



# Synthesis, Characterization and Antimicrobial Screening of Azo Compounds containing 4-hydroxybenzaldehyde Moiety

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## Abstract

Some azo compounds (1a-e) were synthesized by simple diazotization reaction of five different substituted aromatic amines using sodium nitrite and hydrochloric acid followed by coupling with 4-hydroxybenzaldehyde in alkaline medium. Synthesized azo compounds have been confirmed by UV, IR and <sup>1</sup>H NMR spectral data and also screened for their antibacterial activity using disc diffusion method.

**Keywords:** Azo compounds, 4-hydroxybenzaldehyde, Diazotization, Antimicrobial screening.

## Introduction

Out of the different classes of dyes, azo dyes constitutes one of the largest and important class of synthetic organic compounds containing an azo N=N group generally connected to aromatic rings. Mostly, synthesis of azo compounds involves diazotization of substituted primary aromatic amines followed by coupling with nucleophiles. They do not occur naturally but synthesized only through chemical synthesis<sup>1</sup> and have been extensively used in different applications such as dyeing textile fibres, colouring variety of materials, biomedical studies and in advanced organic synthesis as well as shows excellent antibacterial and pesticidal properties<sup>2</sup>. Azo compounds and its derivatives are also known for their use as antifungal, antidiabetics, antineoplastics, anti-inflammatory, antiseptic and other useful chemotherapeutic agents<sup>3-6</sup>. A number of azo compounds particularly synthesized from  $\beta$ -naphthol, m-cresol, resorcinol, tyrosine, aspirin, paracetamol etc have been frequently reported and exhibited impressive biocidal effects. Since compounds with an azo moiety and 4-hydroxybenzaldehyde moiety have been extensively used as dyes but their antimicrobial activity are less reported and hence in the present work, we have prepared five different substituted azo derivatives of 4-hydroxybenzaldehyde namely 1a-e, characterized and also screened for their antibacterial activity at different concentrations by using disc diffusion method.

## Materials and Methods

In present work, chemicals and reagents used were of analytical grade (Merck and Alfa Aesar Company Ltd). Melting points were determined by open capillary method and are uncorrected. The UV spectra of azo compound was determined by Lab India 3000+ spectrophotometer at Vidyabharati Mahavidyalaya, Amravati. IR spectra was recorded in KBr pellets from Shimadzu DRS-8000 IR spectrophotometer at Shri Shivaji

Science College, Amravati and <sup>1</sup>H NMR spectra from Bruker Avance II 400 MHz NMR spectrometer using DMSO as a solvent and TMS as an internal standard at SAIF, Punjab University, Chandigarh.

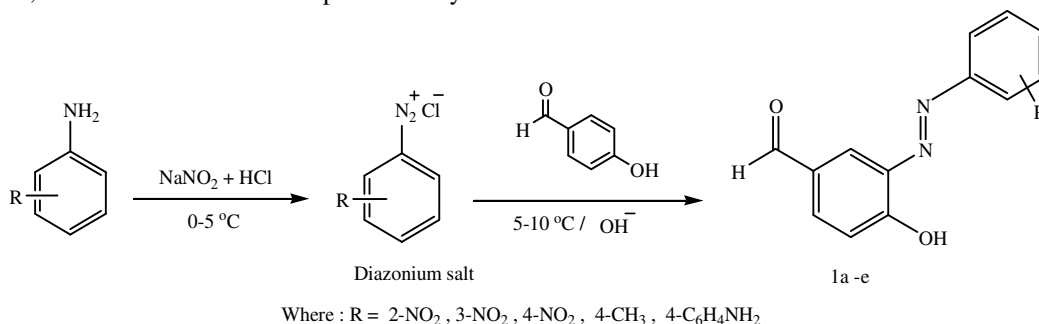
**Experimental procedure for the synthesis of azo compounds<sup>7</sup>:** 4-Nitroaniline (0.01 m) was dissolved in 2.5 ml of conc. HCl and 2.5 ml (4N) cold solution of NaNO<sub>2</sub> was added with constant stirring. During addition, temperature of the reaction mixture was maintained up to 0-5°C. Diazonium salt solution prepared above was added drop by drop to the solution of 4-hydroxybenzaldehyde in 10% NaOH with stirring for 30 to 40 minutes by adjusting the temperature between 5-10°C. The crude product precipitate was filtered, washed with distilled water, dried and recrystallised from hot ethanol to yield brown coloured crystalline solid of 4-hydroxy-3-((4-nitrophenyl) diazenyl) benzaldehyde (1c). M.f. = C<sub>13</sub>H<sub>9</sub>N<sub>3</sub>O<sub>4</sub>; m.w. = 271.23; m.p. = 94-95°C. Its alcoholic solution shows positive test of ferric chloride, showing the presence of phenolic -OH group. Similarly other compounds are prepared by same method and are shown in Scheme-1.

**Spectral data of representative azo compound 1c:** IR: 3707 cm<sup>-1</sup> (Phenolic -OH stretch), 3032 cm<sup>-1</sup> (Aromatic C-H stretch), 1716 cm<sup>-1</sup> (C=O stretch of Ar-CHO), 1539 cm<sup>-1</sup> (Aromatic C=C stretch), 1492 cm<sup>-1</sup> (N=N stretch). <sup>1</sup>H NMR:  $\delta$  3.35 (s, 1H of -OH),  $\delta$  11.7 (s, 1H of -CHO),  $\delta$  6.9-8.4 (m, 7H of Ar-H). UV ( $\lambda_{max}$ ): 360 nm.

**Antimicrobial screening:** All the synthesized azo compounds were screened for their antibacterial activities against three microorganisms viz. *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* by adopting disc diffusion method<sup>8</sup> at Microbial Section, FTL, Krishi Vigyan Kendra, Durgapur (Badnera), Dist. Amravati. The azo compounds were dissolved in ethanol to get solutions of 50 and 100 mcg/ml concentrations.

Sterile discs were dipped in these solutions, dried and placed on nutrient agar plates spreaded with the bacteria. After incubation at 37°C for 24 hr, the zones of inhibition produced by azo

compounds were measured in mm and were compared with streptomycin as a standard.



**Scheme-1**  
The general reaction scheme for synthesis of azo compounds 1a-e

**Table-1**

Compounds codes, names, mol. formulae, mol. weights, melting points and percentage yields of synthesized azo compounds 1a-e

Compds. codes	Compounds names	Mol. formulae	Mol. Wt.	M.p. (°C)	% Yield
1a	(E)-4-hydroxy-3-((2-nitrophenyl)diazenyl) benzaldehyde	C <sub>13</sub> H <sub>9</sub> N <sub>3</sub> O <sub>4</sub>	271.23	120-122	50%
1b	(E)-4-hydroxy-3-((3-nitrophenyl)diazenyl) benzaldehyde	C <sub>13</sub> H <sub>9</sub> N <sub>3</sub> O <sub>4</sub>	271.23	123-124	62%
1c	(E)-4-hydroxy-3-((4-nitrophenyl)diazenyl) benzaldehyde	C <sub>13</sub> H <sub>9</sub> N <sub>3</sub> O <sub>4</sub>	271.23	94-95	71%
1d	(E)-4-hydroxy-3-(p-tolyldiazenyl) benzaldehyde	C <sub>14</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub>	240.26	98-100	56%
1e	(E)-4-hydroxy-3-(4-(4-anilinyl)phenyldiazenyl) benzaldehyde	C <sub>19</sub> H <sub>15</sub> N <sub>3</sub> O <sub>2</sub>	317.34	102-104	52%

**Table-2**

Antimicrobial activity of synthesized azo compounds 1a-e

Compds. codes	Conc. (mcg/ml)	Zones of inhibition in mm		
		<i>E.coli</i>	<i>S.aureus</i>	<i>S.typhi</i>
1a	50	NI	NI	NI
	100	NI	NI	NI
1b	50	NI	NI	NI
	100	NI	NI	NI
1c	50	I(6)	I(13)	I(7)
	100	I(8)	I(14)	I(8)
1d	50	NI	NI	NI
	100	NI	NI	NI
1e	50	NI	NI	NI
	100	NI	NI	NI
Std.	25 mcg/disc	20	26	25

NI=No Inhibition, I=Inhibition (zones of inhibition are given in parenthesis)

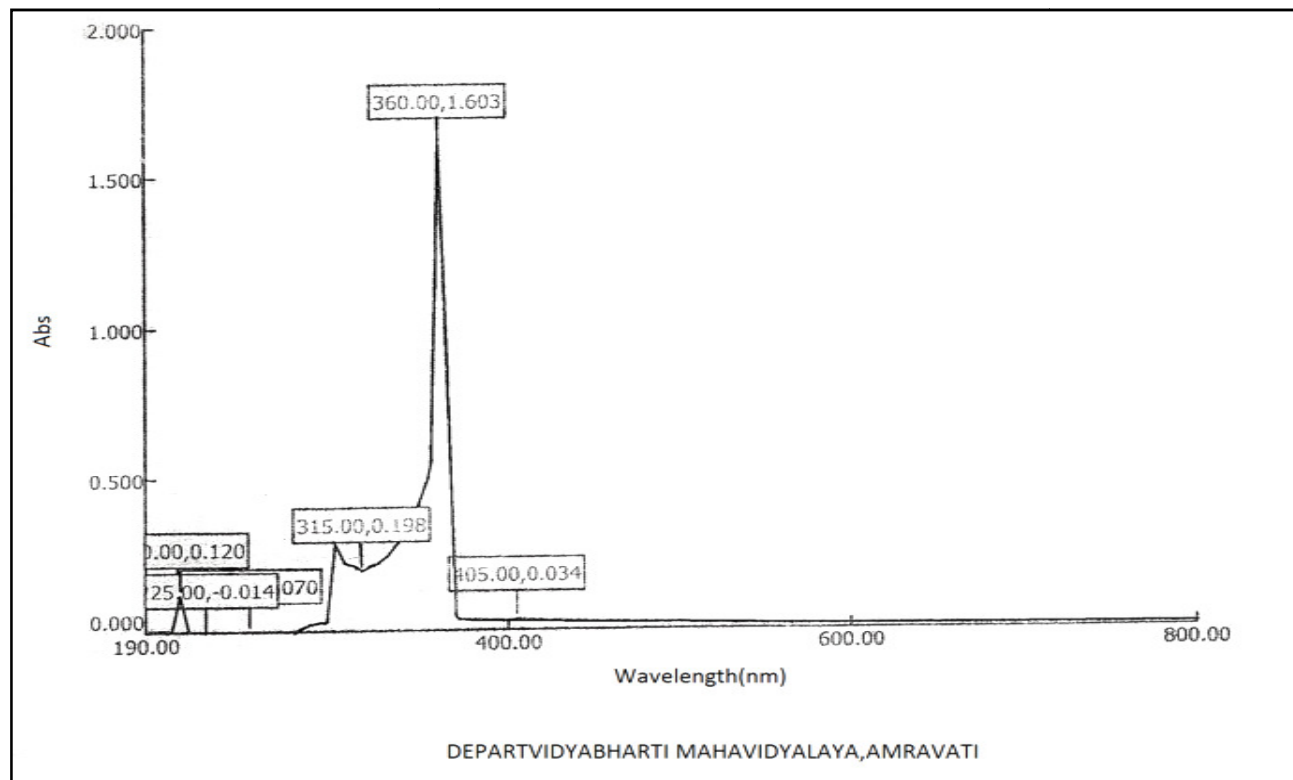


Figure-1  
UV spectra of azo compound 1c

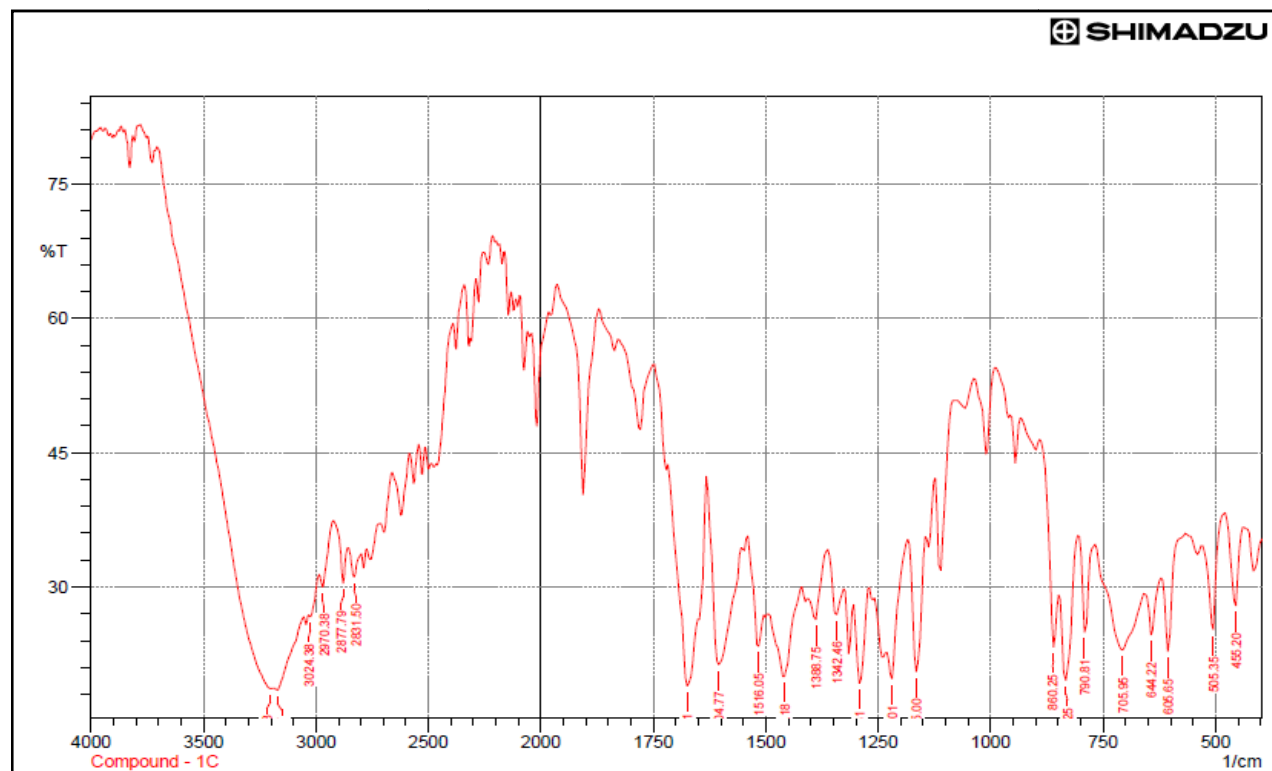


Figure-2  
IR spectra of azo compound 1c

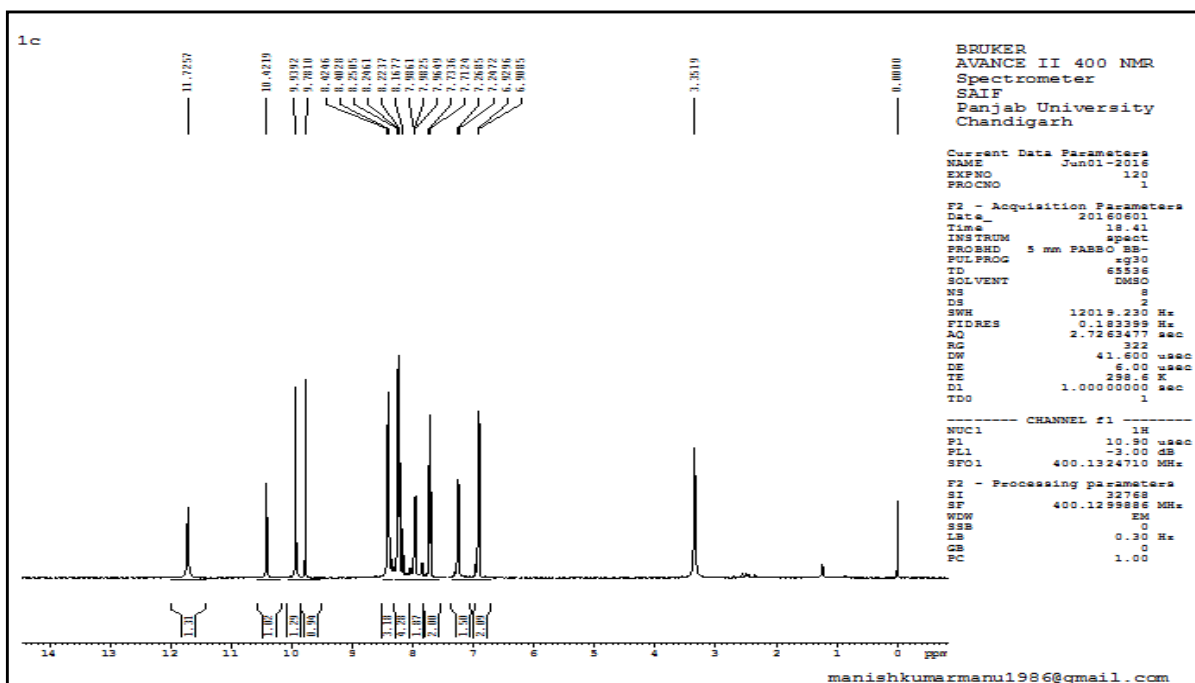


Figure-3  
<sup>1</sup>H NMR spectra of azo compound 1c

## Results and Discussion

In this work, total five substituted azo compounds namely 1a-e were successfully synthesized from five different aromatic amines by simple diazotization-coupling reactions, recrystallised and two different concentrations of each compounds were prepared and further used individually to test their antibacterial activities against three microorganisms viz. *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*. From the data on antimicrobial activities of azo compounds given in Table-2, it was observed that the azo compounds 1a, 1b, 1d and 1e do not showed any inhibitory action and were found to be inactive against all the tested microorganisms at both the concentrations. Only the compound 1c showed inhibitory action against *E. coli*, *S. aureus* and *S.typhi* at both concentrations. At 50 mcg/ml concentration, compound 1c showed 6, 13 and 7 mm of zones of inhibition and at 100 mcg/ml concentrations showed 8, 14 and 8 mm of zones of inhibition against *E. coli*, *S. aureus* and *S.typhi* respectively. The maximum 13 and 14 mm zones of inhibition of compound 1c was found to be against *S. aureus* species at 50 mcg/ml and 100 mcg/ml concentrations respectively. Also Streptomycin at the concentration of 25 mcg/disc showed 20, 26 and 25 mm zones of inhibition against the microorganisms *E. coli*, *S. aureus* and *S.typhi* respectively.

## Conclusion

Out of the five laboratory synthesized substituted azo compounds, the compound 1c is found to be active and showed low to moderate antibacterial activity against all the microorganisms tested. The inhibitory effect of azo compounds

1c recorded especially against *S. aureus* was most satisfactory at both the concentrations and hence it can be used as lead compound against microorganisms *S.aureus*.

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