Synthesis, spectral LFER and antimicrobial activities of some $(E)-N'-(1$-(substituted phenyl)ethylidene)benzohydrazides

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About eleven substituted $(E)-N'-(1$-(substituted phenyl)ethylidene)benzohydrazides have been synthesized. They are characterized by their analytical, ultraviolet, infrared and NMR spectral data. The antibacterial and fungal activities of these hydrazides have been evaluated.

**Keywords:** $(E)-N'-(1$-(substituted phenyl)ethylidene)benzohydrazides, UV, IR and NMR spectra, antibacterial and antifungal activities.

1. **INTRODUCTION**

Nitrogen containing compounds are synthetically challenging models for a number of therapeutically significant products. Azole derivatives occupies a domain of interest in natural and synthetic chemistry. Diazoles are the central building blocks for synthesizing compound libraries in pharmaceutical and agrochemical industries. The organic and medicinal
chemistry consists of design and synthesize molecules that possess potent therapeutic values. The rapid development of resistance to existing antimicrobial drugs generates a serious challenge to the scientific community. It is also interesting to note that the range of bioactivities involved is different substantially from those seems with the benzofused rings [1]. Schiff’s bases are considered as a most important group of compounds in medicinal chemistry. The general method for the synthesis of (E)-N’-(1-(substituted phenyl)ethylidene)benzohydrazides is the reaction of benzo hydrazide with carbonyl compounds such as aldehydes or ketones in solvents like ethanol [2, 3] and so forth. The (E)-N’-(1- (substituted phenyl)ethylidene)benzohydrazides belong to the Schiff’s base family containing azomethine –NHN=CH– protons and they are considered as the important class of compounds for the development of new drugs [4].

Hydrazide-hydrazone derivatives have been claimed to possess interesting bioactive potents such as anti-HIV [5], antimicrobial [6, 7], antidiabetic [8], leishmanicidal [7], antimalarial [8], antimycobacterial [9–12], anticancer [13, 14] anti-inflammatory [15, 16], antiparasitic [17], antiproliferative [18], trypanocidal [19], antitumor [20], analgesic [21], anticonvulsant [22] and antiviral [23] activities. Hence, the development of newer antimycobacterial agents is essential to overcome the rapidly developing drug resistance and side effects with high efficacy. The QSAR and QPR study of spectral data with Hammett equation is interesting to predicting the reactivity on specific functional groups in the aromatic compounds such as carbonyl, vinyl, acetyl, azo, azomethines [24–28].

This study is useful for ground state equilibration behavior of carbonyl and vinyl compounds through infrared spectra [24–28]. The spatial arrangement of vinyl and heterocyclic ring protons were confirmed by proton NMR spectra [29–32]. Recently Rajarajan et al. have studied the spectral correlation study of some (E)-1-benzylidene-2-(4-chlorophenyl)hydrazines [33]. Vijayakumar et al. have studied the spectral correlation analysis of some substituted(E)-2-benzylidene hydrazine-carbothio amides and observed satisfactory and good correlation coefficients in single and multi-parameter correlations [34].

Within the above view, there is no information available in literature in the past for synthesis, spectral correlation and antimicrobial activities of the titled compounds. Therefore the authors have taken efforts for the synthesis of (E)-N’-(1-(substituted phenyl)ethylidene)benzohydrazides to study the quantitative structure activity relationships by spectral correlation through Hammett equation with their UV, IR and NMR
spectral data. Using Bauer-Kriby [35] disc diffusion method for evaluation of antimicrobial activities of synthesized \((E)-N'-(1-(\text{substituted phenyl})\text{ethylidene})\text{benzohydrazides}\).

2. EXPERIMENTAL

2.1. Materials and methods

All chemicals used were purchased from Sigma-Aldrich chemical company Bangalore. Melting points of all chalcones have been determined in open glass capillaries on Suntex melting point apparatus and are uncorrected. The Ultra violet spectra of the chalcones synthesized have been recorded using ELICO-double beam BL222 Bio- Spectrophotometer. Infrared spectra (KBr, 4000–400 cm\(^{-1}\)) have been recorded on AVATAR-300 Fourier transform spectrophotometer. BRUKER-500MHz NMR spectrometers have been utilized for recording \(^1\text{H}\) and \(^{13}\text{C}\) spectra in CDCl\(_3\) solvent using TMS as internal standard. The microanalysis of these hydrazides were performed in Thermofinnigan analyzer.

2.2. General procedure for synthesis of \((E)-N'-(1-(\text{substituted phenyl})\text{ethylidene})\text{benzohydrazides}\)

Appropriate mixture of benzhydrazide (100 mmol) and ortho, meta and para substituted acetophenones (100 mmol) and aqueous solution of sodium hydroxide (20 cm\(^3\) 0.5M) with absolute ethanol (20 cm\(^3\)) (Scheme 1). The reaction mixture was vigorously stirred at room temperature for 30 minutes [24]. After complete conversion of the ketones as monitored by TLC, the mixture was allowed to stand 20 minutes. The reagents were removed by filtration. The filtrate was washed with distilled water and recrystallized from absolute ethanol, dried well and kept in a desiccator.

\[
\begin{align*}
\text{O} & \quad \text{NH}_2 \\
\text{C} & \quad \text{O} \\
\text{N} & \quad \text{H}_3 \\
\text{X} & \quad \text{O} \\
\text{C} & \quad \text{O} \\
\text{N} & \quad \text{H}_3 \\
\text{X} & \quad \text{O} \\
\end{align*}
\]

\(X = \text{H, Br, F, 2-OH, 3-OH, 4-OH, 4-OMe, 4-CH}_3, 3-\text{NO}_2, 4-\text{NO}_2\)

Scheme 1.
<table>
<thead>
<tr>
<th>Entry</th>
<th>X</th>
<th>MF</th>
<th>MW</th>
<th>Yield [%]</th>
<th>Mp [°C]</th>
<th>Found C</th>
<th>(Calcd.) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>C_{15}H_{14}N_{2}O</td>
<td>238</td>
<td>90</td>
<td>141–142</td>
<td>75.54</td>
<td>(75.61) (11.76)</td>
</tr>
<tr>
<td>2</td>
<td>4-Br</td>
<td>C_{15}H_{13}BrN_{2}O</td>
<td>316</td>
<td>89</td>
<td>146–147</td>
<td>56.78</td>
<td>(56.80) (8.83)</td>
</tr>
<tr>
<td>3</td>
<td>4-F</td>
<td>C_{15}H_{13}FN_{2}O</td>
<td>256</td>
<td>91</td>
<td>139–140</td>
<td>70.27</td>
<td>(70.30) (10.93)</td>
</tr>
<tr>
<td>4</td>
<td>2-OH</td>
<td>C_{15}H_{14}N_{2}O_{2}</td>
<td>254</td>
<td>90</td>
<td>134–135</td>
<td>70.88</td>
<td>(70.85) (11.02)</td>
</tr>
<tr>
<td>5</td>
<td>3-OH</td>
<td>C_{15}H_{14}N_{2}O_{2}</td>
<td>254</td>
<td>87</td>
<td>119–120</td>
<td>70.88</td>
<td>(70.85) (11.02)</td>
</tr>
<tr>
<td>6</td>
<td>4-OH</td>
<td>C_{15}H_{14}N_{2}O_{2}</td>
<td>254</td>
<td>96</td>
<td>168–169</td>
<td>70.78</td>
<td>(70.85) (11.02)</td>
</tr>
</tbody>
</table>
cont. Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Substituent</th>
<th>Molecular Formula</th>
<th>Synthesis</th>
<th>Absorption</th>
<th>Emission</th>
<th>Antimicrobial Activity</th>
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</thead>
<tbody>
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<td>7</td>
<td>4-I</td>
<td>C_{15}H_{13}N_{2}O</td>
<td>364 99</td>
<td>182–183</td>
<td>48.98 (49.02)</td>
<td>3.55 (3.60)</td>
</tr>
<tr>
<td>8</td>
<td>4-OCH_{3}</td>
<td>C_{16}H_{16}N_{2}O_{2}</td>
<td>268 80</td>
<td>142–143</td>
<td>71.59 (71.62)</td>
<td>5.96 (6.01)</td>
</tr>
<tr>
<td>9</td>
<td>4-CH_{3}</td>
<td>C_{16}H_{16}N_{2}O</td>
<td>252 91</td>
<td>138–139</td>
<td>76.19 (76.16)</td>
<td>6.27 (6.39)</td>
</tr>
<tr>
<td>10</td>
<td>3-NO_{2}</td>
<td>C_{15}H_{13}N_{3}O_{3}</td>
<td>283 93</td>
<td>108–109</td>
<td>63.54 (63.60)</td>
<td>4.59 (4.63)</td>
</tr>
<tr>
<td>11</td>
<td>4-NO_{2}</td>
<td>C_{15}H_{13}N_{3}O_{3}</td>
<td>283 94</td>
<td>136–137</td>
<td>63.52 (63.60)</td>
<td>4.58 (4.63)</td>
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</tbody>
</table>
Table 2. The ultraviolet absorption maxima ($\lambda_{\text{max}}$, nm), infrared absorptions ($\nu$, cm$^{-1}$) and NMR chemical shifts ($\delta$ ppm) spectral data of substituted (E)-N'-{(1-phenylethylidene)benzohydrazide} compounds.

<table>
<thead>
<tr>
<th>Entry</th>
<th>X</th>
<th>UV $\lambda_{\text{max}}$ [nm]</th>
<th>IR $\nu$ [cm$^{-1}$]</th>
<th>$^1$H NMR [δ, ppm]</th>
<th>$^{13}$H NMR [δ, ppm]</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\lambda_{\text{max}}$</td>
<td>C=O</td>
<td>C=N</td>
<td>N-H</td>
</tr>
<tr>
<td>1</td>
<td>H</td>
<td>245.0</td>
<td>1645.28</td>
<td>1539.20</td>
<td>3452.58</td>
</tr>
<tr>
<td>2</td>
<td>4-Br</td>
<td>243.0</td>
<td>1651.07</td>
<td>1523.76</td>
<td>3277.06</td>
</tr>
<tr>
<td>3</td>
<td>4-F</td>
<td>247.0</td>
<td>1645.28</td>
<td>1543.05</td>
<td>3429.43</td>
</tr>
<tr>
<td>4</td>
<td>2-OH</td>
<td>245.0</td>
<td>1643.35</td>
<td>1537.27</td>
<td>3452.58</td>
</tr>
<tr>
<td>5</td>
<td>3-OH</td>
<td>216.0</td>
<td>1651.07</td>
<td>1535.34</td>
<td>3298.28</td>
</tr>
<tr>
<td>6</td>
<td>4-OH</td>
<td>215.0</td>
<td>1662.64</td>
<td>1517.98</td>
<td>3296.35</td>
</tr>
<tr>
<td>7</td>
<td>4-I</td>
<td>243.0</td>
<td>1664.57</td>
<td>1564.27</td>
<td>3296.35</td>
</tr>
<tr>
<td>8</td>
<td>4-OCH$_3$</td>
<td>242.0</td>
<td>1647.21</td>
<td>1529.55</td>
<td>3217.27</td>
</tr>
<tr>
<td>9</td>
<td>4-CH$_3$</td>
<td>248.0</td>
<td>1651.07</td>
<td>1521.84</td>
<td>3296.35</td>
</tr>
<tr>
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<td>3-NO$_2$</td>
<td>247.0</td>
<td>1656.85</td>
<td>1519.91</td>
<td>3290.56</td>
</tr>
<tr>
<td>11</td>
<td>4-NO$_2$</td>
<td>246.0</td>
<td>1649.14</td>
<td>1523.76</td>
<td>3294.42</td>
</tr>
</tbody>
</table>
The synthesized \((E)-N’-(1\text{-}(\text{substituted phenyl}) \text{ ethylidene})\) benzo-hydrazides have been characterized by their physical constants, elemental analysis and spectral data. The physical constants, analytical and micro analysis data of these \((E)-N’-(1\text{-}(\text{substituted phenyl}) \text{ ethylidene})\) benzo-hydrazides were presented in Table 1. The spectral data of synthesized substituted\((E)-N’-(1\text{-phenylethylidene})\)benzohydrazides are presented in Table 2.

3. RESULTS AND DISCUSSION

3.1. Spectral linearity

In the present investigation, the spectral linearity of synthesized \((E)-N’-(1\text{-phenylethylidene})\) benzohydrazides has been studied by evaluating the substituent effects. The spectral data observed for the benzohydrazides, UV \(\lambda_{\text{max}}\) (nm), infrared \(\nu\text{C}=\text{O}, \nu\text{C}=\text{N}, \nu\text{N-H}\), the proton chemical shifts \(\delta\text{(ppm)}\) of \(\text{N-H}\), \(\text{CH}_3\) and carbon chemical shifts of \(\text{C}=\text{O}, \text{C}=\text{N}\) and \(\text{CH}_3\) have been correlated with various Hammett substituent constants [25, 26, 27, 28].

3.2. UV spectral study

The measured absorption maxima \(\lambda_{\text{max}}\) (nm) values of the synthesized \((E)-N’-(1\text{-phenylethylidene})\)benzohydrazide compounds have been recorded and presented in Table 2. These data are correlated with Hammett substituent constants and \(F\) and \(R\) parameters using single and multi-linear regression analysis [25–28]. Hammett equation employed for the correlation analysis, involving the absorption maxima is as shown below in equation (1).

\[
\lambda = \rho \sigma + \lambda_0
\]

where \(\lambda_0\) is the frequency for the parent member of the series.

From the Table 3, it is evident that the UV absorption maximum \(\lambda_{\text{max}}\) (nm) values of all substituted \((E)-N’-(1\text{-phenylethylidene})\)benzohydrazide compounds, have shown poor correlations (\(r < 0.900\)) with Hammett substituent constants and \(F\) and \(R\) parameters. This is attributed to the weak polar, inductive, field and resonance effects of substituents unable to predicting the reactivity on the UV absorption through electronic effect of substituents as per the conjugative structure as shown in Figure 1.
Table 3. The results of statistical analysis of ultraviolet absorption maxima ($\lambda_{max}$, nm), infrared absorptions ($\nu$, cm$^{-1}$) and NMR chemical shifts ($\delta$ ppm) of substituted(\textit{E})-N'$^\prime$-(1-phenylethylidene)benzohydrazides with Hammett $\sigma$, $\sigma^+$, $\sigma_I$, $\sigma_R$ and $F$ and $R$ parameters.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Const.</th>
<th>R</th>
<th>I</th>
<th>$\rho$</th>
<th>S</th>
<th>n</th>
<th>Correlated derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{max}$</td>
<td>$\sigma$</td>
<td>0.844</td>
<td>237.93</td>
<td>14.811</td>
<td>11.46</td>
<td>11</td>
<td>H, 4-Br, 4-F, 2-OH, 3-OH, 4-OH, 4-I, 4-OCH$_3$, 4-CH$_3$, 3-NO$_2$, 4-NO$_2$</td>
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<tr>
<td></td>
<td>$\sigma^+$</td>
<td>0.869</td>
<td>239.55</td>
<td>15.046</td>
<td>9.13</td>
<td>11</td>
<td>H, 4-Br, 4-F, 2-OH, 3-OH, 4-OH, 4-I, 4-OCH$_3$, 4-CH$_3$, 3-NO$_2$, 4-NO$_2$</td>
</tr>
<tr>
<td></td>
<td>$\sigma_I$</td>
<td>0.821</td>
<td>237.29</td>
<td>7.264</td>
<td>12.65</td>
<td>11</td>
<td>H, 4-Br, 4-F, 2-OH, 3-OH, 4-OH, 4-I, 4-OCH$_3$, 4-CH$_3$, 3-NO$_2$, 4-NO$_2$</td>
</tr>
<tr>
<td></td>
<td>$\sigma_R$</td>
<td>0.838</td>
<td>243.74</td>
<td>17.959</td>
<td>11.78</td>
<td>11</td>
<td>H, 4-Br, 4-F, 2-OH, 3-OH, 4-OH, 4-I, 4-OCH$_3$, 4-CH$_3$, 3-NO$_2$, 4-NO$_2$</td>
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<tr>
<td></td>
<td>F</td>
<td>0.814</td>
<td>236.97</td>
<td>7.271</td>
<td>12.64</td>
<td>11</td>
<td>H, 4-Br, 4-F, 2-OH, 3-OH, 4-OH, 4-I, 4-OCH$_3$, 4-CH$_3$, 3-NO$_2$, 4-NO$_2$</td>
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<tr>
<td></td>
<td>R</td>
<td>0.870</td>
<td>247.46</td>
<td>20.313</td>
<td>9.01</td>
<td>11</td>
<td>H, 4-Br, 4-F, 2-OH, 3-OH, 4-OH, 4-I, 4-OCH$_3$, 4-CH$_3$, 3-NO$_2$, 4-NO$_2$</td>
</tr>
<tr>
<td>$\nu$CO</td>
<td>$\sigma$</td>
<td>0.805</td>
<td>1651.48</td>
<td>0.880</td>
<td>7.38</td>
<td>11</td>
<td>H, 4-Br, 4-F, 2-OH, 3-OH, 4-OH, 4-I, 4-OCH$_3$, 4-CH$_3$, 3-NO$_2$, 4-NO$_2$</td>
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<tr>
<td></td>
<td>$\sigma^+$</td>
<td>0.811</td>
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<td>-1.423</td>
<td>7.34</td>
<td>11</td>
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</table>
cont. Table 3.

<table>
<thead>
<tr>
<th></th>
<th>$\sigma_I$</th>
<th>1649.60</th>
<th>5.938</th>
<th>7.24</th>
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<td>1652.83</td>
<td>5.555</td>
<td>7.23</td>
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<tr>
<td>F</td>
<td>0.813</td>
<td>1650.38</td>
<td>3.184</td>
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<tr>
<td>R</td>
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<td>1652.10</td>
<td>1.336</td>
<td>7.36</td>
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<th>−4.599</th>
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H, 4-Br, 4-F, 2-OH, 3-OH, 4-OH, 4-I, 4-OCH$_3$, 4-CH$_3$, 3-NO$_2$, 4-NO$_2$
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<tr>
<th></th>
<th>vN-H</th>
<th>$\sigma$</th>
<th>335.45</th>
<th>-66.853</th>
<th>79.41</th>
<th>11</th>
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<td></td>
<td>$\sigma_I$</td>
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<td>11</td>
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<tr>
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<tr>
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<th>0.51</th>
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cont. Table 3.

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H, 4-Br, 4-F, 2-OH, 3-OH, 4-OH, 4-I, 4-OCH₃, 4-CH₃, 3-NO₂, 4-NO₂

r = Correlation co-efficient; ρ = slope; I = Intercept; s = Standard deviation; n = Number of substituents.
All the correlations have shown positive $\rho$ values. This indicates the operation of normal substituent effects on UV absorption maximum ($\lambda_{\text{max}}$ nm) values of all substituted (E)-$N'$(1-phenylethylidene)benzohydrazide compounds. All single parameter correlations were failed for the UV absorption maximum ($\lambda_{\text{max}}$ nm) values with Hammett constants and $F$ and $R$ parameter, it is decided to go for multi-regression analysis with Swain-Lupton’s [36]. While seeking the multi regression analysis, there is a satisfactory correlations are observed as shown in the following equations (2) and (3).

\[
\lambda_{\text{max}} \text{ (nm)} = 242.94 (\pm 8.475) + 92.067(\pm 17.554)\sigma_I + 17.470(\pm 15.655)\sigma_R
\]

\[r = 0.938, \text{n} = 11, P > 90\%\]

\[
\lambda_{\text{max}} \text{ (nm)} = 246.09(\pm 6.409) + 3.394(\pm 12.538)F + 20.100(\pm 7.149)R
\]

\[r = 0.971, \text{n} = 11, P > 95\%\]

3.3. IR spectral study

The assigned infrared frequencies (cm$^{-1}$) of $\nu$C=O, $\nu$C=N, $\nu$N–H of substituted (E)-$N'$(1-phenylethylidene)benzohydrazides are presented in Table 2. The measured infrared frequencies (cm$^{-1}$) were correlated [25, 26, 27, 28] with Hammett substituent constants and $F$ and $R$ parameters using single and multi-linear regression analysis. Hammett equation employed for the correlation analysis, involving the absorption maxima is as shown below in equation (4).

\[\nu = \rho\sigma + \nu_o\]

where $\nu_o$ is the frequency for the parent member of the series.
3.3.1. IR Spectral Correlation of $\nu$C=O (cm$^{-1}$)

From the Table 3, it is evident that the IR frequency $\nu$C=O(cm$^{-1}$) values of all substituted (E)-N’-(1-phenylethylidene)benzohydrazide compounds have shown poor correlations ($r < 0.900$) with Hammett substituent constants and $F$ and $R$ parameters. This is attributed to the weak polar, inductive, field and resonance effects of substituents are incapable for predicting the reactivity on the $\nu$C=O(cm$^{-1}$) through electronic effects of substituents and it is associated with the conjugative structure as shown in Figure 1.

All correlations have shown positive $\rho$ values except $\sigma^+$ constant. This indicates that the normal substituent effects operates in all substituted (E)-N’-(1-phenylethylidene)benzohydrazide compounds.

3.3.2. IR Spectral Correlation of $\nu$C=N (cm$^{-1}$)

From the Table 3, it is evident that the IR frequency $\nu$C=N(cm$^{-1}$) values of all substituted (E)-N’-(1-phenylethylidene)benzohydrazide compounds have shown poor correlations ($r < 0.900$) with Hammett substituent constants and $F$ and $R$ parameters. This is attributed to the weak polar, inductive, field and resonance effects of substituents for predicting the reactivity on the IR frequency $\nu$C=N(cm$^{-1}$) through resonance as per the conjugative structure as shown in Figure 1.

All the correlations have shown negative $\rho$ values. This indicates the operation of reverse substituent effect with respect to infrared $\nu$C=N(cm$^{-1}$) frequencies of all substituted (E)-N’-(1-phenylethylidene) benzohydrazide compounds.

3.3.3. IR Spectral Correlation of $\nu$N-H (cm$^{-1}$)

From the Table 3, it is evident that the IR $\nu$N–H(cm$^{-1}$) frequencies of all substituted (E)-N’-(1-phenylethylidene)benzohydrazide compounds have shown poor correlations ($r < 0.900$) with Hammett substituent constants and $F$ and $R$ parameters. This is attributed to the weak polar, inductive, field and resonance effects of substituents for predicting the reactivity on the IR frequency $\nu$N–H(cm$^{-1}$) through resonance-conjugative structure as shown in Figure 1.

All the correlations have shown negative $\rho$ values. This indicates the operation of reverse substituent effects by the electron donating substituents on IR $\nu$N–H(cm$^{-1}$) frequencies of all substituted (E)-N’-(1-phenylethylidene)benzohydrazide compounds.
All the single parameter correlations failed for the single parameter correlation of IR $\nu$C=O, $\nu$C=N and $\nu$N–H, (cm$^{-1}$) frequencies with Hammett constants and $F$ and $R$ parameters. Then the authors decided to go for multi-regression analysis with Swain-Lupton’s [36] parameters. While seeking the multi regression analysis there is a satisfactory correlations have to be found and are shown in the following equations (5) and (10).

\[
\nu_{\text{CO}}(\text{cm}^{-1}) = 1651.05(\pm 5.146) + 4.610(\pm 10.659)\sigma_{I} + 4.465(\pm 9.506)\sigma_{R} \quad (r = 0.925, n = 11, P > 90%)
\]

\[
\nu_{\text{CO}}(\text{cm}^{-1}) = 1650.90(\pm 5.238) + 2.962(\pm 10.246)F + 1.150(\pm 5.842)R \quad (r = 0.912, n = 11, P > 90%)
\]

\[
\nu_{\text{CN}}(\text{cm}^{-1}) = 1529.48(\pm 10.053) + 0.989(\pm 20.823)\sigma_{I} - 11.374(\pm 18.571)\sigma_{R} \quad (r = 0.902, n = 11, P > 90%)
\]

\[
\nu_{\text{CN}}(\text{cm}^{-1}) = 1529.72(\pm 10.099) + 2.010(\pm 19.756)F - 4.900(\pm 11.264)R \quad (r = 0.915, n = 11, P > 90%)
\]

\[
\nu_{\text{NH}}(\text{cm}^{-1}) = 3329.92(\pm 57.648) - 48.873(\pm 119.402)\sigma_{I} - 61.773(\pm 106.491)\sigma_{R} \quad (r = 0.927, n = 11, P > 90%)
\]

\[
\nu_{\text{NH}}(\text{cm}^{-1}) = 334.582(\pm 59.372) - 25.275(\pm 116.143)F - 6.260(\pm 66.223)R \quad (r = 0.908, n = 11, P > 90%)
\]

3.4. NMR spectral study

In nuclear magnetic resonance spectra, the proton and the $^{13}$C chemical shifts ($\delta$) depends on the electronic environment of the nuclei concerned. The assigned chemical shifts (ppm) have been correlated with reactivity parameters using Hammett equation [25, 26, 27, 28] in the form of

\[
\delta = \rho\sigma + \delta_{o}
\]

where $\delta_{o}$ is the frequency for the parent member of the series.
3.4.1. \(^1^H\) NMR Spectral Correlation

3.4.1.1. \(^1^H\) NMR Spectral Correlations of N-H (ppm)

From the Table 3, the assigned N-H chemical shifts (δ ppm) values of (E)-N'-(1-phenylethylidene)benzohydrazide compounds have shown satisfactory correlations with Hammett substituent constants σ (r = 0.900), σ\(^+\) (r = 0.906), σ\(_R\) (r = 0.905), ) , R parameter (r = 0.906). However, (δ ppm)\(^1^H\) NMR values of (E)-N’-(1-phenylethylidene)benzohydrazide compounds have shown poor correlation (r < 0.900) with Hammett \(\sigma_1\) substituent constant and F parameter. The reason for the poor correlation is due to the incapability of weak field and +I and –I effects of substituents are unable to predict the reactivity on the proton chemical shifts (δ, ppm). Also this is associated with the resonance-conjugative structure as shown in Figure 1.

All the correlations have shown positive ρ values. This indicates the operation of normal substituent effects on the proton NMR chemical shifts(δ, ppm) of all substituted (E)-N’-(1-phenylethylidene)benzohydrazide compounds.

3.4.1.2. \(^1^H\) NMR Spectral Correlations of CH\(_3\) (ppm)

From the Table 3, the assigned CH\(_3\) proton chemical shifts (δ ppm) values of (E)-N’-(1-phenylethylidene)benzohydrazide compounds have shown poor correlations (r < 0.900) with Hammett substituent constants and F and R parameter. This due to the absence of electronic effects such as polar, inductive, field and resonance effects of substituents for predicting the reactivity on the CH\(_3\) proton chemical shifts (δ ppm). The substituents effect of substituents are unable to dominates to the methyl proton chemical shifts(δ, ppm) by more than 4 or more carbon atoms of lengths and it is associated with resonance-conjugative structure as shown in Figure 1.

Some of the correlations have shown negative ρ values. This indicates the operation of reversal substituent effects by electron donating substituents on CH\(_3\) proton chemical shifts(δ ppm) of all substituted (E)-N’-(1-phenylethylidene)benzohydrazide compounds.

Some of the single parameter correlations analyses have shown poor correlation coefficients with Hammett substituent constants and F and R parameter, then the authors have decided to go for multi regression analysis with Swain-Lupton’s [36] parameters. While seeking the multi-regression analysis, there is a satisfactory correlation coefficients observed and are shown in the following equations (11) and (14).
\[ \delta N-H(ppm) = 8.858(\pm 0.368) - 0.080(\pm 0.763)\sigma I + \\
+ 1.225(\pm 0.681)\sigma R \quad (11) \]

\[ (r = 0.954, n = 11, P > 90\%) \]

\[ \delta N-H(ppm) = 8.871(\pm 0.320) - 0.097(\pm 0.627)F + \\
+ 0.921(\pm 0.357)R \quad (12) \]

\[ (r = 0.967, n = 11, P > 95\%) \]

\[ \delta CH_3(ppm) = 1.496(\pm 0.145) + 0.253(\pm 0.301)\sigma I - \\
- 0.162(\pm 0.269)\sigma R \quad (13) \]

\[ (r = 0.931, n = 11, P > 95\%) \]

\[ \delta CH_3(ppm) = 1.480(\pm 0.141) + 0.226(\pm 0.277)F - \\
- 0.134(\pm 0.157)R \quad (14) \]

\[ (r = 0.931, n = 11, P > 95\%) \]

3.4.2. $^{13}$C NMR Spectral Correlation

3.4.2.1. $^{13}$C NMR Spectral Correlations of C=O (ppm)

From the Table 3, the assigned $^{13}$C NMR chemical shifts ($\delta$ ppm) of C=O carbons of (E)-N’-(1-phenyl ethylidene)benzohydrazide compounds have shown poor correlations ($r < 0.900$) with Hammett substituent constants and $F$ and $R$ parameter. This is attributed to the weak and absence of polar, inductive, field and resonance effects of substituents for predicting the reactivity and associated with the resonance-conjugative structure as shown in Figure 1.

All the correlations have shown positive $\rho$ values. This indicates the operation of normal substituent effects with respect to $^{13}$C NMR chemical shifts($\delta$ ppm) of C=O carbons of all substituted (E)-N’-(1-phenyl-ethylidene)benzohydrazide compounds.

3.4.2.2. $^{13}$C NMR Spectral Correlations of C=N (ppm)

From the Table 3, the assigned C=N chemical shifts($\delta$ ppm) values of (E)-N’-(1-phenylethylidene)benzohydrazide compounds have shown poor correlations ($r < 0.900$) with Hammett substituent constants and $F$ and $R$ parameter. The reason for the poor correlations was stated earlier and associated with resonance-conjugative structure as shown in Figure 1.

All the correlations have shown negative $\rho$ values. This indicates the operation of reverse substituent effects with respect to $^{13}$C NMR chemical shifts($\delta$ ppm) of C=O carbons of all substituted (E)-N’-(1-phenyl-ethylidene)benzohydrazide compounds.
3.4.2.3. $^{13}$CNMR Spectral Correlations of CH$_3$ (ppm)

From the Table 3, the assigned CH$_3$ chemical shifts ($\delta$ ppm) values of (E)-N’-(1-phenylethylidene)benzohydrazide compounds have shown satisfactory correlations ($r > 0.900$) with Hammett substituent constants and $F$ and $R$ parameter.

All the correlations have shown positive $\rho$ values. This indicates the operation of normal substituent effect with respect to CH$_3$ chemical shifts ($\delta$ ppm) values of all substituted (E)-N’-(1-phenylethylidene) benzohydrazide compounds.

Some of the single parameter correlations analyses have shown poor correlation coefficients with Hammett substituent constants and $F$ and $R$ parameter, then it is decided to go for multi-regression analysis with Swain-Lupton’s [36] parameters. While seeking the multi-regression analysis, there is a satisfactory correlations have to be found and are shown in the multi-regression equations (14) and (19).

\[
\delta C=O \text{ (ppm)} = 162.619(\pm 1.093) + 1.183(\pm 2.264)\sigma_I + \\
+ 1.293(\pm 2.019)\sigma_R \quad (r = 0.932, n = 11, P > 95\%)
\]

\[
\delta C=O \text{ (ppm)} = 162.681(\pm 1.005) + 1.526(\pm 1.966)F + \\
+ 1.400(\pm 1.121) R \quad (r = 0.942, n = 11, P > 95\%)
\]

\[
\delta C=N \text{ (ppm)} = 168.384(\pm 0.898) - 0.216(\pm 1.861)\sigma_I + \\
+ 0.242(\pm 1.660)\sigma_R \quad (r = 0.905, n = 11, P > 95\%)
\]

\[
\delta C=N \text{ (ppm)} = 168.089(\pm 0.891) + 0.311(\pm 1.743)F - \\
- 0.131(\pm 0.994)R \quad (r = 0.907, n = 11, P > 90\%)
\]

\[
\delta C=O \text{ CH}_3 \text{ (ppm)} = 12.943(\pm 0.593) + 2.958(\pm 1.228)\sigma_I + \\
+ 1.650(\pm 1.095)\sigma_R \quad (r = 0.975, n = 11, P > 95\%)
\]

\[
\delta C=O \text{ CH}_3 \text{ (ppm)} = 12.855(\pm 0.625) + 2.894(\pm 1.223)F + \\
+ 1.022(\pm 0.697)R \quad (r = 0.972, n = 11, P > 90\%)
\]
3.5. Anti-microbial activities

All the aryl hydrazides possess a wide range of biological activities. These multi-prolonged activities are associated with different substituents and the unsaturation of C=N moiety in between the aryl rings. Hence, it is intended to examine their antimicrobial activities against their respective microbes—bacterial and fungal strains.

3.6. Antibacterial sensitivity assay

Antibacterial sensitivity assay has been performed using disc diffusion technique [30]. In each Petri plate about 0.5 cm$^3$ of the test bacterial sample has been spreaded uniformly over the solidified Mueller Hinton agar using sterile glass spreader. Then the discs with 5 mm diameter made up of Whatmann No.1 filter paper, impregnated with the solution of the compound have been placed on the medium using sterile forceps. The plates have been incubated for 24 hours at 37°C by keeping the plates upside down to prevent the collection of water droplets over the medium. After 24 hours, the plates have been visually examined and the diameter values of the zone of inhibition were measured. Triplicate results have been recorded by repeating the same procedure.

The antibacterial screening effect of synthesized (E)-N’-(1-phenylethylidene) benzohydrazide is shown in Figure 2 (Plates 1–26). The antibacterial activities of all the synthesized imines have been studied against four gram positive pathogenic strains S. Aureus, S. Pyogenes, M. Luteus, B. Subtilis and seven gram negative strains K. Pneumoniae, S. Typhi, V. Parahaemdyticus, V. Cholerae, K. Oxytoca, P. Mirabilis, S. Paratyphi, E. Coli, P. Aeruginosa. The disc diffusion technique was followed at a concentration of 250 µg/cm$^3$ with Ciprofloxacin taken as the standard.

The mm of zone of inhibition is compared using Table 4 and Table 5 is the corresponding clustered column chart is shown in Figure-3. A good antibacterial activity has been possessed by all substituents on the microorganisms in general. The substituents 4-CH$_3$, 3-NO$_2$and 4-NO$_2$ have very good activity against all bacterial strains. The parent compound (H), 4-Br, 4-F, 4-OCH$_3$ and 4-NO$_2$ substitutes has equal antibacterial activity with standard Ciprofloxacin against B.subtilis bacterial strains.
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<th>(\text{S. Pyogenes})</th>
<th>(\text{M. Luteus})</th>
<th>(\text{B Subtilis})</th>
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Table 5. Antibacterial activity of substituted\((E)\)-\(N'\)-(1-phenylethylidene)benzohydrazides compounds

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<td>6</td>
</tr>
<tr>
<td>Standard</td>
<td>Ciprofloxacin</td>
<td>15</td>
<td>13</td>
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<tr>
<td>Control</td>
<td>DMSO</td>
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Fig. 2. Antibacterial activities of Substituted($E$)-$N'-(1$-phenylethylidene)benzo-hydrazides.

![Plate 25](image1)
![Plate 26](image2)

Fig. 3. Antibacterial activities of substituted($E$)-$N'-(1$-phenylethylidene)benzo-hydrazide-clustered column chart.

### 3.7. Antifungal sensitivity assay

Antifungal sensitivity assay has been performed using disc diffusion technique. PDA medium was prepared and sterilized as above. It has been poured (ear bearing heating condition) in the Petri-plate which has been...
already filled with 1 cm$^3$ of the fungal species. The fungal activities of all the synthesized \((E)-N'-(1\text{-phenylethylidene})\text{benzohydrazide}\) have been studied against three fungal species \textit{A.niger, M.species}, and \textit{T.viride}.

The plates have been rotated clockwise and counter clock-wise for uniform spreading of the species. The discs have been impregnated with the test solution. The test solution has been prepared by dissolving 15mg of the imines in 1ml of DMSO solvent. The medium have been allowed to solidify and kept for 24 h. Then the plates have been visually examined and the diameter values of zone of inhibition have been measured. Triplicate results have been recorded by repeating the same procedure. The antifungal activities of substituted \((E)-N'-(1\text{-phenylethylidene})\text{benzohydrazide}\) have been studied and are shown in Figure 4 for Plates (27–32) and the zone of inhibition values of the effect is given in Table 6. The clustered column chart, shown in Figure-5. It reveals that the compounds with 4-Br and 3-NO$_2$ substituents have moderate antifungal activity against all fungal species namely \textit{A.niger, M.species} and \textit{T.viride}.

### Table 6. Antifungal activities of substituted\((E)-N'-(1\text{-phenylethylidene})\text{benzohydrazides}\)

<table>
<thead>
<tr>
<th>Entry</th>
<th>X</th>
<th>(\text{A.niger})</th>
<th>(\text{M.species})</th>
<th>(\text{T.viride})</th>
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<td>Control</td>
<td>DMSO</td>
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</table>
Fig. 4. Antifungal activities of Substituted\((E)-N'-(1\text{-phenylethylidene})\text{benzohydrazides}\).

Fig. 5. Antifungal activities of substituted\((E)-N'-(1\text{-phenylethylidene})\text{benzohydrazides-clusted column chart.}\)
4. CONCLUSIONS

A series containing eleven numbers of substituted \((E)-N'-(1\text{-}phenylethylidene)\) benzohydrazide compounds have been synthesized by condensation of benzohydrazide and substituted acetophenones. These synthesized substituted \((E)-N'-(1\text{-}phenylethylidene)\) benzo- hydrazide compounds have been characterized by their physical constants, spectral data. The assigned UV, IR, NMR spectral data of these aryl hydrazides has been correlated with Hammett substituent constants, \(F\) and \(R\) parameters. From the results of statistical analyses the effects of substituent on the spectral data have been studied. \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectral correlations produced more number of satisfactory correlations. But UV and IR spectral values give poor correlations with all Hammett substituents constants and Swain-Lupton’s parameters. However, all the multi-regression analyses have shown satisfactory correlations. The antimicrobial activities of all synthesized imines have been studied using Bauer-Kirby method. A very good antibacterial and antifungal activity was possessed by halogens, methoxy, methyl and nitro substituents in the hydrazides to the respective microorganisms.

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REFERENCES


