Chapter 6

Synthesis and characterization of 5-ethyl-2,4-diaryl-1-pyrroline-N-oxides
6.1. Transformations of γ- nitrocarbonyl compounds

γ- Nitrocarbonyl compounds provide an opportunity for researchers to explore their use for the development of novel synthetic methodologies and for various organic transformations. The nitro group can be converted into other useful groups such as amines or carbonyls. Carbonyl groups can be transformed into various other functionalities.

γ-Nitrocarbonyl compounds could be transformed into cyclic nitrones or pyrroline derivatives depending upon the reduction conditions and the nature of the carbonyl groups [1-8]. Various reduction conditions have been experimented for the reductive cyclization of γ-nitrocarbonyl compounds including Fe/AcOH [1], Zn/NH₄Cl [2-5], and catalytic hydrogenation [6,7]. Lee et al. [8] carried out the reductive cyclization of E-benzylidine-5-nitrohexan-2-one 1 under various conditions. The use of Fe/AcOH gave the pyrroline derivative 4 as the major product under refluxing conditions, whereas, the cyclic nitrone derivative 5 was obtained as the major product with Zn/NH₄Cl at low temperature.
The mechanism for the formation of 4 and 5 was proposed as in **Scheme 6.1**. Reduction of the nitro group into amino group leads to 2 and the following condensation gave 5. Partial reduction to hydroxylamine derivative 3 and the following cyclization and dehydration affords 6. Reduction of the adducts between nitroalkanes 6 and enones or enals 7 directly produces cyclic nitrones 9 by an intramolecular reaction of the intermediate hydroxylamines 8 and the carbonyl group.

A series of new pyrroline-1-oxides and their related pyrrolines were prepared by the reductive cyclisation of γ-nitro carbonyl compounds by using various reducing agents. Zinc in aqueous ammonium chloride was found to be a successful reagent in the formation of certain pyrroline-1-oxides whereas reduced iron in a mildly acidic medium was the method of choice for some 2-alkyl substituted compounds.

5,5-Diethoxycarbonyl-1-pyrroline-N-oxide (DECPO) **11** was synthesized by reductive cyclization of the appropriate γ-nitroaldehyde, diethyl-5-oxo-2-nitropentan-2-yl-dicarboxylate **10**, using zinc in the presence of NH₄Cl. **11** was obtained after reduction of the nitro function to the hydroxylamine and subsequent in situ cyclization [9].
Some C-5 mono- and diester-substituted 1-pyrroline-1-oxides 14 have been prepared via reductive cyclisation of the corresponding γ-nitro carbonyl compounds 12 [10].

Cyclic nitrones, which are heterocyclic compounds containing an azomethine N-oxide group, have a wide range of synthetic potentialities. The spin trapping technique, using pyrroline-N-oxide spin traps and EPR spectroscopy, has been widely employed for the identification of free radicals in chemical and biological systems. Suitably substituted pyrroline-N-oxide or cyclic nitrone derivatives has been prepared and used as important synthetic intermediates.

Encouraged by these observations and as a part of our research program in the synthesis of newer heterocyclic systems, a facile route has been described for the synthesis of new pyrroline-N-oxide derivatives.
6.2. Synthesis of 5-ethyl-2,4-diaryl-1-pyrroline-N-oxides 17 - The present work

The analytical and other informational data, available in literature so far, have rendered pyrroline-N-oxides significantly important class of heterocyclic compounds and their applications in ever challenging chemotherapy immensely hiked interests of medicinal chemist and biochemist. The versatile synthetic applicability and biological activity of these heterocycles help the medicinal chemists to plan, organize and implement new approaches towards discovery of novel drugs. Therefore there is a great deal of interest in the synthesis of pyrroline-N-oxides.

For the preparation of nitrones, the most popular method is the condensation of aldehydes or ketones with N-monosubstituted hydroxylamines. Although new synthetic routes to cyclic nitrones continue to emerge [11-13], the reductive cyclisation of γ-nitro carbonyl compounds is most commonly used and was the chosen strategy for this investigation.

The present work was directed towards the synthesis of cyclic nitrones from chalcones as precursors. One useful route towards the synthesis of these compounds is Michael addition of chalcones to nitroalkanes followed by the reductive cyclization of γ-nitroketones with zinc and ammonium chloride.

The starting chalcones 15 were prepared in good yield by conventional Claisen-Schmidt condensation by reacting appropriately substituted benzaldehydes and substituted acetophenones. A mixture of 15, 1-nitropropane and aqueous potassium hydroxide in DMF afforded the corresponding γ-nitroketones 16 in excellent yields, which where promising intermediates for the synthesis of pyrroline-N-oxide derivatives. The γ-nitroketones were heated under reflux with zinc and ammonium chloride in ethanol to assemble the pyrroline-N-oxides 17 (Scheme 6.2).

The reaction was complete within thirty minutes as monitored by TLC. The products were obtained by extracting the reaction mixture with dichloromethane. The yields of the cyclization products are good, varying from 53 -79%.

The procedure described is using inexpensive inorganic base potassium hydroxide as catalyst in Michael addition. Reagents used for cyclization viz zinc and ammonium chloride are also inexpensive. The reaction conditions are mild and involve a straightforward and easy access to the desired compounds with good yields via a simple workup.
Results and discussion

Scheme 6.2 Synthesis of 5-ethyl-2,4-diaryl-1-pyrroline-N-oxides

The 1,4-addition of 1-nitropropane with chalcones 15 leads to the generation of two chiral centers at C-3 and C-4 in the structure of adducts 16. As the reaction is not stereoselective, both configuration of the chiral carbon atoms are expected to be noticed in the synthesized adducts 16, which would have resulted in a mixture of diastereomers. But the spectral data of the crude product suggests that there is only one diastereomer predominantly formed over the other (vide infra).

In this series, only compound 16a [15], is known in the literature. This reported methodology has been modified in the present investigation. The structures of other adducts (16 b, c, d, e, f, g and h) were confirmed on the basis IR, NMR and mass spectral data.

Infrared spectra of these compounds displayed strong peaks at 1550-1535 cm\(^{-1}\) (\(v_{\text{as NO}}\)) and 1350-1370 cm\(^{-1}\) (\(v_{\text{s NO}}\)) indicating the presence of a nitro group. Absence of bands around 1500 cm\(^{-1}\) due to -CH=CH- group and appearance of weak bands at 2980-2900 cm\(^{-1}\) due to methylene and methyl groups also establish the formation of
adducts. The $^1$H NMR data of compounds 16b and 17b are given in Figure 6.1. Infrared spectrum of 16b is given in Figure 6.2.

The $^1$H NMR data substantiated the results of the IR. Observation of new bands in the aliphatic region of $^1$H and $^{13}$C NMR spectra of the adducts confirms the formation of adducts. In the $^1$H NMR spectrum of 16b (Figures 6.3 and 6.4), the protons of phenacetyl methylene gave an AB pattern characteristic of these compounds. H$_A$ proton of the AB system showed a doublet of doublet at 3.36 ($J = 17.7, 7.0$ Hz) and that of H$_B$ a doublet of doublet at 3.56 ($J = 17.7, 7.0$ Hz). The coupling constant value of $17.7$ Hz corresponds to geminal coupling. The other methylene protons being diastereotopic gave two multiplets at 1.81-1.91 ppm and 1.96-2.04 ppm. The methine proton of -CHNO$_2$ appeared as a multiplet in the range 4.76-4.83 ppm. The other methine proton showed a quartet at 3.96 ppm ($J = 6.9$ Hz).

The $^{13}$C NMR spectrum (Figures 6.5 and 6.6) of 16b has five peaks at 10.4, 24.8, 40.4, 43.2 and 93.1 ppm assignable to C-6, C-5, C-3, C-2 and C-4. Eight other peaks are observed in aromatic region attributable to aromatic rings. The peak at 196.7 ppm corresponds to the carbonyl carbon.

The structural identity of the products 17 was established on the basis IR, NMR and mass spectral data. Disappearance of peaks at 1550-1535 cm$^{-1}$and 1350-1370 cm$^{-1}$ corresponding to nitro group and lack of carbonyl peak around 1650 cm$^{-1}$ in the IR spectra confirms that the reductive cyclization subsequent to the Michael addition had taken place. Apart from these, compound 17 displayed new peaks around 1590 and 1210 cm$^{-1}$ corresponding to C=N and N→O groups. Infrared spectrum of 17b is given in Figure 6.7.

Ethyl group showed a diastereotopic splitting of the methylene protons with two well separated multiplets. In the $^1$H NMR spectrum of 17b (Figures 6.8 and 6.9) the methylene protons of ethyl group appeared as multiplets at 1.42-1.48 ppm and 2.05-2.10 ppm. The two geminal methylene ring protons showed two doublet of doublets at 3.28 and 3.46 ppm ($J = 16.5, 8.0$ Hz). The methine protons H-4 and H-5 appeared as quartets at 3.86 and 4.31 ppm respectively with a $J$ value of $8.0$ Hz. Since there is not much difference in the $^1$H NMR spectrum of 16b and 17b, the structure of 17b was purely established by its $^{13}$C NMR spectrum (Figures 6.10 and 6.11). Observation of a new
peak at 79.3 ppm instead of -CHNO₂ peak at 93.1 and disappearance of carbonyl peak at 197.1 ppm revealed the formation of products.

The mass spectra of the synthesized compounds are typical of a cyclic nitrone and the mass spectrum of a representative compound 17b is given in Figure 6.12. In the EI MASS spectrum of 4-(4-chlorophenyl)-5-ethyl-2-phenyl-1-pyrrolone N-oxide 17b, the molecular ion peak is observed at m/z 299.80 with moderate intensity. M16, which is a characteristic peak of nitrone is seen at m/z 283.80 corresponding to the loss of oxygen from the nitrone.

![Figure 6.1](image1.png)

**Figure 6.1** H Chemical shifts in compounds 16b and 17b

![Figure 6.2](image2.png)

**Figure 6.2.** IR Spectrum of 16b
Figure 6.3. $^1$H NMR Spectrum of 16b (CDCl$_3$)

Figure 6.4. $^1$H NMR Spectrum of 16b (expanded)
Figure 6.5 $^1$H NMR Spectrum of 16b (expanded)

Figure 6.6 $^{13}$C NMR Spectrum of 16b
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Figure 6.7. IR Spectrum of 17b

Figure 6.8. 1H NMR Spectrum of 17b(CDC13)
Figure 6.9. $^1$H NMR Spectrum of 17b (expanded)

Figure 6.10. $^1$H NMR Spectrum of 17b (expanded)
Figure 6.11. $^{13}$C NMR Spectrum of 17b(CDCl$_3$)

Figure 6.12. $^{13}$C NMR Spectrum of 17b(expanded)
6.3. Antimicrobial activity of 5-ethyl-2,4-diaryl-1-pyrroline-N-oxides (17)

The development of new antimicrobial agents has become a very important matter for researchers. Most of the research work efforts are because of the unsatisfactory status of present drugs with side effects and the acquirement of the resistance by the infecting organisms to present drugs. The resistance of common pathogens to standard antibiotic therapy is rapidly becoming a major health problem throughout the world. There is an apparent need for the discovery of new compounds endowed with antimicrobial property.

The synthesized compounds were evaluated for their in vitro preliminary antibacterial activity against *E. coli* (ATCC 25922), *S. aureus* (ATCC 11632) and for their antifungal activity against *C. albicans* (ATCC 90028) and *A. Niger* (MTCC 281) by agar disc diffusion method using ciprofloxacin and clotrimazole as reference standards respectively.

The MICs and zone of inhibition were determined for the compounds and the results are summarized along with that of ciprofloxacin and clotrimazole in Table 6.1.
The results show that some of the designed compounds have moderate to good antibacterial and antifungal activities (6.25-50.0µg/ml).

Table 3.2. Antimicrobial activity of 5-ethyl-2,4-diaryl-1-pyrroline-N-oxides (17).

<table>
<thead>
<tr>
<th>Entry</th>
<th>MIC&lt;sup&gt;a&lt;/sup&gt; (ZI)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E.coli (25922)</td>
</tr>
<tr>
<td>17a</td>
<td>25.0(19)</td>
</tr>
<tr>
<td>17b</td>
<td>12.5(22)</td>
</tr>
<tr>
<td>17c</td>
<td>25.0(18)</td>
</tr>
<tr>
<td>17d</td>
<td>50.0(15)</td>
</tr>
<tr>
<td>17e</td>
<td>25.0(20)</td>
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<tr>
<td>17f</td>
<td>25.0(19)</td>
</tr>
<tr>
<td>17g</td>
<td>12.5(20)</td>
</tr>
<tr>
<td>17h</td>
<td>25.0(15)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6.25(28)</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>n.t.</td>
</tr>
</tbody>
</table>

Note: The MIC values were evaluated at concentration range 1.56-50µg/ml.

<sup>a</sup> Minimum inhibitory concentration in µg/mL.

<sup>b</sup> Zone of inhibition in mm for 100 µg/disc of each compound.

<sup>c</sup> n. t. not tested.

Compounds 17b and 17g (12.5 µg/ml) showed promising activity with the zone of inhibition 22.0 and 20.0 mm whereas, 17a, 17c, 17e and 17h (25.0 µg/ml) showed moderate activity with the zone of inhibition 19.0, 18.0, 20.0 and 15.0 mm respectively with ciprofloxacin (6.25 µg/ml and 28 mm) as standard against E. coli. Against S. aureus, compounds 17b, 17c and 17g showed good activity with the zone of inhibition 18.0, 15.0 and 16.0 mm whereas, 17e and 17f showed moderate activity with zone of inhibition 19.0 and 17.0 mm with ciprofloxacin as the standard (6.25 µg/ml and 29 mm). In the antifungal evaluation, compound 17g showed good activity with the zone of inhibition between 14 mm with clotrimazole (6.25 µg/ml and 28 mm) as standard against C. albicans. Compounds 17a, 17b, 17c, 17e and 17f exhibited moderate activity with the zone of inhibition 15.0, 18.0, 16.0, 17.0 and 16.0 mm respectively against C. albicans.
Against *A. Niger*, compounds 17b and 17g showed good activity with the zone of inhibition 18.0 and 16.0 mm while compounds 17a and 17h showed moderate activity with the zone of inhibition 18.0 and 19.0 mm when compared to standard clotrimazole (6.25 μg/ml and 28 mm).

6.4. Conclusion

The synthesis of new sets of novel pyrroline-N-oxide derivatives from the Michael adducts of chalcones with 1-nitropropane has been described. All the synthesized compounds were characterized by using IR, NMR and mass spectral analysis. All the compounds were assayed for antimicrobial activity. Investigation of antibacterial activity of the compounds was done by disc diffusion method using Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Among the compounds tested, two exhibited appreciable antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*. Likewise, two of the tested compounds exhibited good activity, while other compounds showed moderate or weak activity against the fungal strains. Hence the compounds could provide starting materials for the development of new classes of antimicrobial molecules.
6.5. Experimental section

6.5.1. General method

Solid chemicals were used as such without further purification. Liquid chemicals were purified by distillation before use. Standard methods were used for purification of solvents. The melting points were determined through Ajay melting point apparatus using open capillaries and were uncorrected. The TLC method was used to monitor the progress of the reaction and to check the purity of the compounds with a mixture of petroleum ether (60-80 °C) and ethylacetate as eluent. Hand drawn silica gel plates of 0.5-0.7mm thickness were used for TLC.

The NMR spectra were recorded on a JEOL GX 400 Spectrometer or Bruker (Avance) 300 MHz NMR instrument using TMS as internal standard and CDCl₃ as solvent. Chemical shifts are given in parts per million (δ-scale) and the coupling constants are given in Hertz. IR spectra were recorded on a Bruker IFS-66V FT-IR spectrometer or FT-IR-Shimadzu instrument (KBr pellet). The vibrational frequencies are reported in reciprocal centimeter. Mass spectra were recorded on a JEOL GC mate instrument. Elemental analyses were performed on a Perkin Elmer 2400 Series II Elemental CHNS analyzer.

6.5.2. General procedure for the preparation of 1,3-diaryl-4-nitro-1-hexanones (16)

Potassium hydroxide (1.0 M, 10 mL) was added to a stirred solution of chalcone (1 mmol) and 1-nitropropane (1 mmol) at room temperature in DMF (10 mL) and the resulting mixture was stirred until the reaction was complete (monitored by TLC). The product obtained was poured into crushed ice, filtered and dried.

6.5.3. General procedure for the preparation of 5-ethyl-2,4-diaryl-1-pyrroline-N-oxides (17)

1,3-diaryl-4-nitro-1-hexanone (1 mmol) was dissolved in ethanol and aqueous ammonium chloride (1 mmol) was added to it. Zinc dust was slowly added within 30 minutes and the reaction mixture was refluxed for 45 minutes. After completion of the reaction as monitored by TLC the mixture was poured into crushed ice, extracted with dichloromethane and purified by column chromatography using silica gel (60–120 mesh) with 95:5 petroleum ether:ethyl acetate(v/v) as eluent to afford the pure product 17. The analytical data for all the compounds are given below:
6.5.3.1. 5-Ethyl-2,4-diphenyl-1-pyrroline-N-oxide (17a). Light brown solid; Yield-55%; m.p. 124-125 °C; IR (KBr): 1591(C=Н),1210 (N→О) cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δH: 0.83 (t, J = 7.5 Hz, 3H, CH₃), 1.26-1.33, 2.10-2.18 (m, 2H, -CH₂CH₃), 3.05 (dd, J = 16.5, 8.0 Hz,1H, H-3), 3.48 (dd, J = 16.5, 8.0 Hz,1H, H-3'), 3.90 (q, J = 7.5Hz, 1H, H-4), 4.42 (q, J = 5.0 Hz, 1H, H-5) 7.08-7.25 (m, 5H, Ar-H), 7.42-7.56 (m, 3H, Ar-H), 7.81-7.93 (m, 2H, Ar-H); ¹³C NMR (75 MHz, CDCl₃) δC: 10.1, 20.8, 35.6, 40.8, 79.3, 126.7, 127.3, 128.9, 129.1, 129.8, 130.3, 135.1, 136.8, 139.5. MS: m/z 266.33 (M+1); Anal. Calcd. for C₁₈H₁₉NO: C, 81.47; H, 7.22; N, 5.28%. Found: C, 81.40; H, 7.18; N, 5.32%.

6.5.3.2. 4-(4-Chlorophenyl)-5-ethyl-2-phenyl-1-pyrroline-N-oxide (17b). Light brown solid; Yield- 60%; m.p. 138-139 °C; IR (KBr): 1614 (C=N), 1225 (N→O) cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δH: 0.77 (t, J = 7.5 Hz, 3H, CH₃), 1.42-1.48, 2.05-2.10 (m, 2H, CH₂CH₃), 3.28 (dd, J = 16.5, 8.0 Hz, 1H, H-3), 3.46 (dd, J = 16.5, 8.0 Hz, 1H, H-3'), 3.86 (q, J = 7.5 Hz, 1H, H-4), 4.42 (q, J = 5.0 Hz, 1H, H-5) 7.16 (d, J = 8.3 Hz, 2H, Ar-H), 7.30 (d, J = 8.3Hz, 2H, Ar-H), 7.46-7.50 (m, 3H, Ar-H), 8.41 (dd, J = 13.0, 2.0 Hz, 2H, Ar-H); ¹³C NMR (75 MHz, CDCl₃) δC: 10.3, 21.1, 35.7, 40.1, 79.3, 127.4, 128.6, 128.9, 129.1, 129.4, 130.4, 133.2, 137.3, 139.3. MS: m/z 299.80 (M); Anal. Calcd. for C₁₈H₁₈ClNO: C, 72.11; H, 6.05; N, 4.67%. Found: C, 72.05; H, 6.01; N, 4.70%.

6.5.3.3. 5-Ethyl-2-phenyl-4-p-tolyl-1-pyrroline-N-oxide (17c). Brown solid; Yield-56%; m.p. 153-154 °C; IR (KBr): 1594 (C=Н), 1208 (N→O) cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δH: 0.75 (t, J = 7.5 Hz, 3H, CH₃), 1.45-1.52, 1.96-2.06 (m, 2H, CH₂CH₃), 2.34 (s, 3H, Ar-CH₃), 3.34 (dd, J = 16.5, 8.3 Hz, 1H, H-3), 3.50 (dd, J = 16.5, 8.3 Hz, 1H, H-3'), 3.80 (q, J = 7.5 Hz, 1H, H-4), 4.25 (q, J = 5.0 Hz, 1H, H-5) 7.12 (d, J = 8.0 Hz, 2H, Ar-H), 7.44 (d, J = 8.0 Hz, 2H, Ar-H), 8.40-8.44 (m, 5H, Ar-H), 8.41 (dd, J = 13.0, 2.0 Hz, 2H, Ar-H); ¹³C NMR (75 MHz, CDCl₃) δC: 10.4, 21.1, 21.2, 35.7, 40.3, 79.5, 126.9, 127.6, 128.0, 128.5, 129.8, 130.4, 135.5, 139.8. MS: m/z 279.39 (M); Anal. Calcd. for C₁₉H₂₁NO: C, 81.68; H, 7.58; N, 5.01%. Found: C, 81.62; H, 7.53; N, 5.06%.

6.5.3.4. 5-Ethyl-4-(4-methoxyphenyl)-2-phenyl-1-pyrroline-N-oxide (17d). Brown solid; Yield- 63%; m.p. 162-1163 °C; IR (KBr): 1600 (C=Н), 1213 (N→O) cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δH: 0.85 (t, J = 7.5 Hz, 3H, CH₃), 1.52-1.58, 1.94-2.02 (m, 2H,
6.5.3.5. 2-(4-Chlorophenyl)-5-ethyl-4-(4-methoxyphenyl)-1-pyrroline-N-oxide (17e). Brown solid; Yield- 52%; m.p. 176-177 °C; IR (KBr): 1595 (C=N), 1205 (N→O) cm⁻¹; ¹H NMR (300 MHz, CDCI₃) δH: 0.72 (t, J = 7.5 Hz, 3H, CH₃), 1.41-1.47, 1.94-2.02 (m, 2H, CH₂CH₃), 3.23 (dd, J = 16.5, 6.5 Hz, 1H, H-3), 3.46 (dd, J = 16.5, 6.5 Hz, 1H, H-3'), 3.76 (s, 3H, OCH₃), 3.80 (q, J = 7.5Hz, 1H, H-4), 4.22 (q, J = 5.0 Hz, 1H, H-5), 6.38-6.88 (m, 2H, Ar-H), 7.08-7.39 (m, 4H, Ar-H), 8.31-8.35 (m, 2H, Ar-H); ¹³C NMR (75 MHz, CDCI₃) δC: 10.3, 21.2, 35.7, 40.1, 55.2, 79.7, 114.1, 127.7, 128.7, 128.8, 130.3, 136.9, 139.1, 158.9. MS: m/z 329.80 (M); Anal. Calcd. for C₁₉H₂₁NO₂: C, 69.19; H, 6.11; N, 4.25%. Found: C, 69.12; H, 6.08; N, 4.29%.

6.5.3.6. 2-(4-Chlorophenyl)-5-ethyl-4-p-tolyl-1-pyrroline-N-oxide (17f). Brown solid; Yield- 50%; m.p. 176-177 °C; IR (KBr): 1586 (C=N), 1204 (N→O) cm⁻¹; ¹H NMR (300 MHz, CDCI₃) δH: 0.88 (t, J = 7.5 Hz, 3H, CH₃), 1.34-1.42, 1.96-2.02 (m, 2H, CH₂CH₃), 2.30 (s, 3H, Ar-CH₃), 3.15 (dd, J = 16.5, 7.0 Hz, 1H, H-3), 3.26 (dd, J = 16.5, 7.0 Hz, 1H, H-3'), 3.85 (q, J = 6.0 Hz, 1H, H-4), 4.22 (q, J = 5.0 Hz, 1H, H-5), 7.09-7.11 (m, 2H, Ar-H), 7.38-7.44 (m, 2H, Ar-H), 7.76-7.78 (m, 2H, Ar-H); 7.85-7.91 (m, 2H, Ar-H); ¹³C NMR (75 MHz, CDCI₃) δC: 10.2, 21.0, 21.2, 35.7, 40.2, 79.2, 126.5, 127.7, 128.3, 128.6, 129.5, 130.3, 133.1, 135.9, 139.1. MS: m/z 313.80 (M); Anal. Calcd. for C₁₉H₂₀ClNO: C, 72.72; H, 6.42; N, 4.46%. Found: C, 72.66; H, 6.37; N, 4.51%.

6.5.3.7. 2-(4-Chlorophenyl)-5-ethyl-4-phenyl-1-pyrroline-N-oxide (17g). Yellow solid. Yield-52%; m.p. 118-119 °C; IR (KBr): 1592 (C=N), 1225 (N→O) cm⁻¹; ¹H NMR (300 MHz, CDCI₃) δH: 0.75 (t, J = 7.5 Hz, 3H, CH₃), 1.32-1.37, 2.13-2.19 (m, 2H, CH₂CH₃), 3.25 (dd, J = 16.5, 8.0 Hz, 1H, H-3), 3.41 (dd, J = 16.5, 8.0 Hz, 1H, H-3'), 3.87 (q, J = 7.5 Hz, 1H, H-4), 4.32 (q, J = 5.0 Hz, 1H, H-5) 7.18 (d, J = 8.3 Hz, 2H, Ar-
H), 7.40 (d, J = 8.3Hz, 2H, Ar-H), 7.56-7.61 (m, 3H, Ar-H), 8.45(dd, J = 13.0, 2.0 Hz, 2H, Ar-H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$C: 10.2, 21.2, 35.5, 40.3, 78.4, 126.8, 128.2, 128.7, 129.2, 129.7, 131.2, 133.1, 137.1, 139.2. MS: m/z 299.78 (M); Anal. Calcd. for C$_{18}$H$_{18}$ClNO: C, 72.11; H, 6.05; N, 4.67%. Found: C, 72.02; H, 5.96; N, 4.72%.

6.5.3.8. 5-Ethyl-2-(4-methoxyphenyl)-4-p-tolyl-1-pyrroline-N-oxide (17h).

Brown solid; Yield- 63%; m.p. 162-1163 °C; IR (KBr): 1590 (C=N), 1223 (N→O) cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$H: 0.82 (t, J = 7.5 Hz, 3H, CH$_3$), 1.41-1.47, 2.05-2.12 (m, 2H, CH$_2$CH$_3$), 2.23 (s, 3H, Ar-CH$_3$), 3.41 (dd, J = 16.3, 8.0 Hz,1H, H-3), 3.60 (dd, J = 16.3, 8.0 Hz, 1H, H-3'), 3.83 (s, 3H, OCH$_3$), 4.02 (q, J = 6.5 Hz, 1H, H-4), 4.69 (q, J = 5.0 Hz, 1H, H-5) 6.59 (d, J =8.0 Hz, 2H, Ar-H), 6.72 (d, J = 8.0 Hz, 2H, Ar-H), 6.92 (d, J = 8.0 Hz, 2H, Ar-H), 7.31(d, J = 8.0 Hz, 2H, Ar-H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$C: 10.5, 21.0, 21.2, 35.7, 40.6, 55.1, 79.3, 114.0, 126.5, 127.7, 128.0, 128.3, 130.3, 135.4, 139.7, 159.0. MS: m/z 309.39 (M); Anal. Calcd. for C$_{20}$H$_{23}$NO$_2$: C, 77.64; H, 7.49; N, 4.53%. Found: C, 77.56; H, 7.40; N, 4.60%.

6.6. Antibacterial and antifungal activity

The compounds were diluted in dimethylsulfoxide (DMSO) with required concentrations (100μg/disc) for bioassay. Antimicrobial activity was evaluated by screening the compounds by standard agar disc diffusion method against a panel of human pathogenic microorganisms: one Gram positive (S. aureus ATCC 11632) and one Gram negative (E. coli ATCC 25922) bacteria were used for the antibacterial assay, while for the antifungal assay, C. albicans (ATCC 90028) and A. Niger (MTCC 281) were used. Microorganisms were maintained at 37 °C on Mueller Hinton (MH) agar slants. MH agar and sabouraud’s broth were used to evaluate antibacterial and antifungal activity respectively. To make a judgment of antibacterial and antifungal potency of the synthesized compounds, commercial antibiotics such as ciprofloxacin (10μg/disc) and clotrimazole (10μg/disc) in DMSO served as reference standards to compare inhibition of growth. The plates containing bacterial organism were incubated at 37°C for 24 h and the plates containing fungal organism were incubated at 28°C for 48 h. The zone of inhibition was calculated by measuring the diameter of zone of inhibition for bacterial and fungal growth around the disc.
Averages of three independent determinations were recorded. The minimum inhibitory concentration (MIC) of the samples was determined by agar dilution method. MH broth was melted and poured in sterile tubes according to National Committee for Clinical Laboratory Standards (NCCLS, M7-A5 January 2000). Overnight culture were grown at 37 °C by Kirby- Bauer procedure and diluted to Muller Hinton Broth. 0.01ml of culture was added to all the test tubes containing serial double dilution of drugs. All the tubes were incubated at 37 °C for 18-24 h. After incubation, the OD values were observed by spectrophotometric method. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth.