ejpmr, 2022, 9(9), 457-464

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

SYNTHESIS, SPECTRAL CHARACTERIZATION AND ANTIMICROBIAL STUDY OF SOME NEWLY SYNTHESIZED RESORCINOL INCORPORATED AZO DYES

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Article Received on 21/07/2022 **Article Revised on 11/08/2022** **Article Accepted on 01/08/2022**

ABSTRACT

In this work, four azo dyes were synthesized by the diazotization process of four aromatic amines using $NaNO_2$ and HCl, and coupling of these diazonium salts with Resorcinol. The synthesis of azo dyes has been confirmed by FT-IR and H1 NMR spectral data, and their antimicrobial activities have been tested using the disk diffusion method against four different bacteria. These newly synthesized azo dyes are found to have good antimicrobial activity and synthesized in good yield.

KEYWORDS: Azo dyes, Resorcinol, antimicrobial activity.

INTRODUCTION

Azo groups are of great interest because of a wide range of applications. Azo dyes are in use as dyestuffs for wool, leather and synthetic fabrics due to their excellent coloring properties.[1] Compounds containing azo groups are also important structures in medicinal and pharmaceutical chemistry and it has been suggested that the azomethine linkage might be responsible for the biological activities displayed by Schiff bases.[2] These compounds have also received special attention in coordination chemistry due to their mixed hard–soft donor character and versatile coordination behavior.[3,4,5]

Dyes synthesized from heterocyclic amines produce pronounced bathochromic effect, when compared to the corresponding aniline compounds.[6] Azo dyes based on heterocyclic amines have been studied widely due to their excellent thermal[7], optical[8] and medicinal properties, such as antibacterial[9,10,4,11] antiviral[12], antifungal[13] and antioxidant activities.[14]

Recently, the study of azo dyes containing hydroxyl groups has attracted considerable attention.[15,16,17,18,19] Inter- and intramolecular proton transfer from phenolic oxygen to imine nitrogen is very common in polyhydroxy derivatives of azo dyes resulting in a self-isomerisation. Due to proton transfer ability of azo dyes, these systems have been of a special interest from practical viewpoint as the tautomers showing different optical behavior and dyeing properties.

In the present study, Resorcinol is coupled with diazonium salt of eight aromatic amines VIZ: Aniline, o-Nitro aniline, p-Toluedine, α-Naphthylamine, Sulphanilic acid, m-Nitro aniline, Benzedine and Anthranilic acid.

METHODS AND MATERIALS

All the chemicals used in these experiments were of analytical grade. All the melting points were determined by the open capillary method and are uncorrected. The products were confirmed by 1H NMR (Burker avernce II 400 NMR Spectrometer) 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 a solvent.

General procedure for synthesis of azo compounds[20]

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 Resorcinol. 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. The general Scheme for the synthesis of azo dyes of Resorcinol is shown in figure (I).

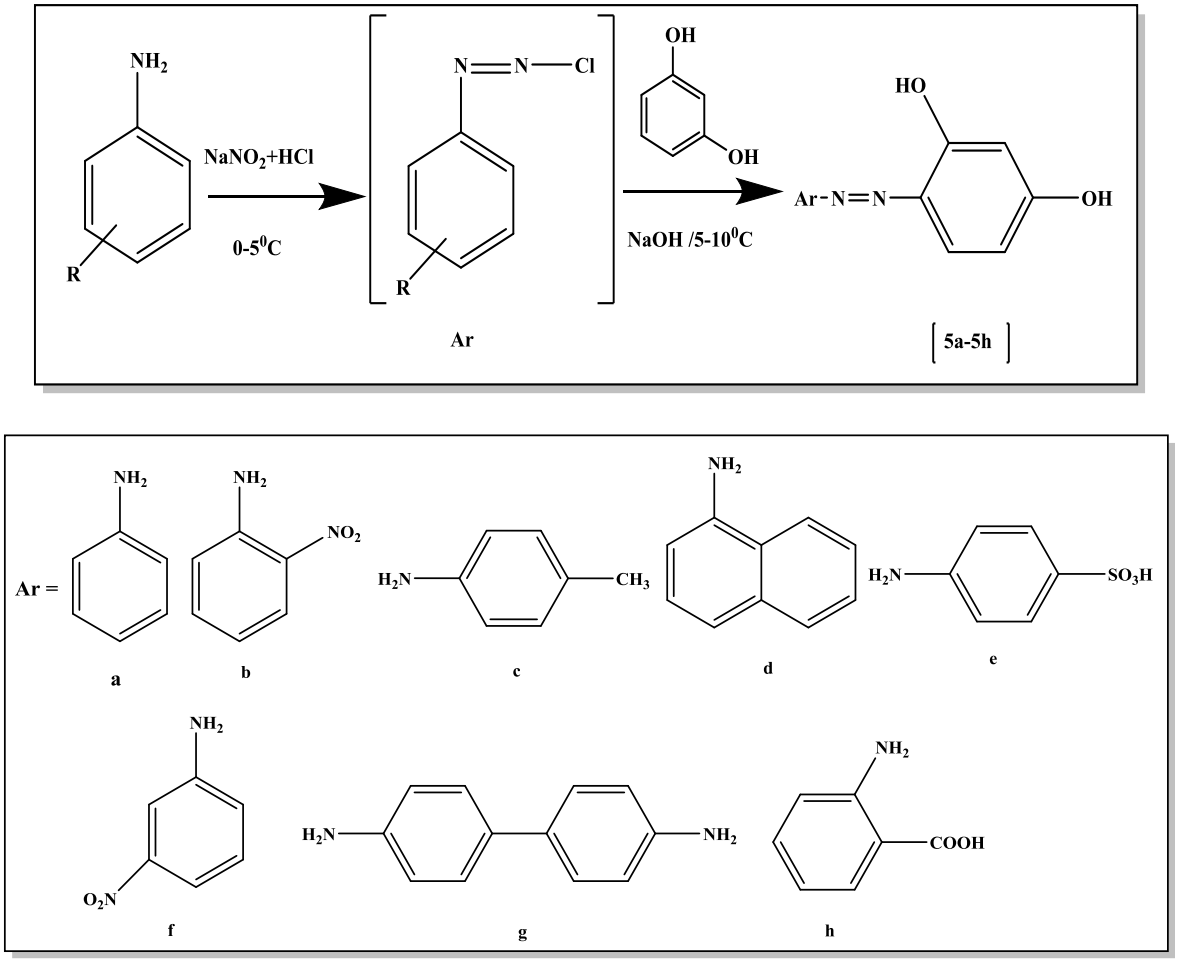


Figure I: General Scheme for the synthesis of azo dyes of Resorcinol.

Table (I): The code, compound name, molecular formula, molecular weight, melting point and percentage yield of synthesized compounds of Resorcinol.

Code	Structure	Molecular Formulae	Molecular Weight	Melting Point (^{0}C)	% Yield
5a	4-(phenyldiazenyl)benzene-1,3-diol	$C_{12}H_{10}N_2O_2$	214	186	45%
5b	4-((2-nitrophenyl)diazenyl)benzene-1,3-diol	$C_{12}H_9N_3O_4$	259	172	62%
5c	4-(p-tolydiazenyl)benzene-1,3-diol	$C_{13}H_{12}N_2O_2$	228	189	58%
5d	4-(naphthalen-1-yldiazenyl)benzene-1,3-diol	$C_{16}H_{12}N_2O_2$	264	195	52%
5e	4-((2, 4-dihydroxyphenyl)diazenyl)benzenesulfonic acid	$C_{12}H_{10}N_2O_5S$	294	201	54%
5f	4-((3-nitrophenyl)diazenyl)benzene-1,3-diol	$C_{12}H_9N_3O_4$	259	174	48%
5g	4-((4'-amino-[1,1'-biphenyl]-4-yl)diazenyl)benzene-1,3-diol	$C_{18}H_{15}N_3O_2$	305	181	56%
5h	2-((2,4-dihydroxyphenyl) diazenyl)benzoic acid	$C_{13}H_{10}N_2O_4$	258	191	62%

Antimicrobial Activity

The newly synthesized azo compounds 5a-5h were analyzed for their antimicrobial activity against four gram positive and gram negative bacteria viz. *Escherichia coli, Staphylococcus aureus, Pseudomanas aeroginosa and Salmonella typhi* by using agar well diffusion method. 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 spreaded 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.

RESULT AND DISCUSSION

The azo dyes synthesized in the present study were characterized by IR and NMR spectroscopic methods. IR and ^{1}H-NMR spectra showed the expected signals which correspond to various groups present in each compounds. The IR and ^{1}H-NMR spectral values for different synthesis dyes are shown in table II.

Table (II): FTIR AND ^{1}H NMR data of azo compounds of Salicyclic acid.

Compound	Spectra	Spectroscopic Data
SDB 5a	IR (KBr. cm-1)	3161cm^{-1} (Phenolic –OH stretch), 3057 cm^{-1} (N-H stretch), 1496 cm^{-1} (C=C Ring stretch), 1612 cm^{-1} (N=N stretch), 1246 cm^{-1}(C-N stretch)
	NMR (δ ppm)	12.47 (s 1H of –OH), 6.38-7.85(m 8H of Ar-H).
SDB 5b	IR (KBr. cm^{-1})	3577 cm^{-1} (Phenolic –OH stretch), 3035 cm^{-1} (N-H stretch), 1552 cm^{-1}(C=C Ring stretch), 1612 cm^{-1} (N=N stretch), 1290 cm^{-1} (C-N stretch), 1498 cm^{-1} (-NO_2 stretch (N-O Asym), 1311 cm^{-1} (-NO_2 stretch (N-O sym)
	NMR (δ ppm)	17.29 (s 1H of –OH), 5.17-8.10 (m 8H of Ar-H).
SDB 5c	IR (KBr. cm^{-1})	3255 cm^{-1} (Phenolic –OH stretch), 3026 cm^{-1}(N-H stretch), 1477 cm^{-1}(C=C Ring stretch), 1614 cm^{-1} (N=N stretch), 1244 cm^{-1} (C-N stretch), 2920 cm^{-1} (C-H stretch of -CH_3)
	NMR (δ ppm)	12.51 (s 1H of –OH), 2.38 (s 3H of –CH_3), 6.33-7.75 (m 7H of Ar-H).
SDB 5d	IR (KBr. cm^{-1})	3419 cm^{-1} (Phenolic –OH stretch), 3051 cm^{-1} (N-H stretch), 1471 cm^{-1}(C=C Ring stretch), 1620 cm^{-1} (N=N stretch), 1244 cm^{-1} (C-N stretch)
	NMR (δ ppm)	11.20 (s 1H of –OH), 6.10-8.39 (m 10H of Ar-H).
SDB 5e	IR (KBr. cm^{-1})	3570 cm^{-1} (Phenolic –OH stretch), 3062 cm^{-1} (N-H stretch), 1481 cm^{-1}(C=C Ring stretch), 1670 cm^{-1} (N=N stretch), 1122 cm^{-1} (C-N stretch), 1197 cm^{-1} (-SO stretch)
	NMR (δ ppm)	12.56 (s 1H of –OH), 3.73 (s 1H of –SO_3H), 6.39-7.85 (m 7H of Ar-H).
SDB 5f	IR (KBr. cm^{-1})	3280 cm^{-1} (Phenolic –OH stretch), 3091 cm^{-1} (N-H stretch), 1487 cm^{-1}(C=C Ring stretch), 1660 cm^{-1} (N=N stretch), 1263 cm^{-1} (C-N stretch), 1527 cm^{-1} (-NO_2 stretch (N-O Asym), 1319 cm^{-1} (-NO_2 stretch (N-O sym)
	NMR (δ ppm)	11.85 (s 1H of –OH), 7.65-8.64 (m 7H of Ar-H).
SDB 5g	IR (KBr. cm^{-1})	3356 cm^{-1} (Phenolic –OH stretch), 3034 cm^{-1} (N-H stretch), 1498 cm^{-1}(C=C Ring stretch), 1616 cm^{-1} (N=N stretch), 1246 cm^{-1} (C-N stretch),
	NMR (δ ppm)	12.56 (s 1H of –OH), 3.36 (s 1H of –NH), 6.9-7.99 (m 11H of Ar-H).
SDB 5h	IR (KBr. cm^{-1})	3456 cm^{-1} (Phenolic –OH stretch), 3066 cm^{-1} (N-H stretch), 2927 cm^{-1} (Carboxylic acid O-H stretch), 1552 cm^{-1}(C=C Ring stretch), 1604 cm^{-1} (N=N stretch), 1321 cm^{-1} (C-N stretch)
	NMR (δ ppm)	4.53 (s 1H of –OH), 12.40 (s 2H of –COOH), 6.26-8.10 (m 7H of Ar-H).

Antimicrobial Activity

Eight azo compounds of Resorcinol have been synthesized, recrystallized and used separately for its study of antimicrobial activity against four gram positive and gram negative bacteria viz. *Escherichia coli, Staphylococcus aureus, Pseudomanas aeroginosa and Salmonella typhi*. The data of antimicrobial activity of these newly synthesized azo dyes of Resorcinol 5a-5h against four pathogens are presented in the tables 1-4.

Antibacterial properties of the synthesized azo compounds of Resorcinol viz 5a – 5h [Zone of inhibition (mm)]

Table (1): Effect of azo compounds of Resorcinol viz. 5a – 5h on the growth response of *Escherichia coli*.

Conc.(mg/ml)	5a	5b	5c	5d	5e	5f	5g	5h
0.5	I (12)	I (10)	I (12)	I (11)	I (11)	I (12)	I (12)	I (10)
1.0	I (14)	I (12)	I (13)	I (10)	I (12)	I (11)	I (14)	I (12)
1.5	I (16)	I (11)	I (10)	I (13)	I (14)	I (14)	I (16)	I (14)
2.0	I (12)	I (10)	I (13)	I (16)	I (14)	I (16)	I (12)	I (16)
2.5	I (10)	I (16)	I (16)	I (13)	I (10)	I (10)	I (10)	I (10)
3.0	I (12)	I (13)	I (13)	I (12)	I (10)	I (12)	I (10)	I (12)

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

Table (2): Effect of azo compounds of Resorcinol viz. 5a – 5h on the growth response of *S. aureus*.

Conc.(mg/ml)	5a	5b	5c	5d	5e	5f	5g	5h
0.5	I (14)	I (14)	I (14)	I (12)	I (12)	I (14)	I (14)	NI
1.0	I (12)	I (12)	I (10)	I (14)	NI	I (18)	I (12)	I (12)
1.5	I (12)	I (16)	I (12)	I (10)	I (14)	I (16)	I (10)	I (14)
2.0	I (16)	I (18)	I (14)	I (10)	I (12)	I (20)	I (12)	I (10)
2.5	I (18)	I (12)	I (16)	I (10)	I (16)	I (10)	I (10)	I (11)
3.0	I (12)	I (14)	I (14)	I (10)	I (12)	NI	I (12)	I (12)

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

Table (3): Effect of azo compounds of Resorcinol viz. 5a – 5h on the growth response of *P. aeroginosa*.

Conc.(mg/ml)	5a	5b	5c	5d	5e	5f	5g	5h
0.5	I (14)	I (12)	I (14)	I (16)	I (11)	I (12)	NI	I (14)
1.0	I (20)	I (14)	I (18)	I (12)	I (14)	I (16)	I (18)	I (12)
1.5	I (22)	I (20)	I (19)	I (14)	I (18)	I (18)	I (20)	I (16)
2.0	I (11)	I (12)	I (12)	I (18)	I (19)	I (11)	I (22)	I (18)
2.5	I (14)	I (22)	I (20)	I (20)	I (10)	I (16)	I (14)	I (19)
3.0	I (16)	I (14)	I (14)	I (22)	I (12)	I (10)	I (12)	I (12)

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

Table (4): Effect of azo compounds of Resorcinol viz. 5a – 5h on the growth response of *Salmonella typhi*.

Conc.(mg/ml)	5a	5b	5c	5d	5e	5f	5g	5h
0.5	I (12)	I (12)	I (18)	I (12)	I (18)	I (18)	I (16)	I (10)
1.0	I (14)	I (18)	I (22)	I (14)	I (12)	I (19)	I (10)	I (12)
1.5	I (20)	I (10)	I (24)	I (18)	I (14)	I (22)	I (18)	I (11)
2.0	I (14)	I (12)	I (18)	I (14)	I (11)	I (12)	I (20)	I (13)
2.5	I (16)	I (11)	I (20)	I (20)	I (20)	I (10)	I (28)	I (10)
3.0	I (18)	I (15)	I (22)	I (14)	I (24)	I (12)	I (22)	NI

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

Figure 1: Effect of azo compounds of Resorcinol viz. 5a– 5h on the growth of *E.Coli.*

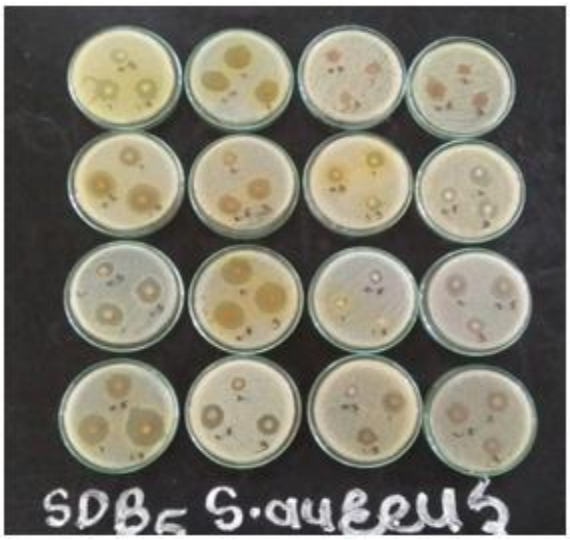
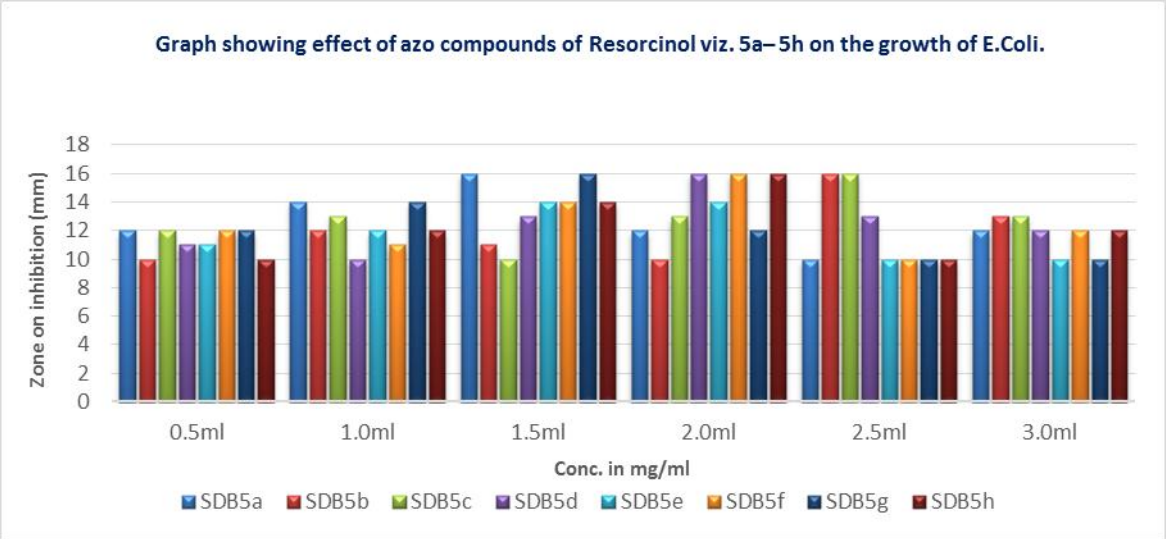
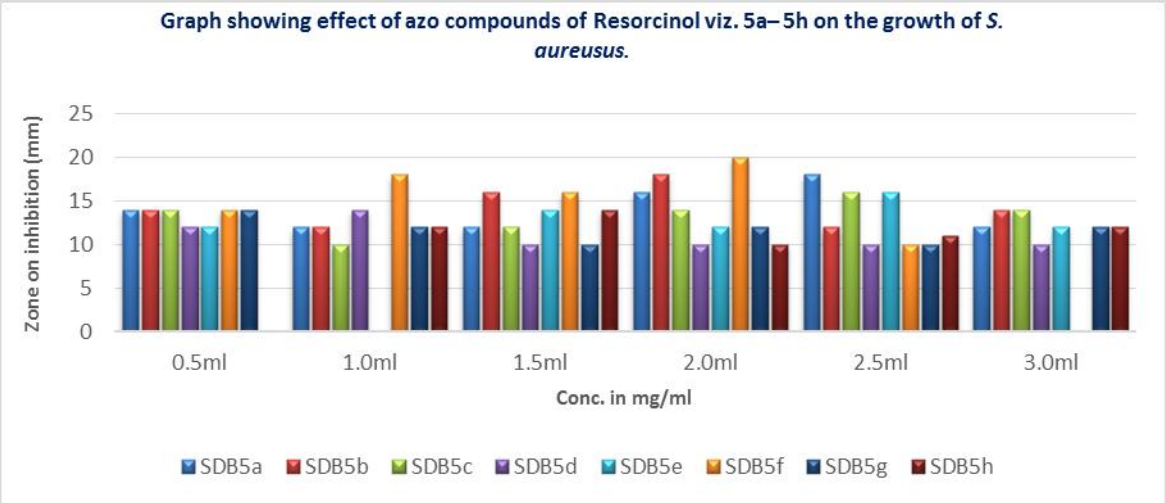


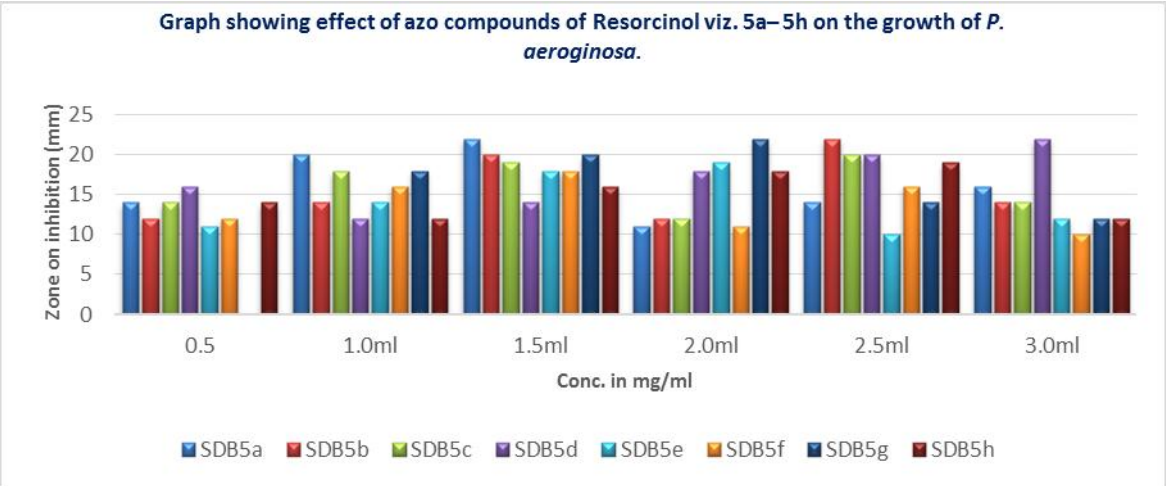
Figure 2: Effect of azo compounds of Resorcinol viz. 5a– 5h on the growth of *Staphylococcus aureusus.*

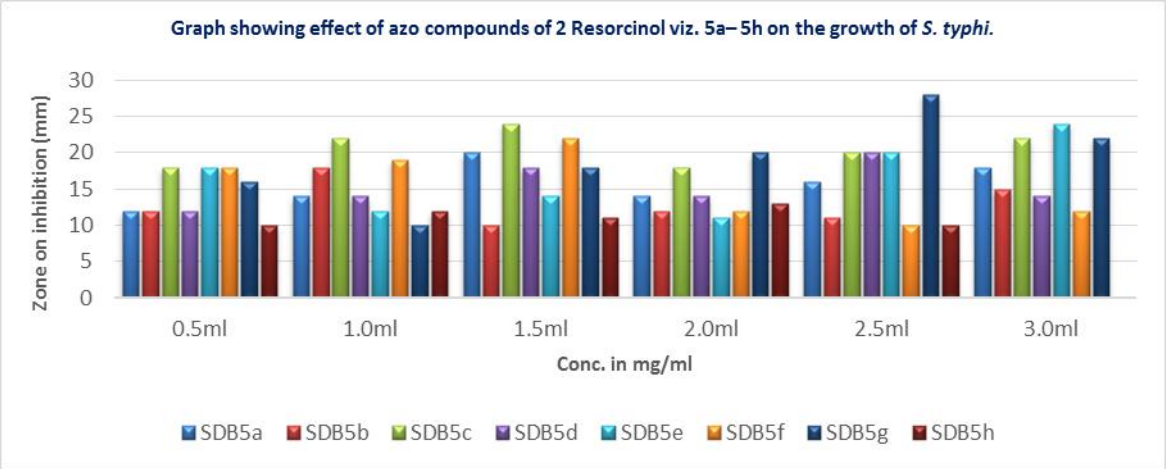
Figure 3: Effect of azo compounds of Resorcinol viz. 5a– 5h on the growth of *Pseudomonas aeroginosa.*

Figure 4: Effect of azo compounds of Resorcinol viz. 5a– 5h on the growth of *Salmonella typhi.*


Graph showing effect of azo compounds of Resorcinol viz. 5a– 5h on the growth of E.Coli.
Zone on inhibition (mm)
18
16
14
12
10
8
6
4
2
0
0.5ml
1.0ml
1.5ml
2.0ml
2.5ml
3.0ml
Conc. in mg/ml
SDB5a SDB5b SDB5c SDB5d SDB5e SDB5f SDB5g SDB5h


Graph showing effect of azo compounds of Resorcinol viz. 5a– 5h on the growth of S. aureusus.
Zone on inhibition (mm)
25
20
15
10
5
0
0.5ml
1.0ml
1.5ml
2.0ml
2.5ml
3.0ml
Conc. in mg/ml
SDB5a SDB5b SDB5c SDB5d SDB5e SDB5f SDB5g SDB5h


Graph showing effect of azo compounds of Resorcinol viz. 5a– 5h on the growth of P. aeroginosa.
Zone on inhibition (mm)
25
20
15
10
5
0
0.5
1.0ml
1.5ml
2.0ml
2.5ml
3.0ml
Conc. in mg/ml
SDB5a SDB5b SDB5c SDB5d SDB5e SDB5f SDB5g SDB5h



The results regarding antibacterial activity of eight azo compounds of Resorcinol against *E. coli* are presented in table (1) and figure (18). The maximum antibacterial activity was observed in case of all the derivative 5a-5h for which all the concentrations used were showed excellent antibacterial activity against *E.Coli* and the average diameter of zone of inhibition ranges from 10 – 16 mm. The peak zone of inhibition recorded 16 mm diameter at 1.5 mg/mL for 5a and 5g derivative, 2.5 mg/mL for 5b and 5c derivatives and 2.0 mg/mL for 5d, 5f and 5h derivatives over control.

The results on antibacterial activity of eight azo compounds of Resorcinol viz. 5a– 5h against *S.aureus* are tabulated in table (2) and figure (19). From the result it was observed that all the compounds showed pronounced antibacterial activity against *S.aureus* at almost all the six different concentrations except 1.0 mg/mL for 5e and 3.0 mg/mL for 5f derivatives. The average diameter of zone of inhibition ranges from 10 – 20 mm. The peak zone of inhibition recorded 20 mm diameter at 2.0 mg/ml for 5f over control.

The antibacterial effect of eight azo compounds viz 5a – 5h against *Pseudomonas aeroginosa* species are recorded in table (3) and figure (20). From the result it was observed that all the compounds showed significant antibacterial effect against *Pseudomonas* species at all the six different concentrations used except 0.5 mg/mL for 5g derivative with average diameter of zone of inhibition ranges from 10 – 22 mm. The peak zone of inhibition recorded 22 mm diameter at 1.5 mg/ml for 5a, 2.5 mg/mL for 5b, 3.0 mg/mL for 5d and 2.0 mg/mL for 5g over control.

The recorded of data on antimicrobial effect of azo compounds viz 5a–5h against *Salmonella typhi* is shown in table (4) and figure (21). The maximum antibacterial activity was recorded at all the six different concentrations in almost all the derivative viz. 5a-5h except 3.0 mg/mL concentration for 5h derivative. The average zone of inhibition ranging from 10 – 28 mm with maximum zone of inhibition 28 mm recorded at 2.5 mg/mL for 5g derivative.

CONCLUSION

All the azo compounds 5a-5h containing Resorcinol moiety were successfully synthesized in good yield and their structures were confirmed using FTIR, & 1HNMR spectroscopy. The results on antimicrobial activity tells that all the eight newly synthesized compounds viz 5a-5h found to have good antibacterial effect against *E.Coli*, *S. aureus*, *Pseudomonas aeroginosa*, and *Salmonella typhi* nearly at all the concentrations analysed. The results shown, the broad spectrum potential of all the compounds in inhibiting the growth of human pathogens, and this finding shows the possible help in drug discovery.

ACKNOWLEDGEMENT

Authors are very thankful to Sophisticated Analytical Instrument Facility (SAIF) Chandigarh and Head department of Chemistry, Shri. Shivaji College, Akola for availing the spectral data. Also thankful to Dr. N. S. Kulkarani, HOD of Microbiology for giving me practical help and guidance for carrying out antibacterial evaluation against micro-organisms.

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