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Synthesis, solvatochromic properties and antimicrobial activities of some novel pyridone-based disperse disazo dyes

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ABSTRACT

In this study, 5-amino-4-arylazo-3-methyl-1H-pyrazoles (**2a–l**) were diazotized and coupled with 3-cyano-6-hydroxy-4-methyl-2-pyridone to give pyridone-based disperse disazo dyes (**3a–l**). The newly synthesized twelve pyridone-based disperse disazo dyes were characterized by elemental analysis and spectral methods. The solvatochromic properties and antimicrobial activities of these disazo disperse dyes were also examined in detail.

1. Introduction

It is well known that nitriles are widely used as intermediates for many heterocyclic compounds. The aminopyrazole compounds have been easily obtained by the reaction of nitrile derivatives with hydrazine hydrate [1–6]. Pyrazole and pyridone derivatives are important intermediates that possess biological and pharmacological activities [7–10]. Some azopyrazole derivatives also find application in dyes and complexes [11–13]. The use of heterocyclic coupling component and diazo components in the synthesis of azo disperse dyes is well established, and the resultant dyes exhibit good tinctorial strength and brighter shade properties than those derived from aniline-based components. For instance, Hallas et al. [14,15] reported the synthesis of azo dyes derived from 2-aminothiophene derivatives and various heterocyclic coupling components, and their application on polyester fibers which leads to excellent results. On the other hand, the use of amino-substituted thiazole and benzothiazole, being very electronegative diazo components, produce a pronounced bathochromic shift when compared to the corresponding benzoid compounds [16–19].

Although, many patents and papers describe the synthesis, tautomeric structures and dyeing properties of monoazo dyes [20–26], very few comparable investigations have been made with disazo dyes [27–31]. In this study, the synthesis and antimicrobial activities of some novel pyridone-based disperse disazo dyes which were derived from 5-amino-4-arylazo-3-methyl-1H-pyrazoles as heterocyclic diazo components were reported. Moreover, solvatochromic properties and tautomeric forms of these dyes were also examined in detail.

2. Experimental

2.1. General

The chemicals which were used for the synthesis of the compounds were obtained from Aldrich and Merck Chemical Company without further purification. The solvents used were in spectroscopic grade. IR spectra were determined using a Mattson 1000 Fourier Transform-infrared (FT-IR) spectrophotometer on a KBr disc. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker-Spectrospin Avance DPX 400 Ultra-Shield in deuterated dimethylsulphoxide (DMSO-d_6) using tetramethylsilane (TMS) as the internal reference; chemical shifts were (δ) given in ppm. Ultraviolet–visible (UV–vis) absorption spectra were recorded on a Shimadzu UV-1601 double beam spectrophotometer at the wavelength of maximum absorption (λ_{max}) in a range of solvents, i.e. DMSO, DMF, acetonitrile, methanol, acetic acid and chloroform at the various concentrations (1×10^{-6}–10^{-8}). Change of λ_{max} was also investigated when 0.1 ml base (potassium hydroxide, 0.1 M) and 0.1 ml acid (hydrochloric acid, 0.1 M) were added to dye solutions in methanol (1 ml). Melting points were determined on an Electrothermal 9100 melting point apparatus and they are uncorrected. Elemental analyses were done on a Leco CHNS-932 analyzer.

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2.2. Synthesis of 2-arylhydrazono-3-ketiminobutyronitriles (**1a–l**) and 5-amino-4-arylazo-3-methyl-1H-pyrazoles (**2a–l**)

2-Arylhydrazone-3-ketiminobutyronitriles (**1a–l**) and 5-amino-4-arylazo-3-methyl-1H-pyrazoles (**2a–l**) were prepared according to the literature procedures [27]. The general route for the synthesis of 2-arylhydrazono-3-ketiminobutyronitriles and 5-amino-4-arylazo-3-methyl-1H-pyrazoles is outlined in Scheme 1.

2.3. Synthesis of pyridone-based disperse disazo dyes (**3a–l**)

5-Amino-4-arylazo-3-methyl-1H-pyrazoles (0.01 mol) were dissolved in a mixture of glacial acetic acid and concentrated hydrochloric acid (20 ml, ratio 1:1) and the solution was then cooled to 0–5 °C. Sodium nitrite (0.69 g, 0.01 mol) in water (10 ml) was then added to this solution dropwise with vigorous stirring, about 1 h, while cooling at 0–5 °C. Then the resulting diazonium solution was added in portions over 30 min to a vigorously stirred solution of 3-cyano-6-hydroxy-4-methyl-2-pyridone (1.50 g, 0.01 mol) in KOH (0.56 g, 0.01 mol) and water (10 ml) between 0 and 5 °C, maintaining the pH at 7–8 by simultaneous addition of sodium acetate solutions. The mixture was then stirred for 2 h between 0 and 5 °C. The precipitated product was separated upon dilution with water (50 ml) and then filtered off, washed with water several times, dried and crystallized from DMF–H_2O, respectively. The general route for the synthesis of disazo dyes **3a–l** is outlined in Scheme 1.

2.3.1. 5-[3′-Methyl–4′-(p-nitrophenylazo)-1′H-pyrazole-5′-ylazo]-3-cyano-6-hydroxy-4-methyl-2-pyridone (**3a**)

Orange crystals; yield 84%; mp. 333–334 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3234–3133 (3 NH), 3069 (Ar-H), 2998 (Al-H), 2225 (CN), 1681, 1668 (2 C=O); ^{1}H NMR (DMSO-d_6): δ = 2.74 (s, 3H, 3-CH_3 pyrazole), 2.90 (s, 3H, 4-CH_3 pyridone), 7.60 (d, 2H, J = 9.2, ArH), 8.13 (d, 2H, J = 9.3, ArH), 12.10 (br, 1H, pyridone NH), 13.25 (br, 1H, pyrazole NH), 15.14 (br, 1H, OH or tautomeric hydrazo NH); Anal. Calcd. for $C_{17}H_{13}N_9O_4$: C: 50.13, H: 3.22, N: 30.95. Found: C: 50.28, H: 3.25, N: 30.74.

2.3.2. 5-[3′-Methyl–4′-(p-methoxyphenylazo)-1′H-pyrazole-5′-ylazo]-3-cyano-6-hydroxy-4-methyl-2-pyridone (**3b**)

Brown crystals; yield 72%; mp. 274–275 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3238–3130 (3 NH), 3050 (Ar-H), 2997 (Al-H), 2223 (CN), 1680, 1666 (2 C=O); ^{1}H NMR (DMSO-d_6): δ = 2.74 (s, 3H, 3-CH_3 pyrazole), 2.90 (s, 3H, 4-CH_3 pyridone), 3.86 (s, 3H, p-OCH_3), 7.12 (d, 2H, J = 8.5, ArH), 7.83 (d, 2H, J = 8.3, ArH), 12.05 (br, 1H, pyridone NH), 13.23 (br, 1H, pyrazole NH), 15.10 (br, 1H, OH or tautomeric hydrazo NH); Anal. Calcd. for $C_{18}H_{16}N_8O_3$: C: 55.10, H: 4.11, N: 28.56. Found: C: 55.23, H: 4.07, N: 28.84.

2.3.3. 5-[3′-Methyl–4′-(p-chlorophenylazo)-1′H-pyrazole-5′-ylazo]-3-cyano-6-hydroxy-4-methyl-2-pyridone (**3c**)

Red crystals; yield 79%; mp. 320–321 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3262–3188 (3 NH), 3058 (Ar-H), 2981 (Al-H), 2225 (CN), 1695, 1676 (2 C=O); ^{1}H NMR (DMSO-d_6): δ = 2.75 (s, 3H, 3-CH_3 pyrazole), 2.92 (s, 3H, 4-CH_3 pyridone), 7.54 (d, 2H, J = 8.4, ArH), 7.77 (d, 2H, J = 8.4, ArH), 12.15 (br, 1H, pyridone NH), 13.42 (br, 1H, pyrazole NH), 15.13 (br, 1H, OH or tautomeric hydrazo NH); Anal. Calcd. for $C_{17}H_{13}ClN_8O_2$: C: 51.46, H: 3.30, N: 28.24. Found: C: 51.26, H: 3.37, N: 28.39.

2.3.4. 5-[3′-Methyl–4′-(p-methlyphenylazo)-1′H-pyrazole-5′-ylazo]-3-cyano-6-hydroxy-4-methyl-2-pyridone (**3d**)

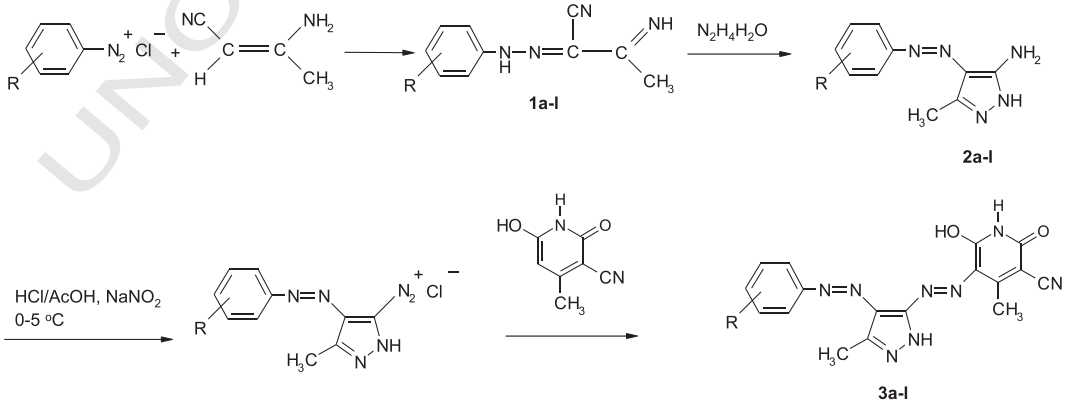
Orange crystals; yield 63%; mp. 228–229 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3246–3121 (3 NH), 3063 (Ar-H), 2993 (Al-H), 2222 (CN), 1689, 1670 (2 C=O); ^{1}H NMR (DMSO-d_6): δ = 2.41 (s, 3H, p-CH_3), 2.74 (s, 3H, 3-CH_3 pyrazole), 2.89 (s, 3H, 4-CH_3 pyridone), 7.39 (d, 2H, J = 7.9, ArH), 7.77 (d, 2H, J = 8.0, ArH), 12.12 (br, 1H, pyridone NH), 13.43 (br, 1H, pyrazole NH), 15.12 (br, 1H, OH or tautomeric hydrazo NH); Anal. Calcd. for $C_{18}H_{16}N_8O_2$: C: 57.44, H: 4.28, N: 29.77. Found: C: 57.62, H: 4.35, N: 29.56.

2.3.5. 5-[3′-Methyl–4′-(m-nitrophenylazo)-1′H-pyrazole-5′-ylazo]-3-cyano-6-hydroxy-4-methyl-2-pyridone (**3e**)

Orange crystals; yield 81%; mp. 324–325 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3226–3136 (3 NH), 3083 (Ar-H), 2954 (Al-H), 2227 (CN), 1677, 1660 (2 C=O); ^{1}H NMR (DMSO-d_6): δ = 2.74 (s, 3H, 3-CH_3 pyrazole), 2.89 (s, 3H, 4-CH_3 pyridone), 7.86–8.64 (m, 4H, ArH), 12.20 (br, 1H, pyridone NH), 13.55 (br, 1H, pyrazole NH), 15.18 (br, 1H, OH or tautomeric hydrazo NH); Anal. Calcd. for $C_{17}H_{13}N_9O_4$: C: 50.13, H: 3.22, N: 30.95. Found: C: 50.31, H: 3.28, N: 31.12.

2.3.6. 5-[3′-Methyl–4′-(m-methoxyphenylazo)-1′H-pyrazole-5′-ylazo]-3-cyano-6-hydroxy-4-methyl-2-pyridone (3f)

Red crystals; yield 69%; mp. 307–308 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3225–3145 (3 NH), 3021 (Ar-H), 2961 (Al-H), 2231 (CN), 1677, 1661 (2 C=O); ^{1}H NMR (DMSO-d_6): δ = 2.74 (s, 3H, 3-CH_3 pyrazole), 2.90 (s, 3H, 4-CH_3 pyridone), 3.86 (s, 3H, m-OCH_3), 7.00–7.98 (m, 4H, ArH), 12.10 (br, 1H, pyridone NH), 13.34 (br, 1H, pyrazole NH), 15.20 (br, 1H, OH or tautomeric hydrazo NH); Anal. Calcd. for



a R = p-NO_2, **b** R = p-OCH_3, **c** R = p-Cl, **d** R = p-CH_3, e R = m-NO_2, f R = m-OCH_3, g R = m-Cl, h R = m-CH_3, i R = o-NO_2, j R = o-OCH_3, k R = o-Cl, l R = o-CH_3

Q2 **Scheme 1.**

$C_{18}H_{16}N_8O_3$: C: 55.10, H: 4.11, N: 28.56. Found: C: 55.19, H: 4.15, N: 28.77.

*2.3.7. 5-[3′-Methyl–4′-(m-chlorophenylazo)-1′H-pyrazole-5′-ylazo]-3-cyano-6-hydroxy-4-methyl-2-pyridone (**3g**)*

Red crystals; yield 73%; mp. 326–327 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3241–3133 (3 NH), 3052 (Ar-H), 2983 (Al-H), 2227 (CN), 1675, 1663 (2 C=O); ^{1}H NMR (DMSO-d_6): δ = 2.73 (s, 3H, 3-CH_3 pyrazole), 2.91 (s, 3H, 4-CH_3 pyridone), 7.00–8.01 (m, 4H, ArH), 12.14 (br, 1H, pyridone NH), 13.45 (br, 1H, pyrazole NH), 15.18 (br, 1H, OH or tautomeric hydrazo NH); Anal. Calcd. for $C_{17}H_{13}ClN_8O_2$: C: 51.46, H: 3.30, N: 28.24. Found: C: 51.59, H: 3.27, N: 28.35.

*2.3.8. 5-[3′-Methyl–4′-(m-methylphenylazo)-1′H-pyrazole-5′-ylazo]-3-cyano-6-hydroxy-4-methyl-2-pyridone (**3h**)*

Red crystals; yield 61%; mp. 314–315 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3224–3138 (3 NH), 3066 (Ar-H), 2946 (Al-H), 2229 (CN), 1670, 1668 (2 C=O); ^{1}H NMR (DMSO-d_6): δ = 2.42 (s, 3H, *m*-CH_3), 2.74 (s, 3H, 3-CH_3 pyrazole), 2.91 (s, 3H, 4-CH_3 pyridone), 7.30–7.99 (m, 4H, ArH), 12.10 (br, 1H, pyridone NH), 13.38 (br, 1H, pyrazole NH), 15.20 (br, 1H, OH or tautomeric hydrazo NH); Anal. Calcd. for $C_{18}H_{16}N_8O_2$: C: 57.44, H: 4.28, N: 29.77. Found: C: 57.32, H: 4.37, N: 29.63.

*2.3.9. 5-[3′-Methyl–4′-(o-nitrophenylazo)-1′H-pyrazole-5′-ylazo]-3-cyano-6-hydroxy-4-methyl-2-pyridone (**3i**)*

Red crystals; yield 78%; mp. 285–286 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3228–3127 (3 NH), 3047 (Ar-H), 2922 (Al-H), 2227 (CN), 1681, 1670 (2 C=O); ^{1}H NMR (DMSO-d_6): δ = 2.75 (s, 3H, 3-CH_3 pyrazole), 2.92 (s, 3H, 4-CH_3 pyridone), 7.65–8.07 (m, 4H, ArH), 12.05 (br, 1H, pyridone NH), 13.52 (br, 1H, pyrazole NH), 14.93 (br, 1H, OH or tautomeric hydrazo NH); Anal. Calcd. for $C_{17}H_{13}N_9O_4$: C: 50.13, H: 3.22, N: 30.95. Found: C: 50.29, H: 3.14, N: 30.81.

*2.3.10. 5-[3′-Methyl–4′-(o-methoxyphenylazo)-1′H-pyrazole-5′-ylazo]-3-cyano-6-hydroxy-4-methyl-2-pyridone (**3j**)*

Brown crystals; yield 71%; mp. 301-302 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3249–3139 (3 NH), 3017 (Ar-H), 2947 (Al-H), 2229 (CN), 1686, 1671 (2 C=O); ^{1}H NMR (DMSO-d_6): δ = 2.73 (s, 3H, 3-CH_3 pyrazole), 2.89 (s, 3H, 4-CH_3 pyridone), 3.94 (s, 3H, *o*-OCH_3), 6.96–7.52 (m, 4H, ArH), 11.94 (br, 1H, pyridone NH), 13.22 (br, 1H, pyrazole NH), 14.75 (br, 1H, OH or tautomeric hydrazo NH); Anal. Calcd. for $C_{18}H_{16}N_8O_3$: C: 55.10, H: 4.11, N: 28.56. Found: C: 55.22, H: 4.07, N: 28.80.

*2.3.11. 5-[3′-Methyl–4′-(o-chlorophenylazo)-1′H-pyrazole-5′-ylazo]-3-cyano-6-hydroxy-4-methyl-2-pyridone (**3k**)*

Dark red crystals; yield 68%; mp. 279–280 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3220–3118 (3 NH), 3038 (Ar-H), 2958 (Al-H), 2238 (CN), 1696, 1676 (2 C=O); ^{1}H NMR (DMSO-d_6): δ = 2.74 (s, 3H, 3-CH_3 pyrazole), 2.90 (s, 3H, 4-CH_3 pyridone), 7.27–7.96 (m, 4H, ArH), 12.05 (br, 1H, pyridone NH), 13.38 (br, 1H, pyrazole NH), 14.88 (br, 1H, OH or tautomeric hydrazo NH); Anal. Calcd. for $C_{17}H_{13}ClN_8O_2$: C: 51.46, H: 3.30, N: 28.24. Found: C: 51.64, H: 3.34, N: 28.37.

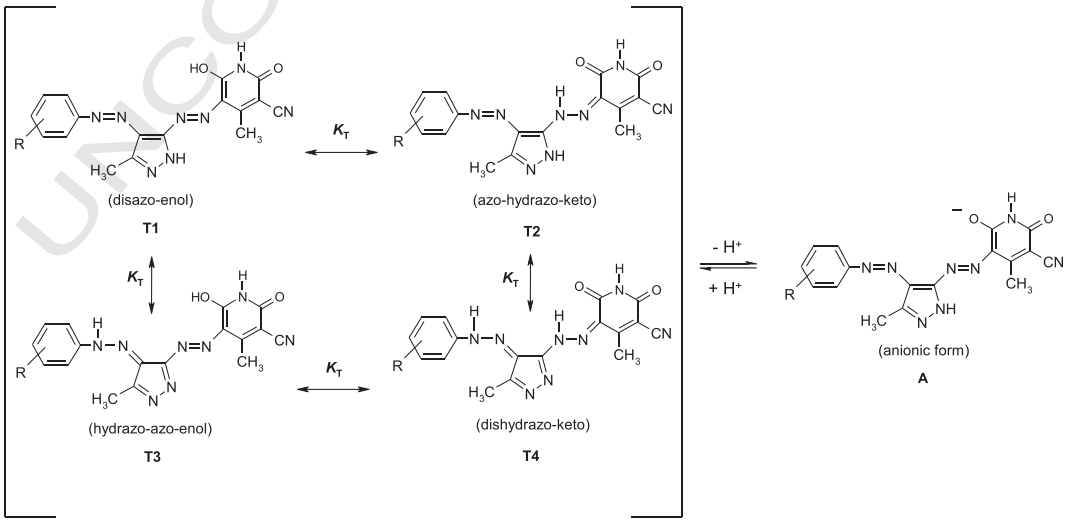
*2.3.12. 5-[3′-Methyl–4′-(o-methylphenylazo)-1′H-pyrazole-5′-ylazo]-3-cyano-6-hydroxy-4-methyl-2-pyridone (**3l**)*

Red crystals; yield 59%; mp. 289–290 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3222–3117 (3 NH), 3037 (Ar-H), 2963 (Al-H), 2238 (CN), 1697, 1662 (2 C=O); ^{1}H NMR (DMSO-d_6): δ = 2.43 (s, 3H, *o*-CH_3), 2.73 (s, 3H, 3-CH_3 pyrazole), 2.89 (s, 3H, 4-CH_3 pyridone), 7.26–7.97 (m, 4H, ArH), 12.00 (br, 1H, pyridone NH), 13.48 (br, 1H, pyrazole NH), 14.82 (br, 1H, OH or tautomeric hydrazo NH); Anal. Calcd. for $C_{18}H_{16}N_8O_2$: C: 57.44, H: 4.28, N: 29.77. Found: C: 57.60, H: 4.30, N: 29.91.

*2.4. Antimicrobial activities of pyridone-based disperse disazo dyes (**3a–l**)*

In vitro antimicrobial activities of the newly synthesized disperse disazo dyes compounds were tested using the microbroth dilution method [32] against a panel of pathogenic and non-pathogenic microorganisms. The panel consisted of *Escherichia coli* ATTC 25922, *Pseudomonas aeruginosa* ATCC 254992, *Salmonella typhimurium* NRRL B-4420, *Staphylococcus aureus* NRRL B-767, *Bacillus subtilis* NRS-744, *Streptococcus faecalis* NRRL B-14617, *Saccharomyces cerevisiae* NRRL Y-12632, and *Candida utilis* NRRL Y-900. Stock solutions of synthesized compounds were sterilized by filtration through 0.45 μm Millipore filters and diluted. A 100 μl from dilutions was transferred to wells of 96-well microtiter plates. Thus, dilution series were prepared ranging from 1 to 0.0009 mg/ml in microtiter plates.

Microorganism cultures prepared from overnight grown microbial suspensions were used as inoculants. These suspensions were standardized to 10^8 CFU/ml using McFarland No: 0.5 standard solutions. A 100 μl from these microorganism suspensions was used as inoculants for each well. The well containing media, sterile distilled water, and inoculum



K_T - tautomeric equilibrium

Q3 **Scheme 2.**

Table 1
Influence of solvent on λ_{max} (nm) of dyes **3a–l**.

Dye no	DMSO	DMF	Acetonitrile	Methanol	Acetic acid	Chloroform
3a	474	476	464	468	465	468
3b	474	474	450	451	453	450
3c	468	470, 524 s	454	456	458	452
3d	467	467, 510 s	457	459	459	459
3e	486	488, 514 s	450	454	454	451
3f	468	469, 512 s	456	458	454	463
3g	467	468, 520 s	454	454	454	459
3h	465	466, 516 s	454	453	454	458
3i	480	482, 530 s	451	453	451	456
3j	468	470, 512 s	454	451	454	456
3k	461	465, 508 s	444	445	444	448
3l	472	473, 520 s	439	434	430	436

Abbreviation: s, shoulder.

served as a positive growth control. The well that does not contain any microorganisms was also used as the control. After incubation at 37 °C for 18–24 h, tetrazolium salts were applied to plates in order to examine microbial growth. The minimal inhibitory concentration (MIC) values were defined as the lowest compound concentration where the absence of growth was recorded. To determine bactericidal or fungicidal activity, a 10 μl aliquot from each well was transferred to the fresh solid media that do not contain any synthesized compounds. Chloramphenicol and ketoconazole were used as reference antibiotics for bacteria and yeasts, respectively. All of the antimicrobial activity studies were performed in triplicate.

3. Results and discussion

3.1. Spectral characteristics and tautomerism

Pyridone-based disperse disazo dyes **3a–l** can exist in four possible tautomeric forms, namely the disazo-enol form (**T1**), the azo-hydrazo-keto form (**T2**), the hydrazo-azo-keto form (**T3**) and the dishydrazo-keto form (**T4**) as shown in Scheme 2. The deprotonation of tautomeric forms of **3a–l** leads to a common anion **A**.

The FT-IR spectra of dyes **3a–l** showed three imino bands (NH) at 3262–3117 cm^{-1} and two carbonyl (C═O) bands at 1697–1660 cm^{-1}. Also, FT-IR spectra of dyes **3a–l** did not show any broad band for hydroxyl group. These suggest that dyes **3a–l** are predominantly in azo-hydrazo-keto form (**T2**) or dishydrazo-keto form (**T4**) as opposed to disazo-enol form (**T1**) and hydrazo-azo-enol form (**T3**), in the solid state (Scheme 2). Numerous investigations were carried out to establish the tautomeric structure of azo pyridone in the solid state using a variety of spectroscopic techniques. The spectral data generally lead to the conclusion that the tautomeric equilibrium of the azo pyridone dyes is in favor of the hydrazone form in the solid state [30,31,33]. The other ν_{max} values of 3083–3017 cm^{-1} (aromatic C–H), 2998–2922 cm^{-1} (aliphatic C–H) and 2238–2222 cm^{-1} (C≡N) were recorded.

^{1}H NMR spectra of dyes **3a–l** showed three broad peaks at 11.94–12.20 ppm (br, 1H, pyridone NH), 13.22–13.55 ppm (br, 1H, pyrazole NH or tautomeric hydrazo NH) and 14.75–15.20 ppm (br, 1H, OH or tautomeric hydrazo NH). These results suggest that dyes **3a–l** are present in a single tautomeric form in DMSO. The other ^{1}H NMR values of 6.96–8.64 ppm (ArH), 2.89–2.92 ppm (3-CH_3 pyrazole) and 2.73–2.75 ppm (4-CH_3 pyridone) were recorded.

3.2. Solvent effects on UV–vis spectra

As the tautomeric equilibria strongly depend on the nature of the media, the behavior of dyes in various solvents was studied. For this purpose, the UV–vis absorption spectra of dyes **3a–l** were recorded over the range of λ between 350 and 700 nm, using a variety of solvents in concentrations (10^{-6}–10^{-8} M). Because of solubility problems, these were run at different concentrations and these results are summarized in Table 1. The visible absorption spectra of the dyes did not correlate with the polarity of solvent.

Dyes **3a** and **3b** gave a maximum absorption peak in all used solvents. This result suggests that dyes **3a** and **3b** are present in a single tautomeric form in all used solvents. Dyes **3c–l** gave a maximum absorption peak with a shoulder in DMF. In the other solvents (DMSO, acetonitrile, methanol, acetic acid and chloroform), dyes **3c–l** gave a maximum absorption peak. These results suggest that dyes **3c–l** are present in the mixture of a tautomeric form and an anionic form in DMF and are present in a single tautomeric form in the other solvents (DMSO, acetonitrile, methanol, acetic acid and chloroform).

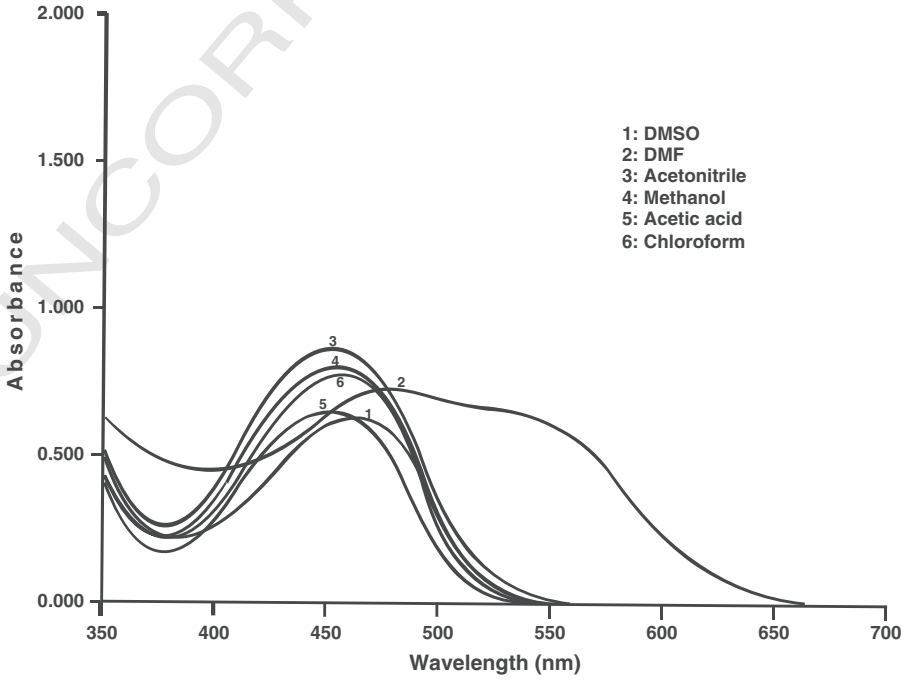


Fig. 1. Absorption spectra of dye **3i** in various solvents.

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Table 2
Absorption maxima of dyes **3a–l** in acidic and basic solutions.

Dye no.	λ_{max} (nm)					
	Methanol	Methanol + KOH	Methanol + HCl	Chloroform	Chloroform + piperidine	Acetic acid
3a	468	497	463	468	479	465
3b	451	492	451	450	451	453
3c	456	502	451	452	465	458
3d	459	496	451	459	470	459
3e	454	512	453	451	470	454
3f	458	500	452	463	473	454
3g	454	504	450	459	470	454
3h	453	493	449	458	469	454
3i	453	508	447	456	487, 525 s	451
3j	451	499	452	456	470, 502 s	454
3k	445	492	442	448	468, 502 s	444
3l	434	498	429	436	470, 517 s	430

Abbreviation: s, shoulder.

It was observed that the λ_{max} of dyes **3a–l** in DMSO and DMF shifted bathochromically with respect to the λ_{max} in chloroform (e.g. for dye **3i** λ_{max} is 456 nm in chloroform, 480 nm in DMSO, 482 nm and 530 nm (shoulder) in DMF) (Fig. 1). On the other hand, λ_{max} values of dyes **3a–l** in acetonitrile, methanol and acetic acid did not change significantly with respect to the λ_{max} in chloroform. It was also observed that absorption ability of these disazo dyes substituted with electron-withdrawing and electron-donating groups at their *o*, *m*- and *p*-position is similar except for dyes **3e** and **3i**. λ_{max} values of *m*-nitro and *o*-nitro derivatives (**3e** and **3i**) shifted bathochromically with respect to the λ_{max} of the other substituents in DMSO and DMF.

3.3. Acid and base effects on UV–vis spectra

The effects of acid and base on the absorption of dye solutions were investigated and the results are shown in Table 2. The absorption spectra of the dyes **3a–l** in methanol was quite sensitive to the addition of base (potassium hydroxide, 0.1 M), with λ_{max} of dyes **3a–l** showing large bathochromic shifts and absorption curves of dyes **3c–l** resembled their shoulders in DMF (e.g. for dye **3i** λ_{max} is 453 nm in methanol, 508 nm in methanol + KOH) (Fig. 2). This indicates that dyes **3a–l** exist in a dissociated state in methanol + KOH. Therefore, the structures of dyes **3a–l** were assigned to a common anionic form (**A**) in strong basic medium (Scheme 2).

When hydrochloric acid (0.1 M) was added to dye solutions in methanol, λ_{max} of dyes **3a–l** did not change significantly with respect to the λ_{max} in methanol (e.g. for dye **3i** λ_{max} is 453 nm in methanol, 447 nm in methanol + HCl) (Fig. 2). This indicates that dyes **3a–l** do not exist in a dissociated state in methanol + HCl.

When piperidine was added to dye solutions in chloroform, λ_{max} of dyes **3a–h** showed bathochromic shifts and absorption curves of the dyes resembled those in DMSO. When piperidine was added to dye solutions in chloroform, λ_{max} of dyes **3i–l** showed bathochromic shifts and absorption curves of the dyes resembled those in DMF (e.g. for dye **3i** λ_{max} is 456 nm in chloroform, 487 nm and 525 nm (shoulder) in chloroform + piperidine) (Fig. 3). These results suggest that dyes **3a–h** are present in a single tautomeric form in chloroform + piperidine and dyes **3i–l** are present in the mixture of a tautomeric form and an anionic form in chloroform + piperidine.

3.4. Antimicrobial activities

Although, antimicrobial activities of monoazo dyes were reported by several authors [34–39], research on disazo dyes can be accepted as

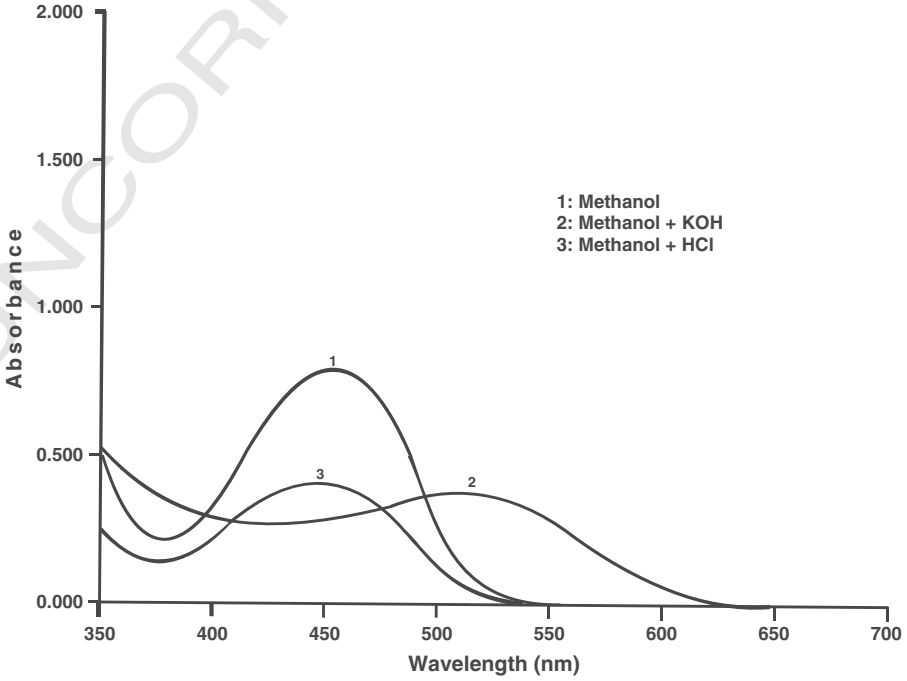

Fig. 2. Absorption spectra of dye **3i** in acidic and basic solutions.

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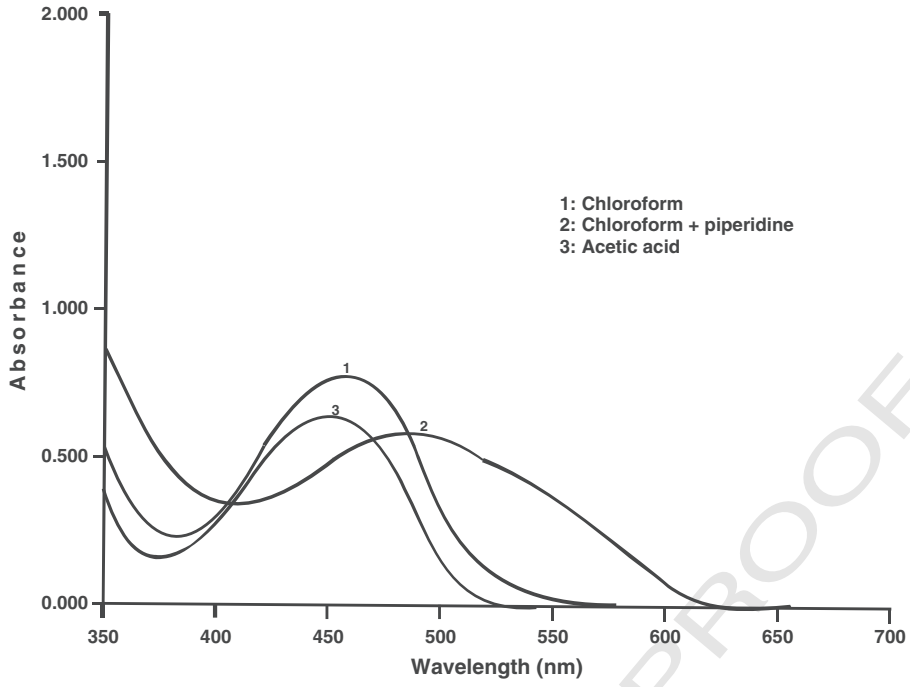

Fig. 3. Absorption spectra of dye **3i** in acidic and basic solutions.

relatively new [40,41]. In the present study, the synthesized disperse disazo dyes **3a–l** were in vitro tested against Gram-positive and negative bacteria and the yeast by using the twofold serial dilution technique. The data presented in Table 3 indicate that the dyes are able to inhibit in vitro growth of the tested microorganisms showing MIC between 125 and 1000 μg/ml except 4k derivative, which was determined active at a MIC 62.5 μg/ml against *S. faecalis*.

It can be seen from Table 3 that most of the synthesized disperse disazo dyes exhibited lower MIC values than the used positive control drugs with the exception of dyes **3d** and **3g**. These dyes have presented only weak antimicrobial activities generally. On the other hand, dye **3c** was the most active compound which exhibited lower MIC values against 6 of the all test microorganisms. Because of the presence in nosocomial infections and resistance to antibiotic therapy, *P. aeruginosa* and *S. faecalis* can be accepted as significant pathogens. Therefore, inhibition of these microorganisms is very important for treatment of secondary infections. Dyes **3a**, **3c**, **3f**, **3j**, and **3k** were presented significant antimicrobial activities against *P. aeruginosa*, exhibiting one dilution step lower potency than the compared control drug, chloramphenicol. On the other hand, dyes **3j** and **3k** showed significant activities against *S. faecalis*, 125 and 62.5 μg/ml MIC concentrations, respectively. Therefore, these dyes may have a potential for nosocomial infections.

It appears that all of the synthesized dyes have static activities against the used test microorganisms other than *C. utilis*. The compounds **3a**, **3c**, **3e**, **3f**, **3j**, and **3k** showed fungicidal activity for *C. utilis* at a MIC 125 μg/ml which is equal to the value of ketoconazole (Table 3).

4. Conclusions

A series of twelve novel pyridone-based disperse disazo dyes was synthesized by coupling diazonium salts of 5-amino-3-methyl-4-arylazo-1H-pyrazoles with 3-cyano-6-hydroxy-4-methyl-2-pyridone. These dyes were characterized by FT-IR, ^{1}H NMR and elemental analysis. Solvent and acid–base influences on the wavelength of maximum

Table 3
Minimal inhibitory concentrations (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentrations (MFC) of dyes **3a–l** and reference antibiotics against test microorganisms.

	MIC (MBC or MFC) μg/ml							
Dye no.	A[a]	B	C	D	E	F	G	H
3a	250 (1000)	250 (1000)	500 (1000)	250 (1000)	500 (>1000)	1000 (>1000)	250 (>1000)	125 (125)
3b	500 (1000)	500 (1000)	500 (1000)	250 (1000)	250 (>1000)	1000 (>1000)	250 (>1000)	250 (250)
3c	250 (>1000)	250 (1000)	250 (1000)	250 (1000)	250 (>1000)	1000 (1000)	250 (>1000)	125 (125)
3d	1000 (1000)	1000 (1000)	1000 (1000)	1000 (>1000)	1000 (>1000)	1000 (>1000)	1000 (>1000)	500 (500)
3e	500 (1000)	500 (1000)	250 (>1000)	250 (1000)	500 (>1000)	1000 (>1000)	250 (>1000)	125 (125)
3f	1000 (1000)	250 (1000)	250 (1000)	500 (1000)	500 (>1000)	1000 (1000)	250 (>1000)	125 (125)
3g	1000 (1000)	1000 (1000)	1000 (1000)	1000 (1000)	1000 (>1000)	1000 (1000)	1000 (>1000)	1000 (1000)
3h	500 (1000)	500 (1000)	500 (1000)	500 (1000)	500 (>1000)	1000 (1000)	250 (>1000)	500 (500)
3i	500 (1000)	500 (1000)	500 (1000)	250 (1000)	250 (>1000)	1000 (>1000)	250 (>1000)	500 (500)
3j	500 (1000)	250 (1000)	500 (1000)	500 (1000)	125 (>1000)	>1000 (>1000)	250 (>1000)	125 (125)
3k	250 (1000)	250 (1000)	500 (1000)	250 (1000)	62.5 (>1000)	1000 (>1000)	125 (>1000)	125 (125)
3l	1000 (1000)	500 (1000)	500 (1000)	500 (1000)	500 (>1000)	1000 (>1000)	500 (>1000)	125 (250)
Reference[b]	1000 (1000)	500 (1000)	500 (1000)	1000 (1000)	1000 (>1000)	1000 (>1000)	1000 (>1000)	125 (125)

[a] A: *Escherichia coli* ATCC 25922, B: *Pseudomonas aeruginosa* ATCC 254992, C: *Salmonella typhimurium* NRRL B-4420, D: *Staphylococcus aureus* NRRL B-767, F: *Bacillus subtilis* NRS-744, E: *Streptococcus faecalis* NRRL B-14617, G: *Saccharomyces cerevisiae* NRRL Y-12632, H: *Candida utilis*, NRRL Y-900.
[b] Reference for A–E: Chloramphenicol and for G, H: Ketoconazole.

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absorption have been studied. Dyes **3a–l** showed bathochromic shifts in most polar solvents, such as DMSO and DMF. It was also observed that the absorption spectra of dyes **3a–l** in methanol were quite sensitive to the addition of base.

As a consequence for antimicrobial activity studies, we can argue that not only bacteria but also yeasts were generally susceptible to all synthesized dyes. Because of its promising antimicrobial activity with broad-spectrum, the newly synthesized heterocyclic disazo dye **3c** could lead for the development of new antimicrobial drug. Dye **3k** revealed a selective activity against *S. faecalis* indicating a potential use for the development of a new selective antimicrobial. On the other hand the most susceptible strain was *C. utilis*. Most of the studied dyes have fungicidal activity against *C. utilis*. Therefore additional studies are needed to determine whether these dyes suitable for other pathogenic *Candida* species such as *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. guilliermondii*.

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