2. Review of the Literature

Despite the continued global threat of *Mycobacterium tuberculosis*, no new class of TB drugs has successfully being discovered in the last 40-50 years which can fight with TB. Pharmaceutical industries have not succeeded in curing tuberculosis without the use of combination of drugs. Use of single drug has always resulted in emergence of resistant strains. Studies related to the survival of this pathogen in human host reveal that the organism uses a complex physiology to adapt to the host environment. The emergence of MDR, XDR and TDR propelled to think beyond the limited number of available pathways for drug target. To develop the concept of new therapeutic interventions against tuberculosis, and to fill the gap in our current understanding of the biology of tubercle bacillus, Cole *et al.* reported in 1998, the complete genome sequence of the most widely used strain H37Rv. A subsequent re-annotation in 2002 successfully assigned functions to almost half of the approximately 4000 genes. This study brought a new ray of hope to discover novel therapeutic agents targeting through a well explored biological path. Consequently, the complete knowledge on Mtb genomic sequence made it feasible to understand the resistance, virulence and metabolic pathways of Mycobacterium. There are many investigational drugs (INDs) with novel mechanism of action which have entered the clinical trial since last couple of years. However, these developments has not changed the scenario completely.

2.1. Potential targets of *Mycobacterium tuberculosis*

A protein which is crucial for the growth and survival of *Mycobacterium tuberculosis*, both in active and latent state could be a potential target in antitubercular drug discovery. With the access of Mtb genome data, research has been focused on identifying an ideal target which can avoid drug resistance. Comparative studies in the host and Mtb enzymatic pathways helped to identify numerous unique pathways which are quite selective in prokaryotes. Eventually, potential drug targets were identified from pathways related to lipid/carbohydrate/amino acid/energy/nucleotide metabolism, and vitamin/cofactor biosynthetic pathways. Of the 185 distinct targets identified till date from these pathways, many are still in various stages of exploration. The complex composition of mycobacterial cell wall and involvement of unique enzymes in its biosynthesis remains a favorite target for the discovery of new drugs since long. It is now driven by the pressing need for alternative drugs to counteract drug-resistant tuberculosis.
Table 1. Promising Drug targets in *Mycobacterium tuberculosis*

<table>
<thead>
<tr>
<th>Name of the pathway</th>
<th>Number of targets</th>
<th>Name of the pathway</th>
<th>Number of targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall biosynthesis</td>
<td>10</td>
<td>Carbohydrate metabolism</td>
<td>40</td>
</tr>
<tr>
<td>Amino acid metabolism</td>
<td>50</td>
<td>Vitamin biosynthesis</td>
<td>35</td>
</tr>
<tr>
<td>Lipid Metabolism</td>
<td>20</td>
<td>Energy metabolism</td>
<td>30</td>
</tr>
</tbody>
</table>

2.2. Mycobacterial cell wall and the enzymes involved in Fatty acid biosynthesis

The core structure of *Mycobacterial* cell wall is composed of covalently linked complex of peptidoglycans, arabinagalactans, mycolic acids, lipids, and carbohydrates. The cell-wall mycolic acids are oriented perpendicular to the plane of the membrane and provide a truly special lipid barrier responsible for the impermeability to hydrophilic antibiotics. Intercalated within this environment, phthiocerol dimycocerosates, cord factor or dimycyltrehalose, sulfolipids, phosphatidyl-inositol mannosides and the related lipomannan and lipoarabinomannan, etc. are responsible for much of the pathogenesis of tuberculosis. Brennan *et al.* have demonstrated that among the 528 proteins in the cell wall of Mtb, 35% are involved in small molecule metabolism and 25% are involved in macromolecule synthesis. They also reported in their study that the Mtb cell wall is actively engaged in Mycobacterial survival and remodeling.

Essential fatty acids required for the biosynthesis of Mtb cell wall undergo four key reactions to elongate their fatty acyl chains. Initially, the acyl chain is extended by two carbon units by β-ketoacyl synthase, which adds a malonyl group to yield a β-ketoacyl chain. The second reaction is catalyzed by a β-ketoacyl reductase, reducing the acyl chain to a β-hydroxyacyl group. This is then converted to a trans-2-enoyl chain by β-hydroxyacyl dehydrase, which is reduced further by an enoyl reductase (Fab I) to complete the elongation cycle (Figure 1). All these biochemical reactions are catalyzed by the Fatty acid synthase (FAS) system. Fatty acid synthase system is of two types, FASI and FAS II, on the basis of whether the reactions are performed by a single polypeptide or by individual enzymes. FAS I is present in eukaryotes and FAS II is generally found in prokaryotes. *Mycobacterium tuberculosis* contains both FAS I and FAS II in their FAS system. The Mycobacterial FASI system is responsible for the synthesis of C16-26 fatty acyl primers which are then passed to the FAS II system and elongated to produce intermediates of the long meromycolate chain. These intermediates can be modified and condensed with the alpha-branched fatty acids to form mycolic acids which is the major component of the cell wall of Mtb.
The type II fatty acid synthase consists of a series of individual enzymes, each encoded by a separate gene, that catalyze discrete steps in chain elongation. Inhibition of such enzymes responsible for mycolic acid biosynthesis is always considered as the most attractive target for antimycobacterial agents. Several literature reports suggest that FAS II enzymes are ideal targets for antitubercular drug discovery. Antimycobacterial agents like Isoniazid, Ethionamide, Isoxyl, Thiolactomycin and Triclosan utilize FAS II system to inhibit the Mtb mycolic acid biosynthesis. Unlike FAS I, FAS II is not present in human beings and thus considered to be a privileged selective target. These major differences between the bacterial and human systems have made it an attractive system for the discovery of novel antibiotic targets. Among many enzymes of FAS II system, Enoyl Acyl Carrier protein reductase is considered to be one of the ideal targets for drug discovery.

**Figure 1.** Pathways of FAS II mediated cell wall biosynthesis of Mtb.

**2.3. Mycobacterial Enoyl Acyl Carrier protein reductase (ENR/ Fab I/ InhA)**

Mycobacterial Acyl carrier proteins (ACPs) play a major role in biosynthesis of fatty acids where the growing substrate is attached via a thioester to the ACP phosphopantetheine group. ACPs involved in FAS II mediated Mtb cell wall biosynthesis are small, highly soluble, acidic proteins with molecular weight of 13kDa. In the last step of fatty acid biosynthesis, Enoyl acyl carrier protein is reduced reversibly to an Acyl acyl carrier protein by an enzyme called Enoyl Acyl Carrier protein reductase (ENR). ENR or InhA belongs to the family of short-chain dehydrogenases/reductases. ENR utilizes NAD to saturate the trans double bond between C2 and C3 of fatty acyl chain linked to the acyl carrier protein (Figure 2). The pyridine nucleotide reduction of the double bond is thought to proceed by conjugate addition of a hydride ion from NADH or NADPH to carbon 3 of the trans-2-acyl group with the intermediate formation of an enzyme-stabilized enolate anion on the C1 carbonyl oxygen.
Collapse of the enolate via protonation at C2 would yield the saturated product with the C2 proton being derived from the hydroxyl group of an active site tyrosine-158 side chain. The tyrosine proton is replenished from solvent via a proton wire involving Lys163 and the ribose hydroxyl groups plus a chain of water molecules.\textsuperscript{35} The pyridine nucleotide cofactor hydride ion utilized by the \textit{Mycobacterium tuberculosis} Enoyl-ACP Reductase is the 4S hydrogen whereas the mammalian FAS-1 uses the 4R hydrogen.\textsuperscript{35}

![Figure 2. The reaction mechanism of ENR.\textsuperscript{34}](image)

The Mtb ENR is one of the extensively studied pathways of FAS II system. Within the field of fatty acid biosynthesis ENRs were rather neglected enzymes until the \textit{Mycobacterium tuberculosis} ENR, was discovered to be the target of the primary anti-tuberculosis drug, Isoniazid\textsuperscript{36} (a successful first line TB drug) and a widely used antibacterial agent Triclosan.\textsuperscript{37}

Though there are already antibiotics in use those targets ENR, still this area continues to be heavily investigated, not only due to the differences in architecture between human and bacterial systems but also due to the essential nature of the ENR.

\textbf{2.4. Inhibitors of Mycobacterial ENR}

Due to the widespread resistance of the pathogenic bacteria to many of the present antibiotics, ENRs are the focus of large scale attempts to find compounds that specifically inhibit these enzymes. Availability of high resolution structures of Mycobacterial ENR helps in structure based design of novel inhibitors. There are many known Mtb inhibitors of fatty acid synthesis which target the Enoyl Acyl Carrier protein reductase. Among them Isoniazid, Thienodiazaborine and Triclosan have been extensively studied (Figure 3).

![Figure 3. Chemical structures of \textit{Mycobacterium tuberculosis} ENR Inhibitors.](image)
Isoniazid is a successful first line antitubercular drug being used since last 50 years in TB therapy. It is a pro-drug that undergoes oxidative activation by the catalase peroxidase (KatG) of *Mycobacterium tuberculosis* to form an isonicotinoyl radical that reacts non-enzymatically with NAD to form adducts.\(^{38,39}\) Among the 12 adducts formed, the acyclic 4'S isomer of INH-NAD is known to be a slow-onset, tight binding inhibitor of ENR.\(^{40}\) In spite of its wide use as an AntiTB drug, Isoniazid has developed resistance rapidly due to inactivating mutations within the catalase/peroxidase gene, which have no effect on *M. tuberculosis* virulence.\(^ {41}\) Thus compounds that inhibit ENR without first requiring KatG activation will be active against the majority of Isoniazid resistant strains of *Mycobacterium tuberculosis*. Broussy et al.\(^ {42}\) (by replacing the pyridine cycle of Isoniazid in INH-NAD adduct by a phenyl cycle) and Lu et al.\(^ {43}\) have attempted to make Isoniazid-NAD\(^+\) analogues with some success, but this work is chemically challenging and at its early stages of exploration.

Diazaborines are a class of heterocyclic boron-containing compounds that inhibit ENR by the formation of a covalent bond between the boron atom and the 2'-hydroxyl of the NAD\(^+\) ribose moiety.\(^ {44}\) The molecule \(\pi\)-stacks with the nicotinamide ring of the coenzyme and also have van der Waals interactions with the hydrophobic substrate-binding pocket. The boron atom and its associated hydroxyl group occupy the space of the enolate in the putative substrate complex. While both Isoniazid and Diazaborines form covalent adducts with the NAD-bound form of enoyl-ACP reductase, the point of attachment is different and the interaction of the two drugs with the target enzyme differs. Diazaborines seem to have been abandoned as a medically useful set of compounds, perhaps due to their undesirable inhibition of RNA processing in eukaryotic cells.\(^ {45}\)

Triclosan is a broad spectrum antibacterial agent, active against a variety of microorganisms such as Gram-positive bacteria, Gram-negative bacteria, fungi and yeasts. Heath and co-workers were the first to demonstrate the inhibitory effect of Triclosan (TCL) on Enoyl-ACP reductase step of fatty acid biosynthesis.\(^ {30}\) Kinetic and structural studies of the interaction of TCL with ENR have shown that TCL forms a tightly associated ternary complex with the protein and the charged nicotinamide cofactor.\(^ {46}\) Triclosan binds by overlapping with the acyl substrate-binding pocket and is quite separate from the site of INH binding. Triclosan is chemically a diphenyl ether (Figure 3), where the phenol ring makes \(\pi\)-stacking interactions with the nicotinamide ring of NAD\(^+\) and the hydroxyl group of the drug forms hydrogen bonding interactions with both the phenolic oxygen of Tyrosine-156 and the 2-hydroxyl of the nicotinamide ribose. In addition to this, it shows extensive van der Waals interactions with the
ENR protein. Hence literature reports suggest that Triclosan binding represents a model for substrate binding.

From the Structure-activity studies of Triclosan analogs, Stewart et al. revealed that the 2-hydroxyl substituent in the moiety is vital for ENR inhibitory effect, but also suggested that replacement of the diphenyl ether bridging oxygen atom with a sulfur atom could decrease the potency since the bulkier sulfur atom will lead to an increase in drug-cofactor distance, thereby disrupting the hydrogen-bond network involving the Triclosan 2-hydroxyl group. 

Unlike INH, Triclosan does not require activation, then also their utility for human treatment is limited due to its poor solubility and sub-optimal bioavailability. However, this small organic molecule, represent reasonable starting point for structure based drug discovery efforts to afford effective ENR inhibitors.

2.5. Diphenyl ether based Antimycobacterial agents

From the literature reports on existing inhibitors of ENR, it can be proposed that blocking the entry of acyl chains from ACP directly by the inhibitors like Triclosan, could be a potential approach to disturb the essential reduction step in the mycolic acid biosynthesis. This would make it more difficult for mycobacterium to develop resistance. Thus the attempts to find Diphenyl ether based AntiTB agents which chemically resemble Triclosan have become a burning chapter in antituberculosis drug discovery.

Sullivan et al. developed the first generation diphenyl ethers by synthesizing a series of alkyl diphenyl ethers as uncompetitive inhibitors of Mycobacterium tuberculosis Enoyl reductase enzyme with best compound of Ki' value of 1 nM for InhA and MIC values of 2-3 µg/mL for both drug-sensitive and drug-resistant strains of Mtb. During the course of this work, they reported the X-ray structures of two Triclosan analogs with 5-alkyl (1a and 1b) moieties, lacking the two B-ring chlorines. They also reported that the overexpression of ENR in Mycobacterium tuberculosis results in a 9-12-fold increase in MIC. This collection of structures provided a precisely defined active site of InhA and a thorough understanding of the ligand-enzyme interactions that render potent enzyme inhibition. However, compounds reported by Sullivan et al. are rapid reversible inhibitors of InhA.

![Figure 4. Chemical structures of compounds 1a and 1b](image-url)
With limited options to do structural variations, modification of phenyl rings in the diphenyl ether derivatives without changing the geometry of the scaffold could be an interesting approach. Hence, through structure-based design, Boyne et al. developed a focused library of A-ring-modified diphenyl ether InhA inhibitors. From this library of analogs, two high-affinity alkyl-substituted diphenyl ethers, 6PP and 8PP, were selected for advanced study into their in vitro activity against *Mycobacterium tuberculosis* clinical isolates, their in vivo properties, and their signature response mode of action. 6PP and 8PP demonstrated enhanced activity against whole bacteria and showed activity in a rapid macrophage model of infection. In addition, transcriptional profiling revealed that the A-ring modifications of 6PP and 8PP increased the specificity of each analog for InhA. Both analogs had substantially longer half-lives in serum than did the parent compound, exhibited a fivefold reduction in cytotoxicity compared to the parent compound, and were well tolerated when administered orally at 300 mg/kg of body weight in animal models. Thus, subtle steric changes to the Triclosan A ring have a dramatic effect on interactions with InhA.

The next step in optimization of the diphenyl ether pharmacophore for preclinical evaluation could be the modification of the B ring to increase their druggability. In an efforts to reduce the lipophilicity and to improve the bioavailability of the previously reported antitubercular alkyl diphenyl ethers, Ende et al. developed a series of B ring analogues of compound 2a (MIC = 1-2 µg/ mL) having either heterocyclic nitrogen rings or phenyl rings with amino, nitro, amide or piperazine functions. Few of their synthesized compounds (2b, 2c and 2d) showed comparable MIC<sub>90</sub> values to that of 1a, but having improved ClogP values.

![Chemical structures of compounds 2a-d](image)

**Figure 5.** Chemical structures of compounds 2a-d with their MIC and ClogP values.

Kini et al. incorporated different heterocycles at ortho, meta and para position in the diphenyl ether ring (compounds 3a-c, 3d-f and 3g-i) to obtain antimycobacterial activity as low as 1 µg/mL against the H<sub>37</sub>Rv strain of *Mycobacterium tuberculosis*. Molecular modeling study carried out by them showed that the synthesized diphenyl ether derivatives occupy the same binding domain as of Triclosan in Enoyl-ACP reductase enzyme.
Heterocycles substituted at α, m and p positions in the diphenyl ring.

Figure 6. Chemical structures of compounds 3a-c, 3d-f, and 3g-i

Using structure-based drug design approach, Freundlich et al. developed a series of 5-substituted Triclosan derivatives. From the SAR study, they observed that, Triclosan derivatives with alkyl and aryl substituents improve the antitubercular potency dramatically. Their most efficacious inhibitor displayed an IC₅₀ value of 21 nM, which was 50-fold more potent than Triclosan. Among the six selected Triclosan derivatives tested against Isoniazid-sensitive and resistant strains of *Mycobacterium tuberculosis*, the best inhibitor (4a) had an MIC value of 4.7 µg/mL (13 µM), which represents a tenfold improvement over the bactericidal activity of Triclosan.

Figure 7. Chemical structure of compound 4a with MIC

Approach to incorporate heterocyclic ring into the diphenyl ether pharmacophore has been given a major attention to improve the druggability of the compounds. A group of researchers from University of Illinois, US synthesized isoxazole-based anti-TB compounds by applying rational drug design approach. Among all of their compounds, isoxazole based diphenyl ether derivative (5a) showed MIC of 0.9 µM against *Mycobacterium tuberculosis* H37Rv and MIC of 13.8 µM against nonreplicating Mtb. This compound was reported to be safe against Vero cells (IC₅₀>128 µM). Hence, they claimed this phenoxy derivative of 5-phenyl-3-isoxazolcarboxylic acid ethyl esters, a highly potent, selective, and versatile series of anti-TB compounds and as such present attractive lead compounds for further TB drug development.

Figure 8. Chemical structure of compound 5a with its biological profile
Incorporation of various amines into the diphenyl ether scaffold through amide linkers (compounds 6a-v) afforded compounds with MIC values ranging from 4-64 μg/mL. While studying the SAR of these compounds, Yang et al. observed that chlorine atoms at 3 and 4 positions in the phenyl ring play a significant role in the antitubercular activity.

![Chemical structures of compounds 6a-v, and MIC of compound 6a](image-url)

**Figure 9.** Chemical structures of compounds 6a-v, and MIC of compound 6a

Telvekar et al. designed various diphenyl ether derivatives by molecular hybridization approach. To achieve a synergistic effect, they synthesized a series of structurally novel, substituted 2-(2-(4-aryloxybenzylidene) hydrazinyl) benzothiazole derivatives (7a-y) by incorporating 2-hydrazinyl benzothiazole and 4-(aryloxy) benzaldehyde. Five of their synthesized compounds exhibited MIC < 3.0 μg/mL against *Mycobacterium tuberculosis* H37Rv, while the best antitubercular activity was obtained at MIC 1.5 μg/mL (7a).

![Chemical structures of compounds 7a-y, and MIC of compound 7a](image-url)

**Figure 10.** Chemical structures of compounds 7a-y, and MIC of compound 7a

### 2.6. Implication of Molecular modeling in designing of Mtb ENR Inhibitors

Structure-based drug design seeks to identify and optimize molecular interactions between ligands and the host proteins, given their three-dimensional structures. Through molecular modelling technique, essential binding interactions and orientations of antitubercular agents with the Mtb ENR protein can be understood at the molecular level. This knowledge can be utilized as a yardstick for the designing of novel inhibitors with promising antimycobacterial activity. Nevertheless, the complete insight into the structural biology of Mtb ENR (Inh) is a must for molecular modelling studies.

X-ray crystallography study has revealed that the C16 fatty acyl chain portion of the natural substrate is completely surrounded by the side chains of hydrophobic residues. The majority of these hydrophobic side chains reside on the substrate binding loop (Ala198, Met199, Ala201, Ile202, Leu207, Ile215, and Leu218). Additional hydrophobic residues,
having side chains that surround the fatty acyl chain but are not part of the substrate binding loop, include Met103, Phe149, Met155, Tyr158, and Met161. The side chain of Phe149 is located near the nicotinamide ring of NAD and appears to help guide the turn of the fatty acyl substrate U-shaped conformation (Figure 11a). There is only one direct hydrogen bond between the fatty acyl substrate and the InhA protein, namely between the thioester carbonyl oxygen and the side chain hydroxyl oxygen of Tyr158. Two additional hydrogen bonds occur between the fatty acyl substrate and NAD. These are between the amide nitrogen of the N-acetyl cysteamine portion of the substrate and phosphate oxygen of NAD and between the substrate thioester carbonyl oxygen and the 2'-hydroxyl oxygen of the nicotinamide ribose of NAD. Furthermore, there is an additional hydrogen bond between the substrate thioester sulfur and an ordered water molecule held by phosphate oxygen of NAD and the side chain of Thr196.63

![Figure 11a](image)

**Figure 11a.** *Mycobacterium tuberculosis* Enoyl-ACP reductase (ENR) in complex with a C16 fatty acyl substrate and NAD; 11b. Triclosan (TCL) in ENR binding pocket.

In course of their research on X-ray crystal structure of the FabI-NAD1-triclosan ternary complex, Heath et al.30 observed that the diphenyl ether ring of Triclosan adopted a conformation with a dihedral angle of about 90° between the two phenyl rings of the inhibitor. The 2’-hydroxyl group of Triclosan was involved in strong hydrogen bonds with the 2’-hydroxyl group of the nicotinamide ribose, and the phenolic hydroxyl group of Tyr-156. The chlorine substituent in the 4-position of the phenyl ring accepted a hydrogen bond from the backbone amide of Ala-95 and formed hydrophobic contacts with the side chain of Met-159 (Figure 4b). Stewart et al.47 contributed in this field by stating that the 2’-OH group of the nicotinamide ribose also forms a hydrogen bond with the bridging oxygen atom of the Triclosan. They also revealed that the Ring B of Triclosan is positioned adjacent to the NADH in a hydrophobic pocket formed by residues like Tyr-146, Tyr-156, Leu100, Tyr146, Tyr156, Met159, Ala196, Ala197, Phe203 and Met206.
Hence Rozwarski et al. reported that the catalytic signatures of *M. tuberculosis* InhA is **Phe-X8-Tyr-X6-Lys**. Thus compounds having interactions with these catalytic sites could be considered as a promising Mtb ENR inhibitor.

Researchers from AIIMS, India, used structure based computer modeling approach to design a tripeptide for the direct inhibition of InhA. Using the X-ray crystal structure of InhA (2H7M) co-crystallized with a known inhibitor, “(3S)-1-cyclohexyl-N-(3,5-dichlorophenyl)-5-oxopyrrolidine-3-carboxamide” they carried out the docking studies which indicated that the best docked peptide has 100 times higher potency than the best known inhibitor. Using computer assisted structure based design of slow onset diphenyl ether, Luckner et al. introduced a methyl group ortho to the diphenyl ether linkage to reduce the conformational flexibility of the lead diphenyl ether. This modification enable ordering of the active site loop, thus in turn resulted in a compound, PT70, which is a slow onset inhibitor of InhA with a *K* value of 22 pM. Molecular docking study with InhA protein (2B37) revealed that the B-ring methyl group of PT70 forms critical van der Waal's interactions with the NAD cofactor and with key amino acids of the formerly disordered substrate-binding loop (Figure 5). These studies provide information on the mechanistic imperatives for slow onset inhibition of Enoyl ACP reductases, and the novel inhibitor has the potential to be effective against both drug-sensitive and drug-resistant strains of *M. tuberculosis*. Yuya et al. attempted to identify novel antibiotics targeting the Mtb enoyl-acyl carrier protein reductase through in silico structure-based drug screening using the crystal structure data for the MTB enoyl-acyl carrier protein reductase (pdb code. 2H71) and a virtual compound library, which includes 152,102 chemicals. By a two-step screening method using DOCK (first screening) and GOLD (second screening), they identified 5 chemical compounds expected to have high binding affinity to the active center of the MTB enoyl-acyl carrier protein reductase. In their report they suggested that a chemical compound, which has a basic skeleton comprised of dibenzofuran, acetoamide, trizol, furyl and methylphenyl groups, completely inhibited the growth of Mycobacterium.

Stigliani et al. reported an ensemble-docking process with four known InhA X-ray crystal structures like 1P44, 1P45, 2H7M and 3FNE employing the Autodock Vina software. They selected five known InhA inhibitors docked sequentially in the substrate cavity of each protein. Through a post-docking optimization, resulting interaction energies combined with the multiple receptor conformations approach allowed them to retrieve the bioactive conformation of each ligand.
During preclinical drug development, selection of a lead molecule with the most appropriate drug-like properties is always considered challenging. The critical drug-like properties can be explored by examining the fate of an orally administered drug. Properties like solubility, lipophilicity, ionization/dissociation, permeability, protein binding and metabolic stability govern the drug-likeness of a NCE in early drug discovery step because they relate to various aspects of absorption, distribution, metabolism, and excretion (ADME). Compounds that fail to demonstrate satisfactory ADME properties and optimum pharmacokinetic profiles can be quickly removed from consideration as drug candidates. It has been estimated that in spite of molecular modeling towards specific molecular target, 30% of compounds fail to show efficacy, while 50% of active compounds fail to become a successful drug due to poor pharmacokinetics or toxicity. However, screening large numbers of compounds for drug-likeness, especially using animals, is a cumbersome process, which limits its utility in drug discovery. Hence, relatively simple in vitro methods have taken a front seat in the study of drug-likeness to provide a rapid selection of compounds with acceptable ADME properties.

Drug-likeness or druggability of a compound can be estimated virtually by using computational tools. This approach is best exemplified by Lipinski’s Rule of Five. Based on a review of the physical and chemical properties of chemical compounds that are drug-like and non-drug-like, Lipinski developed the concept that there are physical and chemical properties that appear to be critical to drug-likeness.

**Figure 12.** Chemical structure of PT70 and 8PP; Docking image of PT70 (black) with InhA with a overlay of different InhA inhibitors (Triclosan, 8PP and JPL) displays the differences in the orientation of the B-ring. 

2.7. Implication of the study of drug-likeness in antitubercular drug discovery
Lipinski’s Rule of Five is stated as follows:

- Molecular weight ≤ 500
- Lipophilicity (Log P) ≤ 5
- No. of H-bond donors ≤ 5
- No. of H-bond acceptors ≤ 5
- The sum of Ns and Os ≤ 10
- No. of rotatable bonds ≤ 10

Compounds with above properties can be considered druggable in the early phase of drug discovery. Rule of five is now widely used to filter out compounds likely to have poor pharmacokinetic properties early in drug discovery. Strategies to correlate the virtual data from computational tools with the experimental data always ensure proper evaluation of drug-likeness of a New Chemical Entity (NCE).

Coming to the challenges posed in further development of antituberculosis drugs, it is useful to evaluate key desirable attributes for a new drug in terms of physicochemical profile and safety. These include optimum oral bioavailability, acceptable protein binding, sufficient lung penetration, a lengthy elimination half life (suitable for once-daily or less frequent dosing), proper metabolic stability and avoidance of toxicities. These are the characteristics that one should pursue for the next generation antitubercular agents.