FULL PAPER

Synthesis, study of structure activity relationship and evaluation of biological activities of substituted (*E*)-2-benzylidene-*N*-methylhyrazinecarbothioamides

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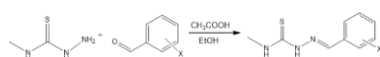[b] Department of Chemistry, Annamalai University, Annamalainagar-608 002, India

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Abstract

A series of ten substituted (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides were synthesized from 4-methyl-3-thiosemicarbazide with substituted benzaldehydes. All the synthesized compounds were in good agreement with elemental and spectral data (UV, FT-IR, ^{1}H NMR and ^{13}C NMR). The assigned UV λ_{max} (nm), IR vC=N (cm^{-1}), NMR δ[1]H (ppm) CH=N and δ^{13}C (ppm) C=N spectral data of (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides correlated with Hammett constants using single and multi-regression analysis. From the results of correlation analysis substituent effects on the spectral data have been discussed. The antibacterial activity of (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides have been studied with three Gram-positive pathogenic bacterial strains namely (*B. subtilis*, *S. aureus* and *S. pyogens*) and two Gram-negative strains (*E. coli* and *P. aeruginosa*). The antifungal activity of (*E*)-2-benzylidene-*N*-methyl hydrazones studied with three fungal species (*A. flavus*, *A. niger* and *T. viride)* using disk diffusion method.

Keywords: hydrazones, biological activity, substituent effect, spectral correlation

INTRODUCTION

Hydrazones are a special group of the Schiff base family. They are consider by the presence of >C=N-N<. In hydrazone moiety have the nitrogen atom behaves as nucleophilic and carbon atom behaves as nucleophilic as well as electrophilic nature [1–3]. Several hydrazones are attained depending on the experimental conditions which have application as biologically active compounds [4] and as analytical reagents [5]. The effect of substituents on the group frequencies through UV–vis, IR, ^{1}H and ^{13}C NMR spectra of ketones [6], unsaturated ketones [7], acid chlorides [8] acyl bromides, and their esters [9] oxazine [10], chalcone [11], hydrazone [12] has been studied.

However, literature survey shows that the study of effect of substituents on the group frequencies of oxiranes is almost absent. As biologically active compounds, hydrazones find applications in the treatment of diseases such as anti-tumor [13], tuberculosis [14], leprosy and mental disorder [4]. The hydrazones antimalarial [15], analgesic [16, 17], antiplatelet [18], antituberculosis [19], anticancer [20, 21] and antioxidant [22] effect. The antimicrobial activity of hydrazones was studied against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. Most of the hydrazone derivatives are possess antibacterial as well as antifungal activities [23, 24]. These potentials are also applied for the study of structure activity relationships [25]. From thorough literature survey there is no report on the effect of substituents-QSAR or QPR study with these compounds, in the past. Therefore, the authors taken effort to synthesised some substituted (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides and study the correlation analysis with their UV, IR and NMR spectral data as well as study of antimicrobial activity using disc diffusion method.

MATERIALS AND METHODS

All the chemicals involved in the present study have been purchased from Sigma–Aldrich chemical company Bangalore. The melting points of all substituted (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamide compounds were recorded by Mettler FP51 melting point apparatus. The electronic spectra of all the synthesized compounds were recorded from ELICO-BL222 spectrophotometer. IR spectra (KBr, 4000–400 cm^{-1}) were recorded from AVATAR-300 Fourier transform spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were obtained using Bruker AV400 NMR spectrometer operating at 400 MHz in DMSO solvent using TMS as internal standard. Microanalyses of all the hydrazones were obtained from Thermo Finnigan analyzer.

Typical Synthesis of substituted (E)-2-benzylidene-N-methylhydrazinecarbothioamides

Equimolar quantities 4-methyl-3-thiosemicarbazide (10 mmol) and substituted benzaldehyde (10 mmol) were dissolved in ethanol (30 ml). Two drops of acetic acid was added and the mixture allowed to refluxing for 6 hours (Scheme 1). After completion of the reaction the compounds was filtered, dried and then was recrystallized from ethanol. The Physical constants and analytical data of substituted (*E*)-2-benzylidene-N-methylhydrazinecarbothioamides are presented in Table 1. The UV, IR and NMR spectral data of substituted (*E*)-2-benzylidene-N-methylhydrazinecarbothioamides are presented in Table 2.
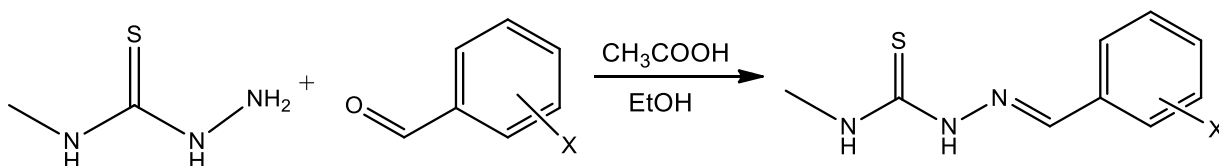
Entry	1	2	3	4	5	6	7	8	9	10
X	H	3-Br	4-Br	3-Cl	4-Cl	4-F	4-OCH$_3$	4-CH$_3$	3-NO$_2$	4-NO$_2$

Scheme 1 Synthesis of substituted (*E*)-2-benzylidene-*N*methylhydrazinecarbothioamides.

Table 1 Physical constants of substituted (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides.

Entry	X	Empirical formula	Formula weight	m.p (°C)	Yield (%)	Microanalysis (calcd)		
						C	H	N
1	H	C$_9$H$_{11}$N$_3$S	193.27	142-143	90	55.93 (55.90)	5.73 (5.74)	21.74 (21.72)
2	3-Br	C$_9$H$_{10}$BrN$_3$S	272.16	137-138	88	39.71 (39.68)	3.70 (3.75)	15.43 (15.13)
3	4-Br	C$_9$H$_{10}$BrN$_3$S	272.16	118-119	92	39.71 (39.68)	3.70 (3.75)	15.43 (15.13)
4	3-Cl	C$_9$H$_{10}$ClN$_3$S	227.21	133-134	95	47.57 (47.44)	4.43 (4.66)	18.49 (18.24)
5	4-Cl	C$_9$H$_{10}$ClN$_3$S	227.21	139-140	91	47.57 (47.44)	4.43 (4.66)	18.49 (18.24)
6	4-F	C$_9$H$_{10}$FN$_3$S	221.26	106-107	94	48.85 (48.78)	4.55 (4.59)	18.99 (18.91)
7	4-CH$_3$	C$_{10}$H$_{13}$N$_3$OS	223.29	163-164	89	53.78 (53.87)	5.86 (5.83)	18.81 (18.96)
8	4-OCH$_3$	C$_{10}$H$_{13}$N$_3$S	207.3	184-185	87	57.93 (57.99)	6.32 (4.42)	20.27 (20.39)
9	3-NO$_2$	C$_9$H$_{10}$N$_4$O$_2$S	238.27	232-233 233[26]	93	45.36 (45.55)	4.23 (4.32)	23.51 (23.57)
10	4-NO$_2$	C$_9$H$_{10}$N$_4$O$_2$S	238.27	231-232 232[26]	92	45.36 (45.55)	4.23 (4.32)	23.51 (23.57)

Table 2 UV, IR and NMR spectral data of substituted (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides

Entry	X	UV λ$_{max}$ (nm)	IR ν(C=N) (cm^{-1})	δ^{1}H NMR (CH=N) (ppm)	δ^{13}C NMR (C=N) (ppm)
1	H	322	1639.38	9.442	149.47
2	3-Br	317	1643.35	9.938	149.40
3	4-Br	318.5	1641.42	9.738	148.92
4	3-Cl	315.5	1645.28	9.829	152.64
5	4-Cl	316.5	1647.21	9.694	149.32
6	4-F	313.5	1639.49	9.815	149.82
7	4-CH$_3$	321	1695.43	9.171	148.83
8	4-OCH$_3$	314.5	1625.99	9.659	149.11

RESULTS AND DISCUSSION

UV spectral study

The assigned characteristic UV absorption maximum λ_{max}(nm) values of all the synthesized (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides under present investigation are presented in Table 2.

These observed spectral data are correlated with Hammett substituent constants and F and R parameters using single and multi-linear regression analyses [27-31]. Hammett equation employed, for the correlation analysis, concerning the absorption maxima is as shown below in equation (1):

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

where λ_O is the frequency for the parent member of the series.

The results of statistical analysis of UV absorption maximum λ_{max} (nm) values with Hammett constants and Swain-Lupton's parameters are presented in Table 3. From Table 3, it is witnessed that the UV absorption maximum λ_{max}(nm) values have shown poor correlation (r < 0.900) with Hammett constants and Swain-Lupton's parameters. This is due to the weak polar, resonance, field and inductive effects of the substituents for the predicting the reactivity on the absorption through resonance-conjugative structure as shown in Figure 1. All the correlations have shown negative ρ values. This shows that the reverse substituent effect operates in all systems. The reason for the poor correlation is attributed to the conjugative structure shown in Figure 1.
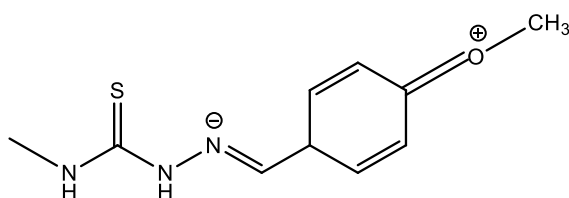

Figure 1 The resonance-conjugative structure.

Since some of the single regression analyses have shown poor correlations with Hammett constants σ_R and F and *R* parameter, it is decided to go for multi regression analysis. The multi regression analysis of the UV absorption maximum λ_{max}C=N(nm) values of all the substituted (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides compounds with inductive, resonance and Swain-Lupton's [32] parameters produce satisfactory correlations as shown in equations (2) and (3)

$$UV\ (\lambda_{max}\ nm) = 319.74(\pm 5.401) + 3.89(\pm 1.082)\sigma_R + 15.48(\pm 2.705)\sigma_R \qquad (2)$$

(R = 0.944, n = 10, P > 90%)

$$UV\ (\lambda_{max}\ nm) = 319.98(\pm 5.30) + 3.92(\pm 1.051)F + 12.78(\pm 2.725)R \qquad (3)$$

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

IR spectral study

The assinged infrared stretching frequency νC=N(cm^{-1}) values of all the substituted (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamide compounds are presented in (Table 2). These observed spectral values are correlated with Hammett substituent constants and F and R parameters using for single and multi-linear regression analyses [27-31].

While seeking Hammett correlation involving group frequencies, the form of the Hammett equation (4) employed is

$$\nu = \rho\sigma + \nu_o \qquad (4)$$

where ν_o is the frequency for the parent member of the series.

From (Table 3), it is evident that the infrared stretching frequency νC=N(cm^{-1}) values of all the (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamide compounds have shown poor correlation with Hammett substituent constants σ, σ^+, σ_I, σ_R and *F* and *R* parameters.

The poor correlation is attributed to weak inductive, resonance and field effect of the substituents unable to predict the reactivity on the frequency through resonance as per the conjugative structure given in (Figure 1).

All the correlations except with σ_I have shown negative ρ values. The multi regression analysis of the stretching frequency νC=N(cm^{-1}) values of all the (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamide compounds with inductive, resonance and Swain-Lupton's [32] parameters produce satisfactory correlations as shown in equations (5) and (6).

$$\nu cm^{-1}(CH=N) = 1637.42(\pm 12.542) + 6.37(\pm 1.513)\sigma_I - 43.23(\pm 3.501)\sigma_R \qquad (5)$$

(R = 0.948, n = 10, P 90%)

$$\nu cm^{-1}(CH=N) = 1639.55(\pm 12.024) - 1.96(\pm 0.728)F - 37.34(\pm 3.297)R \qquad (6)$$

(R = 0.952, n = 10, P > 95%)

^{1}H NMR Spectral study

The assigned ^{1}H NMR chemical shift δCH=N (ppm) values of substituted (*E*)-2-benzylidene-*N*-methyl hydrazinecarbothioamide compounds are presented in (Table 2). These observed spectral values are correlated with Hammett substituent constants and F and R parameters using for single and multi-linear regression analyses [27-31]. In this correlation the structure parameter Hammett equation employed is as shown in equation (7).

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

From the results of statistical analysis, all the substituents except those with 3-NO$_2$ and 4-NO$_2$ substituents have shown satisfactory correlations with Hammett constants σ (r = 0.904), σ^+ (r = 0.903) and R (r = 0.907). The Hammett constant σ_R (r = 0.906) parameter has shown satisfactory correlation for all the substituents except those with 4-F and 4-OCH$_3$. The remaining Hammett constant σ_I and F parameter have shown poor correlations for all the substituents.

The poor correlation is attributed to weak polar, inductive, field and resonance effects of substituents for predicting the reactivity on the ^{1}H NMR chemical shifts through resonance as per the conjugative structure shown in Figure 1. All the correlations with Hammett constants and F and R parameters have shown negative ρ values. This indicates operation of reverse substituent effects with respect to ^{1}H NMR chemical shift values of hydrazone compounds.

In view of the inability of all the Hammett constants and F and R parameters to produce individually satisfactory correlations, it is decided that it is worthwhile to seek multi regression analysis. The multi regression analysis of the ^{1}H NMR chemical shift δCH=N(ppm) values of all hydrazones with inductive, resonance and Swain-Lupton's [32] parameters produce satisfactory correlations as shown in equations. The correlation equations generated are shown in equations (8) and (9).

$$\delta CH=N\ (ppm) = 9.43(\pm 0.327) - 0.63(\pm 0.156)\sigma_I - 1.66(\pm 0.170)\sigma_R \qquad (8)$$

(R = 0.968, n = 10, P > 95%)

$$\delta CH=N\ (ppm) = 9.45(\pm 0.327) - 0.73(\pm 0.148)F + 0.37(\pm 0.161)R \qquad (9)$$

(R = 0.966, n = 10, P > 95%)

Table 3 Results of correlation analyses of UV λ_{max} (nm), IR vC=N (cm^{-1}), NMR δ^1H (ppm) CH=N and δ^{13}C (ppm) C=N of (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides with Hammett substituent constants and Swain-Lupton's *F* and *R* parameters.

Freq.	Constant	r	I	ρ	s	n	Correlated derivatives
λ_{max}	σ	0.903	317.26	8.066	7.617	9	H, 3−Br, 4−Br, 3−Cl,4−Cl,4−F, 4−OCH$_3$, 4−CH$_3$, 3−NO$_2$
	σ$^+$	0.9	318.38	4.538	7.854	9	H,3−Br,4−Br,3−Cl,4−Cl,4−F, 4−OCH$_3$, 4−CH$_3$, 3−NO$_2$
	σ$_I$	0.901	316.96	5.597	8.061	9	H, 3−Br, 4−Br, 3−Cl, 4−Cl, 4−F, 4−OCH$_3$, 4−CH$_3$, 3−NO$_2$, 4−NO$_2$
	σ$_R$	0.84	321.35	16.076	7.388	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	0.811	317.74	3.435	8.137	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	0.841	321.54	12.623	7.493	10	H, 3−Br, 4−Br, 3−Cl, 4−Cl, 4−F, 4−OCH$_3$, 4−CH$_3$, 3−NO$_2$, 4−NO$_2$
C=N	σ	0.835	1650.28	-18.943	18.112	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$^+$	0.881	1649.32	-20.729	16.307	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$_I$	0.802	1654.21	1.6085	19.439	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$_R$	0.798	1640.05	-42.26	17.079	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	0.807	1646.06	-0.545	19.438	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	0.805	1638.76	-37.257	16.817	10	H, 3−Br, 4−Br,3−Cl,4−Cl,4−F, 4−OCH$_3$, 4−CH$_3$
CH=N	σ	0.904	9.617	-0.884	0.512	8	H, 3−Br, 4−Br,3−Cl, 4-Cl, 4-F, 4-OCH$_3$, 4-CH$_3$
	σ$^+$	0.903	9.483	-0.433	0.567	8	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 4-CH$_3$,
	σ$_I$	0.863	9.73	-0.814	0.571	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	9.17	-1.751	0.472	8	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-CH$_3$, 3-NO$_2$, 4-NO$_2$
	F	0.801	9.689	-0.683	0.581	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	0.907	9.155	-1.342	0.497	8	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-OCH$_3$, 4-CH$_3$
C=N	σ	0.823	149.49	-0.111	1.26	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$^+$	0.804	149.44	0.101	1.26	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$_I$	0.709	148.48	-0.046	1.261	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$_R$	0.728	149.25	-1.528	1.215	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	0.803	149.53	-0.162	1.26	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	0.817	149.3	-0.859	1.241	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; ρ = slope; I = intercept; s = standard deviation; n = number of substituents

^{13}C NMR Spectral study

The assigned ^{13}C NMR chemical shift δC=N (ppm) values of substituted (*E*)-2-benzylidene-*N*-methyl hydrazinecarbothioamide compounds are presented in Table 2. These assigned spectral values are correlated with Hammett substituent constants and F and R parameters using for single and multi-linear regression analyses [27-31]. The results of statistical analysis presented in Table 3. From Table 3, it is evident that the ^{13}C NMR chemical shift δCH=N (ppm) values of all the (*E*)-2-benzylidene-*N*-methylhydrazine carbothioamide compounds have shown poor   correlations (r > 0.900) with all the Hammett constants and Swain-Lupton's parameters .

The poor correlation is attributed to weak polar, inductive, field and resonance effects of substituents for predicting the reactivity on the ^{13}C NMR chemical shifts through resonance as per the conjugative structure given in Figure 1. All the correlations with Hammett constants, F and R parameters have shown negative ρ values. This indicates that the reverse substituent effect operates with respect to ^{13}C NMR chemical shift δCH=N (ppm) values of all the substituted (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamide compounds .

The multi regression analysis of the ^{13}C NMR chemical shift δC=N (ppm) values of all (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamide compounds with inductive, resonance and Swain-Lupton's [32] parameters gave satisfactory correlations as shown in equations (10) and (11).

$$\delta_{C=N} \text{ (ppm)} = 149.20(\pm 0.896) + 0.12(\pm 0..079)\sigma_I - 1.50 (\pm 0.210)\sigma_R \qquad (10)$$

(R= 988, n = 10, P > 95%)

$$\delta_{C=N} \text{ (ppm)} = 149.38(\pm 0.886) - 0.19(\pm 0.075)F - 0.86 (\pm 0.079)R \qquad (11)$$

(R = 0.918, n= 10, P 90%)

Antimicrobial activity

Antimicrobial screening of substituted (*E*)-2-benzylidene-*N*-methylhydrazine carbothioamide was done following the Kirby-Bauer [33] disc diffusion technique. All the synthesized hydrazones (1-10) were screened for their in vitro antibacterial activity against three gram positive bacteria namely *B.subtilis, S. aureus* and *S. pyogenes* and two gram negative *bacteria E. coli* and *P. aeruginosa* at 250µg/mL with Ciprofloxacin as the standard drug. The antibacterial screening effects of synthesized (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides is shown in Figure 2(Plates 1-10). The zone of inhibition is compared using Table 4 and the corresponding clustered column chart is shown in Figure 3. The 3-Cl, 4-OCH₃ and 4-NO₂ substituted hydrazone have shown good activity against *B.subtills*. The H, 3-Br, 4-Cl, 4-OCH₃ and 4-NO₂ substituted hydrazone have shown good activity against *S. aureus*. The 3-Cl, 4-CH₃, 3-NO₂ and 4-NO₂ compounds have shown good antibacterial activity against *S.pyogenes*. The 3-Br, 3-NO₂ and 4-NO₂ compounds have shown good activity against *E.Coli*. The H, 4-Cl, 4-CH₃ and 4-NO₂ substituents also have shown good antibacterial activity against *P.aeruginosa.*

Antifungal activity

Antifungal sensitivity assay was performed using Kirby-Bauer [33] disc diffusion technique followed at a concentration of 250µg/mL with amphotericin-B taken as the standard drug. The synthesized compounds were evaluated for their antifungal activity against three fungal species namely *A. flavus, A.niger* and *T. viride* were utilized. The antifungal activities of synthesized (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides is shown in Figure 4 (Plates 11-16). The zone of inhibition is compared using Table 4 and the corresponding clustered column chart is shown in Figure 5. The H, 3-Cl and 4-CH₃ substituents have shown good activities against *A.flavus*. The 3-Br, 3-Cl, 3-NO₂ and 4-NO₂ hydrazone compounds have shown good activities against *A .niger*. The 3-NO₂ compound only has shown good activity against *T.viridi*. The remaining all the As T66 have shown moderate activities against all the three species.

Table 3 Antibacterial activity of substituted (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides

Entry	Subtituent	*B.subtilis*	*S.aureus*	*S.pyogenes*	*E.Coli*	*P.aeruginosa*
1	H	11	13	9	8	12
2	3-Br	9	12	7	9	8
3	4-Br	10	7	8	8	7
4	3-Cl	13	9	12	7	8
5	4-Cl	11	11	11	8	11
6	4-F	8	8	10	8	8
7	4-CH₃	12	10	13	8	12
8	4-OCH₃	10	11	10	7	8
9	3-NO₂	11	7	12	9	10
10	4-NO₂	13	11	13	10	11
Standard	Ciprofloxacin	18	16	20	12	20
Control	DMSO	0	0	0	0	0

Plate-1	Plate-2	Plate-3	Plate-4	Plate-5

Plate-6	Plate-7	Plate-8	Plate-9	Plate-10
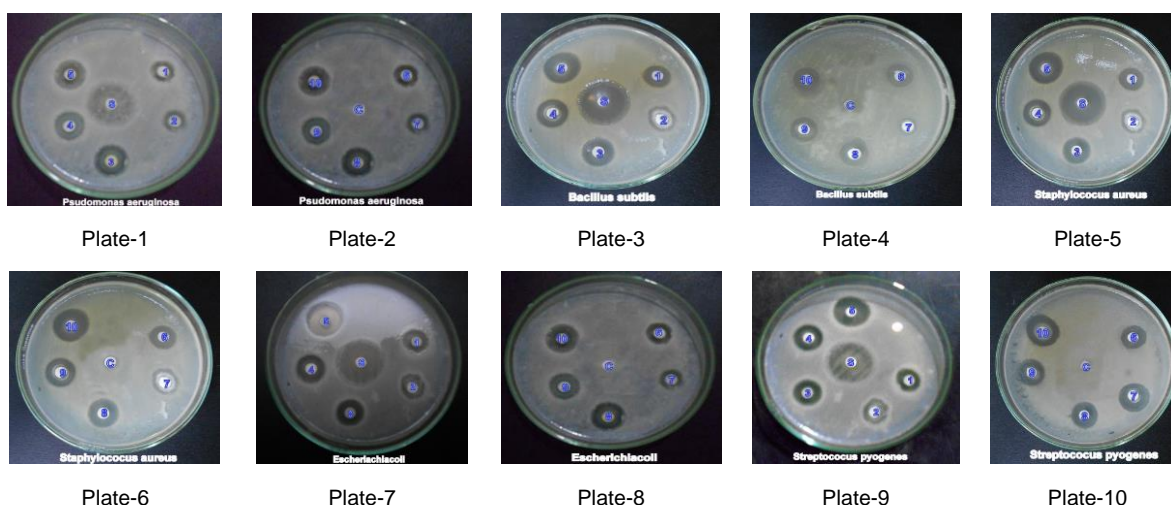
Figure 2 Antibacterial activity of substituted (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides (petri-plates)

1. H
2. 3-Br
3. 4-Br
4. 3-Cl
5. 4-Cl
6. 4-F
7. 4-CH$_3$
8. 4-OCH$_3$
9. 3-NO$_2$
10. 4-NO$_2$

■ *B.subtilis*
■ *S. aureus*
■ *Streptococcus*
■ *E.coli*
■ *P.aeruginosa*

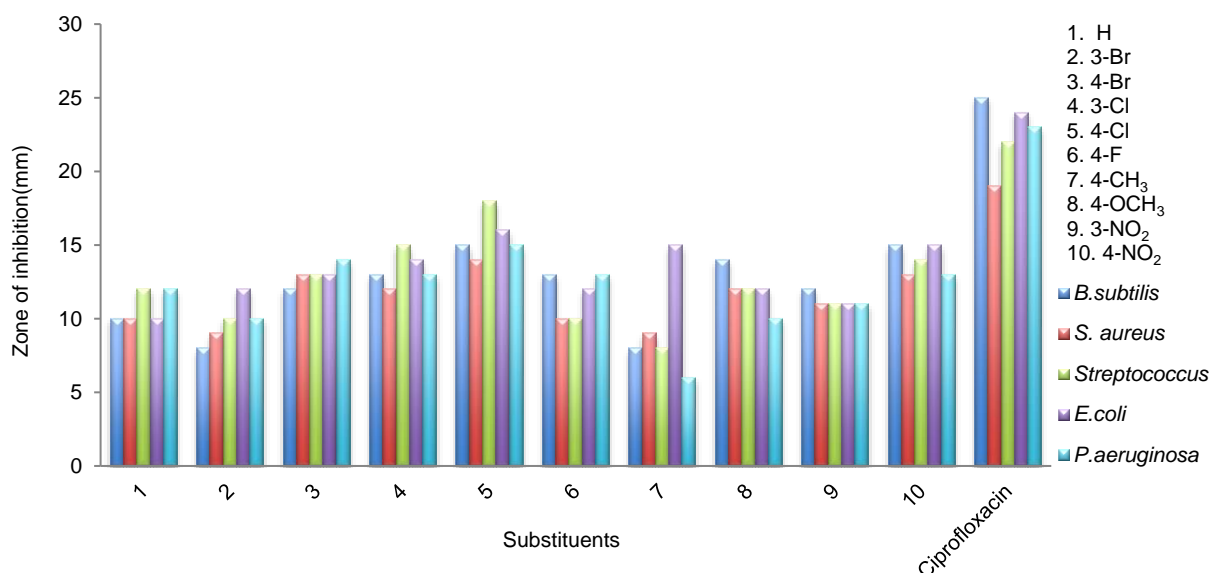**Figure 3:** Antibacterial activity of substituted (*E*)-2-benzylidene-*N*-methyl hydrazinecarbothioamides (clustered column chart)

Plate-11   Plate-12   Plate-13

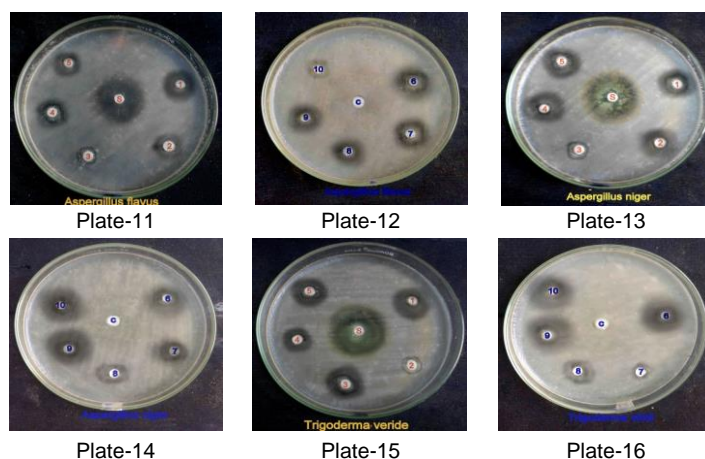Plate-14   Plate-15   Plate-16

Figure 4 Antifungal activity of substituted (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides (petri-plates)

Table 4 Antifungal activity of substituted (*E*)-2-benzylidene-*N*- methyl hydrazinecarbothioamides

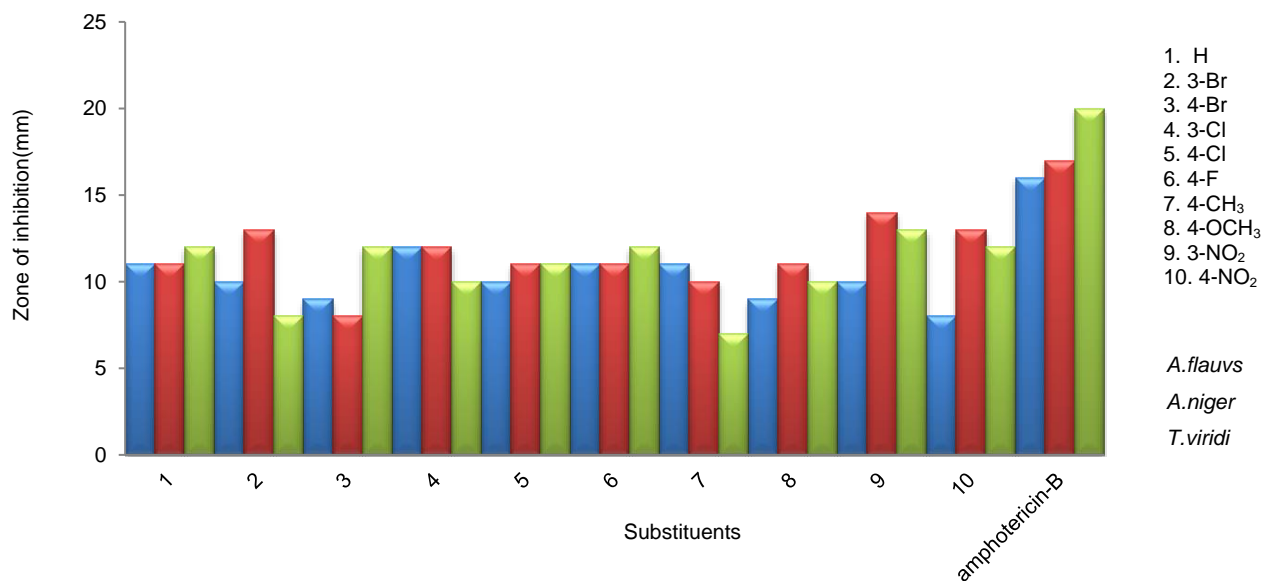| Entry	Subtituents	*A.flavus*	*A.niger*	*T. viridi*
1	H	11	11	12
2	3-Br	10	13	8
3	4-Br	9	8	12
4	3-Cl	12	12	10
5	4-Cl	10	11	11
6	4-F	11	11	12
7	4-CH$_3$	11	10	7
8	4-OCH$_3$	9	11	10
9	3-NO$_2$	10	14	13
10	4-NO$_2$	8	13	12
Standard	amphotericin-B	16	17	20
Control	DMSO	0	0	0

Figure 5: Antifungal activity of substituted (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides (clustered column chart)

CONCLUSIONS

The authors have synthesized a series of ten substituted (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamide compounds using 4-methyl-3-thiosemicarbazide and substituted benzaldehydes. The structure activity relationship of substituted (*E*)-2-benzylidene-*N*-methylhydrazinecarbothioamides have been studied using assigned spectral values with Hammett substituent constants and Swain-Lupton's parameters through single and multi-regression analysis. From the results of some of the single-linear regression analysis have shown satisfactory correlation. All the multi-linear regression analysis produced satisfactory correlations. The biological activities of all synthesized compounds were studied against human pathogenic bacterial and fungal species. The 3-Cl, 4-OCH₃, and 4-NO₂ substituted compounds have shown good activities. The remaining substituted compounds have shown moderate antibacterial activities. The 3-Cl, 3-NO₂ substituted compounds have shown good antifungal activities. The remaining substituted compounds have shown moderate antifungal activities

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REFERENCES

[1] N.P. Belskaya, W. Dehaen and V.A. Bakulev, 2010. Arkivoc (i):275-332.
[2] M. Rajarajan, S. Balaji, R. Vijayakumar, R. Senbagam, V. Manikandan, G. Vanangamudi and G. Thirunarayanan, 2016. JSM Chem., 4(1):1021-1028.
[3] R. Brehme, D. Enders, R. Fernandez and J.M. Lassaletta, 2007. Eur. J. Org. Chem. (34): 5629-5660.
[4] J.P. Corhnelissen, J.H. Van Diemen, L.R. Groeneveld, J.G. Haasnoot, A.L. Spek and J. Reedisk, 1992. Inorg. Chem. (31): 198-202.
[5] S.N. Dubey and B. Kaushik, 1985. Indian J. Chem. (24A): 950 -953.
[6] H.C. Brown and Y. Okamoto, 1957. J. Am. Chem. Soc. (79): 1913.
[7] G. Thirunarayanan, M. Gopalakrishnan and G. Vanangamudi, 2007. Spectrochim. Acta. 67(A): 1106 -1112.
[8] M.S. Flett, 1948. Trans. Faraday Soc. (44): 767-774.
[9] G. Thirunarayanan and G. Vanangamudi, 2011. Indian J. Chem. 50B(4): 593-604

[10] D.R. Richardson and P.V. Bernhardt, 1999. J. Biol. Inorg. Chem. (4): 266-273.
[11] G.Thirunarayanan, V.Sathiyendiran, G.Vanangamudi, R.Arulkumaran, V. Manikandan, R. Suresh, D. Kamalakkannan, S. P. Sakthinathan, R. Sundararajan, K. Sathiyamorthi, S. Balaji, R. Vijayakumar and R. Senbagam, 2015. Int. Lett. Chem. Phys. Astron. (50): 9-16.
[12] S. Balaji, M. Rajarajan, R. Vijayakumar, V. Manikandan, R. Senbagam , G. Vanangamudi and G. Thirunarayanan, 2015. Int. Lett. Chem. Phys. Astron., (57): 145-155.
[13] M. Rajarajan, R. Senbagam, R. Vijayakumar, V. Manikandan, S. Balaji, G. Vanangamudi and G. Thirunarayanan, 2015. World Sci. News (3): 155-171.
[14] L. Ramamohan, R.K. Shikkaragol, S.D. Angadi and V.H. Kulkarni, 1995. Asian. J. Pure Appl. Chem., 1(2): 86
[15] P. Melnyk, V. Leroux, C. Sergheraert and P. Grellier, 2006. Bioorg. Med. Chem. Lett., (16): 31 -35.
[16] L.F.C.C. Leite, M.N. Ramos, J.B.P. da Silva, A.L.P. Miranda, C.A.M. Fraga and E.J. Barreiro, 1999. Farmaco, (54): 747-757.
[17] P.C. Lima, L.M. Lima, K.C.M. da Silva, P.H.O. Leda, A.L.P. de Miranda, C.A.M. Fraga and E.J. Barreiro, 2000. Eur. J. Med. Chem., (35): 187-203.
[18] A.C. Cunha, J.M. Figueiredo, J.L.M. Tributino, A.L.P. Miranda, H.C. Castro, R.B. Zingali, C.A.M. Fraga, M.C.B.V. de Souza, V.F. Ferreira and E.J. Barreiro, 2003. Bioorg. Med. Chem., (11): 2051-2059.
[19] K.K. Bedia, O. Elcin, U. Seda, K. Fatma, S. Nathaly, R. Sevim and A. Dimoglo, 2006. Eur. J. Med. Chem., (41): 1253-1261.
[20] N. Terzioglu and A. Gursoy, 2003. Eur. J. Med. Chem., (38): 781-786.
[21] W. W. Wardakhan, E.-S. Nahed Nasser Eid, and R. M. Mohareb, 2013. Acta Pharm., (63)1: 45-57.
[22] S. Suzen, B. Tekiner-Gulbas, H. Shirinzadeh, D. Uslu, H. Gurer-Orhan, M. Gumustas and S.A. Ozkan, 2013. J. Enzyme Inhib. Med. Chem., (28): 1143-1155.
[23] G. Turan-Zitouni, M.D. Altintop, A. Ozdemir, F. Demirci, U. Abu Mohsen and Z.A. Kaplancikli, 2013. J. Enzyme Inhib. Med Chem., (28): 1211-1216.
[24] N. Tabanca, D.E. Wedge, A. Ali, I.A. Khan, Z.A. Kaplancikli and M.D. Altintop, (2013). Med. Chem. Res., (22): 2602-2609.
[25] G. Thirunarayanan and G. Vanangamudi, 2011. Spectrochim. Acta Mol. Biomol. Spectrosc., (81): 390-396.
[26] P. R. Tenorio, C.S. Carvalho, C.S. Pessanha, J.G. De Lima, A.R. De Faria, A. J. Alves, E.J.T. De Melob and A.J.S. Goesa, 2005. Bioorg. Med. Chem. Lett., (15): 2575-2578.
[27] G. Thirunarayanan, G. Vanangamudi and M. Subramanian, 2013. Org. Chem. Indian. J., 9(1): 1-16.
[28] M. Rajarajan, R. Vijayakumar, R. Senbagam, S. Balaji, V. Manaikandan, G. Vanangamudi and G. Thirunarayan, 2015. Indian J. Chem., (55B): 197-206.
[29] R. Senbagam, R. Vijayakumar, M. Rajarajan, S. Balaji, V. Manikandan, G. Vanangamudi and G. Thirunarayanan, 2016. Karbala Int. J. Mod. Sci., (20): 1-7.

(30) R. Vijayakumar, M. Rajarajan, S. Balaji1, V. Manikandan, R. Senbagam, G. Vanangamudi, G. Thirunarayanan, 2015. World Sci. News, (3): 81-98.

(31) R. Senbagam, M. Rajarajan, R. Vijayakumar, V. Manikandan, S. Balaji, G. Vanangamudi and G. Thirunarayan, 2015. Int. Lett. Chem. Phys. Astron., (53):154-164.

(32) C. G. Swain and E. C. Lupton, 1968. J. Am. Chem. Soc., (90): 4328-4337

(33) A.W. Bauer, W.M.M. Kirby, J.C. Sherris and M. Truck, Am. J. Clin. Pathol., 1966. (45): 493-496.