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The synthesis, antimicrobial activity and absorption characteristics of some novel heterocyclic disazo dyes

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ABSTRACT

Aniline derivatives were diazotized and coupled with 3-aminocrotononitrile to give the corresponding 2-arylhydrazono-3-ketiminobutyronitriles. Cyclization of these arylhydrazono derivatives with hydrazine monohydrate afforded 5-amino-4-arylazo-3-methyl-1*H*-pyrazoles which were subsequently diazotised and coupled with malononitrile to yield a series of pyrazolylhydrazonomalononitriles. These compounds were then reacted with hydrazine monohydrate to provide 10, novel, heterocyclic disazo dyes, which were characterized by elemental analysis and spectral methods. The antimicrobial activity and absorption characteristics of the dyes were also examined in detail.

1. Introduction

The growing interest in the pyrazole chemistry lies in designing new synthetic approach, theoretical calculations and applications of newer spectroscopic techniques. The usage of many pyrazole derivatives has undoubtedly created considerable attention in developing many different synthetic procedures in pharmaceuticals, agrochemicals, dyestuff. The recent developments in the synthetic routes and the chemistry of pyrazoles have been thoroughly reviewed [1–7]. The condensation of β-enaminonitriles and β-ketoesters with hydrazines continues to be the most widely used method for constructing the aminopyrazoles and pyrazolones, respectively [8–11]. The amino derivatives of pyrazoles belong to important compounds used for preparation of other functional derivatives mainly for the synthesis of condensed heterocyclic systems [12–14]. Also, fused pyrazoles are important compounds that have many derivatives with a wide range of interesting properties, such as antihyperglycemic, analgestic, anti-inflammatory, anti-pyretic, anti-bacterial, hypoglycaemic and sedative–hypnotic activities. Recently, some pyrazoles were reported to have non-nucleoside HIV-1 reverse transcriptase inhibitory activity [15–19]. Some azopyrazole derivatives also find application in dyes, biological and pharmacological studies and complexes [20–26].

The use of heterocyclic intermediates in the synthesis of azo disperse dyes is well established and the resultant dyes exhibit good tinctorial strength and brighter dyeing than those derived from aniline-based diazo components. For instance, amino-substituted thiazole, benzothiazole [27–30] and benzoisothiazole [31] compounds afford highly electronegative diazo components and consequently, provide a pronounced bathochromic effect compared to the corresponding benzoid compounds. Moreover, azo disperse dyes containing 3-methyl-1*H*-pyrazole-5-one as coupling component have also been described as having red-violet colours in the literature [32,33].

We have previously reported the synthesis of some pyrazolo[5,1-*c*][1,2,4]triazines [34] and disazo barbituric acid dyes [35]. We report here the synthesis of a series of new heterocyclic disazo dyes based on pyrazole rings. Antimicrobial activity and absorption ability of these dyes substituted with electron-withdrawing and electron-donating groups at their *o*-, *m*- and *p*-position were also examined in detail.

2. Experimental

2.1. General

The chemicals used for the synthesis of the compounds were obtained from Aldrich and Merck Chemical Company without

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further purification. The solvents used were of 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 Schimadzu 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}). Melting points were determined on an Electrothermal 9100 melting point apparatus and are uncorrected. Elemental analysis was done on a Leco CHNS-932 analyzer. LC–ESI–MS analyses were recorded on Agilent 1100 MSD.

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

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

*2.3. Synthesis of pyrazolylhydrazonomalononitriles **3a–3j***

5-Amino-4-arylazo-3-methyl-1*H*-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, during 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 malononitrile (0.66 g, 0.01 mol) in pyridine (10 ml) at 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. at between 0 and 5 °C. The precipitated product separated upon dilution with water (50 ml) was filtered off, washed with water several times, dried and crystallized from DMF–H_2O.

*2.4. Synthesis of heterocyclic disazo dyes **4a–4j***

Equimolar amounts (0.005 mol) of **3a**–**3j** and hydrazine monohydrate in ethanol (30 ml) were heated, under reflux, for 4 h. The reaction mixture was concentrated in vacuo and then triturated with water whereby the resulting solid product was collected by filtration and crystallized from DMF–H_2O. The general route for the synthesis of disazo dyes **4a**–**4j** is shown in Fig. 2.

*2.4.1. 4-(3′-Methyl-4′-phenylazo-1′H-pyrazole-5′-ylazo)-3,5-diamino-1*H-*pyrazole (**4a**)*

Orange crystals; yield 75%; mp. 220–221 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3393, 3337 (2 NH_2), 3280, 3203 (2 NH), 3086 (Ar-H), 2991 (Al-H); ^{1}H NMR (DMSO-d_6): $\delta = 2.47$ (s, 3H, CH_3), 6.00 (b, 2H, NH_2), 6.86 (b, 2H, NH_2), 7.40–7.74 (m, 5H, ArH), 11.15 (b, 1H, NH), 12.61 (b, 1H, NH). Anal. Calcd. for $C_{13}H_{14}N_{10}$: C, 50.32; H, 4.55; N, 45.14. Found: C, 55.46; H, 4.46; N, 44.97; MS: *m*/*z* 310.

*2.4.2. 4-[3′-Methyl-4′-(p-methoxyphenylazo)-1′H-pyrazole-5′-ylazo]-3,5-diamino-1H-pyrazole (**4b**)*

Red crystals; yield 84%; mp. 197–198 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3384, 3339 (2 NH_2), 3284, 3197 (2 NH), 3094 (Ar-H), 2986 (Al-H); ^{1}H NMR (DMSO-d_6): $\delta = 2.45$ (s, 3H, CH_3), 3.85 (s, 3H, *p*-OCH_3), 5.65 (b, 2H, NH_2), 6.23 (b, 2H, NH_2), 7.07–7.72 (dd, 4H, ArH), 10.20 (b, 1H, NH), 12.54 (b, 1H, NH). Anal. Calcd. for $C_{14}H_{16}N_{10}O$: C, 49.41; H, 4.74; N, 41.15. Found: C, 49.28; H, 4.79; N, 41.21; MS: *m*/*z* 340.

*2.4.3. 4-[3′-Methyl-4′-(p-chlorophenylazo)-1′H-pyrazole-5′-ylazo]-3,5-diamino-1H-pyrazole (**4c**)*

Brown crystals; yield 89%; mp. 279–280 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3386, 3341 (2 NH_2), 3276, 3169 (2 NH), 3090 (Ar-H), 2994 (Al-H); ^{1}H NMR (DMSO-d_6): $\delta = 2.48$ (s, 3H, CH_3), 6.25 (b, 2H, NH_2), 7.00 (b, 2H, NH_2), 7.90–8.35 (dd, 4H, ArH), 11.20 (b, 1H, NH), 12.75 (b, 1H, NH). Anal. Calcd. for $C_{13}H_{13}ClN_{10}$: C, 45.29; H, 3.80; N, 40.63. Found: C, 45.14; H, 3.86; N, 40.84; MS: *m*/*z* 344.

*2.4.4. 4-[3′-Methyl-4′-(p-methylphenylazo)-1′H-pyrazole-5′-ylazo]-3,5-diamino-1H-pyrazole (**4d**)*

Yellow crystals; yield 68%; mp. dec. >270 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3381, 3349 (2 NH_2), 3289, 3200 (2 NH), 3022 (Ar-H), 2985 (Al-H); ^{1}H NMR (DMSO-d_6): $\delta = 2.40$ (s, 3H, CH_3), 2.76 (s, 3H, *p*-CH_3), 5.52 (b, 2H, NH_2), 6.23 (b, 2H, NH_2), 7.35–7.73 (dd, 4H, ArH), 9.30 (b, 1H, NH), 10.23 (b, 1H, NH). Anal. Calcd. for $C_{14}H_{16}N_{10}$: C, 51.84; H, 4.97; N, 43.18. Found: C, 51.97; H, 5.08; N, 43.36; MS: *m*/*z* 324.

*2.4.5. 4-[3′-Methyl-4′-(m-methoxyphenylazo)-1′H-pyrazole-5′-ylazo]-3,5-diamino-1H-pyrazole (**4e**)*

Brown crystals; yield 81%; mp. 181–182 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3391, 3347 (2 NH_2), 3294, 3203 (2 NH), 3101 (Ar-H), 2978 (Al-H); ^{1}H NMR (DMSO-d_6): $\delta = 2.46$ (s, 3H, CH_3), 3.82 (s, 3H, *m*-OCH_3), 6.00 (b, 2H, NH_2), 6.89 (b, 2H, NH_2), 6.98–7.44 (m, 4H, ArH), 11.16 (b, 1H, NH), 12.63 (b, 1H, NH). Anal. Calcd. for $C_{14}H_{16}N_{10}O$: C, 49.41; H, 4.74; N, 41.15. Found: C, 49.55; H, 4.77; N, 41.26; MS: *m*/*z* 340.

*2.4.6. 4-[3′-Methyl-4′-(m-chlorophenylazo)-1′H-pyrazole-5′-ylazo]-3,5-diamino-1H-pyrazole (**4f**)*

Red crystals; yield 85%; mp. 240–241 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3376, 3307 (2 NH_2), 3275, 3144 (2 NH), 3069 (Ar-H), 2963 (Al-H); ^{1}H NMR (DMSO-d_6): $\delta = 2.44$ (s, 3H, CH_3), 6.05 (b, 2H, NH_2),

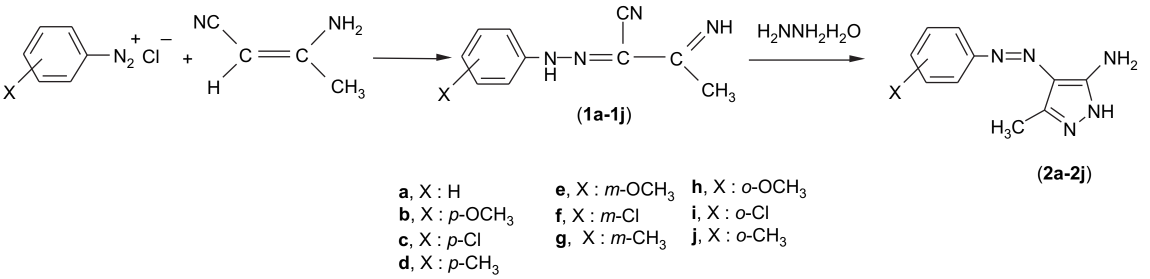

Fig. 1. Synthesis of dyes **2a**–**2j**.

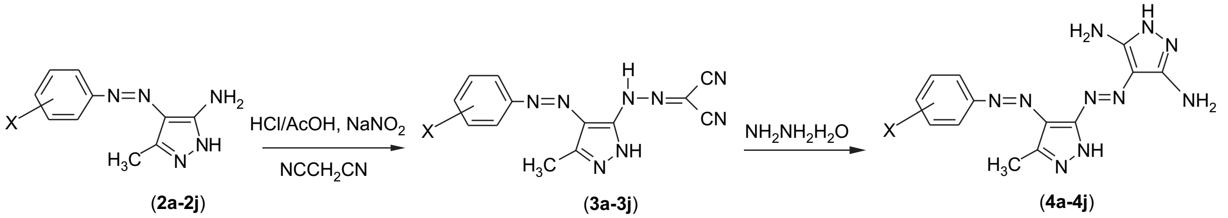

Fig. 2. Synthesis of dyes **4a–4j**.

6.93 (b, 2H, NH_2), 7.45–7.70 (m, 4H, ArH), 11.13 (b, 1H, NH), 12.68 (b, 1H, NH). Anal. Calcd. for $C_{13}H_{13}ClN_{10}$: C, 45.29; H, 3.80; N, 40.63. Found: C, 45.11; H, 3.73; N, 40.47; MS: *m*/*z* 344.

2.4.7. 4-[3′-Methyl-4′-(m-methylphenylazo)-1′H-pyrazole-5′-ylazo]-3,5-diamino-1H-pyrazole (4g)

Red crystals; yield 63%; mp. 218–219 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3392, 3348 (2 NH_2), 3276, 3206 (2 NH), 3078 (Ar-H), 2966 (Al-H); ^{1}H NMR (DMSO-d_6): $\delta = 2.41$ (s, 3H, CH_3), 2.45 (s, 3H, *m*-CH_3), 6.05 (b, 2H, NH_2), 7.00 (b, 2H, NH_2), 7.22–7.53 (m, 4H, ArH), 11.15 (b, 1H, NH), 12.61 (b, 1H, NH). Anal. Calcd. for $C_{14}H_{16}N_{10}$: C, 51.84; H, 4.97; N, 43.18. Found: C, 51.92; H, 4.83; N, 43.40; MS: *m*/*z* 324.

2.4.8. 4-[3′-Methyl-4′-(o-methoxyphenylazo)-1′H-pyrazole-5′-ylazo]-3,5-diamino-1H-pyrazole (4h)

Red crystals; yield 86%; mp. 193–194 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3388, 3343 (2 NH_2), 3284, 3201 (2 NH), 3097 (Ar-H), 2989 (Al-H); ^{1}H NMR (DMSO-d_6): $\delta = 2.46$ (s, 3H, CH_3), 3.91 (s, 3H, *o*-OCH_3), 6.24 (b, 2H, NH_2), 7.00 (b, 2H, NH_2), 7.18–7.52 (m, 4H, ArH), 10.99 (b, 1H, NH), 12.56 (b, 1H, NH). Anal. Calcd. for $C_{14}H_{16}N_{10}O$: C, 49.41; H, 4.74; N, 41.15. Found: C, 49.59; H, 4.83; N, 41.03; MS: *m*/*z* 340.

2.4.9. 4-[3′-Methyl-4′-(o-chlorophenylazo)-1′H-pyrazole-5′-ylazo]-3,5-diamino-1H-pyrazole (4i)

Orange crystals; yield 89%; mp. 245–246 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3388, 3346 (2 NH_2), 3283, 3201 (2 NH), 3081 (Ar-H), 2977 (Al-H); ^{1}H NMR (DMSO-d_6): $\delta = 2.48$ (s, 3H, CH_3), 6.15 (b, 2H, NH_2), 6.72 (b, 2H, NH_2), 7.40–7.66 (m, 4H, ArH), 11.12 (b, 1H, NH), 12.65 (b, 1H, NH). Anal. Calcd. for $C_{13}H_{13}ClN_{10}$: C, 45.29; H, 3.80; N, 40.63. Found: C, 45.46; H, 3.70; N, 40.78; MS: *m*/*z* 344.

2.4.10. 4-[3′-Methyl-4′-(o-methylphenylazo)-1′H-pyrazole-5′-ylazo]-3,5-diamino-1H-pyrazole (4j)

Red crystals; yield 69%; mp. 231–232 °C (DMF–H_2O); IR (KBr): ν (cm^{-1}) = 3389, 3341 (2 NH_2), 3288, 3198 (2 NH), 3093 (Ar-H), 2991 (Al-H); ^{1}H NMR (DMSO-d_6): $\delta = 2.44$ (s, 3H, CH_3), 2.59 (s, 3H, *o*-CH_3), 6.19 (b, 2H, NH_2), 6.94 (b, 2H, NH_2), 7.26–7.53 (m, 4H, ArH), 11.09 (b, 1H, NH), 12.58 (b, 1H, NH). Anal. Calcd. for $C_{14}H_{16}N_{10}$: C, 51.84; H, 4.97; N, 43.18. Found: C, 51.57; H, 5.12; N, 43.37; MS: *m*/*z* 324.

2.5. Antimicrobial activity of heterocyclic disazo dyes 4a–4j

The antimicrobial activities of the newly synthesized compounds were evaluated using the micro broth dilution method [36] against a panel of eight microorganism species. The origin of microbial strains are *Bacillus subtilis* NRRL B-3711, *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* NRRL B-1018, *Enterococcus faecium* NRRL B-2354, *Escherichia coli* ATTC 25922, Proteus vulgaris NRRL B-123, *Candida albicans* NRRL Y-2983, *Candida glabrata* (isolate obtained from Eskisehir Osmangazi University, Medicine Faculty) as yeasts. Stock solutions of synthesized compounds were

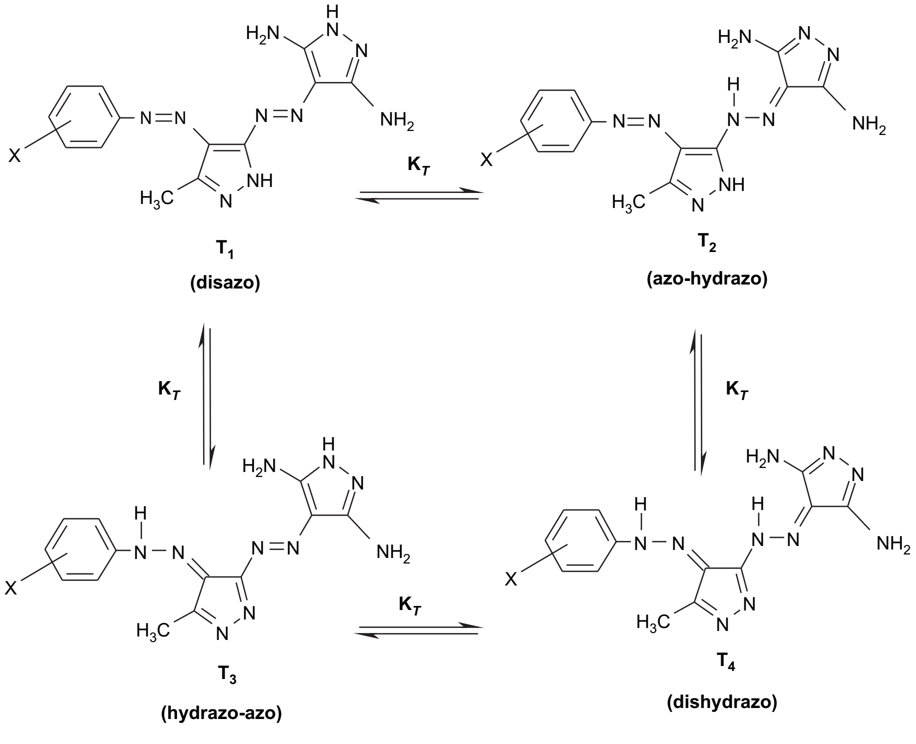

Fig. 3. Tautomeric equilibriums of dyes **4a–4j**.

Table 1
Influence of solvent on λ_{max} (nm) of dyes **4a**–**4j**

Dye no	DMSO	DMF	Acetonitrile	Methanol	Acetic acid	Chloroform
4a	471	467	454	420	436	442
4b	465	463	452	418	433	439
4c	501	502	459	435	448	453
4d	454	451	433	406	408	425
4e	470	466	452	414	416	448
4f	483	480	463	423	422	456
4g	470	466	451	420	418	443
4h	473	466	457	428	425	455
4i	484	479	464	430	426	453
4j	467	462	445	400	416	439

diluted in DMSO to give serial decreasing dilutions ranging from 4 to 0.0009 mg/ml. The dilutions were sterilized by filtration through 0.45 μm millipore filters and were transferred to 96-well microtitre plates. Overnight grown microbial suspensions adjusted to McFarland 0.5 standard solution were used as inoculants. A 100 μl from each microorganism suspension was transferred into the wells. The well containing media, sterile distilled water and inoculum was used for positive growth control. The minimal inhibitory concentration (MIC) values were determined after incubation at 37 °C for 18–24 h. The MIC values were defined as the lowest compound concentration where absence of growth was recorded. Each test was repeated at least twice for all microorganisms. Streptomycin and fluconazole 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

Disazo dyes **4a**–**4j** can exist in four possible tautomeric forms, namely the disazo form **T1**, the azo-hydrazo form **T2**, the hydrazo-azo form **T3** and the dishydrazo form **T4** as shown in Fig. 3. The FT-IR spectra of dyes **4a**–**4j** showed intense two amino (NH_2) bands at 3393–3307 $cm^{-1}$and intense two imino (NH) bands at 3294–3144 cm^{-1}. The other ν_{max} values of 3101–3022 cm^{-1} (aromatic C–H) and 2994–2963 cm^{-1} (aliphatic C–H) were recorded.

^1H NMR spectra of dyes **4a**–**4j** showed four broad peaks at 12.75–10.23 ppm (NH), 11.20–9.30 ppm (NH), 7.00–6.23 ppm (NH_2) and 6.25–5.52 ppm (NH_2), respectively. The other δ values of 2.48–2.40 ppm (CH_3) and 8.35–6.98 ppm (aromatic H) were recorded.

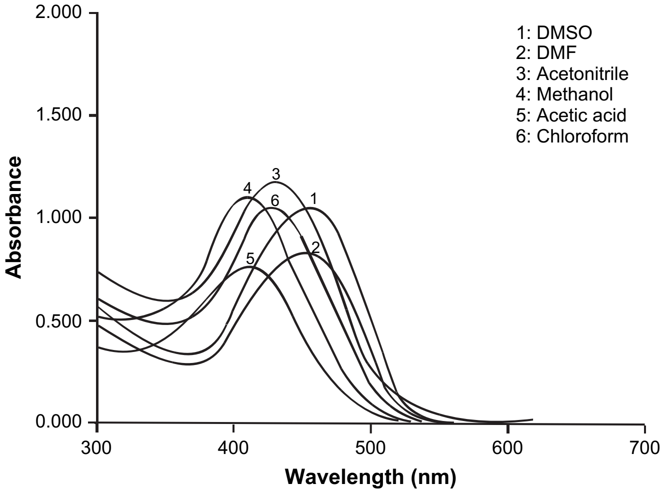

Fig. 4. Absorption spectra of dye **4d** in various solvents.

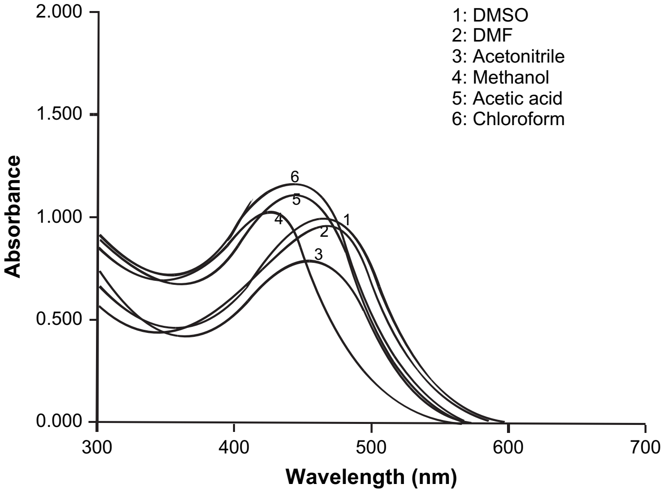

Fig. 5. Absorption spectra of dye **4b** in various solvents.

These results suggest that dyes **4a**–**4j** are present as one of the tautomeric forms in DMSO and the solid state.

Previously, we established that the tautomeric structure of pyrazole dyes in the solid state and solution medium using FT-IR and ^1H NMR. The spectral data generally lead to the conclusion that the tautomeric equilibrium of these dyes was in favour of the hydrazo form [10,11,37]. These suggest that these dyes are predominantly in azo-hydrazo form (**T2**) or dishydrazo form (**T4**) in the solid state and DMSO.

3.2. Solvent effects on UV–vis spectra

The UV–vis absorption spectra of dyes **4a**–**4j** were recorded over the range of λ between 300 and 700 nm, using a variety of solvents in concentrations (10^{-6}–10^{-8} M) and the results are summarised in Table 1. The visible absorption spectra of the dyes did not correlate with the polarity of solvent.

Dyes **4a**–**4j** gave a maximum absorption peak in all used solvents. This result suggests that dyes **4a**–**4j** are present in a single tautomeric form in all used solvents.

It was observed that in DMSO, DMF and acetonitrile, λ_{max} of dyes **4a**–**4j** shifted bathochromically with respect to the λ_{max} in chloroform (e.g. for dye **4d** λ_{max} is 425 nm in chloroform, 454 nm in DMSO, 451 nm in DMF and 433 nm in acetonitrile) (Fig. 4). But, bathochromic shifts of λ_{max} of dyes **4a**–**4j** in acetonitrile are less than bathochromic shifts of λ_{max} of dyes **4a**–**4j** in DMSO and DMF. On the other hand, it was observed that in acetic acid and methanol, λ_{max} of dyes **4a**–**4j** shifted hypsochromically with respect to the λ_{max} in chloroform (e.g. for dye **4b** λ_{max} is 439 nm in chloroform, 433 nm in acetic acid and 418 nm in methanol) (Fig. 5). It was also

Table 2
Absorption maxima of dyes **4a**–**4j** in acidic and basic solutions

Dye no	λ_{max} (nm)					
	Methanol	Methanol + KOH	Methanol + HCl	Chloroform	Chloroform + piperidine	Acetic acid
4a	420	467	433	442	449	436
4b	418	466	431	439	444	433
4c	435	520	446	453	471	448
4d	406	465	407	425	427	408
4e	414	465	417	448	449	416
4f	423	478	423	456	459	422
4g	420	467	421	443	445	418
4h	428	483	427	455	456	425
4i	430	474	427	453	454	426
4j	400	461	422	439	442	416

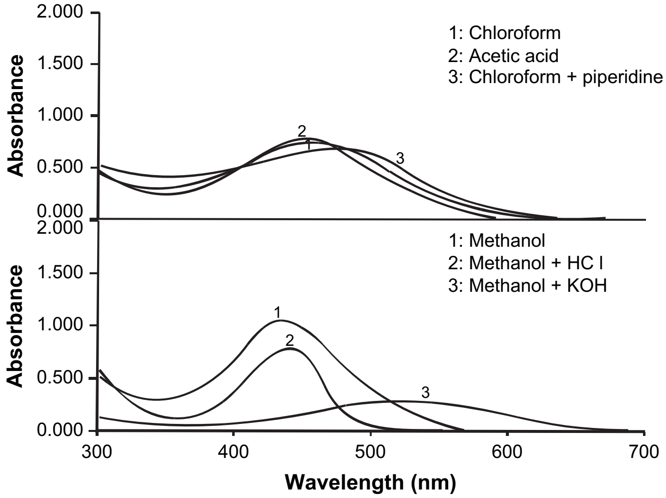

Fig. 6. Absorption spectra of dye **4c** in acidic and basic solutions.

observed that hypsochromic shifts of λ_{max} of dyes **4a**–**4c** and **4j** in acetic acid are less than hypsochromic shifts of λ_{max} of dyes **4a**–**4c** and **4j** in methanol. Hypsochromic shifts of λ_{max} of dyes **4d**–**4i** in acetic acid and methanol are similar.

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 in methanol were also quite sensitive to the addition of base (potassium hydroxide, 0.1 M), with λ_{max} of dyes **4a**–**4j** showing bathochromic shifts and absorption curves of the dyes resembled those in DMSO and DMF (Fig. 6). This result suggests that these dyes are present in a different tautomeric form in methanol + KOH than that in methanol and this tautomeric form resembled those in DMSO and DMF.

When hydrochloric acid (0.1 M) was added to dye solutions in methanol, λ_{max} of dyes **4a**–**4c** and **4j** showed little bathochromic shifts with respect to the λ_{max} in methanol and the absorption spectra of dyes resembled those in acetic acid (Fig. 6). It was also observed that when hydrochloric acid (0.1 M) was added to dye solutions in methanol, λ_{max} of dyes **4d**–**4i** did not change significantly.

When piperidine was added to dye solutions in chloroform, λ_{max} of dyes **4a**–**4j** did not change significantly except for dye **4c** (Fig. 6). λ_{max} of the dye **4c** showed bathochromic shift when a small amount of piperidine was added to dye **4c** solution in chloroform.

3.4. Substituent effects on UV–vis spectra

As seen in Table 1, generally, electron-accepting chloro groups in all positions for dyes **4c**, **4f** and **4i** cause bathochromical shifts in all used solvents when compared with dye **4a**. λ_{max} of dyes **4f** and **4i** in acetic acid hypsochromically shifted when compared with λ_{max} of dye **4a** in acetic acid. Electron-donating methoxy and methyl groups in *para* position for dyes **4b** and **4d** and methyl group in *ortho* position for dye **4j** cause hypsochromical shifts in all used solvents when compared with dye **4a**. Visible absorption spectra of dyes **4e**, **4g** and **4h** did not regularly change with the substituent effect in all used solvents when compared with dye **4a**.

3.5. Antimicrobial activity of the synthesized dyes

Although, there are some reports about antimicrobial activity of monoazo dyes [38–40], research on biological activity of disazo dyes has just started [41]. In the current study, *in vitro* antimicrobial activities of the newly synthesized disazo dyes were also reported. The results of antimicrobial screening of the synthesized compounds and standard antibiotics are given in Table 3.

The MIC values of the dyes are generally within the range 3.90–2000 μg/ml against all tested microorganisms. Results showed that none of the synthesized dyes have important antibacterial activities when compared with control antibiotic, streptomycin. Not only gram negative but also gram-positive bacteria were resistant to all synthesized dyes with the exception of dye **4a**. This compound showed activity against *B. subtilis*, *S. aureus* and *M. luteus* at the dose of 125 μg/ml.

In contrast, different levels of antifungal activities were observed for some dyes against the yeasts. Dyes **4a**–**4c**, **4e** and **4f** exhibited stronger antifungal activity than not only other dyes but also fluconazole against *C. glabrata*. Among the all tested dyes, **4c**, **4e** and **4f** showed the highest antifungal activity against both the yeasts. These dyes had similar activity level with fluconazole against *C. albicans*. On the other hand, as an impressive result, active concentration of these dyes against *C. glabrata* was lower than fluconazole. The rest of dyes had no important inhibitory activity against the yeasts *C. albicans* and *C. glabrata*.

In previous studies about antimicrobial activity of monoazo or disazo dyes, synthesized compound had antibacterial activity [41–45]. It was reported weak or no antimicrobial activity for these compounds against fungi [40,45]. However, in the present study,

Table 3
Biological activities of dyes **4a**–**4j** (μg/ml)

Dye no	Bacteria						Yeasts	
	A[a]	B	C	D	E	F	G	H
4a	125	125	125	1000	2000	2000	500	3.90
4b	2000	2000	2000	2000	2000	1000	2000	3.90
4c	>4000	2000	>4000	>4000	2000	>4000	3.90	3.90
4d	1000	500	1000	1000	1000	1000	1000	1000
4e	1000	2000	1000	500	2000	2000	3.90	3.90
4f	2000	2000	2000	1000	1000	2000	3.90	3.90
4g	2000	2000	2000	1000	2000	2000	2000	1000
4h	2000	2000	2000	1000	2000	2000	2000	2000
4i	2000	2000	2000	1000	1000	2000	2000	1000
4j	2000	2000	2000	2000	1000	2000	2000	2000
Reference	15.62[b]	0.97	1.95	7.81	3.90	31.25	3.90[c]	7.81

[a] A: *Bacillus subtilis* (NRRL B-3711), B: *Staphylococcus aureus* (ATCC-25923), C: *Micrococcus luteus* (NRRL B-1018), D: *Enterococcus faecium* (NRRL B-2354), F: *Escherichia coli* (ATTC-25992), E: *Proteus vulgaris* (NRRL B-123), G: *Candida albicans* (NRRL Y-12983) H: *Candida glabrata* (isolate obtained from Eskişehir Osmangazi University, Medicine Faculty).

[b] Streptomycin for bacteria.

[c] Fluconazole for yeasts.

synthesized dyes were determined more active against fungal organisms. Therefore the results obtained in the present study can be accepted as promising to develop new antifungal compound(s).

4. Conclusions

A series of 10 novel disazo dyes based on heterocyclic rings were synthesized by heating pyrazolylhydrazonomalononitriles with hydrazine monohydrate. Solvent, substituent and acid–base influence on the wavelength of maximum absorption have been studied. In the present paper, heterocyclic disazo dyes showed solvatochromic effects. The absorption maxima of dyes showed bathochromic shifts in DMSO and DMF than the other four solvents. It was also observed that the absorption spectra of these disazo dyes in methanol were quite sensitive to the addition of base.

Our study demonstrated clearly that, novel **4c**, **4e** and **4f** dyes had significant antifungal activity when compared with control antibiotic, fluconazole. As a consequence, we can conclude that especially the newly synthesized **4c**, **4e** and **4f** dyes could be lead for the development of new antifungal drugs.

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