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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL 1, 3-DIPHENYL-2-PROPENE-1-ONES HAVING ANTI-MICROBIAL AND ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT
Chalcones are 1, 3-diphenyl-2-propene-1-one, in which two aromatic rings are linked by a three carbon α, β-unsaturated carbonyl system. Chalcone derivatives constitute an interesting class of compounds due to their synthetic versatility and effective biological activity. In the last few years, considerable attention has been focused on chalcone derivatives due to their interesting biological activities like anti-inflammatory activity, antibacterial activity, antimalarial activity, antitumoral activity, antioxidant activity, cytotoxic activity, anti-histamine activity, antiplasmodial activity etc. In view of the important biological activities, in the present work, four derivatives of novel 1,3-diphenyl-2-propene-1-one derivatives (or) chalcone derivatives (3a-3d) were synthesized by grinding of resacetophenone (1a) with various substituted benzaldehyde derivatives (2a-2d) in the presence of potassium hydroxide/sodium hydroxide (KOH)/(NaOH). The chemical structures of these compounds were confirmed by means of ^{1}H NMR and FTIR spectra. The compounds were assayed for anti-inflammatory activity. Among the compounds tested **3a** shows maximum antibacterial activity towards ***Bacillus*** (Gm +ve) and is effective at 150 µg concentration where as **3c, 3d** shows maximum antibacterial activity towards *Escherichia coli* (Gm -ve) and is effective at 150 µg concentration and 3a showed significant anti inflammatory activity, 3c showed equal anti-inflammatory activity to standard drug diclofenac.

Key words: α, β-unsaturated carbonyl system, anti-inflammatory activity, antibacterial activity, 1H NMR spectra, FTIR spectra

INTRODUCTION
The utility of chalcones due to their usefulness as in synthesis of various heterocyclic compounds, as plant origin [I] and exhibit anti-inflammatory activity [II], antimicrobial activity [III], antibacterial activity [IV], antitumoral activity [V], antioxidant activity [VI], cytotoxic activity [VII], anti-histamine activity [VIII], antiplasmodial [IX], activities.

Classical synthesis of these compounds involves the condensation of acetophenones and aldehydes to give chalcones. The combination of solvents and long reaction time, costly chemicals/catalyst makes this method environmentally hazardous. This provided the stimulus to synthesize some new chalcones using grinding technique [X]. In grinding technique, reaction occurs through generation of local heat by grinding of crystals of substrate and reagent by mortar and pestle. Reactions are initiated by grinding, with the small amount of energy through friction. In some cases, a mixture and reagents turns to a glassy material. Such reaction are simple to handle, reduce pollution, comparatively cheaper to operate and may be regarded as more economical and ecologically favorable procedure in chemistry [XI]. Solid-state reaction occurs more efficiently and more selectively than does the solution reaction, since molecules in the crystal are arranged tightly and regularly [XII]. In present work, grinding technique was used for the synthesis of titled compounds. This method is superior to conventional method; since it is eco-friendly, high yielding, requires no special apparatus, non-hazardous, operationally simple and convenient.

Novel chalcones were prepared by grinding together equivalent amounts of the resacetophenone (1a) with various substituted benzaldehyde derivatives (2a-2d) in the presence of potassium hydroxide/sodium hydroxide (KOH)/(NaOH) in a porcelain mortar under solvent-free conditions for 4-8 min. On completion of reaction (TLC), the reaction mixture was diluted with cold water, neutralized by dilute HCl and recrystalized from acetic acid.

MATERIALS AND METHODS

All the required chemicals used were obtained from Qualikem chemicals and Sd-fine chemicals. All the solvents used were of laboratory grade. Each reaction was monitored by TLC by using appropriate solvent system, which was selected by trial and error method. Pre-coated TLC plates (0.25mm silica gel) were obtained from E. Merck. All the synthesized compounds were purified by recrystallization. Melting points were determined on Fisher Johns melting point apparatus and they were uncorrected. ^{1}H NMR spectrum was recorded on Avance 300 MHz Bruker UX-NMR instrument and the sample was made in $CDCl_3$ and DMSO-d_6 using tetra methyl silane (Me_4Si) as the internal standard. IR spectra were recorded on Shimadzu FTIR thermonicolet instrument by KBr disc method.

The final compounds were synthesized as given below:

I. Preparation of resacetophenone (1a).

II. Preparation of benzaldehyde derivatives (2a-2d).

III. Preparation of chalcone derivatives (3a-3d).

The final compounds were synthesized by grinding of resacetophenone (1a) with various substituted benzaldehyde derivatives (2a-2d) in the presence of potassium hydroxide/sodium hydroxide (KOH)/ (NaOH).

I) Preparation of 1-(2, 4-dihydroxyphenyl) ethanone (1a):

Resacetophenone preparation was carried out by heating a mixture of $ZnCl_2$ (33.0 g, 0.24 mol) with glacial acetic acid (38.0 g, 0.63 mol) at 140 ^{0}C. To this solution, resorcinol (22.0g, 0.18 mol) was added in small portion keeping the temperature below 160 0C. After 20 min. the mixture was diluted with 1:1 (conc. HCl:H_2O) solution and was cooled to 5 ^{0}C. The precipitate thus obtained was filtered and washed with 1:3 (conc. HCl: H_2O) solution, the crude product was dried and was crystallized from dil.HCl.

II) Preparation of m-nitro Benzaldehyde (2a):

Nitric acid (26.6 ml) wast taken in a 250 ml three necked round bottom flask fitted with a thermometer on a mechanical stirrer to this added sulphuric acid (40 ml) with the help of

dropping funnel. The mixture was cooled to 0 ^{0}C in an ice bath, with effective stirring for the preparation of nitration mixture. 10 g of Benzaldehyde was placed in a round bottom flask on a magnetic stirrer, to that added the nitration mixture drop wise with constant stirring below 5 ^{0}C . Stirred the mixture for 1.5 hr and then poured into crushed ice, the crude product was precipitated with continuous stirring. Filtered the precipitate with suction on a funnel. Washed thoroughly with 20ml 5% aqueous sodium carbonate solution and ice water, pressed and dried well. The yield of m-nitro benzaldehyde obtained is 72%, m.p 56-58 ^{0}C.

III). 1) Synthesis of (E)-1-(3-nitrophenyl)-3-phenylprop-2-en-1-one (3a):

Resacetophenone (2g, 0.01mmol) and m-nitro benzaldehyde (0.01mmol) were taken in a mortar and stirred continuously with pestle at room temparature . Then added NaOH pellets (0.002 mmol) to the mixture and stirred for 30 minutes. On completion of reaction it was monitored by TLC, the obtained solid was poured in crushed ice and neutralized by dilute HCl, then recrystalized from acetic acid to obtain the corresponding chalcone.

2) Synthesis of 1,3-diphenyl 2-propene 1- one (3b):

Resacetophenone (2g, 0.01mmol) and benzaldehyde (0.01mmol) were taken in a mortar and stirred continuously with pestle at room temperature. Then added NaOH pellets (0.002 mmol) to the mixture and stirred for 30 minutes. On completion of reaction it was monitored by TLC, the obtained solid was poured in crushed ice and neutralized by dilute HCl, then recrystalized from acetic acid to give the corresponding Chalcone.

3) Synthesis of (E)-1-(4-nitrophenyl)-3-phenylprop-2-en-1-one (3c):

Resacetophenone (2g, 0.01mmol) and p-nitro benzaldehyde (0.01mmol) were taken in a mortar and stirred continuously with pestle at room temparature. Then added NaOH pellets (0.002 mmol) to the mixture and stirred for 30 minutes. On completion of reaction it was monitored by TLC, the obtained solid was poured in crushed ice and neutralized by dilute HCl, then recrystalized from acetic acid to give the corresponding chalcone.

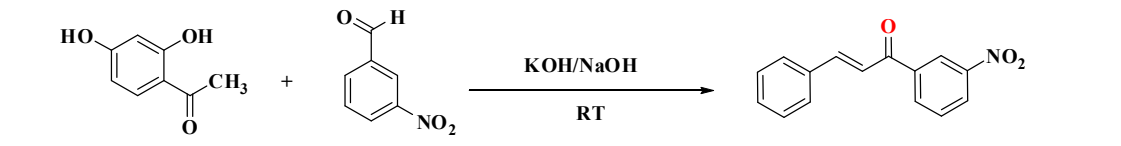
4) **Synthesis of (E)-1-(4-chlorophenyl)-3-phenylprop-2-en-1-one (3d):**

Resacetophenone (2g, 0.01mmol) and p-chloro benzaldehyde (0.01mmol) were taken in a mortar and stirred continuously with pestle at room temperature. Then added NaOH pellets (0.002 mmol) to the mixture and stirred for 30 minutes. On completion of reaction it was monitored by TLC, the obtained solid was poured in crushed ice and neutralized by dilute HCl, then recrystalized from acetic acid to give the corresponding chalcone.

RESULTS AND DISCUSSION

Synthesis of Chalcone derivatives (3a- 3d) incorporating benzaldehyde and its derivatives having nitro (-NO_2), chloro (-Cl), substitutions at 2, 3 and 4 positions has been planned firstly by synthesizing key intermediate Benzaldehyde with nitration mixture which was subsequently grinding with resacetophenone (1a) in the presence of KOH/NaOH.

The reactions are depicted in following scheme.



1-(2,4-dihydroxyphenyl)ethanone

or

Resacetophenone

3-nitrobenzaldehyde

(*E*)-1-(3-nitrophenyl)-3-phenylprop-2-en-1-one (3a)

Table No. 1: Benzaldehyde Derivatives

DERIVATIVES	R1	R2	R3
Benzaldehyde	-H	-H	-H
p-Nitrobenzaldehyde	$-NO_2$	-H	-H
m-Nitrobenzaldehyde	-H	$-NO_2$	-H
p-Chlorobenzaldehyde	$-Cl_2$	-H	-H

Table No. 2: Chemical Formulas of Benzaldehyde Derivatives

S.no	Compound	Chemical formula	Melting point	Yield
1	**1a**	$C_8H_8O_3$	142-143 ^{0}C	81%
2	**2a**	$C_7H_5NO_3$	58-59 ^{0}C	72 %
3	**2b**	C_7H_6O	179 ^{0}C (B.P)	84 %
4	**2c**	$C_7H_5NO_3$	103-106 ^{0}C	70 %
5	**2d**	C_7H_5ClO	45-47 ^{0}C	74 %
6	**3a**	$C_{15}H_{11}NO_3$	150-152 ^{0}C	76 %
7	**3b**	$C_{15}H_{12}O$	160-163 ^{0}C	72 %
8	**3c**	$C_{15}H_{11}NO_3$	160-161 ^{0}C	69 %
9	**3d**	$C_{15}H_{11}ClO$	156-158 ^{0}C	73 %

IR & NMR Data of 1-(2,4-dihydroxyphenyl)ethanone (1a):
It's IR (KBr) cm^{-1} spectrum showed a broad absorption peak at 3299.60 indicating the presence of O-H stretching vibration, peak at 1606.31 is due to C=O stretching.. ^{1}H NMR spectrum showed singlet at δ 2.50 integrating for 3 protons assigned to CH_3, a doublet at 6.25 with coupling constant J=2.2 Hz, integrating for one proton assigned to aromatic proton H-3, a doublet of doublet at 6.32 with coupling constant J=8.7 Hz, 2.2 Hz, integrating for one proton assigned to H-5, another doublet at 7.56 with coupling constant J= 8.7 Hz, integrating for one proton assigned to H-6.

IR & NMR Data of of *m*-nitro Benzaldehyde (2a):
It's IR (KBr) cm^{-1} spectrum showed a short absorption peak at 2871.81 indicating the presence of C-H stretching vibration of aromatic ring, peak at 1702.08 is due to C=O stretching of CHO group and two sharp absorption peaks at 1415.65 and 1352.12 indicating the presence of $–NO_2$ group.

IR Data of (E)-1-(3-nitrophenyl)-3-phenylprop-2-en-1-one (3a):
It's IR (KBr) cm^{-1} spectrum showed a broad absorption peak at 3280.05 indicating the presence of C-H stretching vibration of aromatic ring, peak at 1608.60 is due to C=O stretching and sharp absorption peaks at 1527.52 indicating the C=C stretching. Two sharp absorption peaks at 1442.23-1350.06 indicating the presence of N-O Stretching, sharp absorption peaks at 1064.52 indicating the presence of C-O stretching.

IR Data of 1, 3-diphenyl 2-propene 1- one (Chalcone) (3b):
It's IR (KBr) cm^{-1} spectrum showed a broad absorption peak at 3298.05 indicating the presence of C-H stretching vibration of aromatic ring, peak at 1608.08 is due to C=O stretching and sharp absorption peaks at 1527.52 indicating the C=C stretching.

IR Data of (E)-1-(4-nitrophenyl)-3-phenylprop-2-en-1-one (3c):
It's IR (KBr) cm^{-1} spectrum showed a sharp absorption peak at 2918.16 indicating the presence of C-H stretching vibration of aromatic ring, peak at 1604.65 is due to C=O stretching and sharp absorption peaks at 1546.80 indicating the C=C stretching, and two weak absorption peaks at 1458.66 and 1348.32 indicating the presence of N-O Stretching.
IR Data of (E)-1-(4-chlorophenyl)-3-phenylprop-2-en-1-one (3d):
It's IR (KBr) cm^{-1} spectrum showed a broad absorption peak at 3224.05 indicating the presence of C-H stretching vibration of aromatic ring, peak at 1608.52 is due to C=O stretching and sharp absorption peaks at 1365.51 indicating the C=C stretching.

ANTI MICROBIAL STUDIES:
The antibacterial activity of all the compounds were determined by cup plate method using 24- hr old cultures of

i) Gram-positive bacteria (*Bacillus)*
ii) Gram-negative bacteria (*Escherichia coli).*

Ciprofloxacin was used as a standard drug. **DMSO** was used as a control. Zone of inhibition was measured in mm.

Table.4.6.1
Zone of inhibition of 1, 3-diphenyl 2-propene 1- one derivatives (3a-3d)

	Bacillus			***Escherichia coli***		
compound	50 µg/ml	100 µg/ml	150 µg/ml	50 µg/ml	100 µg/ml	150 µg/ml
standard	**3.6**	**3.8**	**4**	**3.5**	**3.7**	**3.8**
3a	1.9	2.6	**3.9**	0.8	1.6	2.2
3b	1.6	2.4	3.5	0.4	1.3	2.4
3c	0.7	0.9	**3.8**	1.9	2.8	**3.5**
3d	0.5	0.9	3	0.6	0.9	**3.4**

Table.4.6.2
BAR Diagram of Zone of Inhibition *Bacillus* (G +ve) microorganism

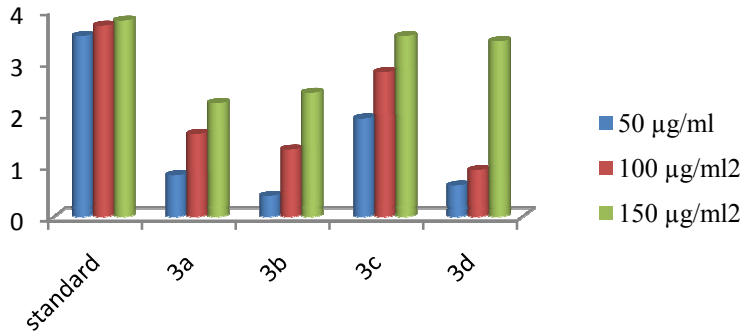
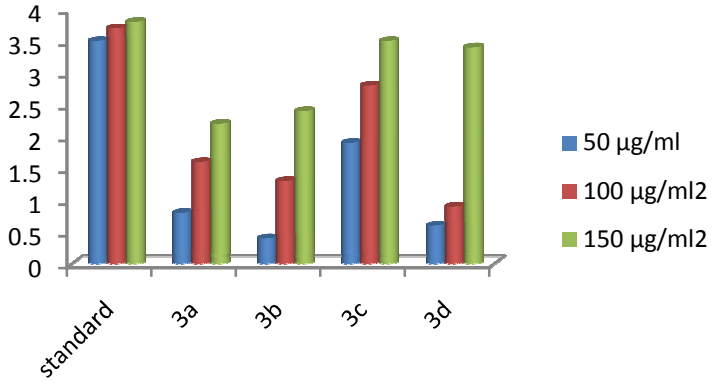


Table.4.6.3
BAR Diagram of Zone of Inhibition *Escherichia coli* (G -ve) microorganism



Discussion on synthesized compounds

Anti microbial study was carried out as per standard procedures and their zone of inhibition was recorded .All the compounds were screened for antimicrobial activity.

In antibacterial study, all the compounds have exhibited minimal activity at the concentration of 1000µg/ml against the two organisms. **3a** shows maximum antibacterial activity towards ***Bacillus*** (Gm +ve) and is effective at 150 µg concentration where as **3c, 3d** shows maximum antibacterial activity towards ***Escherichia coli*** (Gm -ve) and is effective at 150 µg concentration.

Anti-inflammatory activity:

Screening for anti-inflammatory activity was performed for all the chalcone derivatives at dose of 100 mg/kg weight in rats. The drug was administered to the rats, and after 1 hr, the inflammation inducing agent carrageenan was injected into dorsal region of sub plantar surface of hind paw. This allows the evaluation of ability and potency of drug to protect from producing inflammation. The paw volumes were measured after 0.5, 1, 2, 3 and 4 hr of carrageenan administration. Diclofenac at a dose of 100mg/kg was used as standard drug for comparison.

Table No. 3:
Anti-Inflammatory Activity – Mean paw Oedema volume

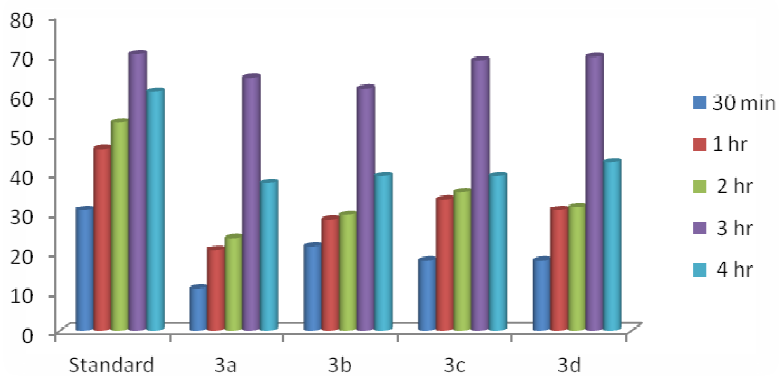
Treatment	Dose mg/kg	MEAN EDEMA VOLUME (ml)				
		30min	1hr	2hr	3hr	4hr
Control	100	0.28±0.002	0.39±0.001	0.51±0.003	0.64±0.002	0.56±0.002
Diclofenac (standard)	100	0.18±0.003	0.21±0.002	0.24±0.001	0.19±0.002	0.22±0.002
3a	100	0.25±0.005	0.31±0.003	0.39±0.001	0.36±0.002	0.35±0.004

3b	100	0.22±0.002	0.28±0.003	0.36±0.002	0.31±0.001	0.34±0.003
3c	100	0.23±0.002	0.26±0.004	0.33±0.001	0.32±0.002	0.34±0.003
3d	100	0.23±0.004	0.27±0.003	0.35±0.005	0.29±0.002	0.32±0.003

Table No. 4:
Anti -Inflammatory activity - Percentage Protection against oedema formation.

Treatment	**Dose mg/kg**	**% Protection**				
		30min	**1hr**	**2hr**	**3hr**	**4hr**
Diclofenac (standard)	100	30.7	46.1	52.9	**70.2**	60.7
3a	100	10.7	20.5	23.5	**64.2**	37.5
3b	100	21.4	28.2	29.4	**61.5**	39.2
3c	100	17.8	33.3	35.2	**68.6**	39.2
3d	100	17.8	30.7	31.3	**69.5**	42.8

Fig. 1: BAR Diagram of Percentage protection against oedema formation



Conclusion on synthesized compounds:
The results obtained in this investigation indicate that **3a** shows maximum antibacterial activity towards ***Bacillus*** (Gm +ve) and is effective at 150 µg concentration where as **3c, 3d** shows maximum antibacterial activity towards ***Escherichia coli*** (Gm -ve) and is effective at 150 µg concentration and the percentage protection against oedema formation with all derivatives, **3d**

was significant and the **3c** derivative showed dose dependent anti-inflammatory activity. From the table it can be observed that the standard drug diclofenac has protected to an extent of 30.7, 46.1, 52.9, 70.2 and 60.7% against inflammation induced by carrageenan at ½, 1, 2, 3rd and 4th hr. Chalcone derivatives have shown maximum activity at 3rd hr. these derivatives have produced less protection than that of standard. From anti-inflammatory studies **3d** showed significant activity to that of standard and **3c** showed moderate activity.

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