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RESEARCH ARTICLE

Synthesis, Characterization and Antimicrobial Activity of Heterocyclic Azodyes Derived from Thiadiazole

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Abstract: 5-Ethyl-1,3,4-thiadiazole-2-amine have been synthesized by single step reaction. A series of heterocyclic azodyes were synthesized by coupling 8-hydroxyquinoline, 2,6-diaminopyridine, *N,N*-dimethyl aniline, 2-napthol and resorcinol with diazotized 5-ethyl-1,3,4-thiadiazol-2-amine in nitrosyl sulphuric acid. These dyes were characterized by UV, IR, ^{1}H NMR, ^{13}C NMR, elemental analysis and mass spectrometry for selected dyes. The synthesized compounds were also screened for biological activity.

Keywords: 5-Ethyl-1,3,4-thiadiazol-2-amine, 8-Hydroxy quinoline, 2,6-Diaminopyridine, *N,N* dimethyl aniline, 2-Napthol, Azo dyes, Antimicrobial activity

Introduction

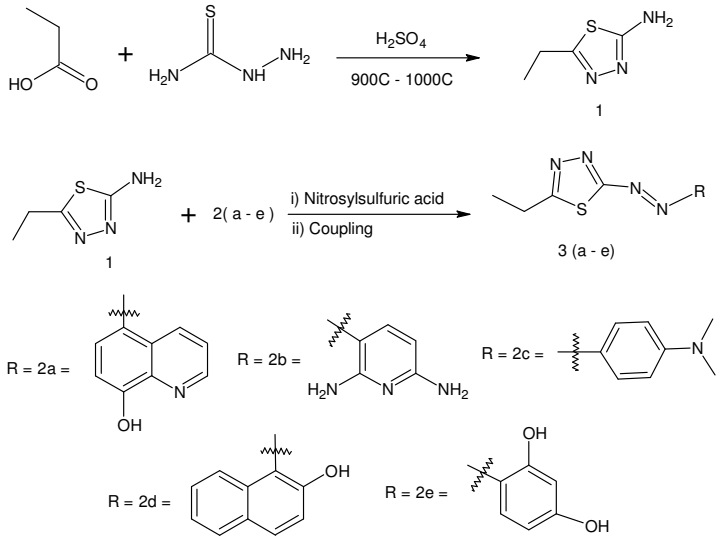
Azo dyes are the most extensively used class of coloring materials because of their massive applications in various fields of science and technology. These are successfully employed as LCD color filters[1], chromophoric substrates for redox enzymes[2], optical switches[3], chemical sensors[4], textile dyes[5], lasers[6], optical data storage[7], non-linear optics[8] and also they have advanced applications in organic synthesis[9]. Azo dyes containing sulfur and/or nitrogen atoms have been the subject of many studies recently. These dyes provide bright and strong shades that range from red to green and blue[10,11]. In this regard, a number of studies have been devoted to the characterization, purification and application of azo dyes as antibacterial agents[12,13]. 1,3,4-Thiadiazole derivatives exhibits a broad spectrum of biocidal activities possibly due to the presence of toxophoric –N-C-S moiety[14].

With these objects in view and also work carried out in our lab on above class of azo dyes[15,16], we now focused on synthesis and screening for antimicrobial activity of heterocyclic azo dyes. 5-Ethyl-1,3,4-thiadiazole-2-amine was synthesized and was transformed

to their corresponding diazonium salt by diazotization reaction and were further coupled with various coupling agents (2-naphthol, 8-hydroxy quinolone, 2,6-diaminopyridine, *N,N*-dimethyl aniline and resorcinol) under suitable experimental reaction.

Experimental

Propionic acid (0.1 mol) and thiosemicarbazide (0.1 mol) in conc. sulphuric acid (20 mL) were refluxed gently for 30 min. The solution was cooled and poured into beaker containing crushed ice. The separated solid was filtered and suspended in water and basified with sodium bicarbonate and kept overnight. The obtained solid was again filtered, washed with water, dried and crystallized from ethanol to obtain a colorless solid with 54% yield. The general scheme for the synthesis is given in the Scheme 1.



Scheme 1

General method for synthesis of hetarylazo dye

5-Ethyl-1,3,4-thiadiazol-2-amine (2.0×10^{-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 min to a cold solution of nitrosyl sulphuric 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 h at 0 ^{0}C. Excess nitrous acid was destroyed 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 8-hydroxyquinoline (2.0×10^{-3} mol) in potassium hydroxide (2.0×10^{-3} mol) and water (20 mL). The solution was stirred at 0-5 ^{0}C for 2 h. After 2 h, the pH of the reaction mixture was maintained at 4-6 by the simultaneous addition of saturated sodium carbonate solution. The mixture was stirred for one day at room temperature. After one day, the resulting solid was filtered, washed with cold water and dried.

Preparation of 5-(5-ethyl-2-thiadiazolylazo)-8-hydroxyquinoline (**3a**)

This dye was synthesized from 5-ethyl-1,3,4-thiadiazol-2-amine and 8-hydroxyquinoline as brown crystals (yield- 50%; m.p: 222 ^{0}C). IR [(KBr) υ_{max}/cm^{-1}]: 3428-3268 (quinoline- OH),

3075 (aromatic C-H), 2973 (aliphatic C-H), 1554 (C=N),1420 (N=N), 1208 (C-O) cm^{-1}; ^1H NMR (DMSO-d_6): 8.8 (d, 1H), 8.6 (d, 1H), 7.8 (t, 1H), 6.7 (d, 1H), 7.2 (d, 1H), 3.1 (q, 2H), 1.4 (t, 3H). ^{13}C NMR (DMSO-d_6, ppm): 14.3(CH_3), 24.5(CH_2), 153.2(C-O), 137.7 (C=N),130.7(C-N), LC-MS: $(M+1)^+$ (m/z): 286 (19.8%), 172 (21.2%), 144(100%). Anal. Calcd. For $C_{13}H_{11}N_5OS$: C,54.72;H, 3.89;N, 24.55; found: C, 54.70; H, 3.86; N, 24.52.

Preparation of 3-[(5-ethyl-1, 3, 4-thiadiazol-2-yl)diazenyl]pyridine-2,6-diamine (**3b**)

This dye was prepared from 5-ethyl-1,3,4-thiadiazol-2-amine and 2,6-diamino pyridine as wine red crystals (yield:62%; m.p: 202 ^{0}C). IR [(KBr) υ_{max}/cm^{-1}]: 3342 cm^{-1}, 3227 cm^{-1} (N-H stretch), 2965 (aliphatic C-H), 1639 cm^{-1} (N-H aromatic) 1427 cm^{-1} (N=N stretch).; ^1H NMR (DMSO-d_6): 7.7 (d, 1H), 6.3 (d, 1H), 3.0 (q, 2H), 1.35 (t, 3H)., ^{13}C NMR (DMSO-d_6, ppm): 14.9(CH_3), 24.9(CH_2), 157.8(C-N), 169.2(C=N), 158.9(C-N), Anal. Calcd. For $C_9H_{11}N_7S$: C,43.36;H, 4.45;N, 39.33; found: C, 43.34; H, 4.42; N, 39.31%.

Preparation of 4-[(5-ethyl-1, 3,4-thiadiazol-2-yl) diazenyl]-N,N-dimethyl aniline (**3c**)

This dye was obtained from 5-ethyl-1,3,4-thiadiazol-2-amine and *N*,*N*-dimethylaniline as dark red crystals (yield-71% , m.p:198). IR [(KBr) υ_{max}/cm^{-1}]: 3015 (aromatic C-H), 2939 (aliphatic C-H), 1546 (C=N) cm^{-1};1435 (N=N) cm^{-1}; ^1H NMR (DMSO-d_6): 7.2 (d, 2H), 6.7 (d, 2H), 2.85 (s, 6H), 3.1 (q, 2H), 1.4 (t, 3H)., ^{13}C NMR (DMSO-d_6, ppm): 14.9(CH_3), 25.1(CH_2), 40.7(CH_3),170.1(C=N), 150.5(C-N), Anal. Calcd. For $C_{12}H_{15}N_5S$: C,55.15;H, 5.79; N, 26.80; found: C, 55.13; H, 5.77; N, 26.78.

Preparation of 1-[(5-ethyl-1, 3, 4-thiadiazol-2-yl)diazenyl]naphthalen-2-ol (**3d**)

This dye was synthesized from 5-ethyl-1,3,4-thiadiazol-2-amine and 2-naphthol as brick red crystals (yield-66% , m.p:219). IR [(KBr) υ_{max}/cm^{-1}]: 3432-3215 (-OH), 2965 (aliphatic C-H), 1542 (C=N),1432 (N=N), ^1H NMR (DMSO-d_6):7.4-7.6 (m, 3H), 7.0-7.2 (m, 3H), 2.9 (q, 2H), 1.3 (t, 3H)., ^{13}C NMR (DMSO-d_6, ppm):15.1(CH_3), 25.4(CH_2), 169.3(C=N), 163.1(C-O), 135.6(C-N). Anal. Calcd. For $C_{14}H_{12}N_4OS$: C,59.14;H, 4.25;N, 19.70; found: C, 59.11; H, 4.22; N, 19.68%.

4-[(5-Ethyl-1,3,4-thiadiazol-2-yl)diazenyl]benzene-1,3-diol (**3e**)

This dye was prepared from 5-ethyl-1,3,4-thiadiazol-2-amine and resorcinol as red crystals (yield-64% , m.p:189). IR [(KBr) υ_{max}/cm^{-1}]: 3428-3248 (-OH), 2965 (aliphatic C-H), 1548 (C=N),1439 (N=N), ^1H NMR (DMSO-d_6):6.4-6.8 (dd, 2H), 6.2 (s, H), 2.9 (q, 2H), 1.4 (t, 3H)., ^{13}C NMR (DMSO-d_6, ppm):14.7(CH_3), 24.4(CH_2), 168.5(C=N), 160.1(C-O). Anal. Calcd. For $C_{10}H_{10}N_4O_2S$: C,47.99;H, 4.03;N, 22.39; found: C, 48.03; H, 4.07; N, 22.42%.

Biological activity

Antibacterial and antifungal activity

The antimicrobial activity of newly synthesized compounds was evaluated using agar disc diffusion assay[17-18]. Briefly, a 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. 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 (24 h) and fungal strains at 25 °C (72 h). Tests were performed in triplicate and the values were expressed as mean ± SD[19-20].

Results and Discussion

As shown in Scheme 1 the hetarylazo dyes **3(a-e)** were prepared through the diazotization of 5-ethyl-1,3,4-thiadiazol-2-amine and coupled with different coupling components 2-napthol, 8-hydroxyquinoline, 2,6-diaminopyridine *N,N* dimethyl aniline and resorcinol. These dyes were in good yield and these are soluble in acetone, DMF and DMSO. The yield, melting point, $\lambda_{max,}$ molar absorptivity (ε), molecular formula and solubility data of all synthesized dyes are tabulated in Table 1. Absorption spectra of the azo dyes **3(a-e)** were recorded in DMSO at a concentration of 10^{-6} mol L^{-1}. The results are summarized in Figure 1. The infrared spectra of all the dyes (in KBr) for the dyes **3a, 3d** and **3e** a broad band has appeared at 3500-3200 which confirms the presence of hydroxyl group (-OH). The dyes **3(a-e)** are showed 1417-1435 cm^{-1} for (N=N) group. The υ_{max} values at 3085-3005 cm^{-1} (aromatic C-H) and at 2986-2851 cm^{-1} (aliphatic C-H) were also observed.

Table 1. Yield, melting point, $\lambda_{max,}$ molar absorptivity (ε), molecular formula and solubility data of dyes **3(a-e)**

Dye	Yield, %	M.P, ^{0}C	λ_{max} in nm	log ε	Molecular formula	Mol. wt	Solubility
3a	50	220-222	522	4.47	$C_{13}H_{11}N_5OS$	285.32	Acetone / DMF Ethanol /DMSO
3b	62	199-202	515	4.29	$C_9H_{11}N_7S$	249.29	Acetone/DMSO DMF/Methanol
3c	71	195-198	513	4.68	$C_{12}H_{15}N_5S$	261.34	Acetone/ DMSO Methanol/DMF
3d	66	215-219	509	4.09	$C_{14}H_{12}N_4OS$	284.33	Acetone/ DMSO Methanol/DMF
3e	64	188-190	517	4.23	$C_{10}H_{10}N_4O_2S$	250.27	Acetone/ DMSO Methanol/DMF

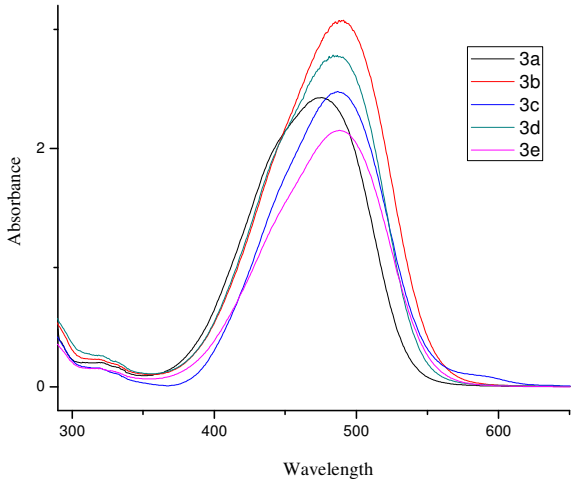


Figure 1. Absorption spectra of dyes **3(a- e)** in Acetone

The ^{1}H NMR spectra was recorded in DMSO-d_6 at room temperature showed a quadrate at 3.0-3.2 ppm (CH_2), a triplet at 1.4- 1.85 ppm (CH_3) for the dyes **3(a-e)**, in dye **3c** singlet at 2.85 ppm are for –$(CH_3)_2$ group of *N,N*-dimethyl aniline, a multiplet from 7.20 to 7.50 ppm for aromatic protons (Aro-H).

Biological activity

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 **3a** and **3b** showed much better activities when compare to the other compounds. The results from the antimicrobial activity of synthesized organic compounds (Table 2) prompted us to investigate their antifungal activity (Table 3) against important pathogens. Although a comparable antibacterial activity was exhibited by few compounds, these compounds failed to show a good response to antifungal activity and the compound **3c** doesn't show any antimicrobial property.

Table 2. *In vitro* antibacterial activities of the compounds **3(a-e)**

S.No.	Compounds	*Escherichia coli*		*Staphylococcus aureus*		*Pseudomonas aeruginosa*	
		Diameter of zone of inhibition, mm					
	Conc. in mg/mL	1	0.5	1	0.5	1	0.5
1	Control	0		0		0	
2	Standard Streptomycin	16±0.4	10±0.5	15±0.4	10±0.4	16±0.5	13±0.4
3	**3a**	8±0.4	4±0.5	7±0.5	4±0.6	8±0.4	4±0.5
4	**3b**	0.7±0.4	4±0.6	6±0.6	4±0.5	7±0.5	4±0.4
5	**3c**	2±0.5	2±0.5	2±0.6	2±0.5	2±0.4	2±0.4
6	**3d**	2±0.4	2±0.5	2±0.5	2±0.5	2±0.4	2±0.5
7	**3e**	2±0.4	2±0.5	2±0.6	2±0.4	2±0.4	2±0.5

Table 3. *In vitro* antifungal activities of the compounds **3(a-e)**

S.No.	Compounds	*Aspergillus Flavus*		*Chrysosporium Keratinophilum*		*Candida Albicans*	
		Diameter of zone of inhibition, mm					
	Conc. in mg/mL	1	0.5	1	0.5	1	0.5
1	Control	0		0		0	
2	Standard Streptomycin	13±0.4	10±0.6	17±0.4	15±0.5	22±0.5	20±0.4
3	**3a**	6±0.6	3±0.5	7±0.4	4±0.6	5±0.5	2±0.6
4	**3b**	2±0.5	1±0.4	2±0.6	1±0.5	2±0.6	1±0.5
5	**3c**	0	0	0	0	0	0
6	**3d**	2±0.6	1±0.4	2±0.6	1±0.5	2±0.6	1±0.5
7	**3e**	2±0.5	1±0.4	2±0.6	1±0.5	2±0.6	1±0.5

Conclusion

In this work, 5 new heterocyclic azo dyes were synthesized by a classical method of diazotizing-coupling. Their structures were confirmed by ^{1}H and ^{13}C NMR, FT-IR and UV-Vis spectra. All the dyes tested had some effect on bacterial growth, most potency being shown with dye number **3a** and **3b**. Dye **3a** is shown the good antifungal activity.

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