



Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis of 4-hydroxybenzoic acid incorporated azo dyes derivatives as potent biological activity molecules

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ABSTRACT

Synthesis of most azo compounds involves diazotization of a primary aromatic amine, followed by coupling with one or more nucleophiles. Thus, benzoic, phenolic, salicylic and naphtholic compounds acts as nucleophiles and undergoes coupling reactions. In this study, a series of azo compounds were synthesized in excellent yields via the diazotization of different aromatic amines followed by coupling with 4-Hydroxybenzoic acid, which is a phenol as well as carboxylic acid. These compounds were characterized by elemental analysis, IR, ¹HNMR and MASS spectroscopic techniques. The synthesized compounds have been tested in vitro against human pathogens in order to assess their antibacterial potential using disk diffusion method. The compounds analysed for its antibacterial action were found to be potent antibacterial agents at all concentrations against all the tested pathogenic cultures.

Keywords: 4-Hydroxybenzoic acid, Azo compounds, Antibacterial Activity, Human pathogens

INTRODUCTION

Azo dyes are the most important, largest and versatile class of synthetic organic compounds with an enormous variety of applications in science and technology owing to their versatility in various fields [1-2].

A survey of the pertinent literature reveals that azo dyes have been found to successfully employed as LCD colour filters [3], chromophoric substrate for redox enzymes [4], optical switches [5], chemical sensors [6], textile dyes [7], lasers [8], optical data storage [9], non-linear optics [10], and in specialized applications, such as food, drug, cosmetic, photochemical production,[11-14] and have advanced applications in organic synthesis [15].

In addition, they have been studied generally because of their outstanding thermal and optical properties in purpose such as toner [16-17], ink-jet printing [18-19]. Azo derivatives are potent biologic agents [20-21], also well known for their medicinal importance and are recognized for their application as antidiabetics [22], antiseptics [23], antitumor [24-26], anti-inflammatory [27-28], anti-tuberculous [29], anti-neoplastics [39], antihistamines [31], insecticides [32] and production of drugs in chemotherapy [33] In this regard, a number of studies have been devoted to the characterization, purification and application of azo dyes derived from thymol [34], aspirin [35], paracetamol [36], m-cresol [37], resorcinol [38] moieties as excellent antimicrobial agents.

As fewer reports on biological activity of azo dye moiety, in view of the above mentioned findings and our previous reports about azo derivatives of vanillin and tyrosine moieties [39- 40] in the present study, we have made an efficient attempt to synthesize azo dyes containing 4-Hydroxybenzoic acid moiety, possessing a potent biological activity. We have synthesized eight azo compounds namely 4a to 4h and characterized by FTIR, ¹HNMR and MASS spectral technique. The antibacterial activities of the synthesized azo compounds were reported in vitro using disc diffusion method.

EXPERIMENTAL SECTION

2.1 Material and Methods:

The chemicals used in the present studies are of synthetic grade, Merck company Ltd. The products were characterized by IR, ¹HNMR and MASS spectral studies. The M.P.s. were determined by open capillary method using digital melting point apparatus model 935/934 by Electronics India and is uncorrected. The IR spectra were recorded on FTIR Spectrophotometer Model RZX (Perkin-Elmer) in the form of KBr pallet. ¹HNMR spectra were recorded in CDCl₃ on a FT-NMR Cryomagnet Spectrometer 400 MHz (Bruker) using TMS as an internal standard and MASS spectra were recorded on LC-MS Spectrometer Model Q-ToF Micro Waters. The purity of compounds was checked by TLC. The crude products were recrystallised from 85% ethanol.

2.2 Experimental procedure for synthesis of azo compounds [41-43]:

Substituted aromatic amines (0.01mole) were mixed with 2.5 ml conc. HCl and 2.5 ml (4N) cold solution of NaNO₂ was added with the stirring. The temperature of the reaction was maintained up to 0–5⁰ C. Diazonium salt solution prepared above was added drop wise to the alkaline 10% NaOH solution of 4-Hydroxybenzoic acid (0.01mole). The reaction mixture stirred for 20 – 40-minutes maintaining the temperature 5 – 10⁰ C. The coloured products obtained is filtered, washed with water and recrystallised from 85% ethanol. The general reaction scheme for synthesis of azo compounds of 4-Hydroxybenzoic acid is shown in figure-(1). Also code, chemical name, molecular formulae, molecular weights, melting points and percentage yield of synthesized azo compounds of 4-Hydroxybenzoic acid are shown in table-(1).

Figure-(1): The general reaction scheme for synthesis of azo compounds of 4-Hydroxy benzoic acid

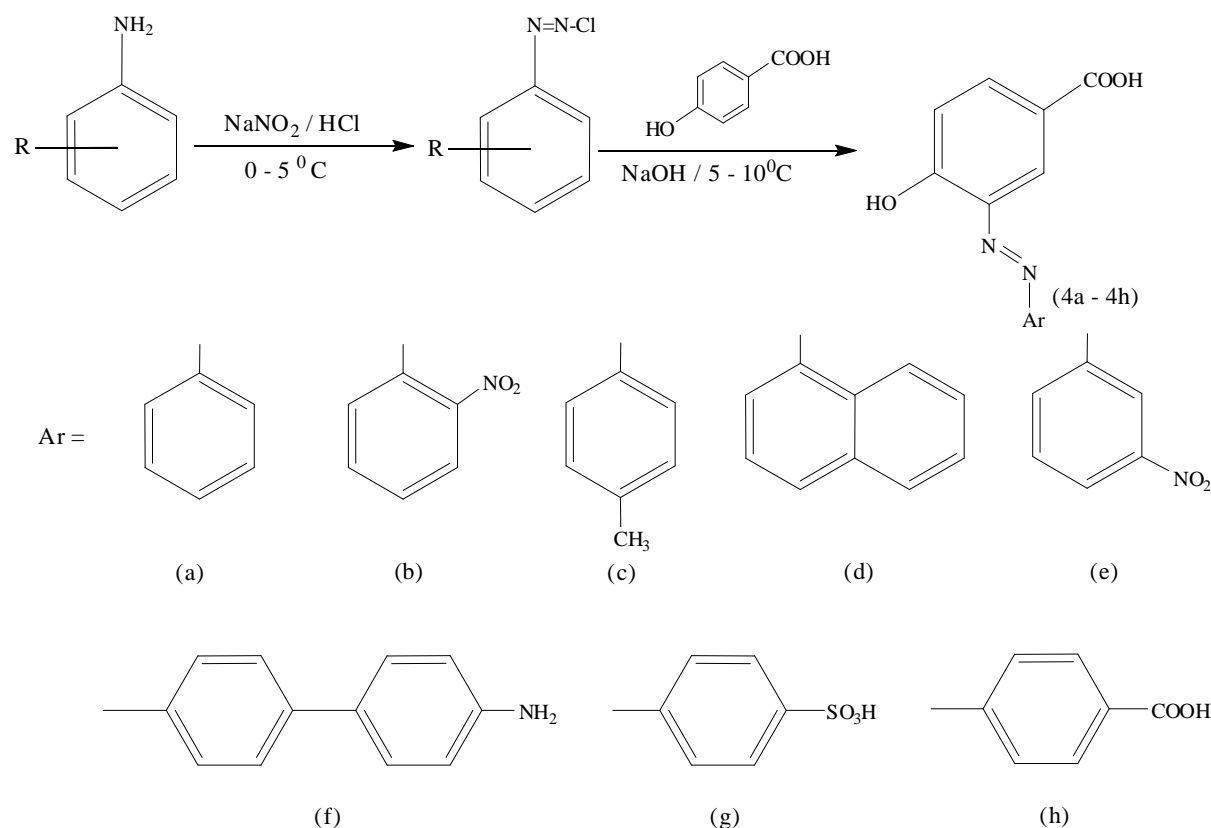


Table-(1)

Code	Chemical Name of Compound	Molecular Formulae	Mol. Wt.	M.P. °C	% Yield
4a	4-hydroxy-3-[(E)-phenyldiazenyl] benzoic acid	C ₁₃ H ₁₀ N ₂ O ₃	242.23	288 – 290	89
4b	4-hydroxy-3-[(E)-(2-nitrophenyl)diazenyl] benzoic acid	C ₁₃ H ₉ N ₃ O ₅	287.23	143 – 145	83
4c	4-hydroxy-3-[(E)-(4-methylphenyl) diazenyl] benzoic acid	C ₁₄ H ₁₂ N ₂ O ₃	256.26	183 – 185	75
4d	4-hydroxy-3-[(E)-naphthalen-1-yl)diazenyl] benzoic acid.	C ₁₇ H ₁₂ N ₂ O ₃	292.29	167 – 169	69
4e	4-hydroxy-3-[(E)-(3-nitrophenyl)diazenyl] benzoic acid	C ₁₃ H ₉ N ₃ O ₅	287.23	129 – 131	84
4f	3-[(E)-(4'-aminobiphenyl-4-yl)diazenyl]-4-hydroxy benzoic acid	C ₁₉ H ₁₅ N ₃ O ₃	333.34	148 – 150	77
4g	4-hydroxy-3-[(E)-(4-sulfophenyl)diazenyl] benzoic acid	C ₁₃ H ₁₀ N ₂ O ₆ S	322.29	166 – 168	82
4h	3-[(E)-(4-carboxyphenyl)diazenyl]-4-hydroxy benzoic acid	C ₁₄ H ₁₀ N ₂ O ₅	286.24	188 – 190	63

2.3 Antimicrobial activity:

The compounds 4a – 4h were screened for the presence of antibacterial constituents against four micro-organisms viz., *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*, adopting disc diffusion method [44, 45]. All the bacterial cultures were obtained from NCL reference laboratory, Pune. The compounds were dissolved in ethanol to give 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/mL solutions. Sterile discs were dipped in solutions, dried and placed on nutrient agar plates spread with the bacteria. The plates were further incubated for 24 hrs at 37°C and the zones of inhibition were measured using antibiotic zone reader (Hi- Media).

RESULTS AND DISCUSSION

3.1 Spectroscopic study:

I.R., ¹HNMR and MASS spectra showed the expected signals / peaks corresponding to various groups present in each of the compounds. The I.R. ¹HNMR and MASS spectral data are shown in Table (2).

Compound	Spectra	Spectroscopic Signals or peaks data
4a	IR (KBr, cm ⁻¹) H ¹ NMR (δ ppm) MASS (m/z, %)	3449 (Phenolic O–H Stretch), 3018 (C–H Stretch aromatic), 2822 (Carboxylic acid O – H Stretch), 1659 (C=O Stretch of –COOH), 1589 (N=N Stretch), 1515 (C=C Aromatic ring stretch), 1282 (C–N Stretch), 1174 (C–O phenol stretch), 769 (C–H def aromatic). 11.14 (s 1H of –COOH), 6.88 to 7.97 (m 8H of Ar–H), 5.32 (s 1H of –OH). LC-MS- 241.1 (M ⁺ , 3.5), 197.1 (HOC ₆ H ₃ N=NC ₆ H ₅ , 100), 93.1 (C ₆ H ₅ OH, 10.22).
4b	IR (KBr, cm ⁻¹) H ¹ NMR (δ ppm) MASS (m/z, %)	3470 (Phenolic O–H Stretch), 3027 (C–H Stretch aromatic), 2825 (Carboxylic acid O–H Stretch), 1657 (C=O Stretch of –COOH), 1589 (N=N Stretch), 1518 (C=C Aromatic ring stretch), 1285 (C–N Stretch), 1172 (C–O phenol stretch), 769 (C–H def aromatic), 1423 (–NO ₂ Stretch, N–O asym), 1320 (–NO ₂ Stretch, N–O sym). 12.14 (s 1H of –COOH), 6.94 to 8.01 (m 7H of Ar–H), 5.14 (s 1H of –OH). LC-MS: 287.0 (M ⁺ , 5.86), 242.0 (HOC ₆ H ₃ N =N C ₆ H ₄ NO ₂ , 100), 196.1 (HOC ₆ H ₃ N=NC ₆ H ₄ , 40), 104.0 (N=NC ₆ H ₄), 92.1 (C ₆ H ₅ OH, 4.3)
4c	IR (KBr, cm ⁻¹) H ¹ NMR (δ ppm) MASS (m/z, %)	3390 (Phenolic O–H Stretch), 2980 (C–H Stretch aromatic), 2833 (C–H stretch aliphatic (–CH ₃), 3202 (Carboxylic acid O–H Stretch), 1676 (C=O Stretch of –COOH), 1595 (N=N Stretch), 1509 (C=C Aromatic ring stretch), 1244 (C–N Stretch), 1168 (C–O phenol stretch), 769 (C–H def aromatic). 3.47 (s 3H of –CH ₃), 5.14 (s 1H of –OH), 6.11 to 7.26 (m 7H of Ar – H), 9.18 (s 1H of –COOH). LC-MS: 255.2 (M ⁺ , 11.3), 137.1 (HOCOOHC ₆ H ₃ , 100), 118.9 (CH ₃ C ₆ H ₄ N=N, 23.0), 92.1 (C ₆ H ₅ OH, 8.1).
4d	IR (KBr, cm ⁻¹) H ¹ NMR (δ ppm) MASS (m/z, %)	3448 (Phenolic O–H Stretch), 3016 (C–H Stretch aromatic), 2823 (Carboxylic acid O–H Stretch), 1661 (C=O Stretch of –COOH), 1590 (N=N Stretch), 1514 (C=C Aromatic ring stretch), 1282 (C–N Stretch), 1173 (C–O phenol stretch), 769 (C–H def aromatic). 5.74 (s 1H – OH), 6.8 to 8.88 (m 10H of Ar – H), δ 9.47 (s 1H –COOH). LC-MS: 292.2 (M ⁺ , 16.64), 154.9 (N=NC ₁₀ H ₇ , 21.99), 137.1 (HOCOOHC ₆ H ₃ , 100), 92.1 (C ₆ H ₅ OH, 6.65).
4e	IR (KBr, cm ⁻¹) H ¹ NMR (δ ppm) MASS (m/z, %)	3395 (Phenolic O–H Stretch), 3081 (C–H Stretch aromatic), 2668 (Carboxylic acid O–H Stretch), 1676 (C=O Stretch of –COOH), 1595 (N=N Stretch), 1526 (C=C Aromatic ring stretch), 1245 (C–N Stretch), 1169 (C–O phenol stretch), 770 (C–H def aromatic), 1423 (–NO ₂ Stretch, N–O asym), 1317 (–NO ₂ Stretch, N–O sym). 5.35 (s 1H – OH), 6.96 to 8.70 (m 7H of Ar – H), 12.85 (s 1H –COOH). LC-MS: 286.1 (M ⁺ , 1.53), 242.0 (HOC ₆ H ₃ N=NC ₆ H ₄ NO ₂ , 100), 104.0 (N=N C ₆ H ₄ NO ₂ , 10.46), 92.1 (C ₆ H ₅ OH, 6.5).
4f	IR (KBr, cm ⁻¹) H ¹ NMR (δ ppm) MASS (m/z, %)	3450 (Phenolic O–H Stretch), 3097 (C–H Stretch aromatic), 2823 (Carboxylic acid O–H Stretch), 1662 (C=O Stretch of –COOH), 1590 (N=N Stretch), 1514 (C=C Aromatic ring stretch), 1283 (C–N Stretch), 1173 (C–O phenol stretch), 770 (C–H def aromatic), 850 (C – C Stretch aromatic). 3.49 (s 2H of –NH ₂), 5.17 (s 1H – OH), 6.26 to 8.01 (m 14H of Ar – H), 11.15 (s 1H –COOH). LC-MS: 333.1 (M ⁺ , 22.95), 195.0 (N=NC ₆ H ₄ C ₆ H ₄ NH ₂ , 4.9), 153.1 (C ₆ H ₄ C ₆ H ₄ , 22.1), 137.0 (HOCC ₆ H ₅ OH, 100), 92.1 (C ₆ H ₅ OH, 6.5).
4g	IR (KBr, cm ⁻¹) H ¹ NMR (δ ppm) MASS (m/z, %)	3390 (Phenolic O–H Stretch), 2980 (C–H Stretch aromatic), 2829 (Carboxylic acid O–H Stretch), 1675 (C=O Stretch of –COOH), 1594 (N=N Stretch), 1510 (C=C Aromatic ring stretch), 1244 (C–N Stretch), 1169 (C–O phenol stretch), 769 (C–H def aromatic), 1317 (S=O Sulfonic acid stretch), 619 (C–S Stretch), 854 (S – O Stretch). 5.37 (s 1H of – OH), 2.5 (s 1H of –SO ₃ H), 6.79 to 8.24 (m 7H of Ar – H), 9.90 (s 1H of –COOH). LC-MS: 321.0 (M ⁺ , 18.18), 277.0 (HOC ₆ H ₃ N=NC ₆ H ₄ SO ₃ H, 100) 196.2 (HOC ₆ H ₃ N=N C ₆ H ₄ , 22.49), 92.1 (C ₆ H ₅ OH, 4.6).
4h	IR (KBr, cm ⁻¹) H ¹ NMR (δ ppm) MASS (m/z, %)	3450 (Phenolic O–H Stretch), 3020 (C–H Stretch aromatic), 2823 (Carboxylic acid O–H Stretch), 1663 (C=O Stretch of –COOH), 1591 (N=N Stretch), 1515 (C=C Aromatic ring stretch), 1282 (C–N Stretch), 1173 (C–O phenol stretch), 770 (C–H def aromatic). 5.35 (s 1H of – OH), 6.79 to 8.12 (m 7H of Ar – H), 9.95 (s H of –COOH), 11.24 (s H of –COOH). LC-MS: 285.1 (M ⁺ , 1.7), 241.0 (HOCHOC ₆ H ₃ N=NC ₆ H ₄ , 100), 137.0 (HOOC HOC ₆ H ₄ , 44.0), 92.1 (C ₆ H ₅ OH, 4.6).

3.2 Antibacterial Evaluation:

A total eight azo compounds of 4-Hydroxybenzoic acid have been synthesized, recrystallised and six different concentrations of each compound were prepared and further used individually to analyze its antibacterial activity against four human pathogens viz. *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*. The data on antimicrobial activity of azo compounds of 4-Hydroxybenzoic acid 4a – 4h against four human pathogens are presented in table-(3) to table (6). From the results it was observed that the azo compounds of 4-Hydroxybenzoic acid have showed enormous antibacterial potential against all four pathogens.

Antibacterial properties of the synthesized azo compounds of 4-Hydroxybenzoic acid viz 4a – 4h [Zone of inhibition (mm)]

Table (3):- Effect of azo compounds of 4-Hydroxybenzoic acid viz. 4a – 4h on the growth response of *Escherichia coli*

Conc. (mg/mL)	4a	4b	4c	4d	4e	4f	4g	4h
0.5	I (10)	I (15)	I (18)	I (12)	I (12)	I (16)	I (10)	I (16)
1.0	I (10)	I (16)	I (14)	I (14)	I (12)	I (12)	I (10)	I (10)
1.5	I (12)	I (18)	I (10)	I (16)	I (12)	I (16)	I (12)	I (10)
2.0	I (11)	I (16)	I (10)	I (12)	I (16)	I (18)	I (10)	I (10)
2.5	I (10)	I (18)	I (18)	I (10)	I (12)	I (18)	NI	I (12)
3.0	I (10)	I (17)	I (15)	I (12)	I (14)	I (11)	I (12)	I (13)

I = Inhibition, values of inhibition are given in parenthesis, NI = Not inhibition

Table (4):-Effect of azo compounds of 4-Hydroxybenzoic acid viz. 4a – 4h on the growth response of *Staphylococcus aureus*

Conc. (mg/mL)	4a	4b	4c	4d	4e	4f	4g	4h
0.5	I (10)	I (12)	I (10)	NI	I (10)	I (10)	I (10)	I (10)
1.0	I (10)	I (16)	I (10)	I (10)	I (12)	I (10)	NI	I (11)
1.5	I (12)	I (10)	I (16)	I (10)	I (10)	NI	I (12)	I (13)
2.0	NI	I (16)	I (18)	I (12)	I (14)	I (12)	I (10)	I (10)
2.5	I (10)	I (18)	I (14)	I (16)	I (12)	I (14)	I (10)	I (10)
3.0	I (10)	I (22)	I (14)	I (12)	I (14)	I (18)	I (10)	I (10)

I = Inhibition, values of inhibition are given in parenthesis, NI = Not inhibition

Table (5):-Effect of azo compounds of 4-Hydroxybenzoic acid viz. 4a – 4h on the growth response of *Pseudomonas aeruginosa*

Conc. (mg/mL)	4a	4b	4c	4d	4e	4f	4g	4h
0.5	NI	I (16)	I (16)	I (12)	I (12)	I (14)	I (10)	I (14)
1.0	I (10)	I (16)	I (18)	I (12)	I (14)	I (12)	I (10)	I (10)
1.5	I (10)	I (16)	I (18)	I (14)	I (16)	I (18)	I (10)	I (10)
2.0	I (10)	I (18)	I (10)	I (11)	I (14)	I (16)	I (12)	I (10)
2.5	I (10)	I (18)	I (18)	I (10)	I (12)	I (14)	I (10)	I (10)
3.0	I (10)	NI	I (10)	I (14)	I (12)	I (18)	I (10)	I (10)

I = Inhibition, values of inhibition are given in parenthesis, NI = Not inhibition

Table (6):-Effect of azo compounds of 4-Hydroxybenzoic acid viz. 4a – 4h on the growth response of *Salmonella typhi*

Conc. (mg/mL)	4a	4b	4c	4d	4e	4f	4g	4h
0.5	NI	I (12)	I (12)	I (10)	I (12)	NI	NI	I (10)
1.0	NI	I (12)	I (14)	I (10)	I (12)	NI	I (10)	I (11)
1.5	I (10)	I (16)	I (14)	I (10)	I (12)	I (10)	I (10)	I (10)
2.0	I (10)	I (14)	I (14)	I (12)	I (14)	I (12)	I (10)	I (10)
2.5	NI	I (14)	I (16)	I (10)	I (12)	I (10)	I (10)	I (12)
3.0	I (10)	I (14)	I (18)	I (10)	I (10)	I (16)	I (12)	I (12)

I = Inhibition, values of inhibition are given in parenthesis, NI = Not inhibition.

The results regarding antibacterial activity of eight azo compounds of 4-Hydroxybenzoic acid against *E.Coli* are presented in table (3) and figure (2). The maximum antibacterial activity was observed in case of derivative 4b, 4c, 4d, 4e, 4f and 4h for which, all the concentrations used were showed remarkable antibacterial effect against *E.Coli* and the average diameter of zone of inhibition ranges from 10 – 18 mm. This is followed by 4a, 4g derivatives for which all the different concentrations showed pronounced antibacterial effect with average diameter of zone of inhibition ranges from 10 – 12 mm recorded except at 2.5 mg/mL for 4g over control where antibacterial activity was not observed.

The results on antibacterial activity of eight azo compounds of 4-Hydroxybenzoic acid viz 4a– 4h against *S.aureus* are tabulated in table (4) and figure (3). From the result it was observed that the compounds 4b, 4c, 4d, 4e, 4f showed excellent antibacterial activity at nearly all the six different concentrations except at 0.5 mg/mL and at 1.5 mg / mL for 4d and 4f respectively. The peak zone of inhibition recorded 22 mm diameter at 3.0 mg/mL for 4b. The zone of inhibition 18 mm at 2.5 mg/mL for 4b, at 2.0 mg/mL for 4c and at 3.0 mg/mL for 4f was recorded over

control. This is followed by 4a, 4g and 4h that exhibited superior antimicrobial activity nearly at all the different concentrations used but with medium inhibition zones ranging from 10 – 13 mm against *S.aureus* with maximum zone of inhibition recorded 13 mm diameter at 1.5 mg/mL for 4h.

The antibacterial effect of eight azo compounds viz 4a – 4h against *Pseudomonas aeruginosa* species are recorded in table (5) and figure (4). From the results it was observed that the azo derivative 4b, 4c, 4d, 4e, and 4f showed significant antibacterial effect against *Pseudomonas* species at all the six different concentrations used with average zone of inhibition ranging from 10 – 18 mm diameter with maximum zone of inhibition 18 mm at 2.0 and 2.5 mg/mL for 4b, at 1.0, 1.5, 2.5 mg/mL for 4c and at 1.5, 3.0 mg/mL for 4f respectively. This is followed by azo derivatives 4a, 4g and 4h which have showed pronounced antibacterial effect at all the different concentrations used but with medium average zone of inhibition ranging from 10 – 14 mm with maximum zone of inhibition 14 mm at 0.5 mg/mL for 4h over control.

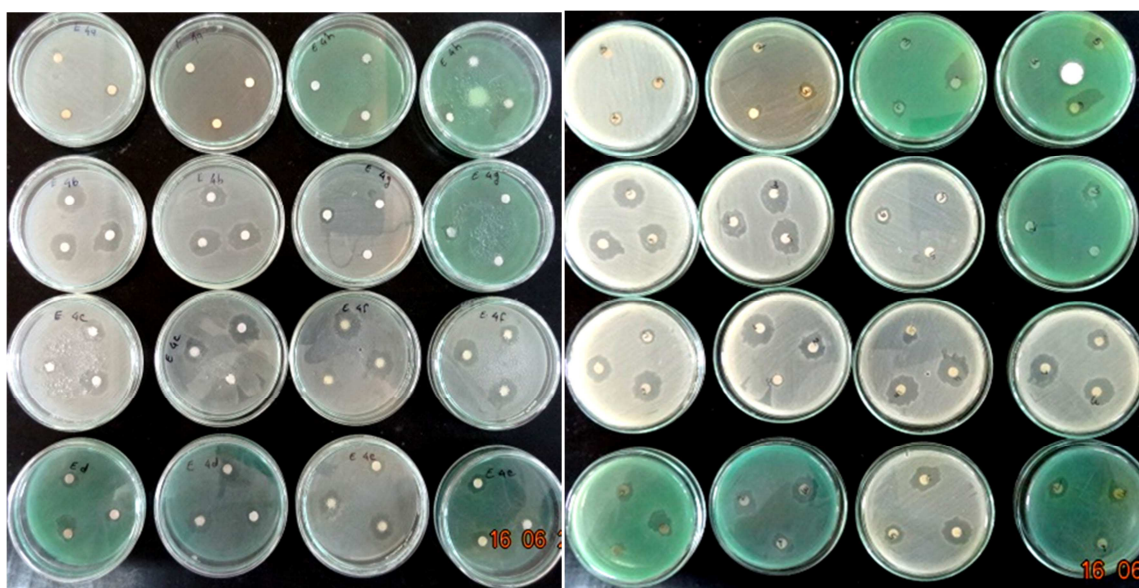


Figure 2: Effect of azo compounds of 4-Hydroxy Benzoic Acid viz. 4a – 4h on the growth response of *E. coli*



Figure 3: Effect of azo compounds of 4-Hydroxy Benzoic Acid viz. 4a – 4h on the growth response of *Staphylococcus aureus*.

The pursuit of data on antimicrobial effect of azo compounds viz 4a – 4h against *Salmonella typhi* is shown in table (6) and figure (5). The maximum antibacterial activity was recorded at all the six different concentrations in

derivative 4b, 4c, 4d, 4e and 4h with average zone of inhibition ranging from 10 – 18 mm with maximum zone of inhibition 18 mm recorded at 3.0 mg/mL for 4c. This is followed by 4g, 4f and 4a which showed very superior antibacterial effect at five, four and three different concentrations respectively with average zone of inhibition ranging from 10 – 16 mm with maximum zone of inhibition 16 mm against *S. typhi* at 3.0 mg/mL for 4f over control.

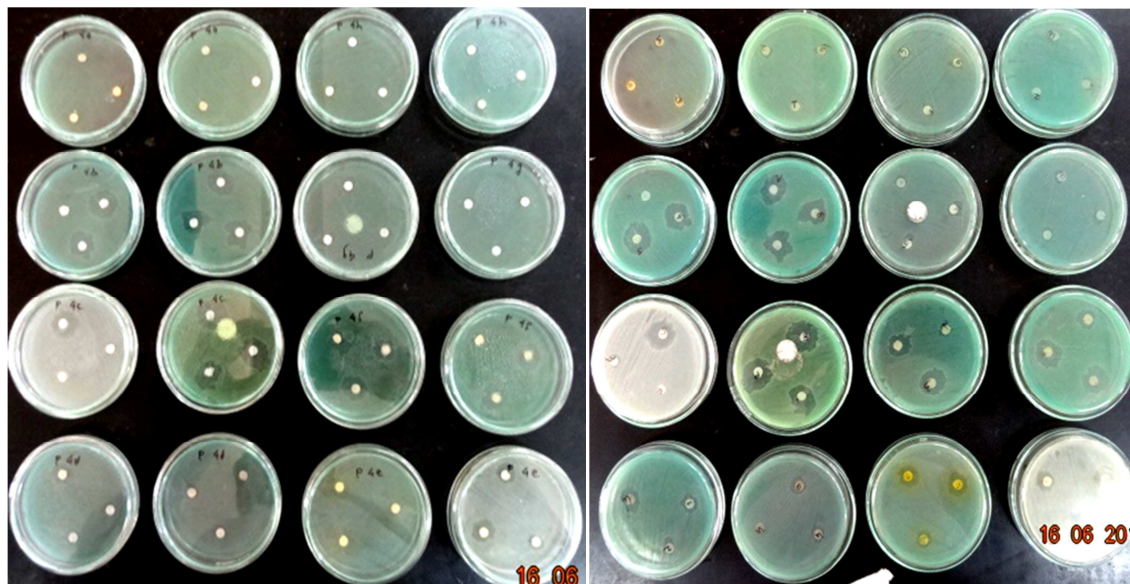


Figure 4: Effect of azo compounds of 4-Hydroxy Benzoic Acid viz. 4a – 4h on the growth response of *Pseudomonas aeruginosa*

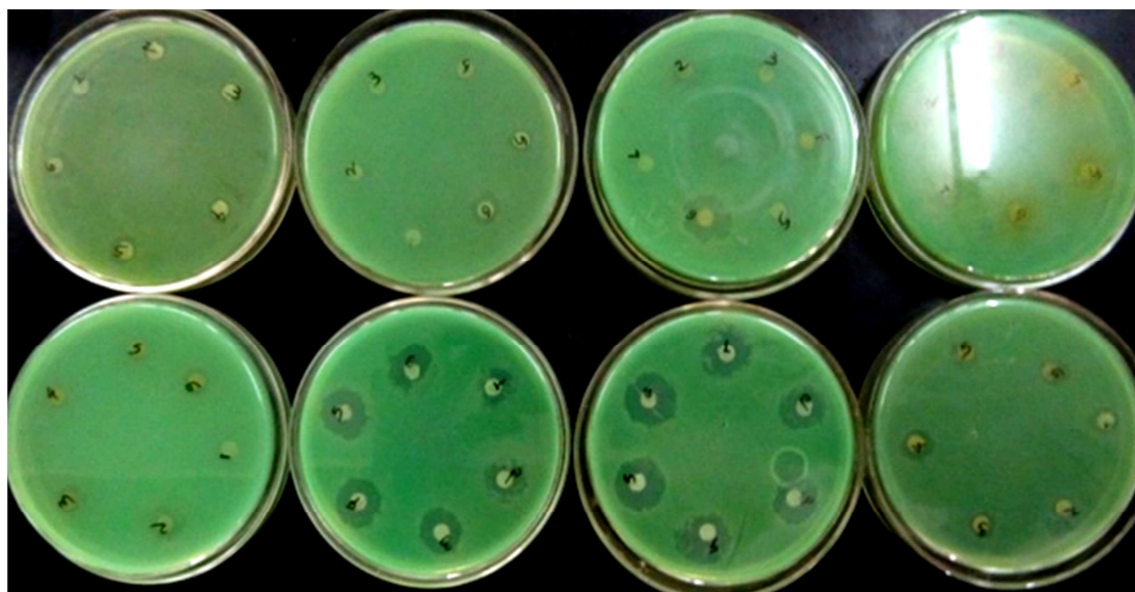


Figure 5: Effect of azo compounds of 4-Hydroxy Benzoic Acid viz. 4a – 4h on the growth response of *Salmonella typhi*

CONCLUSION

All the eight novel azo compounds 4a–4h containing 4-Hydroxybenzoic acid moiety were successfully synthesized in excellent yield and their structures are elucidated using elemental analysis, FTIR, ¹HNMR & MASS spectroscopy.

The results on antimicrobial activity reveals that all the eight newly synthesized compounds viz 4a–4h found to have outstanding antibacterial effect against *E.Coli*, *S. aureus*, *Pseudomonas aeruginosa*, 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.

All the synthesized dyes had significant antimicrobial activity against different bacterial species. As a consequence it is concluded that newly synthesized azo dyes containing 4-Hydroxybenzoic acid moiety, can be used for the development of new antibacterial drugs to cure many disorders caused by the different pathogenic bacterial species. However in this course these compounds should be analysed for its hepatotoxicity and renal toxicity with special interest on drug optimized concentration as well as for pharmacokinetic study.

Acknowledgement

Authors are thankful to Principal, Dr. M. M. Sancheti, of R. A. College, Washim for providing research facilities. Also we are thankful to Dr. N. S. Kulkarani, HOD of Microbiology for giving me practical help and guidance for carrying out antibacterial evaluation against micro-organisms and lastly Prof. L. S. Kabara, HOD of Chemistry.

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