10.1515/umcschem-2015-0019

ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. LXX, 2 SECTIO AA 2015

Synthesis, spectral correlation analysis and evaluation of biological activities of some substituted hydrazones

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Some novel substituted hydrazone derivatives of amino guanidine have been synthesized with different substituted benzaldehydes by condensation method. The synthesized hydrazones were characterized by their physical constants, UV, IR and NMR spectra. The spectral data have been correlated with Hammett substituent constants and Swain–Lupton parameters. From the result of statistical analysis, the effects of substituents on the spectral data have been predicted. The antimicrobial activities of these synthesized hydrazone compounds have been screened by Bauer-Kirby method using human pathogenic bacteria and fungal species. The antimicrobial activities of all synthesized hydrazone compounds have shown significant activity.

***Key words*:** Hydrazones, UV, IR & NMR spectra, Correlation analysis, and Antimicrobial activities.

1. INTRODUCTION

Hydrazones are azomethiens which are characterized by their presence of the triatomic group >C= N-N<. Hydrazones contain two connected nitrogen atoms of different nature and a C–N double bond that is conjugated with a lone electron pair of the terminal nitrogen atom. Both nitrogen atoms of the hydrazone group are nucleophile, although the amino type nitrogen is more reactive. The carbon atom of hydrazone group has both electrophilic and nucleophilic character [1,2]. Hydrazones and their derivatives constitute a versatile class of compounds in organic chemistry. Hydrazones, are used as intermediates in synthesis [3], as functional groups in metal carbonyls [4], in organic compounds [5] and in particular in hydrazone Schiff base ligands [6], which are among others employed in dinuclear catalysts [7]. Recently, a lot of biologically important hydrazone derivatives with a number of functional groups have been synthesized from aromatic and aliphatic compounds [8]. These are found to possess anti-microbial [9-11], anti-mycobacterial [12], anti-convulsant [13], analgesic [14], anti-inflammatory [15], anti-platelet [16], anti-tubercular [17] and anti-tumoral [18] activities. In recent years, correlation analysis is applied by chemists to solve spectral problems. Conformational equilibrium [19] in the ground state of organic molecules has been investigated for *s-cis* and *s-trans* isomers of alkenes, *α, β*-unsaturated ketones, aldehydes, acyl halides and their esters, on the basis of spectral data. Recently, Thirunarayanan et al.[20] have investigated the single and multi-substituent effects on alpha and beta hydrogens and carbons of furyl chalcones. Arulkumaran *et al.* [21,22] have studied the effect of substituents and antimicrobial activities of some substituted styryl 4-nitrophenyl and 3-thienylketones. Similarly, Subramanian *et al.* [23] have investigated the synthesis, effects of substituents and antimicrobial activities of some substituted styryl 3-thienyl and furyl chalcones. Similarly, the effect substituent of compounds like pyrazolines [24] and imines [25] containing C=N moiety have been studied extensively. Literature review reveals that there are no reports available for the study of substituent effects of substituted benzylidineaminoguanidines. Therefore, the authors have taken efforts to synthesize and to study the effect of substituents from spectral data and antimicrobial activities of benzylidineaminoguanidines.

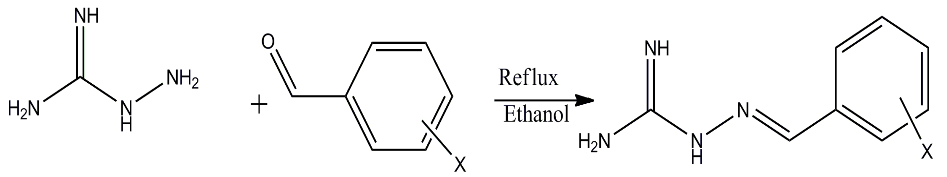
2. METERIAL AND METHODS

2.1. General

All the chemicals involved in the present investigation, have been procured from Sigma–Aldrich chemical company. The UV spectra of all the hydrazones, synthesized, have been recorded with ELICO- BL222 spectrophotometer (λ_{max} nm) in spectral grade methanol solvent. Infrared spectra (KBr, 4000–400 cm^{-1}) have been recorded on AVATAR-300 Fourier transform spectrophotometer. Bruker AV400 NMR spectrometer operating at 400 MHz has been utilized for recording ^{1}H NMR spectra and 100 MHz for ^{13}C NMR spectra in DMSO solvent using TMS as internal standard. Elemental analysis of all compounds were performed in Thermofinnigan analyzer.

2.2. Synthesis of Benzylideneaminoguanidine

A solution of equi-molar quantities of amino guanidine (0.01 mol) and benzaldehydes (0.01 mol) were refluxed for 3h with 20 cm^3 of absolute ethanol [26]. 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. A pale yellow solid was obtained as the final product. This crude product was recrystallized from ethanol. A glittering colourless solid, melting at 62–63°C was obtained. The general scheme for the preparation of substituted benzylidine-aminoguanidines has shown in Scheme 1.



Where X= H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 4-CH$_3$, 3-NO$_2$, 4-NO$_2$

Scheme 1.

The yield, physical constants, analytical and spectral data of all hydrazones are summarized below.

(Benzylideneamino)guanidine (1): Yield: 86%, m.p. 62-63°C.UV (λ_{max}): 311.IR (KBr, cm^{-1}): ν = 1640 (CH=N),937 (N-N),3080 (-NH), 3463(-NH2). ^1H NMR (DMSO, ppm): δ=7.989 (S, 1H.CH=N), 7.22-7.66 (m, 5H Ar-H) 5.934 (S, 1H. C=NH), 7.241 (S, 1H. -NH), 5.552 (S,2H.-NH2). ^{13}C NMR (DMSO, ppm): δ (C_1) = 143.21(CH=N), 136.88 (C_2), 128.29(C_3), 127.67(C_4), 126.17(C_5), 127.67(C_6), 128.2 (C_7), 160.48(CH=NH). Anal.Calcd for $C_8H_{10}N_4$ (162.19): C, 59.24; H, 6.21; N, 34.54%.Found: C,59.25; H, 6 .15; N,34.48%.

(3-Bromobenzylideneamino)guanidine (2): Yield: 92%, m.p. 91-92°C. UV (λ_{max}): 285. IR (KBr, cm^{-1}): ν =1641 (CH=N), 1064 (N-N), 3226 (-NH), 3344 (-NH_2). ^1H NMR (DMSO, ppm): δ=8.219 (S, 1H.CH=N), 7.31-7.64 (m, 4H Ar-H) 7.626 (S, 1H. C=NH), 7.775 (S, 1H.-NH), 7.504 (S, 2H.-NH_2). ^{13}C NMR (DMSO, ppm): δ (C_1) = 141.79 (CH=N), 138.96 (C_2), 134.87 (C_3), 128.25 (C_4), 138.41 (C_5), 133.32 (C_6), 131.34 (C_7), 164.55 (C=NH). Anal.Calcd for C_8H_9N4 (241.09): C, 39.82; H, 3.76; N 23.23%.Found: C, 39.88; H 3.69; N, 23.19%.

(4-Bromobenzylideneamino)guanidine(3): Yield: 89%, m.p. 147-148°C. UV (λ_{max}): 306. IR (KBr, cm^{-1}): ν =1636(CH=N), 1068 (N-N), 3372 (-NH), 3430(-NH_2).^1H NMR (DMSO, ppm): δ=7.937 (S, 1H.CH=N), 7.481-7.764 (m, 4H Ar-H) 5.529 (S, 1H. C=NH), 5.954 (S, 1H.-NH), 4.050 (S, 2H.-NH_2). ^{13}C NMR (DMSO, ppm): δ (C_1) = 141.66 (CH=N), 136.31 (C_2), 131.17 (C_3, C_7), 127.97 (C_4, C_6), 120.40 (C_5), 160.81 (C=NH). Anal.Calcd for C_8H_9N4 (241.09): C, 39.82; H, 3.76; N, 23.23%. Found: C,39.84; H,3.68; N,23.15%.

(3-Chlorobenzylideneamino)guanidine(4): Yield: 93%, m.p. 68-69°C. UV (λ_{max}): 309. IR (KBr, cm^{-1}): ν =1641 (CH=N), 1076 (N-N), 3213 (–NH), 3342 (-NH_2). ^1H NMR (DMSO, ppm): δ = 7.969 (S,1H.CH=N), 7.327-7.831 (m, 4H Ar-H) 7.294 (S, 1H. C=NH), 7.831 (S, 1H.-NH), 7.570 (S, 2H.-NH_2). ^{13}C NMR (DMSO, ppm): δ (C_1) = 141.69(CH=N), 138.96(C_2), 125.27 (C_3), 133.37 (C_4), 130.11 (C_5), 127.39(C_6), 125.14 (C_7) 160.41 (C=NH). Anal.Calcd for $C_8H_9ClN_4$ (196.64): C, 48.82; H, 4.61; N, 28.49%. Found: C, 48.84; H 4.56; N,28.45%.

(4-Chlorobenzylideneamino)guanidine(5): Yield: 89%, m.p. 120-121°C. UV (λ_{max}):286. IR(KBr, cm^{-1}) : ν =1636(CH=N), 1089(N-N), 3218 (-NH), 3371 (–NH_2). ^1H NMR (DMSO, ppm): δ = 7.958 (S, 1H.CH=N), 7.349-7.695 (m, 4H Ar-H) 5.980 (S, 1H. C=NH), 7.349 (S, 1H.-NH), 5.569 (S, 2H.-NH_2). ^{13}C NMR (DMSO, pm): δ (C_1) = 141.69 (CH=N), 131.87 (C_2), 128.30 (C_3, C7), 127.69 (C_4,C_6), 135.88 (C_5), 160.72 (C=NH). Anal.Calcd for $C_8H_9ClN_4$ (196.64): C,48.82; H,4.61; N,28.49%. Found: C,48.85; H,4.59; N,28.42%.

(4-Flulorobenzylideneamino)guanidine(6): Yield: 85%, m.p. 73-74°C. UV (λ_{max}): 290. IR (KBr, cm^{-1}): ν =1603 (CH=N), 1089 (N-N), 3218 (-NH), 3371 (–NH_2). ^{1}H NMR (DMSO, ppm): δ=7.837 (S, 1H.CH=N), 6.970-7.594(m, 4H Ar-H) 6.992 (S, 1H .C=NH), 7.557 (S, 1H.-NH), 6.970 (S,2H.-NH_2). ^{13}C NMR (DMSO, ppm): δ (C_1) = 142.43(CH=N), 128.26(C_2), 133.03(C_3), 115.36(C_4), 163.25(C_5), 115.15 (C_6), 133.01(C_7)160.81(C=NH). Anal.Calcd for $C_8H_9FN_4$ (189.18): C, 59.24; H, 6.21 N 34.54%. Found: C, 59.26; H 6.18; N, 34.45%.

(4-Methoxybenzylideneamino)guanidine(7): Yield:88 %, m.p. 113-114°C.UV (λ_{max}):306. IR (KBr, cm^{-1}): ν =1603 (CH=N), 1026 (N-N), 3007 (-NH), 3402 (–NH_2). ^{1}H NMR (DMSO, ppm): δ=7.962 (S,1H.CH=N), 6.888-7.617 (m, 4H Ar-H) 3.758 (OCH_3),7.596 (S,1H.C=NH), 7.617 (S,1H.–NH), 6.910 (S,2H.-NH_2).^{13}C NMR (DMSO, ppm): δ (C_1) = 143.44(CH=N), 127.63(C_2), 129.44(C_3, C_7), 113.85(C_4, C6), 159.28(C_5), 55.09 (OCH_3), 159.76 (C=NH). Anal.Calcd for $C_9H_{12}N_4O$ (192.22): C, 61.29; H, 6.29; N 31.79%. Found: C,61.18; H 6.17; N,31.72%.

(4-Methylbenzylideneamino)guanidine(8): Yield: 84 %, m.p. 137-138°C. UV (λ_{max}): 286. IR (KBr, cm^{-1}): ν = 1646 (CH=N), 1014 (N-N), 3105 (-NH), 3353 (–NH_2). ^{1}H NMR (DMSO, ppm): δ=7.970 (S,1H.CH=N), 7.131-7.572 (m, 4H Ar-H) 2.292 (CH3), 7.552 (S, 1H.C=NH),7.572 (S,1H.-NH)7.131 (S 2H- NH_2).^{13}C NMR (DMSO, ppm): δ (C_1) =143.49(CH=N), 34.01 (C_2), 127.97 (C_3,C_7), 129.09 (C_4 ,C_6), 137.23 (C_5), 20.88 (CH_3),160.07 (C=NH). Anal.Calcd for $C_9H_{12}N_4$ (176.22): C, 56.19; H, 6.86; N 29.14%. Found: C, 56.22; H 6.83; N, 29.08%.

(3-Nitrobenzylideneamino)guanidine(9): Yield: 96%, m.p. 207-208°C (lit:210[27]). UV (λ_{max}): 307. IR (KBr, cm^{-1}): ν =1600 (CH=N), 937 (N-N), 3363(-NH), 3476(–NH_2).^{1}H NMR (DMSO, ppm): δ=8.452 (S, 1H.CH=N), 7.571-8.130 (m,4H,Ar-H), 7.571 (S,1H.C=NH), 7.610 (S,1H.-NH), 6.082 (S,2H.-NH_2). ^{13}C NMR (DMSO, ppm): δ (C_1) = 141.82 (CH=N), 137.58 (C_2), 126.29 (C_3), 138.97 (C_4), 125.62 (C_5), 129.74 (C_6), 138.40 (C_7) 160.04 (C=NH). Anal.Calcd for $C_8H_9N_5O_2$ (207.19): C, 46.33; H, 4.37; N, 33.80%. Found: C, 46.31; H 4.33; N, 33.83%.

(4-Nitrobenzylideneamino)guanidine(10): Yield: 95%, m.p. 156-157°C. UV (λ_{max}): 247. IR (KBr, cm^{-1}): ν =1636 (CH=N), 988 (N-N), 3198 (-NH), 3469 ($-NH_2$). ^1H NMR (DMSO, ppm): δ=8.156 (S, 1H.CH=N), 7.892-8.134 (m, 4H Ar -H), 7.914 (S, 1H. C=NH), 8.046 (S, 1H.-NH), 7.892 (S, 2H.$-NH_2$). ^{13}C NMR (DMSO, ppm): δ (C_1) = 144.03 (CH=N), 140.01 (C_2), 126.39 (C_3, C_7), 123.68 (C_4, C_6), 145.76 (C_5), 160.0 4(C=NH). Anal.Calcd for $C_8H_9N_5O_2$ (207.19): C, 46.33; H, 4.37; N 33.80%. Found: C, 46.30; H 4.31; N, 33.85.

3. RESULTS AND DISCUSSION

3.1. UV spectral study

The assigned characteristics UV absorption maximum λ_{max}(nm) values of all the synthesized hydrazones under present investigation are presented in Table 1.

Table 1. The characteristics UV absorption (λ_{max}), infrared vibrations (ν, cm^{-1}) and NMR chemical shifts (δ ppm) of substituted (Benzylideneamino)guanidines.

Entry	X	UV Λmax [nm]	IR [ν, cm^{-1}]	^1H NMR [ppm]	^{13}C NMR [ppm]
1	H	311.00	1640.67	7.989	143.21
2	3-Br	285.50	1641.87	8.219	141.79
3	4-Br	306.50	1636.22	7.937	141.66
4	3-Cl	309.50	1641.09	7.969	141.69
5	4-Cl	286.50	1636.43	7.958	141.69
6	4-F	290.50	1603.90	7.837	142.43
7	4-OMe	306.00	1603.50	7.962	143.44
8	4-Me	286.06	1646.83	7.970	143.49
9	$3-NO_2$	307.00	1600.48	8.452	141.82
10	$4-NO_2$	247.50	1636.54	8.046	144.03

These data are correlated with Hammett substituent constants and *F* and *R* parameters using single and multi-linear regression analysis [28–32]. Hammett equation employed, for the correlation analysis, involving the absorption maxima is as shown below in equation (1):

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

Where λ_0 is the frequency for the parent member of the series.

.The results of statistical analysis of UV absorption maximum λmax(nm) values with Hammett substituent constants and *F* and *R* parameters are presented in Table 2. From Table 2, it is observed that the UV absorption maximum λ_{max} (nm) values have shown poor correlation ($r < 0.900$) with Hammett substituent constants and F and R parameters. This is due to the fact that the polar, resonance, filed and inductive effects of the substituents are sufficiently weaker for predicting the reactivity on the absorption through conjugation. All the correlations have shown negative ρ values. This shows that the reverse substituent effect operates in all systems. The failure in correlation is attributed to the conjugative structure shown in Fig 1.

Table-2. The results of statistical analysis of UV λmax (nm), IR ν(cm^{-1}) of CH=N, ^{1}H NMR chemical shift δC=N(ppm) and ^{13}C NMR chemical shift δC=N(ppm) data of substituted (benzylidene amino) guanidine compounds with Hammett constants σ, σ+, σ_I & σ_R and F and R parameters

Freq.	const.	r	I	ρ	s	n	correlated derivatives
λ_{max} [nm]	σ	0.795	298.850	–22.410	18.730	10	H,3–Br,4–Br,3–Cl,4–Cl,4–F, 4–OCH_3,4–CH_3,3–NO_2, 4–NO_2
	σ^+	0.832	295.70	–12.590	19.460	10	H,3–Br,4–Br,3–Cl,4–Cl,4–F, 4–OCH_3,4–CH_3, 3–NO_2, 4–NO_2
	σ_I	0.865	303.210	–24.450	19.510	10	H,3–Br,4–Br,3–Cl,4–Cl,4–F, 4–OCH_3,4–CH_3, 3–NO_2, 4–NO_2

Cont. Table 1.

Freq.	const.	r	I	ρ	s	n	correlated derivatives
	σ_R	0.752	290.020	–26.140	19.680	10	H,3–Br,4–Br,3–Cl,4–Cl,4–F, 4–OCH$_3$,4–CH$_3$, 3–NO$_2$, 4–NO$_2$
	F	0.725	303.760	–24.900	19.390	10	H,3–Br,4–Br,3–Cl,4–Cl,4–F, 4–OCH$_3$,4–CH$_3$, 3–NO$_2$, 4–NO$_2$
	R	0.859	289.890	–19.520	19.850	10	H,3–Br,4–Br,3–Cl,4–Cl,4–F, 4–OCH$_3$,4–CH$_3$, 3–NO$_2$, 4–NO$_2$
ν [cm^{-1}] C=N	σ	0.900	1628.517	1.007	19.430	7	H, 3–Br, 4–Br 3–Cl, 4–Cl, 4–CH$_3$, 4–NO$_2$
	σ^+	0.901	1627.507	7.417	19.060	7	H, 3–Br, 4–Br 3–Cl, 4–Cl, 4–CH$_3$, 4–NO$_2$
	σ_I	0.904	1640.350	–28.962	17.948	7	H, 3–Br, 4–Br 3–Cl, 4–Cl, 4–CH$_3$, 4–NO$_2$
	σ_R	0.903	1632.580	27.937	18.445	8	H, 3–Br, 4–Br, 3–Cl, 4–Cl, 4–F, 4–OCH$_3$, 4–CH$_3$
	F	0.905	1643.915	–37.162	16.718	7	H, 3–Br, 4–Br 3–Cl, 4–Cl, 4–CH$_3$, 4–NO$_2$
	R	0.903	1633.888	27.026	18.107	8	H, 3–Br, 4–Br, 3–Cl, 4–Cl, 4–F, 4–OCH$_3$, 4–CH$_3$
δ [ppm] CH= N	σ	0.906	7.962	0.309	0.147	7	H, 4–Br,3–Cl,4–Cl, 4–OCH$_3$, 4–CH$_3$, 4–NO$_2$

Cont. Table 1.

Freq.	const.	r	I	ρ	s	n	correlated derivatives
	σ^+	0.905	8.001	0.195	0.158	7	H, 4–Br,3–Cl,4–Cl, 4–OCH_3, 4–CH_3, 4–NO_2
	σ_I	0.732	7.935	0.250	0.175	10	H,3–Br,4–Br,3–Cl,4–Cl,4–F, 4–OCH_3,4–CH_3, 3–NO_2, 4–NO_2
	σ_R	0.906	8.108	0.540	0.144	8	H, 4–Br,3–Cl,4–Cl, 4–F, 4–OCH_3, 4–CH_3, 4–NO_2
	F	0.822	7.967	0.163	0.181	10	H,3–Br,4–Br,3–Cl,4–Cl,4–F, 4–OCH_3,4–CH_3, 3–NO_2, 4–NO_2
	R	0.905	8.114	0.426	0.150	8	H, 4–Br,3–Cl,4–Cl, 4–F, 4–OCH_3, 4–CH_3, 4–NO_2
δ [ppm] C=N	σ	0.924	142.679	–0.658	0.949	8	H, 3–Br, 4–Br, 3–Cl, 4–Cl, 4–F, 4–OCH_3, 4–CH_3
	σ^+	0.932	142.638	–0.674	0.918	8	H, 3–Br, 4–Br, 3–Cl, 4–Cl, 4–F, 4–CH_3, 3–NO_2
	σ_I	0.874	143.148	–1.586	0.891	10	H,3–Br,4–Br,3–Cl,4–Cl,4–F, 4–OCH_3,4–CH_3, 3–NO_2, 4–NO_2
	σ_R	0.811	142.602	0.559	0.973	10	H,3–Br,4–Br,3–Cl,4–Cl,4–F, 4–OCH_3,4–CH_3, 3–NO_2, 4–NO_2

Cont. Table 1.

Freq.	const.	r	I	ρ	s	n	correlated derivatives
	F	0.833	143.024	−1.222	0.925	10	H,3–Br,4–Br,3–Cl,4–Cl,4–F, 4–OCH$_3$,4–CH$_3$, 3–NO$_2$, 4–NO$_2$
	R	0.770	142.575	0.263	0.978	10	H,3–Br,4–Br,3–Cl,4–Cl,4–F, 4–OCH$_3$,4–CH$_3$, 3–NO$_2$, 4–NO$_2$

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

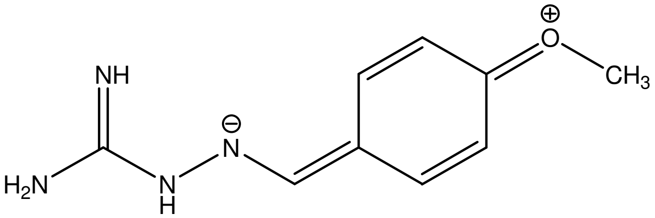


Fig. 1.The resonance – conjugative structure.

In view of the inability of the Hammett constants to produce individually satisfactory correlations with the UV absorption maximum λ_{max} (nm) values, the authors think that, it is worthwhile to seek multiple correlations involving either σ_I and σ_R constants or Swain–Lupton's *F* and *R* parameters. This is shown in the following Equations (2-3):

$$\lambda_{max}(\text{nm}) = 299.10\ (\pm\ 13.918) - 21.937(\pm\ 2.867)\sigma_I - 22.812\ (\pm\ 2.732)\sigma_R \quad (2)$$
$$(R=0.939,\ n = 10,\ P > 90\%)$$

$$\lambda_{max}(\text{nm}) = 300.184\ (\pm\ 11.332) - 25.687\ (\pm\ 3.023)F - 20.535(\pm\ 2.121)R \quad (3)$$
$$(R= 9.041,\ n = 10,\ P >90\%)$$

3.2. IR spectral study

The recorded infrared stretching frequencies (νcm^{-1}) of the synthesized hydrazones (entries 1–10) have been and presented in Table 1. These data are correlated [28-32] with Hammett substituent constants and

Swain–Lupton's parameters shown in Table 2. In this correlation the structure parameter Hammett equation employed is as shown in Eq(4).

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

Where ν_0 is the frequency for the parent member of the series.

From Table 2, it is evident that all the observed IR (νcm^{-1}) frequencies have shown satisfactory correlations with Hammett constants and F and R parameters. All the substituents except 4-F, 4-OCH_3 and 3-NO_2 have shown satisfactory correlations with Hammett constants namely σ(r = 0.900), σ+(r = 0.901) and σ_I(r = 0.904) and F(r = 0.905) parameter. Also, Hammett constant σ_R(r = 0.903) and R(r = 0.903) parameter have shown satisfactory correlations with all the substituents except 3-NO_2 and 4-NO_2.

All the correlations have shown positive ρ values except σR and R parameter. This indicates the operation of normal substituent effect operates in all the synthesized aryl hydrazones. The multi regression analyses have also shown satisfactory correlations as shown in equations (5-6):

$$\nu cm^{-1}{}_{(CH=N)} = 1646.06\ (\pm\ 12.140) - 32.588\ (\pm\ 5.743)\sigma_I + 32.886\ (\pm\ 15.824)\sigma_R \tag{5}$$
$$(R=0.953, n = 10, P > 90\%)$$

$$\nu cm^{-1}{}_{(CH=N)} = 1648.38\ (\pm\ 10.958) - 36.192\ (\pm\ 4.632)F + 25.599\ (\pm\ 4.769)R \tag{6}$$
$$(R=0.961, n = 10, P > 90\%)$$

3.3. NMR spectral study

The proton and carbon chemical shifts (ppm) of all the synthesized hydrazones (CH=N), have been assigned and are presented in Table 1. Attempts have been made to correlate the δCH=N chemical shifts (ppm) with Hammett substituent constants, field and resonance parameters, with the help of single and multi-regression analyses [28-32] to study the reactivity through the effect of substituents. The assigned proton chemical shifts (ppm) have been correlated with reactivity parameters using the Hammett equation as shown in equation (7):

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

Where δ_0 is the chemical shift of unsubstituted system

3.4. ^{1}H NMR spectral study

From the results of statistical analysis, all the substituents except 3-Br, 4-F and 3-NO_2 have shown satisfactory correlations with Hammett constants σ(r = 0.900) and σ^+ (r = 0.901). The Hammett constant σ_R(r = 0.904) and R(r = 0.905) parameter have also shown satisfactory correlations for all the substituents except 3-Br and 3-NO_2. The remaining Hammett constant σ_I and F parameter have shown poor correlations for all the substituents. The reason for the poor correlation was stated earlier.

All the correlations have shown positive ρ values, it indicates the operation of normal substituent effect in all the synthesized aryl hydrazones. The multi regression analyses have also shown satisfactory correlations as shown in equations (8-9):

$$\begin{aligned}\delta_{CH=N}(\text{ ppm}) &= 8.028\ (\pm 0.010) + 0.194\ (\pm 0.082)\sigma_I + \\ &+ 0.510\ (\pm 0.145)\ \sigma_R \\ &(R = 0.968,\ n = 10, P > 95\%)\end{aligned} \tag{8}$$

$$\begin{aligned}\delta_{CH=N}(\text{ ppm}) &= 8.043\ (\pm 0.101) + 0.180\ \ (\pm 0.021)\ F + \\ &+ 0.433\ (\pm 0.154)R \\ &(R=0.964, n = 10, P > 95\%)\end{aligned} \tag{9}$$

3.5. ^{13}C NMR spectral study

The chemical shifts (ppm) of hydrazone (C=N) carbon, have been assigned and are presented in Table 1. Attempts have been made to correlate the δC=N chemical shifts (ppm) with Hammett substituent constants, field and resonance parameters, with the help of single and multi-regression analyses [28-32] to study the reactivity through the effect of substituents.

The results of statistical analysis are presented in Table 2. From the results of statistical analysis, all the substituents except 4-OCH_3 and 4-NO_2 have shown satisfactory correlations with Hammett constants σ(r = 0.900) and σ^+(r = 0.901). The remaining Hammett constant σ_I, σ_R and F and R parameters have shown poor correlations for all the substituents. The reason for the poor correlation was stated earlier.

All the correlations have shown positive ρ values, it indicates the operation of normal substituent effect in all the synthesized aryl hydrazones. The multi regression analysis have also shown satisfactory correlations as shown in equations (10-11):

$$\delta CH{=}N(\text{ ppm}) = 143.295\ (\pm\ 0.644) - 1.676\ (\pm\ 0.454)\sigma I + 0.813\ (\pm\ 0.224)\ \sigma_R \tag{10}$$
$$(R = 945,\ n = 10,\ P > 90\%)$$
$$\delta CH{=}N(\text{ ppm}) = 143.061\ (\pm\ 0.660) - 1.214\ (\pm\ 0.242)F + 0.216\ (\pm\ 0.146)R \tag{11}$$
$$(R = 933,\ n = 10,\ P > 90\%)$$

3.6. Antimicrobial activities

As described in the introduction, hydrazone compounds possess a wide range of multipronged biological activities [33-38]. These multipronged activities such as antimicrobial activity present in different substituted benzylidineamino guanidines and are examined against respective microbes namely bacteria and fungi using Bauer-Kirby *in-vitro* zone of inhibition method.

3.7. Antibacterial sensitivity assay

Measurement of antibacterial sensitivity assay was performed using Kirby-Bauer [39] disc diffusion technique. In each Petri plate about 0.5 mL of the test bacterial sample was spread 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 were placed on the medium using sterile forceps. The plates were incubated for 24 h at 37°C by keeping the plates upside down to prevent the collection of water droplets over the medium. After 24 h, the plates were visually examined and the diameter values of the zone of inhibition were measured. Triplicate results were recorded by repeating the same procedure.

The antibacterial activities of all synthesized hydrazone compounds have been studied against three gram positive pathogenic strains viz., *Streptococcus pyogenes, Bacillus subtilis* and *Staphylococcus aureus* and two Gram-negative bacteria viz., *Escherichia coli* and *Pseudomonas aeruginosa* by using the disc diffusion method. The disc diffusion technique was followed using the Kirby-Bauer [40] method, at a concentration of 250 $\mu g/cm^3$ with Ciprofloxacin as the standard drug. The measured antibacterial activities of all hydrazones are presented in Table 3.

Table 3. Antibacterial activity of substituted hydrazone compounds.

S. No.	X	Zone of inhibition(mm)				
		Gram positive Bacteria			Gram negative Bacteria	
		B. subtilis	*Staphylo -coccus*	*Strepto-coccus*	*E. coli*	*P. aeruginosa*
1	H	12	19	14	10	13
2	3-Br	23	21	22	18	21
3	4-Br	17	12	17	16	12
4	3-Cl	21	19	20	16	15
5	4-Cl	28	20	25	21	27
6	4-F	13	15	14	15	12
7	4-OCH_3	20	20	19	16	17
8	4-CH_3	23	23	25	18	19
9	3-NO_2	19	13	22	18	24
10	4-NO_2	26	22	24	20	21
Standard	Ciprofloaxin	32	32	32	36	32
Control	DMSO	–	–	–	–	–

The antibacterial screening effect of all the synthesized hydrazones are shown in Fig. 2 (Plates 1-10). The zone of inhibition is compared using Table 3 and the clustered column chart is shown in Fig. 3. There is considerable antibacterial activity was possessed by all substituents on the microorganisms in general.

All the compounds showed moderate activities against all the bacterial species under investigation. The compound with substituent 4-Cl has shown excellent activity against *Bacillus subtilis, Streptococcus pyogenes* and *Pseudomonas aeruginosa.* The hydrazone compounds with 3-Br and 4-CH_3 substituents have shown improved activity against *Bacillus subtilis.* The 4-CH_3 substituted hydrazone compound has also shown excellent activity against *Staphylococcus* and *Streptococcus*. The 3-Br, 3-Cl and 4-NO_2 substituted compounds have shown improved activity against all the bacteria under investigation.

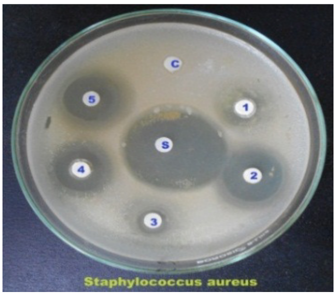


PLATE-1

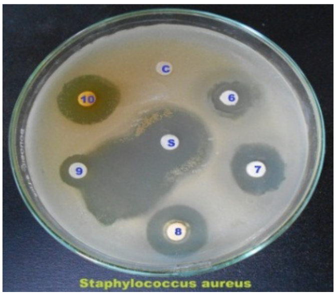


PLATE-2

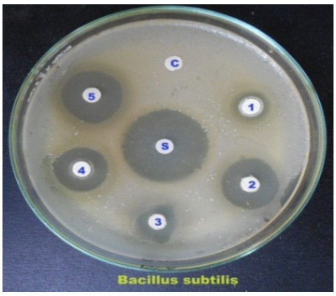


PLATE-3

PLATE-4

PLATE-5

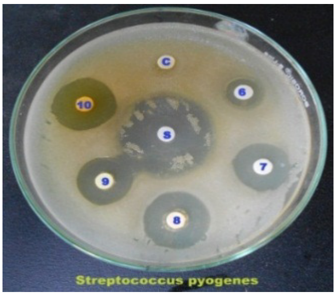


PLATE-6

PLATE-7

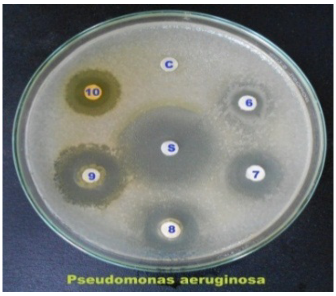


PLATE-8

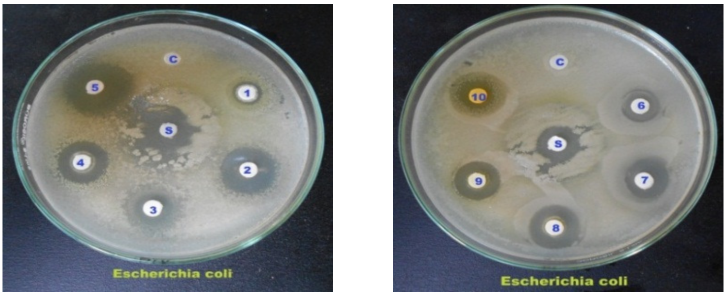


PLATE-9 PLATE-10

Fig. 2. Antibacterial activity of Substituted hydrazone compounds (petri plates).

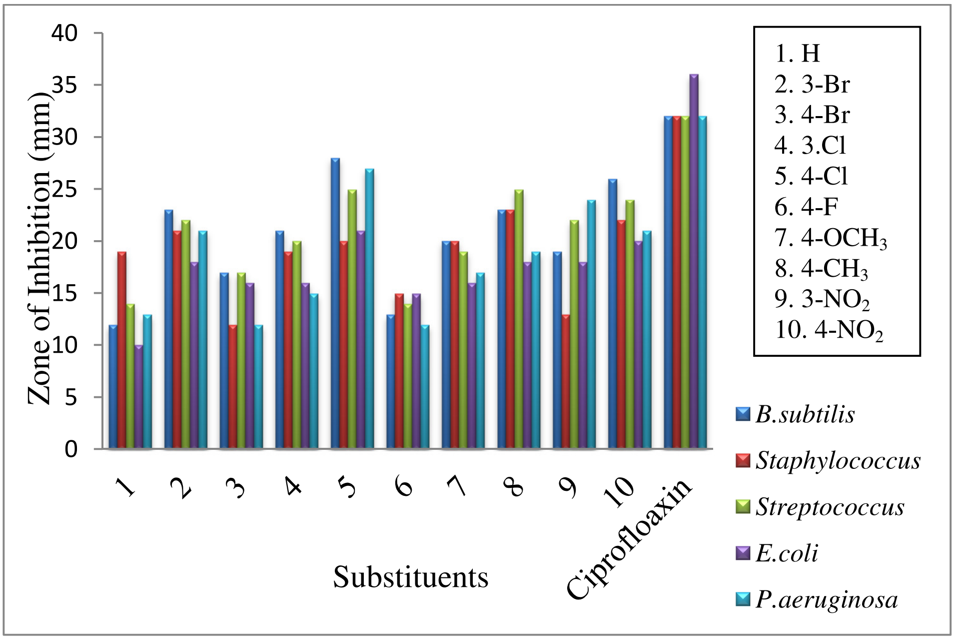


Fig. 3. Antibacterial activity of Substituted hydrazone compounds (clustered column chart)

3.8. Antifungal sensitivity assay

Antifungal sensitivity assay was performed using Kirby-Bauer [40] disc diffusion technique. PDA medium was prepared and sterilized as above. It was poured (ear bearing heating condition) in the Petri- plate which was already filled with 1 cm^3 of the fungal species. The plate was rotated clockwise and counter clock-wise for uniform spreading of the species. The discs were impregnated with the test solution. The test solution was prepared by dissolving 15 mg of the hydrazone compound in 1ml of DMSO solvent. The medium was allowed to solidify and kept for 24 h. Then the plates were visually examined and the diameter values of zone of inhibition were measured. Triplicate results were recorded by repeating the same procedure.

The study of antifungal activities of all hydrazone compounds have been done with *Aspergillus flavus, Aspergillus niger* and *Trigoderma veride* as the fungal strain using the disc diffusion technique. The drug dilution was kept as 50 μg/cm^3. *Ciproflaxin* has been taken as the standard drug. The observed antifungal activities of all hydrazone compounds are presented in Table 4.

Table.4. Antifungal activity of Substituted hydrazone compounds

S. No.	X	Zone of inhibition(mm)		
		Aspergillus flavus	Aspergillus niger	Trigoderma veride
1	H	14	14	13
2	3-Br	14	13	11
3	4-Br	15	16	15
4	3-Cl	16	15	14
5	4-Cl	19	18	14
6	4-F	11	14	13
7	4-OCH_3	11	18	13
8	4-CH_3	13	16	14
9	3-NO_2	13	16	15
10	4-NO_2	14	17	16
Standard	Ciproflaxin	26	22	20
Control	DMSO	–	–	–

The antifungal activities of all substituted hydrazone compounds synthesized in the present study are shown in Fig. 3 (Plates 1-6) and the zone of inhibition values of the effect is given in Table 4. The clustered column chart, shown in Fig-5 reveals that all the compounds showed moderate antifungal activity against *Trigoderma veride.* The compound with substituent 4-Cl has shown excellent activity against

Aspergillus flavus and *Aspergillus niger.* The 4-NO_2 substituted compound has shown excellent activity against *Trigoderma veride*. Also the compounds with substituents 4-Br and 3-NO_2 have shown improved activity against all the three fungal species under investigation

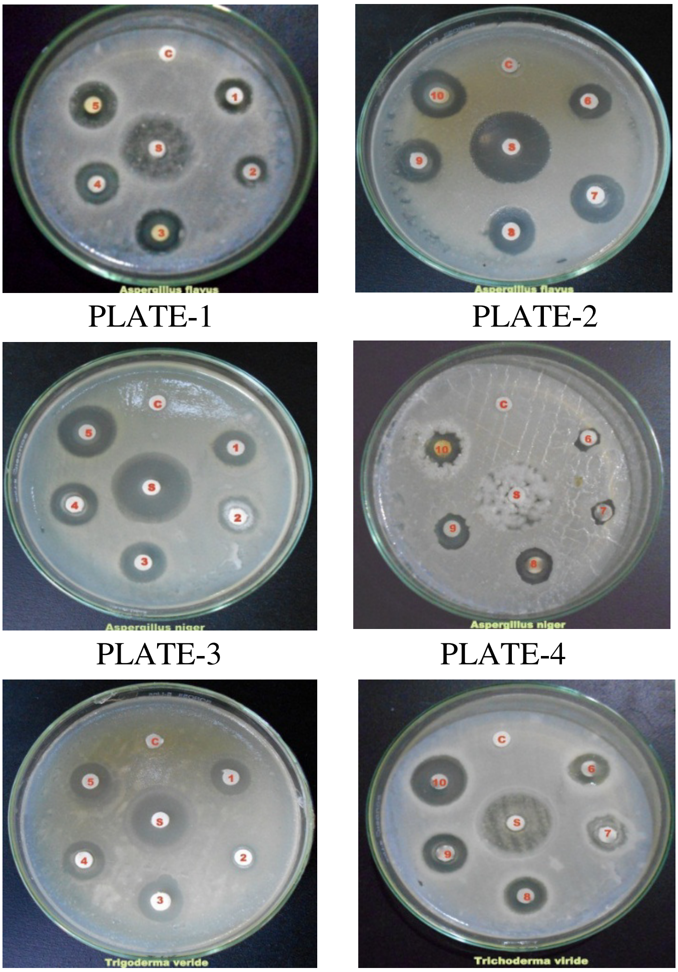


PLATE-1 PLATE-2

PLATE-3 PLATE-4

PLATE-5 PLATE-6

Fig. 4. Antifungal activity of Substituted hydrazone compounds (petri plates).

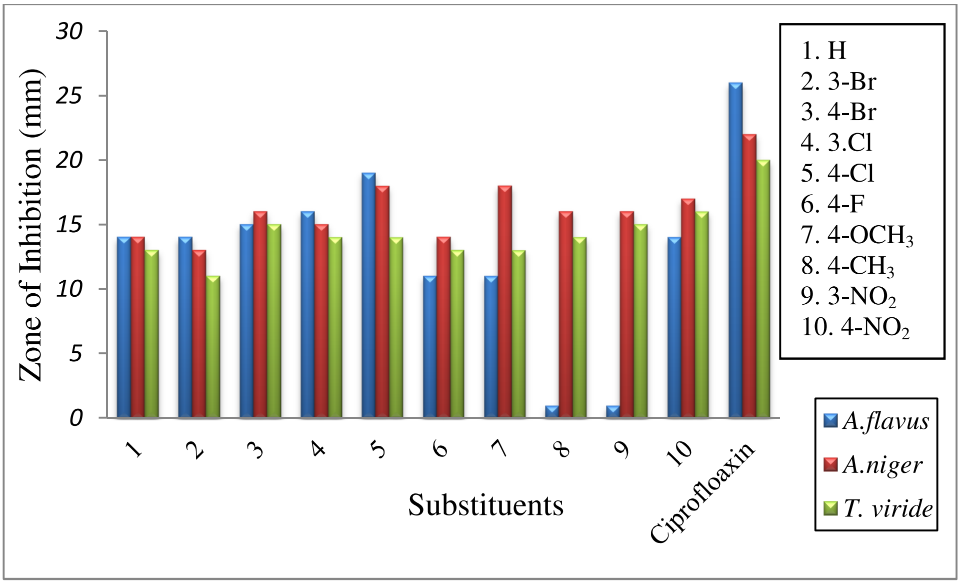


Fig. 5. Antifungal activity of Substituted hydrazone derivatives (clustered column chart)

4. CONCLUSIONS

Some hydrazone compounds have been synthesized by condensation of amino guanidine and benzaldehydes. These hydrazone compounds have been characterized by their physical constants, analytical and spectral data. The UV, IR, NMR spectral data of these hydrazones have 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. In single parameter correlation the UV (λ_{max}) absorption produced poor r values. The infrared νCH=N (cm^{-1}) frequencies produces satisfactory correlation with Hammett substituent constants. The chemical shift (δ ppm) CH=N values of hydrazones gave satisfactory correlation with Hammett σ, σ^+, σ_R constants and R parameters. The ^{13}C NMR chemical shifts (δ ppm) of hydrazones were satisfactorily correlated with hammett σ and σ^+ constants only. The antimicrobial activities of all synthesized hydrazone compounds have been studied using Bauer-Kirby method. The compounds with substituent 4-Cl has shown excellent activity against *Bacillus subtilis, Streptococcus pyogenes* and *Pseudomonas aeruginosa.* The 4-CH_3 substituted hydrazone compound has also shown excellent activity against *Staphylococcus* and *Streptococcus*. *Trigoderma veride*. The compound with substituent 4-Cl has shown excellent activity against *Aspergillus flavus* and *Aspergillus niger*. The 4-NO_2 substituted compound has shown excellent activity against *Trigoderma veride*.

ACKNOWLEDGEMENT

The authors thank DST NMR facility, Department of Chemistry, Annamalai University, Annamalainagar-608 002, India, for recording NMR spectra of all compounds.

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