

Synthesis, assessment of substituent effect and antimicrobial activities of some substituted (*E*)-*N*-benzylidene-5-bromopyridin-2-amines

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A series of substituted (*E*)-*N*-benzylidene-5-bromopyridin-2-amine compounds have been synthesized from 5-bromo-2-aminopyridine with different substituted benzaldehydes. The structure of the adducts was confirmed by their physical constants, UV, IR and NMR spectral data. The observed UV absorption maximum $\lambda_{max}C=N$ (nm), IR frequencies $\nu C=N$ (cm^{-1}), The 1H and ^{13}C NMR δ (ppm) chemical shifts values 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 spectral data has been studied. The antimicrobial activities of all synthesized imines have been studied using Bauer-Kirby method.

Keywords: *E*-imines, UV, IR and NMR spectra, Spectral QSAR study, Antimicrobial activities.

1. INTRODUCTION

The formation of carbon–nitrogen double bond plays an important role in organic synthesis [1]. This can be achieved by the reaction of

aldehydes and amines in acidic medium leading to the formation of imines. The compounds are called “Schiff bases” honor of Schiff who had synthesized such types of compounds first time in the past [2, 3].

Schiff bases have gained importance in medicinal and pharmaceutical fields due to the broad spectrum of biological activities like anti-inflammatory [4], analgesic [5], antimicrobial [6], anticonvulsant [7], anti-tubercular [8], anticancer [9], antioxidant [10], anthelmintic [11], and so forth. Schiff's bases are characterized by the CH=N (imine) group which finds importance in elucidating the mechanism of transamination and racemization reactions in biological systems [12, 13]. Apart from biological activities, Schiff bases are also used as catalysts, intermediates in organic synthesis, dyes, pigments, polymer stabilizers [14] and corrosion inhibitors [15]. In recent years, correlation analysis has been applied by chemists [16–19] for studying the quantitative structure-activity-relationships through Hammett correlations. Literature survey shows that there is no information available regarding the study of UV, IR and NMR spectral correlation and antimicrobial activities of substituted (*E*)-*N*-benzylidene-5-bromopyridin-2-amine compounds. Owing to their interesting characteristic properties and biological properties, the authors synthesizing substituted (*E*)-*N*-benzylidene-5-bromopyridin-2-amine compounds and studying the influence of substitution at benzylidene group on the spectral data and their antimicrobial activities.

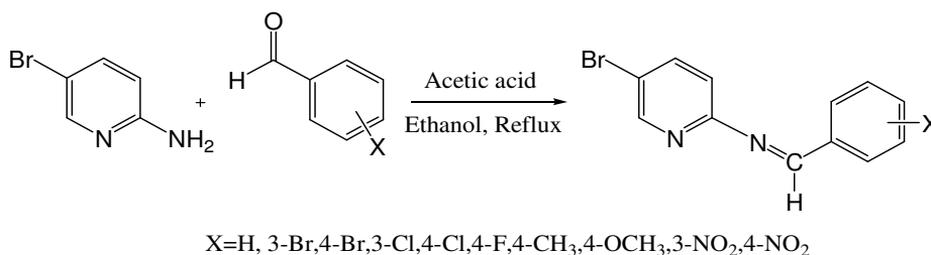
2. EXPERIMENTAL

2.1. General

All chemicals used have been obtained from Sigma–Aldrich and E-Merck chemical companies. Melting points of all (*E*)-*N*-benzylidene-5-bromopyridin-2-amines compound have been determined in open glass capillaries on a Mettler FP51 melting point apparatus and are uncorrected. The UV spectra of all synthesized (*E*)-*N*-benzylidene-5-bromopyridin-2-amine compounds have been recorded using SHIMADZU-1650 SPECTROMETER in spectral grade methanol. Infrared spectra (KBr, 4000–400 cm⁻¹) have been recorded on AVATAR-300 Fourier transform spectrophotometer. The NMR spectra of all (*E*)-*N*-benzylidene-5-bromopyridin-2-amines compound have been recorded in BRUKER 400 spectrometer operating at 500 MHz for ¹H NMR spectra and 125.46 MHz for ¹³C NMR spectra in CDCl₃ solvent using TMS as internal standard.

2.2. Synthesis of substituted (*E*)-*N*-benzylidene-5-bromopyridin-2-amines compound

A mixture of equimolar quantities of 2-amino-5-bromopyridine (0.01 mol), substituted benzaldehydes (0.01 mol) and 0.5 cm³ acetic acid were refluxed for 3h with 20 cm³ of absolute ethanol [16]. The completion of the reaction was monitored by TLC. The resulting mixture was cooled to room temperature. Then the precipitate obtained which was filtered at the filter pump and washed several times with cold water. A pale yellow solid was obtained as the final product. This crude product was recrystallized from ethanol to get glittering colorless solid, and their melting points have been noted. The general reaction is shown in Scheme 1.



Scheme 1. Synthesis of substituted (*E*)-*N*-benzylidene-5-bromopyridin-2-amines.

3. RESULTS AND DISCUSSION

3.1. UV-Vis spectral correlation

The assigned UV absorption maximum λ_{max} (nm) values of all substituted (*E*)-*N*-benzylidene-5-bromopyridin-2-amines compound are presented in Table 1. The UV absorption maximum values are correlated with different Hammett substituent constants and *F* and *R* parameters using single and multi-linear regression analyses [16–19]. While seeking Hammett correlation, involving UV absorption maximum values, the form of the Hammett equation employed is as given in equation (1):

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

where λ_0 is the absorption maximum of the parent member of this series.

Table 1. The UV, IR and NMR spectroscopic data of substituted (*E*)-*N*-benzylidene-5-bromopyridin-2-amine compounds.

Entry	X	Molecular formula	Molecular weight	Melting Point [°C]	λ_{\max} [nm]	$\nu_{\text{C=N}}$ [cm^{-1}]	$\delta^1\text{H}$ CH=N [ppm]	$\delta^{13}\text{C}$ C=N [ppm]
1	H	$\text{C}_{13}\text{H}_{11}\text{BrN}_2$	275.14	83-84	312.00	1627.13	8.088	157.09
2	3-Br	$\text{C}_{12}\text{H}_8\text{Br}_2\text{N}_2$	340.01	82-83	312.50	1626.18	8.182	161.69
3	4-Br	$\text{C}_{12}\text{H}_8\text{Br}_2\text{N}_2$	340.01	106-107	312.10	1626.43	8.087	157.10
4	3-Cl	$\text{C}_{12}\text{H}_8\text{BrClN}_2$	295.56	72-73	312.60	1601.77	8.084	156.96
5	4-Cl	$\text{C}_{12}\text{H}_8\text{BrClN}_2$	295.56	90-91	315.50	1617.56	8.094	161.93
6	4-F	$\text{C}_{12}\text{H}_8\text{BrFN}_2$	279.11	88-89	312.20	1629.71	8.090	157.06
7	4-CH ₃	$\text{C}_{13}\text{H}_{11}\text{BrN}_2$	275.14	100-101	313.50	1601.35	8.062	163.42
8	4-OCH ₃	$\text{C}_{13}\text{H}_{11}\text{BrN}_2\text{O}$	291.14	97-98	312.20	1625.79	8.088	157.02
9	3-NO ₂	$\text{C}_{12}\text{H}_8\text{BrN}_3\text{O}_2$	306.11	98-99	304.50	1592.89	8.241	160.40
10	4-NO ₂	$\text{C}_{12}\text{H}_8\text{BrN}_3\text{O}_2$	306.11	104-105	321.00	1621.86	8.174	160.60

The results of statistical analysis are presented in Table 2.

Table 2. Results of statistical analysis of UV λ_{\max} (nm), $\nu_{\text{C=N}}$ (cm^{-1}) IR, NMR $\delta^1\text{H}$ (ppm) CH=N and $\delta^{13}\text{C}$ (ppm) C=N of substituted (*E*)-*N*-benzylidene-5-bromopyridin-2-amine compounds with Hammett substituent constants σ , σ^+ , σ_{I} , σ_{R} and F and R parameters

Frequency	Constant	r	I	ρ	s	n	Correlated derivatives
λ_{\max}	σ	0.905	312.67	0.585	4.26	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 4-NO ₂
	σ^+	0.902	312.77	0.243	4.26	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 4-NO ₂
	σ_{I}	0.807	312.76	0.116	4.27	10	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
	σ_{R}	0.902	312.87	0.419	4.27	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 4-NO ₂
	F	0.801	312.89	-0.204	4.27	10	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
	R	0.821	312.92	0.586	4.27	10	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂

cont. Table 2.

Frequency	Constant	r	I	ρ	s	n	Correlated derivatives
$\nu_{\text{C=N}}$	σ	0.831	1619.74	-11.440	13.47	10	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
							H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
	σ^+	0.821	1618.30	-7.322	13.62	10	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
							H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
	σ_{I}	0.803	1617.74	-1.720	14.12	10	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
							H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
	σ_{R}	0.795	1613.16	-28.543	12.66	10	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
							H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
	F	0.804	1616.19	-2.150	14.11	10	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
							H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
R	0.904	1612.41	-24.486	12.59	8	3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂	

cont. Table 2.

Frequency	Constant	r	I	ρ	s	n	Correlated derivatives
	σ	0.905	8.08	0.125	0.07	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 3-NO ₂ , 4-NO ₂
	σ^+	0.904	8.10	0.074	0.07	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 3-NO ₂ , 4-NO ₂
	σ_I	0.903	8.06	0.123	0.07	7	H, 3-Br, 4-Cl, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
$\delta\text{CH}=\text{N}$	σ_R	0.904	8.13	0.185	0.07	8	3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
	F	0.906	8.06	0.109	0.07	9	H, 3-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
	R	0.845	8.14	0.145	0.07	10	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂

cont. Table 2.

Frequency	Constant	r	I	ρ	s	n	Correlated derivatives
$\delta\text{C}=\text{N}$	σ	0.857	158.16	3.478	1.83	10	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
	σ^+	0.854	158.58	2.388	1.88	10	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
	σ_I	0.824	158.10	2.245	2.17	10	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
	σ_R	0.841	159.62	4.668	1.99	10	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
	F	0.815	158.43	1.341	2.21	10	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂
	R	0.844	159.71	3.853	2.01	10	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH ₃ , 4-OCH ₃ , 3-NO ₂ , 4-NO ₂

r = correlation coefficient; I = intercept; ρ = slope; s = standard deviation; n = number of correlated derivatives

From Table 2, it is evident that the UV absorption maximum λ_{max} (nm) values of all the (*E*)-*N*-benzylidene-5-bromopyridin-2- amines compound, except that with 3-NO₂ substituent have shown satisfactory correlations with Hammett substituent constants viz., σ ($r = 0.905$), σ^+ ($r = 0.902$) and σ_R ($r = 0.902$). When the 3-NO₂ substituent that has been given exception is included in regression it reduces the correlations considerably.

However UV absorption maximum λ_{max} (nm) values of all the substituted (*E*)-*N*-benzylidene-5-bromopyridin-2-amines compound have shown poor correlations ($r < 0.900$) with the remaining Hammett substituent constant σ_I and *F* and *R* parameters.

The poor correlation is attributed to weak inductive and field effect of the substituents for predicting the reactivity through resonance. This is evident from the resonance conjugative structure shown in Scheme 1.

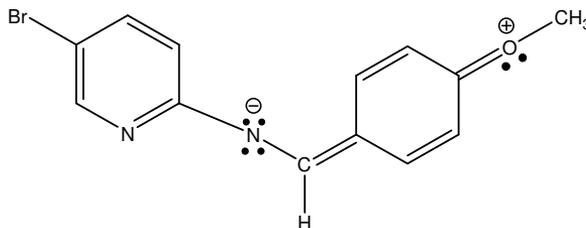


Fig. 1. The resonance-conjugative structure.

All the correlations with Hammett substituent constants and *F* and *R* parameters have shown positive ρ values of the entire substituted (*E*)-*N*-benzylidene-5-bromopyridin-2 amines compound.

Since some of the single regression analyses, have shown poor correlations with few Hammett constants and *F* and *R* parameters. So, it is worthwhile to seek the multi regression analysis. The multi regression analysis of the UV absorption maximum λ_{max} (nm) values of all the (*E*)-*N*-benzylidene-5-bromopyridin-2-amines compound with inductive, resonance and Swain-Lupton's [20] parameters produces satisfactory correlations as shown in equations (2) and (3):

$$\lambda_{max} \text{ (nm)} = 312.838(\pm 3.150) + 0.071(\pm 0.011)\sigma_I + 0.408(\pm 0.055)\sigma_R \quad (2)$$

$(R = 0.900, n = 10, P > 90\%)$

$$\lambda_{max} \text{ (nm)} = 312.995(\pm 3.053) - 0.183(\pm 0.030)F + 0.0579(\pm 0.094)R \quad (3)$$

$(R = 0.900, n = 10, P > 90\%)$

3.2. IR Spectral correlation

The assigned infrared stretching frequency $\nu_{C=N}$ (cm^{-1}) values of all substituted (*E*)-*N*-benzylidene-5-bromopyridin-2 amines compound are presented in Table 1. These infrared stretching frequency values are correlated with different Hammett substituent constants and *F* and *R* parameters using single and multi-linear regression analyses [16–19]. The structure parameter correlation involving group frequencies, the employed Hammett equation is shown in equation (4):

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

where ν_0 is the frequency of the parent member of this series.

The results of the statistical analysis are presented in Table 2. From Table 2, it is evident that the infrared stretching frequency $\nu_{C=N}$ (cm^{-1}) values of all (*E*)-*N*-benzylidene-5-bromopyridin-2- amines compound, except those with H(parent) and 4- NO_2 substituent have shown satisfactory correlations with *R* ($r = 0.904$) parameter only. When the substituents that have been given exception are included in regression they reduce the correlations considerably.

However the infrared stretching frequency $\nu_{C=N}$ (cm^{-1}) values of all (*E*)-*N*-benzylidene-5-bromopyridin-2- amines compound, have shown poor correlations ($r < 0.900$) with the remaining Hammett substituent constants viz., σ , σ^+ , σ_I and *F* parameter.

This is attributed to the polar, inductive and field effect of the substituents unable to predict their electronic effects on the frequency through resonance as per the conjugative structure shown in Figure 1. All correlations have shown negative ρ values. This indicates the operation of reverse substituent effect with respect to infrared stretching frequency $\nu_{C=N}$ (cm^{-1}) values in all (*E*)-*N*-benzylidene-5-bromopyridin-2- amines compound.

Since most of the single regression analyses, have shown poor correlations with Hammett constants and *F* and *R* parameters, it is decided to use multi regression analysis. The multi regression analysis of the stretching frequency $\nu_{C=N}$ (cm^{-1}) values of all aryl imine compounds with inductive, resonance and Swain-Lupton's [20] *F* and *R* parameters produce satisfactory correlations as shown in equations (5) and (6):

$$\begin{aligned} \nu_{C=N}(\text{cm}^{-1}) = & 1612.556(\pm 9.336) + 1.451(\pm 0.0775)\sigma_I - \\ & - 28.763(\pm 3.310)\sigma_R \quad (5) \\ (R = 0.944, n = 10, P > 90\%) \end{aligned}$$

$$\begin{aligned} \nu_{\text{C=N}}(\text{cm}^{-1}) &= 1611.925(\pm 9.004) + 1.224(\pm 0.068)F - \\ &\quad - 24.438(\pm 3.194)R \end{aligned} \quad (6)$$

(R = 0.945, n = 10, P > 90%)

3.3. NMR spectral correlation

The observed chemical shift values (ppm) of all substituted (*E*)-*N*-benzylidene-5-bromopyridin-2- amines compound are presented in Table 1. These chemical shift values (ppm) are correlated with different Hammett substituent constants and *F* and *R* parameters using single and multi-linear regression analyses [16-19]. In this correlation the structure parameter Hammett equation employed is as shown in equation (7).

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

where δ_0 is the chemical shift of the corresponding parent compound.

3.3.1. ¹H NMR spectral correlation

The results of the statistical analysis are presented in Table 2. From Table 2, it is evident that the ¹H NMR chemical shift values of all substituted (*E*)- *N* -benzylidene-5-bromopyridin-2- amines compound have shown satisfactory correlations with σ , σ^+ , σ_I and σ_R and *F* parameter. The remaining *R* parameter has shown poor correlation ($r < 0.900$). All the correlations with Hammett substituent constants and *F* and *R* parameters have shown positive ρ values. This shows that the normal substituent effect operates in all systems. The poor correlation is attributed to the polar, inductive and field effect of the substituents unable to predict their electronic effects on the spectral data through resonance as per the conjugative structure shown in Figure 1. While seeking the multi-correlation, collectively the inductive, resonance and field effects show satisfactory correlation as shown in equations (8) and (9):

$$\begin{aligned} \delta_{\text{CH=N}}(\text{ppm}) &= 8.095(\pm 0.050) + 0.104(\pm 0.1001)\sigma_I + \\ &\quad + 0.169(\pm 0.119)\sigma_R \end{aligned} \quad (8)$$

(R = 0.958, n = 10, P > 95%)

$$\begin{aligned} \delta_{\text{CH=N}}(\text{ppm}) &= 8.095(\pm 0.048) + 0.114(\pm 0.096)F + \\ &\quad + 0.150(\pm 0.098)R \end{aligned} \quad (9)$$

(R = 0.958, n = 10, P > 95%)

3.3.2. ^{13}C NMR spectral correlation

The results of the statistical analysis are presented in Table 2. From Table 2, it is evident that the ^{13}C NMR chemical shift values of all substituted (*E*) - *N*-benzylidene-5-bromopyridin-2- amines compound have shown poor correlations with all the Hammett substituent constants and *F* and *R* parameters along with negative ρ values. This shows that the normal substituent effect gets reversed in all systems. The failure in correlation is attributed to the conjugative structure shown in Figure 1.

Since most of the single regression analyses, have shown poor correlations with Hammett constants and *F* and *R* parameters, it is decided to use multi regression analysis. While seeking the multi regression analysis, satisfactory correlations have been observed with with inductive, resonance and Swain–Lupton's [20] parameters. The correlated multi regression equations are given in equations (10) and (11):

$$\begin{aligned} \delta\text{C}=\text{N}(\text{ppm}) &= 158.890(\pm 1.436) + 1.760(\pm 0.560)\sigma_{\text{I}} + \\ &+ 4.401(\pm 1.303)\sigma_{\text{R}} \end{aligned} \quad (10)$$

($R = 0.904$, $n = 10$, $P > 90\%$)

$$\begin{aligned} \delta\text{C}=\text{N}(\text{ppm}) &= 159.115(\pm 1.406) + 1.490(\pm 0.535)F + \\ &+ 3.912(\pm 1.376)R \end{aligned} \quad (11)$$

($R = 0.904$, $n = 10$, $P > 90\%$)

3.4. Antimicrobial activities

3.4.1. Antibacterial sensitivity assay

The antibacterial activities of all synthesized (*E*)-*N*-benzylidene-5-bromopyridin-2-amines compound have been studied against three Gram – positive pathogenic strains *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus* and two Gram – negative strains *Escherichia coli*, *pseudomonas aurogenosa*. The disc diffusion technique has been followed using the Kirby–Bauer method [21], at a concentration of $250\ \mu\text{g}/\text{cm}^3$ with ciprofloxacin as standard. The antibacterial screening effect of prepared (*E*)-*N*-benzylidene-5-bromopyridin-2- amines compound is shown in Figure 2 (Plates 1–10). The measured zone of inhibition values are given in Table 3 and the corresponding Clustered column Chart is shown in Figure 3. All the compounds showed weak to moderate activity against all five microorganisms. The compound with *p*-tolylbenzylidene group shows excellent activity against *Staphylococcus aureus*. The compound with *p*-tolylbenzylidene group and compound with *m*- nitro benzylidene group have shown good activity against

Pseudomonas aurogenosa. The Compound with benzylidene group and p-bromo benzylidene group has shown good activity against *Micrococcus luteus*. The Compound with m-Chloro benzylidene group shows poor activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* species.



Plate-1

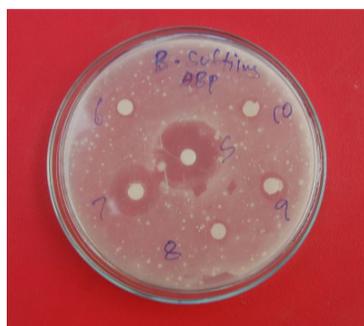


Plate-2



Plate-3



Plate-4



Plate-5

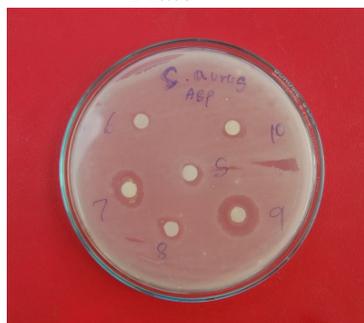


Plate-6

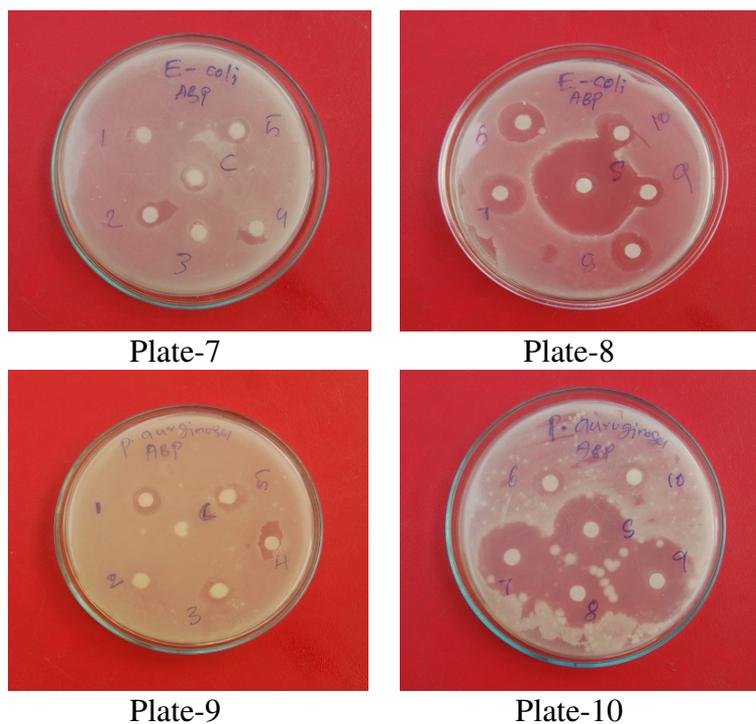


Fig. 2. Antibacterial activity of *(E)*-*N*-benzylidene-5-bromopyridin-2-amine compound (petri-plates).

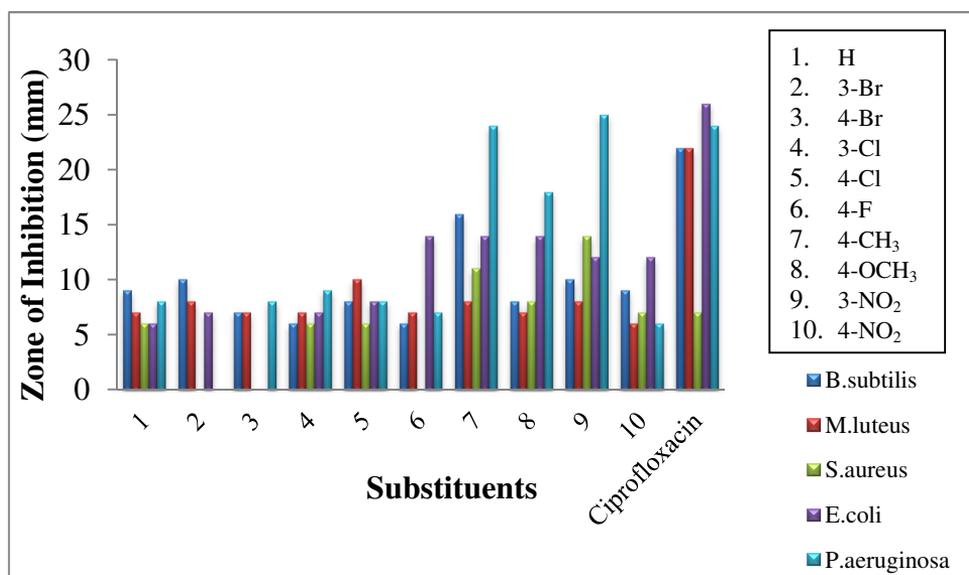


Fig. 3. Antibacterial activity of *(E)*-*N*-benzylidene-5-bromopyridin-2-amine compound (clustered column chart).

Table 3. Zone of inhibition (mm) values of antibacterial activity of substituted (*E*)-*N*-benzylidene-5-bromopyridin-2-amine compounds.

Entry	X	Zone of inhibition(mm)				
		Gramm-positive Bacteria			Gramm-negative Bacteria	
		<i>B. subtilis</i>	<i>M. luteus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
1	H	9	7	6	6	8
2	3-Br	10	8	0	7	0
3	4-Br	7	7	0	0	8
4	3-Cl	6	7	6	7	9
5	4-Cl	8	10	6	8	8
6	4-F	6	7	0	14	7
7	4-CH ₃	16	8	11	14	24
8	4-OCH ₃	8	7	8	14	18
9	3-NO ₂	10	8	14	12	25
10	4-NO ₂	9	6	7	12	6
Standard	Ciprofloxacin	22	22	7	26	24
Control	DMSO	0	0	0	0	0

3.4.2. Antifungal sensitivity assay

The antifungal activities of all synthesized (*E*)-*N*-benzylidene-5-bromopyridin-2-amines compound have been studied against three fungal species namely *Aspergillus niger*, *Mucour species* and *Trichoderma viride*. The disc diffusion technique has been followed using the Kirby–Bauer method [21], at a concentration of 250 µg/cm³ and Micnazole as standard. The antifungal screening effect of prepared (*E*)-*N*-benzylidene-5-bromopyridin-2- amines compound is shown in Figure 4. (Plates 1–4). The measured zone of inhibition values are given in Table 4 and the corresponding Clustered column chart is shown in Figure 5. All compounds have shown moderate to good activity against all three fungal species. Compounds with at benzylidene group (H, 3-Br, 4-Br, 4-OCH₃ and 4-NO₂) have shown very good antifungal activity against *Mucour species*. The Compounds with at benzylidene group(4-F, 4-OCH₃ and 4-NO₂) have shown very good antifungal activity against *Trichoderma viride*. The Compounds m-chloro benzylidene group has shown very

good antifungal activity against *Aspergillus niger*. The compound with p-tolylbenzylidene group a have shown poor activity gainst *Mucour species*.

Table 4. Zone of inhibition (mm) values of antifungal activities of (*E*)-*N*-benzylidene-5-bromopyridin-2-amines compound.

Entry	X	Zone of inhibition(mm)		
		<i>A.niger</i>	<i>M.species</i>	<i>T.viride</i>
1	H	15	14	12
2	3-Br	14	12	13
3	4-Br	15	14	14
4	3-Cl	18	10	8
5	4-Cl	8	14	13
6	4-F	9	16	12
7	4-CH ₃	10	14	8
8	4-OCH ₃	11	16	14
9	3-NO ₂	11	16	12
10	4-NO ₂	9	14	14
Standard	Micnazole	16	14	10
Control	DMSO	0	0	0



Plate-1

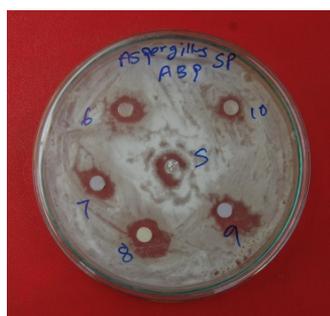


Plate-2

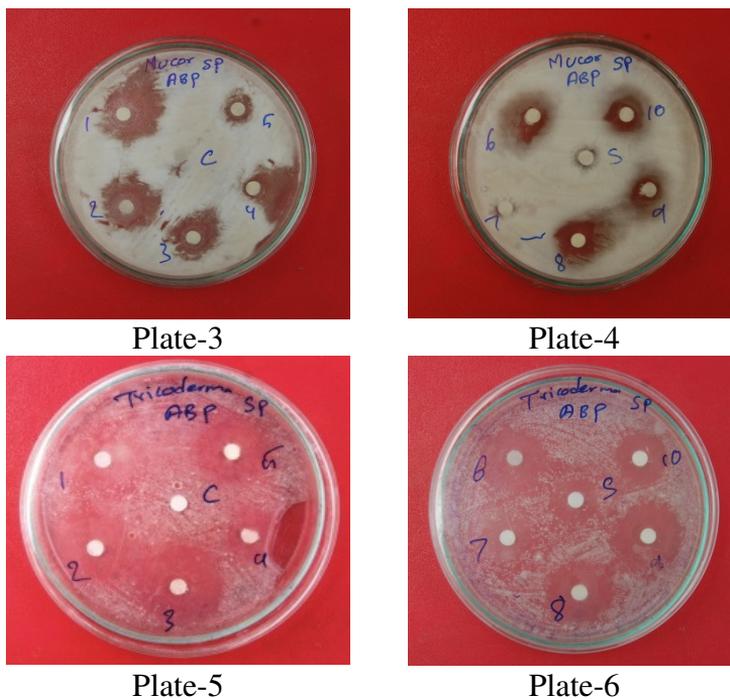


Fig. 4. Antifungal activity of (E)- N -benzylidene-5-bromopyridin-2- amines compound (petri-plates).

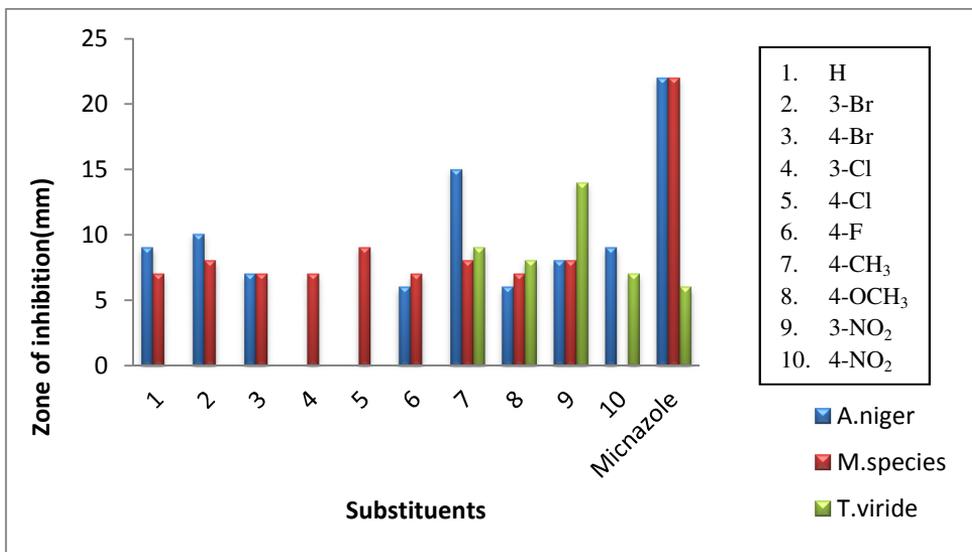


Fig. 5. Antifungal activity of (E)- N -benzylidene-5-bromopyridin-2- amines compound (clustered column chart).

4. CONCLUSIONS

A series of (*E*)-*N*-benzylidene-5-bromopyridin-2-amine compounds have been synthesized by condensation of 2-amino-5-bromopyridine with aromatic aldehydes. The structures of the synthesized compounds were confirmed by their physical constants and spectral data. The spectral data of these compounds have been correlated with Hammett sigma constants and *F* and *R* parameters using single and multi-linear regression analysis. From the results of statistical analysis, the effects of substituents on the spectral data have been discussed. The anti-microbial activities of these compounds have been studied.

ACKNOWLEDGEMENT

The authors thank DST NMR Facility, Department of Chemistry, Annamalai University, Annamalainagar-608002, for recording NMR spectra of all compounds.

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