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Research Article

SYNTHESIS AND THEIR ANTIFUNGAL, ANTIHELMENTIC AND DYING PROPERTIES OF SOME NOVEL AZO DYES

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ABSTRACT
Azo dyes represent the largest class of synthetic dyes and their breakdown products are toxic and/or mutagenic to life. This paper describes the synthesis of some novel azo dyes and evaluation for their resistance against the growth of fungi organisms, antihelmentic properties, and dyeing abilities on cotton fabrics. The products were characterized by UV-Vis, IR, ^{1}H NMR, ^{13}C NMR spectral studies and elemental analysis.

Keywords: Xenobiotic, carcinogenic, biodegradation, anaerobic, colorants, oxidation.

INTRODUCTION

Azo dyes are a class of compounds containing a N=N double bond generally connected to aromatic rings. Due to their ability to absorb visible light, and ease of synthesis, have been extensively used in the textile, fiber, leather, paint and printing industries for more than a century. Synthetic azo dyes produced in large quantities are generally considered as xenobiotic compounds which are very recalcitrant to biodegradation. Textile industry is a major consumer of these dyes. The industrial effluents containing azo dyes released to the eco system undergo reductive cleavage to form aromatic amines that have known mutagenic and/or carcinogenic properties. The usual synthesis of azo dyes involves diazotization of a primary aromatic amine, followed by coupling with nucleophiles.

Azo dyes synthesized have been evaluated for their antibacterial activities against the gram positive and gram negative microorganism. The results of the study revealed that these show moderate antibacterial activity against different organism[1]. A triazo dye synthesized from tri(*p*-aminophenyl)amine and 2-hydroxy-3-napthoic acid had explored the possibilities of its uses in dye sensitized solar cells. The photocells were able to generate reasonably high photocurrent in the presence of the electron donating ionic liquids in the electrolyte composed of redox couple[2]. Naphthalimide-based alkali-clearable azo disperse dyes containing a fluorosulfonyl group were synthesized. These dyes were applied to polyester fabric by the HT method and exhibited good leveling, wash and sublimation fastnesses and moderate light fastness[3].

The Schiff base prepared from ninhydrin and 3-amino phenol was coupled with the dizonium solution of various aromatic amines leads to disperse monoazo dyes. These dyes showed excellent dye bath exhaustion and fixation on the polyester fibers and very good light fastness and washing fastness properties[4]. 1,2-hydroxo phenyl azo dyes and their metal complex with chromium and cobalt salts synthesized showed dyeing protein and polyamide fibers with excellent light and wash fastness. These dyes are used in electrophotographic or sensor applications for photoconductors, lasers, electro-optical devices and ink-jet printers[5].

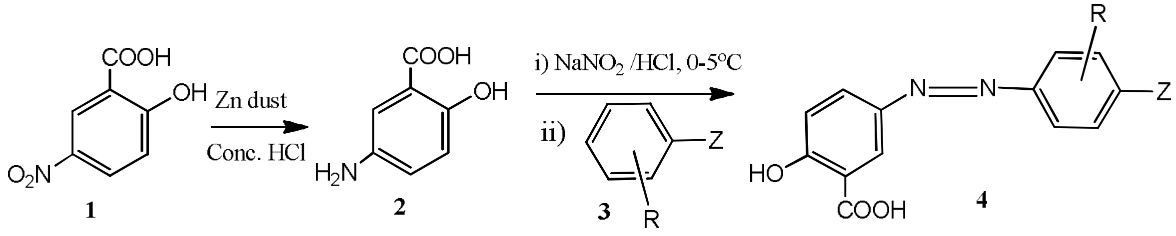
In an environmental context; dye evaluation is complicated due to their relatively low water solubility, do not partition into groundwater to any appreciable extent, and degrade within the environment into more soluble toxic compounds. They degraded products have dermal and immunological effect on human beings. Hence uses of some classes of azo dyes were banned all over the globe. The extensive research was undertaken all over to make these dyes eco-friendly or to transform their carcinogenic degrade products to environment friendly. For instance, the horse radish peroxidase (HRP) in the presence of H_2O_2 was reported to be very effective treatment methodology for degradation of azo dyes[6].

The efficient organisms that degrade azo dyes were isolated from three different sources and were mixed to get consortia. The efficiency of azo dye degradation by consortia was analyzed in presence of various co-substrates, such as glucose, starch, lactose, and sewage water[7]. A sequential anaerobic-aerobic treatment process based on mixed culture of bacteria isolated from textile dye effluent-contaminated soil was devised to degrade reactive azo dyes[8]. A two stage sequential Fenton's oxidation followed by aerobic biological treatment train was used to achieve decolorization and to enhance mineralization of azo dyes at an optimum pH 3[9]. Various mechanisms including enzymatic and low molecular weight redox mediators have been proposed for the non-specific reductive cleavage azo dyes present in industrial waste water[10]. Herein we report the synthesis of some novel azo dyes starting from 5-nitro salicylic acid and the results of their antifungal, antihelmentic activities and their dyeing ability to cotton fabrics.

MATERIALS AND METHODS

The chemicals/reagents used were purchased from Merck Chemicals (India). Electronic absorption studies were carried out by using a solution of the synthesized compounds in dimethyl sulphoxide on UV-Visible spectrometer (UV-160 Shimadzu), the absorption maxima measured in nm and the absorbance values are given in the bracket. IR spectra (KBr pellet) were recorded on Shimadzu 8300 spectrometer. The ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker supercon 400 MHz spectrophotometer using $CDCl_3$ as solvent and chemical shifts are expressed in δ ppm. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyser. Thin layer chromatography (TLC) were performed on a pre-coated Silica Gel sheets (HF 254, sd-fine) using hexane:ethyl acetate (7:1) eluent and visualization of the spots was done in UV light. Chromatographic separations were carried out on silica gel (70-230 mesh, Merck) column using hexane:ethyl acetate (7:1) as eluent.

In a typical procedure, 5-nitro salicylic acid (**1**) is prepared from salicylic acid, then was reduced to 5-amino salicylic acid (**2**). Then 5-amino salicylic acid was subjected to diazotization reaction, the resulting diazonium chloride was coupled with substituted phenols and amines (**3**) to get the products (**4**) in good yield (Scheme-1).



4 a) Z = NH_2, R = H; b) Z = NH_2, R = 2'-CH_3;
c) Z = NH_2, R = 3'-NO_2; d) Z = OH, R = H;
e) Z = OH, R = 2'-CH_3; f) Z = OH, R = 3'-NO_2.

Scheme-1

Synthesis of 5-nitro salicylic acid

Salicylic acid (0.03 mole) was dissolved in glacial acetic acid (40 ml) in an 250 ml Erlenmeyer flask, to this 1:2 mixture of conc. HNO_3 (0.03 mole) and conc. H_2SO_4 (0.06 mole) was added in cold condition with constant stirring. Then reaction mixture was kept aside for 2 hrs at room temperature. The progress of the reaction was monitored by TLC. After the completion of the reaction, the mixture was transferred into crushed ice, with constant stirring. The solid formed was filtered. Then solid obtained was re-dissolved in water under hot condition and the hot solution was filtered, the filtrate was kept aside to attain the room temperature, the precipitate formed was filtered and was recrystalized by 30% alcohol. The isomeric mixture of 3-nitro salicylic acid was separated. The product (**1**) was obtained in 55% yield, mp 230-232°C.

General procedure for the synthesis of azo dyes

Typical procedure for the synthesis of 5-((4-aminophenyl)diazenyl]-2-hydroxybenzoic acid *(4a)*: A mixture of 5-nitro salicylic acid (**1**) (0.01 mole) and zinc powder (0.03 mole) in a 250 ml Erlenmeyer flask fitted with reflux condenser was placed in an ice water bath. To the mixture conc. Hydrochloric acid (18 ml) was added drop wise through condenser and it was refluxed for 4 hrs with occasional shaking. Then the reaction mixture in the flask was cooled to room temperature and filtered. The filtrate was diluted with dil. Hydrochloric acid (15 ml) and with water (25 ml), and then was cooled to 0°C. To this cold solution, a cold solution of $NaNO_2$ (0.01 mole) in water (8 ml) was added to the flask slowly with constant shaking. After the completion of the reaction, a pale yellow solution of diazonium chloride was obtained, to this yellow solution; a cold solution of freshly distilled aniline (**3**a) (0.01 mole) in dil. Hydrochloric acid was added in small intermittent with constant shaking. The progress of the reaction was monitored by TLC. After the completion of the reaction, the solid separated was filtered and recrystalised from dimethyl sulphoxide. The reaction afforded 5-((4-aminophenyl)diazenyl]-2-hydroxybenzoic acid (**4a**) in 51 yield. The same procedure was used in all cases, however in case of **4d-f**, the compounds **3d-f** were dissolved in 5% sodium hydroxide.

RESULTS AND DISCUSSION

5-((4-aminophenyl)diazenyl]-2-hydroxybenzoic acid (4a)

Obtained as solid in 51% yield. UV-Vis: λmax 412 (0.201) nm. IR (KBr): 3454, 3220, 3056, 2908, 1610, 1446 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.25 (s, 1H, OH), 6.15 (s, 2H, NH_2), 6.80 (dd, 2H, Ar-H), 7.62-7.88 (m, 5H, Ar-H), 10.96 (s, 1H, COOH). ^{13}C NMR ($CDCl_3$): δ 113.8 (2C), 115.4 (1C), 122.3 (1C), 124.4 (3C), 129.2 (1C), 133.0 (1C), 144.8 (1C), 150.0 (1C), 155.2 (1C), 171.0 (1C). Anal. Cacld. for $C_{13}H_{11}N_2O_3$; MW 257 : C, 60.70, H, 4.31, N, 16.33%; Found: C, 60.65, H, 4.24, N, 16.28%.

5-((4-amino-3-methylphenyl)diazenyl]-2-hydroxybenzoic acid (4b)

Obtained as solid in 48% yield. UV-Vis: λmax 416 (0.294) nm. IR (KBr): 3458, 3214, 3044, 2919, 1634, 1443 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.26 (s, 3H, CH_3), 5.24 (s, 1H, OH), 6.16 (s, 2H, NH_2), 6.68 (d, 1H, Ar-H), 7.66-7.92 (m, 5H, Ar-H), 10.84 (s, 1H, COOH). Anal. Cacld. for $C_{14}H_{13}N_3O_3$; MW 271 : C, 61.99, H, 4.83, N, 15.49%; Found: C, 61.96, H, 4.84, N, 15.43%.

5-((4-amino-2-nitrophenyl)diazenyl]-2-hydroxybenzoic acid (4c)

Obtained as solid in 44% yield. UV-Vis: λmax 443 (0.312), 544 (0.109) nm. IR (KBr): 3442, 3223, 3062, 2924, 1734, 1637, 1433, 1322 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.32 (s, 1H, OH), 6.26 (s, 2H, NH_2), 7.14 (d, 1H, Ar-H), 7.70-7.96 (m, 5H, Ar-H), 10.78 (s, 1H, COOH). ^{13}C NMR ($CDCl_3$): δ 109.2 (1C), 115.2 (1C), 119.0 (1C), 123.4 (1C), 124.0 (1C), 126.2 (1C), 128.4 (1C), 132.8 (1C)¸ 137.3 (1C), 142.1 (1C), 150.2 (2C), 155.7 (1C), 170.8 (1C) . Anal. Cacld. for $C_{13}H_{10}N_4O_5$; MW 302 : C, 51.66, H, 3.33, N, 18.54%; Found: C, 51.63, H, 3.28, N, 18.47%.

2-Hydroxy-5-[(4-hydroxyphenyl)diazenyl]benzoic acid (4d)

Obtained as solid in 58% yield. UV-Vis: λmax 447 (0.464) nm. IR (KBr): 3458, 3236, 3029, 2920, 1631, 1465 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.33 (s, 2H, OH), 7.18 (dd, 2H, Ar-H), 7.65-7.86 (m, 5H, Ar-H), 10.91 (s, 1H, COOH). ^{13}C NMR ($CDCl_3$): δ 114.0 (2C), 115.0 (1C), 121.2 (2C), 122.2 (2C), 125.7 (2C), 133.8 (1C), 145.6 (1C), 153.3 (1C), 157.0 (1C), 170.9 (1C). Anal. Cacld. for $C_{13}H_{10}N_2O_4$; MW 258 : C, 60.47, H, 3.90, N, 10.85%; Found: C, 60.41, H, 3.88, N, 10.91%.

2-Hydroxy-5-[(4-hydroxy-3-methylphenyl)diazenyl]benzoic acid (4e)

Obtained as solid in 51% yield. UV-Vis: λmax 415 (0.162) nm. IR (KBr): 3460, 3244, 3036, 2924, 1624, 1432 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.18 (s, 3H, CH_3), 5.34 (s, 2H, OH), 7.04 (d, 1H, Ar-H), 7.68-7.95 (m, 5H, Ar-H), 10.98 (s, 1H, COOH). Anal. Cacld. for $C_{14}H_{12}N_2O_4$; MW 272 : C, 61.76, H, 4.44, N, 10.29%; Found: C, 61.72, H, 4.38, N, 10.24%.

2-Hydroxy-5-[(4-hydroxy-2-nitrolphenyl)diazenyl]benzoic acid (4f)

Obtained as solid in 39% yield. UV-Vis: λmax 464 (0.222), 528 (0.077) nm. IR (KBr): 3471, 3243, 3051, 2933, 1730, 1622, 1443, 1322 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.26 (s, 2H, OH), 7.70-8.04 (m, 6H, Ar-H), 10.98 (s, 1H, COOH). Anal. Cacld. for $C_{13}H_9N_3O_6$; MW 304 : C, 51.49, H, 2.99, N, 13.86%; Found: C, 51.46, H, 2.97, N, 13.81%.

The structures of the cycloadducts were provided by UV-Visible, IR, ^{1}H NMR, ^{13}C NMR spectral studies and elemental analysis. For instance, in UV-Visible spectrum, all the compounds showed absorption maxima in the region 415-464 nm. However the compounds **4c** and **4f** showed second absorption maxima peaks at 544 and 528 nm respectively due to the presence of chromophoric (NO_2) group. The results of the electron absorption studies indicating that all synthesized compounds were absorbed in the visible region.

In IR spectra, all the compounds showed a broad band in the range of 3460 cm^{-1}to 3212 cm^{-1} corresponding -OH (str), however, the observed values are lesser than that of the corresponding free -OH group. This suggests that the compounds possess hydrogen bonding between

C=O of carboxylic group and OH group ortho to it. The consistent pattern stretching absorption bands due to –N=N- observed clearly indicates the formation of the compounds.

In ^{1}H NMR spectra, all the synthesized compounds **4a-f** showed the peaks due to aromatic and substituent protons at the expected region. In ^{13}C NMR, all products gave the signals due to aromatic and substituent carbons at the expected region. Further, all showed satisfactorily CHN analysis with a deviation of ±0.02% from the theoretically calculated values. All these observations strongly favor the formation of the products.

Antifungal activity[11-14]

Seed coating: The protection of seeds from the attack of harmful microorganisms is essential to use them for seedling. The fungi developed on the seed surface or in the seeds greatly affect the seedling viability. Melathin powder is commonly used antifungal agent in the preservation of seeds. Otherwise due to attack of fungi on seeds reduces the germination percentage with time.

The anti fungal activities of the synthesized compounds **4a-f** were tested with the seeds of green gram. In a typical experiment, the blotting discs dipped in distilled water were placed in the Petri plates. On the other side, 20 g of green gram were taken in polythene bag, to this, 4-5 drops of water was added and then was thoroughly shaken for 30 minute for the proper distribution of water on the surface of the seeds. By doing so the surface of the seed gets expanded. Now a known quantity of the synthesized compounds was added to the seeds in the polythene bag and shaken well till the compounds were coated uniformly on the surface of the seeds. Then the seeds were transferred to Petri plates and sufficient water was added and kept in incubation for 72 hours. The various fungi developed were noted, control experiments were carried out under similar conditions. The results of the study were summarized in table-1.

Table 1: Resistance of the compounds 4a-f against fungi formation on Green gram

Fungal Species	Fungi Formation Incidence (%)						
	Control	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
A. niger	21	-	31	-	4	-	22
A. flavous	6	16	4	22	-	9	26
R. nigaricans	-	20	-	-	19	-	-
A. nidulens	-	-	6	14	8	-	-
C. herbarum	12	4	-	6	6	6	13
T. roseum	-	-	8	-	-	24	-
A.alternate	-	10	-	8	34	-	40

The experimental result indicates that the synthesized compounds possess moderate to good antifungal activities against many of the fungi species.

Anthelmintic Activity

Helminthiasis is a disease caused by infection of parasitic worms living in the alimentary canals or in the other tissues of the host. Infestation can cause morbidity, and sometimes death, by compromising nutritional status, affecting cognitive processes, inducing tissue reactions, such as granuloma, and provoking intestinal obstruction or rectal prolapsed. Control of helminthiasis is based on drug treatment, improved sanitation and health education. Earthworms have the ability to move by ciliary moment. Outer layer of earthworms is a mucilaginous layer and polysaccharides. This layer being slimy; enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the other layers and this restricts its movement and can cause paralysis and may lead to the death. The drug possessing anthelmentic property will paralyze the worm by causing damage to the mucopolysaccharide layer. This causes irritation leading to paralysis.

The synthesized compounds **4a-f** was evaluated for their antihelmentic activity. In a typical procedure, the uniform sized Earthworms (*Preritima posthuma*) were washed with water to remove adhering materials, and then were kept in 6% dextrose solution for acclimatization. A stock solution of test compounds (100 mg) and 2 drops of surfactant (Tween-80) was prepared by crushing in a mortar and then diluted with water (20 ml). 10 ml and 7.5 ml of the stock solution were transferred to the different petri dishes containing 6% dextrose (5 ml) and were diluted to 25 ml with water. For control, in a third petri dish 6% dextrose (5 ml) was taken and was diluted to 25 ml with water. A solution of antihelmentic drug peperazine citrate was prepared in the same concentrations was placed in separate petri dish as standard. In each petri dish two worms were placed; time taken by each worm for paralysis and death was recorded. The results of antihelmentic activity of the synthesized compounds were summarized in table-2.

Table 2: Antihelmentic activity of the synthesized compounds 4a-f

Compound	Concentration (ml)	Mean paralyzing Time (min)	Mean death time (min)
Standard	7.5	110	160
	10	100	155
Control	-	-	-
4a	7.5	21	31
	10	18	28
4b	7.5	20	30
	10	15	25
4c	7.5	22	28
	10	18	28
4d	7.5	45	65
	10	40	62
4e	7.5	5	13
	10	4	14
4f	7.5	36	45
	10	30	39

The result of the study reveals that synthesized compounds showed significant anthelmintic activity against earth worms.

Textile applications

The synthesized compounds **4a-f** were studied for their ability of dying to cotton fabrics. The color parameters of the dyed fabrics were measured and the dyes have showed excellent absorption, intense and of very good fastness properties on cotton fabrics. Among the compounds **4c** and **4f** have showed excellent dye absorption properties, **4b** and **4d** showed moderate and others showed poorer absorption ability on cotton fabrics.

CONCLUSION

The synthesized compounds showed moderate to excellent antifungal activity against different organisms, good antihelmentic activity. However the structure-activity relationship of the synthesized compounds with the host remains the topic of interest.

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