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Original article

Design, synthesis and antimicrobial activity of novel benzothiazole analogs

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ABSTRACT

In an attempt to design and synthesize a new class of antimicrobials, dialkyne substituted 2-aminobenzothiazole was reacted with various substituted aryl azides to generate a small library of 20 compounds (**3a**–**t**) by click chemistry. Structures of the newly synthesized compounds were established on the basis of spectral data. These compounds were screened for their antibacterial activity against Gram+ bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*), Gram− bacteria (*Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Shigella boydii*) and antifungal activity against *Candida tropicalis*, *Candida albicans*, *Candida krusei*, *Cryptococcus neoformans*) as well as molds (*Aspergillus niger*, *Aspergillus fumigatus*). The compound **3e** showed maximum potency against all Gram+/gram− bacterial strains with MIC value 3.12 μg/ml, which is two fold more active as compared to standard drug ciprofloxacin (MIC 6.25 μg/ml). However, all compounds were found ineffective against *S. boydii* (clinical isolate). Further, only one compound **3n** was found to be the most active against all fungal strains with MIC value in the range of 1.56 μg/ml−12.5 μg/ml while the remaining compounds showed moderate to weak antifungal activity.

1. Introduction

Benzothiazole analogs are one of the most versatile class of compounds which most commonly occur as various marine and terrestrial natural products [1,2]. It is a unique scaffold for further molecular exploration to synthesize novel compounds. It is well documented that benzothiazole derivatives find wide application in non-invasive diagnosis of Alzheimer's disease [3,4], antitubercular [5], antimalarial [6], antihelmintic [7] and also antidiabetic [8] etc. In addition, several benzothiazole analogs have also been screened as potential amyloid-binding diagnostic agents in neurodegenerative disease [9,10] and as selective fatty acid amide hydrolase inhibitors [11].

2-Aminobenzothiazole moiety is present in various bioactive molecules such as imaging agents for β-amyloid plaques [9], photosensitizers [12], inhibitors of stearoyl-coenzyme A δ-9 desaturase [13], antitumor **I** [14], antimicrobial [5], orexin receptor antagonist **II** [15] and the Gram+ selective antibacterial **III** [16]. Antifungal fluconazole **IV** and other marketed drugs, voriconazole **V** and albaconazole **VI** contain 1,2,4-triazole (Fig. 1). However, 1,2,3-triazole ring is not found in any marketed drugs. The click chemistry developed by Sharpless et al. is an excellent approach for regioselective synthesis of 1,2,3-triazole ring system in presence of various functional groups. Sincere efforts have been made to incorporate 1,2,3-triazole in existing drugs, still more research is needed to find lead molecule [17]. Although, 1,2,3-triazole structural moiety does not occur in nature, it is present in several compounds showing various biological activities including anti-HIV [18], anti-bacterial [19], anti-allergic [20], anticonvulsant [21], β-lactamase inhibitory [22], and anti-tuberculosis activities [23]. 1,2,3-triazole has been extensively studied owing to its importance in industrially interesting materials, such as dyes, anticorrosive agents, photo stabilizers, photographic materials, and agrochemicals [17].

We therefore found it interesting to design new molecules within the scope of synthetic procedure using benzothiazole scaffold followed by suitable modification to generate diversified compounds for antimicrobial activity. In this study, we exploited click chemistry for synthesis of diversified benzothiazole compounds mainly for the two reasons; first, it can tolerate wide range of functional groups and easy to do eco-friendly reactions at room temperature either in water or mixture of water and organic solvents, secondly; this approach will generate compounds having 1,2,3-triazole functionality rather than 1,2,4-triazole. These

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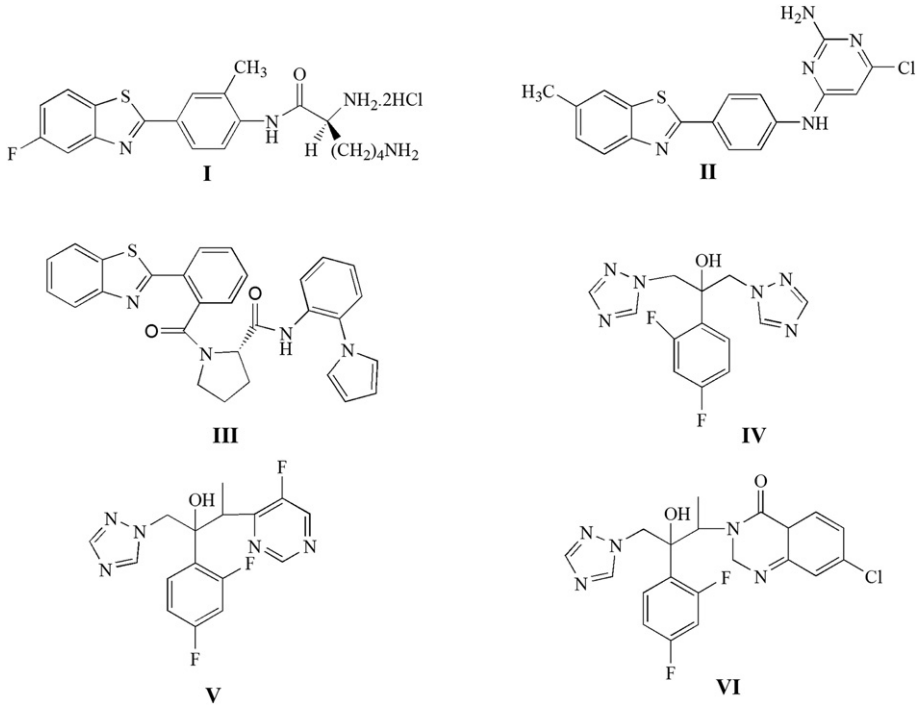


Fig. 1. Chemical structure of pharmacologically important benzothiazoles and fluconazoles.

compounds can be screened for antibacterial and antifungal activities. Structure–activity relationship (SAR) can be done on lead molecule to find the most potent molecule as a clinical candidate. Moreover, synthesis of these compounds will also provide an opportunity to compare antimicrobial activity with compounds containing 1,2,4-triazole, a common moiety found in several clinical antimicrobial drugs.

In view of the above mentioned facts, and in continuation to ongoing research work in our laboratory on design and synthesis of new antimicrobial agents [24–26] and X-rays analysis of small molecules [27–30], we further designed and synthesized small library of 20 compounds of benzothiazole-1,2,3-triazole analogs and screened them for antimicrobial activity.

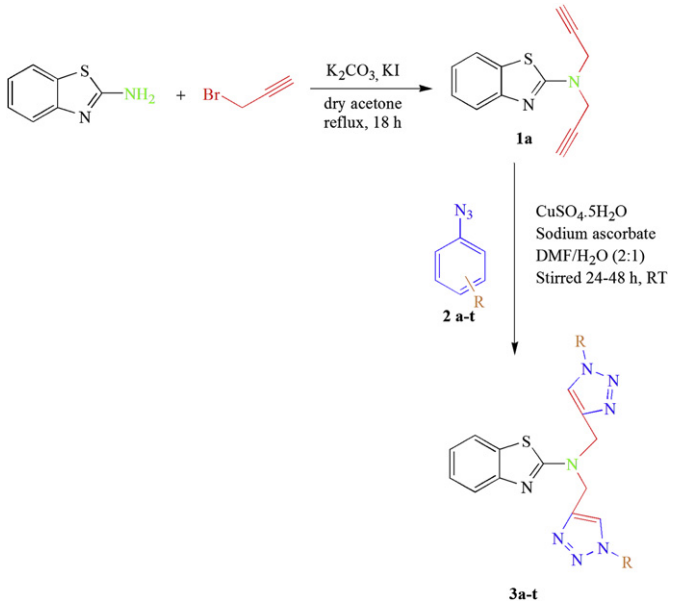
2. Chemistry

The benzothiazol-2-yl-di-prop-2-ynyl-amine (**1a**) was synthesized in good yield by simple alkylation involving reaction of 2-aminobenzothiazole and propargyl bromide as outlined in Scheme 1. The compound **1a** was further reacted with various substituted aromatic azides (**2a**–**t**) to give 20 different compounds (**3a**–**t**). The detailed general synthesis procedure of the compounds is mentioned in the Experimental section. All synthesized compounds (**3a**–**t**) were characterized by ESI-MS, ^{1}H and ^{13}C NMR. The structure of the compound **3e** was also confirmed by X-ray crystallographic analysis and reported elsewhere (CCDC no 896668). The crystal structure of compound **1b** was published earlier [29].

3. Results and discussion

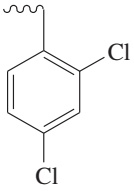
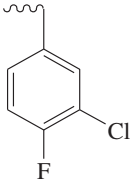
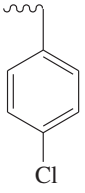
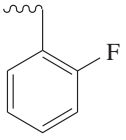
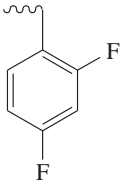
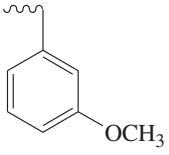
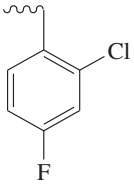
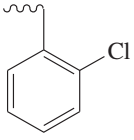
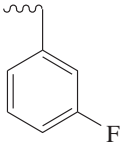
Various compounds were generated by reacting 2-aminobenzothiazole with propargyl bromide in presence of base K_2CO_3 in dry acetone which yielded **1a** (major) and **1b** (minor). The compound **1a** containing propargyl group at 2-position was used as substrate to further generate small 1,4-disubstituted 1,2,3-triazole library of 20-compounds (**3a**–**t**) by reacting various substituted aromatic azides using click chemistry. All compounds were purified by column chromatography or by recrystallization, well characterized by ^{1}H and ^{13}C NMR, ESI-MS etc. Various substituted 1,2,3-triazoles **3a**–**t** were screened for their antibacterial activity (MIC) against various Gram+ and gram– bacterial strains and activity data are summarized in Table 1. We chose two strains of Gram+ bacteria i.e. *Staphylococcus aureus* (ATCC 25323) and *Enterococcus faecalis* (clinical isolate) and five gram– bacterial strains i.e., *Escherichia coli* (ATCC 35218), *Salmonella typhi* (MTCC 3216), *Klebsiella pneumoniae* (clinical isolated), *Pseudomonas aeruginosa* (ATCC 27893) and *Shigella boydii* (clinical isolate) for study.

It is evident from Table 1 that six compounds viz. **3b**, **3c**, **3e**, **3g**, **3h** and **3r** were found more potent with either less or equal MIC as



Scheme 1. Schematic representation of synthesis of benzothiazole 1,2,3-triazole analogs.

Table 1
Antibacterial activity (MIC μg/ml) and % hemolysis of compounds **3a**–**t**.

Compound no.	R	Gram +ve strain		Gram −ve strain					% hemolysis
		S. aureus (ATCC 25323)	*E. faecalis* (clinical isolate)	*S. typhi* (MTCC 3216)	*E. coli* (ATCC 35218)	*K. pneumoniae* (clinical isolate)	*P. aeruginosa* (ATCC 27893)	*S. boydii* (clinical isolate)	
3a		50	50	>100	50	>100	>100	>100	4.54
3b		6.25	3.12	6.25	25	50	3.12	100	5.32
3c		6.25	3.12	6.25	12.5	50	6.25	>100	4.35
3d		>100	>100	>100	>100	>100	>100	>100	26.52
3e		3.12	3.12	3.12	3.12	3.12	3.12	100	2.47
3f		100	>100	>100	50	>100	>100	>100	7.54
3g		50	6.25	6.25	3.12	100	6.25	100	12.31
3h		25	6.25	6.25	12.5	50	12.5	>100	19.48
3i		>100	>100	>100	>100	>100	>100	>100	29.79

(*continued on next page*)

Table 1 (*continued*)

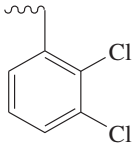
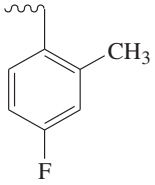
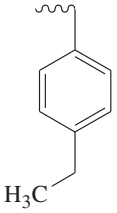
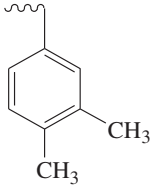
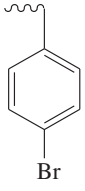
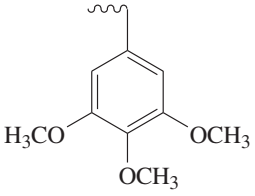
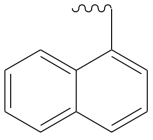
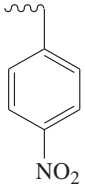
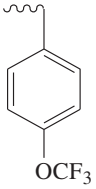
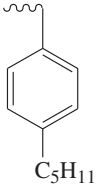
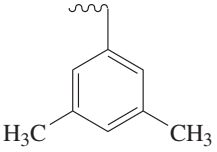
Compound no.	R	Gram +ve strain		Gram −ve strain					% hemolysis
		S. aureus (ATCC 25323)	*E. faecalis* (clinical isolate)	*S. typhi* (MTCC 3216)	*E. coli* (ATCC 35218)	*K. pneumoniae* (clinical isolate)	*P. aeruginosa* (ATCC 27893)	*S. boydii* (clinical isolate)	
3j		50	>100	100	>100	>100	>100	>100	37.66
3k		>100	>100	>100	50	>100	>100	>100	29.79
3l		>100	>100	>100	50	>100	>100	>100	48.27
3m		>100	>100	>100	>100	>100	>100	>100	43.05
3n		>100	>100	>100	>100	>100	>100	>100	12.37
3o		>100	>100	>100	>100	>100	>100	>100	36.60
3p		>100	>100	>100	>100	>100	>100	>100	34.04
3q		>100	>100	>100	>100	>100	>100	>100	27.85

Table 1 (*continued*)

Compound no.	R	Gram +ve strain		Gram −ve strain					% hemolysis
		S. aureus (ATCC 25323)	*E. faecalis* (clinical isolate)	*S. typhi* (MTCC 3216)	*E. coli* (ATCC 35218)	*K. pneumoniae* (clinical isolate)	*P. aeruginosa* (ATCC 27893)	*S. boydii* (clinical isolate)	
3r		12.5	12.5	>100	25	12.5	25	100	9.12
3s		>100	>100	>100	>100	>100	>100	>100	3.28
3t		100	>100	100	100	>100	>100	100	7.42
Ciprofloxacin	–	6.25	6.25	6.25	6.25	6.25	3.12	6.25	–

compared to control drug ciprofloxacin. DMSO was also taken in a control experiment which showed no effect in the experiment.

The compound **3a** containing 2,4-dichlorosubstituted benzene ring showed moderate to weak activity in the range of 50 μg/ml to 100 μg/ml while ciprofloxacin showed MIC 6.25 μg/ml in all strains except *P. aeruginosa* where MIC is 3.12 μg/ml. The compounds **3b** and **3c** having 3-chloro-4-fluoro and 4-chlorosubstituted benzene ring, respectively were found to be the most potent with MIC 3.12 μg/ml in *E. faecalis*. Further, the same compounds also showed MIC 6.25 μg/ml in *S. aureus* and *S. typhi*. Again, the compound **3b** was found to be the most potent with MIC 3.12 μg/ml while the compound **3c** exhibited good activity with MIC 6.25 μg/ml against strain *P. aeruginosa*. Further, the compound **3b** showed MIC 25 μg/ml while compound **3c** showed MIC 12.5 μg/ml against *E. coli*. In other strains, the compounds **3b** and **3c** showed moderate to weak activity in the range of 50 μg/ml to100 μg/ml. The compound **3e** containing 2,4-difluorosubstituted benzene showed excellent potency against six strains having MIC 3.12 μg/ml except *S. boydii*, clinical isolate strain (MIC 100 μg/ml). Further, the compound **3g** having 2-chloro-4-fluorosubstituted benzene ring showed potent inhibitory activity against *E. faecalis*, *S. typhi* and *P. aeruginosa* with MIC 6.25 μg/ml. It also showed two folds strong antibacterial activity against *E. coli* with MIC 3.12 μg/ml while standard drug has MIC 6.25 μg/ml and showed moderate to weak activity in the range of 50 μg/ml to 100 μg/ml against remaining strains. The compound **3h** showed MIC 6.25 μg/ml against *E. faecalis*, *S. typhi* and *E. coli* while against *P. aeruginosa* showed MIC 12.5 μg/ml and against remaining strains showed moderate to weak activity with MIC 25 μg/ml to 100 μg/ml. Again, the compounds **3i** to **3q** showed moderate to weak activity against all strains in the range of 50 μg/ml to 100 μg/ml or >100 μg/ml. The compound **3r** containing 4-OCF_3 substituted benzene ring was found active against three bacterial strains viz. *S. aureus*, *E. faecalis* and *K. pneumoniae* with MIC 12.5 μg/ml while against remaining strains showed moderate to weak susceptibility with MIC in the range of 25 μg/ml–100 μg/ml or >100 μg/ml. Further, the compounds **3s** and **3t** were found inactive with MIC 100 μg/ml or >100 μg/ml against all strains. In general, control drug ciprofloxacin showed MIC 3.12 μg/ml against *P. aeruginosa* while against the remaining strains showed MIC 6.25 μg/ml.

It is obvious from the analysis of activity results that electron withdrawing groups such as fluoro, chloro or combination of these have strong effects in determining the antibacterial activity. This observation is supported by the highest activity shown by the compound **3e** against six bacterial strains (both Gram+ and gram− strains) except *S. boydii*. Further, the position of functional groups in benzene ring also play critical role toward activity. For example, compound **3g** containing chloro and fluoro groups at position-2 & 4 in benzene ring, showed potency while the compound **3i** with fluoro group at position-3 in benzene ring lost activity. The presence of chloro at position-2 & 3 in benzene ring (compound **3j**) also lost activity. However, the presence of Br group at 4-position in benzene ring of compound also lost activity. Thus, we hypothesize that the position-2 is critical for antibacterial activity and fluoro group has significant effect on antibacterial activity as compared to chloro group.

Further, the presence of electron releasing groups such as methyl, methoxy and pentyl groups irrespective of position in the benzene rings have shown strong adverse effect on antibacterial activity and thus the compounds containing these functional groups showed weak or moderate activity as compared to electron withdrawing groups. However, nitro group at position-4 also resulted in decreased activity. In order to ascertain this hypothesis, we need to do more systematic design and synthesis of diversified compounds using multifunctional substituted benzene ring. Once lead compound is identified from in vitro screening, more

systematic structure–activity relationship (SAR) is needed to get molecule for in vivo studies which may eventually clinical candidate for future.

Further, these compounds **3a**–**t** were also screened against five fungal strains viz. *Candida tropicalis* (ATCC 750), *Candida albicans* (clinical), *C. albicans* (ATCC 90028), *Candida krusei* (ATCC 6258), *Cryptococcus neoformans* (clinical) and two molds *Aspergillus niger* (clinical) and *Aspergillus fumigatus* (clinical) for their antifungal activity. These compounds showed weak to moderate antifungal activity as shown in Table 2. Fluconazole was taken as a standard drug in the experiment.

Compound **3a** having 2,4-dichlorosubstituted benzene ring was found to be the most potent. It showed antifungal activity with MIC 1.56 μg/ml against clinical strains *A. niger* and *A. fumigatus* which is comparable to standard drug fluconazole with MIC 1.56 μg/ml. Again, the compound **3a** also showed potent inhibitory activity against strains *C. neoformans* with MIC 3.12 μg/ml while against the remaining strains showed MIC in the range between 6.25 μg/ml and 12.5 μg/ml. The compound **3b** containing 3-chloro-4-fluorobenzene ring showed moderate activity against *C. krusei* and *C. neoformans* with MIC 3.12 μg/ml and 1.56 μg/ml, respectively while against the remaining strains showed MIC in between 6.25 μg/ml and 25 μg/ml.

The compound **3c** containing 4-chlorobenzene ring showed good activity against *C. neoformans* with MIC 3.12 μg/ml while the moderate to weak activity in the range of 3.12 μg/ml–25 μg/ml was observed with other strains. The compound **3f** having 3-methoxysubstituted benzene ring showed good activity against three strains viz. *C. neoformans* with MIC 1.56 μg/ml and *A. niger* and *A. fumigatus* with MIC 3.12 μg/ml while fluconazole showed MIC 0.78 μg/ml and 1.56 μg/m respectively. Again, compound **3g** containing 2-chloro-4-flurosubstituted benzene ring showed good activity against *C. neoformans* with MIC 1.56 μg/ml and showed moderate to weak activity against remaining six strains in between 6.25 μg/ml and 50 μg/ml. Further, the compound **3j** having 2,3-dichlorosubstituted benzene ring showed reasonable good activity against *C. neoformans* with MIC 3.12 μg/ml and weak or no activity against remaining strains with MIC 25 μg/ml to >100 μg/ml. Further, the compound **3n** having 4-bromosubstituted benzene ring had shown good activity against *C. neoformans* with MIC 1.56 μg/ml while against *A. niger* & *A. fumigatus* showed MIC 3.12 μg/ml. Further, the same compound also showed moderate activity in the range of 6.25 μg/ml–12.5 μg/ml against remaining strains.

The compound **3r** containing 4-OCF_3 group in benzene ring also showed reasonable good activity with MIC 3.12 μg/ml against two strains *C. albicans* and *C. neoformans*. Further, the moderate activity was seen with the other remaining strains in between 12.5 μg/ml and 25 μg/ml.

Again, the compound **3s** having 4-pentyl group was found inactive against most of strains except *C. tropicalis* with MIC 12.5 μg/ml. The compound **3t** containing 3,5-dimethyl groups in benzene showed potent activity with MIC 3.12 μg/ml against two strains *C. albicans* & *C. neoformans* while the remaining strains showed moderate activity with MIC in between 12.5 μg/ml or >100 μg/ml. The fluconazole was taken as a control drug which showed MIC value in between 0.78 μg/ml and 3.12 μg/ml against various strains. Thus, it is obvious from the above antifungal screening data that both presence of electron withdrawing groups such as bromo or chloro group and electron releasing groups such as methoxy group on position-2, 3 or 4 showed potent antifungal activity. Further, presence of dihalogen in benzene ring has better antifungal activity than monohalogen. Interestingly, 4-bromosubstituted benzene showed strong antifungal activity while it showed weak antibacterial activity.

We hypothesize that groups such as methoxy, alkyls and halogens present on suitable position in benzene ring enhanced antifungal activity. Dichlorocompounds showed better antifungal activity than difluorocompounds. Moreover, we need to use multifunctional substituted benzene ring in click chemistry to generate large number of diverse compounds for in vitro antifungal screening. Further, the screening of these compounds will also help to predict positional effect of functional groups on antimicrobial activity. Structure–activity relationship (SAR) needs to be done on most potent molecule to find lead molecule which can be evaluated for in vivo activity. Thus, more systematic design and synthesis of second generation compounds with various functional groups are needed to established meaningful structure–activity relation (SAR). We are working in this direction.

Table 2
Antifungal activity (MIC μg/ml) of compounds **3a**–**t**.

Compounds no.	Fungal species					Molds	
	C. tropicalis (ATCC 750)	*C. albicans* (clinical)	*C. albicans* (ATCC 90028)	*C. krusei* (ATCC6258)	*Cryptococcus neoformans* (clinical)	*A. niger* (clinical)	*A. fumigatus* (clinical)
3a	12.5	12.5	6.25	6.25	3.12	1.56	1.56
3b	12.5	25	6.25	3.12	1.56	6.25	25
3c	12.5	25	6.25	6.25	3.12	6.25	6.25
3d	>100	>100	>100	>100	>100	>100	>100
3e	>100	>100	>100	>100	>100	>100	>100
3f	12.5	25	6.25	6.25	1.56	3.12	3.12
3g	6.25	6.25	50	6.25	1.56	25	6.25
3h	>100	>100	>100	>100	>100	>100	>100
3i	>100	>100	>100	>100	>100	>100	>100
3j	>100	25	>100	>100	3.12	>100	>100
3k	>100	>100	>100	>100	>100	>100	>100
3l	>100	>100	>100	>100	>100	>100	>100
3m	>100	>100	>100	>100	>100	>100	>100
3n	6.25	12.5	6.25	6.25	1.56	3.12	3.12
3o	>100	>100	>100	6.25	>100	>100	>100
3p	>100	>100	>100	>100	>100	>100	>100
3q	>100	>100	>100	>100	>100	>100	>100
3r	12.5	12.5	3.12	12.5	3.12	12.5	25
3s	12.5	>100	>100	>100	>100	>100	>100
3t	12.5	12.5	3.12	>100	3.12	12.5	12.5
Fluconazole	1.56	3.12	1.56	1.56	0.78	1.56	1.56

3.1. Hemolytic activity

To test toxicity of synthesized compounds **3a**–**t**, the hemolytic activities were carried out according to the procedure developed by Nielson et al. [31] on human hRBC at a fixed concentration of 100 μM. The analysis results showed that these compounds caused 2–48% hemolysis. Most of the compounds (**3a**–**3c**, **3e**–**3h**, **3n**, and **3r**–**3t**) showed less than 20% hemolysis suggesting that most of the compounds are less toxic. In general, the compounds with better antimicrobial activity showed negligible or very low toxicity profile. The compound **3e** showed only 2.47% hemolysis at very high concentration i.e. 100 μM. Thus, these results further support the significance of the study. The antibacterial as well hemolysis results are depicted in Table 1.

4. Conclusion

The objective of the present work was to design, synthesize and screen the antibacterial and antifungal activities of novel benzothiazole analogs with the hope of discovering new structure leads as the most potent antimicrobial agents. Our aim has been achieved by the synthesis of small benzothiazole library of 20 compounds with diver functionalities by exploiting click chemistry and screened them against various Gram+ and gram− bacterial as well as fungal strains. The compounds **3b**, **3c** and **3e** were found to be the most potent than standard drug ciprofloxacin with MIC 3.12 μg/ml against *E. faecalis* (clinical isolate) and *E. coli* (ATCC 35318) bacterial strains respectively and the compounds viz. **3b**, **3c**, **3g** and **3h** were also active with MIC 6.25 μg/ml, which is same as ciprofloxacin against *S. aureus* (ATCC 25323), *E. faecalis* (clinical isolate), *S. typhi* (MTCC 3216) and *P. aeruginosa* (ATCC 27893). Most of the compounds showed very little toxicity as revealed by hemolysis data.

Further, the compound **3e** was found to be the most active agent against all bacterial strains except *S. boydii* (clinical isolate). The electron withdrawing groups at position-2, 4 in benzene ring enhanced the inhibitory activity while electron releasing groups decrease the inhibitory activity in bacteria. Furthermore, the compound **3a** was found to be the most potent with MIC at 1.56 μg/ml which shows similar activity as fluconazole against *A. niger* (clinical) and *A. fumigatus* (clinical). The only compound **3n** showed good activity against all fungal strains while remaining compounds showed moderate to weak activity. The obtained results clearly demonstrate that the compounds derived from benzothiazole exhibited better antimicrobial activity. This is preliminary results and to reach more appropriate conclusion, 2nd and 3rd generation of compounds should be synthesized in order to establish meaningful structure–activity relationship (SAR).

5. Experimental

Chemicals and solvents used in this study were purchased from E. Merck (India) and Sigma–Aldrich chemicals. Melting points were determined by using open capillary method and are uncorrected. ^{1}H NMR spectral data were recorded on Brucker Advance spectrometer at 300 MHz and Jeol JNM ECX spectrometer at 300 MHz using TMS as an internal standard. The chemical shifts values were recorded on δ scale and the coupling constants (J) in Hertz. The following abbreviations were used in reporting spectra: s = singlet, d = doublet, dd = double doublet, t = triplet, td = triple doublet, q = quartet, m = multiple. ESI-MS spectra were obtained on a Waters Micromass LCT Mass spectrometer. Elemental analysis was done on Elementar GmbH VarioEl analyzer.

5.1. General procedure for the synthesis of benzothiazol-2-yl-di-prop-2-ynyl-amine (**1a**)

The synthesis of alkyne was carried out according to the literature procedure. Briefly, to a solution of amine (6 mmol) in dry acetone was added anhydrous K_2CO_3 (32 mmol) and reaction mixture was further refluxed for 15–30 min. Subsequently, KI (3 mmol) and propargyl bromide (7.2 mmol) were added and the reaction mixture was further refluxed for 18 h. The completion of reaction was monitored by TLC. After completion of the reaction, mixture was cooled, filtered, and the filtrate was evaporated in vacuo to give the two products (**1a** & **1b**), which were purified by column chromatography using hexane and dichloromethane (65:35) as eluent. The major product was compound **1a** which was used for synthesis of next step compounds (**3a**–**t**) by click chemistry while the compound **1b** was crystallized and the structure was established by X-rays analysis. All 20 compounds were screened for antibacterial and antifungal activities.

5.1.1. Benzothiazol-2-yl-di-prop-2-ynyl-amine (**1a**)

Yield: 30%, M.P: 202 °C; MS m/z: 226 (M^+); ^{1}H NMR ($CDCl_3$): δ 7.64–7.61 (dd, 2H, J = 7.5 Hz, 1.2 Hz, Ar–H), 7.35–7.32 (td, 1H, J = 8.2 Hz, 1.2 Hz, Ar–H), 7.15–7.10 (td, 1H, J = 8.2 Hz, 1.2 Hz, Ar–H), 4.48 (d, 4H, J = 2.4 Hz, 2× N–CH_2–), 2.32 (t, 2H, J = 2.4 Hz, 2× –C≡C–H); ^{13}C NMR ($CDCl_3$): δ 39.22, 73.42, 119.82, 120.81, 122.06, 126.11, 131.41, 152.26, 167.02; Elemental analysis: Molecular formula: $C_{13}H_{10}N_2S$: calculated: C-69.00, H-4.45, N-12.38, S-14.17; Found: C-69.08, H-4.51, N-12.31, S-14.10.

5.1.2. Benzothiazol-2-yl-prop-2-ynyl-amine (**1b**)

Yield: 5%, M.P: 215 °C; MS m/z: 188 (M^+); ^{1}H NMR ($CDCl_3$): 8.35 (s, 1H, NH), 7.70–7.67 (d, 1H, J = 7.8 Hz, Ar–H), 7.44–7.42 (d, 1H, J = 7.8 Hz, Ar–H), 7.26–7.21 (t, 1H, J = 7.5 Hz, Ar–H), 7.07–7.02 (t, 1H, J = 7.5 Hz, Ar–H), 4.18–4.17 (d, 2H, J = 2.7 Hz, N–CH_2–), 3.21 (s, 1H, –C≡C–H); Elemental analysis: Molecular formula: $C_{10}H_8N_2S$: calculated for: C-63.8, H-4.2, N-14.9, S-17.1, found C-63.9, H-4.5, N-14.7, S-16.9.

5.2. General procedure for the synthesis of azide **2** (**a**–**t**)

The synthesis of various azides was carried out according to the literature procedure [32]. Briefly, aniline (1 eq, 5 mmol) was dissolved in 6 N HCl solution (20 ml) at room temperature and cooled up to 0 °C, followed by addition of a solution of $NaNO_2$ (1 eq, 5 mmol). The reaction mixture was stirred for 10 min at 0–5 °C. Sodium azide (1.2 eq, 6 mmol) was added and mixture was further stirred at room temperature for 2 h. The reaction was worked up by dilution with ethyl acetate. The organic layer was washed with brine solution and dried over sodium sulfate. After evaporation of the solvent, the crude product **2** (**a**–**t**) was pure enough for further reactions. All the synthesized azides were stored at −20 °C.

5.3. General procedure for the synthesis of compounds (**3a**–**t**)

The synthesis of compounds (**3a**–**t**) was carried out according to the literature procedure [33]. Briefly, benzothiazol-2-yl-di-prop-2-ynyl-amine (**1a**) (1 mmol) and various aromatic azide (2 mmol) were suspended in *N*,*N*′-dimethylformamide (10 ml). Sodium ascorbate (0.3 mmol, in water) was added, followed by copper (II) sulfatepentahydrate (0.03 mmol, in water). The heterogenous mixture was stirred vigorously overnight, and the completion of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with water, cooled in ice, and the precipitate was collected by filtration.

*5.3.1. Benzothiazol-2-yl-bis-[1-(2,4-dichloro-phenyl)-1H-[1,2,3] triazol-4-ylmethyl]-amine (**3a**)*

Yield: 57%, M.P: 135 °C; MS *m/z*: 602 (M^+); ^{1}H NMR ($CDCl_3$): δ 8.10 (s, 2H, 2× C═CH of triazole), 7.62–7.53 (m, 6H, Ar–H), 7.42–7.39 (d, 2H, *J* = 10.5 Hz, Ar–H), 7.33–7.30 (m, 1H, Ar–H), 7.12–7.07 (t, 1H, *J* = 7.5 Hz, Ar–H), 5.04 (s, 4H, 2× N–CH_2–); ^{13}C NMR ($CDCl_3$): δ 46.13, 106.25, 115.57, 112.44, 114.75, 119.20, 120.82, 121.64, 123.62, 126.02, 126.80, 130.46, 138.33, 152.67, 160.52, 167.45; Elemental analysis: Molecular formula: $C_{25}H_{16}Cl_4N_8S$: Calculated: C-49.85, H-2.68, Cl-23.54, N-18.60, S-5.32; Found: C-49.95, H-2.78, Cl-23.50, N-18.50, S-5.26.

*5.3.2. Benzothiazol-2-yl-bis-[1-(3-chloro-4-fluoro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-amine (**3b**)*

Yield: 86%, M.P: 180 °C; MS *m/z*: 569 (M^+); ^{1}H NMR ($CDCl_3$): δ 8.12 (s, 2H, 2× C═CH of triazole), 7.82–7.80 (d, 2H, *J* = 6Hz, Ar–H), 7.62–7.60 (d, 4H, *J* = 6.9 Hz, Ar–H), 7.35–7.25 (m, 3H, Ar–H), 7.13–7.08 (t, 1H, *J* = 7.3 Hz, Ar–H) 5.00 (s, 4H, 2× N–CH_2–); ^{13}C NMR ($CDCl_3$): δ 45.70, 117.30, 119.04, 120.05, 120.64, 121.43, 121.54, 122.77, 125.90, 130.82, 133.29, 143.97, 152.45, 156.28, 159.02, 166.93; Elemental analysis: Molecular formula: $C_{25}H_{16}Cl_2F_2N_8$ S: Calculated: C-52.73, H-2.83, Cl-12.45, F-6.67, N-19.68, S-5.63; Found: C-52.83, H-2.93, Cl-12.40, F-6.62, N-19.61, S-5.60.

*5.3.3. Benzothiazol-2-yl-bis-[1-(4-chloro-phenyl)-1H-[1,2,3] triazol-4-ylmethyl]-amine (**3c**)*

Yield: 56%, M.P: 188 °C; MS *m/z*: 533 (M^+); ^{1}H NMR ($CDCl_3$): δ 8.76 (s, 2H, 2× C═CH of triazole), 7.60–7.588.12 (s, 2H, Ar–H), 7.81–7.79 (d, 3H, *J* = 7.9 Hz, Ar–H), 7.01–6.99 (d, 5H, *J* = 8.5 Hz, Ar–H), 7.26–7.21 (t, 1H, *J* = 7.5 Hz, Ar–H), 7.06–7.01 (t, 1H, *J* = 7.6 Hz, Ar–H), 5.06 (s, 4H, 2× N–CH_2–); ^{13}C NMR ($CDCl_3$): δ 45.79, 119.24, 120.99, 121.91, 123.15, 125.76, 132.89, 135.33, 136.20, 144.45, 152.71, 161.84, 177.93; Elemental analysis: Molecular formula: $C_{25}H_{18}Cl_2N_8S$: Calculated: C-56.29, H-3.40, Cl-13.62, N-21.01, S-6.01; Found: C-56.39, H-3.30, Cl-13.19, N-21.06, S-6.06.

*5.3.4. Benzothiazol-2-yl-bis-[1-(2-fluoro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-amine (**3d**)*

Yield: 92%, M.P: 120 °C; MS *m/z*: 499.85 (M^+); ^{1}H NMR (DMSO): δ 8.60 (s, 2H, 2× C═CH of triazole), 7.83–7.78 (t, 3H, *J* = 7.8 Hz, Ar–H), 7.57–7.51 (t, 5H, *J* = 9 Hz, Ar–H), 7.44–7.40 (t, 2H, *J* = 7.05 Hz, Ar–H), 7.32–7.27 (t, 1H, *J* = 7.5 Hz, Ar–H) 7.12–7.07 (t, 1H, *J* = 7.2 Hz, Ar–H), 5.02 (s, 4H, 2× N–CH_2–); ^{13}C NMR ($CDCl_3$): δ 45.91, 116.89, 117.08, 119.23, 120.80, 121.60, 124.46, 124.93, 125.15, 125.19, 126.00, 130.22, 130.29, 143.58, 152.12, 152.70, 154.44, 167.48; Elemental analysis: Molecular formula: $C_{25}H_{18}F_2N_8S$: Calculated: C-59.99, H-3.62, F-7.59, N-22.39, S-6.41; Found: C-59.94, H-3.67, F-7.54, N-22.49, S-6.36.

*5.3.5. Benzothiazol-2-yl-bis-[1-(2,4-difluoro-phenyl)-1H-[1,2,3] triazol-4-ylmethyl]-amine (**3e**)*

Yield: 90%, M.P: 145 °C; MS *m/z*: 536.04 (M^+); ^{1}H NMR ($CDCl_3$): δ 8.14 (s, 2H, 2× C═CH of triazole), 7.88–7.86 (d, 2H, *J* = 5.1 Hz, Ar–H), 7.61–7.59 (d, 2H, *J* = 7.2 Hz, Ar–H), 7.05–7.03 (d, 6H, *J* = 4.8 Hz, Ar–H), 5.03 (s, 4H, 2× N–CH_2–); ^{13}C NMR ($CDCl_3$): 45.92, 105.33, 112.41, 112.64, 119.23, 120.81, 121.66, 124.32, 126.03, 131.13, 143.70, 152.58, 155.05, 161.26, 163.77, 167.45; Elemental analysis: Molecular formula: $C_{25}H_{16}F_4N_8S$: Calculated: C-55.97, H-3.01, F-14.16, N-20.89, S-5.98; Found: C-55.92, H-3.06, F-14.11, N-20.99, S-5.93.

*5.3.6. Benzothiazol-2-yl-bis-[1-(3-methoxy-phenyl)-1H-[1,2,3] triazol-4-ylmethyl]-amine (**3f**)*

Yield: 54%, M.P: 105 °C; MS *m/z*: 511 (M^+); ^{1}H NMR ($CDCl_3$): δ 8.07 (s, 2H, 2× C═CH of triazole), 7.60–7.58 (m, 4H, Ar–H), 7.36–7.34 (m, 1H, Ar–H), 7.10–7.07 (t, 1H, *J* = 7.5 Hz, Ar–H), 6.95–6.93 (d, 6H, *J* = 7.5 Hz, Ar–H), 5.01 (s, 4H, 2× N–CH_2–), 3.85 (s, 6H, 2× –OCH_3); ^{13}C NMR ($CDCl_3$): δ 46.11, 55.56, 117.90, 118.91, 120.73, 121.91, 122.75, 126.19, 126.79, 129.02, 131.45, 132.89, 135.09, 136.10, 143.99, 152.48, 161.98, 162.79, 167.64; Elemental analysis: Molecular formula: $C_{27}H_{24}N_8O_2S$: Calculated: C-61.82, H-4.61, N-21.36, O-6.10, S-6.11; Found: C-61.92, H-4.55, N-21.32, O-6.05, S-6.16.

*5.3.7. Benzothiazol-2-yl-bis-[1-(2-chloro-4-fluoro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-amine (**3g**)*

Yield: 60%, M.P: 140 °C; MS *m/z*: 569 (M^+); ^{1}H NMR ($CDCl_3$): δ 8.06 (s, 2H, 2× C═CH of triazole), 7.62–7.55 (m, 4H, Ar–H), 7.32–7.25 (m, 2H, Ar–H), 7.17–7.07 (m, 4H, Ar–H), 5.04 (s, 4H, 2× N–CH_2–); ^{13}C NMR ($CDCl_3$): δ 46.06, 115.13, 115.36, 118.18, 119.15, 120.84, 121.61, 125.46, 125.99, 129.24, 130.10, 131.20, 142.72, 152.71, 161.40, 164.02, 167.49; Elemental analysis: Molecular formula: $C_{25}H_{16}Cl_2F_2N_8$ S: Calculated: C-52.73, H-2.83, Cl-12.45, F-6.67, N-19.68, S-5.63; Found: C-52.68, H-2.88, Cl-12.50, F-6.62, N-19.58, S-5.73.

*5.3.8. Benzothiazol-2-yl-bis-[1-(2-chloro-phenyl)-1H-[1,2,3] triazol-4-ylmethyl]-amine (**3h**)*

Yield: 55%, M.P: 135 °C; MS *m/z*: 533 (M^+); ^{1}H NMR (DMSO): δ 8.11 (s, 2H, 2× C═CH of triazole), 7.60–7.58 (m, 6H, Ar–H), 7.43–7.42 (m, 4H, Ar–H), 7.41–7.26 (m, 2H, Ar–H), 5.06 (s, 4H, 2× N–CH_2–); ^{13}C NMR ($CDCl_3$): δ 45.78, 118.38, 119.10, 119.24, 120.56, 121.91, 123.03, 125.94, 127.10, 129.27, 132.76, 135.09, 135.82, 142.66, 152.55, 162.00, 167.67; Elemental analysis: Molecular formula: $C_{25}H_{18}Cl_2N_8S$: Calculated: C-56.29, H-3.40, Cl-13.29, N-21.01, S-6.01; Found: C-56.24, H-3.35, Cl-13.24, N-21.06, S-6.11.

*5.3.9. Benzothiazol-2-yl-bis-[1-(3-fluoro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-amine (**3i**)*

Yield: 82%, M.P: 170 °C; MS *m/z*: 499.74 (M^+); ^{1}H NMR ($CDCl_3$): δ 8.09 (s, 2H, 2× C═CH of triazole), 7.63–7.61 (d, 2H, *J* = 7.5 Hz, Ar–H), 7.46 (s, 6H, Ar–H), 7.11 (s, 4H, Ar–H), 5.01 (s, 4H, 2× N–CH_2–); ^{13}C NMR ($CDCl_3$): δ 45.99, 108.18, 108.45, 115.67, 115.84, 118.99, 121.37, 121.77, 126.29, 131.22, 137.91, 144.22, 152.53, 161.82, 164.15, 167.47; Elemental analysis: Molecular formula: $C_{25}H_{18}F_2N_8S$: Calculated: C-59.99, H-3.62, F-7.59, N-22.39, S-6.41; Found: C-59.89, H-3.72, F-7.69, N-22.34, S-6.36.

*5.3.10. Benzothiazol-2-yl-bis-[1-(2,3-dichloro-phenyl)-1H-[1,2,3] triazol-4-ylmethyl]-amine (**3j**)*

Yield: 76%, M.P: 186 °C; MS *m/z*: 602 (M^+); ^{1}H NMR (DMSO): δ 7.85–7.82 (d, 2H, 2× C═CH of triazole), 7.57–7.52 (d, 2H, *J* = 8.1 Hz, Ar–H), 7.35–7.30 (t, 4H, *J* = 7.5 Hz, Ar–H), 7.17–7.11 (t, 4H, *J* = 7.8 Hz, Ar–H), 4.42 (s, 4H, 2× N–CH_2–); ^{13}C NMR ($CDCl_3$): δ 47.52, 116.20, 119.24, 120.99, 121.86, 126.63, 131.42, 133.59, 134.89, 143.15, 151.84, 157.06, 162.27, 166.19; Elemental analysis: Molecular formula:$C_{25}H_{16}Cl_4N_8S$: Calculated: C-49.85, H-2.68, Cl-23.54, N-18.60, S-5.32; Found: C-49.95, H-2.78, Cl-23.50, N-18.46, S-5.36.

*5.3.11. Benzothiazol-2-yl-bis-[1-(2-methyl-4-fluoro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-amine (**3k**)*

Yield: 79%, M.P: 205 °C; MS *m/z*: 528 (M^+); ^{1}H NMR ($CDCl_3$): δ 8.40 (s, 2H, 2× C═CH of triazole), 8.38–8.34 (d, 3H, *J* = 8.4 Hz, Ar–H), 7.99–7.96 (d, 4H, *J* = 7.2 Hz, Ar–H), 7.63–7.61 (d, 2H, *J* = 7.5 Hz, Ar–H), 7.14–7.10 (t, 1H, *J* = 7.5 Hz, Ar–H), 5.04 (s, 4H, 2× N–CH_2–), 2.19 (s, 6H, 2xCH_3); ^{13}C NMR ($CDCl_3$): δ 30.95, 46.31, 112.03, 119.11, 120.73, 121.34, 121.91, 126.79, 126.99, 131.25, 132.89, 142.56, 152.48, 161.98, 167.64; Elemental analysis: Molecular formula: $C_{27}H_{22}N_8SF_2$: Calculated: C-61.35, H-4.20, N-21.20, S-6.07, F-7.19; Found: C-61.45, H-4.25, N-21.15, S-6.02, F-7.14.

*5.3.12. Benzothiazol-2-yl-bis-[1-(4-ethyl-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-amine (**3l**)*

Yield: 74%, M.P: 195 °C; MS *m/z*: 520 (M^+); ^{1}H NMR ($CDCl_3$): δ 8.04 (s, 2H, 2× C═CH of triazole), 7.62–7.56 (t, 5H, *J* = 8.4 Hz, Ar–H), 7.30–7.28 (d, 6H, *J* = 8.1 Hz, Ar–H), 7.12–7.09 (t, 1H, *J* = 7.5 Hz, Ar–H), 5.01 (s, 4H, 2× N–CH_2–), 2.73–2.66 (q, 4H, *J* = 7.6 Hz, 2× Ar–CH_2–), 1.28–1.23 (t, 6H, *J* = 7.5Hz, 2× CH_3); ^{13}C NMR ($CDCl_3$): δ 28.42, 30.90, 45.95, 119.18, 120.58, 120.81, 121.41, 121.59, 125.98, 129.01, 131.24, 134.72, 143.66, 145.22, 152.51, 167.70; Elemental analysis: Molecular formula: $C_{29}H_{28}N_8S$: calculated: C-66.90, H-5.42, N-21.52, S-6.16; Found: C-67.00, H-5.32, N-21.42, S-6.26.

*5.3.13. Benzothiazol-2-yl-bis-[1-(3,4-dimethyl-phenyl)-1H-[1,2,3] triazol-4-ylmethyl]-amine (**3m**)*

Yield: 76%, M.P: 175 °C; MS *m/z*: 520 (M^+); ^{1}H NMR (DMSO): δ 8.70 (s, 2H, 2× C═CH of triazole), 7.79–7.76 (d, 1H, *J* = 7.8 Hz, Ar–H), 7.64 (s, 2H, Ar–H), 7.57–7.50 (t, 3H, *J* = 10.3 Hz, Ar–H), 7.31–7.29 (d, 3H, *J* = 7.8 Hz, Ar–H), 7.10–7.06 (t, 1H, *J* = 6.7 Hz, Ar–H), 4.99 (s, 4H, 2× N–CH_2–), 2.27 (s, 12H, 4× CH_3); ^{13}C NMR ($CDCl_3$): δ 19.42, 19.81, 45.93, 117.86, 119.17, 120.82, 121.32, 121.56, 121.66, 125.97, 130.54, 131.17, 134.77, 137.54, 138.26, 143.57, 152.69, 167.91; Elemental analysis: Molecular formula: $C_{29}H_{28}N_8S$: Calculated: C-66.90, H-5.42, N-21.52, S-6.16; Found: C-66.96, H-5.52, N-21.42, S-6.10.

*5.3.14. Benzothiazol-2-yl-bis-[1-(4-bromo-phenyl)-1H-[1,2,3] triazol-4-ylmethyl]-amine (**3n**)*

Yield: 72%, M.P: 180 °C; MS *m/z*: 622 (M^+); ^{1}H NMR ($CDCl_3$): δ 8.23 (s, 2H, 2× C═CH of triazole), 7.63–7.57 (m, 9H, Ar–H), 7.45 (s, 1H, Ar–H), 7.33–7.28 (t, 1H, *J* = 7.5 Hz, Ar–H), 7.12–7.07 (t, 1H, *J* = 7.5 Hz, Ar–H), 5.01 (s, 4H, 2× N–CH_2–); ^{13}C NMR ($CDCl_3$): δ 45.79, 119.24, 120.99, 121.91, 123.15, 125.76, 132.89, 135.33, 136.20, 144.45, 152.71, 161.84, 177.93; Elemental analysis: Molecular formula: $C_{25}H_{18}Br_2N_8S$: Calculated: C-48.25, H-2.92, Br-25.68 N-18.01, S-5.15; Found: C-48.35, H-2.82, Br-25.63 N-18.11, S-5.10.

*5.3.15. Benzothiazol-2-yl-bis-[1-(3,4,5-trimethoxy-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-amine (**3o**)*

Yield: 85%, M.P: 110 °C; MS *m/z*: 544 (M^+); ^{1}H NMR (DMSO): δ 8.80 (s, 2H, 2× C═CH of triazole), 7.75–7.76 (d, 2H, *J* = 7.6 Hz), 7.51–7.50 (m, 2H, Ar–H), 7.15 (s, 4H, Ar–H), 5.02 (s, 4H, 2× N–CH_2–), 3.85 (s, 18H, 6× OCH_3); ^{13}C NMR ($CDCl_3$): δ 46.20, 56.39, 60.98, 98.80, 107.49, 108.78, 119.22, 121.84,126.18, 138.35, 152.66, 154.00, 162.69, 167.47; Elemental analysis: Molecular formula: $C_{31}H_{32}N_8O_6S$: calculated: C-57.75, H-5.00, N-17.38, O-14.89, S-4.97; Found: C-57.70, H-5.05, N-17.48, O-14.84, S-4.92.

*5.3.16. Benzothiazol-2-yl-bis-(1-naphthalen-1-yl-1H-[1,2,3]triazol-4-ylmethyl)-amine (**3p**)*

Yield: 60%, M.P: 139 °C; MS *m/z*: 564 (M^+); ^{1}H NMR ($CDCl_3$): δ 8.66 (s, 2H, 2× C═CH of triazole), 7.95–7.94 (d, 4H, *J* = 7.5 Hz, Ar–H), 7.94–7.40 (m, 10H, Ar–H), 7.32–7.2807 (m, 2H, Ar–H), 7.18–7.07 (m, 2H, Ar–H), 5.02 (s, 4H, 2× N–CH_2–); ^{13}C NMR ($CDCl_3$): δ 39.27, 107.08, 119.24, 120.99, 121.42, 126.20, 127.50, 129.24, 133.59, 142.72, 151.41, 167.06; Elemental analysis: Molecular formula: $C_{33}H_{24}N_8S$: calculated: C-70.19, H-4.28, N-19.84, S-5.68; Found: C-70.29, H-4.18, N-19.89, S-5.63.

*5.3.17. Benzothiazol-2-yl-bis-[1-(4-nitro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-amine (**3q**)*

Yield: 95%, M.P: 150 °C; MS *m/z*: 554 (M^+); ^{1}H NMR ($CDCl_3$): δ 9.03 (s, 2H, 2× C═CH of triazole), 8.43–8.40 (d, 4H,*J* = 8.7 Hz, Ar–H), 8.21–8.18 (d, 4H, *J* = 9 Hz, Ar–H),7.80–7.78 (d, 1H, *J* = 7.5 Hz,Ar–H), 7.54–7.52 (d, 1H, *J* = 7.5 Hz, Ar–H), 7.33–7.30 (d, 1H, *J* = 7.8 Hz, Ar–H), 7.12–7.07 (t, 1H, *J* = 8.5Hz, Ar–H), 5.04 (s, 4H, 2× N–CH_2–); ^{13}C NMR ($CDCl_3$): δ 45.78, 112.28, 118.80, 120.54, 120.98, 125.32, 125.52, 126.19, 130.10, 130.53, 133.14, 142.27, 154.01, 160.09, 167.92; Elemental analysis: Molecular formula: $C_{25}H_{18}N_{10}O_4S$: calculated: C-54.15, H-3.27, N-25.26, O-11.54, S-5.78; Found: C-54.20, H-3.22, N-25.36, O-11.50, S-5.72.

*5.3.18. Benzothiazol-2-yl-bis-[1-(4-trifluoromethoxy-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-amine (**3r**)*

Yield: 76%, M.P: 202 °C; MS *m/z*: 632 (M^+); ^{1}H NMR ($CDCl_3$): δ 8.11 (s, 2H, 2× C═CH of triazole), 7.74–7.60 (dd, 6H, J_1 = 7.5 Hz, J_2 = 6.9 Hz, Ar–H), 7.35–7.25 (d, 5H, *J* = 7.2 Hz, Ar–H), 7.11 (s, 1H, Ar–H), 5.02 (s, 4H, 2× N–CH_2–); ^{13}C NMR ($CDCl_3$): δ 30.19, 46.66, 113.60, 114.90, 119.68, 123.59, 126.63, 127.07, 129.76, 134.46, 135.76, 143.15, 151.41, 159.67, 163.15, 166.62; Elemental analysis: Molecular formula: $C_{27}H_{18}F_6N_8O_2S$: calculated: C-51.27, H-2.87, F-18.02, N-17.71, O-5.06, S-5.07; Found: C-51.37, H-2.82, F-18.12, N-17.66, O-5.01, S-5.02.

*5.3.19. Benzothiazol-2-yl-bis-[1-(4-pentyl-phenyl)-1H-[1,2,3] triazol-4-ylmethyl]-amine (**3s**)*

Yield: 98%, M.P: 210 °C; MS *m/z*: 604 (M^+); ^{1}H NMR ($CDCl_3$): δ 8.04 (s, 2H, 2× C═CH of triazole), 7.62–7.55 (m, 10H, Ar–H), 7.28–7.25 (m, 1H, Ar–H), 7.12–7.07 (t, 1H, *J* = 7.6 Hz, Ar–H), 5.01 (s, 4H, 2× N–CH_2–CH_2), 2.67–2.61 (t, 8H, *J* = 7.6 Hz, 2× Ar–CH_2–CH_2–), 1.32 (s, 8H, 2× –CH_2–), 0.89 (s, 6H, 2× –CH_3); ^{13}C NMR ($CDCl_3$): δ 13.98, 22.46, 30.95, 31.31, 35.41, 45.78, 114.89, 118.80, 120.53, 121.57, 126.19, 129.55, 134.88, 144.01, 152.70, 167.92; Elemental analysis: Molecular formula: $C_{35}H_{40}N_8S$: Calculated: C-69.51, H-6.67, N-18.53, S-5.30; Found: C-69.61, H-6.77, N-18.43, S-5.20.

*5.3.20. Benzothiazol-2-yl-bis-[1-(3,5-dimethyl-phenyl)-1H-[1,2,3] triazol-4-ylmethyl]-amine (**3t**)*

Yield: 79%, M.P: 185 °C; MS *m/z*: 520.07 (M^+); ^{1}H NMR ($CDCl_3$): δ 7.99 (s, 2H, 2× C═CH of triazole), 7.63–7.60 (d, 2H, *J* = 8.1 Hz, Ar–H), 7.35–7.25 (m, 5H, Ar–H), 7.12–7.07 (t, 1H, *J* = 7.5 Hz, Ar–H), 7.02 (s, 2H, Ar–H), 5.01 (s, 4H, 2× N–CH_2–), 2.35 (s, 12H, 4× –CH_3); ^{13}C NMR ($CDCl_3$): δ 21.23, 30.92, 45.79, 118.34, 119.24, 121.02, 121.33, 126.20, 130.40, 131.42, 136.63, 139.64, 143.58, 152.71, 163.15, 167.49; Elemental analysis: Molecular formula: $C_{29}H_{28}N_8S$: Calculated: C-66.90, H-5.42, N-21.52, S-6.16; Found: C-66.80, H-5.32, N-21.62, S-6.26.

5.4. Determination of antimicrobial activity

A total of seven bacterial strains viz. *S. aureus* (ATCC 25323), *S. typhi* (MTCC 3216), *E. coli* (ATCC 35318), *E. faecalis* (clinical isolate), *P. aeruginosa* (ATCC 27893), *S. boydii* (clinical isolate) and *K. pneumoniae* (clinical isolate) and seven fungal strains viz. *C. tropicalis* (ATCC 750), *C. albicans* (clinical), *C. albicans* (ATCC 90028), *C. krusei* (ATCC 6258), *A. niger* (clinical), *A. fumigatus* (clinical), *C. neoformans* (clinical) were used in the investigation for antimicrobial assay. All cultures were preserved at Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India which were obtained from American Type Culture Collection (ATCC), MTCC and clinical strain. The fresh microbial broth cultures were prepared in normal saline before the screening procedure. Ciprofloxacin was used as standard drug for antibacterial activity and fluconazole was used as standard drug for antifungal activity.

Minimum inhibitory concentration (MIC) of all compounds was determined by micro-dilution method [34] using serially diluted (8 folds) compounds. MIC of the compounds was determined by series of dilution at various concentrations. Different concentration of the compounds (100 μg/ml, 50 μg/ml, 25 μg/ml, 12.5 μg/ml, 6.25 μg/ml, 3.12 μg/ml, 1.56 μg/ml, 0.78 μg/ml) were serially diluted

in microtiter plate. Specifically, 0.1 ml of standardized inoculums ($1–2 \times 10^7$ cfu/ml) was added in each tube of microtiter plate. The plates were incubated aerobically at 37 °C for 18–24 h. The lowest concentration (highest dilution) of the compounds showed no visible bacterial growth no turbidity in the solution when it was compared with the control was regarded as the MIC. Mueller-Hinton agar and Luria broth (Hi-media, Mumbai, India), was used for antibacterial activity.

Similar protocol was followed for determination of MIC of antifungal compounds except Sabouraud dextrose agar pH 7.3 ± 0.2 (Hi-media) was used.

5.5. Determination of hemolytic activity of compounds on human red blood cells (hRBC)

The hemolysis assay was carried out according to the procedure developed by Nielson et al. [31]. Briefly, the fresh human blood was collected from the hospital and washed three times in sterile phosphate buffered saline (PBS) solution. After each washing step, the cells were centrifuged at 3000 rpm for 7 min at room temperature and supernatant was discarded after each washing. The hRBC were re-suspended in PBS and adjusted the final concentration of 5×10^8 cells/ml. An aliquot (10 μl) of the cell suspension was added in 100 μL buffer solution containing 100 μM test compounds in 1% v/v DMSO in PBS. Further, controls were also taken as 1% v/v DMSO in PBS and sterile water. The cell suspensions were incubated at 37 °C for 1 h with constant shaking. After 1 h, the solution was centrifuged at 1300 rpm for 5 min at room temperature and absorbance was recorded at 540 nm. The UV absorbance values of the test compounds were expressed as a % of the absorbance of sterile water (equivalent to 100% hemolysis) to give % hemolysis results.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.02.027.

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