



Study of IR and NMR spectral correlations and biological evaluation of 4-(6-ethyl-2-(substituted phenyl)-6*H*-pyrido[3,2-*b*]carbazol-4-yl) aniline derivatives

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ABSTRACT

A series of seven titled compounds have been prepared and their purities were examined by literature method. The characteristic infrared stretching frequencies (ν , cm^{-1}) and the NMR chemical shifts (δ , ppm) of these anilines were assigned. These assigned spectral data were correlated with Hammett substituent constants, F and R parameters using single and multi-regression analysis. From the statistical results the effect of substituents on the spectral data have been discussed. The antimicrobial activities of these aniline derivatives have been evaluated by Bauer-Kirby disc-diffusion method.

Keywords: Pyrido[3,2-*b*]carbazol-4-yl) aniline; IR and NMR spectra; Substituent effects; Hammett equation; Antibacterial activity; Antifungal activity

1. INTRODUCTION

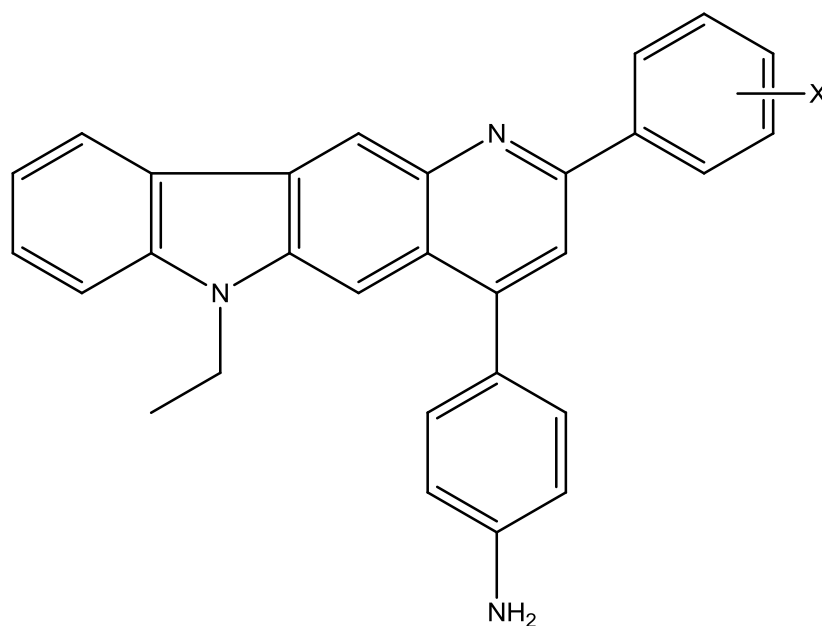
Carbazole derivatives are the three membered heterocyclic compounds comprising of two phenyl rings fused with one pyrrole moiety and the nitrogen atom in pyrrole ring may be substituted. The 9-ethyl carbazole was prepared by substitution of pyrrole nitrogen with ethyl group. These derivatives plays an important role for carbon building blocks and biological activities. Due to the phenyl ring moieties reacts with incoming groups containing polar groups. Presence of polar groups and substitutions in nitrogen atoms shows biological activities.

The correlation study was useful for prediction of geometrical conformers in various organic substrates such as unsaturated carbonyl substrates [1-5], carbon-carbon double bonds [6], triple bonds [7], multi-double bonded linear compounds [8], five, six and eight membered mono and tri heterocyclic compounds [9-11], hydrazine derivatives [12], acyl halides [13], acyl esters [14] and norbornyl systems [15]. Also this study was applied to transition states of reaction mechanisms [16], biological activities [17], and normal co-ordinate [18,19] analysis, long range interactions in the β -sheet structure [20] of oligopeptides, enone-dienol tautomerism [21], Qsar study of substituted benzo[α] phenazines [22] cancer agents, density functional theory [23], Diels-Alder reactions [24], rotational barriers in selenomides [25], gas phase reactivity of alkyl allyl sulphides [26], linear-relationships in α , β -unsaturated carbonyl compounds between the half wave reduction potential [27], the frontier orbital energy and the Hammett σ_p values [27], Chemometric potential analysis [28], toxicological analysis [29], Leishmaniasis-Trypanosomiasis [30] and vector regression for agricultural fields. Manikandan and Thirunarayanan have studied the HPLC method for the simultaneous estimation of olmesartan medoxomil and chlorthalidone in dosage forms, validation of dissolution method for levocetirizine dihydrochloride by isocratic reverse phase HPLC with help of correlation analysis [31,32]. Senbagam et. al., have studied the effect of substituents of some triazole amines [33], 2-pyrimidine Schiff's bases [34] and (4*E*)-4-(benzylidene amino)-1,2-dihydro-2,3-dimethyl-1-phenylpyrazol-5-ones [35].

Recently, Thirunarayanan have studied the effect of substituent and biological activities of some methanones [36]. Within the above view there is no report on the study of effect of substituents and antimicrobial activities of title compounds in the past. Therefore the author have taken effort for the study of substituent effects and biological activities by synthesis, recording the IR and NMR spectra. The antimicrobial activities of these compounds were measured by Bauer-Kirby dis-diffusion methods [37,38].

2. EXPERIMENTAL

In the present investigation, the titled compounds, 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido[3,2-b]carbazol-4-yl)aniline derivatives (Figure 1) was prepared and their purities were examined by literature method [39]. The IR spectra of these derivatives were recorded in AVATAR-300 Fourier transform spectrophotometer. Bruker AV400 NMR spectrometer was used for recording NMR spectra operating at 400 MHz has been utilized for recording ^1H NMR spectra and 100 MHz for ^{13}C NMR spectra in DMSO solvent using TMS as internal standard.



Entry	1	2	3	4	5	6	7
X	H	3-Br	3-Cl	4-F	3-NO ₂	4-SCH ₃	4-CF ₃

Figure 1. General structure of 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido[3,2-b]carbazol-4-yl) anilines.

3. RESULTS AND DISCUSSION

3. 1. Infrared spectral study

The assigned infrared characteristic frequencies of 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido[3,2-b]carbazol-4-yl) anilines have been presented in Table 1. The NH₂ and CN stretches observed in the range of 3052-3063 and 1589-1616 cm⁻¹. These data were correlated with Hammett substituent constants, F and R parameters using single and multi-regression analysis. In infrared spectral correlations the Hammett equation was used in the form as,

$$\nu = \rho\sigma + \nu_0 \quad \dots (1)$$

where ν_0 is the frequency of the parent compound.

The results of statistical analysis is shown in Table 2. From the Table 2, the NH₂ frequencies (ν , cm⁻¹) of 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido[3,2-b]carbazol-4-yl) anilines with Hammett substituent constants, F and R parameters were failed in correlations.

Table 1. Infrared frequencies (ν , cm^{-1}) and NMR spectral chemical shifts (δ , ppm) of 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido[3,2-b]carbazol-4-yl) anilines.

X	IR (ν , cm^{-1})		^1H NMR (δ , ppm)			^{13}C NMR (δ , ppm)				
	NH ₂	C=N	NH ₂	CH ₂ (<i>q</i>)	CH ₃ (<i>t</i>)	CH ₂	CH ₃	C=N	C-NH ₂	C _{ipso}
H	3063	1616	8.70	4.38	1.45	37.73	13.94	158.09	143.82	128.64
3-Br	3052	1610	8.60	4.35	1.45	37.75	13.91	155.85	143.07	123.08
3-Cl	3063	1610	8.62	4.36	1.45	37.75	13.90	156.00	143.11	134.96
4-F	3052	1605	8.63	4.37	1.45	37.73	13.88	156.68	143.59	165.68
3-NO ₂	3052	1616	8.80	4.32	1.45	37.78	13.90	154.20	142.41	148.74
4-SCH ₃	3353	1589	8.58	4.25	1.38	37.54	13.92	151.61	142.62	139.17
4-CF ₃	3052	1605	8.63	4.37	1.45	37.73	13.88	156.68	143.59	165.68

All correlations gave negative ρ values except σ^+ constants. The negative ρ values implies that the electron donating substituents dominates the prediction of substituent effects. The failure in correlation is due to the inability substituents on prediction of substituent effect on the frequencies by polar, inductive, resonance and filed effects of substituents and is associated with the resonance-conjugative effects as shown in Figure 2.

Table 2. The results of statistical analysis of infrared spectral frequencies (ν , cm^{-1}) of 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido[3,2-b]carbazol-4-yl) anilines with Hammett substituent constants, F and R parameters.

Freq.	Constt.	r	I	ρ	s	n	Correlated derivatives
NH ₂	σ	0.847	3154.10	-187.354	108.28	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ^+	0.734	3047.55	142.675	114.29	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ_I	0.813	3122.20	-66.214	122.05	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ_R	0.841	3071.28	-204.359	111.96	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	F	0.849	3163.12	-158.495	115.45	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃

	R	0.715	3089.06	-66.876	121.73	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
C=N	σ	0.904	1603.01	14.303	9.06	6	3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ^+	0.724	1609.59	-6.537	9.89	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ_I	0.900	1605.97	3.626	10.07	6	3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ_R	0.900	1610.47	24.262	8.05	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	F	0.900	1605.88	3.409	10.07	6	3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	R	0.903	1609.13	12.729	9.43	5	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ ,

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

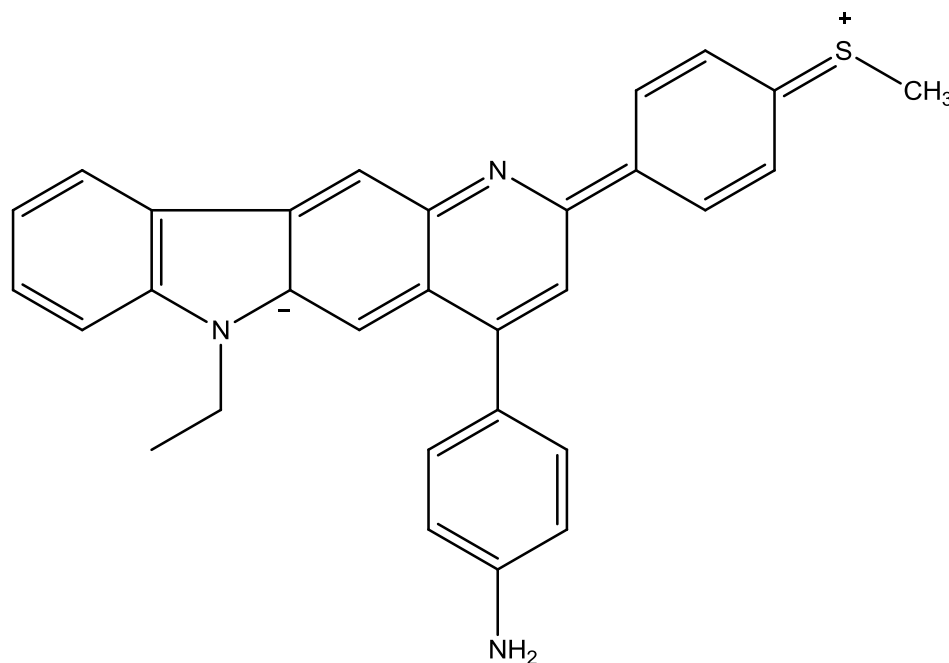


Figure 2. The resonance-conjugative structure of 4-(6-ethyl-2-(4-(methylthio)phenyl)-6H-pyrido[3,2-b]carbazol-4-yl) aniline.

The correlation of CN frequencies (ν , cm⁻¹) of 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido[3,2-b]carbazol-4-yl) anilines with Hammett substituent constants, F and R parameters

gave satisfactory correlation coefficient except σ^+ constant. All constants gave positive ρ values except σ^+ constants. This implies that the normal substituent effect operates in all systems. The failure in correlation was the reason already stated and it is associated with resonance-conjugation structure as shown in Figure 2.

Some of the single parameter correlation was failed in correlation for giving satisfactory correlations. These are worthwhile when seeking in multi-regression analysis with Swain-Lupton's constants [40]. The obtained multi-regression equations are given in (2-5).

$$\text{NH}_2(\nu, \text{cm}^{-1}) = 3110.94(\pm 81.512) - 119.587(\pm 21.619)\sigma_I - 232.745(\pm 22.016)\sigma_R \quad \dots(2)$$

(R = 0.948, n=7, P > 90%)

$$\text{NH}_2(\nu, \text{cm}^{-1}) = 3165.99(\pm 94.547) - 211.653(\pm 21.554)F - 139.442(\pm 20.440)R \quad \dots(3)$$

(R = 0.946, n=7, P > 90%)

$$\text{C=N}(\nu, \text{cm}^{-1}) = 1607.25(\pm 6.373) + 9.706(\pm 1.539)\sigma_I + 26.566(\pm 5.671)\sigma_R \quad \dots(4)$$

(R = 0.965, n=7, P > 95%)

$$\text{C=N}(\nu, \text{cm}^{-1}) = 1605.55(\pm 7.890) + 9.504(\pm 1.970)F + 15.987(\pm 1.705)R \quad \dots(5)$$

(R = 0.943, n=7, P > 90%)

Some of single parameter correlations are shown in Figures 3 and 4.

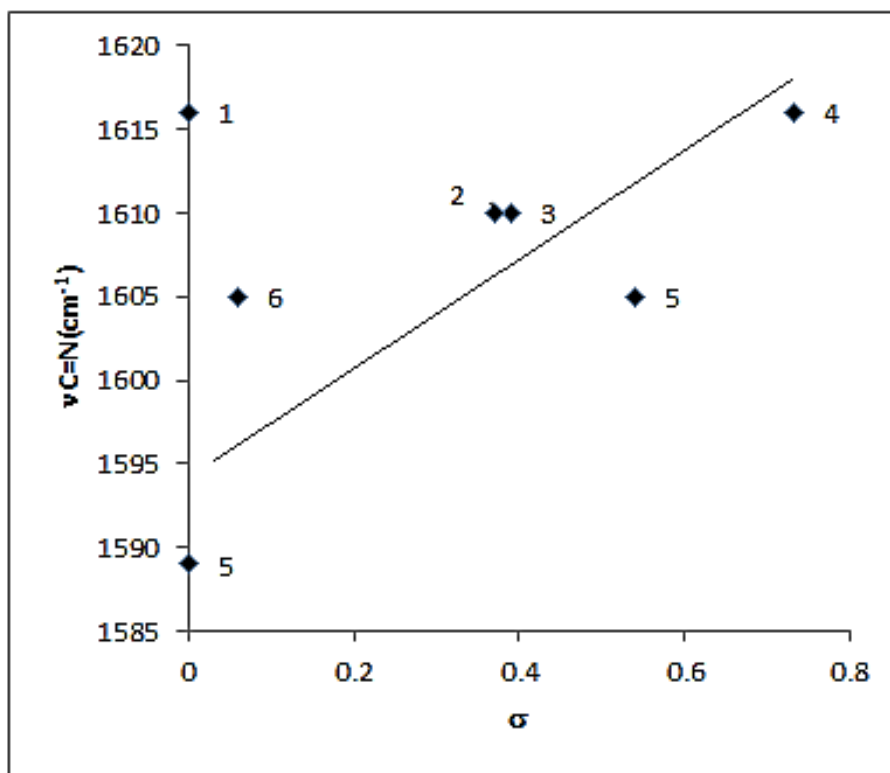


Figure 3. Plot of $\nu_{\text{C=N}}$ (cm^{-1}) of 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido[3,2-b]carbazol-4-yl) anilines Vs σ .

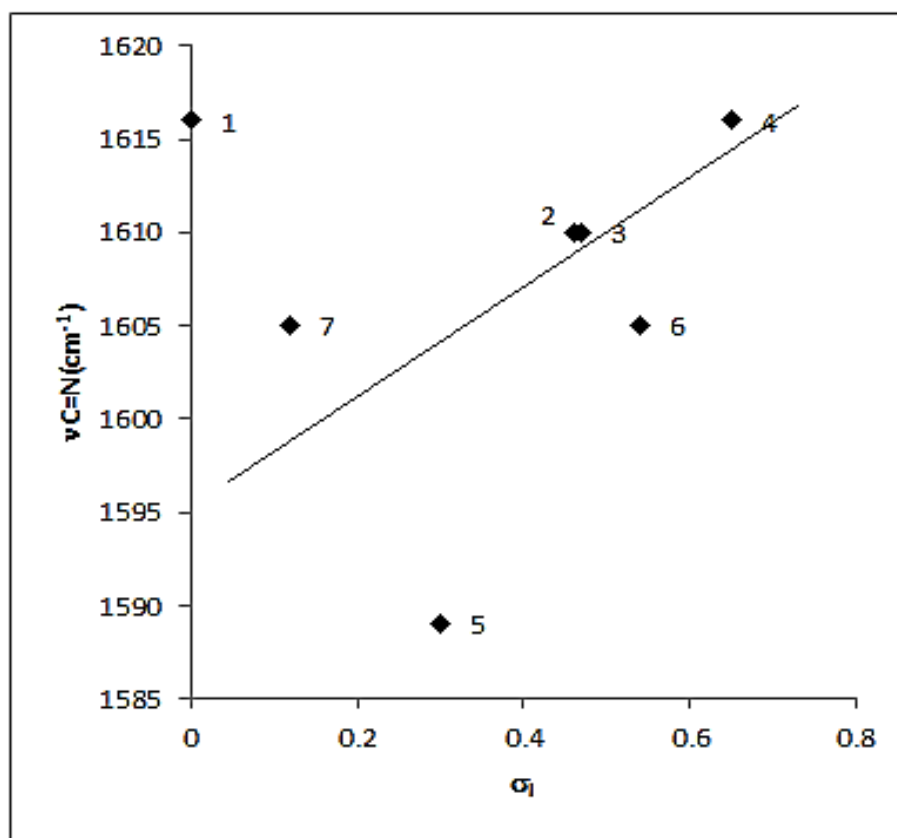


Figure 4. Plot of $\nu_{\text{C=N}}$ (cm^{-1}) of 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido [3, 2-b] carbazol-4-yl) anilines Vs σ_1 .

3. 2. NMR spectral correlations

3. 2. 1. ^1H NMR Spectra

Assigned proton chemical shifts (δ , ppm) of 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido[3,2-b]carbazol-4-yl)anilines were presented in Table 1. The observed NH_2 proton chemical shifts of these anilines are falls in the range of 8.58-8.70 ppm as singlet. The methylene protons gave quartet in the range of 4.25-4.38 ppm. The methyl proton chemical shift of these compounds appeared as triplets in the range of 1.38-1.45ppm. These data were correlated with Hammett substituent constants, F and R parameters. In NMR correlations, the Hammett equation was taken in the form as

$$\delta = \rho\sigma + \delta_0 \quad \dots (6)$$

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

The results of statistical analysis is presented in Table 3. From Table 3, Hammett σ , σ_1 , σ_R constants and R parameters gave satisfactory correlations with NH_2 proton chemical shifts. The σ^+ constants gave poor correlation. The failure in correlation was due to the reason stated earlier and association of resonance-conjugative structure as illustrated in Figure 2.

All correlations gave positive ρ values. This implies that the normal substituent effects in all correlations.

Table 3. The results of statistical analysis of ^1H NMR chemical shifts of (δ , ppm) of 4-(6-ethyl-2-(substituted phenyl)-6*H*-pyrido[3,2-*b*]carbazol-4-yl)anilines with Hammett substituent constants, F and R parameters.

Chemical shift	Constants	r	I	ρ	s	n	Correlated derivatives
NH₂(s)	σ	0.900	8.612	0.129	0.07	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ^+	0.801	8.634	0.046	0.08	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ_I	0.921	8.626	0.067	0.08	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ_R	0.906	8.672	0.217	0.06	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	F	0.617	8.629	0.053	0.08	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	R	0.952	8.662	0.151	0.07	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
CH₂(q)	σ	0.911	4.337	0.101	0.04	6	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-CF ₃
	σ^+	0.968	4.380	-0.105	0.03	6	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-CF ₃
	σ_I	0.921	4.357	-0.041	0.04	6	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-CF ₃
	σ_R	0.732	4.348	0.045	0.04	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	F	0.567	4.387	0.012	0.04	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	R	0.802	4.343	0.003	0.04	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
CH₃(t)	σ	0.345	1.427	0.042	0.02	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ^+	0.346	1.452	0.034	0.02	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃

	σ_I	0.411	1.435	0.013	0.02	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ_R	0.418	1.446	0.048	0.02	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	F	0.319	1.426	0.034	0.02	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	R	0.315	1.442	0.016	0.02	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃

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

The methylene proton chemical shifts gave satisfactory correlations with Hammett σ , σ^+ and σ_I , constants. The Hammett σ_R constant, F and R parameters were fail in correlations. All correlations gave positive ρ values except σ^+ and σ_I , constants. This value implies that the normal substituent effects operates in all systems. The reason for failure correlation was already stated and associated with the conjugation structure in Figure 2.

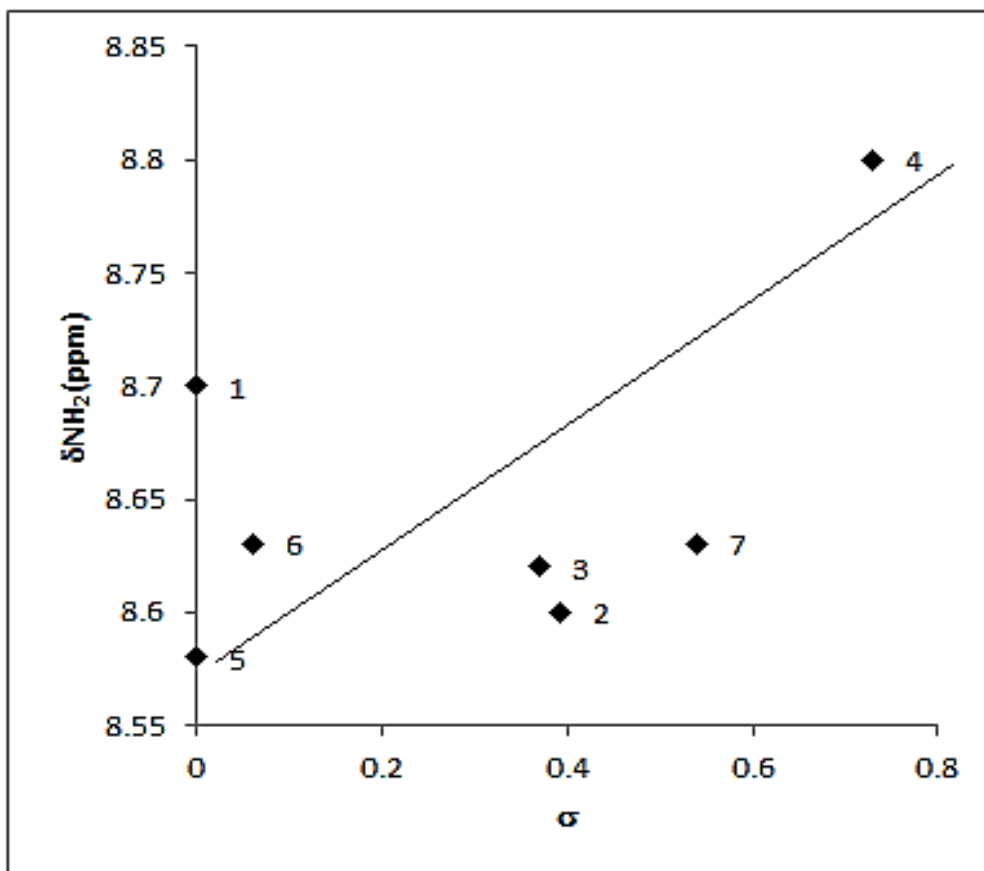


Figure 5. Plot of $\delta\text{NH}_2(\text{ppm})$ of 4-(6-ethyl-2-(substituted phenyl)-6*H*-pyrido [3, 2-*b*] carbazol-4-yl) anilines Vs σ .

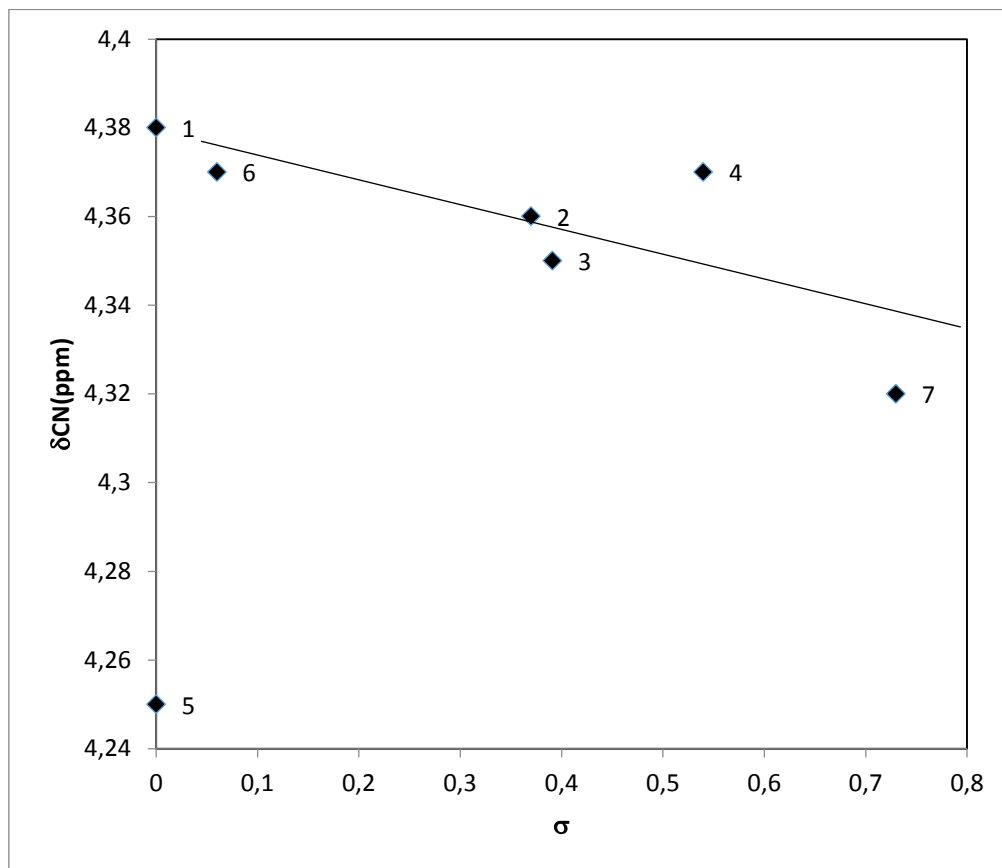


Figure 6. Plot of $\delta_{CN}(\text{ppm})$ of 4-(6-ethyl-2-(substituted phenyl)-6*H*-pyrido[3,2-*b*]carbazol-4-yl) anilines Vs σ .

The methyl proton chemical shifts was failed in correlation for production of satisfactory correlation coefficients. All correlations gave positive ρ values. This value implies that the normal substituent effects operates in all systems. The reason for failure correlation was already stated and associated with the conjugation structure in Figure 2.

Some of single parameter correlation was failed for producing satisfactory correlation coefficient in single parameter regression analysis. These are giving satisfactory correlations in multi-regression analysis using Swain-Lupton's constants [40]. The generated multi-regression analysis equations are illustrated in (6-12).

$$\text{NH}_2(\delta, \text{ppm}) = 8.638(\pm 0.044) + 0.124(\pm 0.106)\sigma_I + 0.246(\pm 0.108)\sigma_R \quad \dots(7)$$

$(R = 0.976, n = 7, P > 95\%)$

$$\text{NH}_2(\delta, \text{ppm}) = 8.625(\pm 0.053) + 0.128(\pm 0.012)F + 0.195(\pm 0.118)R \quad \dots(8)$$

$(R = 0.965, n = 7, P > 95\%)$

$$\text{CH}_2(\delta, \text{ppm}) = 4.359(\pm 0.039) + 0.032(\pm 0.001)\sigma_I + 0.037(\pm 0.004)\sigma_R \quad \dots(9)$$

$(R = 0.928, n = 7, P > 90\%)$

$$\text{CH}_2(\delta, \text{ppm}) = 4.337(\pm 0.042) + 0.016(\pm 0.001)F + 0.097(\pm 0.003)R \quad \dots(10)$$

$(R = 0.908, n = 7, P > 90\%)$

$$\text{CH}_3(\delta, \text{ppm}) = 1.437(\pm 0.025) + 0.025(\pm 0.001)\sigma_I + 0.054(\pm 0.005)\sigma_R \quad \dots(11)$$

$(R = 0.947, n = 7, P > 90\%)$

$$\text{CH}_3(\delta, \text{ppm}) = 1.425(\pm 0.022) + 0.046(\pm 0.002)F + 0.031(\pm 0.005)R \quad \dots(12)$$

$(R = 0.943, n = 7, P > 90\%)$

3. 2. 2. ^{13}C NMR Spectra

The assigned ^{13}C NMR chemical shifts of (δ , ppm) CH_2 , CH_3 , CN, C-NH₂ and C-ipso carbons of 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido[3,2-b]carbazol-4-yl)anilines were presented in **Table 1**. These data were correlated with Hammett substituent constants, F and R parameters. The results of statistical analysis was presented in **Table 4**.

Table 4. The results of statistical analysis of ^{13}C NMR chemical shifts of (δ , ppm) of 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido[3,2-b]carbazol-4-yl)anilines with Hammett substituent constants, F and R parameters.

Chem. shift	Constt.	r	I	ρ	s	n	Correlated derivatives
CH_2	σ	0.905	37.66	6.165	0.06	6	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-CF ₃
	σ^+	0.902	37.73	-0.015	0.08	6	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-CF ₃
	σ_I	0.927	37.68	0.092	0.08	6	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-CF ₃
	σ_R	0.494	37.73	0.170	0.07	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	F	0.903	37.66	0.127	0.07	6	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-CF ₃
	R	0.922	37.32	0.069	0.08	6	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-CF ₃
CH_3	σ	0.904	13.91	-0.034	0.02	6	H, 3-Br, 3-Cl, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ^+	0.902	13.90	-0.007	0.02	6	3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ_I	0.443	13.91	-0.004	0.02	6	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃
	σ_R	0.810	13.90	0.010	0.02	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃

	F	0.907	13.93	-0.069	0.01	6	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃
	R	0.817	13.90	0.014	0.02	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
C=N	σ	0.902	155.64	-0.208	2.30	6	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-CF ₃
	σ^+	0.975	157.50	-5.413	1.50	6	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-CF ₃
	σ_I	0.935	156.75	-3.218	2.15	6	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-CF ₃
	σ_R	0.822	155.85	2.035	2.25	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	F	0.801	155.92	-0.817	2.29	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	R	0.803	155.62	0.285	2.30	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
C-NH ₂	σ	0.994	143.39	-0.750	0.52	5	H, 3-Br, 3-Cl, 3-NO ₂ , 4-CF ₃
	σ^+	0.998	143.73	-1.585	0.26	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ_I	0.960	143.68	-1.407	0.45	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ_R	0.870	143.14	-0.179	0.57	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	F	0.820	143.42	-0.618	0.55	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	R	0.814	143.13	-0.294	0.57	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
C_{ipso}	σ	0.813	140.34	11.261	18.31	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ^+	0.812	146.22	-7.125	18.51	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ_I	0.811	140.73	8.179	18.53	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	σ_R	0.806	143.06	-4.907	18.61	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	F	0.905	138.30	57.857	15.61	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃
	R	0.806	143.12	4.307	18.61	7	H, 3-Br, 3-Cl, 4-F, 3-NO ₂ , 4-SCH ₃ , 4-CF ₃

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

The methylene carbon chemical shifts (δ , ppm) gave satisfactory correlations with Hammett σ , σ^+ and σ_I , σ_R constants, F and R parameters. All correlations gave positive ρ values except σ^+ constants. This value implies that the normal substituent effects operates in all systems. The negative ρ values implies that the electron donating substituents dominates for prediction of reactivity on the chemical shifts.

The methyl carbon chemical shifts (δ , ppm) gave satisfactory correlation coefficients with Hammett σ , σ^+ constants and F parameters. All correlations gave negative ρ values except resonance components. This value implies that the normal substituent effects operates in all systems. The reason for failure correlation was already stated and associated with the conjugation structure in Figure 2.

The CH=N carbon chemical shifts (δ , ppm) gave satisfactory correlations with Hammett σ , σ^+ and σ_I constants. The Hammett σ_R constant, F and R parameters were failed in correlations. All correlations gave negative ρ values except resonance components. This value implies that the electron donating substituents dominates for prediction of substituents effects in operates in all systems. The reason for failure correlation was already stated and associated with the conjugation structure in Figure 2.

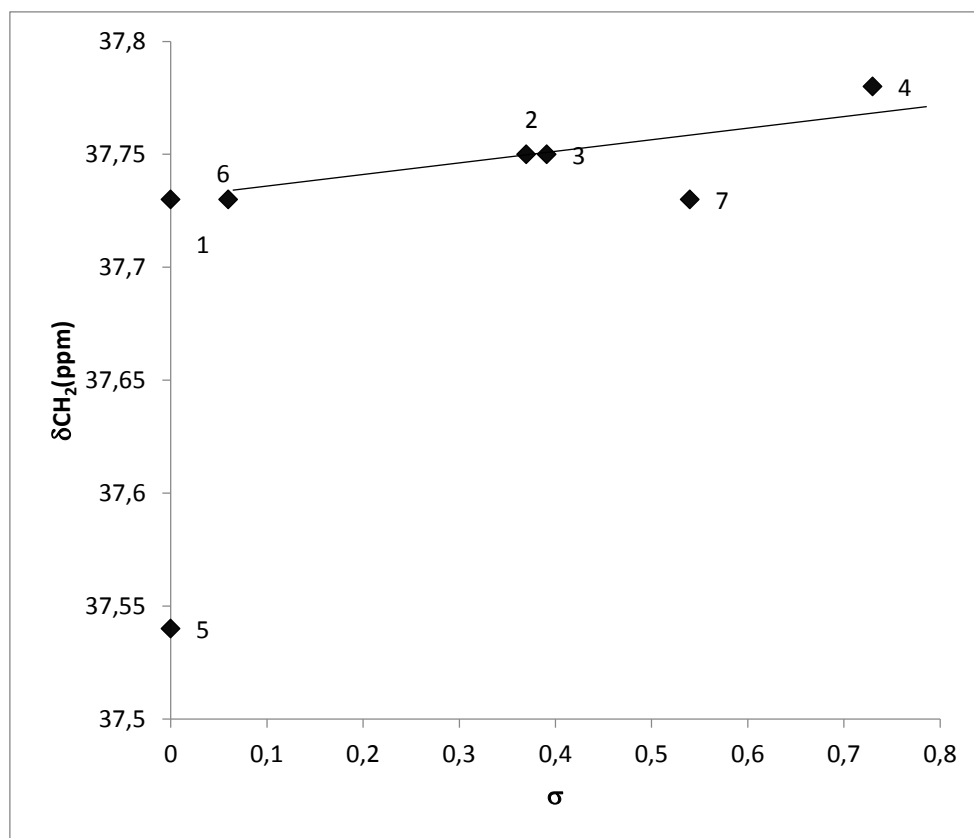


Figure 7. Plot of δ_{CH_2} (ppm) of 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido[3,2-b]carbazol-4-yl) anilines Vs σ .

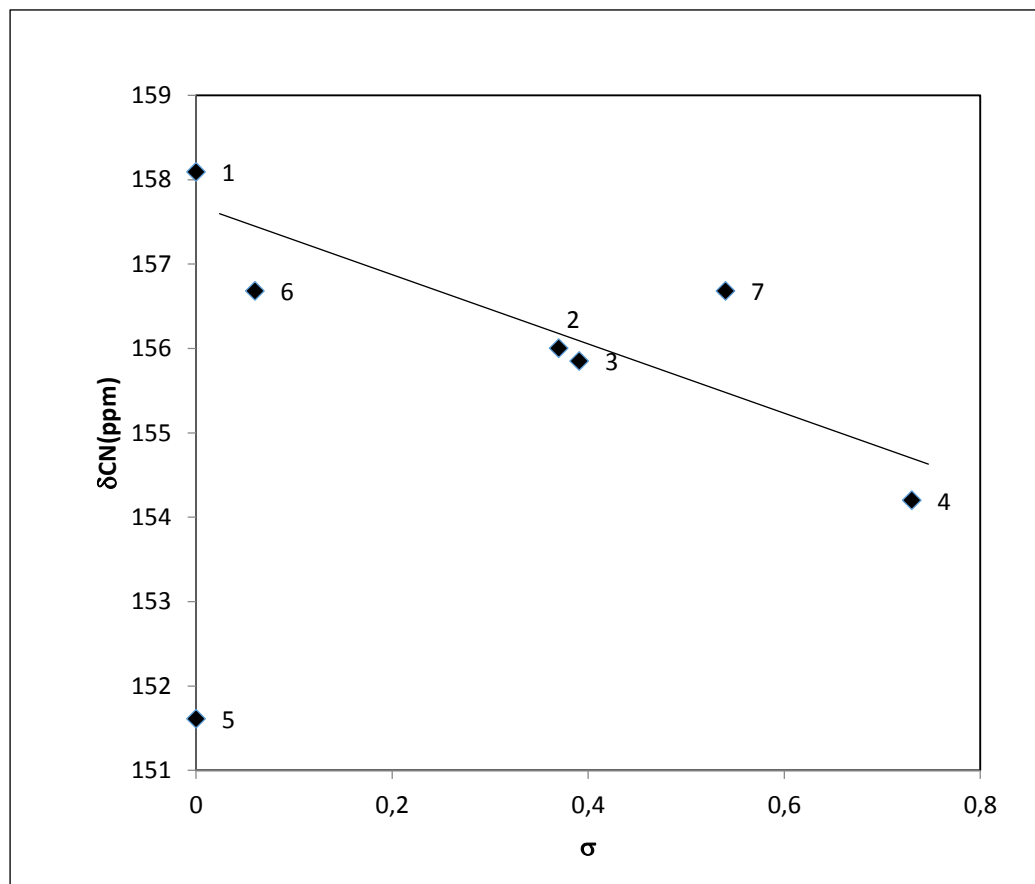


Figure 8. Plot of δ_{CN} (ppm) of 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido[3,2-b]carbazol-4-yl) anilines Vs σ .

The C-NH₂ carbon chemical shifts (δ , ppm) gave satisfactory correlations with Hammett σ , σ^+ and σ_I constants. The Hammett σ_R constant, F and R parameters were failed in correlations. All correlations gave negative ρ values. This value implies that the electron donating substituents dominates for prediction of substituents effects in operates in all systems. The reason for failure correlation was already stated and associated with the conjugation structure in Figure 2.

The F parameter only gave satisfactory correlation with C-ipso carbon chemical shifts (δ , ppm) of 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido[3,2-b]carbazol-4-yl)anilines. All correlations gave positive gave ρ values except polar and resonance components. This value implies that the normal substituents effects in operates in all systems. The reason for failure correlation was already stated and associated with the conjugation structure in Figure 2. Some of the single parameter plots are shown in Figures 7 and 8.

Some of single parameter correlation was failed for producing satisfactory correlation coefficient in single parameter regression analysis in Carbon-13 NMR spectral studies. These are giving satisfactory correlations in multi-regression analysis using Swain-Lupton's constants [40]. The generated multi-regression analysis equations are illustrated in (13-22).

$$\text{CH}_2(\delta, \text{ppm}) = 37.69(\pm 0.055) + 0.138(\pm 0.013)\sigma_{\text{I}} + 0.028(\pm 0.013)\sigma_{\text{R}} \quad \dots(13)$$

$(R = 0.963, n = 7, P > 95\%)$

$$\text{CH}_2(\delta, \text{ppm}) = 37.66(\pm 0.067) + 0.177(\pm 0.014)F + 0.129(\pm 0.013)R \quad \dots(14)$$

$(R = 0.956, n = 7, P > 95\%)$

$$\text{CH}_3(\delta, \text{ppm}) = 13.91(\pm 0.017) - 0.040(\pm 0.002)\sigma_{\text{I}} + 0.105(\pm 0.001)\sigma_{\text{R}} \quad \dots(15)$$

$(R = 0.944, n = 7, P > 90\%)$

$$\text{CH}_3(\delta, \text{ppm}) = 13.932(\pm 0.011) - 0.073(\pm 0.001)F - 0.012(\pm 0.007)R \quad \dots(16)$$

$(R = 0.980, n = 7, P > 95\%)$

$$\text{C}=\text{N}(\delta, \text{ppm}) = 156.81(\pm 1.766) - 2.291(\pm 0.428)\sigma_{\text{I}} + 1.336(\pm 0.043)\sigma_{\text{R}} \quad \dots(17)$$

$(R = 0.938, n = 7, P > 90\%)$

$$\text{C}=\text{N}(\delta, \text{ppm}) = 155.92(\pm 1.987) - 0.810(\pm 0.045)F + 0.072(\pm 0.004)R \quad \dots(18)$$

$(R = 0.954, n = 7, P > 95\%)$

$$\text{C}-\text{NH}_2(\delta, \text{ppm}) = 143.65(\pm 0.357) - 1.532(\pm 0.862)\sigma_{\text{I}} - 0.542(\pm 0.087)\sigma_{\text{R}} \quad \dots(19)$$

$(R = 0.966, n = 7, P > 95\%)$

$$\text{C}-\text{NH}_2(\delta, \text{ppm}) = 143.43(\pm 0.458) - 0.840(\pm 0.104)F - 0.582(\pm 0.009)R \quad \dots(20)$$

$(R = 0.939, n = 7, P > 90\%)$

$$\text{C}_{\text{ipso}}(\delta, \text{ppm}) = 140.58(\pm 15.364) + 7.461(\pm 3.710)\sigma_{\text{I}} - 3.135(\pm 0.037)\sigma_{\text{R}} \quad \dots(21)$$

$(R = 0.911, n = 7, P > 90\%)$

$$\text{C}_{\text{ipso}}(\delta, \text{ppm}) = 138.28(\pm 13.314) + 4.315(\pm 3.017)F + 9.857(\pm 2.871)R \quad \dots(22)$$

$(R = 0.956, n = 7, P > 90\%)$

3. 3. Antimicrobial activities

Antimicrobial activities of these synthesized 4-(6-ethyl-2-(substituted phenyl)-6H-pyrido[3,2-b]carbazol-4-yl) anilines were evaluated using Bauer-Kirby [37] disc diffusion method. In this investigation the author selected the two gram positive and two gram negative bacterial strains such as *B. subtilis*, *M. luteus*, *S. aureus* and *P. argenosea*. The selected fungal strains are *A. niger* and *T. viridi*. In this experiment 250 µg/mL concentration of test compounds were taken. Ampicillin and Miconazole are the standard and DMSO is the control for this experiment.

3. 3. 1. Antibacterial activity assay

The observed antibacterial activity by mean of mm of zone of inhibition was presented in Table 5. From the table compounds 2-4 and 7 shows good antibacterial activities against *B. subtilis* bacterial strains about 20-22 mm maximum zone of inhibition. Compounds 5 and 6 shows satisfactory antibacterial activity against the same strain within 13-19 mm of zone of inhibition. The parent compound active against 9-12 mm of zone of inhibition.

Table 5. Antimicrobial activities of 4-(6-ethyl-2-(substituted phenyl)-6*H*-pyrido[3,2-*b*]carbazol-4-yl) anilines.

Entry	X	Antibacterial activity ^a				Antifungal activity ^b	
		Gram +ve bacteria		Gram -ve bacteria		<i>E. coli</i>	<i>T. viridi</i>
		<i>B. subtilis</i>	<i>M. luteus</i>	<i>S. aureus</i>	<i>P. argenosea</i>		
1	H	±	+	---	±	+	---
2	3-Br	++	+	+	++	±	+
3	3-Cl	++	++	++	++	±	---
4	4-F	++	++	+	++	+	±
5	3-NO ₂	+	±	---	---	++	++
6	4-SCH ₃	+	±	---	---	++	++
7	4-CF ₃	++	+	+	±	±	+

a: referred with Ampicillin standard drug. ++: Good; + Satisfactory; ±: Moderate; --- No activity.
b: referred with Miconazole standard drug. ++: No fungal colony; +: 1 Fungal colony; ± 1 or 2 fungal colonies; --- Heavy fungal colonies

The carbazole derivatives 3 and 4 shows good antibacterial activity against *M. luteus* strain within 20-22 mm of zone of inhibition. Aniline derivatives 1, 2 and 7 shows satisfactory activity within 13-19 mm of zone of inhibition. The compounds 5 and 6 shows moderate activity within 9-12 mm of zone of inhibition.

The compounds 3 and 7 shows good antibacterial activity against *S.aureus* strain within 20-22 mm of zone of inhibition. The carbazole compounds 2 and 4 shows satisfactory antibacterial activity against the same strains with 13-19 mm of zone of inhibition. The remaining compounds 1, 5 and 6 have no antibacterial activity.

The aniline derivatives 2-4 shows good antibacterial activities against *P.argenosea* strain within 20-22 mm of zone of inhibition. The compounds 1 and 7 shows moderate antibacterial activities against the same strain within 9-12 mm of zone of inhibition. The carbazole derivatives 5 and 6 has no activity.

3. 3. 2. Antifungal activity assay

The 4-(6-ethyl-2-(substituted phenyl)-6*H*-pyrido[3,2-*b*]carbazol-4-yl) anilines 5 only shows good antifungal activity against *A.niger* fungal strain within 20-22 mm of zone of inhibition. Compounds 1 and 4 shows satisfactory antifungal activity against *A.niger* strain by formation of 2-3 fungal colonies with 13-19 mm of zone of inhibition. Anilines 2, 3 and 7 shows moderate antifungal activity against the same fungi strain by formation one fungal colony within 9-12 mm of zone of inhibition.

The titled compounds 5 and 6 shows good antifungal activities against *T. viride* fungal strains by no fungal colony formation within 20-22 mm of zone of inhibition. The compounds 3 and 7 shows satisfactory antifungal activity within 13-19 mm of zone of inhibition against *T. viride* fungal strains by one fungal colony formation. The aniline derivative 4 only shows moderate antifungal activity against the same strain with 9-12mm of zone of inhibition by the formation 2 or 3 fungal colonies. The parent compound and 3 were possess heavy fungal colonies and no antifungal activity against the *T. viride* strains.

4. CONCLUSIONS

Some 4-(6-ethyl-2-(substituted phenyl)-6*H*-pyrido[3,2-*b*]carbazol-4-yl) anilines were synthesized and their purities were examined by their physical constants and spectroscopic data. From the IR and NMR spectra, the characteristic frequencies were assigned. These frequencies were correlated with Hammett substituent constants, F and R parameters. From the results of statistical analysis the infrared spectral data gave satisfactory correlations with C=N stretches. The NH₂ and CH₂ proton chemical shifts of these aniline derivatives shows satisfactory correlations with Hammett substituent constants, F and R parameters. Many correlations involving ¹³C carbon chemical shifts gave satisfactory correlations. All spectral data gave satisfactory correlation in multi-regression analysis. The halo substituted compounds shows good antibacterial activity against their bacterial strains within the prescribed mm of zone of inhibition. The nitro and thio methyl substituted compounds shows good antifungal activities against their fungal strains within the prescribed mm of zone of inhibitions.

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