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Novel synthetic analogues of Fluoxetine as potent and selective anti-TB agents

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ABSTRACT

We have recently identified potent anti-TB activity in several CNS drugs. Most prominently, the phenothiazine antipsychotics (Thioridazine-MIC 3.125 µg/mL) and anti-depressant drugs (sertraline-MIC 1.6 µg/mL) have shown anti-TB activity against *Mycobacterium tuberculosis* H37Rv. In continuation, we have synthesized a series of 1-(3-aryloxy-3-phenylpropyl) amine analogues of fluoxetine to optimize its anti-TB activity. Identities of the synthesized compounds were confirmed by FTIR, ^{1}H NMR and mass spectral analysis. They were tested for in vitro antitubercular activity by MABA Assay. To determine selective TB activity, they were also tested for antimicrobial activity. Among the synthesized compounds, 1-(3-(4-fluorophenoxy)-3-phenylpropyl) piperidine (AM3e) has shown highest anti-TB activity (MIC 1.6 µg/mL) against MtbH37Rv and is free from antibacterial/antifungal activity (MIC >100 µg/mL).

INTRODUCTION

As per WHO report released recently, drug resistance has been noted for almost every antibiotic in use today. The list includes aminoglycosides, peptides, β-lactams, sulfonamides, nitroimidazoles, quinolones, tetracyclines, chloramphenicol and macrolides (WHO report, 2017). It is becoming a major threat to concern for the development of modern medicine (Willyard *et al.*, 2017). *Mycobacterium tuberculosis* (Mtb) is the causative agent for one of the deadliest infectious diseases, tuberculosis. It is known for its "signature" lipid-rich cell wall, which offers a formidable barrier to most of the antibiotics. Further, it is also known to develop drug resistance to a multitude of chemotherapeutic agents. In 2016-17 alone, a total of 600 000 cases with resistance to rifampicin (RRTB) were reported, of which 490 000 were with multidrug resistance. Drug resistance in Mtb arises predominantly from a change of intracellular target protein/enzyme (WHO report, 2017; Juan Carlos *et al.*, 2014).

Hence, development of a novel agent targeting an essential mycobacterial biochemical pathway only can offer a dependable drug molecule. In this regard, search for structurally diverse anti-Tb agents, especially those with a novel mechanism of action has received a great deal of attention in the recent past. Repurposing of FDA approved "non-antibiotic" drugs has also aroused immense interest in anti-TB drug researchers, as it presumed to inflict less financial/clinical trial burden. Several CNS agents including antipsychotics (viz. phenothiazines) and antidepressant agents (viz. fluothe xetine) were reported to have potent antimicrobial activity against bacteria including Mtb. (Marta Martins *et al.*, 2008; Lass-Florl *et al.*, 2001; Kristiansen *et al.*, 2007; Murali Krishna Kumar *et al.*, 2015; Munoz-Bellido *et al.*, 2000). In view of its potential antimicrobial properties, fluoxetine analogues have been explored for antifungal activity against *Candida* sp. (Romano Silvestri *et al.*, 2004). These reports and our personal interest in anti-TB drug discovery (Harian Babu Bolikala *et al.*, 2017; Murali Krishna Kumar *et al.*, 2015), has induced us to do ligand optimization studies on fluoxetine by altering substitutions on aryloxy group and replacing methyl amino group with piperidine/piperazine.

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MATERIALS AND METHODS

Instruments and reagents

Melting points (m.p.) were recorded on Digimelt (Stanford Research Systems, USA) Automatic melting point apparatus by the one-end-open capillary method and are uncorrected. Biotage Initiator was used for microwave synthesis. The reaction was conducted at 100°C (40W) with absorption level high and fixed holding time switched ON, in a crimped 10ml microwave reaction vessel. Crimped microwave vial. IR spectra were recorded on a Bruker-Alpha FTIR spectrometer using KBr pellet method. NMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer in a proper deuterated solvent using TMS as an internal standard. Mass spectra were recorded on Agilent QQQ LCMS-6410 mass spectrometer. Pre-coated TLC plates were used for monitoring synthetic reactions and also to find out uniformity of synthesized compounds. All laboratory grade reagents were procured and used as received.

Synthesis of compounds AM1-6 (Ali S *et al.*, 2002)

Acetophenone mannich bases were obtained by a solid phase microwave synthetic method (Scheme 1). Acetophenone (1 mmol), paraformaldehyde (1 mmol) and appropriate amine (1.1 mmol) were thoroughly mixed and adsorbed on acidic alumina (2 grams) in a china dish. This reaction mixture was then transferred to a 10 ml reaction vial and crimped. It was then subjected to microwave irradiation (100°C, 40 Watts) using Biotage Initiator for 5 minutes. Upon completion of the reaction, as indicated by TLC, the product was obtained via extracting the reaction mixture with ethyl acetate. The products AM2 to AM6 were obtained in sufficiently pure form after usual work-up and washed with hexanes. AM1 was purified by column chromatography using eluent ethyl acetate: hexane (9:1).

*3-(Diethylamino)-1-phenylpropan-1-one (**AM1**)*

Colorless oil, Yield: 85%. IR (KBr) ν_{max} cm^{-1}: 1281, 1602, 1675, 2815, 3072. ^{1}H NMR (400 MHz, $CDCl_3$): 1.09 (t, 6H, *J* = 7Hz), 2.45 (m, 4H, *J* = 7Hz), 2.65 (t, 2H, *J* = 6Hz), 2.98 (t, 2H, *J* = 6Hz), 7.31 (dd. 2H, *J* = 8Hz), 7.45 (m, 1H), 7.99 (d, 2H *J* = 8.2Hz). ESIMS $(M+H)^+$ *m*/*z*: 206.

*3-Morpholino-1-phenylpropan-1-one (**AM2**)*

Colorless solid, Yield = 78%, mp 177-179°C. IR (KBr) ν_{max} cm^{-1}: 1194, 1285, 1585, 1680, 2969, 3082. ^{1}H NMR (400 MHz, $CDCl_3$): 2.42 (m, 4H), 2.79 (t, 2H, *J* = 4Hz), 3.12 (t, 2H, *J* = 4Hz), 3.69 (m, 4H), 7.35 (m, 2H), 7.45 (m, 1H), 7.88 (m, 2H). ESIMS $(M+H)^+$ *m*/*z*: 220.

*1-Phenyl-3-(piperidin-1-yl)propan-1-one (**AM3**)*

White colored Semi solid,Yield = 92%. IR (KBr) ν_{max} cm^{-1}: 1282, 1601, 1670, 2913, 3079. ^{1}H NMR (400 MHz, $CDCl_3$): 1.61 (m, 6H), 2.55 (t, 4H, *J* = 4Hz), 2.89 (t, 2H, *J* = 6Hz), 2.96 (t, 2H, *J* = 6Hz), 7.35 (m, 2H), 7.42 (m, 1H), 7.84 (m, 2H). ESIMS $(M+H)^+$ *m*/*z*: 218.

*1-Phenyl-3-(4-phenylpiperazin-1-yl)propan-1-one (**AM4**)*

Colorless solid, Yield = 90, 116-117°C. IR (KBr) ν_{max} cm^{-1}: 1258, 1595, 1682, 2962, 3056. ^{1}H NMR (400 MHz, $CDCl_3$): 2.69 (m, 4H), 2.92 (t, 2H, *J* = 5.8Hz), 3.01 (t, 2H, *J* = 5.8Hz), 3.29 (m, 4H), 6.83-6.95 (m, 3H), 7.13 (m, 2H), 7.42 (m, 3H), 7.95 (dd, 2H, *J* = 7.8Hz). ESIMS $(M+H)^+$ *m*/*z*: 295.

*3-(4-Benzylpiperazin-1-yl)-1-phenylpropan-1-one (**AM5**)*

Colorless solid, Yield = 90, 136-139°C. IR (KBr) ν_{max} cm^{-1}: 1296, 1595, 1643, 2962 , 3093. ^{1}H NMR (400 MHz, $CDCl_3$): 2.29 (m, 4H), 2.54 (m, 4H), 2.61 (t, 2H, *J* = 5.7 Hz), 2.92 (t, 2H, *J* = 5.7 Hz), 3.52 (s, 2H) 7.21-7.55 (m, 8H), 7.96 (d, 2H, *J* = 7.9 Hz). ESIMS $(M+H)^+$ *m*/*z*: 309.

*3-(4-Benzylpiperidin-1-yl)-1-phenylpropan-1-one (**AM6**)*

Colorless solid, Yield = 88, 128-129°C. IR (KBr) ν_{max} cm^{-1}: 1281, 1597, 1679, 2846, 3082. ^{1}H NMR (400 MHz, $CDCl_3$): 1.08 (m, 2H), 1.41 (m, 2H), 1.61 (m, 1H), 1.87 (t, 2H, *J* = 4Hz), 2.46 (d, 2H, *J* = 4.8Hz), 2.83 (t, 2H, *J* = 4.6Hz), 2.72 (t, 2H, *J* = 4Hz), 7.08 (d, 2H, *J* = 7.8Hz), 7.17 (dd, 1H, *J* = 7Hz), 7.24 (d, 2H, *J* = 7.4Hz), 7.44 (dd, 2H, *J* = 7.8Hz), 7.54 (dd, 1H, *J* = 7.6Hz), 7.93 (d, 2H, *J* = 7.4Hz). ESIMS $(M+H)^+$ *m*/*z*: 308.

Synthesis of compounds AM1a-6a: Sodium borohydride (30 mmol) was added in portions to a stirred and cooled solution of Mannich bases (**AM1-AM6**) (10 mmol) in methanol (25 mL) over a period of 30 min. The reaction mixture was further stirred at room temperature for 4 h. Methanol was distilled under reduced pressure. The residue was triturated with water (25 mL) and extracted 3 times with dichloromethane (15 mL). The combined organic layer was dried over sodium sulfate and concentrated to give the hydroxyl compounds **AM1a-AM6a** in >90% yield as colorless oils.

*3-(Diethylamino)-1-phenylpropan-1-ol (**AM1a**)*

Yield: 90%; IR (KBr) ν_{max} cm^{-1}: 3259, 3081, 2924, 2827, 2788, 1605, 1466, 1382, 1259, 1042. ^{1}H NMR (400 MHz, $CDCl_3$): 1.08 (t, 6H, *J* = 7.2Hz), 1.78-1.96 (m, 2H), 2.62-2.85 (m, 6H), 4.95-5.01 (m, 1H), 7.21-7.39 (m, 5H). ESIMS $(M+H)^+$ *m*/*z*: 208.

*3-Morpholino-1-phenylpropan-1-ol (**AM2a**)*

Yield: 92%; IR (KBr) ν_{max} cm^{-1}: 3206, 3075, 2997, 2929, 2831, 2792, 1501, 1466, 1254, 1219, 1037. ^{1}H NMR (400 MHz, $CDCl_3$): 1.88 (m, 2H), 2.41-2.46 (t, 2H, *J* = 2.8Hz), 2.58-2.65 (m, 4H), 3.65-3.72 (m, 4H), 4.94 (m, 1H), 7.22-7.36 (m, 5H). ESIMS $(M+H)^+$ *m*/*z*: 222.

*1-Phenyl-3-(piperidin-1-yl)propan-1-ol (**AM3a**)*

Yield: 93%; IR (KBr) ν_{max} cm^{-1}: 3260, 3078, 2906, 2815, 1618, 1512, 1156. ^{1}H NMR (400 MHz, $CDCl_3$): 1.35-1.52 (m, 6H), 1.78 (m, 2H), 2.32-2.35 (m, 2H), 2.45-2.48 (m, 2H), 2.53 (m, 2H), 4.88 (t, 1H, *J* = 5.6Hz), 7.18-7.28 (m, 5H). ESIMS $(M+H)^+$ *m*/*z*: 220.

*1-Phenyl-3-(4-phenylpiperazin-1-yl)propan-1-ol (**AM4a**)*

Yield: 88%; IR (KBr) ν_{max} cm^{-1}: 3341, 3224, 3065, 2925, 2835, 1614, 1523, 1465, 1242. ^{1}H NMR (400 MHz, $CDCl_3$): 1.95-1.98 (m, 2H), 2.58-2.62 (m, 6H), 3.48-3.52 (m, 4H), 5.05-5.11 (t, 1H, *J* = 5.6Hz), 6.63-6.75 (m, 3H), 7.08 (d, 2H, *J* = 7.6Hz), 7.18-7.31 (m, 5H). ESIMS $(M+H)^+$ *m*/*z*: 297.

*3-(4-Benzylpiperazin-1-yl)-1-phenylpropan-1-ol (**AM5a**)*

Yield: 90%; IR (KBr) ν_{max} cm^{-1}: 3364, 3226, 3072, 2928, 2812, 1611, 1516, 1446, 1244. ^{1}H NMR (400 MHz, $CDCl_3$): 1.91-1.96 (m, 2H), 2.47 (m, 4H), 2.53-2.56 (m, 6H), 3.59 (s, 2H), 4.85 (t, 1H, *J* = 5.8Hz), 7.21-7.35 (m, 10H). ESIMS $(M+H)^+$ *m/z*: 311.

*3-(4-Benzylpiperidin-1-yl)-1-phenylpropan-1-ol (**AM6a**)*

Yield: 90%; IR (KBr) ν_{max} cm^{-1}: 3223, 3082, 2921, 2877, 2824, 2364, 1611, 1498, 1450, 1361, 1311, 1250, 1163, 1105. ^{1}H NMR (400 MHz, $CDCl_3$): 1.29-1.45 (m, 4H), 1.62-1.91 (m, 3H), 2.27-2.31 (m, 4H), 2.55 (brd, 2H), 2.72 (t, 2H, *J* = 5Hz), 5.12 (m, 1H), 7.14-7.27 (m, 10H). ESIMS $(M+H)^+$ *m/z*: 310.

Synthesis of compounds AM1b-6g: The target compounds were obtained by Mitsunobu reaction between the secondary alcohol and substituted phenol. A solution of diethyl azo di carboxylate (DEAD) (55 mmol) in 25 mL of THF was added dropwise to a stirred solution of the alcohol (**1a-6a**, 50 mmol), phenol/substituted phenol (55 mmol) and triphenylphosphine (55 mmol) in 50mL of THF. Stirring was continued at room temperature until thin layer chromatography indicated the absence of alcohol (12-18 hours). The mixture was then concentrated under reduced pressure. The crude mixture was subjected to flash chromatography to obtain the pure product as viscous, colorless to brown colored oil with an overall yield of 65-88%.

*N,N-Diethyl-3-phenoxy-3-phenylpropan-1-amine (**AM1b**)*

Yield: 83%; IR (KBr) ν_{max} cm^{-1}: 3259, 3081, 2924, 2827, 2788, 1605, 1466, 1382, 1259, 1042. ^{1}H NMR (400 MHz, $CDCl_3$): 1.06 (t, 6H, *J* = 7.2Hz), 2.22-2.28 (m, 2H), 2.48 -2.61 (m, 6H), 5.29-5.38 (m, 1H), 6.88 (d, 2H, *J* = 8Hz), 7.25-7.52 (m, 8H). ESIMS $(M+H)^+$ *m/z*: 284.

*N,N-Diethyl-3-phenyl-3-(p-tolyloxy)propan-1-amine(**AM1c**)*

Yield: 70%; IR (KBr) ν_{max} cm^{-1}: 3075, 2889, 1601, 1466, 1265, 1065. ^{1}H NMR (400 MHz, $CDCl_3$): 1.08 (t, 6H, *J* = 7.2Hz), 2.18-2.26 (m, 2H), 2.42-2.55 (m, 6H), 5.09-5.21 (m, 1H), 6.78 (d, 2H, *J* = 8Hz), 7.11 (d, 2H, *J* = 8.2Hz), 7.22-7.30 (m, 5H). ESIMS $(M+H)^+$ *m/z*: 298.

*3-(4-Bromophenoxy)-N,N-diethyl-3-phenylpropan-1-amine (**AM1d**)*

Yield:72%; IR (KBr) ν_{max} cm^{-1}: 3085, 2911, 2872, 1596, 1461, 1385, 1245, 1070. ^{1}H NMR (400 MHz, $CDCl_3$): 1.08 (t, 6H, *J* = 7.2Hz), 2.24-2.36 (m, 2H), 2.48-2.62 (m, 6H), 5.22-5.35 (m, 1H), 6.81 (d, 2H, *J* = 7.6Hz), 7.19-7.31 (m, 5H), 7.33 (d, 2H, *J* = 7.6Hz). ESIMS $(M+H)^+$ *m/z*: 362.

*N,N-Diethyl-3-(4-fluorophenoxy)-3-phenylpropan-1-amine (**AM1e**)*

Yield: 82%; IR (KBr) ν_{max} cm^{-1}: 3068, 2941, 2850, 2745, 1608, 1391, 1268, 1065. ^{1}H NMR (400 MHz, $CDCl_3$): 1.06 (t, 6H, *J* = 7.2Hz), 2.28-2.35 (m, 2H), 2.42 -2.51 (m, 6H), 5.30-5.44 (m, 1H), 6.88 (d, 2H, *J* = 8.2Hz), 6.92 (d, 2H, *J* = 8.4Hz), 7.21-7.38 (m, 5H). ESIMS $(M+H)^+$ *m/z*: 302.

*3-(4-Chlorophenoxy)-N,N-diethyl-3-phenylpropan-1-amine (**AM1f**)*

Yield: 85%; IR (KBr) ν_{max} cm^{-1}: 3105, 3082, 2908, 2857, 1608, 1461, 1373, 1275, 1068. ^{1}H NMR (400 MHz, $CDCl_3$): 1.08 (t, 6H, *J* = 7.2Hz), 2.24-2.36 (m, 2H), 2.44 -2.56 (m, 6H), 5.25-5.39 (m, 1H), 6.80 (d, 2H, *J* = 7.8Hz), 7.19-7.38 (m, 7H). ESIMS $(M+H)^+$ *m/z*: 318.

*N,N-Diethyl-3-phenyl-3-(4-(trifluoromethyl)phenoxy)propan-1-amine (**AM1g**)*

Yield: 85%; IR (KBr) ν_{max} cm^{-1}: 3101, 3085, 2911, 2870, 1605, 1599, 1380, 169, 1073.^{1}H NMR (400 MHz, $CDCl_3$): 1.08 (t, 6H, *J* = 7.2Hz), 2.32-2.36 (m, 2H), 2.48-2.55 (m, 6H), 5.28-5.42 (m, 1H), 6.82 (d, 2H, *J* = 8Hz), 7.25-7.42 (m, 5H), 7.43-7.55 (d, 2H, *J* = 7.9Hz). ESIMS $(M+H)^+$ *m/z*: 352.

*4-(3-Phenoxy-3-phenylpropyl)morpholine (**AM2b**)*

Yield: 80%; IR (KBr) ν_{max} cm^{-1}: 3108, 2912, 1612, 1459, 1385, 1252, 1071.^{1}H NMR (400 MHz, $CDCl_3$): 1.93 (m, 1H), 2.14 (m, 1H), 2.35-2.69 (m, 6H), 3.56 (t, 4H, *J* = 6.2Hz), 5.11 (m, 1H), 6.82 (dd, 2H, *J* = 8.2Hz), 7.12-7.35 (m, 7H). ESIMS $(M+H)^+$ *m/z*: 298.

*4-(3-Phenyl-3-(p-tolyloxy)propyl)morpholine (**AM2c**)*

Yield: 72%; IR (KBr) ν_{max} cm^{-1}: 3081, 2095, 1608, 1462, 1261, 1068 .^{1}H NMR (400 MHz, $CDCl_3$): 1.90 (t, 2H, *J* = 5.2Hz), 2.11 (m, 4H), 2.30 (s, 3H), 2.34-2.70 (m, 4H), 3.52 (t, 2H, *J* = 5.6Hz), 5.19 (m, 1H), 6.78 (d,2H, *J* = 8Hz), 7.08 (d, 2H *J* = 7.2Hz), 7.21-7.32 (m, 5H). ESIMS $(M+H)^+$ *m/z*: 312.

*4-(3-(4-Bromophenoxy)-3-phenylpropyl)morpholine (**AM2d**)*

Yield: 65%; IR (KBr) ν_{max} cm^{-1}: 3088, 2915, 2872, 1611, 1465, 1385, 1242, 1068. ^{1}H NMR (400 MHz, $CDCl_3$): 1.92 (t, 2H, *J* = 5.2Hz), 2.12 (m, 4H), 2.35 (s, 3H), 3.55 (t, 2H, *J* = 5.2Hz), 5.08-5.15 (m, 1H), 6.86 (d, 2H, *J* = 7.8Hz), 7.18 (d, 2H *J* = 7.9Hz), 7.20-7.32 (m, 5H). ESIMS $(M+H)^+$ *m/z*: 376.

*4-(3-(4-Fluorophenoxy)-3-phenylpropyl)morpholine (**AM2e**)*

Yield: 70%; IR (KBr) ν_{max} cm^{-1}: 3069, 2948, 2865, 1605, 1390, 1262, 1065. ^{1}H NMR (400 MHz, $CDCl_3$): 1.90 (t, 2H, *J* = 5.2Hz), 2.14 (m, 4H), 2.34 (s, 3H), 3.56 (t, 2H, *J* = 5.6Hz), 5.21-5.28 (m, 1H), 6.81 (d, 2H, *J* = 8Hz), 7.16 (d, 2H *J* = 7.2Hz), 7.19-7.32 (m, 5H). ESIMS $(M+H)^+$ *m/z*: 316.

*4-(3-(4-Chlorophenoxy)-3-phenylpropyl)morpholine (**AM2f**)*

Yield: 70%; IR (KBr) ν_{max} cm^{-1}: 3101, 3088, 2911, 2852, 1608, 1463, 1373, 1265, 1069. ^{1}H NMR (400 MHz, $CDCl_3$): 1.92 (t, 2H, *J* = 5.2Hz), 2.12 (m, 4H), 2.31 (s, 3H), 3.54 (t, 2H, *J* = 5.6Hz), 5.14 (m, 1H), 6.79 (d, 2H, *J* = 7.6Hz), 7.20 (d, 2H *J* = 7.2Hz), 721-7.35 (m, 5H). ESIMS $(M+H)^+$ *m/z*: 332.

*4-(3-Phenyl-3-(4-(trifluoromethyl) phenoxy)propyl)morpholine (**AM2g**)*

Yield: 72%; IR (KBr) ν_{max} cm^{-1}: 3095, 3069, 2908, 2811, 1605, 1609, 1385, 1262, 1085. ^{1}H NMR (400 MHz, $CDCl_3$): 1.91 (t, 2H, *J* = 5.2Hz), 2.13 (m, 4H), 2.36-2.68 (m, 4H), 3.59 (t, 2H, *J* = 5.2Hz), 5.26 (m, 1H), 6.78 (d, 2H, *J* = 7.2Hz), 7.31-7.49 (m,

5H), 7.61 (d, 2H *J* = 7.2Hz). ESIMS $(M+H)^+$ *m*/*z*: 366.

*1-(3-Phenoxy-3-phenylpropyl)piperidine (**AM3b**)*

Yield: 85%; IR (KBr) ν_{max} cm^{-1}: 3115, 2928, 2854, 1568, 1357, 1249, 1069. ^1H NMR (400 MHz, $CDCl_3$): 1.22-1.40 (m, 6H), 1.85 (m, 1H), 2.09 (m, 1H), 2.21 (m, 4H), 2.28 (m, 2H), 4.98 (m, 1H), 6.74 (d, 2H, *J* = 8Hz), 7.11-7.26 (m, 8H). ESIMS $(M+H)^+$ *m*/*z*: 296.

*1-(3-Phenyl-3-(p-tolyloxy)propyl)piperidine (**AM3c**)*

Yield: 75%; IR (KBr) ν_{max} cm^{-1}: 3098, 2892, 1608, 1456, 1261, 1071. ^1H NMR (400 MHz, $CDCl_3$): 1.20-1.35 (m, 6H), 1.83 (m, 1H), 2.10 (m, 1H), 2.19-2.23 (m, 4H), 2.25 (m, 2H), 2.32 (s, 3H), 5.02 (m, 1H), 6.76 (d, 2H, *J* = 7.6Hz), 7.06 (d, 2H, *J* = 7.6Hz), 7.19-7.30 (m, 5H). ESIMS $(M+H)^+$ *m*/*z*: 310.

*1-(3-(4-Bromophenoxy)-3-phenylpropyl)piperidine (**AM3d**)*

Yield: 68%; IR (KBr) ν_{max} cm^{-1}: 3085, 2908, 2875, 1606, 1460, 1391, 1253, 1074. ^1H NMR (400 MHz, $CDCl_3$): 1.18-1.36 (m, 6H), 1.85 (m, 1H), 2.12 (m, 1H), 2.19-2.24 (m, 4H), 2.28 (m, 2H), 5.11 (m, 1H), 6.81 (d, 2H, *J* = 8.6Hz), 7.16 (d, 2H, *J* = 8.2Hz), 7.21-7.33 (m, 5H). ESIMS $(M+H)^+$ *m*/*z*: 374.

*1-(3-(4-Fluorophenoxy)-3-phenylpropyl)piperidine (**AM3e**)*

Yield: 70%; IR (KBr) ν_{max} cm^{-1}: 3072, 2909, 2862, 1608, 1408, 1272, 1085. ^1H NMR (400 MHz, $CDCl_3$): 1.22-1.39 (m, 6H), 1.81 (m, 1H), 2.09 (m, 1H), 2.20-2.24 (m, 4H), 2.29 (m, 2H), 5.14 (m, 1H), 6.75 (d, 2H, *J* = 6.8Hz), 7.13 (d, 2H, *J* = 6.6Hz), 7.21-7.33 (m, 5H). ESIMS $(M+H)^+$ *m*/*z*: 314.

*1-(3-(4-Chlorophenoxy)-3-phenylpropyl)piperidine (**AM3f**)*

Yield: 65%; IR (KBr) ν_{max} cm^{-1}: 3112, 3088, 2915, 2806, 1611, 1455, 1369, 1271, 1058. ^1H NMR (400 MHz, $CDCl_3$): 1.18-1.36 (m, 6H), 1.85 (m, 1H), 2.12 (m, 1H), 2.19-2.24 (m, 4H), 2.28 (m, 2H), 5.11 (m, 1H), 6.81 (d, 2H, *J* = 7.6Hz), 7.16 (d, 2H, *J* = 7.56Hz), 7.21-7.33 (m, 5H). ESIMS $(M+H)^+$ *m*/*z*: 330.

*1-(3-Phenyl-3-(4-(trifluoromethyl)-phenoxy)propyl)piperidine (**AM3g**)*

Yield: 75%; IR (KBr) ν_{max} cm^{-1}: 3109, 3065, 2962, 2829, 1601, 1429, 1378, 1268, 1071. ^1H NMR (400 MHz, $CDCl_3$): 1.24 (m, 2H), 1.41 (m, 4H), 1.85 (m, 1H), 2.07 (m, 1H), 2.20- 2.28 (m, 6H), 5.23 (m, 1H), 6.74 (d, 2H, *J* = 8Hz), 7.11 (d, 2H, *J* = 7.92Hz), 7.20 (m, 5H). ESIMS $(M+H)^+$ *m*/*z*: 364.

*1-(3-Phenoxy-3-phenylpropyl)-4-phenylpiperazine (**AM4b**)*

Yield: 67%; IR (KBr) ν_{max} cm^{-1}: 3109, 3082, 2961, 2837, 1611, 1445, 1384, 1271, 1068. ^1H NMR (400 MHz, $CDCl_3$): 1.97-2.05 (m, 2H), 2.56-2.63 (m, 6H), 3.45-3.52 (m, 4H), 5.11-5.14 (m, 1H), 6.68 (d, 2H, *J* = 8Hz), 6.81 (d, 2H, *J* = 7.6Hz), 7.08-7.31 (m, 11H). ESIMS $(M+H)^+$ *m*/*z*: 373.

*1-Phenyl-4-(3-phenyl-3-(p-tolyloxy)propyl)piperazine (**AM4c**)*

Yield: 80%; IR (KBr) ν_{max} cm^{-1}: 3115, 3075, 2889, 1601, 1465, 1265, 1068. ^1H NMR (400 MHz, $CDCl_3$): 1.92-2.01 (m, 2H), 2.35 (s, 3H), 2.54-2.62 (m, 6H), 3.46-3.55 (m, 4H), 5.04-5.10 (m, 1H), 6.65 (d, 2H, *J* = 7.8Hz), 6.82 (d, 2H, *J* = 8Hz), 7.08 (d, 2H, *J* = 8Hz), 7.10-7.30 (m, 8H). ESIMS $(M+H)^+$ *m*/*z*: 387.

*1-(3-(4-Bromophenoxy)-3-phenylpropyl)-4-phenylpiperazine (**AM4d**)*

Yield: 82%; IR (KBr) ν_{max} cm^{-1}: 3105, 3046, 2925, 2883, 1598, 1461, 1388, 1252, 1074. ^1H NMR (400 MHz, $CDCl_3$): 1.94-2.05 (m, 2H), 2.55-2.62 (m, 6H), 3.48-3.52 (m, 4H), 5.09-5.13 (m, 1H), 6.72 (d, 2H, *J* = 8Hz), 6.81 (d, 2H, *J* = 8.6Hz), 7.08 (m, 1H), 7.16 (d, 2H, *J* = 8.4Hz), 7.21-7.34 (m, 7H). ESIMS $(M+H)^+$ *m*/*z*: 451.

*1-(3-(4-Fluorophenoxy)-3-phenylpropyl)-4-phenylpiperazine (**AM4e**)*

Yield: 85%; IR (KBr) ν_{max} cm^{-1}: 3115, 3068, 2950, 2842, 2721, 1601, 1390, 1275, 1068. ^1H NMR (400 MHz, $CDCl_3$): 1.89-2.01 (m, 2H), 2.55-2.63 (m, 6H), 3.46-3.58 (m, 4H), 5.21-5.28 (m, 1H), 6.75 (d, 2H, *J* = 7.54Hz), 6.79 (d, 2H, *J* = 7.6Hz), 7.13 (m, 3H), 7.23-7.42 (m, 7H). ESIMS $(M+H)^+$ *m*/*z*: 391.

*1-(3-(4-Chlorophenoxy)-3-phenylpropyl)-4-phenylpiperazine (**AM4f**)*

Yield: 80%; IR (KBr) ν_{max} cm^{-1}: 3105, 3085, 2918, 2855, 1611, 1465, 1372, 1276, 1074. ^1H NMR (400 MHz, $CDCl_3$): 1.92-2.05 (m, 2H), 2.55-2.62 (m, 6H), 3.48-3.52 (m, 4H), 5.09-5.13 (m, 1H), 6.74 (d, 2H, *J* = 8.2Hz), 6.81 (d, 2H, *J* = 8Hz), 7.07 (m, 1H), 7.18 (d, 2H, *J* = 8.6Hz), 7.21-7.33 (m, 7H). ESIMS $(M+H)^+$ *m*/*z*: 407.

*1-Phenyl-4-(3-phenyl-3-(4-(trifluoromethyl)phenoxy)propyl) piperazine (**AM4g**)*

Yield: 85%; IR (KBr) ν_{max} cm^{-1}: 3101, 3082, 2911, 2870, 1605, 1605, 1380, 1265, 1071. ^1H NMR (400 MHz, $CDCl_3$): 1.93-2.01 (m, 2H), 2.55-2.62 (m, 6H), 3.46-3.55 (m, 4H), 5.17-5.21 (m, 1H), 6.74 (d, 2H, *J* = 7.2Hz), 6.81 (d, 2H, *J* = 7.6Hz), 7.03-7.09 (m, 2H), 7.20-7.31 (m, 7H). ESIMS $(M+H)^+$ *m*/*z*: 441.

*1-Benzyl-4-(3-phenoxy-3-phenylpropyl)piperazine (**AM5b**)*

Yield: 70%; IR (KBr) ν_{max} cm^{-1}: 3128, 3081, 2924, 2827, 2795, 1612, 1382, 1255, 1069. ^1H NMR (400 MHz, $CDCl_3$): 1.94-2.05 (m, 2H), 2.47 (m, 4H), 2.53-2.56 (m, 6H), 3.59 (s, 2H), 5.12-5.25 (m, 1H), 6.82 (d, 2H, *J* = 8.2Hz), 7.16-7.35 (m, 13H). ESIMS $(M+H)^+$ *m*/*z*: 387.

*1-Benzyl-4-(3-phenyl-3-(p-tolyloxy)propyl)piperazine (**AM5c**)*

Yield: 70%; IR (KBr) ν_{max} cm^{-1}: 3095, 3012, 2924, 2805, 1615, 1422, 1275, 1076. ^1H NMR (400 MHz, $CDCl_3$): 1.93-2.01 (m, 2H), 2.31 (s, 3H), 2.44 (m, 4H), 2.52-2.56 (m, 4H), 3.59 (s, 2H), 4.85-4.91 (m, 1H), 6.68 (d, 2H, *J* = 7.8Hz), 6.81 (d, 2H, *J* = 7.2Hz), 7.10 (d, 2H, *J* = 8Hz), 7.13-7.30 (m, 8H). ESIMS $(M+H)^+$ *m*/*z*: 401.

*1-Benzyl-4-(3-(4-bromophenoxy)-3-phenylpropyl)piperazine (**AM5d**)*

Yield: 75%; IR (KBr) ν_{max} cm^{-1}: 3085, 2911, 2872, 1614, 1437, 1365, 1258, 1075. ^1H NMR (400 MHz, $CDCl_3$): 1.91-2.02 (m, 2H), 2.41 (m, 4H), 2.49-2.55 (m, 4H), 3.55 (s, 2H), 5.02-5.16 (m, 1H), 6.81 (d, 2H, *J* = 8.4Hz), 7.16 (d, 2H, *J* = 7.6Hz), 7.21-7.34 (m, 10H). ESIMS $(M+H)^+$ *m*/*z*: 465.

*1-Benzyl-4-(3-(4-fluorophenoxy)-3-phenylpropyl)piperazine **(AM5e)***

Yield: 75%; IR (KBr) ν_{max} cm^{-1}: 3068, 2941, 2850, 2745, 1608, 1391, 1268, 1065. ^1H NMR (400 MHz, $CDCl_3$): 1.89-2.01 (m, 2H), 2.52-2.63 (m, 6H), 3.46-3.53 (m, 4H), 3.56 (s, 2H), 5.15-5.19 (m, 1H), 6.78 (d, 2H, *J* = 8Hz), 6.95 (d, 2H, *J* = 7.5Hz), 7.19-7.33 (m, 10H). ESIMS $(M+H)^+$ *m/z*: 405.

*1-Benzyl-4-(3-(4-chlorophenoxy)-3-phenylpropyl)piperazine **(AM5f)***

Yield: 72%; IR (KBr) ν_{max} cm^{-1}: 3115, 3083, 2905, 2863, 1605, 1452, 1373, 1278, 1075. ^1H NMR (400 MHz, $CDCl_3$): 1.92-2.05 (m, 2H), 2.55-2.62 (m, 6H), 3.48-3.52 (m, 4H), 5.09-5.13 (m, 1H), 6.83 (d, 2H, *J* = 8Hz), 7.16 (d, 2H, *J* = 8.2Hz), 7.21-7.33 (m, 10H); ESIMS $(M+H)^+$ *m/z*: 421.

*1-Benzyl-4-(3-phenyl-3-(4-(trifluoromethyl)phenoxy)propyl) piperazine **(AM5g)***

Yield: 80%; IR (KBr) ν_{max} cm^{-1}: 3101, 3085, 2911, 2870, 1605, 1599, 1380, 1269, 1073. ^1H NMR (400 MHz, $CDCl_3$): 1.85-2.04 (m, 2H), 2.34 (m, 4H), 2.51-2.60 (m, 6H), 3.58 (s, 2H), 5.14-5.19 (1H, m), 6.74 (d, 2H, *J* = 9.2Hz), 6.81 (d, 2H, *J* = 8.6Hz), 7.03-7.09 (m, 2H), 7.20-7.31 (m, 10H); ESIMS $(M+H)^+$ *m/z*: 455.

*4-Benzyl-1-(3-phenoxy-3-phenylpropyl)piperidine **(AM6b)***

Yield: 80%; IR (KBr) ν_{max} cm^{-1}: 3119, 3081, 2924, 2827, 2792, 1613, 1592, 1375, 1281, 1078; ^1H NMR (400 MHz, $CDCl_3$): 1.68-1.73 (m, 5H), 1.84-1.91 (m, 2H), 2.26-2.32 (m, 4H), 2.62 (d, 2H, *J* = 5.6Hz), 2.65 (d, 2H, *J* = 4.8Hz), 5.08-5.15 (1H, m), 6.85 (d, 2H, *J* = 7.6Hz), 7.14-7.33 (m, 13H). ESIMS $(M+H)^+$ *m/z*: 386.

*4-Benzyl-1-(3-phenyl-3-(p-tolyloxy)propyl)piperidine **(AM6c)***

Yield: 85%;, IR (KBr) ν_{max} cm^{-1}: 3086, 2889, 1611, 1589, 1455, 1276, 1082, ^1H NMR (400 MHz, $CDCl_3$): 1.61-1.72 (m, 5H), 1.79-1.88 (m, 2H), 2.21-2.30 (m, 4H), 2.3 2 (s, 3H), 2.62 (brd, 2H), 2.67 (t, 2H, *J* = 4.8Hz), 5.03-5.15 (1H, m), 6.88 (2H, *J* = 8.2Hz), 7.08 (2H, *J* = 7.8Hz), 7.16-7.31 (m, 10H). ESIMS $(M+H)^+$ *m/z*: 400.

*4-Benzyl-1-(3-(4-bromophenoxy)-3-phenylpropyl)piperidine **(AM6d)***

Yield: 70%; IR (KBr) ν_{max} cm^{-1}: 3115, 3082, 2905, 2864, 1609, 1588, 1461, 1385, 1262, 1078. ^1H NMR (400 MHz, $CDCl_3$): 1.63-1.75 (m, 5H), 1.80-1.87 (m, 2H), 2.18-2.27 (m, 4H), 2.66 (brd, 2H), 2.65 (t, 2H, *J* = 5.2Hz), 5.12-5.19 (1H, m), 6.82 (t, 2H, *J* = 6.8Hz), 7.18 (t, 2H, *J* = 7Hz), 7.16-7.31 (m, 10H). ESIMS $(M+H)^+$ *m/z*: 464.

*4-Benzyl-1-(3-(4-fluorophenoxy)-3-phenylpropyl)piperidine **(AM6e)***

Yield: 85%; IR (KBr) ν_{max} cm^{-1}: 3068, 2941, 2850, 2745, 1608, 1391, 1268, 1065. ^1H NMR (400 MHz, $CDCl_3$): 1.65-1.76 (m, 5H), 1.80-1.87 (m, 2H), 2.18-2.37 (m, 4H), 2.64 (brd, 2H), 5.14-5.19 (1H, m), 6.79 (t, 2H, *J* = 7.2Hz), 7.01 (t, 2H, *J* = 7Hz), 7.19-7.32 (m, 10H). ESIMS $(M+H)^+$ *m/z*: 404.

*4-Benzyl-1-(3-(4-chlorophenoxy)-3-phenylpropyl)piperidine **(AM6f)***

Yield: 82%; IR (KBr) ν_{max} cm^{-1}: 3105, 3075, 2918, 2844, 1608, 1423, 1384, 1275, 1082. ^1H NMR (400 MHz, $CDCl_3$): 1.63-1.78 (m, 5H), 1.80-1.85 (m, 2H), 2.20-2.31 (m, 4H), 2.62 (brd, 2H), 2.64 (t, 2H, *J* = 5.2Hz), 5.06-5.14 (1H, m), 6.84 (*J* = 7.4Hz), 7.13 (*J* = 7.2Hz), 7.16-7.29 (m, 10H); ESIMS $(M+H)^+$ *m/z*: 420.

*4-Benzyl-1-(3-phenyl-3-(4-(trifluoromethyl)phenoxy)propyl) piperidine **(AM6g)***

Yield: 88%; IR (KBr) ν_{max} cm^{-1}: 3105, 3085, 2911, 2854, 1609, 1599, 1380, 1257, 1078. ^1H NMR (400 MHz, $CDCl_3$): 1.61-1.76 (m, 5H), 1.83-185 (m, 1H), 2.19-2.28 (m, 4H), 2.60 (d, 2H, *J* = 4.8Hz), 2.65 (t, 2H, *J* = 5.2Hz), 5.11-5.15 (m, 1H), 6.82 (d, 2H, *J* = 7.2Hz), 7.18 (d, 2H, *J* = 7.4Hz), 7.21-7.33 (m, 10H). ESIMS $(M+H)^+$ *m/z*: 454.

Bioactivity screening

The synthesized compounds were tested for antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) by Microplate Alamar Blue Assay (MABA) method (Franzblau *et al.*, 1998). To determine selective TB activity, they were also tested for antimicrobial activity. They were tested against *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) *Escherichia coli* (ATCC 26), *Aspergillus niger* (ATCC 9642) and *Candida albicans* (ATCC 10231). Antimicrobial activity (MIC) of the test compounds were determined by the broth microdilution method, with minor modifications as described below (Daouk *et al.*, 1995; Hanel *et al.*, 1988).

Antitubercular activity

The method used for antitubercular activity (Franzblau *et al.*, 1998): Growth on Löwenstein Jensen (LJ) medium was suspended in sterile Middle brook 7H9 broth supplemented with 0.2% glycerol and 10% OADC (oleate-albumin-dextrose-catalase) enrichment and a 1:20 dilution used as the inoculum for MABA. All manipulations were performed with appropriate safety hoods. 200 µL of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100µL of the Middle brook 7H9 broth and serial dilution of compounds were made directly on the plate. The final drug concentrations tested were 0.01 to 32 µg/mL**.** Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µL of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of the compound required to give complete inhibition of bacterial growth. The results were depicted in Table 1.

Table 1: The Antitubercular activity of the synthesized compounds.

Compound code	MIC (µg/mL)	Compound code	MIC (µg/mL)	Compound code	MIC (µg/mL)
AM1	12.5	AM2g	6.25	AM5b	50
AM2	3.12	AM3b	12.5	AM5c	50
AM3	3.25	AM3c	12.5	AM5d	25
AM4	6.25	AM3d	3.25	AM5e	12.5
AM5	6.25	AM3e	1.62	AM5f	12.5
AM6	25	AM3f	3.25	AM5g	6.25
AM1a	50	AM3g	1.62	AM6b	>100
AM1b	>100	AM4b	12.5	AM6c	>100
AM1c	>100	AM4c	12.5	AM6d	100
AM1d	100	AM2a	25	AM6e	50
AM1e	50	AM3a	25	AM6f	100
AM1f	50	AM4a	50	AM6g	50
AM1g	50	AM5a	50	INH	1.6
AM2b	50	AM6a	>100	Pyrazinamide	3.125
AM2c	50	AM4d	6.25	Streptomycin	6.25
AM2d	25	AM4e	3.25	Ciprofloxacin	3.125
AM2e	3.25	AM4f	6.25		
AM2f	6.25	AM4g	3.25		

Antimicrobial activity

Antibacterial activity

The test compounds were dissolved in DMSO, and further diluted to 1:50 in RPMI-1640 medium and each resulting solution was used for a doubling dilution series. Microtiter plates were prepared to contain 100 µL of undiluted extracts in the first well, followed by doubling dilutions of extracts from second well onwards. A standardized inoculum of each bacterial species was added to the respective dilution wells including the first well. The final concentrations of the compounds ranged from 100 to 0.8 µg/mL. For each test, there were sterility control wells containing test compound in RPMI-1640 broth plus DMSO and a growth control well containing bacterial suspension without test compound. The microtiter plates were incubated at 35 ± 2°C for 24 hours with their upper surface covered by sterile sealers. The lowest concentration of the test compound that did not show any visible growth was considered MIC of the compound for that bacterial species. All the experiments were carried out in triplicate. The results were depicted in Table 3.

Antifungal activity

Antifungal activity was evaluated by using agar well diffusion method against fungi *Aspergillus niger-NCIM 652 and Candida albicans-NCIM 3102*. 1 mg/mL stock solution of the selected compounds and Ketoconazole (antifungal standard) were prepared using methanol as a vehicle. Sabouraud's medium was used for fungal studies. 100 µL of the inoculum was added to the sterilized agar medium, mixed, poured into sterile Petri plates and allowed to solidify. Wells of 6mm diameter were made by using a borer and 100µL of the synthesized compounds, control and standards were transferred to them using micropipette. The Petri plate was incubated at 26ºC for about 48 hrs to determine the zone of inhibition. None of the compounds showed fungal activity.

Drug-inhibitor combination studies (Rastogi *et al.*, 1994)

The *x/y* quotient calculation method was used with slight modification to evaluate the combined drug (plus inhibitor) action. For this procedure, all the drugs and inhibitors were used at sub-lethal concentrations. The combined drug-inhibitor activity was assessed by calculating *x/y* quotients as follows. The y value was the MIC obtained with the combination of drug-inhibitor by using the MABA method, whereas the *x* value was the lowest MIC obtained at the same time with the drug or the inhibitor used alone. For combinations, an *x/y* value of 1 indicated that there was no interaction between the two drugs, a quotient of <0.5 indicated enhanced drug action, whereas an *x/y* quotient of >2 indicated the presence of antagonism between the drug and the inhibitor. The drugs or inhibitors were added at final concentrations 12.5, 6.12, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1. 0.05 and 0.025 µg/mL in 1:1 ratio. MIC of the drug-inhibitor combination is compared with the MIC of the drug. The results were depicted in Table 2.

Table 2: Combined drug-inhibitor activity against *M. tuberculosis* H37Rv assessed by MABA method.

S.No	Compound Code	Enhancement of drug activities (x/y coefficients)
1	AM3e+ INH	+++ (0.031)
2	AM3e + RIF	+ (0.50)
3	AM3e + CIP	+ (0.125)
4	AM3e + STR	+ (0.25)

INH - Isonicotinic acid hydrazide; RIF- Rifampicin; CIP - Ciprofloxacin, STR - Streptomycin.

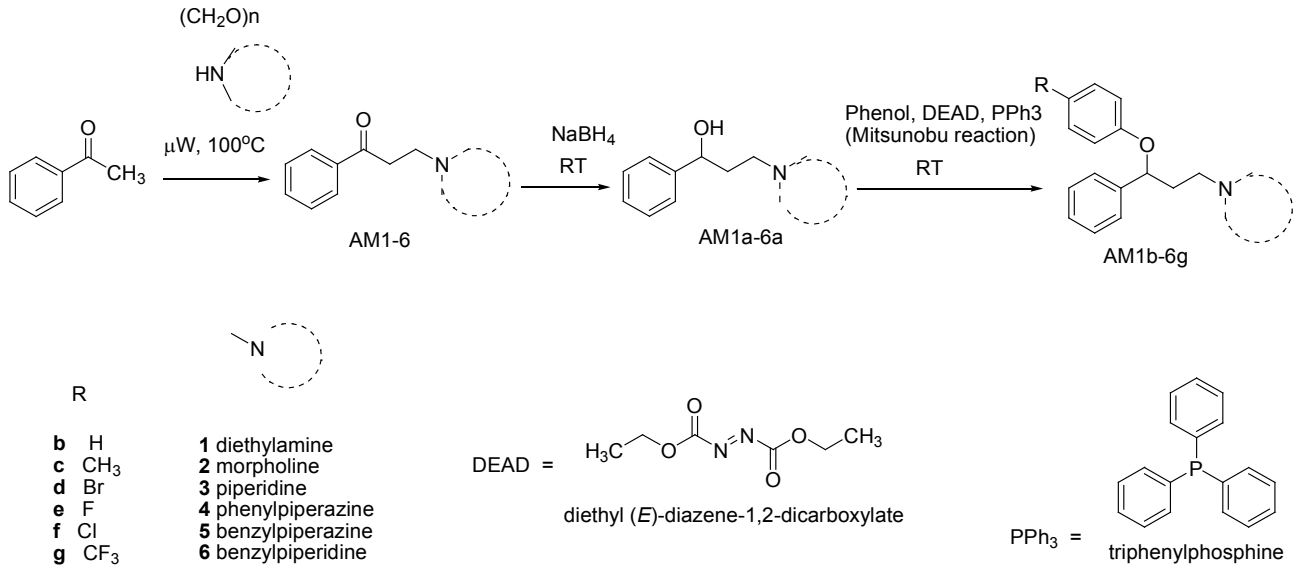
RESULTS AND DISCUSSION

Our group has recently reported anti-TB activity for fluoxetine. This drug is a very important ingredient in antidepressant formulations currently used around the world. Hence, it cannot be considered for drug repurposing in anti-TB category because of its CNS activity and current clinical applications. As per the reported structure-activity relationship (SAR) data, we designed fluoxetine analogues with increased bulk on nitrogen to abolish CNS activity and obtain selective anti-TB agents (Andersen *et al.*, 2009; Wenthur *et al.*, 2014; Larsen *et al.*, 2016).

The precursors of the target compounds, the aryloxyphenyl propanolamines (AM1b-6g), were obtained (Scheme 1) via synthesis of appropriate acetophenone mannich bases (**AM1-6**) followed by reduction (**AM1a-6a**) using sodium borohydride (Ali S *et al.*, 2002; Ianni A *et al.*, 2006). The resultant 3-(alkyl/arylalkyl-amino)-1-phenylpropan-1-ols (**AM1a-AM6a**), were then made to react with appropriate phenol using Mitsunobu reaction to obtain a total of 36 compounds (**AM1b-AM6g**) (Mitsunobu O, 1981).

Table 3: Antibacterial activity of the synthesized compounds.

Compound code	Minimum Inhibitory Concentration (MIC90) in µg/mL			
	S. aureus	***B. subtilis***	***E. coli***	***P. vulgaris***
AM1	50	50	>100	>100
AM2	25	25	100	100
AM3	25	25	100	100
AM4	50	25	>100	>100
AM5	50	50	>100	>100
AM6	50	50	>100	>100
AM1a	>100	100	>100	>100
AM2a	100	50	>100	>100
AM3a	>100	100	>100	>100
AM4a	100	100	>100	>100
AM5a	100	100	>100	>100
AM6a	>100	>100	>100	>100
AM1b	100	>100	>100	>100
AM1c	100	50	>100	>100
AM1d	50	50	>100	100
AM1e	50	50	>100	100
AM1f	100	100	>100	100
AM1g	50	50	>100	>100
AM2b	100	>100	>100	>100
AM2c	50	50	>100	100
AM2d	50	50	100	>100
AM2e	25	25	50	>100
AM2f	50	50	100	100
AM2g	25	25	50	>100
AM3b	100	100	>100	100
AM3c	50	50	>100	>100
AM3d	25	25	100	>100
AM3e	25	25	100	100
AM3f	50	50	100	100
AM3g	25	25	100	100
AM4b	100	100	100	100
AM4c	50	50	100	100
AM4d	50	50	100	100
AM4e	25	25	100	>100
AM4f	50	50	100	100
AM4g	50	50	100	>100
AM5b	100	100	100	>100
AM5c	50	50	100	100
AM5d	50	50	>100	100
AM5e	50	25	>100	100
AM5f	100	50	100	100
AM5g	50	25	>100	100
AM6b	>100	>100	>100	>100
AM6c	50	50	100	100
AM6d	50	50	100	100
AM6e	50	50	100	100
AM6f	50	100	100	100
AM6g	50	50	100	100



Scheme 1: Plan of synthesis for the target compounds.

The IR spectrum of the mannich bases (**AM1a-AM6a**) showed characteristic signals for ketone (C=O str, 1670-1690 cm^{-1}), amine (C-N str, 1280-1370), aromatic ring (-C=C- 1610-1640 cm^{-1}, C-H str, 2850-2980 cm^{-1}). The ^{1}HNMR of AM1-6 showed signals for the aromatic ring in the range δ 7.16 to 7.35. Characteristic signals of the deshielded protons present on C-2 and C-6 of the benzene ring (ortho to C=O group of the mannich base) at 7.85 to 7.98 as a doublet.

Further, the 3-(alkyl/arylalkyl-amino)-1-phenylpropan-1-ols the precursors needed for fluoxetine analogue synthesis were obtained in quantitative yield. The compounds were obtained in the nearly pure state as viscous oils. The IR spectrum clearly showed the absence of signal for ketone (1670-1690 cm^{-1}) and appearance of a new broad signal at 3240-3310 cm^{-1} indicating the formation of alcohol. The ^{1}HNMR also showed a -CH-OH signal at δ 4.3 to 5.0. The compounds were obtained as racemic mixtures, which were used as such for the next reaction. Formation of the target molecules (3-phenyl-3-(4-aryloxy)propyl)amines) were confirmed by the appearance of proton present on carbon bearing ether group at δ 4.80-5.25 and disappearance of IR signal for -OH at 3200-3350 cm^{-1}.

The synthesized compounds were screened for antimicrobial and antitubercular activity to obtain their selective toxicity profile. Among these (Table 1), AM3e showed four times more in vitro anti-TB activity (MIC 1.62 μg/mL) than fluoxetine (MIC 6.25 μg/mL) and is virtually free from antibacterial and antifungal activity (Table 3).

Among the others, the aryl ethers synthesized using 4F-phenol and 4-CF_3-phenol showed the highest potency. Change in the amine altered the activity in the order: morpholine = piperidine>phenylpiperazine = benzylpiperazine>diethylamine = benzylpiperidine (Figure 1). The derivatives obtained from AM2 (morpholine), AM3 (piperidine), AM4 (phenylpiperazine) showed the highest potency. This study clearly showed the importance of electron withdrawing substituent at the C-4 position of the phenoxy ring.

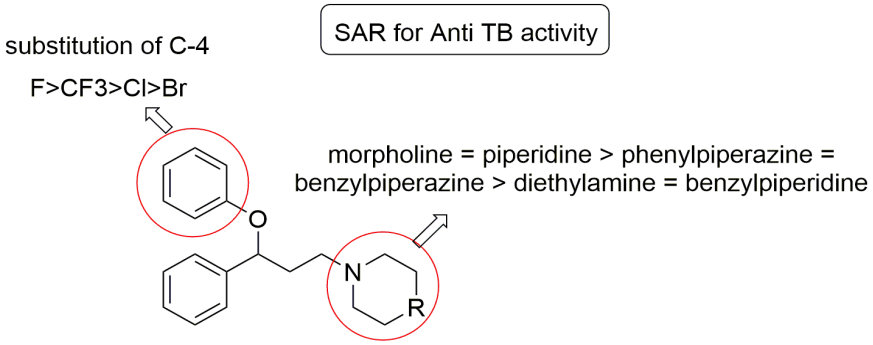


Fig. 1: SAR observed in anti-TB activity studies of the test compounds.

Among the synthesized compounds, mannich bases (**AM1-6**) showed feeble antibacterial activity against gram +ve test organisms with a MIC of 25-100 μg/mL. Reduction of the keto group on mannich bases significantly reduced their antibacterial activity. The aryloxyphenyl-propylamine (**AM1b-6g**) derivatives also showed antimicrobial activity with a MIC of 25-100 μg/

mL. None of the synthesized compounds have shown antifungal activity below 100 µg/mL. These observations ratified selective toxicity profile of the synthesized compounds.

Further, combined drug-inhibitor studies were performed to obtain mechanistic information. In these studies, AM3e showed synergism with INH, Rifampicin, Ciprofloxacin, and Streptomycin. This compound has also shown synergistic activity with the first line anti-TB drugs (Table 2) suggesting a new molecular target for this compound.

CONCLUSION

In our study, we identified potent and selective anti-TB activity in 1-(3-(4-fluorophenoxy)-3-phenylpropyl) piperidine (**AM3e,** MIC 1.6 µg/mL) via optimizing the structure of fluoxetine. This compound has also shown synergism with first line anti-TB drugs. Further, our strategy could be used for efficient drug repurposing and to discover new leads for different diseases using known pharmacophores.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

We have no conflict of interest.

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