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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antimicrobial, and antioxidant activities of some new indole analogues containing pyrimidine and fused pyrimidine systems

Saundane Anand Raghunath · Yarlakatti Manjunatha · Kalpana Rayappa

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Abstract To examine new leads with potential antimicrobial and antioxidant activities, a new series of tetrahydropyrimidines (**2a–c**, **3a–c** and **4a–c**), pyrazolo[3,4-d]pyrimidines (**5a–c**), and ditetrazolo[1,5-a;1′,5′-c] pyrimidines (**6a–c**) were synthesized in this study using appropriate synthetic routes. The newly synthesized compounds have been tested for their antimicrobial and antioxidant activities against DPPH stable free radical. In the case of antibacterial activity, compounds **2a**, **6a**, and **6c** exhibited the maximum zone of inhibition against *Staphylococcus aureus*; compound **6c** exhibited maximum zone of inhibition against *Pseudomonas aeruginosa*; and compound **2a** showed maximum inhibitory growth against *Klebsiella pneumonia*. While in the case of antifungal activities, compound **5a** showed good zone of inhibition against *Aspergillus oryzae*, compounds **2b** and **6a** exhibited maximum zone of inhibition against *Aspergillus niger*. In case of antioxidant activities, compound **2a** showed the highest DPPH radical scavenging activity.

Keywords Indole · Pyrazolopyrimidine · Tetrazolopyrimidines · Antimicrobial · Antioxidant

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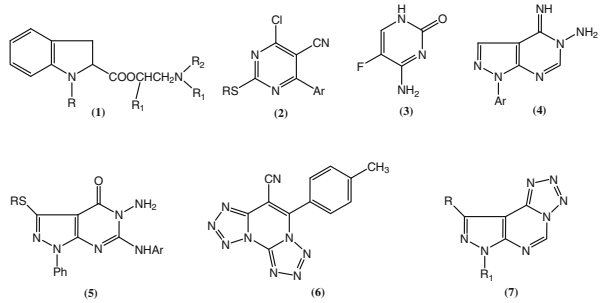
Introduction

Food oxidation by atmospheric oxygen and free radicals is a destructive process, causing the loss of nutritional values and changes in chemical composition. The formation of reactive oxygen species (ROS) is a natural consequence of aerobic metabolism and is an integral part of tissue oxygen homeostasis maintenance (Castro and Freeman, 2001). When present in high concentrations, these compounds can damage cellular proteins, lipids, and form carcinogenic DNA adducts. Although ROS play crucial roles in normal physiological processes, such as the apoptotic elimination of damaged cells, aberrant production or regulation of ROS activity has been demonstrated to contribute to the development of some prevalent diseases, including cancer and cardiovascular (Seifried *et al.,* 2007). The purpose of antioxidants is to prevent ROS concentrations from reacting harmful intracellular levels and degenerative processes by various mechanisms including scavenging of free radicals.

Indole is an important substance used in organic synthesis (Bajtos *et al.*, 2007), and its physiological activities attracted a lot of scientific attention as well (Bittner *et al.*, 2007). The antioxidant effect of indole derivatives, such as melatonin, tryptophan, and serotonin on cisplatin-induced ROS have been reported (Fukutomi *et al.*, 2006; Matuszak *et al.*, 1997). In vitro radical scavenging activity (RSA) of several N-substituted indole-2-carboxylic acid esters (**1**) was studied (Kruk *et al.*, 2007). This property of indole attracted researchers' attention because of the correlation of serious diseases with the oxidative damage of the membrane, DNA- and RNA-induced radicals (Ratnam *et al.*, 2006). Several indole derivatives are well known for their manifold uses because of their potential application in medicinal chemistry as antitumor (Sunjoo *et al.*, 2011;

Mohamed and Mahmoud, 2007) antivascular (Nancy *et al.*, 2008), antituberculosis (Basavarajaiah and Mruthyunjayaswamy, 2009), and anti-inflammatory (Mohamed *et al.*, 2007) activities.

Pyrimidine and purine compounds play an essential role in several biological processes and have very important chemical and pharmacological properties. Some of the pyrimidine analogues (**2**) (Agarwal *et al.*, 2002), and (**3**) (Polak and Scholer, 1975) have been reported as potential antimicrobial agents. Also, pyrazolopyrimidine systems, as they are structurally related to purine, considered as typical examples of purine analogues (**4**) (Aly and Gad El Karim, 2011) and (**5**) (Wang *et al.*, 2008), have been reported as antibacterial agents, inhibitors for the syntheses of DNA and RNA in cells of some kind of cancers (Seela *et al.*, 2000), and viruses (Tomonaga *et al.*, 1990). The literature survey has revealed that, several tetrazolopyrimidines have been reported as antimicrobial agents (**6**) (El-Assiery and El-Haiza, 1998) and (**7**) (Dave and Shah, 2002). Tetrazolopyrimidines are also used in the treatment of obesity, atherosclerosis, glaucoma (Aspens *et al.*, 2002), hypertension (Takaya *et al.*, 1988; Uehata *et al.*, 2001), coronary heart disease (Fujii *et al.*, 2005), and diabetic retinotherapy (Takayama *et al.*, 2006).



Literature Compounds **1-7**

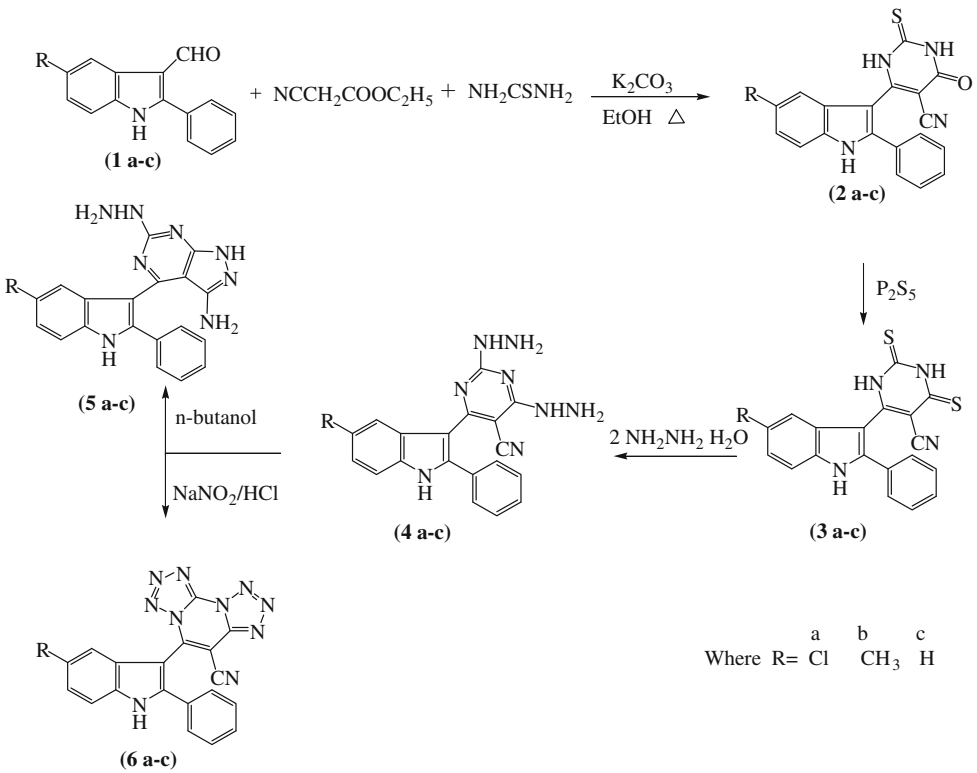
As a result, encouraged by these pharmacological properties of indole, pyrozolopyrimidine, and tetrazolopyrimidines and guided by the observation that many times the combination of one or more heterocyclic nuclei in a molecule enhances the biological profile manifold, and in continuation of our interest in the synthesis of biologically active indole analogues (Saundane *et al.*, 2009; Saundane and Manjunatha, 2011), we have synthesized the title compounds and screened them for their antimicrobial and antioxidant activities.

Results and discussion

The reaction sequence employed for the synthesis of title compounds is shown in Scheme 1. Starting material 6-(2′,5′-disubstituted 1*H*-indol-3′-yl)-4-oxo-1,2,3,4-tetrahydro-2-thioxo pyrimidin-5-carbonitriles (**2.a–2.c**) was conveniently prepared by the reaction of 2,5-disubstituted indole-3-carboxaldehydes (**1.a–1.c**) (Hiremath *et al.*, 1982) with ethyl acetoacetate, thiourea, and anhydrous potassium carbonate in dry ethanol under reflux conditions (Kambe *et al.*, 1979; Ram *et al.*, 1988). The IR spectrum of compound **2.a** showed absorption bands at 3259 (NH), 3056 (indole NH), 2210 (CN), 1693 (C=O) cm^{-1} functions, and its ^1H NMR spectrum revealed the signals at δ ppm 11.71 (s, 1H, indole NH), 9.92 (s, 1H, NH), 7.78 (s, 1H, Ar–H), 7.70 (d, 1H, Ar–H), 7.65 (d, 1H, Ar–H), 7.59 (t, 1H, Ar–H), 7.30 (t, 2H, Ar–H), 7.01 (dd, 2H, Ar–H), and 5.13 (s, 1H, SH). Its mass spectrum exhibited isotopic molecular ion peak at m/z 378 and 380. Compounds (**2.a–2.c**) on reaction with phosphorus pentasulfide in boiling pyridine gave 2.4-dithiopyrimidines (**3.a–3.c**). Compound **3.a** in its IR spectrum exhibited absorption bands at 3289, 3057, 2210, and 1239 cm^{-1} due to NH, indole NH, CN, and C=S functions, respectively. Further, its ^1H NMR spectrum showed signals at δ ppm 12.24 (s, 1H, indole NH), 10.01 (s, 1H, NH), 8.11 (s, 1H, Ar–H), 8.02 (d, 1H, Ar–H), 7.83 (d, 1H, Ar–H), 7.45 (t, 2H, Ar–H), 7.38 (t, 1H, Ar–H), 7.09 (dd, 2H, Ar–H), and 5.23 (s, 1H, SH). Its mass spectrum showed isotopic molecular ion peak at m/z 394 and 396, which confirms the formation of **3.a** from **2.a**. Compounds (**3.a–3.c**) upon refluxing with hydrazine hydrate in ethanol afforded 6-(2′,5′-disubstituted 1*H*-indole-3′-yl)-2,4-dihydrazinylpyrimidin-5-carbonitriles (**4.a–4.c**). Compound **4.a** in its IR spectrum exhibited absorption bands at 3434, 3322, and 3150 cm^{-1} due to indole NH, NH, and NH_2 functions, respectively. While in its ^1H NMR spectrum, signals due to various protons appeared at δ ppm 11.72 (s, 1H, indole NH), 8.91 (bs, 2H, NH), 8.45 (s, 1H, Ar–H), 8.03 (d, 1H, Ar–H), 7.74 (d, 1H, Ar–H), 7.53 (t, 2H, Ar–H), 7.32 (t, 1H, Ar–H), 7.08 (dd, 2H, Ar–H), and 6.83 (s, 2H, NH_2). In mass spectrum, isotopic molecular ion peaks at m/z 390 and 392 were observed which confirmed the formation of **4.a** from **3.a**.

Then, compounds (**4.a–4.c**) were invested in the synthesis of pyrazolopyramidine and tetrazolopyrimidine systems. Thus, compounds (**4.a–4.c**) when subjected to thermal cyclization in 1-butanol under reflux conditions for 5 h, yielded pyrazolopyrimidines (**5.a–5.c**). The structure of compound (**5.a**) was confirmed by its spectral data. Compound (**5.a**) in its IR spectrum showed the absence of CN at 2210 cm^{-1} function, and fresh appearances of the absorption bands at 3308, 3286, 3186, 3114, and 3056 cm^{-1} due to NH_2, NH_2, NH, NH, and indole NH functions, respectively were noticed. Also, the ^1H NMR spectrum showed the presence of signals at δ ppm 12.02 (s, 1H, indole NH). 9.04 (s, 1H, pyrazole NH), 8.41 (1H, NH, and hydrazinyl NH), 8.12 (s, 1H, Ar–H), 7.97 (d, 1H,

Scheme 1 Pathway for synthesis of compounds **2–6**



Ar–H), 7.84 (d, 1H, Ar–H), 7.75 (t, 2H, Ar–H), 7.67 (t, 1H, Ar–H), 7.54 (dd, 2H, Ar–H), 7.32 (s, 2H, NH_2), and 7.11 (s, 2H, NH_2). The structure of this pyrazolopyrimidine was further confirmed by recording on mass spectrum, which showed that isotopic molecular ion peaks at m/z 390 and 392 confirmed the formation of **5.a** from **4.a**. Again, synthons (**4.a–4.c**) on diazotization with sodium nitrite at 0–5°C afforded ditetrazolo[1,5-a;1′,5′-c]pyrimidines (**6.a–6.c**). In the IR spectrum of compound **6.a**, the bands due to indole NH and CN, which are present in all studied compounds, were observed at about 3053 and 2210 cm^{-1} functions, respectively. The bands at 1240 and 1012 cm^{-1} were characteristic of the tetrazole ring. In ^1H NMR spectrum, different protons were resonated at δ ppm: 12.12 (s, 1H, indole NH) and 8.02 (s, 1H, Ar–H), 7.95 (d, 1H, Ar–H), 7.83 (d, 1H, Ar–H), 7.65 (t, 2H, Ar–H), 7.59 (t, 1H, Ar–H), 7.24 (dd, 2H, Ar–H), and the absence of signals due to NH/NH_2 functions confirmed the formation of **6.a** from **4.a**. Further, formation of **6.a** was confirmed by its mass spectrum, which showed isotopic molecular ion peaks at m/z 412 and 414.

Biological results

Antimicrobial activity

All the synthesized compounds (**2–6**) were evaluated for their antibacterial activities against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*, whereas antifungal activities against *Aspergillus niger*, *A. terrus*, and *A. oryzae* were evaluated by cup plate method at a concentration of 1 mg/ml following the literature procedure (Indian pharmacopoeia, 1985). The zone of inhibition (in mm) was compared with the standard gentamycin and fluconazole for antibacterial and antifungal activities, respectively. The results are tabulated in Table 1. In case of antibacterial activities, compounds **2.a** and **6.a** showed maximum zone of inhibition against *S. aureus* and *K. Pneumonia*, whereas, compounds **5.a** and **6.c** exhibited maximum zone of inhibition against *S. aureus* and *P. aeruginosa*. Compound **5.c** showed maximum inhibitory growth against *S. aureus* and *K. pneumonia*. Compound **4.a** showed maximum zone of inhibition against *P. aeruginosa,* whereas compounds **2.c**, **4.b**, and **5.b** exhibited good zone of inhibition against *K. pneumonia*.

On the other hand, antifungal activities of test compounds revealed that, compound **2.b** showed maximum fungal growth inhibition against all the three fungi. Compounds **2.a**, **5.a**, and **5.c** exhibited maximum inhibitory growth against *A. oryzae*. Compound **6.b** exhibited maximum inhibition against *A. terrus*, and compound **6.a** possessed good zone of inhibition against *A. niger*.

From the results of antimicrobial activities, it is revealed that, the majority of the synthesized compounds having chloro substitution exhibited the maximum growth

Table 1 Antimicrobial activity results of compounds **2–6**

Comp. no.	Antibacterial activity (zone of inhibition in mm)			Antifungal activity (zone of inhibition in mm)		
	S. aureus	*P. aeruginosa*	*K. pneumonia*	*A. oryzae*	*A. terrus*	*A. niger*
2.a	14	10	14	12	09	10
2.b	12	10	11	11	11	13
2.c	11	11	12	10	10	10
3.a	12	10	09	11	10	11
3.b	09	10	08	09	10	09
3.c	08	07	08	10	11	11
4.a	11	13	10	10	10	08
4.b	10	10	12	09	11	11
4.c	08	10	11	10	11	10
5.a	13	13	09	13	10	09
5.b	11	12	13	09	10	10
5.c	12	11	12	12	10	12
6.a	14	12	13	10	11	13
6.b	10	10	11	09	12	09
6.c	14	14	12	07	10	10
Gentamycin	15	16	15	–	–	–
Fluconazole	–	–	–	14	15	14

inhibitory activity. In general, it is worth mentioning that compounds **2.a**, **3.a**, **4.a**, **5.a**, and **6.a** contain chloro substitution at position-5 of the indole nucleus. The electronegative nature of the chloro group may be responsible for inhibiting the growth of the microbes. Antimicrobial activities of compounds (**5**) and (**6**) may be due to the presence of pyrazolopyrimidine and diterazolopyrimidine systems, respectively. However, none of the compounds exhibited zone of inhibition more than that of the standards.

Radical scavenging activity (RSA)

Free radical scavenging is one of the best known mechanisms by which antioxidants inhibit lipid oxidation. 1,1-diphenyl-2-picrylhydrazyl (DPPH) RSA evaluation is standard assay in antioxidant activity studies and offers a rapid technique for screening the RSA of specific compounds. The RSA of synthesized compounds (**2–6**) were carried out using methanolic solution of the stable free radical DPPH, and the results are shown in Figs. 1 and 2. The freshly prepared DPPH solution exhibits a deep purple color with absorption maxima at 517 nm. The purple color generally fades/disappears when an antioxidant is present in the medium. Thus, antioxidant molecule can quench DPPH free radical (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free radical attack on DPPH molecule) and convert them to a colorless/bleached

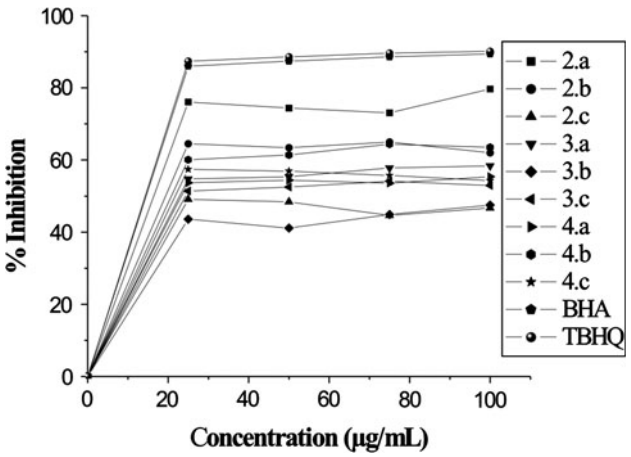


Fig. 1 Antioxidant activities of compounds **2–4**

product, resulting in the absorbance at 517 nm. Therefore, the more rapidly the absorbance decreases, the more potent the Antioxidant activities of the compounds. Hence, this method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction.

In the case of 6-(2′,5′-disubstituted 1*H*-indol-3′-yl)-4-oxo-1,2,3,4-tetrahydro-2-thioxopyrimidin-5-carbonitriles (**2a–c**), derivative **2a** showed the highest DPPH scavenging activity with percent inhibition of 79.7 at concentration of 100 µg/ml (IC_{50} 16.43 µg/ml), when compared with the

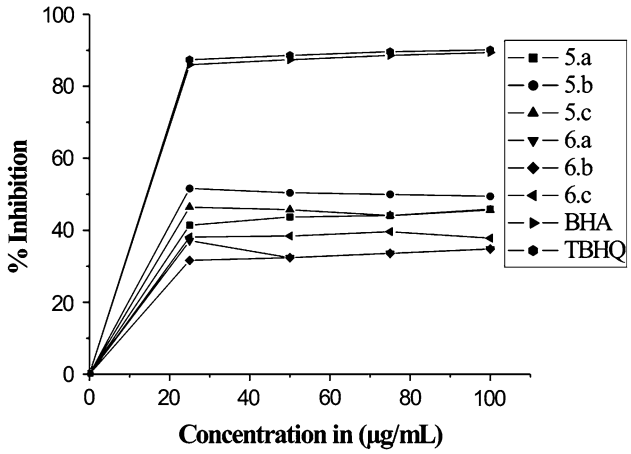


Fig. 2 Antioxidant activities of compounds **5** and **6**

other compounds. The higher activity of this compound may be due to the presence of enolizable thio and oxo groups. These groups may be responsible for stabilization of free radicals formed after donating hydrogen or electron to the stable DPPH radical as shown in Scheme 2. However, none of the compounds showed better RSA activity than standards BHA (IC_{50} 14.35 µg/ml) and TBHQ (IC_{50} 14.46 µg/ml).

Conclusion

In general, it was found that the compounds having chloro substitution along with the pyrazolopyrimidine and ditetrazolopyrimidine systems exhibited good antioxidant and antimicrobial activities.

Experimental procedures

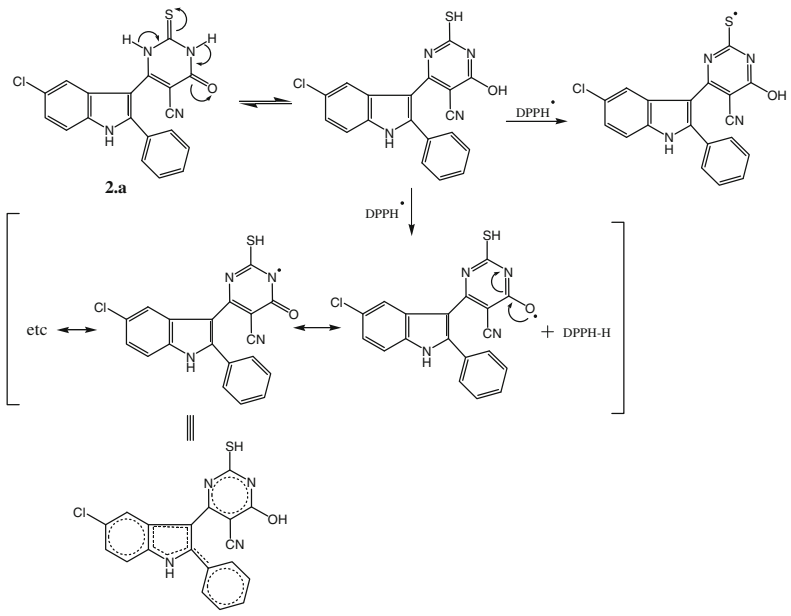
Chemistry

Materials and methods

All the reagents were obtained commercially and used by further purification. Melting points were determined by an open capillary method and are uncorrected. The IR (KBr) spectra were recorded with a Perkin-Elmer spectrum one FT-IR spectrometer. The ^{1}H NMR (DMSO-d_6) spectra were recorded using Bruker NMR (500 MHz), and the chemical shifts were expressed in ppm (δ scale) downfield from TMS. Mass spectra were recorded with a JEOL GCMATE II GC–MS mass spectrometer. Elemental analysis carried out using Flash EA 1112 series elemental analyzer. The progress of the reaction was monitored by TLC, and the purities of the synthesized compounds were also checked by TLC.

*General procedure for the synthesis of 6-(2′,5′-disubstituted 1H-indol-3′-yl)-4-oxo-1,2,3,4-tetrahydro-2-thioxopyrimidin-5-carbonitriles (**2.a–2.c**)*

A mixture of 2,5-disubstitutedindole-3-carboxaldehyde (**1.a–1.c**) (Hiremath *et al.*, 1982) (0.01 mol), ethyl cyanoacetate (0.01 mol), thiourea (0.01 mol), and potassium carbonate (0.01 mol) in absolute ethanol (30 ml) was refluxed for 20 h. The reaction mixture was cooled to room temperature and acidified with dil. HCl. The precipitate formed was filtered off, washed with water, dried, and crystallized from ethanol to give compound (**2.a–2.c**).



Scheme 2 DPPH RSA: probable mechanism for hydrogen donation from compound **2a** to DPPH radical and stabilization of free radical formed

6-(5′-Chloro-2′-phenyl-1*H*-indol-3′-yl)-4-oxo-1,2,3,4-tetrahydro-2-thioxopyrimidin-5-carbonitrile (**2.a**): Yield: 71%, mp 265–266°C. FTIR (KBr) cm^{-1}: 3259 (NH); 3056 (indole NH); 2210 (CN); 1693 (C=O); ^{1}HNMR (DMSO-d_6, δ ppm) 11.71 (s, 1H, indole NH); 9.92 (s, 1H, NH); 7.78 (s, 1H, Ar–H), 7.70 (d, 1H, Ar–H), 7.65 (d, 1H, Ar–H), 7.59 (t, 1H, Ar–H), 7.30 (t, 2H, Ar–H), 7.01 (dd, 2H, Ar–H); 5.13 (s, 1H, SH); MS (EI) *m/z* 378 (M^+); 380 (M^++2). Anal. % $C_{19}H_{11}N_4OSCl$: C, 60.32; H, 2.91; N, 14.81. Found: C, 60.54; H, 2.72; N, 14.71.

6-(5′-Methyl-2′-phenyl-1*H*-indol-3′-yl)-4-oxo-1,2,3,4-tetrahydro-2-thioxopyrimidin-5-carbonitrile (**2.b**): Yield: 69%, mp 269–270°C. FTIR (KBr) cm^{-1}: 3255 (NH); 3059 (indole NH); 2210 (CN); 1693 (C=O); ^{1}HNMR (DMSO-d_6, δ ppm): 11.82 (s, 1H, indole NH); 9.91 (s, 1H, NH); 7.83 (s, 1H, Ar–H), 7.75 (d, 1H, Ar–H), 7.68 (d, 1H, Ar–H), 7.58 (t, 2H, Ar–H), 7.35 (t, 1H, Ar–H),7.04 (dd, 2H, Ar–H); 5.11 (s, 1H, SH); 2.15 (s, 3H, CH_3); Anal. % $C_{20}H_{14}N_4OS$: C, 67.03; H, 3.91; N, 15.64. Found: C, 67.32; H, 4.12; N, 15.59.

6-(2′-Phenyl-1*H*-indol-3′-yl)-4-oxo-1,2,3,4-tetrahydro-2-thioxopyrimidin-5-carbonitrile (**2.c**): Yield 69%, mp 258–259°C. FTIR (KBr) cm^{-1}: 3257 (NH); 3059 (indole NH); 2216 (CN); 1690 (C=O); ^{1}HNMR (DMSO-d_6, δ ppm): 11.84 (s, 1H, indole NH); 9.83 (s, 1H, NH); 7.82 (d, 1H, Ar–H), 7.73 (t, 1H, Ar–H), 7.70 (t, 1H, Ar–H), 7.62 (d, 1H, Ar–H), 7.54 (t, 2H, Ar–H), 7.30 (t, 1H, Ar–H),7.10 (dd, 2H, Ar–H); 5.23 (s, 1H, SH); Anal. % $C_{19}H_{12}N_4OS$: C, 66.28; H, 3.49; N, 16.28. Found: C, 66.52; H, 3.71; N, 16.53.

*General procedure for the synthesis of 6-(2′,5′-disubstituted 1H-indol-3′-yl)-2-4-dithioxo-1,2,3,4,-tetrahydropyrimidin-5-carbonitriles (**3.a–3.c**)*

A solution of (**2.a–2.c**) (0.01 mol) in pyridine (10 ml) and P_2S_5 (0.01 mol) was refluxed for 5 h, then cooled, poured to crushed ice, filtered, washed with water, dried, and re-crystallized from ethyl acetate to obtain (**3.a–3.c**).

6-(5′-Chloro-2′-phenyl-1*H*-indol-3′-yl)-2-4-dithioxo-1,2,3,4,-tetrahydropyrimidin-5-carbonitrile (**3.a**): Yield 70%, mp 235–236°C. FTIR (KBr) cm^{-1}: 3289 (NH); 3057 (indole NH); 2210 (CN); 1293 (C=S); ^{1}HNMR (DMSO-d_6, δ ppm): 12.24 (s, 1H, indole NH); 10.01 (s, 1H, NH); 8.11 (s, 1H, Ar–H), 8.02 (d, 1H, Ar–H), 7.83 (d, 1H, Ar–H), 7.45 (t, 2H, Ar–H), 7.38 (t, 1H, Ar–H), 7.09 (dd, 2H, Ar–H); 5.23 (s, 1H, SH); MS (EI) *m/z* 394 (M^+), 396 (M^++2). Anal. % $C_{19}H_{11}N_4S_2Cl$: C, 57.87; H, 2.79; N, 14.21. Found: C, 57.79; H, 2.57; N, 14.53.

6-(5′-Methyl-2′-phenyl-1*H*-indol-3'-yl)-2-4-dithioxo-1,2,3,4,-tetrahydropyrimidin-5-carbonitrile (**3.b**): Yield 68%, mp 239–240°C. FTIR (KBr) cm^{-1}: 3291 (NH), 3060 (indole NH), 2215 (CN), 1283 (C=S). ^{1}HNMR (DMSO-d_6, δ ppm): 12.12 (s, 1H, indole NH); 10.13 (s, 1H, NH); 8.25 (s, 1H, Ar–H), 8.05 (d, 1H, Ar–H), 7.83 (d, 1H, Ar–H), 7.52 (t, 2H, Ar–H), 7.39 (t, 1H, Ar–H), 7.11 (dd, 2H, Ar–H); 5.27 (s, 1H, SH); 2.26 (s, 3H, CH_3); Anal. % $C_{20}H_{14}N_4S_2$: C, 64.17; H, 3.74; N, 14.97. Found: C, 65.35; H, 3.97; N, 15.15.

6-(2′-Phenyl-1*H*-indol-3′-yl)-2-4-dithioxo-1,2,3,4,-tetrahydropyrimidin-5-carbonitrile (**3.c**): Yield 65%, mp 249–250°C. FTIR (KBr) cm^{-1}: 3287 (NH); 3059 (indole NH); 2219 (CN); 1285 (C=S); ^{1}HNMR (DMSO-d_6, δ ppm): 12.19 (s, 1H, indole NH); 10.02 (s, 1H, NH); 8.28 (d, 1H, Ar–H), 8.10 (t, 1H, Ar–H), 7.87 (t, 1H, Ar–H), 7.55 (d, 1H, Ar–H), 7.41 (t, 2H, Ar–H), 7.25 (t, 1H, Ar–H), 7.14 (dd, 2H, Ar–H); 5.17 (s, 1H, SH); Anal. % $C_{19}H_{12}N_4S_2$: C, 63.33; H, 3.33; N, 15.56. Found: C, 63.57; H, 3.51; N, 15.78.

*General procedure for the synthesis of 6-(2′,5′-disubstituted-1H-indol-3′-yl)-2,4-dihydrazinylpyrimidin-5-carbonitriles (**4.a–4.c**)*

An equimolar mixture of compounds (**3.a–3.c**) (0.01 mol) and hydrazine hydrate (0.01 mol, 99–100%) in ethanol (15 ml) was refluxed for 4 h. After cooling, the resulting solid was collected by filtration, washed with cold ethanol, and recrystallized from ethanol to give (**4.a–4.c**).

6-(5′-Chloro-2′-phenyl-1*H*-indol-3′-yl)-2,4-dihydrazinylpyrimidin-5-carbonitrile (**4.a**): Yield 69%, mp 210–211°C. FTIR (KBr) cm^{-1}: 3434 (NH_2), 3322 (NH) and 3150 (indole NH); 2195 (CN); ^{1}HNMR (DMSO-d_6, δ ppm): 11.72 (s, 1H, indole NH); 8.91 (bs, 2H, 2 NH); 8.45 (s, 1H, Ar–H), 8.03 (d, 1H, Ar–H), 7.74 (d, 1H, Ar–H), 7.53 (t, 2H, Ar–H), 7.32 (t, 1H, Ar–H), 7.08 (dd, 2H, Ar–H), 6.83 (s, 2H,NH_2); MS (EI) *m/z* 390 (M^+), 392 (M^++2). Anal. % $C_{19}H_{15}N_8Cl$: C, 58.46; H, 3.85; N, 28.72. Found: C, 58.71; H, 3.99; N, 28.93.

6-(5′-Methyl-2′-phenyl-1*H*-indole-3′-yl)-2,4-dihydrazinylpyrimidin-5-carbonitrile (**4.b**): Yield 65%, mp 215–216°C. FTIR (KBr) cm^{-1}: 3435 (NH_2), 3320 (NH) 3125 (indole NH); 2196 (CN); ^{1}HNMR (DMSO-d_6, δ ppm): 11.84 (s, 1H, indole NH); 8.88 (bs, 2H, 2 NH); 8.35 (s, 1H, Ar–H), 8.05 (d, 1H, Ar–H), 7.71 (d, 1H, Ar–H), 7.49 (t, 2H, Ar–H), 7.30 (t, 1H, Ar–H), 7.15 (dd, 2H, Ar–H), 6.82 (s, 2H,NH_2); 2.11 (s, 3H, CH_3); Anal. % $C_{20}H_{18}N_8$: C, 64.86; H, 4.86; N, 30.27. Found: C, 64.95; H, 4.95; N, 30.55.

6-(2′-Phenyl-1*H*-indol-3′-yl)-2,4-dihydrazinylpyrimidin-5-carbonitrile (**4.c**): Yield 67%, mp 201–203°C. FTIR (KBr) cm^{-1}: 3430 (NH), 3315 (NH) 3125 (indole NH); 2198 (CN); ^{1}HNMR (DMSO-d_6, δ ppm): 11.71 (s, 1H, indole NH); 8.95 (bs, 2H, 2 NH); 8.35 (d, 1H, Ar–H), 8.06 (t, 1H, Ar–H),8.01 (t, 1H, Ar–H), 7.89 (d, 1H, Ar–H), 7.54

(t, 2H, Ar–H), 7.43 (t, 1H, Ar–H), 7.31 (dd, 2H, Ar–H), 6.72 (s, 2H,NH_2); Anal. % $C_{19}H_{16}N_8$: C, 64.04; H, 4.49; N, 31.46.Found: C, 64.35; H, 4.67; N, 31.71.

*General procedure for the synthesis of 4-(2′,5′-disubstituted-1H-indol-3′-yl)-6-hydrazino-1H-pyrazolo[3,4-d]pyrimidin-3-amines (**5.a–5.c**)*

A solution of compounds (**4.a–4.c**) (0.001 mol) in 1-butanol (20 ml) was refluxed for 7 h. The excess solvent was removed under reduced pressure, and the residue was recrystallized form ethanol to give compound (**5.a–5.c**).

4-(5′-Chloro-2′-phenyl-1*H*-indol-3′-yl)-6-hydrazino-1*H*-pyrazolo[3,4-d]pyrimidin-3-amine (**5.a**): Yield 63%, mp 186–187°C. FTIR (KBr) cm^{-1}: 3308 (NH_2); 3286 (NH_2); 3186 (NH); 3114 (NH); 3056 (indole NH); ^{1}HNMR (DMSO-d_6, δ ppm): 12.02 (s, 1H, indole NH); 9.04 (s, 1H, pyrazole NH); 8.91 (s, 1H, hydrazinyl NH); 8.12 (s, 1H, Ar–H), 7.97 (d, 1H, Ar–H), 7.84 (d, 1H, Ar–H), 7.75 (t, 2H, Ar–H), 7.67 (t, 1H, Ar–H), 7.54 (dd, 2H, Ar–H), 7.32 (s, 2H, NH_2), 7.11 (s, 2H, NH_2); MS (EI) *m/z* 390 (M^+), 392 (M^++2); Anal. % $C_{19}H_{15}N_8Cl$: C, 58.46; H, 3.85; N, 28.72. Found: C, 58.69; H, 3.99; N, 28.93.

4-(5′-Methyl-2′-phenyl-1*H*-indol-3′-yl)-6-hydrazino-1*H*-pyrazolo[3,4-d]pyrimidin-3-amine (**5.b**): Yield 65%, mp 190–191°C. FTIR (KBr) cm^{-1}: 3310 (NH_2); 3289 (NH_2); 3189 (NH); 3116 (NH); 3060 (indole NH); ^{1}HNMR (DMSO-d_6, δ ppm): 11.91 (s, 1H, indole NH); 8.92 (s, 1H, pyrazole NH); 8.81 (s, 1H, hydrazinyl NH); 8.16 (s, 1H, Ar–H), 7.95 (d, 1H, Ar–H), 7.82 (d, 1H, Ar–H), 7.71 (t, 2H, Ar–H), 7.65 (t, 1H, Ar–H), 7.52 (dd, 2H, Ar–H), 7.30 (s, 2H, NH_2), 7.05 (s, 2H, NH_2); 2.21 (s, 3H, CH_3); Anal. % $C_{20}H_{18}N_8$: C, 64.86; H, 4.86; N, 30.27. Found: C, 64.97; H, 5.01; N, 30.53.

4-(2′-Phenyl-1*H*-indol-3′-yl)-6-hydrazino-1*H*-pyrazolo [3,4-d]pyrimidin-3-amine (**5.c**): Yield 63%, mp 196–197°C. FTIR (KBr) cm^{-1}: 3305 (NH_2); 3284 (NH_2); 3180 (NH); 3119 (NH); 3050 (indole NH); ^{1}HNMR (DMSO-d_6, δ ppm): 12.11 (s, 1H, indole NH); 9.15 (s, 1H, pyrazole NH); 8.89 (s, 1H, hydrazinyl NH); 8.11 (d, 1H, Ar–H), 7.96 (t, 1H, Ar–H), 7.83 (t, 1H, Ar–H), 7.79 (d, 1H, Ar–H), 7.67 (t, 2H, Ar–H), 7.63 (t, 1H, Ar–H), 7.56 (dd, 2H, Ar–H), 7.35 (s, 2H, NH_2), 7.04 (s, 1H, NH_2); Anal. % $C_{19}H_{16}N_8$: C, 64.04; H, 4.50; N, 31.46. Found: C, 64.35; H, 4.73; N, 31.78.

*General procedure for the synthesis of 5-(2′,5′-disubstituted 1H-indol-3′-yl)ditetrazolo[1,5-a;1′,5′-c]pyrimidin-6-carbonitriles (**6.a–6.c**)*

A solution of sodium nitrite (0.001 mol) in HCl (8 ml) was added to the stirred solution of compounds (**4.a–4.c**) (0.001 mol) in 20% aqueous hydrochloric acid (10 ml) at 0–5°C. The mixture was then allowed to react at same temperature for 3 h. The formed solid was filtered, washed with water, dried, and recrystallized from alcohol to obtain (**6.a–6.c**).

5-(5′-Chloro-2′-phenyl-1*H*-indol-3′-yl)ditetrazolo[1,5-a;1′,5′-c]pyrimidin-6-carbonitrile (**6.a**): Yield 61%, mp 278–279°C. FTIR (KBr) cm^{-1}: 3053 (indole NH); 2210 (CN); ^{1}HNMR (DMSO-d_6, δ ppm): 12.12 (s, 1H, indole NH), 8.02 (s, 1H, Ar–H), 7.95 (d, 1H, Ar–H), 7.83 (d, 1H, Ar–H), 7.65 (t, 2H, Ar–H), 7.59 (t, 1H, Ar–H), 7.24 (dd, 2H, Ar–H); MS (EI) *m/z* 412 (M^+), 414 (M^++2); Anal. % $C_{19}H_9N_{10}Cl$: C, 55.34; H, 2.18; N, 33.98. Found: C, 55.59; H, 2.39; N, 33.71.

5-(5′-Methyl-2′-phenyl-1*H*-indol-3′-yl)ditetrazolo[1,5-a;1′,5′-c]pyrimidin-6-carbonitrile (**6.b**): Yield 64%, mp 282–283°C. FTIR (KBr) cm^{-1}: 3050 (indole NH), 2214 (CN). ^{1}HNMR (DMSO-d_6, δ ppm): 12.0 (s, 1H, indole NH); 8.05 (s, 1H, Ar–H), 7.93 (d, 1H, Ar–H), 7.85 (d, 1H, Ar–H), 7.61 (t, 2H, Ar–H), 7.57 (t, 1H, Ar–H), 7.14 (dd, 2H, Ar–H); 2.12 (s, 3H, CH_3); Anal. % $C_{20}H_{12}N_{10}$: C, 61.22; H, 3.06; N, 35.71. Found: C, 61.15; H, 3.31; N, 35.97.

5-(2′-Phenyl-1*H*-indol-3′-yl)ditetrazolo[1,5-a;1′,5′-c] pyrimidin-6-carbonitrile (**6.c**): Yield 60%, mp 275–276°C. IR: ν/cm^{-1}: 3062 (indole NH), 2214 (CN). ^{1}HNMR (DMSO-d_6, δ ppm): 12.11 (s, 1H, indole NH); 8.25 (d, 1H, Ar–H), 8.02 (t, 1H, Ar–H), 7.78 (t, 1H, Ar–H), 7.85 (d, 1H, Ar–H), 7.77 (t, 2H, Ar–H), 7.53 (t, 1H, Ar–H),7.15 (dd, 2H, Ar–H); MS, 378 (M^+); Anal. % $C_{19}H_{10}N_{10}$: C, 60.32; H, 2.64; N, 37.04. Found: C, 60.55; H, 2.81; N, 36.95.

Biology

Antioxidant activity: 1,1-diphenyl-2-picryl hydrazyl (DPPH) RSA

The free RSAs of compounds **2–6** were carried out in the presence of the stable free radical DPPH following the literature procedure. (Hatano *et al.*, 1988) using 2-tert-butyl-4-methoxyphenol (butylated hydroxyl anisole, BHA) and 2-(1,1-dimethylethyl)-1,4-benzenediol (2-tert. butyl hydroquinone, TBHQ) as standards. The RSAs for methanolic solutions of compounds **2–6** at concentrations 25, 50, 75, and 100 μg/ml containing freshly prepared DPPH solution (0.004% w/v) were carried out and compared to those of standards, BHA and TBHQ. All the test analyses were performed on three replicates, and the results are averaged. The results in percentage are expressed as the ratio of the decrease in the absorption of DPPH in the presence of test compounds and the absorption of DPPH in the absence of test compounds at 517 nm using ELICO SL 171 Mini Spec spectrophotometer. The percentage

scavenging activity of the DPPH free radical was measured using the following equation

$$\%\text{DPPH raddical scavenging} = \frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

The results are shown in Figs. 1 and 2.

Antimicrobial activities

The antimicrobial activities (Indian pharmacopoeia, 1985) of the synthesized compounds were evaluated against *P. aeruginosa*, *S. aureus* (Gram negative bacteria), *K. pneumoniae* (gram negative bacteria), *A. oryzae*, and *A. niger* (fungi). An aliquot 0.1 ml of each bacterial strain was spread on nutrient agar, while 0.1 ml of the fungal spore suspension was spread on potato dextrose agar (PDA). An agar-well diffusion test was performed in each case. In these tests, 6 mm wells were produced using a sterile cork borer, and each well was then inoculated with 100 µl of each key substance in DMF. Nutrient agar plates were incubated at 37°C for 24 h, while the PDA plates incubated at 25°C for 72 h. The zone of inhibition around the well was determined. Gentamycin and fluconazole were used as the reference standards for antibacterial and antifungal activity, respectively.

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