CHAPTER 1

Drug Development Against Tuberculosis:
An Overview
1.1 INTRODUCTION

Tuberculosis (TB) an infectious and airborne disease commonly found in third world countries is linked to dense population, poor nutrition and sanitation. TB is a single largest infectious disease in the world causing nearly two million deaths annually. \(^{1-7}\) 95\% of new TB cases every year occur in developing countries and it is single biggest killer of young women, one million per year in the developing world followed by malaria and maternal mortality.\(^{8}\)

History of *Mycobacterium tuberculosis* is very old and it has been known since 2400 BC when antiquity-fragments of the spinal column from Egyptian mummies show definite pathological signs of tubercular decay.\(^{9}\) Exact pathological and anatomical description of the disease was appeared in 17th century. A new theory of the disease by English physician B. Manter (1720) was proposed that TB could be caused by, “wonderfully minute lung creatures”, which once had foothold in the body, could generate lesions and symptoms of the disease. A close association of patients with normal individuals was thought to be reason for rapid spread of disease. Introduction of sanitorium was the first real step against tuberculosis. In 1854, H. Brehmer presented his doctoral dissertation bearing the auspicious title ‘*Tuberculosis is a curable disease*’. New advances then followed in rapid succession and in 1865, a French military doctor J-A Villemin postulated that a *specific microorganism is the cause of the disease*. In 1882, Robert Koch’s scientific brilliance led to the discovery of *Mycobacterium tuberculosis* as the causative agent of the disease. Different means of tuberculosis curtailment were developed from time to time. In 19th century a French bacteriologist Calmette together with Guerin created the basis for the BCG vaccine, even though relatively ineffective still in wide spread use. In the 20th century during the World War II came the final breakthrough, the greatest challenge to the bacterium that had threatened humanity for thousand of years- *chemotherapy*.\(^{9,10}\)

India accounts for nearly one third of global TB problem and more than 20 million people become infected with this disease, more than 5000 develop disease and more than 1000 die from the disease. In India tuberculosis kills 14 times more people than all tropical diseases. India had over 4.5 million TB cases with 1.8 million new cases being reported each year. Approximately 50\% of the India’s population is reported to be
tuberculin test positive. Annually about 0.4 million death and one million new cases of TB are reported. As per RNTPC status report every year additional 20-lakh people develop this disease and in India, one person dies every minute by TB.

History of tuberculosis in India also dates as 600 BC where in a Sushruta Samhita, a compendium of ancient medicine and surgery. In Sanskrit the disease is known as Kshaya, 'wasting disease', or Raja Yakshmaa, "the king of diseases". There were said to be four causes of the disease: over strain, suppression of natural urges, wasting (for example, due to grief, anxiety or longing) and a promiscuous diet, any of which could cause the three morbid humours Vata, Pitta and Kapha to flare up. A treatment based on the principles of Ayurveda, the classical Indian system of health and healing, was provided. Besides medicines, dietary prescriptions were detailed; alcohol in moderate quantities, the flesh of birds and animals which inhibit dry areas and goat's milk were recommended among the items. Some sages were of the opinion that the disease could be spread from person to person and listed it in the class of communicable diseases along with leprosy, fevers, conjunctivitis and syphilis. All said and done, TB was rare until the second half of the 19th century. Concomitant with the growing population density caused by industrialization, its incidence has increased progressively.

In 1993, World Health Organization has declared this disease as global emergency and lot of funds are pouring in to combat this disease from developed countries and many non-governmental organizations. An estimated one-third of the 42 million people living with HIV/AIDS worldwide were co-infected with tuberculosis. As per WHO reports about 90% of patients having TB and HIV both died within a few months after clinical symptoms appeared for the disease. Therefore, WHO warned the world for "even greater TB / HIV crisis" and called for wide availability of free antituberculosis drugs to those living with HIV. Starting that an even greater TB/HIV crisis might be emerging in India and as per WHO, HIV is spreading rapidly in the country, which has the largest number of TB cases in the world.

The emergence of multi drug resistant (MDR) tuberculosis has focused the attention of scientific community throughout the world on the urgent need of new antitubercular agents. Antitubercular agents have not perceived high priority by pharmaceutical companies over the last 40 years because of non-profit nature of this disease and
therefore the main responsibility lies on government and social organizations to combat this disease. Due to its emergence in developed countries now a coordinated effort is being made to develop new mechanism based antituberculosis agents. The recent developments in genetic engineering of \textit{M. tuberculosis} have now offered many targets to be validated and screen library of organic compounds to develop new antituberculosis targets. In selecting targets for antituberculosis agents, it is advantageous to avoid targets, which are close to the counterparts in mammalian cells. It is also desirable that new targets should be specific to \textit{Mycobacteria} in order to limit the transfer of resistance factors from other bacteria. Further, new drugs must act on a target that is essential for bacterial survival and ideally they should be active against \textit{mycobacterium} through out their growth cycle both inside and outside mammalian cells during infection.

In India, already 180000 people living with HIV were co-infected with tuberculosis and because of that government and non-government health monitoring agencies are popularizing the directly observed therapy for tuberculosis (DOTS) programme for tuberculosis control. \textsuperscript{28} In 2002, 55 \% of the Indian population had access to DOTS. \textsuperscript{29-31} Anti-TB drugs used through the DOTS strategy could prolong the lives of people living with HIV at a cost of approximately $200 for the entire 6-8 month treatment period, including health service and staff costs. However, only 32 \% of TB patients worldwide were being treated under the DOTS programmes. WHO predicts it would be 2013 by the time 70 \% case detection and 85 \% cure rates- were met. The report said that in countries like India, private practioners might see DOTS as a threat as patients enrolled in a free DOTS scheme were lost income. \textsuperscript{32}

1.2 DIFFERENT APPROACHES TO CONTROL TUBERCULOSIS

Three major approaches to control tuberculosis existed since the disease appeared; the Sanitorium development for fresh air, cleanliness and nutritious diet; the vaccination and the chemotherapy.

1.2.1 Vaccines: In general the efforts to make new vaccine candidates are focusing on four classes including rationally attenuated strains of \textit{Mycobacterium tuberculosis}, \textsuperscript{33-40} bacilli calmette- guerin (BCG) vaccine, \textsuperscript{41-44} protein subunit vaccines \textsuperscript{45, 46} and nucleic acid vaccines. \textsuperscript{47-49}
1.2.1.1 Rationally attenuated strains of Mycobacterium tuberculosis: Generation of rationally attenuated *M. tuberculosis* i.e. strains lacking virulence genes will not cause disease but still elicit a protective immune response and this is achieved by genetic manipulation of the *Mycobacterial* chromosome. For the application to a wide variety of specific genes, several methods were developed. A library of mutants can be generated using transposons, mobile genetic elements that can 'hop', disrupting genes. A leucine auxotroph, a strain requires exogenous leucine for growth, was isolated from a transposon mutant library of BCG, which was unable to survive in mice or in cultured macrophages.

1.2.1.2 Bacilli Calmette- Guerin (BCG) vaccine: BCG vaccine was improved by several ways. One strategy is to introduce into BCG the *Mycobacterium tuberculosis* genes encoding antigens which have high reactivity to memory immune T- cells, but were deleted from BCG in the mutations led to attention. Alternatively, the immune responses to purified protein derivative have been enhanced in mice by injecting BCG clones expressing various immune stimulating proteins. Finally, mice with severe immuno deficiency disease, which lack both T- & B- cells, survived inoculation with an auxotrophic Leu- D mutant of BCG, but not with normal BCG. The same auxotrophs protected immuno competent mice at a level comparable with normal BCG, suggesting that the auxotrophs may be used as a safe alternative to BCG in individuals with a compromised immune system.

1.2.1.3 Protein subunit vaccine: Utilization of purified protein subunits as vaccines offers a number of advantages over attenuated organisms. They are inherently safe; having no propensity to cause disease, an important consideration when vaccinating individuals have been exposed to HIV. Immunization with short-term culture filtrate proteins and their protection from virulent *M. tuberculosis* has been well demonstrated in mice. Those protection was magnitudically similar to that afforded by BCG, and so confirming the hypothesis that *M. tuberculosis* releases protective antigens during the early phase of infection. Immunization of guinea pigs with one antigen alone or in combination with other secreted proteins also induced a strong protective response, however in this case, the reduction in viable *M. tuberculosis* upon subsequent challenge was less than that expected with BCG-vaccinated animals.
1.2.1.4 Nucleic acid vaccines: ‘Naked DNA vaccines’ followed the hypothesis that mice became immune to influenza when vaccinated with only the DNA-encoding influenza, a nucleoprotein. The above hypothesis has now been applied to *M. tuberculosis* as vaccination with DNA encoding either the *M. Leprae* 65 KDa heat shock protein or the *M. tuberculosis* antigen 85 A protein protect from subsequent infection with virulent *M. tuberculosis*.

1.2.2 Genomics: The most significant developments in the area of tuberculosis are perhaps the sequencing of the mycobacterial genome. Sequencing of the H37Rv strains of *M. tuberculosis* and a highly virulent clinical isolation is well documented. Among the estimated 4500 genes, every drug target and every antigen, or protein elicits an immune response. The challenge for the tuberculosis research programme is to validate and prioritize those, which will most rapidly lead to new treatments. Comparing the two *mycobacterium tuberculosis* genome sequences may be particularly useful in identifying genes associated with virulence.

1.2.3 Immunotherapy: A radical, alternative approach to Tuberculosis therapy has been pioneered by John Stanford and Graham Rook in which, patients are treated with a heat-killed preparation of the saprophyte *Mycobacterium vaccae*, a non pathogenic organism isolated from soil, at the outset of the normal course of drug therapy. They found that while there was little difference in overall cure rate, there was a significant improvement in difficult to treat MDR-TB cases and where chemotherapy was incomplete or intermittent, as is the case in many parts of the developing world. This improvement is thought to depend on stimulation of a particular immune response that helps the host to eliminate persisting, or non-dividing bacteria which are unaffected by the drugs used. Thus, it may be possible to reduce the overall duration of therapy by this approach.

1.2.4 Chemotherapy: Chemotherapy of tuberculosis recently started in between and after World War II. In 1943, anti-TB research resulted in discovery of the active anti-TB agent streptomycin. A number of agents have been discovered since that time, including *para*-aminosalicylic acid (1946), isoniazid (1952), pyrazinamide (1952), cycloserine
(1952), ethionamide (1956), rifampicin (1957) and ethambutol (1962). The majority of these drugs were discovered through broad screening; very little optimization was undertaken and little regard was given to the targets of drug action since the biochemical tools for these studies were not as sophisticated as they are now. This lack of understanding of drug action because of ignorance in the biochemistry of the *Mycobacterium* and urgency to develop drugs against this devastating disease led to random screening of compound libraries. In fact, among other reasons, the difficulty in manipulating *M. tuberculosis* has hindered efforts to delineate the mode of action of these agents. Recent improvements in biological techniques have allowed the mechanisms of action of many of these agents to be uncovered and more carefully studied.

1.3 TARGETS FOR ANTITUBERCULAR DRUG DEVELOPMENT

Generally the targets for anti tuberculosis drug, involves the biosynthetic pathways involved in the production of macromolecules (the proteins, the nucleic acids or cell wall polymers). Many well known antitubercular drugs, target the biosynthesis of these macromolecules.

1.3.1 Protein Synthesis

Streptomycin, an aminoglycoside for widespread use in the treatment of tuberculosis, disrupts the protein synthesis in bacteria.\(^{59,60}\) Streptomycin resistance in *M. tuberculosis* is due to the mutation altering the ribosomal 16S RNA molecule.\(^{61}\) Most of the aminoglycosides act through this mechanism. The oxazolidinones also inhibits bacterial protein synthesis.\(^{70}\) Many other inhibitors of protein synthesis including tetracycline, chloramphenicol and macrolides (erythromycin) do not show activity against *M. tuberculosis*. The intensive effort of the medicinal chemists to develop antitubercular agents based on inhibition of protein synthesis suggests that the ribosome may not be particularly an attractive target for new antituberculosis drugs.

1.3.2 Nucleic Acids

Sulphonamides, the structural analogs of p-amino benzoic acid inhibit biosynthesis of tetrahydrofolic acid, and thereby block the production of purine and pyrimidine bases required for nucleic acid synthesis in microbes. The antituberculosis drug *p*-amino salicylic acid initially designed for as competitive inhibitor of salicylic acid may act on
the tetrahydrofolate pathway as well as salicylate dependent biosynthesis of mycobactins, required for iron transport. Efforts have been made to enhance the efficacy of sulphonamides in combination with other drugs (trimethoprim) inhibiting subsequent steps in tetrahydrofolate pathway catalysed by the enzyme dihydrofolate reductase. A detailed study of enzymes involved in tetrahydrofolate biosynthesis may lead to a rational design of new and novel antituberculosis drugs.62,63

Another promising target for inhibition of growth of *M. tuberculosis* is DNA topoisomerase particularly DNA gyrase, a type II topoisomerase. DNA gyrase involved in many reactions including ATP-dependent negative supercoiling of closed circular double stranded DNA; ATP-independent relaxation of negatively supercoiled DNA, nucleotide-dependent relaxation of negatively supercoiled DNA; formation and resolution of catenated DNA; resolution of knotted DNA; quinoline or calcium ion induced double stranded breakage of DNA; DNA dependent ATP hydrolysis. Fluoroquinolone class of drugs acts by inhibiting this enzyme.64,65 Recently gyr A and gyr B have been cloned from *M. tuberculosis* and *M. smegmatis*. A stretch of 165 amino acids found in *E.coli* gyr B is absent from mycobacterial gyr B and thus any drug acting against the latter would be uniquely specific to mycobacteria. Inhibition of its activity prevents supercoiling, as subsequent process such as replication and transcription are DNA topological dependent.66,63 Topo-IV is responsible for resolution of daughter molecules after chromosomal replication and inhibition of its activity prevents resolution of replicated DNA. The bactericidal effects of such agents involve the interaction of these agents with deoxyribonucleic acid (DNA) and DNA topoisomerase IV. In some organisms such as E. Coli, DNA-gyrase is the primary target, where as in other organisms particularly the gram positive Cocci DNA-topo IV will be primary target.66

Biosynthesis of nucleotides has recently been reported to be a good target particularly for tuberculosis in HIV cases. Very recently, thymidine monophosphate kinase (*dTMKase*) has been suggested as validated target to develop new antitubercular agents particularly for the treatment of MDR TB and tuberculosis in HIV infected patients. This enzyme is an essential enzyme of nucleotide metabolism that catalyses the reversible phosphorylation of thymidine monophosphate (*dTMP*) to thymidine diphosphate (*dTDP*). Detailed structural elucidation of this enzyme is known and the well
known anti HIV drug AZT has low affinity and this has led to the design and synthesis of more potent nucleoside analogs.\textsuperscript{67}

1.3.3 Cell wall macromolecules biosynthesis

Based upon most recent developments in the ultra structure\textsuperscript{68} and biochemistry of \textit{M. tuberculosis} its cell envelope\textsuperscript{69} consisting in three structural components, the plasma membrane, the cell wall and the capsule\textsuperscript{70} has been identified as the most the important validated targets to develop new drugs.\textsuperscript{71-73} Plasma membrane appears to be a typical bacterial membrane contributing very little towards the pathological processes.\textsuperscript{74, 75} Cell wall in mycobacteria, members of which cause tuberculosis and leprosy is very complex and of very poor permeability that contribute to their resistance to therapeutic agents. It consists of large amounts of C\textsubscript{60}-C\textsubscript{90} fatty acids; mycolic acids that are covalently linked to arabinogalactan which in turn are linked to peptidoglycan.\textsuperscript{79} Thus the complex cell wall of \textit{M. tuberculosis} may be described as a peptidoglycan to which polysaccharide side chains esterified at their distal ends with mycolic acids are linked (mycolyl-AG-peptidoglycan complex), 40 \% of which is lipids in the form of mycolic acids.\textsuperscript{76} The peptidoglycan consists of peptide side chains of L-alanine-D-isoglutaminyl-meso-diaminopimelyl-D-alanine, in which diaminopimelic acids are amidated.\textsuperscript{77} The mycobacterial peptidoglycan differs in two ways from that commonly found in other bacteria; the muramic acid is \textit{N}-glycolylated and that the cross links include bonds between two residues of dianaminopimelic acid as well as between dianaminopimelic acid and D-alanine.\textsuperscript{78}

It is known that major wall polysaccharide in \textit{M. tuberculosis} is a serologically active branched chain AG, resulting in non-reducing termini of the chains. AG is attached to peptidoglycan through a phosphoryl group to the position 6 of about 10-12 \% of muramic acid residues.\textsuperscript{79} All the arabinose and galactose residues are in the furanose form,\textsuperscript{80} the non reducing terminal of arabinan consists of a branched hexafuranosyl structure [\(\beta-D\text{-Araf}\ -(1\rightarrow2)-\alpha-D\text{- Araf}\;)\text{ }-3,5-\alpha-D\text{- Araf}\text{ }(1,5-\alpha-D\text{- Araf}\); the majority of arabinan chain consists of 5-linked \(\alpha-D\text{- Araf\ with branching introduced by 3 ,5-\alpha-D\text{- Araf\ replaced at both branch positions with 5 -\alpha-D\text{- Araf\; arbinan chains are attached to the galactan core through of some of the 6-linked Ga\textit{f} units; the galactan region consists of linear alternating 5- and 6-linked [\(\beta-D\text{- Galf}\text{residues, the galactan of AG is linked to C-
6 of some muramyl residues of peptidoglycan via the diglycosylphosphoryl bridge, L-Rahp (1-3)-D-GlcNAc-(1-P) and mycolic acids are located in clusters of four on the terminal hexaarabinofuranosyl units, but only about two thirds of these arrangements are mycolated. 81, 82

![Chemical structure of mycolyl-AG peptidoglycan complex of mycobacteria](image)

**Figure 1: Chemical structure of mycolyl-AG peptidoglycan complex of mycobacteria**

Mycolic acids are high molecular weight α-alkyl-β-hydroxy fatty acids, present mostly as bound clusters of AG, where they appear primarily as tetramycolylpenta-arabinosyl clusters, but also in extractable lipids mainly as trehalose 6,6'-dimycolate. The main part of the branched chain is called as “meromycolic acid” and the other part as α-branch. 83 Characteristic feature of these mycolic acids are that they are the largest (C_{60}-C_{90}); they have the largest α-branch (C_{20}-C_{25}); the main chain contain one or two functionalities- which may be double bond or cyclopropane rings-that are capable of
producing "kinks" in the molecule; they may contain oxygen functions additional to β-hydroxy group; and they may have methyl branches in the main carbon backbone.84

![chemical structures]

Figure 2a: Structures of *M. tuberculosis* complex

![chemical structures]

Figure 2b: Structures of *M. smegmatis* complex

Apart from the above macromolecules cell wall also contains many other macromolecules and those identified till date include, lipoarbinomannan (LAM),85, 86
many extractable lipids including glycolipids (glycopeptidolipids, GPL; \textsuperscript{87, 88} trehalose containing lipopolysaccharides, LOS; \textsuperscript{89, 90} phenolic glycolipids, PGL) \textsuperscript{91, 92} and other classes of free lipids (SL, sulpholipids; PDM, phthiocerol dimycocerosate). These lipids are very important in pathogenesis and survival of the \textit{M. tuberculosis} in the host macrophages. LAM exhibits a wide spectrum of immunoregulatory functions. It suppresses the immune responses, thus contributing to the pathogenesis.

Figure 3a: Generic structures of Lipooligosaccharides (LOS)

Figure 3b: Generic structures of Glycopeptidolipids (GPL)

Figure 3c: Generic structures of Phenolic Glycolipids (PGL)
LAM induced abrogation of T-cell activation, inhibiton of γ-interferon mediated activation of murine macrophages, scavenging of potentially cytotoxic oxygen free radicals and inhibition of protein kinase C activity are the main factors which make LAM and arbinomannan as the most important for pathogenesis and survival of the bacterium in the hostile environment of the macrophages. GPLs or PGLs located at cell surface or outside the bilayer structure of cell wall have been also been implicated in pathogenesis. Carbohydrate layers on cell surfaces usually contribute to virulence by preventing nonspecific phagocytosis but the survival within the macrophages had led some doubt earlier but now days role of carbohydrates in the protection of bacterium within macrophages has also been elucidated. Biosynthesis of these macromolecules particularly mycolic acids, arablingalactan, lipoarabinomannan and peptidoglycan have been the targets for development of new drugs.

Arabinogalactan biosynthesis (Figure 30) begins with the transfer of GlcNAc-1-P moiety from UDP-GlcNac onto polyisoprenoid lipid carrier catalysed by translocases resulting in polyprenyl-P-P-N-acetylglucosamine-1-phosphate. This is followed by the transfer of N-rhamnosyl moiety (presumably from (dTDP-Rha) and then by transfer of galfuranose unit probably from UDP Galf Tunicamycin group of antibiotics inhibit this translocation. Arabinose sugars are also added to growing polysaccharide chain by same sequence of reactions while it is still linked to the carrier. Transfer of arabinosyl unit to growing chain is catalysed by arabinosyl transferases (α and β-arabinosyl transferases). Probably many antituberculosis drugs (ethambutol) target this pathway for their effect. Ethambutol inhibits the biosynthesis of arabinan in both AG and LAM.

Mycolic acids (Figure 4) are biosynthesized by Claisen type condensation and reduction of C16 fatty acids. The four distinct steps involved in the biosynthesis include, synthesis of straight chain C24-C26 fatty acids to provide C1 and C2 atoms and the α-alkyl chain; synthesis of backbone of meromycolic acids of C40 - C60; modification of meromycolic acids to introduce functional groups other than β-hydroxy; and finally the condensation step to provide mycolic acids. Many enzymes involved in the catalyzing different steps are target to develop mechanism based antituberculosis drugs.
Figure 4: Biosynthetic pathway of mycolic acid

Biosynthesis of peptidoglycan can be divided into three distinct steps. (a) The first step involves the synthesis of UDP-N-acetyl muramyl pentapeptide. (b) Synthesis of UDP-N-acetyl-glucosamine, and finally (c) attachment of undecaprenyl phosphate. All three combine together (as depicted in Figure 5) to generate the cross-linked complex structure of peptidoglycan.
HOH₂C
HO~q
HO~

0 0
NH

UDP-N-acetyl-D-glucosamine

phosphoenolpyruvate

UDP-N-acetyl-3-O-(1-carboxyvinyl)-D-glucosamine

ADP + P_i
L-Ala + ATP

UDP-N-acetyl-3-O-((R)carboxyethyl)-D-glucosamine-
N-acetyl muramic acid UDP-Mur₂AC

D-Glu + ATP

ADP + P_i

UDP-Mur₂ AC-N

UDP-N-acetylmuramoyl-L-alanine

UDP-Mur₂ Ac-L-Ala-D-Glu

Lys + ATP

ADP + P_i

UDP-Mur₂Ac-L-Ala-D-Glu

D-Ala + D-Ala

ATP

ADP + P_i

Kaa = Lys

UDP-Mur 2 Ac - L-Ala - D-Ala - D-Glu - Xaa - D-Ala - D-Ala; UDP-Mur 2 Ac-pentapeptide

UDP-Mur₂Ac-L-Ala-D-Glu-A₂pm - D-Ala-D-Ala; UDP-Mur₂Ac-pentapeptide

UDP-Mur₂Ac-L-Ala-D-Glu-A₂pm - D-Ala-D-Ala; UDP-Mur₂Ac-pentapeptide

UDP-Mur₂Ac-L-Ala-D-Glu-A₂pm - D-Ala-D-Ala; UDP-Mur₂Ac-pentapeptide
Figure 5: Biosynthetic pathway of Peptidoglycan
1.3.4 Isocitrate lyase as target for combating the latent infection

During the latency the bacillus appears to coincide with the immune response and the formation of granulomas, which encase the bacterium. It is known that in activated macrophages the bacterium shifts its metabolic priorities and turns on the glyoxylate cycle - presumably to adapt to an inhospitable environment where carbohydrates are limiting and lipids (from, perhaps, dying cells in the granuloma) are more abundant. An enzyme isocitrate lyase (absent in mammals) responsible for conversion of isocitrate to glyoxylate is a very hot molecule and is considered a very promising target for drug therapy of tuberculosis. It has the very attractive feature of being bacterium-specific. Known inhibitors of this enzyme are aconitate or its derivative including aconitic anhydride, nitropropionic acid and bromo propionic acid. Specific inhibitors with good pharmacokinetic parameters would presumably not have side effects for the host and prove to be good antitubercular drugs.

![Known Isocitrate Lyase Inhibitors](image)

Besides above, several other enzymes as linkage region biosynthesis, UDP-galactopyranose mutase, galactofuranose biosynthesis, enoyl reductase, cyclopropanase, glucosyl transferase, fucosyl transferase, xylose isomerase, fatty acid synthetase and peptide deformylase have now been identified and inhibitors of these enzymes taking both rational design and random, high throughput screening may be approach to develop new chemical entities against tubercular infections.

1.4 CURRENT THERAPY:

The drugs used to treat tuberculosis include broad spectrum and narrow spectrum agents including isoniazid (INH), rifampicin (RMP), pyrazinamide (PZA), ethambutol
(EMB), streptomycin (SM), p-amino salicylic acid (PAS), thiolactomycin (TLM) ethionamide, cycloserine, capreomycin, kannamycin, thioactazone etc. 122-126 The mode of action of important drugs and drawbacks associated with them are discussed below.

**Streptomycin** (*Figure 7*) is an aminoglycoside antibiotic isolated from *Streptomyces griseus* and made of three structural components; streptidine, streptose and N- methyl-L-glucosamine. Because of its poor absorbance from gastrointestinal tract, it is administered intramuscularly and very occasionally by intrathecal route. SM was the first really effective drug against tuberculosis with MIC value of 1μg/mL. It has 50-60 % plasma protein bound having plasma half-life 5-7 hours. It penetrates the inner membrane of *M. tuberculosis* and binds to the 30S subunit of the ribosome.$^{127}$

**Figure 7: Structure of Streptomycin and Kanamycin**

R₁ = CHO, Streptomycin  
R₁ = CH₂OH, DihydroStreptomycin  
Kanamycin, R = H, Active  
R= PO₃H₂, Inactive

SM has many toxic effects on peripheral, central nervous system at higher doses and hypersensitivity reactions and so is not a drug of popular choice. Dihydrostreptomycin, once thought to be less toxic causes severe damage to eighth cranial nerve, inducing irreversible impairment of auditory function.$^{128-130}$

**Isoniazid** (*Figure 8a*) is a prodrug that requires activation by the mycobacterial catalase peroxidase enzyme (kat G), which confers sensitivity in *M. tuberculosis* to INH.$^{131}$ It is orally active and exhibits bactereostatic action on the resting bacilli and is highly active against the *M. tuberculosis* complex (*M. Tb, M. bovis, M. africanum* and *M. Microti*) with very low MICs (0.02-0.06 μg/ml).$^{132}$ INH enters the organism by diffusion and
oxygen-dependent active transport and it has been reported to affect almost every aspect of mycobacterial metabolism.\textsuperscript{133, 134} INH inhibits the mycolic acid biosynthesis in mycobacterium tuberculosis by affecting an enzyme \textit{mycolase synthatase}, unique for mycobacteria.\textsuperscript{135-137} A mutation within mycobacterial \textit{inhA} gene was shown to confer resistance to both INH and ethionamide in \textit{M. smegamatis} and in \textit{M. bovis} suggesting that \textit{inhA} is likely target of this drug and related agents.\textsuperscript{138, 139} It is believed that the mechanism of action of ethionamide (\textit{Figure 8b}) is similar to that of INH, including with no-drug activation step.\textsuperscript{140}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig8.png}
\caption{(a) Isoniazid (b) Ethionamide (c) Prothionamide}
\end{figure}

\textbf{Rifamycins} (\textit{Figure 9}) are a group of semisynthetic antibiotics of rifamycin B, isolated from \textit{Streptomyces mediterrani} with characteristic ansa structure (chromophoric naphthaquinone group spanned by a long aliphatic bridge). They inhibit prokaryotic DNA-dependent RNA polymerase, an enzyme necessary for RNA synthesis.\textsuperscript{141} Rifampicin acts on B subunit of this enzyme resulting into formation of stable complex. This in turn, causes inhibition of bacterial RNA synthesis. Mammalian enzymes are not affected by rifampicin and the lipophilic properties of the molecule are important for binding of the drug to the polymerase and to help the drug in penetration across the mycobacterial cell wall. To avoid rapid development of bacterial resistance, RMP is recommended in combination with other first line agents either INH or EMB. However, combination of INH and rifampicin may increase risk of hepatotoxicity. Rifampicin is effective against \textit{M. tuberculosis} with MIC ranging from 0.1 to 0.2 \textmu g/ml.\textsuperscript{142}

Redesigning of rifampicin as conversion to KRM-1648, a benzoxazinorifamycin was found to be more potent than RF.\textsuperscript{143} It is well documented in literature that by inclusion of KRM-1648 in standard combination therapy duration of treatment would be shortened significantly.\textsuperscript{144-148} Since drug resistance has a very close relation with the pharmako kinetics (PK) properties of the molecule. So, a restructured rifamycin with substantially
reduced side effects may produce peak plasma concentration within a short period of time to render a bolus bactericidal dose and its quick bioclearance would leads least exposure to mycobacteria bacilli for evoking mutation necessary for drug resistance.

R = CH₃, Rifampicin
R = C₄H₉, Rifabutin
R = ——— , Rifapentine

Figure 9: Structure of Rifamycins

Ethambutol (Figure 10), a synthetic amino alcohol, is orally effective bacteriostatic agent and active against most strains of *Mycobacterium*. The proposed site of action of this first line drug ranged from trehalose dimycolate, mycolate and glucose metabolism to spermidine biosynthesis. However, recent studies have evidenced the primary site of action to be arabinan bisynthesis both in arabinogalactan (AG) and lipoarabinomannan (LAM). Activity of EMB is stereospecific as dextro isomer exhibited maximum antitubercular activity (S, S form is 600 times more active than R, R). Mechanism of action of EMB is still not known completely, but probably it interferes in the synthesis of proteins and nucleic acids by acting as antimetabolite. Its complex forming ability is also a contributing feature to its bacteriostatic activity. Disruption of the arabinogalactan synthesis inhibits the formation of Mycolyl arabinogalactan peptidoglycan (MAGP) complex and thus may lead to increased permeability of the cell wall. It has been suggested that this drug inhibits the enzyme arabinosyl transferase and
thereby affect the biosynthesis of AG and LAM.\textsuperscript{152} The target of EMB lies in the pathway for the biosynthesis of cell wall AG through arabinosyl transfer, as \textit{arabinosyl transferase} is the primary cellular target for EMB. Arabinosyl transferase III is responsible for the polymerization of arabinose into arabinan of AG during cell wall biosynthesis.\textsuperscript{153}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figures/figure10.png}
\caption{Different isomers of Ethambutol}
\end{figure}

The \textit{macrolides} (\textit{Figure 11}) comprise a family of antibiotics ranging from erythromycin (1952) to analogs synthesized more recently. Erythromycin is 14 membered macrolide consisting of a macrocyclic lactone ring attached to two sugar residues.\textsuperscript{154} Newer derivatives differ from the parent erythromycin in the size and/or substitution pattern of the lactose ring and include; roxithromycin, clarithromycin, azithromycin, rokitamycin and spiramycin.\textsuperscript{155-160}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figures/figure11.png}
\caption{Structure of Macrolides}
\end{figure}

Roxithromycin $X = \text{NOCH}_2\text{OCH}_2\text{CH}_2\text{OCH}_3$, $R = \text{Me}$;  
Clarithromycin $X = \text{O}$, $R = \text{Me}$;  
Erythromycin
Although some of the macrolides display poor antimicrobial activity against enterobacteria, they can generally be regarded as broad-spectrum agents inhibiting mycobacterial growth also.\textsuperscript{161} A number of semisynthetic derivatives with improved PK properties appear to be promising in the treatment of mycobacterial infections particularly those caused by non-tuberculosis species.\textsuperscript{162}

These antibiotics bind to high affinity site in the petidyl \textit{t}-RNA binding region of the bacterial 50S ribosome subunit, causing dissociation of peptidyl-Trna form ribosomes and inhibition of bacterial protein synthesis.\textsuperscript{163}

\textbf{Figure 13: Structure of Capreomycin}

Capreomycin (\textit{Figure 13}), a macrocyclic polypeptide, is active against \textit{M. tuberculosis} and occasionally used in the treatment of tuberculosis.\textsuperscript{164,165} Although the exact mode of
action of capreomycin is uncertain. However, since it is structurally related to the viomycin, which is known to inhibit protein synthesis, so it is believed that capreomycin also inhibits protein synthesis. This conclusion is further supported by the observation that viomycin-resistant strains of *M. tuberculosis* are generally cross-resistant to capreomycin.\textsuperscript{166}

A number of diacyl thioureas have shown activity in experimental tuberculosis. One of such agent *isoxy* (Figure 39, 4,4'-diisoamyloxydiphenylthiourea, 4,4'-diisoamyloxydiphenyl thiocarbanitide, thiocarlide, ISO) has been reported to be clinically useful.\textsuperscript{167-169} Inhibition of mycolic biosynthesis in *M. bovis* has been shown to be the inhibition of mycolic acid.

**Thioacetazone** (Figure 39), a member of thiosemicarbazones reported to be specific anti-mycobacterial agents and exhibits potent *in vitro* activity against *M. tuberculosis* and *M. avium*.\textsuperscript{170-174} It was introduced as a cheap and effective substitute for PAS in combined chemotherapy for tuberculosis. Although the exact mode of action of thioacetazone is not known however, since some thiacetazone-resistant strains of *M. tuberculosis* exhibits cross-resistance to ethionamide. It has been suggested that like ethionamide thiacetazone might inhibit mycolic acid biosynthesis.\textsuperscript{166}

The antimycobacterial activity of *p*-aminosalicylic acid (Figure 14a) was reported only in 1946 although it was synthesized a long back.\textsuperscript{175} PAS is highly effective against *M. tuberculosis*, but has no effect against other bacteria.\textsuperscript{176} Following DOTS it is rarely used today. However, it is occasionally used in the regimen for the treatment of tuberculosis caused by MDR TB.\textsuperscript{177} The exact mode of action of PAS drug is still unclear but it has been suggested that it interferes with the salicylate-dependent biosynthesis of the iron chelating mycobactins involved in iron assimilation.\textsuperscript{178}

**Pyrazinamide** (Figure 14b), a structural analogue of nicotinamide, is first line drug of short course tuberculosis therapy. It is also active against semidormnant bacilli not affected by any other drug. Its inclusion with INH and RMP considerably shortens the treatment period from 12-18 months to 6 months and these three drugs together form the basis of the current DOTS.\textsuperscript{179,180} The drug has no significant bactericidal effect and is thought o act by sterilizing effect. The activity of PZA depends on the presence of bacterial amidase, which converts PZA to pyrazinoic acid, the active antituberculosis
molecule and this activity is highly specific to *M. tuberculosis*.\textsuperscript{181-183} Mutation in the *pncA* gene responsible for the production of pyrazinamidase has been shown to be the reason for resistance against this drug.\textsuperscript{184}

\begin{center}
\begin{tabular}{ccc}
\textbf{COOH} & \textbf{N} & \textbf{H2} \\
\textbf{NH2} & \textbf{HO} & \textbf{OH} \\
\end{tabular}
\end{center}

\textbf{Figure 14: (a) PAS (b) Pyrazinamide (c) D-Cycloserine}

D-Cycloserine (\textit{Figure 14c}), a structural analogue of D-alanine possesses activity against a wide range of bacteria\textsuperscript{185,186} and inhibits *M. tuberculosis* at concentrations of 5-20 \(\mu\text{g/mL}\). It blocks peptidoglycan biosynthesis by inhibition of D-alanine racemase and D-alanine synthetase.\textsuperscript{163, 187} Microorganisms treated with cycloserine accumulate a muramic-uridine-nucleotide-petide, which differs from that produced by mycobacteria in the absence of terminal D-alanine dipeptide.\textsuperscript{188-192} Cycloserine produces severe side effects in the central nervous system that can also generate psychotic states with suicidal tendencies and epileptic convulsion.

The \textbf{Fluoroquinolones (Figure 15)}, a synthetic derivative of nalidixic acid, display broad-spectrum antimycobacterial activity.\textsuperscript{193-195} When ciprofloxacin and ofloxacin were used as part of multi drug regimens, they resulted in clinical and microbiological cure of patients infected with *M. tuberculosis* and *M. avium*.\textsuperscript{193-196} Structural modification of FQ to optimize antimycobacterial activity may produce develop candidates that are more efficacious than earlier FQ.\textsuperscript{197} Their bactericidal effects involve an interaction of the drugs with DNA-gyrase and DNA – topoisomerase IV.\textsuperscript{197-200}

\begin{center}
\begin{tabular}{ccc}
\textbf{Ciprafloxacin} & \textbf{Levofloxacin} & \textbf{Moxifloxacin} \\
\end{tabular}
\end{center}

\textbf{Figure 15: Structures of Fluoroquinolones}
Thiolactomycin (Figure 16) [(4R) (2E, 5E) 2,4,6-trimethyl-3-hydroxy-2,5,7-octatriene-4-thiolide] belonging to small group of thioteric acid antibacterials is an unique thiolactone exhibiting antitubercular activity by inhibiting mycolic acid biosynthesis. It inhibits FAS-II of plant and bacterial, KasA and KasB consistent to the fact that both enzymes belong to FAS-II involved in fatty acid and mycolic acid biosynthesis. It has high MIC of 5μg/ml but in absence of in vivo toxicology and in vitro cytotoxicity data it is difficult to judge whether these concentrations are far below the toxic concentrations.

![Figure 16: Structure of Thiolactomycin](image)

Tryptanthrin (Figure 17) is a potent structurally novel indoloquinazolinone alkaloid, active against MDR TB with an MIC of 0.5-1.0 μg/ml. But in vivo data and in vitro toxicity are needed before this structural prototype is applied in MDR TB.

![Figure 17: Structure of Tryptanthrin](image)

Clofazimine analogs [Image] B 4157  B 4154

![Figure 18: Structure of Clofazimine analogs](image)
Clofazimine (Figure 18) is active in vivo against M. tuberculosis, M. bovis, M. leprae and M. avium complex with an MIC of 0.01 to 3.3 mg/L. Few of the tetramethyl piperidine substituted phenazines (TMP phenazines) were found to possess significantly more activity against M. tuberculosis, including MDR clinical strains than clofazimine Analogs. The most important virtues of friminophenazines, their intracellular accumulation in mononuclear phagocytic cells, anti-inflammatory activity, a low incidence of drug resistance and slow metabolic elimination make them attractive candidate for the treatment of mycobacterial infections.

Antimycobacterial and antibacterial activities reported earlier in nitroimidazofurans (Figure 19) could not be pursued further because of mutagenic side effects in this class of compounds. Bicyclic nitroimidazopyrans (NAP) a narrow spectrum, have recently been reported to possess antitubercular activity and one of the compounds PA-824 emerged as a lead molecule and it was effective both in the replicating and latent M. tuberculosis cells with MIC from 0.015 to 0.25 µg/ml. Poly and multi drug resistant strains of this bacterium were susceptible to PA-824, indicating that there is no cross-resistance with current drugs. The mode of action of this class of compound has been by a mechanism dependent on M. tuberculosis F420 cofactor, inhibition of protein biosynthesis and cell wall lipid. Another orally active analog of NAP (PA 1343) has been developed and is in preclinical studies with MIC of 0.015 µg/ml.

![Figure 19: Structure of Nitroimidazofurans and Nitroimidazopyrans](attachment://image.png)

Nitroimidazopyrans (Figure 19) inhibited the biosynthesis of protein and cell wall lipid. Effects on cell wall lipids accounts for an accumulation of hydroxy mycolic acid with a concomitant reduction in keto mycolate. Since hydroxy mycolate is a precursor of
ketomycolate in mycolic acid biosynthesis hence an enzyme responsible for the oxidation of hydroxymycolate to ketomycolate might be inhibited or depleted by this class of compound. Several modifications were carried out for good pharmacokinetic and to lower the toxicity observed with original compounds. One such lead molecule was PA-1343 with good water solubility better oral bioavailability.\textsuperscript{212}

**Oxazolidinones (Figure 20),** totally synthetic, orally active antibacterial agents were discovered by Du Pont company.\textsuperscript{213-218} They are inhibitors of bacterial protein synthesis, with inhibition uniquely in the initiation phase of protein synthesis. Thiomorpholine analogues of U-100480 with the biphenyl methyl group replacing the acetamido methyl oxazolidinone moiety showed potent \textit{in vitro} activity against \textit{M. tuberculosis}.\textsuperscript{217} However, this class of molecule could not enter into clinics because of toxicity in mice.

![Figure 20: Structures of Oxazolidinones](image)

**Diterpenoids (Figure 21)** known for various medicinal values have recently been screened for antituberculosis activities against \textit{M. tuberculosis}. Benzooxazole alkaloids isolated from Indian sea whip \textit{Pseudopterogorgia elisabethae} were tested against the bacterium and it was found that pseudopteroxazole has potent inhibitory activity (97 \%) at 12.5 \(\mu\)g/ml in \textit{M. tuberculosis} \textit{H37 Rv} strain.\textsuperscript{219-221} Many other analogs have also shown potent antitmycobacterial activity and it has also been established that benzooxazole moiety is not essential for activity. The structures of potent molecules have been given below and all of them were isolated from natural sources.\textsuperscript{222, 223}
9-Benzyl purines (Figure 22a) with a variety of substituents in the 2-, 6- and/or 8-position have been shown to possess high inhibitory activities against *M. tuberculosis*. One of the compounds belonging to the above class carrying trans-styryl or aryl substituents in the 6-position and generally chlorine in the 2-position tends to increase the activity and has MIC of 0.78 \( \mu g/ml \) *in vitro*.\(^{224}\) Antimycobacterial Activity of 6-Aryl purines \(^{225}\) and 9-Sulphonylated or Sulphenylated -6-Mercaptopurines \(^{226}\) were also known in literature.
Imidazo[4,5-c]pyridines (Figure 22b) prepared as antimitotic agents have recently displayed anti-TB activities. One of the compounds shown below has MIC of 6.25 µg/ml against *M. tuberculosis* H37 Rv. But the activity in this class of molecule may be due to cytotoxicity.

![Glycylcycline and Doxycycline](image)

**Figure 23: Structure of tetracycline**

Antitubercular activity of Benzopyran-2-ones was reported while investigating the anti HIV activity in natural products (+) calanolide and (-) calanolide A (Figure 24a), where these compounds demonstrated antimycobacterial activity against *M. tuberculosis* H37 Rv to the extent of 96 and 98 % with MIC values as low as 3.13 µg/ml. A series of novel novobiocin-like coumarins (Figure 24b) were discovered as inhibitors of DNA gyrase with promising *in vitro* antibacterial activity.

![Figure 23: (a) calanolide A (b) Novobiocin-like coumarins](image)
Antitubercular activity of several **Marine natural products** (*Figure 25*) marine natural products are well documented in literature. Massetolide A and Viscosin B are cyclic depsipeptides isolated from cultures of two pseudomonans, a marine alga and tune worm when tested against *M. avium-intracellulase* showed MIC 2.5-5 and 5-10 µg/ml respectively. Kahalalides A, a known polypeptides isolated from the Sacoglossan mollusk *Elysia rufescens*, inhibited 83% of the growth of *M. tuberculosis H37 Rv* at 12 µg/ml. Litosterol and Nephsterol C, are known C19 hydroxy steroids isolated from a red sea Nephtheasp; inhibited 90 and 96% of the growth of *M. tuberculosis H37 Rv* with MIC 3.13 and 12.5 µg/ml respectively. Heteronemin is a scalarin type Sesterterpene isolated from a red sea sponge; displayed a 99% inhibition of *M. tuberculosis H37 Rv* with an MIC 6.25 µg/ml and IC$_{50}$ 1.3 µg/ml.

**Figure 25: Structures of Marine Natural products**
Thiazidine thiones (Figure 26), a derivative of dithiocarbamic acids have been screened against *M. Tuberculosis* because of their well-known biological activities including antifungal and antibacterial activities. We have very recently reinvestigated the antitubercular activities in this class of molecule and one of the compounds have shown potent *in vitro* antitubercular activity against *M. tuberculosis* H₃₇ Rv even in resistant strains.²³¹ The compound, when tested in mice model also protected the mice marginally.

![Figure 26: Structure of Thiazidine thione derivatives](image)

**Simple Carbohydrate derivatives:** Based on the observation that simple sugar derivatives possess inhibitory activity against the enzymes involved in cell wall biosynthesis, many simple monosachharide derivatives have been tested against *M. Tuberculosis* H₃₇ Rv and have shown potent activity *in vitro*. Our group has synthesized a number of derivatives from monosachharides and evaluated against the bacterium.²³²-²³⁸ Few of the compounds have shown potent *in vitro* activity even in many clinical MDR strains of *M. tuberculosis*.²³⁹ However, many compounds displayed toxicity in the animals and an effort in this direction is continued.

![Figure 27: Structure of Tunicamycins](image)

In addition to the above other class of molecules which have been evaluated for their antitubercular activities comprise 4-(coumarinyl)-4-thiozolin-2-one-benzylidene-
hydrazones, 3-hydrazono-1 H-2-indolinones, quinoxaline-1, 4-di-N-oxides, cyclohexadienes, N- (2-naphthyl) glycin hydrazide analogous, isoxazoles, cycopyridines, 4-phenyl-1, 8-naphthyridine, (E) phytol derivatives, pyridinecarboxamidrazone derivatives, quinazilidines, aminolupinanes, benzoxy thiozole-2-carbamates, thiosemicarbazones, dithiocarbamates, thiazolidinones, succinamides, diarylsuccinamides, glutaconylthiosemicarbazides, 2', 2'-dithio-bis (benzamides), 1,3,5-triazines, trimethyl silyl-3-(carbethoxy carbamoyl) propionates, pyrrolinitrin and pyrrole derivatives diguanidino and “reversed” diamidino, diaryl- furans, thiazole, thia diazole, quinazolines, quinolines, 3- Me N'(disubstituted-quinolinyl) pyrazol-5-ones, 2-quinoxalinecarbonitriles, 6/7- Tri fluoro methyl (nitro)- 6,7- difluoro-3-alkyl(aryl)- substituted- quinoxalin-2-ones, quinoxaline 1,4 dioxides, quinoxaline-2-carboxamide 1,4-di-N-oxide, nitroquinolones, 2,4- dihydroxy quinolines, 3-nitro and 3-bromo 4-hydroxy-2 quinonines, quinazolinones, 6-nitro quinazolones, 1,8- naphthyridines, benzimidazole, benzazole, substituted 2-(4-amino phenyl sulphonamido)-benzothiazoles, 3-aryl substituted -2-[(1H(2H) benzotriazol-1(2)-yl)- acrylonitrile, 3-aryl, 3-cyclohexyl and 3-heteroaryl- substituted -2-[(1H(2H)- Benzotriazol-1(2)-yl)- prop-2-enenitriles, 2-azetidinones, pyrimidines, pyrazine carboxylic acid, pyrazines, α- oxo- ketene dithioacetals, hydantoins, pyridines, dihydropyridine, hydrazones, spirothiazolidinones, N'-[3-Aryl-1-(Pyridin-2-,3-or 4yl)-3-oxo]-propyl]-2-pyridinecarboxamidrazone, isonicotinoyl hydrazones, S-alkyllisothiocarbazone, thiocarboxamidrazone, hydrazinecarboxamides, pyrazolines, 4-aryl hydrazono-2-pyrazoline-5-ones, 5-aryl-1-isonicotinoyl-3-(pyridin-2-yl)-4,5-dihydro-1H-pyrazoles, 5-alkyl-6-alkylsulfanyl or 5-alkyl-6-arylsulfanyl pyrazine-2-carboxamides, N-alkyl-1,2- dihydro-2-thiao- 3-pyridine carbothioamides, thiobenzanilides, 2- benzylthiopyridine-4-carbothioamides, 2-substituted pyrazines, disubstituted 1,3,4-oxadiazoles, 6-chloro cinnolinothiazoles, imidazo[2,1-b]-thiazoles, sydones, pyrazinoyl heterocycles, pyrazinoic acid hydrazide, pyrazinamie derivatives, azetidone, pyrazine carboxylic Acids isosteres, ureas and thioureas derivatives.
1.6 THE FUTURE PERSPECTIVES

The two key factors behind effective public health measures to combat TB are the prompt identification of infected individuals and a rapid laboratory confirmation of the infection. A survey predicts that there will be a total of 225 million new cases and 79 million deaths from TB between 1998 and 2030. Active screening by sputum examination combined with a single contact treatment with INH could avert 24 million cases and lower mortality by nearly 40%. More attention needs to be paid to the behaviors of patients as also the health provider. There is often considerable delay on the part of the physician in making a prompt diagnosis and making drugs available to the patient at the first point of contact. It has been reported that 80% of the chest symptomatics who reported to the nearest health facility did not get adequate and timely diagnosis; at times the delay in diagnosis was as long as 35 days. The same study also revealed that 75% of the chest symptomatics were simply turned away without subjecting them for sputum examination.

The control of TB at the population level depends to a large extent on the improvement of social, environmental and educational conditions. The health of slum dwellers of industrial and many developing countries with the introduction of clean water supplies, sewage disposal and hygiene education would be beneficial in curtailing the disease. Industrial pollution particularly the smoke, as also smoke from active or passive smoking, weaken the lungs and make them susceptible to bacterial infection. In India an increase in the extent of female literacy is crucial for the improvement of public health because it turns out that whether women are literate or not influences acceptance of hygienic measures in the entire family, incidentally, it is ironic that young females, in general, are more susceptible to infection by \textit{M. tuberculosis} than young males of the same age.

Given a degree of fallibility in public Health measures, research into drug based and clinical therapies will have to continue. However, the production of a new drug, which is ready for clinical use in India, poses a serious financial problem. Selection for drug-resistant strains is developing into a major problem and the population biology of \textit{Mycobacterium} will need to be understood much better.

In view of the difficulties faced by developing countries, including India, to effectively treat cases of TB with drugs, and also because of the toxicity of the available
drugs in certain cases, the use of a vaccine to prevent the onset of the disease appears to offer the best prospect for controlling TB world-wise. The model referred to above predicts that a vaccine with 50% efficacy would reduce by 36 million the expected number of new TB cases between now and 2030 and prevent 11 million deaths. Although the prospects for development of antitubercular vaccines have never been better, yet a major hurdle remains to be overcome before any candidates will be taken into clinical studies in man. Ways of monitoring protective immunity must be identified to avoid lengthy and expensive clinical trials. These finding must be developed into robust tools and be fully validated in field trials with BCG before being used to evaluate any new vaccine. The development of a more effective vaccine than BCG, which works well on infants and young children, has to await a clearer understanding of the virulence of \textit{M. tuberculosis} and the immunology of the infection. \textsuperscript{349}

Curiously, during the same period in which the situation with regard to TB has worsened, the incidence of leprosy, caused by the related organism \textit{M. leprae}, has registered a steep fall according to a WHO report, the drop has been from 12 million symptomatic individuals worldwide to 172 million over the last 20 years. By strictly adhering to the triple drug regimen of dapsone, isoniazid and rifampicin for extended periods, the incidence of leprosy has been drastically reduced worldwide, and the disease may disappear from India in the course of the next few years (by 2002, according to a WHO forecast). This may be an overly optimistic view, but it remains a fact that chemotherapy has brought down the prevalence of leprosy in India significantly, from 20 per 10,000 in 1981 to 2 per 10,000 in 1997. Perhaps we can take a leaf from the methods and precautions taken to control leprosy to make the fight against tuberculosis too. These include simultaneous treatment with at least three different drugs and continuation of treatment till smear microscopy of sputum shows absence of organisms. \textsuperscript{350}

The alliance between TB and AIDS is merging as a serious problem. HIV is thought to have entered India in the early 1980s, the first case being reported in March 1986. The results of several sero-surveillance surveys in different parts of the country have helped to provide an insight into the main modes of HIV transmission as well as identification of high-risk groups within society. The profile of the epidemic varies widely from one region to another, reflecting the country's great diversity. Official estimates indicate that
HIV had infect 1.5 million persons by the end of 1994 and this number is expected to go up to 2 million by the year 2000. There is no mystery about the powerful effect of HIV infection on the natural history of TB. The link between the two has to do with the well established, though not widely appreciated, fact that 90% of the otherwise healthy persons infected with M. tuberculosis do with the well established, though not widely appreciated, fact that 90% of the otherwise healthy persons carry the infection but do not display the disease. Progression from infection to disease is ordinarily prevented by an intact immune system, particularly by cell-mediated immunity; and it is the immune response that is primarily compromised by HIV infection. A study undertaken in 1991 by the TB Research Centre, Madras, shows that the average risk of developing TB among HIV positives was as high as 2% per year; and 67% of the cases were detected within 30 months of registration. It is unfortunate that the funds available for controlling TB, both nationally and internationally, have been seriously affected in recent years by being partly siphoned off for the control of HIV.

Because of the HIV-TB nexus, it becomes important and urgent that the highest priority be given to further strengthening the TB programme in India. A portent of what can happen otherwise can be seen from the situation in the USA. From 1953 to 1984 the number of TB cases reported in the USA dropped by an average of 6% each year. In contrast, from 1984 to 1997 there was an overall increase of 14% in new cases. Part of this reversal in a trend of long duration is undoubtedly due to the HIV epidemic: not only have the areas most affected by HIV been the same ones that have reported the largest increase in TB cases, the largest increase in TB cases has occurred among people in the age group (25 to 44) most affected by AIDS. Finally, the question: Where do we stand now? We started at the "foot of the ladder" prior to 1950 when there was no treatment for TB and went up from then to a maximum around 1984 during which time streptomycin and other antitubercular drugs were discovered. Since the, it has been a gradual fall due to drug resistance and HIV infection to a low "rung in the ladder". Whether we can go up the ladder again and how far depends on the efficacy of the methods followed in future in pursuit of this goal. The methods would include simultaneous treatment by at least three, preferably four, drugs for which the infective organism is sensitive; continuation of the therapy without break till smear microscopy shows the absence of the infection, and
finally, control in the spread of HIV. For the authors, there is the disheartening realization that in spite of their best efforts and those of their colleagues, the aims with which they began almost 40 years ago have not been realized. Tuberculosis is prevalent in this country almost to the same extent as when they started their work. Hopes for ending this pathetic state of affairs rest on the development of a vaccine and the adherence by physicians and patients to a scientific attitude are equally important, simultaneously the government has to strengthen public measure programmes like control of pollution both in air and water, provision of save drinking water and hygienic sewage disposal as well as promoting hygiene education among the masses, particularly among women, in order to make a success of the medical efforts to control tuberculosis.

1. 6 BASIS OF WORK

Based on the above discussion, as cell wall biosynthesis is very important target in antitubercular drug development, the synthesis of mechanism based new chemical entities are undertaken. The proposed sugar derivatives as β- glycosylated amino acids derivatives, β- glycosylated amino alcohols, glycoconjugates, β- glycosylated mercapto esters and glycosyl peptides derivatives have been synthesized keeping in view the following targets of cell wall biosynthesis.

1.6.1 Peptidoglycan biosynthesis: The biosynthesis of peptidoglycan (munamyl penta peptide) is a crucial step in cell wall biosynthesis. In the fifth step of its biosynthesis D-alanyl alanine is attached to a tripeptide to give pentapeptide. (Figure 28)

![Figure 28](image)

Natural amino acids are always of L- stereochemistry. Mycobacteria and many other bacteria convert L alanine to D-alanine with the help of cytoplasmic enzyme D- alanine racemase and with the help of D- alanine synthetase D-alanine is converted to D- alanyl...
alanine. Since D-alanine is abundant only in bacteria and almost absent in host, it is crucial target for antimycobacterial drug development.\textsuperscript{186,354} Cyclic analogue of amino acid, D-cycloserine is very potent antitubercular drug both \textit{in vitro} and \textit{in vivo}.\textsuperscript{355,356} But due to neurotoxicity, it could not find its prominent place in clinics.\textsuperscript{357} Further, certain β-amino acids (β-alanine derivatives) are known to possess antitubercular activity. It is presumed that proposed β-amino acids being glycosylated would not only reduce toxicology only but also would offer better pharmacokinetic parameters, better stability and better transport too.\textsuperscript{258-360}

1.6.2 Mycolyl transferase: Mycolyl transferase is an enzyme, which is responsible for the trans esterification of mycolic acids with sugars.\textsuperscript{361-364} Azido deoxy trehalose (ADT) is known inhibitor of mycolyl transferase. Few amino glycosides are also known for mycolyl transferase inhibitory activity.

![Figure 29](image)

Apart from this 5-Amino-5-deoxy sugar derivatives are the compounds having components of many antibiotics as discussed above (Figure 29). Since compounds having thiourea functionalities (ISO) are known to inhibit biosynthesis of mycolic acid.\textsuperscript{365} Keeping in view this, it was thought to synthesize selective and potent glycohybrid molecules with thioureidyl functionalities as antitubercular agents.
1.6.3 Glycosyl transferase: The third enzyme, which might be the target of the proposed compounds, is glycosyl transferase, as certain unnatural sugar derivatives bearing long alkyl chain possess galactosyl transferase and arabinosyl transferase inhibitory activity. (Figure 30)

![Figure 30: Biosynthesis of Arabinoglycan](image)

![Figure 31: Known inhibitors of Arabinosyl transferases](image)

Ethambutol (diamino dialcohol) is known inhibitor of arabinosyl transferase and it is presumed that if diamino functionality is looked with glycofuranoses in such a manner that amino and hydroxy (ester, acids) functionalities are available for binding with the enzyme, the compounds might be potent antitubercular drug more particularly for resistance cases. The proposed compounds might be useful synthon for the synthesis of glycosylated β-lactam antibiotics and scaffold for the synthesis of combinatorial library of glycopeptides or peptidomimetics (Chaptar-3 & 4).

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