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Synthesis, Absorption Properties and Biological Evaluation of Some Novel Disazo Dyes Derived from Pyrazole Derivatives

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In this study, 20 novel disazo dyes containing pyrazole derivatives were synthesized by a convenient method. Diazotized aniline and some aniline derivatives were reacted with malononitrile to give 2-arylazomalononitrile derivatives **1**(**a-e**). The synthesized 2-arylazomalononitrile derivatives were reacted with hydrazine and phenyl hydrazine to afford the corresponding 3,5-diamino-4-arylazo-1*H*-pyrazole **2**(**a-e**) and 3,5-diamino-4-arylazo-1-phenylpyrazole **3**(**a-e**). Diazotized compounds of **2**(**a-e**)and **3**(**a-e**) reacted with ethylacetoacetate to give 4-arylazo-3-amino-1*H*-pyrazole-5-ylazo-ethylacetoacetate **4**(**a-e**) and 4-arylazo-3-amino-1-phenylpyrazole-5-ylazo-ethylacetoacetate **7**(**a-e**). The obtained **4**(**a-e**) and **7**(**a-e**) were reacted with hydrazine and phenyl hydrazine to give disazo dyes **5**(**a-e**), **6**(**a-e**), **8**(**a-e**) and **9**(**a-e**), respectively. The synthesized disazo dyes were characterized by spectroscopic techniques as well as elemental analysis. The solvatochromic behaviours of these dyes in various solvents were examined. Acid-base effects on the absorption maxima of the dyes were also reported. Antimicrobial activities of the synthesized novel disazo dyes were investigated.

Keywords: Pyrazolone, Diazotization, Solvatochromism, Pyrazole, Disazo dyes, Antimicrobial activity.

INTRODUCTION

Azo dyes and pigments generate the largest and most varied group of synthetic organic colorants in use today, having wide applications in textile, food, paper printing, ink, biomedical and cosmetics industries. They have characteristically good tinctorial strength as well as stability. Their preparation procedures by the classic diazotization and coupling reactions, is very simple and low cost[1-7].

Pyrazole and their substituted derivatives are used as potential pharmaceuticals and intermediates in dye industry. Azo pyrazoles were exhibited a wide variety of biological and pharmaceutical activities and therefore they play important role in medicinal chemistry. An exciting development in the synthesis of nitrogen heterocycles like azopyrazoles has commenced in last few years[8,9]. The pyrazole nucleus has been reported to possess a wide spectrum of biological properties such as anti-inflammatory, antibacterial, analgesic, antifungal and antiviral[10-14]. Pyrazoles having azo group have been found to exhibit a wide range of biological activities like antibacterial, CNS depressant, antitumor, potent local anesthetics and dyes[9,15,16].

Diazo compounds are widely used as coloring materials. The antimicrobial activity of these compounds can provide a great advantage in practice. This study has been carried out by considering the fact that many of pyrazole derivatives show antimicrobial activity, disazo dyes containing pyrazole ring can also show antimicrobial activity.

In the present study, 20 novel disazo dyes containing pyrazole derivatives were synthesized and investigated for their absorption properties and antimicrobial activity. The structural characterization, absorption properties and preliminary biological evaluation of these novel compounds could be interesting for screening potent dyes having antimicrobial activity.

EXPERIMENTAL

The chemicals were purchased from Aldrich, Sigma and Merck Chemical Company without further purification. Solvents were of spectroscopic grade. Melting points of the synthesized dyes were determined by using stuart smp 30 melting point apparatus (UK). Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker (Germany) spectrosp in avance DPX 400 ultra-shield 400 MHz spectrometer at room temperature by using tetramethylsilane (TMS) as the internal standard. Chemical shifts were (δ) given in ppm. IR spectra were recorded on a Perkin Elmer FT-IR spectrometer (USA). Mass spectra were obtained from waters LCT premier XE LTOF (TOF MS) instruments (USA). Elemental analyses were done on a Leco CHNS-932 analyzer (USA). Absorption spectra were recorded on an

ATI (UK) Unicam UV-100 spectrophotometer over the range of λ between 300-700 nm. The wavelengths of maximum absorption (λ_{max}) were investigated in various solvents such as dimethylsulfoxide (DMSO), dimethylformamide (DMF), acetonitrile, methanol, acetic acid and chloroform at various concentrations (1×10^{-6}-1×10^{-8} M). λ_{max} change of 1 mL the dye solution in methanol due to addition of 0.1 mL of base (potassium hydroxide, 0.1 M) or 0.1 mL of acid (hydrochloric acid, 0.1 M) was investigated.

Synthesis: 2-Arylazomalononitriles **1**(**a-e**) were synthesized as described by reported method[17]. The general route for the synthesis of 2-arylazomalononitriles **1(a-e)** is showed in Fig. 1.

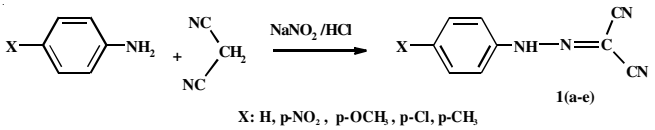

Fig. 1. Synthesis of 2-arylazomalononitriles **1**(**a-e**)

Synthesis of 3,5-diamino-4-arylazo-1*H*-pyrazoles and 3,5-diamino-4-arylazo-1-phenylpyrazoles: 3,5-Diamino-4-arylazo-1*H*-pyrazole **2**(**a-e**) and 3,5-diamino-4-arylazo-1-phenylpyrazole **3**(**a-e**) were prepared according to the procedures given in literatures[18-20]. The general route for the synthesis of 3,5-diamino-4-arylazo-1*H*-pyrazoles and 3,5-diamino-4-arylazo-1-phenylpyrazoles is outlined in Fig. 2.

General synthesis of disazo dyes 5(a-e), 6(a-e), 8(a-e) and 9(a-e)

Synthesis of 4-(4'-phenylazo-3'-amino-1'*H*-pyrazole-5-ylazo)-3-methyl-1*H*-pyrazole-5-one (5a): 3,5-Diamino-4-phenylazo-1*H*-pyrazole (**2a**) 1 g (4.95 mmol) was dissolved in a mixture of glacial acetic acid (10 mL) and concentrated hydrochloric acid (5 mL).The solution was cooled to 0-5 °C. Sodium nitrite 0.35 g (4.95 mmol) was dissolved in water (10 mL) then added to this solution dropwise with vigorous stirring, during about 1 h, while cooling at 0-5 °C. The clear diazonium salt solution was poured in portions over 0.5 h. into well-cooled (0-5 °C) and stirred solution of ethylacetoacetate 0.64 g (4.95 mmol) in pyridine. Stirring continued for 2 h at 0-5 °C and the precipitated products separated upon dilution with cold water (50 mL) were filtered off, washed with water several times and dried. The obtained product 4-phenylazo-3-amino-1*H*-pyrazole-5-ylazo-ethylacetoacetate (**4a**) was dissolved in ethanol (50 mL) and then hydrazine monohydrate 0.74 g (14.85 mmol) was added into this solution. The reaction mixture was heated under reflux for 3-4 h, then cooled up to the room temperature and the precipitated products were separated upon dilution with water (50 mL), filtered off, washed with water several times and dried. The obtained product was crystallized from DMF-H_2O mixture (2:3 by volume) to give 4-(4'-phenylazo-3'-amino-1'*H*-pyrazole-5-ylazo)-3-methyl-1*H*-pyrazole-5-on (**5a**) as dark red solid crystals, yield 1.17 g (76 %), m.p. > 291 °C dec. Anal. Calcd. for $C_{13}H_{13}N_9O$; C: 50.16 %; H: 4.18 %; N: 40.51 %; found: C: 50.21 %; H:4.21 %; N: 40.54 %. FT-IR (ν_{max}, cm^{-1}): 3251 (-NH_2); 3147 (-NH); 3020 (Ar-H); 2990 (Aliphatic C-H); 1672 (C=O); 1532, 1482 (N=N). ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.18 (s, 3H, pyrazolone-CH_3); 6.79 (s, 2H, -NH_2); 7.31-8.19 (m, 5H, Ar-H); 11.62-11.89 (g, -NH); 13.70-14.48 (s, -OH). HR-MS: 311.3026 ($[M + H]^+$, calcd. 311.3024.

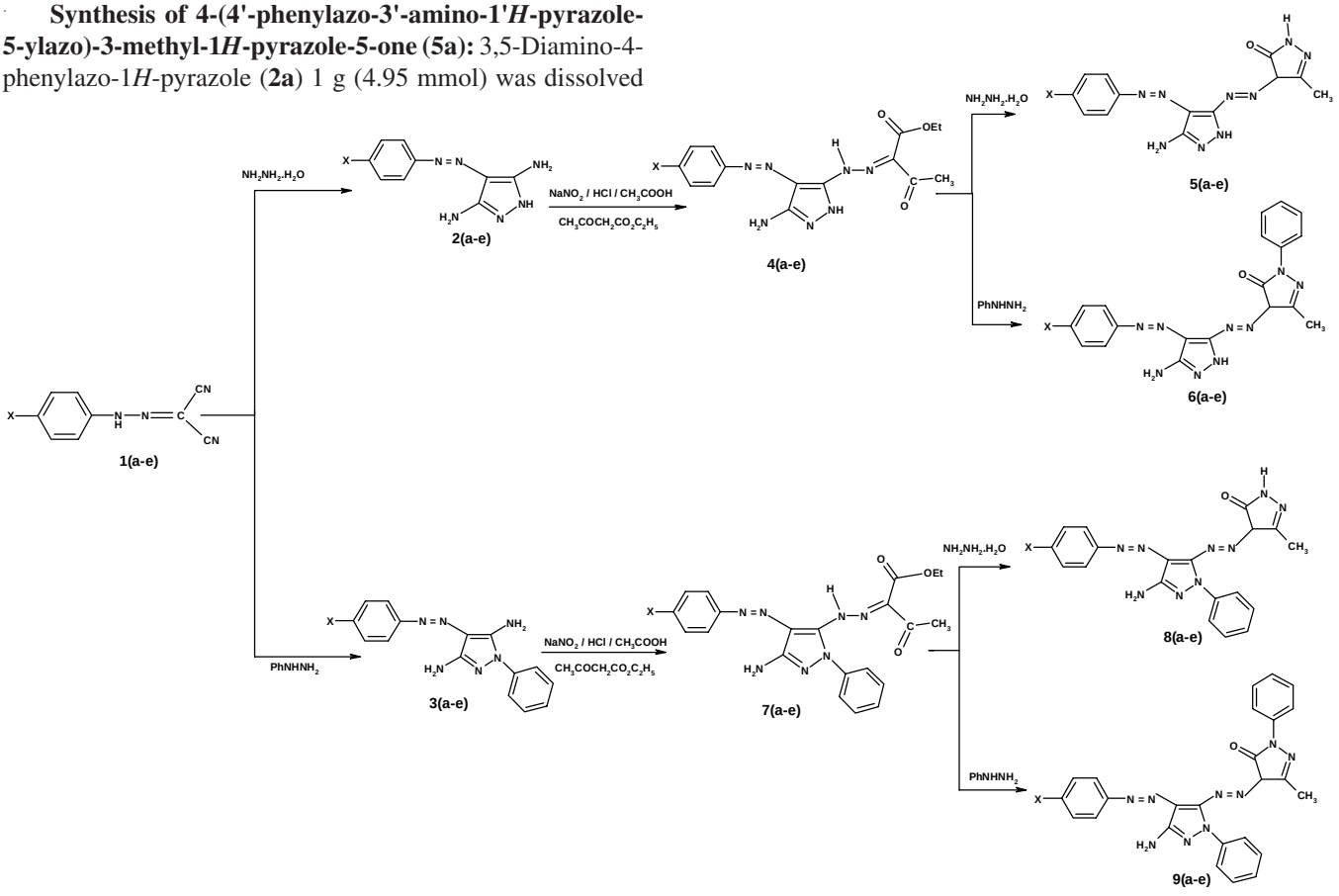

Fig. 2. General route of synthesized dyes

The above procedure was also used to synthesize dye **5**(**b-e**). The general route of synthesized dyes is outlined in Fig. 2.

4-[4'-(4''-Nitrophenyl)azo-3'-amino-1'*H*-pyrazole-5-ylazo]-3-methyl-1*H*-pyrazole-5-one (5b): Dark-orange solid crystals, yield 1.05 g (73 %), m.p. > 340 °C dec. Anal. Calcd. for $C_{13}H_{12}N_{10}O_3$; C: 43.82 %; H: 3.37 %; N: 39.325 %; found: C: 43.83 %; H:3.36 %; N: 39.37 %. FT-IR (ν_{max}, cm^{-1}): 3255 ($-NH_2$); 3187 (-NH); 3081 (Ar-H); 2980 (Aliphatic C-H); 1667 (C=O); 1547,1413 (N=N); 1513,1336 (NO_2). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.18 (s, 3H, pyrazolone-CH_3); 7.15 (s, 2H, $-NH_2$); 8.01-8.65 (m, 4H, Ar-H); 11.95 (b, -NH); 14.27 (s, -OH). HR-MS: 356.310 ($[M + H]^+$, calcd. 356.2996.

4-[4'-(4''-Methoxyphenyl)azo-3'-amino-1'*H*-pyrazole-5-ylazo]-3-methyl-1*H*-pyrazole-5-one (5c): Burgundy red solid crystals, yield 1.03 g (70 %), mp: 188 °C. Anal. Calcd. for $C_{14}H_{15}N_9O_2$; C: 49.26 %; H: 4.40 %; N: 36.95 %; found: C: 49.27 %; H:4.41 %; N: 36.96 %. FT-IR (ν_{max}, cm^{-1}): 3256 ($-NH_2$); 3195 (-NH); 3054 (Ar-H); 2997 (Aliphatic C-H); 1670 (C=O); 1542, 1491 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.15 (s, 3H, pyrazolone-CH_3); 3.78 (s, 3H, p-OCH_3); 7.05 (s, 2H, $-NH_2$); 7.01-8.10 (m, 4H, Ar-H); 11.51-11.67 (b, -NH); 13.71-14.26 (s, -OH). HR-MS: 341.3165 ($[M + H]^+$, calcd. 341.3284.

4-[4'-(4''-Chlorophenyl)azo-3'-amino-1'*H*-pyrazole-5-ylazo]-3-methyl-1*H*-pyrazole-5-on (5d): Dark-brown solid crystals, yield 1.07 g (73 %), m.p. > 340 °C dec. Anal. Calcd. for $C_{13}H_{12}N_9OCl$; C: 45.15 %; H: 3.47 %; N: 36.47 %; found: C: 45.17 %; H:3.48 %; N: 36.47 %. FT-IR (ν_{max}, cm^{-1}): 3245 ($-NH_2$); 3086 (-NH); 3040 (Ar-H); 2918 (Aliphatic C-H); 1670 (C=O); 1550,1409 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.17 (s, 3H, pyrazolone-CH_3); 6.95 (s, 2H, $-NH_2$); 7.63-8.01 (m, 4H, Ar-H); 11.89 (b, -NH); 14.21 (s, -OH). HR-MS: 345.7472 ($[M + H]^+$, calcd. 345.7471.

4-[4'-(4''-Methylphenyl)azo-3'-amino-1'*H*-pyrazole-5-ylazo]-3-methyl-1*H*-pyrazole-5-one (5e): Dark-red solid crystals, yield 1.06 g (70 %), m.p. > 278 °C dec. Anal. Calcd. for $C_{14}H_{15}N_9O$; C: 51.69 %; H: 4.61 %; N: 38.77 %; found: C: 51.70 %; H:4.63 %; N: 38.79 %. FT-IR (ν_{max}, cm^{-1}): 3230 ($-NH_2$); 3115 (-NH); 3050 (Ar-H); 2948 (Aliphatic C-H); 1673 (C=O); 1540, 1420 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.17 (s, 3H, pyrazolone-CH_3); 2.31 (s, 3H, p-CH_3); 7.05 (s, 2H, $-NH_2$); 7.22-7.45 (m, 4H, Ar-H); 11.54 (b, -NH); 13.45 (s, -OH). HR-MS: 325.3290 ($[M + H]^+$, calcd. 325.3286.

Synthesis of 4-(4'-[phenyl)azo-3'-amino-1'*H*-pyrazole-5-ylazo]-3-methyl-1-phenylpyrazole-5-one (6a)

Compounds **6(a-e)** were prepared as described above for **5a** using phenylhydrazine: Dark-red solid crystals, yield 1.38 g (72 %), m.p.: 276-277 °C. Anal. Calcd. for $C_{19}H_{17}N_9O$; C: 58.91 %; H: 4.39 %; N: 32.558 %; found: C: 58.92 %; H: 4.41 %; N: 32.56 %. FT-IR (ν_{max}, cm^{-1}): 3261 ($-NH_2$); 3113 (-NH); 3064 (Ar-H); 2959 (Aliphatic C-H); 1672 (C=O); 1531-1491 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.29 (s, 3H, pyrazolone-CH_3); 6.85 (s,pyrazole-NH_2); 7.25-8.05 (m, 10H, Ar-H); 11.95 (b,pyrazole-NH); 14.10 (s, -OH). HR-MS: 387.3981 ($[M + H]^+$, calcd. 387.3980.

4-[4'-(4''-Nitrophenyl)azo-3'-amino-1'*H*-pyrazole-5-ylazo]-3-methyl-1-phenylpyrazole-5-one (6b): Red solid crystals, yield 1.21 g (69 %), m.p. > 330 °C dec. Anal. Calcd. for $C_{19}H_{16}N_{10}O_3$; C: 52.278 %; H: 3.70 %; N: 32.41 %; found: C: 52.90 %; H: 3.71 %; N: 32.43 %. FT-IR (ν_{max}, cm^{-1}): 3241 ($-NH_2$); 3120 (-NH); 3048 (Ar-H); 2960 (Aliphatic C-H); 1672 (C=O); 1515, 1324 (NO_2); 1531, 1498 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.29 (s, 3H, pyrazolone-CH_3); 6.95 (s,pyrazole-NH_2); 7.21-8.28 (m, 9H, Ar-H); 11.75 (b, pyrazole-NH); 13.20 (s, -OH). HR-MS: 432.3954 ($[M + H]^+$, calcd. 432.3955.

4-[4'-(4''-methoxyphenyl)azo-3'-amino-1'*H*-pyrazole-5-ylazo]-3-methyl-1-phenylpyrazole-5-one (6c): Light orange solid crystals, yield 1.26 g (70 %), m.p.: 305-306 °C. Anal. Calcd. for $C_{20}H_{19}N_9O_2$; C: 57.55 %; H: 4.56 %; N: 30.21 %; found: C: 57.57 %; H: 4.57 %; N: 30.23 %. FT-IR (ν_{max}, cm^{-1}): 3230 ($-NH_2$); 3105 (-NH); 3065 (Ar-H); 2914 (Aliphatic C-H); 1670 (C=O); 1551-1496 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.28 (s, 3H, pyrazolone-CH_3); 3.58 (s, 3H, p-OCH_3); 6.69 (s, pyrazole-NH_2); 7.01-8.01 (m, 9H, Ar-H); 11.88 (b, pyrazole-NH); 14.15 (s, -OH). HR-MS: 417.4239 ($[M + H]^+$, calcd. 417.4240.

4-[4'-(4''-Chlorophenyl)azo-3'-amino-1'*H*-pyrazole-5-ylazo]-3-methyl-1-phenylpyrazole-5-on (6d): Orange solid crystals, yield 1.28 g (72 %), m.p.: 288-289 °C. Anal. Calcd. for $C_{19}H_{16}N_9OCl$; C: 54.09 %; H: 3.795 %; N: 29.89 %; found: C: 54.11 %; H: 3.80 %; N: 29.95 %. FT-IR(ν_{max}, cm^{-1}): 3235 ($-NH_2$); 3104 (-NH); 3064 (Ar-H); 2920 (Aliphatic C-H); 1671 (C=O); 1553-1499 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.29 (s, 3H, pyrazolone-CH_3); 6.79 (s, pyrazole-NH_2); 7.22-8.05 (m, 9H, Ar-H); 14.01 (b, pyrazole-NH); 14.28 (s, -OH). HR-MS: 421.8431 ($[M + H]^+$, calcd. 421.8430.

4-[4'-(4''-Methylphenyl)azo-3'-amino-1'*H*-pyrazole-5-ylazo]-3-methyl-1-phenylpyrazole-5-one (6e): Brick red solid crystals, yield 1.32 g (71 %), m.p.: 309-310 °C. Anal. Calcd. for $C_{20}H_{19}N_9O$; C: 59.85 %; H: 4.738 %; N: 31.42 %; found: C: 59.88 %; H: 4.74 %; N: 31.45 %. FT-IR (ν_{max}, cm^{-1}): 3240 ($-NH_2$); 3110 (-NH); 3002 (Ar-H); 2918 (Aliphatic C-H); 1651 (C=O); 1541-1498 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.31 (s, 3H, pyrazolone-CH_3); 2.35 (s, 3H, p-CH_3); 6.82 (s, pyrazole-NH_2); 7.21-7.95 (m, 14H, Ar-H); 13.33 (b, pyrazole-NH); 14.29 (s, -OH). HR-MS: 401.4245 ($[M + H]^+$, calcd. 401.4246.

Synthesis of 4-[4'-(phenyl)azo-3'-amino-1'-phenylpyrazole-5-ylazo]-3-methyl-1*H*-pyrazole-5-one (8a)

Compounds **8(a-e)** were prepared as described above for **5a** using 3,5-diamino-4-arylazo-1-phenylpyrazole **3(a-e)**: Silvery red solid crystals, yield 1.02 g (73 %), m.p.: 276-277 °C. Anal. Calcd. for $C_{19}H_{17}N_9O$; C: 58.91 %; H: 4.39 %; N: 32, 558 %; found: C: 58.88 %; H: 4.41 %; N: 32.539 %. FT-IR (ν_{max}, cm^{-1}): 3310 ($-NH_2$); 3191 (-NH); 3054 (Ar-H); 2996 (Aliphatic C-H); 1664 (C=O); 1528-1497 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.14 (s, 3H, pyrazolone-CH_3); 7.17 (g, 2H, pyrazole-NH_2); 7.35-8.09 (m, 10H, Ar-H); 11.71 (b, pyrazolone-NH); 14.21 (s, -OH). HR-MS: 387.3981 ($[M + H]^+$, calcd. 387.3980.

4-[4'-(4''-Nitrophenyl)azo-3'-amino-1'-phenylpyrazole-5-ylazo]-3-methyl-1*H*-pyrazole-5-one (8b): Dark red solid

crystals,yield 0.92 g (69 %), m.p. > 280 °C dec. Anal. Calcd. for $C_{19}H_{16}N_{10}O_3$; .C: 52.278 %; H: 3.70 %; N: 32.41 %; found: C: 52.28 %; H: 3.73 %; N: 32.42 %. FT-IR (ν_{max}, cm^{-1}): 3381 ($-NH_2$); 3231 (-NH); 3174 (Ar-H); 2960 (Aliphatic C-H); 1669 (C=O); 1535-1521 (N=N); 1504 and 1337 ($-NO_2$). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.14 (s, 3H, pyrazolone-CH_3); 7.17 (g, 2H, pyrazole-NH_2); 7.58-8.37 (m, 9H, Ar-H); 11.77 (b, pyrazolone-NH); 14.25 (s, -OH). HR-MS: 432.3954 ($[M + H]^+$, calcd. 432.3955

4-[4'-(4''-Methoxyphenyl)azo-3'-amino-1'-phenylpyrazole-5-ylazo]-3-methyl-1*H*-pyrazole-5-one (8c): Dark red crystals, yield 0.95 g (70 %), m.p.: 285-286 °C. Anal. Calcd. for $C_{20}H_{19}N_9O_2$; C: 57.55 %; H: 4.56 %; N: 30.21 %; found: C: 57.56 %, H: 4.58 %; N: 30.20 %. FT-IR (ν_{max}, cm^{-1}): 3307 ($-NH_2$); 3171 (-NH); 3094 (Ar-H); 2990 (Aliphatic C-H); 1671 (C=O); 1523-1496 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.14 (s, 3H, pyrazolone-CH_3); 3.84 (s, 3H, p-OCH_3); 7.00 (g, 2H, pyrazole-NH_2); 7.06-8.09 (m, 9H, Ar-H); 11.69 (b, pyrazolone-NH); 14.20 (s, -OH). HR-MS: 417.4239 ($[M + H]^+$, calcd. 417.4240.

4-[4'-(4''-Chlorophenyl)azo-3'-amino-1'-phenylpyrazole-5-ylazo]-3-methyl-1*H*-pyrazole-5-one (8d): Orange solid crystals, yield 0.97 g (72 %), m.p. > 325 °C. Anal. Calcd. for $C_{19}H_{16}N_9OCl$; C: 54.09 %; H: 3.795 %; N: 29.89 %. found: C: 54.11 %; H: 3.78 %; N: 29.86 %. FT-IR (ν_{max}, cm^{-1}): 3304 ($-NH_2$); 3108 (-NH); 3079 (Ar-H); 2929 (Aliphatic C-H); 1669 (C=O); 1521-1502 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.14 (s, 3H, pyrazolone-CH_3); 6.85 (g, 2H, pyrazole-NH_2); 7.22-8.11 (m, 9H, Ar-H); 11.70 (b, pyrazolone-NH); 14.22 (s, -OH). HR-MS: 421.8431 ($[M + H]^+$, calcd. 421.8430.

4-[4'-(4''-Methylphenyl)azo-3'-amino-1'-phenylpyrazole-5-ylazo]-3-methyl-1*H*-pyrazole-5-one (8e): Brown solid crystals, yield 0.98 g (71 %), m.p.: 283-284 °C. Anal. Calcd. for $C_{20}H_{19}N_9O$; C: 59.85 %; H: 4.738 %; N: 31.42 %; found: C: 59.82 %; H: 4.74 %; N: 31.43 %. FT-IR (ν_{max}, cm^{-1}): 3370 ($-NH_2$); 3191 (-NH); 3062 (Ar-H); 2980 (Aliphatic C-H); 1671 (C=O); 1528-1404 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.14 (s, 3H, pyrazolone-CH_3); 2.38 (s, 3H, p-CH_3); 7.22 (g, 2H, pyrazole-NH_2); 7.30-8.02 (m, 9H, Ar-H); 11.70 (b, pyrazolone-NH); 14.20 (s, -OH). HR-MS: 401.4245 ($[M + H]^+$, calcd. 401.4246.

Synthesis of 4-[4'-(phenyl)azo-3'-amino-1'-phenylpyrazole-5-ylazo]-3-methyl-1-phenylpyrazole-5-one (9a)

Compound **9(a-e)** were prepared as described above for **5a** using 3,5-diamino-4-arylazo-1-phenylpyrazole **3(a-e)** and phenylhydrazine: Light orange solid crystals, yield 1.18 g (71 %), m.p.: 220-221°C. Anal. Calcd. for $C_{25}H_{21}N_9O$; C: 64.79 %; H: 4.53 %; N: 27.21 %; found: C: 64.81 %; H: 4.57 %; N: 27.24 %. FT-IR (ν_{max}, cm^{-1}): 3338 ($-NH_2$); 3063 (Ar-H); 2980 (Aliphatic C-H); 1667 (C=O); 1538-1494 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.29 (s, 3H, pyrazolone-CH_3); 7.22 (b, 2H, pyrazole-NH_2); 7.26-8.13 (m, 15H, Ar-H); 14.23 (s, -OH). HR-MS: 463.4940 ($[M + H]^+$, calcd. 463.4939.

4-[4'-(4''-Nitrophenyl)azo-3'-amino-1'-phenylpyrazole-5-ylazo)-3-methyl-1-phenylpyrazole-5-one (9b): Red solid crystals, yield 1.07 g (68 %), m.p.: 282-283 °C. Anal. Calcd. for $C_{25}H_{20}N_{10}O_3$; C: 59.05 %; H: 3.93 %; N: 27.56 %; found: C: 58.97 %; H: 3.95 %; N: 27.53 %. FT-IR (ν_{max}, cm^{-1}): 3353 ($-NH_2$); 3063 (Ar-H); 2930 (Aliphatic C-H); 1661 (C=O); 1553-1493 (N=N); 1537, 1324 (NO_2). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.26 (s, 3H, pyrazolone-CH_3); 7.26 (b, 2H, pyrazole-NH_2); 7.48-8.38 (m, 14H, Ar-H); 14.29 (s, -OH). HR-MS: 508.4916 ($[M + H]^+$, calcd. 508.4915.

4-[4'-(4''-Methoxyphenyl)azo-3'-amino-1'-phenylpyrazole-5-ylazo]-3-methyl-1-phenylpyrazole-5-one (9c): Light pinksolid crystals, yield 1.12 g (70 %), m.p.: 231-232 °C. Anal. Calcd. for $C_{26}H_{23}N_9O_2$; C: 63.28 %; H: 4.66 %; N: 25.558 %; found: C: 63.25 %; H: 4.69 %; N: 25.56 %. FT-IR (ν_{max}, cm^{-1}): 3360 ($-NH_2$); 3062-3024 (Ar-H); 2964 (Aliphatic C-H); 1671 C=O); 1525-1498 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.27(s, 3H, pyrazolone-CH_3); 3.85; (s, 3H, p-OCH_3); 7.00 (g, 2H, pyrazole-NH_2); 7.08-8.10 (m, 14H, Ar-H); 14.20 (s, -OH). HR-MS: 493.5198 ($[M + H]^+$, calcd. 493.5199.

4-[4'-(4''-Chlorophenyl)azo-3'-amino-1'-phenylpyrazole-5-ylazo]-3-methyl-1-phenylpyrazole-5-one (9d): Claret red solid crystals, yield 1.15 g (72 %), m.p.: 252-253 °C. Anal. Calcd. for $C_{25}H_{20}N_9OCl$; C: 60.36 %; H: 4.02 %; N: 25.35 %; found: C: 60.35 %; H: 4.05 %; N: 25.37 %. FT-IR (ν_{max}, cm^{-1}): 3349 ($-NH_2$); 3059 (Ar-H); 2923 (Aliphatic C-H); 1663 C=O); 1538-1493 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.27 (s, 3H, pyrazolone-CH_3); 7.29 (g, 2H, pyrazole-NH_2); 7.43-8.13 (m, 14H, Ar-H); 14.22 (s, -OH). HR-MS: 497.9395 ($[M + H]^+$, calcd. 497.939.

4-[4'-(4''-Methylphenyl)azo-3'-amino-1'-phenylpyrazole-5-ylazo]-3-methyl-1-phenylpyrazole-5-one (9e): Dark red solid crystals, yield 1.16 g (71 %), m.p.: 240 °C. Anal. Calcd. for $C_{26}H_{23}N_9O$; C: 65.408 %; H: 4.82 %; N: 26.415 %; found: C: 65.410 %; H: 4.83 %; N:26.42 %. FT-IR (ν_{max}, cm^{-1}): 3419 ($-NH_2$); 3023 (Ar-H); 2917 (Aliphatic C-H); 1668 C=O); 1525-1496 (N=N). ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ (ppm): 2.27 (s, 3H, pyrazolone-CH_3); 2.39; (s, 3H, p-CH_3); 7.12 (g, 2H, pyrazole-NH_2); 7.27-8.03 (m, 14H, Ar-H); 14.21 (s, -OH). HR-MS: 477.522 ($[M + H]^+$, calcd. 477.520

Antimicrobial evaluation: Newly synthesized compounds were individually tested against a panel of Gram-positive and Gram-negative bacterial pathogens, yeast and fungi. Antimicrobial tests were carried out by the agar well diffusion method[21] using 100 µL of suspension containing 1×10^6 CFU/mL of pathological tested bacteria, 1×10^6 CFU/mL of yeast spread on nutrient agar (NA) and Sabouraud dextrose agar (SDA), respectively. After the media had cooled and solidified, wells (10 mm in diameter) were made in the solidified agar and loaded with 100 µL of tested compound solution prepared by dissolving 100 µg of the chemical compound in one mL of DMSO. The inculcated plates were then incubated for 24 h. at 37 °C for bacteria and 48 h. at 28 °C for fungi. Negative controls were prepared using DMSO employed for dissolving the tested compound. Ciprofloxacin (50 µg/mL) and ketoconazole (50 µg/mL) were used as standard for antibacterial and antifungal activities, respectively. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated.

The minimum inhibitory concentration (MIC) measurement was determined for compounds using twofold serial dilution method[22].

The microdilution susceptibility test in Müeller Hinton Broth (Oxoid) was used for the determination of antibacterial activity and sabouraud liquid medium (Oxoid) was used for the determination of antifungal activity. Stock solutions of the tested compounds, ciprofloxacin and ketoconazole were prepared in DMF at concentration of 1000 µg/mL. Two-fold serial dilutions of the tested compounds solutions were prepared using the proper nutrient broth. The final concentration of the solutions was 132, 66, 33, 16.5 and 8.25 µg/mL. The tubes were then inoculated with the test organisms, grown in their suitable broth at 37 °C for 24 h. for bacteria (about 1 × 10^6 CFU/mL), each 5 mL received 0.1 mL of the previous inoculums and incubated at 37 °C for 24 h. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC). Control experiments with DMF and uninoculated media were run parallel to the test compounds under the same conditions. The MIC (µg/mL) and inhibition zone diameters values are recorded.

RESULTS AND DISCUSSION

In continuation of our previous works[23-25], we report here the synthesis of new disazo dyes **5**(**a-e**), **6**(**a-e**), **8**(**a-e**), **9**(**a-e**) in this study. By purification of the reaction mixtures, 20 new disazo dyes were obtained in 68-76 % yield. These compounds were characterized by elemental analysis, absorption spectra analysis, FT-IR, ^{1}H NMR and mass spectral data. The synthesized new dyes **5**(**a-e**) may exist in eight possible tautomeric forms and **6**(**a-e**) in six possible tautomeric forms. Similarly, **8**(**a-e**) may exist in four possible tautomeric forms and **9**(**a-e**) in three possible tautomeric forms. These tautomeric forms are disazo-keto form, disazo-enol form, azo-hydrazo-keto form, azo-hydrazo-enol form, dishydrazo-keto form, dishydrazo-enol form, hydrazo-azo-keto form and hydrazo-azo-enol form, respectively. Typical example for dyes **5**(**a-e**) is given in Fig. 3.

The infrared spectra of all the compounds showed azo band located at 1550-1409 cm^{-1} and carbonyl bands at 1672-1651 cm^{-1}. These results confirm that all the dyes in the solid state exist as disazo-keto form, azo-hydrazo-keto form and hydrazo-azo-keto form. The values of ν_{max} of the others are

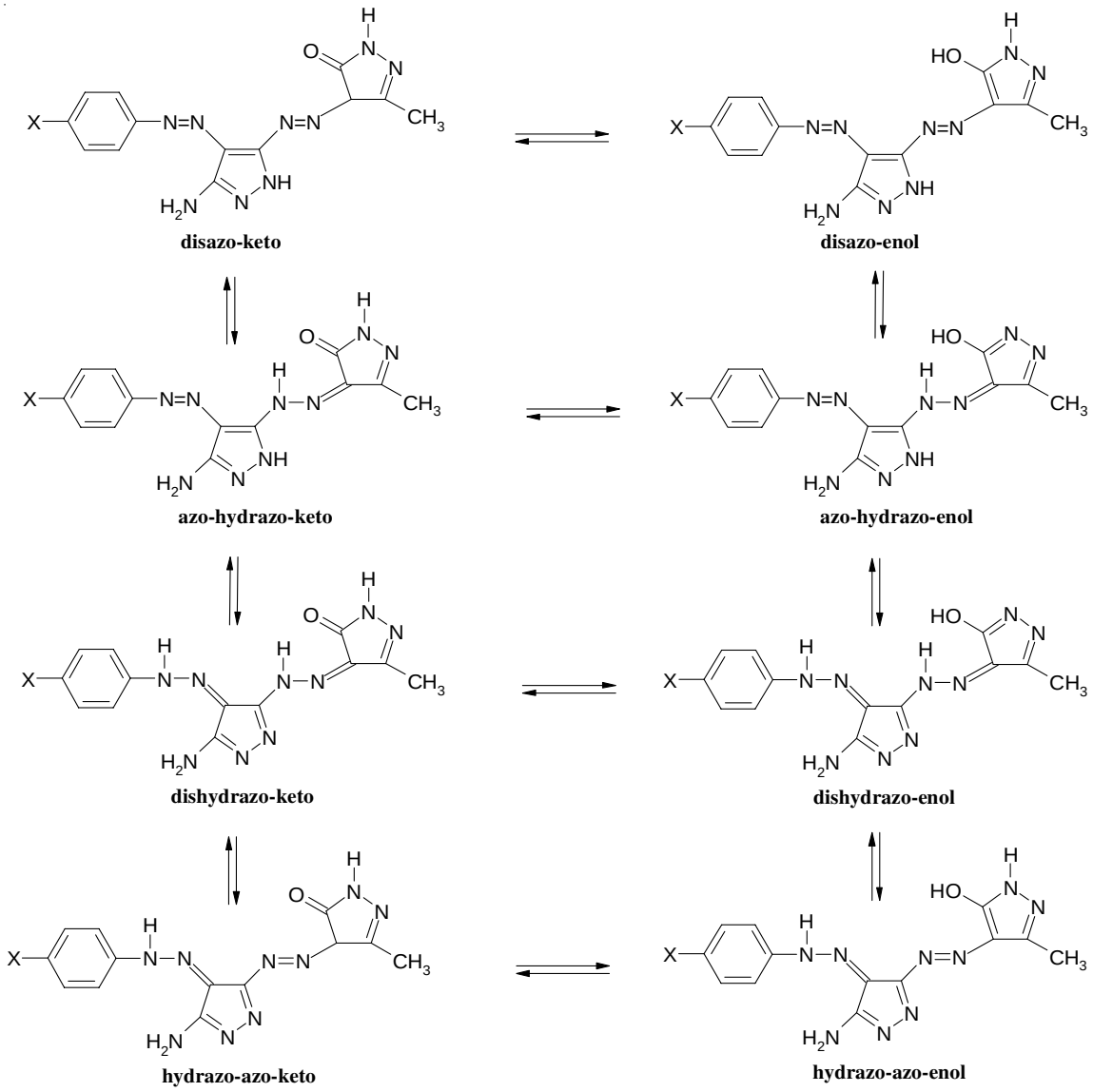

Fig. 3. Tautomeric forms of dyes **5**(**a-e**)

located at 3419-3245 cm^{-1} assigned to NH_2, 3231-3086 cm^{-1} assigned to NH except for **9**(**a-e**), 3174-3002 cm^{-1} assigned to aromatic C-H, 2990-2917 cm^{-1} assigned to aliphatic C-H and a band located at 1537-1324 cm^{-1} assigned to (NO_2) for the dyes **5b**, **6b**, **8b** and **9b** were also recorded.

The ^{1}H NMR spectra of all compounds showed a singlet peak for methyl protons (pyrazolone-CH_3) at between 2.14-2.31 ppm. The ^{1}H NMR spectra of dyes **5c**, **6c**, **8c** and **9c** showed a singlet peaks for methoxy protons (Ph-OCH_3) at between 3.78-3.85 ppm and **5e**, **6e**, **8e** and **9e**, showed a singlet peaks for methyl protons (Ph-CH_3) at between 2.31-2.39 ppm. ^{1}H NMR spectra of the all compounds demonstrated a broad peak for NH_2 protons at between 6.69-7.29 ppm. The ^{1}H NMR spectra of the all compounds showed a multiple peak at between 7.01-8.65 ppm for aromatic protons (Ar-H). The ^{1}H NMR spectra of all the dyes showed a broad peak for hydroxyl protons (pyrazole-OH) at between 13.20-14.48 ppm. The ^{1}H NMR spectra of the all dyes showed a broad peak for -NH protons except for **9**(**a-e**). The structure of compounds **9**(**a-e**) was confirmed by ^{1}H-NMR spectra through the loss of -NH protons signal belonging to other compounds. As a typical example, the ^{1}H-NMR spectra of **9c** in DMSO-d_6, is shown in Fig. 4. The ^{1}H-NMR spectra of dyes showed only -NH proton, but did not show hydrazo -NH proton except for **5a** and **5c**. According to ^{1}H-NMR results, all of the dyes have enol tautomeric forms, but not keto tautomeric forms. The dyes named as **5a** and **5c** can be present asazo-hydrazo-enol form, dishydrazo-enol form and hydrazo-azo-enol form in DMSO-d_6, as depicted in Fig. 3.

Absorption spectra analysis: In general, tautomeric equilibrium strongly depends on the nature of the media. Therefore, the behaviours of disazo dyes were studied in various solvents. The absorption spectra of disazo dyes were measured in various solvents at a concentration of (10^{-6}-10^{-8} M). Solvents used for the absorption spectra measurements have different dielectric constants (ε), *i.e.* DMSO (ε, 46.45), DMF (ε, 36.71), acetonitrile (ε, 35.94), methanol (ε, 32.66), acetic acid (ε, 6.17) and chloroform (ε, 4.89)[26].

UV spectra of dye **6b** in DMF (2×10^{-3} mol L^{-1}) titrated with diluted NaOH (3×10^{-3} mol L^{-1}) and HCl (3×10^{-3} mol L^{-1}) at room temperature. The color changes are depicted in Fig. 5.

The dye **6b** has responded to H^+ in a wide pH range from 1.84 to 12.96. The reversible conversion may be attributed to the protonation or deprotonation[27]. The cationic and anionic form of dye **6b** is given in Fig. 6. as a typical example.

Usually, the ground state nearly all of molecules is less polar, than excited state so that a polar solvent will tend to stabilize in the excited state more than ground state. It was determined that, meanwhile the polarity of the solvents was increased with the increasing dielectric constant of the solvents, the absorption maximum of the dyes mostly shows small batho-chromic shifts[28,29]. The obtained results from the absorption measurements are given in Table-1.

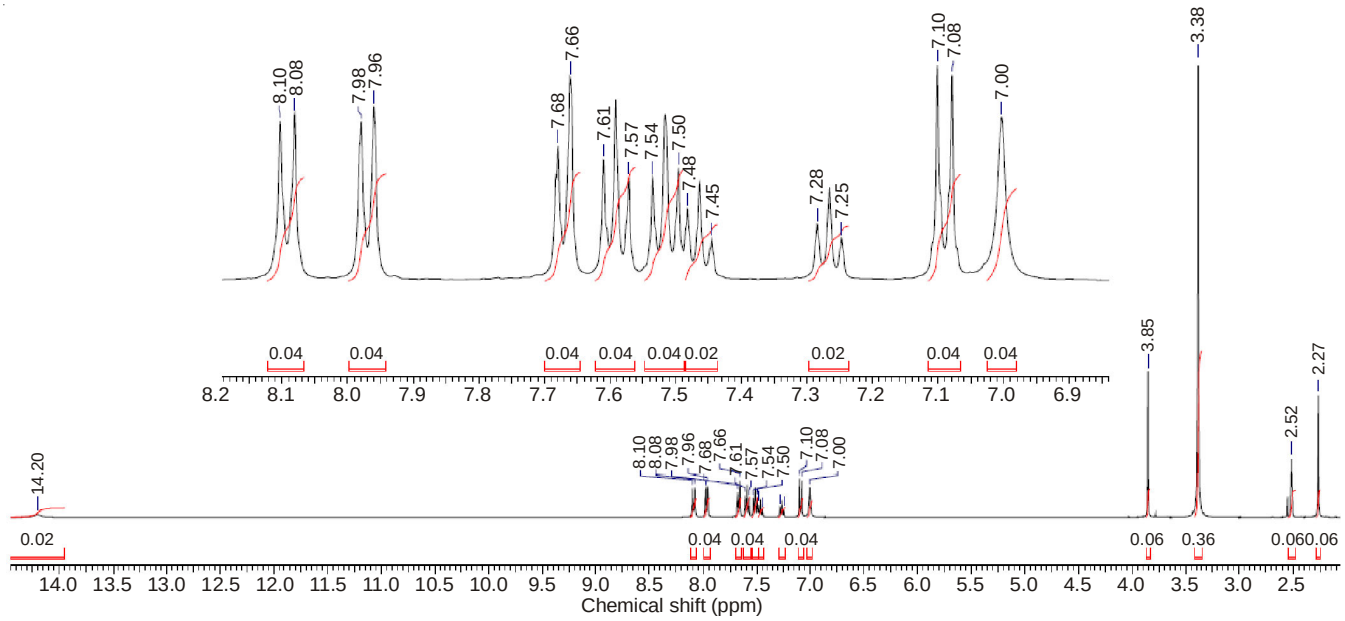

Fig. 4. ^{1}H NMR spectra of the dye **9c**

Fig. 5. Color changes corresponding to different pH values

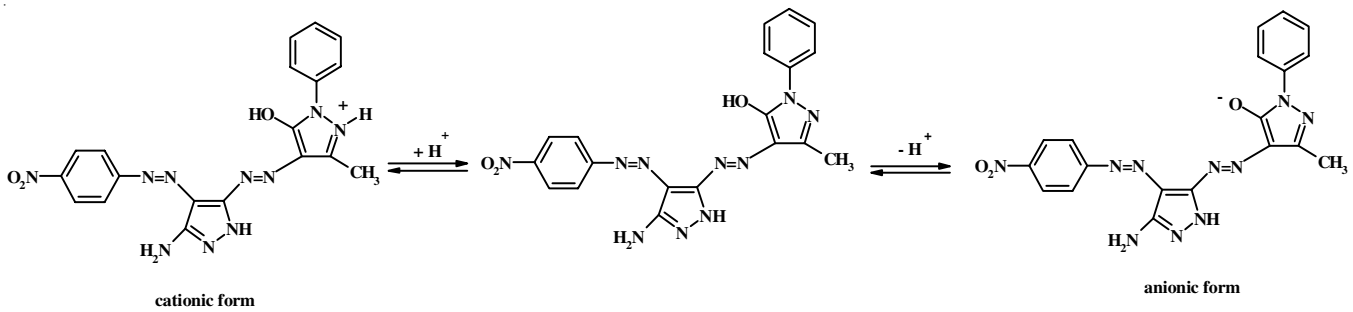

Fig. 6. Cationic and anionic form of dye **6b**

TABLE-1
INFLUENCE OF SOLVENT ON λ_{max} (nm) OF DYES

Dye	DMSO	DMF	Acetonitrile	Methanol	Acetic acid	Chloroform
5a	360, 451	364, 460, 564s	353, 435	350, 433	355, 442	342, 434
5b	350, 480	366, 430	341, 469	342, 466	341, 464	343, 470
5c	432, 360s	345, 461	425, 355s	433	437	433, 348s
5d	342, 464	348, 469	333, 438	334, 434	334, 405	339, 417
5e	362, 455	362, 456	352, 428	355, 437	357, 434	426
6a	355, 438	341, 411	360, 434	347, 414	338, 416	343, 414
6b	355, 483	337, 456	346, 470	338, 451	337, 446	342, 455
6c	469, 410s	383, 489	423, 358s	435	425	433
6d	337, 447	342, 508	330,426	332, 414	335, 405	337, 428
6e	407	403, 528	398	400	405	435
8a	353, 447	352, 438	347, 429	344, 429	346, 441	350, 429
8b	387, 471	379, 470	388, 447	378, 448	373, 448	382, 443
8c	350, 429	361, 443	356, 432	356, 435	357, 446	361, 437
8d	384, 473	382, 468	374, 452	369, 453	370, 451	375, 450
8e	356, 444	356, 438	350, 429	351, 430	351, 443	353, 429
9a	384, 470	351, 431	346, 417	346, 416	346, 423	351, 416
9b	385, 473	384, 464	375, 448	373, 456	370, 446	351, 414
9c	365, 442	363, 438	358, 428	356, 426	357, 434	361, 426s
9d	357, 438	356, 434	352, 421	350, 419	350, 420	355, 423
9e	360, 438	355, 433	352, 416	348, 420	351, 426	352, 421

s : shoulder

According to the absorption results, it is concluded that the absorption spectra of the dyes have not correlated with the polarity of solvents. It was observed that λ_{max} of the dyes **5e** and **6e** shifted bathochromically in chloroform with respect to the λ_{max} in DMSO and DMF. The explanation for this irregular behaviour may be due to presence of nonbonding electron pairs of the carbonyl oxygen and nitrogen atoms in the molecule ring[29]. The molecular structure of the dyes with intramolecular hydrogen bonding, have great potential of interacting with the solvent molecules through non-covalent or non-conventional interactions[28]. In polar protic solvents the lone pair of electron is engaged in hydrogen bonding and the promotion of these electrons to a π^* orbital requires energy to weaken or break the hydrogen bond in addition normal transition energy. This results in absorption spectra at shorter wavelength or rather a blue shift in going from polar to non-polar solvents[29, 30]. Absorption measurements showed that the λ_{max} of dye **5e** did not change in DMSO and DMF. We can conclude from the absorption measurements that the stability of excited state in going from DMSO to DMF is not change remarkably. From the absorption spectra of dyes **5**(**b**,**d**), **6d**, **8**(**a-d**) and **9**(**c-e**) in DMSO and DMF, little bathochromic shifting with respect to the absorption spectra in chloroform has been recorded. For example for dye **5b**, 29, λ_{max} is 343 and 470 nm in $CHCl_3$, 350 and 480 nm in DMSO. For dye **9e**, λ_{max} is 352 and 421 nm in $CHCl_3$, 360 and 438 nm in DMSO, 355 and 433 nm in DMF. It may conclude that there is no significant change in stability of excited state in going from DMSO to DMF and $CHCl_3$. We can say that the stabilization is less in going from DMSO, DMF to $CHCl_3$. The absorption spectra of dyes **5**(**a**,**b**,**d**), **6**(**a**,**b**,**d**), **8**(**a-e**), **9**(**a**,**b**,**d**,**e**) showed two absorbance. Dye **6e** showed single absorbance in all used solvents. It can be suggested that all of the dyes except for **6e** may be a mixture of tautomeric forms in various solvents. Dye **6e** is predominantly in single tautomeric form in all solvents. The absorption spectra of dye **5a** showed two maximum absorption peaks with a shoulder in DMF. The dye **5c** showed only one absorption peak in all used solvents except for DMF. Dye **5c** has one maximum absorption peak with a shoulder in DMSO, acetonitrile and chloroform. The absorption spectra of dye **6c** showed one maximum absorption peak in all used solvents except for DMF. Dye **6c** has one maximum absorption peak with a shoulder in DMSO and acetonitrile. The dye **9c** showed a maximum absorption peak with a shoulder in chloroform. There is no significant change in the absorption spectra of all the dyes in acetonitrile, methanol and acetic acid. The spectral shifts of dye **5b** in various solvents are depicted in Fig. 7.

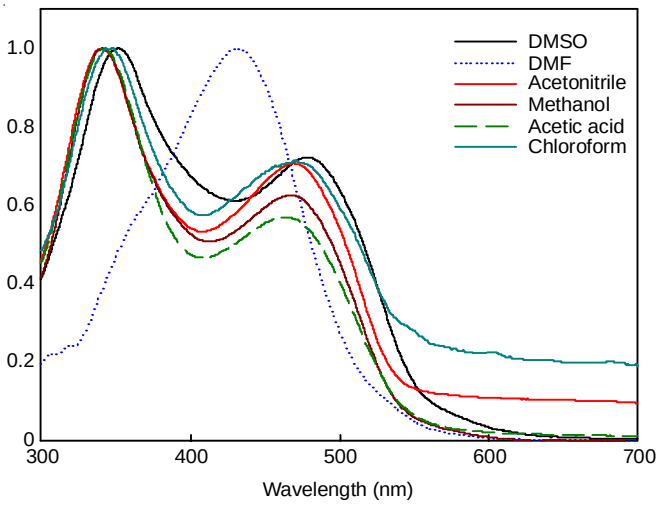

Fig. 7. Absorption spectra of dye 5b in various solvents

The effects of the acid and base on the absorption spectra of the dye solutions were investigated and the results are depicted in Table-2.

The absorption spectra of the dyes in methanol were sensitive to the addition of base (potassium hydroxide, 0.1 M). Therefore, λ_{max} of all the dyes except for **6c** and **6e** showed a bathochromic shifts with the addition of base to methanol. For example; λ_{max} of **5e** was recorded at 355 and 437 nm in methanol, 461 and 453 nm in methanol + KOH. The λ_{max} of **6e** showed a hypsochromic shift with a shoulder at longer wavelength in basic solution.

When hydrochloric acid (0.1 M) was added to the dye solutions in methanol hypsochromic shifts were detected, except for **5a**, **5c**, **5d**, **6e** and **8b**. The λ_{max} of **5b** in methanol did not change when 0.1 M HCl was added. The λ_{max} of **6b** was observed at 338 and 451 nm in methanol and 333 and 443 nm in methanol + HCl. The λ_{max} of **5b** and **5d** showed a bathochromic shift with a shoulder at shorter wavelength, **9d** hypsochromic shift with a shoulder at longer wavelength in acidic solution. These results indicate that the tautomeric form in methanol changed with another tautomeric form in acidic and basic solution. Typical example is given in Fig. 8 for the dye **6b**.

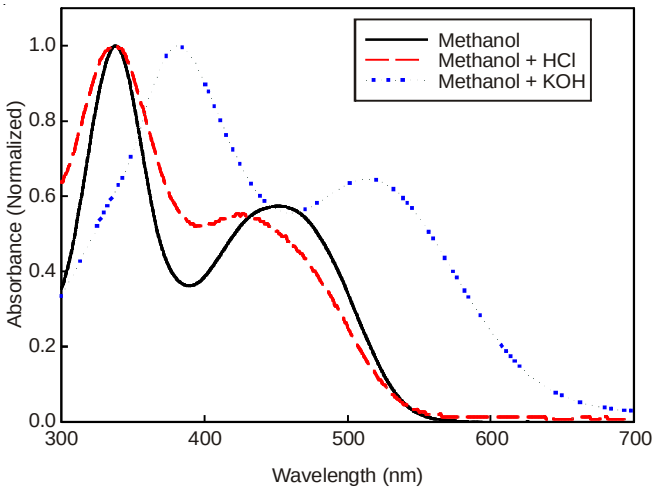

Fig. 8. Absorption spectra of dye **6b** in acidic and basic solutions

The effects of substituent on the absorption spectra of the dye solution were investigated and the results are given in Table-1. The electron-donating groups (-OCH_3, CH_3) are attached to the benzene ring. Dye **5c** resulted in bathochromic shifts with a shoulder in shorter wavelength in DMSO, acetonitrile and chloroform when compared with dye **5a**. For example, for dye **5c** λ_{max} is 432 nm ($\Delta\lambda$:72) in DMSO, 425 nm ($\Delta\lambda$:72) in acetonitrile, 433 nm ($\Delta\lambda$:91) in chloroform with a shoulder. Dye **5c** resulted in bathochromic shifts in methanol and acetic acid and has only one maximum absorption peak when compared with dye **5a**. For example, **5c** λ_{max} is 433 nm ($\Delta\lambda$:73) in methanol, λ_{max} is 437 nm ($\Delta\lambda$:82) in acetic acid. As

TABLE-2
ABSORPTION MAXIMA OF DYES IN ACIDIC AND BASIC SOLUTIONS

Dye	Methanol	Methanol + HCl	Methanol +KOH	Chloroform	Acetic acid
5a	350, 433	356, 435	373, 480	342, 434	355, 442
5b	342, 466	342, 466	380, 506	343, 470	341, 464
5c	433	458, 359s	379, 415	433, 348s	437
5d	334, 434	351, 414s	353, 467	339, 417	334, 405
5e	355, 437	342, 438	361, 453	426	357, 434
6a	347, 414	332, 400	351, 465	343, 414	338, 416
6b	338, 451	333, 443	379, 515	342, 455	337, 446
6c	435	428	379, 488	433	425
6d	332, 414	329, 403	352, 488	337, 428	335, 405
6e	400	402	380, 494s	435	405
8a	344, 429	332, 447	357, 435	350, 429	346, 441
8b	378, 448	387	391, 458	382, 443	373, 448
8c	356, 435	348, 471	362, 437	361, 437	357, 446
8d	369, 453	357, 448	385, 462	375, 450	370, 451
8e	351, 430	335, 452	358, 432	353, 429	351, 443
9a	346, 416	337, 420	352, 435	351, 416	346, 423
9b	373, 456	365, 433s	382, 464	351, 414	370, 446
9c	356, 426	347, 453	357, 439	361, 426s	357, 434
9d	350, 419	346, 432	358, 434	355, 423	350, 420
9e	348, 420	342, 435	352, 439	352, 421	351, 426

s : shoulder

seen in Table-1, dye **5a** showed two absorption peaks in all solvents. Dye **5e** resulted in bathochromic shift in DMSO and methanol when compared with **5a**. Dye **5e** showed bathochromic shift and has only one absorption peak in chloroform. In contrast, dye **5b** which include electron-withdrawing groups (NO_2), resulted in hypsochromic shifts in shorter wavelength absorption peak on the other hand bathochromic shift in longer wavelength in all solvents except for DMF when compared with dye **5a**. Dye **5d** which include electron-withdrawing groups (Cl), resulted in hypsochromic shift in acetic acid and chloroform with respect to dye **5a**. Dye **5d** showed hypsochromic shift in shorter wavelength absorption peak and bathochromic shift in longer wavelength in all solvents except for acetic acid and chloroform with respect to dye **5a**. It was observed that λ_{max} of dyes have electron-donating groups **6c** and **6e** resulted bathochromic shifts in all used solvents except for DMF with respect to dye **6a**. Additionally, λ_{max} is **6c** showed a bathochromic shift with a shoulder at longer wavelength in DMSO and acetonitrile and has one absorption peak in methanol, acetic acid and chloroform. On the other hand, λ_{max} of dyes, **6b** and **6d**, have electron-withdrawing groups showed generally hypsochromic shift when compared with dye **6a**. For example, dye **6b** showed hypsochromic shift in shorter wavelength absorption peak and bathochromic shift in longer wavelength absorption peak in DMF, acetonitrile, methanol, acetic acid and chloroform when compared with **6a**. Dye **6d** showed hypsochromic shift in acetonitrile and acetic acid. Dye **6d** showed hypsochromic shift in shorter wavelength absorption peak and bathochromic shift in longer wavelength absorption peak in DMSO, DMF and chloroform. On the contrary the absorption maxima of dyes **8**(**b-e**) have electron-donating groups and electron-withdrawing groups showed bathochromic shifts in all solvents except for **8c** and **8e** in DMSO when compared with dye **8a**. The λ_{max} of **9**(**b-e**) have electron-donating groups and electron-withdrawing groups showed bathochromic shift in all solvents except for DMSO when compared with dye **9a**. The λ_{max} of **9**(**c-e**) have electron-donating groups and electron-withdrawing groups showed hypsochromic shift in DMSO when compared with dye **9a**. On the other hand, λ_{max} of dye **9b** which include electron-withdrawing groups (NO_2), resulted bathochromic shift in DMSO when compared with dye **9a**. The dye **9c** showed a bathochromic shift with a shoulder at longer wavelength in chloroform.

Antimicrobial activity: Synthesized 20 novel dyes were evaluated for their *in vitro* antimicrobial activities at 100 mg/mL concentration against *Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* ATCC 6633 as examples of Gram-positive bacteria, *Klebseilla pneumonia* ATCC13883 and *Escherichia coli* ATCC 25922 as examples of Gram-negative bacteria and *Saccharomyces cerevisiae* and *Candida albicans* NRRL Y-477 fungal strains. Agar-diffusion method was used for the determination of the preliminary antibacterial and antifungal activity. Also the minimum inhibitory concentration (MIC) measurement was determined for compounds using twofold serial dilution method. Ciprofloxacin and ketoconazole were used as reference drugs. The results of antimicrobial screening of newly prepared compounds are summarized in Table-3.

Table-3 revealed that the majority of the synthesized compounds showed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains and also against antifungal strains. In general, most of the test compound revealed better antibacterial potency than the

TABLE-3
MINIMAL INHIBITORY CONCENTRATIONS (MIC, µg/mL) AND
INHIBITION ZONE (MM) OF SOME NEW SYNTHESIZED COMPOUNDS

Compound	MIC in µg/mL, and zone of inhibition (mm)					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	S. aureus	*B. subtilis*	*K. pneumoniae*	*E. coli*	*S. cerevisiae*	*C. albicans*
5a	66 (22)	132 (16)	66 (19)	33 (24)	132 (16)	132 (19)
5b	132 (18)	132 (16)	66 (20)	132 (18)	132 (14)	132 (12)
5c	132 (17)	66 (21)	33 (25)	33 (26)	66 (22)	66 (20)
5d	132 (18)	132 (14)	33 (24)	16.5 (28)	132 (15)	132 (15)
5e	66 (22)	132 (14)	33 (24)	132 (15)	132 (15)	132 (15)
6a	132 (18)	66 (20)	33 (24)	16.5 (28)	132 (14)	132 (13)
6b	16.5 (28)	132 (14)	33 (26)	8.25 (33)	33 (25)	16.5 (27)
6c	16.5 (28)	66 (20)	8.25 (30)	8.25 (32)	132 (16)	16.5 (28)
6d	66 (23)	132 (16)	8.25 (29)	8.25 (30)	132 (17)	33 (25)
6e	66 (22)	33 (24)	16.5 (28)	16.5 (29)	132 (18)	66 (21)
8a	132 (17)	132 (18)	132 (18)	132 (14)	132 (15)	33 (24)
8b	66 (21)	33 (24)	132 (15)	33 (24)	66 (18)	66 (22)
8c	33 (24)	132 (14)	16.5 (27)	8.25 (30)	66 (20)	16.5 (28)
8d	132 (16)	132 (18)	66 (20)	33 (26)	33 (26)	33 (24)
8e	132 (13)	132 (16)	33 (23)	132 (18)	66 (21)	33 (25)
9a	66 (20)	33 (23)	16.5 (28)	33 (26)	132 (18)	132 (16)
9b	8.25 (31)	16.5 (28)	16.5 (29)	16.5 (28)	33 (24)	66 (22)
9c	16.5 (28)	8.25 (30)	8.25 (33)	8.25 (34)	33 (26)	8.25 (30)
9d	66 (25)	33 (25)	8.25 (30)	8.25 (30)	132 (19)	132 (18)
9e	16.5 (30)	16.5 (29)	16.5 (28)	33 (25)	66 (21)	16.5 (28)
Ciprofloxacin	8.25 (32)	8.25 (31)	8.25 (29)	16.5 (28)	NT	NT
Ketoconazole	NT	NT	NT	NT	8.25 (30)	8.25 (31)

Experiment was carried out in triplicate and the average zone of inhibition was calculated; NT: Not tested

antifungal potency. Furthermore, among all the studied compound, **9c** displayed the highest antibacterial and antifungal activities. In case of Gram-negative bacteria, compounds **6c**, **6d**, **9c** and **9d** were found to be most effective against *K. pneumonia* ATCC13883 with zone of inhibition ranging between 29 and 33 mm and the compounds **6b**, **6c**, **6d**, **6e**, **8c**, **9c** and **9e** were most effective against *E. coli* ATCC 25922 with zone of inhibition ranging between 29 and 34 mm.

Compound **9c** inhibited the growth of *B. subtilis* ATCC6633, *K. pneumonia* ATCC13883, *E. coli* ATCC 25922 and *C. albicans* NRRL Y-477 with inhibition zones 30, 33, 34 and 30 mm, respectively. Also, compound **9b** showed highest activity against *S. aureus* ATCC 29213, with inhibition zone 30 mm. Compounds **9c** exhibited low MIC 8.25 mg/mL against *B. subtilis* ATCC6633, *K. pneumonia* ATCC13883, *E. coli* ATCC 25922 and *C. albicans* NRRL Y-477. Compounds **6c**, **6d** and **9b** showed MIC 8.25 µg/mL against *K. pneumonia* ATCC13883 and *E. coli* ATCC 25922. Additionally, compounds **6b** and **8c** exhibited MIC 8.25 µg/mL against *E. coli* ATCC 25922 and also compound **9b** showed MIC 8.25 µg/mL against *Staphylococcus aureus* ATCC 29213.

Conclusion

In this work, 20 novel disazo dyes have been synthesized, by the reaction of diazotization of **2**(**a-e**) with **3**(**a-e**) and cyclization of hydrazine and phenylhydrazine. These dyes were characterized by UV, FT-IR, ^{1}H NMR, mass spectroscopic techniques as well as elemental analysis. The absorption spectra of all the dyes may be a mixture of tautomeric forms except for **6e**. The spectral characterization of the synthesized dyes assessed with respect to absorption properties in various solvents. The dyes generally demonstrated bathochromic shifts in polar solvent, such as DMSO or DMF. The effects of electron donating and withdrawing groups are not consistent on absorption maximum especially for the 8 and 9 series dyes. In addition, the new dyes were tested for their antimicrobial activities and most of them show significant activities. The results clearly indicate that the presence of the methoxy or chloro group at the phenyl ring increases the antibacterial activity. The activity, however, was maximum for a compound with methoxy groups. It is interesting to point out that the incorporation of pyrazolone to phenyl ring produced a high antimicrobial activity. Biological activity results for the newly synthesized compounds obtained from this study indicate that these dyes can be used as antimicrobial reagent.

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