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REGULAR ARTICLE

Synthesis of some novel piperidine fused 5-thioxo-1*H*-1,2,4-triazoles as potential antimicrobial and antitubercular agents

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Abstract. A novel series of analogues based on 5-(1-(4-chloro-3-methoxyphenyl)piperidin-4-yl)-4-phenyl-2*H*-1,2,4-triazole-3(4*H*)-thione core have been synthesized and their potential as antibacterial, antifungal and antitubercular agents was examined. The structure-activity relationship (SAR) studies of these derivatives **5** (**a–k**) clearly indicate the vital role of lipophilicity as a major factor in enhancing the biological activity of these compounds. Among the compounds screened, **5a**, **5c**, **5d**, **5j** and **5k** displayed significant activity against *Mycobacterium tuberculosis* H37Rv strain.

Keywords. Piperidine; Triazole; Antibacterial activity; Antimycobacterial.

1. Introduction

Microorganisms like bacteria, fungi and viruses have been identified to pose a severe threat and global health hazards around the world.[1] Despite the development of several anti-microbial agents, multidrug resistance developed by these microorganisms demands the need for new therapeutics to target these disease-causing agents.[2] Hence there is a continuous need to discover new drugs with good antimicrobial potency, particularly against the drug-resistant strains.[3] Tuberculosis (TB) is a disease caused by bacteria called *Mycobacterium tuberculosis* and is globally considered as one of the severe life-threatening diseases after Acquired Immunodeficiency Syndrome (AIDS).[4] This bacterium usually targets the lungs but can also cause severe damage to other parts

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of the body. The reports from world health organization (WHO) indicates that till 2016, TB has resulted in more than 1.4 million deaths and 10.4 million clinical cases.[5] Over the past five decades, researchers across the globe have laid all their hard work for the development of new anti-TB agents. Despite these efforts, only a few compounds have entered the human trials. This has enforced the use of a number of second-line anti-TB agents such as cycloserine and ethionamide to treat TB infected patients. It is noteworthy that sirturo (bedaquiline/TMC207) and delamanid have received approval for using against *MDR-TB* recently. However, taking into account their adverse side-effects, these drugs are only recommended to *MDR-TB* patients who don't respond to any other treatment options.[6] All the aforementioned facts demanded a vital requirement to develop new anti-TB agents with an exceptional mechanism of action, high potency, well-tolerance, low toxicity and short therapy duration profiles.[7]

The presence of heterocyclic framework in biologically active molecules highlights the significance of synthesizing novel compounds by incorporating these architectures for developing new anti-tubercular agents. Fully decorated heterocyclic architectures involving derivatives of pyrrole,[8] benzimidazole,[9] indole,[10] imidazole,[11] furan,[12] and benzotriazole,[13] are reported to show excellent antimycobacterial properties. Among these frameworks, triazole derivatives are of immense potential because of their wide spectrum of biological activities.[14–18] These vital pharmacophores can allow better binding interactions with the target proteins and additionally can display excellent stability profiles. Accordingly, our efforts were focused on the synthesis and preliminary pharmacological screening of various triazole molecules, in particular, 1,2,4- triazoles.

As a part of our continued research work on the synthesis of potentially active molecules for medicinal chemistry screening,[19–24] and for the development of new antimicrobial and antitubercular agents,[25–29] it was planned to introduce piperidine fused 5-thioxo-1*H*-1,2,4-triazole molecule using electronically diverse aryl groups. A detailed literature survey revealed the fact that the synthesis of such fused 1,2,4-triazole derivatives containing thioxo group requires the use of semicarbazides or carbon disulphide in hydrazine.[30,31] Moreover, the need for harsh reaction conditions enlightened us the necessity of developing a facile and efficient protocol for the synthesis of these pharmacologically relevant molecules. In the modern era of organic synthesis, microwave technology has played a prominent role in functionalizing, decorating or constructing any heterocyclic architecture.[22,23] Microwave-assisted technology allows rapid and facile construction of a potential heterocyclic core with excellent yields. Accordingly, we utilized microwave irradiation for the synthesis of targeted 1,2,4-triazole derivatives.

In our successful trials, the parent core 1-(1-(4-chloro-3-methoxyphenyl)piperidine-4-carbonyl)-4-phenylthiosemicarbazides **4(a–k)** was initially synthesized and screened for the antimicrobial studies. Surprisingly, most of the compounds failed to show any promising activity. Conversely, 1,2,4-triazole fused molecules **5(a–k)**, obtained after the cyclization of intermediates **4(a–k)** under microwave irradiation displayed enhanced activity. In this article, we describe the design, synthesis and evaluation of antimicrobial and antitubercular activities of a series of novel piperidine fused 5-thioxo-1*H*-1,2,4-triazole derivatives **5(a–k)**. We have also focused on substantiating our in vitro screening results by explaining the structure-activity relationships (SAR) studies.

2. Experimental

2.1 *Materials and methods*

All reagents were purchased from Aldrich. Solvents used were extra dried. Final purifications were carried out using Quad biotage Flash purifier (A Dyax Corp. Co.). Microwave-assisted syntheses were performed in Biotage initiator. Thin Layer Chromatography (TLC) experiments were performed on alumina backed silica gel 40 F254 plates (Merck, Darmstadt, Germany). The plates were illuminated under UV (254 nm) and molesybidinic acid. All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-300 (300.12 MHz) and AM-400 (400.13 MHz), Bruker Biospin Corp., Germany. Molecular weights of unknown compounds were checked by LCMS 6200 series Agilent Technology. Chemical shifts are reported in ppm (d) with reference to internal standard TMS. The signals are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet.

2.2 *General procedure for the synthesis of derivatives*

2.2a *Procedure for the preparation of 1-(4-chloro-3-methoxyphenyl)piperidine-4-carbohydrazide (**2**)*: A mixture of ethyl 1-phenylpiperidine-4-carboxylate (0.01 mole) in ethanol (20 mL) and hydrazine hydrate (0.01 mole) were refluxed for 3 h. The reaction progress was monitored by TLC. Upon completion of the reaction, the reaction mixture was cooled to ambient temperature, poured to ice cold water to obtain a solid precipitate. The

separated solid was filtered under vacuum, dried and recrystallized in ethanol to afford (**2**) as white solid in 90% yield. M.p. 124–126 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.95–2.00 (m, 4H), 2.52–2.59 (m, 1H), 3.23 (m, 2H), 3.59–3.62 (m, 2H), 3.87 (s, 3H), 7.00 (m, 1H), 7.20 (m, 1H), 7.42 (d, 1H, *J* = 8.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.2, 154.7, 149.5, 130.7, 113.4, 109.3, 98.4, 54.2, 54.6, 42.6, 28.8; LCMS (ESI-MS) m/z = 284.1 (M+1); Anal. Calculated for $C_{13}H_{11}ClN_3O_2$: C, 55.03; H, 6.39; N, 14.81 Found: C, 55.02; H, 6.40; N, 14.82.

2.2b *General procedure for the Synthesis of 1-(1-(4-chloro-3-methoxyphenyl)piperidine-4-carbonyl)-4-phenylthiosemicarbazide **4(a–k)***: To an oven-dried flask, 1-(4-chloro-3-methoxyphenyl)piperidine-4-carbohydrazide (**2**) (0.01 mole) and DIPEA (0.015 mole) were added in THF (20 mL) and stirred for about 5 min. Substituted isothiocyanatobenzene (**3**) (0.01 mole) was then added and allowed to stir at RT for 2 h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the reaction mixture was quenched with ice and the resulting solution extracted with Ethyl acetate, (25 mL x 2) washed with brine solution. Then the organic phase was dried over anhydrous Na_2SO_4 and evaporated under vacuum, the obtained crude product was further purified by flash chromatography on silica gel (230–400 mesh) using ethylacetate (40–50%) in petroleum ether as eluent to afford the entitled compounds **4** (**a–k**).

2.2.2a *2-(1-(4-chloro-3-methoxyphenyl)piperidine-4-carbonyl)-N-(3,5-dichlorophenyl)hydrazinecarbothioamide **(4a)*** White solid; M.p. 135–137 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.64–1.72 (m, 2H), 1.88–1.91 (m, 2H), 2.40–2.43 (m, 1H), 2.73 (t, 2H, *J* = 11.2 Hz), 3.76–3.79 (m, 2H), 3.83 (s, 3H), 6.52 (dd, 1H, *J* = 2.8, 8.8 Hz), 6.66 (d, 1H, *J* = 2.8 Hz), 7.18 (d, 1H, *J* = 8.8 Hz), 7.37 (s, 1H), 7.68 (s, 2H), 9.96 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 181.2, 172.7, 149.2, 140.0, 139.5, 138.6, 137.3, 130.6, 130.1, 128.7, 125.3, 119.8, 117.8, 116.3, 61.8, 50.8, 43.6, 30.0; LCMS (ESI-MS) m/z = 487.0 (M+1); Anal. Calculated for $C_{20}H_{21}Cl_3N_4O_2S$: C, 49.24; H, 4.34; N, 11.48; Found: C, 49.26; H, 4.36; N, 11.46%.

2.2.2b *2-(1-(4-chloro-3-methoxyphenyl)piperidine-4-carbonyl)-N-(3-fluorophenyl)hydrazinecarbothioamide **(4b)*** White solid; M.p. 127–129 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.64–1.73 (m, 2H), 1.87–1.90 (m, 2H), 2.40–2.46 (m, 1H), 2.67–2.75 (m, 2H), 3.76–3.82 (m, 2H), 3.83 (s, 3H), 6.52 (dd, 1H, *J* = 2.4, 8.8 Hz), 6.66 (d, 1H, *J* = 2.4 Hz), 7.18 (m, 1H), 7.29 (d, 1H, J = 8.8 Hz), 7.35 (m, 1H), 7.39 (m, 1H), 7.52 (m, 1H), 9.67 (bs, 1H), 9.94 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 179.4, 172.8, 162.8, 154.2, 147.6, 137.2, 130.6, 122.3, 114.2, 111.4, 109.6, 99.2, 54.2, 52.4, 42.6, 28.8; LCMS (ESI-MS) m/z = 437.0 (M+1); Elemental analysis for $C_{20}H_{22}ClFN_4O_2S$ Theoretical: C, 54.98; H, 5.08; N, 12.82; Found: C, 54.99; H, 5.10: N, 12.80.

2.2.2c *2-(1-(4-chloro-3-methoxyphenyl)piperidine-4-carbonyl)-N-(3-(trifluoromethyl)phenyl)hydrazinecarbothioamide **(4c)*** Off white solid; M.p. 128–130 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.64–1.73 (m, 2H), 1.88–1.91 (m, 2H), 2.44 (m, 1H), 2.73 (t, 2H, *J* = 11.2 Hz), 3.79 (d, 2H, *J* = 12.0 Hz), 3.83 (s, 3H), 6.52 (dd, 1H, *J* = 2.4, 8.8 Hz), 6.66 (d, 1H, *J* = 2.4 Hz), 7.14 (d, 1H, *J* = 8.8 Hz), 7.70 (d, 2H, *J* = 8.8 Hz), 7.78 (d, 2H, *J* = 8.0 Hz), 9.80 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.8, 172.3, 149.4, 141.4, 139.9, 131.1, 130.4, 130.1, 129.6, 125.3, 124.0, 119.1, 117.7, 116.3, 61.8, 50.8, 43.6, 29.2; LCMS (ESI-MS) m/z = 487.0 (M+1); Elemental analysis for $C_{21}H_{22}ClF_3N_4O_2S$ Theoretical: C, 51.80; H, 4.55; N, 11.51; Found: C, 51.82; H, 4.56: N, 11.50.

2.2.2d *2-(1-(4-chloro-3-methoxyphenyl)piperidine-4-carbonyl)-N-(4-cyanophenyl)hydrazinecarbothioamide **(4d)**:* White solid; M.p. 147–149 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.67–1.72 (m, 2H), 1.87–1.90 (m, 2H), 2.44 (m, 1H), 2.76 (t, 2H, *J* = 11.6 Hz), 3.79 (d, 2H, *J* = 12.4 Hz), 3.83 (s, 3H), 6.52 (dd, 1H, *J* = 2.8, 8.8 Hz), 6.66 (d, 1H, *J* = 2.4 Hz), 7.18 (d, 1H, *J* = 8.8 Hz), 7.77–7.82 (m, 4H), 9.72–10.04 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.4, 172.5, 149.3, 139.5, 137.4, 133.9, 130.5, 125.9, 118.5, 116.8, 106.5, 61.5, 50.2, 43.6, 30.1; LCMS (ESI-MS) m/z = 444.0 (M+1); Elemental analysis for $C_{21}H_{22}ClN_5O_2S$ Theoretical: C, 56.81; H, 4.99; N, 15.78; Found: C, 56.82; H, 4.98: N, 15.78.

2.2.2e *2-(1-(4-chloro-3-methoxyphenyl)piperidine-4-carbonyl)-N-(3-methoxyphenyl)hydrazinecarbothioamide **(4e)*** Off white solid; M.p. 123–124 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.64–1.72 (m, 2H), 1.87–1.90 (m, 2H), 2.40–2.46 (m, 1H), 2.76 (t, 2H, *J* = 12.0 Hz), 3.79 (m, 2H), 3.83 (s, 3H), 6.52 (dd, 1H, *J* = 2.8, 8.8 Hz), 6.66 (d, 1H, *J* = 2.4 Hz), 6.74 (d, 1H, *J* = 6.8 Hz), 7.03 (dd, 1H, *J* = 1.2, 8.0 Hz), 7.17 (d, 1H, *J* = 8.8 Hz), 7.21–7.25 (m, 3H), 9.52 (bs, 1H), 9.90 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.7, 172.5, 160.9, 149.3, 140.4, 139.5, 130.6, 130.8, 125.9, 119.2, 116.5, 108.6, 106.2, 61.8, 55.6, 50.4, 43.6, 30.1; LCMS (ESI-MS) m/z = 449.0 (M+1); Elemental analysis for $C_{21}H_{25}ClN_4O_3S$ Theoretical: C, 56.18; H, 5.61; N, 12.48; Found: C, 56.20; H, 5.62: N, 12.46.

2.2.2f *2-(1-(4-chloro-3-methoxyphenyl)piperidine-4-carbonyl)-N-(2-cyanophenyl)hydrazinecarbothioamide **(4f)*** Pale yellow solid; M.p. 141–143 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.89–2.00 (m, 2H), 2.15 (m, 2H), 2.92–2.99 (m, 2H), 3.14–3.19 (m, 1H), 3.82 (m, 2H), 3.84 (s, 3H), 6.57 (dd, 1H, *J* = 2.8, 8.8 Hz), 6.70 (s, 1H), 7.20 (d, 1H, *J* = 8.8 Hz), 7.53 (dd, 1H, *J* = 2.4, 8.0 Hz), 7.76–7.78 (m, 1H), 7.80 (m, 1H), 8.22 (dd, 1H, *J* = 8.0 Hz), 13.94 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.6, 172.5, 149.2, 141.1, 139.5, 132.8, 131.5, 130.6, 130.1, 125.3, 121.9, 117.7, 116.3, 97.2, 61.8, 50.4, 43.6, 30.1; LCMS (ESI-MS) m/z = 444.0 (M+1); Elemental analysis for $C_{21}H_{22}ClN_5O_2S$ Theoretical: C, 56.81; H, 4.99; N, 15.78; Found: C, 56.80; H, 5.00: N, 15.76.

2.2.2g *2-(1-(4-chloro-3-methoxyphenyl)piperidine-4-carbonyl)-N-(3-cyanophenyl)hydrazinecarbothioamide (**4g**)* White solid; M.p. 150–152 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.64–1.72 (m, 2H), 1.88–1.91 (m, 2H), 2.40–2.46 (m, 1H), 2.72 (t, 2H, *J* = 12.0 Hz), 3.79 (d, 2H, *J* = 12.4 Hz), 3.83 (s, 3H), 6.52 (dd, 1H, *J* = 2.8, 8.8 Hz), 6.66 (d, 1H, *J* = 2.4 Hz), 7.18 (d, 1H, *J* = 8.8 Hz), 7.53–7.61 (m, 2H), 7.82 (d, 1H, *J* = 8.0 Hz), 7.96 (bs, 1H), 9.80 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 181.2, 172.7, 149.2, 140.0, 139.5, 130.6, 130.8, 125.3, 124.6, 123.7, 119.1, 118.4, 116.3, 112.3, 61.8, 50.2, 43.6, 30.4; LCMS (ESI-MS) m/z = 444.0 (M+1); Elemental analysis for $C_{21}H_{22}ClN_5O_2S$ Theoretical: C, 56.81; H, 4.99; N, 15.78; Found: C, 56.82; H, 5.01: N, 15.78.

2.2.2h *2-(1-(4-chloro-3-methoxyphenyl)piperidine-4-carbonyl)-N-(4-nitrophenyl)hydrazinecarbothioamide (**4h**)* Off white solid; M.p. 173–175 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.63–1.74 (m, 2H), 1.88–1.92 (m, 2H), 2.40–2.47 (m, 1H), 2.72 (t, 2H, *J* = 11.2 Hz), 3.80 (d, 2H, *J* = 10.5 Hz), 3.82 (s, 3H), 6.52 (dd, 1H, *J* = 2.4, 8.7 Hz), 6.66 (d, 1H, *J* = 2.4 Hz), 7.18 (dd, 1H, *J* = 3.6, 8.7 Hz), 7.62 (t, 2H, *J* = 8.1 Hz), 7.99 (bs, 1H), 8.47 (bs, 1H), 9.88 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.8, 172.2, 148.2, 140.6, 138.2, 137.6, 130.6, 130.1, 125.3, 118.5, 117.9, 116.3, 61.8, 50.1, 43.1, 30.1; LCMS (ESI-MS) m/z = 464.0 (M+1); Elemental analysis for $C_{20}H_{22}ClN_5O_4S$ Theoretical: C, 51.78; H, 4.78; N, 15.10; Found: C, 51.79; H, 4.80: N, 15.12.

2.2.2j *2-(1-(4-chloro-3-methoxyphenyl)piperidine-4-carbonyl)-N-(3-chlorophenyl)hydrazinecarbothioamide (**4j**)* Pale orange solid; M.p. 134–135°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.61–1.72 (m, 2H), 1.87–1.91 (m, 2H), 2.38–2.46 (m, 1H), 2.71 (t, 2H, *J* = 12.0 Hz), 3.75–3.82 (m, 2H), 3.82 (s, 3H), 6.52 (dd, 1H, *J* = 2.7, 9.0 Hz), 6.66 (d, 1H, *J* = 2.4 Hz), 7.15–7.22 (m, 2H), 7.42 (t, 2H, *J* = 8.1 Hz), 7.63 (bs, 1H), 9.70 (bs, 1H), 9.95 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 181.0, 172.4, 149.5, 139.8, 130.4,, 30.8, 129.2, 125.6, 124.4, 123.3, 116.4, 61.5, 50.6, 43.6, 30.5; LCMS (ESI-MS) m/z = 453.0 (M+1); Elemental analysis for $C_{20}H_{22}Cl_2N_4O_2S$ Theoretical: C, 52.98; H, 4.89; N, 12.36; Found: C, 52.96; H, 4.90: N, 12.38.

2.2.2k *2-(1-(4-chloro-3-methoxyphenyl)piperidine-4-carbonyl)-N-(4-chlorophenyl)hydrazinecarbothioamide (**4k**)* Off white solid; M.p. 120–122 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.61–1.71 (m, 2H), 1.87–1.92 (m, 2H), 2.39–2.46 (m, 1H), 2.72 (t, 2H, *J* = 11.1 Hz), 3.75–3.79 (d, 2H, *J* = 12.6 Hz), 3.82 (s, 3H), 6.51 (dd, 1H, *J* = 2.7, 9.0 Hz), 6.65 (d, 1H, *J* = 2.4 Hz), 7.18 (d, 1H, *J* = 9.0 Hz), 7.37–7.40 (m, 2H), 7.49 (m, 2H), 9.63 (bs, 2H), 9.99 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 181.1, 172.7, 149.2, 141.1, 139.5, 130.6, 130.8, 129.4, 125.5, 123.3, 122.8, 121.5, 116.5, 61.8, 50.4, 43.6, 30.4; LCMS (ESI-MS) m/z = 453.0 (M+1); Elemental analysis for $C_{20}H_{22}Cl_2N_4O_2S$ Theoretical: C, 52.98; H, 4.89; N, 12.36; Found: C, 52.97; H, 4.90: N, 12.39.

2.2c *General procedure for the Synthesis of 5-(1-(4-chloro-3-methoxyphenyl)piperidin-4-yl)-4-phenyl-2H-1,2,4-triazole-3(4H)-thione derivatives **5(a–k)***: The substituted phenylthiosemicarbazides **4**(**a–k**) (100 mg) and 2 mL of 2% NaOH solution was taken in 8 mL vials and irradiated in a Biotage Microwave Oven at 100°C for 5-10 min. The reaction progress was monitored by TLC. Upon completion of the reaction, the reaction mixture was cooled to ambient temperature and quenched with water and the precipitated solid was filtered, dried and then crystallized in methanol to obtain the titled compounds **5**(**a–k**).

2.2.3a *5-(1-(4-chloro-3-methoxyphenyl)piperidin-4-yl)-4-(3,5-dichlorophenyl)-2H-1,2,4-triazole-3(4H)-thione (**5a**)* White solid; M.p. 123–125 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.67–1.79 (m, 4H), 2.63–2.71 (m, 3H), 3.66–3.67 (m, 2H), 3.79 (s, 3H), 6.48 (dd, 1H, *J* = 3.6, 12.0 Hz), 6.60 (d, 1H, *J* = 3.6 Hz), 7.12 (d, 1H, *J* = 12 Hz), 7.65 (s, 1H), 7.80 (s, 1H), 13.82 (s,br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.1, 154.9, 152.8, 150.7, 144.7, 135.1, 128.8, 125.3, 123.0, 121.2, 118.3, 100.9, 56.7, 50.7, 40.9, 30.0; LCMS (ESI-MS) m/z = 469.0 (M+1); Anal. Calculated for $C_{20}H_{19}Cl_3N_4OS$: C, 51.13; H, 4.08; N, 11.93 Found: C, 51.10; H, 4.16; N, 11.95 %.

2.2.3b *5-(1-(4-chloro-3-methoxyphenyl)piperidin-4-yl)-4-(3-fluorophenyl)-2H-1,2,4-triazole-3(4H)-thione (**5b**)* Off white solid; M.p. 112–114 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.65–1.80 (m, 4H), 2.49–2.68 (m, 3H), 3.69–3.69 (m, 2H), 3.79 (s, 3H), 6.47 (dd, 1H, *J* = 3.6, 12.0 Hz), 6.60 (d, 1H, *J* = 3.6 Hz), 7.15 (d, 1H, *J* = 12 Hz), 7.32–7.56 (m, 2H), 7.62–7.73 (m, 1H), 13.80 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.8, 155.3, 151.4, 150.4, 145.2, 134.1, 130.2, 127.2, 124.6, 121.1, 119.8, 102.1, 56.2, 50.4, 41.0, 30.2; LCMS (ESI-MS) m/z = 419.0 (M+1); Elemental analysis Theoretical: C, 57.34; H, 4.81; N, 13.37; Found: C, 57.32; H, 4.82: N, 13.38.

2.2.3c *5-(1-(4-chloro-3-methoxyphenyl)piperidin-4-yl)-4-(3-(trifluoromethyl)phenyl)-2H-1,2,4-triazole-3(4H)-thione (**5c**)* White solid; M.p. 126–128 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.67–1.78 (m, 4H), 2.57–2.66 (m, 3H), 3.64–3.68 (m, 2H), 3.79 (s, 3H), 6.45 (dd, 1H, *J*=3.6 Hz, 12.6 Hz), 6.59 (d, 1H, *J* = 3.6 Hz), 7.12–7.15 (d, 1H, *J* = 12 Hz), 7.75 (d, 2H, *J* = 11.2 Hz), 7.96–7.99 (d, 2H, *J* = 11.2 Hz), 13.84 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.2, 154.9, 152.8, 150.7, 144.7, 131.4, 129.8, 128.4, 125.9, 124.0, 123.9, 122.1, 117.7, 112.1, 100.9, 56.7, 50.6, 40.5, 30.1; LCMS (ESI-MS) m/z = 469.2 (M+1); Elemental analysis Theoretical: C, 53.79; H, 4.30; N, 11.95; Found C, 53.78; H, 4.32; N, 11.94.

2.2.3d *4-(3-(1-(4-chloro-3-methoxyphenyl)piperidin-4-yl)-5-thioxo-1H-1,2,4-triazol-4(5H)-yl)benzonitrile (**5d**)* Off white solid; M.p. 144–146 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.78 (m, 4H), 2.71–2.72 (m, 3H), 3.62–3.66 (m, 3H), 3.79 (s, 3H), 6.67 (dd, 1H, *J* = 3.6, 12.0 Hz), 7.16–7.19 (d, 1H, *J* = 11.6 Hz), 7.87–7.92 (m, 1H), 8.00–8.03 (m, 1H),

8.40–8.44 (m, 1H), 8.49–8.50 (m, 1H), 13.89 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.2, 155.1, 153.8, 149.4, 144.7, 133.9, 131.7, 128.6, 125.6, 123.4, 118.2, 114.6, 113.6, 106.7, 100.6, 56.9, 50.4, 40.9, 30.4; LC/MS (ESI-MS)m/z = 426.0 (M+1); Anal. Calculated for $C_{21}H_{20}ClN_5OS$: C, 59.22; H, 4.73; N, 16.44; Found: C, 59.24; H, 4.74; N, 16.42.

2.2.3e *5-(1-(4-chloro-3-methoxyphenyl)piperidin-4-yl)-4-(3-methoxyphenyl)-2H-1,2,4-triazole-3(4H)-thione* ***(5e)*** Off white solid; M.p. 132–133 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.68–1.78 (m, 4H), 2.58–2.64 (m, 3H), 3.69 (d, 3H, *J* = 12 Hz), 3.80 (s, 6H), 6.46 (dd, 1H, *J* = 2.4, 8.8 Hz), 6.60 (d, 1H, *J* = 2.4 Hz), 6.93 (d, 1H, *J* = 8.0 Hz), 6.97 (s, 1H), 7.06 (dd, 1H, *J* = 2.4, 8.0 Hz), 7.14 (d, 1H, *J* = 8.8 Hz), 7.42–7.46 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 29.43, 33.12, 48.59, 55.89, 56.26, 101.42, 108.83, 110.72, 114.65, 114.92, 121.01, 130.02, 130.32, 136.94, 151.71, 154.35, 155.32, 160.05, 167.81; LC/MS (ESI-MS) m/z = 431.0 (M+1); Anal. Calculated for $C_{21}H_{23}ClN_4O_2S$: C, 58.53; H, 5.38; N, 13.00; Found: C, 58.54; H, 5.40; N, 12.98.

2.2.3f *2-(3-(1-(4-chloro-3-methoxyphenyl)piperidin-4-yl)-5-thioxo-1H-1,2,4-triazol-4(5H)-yl)benzonitrile* ***(5f)*** Brown solid; M.p. 149–151 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.67–1.80 (m, 4H), 2.59–2.69 (m, 3H), 3.69 (d, 3H, *J* = 12 Hz), 3.80 (s, 3H), 6.47 (dd, 1H, *J* = 2.4, 8.8 Hz), 6.60 (d, 1H, *J* = 2.4 Hz), 700–7.14 (m 4H), 7.47–4.51 (m, 1H), 13.69 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 30.67, 35.87, 48.98, 5.30, 56.26, 101.35, 108.83, 110.57, 114.54, 122.76, 123.33, 124.46, 130.09, 130.24, 144.46, 149.58, 151.94, 155.40, 166.49, 167.05; LC/MS (ESI-MS) m/z = 426.0 (M+1); Anal. Calculated for $C_{21}H_{20}ClN_5OS$: C, 59.22; H, 4.73; N, 16.44; Found: C, 59.24; H, 4.71; N, 16.46.

2.2.2g *3-(3-(1-(4-chloro-3-methoxyphenyl)piperidin-4-yl)-5-thioxo-1H-1,2,4-triazol-4(5H)-yl)benzonitrile* ***(5g)*** Off white solid; M.p. 144–146 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.69–1.78 (m, 4H), 2.58–2.66 (m, 3H), 3.69 (d, 2H, *J* = 20.0 Hz), 3.79 (s, 3H), 6.46 (dd, 1H, *J* = 3.2, 12.0 Hz), 6.59 (d, 1H, *J* = 3.2 Hz), 7.14 (d, 1H, *J* = 12.0 Hz), 7.32 (d, 1H, *J* = 10.0 Hz), 7.36–7.45 (m, 2H), 7.56–7.65 (m, 1H), 13.78 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.1, 154.1, 152.8, 150.7, 144.7, 128.8, 127.1, 125.3, 124.1, 123.0, 122.1, 118.4, 116.3, 111.4, 100.9, 56.7, 50.6, 40.9, 30.2; LC/MS (ESI-MS) m/z = 426.0 (M+1); Anal. Calculated for $C_{21}H_{20}ClN_5OS$: C, 59.22; H, 4.73; N, 16.44; Found: C, 59.27; H, 4.74; N, 16.47.

2.2.2h *5-(1-(4-chloro-3-methoxyphenyl)piperidin-4-yl)-4-(4-nitrophenyl)-2H-1,2,4-triazole-3(4H)-thione* ***(5h)*** Pale yellow solid; M.p. 170–173 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.78–1.81 (m, 4H), 2.67–2.73 (m, 3H), 3.66 (d, 2H, *J* = 12.0 Hz), 3.78 (s, 3H), 6.51 (s, 1H), 6.65 (s, 1H), 7.17 (d, 1H, *J* = 8.0 Hz), 7.90 (m, 1H), 8.01–8.03 (m, 1H), 8.40–8.43 (m, 1H), 8.48–8.93 (m, 1H), 13.88 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.6, 153.6, 150.8, 149.9, 144.9, 141.4, 129.6, 126.6, 123.0, 117.6, 117.9, 100.9, 56.7, 50.2, 40.9, 30.4; LC/MS (ESI-MS) m/z = 446.0 (M+1); Elemental analysis Theoretical: C, 53.87; H, 4.52; N, 15.71; Found C, 53.88; H, 4.54; N, 15.72.

2.2.2i *3-(1-(4-chloro-3-methoxyphenyl)piperidin-4-yl)-4-phenyl-1H-1,2,4-triazole-5(4H)-thione* ***(5i)*** Yellow solid; M.p. 131–133 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.70–1.77 (m, 4H), 2.54–2.66 (m, 3H), 3.68 (d, 2H, *J* = 16.0 Hz), 3.78 (s, 3H), 6.46 (dd, 2H, *J* = 3.2, 12.0 Hz), 6.58 (d, 1H, *J* = 3.6 Hz), 7.14 (d, 1H, *J* = 12.0), 7.75 (d, 2H, *J* = 10.8 Hz), 7.99 (d, 2H, *J* = 10.8 Hz), 13.84 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.1, 155.5, 153.4, 150.7, 144.3, 129.5, 129.2, 128.8, 125.3, 124.7, 123.0, 122.1, 122.1, 100.9, 56.7, 50.4, 40.9, 30.0; LC/MS (ESI-MS) m/z = 401.1 (M+1); Elemental analysis Theoretical: C, 59.91; H, 5.28; N, 13.97; Found C, 59.92; H, 5.30; N, 13.96.

2.2.2j *5-(1-(4-chloro-3-methoxyphenyl)piperidin-4-yl)-4-(3-chlorophenyl)-2H-1,2,4-triazole-3(4H)-thione* ***(5j)*** White solid; M.p. 125–127 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.74–1.80 (m, 4H), 2.59–2.64 (m, 3H), 3.68 (d, 2H, *J* = 12.0 Hz), 3.79 (s, 3H), 6.46 (dd, 1H, *J* = 2.8, 8.8 Hz), 6.60 (d, 1H, *J* = 2.8 Hz), 7.14 (d, 1H, *J* = 8.8 Hz), 7.33–7.36 (m, 2H), 7.54–7.56 (m, 2H), 13.80 (br, s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.0, 153.5, 152.8, 150.7, 144.0, 134.6, 130.2, 128.6, 125.6, 123.4, 123.0, 122.1, 118.3, 100.9, 56.7, 50.4, 40.5, 30.5; LC/MS (ESI-MS) m/z = 435.0 (M+1); Elemental analysis Theoretical: C, 55.17; H, 4.63 N, 12.87; O, 3.67; Found C, 55.18; H, 4.63 N, 12.86; O, 3.68.

2.2.2k *5-(1-(4-chloro-3-methoxyphenyl)piperidin-4-yl)-4-(4-chlorophenyl)-2H-1,2,4-triazole-3(4H)-thione* ***(5k)*** Off white solid; M.p. 136–138 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.68–1.73 (m, 4H), 2.61–2.51 (m, 3H), 3.66–3.70 (d, 2H, *J* = 16 Hz), 3.79 (s, 3H), 6.44–6.47 (m, 1H), 6.59–6.60 (d, 1H, *J* = 4 Hz), 7.13–7.15 (d, 1H, *J* = 8 Hz), 7.48–7.50 (m, 1H), 7.51–7.64 (m, 2H), 7.65–7.79 (m, 1H),13.80 (br, s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.8, 155.5, 151.8, 150.7, 143.9, 129.2, 128.1, 127.8, 125.6, 125.3, 123.0, 100.9, 56.7, 50.8, 40.9, 30.5; LC/MS (ESI-MS) m/z = 435.0 (M+1); Elemental analysis Theoretical: C, 55.17; H, 4.63; N, 12.87; O, 3.67; Found C, 55.16; H, 4.65; N, 12.88.

2.3 *Antibacterial studies*

The synthesized compounds were screened for their antibacterial activity against *E. coli* (ATCC-25922), *S. aureus* (ATCC-25923) and *K. pneumoniae* (ATCC-BBA-1705) bacterial strains by serial plate dilution method. Serial dilutions of the drug in Muellere Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16–18 h. at 37 °C. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth. A number of

antimicrobial discs are placed on the agar for the sole purpose of producing zone of inhibition in the bacterial lawn. Twenty millilitres of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 °C for an hour. Using an agar punch, wells were made on these seeded agar plates and minimum inhibitory concentration of the test compounds in dimethyl sulfoxide (DMSO) were added into each labeled well. A control was also prepared for the plates, in the same way, using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37 °C for 3–4 days. Antibacterial activity was determined by measuring the diameter of the inhibition zone. The activity of each compound was compared with ciprofloxacin as standard. MIC (μg/mL) and Zone of inhibition (mm) was determined for all the synthesized compounds.

2.4 *Antifungal studies*

Newly prepared compounds were screened for their antifungal activity against *A. flavus* (NCIM No. 524), *Rhizopus. sp* and *C. albicans* in DMSO by serial plate dilution method. Sabouraud agar media was prepared by dissolving peptone (1 g), D-Glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal stain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty millilitres of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 °C for 1 h. Using a punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37 °C for 3–4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. The activity of each compound was compared with Amphotericin B as standard. MIC (μg/mL) and Zone of inhibition (mm) were determined for all the synthesized compounds.

2.5 *Antituberculosis study*

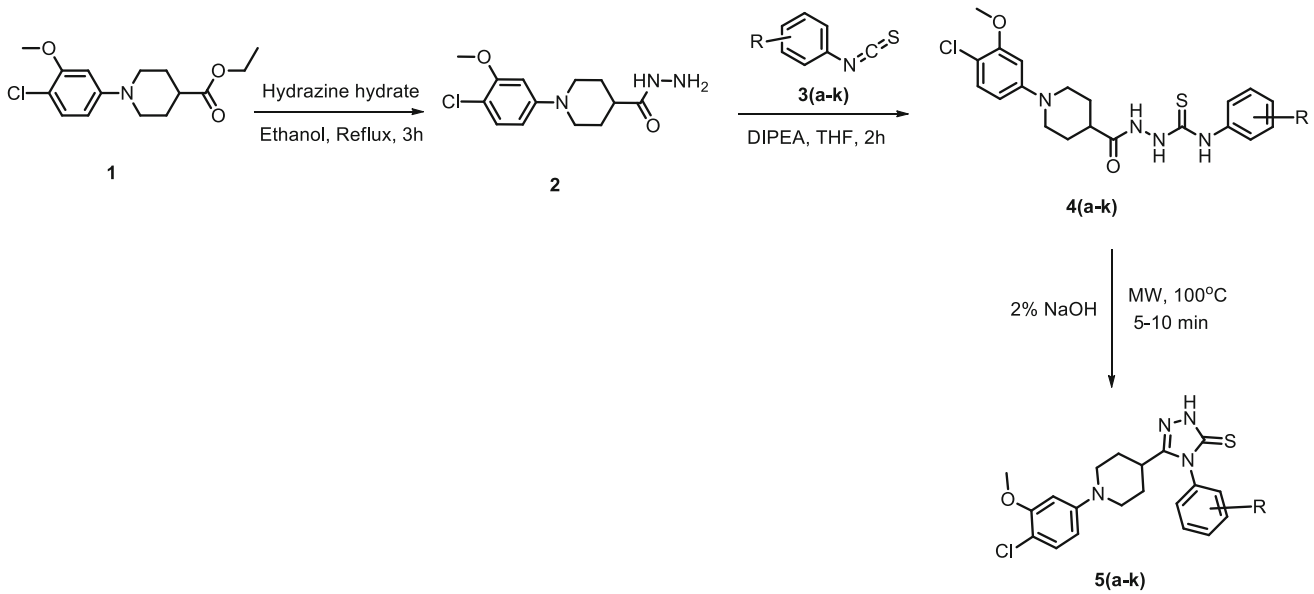
The compounds were screened for their in vitro antimycobacterial activity against *M. tuberculosis H37Rv* ATCC 27294 and non-tubercular mycobacterial (NTM) species like *M. smegmatis* (MC2) ATCC 19420, *M. fortuitum* ATCC 19542 and *MDR-TB* strains by Resazurin Assay method and their MIC values were determined. The standard drugs, *viz.,* isoniazid and rifampicin were used for comparison. *M. tuberculosis* strains were grown in Middlebrook 7H9 broth (Difco BBL, Sparks, MD, USA) supplemented with 10% OADC (Becton Dickinson, Sparks, MD, USA). The culture was diluted to McFarland 2 standard with the same medium. From this, 50 mL of the culture was added to 150 mL of fresh medium in 96 well microtitre plates. Stock solutions (2 mg/mL) of the test compounds were prepared in N,N-Dimethyl formamide (DMF). The compounds were tested at 1, 10 and 100 μg/mL concentrations. Further, the second level testing was carried out at 0.3125, 0.625, 1.25, 2.5 and 5 μg/mL concentrations. Control tubes had the same volumes of DMF without any substrate. Rifampicin and isoniazid were used as the reference compounds. After incubating at 37 °C for 7 days, 20 mL of 0.01% Resazurin (Sigma, St. Louis. MO, USA) in water was added to each tube. Resazurin, a redox dye, is blue in the oxidized state and turns pink when reduced by the growth of viable cells. The control tubes showed a color change from blue to pink after 1 h. at 37 °C. Compounds which prevented the change of colour of the dye were considered to be inhibitory to the tested bacteria.

3. Results and Discussion

3.1 *Chemistry*

The synthetic route adopted for getting the target compounds has been detailed in Scheme 1. The intermediate **1** was converted into 1-(4-chloro-3-methoxyphenyl)piperidine-4-carbohydrazide (**2**) by refluxing a mixture of ethyl-1-phenylpiperidine-4-carboxylate (**1**) and hydrazine hydrate in ethanol. The intermediates **4(a–k)** were prepared by the condensation reaction between 1-(4-chloro-3-methoxyphenyl)piperidine-4-carbohydrazide (**2**) and various substituted 1-isothiocyanatobenzene **3(a–k)** that was obtained by a previously reported method.[32] The targeted final derivatives **5(a–k)** were synthesized by the base mediated cyclization of intermediates **4(a–k)** under microwave irradiation. The newly synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and LC-MS analysis.

The formation of intermediate **2** was confirmed by the presence of a singlet peak at δ 8.17 ppm (–NH) and the disappearance of $–CH_2$ and $–CH_3$ peaks at δ 4.22 ppm and δ 1.18 ppm respectively. The formation of **4(a–k)** was confirmed by its ^{1}H NMR and LCMS spectra. The formation of the target molecule was confirmed by its NMR spectrum and was also evidenced by LCMS spectral data. The detailed experimental procedures and spectral data for all the synthesized compounds are given in supplementary data. The newly synthesized target molecules **4(a–k)** and **5(a–k)** along with their isolated yields are detailed in Table 1.



Scheme 1. Synthesis of target compounds **5**(**a–k**).

3.2 *Structure activity relationships*

Our initial studies was aimed at the synthesis of semicarbazide analogues **4**(**a–k**) and their subsequent diversification at **region 2** of 5-(1-(4-chloro-3-methoxyphenyl)piperidin-4-yl)-4-phenyl-2*H*-1,2,4-triazole-3(4*H*)-thione derivatives **5**(**a–k**) (Figure 1).

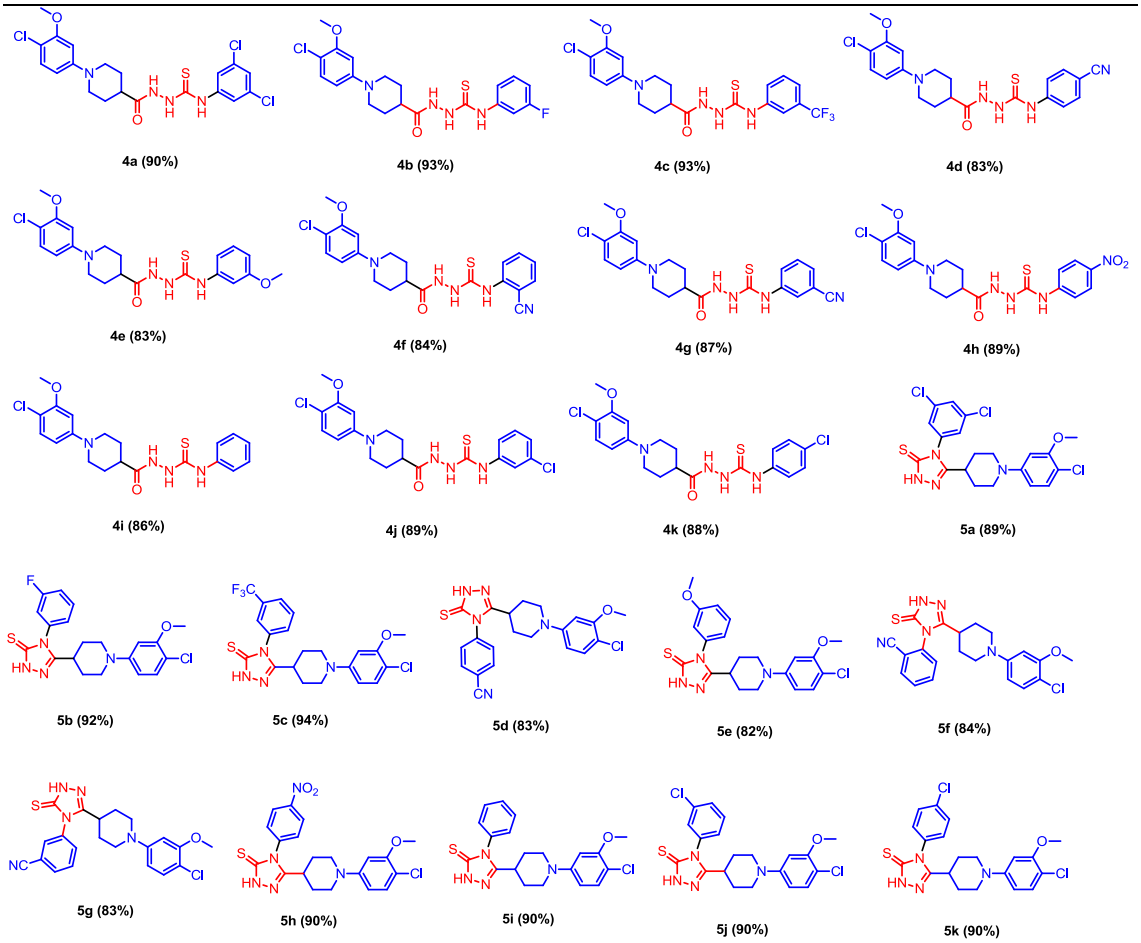
Initial screening data of the newly synthesized molecules for antibacterial activity displayed a reasonable activity for compound **4k**. Surprisingly, the compound **5k** displayed enhanced activity when compared to the corresponding semicarbazide. This could be attributed to the enhancement in lipophilicity which increases the cell permeability. Additionally, the formation of triazole molecule could have possibly increased the binding affinity. Encouraged by these results, we synthesized a new series of triazole derivatives and explored their potential as antimicrobial and antitubercular agents. Different substitutions were attempted at the **region 1** and **region 2** of the synthesized molecules with the intention of increasing the activity profile of the target molecules. Our initial diversification was focused on **region 2** and the biological results showed that the compounds **5a**, **5c**, **5k** and **5j** displayed good and comparable activities with the standard drugs. One of the major reasons for this superior activity could be the presence of halogen group attached to the phenyl ring whereas when $-CF_3$ group is attached to the ring, it is presumed to increase the lipophilic nature of the compound, thereby making the molecule more cell-permeable. The presence of electron-donating or withdrawing groups on the aryl ring is known to play a beneficial effect on the activity of these types of molecules. Accordingly, our efforts were focused on attaching groups with varied electronic properties on the aryl rings as a part of our late-stage diversification. The biological results indicated that the compound with the electron-withdrawing group at the phenyl ring of the **region 2** were more active. It is evident that the biological activity decreased when an electron-donating group was attached to the ring (**5e**). It has been observed that the introduction of electron-withdrawing substitutions at the ring increases the lipophilicity of the molecule, thereby making the molecule more cell-permeable and hence enhance the potency of the compounds. The SAR studies of the newly synthesized molecules revealed the fact that the electron-donating substitution at the phenyl ring in the **region 2** of these molecules were found to be less active when compared to the electron-withdrawing substituents.

3.3 *Biological results*

All the newly synthesized molecules were tested for their *in vitro* antibacterial, antifungal and antitubercular properties by ensuing standard test methods.

3.3a *Antibacterial activity*: *In vitro* antibacterial activity of the newly synthesized molecules were explored against three different bacterial strains, *Staphylococcus aureus*, (Gram-positive), *Escherichia coli* and *Klebsiella pneumoniae* (Gram-negative) using

Table 1. Synthesized compounds **4**(**a–k**) and **5**(**a–k**).



ciprofloxacin as a reference, by serial dilution method.[33, 34] Table 2 describes the antibacterial screening results (MIC in μg/mL) of synthesized compounds, **4**(**a–k**) and **5**(**a–k**).

After the investigation of antibacterial screening (Table 2), it has been noticed that the newly synthesized compounds exhibited moderate to good inhibition (4-256 μg/mL in DMSO) against the tested two Gram-negative and one Gram-positive bacteria except **4a** against *S. aureus*, **5e** against *E. coli* and **5f** against *K. pneumoniae*. Some of the compounds exhibited significant activity against *S. aureus* as exemplified by compounds **5a**, **5b**, **5c**, **5j** and **5k**. Unexpectedly, the inhibitory activity of the compounds **5k** and **5c** against *S. aureus* exhibited MIC value about one fold times lower than the reference, Ciprofloxacin. Similarly, the inhibitory activity of the compound **5j** and **5c** against *E. coli* was found to be same as that of the reference. The MIC value of the compound **5k** against *K. pneumonia* was found to be about two-fold times lower than the reference whilst the compounds **5a**, **5c** and **5j** possessed inhibitory activity about one fold times lower than Ciprofloxacin.

3.3b *Antifungal activity*: *In vitro* antifungal activity of the synthesized molecules was explored against three different fungal strains, *Candida albicans*, *Aspergillus flavus* and *Rhizopus sp* by serial dilution method using Amphotericin B as the reference.[35,36] Table 2 describes the antifungal screening results (MIC in μg/mL) of synthesized compounds, **4**(**a–k**) and **5**(**a–k**). The investigation of antifungal screening data has shown that only a few of the compounds produced extensive and diverse results. It is deceptive from Table 2 that the compounds **4a** and **5e** failed to exert comparable activity against all the three pathogenic strains at MIC 256 μg/mL. The compound **5k** against *C. albicans* and the compound **5j** against *A. flavus* showed equivalent potency in comparison with the reference, Amphotericin B. The compounds **5j** and **5k** showed MIC value about one

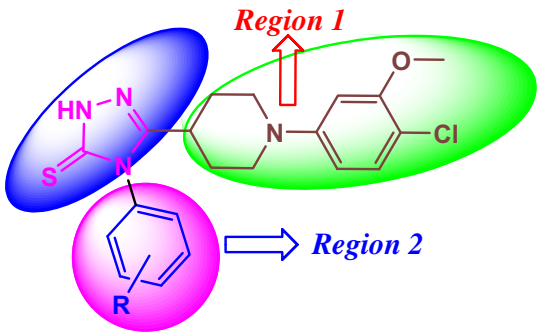


Figure 1. Possible diversification at the target molecules.

fold times lower than the reference against *C. albicans* and *A. flavus* respectively whereas against *Rhizopus sp*, both the compounds displayed MIC value about one fold times lower than the reference.

3.3c *Antitubercular study*: After the evaluation of antimicrobial screening results, it was evident that the pre-final molecules **4(a–k)** do not possess much activity towards the tested microorganisms. On the other hand, after the base mediated cyclization reaction, the formation of the triazole ring enhanced the biological activity of the final molecules **5(a–k)**. Therefore, we selected the final triazole incorporated molecules **5(a–k)** for further antimycobacterial evaluation by Resazurin Assay method using rifampicin and isoniazid as reference standards.[37] Our initial screening results of the in vitro antitubercular activity of the target molecules are summarized in Table 3. The antimycobacterial evaluations were primarily carried out at 1, 10 and 100 µg/mL concentrations against *MDR-TB* strain and other three different TB strains. To our delight, it was

Table 2. Antimicrobial activity data of the synthesized compounds **4(a–k)** and **5(a–k)**.

	Minimum inhibitory concentration (MIC in µg/mL)[a]					
	Bacterial strains			Fungal strains		
Entry	*S. aureus*	*E. coli*	*K. pneumoniae*	*C. albicans*	*A. flavus*	*Rhizopus sp*
Control	00	00	00	00	00	00
4a	>256	256	256	>256	>256	256
4b	256	128	128	64	128	64
4c	128	64	64	64	64	128
4d	64	64	32	64	32	32
4e	32	32	16	16	32	32
4f	64	64	32	64	64	32
4g	64	32	64	32	32	64
4h	16	32	16	32	32	32
4i	64	64	128	64	128	128
4j	128	128	256	128	256	256
4k	128	64	64	64	128	128
5a	16	8	8	16	32	32
5b	16	32	16	32	16	32
5c	4	4	8	8	4	8
5d	64	32	32	64	64	32
5e	256	>256	256	256	256	>256
5f	128	64	>256	128	64	256
5g	64	128	128	128	64	128
5h	32	32	16	32	16	16
5i	64	128	64	64	28	28
5j	8	4	8	4	8	8
5k	4	8	4	8	4	8
Cfn	8	4	16	–	–	–
Am B	–	–	–	8	8	16

[a]MIC values were evaluated at a concentration ranging between 4-256 µg/mL. The figures in the table show the MIC values in µg/mL; MIC (µg/mL) = minimum inhibitory concentration, i.e., lowest concentration to completely inhibit bacterial growth.

Table 3. Antitubercular activity data of the synthesized compounds **5(a–k)**

Compound	Preliminary *in vitro* screening results, MIC(μg/mL)				Second level screening results, MIC(μg/mL)			
	MTB[a]	MS[b]	MF[c]	%[d]	MTB	MS	MF	MDR-TB
5a	10	1	1	95	0.625	1.25	10	6.25
5b	10	1	10	90	–	10	–	50
5c	10	10	10	90	1.25	5	10	6.25
5d	1	1	10	90	0.625	10	10	12.5
5e	>100	>100	>100	0	–	–	–	–
5f	10	10	>100	<90	10	–	–	>50
5g	>100	10	>100	<90	–	–	–	25
5h	>100	>100	>100	0	–	–	–	–
5i	10	10	>100	<90	10	–	10	>50
5j	1	10	10	95	0.625	10	5.0	6.25
5k	1	1	10	90	0.625	10	10	12.5
Isoniazid	0.7	50	12.5	95	0.7	50	12.5	12.5
Rifampicin	0.5	1.5	1.5	95	0.5	1.5	1.5	25

[a]*Mycobacterium tuberculosis* H37Rv.

[b]*Mycobacterium smegmatis* (ATCC 19420).

[c]*Mycobacterium fortuitum* (ATCC 19542).

[d]percentage of inhibition against *M. tuberculosis* H37Rv.

'–' not detected.

identified that some of the molecules were potent at the range of 1 and 10 μg/mL concentrations against *Mycobacterium tuberculosis H37Rv* strain. The more active molecules from the preliminary examination were further subjected to the next level of screening. At this phase, 10 μg/mL concentrations were taken as the cut off and the compounds that were active at 100 μg/mL or more were not considered. In this second level of testing, all the selected compounds were evaluated at 0.3125, 0.625, 1.25, 2.5 and 5.0 μg/mL concentrations. Amongst the tested compounds, **5a**, **5d**, **5j** and **5k** were identified to be potent at 0.625 μg/mL concentrations against *Mycobacterium tuberculosis H37Rv* strain and the target molecule **5c** was found to be active at 1.25 μg/mL concentrations. However, the compound **5a** exhibited significant antitubercular activity against *Mycobacterium smegmatis* (ATCC 19420) at 1.25 μg/mL. It is noteworthy that most of the newly synthesized compounds showed either enhanced activity or comparable activity against *Mycobacterium fortuitum* (ATCC 19542) when compared with the standard drug, isoniazid. Furthermore, the compounds **5a** and **5j** displayed promising antitubercular activity against the *MDR-TB* strain at 6.25 μg/mL. The studies for understanding the mechanism of action of these most active compounds would be done in due course.

4. Conclusions

We herein report the successful design and synthesis of novel derivatives of 5-(1-(4-chloro-3-methoxyphenyl)piperidin-4-yl)-4-phenyl-2*H*-1,2,4-triazole-3(4*H*)-thione as potent antimicrobial and antitubercular agents. The halogenated compounds possess a major role in the enhancement of biological activity because of their lipophilic nature and the methoxy derivatives act as electron donors which could be the plausible reason for its diminished activity. The above-mentioned properties of these pharmacophores could be responsible for the promising activities of the title compounds. The compounds synthesized in this research work can be taken for further derivatization in order to find the lead in these series.

Supplementary Information (SI)

Supplementary information (characterization data such as NMR spectra for the synthesized compounds) associated with this article are available at www.ias.ac.in/chemsci.

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