Synthesis Characterization and Antibacterial Activity of 4-Ethynyl Chalcone Derivatives

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Abstract: Chalcones are polyhydroxylated aryl rings with most of their biological importance. The chemistry of chalcones remained fascinating among researchers due to their simple chemistry, ease of synthesis, large number of replaceable hydrogens and variety of pharmacological activities. In this present investigation our aim is to synthesize a set of 4-ethynylchalcones (3a-j) and to evaluate their Anti-bacterial activity. Synthesis of chalcone derivatives 3a-j was achieved using the classical Claisen-Schmidt reaction. The synthesized chalcone derivatives (3a-j) were tested against Gram negative strains of (i) Escherichia coli (MTCC 443) and (ii) Pseudomonas aeruginosa (MTCC 424) and Gram-positive strains of (iii) Staphylococcus aureus (MTCC 96) and (iv) Streptococcus pyogenes (MTCC 442) using agar well diffusion method. The compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain solutions of concentration 250 µg mL⁻¹. Ciprofloxacin was used as the reference antibacterial drug. Yields of the chalcone derivatives differed from 70 to 85%. The structures of newly synthesized chalcone derivatives 3a-j were established by spectroscopic techniques like ¹H NMR, mass and IR spectra of the chalcone derivatives 3a-j are in agreement with the desired structures. Chalcone derivatives (3a-j) characterized by Infrared, ESI-Mass and NMR spectroscopy. Chalcones (3b, 3g, 3h and 3j) with R = 4-OMe, 3-OH, 3-CN and 3,4,5-OMe exhibit good antibacterial activity, the chalcones (3e and 3f) with R = H and 3-NO₂ show moderate antibacterial activity and the remaining chalcones in the series such as 3a, 3c, 3d and 3i with R = 4-Br, 3-Br, (3-Br, 4-F) and 4-CH₃ display weak antibacterial activity against tested bacterial strains. Now a days health care became a more necessity thing in every one life. So, these chalcone derivatives are may helpful in development of less side effect radical quenching drugs.

Keywords: 4-Ethynylchalcones; Anti-bacterial activity; Agar well diffusion method; Ciprofloxacin.
1. INTRODUCTION

Chalcones, 1,3-diaryl-2-propen-1-ones, belong to the flavonoid family. They are abundant in edible plants. Large number of naturally occurring chalcones are polyhydroxylated in their aryl rings. Radical quenching properties of the phenolic groups of chalcones raised interest in using the chalcone rich plant extracts as drugs and food preservatives. They exhibit broad spectrum of biological activities such as anti-cancer, anti-inflammatory, anti-protozoal, anti-HIV, antitubercular, anti-diabetic and antioxidant activities. Chalcones are very important reaction intermediates for the synthesis of various heterocyclic compounds such as isoxazolines, pyrazoles, pyrimidines, pyrans, oxiranes, pyridines, oxazoles, and benzothiazepines. Chalcone and its derivatives can be synthesized classically by Claisen-Schmidt condensation between aromatic aldehyde and acetophenone employing sodium hydroxide solution as catalyst. The chemistry of chalcones remained fascinating among researchers due to their simple chemistry, ease of synthesis, large number of replaceable hydrogens and variety of pharmacological activities. Synthetic manipulations of chalcones or their isolation from natural products are being investigated worldwide for the development of more potent and efficient drugs for the treatment of several dreadful diseases such as cancer, diabetes, tuberculosis, malaria etc. Acetylenic metabolites belong to a class of molecules containing triple bond(s). They are found in plants, fungi, microorganisms, and marine invertebrates. In the last three decades, biologically active polycyclics having unusual structural features have been reported from plants, cyanoacteria, algae, invertebrates, and other sources. Many of the naturally occurring aquatic acetylenes display important biological activities such as antitumor, antibacterial, antimicrobial, antifungal, phototoxic, HIV inhibitory and immunosuppressive properties. Acetylenic drugs are frequently more active, less toxic and more easily absorbed into the body than their olefinic and saturated analogs. The acetylenic moiety functions as a key pharmacophoric unit in acetylenic antibiotics and its presence in anticancer and antitubercular agents is noteworthy. Acetylenic chalcones have been reported to possess antimalarial and antitubercular activities. In addition, acetylenic compounds play an important role as building blocks in many synthetic transformations and in new materials. In view of the biological importance of the chalcones and acetylene compounds, our aim is to prepare a set of 4-ethynylchalcones and to evaluate their antibacterial activity.

2. MATERIALS AND METHODS

Chemicals and solvents were purchased from Sigma-Aldrich and Merck. All the reagents were of analytical grade. Thin-layer chromatography (TLC) was performed. Merck AL silica gel 60 F254 plates and visualized under UV light. IR spectra were recorded as KBr pellets with the Perkin-Elmer Spectrum GX FTIR instrument and only diagnostic and/or intense peaks were reported. ‘H NMR spectra were recorded in CDCl3 with Varian Mercury plus 400 MHz instrument, Bruker Biospin Gmbh-300 MHz, 400MHz or 500MHz instruments. Signals due to the residual protonated solvent (‘H NMR) served as the internal standard. All the chemical shifts were reported in δ (ppm) using TMS as the internal standard. The ‘H NMR chemical shifts and coupling constants were determined assuming first-order behavior. Mass spectra were recorded with a PE Sciex model API 3000 instrument. All the reactions were carried out under a nitrogen atmosphere.

2.1 Synthesis

In organic chemistry, the Claisen–Schmidt condensation is the reaction between an aldehyde or ketone having an α-hydrogen with an aromatic carbonyl compound lacking an α-hydrogen. This reaction is named after two of its pioneering investigators Rainer Ludwig Claisen and J. G. Schmidt. In this present investigation Synthesis of chalcone derivatives 3a-j was achieved using the classical Claisen–Schmidt reaction as illustrated in Scheme; I. To a stirred solution of NaOH (0.16g, 4 mmol) in methanol at room temperature was added a corresponding acetophenones 2a-j (1 mmol) and stirred at the same temperature for 15 min. To the above homogeneous reaction mixture was added aldehyde 1 (0.13g, 1 mmol) and stirred for 5-6 h. The reaction mixture was diluted with water and the precipitated solids were filtered and dried at the pump to obtain the corresponding chalcones 3a-j. All the precipitates were Recrystallized by ethanol. Note: In case of the chalcone derivative 3g (R = 3-hydroxy), 3N HCl was used to neutralize the reaction mixture to isolate compound 3g. Yields of the chalone derivatives differed from 70 to 85%. The synthesized chalcones, their structures and yields are given in the Table I.

![Scheme-1: Synthesis of 4-ethynyl chalcones.](image)

**2.2 Antibacterial Bioassay**

The synthesized chalone derivatives 3a-j were tested against Gram negative strains of (i) *Escherichia coli* (MTCC 443) and (ii) *Pseudomonas aeruginosa* (MTCC 424) and Gram-positive strains of (iii) *Staphylococcus aureus* (MTCC 96) and (iv) *Streptococcus pyogenes* (MTCC 442) using agar well diffusion method. Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly to the procedure used in disk-diffusion method, the
agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. The compounds were dissolved in dimethyl sulphoxide (DMSO) to obtain solutions of concentration 250 µg mL\(^{-1}\). Ciprofloxacin was used as the reference antibacterial drug\(^{11}\). Antibacterial activity of the compounds was determined by zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control. All the samples were taken in triplicate.

3. STATISTICAL ANALYSIS

4. RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Structure</th>
<th>Formula</th>
<th>Yield [%]</th>
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<td>C(<em>{17}H</em>{11}BrO)</td>
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<td>C(<em>{17}H</em>{11}BrO)</td>
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</tr>
<tr>
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<td>3-Br, 4-F</td>
<td><img src="image" alt="Structure" /></td>
<td>C(<em>{17}H</em>{10}BrFO)</td>
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</tr>
<tr>
<td>3e</td>
<td>H</td>
<td><img src="image" alt="Structure" /></td>
<td>C(<em>{17}H</em>{12}O)</td>
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<tr>
<td>3f</td>
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<td><img src="image" alt="Structure" /></td>
<td>C(<em>{18}H</em>{11}NO)</td>
<td>74</td>
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</table>

Results were expressed as mean value ± standard error of the mean of growth inhibition zones diameters obtained with chalcone derivatives. Statistical differences between the standard drug and chalcone derivatives were detected by analysis of variance (ANOVA) followed by Duncan test\(^{15}\) when required. Duncan's new multiple range test (MRT) is a multiple comparison procedure developed by David B. Duncan in 1955. Duncan's MRT belongs to the general class of multiple comparison procedures that use the studentized range statistic \(q\) to compare sets of means\(^{21}\).
4.1 Spectral Interpretation

The structures of newly synthesized chalcone derivatives 3a-j were established by spectroscopic techniques like $^1$H NMR, mass and IR spectra. $^1$H NMR spectra of the chalcone derivatives 3a-j are in agreement with the desired structures. Olefin protons H-7 and H-8 of the compounds resonate as doublets in the regions $\delta$ 7.87-7.76 ppm and $\delta$ 7.56-7.47 ppm respectively with $J$= 15.3-15.9 Hz, H-2 and H-6 protons resonate as doublet around $\delta$ 7.66-7.60 ppm, protons H-3 and H-5 resonate as doublet around $\delta$ 7.56-7.53 ppm and acetylenic proton resonates around $\delta$ 3.20-3.24 ppm (in CDCl$_3$) and 3.34-3.39 ppm (in CDCl$_3$ + DMSO). Coupling constants ($J$ = 15.3-15.9 Hz) of doublets of olefin protons confirm the existence of compounds 3a-j as E-isomers. The mass spectra of the compounds showed (M+1) peaks and are in agreement with their molecular formulae. The IR spectra of the compounds 3a-j represented the characteristic peaks that conform to the desired functional groups in the structure. Sharp bands around 3302-3234 cm$^{-1}$ and 2105-2101 cm$^{-1}$ correspond to $\equiv$C-H and -C≡C- groups respectively. The characteristic $\alpha$, $\beta$-unsaturated carbonyl stretching bands appeared in the regions 1606-1572 cm$^{-1}$ (C=O, conjugated with C=C and benzene ring).

4.2 Spectral characteristics of (E)-1-(4-bromophenyl)-3-(4-ethynylphenyl) prop-2-en-1-one (3a)

Yellow solid; Yield: 85%; M.p: 95-97°C; IR (KBr): $\nu_{\text{max}}$ (cm$^{-1}$) 3295 (s, $\equiv$C-H), 1655 (s, C=O), 1606 (s, C=C); $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.89 (d, $J$ = 8.5 Hz, 2H), 7.78 (d, $J$ = 15.7 Hz, 1H), 7.65 (d, $J$ = 8.5 Hz, 2H), 7.60 (d, $J$ = 8.2 Hz, 2H), 7.54 (d, $J$ = 8.2 Hz, 2H), 7.47 (d, $J$ = 15.7 Hz, 1H), 3.22 (s, 1H); ESI MS: m/z 311 (M+H)$^+$. 

4.3 Spectral characteristics of (E)-3-(4-ethynylphenyl)-1-(4-methoxyphenyl) prop-2-en-1-one (3b)

Pale yellow solid; Yield: 80%; M.p: 114-115°C ; IR (KBr): $\nu_{\text{max}}$(cm$^{-1}$) 3302(w), 3263 (s, C=O), 3070(w), 3027 (w, Ar-H), 2839(w, OCH$_3$), 1654 (s, C=O), 1595 (s, C=C); $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.04 (d, $J$ = 8.9 Hz, 2H), 7.77 (d, $J$ = 15.7 Hz, 1H), 7.60 (d, $J$ = 8.3 Hz, 2H), 7.55 (d, $J$ = 15.7 Hz, 1H), 7.53 (d, $J$ = 8.3 Hz, 2H), 6.99 (d, $J$ = 8.9 Hz, 2H), 3.90 (s, 3H), 3.20 (s, 1H); ESI MS: m/z 262.8 (M+H)$^+$. 

4.4 Spectral characteristics of (E)-1-(3-bromophenyl)-3-(4-ethynylphenyl) prop-2-en-1-one (3c)
4.5 **Spectral characteristics of (E)-1-(3-bromo-4-fluorophenyl)-3-(4-ethynylphenyl) prop-2-en-1-one (3d)**

Yellow orange solid; Yield: 80%; M.p: 117-118 °C ; IR (KBr): $\nu_{max}(\text{cm}^{-1})$ 3289(w), 3252 (s, $\equiv$C-H), 3048 (w, Ar-H), 2101 (w, C≡C), 1657 (s, C=O), 1605 (s, C=C); $^1$H NMR (300 MHz, CDCl$_3$+DMSO-d$_6$): $\delta$ 8.28 (dd, $J$ = 8.2, 1.4 Hz, 1H), 8.12 – 7.95 (m, 1H), 7.79 (d, $J$ = 15.6 Hz, 1H), 7.66 (d, $J$ = 8.3 Hz, 2H), 7.56 (d, $J$ = 15.6 Hz, 1H), 7.54 (d, $J$ = 8.3 Hz, 2H), 7.29 (t, $J$ = 8.3 Hz, 1H), 3.34 (s, 1H); ESI MS: m/z 329 (M+H)$^+$.

4.6 **Spectral characteristics of (E)-3-(4-ethynylphenyl)-1-phenylprop-2-en-1-one (3e)**

Pale yellow solid; Yield: 84%; M.p: 120-121 °C; IR (KBr): $\nu_{max}(\text{cm}^{-1})$ 3291(w), 3260 (s, $\equiv$C-H), 2105 (w, C≡C), 1659 (s, C=O), 1603 (s, C=C); $^1$H NMR (300 MHz, CDCl$_3$+DMSO-d$_6$): $\delta$ 8.04 (d, $J$ = 7.2 Hz, 2H), 7.76 (d, $J$ = 15.7 Hz, 1H), 7.70 – 7.39 (m, 8H), 3.39 (s, 1H); ESI MS: m/z 233 (M+H)$^+$.

4.7 **Spectral characteristics of (E)-3-(4-ethynylphenyl)-1-(3-nitrophenyl) prop-2-en-1-one (3f)**

Dark yellow solid; Yield: 76%; M.p: 104-105 °C ; IR (KBr): $\nu_{max}(\text{cm}^{-1})$ 3287 (w), 3234 (s, $\equiv$C-H), 3082(w), 3031(w, Ar-H), 2102 (w, C≡C), 1659 (s, C=O), 1595 (s, C=C), 1530 (vs, NO$_2$) 1348(vs, NO$_2$); $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.85 (t, $J$ = 1.7 Hz, 1H), 8.46 (dd, $J$ = 8.0, 1.7 Hz, 1H), 8.36 (d, $J$ = 8.0 Hz, 1H), 7.88 (d, $J$ = 15.6Hz, 1H), 7.74 (t, $J$ = 8.0 Hz, 1H), 7.65 (d, $J$ = 8.3 Hz, 2H), 7.60 – 7.50 (m, 3H), 3.24 (s, 1H); ESI MS: m/z 277.9 (M+H)$^+$.

4.8 **Spectral characteristics of (E)-3-(4-ethynylphenyl)-1-(3-hydroxyphenyl) prop-2-en-1-one (3g)**
Fig: 8. (E)-3-(4-ethynylphenyl)-1-(3-hydroxyphenyl) prop-2-en-1-one (3g)

Yellow solid; Yield: 78%; M.p: 130-131°C; IR (KBr): \(v_{\text{max}}(\text{cm}^{-1})\) 3347 (br s, OH), 3250 (s, C=H), 3066(w), 3036 (w, Ar-H), 2104 (w, C≡C), 1652 (s, C=O), 1572(s, C≡C); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.78 (d, \(J = 15.7\) Hz, 1H), 7.66 – 7.56 (m, 3H), 7.56 – 7.46 (m, 4H), 7.40 (t, \(J = 7.9\) Hz, 1H), 7.09 (dd, \(J = 7.9\), 2.4 Hz, 1H), 5.16 (s, 1H), 3.21 (s, 1H); ESI MS: \(m/z\) 248.9 (M+H)\(^+\) and 246.9 (M-H)\(^+\).

4.9 Spectral characteristics of (E)-3-(3-(4-ethynylphenyl) acryloyl) benzonitrile (3h)

Fig: 9. (E)-3-(3-(4-ethynylphenyl) acryloyl) benzonitrile (3h)

Pale yellow solid; Yield: 74%; M.p: 119-120°C; IR (KBr): \(v_{\text{max}}(\text{cm}^{-1})\) 3297(w), 3248 (s, C≡C-H), 3077 (w, Ar-H), 2231 (m, C≡N), 2103 (w, C≡C), 1664 (s, C=O), 1605 (s, C≡C); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.30 (s, 1H), 8.25 (d, \(J = 7.9\) Hz, 1H), 7.88 (d, \(J = 7.9\) Hz, 1H), 7.84 (d, \(J = 15.6\) Hz, 1H), 7.70 – 7.60 (m, 3H), 7.56 (d, \(J = 8.0\)Hz, 2H), 7.48 (d, \(J = 15.6\) Hz, 1H), 3.24 (s, 1H); ESI MS: \(m/z\) 257.8 (M+H)\(^+\).

4.10 Spectral characteristics of (E)-3-(4-ethynylphenyl)-1-(p-tolyl) prop-2-en-1-one (3i)

Fig: 10. (E)-3-(4-ethynylphenyl)-1-(p-tolyl) prop-2-en-1-one (3i)

Pale yellow solid; Yield: 74%; M.p: 108-109°C; IR (KBr): \(v_{\text{max}}(\text{cm}^{-1})\) 3298(w), 3250 (s, C=H), 3077 (w, Ar-H), 2970 (w, CH\(_3\)), 2102 (w, C≡C), 1660 (s, C=O), 1603(s, C≡C); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.94 (s, 2H), 7.78 (d, \(J = 15.5\) Hz, 1H), 7.70-7.44 (m, 4H), 7.43-7.05 (m, 3H), 3.21 (s, 1H), 2.45 (s, 3H); ESI MS: \(m/z\) 246.9 (M+H)\(^+\).

4.11 Spectral characteristics of (E)-3-(4-ethynylphenyl)-1-(3,4,5-trimethoxy phenyl) prop-2-en-1-one (3j)

Fig: 11. (E)-3-(4-ethynylphenyl)-1-(3,4,5-trimethoxy phenyl) prop-2-en-1-one (3j)

Pale yellow solid; Yield: 80%; M.p: 123-124°C; IR (KBr): \(v_{\text{max}}(\text{cm}^{-1})\) 3293 (s, C=H), 3076 (w), 3031 (w, Ar-H), 2836 (w, OCH\(_3\)), 1656 (s, C=O), 1601 (s, C=O); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.79 (d, \(J = 15.7\) Hz, 1H), 7.62 (d, \(J = 8.1\) Hz, 2H), 7.55 (d, \(J = 8.1\) Hz, 2H), 7.49 (d, \(J = 15.7\) Hz, 1H), 7.28 (s, 2H), 3.96 (s, 6H), 3.95 (s, 3H), 3.22 (s, 1H); ESI MS: \(m/z\) 322.8 (M+H)\(^+\).

4.12 Antibacterial activity of chalcone derivatives 3a-j

Naturally occurring acetylenes display important biological activities such as antitumor, antibacterial, antimicrobial, antifungal, phototoxic,\(^9\) The newly synthesized compounds 3a-j were evaluated for in-vitro antimicrobial activity against two Gram-negative bacterial strains, Escherichia coli and Pseudomonas aeruginosa and two Gram-positive bacterial strains, Staphylococcus aureus and Streptococcus pyogenes.\(^9\) The results of the antibacterial activity data are compiled in Table-2. From the table, it is observed that chalcones 3b, 3g, 3h and 3j with R = 4-OMe, 3-OH, 3-CN and 3,4,5-OMe exhibit good antibacterial activity, the chalcones 3e and 3f with R = H and 3-NO\(_2\) show moderate antibacterial activity and the remaining chalcones in the series such as 3a, 3c, 3d and 3i with R = 4-Br, 3-Br, (3-Br, 4-F) and 4-CH\(_3\) display weak antibacterial activity against tested bacterial strains. When compared both natural acetylenes and new substituted synthetic acetylenes, natural acetylenes showed increased antibacterial activity against selected strains.
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Fig. 12: Graphical representation of anti-bacterial activity of compounds 3a-j

Table 2: Antibacterial activity of compounds 3a-j

<table>
<thead>
<tr>
<th>Compound number</th>
<th>R</th>
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<td></td>
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<tr>
<td>3a</td>
<td>4-Br</td>
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<td>4-CH₃</td>
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<tr>
<td>3j</td>
<td>3,4,5-OMe</td>
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<tr>
<td><em>Standard Drug</em></td>
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</table>

* Ciprofloxacin (250 µg mL⁻¹ of DMSO); * Zone of inhibition – (i) Gram negative bacteria: good activity: 22-26 mm; moderate activity: 16-21 mm; weak activity: < 16 mm; (ii) Gram positive bacteria: good activity: 16-21 mm; moderate activity: 10-15 mm; weak activity: < 10 mm

5. CONCLUSION

In this present study our interest is to synthesize substituted chalcone derivatives (3a-j). The chalcones with R = 4-OMe, 3-OH, 3-CN and 3,4,5-OMe exhibit good antibacterial activity, the chalcones with R = H and 3-NO₂ show moderate antibacterial activity and the remaining chalcones with R = 4-Br, 3-Br, (3-Br, 4-F) and 4-CH₃ display weak antibacterial activity against tested bacterial strains. From this study it was very clear that electron donating substituents increases the antibacterial activity of chalcones, when compared withdrawing substituents. these compounds shown lesser activity when compared to natural chalcones. Our study is new addition to chalcone chemistry.

6. AUTHORS CONTRIBUTION STATEMENT

Dachepally Raju and J. Sriramulu conceptualized and designed the study and P. Malleswarareddy analyzed the data and necessary inputs were given. All the authors discussed the Methodology and results and final manuscript.

7. ACKNOWLEDGEMENT

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8. CONFLICT OF INTEREST

Conflict of interest declared none.
9. REFERENCES


