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## Synthesis And Biological Evaluation Of Novel Series Of 2, 4, 6-Trimethoxy Chalcones.

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### ABSTRACT

A novel series of 2,4,6-trimethoxy substituted chalcones have been prepared by the Claisen-Schmidt condensation. Firstly, synthesized 2,4,6-trimethoxybenzaldehyde from 3,4,5 trimethoxy benzene and then condense the obtained 2,4,6-trimethoxybenzaldehyde with substituted acetophenone to get different chalcones. The structural interpretations of newly synthesized compounds were based on spectroscopic evidences. All compounds (d1-d10) have been tested for their antimicrobial activities (agar disc-diffusion method) and antioxidant activities (1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method and NO). Among the synthesized compounds, d3, d5 and d8 were found to be moderate active antibacterial and d6 was observed to be active antifungal. Compounds d2, d5 and d6 were found to be moderate active antioxidant.

**Keywords:** chalcones, synthesis, antimicrobial and antioxidant activities.

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## INTRODUCTION

Chalcones (1,3-diphenyl-propene-1-one) are belong to the flavonoid family [1]. Flavonoids are a large family of plant-derived poly- phenolic compounds classified as flavonols, chalcones, aurones, flavanones, isoflavones, flavanols, and they are differencing from each other in their structural group arrangements [2]. Chalcones containing a central 1,3-diphenyl-prop-2-en-1-one structure called as “chalconecore; in which, two phenyl rings are joined by the three-carbon  $\alpha$ ,  $\beta$ -unsaturated carbonyl system [3]. Chalcones containing compounds have become of particular curiosity to chemists and researchers because of their simple synthetic procedure and wide range of different biological activities such as anti-inflammatory [4], antioxidant [5], anticancer [6] antimicrobial [7], antipyretic [8], antianalgesic [9], antimalarial [10], antileishmanicidal [11] and anti-allergic activities [12]. Different substituted chalcones are available in the market with versatile biological activity like, 4-hydroxy, 2,4 dihydroxy, methyl, methoxy, methoxy amino[13-19]. In spite of these substituted chalcone, methoxy substituted chalcones (3-methoxy, 4-methoxy, 2,4 dimethoxy, 2,4,5-trimethoxy and 3,4,5 trimethoxy) have wide spread of biological activity like, anticancer, antimicrobial, antioxidants and anti-inflammatory [20-22]. The literature survey revealed that no attempts were made towards the synthesis of 2,4,6 trisubstituted chalcones from 2,4,6-trimethoxybenzaldehyde and to verify its effects on the biological activity. Considering these facts, we are synthesized novel 2,4,6-trimethoxy substituted chalcones from 2,4,6 trimethoxy benzaldehyde and substituted acetophenone to get different chalcones having antibacterial, antifungal and antioxidant activity.

## MATERIALS AND METHODS

Instrumentation, (TLC) on silica gel plates (Merck 60 F245) using cyclohexane/ethyl acetate (7:3 v/v) as eluent. Column chromatography was performed with Merck 60 silica gel (0.040-0.063 mm, 230-400 mesh) or <sup>1</sup>H NMR spectra were acquired on a Bruker DRX400 spectrometers in CDCl<sub>3</sub> or DMSO as solvent (unless otherwise stated). Chemical shifts were reported in parts per million (ppm) relative to the solvent peak (CDCl<sub>3</sub>, 7.26 ppm and DMSO, 1.2 ppm). <sup>13</sup>C NMR spectra were recorded on Bruker 300 MHz. Melting points were determined in open capillaries with electrical melting point apparatus.

## EXPERIMENTAL SECTION

### Procedure for the synthesis of 2,4,6-trimethoxybenzaldehyde

Phosphorus oxychloride (1.38 mol) was added in solvent dimethylformamide (2.5 vol) at 10-20°C. 1,3,5-trimethoxybenzene was added lot wise in the corresponding mixture. The reaction mixture was heated to a temp. 75-80 °C for 1 h and reaction progress is monitored on TLC; then allowed to cool to room temperature and quenched product in water and adjusted pH to neutral by using sodium carbonate. The obtained crystals were filtered off and washed water with water. The obtained 2,4,6-trimethoxybenzaldehyde crude was re-crystallized from methanol. Yield: 55.4%. m.p. 218-220°C. C<sub>10</sub>H<sub>12</sub>O<sub>4</sub>: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): <sup>1</sup>H NMR: 3.75 (3H, s), 3.76 (6H, s), 6.11 (2H, d), 8.85 (1H, s), <sup>13</sup>C NMR (CDCl<sub>3</sub>, 300 MHz): 56, 90, 55, 162, 164, 108, 186. MS m/z: 197.2 (M+1).

### General procedure for the preparation of chalcones (d)

To a stirred solution of the sodium hydroxide (1mol) in methanol (5 vol), (1mol) 2,4,6-trimethoxybenzaldehyde, followed by substituted acetophenone (1mol) were added at 25 to 35°C. The reaction mixture was stirred at 30-35°C for 3-4hr (monitored by TLC). The reaction was, quenched by the addition chilled water (10 vol) to get yellow precipitate. The reaction was stirred at same temperature for 1h and filtered off and wash with water to get crude product. Crude product was suspended in the methanol (5 vol) to reflux temperature for 1h and then filter at ambient temperature. Product was washed with methanol to get pure dried product.

### Preparation of 1-(3-methoxyphenyl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one(d1).

General procedure mentioned in example (d), 2,4,6-trimethoxybenzaldehyde condensation with (1.0 mole) 3-methoxy acetophenone. Yield: 73-74%, m.p. 151-153°C, C<sub>19</sub>H<sub>20</sub>O<sub>5</sub> <sup>1</sup>H NMR:  $\delta$  3.74 (3H, s), 3.78 (3H, s),

3.84 (6H, s), 6.17 (2H, d), 6.30 (1H, d), 6.96 (1H, dd), 7.42 (2H, dd), 7.59 (1H, dd), 7.79 (1H, dd).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 56, 92, 55, 157, 110, 140, 122, 189, 140, 112, 160, 130, 120. MS m/z: 329.35(M+1).

#### Preparation of 1-(3,4-dimethoxyphenyl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one(d2)

General procedure mentioned in example (d), 2,4,6-trimethoxybenzaldehyde condensation with (1.0 mole) 3,4 dimethoxy acetophenone. Yield: 74.5%, m.p. 169-171°C,  $\text{C}_{20}\text{H}_{22}\text{O}_6$   $^1\text{H}$  NMR:  $\delta$  3.78 (3H, s), 3.81 (3H, s), 3.87 (6H, s), 3.93 (3H, s), 6.18 (2H, d), 6.27 (1H, d), 6.88 (1H, dd), 7.42 (1H, d), 7.49 (1H, dd), 7.57 (1H, dd).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 56, 92, 55, 157, 110, 140, 122, 189, 133, 110, 150, 149, 110, 121. MS m/z: 359.32 (M+1).

#### Preparation of 1-phenyl-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one(d3)

General procedure mentioned in example (d), 2,4,6-trimethoxybenzaldehyde condensation with (1.0 mole) acetophenone. Yield: 80.5%, m.p.: 108-110°C,  $\text{C}_{18}\text{H}_{18}\text{O}_4$   $^1\text{H}$  NMR:  $\delta$  3.79 (3H, s), 3.86 (6H, s), 6.17 (2H, d), 6.28 (1H, d), 7.40-7.57 (4H, dddd), 7.76 (2H, ddd).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 56, 92, 55, 157, 110, 140, 122, 189, 136, 127, 128, 129. MS m/z: 300.3(M+2).

#### Preparation of 1-(3-Nitro phenyl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one(d4)

General procedure mentioned in example (d), 2,4,6-trimethoxybenzaldehyde condensation with (1.0 mole) 3-nitro acetophenone. The compound was crystallized in methanol. Yield: 83%, M.P. : 159-160°C,  $\text{C}_{18}\text{H}_{17}\text{NO}_6$ :  $^1\text{H}$  NMR:  $\delta$  3.79 (3H, s), 3.86 (6H, s), 6.17 (2H, d), 6.28 (1H, d), 7.40-7.59 (3H, 7.54 (ddd), 7.76 (2H, dd).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 56, 92, 55, 157, 110, 140, 122, 186, 136, 127, 128, 129. MS m/z: 344.38(M+1).

#### Preparation of 1-(4-methoxyphenyl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (d5)

General procedure mentioned in example (d), 2,4,6-trimethoxybenzaldehyde condensation with (1.0 mole) 4-methoxy acetophenone. The compound was crystallized in methanol. Yield: 84.3%, m.p. 146-147°C,  $\text{C}_{19}\text{H}_{20}\text{O}_5$   $^1\text{H}$  NMR:  $\delta$  3.79 (3H, s), 3.84 (3H, s), 3.87 (6H, s), 6.17 (2H, d), 6.30 (1H, d), 7.05 (2H, ddd), 7.41 (1H, d), 7.55 (2H, ddd),  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 56, 92, 55, 157, 110, 140, 122, 189, 133, 130, 114, 160. MS m/z: 329.35(M+1).

#### Preparation of 1-(4-fluorophenyl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one(d6)

General procedure mentioned in example (d), 2,4,6-trimethoxybenzaldehyde condensation with (1.0 mole) 4-fluoro acetophenone. Yield: 78%, m.p.: 151-152°C,  $\text{C}_{18}\text{H}_{17}\text{FO}_4$   $^1\text{H}$  NMR:  $\delta$  3.79 (3H, s), 3.87 (6H, s), 6.17 (2H, d), 6.30 (1H, d), 7.37-7.46 (3H, 7.41 (ddd), 7.62 (2H, ddd).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 56, 92, 55, 157, 110, 140, 122, 189, 133, 131, 115, 163. MS m/z: 317.1 (M+1), 318.23(M+2), 339.16(M+23).

#### Preparation of 1-(4-methylphenyl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (d7)

General procedure mentioned in example (d), 2,4,6-trimethoxybenzaldehyde condensation with (1.0 mole) 4-methyl acetophenone. Yield: 80%, m.p.: 131-133°C,  $\text{C}_{19}\text{H}_{20}\text{O}_4$   $^1\text{H}$  NMR:  $\delta$  2.31 (3H, s), 3.79 (3H, s), 3.85 (6H, s), 6.17 (2H, d), 6.29 (1H, d), 7.14 (2H, ddd), 7.44 (1H, d), 7.78 (2H, ddd).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 56, 92, 55, 157, 110, 140, 122, 189, 137, 128, 130, 140, 22. MS m/z: 314.35(M+2).

#### Preparation of 1-(4-bromophenyl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one(d8)

General procedure mentioned in example (d), 2,4,6-trimethoxybenzaldehyde condensation with (1.0 mole) 4-bromo acetophenone. Yield: 74.8%, m.p.: 153-154°C,  $\text{C}_{18}\text{H}_{17}\text{BrO}_4$   $\delta$  3.79 (3H, s), 3.87 (6H, s), 6.17 (2H, d), 6.26 (1H, d), 7.42 (1H, d), 7.64 (2H, ddd), 7.81 (2H, ddd).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 56, 92, 55, 157, 110, 140, 122, 189, 136, 132, 124. MS m/z: 377.23 (M), 379.3(M+2).

#### Preparation of 1-(4-chlorophenyl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one(d9)

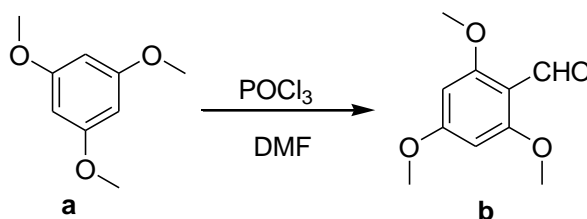
General procedure mentioned in example (d), 2,4,6-trimethoxybenzaldehyde condensation with (1.0 mole) 4-chloro acetophenone. Yield: 70%, m.p.: 156-157°C,  $\text{C}_{18}\text{H}_{17}\text{ClO}_4$   $\delta$  3.79 (3H, s), 3.87 (6H, s), 6.17 (2H, d),

6.26 (1H, d), 7.42 (1H, d), 7.64 (2H, ddd), 7.81 (2H, ddd).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 56, 92, 55, 157, 110, 140, 122, 189, 136, 130, 131, 136. MS m/z: 333.81(M+1).

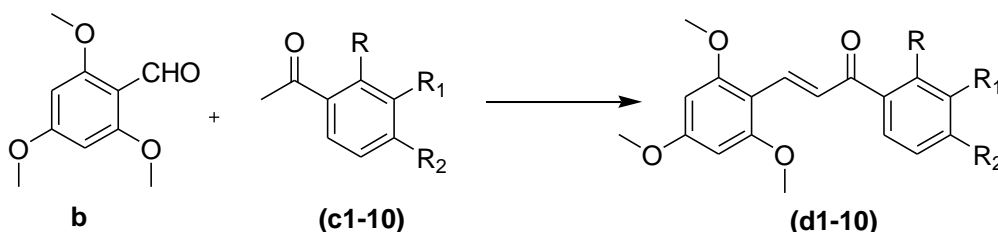
### Preparation of 1-(2,4-dichlorophenyl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one(d10)

General procedure mentioned in example (d), 2,4,6-trimethoxybenzaldehyde condensation with (1.0 mole) 2,4-dichloro acetophenone. Yield: 80.0%, m.p.: 178-180°C,  $\text{C}_{18}\text{H}_{16}\text{Cl}_2\text{O}_4$   $^1\text{H}$  NMR:  $\delta$  3.79 (3H, s), 3.88 (6H, s), 6.17 (2H, d), 6.28 (1H, d), 7.43 (1H, d), 7.73-7.81 (2H, dd), 7.90 (1H, dd).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 56, 92, 55, 157, 110, 140, 122, 192, 136, 132, 130, 135, 127. MS m/z: 368.31(M+1).

### Reaction Scheme



Scheme I: Preparation of 2,4,6-trimethoxybenzaldehyde



**R, R<sub>1</sub>, R<sub>2</sub> = Hydrogen, Methyl, Methoxy, Halide, Amine**

Scheme II: Preparation of substituted 2,4,6-trimethoxy chalcones

A detail of substituted groups are mentioned below in table no. 1

Table 1: 2, 4, 6 trimethoxy chalcones (d1-d10)

Sr. No.	Sample code	R	R <sub>1</sub>	R <sub>2</sub>
1	d1	H	OCH <sub>3</sub>	H
2	d2	H	OCH <sub>3</sub>	OCH <sub>3</sub>
3	d3	H	H	H
4	d4	H	NO <sub>2</sub>	H
5	d5	H	H	OCH <sub>3</sub>
6	d6	H	H	F
7	d7	H	H	CH <sub>3</sub>
8	d8	H	H	Br
9	d9	H	H	Cl
10	d10	Cl	H	Cl

### BIOLOGICAL ACTIVITY

#### Antibacterial and antifungal activity evaluation

Antibacterial activity of the various synthesized compounds was evaluated by pour plate method. Briefly, the test compounds were dissolved in dimethyl sulphoxide (DMSO) to produce 1 mg/ml stock solutions. All bacterial strains were thawed, then bacteria, *Staphylococcus aureus* in soya broth. *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* in nutrient and *Candida albicans* (ATCC 1023) in potato

dextrose agar medium were cultured respectively. Broth solution was prepared in bacterial cultures on media at  $37 \pm 2$  °C. The bacterial inoculum was uniformly spread using sterile cotton swab on a sterile Petri dish agar. To this Petri dish, 50 µL of test compounds at various concentrations (0.1 to 1000 µg/ml) were added to each of the 5 wells (7 mm diameter holes cut in the agar gel, having distance of 20-30 mm from another hole). The plates were incubated for 24 h at  $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ , under aerobic conditions. After incubation, confluent bacterial growth was observed. The zone of inhibition in mm was measured for the test compound and recorded. From these values, the area of inhibition was calculated. Streptomycin and gentamicin were used as standard antibacterial agents for comparison [23]

### Minimum Inhibitory concentration (MIC)

The antibacterial activity of test compounds was determined by microdilution method. Test compounds were dissolved in DMSO. The 96 well plates was numbered as per the test compounds and the microorganism used, to which 50µl of various concentrations of test compounds were added to this, 50µl of test organism suspension was added. To this inoculated broth was added. The plate was sealed and incubated for 24 hr at room temperature.

### RESULTS

Our test compounds mainly d3, d5 and d8 exhibited significant antibacterial activity against both gram-positive and gram-negative bacteria. Inhibitory concentration (IC50) of d3, d5 and d8 were found to be 55, 470 and 60µg/ml against *S. aureus*; 250, 228 and 326µg/ml against *B. subtilis*; respectively and Inhibitory concentration (IC50) of d2 was found to be 370 µg/ml against *E. Coli*. Thus, it is possible that these test compounds showed moderate antibacterial activity against these microorganism. Furthermore, minimum inhibitory concentrations of these compounds were also found to have significant compared to control plates found as shown in table 2. Therefore, it is possible that compound d3, d5 and d8 have antibacterial potential against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. For antifungal activity, compounds d6 exhibited moderate activity against *Candida albicans* (ATCC 1023) strain as shown in table no.2.

**Table 2: Antimicrobial activity of the compounds (d1-d10)**

Test compounds	S. aureus		B. subtilis		P. auroginosa		E. Coli		Candida albicans	
	IC50	MIC	IC50	MIC	IC50	MIC	IC50	MIC	IC50	MIC
(d1)					-	-			-	-
(d2)					-	-	370	1000	-	-
(d3)	55	50	250	50	-	-			-	-
(d4)					-	-			-	-
(d5)	470	500	228	500	-	-			-	-
(d6)					-	-			2475	500
(d7)										
(d8)	60	100	326	100	-	-			-	-
(d9)					-	-			-	-
(10)					-	-				
Stretomycin	<b>0.65</b>	<b>0.5</b>	<b>0.71</b>	<b>2</b>	-	-	-	-	-	-
Gentamycin	-	-	-	-	<b>11</b>	<b>1</b>	<b>14</b>	<b>1</b>	-	-
Griseofulvin	-	-	-	-	-	-	-	-	<b>67</b>	<b>1</b>

### Antioxidant activity

#### 1,1-diphenyl-2-picrylhydrazyl (DPPH) Scavenging activity

The free radical scavenging activity of test compounds were determined by DPPH scavenging method as per procedure described earlier by Shen [24]. In this method, 0.1mM DPPH solution was prepared in methanol by adding 39.4 mg of DPPH in 1000 ml of methanol, and to 0.5 mL of this solution, 1.5 mL of test compounds of the dissolved in DMSO were added at various concentrations of all (1, 10, 100, 500 & 1000

µg/mL). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Shimadzu, spectrophotometer). Vitamin C was used as standard compound. Reduction in absorbance by test compounds and indicates radical scavenging activity. The scavenging activity by the DPPH radical was determined by

$$\text{DPPH scavenging effect (\% inhibition)} = \{(A_0 - A_1)/A_0\} \times 100$$

Where, A<sub>0</sub> is the absorbance of the control reaction, and A<sub>1</sub> is the absorbance test compound and vitamin C.

**Nitric oxide (NO) radical scavenging Activity**

Nitric oxide (NO) radical scavenging activity of various test compounds was determined as per the procedure described by Balakrishnan[25]. Briefly, various concentrations of test compounds (as 1, 10, 100, 500, and 1000 µg/ml) were prepared in ethanol. To 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline, to this, 1 ml of various concentrations of test compounds were mixed, and to this equal volume of freshly prepared Griess reagent was added, solution was then incubated at 25°C for 3 hours. Form this, 100 µl of the reaction mixture was transferred to a 96-well plate, and the absorbance was read at 546 nm using a microplate reader (Biotek, Italy). Ascorbic acid was used as standard control.

The percentage of nitrite radical scavenging activity of test compounds was calculated by

$$\text{Nitric oxide scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test compounds} \times 100}{\text{Absorbance of control}}$$

**Table 3: Antioxidant activity of the compounds (d1-d10)**

Test compounds	Concentration (µg/mL)	IC50 (µg/mL)	
		DPPH	NO
d1	1-1000		
d2	1-1000	134	131
d3	1-1000		
d4	1-1000		
d5	1-1000	174	56
d6	1-1000	305.5	10
d7	1-1000		
d8	1-1000		
d9	1-1000		
d10	1-1000	326	307
<b>Ascorbic Acid</b>	1-1000	<b>6.1</b>	<b>6.4</b>

Antioxidant potential of synthesized compounds scavenged DPPH radicals by providing hydrogen atom or electron donation and decreases characteristic absorption of radical DPPH at 517 nm. A decreased absorbance at 517 nm suggested as higher radical scavenging property of test compounds. The scavenging effects of the test compounds and ascorbic acid on the DPPH radicals were calculated as half maximal inhibitory concentration (IC<sub>50</sub>) values. The potent free radical scavenging activity elicited by test compounds, d2 (IC<sub>50</sub> 134 µg/mL), d5 (IC<sub>50</sub> 174µg/mL), d6 (305.5 µg/mL) and d10 (326 µg/mL) whereas ascorbic acid showed the highest DPPH radical scavenging activity (IC<sub>50</sub> 6.1 µg/mL).

In NO scavenging activity, test compounds d2 (IC<sub>50</sub> 131 µg/mL), d5 (IC<sub>50</sub> 56µg/mL), d6 (10 µg/mL), d10 (307 µg/mL) and standard ascorbic acid showed highest scavenging activity i.e. 6.5 µg/mL .Thus, it can be concluded that, compounds mainly d2, d5, d6 and d10 exhibited significant antioxidant activity by scavenging free radicals generated in DDPH and NO method.

**CONCLUSION**

A novel series of 2,4,6-trimethoxy substituted chalcones have been prepared by the Claisen-Schmidt condensation. Their structures were identified using  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and MS spectral data. The entire target compounds were also investigated for their antioxidant and antimicrobial potential. Considering both the antimicrobial and the antioxidant evaluation, compounds exhibited the moderate effect. The present investigation has provides impetus for development of more potent 2,4,6-trimethoxy substituted chalcone derivatives with different biological activity.

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