Original Article

Identification of a New Potential Reductase Inhibitor as an Anti-Tubercular Agent for Enoyl-Acp Reductase Inha Gene of *Mycobacterium tuberculosis* in Comparison with PT70 (5-Hexyl-2-(2-Methylphenoxy) Phenol)

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ABSTRACT

Bacterium *Mycobacterium tuberculosis* is the causative agent for the disease tuberculosis (TB) and is responsible for more than ten million different infections with an additional accountability for about two million deaths every year. PT70 molecule as described in the literature acts as a drug in the market, which has been utilized as a curative agent for the disease. However, for these commercialized anti-tuberculotic drugs the causative agent that is *Mycobacterium tuberculosis* is becoming drug resistant in a progressive manner. Therefore, to combat the metabolic activity of the bacteria there is a need for a potent drug that could be subjected to cure TB. The present study deals with the perspective of designing a novel inhibitor as an anti-tuberculotic agent for which PT70 has been taken as a base molecule, which is henceforth used in molecular docking with the target INHA gene, and as a result, the process observed a binding energy as -10.133. Comparative molecules were selected based on the process of pharmacophore modeling which were then docked with the receptor molecule. On concluding remarks, top five molecules were prioritized on the bases of their binding energy (highest as -10.881) as compared to the PT70 molecule. Therefore, all such molecules selected will be taken as drug like molecules in future, which can be used for inhibiting the INHA gene. The aforementioned five molecules passed that ADMET analysis, membrane permeability test, pKa, and density function theory.

Key words: Anti-tuberculotic, PT70, Pharmacophore, Binding energy, Membrane Permeability, Drug discovery.

INTRODUCTION

Tuberculosis (TB), a disease that is caused by the bacterium *Mycobacterium tuberculosis* (MTB), it is an airborne infection that spreads from a single person to another through sneezing and coughing, further killing 13 lakh people every single year.[1] As per WHO (World Health Organization) data, in 2015, worldwide TB cases were estimated as 1.04 crores new (incident), of which 59 lakh that is 56% were among men, 35 lakh that is 34% were women and 10 lakh (10%) were children. Individuals who were living with HIV accounted for 12 lakh (11%) of all the new TB cases.[2] Of all the infectious ailments, only HIV kills more individuals than TB, and it is henceforth one of the primary causes of death for AIDS patients. Meanwhile, penicillin and sulfonamides were the first antibiotics in consumption that were very ineffectual against TB. On the other hand, primary effective drug against tuberculosis was streptomycin. In the year 1943 in Selman laboratory this efficient drug was isolated from *Streptomyces*

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griseus and was effectively administered to a tuberculosis patient in the year 1944.[3] In later years after the innovation of streptomycin, numerous antibiotics were presented as anti TB agents: p-aminosalicylic acid in 1949, isoniazid in 1952, pyrazinamide in 1954, consecutively cycloserine in 1955, ethambutol in 1962, and rifampicin in the year 1963. These medicines were discovered for more than 40 years ago and are still in use today as the standard treatment for TB, which entails the intake of isoniazid, ethambutol, pyrazinamide, and rifampicin for two months followed by rifampicin and isoniazid for four months.

Until now, the strain that involves drug resistance represents a large problem in the present day scenario. When the prolonged, complex TB drug treatment is inappropriate or incomplete, for example, when patients discontinue their medications before the removal of disease from their body, or the access to the medicines becomes limited due to which patient is compelled to take only a single drug dosage, the progress of drug resistance is very plausible. The drug resilient strains of MTB can then spread from one person to other person like drug susceptible bacteria. Strains of *M. tuberculosis* that are unaffected by isoniazid and rifampicin, the best effective drugs against tuberculosis, are defined as multi drug resistant (MDR-TB).[4] INHA, an MTB enoyl acyl-carrier protein reductase, is the fundamental object of the front-line drug isoniazid (INH).[5] Although it is one of the utmost significant anti-tubercular drugs and the only drug that is used for TB prophylaxis, INH suffers from resistance that continues to increase.[1,5b,6]

WHO data indicate up to 28% of all TB cases are INH-resistant, and in earlier treated TB patients, up to 60% exhibit resistance, creating it extremely difficult, time-consuming, and costly to treat them (if they can be treated at all).[1,7]

INHA is clinically confirmed as target, which is based on the attainment of isoniazid (INH) in treating patients suffering from TB.[5b,8] INH is a pro-drug and is activated by KatG, a catalase−peroxidase enzyme. This catalase−peroxidase enzyme oxidizes INH to an acyl radical that formerly forms a covalent adduct (INH-NAD) in association with NAD known as nicotinamide adenine dinucleotide.[8a] The active drug is an INH-NAD covalent adduct[1] and one of the resistance mechanisms to INH is a complete precise mutation in the KatG gene.[8a]

Luckner and co-workers of his laboratory[9] in their study had prepared a triclosanlike inhibitor that is PT70, by the addition of a single methyl (CH_4) group explicitly, which further was designed to act together with the ordered INHA helix along with the NAD+ cofactor. This simple modification was originated to intensify the residence time of PT70 by numerous orders of magnitude associated with the triclosan itself.[10] Inhibition of the mycobacterial gene, enoyl reductase INHA is one of the most effective targets of killing MTB, as clinically confirmed by isoniazid, the most powerful TB drug. Unfortunately, both MDR and Extensive Drug-Resistant (XDR) MTB isolates are resistant to isoniazid, mostly due to mutations in KatG, the catalase-peroxidase involved in the activation of isoniazid.[11] This has led to broad efforts to recognize the direct INHA inhibitors.[8c,12]

MATERIALS AND METHODS

Data selection and Software

Protein 3D structure of INHA protein (PDB ID - 2X22) were retrieved from PDB (PROTEIN DATA BANK), and PT70 (5-Hexyl-2-(2-Methylphenoxy) Phenol) (TCU – PDB entry name) (Figure 1) taken from PDB.[9,13] All other molecular structures for docking were retrieved from zinc database.[13f] In the present research, Schrödinger software[13e] tool was used for all processes like docking, pharmacophore, ADMET prediction, membrane permeability and pKa analysis.

Molecular Docking

Lead discovery part is one of the most essential methods in rational drug design. The docking procedure inside a targeted binding site includes the prediction of ligand conformation and orientation (or posing). In common, there are two foremost objectives of the docking studies: correct prediction of activity and accurate structural modeling.[14] Nevertheless, the identification of molecular features those are accountable for exact prediction of compound variations or biological recognition with those having developed potency, are complex issues that are often difficult to understand and even more consequent to simulate on a computer.[15]

The procedure of binding a small molecule with its protein target is not easy; numerous entropic and enthalpy factors affect the interactions between them.

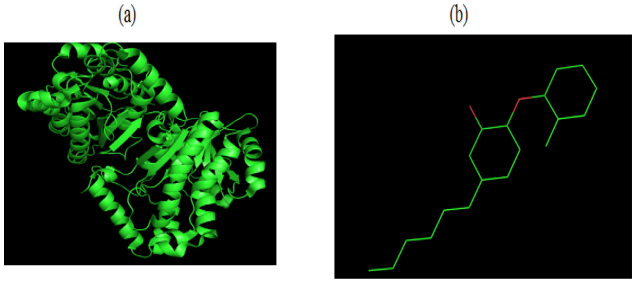


Figure 1 : (a) INHA protein 3D structure. (b) pt70 molecule structure

The flexibility of both ligand and receptor molecule, the consequence of the protein environment on the charge distribution over the ligand,[16] and their interactions with the close water molecules, further complicate the quantitative description of the processes occurring.[17]

Docking the ligand with individually protein in the ensemble establishes the best comprehensive, although costly, approach. Although this approach is not a representative option for the virtual screening of a huge library, it is an effective method for difficult docking problems where even slight conformational changes of the receptor molecule are expected to have a major influence on the binding process involved.[18]

Pharmacophore

Pharmacophore-based screening is not suitable when the three-dimensional structure of the desired protein is not known. Moreover, if the three dimensional structure of a target is known, both docking-based and pharmacophore-based virtual screening methods can be utilized.[19] A screened molecule is counted as a hit if it contains the important pharmacophore features of the proposed model. Pharmacophore-based screenings are nearby 10K times faster than docking-based screenings, thus they are usually preferred over docking as a primary filter to remove the molecules, which do not have important features for binding.[20] Docking can be used in advanced stages for a more detailed evaluation of the hit compounds. In the present study, the ZINC database library was created for screening of the molecules via pharmacophore-based virtual screening model.[21]

Membrane permeability

Molecular weight plays an important role in drug discovery because of the poorer intestinal and blood brain barrier permeability.[13b,13c] Too many hydrogen bond acceptor molecules hinder the permeability across a membrane bi-layer.[13d]

Pka (aqueous dissociation constant)

The cosolvent method (mixed solvent procedure) is the most broadly used way for the pKa determination of water-insoluble compounds. The solubility of unionized molecules can be improved by mixing solvents such as dioxane, methanol or acetonitrile with water, but not entirely compounds dissolve in any particular solvent-water mixture.[22] On the other hand, ionized molecules are more soluble in water than neutral ones. For the reason that aqueous solubility is critical for oral bioavailability, most drugs contain ionizable groups (Figure 2).[23] Electrostatic attraction of ionized functionalities is similarly an important contributor to target binding.[23]

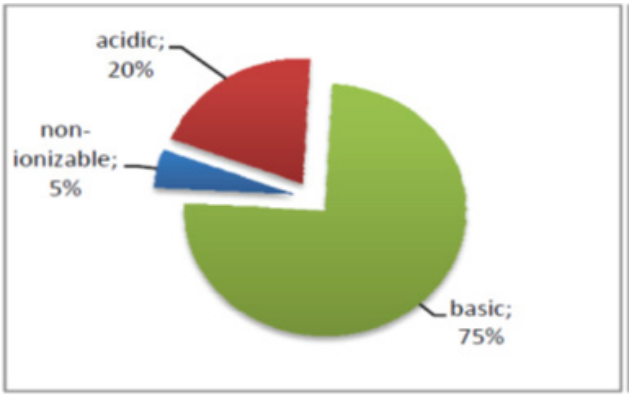


Figure 2 : Most drugs contain ionizable functionalities[23]

The extent to which a compound is ionized in solution is measured by the pKa. It is well defined as the negative decadic logarithm of the heterolytic dissociation constant. Accordingly, strong acids have a low pKa and are more ionized at a given pH. Strong bases have a high pKa, as their conjugate acids are weak.

The formula for the raw pKa is:

$$pKa_{raw} = \frac{\Delta G}{2.3RT} \qquad \text{(EQ 1)}$$

Where ΔG is the free energy of the deprotonation of the molecule in solution, R is the universal gas constant, and T is the temperature.[24]

Density functional theory analysis

The relative energies and electron occupancy of molecular orbitals can deliver insights into photophysical processes. Of particular importance are the HOMO (Highest occupied molecular orbital) and LUMO (Lowest unoccupied molecular orbital) (HOMO and LUMO, respectively) which are known as Frontier Molecular Orbital theory.[25]

Two fundamental aspects for describing chemical reactivity are electron transfer effects and electrostatic interactions. Electron transfer effects relate to frontier orbitals and the capacity of a chemical group to be a good donor or acceptor while a reaction takes place. Frontier molecular orbital theory can predict whether a reaction will take place by focusing on the shape and the symmetry of HOMO and LUMO. When the HOMO of one molecule (as an electron donor) and the LUMO of another molecule (as an electron acceptor) have the same shape and phase, the electrons can transfer from the HOMO to the LUMO and form a chemical bond. A strong chemical bond take place when the energy gap in the middle of the HOMO of the electron donor and the LUMO of the electron acceptor is minor.[26] As a result, a good electron donor should have a high

HOMO whereas a good electron acceptor should have a low LUMO (Figure 3). The smaller this energy gap is, the easier the chemical bond will be to form.[26]

RESULTS AND DISCUSSION

To design a better drug than pt70, first dock pt70 dock with INHA protein than with pharmacophore help to find better molecules, which can replace the pt70 drug. To dock with receptor first find the binding site where molecule can bind and high affinity with protein. The binding site was TYR 158A, ILE 202A, ALA 198A, MET 199A, MET 161A, TYR 158A, VAL 203A, PHE 149A. Receptor was prepared with LigPrep tool[27] of Schrödinger software. That prepared ligand was docked with pt70 result shown in Table 1.

Docking score was -10.133, which is a very good score with receptor. For designing a better drug, compare to pt70 was taken as a base molecule and designing a pharmacophore model (Figure- 4) to find similar drug was done.

Nine lacs molecules were retrieved from zinc database to screen out all the desired molecules through pharmacophore model screening from pharmacophore model to aid in the identification of similar molecule equivalent to pt70. This process was done with phase tool.[28] All the selected molecules were docked with the main receptor and there then the docking score got better than the base molecules. Top five molecules (on the bases of binding energy) were selected for further process analysis. These five molecules were found potent to replace the pt70 drug (Table 2).

For the analysis of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) done with QikProp tool.[29] All five molecules ADMET analysis result given into Table 3. All five molecules passed the ADMET result.

Optimizing membrane permeability is a critical component in the discovering of small molecule drugs.[30] Many relevant biological processes, such as intestinal absorption, blood brain barrier permeation, or skin penetration, involve permeation of molecules across biological membrane via passive transmembrane diffusion and/or active transport mechanisms.[30,31] Prime tool[32] was used for membrane permeability analysis.

Membrane permeability had shown the better result with all the five molecules (Table 4).

These outcomes indicated that, higher hydrophobicity leads to higher membrane permeation, additional factors such as size and conformation of the ester side chain may show similar affect delivery of the compound into the cell and accordingly the inhibition activity. Consequently, the activity of these compounds possibly reflects a balance between the ease of membrane permeability, stability against extracellular hydrolytic activity (in serum and red blood cell), and hydrolysis rate by esterases in the parasite cells.[33]

The pKa's of a drug's numerous functional groups play a critical role in determining its bioavailability and pharmacokinetic profile, although virtual screening software depends correctly on protonated structures in order to observe the discrete interactions that drive ligand binding.[34] All molecules active site shown into the

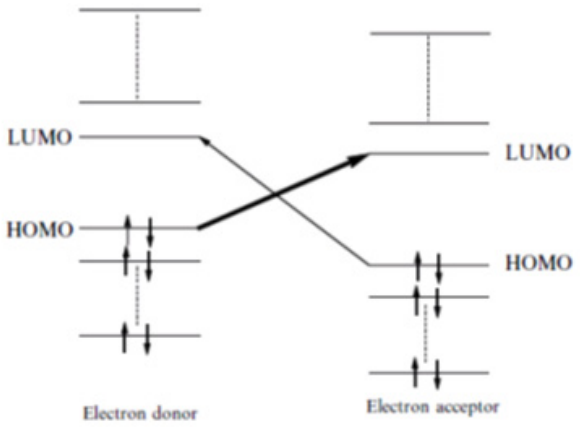


Figure 3 : Frontier orbital interactions[26]

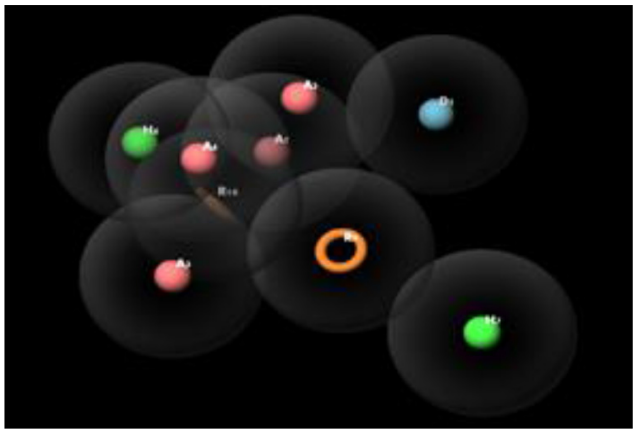


Figure 4 : pharmacophore hypothesis

Table 1: Docking with pt70.

Title	Chain ID	Residue Number	Formula	Molecular Weight	docking score	XP GScore	glide gscore
2X22	A	1271	C19 H24 O2	284.393	-10.133	-10.147	-10.147

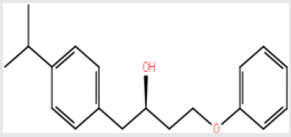
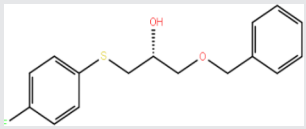
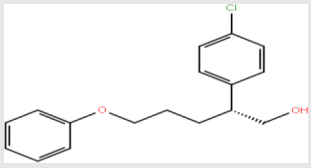
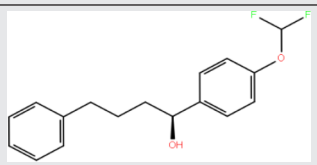
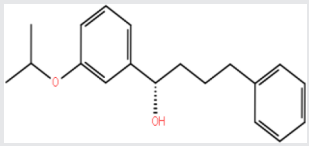
Table 2: Through Pharmacophore models generated molecules result.					
Title	structure	Energy	docking score	XP GScore	glide gscore
ZINC53781180		14.631	-10.881	-10.881	-10.881
ZINC70193107		14.981	-10.648	-10.648	-10.648
ZINC93154378		14.697	-10.478	-10.478	-10.478
ZINC87174488		10.728	-10.288	-10.288	-10.288
ZINC87173841		14.619	-10.203	-10.203	-10.203

Table 3: ligand based ADMET prediction.					
Title	ZINC53781180	ZINC70193107	ZINC93154378	ZINC87174488	ZINC87173841
SASA	595.831	558.822	575.483	544.9	602.615
FOSA	262.539	102.8	133.905	123.511	255.923
FISA	46.822	47.884	45.523	44.444	45.348
PISA	286.469	342.973	324.496	301.347	301.344
WPSA	0	65.165	71.559	75.598	0
QPlogHERG	-5.522	-5.712	-5.846	-5.289	-5.289
QPPCaco	3563.547	3481.899	3666.118	3666.118	3680.136
QPlogBB	-0.363	-0.208	-0.185	-0.077	-0.361
QPPMDCK	1953.753	4334.741	4968.092	5362.585	2022.935
QPlogKp	-0.605	-0.426	-0.447	-0.605	-0.526
QPlogKhsa	0.661	0.143	0.467	0.569	0.648
RuleOfFive	0	0	0	0	0

SASA Total solvent accessible surface area (SASA) in square angstroms using a probe with a 1.4 Å radius. - **300.0 – 1000.0**
FOSA Hydrophobic component of the SASA (saturated carbon and attached hydrogen). - **0.0 – 750.0**
FISA Hydrophilic component of the SASA (SASA on N, O, and H on heteroatoms). - **7.0 – 330.0**
PISA π (carbon and attached hydrogen) component of the SASA. - **0.0 – 450.0**
WPSA Weakly polar component of the SASA (halogens, P, and S). - **0.0 – 175.0**
QPlogHERG Predicted IC50 value for blockage of HERG K+ channels. - **concern below –5**
QPPCaco Predicted apparent Caco-2 cell permeability in nm/sec. Caco- 2 cells are a model for the gut-blood barrier. QikProp predictions are for non-active transport. - **<25 poor, >500 great**
QPlogBB Predicted brain/blood partition coefficient. Note: QikProp predictions are for orally delivered drugs so, for example, dopamine and serotonin are CNS negative because they are too polar to cross the blood-brain barrier. - **–3.0 – 1.2**
QPPMDCK Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the bloodbrain barrier. QikProp predictions are for non-active transport. - **<25 poor, >500 great**
QPlogKp Predicted skin permeability, log *Kp*. - **–8.0 – –1.0**
QPlogKhsa Prediction of binding to human serum albumin. - **–1.5 – 1.5**

RuleOfFive Number of violations of Lipinski's rule of five [3]. The rules are mol_MW < 500, QPlogPo/w < 5, donorHB ≤ 5, accptHB ≤ 10. Compounds that satisfy these rules are considered druglike.(The "five" refers to the limits, which are multiples of 5.) - **Maximum is 4.**

Table 4 - Member Permeability.			
Title	Membrane HDLD	Membrane dG Insert	Log Perm RRCK
ZINC53781180	-3.457	-3.457	-4.281
ZINC70193107	0.29	0.29	-4.365
ZINC93154378	-1.482	-1.482	-4.309
ZINC87174488	-1.296	-1.296	-4.315
ZINC87173841	-1.865	-1.865	-4.349

Membrane HDLD The free energy penalty for the neutral form of the ligand in its conformation inside the membrane to enter the membrane (i.e., move from the high dielectric region to the low dielectric region, hence HDLD).
Membrane dG Insert The total free energy penalty for the ligand to change state and enter the membrane. This is the sum of Membrane HDLD and Membrane State-Penalty, as described below.
Log Perm RRCK Logarithm of the RRCK permeability in cm/s. This property is optimized to reproduce RRCK permeability assay results, with fitted energy and volume terms.

Table 5 - Pka (aqueous dissociation constant).	
Title	pKa water
ZINC53781180 -OH	-0.2
ZINC93154378 -OH	0.3
ZINC70193107 -C	-0.8
ZINC53781180 -C	2.6
ZINC93154378 -O	2.3
ZINC87174488 -O	8
ZINC93154378 -CL	-17.9
ZINC70193107 -OH	-0.7
ZINC87174488 -OH	-1.6
ZINC87173841 -OH	1.3
ZINC87173841 -O	4.6

Table 6 - HOMO LUMO analysis.			
Title	HOMO	LUMO	HLG (eV)
ZINC53781180 -OH	-0.20996	-0.00577	- 0.20419
ZINC93154378 -OH	-0.21068	-0.01921	-0.19147
ZINC70193107 -C	-0.22617	-0.02213	-0.20404
ZINC53781180 -C	-0.20996	-0.00577	-0.20419
ZINC93154378 -O	-0.21068	-0.01921	-0.19147
ZINC87174488 -O	-0.23256	-0.00811	-0.22445
ZINC93154378 -CL	-0.21068	-0.01921	-0.19147
ZINC70193107 -OH	-0.22617	-0.02213	-0.20404
ZINC87174488 -OH	-0.23256	-0.00811	-0.22445
ZINC87173841 -OH	-0.21571	-0.00376	-0.21195
ZINC87173841 -O	-0.21571	-0.00376	-0.21195

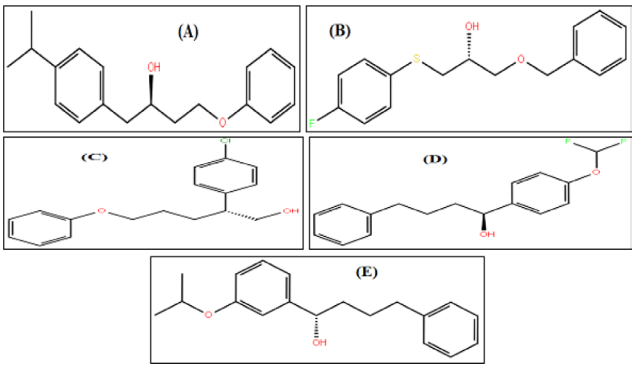


Figure 5 : All five ligand 2D structure. (A) ZINC53781180, (B) ZINC70193107, (C) ZINC93154378, (D) ZINC87174488, (E) ZINC87173841

Figure 5. Epik[35] is a software package for calculating pKa values for drug-like molecules. Epik use competence in combination with technology for tautomerization to modify the protonation state of small drug-like molecules to automatically produce one or more of the most possible forms for use in advance molecular modeling studies.[36] pKa result shown into the Table 5.

Molecules with large HOMO-LUMO gaps have been shown to be stable and unreactive. The gap between HOMO–LUMO is the potential energy difference between the HOMO and the LUMO. A small gap in HOMO–LUMO means slight excitation energies to the manifold of excited states. Hence, small gap molecules will be more polarizable than the large gap molecules.[37] HOMO LUMO result shown into Table 6 with between HOMO and LUMO.

CONCLUSION

Designing a new drug better than the existing drug for *Mycobacterium tuberculosis* is very challenging and important for cure. In the present research, PT70 had been taken as base molecule and was docked with the main receptor INHA gene and the binding energy came out to be -10.133. Pharmacophore model of PT70 design by phase tool and screen out lac of molecules through this model and thousands of molecules selected for final docking with INHA gene and result got

better than PT70 molecule (1. ZINC53781180 -10.881, 2. ZINC70193107 -10.648, 3. ZINC93154378 -14.697, 4. ZINC87174488 -10.288, 5. ZINC87173841 -10.203) and pass the ADMET analysis conditions and membrane permeability conditions. These molecules can be used for further analysis for inhibiting INHA gene and these molecules can replace old drug.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

TB: Tuberculosis; **ADMET:** Absorption, Distribution, Metabolism, and Excretion; **MTB:** Mycobacterium Tuberculosis; **WHO:** World Health Organization; **HIV:** Human immunodeficiency virus; **MDR-TB:** Multi-Drug-Resistant Tuberculosis; **XDR-TB:** Extensively Drug-Resistant TB; **pKa:** Aqueous dissociation constant; **pH:** Potential of hydrogen; **HOMO:** Highest occupied molecular orbital; **LUMO:** Lowest unoccupied molecular orbital.

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SUMMARY

Tuberculosis, is a disease which is instigated by the bacterium Mycobacterium Tuberculosis (MTB), it is an air-borne infection that spreads from a one person to another via sneezing and coughing, promoting the killing of 13 lakh people every single year as per WHO. Supplementary, the literature survey says that the PT70 molecule has been described as a potent drug in the market that has been exploited as a therapeutic agent for TB. Yet, for these commercialized anti-TB drugs, the causative agent has become drug resistant in a tolerant modus. Hence, to fight the metabolic activity of the Mycobacterium tuberculosis there is a necessity for a potent drug that could be subjected in the treatment of TB. Henceforth, a novel inhibitor as an anti-tuberculotic agent has been designed for which PT70 has been taken as a base molecule with the target INHA gene, and as a result, the process observed a minimal binding energy. However, both MDR and Extensive Drug Resistant (XDR) MTB isolates are resistant to isoniazid, mostly due to mutations in KatG, the catalase peroxidase involved in the activation of isoniazid. This has led to broad efforts to recognize the direct INHA inhibitors. Moreover, a comparative analysis was done using pharmacophore modeling. In common, there were two foremost objectives of the docking studies: correct prediction of activity and accurate structural modeling. Ultimately, top five molecules were arranged on the bases of their binding energy highest being-10.881. Aforementioned molecules were then passed via ADMET analysis, membrane permeability test, pKa, and density function theory, these tests were passed successfully.

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