Supplementary Information

In vitro antitubercular screening

Synthesized compounds were screened against *Mycobacterium tuberculosis* H37Rv using Micro plate Alamar Blue Assay (MABA)]. Culture suspension of *Mycobacterium tuberculosis* H37Rv strain was used as the inoculum in the MABA assay. Drugs were dissolved in DMSO to prepare the stock solutions. Dilutions of *M. Mycobacterium tuberculosis* H37Rv culture (ATTCC 27294, $2x10^5$ cfu/mL) were added onto media (Middlebrook 7H9 broth, Bactocasitone, Difco polysorbate-80 and OADC) containing a range of drug concentrations (1.56–100 μg/mL) in 96 well plates and incubated at 37 °C for 7 days under aeration. Isoniazid and triclosan were used as standards and DMSO as blank for comparison. Positive control (only inoculum) and negative control (only media) were also applied in the plate to minimize the error. On the seventh day of incubation, 12.5 μL of 20% Tween-80 and 20 μL of Alamar blue were added to each well of the plate and incubated for another 24 h at 37 °C. A change in color from blue to pink was considered as the growth of the Mycobacterium at that concentration of the drug. For better interpretation of the results, the color was compared with the color present in the growth control wells. The MIC is defined as the lowest concentration of drug that inhibited bacterial growth.

In vitro cell cytotoxicity screening

Potential toxicity of the synthesized diphenyl ether derivatives towards mammalian Vero cell-lines and HepG2 cell lines was investigated up to concentrations of 300 μg/mL. Test sample dilutions were distributed at a volume of 100 μL to each wells containing monolayer of Vero cells (10^4 cells/well) in a 96-well plate. The plates were 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, the supernatant 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 3 h. After the incubation, the supernatant was removed carefully from each wells and 50 µL of sterile DMSO (filtered through 0.22 µm syringe filter) was added. The plate was transferred to an incubator and kept at 37°C for 2h. Optical density (OD) of the wells was measured at 540 nm using Elisa Reader.

In case of HepG2 cells, $5x10^3$ cells/well was added and MTT was added after 24 h of incubation. Optical Density (OD) readings from each well were entered into 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

Molecular docking study

Structures of designed diphenyl ethers were drawn using ChemDraw Ultra-08 and converted to 3D structures. Generation of ionization state (through Epik) of the ligands was done using Ligprep tool of Schrodinger-2010. OPLS-2005 force field was used for the energy minimization of 3D structures of the ligands. The X-ray structure of *Mycobacterium tuberculosis* ENR (pdb 1P45) as used in all the docking experiments. The InhA protein was optimized using the Protein preparation wizard of Schrodinger-2010. Protein was preprocessed by assigning bond order, adding hydrogens and treating disulfide bonds. Water molecules were removed within 5 Å of the binding site. Hydrogen bond was assigned by exhaustive sampling and energy was minimized to RMSD 0.30 Å by OPLS-2005. Receptor grid was generated around the co-crystallized ligand (triclosan) in Schrödinger-2010 suit by using default parameters. Ligand was

excluded from the protein and it was confined to the enclosing box at the centroid. The grid was generated to fit the ligands similar in size to Triclosan. 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 compounds were docked with the Mtb InhA protein at the triclosan binding site. Then all ligand docking results from SP docking was studied.

Determination of logP

All the chromatographic runs were conducted on Shimadzu HPLC at room temperature using ODS-4 (Intersil ODS-4, 5 µm, 4.6 x 150 mm, GL Science Inc.) column and UV-Visible detector. Numerical analysis and data processing were done using Lab solution-2013 software. 3-Morpholinopropane-1-sulfonic acid (MOPS, 4.18 g) was added to 900 mL of octanol saturated MilliQ water, and the volume was made up to 1L. pH of the buffer was adjusted to 7.4. A mixture of methanol (0.25% v/v octanol) and buffer at the ratio of 60:40, 65:45 and 70:30, were used to elute the test sample. 5 µL of the sample was injected, and the flow rate was kept at 1 mL/min. Signal was detected at λmax 270 nm. Sample run time was kept in between 15 min. to 1h. Capacity factor (*k'*) was calculated for each run by using the equation given below.

$$k' = t_R - t_0 / t_0$$

Where t_R is the retention time of sample, t_0 is the retention time of blank (methanol). A graph was plotted by taking log *k′* (y axis) and % methanol (3-4 percentage concentrations) (x-axis). The logarithm of *k′* was extrapolated to a 0% concentration of methanol in the graph. Log *k′* at 0% methanol was calculated from the regression equation ($R^2 = 0.99$) generated from the graph to determine logP.

Determination of p*K*a

All the chromatographic runs were conducted on a Shimadzu HPLC at room temperature using C18 column (Gemini 5 µ C18 110 Å, 4.6 x 150 mm, Phenomenex) and UV-Visible detector. Numerical analysis and data processing were done using Lab solution-2013 software. Buffers of pH 2, 5, 7.4 and 10 were prepared by mixing universal buffer I [Phosphoric acid (1.96 g), glacial acetic acid (1.2 g, 1.14 mL), and boric acid (1.36 g) were added to MilliQ water, and volume was made up to 5 L] and universal buffer II (0.02 M NaOH) at different proportions. Mobile phase contained acetonitrile as the organic modifier. Chromatographic measurements were done at 25-27 °C with eluent flow rate of 1 mL/min. Compound was eluted using acetonitrile: buffer in the ratio of 50:50 in each different pH points (pH 2, 5, 7.4 and 10). Signal was detected at λmax 270 nm. Sample run time was kept in between 15 min. to 1h. 3 µL of samples were injected using autosampler. Acetonitrile was used as blank. pH gradient run was applied with a fixed concentration of organic modifier providing complete suppression of ionization of the test sample at the beginning of the gradient and its full ionization at its end. The retention time values, t_R of compound **10b** was determined from three separate injections. Capacity factor (*k′*) was calculated for each run by using the equation given below.

$$k' = t_R - t_0 / t_0$$

Where t_R is retention time of sample, t_0 is the retention time of blank (acetonitrile). p*K*a was calculated by inserting the data in the following equation.

p*K*a = pH – log(kHA–*k*)/*k* - kA

Where *k* is the retention factor at a given pH, kHA and kA are the retention factors of unionized and fully ionized form.

Evaluation of extent of protein binding

Extent of binding of compound **10b** to serum protein was determined over a HSA (human serum albumin) column using reverse phase HPLC technique. All the chromatographic runs were conducted on a Shimadzu HPLC at room temperature using Thermo-HSA column (4.6 x 150 mm, 5 µm/ 025) and UV -Visible detector. Numerical analysis and data processing were done using Lab solution-2012 software. Mobile phase contained isopropyl alcohol as the organic modifier and potassium phosphate buffer (0.067 M, pH 7.4) as the aqueous phase. Compound **10b** was dissolved in isopropyl alcohol to prepare test sample of concentration 10 µg/mL. Chromatographic measurements were done at 25-27 °C with eluent flow rate of 1-2 mL/min. The HSA column retention characteristics were calibrated using verapamil and metoprolol. Compound **10b** was then eluted using buffer and acetonitrile in gradient run at the ratio of 90:10. Signals were detected at λmax 256 nm. Retention time (RT) of isopropanol (IPA) was used as t_0. The retention time values, t_R of compound **10b** was determined from three separate injections.Capacity factor (*k'*) was calculated for each run by using the equation: $\boldsymbol{k' = t_R\text{-}t_0/t_0}$

Where t_R is retention time of sample, t_0 is the retention time of blank (acetonitrile). Capacity factor (*k*') is used to calculate the percentage of protein binding. The % protein binding (P) is calculated by

P = 100(*k*'/(*k*' +1))

where *k'* is the capacity factor

Human microsomal stability assay

Compound **10b** was incubated with human liver microsomes to probe its stability. Liver microsomes (20 mg of protein per mL), and NADPH regenerating system solutions A and B were kept in wet ice. Purified water (356 µL), potassium phosphate (100 µL 0.5 M, pH 7.4) NADPH regenerating system solution A (25 µL), NADPH regenerating System Solution B (5 µL) and substrate (1 µL, 10 µM final concentration) were added to micro centrifuge tube (1.5 mL) and warmed to 37 ^{o}C for 5 minutes in a water bath. To it, 12.5 µL (0.25 mg) of liver microsome was added, mixed well and placed inside a water bath at 37^{o}C. After 0, 15, 30, 60 and 120 minutes of incubation, 100 µL of the mixture was withdrawn and added to a micro centrifuge tube containing 100 µL ice cold acetonitrile and losartan as internal standard (2 µM). It was mixed well and centrifuged at 10,000 x g for 15 minutes. Supernatant from the protein pellet was withdrawn, and then the protein pallet was extracted with acetonitrile. Both the supernatant and acetonitrile extract were mixed together and analyzed by HPLC.

A positive control (testosterone) was used to confirm that the assay is working properly. A negative control (without NADPH) for each test compound was used to detect problems such as nonspecific protein binding or heat instability.

The percentage of compound remaining after 15, 30, 60 and 120 min. of incubation was calculated as:

% remaining = 100 × (mean $PART_R$/mean $PART_0$)

Where PAR = Peak area of analyte/IS peak area ratio