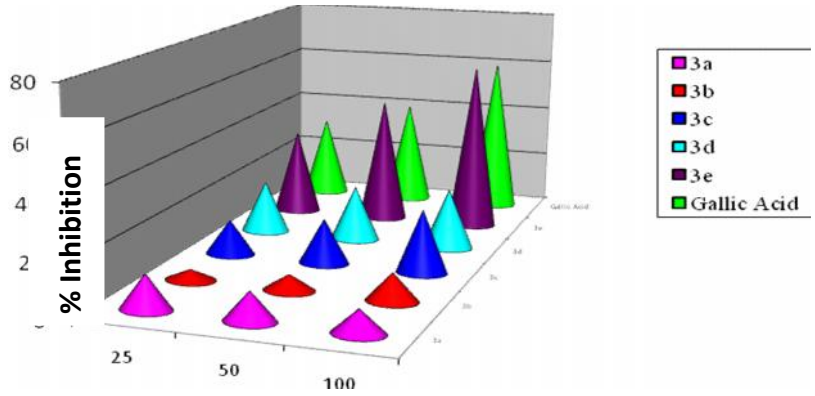
Table 2: Cytotoxic activity of chalcones by using Brine shrimp lethality test (compounds 3a-3e)

Compounds	Quantity (µg/ml) Percentage inhibition			
	25 µg/ml	**50 µg/ml**	**100 µg/ml**	**IC_{50} µg/ml**
3a	12.74	10.62	8.47	>100
3b	3.97	5.91	10.29	62.18
3c	13.16	16.73	23.47	>100
3d	19.71	20.63	22.62	63.04
3e	32.66	47.83	63.82	68.16
Gallic acid	31.21 0.25µg/ml	40 0.5 µg/ml	59.83 0.75µg/ml	0.61 µg/ml

Table 3: Percentage inhibition of superoxide radicals using NBT-riboflavin photo reduction method (Compounds 3a-3e).

S.NO	COMPOUNDS	Solubility	ED_{50} µg/ml
3a	4''- nitro phenyl	DMSO	42.05
3b	4''- chloro-3''- nitro phenyl	-	43.62
3c	3''-bromo-4''-methoxyphenyl	-	27.14
3d	4''- methyl phenyl	-	3.73
3e	2'',5''- dimethyl phenyl	-	4.51
Standard	(Podophyllotoxin)	-	3.88

Figure 1: Anti-oxidant activity



Quantity (µg/ml)

Graph showing percentage inhibition of free radicals by compounds (3a-3e).

CONCLUSION

The synthesis of chalcones by microwave irradiation method showed that MWI is a preferable method because of rapid synthesis and also higher product yield. All the synthesized compounds were purified by recrystallization or by column chromatography. The characterization of compounds was established by single spot TLC, melting point and by spectral analysis involving IR, ^{1}H NMR, Mass and elemental analysis. Since chalcones were widely reported to possess great cytotoxic activity and antioxidant activity. All the chalcone derivatives **(3a-3e)** were evaluated for cytotoxic activity and found that **3c, 3d, 3e** showed dose dependent cytotoxic inhibition at different concentrations as per BSLT test. So also, all the chalcone derivatives **(3a-3e)** were evaluated for the antioxidant activity and found that **3b, 3d, 3e** showed dose dependent inhibition at different concentrations and **3b** showed maximum inhibition of superoxide radicals, as per NBT-superoxide radical scavenging activity.

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