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Applications on Acrylic Fabrics and Antimicrobial Assessment of Synthesized Azo Derivatives of 4-hydroxy-6-methyl-2-mercaptopyrimidine

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Abstract

The study presents the synthesis of novel pyrimidine azo dyes achieved through systematic diazotization and coupling reactions, using 4-hydroxy-6-methyl-2-mercaptopyrimidine as the core substrate. The molecular structures of the synthesized dyes were characterized using advanced spectroscopic techniques. These included Fourier transform infrared (FTIR) spectroscopy for functional group analysis, ultraviolet-visible (UV-Vis) spectroscopy for electronic transitions and both proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy for structural elucidation. The azo compounds were tested for their effectiveness in dyeing acrylic fabrics. Four derivatives yielded vibrant pink colouration (except for compound 8), demonstrating uniform dye uptake and excellent colour fastness properties, with ratings ranging between 4 and 4/5, indicative of high durability and stability under standard washing, light, and rubbing conditions. Furthermore, the biological activity of the synthesized dyes was evaluated against a range of bacterial and fungal strains. Compounds 5 (5-[1H,3-methyl-2,6-dioxo(pyrimidyl)diazenyl]-4-hydroxy-6-methyl-2-mercaptopyrimidine) and 6 (5-[3H,4-hydroxy-2-oxo(pyrimidyl)diazenyl]-4-hydroxy-6-methyl-2-mercaptopyrimidine) exhibited remarkable antimicrobial efficacy, displaying potent inhibitory effects against both gram-positive and gram-negative bacteria as well as fungal pathogens.

Keywords: Pyrimidine dyes, acrylic fibre, pharmacological properties, metallic complexes

Introduction

The design and development of heterocyclic dyes have gained significant attention due to their remarkable properties, such as high molar extinction coefficients, high tinctorial strength, brilliant colours and high fastness on textile substrates [1-4]. These heterocyclic based dyes are widely applied in many areas of human endeavor. For instance, the performance of heterocyclic colourants in the field of corrosion inhibition has been reported [5-8]. They have also been utilized in the area of biomedicine for the detection of microbes [9,10]. Among the heterocyclic dyes, pyrimidine-based dyes stand out for their unique properties. These dyes, which incorporate hydroxyl substituents as coupling units and diazo components, have been reported to produce a range of vibrant shades, including golden yellow, orange,

red and pink shades [11]. Despite the extensive research on heterocyclic dyes, there remains a gap in the literature regarding the synthesis and application of dyes based on the 4-hydroxy-6-methyl-2-meracaptopyrimidine skeleton. This study aims to address this gap by focusing on the synthesis of monoazo disperse dyes derived from 4-hydroxy-6-methyl-2-meracaptopyrimidine and their application on acrylic fibers. The objectives of this work are to synthesize and characterize monoazo disperse dyes based on the 4-hydroxy-6-methyl-2-meracaptopyrimidine skeleton, investigate the dyeing properties of these dyes on acrylic fibres, synthesize the aluminum complexes of these dyes.

Materials and Methods

The coupling unit, 4-hydroxy-6-methyl-2-mercaptopyrimidine and all the aniline derivatives were procured from Sigma-Aldrich (Germany) A1l other reagents and solvents are of good quality analytical grades purchased and used as received. The chemical reactions were monitored using thin-layer chromatograph, using 0.25 mm Merck Silica gel 60 F254 precoated plates. The melting points of the dyes were performed in a Gallenkamp instrument and are presented uncorrected. The IR absorption spectra were measured on a Schimadzu FTIR-8400S spectrophotometer (Japan) using potassium bromide (KBr) disc and the absorbance were expressed in nm (nanometers)

The nuclear magnetic resonance (NMR) spectra (^{1}H NMR and ^{13}C NMR) were measured at 200 MHz and 50 MH respectively on a mercury 200 BB series spectrometer (USA). Tetramethylsilance (TMS) was used as the internal standard reference and deuterated dimethylsulfoxide (DMSO-δ_6) as solvent. The NMR analysis was carried out at the central laboratory, Obafemi Awolowo University, Nigeria. The ultraviolet-visible (UV-Vis) absorption spectra were recorded using UV-2500PC series instrument. The antimicrobial activities were carried out at Obafemi Awolowo University, Department of Pharmacognosy, Nigeria.

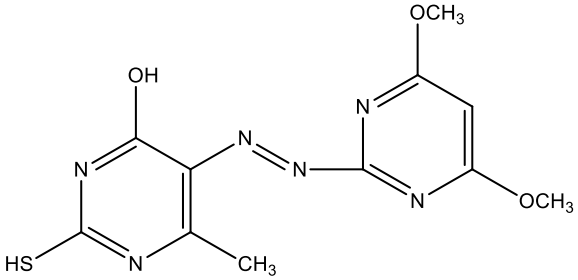
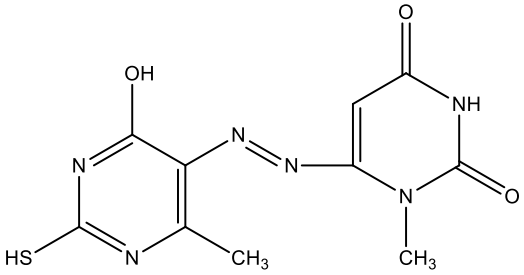
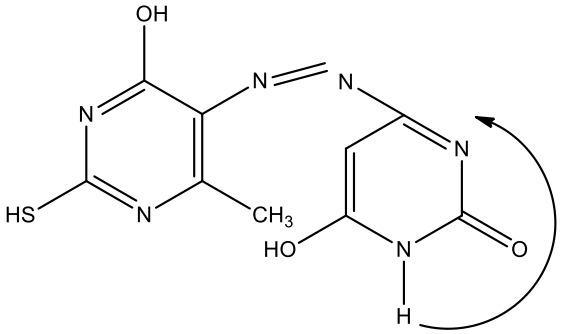
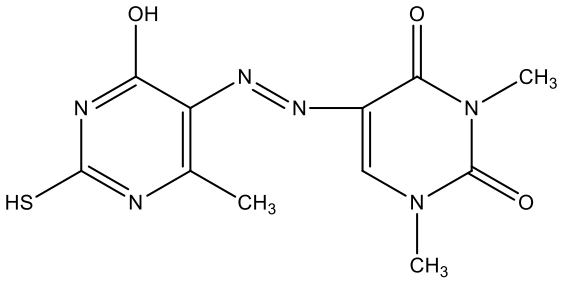
General procedure for the synthesis of dyes 4 to 8

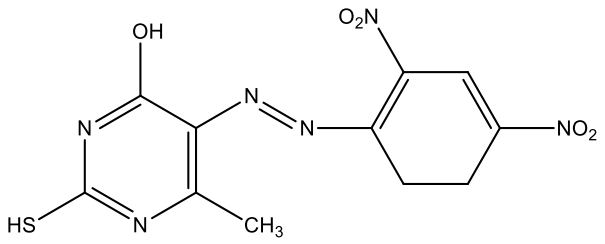
Each of the aniline derivatives (0.01 mol) was dissolved in concentrated hydrochloric acid (36%, 25 mL) and the solution was cooled to 0-5 °C in an ice bath. Sodium nitrite (0.90 g, 0.013 mol) was dissolved in water (10 mL) and the solution was slowly added to the above amine solution. A strip of starch-potassium iodide paper was exposed to the reaction mixture to detect nitrous acid, with a blue black or brown colour indicating a positive result. The excess nitrous acid was removed by adding a small quantity of sulfamic acid.

The 2,4-dinitroaniline was however diazotized in nitrosyl sulphuric acid, generated in-situ from sodium nitrite and concentrated sulphuric acid. The coupling compound, 4-hydroxy-6-methyl-2-mercaptopyrimidine was dissolved in sodium hydroxide (15 mL, 1.0 g) and added dropwise to the active diazonium salt solution with mechanical

stirring for 20 min. The reaction continued for 3 hours and the precipitated product was isolated by filtration, washed several times with water and dried in an oven at 50 ^{o}C to constant weight. The pure compounds 4 to 8 were recrystallized from ethanol to afford the final products.

Table 1: IUPAC names and structures of the synthesized dyes 4 to 8

Dyes	IUPA names	Structures
4	5-[4,6-dimethyoxy(pyrimidyl)diazenyl]-4-hydroxy-6-methyl-2-mercaptopyrimidine	
5	5-[1H,3-methyl-2,6-dioxo(pyrimidyl)diazenyl]-4-hydroxy-6-methyl-2-mercaptopyrimidine	
6	5-[3H,4-hydroxy-2-oxo(pyrimidyl)diazenyl]-4-hydroxy-6-methyl-2-mercaptopyrimidine	
7	5-[1,3-dimethyl-4,6-dioxo(pyrimidyl)diazenyll-4-hydroxy-6-methyl-2-mercaptopyrimidine	

8	5-[2,4-dinitro(phenyl)diazenyl]-4-hydroxy-6-methyl-2-mercaptopyrimidine	

Synthesis of metallic complexes of dyes 9 to 12

Each ligand (of 4 to 7) (0.2 g, 0.90 mmoles) was mixed with potassium hydroxide (10 mL of 0.10 M) and stirred until it dissolved. A quantity (10 mL) of aluminum sulphate (1.62 mmoles, 0.57 g) was added slowly with simultaneous mechanical stirring until a precipitate was obtained. The precipitate was centrifuged and washed three times with distilled water to afford a brown solid with a product yield of 48% [12-14].

Synthesis of metallic complex 13

Ligand 8 (0.2 g, 0.90 mmoles) was dissolved in sodium hydroxide solution (0.10 M, 10 mL) and the rest procedure used to obtain metallic complex 13 was similar to that described for metallic complex 4, to give a product yield of 45 %

Antibacterial assessment

Ligands (4-8) and their corresponding metal complexes (9-13) were dissolved in dimethylformamide at 100 and 50 mg/mL concentrations, and their antimicrobial activities were evaluated against bacterial strains *Salmonella typhi*, *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6635) and *Staphylococcus aureus* (ATCC 25923) and Fungal strain *Candida albicans*, which were obtained from Microbial Type Culture Collection and characterized by standard biochemical tests and 16S rRNA sequencing. Each microorganism (adjusted to 10^8 cells/mL) was inoculated on Mueller-Hinton agar plates using a sterile cotton swab, and 6 mm diameter filter paper discs containing10 µL of compound solutions (1 and 0.5 mg disc-1) were placed on the inoculated plates. The experimental setup included a positive control (chloramphenicol for bacteria), a negative control (cephalothin), cycloheximide for fungus and the test compounds, with plates incubated at 36°C for 24 hours and inhibition zones measured using a transparent ruler in three replicates for each test organism and compound [15-17].

Antifungal assessment

From the prepared stock cultures, the active inoculum of the experiments was prepared and many loopfuls of spores were transferred to test tubes containing sterile distilled water which were then shaken and diluted with distilled water to bring the optical density to 2.0×10^5 spores mL-1. Subsequently, an inoculum of 0.1% suspension was swabbed uniformly and allowed to dry for 5 mm and following a similar procedure as mentioned above [18].

Dye Application

Dyeing of acrylic fabrics

The dyes (1.0 g) were dissolved in dimethylformamide and a dispersing agent (Lignosulfonate-sulfite liquors) was added and stirred to form a homogeneous dispersion respectively. The dyebath was prepared to contain 3% shade of dye, liquor ratio 1:50, a pH of 4.5, adjusted using 30% acetic acid. The acrylic fabric (2.0, g) was wetted and dipped into the dyebath at 50°C and dyeing commenced in an Aluba Texomat dyeing machine. The temperature of the dyebath was raised to 100°C at a rate of 2 °C/min and then maintained at this temperature for 60 minutes. After dyeing, the temperature of the dyebath was allowed to cool slowly to 60°C and the dyed sample was removed, soaped off, rinsed with cold water and dried in air.

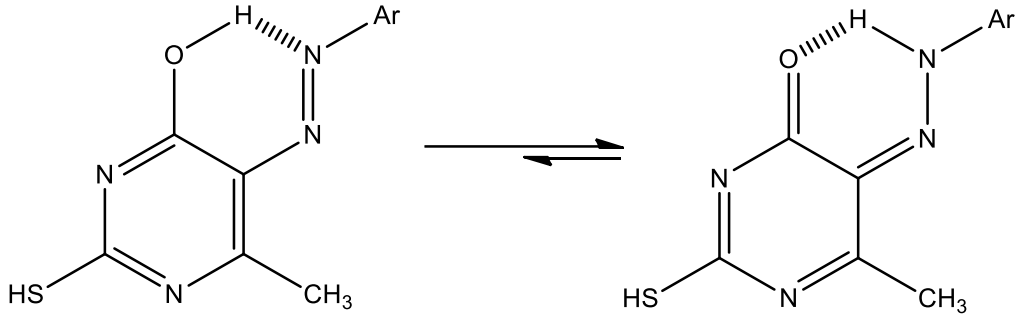
Colour fastness tests

The wash fastness of the fabric samples was determined according to ISO 106, [19] standard method. The light fastness was tested using ISO 105-B02: 2013 [20] test method using an Atlas Xenotest Alpha fastness tester (SDL, Atlas, USA). Rubbing fastness was evaluated using ISO 105-X12: 2013 [21] standard protocol. Sublimation fastness was carried out according to ISO 105-POl: 2013 [22].

Results and Discussion

The coupling reactions of 4-hydroxy-6-methyl-2-mercapptopyrimidine (1), in an alkaline solution (5%, 20 mL) at 0-5 °C with a number of aromatic diazonium salts of 2-amino-4,6-dimethoxypyrimidine, **2a**, 4-amino. 1H-3-mmethyl-2,6-dihydroxypyrimidine **2b**, 6-amino2,4- dihydroxypyrimidine **2c**, 5-amino-I ,3-dimethylpyrimidine **2d**, 2,4-dinitroaniline **2e** afforded azo compounds 4 to 8 respectively (Scheme 1). The structures were confirmed based on the ^{1}H and ^{13}C NMR and FTIR spectroscopic analyses which showed a stable tautometric structure (Figure 1) [23]. The FTIR spectral analysis and proton (^{1}H) and ^{13}C NMR studies carried out agree with the structures of the compounds.

In the FTIR spectra of the compounds (Table 4), we observed O-H and NH bands between 3754 and 3436 cm^{-1} [24]. There is, however, the absence of bands that could be attributed to the C=N stretching vibrations. This suggests that the dyes 4 – 7 exist as the tautomeric forms in the solid state (Fig. 1) [16; 25]. The presence of absorption bands observed at 3556 – 3756 cm^{-1} for the dyes indicates N-H stretching vibrations of the hydrazine form. The symmetric and asymmetric stretching vibrations of $–NO_2$ was observed between 1525 and 1344 cm^{-1} in compound 8. The absorption bands located at 1316, 1433 and 1497 cm^{-1} were assigned to C=C stretching vibrations. The absorption bands between 1063 and 1037 cm^{-1} were assigned to C-O group in the azo compounds [26; 27].



Azo form Hydrozone form

Equilibrium between the azo form and hydrozone form

Figure 1: Structure of compounds 4 to 8 in the solid state

Table 1 presents a detailed analysis of five synthesized chemical compounds (4-8), primarily dyes with distinctive physical and chemical properties. These compounds share similar molecular structures, predominantly composed of carbon, hydrogen, nitrogen, oxygen and sulphur, with molecular weights ranging from 267 to 325 g/mol. Most of the compounds exhibit a pink hue, except for compound 8, which is distinctly brown. The wavelength of maximum absorption (ʎmax) varies significantly across the compounds, spanning from 455 nm to 765 nm, reflecting diverse optical properties that could be valuable in various applications.

The retention factor (Rf) values obtained via thin-layer chromatography (TLC), demonstrate the compounds' different mobilities in the chromatographic system, ranging from 0.36 to 0.63. In terms of synthesis efficiency, the yield percentages fluctuate between 45% and 66%, with compound 7 proving to be the most efficiently produced. The melting points of these compounds are notably high, ranging from 210°C to over 300°C, with compound 8 showing the most remarkable thermal stability by maintaining its structure beyond 300°C. This comprehensive data set provides insights into the nuanced chemical and physical properties of these synthesized dyes. The ^{1}H NMR spectra of compounds 5 and 6 (Table 3) show that there are exchangeable broad singlet signals in the range δ 13.05 - 13.22 ppm, attributed to the NH protons of the pyrimidines. In addition, the presence of the protons at δ 6.82 - 6.92 ppm suggests that compounds 4 to 8 only exist in the solid state. The ^{1}H NMR spectrum of dye 8 showed doublet signals at δ 7.78 and 8.18 ppm, which is attributed to the protons of aromatic rings close to the electromagnetic atoms [28].

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Table 2: Physical Characteristics, Wavelength (m) of Synthesized Dyes 4 to 8

Compound	Molecular formula (molecular weight)	Colour	λ_{max} (nm)	Rf	Yield (%)	Mp
4	$C_{11}H_{12}N_6O_3S$ (297)	Pink	455	0.45	54	280-282
5	$C_{10}H_{12}N_6O_3S$ (294)	Pink	570	0.36	55	216
6	$C_8H_7N_6O_3S$ (267)	Pink	583	0.47	63	210-212
7	$C_{11}H_{12}N_6O_3S$ (297)	Pink	569	0.51	66	234
8	$C_{11}H_8N_6O_5S$ (325)	Brown	765	0.63	45	>300

Table 3: ^{1}H NMR and ^{13}C NMR Assignments of Dyes 4 to 8

Dyes	^{1}H NMR (200 MHz δ, ppm)	^{13}C NMR (50 MHz, δ, ppm)
4	1.62 (s, 1H CH_3), 2.45 (s, 1H, SH), 3.12 (s, 1H, OH), 4.62 (s, 6H, OCH_3x2) 6.97 (s, IH, CH)	167.6, 162.4, 154.7, 151.9, 135.3, 122.8, 121.6, 115.9, 54.2, 45.5
5	1.26 (s, 311, CH_3), 2.43 (s, 1H, SH), 4.52 (s, 1H, OH, D_2O exchangeable) 6.87 (s, 1H, CH), 13.21 (s, 1H, NH)	181.4, 172.3, 155.5, 132.6, 131.5, 124.6, 110.8, 115.0, 46.7
6	1.32 (s, 3H, CH_3), 2.45 (s, 1H, SH), 4.51 (s, 1H, OH, D_2O exchangeable) 6.82 (s, 1H, CH), 13.25 (s, 1H, NH)	165.7, 161.5, 155.0, 142.4, 132.8, 128.6, 127.8, 105.6, 50.3
7	1.34 (s, 9H, CH_3x3,), 2.52 (s, 1H, SH), 4.32 (s, 1H, OH D_2O exchangeable), 6.85 (s, 1H, CH)	182.0, 175.6, 156.7, 157.8, 146.4, 145.2, 144.9, 136.6, 49.3
8	1.31 (s, 3H, CH_3), 2.44 (s, 1H, SH), 4.61 (s, 1H, OH), 7.78 (d, 1H, J=7.8 Hz, ArH), 8.56 (m, 1H, ArH), 8.15 (d, 1H, J8.2Hz, ArH)	172.4, 158.8, 154.0, 135.2, 127.8, 127.5, 118.9, 115.3, 112.7, 106.8, 47.5

All ^{1}H and ^{13}C NMR spectra were measured in DMSO-d_6, s; singlet, m; multiplet, d; doublet

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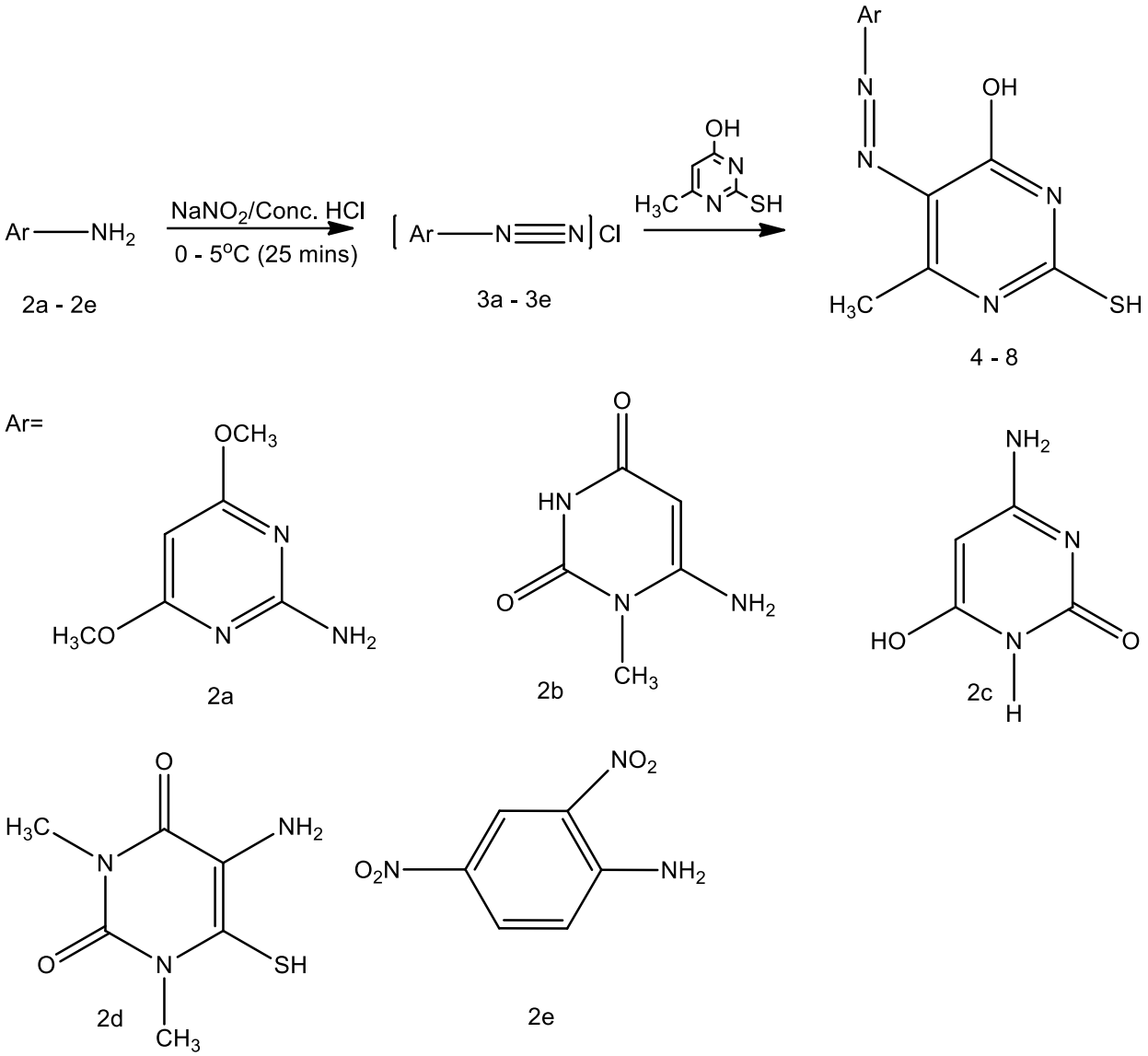
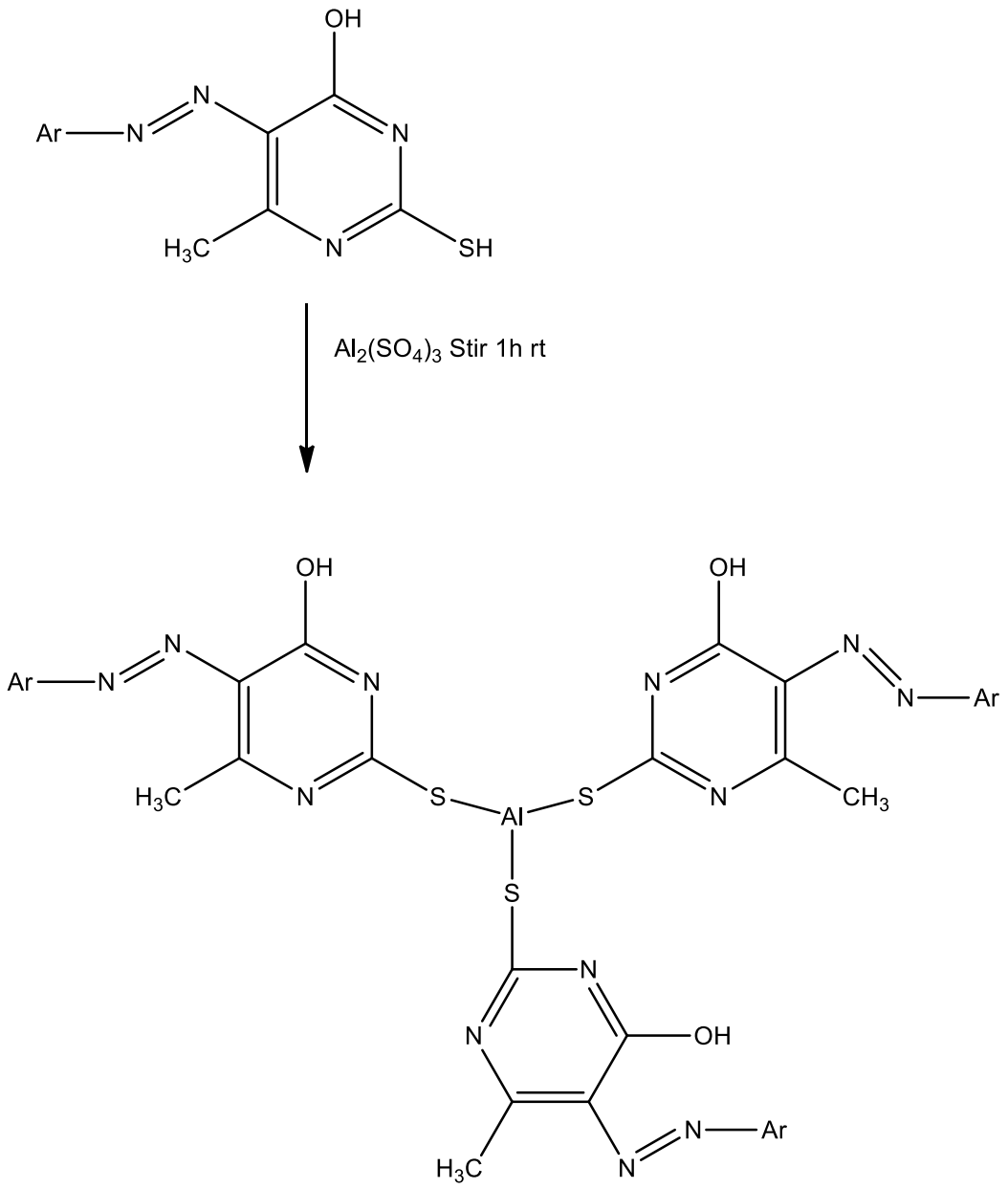
Table 4: Infrared results of Dyes 4 to 8

Dyes	FTIR (Vmax, cm^{-1})
4	3486 (O-H), 2873-2932 (CH), 2528 (S-H), 1658, 1497, 1440, 1390 (C=C), 1255, 1090 (C-O-C), 1063 (C-O)
5	3514 (O-H), 3225 (N-H), 2937- 3022 (C-H), 2594 (S-H), 1668 (C=O), 1350, 1448 (C=C), 1108 (C-O)
6	3743-3665 (O-H), 3216 (N-H), 3117-2981 (C-H), 2535 (S-H), 1707 (C=O), 1654, 1469, 1390 (C=C), 1068 (C-O)
7	3553 (O-H), 3119-3082 (C-H), 2504 (S-H), 1670 C=O), 1553, 1516, 1433 (C=C), 1047 (C-O)
8	3754-3622 (O-H), 2953-3021 (C-H), 564 (S-H), 1652, 1471 (C=C), 1525 NO_2 symmetric stretch, 1344 (NO_2 asymmetric stretch, 1037 (C-O)



Scheme 1: Synthesis and structures of azo compounds 4 – 8



Scheme 2: Synthesis and structure of azo aluminum complex dyes 9-13

Antimicrobial activity

The five synthesized azo compounds (Table 4) were tested for their antibacterial activity against *Salmonella typhi* (ATCC 14028) and *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6635) and *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 10231) The disc-agar diffusion standard method was used and the bacterial activity was evaluated by measuring the diameter of the inhibition zone. The results are listed in Table 3 and it indicates that all the screened compounds showed different inhibitory

effects against the growth of the tested micro-organisms (gram-positive and gram-negative bacterial strains and fungal strain). However, of all the tested compounds 5, 5-[1H,3-methyl-2,6-dioxo(pyrimiyl) diaenyl]-4-hydroxy-6-methyl-2-mercaptopyrimidine and 6, 5-[3H,4-hydroxy-2-oxo(pyrimiyl) diazenyl]-4-hydroxy-6-methyl-2-mercaptopyrimidine were found to be the most potent antibacterial agents against gram-negative bacteria instead of the gram-positive bacteria. Compounds 5 and 6 demonstrated notable antimicrobial activity, showing enhanced potency against *Candida albicans* compared to the standard reference drugs. The observed biological efficacy can be attributed to the structural characteristics of these molecules, specifically the NH groups, which facilitate molecular interactions through hydrogen bonding. These NH groups potentially function as proton donors, interacting with glycosidic bonds and potentially disrupting molecular interactions within the fungal cell substrate. The mechanism likely involves the ability of these proton-donating groups to weaken or cleave glycosidic linkages, which may comprise the structural integrity of the fungal cell wall or interfere with critical enzymatic processes.

Table 5: Antibacterial and Antifungal Activity of the Synthesized compounds expressed as MIC and MBC in mm

Dyes	**Test organisms**									
	Gram-positive bacteria				**Gram-negative bacteria**				**Fungi**	
	Staphylococcus aureus (ATCC 25923)		**Bacillus subtilis (ATCC 6635)**		**Salmonella typhi (ATCC 14028)**		**Escherichia coli (ATCC 25922)**		**Candida albicans (ATCC 10231)**	
	1 mgmL^{-1}	0.5 mgmL^{-1}	1 mgmL^{-1}	0.5 mgmL^{-1}	1 mgmL^{-1}	0.5 mgmL^{-1}	1 mgmL^{-1}	0.5 mgmL^{-1}	1 mgmL^{-1}	0.5 mgmL^{-1}
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
4	8	6	9	8	10	11	9	8	9	7
5	9	8	21	16	17	12	14	9	27	23
6	8	7	8	7	20	15	13	10	26	22
7	9	6	7	6	11	9	8	7	10	8
8	8	6	1	9	7	6	10	8	8	6
Standard	35	26	35	25	36	28	38	27	35	28

Standards: Chloranphenical for gram-positive bacteria, Cephalothin for gram-negative bacteria and cycloheximide for fungus. MBC: Minimum bacterial concentration, MIC: Minimum inhibitory concentratrion

Fastness properties

The results for washing and rubbing fastness properties of acrylic fabrics dyed with the azo compounds are presented in Table 5. The washing fastness ratings of the dyed fabrics ranged from 4 to 4/5 on the grey scale, this observation aligns with findings from prior research [29] which suggest that glycoside bond plays a significant role in cleaving the C-O bond within the substrate. Additionally, the NH group, with its lone pair of electrons contributes to this process by donating electrons to the C-O bond. This interaction disrupts the structural integrity of microorganisms[16].

The shade of the dyed fabrics only altered slightly during washing, implying that only a small amount of dye was washed off during the laundering. The removed dye molecules were not able to attach to the undyed adjacent fabrics, since the staining values were good.

The dry and wet rubbing fastness ratings were good to very good from 3/4 to 4/5, which indicates that a small staining occurred during the rubbing. However, the wet rubbing fastness ratings were lower for dyes 7 and 8 (from 3/4 to 4) compared with that of dry rubbing (grade 4/5) for the same dye.

The light and sublimation fastness results are shown in Table 6. The dyed fabric sample shows a light fastness rating of 4 to 6/7 based on the blue wool scale. An inspection of the results show that dye **7** recorded the highest light fastness, which may be due to the two alkylated nitrogen atoms [30] in the dye structure. Dye 5, which is the next higher in fastness ratings of grade 6, had only one of the nitrogens alkylated. Alkylated nitrogens in dye structures have been found to confer high light fastness to dye fabrics. The number of alkyl groups bonded to the nitrogen also affects the light fastness. The greater the number of alkyl groups present, the higher the light fastness rating as reported in [31]. The fastness to sublimation of the dyed fabric samples showed good to very good ratings (3/4 to 4).

Table 6: Fastness Properties of the Synthesized Dyes 4 to 8 on Acrylic Fabrics

Compounds	Washing					Rubbing	
	SA	SP	SN	SW	Alt	Dry	Wet
4	4	4/5	4	4/5	4	¾	¾
5	4	4/5	4	4/5	4	¾	¾
6	4	4/5	4	4/5	4	¾	¾
7	4	4/5	4	4/5	4	4/5	¾
8	4	4/5	4	4/5	4/5	4/5	4

SA; staining on acrylic, SP; staining on polyester, SN; staining on nylon, SW; staining on wool, Alt; Alteration (Change in colour)

Table 7: Fastness Properties of the Synthesized Dyes 4 to 8 on Acrylic fabrics

Compound	Sublimation	Light	
		35 h	80 h
4	¾	4	4
5	4	6	5/6
6	¾	4/5	4
7	4	6/7	5/6
8	4	5/6	5/6

Sublimation fastness, 1-5 scale and light fastness 1-8 scale

Conclusion

This study synthesized monoazo dyes derived from 4-hydroxy-6- methyl-2-mercaptopyrimidine, characterized by ^{1}H and ^{13}C NMR spectroscopy showing stable tautomeric structures with absorption bands in the 455-765 nm range and evaluated their performance on acrylic fabrics through exhaustion dyeing technique, revealing pink shades with excellent fastness properties (light, rubbing, sublimation and washing), while antimicrobial screening demonstrated that compound 5 and 6 exhibited the most potent antibacterial and antifungal activities, suggesting their potential as innovative textile colourants with multifunctional properties.

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