

## Full Length Research Paper

# Synthesis, Spectral Correlations and Biological Evaluation of Some Aryl (*E*)-*N*-Benzylidene-3-nitrobenzenamines

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**Abstract.** Assessment of substituent effects in a series of aryl imines through spectral correlation has been studied since they have close pharmacological association with diverse pharmacological properties. A series of aryl imines have been synthesized from 3-Nitro aniline with various substituted benzaldehydes were refluxed for 4h with 20 mL of absolute ethanol. The purity of all imines has been checked using their physical constants and spectral data. The UV  $\lambda_{max}$ (nm), infrared  $\nu_{C=N}$ ( $cm^{-1}$ ), NMR  $\delta$ (ppm) of CH=N and C=N spectral data have been correlated with Hammett substituent constants and F and R parameters using single and multi-linear regression analysis. From the results of statistical analysis, the effect of substituents on the above spectral data has been studied. The single parameter correlation with few Hammett constants and F and R parameters gave satisfactory correlation coefficients whereas all multiple correlations gave satisfactory correlation coefficients with Resonance, Field and Swain-Lupton's parameters. The antimicrobial activities of all imines have been studied using Bauer-Kirby method. Aryl imine compounds with 2-Cl, 4-Br and 2-OCH<sub>3</sub> substituents have shown good antibacterial activity against *S.aureus* and those with 3-NO<sub>2</sub> and 4-NO<sub>2</sub> substituents have shown good antifungal activity against *T.viridi*.

**Key words:** *E*-Aryl imines; UV, IR and NMR spectra; Substituent effects.

## 1. INTRODUCTION

The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. However, the increasing microbial resistance to antibiotics in use nowadays necessitates the search for new compounds with potential effects against pathogenic bacteria. The most spectacular advances in medicinal chemistry have been made when heterocyclic compounds played an important role in regulating biological activities. Benzylidene anilines belong to a class of compound called aldimines, which are the condensed products of aldehydes or ketones with primary amines and have azomethine group (CH=N) as the characteristic functional moiety. Interests in these compounds are largely due to their structural similarities with natural biological substances and relatively simple procedures of synthesis as well as synthetic flexibility that enable the design of suitable structural properties (Patai 2009; Jungreis et al., 1969). They are well known intermediate for the preparation of azetidinone (Bongini et al., 2000), thiazolidinone (Mulwad et al., 2002), formazone (Weber et al., 2005), arylacetamide (Fukumura 2008), metal complexes (Singh 2007; Zhu

et al., 2008; Zhu et al., 2009) and many other derivatives (Wang et al., 2008; Cheng et al., 2009).

An interesting application of Schiff bases is their use as an effective corrosion inhibitor which is based on their ability to spontaneously form a monolayer on the surface to be protected (Hosseini et al., 2008). Schiff bases have been found to possess more inhibitor efficiency than their constituent carbonyls and amines (Chitra et al., 2010). The Schiff bases constitute one of the most active classes of the compounds possessing diversified biological applications. The Schiff bases have been reported to possess higher degree of antitubercular (Solak et al., 2006), anticancer (Kuzmin et al., 2005), antibacterial (Shlyakhov et al., 1989), anti-inflammatory (Bawa et al., 2009), antifungal (Hothi et al., 2008). Several Schiff bases have been reported to possess remarkable antitumor (Kato, 1985), diuretic (Mishra et al., 1995), insecticidal (Zhu et al., 2000), anti-HIV (Sridhar et al., 2001) and antiparasitic (Forbes et al., 2003) activities. Therefore the authors have taken efforts for the synthesis of substituted benzylidene-3-nitroamines in condensation reaction. Also there is no information in the literature regarding the correlation study of the quantitative structure property relationship from UV, IR and NMR spectroscopic data with Hammett equation. The biological activities of these imine

derivatives have been studied using Bauer–Kirby (Bauer et al., 1966) method.

## 2. MATERIALS AND METHODS

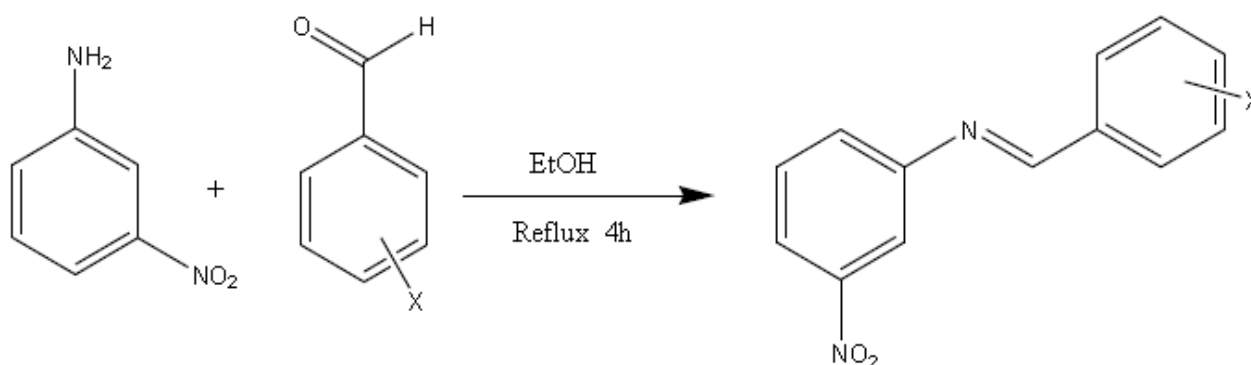
### 2.1. General

All the chemicals involved in the present investigation, have been procured from Sigma-Aldrich and E-Merck chemical companies. Melting points of all imines have been determined in open glass capillaries on SUNTEX melting point apparatus and are uncorrected. The UV spectra of all the imines, synthesized, have been recorded with ELICO-BL222 spectrophotometer  $\lambda_{\max}$  (nm) in spectral grade methanol solvent. Infrared spectra (KBr, 4000-400  $\text{cm}^{-1}$ ) have been recorded on AVATAR-300 Fourier transform spectrophotometer. The NMR spectra were recorded in Bruker AV400 NMR spectrometer

operating at 400 MHz has been utilized for recording  $^1\text{H}$  NMR spectra and 100 MHz for  $^{13}\text{C}$  spectra in  $\text{CDCl}_3$  solvent using TMS as internal standard.

### 2.2. Preparation of Schiff base

Equimolar quantities of benzaldehyde (0.01mol) and 3-nitroaniline (0.01mol) were refluxed for 4h with 20 mL of absolute ethanol (Issa et al., 2008) and it is shown in (Scheme-1). After the completion of the reaction, as monitored by TLC, the mixture was cooled at room temperature. The resulting precipitate was filtered and washed with cold water. The product appeared as pale yellow solid. Then this was recrystallized using ethanol to obtain pale yellow glittering solid. The analytical and physical constants of these synthesized schiff's bases are presented in Table 1.



X = H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH<sub>3</sub>, 2-OCH<sub>3</sub>, 4-OCH<sub>3</sub>, 3-NO<sub>2</sub>.

**Scheme 1:** Synthesis of (*E*)-*N*-Benzylidene-3-nitrobenzenamines

**Table 1:** Physical constants and mass spectral data of substituted benzylidene-3-nitroanilines

| Entry | X                  | M. F.  | M. W.  | m.p.(°C)               |
|-------|--------------------|--|--------|------------------------|
| 1     | H                  | C <sub>13</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub>  | 226.23 | 101-102 [74](32)       |
| 2     | 4-Br               | C <sub>13</sub> H <sub>9</sub> BrN <sub>2</sub> O <sub>2</sub> | 305.13 | 108-109                |
| 3     | 2-Cl               | C <sub>13</sub> H <sub>9</sub> ClN <sub>2</sub> O <sub>2</sub> | 260.68 | 91-92[114-116](33)     |
| 4     | 4-Cl               | C <sub>13</sub> H <sub>9</sub> ClN <sub>2</sub> O <sub>2</sub> | 260.68 | 120-121[124-125.5](33) |
| 5     | 4-F                | C <sub>13</sub> H <sub>9</sub> FN <sub>2</sub> O <sub>2</sub>  | 244.22 | 96-97                  |
| 6     | 4-CH <sub>3</sub>  | C <sub>14</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub>  | 240.26 | 112-113                |
| 7     | 2-OCH <sub>3</sub> | C <sub>14</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub>  | 256.26 | 73-74                  |
| 8     | 4-OCH <sub>3</sub> | C <sub>14</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub>  | 256.26 | 104-105[109](34)       |
| 9     | 3-NO <sub>2</sub>  | C <sub>13</sub> H <sub>9</sub> N <sub>3</sub> O <sub>4</sub>   | 377.40 | 141-142[158](35)       |

## 3. RESULTS AND DISCUSSION

### 3.1. Spectral linearity

In the present study the spectral linearity of synthesized imines has been studied by evaluating the substituent effects. The spectral data observed for the imines, UV  $\lambda_{\max}$ (nm), infrared  $\nu\text{C}=\text{N}$ , the proton

chemical shifts  $\delta$ (ppm) of C-H and carbon chemical shifts of C=N are correlated with various substituent constants.

#### 3.1.1. UV-Vis spectral study

The measured absorption maxima ( $\lambda_{\max}$  nm) values of the synthesized imines 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 (Sakthinathan et al., 2012, Suresh et al., 2013, Sathiyamoorthi et al., 2013, Jovanovic et al., 2002 and Drmanic et al., 2002). Hammett equation employed,

for the correlation analysis, involving the absorption maxima is as shown below in equation (1).

$$\lambda = \rho\sigma + \lambda_0 \quad (1)$$

Where  $\lambda_0$  is the frequency for the parent member of the series.

**Table 2:** The UV, IR and NMR spectroscopic data of substituted benzylidene-3-nitroanilines

| Entry | X                  | UV $\lambda_{max}$ | $\nu$ IR         | $\delta^1H$ | $\delta^{13}C$ |
|-------|--------------------|--------------------|------------------|-------------|----------------|
|       |                    | (nm)               | C=N( $cm^{-1}$ ) | CH=N(ppm)   | C=N(ppm)       |
| 1     | H                  | 300.89             | 1525.69          | 8.38        | 161.09         |
| 2     | 4-Br               | 332.50             | 1521.84          | 8.46        | 161.19         |
| 3     | 2-Cl               | 329.95             | 1525.84          | 8.37        | 159.25         |
| 4     | 4-Cl               | 337.50             | 1510.26          | 8.40        | 160.79         |
| 5     | 4-F                | 329.81             | 1512.19          | 8.41        | 161.03         |
| 6     | 4-CH <sub>3</sub>  | 295.24             | 1525.69          | 8.38        | 160.73         |
| 7     | 2-OCH <sub>3</sub> | 357.50             | 1519.91          | 8.90        | 159.86         |
| 8     | 4-OCH <sub>3</sub> | 349.86             | 1517.98          | 8.70        | 161.90         |
| 9     | 3-NO <sub>2</sub>  | 339.96             | 1519.91          | 8.95        | 159.65         |

The results of statistical analysis of these values with Hammett substituent constants are presented in Table 3. From Table 3, it is observed that the UV

absorption maximum  $\lambda_{max}(nm)$  values have shown satisfactory correlation with Hammett substituent constants  $\sigma_I$  ( $r = 0.906$ ),  $\sigma_R$  ( $r=0.900$ ).

**Table 3:** Results of statistical analysis of UV  $\lambda_{max}$  (nm),  $\nu$  C=N ( $cm^{-1}$ ) IR, NMR  $\delta^1H$  (ppm) CH=N and  $\delta^{13}C$  (ppm) C= N of substituted benzylidene-3-nitroanilines with Hammett substituent constants  $\sigma$ ,  $\sigma^+$ ,  $\sigma_I$ ,  $\sigma_R$ , F and R parameters

| Frequency       | Constants  | r     | I       | $\rho$ | s     | n | Correlated derivatives  |
|-----------------|------------|-------|---------|--------|-------|---|---|
| $\lambda_{max}$ | $\sigma$   | 0.802 | 330.44  | -01.24 | 21.92 | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
|                 | $\sigma^+$ | 0.705 | 330.38  | -04.70 | 21.79 | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
|                 | $\sigma_I$ | 0.906 | 311.22  | 55.20  | 16.87 | 7 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 3-NO <sub>2</sub>   |
|                 | $\sigma_R$ | 0.900 | 320.23  | -44.86 | 18.75 | 8 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub>                     |
|                 | F          | 0.855 | 313.96  | 45.11  | 18.27 | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
|                 | R          | 0.845 | 320.24  | -37.76 | 19.32 | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
| $\nu$ C=N       | $\sigma$   | 0.809 | 1520.03 | 01.56  | 6.09  | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
|                 | $\sigma^+$ | 0.812 | 1519.91 | 01.43  | 6.07  | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
|                 | $\sigma_I$ | 0.923 | 1524.32 | 12.67  | 5.20  | 7 | H, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub>                           |
|                 | $\sigma_R$ | 0.835 | 1521.87 | 08.62  | 5.71  | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
|                 | F          | 0.938 | 1524.92 | 13.72  | 4.87  | 7 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
|                 | R          | 0.840 | 1522.33 | 08.98  | 5.59  | 9 | H, 4-Br, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub>             |
| $\delta$ CH=N   | $\sigma$   | 0.808 | 8.66    | -0.05  | 0.25  | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
|                 | $\sigma^+$ | 0.806 | 8.65    | -0.03  | 0.25  | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
|                 | $\sigma_I$ | 0.822 | 8.56    | 0.26   | 0.24  | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
|                 | $\sigma_R$ | 0.823 | 8.58    | -0.29  | 0.24  | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
|                 | F          | 0.814 | 8.60    | 0.13   | 0.25  | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
|                 | R          | 0.823 | 8.60    | -0.18  | 0.24  | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
| $\delta$ C=N    | $\sigma$   | 0.935 | 160.67  | -0.96  | 0.84  | 7 | H, 4-Br, 4-Cl, 4-F, 4-CH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub>                            |
|                 | $\sigma^+$ | 0.913 | 160.61  | -0.96  | 0.76  | 7 | H, 4-Br, 4-Cl, 4-F, 4-CH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub>                            |
|                 | $\sigma_I$ | 0.831 | 161.04  | -1.24  | 0.85  | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
|                 | $\sigma_R$ | 0.732 | 169.49  | -0.52  | 0.89  | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
|                 | F          | 0.822 | 160.89  | -0.77  | 0.88  | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |
|                 | R          | 0.831 | 160.33  | -1.04  | 0.86  | 9 | H, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH <sub>3</sub> , 2-OCH <sub>3</sub> , 4-OCH <sub>3</sub> , 3-NO <sub>2</sub> |

r = Correlation co-efficient;  $\rho$  = Slope; I = Intercept; s = Standard deviation; n = Number of substituents

The remaining Hammett substituent constants namely  $\sigma$ ,  $\sigma^+$  and F and R parameters have shown poor correlations. This is attributed to the weak polar, field and resonance effects of the substituents for predicting the reactivity on the UV absorption through resonance as per the conjugative structure as shown in Fig-1.

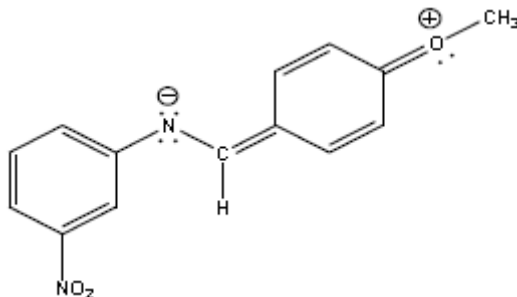
The multi regression analysis of these UV spectral data of all imines with inductive, resonance and Swain – Lupton’s (Swain et al., 1968) constants produce satisfactory correlations as shown in equations (2) and (3).

$$\lambda_{max}(nm)=301.989(\pm 9.641)+53.788(\pm 17.741)\sigma_I-43.097(\pm 14.079)\sigma_R \dots (2)$$

( $R = 0.980, n = 9, P > 95\%$ )

$$\lambda_{max}(nm)=307.517(\pm 11.792)+39.795(\pm 13.790)F-31.298(\pm 11.390)R \dots (3)$$

( $R = 0.965, n = 9, P > 95\%$ )



**Fig. 1:** The resonance-conjugative structure

### 3. 1. 2. IR Spectral study

The infrared  $\nu_{C=N}$  stretching frequencies ( $\text{cm}^{-1}$ ) of the synthesized imines have been recorded and presented in Table-2. These data are correlated (Sakthinathan et al., 2012, Suresh et al., 2013, Sathiyamoorthi et al., 2013, Jovanovic et al., 2002 and Drmanic et al., 2002) with Hammett substituent constants and Swain-Lupton's parameters. In this correlation the structure parameter Hammett equation employed is as shown in equation (4).

$$\nu = \rho\sigma + \nu_0 \quad (4)$$

Where  $\nu_0$  is the frequency for the parent member of the series.

The assigned  $\nu_{C=N}$  stretching frequencies ( $\text{cm}^{-1}$ ) are correlated with various Hammett substituent constants and  $F$  and  $R$  parameters through single and multi-

$$\nu_{C=N}(\text{cm}^{-1}) = 1526.352(\pm 3.573) - 12.402(\pm 4.132)\sigma_I + 8.216(\pm 2.699)\sigma_R \dots (5)$$

( $R = 0.962, n = 9, P > 95\%$ )

$$\nu_{C=N}(\text{cm}^{-1}) = 1526.353(\pm 3.278) - 12.571(\pm 4.152)F - 6.943(\pm 2.731)R \dots (6)$$

( $R = 0.967, n = 9, P > 95\%$ )

### 3.1.3. $^1\text{H}$ NMR Spectral study

The  $^1\text{H}$  NMR spectra of the imine derivatives under investigation have been recorded in deuteriochloroform solution employing tetramethylsilane (TMS) as internal standard and presented in Table-2. In nuclear magnetic resonance spectra, the  $^1\text{H}$  or the  $^{13}\text{C}$  chemical shifts ( $\delta$  ppm) depend on the electronic environment of the nuclei concerned. These chemical shifts have been correlated with reactivity parameters. Thus the Hammett equation has been used in the form as shown in (7).

$$\delta = \delta_0 + \rho\sigma \quad \dots (7)$$

Where  $\delta_0$  is the chemical shift of the corresponding parent compound.

regression analyses including Swain-Lupton's (Swain et al., 1968) parameters. The results of statistical analysis of single parameter correlation are shown in Table 3. From Table 3, it is observed that the infrared stretching frequency  $\nu_{C=N}(\text{cm}^{-1})$  values have shown satisfactory correlation with Hammett substituent constant  $\sigma_I$  ( $r = 0.923$ ) and  $F$  parameter ( $r = 0.938$ ). The remaining Hammett substituent constants namely  $\sigma$ ,  $\sigma^+$  and  $\sigma_R$  and  $R$  parameter have shown poor correlations. This is attributed to the weak polar, resonance effects of the substituents for predicting the reactivity on the stretching frequency through resonance as per the conjugative structure as shown in Fig-1. So, the authors think that it is worthwhile to seek the multi regression analysis which may produce a satisfactory correlation with Resonance, Field and Swain-Lupton's (Swain et al., 1968) constants. This is shown in the following equations (5) and (6).

The assigned proton chemical shifts (ppm) of imines have been correlated (Sakthinathan et al., 2012, Suresh et al., 2013, Sathiyamoorthi et al., 2013, Jovanovic et al., 2002 and Drmanic et al., 2002) with various Hammett sigma constants,  $F$  and  $R$  parameters. The results of statistical analysis are presented in Table-3. From Table-3, it is observed that the  $^1\text{H}$  NMR chemical shift  $\delta_{\text{CH=N}}$  (ppm) values have shown poor correlation ( $r < 0.900$ ) with Hammett substituent constants and  $F$  and  $R$  parameters. This is attributed to the weak polar, inductive, resonance and field effects of the substituents for predicting the reactivity on the  $^1\text{H}$  NMR chemical shift through resonance as per conjugative structure as shown in Fig-1. In view of the inability of the Hammett  $\sigma$  constants to produce individually satisfactory

correlations with the imine proton chemical shifts, the authors think that, it is worthwhile to seek multiple correlations involving either  $\sigma_I$  and  $\sigma_R$  constants or

$$\delta_{CH=N}(\text{ppm}) = 8.501(\pm 0.174) - 0.259(\pm 0.175) \sigma_I - 0.289(\pm 0.0764) \sigma_R \dots (8)$$

$(R = 0.935, n = 9, P > 95\%)$

$$\delta_{CH=N}(\text{ppm}) = 8.573(\pm 0.180) + 0.104(\pm 0.073) F - 0.166(\pm 0.072) R \dots (9)$$

$(R = 0.922, n = 9, P > 95\%)$

### 3.1.4. $^{13}\text{C}$ NMR spectra

In the present study, the chemical shifts (ppm) of imines  $\delta_{C=N}$  carbon, have been assigned and are presented in Table-2. Attempts have been made to correlate the  $\delta_{C=N}$  chemical shifts (ppm) with Hammett substituent constants, field and resonance parameters, with the help of single and multi-regression analyses to study the reactivity through the effect of substituents. The chemical shifts (ppm) observed for the  $\delta_{C=N}$  have been correlated with Hammett constants and the results of statistical analysis (Sakthinathan et al., 2012, Suresh et al., 2013, Sathiyamoorthi et al., 2013, Jovanovic et al., 2002 and Drmanic et al., 2002) are presented in Table-3. From Table-3, it is observed that the  $^{13}\text{C}$  chemical shift  $\delta_{C=N}(\text{ppm})$  values have shown satisfactory

$$\delta_{C=N}(\text{ppm}) = 160.869(\pm 1.569) - 2.240(\pm 0.726) \sigma_I - 3.962(\pm 1.144) \sigma_R \dots (10)$$

$$(R = 0.953, n = 9, P > 95\%)$$

$$\delta_{C=N}(\text{ppm}) = 160.066(\pm 1.006) + 0.930(\pm 0.054) F - 1.001(\pm 0.072) R \dots (11)$$

$$(R = 0.955, n = 9, P > 95\%)$$

## 3.2. Anti-microbial activities

Aryl imines 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.

### 3.2.1. Antibacterial sensitivity assay

Antibacterial sensitivity assay has been performed by using disc diffusion (Bauer et al., 1996) technique. In each Petri plate about 0.5 ml of the test bacterial sample has been spread uniformly over the solidified Mueller Hinton agar using sterile glass spreader. Then the discs with 5mm 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

Swain-Lupton's (Swain et al., 1968)[6], F and R parameters. This is shown in the following equations (8) and (9).

correlation with Hammett substituent constants  $\sigma$  ( $r = 0.900$ ),  $\sigma^+$  ( $r = 0.927$ ),  $\sigma_R$  ( $r = 0.932$ ) and  $R$  ( $r = 0.902$ ) parameter. The remaining Hammett substituent constant  $\sigma_I$  and F parameter have shown poor correlations ( $r < 0.900$ ). This is attributed to weak inductive and field effects of the substituents for predicting the reactivity on the  $^{13}\text{C}$  chemical shift through resonance as per the conjugative structure as shown in Fig-1. Since some of the single regression analyses, have shown poor correlations with a few Hammett substituent constants and F parameter, it is decided to go for multi regression analysis. The multi regression analysis of chemical shift  $\delta_{C=N}(\text{ppm})$  values of all aryl imine compounds with inductive, resonance and Swain-Lupton's (Swain et al., 1968) parameters produce satisfactory correlations as shown in equations (10) & (11).

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 imines is shown in Fig-2 (Plates 1-10). The antibacterial activities of all the synthesized imines have been studied against three gram positive pathogenic strains *Micrococcus luteus*, *Bacillus subtilis*, *Staphylococcus aureus* and two gram negative strains *Escherichia coli* and *Klebsiella species*. The disc diffusion technique was followed at a concentration of 250  $\mu\text{g/mL}$  with Ampicillin taken as the standard drug. The zone of inhibition is compared using Table-4 and the corresponding clustered column chart is shown in Fig-3. A good antibacterial activity has been possessed by all substituents on the microorganisms in general. The substituents H and 2-Cl have very good activity against *Micrococcus luteus*. The substituent 4-Cl has improved antibacterial activity against *B. subtilis*. The substituents 2-Cl, 4-Br and 2-  $\text{OCH}_3$  have very good activity against *S. aureus*.

**Table 4:** Antibacterial activity of substituted benzylidene-3-nitroanilines

| S.No     | X                  | Zone of Inhibition (mm) |                    |                 |                        |                    |
|----------|--------------------|-------------------------|--------------------|-----------------|------------------------|--------------------|
|          |                    | Gram positive bacteria  |                    |                 | Gram negative bacteria |                    |
|          |                    | <i>B.subtilis</i>       | <i>Micrococcus</i> | <i>S.aureus</i> | <i>E.coli</i>          | <i>Pseudomonas</i> |
| 1        | H                  | -                       | 9                  | 7               | 8                      | 8                  |
| 2        | 4-Br               | -                       | 8                  | 9               | 7                      | -                  |
| 3        | 2-Cl               | 7                       | 12                 | 9               | -                      | 7                  |
| 4        | 4-Cl               | 9                       | -                  | 7               | -                      | 7                  |
| 5        | 4-F                | 8                       | 7                  | 8               | 7                      | -                  |
| 6        | 4-CH <sub>3</sub>  | -                       | -                  | -               | 8                      | -                  |
| 7        | 2-OCH <sub>3</sub> | -                       | -                  | 11              | 8                      | 8                  |
| 8        | 4-OCH <sub>3</sub> | 8                       | 7                  | -               | -                      | 7                  |
| 9        | 3-NO <sub>2</sub>  | 7                       | 7                  | 7               | -                      | 7                  |
| Standard | Ampicillin         | 14                      | 14                 | 14              | 16                     | 15                 |
| Control  | DMSO               | -                       | -                  | -               | -                      | -                  |

### 3.2.2. Antifungal sensitivity assay

Antifungal sensitivity assay has been performed using disc diffusion technique (Bauer et al., 1996). 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 1ml of the fungal species. 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 imines have been studied and are shown in Fig. 4 for Plates (1-4) and the zone of inhibition values of the effect is given in Table-5. The clustered column chart, shown in Fig-5. It reveals that the compounds with H, 2-Cl and 4-CH<sub>3</sub> substituents have moderate antifungal activity against *A.niger*. The compounds with substituents 3-NO<sub>2</sub> and 4-NO<sub>2</sub> have good activity against *T.viride*.

**Table 5:** Antifungal activities of substituted benzylidene-3-nitroanilines

| Entry    | X                  | Zone of Inhibition (mm) |                 |
|----------|--------------------|-------------------------|-----------------|
|          |                    | <i>A. niger</i>         | <i>T.Viride</i> |
| 1        | H                  | -                       | 11              |
| 2        | 4-Br               | -                       | 9               |
| 3        | 2-Cl               | 8                       | 12              |
| 4        | 4-Cl               | 8                       | 10              |
| 5        | 4-F                | -                       | 11              |
| 6        | 4-CH <sub>3</sub>  | 8                       | 9               |
| 7        | 2-OCH <sub>3</sub> | 12                      | 11              |
| 8        | 4-OCH <sub>3</sub> | 8                       | -               |
| 9        | 3-NO <sub>2</sub>  | 10                      | 11              |
| Standard | Miconazole         | 14                      | 13              |
| Control  | DMSO               | ---                     | ---             |



Plate-1



Plate- 2



Plate-3



Plate-4



Plate-5



Plate-6



Plate-7



Plate-8



Plate 9



Plate 10

Fig. 2: Antibacterial activities of Substituted(*E*)-*N*-benzylidene-3-nitro benzenamines-petri dishes



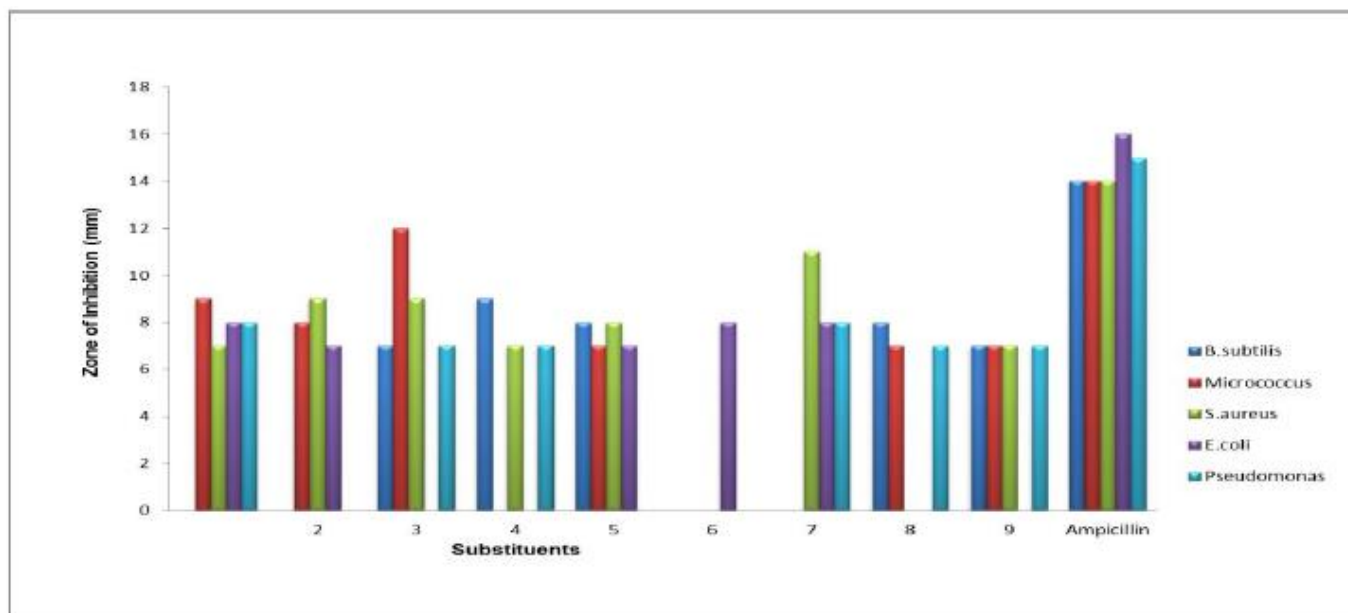


Fig. 3: Antibacterial activities of substituted(*E*)-*N*-benzylidene-3-nitro benzenamines-clustered column chart

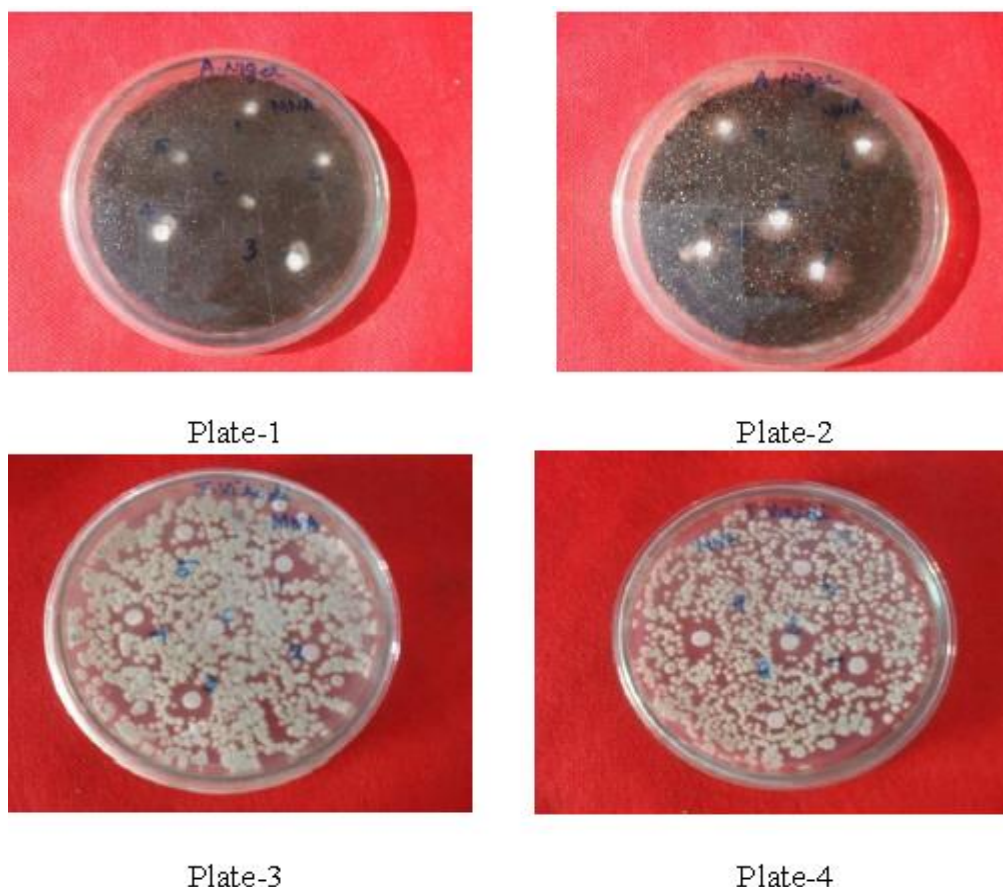


Fig. 4: Antifungal activities of substituted (*E*)-*N*-benzylidene-3-nitrobenzenamine compounds-petri plates

#### 4. CONCLUSION

Some imines have been synthesized by condensation of amines and benzaldehydes. These imines have been characterized by their physical constants, spectral data. The UV, IR, NMR spectral data of these imines

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. The antimicrobial activities of all synthesized imines have been studied using Bauer-Kirby method.



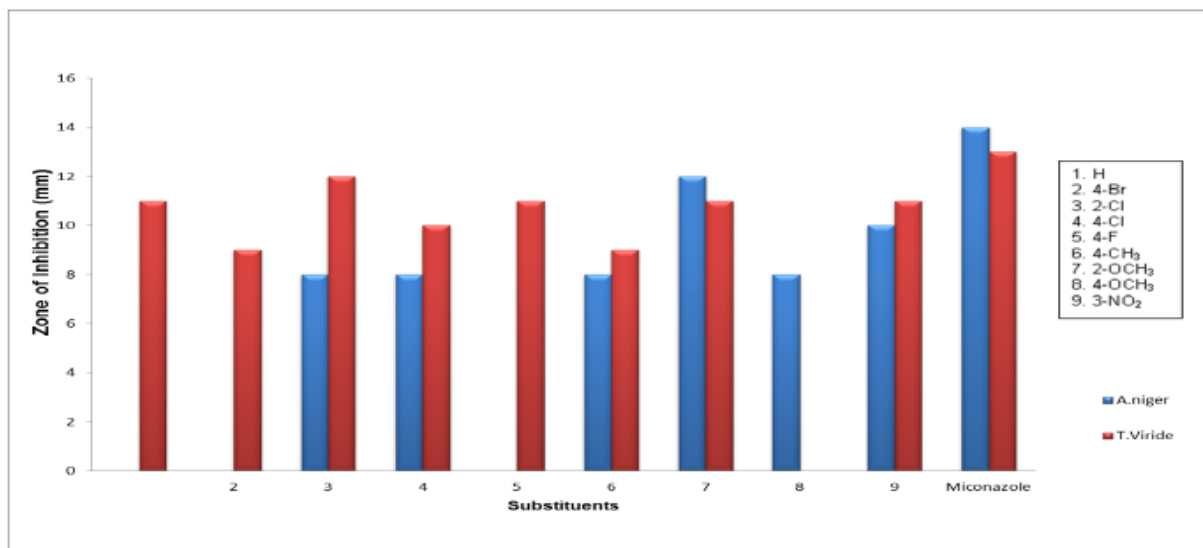


Fig. 5: Antifungal activities of substituted (E)-N-benzylidene-3-nitro benzenamine compounds-clustered column chart

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