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Original article

Pharmacological evaluation and characterizations of newly synthesized 1,2,4-triazoles

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ABSTRACT

The triazole analogs were obtained via. multistep synthesis sequence beginning with ethyl nicotinoate **3** which on treatment with hydrazine hydrate yields nicotinoyl hydrazide **4**. Intermolecular cyclisation of **4** with 4-methylbenzoic acid in presence of phosphorous oxy chloride affords 2-(3-pyridyl)-5-(4-methylphenyl)-1,3,4-oxadiazole **5**. Condensation of **5** with various substituted 2-hydrazino benzothiazole **2a**–**j** results in 3-(3-pyridyl)-5-(4-methylphenyl)-4-(*N*-substituted-1,3-benzothiazol-2-amino)-4*H*-1,2,4-triazole **6a**–**j** analogs. All the compounds have been characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. *In vitro* antitubercular activity was carried out against *Mycobacterium tuberculosis H$_{37}$Rv* strain using Lowenstein-Jensen medium and antimicrobial activity against various bacteria and fungi using broth microdilution method. Compounds **2e**, **6a**, **6b**, **6c**, **6d**, **6g**, **6h** and **6i** emerged as promising antimicrobials. It was also observed that the promising antimicrobials have proved to be better antituberculars. Compound **6j** showed better antitubercular activity compared to rifampicin.

1. Introduction

Tuberculosis (TB), a disease long considered substantially eradicated in the developed countries, has resurged dramatically in the last decades, establishing itself as one of the infectious diseases resulting in the highest number of human deaths worldwide [1–3]. Likely, the first underlying reasons for such escalation in numbers of infections with the TB pathogen, *Mycobacterium tuberculosis* (MT), is the deadly synergy with human immunodeficiency virus (HIV) indeed, an impressive number of HIV-infected individuals succumb to MT aggression [4]. The second, important cause is the emergence of multi-drug resistant strains (MDR) of MT [2–7], together with the spread of severe opportunistic disseminated infections produced by Mycobacterium other than tuberculosis (MOTT), particularly Mycobacterium avium [8].

The standard therapy [3] for TB includes isoniazid, targeting both the NADH-dependent enoyl reductase (InhA) and the 3-oxoacyl ACP synthase (KasA) [9,10] and rifampicin, a well characterized inhibitor of the DNA-dependent RNA-polymerase [11]. There are two basic approaches to develop a new drug for TB: (i) synthesis of analogs, modifications or derivatives of existing compounds for shortening and improving TB treatment and, (ii) searching novel structures, that the TB organism has never been presented with before, for the treatment of multi-drug resistant TB [12].

To pursue this goal, our research efforts are directed to find new chemical classes of antitubercular active agent with different mode of action. The azole antitubercular may be regarded as a new class providing truly effective drugs which are reported to inhibit the bacteria by blocking the biosynthesis of certain bacterial lipids and/or by additional mechanism [13,14]. Triazoles in particular, substituted-1,2,4-triazole are among various heterocycles that have received the most attention during last two decades as potential antimicrobial agents, antifungal, antitubercular, anti-HIV, anti-inflammatory, CNS stimulants, sedatives, antianxiety [15–23]. Benzothiazole moiety has already been reported for its antimicrobial and antitubercular activity [24,25] along with antitumor, anti-inflammatory, analgesic, anticonvulsant, diuretic, antimalarial, antidibetic [26–33]. After extensive literature search, it was observed that, till date enough effort has not been made to combine this two moieties as a single molecular scaffold and to identify new candidates that may be value in designing new, potent, selective and less toxic antitubercular and antimicrobial agents. In view of this data, we reported the synthesis of new 1,2,4-triazoles incorporated with benzothiazole and pyridine which possessed wide variety of biological activity encouraging antitubercular activity against *M. tuberculosis* H$_{37}$Rv and antimicrobial activity.

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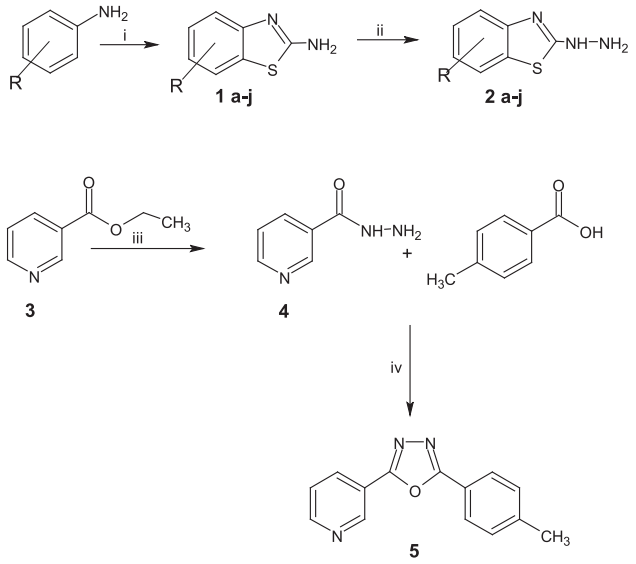
2. Chemistry

Substituted 2-hydrazino-1,3-benzothiazole **2a**–**j** were prepared from substituted 2-amino-1,3-benzothiazole according to described process as in Scheme 1. Nicotinic acid was converted to ethyl nicotinoate **3** using ethanol and catalytic amount of sulphuric acid. Ethyl nicotinoate on treatment with hydrazine hydrate yielded nicotinoyl hydrazide **4** by reported method [34], which on intermolecular cyclisation with 4-methylbenzoic acid in presence of phosphorous oxy chloride afforded 2-(3-pyridyl)-5-(4-methylphenyl)-1,3,4-oxadiazole **5**, this on further condensation with substituted 2-hydrazino-1,3-benzothiazoles **2a**–**j** in dry pyridine resulted to 3-(3-pyridyl)-5-(4-methylphenyl)-4-(*N*-substituted-1,3-benzothiazol-2-amino)-4*H*-1,2,4-triazoles **6a**–**j** (Scheme 2).

3. Biology

The MICs of synthesized compounds were carried out by broth microdilution method as described by Rattan [35]. Antibacterial activity was screened 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 by using ampicillin as a standard antibacterial agent. Antifungal activity was screened against three fungal species *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282 and *Aspergillus clavatus* MTCC 1323 and greseofulvin was used as a standard antifungal agent. The antimicrobial screening data are shown in Table 1 and Table 2.

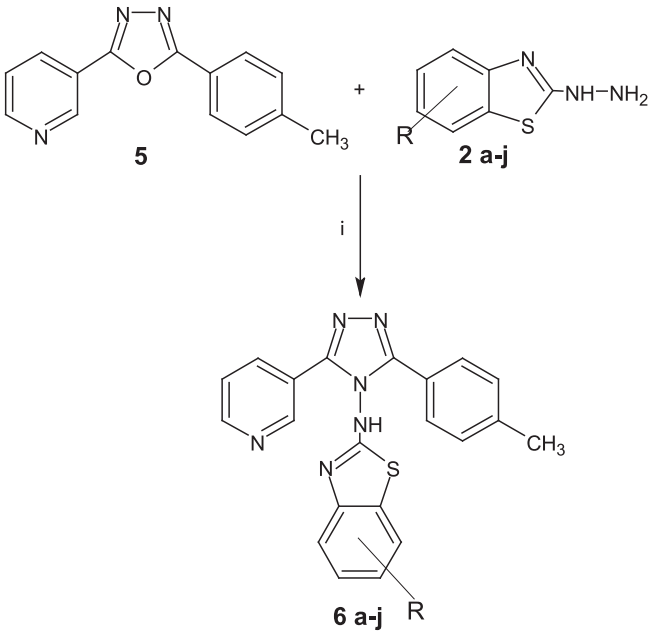
All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh and tested against above mentioned known drugs. Mueller Hinton broth was used as nutrient medium to grow and dilute the drug suspension for the test. Inoculum size for test strain was adjusted to 10^8 CFU (Colony Forming Unit) per milliliter by comparing the turbidity. DMSO was used as diluents



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. $POCl_3$, refluxed 9h

R = a. 6-F, d. 6-CH_3, g. 4-CH_3, j. 4-Cl
b. 6-Br, e. 6-OCH_3, h. 4-NO_2,
c. 6-NO_2, f. 6-Cl, i. 5-Cl,6-Cl,

Scheme 1. Synthetic protocol for 2-hydrazino benzothiazoles **2a**–**j** and 2-(3-pyridyl)-5-(4- methylphenyl)-1,3,4-oxadiazole **5**.



i. dry pyridine, refluxed

R = a. 6-F, d. 6-CH_3, g. 4-CH_3, j. 4-Cl
b. 6-Br, e. 6-OCH_3, h. 4-NO_2,
c. 6-NO_2, f. 6-Cl, i. 5-Cl,6-Cl,

Scheme 2. Synthetic protocol for 3-(3-pyridyl)-5-(4-methylphenyl)-4-(N-substituted-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole **6a**–**j**.

to get desired concentration of drugs to test upon standard bacterial strains.

MIC of compounds was determined against *M. tuberculosis* $H_{37}Rv$ strain by using Lowenstein-Jensen medium (conventional method) as described by Rattan [35].

Table 1
Minimum inhibitory concentrations (MICs, μg/ml).

Compound	Gram positive bacteria		Gram negative bacteria	
	S. aureus	*S. pyogenus*	*E. coli*	*P. aeruginosa*
	MTCC-96	MTCC-443	MTCC-442	MTCC-2488
2a	500	250	250	250
2b	250	500	500	500
2c	500	500	250	250
2d	250	250	100	500
2e	200	250	62.5	125
2f	500	500	100	125
2g	500	500	250	250
2h	500	250	500	250
2i	250	500	100	125
2j	500	500	250	250
5	100	250	250	100
6a	125	100	250	250
6b	125	125	100	100
6c	250	125	100	200
6d	100	100	125	250
6e	250	250	250	125
6f	500	500	250	125
6g	500	250	250	500
6h	500	250	12.5	25
6i	250	500	100	500
6j	250	500	125	500
Ampicillin	250	100	100	100

Table 2
Minimum inhibitory concentrations (MICs, μg/ml).

Compound	Fungal species		
	C. albicans	*A. niger*	*A. clavatus*
	MTCC-227	MTCC-282	MTCC-323
2a	250	>1000	>1000
2b	500	500	500
2c	500	500	1000
2d	250	1000	>1000
2e	500	500	250
2f	250	200	250
2g	500	250	200
2h	200	250	200
2i	500	500	1000
2j	1000	500	500
5	1000	500	500
6a	500	500	250
6b	1000	500	1000
6c	500	500	500
6d	500	1000	>1000
6e	250	500	1000
6f	500	500	250
6g	100	250	200
6h	1000	500	500
6i	200	500	250
6j	500	>1000	1000
Greseofulvin	500	100	100

4. Result and discussion

4.1. Analytical results

A series of 1,2,4-triazole analogs has been synthesized in good yields by using the synthetic route as outlined in Scheme 1 and 2. IR, ^{1}H NMR, ^{13}C NMR and mass spectral data are in well agreement with the proposed structures of all newly synthesized compounds.

The IR spectra of **1a** showed the broad stretching band around 3425 cm^{-1} due to amine and ^{1}H NMR spectrum showed a singlet at δ 6.8 which accounted for NH_2; whereas **2a** showed broad stretching band around 3425 and 3200 cm^{-1} for NH and NH_2 with ^{1}H NMR a singlet at δ 4.8 and δ 8.9 accounted for NH_2 and NH, vanished on D_2O exchange. Nicotinoyl hydrazide **4** showed IR stretching band around 3335 and 3278 cm^{-1} due to amine NH_2/amide NH while strong stretching band at 1610 cm^{-1} was attributed to amide carbonyl with agreement of a singlet at δ 4.51 and δ 9.81 accounted for NH_2 and NH and vanished on D_2O exchange in ^{1}H NMR. Disappearance of NH and NH_2 signals of **5** with ^{1}H NMR proved the ring closure resulted in the formation of 1,3,4-oxadiazole ring from **4**. This was further substantiated by the ^{13}C NMR data of **5**, showed a signal at δ 157.50 and δ 158.82 due to C_2 and C_5 of oxadiazole. Mass spectrum of **5** displayed a molecular ion peak at *m*/*z* 237, confirmed its molecular weight. Absence of NH_2 signal of 2-hydrazino benzothiazoles and appearance of signal at δ 7.82 for NH were observed in ^{1}H NMR of **6a** proved the condensation of **2a** and **5** resulted in the formation of 1,2,4-triazole ring. This was substantiated by ^{13}C NMR, showed a signal at δ 163.48 and δ 163.35 due to C_3 and C_5 of triazole. Mass spectrum of **6a** displayed a molecular ion peak at *m*/*z* 402, confirmed its molecular weight.

4.2. Biological results

The antibacterial screening results are summarized in Table 1. The results revealed that substituted 2-hydrazino benzothiazoles showed moderate activity against all the bacterial strain except compound **2e** having 6-methoxy substituent showed good activity against *S. aureus* and *E. coli*, while 1,3,4-oxadiazole **5** exhibited good activity against *S. aureus* and *P. aeruginosa*. Most of 1,2,4-triazoles showed good activity (250 μg/ml) while compounds **6a**, **6b** and **6d** containing 6-flouro, 6-bromo and 6-methyl substituents possessed pronounced activity (100–125 μg/ml) against *S. aureus*. Compounds **6a** and **6d** having 6-flouro and 6-methyl substituents showed good activity (100 μg/ml) then other compounds against *S. pyogenes*. Compound **6h** containing electron withdrawing 4-nitro substituent has higher activity (12.5 μg/ml) against *E. coli* and (25 μg/ml) against *P. aeruginosa*. Good activity was observed with compound **6b**, **6c** and **6i** containing 6-bromo, 6-nitro and 5,6-dichloro substituents while other displayed moderate activity against *E. coli*. Compound **6b** having 6-bromo substituent showed good activity (100 μg/ml) whereas other displayed moderate activity against *P. aeruginosa*. Compound **6a** and **6d** exhibited very good activity against gram positive bacteria whereas **6b** and **6h** showed very good activity towards gram negative bacteria. Compounds **2e** and **5** were found active against gram positive and gram negative bacteria.

The results of antifungal activity are summarized in Table 2. The results showed that 2-hydrazino benzothiazoles **2a**–**i** possessed good activity (200–500 μg/ml) against *C. albicans* except **2j**. Compounds **2a**–**j** displayed moderate to weak activity (250–500 μg/ml) against *A. niger* and *A. clavatus* while 1,3,4-oxadiazole **5** exhibited weak activity against all three fungi. Compound **6g** having electron releasing 4-methyl substituent exhibited better activity (100 μg/ml) whereas other compounds showed good activity (200–500 μg/ml) except **6j** and **6h** against *C. albicans*. All the compounds showed weak activity against *A. niger* and *A. clavatus*.

The encouraging results from the antibacterial studies impelled us to go for preliminary screening of synthesized compounds against *M. tuberculosis* is summarized in Table 3. Compound **2e** containing 4-methoxy benzothiazole, showed better activity (50 μg/ml) against *M. tuberculosis* and compound **6a**, **6b**, **6d**, **6e** and **6h** showed good activity (50–62.5 μg/ml) which is attributed due to 6-flouro, 6-bromo, 6-methyl, 6-methoxy and 4-nitro substituents whereas compound **6j** which is having inductively electron withdrawing but mesomerically electron releasing 4-chloro substituent on benzothiazole ring showed better 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.

Table 3
Minimum inhibitory concentrations (MICs, μg/ml).

Compound	MIC values (μg/ml) of *M. tuberculosis* H_{37}Rv	% Inhibition
2a	250	98%
2b	500	97%
2c	250	99%
2d	1000	98%
2e	50	99%
2f	200	99%
2g	500	98%
2h	500	98%
2i	250	99%
2j	100	99%
5	500	98%
6a	200	98%
6b	50	99%
6c	200	98%
6d	50	99%
6e	62.5	99%
6f	100	99%
6g	500	98%
6h	50	99%
6i	100	99%
6j	25	99%
Rifampicin	40	98%

5. Conclusion

A series of newer analogs of 1,2,4-triazoles were synthesized by introduction of 2-hydrazino benzothiazoles to 1,3,4-oxadiazole and assessed for their antimicrobial and antituberculosis activity. Modification of substituents on benzothiazole ring with various electron releasing and electron withdrawing substituents improved the activity. It is important to point out that activity is not affected by electronic properties of the substituents. The antibacterial data indicates that the analogs with halogen, methyl and nitro substituents emerged as promising antimicrobials showing better to moderate activity while analogs bearing methyl substituent showed better antifungal activity. It was also observed that the promising antimicrobials have proved to be better antituberculars. Specially, compound **6j** due to their better activity against $H_{37}Rv$ strain, are the best choice for the preparation of new derivatives in order to improve antitubercular activity in future.

6. Experimental

6.1. 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 purity of compounds were checked by TLC using Merck silica gel 60 F_{254} and visualized by exposure to iodine vapours or UV light. IR spectra were recorded on a Perkin–Elmer RX 1 FTIR spectrophotometer, using potassium bromide pellets, the frequencies are expressed in cm^{-1}. The ^1H NMR and ^{13}C NMR spectra were recorded with a Bruker Avance II 400 NMR spectrometer, using tetramethylsilane as the internal reference, with chloroform ($CDCl_3$) as solvent. The chemical shifts are reported in parts per million (δ ppm). Elemental analyses were performed on a Heraeus Carlo Erba 1180 CHN analyzer. The mass spectra were recorded on micromass Q-T of micro (TOF MS ES^+).

All spectral data were consistent with the proposed structure and micro analysis within ±0.4% of theoretical values.

Substituted 2-hydrazino-1,3-benzothiazole **2a**–**j** were prepared by the literature procedure [36].

6.1.1. 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 substituted 2-amino-1,3-benzothiazole **1a**–**j** (20 mmol) were 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 to obtain crystalline product. The other compounds of the series were prepared by similar procedure.

*6.1.1.1. 2-Hydrazino-6-flouro-1,3-benzothiazole **2a**.* Greyish brown solid (70%); m.p. 204–206 °C; IR (KBr): 3200–3435 (–$NHNH_2$), 1631 (C=N), 1442 (thiazole) cm^{-1}; ^1H NMR ($CDCl_3$) δ 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, 3CH) ppm. 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%.

*6.1.1.2. 2-Hydrazino-6-bromo-1,3-benzothiazole **2b**.* Yellowish brown solid (61%); m.p. 200–202 °C; IR (KBr): 3212–3449 (–$NHNH_2$), 1623 (C=N), 1451 (thiazole) cm^{-1}; ^1H NMR ($CDCl_3$) δ 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, 3CH) ppm. 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%.

*6.1.1.3. 2-Hydrazino-6-nitro-1,3-benzothiazole **2c**.* Brownish yellow solid (67%); m.p. 210–212 °C; IR (KBr): 3220–3445 (–$NHNH_2$), 1640 (C=N), 1448 (thiazole) cm^{-1}; ^1H NMR ($CDCl_3$) δ 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, 3CH) ppm. 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%.

*6.1.1.4. 2-Hydrazino-6-methyl-1,3-benzothiazole **2d**.* Yellowish brown solid (62%); m.p. 198–200 °C; IR (KBr): 3222–3449 (–$NHNH_2$), 1620 (C=N), 1439 (thiazole) cm^{-1}; ^1H NMR ($CDCl_3$) δ 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, 3CH) ppm. 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%.

*6.1.1.5. 2-Hydrazino-6-methoxy-1,3-benzothiazole **2e**.* Reddish brown solid (65%); m.p. 193–195 °C; IR (KBr): 3208–3439 (–$NHNH_2$), 1628 (C=N), 1448 (thiazole) cm^{-1}; ^1H NMR ($CDCl_3$) δ 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, 3CH) ppm. 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%.

*6.1.1.6. 2-Hydrazino-6-chloro-1,3-benzothiazole **2f**.* Khaki solid (68%); m.p. 198–200 °C; IR (KBr): 3218–3445 (–$NHNH_2$), 1624 (C=N), 1428 (thiazole) cm^{-1}; ^1H NMR ($CDCl_3$) δ 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, 3CH) ppm. 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%.

*6.1.1.7. 2-Hydrazino-4-methyl-1,3-benzothiazole **2g**.* Pale green solid (69%); m.p. 167–169 °C; IR (KBr): 3220–3439 (–$NHNH_2$), 1638 (C=N), 1435 (thiazole) cm^{-1}; ^1H NMR ($CDCl_3$) δ 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, 3CH) ppm. 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%.

*6.1.1.8. 2-Hydrazino-4-nitro-1,3-benzothiazole **2h**.* Yellowish brown solid (60%); m.p. 199–201 °C; IR (KBr): 3200–3440 (–$NHNH_2$), 1631 (C=N), 1445 (thiazole) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.88 (s, 2H, NH_2, disappeared on D_2O exchange), 8.95 (s, 1H, NH, disappeared on D_2O exchange), 7.06–8.82 (m, 3H, 3CH) ppm. 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%.

*6.1.1.9. 2-Hydrazino-(5,6-dichloro)-1,3-benzothiazole **2i**.* Dark green solid (68%); m.p. 248–250 °C; IR (KBr): 3212–3449 (–$NHNH_2$), 1640 (C=N), 1439 (thiazole) cm^{-1}; ^1H NMR ($CDCl_3$) δ 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) ppm. 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%.

*6.1.1.10. 2-Hydrazino-4-chloro-1,3-benzothiazole **2j**.* Yellow solid (63%); m.p. 239–241 °C; IR (KBr): 3220–3449 (–$NHNH_2$), 1640 (C=N), 1445 (thiazole) cm^{-1}; ^1H NMR ($CDCl_3$) δ 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, 3CH) ppm. 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%.

6.1.2. 2-(3-Pyridyl)-5-(4-methylphenyl)-1,3,4-oxadiazole ***5***

A mixture of nicotinoyl hydrazide **4** (5 mmol) and 4-methylbenzoic acid (5 mmol) in phosphorus oxy chloride (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.

Off-white solid (65%); m.p. 266–270 °C; IR (KBr): 1667 (C=N), 1287, 1073 (C–O–C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.44 (s, 3H, CH_3), 9.37 (s, 1H, CH), 8.81 (dd, 1H, J = 3.48 Hz, CH), 8.44 (d, 1H, J = 8.08 Hz, CH), 7.51 (t, 1H, CH), 8.03 (d, 2H, J = 8.16 Hz, 2CH), 7.35 (d, 2H, J = 8.04 Hz, 2CH); ^{13}C NMR ($CDCl_3$) δ 158.82 (C_5-oxadiazole), 157.50 (C_2-oxadiazole), 24.96 (CH_3), 147.5, 143.02, 138.02, 129.42, 125.16, 125.01, 122.29, 122.10, 115.97 (aromatic ring) ppm; MS (m/z): 237 (M^+); Anal. Calcd. for $C_{14}H_{11}N_3O$: C, 70.87; H, 4.67; N, 17.71; Found: C, 70.84; H, 4.71; N, 17.68%.

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

A mixture of 2-(3-pyridyl)-5-(4-methylphenyl)-1,3,4-oxadiazole **5** (5 mmol) and substituted 2-hydrazino-1,3-benzothiazole **2a–j** (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.

6.1.3.1. 3-(3-Pyridyl)-5-(4-methylphenyl)-4-(N-6-flouro-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6a***. Pink solid (62%), m.p. 165–167 °C; IR (KBr): 3437 (NH), 1661 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.42 (s, 3H, CH_3), 7.82 (s, 1H, NH), 9.36 (s, 1H, CH), 8.80 (dd, 1H, J = 4.0 Hz, CH), 8.43 (d, 1H, J = 8.35 Hz, CH), 7.49 (t, 1H, CH), 8.03 (d, 2H, J = 8.16 Hz, 2CH), 7.35 (d, 2H, J = 8.04 Hz, 2CH), 7.06–7.26 (m, 3H, benzothiazole-H); ^{13}C NMR ($CDCl_3$) δ 163.48 (C_3-triazole), 163.35 (C_5-triazole), 24.44 (CH_3), 152.91, 149.66, 147.94, 145.66, 142.02, 134.42, 132.66, 132.51, 131.12, 129.31, 129.12, 124.85, 124.47, 121.91, 113.66, 109.85 (aromatic ring) ppm; MS (m/z): 402 (M^+); Anal. Calcd. for $C_{21}H_{15}N_6FS$: C, 62.67; H, 3.76; N, 20.88; Found: C, 62.63; H, 3.79; N, 20.91%.

6.1.3.2. 3-(3-Pyridyl)-5-(4-methylphenyl)-4-(N-6-bromo-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6b***. Light brown solid (59%), m.p. 149–151 °C; IR (KBr): 3432 (NH), 1646 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.41 (s, 3H, CH_3), 7.84 (s, 1H, NH), 9.37 (s, 1H, CH), 8.82 (dd, 1H, J = 4.0 Hz, CH), 8.43 (d, 1H, J = 8.35 Hz, CH), 7.49 (t, 1H, CH), 8.05 (d, 2H, J = 8.08 Hz, 2CH), 7.29 (d, 2H, J = 8.16 Hz, 2CH), 7.61–7.76 (m, 3H, benzothiazole-H); ^{13}C NMR ($CDCl_3$) δ 163.21 (C_3-triazole), 162.69 (C_5-triazole), 24.39 (CH_3), 151.93, 149.82, 147.69, 145.60, 136.69, 134.28, 131.98, 133.15, 128.83, 129.03, 127.78, 124.49, 124.23, 123.09, 118.99, 111.63 (aromatic ring) ppm; MS (m/z): 463 (M^+), 465 (M + 2); Anal. Calcd. for $C_{21}H_{15}N_6BrS$: C, 54.43; H, 3.26; N, 18.14; Found: C, 54.39; H, 3.22; N, 18.17%.

6.1.3.3. 3-(3-Pyridyl)-5-(4-methylphenyl)-4-(N-6-nitro-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6c***. Yellow solid (63%), m.p. 163–165 °C; IR (KBr): 3449 (NH), 1652 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.43 (s, 3H, CH_3), 7.90 (s, 1H, NH), 9.38 (s, 1H, CH), 8.82 (dd, 1H, J = 3.8 Hz, CH), 8.43 (d, 1H, J = 8.5 Hz, CH), 7.51 (t, 1H, CH), 8.35 (d, 2H, J = 8.16 Hz, 2CH), 7.31 (d, 2H, J = 8.16 Hz, 2CH), 7.58 (d, 1H, J = 7.85 Hz, CH), 8.05 (d, 1H, J = 8.04 Hz, CH), 8.74 (s, 1H, CH); ^{13}C NMR ($CDCl_3$) δ 162.94 (C_3-triazole), 162.36 (C_5-triazole), 24.37 (CH_3), 150.96, 148.83, 146.74, 146.61, 141.77, 134.48, 132.86, 132.60, 132.32, 129.53, 129.31, 124.61, 123.97, 121.98, 110.58, 119.72 (aromatic ring) ppm; MS (m/z): 429 (M^+); Anal. Calcd. for $C_{21}H_{15}N_7O_2S$: C, 58.73; H, 3.52; N, 22.83; Found: C, 58.69; H, 3.48; N, 22.87%.

6.1.3.4. 3-(3-Pyridyl)-5-(4-methylphenyl)-4-(N-6-methyl-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6d***. Redish brown solid (66%), m.p. 156–158 °C; IR (KBr): 3442 (NH), 1659 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.40 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 7.88 (s, 1H, NH), 9.36 (s, 1H, CH), 8.82 (dd, 1H, J = 3.4 Hz, CH), 8.43 (d, 1H, J = 8.48 Hz, CH), 7.45 (t, 1H, CH), 8.01 (d, 2H, J = 8.04 Hz, 2CH), 7.58 (d, 2H, J = 8.38 Hz, 2CH), 7.04–7.48 (m, 3H, benzothiazole-H); ^{13}C NMR ($CDCl_3$) δ 163.01 (C_3-triazole), 162.58 (C_5-triazole), 24.42 (CH_3), 22.91 (CH_3), 151.93, 148.85, 146.97, 144.75, 135.28, 135.17, 131.31, 131.15, 129.92, 128.79, 128.52, 124.61, 124.21, 124.02, 121.27, 121.38 (aromatic ring) ppm; MS (m/z): 398 (M^+); Anal. Calcd. for $C_{22}H_{18}N_6S$: C, 66.31; H, 4.55; N, 21.09; Found: C, 66.34; H, 4.51; N, 21.05%.

6.1.3.5. 3-(3-Pyridyl)-5-(4-methylphenyl)-4-(N-6-methoxy-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6e***. Red solid (63%), m.p. 241–243 °C; IR (KBr): 3449 (NH), 1652 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.44 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 7.86 (s, 1H, NH), 9.37 (s, 1H, CH), 8.82 (dd, 1H, J = 4.0 Hz, CH), 8.43 (d, 1H, J = 8.08 Hz, CH), 7.51 (t, 1H, CH), 8.05 (d, 2H, J = 8.16 Hz, 2CH), 7.37 (d, 2H, J = 8.16 Hz, 2CH), 6.84–7.28 (m, 3H, benzothiazole-H); ^{13}C NMR ($CDCl_3$) δ 163.38 (C_3-triazole), 162.82 (C_5-triazole), 24.32 (CH_3), 52.69 (OCH_3), 152.91, 149.56, 147.83, 147.35, 143.02, 134.36, 132.60, 132.44, 131.13, 127.61, 127.42, 124.23, 124.08, 119.15, 113.98, 106.25 (aromatic ring) ppm; MS (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, 4.42; N, 20.24%.

6.1.3.6. 3-(3-Pyridyl)-5-(4-methylphenyl)-4-(N-6-chloro-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6f***. Redish black solid (69%), m.p. 247–249 °C; IR (KBr): 3438 (NH), 1661 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.41 (s, 3H, CH_3), 7.85 (s, 1H, NH), 9.36 (s, 1H, CH), 8.48 (dd, 1H, J = 3.8 Hz, CH), 8.43 (d, 1H, J = 8.35 Hz, CH), 7.49 (t, 1H, CH), 8.03 (d, 2H, J = 8.20 Hz, 2CH), 7.35 (d, 2H, J = 8.04 Hz, 2CH), 7.59–7.80 (m, 3H, benzothiazole-H); ^{13}C NMR ($CDCl_3$) δ 163.24 (C_3-triazole), 162.76 (C_5-triazole), 24.38 (CH_3), 151.91, 148.97, 146.32, 144.44, 134.08, 131.52, 131.36, 131.05, 127.81, 127.62, 126.85, 125.47, 124.33, 123.84, 121.95, 121.52 (aromatic ring) ppm; MS (m/z): 418 (M^+), 420 (M + 2); Anal. Calcd. for $C_{21}H_{15}N_6ClS$: C, 60.21; H, 3.61; N, 20.06; Found: C, 60.25; H, 3.58; N, 20.03%.

6.1.3.7. 3-(3-Pyridyl)-5-(4-methylphenyl)-4-(N-4-methyl-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6g***. Pale yellow solid (61%), m.p. 159–161 °C; IR (KBr): 3435 (NH), 1660 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.39 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 7.88 (s, 1H, NH), 9.36 (s, 1H, CH), 8.80 (dd, 1H, J = 3.8 Hz, CH), 8.43 (d, 1H, J = 8.38 Hz, CH), 7.50 (t, 1H, CH), 8.04 (d, 2H, J = 8.16 Hz, 2CH), 7.32 (d, 2H, J = 8.12 Hz, 2CH), 7.09–7.28 (m, 3H, benzothiazole-H); ^{13}C NMR ($CDCl_3$) δ 163.18 (C_3-triazole), 162.65 (C_5-triazole), 24.35 (CH_3), 20.49 (CH_3), 151.88, 148.93, 146.88, 143.47, 134.11, 132.32, 132.15, 131.07, 129.18, 128.96, 128.82, 127.57, 124.53, 123.97, 122.04, 120.96 (aromatic ring) ppm; MS (m/z): 398 (M^+); Anal. Calcd. for $C_{22}H_{18}N_6S$: C, 66.31; H, 4.55; N, 21.09; Found: C, 66.27; H, 4.58; N, 21.12%.

6.1.3.8. 3-(3-Pyridyl)-5-(4-methylphenyl)-4-(N-4-nitro-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6h***. Yellow solid (64%), m.p. 190–192 °C; IR (KBr): 3447 (NH), 1662 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.40 (s, 3H, CH_3), 7.88 (s, 1H, NH), 9.36 (s, 1H, CH), 8.81 (dd, 1H, $J = 4.0$ Hz, CH), 8.43 (d, 1H, $J = 8.35$ Hz, CH), 7.49 (t, 1H, CH), 8.01 (d, 2H, $J = 8.40$ Hz, 2CH), 7.35 (d, 2H, $J = 8.04$ Hz, 2CH), 6.63 (t, 1H, CH), 8.22 (d, 1H, $J = 8.16$ Hz, CH), 8.56 (d, 1H, $J = 8.16$ Hz, CH); ^{13}C NMR ($CDCl_3$) δ 162.94 (C_3-triazole), 162.42 (C_5-triazole), 24.41 (CH_3), 152.21, 148.83, 146.89, 144.36, 138.06, 134.51, 132.10, 131.98, 131.81, 129.12, 128.07, 127.60, 124.19, 123.89, 122.55, 120.65 (aromatic ring) ppm; MS (*m*/*z*): 429 (M^+); Anal. Calcd. for $C_{21}H_{15}N_7O_2S$: C, 58.73; H, 3.52; N, 22.83; Found: C, 58.76; H, 3.56; N, 22.79%.

6.1.3.9. 3-(3-Pyridyl)-5-(4-methylphenyl)-4-(N-(5,6-dichloro)-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6i***. Redish brown solid (65%), m.p. 172–174 °C; IR (KBr): 3449 (NH), 1655 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.41 (s, 3H, CH_3), 7.88 (s, 1H, NH), 9.36 (s, 1H, CH), 8.81 (dd, 1H, $J = 3.8$ Hz, CH), 8.43 (d, 1H, $J = 8.04$ Hz, CH), 7.48 (t, 1H, CH), 8.01 (d, 2H, $J = 8.48$ Hz, 2CH), 7.35 (d, 2H, $J = 8.16$ Hz, 2CH), 7.59 (s, 1H, CH), 7.66 (s, 1H, CH); ^{13}C NMR ($CDCl_3$) δ 163.14 (C_3-triazole), 162.61 (C_5-triazole), 24.39 (CH_3), 152.35, 149.74, 147.82, 144.86, 134.21, 133.66, 133.49, 129.60, 129.41, 128.02, 128.75, 124.48, 124.02, 123.37, 123.78, 121.74 (aromatic ring) ppm; MS (*m*/*z*): 453 (M^+), 455 (M + 2); Anal. Calcd. for $C_{21}H_{14}N_6Cl_2S$: C, 55.64; H, 3.11; N, 18.54; Found: C, 55.61; H, 3.08; N, 18.50%.

6.1.3.10. 3-(3-Pyridyl)-5-(4-methylphenyl)-4-(N-4-chloro-1,3-benzothiazol-2-amino)-4H-1,2,4-triazole ***6j***. Pale yellow solid (63%), m.p. 188–190 °C; IR (KBr): 3442 (NH), 1657 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.44 (s, 3H, CH_3), 7.87 (s, 1H, NH), 9.37 (s, 1H, CH), 8.81 (dd, 1H, $J = 4.0$ Hz, CH), 8.43 (d, 1H, $J = 8.40$ Hz, CH), 7.50 (t, 1H, CH), 8.03 (d, 2H, $J = 8.36$ Hz, 2CH), 7.35 (d, 2H, $J = 8.08$ Hz, 2CH), 6.91–7.30 (m, 3H, benzothiazole-H); ^{13}C NMR ($CDCl_3$) δ 163.31 (C_3-triazole), 162.80 (C_5-triazole), 24.43 (CH_3), 152.59, 148.96, 146.89, 144.86, 134.01, 131.56, 131.42, 131.59, 128.02, 127.81, 124.47, 124.08, 123.18, 120.29, 120.18, 118.79 (aromatic ring) ppm; MS (*m*/*z*): 418 (M^+), 420 (M + 2); Anal. Calcd. for $C_{21}H_{15}N_6ClS$: C, 60.21; H, 3.61; N, 20.06; Found: C, 60.17; H, 3.64; N, 20.10%.

6.2. Biological assay

6.2.1. In vitro evaluation of antimicrobial activity

The MICs of synthesized compounds were carried out by broth microdilution method. 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.

6.2.2. In vitro evaluation of antitubercular activity

Drug susceptibility and determination of MIC of the test compounds against *M. tuberculosis* $H_{37}Rv$ were performed by L. J. agar (MIC) method [37] where primary 1000, 500 and 250 μg/ml and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25 and 3.25 μg/ml dilutions of each test compound were added liquid L. J. Medium and then media were sterilized by inspissation method. A culture of *M. tuberculosis* $H_{37}Rv$ growing on L. J. medium was harvested in 0.85% saline in bijou bottles. All test compound make first stock solution of 2000 μg/ml concentration of compounds were 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 days, 22 days 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.

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Appendix. Supplementary data

Supplementary data associated with article can be found in online version at doi:10.1016/j.ejmech.2010.06.031.

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