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Synthesis and biological evaluation of new 2-aryl-4-((4-aryl-1*H*-1,2,3-triazol-1-yl)methyl)thiazole derivatives

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Abstract A series of 2-aryl-4-((4-aryl-1*H*-1,2,3-triazol-1-yl)methyl)thiazole derivatives (**8a–p**) have been synthesized. The structure of the newly synthesized compounds was determined by spectral analysis. The title compounds were screened for their preliminary antitubercular activity against *Mycobacterium tuberculosis* H37Ra (MTB, ATCC 25177) and *Mycobacterium bovis* BCG (BCG, ATCC 35743). Further, the synthesized compounds were screened for antimicrobial activity against standard Gram-negative bacteria *Escherichia coli* (NCIM 2576) and *Pseudomonas flurescence* (NCIM 2059) and Gram-positive bacteria *Staphylococcus aureus* (NCIM 2602) and *Bacillus subtilis* (NCIM 2162). Among all the synthesized compounds, **8a–c**, **f–h**, **m** exhibited good activity against dormant *M. bovis* BCG strain. Compounds **8h**, **j** exhibited good activity against all tested bacterial strains. All active compounds were screened for cytotoxicity and found inactive. Their high potency and promising antimycobacterial activity suggest that these compounds could serve as good leads for further optimization and development.

Keywords 1,2,3-Triazole · Thiazole · Antitubercular activity · Antibacterial activity

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Introduction

Mycobacterium tuberculosis (MTB) is among the most challenging bacterial infections. TB was one of the top 10 causes of death worldwide in 2015, responsible for more deaths than human immunodeficiency virus (HIV) and malaria [1]. In addition, *M. bovis* BCG is among the most commonly administered vaccines worldwide [2]. Due to emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens in recent decades, new classes of antimicrobial agents are required. The increase in antibiotic resistance due to multiple factors has encouraged the search for new compounds which are active against multidrug-resistant pathogens.

Synthesis of motifs containing more than one heterocycle ring has received much attention in recent years. Triazole and its derivatives are an important class of bioactive molecules, exhibiting significant pharmacological activities including antimicrobial [3, 4], antiinflammatory, anesthetic [5], antineoplastic [6], anticonvulsant [7], antiproliferative [8], anticancer [9], antimalarial [10], and antiviral activity [11]. Also, they show phosphodiesterase enzyme inhibition [12], anti-hepatitis C [13], β-lactamase inhibition [14], fungicidal [15], insecticidal [16], and plant growth inhibition [17] activity, as well as many more. Among heterocyclic derivatives, triazole compounds were reported to be the most promising candidates towards anti-TB activity [18–33].

Thiazole and its derivatives are important structures in medicinal chemistry that could provide a rich spectrum of biological activities [34–59]. The structural diversity and biological importance of triazole and thiazoles have made them attractive targets for synthesis. 1,2,3-Triazole and thiazole rings present in the same molecule could represent a convenient model for investigation of their biological activity. Bearing in mind the biological significance of triazole and thiazole derivatives and in continuation of our study on synthesis and biological evaluation of various compounds [58, 59], we report herein synthesis of 2-aryl-4-((4-aryl-1*H*-1,2,3-triazol-1-yl)methyl)thiazole derivatives **8a–p** as potential antimycobacterial agents.

Experimental

All reactions were monitored and the purity of products checked by thin-layer chromatography (TLC) on Merck 60 F-254 silica gel plates with visualization by ultraviolet (UV) light. Melting points were determined in capillary tubes in silicone oil bath using a Veego melting point apparatus and are uncorrected. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance II 500 NMR spectrometer (Bruker Instruments Inc., Billerica, MA, USA) at either 500 MHz (^{1}H NMR) or 126 MHz (^{13}C NMR). Chemical shifts are reported from internal tetramethylsilane standard and are given in δ units. Column chromatography was performed on silica gel (100–200 mesh) supplied by Acme Chemical Co. (Mumbai, Maharashtra, India). Starting (2-arylthiazol-4-yl)methanol compounds

3a–d were synthesized by known literature method [56]. Chemicals and solvents used were of laboratory grade and purified as per literature methods.

Synthesis of (2-phenylthiazol-4-yl)methyl methanesulfonate (5a)

To a mixture of (2-phenylthiazol-4-yl)methanol (**3a**, 2 g, 0.01 mol), triethylamine (2.02 mL, 0.02 mol) in dichloromethane (DCM, 20 mL) at 0 °C, and methanesulfonyl chloride (1.71 g, 0.015 mol) was added dropwise at 0 °C and the reaction mixture stirred for 3 h. After completion of reaction (TLC), the mixture was extracted with water. Organic layer was dried over sodium sulfate and evaporated on rotary evaporator to obtain (2-phenylthiazol-4-yl)methyl methanesulfonate (**5a**) as thick oil in yield of 2.42 g (90 %).

Synthesis of 4-(azidomethyl)-2-phenylthiazole (6a)

A mixture of (2-phenylthiazol-4-yl)methyl methanesulfonate (**5a**, 2.0 g, 0.007 mol) and sodium azide (0.58 g, 0.009 mol) in dimethyl sulfoxide (DMSO) was stirred for 3 h at 70 °C. After completion of reaction (TLC), the mixture was quenched in water and the product extracted by ethyl acetate (3 × 25 mL); organic layer was dried over sodium sulfate and evaporated under vacuum to give 4-(azidomethyl)-2-phenylthiazole (**6a**) as thick oil in yield of 1.3 g (86 %).

General procedure for synthesis of 2-phenyl-4-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)thiazole (8a)

A reaction mixture of 4-(azidomethyl)-2-phenylthiazole (**6a**, 0.2 g, 0.0009 mol), ethynylbenzene (0.094 g, 0.0009 mol), copper sulfate (0.035 g, 0.00022 mol), and sodium ascorbate (0.045 g, 0.00022 mol) in dimethylformamide (DMF):water (6 mL, 3:1) was stirred overnight. After completion of reaction (TLC), the reaction mixture was quenched in water and extracted by ethyl acetate (3 × 15 mL). Organic layer was dried over sodium sulfate and evaporated on rotary evaporator. The crude product was purified by column chromatography (ethyl acetate:hexane) to furnish target compound 2-phenyl-4-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)thiazole (**8a**). Compounds **8b–p** were synthesized by similar procedure.

*2-Phenyl-4-((4-phenyl-1H-1,2,3-triazol-1-yl)methyl)thiazole (**8a**)* Yield: 70 %; m.p.: 138 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 5.76 (s, 2H), 7.25 (s, 1H), 7.37–7.43 (m, 3H), 7.45–7.51 (m, 3H), 7.85–7.87 (m, 2H), 7.99–7.93 (m, 2H), 8.02 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 50.0, 117.7, 120.1, 125.8, 126.6, 128.2, 128.8, 129.1, 130.5, 130.6, 133.1, 148.1, 150.6, 169.5; HRMS: 319.1021 $(M + H)^+$, 341.0840 $(M + Na)^+$.

*4-((4-(4-Fluorophenyl)-1H-1,2,3-triazol-1-yl)methyl)-2-phenylthiazole (**8b**)* Yield: 76 %; m.p.: 146 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 5.75 (s, 2H), 7.11 (t, J = 8.7 Hz, 2H), 7.26 (s, 1H), 7.49–7.44 (m, 3H), 7.82 (dd, J = 8.8 and 3.3 Hz, 2H), 7.97–7.92 (m, 2H), 7.98 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 50.0, 115.7,

115.9, 117.8, 119.9, 126.6, 126.8, 127.5, 127.5, 129.1, 130.6, 133.1, 147.3, 150.5, 161.7,163.7, 169.5; HRMS: 337.0930 $(M + H)^+$, 359.0748 $(M + Na)^+$.

*4-((4-(4-Methoxyphenyl)-1H-1,2,3-triazol-1-yl)methyl)-2-phenylthiazole (**8c**)* Yield: 74 %; m.p.: 120 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 3.84 (s, 3H), 5.74 (d, J = 0.5 Hz, 2H), 6.98–6.93 (m, 2H), 7.23 (s, 1H), 7.48–7.43 (m, 3H), 7.80–7.74 (m, 2H), 7.93 (s, 1H), 7.95 (dd, J = 4.7 and 2.3 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 50.0, 55.3, 114.2, 117.6, 119.3, 123.3, 126.6, 127.1, 129.1, 130.5, 133.1, 148.0, 150.7, 159.6, 169.4; HRMS: 349.1124 $(M + H)^+$, 371.0942 $(M + Na)^+$.

*4-((4-(3,4-Dimethoxyphenyl)-1H-1,2,3-triazol-1-yl)methyl)-2-phenylthiazole (**8d**)* Yield: 80 %; m.p.: 138 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 3.90 (s, 3H), 3.95 (s, 3H), 5.74 (s, 2H), 6.90 (d, J = 8.3 Hz, 1H), 7.23 (s, 1H), 7.29 (dd, J = 8.1 and 1.8 Hz, 1H), 7.44–7.46 (m, 3H), 7.50 (d, J = 1.8 Hz, 1H), 7.94 (dd, J = 6.8 and 2.7 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 50.0, 56.0, 56.0, 109.0, 111.3, 117.6, 118.3, 119.5, 123.6, 126.6, 129.1, 130.5, 133.1, 148.0, 149.1, 149.3, 150.7, 169.4; HRMS: 379.1231 $(M + H)^+$, 401.1050 $(M + Na)^+$.

*2-(4-Bromophenyl)-4-((4-phenyl-1H-1,2,3-triazol-1-yl)methyl)thiazole (**8e**)* Yield: 72 %; m.p.: 122 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 5.76 (s, 2H), 7.29 (s, 1H), 7.35 (d, J = 7.6 Hz, 2H), 7.62 - 7.56 (m, 3H), 7.89–7.84 (m, 2H), 7.91 (d, J = 7.9 Hz, 2H), 8.13 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 49.9, 118.3, 120.1, 125.2, 125.8, 128.0, 128.9, 129.4, 130.6, 132.3, 133.4, 148.2, 150.9, 167.6; HRMS: 397.0124 $(M + H)^+$, 418.9942 $(M + Na)^+$.

*2-(4-Bromophenyl)-4-((4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl)methyl)thiazole (**8f**)* Yield: 75 %; m.p.: 98 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 5.76 (s, 2H), 7.12 (t, J = 8.6 Hz, 2H), 7.32 (s, 1H), 7.35 (d, J = 8.7 Hz, 2H), 7.75–7.78 (m, 2H), 7.89 (d, J = 8.7 Hz, 2H), 8.13 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 49.9, 115.8, 115.9, 118.4, 119.9, 125.2, 127.5, 127.6, 129.4, 130.6, 132.3, 133.4, 147.4, 150.8, 161.7, 163.7, 167.6; HRMS: 415.0030 $(M + H)^+$, 436.9848 $(M + Na)^+$.

*2-(4-Bromophenyl)-4-((4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)methyl)thiazole (**8g**)* Yield: 80 %; m.p.: 98 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 3.84 (s, 3H), 5.74 (s, 2H), 6.96 (d, J = 8.6 Hz, 2H), 7.27 (s, 1H), 7.33 (d, J = 7.9 Hz, 2H), 7.79 (d, J = 7.9, Hz, 2H), 7.91 (d, J = 7.9 Hz, 2H), 8.12 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 49.9, 55.3, 114.3, 118.2, 119.3, 123.2, 123.2, 125.2, 127.1, 129.4, 130.6, 133.3, 148.1, 151.0, 159.7, 167.5; HRMS: 427.0226 $(M + H)^+$, 449.0041 $(M + Na)^+$.

*2-(4-Bromophenyl)-4-((4-(p-tolyl)-1H-1,2,3-triazol-1-yl)methyl)thiazole (**8h**)* Yield: 68 %; m.p.: 110 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 2.84 (s, 3H), 5.74 (s, 2H), 7.15 (d, J = 8.6 Hz, 2H), 7.24 (s, 1H), 7.35 (d, J = 8.1 Hz, 2H), 7.77 (d, J = 8.6 Hz, 2H), 7.87 (d, J = 8.1 Hz, 2H), 7.92 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 21.3, 49.9, 118.2, 119.7, 125.2, 125.7, 129.5, 130.6, 132.3, 133.3, 134.9, 138.1, 148.3, 151.0, 167.5; HRMS: 411.0269 $(M + H)^+$, 433.0083 $(M + Na)^+$.

*2-(4-Chlorophenyl)-4-((4-phenyl-1H-1,2,3-triazol-1-yl)methyl)thiazole (**8i**)* Yield: 72 %; m.p.: 138 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 5.75 (s, 2H), 7.26 (s, 1H), 7.34 (d, J = 7.3 Hz, 2H), 7.42-7.44 (m, 3H), 7.85 (d, J = 8.0 Hz, 2H), 7.88 (d, J = 7.3 Hz, 2H), 8.00 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 50.0, 117.9, 120.1, 125.8, 127.8, 128.2, 128.9, 129.3, 130.5, 131.6, 136.5, 148.2, 150.8, 168.1; HRMS: 353.0630 $(M + H)^+$, 375.0448 $(M + Na)^+$.

*2-(4-Chlorophenyl)-4-((4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl)methyl)thiazole (**8j**)* Yield: 66 %; m.p.: 120 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 5.73 (s, 2H), 7.08 (t, J = 8.6 Hz, 2H), 7.25 (s, 1H), 7.34 (d, J = 8.7 Hz, 2H), 7.76–7.78 (m, 2H), 7.88 (d, J = 8.7 Hz, 2H), 7.91 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 50.0,115.7, 115.9, 118.0, 119.8, 126.8, 127.5, 127.5, 127.8, 129.3, 131.5, 136.6, 147.3, 150.7, 161.7, 163.7, 168.1; HRMS: 371.0534 $(M + H)^+$, 393.0352 $(M + Na)^+$.

*2-(4-Chlorophenyl)-4-((4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)methyl)thiazole (**8k**)* Yield: 76 %; m.p.: 122 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 3.83 (s, 3H), 5.74 (s, 2H), 6.96 (d, J = 8.9 Hz, 2H), 7.25 (s, 1H), 7.3 (d, J = 8.1 Hz, 2H), 7.77 (d, J = 8.9 Hz, 2H), 7.88 (d, J = 8.1 Hz, 2H), 7.91 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 49.9, 55.3, 114.3, 117.8, 119.3, 123.2, 127.1, 127.8, 129.3, 131.6, 136.5, 148.0, 150.9, 159.7, 168.0; HRMS: 383.0735 $(M + H)^+$, 405.0553 $(M + Na)^+$.

*2-(4-Chlorophenyl)-4-((4-(3,4-dimethoxyphenyl)-1H-1,2,3-triazol-1-yl)methyl)thiazole (**8l**)* Yield: 80 %; m.p.: 120 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 3.89 (s, 3H), 3.94 (s, 3H), 5.72 (s, 2H), 6.88 (d, J = 8.3 Hz, 1H), 7.25 (s, 1H),7.27 (dd, J = 8.3 Hz and 1.5 Hz, 2H), 7.40 (d, J = 8.5 Hz, 2H), 7.47 (d, J = 1.5 Hz, 1H), 7.85 (d, J = 8.5 Hz, 2H), 7.92 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 49.9, 55.9, 56.0, 109.0, 111.3, 117.9, 118.2, 119.5, 123.5, 127.8, 129.3, 131.5, 136.5, 148.0, 149.1, 149.3, 150.8, 168.0; HRMS: 413.0840 $(M + H)^+$, 435.0658 $(M + Na)^+$.

*2-(4-Fluorophenyl)-4-((4-phenyl-1H-1,2,3-triazol-1-yl)methyl)thiazole (**8m**)* Yield: 70 %; m.p.: 96 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 5.75 (s, 2H),7.16 (dd, J = 12.6 and 4.7 Hz, 2H), 7.24 (s, 1H), 7.37–7.31 (m, 1H), 7.43 (m, 2H), 7.85 (m, 2H), 7.97–7.91 (m, 2H), 8.01 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 50.0, 116.1, 116.3, 117.6, 120.1, 125.8, 128.2, 128.5, 128.6, 128.9, 129.5, 130.5, 148.2, 150.6,163.1, 165.1, 168.2; HRMS: 373.0930$(M + H)^+$, 359.0748 $(M + Na)^+$.

*2-(4-Fluorophenyl)-4-((4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl)methyl)thiazole (**8n**)* Yield: 66 %; m.p.: 154 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 5.74 (s, 2H), 7.13–7.15 (m, 4H), 7.26 (s, 1H), 7.84–7.78 (m, 2H), 7.95–7.91 (m, 2H), 7.96 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 50.0, 115.7, 115.9, 116.1, 116.3, 117.7, 119.8, 126.8, 127.5, 127.5, 128.5, 128.6, 129.4, 147.3, 150.5, 161.7, 163.1, 163.7,165.1, 168.3; HRMS: 355.0831 $(M + H)^+$, 377.0649 $(M + Na)^+$.

*2-(4-Fluorophenyl)-4-((4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)methyl)thiazole (**8o**)* Yield: 74 %; m.p.: 118 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 3.84 (s, 3H), 5.73 (s, 2H), 6.98–6.94 (m, 2H), 7.15 (t, J = 8.6 Hz, 2H), 7.23 (s, 1H), 7.79–7.75 (m, 2H), 7.91 (s, 1H), 7.96–7.92 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 49.9, 55.3, 114.2, 116.1, 116.3, 117.6, 119.3, 123.3, 127.1, 128.5, 128.6, 129.5, 148.0, 150.8, 159.64, 163.1, 165.1, 168.2; HRMS: 367.1031 $(M + H)^+$, 389.0849 $(M + Na)^+$.

*4-((4-(3,4-Dimethoxyphenyl)-1H-1,2,3-triazol-1-yl)methyl)-2-(4-fluorophenyl)thiazole (**8p**)* Yield: 76 %; m.p.: 136 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H ppm = 3.91 (s, 3H) 3.96 (s, 3H), 5.74 (s, 2H), 6.91 (d, J = 8.3 Hz, 1H), 7.15 (t, J = 8.6 Hz, 2H), 7.23 (s, 1H), 7.29 (t, J = 4.1 Hz, 2H), 7.50 (d, J = 1.8 Hz, 1H), 7.97–7.90 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ_C ppm = 50.0, 55.9, 56.0, 109.0, 111.3, 116.1, 116.3, 117.6, 118.2, 119.5, 123.5, 128.5, 128.6, 129.4, 148.1, 149.1, 149.3, 150.7, 163.1, 165.1, 168.2; HRMS: 397.1136 $(M + H)^+$, 419.0954 $(M + Na)^+$.

Experimental protocol for biological activity

Antitubercular activity

All synthesized compounds were screened for their in vitro activity against *M. tuberculosis* H37Ra (ATCC 25177) and *M. bovis* BCG (ATCC 35743) using twofold dilution technique to determine the actual minimum inhibitory concentration (MIC). Activity against MTB was determined through XTT reduction menadione assay (XRMA), reading absorbance at 470 nm as per the protocol described in literature [60–64]. Nitrate reductase (NR) assay was performed to estimate inhibition of *M. bovis* BCG [60–64], measuring absorbance at 540 nm. In vitro activity against MTB and *M. bovis* BCG at dormant (12 days) stage was performed using the XRMA and NR assay, respectively, as described above. Percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = [(\text{control} - \text{CMP})/(\text{control} - \text{blank})] \times 100,$$

where "control" is the activity of mycobacteria without compounds, "CMP" is the activity of mycobacteria in presence of compound, and "blank" is the activity of culture medium without mycobacteria.

Cytotoxicity

To check the selectivity, selected 2-aryl-4-((4-aryl-1*H*-1,2,3-triazol-1-yl)methyl)thiazole derivatives (**8a**–**p**) were assayed for their cytotoxic effects in two different cell lines (HeLa, HCT) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [65, 66]. The cell lines were maintained under standard cell culture conditions under 5 % CO_2 at 37 °C in 95 % air humidified environment. Each concentration was tested in duplicates in a single experiment.

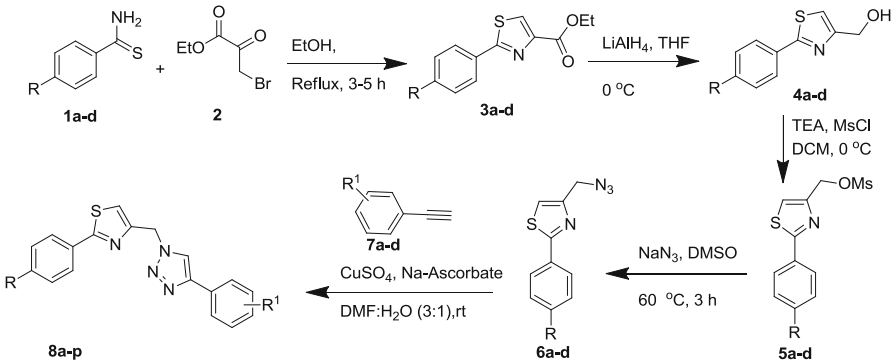
Antibacterial activity

All bacterial cultures were first grown in Luria–Bertani medium at 37 °C at 180 rpm. Once the culture reached 1 OD, it was used for antibacterial assay. Bacterial strains *Escherichia coli* (NCIM 2576), *Pseudomonas flurescence* (NCIM 2059) as Gram-negative and *Staphylococcus aureus* (NCIM 2602) and *Bacillus subtilis* (NCIM 2162) as Gram-positive were obtained from NCIM (NCL, Pune) and grown in Luria–Bertani medium from HiMedia, India. The assay was performed in 96-well plates after 8 and 12 h for Gram-negative and Gram-positive bacteria, respectively. Screening was carried out using 0.1 % of 1-OD culture at 620 nm. Inoculated culture was added into each well of 96-well plate containing the compounds to be tested. Optical density for each plate was measured at 620 nm after 8 h for Gram-negative bacteria and after 12 h for Gram-positive bacteria.

Results and discussion

A series of 2-aryl-4-((4-aryl-1*H*-1,2,3-triazol-1-yl)methyl)thiazole derivatives (**8a–p**) were synthesized according to Scheme 1. Ethyl 3-bromo-2-oxopropanoate **2** on cyclocondensation reaction with aryl thioamides **1a–d** gave ethyl 2-arylthiazole-4-carboxylates **3a–d**. Ester **3a–d** on reduction with lithium aluminum hydride in tetrahydrofuran (THF) gave (2-arylthiazol-4-yl)methanol **4a–d**; subsequent reaction with methanesulfonyl chloride and triethylamine in DCM furnished (2-phenylthiazol-4-yl)methyl methanesulfonate **5a–d**, which on reaction with sodium azide in DMSO gave 4-(azidomethyl)-2-arylthiazole **6a–d**. Azide **6a–d**, on click reaction with substituted alkynes **7a–d**, furnished target compounds 2-aryl-4-((4-aryl-1*H*-1,2,3-triazol-1-yl)methyl)thiazole **8a–p**.

The structure of the title compounds **8a–p** was confirmed by NMR and high-resolution mass spectrometry (HRMS). As a representative analysis of compound 4-((4-(4-fluorophenyl)-1*H*-1,2,3-triazol-1-yl)methyl)-2-phenylthiazole (**8b**), the ^{1}H NMR spectrum of compound **8b** displayed one singlet in aliphatic region at δ 5.75 (thiazole-CH_2-triazole). A triplet at δ 7.11 and multiplet at δ 7.80–7.83 were attributed to protons of fluoro-substituted phenyl ring, while multiplet at δ 7.44–7.49 and δ 7.92–7.97 corresponds to protons of phenyl ring. Thiazole and triazole protons resonated as singlet at δ 7.26 and 7.98, respectively. The ^{13}C NMR spectrum of compound **8b** showed one signal of thiazole-CH_2-N carbon at δ 50.0. Aromatic carbons of fluoro-substituted phenyl showed typical fluoro-coupling [$\underline{C}_1$–F δ 163.66, 161.69 ($^{1}J = 248$ Hz), $\underline{C}_2$-F δ 115.89, 115.72 ($^{2}J = 21.42$ Hz), $\underline{C}_3$-F δ 127.54, 127.48 ($^{3}J = 7.56$ Hz)]. Structure of compound **8b** was further confirmed by HRMS, which showed molecular ion peak at *m/z* 337.0930 $(M + H)^+$, 359.0748 $(M + Na)^+$. Structures of all derivatives were ascertained similarly.



Comp	R	R^1	Comp	R	R^1	Comp	R	R^1	Comp	R	R^1
8a	H	H	**8e**	Br	H	**8i**	Cl	H	**8m**	F	H
8b	H	4-F	**8f**	Br	4-F	**8j**	Cl	4-F	**8n**	F	4-F
8c	H	4-OMe	**8g**	Br	4-OMe	**8k**	Cl	4-OMe	**8o**	F	4-OMe
8d	H	3,4-OMe	**8h**	Br	4-Me	**8l**	Cl	3,4-OMe	**8p**	F	4-CH_3

Scheme 1 Synthetic route for 2-aryl-4-((4-aryl-1*H*-1,2,3-triazol-1-yl)methyl)thiazoles **8a**–**p**

Biological evaluation

Antitubercular activity

The antitubercular activity of each synthesized compound was determined by measuring inhibition of growth against a virulent dormant-stage strain of *M. tuberculosis* H37Ra (MTB, ATCC 25177) and *M. bovis* (BCG, ATCC 35743) in liquid medium. In preliminary screening, the antimycobacterial activity of these compounds was assessed at concentrations of 30, 10, and 3 μg/mL using first-line antitubercular drug rifampicin as reference standard. In vitro activity studies against dormant stage of MTB and *M. bovis* BCG were performed using XRMA [60–63] and NR assay [60–63], respectively. The results of antitubercular activity are reported in Table 1.

The in vitro antitubercular assay against *M. tuberculosis* H37Ra and *M. bovis* BCG revealed that compounds **8a**–**c**, **f**–**h**, **m** (MIC 0.03–2.47 μg/mL) exhibited good activity against dormant-stage BCG strain. The preliminary structure–activity relationship study revealed that replacement of hydrogen atom of phenyl ring A and B (Fig. 1) by substituent groups such as Br, Cl, F, OMe, and CH_3 affected the antitubercular activity.

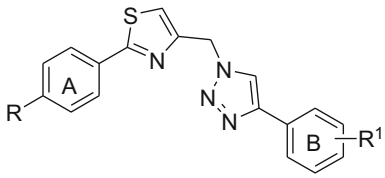
Further, it was also noted that, among the compounds **8a**–**d** with unsubstituted phenyl ring A and substituted phenyl ring B, compound **8a** (R^1 = H) showed good activity against *M. tuberculosis* H37Ra (MIC 9.28 μg/mL) and *M. bovis* (MIC 0.88 μg/mL), compound **8b** (R^1 = 4-F) exhibited good activity against H37Ra (MIC 8.99 μg/mL) and *M. bovis* (MIC 1.69 μg/mL), whereas compound **8c** (R^1 = 4-OMe) showed good activity against *M. bovis* (MIC 1.37 μg/mL). Among the compounds **8e**–**h** with 4-bromo-substituted phenyl ring A and substituted phenyl ring B, compound **8f** (R^1 = 4-F) and **8h** (R^1 = 3,4-OMe) showed excellent activity

Table 1 Antitubercular (dormant stage) activity (MIC in μg/mL) of compounds **8a–p**

Compound	*M. tuberculosis* H37Ra		*M. bovis* BCG	
	IC_{50}	MIC_{90}	IC_{50}	MIC_{90}
8a	1.4	9.28	0.24	0.88
8b	0.92	8.99	0.15	1.69
8c	>30	>30	0.08	1.37
8d	>30	>30	30	>30
8e	22.01	>30	>30	>30
8f	18.33	>30	0.05	0.67
8g	>30	>30	1.51	2.25
8h	4.85	>30	0.03	0.35
8i	16.88	>30	>30	>30
8j	23.16	>30	>30	>30
8k	>30	>30	>30	>30
8l	12.62	>30	>30	>30
8m	>30	>30	0.25	2.47
8n	25.52	>30	>30	>30
8o	>30	>30	>30	>30
8p	>30	>30	>30	>30
Rifampicin	0.85	0.015	0.77	0.021

MIC90 minimum inhibitory concentration, *IC50* 50 % inhibitory concentration

Fig. 1 Compounds **8a–p**



against *M. bovis* (MIC 0367 and 0.35 μg/mL respectively) and **8g** (R^1 = 4-OMe) exhibited good activity with MIC of 2.25 μg/mL Among the compounds **8i–l** with 4-chloro-substituted phenyl ring A and substituted phenyl ring B, all compounds were found less active. From the compounds **8m–p** with 4-fluoro-substituted phenyl ring A and substituted phenyl ring B, compound **8m** exhibited good activity against *M. bovis* (MIC 2.47 μg/mL), whereas compounds **8n–p** were found less active. It was notable that compounds with unsubstituted or 4-bromo-substituted phenyl ring A and substituted phenyl ring B showed good antitubercular activity against BCG.

Compounds **8a–p** were further evaluated against two human cancer cell lines (HeLa and HCT116) and against primary human umbilical vein endothelial cells (HUVECs) to check the toxicity of these compounds (Table S-2). All active compounds were relatively nontoxic up to 100 μg/mL against HUVECs; the results of cytotoxicity screening are presented in Table 2.

According to the study on drug susceptibility of TB, the antimycobacterial activity was considered to be specific for selectivity index >10. The selectivity

Table 2 In vitro cytotoxicity and selectivity index (SI) of selected compounds against cancer cells

Entry	HeLa cells (cervix)		SI on HeLA		HCT116 (colorectal)		SI on HCT 116		HUVECs (primary cells)		SI on HUVECs	
	GI_{50} (μg/mL)	GI_{90} (μg/mL)	Against H37Ra	Against BCG	GI_{50} (μg/mL)	GI_{90} (μg/mL)	Against H37Ra	Against BCG	GI_{50} (μg/mL)	GI_{90} (μg/mL)	Against H37Ra	Against BCG
8a	2.99	>100	0	3	12.9	>100	1	15	>100	>100	>11	>114
8b	16.11	>100	2	10	15.14	>100	2	9	>100	>100	>11	>59
8c	5.76	>100	0	4	>100	>100	>3	>73	>100	>100	>3	>73
8f	26.21	>100	1	39	>100	>100	>3	>149	>100	>100	>3	>149
8g	18.8	>100	1	8	9.36	>100	0	4	>100	>100	>3	>44
8h	27.78	>100	1	79	9.16	>100	0	26	>100	>100	3	>286
8m	7.84	>100	0	3	9.26	>100	0	4	>100	>100	>3	40
[a]Rifampicin	>100	>100	>118	>130	>100	>100	>118	>130	>100	>100	>118	>130
[b]Paclitaxel 0.0055	0.083	–	–	0.1400	5.89	–	–	0.1279	5.715	–	–	

HeLa Homo sapiens cervix adenocarcinoma, *HCT116 Homo sapiens* colon colorectal carcinoma, *HUVECs* human umbilical vein endothelial cells, *M. tuberculosis* H37Ra and *M. bovis* BCG

[a]Positive control for antitubercular activity

[b]Positive control for cytotoxicity

index (SI) was calculated by dividing GI_{50} for cell lines (HeLa, HCT 116, and HUVEC) by the MIC for in vitro activity against dormant MTB and *M. bovis* BCG. For dormant state of *M. bovis* BCG, all compounds showed SI higher than >40 against primary HUVEC cells (Table 2).

Cytotoxic activity evaluation

The results of this evaluation are presented in Table 2.

Antibacterial activity

The antibacterial activity of synthesized compounds was determined against standard Gram-negative bacteria *E. coli* (NCIM 2576) and *P. flurescence* (NCIM 2059) and Gram-positive bacteria *S. aureus* (NCIM 2602) and *B. subtilis* (NCIM 2162). Ampicillin served as positive control for antibacterial activity [64]. The in vitro preliminary screening values (% inhibition) against the tested microorganisms are summarized in Table S-2. The antibacterial activity results are reported as minimum inhibitory concentration in Table 3.

Analysis of the antibacterial activity results presented in Table 3 provides some lead molecules with good antibacterial activity. Among the compounds **8a–p** tested, it was observed that the synthesized compounds showed moderate to good activity. It is worthwhile to mention that compounds **8h** (R = Br, $R^1 = CH_3$) and **8j** (R = Cl, R^1 = F) exhibited good activity with MIC values of 4.5 to 20.2 μg/mL

Table 3 Antibacterial activity in MIC_{90} (μg/mL) of compounds **8a–p**

Comp.	*E. coli*	*P. flurescence*	*S. aureus*	*B. subtilis*
8a	>30	>30	>30	>30
8b	>30	>30	>30	>30
8c	>30	>30	>30	>30
8d	>30	>30	>30	>30
8e	>30	>30	>30	>30
8f	>30	>30	>30	>30
8g	>30	>30	>30	>30
8h	**9.5**	**7.2**	**6.7**	**12.2**
8i	>30	>30	>30	>30
8j	**4.5**	**5.4**	**20.2**	**12.5**
8k	>30	>30	>30	>30
8l	>30	>30	**25.6**	>30
8m	>30	>30	>30	>30
8n	>30	>30	>30	>30
8o	**23.3**	>30	**28.5**	>30
8p	>30	>30	>30	>30
Ampicillin	1.46	4.36	1.0	10.32
Kanamycin	1.62	0.49	>30	1.35

against all tested strains. Compounds **8l** (R = Cl, $R^1 = CH_3$) and **8o** (R = F, $R^1 = OCH_3$) showed good antibacterial activity against *S. aureus* with MIC of 25.6 and 28.5 μg/mL, respectively, while compound **8o** also exhibited good activity against *E. coli* with MIC of 23.3 μg/mL. Thus, it is concluded that compounds with R = Br, $R^1 = CH_3$ and R = Cl, R^1 = F group showed good antibacterial activity.

Conclusions

We describe synthesis and biological screening of 2-aryl-4-((4-aryl-1*H*-1,2,3-triazol-1-yl)methyl)thiazole derivatives **8a**–**p**. Most of the synthesized compounds with unsubstituted or 4-bromo-substituted phenyl ring at 2-position of thiazole and substituted phenyl ring at 4-position of 1,2,3-triazole showed good antitubercular activity against *M. bovis*. Most of the synthesized compounds exhibited good antibacterial activity. These results warrant synthesis of similar libraries with other substituents to confirm the trend described herein.

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