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Synthesis and Antimicrobial Activity of 2-Benzylidene-1,3 Indandiones: A Structure-Reactivity Study

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ABASTRACT

Substituted 2-benzylidene-1,3-indandiones have been prepared and characterized by UV, IR, ^{1}H and ^{13}C NMR spectral analysis. The antimicrobial activities and structure reactivity correlation of the compounds have been studied.

Keywords: Substituted 2-benzylidene-1,3-indandiones; Antimicrobial; Correlation studies

INTRODUCTION

Different methods have been used for the synthesis of 1,3-indandione derivatives with substitution at position 2. Previous studies [1,2] reported phenylation of 1,3-indandione with diaryliodonium salts and α-alkenylation of β-dicarbonyl compounds with alkenyltriarylbismuthonium salts. The Friedel-Crafts methods were also reported for the derivatization of 1,3-indandione at position 2 [3]. In addition to these conventional methods, the electrochemical synthesis has also been used for preparation of indandione derivatives with catechol or 2,3-dimethylhydroquinone ring on their position 2 [4-6].

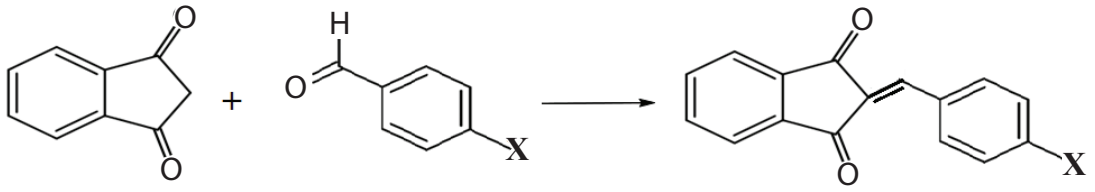
Studies of substituent effects on zone of inhibition against the growth of microorganisms in various substituted N-(1-piperidino benzyl) nicotinamide [7] and substituted N-(1-piperidinobenzyl) acetamide and substituted N-(1-morpholinobenzyl) acetamide [8] have been reported. The literature reveals that there is a little work done on the antimicrobial study of activated olefinic compounds. As a part of our interest in the structure-reactivity study, we have synthesized 2-benzylidene-1,3-indandiones and studied the antimicrobial activity to find out the substituent effect on 2-benzylidene-1,3-indandione.

MATERIALS AND METHODS

All chemicals used were purchased from Sigma Aldrich. Purity of the compounds was checked by TLC on silica gel G plates. UV-Visible spectra were recorded on a CARY VARION (V 550), $CHCl_3$ was used as a solvent. Infrared spectra were obtained on a PARAGON 500 spectrometer on KBr pellets. ^{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 $CDCl_3$ at δ 7.29 as the internal reference, while those of ^{13}C were recorded with the central peak of $CDCl_3$ at δ 77.03 as the internal reference.

General procedure for the synthesis of 2-benzylidene-1,3-indandiones (1 to 6)

Preparation of 2-Benzylidene-1,3-indandione and its substituted compounds (1 to 6) were done as per the reported procedure [9]. To the calculated amount of the appropriate benzaldehyde (12.5 mM) and 1,3-indandione (12.5 mM) in warm ethyl alcohol was added a 10% solution of sodium hydroxide (catalytic amount) and the reaction mixture stirred for 2 h to 3 h and left overnight (**Scheme 1**). The solvent was removed in vacuum.



1,3- indandione **Substituted benzaldehyde** (X= OCH_3, CH_3, H, Cl, Br, COOH) **Substituted 2-benzylidene-1,3-indandiones**

Scheme 1: Synthesis of 2-benzylidene-1,3-indandiones

Spectral analysis of compounds (1 to 6)

Compound 1: 2-(4'-Methoxybenzylidene)-1,3-indandione

UV: 252,386 nm; **IR**: 526.21, 731.99, 839.46, 1022.34, 1174.91, 1266.32, 1505.89, 555.70, 1581.14, 2839.62, 3439.07 cm^{-1}; **^{1}H NMR**: δ 3.917(s,3H), 6.998-7.027(m,2H), 7.766-7.842(m,2H), 7.958-8.00(m,2H), 8.529-8.558(m,2H); **^{13}C NMR:** δ 55.60,114.38, 123.07, 126.46, 134.87, 146.86, 189.54, 190.86.

Compound 2: 2-(4'-Methylbenzylidene)-1,3-indandione

UV: 264,357 nm; **IR:** 739.74, 818.12, 989.52, 1080.52, 1189.24, 1332.42, 1351.89, 1376.46, 1588.08, 1683.93, 1725.29, 3489.89 cm^{-1}; **^{1}H NMR**: δ 2.343(s,3H), 7.178(d,2H), 7.689-7.710(m,2H), 7.766(s,1H), 7.899(dd,1H), 8.287(d,1H); **^{13}C NMR**: δ 22.01, 123.22, 123.22, 128.17, 135.04, 147.10, 189.20, 190.53.

Compound 3: 2-Benzylidene-1,3-indandione

UV: 264,345 nm; **IR**: 568.09, 682.38, 732.69, 981.07, 1198.94, 1244.02, 1345.96, 1376.53, 1586.17, 1683.75, 1724.93, 2364.96, 3063.50, 3783.09 cm^{-1}; **^{1}H NMR:** δ 7.519-7.580(m,4H), 7.824-7.846(m,2H), 7.9249(s,1H), 8.020-8.047(m,1H), 8.465-8.4869(dd,2H); **^{13}C NMR:** δ 123.26, 128.82,129.17, 135.25, 135.45, 189.04, 190.32.

Compound 4: 2-(4'-Chlorobenzylidene)-1,3-indandione

UV: 265,351 nm; **IR:** 830.56, 1074.32, 1092.42, 1203.63, 1411.93, 1486.02, 1582.35, 1609.80, 1689.99, 1726.37, 2363.08, 3092.11, 3696.20 cm^{-1}; **^{1}H NMR**: δ 7.474-7.495,(m,2H), 7.827(s,1H), 7.834-7.848(m,2H), 7.997-8.018(m,2H), 8.417-8.438(m,2H); **^{13}C NMR:** δ 123.44, 129.14, 131.54, 135.39, 145.20, 189.01, 189.98.

Compound 5: 2-(4'-Bromobenzylidene)-1,3-indandione

UV: 265,353 nm; **IR:** 736.35, 828.32, 1072.53, 1162.92, 1202.37, 1248.20, 1408.55, 1485.35, 1577.50, 1612.90, 1689.61, 1726.98, 3086.69 cm^{-1}; **^{1}H NMR:** δ 7.640-7.662(dd,2H), 7.811(s,1H), 7.829-7.851(m,2H), 8.011-8.035(m,2H), 8.322-8.353(dd,2H); **^{13}C NMR:** δ 123.44, 129.63, 132.16, 135.57, 145.27, 189.01, 189.96.

Compound 6: 2-(4'-Carboxybenzylidene)-1,3-indandione

UV: 263,340 nm; **IR:** 734.01, 988.89, 1156.73, 1202.53, 1248.65, 1293.26, 1428.79, 1592.13, 1618.67, 1687.05, 1731.80, 2549.02, 2825.28 cm^{-1}; **^{1}H NMR:** δ 7.851(s,1H), 7.902-7.923(m,2H), 7.955-7.992(m,2H), 8.039(d,2H), 8.465(d,2H); **^{13}C NMR:** δ 39.05, 123.64, 129.70, 134.37, 136.36, 144.36, 188.60, 189.34.

Antimicrobial activity

Agar well-diffusion method was followed to determine the antimicrobial activity [10]. Nutrient agar (NA) and potato dextrose agar (PDA) plates were swabbed (sterile cotton swabs) with 8 h old broth culture of respective bacteria. Wells (6 mm) 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 h. After the incubation period, the radii of the growth of inhibition zones were measured. The distance between centers of the well to the edge of the zone was determined to be the inhibition zone radius. Three inhibition zone radii 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 radii for the various bacteria are shown in **Table 1.**

RESULTS AND DISCUSSION

In this study, gram-positive bacteria (*Staphylococcus aureus*) and five gram-negative bacteria (*Aeromonas hydrophila*,

Escherichia coli, *Psedomonas aeruginosa*, *Proteus mirabilis*, and *Vibrio paraheamolyticus*) were used. The result of the present study showed a broad range of antimicrobial activity. The data found in the literature, that the compounds with halogen substituent are the most efficient against gram-positive bacteria, particularly against *S. aureus* [11,12]. But in this study, we found more or less equal zone of inhibition values for all gram-positive and gram-negative bacteria **(Figure 1**). It shows that the antibacterial activity depends upon substituent only. Compound 6 exhibited excellent antibacterial activity. It has been established that the –COOH group has an excellent metal-binding capacity [13]. This explains the higher antibacterial activity. The results also reveal that the antibacterial activity is affected by the nature of the substituent group (X) found in the aryl ring. The chloride derivative is characterized by greater antibacterial activity than that of the methyl and methoxy derivatives. According to Mohamed et al. [13] this may be attributed to the electron-withdrawing character of the chlorine group that decreases the electron density in the indandiones group, increasing its cationic character. The derivatives with electron withdrawing groups showed strong antibacterial activity than those of electron donating group [14]. Electron-withdrawing substituent increases acidity also. Bacterial growth is inhibited by increasing the acidity of the substituents. The order of antibacterial activity of compounds (1 to 6) for all the microorganism were in the following sequence:

$$–OCH_3<–CH_3<–H<–Cl<–Br<–COOH$$

Table 1: Antimicrobial activity (zone of inhibition (mm) values) of substituted 2-benzylidene-1, 3-indandiones.

S. No.	Name of the microorganisms	Inhibition zone radius (mm)						
		Standard (Amphotericin-B)	–OCH3	–CH3	–H	–Cl	–Br	–COOH
1	*Aeromonas hydrophila*	21	5	6	7	8	9	12
2	*Escherichia coli*	16	6	7	8	9	10	12
3	*Psedomonas aeruginosa*	21	5	6	8	8	9	11
4	*Proteus mirabilis*	18	5	6	7	9	10	12
5	*Staphylococcus aureus*	16	6	7	8	9	9	11
6	*Vibrio paraheamolyticus*	18	5	7	9	10	10	12

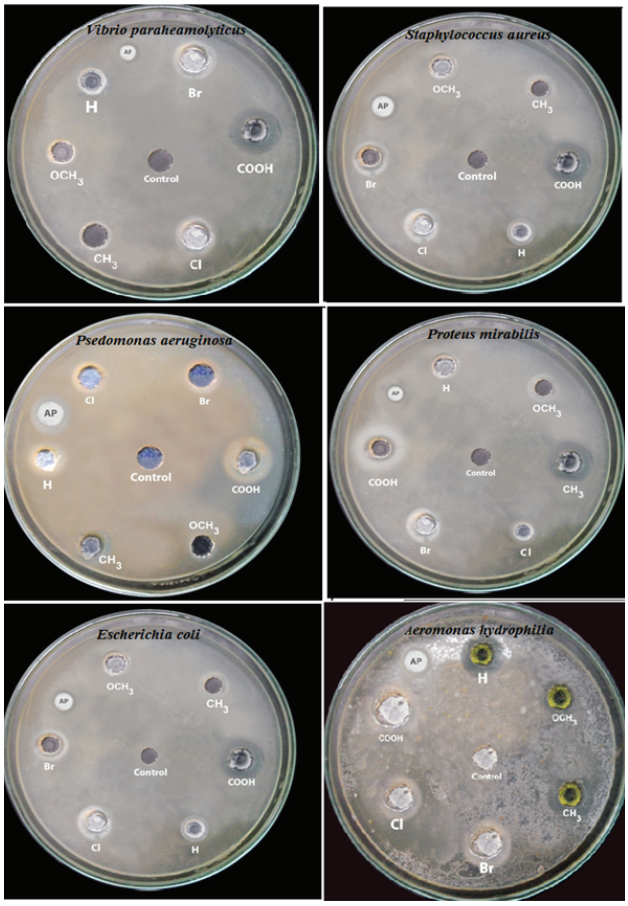


Figure 1: Antimicrobial activity of substituted 2-benzylidene-1,3-indandiones.

If atom or group attracts electrons less strongly than hydrogen, it is said to have +I effect (electron repelling or electron releasing) viz., $–OCH_3$, $–CH_3$ groups 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 radius (IZR) of 1 to 6 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 *V. paraheamolyticus* is shown in **Figure 2**.

The positive value of the reaction constant (ρ) equation 1:

$$\log IZR=(0.32 \pm 0.01)\sigma_p^{+}+(0.952 \pm 0.003) \quad (1)$$

(r=0.998, n=6, F=1337.04)

Indicates that electron withdrawing substituents increase the antimicrobial activity and electron releasing substituents retard it.

DSP analysis has been performed for each of the resonance scale (σ_R, σ_R^{+}, σ_R^{-}). The best fit of DSP analysis for *A. hydrophila* 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.**

Table 2: Results of statistical treatment of log IZR (mm) with σ_p, σ_p^{o}, σ_p^{+}, σ_p^{+}/σ_p, $\sigma_p^{+}/\sigma_p^{-}$, $\sigma_p^{+}/\sigma_p/\sigma_p^{-}$ substituent constants using single parameter Equation 1.

S. No.	Bacteria	Scale	ρ	r	s	F	Log(IZR)^{o}	N
1	*Aeromonas hydrophila*	σ_p	0.48 ± 0.04	0.981	0.03	104.12	0.839 ± 0.012	6
		σ_p^{o}	0.50 ± 0.11	0.915	0.06	20.56	0.815 ± 0.028	6
		σ_p^{+}	0.30 ± 0.05	0.95	0.05	37.47	0.897 ± 0.019	6
		σ_p^{+}/σ_p	0.29 ± 0.06	0.927	0.06	24.42	0.878 ± 0.023	6
		$\sigma_p^{+}/\sigma_p^{-}$	0.26 ± 0.03	0.981	0.03	102.65	0.879 ± 0.012	6
		$\sigma_p^{+}/\sigma_p/\sigma_p^{-}$	0.25 ± 0.03	0.972	0.04	67.47	0.865 ± 0.015	6
2	*Escherichia coli*	σ_p	0.39 ± 0.03	0.985	0.02	134.03	0.896 ± 0.008	6
		σ_p^{o}	0.40 ± 0.09	0.918	0.05	21.5	0.877 ± 0.022	6
		σ_p^{+}	0.25 ± 0.04	0.96	0.03	47.81	0.944 ± 0.014	6
		σ_p^{+}/σ_p	0.23 ± 0.04	0.94	0.04	30.76	0.928 ± 0.017	6
		$\sigma_p^{+}/\sigma_p^{-}$	0.20 ± 0.02	0.975	0.03	78.24	0.929 ± 0.011	6
		$\sigma_p^{+}/\sigma_p/\sigma_p^{-}$	0.20 ± 0.03	0.97	0.03	64.73	0.917 ± 0.012	6
3	*Pseudomonas aeruginosa*	σ_p	0.45 ± 0.05	0.976	0.03	81.07	0.853 ± 0.013	6
		σ_p^{o}	0.47 ± 0.10	0.916	0.06	21.05	0.83 ± 0.026	6
		σ_p^{+}	0.30 ± 0.03	0.986	0.02	140.74	0.909 ± 0.01	6
		σ_p^{+}/σ_p	0.28 ± 0.04	0.963	0.04	52.47	0.89 ± 0.015	6
		$\sigma_p^{+}/\sigma_p^{-}$	0.24 ± 0.03	0.968	0.04	61.35	0.891 ± 0.014	6
		$\sigma_p^{+}/\sigma_p/\sigma_p^{-}$	0.24 ± 0.03	0.965	0.04	54.32	0.877 ± 0.015	6
4	*Proteus miratrilis*	σ_p	0.52 ± 0.03	0.992	0.02	257.61	0.852 ± 0.008	6
		σ_p^{o}	0.55 ± 0.10	0.943	0.05	32.39	0.824 ± 0.025	6
		σ_p^{+}	0.32 ± 0.05	0.952	0.05	38.53	0.915 ± 0.02	6
		σ_p^{+}/σ_p	0.31 ± 0.06	0.94	0.05	38.53	0.895 ± 0.022	6
		$\sigma_p^{+}/\sigma_p^{-}$	0.27 ± 0.04	0.953	0.05	30.39	0.895 ± 0.02	6
		$\sigma_p^{+}/\sigma_p/\sigma_p^{-}$	0.27 ± 0.04	0.956	0.05	40.009	0.88 ± 0.019	6
5	*Staphylococcus aureus*	σ_p	0.33 ± 0.03	0.987	0.02	162.4	0.887 ± 0.007	6
		σ_p^{o}	0.35 ± 0.07	0.931	0.04	25.9	0.869 ± 0.018	6
		σ_p^{+}	0.21 ± 0.02	0.979	0.02	92.15	0.927 ± 0.009	6
		σ_p^{+}/σ_p	0.20 ± 0.03	0.962	0.03	49.6	0.914 ± 0.011	6
		$\sigma_p^{+}/\sigma_p^{-}$	0.18 ± 0.14	0.988	0.16	164.59	0.914 ± 0.006	6
		$\sigma_p^{+}/\sigma_p/\sigma_p^{-}$	0.18 ± 0.01	0.986	0.02	143.3	0.904 ± 0.007	6
6	*Vibrio paraheamolyticus*	σ_p	0.47 ± 0.08	0.945	0.05	33.62	0.893 ± 0.023	6
		σ_p^{o}	0.48 ± 0.14	0.865	0.08	11.91	0.87 ± 0.036	6
		σ_p^{+}	0.32 ± 0.01	0.998	0.01	1337.04	0.952 ± 0.003	6
		σ_p^{+}/σ_p	0.31 ± 0.02	0.99	0.02	200.93	0.932 ± 0.009	6
		$\sigma_p^{+}/\sigma_p^{-}$	0.26 ± 0.04	0.964	0.04	52.37	0.932 ± 0.017	6
		$\sigma_p^{+}/\sigma_p/\sigma_p^{-}$	0.26 ± 0.03	0.973	0.03	72.13	0.917 ± 0.014	6

log IZR=(0.61 ± 0.05) σ_I+(0.68 ± 0.06)σ_R-+(0.86 ± 0.02) (2)

(R=0.991, SE=0.02, n=6, F=84.98)

log IZR=(0.41 ± 0.08)F+(0.46 ± 0.08)R+(0.84 ± 0.02) (3)

(R=0.979, SE=0.03, n=5, F=22.27)

The sign of ρ_I and ρ_R are positive, reveals that the normal substituent effects operates on IZR, i.e. An electron releasing substituents decrease the IZR and electron withdrawing substituents increase the IZR. The ρ_R values are rather smaller than ρ_I values and this reveals the importance of polar component.

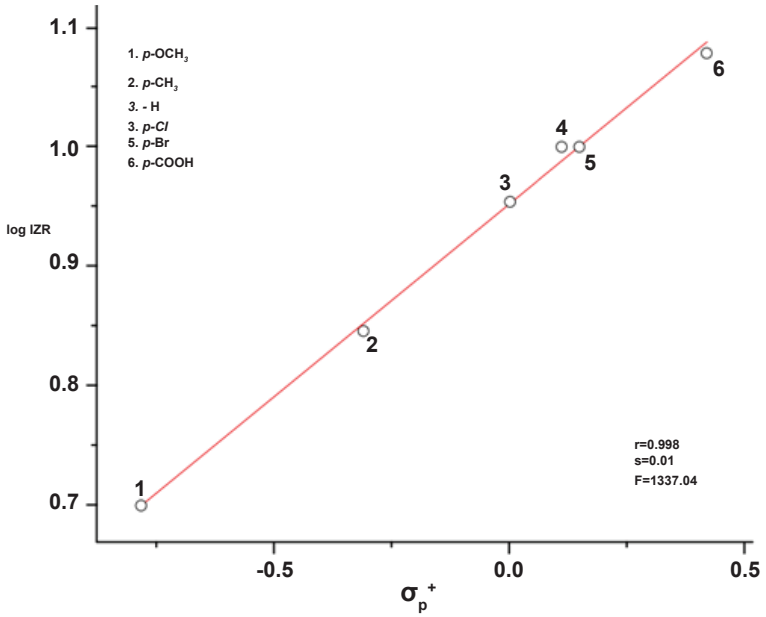


Figure 2: Hammet plot for vibrio paraheamolyticus

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

S. No.	Bacteria	Scale	ρ_I	ρ_R	R	SE	F	Log(IZR)°	N	$\lambda=\rho_R/\rho_I$
1	*Aeromonas hydrophila*	σ_I, σ_R	0.44 ± 0.17	0.50 ± 0.17	0.89	0.08	5.72	0.87 ± 0.05	6	1.14
		σ_I, σ_R^+	0.08 ± 0.18	0.17 ± 0.06	0.876	0.08	4.97	0.92 ± 0.07	6	2.12
		σ_I, σ_R^-	0.61 ± 0.05	0.68 ± 0.06	0.991	0.02	84.98	0.86 ± 0.02	6	1.11
		F, R	0.41 ± 0.08	0.46 ± 0.08	0.979	0.03	22.27	0.84 ± 0.02	5	1.12
2	*Escherichia coli*	σ_I, σ_R	0.37 ± 0.12	0.42 ± 0.12	0.918	0.06	8.04	0.92 ± 0.04	6	1.14
		σ_I, σ_R^+	0.08 ± 0.15	0.14 ± 0.05	0.88	0.07	5.17	0.96 ± 0.05	6	1.75
		σ_I, σ_R^-	0.50 ± 0.06	0.53 ± 0.06	0.986	0.02	50.92	0.91 ± 0.02	6	1.06
		F, R	0.36 ± 0.07	0.39 ± 0.07	0.978	0.03	21.88	0.90 ± 0.02	5	1.08
3	*Pseudomonas aeruginosa*	σ_I, σ_R	0.43 ± 0.10	0.53 ± 0.11	0.956	0.05	16.19	0.89 ± 0.03	6	1.23
		σ_I, σ_R^+	0.06 ± 0.14	0.17 ± 0.05	0.92	0.06	8.35	0.94 ± 0.05	6	2.83
		σ_I, σ_R^-	0.56 ± 0.1	0.62 ± 0.11	0.966	0.04	20.96	0.87 ± 0.03	6	1.11
		F, R	0.43 ± 0.22	0.56 ± 0.23	0.999	0.009	341.28	0.88 ± 0.007	5	1.3
4	*Proteus miratrilis*	σ_I, σ_R	0.54 ± 0.13	0.53 ± 0.14	0.944	0.06	12.26	0.87 ± 0.04	6	0.98
		σ_I, σ_R^+	0.18 ± 0.19	0.17 ± 0.07	0.883	0.09	5.29	0.91 ± 0.07	6	0.94
		σ_I, σ_R^-	0.69 ± 0.09	0.66 ± 0.09	0.98	0.04	36.34	0.86 ± 0.02	6	0.96
		F, R	0.54 ± 0.80	0.54 ± 0.08	0.984	0.03	31.31	0.85 ± 0.03	5	1
5	*Staphylococcus aureus*	σ_I, σ_R	0.31 ± 0.09	0.37 ± 0.10	0.931	0.04	9.72	0.91 ± 0.03	6	1.19
		σ_I, σ_R^+	0.05 ± 0.12	0.12 ± 0.04	0.889	0.05	5.65	0.95 ± 0.04	6	2.4
		σ_I, σ_R^-	0.41 ± 0.05	0.47 ± 0.05	0.985	0.02	47.3	0.90 ± 0.01	6	1.15
		F, R	0.29 ± 0.19	0.36 ± 0.19	0.998	0.007	216.31	0.90 ± 0.006	5	1.24
6	*Vibrio paraheamolyticus*	σ_I, σ_R	0.42 ± 0.08	0.61 ± 0.08	0.98	0.03	36.82	0.95 ± 0.02	6	1.45
		σ_I, σ_R^+	0.02 ± 0.19	0.18 ± 0.07	0.865	0.09	4.45	0.99 ± 0.07	6	9
		σ_I, σ_R^-	0.55 ± 0.15	0.68 ± 0.16	0.934	0.06	10.3	0.93 ± 0.05	6	1.24
		F, R	0.42 ± 0.03	0.66 ± 0.03	0.998	0.01	314.17	0.95 ± 0.008	5	1.57

Table 4: Results of multiple regression analysis of log IZR (mm) with σ_p, $(\sigma_p^+-\sigma_p)$ and σ_p^o, $(\sigma_p^+-\sigma_p^o)$ constants using Yukava-Tsuno Equation 4.

S. No.	Bacteria	Scale	ρ	r	R	SE	F	N
1	*Aeromonas hydrophila*	σ_p, $(\sigma_p^+-\sigma_p)$	0.45 ± 0.07	0.07 ± 0.01	0.984	0.03	45.17	6
		σ_p^o, $(\sigma_p^+-\sigma_p^o)$	0.41 ± 0.09	0.2 ± 0.9	0.97	0.04	23.99	6
2	*Escherichia coli*	σ_p, $(\sigma_p^+-\sigma_p)$	0.35 ± 0.04	0.07 ± 0.04	0.992	0.02	94.85	6
		σ_p^o, $(\sigma_p^+-\sigma_p^o)$	0.33 ± 0.06	0.17 ± 0.06	0.978	0.03	32.96	6
3	*Pseudomonas aeruginosa*	σ_p, $(\sigma_p^+-\sigma_p)$	0.38 ± 0.04	0.12 ± 0.05	0.993	0.02	110.24	6
		σ_p^o, $(\sigma_p^+-\sigma_p^o)$	0.37 ± 0.03	0.23 ± 0.03	0.996	0.02	173.63	6
4	*Proteus miratrilis*	σ_p, $(\sigma_p^+-\sigma_p)$	0.50 ± 0.04	0.04 ± 0.05	0.994	0.02	122.79	6
		σ_p^o, $(\sigma_p^+-\sigma_p^o)$	0.46 ± 0.07	0.18 ± 0.07	0.983	0.03	44.05	6
5	*Staphylococcus aureus*	σ_p, $(\sigma_p^+-\sigma_p)$	0.29 ± 0.02	0.07 ± 0.02	0.0998	0.008	311.31	6
		σ_p^o, $(\sigma_p^+-\sigma_p^o)$	0.28 ± 0.02	0.15 ± 0.02	0.995	0.01	145.43	6
6	*Vibrio paraheamolyticus*	σ_p, $(\sigma_p^+-\sigma_p)$	0.34 ± 0.04	0.22 ± 0.05	0.993	0.02	115.03	6
		σ_p^o, $(\sigma_p^+-\sigma_p^o)$	0.33 ± 0.02	0.31 ± 0.02	0.998	0.009	556.9	6

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

$$\log IZR=(0.29 \pm 0.02)\sigma_p+(0.07 \pm 0.02)(\sigma_p^+-\sigma_p)+(0.90 \pm 0.06) \quad (4)$$

(R=0.998, SE=0.008, n=6, F=311.31)

CONCLUSION

To summarize, substituted 2-benzylidene-1,3-indandiones have 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 antimicrobial activities of all synthesized compounds have been studied. The inhibition zone radii 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 antimicrobial activity of compounds have been studied.

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