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Design, Synthesis and Evaluation of Diphenyl ether Derivatives as Antitubercular and Antibacterial Agents

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ABSTRACT

A new series of diphenyl ether derivatives were synthesized from meta phenoxy benzaldehyde and aniline. The synthesized compounds were screened for *in-vitro* antimycobacterial, antibacterial and cytotoxicity studies. The synthesized compound 2a have shown potential activity against Mycobacterium tuberculosis H37Rv strain with MIC 12.5. These diphenyl ether derivatives were subjected to docking studies and virtual screening. The receptor specificity of the synthesized compounds were shown from the antibacterial activity study since none of the synthesized compounds showed activity, so it have been proved that newly synthesized compounds were specifically act on the enoyl acyl carrier protein receptor.

INTRODUCTION

Tuberculosis (TB) is an ancient infectious disease of global influence, re-emerged with multi-drug resistant strains (MDR-TB) and acquired immune deficiency syndrome (AIDS).

Tuberculosis typically attacks the lungs, but can also affect other parts of the body. Symptoms of TB disease depend on where in the body the TB bacteria are growing. TB disease symptoms may include: a bad cough that lasts 3 weeks or longer, coughing up blood or sputum, pain in the chest, weakness or fatigue, weight loss, chills and fever. It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit respiratory fluids through the air. Most infections do not have symptoms, known as latent tuberculosis. About one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected. *Mycobacterium tuberculosis (M.tuberculosis*) is the etiological agent for the tuberculosis (TB). The incidence of TB has steadily risen in the last years and TB is the world's second most common cause of death from infectious

diseases, after acquired immunodeficiency syndrome (AIDS). Furthermore, association of TB and AIDS is wide-spread. This resurgence affects both developed and developing countries with high rates of human immunodeficiency virus infection. Current chemotherapeutic treatments are based on the use of antibiotics, the most important being isoniazid (INH), rifampicin, pyrazinamide, ethambutol and streptomycin. The effectiveness of current anti-tuberculosis drugs to combat this infection is severely compromised by the emergence of multi-and extensively drug resistant tuberculosis (MDR TB and XDR TB).

Today's tuberculosis (TB) drug regimen takes too long to be effective and requires too many medications. Treatment of drug sensitive disease requires 6-9 months whereas treatment of drug-resistant TB is even lengthier, taking 18-24 months or longer. Second line drugs are also much more toxic and considerably more expensive than the standard first-line anti-TB regimen. Furthermore, current first-line treatment regimens are not compatible with the common antiretroviral therapies used to treat HIV/AIDS. Therefore, new drugs are needed which will be effective in treating active, resistant and latent TB infections, and will also be compatible with antiretroviral therapy. Treatment of MDR-TB is extremely expensive, toxic, arduous and often unsuccessful. MDR-TB is slowly tightening its deadly grip on India because TB patients often do not take the full course of treatment, as it is cumbersome. Consequently it helps the bacteria rejuvenate, learn how to neutralise the drug and survive. Drug resistance of *M.tuberculosis* is caused by mutations in relatively restricted regions of the genome. Various molecular methods have been developed to detect mutations in the genes coding for drug resistance of *M.tuberculosis*. PCR based DNA sequencing targeting the genes coding for drug resistance is one of the most reliable molecular methods to detect the mutations responsible for drug resistance.

In the last few years, *M.tuberculosis* enzymes involved in the fatty acid synthase type- 11 (FAS-11) systems have been identified and validated as relevant drug targets. Among the FAS-11 enzymes, InhA, a trans-2-enoyl-ACP reductase, is one of the most druggable targets in tuberculosis field. Despite enormous efforts have been made in the hunt for new drugs, tuberculosis (TB) still remains the first bacterial cause of mortality worldwide. Resistance of *Mycobacterium tuberculosis* (MTB) (pathogen agent of tuberculosis) strains to the two main drugs, isoniazid (INH) and rifampicin, used in therapy regimen of tuberculosis is a burning problem nowadays. Indeed, no new first line antibiotics have been introduced into the pharmaceutical field against this disease in the last 50 years. Thus it is well recognised that there is a pressing need for the development of more effective antimicrobial agents.

MATERIALS AND METHODS

Molecular modelling

Structure based drug design approach was utilized for the initial designing of the molecules. Structure Activity Relationships of diphenyl ether based antitubercular agents from published journals were also taken in to consideration. Modern medicinal chemistry strategies like Bioisosteric replacement and Scaffold hopping techniques were taken as methods for designing a diphenyl ether library. Prepared ligands were subjected to physicochemical properties screening by QikProp tool of Schrodinger-2011. Compounds with undesirable features like reactive groups and poor pharmacokinetic properties were removed. Descriptors like lipophilicity (logP), molecular weight, number of nitrogen and oxygen, hydrogen bond donor/ acceptor, solubility, % human absorption, number of rotors, polar surface area (PSA) were taken in to consideration for the virtual screening. Lipinski's parameter was also considered to select drug-like compounds. About 1000 druggable ligands were selected by virtual screening and subjected to molecular docking study. In all docking experiments, the X-ray structure of Mycobacterial tuberculosis ENR (pdb 1P45) [6] was used. In this crystal structure InhA protein was a heterodimer and the Chain-A consisted of two Triclosan moieties in the binding site. Among the two ligands, one was showing the required catalytic interactions with the amino acid residues while the other ligand was just lying above, showing some van der Waals interactions. Chain-B contained one Triclosan moiety and showed required interactions at the catalytic site of the protein. Hence chain-B was chosen for docking study to get better binding mode. Flexible docking

study was conducted with standard precision (SP). Epik state penalty was added to the docking score. Docking was limited to the ligands having < 300 atoms and < 50 rotable bonds. Van der Waal radii of the ligand atoms were scaled to 0.8 and the partial charge cut off was kept less than 0.15. The position of ligand was kept at the center of a 10 Å docking sphere. Default settings were used in Glide for all dockings. At the beginning, co-crystallized ligand (Triclosan) was extracted from the optimized protein and redocked to probe the RMSD and docking parameters. Then all the 1200 ligands qualified in virtual screening were docked with the Mtb InhA protein at the Triclosan binding site. Then all ligand docking results from SP docking was studied. Diphenyl ether derivatives incorporating the features of Triclosan at the Mtb ENR binding site were given priority for synthesis.

Synthesis

All the chemicals used as starting materials and catalysts in this study were purchased from NICE (Edappally), SIGMA ALDRICH, MERK (Mumbai). All of the solvents used were obtained from NICE (Edappally), HIMEDIA (Mumbai), MERK (Mumbai). All commercially available reagents procured were used without further purification. Column chromatography was carried out on 100-200 mesh silica gel. Progress of the reaction was monitored by TLC using aluminium backed sheets of silica gel from Merk. Melting points were recorded with a laboratory melting point apparatus (BOROSIL). 1H NMR and C13NMR spectra were recorded on a NMR spectrometer (400MHZ high resolution FTNMR spectrometer, BRUKER) using DMSOD6 as the solvent. Mass spectroscopy was performed using GC-MS (shimadzu GC-MS, QP-5050). IR spectrum was taken in FTIR spectrophotometer (FTIR spectrum model 400). UV absorbance of the compound was monitored by UV-VISIBLE spectrophotometer and λmax was recorded (Shimadzu UV-1800).

General method for synthesis of compounds (2a-h).

To the stirred solution of 3phenoxy-benzaldehyde [1] (0.5 mL, 2.5 mmol) in methanol (10mL), aniline (3 mmol) was added. The resulting reaction mixture was refluxed at 75-80 °C for 7h. Progress of the reaction was monitored by TLC, using pet.ether: ethyl acetate (9:1). After the completion of reaction $NaBH_4$ was added and refluxing was continued for 3 hours. When the reaction was completed, the solvent was evaporated; water was added and extracted with ethyl acetate (20x3 mL). The organic layers were combined 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 pet.ether (60-80): ethyl acetate (9:1) as the mobile phase to afford the target compound.

3-phenoxy benzyl) phenyl amine (2a)

Yield = 0.6 g (86%); R_f = 0.79 (pet.ether: ethyl acetate = 9:1); λmax = 240nm (MeOH);mp = 60-62 °C; IR (KBr, cm^{-1}) = 3421 (N-H str.), 3062 (Ar-H str.), 1602, 1591, 1579 (Ar-C=C str.), 1325 (Asym. C-O-C str.), 1232 (C-N str.), 1165 (Sym. C-O-C str.); ^{1}HNMR (400 MHz, DMSO-d_6): 7.38-7.31 (m, 3H), 7.14-7.10(m, 2H), 7.05-7.01 (dd, *J* =6.4Hz &*J*= 8 Hz, 5H), 6.84 (d, *J* =8Hz, 1H), 6.55- 6.49 (dd, *J* =8Hz &*J* = 7.2Hz, 3H), 6.23 (t, *J*=6Hz, 1H), 4.25 (d,*J* = 6Hz, 2H); ^{13}CNMR (100.62 MHz, DMSO-d_6): GCMS (EI, m/z): 276 $(M)^+$.

(4-Chloro phenyl) (3-phenoxy benzyl) amine (2b)

Yield = 0.385 g (49%); R_f = 0.84 (pet.ether: ethyl acetate = 9:1); λmax = 253nm (MeOH); IR (KBr, cm^{-1}) = 3388 (N-H str.), 3061, 2901 (Ar-H str.), 1581, 1495(Ar-C=C str.), 1315 (Asym. C-O-C str.), 1244 (C-N str.), 1163 (Sym. C-O-C str.); ^{1}HNMR (400 MHz, DMSO-d_6): 7.29 (t, *J* =6.6 Hz, 2H), 7.07 (d,*J*= 6.8 Hz, 2H), 6.99 (t, *J* =7 Hz, 1H), 6.94- 6.90 (m, 2H), 6.82-6.77 (m, 3H), 6.57 (d, *J*=7.2Hz, 2H), 6.44 (s, 1H), 4.182 (d,*J*= 4 Hz, 2H); ^{13}CNMR (100.62 MHz, DMSO-d_6): 38.86, 39.69, 40.11, 45.99,113.49, 115.94, 117.0, 120.98, 122.980, 128.50, 129.48, 132.77, 149.28, 157.09, 158.07; GCMS (EI, m/z): 309 $(M)^+$.

(4-Flouro phenyl) (3-phenoxy benzyl) amine (2c)

Yield = 0.70 g (94%); R_f = 0.86 (pet.ether:ethyl acetate = 9:1); λmax = 271nm (MeOH); IR (KBr, cm^{-1}) = 3425 (N-H str.), 3059, 2852 (Ar-H str.), 1583, 1508, 1444 (Ar-C=C str.), 1244 (Asym. C-O-C str.), 1209 (C-N str.), 1161 (Sym. C-O-C str.); ^{1}HNMR (400 MHz, DMSO-d_6): 7.37-7.30 (m, 4H),

7.12 (d, *J* = 7.6Hz, 1H), 6.95 (d, *J* = 8Hz, 2H), 6.89-6.82 (m, 4H), 6.53- 6.50 (m, 2H), 6.17 (t, *J* = 6Hz, 1H), 4.22 (d, *J* = 6.4Hz, 2H); ^{13}CNMR (100.62 MHz, DMSO-d_6): 38.86, 39.49, 40.11, 46.56, 55.26, 112.86, 115.93, 118.28, 120.01, 121.76, 123.37, 129.74, 137.49, 140.99, 145.39, 149.21, 153.36, 155.36, 158.08;GCMS (EI, m/z): 293 $(M)^+$.

(2-Flouro phenyl) (3-phenoxy benzyl) amine (2d)

Yield = 0.18 g (63%); λmax = 240nm (MeOH); IR (KBr, cm^{-1}) = 3516 (N-H str.), 3442 (O-H str.), 3055, 2860 (Ar-H str.), 1589, 1510 (Ar-C=C str.), 1298 (Asym. C-O-C str.), 1226 (C-N str.), 1166 (Sym. C-O-C str.); ^{1}HNMR (400 MHz, DMSO-d_6): 7.28 (t, *J* =7.4Hz, 2H), 7.03-6.877 (m, 5H), 6.80 (d, *J* = 7.2Hz, 3H), 6.60-6.51 (m, 2H), 6.16 (s, 1H), 4.27 (d, *J* = 5.2Hz, 2H); ^{13}CNMR (100.62 MHz, DMSO-d_6): 38.88, 39.93, 40.14, 45.46, 112.11, 114.32, 115.96, 118.00, 121.74, 124.60, 129.46, 136.58, 137.38, 141.00, 149.65, 152.01, 158.07.

(4-Bromo phenyl) (3-phenoxy benzyl) amine (2e)

Yield = 0.49 g (54%); R_f = 0.81 (pet.ether: ethyl acetate = 9:1); λmax = 307nm (MeOH); IR (KBr, cm^{-1}) = 3423 (N-H str.), 3062 (Ar-H str.), 1602, 1581, 1485(Ar-C=C str.), 1290 (Asym. C-O-C str.), 1245 (C-N str.), 1163 (Sym. C-O-C str.); ^{1}HNMR (400 MHz, DMSO-d_6): 7.27 (t, *J* =7.8Hz, 2H), 7.05-6.96 (m, 4H), 6.89 (d, *J* =8.4Hz, 2H), 6.79 (t, *J* =6.6Hz, 3H), 6.57 (d, *J* =8Hz, 2H), 6.50 (t, *J*=7Hz, 1H), 6.17 (s, 1H), 4.17 (s, 2H); ^{13}CNMR (100.62 MHz, DMSO-d_6): 38.86, 39.48, 40.11, 46.88, 55.26, 116.09, 117.84, 120.01, 122.93, 123.37, 129.93, 132.85, 149.18, 156.28; GCMS (EI, m/z): 354 $(M)^+$.

(3-Chloro 4-fluoro phenyl) (3-phenoxy benzyl) amine (2f)

Yield = 0.462 g (55%); R_f = 0.84 (pet.ether: ethyl acetate = 9:1); λmax = 312nm (MeOH); IR (KBr, cm^{-1}) = 3388 (N-H str.), 3061, 2901 (Ar-H str.), 1583, 1487(Ar-C=C str.), 1315 (Asym. C-O-C str.), 1244 (C-N str.), 1163 (Sym. C-O-C str.); GCMS (EI, m/z): 327 $(M)^+$.

(3-Chloro phenyl) (3-phenoxy benzyl) amine (2g)

Yield = 0.69 g (88%); R_f = 0.84 (pet.ether: ethyl acetate = 9:1); λmax = 312nm (MeOH); IR (KBr, cm^{-1}) = 3415 (N-H str.), 3061, 2901 (Ar-H str.), 1595, 1483(Ar-C=C str.), 1323 (Asym. C-O-C str.), 1246 (C-N str.), 1163 (Sym. C-O-C str.);

(4-Methoxy phenyl) (3-phenoxy benzyl) amine (2h)

Yield = 0.68 g (88%); R_f = 0.84 (pet.ether: ethyl acetate = 8:2); λmax = 239nm (MeOH); IR (KBr, cm^{-1}) = 3417 (N-H str.), 2831(Ar-H str.), 1581, 1510(Ar-C=C str.), 1315 (Asym. C-O-C str.), 1232 (C-N str.), 1211 (Sym. C-O-C str.);^{1}HNMR (400 MHz, DMSO-d_6): 7.28 (t, *J* = 8 Hz, 2H) 7.00- 6.77 (m, 8H), 6.49 (d, *J* = 8Hz, 2H), 5.98 (t, *J* = 5.8Hz, 1H), 4.15 (d, *J* = 6 Hz, 2H), 2.12 (s, 3H); ^{13}CNMR (100.62 MHz, DMSO-d_6): 46.88, 55.26, 113.16, 115.92, 118.27, 121.71, 129.46, 137.98, 140.88, 142.94, 149.16, 158.11.

BIOLOGICAL ACTIVITY

Anti-tubercular activity

The anti-mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. It is a fluorescence reduction assay of alamar blue (a resazurin) in a microplate format. It is designed to quantitatively measure the proliferation of human and animal cell lines, bacteria and fungi. Using the redox indicator resazurin (oxidised form), it is possible to spectrophotometrically measure the cellular proliferation. Resazurin is blue and non-fluorescent; whereas resorufin (reduced form) is red and highly fluorescent. Thus, measuring the changes in the fluorescence of the dye in the intracellular environment, modifications in the number of metabolic active cells can be detected [7]. 200µl of sterile deionised water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The

final drug concentrations tested were 100 to 0.2 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 Almar 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 MIC was defined as lowest drug concentration which prevented the color change from blue to pink (Maria C.S. Lourenco et al., 2007).

In vitro cytotoxicity

A number of methods have been developed to study the cell cytotoxicity of NCEs. Microplate assay using MTT is the most convenient method implemented widely in modern drug discovery. MTT Cell Assay is a safe, sensitive, in vitro colorimetric assay for the measurement of cell viability. This assay is based on enzyme (cytoplasmic NADH/ NADPH and succinate-tetrazolium reductase of mitochondrial respiratory chain) mediated reduction of yellow tetrazolium compound MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) to formazan, a purple dye by the viable cells. The optical density of formazan is directly proportional to the number of living cells in the culture. [10] Vero cells are the most common mammalian continuous cell lines used to study the cytotoxicity of NCEs. They are nothing but the kidney epithelial cells of African green monkey (*Cercopithecusaethiops*). [11] Outer perimeter wells of the 96 well plates were filled with sterile deionized water to prevent dehydration in test wells during incubation. Test sample dilutions were distributed at a volume of 100 µL to each wells containing monolayer of Vero cells in a 96-well plate. The plate was incubated at 37 °C with 5% of CO_2 inside an incubator. DMSO was used as blank. Positive control (only inoculum) and negative control (only media) were also maintained in the plate. After 72 h of incubation, supernant was removed from the wells and added with 50 µL of MTT (2 mg/mL) in dark. It was further incubated at 37°C for 3h. After the incubation, supernant was removed carefully from each well. 50 µL of sterile DMSO (filtered through 0.22 µm syringe filter) was added to each well. The plate was transferred to an incubator and kept at 37°C for 2h. Then the plate was placed in an Elisa Reader and the optical density (OD) of the wells was measured at 540 nm.

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

$$\% \text{ CellViability} = \frac{\text{Optical Density of Test}}{\text{Optical Density of Control}} \times 100$$

% Cell Inhibition = 100 - % Cell Viability

The CC_{50} values were determined using a curve-fitting program. The CC_{50} values represent an average of two individual measurements.

In vitro hepatotoxicity

Drug-induced hepatotoxicity is the most common side effect of current antitubercular drugs. [14, 15] It is also one of the chief contributors to high attrition rates during preclinical and clinical drug development. Hepatotoxicity screening of NCEs at the early phase of drug discovery can provide a better idea about the safety profile as most of the drugs metabolized by liver. However, microplate assay using MTT against cultures of HepG2 is the most efficient method reported to be used for in vitro hepatotoxicity screening. [16] This is an easy to handle reliable assay method which can provide more accurate human toxicity data by avoiding species variations [17] and less time consuming than animal models. [18] MTT assay against HepG2 cells was performed by following the same method described for in vitro cytotoxicity screening against Vero cells. HepG2 cells added to each well at a concentration of 7,00,00 cells/mL. MTT was added after 24h and 48h of incubation to visualize the morphological changes. [19] CC_{50} was calculated from following equations.

$$\% \text{ Cell Viability} = \frac{\text{Optical Density of Test}}{\text{Optical Density of Control}} \times 100$$

% Cell Inhibition = 100 - % Cell Viability

Antibacterial activity

Agar diffusion refers to the movement of molecules through the matrix that is formed by the gelling of agar. When performed under controlled conditions, the degree of the molecule's movement can be related to the concentration of the molecule. This phenomenon forms the basis of the agar diffusion assay that is used to determine the

susceptibility or resistance of a bacterial strain to an antibacterial agent, (e.g., including antibiotics). When the seaweed extract known as agar is allowed to harden, the resulting material is not impermeable. Rather, there are spaces present between the myriad of strands of agar that comprise the hardened polymer. Small molecules such as antibiotics are able to diffuse through the agar. Typically, an antibiotic is applied to a well that is cut into the agar. Thus, the antibiotic will tend to move from this region of high concentration to the surrounding regions of lower antibiotic concentration. If more material is present in the well, then the zone of diffusion can be larger. This diffusion was the basis of the agar diffusion assay devised in 1944. A bacterial suspension is spread onto the surface of the agar. Then, antibiotic is applied to a number of wells in the plate. There can be different concentrations of a single antibiotic or a number of different antibiotics present. Following a time to allow for growth of the bacteria then agar is examined. If bacterial growth is right up to the antibiotic containing well, then the bacterial strain is deemed to be resistant to the antibiotic. If there is a clearing around the antibiotic well, then the bacteria have been adversely affected by the antibiotic. The size of the inhibition zone can be measured and related to standards, in order to determine whether the bacterial strain is sensitive to the antibiotic. This technique can also be done by placing disks of an absorbent material that have been soaked with the antibiotic of interest directly onto the agar surface. The antibiotic will subsequently diffuse out of the disk into the agar. This version of agar diffusion is known as the Kirby-Bauer disk-diffusion assay. The agar diffusion assay allows bacteria to be screened in a routine, economical and easy way for the detection of resistance. More detailed analysis to ascertain the nature of the resistance can then follow [8, 9]. The organism was grown to logarithmic phase and the inoculum was prepared by adjusting the turbidity of the bacterial suspension to 0.5 M McFarland's standard tube. Petriplates containing 20ml Muller Hinton medium were seeded with the bacterial strain*; namely E.coli and Staphylococcus aureus*. Wells of approximately 10mm was bored using a well cutter and samples in 25, 50 and 100 µl conc. were added,. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993).

RESULTS AND DISCUSSION

Rationale behind design

Diphenyl ether nucleus is an important unit found in several synthetic and natural agents possessing wide range of pharmacological activities. A classic chemical with this nucleus is Triclosan. It is widely used in a variety of cleaning and personal care products for its broad spectrum antimicrobial activity against a variety of Gram positive and Gram negative bacteria. Continuous studies have been focused on diphenyl ether derivatives without altering its basic nucleus. They inhibit enoyl acyl carrier protein reductase (inhA), enzyme of mycobacterial cell wall, thisinhA is an essential enzyme of mycolic acid synthesis of mycobacterial cell wall. InhA is the primary target for the miraculous TB drug Isonicotinicacid. Resistance develop to INH because of mutations to genes responsible for the activity of INH. Triclosan also directly inhibit inhA. Triclosan, because of its inherent high lipophilicity and toxicity, cannot be used internally; hence we decided to modify the structure of Triclosan by decreasing the lipophilicity and toxicity while maintaining the antibacterial and antitubercular potency.

Molecular modelling

Designed compounds were subjected to virtual screening for predicting drug-likeness and molecular docking studies was done against Mtb ENR. Virtual screening and docking results of compounds **2a-h** are depicted in table and respectively. All of the compounds except **2g** violates Lipinski's filter in the case of logP values. Their oral absorption was predicted to be in the range of 100% in human. All the synthesized compounds were docked against Mycobacterial ENR (pdb 1P45) using Schrodinger suit-2011. Co-crystallized Triclosan was redocked at the binding site of the receptor to validate the docking parameters. Docking score of the molecules of these schemes were found to be superior to Triclosan. All the molecules were showing the same orientation in the binding pocket of InhA as that of Triclosan.

Chemistry

Analogues designed through *in silico* methods were chosen for wet lab synthesis based on the availability of chemicals and reagents. Out of the designed compounds, 8 molecules were selected to synthesize as mentioned in scheme-1. Synthesis is achieved via reductive amination reactionby the condensation of 3-phenoxy benzaldehyde and aniline in thepresence of glacial acetic acid. Here the solvent used wasmethanol. Schiff base was formed initially which was reduced insitu with $NaBH_4$, a reducing agent. All the compounds were separated from the reaction mixture and purified by column chromatography using petroleum ether (60-80) and ethyl acetate as the solvent system.

Biological evaluation

After confirming the structures through preliminary analysis and spectral characterization, the synthesized derivatives were subjected to preliminary biological screening for their antitubercular activity through microplate alamar blue assay. The assay includes the preliminary screening and determination of Minimum Inhibitory Concentration (MIC) of each sample. The concentrations of samples were taken in µg/ml ranging from 100- 3.125. The minimum concentration of each sample at which the mycobacteria found sensitive were recorded as MIC. The standards used were Triclosan and INH. The synthesized compounds were screened for their antitubercular efficacy against M. tuberculosis H37Rv (ATCC-27294) using the standard Microplate Alamar Blue Assay (MABA). It is evident from results (Table 5.4) that most of the synthesized compounds displayed antitubercular activity ranging from 25- 12.5µg/ml against M. tuberculosis H37Rv strain. Among the compounds tested, compound**2a,** the unsubstituted derivative has shown the most significant antitubercular activity than the other compounds. Compounds **2c, 2d, 2e, 2f, 2g, and 2h** showed similar antitubercular activity. Compound **2b** is comparatively weaker in activity than the other compounds. The activity of the most potent compound was similar to the activity of Triclosan, but the polarity was significantly lower than Triclosan. The cytotoxicity of all the synthesised compounds was evaluated by MTT assay using vero cells. The results are shown in table 5.4. All the compounds were found to be safe against verocell line even at 300µg/mL concentration. The selectivity index (SI index) of most of the compounds was greater than 10 indicating that all of them are safe for administration. Since hepatotoxicity is the major side effect of most of the antitubercular drugs, the hepatotoxicity of the synthesised compounds was also evaluated by MTT assay using HepG2 cells. None of the compounds showed any signs of toxicity even at the highest concentration evaluated, ie, at 300µg/mL. Antibacterial activity study was carried out by agar well diffusion method. In this study the medium selected is mullerhinton and the bacterial strains used are *E.coli* and *Staphylococcus aureus*. Activity was measured by means of zone of inhibition at concentrations 100, 50 and 25µg/ml. There is no antibacterial activity for the synthesized compounds. The receptor specificity of the synthesized compounds were shown from the antibacterial activity study since none of the synthesized compounds showed activity, so it have been proved that newly synthesized compounds specifically act on the enoyl acyl carrier protein reductase enzyme.

Structure activity relationship

The InhA inhibition activities of the diphenyl ether seriesof compounds are summarized in the table. Compound**2a**, without any substituent on ring C, displayed an activity of 12.5µg/ml and serves as themost active compound. The activity of **2b** decreased twofold when a –Cl- was introducedat the 4^{th} position of the phenyl ring C. The presenceof additional electron-withdrawing groups 4-F, 2-F, 4-Br, 3-Cl-4-F, 3-Cl on ring C of **2c, 2d, 2e, 2f, 2g, 2h** respectively, slightly increased the potency comparable to that of **2b**, but they had activity lesser than **2a**.The presence of 0-CH_3 group on ring C of **2h** also decreased the activity. The electron-withdrawing nature of the groupat the ortho, meta, and para-position of ring C is an important determinant of activity, as many analogues with para- orortho-substituents on ring C were found to be inactive or less active in the MIC determination.

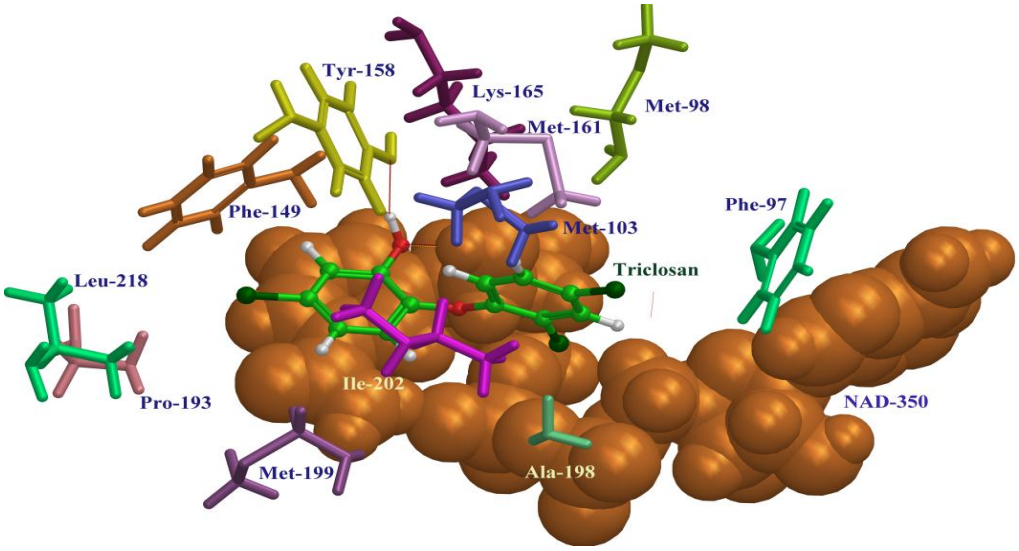


Figure 1. Molecular docking poses of TCL

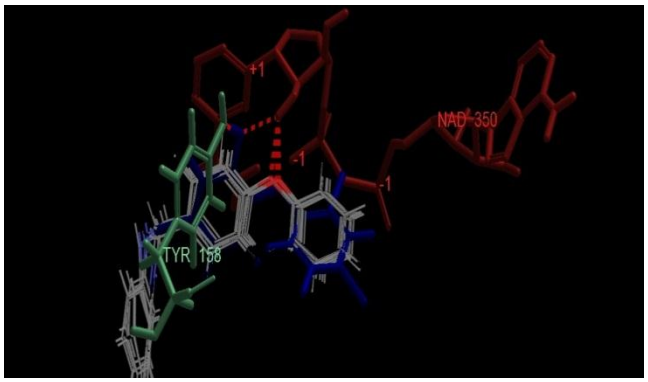


Figure 2. Overlapping of the molecules with Triclosan in the binding pocket of InhA

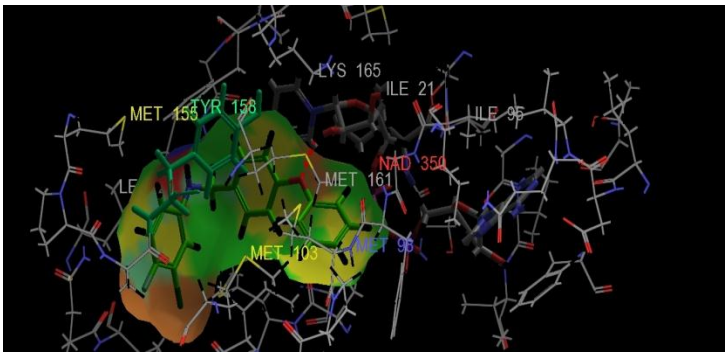


Figure 3. Docking pose of compound **2f** in the binding pocket of InhA.

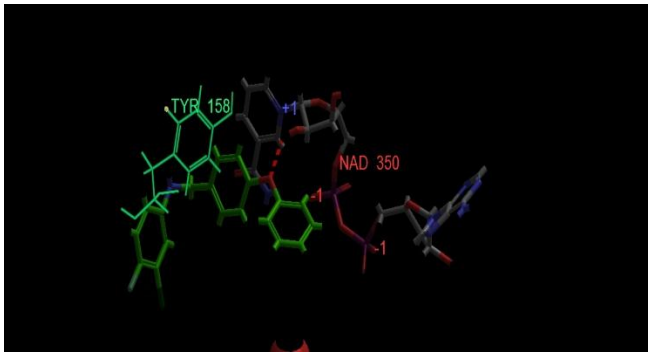
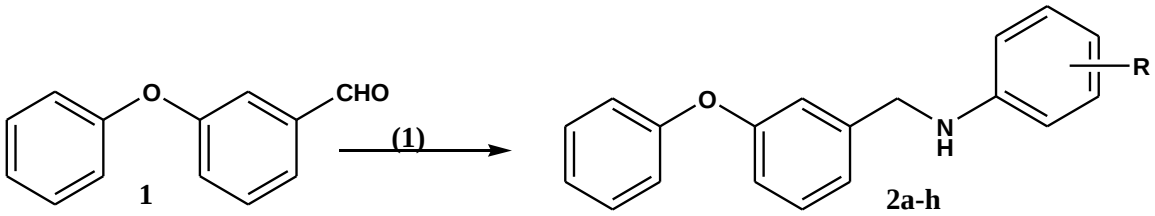


Figure 4. Hydrogen bonding interaction of compound **2f** with NAD-350.



2a. R = H **2e. R = 4-Br**
2b. R = 4-Cl **2f. R = 3-Cl, 4-F**
2c. R = 4-F **2g. R = 3-Cl**
2d. R = 2-F **2h. R = 4-OCH_3**

Reagents & Conditions:- (1) $ArNH_2$, $NaBH_3CN$, CH_3COOH, CH_3OH, 70-80^{0}C, 6-8h

Figure 5. Scheme1

Table1. Virtual physicochemical properties of compounds**2**

Compd	MW	Hd[a]	Ha[b]	QPlogPo/w[c]	QPlogS[d]	Oral Abs (%)[e]	PSA[f]	Rule of five[g]
2a	327.785	1	1.5	5.897	-6.372	100	19.959	1
2b	293.34	1	1.5	5.418	-5.54	100	19.957	1
2c	309.794	1	1.5	5.68	-5.954	100	19.923	1
2d	293.34	1	1.5	5.478	-5.782	100	18.674	1
2e	309.794	1	1.5	5.731	-6.085	100	19.536	1
2f	354.245	1	1.5	5.746	-6.106	100	19.5	1
2g	289.545	1	1.25	4.758	-4.526	100	29.028	0
2h	275.349	1	1.5	5.208	-5.341	100	18.991	1

Table 2. Molecular docking studies of compounds **2a-h.**

Sl.No.	Compound	Docking score	H-Bonding Interactions		Distance (A^0)	
			NAD- 350	TYR-158	NAD-350	TYR-158
1	**2a**	-8.09	-0.30	0.0	2.07	2.83
2	**2b**	-8.33	-0.49	0.0	2.01	2.62
3	**2c**	-8.46	-0.55	0.0	2.02	2.61
4	**2d**	-9.34	-0.70	0.0	1.99	2.75

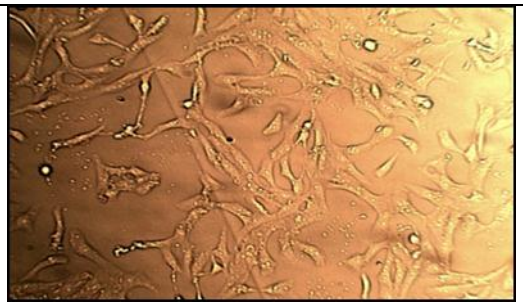
5	**2e**	-8.48	-0.55	0.0	2.03	2.65
6	**2f**	-9.00	-0.68	0.0	2.00	2.82
7	**2g**	-8.92	-0.66	0.0	2.03	2.85
8	**2h**	-8.44	-0.58	0.0	1.98	2.57

Table3. In Vitro Antimycobacterial activity, Cytotoxicity and hepatotoxicity of Compounds **2a-h**

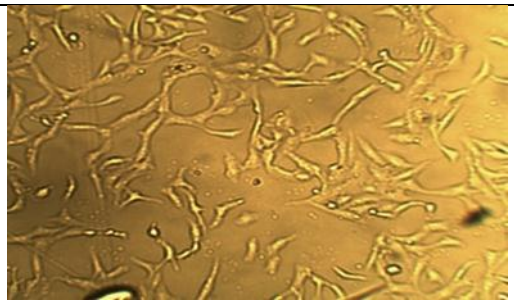
Compound	**MIC(µg/mL)**	**Cytotoxicity(µg/mL)**		**Selectivity index**
		Vero	**HepG2**	
2a	12.5	>300	>300	>10
2b	50	>300	>300	-
2c	25	>300	>300	>10
2d	25	>300	>300	>10
2e	25	>300	>300	>10
2f	25	>300	>300	>10
2g	25	>300	>300	>10
2h	25	>300	>300	>10
Triclosan	12.5	>300	>300	>10
INH	0.05	-	-	-

Table 4. Antibacterial activity of compounds 2a-h

Sl.no	**Compound code**	**Antibacterial activity**					
		Zone of inhibition(mm) (*E.coli*)			Zone of inhibition(mm)		(*Staphylococcusaureus)*
		100µg/ml	50 µg/ml	25 µg/ml	100 µg/ml	50 µg/ml	25 µg/ml
1	**2a**	Nil	Nil	Nil	Nil	Nil	Nil
2	**2b**	12	Nil	Nil	12	Nil	Nil
3	**2c**	Nil	Nil	Nil	Nil	Nil	Nil
4	**2d**	Nil	Nil	Nil	Nil	Nil	Nil
5	**2e**	Nil	Nil	Nil	Nil	Nil	Nil
6	**2f**	Nil	Nil	Nil	Nil	Nil	Nil
7	**2g**	Nil	Nil	Nil	Nil	Nil	Nil
8	**2h**	Nil	Nil	Nil	Nil	Nil	Nil
9	**Streptomycin**	-	-	26	-	-	36



A



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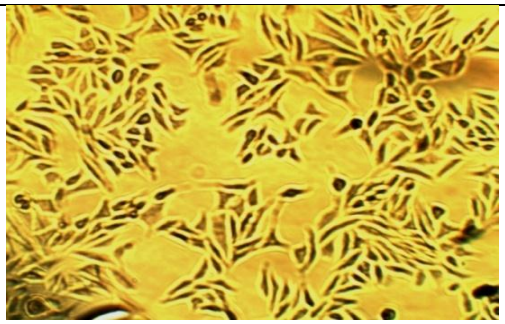

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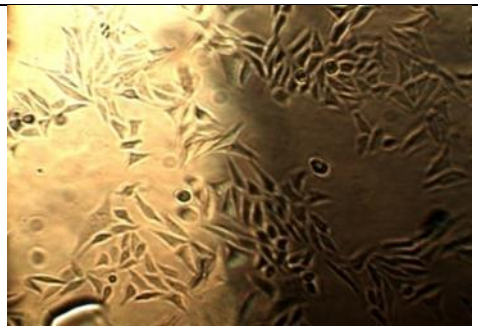
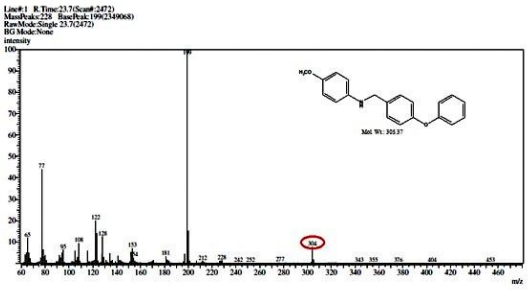
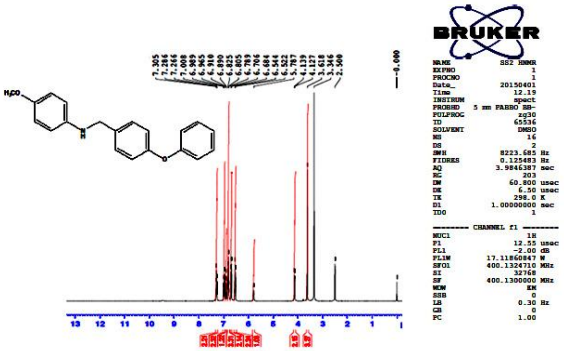


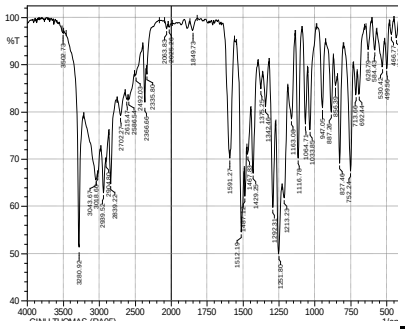
D

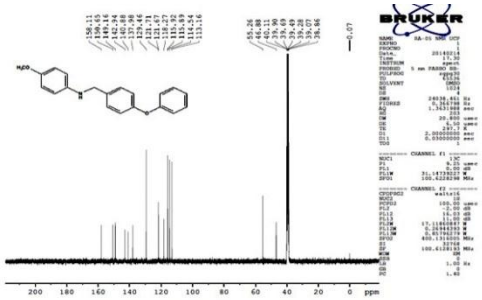
Figure 7. .**a**. DMSO (0.1%) treated vero cells after 72 h of incubation; **b**. Compound **2a (**300 µg/ mL)treated vero cells after 72 h of incubation; **c**. DMSO (0.1%) treated HepG2 cells after 24 h of incubation; **d.**Compound**2a (**300 µg/ mL)treated HepG2cells after 24 h of incubation.

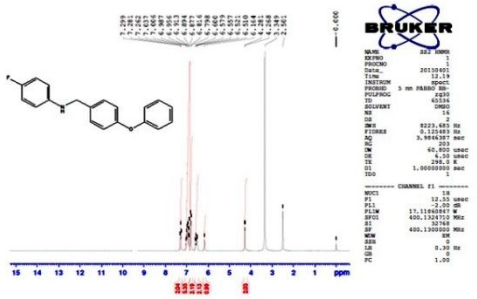
Spectra

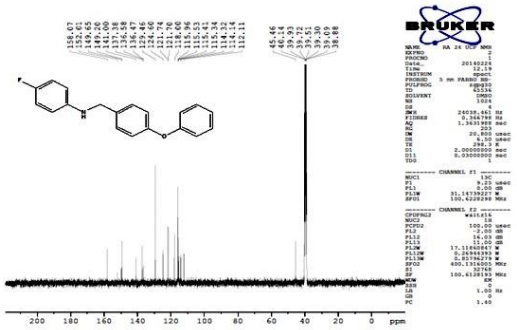


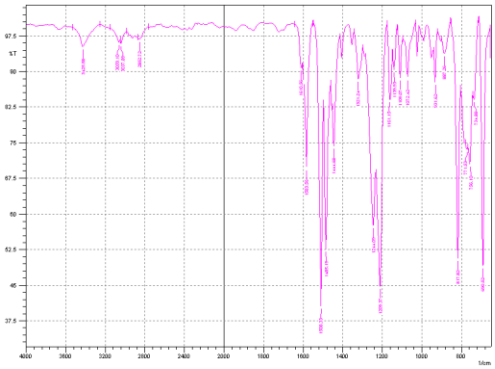












Conclusions

The present study established the design, synthesis and evaluation of diphenyl ether derivatives as antitubercular and antibacterial agents. Here the basic nucleus selected is triclosan. Because of its high lipophilicity triclosan cannot be used internally. So we decided to modify the structure of triclosan by decreasing its lipophilicity while maintaining the activity. Compound **2a** exhibited an MIC of 12.5µg/ml that is similar to that of triclosan, all other compounds showed comparable activity to that of triclosan. So it can be concluded that our compound **2a** has significant activity like triclosan but the lipophilicity is lesser than triclosan. The receptor specificity of the synthesized compounds were shown from the antibacterial activity study since none of the synthesized compounds showed activity, so it have been proved that newly synthesized compounds were specifically act on the enoyl acyl carrier protein receptor.

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