# Synthesis, Characterization, Solvatochromic Properties, and Antimicrobial-radical Scavenging Activities of New Diazo Dyes Derived from Pyrazolo[1,5-a]pyrimidine

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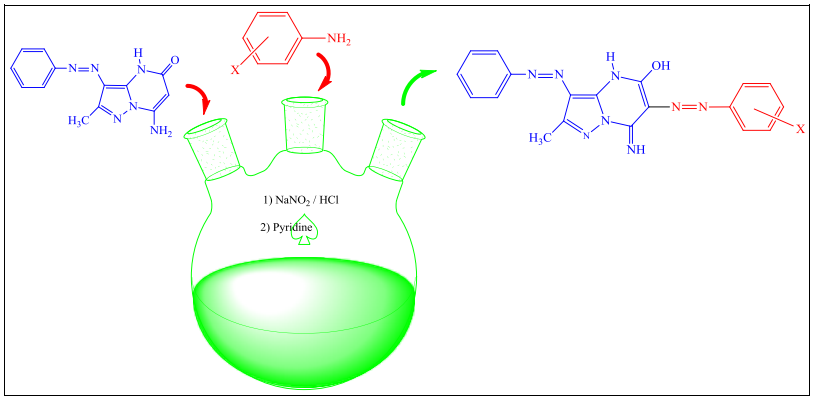
Received May 30, 2017
DOI 10.1002/jhet.2977
Published online 00 Month 2017 in Wiley Online Library (wileyonlinelibrary.com).




5-Amino-3-methyl-4-phenylazo-1*H*-pyrazole and ethyl cyanoacetate reacted in solvent-free media at 150°C to produce 7-amino-3-phenylazo-2-methyl-4*H*-pyrazolo[1,5-a]pyrimidine-5-one (**3**). A series of aromatic amines was coupled using this compound (**3**) and nitrous acid to produce new pyrazolo[1,5-a] pyrimidine derivatives with two arylazo groups **4(a-m)**. The structures of these dyes were determined *via* UV–vis, Fourier transform infrared, proton nuclear magnetic resonance, high-resolution mass spectral data, and elemental analysis. After synthesis, the solvent and acid–base effects of the dyes were investigated within the UV–vis region. The antimicrobial properties of the dyes were also studied. All dyes exhibited activity against Gram-positive and Gram-negative bacteria, and even against fungi. The results were compared to conventional reference results from the antibiotics ciprofloxacin and ketoconazole. Antioxidant potentials were analyzed using *in vitro* antioxidant models on the basis of DPPH (1,1-*d*iphenyl-2-picrylhydrazyl) radical scavenging activities. Most of the compounds exhibited excellent antioxidant activities. In particular, compound **4b** had a higher activity than Vitamin C.



## INTRODUCTION

Nitrogen-containing heterocyclic compounds are of great interest to researchers owing to their biological activities. Of these, heterocycles with pyrimidine molecules represent a continuously expanding field of research in heterocyclic chemistry because of their syntheses, reactions, and biological activities. Pyrimidine structures are seen in numerous pharmaceutical agents and natural products [1–8].

Nitrogen heterocycles are well known for their special value as pharmaceuticals. The pyrimidine nucleus is the key feature of various drugs. Several studies of dyes that include pyrimidine note that they have been used as hypnotic drugs for the nervous system [9], to detect cancer, as chemotherapeutic components, and within the structures of nucleic acids in living cells [10]. Some of these molecules have pharmacological and biological activities [1–3,5]. In addition, pyridyl-benzimidazole analogues were evaluated *in vitro* for urease inhibitory activity [11]. Indole-2-hydrazones were synthesized and evaluated for their antileishmanial activities [12]. Oxindole derivatives were analyzed for their alpha-glucosidase[13] and urease inhibitory activities [14], and the molecular interactions of the active compounds within the urease enzyme binding sites were studied *via* molecular docking simulations.

Aminopyrazole compounds can be produced *via* reactions between nitriles and hydrazine hydrate [15–21]. Pyrazole derivatives exhibit biological and pharmacological activities [22–24]. Pyrazolopyrimidines and related fused heterocycles are potential bioactive molecules and are of great interest to researchers [25,26]. They have been proven to exhibit pharmacological

activities such as central nervous system depression, reduction of nervous tension *via* depression of nerve functions, and inhibition of tubercle mycobacteria growth [27].

In this study, we synthesized various pyrazolo[1,5-a] pyrimidine derivatives **4(a-m)** and investigated their spectral properties. The effects of solvents, acids, and bases on their visible absorption spectra and antimicrobial activities were evaluated.

## RESULT AND DISCUSSION

**Chemistry.** This work is the continuation of our previous studies [28,29]. We report here synthesis of 7-imino-3-phenylazo-6-arylazo-2-methyl-4*H*-pyrazolo[1,5-a]pyrimidine-5-ole derivatives **4(a-m)**. Dye structures were identified *via* several spectroscopic methods, including proton nuclear magnetic resonance ($^1$H-NMR), Fourier transform–infrared (FT-IR) spectroscopy, mass spectroscopy (MS), and elemental analysis. Compounds **1**–**2** were synthesized according to the previously reported procedure [15,16,30], shown in Scheme 1.

7-Amino-3-phenylazo-2-methyl-4*H*-pyrazolo[1,5-a] pyrimidine-5-one (**3**) was produced *via* a reaction between 5-amino-4-arylazo-3-methyl-1*H*-pyrazole and ethyl cyanoacetate at 150°C in solvent free media. The reaction is depicted in Scheme 2**.**

Aniline derivatives were diazotized with nitrous acid in the presence of sodium nitrite and hydrochloric acid at 0–5°C, as shown in Scheme 3. Then a reaction between diazonium salts and 7-amino-3-phenylazo-2-methyl-4*H*-pyrazolo[1,5-a]pyrimidine-5-one produced corresponding 7-imino-3-phenylazo-6-arylazo-2-methyl-4*H*-pyrazolo[1,5-a]pyrimidine-5-ole derivatives. In this way, 13 new azo pyrazolo[1,5-a]pyrimidine derivatives were synthesized.

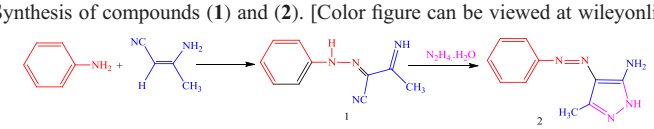
Compounds **4(a–m)** have six different tautomeric forms. As shown in Scheme 4**,** the forms are imino-diazo-keto **(T1)**, imino-hydrazo-keto-azo**(T2)**, imino-diazo-keto (**T3)**, imino-azo-keto-hydrazo**(T4)**, amino-diazo-keto **(T5)**, imino-diazo-enol **(T6)**. The FT-IR spectrum of compound **3** includes –OH stretching vibrations at 3580 cm$^{-1}$. While a band situated at 3334 cm$^{-1}$ was assigned to –NH stretching vibrations, ═NH stretching vibrations were also seen at 3095 cm$^{-1}$. Another vibration at 1624 cm$^{-1}$ resulted from C═N stretching. No carbonyl (C═O) stretching FT-IR band is observed from compound **3.** Its NMR results include a singlet signal at δ 2.52 ppm from –$CH_3$ protons, as well as singlet peaks at δ 5.20 ppm and at δ 5.75 ppm attributed to –CH and –$NH_2$ protons, respectively. The broad peak at δ 9.82 ppm was due to –NH protons. The FT-IR spectra of dyes **4(a–m)** exhibited –NH bands at 3414–3265 cm$^{-1}$, as well as ═NH bands at 3328–3062 cm$^{-1}$. Compounds other than **4i** and **4k** exhibited an intense band at 3569–3319 cm$^{-1}$ resulting from –OH stretching vibrations. We think that this solid state compound was present only in the imino-diazo-enol form **(T6)**. However, **4i** and **4k** exhibited bands from carbonyl (C═O) groups at 1646 cm$^{-1}$ and 1636 cm$^{-1}$, respectively. We suggest that all of the compounds, except for **4i** and **4k**, were in imino-diazo-keto **(T1)**, imino-hydrazo-keto-azo**(T2)**, imino-diazo-keto **(T3)**, imino-azo-keto-hydrazo**(T4),** and amino-diazo-keto **(T5)** forms in the solid state.

$^1$H-NMR spectra of azopyrazolo[1,5-a]pyrimidine derivatives **4(b–m)** exhibit a broad peak at δ 13.41–9.36 ppm attributed to ═NH and –NH protons. The broad peak at δ 11.61–16.09 ppm resulted from tautomeric hydroxyl –OH protons. $^1$H-NMR spectra of **4a** showed a singlet peak at δ 4.97 ppm resulting from –CH protons, a broad peak at δ 10.12 ppm attributed to ═NH protons, and a broad peak at δ 12.23 ppm from –NH protons. The $^1$H-NMR results show that the dyes **4(b-m)** prefer imino-diazo-enol form **(T6)**. As seen in Scheme 4, the dye **4a** may exist in various tautomeric forms including imino-diazo-keto **(T1)**, imino-diazo-keto **(T3),** and imino-azo-keto-hydrazo**(T4)** forms in DMSO-$d_6$. The mass spectra of the dyes match the molecular weights of the respective compounds.

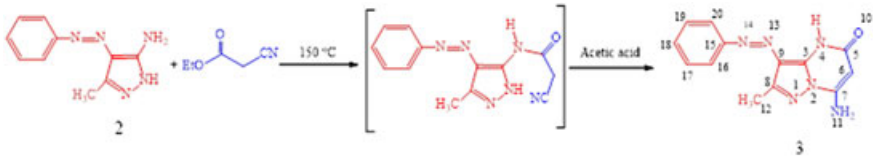
**Absorption spectra.** All molecules have ground states that are less polar than their excited states. Therefore, polar solvents in excited states tend to stabilize more than those in ground states. In addition, the relationship between solvent polarity and dielectric constant is proportional. [31,32]. For the UV measurements, we used the following solvent dielectric constants (ε): DMSO (46.45), DMF (36.71), acetonitrile (35.94), methanol (32.66), acetic acid (6.17), and chloroform (4.89) [33]. The UV–vis absorption spectra of the synthesized dyes were scanned from λ = 300 to λ = 700 nm at various solvent concentrations ($10^{-8}$ to $10^{-6}$ M). Spectral data from all dyes are shown in Table 1.

Generally, the visible absorption spectra of all dyes except for chloroform correlate with solvent polarity. Dye **4m** exhibits a bathochromic shift in chloroform relative

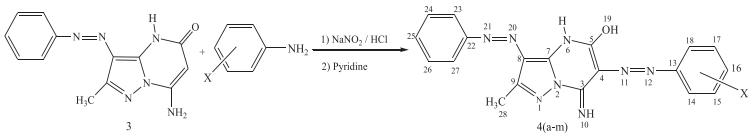
**Scheme 1.** Synthesis of compounds (**1**) and (**2**). [Color figure can be viewed at wileyonlinelibrary.com]



**Scheme 2.** Synthesis of compound **(3).** [Color figure can be viewed at wileyonlinelibrary.com]

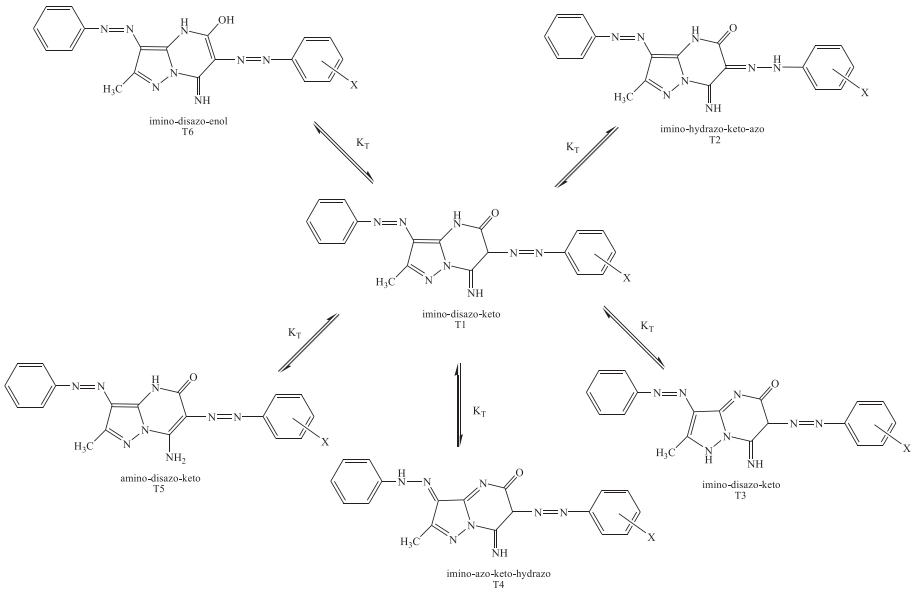


**Scheme 3.** Synthesis of compounds **4(a–m).**



a; X: H
b; X: *p*-$NO_2$
c; X: *p*-$OCH_3$
d; X: *p*-Cl
e; X: *p*-$CH_3$
f; X: *m*-$NO_2$
g; X: *m*-$OCH_3$
h; X: *m*-Cl
i; X: *m*-CH3
j; X: *o*-$NO_2$
k; X: *o*-$OCH_3$
l; X: *o*-Cl
m; X: *o*-$CH_3$

**Scheme 4.** The tautomeric form of azo pyrazolo[1,5-a]pyrimidine derivatives.




to DMSO and DMF. For instance, dye **4m** exhibited absorption maxima at 397 nm in DMSO and 394 nm in DMF but 398 nm in chloroform. Absorption measurements showed that the $\lambda_{max}$ of the dye **4a** was the same in DMSO as in DMF. While the other dyes exhibited bathochromic shifts in most polar solvents such as DMSO and DMF, dyes **4f**, **4g**, and **4i** did not. For example, the maximum absorption of dye **4g** was observed at 373 nm in DMSO and 375 nm in DMF. Dye **4d** exhibits absorption maxima at 402 nm in DMSO, 401 nm in DMF and acetonitrile, 392 nm in methanol, 396 nm in acetic acid, and 400 nm in chloroform. The maximum absorption shifts of dye **4d** in various solvents are indicated in Figure 1**.**

The absorption spectrum of **4k** exhibited two absorbance peaks in acetonitrile. While dyes such as **4b** and **4m** exhibited two absorption peaks with a shoulder in DMF and acetonitrile, the others had either a single absorption peak or a single absorption peak with shoulders. Dyes **4a** and **4g** also had one absorption maximum each but gave different results in acetic acid and chloroform. With dyes **4c** and **4e**, a single absorption peak was observed in all solvents but was not observed for these compounds in chloroform and in acetonitrile,

**Table 1**

Influence of solvent on $\lambda_{max}$ (nm) of dyes.

| Dye no. | DMSO | DMF | Acetonitrile | Methanol | Acetic acid | Chloroform |
|---|---|---|---|---|---|---|
| **4a** | 374 | 374 | 369 | 365 | 352, 430[a] | 362, 438[a] |
| **4b** | 373, 412[a], 466[a] | 371, 410, 457[a] | 408, 362[a], 452[a] | 361, 405[a] | 355, 403[a], 448[a] | 366, 407[a], 458[a] |
| **4c** | 371 | 364 | 364 | 364 | 353 | 362, 370[a] |
| **4d** | 402, 430[a] | 401, 434[a] | 401, 435[a] | 392, 438[a] | 396, 337[a], 438[a] | 400, 426[a], 444[a] |
| **4e** | 373 | 371 | 365 | 361 | 358, 467[a] | 364 |
| **4f** | 400, 473[a] | 402, 441[a] | 397, 441[a] | 393, 433[a] | 392, 435[a] | 397, 441[a] |
| **4g** | 373 | 375 | 367 | 365 | 362, 461[a] | 369, 406[a] |
| **4h** | 402, 443[a] | 397, 447[a] | 396, 438[a] | 396, 437[a] | 394, 433[a] | 397, 423[a], 443[a] |
| **4i** | 393, 441[a] | 394, 439[a] | 394, 437[a] | 388, 435[a] | 342, 392[a], 435 | 394, 442[a] |
| **4j** | 432, 360[a] | 424, 360[a] | 419, 350[a] | 414, 348[a] | 419, 342[a] | 423, 348[a] |
| **4k** | 414, 337[a], 438[a] | 411, 435[a], 460[a], 337[a] | 410, 433[a], 338[a] | 406, 430[a], 455[a] | 334, 485 | 411, 432[a], 457[a], 338[a] |
| **4l** | 410, 437[a] | 403, 433[a], 453[a] | 406, 428[a], 448[a] | 401, 441[a] | 401, 330[a], 435[a] | 403, 424[a], 448[a] |
| **4m** | 397, 448[a] | 394, 448[a] | 384, 443[a] | 391, 442[a] | 350, 393, 437[a] | 398, 443[a] |

[a]Shoulder.

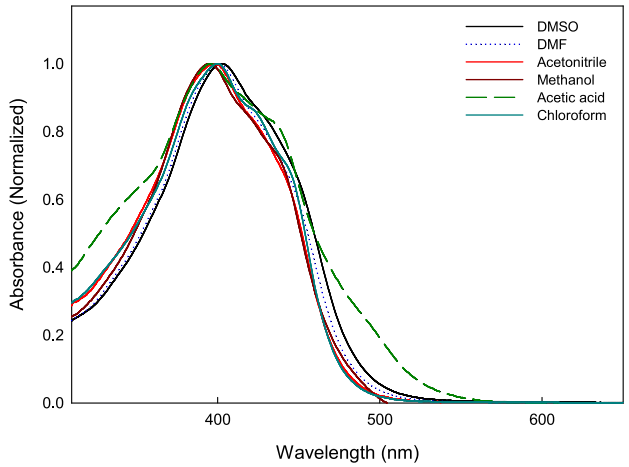



**Figure 1.** Absorption spectra of dye **4d** in various solvents. [Color figure can be viewed at wileyonlinelibrary.com]

**Table 2**

Absorption maxima of dyes in acidic and basic solutions.

| Dye no. | Methanol | Methanol + HCl | Methanol + KOH |
|---|---|---|---|
| **4a** | 365, 433[a] | 334, 453 | 385, 430[a] |
| **4b** | 361, 405[a] | 341, 452 | 391, 426[a], 515[a] |
| **4c** | 364 | 341, 455 | 388, 425[a] |
| **4d** | 392, 438[a] | 332, 453 | 394, 441 |
| **4e** | 361 | 348, 453 | 388, 430[a] |
| **4f** | 393, 433[a] | 334, 425 | 391, 447 |
| **4 g** | 365 | 355, 461 | 393 |
| **4 h** | 396, 437[a] | 333, 441 | 391, 443 |
| **4i** | 388, 435[a] | 337, 455 | 393, 438[a] |
| **4j** | 414, 348[a] | 341, 447 | 389, 461 |
| **4k** | 406, 430[a], 455[a] | 337, 478 | 444, 414[a] |
| **4l** | 401, 441[a] | 338, 446 | 398, 447 |
| **4m** | 391, 442[a] | 334, 458 | 396, 435[a] |

[a]Shoulder

respectively. On the basis of these results, one can suggest that dyes **4a** and **4g** exist predominantly in only one tautomeric form in solvents other than acetic acid and chloroform. Likewise, dyes **4c** and **4e** exist predominantly in one tautomeric form in all solvents except for chloroform and acetonitrile, respectively. It is likely that other synthesized dyes exist in different tautomeric forms in various solvents. The tautomeric forms of dyes **4(a-m)** are shown in Scheme 4**.**

A study of the effects of acids and bases on dye absorption has also been performed. The results are shown in Table 2.

Addition of acids and bases to the dyes in methanol caused some changes. Most dyes exhibited a single absorption maximum with a shoulder in methanol except for **4c**, **4e**, and **4g**. It is possible that these dyes exist in a single tautomeric form in this solution. However, the situation is different for dyes such as **4a**, **4b**, **4d**, **4f**, **4h**, **4i**, **4j**, **4k**, **4l**, and **4m**, which are present more than one tautomeric form. Adding HCl (0.1 M) to the methanol solution causes a hypsochromic shift, and the formation of two absorption maxima. It is thought that all dyes are present primarily in two tautomeric forms. Upon adding KOH (0.1 M) in methanol, a bathochromic shift in the $\lambda_{max}$ values is detected and one absorption maximum with a shoulder appears. Different behavior is observed for **4d**, **4f**, **4g**, **4h**, **4j**, and **4k.** These results show that dyes **4a**, **4b**, **4c**, **4e**, **4i**, **4l**, and **4m** exist as a mixture of tautomers. In a basic solution, dyes **4d**, **4f**, **4h**, **4j**, and **4l** are predominantly present in two tautomeric forms, but dye **4g** is present as a single tautomer. Spectral shifts of compound **4d** are seen in acidic and basic solutions and are shown in Figure 2.

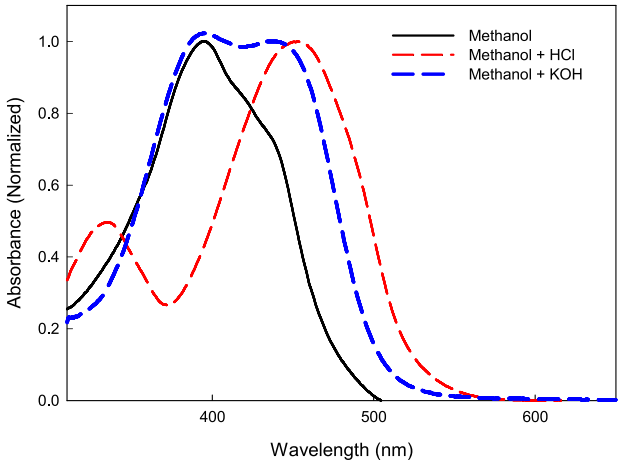



**Figure 2.** Absorption spectra of dye **4d** in acidic and basic solutions. [Color figure can be viewed at wileyonlinelibrary.com]

**Antimicrobial activity.** In this study, 13 of the newly synthesized compounds were tested for antimicrobial and antifungal properties. We used concentrations of 100 μg/mL for each compound. In these analyses, various microorganisms such as *Staphylococcus aureus* ATCC 29213, Gram (+) *Bacillus subtilis* ATCC 6633, Gram (−) *Klebseilla pneumonia* ATCC13883 and *Escherichia coli* ATCC 25922, *Saccharomyces cerevisiae*, and fungus *Candida albicans* NRRL Y-477 were used. The agar-diffusion method was used to determine the preliminary antibacterial and antifungal activities. Ciprofloxacin and ketoconazole were used as antibacterial and antifungal references, respectively. All results were collected and average inhibition zone diameters with no bacterial or fungal growth were measured in millimeter.

All dyes obtained from this study exhibited excellent antimicrobial and antifungal activities when compared with the reference drugs. These dyes had inhibition zones of various sizes when exposed to bacteria (Table 3). In addition, the minimum inhibitory concentrations (MICs) of these compounds were determined to elucidate their inhibitory effects on various bacterial and fungal strains as seen in Table 4.

The antibacterial screening data showed that that all tested dyes exhibited significant and diverse activities against the four human pathogenic bacteria. In general, most of the target compounds exhibited better activities against the Gram-negative bacteria than the Gram-positive bacteria. Compounds **4b**, **4d**, **4e**, **4j**, and **4l** exhibited the highest potencies against the organisms considered. The highest anti-bacterial activities were achieved by compounds **4b**, **4d**, **4e**, and **4j**. This may stem from the presence of aromatic rings, chlorine, and nitro groups in *para* and *ortho* positions.

These compounds are better antibacterial agents than antifungal agents. When potency against the various organisms was compared with those of reference drugs, good antimicrobial activity was observed with compound **4l**. Dye **4d** inhibited the growth of *S. aureus* ATCC 29213, *B. subtilis* ATCC6633, and *K. pneumonia*

**Table 3**

Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm) of chemical compounds against the pathological strains based on well-diffusion assay.

| | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Bacteria | | | | Fungi | |
| | Gram-positive | | Gram-negative | | | |
| Compound | *Staphylococcus aureus* | *Bacillus subtilis* | *Klebseilla pneumoniae* | *Escherichia coli* | *Saccharomyces cerevisiae* | *Candida albicans* |
| **4a** | 18 ± 0.4 | 23 ± 0.6 | 23 ± 0.7 | 20 ± 1.1 | 21 ± 0.4 | 19 ± 0.6 |
| **4b** | 24 ± 0.8 | 24 ± 0.3 | 31 ± 0.8 | 26 ± 0.6 | 26 ± 1.3 | 28 ± 0.7 |
| **4c** | 22 ± 1.1 | 26 ± 0.4 | 24 ± 0.6 | 23 ± 0.4 | 23 ± 0.6 | 23 ± 0.2 |
| **4d** | 32 ± 0.5 | 29 ± 0.6 | 32 ± 0.9 | 27 ± 0.4 | 22 ± 0.4 | 20 ± 0.4 |
| **4e** | 20 ± 0.3 | 23 ± 0.9 | 31 ± 0.5 | 15 ± 0.7 | 19 ± 0.1 | 21 ± 0.3 |
| **4f** | 18 ± 0.7 | 16 ± 1.2 | 26 ± 1.0 | 24 ± 0.6 | 14 ± 0.3 | 19 ± 0.5 |
| **4g** | 26 ± 0.7 | 21 ± 0.4 | 24 ± 1.1 | 20 ± 0.3 | 18 ± 0.5 | 15 ± 1.3 |
| **4h** | 21 ± 0.3 | 17 ± 0.7 | 18 ± 0.9 | 16 ± 1.4 | 21 ± 0.8 | 24 ± 0.6 |
| **4i** | 24 ± 0.4 | 18 ± 0.5 | 16 ± 0.5 | 15 ± 0.3 | 20 ± 0.9 | 23 ± 0.9 |
| **4j** | 30 ± 0.6 | 23 ± 0.2 | 29 ± 0.6 | 28 ± 0.6 | 26 ± 0.3 | 20 ± 0.4 |
| **4k** | 26 ± 0.8 | 21 ± 0.5 | 28 ± 0.7 | 21 ± 0.4 | 18 ± 1.1 | 23 ± 0.3 |
| **4l** | 27 ± 1.0 | 29 ± 0.6 | 26 ± 0.7 | 29 ± 0.9 | 27 ± 0.8 | 30 ± 0.6 |
| **4m** | 24 ± 0.3 | 16 ± 0.4 | 20 ± 1.5 | 16 ± 0.7 | 21 ± 0.7 | 25 ± 0.3 |
| **Ciprofloxacin** | 29 ± 0.4 | 28 ± 0.3 | 29 ± 0.8 | 25 ± 0.6 | NT | NT |
| **Ketoconazole** | NT | NT | NT | NT | 27 ± 0.4 | 29 ± 0.6 |

Zone of inhibition values are presented as the mean ± SEM from at least three separated experiments NT not tested.

**Table 4**

Minimum inhibitory concentration (μg/mL) against the pathological strains based on two fold serial dilution technique.

| | MIC in μg/mL | | | | | |
|---|---|---|---|---|---|---|
| | Bacteria | | | | Fungi | |
| | Gram-positive | | Gram-negative | | | |
| Compound | *Staphylococcus aureus* | *Bacillus subtilis* | *Klebseilla pneumoniae* | *Escherichia coli* | *Saccharomyces cerevisiae* | *Candida albicans* |
| **4a** | 66 | 33 | 33 | 66 | 66 | 66 |
| **4b** | 33 | 33 | 8.25 | 16.5 | 16.5 | 16.5 |
| **4c** | 33 | 16.5 | 33 | 66 | 33 | 33 |
| **4d** | 8.25 | 8.25 | 8.25 | 16.5 | 66 | 66 |
| **4e** | 66 | 33 | 8.25 | 132 | 132 | 66 |
| **4f** | 132 | 132 | 16.5 | 33 | 132 | 66 |
| **4g** | 33 | 66 | 33 | 66 | 132 | 132 |
| **4h** | 66 | 132 | 132 | 132 | 66 | 33 |
| **4i** | 33 | 132 | 132 | 132 | 66 | 33 |
| **4j** | 8.25 | 33 | 8.25 | 16.5 | 16.5 | 66 |
| **4k** | 16.5 | 66 | 16.5 | 66 | 132 | 33 |
| **4l** | 16.5 | 16.5 | 16.5 | 8.25 | 16.5 | 8.25 |
| **4m** | 33 | 132 | 66 | 132 | 66 | 33 |
| **Ciprofloxacin** | 8.25 | 16.5 | 8.25 | 16.5 | NT | NT |
| **Ketoconazole** | NT | NT | NT | NT | 16.5 | 8.25 |

NT, not tested.

ATCC13883. In addition, compound **4j** exhibited good activity against *S. aureus* ATCC 29213 and *K. pneumonia* ATCC13883. Compound **4 l** showed highest activities against S. cerevisiae and the *C. albicans* NRRL Y-477 fungal strain.

**DPPH radical scavenging activity.** Free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) provides a rapid, simple, and inexpensive method of evaluating the antioxidant capacities of various substances. In this method, a stable radical is generated directly before exposure to and reaction with putative antioxidants. This method was used with all of the compounds obtained. Ascorbic acid was used as a positive control material. In Table 5, 50% inhibitory concentrations are expressed as IC50s**.** All compounds exhibit DPPH radical quenching activities in a concentration dependent manner. Compound **4b** exhibits better activity (IC50 = 24.44 μg/mL) than the vitamin C used as a reference (IC50 = 26.71 μg/mL).

The test compounds, such as **4c**, **4e**, **4h**, **4i**, **4j**, **4l**, and **4m** exhibit IC50s of 57.91, 63.85, 64.32, 57.69, 32.33, 42.87, and 53.24 μg/mL, respectively. In addition, the remaining test compounds exhibit IC50 values above 100 μg/mL.

Vitamin C was used as a positive control. The IC50 value was determined as the effective concentration at which 50% of DPPH radicals were removed. The IC50 value was determined *via* interpolation after linear regression analysis.

**Table 5**

DPPH radical scavenging activity of compounds **4(a-m).**

| Compound | $IC_{50}$ (μM) | Compound | $IC_{50}$ (μM) |
|---|---|---|---|
| **4a** | 94.56 ± 1.41 | **4h** | 64.32 ± 1.53 |
| **4b** | 24.45 ± 0.96 | **4i** | 57.69 ± 0.62 |
| **4c** | 57.91 ± 2.24 | **4j** | 32.33 ± 1.90 |
| **4d** | 145.32 ± 1.55 | **4k** | 108.41 ± 1.66 |
| **4e** | 63.85 ± 1.39 | **4l** | 42.87 ± 1.47 |
| **4f** | 81.24 ± 0.67 | **4m** | 53.24 ± 1.23 |
| **4g** | 110.87 ± 2.01 | **Vitamin C** | 26.71 ± 1.62 |

## CONCLUSION

In this work, new azopyrazolo[1,5-a]pyrimidine derivatives **4(a-m)** were synthesized. The resulting dyes were evaluated for antimicrobial activity and compared to various standards. Some of the compounds exhibited significant antibacterial and antifungal activities. Furthermore, the antioxidant activities of the dyes were investigated. All of the compounds tested exhibited DPPH radical quenching activities.

## EXPERIMENTAL

**General.** All chemicals were purchased and were used as received. Solvents were spectroscopic grade. A melting point apparatus (Stuart SMP 30, UK) was used to

determine the dye melting points. $^1$H-NMR spectra were recorded using a Bruker (Germany) Ultra Shield Plus 400-MHz spectrometer at room temperature in deuterated dimethyl sulphoxide (DMSO-$d_6$). Chemical shifts are (δ) given in ppm. FT-IR spectra were recorded on a Perkin Elmer Mattson (USA) 1000 FT-IR spectrometer. A Waters LCT Premier XE LTOF (TOFMS) instrument (Waters Corporation, Milford MA, USA) was used for MS. A Leco CHNS-932 analyzer (USA) was used for elemental analysis. UV–vis absorption spectra were recorded on a Shimadzu (Japan) UV-1601 UV–visible spectrophotometer over the λ range of 300 to 700 nm. The wavelengths of maximum absorption ($\lambda_{max}$) were determined with DMSO, DMF, acetonitrile, methanol, acetic acid, and chloroform as solvents using concentrations that ranged from $1 \times 10^{-8}$ to $1 \times 10^{-6}$ M. Changes in absorption maxima were investigated by adding 0.1 mL of hydrochloric acid and 0.1 mL of potassium hydroxide to 1 mL of the dye solution in methanol.

**Synthesis.** ***Synthesis of 2-phenylhydrazone-3-ketimino butyronitrile (1).*** A solution of aniline (0.93 g, 10 mmol) in hydrochloric acid (10 mL) reacted with a sodium nitrite (10 mmol) solution in water (3 mL) at 0–5°C for 2 h. The resulting diazonium salt solution was then added to 3-aminocrotononitrile (0.82 g, 10 mmol) in sodium acetate (2 g, dissolved in 10 mL of 1:1 ethanol/water) under stirring, at the same temperature. Stirring continued for 2 h and cold water (50 mL) was added. Solids were filtered off, dried and recrystallized from aqueous ethanol. Greenish-yellow crystals were obtained (1.45 g, 78% yield), mp: 166–167°C.

***Synthesis of 5-amino-3-methyl-4-phenylazo-1H-pyrazole (2).*** Compound **1** (1.86 g, 10 mmol) was dissolved in 30 mL of ethanol and hydrazine hydrate (0.5 g, 10 mmol) was added. The reaction balloon was heated under reflux for 4 h, and then cooled to room temperature. The precipitated products separated upon dilution with water (50 mL) and were filtered off, dried, and recrystallized from aqueous ethanol. Yellow crystals were obtained (1.52 g, 76% yield), mp: 165–166°C.

***Synthesis of 7-amino-3-phenylazo-2-methyl-4H-pyrazolo [1,5-a]pyrimidine-5-one (3).*** Compound (**3**) was synthesized *via* a reaction between 5-amino-3-methyl-4-phenylazo-1*H*-pyrazole and ethyl cyanoacetate. 5-Amino-3-methyl-4-phenylazo-1*H*-pyrazole (2.01 g, 10 mmol) reacted with ethyl cyanoacetate 6.0 mL (50 mmol) without a solvent. The reaction was heated for 2 h at 150°C and then allowed to cool to 60–70°C. It was then heated under reflux in 20–30 mL of acetic acid. The mixture was cooled and added to a 100 mL mixture of ethanol and water (1:1). The precipitated brown product was filtered off, washed with water several times, and dried. The obtained product was crystallized from DMSO : water mixture as brown crystals, yield (82%), decomp > 130°C; FT–IR (cm$^{-1}$) $\nu_{max}$: 3580 (−OH), 3334 (−NH), 3095 and 3073 (Ar–H), 2927 (Aliphatic C–H), 1624 (C═N), 1560 (N═N); $^1$H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.52 (3H, s, $-CH_3$), 5.20 (1H, s, −CH), 5.75 (2H, s, $NH_2$), 7.31 (1H, t, *J* = 7.3 Hz, H-18), 7.38 (2H, t, *J* = 7.4 Hz, H-17 and H-19), 7.68 (2H, d, *J* = 7.5 Hz, H-16 and H-20), 9.82 (b, pyrimido –NH); $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ:12.37 ($CH_3$, C-12), 83.49 (CH, C-6), 109.84 (C, C-9), 125.66 (CH, C-16, C-20), 127.34 (CH, C-17, C-19), 129.37 (CH, C-18), 143.35 (C, C-8), 146.91 (C, C-7), 150.32 (C, C-15), 162.21 (C, C-5), 169.90 (C, C-3); HR-MS: 268.2816 [M + H]$^+$, calcd 268.2819. *Anal*. Calcd for $C_{13}H_{12}N_6O$: C, 58.20%; H, 4.51%; N, 31.33%. Found: C, 58.22%; H, 4.50%; N, 31.32%.

**General procedure for the synthesis of azo dyes 4(a–m).** ***7-Imino-2-methyl-3, 6-diphenylazo-6, 7-dihydro-1H-pyrazolo[1,5-a]pyrimidine-5-one (4a).*** A solution of aniline (1.04 g, 11.19 mmol) in hydrochloric acid (7.5 mL) was reacted with a solution of sodium nitrite (1.16 g, 16.80 mmol) in water (10 mL) at 0–5°C. This mixture was stirred over 1 h. The resulting diazonium solution was added to a vigorously stirred solution of 7-amino-3-phenylazo-2-methyl-4*H*-pyrazolo[1,5-a]pyrimidine-5-one (3.00 g, 11.19 mmol), which was dissolved in pyridine (10 mL). The reaction was stirred for 2 h at 0–5°C. The product separated upon dilution with water (50 mL). It was filtered, dried, and crystallized from a mixture of DMSO and water (2:3) to obtain 7-imino-2-methyl-3,6-diphenylazo-6,7-dihydro-1*H*-pyrazolo[1,5-a]pyrimidine-5-one (**4a**) as a brown crystals, yield (75%), mp: 262–264°C; FT–IR (cm$^{-1}$) $\nu_{max}$: 3319 (−OH), 3265 (−NH), 3062 (═NH), 3062 (Ar–H), 2926 (Aliphatic C–H), 1597 (C═N), 1563 (N═N); $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 2.58 (3H, s, $-CH_3$), 4.97 (1H, s, −CH),7.42 (1H, t, *J* = 7.3 Hz, H-25), 7.47-7.57 (5H, m, H-16, H-15, H-17, H-24, H-26), 7.83 (1H, d, *J* = 7.3 Hz, H-14), 7.94 (2H, d, *J* = 7.3 Hz, H-18, H-27), 8.01 (1H, d, *J* = 7.3 Hz, H-23), 10.12 (1H, b, pyrimido ═NH), 12.23 (1H, b, pyrazole–NH); $^{13}$C-NMR(100 MHz, DMSO-$d_6$) δ:12.37 ($CH_3$, C-28), 92.78 (CH, C-4), 118.22 (C, C-8), 123.24 (CH, C-14, C-18), 125.66 (CH, C-23, C-27), 127.34 (CH, C-24, C-26), 129.37 (CH, C-25), 129.64 (C, C-16), 130.01 (CH, C-15, C-17), 140.92 (C, C-3), 146.90 (C, C-9), 151.41 (C, C-13), 151.44 (C, C-22), 162.51 (C, C-7), 163.72 (C, C-5); HR-MS: 372.3954 [M + H]$^+$, calcd 372.3955. *Anal*. Calcd for $C_{19}H_{16}N_8O$: C, 61.28%; H, 4.33%; N, 30.09%. Found: C, 61.24%; H, 4.36%; N, 30.11%.

The above procedure was also used to synthesize dye **4(b–m)**. The general route of synthesized dyes is outlined in Scheme 3**.**

***7-Imino-3-phenylazo-6-(4′-nitrophenylazo)-2-methyl-4H-pyrazolo[1,5-a]pyrimidine-5-one (4b).*** Dark brown solid crystals, (78%), decomp > 287°C; FT–IR (cm$^{-1}$) $\nu_{max}$**:** 3371 (−OH), 3371 (−NH), 3063 (═NH), 3063 (Ar–H),

2925(Aliphatic C–H), 1641 (C═N), 1599 (N═N), 1505 and 1315 (−$NO_2$); $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm):2.57 (3H, s, −$CH_3$), 7.41 (1H, t, *J* = 7.3 Hz, H-25), 7.52 (2H, t, *J* = 7.4 Hz, H-24, H-26), 7.83 (2H, d, *J* = 7.3 Hz, H-23, H-27), 7.92 (2H, d, *J* = 8.0 Hz, H-14, H-18), 8.10 (2H, d, *J* = 8.0 Hz, H-15, H-17), 9.82 (1H, b, pyrimido ═NH), 11.05 (1H, b, pyrimido –NH), 12.32 (1H, b, −OH); $^{13}$C-NMR(100 MHz, DMSO-$d_6$) δ:12.40 ($CH_3$, C-28), 92.75 (C, C-4), 118.22 (C, C-8), 123.24 (CH, C-14, C-18), 125.66 (CH, C-23, C-27), 125.77 (CH, C-15, C-17), 127.34 (CH, C-24, C-26), 129.37 (CH, C-25), 140.92 (C, C-3), 146.89 (C, C-9), 148.40 (C, C-16), 151.44 (C, C-22), 154.60 (C, C-13), 162.52 (C, C-7), 163.72 (C, C-5); HR-MS: 417.3951 $[M + H]^+$, calcd 417.3955. *Anal*. Calcd for $C_{19}H_{15}N_9O_3$: C, 54.68%; H, 3.62%; N, 30.20%. Found: C, 54.65%; H, 3.63%; N, 30.24%.

***7-Imino-3-phenylazo-6-(4′-methoxyphenylazo)-2-methyl-4H-pyrazolo[1,5-a]pyrimidine-5-one (4c).*** Brick red solid crystals, (74%), mp: 255–257°C; FT–IR ($cm^{-1}$) $\nu_{max}$**:** 3569 (−OH), 3318 (−NH), 3068 (═NH), 3068 (Ar–H), 2927 (Aliphatic C-H), 1661 (C═N), 1622 (N═N); $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 2.57 (3H, s, −$CH_3$), 3.77 (3H, s, −$OCH_3$), 7.19 (2H, d, *J* = 7.5 Hz, H-15, H-17), 7.43 (1H, t, *J* = 7.3 Hz, H-25),7.56 (2H, t, *J* = 7.3 Hz, H-24, H-26), 7.74 (2H, d, *J* = 8.0 Hz, H-14, H-18), 7.91 (2H, d, *J* = 7.3 Hz, H-23, H-27), 12.18 (2H, b, pyrimido ═NH, pyrimido –NH), 15.83 (1H, b, −OH); $^{13}$C-NMR(100 MHz, DMSO-$d_6$) δ:12.37 ($CH_3$, C-28), 55.40 ($OCH_3$, C-30), 101.71 (C, C-4), 116.20 (CH, C-15, C-17), 121.83 (C, C-8) 122.00 ppm (CH, C-14, C-18), 124.85 (CH, C-23, C-27), 127.34 (CH, C-24, C-26), 129.37 (CH, C-25), 140.92 (C, C-3), 144.36 (C, C-9), 147.93 (C, C-13), 156.83 (C, C-22), 159.08 (C, C-16),160.53 (C, C-7), 194.63 (C, C-5); HR-MS: 402.4231 $[M + H]^+$, calcd 402.4236. *Anal*. Calcd for $C_{20}H_{18}N_8O_2$: C, 59.69%; H, 4.51%; N, 27.85%. Found: C, 59.72%; H, 4.50%; N, 27.81%.

***7-Imino-3-phenylazo-6-(4′-chlorophenylazo)-2-methyl-4H-pyrazolo[1,5-a]pyrimidine-5-one (4d).*** Brick red solid crystals, (77%), mp: 276–278°C; FT-IR ($cm^{-1}$) $\nu_{max}$**:** 3507 (−OH), 3414 (−NH), 3328 (═NH), 3062 (Ar–H), 2927 (Aliphatic C–H), 1624 (C═N), 1576 (N═N); $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 2.61 (3H, s, −$CH_3$), 7.44 (1H, t, *J* = 7.3 Hz, H-–25), 7.51–7.56 (4H, m, H-23, H-24, H-26, H-27), 7.81 (2H, d, *J* = 8.8 Hz, H-14, H-18), 7.99 (2H, d, *J* = 7.5 Hz, H-15, H-17), 9.51 (1H, b, pyrimido ═NH), 10.82 (1H, b, pyrimido –NH), 12.10 (1H, b, −OH); $^{13}$C-NMR(100 MHz, DMSO-$d_6$) δ:12.37 ($CH_3$, C-28), 101.71 (C, C-4), 121.83 (C, C-8), 124.82 (CH, C-14, C-18), 124.85 (CH, C-23, C-27), 127.34 (CH, C-24, C-26), 130.08 (CH, C-25),130.40 (CH, C-15, C-17), 139.74 (C, C-16), 140.52 (C, C-3), 144.36 (C, C-9), 150.83 (C, C-22), 153.02 (C, C-13), 160.53 (C, C-7), 194.63 (C, C-5); HR-MS:407.1132 $[M + H]^+$, calcd 407.1136. *Anal*. Calcd for $C_{19}H_{15}ClN_8O$: C, 56.09%; H, 3.72%; N, 27.54%. Found: C, 56.07%; H, 3.75%; N, 27.52%.

***7-Imino-3-phenylazo-6-(4′-methylphenylazo)-2-methyl-4H-pyrazolo[1,5-a]pyrimidine-5-one (4e).*** Dark mustard yellow solid crystals, (73%), mp: 213°C; FT–IR ($cm^{-1}$) $\nu_{max}$**:** 3332 (−OH), 3332 (−NH), 3094 (═NH), 3071 (Ar–H), 2926 (Aliphatic C–H), 1623 (C═N), 1597 (N═N); $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 2.60 (3H, s, Ar–$CH_3$), 2.69 (3H, s, pyrazole–$CH_3$), 7.18 (1H, t, *J* = 7.3 Hz, H-25), 7.47 (2H, d, *J* = 7.5 Hz, H-15, H-17), 7.52 (2H, t, *J* = 7.3 Hz, H-24, H-26), 7.65 (2H, d, *J* = 8.0 Hz, H-14, H-18), 7.98 (2H, d, *J* = 7.3 Hz, H-23, H-27),11.76 (2H, b,pyrimido ═NH, pyrimido –NH), 16.09 (1H, b, −OH); $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ: 12.37 ($CH_3$, C-28), 22.21 ($CH_3$, C-29), 101.71 (C, C-4), 112.04 (CH, C-14, C-18), 121.83 (C, C-8), 124.85 (CH, C-23, C-27), 127.34 (CH, C-24, C-26), 129.37 (CH, C-25), 130.70 (CH, C-15, C-17), 132.32 (C, C-16), 140.52 (C, C-3), 144.36 (C, C-9),149.61 (C, C-13),151.48 (C, C-22), 160.53 (C, C-7), 194.63 (C, C-5); HR-MS: 386.4223 $[M + H]^+$, calcd 386.4227. *Anal*. Calcd for $C_{20}H_{18}N_8O$: C, 62.17%; H, 4.70%; N, 29.00%. Found: C, 62.12%; H, 4.72%; N, 29.04%.

***7-Imino-3-phenylazo-6-(3′-nitrophenylazo)-2-methyl-4H-pyrazolo[1,5-a]pyrimidine-5-one (4f).*** Bright dark brown solid crystals, (74%), mp: 308–309°C; FT–IR ($cm^{-1}$) $\nu_{max}$**:** 3427 (−OH), 3341 (−NH), 3265 (═NH), 3090 and 3058 (Ar–H), 2924 (Aliphatic C–H), 1640 (C═N), 1595 (N═N), 1524 and 1348 (−$NO_2$); $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 2.59 (3H, s, −$CH_3$), 7.44 (1H, t, *J* = 7.3 Hz, H-25), 7.53 (2H, t, *J* = 7.5 Hz, H-24, H-26), 7.76 (1H, t, *J* = 8.0 Hz, H-17), 7.98 (2H, d, *J* = 7.5 Hz, H-23, H-27), 8.18 (1H, d, *J* = 7.8 Hz, H-18), 8.23 (1H, d, *J* = 8.0 Hz, H-16), 8.61 (1H, s, H-14), 9.66 (1H, b, pyrimido ═NH), 10.84 (1H, b, pyrimido –NH), 12.12 (1H, b, −OH); $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ:12.37 ($CH_3$, C-28), 101.71 (C, C-4), 111.05 (CH, C-18), 117.00 (CH, C-16),118.07 (CH, C-14),121.83 (C, C-8), 124.85 (CH, C-23, C-27), 127.34 (CH, C-24, C-26), 129.37 (CH, C-25),133.42 (CH, C-15), 140.52 (C, C-3), 144.36 (C, C-9), 150.50 (C, C-13), 150.83 (C, C-22), 151.50 (C, C-17), 160.53 (C, C-7), 194.63 (C, C-5); HR-MS: 417.3951$[M + H]^+$, calcd 417.3958. *Anal*. Calcd for $C_{19}H_{15}N_9O_3$: C, 54.68%; H, 3.62%; N, 30.20%. Found: C, 54.63%; H, 3.64%; N, 30.24%.

***7-Imino-3-phenylazo-6-(3′-methoxyphenylazo)-2-methyl-4H-pyrazolo[1,5-a]pyrimidine-5-one (4g).*** Dark brown solid 1crystals, (80%), decomp > 292°C; FT-IR ($cm^{-1}$) $\nu_{max}$**:** 3354 (−OH), 3281 (−NH), 3144 (=NH), 3033 (Ar–H), 2978 and 2939 (Aliphatic C–H), 1646 (C═N), 1602 (N═N); $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 2.65 (3H, s, −$CH_3$), 5.00 (3H, s, −$OCH_3$),

7.35 (1H, d, *J* = 8.0 Hz, H-16), 7.37 (1H, s, H-14), 7.41 (1H, t, *J* = 7.3 Hz, H-25), 7.46 (1H, t, *J* = 8.0 Hz, H-17), 7.56 (2H, t, *J* = 7.3 Hz, H-24, H-26), 7.84 (1H, d, *J* = 7.5 Hz, H-18), 7.93 (2H, d, *J* = 7.5 Hz, H-23, H-27), 11.26 (3H, b, pyrimido ═NH, pyrimido –NH, −OH); $^{13}$C-NMR(100 MHz, DMSO-$d_6$) δ: 12.37 ($CH_3$, C-28), 55.71 ($CH_3$, C-30),101.71 (C, C-4), 106.36 (CH, C-18), 109.62 (CH, C-16), 112.08 (CH, C-14), 121.83 (C, C-8),124.85 (CH, C-23, C-27), 127.34 (CH, C-24, C-26), 129.37 (CH, C-25), 133.32 (CH, C-15), 140.52 (C, C-3), 144.36 (C, C-9),150.83 (C, C-22), 151.98 (C, C-13), 160.53 (C, C-7), 162.88 (CH, C-17), 194.63 (C, C-5); HR-MS: 402.4231 $[M + H]^+$, calcd 402.4339. *Anal*. Calcd for $C_{20}H_{18}N_8O_2$: C, 59.69%; H, 4.51%; N, 27.85%. Found: C, 59.72%; H, 4.50%; N, 27.81%.

***7-Imino-3-phenylazo-6-(3′-chlorophenylazo)-2-methyl-4H-pyrazolo[1,5-a]pyrimidine-5-one (4h).*** Reddish brown solid crystals, (77%), mp: 282–284°C; FT-IR ($cm^{-1}$) $\nu_{max}$**:** 3428 (−OH), 3337 (−NH), 3242 (═NH), 3064 (Ar–H), 2927, and 2853 (Aliphatic C–H), 1645 (C═N), 1595 (N═N); $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 2.62 (3H, s, $-CH_3$), 7.41 (2H, m, H-25, H-16), 7.53 (3H, t, *J* = 7.4 Hz, H-17, H-24, H-16), 7.77 (1H, d, *J* = 8.0 Hz, H-18), 7.85 (1H, s, H-14), 7.99 (2H, d, *J* = 7.5 Hz, H-23, H-27), 9.53 (1H, b, pyrimido ═NH), 10.81 (1H, b, pyrimido –NH), 12.22 (1H, b, −OH); $^{13}$C-NMR(100 MHz, DMSO-$d_6$) δ:12.37 ($CH_3$, C-28), 101.71 (C, C-4), 114.13 (CH, C-18), 114.37 (CH, C-14), 121.83 (C, C-8), 122.93 (CH, C-16), 124.85 (CH, C-23, C-27), 127.34 (CH, C-24, C-26), 129.37 (CH, C-25), 131.67 (CH, C-17), 138.84 (C, C-15), 140.52 (C, C-3), 144.36 (C, C-9), 150.83 (C, C-22), 151.98 (C, C-13), 160.53 (C, C-7), 194.63 (C, C-5); HR-MS: 407.1132 $[M + H]^+$, calcd 407.1143. *Anal*. Calcd for $C_{19}H_{15}ClN_8O$: C, 56.09%; H, 3.72%; N, 27.54%. Found: C, 56.05%; H, 3.75%; N, 27.55%.

***7-Imino-3-phenylazo-6-(3′-methylphenylazo)-2-methyl-4H-pyrazolo[1,5-a]pyrimidine-5-one (4i).*** Bright dark red solid crystals, **(**75%), mp: 252–254°C; FT-IR ($cm^{-1}$) $\nu_{max}$**:** 3312 (−NH), 3259 (═NH), 3060 (Ar–H), 2921 (Aliphatic C–H),1646 (C═O),1610 (C═N), 1592 (N═N); $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 2.39 (3H, s, Ar–$CH_3$), 2.63 (3H, s, pyrazole–$CH_3$), 7.21 (1H, d, *J* = 7.5 Hz, H-16), 7.39 (1H, t, *J* = 7.6 Hz, H-25), 7.45 (1H, t, *J* = 7.1 Hz, H-17), 7.52 (1H, d, *J* = 7.8 Hz, H-24), 7.57 (1H, d, *J* = 7.8 Hz, H-26), 7.61 (1H, s, H-14), 7.93 (1H, d, *J* = 7.5 Hz, H-18), 7.99 (2H, d, *J* = 7.5 Hz, H-23, H-27), 9.36 (1H, b, pyrimido ═NH), 10.86 (1H,s, pyrimido –NH), 11,79 (1H, b, −OH); $^{13}$C-NMR(100 MHz, DMSO-$d_6$) δ:12.37 ($CH_3$, C-28), 21.02 ($CH_3$, C-29), 101.71 (C, C-4), 118.63 (CH, C-18), 121.78 (CH, C-14), 121.83 (C, C-8), 124.85 (CH, C-23, C-27), 126.54 (CH, C-16), 127.34 (CH, C-24, C-26), 129.37 (CH, C-25), 137.13 (CH, C-17), 139.72 (C, C-15), 140.52 (C, C-3), 144.36 (C, C-9), 150.83 (C, C-22), 158.29 (C, C-13), 160.53 (C, C-7), 194.63 (C, C-5); HR-MS: 386.4223 $[M + H]^+$, calcd 386.4228. *Anal*. Calcd for $C_{20}H_{18}N_8O$: C, 62.17%; H, 4.70%; N, 29.00%. Found: C, 62.20%; H, 4.71%; N, 28.97%.

***7-Imino-3-phenylazo-6-(2′-nitrophenylazo)-2-methyl-4H-pyrazolo[1,5-a]pyrimidine-5-one (4j).*** Burgundy solid crystals, **(**72**%**), mp: 298–299°C; FT–IR ($cm^{-1}$) $\nu_{max}$**:** 3495 (−OH), 3394 (−NH), 3278 (═NH), 3127 (Ar–H), 2920 (Aliphatic C–H), 1643 (C═N), 1579 (N═N), 1507 and 1348 ($-NO_2$); $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 2.61 (3H, s, $-CH_3$), 7.45 (1H, t, *J* = 7.3 Hz, H-25), 7.52–7.60 (3H, m, H-17, H-24, H-26), 7.82 (1H, t, *J* = 7.6 Hz, H-16), 7.90 (1H, d, *J* = 7.8 Hz, H-18), 8.00 (2H, d, *J* = 7.3 Hz, H-23, H-27), 8.09 (1H, d, *J* = 8.0 Hz, H-15), 10.05 (1H, b, pyrimido ═NH), 11.02 (1H, b, pyrimido –NH), 12.08 (1H, b, −OH); $^{13}$C-NMR(100 MHz, DMSO-$d_6$) δ:12.37 ($CH_3$, C-28), 101.71 (C, C-4), 121.83 (C, C-8), 121.96 (CH, C-18), 122.96 (CH, C-16), 124.85 (CH, C-23, C-27), 127.34 (CH, C-24, C-26), 129.08 (CH, C-15), 129.37 (CH, C-25), 133.64 (C, C-14), 136.42 (CH, C-17), 140.52 (C, C-3), 144.36 (C, C-9), 144.58 (C, C-13), 150.83 (C, C-22); 160.53 (C, C-7), 194.63 (C, C-5); HR-MS: 417.3951 $[M + H]^+$, calcd 417.3963. *Anal*. Calcd for $C_{19}H_{15}N_9O_3$: C, 54.68%; H, 3.62%; N, 30.20%. Found: C, 54.68%; H, 3.62%; N, 30.20%.

***7-Imino-3-phenylazo-6-(2′-methoxyphenylazo)-2-methyl-4H-pyrazolo[1,5-a]pyrimidine-5-one (4k).*** Dark burgundy solid crystals, **(**76%), mp: 275–276°C; FT-IR ($cm^{-1}$) $\nu_{max}$**:** 3300 (−NH), 3166 (═NH), 3066 and 3010 (Ar–H), 2942 (Aliphatic C–H), 1636 (C═O), 1580 (C═N), 1523 (N═N); $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 2.63 (3H, s, $CH_3$), 3.96 (3H, s, $-OCH_3$), 7.07 (1H, t, *J* = 7.6 Hz, H-16), 7.25 (1H, d, *J* = 7.5 Hz, H-15), 7.40 (1H, t, *J* = 7.8 Hz, H-25), 7.45 (1H, t, *J* = 7.3 Hz, H-17), 7.54 (2H, t, *J* = 7.5 Hz, H-24, H-26), 7.65 (1H, d, *J* = 7.5 Hz, H-18), 7.99 (2H, d, *J* = 7.5 Hz, H-23, H-27), 9.46 (1H, s, pyrimido ═NH), 11.43 (1H, s, pyrimido –NH), 12.01 (1H, b, −OH); $^{13}$C-NMR(100 MHz, DMSO-$d_6$) δ:12.37 ($CH_3$, C-28), 56.10 ($CH_3$, C-30), 101.71 (C, C-4), 112.58 (CH, C-15), 117.08 (CH, C-18), 121.83 (C, C-8), 121.96 (CH, C-16), 124.68 (CH, C-17), 124.85 (CH, C-23, C-27), 127.34 (CH, C-24, C-26), 129.37 (CH, C-25), 140.52 (C, C-3), 140.60 (C, C-13), 144.36 (C, C-9), 150.73 (C, C-14), 150.83 (C, C-22), 160.53 (C, C-7), 194.63 (C, C-5); HR-MS: 402,4231 $[M + H]^+$, calcd 402.4335. *Anal*. Calcd for $C_{20}H_{18}N_8O_2$: C, 59.69%; H, 4.51%; N, 27.85%. Found: C, 59.67%; H, 4.54%; N, 27.82%.

***7-Imino-3-phenylazo-6-(2′-chlorophenylazo)-2-methyl-4H-pyrazolo[1,5-a]pyrimidine-5-one (4l).*** Bright light brown solid crystals, **(**75%), mp: 300–301°C; FT–IR ($cm^{-1}$)

$\nu_{max}$: 3446 (−OH), 3322 (−NH), 3145 (=NH), 3064 (Ar–H), 2926 (Aliphatic C–H), 1637 (C=N), 1578 (N=N); $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 2.63 (3H, s, $-CH_3$), 7.41 (1H, t, $J$ = 7.5 Hz, H-25), 7.47 (2H, m, H-16, H-17), 7.54 (2H, t, $J$ = 7.6 Hz, H-24, H-26), 7.67 (1H, d, $J$ = 7.8 Hz, H-18), 7.80 (1H, d, $J$ = 7.8 Hz, H-15), 8.01 (2H, d, $J$ = 7.8 Hz, H-23, H-27), 9.91 (1H, s, pyrimido =NH), 11.25 (1H, b, pyrimido –NH), 12.11 (1H, b, −OH); $^{13}$C-NMR(100 MHz, DMSO-$d_6$) δ:12.37 ($CH_3$, C-28), 101.71 (C, C-4), 116.62 (CH, C-18), 117.26 (C, C-14),120.86 (CH, C-16), 121.83 (C, C-8), 124.85 (CH, C-23, C-27), 127.34 (CH, C-24, C-26), 128.17 (CH, C-17), 129.37 (CH, C-25), 130.82 (CH, C-15), 140.52 (C, C-3), 143.35 (C, C-13), 144.36 (C, C-9), 150.83 (C, C-22), 160.53 (C, C-7), 194.63 (C, C-5); HR-MS: 407, 1132 $[M + H]^+$, calcd 407.1137. *Anal*. Calcd for $C_{19}H_{15}ClN_8O$: C, 56.09%; H, 3.72%; N, 27.54%. Found: C, 56.12%; H, 3.73%; N, 27.52%.

***7-Imino-3-phenylazo-6-(2′-methylphenylazo)-2-methyl-4H-pyrazolo[1,5-a]pyrimidine-5-one (4m)***. Bright dark brown solid crystals, (79%), mp: 269–271°C; FT-IR ($cm^{-1}$) $\nu_{max}$: 3415 (−OH), 3294 (−NH), 3146 (=NH), 3063 (Ar–H), 2962 and 2925 (Aliphatic C–H), 1634 (C=N), 1581 (N=N); $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 2.56 (3H, s, Ar–$CH_3$), 2.67 (3H, s, pyrazole–$CH_3$), 7.34 (1H, t, $J$ = 8.0 Hz, H-16), 7.41 (1H, t, $J$ = 7.3 Hz, H-25), 7.45 (1H, d, $J$ = 7.8 Hz, H-15), 7.51 (2H, t, $J$ = 7.5 Hz, H-24, H-26), 7.62 (1H, t, $J$ = 8.0 Hz, H-17), 7.74 (1H, d, $J$ = 7.8 Hz, H-18), 7.96 (2H, d, $J$ = 7.5 Hz, H-23, H-27), 9.57 (1H, s, pyrimido =NH), 10.95 (1H, b, pyrimido –NH), 12.01 (1H, b, −OH); $^{13}$C-NMR(100 MHz, DMSO-$d_6$) δ:12.37 ($CH_3$, C-28), 17.70 ($CH_3$, C-29), 101.71 (C, C-4), 110.51 (CH, C-18), 121.22 (CH, C-16), 121.83 (C, C-8), 124.85 (CH, C-23, C-27), 127.17 (CH, C-17), 127.34 (CH, C-24, C-26), 128.14 (C, C-14), 129.37 (CH, C-25), 132.07 (CH, C-15), 140.52 (C, C-3), 144.36 (C, C-9), 150.14 (C, C-13), 150.83 (C, C-22), 160.53 (C, C-7), 194.63 (C, C-5); HR–MS: 386.4223 $[M + H]^+$, calcd 386.4231. *Anal*. Calcd for $C_{20}H_{18}N_8O$: C, 62.17%; H, 4.70%; N, 29.00%. Found: C, 62.21%; H, 4.72%; N, 28.95%.

**Antimicrobial evaluation.** The compounds were tested against a panel of Gram-positive and Gram-negative bacterial pathogens, fungi, and yeast. Antimicrobial experiments were performed using the agar diffusion method [34,35] with 100 μL 1 × $10^6$ CFU/mL of pathological organisms tested and 1 × $10^6$/mL of yeast spread on nutrient agar and Sabouraud dextrose agar, respectively. After cooling and solidification, the media (10 mm in diameter) were placed in the solidified agar and loaded with a solution of the compound to be tested (100 μL) prepared by dissolving the chemical (100 mg) in DMSO (1 mL). The inoculcated plates were then incubated for 24 h at 37°C for bacteria and 48 h at 28°C for fungi. Negative controls were prepared by using DMSO to dissolve the tested compound. Ciprofloxacin (50 μg/mL) and ketoconazole (50 μg/mL) were used as references for antibacterial and antifungal activities, respectively. Antimicrobial activities were measured as inhibition diameter zones in millimeters (mm). The results are shown in Table 3. The dyes produced significant growth inhibition zones (>14 mm) using the twofold serial dilution technique. Their MICs were measured as well.

**Minimal inhibitory concentration measurement.** The micro dilution susceptibility test in Müueller Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) were used to determine antibacterial and antifungal activities, respectively. Ciprofloxacin and ketoconazole, as well as stock solutions of the tested compounds, were prepared at concentrations of 1000 mg/mL in DMF. Dye solutions were prepared by using the proper nutrient broth twice, and the final solution concentrations were 132, 66, 33, 16.5, and 8.25 μg/mL. Test tubes were prepared with the target bacteria grown in appropriate broths for 24 h at 37°C (about 1 × $10^6$ CFU/mL). Each 5 mL of broth received 0.1 mL of the previous inoculum. The samples were then incubated for 24 h at 37°C. The lowest concentration that showed no growth was taken to be the MIC. Control experiments with DMF and uninoculated media were run parallel and in the same conditions as the test compounds. The MICs (mg/mL) and inhibition zone diameters are shown in Table 4.

**DPPH radical scavenging activity.** The antioxidant activities of the test compounds were determined using a previously published diphenylpicrylhydrazyl (DPPH) radical scavenging method [36]. Various concentrations of the test compounds (10–1000 μg/mL) in methanol were mixed with a methanolic DPPH solution (1 mM) to produce final volume of 2.0 mL. The mixtures were incubated at room temperature in the dark, and their absorbances were recorded at 517 nm. A control was tested under the same conditions without a test compound, and ascorbic acid was used as a reference. The experiment was performed in triplicate, and the percentage of radicals scavenged was determined as follows;

$$[(\text{Absorbance of blank} - \text{Absorbance of test})/\text{Absorbance of blank}] \times 100.$$

The radical-scavenging activities of the compounds were presented as IC50s. The IC50 is the concentration in micrometer required to produce a 50% decrease in absorbance by DPPH radicals.

**Acknowledgments.** The authors are grateful to the Scientific Research Projects Council of Pamukkale University (PAU. BAP, 2012FBE066 and Kastamonu University).

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## SUPPORTING INFORMATION

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