SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SOME NEW AZO DYES OF 4-HYDROXY BENZOIC ACID.

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Abstract : In the present study azo dyes compounds of 4-hydroxy benzoic acid were prepared from the reaction of seven different substituted aromatic amines and 4-hydroxy benzoic acid by using diazotization reaction. The structures of all the newly synthesized compounds were characterized by NMR and IR spectroscopy. The antimicrobial activity of compounds was evaluated against both gram-positive and gram-negative bacteria by disc diffusion menthod. All the compounds were found to active against all tested bacteria.

Keywords - Azo dyes, 4-hydroxy benzoic acid and antimicrobial activity.

I. INTRODUCTION

Compounds containing azo groups are having great interest because of having variety of application like organic dye[1], radical reaction intiator[2], indicators[3] and therapeutic agents[4]. Azo dyes compounds used for dyeing wool, leather and synthetic material and also used in photonic devices. Many azo- azomethine compounds were studied as ion selective electrodes and sensor for metal ions[5, 6]. Biological studies of 3,4-diamino-1,2,5-oxadiazole shows significant antimicrobial activity and also shows active against cancer treatment, angiogenesis, inflammatory diseases and neurodegenerative studies[7, 8].
In the present study some azo dyes of 4-hydroxy bezoic acid were synthesized by diazotization reaction, in which 4-hydroxy benzoic acid was coupled with diazonium salt of eight different aromatic amines VIZ: Aniline, o-Nitro aniline, p-Toluedine, α-Naphthylamine, Sulphanilic acid, m-Nitro aniline, Benzedine and Anthranilic acid.

II. MATERIAL AND METHODS

All the chemicals used in these experiments were of analytical grade. All the melting points were determined by open capillary method and are uncorrected. The products were confirmed by ^{1}H NMR (Burkeravernce II 400 NMR Specrtometer) and IR technique (Shimatzu). The biological activity was evaluated against two kinds of bacteria gram positive and gram negative. The products were recrystallized by ethanol as solvent.
Synthesis[9,10]: Substituted aromatic amines (0.01mole) were mixed with 2.5 ml conc. HCl and 2.5 ml (4N) cold solution of $NaNO_2$ was added with the stirring. The temperature of the reaction was maintained up to 0-5^0 C. Diazonium salt solution prepared above was added drop wise to the alkaline solution of 4-hydroxy benzoic acid. The reaction mixture stirred for 10–20 minutes maintaining the temperature 5-10^0 C. The colored product so obtained is filtered washed with water and recrystallized from 80% ethanol. Scheme for the synthesis is shown in figure (I).

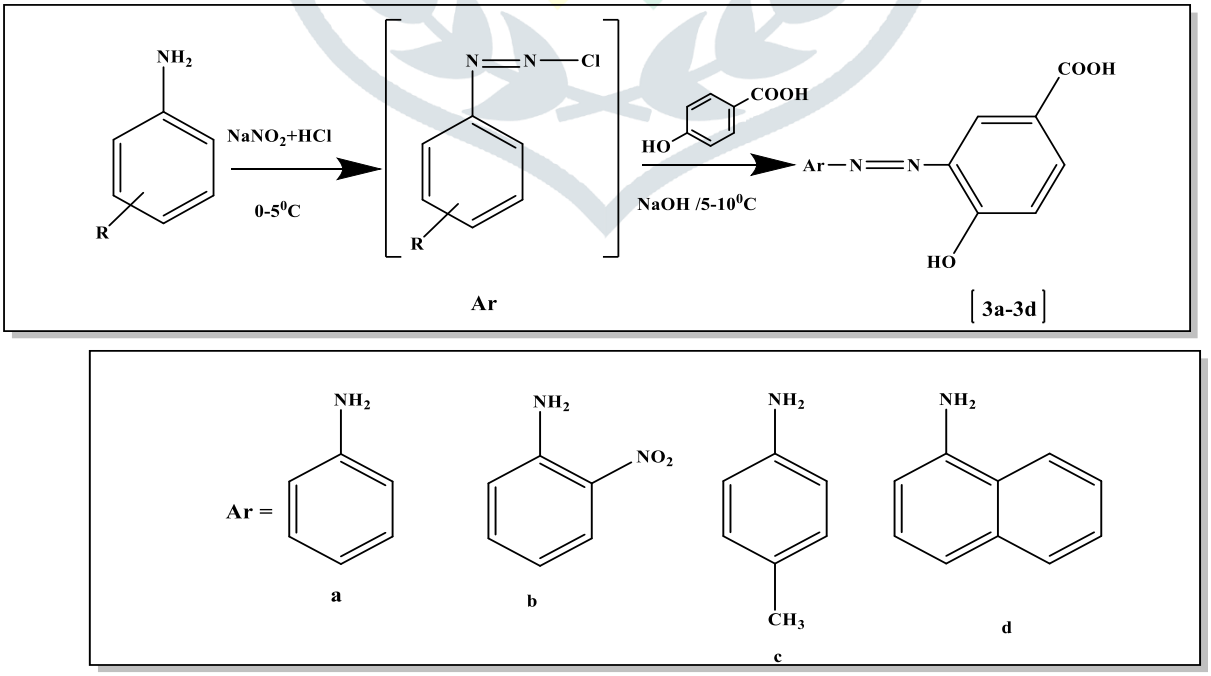


Figure (I): Scheme for the synthesis of 4-hydroxy bezoic acid azo dyes.

Table (I): The code, compound name, molecular formula, molecular weight, melting point and percentage yield of synthesized 4-hydroxy-3-(phenyldiazenyl)bezoic acid compounds.

Sr. No.	Structure	Molecular Formula	Molecular Weight	Melting Point	Yield
3a	4-hydroxy-3-(phenyldiazenyl)benzoic acid.	$C_{13}H_{10}N_2O_3$	242	285^0 C	56%
3b	4-hydroxy-3-(2-nitrophenyl)phenyldiazenyl)benzoic acid.	$C_{13}H_9N_3O_5$	287	142^0 C	66%
3c	4-hydroxy-3-*p*-tolyldiazenyl)benzoic acid	$C_{14}H_{12}N_2O_3$	256	179^0 C	68%
3d	4-hydroxy-3-naphthalen-1-yldiazenyl)benzoic acid	$C_{17}H_{12}N_2O_3$	292	167^0 C	55%

Antimicrobial activity: The synthesized compounds 3a-3d were analyzed for their antimicrobial activity against four gram positive and gram negative bacteria viz. *Escherichia coli, Staphylococcus aureus, Pseudomanasaeroginosa and Salmonella typhi*by using agar well diffusion method[11]. These compounds were mixed in Ethanol to form the solution of concentration 1mg/ml. sterile disc were dipped in the solutions, dried it and placed on the nutrient agar medium spread with the bacteria. The plates were further incubated for 24 to 48 hours at 37^0 C and the diameter of zones of inhibition was measured in millimeter.

III. RESULT AND DISCUSSION

The synthesized azo dyes compounds of 4-hydroxy bezoicacid were characterized by IR and ^{1}H-NMR spectroscopic techniques. The IR and ^{1}H-NMR spectra showed the expected signals for the corresponding groups in each compound. The spectral values for different compounds were shown in table below (II).

Table (II): FTIR and ^{1}H-NMR spectral data 4-hydroxy bezoic acid azo dyes compounds.

Compound	Spectra	Spectroscopic Data
SDB 3a	IR (KBr. cm^{-1})	3062 (Phenolic –OH stretch), 2821 (Carboxylic acid O-H stretch), 1490 (C=C Ring stretch), 1589 (N=N stretch), 1278 (C-N stretch), 1762 (C=O stretch of –COOH).
	NMR (δ ppm)	10.63 (s 1H of –COOH), 6.93 (s 1H of –OH), 7.18-7.90 (m 8H of Ar-H).
SDB 3b	IR (KBr. cm^{-1})	3377 (phenolic –OH stretch), 1485 (C=C Aromatic), 1577 (N=N), 3086 (N-H stretch), 1348 (C-N Stretch), 2808 (Carboxylic acid O-H stretch), 1820 (C=O stretch of –COOH), 1523 (-NO_2 stretch (N-O Asym), 1348 (-NO_2 stretch (N-O sym).
	NMR (δ ppm)	8.01 (s 1H of –COOH), 6.84 (s 1H of –OH), 7.00-7.99 (m 7H of Ar-H).
SDB 3c	IR (KBr. cm^{-1})	3203 (phenolic –OH stretch), 1494 (C=C Aromatic), 1600 (N=N), 3026 (N-H stretch), 1242 (C-N Stretch), 2864 (Carboxylic acid O-H stretch), 1770 (C=O stretch of –COOH), 2916 (C-H of CH_3 stretch).
	NMR (δ ppm)	8.18 (s 1H of –COOH), 6.96 (s 1H of –OH), 2.29 (s 3H of –CH_3), 7.17-7.91 (m 7H of Ar-H).
SDB 3d	IR (KBr. cm^{-1})	3313 (phenolic –OH stretch), 1496 (C=C Aromatic), 1591 (N=N), 3053 (N-H stretch), 1274 (C-N Stretch), 2806 (Carboxylic acid O-H stretch).
	NMR (δ ppm)	8.93 (s 1H of –COOH), 7.10 (s 1H of –OH), 7.19-8.22 (m 10H of Ar-H).

Antimicrobial activity: A total four 4-hydroxy-3-(phenyldiazenyl)bezoic acid azo dyes compounds have been synthesized, recrystallized and used separately for antimicrobial activity two gram positive and gram negative bacteria viz.*Escherichia coli, Staphylococcus aureus, Pseudomonas aeroginosa* and *Salmonella typhi*. The results of antimicrobial activity of these compounds were shown in table III-VI.

Table (III): Effect of compounds 3a-3d on growth response of *Escherichia coli.*

Conc.(mg/ml)	3a	3b	3c	3d
0.5	I (>10)	I (16)	I (14)	I (26)
1.0	I (10)	I (12)	I (19)	I (20)
1.5	I (11)	I (27)	I (16)	I (17)
2.0	I (11)	I (24)	I (15)	I (19)
2.5	I (12)	I (22)	I (23)	I (22)
3.0	I (10)	I (23)	I (16)	I (19)

I = Inhibition, values of inhibition are given in parenthesis, NI = No inhibition

Table (IV): Effect of compounds 3a-3d on growth response of *Staphylococcus aureus.*

Conc.(mg/ml)	3a	3b	3c	3d
0.5	I (10)	I (>10)	I (13)	I (>10)
1.0	I (10)	I (>10)	NI	I (10)
1.5	I (>10)	I (12)	I (>10)	I (11)
2.0	I (10)	I (15)	I (11)	I (10)
2.5	I (>10)	I (10)	I (15)	I (>10)
3.0	I (10)	I (11)	I (12)	I (11)

I = Inhibition, values of inhibition are given in parenthesis, NI = No inhibition

Table (V): Effect of compounds 3a-3d on growth response of *Pseudomonas aeroginosa.*

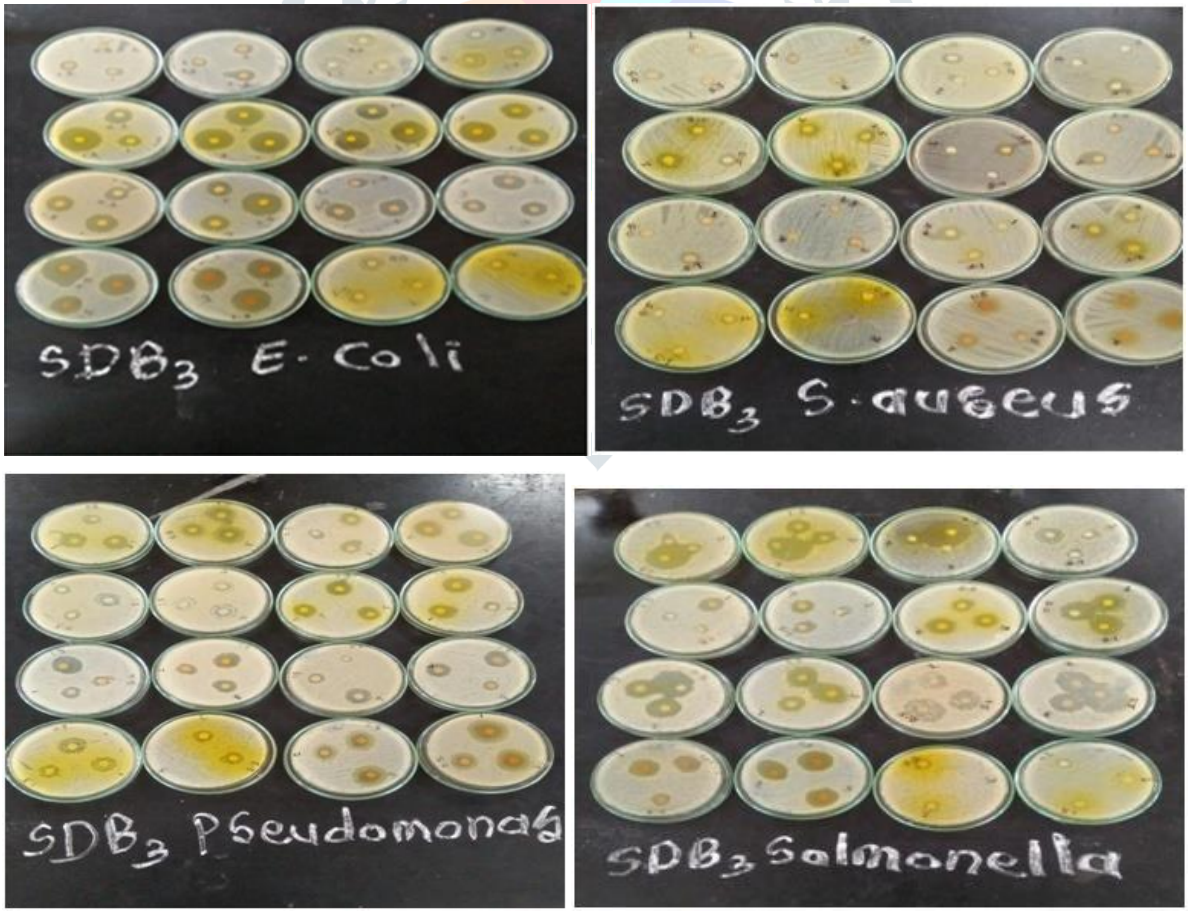
Conc.(mg/ml)	3a	3b	3c	3d
0.5	I (11)	I (10)	I (10)	I (12)
1.0	I (16)	I (11)	I (13)	I (15)
1.5	I (14)	I (16)	I (12)	I (13)
2.0	I (17)	I (11)	I (15)	I (11)
2.5	I (13)	I (16)	I (16)	I (12)
3.0	I (14)	I (13)	I (13)	I (16)

I = Inhibition, values of inhibition are given in parenthesis, NI = No inhibition

Table (VI): Effect of compounds 3a-3d on growth response of *Salmonella typhi.*

Conc.(mg/ml)	3a	3b	3c	3d
0.5	I (10)	I (>10)	I (15)	I (11)
1.0	I (10)	I (15)	I (19)	I (16)
1.5	I (24)	I (>10)	I (18)	I (15)
2.0	I (13)	I (10)	I (15)	I (16)
2.5	I (14)	I (11)	I (18)	I (19)
3.0	I (15)	I (13)	I (18)	I (18)

I = Inhibition, values of inhibition are given in parenthesis, NI = No inhibition



IV. CONCLUSION

All the four azo compounds 3a–3d were successfully synthesized in good yield and their structures are confirmed using elemental analysis, FTIR &^{1}HNMR. The results on antimicrobial activity reveal that all the eight newly synthesized compounds viz 3a–d

found to have outstanding antibacterial effect against *E.Coli*, *S. aureus*, *Pseudomonas aeroginosa*, and *Salmonella typhi*nearly at all the concentrations analysed. The results revealed, the broad spectrum potential of all the compounds in inhibiting the growth of human pathogens, and this finding enlighten the possible help in drug discovery.

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