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Synthesis and *in Vitro* Antibacterial Evaluation of 4-Chlorophenol Incorporated Azo Dyes 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 four azo compounds were synthesized in excellent yields via the diazotization of 4 different aromatic amines followed by coupling with 4-chlorophenol. These compounds were characterized by IR, ^{1}HNMR 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 showed moderate to significant inhibitory effect at some specific concentrations against the tested pathogens.

Keywords: 4-chlorophenol, Azo compounds, Antibacterial Activity, Human pathogens

1. Introduction

Azo compounds are the most fundamental class of commercial dyes and are well coloured that have been used as dyes and pigments [1-2]. Organic colour chemistry is undergoing very exciting development as a result of the opportunities presented by dye applications in science and technology fields such as, pharmaceutical, cosmetic, textile and leather industries, electronic devices, linear and non-linear optics [3-7]. It has been found that, existence of an azo (-N=N-) moiety in different types of azo derivatives has caused them to show antibacterial and pesticidal activity [8-9]. Hence in the present time, exploration of azo dye as antibacterial and antifungal agents has received considerable attention [10-12].

For the reason of variety applications of azo compounds, it is interesting to study synthesis of such new azo compounds and their derivatives in order to explore the newer potentials of such compounds. Several azo compounds derived from 4-Hydroxybenzoic acid[13], aspirin[14], paracetamol[15], 2,4-Dintrophenol[16], tyrosine[17] & vanillin[18] moieties have been frequently reported and exhibited excellent biological properties.

In view of the above relevant literature survey, it can be said that, compounds with azo moiety have been extensively used as dyes due to their utility in colorings functions. But their variety in biological activity potential is less reported and as a consequence still have a huge scope to synthesize new azo compounds and to test their biological activities. So, in the present work, we have synthesized four new azo compounds namely 5A to 5D containing 4-chlorophenol moiety and characterized by FTIR, 1HNMR and MASS spectral technique. The antibacterial activities of the synthesized

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azo compounds were reported in *vitro* using disc diffusion method.

2. Material and Methods

The chemicals used in the present studies are of synthetic grade, Merck company ltd. The products were characterized by IR, ^{1}HNMR and MASS spectral studies. The M.P. 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. ^{1}HNMR spectra were recorded in $CDCl_3$ 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.

Experimental procedure for synthesis of azo compounds [19-21]:

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 so prepared was added drop wise to the alkaline 10% NaOH solution of 4-chlorophenol (0.01mole). The reaction mixture was stirred for 30-45 minutes maintaining the temperature 5-10^0 C. The coloured products obtained was filtered, washed with water and recrystallized from 50% ethanol. The general reaction scheme for synthesis of azo compounds of p-chlorophenol is shown in figure-(1). Code, chemical name, formulae, mol.wt. m.p. and % yield of synthesized azo compounds of 4-chlorophenol is shown in table-(1).

Figure 1:The general reaction scheme for synthesis of azo compounds of 4-chlorophenol

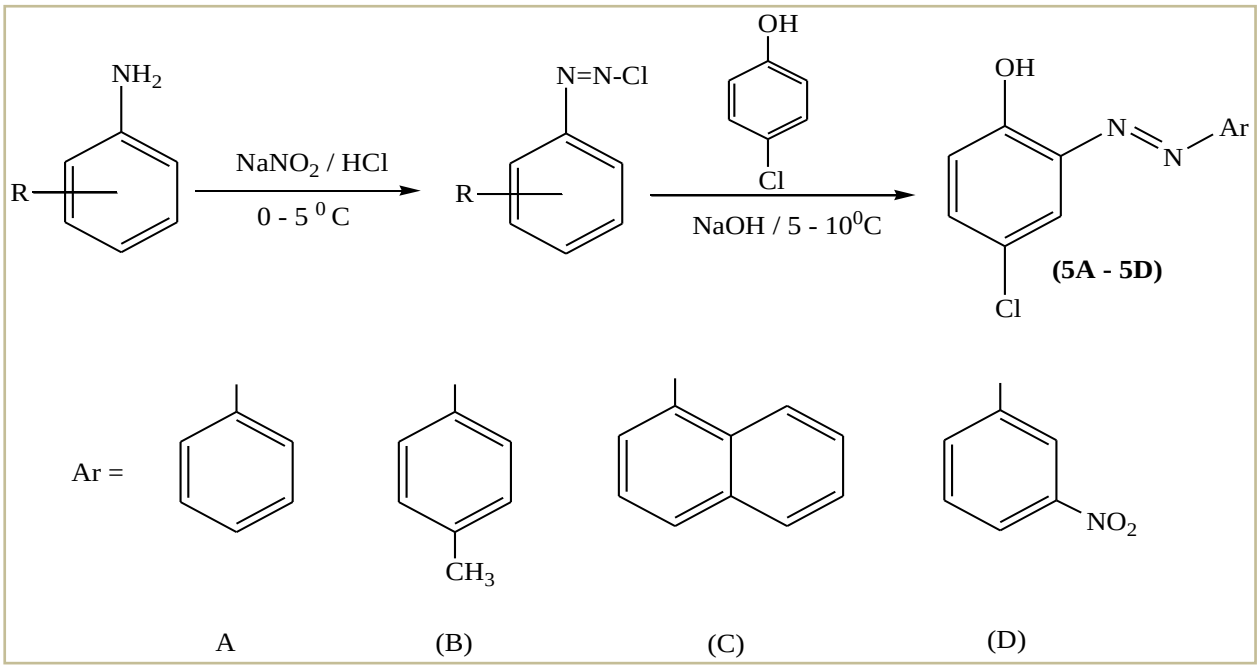

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Table 1:

Code	Name of Compound	Mol. Formula	Mol.t	M.P. ^{0}C	% Yield	Colour
5A	(E)-2-(2-phenyldiazenyl)-4-chlorophenol	$C_{12}H_9ClN_2O$	232.67	103.5	69.00	Orange Red
5B	4-chloro-2-[(*Z*)-(4-methylphenyl)diazenyl] phenol	$C_{13}H_{11}ClN_2O$	246.69	110.5	74.58.	Brick Red
5C	(E)-2-(2-(naphthalen-1-yl)diazenyl)-4-chlorophenol.	$C_{16}H_{11}ClN_2O$	282.72	91.00	76.14	Brick Red
5D	4-chloro-2-[(*Z*)-(3-nitrophenyl) diazenyl] phenol	$C_{12}H_8ClN_3O_3$	278.67	95.50	87.03	Orange Red

3. Result and discussion

Spectroscopic study: I.R., ^{1}HNMR and MASS Spectra Showed the expected signals / peaks which correspond to various groups present in each compounds. The I.R. ^{1}HNMR and MASS spectral data are shown in Table (2).

Table (2):- IR, ^{1}HNMR and MASS SPECTRAL DATA:

5A IR- 3581cm^{-1} (Phenolic –OH stretch), 3057cm^{-1}(Ar–H Stretch), 1598cm^{-1} (N=N Stretch), 1604cm^{-1} (C=C Ring or aromatic stretch), 1384cm^{-1} (C–N Stretch), 1261cm^{-1} (C–O Phenol stretch), 764cm^{-1}(C–H aromatic def), 731cm^{-1} C–Cl stretching.

NMR- δ 6.9-7.8 (m 8H of Ar-H), δ-7.9 (s 1H of –OH).

MASS- The mass spectrum displayed the m/z molecular ion peak at 232

5B IR- 3467cm^{-1} (Phenolic –OH stretch), 2992cm^{-1} (C–H Ar stretching), 1610cm^{-1} (N=N Stretch), 1775 cm^{-1} (C=C Ring or aromatic stretch), 1492 cm^{-1}(C–H def of CH_3 group), 1384 cm^{-1} (C–N Stretch), 1342 cm^{-1}(C–O Phenol stretch), 1140 cm^{-1}(C–C aliphatic chain stretch), 809cm^{-1} C–Cl stretching.

NMR- δ 2.4 (s 3H of–CH_3), δ 6.96 to 7.70 (m 7H of Ar-H), δ-7.9 (s 1H of –OH).

5C IR- 3046cm^{-1} (Phenolic –OH stretch), 3046cm^{-1}(Ar–H Stretch, sp^2 plane), 1591 cm^{-1}(N=N Stretch), 1617cm^{-1} (C=C Ring or aromatic stretch), 1218cm^{-1} (C–N Stretch), 1243 cm^{-1}(C–O Phenol stretch), 838cm^{-1}(C–C aliphatic chain stretch), 772cm^{-1}(C–Cl stretching)

NMR- δ 6.50 to 8.90 (m 7H of Ar-H), δ-8.8 (s 1H of –OH).

5D IR- 3432cm^{-1} (Phenolic –OH stretch), 3091cm^{-1}(Ar–H Stretch, sp^2-plane), 1591cm^{-1}(N=N Stretch), 1620cm^{-1} (C=C Ring or aromatic stretch), 1218 cm^{-1} (C–N Stretch), 1243 cm^{-1}(C–O Phenol stretch), 838 cm^{-1}(C–C aliphatic chain stretch), 737cm^{-1}(C–Cl stretching)

NMR- δ 7.00 to 8.30 (m 7H of Ar-H), δ-8.7 (s 1H of –OH).

ANTIMICROBIAL ACTIVITY:

The compounds 5A–5D were screened for the presence of antibacterial constituents against four micro-organisms viz., *Escherichia coli, Staphylococcus aureus, Pseudomonas aeroginosa* and *Salmonella typhi*, adopting

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disc diffusion method[22,23]. All the bacterial cultures were obtained from University recognized Microbiology Research Laboratory, R. A. College Washim. 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 37^0C and the zones of inhibition were measured using antibiotic zone reader (Hi- Media).

The data on antimicrobial activity of azo compounds of 4-chlorophenol viz. 5A–5D against four human pathogens are presented in table (5.1) – (5.4) as follows.

Table (5.1):- Effect of azo compounds of 4-chlorophenol viz. 5A–5D on the growth response of *Escherichia coli*

Conc. (mg/mL)	5A	5B	5C	5D
0.5	I (10.1)	I (10)	I (8.3)	I (9.3)
1.0	I (10.1)	I(11.1)	I (10.0)	I (9.0)
1.5	I (11.0)	I(11.3)	I (10.2)	I (10.5)
2.0	I (10.6)	I(11.2)	I (10.4)	I (10.2)
2.5	I (10.3)	I(11.6)	I (10.1)	I (10.4)
3.0	I (10.3)	I (11.7)	I (10.2)	I (10.6)

Table (5.2):- Effect of azo compounds of 4-chlorophenol viz. 5A–5D on the growth response of *Staphylococcus aureus***:**

Conc. (mg/mL)	5A	5B	5C	5D
0.5	NI	I (10)	I (12.0)	I (17.0)
1.0	NI	I(11.5)	I (12.4)	I (17.5)
1.5	NI	I(11.4)	I (12.6)	I (17.6)
2.0	NI	I(11.2)	I (12.8)	I (17.7)
2.5	NI	I(11.6)	I (12.5)	I (17.8)
3.0	NI	I (11.8)	I (12.9)	I (17.9)

Table (5.3):- Effect of azo compounds of 4-chlorophenol viz. 5A–5D on the growth response of *Salmonella typhi***:**

Conc. (mg/mL)	5A	5B	5C	5D
0.5	I (10.0)	I (11.3)	NI	I (11.0)
1.0	I (10.1)	I(11.5)	NI	I (11.3)
1.5	I (10.3)	I(11.6)	NI	I (11.4)
2.0	I (10.4)	I(11.8)	NI	I (11.3)
2.5	I (10.2)	I(11.7)	NI	I (11.5)
3.0	I (10.5)	I (11.9)	NI	I (11.6)

Table (5.4):- Effect of azo compounds of 4-chlorophenol viz. 5A–5D on the growth response of *Pseudomonas aeroginosa*:

Conc. (mg/mL)	5A	5B	5C	5D
0.5	NI	NI	I (16.0)	NI
1.0	NI	NI	I (16.2)	NI
1.5	NI	NI	I (16.4)	NI
2.0	NI	NI	I (16.6)	NI
2.5	NI	NI	I (16.5)	NI
3.0	NI	NI	I (16.2)	NI

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The results regarding antibacterial activity of four azo compounds of 4-chlorophenol viz 5A–5D against *E.Coli* are presented in table (5.1). The maximum antibacterial activity was observed in case of derivative 5A and 5B for which nearly all six concentrations used were showed significant antibacterial effect against *E.Coli* and the average diameter of zone of inhibition ranges from 10–11.7mm with maximum zone of inhibition recorded 11.7mm diameter for 5B at 3.00 mg/mL over control. This is followed by 5D and 5C derivatives for which most of the different concentrations showed moderate antibacterial effect with average zone of inhibition ranges from 10–10.6 mm with peak zone of inhibition recorded 10.6mm diameter at 3.00 mg/mL for 5D over control where antibacterial activity was not observed.

The results on antibacterial activity of four azo compounds of 4-chlorophenol viz 5A–5D against *S.aureus* are tabulated in table (5.2). From the result it was observed that the compounds 5B, 5C and 5D showed pronounced antibacterial activity at nearly all the six different concentrations. The peak zone of inhibition recorded 17.9 mm diameter at 3.00 mg/mL for 5D, 12.9 mm diameter at 3.00 mg/mL for 5C and 11.8 mm diameter at 3.00 mg/mL for 5D over control where no activity is recorded against S.aureus. The compound 5A found scarce to inhibit the growth of *S.aureus* species

The results regarding antibacterial activity of four azo compounds of 4-chlorophenol viz 5A–5D against *Salmonella typhi* are tabulated in table (5.3). From the result it was observed that the compounds 5A, 5B and 5D shows significant antibacterial properties against *Salmonella typhi* at all the six different concentrations used with average zone of inhibition ranging from 10 – 11.9 mm diameter with peak zone of inhibition 11.9 mm at 3.0 mg/mL for 5B, 11.6 mm at 3.0 mg/mL for 5D and 10.5 mm at 3.00 mg/mL for 5A respectively. The compounds 5C was found insufficient to inhibit the growth of *Salmonella typhi* species

The pursuit of data on antibacterial effect of azo compounds viz 5A–5D against *Pseudomonas aeroginosa* species is tabulated in table (5.4). The excellent antibacterial activity was recorded at all the six different concentrations in derivative 5C where average zone of inhibition ranges from 16.00 – 16.6 mm with peak zone of inhibition 16.6mm recorded at 2.0 mg/mL. The remaining three azo derivatives 5A, 5B and 5d showed no antibacterial effect at any concentrations against *Pseudomonas aeroginosa* species.

5. Conclusion

The entire four novel azo compounds 5A–5D containing 4-chlorophenol moiety were successfully synthesized in average yield and their structures are elucidated using IR, NMR & MASS spectroscopy.

The results on antibacterial evaluation study reveals that all the four newly synthesized compounds viz 5A-5D found to have moderate to significant antibacterial effect against *E.Coli*, *S. aureus*, *Pseudomonas aeroginosa*, and *Salmonella typhi* at two or more different concentrations analysed.

All the four azo derivatives and specially 5B is extraordinarily effective against *E.Coli*, azo derivative 5B, 5C and 5D are profoundly active against *S. aureus*, 5A, 5B and 5D are significantly active against *Salmonella typhi* whereas only 5C has pronounced antibacterial effect against *Pseudomonas aeroginosa* at all the six different concentrations used for analysis. As a consequence it can be concluded that 5B, 5C and 5D synthesized azo dyes containing 4-chlorophenol moiety, may be used for the development of new antibacterial drugs to cure many disorders caused by the different pathogenic bacteria species. However in this course these compounds should be analysed for its hepatic-toxicity and renal toxicity with special interest on drug optimized concentration as well as for pharmaco-kinetic study.

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