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Synthesis and Antibacterial Activity of 5-benzylidenebarbituric acids : A structure - reactivity Study.

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Abstract : A very simple and highly efficient synthesis was established for the reaction of barbituric acid and substituted benzaldehydes to provide novel substituted 5-benzylidenebarbituric acids. Synthetized substituted 5-benzylidenebarbituric acids were characterized by ^{1}H and ^{13}C NMR spectral analysis. The antibacterial activities and structure reactivity correlation of the compounds have been studied.
Keywords : Substituted 5-benzylidenebarbituric acids; antibacterial, correlation studies.

Introduction

The barbituric acid derivatives are clinically useful. By substituting two protons in C-5 position during barbiturate synthesis, acidity of the whole molecule can be reduced and an unsaturated group can be added for the later incorporation of parahydrogen into the molecule [1]. Benzylidene barbituric acids as potential organic oxidizers [2] are applied for preparing pyrimidine derivatives [3].The benzylidenebarbituric acids are the important building blocks in synthesizing pyrazolo[3,4-d]pyrimidines and pyrido[2,3-d]pyrimidines [4,5]. They also have a broad range of biological activitiesSome barbituric acid derivatives have been widely used as sedative, hypnotic, anticonvulsant, antispasmodic, as well as local anesthetic agents [6]. Benzylidenebarbituric acids are useful as potential organic oxidizers, for the preparation of oxadeazaflavines [7] and for the unsymmetrical synthesis of disulfides [8]. Some of them have been recently studied as nonlinear optical materials [9]. Several 5-benzylidenebarbituric acids were prepared in the absence of solvent by the influence of infrared irradiation. These molecules were obtained by means of a Knoevenagel condensation between barbituric acid and various benzaldehydes [10]. Recently we have reported the substituent effects on zone of inhibition against the growth of microorganisms in various substituted 2-benzylidene-1,3-indandiones [11]. In continuation of our research interest in the structure - reactivity study, we have synthesized substituted 5-benzylidenebarbituric acids and studied the antibacterial activity to find out the substituent effect on 5-benzylidenebarbituric acid.

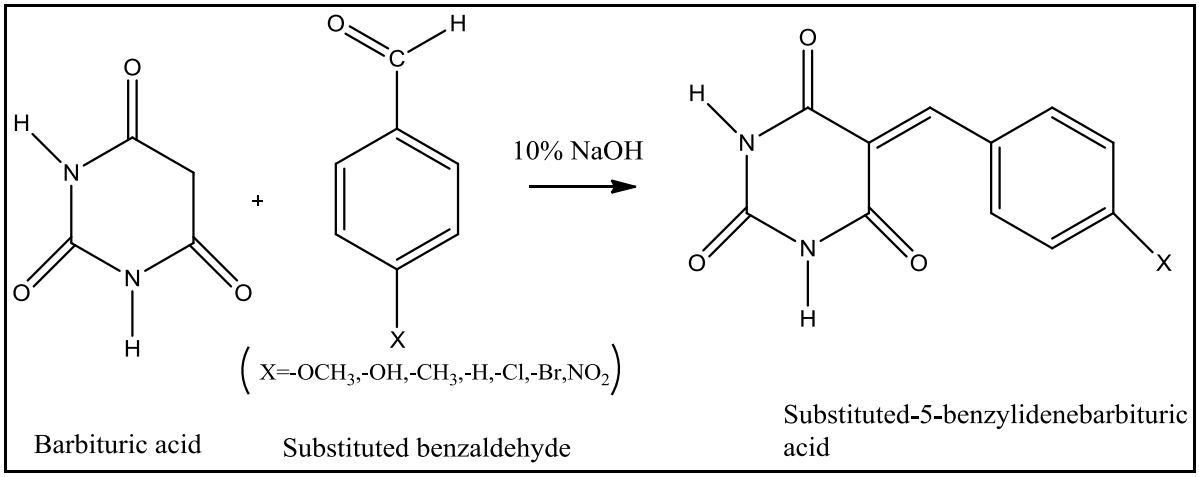
Experimental

All chemicals used were purchased from Sigma Aldrich. Purity of the compounds was checked by TLC on silica gel G plate. ^{1}H and ^{13}C spectra were obtained on a BRUKER AMX 400 MHz spectrometer. Chemical shift of ^{1}H were measured with the peak of DMSO at δ 2.51 as the internal reference, while those of ^{13}C were recorded with the central peak of DMSO at δ 39.90 as the internal reference.

General procedure for the synthesis of 5-benzylidenebarbituric acids (1 to 7)

5-benzylidenebarbituric acid and its substituted compounds (1 to 7) were prepared by the modified procedure of Branko Jursic(2001) [12].

To the calculated amount of the pure benzaldehyde (2 g, 0.015mol) and barbituric acid (1.55g, 0.015 mol) in warm ethyl alcohol was added a 10% solution of sodium hydroxide (catalytic amount) and the reaction mixture stirred for 2 hours. After completion of the reaction as indicated by TLC, the reaction mixture was left overnight (scheme I). Solid product was separated by filtration and washed several times with cold methanol.



Scheme I: Synthesis of 5-benzylidenebarbituric acids.

Spectral analysis of compounds (1 to 7)

Compound 1:*5-(4'-methoxybenzylidene)barbituric acid*

^{1}H NMR: δ 3.877 (s,3H), 7.065 (d,2H), 8.252(s,1H), 8.369 (d,2H), 11.175 (s,1H), 11.302 (s,1H);**^{13}C NMR:** δ 56.22,114.41,116.00,125.62,137.96,150.67,155.46,162.64,163.92,164.39.

Compound 2:*5-(4'-hydroxybenzylidene)barbituric acid*

^{1}H NMR: δ 6.878 (d,2H), 8.213 (s,1H), 8.320 (d,2H), 10.851 (s,1H), 11.117(s,1H), 11.249 (s,1H);

^{13}C NMR: δ 114.61, 115.97, 124.24, 138.77, 150.70, 156.05,162.75,163.48,164.59.

Compound 3:*5-(4'-methylbenzylidene)barbituric acid*

^{1}H NMR: δ 2.385 (s,3H), 7.304 (d,2H), 8.094 (d,2H), 8.255 (s,1H), 11.218 (s,1H), 11.365 (s,1H);

^{13}C NMR: δ 118.30, 129.33, 130.31, 134043, 143.96, 150.68, 155.46, 162.26, 164.08.

Compound 4:*5-benzylidenebarbituric acid*

^{1}H NMR: δ 7.485 (m,3H), 8.073 (d,2H), 8.285 (s,1H), 11.238 (s,1H), 11.397 (s,1H);

^{13}C NMR: δ 119.55, 128.52, 132.69, 133.11, 133.54, 150.69, 155.20, 162.03, 163.87.

Compound 5: *5-(4'-chlorobenzylidene)barbituric acid*

^{1}H NMR: δ 7.518 (d,2H), 8.069 (d,2H), 8.243 (s,1H), 11.275 (s,1H), 11.425 (s,1H);

^{13}C NMR: δ 120.09, 128.55, 132.01, 135.15, 137.21, 150.65, 153.52, 162.04, 163.67.

Compound 6: *5-(4'-bromobenzylidene)barbituric acid*

^{1}H NMR: δ 7.670 (d,2H), 7.979 (d,2H), 8.223 (s,1H), 11.272 (s,1H), 11.421 (s,1H);

^{13}C NMR: δ 120.24, 126.29, 131.51, 132.40, 135.15, 150.65, 153.56, 162.04, 163.67.

Compound 7: *5-(4'-nitrobenzylidene)barbituric acid*

^{1}H NMR: δ 8.017 (d,2H), 8.245 (d,2H), 8.324 (s,1H), 11.329 (s,1H), 1.504 (s,1H);

^{13}C NMR: δ 123.15, 123.37, 132.69, 140.48, 148.49, 150.68, 151.63, 161.62, 163.13.

Antibacterial Activity:

Agar well-diffusion method was followed to determine the antibacterial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 hours old -broth culture of respective bacteria. Wells (6mm) were made in each of these plates using sterile cork borer. Briefly, agar plates were inoculated with bacterial strain under aseptic conditions and wells (diameter = 6 mm) were filled with 50 µl of the test samples and incubated at 37°C for 24 hours. After the incubation period, the diameter of the growth of inhibition zones were measured. Three inhibition zone diameter measurements were taken for each well and averaged, for each replicates the readings were taken in three different fixed directions and the average values were recorded. The average inhibition zone diameter for the various bacteria are shown in Table 1.

Results and Discussion

In thisstudy, gram-positive bacteria (*Staphylococcus aureus*) and five gram-negative bacteria (*Escherichia coli, Klebsiella oxytoca, Proteus mirabilis,Pseudomonas aeruginosa and Shigella sonnei)*were used. The result of the present study showed a broad range of antibacterial activity, shown in Figure 1. The order of antibacterial activity of compounds (1 to 7) for all the microorganism were in the following sequence.

$-OCH_3$<-OH < $-CH_3$< -H < -Cl < -Br< $-NO_2$

Table 1. Antibacterial activity (zone of inhibition(mm) values) of substituted 5-benzylidenebarbituric acid

S.No.	Name of the microorganism	Inhibition zone diameter							
		Standard (Amphotericin-B)	$-OCH_3$	-OH	$-CH_3$	-H	-Cl	-Br	$-NO_2$
1	*Escherichia coli*	21	9	10	12	14	16	17	20
2	*Klebsiella oxytoca*	16	8	12	14	16	20	21	28
3	*Proteus mirabilis*	18	7	9	10	11	16	19	22
4	*Pseudomonas aeruginosa*	21	11	13	15	16	20	23	28
5	*Shigella Sonnei*	16	8	10	11	12	14	18	21
6	*Staphylococcus aureus*	18	7	12	13	14	16	23	28

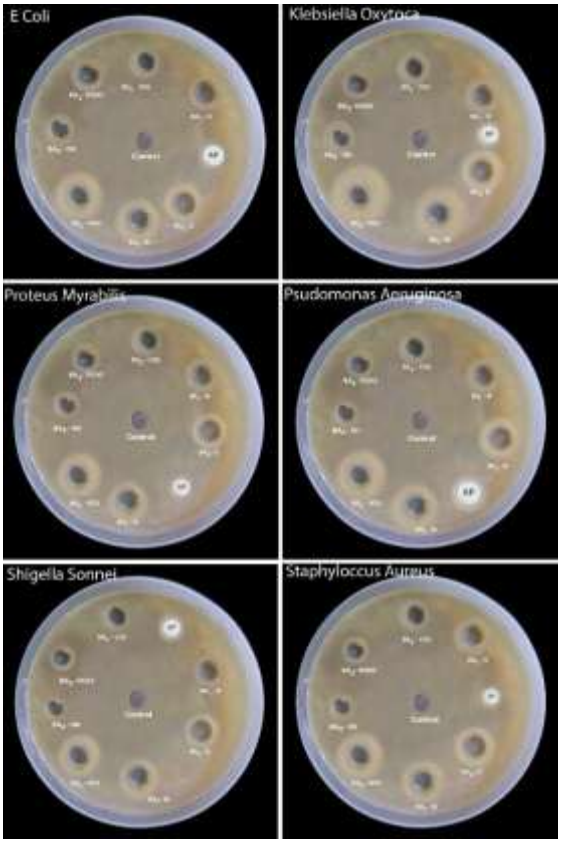


Figure 1: Antibacterial activity of substituted 5-benzylidenebarbituric acids

If atom or group attracts electrons less strongly than hydrogen, it is said to have +I effect (electron repelling or electron releasing) viz., -OCH_3, -OH, -CH_3groups showing lesser zone inhibition values compared to unsubstituted phenyl ring (-H).

In order to express the effect of substituents quantitatively it was considered worthwhile to correlate the logarithm of inhibition zone diameter (IZD) of (1 to 7) at the same concentration with the Hammett substituent constants for all the microorganisms. The results of statistical SSP analysis are given in Table 2. The corresponding Hammett plot for *Klebsiella oxytoca* is shown in Figure2.

The positive value of the reaction constant (ρ) equation 1 indicates that electron withdrawing substituents increase the antibacterial activity and electron releasing substituents retard it.

$$\log (IZD) = (0.36\pm 0.02)\ \sigma_p^+ / \sigma_p + (1.20\pm 0.01) \quad (1)$$

(r = 0.993, n=7, F = 360.60)

Table 2: Results of statistical treatment of log IZD (mm)with σ_p,σ_p^o,σ_p^+,σ_p^+/σ_p,σ_p^+/σ_p^-,$\sigma_p^+/\sigma_p/\sigma_p^-$substituent constants using single parameter equation

S.No.	Bacteria	Scale	ρ	r	s	F	Log(IZD)^o	n
1	*Escherichia coli*	σ_P	0.31±0.05	0.937	0.048	36.19	1.113±0.02	7
		σ_P^o	0.29±0.09	0.854	0.07	10.78	1.10±0.03	6 [Excluding -OH]
		σ_P^+	0.21±0.02	0.969	0.034	76.36	1.159±0.01	7

		σ_P^+ / σ_P	0.25±0.02	0.977	0.03	104.92	1.135±0.01	7
		σ_P^+ / σ_P^-	0.16±0.03	0.934	0.05	35.95	1.14±0.02	7
		$\sigma_P^+ / \sigma_P / \sigma_P^-$	0.18±0.03	0.929	0.05	31.38	1.12±0.02	7
2	*Klebsiella oxytoca*	σ_P	0.42±0.09	0.990	0.08	21.23	1.18±0.03	7
		σ_P^o	0.43±0.14	0.837	0.11	9.33	1.14±0.05	6 [Excluding -OH]
		σ_P^+	0.28±0.05	0.926	0.07	29.94	1.23±0.03	7
		σ_P^+ / σ_P	0.36±0.02	0.993	0.02	360.60	1.20±0.01	7
		σ_P^+ / σ_P^-	0.22±0.04	0.901	0.08	21.81	1.22±0.03	7
		$\sigma_P^+ / \sigma_P / \sigma_P^-$	0.26±0.04	0.945	0.06	41.80	1.18±0.02	7
3	*Proteus mirabilis*	σ_P	0.44±0.08	0.922	0.08	28.2	1.07±0.03	7
		σ_P^o	0.45±0.13	0.868	0.1	12.26	1.03±0.05	6 [Excluding -OH]
		σ_P^+	0.28±0.06	0.911	0.08	25.5	1.13±0.03	7
		σ_P^+ / σ_P	0.36±0.05	0.959	0.06	57.12	1.1±0.02	7
		σ_P^+ / σ_P^-	0.22±0.05	0.887	0.09	18.47	1.11±0.04	7
		$\sigma_P^+ / \sigma_P / \sigma_P^-$	0.26±0.05	0.915	0.08	25.73	1.08±0.03	7
4	Pseudomonas aeruginosa	σ_P	0.33±0.05	0.953	0.04	49.84	1.21±0.02	7
		σ_P^o	0.33±0.08	0.889	0.07	15.15	1.19±0.03	6 [Excluding -OH]
		σ_P^+	0.21±0.03	0.940	0.05	38.35	1.26±0.02	7
		σ_P^+ / σ_P	0.26±0.04	0.950	0.05	46.15	1.24±0.02	7
		σ_P^+ / σ_P^-	0.17±0.03	0.940	0.05	37.76	1.25±0.02	7
		$\sigma_P^+ / \sigma_P / \sigma_P^-$	0.19±0.03	0.944	0.05	40.59	1.22±0.02	7
5	Shigella sonnei	σ_P	0.35±0.06	0.923	0.06	28.74	1.09±0.02	7
		σ_P^o	0.36±0.11	0.860	0.09	11.32	1.06±0.04	6 [Excluding -OH]

		σ_P^+	0.23±0.04	0.920	0.06	27.4	1.14±0.02	7
		σ_P^+ / σ_P	0.29±0.03	0.965	0.04	67.55	1.11±0.02	7
		σ_P^+ / σ_P^-	0.18±0.04	0.908	0.07	23.36	1.12±0.03	7
		$\sigma_P^+ / \sigma_P / \sigma_P^-$	0.21±0.04	0.940	0.06	35.65	1.09±0.02	7
6	Staphylococcus aureus	σ_P	0.44±0.12	0.861	0.11	14.35	1.15±0.04	7
		σ_P^o	0.47±0.17	0.810	0.14	7.53	1.1±0.07	6 [Excluding -OH]
		σ_P^+	0.29±0.07	0.873	0.1	16.09	1.21±0.04	7
		σ_P^+ / σ_P	0.39±0.05	0.962	0.06	62.09	1.18±0.02	7
		σ_P^+ / σ_P^-	0.23±0.06	0.862	0.11	14.49	1.19±0.04	7
		$\sigma_P^+ / \sigma_P / \sigma_P^+$	0.28±0.05	0.924	0.08	29.4	1.15±0.03	7

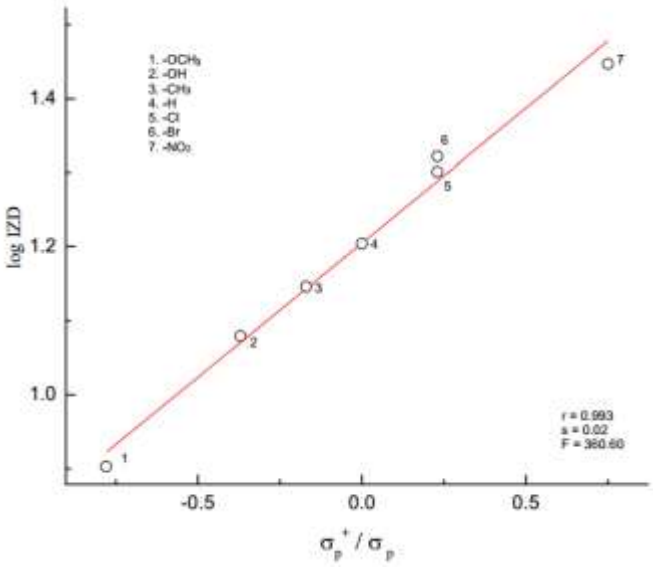


Figure 2. Hammett plot for Klebsiella oxytoca

DSP analysis has been performed for each of the resonance scale (σ_R, σ_R^+, σ_R^-). The best fit of DSP analysis for *Pseudomonas aeruginosa* is taken from satisfactory correlation coefficient (R) and least standard error (SE) of the regression equations (2) and (3) and the result obtained given in Table 3.

$$\log (IZD) = (0.33 \pm 0.08)\sigma_I + (0.37 \pm 0.08)\ \sigma_R + (1.22 \pm 0.04) \qquad (2)$$

R = 0.963, SE = 0.05, n = 6, F = 19.09
log (IZD) = (0.35 ± 0.10) F + (0.34 ± 0.08) R + (1.21 ± 0.05) (3)
R = 0.941, SE = 0.06, F = 15.54

The sign of $\rho_I$and$\rho_R$are positive, reveals that the normal substituent effects operates on IZD, i.e. electron releasing substituents decrease the IZD and electron withdrawing substituents increase the IZD. The $\rho_R$values are rather smaller than $\rho_I$values and this reveals the importance of polar component.

Table 3: DSP analysis of log IZD (mm) with dual parameter equations 2 and 3.

S.No	Bacteria	Scale	ρ_I	ρ_R	R	SE	F	Log(IZD)^o	n	$\lambda=\rho_R/\rho_I$
1	*Escherichia coli*	σ_I,σ_R	0.24±0.08	0.35±0.08	0.954	0.05	15.2	1.14±0.03	6	1.46
		σ_I,σ_R^+	0.11±0.20	0.15±0.2	0.761	0.1	2.75	1.16±0.09	7	1.36
		σ_I,σ_R^o	0.28±0.18	0.15±0.17	0.722	0.1	1.64	1.10±0.07	6	0.54
		σ_I,σ_R^-	0.23±0.16	0.18±0.14	0.790	0.1	2.50	1.11±0.07	6	0.78
		F,R	0.24±0.09	0.34±0.07	0.945	0.05	16.8	1.14±0.04	7	1.42
2	*Klebsiella oxytoca*	σ_I,σ_R	0.33±0.11	0.55±0.12	0.956	0.07	15.83	1.21±0.05	6	1.66
		σ_I,σ_R^+	0.15±0.28	0.21±0.13	0.760	0.14	2.74	1.24±0.13	7	1.40
		σ_I,σ_R^o	0.40±0.28	0.26±0.26	0.707	0.17	1.50	1.15±0.12	6	0.65
		σ_I,σ_R^-	0.31±0.25	0.30±0.21	0.777	0.15	2.29	1.16±0.10	6	0.97
		F,R	0.35±0.17	0.25±0.13	0.903	0.09	8.86	1.20±0.05	7	0.71
3	*Proteus mirabilis*	σ_I,σ_R	0.45±0.13	0.44±0.14	0.940	0.08	11.40	1.06±0.06	6	0.98
		σ_I,σ_R^+	0.31±0.26	0.17±0.12	0.801	0.13	03.60	1.08±0.13	7	0.55
		σ_I,σ_R^o	0.51±0.25	0.14±0.24	0.763	0.16	02.09	0.996±0.1	6	0.28
		σ_I,σ_R^-	0.44±0.24	0.21±0.19	0.814	0.14	02.94	1.01±0.09	6	0.48
		F,R	0.47±0.16	0.40±0.12	0.918	0.09	10.67	1.04±0.07	7	0.85
4	*Pseudomonas aeruginosa*	σ_I,σ_R	0.33±0.08	0.37±0.08	0.963	0.05	19.09	1.22±0.04	6	1.12
		σ_I,σ_R^+	0.22±0.21	0.13±0.10	0.777	0.11	03.06	1.23±0.10	7	0.59
		σ_I,σ_R^o	0.37±0.19	0.15±0.19	0.767	0.12	02.15	1.17±0.08	6	0.41
		σ_I,σ_R^-	0.31±0.17	0.21±0.14	0.839	0.1	03.58	1.18±0.07	6	0.68
		F,R	0.35±0.10	0.34±0.08	0.941	0.06	15.54	1.21±0.05	7	0.97
5	*Shigella sonnei*	σ_I,σ_R	0.33±0.01	0.39±0.1	0.950	0.06	13.88	1.09±0.04	6	1.18
		σ_I,σ_R^+	0.21±0.22	0.15±0.1	0.782	0.11	03.15	1.11±0.10	7	0.71
		σ_I,σ_R^o	0.38±0.22	0.14±0.2	0.731	0.13	01.72	1.04±0.09	6	0.37
		σ_I,σ_R^-	0.31±0.18	0.22±0.15	0.825	0.11	03.20	1.06±0.08	6	0.71
		F,R	0.35±0.13	0.33±0.1	0.920	0.07	10.98	1.08±0.06	7	0.94
6	*Staphylococcus aureus*	σ_I,σ_R	0.38±0.15	0.61±0.16	0.940	0.09	11.34	1.18±0.07	6	1.61
		σ_I,σ_R^+	0.19±0.32	0.22±0.15	0.745	0.16	2.49	1.20±0.15	7	1.16
		σ_I,σ_R^o	0..45±0.33	0.23±0.32	0.650	0.2	1.14	1.09±0.14	6	0.51
		σ_I,σ_R^-	0.35±0.24	0.35±0.28	0.780	0.17	2.32	1.13±0.12	6	1.00
		F,R	0.42±0.22	0.44±0.17	0.860	0.12	5.63	1.15±0.09	7	1.05

(* when n=6, the -OH substituent was excluded.)

The Yukawa-Tsuno equation 4 and Table 4for *Escherichia coli*proved the less contribution of resonance effect.

$\log IZD = (0.171\pm0.05)\ \sigma_p^{o} + (0.211 \pm 0.05)\ (\sigma_p^{+} - \sigma_p^{o}) + (1.18 \pm 0.02)$ (4)

(R = 0.981, SE = 0.03, n = 6, F = 38.16)

Table 4:Results of multiple regression analysis of log IZR (mm) with σ_p,(σ_p^{+}- σ_p) and σ_p^{o},(σ_p^{+}- σ_p^{o}) constants using Yukava – Tsuno equation (4).

S.No.	Bacteria	Scale	ρ	r	R	SE	F	n
1	*Escherichia coli*	σ_p,(σ_p^{+}- σ_p)	0.205±0.06	0.279±0.11	0.975	0.04	28.35	6
		σ_p^{o},(σ_p^{+}- σ_p^{o})	0.171±0.05	0.211±0.05	0.981	0.03	38.16	6
2	*Klebsiella oxytoca*	σ_p,(σ_p^{+}- σ_p)	0.282±0.09	0.484±0.16	0.977	0.05	31.13	6
		σ_p^{o},(σ_p^{+}- σ_p^{o})	0.248±0.09	0.325±0.09	0.969	0.06	23.16	6
3	*Proteusmirabilis*	σ_p,(σ_p^{+}- σ_p)	0.386±0.14	0.237±0.27	0.938	0.08	11.02	6
		σ_p^{o},(σ_p^{+}- σ_p^{o})	0.316±0.13	0.243±0.13	0.841	0.08	11.55	6
4	*Pseudomonas aeruginosa*	σ_p,(σ_p^{+}- σ_p)	0.294±0.08	0.133±0.15	0.959	0.05	16.95	6
		σ_p^{o},(σ_p^{+}- σ_p^{o})	0.242±0.09	0.15±0.09	0.945	0.06	12.42	6
5	*Shigella sonnei*	σ_p,(σ_p^{+}- σ_p)	0.294±0.1	0.239±0.19	0.954	0.06	15.04	6
		σ_p^{o},(σ_p^{+}- σ_p^{o})	0.239±0.09	0.215±0.1	0.948	0.06	12.24	6
6	*Staphylococcus albus*	σ_p,(σ_p^{+}- σ_p)	0.326±0.14	0.504±0.26	0.953	0.08	15.01	6
		σ_p^{o},(σ_p^{+}- σ_p^{o})	0.272±0.14	0.637±0.26	0.942	0.09	11.74	6

(* The -OH substituent was excluded)

Conclusion

To summarize, substituted 5-benzylidenebarbituric acidshave been synthesized and evaluated for their antibacterial activities. This reaction protocol offers a simple, easier work-up procedure and good yields. The compounds have been characterized by their spectral data. The antibacterial activities of all synthesized compounds have been studied. The inhibition zone diameters of these compounds have been correlated with Hammett substituent constants, F and R parameters. From the results of statistical analysis, the effects of substituent on the antibacterial activity of compounds have been studied.

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