Synthesis, Hammett spectral correlations and antimicrobial activities of (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines

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ABSTRACT

In the present study, a series of nine number of (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazine compounds have been synthesized by condensation reaction of meta and para substituted benzaldehydes with 4-chlorophenylhydrazine using acetic acid catalyst. They are characterized by their physical constants, UV (200-450 nm), Infra-Red (KBr, 4000-400 cm^{-1}) and NMR (^{1}H and ^{13}C) spectral data. The observed UV, IR and NMR spectral data have been correlated with Hammett substituent constants and Swain-Lupton's *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 antimicrobial activities of all the synthesized (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazine compounds have been studied using Bauer–Kirby method.

1. Introduction

Hydrazones are a class of organic compounds in the Schiff base family [1]. They are constitute a versatile compound of organic class having the basic structure $R_1R_2C=NNR_3R_4$ [2-3]. The two nitrogen atoms of hydrazone are nucleophilic but the amino type nitrogen is more reactive, whereas the carbon atom possesses both characters, that is, nucleophilic and electrophilic. The active centres of hydrazine, that is, carbon and nitrogen, are mainly responsible for the physical and chemical properties of the hydrazones and due to the reactivity toward electrophiles and nucleophiles, hydrazones are used for the synthesis of organic compound such as heterocyclic compounds [4-5] .

The general method for the synthesis of the hydrazones is the reaction of hydrazine with carbonyl compounds such as aldehydes or ketones in solvents like ethanol, methanol, butanol [6-8], and so forth. Hydrazone containing azomethine –NHN=CH protons constitute an important class of compounds for new drug development [9]. Many researchers have been synthesized these compounds as target structures and evaluated their antimicrobial [10], antitubercular [11], anticonvulsant [12], analgesic [13], anti-inflammatory [14], antiplatelet [15], anticancer [16], antiviral [17], antitumoral [18], antimalarial [19], antioxidant [20], analgesic [21], ntiprotozoal [22], antiparasitic [23], cardioprotective [24], antidepressant [25] and anti-HIV [26]. They are also used to couple with certain drugs and the bonds based on hydrazones are stable at the neutral PH [27]. Hydrazones have been investigated as potential charge-transport materials for organic photoconductors [28] and nonlinear optical polymers [29]. From through literature survey it is observed that there is no report on the effect of substituents QSAR or QPR study with these (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines, in the past. Therefore the authors take effort to synthesis some substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines and study the correlation analysis with their UV, IR and NMR spectral data and study their antimicrobial activities by using disc diffusion technique.

2.   Experimental Section
2.1.   Materials and Methods

All the chemicals used in the present investigation have been purchased from Sigma–Aldrich and E-Merck chemical companies. Melting points of all the substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazine compounds have been determined in open glass capillaries on a Mettler FP51 melting point apparatus and are uncorrected. The electronic spectra of all the synthesized (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines have been recorded on a SHIMADZU-1650 SPECTROMETER ($\lambda_{max,}$ nm) in spectral grade methanol. SHIMADZU-2010 FT-IR SPECTROMETER has been utilized for recording FT-IR (KBr pellet, 4000–400 cm^{-1}) spectra. The ^{1}H NMR and ^{13}C NMR spectra of all the synthesized (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines compounds have been recorded with BRUKER AV400 NMR spectrometer using CDCl$_3$ as a solvent and TMS as an internal standard.

2.2.   Synthesis of substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines

A mixture of equimolar quantities of substituted benzaldehydes (0.01mol) and 4-chlorophenylhydrazine (0.01mol), acetic acid (0.5 mL) and 20 mL of ethanol were taken in 100 mL round bottom flask the mixture was refluxed [30] for 3 hours as shown in **Scheme-1**. The completion of the reaction was monitored by TLC continuously. The resultant mixture was cooled at room temperature. Then the precipitate obtained, was filtered at the filter pump and washed several times with cold water. This crude product was recrystallized from ethanol and their melting points have been noted. All the synthesized (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines have been identified by their physical constants, UV, IR and NMR spectral data as shown in **Table 1**. The spectral data of all of substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines have been correlated with Hammett substituent constants and *F* and *R* parameter and are shown in **Table 2.**

X= H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
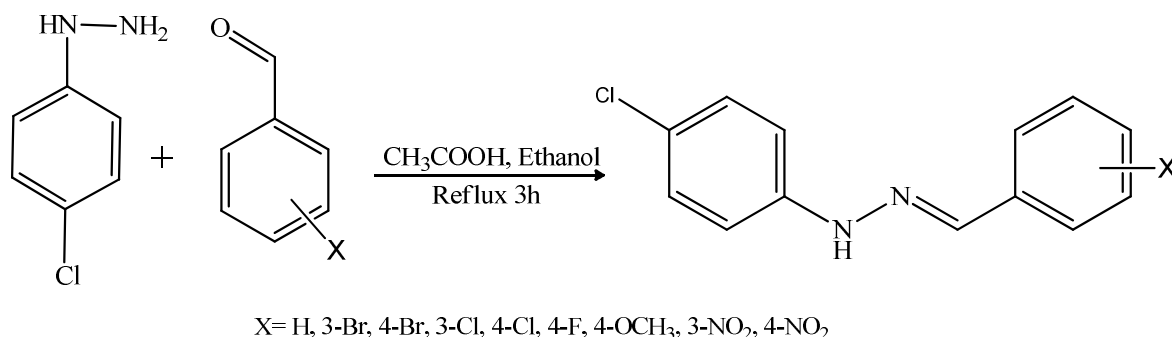
Scheme 1. Synthesis of substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines.

Table1. The physical constants, UV, IR and NMR data of (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines

Entry	X	m.p (°C)	M. F	M. W	Yield (%)	λ_{max} (nm)	ν C=N (cm^{-1})	δ^1H CH=N (ppm)	δ^{13}C C=N (ppm)
1	H	103-104 [103-105][31]	$C_{13}H_{11}N_2Cl$	230	80	348	1583.56	8.318	143.31
2	3-Br	80-81	$C_{13}H_{10}N_2BrCl$	309	82	354.5	1591.27	8.105	150.85
3	4-Br	108-109	$C_{13}H_{10}N_2BrCl$	309	84	355.5	1587.42	7.78	142.99
4	3-Cl	79-80	$C_{13}H_{10}N_2Cl_2$	265	86	354	1587.42	7.923	150.88
5	4-Cl	101-102[32]	$C_{13}H_{10}N_2Cl_2$	265	83	348	1587.42	8.386	151.1
6	4-F	104-105	$C_{13}H_{10}N_2FCl$	248	86	347.5	1595.13	7.634	144.74
7	4-OCH$_3$	151-152	$C_{14}H_{13}N_2OCl$	260	85	350	1600.92	7.847	143.63
8	3-NO$_2$	123-124	$C_{13}H_{10}N_3O_2Cl$	275	87	350.5	1593.2	7.686	147.93
9	4-NO$_2$	153-154	$C_{13}H_{10}N_3O_2Cl$	275	81	340.5	1575.84	7.661	147.19

Table 2. Results of statistical analysis of UV λ_{max} (nm), $\nu_{C=N}$ (cm^{-1}) IR, NMR δ^1H (ppm) CH=N and δ^{13}C (ppm) C= N data of substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with Hammett substituent constants σ, σ^+, σ_I, σ_R and *F* and *R* parameters.

Frequency	Constants	r	I	ρ	s	n	Correlated derivatives
λ_{max} (nm)	σ	0.862	350.67	-3.01	4.81	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	σ^+	0.802	349.97	-0.65	4.93	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	σ_I	0.816	351.46	-3.75	4.87	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	σ_R	0.827	349.02	-5.76	4.75	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	F	0.820	351.76	-4.26	4.83	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	R	0.826	348.98	-4.63	4.76	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
νC=N (cm^{-1})	σ	0.905	1592.44	-11.87	6.341	7	3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 4-NO$_2$
	σ^+	0.906	1591.28	-9.74	5.83	7	3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 4-NO$_2$
	σ_I	0.809	1590.58	-3.34	7.64	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	σ_R	0.906	1585.98	-22.48	5.57	8	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 4-NO$_2$
	F	0.804	1588.42	-1.57	7.66	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	R	0.907	1585.34	19.79	5.31	8	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 4-NO$_2$
δCH=N (ppm)	σ	0.805	7.907	0.052	0.32	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	σ^+	0.832	7.917	0.019	0.32	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	σ_I	0.803	7.914	-0.051	0.32	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	σ_R	0.813	7.914	-0.051	0.32	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	F	0.817	8.028	-0.235	0.31	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	R	0.804	7.932	0.055	0.32	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
δC=N (ppm)	σ	0.813	146.57	1.388	3.62	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	σ^+	0.817	146.68	1.237	3.60	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	σ_I	0.816	148.14	-2.745	3.61	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	σ_R	0.808	147.14	1.322	3.64	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	F	0.824	148.64	-3.739	3.55	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$
	R	0.817	147.38	2.254	3.60	9	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$, 4-NO$_2$

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

3.   RESULTS AND DISSCUSION

3.1.   UV-visible spectral correlations:

The assigned UV absorption maximum λ_{max} (nm) values of all the substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines are presented in **Table 1**. These UV absorption maximum λ_{max} (nm) values are correlated with different Hammett substituent constants and *F* and *R* parameters using single and multi-linear regression analyses according to the approach of John Shorter [33]. Hammett equation employed, for the correlation analysis, involving the UV absorption maximum is shown in equation (1).

$$\lambda = \rho\,\sigma + \lambda_0 \tag{1}$$

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

The results of statistical analysis [34-37] are shown in **Table 2**. From **Table 2**, it is evident that the UV absorption maximum λ_{max} (nm) values of all the substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines have shown poor correlations (r < 0.900) with Hammett substituent constants namely, σ, σ^+, σ_I, σ_R and *F* and R parameters. This is attributed to the weak polar, inductive, field and resonance effects of the substituents for predicting the reactivity on the UV absorption maximum λ_{max} (nm) values of all the (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines through resonance as per the conjugative structure shown in **Fig. 1**.
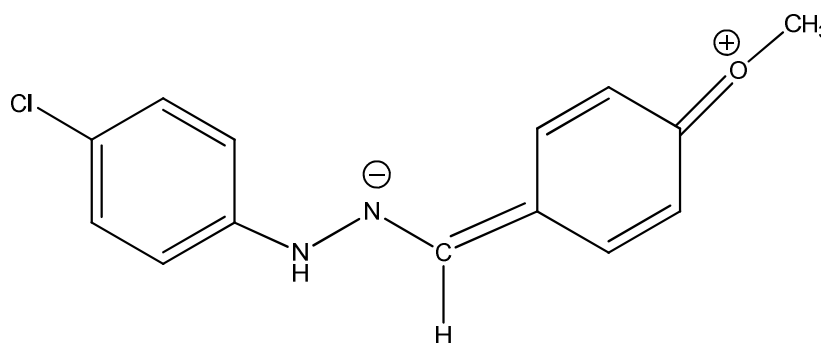

Fig. 1. The resonance-conjugative structure

All the correlations have shown negative ρ values. This indicates the operation of reverse substituent effect with respect to UV absorption maximum λ_{max} C=N (nm) values in all the (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazine compounds. In this case all the single regression analyses have shown poor correlations with Hammett substituent constants namely, σ, σ^+, σ_I, σ_R and *F* and *R* parameters, it is decided to go for multi-regression analysis. The multi-regression analysis of the UV absorption maximum λ_{max} (nm) values of all the substituted (*E*)-1-benzylidene-2-(4-chlorophenyl) hydrazine compounds with inductive, resonance and Swain-Lupton's [38] *F* and *R* parameters produce satisfactory correlations as shown in equations **(2)** and **(3)**.

$$\lambda_{max}\ C{=}N\ (nm) = 350.21(\pm 4.69) - 2.57(\pm 0.716)\sigma_I - 5.281\ (\pm 1.397)\sigma_R \tag{2}$$
$$(R = 0.929,\ n{=}9,\ P{>}90\%)$$

$$\lambda_{max}\ C{=}N\ (nm) = 350.945(\pm 4.153) - 4.476\ (\pm 1.007)F - 4.771\ (\pm 1.791)R \tag{3}$$
$$(R = 0.933,\ n{=}9,\ P{>}90\%)$$

3.2.   IR Spectral correlation

The assigned infrared stretching frequency νC=N (cm^{-1}) values of all the substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines are presented in **Table-1.** These IR frequency νC=N (cm^{-1}) values are correlated with different Hammett substituent constants and *F* and *R* parameters using single and multi-linear regression analyses. The structure parameter correlation involving group frequencies, the employed Hammett equation is shown in equation (4).

$$\nu = \rho\,\sigma + \nu_0 \tag{4}$$

Where ν_0 is the frequency of the parent member of this series

The results of the statistical analysis [34-37] are presented in **Table 2**, it is evident that the infrared stretching frequency $\nu C=N$ (cm^{-1}) values of all the substituted (E)-1-benzylidene-2-(4-chlorophenyl)hydrazines, except those with parent (H) and 3-NO$_2$ substituents have shown satisfactory correlations with Hammett constants namely, σ (r =0.905) and σ^+ (r =0.906). The infrared stretching frequency $\nu C=N$ (cm^{-1}) values of all the substituted (E)-1-benzylidene-2-(4-chlorophenyl)hydrazines, except those with 3-NO$_2$ substituent have shown satisfactory correlations with Hammett constant σ_R (r =0.906) and Swain-Lupton R (r =0.907) parameters. When these 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 the substituted (E)-1-benzylidene-2-(4-chlorophenyl)hydrazines, have shown poor correlations (r < 0.900) with the remaining Hammett constant σ_I and F parameters. This is attributed to weak polar, inductive and field effect of the substituents to predict their electronic effects through resonance as per conjugative structure shown in **Fig. 1**.

Since some of the single regression analyses, have shown poor correlations with Hammett constant σ_I and F parameters. It is decided to go for multi regression analysis. The multi regression analysis of the stretching frequency ν C=N (cm^{-1}) values of all the substituted (E)-1-benzylidene-2-(4-chlorophenyl)hydrazines with inductive, resonance and Swain-Lupton's [38] parameters produce satisfactory correlations as shown in equations (4) and (5).

$$\nu_{C=N} \text{ (cm}^{-1}) = 1585.17(\pm 5.530) + 1.757(\pm 0.380) \, \sigma_I - 22.817(\pm 4.895)\sigma_R \tag{5}$$
$$(R = 0.968, \text{n} = 9, \text{P} > 95\%)$$

$$\nu_{C=N} \text{ (cm}^{-1}) = 1585.03(\pm 4.746) + 0.708(\pm 0.021) \, F + 19.777(\pm 3.761)R \tag{6}$$
$$(R = 0.972, \text{n} = 9, \text{P} > 95\%)$$

3.3. NMR spectral correlation

The assigned chemical shift values (ppm) of all the substituted (E)-1-benzylidene-2-(4-chlorophenyl)hydrazines are presented in **Table 1**. These CH=N (δ ppm) chemical shift values are correlated with different Hammett substituent constants and F and R parameters using single and multi-linear regression analyses [34-37]. In this correlation, the structure-parameter equation employed is shown in equation (7).

$$\delta = \rho \, \sigma + \delta_0 \tag{7}$$

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

3.3.1. ^{1}H NMR spectral correlation

The chemical shift CH=N (δ ppm) values of all the substituted (E)-1-benzylidene-2-(4-chlorophenyl)hydrazines are presented in **Table-1**. The ^{1}H NMR spectral correlations of all the substituted (E)-1-benzylidene-2-(4-chlorophenyl)hydrazines are presented in **Table 2**.

From the results of the statistical analysis [34-37] the ^{1}H NMR chemical shift δ CH =N (ppm) values of all the substituted (E)-1-benzylidene-2-(4-chlorophenyl)hydrazines have shown poor correlations (r < 0.900) with different Hammett constants and Swain-Lupton's F and R parameters. The failure in correlation is due to the reason that has been stated earlier.

All the correlations with Hammett substituent constants namely σ, $\sigma+$ and R parameters have shown positive ρ values. It indicates the operation of normal substituent effect with respect to ^{1}H NMR spectral data of all the compounds.

All the single regression analyses have shown poor correlations. Hence, it is decided to go for multi-regression analysis. While seeking the multi–correlation collectively the Hammett constants namely σ_I and σ_R and F and R parameters [38] have shown satisfactory correlation as shown in the equations (8) and (9).

$$CH=N \, (\delta_{ppm}) = 7.925(\pm 0.320) - 0.023 \, (\pm 0.625) \, \sigma_I - 0.047(\pm 0.572) \, \sigma_R \tag{8}$$
$$(R = 0.904, \text{n}= 9, P > 95\%)$$

$$CH=N \, (\delta_{ppm}) = 8.037(\pm 0.283) - 0.233 \, (\pm 0.546) \, F + 0.048(\pm 0.463) \, R \tag{9}$$
$$(R = 0.984, \text{n}= 9, P > 95\%)$$

3.3.2. ^{13}C NMR spectral correlation

The results of the statistical analysis [34-37] are presented in **Table-2**. It is evident that the ^{13}CNMR chemical shift δ C=N (ppm) values of all the substituted (E)-1-benzylidene-2-(4-

chlorophenyl)hydrazines have shown poor correlations (r < 0.900) with different Hammett constants and Swain-Lupton's *F* and *R* parameters. The failure in correlation is due to the reason that has been stated earlier with resonance conjugative structure as shown in **Fig. 2.** The Hammett constants σ, σ+, σ_R and *R* parameters have shown positive ρ values. This shows that the normal substituent effect operates in all systems. While seeking the multi-correlation, collectively with inductive, resonance and field effects [38] satisfactory correlation as shown in equations (10) and (11).

$$\text{C=N } (\delta_{ppm}) = 148.60(\pm 3.562) - 3.174(\pm 1.957)\sigma_I + 1.918(\pm 0.437)\sigma_R \quad (10)$$
$$(R = 0.973, \text{ n} = 9, \text{ P>95\%})$$

$$\text{C=N}(\delta_{ppm}) = 149.01(\pm 3.125) -3,646(\pm 1.026)F + 2.139(\pm 0.711)R \quad (11)$$
$$(R = 0.929, \text{ n} = 9, \text{ P>95\%})$$

4.   Antimicrobial activities of (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines
4.1.   Antibacterial sensitivity assay

The antibacterial activities of all the (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines was done following the disc diffusion technique [39]. All the (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazine compounds were screened for their in antibacterial activity against *B. subtilis*, *M. luteus, S. aureus, E. coli* and *P. aeruginosa* at a concentration 250 µg/mL with ciprofloxacin as the standard drug. The antibacterial activity of all the (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines is shown in **Fig. 2** (Plates 1–10). The measured zone of inhibition values are given in **Table 3** and the corresponding clustered column chart is shown in **Fig. 3**. Analysis of the zone of inhibition (mm) values reveals that the (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with 3-Cl, 4-F, 4-OCH$_3$ and 3-NO$_2$ substituents have shown good antibacterial against *B. subtilis, M. luteus* species. The (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with parent (H), 3-Br, 4-Br, 4-Cl and 4-NO$_2$ substituents showed moderate antibacterial activity against *B. subtilis, M. luteus* species. The (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with 4-NO$_2$ substituent have shown excellent activity against *M. luteus species*. The (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$ and 4-NO$_2$ substituents have shown good antibacterial activity against *S. aureus species*. The remaining three (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with parent (H), 3-Br and 4-Br substituents has shown moderate antibacterial activity against *S. aureus species*   .

The eight (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with parent (H), 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 3-NO$_2$ and 4-NO$_2$ substituents has shown good antibacterial activity against *E. coli* species. The remaining one (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with 3-Br substituent have shown moderate antibacterial activity against *E. coli* species. The four (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with 3-Cl, 4-Cl, 4-F and 4-NO$_2$ substituents have shown good antibacterial activity *S. aureus* species. The three (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with parent (H), 3-Br and 3-NO$_2$ substituents has shown moderate antibacterial activity *S. aureus* species. The remaining two (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines compounds with 4-Br and 4-OCH$_3$ substituents has shown poor antibacterial activity against *S. aureus* species.
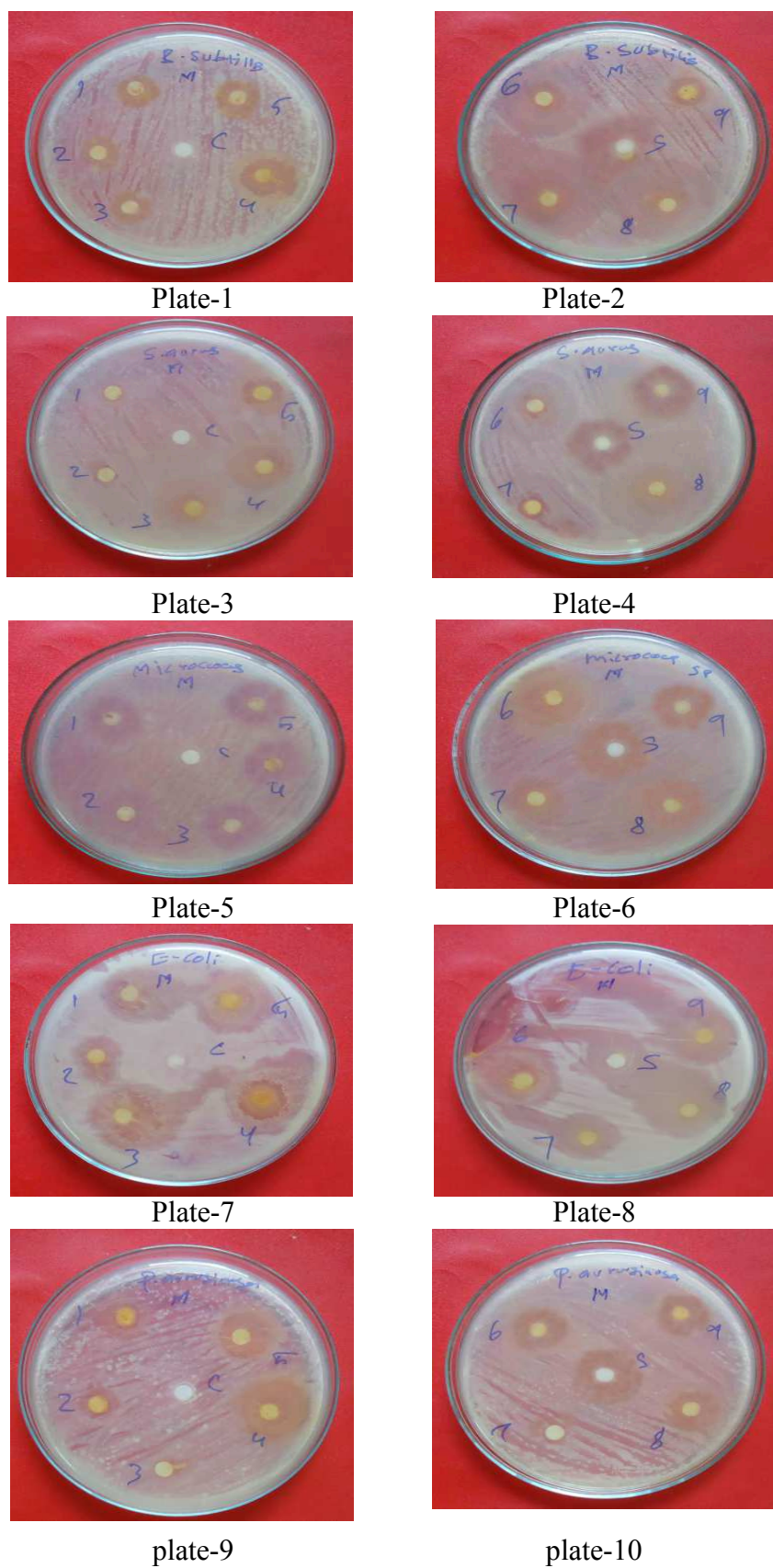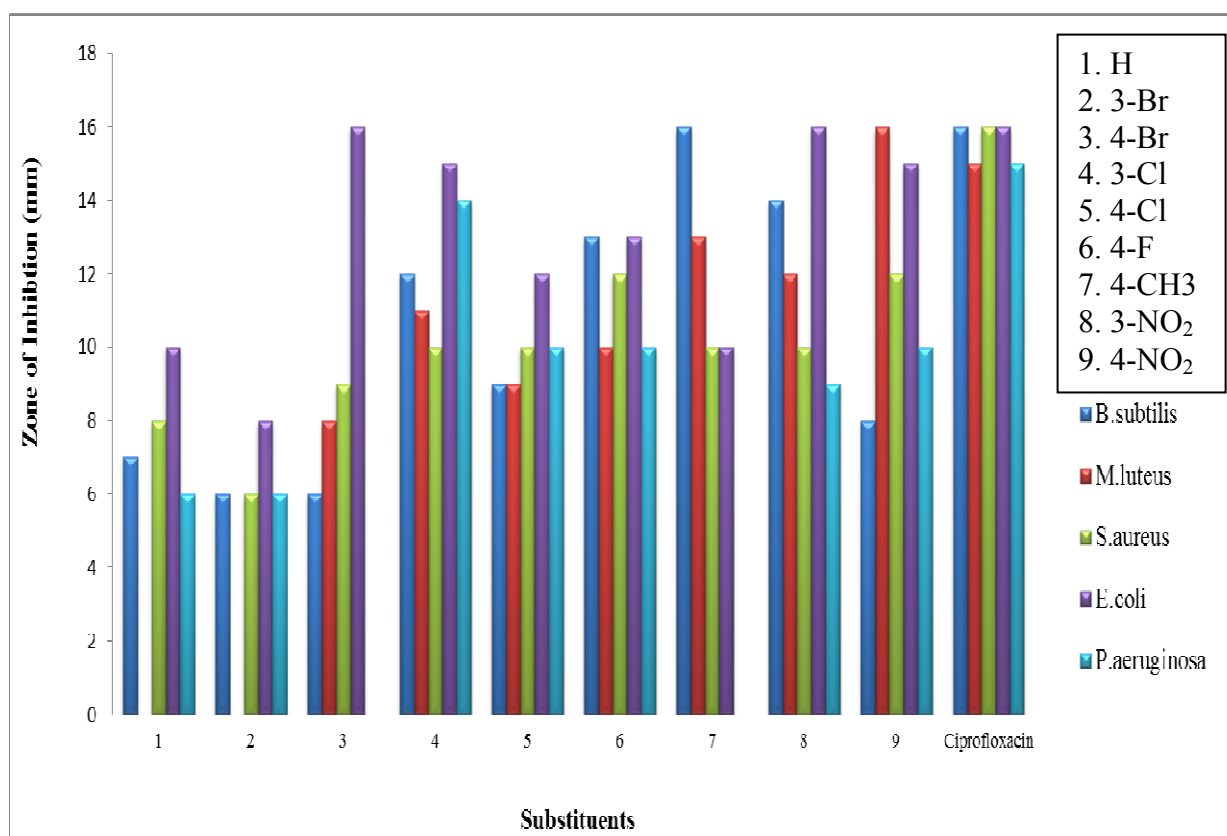
Fig. 2. Antibacterial activity of substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines (petri-plates)

Table 3. The zone of inhibition (mm) values of antibacterial activity of (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines

S.No.	Substituents	Zone of inhibition(mm)				
		Gram positive Bacteria			Gram negative Bacteria	
		B.subtilis	*M.luteus*	*S.aureus*	*E.coli*	*P.aeruginosa*
1	H	7	0	8	10	6
2	3-Br	6	0	6	8	6
3	4-Br	6	8	9	16	0
4	3-Cl	12	11	10	15	14
5	4-Cl	9	9	10	12	10
6	4-F	13	10	12	13	10
7	4-OCH$_3$	16	13	10	10	0
8	3-NO$_2$	14	12	10	16	9
9	4-NO$_2$	8	16	12	15	10
Standard	Ciprofloxacin	16	15	16	16	15
Control	DMSO	0	0	0	0	0

Fig. 3. Antibacterial activity of substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines (clustered column chart)

4.2. Antifungal activity

Antifungal assay has been performed using Kirby–Bauer [39] disc diffusion technique. The antifungal activities of all the substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines have been studied and are shown in **Fig. 4** for Plates (1–4). The zone of inhibition values of the antifungal activities is given in **Table 4.** The clustered column chart was shown in **Fig. 5** and it reveals that the (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazine compounds with Parent (H), 3-Br, 4-Br, 4-Cl and 4-F substituents have shown moderate antifungal activity against *A. niger species*. The (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines having substituents 4-OCH$_3$ and 4-NO$_2$ have shown good antifungal activity against *A. niger* alone. The (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with 3-NO$_2$ substituent have shown excellent antifungal activity against *A. niger species*. The (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with 3-Br, 4-Br and 3-Cl substituents have shown improve antifungal activity against *Mucor Species*. The (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with 4-Cl, 4-F, 3-NO$_2$ and 4-NO$_2$ substituents have shown good antifungal activity against *Mucor Species*. The (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with 4-OCH$_3$ substituent have shown excellent antifungal activity against *Mucor Species*. The (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with Parent (H), 3-Br, 3-Cl, 4-F, 3-NO$_2$ and 4-NO$_2$ substituents have shown improve antifungal activity against *T.viride Species*. The (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines with 4-OCH$_3$ substituent have shown good antifungal activity against *T.viride Species*.
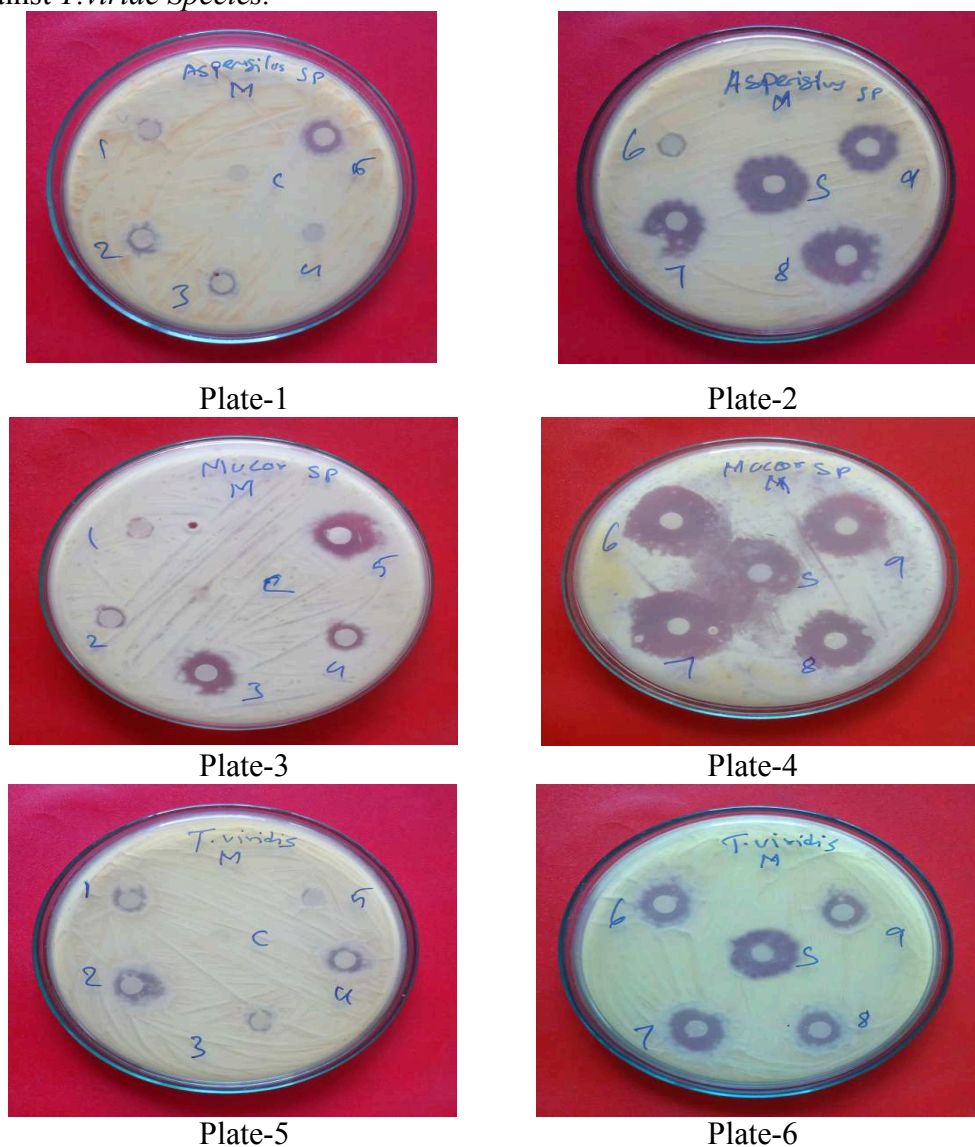

Plate-1   Plate-2

Plate-3   Plate-4

Plate-5   Plate-6

Fig. 4. Antifungal activity of substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines (petri-plates)

Table 4.   The zone of inhibition (mm) values of antifungal activities of (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines

S.No	Compounds	Zone of Inhibition (mm)		
		A.niger	*M.species*	*T.viride*
1	H	6	0	6
2	3-Br	7	6	7
3	4-Br	6	8	0
4	3-Cl	0	7	6
5	4-Cl	8	10	0
6	4-F	6	18	8
7	4-OCH$_3$	13	24	10
8	3-NO$_2$	21	19	8
9	4-NO$_2$	14	20	9
Standard	Micnazole	20	23	18
Control	DMSO	0	0	0
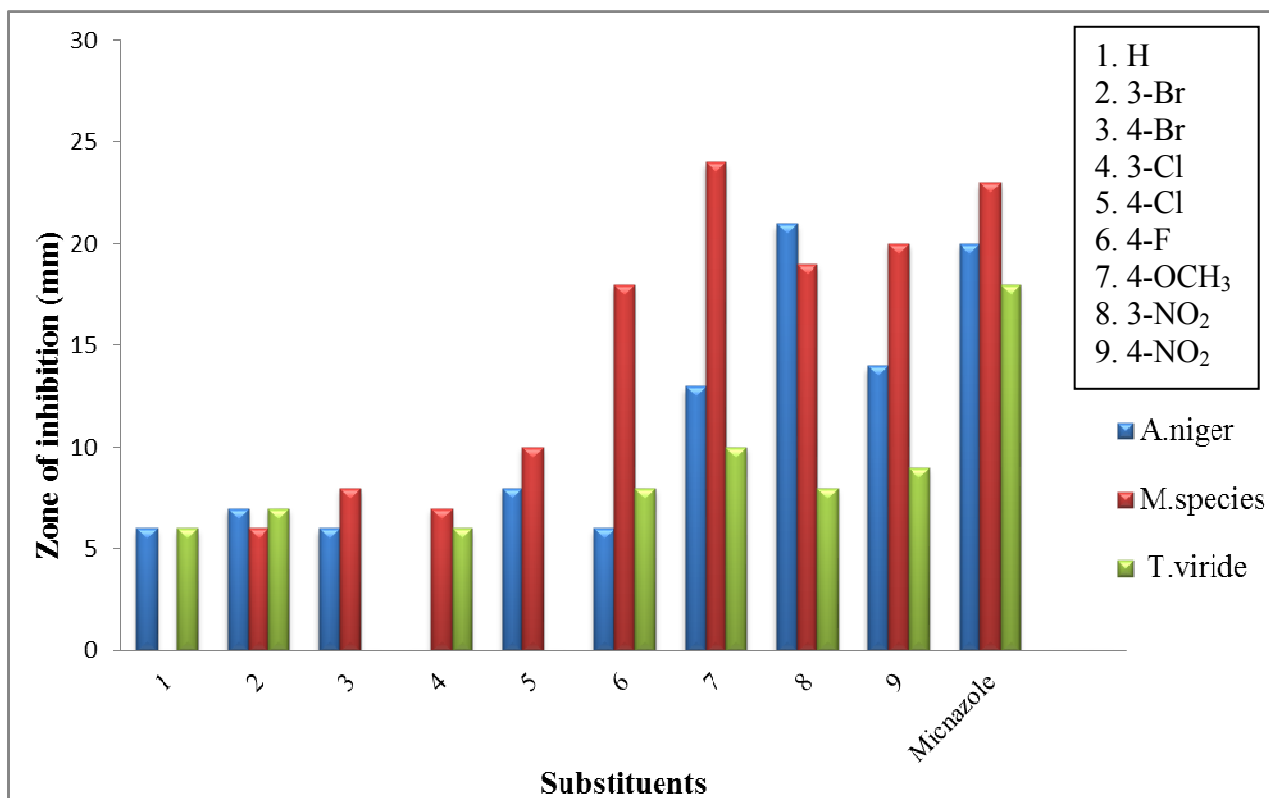
Fig. 5. Antifungal activity of substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines (clustered column chart)

5. CONCLUSION

A series of nine number of (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines have been synthesized by condensation reaction of various meta- and para- substituted benzaldehydes containing either electron-releasing or electron-withdrawing groups, 4-chlorophenylhydrazine with acetic acid acid. The yield of the product is more than 80%. The synthesized substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines are characterized by their physical constants and the UV, IR, and NMR spectral data. The UV, IR, and NMR spectral data of the substituted (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines have been correlated with Hammett constants σ, σ^+, σ_I, σ_R and swain-Lupton's *F* and *R* parameters using single and multi-regression analyses. The single parameter correlation with few Hammett constants and *F* and *R* parameters gave satisfactory correlation whereas all multiple correlations gave satisfactory correlation coefficients with Resonance, Field and Swain-Lupton's parameters. The Antimicrobial activities of all synthesized (*E*)-1-benzylidene-2-(4-chlorophenyl)hydrazines have been studied using Bauer-Kirby method.

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