Research Article

Synthesis, Characterization, Biological Activity and Electrochemical studies of Heterocyclic Azo Dye

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Abstract

A series of heterocyclic azo dyes were synthesized by diazotization of 2-Amino-5-(4-nitrophenyl)-1,3,4-thiadiazole by propionic acid and acetic acid in the ratio 2:3, followed by coupling with different coupling components. Synthesized heterocyclic azo dyes were characterized by UV-Vis, IR, ^{1}H-NMR, ^{13}C-NMR, element analysis and Mass spectral techniques. Electrochemical property of synthesized thiadiazole substituted azo dyes was studied by cyclic voltammetric technique. Synthesized azo dyes showed two reduction peaks indicating the two step reduction process. Probable mechanism for the reduction of azo dyes was proposed. The synthesized heterocyclic azo dyes were screened for biological activity. The results of these investigations revealed that the newly synthesized compounds are potent antimicrobial agents. Some of the synthesized compounds exhibit significant antimicrobial activity.

Keywords: 2-Amino-5-(4-nitrophenyl)-1,3,4-thiadiazole, azo dyes, cyclic voltammetry, biological activity.

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Introduction

Azo dyes are the most widely used synthetic organic compounds, with an enormous variety of applications [1]. They can be synthesized easily and inexpensively using a large variety of diazo and coupling components. These showed high dyeing and good fastness properties and wide applications in areas such as dyeing of textile fibers, plastics, leather, paper and biomedical studies [2].

In recent, years heterocyclic based azo dyes, have found great success due to their higher tinctorial strength, brighter dyeing and excellent light, washing and sublimation fastness, and chromophoric strength in relation to diazo dyes based uniquely on azobenzene derivatives [3]. Heterocyclic azo dyes have wide applications as high level-dying agents in the dyestuff industries [4-6]. The increasing usage of these dyes in electronic industry, such as colorimetric sensors, nonlinear optical (NLO) devices and liquid crystalline displays (LCDs) have been investigated as potential sensitizers for photodynamic therapy (PDT) and has attracted much attention [7-9].These are successfully employed as LCD color filters, chromophoric substrates for redox enzymes, optical switches, chemical sensors, textile dyes, lasers and also they have advanced applications in organic synthesis [10-14].

With these objects in view and also work carried out in our lab on above class of azo dyes [15-17] we now focused on synthesis, screening for antimicrobial activity and electrochemical studies of heterocyclic azo dyes. 2-Amino-5-(4-nitrophenyl)-1,3,4-thiadiazole was transformed to their corresponding diazonium salt by diazotization reaction and were further coupled with various coupling agents 2-naphthol, 8-hydroxy quinolone and 3-hydroxy-*N*-phenylnaphthalene-2-carboxamide (naphthol-AS) under suitable experimental reaction.

Materials and Methods

2-Amino-5-(4-nitrophenyl)-1,3,4-thiadiazole was purchased from Sigma Aldrich and used without further purification. All other chemicals used were of analytical grade. Melting point was taken in an open capillary tube and was uncorrected. The purity of the compound was confirmed by thin layer chromatography (TLC) using Merck silica

gel 60 F254 coated aluminum plates. Infrared spectra of azo dyes were recorded in the region of 4000 cm^{-1} – 400cm^{-1} on a FT-IR 8400s SHIMADZU spectrometer in KBr pellets. The ^{1}H and ^{13}C- NMR spectra were recorded in DMSO-d_6, using Bruker 500 MHz instrument. LC-MS was obtained using Agilent 1200 series LC and MicromasszQ spectrometer. The UV-Visible absorption was recorded in DMSO solvent with SHIMADZU UV-Visible 1650 spectrometer in a wavelength range 200 - 800 nm. Elemental analysis was carried out by using VARIO EL-III (Elementar analysensysteme GmbH). Electrochemical properties and redox properties of dyes was studied in Electroanalyser-201Chemi link.

Procedure for the synthesis of heterocyclic azo dyes: 2(a-c)

2-Amino-5-(4-nitrophenyl)-1,3,4-thiadiazole ($2.0X10^{-3}$mol) was dissolved in hot glacial acetic acid-propionic acid mixture (2:1, 6.0 ml) and was rapidly cooled in an ice/salt bath to -5 ^{0}C. The liquor was then added in portions during 30 minutes to a cold solution of nitrosylsulphuric acid (prepared from sodium nitrite (0.15 g) and concentrated sulphuric acid (3 ml at 50 ^{0}C). The mixture was stirred for an additional 2 hours at $0^{0}C$. Excess nitrous acid was neutralized by addition of urea. The resulting diazonium salt was cooled in salt/ice mixture. After diazotization was complete the diazo liquor was slowly added to vigorously stirred solution of coupling component (0.002 mol) in potassium hydroxide (0.002 mol) and water (2 ml). The solution was stirred at 0-5 ^{0}C for 2 hours. After 2 hours the pH of the reaction mixture was maintained in the range of 4-6 by the simultaneous addition of saturated sodium carbonate solution. The mixture was stirred for 4 hours at room temperature and the resulting solid was filtered, washed with cold water and dried.

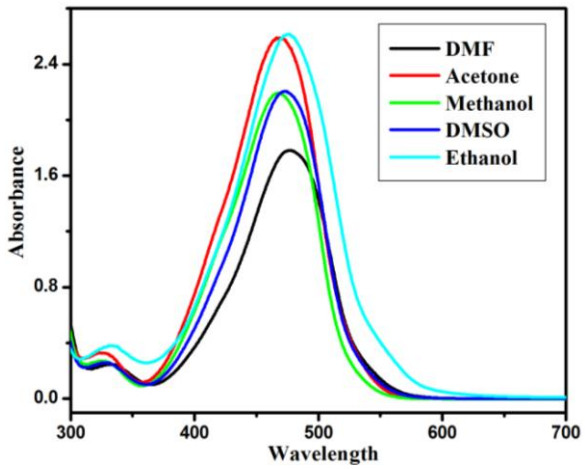


Figure 1 UV-visible spectra of dye 2a in different solvents.

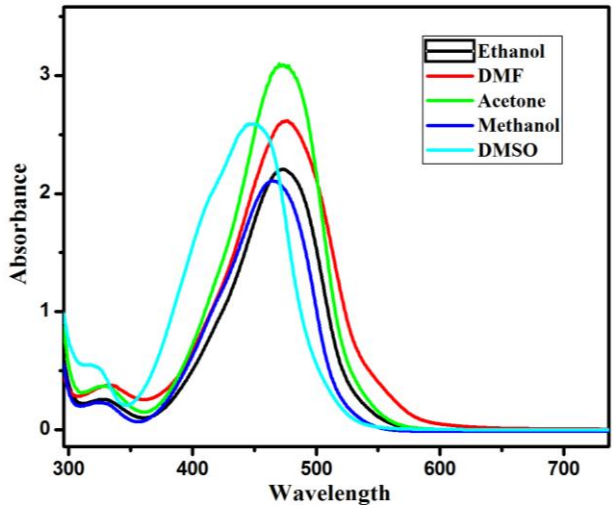


Figure 2 UV-visible spectra of dye 2b in different solvents.

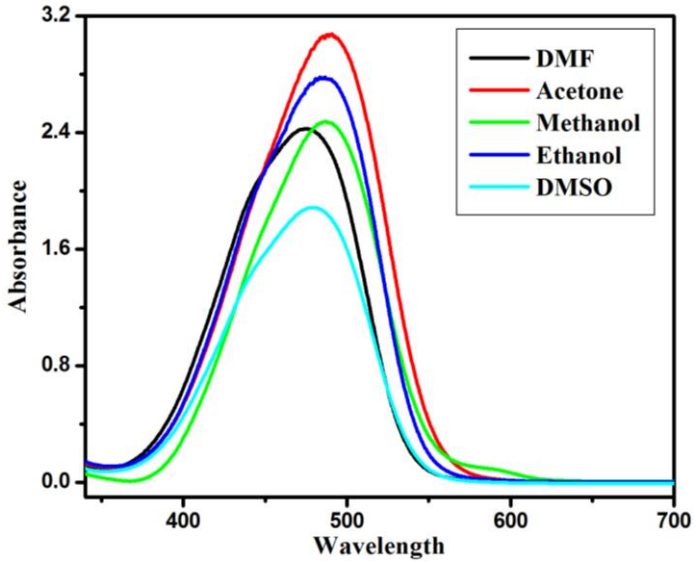


Figure 3 UV-visible spectra of dye 2c in different solvents.

Spectral data of 1-[5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl]diazenyl}-2-naphthol:[2a]

The dye was obtained from 2-Amino-5-(4-nitrophenyl)-1,3,4-thiadiazole and 2-naphthol as brick red colour are (yield-61% ,m.p:225). IR [(KBr) υ $_{max}$/cm^{-1}]: 3450-3410 cm^{-1} (broad -OH group), 3063 (aromatic C-H), 1573(C=N), 1510 (-N=N-) cm^{-1};^{1}H NMR (DMSO-d_6): 8.5 (d, 1H), 7.9 (d, 1H), 7.8 (t, 2H), 6.80 (d, 1H),7.2 - 7.5 (m, 5H), ^{13}C NMR (DMSO-d_6): 153.9(C-O), 130.4(C-N), 174.6(C=N), 150.8 (C-N,Quinoline), MS *m/z = 3*78(M^{+}); Anal. calcd. for $C_{18}H_{11}N_5O_3S$: C,57.29; H,2.94; N,18.56; Found: C,57.25; H,2.90; N,18.51%.

Spectral data of 5-[5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl]diazenyl)quinolin-8-ol:[2b]

This dye was obtained from 2-Amino-5-(4-nitrophenyl)-1,3,4-thiadiazole and 8-hydroxy qinoline as brown colour (yield-48% , m.p:237) IR [(KBr) υ $_{max}$/cm^{-1}]: 3450-3400 cm^{-1} (broad -OH group), 3030 cm^{-1} (aromatic C-H), 1545 (N=N) cm^{-1},1590 (C=N) cm $^{-1}$; ^{1}H NMR (DMSO-d_6): 14.6(s,1H) (-OH), 7.1 (d, 1H), 7.5 - 7.6 (d, 3H), 7.7 - 8.5 (m, 7H); ^{13}C NMR (DMSO-d_6): 162.9(C-O), 130 (C-N), 172 (C=N), MS *m/z = 3*79(M^{+}); Anal. calcd. for $C_{17}H_{10}N_6O_3S$: C,53.96; H,2.66; N,22.21; Found: C,53.91; H,2.62; N,22.17%.

Preparation of heterocyclic azo dye (2c)

This dye was obtained from 5-phenyl-1,3,4-thiadiazol-2-amine and naphthol-AS as red colour. (Yield 57%, m.p:230);IR [(KBr) υ $_{max}$/cm^{-1}]: 3069.21cm^{-1}(Ar-CH), 1511.26cm^{-1}(-N=N-), 1641cm^{-1}(C=N); ^{1}H NMR (400 MHz, DMSO-d_6): 14.29(s, 1H, -OH), 11.2(s, 1H, NH), 8.1(s, 1H,), 7.8(d,2H, Ar H), 7.7(d, 2H, Ar H), 7.6(d, 2H, Ar H), 7.5(t, 2H, Ar H), 7.26(s, 1H, Ar H); ^{13}C NMR (DMSO-d_6): 126(C-O), 153 (C-N), 127(C=N)(azo), 153 (C-S),164(C-NH); MS *m/z* =497.5 (M^{+}), Anal. calcd. for $C_{25}H_{16}N_6O_4S$: C,60.48; H,3.25;N,16.93; Found: C, 60.01; H, 3.19; N, 16.89%.

Results and Discussions

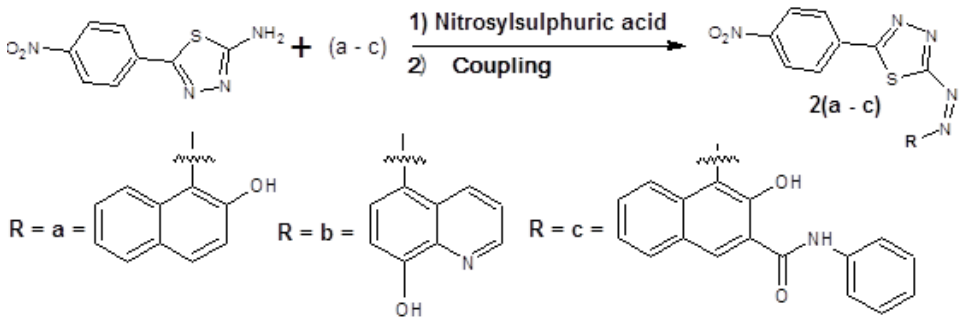
As shown in **Scheme 1**, the hetarylazo dyes 2(a-c) were prepared through the diazotization of 2-Amino-5-(4-nitrophenyl)-1,3,4-thiadiazole and coupled with different coupling components such as 2-naphthol, 8-hydroxyquinoline and naphthol AS . The synthesized dyes were characterized by FT-IR, UV-Vis, ^{1}H and ^{13}C NMR, mass and elemental analysis.

Infrared spectra of synthesized dyes (in KBr) 2a, 2b and 2c, a broad peak has appeared at the region 3500-3200 cm^{-1} which confirms the presence of hydroxyl group (-OH). The dyes 2(a-c) are showed 1450-1530 cm^{-1}for (-N=N-) group and at 3085-3005 cm^{-1} (aromatic C-H) were also observed. Electronic absorption spectra of all compounds 2(a-c) were recorded in different solvents DMSO, DMF, acetone, ethanol and methnol at a concentration of 10^{-4} - 10^{-6}mol

L^{-1} were showed in figure 1, figure 2 and figure 3 respectively. Azo dyes showed an intense lowest energy charge-transfer absorption band in the UV-visible region. Synthesized azo dyes are exhibit λ_{max} values around 400 to 550nm.^{1}H NMR spectra was recorded in DMSO-d_6 in dye 2a singlet at 14.3 ppm for hydroxyl group(-OH) of 2-naphthol, a multiplet from 7.20 to 7.50 ppm for aromatic protons (Aro-H). In 2b singlet at 14.6 for hydroxyl group(-OH). In dyes 2c the signals within the range of 7.25–7.6 ppm are attributable to aromatic protons and –NH proton at the range of 9.6.

^{1}H NMR, ^{13}C NMR, Elemental analysis and Mass spectral data was found in good agreement with the newly synthesized compounds.

SCHEME - 1



Biological Activity

Antibacterial and Antifungal activity

The antimicrobial activity of newly synthesized compounds was evaluated using agar disc diffusion assay [18]. Briefly, 24 hours old culture of bacteria and 48 hours old culture of fungi was mixed with sterile physiological saline (0.9%) and the turbidity was adjusted to the standard inoculum of MacFarland scale 0.5 (10^6 colony forming units (CFU) per ml). Petri plates containing 20 ml of Mueller Hinton Agar and Sabouraud-dextrose agar was used for antibacterial and antifungal activity respectively. The inoculums was spread on the surface of the solidified media and Whatman No. 1 filter paper discs (5 mm in diameter) impregnated with the test compound (20 µl/disc) were placed on the solidified media. Streptomycin (5 mg/disc) and Fluconazole (5 mg/disc) were used as positive control for bacteria and fungi respectively along with DMSO disc as negative control. Zone of inhibition was recorded in millimeters after incubating bacterial strains at 37°C (24hr) and fungal strains at 25°C (72hr). Tests were performed in triplicate and the values were expressed as mean ± SD [19, 20].

Synthesized organic compounds were evaluated for the antimicrobial activity with standard drugs (Streptomycin and fluconazole**)**. The closer look into the biological studies of these synthesized dyes revealed that compound **2b** showed much better activities when compare to the other compounds. The results from the antimicrobial activity of synthesized organic compounds (**Table 1**) prompted us to investigate their antifungal activity (**Table 2**) against important pathogens. Although a comparable antibacterial activity was exhibited by all compounds.

Table 1 *In vitro* antibacterial activities of the compounds 2(a-c).

Sl. No		**Diameter of zone of inhibition (mm)**					
		Escherichia coli		***Staphylococcus aureus***		***Pseudomonas aeruginosa***	
	Conc. in mg/ml	1	0.5	1	0.5	1	0.5
1	**Control**	00	00	00	00	00	00
2	**Standard Streptomycin**	16±0.2	10±0.3	15±0.4	10±0.5	16±0.3	13±0.6
3	2a	06±0.5	04±0.7	08±0.6	07±0.3	07±0.5	05±0.5
4	2b	12±0.4	09±0.6	12±0.4	10±0.5	13±0.3	11±0.2
5	2c	04±0.2	01±0.2	02±0.5	00	03±0.3	01±0.1

Minimal inhibitory concentrations (MIC)

The in vitro determination of the Minimum inhibitory concentration (MIC) against selected bacterial strains was

carried out using serial dilution method. The agar dilution susceptibility test was performed based on the reported method to determine the MIC of the synthesized compounds [21]. The test compounds 2(a–c) dissolved in sterilized 5% DMSO (400 mg/mL concentration) were taken as standard stock. A series of two fold dilutions of each compound in the final concentrations of 400, 300, 200, 100, 50, 25, 13 and 7 mg/mL were prepared in nutrient agar for bacteria. After solidification, the plates were spotted with 100 µL of overnight grown bacterial cultures approximately containing $1X10^4$ CFU/mL. The test was carried out in triplicates. The plates of bacterial culture were incubated at 37^0C for 18-24. After incubation, the MIC was determined.

Table 2 *In vitro* antifungal activities of the compounds 2(a-c).

Sl. No		**Diameter of zone of inhibition (mm)**					
		Aspergillus flavus		***Chrysosporium keratinophilum***		***Candida albicans***	
	Conc. in mg/ml	1	0.5	1	0.5	1	0.5
1	**Control**	00	00	00	00	00	00
2	**Standard Flucanazole**	18±0.5	15±0.3	16±0.7	14±0.2	23±0.8	20±0.3
3	2a	05±0.6	03±0.3	04±0.6	01±0.4	04±0.2	01±0.1
4	2b	09±0.5	06±0.3	10±0.7	08±0.8	11±0.5	07±0.2
5	2c	04±0.3	02±0.2	05±0.3	03±0.6	03±0.3	02±0.1

The capacity of antimicrobial activity of the three newly synthesized compounds against six (3 bacteria and 3 fungi) human pathogen microorganisms were assessed both qualitatively and quantitatively by the presence or the absence of inhibition zone and MIC values with standard drugs (Streptomycin and Fluconazole**)**. The results of antibacterial activities reveals that the synthesized compounds showed lower antimicrobial activities than that of standard. The compound 2a (100, 50, 50 of MIC), 2b (50, 25, 50 of MIC) and 2c (200, 100, 100 of MIC) show good and comparable activity that of standard the compounds shows an MIC less than 75 µg/ml considered to have strong antimicrobial activity, from 75 to 150 µg/ml the activity is considered as moderate, from 150 to 250 µg/ml the antimicrobial activity was weak and over 250 µg/ml, the compounds was considered as inactive (**Table 3**). Compound 2b showed good activity comparing to remaining synthesized dyes.

Table 3 Minimum Inhibitory Concentration of 2(a-c).

Sl No	**Compounds**	**Concentration in µg/ml**		
		Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
1	2a	100	50	50
2	2b	50	25	50
3	2c	200	100	100

Cyclic voltammetry studies

The redox property of synthesized thiadiazole substituted azo dyes were studied by cyclic voltammetry. All the dyes contain electroactive species with an azo group (-N=N-). The azo group easily reduced on Glassy carbon electrode. All the experiment was carried out in Electroanalyser-201 cyclic voltammetry using $1X10^{-2}$M H_2SO_4 as a supporting electrolyte and DMF as a solvent. Electrochemical cell consists of glass container with cap having holes for introducing electrodes. The reference electrode used was saturated calomel (SCE), the auxiliary and working electrode were platinum wire and glassy carbon electrode respectively. The cyclic voltammetric studies of the dye were carried out in the potential range +800 to -1000mV at scan rate 50 mVs^{-1}. The cyclic voltammogram of $1X10^{-3}$ M concentrated dyes 2(a-c) recorded in 0.01M H_2SO_4. Cyclic voltammogram for dye 2a, 2b and 2c were shown in the **Figures 4-8**. It gives two reduction peaks for assigned potential range. But the irreversibility was confirmed by the absence of anodic peak in voltammogram between the potential of +800 to -1000mV. The different dye showed reductive peak (E_{Pc1}) and (E_{Pc2}) mVs^{-1} potentials. The values are given in the **Table 4**. The general mechanism of reduction is two step two electron change of the azo group. The scan rate effect showed that by increasing the scan rate from $50mVs^{-1}$ to $150mVs^{-1}$ the current also increases with peak shift towards the less positive direction. The probable mechanism of reduction of azodyes was showed in **Scheme 2.**

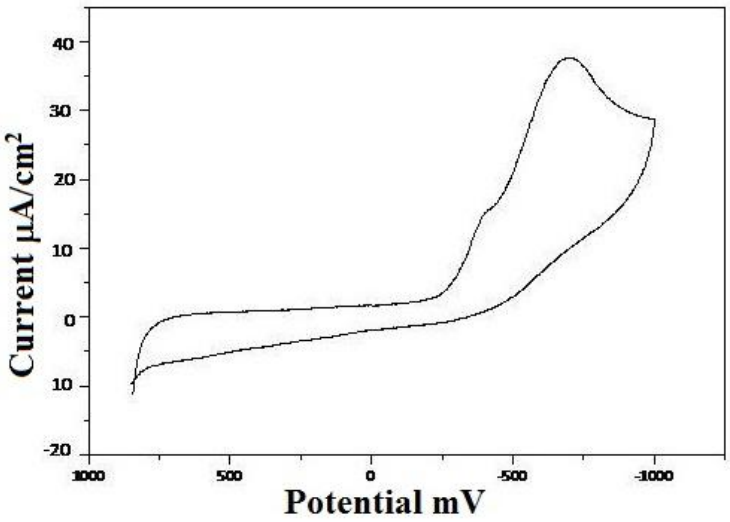


Figure 4 Cyclic voltammogram of 2a at 50mVs^{-1} scan rate.

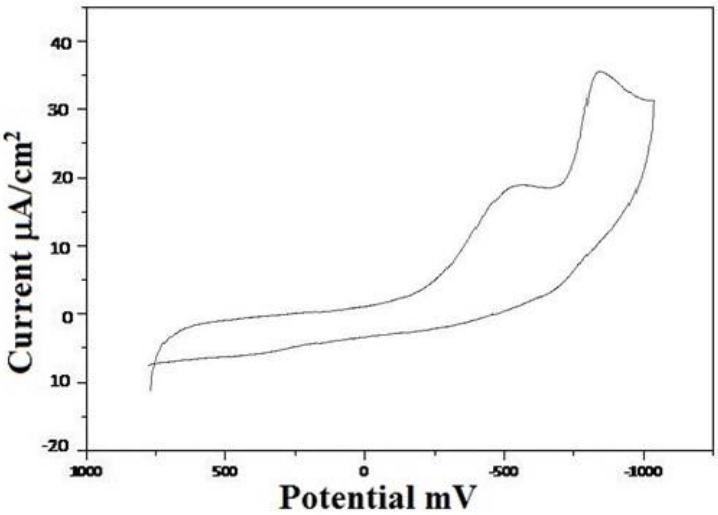


Figure 5 Cyclic voltammogram of 2b at 50mVs^{-1} scan rate.

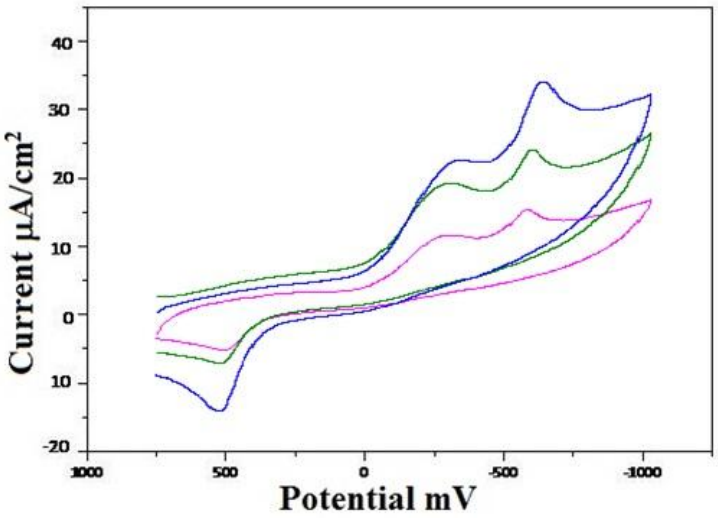


Figure 6 Cyclic voltammograms of 2a at 50mVs^{-1},100mVs^{-1} and 150 mVs^{-1}scan rate.

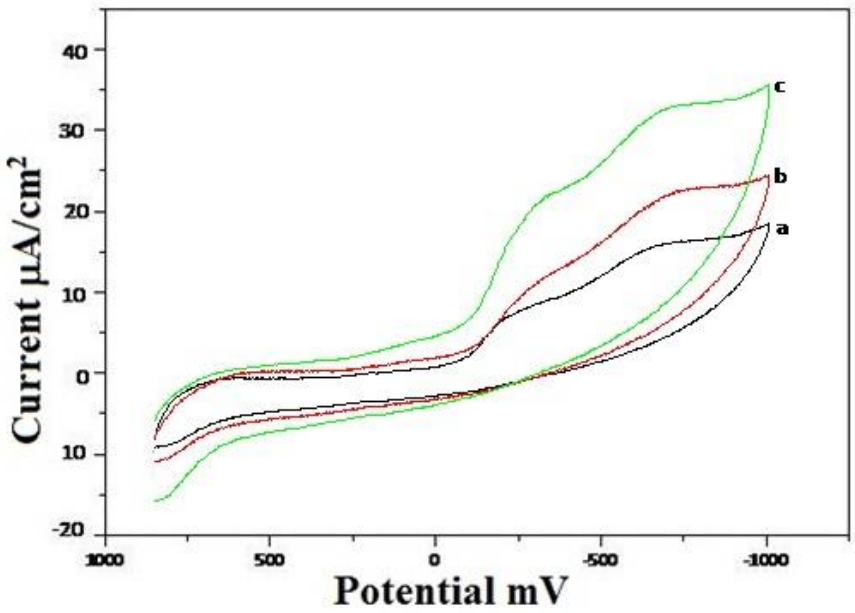


Figure 7 Cyclic voltammograms of 2b at 50mVs^{-1},100mVs^{-1} and 150 mVs^{-1}scan rate.

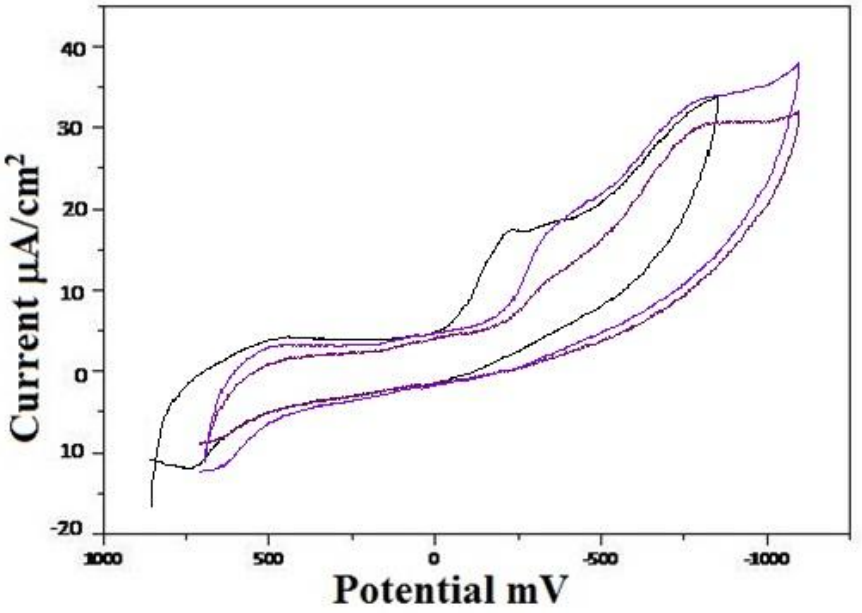
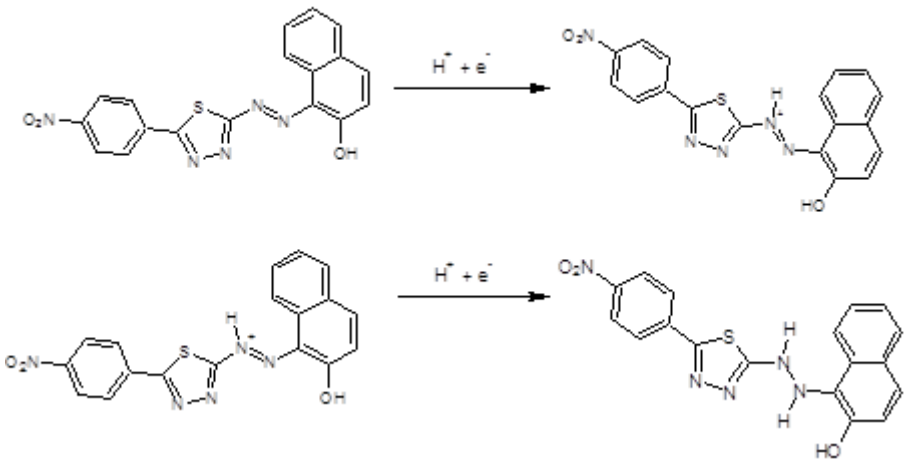


Figure 8 Cyclic voltammograms of 2c at 50mVs^{-1},100mVs^{-1} and 150 mVs^{-1}scan rate.

Table 4 Data Regarding variation of scan rate from 50-150 mVs^{-1} for 2a-2c azodyes

Dye	Scan rate (mV/s)	E_{pc1} (mV)	I_{pc1} (µA)	E_{pc2} (mV)	I_{pa2} (µA)
2a	50	-208	7.43	-510	22.56
	100	-217	13.96	-526	29.28
	150	-218	24.17	-528	34.13
2b	50	-200	6.26	-505	20.00
	100	-210	10.00	-528	27.49
	150	-210	17.10	-529	28.26
2c	50	-202	6.95	-508	21.14
	100	-212	11.32	-524	28.46
	150	-214	19.91	-525	30.21

SCHEME - 2



Conclusion

In this work, 3 new heterocyclic azo dyes were synthesized by diazotizing-coupling. Their structures were confirmed by ^{1}H and ^{13}C NMR, Mass spectral date, FT-IR and UV-Vis spectra. All the dyes tested had some effect on bacterial growth, most potency being shown with dye number **2b**. Eelectrochemical studies of synthesized azo dyes were carried out by cyclic voltammetry. All the dyes undergo two step reduction and showed irreversible two reduction peaks. Probable electrochemical mechanism of the azo dyes also described.

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