Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 95 (2012) 693–700

Contents lists available at SciVerse ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Synthesis, spectral studies and antimicrobial activities of some 2-naphthyl pyrazoline derivatives

S.P. Sakthinathan [a], G. Vanangamudi [a], G. Thirunarayanan [b,*]

[a] PG & Research Department of Chemistry, Government Arts College, C-Mutlur 608102, Chidambaram, India
[b] Department of Chemistry, Annamalai University, Annamalainagar 608002, India

ARTICLE INFO

Article history:
Received 3 February 2012
Received in revised form 13 April 2012
Accepted 18 April 2012
Available online 25 April 2012

Keywords:
2-Naphthyl pyrazolines
IR and NMR spectra
Hammett correlation
Antimicrobial activities

ABSTRACT

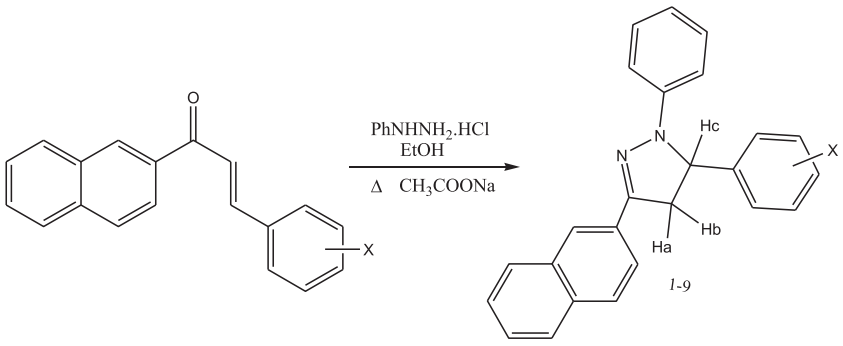
A series of 2-naphthyl pyrazolines were synthesized by the cyclization of 2-naphthyl chalcones and phenylhydrazine hydrochloride in the presence of sodium acetate. The yields of pyrazoline derivatives are more than 80%. The synthesized pyrazolines were characterized by their physical constants, IR, ^{1}H, ^{13}C and MS spectra. From the IR and NMR spectra the C=N (cm^{-1}) stretches, the pyrazoline ring proton chemical shifts (ppm) of δ_{H_a}, H_b and H_c and also the carbon chemical shifts (ppm) of δC=N are correlated with Hammett substituent constants, *F* and *R*, and Swain–Lupton's parameters using single and multi-regression analyses. From the results of linear regression analysis, the effect of substituents on the group frequencies has been predicted. The antimicrobial activities of all synthesized pyrazolines have been studied.

Introduction

Pyrazoline refers to both the classes of simple aromatic ring organic compounds of the heterocyclic series characterized by a 5-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions, and the unsubstituted parent compound. So these compounds with pharmacological effects on humans, they are classified as alkaloid, although they are rare in nature. Many pyrazolines show various pharmacological properties [1]. Some pyrazoline derivatives are used as pesticides [2], fungicides [3], antibacterial [4], antifungal [5], antiamoebic [6], and antidepressant activity [7] and insecticides. Heterocyclic of the type 3-hetaryl-1H-4,5-dihydropyrazoles arouse particular interest because the properties determined by the pyrazoline fragment are combined with the features of the corresponding hetarene [7,8]. Therefore, it should be noted that 3-(4-hydroxy-3-coumarinyl)-1H-4,5-dihydropyrazoles are structural analogues of 3-substituted 4-hydroxy-coumarins some representatives of which are effective blood anticoagulants. The pyrazoline function is quite stable, and has inspired chemists to utilize the mentioned stable fragment in bioactive moieties to synthesize new compounds possessing biological activity. Some related compounds were evaluated for anticonvulsant activity [9]. The antidepressant activity of these compounds was evaluated by the "Porsolt Behavioural Despair Test" on Swiss-Webster mice [10]. The α,β-unsaturated ketones can play the role of versatile precursors in the synthesis of the corresponding pyrazoline derivatives [11,12]. The reaction of hydrazine and its derivatives with α,β-unsaturated ketones and α,β-epoxy ketones is one of the preparative methods for the synthesis of pyrazolines and pyrazoles [13]. Alternatively, the reaction of substituted hydrazines with α,β-unsaturated ketones has been reported to form regioselective pyrazolines [14]. The synthesis of pyrazoline rings from chalcone derivatives containing anisole and the 3,4-methylenedioxyphenyl ring by the conventional method using acetic acid was reported with low yields [15]. Some 1-(4-arylthiazol-2-yl)-3,5-diaryl-2-pyrazoline derivatives have been synthesized by the reaction of 1-thiocarbamoyl-3,5-diaryl-2-pyrazoline derivatives with phenacetylbromide in ethanol. The structural elucidations of the compounds were performed by IR, ^{1}HNMR and mass spectral data and elemental analysis [16]. Semicarbazide (hydrochloride) and thiosemicarbazide on reaction with α,β-unsaturated ketones of the ferrocene series in excess of *t*-But-OK gave 1-carbamoyl and 1-thiocarbamoyl (ferrocenyl)-4,5-dihydropyrazoles. Ten new fluorine-containing 1-thiocarbamoyl-3,5-diphenyl-2-pyrazolines have been synthesized in 80–85% yields by a microwave-promoted solvent-free condensation of 2,4-dichloro-5-fluoro chalcones with thiosemicarbazide over potassium carbonate[17]. Nanoparticles of 1-phenyl-3-naphthyl-5-(dimethylamino phenyl)-2-pyrazolines ranging from tens to hundreds of nanometres have been prepared by the reprecipitation method [18]. Five new 1,3,5-triphenyl-2-pyrazolines have been synthesized by reacting 1,3-diphenyl-2-propene-1-one with phenylhydrazine hydrochloride and another five new 3-(2″-hydroxy naphthalen-1″-yl)-1,5-diphenyl-2-pyrazoline have been synthesized by reacting 1-(2′-hydroxylnaphthyl)-3-phenyl-2-propene-1-one with

* Corresponding author. Tel.: +91 4144 220015.
E-mail address: drgtnarayanan@gmail.com (G. Thirunarayanan).

1386-1425/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.saa.2012.04.082



Where X= H, 3-Br, 4-Br, 2-Cl, 3-Cl, 4-Cl, 4-F, 2-OCH_3, 4-CH_3

Scheme 1. Synthesis of 1-phenyl-3-(2-naphthyl)-5-(substituted phenyl)-2-pyrazolines.

Table 1
Analytical and mass spectral data of 1-phenyl-3-(2-naphthyl)-5-(substituted phenyl)-2-pyrazolines.

Entry	X	Mol. formula	Mol. weight	Yield (%)	m.p. (°C)	Mass (*m*/*z*)
1	H	$C_{25}H_{20}N_2$	348	85	116–117	348[M^+], 272, 271, 195, 181, 168, 167, 154, 147, 127, 77, 68, 41
2	3-Br	$C_{25}H_{19}BrN_2$	426	80	64–65	426[M^+], 428[M^{2+}], 299, 271, 155, 154, 145, 127, 79, 77
3	4-Br	$C_{25}H_{19}BrN_2$	456	85	110–111	426[M^+], 428[M^{2+}], 299, 271, 194, 168, 154, 145, 127, 77
4	2-Cl	$C_{25}H_{19}ClN_2$	382	83	81–82	382[M^+], 384[M^{2+}], 347, 271, 195, 154, 111, 77, 68, 35
5	3-Cl	$C_{25}H_{19}ClN_2$	382	83	91–92	382[M^+], 384[M^{2+}], 346, 271, 197, 195, 154, 145, 77, 68, 43, 35
6	4-Cl	$C_{25}H_{19}ClN_2$	382	87	62–63	382[M^+], 384[M^{2+}], 345, 270, 197, 195, 155, 154, 145, 77, 68, 35, 28
7	4-F	$C_{25}H_{19}FN_2$	366	80	65–66	366[M^+], 368[M^{2+}], 347, 289, 244, 239, 163, 155, 127, 122, 95, 77, 41, 28, 18
8	2-OCH_3	$C_{26}H_{21}N_2O$	378	85	104–105	378[M^+], 347, 301, 271, 251, 225, 145, 127, 107, 105, 91, 77, 31, 27
9	4-CH_3	$C_{26}H_{21}N_2$	362	85	70–71	362[M^+], 347, 285, 271, 257, 235, 195, 167, 165, 127, 91, 77, 28

Table 2
IR and NMR spectral data of 1-phenyl-3-(2-naphthyl)-5-(substituted phenyl)-2-pyrazolines.

Entry	X	*ν*CN (cm^{-1})	δ_{H_a} (ppm)	δ_{H_b} (ppm)	δ_{H_c} (ppm)	*δ*CN (ppm)
1	H	1671.38	3.286	3.954	5.333	153.01
2	3-Br	1590.34	3.254	3.960	5.290	169.77
3	4-Br	1677.78	3.253	3.955	5.303	153.14
4	2-Cl	1681.12	3.187	4.082	5.705	159.07
5	3-Cl	1683.12	3.248	3.962	5.278	152.81
6	4-Cl	1677.88	3.243	3.963	5.316	153.16
7	4-F	1678.88	3.253	3.953	5.319	153.10
8	2-OCH_3	1596.70	3.252	3.906	5.294	159.07
9	4-CH_3	1681.12	3.256	3.935	5.304	153.26

phenylhydrazine hydrochloride [19]. Also some new 1,3,5-triphenyl-2-pyrazolines have been synthesized by reacting 1,3-diphenyl-2-propene-1-one with phenylhydrazine hydrochloride and another five new 3-(2″-hydroxy naphthalen-1″-yl)-1,5-diphenyl-2-pyrazoline have been synthesized by reacting 1-(2′-hydroxylnaphthyl)-3-phenyl-2-propene-1-one with phenylhydrazine hydrochloride [20]. The effect of substituents on the group frequencies have been studied, through UV–vis, IR, ^1H and ^{13}C NMR spectra of ketones [21] unsaturated ketones [22–24], acid chlorides [14] acyl bromides, and their esters [25]. The effect of substituents on the infrared, proton and carbon-13 group frequencies of pyrazoline derivatives are not been studied so far. Hence the authors have taken efforts to synthesise some 2-naphthyl pyrazoline derivatives by cyclization of 2-naphthyl chalcones and phenylhydrazine hydrochloride in the presence of anhydrous sodium acetate and to study the spectral linearity and also the antimicrobial activities.

Experimental

All chemicals used were procured from Sigma–Aldrich and E-Merck. Melting points of all pyrazoles have been determined in open glass capillaries on Mettler FP51 melting point apparatus and are uncorrected. Infrared spectra (KBr, 4000–400 cm^{-1}) have been recorded on BRUKER (Thermo Nicolet) Fourier transform spectrophotometer. The NMR spectra of all pyrazolines have been recorded on JEOL-400 spectrometer operating at 400 MHz for recording ^1H spectra and 100 MHz for ^{13}C spectra in $CDCl_3$ solvent using TMS as internal standard. Mass spectra have been recorded on SHIMADZU spectrometer using chemical ionization technique.

Synthesis of 2-naphthyl pyrazolines derivatives: [1-phenyl-3-(2-naphthyl)-5-(substituted phenyl)-2-pyrazolines]

An appropriate equi-molar quantities of substituted styryl 2-naphthyl ketones (0.20 mmol), phenylhydrazine hydrochloride (0.20 mmol) and anhydrous sodium acetate (0.5 g) was refluxed [26] in (15 mL) ethanol for 8 h (Scheme 1). The completion of the reaction was monitored by TLC. The reaction mixture was cooled, and poured into ice cold water. The precipitate was filtered, dried and subjected to column chromatography using hexane and ethyl acetate (3:1) as eluent. The analytical and mass spectral data are presented in Table 1. The IR and NMR spectral data are given in Table 2.

Table 3
Results of statistical analysis of IR and NMR spectral data of 1-phenyl-3-(2-naphthyl)-5-(substituted phenyl)-2-pyrazolines with Hammett substituent constants σ, σ^+, σ_I, σ_R and F and R parameters.

Frequency	Constants	r	I	ρ	s	n	Correlated derivatives
ν_{CN} (cm^{-1})	σ	0.901	1656.50	29.537	3.91	8	H, 3-Br, 2-Cl, 3-Cl, 4-Cl, 4-F, 2-OCH_3, 4-CH_3
	σ^+	0.900	1657.14	23.092	3.90	8	
	σ_I	0.800	1662.21	7.971	3.90	9	H, 3-Br, 4-Br, 2-Cl, 3-Cl, 4-Cl, 4-F, 2-OCH_3, 4-CH_3
	σ_R	0.810	1680.68	8.164	3.20	9	
	F	0.871	1649.93	8.329	3.01	9	
	R	0.819	1898.95	9.321	3.00	9	
δ_{H_a} (ppm)	σ	0.801	3.255	2.251	3.91	9	H, 3-Br, 4-Br, 2-Cl, 3-Cl, 4-Cl, 4-F, 2-OCH_3, 4-CH_3
	σ^+	0.821	3.654	2.092	6.90	9	
	σ_I	0.791	3.251	7.978	6.98	9	
	σ_R	0.851	3.268	8.166	10.23	9	
	F	0.842	3.512	8.264	9.23	9	
	R	0.815	3.812	9.002	8.36	9	
δ_{H_b} (ppm)	σ	0.904	3.954	0.089	0.31	8	H, 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 2-OCH_3, 4-CH_3
	σ+	0.913	3.954	0.917	0.89	6	H, 2-Cl, 4-Cl, 4-F, 2-OCH_3, 4-CH_3
	σ_I	0.803	3.937	0.077	0.73	9	H, 3-Br, 4-Br, 2-Cl, 3-Cl, 4-Cl, 4-F, 2-OCH_3, 4-CH_3
	σ_R	0.851	3.268	8.166	10.23	9	
	F	0.862	3.262	8.021	10.03	9	
	R	0.880	3.541	9.922	9.96	9	
δ_{H_c} (ppm)	σ	0.811	5.343	6.123	1.40	9	H, 3-Br, 4-Br, 2-Cl, 3-Cl, 4-Cl, 4-F, 2-OCH_3, 4-CH_3
	σ+	0.831	5.335	1.131	1.32	9	
	σ_I	0.817	5.331	2.102	1.52	9	
	σ_R	0.802	5.351	3.201	1.41	9	
	F	0.871	5.921	4.021	2.03	9	
	R	0.808	5.624	5.027	2.30	9	
δ_{CN} (ppm)	σ	0.902	155.79	4.568	0.57	7	H, 3-Br, 4-Br, 2-Cl, 4-Cl, 4-F, 4-CH_3
	σ^+	0.822	155.96	3.826	0.86	9	H, 3-Br, 4-Br, 2-Cl, 3-Cl, 4-Cl, 4-F, 2-OCH_3, 4-CH_3
	σ_I	0.722	154.40	5.372	0.88	9	
	σ_R	0.818	154.92	5.611	0.95	9	
	F	0.881	156.34	4.521	1.02	9	
	R	0.821	159.32	5.116	1.11	9	

r, correlation co-efficient; I, intercept; ρ, slope; s, standard deviation; n, number of substituents.

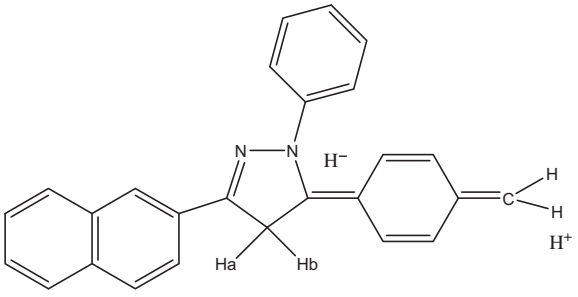


Fig. 1. The resonance conjugative structure.

Results and discussion

IR spectral study

The synthesized 2-naphthyl pyrazoline derivatives are shown in Scheme 1.The infrared C=N stretching frequencies (cm^{-1}) of these pyrazolines have been recorded and are presented in Table 2. These data are correlated with Hammett substituent constants and Swain–Lupoton's [27] parameters. In this correlation the structure parameter Hammett equation employed is as shown in Eq. (1).

$$v = \rho\sigma + v_o \quad (1)$$

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

The observed νC=N stretching frequencies (cm^{-1}) are correlated with various Hammett substituent constants and F and R parameters through single and multi-regression analyses including Swain–Lupoton's parameters. The results of statistical analysis of single parameter correlation are shown in Table 3. The correlation of νC=N (cm^{-1}) frequencies of pyrazolines with Hammett σ and σ^+ substituent constants is found to be satisfactory. All correlations produce positive ρ values. This implies that there is a normal substituent effect operates in all systems. The Hammett σ_I and σ_R constants fail in correlation. This is due to the absence of inductive and resonance effects of the substituent and is associated with the conjugated structure shown in (Fig. 1). In short some of the single parameter correlations of νC=N (cm^{-1}) frequencies with Hammett substituent constants of resonance and inductive effects fail. So, the authors think that it is worthwhile to seek the multi regression analysis and which produce a satisfactory correlation with resonance, field and Swain–Lupoton's [27] constants. The corresponding equations are given in (2) and (3).

$$\nu_{CN}\ (cm^{-1}) = 1673.93(\pm 26.670) + 29.957\sigma_I(\pm 0.694) + 104.181\sigma_R(\pm 8.148) \quad (R = 0.916, P > 90\%,\ n = 9) \quad (2)$$

$$\nu_{CN}\ (cm^{-1}) = 1669.21(\pm 26.617) + 44.895F(\pm 7.681) + 96.718R(\pm 9.194) \quad (R = 0.939, P > 90\%,\ n = 9) \quad (3)$$

^{1}H NMR spectral study

The ^{1}H NMR spectra of nine pyrazoline derivatives under investigation have been recorded in deuteriochloroform solution employing tetramethylsilane (TMS) as internal standard. The signals of the pyrazoline ring protons have been assigned. They have been calculated as AB or AA′ systems, respectively. The chemical shifts (ppm) of H_a are at higher fields than those of H_b and H_c in this series of pyrazolines. This is due to the deshielding of H_b and

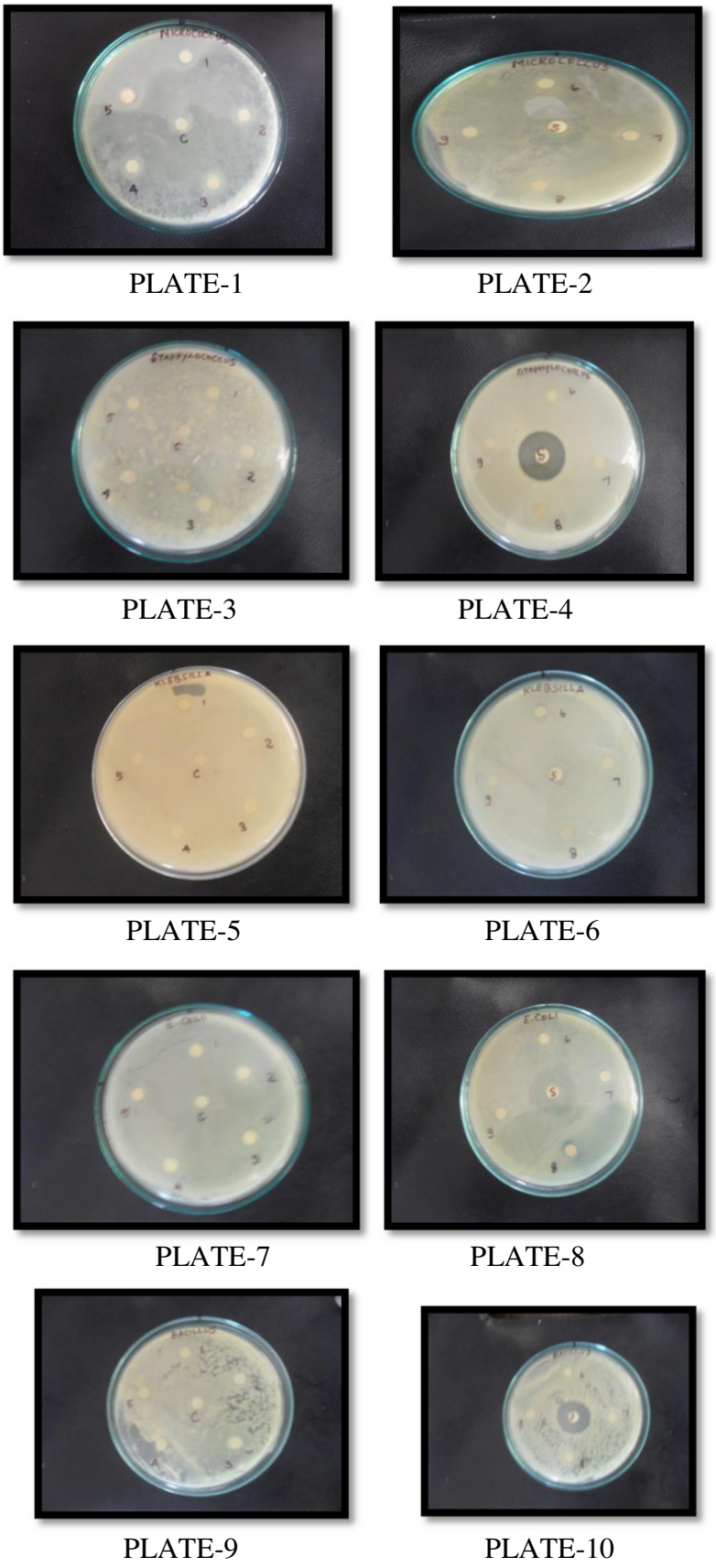


Fig. 2. Antibacterial activities of 1-phenyl-3-(2-naphthyl)-5-(substituted phenyl)-2-pyrazolines.

Table 4
Antibacterial activities of 1-phenyl-3-(2-naphthyl)-5-(substituted phenyl)-2-pyrazolines.

S. No.	Compound	X	Zone of Inhibition (mm)				
			Gram positive bacteria			Gram negative bacteria	
			B. subtilis	*M. luteus*	*S. aureus*	*E. coli*	*K. pneumoniae*
1	K_1	H	–	7	8	6	7
2	K_2	3-Br	–	7	7	6	8
3	K_3	4-Br	8	8	7	7	7
4	K_4	2-Cl	8	8	8	7	8
5	K_5	3-Cl	7	7	8	6	–
6	K_6	4-Cl	8	7	–	10	7
7	K_7	4-F	9	7	6	9	7
8	K_8	2-OCH_3	–	–	6	10	7
9	K_9	4-CH_3	6	–	–	12	7
	Standard	Ampicillin	20	18	18	18	12
	Control	DMSO	–	–	–	–	–

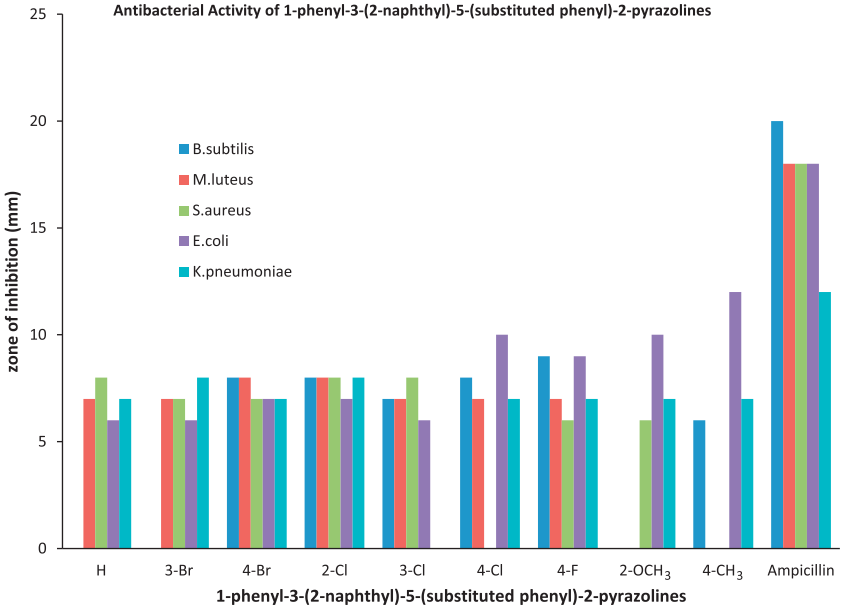


Fig. 3. Clustered column chart of antibacterial activities of 1-phenyl-3-(2-naphthyl)-5-(substituted phenyl)-2-pyrazolines.

H_c which are in different chemical as well as magnetic environment. These H_a protons gave an AB pattern and the H_b proton doublet of doublet in most cases was well separated from the signals H_c and the aromatic protons. The assigned chemical shifts (ppm) of the pyrazoline ring H_a, H_b and H_c protons are presented in Table 2.

In nuclear magnetic resonance spectra, the ^1H or the ^{13}C chemical shifts (δ) (ppm) depend on the electronic environment of the nuclei concerned. These chemical shifts have been correlated with reactivity parameters. Thus the Hammett equation may be used in the form as shown in (4).

$$\mathrm{Log}\,\delta = \mathrm{Log}\,\delta_0 + \rho\sigma \tag{4}$$

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

The assigned H_a, H_b and H_c proton chemical shifts (ppm) of pyrazoline ring have been correlated with various Hammett sigma constants. The results of statistical analysis are presented in Table 3.The H_a proton chemical shifts (ppm) with Hammett substituent constants and F and R parameters fail in correlation. All correlations give positive ρ values. This shows that the normal substituent effect operates in all systems. The failure in correlation is associated with the conjugative structure shown in Fig. 1.

The results of statistical analysis of H_b proton chemical shifts (ppm) with Hammett substituents are shown in Table 3. The H_b proton chemical shifts with Hammett σ and σ^+ constants give satisfactory correlation excluding 2-Cl, 3-Cl, 3-Br and 4-Br substituents. The σ_I and σ_R constants fail in correlation. This is due to the absence of inductive and resonance effect of substituents and it is associated with the conjugative structure shown in Fig. 1.

The results of statistical analysis of H_c proton chemical shifts (ppm) with Hammett substituents are presented in Table 3. The H_c proton chemical shifts with Hammett σ constants and F and R parameters fail in correlation. All correlations produce positive ρ values. This means that the normal substituent effect operates in all systems. This failure in correlation is associated with conjugative structure shown in Fig. 1.

In view of the inability of the Hammett σ constants to produce individually satisfactory correlation, the authors think that it is worthwhile to seek multiple correlations involving either σ_I and σ_R constants or Swain–Lupton's [27] F and R parameters. The correlation equations for H_a–H_c protons are given in (5)–(10).

$$\delta_{H_a}^{(ppm)} = 3.268(\pm 0.018) + 0.059(\pm 0.004)\sigma_I + 0.184(\pm 0.005)\sigma_R \quad (R = 0.905, P > 90\%\ n = 9) \tag{5}$$

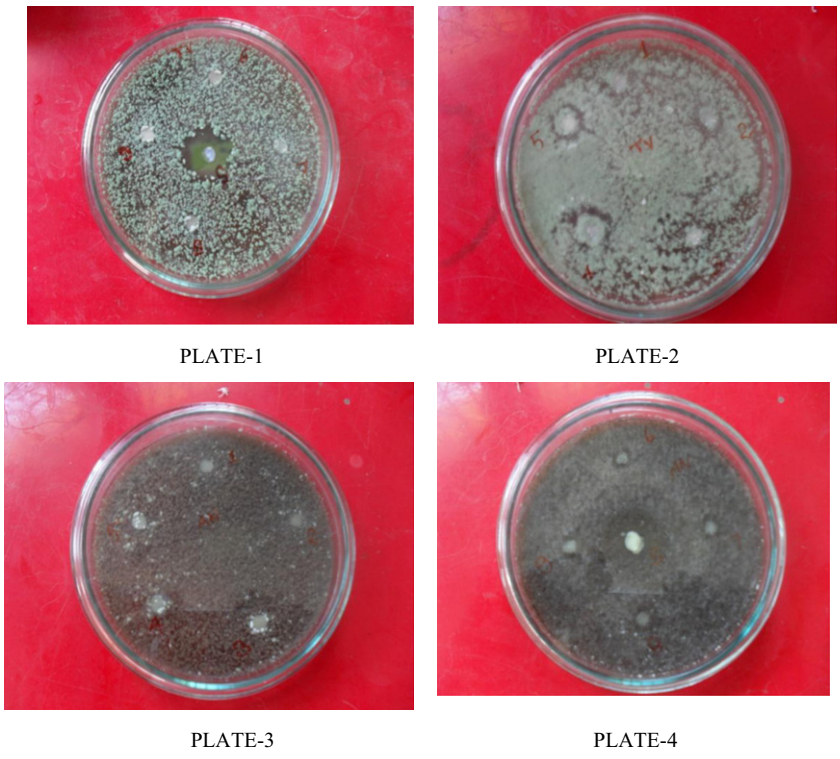


Fig. 4. Antifungal activity of 1-phenyl-3-(2-naphthyl)-5-(substituted phenyl)-2-pyrazolines.

Table 5
Antifungal activities of 1-phenyl-3-(2-naphthyl)-5-(substituted phenyl)-2-pyrazolines.

S. No.	Compound	X	Zone of Inhibition (mm)	
			A. niger	*T. viride*
1	K_1	H	–	7
2	K_2	3-Br	–	8
3	K_3	4-Br	–	8
4	K_4	2-Cl	–	10
5	K_5	3-Cl	–	10
6	K_6	4-Cl	9	–
7	K_7	4-F	9	7
8	K_8	2-OCH_3	8	–
9	K_9	4-CH_3	8	–
	Standard	Miconazole	14	16
	Control	DMSO	–	–

$$\delta_{H_a}^{(ppm)} = 3.261(\pm0.018) + 0.049(\pm0.003)F + 0.158(\pm0.006)R \quad (R = 0.908, P > 90\%\ n = 9) \tag{6}$$

$$\delta_{H_b}^{(ppm)} = 3.954(\pm0.033) + 0.1159(\pm0.008)\sigma_I + 0.111(\pm0.001)\sigma_R \quad (R = 0.951, P > 95\%,\ n = 9) \tag{7}$$

$$\delta_{H_b}^{(ppm)} = 3.960(\pm0.031) + 0.122(\pm0.009)F + 0.158(\pm0.001)R \quad (R = 0.950, P > 95\%,\ n = 9) \tag{8}$$

$$\delta_{H_c}^{(ppm)} = 5.319(\pm0.105) + 1.135(\pm0.027)\sigma_I + 0.621(\pm0.003)\sigma_R \quad (R = 0.901, P > 90\%,\ n = 9) \tag{9}$$

$$\delta_{H_c}^{(ppm)} = 5.319(\pm0.105) + 0.121F(\pm0.002) + 0.154(\pm0.003)R \quad (R = 0.900, P > 90\%,\ n = 9) \tag{10}$$

^{13}C NMR spectra

Organic chemists and researchers [25,28,29]have made extensive study of ^{13}C NMR spectra for a large number of different ketones, styrenes and keto-epoxides. They have studied linear correlation of the chemical shifts (ppm) of C_α, C_β and CO carbons with Hammett σ constants in alkenes, alkynes, acid chlorides and styrenes. In the present study, the chemical shifts (ppm) of pyrazoline ring 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 to study the reactivity through the effect of substituents.

The chemical shifts (ppm) observed for the δC=N have been correlated with Hammett constants and the results of statistical analysis are presented in Table 3. The δC=N chemical shifts (ppm) give satisfactory correlation with Hammett σ constants except 3-Cl and 2-OCH_3 substituents. When these are included in the correlation they reduce the correlation co-efficient considerably. The remaining Hammett σ^+, σ_I, σ_R, F and R parameters fail in correlation. This is due to the reason stated earlier with resonance conjugative structure shown in Fig. 1.

In view of inability of some of the σ constants to produce individually satisfactory correlation, the authors think that it is worthwhile to seek multiple correlation involving all σ_I, σ_R, F and R parameters. The correlation equations are given in (11) and (12).

$$\delta_{CN}^{(ppm)} = 154.008(\pm4.452) + 4.07(\pm1.150)\sigma_I + 3.565(\pm0.100)\sigma_R \quad (R = 0.922, P > 90\%,\ n = 9) \tag{11}$$

$$\delta_{CN}^{(ppm)} = 154.995(\pm4.361) + 3.401(\pm1.025)F + 2.205(\pm0.054)R \quad (R = 0.914, P > 90\%,\ n = 9) \tag{12}$$

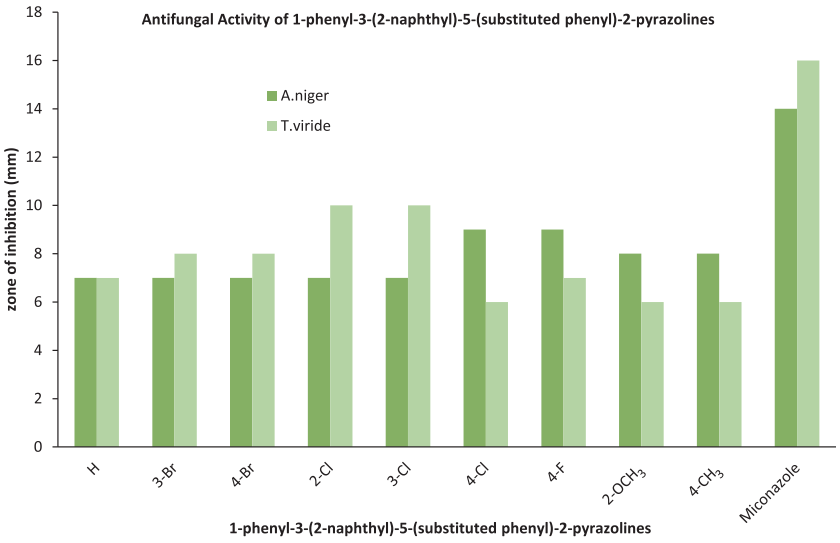


Fig. 5. Clustered column chart of antifungal activities of 1-phenyl-3-(2-naphthyl)-5-(substituted phenyl)-2-pyrazolines.

Microbial activities

Pyrazoline derivatives possess a wide range of biological activities [2,4,6,8–10,29–31]. These multipronged activities are associated with different pyrazoline rings. Hence, it is intended to examine their activities against respective microbes-bacteria's.

Antibacterial sensitivity assay

The antibacterial screening effect of synthesized pyrazoline is shown in Fig. 2 (plates 1–10). The antibacterial activities of all the synthesized pyrazolines have been studied against three gram positive pathogenic strains *Micrococcous luteus, Bacillus substilis Staphylococcus aureus and* two gram negative strains *Escherichia coli and Klebsiella species*. The disc diffusion technique was followed using the Kirby–Bauer [32]method, at a concentration of 250 μg/mL with Ampicillin taken as the standard drug. The measured zone of inhibition is shown in Table 4 and the clustered column chart is shown in Fig. 3. All the compounds showed moderate to high activity against *S. aureus*, *E. coli* and *Klebsiella.* While weak to moderate activity was observed against *M. luteus* and *B. substilis.* The compounds K_6, K_7, K_8 and K_9 were high activity against *E. coli* and *B. substilis.* The compounds K_3 and K_4 were highly activity against *M. luteus.* K_1, K_2 and K_5 were highly active against *S. aureus*, whereas compounds K_1, K_2, K_7, K_8 and K_9 were moderately active against *B. substilis, S. aureus and E. coli.* The rest of the compounds displayed weak activity against all the microorganisms. However the activities of the test compounds are less than that of standard antibacterial agent used.

Antifungal sensitivity assay

Antifungal sensitivity assay was performed using Kirby–Bauer [32] 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 ml 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 pyrazoline in 1 ml 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 antifungal activity of substituted pyrazoline synthesized in the present study are shown in Fig. 4 for plates (1–4) and the zone of inhibition values of the effect is given in Table 5. The clustered column chart, shown in Fig. 5 reveals that all the compounds K_6, K_7, K_8, K_9 have moderate antifungal activity against *Aspergillus niger* and K_2, K_3, K_4, K_5 have good antifungal activity against *Trichoderma viride.* The pyrazoline with 3-Cl, 4-Cl and 4-F, substituents have shown greater antifungal activity than those with the other substituents present in the series.

Acknowledgement

The authors thank to SAIF, IIT Chennai-600036 for recording NMR spectra of all compounds.

References

[1] (a) V.H. Babu, C.H. Sridevi, A. Joseph, K.K. Srinivasan, Indian J. Pharm. Sci. 69 (2007) 470;
(b) R.A. Nugent, M. Murphy, S.T. Schlachter, C.J. Dunn, J. Med. Chem. 36 (1993) 134;
(c) V. Rangari, V.N. Gupta, C.K. Atal, Indian J. Pharm. Sci. 52 (1990) 158.
[2] M.A. Berghot, E.B. Moawad, Eur. J. Pharm. Sci. 20 (2003) 173.
[3] D. Nauduri, G.B. Reddy, Chem. Pharm. Bull. 46 (1998) 1254.
[4] S.S. Korgaokar, P.H. Patil, M.T. Shah, H.H. Parekh, Indian J. Pharm. Sci. 58 (1996) 222.
[5] R.H. Udupi, A.R. Kushnoor, A.R. Bhat, Indian J. Heterocycl. Chem. 8 (1998) 63.
[6] M. Abid, A. Azam, Bioorg. Med. Chem. 15 (2005) 2213.
[7] A. Bilgin, E. Palaska, R. Sunal, Arzneim. Forsch. 43 (1993) 1041.
[8] M. Abid, A. Azam, Bioorg. Med. Chem. 16 (2006) 2812.
[9] K.S. Guniz, S. Rollas, H. Erdeniz, M. Kiraz, E.A. Cevdet, A. Vidin, Eur. J Med. Chem. 35 (2000) 761.
[10] E. Palaska, M. Aytemir, T. Uzbay, D. Erol, Eur. J. Med. Chem. 36 (2001) 539.
[11] R.J. Cremlyn, F.J. Swinbourne, E. Mookerjee, Indian J. Chem. 25B (1986) 562.
[12] N.G. Gawande, M.S. Shingare, Indian J. Chem. 26B (1987) 351.
[13] Y.R. Huang, J.A. Katzenellenbogen, Org. Lett. 2 (2000) 2833.
[14] A.R. Katritzky, M.Y. Wang, S.M. Zhang, A.V.V. Vonkov, P.J. Steel, J. Org. Chem. 66 (2001) 6787.
[15] M. Kidwai, P. Misra, Synth. Commun. 29 (1999) 3237.
[16] G.T. Zitouni, P. Chevallet, F.S. Kilic, K. Erol, Eur. J. Med. Chem. 35 (2000) 635.
[17] V.M. Patel, K.R. Desai, Arkivoc. 10 (2004) 123.
[18] S.W. Oh, D.R. Zhan, Y.S. Kang, Mat. Sci. Eng. 24 (2004) 131.
[19] J.S. Ghomi, A.H. Bamoniri, M.S. Telkabadi, Chem. Hetro. Compd. 42 (2006) 7.
[20] Y.R. Prasad, A.L. Rao, K. Prasoona, K. Murali, P. Ravikumar, Bioorg. Med. Chem. Lett. 15 (2005) 5030.
[21] N. Owen, M.V.S. Sultanbawa, J. Chem. Soc. (1949) 3098.
[22] G. Thirunarayanan, P. AnanthakrishnaNadar, J. Korean Chem. Soc. 50 (3) (2006) 183.
[23] (a) G. Thirunarayanan, Indian J. Chem. 46B (2007) 1551;
(b) G. Thirunarayanan, J. Korean Chem. Soc. 51 (2) (2007) 115.

[24] (a) G. Thirunarayanan, J. Indian Chem. Soc. 84 (2008) 47;
(b) G. Thirunarayanan, J. Korean Chem. Soc. J. Korean Chem. Soc. 52 (4) (2008) 369.
[25] G. Thirunarayanan, G. Vanangamudi, V. Sathiyendiran, K. Ravi, Indian J. Chem. 50 (4) (2011) 593.
[26] R. Sasikala, M.Phil. Thesis, Annamalai University, 2010.
[27] C.G. Swain, E.C. Lupton Jr., J. Am. Chem. Soc. 90 (1968) 4328.
[28] (a) K.S. Dhami, J.B. Stothers, Can. J. Chem. 43 (1963) 479;
(b) K.S. Dhami, J.B. Stothers, Can. J. Chem. 43 (1963) 10.
[29] (a) G. Thirunarayanan, G. Vanangamudi, Spectrochim. Acta 81A (2011) 390;
(b) G. Thirunarayanan, M. Gopalakrishnan, G. Vanangamudi, Spectrochim. Acta 67A (2007) 1106.
[30] J. Elguero, BultonMckillop (Eds.), Comprehensive Heterocyclic Chemistry, vol. 5, Pergamon Press, 1984, p. 293.
[31] D.B. Dambal, P.P. Pattanashetti, R.K. Tikare, B.V. Badami, G.S. Puranik, Indian J. Chem. 23B (1984) 186.
[32] A.W. Bauer, W.M.M. Kirby, J.C. Sherris, M. Truck, Am. J. Clin. Pathol. 45 (1996) 493.