Design, synthesis, and evaluation of diphenyl ether analogues as antitubercular agents

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Contents of supplementary information

1. Log P calculation of compound DE-3:

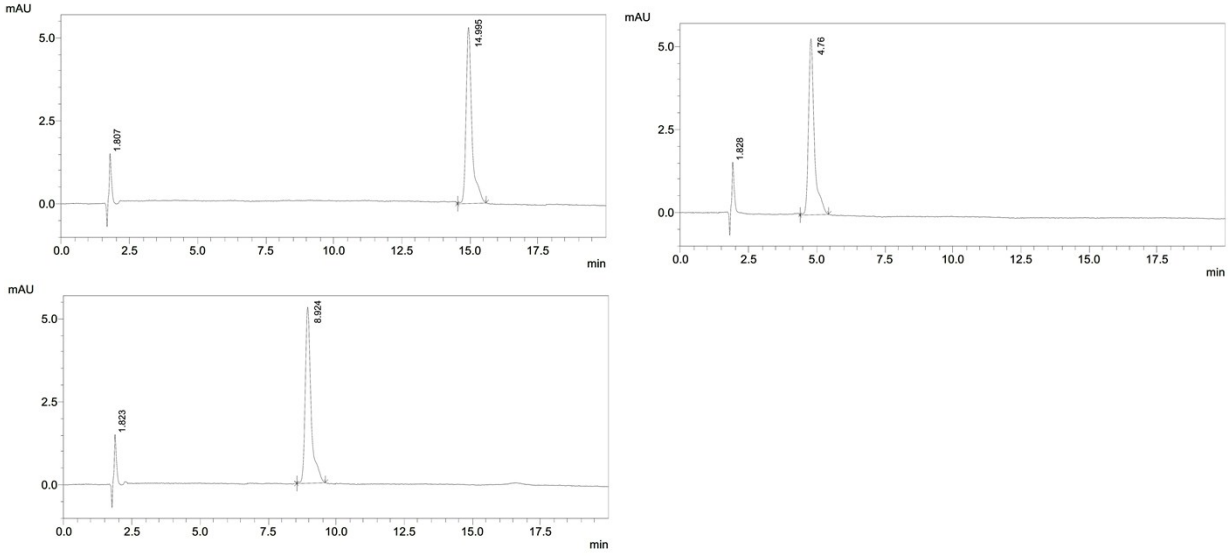
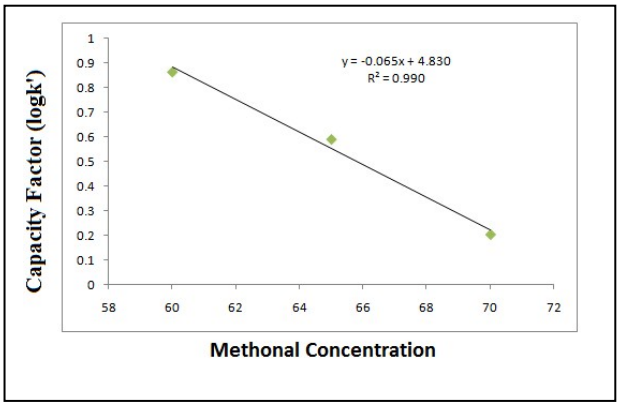


Figure-1: Chromatograms of Compound **DE3**.

Mobile Phase MeOH: MOPS	t_0	t_R	K'	logk'
60:40	1.807	14.995	7.298	0.818
65:35	1.823	8.924	3.895	0.588
70:30	1.828	4.76	1.6033	0.322



2. MM-PB/SA Calculations

The MM-PBSA method[34] was used to calculate the binding free energy of the protein-ligand complex. The binding free energy is estimated by the following equation:

$$\Delta G = \Delta H - T\Delta S$$

T is the temperature of the system at 300 K. The binding free energy (Δ*G*) of the protein-ligand complex is computed as:

$$\Delta G = G_{complex} - [\, G_{protein} + G_{ligand} \,]$$

In equation 4, *G***complex** is the absolute free energy of the complex, *G***protein** is the absolute free energy of the protein, and *G***ligand** is the absolute free energy of the ligand. We taken 2500 snapshots (at time intervals of 2 fs) for all complexes (complex, protein and ligand). The enthalpy term in equation 1 is dissected into sub-energy terms:

$$H_{tot} = H_{gas} + G_{solv}$$

$H_{\mathbf{gas}}$ is the potential energy of the solute which is determined as the sum of van der Waals (*E*vdw), electrostatic (*E*el) and internal energies (*E*int) in gas phase by using the SANDER module of AMBERtools 15. *G***solv** is the solvation free energy for transferring the solute from vacuum into solvent and is a sum of electrostatic (*G***el**) and non-electrostatic (hydrophobic) contributions (*G***nonel**) as shown in equation 7:

$$G_{solv} = G_{el} + G_{nonel}$$

Gel in equation 7 was computed at 0.15 M salt concentration by the PBSA module of Amber 15 .0 by dividing implicitly solvated solute species into 0.4Å cubic grid points and summing up the electrostatic potentials computed at each grid point. Electrostatic potential Ø(r) at a grid point r

that is not at the solvent-solute boundary was computed by a linear Poisson Boltzmann (PB) equation, which is a three dimensional vector differential equation as shown in equation 8:

$$\varepsilon(\mathbf{r})\varnothing(\mathbf{r}) = -4\pi \cdot \rho(\mathbf{r})$$

In equation 8 $\varepsilon(\mathbf{r})$ is the dielectric constant ($\varepsilon = 1$ for the solute interior and $\varepsilon = 80$ for implicit PB water) and $\rho(\mathbf{r})$is the charge density. The grid point potentials were then summed up for each atom ***i*** to yield atomic potentials $\varnothing i$**.** The PB implicit solvent molecules at the solute solvent boundary were allowed to energetically converge over 1000 iterations before the single-point Poisson computations (PBSA) were applied for each snapshot. The total entropy (S**tot**), as formulated in equation 9 arose from changes in the degree of freedom:

$$S_{\mathbf{tot}} = S_{\mathbf{trans}} + S_{\mathbf{rot}} + S_{\mathbf{vib}}$$

In equation 9 (Strans) is the translational, (Srot) the rotational, (Srot), and the vibrational (Svib)] entropy of each species. Considering all absolute energy terms as given in equation 2, the binding free energy ΔG takes the following form:

$$\Delta G_{\mathbf{binding}} = [\Delta H_{\mathbf{gas}} + \Delta G_{\mathbf{solv}}] - T\Delta S_{\mathrm{tot}}$$

3. **ADMET properties of synthesized compounds.**

Cpd code	HIA	PPB	BBB	Caco2
DE1	94	91	0.043	20.4
DE2	94	91	0.70	27.7
DE3	95	100	1.66	21.8
DE4	95	96	1.62	21.0
DE5	96	92	0.01	38.0
DE6	94	91	0.01	17.2
DE7	92	94	0.60	14.5
DE8	94	89	1.33	30.5
DE9	94	89	1.32	30.5
DE10	94	91	0.78	32.1

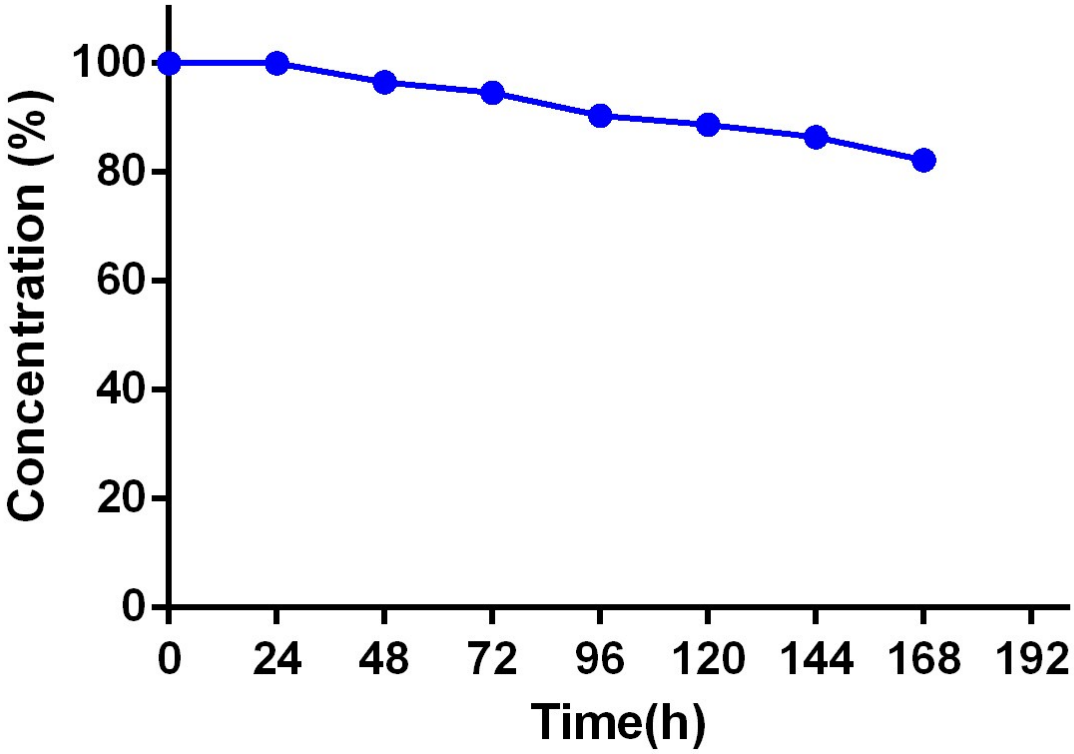
(HIA: Human Intestinal Absorption, **PPB**: Plasma protein binding,

BBB: Blood brain barrier penetration, **Cac02**: Caco2 cell permeability.)

4. Chemical Stability Assay:

The Chemical Stability of the active compound (**DE3**) was studied on Shimadzu LC-20AD Prominence Liquid Chromatography with SIL-20AC HT Auto sampler. The HPLC separation was carried out in phenomenex C18 coloumn using mixture of phosphate buffer 0.05 M (pH: 7.4) and acetonitrile (60:40) was used as a mobile phase. The rate flow of the mobile phase was 1.0 mL/min, with an injection volume of 20µL, the column oven temperature was maintained at 37 ^{o}C. The compound was detected at 254 nm using PDA detector with a retention time(Rt) of 20 min. Sample solution of compound (50 µM) was prepared using Phosphate Buffer pH 7.4 and stored at 37^{o}C for 6 days (These conditions resembles MABA assay conditions.) The samples were analysed at 0h, 24h, 48h, 72h, 96h, 120h, 144h and 168h for RP-HPLC analysis. The results proves that compound is stable(>80%) in the assay conditions. The stability graph of **DE-3** was plotted against Time Vs Concentration shown in below figure.

Time(h)	Concentration(%)
0	100
24	100
48	96.5
72	94.6
96	90.3
120	88.7
144	86.4
168	82.2


Concentration (%)
100
80
60
40
20
0
0
24
48
72
96
120
144
168
192
Time(h)