Full Paper

Antimycobacterial and antimicrobial study of new 1,2,4-triazoles with benzothiazoles

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In this study, we report the antimycobacterial and antimicrobial evaluation of newly synthesized 3-(3-pyridyl)-5-(4-methoxyphenyl)-4-(*N*-substituted-1,3-benzothiazol-2-amino)-4*H*-1,2,4-triazole **6a–j** in good yields. All the synthesized compounds have been established by elemental analysis, IR, ^{1}H NMR, ^{13}C-NMR and Mass spectral data. *In-vitro* antimycobacterial activity was carried out against (*Mycobacterium tuberculosis*) H_{37}Rv strain using Lowenstein-Jensen medium and antimicrobial activity against two Gram positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*), two Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and three fungal species (*Candida albicans*, *Aspergillus niger*, *Aspergillus clavatus*) using the broth microdilution method. Compounds **2e**, **6a**, **6g**, **6h**, and **6j** exhibited promising antimicrobial activity whereas compound **6j** showed very good antimycobacterial activity.

Keywords: 1,2,4-Triazole / Antimycobacterial activity / Antimicrobial activity

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Introduction

Among infectious diseases, tuberculosis (TB) is the leading killer with over two million casualties annually worldwide. The WHO considers tuberculosis to be the most dangerous chronic communicable disease in the world [1]. The emergence of AIDS, decline of socioeconomic standards and a reduced emphasis on tuberculosis control programs contribute to the disease's resurgence in industrialized countries [2]. Resistance of Mycobacterium tuberculosis strains to antimycobacterial agents is an increasing problem worldwide [3–6]. In spite of severe toxicity on repeated dosing of isoniazid (INH), it is still considered to be a first line drug for the chemotherapy of tuberculosis. The azole antitubercular may be regarded as a new class providing truly effective drugs, which is reported to inhibit the bacteria by blocking the biosynthesis of certain bacterial lipids and/or by additional mechanism [7–9]. 1,2,4-Triazole derivatives are active against many mycobacteria [10–13] and also possess wide variety of biological activity [14–18]. Benzothiazole moiety has already been reported for its mycobacterial activity [19, 20] and also possesses a wide range of biological activities [21–26]. After extensive literature survey, it was observed that, till date enough effort have not been to combine this two moieties as a single scaffold and to identify new candidates that may be value in designing new, potent, selective and less toxic antimycobacterial and antimicrobial agents. We reported here the synthesis of new 1,2,4-triazoles incorporated with benzothiazole at N-4 and pyridine at C-3 position encouraging antimycobacterial activity against *Mycobacterium tuberculosis* H_{37}Rv and antimicrobial activity.

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Result and discussion

Chemistry

2-Amino-6-flouro-1,3-benzothiazole **1a** on treatment of hydrazine hydrate, concentrated hydrochloric acid and ethylene glycol yields 2-hydrazino-6-fluoro-1,3-benzothiazole **2a**. IR spectra of **2a** showed broad stretching band around 3425 and 3200 cm^{-1} for NH and NH_2. ^{1}H-NMR spectrum showed a singlet at δ 4.83 and δ 8.93 which were accounted for NH_2 and NH which vanished on D_2O exchange. Ethyl nicotinoate **3** on treatment with hydrazine hydrate yields nicotinoyl hydrazide **4**, the IR spectra of **4** showed stretching band around 3335 and 3278 cm^{-1} where due to amine/amide NH while strong stretching band at 1610 cm^{-1} was attributed to amide

carbonyl. ^{1}H-NMR spectrum showed a singlet at δ 4.51 and δ 9.81 which were accounted for NH_2 and NH which vanished on D_2O exchange. Intermolecular cyclization of nicotinoyl hydrazide **4** with 4-methoxybenzoic acid in presence of phosphorous oxy chloride affords 2-(3-pyridyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole **5**. Disappearance of ^{1}H-NMR resonances observed with NH and NH_2 functions in the ^{1}H-NMR spectrum of **5** proved that ring closure starting from **4** resulted in the formation of 1,3,4-oxadiazole ring. This was further substantiated by the ^{13}C-NMR data of **5** which showed a peak at δ 157.30 and δ 157.19 due to C_2 and C_5 of oxadiazole. Mass spectrum of **5** displayed a molecular ion peak at *m*/*z* 253 which confirmed its molecular weight. Condensation of **5** with various substituted 2-hydrazino-1,3-benzothiazole **2a–j** in pyridine results in 3-(3-pyridyl)-5-(4-methoxyphenyl)-4-(*N*-substituted-1,3-benzothiazol-2-amino)-4*H*-1,2,4-triazole **6a–j**. Absence of ^{1}H-NMR resonances observed with NH_2 function of **2a** and appearance of signal at δ 7.82 for NH was observed in ^{1}H-NMR of **6a** proved the condensation of **2** and **5** resulted in the formation of 1,2,4-triazole ring. This was substantiated by ^{13}C-NMR data of **6a** which showed a peak at δ 162.58 and δ 161.97 due to C_3 and C_5 of triazole. Mass spectrum of **6** displayed a molecular ion at *m*/*z* 418 which confirmed its molecular weight.

Pharmacology

Antimycobacterial activity of all the synthesized compounds were accessed by L.J.-method and antimicrobial activity were assessed by broth microdilution method.

The minimum inhibitory concentrations (MICs) of antibacterial screening are shown in Table 1. All the compounds were tested for *in-vitro* antibacterial activity against two Gram positive (*Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 442) and two Gram negative (*Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 2488) bacteria. Ampicillin was used as a standard drug. The results revealed that substituted 2-hydrazino benzothiazoles were moderately active against bacteria except **2e**, which showed good activity as compared with ampicillin against *S. aureus* and *E. coli* while 1,3,4-oxadiazole **5** exhibited quite good activity to some extent. Most of 1,2,4-triazole derivative were found good activity (100–250 μg/mL) against *S. aureus*. Compounds **6a**, **6g**, and **6h** possessed very good activity (100–200 μg/mL) against *S. aureus*. Most of the compounds exhibited moderate activity (150–250 μg/mL) *S. pyogenes*. Compounds **6a**, **6h**, and **6j** possessed good activity (100–125 μg/mL) while others displayed moderate activity against *E. coli*. Compounds **6a**, **6g**, and **6j** showed good activity (62.5–125 μg/mL) except **6h** showed very good activity (25 μg/mL) while others possessed

Table 1. Antibacterial activtiy of compounds **2a–j**, **5**, **6a–j**.

Compound	R	Minimum Inhibitory Concentration (MIC) (μg/mL)			
		Gram positive bacteria		Gram negative bacteria	
		S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*
		MTCC-96	MTCC-442	MTCC-443	MTCC-2488
2a	6-F	500	250	250	250
2b	6-Br	250	500	500	500
2c	6-NO_2	500	500	250	250
2d	6-CH_3	250	250	100	500
2e	6-OCH_3	200	250	62.5	125
2f	6-Cl	500	500	100	125
2g	4-CH_3	500	500	250	250
2h	4-NO_2	500	250	500	250
2i	5-Cl, 6-Cl	250	500	100	125
2j	4-Cl	500	500	250	250
5	–	500	500	100	50
6a	6-F	125	250	100	62.5
6b	6-Br	500	500	500	500
6c	6-NO_2	500	200	250	250
6d	6-CH_3	250	250	500	500
6e	6-OCH_3	500	500	500	250
6f	6-Cl	250	250	1000	500
6g	4-CH_3	100	250	500	125
6h	4-NO_2	200	250	125	25
6i	5-Cl, 6-Cl	500	250	500	250
6j	4-Cl	250	500	100	62.5
Ampicillin	–	250	100	100	100

moderate activity against *P. aeruginosa*. Compounds **6a**, **6g**, and **6h** exhibited very good activity against Gram positive bacteria whereas **6a**, **6h**, and **6j** showed very good activity towards Gram negative bacteria. Compound **2e** was found to be active against Gram positive and Gram negative bacteria.

The MICs of antifungal results are shown in Table 2. All the compounds were screened for antifungal activity against three fungal species *Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus*. The results showed that 2-hydrazino benzothiazoles **2a–j** possessed good activity (250–500 μg/mL) compared to greseofulvin against *C. albicans*, except **2j** (1000 μg/mL). Compounds **2a–j** displayed moderate to poor activity (250–500 μg/mL) against *A. niger* and *A. clavatus* compared with greseofluvin while 1,3,4-oxadiazole **5** exhibited good activity (250 μg/mL) weak activity against *A. niger* and *A. clavatus*. Compounds **6a**, **6c**, **6d**, **6e**, **6g**, **6i**, and **6j** showed very good activity (200–500 μg/mL) whereas **6b**, **6f**, and **6h** exhibited pronounced activity (100 μg/mL) against *C. albicans*. Compounds **6g** and **6i** exhibited moderate activity (200–250 μg/mL) while remaining compounds showed weak activity against *A. niger*. Compounds **6f**, **6g**, and **6i** displayed moderate activity (200–250 μg/mL) while rest of the compounds showed weak activity against *A. clavatus*. Compounds **2f**, **2g**, **2h**, **6g**, and **6i** were found to be active against all the three fungal species.

From first preliminary examination of the antimycobacterial activity results are summarized in Table 3. Compound **2e** containing hydrazide group, shows better activity (50 μg/mL) against *M. tuberculosis* and compounds **6b**, **6d**, **6e**, and **6h** showed good activity (50–62.5 μg/mL) whereas compound **6j** showed pronounced activity (25 μg/mL). Due to the better activity against tested microorganisms and mycobacteria, compound **6j** has been selected for further development and studies to acquire more information about structure–activity relationships are in progress in our laboratories.

MICs of tested compounds showed that 2-hydrazino benzothiazoles possessed poor to good activity but when 2-hydrazino benzothiazoles were introduced to 1,3,4-oxadiazole to form 1,2,4-triazole, the activity increased or decreased depending upon the substituents. Flouro and chloro containing 1,2,4-triazoles were found with good activity towards bacterial and fungal species. Moreover methyl group containing 1,2,4-triazoles also showed good bacterial and fungal activity, whereas nitro containing 1,2,4-triazole compounds showed good bacterial activity.

Bromo, chloro, nitro, methoxy, and methyl containing 1,2,4-triazoles showed good activity against *M. tuberculosis*.

Table 2. Antifungal activtiy of compounds **2a–j**, **5**, **6a–j**.

Compound	R	Minimum Inhibitory Concentration (MIC) (μg/mL)		
		Fungal species		
		C. albicans	***A. niger***	***A. clavatus***
		MTCC-227	**MTCC-282**	**MTCC-323**
2a	6-F	250	>1000	>1000
2b	6-Br	500	500	500
2c	6-NO_2	500	500	1000
2d	6-CH_3	250	1000	>1000
2e	6-OCH_3	500	500	250
2f	6-Cl	250	200	250
2g	4-CH_3	500	250	200
2h	4-NO_2	200	250	200
2i	5-Cl, 6-Cl	500	500	1000
2j	4-Cl	1000	500	500
5	–	500	1000	1000
6a	6-F	200	500	1000
6b	6-Br	100	500	500
6c	6-NO_2	250	500	500
6d	6-CH_3	500	1000	>1000
6e	6-OCH_3	250	500	1000
6f	6-Cl	100	500	250
6g	4-CH_3	200	250	200
6h	4-NO_2	100	500	500
6i	5-Cl, 6-Cl	200	250	250
6j	4-Cl	200	500	1000
Greseofulvin	–	500	100	100

Table 3. Antimycobacterial activtiy of compounds **2a–j**, **5**, **6a–j**.

Compound	R_1	MIC values (μg/mL) of *M. tuberculosis* $H_{37}Rv$	% Inhibition
2a	6-F	250	98%
2b	6-Br	500	97%
2c	6-NO_2	250	99%
2d	6-CH_3	1000	98%
2e	6-OCH_3	50	99%
2f	6-Cl	200	99%
2g	4-CH_3	500	98%
2h	4-NO_2	500	98%
2i	5-Cl, 6-Cl	250	99%
2j	4-Cl	100	98%
5	–	1000	99%
6a	6-F	200	98%
6b	6-Br	50	99%
6c	6-NO_2	250	96%
6d	6-CH_3	62.5	98%
6e	6-OCH_3	62.5	92%
6f	6-Cl	250	94%
6g	4-CH_3	500	90%
6h	4-NO_2	50	99%
6i	5-Cl, 6-Cl	200	98%
6j	4-Cl	25	99%
Rifampicin	–	40	98%

Experimental

Chemistry

All chemical were of analytical grade and used directly. Melting points were determined in PMP-DM scientific melting point apparatus and are uncorrected. The purities of compounds were checked by TLC using Merck silica gel 60 F254. IR spectra were recorded on Perkin-Elmer RX 1 FTIR spectrophotometer in KBr (γ_{max} in cm^{-1}). ^{1}H- and ^{13}C-NMR spectra were measured with Bruker Avance II 400 NMR spectrometer (400 MHz) in $CDCl_3$ using TMS as internal standard (δ in ppm). The microanalyses were performed on a Heraeus Carlo Erba 1180 CHN analyzer. The mass spectra were obtained with micromass Q-T of micro (TOF MS ES+).

Substituted 2-amino-1,3-benzothiazole **2a–j** were prepared accordant to literature [27, 28].

*General procedure for synthesis of 2-hydrazino benzothiazoles **2a–j***

Concentrated hydrochloric acid (0.067 M) was added drop wise with stirring to hydrazine hydrate (0.12 M) at 5–6°C followed by ethylene glycol (30 mL). Thereafter, 6-fluoro-2-amino-1,3-benzothiazole **1a** (20 mmol) was added in portions and the resultant mixture was refluxed for 2–3 h and cooled at room temperature. The reaction progress was monitored by TLC using toluene/ethylacetate (75:25) as mobile phase. The reaction mixture was filtered and resulting precipitate was washed with distilled water. The resulting crude was crystallized from ethanol. The other compounds of the series were prepared by similar procedure.

*2-Hydrazino-6-fluoro-1,3-benzothiazole **2a***

Yield 70%; m.p. 204-206°C; IR (KBr, γ_{max}, cm^{-1}): 3435 (NH_2), 3200 (NH), 1631 (C=N), 1442 (thiazole); ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.83 (s, 2H, NH_2, disappeared on D_2O exchange), 8.94 (s, 1H, NH, disappeared on D_2O exchange), 7.28–7.64 (m, 3H, 3 CH); Anal. calcd. for $C_7H_6N_3FS$: C, 45.89; H, 3.30; N, 22.94. Found: C, 45.91; H, 3.31; N, 22.98.

*2-Hydrazino-6-bromo-1,3-benzothiazole **2b***

Yield 61%; m.p. 200–202°C; IR (KBr, γ_{max}, cm^{-1}): 3449 (NH_2), 3212 (NH), 1623 (C=N), 1451 (thiazole); ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.83 (s, 2H, NH_2, disappeared on D_2O exchange), 8.90 (s, 1H, NH, disappeared on D_2O exchange), 7.63–7.93 (m, 3H, 3 CH); Anal. calcd. for $C_7H_6N_3SBr$: C, 34.44; H, 2.48; N, 17.21. Found: C, 34.40; H, 2.45; N, 17.19.

*2-Hydrazino-6-nitro-1,3-benzothiazole **2c***

Yield 67%; m.p. 210–212°C; IR (KBr, γ_{max}, cm^{-1}): 3445 (NH_2), 3220 (NH), 1640 (C=N), 1448 (thiazole); ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.81 (s, 2H, NH_2, disappeared on D_2O exchange), 8.92 (s, 1H, NH, disappeared on D_2O exchange), 7.04–7.79 (m, 3H, 3 CH); Anal. calcd. for $C_7H_6N_4O_2S$: C, 40.00; H, 2.88; N, 26.65. Found: C, 39.97; H, 2.90; N, 26.68.

*2-Hydrazino-6-methyl-1,3-benzothiazole **2d***

Yield 62%; m.p. 198–200°C; IR (KBr, γ_{max}, cm^{-1}): 3449 (NH_2), 3222 (NH), 1620 (C=N), 1439 (thiazole); ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.82 (s, 2H, NH_2, disappeared on D_2O exchange), 8.94 (s, 1H, NH, disappeared on D_2O exchange), 2.45 (s, 3H, CH_3), 7.26–7.71 (m, 3H, 3 CH); Anal. calcd. for $C_8H_9N_3S$: C, 53.61; H, 5.06; N, 23.44. Found: C, 53.57; H, 5.08; N, 23.47.

*2-Hydrazino-6-methoxy-1,3-benzothiazole **2e***

Yield 65%; m.p. 193–195°C; IR (KBr, γ_{max}, cm^{-1}): 3439 (NH_2), 3208 (NH), 1628 (C=N), 1448 (thiazole); ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.80 (s, 2H, NH_2, disappeared on D_2O exchange), 8.94 (s, 1H, NH, disappeared on D_2O exchange), 3.86 (s, 3H, OCH_3), 7.16–7.65 (m, 3H, 3 CH); Anal. calcd. for $C_8H_9N_3OS$: C, 49.21; H, 4.65; N, 21.52. Found: C, 49.25; H, 4.62; N, 21.48.

*2-Hydrazino-6-chloro-1,3-benzothiazole **2f***

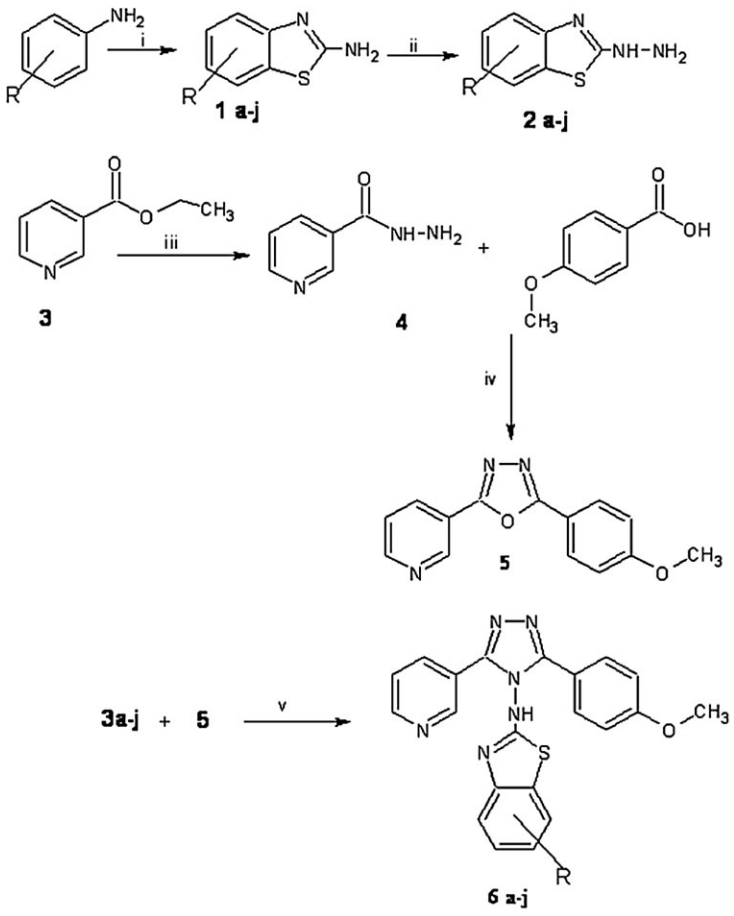
Yield 68%; m.p. 198–200°C; IR (KBr, γ_{max}, cm^{-1}): 3445 (NH_2), 3218 (NH), 1624 (C=N), 1428 (thiazole); ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.79 (s, 2H, NH_2, disappeared on D_2O exchange), 8.90 (s, 1H, NH, disappeared on D_2O exchange), 7.82–8.21 (m, 3H, 3 CH); Anal. calcd. for $C_7H_6N_3ClS$: C, 42.11; H, 3.03; N, 17.76. Found: C, 42.15; H, 3.07; N, 17.79.

*2-Hydrazino-4-methyl-1,3-benzothiazole **2g***

Yield 69%; m.p. 167–169°C; IR (KBr, γ_{max}, cm^{-1}): 3439 (NH_2), 3220 (NH), 1638 (C=N), 1435 (thiazole); ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.83 (s, 2H, NH_2, disappeared on D_2O exchange), 8.92 (s, 1H, NH, disappeared on D_2O exchange), 2.83 (s, 3H, CH_3), 7.31–7.69 (m, 3H, 3 CH); Anal. calcd. for $C_8H_9N_3S$: C, 53.61; H, 5.06; N, 23.44. Found: C, 53.65; H, 5.01; N, 23.38.

*2-Hydrazino-4-nitro -1,3-benzothiazole **2h***

Yield 60%; m.p. 199–201°C; IR (KBr, γ_{max}, cm^{-1}): 3440 (NH_2), 3200 (NH), 1631 (C=N), 1445 (thiazole); ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.88 (s, 2H, NH_2, disappeared on D_2O exchange), 8.95 (s, 1H, NH,



i. NH_4SCN,Br_2, Glacial acetic acid, NH_3; ii. Hydrazine Hydrate, conc. HCl, Ethylene Glycol;
iii. $NH_2NH_2\,H_2O$, ethanol, refluxed; iv. $POCl_3$, reflux 9h;
v. dry pyridine, refluxed

R = a. 6-F, d. 6-CH3, g. 4-CH3, j. 4-Cl
b. 6-Br, e. 6-OCH3, h. 4-NO2,
c. 6-NO2, f. 6-Cl, i. 5-Cl,6-Cl,

Scheme 1. Synthetic protocol for the compounds **6a–j**.

disappeared on D_2O exchange), 7.06–8.82 (m, 3H, 3 CH); Anal. calcd. for $C_7H_6N_4O_2S$: C, 40.00; H, 2.88; N, 26.65. Found: C, 40.04; H, 2.85; N, 26.61.

*2-Hydrazino-(5,6-dichloro)-1,3-benzothiazole **2i***

Yield 68%; m.p. 248–250°C; IR (KBr, γ_{max}, cm^{-1}): 3449 (NH_2), 3212 (NH), 1640 (C=N), 1439 (thiazole); ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.86 (s, 2H, NH_2, disappeared on D_2O exchange), 8.94 (s, 1H, NH, disappeared on D_2O exchange), 7.81 (s, 1H, CH), 7.98 (s, 1H, CH); Anal. calcd. for $C_7H_5N_3Cl_2S$: C, 35.91; H, 2.15; N, 17.95. Found: C, 35.88; H, 2.19; N, 17.92.

*2-Hydrazino-4-chloro-1,3-benzothiazole **2j***

Yield 63%; m.p. 239–241°C; IR (KBr, γ_{max}, cm^{-1}): 3449 (NH_2), 3220 (NH), 1640 (C=N), 1445 (thiazole); ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.82 (s, 2H, NH_2, disappeared on D_2O exchange), 8.92 (s, 1H, NH, disappeared on D_2O exchange), 7.55–7.87 (m, 3H, 3 CH); Anal. calcd. for $C_7H_6N_3ClS$: C, 42.11; H, 3.03; N, 17.76. Found: C, 42.07; H, 3.01; N, 18.00.

*2-(3-Pyridyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole **5***

A mixture of nicotinoyl hydrazide **4** (5 mmol) and 4-methoxy benzoic acid (5 mmol) in phosphorus oxychloride (5 mL) was refluxed on water bath for 9 h. The progress of the reaction was monitored by TLC using toluene/ethylacetate/methanol (70:20:10) as mobile phase. After the completion of reaction, it was cooled and poured onto crushed ice with continuous stirring. The solid mass separated was neutralized with sodium bicarbonate solution (10% w/v). The resulting solid thus obtained was collected by filtration, washed well with cold water, dried and crystallized from absolute ethanol.

Yield 67%; m.p. 111–113°C; IR (KBr, γ_{max}, cm^{-1}): 1667 (C=N), 1287, 1073 (C–O–C) cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.86 (s, 3H, OCH_3), 9.32 (s, 1H, CH), 8.77 (dd, 1H, J = 3.48 Hz, CH), 8.40 (d, 1H, J = 8.08 Hz, CH), 7.48 (t, 1H, CH), 8.06 (d, 2H, J = 8.16 Hz, 2 CH), 7.02 (d, 2H, J = 8.04 Hz, 2 CH); ^{13}C-NMR ($CDCl_3$, δ, ppm): 157.30 (C_5-oxadiazole), 157.19 (C_2-oxadiazole), 50.71 (OCH_3), 160.25, 147.37, 142.88, 129.31, 127.16, 124.06, 115.83, 119.11, 109.84 (aromatic ring); MS (*m*/*z*): 253 (M^+); Anal. calcd. for $C_{14}H_{11}N_3O_2$: C, 60.40; H, 4.84; N. 16.59; Found: C, 60.35; H, 4.91; N, 16.51.

General procedure for the synthesis of 3-(3-pyridyl)-5-(4-methoxyphenyl)-4-(N-substituted-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6a–j***

A mixture of 2-(3-pyridyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole **5** (5 mmol) and 2-hydrazino-6-fluoro-1,3-benzothiazole **2a** (5 mmol) in dry pyridine (10 mL) was refluxed for 18–24 h. The reaction was monitored by TLC on silica gel using ethyl acetate/toluene (2.5:7.5). It was then cooled and poured on to crushed ice. The reaction mass was neutralized by dilute hydrochloric acid and resulting solid was washed with cold water, dried and crystallized from absolute ethanol.

The other compounds of the series were prepared by similar procedure.

3-(3-Pyridyl)-5-(4-methoxyphenyl)-4-(N-6-fluoro-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6a***

Yield 65%; m.p. 168–170°C; IR (KBr, γ_{max}, cm^{-1}): 3434 (NH),1649 (C=N); ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.88 (s, 3H, OCH_3), 7.82 (s, 1H, NH), 9.32 (s, 1H, CH), 8.78 (dd, 1H, J = 4.0 Hz, CH), 8.41 (d, 1H, J = 8.35 Hz, CH), 7.52 (t, 1H, CH), 8.05 (d, 2H, J = 8.16 Hz, 2 CH), 6.98 (d, 2H, J = 8.04 Hz, 2 CH), 7.07–7.24 (m, 3H, benzothiazole-H); ^{13}C-NMR ($CDCl_3$, δ, ppm): 162.58 (C_3-triazole), 161.97 (C_5-triazole), 55.53 (OCH_3), 162.94, 152.22, 147.37, 146.54, 142.91, 133.96, 129.33, 128.78, 125.78, 123.94, 121.88, 120.18, 115.78, 114.65, 112.01, 108.21; (*m*/*z*): 418 (M^+); Anal. calcd. for $C_{21}H_{15}N_6OFS$: C, 60.28; H, 3.61; N, 20.08. Found: C, 60.32; H, 3.63; N, 20.11.

3-(3-Pyridyl)-5-(4-methoxyphenyl)-4-(N-6-bromo-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6b***

Yield 62%; m.p. 153–155°C; IR (KBr, γ_{max}, cm^{-1}): 3432 (NH), 1646 (C=N); ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.85 (s, 3H, OCH_3), 7.86 (s, 1H, NH), 9.35 (s, 1H, CH), 8.80 (dd, 1H, J = 3.8 Hz, CH), 8.45 (d, 1H, J = 8.35 Hz, CH), 7.50 (t, 1H, CH), 8.05 (d, 2H, J = 8.04 Hz, 2 CH), 7.02 (d, 2H, J = 8.16 Hz, 2 CH), 7.61–7.76 (m, 3H, benzothiazole-H); ^{13}C-NMR ($CDCl_3$, δ, ppm): 162.37 (C_3-triazole), 161.85 (C_5-triazole), 55.48 (OCH_3), 162.31, 152.54, 147.32, 142.91, 133.85, 129.33, 128.78, 126.58, 125.91, 124.01, 121.09, 120.18, 116.99, 115.78, 114.88, 109.79; (*m*/*z*): 479 (M^+), 481 (M + 2); Anal. calcd. for $C_{21}H_{15}N_6OBrS$: C, 52.62; H, 3.15; N, 17.53. Found: C, 52.59; H, 3.18; N, 17.49.

3-(3-Pyridyl)-5-(4-methoxyphenyl)-4-(N-6-nitro-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6c***

Yield 68%; m.p. 159–161°C; IR (KBr, γ_{max}, cm^{-1}): 3445 (NH), 1652 (C=N); ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.82 (s, 3H, OCH_3), 7.88 (s, 1H, NH), 9.36 (s, 1H, CH), 8.81 (dd, 1H, J = 4.0 Hz, CH), 8.44 (d, 1H, J = 8.38 Hz, CH), 7.54 (t, 1H, CH), 8.03 (d, 2H, J = 8.20 Hz, 2 CH), 7.04 (d, 2H, J = 8.16 Hz, 2 CH), 7.63 (d, 1H, J = 8.04 Hz, CH), 8.12 (d, 1H, J = 7.84 Hz, CH), 8.74 (s, 1H, CH); ^{13}C-NMR ($CDCl_3$, δ, ppm): 162.38 (C_3-triazole), 161.79 (C_5-triazole), 55.41 (OCH_3), 163.01, 152.38, 147.28, 142.91, 139.21, 134.21, 129.38, 128.65, 126.29, 124.17, 121.75, 119.84, 118.58, 117.72, 116.32, 115.19; (*m*/*z*): 445 (M^+); Anal. calcd. for $C_{21}H_{15}N_7O_3S$: C, 56.62; H, 3.39; N, 22.01. Found: C, 52.65; H, 3.43; N, 21.98.

3-(3-Pyridyl)-5-(4-methoxyphenyl)-4-(N-6-methyl-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6d***

Yield 59%; m.p. 162–165°C; IR (KBr, γ_{max}, cm^{-1}): 3438 (NH), 1661 (C=N); ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.85 (s, 3H, OCH_3), 2.33 (s, 3H, CH_3), 7.86 (s, 1H, NH), 9.34 (s, 1H, CH), 8.80 (dd, 1H, J = 3.8 Hz, CH), 8.42 (d, 1H, J = 8.38 Hz, CH), 7.54 (t, 1H, CH), 8.03 (d, 2H, J = 8.04 Hz, 2 CH), 7.01 (d, 2H, J = 8.16 Hz, 2 CH), 7.05–7.48 (m, 3H, benzothiazole-H); ^{13}C-NMR ($CDCl_3$, δ, ppm): 162.97 (C_3-triazole), 162.31 (C_5-triazole), 55.49 (OCH_3), 22.91 (CH_3), 162.86, 152.38, 147.29, 142.83, 134.32, 129.24, 128.33, 128.63, 127.85, 123.53, 122.30, 120.58, 120.69, 119.93, 116.21, 114.98; (*m*/*z*): 414 (M^+); Anal. calcd. for $C_{22}H_{18}N_6OS$: C, 63.75; H, 4.38; N, 20.28. Found: C, 63.72; H, 4.44; N, 20.32.

3-(3-Pyridyl)-5-(4-methoxyphenyl)-4-(N-6-methoxy-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6e***

Yield 64%; m.p. 229–231°C; IR (KBr, γ_{max}, cm^{-1}): 3449 (NH), 1649 (C=N); ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.85 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 7.86 (s, 1H, NH), 9.36 (s, 1H, CH), 8.80 (dd, 1H, J = 4.0 Hz, CH), 8.43 (d, 1H, J = 8.24 Hz, CH), 7.48 (t, 1H, CH), 8.03 (d, 2H, J = 8.08 Hz, 2 CH), 7.01 (d, 2H, J = 8.04 Hz, 2 CH), 7.05–7.28 (m, 3H, benzothiazole-H); ^{13}C-NMR ($CDCl_3$, δ, ppm): 162.36 (C_3-triazole), 162.02 (C_5-triazole), 55.43 (OCH_3), 52.69 (OCH_3), 162.78, 152.91, 147.35, 147.32, 144.96, 142.88, 131.13, 129.24, 128.68, 123.78, 120.21, 119.15, 115.52, 114.65, 113.98, 106.25; (*m*/*z*): 430 (M^+); Anal. calcd. for $C_{22}H_{18}N_6O_2S$: C, 61.38; H, 4.21; N, 19.52. Found: C, 61.42; H, 4.24; N, 19.49.

3-(3-Pyridyl)-5-(4-methoxyphenyl)-4-(N-6-chloro-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6f***

Yield 72%; m.p. 241–243°C; IR (KBr, γ_{max}, cm^{-1}): 3442 (NH), 1659 (C=N); ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.85 (s, 3H, OCH_3), 7.89 (s, 1H, NH), 9.32 (s, 1H, CH), 8.74 (dd, 1H, J = 3.8 Hz, CH), 8.42 (d, 1H, J = 8.40 Hz, CH), 7.49 (t, 1H, CH), 8.05 (d, 2H, J = 8.04 Hz, 2 CH), 7.09 (d, 2H, J = 8.28 Hz, 2 CH), 7.59–7.80 (m, 3H, benzothiazole-H); ^{13}C-NMR ($CDCl_3$, δ, ppm): 162.12 (C_3-triazole), 161.99 (C_5-triazole), 55.46 (OCH_3), 162.85, 152.16, 147.32, 144.44, 142.69, 132.96, 129.31, 128.63, 126.85, 123.84, 125.47, 121.95, 120.24, 119.52, 115.65, 114.58; (*m*/*z*): 434 (M^+), 436 (M + 2); Anal. calcd. for $C_{21}H_{15}N_6OClS$: C, 58.00; H, 3.48; N, 19.32. Found: C, 57.96; H, 3.51; N, 19.36.

3-(3-Pyridyl)-5-(4-methoxyphenyl)-4-(N-4-methyl-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6g***

Yield 62%; m.p. 167–169°C; IR (KBr, γ_{max}, cm^{-1}): 3435 (NH), 1660 (C=N); ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.61 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 7.87 (s, 1H, NH), 9.36 (s, 1H, CH), 8.81 (dd, 1H, J = 4.0 Hz, CH), 8.43 (d, 1H, J = 8.02 Hz, CH), 7.54 (t, 1H, CH), 8.07 (d, 2H, J = 8.34 Hz, 2 CH), 7.02 (d, 2H, J = 8.24 Hz, 2 CH), 7.09–7.28 (m, 3H, benzothiazole-H); ^{13}C-NMR ($CDCl_3$, δ, ppm): 161.98 (C_3-triazole), 161.53 (C_5-triazole), 20.49 (CH_3), 55.43 (OCH_3), 162.89, 152.12, 147.34, 143.47, 142.87, 132.87, 129.29, 128.82, 128.64, 127.57, 123.59, 122.04, 120.96, 119.98, 115.78, 114.65; (*m*/*z*): 414

(M^+); Anal. calcd. for $C_{22}H_{18}N_6OS$: C, 63.75; H, 4.38; N, 20.28. Found: C, 63.79; H, 4.35; N, 20.24.

3-(3-Pyridyl)-5-(4-methoxyphenyl)-4-(N-4-nitro-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6h***

Yield 65%; m.p. 198–200°C; IR (KBr, γ_{max}, cm^{-1}): 3442 (NH), 1657 (C=N); ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.84 (s, 3H, OCH_3), 7.88 (s, 1H, NH), 9.34 (s, 1H, CH), 8.80 (dd, 1H, J = 3.8 Hz, CH), 8.43 (d, 1H, J = 8.08 Hz, CH), 7.50 (t, 1H, CH), 8.03 (d, 2H, J = 8.16 Hz, 2 CH), 7.01 (d, 2H, J = 8.34 Hz, 2 CH), 6.63 (t, 1H, CH), 8.22 (d, 1H, J = 8.24 Hz, CH), 8.56 (d, 1H, J = 8.24 Hz, CH); ^{13}C-NMR ($CDCl_3$, δ, ppm): 162.45 (C_3-triazole), 161.99 (C_5-triazole), 55.40 (OCH_3), 162.83, 152.22, 147.41, 144.36, 142.93, 138.06, 132.10, 129.37, 128.82, 128.07, 123.85, 122.55, 120.65, 119.97, 115.82, 114.74; (*m*/*z*): 445 (M^+); Anal. calcd. for $C_{21}H_{15}N_7O_3S$: C, 56.62; H, 3.39; N, 22.01. Found: C, 56.59; H, 3.44; N, 22.05.

3-(3-Pyridyl)-5-(4-methoxyphenyl)-4-(N-(5,6-dichloro)-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6i***

Yield 69%; m.p. 176–178°C; IR (KBr, γ_{max}, cm^{-1}): 3449 (NH), 1655 (C=N); ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.82 (s, 3H, OCH_3), 7.85 (s, 1H, NH), 9.36 (s, 1H, CH), 8.82 (dd, 1H, J = 4.0 Hz, CH), 8.45 (d, 1H, J = 8.48 Hz, CH), 7.54 (t, 1H, CH), 8.06 (d, 2H, J = 8.38 Hz, 2 CH), 7.03 (d, 2H, J = 8.16 Hz, 2 CH), 7.59 (s, 1H, CH), 7.66 (s, 1H, CH); ^{13}C-NMR ($CDCl_3$, δ, ppm): 162.58 (C_3-triazole), 161.97 (C_5-triazole), 55.53 (OCH_3), 162.86, 152.35, 147.32, 144.86, 142.84, 133.96, 129.35, 128.02, 128.75, 128.69, 123.86, 123.78, 123.37, 121.74, 119.89, 115.78, 114.65, 112.01, 108.21; (*m*/*z*): 469 (M^+), 471 (M + 2), 473 (M + 4); Anal. calcd. for $C_{21}H_{14}N_6OCl_2S$: C, 53.74; H, 3.01; N, 17.91. Found: C, 53.79; H, 3.05; N, 17.87.

3-(3-Pyridyl)-5-(4-methoxyphenyl)-4-(N-4-chloro-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6j***

Yield 64%; m.p. 192–194°C; IR (KBr, γ_{max}, cm^{-1}): 3447 (NH), 1661 (C=N); ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.85 (s, 3H, OCH_3), 7.88 (s, 1H, NH), 9.36 (s, 1H, CH), 8.82 (dd, 1H, J = 3.4 Hz, CH), 8.45 (d, 1H, J = 8.08 Hz, CH), 7.53 (t, 1H, CH), 8.07 (d, 2H, J = 8.24 Hz, 2 CH), 6.96 (d, 2H, J = 8.04 Hz, 2 CH), 7.03–7.35 (m, 3H, benzothiazole-H); ^{13}C-NMR ($CDCl_3$, δ, ppm): 162.12 (C_3-triazole), 161.57 (C_5-triazole), 55.45 (OCH_3), 162.85, 152.59, 147.36, 144.86, 142.88, 132.96, 129.28, 128.71, 123.94, 123.18, 121.52, 119.98, 118.79, 115.82, 114.78; (*m*/*z*): 434 (M^+), 436 (M + 2); Anal. calcd. for $C_{21}H_{15}N_6OClS$: C, 58.00; H, 3.48; N, 19.32. Found: C, 58.04; H, 3.45; N, 19.37.

Biological assay

In-vitro evaluation of antimicrobial activity

The MICs of synthesized compounds were carried out by broth microdilution method [29]. DMSO was used as diluents to get desired concentration of drugs to test upon standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately sub cultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37°C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism was recorded as the MIC. All the tubes not showing visible growth (in the same manner as control tube described above) was sub cultured and incubated overnight at 37°C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show similar number of colonies indicating bacteriostatic; a reduced number of colonies indicating a partial or slow bactericidal activity and no growth if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. Each synthesized drug was diluted obtaining 2000 μg/mL concentration, as a stock solution. In primary screening 500, 250, and 125 μg/mL concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 100, 50, 25, 12.5, 6.250, 3.125, and 1.5625 μg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC.

In-vitro evaluation of antimycobacterial activity

Drug susceptibility and determination of MIC of the test compounds against *M. tuberculosis* $H_{37}Rv$ were performed by Lowenstein-Jensen (LJ) MIC method [29–32] where primary 1000, 500, 250, and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25, 3.25 μg/mL dilutions of each test compound were added liquid Lowenstein-Jensen Medium and then media were sterilized by inspissation method. A culture of *M. tuberculosis* $H_{37}Rv$ growing on Lowenstein-Jensen Medium was harvested in 0.85% saline in bijou bottles. All test compound make first stock solution of 2000 μg/mL concentration of compounds was prepared in DMSO. These tubes were then incubated at 37°C for 24 h followed by streaking of *M. tuberculosis* $H_{37}Rv$ (5×104 bacilli per tube). These tubes were then incubated at 37°C. Growth of bacilli was seen after 12, 22, and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with M. tuberculosis $H_{37}Rv$. The concentration at which no development of colonies occurred or <20 colonies was taken as MIC concentration of test compound. The standard strain *M. tuberculosis* $H_{37}Rv$ was tested with known drug rifampicin.

The authors have declared no conflict of interest.

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