

Synthesis, characterization, and biological activities of 4-imino-3-arylazo-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-2-oles

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Abstract 4-Imino-3,4-dihydro-2*H*-pyrimido[2,1-*b*][1,3]benzothiazole-2-one (**3**) was synthesized by the reaction of 2-aminobenzothiazole with ethyl cyanoacetate in solvent free conditions at 150 °C. A series of pyrimido benzothiazole-based azo dyes **4(a–m)** were obtained by the coupling of carbocyclic amine-based diazonium chloride with compound (**3**). The synthesized dyes were purified and characterized by elemental analysis, FT-IR, ¹H NMR, and high-resolution mass spectral data. The solvatochromic behaviors of dyes in various solvents were examined. All the azo dyes exhibited pronounced in vitro antibacterial activities against Gram-positive and Gram-negative bacteria, as well as fungi. The results revealed that most of the compounds exhibited good levels of antibacterial activity. Compounds **4d** and **4h** showed excellent levels of antimicrobial activity with MIC values of 8.25 µg/mL.

Keywords Solvatochromism · Pyrimido benzothiazole · Diazo-coupling reaction · Azo dyes · Antimicrobial activity

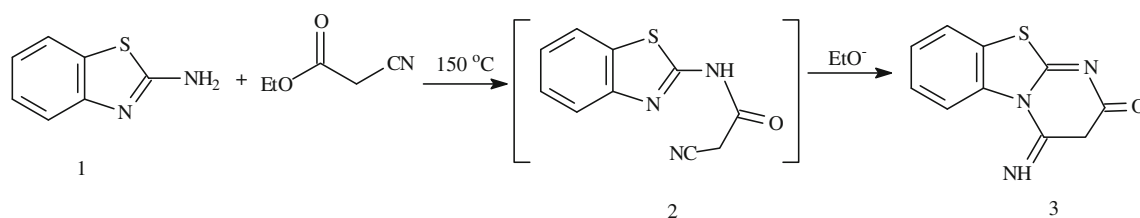
Introduction

Fused heterocyclic compounds are very important compounds partially because of their pharmacological properties which include wide applications in medicinal chemistry (Ellis, 2008). Pyrimido benzothiazoles are very important class of heterocyclic because of their wide range of biological pharmacological properties. Some of which have antiviral, antitumor, anti-inflammatory, antihypertensive, and anti-allergy (Kappe, 1993; Atwal *et al.*, 1989, 1991; Rovnyak *et al.*, 1992; El-Sherbeny, 2000; Glennon *et al.*, 1981; Bartovic *et al.*, 1995). Pyrimido benzothiazole derivatives have also been known for their antimicrobial activities (Wade *et al.*, 1983; Alaimo, 1973; Gupta and Rawat, 2010; Lanjewar *et al.*, 2009; Heravi *et al.*, 2008; Chang *et al.*, 2008; Shah *et al.*, 2009; Kumar *et al.*, 2009; Chaitanya *et al.*, 2010; Sahu *et al.*, 2012). As a continuation of our previous work (Karcı *et al.*, 2006, 2009; Öztürk *et al.*, 2012; Şener *et al.*, 2006; Yavuz and Yildirim, 2013). 4-imino-3,4-dihydro-2*H*-pyrimido[2,1-*b*][1,3]benzothiazole-2-one have been reported in the literature, by the cyclocondensation of benzothiazole with ethyl cyanoacetate in the presence of sodium ethoxide/ethanol, or phosphoric acid (Tadakazu, 1991). We wish to give new report the synthesis of 4-imino-3,4-dihydro-2*H*-pyrimido[2,1-*b*][1,3]benzothiazole-2-one by the reaction of 2-aminobenzothiazole with ethyl cyanoacetate in solvent free conditions at 150 °C. Although a number of paper have been published concerning the synthesis of pyrimidine and azopyrimidine derivatives, those containing an azo pyrimidobenzothiazole system have not yet been reported. In the present study, we reported the synthesis of 4-imino-3,4-dihydro-2*H*-pyrimido[2,1-*b*][1,3]benzothiazole-2-one and some 4-imino-3-arylazo-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-2-ol derivatives. The synthesized azo dyes were evaluated biological and antimicrobial activities.

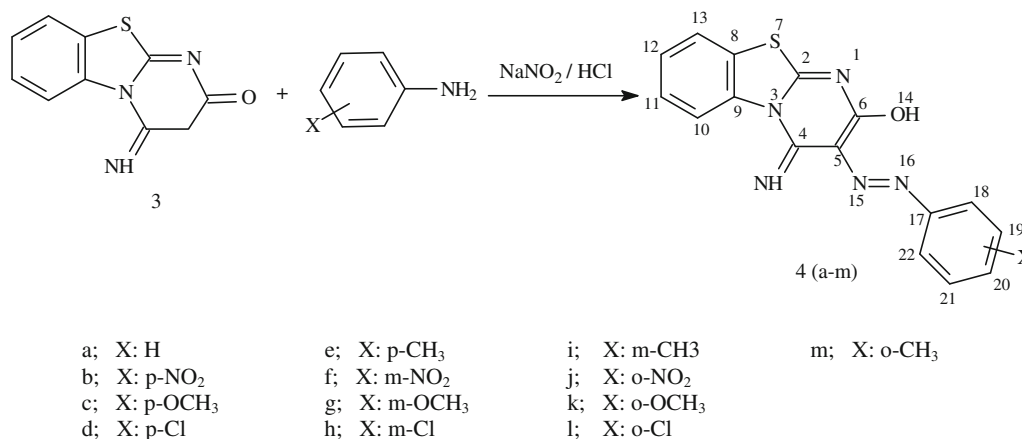
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Scheme 1 Synthesis of compound (3)



Scheme 2 Synthesis of compounds 4(a–m)

Results and discussion

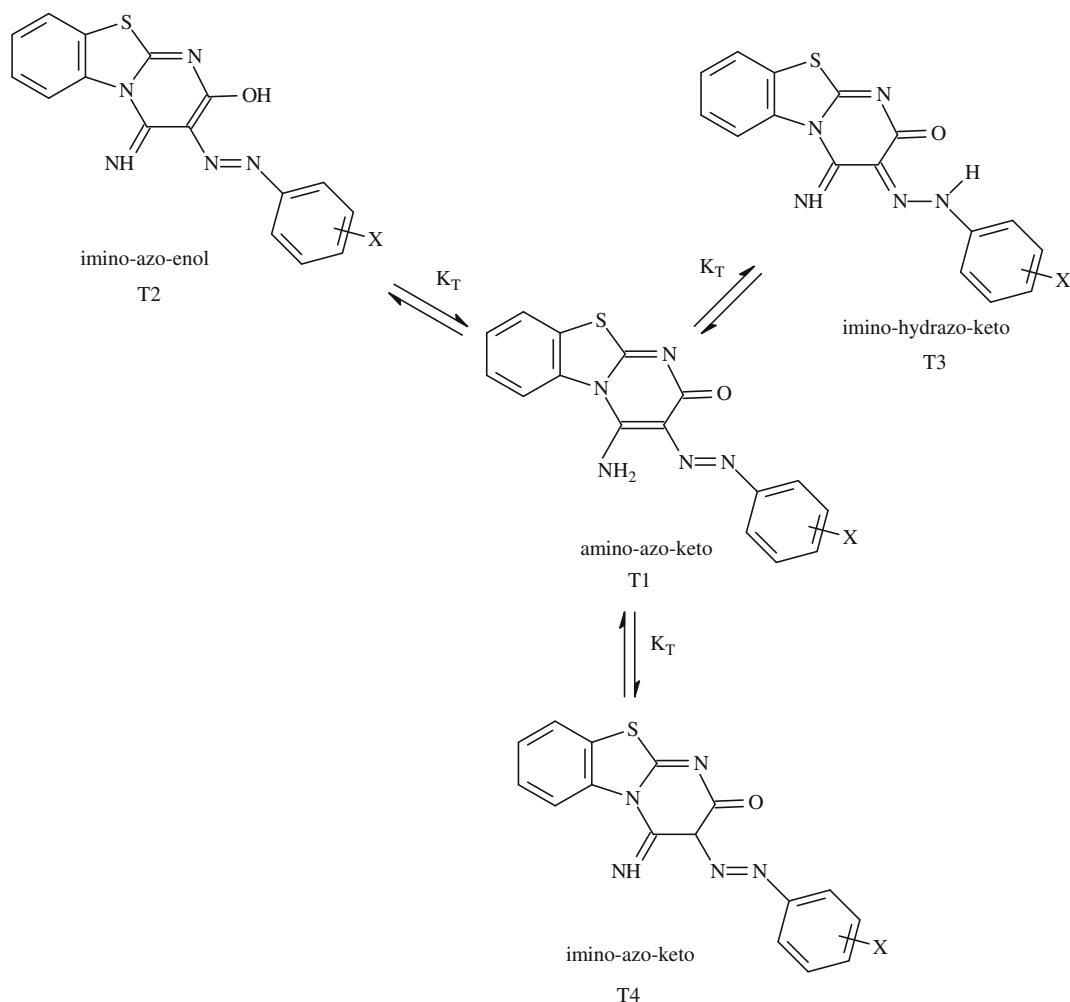
Chemistry

In this study, 4-imino-3-arylazo-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-2-ol derivatives have been reported. The structures of the synthesized dyes were determined by analysis of their FT-IR, NMR, high-resolution mass spectra for the molecular weights and elemental analysis. As shown in Scheme 1, firstly, 4-imino-3,4-dihydro-2*H*-pyrimido[2,1-*b*][1,3]benzothiazole-2-one was prepared by the reaction of 2-aminobenzothiazole with ethyl cyanoacetate in solvent free conditions at 150 °C.

Secondly, aniline derivatives were diazotized using sodium nitrite in hydrochloric acid in ice bath at between 0 and 5 °C. The synthesized carbocyclic diazonium salts were then coupled with 4-imino-3,4-dihydro-2*H*-pyrimido[2,1-*b*][1,3]benzothiazole-2-one to give corresponding 4-imino-3-arylazo-4*H*-pyrimido[2,1-*b*][1,3]benzothiazole-2-ol derivatives. This reaction route is depicted in Scheme 2. By the purification of the reaction mixture, the thirteen novel azo pyrimido[2,1-*b*][1,3]benzothiazole derivatives have been obtained.

The obtained product 4(a–m) can exist in possible four tautomeric forms namely amino-azo-keto form **T1**, imino-azo-enol form **T2**, imino-hydrazo-keto form **T3**, imino-azo-keto form **T4** as shown in Scheme 3. The FT-IR

spectra of synthesized compound (3) showed an intense (–OH) band at 3375 cm^{–1}, and a band (=NH) located at 3167–3133 cm^{–1}. The other band at 1697 cm^{–1} assigned to (C=N). FT-IR spectra of compound (3) did not appear any band for (C=O) group. The ¹H NMR spectra of compounds (3) exhibited singlet signal at δ 4.15 ppm attributed to (–CH₂) protons and broad peak at δ 12.75 ppm, which was attributed to (=NH) proton. The IR spectra of dyes 4(a–m) showed characteristic hydroxyl (–OH) bands at 3211–3385 cm^{–1}, but did not showed any bands for carbonyl (C=O) group. We can suggest that these compounds were only in imino-azo-enol form (**T2**) in solid state. ¹H NMR spectra of synthesized azo pyrimido[2,1-*b*][1,3]benzothiazole derivatives 4(a–d) exhibit a broad peak at 12.01–12.28 ppm, which was attributed to =NH protons, a broad peak at 12.58–13.25 ppm which was attributed hydrazo (–NH) protons and showed a broad peak at 13.50–14.22 ppm attributed to tautomeric hydroxyl (–OH). ¹H NMR spectra of dyes did not showed –NH₂ protons except for 4j. ¹H NMR spectra of 4g, 4k, 4l, and 4m showed only =NH proton, but did not showed hydrazo –NH protons. ¹H NMR spectra of 4e, 4f, 4h, and 4i showed only =NH proton, and hydrazo –NH protons. ¹H NMR spectra of 4j showed only –NH₂ protons. According to the ¹H NMR results, suggest that dyes 4(a–d) a mixture of predominantly in imino-azo-enol form (**T2**) and imino-hydrazo-keto form (**T3**), the dyes of 4e, 4f, 4h, and 4i have



Scheme 3 The tautomeric form of azo pyrimido[2,1-*b*][1,3] benzothiazole derivatives

only in imino-hydrazo-keto form (**T3**), the dyes of **4g**, **4k**, **4l**, and **4m** have only in imino-azo-enol form (**T2**). The dye of **4j** has in amino-azo-keto (**T1**). All the synthesized dyes did not have in imino-azo-keto (**T4**) tautomeric forms in DMSO-*d*₆ as showed in Scheme 3. The mass spectra of dyes have molecular ion peak which corresponds to molecular weight of respective compounds.

Absorption spectra

The UV–Vis absorption spectra of the dyes **4(a–m)** were recorded over the range of λ between 350 and 700 nm, using variety of solvents in concentration 10^{-6} to 10^{-8} M. The spectral data of the synthesized dyes are depicted in Table 1.

The visible absorption spectra of the dyes did not correlate with the polarity of solvent. The dyes showed color ranging from dark red (λ_{\max} 357 nm) to yellow (λ_{\max} 592 nm). It was observed that λ_{\max} of *p*-substitute dyes **4(a,c,d)** shifted bathochromically in chloroform with

respect to the λ_{\max} in dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF) except for **4b**. For example, for the dye **4a** λ_{\max} is 385 nm in DMSO, 394 nm in chloroform. For the dye **4d** λ_{\max} is 391 nm in DMSO 396 nm in chloroform. The explanation for this irregular behavior may be due to the presence of nonbonding electron pairs of carbonyl, oxygen, and nitrogen atoms in the molecule ring (Harikrishnan and Menon, 2008). General observation made is that most of the substituted dyes have higher absorption maxima when compared to their unsubstituted analogs. The absorption spectra of dye **4j** showed two absorbance in all solvent except for acetic acid. The other spectra of the dyes showed single absorption peak with a shoulder or two absorption peak with a shoulder. It can be suggested that all dyes have a mixture of tautomer forms in various solvent. The spectral shifts of the dye **4b** in various solvents are shown in Fig. 1.

The effects of the acid and base on the absorption spectra of the dyes were investigated and these results are given in Table 2.

Table 1 Influence of solvent on λ_{\max} (nm) of dyes

Dye no.	DMSO	DMF	Acetonitrile	Methanol	Acetic acid	Chloroform
4a	385,325 ^a	387	403	409,331 ^a	392	394
4b	417,592,332 ^a	420,590	419,584 ^a	422,334 ^a	411,333 ^a	414,552 ^a
4c	403,319 ^a	401	416,341 ^a	426,338 ^a	408,325 ^a	405
4d	391,320 ^a	393,496 ^a	404,330 ^a	413,336 ^a	396,323 ^a	396
4e	389,328 ^a	395	385	398,335 ^a	381,320 ^a	385
4f	392,322 ^a	393	387	389,330 ^a	383,322 ^a	387
4g	386,320 ^a	391	380	390,328 ^a	378,319 ^a ,478 ^a	380
4h	399,333 ^a ,487 ^a	396,491 ^a	403,485 ^a	407,332 ^a	392,325	394
4i	387,320 ^a	390	381	386,330 ^a	378,318 ^a	382
4j	365,425	363,416	365,422	359,420	409,345 ^a	357,414
4k	416,336 ^a	413	420	420,338	410,328 ^a	414
4l	405,327 ^a	405,491 ^a	405	405,334 ^a	389,328 ^a	401
4m	408,330 ^a	407	411	414,341 ^a	400,324 ^a	405

^a Shoulder

The absorption spectra of the dyes were sensitive in methanol to the addition of acid and base. With the exception of dyes **4c**, **4k** and **4m**, when small amount of potassium hydroxide (0.1 M) was added to methanol solution, the λ_{\max} values showed bathochromic shift. When hydrochloric acid (0.1 M) was added in methanol hypsochromic shifts were detected except for **4j**. The spectral shifts of **4b** in acidic and basic solution are shown in Fig. 2.

Antimicrobial activity

In the present study, a total of thirteen diazo derivatives **4(a–m)** was evaluated for their in vitro antibacterial and antifungal activities at 100 $\mu\text{g/mL}$ concentration against microbial strains such as two Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* ATCC 6633), two Gram-negative bacteria (*Klebsiella pneumonia* ATCC13883 and *Escherichia coli* ATCC 25922), and two fungi (*Saccharomyces cerevisiae* and *Candida albicans* NRRL Y-477). Agar-diffusion method was used for the determination of the preliminary antibacterial and antifungal activity. Ciprofloxacin and Ketoconazole were used as standard antibacterial and antifungal reference, respectively. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the disks in mm. Most of the newly synthesized compounds showed excellent antimicrobial activities with respect to the control drugs. The results in Table 3 revealed that the majority of the synthesized compounds showed variable inhibition activities against the tested strains. Data in Table 3 revealed that the most of tested compounds displayed

variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains, and also against antifungal strains. In general, the results revealed that para phenyl substituted compounds exhibited better activities than *ortho* and *meta* phenyl substituted compounds. Furthermore, compound **4d** having chlorine substituent at *para* and **4h** *meta* positions of the phenyl ring exhibited potent antimicrobial activities against Gram-positive and Gram-negative bacteria, as well as fungi. Most of pyrimido benzothiazole derivatives have superior significant antibacterial potency than antifungal potency. One of the tested compound **4d** exhibited good antimicrobial activity against all tested organisms with respect to reference drugs. Compound **4d** inhibited the growth of *S. aureus* ATCC 29213, *B. subtilis* ATCC6633, and *C. albicans* NRRL Y-477 with IZs 30, 32, and 31 mm, respectively. Also compound **4h** showed excellent activity against *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 with IZ 31 and 30 mm, respectively.

The minimum inhibitory concentration (MIC) of the synthesized compounds against highly inhibited organisms is reported in Table 4. Compound **4d** exhibited low MIC (8.25 $\mu\text{g/mL}$) against *S. aureus* ATCC 29213, *B. subtilis* ATCC 6633, *K. pneumonia* ATCC13883, *S. cerevisiae*, and *C. albicans* NRRL Y-477. In addition, compound **4h** showed MIC 8.25 $\mu\text{g/mL}$ against *S. aureus* ATCC 29213, *K. pneumonia* ATCC13883, *E. coli* ATCC 25922, *S. cerevisiae*, and *C. albicans* NRRL Y-477. Additionally, compounds **4f** and **4l** exhibited MIC 8.25 $\mu\text{g/mL}$ against *S. aureus* ATCC 29213 and also compounds **4b** and **4j** showed good inhibitory activities against *B. subtilis* ATCC 6633 (MIC 8.25 $\mu\text{g/mL}$).

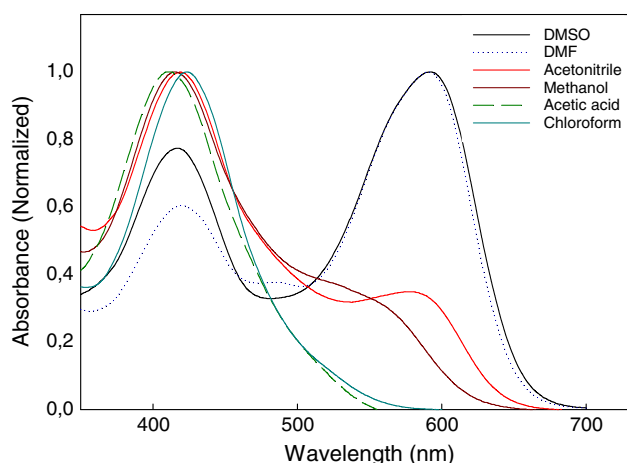


Fig. 1 Absorption spectra of dye **4b** in various solvents

Table 2 Absorption maxima of dyes in acidic and basic solutions

Dye no.	Methanol	Methanol + KOH	Methanol + HCl
4a	409,331 ^a	410	392
4b	422,334 ^a	408 ^a , 542	415
4c	426,338 ^a	413	415
4d	413,336 ^a	430	396
4e	398, 335 ^a	406	388
4f	389,330 ^a	410	361 ^a , 386
4g	390,328 ^a	416	381
4h	407,332 ^a	445, 498 ^a	391
4i	386,330 ^a	406	385
4j	359,420	365 ^a , 466	360 ^a , 418
4k	420,338	411	416
4l	405,334 ^a	424	404
4m	414,341 ^a	402	408

^a Shoulder

Experimental

General

All chemicals were purchased and were used without further purification. Solvents were of spectroscopic grade. Melting points of the synthesis dyes were determined using Stuart smp 30 melting point apparatus (UK). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker (Germany) Spectrospin Avance DPX 400 Ultra-Shield spectrometer at room temperature by using tetramethylsilane (TMS) as the internal standard. Chemical shifts were (δ) given in ppm. FT-IR spectra were recorded on a Perkin Elmer (USA) spectrometer. MS analyses were recorded on from Waters LCT Premier XE LTOF (TOF

MS) instruments (Waters Corporation, Milford MA, USA). Elemental analyses were done on a Leco CHNS-932 analyzer (USA). UV-Vis absorption spectra were recorded on an ATI (UK) Unicam UV-100 spectrophotometer over the range of λ between 300 and 700 nm. The wavelengths of maximum absorption (λ_{\max}) were investigated in various solvents such as DMSO, DMF, acetonitrile, methanol, acetic acid, and chloroform at various concentrations (1×10^{-6} M). Change of (λ_{\max}) was also investigated when 0.1 mL of base (potassium hydroxide, 0.1 M) or 0.1 mL of acid (hydrochloric acid, 0.1 M) was added to 1 mL of the dye solution in methanol.

Synthesis

Preparation of 4-imino-3,4-dihydro-2H-pyrimido[2,1-b][1,3]benzothiazole-2-one (**3**)

The starting compounds were synthesized by the reaction of 2-aminobenzothiazole with ethyl cyanoacetate. 2-aminobenzothiazole 0.2 g (1.33 mmol) reacted with ethyl cyanoacetate 0.7 mL (6.65 mmol) in solvent free conditions. The reaction mixture was heated at 150 °C for 2 h, and then cooled at room temperature and upon dilution with 100 mL ethanol:water mixture (1:1 by volume). The precipitated yellow products separated were filtered off, washed with water several times, and dried. The obtained product was crystallized from DMF:water mixture as yellow crystals, yield (90 %), mp: 236–238 °C; IR (cm^{-1}) ν_{\max} : 3375 (OH), 3167–3133 (=NH), 3055 (Ar-H), 2944 (Aliphatic C-H), 1697 (C=N); ¹H NMR (DMSO-*d*₆) δ (ppm): 4.15 (s, 2H, CH₂), 7.31 (d, 1H, *J* = 7.47 Hz, H-10), 7.45 (1H, *J* = 7.47 Hz, H-13), 7.78 (1H, t, *J* = 7.47 Hz, H-11), 8.05 (1H, t, *J* = 7.47, H-12), 12.75 (b, 1H, pyrimidone =NH); ¹³C NMR (DMSO-*d*₆) δ : 40.09 (CH₂, C-5), 110.49 (CH, C-10), 122.05 (CH, C-12), 122.68 (CH, C-13), 124.8 (C, C-8), 127.11 (CH, C-11), 147.6 (C, C-2), 147.82 (C, C-9), 166.9 (C, C-6), 166.96 (C, C-2); HR-MS: 217.2482 [M+H]⁺, calcd. 217.2485. Anal. Calcd. for C₁₀H₇N₃OS: C, 55.2 %; H, 3.25 %; N, 19.34 %. Found: C, 55.28 %; H, 3.26 %; N, 19.34 %.

General procedure for the synthesis of azo dyes **4(a–m)**

Preparation of 4-imino-3-phenylazo-4H-pyrimido[2,1-b][1,3]benzothiazole-2-ole (**4a**)

Aniline 0.93 g (10 mmol) was dissolved in hydrochloric acid (7.5 mL) and water (15 mL) was added. The solution was cooled to 0–5 °C. Sodium nitrite 0.69 g (10 mmol) in water (10 mL) was then added to this cold solution drop

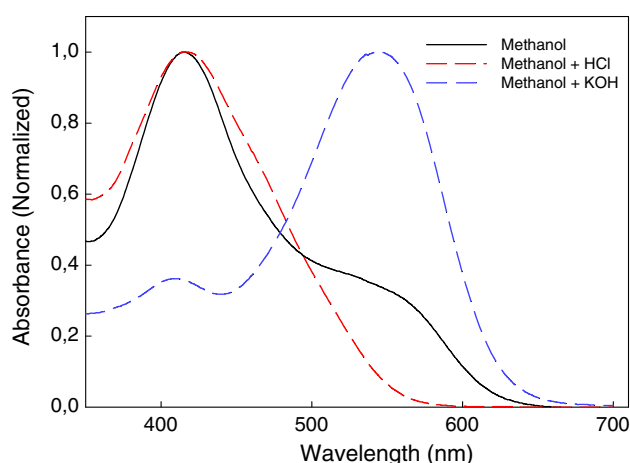


Fig. 2 Absorption spectra of dye **4b** in acidic and basic solutions

wise with vigorous stirring over 1 h. Excess nitrous acid was destroyed by the addition of the urea. The resulting diazonium solution was then added in portion over 30 min. to a vigorously stirred solution of 4-imino-3,4-dihydro-2*H*-pyrimido [2,1-*b*][1,3]benzothiazole-2-one 2.17 g (10 mmol), which was dissolved in potassium hydroxide (20 mmol) and water (20 mL). The solution was stirred at 0–5 °C for 2 h and the pH of the reaction mixture was maintained at 6–7 by the simultaneous addition of sodium acetate solutions. The precipitated product separated upon dilution with water (50 mL) was filtered, washed with water several times, dried and crystallized from DMF:water (2:3 by volume) to give 4-imino-3-phenylazo-4*H*-pyrimido[2.1-*b*][1,3]benzothiazole-2-ole as a yellow crystals, yield (88 %), mp: 249–250 °C. IR (cm⁻¹) ν_{\max} : 3385 (–OH), 3167–3132 (=NH), 3039 (Ar–H), 2924 (Aliphatic C–H), 1641 (C=N), 1510, 1453 (N=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 7.16 (1H, t, *J* = 7.24 Hz, H-20), 7.45 (2H, t, *J* = 7.71 Hz, H-19, H-21), 7.51 (2H, *J* = 6.95 Hz, H-11, H-12), 7.51 (1H, d, *J* = 7.45 Hz, H-10), 7.57 (1H, d, *J* = 7.43 Hz, H-13), 8.03 (2H, d, *J* = 9.24 Hz, H-18, H-22), 12.25 (1H, b, =NH), 12.72 (1H, b, hydrazo-NH), 14.22 (s, tautomeric –OH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ : 104.90 (CH, C-5), 108.71 (CH– C-10), 112.19 (CH, C-18, C-22), 118.22 (CH, C-20), 122.20 (CH, C-12), 123.27 (C, C-8), 125.18 (CH, C-13), 127.46 (CH, C-11), 129.97 (CH, C-19, C-21), 131.57 (C, C-4), 151.72 (C, C-6), 152.37 (C, C-9), 153.59 (C, C-17), 168 (C, C-2); HR-MS: 321.3576 [M+H]⁺, calcd. 321.3577. Anal. Calcd. for C₁₅H₁₁N₃OS: C, 59.80 %; H, 3.45 %; N, 21.79 %; Found: C, 59.81 %; H, 3.46 %; N, 21.78 %.

The above procedure was also used to synthesize dye **4(b–g)**. The general route of synthesized dyes is outlined in Scheme 1.

Preparation of 4-imino-3-(4'-nitrophenylazo)-4*H*-pyrimido[2.1-*b*][1,3]benzothiazole-2-ole (**4b**)

Brown solid crystals, (82 %), mp: 232–234 °C. IR (cm⁻¹) ν_{\max} : 3211 (–OH), 3104 (=NH), 3088 (Ar–H), 2987 (Aliphatic C–H), 1661 (C=N), 1598–1455 (N=N), 1514, 1336 (NO₂); ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 7.37 (1H, t, *J* = 8.1 Hz, H-12), 7.54 (1H, t, *J* = 8.1 Hz, H-11), 7.62 (1H, d, *J* = 8.74 Hz, H-13), 7.70 (1H, d, *J* = 8.75 Hz, H-10), 8.01 (1H, d, *J* = 7.24 Hz, H-22), 8.04 (1H, d, *J* = 7.24 Hz, H-18), 8.30 (2H, d, *J* = 8.74 Hz, H-19, H-21), 12.01 (1H, b, =NH), 13.25 (1H, b, hydrazo-NH), 14.13 (s, tautomeric –OH). ¹³C NMR (100 MHz, DMSO-*d*₆) δ : 104.90 (C, C-5), 108.71 (CH, C-10), 122.2 (CH, C-12), 122.9 (CH, C-18, C-22), 123.27 (C, C-8), 125.18 (CH, C-13), 126.01 (CH, C-21), 127.46 (CH, C-11), 131.57 (C, C-4), 149.65 (C, C-20), 151.72 (C, C-6), 152.37 (C, C-9), 155.28 (C, C-17), 168 (C, C-2); HR-MS: 366.3552 [M+H]⁺, calcd. 366.3553. Anal. Calcd. For C₁₆H₁₀N₆O₃S: C, 52.45 %; H, 2.75 %; N, 22.94 %; Found: C, 52.46 %; H, 2.76 %; N, 22.93 %.

Preparation of 4-imino-3-(4'-methoxyphenylazo)-4*H*-pyrimido[2.1-*b*][1,3]benzothiazole-2-ole (**4c**)

Orange solid crystals, (79 %), mp: 216–218 °C. IR (cm⁻¹) ν_{\max} : 3251 (–OH), 3128 (=NH), 3028 (Ar–H), 2995 (Aliphatic C–H), 1637 (C=N), 1509–1455 (N=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 3.79 (3H, s, –OCH₃), 7.35 (1H, t, *J* = 7.79 Hz, H-12); 7.39 (1H, t, *J* = 7.79 Hz, H-11); 7.47 (1H, d, *J* = 8.27 Hz, H-13); 7.64 (1H, d, *J* = 7.79 Hz, H-10), 7.78 (1H, d, *J* = 7.79 Hz, H-21), 7.87 (2H, d, *J* = 7.79 Hz, H-18, H-22), 8.03 (1H, d, *J* = 7.79 Hz, H-19), 12.20 (1H, b, =NH), 12.58 (1H, b, hydrazo-NH), 14.06 (s, tautomeric –OH). ¹³C NMR (100 MHz, DMSO-*d*₆) δ : 55.46 (CH₃, C-25), 104.90 (C, C-5), 108.71 (CH– C-10), 115.31 (CH, C-19, C-21), 122.2 (CH, C-12), 123.27 (C, C-8), 124.16 (CH, C-18, C-22), 125.18 (CH, C-13), 127.46 (CH, C-11), 131.57 (C, C-4), 147.46 (C, C-17), 151.72 (C, C-6), 152.37 (C, C-9), 160.04 (C, C-20), 168 (C, C-2); HR-MS: 351.3836 [M+H]⁺, calcd. 351.3837. Anal. Calcd. for C₁₇H₁₃N₅O₂S: C, 58.11 %; H, 3.73 %; N, 19.93 %; Found: C, 58.12 %; H, 3.74 %; N, 19.92 %.

Preparation of 4-imino-3-(4'-chlorophenylazo)-4*H*-pyrimido[2.1-*b*][1,3]benzothiazole-2-ole (**4d**)

Dark orange solid crystals, (81 %), mp: 248–250 °C. IR (cm⁻¹) ν_{\max} : 3260 (–OH), 3186 (=NH), 3062 (Ar–H), 2940 (Aliphatic C–H), 1637 (C=N), 1495–1454 (N=N); ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 7.41 (1H, t, *J* = 7.47 Hz, H-12), 7.54 (1H, t, *J* = 7.47 Hz, H-11), 7.63 (1H, d,

Table 3 Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm) of chemical compounds against the pathological strains based on well-diffusion assay

Compounds	Zone of inhibition (mm)					
	Bacteria				Fungi	
	Gram-positive		Gram-negative		<i>S. cerevisiae</i>	<i>C. albicans</i>
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>K. pneumoniae</i>	<i>E. coli</i>		
4a	22 ± 0.5	19 ± 1.7	16 ± 1.1	18 ± 0.7	14 ± 1.2	15 ± 1.6
4b	28 ± 1.8	32 ± 1.3	26 ± 0.6	16 ± 0.9	14 ± 1.4	18 ± 1.4
4c	22 ± 1.2	22 ± 0.8	25 ± 1.3	28 ± 2.0	16 ± 1.1	16 ± 1.2
4d	30 ± 1.6	32 ± 0.9	29 ± 0.8	28 ± 1.9	29 ± 0.3	31 ± 0.3
4e	24 ± 0.7	22 ± 1.5	24 ± 1.6	20 ± 1.3	16 ± 0.6	15 ± 2.1
4f	32 ± 0.9	28 ± 1.4	24 ± 1.3	25 ± 1.5	21 ± 1.1	19 ± 1.4
4g	25 ± 2.1	27 ± 1.5	30 ± 0.9	31 ± 1.7	24 ± 1.4	28 ± 1.6
4h	31 ± 0.4	28 ± 0.9	29 ± 0.8	30 ± 1.8	23 ± 1.2	29 ± 0.8
4i	18 ± 0.7	16 ± 0.3	20 ± 1.6	19 ± 0.8	12 ± 0.5	13 ± 0.7
4j	28 ± 1.2	30 ± 1.3	24 ± 1.4	22 ± 1.3	18 ± 0.7	18 ± 1.5
4k	22 ± 1.6	24 ± 2.4	24 ± 1.2	26 ± 0.6	29 ± 1.2	28 ± 0.6
4l	30 ± 1.0	24 ± 1.8	18 ± 0.5	22 ± 1.4	19 ± 1.6	23 ± 1.2
4m	19 ± 1.4	18 ± 1.4	12 ± 1.7	12 ± 1.1	12 ± 1.4	14 ± 2.2
Ciprofloxacin	28 ± 1.2	29 ± 0.6	29 ± 1.3	24 ± 0.7	NT	NT
Ketoconazole	NT	NT	NT	NT	28 ± 1.2	28 ± 1.4

Zone of inhibition values are presented as the mean ± SEM from at least three separated experiments

NT not tested

$J = 7.47$ Hz, H-13), 7.65 (1H, d, $J = 7.47$ Hz, H-10), 7.85 (1H, d, $J = 8.18$ Hz, H-21), 8.97 (2H, d, $J = 7.44$ Hz, H-22, H-18), 8.02 (1H, $J = 7.43$ Hz, H-19), 12.28 (1H, b, =NH), 12.79 (1H, b, hydrazo-NH), 13.50 (b, tautomeric -OH); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 104.90 (C, C-5), 108.71 (CH-C-10), 122.2 (CH, C-12), 123.27 (C, C-8), 123.75 (CH, C-18, C-22), 125.18 (CH, C-13), 127.46 (CH, C-11), 130.4 (CH, C-21, C-19), 131.57 (C, C-4), 133.63 (C, C-20), 151.72 (C, C-6), 152.28 (C, C-17), 152.37 (C, C-9), 168 (C, C-2); HR-MS: 355.8024 [M+H] $^+$, calcd. 355.8023. Anal. Calcd. For $\text{C}_{16}\text{H}_{10}\text{ClN}_5\text{OS}$: C, 54.01 %; H, 2.83 %; N, 19.68 %; Found: C, 54.01 %; H, 2.84 %; N, 19.67 %.

Preparation of 4-imino-3-(4'-methylphenylazo)-4H-pyrimido[2.1-b][1,3]benzothiazole-2-ole (4e)

Orange solid crystals, (83 %), mp: 251–252 °C. IR (cm^{-1}) ν_{max} : 3285 (-OH), 3168 (=NH), 3055 (Ar-H), 2930 (Aliphatic C-H), 1683 (C=N), 1428–1487 (N=N); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.32 (3H, s, -CH $_3$), 7.27 (2H, d, $J = 7.08$ Hz, H-19, H-21), 7.35 (1H, t, $J = 7.08$ Hz, H-12), 7.57 (1H, t, $J = 7.09$ Hz, H-11), 7.79 (1H, d, $J = 7.08$ Hz, H-13), 7.81 (1H, d, $J = 7.09$ Hz, H-10), 7.97 (1H, d, $J = 7.09$ Hz, H-22), 8.03 (1H, d, $J = 7.09$ Hz, H-18), 12.18 (1H, b, =NH), 12.62 (1H, b,

hydrazo-NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 20.65 (CH $_3$, C-25), 108.98 (CH, C-10), 117.05 (CH-C-18, C-22), 123.18 (CH, C-12), 123.49 (CH, C-13), 124.3 (C, C-8), 127.92 (CH, C-11), 130.51 (CH, C-19, C-21), 133.88 (C, C-4), 136.95 (C, C-20), 140.04 (C, C-17), 141.96 (C, C-5), 147.11 (C, C-9), 153.35 (C, C-6), 168.9 (C, C-2); HR-MS: 335.3842 [M+H] $^+$, calcd. 335.3841. Anal. Calcd. for $\text{C}_{17}\text{H}_{13}\text{N}_5\text{OS}$: C, 60.88 %; H, 3.91 %; N, 20.88 %; Found: C, 60.89 %; H, 3.92 %; N, 20.87 %.

Preparation of 4-imino-3-(3'-nitrophenylazo)-4H-pyrimido[2.1-b][1,3]benzothiazole-2-ole (4f)

Burgundy red solid crystals, (79 %), mp: 250–251 °C. IR (cm^{-1}) ν_{max} : 3290 (-OH), 3160 (=NH), 3065 (Ar-H), 2985 (Aliphatic C-H), 1665 (C=N), 1520–1455 (N=N), 1510, 1332 (NO $_2$); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 7.23 (1H, t, $J = 6.52$ Hz, H-21), 7.35 (1H, t, $J = 7.73$ Hz, H-12), 7.39 (1H, t, $J = 7.73$ Hz, H-11), 7.47 (1H, d, $J = 7.32$ Hz, H-13), 7.79 (1H, d, $J = 7.33$ Hz, H-10), 7.81 (1H, d, $J = 7.33$ Hz, H-22), 7.97 (1H, d, $J = 7.33$ Hz, H-20), 8.03 (1H, s, H-18), 12.18 (1H, b, =NH), 12.63 (1H, b, hydrazo-NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 109.98 (CH, C-10), 113.73 (CH, C-22), 115.14 (CH, C-20), 120.6 (CH, C-18), 123.18 (CH, C-12), 123.48 (CH, C-13),

Table 4 MIC ($\mu\text{g/mL}$) against the pathological strains based on two fold serial dilution technique

Compounds	MIC in $\mu\text{g/mL}$					
	Bacteria				Fungi	
	Gram-positive		Gram-negative		<i>S. cerevisiae</i>	<i>C. albicans</i>
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>K. pneumoniae</i>	<i>E. coli</i>		
4a	33	66	132	66	132	132
4b	16.5	8.25	33	132	132	66
4c	33	33	33	16.5	132	132
4d	8.25	8.25	8.25	16.5	8.25	8.25
4e	33	66	33	66	132	132
4f	8.25	16.5	33	33	66	66
4g	33	16.5	8.25	8.25	33	16.5
4h	8.25	16.5	8.25	8.25	33	8.25
4i	66	132	66	66	132	132
4j	16.5	8.25	33	33	132	132
4k	33	33	33	16.5	16.5	16.5
4l	8.25	33	66	33	66	33
4m	66	66	132	132	132	132
Ciprofloxacin	8.25	8.25	8.25	16.5	NT	NT
Ketoconazole	NT	NT	NT	NT	8.25	16.5

NT not tested

124.3 (C, C-8), 127.92 (CH, C-11), 130.42 (CH, C-19), 133.88 (C, C-4), 141.96 (C, C-5), 146.37 (C, C-17), 147.11 (C, C-9), 149.6 (C, C-21), 153.35 (C, C-6), 168.9 (C, C-2); HR-MS: 366.3552 $[\text{M}+\text{H}]^+$, calcd. 366.3551. Anal. Calcd. for $\text{C}_{16}\text{H}_{10}\text{N}_6\text{O}_3\text{S}$: C, 52.45 %; H, 2.75 %; N, 22.94 %; Found: C, 52.43 %; H, 2.75 %; N, 22.94 %.

Preparation of 4-imino-3-(3'-methoxyphenylazo)-4H-pyrimido[2.1-b][1,3]benzothiazole-2-ole (4g)

Yellow solid crystals, (83 %), mp: 219–221 °C. IR (cm^{-1}) ν_{max} : 3311 (–OH), 3175 (=NH), 3062 (Ar–H), 2993 (Aliphatic C–H), 1644 (C=N), 1514–1454 (N=N); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 3.82 (3H, s, –OCH₃), 6.76 (1H, t, $J = 6.15$ Hz, H-21), 7.08 (2H, t, $J = 6.15$ Hz, H-11, H-12), 7.34 (1H, d, $J = 6.77$ Hz, H-13), 7.40 (1H, d, $J = 6.77$ Hz, H-10), 7.62 (1H, d, $J = 6.77$ Hz, H-22), 7.97 (1H, d, $J = 6.77$ Hz, H-20), 8.00 (1H, s, H-18), 14.01 (1H, b, =NH), 14.16 (1H, b, –OH); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ : 55.71 (CH₃, C-25), 104.9 (C, C-5), 107 (CH, C-18), 108.71 (CH, C-10), 109.62 (CH, C-20), 111.72 (CH, C-22), 122.2 (CH, C-12), 123.27 (C, C-8), 125.18 (CH, C-13), 127.48 (CH, C-11), 131.57 (C, C-4), 133.32 (CH, C-21), 151.72 (C, C-6), 152.15 (C, C-17), 152.37 (C, C-9), 162.88 (C, C-19), 168 (C, C-2); HR-MS: 351.3836 $[\text{M}+\text{H}]^+$, calcd. 351.3837. Anal. Calcd. for $\text{C}_{17}\text{H}_{13}\text{N}_5\text{O}_2\text{S}$: C, 58.11 %; H, 3.73 %; N, 19.93 %; Found: C, 58.10 %; H, 32.74 %; N, 19.91 %.

Preparation of 4-imino-3-(3'-chlorophenylazo)-4H-pyrimido[2.1-b][1,3]benzothiazole-2-ole (4h)

Light orange solid crystals, (84 %), mp: 215–217 °C. IR (cm^{-1}) ν_{max} : 3212 (–OH), 3074 (=NH), 3006 (Ar–H), 2922 (Aliphatic C–H), 1639 (C=N), 1515–1456 (N=N); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 7.42 (1H, t, $J = 7.85$ Hz, H-21), 7.52 (2H, t, $J = 7.85$ Hz, H-11, H-12), 7.75 (1H, d, $J = 7.43$ Hz, H-13), 7.83 (1H, d, $J = 7.44$ Hz, H-10), 7.97 (2H, d, $J = 7.43$ Hz, H-20, H-22), 9.49 (1H, s, H-18), 10.79 (1H, s, =NH), 11.93 (1H, b, hydrazo-NH); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ : 104.9 (C, C-5), 108.71 (CH, C-10), 114.78 (CH, C-22), 115.02 (CH, C-18), 122.2 (CH, C-12), 122.93 (CH, C-20), 123.27 (C, C-8), 125.18 (CH, C-13), 127.46 (CH, C-11), 131.57 (C, C-4), 131.67 (CH, C-21), 138.84 (C, C-19), 151.72 (C, C-6), 152.37 (C, C-9), 152.59 (C, C-17), 168 (C, C-2); HR-MS: 355.8024 $[\text{M}+\text{H}]^+$, calcd. 355.8023. Anal. Calcd. for $\text{C}_{16}\text{H}_{10}\text{ClN}_5\text{OS}$: C, 54.01 %; H, 2.83 %; N, 19.68 %; Found: C, 54.02 %; H, 2.84 %; N, 19.69 %.

Preparation of 4-imino-3-(3'-methylphenylazo)-4H-pyrimido[2.1-b][1,3]benzothiazole-2-ole (4i)

Pink solid crystals, (81 %), mp: 242–243 °C. IR (cm^{-1}) ν_{max} : 3221 (–OH), 3168 (=NH), 3035 (Ar–H), 2960 (Aliphatic C–H), 1683 (C=N), 1508–1489 (N=N); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ (ppm): 2.38 (3H, s, –CH₃), 7.01

(1H, d, $J = 7.47$ Hz, H-20), 7.31 (1H, t, $J = 7.47$ Hz, H-12), 7.36 (1H, t, $J = 7.47$ Hz, H-11), 7.49 (1H, t, $J = 7.47$ Hz, H-21), 7.68 (1H, d, $J = 7.47$ Hz, H-13), 7.80 (1H, d, $J = 7.47$ Hz, H-10), 7.96 (1H, s, H-18), 8.03 (1H, d, $J = 7.47$ Hz, H-22), 12.15 (1H, b, 1H, =NH), 12.56 (1H, b, hydrazo-NH); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 20.94 (CH₃, C-24), 109.98 (CH, C-10), 113.19 (CH, C-18), 118.68 (CH, C-22), 123.18 (CH, C-12), 123.49 (CH, C-13), 124.3 (C, C-8), 126.67 (CH, C-20), 127.92 (CH, C-11), 129.04 (CH, C-19), 133.88 (C, C-4), 140.9 (C, C-21), 141.96 (C, C-5), 147.11 (C, C-9), 147.28 (C, C-17), 153.59 (C, C-6), 168.9 (C, C-2); HR-MS: 335.3842 [M+H]⁺, calcd. 335.3841. Anal. Calcd. for C₁₇H₁₃N₅OS: C, 60.88 %; H, 3.91 %; N, 20.88 %; Found: C, 60.88 %; H, 3.93 %; N, 20.89 %.

Preparation of 4-imino-3-(2'-nitrophenylazo)-4H-pyrimido[2.1-b][1,3]benzothiazole-2-ole (4j)

Red solid crystals, (81 %), mp: 264–265 °C. IR (cm⁻¹) ν_{max} : 3260 (–OH), 3150 (=NH), 3076 (Ar–H), 2935 (Aliphatic C–H), 1641 (C=N), 1506–1453 (N=N), 1515, 1337 (NO₂); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.90 (2H, s, –NH₂), 7.35 (1H, t, $J = 7.73$ Hz, H-12), 7.43 (1H, t, $J = 7.73$ Hz, H-11), 7.58 (2H, t, $J = 7.73$ Hz, H-21, H-20), 7.86 (1H, d, $J = 7.32$ Hz, H-13), 7.97 (1H, d, $J = 7.32$ Hz, H-10), 8.03 (1H, d, $J = 7.32$ Hz, H-22), 8.27 (1H, d, $J = 7.32$ Hz, H-19); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 109.86 (CH, C-9), 116.68 (C, C-12), 119.98 (C, C-5), 121.53 (CH, C-6), 122.98 (CH, C-20), 124.71 (CH, C-7), 125.3 (CH, C-22), 125.88 (CH, C-19), 128.62 (CH, C-8), 133.3 (CH, C-21), 136.98 (C, C-18), 143.7 (C, C-4), 143.79 (C, C-17), 147.67 (C, C-11), 162.83 (C, C-2), 175.22 (C, C-13); HR-MS: 366.3552 [M+H]⁺, calcd. 366.3551. Anal. Calcd. for C₁₆H₁₀N₆O₃S: C, 52.45 %; H, 2.75 %; N, 22.94 %; Found: C, 52.44 %; H, 2.76 %; N, 22.95 %.

Preparation of 4-imino-3-(2'-methoxyphenylazo)-4H-pyrimido[2.1-b][1,3]benzothiazole-2-ole (4k)

Dark red solid crystals, (81 %), mp: 163–165 °C. IR (cm⁻¹) ν_{max} : 3210 (–OH); 3132 (=NH), 3042 (Ar–H), 2963 (Aliphatic C–H), 1651 (C=N), 1506–1454 (N=N); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 3.98 (3H, s, –OCH₃), 7.05 (1H, t, $J = 7.66$ Hz, H-12), 7.17 (1H, t, $J = 7.66$ Hz, H-11), 7.19 (1H, t, $J = 7.66$ Hz, H-20), 7.40 (1H, t, $J = 7.95$ Hz, H-21), 7.63 (1H, d, $J = 7.66$ Hz, H-13), 7.67 (1H, d, $J = 7.66$ Hz, H-10), 7.98 (1H, d, $J = 7.65$ Hz, H-19, H-22), 14.12 (1H, b, =NH), 14.25 (1H, b, –OH); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 56.1 (CH₃, C-25), 104.9 (C, C-5), 108.71 (CH, C-10), 112.58 (CH, C-19), 117.72 (CH, C-22), 121.96 (CH, C-20), 122.2 (CH, C-12), 123.27 (C, C-8), 124.68 (CH, C-21), 125.18 (CH, C-13), 127.46 (CH, C-11), 131.57 (C, C-4), 138.03 (C, C-17), 150.91 (C,

C-6), 151.37 (C, C-8), 152.37 (C, C-9), 168 (C, C-2); HR-MS: 351.3836 [M+H]⁺, calcd. 351.3837. Anal. Calcd. for C₁₇H₁₃N₅O₂S: C, 58.11 %; H, 3.73 %; N, 19.93 %; Found: C, 58.12 %; H, 3.72 %; N, 19.92 %.

Preparation of 4-imino-3-(2'-chlorophenylazo)-4H-pyrimido[2.1-b][1,3]benzothiazole-2-ole (4l)

Orange solid crystals, (80 %), mp: 159–162 °C. IR (cm⁻¹) ν_{max} : 3223 (–OH), 3123 (=NH), 3062 (Ar–H), 2979 (Aliphatic C–H), 1644 (C=N), 1500–1452 (N=N); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm) 7.20 (1H, t, $J = 7.50$ Hz, H-12), 7.41 (1H, t, $J = 7.52$ Hz, H-11), 7.45 (1H, t, $J = 7.52$ Hz, H-20), 7.55 (1H, t, $J = 7.50$ Hz, H-21), 7.59 (1H, d, $J = 7.50$ Hz, H-10), 7.71 (1H, d, $J = 7.50$ Hz, H-13), 7.97 (1H, d, $J = 7.50$ Hz, H-22), 8.00 (1H, d, $J = 7.50$ Hz, H-19), 14.21 (1H, b, =NH), 14.48 (1H, b, –OH); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 104.9 (C, C-5), 108.71 (CH, C-10), 117.26 (CH, C-22), 117.91 (C, C-18), 120.86 (CH, C-20), 122.2 (CH, C-12), 123.27 (C, C-8), 125.18 (CH, C-13), 127.46 (CH, C-11), 128.12 (CH, C-21), 130.82 (CH, C-19), 131.57 (C, C-4), 143.20 (C, C-17), 151.69 (C, C-6), 152.37 (C, C-9), 168 (C, C-2); HR-MS: 355.8024 [M+H]⁺, calcd. 355.8023. Anal. Calcd. for C₁₆H₁₀ClN₅OS: C, 54.01 %; H, 2.83 %; N, 19.68 %; Found: C, 54.03 %; H, 2.82 %; N, 19.67 %.

Preparation of 4-imino-3-(2'-methylphenylazo)-4H-pyrimido[2.1-b][1,3]benzothiazole-2-ole (4m)

Light red solid crystals, (79 %), mp: 222–224 °C. IR (cm⁻¹) ν_{max} : 3228 (–OH), 3120 (=NH), 3037 (Ar–H), 2974 (Aliphatic C–H), 1661 (C=N), 1497–1453 (N=N); ^1H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.40 (3H, s, –CH₃), 7.31 (1H, t, $J = 7.22$ Hz, H-12), 7.35 (1H, t, $J = 7.22$ Hz, H-11), 7.40 (1H, t, $J = 6.5$ Hz, H-20), 7.54 (1H, t, $J = 6.5$ Hz, H-21), 7.58 (1H, d, $J = 7.22$ Hz, H-13), 7.97 (1H, d, $J = 7.21$ Hz, H-10), 7.99 (1H, d, $J = 7.22$ Hz, H-22), 14.21 (1H, b, 1H, =NH), 14.34 (1H, b, –OH); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 16.7 (CH₃, C-24), 104.9 (C, C-5), 108.71 (CH, C-10), 111.15 (CH, C-22), 121.22 (CH, C-20), 122.2 (CH, C-12), 123.27 (C, C-8), 125.18 (CH, C-13), 127.07 (CH, C-21), 127.46 (CH, C-11), 128.78 (C, C-18), 131.57 (C, C-4), 132.07 (C, C-19), 150.32 (C, C-17), 152.17 (C, C-6), 152.37 (C, C-9), 168 (C, C-2); HR-MS: 335.3842 [M+H]⁺, calcd. 335.3841. Anal. Calcd. for C₁₇H₁₃N₅OS: C, 60.88 %; H, 3.91 %; N, 20.88 %; Found: C, 60.87 %; H, 3.92 %; N, 20.87 %.

Antimicrobial evaluation

Newly synthesized compounds **4(a–m)** were individually tested against a panel of Gram-positive and Gram-negative bacterial pathogens, yeast, and fungi. The antimicrobial

activity of synthesized compounds was evaluated by the agar well-diffusion method (Yavuz and Yildirim, 2013; Scott, 1989) using 100 μL of suspension containing 1×10^6 CFU/mL of pathological tested bacteria and 1×10^6 /mL of yeast spread on nutrient agar (NA) and Sabourand 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 mg of the chemical compound in 1 mL of DMSO. The inoculated 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 dimethyl sulphoxide employed for dissolving the tested compound. Ciprofloxacin (50 $\mu\text{g}/\text{mL}$) and Ketoconazole (50 $\mu\text{g}/\text{mL}$) were used as standard for antibacterial and antifungal activity, 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.

Determination of minimal inhibitory concentration (MIC)

MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. The micro dilution susceptibility test in MüllereHinton 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 1,000 $\mu\text{g}/\text{mL}$. Twofold 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 $\mu\text{g}/\text{mL}$. 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 above inoculum and incubated at 37 °C for 24 h. The lowest concentration showing no growth was taken as the MIC. Control experiments with DMF and uninoculated media were run parallel to the test compounds under the same conditions. The MIC ($\mu\text{g}/\text{mL}$) values are recorded in Table 4.

Conclusion

In summary, based on our previous work, we have synthesized new series of azo pyrimido[2,1-*b*][1,3]benzothiazole derivatives. The antimicrobial activities of these

compounds were evaluated and compared with standard drugs. The results revealed that the most of the compounds exhibited good levels of antibacterial activity against Gram-positive bacteria, Gram-negative bacteria, as well as fungi. In particular, compounds **4d** and **4h** showed excellent levels of antimicrobial activity with MIC values of 8.25 $\mu\text{g}/\text{mL}$. Many of the synthesized motifs, possessing electron withdrawing atom/group such as chlorine and nitro were identified as the most potent antimicrobial activity.

The mechanism of action of the compounds tested in this study currently remains unknown. Thus, further studies of related compounds in the context of their structure–activity relationship, toxicity, and other biological effects might be helpful in designing new antimicrobials for therapeutic use.

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