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SYNTHESIS, CHARACTERIZATION, DYEING ASSESSMENT AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF DERIVATIVES OF 3,3'-DICHLORO BENZIDINE

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Abstract: A series of bisazo reactive dyes have been synthesized via efficient methodology. In this paper we report the synthesis of a series of reactive dyes by coupling of tetrazotized 3, 3'-dichloro benzidine with various 2-amino 5(4'-nitro phenyl) 1,3,4-thiadiazole cyanurated coupling component viz, H-acid, J-acid, N-methyl J-acid, N-phenyl J-acid, sulfo tobias acid, tobias acid, peri acid, S-acid, K-acid, N-benzoyl H-acid, sulfo C-acid, N-phenyl peri acid, laurent acid and Chicago acid dyeing performance of these dyes was evaluated on cotton, wool and silk fabric. The purity of dyes was checked by thin layer chromatography (TLC). All synthesized dyes were characterized by FTIR and ¹H NMR spectra, fastness properties and antimicrobial activity of these dyes were evaluated.

Keywords: 3,3'-dichloro benzidine, bis azo reactive dyes, Thiadiazole, fastness properties, antimicrobial activity.

Introduction:

Reactive dyes are colored compounds with one or two groups capable of creating a covalent link between a carbon atom or phosphorous atom of the dye ion or molecule and an oxygen atom, nitrogen atom or sulphur atom of the substrate's hydroxyl, amino or mercapto group respectively [1-6].

These colors are typically used on higher end garments that are mercerized [7].

Reactive dyes are well-known and

used to color a variety of materials [8]. Triazine derivatives are one of the most important among them. S-triazine is a key component of synthetic colors. For reactive dyes cyanuric chloride is essential intermediate, which has two reactive groups in its structure and provides high fixation yields, great wet fastness, dazzling shade, and easy application procedures in textile printing. Due to high fixation yield on a variety of fibers, hot brand reactive dyes have been highly considered [9].

This paper deals with synthesis of new hot

brand reactive dyes (hot brand reactive dyes having low reactivity towards cotton, high dyeing temperature is required and dyeing is performed at > 80-90°C, they require long steaming time.) by coupling of tetrazotized 3,3'-dichloro benzidine with various 2-amino 5(4'-nitro phenyl) 1,3,4-thiadiazole cyanurated coupling component such as H-acid, J-acid, N-methyl J-acid, N-phenyl J-acid, sulpho tobias acid, tobias acid, peri acid, S-acid, K-acid, N-benzoyl H-acid, sulfo C-acid, N-phenyl peri acid, laurent acid and Chicago acid. 2-amino 5(4'-nitro phenyl) 1,3,4-thiadiazole was prepared according to reported method [10-11]. Cyanuric chloride was condensed with naphthalene based acid couplers and then condensed with 2-amino 5(4'-nitro phenyl) 1,3,4-thiadiazole. Finally tetrazotization of 3,3'-dichloro benzidine and its coupling with 2-amino 5(4'-nitro phenyl) 1,3,4-thiadiazole cyanurated naphthalene based couplers yielded a series of dyes (VP₁-VP₁₄) [12-16].

Materials and methods:

All the materials used were analytical grade reagents and were used directly. The melting points were determined using Stuart SMP 10 melting point equipment and are uncorrected. Thin-layer chromatography (TLC) was used to check the purity and R_f value of all dyes using silica gel-G coated Al-plates (0.5 mm thickness, Merck) and a solvent system of methanol and toluene (4:1) was used, spots were visualized using UV illumination.

UV-1800 Shimadzu Spectrophotometer was used to measure UV spectra. IR spectra were taken on a Perkin-Elmer 1600 FTIR in KBr disc in the range of

4000 cm⁻¹ to 400 cm⁻¹, and ¹H NMR spectra were taken in DMSO-d₆ as solvent and TMS as internal standard on a Bruker Avance II 500 MHz NMR. All reactive dyes were applied on cotton, wool and silk fabric at a 2% depth. In compliance with ISO 105 [17-18], the fastness qualities against light, wash, sublimation, perspiration and rubbing were examined. A reflectance spectrometer was used to record the Computer Color Matching (CCM) characteristics (L*, a*, b*, c*, h° and K/S). % Exhaustion and % Fixation values were calculated following reported procedure [17-21]. Antimicrobial activity (antibacterial and antifungal) was evaluated using broth dilution method [22].

Reaction process:

Step-1: Synthesis of 2-amino 5-(4'-nitro phenyl)1,3,4-thiadiazole:

Mixture of 4-Nitro benzoic acid (0.01 mole; 1.67 gm) and thiosemicarbazide (0.01 mole; 0.91 gm) was dissolved in ethanol (70 mL) and 10 mL conc. H₂SO₄ was added as a cyclizing agent, reaction mixture was refluxed in water bath at 90°C for 7 hours, the progress of the reaction was monitored by TLC (n-hexane: ethyl acetate), after completion of reaction, the mixture was poured into crushed ice and basified with liq. ammonia to give white solid, which was filtered, dried and recrystallized from ethanol to give pure compound.

(Scheme-1) (R_f = 0.1923) White solid, yield 82%, m.p. 218-220°C; Anal. Calc. For C₈H₆N₄O₂S (222.0 gm/mole); N, 25.21%; Found: 24.12%. IR (KBr, cm⁻¹): 718.02 (C-S-C), 1410.08 (Ar-NO₂), C=C (1562.40), C-H (2850), N-H (3442.70).

^1H NMR (500MHz, DMSO- d_6 , δ_{ppm}): 4.57 (m, 4H, -Ar), 6.6-7.9 (s, 2H, -NH $_2$).

Step-2: Synthesis of 5-(4'-nitro phenyl) thiadiazole-2-amino cyanurated H-acid:

Cyanuration of H-acid:

Cyanuric chloride (0.01 mole; 1.84 gm) was dissolved in acetone (25 mL) and stirred at a temperature below 5°C for a period of an hour. A neutral solution of H-acid (0.01 mole; 3.19 gm) in aqueous sodium carbonate solution (10% w/v) was then added in small lots in about an hour. The pH was maintained neutral by simultaneous addition of sodium carbonate solution (1% w/v). The reaction mass was then stirred at 0-5°C for further 4 hours.

The cyanurated H-acid solution was used for subsequent coupling reaction.

Condensation with 2-amino-5(4'-nitro phenyl) 1,3,4-thiadiazole:

The temperature of ice-cooled well stirred solution of cyanurated H-acid was gradually raised to 45°C.

The solution of 2-amino-5(4'-nitro phenyl) 1,3,4-thiadiazole in acetone was added slowly to the cyanurated H-acid solution at same temperature, during a period of 30 minutes, maintaining the pH neutral by simultaneous addition of sodium bicarbonate solution (1% w/v). After the addition was completed, stirring was carried out for 3 hours. Solution of 5-(4'-nitro phenyl) thiadiazole-2-amino cyanurated H-acid was obtained and solution used for coupling reaction (Scheme-2).

Step-3: Tetrazotization of 3,3'-dichloro benzidine:

3,3'-dichloro benzidine (0.005 mole; 1.265 gm) was mixed with H $_2$ O and hydrochloric acid (5 mL) was dropwise added with continuous stirring. The mixture was gradually heated up to 70°C, till clear solution was obtained. The solution was cooled to 0-5°C in an ice-bath. A solution of NaNO $_2$ (0.01 mole; 0.69 gm) in water (8 mL) previously cooled to 0°C, was then added over a period of 5 minutes with stirring. The stirring was carried out for an hour, maintaining the same temperature, with positive test for nitrous acid on starch iodide paper. After removal of excess of nitrous acid with required amount of solution of sulphamic acid, the clear tetrazotized solution at 0-5°C was used for subsequent condensation reaction (Scheme-3).

Step-4: Coupling of tetrazotized solution with 5-(4'-nitro phenyl) thiadiazole 2-amino cyanurated H-acid:

Formation of Dyes (VP $_1$):

A freshly prepared tetrazotized solution (0.005 mole) was added dropwise over a period of 10-15 minutes ice cold well stirred solution of 5-(4'-nitro phenyl) thiadiazole 2-amino cyanurated H-acid (0.01 mole). The pH was maintained 7.5 to 8.5 by simultaneous addition of sodium carbonate solution (10% w/v). During coupling, a purple solution was formed. The stirring was continued for 3-4 hours, maintaining the temperature below 5°C. Then reaction mixture was stirred at room temperature and sodium chloride was added until the above colouring

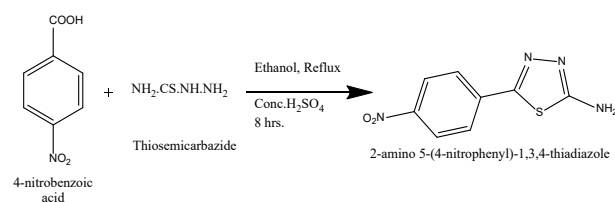
material was solidified. It was further stirred for an hour, filtered and washed with a small amount of sodium chloride solution (5% w/v). The solid was dried at 80-90°C and extracted with DMF. The dye was obtained by diluting the DMF - extract with excess of chloroform.

A violet dye(VP₁) formed, was then filtered, washed with chloroform and dried at 60°C, Yield 85% (Scheme-4).

Following the above procedure reactive dyes VP₂-VP₁₄ were synthesized using various 5-(4'-nitro phenyl) thiadiazole 2-amino cyanurated coupling components viz J-acid, N-methyl J-acid, N-phenyl J-acid, Sulpho tobias acid, Tobias acid, Peri acid, S-acid, K-acid, N-benzoyl H-acid, Sulpho C-acid, N-phenyl peri acid, laurent acid and Chicago acid.

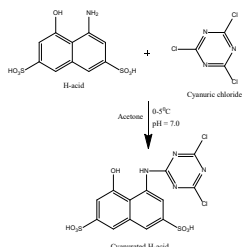
Reaction Scheme:

Step-1: Synthesis of 2-amino 5-(4'-nitro phenyl)1,3,4-thiadiazole:

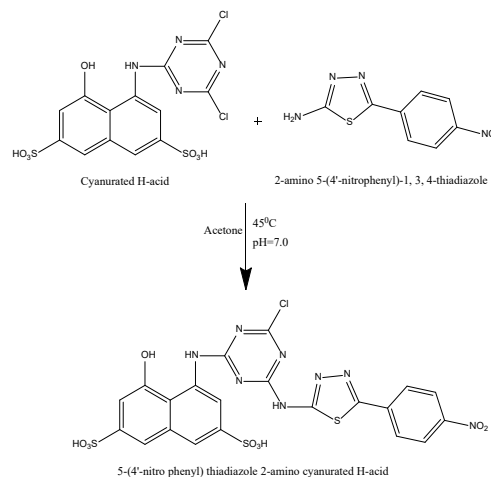


Step-2: Synthesis of 5-(4'-nitro phenyl) thiadiazole-2-amino cyanurated H-acid:

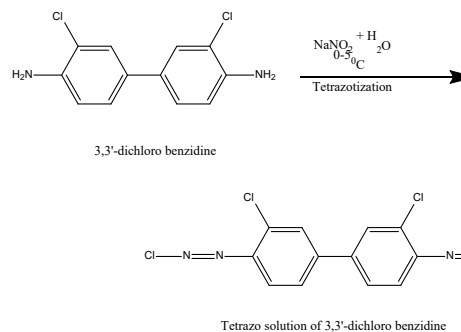
Cyanuration of H-acid:



Condensation with 2-amino-5(4'-nitro phenyl) 1,3,4-thiadiazole:

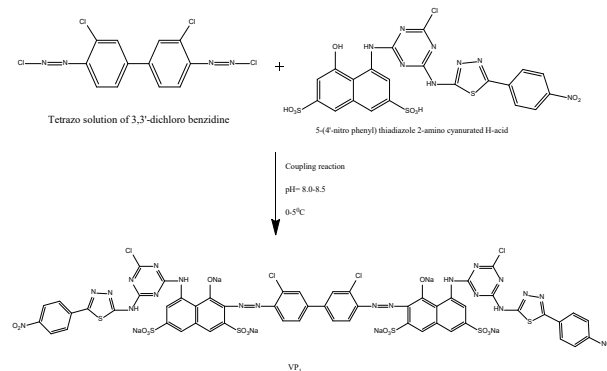


Step-3: Tetrazotization of 3,3'-dichloro benzidine:



Step-4: Coupling of tetrazotized solution with 5-(4'-nitro phenyl) thiadiazole 2-amino cyanurated H-acid:

Formation of Dyes (VP₁):



Results and Discussion:

Cyanuric chloride was condensed with naphthalene based acid couplers at 0-5°C, then 2-amino 5(4'-nitro phenyl) 1,3,4-thiadiazole was condensed with cyanurated naphthalene based couplers. Finally tetrazotization of 3, 3'-dichloro benzidine and coupling with above couplers yielded a series of bis azo reactive dyes VP₁-VP₁₄. The physical appearance of all dyes are violet, orange, red and brown color solid. The newly synthesized dyes were confirmed by elemental analysis, UV-Visible, IR, ¹H NMR spectral analysis. The synthesized dyes were evaluated for their antimicrobial activity also. The physical properties of synthesized dyes (VP₁-VP₁₄) are given in Table-1.

Spectral Analysis:**IR and ¹H NMR spectra:**

The IR spectra of synthesized dyes showed the absorption band at 3414-3450 cm⁻¹ corresponding to the secondary -NH group, C=C stretching vibration of azo compound appeared at 1593-1626 cm⁻¹, N=N stretching vibration at 1519-1526 cm⁻¹, aromatic -NO₂ stretching vibration appeared at 1369-1396 cm⁻¹, S=O stretching vibration of -SO₃Na group appeared at 1196-1279 cm⁻¹, stretching vibration of p-substituted benzene ring appeared at 869-877 cm⁻¹, C-Cl stretching in aromatic ring appeared at 779-786 cm⁻¹, C-S-C stretching in thiadiazole ring appeared at 714-719 cm⁻¹, C-Cl stretching in triazine ring vibration appeared at 669-680 cm⁻¹.

¹H NMR spectra of all the dyes were recorded using DMSO-D₆. Dye VP₁

Table-1: The physical properties of dyes VP₁-VP₁₄:

Dye No.	Substituent (R)	M. F.	M.W.	Melting point (°C)	Yield %	R _f	%Nitrogen	
							Found	Calc.
VP ₁	H-acid	C ₅₄ H ₂₄ Cl ₄ N ₂₀ Na ₆ O ₁₈ S ₆	1713.01	>300°C	83.00	0.40	16.20	16.35
VP ₂	J-acid	C ₅₄ H ₂₆ Cl ₄ N ₂₀ Na ₄ O ₁₂ S ₄	1508.93	>300°C	84.02	0.41	17.50	18.57
VP ₃	N-Methyl J-acid	C ₅₆ H ₃₀ Cl ₄ N ₂₀ Na ₄ O ₁₂ S ₄	1536.98	>300°C	75.21	0.38	17.80	18.23
VP ₄	N-Phenyl J-acid	C ₆₆ H ₃₄ Cl ₄ N ₂₀ Na ₄ O ₁₂ S ₄	1661.13	>300°C	78.12	0.42	16.20	16.86
VP ₅	Sulpho tobias acid	C ₅₄ H ₂₆ Cl ₄ N ₂₀ Na ₄ O ₁₆ S ₆	1637.05	>300°C	67.30	0.38	16.90	17.11
VP ₆	Tobias acid	C ₅₄ H ₂₈ Cl ₄ N ₂₀ Na ₂ O ₁₀ S ₄	1432.97	>300°C	62.50	0.36	19.00	19.55
VP ₇	Peri acid	C ₅₄ H ₂₈ Cl ₄ N ₂₀ Na ₂ O ₁₀ S ₄	1432.97	>300°C	82.25	0.37	19.10	19.55
VP ₈	S-acid	C ₅₄ H ₂₆ Cl ₄ N ₂₀ Na ₄ O ₁₂ S ₄	1508.93	>300°C	87.90	0.44	18.10	18.57
VP ₉	K-acid	C ₅₄ H ₂₄ Cl ₄ N ₂₀ Na ₆ O ₁₈ S ₆	1713.01	>300°C	74.12	0.48	16.00	16.35
VP ₁₀	N-Benzoyl H-acid	C ₆₈ H ₃₂ Cl ₄ N ₂₀ Na ₆ O ₂₀ S ₆	1921.22	>300°C	78.32	0.39	14.00	14.58
VP ₁₁	Sulpho C-acid	C ₅₄ H ₂₄ Cl ₄ N ₂₀ Na ₆ O ₂₂ S ₈	1841.12	>300°C	78.50	0.40	14.90	15.22
VP ₁₂	N-Phenyl periacid	C ₆₆ H ₃₆ Cl ₄ N ₂₀ Na ₂ O ₁₀ S ₄	1585.16	>300°C	68.20	0.38	17.00	17.67
VP ₁₃	Laurent acid	C ₅₄ H ₂₈ Cl ₄ N ₂₀ Na ₂ O ₁₀ S ₄	1432.97	>300°C	72.25	0.35	19.00	19.55
VP ₁₄	Chicago acid	C ₅₄ H ₂₄ Cl ₄ N ₂₀ Na ₆ O ₁₈ S ₆	1713.01	>300°C	83.00	0.38	16.15	16.35

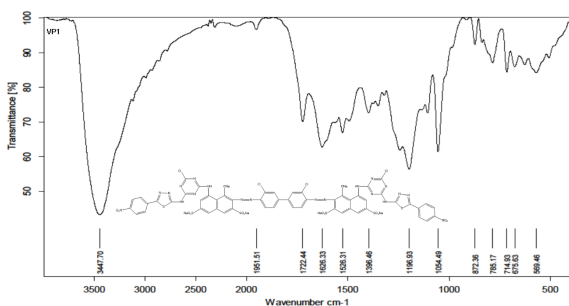
showed multiplet at δ 6.6-9.0 ppm for 20 proton of aromatic part. It also showed singlet at δ 8.8 ppm for two protons of -NH group, two protons of -NH show singlet at δ 11.1 ppm, higher δ value of this proton indicates deshielding of thiadiazole ring.

^1H NMR of dye VP_4 showed multiplet at

δ 6.5-8.5 ppm for 32 proton of aromatic region. It also showed singlet at δ 13.3 ppm for two protons of -NH group, higher value of δ indicate deshielding effect of thiadiazole ring. IR and ^1H NMR data of synthesized dyes (VP_1 - VP_{14}) are given in Table-2 and graph of IR and ^1H NMR data of dyes VP_1 and VP_4 are given in Figure-1 to 4.

Table-2: IR and ^1H NMR spectral data of dyes VP_1 - VP_{14} :

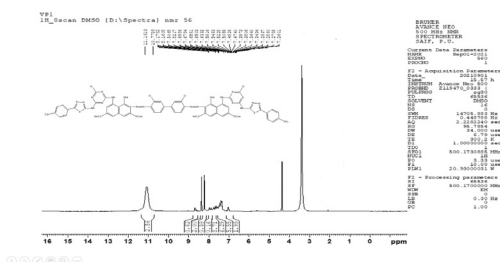
Dye No.	IR (KBr, cm^{-1})	^1H NMR (500MHz, DMSO-D_6), chemical shift in δ_{H} ppm
VP_1	3447 (N-H), 1626 (C=C), 1526 (N=N), 1396 (-NO ₂), 1196 (S=O), 872 (p-C ₆ H ₆), 785 (Ar-Cl), 714 (C-S-C), 675 (C-Cl in triazine)	6.6-9.0 (20H, m, Ar-H), 8.8 (2H, s, -NH), 11.1 (2H, s, -NH)
VP_2	3448 (N-H), 1620 (C=C), 1525 (N=N), 1392(-NO ₂), 1210 (S=O), 873 (p-C ₆ H ₆), 780 (Ar-Cl), 715 (C-S-C), 673 (C-Cl in triazine)	6.76-9.50 (22H, m, Ar-H), 9.0 (2H, s, -NH), 12.5 (2H, s, -NH)
VP_3	3450 (N-H), 1599 (C=C), 1520 (N=N), 1397(-NO ₂), 1200 (S=O), 874 (p-C ₆ H ₆), 779 (Ar-Cl), 715 (C-S-C), 672 (C-Cl in triazine)	3.5 (6H, s, 2-CH ₃), 6.5-8.9 (22H, m, Ar-H), 11.9 (2H, s, -NH)
VP_4	3414 (N-H), 1593 (C=C), 1526 (N=N), 1369(-NO ₂), 1279 (S=O), 872 (p-C ₆ H ₆), 783 (Ar-Cl), 717 (C-S-C), 672 (C-Cl in triazine)	6.5-8.5 (32H, m, Ar-H), 13.3 (2H,s, -NH)
VP_5	3420 (N-H), 1595 (C=C), 1522 (N=N), 1375(-NO ₂), 1250 (S=O), 870 (p-C ₆ H ₆), 780 (Ar-Cl), 715 (C-S-C), 678 (C-Cl in triazine)	6.7-9.3 (22H, m, Ar-H), 8.6 (2H, s, -NH), 12.1 (2H, s, -NH)
VP_6	3425 (N-H), 1610 (C=C), 1525 (N=N), 1380(-NO ₂), 1200 (S=O), 870 (p-C ₆ H ₆), 784 (Ar-Cl), 718 (C-S-C), 678 (C-Cl in triazine)	6.8-9.2 (24H, m, Ar-H), 8.2 (2H, s, -NH), 12.3 (2H, s, -NH)
VP_7	3435 (N-H), 1598 (C=C), 1521 (N=N), 1380(-NO ₂), 1250 (S=O), 869 (p-C ₆ H ₆), 780 (Ar-Cl), 718 (C-S-C), 680 (C-Cl in triazine)	6.3-9.5 (24H, m, Ar-H), 9.5 (2H, s, -NH), 12.3 (2H, s, -NH)
VP_8	3450 (N-H), 1615 (C=C), 1519 (N=N), 1372 (-NO ₂), 1258 (S=O), 870 (p-C ₆ H ₆), 785 (Ar-Cl), 719 (C-S-C), 679 (C-Cl in triazine)	6.6-9.2 (22H, m, Ar-H), 9.4 (2H, s, -NH), 12.2 (2H, s, -NH)
VP_9	3448 (N-H), 1625 (C=C), 1520 (N=N), 1380(-NO ₂), 1250 (S=O), 877 (p-C ₆ H ₆), 786 (Ar-Cl), 718 (C-S-C), 680 (C-Cl in triazine)	6.3-9.7(20H, m, Ar-H), 8.4(2H, s, -NH), 12.0 (2H, s, -NH)
VP_{10}	3446 (N-H), 1596 (C=C), 1522 (N=N), 1374 (-NO ₂), 1260 (S=O), 870 (p-C ₆ H ₆), 785 (Ar-Cl), 715 (C-S-C), 678 (C-Cl in triazine)	6.8-9.2 (30H, m, Ar-H), 12.5 (2H, s, -NH)
VP_{11}	3440 (N-H), 1598 (C=C), 1524 (N=N), 1380(-NO ₂), 1274 (S=O), 870 (p-C ₆ H ₆), 785 (Ar-Cl), 718 (C-S-C), 669 (C-Cl in triazine)	6.5-9.2 (20H, m, Ar-H), 8.8 (2H, s, -NH), 12.1 (2H, s, -NH)
VP_{12}	3442 (N-H), 1593 (C=C), 1520 (N=N), 1369 (-NO ₂), 1279 (S=O), 870 (p-C ₆ H ₆), 785 (Ar-Cl), 716 (C-S-C), 670 (C-Cl in triazine)	6.4-9.8 (34H, m, Ar-H), 12.3 (2H, s, -NH)
VP_{13}	3444 (N-H), 1596 (C=C), 1522 (N=N), 1372(-NO ₂), 1274 (S=O), 874 (p-C ₆ H ₆), 784 (Ar-Cl), 718 (C-S-C), 670 (C-Cl in triazine)	6.5-8.5 (24H, m, Ar-H), 8.9 (2H, s, -NH), 12.1 (2H, s, -NH)
VP_{14}	3443 (N-H), 1594 (C=C), 1525 (N=N), 1370 (-NO ₂), 1275 (S=O), 873 (p-C ₆ H ₆), 781 (Ar-Cl), 715 (C-S-C), 671 (C-Cl in triazine)	6.5-8.6 (20H, m, Ar-H), 8.5 (2H, s, -NH), 12.4 (2H, s, -NH)

Figure-1: IR spectrum of dye VP₁:

IR (KBr, cm⁻¹): 3447.70 (N-H str. asym.), 1626.33 (C=C str.), 1526.31 (N=N str.), 1396.46 (Ar-NO₂ str.), 1196.93 (S=O str. of -SO₃Na), 872.36 (p-sub. in C₆H₆), 785.17 (C-Cl str. in Ar. ring), 714.93 (C-S str.), 675.63 (C-Cl str. in triazine ring).

Figure-2: IR spectrum of dye VP₄:

IR (KBr, cm⁻¹): 3414.70 (N-H str. asym.), 1593.26 (C=C str.), 1526.84 (N=N str.), 1369.40 (Ar-NO₂ str.), 1279.17 (S=O str. of SO₃Na), 872.25 (p-sub. in C₆H₆), 783.10 (C-Cl str. in Ar. ring), 717.86 (C-S str.), 672.33 (C-Cl str. in triazine ring).

Figure-3: ¹H NMR spectrum of dye VP₁:

¹H NMR (500 MHz, DMSO-d₆, δ_H ppm): 6.6-9.0 (20H, m, Ar-H), 8.8 (2H, s, -NH), 11.1 (2H, s, -NH).

Figure-4: ¹H NMR spectrum of dye VP₄:

¹H NMR (500 MHz, DMSO-d₆, δ_H ppm): 6.5-8.5 (32H, m, Ar-H), 13.3 (2H, s, -NH).

Dyeing study:

All the dyes VP₁-VP₁₄ were applied at 2% depth on cotton, wool and silk fibers according to following procedure. The hues of dyes ranged from purple to yellow depending upon the coupling component used, e.g. H-acid gave purple hue, J-acid gave yellow hue, N-methyl J-acid gave orange hue and N-Phenyl J-acid gave red hue on different fibers.

Dyeing of cotton:

The dye (0.2 gm) was pasted with a few drops of cold water, then 80 mL cold water was added, mixed and the mixture was made up to 100 mL with dye solution (20 mL), glauher's salt solution (4 mL of 10%w/v) and water (14.4 mL). At 30°C the cotton fabric (2 gm) was placed in the dye bath, and the temperature was gradually increased to 80°C over 20 minutes. The dyeing was then continued for another 40 minutes at

the same temperature with the addition of a soda ash (Na_2CO_3) solution (0.4 mL of 10% v/v). After that, the material was removed, washed with cold water, soaped and dried. Shade and color coordinates of dyes (VP_1 - VP_{14}) on cotton fabrics are given in Table-3 and graph of b^* vs a^* for cotton fabric is given in Figure-5.

Dyeing of Wool:

The dye (0.2 gm) was pasted with a few drops of cold water, then 80 mL cold water was added, mixed and the mixture was made up to 100 mL, acetic acid (1.5 mL of 10% v/v), glauher's salt solution (4 mL of 10% w/v) and water (14.4 mL). At 30°C, the wool fabric (2 gm) was placed in the dye bath and the temperature was gradually increased to 80°C over 20 minutes. Sulphuric acid (0.4 mL, 10% v/v) was then added and the dyeing was continued at the same temperature for another 40 minutes. After that, the material was removed, washed with cold water, soaped and dried. Shade and color coordinates of dyes (VP_1 - VP_{14}) on wool fabric are given in Table-4 and graph of b^* vs a^* for wool fabric is given in Figure-6.

Dyeing of Silk:

The dye (0.2 gm) was pasted with a drop of cold water, then 80 mL of cold water was added and thoroughly mixed to produce a clear solution. The dye solution (20 mL), acetic acid (2 mL of 10% v/v), and water (18 mL) were mixed to make a dye solution with a volume up to 100 mL. The temperature of the dye bath was kept at 30°C while the silk fabric (2 gm) was added and the temperature was gradually increased to 80°C over 20 minutes. To achieve satisfactory exhaustion, formic

acid (1.5 mL of 40%v/v) was added to the dye bath at this temperature. After another 40 minutes of dyeing the dyed material was washed with cold water, soaped and dried. Shade and color coordinates of dyes (VP_1 - VP_{14}) on silk fabric given in Table-5 and graph of b^* vs a^* for silk fabric is given in Figure-7.

Wash-off process:

The dyed fabrics (cotton, wool and silk) was rinsed in warm water, scoured with 2 gm/L Lissapol detergent at 90°C for 5 minutes and rinsed again in warm water. The dyed fabrics afforded color in the first warm water rinse, less color in the scouring bath and practically no color in the second water rinse. This indicated that the unfixed dye was easily removed from the fiber surface. The amount of the hydrolyzed dye having low substantivity released easily from the substrate after two or three washes. The higher molecular size greatly increased the wash fastness.

Colorimetric data (CIE lab data):

The color of a dyeing on cotton, wool and silk fabrics are expressed in terms of CIE lab values and the following CIE lab coordinates were measured and lightness (L^*), Chroma (C^*), hue angle from 0° to 360° (h°), a^* value represent the degree of redness (positive) and greenness (negative) and b^* represents the degree of yellowness (positive) and blueness (negative). A reflectance spectrophotometer was used for the colorimetric measurements of the dyed samples. K/S values given by the reflectance spectrophotometer were calculated at λ_{max} and are directly correlated with the dye concentration on the substrate according to the Kubelka-

Munk equation [22]. Graph of dye No. $(VP - VP_1) \rightarrow K/S$ value for cotton, wool $K/S = \frac{(1 - R)^2}{2R}$ are shown in Figure-8.

Where K is absorbance coefficient, S is scattering coefficient, and R is reflectance ratio.

Table-3: Shade and color coordinates of the dyes VP₁-VP₁₄ on cotton fabric:

Dye No.	Shade on cotton fabric	L*	a*	b*	C*	h°
VP ₁	Air force (blue)	46.44	1.20	-20.83	20.87	273.31
VP ₂	Cream	76.18	11.47	20.23	23.25	60.44
VP ₃	Baby pink	75.89	11.80	9.68	15.26	39.34
VP ₄	Purple	41.52	17.37	-14.35	22.53	320.43
VP ₅	Light peach	71.97	24.49	20.35	31.84	39.72
VP ₆	Off white	89.83	3.05	11.07	11.48	74.59
VP ₇	Grey	77.10	4.73	6.95	8.40	55.78
VP ₈	Pearl river	69.06	-0.24	-7.74	7.75	268.24
VP ₉	Cocoon white	81.61	4.27	8.45	9.47	63.21
VP ₁₀	Grey	74.59	0.04	1.41	1.41	88.22
VP ₁₁	Pastel green	73.10	-1.60	13.56	13.66	96.74
VP ₁₂	Violet	59.70	12.40	-13.38	18.24	312.82
VP ₁₃	Light brown	71.58	4.72	8.56	9.78	61.13

Table-4: Shade and color coordinates of the dyes VP₁-VP₁₄ on wool fabric:

Dye No.	Shade on wool fabric	L*	a*	b*	C*	h°
VP ₁	Grey	47.24	5.74	-9.49	11.09	301.18
VP ₂	Cream	70.16	15.05	17.11	22.79	48.65
VP ₃	Malaga	70.89	18.70	19.68	28.26	44.34
VP ₄	Mulberry	33.95	24.44	-6.27	25.23	345.61
VP ₅	Fire	45.65	38.00	34.31	51.20	42.07
VP ₆	Pink	80.73	7.25	22.07	31.48	83.59
VP ₇	Light brown	59.46	9.07	6.97	11.44	37.57
VP ₈	Off white	70.07	4.99	18.75	19.40	75.11
VP ₉	Maroon	70.61	7.27	12.55	17.84	73.21
VP ₁₀	Grey	62.25	2.86	10.25	10.64	74.41
VP ₁₁	Light yellow	77.40	3.13	24.13	24.34	82.61

VP ₁₂	Grey	55.70	15.39	-7.38	27.57	325.82
VP ₁₃	Cyber purple	60.89	9.78	18.56	19.82	72.13
VP ₁₄	Cream	71.21	8.07	11.89	14.37	55.83

Table-5: Shade and color coordinates of the dyes VP₁-VP₁₄ on silk fabric:

Dye No.	Shade on silk fabric	L*	a*	b*	C*	h°
VP ₁	Light purple	74.21	3.59	-5.55	6.61	302.89
VP ₂	White	89.24	-0.03	9.46	9.46	90.2
VP ₃	Mellow	83.46	6.55	14.84	16.22	66.2
VP ₄	Rosewood	55.31	24.80	-8.19	26.12	341.72
VP ₅	Aerospace orange	65.07	33.14	30.21	44.85	42.35
VP ₆	Yellowish white	85.27	6.69	16.37	17.69	67.77
VP ₇	Linen	76.06	6.48	6.93	9.49	46.95
VP ₈	Goldenrod	81.88	7.04	20.87	22.02	71.35
VP ₉	Yellowish white	84.35	0.20	13.52	13.52	89.17
VP ₁₀	Cream	82.14	-1.89	11.48	11.63	99.37
VP ₁₁	Light yellow	88.16	-1.59	19.21	19.28	94.74
VP ₁₂	Violet	46.59	14.76	-15.10	21.11	314.34
VP ₁₃	Mauve	74.21	3.59	-5.55	6.61	302.89
VP ₁₄	Light yellow	82.83	5.99	5.80	8.34	44.11

Figure-5: Graph of b* vs a* of dyes (VP₁-VP₁₄) on cotton fiber:

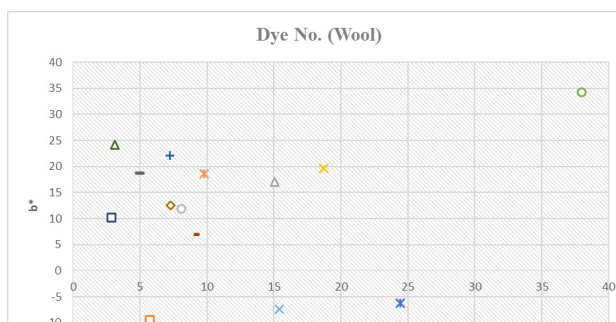


Figure-6: Graph of b* vs a* of dyes (VP₁-VP₁₄) on wool fiber:

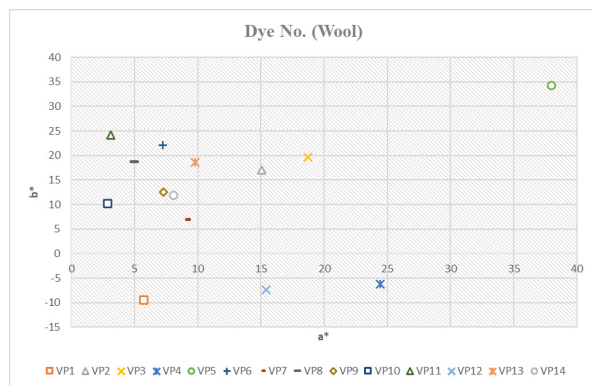


Figure-7: Graph of b* vs a* of dyes (VP₁-VP₁₄) on silk fiber:

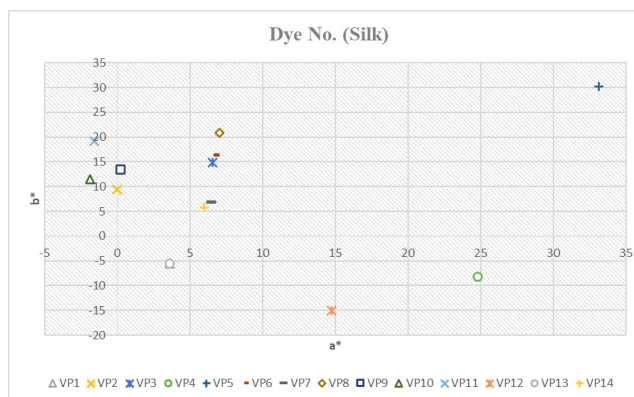
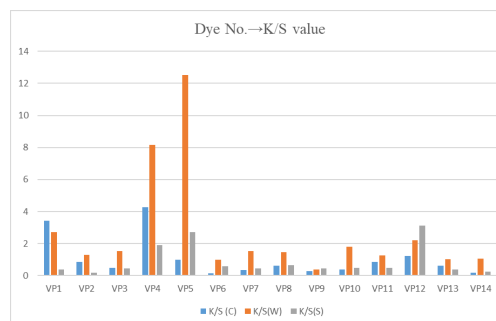


Figure-8: Graph of K/S Value of dyes (VP₁-VP₁₄) on cotton, wool and silk fiber:



% Exhaustion and % Fixation of Dyes:

% Exhaustion and % Fixation data were calculated according to reported method [23-25]. % Exhaustion of VP₁-VP₁₄ dyes for cotton fabric ranges from 64.20% to 82.23%, for wool fabric ranges from 60.22% to 80.32%, for silk fabric ranges from 70.21% to 84.25%. % Fixation of VP₁-VP₁₄ dyes for cotton fabric ranges from 70.30% to 91.52%, for wool fabric ranges from 70.30% to 85.52% and for silk fabric ranges from 72.30% to 91.80%. Here, the value of % Exhaustion and % Fixation clearly indicates that for wool and silk fabric it is higher than cotton fabric, due to lower substantivity of the dyes with cotton fabric. UV-Visible spectroscopic data, % Exhaustion and % Fixation value of dyes (VP₁-VP₁₄) are shown in Table-6.

Table-6: UV-Visible spectroscopic data, % Exhaustion and % Fixation of dyes VP₁-VP₁₄:

Dye No.	λ_{max}	K/S value			% Exhaustion			% Fixation		
		C	W	S	C	W	S	C	W	S
VP ₁	586.36	3.44	2.71	0.37	72.23	68.33	74.25	75.30	70.30	85.30
VP ₂	444.25	0.86	1.29	0.18	68.12	65.12	78.12	78.23	74.32	82.25
VP ₃	453.88	0.48	1.52	0.45	68.25	64.52	70.21	74.20	72.19	80.20
VP ₄	564.59	4.26	8.17	1.9	75.20	72.29	80.20	80.23	75.32	85.23
VP ₅	516.77	1.0	12.53	2.71	76.30	71.02	82.30	82.50	80.50	88.05
VP ₆	474.41	0.14	0.98	0.6	64.20	60.22	74.20	70.30	65.30	72.30
VP ₇	458.80	0.36	1.53	0.47	80.12	75.12	84.25	90.12	82.21	90.12
VP ₈	614.84	0.61	1.47	0.67	82.23	80.32	84.25	91.52	85.52	91.80
VP ₉	456.94	0.3	0.38	0.44	76.56	72.58	78.80	81.23	78.23	84.23
VP ₁₀	452.79	0.38	1.79	0.5	78.80	74.88	81.20	84.20	80.20	85.03
VP ₁₁	438.92	0.87	1.28	0.5	70.20	65.20	75.30	78.30	74.30	79.98
VP ₁₂	583.75	1.22	2.21	3.12	69.12	64.20	72.20	75.62	72.54	78.80
VP ₁₃	449.39	0.62	1.04	0.37	78.52	73.27	80.82	83.32	80.02	90.02
VP ₁₄	467.00	0.18	1.06	0.24	74.20	68.70	75.20	80.20	76.30	80.20

Abbreviations: C: Cotton, W: Wool, S: Silk

Fastness properties:

Light fastness properties were tested according to BS:1006-1978 [26]. Evaluation of rubbing fastness was carried out with Crock meter (Atlas) in accordance with AATCC-1961 [27] and wash fastness checked as per IS: 765-1979 [28].

All reactive dyes (VP₁-VP₁₄) showed moderate to good light fastness properties. Dye VP₄, VP₆ and VP₈ showed fair to excellent and other dyes showed good to excellent washing fastness properties. Dye VP₃, VP₅, VP₆ and VP₁₂ showed fair to excellent and other dyes showed good to excellent rubbing fastness properties on cotton, wool and silk fabric. High light fastness is due to higher attraction between dye and fiber and higher stability of resonance in the conjugated system. Data of fastness properties of dyes (VP₁-VP₁₄) are summarized in table-7.

Abbreviations:

Light fastness: 1- poor, 2-slight, 3-moderate, 4-fair, 5-good, 6-very good, 7-excellent.

Fastness of washing, rubbing: 1-poor, 2-fair, 3-good, 4-very good, 5-excellent.

Antimicrobial activity:

All the synthesized dyes (VP₁-VP₁₄) were tested for their antibacterial and antifungal activity (MIC) in vitro by broth dilution method with two Gram positive bacteria *Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 442 and two Gram negative bacteria *Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 1688 and fungi *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282 and *Aspergillus clavatus* MTCC 1323 against Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin and Griseofulvin as standard drugs. All MTCC cultures were collected from Institute of

Table-7: Fastness properties of the dyes VP₁-VP₁₄:

Dye No.	Fastness to light			Fastness to washing			Rubbing					
	C	W	S	C	W	S	Dry			Wet		
							C	W	S	C	W	S
VP ₁	5	4	4	3-4	5	5	5	4	4	5	3	3-4
VP ₂	4	3	3	4	4	4	4	5	3	4	4	4
VP ₃	3	3-4	5	5	4	3-4	3	2-3	5	2-3	5	3
VP ₄	4	4	4-5	2-3	3	4	4	4	4-5	4	3-4	4
VP ₅	5	5	3-4	5	4	5	3-4	5	3-4	5	4	2-3
VP ₆	5	4	5	4	3-4	2-3	5	2-3	5	2-3	4	5
VP ₇	4-5	5	4	4-5	4	4	4-5	4	4	4	3	4-5
VP ₈	3-4	3	3	3	2-3	5	3-4	4-5	3	5	3	3-4
VP ₉	5	4-5	5	5	5	4	5	4	5	3-4	5	5
VP ₁₀	5	3	4	4	4	4	3-4	4	4	4	3-4	4-5
VP ₁₁	4	4	5	5	3	3	4	3	5	3	5	4
VP ₁₂	3	5	3-4	4	4	4	3	4	3-4	4	2-3	3
VP ₁₃	5	4	4	4	5	5	5	4	4	4-5	4	4
VP ₁₄	4	3	3	4	3	3	5	4	3	4	3	2-3

Microbial Technology, Chandigarh and tested against known drugs ampicillin and griseofulvin. Muller-Hinton broth was used as nutrient medium to grow and dilute the drug suspension for the test [29]. The data of antimicrobial activity of all the dyes (VP₁-VP₁₄) are mentioned in table-8.

Antibacterial activity:

Dye VP₁, VP₆ and VP₁₂ showed excellent activity against *E. coli* and very good activity against *S. aureus* with respect to standard drug Ampicillin. Dye VP₂ and VP₈ showed excellent activity against *S. Aureus* with respect to standard

drug Ampicillin. Dye VP₅ and VP₁₃ showed equipotential activity against *E. coli* with respect to standard drug Chloramphenicol. Dye VP₅, VP₉ and VP₁₃ showed equipotential activity against *S. aureus* with respect to standard drug Ampicillin. Dye VP₄ and VP₁₀ showed equipotential activity against *S. pyogenes* with respect to standard drug Ampicillin.

Antifungal activity:

Dye VP₃ and VP₉ showed excellent activity against *C. albicans* and dye VP₅ showed equipotential activity against *C. albicans* with respect to standard drug Nystatin. Dye VP₁, VP₆ and VP₁₂ showed

Table-8: Antimicrobial data of dyes VP₁-VP₁₄:

Dye No.	Minimal bactericidal concentration (µg/mL)				Minimal fungicidal concentration (µg/mL)		
	Gram-negative		Gram-positive		<i>A.Niger</i>		
	<i>E. coli</i> MTTC443	<i>P. aeruginosa</i> MTTC 1688	<i>S.aureus</i> MTCC 96	<i>S.pyogenes</i> MTCC 442	<i>C.albicans</i> MTCC 227	282	<i>Clavatus</i> MTCC 1323
VP ₁	62.5	100	200	200	500	500	500
VP ₂	500	500	100	500	1000	1000	1000
VP ₃	100	200	1000	200	62.5	500	200
VP ₄	200	100	200	100	200	1000	500
VP ₅	50	100	250	250	100	500	500
VP ₆	62.5	100	200	200	500	500	500
VP ₇	100	100	1000	500	>1000	>1000	>1000
VP ₈	500	200	100	200	500	500	500
VP ₉	200	200	250	200	62.5	200	200
VP ₁₀	200	500	500	100	>1000	>1000	>1000
VP ₁₁	500	100	1000	500	>1000	>1000	>1000
VP ₁₂	62.5	100	200	1000	500	500	200
VP ₁₃	50	100	250	250	200	200	500
VP ₁₄	100	200	1000	200	>1000	>1000	>1000
Ampicilin	100	100	250	100	-	-	-
Chloram-phenicol	50	50	50	50	-	-	-
Nystatin	-	-	-	-	100	100	100
Griseofulvin	-	-	-	-	500	100	100

equipotential activity against *C. albicans* and VP₄ and VP₁₃ showed very good activity against *C. albicans* with respect to standard drug Griseofulvin.

Conclusion:

Tetrazotization of 3,3'-dichloro benzidine and coupling with 5(4'-nitro phenyl)

thiadiazole 2-amino cyanurated coupling components yielded a series of bis azo reactive dyes (VP₁-VP₁₄) with excellent color. All synthesized dyes were solid in nature and their physical appearance was violet, orange, brown and red color. All dyes gave brighter shade on cotton, wool and silk fibers. Dyes VP₁-VP₁₄ showed moderate to good light fastness, fair to excellent washing and rubbing fastness on cotton, wool and silk fabrics. All synthesized dyes gave remarkable % Exhaustion, % Fixation and K/S values. Some of the dyes showed excellent to very good antimicrobial activities.

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