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ORIGINAL ARTICLE

Synthesis of 1,2,4-triazole derivatives containing benzothiazoles as pharmacologically active molecule

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Abstract

In attempt to make significant pharmacologically active molecule, we report here the synthesis and *in vitro* antimicrobial and antitubercular activity of various series of 3-(3-pyridyl)-5-(4-nitrophenyl)-4-(*N*-substituted-1,3-benzothiazol-2-amino)-4*H*-1,2,4-triazole. The antimicrobial activity of title compounds were examined against two Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*), two Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and three fungi (*Candida albicans*, *Aspergillus niger*, *Aspergillus clavatus*) using the broth microdilution method and antitubercular activity $H_{37}Rv$ using Lowenstein-Jensen agar method.

Keywords: 1,2,4-Triazole, antimicrobial activity, antitubercular activity

Introduction

Tuberculosis (TB) is the leading infectious cause of death in the world today, with ~3 million deceasing every year. An increase in the global burden of TB with the worldwide mortality rate of 23% is a major concern in the socioeconomic and health sectors.[1–5] The synergy of this disease with HIV infection and the emergence of multidrug resistance and extensively drug resistance tuberculosis (MDRTB and XDRTB) pose a threatening global challenge.[6–8] Although a number of lead molecules exist today to develop new drugs, no new chemical entity has emerged for clinical use for over the last 45 years in the treatment of this disease.[9,10] Therefore, there is an urgent need to develop new drugs, acting through a novel mechanism of action for the chemotherapy of TB.

Recently, certain triazole-based compounds were reported to possess antimicrobial activities.[11–13] It is believed that aryl-azolyl-ethane moiety, present in many azole antifungal drugs, serves as pharmacophore in compounds having *Mycobacterium* killing activity.[14,15] Many azole derivatives have also been shown to possess interesting antimycobacterial activity in addition to antifungal activity.[16–18] In addition, closer analogues of our oxadiazole were reported to possess antitubercular activity.[19,20] It is established that these compounds target the sterol demethylase, a mixed-function oxidase involved in sterol synthesis in eukaryotic organisms.[21] The unraveling of *Mycobacterium* genome sequence has revealed that a protein having homology to one of the above mixed oxidase function is present in *Mycobacterium tuberculosis*.[22] In view of this data, we aimed the synthesis, antimicrobial, and antitubercular evaluation of new substituted 1,2,4-triazole derivatives. We have incorporated triazoles with pyridine and benzothiazole derivatives, which possess wide variety of biological activity.

Experimental section

Chemistry

All chemicals were of analytical grade and use directly. All melting points were determined in PMP-DM scientific melting point apparatus and are uncorrected. The completion of the reaction was checked by TLC using Merck silica gel 60 F254 and spots were visualized under UV radiation. IR spectra were recorded on Perkin-Elmer RX 1 FT-IR spectrophotometer in KBr (γ_{max} in cm^{-1}). ^1H

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NMR spectra were recorded in $CDCl_3$ on a Bruker Avance II 400 NMR spectrometer (400 MHz) using TMS as internal standard (δ in ppm). ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker Avance II 400 NMR spectrometer operating at 400 MHz (δ in ppm). The microanalyses were performed on a Heraeus Carlo Erba 1180 CHN analyzer. The mass spectra were recorded on micromass Q-T of micro (TOF MS ES+).

Substituted 2-hydrazino-1,3-benzothiazole (**2a–j**) was prepared by the literature procedure.[23,24]

General procedure for synthesis of 2-hydrazino benzothiazoles

(**2a–j**). Concentrated hydrochloric acid (0.067 mol) was added drop wise with stirring to hydrazine hydrate (0.12 mol) at 5–6°C followed by ethylene glycol (30 mL); thereafter substituted 2-amino-1,3-benzothiazole (**1a–j**) (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.

(**2a**). Yield 70%: m.p. 204–206°C. IR (KBr): 3435 (NH_2), 3200 (NH), 1631 (C=N), 1442 (thiazole) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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%.

(**2b**). Yield 61%; m.p. 200–202°C; IR (KBr): 3449 (NH_2), 3212 (NH), 1623 (C=N), 1451 (thiazole) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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%.

(**2c**). Yield 67%; m.p. 210–212°C; IR (KBr): 3445 (NH_2), 3220 (NH), 1640 (C=N), 1448 (thiazole) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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%.

(**2d**). Yield 62%; m.p. 198–200°C; IR (KBr): 3449 (NH_2), 3222 (NH), 1620 (C=N), 1439 (thiazole) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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%.

(**2e**). Yield 65%; m.p. 193–195°C; IR (KBr): 3439 (NH_2), 3208 (NH), 1628 (C=N), 1448 (thiazole) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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, 1H, 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%.

(**2f**). Yield 68%; m.p. 198–200°C; IR (KBr): 3445 (NH_2), 3218 (NH), 1624 (C=N), 1428 (thiazole) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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%.

(**2g**) Yield 69%; m.p. 167–169°C; IR (KBr): 3439 (NH_2), 3220 (NH), 1638 (C=N), 1435 (thiazole) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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%.

(**2h**). Yield 60%; m.p. 199–201°C; IR (KBr): 3440 (NH_2), 3200 (NH), 1631 (C=N), 1445 (thiazole) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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%.

(**2i**). Yield 68%; m.p. 248–250°C; IR (KBr): 3449 (NH_2), 3212 (NH), 1640 (C=N), 1439 (thiazole) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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%.

(**2j**). Yield 63%; m.p. 239–241°C; IR (KBr): 3449 (NH_2), 3220 (NH), 1640 (C=N), 1445 (thiazole) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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%.

2-(3-Pyridyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (5)

A mixture of nicotinoyl hydrazide (**4**) (5 mmol) and 4-nitro 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.

(**5**). Yield 65%; m.p. 121–123°C; IR (KBr): 1667 (C=N), 1284, 1078 (C-O-C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.38 (s, 1H, CH), 8.83 (dd, 1H, J = 3.48 Hz, CH), 8.39–8.48 (m, 5H, 5CH), 7.61 (t, 1H, CH) ppm; ^{13}C NMR (400 MHz, $CDCl_3$) δ 160.22 (C_2-oxadiazole), 159.58 (C_5-oxadiazole), 149.50, 146.88, 145.38, 135.83, 129.65, 128.68, 124.68, 124.29, 123.34 (aromatic ring) ppm; MS (m/z): 268 (M^+);

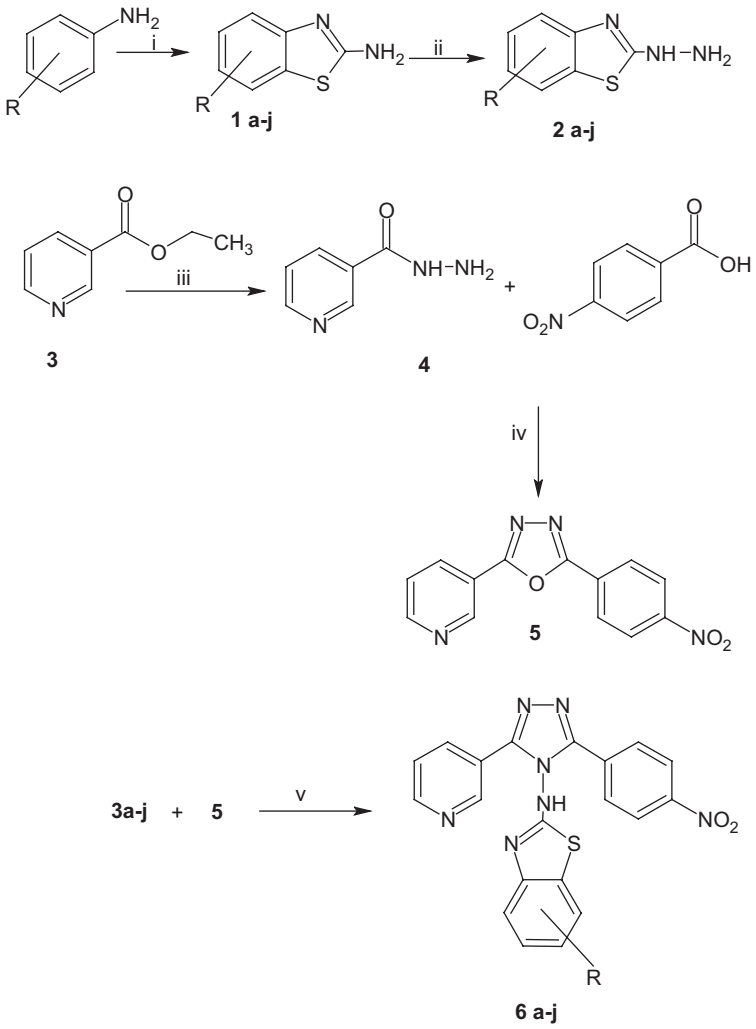
Anal. calcd. for $C_{13}H_8N_4O_3$: C, 58.21; H, 3.01; N, 20.89; Found: C, 58.17; H, 3.04; N, 20.85%.

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

A mixture of 2-(3-pyridyl)-5-(4-nitrophenyl)-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 (Scheme 1).

(**6a**). Yield 66%; m.p. 210–212°C; IR (KBr): 3434 (NH), 1649 (C=N) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 (s, 1H, NH), 9.38 (s, 1H, CH), 8.84 (dd, 1H, J = 4.0 Hz, CH), 8.39–8.49 (m, 5H, 5CH), 7.60 (t, 1H, CH), 7.06–7.26 (m, 3H, benzothiazole-H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 163.48 (C_3-triazole), 163.35 (C_5-triazole), 152.91, 149.66, 147.94, 145.66, 138.43, 134.42, 132.66, 131.12, 129.03, 129.12, 128.15, 124.55, 124.19, 121.91, 113.66, 109.85 (aromatic ring) ppm; MS (m/z): 433 (M^+); Anal. calcd. for $C_{20}H_{12}N_7O_2FS$: C, 55.42; H, 2.79; N, 22.62; Found: C, 55.38; H, 2.81; N, 22.71%.

(**6b**). Yield 62%; m.p. 199–201°C; IR (KBr): 3432 (NH), 1646 (C=N) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.79 (s,



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

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

1H, NH), 9.37 (s, 1H, CH), 8.82 (dd, 1H, *J* = 4.0 Hz, CH), 8.40–8.51 (m, 5H, 5CH), 7.56 (t, 1H, CH), 7.61-7.76 (m, 3H, benzothiazole-H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 163.28 (C_3-triazole), 162.69 (C_5-triazole), 153.03, 149.82, 147.69, 145.60, 136.69, 134.28, 133.15, 131.43, 129.03, 128.15, 127.78, 124.49, 124.16, 123.09, 118.99, 111.63 (aromatic ring) ppm; MS (*m*/*z*): 494 (M^+), 496 (M+2); Anal. calcd. for $C_{20}H_{12}N_7O_2BrS$: C, 48.59; H, 2.45; N, 19.83; Found: C, 48.63; H, 2.41; N, 19.79%.

(**6c**). Yield 69%; m.p. 186–188°C; IR (KBr): 3449 (NH), 1652 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) 7.80 (s, 1H, NH), 9.38 (s, 1H, CH), 8.83 (dd, 1H, *J* = 3.8 Hz, CH), 8.36–8.47 (m, 5H, 5CH), 7.62 (t, 1H, CH), 7.33 (d, 1H, *J* = 8.16 Hz, CH), 7.56 (d, 1H, *J* = 7.85 Hz, CH), 8.74 (s, 1H, CH); ^{13}C NMR (400 MHz, $CDCl_3$) δ 162.94 (C_3-triazole), 162.53 (C_5-triazole), 152.96, 148.83, 147.74, 146.61, 141.77, 134.48, 132.86, 132.32, 129.03, 129.53, 129.31, 124.61, 124.19, 121.98, 110.58, 119.72 (aromatic ring) ppm; MS (*m*/*z*): 460 (M^+); Anal. calcd. for $C_{20}H_{12}N_8O_4S$: C, 52.17; H, 2.63; N, 24.34; Found: C, 52.21; H, 2.59; N, 24.31%.

(**6d**). Yield 70%; m.p. 179–180°C; IR (KBr): 3447 (NH), 1661 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34 (s, 3H, CH_3), 7.82 (s, 1H, NH), 9.37 (s, 1H, CH), 8.82 (dd, 1H, *J* = 4.0 Hz, CH), 8.39–8.52 (m, 5H, 5CH), 7.56 (t, 1H, CH), 7.05-7.50 (m, 3H, benzothiazole-H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 163.07 (C_3-triazole), 162.61 (C_5-triazole), 22.91 (CH_3), 152.93, 149.57, 148.01, 144.75, 135.28, 135.07, 131.31, 129.05, 129.92, 128.79, 128.52, 124.61, 124.21, 124.02, 121.27, 121.38 (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, 58.77; H, 3.47; N, 22.78%.

(**6e**). Yield 71%; m.p. 189–191°C; IR (KBr): 3449 (NH), 1655 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.82 (s, 3H, OCH_3), 7.76 (s, 1H, NH), 9.36 (s, 1H, CH), 8.81 (dd, 1H, *J* = 4.0 Hz, CH), 8.39–8.48 (m, 5H, 5CH), 7.59 (t, 1H, CH), 6.84–7.28 (m, 3H, benzothiazole-H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 163.38 (C_3-triazole), 162.82 (C_5-triazole), 52.59 (OCH_3), 152.91, 149.99, 147.76, 147.35, 143.02, 134.36, 132.60, 132.44, 131.13, 129.13, 128.42, 124.23, 124.08, 119.15, 113.98, 106.25 (aromatic ring) ppm; MS (*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, 56.66; H, 3.41; N, 21.98%.

(**6f**). Yield 65%; m.p. 204–206°C; IR (KBr): 3442 (NH), 1657 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H, NH), 9.36 (s, 1H, CH), 8.82 (dd, 1H, *J* = 3.8 Hz, CH), 8.37–8.48 (m, 5H, 5CH), 7.52 (t, 1H, CH), 7.59–7.76 (m, 3H, benzothiazole-H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 163.34 (C_3-triazole), 162.85 (C_5-triazole), 152.87, 148.97, 147.32, 144.44, 134.08, 131.52, 131.05, 129.09, 127.81, 127.62, 126.85, 125.47, 124.33, 123.84, 121.95, 121.52 (aromatic ring) ppm; MS (*m*/*z*): 449 (M^+), 451 (M+2); Anal. calcd. for $C_{20}H_{12}N_7O_2ClS$: C, 53.40; H, 2.69; N, 21.79; Found: C, 53.42; H, 2.73; N, 21.83%.

(**6g**). Yield 65%; m.p. 192–194°C; IR (KBr): 3435 (NH), 1660 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.61 (s, 3H, CH_3), 7.76 (s, 1H, NH), 9.36 (s, 1H, CH), 8.82 (dd, 1H, *J* = 3.8 Hz, CH), 8.38–8.49 (m, 5H, 5CH), 7.59 (t, 1H, CH), 7.09–7.28 (m, 3H, benzothiazole-H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 163.18 (C_3-triazole), 162.65 (C_5-triazole), 20.82 (CH_3), 152.88, 148.93, 146.98, 143.47, 134.28, 132.47, 132.25, 131.07, 129.18, 128.96, 128.82, 127.57, 124.53, 123.98, 122.04, 120.96 (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, 58.81; H, 3.57; N, 22.87%.

(**6h**). Yield 67%; m.p. 171–173°C; IR (KBr): 3442 (NH), 1659 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H, NH), 9.37 (s, 1H, CH), 8.82 (dd, 1H, *J* = 4.0 Hz, CH), 8.39–8.48 (m, 5H, 5CH), 7.60 (t, 1H, CH), 8.22 (d, 1H, *J* = 8.16 Hz, CH), 8.56 (d, 1H, *J* = 8.14 Hz, CH), 6.63 (t, 1H, CH); ^{13}C NMR (400 MHz, $CDCl_3$) δ 162.94 (C_3-triazole), 162.45 (C_5-triazole), 152.21, 148.83, 147.74, 144.36, 138.06, 134.67, 132.10, 131.98, 131.81, 129.23, 128.07, 127.60, 124.20, 123.91, 122.55, 120.65 (aromatic ring) ppm; MS (*m*/*z*): 460 (M^+); Anal. calcd. for $C_{20}H_{12}N_8O_4S$: C, 52.17; H, 2.63; N, 24.34; Found: C, 52.21; H, 2.67; N, 24.37%.

(**6i**). Yield 68%; m.p. 205–207°C; IR (KBr): 3449 (NH), 1649 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H, NH), 9.36 (s, 1H, CH), 8.81 (dd, 1H, *J* = 3.8 Hz, CH), 8.38–8.50 (m, 5H, 5CH), 7.59 (t, 1H, CH), 7.59 (s, 1H, CH), 7.66 (s, 1H, CH); ^{13}C NMR (400 MHz, $CDCl_3$) δ 163.34 (C_3-triazole), 162.89 (C_5-triazole), 152.35, 149.74, 147.82, 144.86, 134.29, 133.72, 133.50, 129.69, 129.41, 128.02, 128.75, 124.46, 124.02, 123.37, 123.78, 121.74 (aromatic ring) ppm; MS (*m*/*z*): 484 (M^+), 486 (M+2), 488 (M+4); Anal. calcd. for $C_{20}H_{11}N_7O_2Cl_2S$: C, 49.60; H, 2.29; N, 20.24; Found: C, 49.64; H, 2.26; N, 20.21%.

(**6j**). Yield 65%; m.p. 191–193°C; IR (KBr): 3438 (NH), 1661 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H, NH), 9.37 (s, 1H, CH), 8.81 (dd, 1H, *J* = 4.0 Hz, CH), 8.38–8.51 (m, 5H, 5CH), 7.61 (t, 1H, CH), 6.91–7.30 (m, 3H, benzothiazole-H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 163.42 (C_3-triazole), 162.96 (C_5-triazole), 152.59, 148.96, 146.89, 144.86, 134.12, 131.65, 131.32, 131.59, 128.02, 127.81, 124.47, 124.08, 123.18, 120.29, 120.18, 118.79 (aromatic ring) ppm; MS (*m*/*z*): 449 (M^+), 451 (M+2); Anal. calcd. for $C_{20}H_{12}N_7O_2ClS$: C, 53.40; H, 2.69; N, 21.79; Found: C, 53.34; H, 2.72; N, 21.83%.

Antimicrobial activity

The minimum inhibitory concentrations (MICs) of synthesized compounds were carried out by broth microdilution method as described by Rattan.[25] Antibacterial activity was screened against two Gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Streptococcus pyogenes* MTCC 442) and two Gram-negative bacteria (*Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 2488). Ampicillin was used 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. Griseofulvin was used as a standard antifungal agent.

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 colony-forming unit (CFU) per millilitre by comparing the turbidity. Dimethyl sulphoxide (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 subcultured (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 earlier) was subcultured 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 compounds 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.

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[25–28] 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 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 compounds that 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* $H_{37}Rv$ (5×10^4 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.

Results 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-flouro-1,3-benzothiazole **2a**. IR spectra of **2a** showed broad stretching band around 3425 and 3200 cm^{-1} for NH and NH_2. ^1H 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 nicotinate **3** on treatment with hydrazine hydrate yields nicotinoyl hydrazide **4**; the IR spectra of **4** showed stretching band around 3335 and 3278 cm^{-1} due to amine/amide NH, whereas strong stretching band at 1610 cm^{-1} was attributed to amide carbonyl. ^1H 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-nitrobenzoic acid in presence of phosphorus oxy chloride affords 2-(3-pyridyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole **5**. Disappearance of ^1H NMR resonances observed with NH and NH_2 functions in the ^1H 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 160.22 and δ 159.58 due to C_2 and C_5 of oxadiazole. Mass spectrum of **5** displayed a molecular ion peak at *m*/*z* 268 that 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-nitrophenyl)-4-(*N*-substituted-1,3-benzothiazol-2-amino)-4*H*-1,2,4-triazole **6a–j**. Absence of ^1H NMR resonances observed with NH_2 function of **2a** and appearance of signal at δ 7.73 for NH was observed in ^1H 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** that showed a peak at δ 163.48 and δ 163.35 due to C_3 and C_5 of triazole. Mass spectrum of **6** displayed a molecular ion at *m*/*z* 433 that confirmed its molecular weight.

Antibacterial activity

The MICs of the tested compounds are shown in Tables 1 and 2. The results revealed that substituted 2-hydrazino benzothiazoles were moderately active against bacteria except **2e**, which showed good activity 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 (62.5–250 µg/mL) against *S. aureus*. Compounds **6b**, **6c**, **6f**, **6g**, and **6j** exhibited pronounced activity (62.5–125 µg/mL) against *S. aureus*. All the compounds exhibited moderate activity (150–250 µg/mL) except **6b** and **6j** (62.5 µg/mL) against *S. pyogenes*. Compounds **6b**, **6f**, **6g**, **6h**, and **6j** possessed good activity (100–125 µg/mL) except **6c** showed pronounced activity (62.5 µg/mL) while others displayed moderate activity (150–250 µg/mL) against *E. coli*. Compounds **6c** and **6d** showed good activity (100 µg/mL) except **6i**

showed very good activity (62.5 μg/mL) while others possessed moderate activity (150–250 μg/mL) against *P. aeruginosa*. Compounds **6b**, **6c**, **6f**, **6g**, and **6j** exhibited good activity against Gram-positive bacteria, whereas **6c**, **6g**, **6i**, and **6j** showed good activity toward Gram-negative bacteria. Compounds **2e**, **6c**, **6g**, and **6j** were found active against Gram-positive and Gram-negative bacteria.

Table 1. Minimum inhibitory concentrations (MICs, μg/mL) for the title compounds.

		Gram-positive bacteria		Gram-negative bacteria	
		S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*
Compound	R	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	—	100	125	500	250
6a	6-F	250	250	250	500
6b	6-Br	100	62.5	125	250
6c	6-NO_2	62.5	500	62.5	100
6d	6-CH_3	250	250	150	100
6e	6-OCH_3	250	125	500	250
6f	6-Cl	125	500	125	250
6g	4-CH_3	100	1000	100	250
6h	4-NO_2	500	250	125	200
6i	5-Cl, 6-Cl	1000	250	500	62.5
6j	4-Cl	100	62.5	100	250
Ampicillin	—	250	100	100	100

Table 2. Minimum inhibitory concentrations (MICs, μM) for the title compounds.

		Gram-positive bacteria		Gram-negative bacteria	
		S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*
Compound	R	MTCC 96	MTCC 442	MTCC 443	MTCC 2488
2a	6-F	2732	1366	1366	1366
2b	6-Br	1024	2049	2049	2049
2c	6-NO_2	2380	2380	1190	1190
2d	6-CH_3	1396	1396	558.6	2793
2e	6-OCH_3	1025	1282	320.5	641.0
2f	6-Cl	2512	2512	502.5	628.1
2g	4-CH_3	2793	2793	1396	1396
2h	4-NO_2	2380	1190	2380	1190
2i	5-Cl, 6-Cl	1068	2136	427.3	534.2
2j	4-Cl	2512	2512	1256	1256
5	—	373.1	466.4	1865	932.8
6a	6-F	577.3	577.3	577.3	1154
6b	6-Br	202.4	126.5	253.0	506.0
6c	6-NO_2	135.8	1086	135.8	217.4
6d	6-CH_3	582.7	582.7	349.6	233.1
6e	6-OCH_3	561.8	280.9	1123	561.8
6f	6-Cl	278.4	1113	278.4	556.6
6g	4-CH_3	233.1	2331	233.1	582.7
6h	4-NO_2	1086	543.4	271.7	434.7
6i	5-Cl, 6-Cl	2066	516.5	1033	124.1
6j	4-Cl	222.7	139.2	222.7	556.8
Ampicillin	—	716.3	286.5	286.5	286.5

Antifungal activity

In vitro antifungal activities (MICs) of the synthesized compounds are shown in Tables 3 and 4. The results showed that 2-hydrazino benzothiazoles **2a–i** possessed good activity (250–500 μg/mL) against *C. albicans* except **2j** (1000 μg/mL). Compounds **2a–j** displayed moderate to weak activity (250–500 μg/mL) against *A. niger* and *A. clavatus*, whereas 1,3,4-oxadiazole **5** exhibited weak activity against all three fungi. Compounds **6c**, **6d**, **6e**, **6f**, **6i**, and **6j** showed good activity (250–500 μg/mL), whereas **6a** and **6h** exhibited pronounced activity (100 μg/mL) against *C. albicans*. Compounds **6a**, **6h**, and **6i** exhibited moderate activity (200–250 μg/ml), whereas remaining compounds showed weak activity against *A. niger*. Compounds **6d** and **6h** displayed moderate activity (200–250 μg/mL), whereas rest of the compounds showed weak activity against *A. clavatus*. Compounds **2f**, **2g**, **2h**, **6a**, **6d**, and **6h** were found active against all the three fungal species.

Antitubercular activity

The encouraging results from the antibacterial studies impelled us to go for preliminary screening of synthesized compounds against *M. tuberculosis* are summarized in Table 5. From the preliminary examination of the antitubercular activity results, compound **2e** containing hydrazide group showed better activity (50 μg/mL) against *M. tuberculosis* and compounds **6a**, **6e**, and **6j** showed good activity (50–62.5 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) for the title compounds.

		Fungal species		
		C. albicans	*A. niger*	*A. clavatus*
Compound	R	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	500	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	—	>1000	>1000	>1000
6a	6-F	100	250	1000
6b	6-Br	>1000	>1000	>1000
6c	6-NO_2	500	500	1000
6d	6-CH_3	250	500	250
6e	6-OCH_3	250	500	1000
6f	6-Cl	500	1000	1000
6g	4-CH_3	>1000	>1000	>1000
6h	4-NO_2	100	200	200
6i	5-Cl, 6-Cl	500	250	500
6j	4-Cl	500	>1000	>1000
Griseofulvin	—	500	100	100

Table 4. Minimum inhibitory concentrations (MICs, μM) for the title compounds.

		Fungal species		
		C. albicans	*A. niger*	*A. clavatus*
Compound	R	MTCC 227	MTCC 282	MTCC 323
2a	6-F	1366	5464	5464
2b	6-Br	2049	2049	2049
2c	6-NO_2	2380	2380	4761
2d	6-CH_3	1396	2793	5586
2e	6-OCH_3	2564	2564	1282
2f	6-Cl	1265	1005	1265
2g	4-CH_3	2793	1396	1117
2h	4-NO_2	952.3	1190	952.3
2i	5-Cl, 6-Cl	2136	2136	4273
2j	4-Cl	5025	2512	2512
5	—	>2731	>2731	>2731
6a	6-F	230.9	577.3	2309
6b	6-Br	>2024	>2024	>2024
6c	6-NO_2	1086	1086	2173
6d	6-CH_3	582.7	1165	582.7
6e	6-OCH_3	561.8	1123	2247
6f	6-Cl	1113	2227	2227
6g	4-CH_3	>2331	>2331	>2331
6h	4-NO_2	217.4	434.8	434.8
6i	5-Cl, 6-Cl	1033	0516	1033
6j	4-Cl	1113	>2227	>2227
Griseofulvin	—	1420	284.0	284.0

Table 5. Minimum inhibitory concentrations (MICs, μg/mL and μM) for the title compounds.

		MIC values of *M. tuberculosis* $H_{37}Rv$		
Compound	R	μg/mL	μM	% Inhibition
2a	6-F	250	1366	98%
2b	6-Br	500	2049	96%
2c	6-NO_2	250	1109	98%
2d	6-CH_3	1000	5586	95%
2e	6-OCH_3	50	256.4	98%
2f	6-Cl	200	1005	96%
2g	4-CH_3	500	2793	95%
2h	4-NO_2	500	2380	97%
2i	5-Cl, 6-Cl	250	1068	98%
2j	4-Cl	100	502.5	96%
5	—	1000	3731	95%
6a	6-F	200	461.6	96%
6b	6-Br	62.5	126.5	98%
6c	6-NO_2	250	543.4	96%
6d	6-CH_3	100	233.1	98%
6e	6-OCH_3	62.5	140.44	96%
6f	6-Cl	250	556.8	94%
6g	4-CH_3	1000	2331	97%
6h	4-NO_2	100	217.4	96%
6i	5-Cl, 6-Cl	200	413.2	98%
6j	4-Cl	50	111.3	98%
Rifampicin	—	40	48.66	98%

Conclusion

A series of newer analogues 1,2,4-triazoles were synthesized by the introduction of 2-hydrazino benzothiazoles to 1,3,4-oxadiazoles and accessed for antimicrobial and antitubercular activity. Modification of substituents on benzothiazoles ring with various electron-releasing and electron-withdrawing substituents improved the activity. The analogues with halogen, methyl, and nitro substituents emerged as promising antibacterials showing better to moderate activity, whereas analogues bearing nitro 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, is the best choice for the preparation of new derivatives in order to improve antitubercular activity in future.

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Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article.

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