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ORIGINAL PAPER

Synthesis and antimicrobial evaluation of 1,4-disubstituted 1,2,3-triazoles containing benzofused *N*-heteroaromatic moieties

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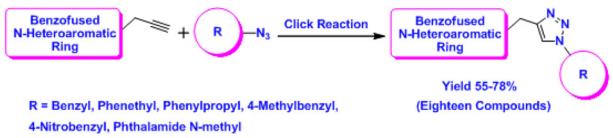
Abstract Synthesis of a small library of 1,4-disubstituted 1,2,3-triazoles containing benzofused *N*-heteroaromatic moieties was carried out through click reaction of various benzofused *N*-heteroaromatic alkynes with aromatic azides. All the synthesized compounds were characterized by spectroscopic techniques like IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry and evaluated in vitro for antimicrobial activity against two Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), one Gram-negative bacteria (*Escherichia coli*) and two fungi (*Candida albicans*, *Aspergillus niger*). Most of the synthesized 1,4-disubstituted 1,2,3-triazoles were found to possess significant antibacterial and antifungal activity against tested microbial species. Moreover, docking simulation of the compound 1-[(1-(4-nitrobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl]-1*H*-benzo[*d*]imidazole was also carried out against *E. coli* topoisomerase II DNA gyrase B enzyme to study the binding modes and mechanism of action.

Graphical abstract



Keywords Heterocycles · 1,4-Disubstituted 1,2,3-triazoles · Antibacterial · Fungicidal · Cycloaddition · Docking

Introduction

Nitrogen heterocyclic compounds play key roles in the field of medicinal chemistry [1]. Among these heterocycles, much importance has been given to the triazole nucleus which possess a wide range of pharmacological activities like antimicrobial [2, 3], antitrypanosomal [4], antimalarial [5, 6], anti-HIV [7], anticancer [8–10], antitubercular [11, 12], anticonvulsant [13], antiallergic [14], etc. The regioselective synthesis of 1,4-disubstituted 1,2,3-triazoles through copper(I) catalyzed 1,3-dipolar cycloaddition was independently reported by Sharpless [15] and Meldal [16] in 2002, led to great success over Huisgen's classical thermal method [17] which yields 1,4- and 1,5-disubstituted isomers. This copper(I) catalyzed 1,3-dipolar cycloaddition is one of the finest reaction of click chemistry due to better regioselectivity, versatility, and compatibility.

In addition to above clinical utility, the importance of 1,2,3-triazoles have also been recognized as proton transport agents [18], glycoside cluster arrays [19], linkers [20],

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dendrimers [21], hyper-branched polymers [22], and liquid crystals [23]. Likewise, benzofused *N*-heteroarenes such as benzimidazole [24, 25], benzotriazole [26, 27], and carbazole derivatives [28] have received considerable attention owing to their presence in many medicinal agents.

Therefore, owing to medicinal utility of above class of compounds, we have synthesized a small library of 1,4-disubstituted 1,2,3-triazoles containing benzofused N-heteroaromatic moieties. To the best of our knowledge, most of the synthesized compounds are new except **6a** [29], **9a**, and **9e** [30]. All the synthesized compounds were characterized by spectroscopic techniques like IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry and screened in vitro for antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*. Molecular docking studies give information about interactions of the molecules with biological targets to support experimental data [31]. Therefore, to study the binding modes, docking simulation of the broadly active compound 1-[(1-(4-nitrobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl]-1*H*-benzo[*d*]imidazole (**3e**) was also carried out against *E. coli* topoisomerase II DNA gyrase B enzyme.

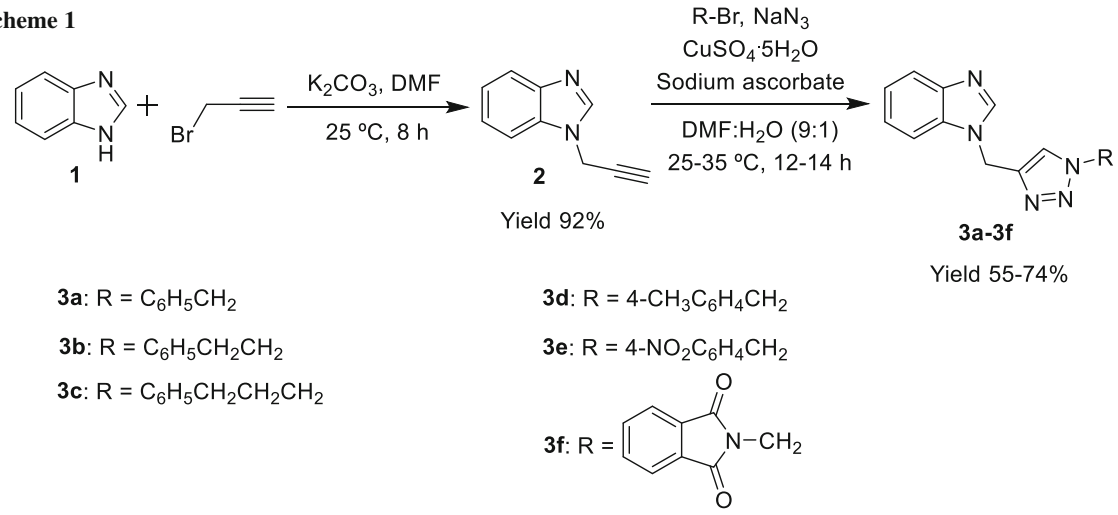
Results and discussion

Chemistry

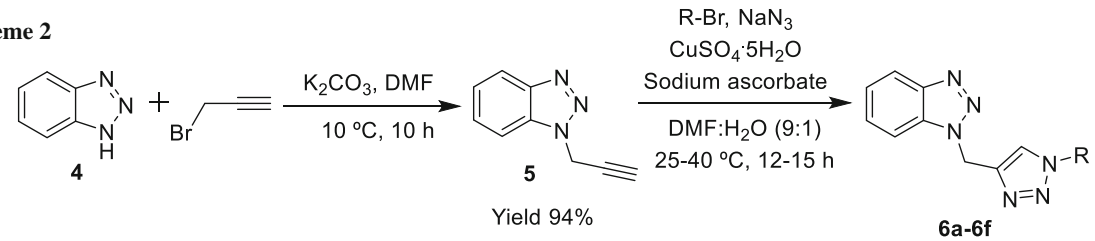
The synthetic strategy of the target compounds is given in Schemes 1, 2, and 3. Benzofused *N*-heterocycles, i.e., benzimidazole, benzotriazole, and carbazole were used as starting materials. The benzofused *N*-heteroaromatic alkynes were synthesized [32] by reacting benzimidazole, benzotriazole, or carbazole with propargyl bromide in the presence of potassium carbonate in dimethylformamide at ambient temperature. 1,4-Disubstituted 1,2,3-triazoles containing benzofused *N*-heteroaromatic moieties were synthesized via click reaction of benzofused *N*-heteroaromatic alkynes with aromatic azides which in turn were prepared by in situ reaction of aromatic bromides with sodium azide, as per literature procedure [33].

The synthesized 1,4-disubstituted 1,2,3-triazoles **3a–3f**, **6a–6f**, and **9a–9f** were characterized by spectroscopic techniques like IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. The ^{1}H NMR spectra of the compounds **3a–3f** displayed one characteristic singlet in the region at $\delta = 7.77$–8.26 ppm due to triazolyl proton and another singlet appeared in the region at 7.84–8.33 ppm was attributed to C_2-H proton of benzimidazole moiety. A singlet appeared in the range at 4.18–6.11 ppm integrating two protons which was assigned to methylene group attached to N_1 of triazole ring, while methylene protons attached to C_4 of the triazole ring were observed in the region at 5.40–5.59 ppm. The remaining aromatic protons were observed in the region at 6.91–8.23 ppm. In ^{13}C NMR spectra of the compounds **3a–3f**, signal due to C_4 of the triazole moiety appeared in the region at $\delta = 142.8$–143.8 ppm, whereas peak owing to C_5 of triazole ring resonated in the range at 126.3–128.0 ppm. The peak observed in the region at 40.3–40.7 ppm was assigned to methylene carbon attached to N_1 of benzimidazole ring, while the signal due to methylene carbon linked to N_1 of the triazole ring was resonated in the region at 49.5–54.2 ppm. The carbonyl carbon of **3f** appeared at 166.3 ppm. The remaining aliphatic and aromatic carbons appeared in their usual range. The IR spectra of the compounds **3a–3f** exhibited absorption band in the region at

Scheme 1



Scheme 2



6a: R = $C_6H_5CH_2$

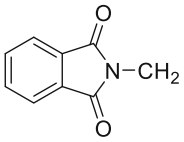
6b: R = $C_6H_5CH_2CH_2$

6c: R = $C_6H_5CH_2CH_2CH_2$

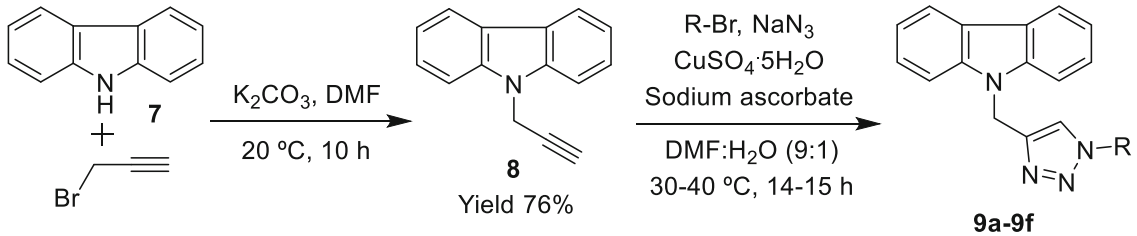
6d: R = 4-$CH_3C_6H_4CH_2$

6e: R = 4-$NO_2C_6H_4CH_2$

6f: R =



Scheme 3



9a: R = $C_6H_5CH_2$

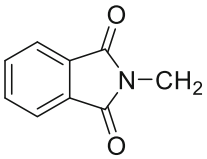
9b: R = $C_6H_5CH_2CH_2$

9c: R = $C_6H_5CH_2CH_2CH_2$

9d: R = 4-$CH_3C_6H_4CH_2$

9e: R = 4-$NO_2C_6H_4CH_2$

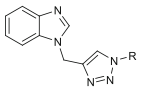
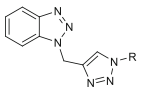
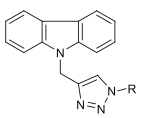
9f: R =



3136–3113 cm^{-1} due to C–H stretching vibrations of triazole ring, while C–H stretching vibrations of aromatic rings were absorbed in the range at 3089–3072 cm^{-1}. In case of **3f**, absorption band at 1720 cm^{-1} was attributed to carbonyl carbon. The absorption bands owing to C=C stretching vibrations of aromatic rings were observed in their common range. The mass spectra of the compounds **3a**–**3f** showed signals because of $[M^+]$ and $[M^+ + 1]$ ions, which are in good agreement with their calculated values.

Likewise, the ^{1}H NMR spectra of the compounds **6a**–**6f** displayed one characteristic singlet in the region at 7.42–8.36 ppm due to triazolyl proton. A singlet observed in the region at 5.40–5.97 ppm integrating two protons was assigned to methylene protons attached to N_1 of the benzotriazole ring, while another singlet appeared in the region at 4.28–6.14 ppm was attributed to methylene protons linked to N_1 of the triazole ring. Further, remaining aromatic protons were observed in the region at 6.93–8.22 ppm. In ^{13}C NMR spectra of the compounds **6a**–**6f**, signal due to C_4 carbon atom of the triazole ring appeared in the region at 141.7–143.7 ppm, whereas C_5 carbon atom absorbed in the range at 127.2–127.9 ppm. The signal appeared in the region at 49.7–54.2 ppm was assigned to methylene carbon attached to N_1 of the triazole ring, while signal due to methylene carbon linked to N_1 of the benzotriazole ring was observed in the region at

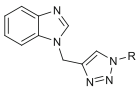
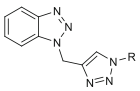
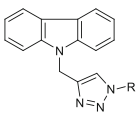
Table 1 Antibacterial activity of 1,4-disubstituted 1,2,3-triazoles in terms of MIC/μmol/$cm^3 \times 10^{-2}$

Compounds	Representative structure	R	*E. coli*	*B. subtilis*	*S. aureus*
3a		$C_6H_5CH_2$	17.28	8.64	17.28
3b		$C_6H_5CH_2CH_2$	8.24	8.24	16.48
3c		$C_6H_5CH_2CH_2CH_2$	15.75	3.94	3.94
3d		4-$CH_3C_6H_4CH_2$	4.12	4.12	8.24
3e		4-$NO_2C_6H_4CH_2$	1.86	3.73	1.86
3f		$C_6H_4(CO)_2NCH_2$	3.48	13.95	1.74
6a		$C_6H_5CH_2$	8.61	8.61	17.22
6b		$C_6H_5CH_2CH_2$	16.43	2.05	16.43
6c		$C_6H_5CH_2CH_2CH_2$	7.85	15.70	3.92
6d		4-$CH_3C_6H_4CH_2$	4.10	16.43	4.10
6e		4-$NO_2C_6H_4CH_2$	1.86	3.72	7.45
6f		$C_6H_4(CO)_2NCH_2$	13.91	6.95	13.91
9a		$C_6H_5CH_2$	14.77	7.38	7.38
9b		$C_6H_5CH_2CH_2$	7.09	7.09	7.09
9c		$C_6H_5CH_2CH_2CH_2$	6.82	1.70	1.70
9d		4-$CH_3C_6H_4CH_2$	1.77	14.18	3.54
9e		4-$NO_2C_6H_4CH_2$	6.52	3.26	6.52
9f		$C_6H_4(CO)_2NCH_2$	12.27	3.06	6.13
Norfloxacin		-----	3.91	1.95	3.91

43.3–43.8 ppm. The carbonyl carbon of **6f** appeared at 166.4 ppm. The remaining aliphatic and aromatic carbons displayed peaks in the normal range. The IR spectra of the compounds **6a**–**6f** showed absorption band in the region at 3142–3126 cm^{-1} because of C–H stretching vibrations of triazole ring, while bands due to C–H stretching vibrations of aromatic rings observed in the region at 3074–3026 cm^{-1}. In the compound **6f**, band absorbed at 1720 cm^{-1} was assigned to carbonyl carbon of phthalimide moiety. The bands due to aromatic ring stretching vibrations were absorbed in their general range. The mass spectra of the compounds **6a**–**6f** displayed peaks corresponding to $[M^+]$ and $[M^+ + 1]$ ions, which are in good agreement with predicted values.

Further, ^{1}H NMR spectra of the compounds **9a**–**9f** exhibited characteristic peak in the region at 7.36–8.08 ppm due to triazolyl proton. A singlet corresponding to two protons in the region at 5.28–5.62 ppm was attributed to methylene protons attached to nitrogen atom of the carbazole ring, while another singlet observed in the region at 4.11–6.00 ppm integrating two protons was due to methylene group linked to N_1 of triazole ring. The remaining aromatic protons appeared in the region at 6.62–8.14 ppm. In the ^{13}C NMR spectra of the compounds **9a**–**9f**, two characteristic signals due to C_4 and C_5 of the triazole moiety appeared in the region at 143.8–145.0 ppm and 126.0–126.9 ppm, respectively. The singlet appeared in the region at 38.1–39.0 ppm was attributed to methylene carbon linked to N_1 of carbazole ring; whereas, the peak due to methylene carbons linked to N_1 of the triazole ring resonated in the region at 49.5–54.0 ppm. The carbonyl carbon of **9f** was displayed at 166.4 ppm. The remaining aliphatic and aromatic carbons resonated in their normal range. The IR spectra of the

Table 2 Antifungal activity of 1,4-disubstituted 1,2,3-triazoles in terms of MIC/μmol/$cm^3 \times 10^{-2}$

Compounds	Representative structure	R	*C. albicans*	*A. niger*
3a		$C_6H_5CH_2$	4.32	17.28
3b		$C_6H_5CH_2CH_2$	4.12	4.12
3c		$C_6H_5CH_2CH_2CH_2$	7.87	7.87
3d		4-$CH_3C_6H_4CH_2$	4.12	8.24
3e		4-$NO_2C_6H_4CH_2$	14.95	3.73
3f		$C_6H_4(CO)_2NCH_2$	13.95	13.95
6a		$C_6H_5CH_2$	17.22	2.15
6b		$C_6H_5CH_2CH_2$	2.05	2.05
6c		$C_6H_5CH_2CH_2CH_2$	3.92	15.70
6d		4-$CH_3C_6H_4CH_2$	8.21	8.21
6e		4-$NO_2C_6H_4CH_2$	14.91	3.72
6f		$C_6H_4(CO)_2NCH_2$	6.95	6.95
9a		$C_6H_5CH_2$	7.38	7.38
9b		$C_6H_5CH_2CH_2$	1.77	3.54
9c		$C_6H_5CH_2CH_2CH_2$	3.41	3.41
9d		4-$CH_3C_6H_4CH_2$	3.54	14.18
9e		4-$NO_2C_6H_4CH_2$	6.52	1.63
9f		$C_6H_4(CO)_2NCH_2$	6.13	3.06
Fluconazole		-----	2.04	4.08

compounds **9a**–**9f** showed absorption bands in the region at 3128–3122 and 3074–3057 cm^{-1} owing to C–H stretching vibrations of triazole and aromatic rings, respectively. Three strong bands were absorbed in the region at 1601–1448 cm^{-1} because of C=C stretching vibrations of aromatic rings. The compound **9f** displayed strong absorption band at 1724 cm^{-1} owing to presence carbonyl carbon in the molecule. The results of mass spectral analysis of compounds **9a**–**9f** showed presence of signals with respect to $[M^+]$ and $[M^+ + 1]$ ions, which are in good agreement with expected values.

Antibacterial activity

The antibacterial activity of synthesized 1,4-disubstituted 1,2,3-triazoles was evaluated in vitro against two Gram-positive bacteria, i.e., *B. subtilis* (MTCC 441), *S. aureus* (MTCC 3160) and one Gram-negative bacteria, viz. *E. coli* (MTCC 443) by standard serial dilution method [34]. *B. subtilis* produces a proteolytic enzyme subtilisin, which causes ropiness. *S. aureus* causes boils, sties, and skin infection. *E. coli*, in a number of patients is responsible for gastroenteritis, urinary tract infections, and neonatal meningitis [35]. The double-strength nutrient broth was used as culture media. Dimethylsulfoxide was used as negative control. Norfloxacin, a broad spectrum antibiotic that is effective against both Gram-positive and Gram-negative bacteria was used as reference drug for antibacterial activity assay. Minimal inhibitory concentration (MIC) values of standard drug and synthesized compounds were determined in terms of μmol/$cm^3 \times 10^{-2}$ as shown in Table 1.

Most of the synthesized compounds displayed moderate-to-good antibacterial activity against tested bacterial

strains as depicted by MIC values expressed in μmol/$cm^3 \times 10^{-2}$. Among synthesized triazoles, compounds **3e** (MIC, 1.86), **3f** (MIC, 1.74) and **9c** (MIC, 1.70) against *S. aureus* while **3e** (MIC, 1.86), **6e** (MIC, 1.86), and **9d** (MIC, 1.77) against *E. coli* displayed almost twofold antibacterial potency as compared to reference drug, norfloxacin. However, some of the compounds like **6b** (MIC, 2.05) and **9c** (MIC, 1.70) against *B. subtilis*; **3c** (MIC, 3.94), **6c** (MIC, 3.92), **6d** (MIC, 4.10) and **9d** (MIC, 3.54) against *S. aureus*; **3d** (MIC, 4.12), **3f** (MIC, 3.48), and **6d** (MIC, 4.10) against *E. coli* exhibited comparable antibacterial activity to the reference drug used.

From the above results, it has been generalized that the presence of electron withdrawing group on phenyl ring improved the antibacterial activity of synthesized triazoles. Similarly, the increase in length of carbon chain at N_1 position of triazole ring also enhanced the antibacterial potency of the synthesized compounds containing benzimidazolyl and carbazolyl moieties. Substitution of benzyl group by phthalimide-NCH_2 at N_1 position of triazole ring in the compound (**3f**) with benzimidazolyl moiety leads to improved bactericidal efficiency against *E. coli* and *S. aureus*.

Antifungal activity

The in vitro antifungal activity of the synthesized triazoles was tested against two fungal strains, *C. albicans* (MTCC 227) and *A. niger* (MTCC 281) by standard serial dilution method [34]. *C. albicans* generally causes mucosal infections in immunosuppressant patients which results in life-threatening diseases. *A. niger*, a xerophilic fungus also causes many serious infections in humans and other organisms [35]. Sabouraud dextrose broth was used as nutrient media while dimethylsulfoxide as a solvent control. Fluconazole, an antifungal medicine possessing triazole moiety was used as standard drug for antifungal activity assay. MIC values were reported in μmol/$cm^3 \times 10^{-2}$ as given in Table 2.

All the synthesized compounds exhibited moderate-to-good antifungal activity against tested fungal strains as depicted by MIC values expressed in μmol/$cm^3 \times 10^{-2}$. The compounds **6a** (MIC, 2.15), **6b** (MIC, 2.05), and **9e** (MIC, 1.63) exhibited almost twofold antifungal activity against *A. niger* as compared to standard drug, fluconazole. However, in case of *C. albicans*, some of the compounds like **6b** (MIC, 2.05) and **9b** (MIC, 1.77) displayed antifungal efficacy comparable to reference drug. Further, the compounds **3b** (MIC, 4.12), **3e** (MIC, 3.73), **6e** (MIC, 3.72), **9b** (MIC, 3.54), **9c** (MIC, 3.41), and **9f** (MIC, 3.06) showed good antifungal activity like standard drug, against *A. niger*.

The results clearly indicated that the presence of electron withdrawing groups on phenyl ring improved the antifungal activity of synthesized compounds against *A. niger*; whereas, presence of electron donating groups on phenyl ring increased the fungicidal activity of synthesized triazoles against *C. albicans*. Moreover, replacement of benzyl group by phthalimide-NCH_2 group at N_1 position of triazole ring of compound (**9f**) having carbazolyl moiety increased the antifungal efficiency against *A. niger*.

Docking studies

E. coli topoisomerase II DNA gyrase B enzyme was used as target for envisaging antimicrobial potential of the

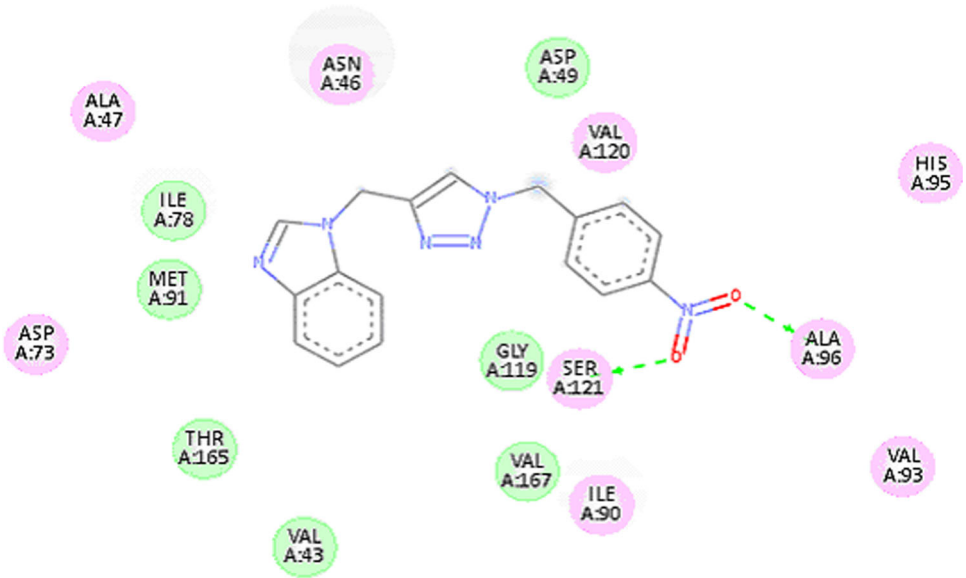


Fig. 1 2D schematic diagram of compound **3e** docked in active site of *E. coli* topoisomerase II DNA Gyrase B (color figure online)

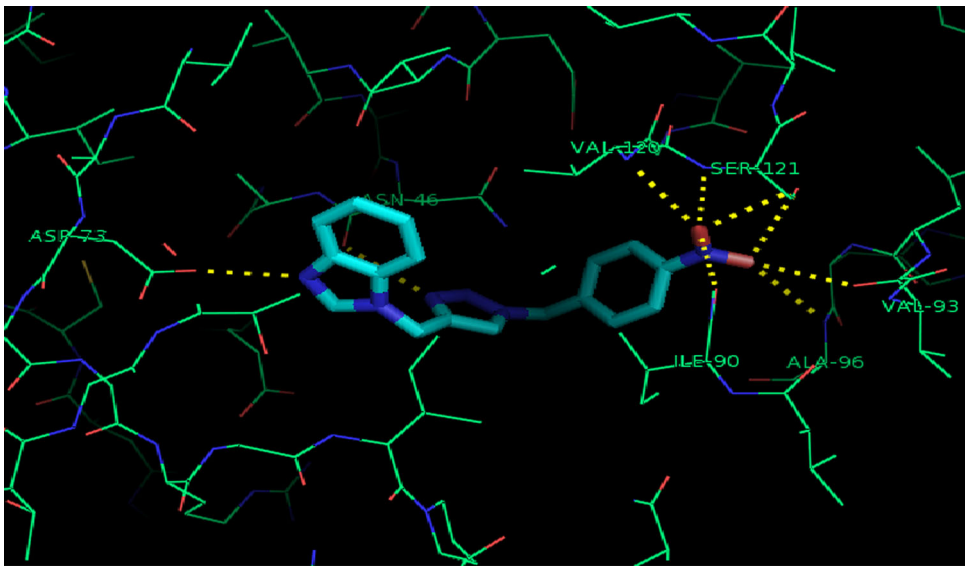


Fig. 2 Docked pose of compound **3e** showing hydrogen bonding with active site residues of *E. coli* topoisomerase II DNA Gyrase B

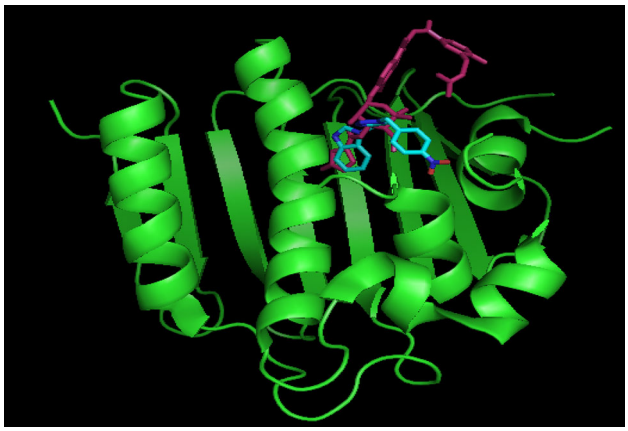


Fig. 3 Secondary structure of *E. coli* topoisomerase II DNA gyrase B along with docked compound **3e**

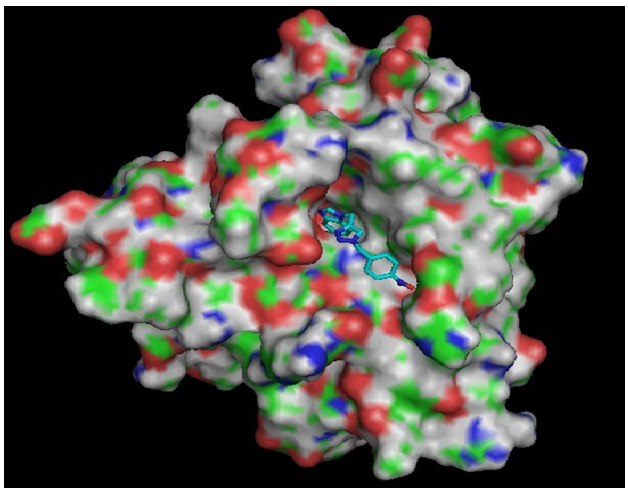


Fig. 4 Surface diagram showing docked molecule **3e** with enzyme *E. coli* topoisomerase II DNA Gyrase B

synthesized 1,4-disubstituted 1,2,3-triazoles containing benzofused N-heteroaromatic moiety, i.e., benzimidazole, benzotriazole, and carbazole [36, 37]. As the compounds under study contains these nuclei, docking simulations of the compound **3e** (MIC, 1.86) was performed into binding site of *E. coli* topoisomerase II DNA gyrase B enzyme in order to find out a rational mechanism of action for antimicrobial activity of the compounds and to predict the best binding orientation of this compound. The protein of interest was taken from RCSB Protein Data Bank (PDB ID: 1KZN) and Autodock Vina docking program [38] was used for docking simulations. Best ranking binding mode of compound **3e** in the active site of the enzyme showing hydrogen bonding (green dotted lines), van der Waals (green spheres), and electrostatic (magenta) interactions is depicted as two-dimensional diagram in Fig. 1. It was involved in hydrogen bond interactions with the active site residue Ser121 and Ala96. One oxygen atom of nitro group of compound **3e** created a strong hydrogen bond with Ser121 (H···O distance = 2.173 Å) while another oxygen atom of nitro group a formed hydrogen bond with Ala96 (H···O distance = 2.453 Å) as depicted in Fig. 2. Several active site residues, i.e., Val43, Asp49, Ile78, Met91, Gly119, Thr165, and Val167 were involved in van der Waal's interactions with the compound. Further, residues Asn46, Ala47, Asp73, Ile90, Val93, His95, and Val120 exhibited electrostatic interactions with the target compound. These residues except Val93, His95, Ala96, Gly119, and Ser121, also assist in fixing of co-crystallized ligand clorobiocin in the enzyme active site. For these reasons, it can be accepted that the compound under study inhibits topoisomerase II DNA gyrase B enzyme in a successful manner which could be the likely cause of their antimicrobial action. Docked conformation of compound

3e along with co-crystallized ligand clorobiocin in ribbon diagram of protein is shown in Fig. 3 and surface diagram also shown in Fig. 4.

Conclusion

In the present case, we have synthesized a small library of 1,4-disubstituted 1,2,3-triazoles containing benzofused *N*-heteroaromatic moieties through copper(I)-catalyzed click reaction of benzofused N-heteroaromatic alkynes with aromatic azides. The synthesized compounds were screened in vitro for antimicrobial activity. Among synthesized compounds **3e**, **3f**, and **9c** against *S. aureus*; **3e**, **6e**, and **9d** against *E. coli*; **6a**, **6b**, and **9e** against *A. niger* displayed almost twofold antimicrobial efficiency as compared to reference drugs. The docking simulation revealed that the compound **3e** inhibits *E. coli* topoisomerase II DNA gyrase B enzyme through hydrogen bonding, electrostatic, and van der waal interactions. This work can be further explored for designing of new 1,4-disubstituted 1,2,3-triazoles as potential antimicrobials.

Experimental

Chemicals used in present work were purchased from Himedia/Alfa-Aesar and were used without further purification. Solvents were dried as per standard literature procedures. Melting points of synthesized compounds were recorded in °C by applying open capillary method. The IR spectra were scanned on Shimazdu IR Affinity-I IR spectrophotometer using potassium bromide (KBr) powder and values are given in cm^{-1}. The ^{1}H NMR spectra were recorded on Bruker Avance II 400 MHz or Bruker 300 MHz spectrometer and ^{13}C NMR on Bruker Avance II 400 at 100 MHz or Bruker 300 at 75 MHz, in deuterated chloroform or dimethylsulfoxide-d_6 using tetramethylsilane (TMS) as an internal standard (chemical shift in δ/ppm). Coupling constant (J) values are given in Hertz (Hz). Mass spectra were recorded on Waters Micromass Q-Tof Micro (ESI) spectrophotometer. The completion of reactions and the purity of the compounds were analyzed by thin layer chromatography (TLC) using silica gel plates (SIL G/UV_{254}, ALUGRAM) in ethyl acetate:hexane mixture and visualized under ultraviolet lamp.

General method for the synthesis of 1,4-disubstituted 1,2,3-triazoles 3a–3f, 6a–6f, 9a–9f

The benzofused N-heteroaromatic alkynes **2**, **5**, and **8** were synthesized by reacting benzimidazole (1.0 mmol), benzotriazole (1.0 mmol), or carbazole (1.0 mmol) with propargyl bromide (1.2 mmol) in the presence of potassium carbonate (2.0 mmol) using dimethylformamide by continuous stirring at 10–25 °C up to 8–10 h [32]. For the synthesis of target compounds, solution of aromatic bromides (1.0 mmol) in dimethylformamide was taken in a round-bottomed flask and aqueous solution of sodium azide (3.0 mmol) was added, thereafter, stirred the reaction mixture for 1 h at 25–40 °C. To the above reaction mixture, benzofused *N*-heteroaromatic alkyne **2** (1.0 mmol), **5** (1.0 mmol), or **8** (1.0 mmol) was added followed by copper sulfate pentahydrate (5 mol %) and sodium ascorbate (10 mol %) [33], then, stirred the reaction contents overnight. The progress of reaction was monitored by thin layer chromatography. After the completion of reaction, ice-cold distilled water was added to the reaction mixture and product was extracted with ethyl acetate (50 $cm^3 \times 3$). The organic layer was washed with aqueous ammonia solution followed by brine solution and dried using anhydrous sodium sulfate. Filtered and evaporated the solvent under vacuum to get crude product which was further purified by column chromatography using hexane:ethyl acetate (7:3) as eluent.

1-[(1-Benzyl-1H-1,2,3-triazol-4-yl)methyl]-1H-benzo[d]imidazole (**3a**, $C_{17}H_{15}N_5$)
Yellowish-white solid; yield: 64 %; m.p.: 130–134 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.46$ (s, 4H, CH_2), 7.20–7.36 (m, 9H, Ar–H), 7.79 (s, 1H, C-H triazole), 7.97 (s, 1H, C_2–H benzimidazole) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 40.5$, 54.2, 109.8, 120.4, 121.8, 122.4, 123.1, 127.9 (C_5 triazole), 128.8, 129.1, 133.4, 134.2, 142.6, 143.2, 143.8 (C_4 triazole) ppm; IR (KBr): $\bar{\nu} = 3136$ (C–H str., triazole ring), 3088 (C–H str., aromatic), 1656 (C=N str., aromatic), 1605, 1494, 1450 (C=C str., aromatic) cm^{-1}; MS: *m/z* for $C_{17}H_{15}N_5$: 290.0 [M^+], 291.0 [M^++1].

1-[(1-Phenethyl-1H-1,2,3-triazol-4-yl)methyl]-1H-benzo[d]imidazole (**3b**, $C_{18}H_{17}N_5$)
Yellowish-white solid; yield: 58 %; m.p.: 122–126 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.11$ (t, $J = 10.5$ Hz, 2H, CH_2), 4.50 (t, $J = 10.5$ Hz, 2H, CH_2), 5.40 (s, 2H, CH_2), 6.91 (t, $J = 6$ Hz, 3H, Ar–H), 7.11–7.32 (m, 6H, Ar–H), 7.79 (s, 1H, C–H triazole), 7.88 (s, 1H, C_2-H benzimidazole) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 36.6$, 40.5, 51.8, 109.8, 120.4, 121.8, 122.4, 123.2, 127.1 (C_5 triazole), 128.5, 128.7, 133.4, 136.6, 142.4, 143.8 (C_4 triazole) ppm; IR (KBr): $\bar{\nu} = 3128$ (C–H str., triazole ring), 3089 (C–H str., aromatic), 1656 (C=N str., aromatic), 1609, 1505, 1442 (C=C str., aromatic) cm^{-1}; MS: *m/z* for $C_{18}H_{17}N_5$: 304.0 [M^+], 305.0 [M^++1].

1-[[1-(3-Phenylpropyl)-1H-1,2,3-triazol-4-yl]methyl]-1H-benzo[d]imidazole (**3c**, $C_{19}H_{19}N_5$)
Brown solid; yield: 72 %; m.p.: 96–98 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.12$ (p, $J = 10.5$ Hz, 2H, CH_2), 2.53 (t, $J = 10.5$ Hz, 2H, CH_2), 4.18 (t, $J = 10.5$ Hz, 2H, CH_2), 5.41 (s, 2H, CH_2), 7.01–7.05 (m, 3H, Ar–H), 7.17 (t, $J = 12$ Hz, 3H, Ar–H), 7.24-7.28 (m, 3H, Ar–H), 7.77 (s, 1H, C–H triazole), 7.84 (s, 1H, C_2–H benzimidazole) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 31.3$, 32.3, 40.7, 49.5, 109.9, 120.5, 121.8, 123.2, 126.3 (C_5 triazole), 128.2, 128.5, 139.6, 142.8 (C_4 triazole) ppm; IR (KBr): $\bar{\nu} = 3113$ (C–H str., triazole ring), 3082 (C–H str., aromatic), 1670 (C=N str., aromatic), 1615, 1525, 1438 (C=C str., aromatic) cm^{-1}; MS: *m/z* for $C_{19}H_{19}N_5$: 318.0 [M^+], 319.0 [M^++1].

1-[[1-(4-Methylbenzyl)-1H-1,2,3-triazol-4-yl]methyl]-1H-benzo[d]imidazole (**3d**, $C_{18}H_{17}N_5$)
Light yellow solid; yield: 74 %; m.p.: 150–154 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.00$ (s, 3H, CH_3), 5.50 (s, 2H, CH_2), 5.57 (s, 2H, CH_2), 7.27–7.29 (m, 2H, Ar–H), 7.36 (t, $J = 8.8$ Hz, 3H, Ar–H), 7.40–7.43 (m, 1H, Ar–H), 7.80 (s, 1H, C–H triazole), 7.99 (s, 1H, C_2–H benzimidazole), 8.17 (d, $J = 8.8$ Hz, 2H, Ar–H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 21.1$, 40.5, 54.0, 109.8, 120.4, 121.6, 122.3, 123.1, 128.0 (C_5 triazole), 129.8, 131.1, 138.7, 143.1, 143.7 (C_4 triazole) ppm; IR (KBr): $\bar{\nu} = 3118$ (C–H str., triazole ring), 3072 (C–H str., aromatic), 1658 (C=N str., aromatic), 1614, 1550, 1431 (C=C str., aromatic) cm^{-1}; MS: *m/z* for $C_{18}H_{17}N_5$: 304.0 [M^+], 305.0 [M^++1].

1-[[1-(4-Nitrobenzyl)-1H-1,2,3-triazol-4-yl]methyl]-1H-benzo[d]imidazole (**3e**, $C_{17}H_{14}N_6O_2$)
Light yellow solid; yield: 70 %; m.p.: 144–148 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 5.59$ (s, 2H, CH_2), 5.75 (s, 2H, CH_2), 7.19–7.26 (m, 2H, Ar–H), 7.50 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.64 (t, $J = 8.8$ Hz, 2H, Ar–H), 8.20-8.23 (m, 2H, Ar–H), 8.26 (s, 1H, C–H triazole), 8.33 (s, 1H, C_2–H benzimidazole) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 40.6$, 52.4, 111.1, 119.9, 122.1, 122.8, 124.6, 127.1 (C_5 triazole), 129.5, 131.1, 143.6, 143.8 (C_4 triazole), 147.7 ppm; IR (KBr): $\bar{\nu} = 3113$ (C–H str., triazole ring), 3082 (C–H str., aromatic), 1656 (C=N str., aromatic), 1606, 1555, 1492 (C=C str., aromatic), 1523 (N–O str., asym., NO_2), 1348 (N–O str., sym., NO_2) cm^{-1}; MS: *m/z* for $C_{17}H_{14}N_6O_2$: 335.0 [M^+], 336.0 [M^++1].

2-[[4-[(1H-Benzo[d]imidazol-1-yl)methyl]-1H-1,2,3-triazol-4-yl]methyl]isoindoline-1,3-dione (**3f**, $C_{19}H_{14}N_6O_2$)
Dark brown solid; yield: 55 %; m.p.: 152–156 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.45$ (s, 2H, CH_2), 6.11 (s, 2H, phthalimide NCH_2), 7.23–7.27 (m, 3H, Ar–H), 7.73–7.77 (m, 4H, Ar–H), 7.85–7.89 (m, 2H, Ar–H), 7.99 (s, 1H, C_2–H benzimidazole) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 40.3$, 49.7, 109.9, 120.4, 122.5, 123.2, 123.3, 124.2, 127.4 (C_5 triazole), 131.3, 134.1, 134.8, 142.6, 143.5 (C_4 triazole), 166.3 (C=O) ppm; IR (KBr): $\bar{\nu} = 3134$ (C–H str., triazole ring), 3078 (C–H str., aromatic), 1720 (C=O str.), 1654 (C=N str., aromatic), 1612, 1492 (C=C str., aromatic) cm^{-1}; MS: *m/z* for $C_{19}H_{14}N_6O_2$: 359.0 [M^+], 360.0 [M^++1].

1-[(1-Benzyl-1H-1,2,3-triazol-4-yl)methyl]-1H-benzo[d]-[1,2,3]triazole (**6a**, $C_{16}H_{14}N_6$)
Light brown crystalline solid; yield: 74 %; m.p.: 156–160 °C (Ref. [29] 158–160 °C).

1-[(1-Phenethyl-1H-1,2,3-triazol-4-yl)methyl]-1H-benzo[d]-[1,2,3]triazole (**6b**, $C_{17}H_{16}N_6$)
Light yellow solid; yield: 70 %; m.p.: 120–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.12$ (t, $J = 7.2$ Hz, 2H, CH_2), 4.51 (t, $J = 7.2$ Hz, 2H, CH_2), 5.91 (s, 2H, CH_2), 6.93 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.07–7.16 (m, 4H, Ar–H), 7.37 (t, $J = 8.4$ Hz, 1H, Ar–H), 7.47 (s, 1H, C–H triazole), 7.66 (d, $J = 8.4$ Hz, 1H, Ar–H), 8.04 (d, $J = 8.4$ Hz, 1H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 36.6$, 43.8, 51.9, 110.1, 119.9, 123.2, 124.1, 127.2 (C_5 triazole), 127.6, 128.5, 132.6, 136.6, 141.7 (C_4 triazole), 146.2 ppm; IR (KBr): $\bar{\nu} = 3142$ (C–H str., triazole ring), 3026 (C–H str., aromatic), 1612, 1494, 1450 (C=C str., aromatic) cm^{-1}; MS: *m/z* for $C_{17}H_{16}N_6$: 305.0 [M^+], 306.0 [M^++1].

1-[[1-(3-Phenylpropyl)-1H-1,2,3-triazol-4-yl]methyl]-1H-benzo[d][1,2,3]triazole (**6c**, $C_{18}H_{18}N_6$)
Creamy white solid; yield: 72 %; m.p.: 92–96 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.19$ (p, $J = 7.2$ Hz, 2H, CH_2), 2.60 (t, $J = 7.2$ Hz, 2H, CH_2), 4.28 (t, $J = 7.2$ Hz, 2H, CH_2), 5.97 (s, 2H, CH_2), 7.08 (d, $J = 7.2$ Hz, 2H, Ar–H), 7.16 (d, $J = 7.2$ Hz, 1H, Ar–H), 7.25 (t, $J = 7.2$ Hz, 2H, Ar–H), 7.36 (t, $J = 7.2$ Hz, 1H, Ar–H), 7.45 (s, 1H, C–H triazole), 7.48 (d, $J = 7.2$ Hz, 1H, Ar–H), 7.71 (d, $J = 8.4$ Hz, 1H, Ar–H), 8.04 (d, $J = 8.4$ Hz, 1H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 31.4$, 32.4, 43.8, 49.7, 110.1, 119.9, 122.8, 124.2, 126.4, 127.7 (C_5 triazole), 128.4, 128.6, 132.7, 139.8, 142.1 (C_4 triazole), 146.2 ppm; IR (KBr): $\bar{\nu} = 3134$ (C–H str., triazole ring), 3070 (C–H str., aromatic), 1604, 1494, 1448 (C=C str., aromatic) cm^{-1}; MS: *m/z* for $C_{18}H_{18}N_6$: 319.0 [M^+], 320.0 [M^++1].

1-[[1-(4-Methylbenzyl)-1H-1,2,3-triazol-4-yl]methyl]-1H-benzo[d][1,2,3]triazole (**6d**, $C_{17}H_{16}N_6$)
Light brown solid; yield: 70 %; m.p.: 152–156 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.32$ (s, 3H, CH_3), 5.40 (s, 2H, CH_2), 5.92 (s, 2H, CH_2), 7.09–7.15 (m, 4H, Ar–H), 7.35 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.42 (s, 1H, C–H triazole), 7.46 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.71 (d, $J = 8.0$ Hz, 1H, Ar–H), 8.01 (d, $J = 8.0$ Hz, 1H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 21.2$, 43.8, 54.2, 110.2, 119.8, 122.7, 124.1, 127.0, 127.6 (C_5 triazole), 128.5, 131.0, 132.7, 139.0, 142.3 (C_4 triazole), 146.1 ppm; IR (KBr): $\bar{\nu} = 3134$ (C–H str., triazole ring), 3072 (C–H str.,

aromatic), 1614, 1508, 1438 (C=C str., aromatic) cm^{-1}; MS: *m/z* for $C_{17}H_{16}N_6$: 305.0 [M^+], 306.0 [M^++1].

1-[[1-(4-Nitrobenzyl)-1H-1,2,3-triazol-4-yl]methyl]-1H-benzo[d][1,2,3]triazole (**6e**, $C_{16}H_{13}N_7O_2$)
Light yellow solid; yield: 76 %; m.p.: 220–226 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 5.76 (s, 2H, CH_2), 6.07 (s, 2H, CH_2), 7.41 (t, J = 7.2 Hz, 1H, Ar–H), 7.50–7.58 (m, 3H, Ar–H), 7.91 (d, J = 8.4 Hz, 1H, Ar–H), 8.05 (d, J = 8.4 Hz, 1H, Ar–H), 8.22 (d, J = 8.4 Hz, 2H, Ar–H), 8.36 (s, 1H, C–H triazole) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 43.3, 52.4, 111.3, 119.6, 124.4, 125.1, 127.9 (C_5 triazole), 129.5, 133.1, 142.5, 143.7 (C_4 triazole), 145.7, 147.7 ppm; IR (KBr): $\bar{\nu}$ = 3126 (C–H str., triazole ring), 3074 (C–H str., aromatic), 1602, 1556, 1445 (C=C str., aromatic), 1523 (N–O str., asym., NO_2), 1344 (N–O str., sym., NO_2) cm^{-1}; MS: *m/z* for $C_{16}H_{13}N_7O_2$: 336.0 [M^+], 337.0 [M^++1].

2-[[4-[(1H-benzo[d][1,2,3]triazol-1-yl)methyl]-1H-1,2,3-triazol-1-yl]methyl]isoindoline-1,3-dione (**6f**, $C_{18}H_{13}N_7O_2$)
Light yellow solid; yield: 55 %; m.p.: 148–152 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.95 (s, 2H, CH_2), 6.14 (s, 2H, phthalimide NCH_2), 7.34 (t, J = 8.4 Hz, 1H, Ar–H), 7.45 (t, J = 8.4 Hz, 1H, Ar–H), 7.75–7.89 (m, 5H, Ar–H), 7.92 (s, 1H, C–H triazole), 8.00 (d, J = 7.6 Hz, 1H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 43.5, 49.8, 110.2, 119.8, 123.6, 124.0, 124.2, 127.7 (C_5 triazole), 131.3, 132.7, 135.0, 142.7 (C_4 triazole), 146.1, 166.4 (C=O) ppm; IR (KBr): $\bar{\nu}$ = 3126 (C–H str., triazole ring), 3026 (C-H str., aromatic), 1720 (C=O str.), 1608, 1456 (C=C str., aromatic) cm^{-1}; MS: *m/z* for $C_{18}H_{13}N_7O_2$: 360.0 [M^+], 361.0 [M^++1].

9-[(1-Benzyl-1H-1,2,3-triazol-4-yl)methyl]-9H-carbazole (**9a**, $C_{22}H_{18}N_4$)
White solid; yield: 78 %; m.p.: 158–162 °C (Ref. [30] 160–162 °C).

9-[(1-Phenethyl-1H-1,2,3-triazol-4-yl)methyl]-9H-carbazole (**9b**, $C_{23}H_{20}N_4$)
Dull-white solid; yield: 62 %; m.p.: 132–136 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.96 (t, J = 7.0 Hz, 2H, CH_2), 4.30 (t, J = 7.0 Hz, 2H, CH_2), 5.51 (s, 2H, CH_2), 6.62 (t, J = 8.0 Hz, 1H, Ar–H), 6.80 (t, J = 8.0 Hz, 2H, Ar–H), 7.01–7.04 (m, 3H, Ar–H), 7.20 (t, J = 8.0 Hz, 2H, Ar–H), 7.36 (s, 1H, C–H triazole), 7.36–7.39 (m, 3H, Ar–H), 8.05 (d, J = 8.0 Hz, 2H, Ar–H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 36.6, 38.7, 51.6, 108.7, 119.4, 120.4, 121.7, 123.1, 125.9, 126.9 (C_5 triazole), 128.6, 136.6, 140.0, 143.8 (C_4 triazole) ppm; IR (KBr): $\bar{\nu}$ = 3124 (C–H str., triazole ring), 3064 (C–H str., aromatic), 1593, 1490, 1448 (C=C str., aromatic) cm^{-1}; MS: *m/z* for $C_{23}H_{20}N_4$: 353.0 [M^+], 354.0 [M^++1].

9-[[1-(3-Phenylpropyl)-1H-1,2,3-triazol-4-yl]methyl]-9H-carbazole (**9c**, $C_{24}H_{22}N_4$)
White solid; yield: 66 %; m.p.: 140–146 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.07 (p, J = 7.2 Hz, 2H, CH_2), 2.49 (t, J = 7.2 Hz, 2H, CH_2), 4.11 (t, J = 7.2 Hz, 2H, CH_2), 5.62 (s, 2H, CH_2), 6.90–7.45 (m, 11H, Ar–H), 7.46 (s, 1H, C–H triazole), 8.10 (d, J = 7.6 Hz, 2H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 31.3, 32.3, 39.0, 49.5, 108.8, 119.5, 120.3, 120.5, 121.4, 123.2, 126.0 (C_5 triazole), 128.4, 128.6, 139.9, 140.1, 144.5 (C_4 triazole) ppm; IR (KBr): $\bar{\nu}$ = 3122 (C–H str., triazole ring), 3062 (C–H str., aromatic), 1597, 1489, 1452 (C=C str., aromatic) cm^{-1}; MS: *m/z* for $C_{24}H_{22}N_4$: 367.0 [M^+], 368.0 [M^++1].

9-[[1-(4-Methylbenzyl)-1H-1,2,3-triazol-4-yl]methyl]-9H-carbazole (**9d**, $C_{23}H_{20}N_4$)
White solid; yield: 58 %; m.p.: 158–162 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.28 (s, 3H, CH_3), 5.28 (s, 2H, CH_2), 5.57 (s, 2H, CH_2), 7.01 (d, J = 7.6 Hz, 3H, Ar–H), 7.07 (d, J = 7.6 Hz, 2H, Ar–H), 7.20–7.26 (m, 2H, Ar–H), 7.41–7.43 (m, 4H, Ar–H), 8.07 (d, J = 8.0 Hz, 2H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.1, 39.0, 54.0, 108.8, 119.4, 120.4, 121.3, 123.1, 126.0 (C_5 triazole), 128.0, 129.7, 131.4, 138.6, 140.1, 144.8 (C_4 triazole) ppm; IR (KBr): $\bar{\nu}$ = 3122 (C–H str., triazole ring), 3074 (C–H str., aromatic), 1597, 1485, 1450 (C=C str., aromatic) cm^{-1}; MS: *m/z* for $C_{23}H_{20}N_4$: 353.0 [M^+], 354.0 [M^++1].

9-[[1-(4-Nitrobenzyl)-1H-1,2,3-triazol-4-yl]methyl]-9H-carbazole (**9e**, $C_{22}H_{17}N_5O_2$)
Dull-yellow solid; yield: 60 %; m.p.: 148–152 °C (Ref. [30] 150–153 °C).

2-[[4-[(9H-Carbazol-9-yl)methyl]-1H-1,2,3-triazol-1-yl]methyl]isoindoline-1,3-dione (**9f**, $C_{24}H_{17}N_5O_2$)
Brown solid; yield: 56 %; m.p.: 154–158 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.57 (s, 2H, CH_2), 6.00 (s, 2H, phthalimide NCH_2), 7.12–7.25 (m, 3H, Ar–H), 7.40–7.48 (m, 3H, Ar–H), 7.70–7.75 (m, 2H, Ar–H), 7.80–7.82 (m, 2H, Ar–H), 7.99 (d, J = 7.6 Hz, 1H, Ar–H), 8.05 (d, J = 7.6 Hz, 2H, Ar–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 38.7, 49.6, 108.8, 119.9, 120.3, 122.5, 123.1, 126.2 (C_5 triazole), 131.3, 134.8, 139.3, 145.0 (C_4 triazole), 166.4 (C=O) ppm; IR (KBr): $\bar{\nu}$ = 3128 (C–H str., triazole ring), 3057 (C–H str., aromatic), 1724 (C=O), 1601, 1456 (C=C str., aromatic) cm^{-1}; MS: *m/z* for $C_{24}H_{17}N_5O_2$: 408.0 [M^+], 409.0 [M^++1].

Antibacterial activity

All the synthesized triazoles were screened for their in vitro antibacterial activity against two Gram-positive bacteria, i.e., *B. subtilis* (MTCC 441), *S. aureus* (MTCC 3160) and one Gram-negative bacteria, *E. coli* (MTCC 443) by

standard serial dilution method [34] using a stock solution of 100 μg/cm^3. Dimethylsulfoxide was employed as a solvent control. Dilutions of test compounds were prepared in double-strength nutrient broth. One cm^3 nutrient broth was taken in each of seven test tubes. To the first test-tube 1.0 cm^3 of drug solution (100 μg/cm^3) was added aseptically to get the concentration of 50 μg/cm^3. From this dilution, other concentrations were prepared by serial dilution to get final concentrations of 25, 12.5, 6.25, 3.12, 1.56, 0.78 μg/cm^3 in test-tube number two to seven. All the test tubes were aseptically inoculated by 0.1 cm^3 of desired bacterial strain in sterile saline. The inoculated test samples were then incubated at 37 ± 1 °C for 1 day. Norfloxacin, a broad spectrum antibiotic, was used as standard drug and also tested under similar experimental conditions for comparison with synthesized triazoles.

Antifungal activity

The in vitro antifungal activity of synthesized triazoles was performed against two fungal strains, i.e., *C. albicans* (MTCC 227) and *A. niger* (MTCC 281) by serial dilution method [34] using a stock solution of 100 μg/cm^3 concentration of compounds. Sabouraud dextrose broth was used as a fungal culture media and DMSO as solvent control. One cm^3 of freshly prepared sterile culture media was added aseptically in each test tube followed by serial dilution with synthesized compounds to prepare concentrations of 25–0.78 μg/cm^3. Further these dilutions of triazole compounds were inoculated with 0.1 cm^3 of suspension of respective microorganism contained in sterilized saline. Then samples of compounds loaded with microorganisms incubated at 27 ± 1 °C for 2 days in case of *C. albicans* and at 25 ± 1 °C for 7 days in case of *A. niger*. Fluconazole, a widely accepted antifungal drug, was used as standard drug.

Computational details

The docking procedure chosen in the present study was in accordance with the procedure given by Kumar [39–41]. Structures of the compounds were sketched with Mavin Sketch 5.10 [42] which were optimized and cleaned with gradient optimization. The X-ray crystallographic structure of *E. coli* topoisomerase II DNA gyrase B enzyme along with co-crystallized ligand CBN (PDB ID: 1KZN) was obtained from Brookhaven Protein Databank (http://www.rcsb.org/pdb). Preparation of protein was accomplished with UCSF Chimera 1.9 [43]. Incomplete side chains were completed with Dunbrack rotamer library [44] and Gasteiger charges were assigned with Antechamber [45]. Structures of ligands and proteins were transformed into pdbqt format with the aid of AutoDock tools [46]. Docking simulations were carried out by AutoDock Vina program. The Vina search space taken was center_x = 19.7572768649, center_y = 30.6958566405, center_z = 36.3020605554, size_x = 25.0, size_y = 24.2096062606, size_z = 21.3564769495. The exhaustiveness for docking was set to be 8. Validation of docking protocols was made by means of reported crystal structure of protein–ligand complex. Protocols selected for the AutoDock Vina docking studies were realistic to mimic the X-ray structure as the root-mean square deviation (RMSD) between the conformations of the CBN from the X-ray crystal structure and that from AutoDock Vina was less than 2 Å. These protocols were used for docking of the compound under study into the active site of DNA Gyrase B. Results were pictured with the help of PyMol [47] and Discovery Studio version 3.5 [48].

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