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Synthesis and Evaluation of Antitubercular Activity of Novel Diphenyl Ether Derivatives

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ABSTRACT: Novel derivatives of diphenyl ether scaffold were synthesized and evaluated for the antitubercular activity. All the synthesized molecules were characterized by spectral analysis. Evaluation of antitubercular activity of the molecules by Microplate Alamar Blue Assay (MABA) showed less to moderate activity in comparison with Triclosan. All the molecules exhibited an acceptable safety profile in the MTT assay against vero and HepG2 cell lines.

KEYWORDS: Diphenyl Ether; Antitubercular Activity; Triclosan; Safety Profile.

INTRODUCTION

Tuberculosis, or TB, is an communicable disease caused by *Mycobacterium tuberculosis*, considered as one of the most threatening diseases for public health[1]. The majority of new cases and deaths occur in Asia AfricaandAsia: in particular,ChinaandIndia together account fornearly 40% of the world's TB burden[2]. The current therapy is around 50 years old and requires 6 months of treatment and 20 months in the case of MDR-TB.

The problems of current therapy are ever increasing resistance, serious side effects of some anti TB drugs, long term treatment and incompatibility of antiretroviral therapies to current TB regimen. To address these problems the development of novel anti-TB agents with stronger efficacy have become an urgent priority.Mycobacterial Enoyl Acyl Carrier protein Reductase (Mtb ENR or InhA) is acrucial enzyme of mycolic acid biosynthesis pathway which is needed for the formation of mycobacterial cell wall.InhA has already been known as the molecular target of the frontline antitubercular drugs like Isoniazid (INH) and Ethionamide (ETA).[3,4] Well-known local antibacterial agent Triclosan has been reported to be an InhA inhibitor[5]. Hence in this study, we report synthesis, antitubercular activity and in vitro safety evaluation of some Triclosan like diphenyl ether derivatives. The drug-likeness of Triclosan has always been seriously questioned. Hence novel diphenyl ethers derivatives were designed by modifying the Triclosan template to afford better druggability (Figure 1).

MATERIALS & METHODS

All the chemicals used as starting materials and catalysts in this study were purchased from Aldrich Chemical Co., Spectrochem Ltd., TCI Chemicals and Himedia. All the

solvents used were obtained from S.D. Fine Chemicals Ltd. Column chromatography was performed using100-200 mesh silica gel. Progress of the reactions was monitored by TLC using Aluminum backed sheets of silica gel 60 F24 (Merck). Melting points were recorded with a laboratory melting point apparatus and are uncorrected. ^{1}HNMR and ^{13}CNMR spectra were recorded on a NMR Spectrometer (AV400 - 400 MHz High Resolution Multinuclear FT-NMR Spectrometer, Bruker) using DMSO-d_6 as the solvent. Mass spectroscopy was performed using LC-MS (Agilent 6520 series, Q-TOF LC/MS) and GC-MS (Shimadzu, GCMS-QP5050A). IR spectrum was obtained using FT IR Spectrophotometer (IR Affinity-1, Shimadzu) using KBr pellets.

Synthesis of 1-(3-methoxy-4-phenoxyphenyl) ethanone (1)

To the stirred solution of 4-hydroxy-3-methoxy-acetophenone (3 g, 18.05 mmol) in anhydrous dichloromethane (90 mL), activated molecular sieves (4A°, 3 g) were added. Phenylboronic acid (4.49 g, 36.82 mmol), copper (II) acetate (7.36g, 40.58 mmol) and anhydrous pyridine (5.70 g, 72.15 mmol, 5.82 mL) were added successively to the reaction mixture. The resultant suspension was stirred at 25-27 °C. Progress of the reaction was scrutinised by TLC, using hexane: ethyl acetate (8:2). When the reaction was completed(72h), it was diluted with dichloromethane (40 mL) and filtered under vacuum. The filtrate was washed with dilute HCl (2 M, 50 mL), followed by water (50 mL), dried over anhydrous $MgSO_4$ and evaporated under vacuum. The crude compound was purified by column chromatography over silica 100-200 with hexane: ethyl acetate (8:2) as the mobile phase to afford the target compound as white crystalline solid.

Yield= 4.12 g (94%); mp = 82-84 °C R_f = 0.9 (hexane: ethyl acetate = 8:2); λmax = 269.6 nm (MeOH); IR (KBr, cm^{-1}): 3051 (Ar-H str.), 2928 (C-H str.), 1674 (C=O str.), 1583, 1489, 1413 (Ar-C=C str.), 1276 (Asym. C-O-C str.), 1134 (Sym.C-O-C str.); ^{1}HNMR (400 MHz, DMSO-d_6): 7.60 (d, *J* = 8 Hz, 2H), 7.36 (t, *J* = 7.8 Hz, 2H), 7.12 (t, *J* = 7.2 Hz, 1H), 7.00 (d, *J* = 8 Hz, 1H), 6.94 (d, *J* = 8 Hz, 2H), 3.83 (s, 3H), 2.56 (s, 3H).

Synthesis of 1-(3-hydroxy-4-phenoxyphenyl) ethanone (2)

To a stirred solution of 1-(3-methoxy-4-phenoxyphenyl) ethanone (4 g, 16.52 mmol) in anhydrous dichloromethane (60 mL), BBr_3 (1M, 8.28 g, 33.04 mmol) was added in presence of N_2at -78 °C. The resulting reaction mixture was stirred for 2h at -78 °C. Then the temperature of the reaction mixture was allowed to increase up to 10 °C and stirred continuously. Progress of the reaction was monitored by TLC, using hexane: ethyl acetate (6:4). When the reaction was completed (6h), the reaction was quenched by pouring it into ice cold saturated sodium bicarbonate solution with continuous stirring. The DCM layer was separated, washed with water, brine, dried over anhydrous $MgSO_4$ and evaporated under vacuum. The crude compound was purified by column chromatography over silica 100-200 using hexane: ethyl acetate (6:4) as the mobile phase to give, off white crystalline compound.

Yield= 3.6 g (96%); mp = 87-89 °C; R_f = 0.73 (hexane: ethyl acetate = 8:2); λmax = 269.4 nm (MeOH); ^{1}HNMR (400 MHz, DMSO-d_6): 9.89 (s, 1H), 7.51 (d, *J* = 2 Hz, 1H), 7.45-7.43 (dd, *J* = 8.4 Hz and 2.4 Hz, 1H), 7.37-7.33 (m, 2H), 7.11-7.07 (m, 1H), 6.96 (s, 1H), 6.94-6.92 (m, 2H), 2.50 (s, 3H); ^{13}CNMR (100.64 MHz, DMSO-d_6): 27.02, 116.81, 117.90, 120.57, 121.27, 123.51, 130.33, 134.03, 148.15, 149.23, 157.17, 197.14; LCMS (+ESI, m/z): 229.085 $(M+H)^+$.

General method for synthesis of 2-Phenoxy-5-(1-arylamino -ethyl)-phenol (3a-e)

To the stirred solution of 1-(3-methoxy-4-phenoxyphenyl) ethanone (0.2 g, 0.93 mmol) in methanol (10mL), amine (1.12 mmol) was added. The resulting reaction mixture was refluxed at 64-65 °C for 7h. Progress of the reaction was monitored by TLC, using hexane: ethyl acetate (8:2). After the completion of reaction,$NaBH_3CN$ (sodium cyanoborohydride)was added in three portions and refluxed at 64-65 °C for 3h. Progress of the reaction was supervised by TLC, using hexane: ethyl acetate (8:2). When the reaction was completed, excess solvent was evaporated from it under vacuum. The obtained residue was added with water and extracted with ethyl acetate (3x20 mL). The organic layers were separated, pooled and washed with saturated solution of $NaHCO_3$.The ethyl acetate layer was dried over anhydrous $MgSO_4$ and evaporated under vacuum. The crude compound was purified by column chromatography over silica 100-200 with hexane: ethyl acetate (8:2) as the mobile phase to afford the target compound as off white crystalline solid.

2-Phenoxy-5-(1-phenylamino-ethyl)-phenol (3a)

Yield= 0.13 g (49%); mp = 96-98°C; R_f = 0.52 (hexane: ethyl acetate = 7:3); λmax = 243 nm (MeOH); IR (KBr, cm^{-1}): 3305 (O-H str.), 3059, 2974 (Ar-H str.), 1595, 1498 (Ar-C=C str.), 1271 (Asym. C-O-C str.), 1219 (C-N str.), 1180 (Sym. C-O-C str.); LCMS (-APCI, m/z): 304.17 $(M-H)^-$.

2-Phenoxy-5-(1-p-tolylamino-ethyl)-phenol (3b)

Yield= 0.128 g (43%); mp = 114 -116°C; R_f = 0.71 (hexane: ethyl acetate = 7:3); λmax = 242 nm (MeOH); IR (KBr, cm^{-1}) = 3346 (O-H str.), 3030, 2924 (Ar-H str.), 1593, 1508 (Ar-C=C str.), 1282 (Asym. C-O-C str.), 1224 (C-N str.), 1180 (Sym. C-O-C str.); ^{1}HNMR (400 MHz, DMSO-d_6): 9.78 (s, 1H), 7.70 (s, 2H), 7.41 -7.36 (m, 2H), 7.27 – 7.22 (m, 5H), 6.83 (d, *J*= 6.4 Hz, 2H), 6.29 (s, 1H), 4.75 (s, 1H), 2.92 (s, 3H), 1.81 (s, 3H);^{13}CNMR (100.64 MHz, DMSO-d_6): 20.92, 25.56, 39.82, 40.23, 41.27,

52.78, 113.71, 115.51, 117.00, 118.01, 122.64, 130.38, 144.54, 149.93; LCMS (-APCI, m/z): 318.17 $(M-H)^-$.

5-[1-(4-Fluoro-phenylamino)-ethyl]-2-phenoxy-phenol (3c)

Yield= 0.15 g (53%); mp = 104 -106°C; R_f = 0.65 (hexane: ethyl acetate = 7:3); λmax = 237 nm (MeOH); IR (KBr, cm^{-1}): 3350 (O-H str.), 3068, 2970 (Ar-H str.), 1591, 1506 (Ar-C=C str.), 1282 (Asym. C-O-C str.), 1219 (C-N str.), 1163 (Sym. C-O-C str.); LCMS (-APCI, m/z): 322.25 $(M-H)^-$.

5-[1-(4-Chloro-phenylamino)-ethyl]-2-phenoxy-phenol (3d)

Yield= 0.1 g (33%); mp = 104 -106°C; R_f = 0.75 (hexane: ethyl acetate = 7:3); λmax = 256 nm (MeOH); IR (KBr, cm^{-1}): 3354 (O-H str.), 3062, 2972 (Ar-H str.), 1593, 1496 (Ar-C=C str.), 1280 (Asym. C-O-C str.), 1222 (C-N str.), 1180 (Sym. C-O-C str.); ^{1}HNMR (400 MHz, DMSO-d_6): 9.40 (s, 1H), 7.28 (s, 2H), 7.02 -7.00 (m, 3H), 6.93 (s, 1H), 6.87 (d, *J*= 7.2 Hz, 1H), 6.81 (s, 3H), 6.49 (d, *J*= 7.6 Hz, 2H), 6.34 (d, *J*= 4.8 Hz, 1H), 4.35 (s, 1H), 1.40 (d, *J* = 5.2 Hz, 3H);^{13}CNMR (100.64 MHz, DMSO-d_6): 24.47, 38.90, 39.52, 40.15, 51.72, 113.94, 114.50, 116.11, 117.04, 118.78, 121.77, 128.35, 129.48, 142.91, 146.83, 149.09, 157.99; LCMS (-APCI, m/z): 338.00 $(M-H)^-$.

5-[1-(2-Fluoro-phenylamino)-ethyl]-2-phenoxy-phenol (3e)

Yield= 0.15 g (33%);R_f = 0.63 (hexane: ethyl acetate = 7:3); λmax = 236nm (MeOH); IR (KBr, cm^{-1}) = 3527 (N-H str.), 3429 (O-H str.), 3066, 2970 (Ar-H str.), 1593, 1510 (Ar-C=Cstr.), 1271 (Asym. C-O-C str.), 1219 (C-N str.), 1192 (Sym. C-O-C str.); LCMS (-APCI, m/z): 322.00 $(M-H)^-$.

2-Bromo-1-(3-hydroxy-4-phenoxy-phenyl)-ethanone (4)

To a refluxing solution of cupric bromide ($CuBr_2$) (0.89 g, 4 mmol) in ethyl acetate (10 mL), 1-(3-hydroxy-4-phenoxyphenyl) ethanone (**2**)(0.5 g, 2mmol)dissolved in chloroform (5 mL) was added. The reaction mixture was refluxed for 8h. Completion of the reaction was probed by TLC, using hexane: ethyl acetate (8:2). The reaction mixture was cooled, filtered and the filtrate was evaporated under vacuum. The crude compound was purified by column chromatography over silica 100-200 with hexane: ethyl acetate (8:2) as the mobile phase to afford the target compound as off white crystalline solid.

Yield= 0.45 g (68%); mp = 74-76 °C; R_f = 0.78 (hexane: ethyl acetate = 8:2); λmax = 269.4 nm (MeOH); IR (KBr, cm^{-1}): 3346 (O-H str.), 3076 (Ar-H str.), 2966 (C-H str.), 1662 (C=O str.), 1577, 1496, (Ar-C=C str.), 1273 (Asym. C-O-C str.), 1215 (Sym.C-O-C str.); ^{1}HNMR (400 MHz, DMSO-d_6): 9.80 (s, 1H), 7.67-7.62 (m, 2H),7.39 (t, *J* = 8 Hz, 2H), 7.15 (t, *J* = 7.2 Hz, 1H), 6.99 (t, *J* = 8 Hz, 3H), 4.94 (s, 2H); LCMS (+APCI, m/z): 307.08 $(M+H)^+$.

General method for synthesis of 1-(3-Hydroxy-4-phenoxy-phenyl)-2-p-aryl oxy-ethanone (5a-b)

To a solution of 2-Bromo-1-(3-hydroxy-4-phenoxy-phenyl)-ethanone(**4**)(0.4g, 1.3mmol) in dry acetonitrile (10 mL), anhydrous Na_2CO_3 (0.275g, 2.6 mmol) and substituted phenol (1.3 mmol) were added. The reaction mixture was stirred for 2 h at 25-27 °C. Progress of the reaction was observed by TLC, using hexane: ethyl acetate (8:2). After the completion of reaction, the solvent was evaporated, added water, and extracted with ethyl acetate (3x20 mL). The organic layers were separated, pooled, washed with water, dried over anhydrous $MgSO_4$ and evaporated under vacuum. The crude compound was purified by column chromatography over silica 100-200 with hexane: ethyl acetate (8:2) as the mobile phase to afford the target compound as off white crystalline solid.

1-(3-Hydroxy-4-phenoxy-phenyl)-2-p-tolyloxy-ethanone (5a)

Yield = 0.30 g (68%); mp = 70 - 72 °C; R_f = 0.73 (hexane: ethyl acetate = 7:3); λmax = 276 nm (MeOH); IR (KBr, cm^{-1}): 3348 (O-H str.), 3080, 2966 (Ar-H str.), 1666 (C=O str.), 1577, 1489 (Ar-C=C str.), 1273 (Asym. C-O-C str.), 1215 (Sym. C-O-C str.); ^{1}HNMR (400 MHz, DMSO-d_6): 10.04 (s, 1H), 7.55-7.50 (m, 3H), 7.38 (t, *J* = 7.8 Hz, 3H), 7.13 (t, *J* = 7.4 Hz, 2H), 6.97 (t, *J* = 3.8 Hz, 4H), 4.84 (s, 2H), 3.19 (s, 3H);^{13}CNMR (100.64 MHz, DMSO-d_6): 33.68, 38.90, 39.53, 40.15, 116.91, 117.98, 119.75, 121.40, 123.30, 129.98, 130.36, 148.77, 156.37, 190.46; LCMS (+APCI, m/z): 335.25 $(M+H)^+$.

2-(4-Fluoro-phenoxy)-1-(3-hydroxy-4-phenoxy-phenyl)-ethanone (5b)

Yield = 0.32 g (72%); mp = 70 - 72 °C; R_f = 0.76 (hexane: ethyl acetate = 7:3); λmax = 276 nm (MeOH); IR (KBr, cm^{-1}) = 3350 (O-H str.), 3076, 2966 (Ar-H str.), 1666 (C=O str.), 1579, 1504 (Ar-C=C str.), 1313, 1273 (Asym. C-O-C str.), 1215 (Sym. C-O-C str.); ^{1}HNMR (400 MHz, DMSO-d_6): 10.03 (s, 1H), 7.55- 7.50 (m, 3H), 7.38 (t, *J* = 7.8 Hz, 3H), 7.13 (t, *J* = 7.4 Hz, 2H), 7.01-6.95 (m, 4H), 4.84 (s, 2H); ^{13}CNMR (100.64 MHz, DMSO-d_6): 33.68, 38.90, 39.53, 40.15, 116.91, 117.98, 119.75, 120.63, 121.40, 123.30, 129.89, 130.36, 148.77, 156.37, 190.46; LCMS (-APCI, m/z): 337.25 $(M+H)^-$.

General method for synthesis of 5-(1-Hydroxy-2-aryl oxy-ethyl)-2-phenoxy-phenol(6a-b)

To a solution of ketone (**5a-b)** (0.59 mmol) in THF (4 mL), methanol (4 mL) was added as a co-solvent. Resulting solution was added with $NaBH_4$ (1.08 mmol) in three portions and stirred at ambient temperature. Progress of the reaction was monitored by TLC, using hexane: ethyl acetate (8:2) as the mobile phase. After the completion of reaction (2h), the reaction mixture was evaporated under vacuum to remove the volatiles. The residue obtained was treated with ice cold water (25 mL)and extracted with ethyl acetate (3x25 mL). The

combinedorganic layers were washed with brine, driedover anhydrous $MgSO_4$ and concentrated under vacuum. The crude compound was purified by column chromatography over silica 100-200 with hexane: ethyl acetate (8:2) as the mobile phase to afford the target compound in quantitative yield.

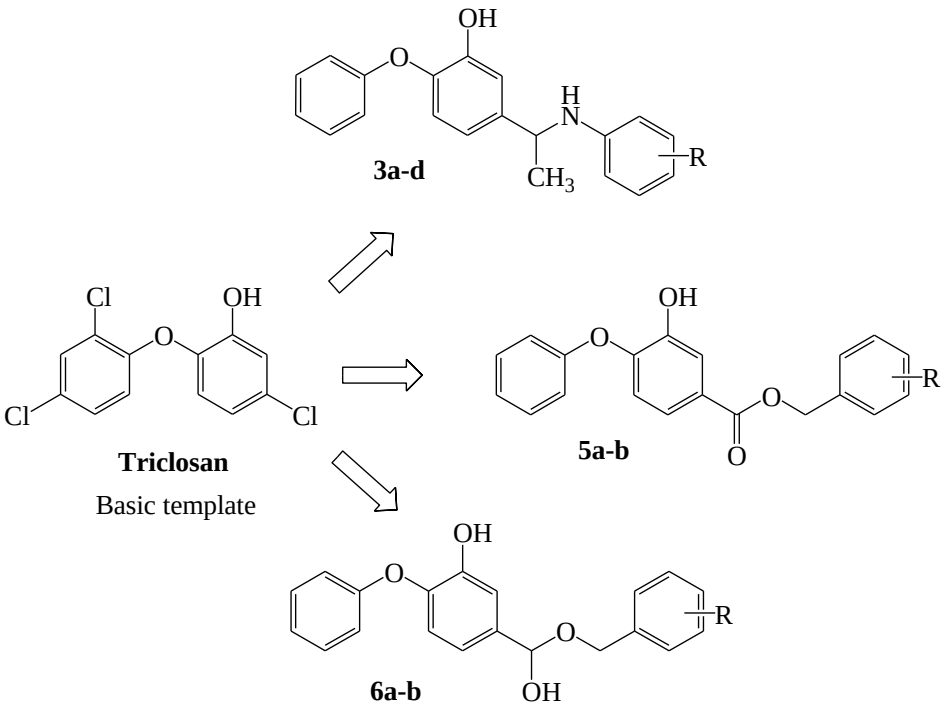
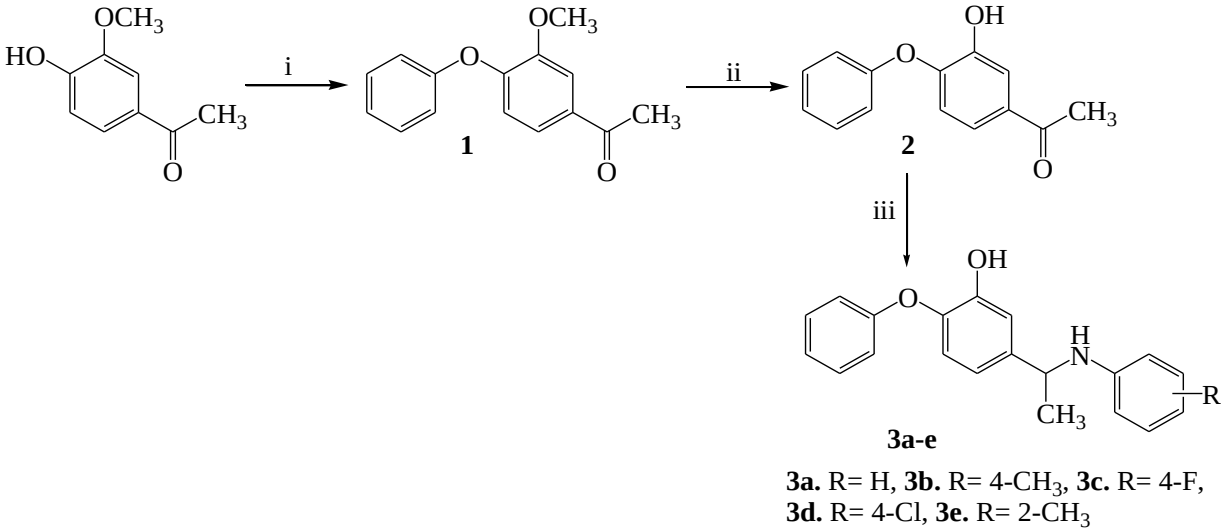


Figure 1. Design of Diphenyl Ether Derivatives.

Scheme -1[a]

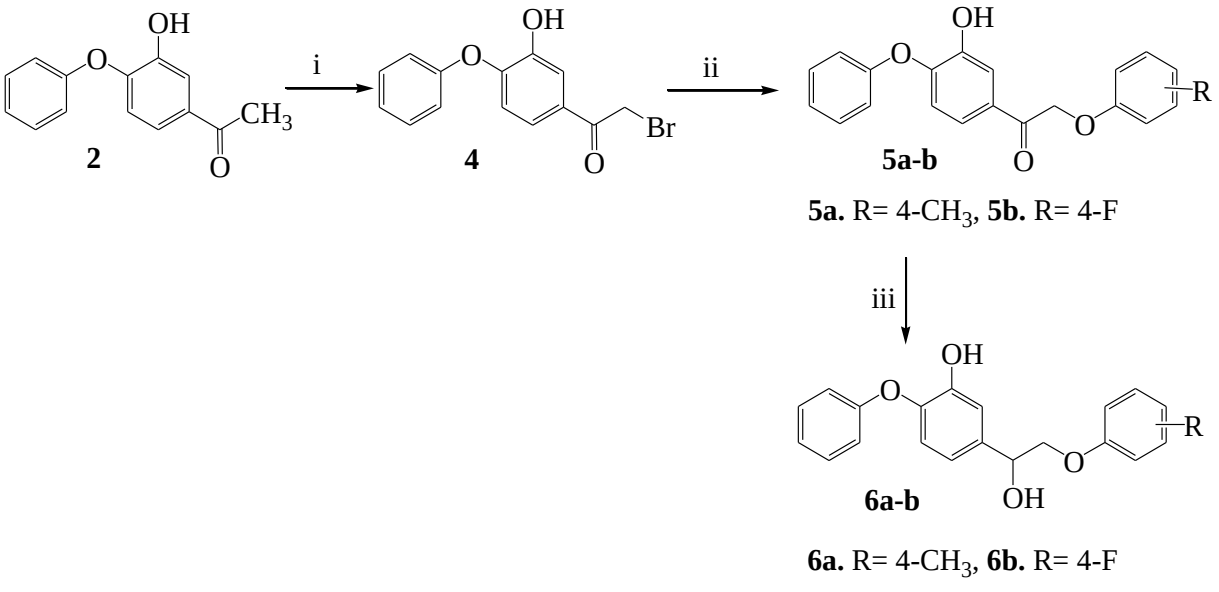


3a. R= H, **3b.** R= 4-CH_3, **3c.** R= 4-F, **3d.** R= 4-Cl, **3e.** R= 2-CH_3

[a]**Reagents and conditions :** (i). $PhB(OH)_2$, $Cu(OAc)_2$, C_5H_5N, CH_2Cl_2, 25-27 ^oC, 72h; (ii). BBr_3 (1M, CH_2Cl_2), CH_2Cl_2, -78^{0}C to 25-27^{0}C, 3h; (iii). $ArCH_2NH_2$, $NaBH_3CN$, MeOH, 75-80 ^oC, 48h;

Figure 1: Scheme for the Synthesis of Compounds 3a-e.

Scheme-2[a]



[a]**Reagents and conditions:** (i). Ar-OH, Na_2CO_3, MeCN, 25-27 ^oC, 2h; (ii). $NaBH_4$, MeOH, THF, 25-27 ^oC, 2h

Figure 2: Scheme for the Synthesis of Compounds 5a-b and 6a-b.

5-(1-Hydroxy-2-p-tolyloxy-ethyl)-2-phenoxy-phenol (6a)

Yield = 0.10 g (50%); mp = 90 - 92 °C; R_f = 0.43 (hexane: ethyl acetate = 7:3); λmax = 276 nm (MeOH); IR (KBr, cm^{-1}): 3398, 3307 (O-H str.), 3059(Ar-H str.), 2987(C-H str.), 1593, 1498 (Ar-C=C str.), 1271 (Asym. C-O-C str.), 1215 (Sym. C-O-C str.); ^{1}HNMR (400 MHz, DMSO-d_6): 9.49 (s, 1H), 7.30 (t, *J* = 8 Hz, 3H), 7.05- 6.99 (m, 3H), 6.90 (t, *J* = 5.4 Hz, 2H), 6.84 (d, *J* = 8 Hz, 1H), 6.77- 6.72 (m, 3H), 4.80 (t,*J* = 5.8 Hz, 1H), 4.10 (t, *J* = 5.6 Hz, 1H), 3.52- 3.48 (m, 2H), 3.19 (s, 3H);^{13}CNMR (100.64 MHz, DMSO-d_6): 38.90, 39.53, 40.15, 56.32, 65.78, 84.17, 115.53, 116.17, 118.17, 121.84, 129.50, 137.04, 141.81, 149.03, 157.94; LCMS (-APCI, m/z): 335.25 $(M+H)^-$.

5-[2-(4-Fluoro-phenoxy)-1-hydroxy-ethyl]-2-phenoxy-phenol (6b)

Yield = 0.05 g (60%); R_f = 0.47 (hexane: ethyl acetate = 7:3); λmax = 276 nm (MeOH); IR (KBr, cm^{-1}): 3332 (O-H str.), 3029 (Ar-H str.), 1595, 1500 (Ar-C=C str.), 1292 (Asym. C-O-C str.), 1219, 1114 (Sym. C-O-C str.).

Antitubercular activity

Standard Microplate Alamar Blue Assay (MABA) protocol[6,7] was followed to determine the minimum inhibitory concentration (MIC) of all the synthesised diphenyl ether derivatives. The synthesised compounds were screened against *Mycobacterium tuberculosis* H37Rv using serial dilution technique in middlebrook 7H9 broth medium. A blue colour in the well was interpreted as no bacterial growth and pink colour was scored as growth. The MIC was defined as the lowest drug concentration, which prevented colour change from blue to pink.

In vitro Cytotoxicity and Hepatotoxicity

Synthesized compounds were assessed for their *in vitro* safety profile against Vero and HepG2 cells using MTT assay.The cellular conversion of MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide] into a formazan product was used as an indicator to evaluate the cytotoxic effect of the synthesised compounds against a mammalian Vero cell line from the kidney of African Green monkey[8].MTT assay against HepG2 cells was performed by following the same method described for *in vitro* cytotoxicity screening against Vero cell line.In case of HepG2 cells, $5x10^3$ cells/well was added and MTT was added after 24h of incubation.

Optical density (OD) of the wells was measured at 540 nm using Elisa Reader. The CC_{50} values were determined using a curve-fitting program.

Optical Density (OD) readings from each well were entered in to the equations shown below to determine % Cell Viability and % Cell Inhibition.

% Cell Viability = (Optical Density of Test/Optical Density of Control) x100

% Cell Inhibition = 100 - % Cell Viability

RESULTS & DISCUSSION

Chemistry

The synthetic route employed for the preparation of **3a-e** is shown in scheme 1.Acetovanillone was used as starting material and condensed with phenyl boronic acid by Chan-Lam O-arylation reaction. The 1-(3-methoxy-4-phenoxyphenyl) ethanone**(1)** obtained was subjected to BBr_3 assisted O-demethylation reaction[9]to afford compound **2**.One pot reductive amination reaction[10]of compound **2**with different aromatic amines followed by reduction with mild selective reducing agent $NaBH_3CN$, affordedthe desired compounds **3a-e**.All the new compounds were characterized by their IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. Singlet in between δ 9-10 was assigned to phenolic –OH of diphenyl ether. Appearance of 8 aromatic protons in the region of δ 6-8 confirmed the formation of diphenyl ether ring. Presence of a singlet in between δ 3-5 indicates the methylene protons which confirm the formation of the desired compounds.

The synthetic route of compounds **5a-b** and **6a-b**are shown in scheme 2. Compound **2**was converted to α-bromo derivative by refluxing with cupric bromide in chloroform-ethyl acetate mixture.Sodium carbonate assisted O-alkylation of phenols having strategically placed functional groups with 2-Bromo-1-(3-hydroxy-4-phenoxy-phenyl)-ethanone (compound **4**) afforded compounds **5a-f** with good yield.In order to decrease the lipophilicity, the ketofunctionality of these compounds (**5a-f**) was reduced conventionally with $NaBH_4$to –OH(**6a-b).** All the compounds of this scheme were characterized by their IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. The carbonyl stretching of compounds **5a-b** at1666 $cm^{-1}$was disappeared in the IR spectra of compounds **6a-b**to confirm the complete reduction of keto group to -OH. A triplet for –CH proton and a singlet for –OH proton in between δ 4-5 in the ^{1}HNMR spectra of compounds **6a-b** confirm the formation of the desired compounds.

Biological evaluation

Compounds **3a-e, 5a-b** and **6a-b** were evaluated for antitubercular activity against *Mycobacterium tuberculosis* H37Rv using MABA technique (Table 1). The MIC is reported as the lowest concentration (µg/mL) of drug that visually inhibited the growth of organism. Triclosan and Isoniazid were used as standards. Safety profiles of the synthesized compounds are also presented in Table 1. Results show that most of the compounds exhibited less to moderate MIC values rangingfrom 25 to 100 µg/mL. In scheme 1, compound **3b**was found to be most active (MIC= 50 µg/mL) in which an electron donating methyl group was attached to the distal aromatic ring. When the methyl group was replaced with electron withdrawing groups (**3c**. R= 4-F, **3d**. R= 4-Cl), the activity was decreased two fold. To our surprise, changing the –Me group from para toortho position of the phenyl ring also abrogated the activity. In scheme 2,bioisosteric replacement of the nitrogen atom of the linker group with oxygen and introduction ofhydrogen acceptor/donor groups adjacent to the diphenyl ether nucleus, were envisaged to improve the antitubercular activity and druggability.

Table 1: In vitro Antitubercular Activity (MIC) and Cytotoxicity (CC_{50}) of Triclosan and Compounds 3a-e, 5a-b, and 6a-b.

Compounds	MIC (µg/mL)[a]	CC_{50} (µg/mL)[b]		ClogP[c]
		Vero	HepG2	
3a	100	>300	>300	4.579
3b	50	>300	>300	5.078
3c	100	>300	>300	5.024
3d	100	>300	>300	5.594
3e	100	>300	>300	5.078
5a	25	>300	>300	5.239
5b	50	>300	>300	5.023
6a	25	>300	>300	4.438
6b	25	>300	>300	4.222
Triclosan	12.5	-	-	5.528
INH	0.05	-	-	

[a]MIC = minimal drug concentration required to stop the growth of *Mycobacterial tuberculosis* H37Rv; [b]CC_{50} = minimal drug concentration required for 50% death of viable cells; [c]ClogP predicted from ChemDraw Ultra-2008.

Gratifyingly, compounds (**5a, 6a**and **6b**)of scheme 2 demonstrated better antiTB activity (MIC= 25µg/mL) when compared to the activity of the molecules of scheme 1. It was also observed that conversion of keto functionality of compound **5b** to hydroxyl group in compound **6b**,resulted a twofold improved activity (MIC= 25µg/mL). Interestingly, the inhibitory effect of electron withdrawing group (-F) on the activity of compound **6b** was not noticed. Even though the activity was lesser than the standard compounds, they had lesser lipophilicity (ClogP) than Triclosan. Notably, all the synthesized diphenyl ether derivatives displayed acceptable safety profile in the MTT assay against mammalian epithelial cells (Vero) and hepatocytes (HepG2).

CONCLUSION

A group of novel diphenyl ether derivatives has been designed by taking the Mtb ENR blocker Triclosan as the template. All the designed compounds were synthesized and evaluated for antitubercular activity against *Mycobacterium tuberculosis* H37Rv. Most of the compounds showed weak to moderate antitubercular activity. Most promising compounds **5a, 6a** and **6b** exhibited MIC at 25 µg/mL against *Mycobacterium tuberculosis* H37Rv. All of our synthesized compounds showed an excellent safety profile against vero and HepG2 cells. These compounds represent a crucial tool for developing prospective druggable diphenyl ether based antiTB agents.

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