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# Synthesis and in vitro study of antibacterial activity of some novel chalcones

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**Abstract :** Some chalcones derivatives have been synthesized and their structures have been confirmed by IR, $^1$H NMR, and Mass spectral data. The antibacterial activity of these synthesized Chalcones has been studied against some Gram positive and Gram negative bacteria in dimethyl sulphoxide using agar well diffusion method. It is observed that inhibition depends on compound structure and bacterial strain.


**Keywords :** Chalcone derivatives; Dimethyl sulphoxide; Antibacterial activity.

## INTRODUCTION

Biological activity is an expression describing the beneficial or adverse effects of a drug on living matter. Biological activity spectrum of a compound represents the pharmacological effects, physiological and biochemical mechanisms of action, specific toxicity which can be revealed in compound's interaction with biological systems.

The chalcones and their derivatives are an interesting class of compounds which are extensively investigated due to their biological and industrial applications. Many of these have been used as important intermediates in organic synthesis[1,2]. They serve as starting material for the synthesis of variety of heterocyclic compounds which are of physiological importance. The compounds with the backbone of chalcones have been reported to possess various biological activities such as antimicrobial[3-5], antiinflammatory[6,7], antifungal[8,9], antioxidant[10, 11], antileishmanial[12], antimalarial[13,14], antituberculosis[15,16], analgesic[17], anti HIV[18], antioxidant[19], antitumor[20, 21] etc.

In the present work, some new Chalcone compounds have been synthesized and their structure confirmation was done by IR, $^1$H NMR, and mass spectral data. The antibacterial activity of these compounds was studied in dimethyl sulphoxide.

## EXPERIMENTAL

### Synthesis

Synthesis of 3,-methoxy4-ethoxy benzaldehyde: An aqueous solution of 4-hydroxy, 3-methoxy benzaldehyde was refluxed at 95-97$^0$C for half an hour with stirring and to this solution, few drops of sodium hy-

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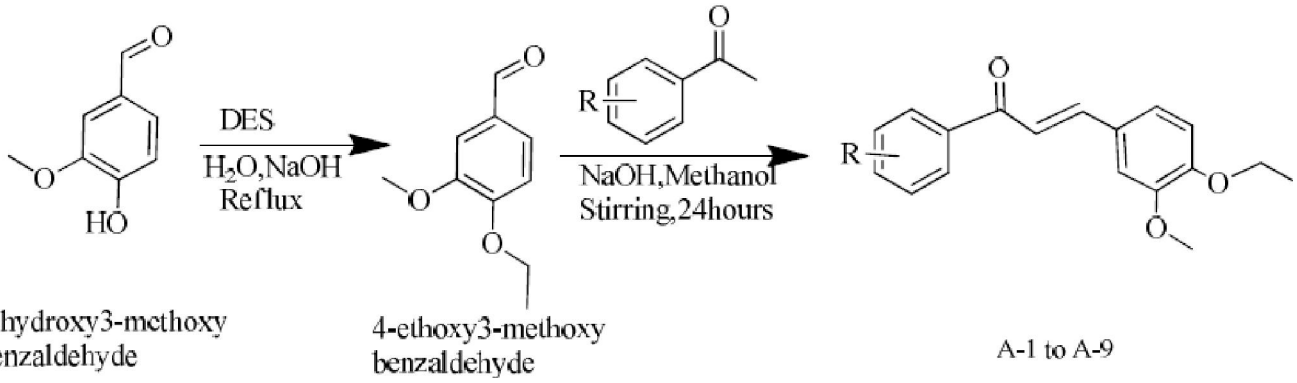


droxide and diethyl sulphate were added. The reaction mixture was refluxed for 5 to 7 hours with stirring. The organic layer was isolated and was cooled at room temperature. The solid crude product was isolated and crystallized from absolute ethanol.

Synthesis of chalcone derivatives: A mixture of 3-methoxy 4-ethoxy benzaldehyde and substituted acetophenone in methanol was stirred for 24 hours in presence of few drops of sodium hydroxide solution. The product was filtered and dried. The recrystalisation was done in ethanol.

Overall, nine Chalcones (A-1 to A-9) were synthesized and the reaction is as under:

All the synthesized compounds were recrystallized. The purity of the synthesized compounds was checked by Thin Layer Chromatography. The melting points of compounds were taken by open capillaries.

The characterization of synthesized compounds was done by IR, $^1$H NMR and mass spectral data. The IR spectra were recorded by SHIMADZU-FTIR-8400 Spectrophotometer in the frequency range of 4000-400cm$^{-1}$ by KBr powder method. The NMR spectra were recorded by BRUKER Spectrometer (400 MHz) using internal reference TMS and solvent CDCl3/DMSO. The chemical shifts are reported as parts per milloian (ppm). The Mass spectra were recorded by GCMS-SHIMADZU-QP2010.

### Biological Activity

For all the compounds, agar well diffusion method was used.

### Microorganism used

Gram positive bacteria *Streptococcus pyogenes* (NCIE 1925), *Bacillus subtilis* (ATCC 2274) and Gram negative bacteria viz. *proteus mirabilis* (NCIM 2241), *salmonella typhimurium* (ATCC 23564) were obtained from National Chemical Laboratory (NCL), Pune, India and were maintained at 4°C on nutrient agar slants.

### Preparation of test compounds

The solutions were prepared at a concentration of 1 mg/µl for all the compounds.

### Preparation of the plates and microbiological assay

The antibacterial evaluation was done by agar well diffusion method using Mueller Hinton Agar No.2 as the nutrient medium. The bacterial strains were activated by inoculating a loop full of test strain in 25 ml of N-broth and the same was incubated for 24 hours in an incubator at 37°C. 0.2 ml of the activated strain was inoculated in Mueller Hinton Agar kept at 45°C. It was then poured in the Petri dishes and was allowed to solidify. After solidification of the media, 0.85 cm ditch was made in the plates using a sterile cork borer and these were completely filled with the test solution. The plates were incubated for 24 hours at 37°C. The mean value obtained for the three wells was used to calculate the zone of growth inhibition of each sample. The controls were maintained for each bacterial strain and each solvent. The inhibition zone formed by these compounds against the particular test bacterial strain determined the antibacterial activities of these synthesized compounds.

## RESULTS AND DISSCUSSION

TABLE 1 shows physical parameters of the synthesized compounds.

### Spectral data

# ORIGINAL ARTICLE

**TABLE 1 : Physical constant of chalcones**

| Sr No. | Code | -R | M.F. | M.Wt. (g/mol) | M.P.$^0$C | Yield % |
|---|---|---|---|---|---|---|
| 1 | A-1 | 4 –Cl | $C_{18}H_{17}ClO_3$ | 316 | 153 | 73 |
| 2 | A-2 | 4 -$NO_2$ | $C_{18}H_{17}NO_5$ | 327 | 153 | 59 |
| 3 | A-3 | -H | $C_{18}H_{18}O_3$ | 282 | 132 | 66 |
| 4 | A-4 | 4-$CH_3$ | $C_{18}H_{20}O_3$ | 296 | 124 | 67 |
| 5 | A-5 | 4-$OCH_3$ | $C_{18}H_{20}O_4$ | 312 | 151 | 73 |
| 6 | A-6 | 4 Br | $C_{18}H_{17}BrO_3$ | 361 | 134 | 57 |
| 7 | A-7 | 4-OH | $C_{18}H_{18}O_4$ | 298 | 133 | 59 |
| 8 | A-8 | 2.4 –$OCH_3$ | $C_{20}H_{22}O_5$ | 343 | 129 | 64 |
| 9 | A-9 | 3-Cl | $C_{18}H_{17}ClO_3$ | 316 | 170 | 75 |

**A-1**

IR (KBr, cm$^1$): 1512.32 (C=C stretching), 1653.73 (C=O stretching), 1232.54 (C-O-C stretching), 2958 (C-H stretching), 844 (C-Cl stretching), $^1$H NMR (BRUCKER Spectrometer 400 MHz DMSO-$d_6$, δppm): 3.34(3H, s, -$OCH_3$), 4.01-4.08(2H, q, -$OCH_2$), 1.30-1.35 (3H, t, -$CH_3$), 6.96-6.99 (1H, d, aromatic), 7.34-7.37 (1H, d, aromatic), 7.53 (1H, s, aromatic), 8.14-8.17 (2H, s, -CH-CH), 7.60-7.63 (2H, d, aromatic), 7.72-7.83 (2H, d, aromatic); MS: (m/z): 316 (M$^+$ BP, 100 %), 288, 282, 254, 180, 155;

**A-2**

IR (KBr, cm$^1$): 1495 (C=Cstretching), 1678 (C=Ostretching), 1248 (C-O-Cstretching), 2953 (C-Hstretching),1318 (C-N stretching); $^1$H NMR (BRUCKER Spectrometer 400 MHz DMSO-$d_6$, δ ppm): 3.28(3H, s, -$OCH_3$), 3.98-4.05 (2H, q, -$OCH_2$), 1.32-1.37 (3H, t, -$CH_3$), 6.92-7.03 (1H, d, aromatic), 7.32 (1H, d, aromatic), 7.33 (1H, s, aromatic), 7.86-8.18 (2H, s, -CH-CH), 7.93(2H, d, aromatic), 8.02 (2H, d, aromatic); MS: (m/z): 327 (M$^+$ BP,100 %), 298, 281, 176, 151;

**A-3**

IR(KBr, cm$^1$): 1488.64 (C=Cstretching), 1682.32 (C=O stretching), 1257 (C-O-Cstretching), 2955 (C-H asymmetrical stretching), $^1$H NMR (BRUCKER Spectrometer 400 MHz DMSO-$d_6$, δ ppm): 3.23 (3H, s, -$OCH_3$), 4.05-4.10 (2H, q, -$OCH_2$), 1.23-1.28 (3H, t, -$CH_3$), 7.01-7.03 (1H, d, aromatic), 7.40-7.43 (1H, d, aromatic), 7.58 (1H, s, aromatic), 8.24-8.27(2H, s, -CH-CH), 7.60-7.63 (2H, d, aromatic), 7.65-7.73 (2H, d, aromatic), 7.59 (1H, s, aromatic); MS: (m/z): 282 (M$^+$BP,100%), 282, 267, 253, 237, 151, 131;

**A-4**

IR (KBr, cm$^{-1}$): 1522.31 (C=Cstretching), 1642.47 (C=Ostretching), 1269.05 (C-O-Cstretching), 2922 (C-Hstretching), 2953 (C-Cretching), $^1$H NMR (BRUCKER Spectrometer 400 MHz DMSO-$d$ δ ppm): 3.29 (3H, s, -$OCH_3$), 4.03-4.10 (2H, q, $OCH_2$), 1.21-1.32 (3H, t, $CH_3$), 6.90-6.96 (1H, d, aromatic), 728-7.32 (1H, d, aromatic), 7.49 (1H, s, aromatic), 8.12-8.15 (2H, s, -CH-CH), 7.58-7.61 (2H, d, aromatic), 7.55-7.62 (2H, d, aromatic); 2.52 (3H, d, -$CH_3$); MS: (m/z): 296 (M$^+$ BP,100 %), 281, 267, 251, 151;

**A-5**

IR(KBr, cm$^1$): 1512.32 (C=Cstretching), 1665.14 (C=Ostretching), 1273.05 (C-O-Cstretching), 2977 (C-Hstretching), 1276 (C-O-Cstretching); $^1$H NMR (BRUCKER Spectrometer 400MHz DMSO-$d_6$, δ ppm): 3.26 (3H, s, -$OCH_3$), 3.96-4.03 (2H, q, -$OCH_2$), 1.33-1.38 (3H,t, $CH_3$), 6.90-6.93 (1H, d, aromatic), 7.30-7.34 (1H, d, aromatic), 7.58 (1H, s, aromatic), 8.13-8.16 (2H, s, -CH-CH), 7.63-7.66 (2H, d, aromatic), 7.90- 7.93 (2H,d, aromatic), 3.93 (3H, s, -$OCH_3$); MS: (m/z): 312(M$^+$BP,100%), 283, 281, 197, 168, 151;

**A-6**

IR(KBr,cm$^1$): 1524.85 (C=Cstretching), 1654.84 (C=Ostretching), 1248.21 (C-O-Cstretching), 2967 (C-Hstretching), 778 (C-Br stretching); $^1$H NMR (BRUCKER Spectrometer 400 MHz DMSO-$d_6$, δ ppm): 3.23 (3H, s, -$OCH_3$), 4.05-4.10 (2H, q, $OCH_2$), 1.23-1.28 (3H,t, $CH_3$), 7.01-7.03 (1H, d,aromatic), 7.40-7.43 (1H, d, aromatic), 7.58 (1H, s, aromatic), 8.24-8.27 (2H, s, -CH-CH), 7.60-7.63 (2H, d, aro-

ORIGINAL ARTICLE

matic), 7.65-7.73 (2H,d, aromatic), 7.59 (1H, s, aromatic); MS: (m/z): 361 ($M^+$BP,100 %), 345, 331, 329, 208, 151;

**A-7**

IR (KBr,cm$^1$): 1502.32 (C=Cstretching), 1662.14 (C=Ostretching), 1270.05 (C-O-Cstretching), 2973 (C-H stretching), 3412(O-H stretching); $^1$H NMR (BRUCKER Spectrometer 400 MHz DMSO-$d_6$, δppm): 3.44 (3H, s, -$OCH_3$), 4.03-4.12 (2H, q, -$OCH_2$), 1.38-1.42 (3H, t $CH_3$), 7.02-7.10 (1H, d, aromatic), 7.25-7.28(1H, d, aromatic), 7.50 (1H, s, aromatic), 8.16-8.20 (2H, s, -CH-CH), 7.58-7.68 (2H, d, aromatic), 7.75-7.86 (2H, d, aromatic), 5.40 (1H, s, -OH); MS: (m/z): 281 ($M^+$ BP,100 %), 269, 205, 151, 157;

**A-8**

IR (KBr,cm$^1$): 1518.12 (C=C stretching), 1677.65 (C=O stretching), 1272.42 (C-O-C stretching), 2912 (C-H stretching), 1231 (C-O-C stretching); $^1$H NMR (BRUCKER Spectrometer 400 MHz DMSO-$d_6$, δ ppm): 3.32 (3H, s, -$OCH_3$), 4.15-4.21 (2H, q, -$OCH_2$), 1.28-1.32 (3H, t, $CH_3$), 7.03-7.12 (1H, d, aromatic), 7.34-7.37 (1H,d,aromatic), 7.53 (1H, s, aromatic), 8.20-8.24 (2H, s, -CH-CH), 7.58-7.62 (1H, d, aromatic), 7.72-7.83(2H, d, aromatic), 7.70-7.75 (1H, s, aromatic), 3.88 (3H, s, -$OCH_3$), 3.83 (3H, s, -$OCH_3$) ; MS: (m/z): 327 ($M^+$ BP,100 %), 297, 280, 311, 151;

**A-9**

IR(KBr,cm$^1$): 1498.64 (C=Cstretching), 1679.12 (C=Ostretching), 1243.65 (C-O-Cstretching), 2970 (C-H stretching), 776(C-Cl stretching); $^1$H NMR (BRUCKER Spectrometer 400 MHz DMSO-$d_6$, δ ppm): 3.43 (3H, s, -$OCH_3$), 4.13-4.19 (2H, q, -$OCH_2$), 1.26-1.31(3H, t, -$CH_3$), 6.84-6.92 (1H, d, aromatic), 730-7.35(1H, d, aromatic), 7.47(1H, s,aromatic), 8.12-8.16(2H,s, -CH-CH), 7.60-7.63 (1H, d, aromatic), 7.72-7.83(2H, d, aromatic) 7.79-7.83(1H, t, aromatic), 7.65 (1H, d, aromatic), 7.52 (1H, s, aromatic); MS:(m/z):327 ($M^+$ BP,100 %),288, 282, 254, 180, 155;

**Biological activity**

Figure 1 shows zone of inhibition against Gram positive bacteria in DMSO. It is observed from Figure 1 [A] that against *Streptococcus pyogenes*, A-9 showed maximum inhibition whereas A-2 and A-3 showed minimum inhibition. A-1, A-4 and A-5 showed no inhibition at all. Other compounds exhibited intermediate inhibition. For *Bacillus subtilis*, all the compounds showed inhibition and maximum inhibition is observed by A-9. Whereas A-1, A-3 and A-4 exhibited minimum inhibition. Thus, for both the selected Gram positive bacterial strains, A-9 is the most effective compound.

Figure 2 shows inhibition against Gram negative bacteria in DMSO. It is observed that against both *Proteus mirabilis*, A-5 showed maximum inhibition whereas A-4 had no effect. For *Salmonella typhimurium*, A-8 showed maximum inhibition whereas A-2 exhibited minimum inhibition. There is no effect of A-6 and A-4 on this strain.

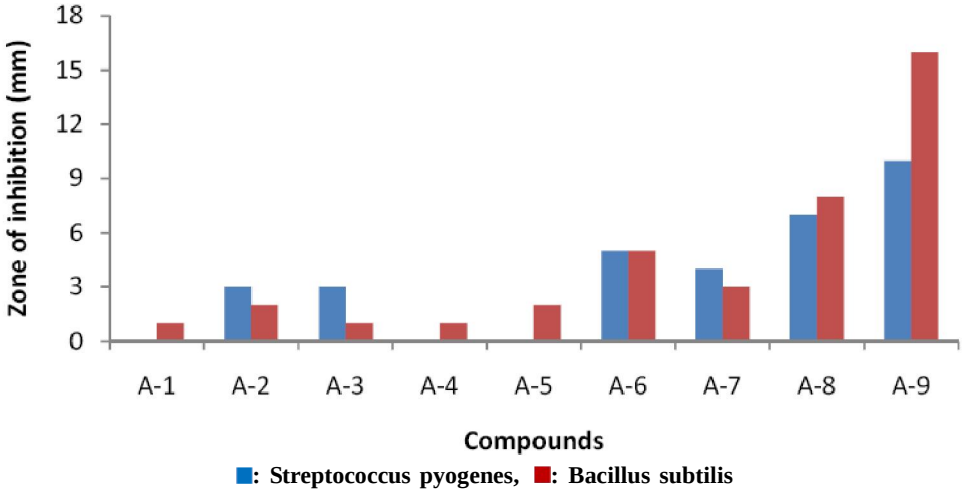


**Figure 1 : Antibacterial activity of chalcones against Gram positive bacteria**

ORIGINAL ARTICLE

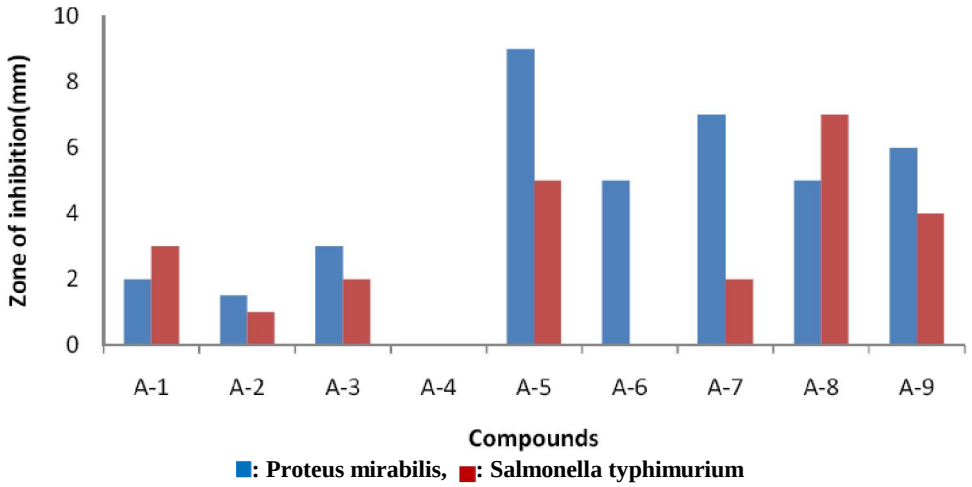


**Figure 2 : Antibacterial activity of chalcones against Gram negative bacteria in DMSO.**

The inhibition depends on solvent, compound structure and bacterial strain. In the present study, only one solvent is selected. A-9 contains 3-chloro group, which is found to be effective for both Gram positive bacteria viz *Streptococcus pyogenes* and *Bacillus subtilis*. A-1 also contains chloro group but at 4th position. But, this compound had no effect on *Streptococcus pyogenes*. This suggests that the position of group also plays an important role in inhibition. The 4-methyl group (as in A-4) and 4-methoxy group (as in A-5) could not affect *Streptococcus* pyogenes.

However, for Gram negative bacteria, 4-methoxy group present in A-5 is most effective against *Proteus mirabilis* whereas for *Salmonella typhimurium*, 2, 4-dimethoxy group present in A-8 is found to most effective. Again, position of group effects inhibition. A-4 containing 4-methyl group had no effect on both the selected Gram negative bacterial strains. Further, *salmonella typhimurium* could not be affected by 4-bromo group which is present in A-6.

Thus, it can be concluded that for the studied chalcone derivatives, for Gram positive bacteria the chloro group showed maximum inhibition provided, it was at 3-position while the chloro group at 4th position decreased the inhibition. However, for Gram negative bacteria, methoxy group showed best activity at 4th position. But when methoxy groups are at 3rd and 4th positions, inhibition is decreased.

## CONCLUSION

The type and position of substitution affects the inhibition to a larger extent. For the studied compounds, 3-chloro group is most effective against Gram positive bacteria whereas 4-methoxy group is most effective against Gram negative bacteria.

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