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Synthesis, antimicrobial activity and absorption studies of some novel heterocyclic dyes based on 4-hexylbenzene-1,3-diol

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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 diazotized and coupled with malononitrile to yield a series of pyrazolylhydrazonomalononitriles. These compounds were then reacted with hydrazine monohydrate to provide, heterocyclic dyes, which were further diazotized and coupled with 4-hexylbenzene-1,3-diol to produce novel heterocyclic tetraazo 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 [1-5]. 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 [6,7]. The amino derivatives of pyrazoles belong to important compounds used for preparation of other functional derivatives mainly for the synthesis of condensed heterocyclic systems [8-12]. 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 [13-15].

Some azopyrazole derivatives also find application in dyes, biological and pharmacological studies and complexes [16-18]. 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 [19,20] and benzoisothiazole [21] 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 and 4-hexylbenzene-1,3-diol as coupling component have also been described as having red-violet colours in the literature [22-25].

We report here the synthesis of a series of new heterocyclic tetraazo dyes based on 4-hexylbenzene-1,3-diol. 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 Synthesis

All the chemicals used for the synthesis of the compounds were obtained from various companies (commercial grade) and were further purified by crystallization. The solvents used were of spectroscopic grade.

IR spectra were determined using a Perkin-elmer Spectrum GX FT-IR model, on a KBr disc. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Hitachi R-1500 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 Carl Zeiss UV/VIS Specord spectrometer 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 ($1x10^{-6}$ - $1x10^{-8}$ M). Melting points were determined by open capillary method and are uncorrected. Elemental analysis was done on a Perkin Elmer CHNS/O Analyzer 2400 Series II were recorded on Agilent 1100 MSD.

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

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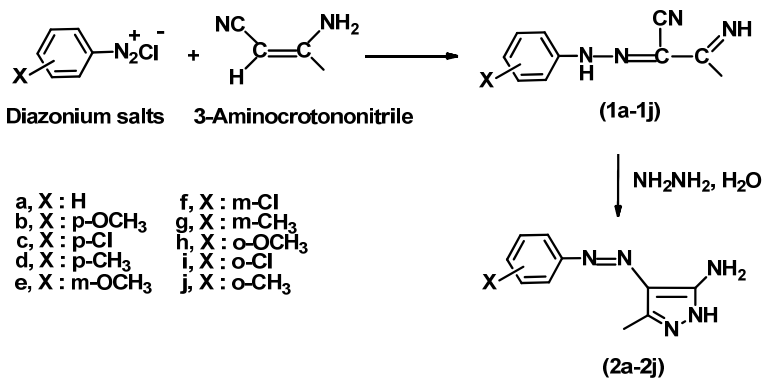
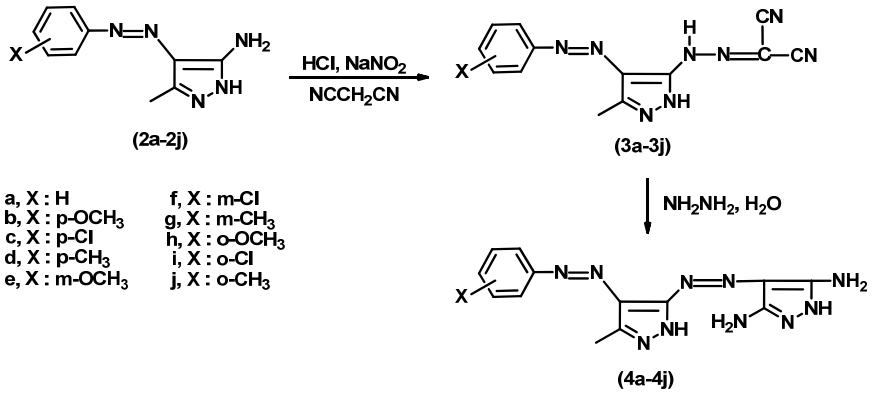


Scheme 1



Scheme 2

2-Arylhydrazone-3-ketiminobutyronitriles (**1a-1j**) and 5-amino-4-arylazo-3-methyl-1H-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 (Scheme 1).

2.1.2. 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 273.15-278.15 K. Sodium nitrite (0.69 g, 0.01 mol) in water (10 mL) was then added to this solution drop wise with vigorous stirring, during about 1 h, while cooling at 273.15 - 278.15 K. 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 273.15 and 278.15 K, maintaining the pH at 7-8 by simultaneous addition of sodium acetate solutions. The mixture was then stirred for 2 h. at between 273.15 and 278.15 K. 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.

2.1.3. 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 (Scheme 2).

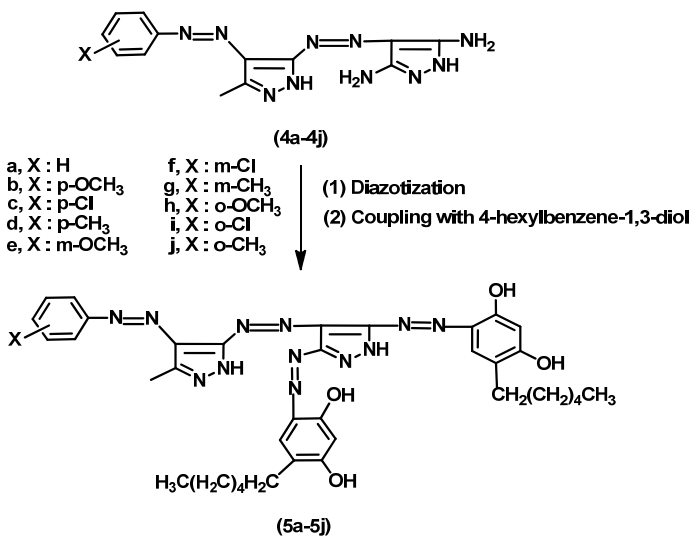
2.1.4. Synthesis of heterocyclic tetraazo dyes 5a-5j

Diazotization of **4a-4j** and coupling with 4-hexylbenzene-1,3-diol were prepared according to the literature procedure [26]. The general route for the synthesis of heterocyclic tetraazo dyes **5a-5j** is shown in (Scheme 3).

4-(3'-Methyl-4'-phenylazo-1'H-pyrazole-5'-ylazo)-3,5-[4,4'-dihexyl-1,1',3,3'-tetrahydroxy-diphenylazo]-1H-pyrazole (**5a**): yellowish Orange. Yield: 77%. M.p.: 186-187 °C. FT-IR (KBr, cm^{-1}): 3460, 3449 (2 OH), 3280, 3203 (2 NH), 3086 (Ar-H), 2991 (Ar-H). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.90 (t, 3H, CH_3),1.31-1.59 (m, 6H, -CH_2), 2.45 (s, 3H, CH_3), 2.62 (s, 2H, -CH_2), 6.45 (s, 1H, ArH), 6.91 (s, 1H, ArH), 7.40-7.74 (m, 5H, ArH), 8.2 (s, 1H, Ar-OH), 10.5 (s, 1H, Ar-OH), 11.15 (b,1H, NH),12.61 (b, 1H, NH). Anal. calcd. for $C_{37}H_{44}O_4N_{12}$: C, 61.60; H, 6.10; N, 23.33. Found: C, 61.51; H, 5.98; N, 23.28%.

4-[3'-Methyl-4'-(p-methoxyphenylazo)-1'H-pyrazole-5'-ylazo]-3,5-[4,4'-dihexyl-1,1',3,3'-tetrahydroxy-diphenylazo]-1H-pyrazole (**5b**): Red. Yield: 81%. M.p.: 167-168 °C. FT-IR (KBr, cm^{-1}): 3462, 3453 (2 OH), 3284, 3197 (2 NH), 3094 (Ar-H), 2986 (Ar-H). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.90 (t, 3H, CH_3),1.31-1.59 (m, 6H, -CH_2), 2.45 (s, 3H, CH_3), 2.62 (s, 2H, -CH_2), 6.45 (s, 1H, ArH), 6.91 (s, 1H, ArH), 7.40-7.74 (m, 5H, ArH), 8.2 (s, 1H, Ar-OH), 10.5 (s, 1H, Ar-OH), 11.15 (b,1H, NH),12.61 (b, 1H, NH). Anal. calcd. for $C_{38}H_{46}O_5N_{12}$: C, 60.80; H, 6.10; N, 22.41. Found: C, 60.20; H, 6.88; N, 22.37%.

4-[3'-Methyl-4'-(p-chlorophenylazo)-1'H-pyrazole-5'-ylazo]-3,5-[4,4'-dihexyl-1,1',3,3'-tetrahydroxy-diphenylazo]-1H-pyrazole (**5c**): Reddish Brown. Yield: 84%. M.p.: 249-250 °C.



Scheme 3

FT-IR (KBr, cm^{-1}): 3480, 3460 (2 OH), 3276, 3169 (2 NH), 3090 (Ar- H), 2994 (Ar-H). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.90 (t, 3H, CH_3), 1.31-1.59 (m, 6H, -CH_2), 2.45 (s, 3H, CH3), 2.62 (s, 2H, -CH_2), 6.45 (s, 1H, ArH), 6.91 (s, 1H, ArH), 7.40-7.74 (m, 5H, ArH), 8.2 (s, 1H, Ar-OH), 10.5 (s, 1H, Ar-OH), 11.15 (b, 1H, NH),12.61 (b, 1H, NH). Anal. calcd. for $C_{37}H_{43}O_4N_{12}Cl$: C, 58.88; H, 5.71; N, 22.28. Found: C, 58.82; H, 5.69; N, 22.23%.

4-[3'-Methyl-4'-(p-methylphenylazo)-1'H-pyrazole-5'-ylazo]-3,5-[4,4'-dihexyl-1,1',3,3'-tetrahydroxy-diphenylazo]-1H-pyrazole (**5d**): Yellow Brown. Yield: 62%. M.p.: 275 °C. FT-IR (KBr, cm^{-1}): 3491, 3480 (2 OH), 3289, 3200 (2 NH), 3022 (Ar-H), 2985 (Ar-H). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.88 (t, 3H, CH_3),1.26-1.79 (m, 2H, CH_2),2.40 (s, 3H, CH_3), 2.76 (s, 3H, p-CH_3), 7.35-7.73 (dd, 4H, ArH), 8.9 (s, 1H, Ar-OH), 10.9 (s, 1H, Ar-OH), 9.30 (b, 1H, NH), 10.23 (b, 1H, NH). Anal. calcd. for $C_{38}H_{46}O_4N_{12}$: C, 62.10; H, 6.23; N, 22.28. Found: C, 61.92; H, 6.18; N, 22.82%.

4-[3'-Methyl-4'-(m-methoxyphenylazo)-1'H-pyrazole-5'-ylazo]-3,5-[4,4'-dihexyl-1,1',3,3'-tetrahydroxy-diphenylazo]-1H-pyrazole (**5e**): Yellowish. Yield: 78%. M.p.: 168-169 °C. FT-IR (KBr, cm^{-1}): 3464, 3450 (2 OH), 3294, 3203 (2 NH), 3101 (Ar-H), 2978 (Ar-H). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.85 (t, 3H, CH_3), 1.31-1.68 (m, 2H, CH_2),2.46 (s, 3H, CH_3), 3.82 (s, 3H, m-OCH_3), 6.98-7.44 (m, 4H, ArH), 8.4 (s, 1H, Ar-OH), 11.0 (s, 1H, Ar-OH), 11.16 (b, 1H, NH), 12.63 (b, 1H, NH). Anal. calcd. For $C_{38}H_{46}O_5N_{12}$: C, 60.80; H, 6.10; N, 22.40. Found: C, 60.60; H, 6.01; N, 22.10%.

4-[3'-Methyl-4'-(m-chlorophenylazo)-1'H-pyrazole-5'-ylazo]-3,5-[4,4'-dihexyl-1,1',3,3'-tetrahydroxy-diphenylazo]-1H-pyrazole (**5f**): Red. Yield: 81%. M.p.: 224-225 °C. FT-IR (KBr, cm^{-1}): 3482, 3459 (2 OH), 3275, 3144 (2 NH), 3069 (Ar-H), 2963 (Ar-H). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.89 (t, 3H, CH_3), 1.33-1.89 (m, 2H, CH_2),2.44 (s, 3H, CH_3), 7.45-7.70 (m, 4H, ArH), 8.7 (s, 1H, Ar-OH), 11.2 (s, 1H, Ar-OH), 11.13 (b, 1H, NH), 12.68 (b,1H, NH). Anal. calcd. for $C_{37}H_{43}O_4N_{12}$: C, 58.88; H, 5.70; N, 22.28. Found: C, 58.81; H, 5.60; N, 22.22%.

4-[3'-Methyl-4'-(m-methylphenylazo)-1'H-pyrazole-5'-ylazo]-3,5-[4,4'-dihexyl-1,1',3,3'-tetrahydroxy-diphenylazo]-1H-pyrazole (**5g**): Red. Yield: 73%. M.p.: 190-191 °C. FT-IR (KBr, cm^{-1}): 3494, 3483 (2 OH), 3276, 3206 (2 NH), 3078 (Ar-H), 2966 (Ar-H). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.85 (t, 3H, CH_3), 1.37-1.79 (m, 2H, CH_2), 2.41 (s, 3H,CH_3), 2.45 (s, 3H, m-CH_3), 7.22-7.53 (m, 4H, ArH), 8.3 (s, 1H, Ar-OH), 10.6 (s, 1H, Ar-OH), 11.15 (b, 1H, NH), 12.61 (b, 1H, NH). Anal. calcd. for $C_{38}H_{46}O_4N_{12}$: C, 62.10; H, 6.23; N, 22.88. Found: C, 61.89; H, 6.18; N, 22.85%.

4-[3'-Methyl-4'-(o-methoxyphenylazo)-1'H-pyrazole-5'-ylazo]-3,5-[4,4'-dihexyl-1,1',3,3'-tetrahydroxy-diphenylazo]-1H-pyrazole (**5h**): Red. Yield: 84%. M.p.: 183-184 °C. FT-IR (KBr, cm^{-1}): 3467, 3456 (2 OH), 3284, 3201 (2 NH), 3097 (Ar-H), 2989 (Ar-H). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.86 (t, 3H, CH_3), 1.41-1.67 (m, 2H, CH_2), 2.46 (s, 3H,CH_3), 3.91 (s, 3H, o-OCH_3), 7.18-7.52 (m, 4H, ArH), 7.9 (s, 1H, Ar-OH), 11.0 (s, 1H, Ar-OH), 10.99 (b, 1H, NH), 12.56 (b, 1H, NH). Anal. Calcd. for $C_{38}H_{46}O_5N_{12}$: C, 60.8; H, 6.10; N, 22.40. Found: C, 60.50; H, 5.89; N, 22.31%.

4-[3'-Methyl-4'-(o-chlorophenylazo)-1'H-pyrazole-5'-ylazo]-3,5[4,4'-dihexyl-1,1',3,3'-tetrahydroxy-diphenylazo]-1H-pyrazole (**5i**): Yellowish Orang. Yield: 85%. M.p.: 235-236 °C. FT-IR (KBr, cm^{-1}): 3479, 3465 (2 OH), 3283, 3201 (2 NH), 3081 (Ar-H), 2977 (Ar-H). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.88 (t, 3H, CH_3), 1.49-1.89 (m, 2H, CH_2), 2.48 (s, 3H, CH_3), 7.40-7.66 (m, 4H, ArH), 8.4 (s, 1H, Ar-OH), 10.7 (s, 1H, Ar-OH), 11.12 (b, 1H, NH), 12.65 (b, 1H, NH). Anal. calcd. for $C_{37}H_{43}O_4N_{12}Cl$: C, 58.88; H, 5.70; N, 22.28. Found: C, 58.80; H, 5.63; N, 22.23%.

4-[3'-Methyl-4'-(o-methylphenylazo)-1'H-pyrazole-5'-ylazo]-3,5-[4,4'-dihexyl-1,1',3,3'-tetrahydroxy-diphenylazo]-1H-pyrazole (**5j**): Redish Brown. Yield: 72 %. M.p.: 221-222 °C. FT-IR (KBr, cm^{-1}): 3497, 3486 (2 OH), 3288, 3198 (2 NH), 3093 (Ar-H), 2991 (Ar-H). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.90 (t, 3H, CH_3), 1.35-1.76 (m, 2H, CH_2), 2.44 (s, 3H, CH_3), 2.59 (s, 3H, -CH_3), 7.26-7.53 (m, 4H, ArH), 8.8 (s, 1H, Ar-OH), 11.2 (s, 1H, Ar-OH), 11.09 (b, 1H, NH), 12.58 (b, 1H, NH). Anal. calcd. for $C_{38}H_{46}O_4N_{12}$: C,62.10; H, 6.23; N, 22.88. Found: C, 61.81; H, 6.19; N, 22.84%.

2.2. Antimicrobial activity of heterocyclic tetraazo dyes 5a-5j

The antimicrobial activities of the newly synthesized compounds were evaluated using the micro broth dilution method [27] against a panel of eight microorganism species. The origin of microbial strains are *Bacillus subtilis* (NRRL B-3711), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATTC 25922), *Proteus vulgaris* (NRRL B-123), *Candida albicans* (NRRL Y-2983), *Candida glabrata* as yeasts. Stock solutions of synthesized compounds were 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.

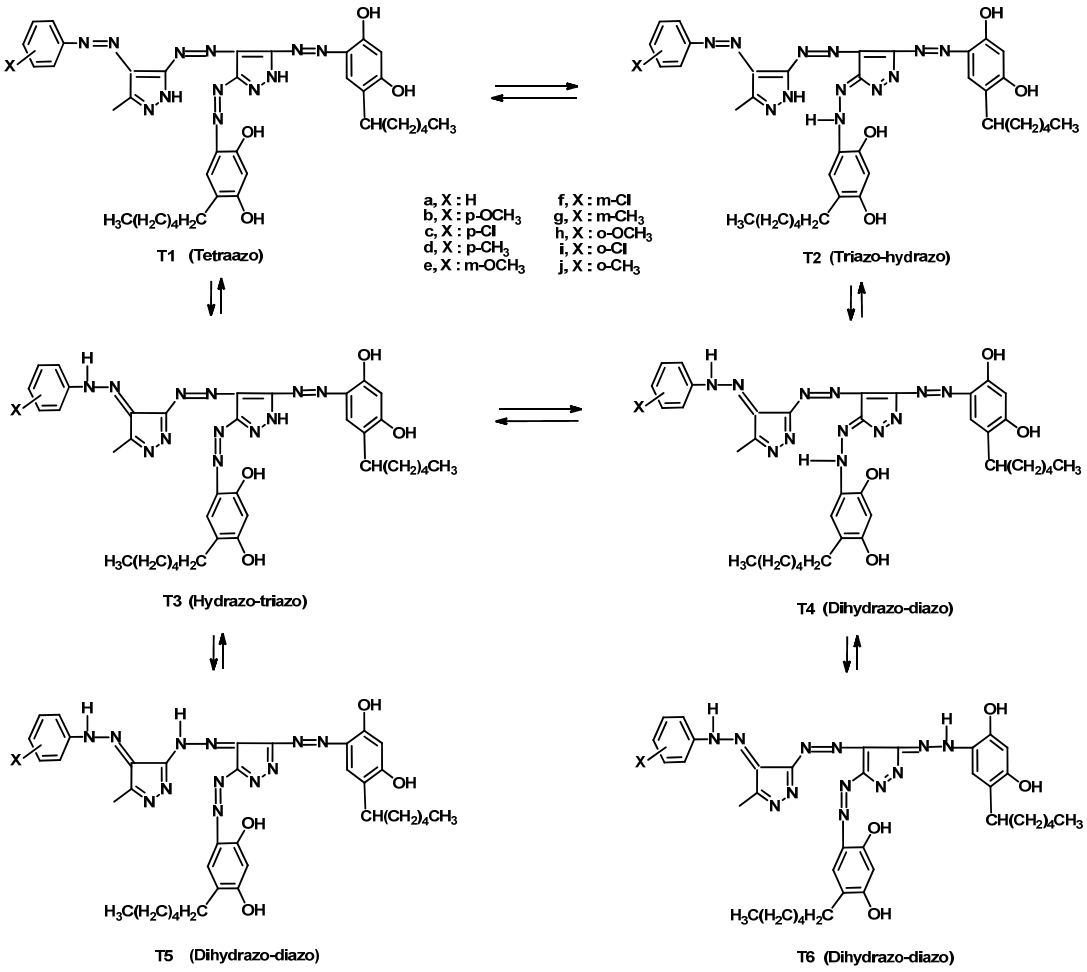


Figure 1. Tautomeric equilibriums of dyes **5a-5j**.

Overnight grown microbial suspensions adjusted to McFarland 0.5 standard solutions were used as inoculants. A 100 µl from each microorganism suspension was transferred into the wells. The well containing media, sterile distilled water and inoculums were used for positive growth control. The minimal inhibitory concentration (MIC) values were determined after incubation at 310.15 K 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

Tetraazo dyes **5a-5j** can exist in six possible tautomeric forms, namely the Tetraazo form **T1**, the triazo-hydrazo form **T2**, the hydrazo-triazo form **T3,** the dihydrazo-diazo form **T4**, **T5** and the dihydrazo-diazo form **T6** as shown in (Figure 1). The FT-IR spectra of dyes **5a-5j** showed intense intense two imino (NH) bands at 3294-3144 cm^{-1}. The other λ_{max} values of 3101-3022 cm^{-1} (aromatic C-H) and 2994-2963 cm^{-1} (aliphatic C-H) were recorded.

^{1}H NMR spectra of dyes **5a-5j** showed four broad peaks at 12.75-10.23 ppm (NH), 11.20-9.30 ppm (NH) and two broad peaks at 7.9-8.9 ppm (OH), 10.5-11.2 ppm (OH), 0.86-0.96 ppm (CH_3), 1.24-1.89 ppm (CH_2), respectively. The other δ values of 2.48-2.40 ppm (CH_3) and 8.35-6.98 ppm (aromatic H) were recorded.

These results suggest that dyes **5a-5j** is 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 ^{1}H NMR. The spectral data generally lead to the conclusion that the tautomeric equilibrium of these dyes was in favour of the hydrazo form [28-30]. These suggest that these dyes are predominantly in triazo-hydrazo form (**T2**), (**T3**) or dihydrazo-diazo form (**T4**), (**T5**) and (**T6**) in the solid state and DMSO.

3.2. Solvent effects on UV-Vis spectra

The UV-Vis absorption spectra of dyes **5a-5j** 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 summarized in (Table 1). The visible absorption spectra of the dyes did not correlate with the polarity of solvent.

Dyes **5a-5j** gave a maximum absorption peak in all used solvents. This result suggests that dyes **5a-5j** is present in a single tautomeric form in all used solvents.

Table 1. Influence of solvent on λ_{max} (nm) of dyes **5a-5j**.

Dye no	DMSO	DMF	Acetonitrile	Methanol	Acetic acid	Chloroform
5a	473	468	454	422	437	444
5b	467	464	455	420	432	440
5c	503	505	459	437	449	455
5d	457	449	436	408	406	432
5e	472	463	452	414	416	445
5f	483	482	463	423	422	456
5g	472	463	453	422	418	445
5h	475	466	455	429	427	458
5i	487	476	466	433	428	457
5j	467	465	447	403	414	440

Table 2. Absorption maxima (λ_{max}, nm) of dyes **5a-5j** in acidic and basic solutions.

Dye no	Methanol	Methanol + KCl	Methanol + HCl	Chloroform	Chloroform + Piperidine	Acetic acid
5a	473	468	454	422	437	444
5b	467	464	455	420	432	440
5c	503	505	459	437	449	455
5d	457	449	436	408	406	432
5e	472	463	452	414	416	445
5f	483	482	463	423	422	456
5g	472	463	453	422	418	445
5h	475	466	455	429	427	458
5i	487	476	466	433	428	457
5j	467	465	447	403	414	440

It was observed that in DMSO, DMF and acetonitrile, λ_{max} of dyes **5a-5j** shifted bathochromically with respect to the λ_{max} in chloroform (e.g. for dye **5d** λ_{max} is 432 nm in chloroform, 457 nm in DMSO, 449 nm in DMF and 436 nm in acetonitrile) (Figure 2). But, when we compare the bathochromic shifts of λ_{max} of dyes **5a-5j** in acetonitrile are less than in DMSO and DMF solvent. On the other hand, it was observed that in acetic acid and methanol, λ_{max} of dyes **5a-5j** shifted hypsochromically with respect to the λ_{max} in chloroform (e.g. for dye 5**b** λ_{max} is 440 nm in chloroform, 432 nm in acetic acid and 420 nm in methanol) (Figure 3). It was also observed that hypsochromic shifts of λ_{max} of dyes **5a-5c** and **5j** in acetic acid are less than hypsochromic shifts of λ_{max} of dyes **5a-5c** and **5j** in methanol. Hypsochromic shifts of λ_{max} of dyes **5d-5i** in acetic acid and methanol are similar.

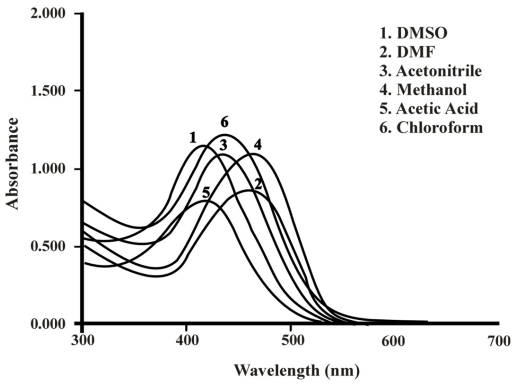


Figure 2. Absorption spectra of dye **5d** in various solvent.

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 **5a-5j** showing bathochromic shifts and absorption curves of the dyes resembled those in DMSO and DMF (Figure 3). 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 piperidine was added to dye solutions in chloroform, λ_{max} of dyes **5a-5j** did not change significantly except for dye **5c** (Figure 4). λ_{max} of the dye **5c** showed bathochromic shift when a small amount of piperidine was added to dye **5c** solution in chloroform.

When hydrochloric acid (0.1 M) was added to dye solutions in methanol, λ_{max} of dyes **5a-5c** and **5j** showed little bathochromic shifts with respect to the λ_{max} in methanol and the absorption spectra of dyes resembled those in acetic acid (Figure 5). It was also observed that when hydrochloric acid (0.1 M) was added to dye solutions in methanol, λ_{max} of dyes **5d-5i** did not change significantly.

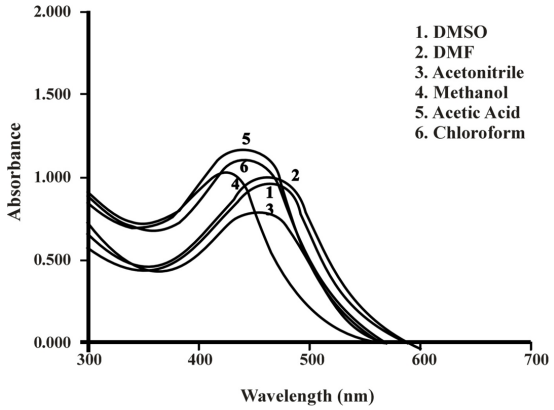


Figure 3. Absorption spectra of dye 5b in various solvent.

3.4. Substituent effects on UV-Vis spectra

As seen in (Table 1), generally, electron-accepting chloro groups in all positions for dyes **5c**, **5f** and **5i** cause bathochromical shifts in all used solvents when compared with dye **5a**. λ_{max} of dyes **5f** and **5i** in acetic acid hypsochromically shifted when compared with λ_{max} of dye **5a** in acetic acid. Electron-donating methoxy and methyl groups in *para* position for dyes **5b** and **5d** and methyl group in ortho position for dye **5j** because hypsochromical shifts in all used solvents when compared with dye **5a**. Visible absorption spectra of dyes **5e**, **5g** and **5h** did not regularly change with the substituent effect in all used solvents when compared with dye **5a**.

Table 3. Biological activities of dyes **5a-5j** (μg/mL).

Dye no	Bacteria				Yeasts	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Proteus vulgaris*	*Candida albicans*	*Candida glabrata*
5a	125	125	1000	1000	500	3.90
5b	2000	1000	2000	1000	2000	3.90
5c	>4000	2000	1000	>4000	3.90	3.90
5d	1000	5000	1000	1000	1000	1000
5e	1000	1000	2000	2000	3.90	3.90
5f	1000	2000	1000	2000	3.90	3.90
5g	2000	1000	1000	1000	1000	1000
5h	1000	2000	2000	2000	2000	2000
5i	2000	2000	1000	2000	2000	1000
5j	2000	2000	1000	2000	2000	2000
Streptomycin	15.62	0.97	3.90	31.25	-	-
Fluconazole	-	-	-	-	3.90	7.81

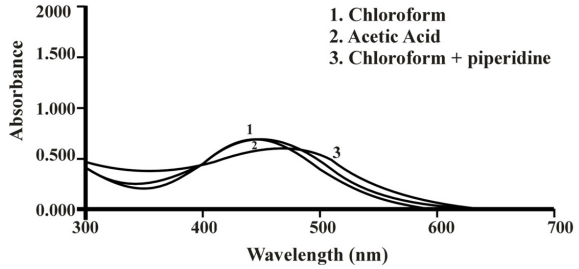


Figure 4. Absorption spectra of dye **5c** in different solutions.

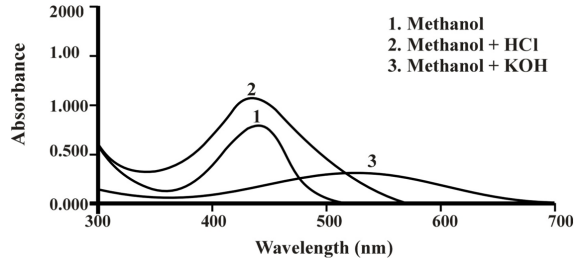


Figure 5. Absorption spectra of dye **5c** in basic solutions.

3.5. Antimicrobial activity of the synthesized dyes

Although, there are some reports about antimicrobial activity of monoazo dyes [31-33], research on biological activity of disazo dyes has just started [34]. 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 **5a**. This compound showed activity against *B. subtilis* and *S. aureus* at the dose of 125 mg/mL.

In contrast, different levels of antifungal activities were observed for some dyes against the yeasts. Dyes **5a**-**5c**, **5e** and **5f** exhibited stronger antifungal activity than not only other dyes but also fluconazole against *C. glabrata*. Among the all tested dyes, **5c**, **5e** and **5f** 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 [35,36]. It was reported weak or no antimicrobial activity for these compounds against fungi [37]. However, in the present study, 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. Conclusion

A series of novel disazo dyes **4a-4j** based on heterocyclic rings were synthesized by heating pyrazolylhydrazonomalono nitriles with hydrazine monohydrate and disazo dyes **4a-4j** were further diazotized and coupled with 4-hexylbenzene-1,3-diol to produce the tetraazo dyes **5a-5j.** All newly synthesized tetraazo dyes were well characterized and acid-base influence on the wavelength of maximum absorption have been studied. In the present paper, heterocyclic tetraazo dyes showed solvatochromic effects. When we compare the absorption maxima of such dyes in different solvent then it clearly indicate bathochromic shifts in DMSO and DMF rather than the other four solvents. It was also observed that the absorption spectra of these tetraazo dyes in methanol were quite sensitive to the addition of base.

Our study demonstrated clearly that, novel **5c**, **5e** and **5f** tetraazo dyes had significant antifungal activity when compared with control antibiotic, fluconazole. As a consequence, we can conclude that especially the newly synthesized **5c**, **5e** and **5f** dyes could be lead for the development of new antifungal drugs.

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