**Substituted 5-benzylidenebarbituric acid.**

**INTRODUCTION**

**MATERIALS AND METHODS**

5-benzylidenebarbituric acid and its *p*-substituted compounds were prepared and their characterization were done using 1H and 13C spectral techniques.

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

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.



Spectral analysis of compounds (1 to 7)

*Compound 1: 5-(4-methoxy-benzylidene)-pyrimidine-2,4,6-trione*

*Compound 2: 5-(4-methyl-benzylidene)-pyrimidine-2,4,6-trione*

*Compound 3: 5-(4-hydroxy-benzylidene)-pyrimidine-2,4,6-trione*

*Compound 4: 5-benzylidene-pyrimidine-2,4,6-trione*

*Compound 5: 5-(4-chloro-benzylidene)-pyrimidine-2,4,6-trione*

*Compound 6: 5-(4-bromo-benzylidene)-pyrimidine-2,4,6-trione*

*Compound 7: 5-(4-nitro-benzylidene)-pyrimidine-2,4,6-trione*

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 hours 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 hours. After the incubation  
period, the diameter of the growth of inhibition zones were measured. The distance between center  
of the well to the edge of the zone was determined to be the inhibition zone radii. 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 this study, 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.

The order of antibacterial activity of compounds (1 to 7) for all the microorganism were ub tge following sequence.

-OCH3 < -CH3 < -OH < -H < -Cl < -Br< -NO2

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)** | **-OCH3** | **-OH** | **-CH3** | **-H** | **-Cl** | **-Br** | **-NO2** |
| 1 | Escherichia coli | 21 | 9 | 10 | 12 | 14 | 16 | 17 | 20 |
| 2 | Klebsiella oxytoca | 16 | 8 | 12 | 14 | 16 | 20 | 24 | 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 acid

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:

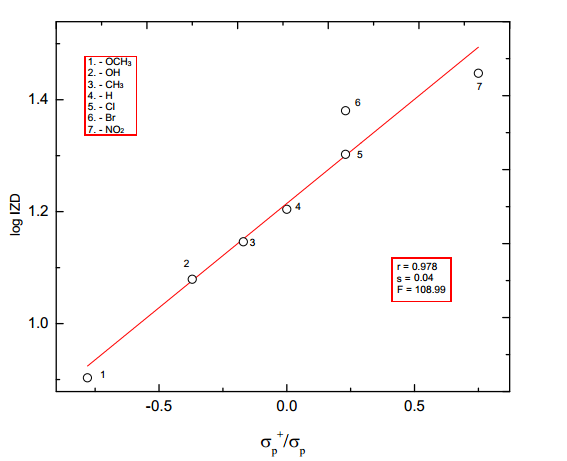
Log (IZD) = (0.37± 0.04) σp+ / σp + (1.21± 0.02) (1)

(r = 0.978, n=7, F = 108.99)

indicates that electron withdrawing substituents increase the antibacterial activity and electron releasing substituents retard it.

**Table 2**: Results of statistical treatment of log IZD (mm) with σp, σpo, σp+, σp+/ σp,σp+/ σp-, σp+/ σp/ σp-substituent constants using single parameter equation

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S.No. | Bacteria | Scale | ρ | r | s | F | Log(IZD)o | n |
| 1 | *Escherichia coli* | σP | 0.31±0.05 | 0.937 | 0.048 | 36.19 | 1.113±0.02 | 7 |
|  | σPo | 0.29±0.09 | 0.854 | 0.07 | 10.78 | 1.10±0.03 | 6 |
|  | σP+ | 0.21±0.02 | 0.969 | 0.034 | 76.36 | 1.159±0.01 | 7 |
|  | σP+/ σP | 0.25±0.02 | 0.977 | 0.03 | 104.92 | 1.135±0.01 | 7 |
|  | σP+/ σP- | 0.16±0.03 | 0.934 | 0.05 | 35.95 | 1.14±0.02 | 7 |
|  | σP+/ σP/ σP- | 0.18±0.03 | 0.929 | 0.05 | 31.38 | 1.12±0.02 | 7 |
| 2 | *Klebsiella oxytoca* | σP | 0.43±0.10 | 0.885 | 0.09 | 18.17 | 1.18±0.04 | 7 |
|  | σPo | 0.44±0.16 | 0.810 | 0.13 | 7.61 | 1.15±0.06 | 6 |
|  | σP+ | 0.29±0.06 | 0.912 | 0.08 | 24.87 | 1.13±0.03 | 7 |
|  | σP+/ σP | 0.37±0.04 | 0.978 | 0.04 | 108.99 | 1.21±0.02 | 7 |
|  | σP+/ σP- | 0.23±0.05 | 0.880 | 0.1 | 17.17 | 1.22±0.04 | 7 |
|  | σP+/ σP/ σP- | 0.27±0.05 | 0.920 | 0.05 | 27.41 | 1.19±0.03 | 7 |
| 3 | *Proteus mirabilis* | σP | 0.44±0.08 | 0.922 | 0.08 | 28.2 | 1.07±0.03 | 7 |
|  | σPo | 0.45±0.13 | 0.868 | 0.1 | 12.26 | 1.03±0.05 | 6 |
|  | σP+ | 0.28±0.06 | 0.911 | 0.08 | 25.5 | 1.13±0.03 | 7 |
|  | σP+/ σP | 0.36±0.05 | 0.959 | 0.06 | 57.12 | 1.1±0.02 | 7 |
|  | σP+/ σP- | 0.22±0.05 | 0.887 | 0.09 | 18.47 | 1.11±0.04 | 7 |
|  | σP+/ σP/ σP- | 0.26±0.05 | 0.915 | 0.08 | 25.73 | 1.08±0.03 | 7 |
| 4 | Pseudomonas aeruginosa | σP | 0.33±0.05 | 0.953 | 0.04 | 49.84 | 1.21±0.02 | 7 |
|  | σPo | 0.33±0.08 | 0.889 | 0.07 | 15.15 | 1.19±0.03 | 6 |
|  | σP+ | 0.21±0.03 | 0.940 | 0.05 | 38.35 | 1.26±0.02 | 7 |
|  | σP+/ σP | 0.26±0.04 | 0.950 | 0.05 | 46.15 | 1.24±0.02 | 7 |
|  | σP+/ σP- | 0.17±0.03 | 0.940 | 0.05 | 37.76 | 1.25±0.02 | 7 |
|  | σP+/ σP/ σP- | 0.19±0.03 | 0.944 | 0.05 | 40.59 | 1.22±0.02 | 7 |
| 5 | Shigella sonnei | σP | 0.35±0.06 | 0.923 | 0.06 | 28.74 | 1.09±0.02 | 7 |
|  | σPo | 0.36±0.11 | 0.860 | 0.09 | 11.32 | 1.06±0.04 | 6 |
|  | σP+ | 0.23±0.04 | 0.920 | 0.06 | 27.4 | 1.14±0.02 | 7 |
|  | σP+/ σP | 0.29±0.03 | 0.965 | 0.04 | 67.55 | 1.11±0.02 | 7 |
|  | σP+/ σP- | 0.18±0.04 | 0.908 | 0.07 | 23.36 | 1.12±0.03 | 7 |
|  | σP+/ σP/ σP- | 0.21±0.04 | 0.940 | 0.06 | 35.65 | 1.09±0.02 | 7 |
| 6 | Staphylococcus aureus | σP | 0.44±0.12 | 0.861 | 0.11 | 14.35 | 1.15±0.04 | 7 |
| σPo | 0.47±0.17 | 0.810 | 0.14 | 7.53 | 1.1±0.07 | 6 |
| σP+ | 0.29±0.07 | 0.873 | 0.1 | 16.09 | 1.21±0.04 | 7 |
| σP+/ σP | 0.39±0.05 | 0.962 | 0.06 | 62.09 | 1.18±0.02 | 7 |
| σP+/ σP- | 0.23±0.06 | 0.862 | 0.11 | 14.49 | 1.19±0.04 | 7 |
| σP+/ σP/ σP+ | 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 (σR, σR+, σR-). 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 ± 0.08) σI  + (0.37±0.08) σR + (1.22 ± 0.04) (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 ρI and ρR 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 ρR values are rather smaller than ρI 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 | ρI | ρR | R | SE | F | Log(IZD)o | n | λ=ρR/ρI |
|
| 1 | Escherichia coli | σI ,σR | 0.24±0.08 | 0.35±0.08 | 0.954 | 0.05 | 15.2 | 1.14±0.03 | 6 | 1.46 |
|  | σI ,σR+ | 0.11±0.20 | 0.15±0.2 | 0.761 | 0.1 | 2.75 | 1.16±0.09 | 7 | 1.36 |
|  | σI ,σRo | 0.28±0.18 | 0.15±0.17 | 0.722 | 0.1 | 1.64 | 1.10±0.07 | 6 | 0.54 |
|  | σI ,σR- | 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 | σI ,σR | 0.36±0.15 | 0.55±0.16 | 0.931 | 0.09 | 9.81 | 1.21±0.07 | 6 | 1.53 |
|  | σI ,σR+ | 0.17±0.29 | 0.21±0.14 | 0.757 | 0.15 | 2.69 | 1.25±0.14 | 7 | 1.24 |
|  | σI ,σRo | 0.42±0.30 | 0.2±0.29 | 0.663 | 0.18 | 1.18 | 1.14±0.13 | 6 | 0.48 |
|  | σI ,σR- | 0.34±0.28 | 0.28±0.23 | 0.747 | 0.17 | 1.89 | 1.16±0.11 | 6 | 0.82 |
|  | F,R | 0.37±0.19 | 0.45±0.15 | 0.885 | 0.1 | 7.23 | 1.20±0.08 | 7 | 1.22 |
|  |  |  |  |  |  |  |  |  |  |
| 3 | Proteus mirabilis | σI ,σR | 0.45±0.13 | 0.44±0.14 | 0.940 | 0.08 | 11.40 | 1.06±0.06 | 6 | 0.98 |
|  | σI ,σR+ | 0.31±0.26 | 0.17±0.12 | 0.801 | 0.13 | 03.60 | 1.08±0.13 | 7 | 0.55 |
|  | σI ,σRo | 0.51±0.25 | 0.14±0.24 | 0.763 | 0.16 | 02.09 | 0.996±0.1 | 6 | 0.28 |
|  | σI ,σR- | 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 | σI ,σR | 0.33±0.08 | 0.37±0.08 | 0.963 | 0.05 | 19.09 | 1.22±0.04 | 6 | 1.12 |
|  | σI ,σR+ | 0.22±0.21 | 0.13±0.10 | 0.777 | 0.11 | 03.06 | 1.23±0.10 | 7 | 0.59 |
|  | σI ,σRo | 0.37±0.19 | 0.15±0.19 | 0.767 | 0.12 | 02.15 | 1.17±0.08 | 6 | 0.41 |
|  | σI ,σR- | 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 | σI ,σR | 0.33±0.01 | 0.39±0.1 | 0.950 | 0.06 | 13.88 | 1.09±0.04 | 6 | 1.18 |
|  | σI ,σR+ | 0.21±0.22 | 0.15±0.1 | 0.782 | 0.11 | 03.15 | 1.11±0.10 | 7 | 0.71 |
|  | σI ,σRo | 0.38±0.22 | 0.14±0.2 | 0.731 | 0.13 | 01.72 | 1.04±0.09 | 6 | 0.37 |
|  | σI ,σR- | 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 albus | σI ,σR | 0.38±0.15 | 0.61±0.16 | 0.940 | 0.09 | 11.34 | 1.18±0.07 | 6 | 1.61 |
|  | σI ,σR+ | 0.19±0.32 | 0.22±0.15 | 0.745 | 0.16 | 2.49 | 1.20±0.15 | 7 | 1.16 |
|  | σI ,σRo | 0..45±0.33 | 0.23±0.32 | 0.650 | 0.2 | 1.14 | 1.09±0.14 | 6 | 0.51 |
|  | σI ,σR- | 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 |

**Table 4:** Results of multiple regression analysis of log IZR (mm) with σp,( σp+- σp)  and σpo,

(σp+- σpo) constants using Yukava – Tsuno equation (4).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S.No. | Bacteria | scale | ρ | r | R | SE | F | n |
| 1 | *Escherichia coli* | σp,( σp+- σp) | 0.205±0.06 | 0.279±0.11 | 0.975 | 0.04 | 28.35 | 6 |
|  |  | σpo,( σp+- σpo) | 0.171±0.05 | 0.211±0.05 | 0.981 | 0.03 | 38.16 | 6 |
| 2 | *Klebsiella oxytoca* | σp,( σp+- σp) | 0.287±0.12 | 0.494±0.23 | 0.956 | 0.07 | 16.09 | 6 |
|  |  | σpo,( σp+- σpo) | 0.236±0.11 | 0.353±0.12 | 0.957 | 0.07 | 16.21 | 6 |
| 3 | *Proteus mirabilis* | σp,( σp+- σp) | 0.386±0.14 | 0.237±0.27 | 0.938 | 0.08 | 11.02 | 6 |
|  |  | σpo,( σp+- σpo) | 0.316±0.13 | 0.243±0.13 | 0.841 | 0.08 | 11.55 | 6 |
| 4 | *Pseudomonas aeruginosa* | σp,( σp+- σp) | 0.294±0.08 | 0.133±0.15 | 0.959 | 0.05 | 16.95 | 6 |
|  |  | σpo,( σp+- σpo) | 0.242±0.09 | 0.15±0.09 | 0.945 | 0.06 | 12.42 | 6 |
| 5 | *Shigella sonnei* | σp,( σp+- σp) | 0.294±0.1 | 0.239±0.19 | 0.954 | 0.06 | 15.04 | 6 |
|  |  | σpo,( σp+- σpo) | 0.239±0.09 | 0.215±0.1 | 0.948 | 0.06 | 12.24 | 6 |
| 6 | *Staphylococcus albus* | σp,( σp+- σp) | 0.326±0.14 | 0.504±0.26 | 0.953 | 0.08 | 15.01 | 6 |
|  |  | σpo,( σp+- σpo) | 0.272±0.14 | 0.637±0.26 | 0.942 | 0.09 | 11.74 | 6 |
|  |  |  |  |  |  |  |  |  |

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

log IZD = (0.171±0.05) σpo + (0.211 ± 0.05) (σp+ - σpo) + (1.18 ± 0.02) (4)

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

CONCLUSION