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ORIGINAL PAPER

Synthesis and biological evaluation of some novel indole analogues containing triazolopyrimidine moiety

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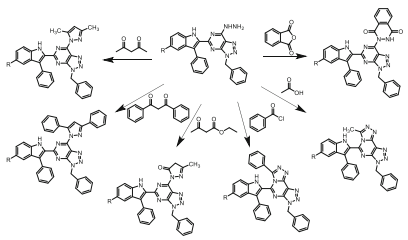
Abstract To examine new leads with potent antimicrobial and antioxidant activities, in this study, new indole analogues containing triazolopyrimidine moiety are synthesized and their structures have been established on the basis of their spectral studies and elemental analyses. These compounds were evaluated for their in vitro antimicrobial and antioxidant activities. Some of the compounds revealed good antimicrobial and antioxidant activity.

Graphical abstract



Keywords Indole · Triazolopyrimidine · Antimicrobial · Antioxidant

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Introduction

The potentiality of biologically active compound can be greatly altered by its minor structural modifications. This implies that a major line of approach is served by promising lead compounds substantiating molecular manipulation which involves an effort to combine separate pharmacophoric groups into one compound to achieve more potent molecule.

It is well known that the indole moiety which is probably the most widely spread nitrogen heterocycle in nature is very important for its medicinal and biological aspects, thus attracting a lot of scientific attention. It has been found to possess pharmacological and chemotherapeutic properties such as anticancer [1], antidiabetic [2], antinflammatory [3], antimalarial [4], antibacterial [5–7], antifungal [8], antiviral [9, 10], and so forth.

A large number of fused pyrimidines have been the focus of great importance and have reflected significant biological activities with appreciably wider spectrum like antiplatelet activity [11], anticancer [12], antimicrobial [13], *Escherichia coli* and *Staphylococcus aureus* SecA inhibitors [14], c-Met kinase inhibitors [15], and serotonin 5-HT_6 receptor antagonists [16]. The versatile applicability of these heterocycles has helped long to plan, organize, and implement new approaches towards discovery of novel drugs.

Hence, the synthesis of triazolopyrimidine derivatives has become a topic of particular interest because of their broad spectrum of biological properties. Some of these derivatives have shown remarkable biological properties such as antiviral, antitumor [17], adenosine A_{2A} receptor [18], analgesic, anticonvulsant, anti-inflammatory [19], antineoplastic [20], anticancer [21], and herbicidal [22]. Thus, fusion of pyrimidine moiety with different

heterocyclic scaffolds gives rise to a new class of hybrid heterocycles possessing improved activities.

Owing to the important role played by indole and evaluating the pharmacological profile of pyrimidine we have designed the program for the synthesis of indole fused to triazolopyrimidine so as to get biologically more potent molecules.

Results and discussion

Chemistry

The synthetic strategy to synthesize the target indolyl-triazolopyrimidine compounds is depicted in Scheme 1. The starting material 1-benzyl-5-amino-1*H*-1,2,3-triazole-4-carboxamide (**1**) was prepared by the 1,3-dipolar cycloaddition of benzyl azide and cyanoacetamide as previously reported method [23]. Reaction of **1** with ethyl 5-substituted-3-phenylindole-2-carboxylates **2a–2c** afforded the annulation product 3-benzyl-5-(5-substituted-3-phenyl-1*H*-indol-2-yl)-3*H*-[1,2,3] triazolo[4,5-*d*]pyrimidin-7-ols **3a–3c**. The triazolopyrimidine compounds **3a–3c** when subjected to chlorination with $POCl_3$ gave 3-benzyl-7-chloro-5-(5-substituted-3-phenyl-1*H*-indol-2-yl)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidines **4a–4c** which on reaction with hydrazine hydrate in refluxing ethanol afforded the key intermediate 3-benzyl-5-(5-substituted-3-phenyl-1*H*-indol-2-yl)-7-hydrazinyl-3*H*-[1,2,3] triazolo[4,5-*d*]pyrimidines **5a–5c**. The hydrazines **5a–5c** on cyclocondensation with acetyl acetone, dibenzoyl methane, and ethyl acetoacetate in refluxing methanol gave the respective pyrazole derivatives **6a–6c**, **7a–7c**, and **8a–8c**. Similarly, the hydrazines **5a–5c** on cyclization with benzoyl chloride and acetic acid under reflux conditions yielded the respective cyclization products **9a–9c** and **10a–10c**. Further the hydrazines **5a–5c** when reacted with phthalic anhydride in dimethyl formamide at reflux temperature gave the compounds 2-[3-benzyl-5-(5-substituted-3-phenyl-1*H*-indol-2-yl)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7-yl]-2,3-dihydrophthalazine-1,4-diones **11a–11c**. The structures of all the new compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and mass spectral studies and elemental analyses.

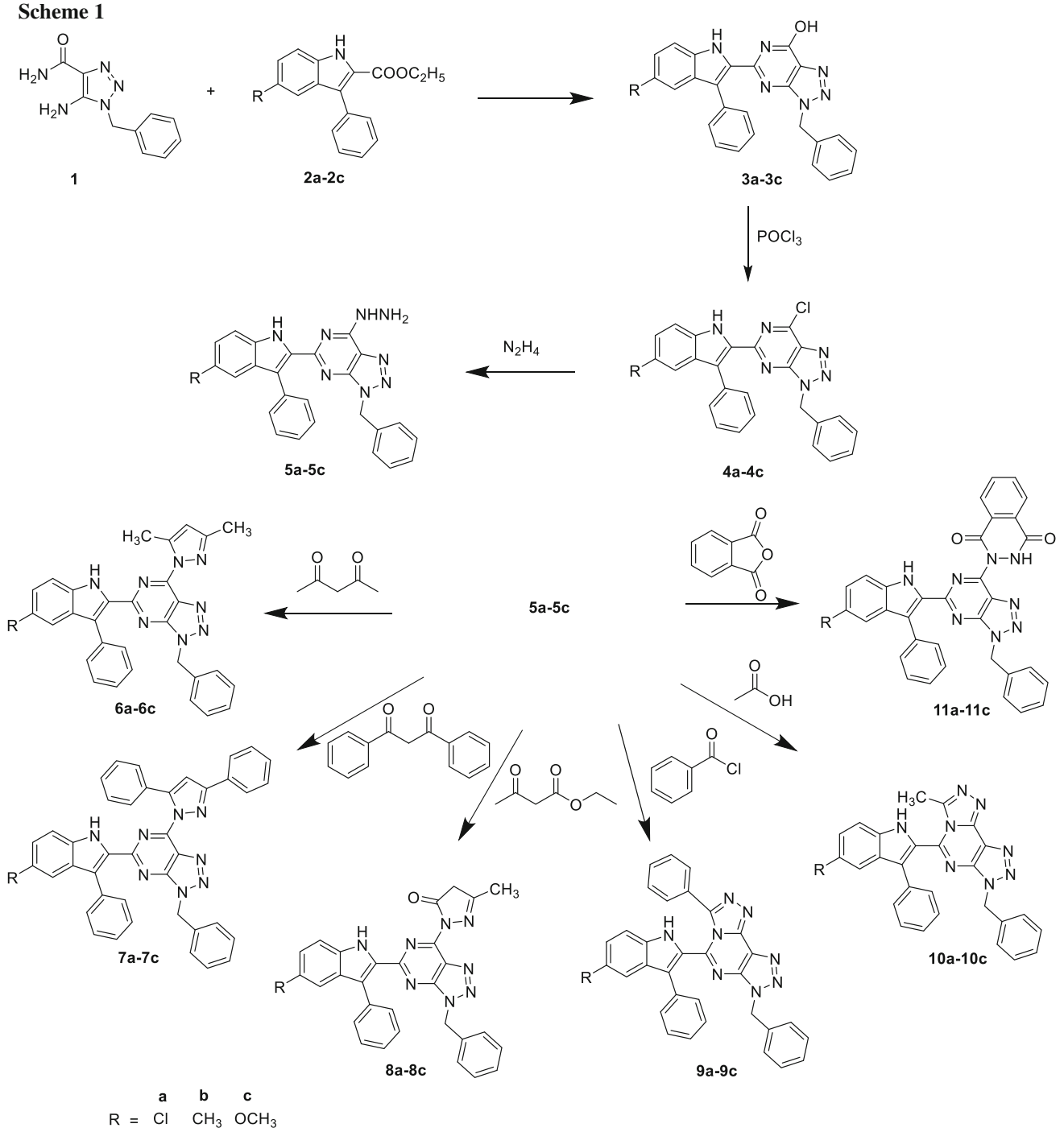
In the IR spectrum of **3a**, absorption bands at 3351 and 3318 cm^{-1} were observed due to OH and indole NH groups, respectively. Furthermore, its ^{1}H NMR spectrum exhibited characteristic signals at δ = 11.86, 5.64, and 5.35 ppm due to the protons of indole NH, CH_2 of benzyl moiety, and OH, respectively. Formation of **4a** was confirmed by the disappearance of OH stretching frequency at 3351 cm^{-1} and the appearance of new C–Cl stretching frequency at 761 cm^{-1} in its IR spectrum. The absence of the signal due to OH proton at 5.35 ppm in its ^{1}H NMR spectrum is the additional evidence for the formation of **4a**. The IR spectrum of **5a** exhibited absorption bands at 3407/3261 cm^{-1} and in its ^{1}H NMR spectrum, signals at 8.93 and 4.50 ppm corresponding to NH/NH_2, respectively. In the IR spectrum of compounds **6a**, **7a**, and **8a** the absorption bands due to NH/NH_2 groups at 3407/3261 cm^{-1} were missing. In its ^{1}H NMR spectrum, compound **6a** revealed signals at 5.93, 2.40, and 2.01 ppm due to the protons of pyrazole CH and the two CH_3 groups. While in the ^{1}H NMR spectrum of **7a**, signal at 6.75 ppm resonated due to pyrazole CH. In the IR spectrum of **8a**, characteristic absorption band at 1732 cm^{-1} was observed due to C=O group and its ^{1}H NMR spectrum exhibited signals at 4.10 and 3.10 ppm due to pyrazole CH_2 and CH_3, respectively. In the IR spectra of compounds **9a** and **10a**, the absorption bands at 3407/3261 cm^{-1} due to NH/NH_2 groups were missing. The ^{1}H NMR spectrum of **10a** showed signal at 1.25 ppm which was attributed to the protons of CH_3. Compound **11a** in its IR spectrum exhibited absorption band at 3363 cm^{-1} corresponding to the NH group and in its ^{1}H NMR spectrum a signal at 10.88 ppm appeared due to NH. The structures of all the compounds were further established by ^{13}C NMR and mass spectral studies and elemental analyses.

In vitro antimicrobial activity

The newly synthesized compounds were screened for their in vitro antimicrobial activity by cup-plate method (Indian Pharmacopoeia, 1985). This method depends on the diffusion of antibiotic from a cavity through the solidified agar layer in a petri-dish to an extent such that the growth of the added microorganism is prevented in a circular zone around the cavity containing a solution of antibiotic. For antibacterial activity *S. aureus* (ATCC-29513), *E. coli* (MTCC-723), and *Pseudomonas aeruginosa* (MTCC-1688) were used as pathogenic representatives, whereas *Aspergillus niger* (MTCC-281), *Aspergillus flavus* (MTCC-1973), and *Aspergillus oryzae* (MTCC-3567^T) were used for antifungal activity. Streptomycin and fluconazole were used as standards for antibacterial and antifungal activities, respectively.

The investigation of antibacterial screening (Table 1) revealed that all tested compounds have moderate to high antibacterial activity as compared to the standard drug streptomycin. Compounds **4a**, **8a**, and **11a** showed excellent antibacterial activity against the tested microorganism *S. aureus*. Compounds **4c**, **7a**, and **10c** exhibited good activity against *E. coli*. While the compounds **5a**, **8c**, and **11a** displayed good activity against *P. aeruginosa*.

Scheme 1



On the other hand, the antifungal activity results (Table 2) suggested that the compounds **5a**, **6c**, and **8a** revealed profound activity against *A. niger*. Compound **3c** showed good activity against *A. flavus*. Whereas the compounds **3a** and **11c** exhibited good activity against *A. oryzae*. Rest of the compounds was either moderately active or inactive against the bacterial and fungal strains.

Antioxidant activities

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (RSA)

1,1-Diphenyl-2-picrylhydrazyl, a stable free radical, has an odd electron and thus a strong absorption at 517 nm. When this electron becomes paired off, the absorption decreases

Table 1 In vitro antibacterial activity of compounds **3–11**

Comp.	Zone of inhibition/mm (concentration in μg cm^{-3})											
	S. aureus				*E. coli*				*P. aeruginosa*			
	1000	500	250	125	1000	500	250	125	1000	500	250	125
3a	10	09	08	10	10	11	12	12	11	10	10	10
3b	09	08	07	09	10	11	11	12	10	09	–	–
3c	10	13	15	15	11	10	12	12	08	08	11	12
4a	20	19	16	13	11	13	10	10	09	10	12	11
4b	–	–	–	–	08	07	–	07	–	–	–	–
4c	09	10	10	11	12	13	12	10	10	08	08	09
5a	10	12	13	12	11	10	10	10	13	12	11	11
5b	08	06	06	07	09	08	07	09	09	10	10	09
5c	12	11	09	10	11	11	10	09	09	10	11	11
6a	09	08	08	09	10	07	08	07	06	05	05	06
6b	04	02	03	03	05	04	04	04	06	07	07	07
6c	10	09	10	10	08	07	08	06	06	05	05	04
7a	13	12	12	12	11	12	12	10	12	10	10	09
7b	09	08	08	07	06	07	07	07	10	09	08	08
7c	12	11	10	10	10	09	10	11	11	12	12	10
8a	13	13	12	12	11	10	09	10	11	09	11	11
8b	09	08	07	07	09	10	10	09	08	07	06	07
8c	11	10	11	11	11	12	10	10	11	11	12	11
9a	12	10	11	11	10	09	10	11	08	09	08	08
9b	09	07	07	06	09	09	08	09	07	09	10	08
9c	06	05	04	05	07	08	06	07	10	09	09	08
10a	09	09	09	08	10	09	09	09	05	04	05	06
10b	03	03	02	03	05	04	03	05	07	09	07	06
10c	10	11	11	10	12	12	11	12	09	10	10	09
11a	14	13	13	12	10	09	10	10	12	11	12	12
11b	10	09	10	10	08	09	08	08	10	09	09	09
11c	12	11	11	10	10	08	08	09	11	11	10	09
Streptomycin	15	15	15	15	15	14	14	13	14	13	13	13

stoichiometrically with respect to the number of electrons or hydrogen atoms taken up. Such a change in absorption by this reaction has been extensively adopted to test the capacity of several molecules to act as free radical scavengers. The scavenging effects of the synthesized compounds on the DPPH radical were evaluated. The results were compared with the standards 2-*tert*-butyl-4-methoxyphenol (butylated hydroxyanisole, BHA), 2-(1,1-dimethylethyl)-1,4-benzenediol (2-*tert*-butyl hydroquinone, TBHQ), and ascorbic acid (AA). The antioxidant capacity was expressed as the 50% inhibitory concentration (IC50) based on the amount of compound required for a 50% decrease of the initial DPPH radical concentration. The values of IC50, the effective concentration at which 50% of the radicals scavenged, were calculated to evaluate the antioxidant activity. The compounds **3–11** showed minimum IC50 value, i.e., less than the 25 μg cm^{-3} concentration. The results, as depicted in Table 3 (Figs. 1, 2, 3, 4, 5) suggested that compounds **5a** and **8a** showed promising RSA at all concentrations. Compound **8c** was found to enhance the RSA 72.17, 76.95, and 77.82% at 50, 75, and 100 μg cm^{-3}, respectively. Compound **11a** showed good activity, i.e., 71.73 and 72.17 at 25 and 100 μg cm^{-3}, respectively. While compound **3c** showed good activity, i.e., 72.54% at 25 μg cm^{-3} and **5c** showed 76.95% activity at 75 μg cm^{-3}. Rest of the compounds was found to augment the RSA to a lesser extent.

Ferric ion (Fe^{3+}) reducing antioxidant power (FRAP)

The reductive ability of the synthesized compounds was assessed by the extent of conversion of Fe^{3+}/ferricyanide complex to the Fe^{2+}/ferrous form, at different concentrations (25, 50, 75, 100 μg cm^{-3}) and the results were compared with standards BHA, TBHQ, and AA. Reductive ability results (Figs. 6, 7, 8, 9 10) suggested that compounds **6a**, **7a**, **7c**, and **8a** reduced metal ion complex to its

Table 2 In vitro antifungal activity of compounds **3–11**

Comp.	Zone of inhibition/mm (concentration in μg cm^{-3})											
	A. niger				*A. flavus*				*A. oryzae*			
	1000	500	250	125	1000	500	250	125	1000	500	250	125
3a	10	09	09	08	11	09	10	09	13	12	12	10
3b	11	11	10	09	12	12	11	10	10	12	11	11
3c	11	10	10	10	13	11	12	11	09	07	08	10
4a	13	12	10	10	12	12	12	10	12	11	10	10
4b	–	10	08	–	12	11	09	–	10	10	09	08
4c	11	08	08	07	08	10	09	09	07	06	05	06
5a	12	10	13	12	07	12	09	–	09	11	11	10
5b	08	–	–	–	07	07	08	08	12	12	09	–
5c	07	08	07	–	10	11	11	12	09	12	10	09
6a	12	11	09	–	09	10	10	11	12	12	11	10
6b	12	10	08	09	13	10	07	08	09	08	–	–
6c	13	11	10	11	13	12	11	10	12	10	11	09
7a	07	05	05	06	04	03	02	02	09	07	08	08
7b	05	05	04	05	06	07	07	07	09	09	09	08
7c	10	10	11	10	09	10	10	11	12	11	10	10
8a	13	12	12	10	11	10	10	09	12	10	10	11
8b	10	09	09	10	08	07	07	06	10	11	10	11
8c	12	12	11	10	06	07	09	08	10	10	09	08
9a	07	09	08	–	12	10	09	09	06	07	07	05
9b	09	08	08	07	08	09	07	05	10	09	09	08
9c	12	11	11	10	11	11	10	–	10	10	11	10
10a	11	10	10	–	13	12	10	10	10	11	12	11
10b	10	11	–	–	12	09	07	–	10	10	11	09
10c	08	06	07	06	10	09	10	09	11	10	09	09
11a	12	12	11	10	12	11	09	09	07	08	08	07
11b	10	09	09	08	05	06	07	07	12	10	10	09
11c	11	11	11	10	09	10	11	11	12	12	11	12
Fluconazole	14	13	12	12	14	13	13	12	14	14	13	13

lower oxidation state or took part in electron transfer reaction to a quite good extent. Rest of the compounds showed comparatively lesser reducing ability. In other words, these compounds showed the ability of electron donor to scavenge free radicals. The reducing ability of the synthesized compounds indicated that increase in the concentration of samples increases the reductive ability. Thus, higher the absorbance of compounds, greater is the reducing power.

Ferrous (Fe^{2+}) ion metal chelating activity

Ferrous ion is the prooxidant among the various species of metal ion. Minimizing ferrous ions afford protection of reactive oxygen species and lipid production. The effective ferrous ion chelators protects by removing ferrous ion (Fe^{2+}) that may otherwise participate in the generation of hydroxyl radical. The chelating effect of ferrous ion with test compounds was determined and results were compared with the standards BHA, TBHQ, and AA. Ferrozine can quantitatively form complex with ferrous ion in this method. In the presence of chelating agents the complex formation is disrupted resulting in a decrease in red color of the complex. Measurement of color reduction, therefore, allows estimating the metal chelating activity of the coexisting chelators. Lower absorption indicates higher metal chelating activity. In this assay, synthesized compounds interfere with the formation of ferrous and ferrozine complex. These results (Figs. 11, 12, 13, 14, 15) suggested that the compounds **6a**, **7a**, and **7b** exhibited enhanced activity at all concentrations. Compound **8a** displayed good activity, i.e., 71.46 and 72.82% at 50 and 75 μg cm^{-3}. Compound **4a** showed good activity, i.e., 76.34 and 79.03% at 75 and 100 μg cm^{-3}, respectively. While compounds **3a** and **3c** exhibited good activity, i.e., 75.26 and 78.76%, respectively, at

Table 3 In vitro antibacterial activity with IC 50% values of compounds **3–11**

Comp.	Concentration				
	25 μg cm^{-3}/%	50 μg cm^{-3}/%	75 μg cm^{-3}/%	100 μg cm^{-3}/%	IC 50/μg cm^{-3}
3a	66.72	63.12	66.13	65.13	<25
3b	66.53	66.13	65.73	60.92	<25
3c	69.33	72.54	63.12	66.93	<25
4a	62.52	59.11	63.52	70.14	<25
4b	44.28	44.88	43.68	46.89	<25
4c	48.49	49.69	50.30	51.90	<25
5a	73.04	70.43	79.13	74.34	<25
5b	69.13	65.21	71.30	70.87	<25
5c	68.26	66.52	76.95	70.00	<25
6a	51.30	60.43	57.39	54.78	<25
6b	44.34	40.87	47.82	49.13	<25
6c	48.69	50.43	46.95	53.91	<25
7a	60.13	62.87	59.16	68.24	<25
7b	56.65	57.39	62.17	59.13	<25
7c	66.95	64.24	69.13	58.26	<25
8a	86.52	86.95	87.39	87.82	<25
8b	70.00	69.03	65.65	69.50	<25
8c	70.87	72.17	76.95	77.82	<25
9a	55.21	57.82	58.69	59.13	<25
9b	51.30	51.73	46.00	43.04	<25
9c	57.39	53.04	50.00	56.95	<25
10a	69.13	70.00	67.82	61.30	<25
10b	63.47	66.95	59.13	60.87	<25
10c	64.78	60.60	67.39	58.26	<25
11a	71.73	64.34	69.56	72.17	<25
11b	59.13	53.91	45.65	57.82	<25
11c	42.78	40.43	46.08	49.13	<25

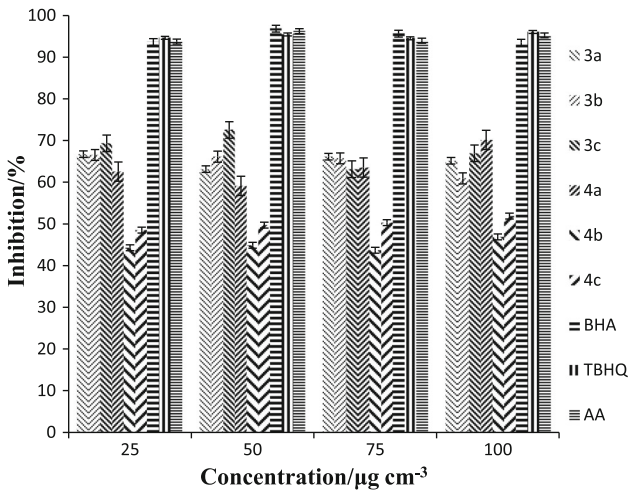


Fig. 1 Graphical representation for DPPH radical scavenging activity of compounds **3a–3c** and **4a–4c**

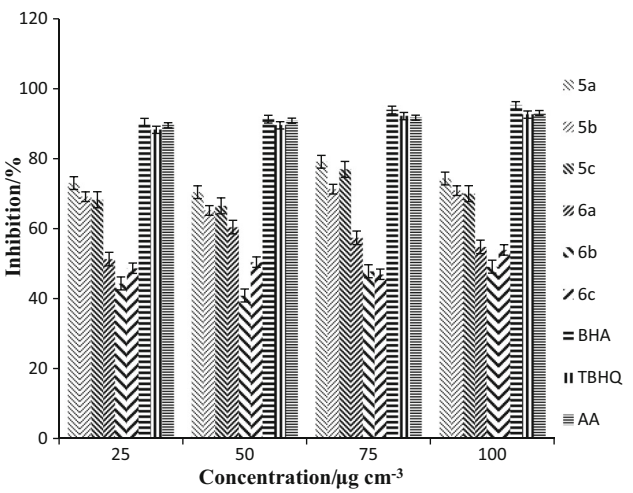


Fig. 2 Graphical representation for DPPH radical scavenging activity of compounds **5a–5c** and **6a–6c**

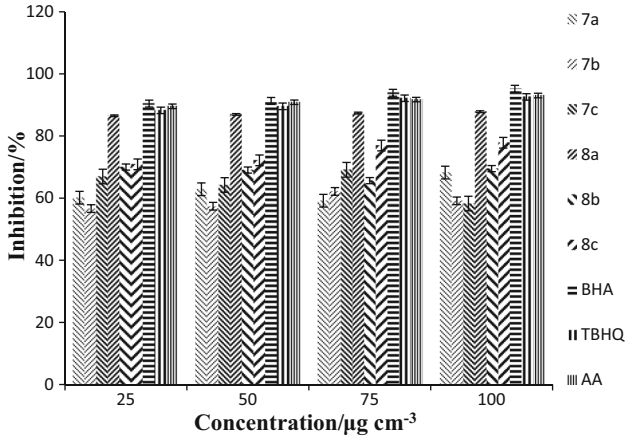


Fig. 3 Graphical representation for DPPH radical scavenging activity of compounds **7a–7c** and **8a–8c**

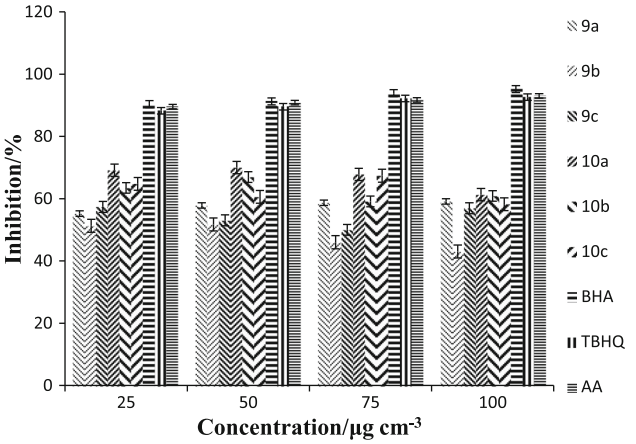


Fig. 4 Graphical representation for DPPH radical scavenging activity of compounds **9a–9c** and **10a–10c**

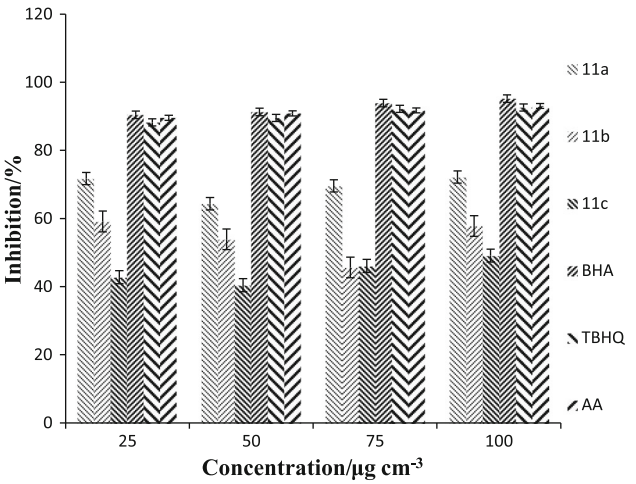


Fig. 5 Graphical representation for DPPH radical scavenging activity of compounds **11a–11c**

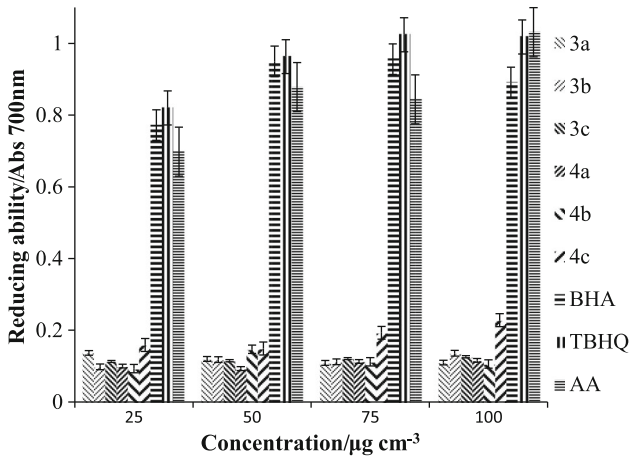


Fig. 6 Graphical representation for ferric ion (Fe^{3+}) reducing antioxidant power (FRAP) of compounds **3a–3c** and **4a–4c**

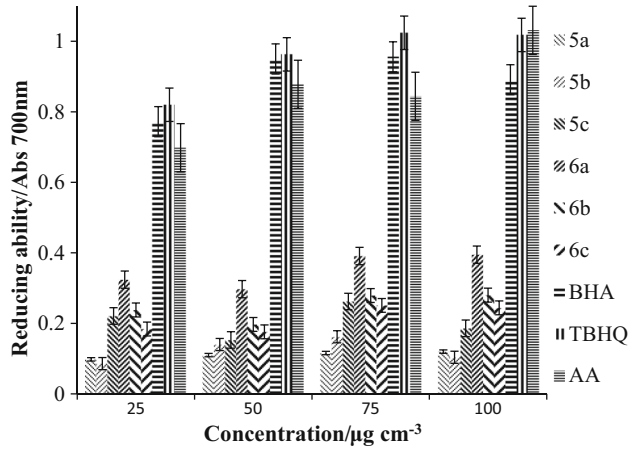


Fig. 7 Graphical representation for ferric ion (Fe^{3+}) reducing antioxidant power (FRAP) of compounds **5a–5c** and **6a–6c**

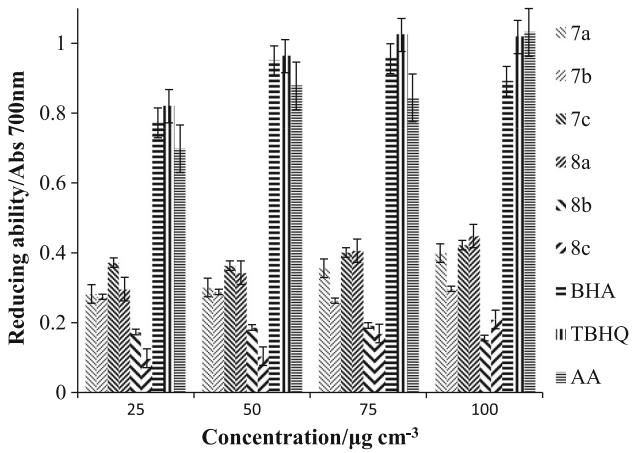


Fig. 8 Graphical representation for ferric ion (Fe^{3+}) reducing antioxidant power (FRAP) of compounds **7a–7c** and **8a–8c**

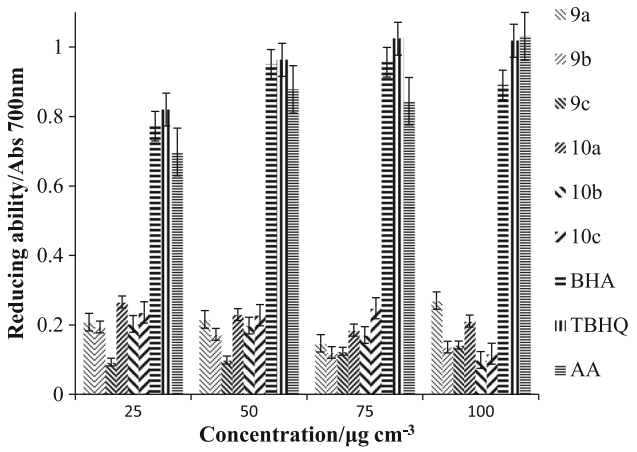


Fig. 9 Graphical representation for ferric ion (Fe^{3+}) reducing antioxidant power (FRAP) of compounds **9a–9c** and **10a–10c**

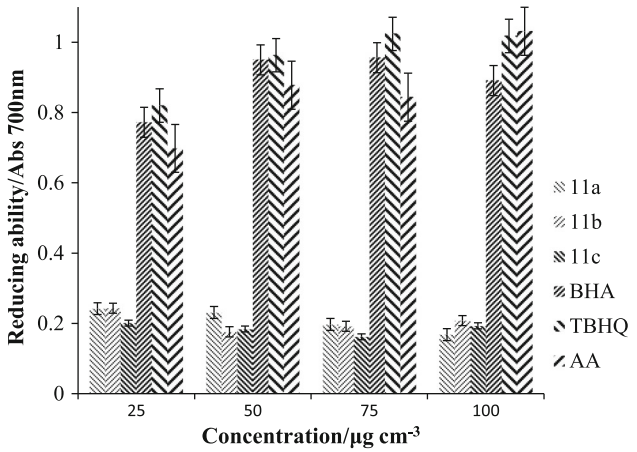


Fig. 10 Graphical representation for ferric ion (Fe^{3+}) reducing antioxidant power (FRAP) of compounds **11a–11c**

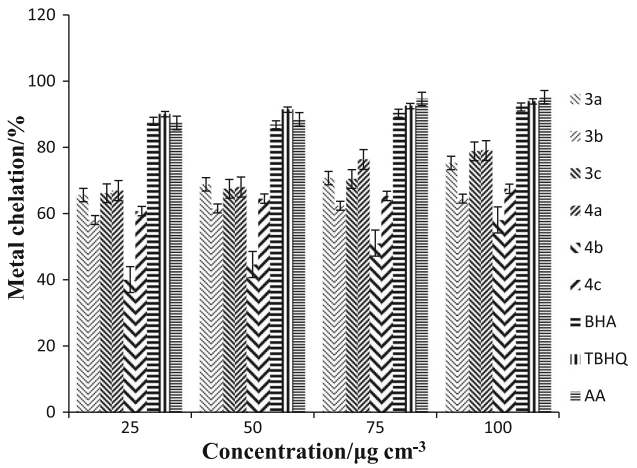


Fig. 11 Graphical representation for ferrous (Fe^{2+}) ion metal chelating activity of compounds **3a–3c** and **4a–4c**

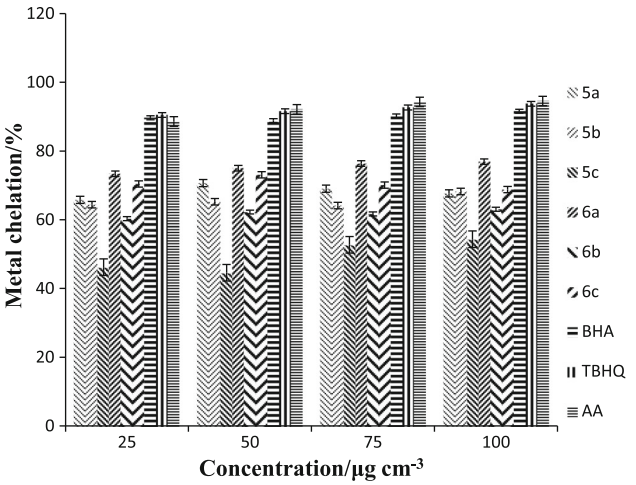


Fig. 12 Graphical representation for ferrous (Fe^{2+}) ion metal chelating activity of compounds **5a–5c** and **6a–6c**

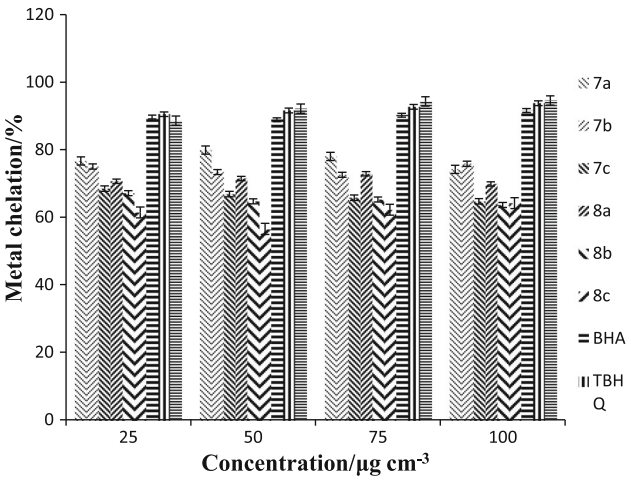


Fig. 13 Graphical representation for ferrous (Fe^{2+}) ion metal chelating activity of compounds **7a–7c** and **8a–8c**

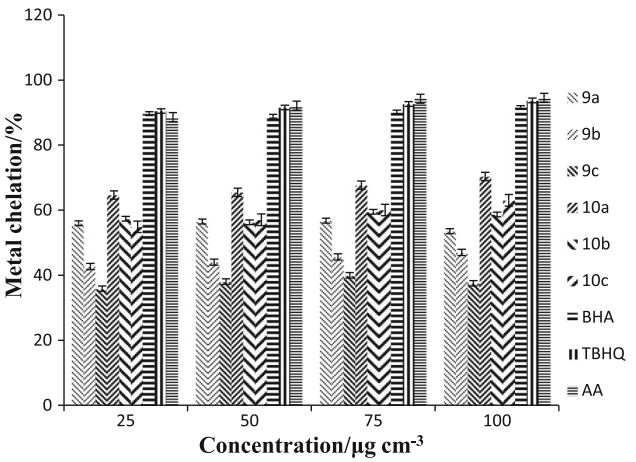


Fig. 14 Graphical representation for ferrous (Fe^{2+}) ion metal chelating activity of compounds **9a–9c** and **10a–10c**

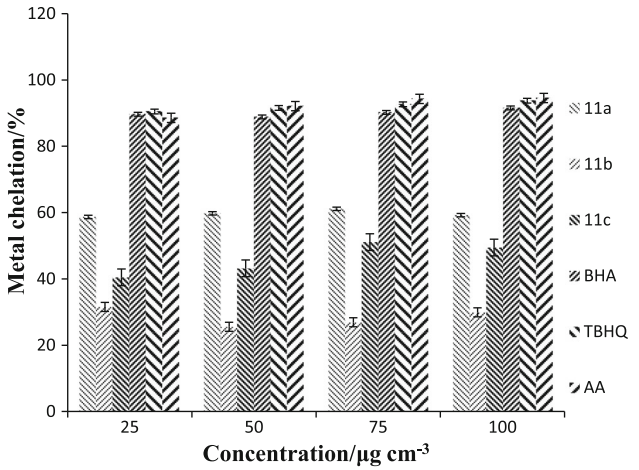


Fig. 15 Graphical representation for ferrous (Fe^{2+}) ion metal chelating activity of compounds **11a–11c**

100 µg cm^{-3}, compound **6c** showed 73.09% activity at 50 µg cm^{-3}. Of the remaining, some compounds were moderately active and some were less active.

Conclusion

The objective of the present study was to synthesize the entitled compounds, i.e., indole derivatives incorporated with triazolopyrimidine and evaluate their antimicrobial and antioxidant activities which were done efficiently and the structures for all of which have been established on the basis of their spectral studies. From the SAR study, we can conclude that the antimicrobial and antioxidant activities may be due to:

- The presence of chloro and methoxy substitutions; evidently by the presence of which enhanced the activity in contrast to the same devoid of any substitution.
- The triazolopyrimidine generally augmenting the activity.
- The indole moiety proving essential for activity.

Furthermore, by the integration of triazolopyrimidine with the parent indole moiety, we have added a new class of compounds to the unit of bioactive molecules.

Experimental

All the reagents were obtained commercially and used by further purification. Melting points were determined by an open capillary method. Purity of the compounds was checked by thin layer chromatography using silica gel-G coated aluminium plates (Merck). The IR spectra were recorded on Thermo Fischer (id S-5) FT-IR spectrometer. The ^{1}H NMR spectra were recorded with a BRUKER NMR (400 or 500 MHz), ^{13}C NMR spectra were recorded by BRUKER AVANCE II (100 MHz) spectrometer and the mass spectral data were recorded by electron impact method on JEOL GCMATE II GC–MS mass spectrometer. Elemental analysis was carried out using Flash EA 1112 series elemental analyzer.

1-Benzyl-5-amino-1*H*-1,2,3-triazole-4-carboxamide (**1**) was synthesized by the reported literature method [23]. Ethyl 5-substituted-3-phenylindol-2-carboxylates **2a–2c** were prepared as per the reported literature procedure [24].

*General procedure for the synthesis of 3-benzyl-5-(5-substituted-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-ols **3a–3c***

To a solution of compound **1** (1 mmol) in anhydrous ethyl alcohol ethyl-5-substituted-3-phenylindol-2-carboxylates **2a–2c** (2 mmol) was added and refluxed for 40 h. Further the excess of solvent was removed under reduced pressure and the residue was decomposed in crushed ice followed by neutralization with acetic acid to get the product which was collected by filtration, washed with water, dried, and recrystallized from ethanol.

3-Benzyl-5-(5-chloro-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]-triazolo[4,5-d]pyrimidin-7-ol (**3a**, $C_{25}H_{17}ClN_6O$)
Pale green crystals; 0.36 g (72%); m.p.: 195 °C; $R_f = 0.72$ (benzene/ethyl acetate 8:2 v/v); ^{1}H NMR (500 MHz, DMSO-d_6): $\delta = 11.86$ (s, 1H, indole NH), 7.18-7.80 (m, 13H, Ar–H), 5.64 (s, 2H, CH_2), 5.35 (s, 1H, OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 165.13$, 159.19, 154.18, 152.15, 139.13, 132.88, 132.44, 132.36, 131.46, 131.12, 130.85, 130.00, 129.49 128.93, 128.55, 126.25, 125.14, 122.72, 121.84, 113.14, 46.55 ppm; IR (thin film): $\bar{\nu} = 3351$ (OH), 3318 (indole NH), 1666 (C=N) cm^{-1}; MS (70 eV): $m/z = 452$ (M^+), 454 ([M + 2]$^+$).

3-Benzyl-5-(5-methyl-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]-triazolo[4,5-d]pyrimidin-7-ol (**3b**, $C_{26}H_{20}N_6O$)
Pale green crystals; 0.32 g (65%); m.p.: 120 °C; $R_f = 0.70$ (benzene/ethyl acetate 8:2 v/v); ^{1}H NMR (500 MHz, DMSO-d_6): $\delta = 11.62$ (s, 1H, indole NH), 7.15–7.79 (m, 13H, Ar–H), 5.61 (s, 2H, CH_2), 5.26 (s, 1H, OH), 2.8 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 164.55$, 160.05, 154.25, 152.55, 140.02, 133.01, 132.54, 132.39, 131.52, 131.09, 130.94, 130.09, 129.68 129.03, 128.35, 126.75, 125.38, 122.58, 121.66, 113.16, 46.98, 27.89 ppm; IR (thin film): $\bar{\nu} = 3345$ (OH), 3310 (indole NH), 1658 (C=N) cm^{-1}.

3-Benzyl-5-(5-methoxy-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-ol (**3c**, $C_{26}H_{20}N_6O_2$)
Green crystals; 0.33 g (67.7%); m.p.: 177 °C; $R_f = 0.69$ (benzene/ethyl acetate 8:2 v/v); ^{1}H NMR (500 MHz, DMSO-d_6): $\delta = 11.89$ (s, 1H, indole NH), 7.20–7.88 (m,

13H, Ar–H), 5.68 (s, 2H, CH_2), 5.40 (s, 1H, OH), 3.82 (s, 3H, OCH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 164.98$, 159.99, 154.15, 152.98, 140.42, 133.25, 132.64, 132.40, 131.48, 131.19, 130.86, 130.10, 129.71 129.26, 128.35, 126.58, 125.42, 122.60, 121.70, 113.20, 50.55, 49.42 ppm; IR (thin film): $\bar{\nu} = 3352$ (OH), 3320 (indole NH), 1664 (C=N) cm^{-1}.

*General procedure for the synthesis of 3-benzyl-7-chloro-5-(5-substituted-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidines **4a–4c***

Compound **3** (1 mmol) in 5 cm^3 $POCl_3$ was heated under reflux for 8 h. The reaction mixture was cooled to room temperature and decomposed in crushed ice. The product separated was filtered, washed with water, dried, and recrystallized from benzene.

3-Benzyl-7-chloro-5-(5-chloro-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidine (**4a**, $C_{25}H_{16}Cl_2N_6$)

Pale yellow crystals; 0.36 g (73%); m.p.: 230 °C; $R_f = 0.54$ (chloroform/ethyl acetate 7:3 v/v);^1H NMR (500 MHz, DMSO-d_6): $\delta = 12.04$ (s, 1H, indole NH), 7.25–7.70 (m, 13H, Ar–H), 5.68 (s, 2H, CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 167.25$, 160.39, 156.25, 153.18, 140.09, 134.55, 132.46, 130.41, 130.09, 129.70, 129.43, 129.19, 128.85, 128.55, 128.30, 127.18, 126.86, 125.14, 122.12, 113.14, 48.11 ppm; IR (thin film): $\bar{\nu} = 3319$ (indole NH), 1678 (C=N), 761 (C–Cl) cm^{-1}; MS (70 eV): $m/z = 470$ (M^+), 472 ($[M + 2]^+$), 474 ($[M + 4]^+$).

3-Benzyl-7-chloro-5-(5-methyl-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidine (**4b**, $C_{26}H_{19}ClN_6$)

Pale yellow crystals; 0.31 g (63%); m.p.: 220 °C; $R_f = 0.55$ (chloroform/ethyl acetate 7:3 v/v); ^1H NMR (500 MHz, DMSO-d_6): $\delta = 12.01$ (s, 1H, indole NH), 7.25–7.69 (m, 13H, Ar–H), 5.65 (s, 2H, CH_2), 2.52 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 167.48$, 160.42, 156.30, 153.10, 140.15, 134.68, 132.49, 130.57, 130.21, 129.85, 129.44, 129.21, 128.96, 128.32, 128.29, 127.15, 126.46, 125.11, 122.42, 113.23, 50.56, 29.28 ppm; IR (thin film): $\bar{\nu} = 3315$ (indole NH), 1672 (C=N), 758 (C–Cl) cm^{-1}.

3-Benzyl-7-chloro-5-(5-methoxy-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidine (**4c**, $C_{26}H_{19}ClN_6O$)

Pale yellow crystals; 0.34 g (69%); m.p.: 196 °C; $R_f = 0.54$ (chloroform/ethyl acetate 7:3 v/v); ^1H NMR (500 MHz, DMSO-d_6): $\delta = 12.09$ (s, 1H, indole NH), 7.35–7.72 (m, 13H, Ar–H), 5.71 (s, 2H, CH_2), 3.53 (s, 3H, OCH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 167.05$, 160.15, 156.10, 153.05, 140.02, 134.48, 132.39, 130.38, 130.00, 129.60, 129.28, 129.14, 128.67, 128.35, 128.10, 127.08, 126.14, 125.02, 122.01, 113.41, 51.58, 49.99 ppm; IR (thin film): $\bar{\nu} = 3322$ (indole NH), 1680 (C=N), 765 (C–Cl) cm^{-1}.

*General procedure for the synthesis of 3-benzyl-5-(5-substituted-3-phenyl-1H-indol-2-yl)-7-hydrazinyl-3H-[1,2,3]-triazolo[4,5-d]pyrimidines **5a–5c***

Compounds **4a–4c** (10 mmol) and 5 cm^3 of hydrazine hydrate (98%) were refluxed in absolute alcohol for 9 h. The hydrazine derivative separated after cooling was filtered, washed with alcohol, dried, and crystallized using benzene to afford the compounds **5a–5c**.

3-Benzyl-5-(5-chloro-3-phenyl-1H-indol-2-yl)-7-hydrazinyl-3H-[1,2,3]triazolo[4,5-d]pyrimidine (**5a**, $C_{25}H_{19}ClN_8$)

Brown crystals; 0.41 g (82%); m.p.: 170 °C; $R_f = 0.54$ (chloroform/ethyl acetate 8:2 v/v); ^1H NMR (500 MHz, DMSO-d_6): $\delta = 11.95$ (s, 1H, indole NH), 8.93 (s, 1H, NH), 7.22–7.47 (m, 13H, Ar–H), 5.49 (s, 2H, CH_2), 4.50 (s, 2H, NH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 169.54$, 161.22, 158.35, 150.06, 133.08, 133.00, 132.90, 130.68, 129.91, 129.84, 129.24, 128.86, 128.35, 126.69, 126.68, 126.45, 125.92, 120.06, 119.59, 113.33, 49.28 ppm; IR (thin film): $\bar{\nu} = 3407/3261$ (NH/NH_2), 3056 (indole NH), 1602 (C=N) cm^{-1}; MS (70 eV): $m/z = 466$ (M^+), 468 ($[M + 2]^+$).

3-Benzyl-5-(5-methyl-3-phenyl-1H-indol-2-yl)-7-hydrazinyl-3H-[1,2,3]triazolo[4,5-d]pyrimidine (**5b**, $C_{26}H_{22}N_8$)

Pale brown crystals; 0.35 g (71%); m.p.: 214 °C; $R_f = 0.55$ (chloroform/ethyl acetate 8:2 v/v); ^1H NMR (500 MHz, DMSO-d_6): $\delta = 11.92$ (s, 1H, indole NH), 8.87 (s, 1H, NH), 7.24–7.45 (m, 13H, Ar–H), 5.45 (s, 2H, CH_2), 4.48 (s, 2H, NH_2), 2.74 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 169.89$, 161.55, 158.25, 150.04, 133.10, 133.02, 132.70, 130.55, 129.84, 129.56, 129.14, 128.75, 128.28, 126.70, 126.48, 126.35, 125.81, 120.02, 119.60, 113.35, 51.52, 29.88 ppm; IR (thin film): $\bar{\nu} = 3405/3260$ (NH/NH_2), 3052 (indole NH), 1589 (C=N) cm^{-1}.

3-Benzyl-5-(5-methoxy-3-phenyl-1H-indol-2-yl)-7-hydrazinyl-3H-[1,2,3]triazolo[4,5-d]pyrimidine (**5c**, $C_{26}H_{22}N_8O$)

Brown crystals; 0.31 g (63%); m.p.: 156 °C; $R_f = 0.55$ (chloroform/ethyl acetate 8:2 v/v); ^1H NMR (500 MHz, DMSO-d_6): $\delta = 11.98$ (s, 1H, indole NH), 8.95 (s, 1H, NH), 7.50–7.72 (m, 13H, Ar–H), 5.52 (s, 2H, CH_2), 4.51 (s, 2H, NH_2), 3.98 (s, 3H, OCH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.01$, 161.85, 158.55, 150.45, 133.45, 133.34, 132.89, 130.65, 129.98, 129.65, 129.34, 128.91 128.48, 126.82, 126.51, 126.41, 125.95, 120.21, 119.55, 113.48, 51.98, 49.98 ppm; IR (thin film): $\bar{\nu} = 3410/3264$ (NH/NH_2), 3059 (indole NH), 1610 (C=N) cm^{-1}.

General procedure for the synthesis of 3-benzyl-5-(5-substituted-3-phenyl-1H-indol-2-yl)-7-(3,5-dimethyl-1H-pyrazol-1-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidines ***6a–6c****, 3-benzyl-5-(5-substituted-3-phenyl-1H-indol-2-yl)-7-(3,5-diphenyl-1H-pyrazol-1-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidines* ***7a–7c****, and 1-[3-benzyl-5-(5-substituted-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl]-3-methyl-1H-pyrazol-5(4H)-ones* ***8a–8c***

A mixture of hydrazine derivatives **5a**–**5c** (1 mmol), acetylacetone (or dibenzoylmethane or ethyl acetoacetate) (1 mmol) in 20 cm^3 of dry methanol containing 4–5 drops of conc. HCl was refluxed for 4 h on steam bath. The excess of methanol was removed under vacuum. The reaction mixture was cooled to room temperature. The separated solid was filtered off, washed with little methanol, dried, and recrystallized using ethanol to furnish compounds **6a**–**6c**, **7a**–**7c**, and **8a**–**8c**.

3-Benzyl-5-(5-chloro-3-phenyl-1H-indol-2-yl)-7-(3,5-dimethyl-1H-pyrazol-1-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidine (**6a**, $C_{30}H_{23}ClN_8$)

Grey crystals; 0.33 g (66%); m.p.: 118 °C; $R_f = 0.62$ (toluene/ethyl acetate 6:4 v/v); ^{1}H NMR (500 MHz, DMSO-d_6): $\delta = 11.50$ (s, 1H, indole NH), 7.26–7.75 (m, 13H, Ar–H), 5.93 (s, 1H, pyrazole CH), 5.24 (s, 2H, CH_2), 2.40 (s, 3H, CH_3), 2.01 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.16$, 158.81, 152.39, 146.46, 134.41, 134.28, 133.96, 133.81, 133.76, 132.93, 131.30, 130.96, 130.69, 129.47, 128.75, 128.62, 127.93, 126.92, 126.25, 125.27, 123.19, 113.11, 111.41, 60.06, 15.09, 14.19 ppm; IR (thin film): $\bar{\nu} = 3289$ (indole NH), 1603 (C=N) cm^{-1}; MS (70 eV): $m/z = 531$ (M^+), 533 ($[M + 2]^+$).

3-Benzyl-5-(5-methyl-3-phenyl-1H-indol-2-yl)-7-(3,5-dimethyl-1H-pyrazol-1-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidine (**6b**, $C_{31}H_{26}N_8$)

Dark blue crystals; 0.29 g (58%); m.p.: 98–100 °C; $R_f = 0.62$ (toluene/ethyl acetate 6:4 v/v); ^{1}H NMR (500 MHz, DMSO-d_6): $\delta = 11.49$ (s, 1H, indole NH), 7.25-7.73 (m, 13H, Ar–H), 5.90 (s, 1H, pyrazole CH), 5.24 (s, 2H, CH_2), 2.62 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.01 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.99$, 158.75, 152.30, 146.45, 134.50, 134.41, 133.85, 133.75, 133.68, 132.89, 131.35, 130.85, 130.71, 129.52, 128.68, 128.51, 127.89, 126.85, 126.34, 125.30, 123.51, 113.18, 111.38, 60.15, 29.98, 15.55, 14.86 ppm; IR (thin film): $\bar{\nu} = 3285$ (indole NH), 1598 (C=N) cm^{-1}.

3-Benzyl-5-(5-methoxy-3-phenyl-1H-indol-2-yl)-7-(3,5-dimethyl-1H-pyrazol-1-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidine (**6c**, $C_{31}H_{26}N_8O$)

Dark blue crystals; 0.34 g (69%); m.p.: 135 °C; $R_f = 0.66$ (toluene/ethyl acetate 6:4 v/v); ^{1}H NMR (500 MHz, DMSO-d_6): $\delta = 11.52$ (s, 1H, indole NH), 7.25-7.75 (m, 13H, Ar–H), 5.96 (s, 1H, pyrazole CH), 5.25 (s, 2H, CH_2), 3.42 (s, 3H, OCH_3), 2.43 (s, 3H, CH_3), 2.04 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.88$, 158.77, 152.40, 146.51, 134.45, 134.30, 133.85, 133.99, 133.80, 132.95, 131.42, 131.05, 130.95, 129.51, 128.84, 128.72, 127.95, 126.95, 126.45, 125.44, 123.33, 113.18, 111.84, 60.58, 50.55 15.78, 14.55 ppm; IR (thin film): $\bar{\nu} = 3291$ (indole NH), 1609 (C=N) cm^{-1}.

3-Benzyl-5-(5-chloro-3-phenyl-1H-indol-2-yl)-7-(3,5-diphenyl-1H-pyrazol-1-yl)-3H-[1,2,3]-triazolo[4,5-d]pyrimidine (**7a**, $C_{40}H_{27}ClN_8$)

Yellow crystals; 0.44 g (88%); m.p.: 154 °C; $R_f = 0.68$ (toluene/ethyl acetate 6:4 v/v); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.21$ (s, 1H, indole NH), 7.20-7.92 (m, 23H, Ar–H), 6.75 (s, 1H, pyrazole CH), 4.54 (s, 2H, CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.10$, 167.87, 153.96, 152.09, 150.52, 142.39, 137.86, 136.09, 135.29, 133.76, 133.29, 132.96, 131.49, 130.95, 130.66, 130.55, 130.11, 129.31, 128.91, 128.82, 128.78, 128.51, 128.10, 127.86, 127.77, 127.50, 127.38, 126.15, 111.44, 105.55, 55.95 ppm; IR (thin film): $\bar{\nu} = 3061$ (indole NH), 1597 (C=N) cm^{-1}; MS (70 eV): $m/z = 654$ (M^+), 656 ($[M + 2]^+$).

3-Benzyl-5-(5-methyl-3-phenyl-1H-indol-2-yl)-7-(3,5-diphenyl-1H-pyrazol-1-yl)-3H-[1,2,3]-triazolo[4,5-d]pyrimidine (**7b**, $C_{41}H_{30}N_8$)

Yellow crystals; 0.34 g (69%); m.p.: 261 °C; $R_f = 0.78$ (toluene/ethyl acetate 6:4 v/v); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.18$ (s, 1H, indole NH), 7.20–7.90 (m, 23H, Ar–H), 6.74 (s, 1H, pyrazole CH), 4.52 (s, 2H, CH_2), 2.35 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.08$, 167.84, 153.89, 152.06, 150.48, 142.25, 137.75, 136.05, 135.18, 133.69, 133.19, 132.88, 131.98, 130.88, 130.51, 130.48, 130.05, 129.25, 128.84, 128.78, 128.69, 128.49, 128.05, 127.77, 127.68, 127.41, 127.29, 126.09, 111.35, 105.45, 55.85, 30.05 ppm; IR (thin film): $\bar{\nu} = 3059$ (indole NH), 1595 (C=N) cm^{-1}.

3-Benzyl-5-(5-methoxy-3-phenyl-1H-indol-2-yl)-7-(3,5-diphenyl-1H-pyrazol-1-yl)-3H-[1,2,3]-triazolo[4,5-d]pyrimidine (**7c**, $C_{41}H_{30}N_8O$)

White crystals; 0.37 g (74%); m.p.: >300 °C; $R_f = 0.62$ (toluene/ethyl acetate 6:4 v/v); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.25$ (s, 1H, indole NH), 7.22–7.94 (m, 23H, Ar–H), 6.76 (s, 1H, pyrazole CH), 4.56 (s, 2H, CH_2), 3.27 (s, 3H, OCH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.25$, 167.99, 154.06, 152.51, 150.62, 142.45, 137.91, 136.25, 135.34, 133.95, 133.40, 132.99, 131.65, 130.98, 130.75, 130.60, 130.15, 129.45, 128.89, 128.79, 128.68, 128.60, 128.20, 127.95, 127.85, 127.65, 127.43,

126.25, 111.48, 105.60, 56.10, 50.94 ppm; IR (thin film): $\bar{\nu}$ = 3063 (indole NH), 1602 (C=N) cm^{-1}.

1-[3-Benzyl-5-(5-chloro-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl]-3-methyl-1H-pyrazol-5(4H)-one (**8a**, $C_{29}H_{21}ClN_8O$)
Purple crystals; 0.37 g (75%); m.p.: 100 °C; R_f = 0.62 (*n*-hexane/ethyl acetate 8:2 v/v); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.91 (s, 1H, indole NH), 7.21–7.51 (m, 13H, Ar–H), 5.36 (s, 2H, CH_2), 4.10 (s, 2H, pyrazole CH_2), 3.10 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.73, 162.72, 159.19, 154.10, 152.75, 139.41, 134.32, 132.46, 130.47, 130.09, 129.70, 129.43, 129.19, 128.85, 128.55, 128.38, 128.17, 127.78, 126.25, 125.14, 122.72, 116.10, 46.55, 40.09, 15.70 ppm; IR (thin film): $\bar{\nu}$ = 3233 (indole NH), 1732 (C=O), 1660 (C=N) cm^{-1}; MS (70 eV): *m/z* = 532 (M^+), 534 ($[M + 2]^+$).

1-[3-Benzyl-5-(5-methyl-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl]-3-methyl-1H-pyrazol-5(4H)-one (**8b**, $C_{30}H_{24}N_8O$)
Blue crystals; 0.34 g (68%); m.p.: 274 °C; R_f = 0.55 (*n*-hexane/ethyl acetate 8:2 v/v); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.88 (s, 1H, indole NH), 7.19–7.50 (m, 13H, Ar–H), 5.33 (s, 2H, CH_2), 3.97 (s, 2H, pyrazole CH_2), 3.08 (s, 3H, CH_3), 2.56 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.65, 162.61, 159.15, 154.05, 152.68, 139.35, 134.28, 132.35, 130.39, 130.01, 129.65, 129.35, 129.11, 128.77, 128.50, 128.26, 128.06, 127.66, 126.15, 125.11, 122.68, 116.01, 46.59, 40.95, 30.11, 15.68 ppm; IR (thin film): $\bar{\nu}$ = 3231 (indole NH), 1730 (C=O), 1658 (C=N) cm^{-1}.

1-[3-Benzyl-5-(5-methoxy-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl]-3-methyl-1H-pyrazol-5(4H)-one (**8c**, $C_{30}H_{24}N_8O_2$)
Blue crystals; 0.34 g (69%); m.p.: 140 °C; R_f = 0.60 (*n*-hexane/ethyl acetate 8:2 v/v); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.97 (s, 1H, indole NH), 7.28-7.56 (m, 13H, Ar–H), 5.39 (s, 2H, CH_2), 4.12 (s, 2H, pyrazole CH_2), 3.62 (s, 3H, OCH_3), 3.12 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.95, 162.88, 159.48, 154.32, 152.98, 139.65, 134.41, 132.64, 130.71, 130.25, 129.84, 129.51, 129.28, 128.92, 128.63, 128.55, 128.27, 127.95, 126.51, 125.29, 122.84, 116.22, 51.05, 47.05, 40.98, 15.95 ppm; IR (thin film): $\bar{\nu}$ = 3235 (indole NH), 1736 (C=O), 1664 (C=N) cm^{-1}.

General procedure for the synthesis of 3-benzyl-5-(5-substituted-3-phenyl-1H-indol-2-yl)-7-phenyl-3H-[1,2,3]triazolo[4,5-e][1,2,4] triazolo[4,5-c]pyrimidines ***9a–9c***
A mixture of compounds **5a–5c** (2 mmol) and 20 cm^3 of benzoyl chloride was heated under reflux for 8 h. Excess of acid chloride was removed under reduced pressure. After cooling the product separated was filtered, washed with hot petroleum ether (40–60 °C), dried, and recrystallized from acetic acid to obtain **9a–9c**.

3-Benzyl-5-(5-chloro-3-phenyl-1H-indol-2-yl)-7-phenyl-3H-[1,2,3]triazolo[4,5-e][1,2,4]triazolo[4,5-c]pyrimidine (**9a**, $C_{32}H_{21}ClN_8$)
Dark green crystals; 0.34 g (68%); m.p.: 120 °C; R_f = 0.82 (benzene/ethyl acetate 8:2 v/v); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.99 (s, 1H, indole NH), 7.49–7.97 (m, 18H, Ar–H), 5.24 (s, 2H, CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.05, 154.02, 152.5, 152.3, 141.12, 140.03, 133.91, 133.25, 132.44, 132.00, 132.36, 131.34, 131.12, 130.94, 130.20, 129.95, 129.83, 128.98, 128.82, 128.61, 128.35, 128.19, 127.96, 126.65, 114.00, 54.02 ppm; IR (thin film): $\bar{\nu}$ = 3071 (indole NH), 1601 (C=N) cm^{-1}; MS (70 eV): *m/z* = 553 (M^+), 555 ($[M + 2]^+$).

3-Benzyl-5-(5-methyl-3-phenyl-1H-indol-2-yl)-7-phenyl-3H- [1,2,3]triazolo[4,5-e][1,2,4]triazolo[4,5-c]pyrimidine (**9b**, $C_{33}H_{24}N_8$)
Green crystals; 0.26 g (53%); m.p.: 220 °C; R_f = 0.82 (benzene/ethyl acetate 8:2 v/v); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.91 (s, 1H, indole NH), 7.49–7.95 (m, 18H, Ar–H), 5.23 (s, 2H, CH_2), 2.41 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.02, 154.00, 152.48, 152.28, 141.09, 140.00, 133.85, 133.04, 132.25, 132.11, 132.28, 131.19, 131.02, 130.88, 130.15, 129.86, 129.77, 128.78, 128.78, 128.55, 128.28, 128.05, 127.88, 126.59, 114.05, 54.18, 29.55 ppm; IR (thin film): $\bar{\nu}$ = 3067 (indole NH), 1595 (C=N) cm^{-1}.

3-Benzyl-5-(5-methoxy-3-phenyl-1H-indol-2-yl)-7-phenyl-3H-[1,2,3]triazolo[4,5-e][1,2,4]triazolo[4,5-c]pyrimidine (**9c**, $C_{33}H_{24}N_8O$)
Pale green crystals; 0.38 g (77%); m.p.: 130 °C; R_f = 0.87 (benzene/ethyl acetate 8:2 v/v); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 13.01 (s, 1H, indole NH), 7.52–7.99 (m, 18H, Ar–H), 5.26 (s, 2H, CH_2), 3.02 (s, 3H, OCH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.35, 154.22, 152.45, 152.13, 141.25, 140.43, 134.01, 133.55, 132.61, 132.19, 132.01, 131.54, 131.22, 130.99, 130.40, 129.88, 129.79, 128.99, 128.90, 128.75, 128.41, 128.25, 127.98, 126.81, 114.24, 54.41, 51.56 ppm; IR (thin film): $\bar{\nu}$ = 3077 (indole NH), 1605 (C=N) cm^{-1}.

General procedure for the synthesis of 3-benzyl-5-(5-substituted-3-phenyl-1H-indol-2-yl)-7-methyl-3H-[1,2,3]triazolo[4,5-e][1,2,4] triazolo[4,5-c]pyrimidines ***10a–10c***
A mixture of hydrazines **5a–5c** (10 mmol) and 30 cm^3 of acetic acid was heated under reflux for 10 h, cooled to room temperature, and poured into crushed ice. The product obtained was filtered, washed with water, dried, and recrystallized from ethanol to yield compounds **10a–10c**.

3-Benzyl-5-(5-chloro-3-phenyl-1H-indol-2-yl)-7-methyl-3H-[1,2,3]triazolo[4,5-e][1,2,4]triazolo[4,5-c]pyrimidine (**10a**, $C_{27}H_{19}ClN_8$)

Black crystals; 0.40 g (80%); m.p.: 164 °C; $R_f = 0.79$ (benzene/ethyl acetate 6:4 v/v); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 10.19$ (s, 1H, indole NH), 7.35–7.53 (m, 13H, Ar–H), 5.36 (s, 2H, CH_2), 1.25 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.88$, 154.76, 152.36, 148.93, 139.49, 133,76, 133.25, 132.88, 132.52, 130.85, 130.00, 129.62, 129.13, 128.69, 128.19, 127.34, 126.48, 126.40, 123.96, 123.19, 113.96, 52.00, 12.96 ppm; IR (thin film): $\bar{\nu} = 3213$ (indole NH), 1635 (C=N) cm^{-1}; MS (70 eV): $m/z = 490$ (M^+), 492 ([M + 2]$^+$).

3-Benzyl-5-(5-methyl-3-phenyl-1H-indol-2-yl)-7-methyl-3H-[1,2,3]triazolo[4,5-e][1,2,4]triazolo[4,5-c]pyrimidine (**10b**, $C_{28}H_{22}N_8$)

Brown crystals; 0.29 g (59%); m.p.: 86 °C; $R_f = 0.83$ (benzene/ethyl acetate 6:4 v/v); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 10.15$ (s, 1H, indole NH), 7.32-7.53 (m, 13H, Ar–H), 5.34 (s, 2H, CH_2), 2.23 (s, 3H, CH_3), 1.23 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.75$, 154.69, 152.28, 148.85, 139.35, 133,69, 133.15, 132.75, 132.48, 130.77, 130.05, 129.55, 129.11, 128.55, 128.15, 127.28, 126.35, 126.20, 123.85, 123.09, 113.85, 52.55, 28.98, 12.89 ppm; IR (thin film): $\bar{\nu} = 3210$ (indole NH), 1631 (C=N) cm^{-1}.

3-Benzyl-5-(5-methoxy-3-phenyl-1H-indol-2-yl)-7-methyl-3H-[1,2,3] triazolo[4,5-e][1,2,4]triazolo[4,5-c]pyrimidine (**10c**, $C_{28}H_{22}N_8O$)

Pale brown crystals; 0.38 g (76%); m.p.: 120 °C; $R_f = 0.66$ (benzene/ethyl acetate 6:4 v/v); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 10.23$ (s, 1H, indole NH), 7.38–7.54 (m, 13H, Ar–H), 5.39 (s, 2H, CH_2), 3.41 (s, 3H, OCH_3), 1.26 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.92$, 154.85, 152.42, 148.99, 139.55, 133,84, 133.44, 132.91, 132.65, 130.92, 130.18, 129.65, 129.25, 128.71, 128.21, 127.42, 126.55, 126.52, 123.89, 123.25, 114.00, 52.58, 51.55, 12.88 ppm; IR (thin film): $\bar{\nu} = 3215$ (indole NH), 1639 (C=N) cm^{-1}.

General procedure for the synthesis of 2-(3-benzyl-5-(5-substituted-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl)-2,3-dihydrophthalazine-1,4-diones ***11a–11c***

A mixture of compounds **5a–5c** (1 mmol) and phthalic anhydride (1 mmol) was refluxed in dimethyl formamide for 12 h. After completion of the reaction, the reaction mixture was cooled to room temperature and poured into crushed ice. The solid obtained was filtered, washed with water, dried, and recrystallized from methanol to yield compounds **11a–11c**.

2-[3-Benzyl-5-(5-chloro-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl]-2,3-dihydrophthalazine-1,4-dione (**11a**, $C_{33}H_{21}ClN_8O_2$)

Pale yellow crystals; 0.42 g (85%); m.p.: 154 °C; $R_f = 0.66$ (benzene/ethyl acetate 6:4 v/v); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.50$ (s, 1H, indole NH), 10.88 (NH), 7.24–7.48 (m, 17H, Ar–H), 4.88 (s, 2H, CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.67$, 167.85, 164.44, 160.19, 154.35, 154.10, 140.03, 135.12, 134.24, 133.91, 132.88, 131.46, 130.94, 130.00, 129.62, 128.98, 128.61, 128.19, 127.34, 126.65, 124.44, 123.96, 123.49, 123.36, 113.88, 48.10 ppm; IR (thin film): $\bar{\nu} = 3363$ (NH), 3291 (indole NH), 1742, 1702 (C=O), 1660 (C=N) cm^{-1}; MS (70 eV): $m/z = 597$ (M^+), 599 ([M + 2]$^+$).

2-[3-Benzyl-5-(5-methyl-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl]-2,3-dihydrophthalazine-1,4-dione (**11b**, $C_{34}H_{24}N_8O_2$)

Yellow crystals; 0.39 g (79%); m.p.: 76 °C; $R_f = 0.74$ (benzene/ethyl acetate 8:2 v/v); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.47$ (s, 1H, indole NH), 10.85 (NH), 7.23–7.48 (m, 17H, Ar–H), 4.87 (s, 2H, CH_2), 2.51 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.55$, 167.78, 164.35, 160.10, 154.28, 154.05, 140.00, 135.03, 134.12, 133.85, 132.77, 131.41, 130.86, 130.05, 129.59, 128.88, 128.58, 128.09, 127.24, 126.53, 124.39, 123.88, 123.35, 123.28, 113.79, 48.56, 29.96 ppm; IR (thin film): $\bar{\nu} = 3361$ (NH), 3289 (indole NH), 1741, 1698 (C=O), 1657 (C=N) cm^{-1}.

2-[3-Benzyl-5-(5-methoxy-3-phenyl-1H-indol-2-yl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl]-2,3-dihydrophthalazine-1,4-dione (**11c**, $C_{34}H_{24}N_8O_3$)

Pale yellow crystals; 0.36 g (73%); m.p.: 282 °C; $R_f = 0.77$ (benzene/ethyl acetate 8:2 v/v); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.52$ (s, 1H, indole NH), 10.92 (NH), 7.26–7.50 (m, 17H, Ar–H), 4.91 (s, 2H, CH_2), 3.63 (s, 3H, OCH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.85$, 167.91, 164.64, 160.32, 154.55, 154.25, 140.23, 135.32, 134.32, 133.78, 132.91, 131.66, 130.99, 130.21, 129.75, 128.89, 128.71, 128.25, 127.44, 126.74, 124.51, 123.85, 123.51, 123.41, 113.91, 51.23, 48.15 ppm; IR (thin film): $\bar{\nu} = 3366$ (NH), 3294 (indole NH), 1743, 1705 (C=O), 1662 (C=N) cm^{-1}.

In vitro antimicrobial activities

The in vitro biological screening of the synthesized compounds **3–11** was carried out against bacteria species namely, *S. aureus, E. coli*, and *P. aeruginosa* and fungal species namely, *A. niger*, *A. flavus*, and *A. oryzae* by Cup-plate method [25]. An aliquot of 0.1 cm^3 of each bacterial strain was spread on nutrient agar, while 0.1 cm^3 of the

fungal spore suspension was spread on potatoes dextrose agar. 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 1000, 500, 250, and 125 μg cm^{-3} of each test compound in DMF. The plates were incubated at 37 °C for 24 and 72 h in case of antibacterial and antifungal activity, respectively. The zone of inhibition around the well was determined (in mm). Streptomycin and fluconazole were used as standards for antibacterial and antifungal activities, respectively.

Antioxidant activity

1,1-Diphenyl-2-picryl hydrazil (DPPH) Radical Scavenging Activity (RSA)

The RSA of the test compounds in methanolic solution at concentrations 25, 50, 75, and 100 μg cm^{-3} containing freshly prepared DPPH solution (0.004% w/v) was carried out and compared with those of standards BHA, TBHQ, and AA using reported method [26]. All the test analyses were performed on three replicates were averaged. The results in percentage are expressed as the ratio of absorption decrease in the presence of test compounds and absorption of DPPH solution in the absence of test compounds at 517 nm on ELICO SL 171 minispect spectrometer. The percentage scavenging activity of the DPPH free radical was determined using the following equation:

$$\%\text{DPPH RSA} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100.$$

Ferric ion (Fe^{3+}) reducing antioxidant power (FRAP)

The reducing power of the synthesized compounds was determined according to the literature method [27]. Different concentrations of samples (25, 50, 75, and 100 μg cm^{-3}) in 1 cm^3 DMSO were mixed with 2.5 cm^3 of phosphate buffer (0.2 M, pH 6.6) and 2.5 cm^3 of potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 min after which 2.5 cm^3 of trichloroacetic acid (10%) was added to the mixture and centrifuged for 10 min at 1000 rpm. The upper layer solution (2.5 cm^3) was mixed with 2.5 cm^3 of distilled water and 0.5 cm^3 of ferric chloride (0.1%). Then the absorbance at 700 nm was measured in spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Ferrous (Fe^{2+}) ion metal chelating activity

The chelating activity of the ferrous ions by the synthesized compounds and standards was estimated by following reported method [28]. The test samples (25, 50, 75, and 100 μg cm^{-3}) in 0.4 cm^3 of ethanolic solution were added to a solution of ferrous chloride (0.05 cm^3, 2 mM). The reaction was initiated by the addition of 0.2 cm^3 ferrozine (5 mM) and the total volume was adjusted to 4 cm^3 with ethanol. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. The mixture was shaken vigorously and kept at room temperature for 10 min. Then the absorbance of the solution was measured spectrophotometrically at 562 nm. All test analyses were run in triplicate and averaged. The percentage of the inhibition of the ferrozine Fe^{2+} complex formations was calculated using the formula:

$$\%\text{Ferrous ion chelation} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100.$$

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References

1. Rufine AG, Eric D, Yoan F, Laurent M, Michelle P, Fabrice A, Pascale M (2009) Bioorg Med Chem 17:4420
2. James FD, Taro A, Monica E, Bahanu H, Tom D, Joel PB, Peter TM, Guo QS (2005) Bioorg Med Chem 15:5035
3. Karg EM, Luderer S, Pergola C, Bühring U, Rossi A, Northoff H, Sautebin L, Troschütz R, Werz O (2009) J Med Chem 52:3474
4. Anu A, Kumkum S, Puri SK, Prem MSC (2005) Bioorg Med Chem Lett 15:3133
5. Yasuo Y, Mizuyo K (2007) Bioorg Med Chem Lett 17:1626
6. Mahboobi S, Eichhorn E, Popp A, Sellmer A, Elz S, Möllmann U (2006) Eur J Med Chem 41:176
7. Chung-Kyu R, Jung YL, Rae-Eun P, Mi-Young M, Ji-Hee N (2007) Bioorg Med Chem Lett 17:127
8. Williams JD, Drach JC, Townsend LB (2005) Nucleosides Nucleotides Nucleic Acids 24:1613
9. Chen JJ, Wei J, Williams JD, Drach JC, Townsend LB (2005) Nucleosides. Nucleotides Nucleic Acids 24:1417
10. Huifang C, Yanfang Z, Chunshen Z, Ping G (2006) Bioorg Med Chem 14:911
11. Di Braccio M, Roma G, Leoncini G (1995) Eur J Med Chem 30:27
12. Aymn ER, Abeer EM, Mamdouh MA (2011) Eur J Med Chem 46:1019
13. Bhuiyan MMH, Khandker MMR, Hossain MK, Rahim MA, Hossain MI (2005) Croat Chem Acta 78:633
14. Mi-Yeon J, Steven DJ, Kenneth S, Jozef A, Piet H (2011) Bioorg Med Chem 19:702
15. Ailing Z, Xin G, Yuanxiang W, Jing A, Ying W, Chen Y, Meiyu G, Zhang Ao (2011) Bioorg Med Chem 19:3906
16. Aivachtchenko AV, Golovina ES, Kadieva MG, Koryakova AG, Mitkin OD, Tkachenko SE, Kysil VM, Okun I (2011) Eur J Med Chem 46:1189

17. Santana L, Teijeira M, Uriarte E, Balzarini J, De Clercq E (2002) Eur J Med Chem 37:755
18. Hairuo P, Gnanasambandam K, Gang Y, Sha L, Joy W, Herman VV, Tonika B, Carol H, Chi BV, Carol LE, Hexi C, Thomas ME, Eric TW, Russel CP (2004) J Med Chem 47:6218
19. Said AS, Abd El-Galil EA, Nermien MS, Mohamed MA (2009) Eur J Med Chem 44:4787
20. Blanco JM, Caamano O, Fernandez F, Garcia-Mera X, Hergueta AR, Lopez C, Rodriguez-Borges JE, Balzarini J, De Clerc E (1999) Chem Pharm Bull 47:1314
21. Zhang N, Ayral-Kaloustian S, Nguyen T, Afragola J, Hernandez R, Lucas J, Gibbons J, Beyer C (2007) J Med Chem 50:319
22. Kleschick WA, Costales MJ, Dunbar JE, Meikle RM, Monte WT, Pearson NR, Snider SW, Vinogradoff AP (1990) Pestic Sci 29:341
23. Giorgi I, Biagi G, Bianucci AM, Borghini A, Livi O, Leonardi M, Pietra D, Calderone V, Martelli A (2008) Eur J Med Chem 43:1639
24. Shivayogi PH, Mruthyunjayswamy BHM, Muralidhar GP (1978) Ind J Chem 16B:789
25. Pharmacopoeia Indian (1985) Appendix IV, 90, 3rd edn. Government of India, New Delhi
26. Hatano T, Kagawa H, Yasuhara T, Okuda T (1988) Chem Pharm Bull 36:2090
27. Oyaizu M (1986) Jpn J Nutr 44:307
28. Dinis TCP, Maderia VMC, Almeida LM (1994) Arch Biochem Biophys 315:161