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REGULAR ARTICLE

Synthesis and biological evaluation of some bicyclic [2-(2,4-dimethylphenylthio)phenyl] aniline and its amide derivatives as potential antitubercular agents

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Abstract. In the present investigation, a series of bicyclic [2-(2,4-dimethylphenylthio)phenyl] aniline analogues were synthesized and characterized by IR, NMR (^{1}H and ^{13}C) and mass spectra. All newly synthesized 15 compounds were inspected for their in vitro antitubercular activity against *Mycobacterium tuberculosis* (MTB) H_{37}Ra in both active and dormant state using an established XTT Reduction Menadione assay (XRMA). The titled compounds exhibited minimum inhibitory concentration (MIC90) ranging from 0.05 to >30 (μg/mL). The potent four compounds were further evaluated in THP-1 infection model where they demonstrated significant antitubercular activity. All the ex vivo active were further evaluated for cytotoxic activity against THP-1, MCK-7 and HeLa cell lines in order to check selectivity index. All compounds were further screened against four different bacteria to assess their selectivity towards MTB. These derivatives could be considered as a precursor structure for further design of antituberculosis agent.

Keywords. [2-(2,4-dimethylphenylthio)phenyl] aniline; antituberculosis activity; cytotoxicity.

1. Introduction

Tuberculosis (TB) is a contagious disease caused by the Mycobacterium tuberculosis (MTB). It is one of the top ten diseases and now ranks alongside Human Immunodeficiency Virus (HIV), as the main cause of death from a single infectious disease.[1] One third of the world population is infected with latent tuberculosis has a major threat to humankind.[2] Patient with latent tuberculosis is in class of high risk may be converted into tuberculosis at a later stage. Majority of TB cases were reported into Asian, African and western pacific regions. Two Asian countries, China and India together accounted 40% of the total TB cases worldwide.[3] Side effects and adverse drug reactions of current anti-TB drugs coupled with combination drug regimens and lengthy treatment duration often complicate the therapy along exploding cost of the treatment.[4] Hence, there is an urgent need to develop potent and cost effective anti-TB drug.

Moreover, the literature reveals that sulfides[5] and amides[6] are extremely attractive and rewarding research targets. Fatty acid amide,[7] pyridine containing amide,[8] glycine containing amide,[9] α, β unsaturated amide,[10] and sulphide containing amide[11] were found to show broad spectrum of biological activity. Amide bond may be prone to hydrolysis *in vivo* due to the presence of plasma amidases, leading to rapid clearance.[12]

Hence, in a search of new compounds sulphide based amide derivatives were synthesized. These derivatives screened for *Mycobacterium tuberculosis* (MTB) and antimicrobial activity. Furthermore, molecular docking studies of most active compounds against enoyl-acyl carrier protein reductase (InhA) enzyme helped in revealing the potential mode of action through their interactions.

2. Experimental

2.1 *Materials and methods*

All reagents, solvents and raw materials are commercially available used without further purification. Melting points

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were determined by open capillary method and are uncorrected. IR spectra (neat in cm^{-1}) were recorded using Perkin Elmer Spectrum-100 analyzer. Spectrophotometer. NMR (^1H and ^{13}C) spectra were recorded in $CDCl_3$ and DMSO-d_6 using a JEOL 400 MHz FT NMR spectrometer; the chemical shifts are reported in δ ppm relative to TMS. The following abbreviations were used for spin multiplicity: s = singlet = doublet, t = triplet, dd = double doublet, m = multiplet, brs = broad singlet. Chemical shifts for ^{13}C NMR reported in ppm relative to the solvent peak. Mass spectrometry was carried out using an Agilent LC/MSD Trap 1100 series. The reaction monitoring and purity of the compounds were checked by thin layer chromatography (TLC). Silica gel coated aluminium sheets (Silica Gel 60 F254). TLC check with hexane/ethyl acetate 9:1.

All the chemicals such as sodium salt XTT and MTT, DMSO, ampicillin and rifampicin were purchased from Sigma-Aldrich, USA. Dubos medium was purchased from DIFCO, USA. Synthesized compounds were dissolved in DMSO and it was used as stock solution (10 mg/mL) for further biological testing.

2.2 *Synthesis*

2.2a *Synthesis of 2, 4 Dimethyl thiophenol* **(2)**: In an oven-dried 3-necked round-bottomed flask, a mixture of 2, 4 dimethyl aniline (**1**, 10 g, 82 mmol) in aqueous HCl (50 mL, 5 N) at 0–5 °C followed by dropwise addition of sodium nitrite (10.5 g, 120 mmol) in water (30 mL). After 1 h, the diazonium reaction mass was added into potassium ethyl xanthate (19.5 g, 120 mmol), in water (50 mL) solution. The mixture was heated to 40–45 °C for 2 h. The compound extracted with MTBE (2 × 50 mL) and washed with 10% NaOH solution (80 mL). Organic layer dried over $CaCl_2$ and evaporated in vacuo to afford 2, 4dimethyl ethyl xanthate (**2a)**. It was further refluxed into ethanolic KOH solution (100 mL in 15 gm) for 4–5 h (TLC check with hexane/ethyl acetate 9:1). The reaction was allowed to cool, quenched with water (400 mL) and evaporated *in vacuo*. After acid and base treatment and vacuum distillation afforded compound **2.** Faint yellow liquid; yield: 8.2 g, 72%; B.p.: 207–209 °C; IR (Neat) υ: 806 (o, p-substituted Ar), 1477 (C=C for Ar) and 2567 (-SH substituted on Ar) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.27 (s, $-CH_3$), 2.30 (s, $-CH_3$), 3.20 (s,-SH), 6.88 (d, J = 8.0 Hz, Ar-H), 6.99 (s, Ar-H) and 7.17 (1H, d, J = 8.0 Hz, Ar-H) ppm; ^{13}C NMR ($CDCl_3$): δ 20.79, 20.97, 126.86, 127.26, 130.27, 131.15, 135.77 and 136.22 ppm; MS (EI): m/z 137agreed for $C_8H_{10}S$ $[M-H]^+$ of compound **2**.

2.2b *Synthesis of (2,4-dimethylphenyl)(2-nitrophenyl) sulfane* **(4)**: A mixture of 2,4-dimethyl thiophenol (**2,** 10 g, 72 mmol), 2-chlro nitrobenzene (**3**, 11.5 g, 72 mmol), and anhydrous potassium carbonate (15 g, 108 mmol) in acetonitrile (80 mL) was stirred and refluxed for 8–10 h (TLC check with hexane/ethyl acetate 9:1). The mixture was allowed to cool and filtered. Recrystallized solid in methanol (50 mL) to afford compound **4.** Bright yellow solid; yield:17 g, 92%; M.p.: 97–98 °C; IR (Neat) υ: 733 (o-substituted Ar), 810 (o, p-substituted Ar), 1332 ($-NO_2$), 1518 ($-NO_2$) and 1567 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.36 (s, $-CH_3$), 2.45 (s, $-CH_3$), 6.76 (dd, J = 8.0 Hz, Ar-H), 7.17 (dd, J = 7.0 Hz, Ar-H), 7.26 (dd, Ar-2H), 7.36 (m, Ar-H), 7.52 (d, J = 7.0 Hz, Ar-H) and 8.31 (dd, J = 8.0 Hz, Ar-H) ppm; ^{13}C NMR ($CDCl_3$): δ 20.34, 21.24, 124.52, 125.91, 126.19, 127.25, 128.35, 132.16, 133.44, 137.12, 139.26, 141.01, 143.06 and 144.85 ppm; MS (EI): m/z calculated for $C_{14}H_{13}NO_2S$ $[M+H]^+$: 260.1; found 260.0.

2.2c *Synthesis of 2-(2,4-dimethylphenylthio)benzenamine* **(5)**: In an oven-dried 3-necked round-bottomed flask, a mixture of (2, 4-dimethylphenyl) (2-nitrophenyl) sulfane (**4**, 15 g, 58 mmol) and Pd/C (wet, 0.75 g, 5%) in methanol (75 mL) was stirred for 6h at 25–30 °C under H_2 atmosphere (1 atm). Reaction monitored on TLC with hexane/ethyl acetate 9:1. After completion, reaction mixture was filtered through a celite pad and filter cake washed with methanol (15 mL). The combined filtrate was evaporated *in vacuo* to furnish compound **5.** Brown semi solid; yield:13 g, 98%; M.p.: 34–36 °C; IR (Neat) υ: 750 (o-substituted Ar), 807 (o, p-substituted Ar), 1608 (C=C), 3369 ($-NH_2$) and 3446 ($-NH_2$) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.26 (s, CH_3), 2.39 (s, CH_3), 4.22 (s, NH_2), 6.69 (d, J = 8.0 Hz, Ar-H), 6.73–6.80 (m, Ar-2H), 6.85 (d, J = 8.0 Hz, Ar-H), 7.00 (s, Ar-H), 7.19–7.23 (m, Ar-H) and 7.36 (dd, J = 7.0 Hz, Ar-H) ppm; ^{13}C NMR ($CDCl_3$): δ 19.98, 20.73, 115.01, 115.24, 118.78, 126.49, 127.26, 130.41, 131.13, 131.68, 135.28, 135.71, 136.55 and 148.13 ppm; MS (EI): m/z calculated for $C_{14}H_{15}NS$ $[M+H]^+$: 230.1; found 230.0.

2.2d *General procedure for the preparation of compound* **(6a-h)**: In an oven-dried two neck round-bottomed flask, a mixture of 2-(2,4-dimethylphenylthio)benzenamine (**5**, 8.7 mmol) and sodium hydroxide (17.5 mmol in 2 mL water) in acetonitrile (10 mL) cooled 0–5 °C. Acetyl chloride (10 mmol in 5 mL) added into the reaction mixture and allowed to warm at room temperature. Stirred the reaction mixture for 1–2 h, for completion (TLC check with hexane/ethyl acetate 8:2) and evaporated *in vacuo*. Reaction mass quenched with water (15 mL) and solid precipitated out. Filtation followed by recrystallization from ethanol afforded desired product (**6a**–**h**).

2.2d1 N-(2-(2,4-dimethylphenylthio)phenyl) acetamide (**6a**): White solid; yield: 95%; M.p.: 42–44 °C; IR (Neat) υ:1575 (C=C), 1678 (C=O), and 3107 (-NH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.08 (s, CH_3), 2.28 (s, CH_3), 2.39 (s, CH_3), 6.72 (d, J = 7.0 Hz, Ar-H), 6.87 (d, J = 7.0 Hz, Ar-H), 7.06 (m, Ar-2H), 7.39 (q, J = 8.0 Hz, Ar-2H), 8.06 (s, NH) and 8.39 (d, J = 8.0 Hz, Ar-H) ppm; ^{13}C NMR ($CDCl_3$): δ 20.16, 20.78, 24.70, 120.93, 121.21, 124.44, 127.66, 128.31, 129.93, 130.51, 131.41, 135.03, 136.66, 139.19, 139.25 and 168.24 ppm; MS (EI): m/z calculated for $C_{16}H_{17}NOS$ $[M+H]^+$: 272.1; found 272.1.

2.2d2 N-(2-(2,4-dimethylphenylthio)phenyl)-2,2,2-trifluoroacetamide (**6b**): White solid; yield: 98%; M.p.: 52–54 °C; IR (Neat) υ: 1576 (C=C), 1651 (C=O), and 3107 (-NH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.28 (s, CH_3), 2.38 (s, CH_3), 6.75 (d, J = 7.0 Hz, Ar-H), 6.88 (d, J = 7.0 Hz, Ar-H), 7.04 (s, Ar-H), 7.22 (t, J = 7.0 Hz, Ar-H), 7.45 (t, J = 7.0 Hz, Ar-H), 7.51 (m, Ar-H), 8.35 (d, J = 8.0 Hz, Ar-H) and 8.95 (s, NH) ppm; ^{13}C NMR ($CDCl_3$): δ 20.16, 20.82, 114.12, 116.99 (CF_3), 121.16, 121.21, 123.5, 126.44, 127.82, 128.94, 129.45, 130.09, 131.64, 135.38, 136.27, 137.31, 137.43 and 154.31, 154.41, 154.6, 154.77 (q, C=O) ppm; MS (EI): m/z calculated for $C_{16}H_{14}F_3NOS$ $[M+H]^+$: 226.1; found 326.0.

2.2d3 N-(2-(2,4-dimethylphenylthio)phenyl) isobutyramide (**6c**): White solid; yield: 90%; M.p.: 38–40 °C; IR (Neat) υ: 1528 (C=C), 1651 (C=O) and 3125 (-NH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.08 (s, CH_3), 1.10 (s, CH_3), 2.26 (s, CH_3), 2.36–2.46 (m, CH_3 and CH), 6.65 (d, J = 8.0 Hz, Ar-H), 6.84 (d, J = 8.0 Hz, Ar-H), 7.02 (s, Ar-H), 7.08 (m, Ar-H), 7.38–7.46 (m, Ar-2H), 8.16 (s, NH) and 8.45 (d, J = 8.0 Hz, Ar-H) ppm; ^{13}C NMR ($CDCl_3$): δ 19.26, 20.10, 20.77, 36.92, 120.77, 124.26, 127.52, 127.69, 130.26, 130.59, 131.35, 135.48, 136.14, 136.37, 139.46, 139.50 and 175.07 ppm; MS (EI): m/z calculated for $C_{17}H_{19}NOS$ $[M+H]^+$: 300.1; found 300.2.

2.2d4 N-(2-(2,4-dimethylphenylthio)phenyl) propionamide (**6d**): White solid; yield: 92%; M.p.: 46–48 °C; IR (Neat) υ: 1575 (C=C), 1678 (C=O) and 3125 (-NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.06 (t, CH_3), 2.20–2.31 (m, CH_3 and CH_2), 6.90 (d, J = 7.0 Hz, Ar-H), 7.0 (d, J = 7.0 Hz, Ar-H), 7.07–7.10 (m, Ar-2H), 7.15 (s, Ar-H), 7.30 (t, J = 8.0 Hz, Ar-H), 7.37 (m, Ar-H), and 9.30 (s, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 9.76, 20.05, 20.77, 29.02, 31.16, 126.03, 127.17, 127.82, 129.58, 130.23, 131.62, 132.83, 135.90, 136.63, 138.04, 139.55, 141.68 and 175.50 ppm; MS (EI): m/z calculated for $C_{17}H_{19}NOS$ $[M+H]^+$: 286.1; found 286.2.

2.2d5 N-(2-(2,4-dimethylphenylthio)phenyl) pentanamide (**6e**): White solid; yield: 90%; M.p.: 56–58 °C; IR (Neat) υ:1651 (C=C), 1678 (C=O) and 3125 (-NH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (t, J = 7.0 Hz, CH_3), 1.27 (m, J = 7.0 Hz, CH_2), 1.52 (m, CH_2), 2.24 (t, CH_2), 2.26 (s, CH_3), 2.39 (s, CH_3), 6.67 (d, J = 8.0 Hz, Ar-H), 6.85 (d, J = 8.0 Hz, Ar-H), 7.02 (s, Ar-H), 7.08 (m, J = 8.0 Hz, Ar-H), 7.38–7.45 (m, Ar-2H), 8.08 (s, NH) and 8.43 (d, J = 8.0 Hz, Ar-H) ppm; ^{13}C NMR ($CDCl_3$): δ 13.69, 20.14, 20.78, 22.20, 27.49, 37.80, 120.84, 124.31, 127.71, 127.78, 130.18, 130.64, 131.39, 131.42, 135.39, 136.32, 136.46, 139.39 and 171.38 ppm; MS (EI): m/z calculated for $C_{19}H_{23}NOS$ $[M+H]^+$: 314.2; found 314.3.

2.2d6 Ethyl(2-(2,4-dimethylphenylthio)phenylcarbamoyl)formate (**6f**): White solid; yield: 90%; M.p.: 50–52 °C; IR (Neat) υ: 1639 (C=C), 1651 (C=O) and 3125 (-NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.29 (t, J = 7.0 Hz, CH_3), 2.25 (s, CH_3), 2.28 (s, CH_3), 4.29 (q, J = 7.0 Hz, CH_2), 6.99 (m, Ar-2H), 7.14 (d, J = 6.0 Hz, Ar-2H), 7.21 (t, J = 8.0 Hz, Ar-H), 7.37 (t, J = 6.0 Hz, Ar-H), 7.78 (d, J = 8.0 Hz, Ar-H) and 10.26 (s, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 13.76, 19.92, 20.49, 62.69, 123.89, 126.75, 127.77, 128.22, 128.37, 129.02, 131.52, 131.60, 132.12, 135.81, 137.65, 138.66, 154.96 and 160.17 ppm; MS (EI): m/z calculated for $C_{18}H_{19}NO_3S$ $[M+H]^+$: 330.1; found 330.1.

2.2d7 N-(2-(2,4-dimethylphenylthio)phenyl) pivalamide (**6g**): White solid; yield: 88%; M.p.: 38–40 °C; IR (Neat) υ: 1575 (C=C), 1651 (C=C), 1678 (C=O), and 3125 (-NH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.11 (s, $3CH_3$), 2.25 (s, CH_3), 2.39 (s, CH_3), 6.56 (d, J = 8.0 Hz, Ar-H), 6.82 (d, J = 8.0 Hz, Ar-H), 7.01 (s, Ar-H), 7.09 (m, J = 7.0 Hz & 1.5 Hz, Ar-H), 7.40–7.44 (m, Ar-H), 7.48 (m, J = 7.0 Hz & 1.5 Hz, Ar-H) and 8.44–8.49 (m, Ar-2H) ppm; ^{13}C NMR ($CDCl_3$): δ 19.96, 20.72, 27.20, 39.97, 120.39, 120.62, 124.18, 126.60, 127.66, 130.45, 130.57, 131.27, 135.54), 135.75, 136.04, 139.64 and 176.64 ppm; MS (EI): m/z calculated for $C_{19}H_{23}NOS$ $[M+H]^+$: 314.4; found 314.3.

2.2d8 N-(2-(2,4-dimethylphenylthio)phenyl) dibenzamide (**6h**): White solid; yield: 95%; M.p.: 154–156 °C; IR (Neat) υ: 1580 (C=C), 1660 (C=O) and 3057 (-NH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.34 (s, $2CH_3$), 6.71 (d, J = 7.0 Hz, Ar-H), 7.03–7.09 (m, Ar-4H), 7.15 (s, Ar-H), 7.33-7.40 (m, Ar-5H), 7.44 (dd, J = 7.0 Hz, Ar-2H) and 7.91 (m, Ar-4H) ppm; ^{13}C NMR ($CDCl_3$): δ = 20.52, 21.10, 126.06, 126.30, 127.61, 127.89, 128.33, 128.73, 129.10, 129.73, 131.85, 132.44, 134.83, 135.91, 136.76, 136.98, 139.69, 142.07 and 173.26 ppm; MS (EI): m/z calculated for $C_{28}H_{23}NO_2S$ $[M+H]^+$: 437.57; found 437.6.

2.2e *General procedure for the preparation of compound* (**6i-o**): To a mixture of 2-(2,4-dimethylphenylthio) benzenamine (**5**, 8.7 mmol) and acid derivative (10 mmol) in DMF (10 mL) at 0–5 °C, was added dropwise coupling agent solution CDI (17.5 mmol in 5 mL DMF) The reaction mass, allowed to warm at room temperature and stirred for an 1 h (TLC check with hexane/ethyl acetate 9:1). Quenched reaction mass with water (15 mL) and filtered to afford desired product (**6i-o**).

2.2e1 N-(2-(2,4-dimethylphenylthio)phenyl)-2-phenylacetamide (**6i**): White solid; yield: 82%; M.p.: 78–80 °C; IR (Neat) υ: 1521 (C=C), 1677 (C=O) and 3245 (-NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.22 (s, CH_3), 2.27 (s, CH_3), 3.67 (s, CH_2), 6.96 (m, Ar-3H), 7.10 (t, J = 7.0 Hz, Ar-H), 7.14 (s, Ar-H), 7.28–7.22 (m, Ar-6H), 7.61 (d, J = 7.0 Hz, Ar-H) and 9.47 (s, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 20.09, 20.75, 42.99, 125.25, 126.21, 126.83, 127.78, 127.89, 128.54, 128.86, 129.26, 129.38, 131.13, 131.74, 131.96, 135.72, 136.93, 137.88, 139.08 and 169.63 ppm; MS (EI): m/z calculated for $C_{22}H_{21}NOS$ $[M+H]^+$: 348.1; found 348.1.

2.2e2 (2-(2,4-dimethylphenylthio)phenylcarbamoyl)(phenyl)methyl acetate (**6j**): White solid; yield: 80%; M.p.:

86–88 °C; IR (Neat) υ: 1516 (C=C), 1578 (C=C), 1693 (C=O), 1744 (C=O) and 3333 (-NH) cm^{-1}; ^1H NMR ($CDCl_3$): δ = 2.06 (s, CH_3), 2.29 (s, CH_3), 2.39 (s, CH_3), 6.12 (s, CH), 6.53 (d, J = 8.0 Hz, Ar-H), 6.83 (d, J = 8.0 Hz, Ar-H), 7.04 (s, Ar-H), 7.12 (m, Ar-H), 7.28 (m, Ar-6H), 7.39–7.44 (m, Ar-H), 7.48 (m, Ar-H), 8.49 (d, J = 8.0 Hz, Ar-H), and 9.09 (s, -NH) ppm; ^{13}C NMR ($CDCl_3$): δ 20.01, 20.59, 20.79, 75.81, 120.43, 125.0, 126.66, 127.26, 127.80, 128.70, 128.97, 130.59, 130.65, 131.39, 135.05, 135.52, 135.95, 136.14, 136.20, 138.91, 166.37 and 168.72 ppm; MS (EI): m/z calculated for $C_{24}H_{23}NO_3S$ $[M+H]^+$: 406.1; found 406.1.

2.2e3 N-(2-(2,4-dimethylphenylthio)phenyl)-5-methylpyrazine-2-carboxamide (**6k**): White solid; yield: 85%; M.p.: 100–102 °C; IR (Neat) υ: 1576 (C=C), 1687 (C=O) and 3289 (-NH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.22 (s, CH_3), 2.46 (s, CH_3), 2.66 (s, CH_3), 6.82 (m, Ar-2H), 6.99 (s, Ar-H), 7.11–7.15 (m, Ar-H), 7.46 (m, Ar-2H), 8.39 (s, Ar-H), 8.63 (d, J = 8.0 Hz, Ar-H), 9.30 (s, Ar-H) and 10.65 (s, -NH) ppm; ^{13}C NMR ($CDCl_3$): δ 20.27, 20.77, 21.80, 120.68, 122.80, 124.73, 127.48, 128.96, 129.85, 130.66, 131.17, 135.32, 136.60, 137.21, 138.55, 141.88, 142.14, 143.41, 157.22 and 161.10 ppm; MS (EI): m/z calculated for $C_{20}H_{19}N_3OS$ $[M+H]^+$: 350.1; found 349.9.

2.2e4 N-(2-(2,4-dimethylphenylthio)phenyl)furan-2-carboxamide (**6l**): White solid; yield: 88%; M.p.: 120–122 °C; IR (Neat) υ: 1578 (C=C), 1651 (C=C), 1675 (C=O), 1702 (C=O), and 3309 (-NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.25 (s, CH_3), 2.28 (s, CH_3), 6.67 (s, Ar-H), 6.96 (s, Ar-H), 7.03 (m, Ar-2H), 7.08 (d, J = 8.0 Hz, Ar-H), 7.15 (t, J = 7.0 Hz, Ar-H), 7.29 (m, Ar-2H), 7.71 (t, J = 8.0 Hz, Ar-H), 7.88 (s, Ar-H) and 9.75 (s, -NH) ppm; ^{13}C NMR (DMSO-d_6): δ 19.92, 20.48, 112.28, 114.94, 125.21, 126.27, 127.67, 127.72, 128.99, 129.87, 131.01, 131.46, 132.24, 136.14, 137.77, 139.12, 145.74, 147.14 and 156.0 ppm; MS (EI): m/z calculated for $C_{18}H_{15}NO_2S$ $[M+H]^+$: 324.1; found 324.3.

2.2e5 2-(2-(2,4-dimethylphenylthio)phenylcarbamoyl) benzoic acid (**6m**): White solid; yield: 87%; M.p.: 126–128 °C; IR (Neat) υ: 1513 (C=C), 1690 (C=O), 1702 (C=O), and 3301 (-NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.24 (s, CH_3), 2.29 (s, CH_3), 6.88 (d, J = 7.0 Hz, Ar-H), 7.03 (d, J = 7.0 Hz, Ar-H), 7.15 (m, Ar-3H), 7.28 (t, J = 6.0 Hz, Ar-H), 7.42 (d, J = 6.0 Hz, Ar-H) 7.56–7.64 (m, Ar-3H), 7.86 (d, J = 7.0 Hz, Ar-H), 9.86 (s, -NH) and 13.06 (s, -COOH) ppm; ^{13}C NMR (DMSO-d_6): δ 20.10, 20.80, 115.3, 118.91, 125.25, 126.21, 126.83, 127.8, 128.7, 128.86, 129.26, 129.38, 131.13, 131.74, 131.96, 135.72, 136.93, 137.88, 139.08, 167.46 and 169.88 ppm; MS (EI): m/z (%) 378 (M^++ 1, 100).

2.2e6 N-(2-(2,4-dimethylphenylthio)phenyl)-3-methylisoxazole-4-carboxamide (**6n**): White solid; yield: 85%; M.p.: 80–82 °C; IR (Neat) υ: 1578 (C=C), 1702 (C=O) and 3028 (-NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.22 (s, CH_3), 2.27 (s, CH_3), 2.65 (s, CH_3), 6.87 (d, J = 7.0 Hz, Ar-H), 7.01 (d, J = 7.0 Hz, Ar-H), 7.14 (s, Ar-H), 7.16–7.19 (m, Ar-2H), 7.26 (t, J = 6.0 Hz, Ar-H), 7.42 (d, J = 6.0 Hz, Ar-H), 8.99 (s, Ar-H) and 9.87 (s, -NH) ppm; ^{13}C NMR (DMSO-d_6): δ 12.02, 20.0, 20.55, 111.53, 126.60, 127.07, 127.61, 127.74, 128.46, 129.12, 131.53, 133.24, 133.70, 135.16, 138.28, 140.06, 149.01, 159.37 and 172.60 ppm; MS (EI): m/z calculated for $C_{19}H_{18}N_2O_2S$ $[M+H]^+$: 339.1; found 339.0.

2.2e7 N-(2-(2,4-dimethylphenylthio)phenyl)-5-chlorothiophene-2-carboxamide (**6o**): White solid; yield: 90%; M.p.: 74–76 °C; IR (Neat) υ: 1536 (C=C), 1633 (C=O) and 3250 (-NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.22 (s, CH_3), 2.26 (s, CH_3), 6.85 (d, J = 7.0 Hz, Ar-H), 7.01 (s, Ar-H), 7.14 (d, J = 7.0 Hz, Ar-H), 7.19 (m, Ar-2H), 7.25 (m, Ar-2H), 7.41 (d, J = 8.0 Hz, Ar-H), 7.86 (d, J = 4.0 Hz, Ar-H) and 10.20 (s, -NH) ppm; ^{13}C NMR (DMSO-d_6): δ 20.03, 20.58, 126.54, 127.24, 127.74, 127.78, 128.21, 128.33, 128.87, 129.14, 131.56, 133.78, 133.85, 133.98, 134.97, 138.42, 140.24, and 159.07 ppm; MS (EI): m/z calculated for $C_{19}H_{16}ClNOS_2[M]^+$: 373.8; found 373.6, 375.5 (Table 1).

3. Results and Discussion

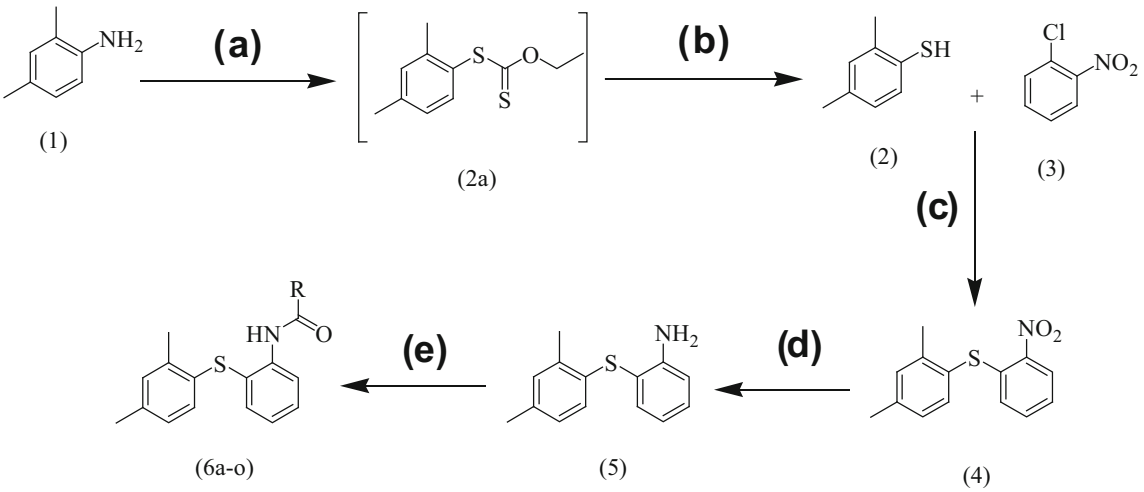
3.1 *Chemistry*

Synthesis of the target compounds **6a–o** were achieved through the straight pathway illustrated in Scheme 1 starting from commercially available 2,4 dimethyl aniline (**1**). In the first step, Leuckart reaction [13,14] allows the preparation of 2, 4 dimethyl thiophenol (**2**). It involved formation of an aryl diazonium salt by using sodium nitrite in acidic condition. Further reaction with potassium alkyl xanthate produced 2, 4 dimethyl aryl xanthate (**2a**), in situ basic hydrolysis produced 2, 4 dimethyl thiophenol (**2**). This compound was purified by vacuum distillation and identity confirmed by spectral data. The IR spectra suggest –SH characteristic band 2567 cm^{-1}, ^1H NMR suggest four singlet at δ 2.27, 2.30, 3.20 and 6.99 belongs to aromatic C-2 and C-4 methyl substitution, D_2O exchangeable –SH and aromatic C-3 respectively. Remaining two δ 6.88 and 7.17 belong to C-5 and C-6 doublet with ortho coupling J = 8.0 Hz.

Further nucleophilic substitution on 2-chloro nitrobenzene (**3**) with 2, 4 dimethyl thiophenol (**2**) yielded (2, 4-Dimethylphenyl) (2-nitrophenyl) sulfane (**4**). [15,16] This on further catalytic hydrogenation produced [2-(2,4-dimethylphenylthio)phenyl] aniline (**5**). [17] The intermediate amine compound formed amide bond with different acyl chloride or acid derivatives in presence of inorganic base or coupling agent with suitable solvent to get desired compounds **6a–o** with moderate to good yields. The structures of all newly synthesized compounds **6a–o** were confirmed by IR, NMR, Mass spectral analyses and were in full agreement with

Table 1. Physical data of bicyclic [2-(2,4-dimethylphenylthio)phenyl] aniline derivatives (**6a–o**).

Entry	Compound	R	Yields (%)	Melting points (°C)
1	**6a**	Methyl	95	42-44
2	**6b**	Trifluoro methyl	98	52-54
3	**6c**	2-propane	90	38-40
4	**6d**	Ethyl	92	46-48
5	**6e**	Butyl	90	56-58
6	**6f**	Ethyl Oxalate	90	50-52
7	**6g**	tButyl	88	38-40
8	**6h**	Dibenzoyl	95	154-156
9	**6i**	Phenyl	82	78-80
10	**6j**	O-acetyl mandelic	80	86-88
11	**6k**	4-methlyl Pyrazine	85	100-102
12	**6l**	2-Furan	88	120-122
13	**6m**	Phthalic acid	87	126-128
14	**6n**	3-methyl Isoxazole	85	80-82
15	**6o**	5-chloroThiophene	90	74-76



Scheme 1. Synthetic route for the preparation of title compounds **6a–o**. Reagents and conditions: (a) $NaNO_2$, HCl: water, potassium xhanthate, 25–30 °C, (b) KOH, Ethanol, 76–78 °C, 72%; (c) K_2CO_3, Acetonitrile, 78–82 °C, 92%; (d) Pd/C, H_2, methanol, 25–30 °C, 98%; (e) Acyl chloride derivative, NaOH, ACN, 5 − 30°C or acid derivative, CDI, DMF, 5–30 °C, 80–98%.

proposed structures. The IR spectra of synthesized compound **6a–o** showed band 3050–3107 $cm^{-1}$and 1660–1675 cm^{-1}corresponding to –NH and >C=O stretching of amide. In ^{1}H NMR aromatic protons were observed at δ 6.5–8.0 ppm. Characteristic peak around 160–170 ppm in ^{13}C NMR confirmed the presence of >C=O group.

3.2 *Biological evaluation*

In a standard primary screening, all the newly synthesized compounds (**6a–o**) were tested for their in vitro antitubercular activity against *M. tuberculosis* H37Ra (ATCC 25177) at 3 μg/mL concentrations using an established XTT Reduction Menadione Assay (XRMA). Among the synthesized compounds, **6a**, **6b**, **6d**, **6g**, **6j**, **6l, 6m** and **6n** displayed activity towards *M. tuberculosis* H37Ra (Dormant) value less than 30 μg/mL. The results of the screening are tabulated in Table S1.

Active compounds from in vitro screening were screened in THP-1 infection model. Compounds exhibiting MIC90 less than 10 μg/mL was considered as potent compound. Compound **6a**, **6j**, **6n** showed excellent activity in infection models (Table S2). Moreover, compound **6n** showed significant activity with MIC90 6.04 and 2.72 μg/mL respectively in the active and dormant infection models.

After performing antitubercular screening, the active compounds from ex-vivo infection model were evaluated for cytotoxicity on three human cancerous cell lines THP-1, HeLa, MCF-7 using MTT assay and IC_{50} values were determined. The results are recorded in Table S4. It has been noticed that compounds **6j** and **6m** showed GI_{90} values >100 μg/mL in all the cancer cell lines.

Further, the selectivity of the compounds (**6a, 6j** and **6n**) towards human cell lines against MTB was determined via their selectivity index (Table S5). The selectivity index reflects the quantity of the compound that is active against MTB but non-toxic towards host cells. A higher selectivity index indicates that the compound can be used as a therapeutic agent. It was found that **6n** have a selectivity index of ≥10 at In vitro and Ex vivo model of MTB when compared to both HeLa and THP-1 cells.

Compounds **6a-o** were further screened against (Gram positive *B. subtilis* and *S. aureus*, Gram negative *E. coli* and *P. aeroginosa*) at 3 μg/mL concentration, to assess their selectivity towards MTB. The antimicrobial activity is summarized in Table S3. None of the compounds showed significant activity towards any of the screened bacterial strain.

3.3 *Molecular docking and ADME study*

In addition, computational molecular docking and silico ADME study were performed to rationalize the observed biological results. To gain an insight into binding mode and the thermodynamic interactions which govern the binding of the most active bicyclic [2-(2,4-dimethylphenylthio)phenyl] aniline derivative. Summarized docking and ADME study available in supporting information.

4. Conclusion

In summary, a series of bicyclic [2-(2,4-dimethylphenylthio)phenyl] aniline and its amide derivatives containing aliphatic, aromatic and heterocyclic moieties were synthesized, characterized and evaluated for antituberculosis activity. Most of the compounds displayed good antituberculosis activity. Among all tested compounds, **6j** (active state MIC90: 24.9 μg/mL, dormant state MIC90: 5.78 μg/mL) and **6n** (active state MIC90: 6.04 μg/mL, dormant state MIC90: 2.72 μg/mL) demonstrated significant inhibition against all the strains tested. It may be helpful for further screening, designing and developing more potent antituberculosis agents.

Supplementary Information (SI)

All additional information pertaining to molecular biological activity, docking study, ADME study and spectral data for the characterization of compounds are given in the supporting information. Supplementary Information is available at www.ias.ac.in/chemsci.

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References

1. The World Health Organization (WHO) 2014 *Global Tuberculosis Report* https://doi.org/10.1016/S2214109X(14)70361-4 (accessed on January 2015)
2. Abubakar I, Zignol M, Falzon D, Raviglione M, Ditiu L, Masham S, Adetifa I, Ford N, Cox H and Lawn S D 2013 Drug-resistant tuberculosis: time for visionary political leadership *Lancet Infect. Dis.* **13** 529
3. The World Health Organization (WHO) 2015 *Global Tuberculosis Report* http://www.who.int/tb/publications/global_report/en/ (accessed on November 2015)
4. Espinal M A 2003 The global situation of MDR-TB *Tuberculosis* **83** 44
5. Jones T R, Webber S E, Varney M D, Reddy M R, Lewis K K, Kathardekar V, Mazdiyasni H, Deal J, Nguyen D, Welsh K M, Webber S, Johnston A, Matthews D A, Smith W W, Janson C A, Bacquet R J, Hwland E F, Booth C L J, Herrmann S M, Ward R W, White J, Bartlett C A and Morse C A 1997 Structure-based design of substituted diphenyl sulfones and sulfoxides as lipophilic inhibitors of thymidylate synthase *J. Med. Chem.* **40** 677
6. Sreekanth A, Ramachandran P S, Jadhavar S K, Miglani M P, Singh D P, Kalane A K, Agarwal B D, Sathe K M, Gupta A, Haldar S, Mohd R, Singh S, Pham S M, Chakravarty S, Kevin Q, Sebastian B, Ivan E A, Higgs C, Francisco J and Herrera R 2017 Design, synthesis and optimization of bis-amide derivatives as CSF1R inhibitors *Bioorg. Med. Chem. Lett.* **27** 2153
7. Caroline R, Montes T C, Tamara G M, Carolina R L, Hack R D, Pedro A S, Marcelo G and Montes D 2010 Synthesis and antituberculosis activity of new fatty acid amides *Bioorg. Med. Chem. Lett.* **20** 5255
8. Yang Z B, Hu D Y, Zeng S and Bao A S 2016 Novel hydrazone derivatives containing pyridine amide moiety: Design, synthesis, and insecticidal activity *Bioorg. Med. Chem. Lett.* **26** 1161
9. Yamaki S, Suzuki D, Fujiyasu J, Neya M, Nagashima A, Kondo M, Takafumi A, Keitaro K, Ayako M and Yoshihara Y 2017 Synthesis and pharmacological evaluation of glycine amide derivatives as novel vascular adhesion

protein-1 inhibitors without CYP3A4 and CYP2C19 inhibition *Bioorg. Med. Chem.* **25** 4110
10. Francisco G A A, Eugenio H F, Pilar C R, Susana L C, Jorge H F, Mario O, Nancy E G D, Alejandro M V, Víctor M V M and Maria G S M 2017 Synthesis, antimycobacterial and cytotoxic activity of α, β-unsaturated amides and 2, 4-disubstituted oxazoline derivatives *Bioorg. Med. Chem. Lett.* **27** 821
11. Yang K, Guo S S, Geng Z F, You C X, Zhang W J, Li Y P, Wang C F, Du S S and Deng Z W 2015 Five new sulphur-containing amides from Glyco smislucida with antifeedant activity against *Tribolium castaneum* *Ind. Crops Prod.* **74** 628
12. Greger H and Zechner G 1996 Bioactive Amides from *Glycosmis* species *J. Nat. Prod.* **59** 1163
13. Tarbell D S and Fukushima D K 1947 M-Thiocresol *Org. Synth.* **27** 81
14. Leuckart R J 1890 Eine neue Methode zur Darstellung aromatischer Mercaptane *J. Prakt. Chem.* **41** 179
15. Molly S H, Alice L P and Atli T 1999 Palladium catalyzed bispyrimidine thioether synthesis *Synlett* **10** 1579
16. Mao Y, Jiang L, Chen T, He H, Liu G and Wang H 2015 A new and practical synthesis of Vortioxetine Hydrobromide *Synthesis* **47** 1387
17. Qunfeng Z, Wei X, Xiaonian Li, Dahao J, Yizhi X, Jianguo W, Jie C, Stephen R and Jun N 2015. Catalytic hydrogenation of sulfur-containing nitrobenzene over Pd/C catalysts: In situ sulfidation of Pd/C for the preparation of Pd_xS_y catalysts *Appl. Catal. A Gen.* **497** 17