

Fighting Tuberculosis: An old disease with new challenges

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1. INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, a slow growing bacterium, evolved from soil bacterium more than 10,000 years ago is a respiratory transmission infectious disease. It is a single largest infectious disease in the world infecting nearly 32% of the world's population is infected with TB. Among the infected individuals approximately 8 million develop active TB, and almost 2 million of these die from this disease and 95 % of new TB cases occur in developing countries every year.¹⁻⁵ The young women, about one million per year are victimized with this disease in the developing world after malaria and maternal mortality. Occurrence of this disease is linked to dense population, poor nutrition and sanitation.⁶ India having 2% of land area and 15% of total population of the world accounts a disproportionately high 30% of the TB burden. In India TB kills 14 times more people than all tropical diseases and over 4.5 million TB cases with 1.8 million new cases being reported each year where nearly 50 % of the population is reported to be tuberculin test positive.⁷⁻⁸ Here one person dies from tuberculosis every minute.⁹

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History of *Mycobacterium tuberculosis* is very old¹⁰ and it has been known since 2400 BCE when antiquity-fragments of the spinal column from Egyptian mummies show definite pathological signs of tubercular decay. Exact pathological and anatomical description of the disease appeared in 17th century. Introduction of sanatorium was the first real step against TB. In 1854 Hermann 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 Jean-Antoine 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 approaches to control this disease 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. Most of the drugs in current use were developed in the 20th century during the World War II.

History of tuberculosis in India¹¹⁻¹² also dates back to 600 BC where in a Sushruta Samhita, the disease is known as Kshaya, 'wasting disease', or Raja Yakshmaa, "the king of diseases". The four causes of the disease identified were: overstrain, 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 inhabit dry areas and goat's milk were among the items recommended. TB was rare until the second half of the nineteenth century. Concomitant with the growing population density caused by industrialization, its incidence has increased progressively since then¹³⁻¹⁴

2. PATHOGENESIS

The primary source of infection are viable tubercular bacilli, expelled in the environment by coughing, sneezing, shouting and singing of a patient with active tuberculosis and the contaminated air. Inhaled bacilli in other person are inoculated into his respiratory bronchioles and alveoli usually towards the apex of the lung. The inhaled microorganism multiply to the sufficient extent and consequently an antigen –antibody interaction is evoked by the cell mediated T-lymphocytes. Tubercles are then formed due to accumulation of macrophages at the site of infection.^{15, 16,17} This may lead to either permanent suppression of infection or some microbes may survive in the foci and may become the source of post primary infection when these foci breakdown under the conditions of weak host defense mechanisms.^{18,19} This may happen immediately or months or year later. The *hilar lymph nodes* may get easily infected due to spreading of infected macrophages having active bacilli. The released microorganism are circulated through lymph and blood to different parts of the body and infects (i) reticuloendothelial system (e.g. liver, spleen and lymph nodes), (ii) serosal surfaces and sites with high oxygen pressure (apices of lungs, renal cortex and epiphyses of growing bones). Due to multiplication of organisms at these sites, numerous small foci develop throughout the body.^{20,21} This type of wide- spread of infection is known as millitary tuberculosis.

In some patients, the foci formation leads to temporary suppression of the infection, while microorganism may still be present in the foci. During coughing, the caseous material containing microorganisms is expelled out leaving cavities in the lungs. These active bacilli may then be swallowed by same patient or inhaled by healthy patient resulting into infection of trachea, larynx or bronchi in a more crowded and poor personal and public hygiene condition. Infection of the oropharynx, larynx and tracheobronchial tree respond fairly well to antituberculosis drugs while infections in gastrointestinal tract, urinary tract or lymph nodes respond partially to the treatment.^{22,23} The main symptoms are cough trachycardia, cyanosis and respiratory failure.

3. MDR TB AND TB IN HIV CASES:

An estimated one-third of the 42 million people living with HIV/AIDS worldwide are co-infected with tuberculosis.²⁴ 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 anti- TB drugs to those living with HIV. Starting that an even greater TB/HIV crisis might be emerging in developing countries. As per WHO HIV is spreading rapidly in the country with the largest number of TB cases in the world.²⁵⁻²⁹ In India already 180,000 people living with HIV were co-infected with tuberculosis.

The emergence of multiple drug resistance (MDR) tuberculosis has focused the attention of scientific community throughout the world on the urgent need of new antitubercular drugs. Resistance has been developed against almost every front line drug.³⁰⁻³⁷ Different mechanisms have been put forward for the development of resistance against the existing drugs. The main causes are impermeability of the highly hydrophobic cell envelop to many drugs,³⁸ a well developed drug efflux system,³⁹ production of certain enzymes to inactivate the drugs (β -lactamases, aminoglycoside acyl transferase)⁴⁰⁻⁴¹ and at the molecular level acquisition of resistance in *M. tuberculosis* is due to mutational events in the chromosomes.⁴²

4. MANAGEMENT OF TUBERCULOSIS

Three major approaches have been in practice since the first appearance of this disease; the Sanitorium development for fresh air, cleanliness and nutritious diet; the vaccination and the chemotherapy.

4.1 Vaccines: Active immunization is one of the essential components to control tuberculosis, although the vaccination is ineffective nowadays. Till now one billion people have been vaccinated with BCG. In general, four classes of vaccine candidates are being focussed and these are; rationally attenuated strains of *Mycobacterium tuberculosis*, bacilli galmette- guerin (BCG) vaccine, protein subunit vaccines and nucleic acid vaccines.

4.1.1 Rationally attenuated strains of *Mycobacterium tuberculosis*: The genetic manipulation of *Mycobacterium tuberculosis* chromosome generates bacterial strains, which lack the pathogenicity but elicit a protective immune response; and this has been achieved by several techniques.⁴³⁻⁴⁶ A library of mutants can be generated using transposons, mobile genetic elements that can ‘hop’, disrupting genes.^{47,48}

4.1.2 Bacilli Calmette- Guerin (BCG) vaccine: Advances in the discovery and characterization of genes and antigens of *M. tuberculosis* has led to substantial progress towards the development of improved vaccines since BCG vaccine was first used successfully. This BCG vaccine is cheap and associated with minor side effects can safely be given to children. It is proven now that while BCG does have some protective effects in children, for few tuberculosis such as meningitis tuberculosis, it does not prevent the emergence of pulmonary tuberculosis, particularly as the individual approaches adulthood. Variation in the efficacy of BCG has been due to interference of BCG take due to previous exposure to environmental Mycobacteria. *Mycobacterium tuberculosis* genes encoding antigens which have high reactivity to memory immune T- cells, but were deleted from BCG in the mutations led to attenuation.^{49, 50} Alternatively, the immune responses to purified protein derivative have been enhanced in mice by injecting BCG clones expressing various immune stimulating proteins⁵¹ and this approach has been very useful in the treatment of experimental tuberculosis in immunodeficient animals which did not respond to normal BCG.⁵²

Protein subunit vaccines: Purified proteins as vaccines have several advantages over attenuated organisms. They are inherently safe and have no propensity to cause disease, which is an important consideration when vaccinated individuals have been exposed to HIV and the efficacy of such vaccines have been demonstrated in mice and guinea pigs.^{53, 54}

Nucleic acid vaccines: The hypothesis that ‘Naked DNA vaccines’ and DNA-encoding influenza nucleoprotein lend immunity to influenza in mice⁵⁵ has been applied to *M. tuberculosis* also. Vaccination with DNA encoding either the *M. leprae* 65 KDa heat shock protein⁵⁶ or the *M. tuberculosis* antigen 85A protein⁵⁷ protect from subsequent

infection with virulent *M. tuberculosis*. Many nucleic acid vaccines have shown efficacy in experimental tuberculosis.

Genomics: The most significant developments in the area of tuberculosis are perhaps the sequencing of the mycobacterial genome. Sequencing of the H37Rv strains of mycobacterium tuberculosis and a highly virulent clinical isolate is well documented.^{61, 62} Among the estimated 4500 genes, every drug target and every antigen, or protein elicits an immune response. Among these precise biochemical functions of only 40 % of the above is known, another 44 % have some sequence homology while functions of 16 % are completely unknown and they may account for specific mycobacterial functions. The challenge for the tuberculosis research programme is to validate and prioritize those, which will most rapidly lead to new treatments. Comparison of the two *Mycobacterium* genome sequences *M. tuberculosis* and *M. leprae* has been shown to be useful in identifying genes associated with virulence^{61, 62} and this will give new insight in identifying the targets and development of new control strategies.

Immunotherapy: Immunotherapy is a therapeutic means whereby the immune system is stimulated by the injection of inactivated Mycobacteria, resulting in the activation of TH1 cells and inactivation of TH2 cells. It represents an important adjunct to modern chemotherapy to overcome the problems of non-compliance and drug resistance, as well as improving cure rates and reducing mortality. Cell mediated immune (CMI) and delayed type of hypersensitive (DTH) responses play very important role in host during *M. tuberculosis* infection. The CD4 T lymphocyte is divided into TH1 and TH2 subsets depending on the type of cytokines produced and TH1 cells produce the cytokines interferon-gamma and interleukin-2, an important event for activation of antimycobacterial activities and essential for the DTH response.⁶³ Several studies on the effectiveness of *Mycobacterium vaccae*, as an immunotherapeutic agent for tuberculosis have recently been carried out.⁶⁴⁻⁶⁷ It is thought to be a powerful TH1 adjuvant. and has a beneficial effect with enough evidence now to show that its use as an immunotherapeutic agent, as an adjunct to chemotherapy in the treatment of tuberculosis especially in MDR-TB and cases where chemotherapy was incomplete or intermittent, in many parts of the developing world has been reported.⁶³⁻⁶⁷

CHEMOTHERAPY

Chemotherapy of tuberculosis started in between and after World War II. In 1943, anti-TB research resulted in discovery of the active anti-TB agents and strategies have been devised to treat tuberculosis from time to time.⁶⁸⁻⁷¹ A number of agents have been discovered since that time, including *para*-aminosalicylic acid (PAS), isoniazid (INH), pyrazinamide (Pz), cycloserine, ethionamide, rifampicin (RMP), and ethambutol. The majority of these drugs were discovered through broad random screening. Very little optimization was undertaken with insignificant to the targets of drug action, as no biochemical tools for these studies were known at that time. 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. Current treatments involve multiple drug regimens that extend for months at a time, and the pharmacology of these treatment regimens can be complex, especially for the treatment of multiple drug-resistant forms of TB. Isoniazid and rifampicin are the keystones to modern chemotherapy. Once the treatment is initiated, a good physician pays attention to the sign and symptoms of poor progression of the disease and this alerts him to a therapeutic failure: prolonged or recurrent fever; and (ii) failure to convert acid fast stains to negative (median time to conversion of sputum smear is 22 days for non cavitary TB and 48 days for cavitary TB).^{72,73} Detailed discussion of the strategies in the chemotherapy is discussed under the head current therapy. To understand chemotherapy and develop new drugs against this disease it is essential to know the targets to which the drugs act and the targets which can be utilized in development of new chemical entities.

VALIDATED 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 anti TB drugs target the biosynthesis of these macromolecules. The recent developments in genetic engineering of *M. tuberculosis* have now offered many targets to be validated and screen libraries of compounds against them to develop new antituberculosis agents. In selecting targets for antitubercular 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 *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 mycobacterium through out their growth cycle both inside and outside mammalian cells during infection.

Protein Synthesis

Streptomycin, an aminoglycoside for widespread use in the treatment of tuberculosis, disrupts the protein synthesis in bacteria. Streptomycin resistance in *M. tuberculosis* is due to the mutation altering the ribosomal 16s RNA molecule. Most of the aminoglycosides act through this mechanism.⁷⁴ 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.⁷⁵

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 biosynthesis in microbes.⁷⁶ The antituberculosis drug p-amino salicylic acid initially designed as competitive inhibitor of salicylic acid has been reported to 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.⁷⁷

DNA Topoisomerases

Another promising target for inhibition of growth of *M. tuberculosis* is DNA topoisomerases particularly DNA gyrase, a type II topoisomerase.⁷⁸ 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. Many quinolone drugs act by inhibiting DNA gyrase. 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. 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.^{79,80}

Biosynthesis of nucleotides has recently been reported to be a good target particularly for tuberculosis in HIV cases. Very recently, thymidine monophosphate kinase (*dTMKase*)⁸¹ 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 to develop new antituberculosis agents.⁸²

Cell wall macromolecules biosynthesis

Based upon most recent developments in the ultra structure^{83,84} and biochemistry of *M. tuberculosis* its cell envelope consisting in three structural components,⁸⁵ the plasma membrane, the cell wall and the capsule have been identified as the most important to develop new drugs.⁸⁶⁻⁹⁰ Plasma membrane appears to be a typical bacterial membrane contributing very little towards the pathological processes. Cell wall in mycobacteria, members of which cause tuberculosis and leprosy is very complex and of very poor permeability, which contribute to their resistance to therapeutic agents. It consists of two segments, the upper and lower. Beyond the membrane peptidoglycan (PG) covalently linked to arabinogalactan (AG), which in turn, is attached to large mycolic acids with their long meromycolate and short α -chains. These three constitute the cell wall core-the mycolyl arabinogalactan-peptidoglycan (*mAGP*) complex.

The mAGP Complex: The peptidoglycan in bacterial cell wall in general is thought to consist of alternating units of N-acetyl glucosamine (GlcNAc) and a modified muramic acid (Mur). The tetrapeptide side chains of PG consist of L-alanine-D-isoglutaminyl-meso-diaminopimelyl-D-alanine (L-Ala-D-Glu-A₂pm-D-Ala), with the Glu being further amidated.⁹¹⁻⁹⁴ The mycobacterial peptidoglycan differs in two ways from that commonly found in other bacteria; some or all the muramic acid residues are *N*-glycolylated with glycolic acid (MurNGly), and that the cross links include bonds between two residues of diaminopimelic acid as well as between diaminopimelic acid and D-alanine.^{92,93}

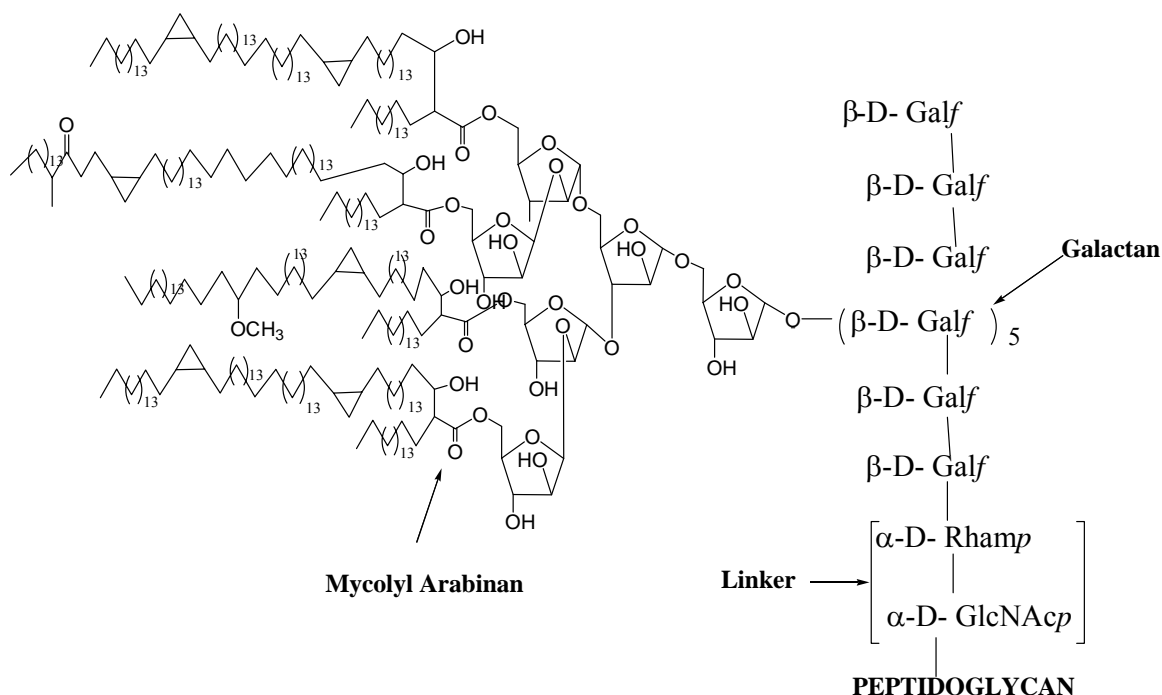


Fig 1. Generalised structure of mAGP complex

It is known that major wall polysaccharide in *M. tuberculosis* is a serologically active branched chain AG, resulting in nonreducing termini of the chains. AG is attached to peptidoglycan through a phosphodiester link to the position 6 of about 10-12 % of muramic acid residues.⁹⁵ All the arabinose and galactose residues are in the furanose form,⁹⁶ the non reducing termini of arabinan consists of a branched hexafuranosyl structure $[\beta\text{-D-Araf}-(1\rightarrow2)\text{-}\alpha\text{-D-Araf}]_2-3,5\text{-}\alpha\text{-D-Araf}(1,5\text{-}\alpha\text{-D-Araf}$; the majority of arabinan chain consists of 5-linked $\alpha\text{-D-Araf}$ with branching introduced by 3,5- $\alpha\text{-D-Araf}$ replaced at both branch positions with 5- $\alpha\text{-D-Araf}$; arbinan chains are attached to the galactan core through some of the 6-linked Galf units,; the galatan region consists of linear alternating 5- and 6-linked $[\beta\text{-D-Galf}]$ residues, the galactan of AG is linked to C-6 of some muramyl residues of peptidoglycan via the diglycosylphosphoryl bridge, L-Rhap(1-3)-D-GlcNAc-(1-P)⁹⁷⁻⁹⁸ and mycolic acids are located in clusters of four on the terminal hexaarabinofuranosyl units, but only about two thirds of these arrangements are mycolated.⁹⁹⁻¹⁰⁰ Very recently oligosaccharides consisting of 26 glycosyl residues with alkylation patterns have been reported.¹⁰¹ The extended nonreducing ends of the arabinan were shown to be a tricosarabinoside (23 mer), with three such units attached to the

galactan unit. Galactans were also isolated and consisted of 23 Gal residues of the repeating linear structure, $[\beta\text{-D-Galf-(1-5)-} [\beta\text{-D-Galf -(1-6-[}\beta\text{-D-Galf}]_n$, devoid of any branching, thereby demonstrating that the points of attachment of the arabinan are close to the reducing end of the galactan, itself linked to PG via the linker disaccharide-P.

Understanding the biosynthesis of the mAGP complex is very important aspect in the design of new drugs against tuberculosis. One of the most important targets is biosynthesis of diglycosyl-P bridge lying between the linear PG and the linear galactan. The synthesis of entire core unit takes place on a polyprenyl-P carrier lipid and there is concomitant extension of the galactan and arabinan chains while this intermediate is transported through the cytoplasmic membrane.^{102,103} Arabinogalactan biosynthesis 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.¹⁰²⁻¹⁰³ This is followed by the transfer of N-rhamnosyl moiety (presumably from dTDP-Rha).¹⁰⁴ The Glaf units of the galactofuranose are synthesized from UDP Galf, which in turn, originate from UDPGalp catalysed by UDPgalactopyranose mutase¹⁰⁴ the Rv3808c gene product.¹⁰⁵ The galactosyl transferase responsible for the polymerization of bulk of the galactofuran has been reported to be the gene product Rv 3809c.¹⁰⁵ Tunicamycin group of antibiotics inhibits this translocation. The intermediate precursor of Araf units of arabinofuran has also been identified as decaprenyl-P-Araf¹⁰⁶ and not a nucleotide precursor as previously thought. This intermediate probably arises in the pentose phosphate pathway.¹⁰⁷⁻¹⁰⁸ Arabinose sugars are also added to growing polysaccharide chain by same sequence of reactions while it is still linked to the carrier. Transfer of arbinosyl unit to growing chain is catalysed by arbinosyl transferases (α and β -arbinosyl transferases).^{109,110} Probably many antituberculosis drugs (ethambutol) target this pathway for their effect. Ethambutol inhibits the biosynthesis of arabinan in both AG and LAM.^{107, 110} The final steps in biosynthesis of mAGP complex, the attachment of mycolic acids and ligation to PG, which awaits further research and it will prove to be excellent drug target for new generation of anti-TB drugs.

Mycolic Acids and other lipids:

Mycolic acids are high molecular weight α -alkyl- β -hydroxy fatty acids, present mostly as bound clusters of AG, where they appear primarily as tetramycolylpentaarbinosyl clusters, but also in extracable lipids mainly as trehalose 6,6'-dimycolate (cord factor). The main part of the branched chain is called as "meromycolic acid" and the other part as α -branch.¹¹¹ 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 -hydroxyl group; and they may have methyl branches in the main carbon backbone.¹¹²

(i): *M. tuberculosis* complex

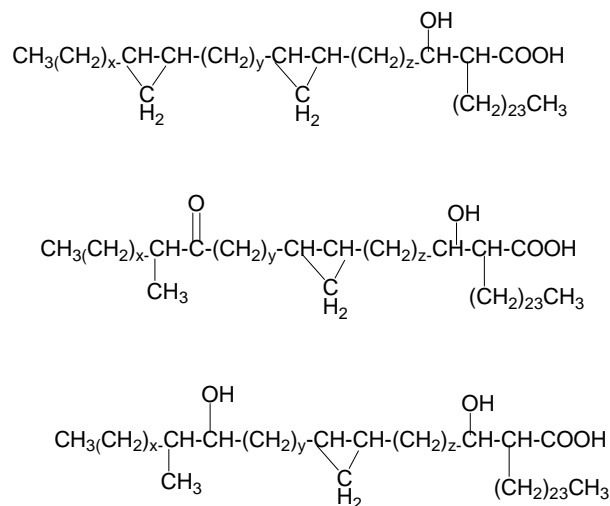


Fig.2 Few mycolic acids from *M. tuberculosis*

(ii): *M. smegmatis*

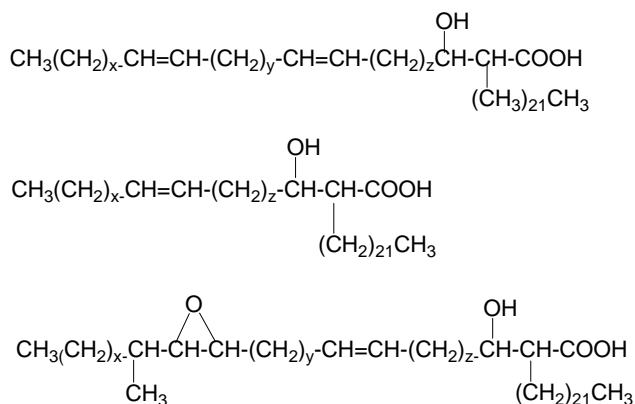


Fig. 3 Few mycolic acids from *M.segmatis*

Apart from the above macromolecules cell wall also contains many other macromolecules and those identified till date include, liparbinomannan (LAM),¹¹³⁻¹¹⁴ many extractable lipids including glycolipids, glycopeptidolipids (GPL),¹¹⁵⁻¹¹⁶ LOS, trehalose containing lipopolysaccharides;¹¹⁷⁻¹¹⁸ phenolic glycolipids (PGL)¹¹⁹⁻¹²³ and other classes of free lipids sulpholipids (SL), phthiocerol dimycocerosate (PDM).¹²⁴⁻¹²⁷ These lipids are very important in pathogenesis and survival of the *M. tuberculosis* in the host macrophages. LAM exhibits a wide spectrum of immunoregulatory functions. It suppresses the immune responses, thus contributing to the pathogenesis. LAM induced abrogation of T-cell activation, inhibition 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.¹²⁷ 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.

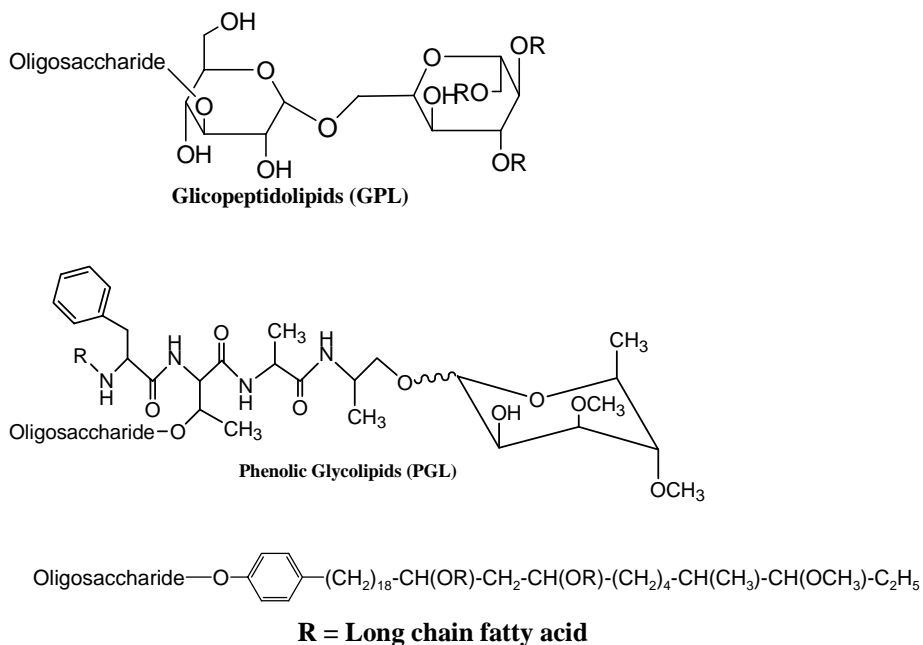


Fig 4. Extractable Lipids from *M. tuberculosis*

Mycolic acids are biosynthesized by Claisen type condensation and reduction of C_{16} fatty acids.¹²⁸ The four distinct steps involved in the biosynthesis include, synthesis of straight chain C_{24} - C_{26} fatty acids to provide C_1 and C_2 atoms and the α -alkyl chain; synthesis of backbone of meromycolic acids of C_{40} - C_{60} ; modification of meromycolic acids to introduce functional groups other than β -hydroxy; and the final condensation step to provide mycolic acids. Many enzymes involved in the catalyzing different steps involved in the biosynthesis of these molecules are targets to develop mechanism based antituberculosis drugs. Many of the genes in the mycolic acid biosynthesis have been identified and characterized.¹²⁹⁻¹³²

Acyl CoA carboxylase (Acc) gives rise to malonyl CoA. FabD exchanges CoA for ACP. FabH is responsible for the coupling of fatty acid synthases I and II (Fas I and Fas II, giving rise to the first precursor of mycolic acid biosynthesis, a β -keto acyl-ACP, which then undergoes reduction by MabA, dehydration (the dehydratase has not yet been identified), enoyl ACP reduction catalysed by InhA, the target of INH, and then another round of elongation catalysed by KasA and KasB.¹³²⁻¹³⁵ These targets have recently been introduced into high throughput screen for the identification new drugs against TB.

The carrier lipid polyprenyl-P identified as decaprenyl-P, responsible for the synthesis PG and also for the synthesis of the linker unit-Rha-GlcNAc-P and subsequently arabinan and galactan formation. This carrier lipid is also responsible for the polyprenyl linked Araf and also for the attachment of a new mycolic acid to the cell wall. This lipid is not synthesized by mevalonic acid pathway but by a new nonmevalonate pathway using deoxy xylulose-5-P, a pathway that provides another good target Genes responsible for their biosynthesis are being identified and have been reported as very good target for new drug development.¹³⁶⁻¹⁴¹

The importance of phthiocerol dimycocersate (DIM) and related lipids as well as methyl branched fatty acids has been the much recent progress in understanding the biosynthesis and their role in disease process.^{133, 134, 135, 142} Polyketide synthase (type-I modular) has been shown to be present in *M. tuberculosis* although evidence of classical polyketides has not been evidenced by chemists in this organism. It has been proved that polyketide synthase acts like FasI, but produces mycocerocic acids after four rounds of extension of a C₁₈ fatty acid but using methyl malonyl CoA instead of malonyl-CoA, which is source of methyl branches of mycocerocic acid. This enzyme is also a very good target to develop new drugs.

Other group of free lipids important for understanding the disease process and developing new drugs are PIMs, LM, and LAM. PIMs are based on phosphatidylinositol (PI) and attached to inositol having one to six mannose residues.^{143, 144, 144, 145, 146} The PIMs with two mannose residues are most common in *M. tuberculosis* Lipomannans (LM) and lipoarabinomannanas (LAM) are the extension of PIMs, while in LM mannan chain is extended and branched, in the case of LAM, an additional arabinan is attached. LAM is very important in TB disease process and it has been shown that mannose capped oligosaccharides allow it to bind to mannose receptor on macrophages in *M. tuberculosis* unlike *M. smegmatis* where mannose caps are absent. LAM can bind all sorts of receptors it can enter into membranes and induces signaling events in the host response in TB. Genes responsible for the synthesis of phosphatidyl inositols capped with mannose have been characterised. PI synthase allows the condensation of inositol and the diacylglycerol of the CDP-diacylglycerol derivative. Pims have been shown to allow mannose units to

attach with alcoholic groups. Evidences are emerging on the roles of these macromolecules in survival of bacterium as well as their latency in the macrophages in persistent TB.^{147,148, 149,150,151}

Isocitrate lyase and malate synthase targets for perisistence

During the latency *M. tuberculosis* appears to coincide with the immune response of the host and the formation of granulomas, which encase the bacterium for its survival. The strategy for survival during chronic stages of infection entails a metabolic shift in the bacteria's carbon source to C₂ substrates generated by β-oxidation of fatty acids.¹⁵² 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.^{152,153} Eenzyme isocitrate lyase and malate synthase¹⁵⁴⁻¹⁵⁶ (absent in mammals) responsible for conversion of isocitrate to glyoxylate is a very hot molecule and is considered to be a very promising target for new drug development 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 as well as propionic acid and 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

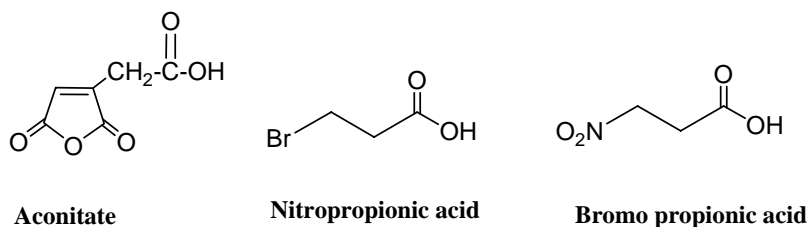


Fig 5. Known Isocitrate lyase inhibitors

drugs.

CURRENT THERAPY

The drugs used to treat tuberculosis include broad spectrum and narrow spectrum agents and different drug combinations are used in different type of tuberculosis discussed briefly below. The aims of the current chemotherapy are conversion of the sputum cultures to negative in shortest time; prevention of emergence of drug resistance and assurance of a complete cure without relapse.¹⁵⁷

Most effective Drug Combination in Pulmonary tuberculosis

Acid-Fast Bacilli (AFB) Smear and Culture Positive:

The best short course regimen should contain isoniazid (300 mg daily) and rifampicin (600 mg or 450 mg daily). The earlier short course therapy for 9 months included the above two drugs as well as streptomycin (750 mg daily 6 days a week) or ethambutol (25 mg/kg daily) with 96 to 99 % cure rate.¹⁵⁸ Later addition of pyrazinamide in lower dose (to avoid hepatotoxicity) of 20 to 40 mg/kg/day 9-up to 2500mg/day) to the isoniazid, rifampicin, streptomycin or ethambutol regimen shortened the treatment period to 6 months with the same cure rate.¹⁵⁸ Further studies proved that Pyrazinamide was necessary for only two months. This regimen of 6 months consisting of isoniazid plus rifampicin throughout, supplemented during first two months with pyrazinamide and ethambutol or streptomycin, have offered great flexibility. Different mechanisms of these adjunct chemotherapeutic agents have been proposed.^{159, 160}

AFB Smear negative but Culture positive: In such patients bacillary load is lower than those with positive smear sputum and therefore, regimens shorter than 6 months may be effective. The study has been carried out and it has been proved that therapy with streptomycin, isoniazid, rifampicin and pyrazinamide strictly had cure rate of 98 %.¹⁶¹

AFB Smear and Culture negative:

In such cases with no bacteriological confirmation a regimen with streptomycin, isoniazid, rifampicin plus pyrazinamide for 3 to 4 months have been reported to be effective.¹⁶¹

INH and Multiple drug resistant TB:

In pulmonary tuberculosis with INH resistance a regimen of 12 month consisting of rifampicin, pyrazinamide and ethambutol or 4 to 5 drug combination including isoniazid, rifampicin, pyrazinamide, ethambutol and or streptomycin for a period of 6 to 8 months led to failure rate of only 2 % and relapse was slightly higher than those with initial susceptible to the disease.¹⁶² The risk for secondary or MDR TB is high in patients who have remained culture positive while receiving multiple drug combination for a prolonged period. In such cases it has been recommended at least three drugs that they have not received before or and to which tubercle bacilli are likely to be susceptible. In such cases no single drug should be given and retreatment should be started in hospitals and for out door patients, retreatment should be directly supervised daily as much as possible. Intermittent therapy 2 or 3 times weekly is usually not effective unless both rifampicin and ethambutol can be used. Tuberculosis resistant to both INH and rifampicin both is a serious problem. A 3 drugs regimen containing pyrazinamide or ethambutol or ethionamide (if they have not been previously used) and 1 injecable drug (usually streptomycin or capreomycin) should be used. However, in some case a fourth drug (cycloserine, ciprofloxacin or amino salicylic acid) is preferred. The treatment should be continued till the sputum is culture negative for two years and despite the risk of toxicity the dosage should be increased to maximum tolerated dose to achieve best result. The treatment success rate with such a strategy may be 85-90% and treatment effort should be continued for at least 6 months before declaring failure. Many other drugs such as clofazimine, ciprofloxacin, ofloxacin and many other investigational agents (rifabutin) have also been used in very highly resistant cases.¹⁶³

Tuberculosis in AIDS patients:

HIV induced immunosuppression has led to comprehensive studies on the immunology of the disease and its treatment in immunosuppressed patients. Although a regimen of three drugs, ethambutol, isoniazid and ethambutol for 6 months supplemented with pyrazinamide during first two months is quite effective¹⁶⁴ yet the relapse has also been reported. Corticosteroids added to antituberculosis chemotherapy has been shown to have dramatic clinical improvement.¹⁶⁵ Even though the optimal duration for the treatment of TB in HIV patients is unknown, it should be continued for a minimum period of 9 months. INH preventive therapy for 12 months is recommended for any person, regardless of the age, who has a positive skin test reaction and is HIV seropositive.¹⁶⁶

Extrapulmonary tuberculosis:

It is handled as pulmonary tuberculosis but because rifampicin and ethambutol cross the blood brain barrier it has been recommended for the treatment of tubercular meningitis. Pyrazinamide should be continued along with INH and rifampicin and the duration for the treatment should be extended to 9 months. The mode of action of important drugs and drawbacks associated with them are discussed below.

Tuberculosis in Pregnancy:

INH, Rifampicin and ethambutol have been used in pregnant women and it is safe to foetus.¹⁶⁷ Ethionamide is teratogenic in mice at higher dose but no reports exist in therapeutic dose for humans. In case of INH and rifampicin resistant TB it can be used during last months of pregnancy. Streptomycin and aminoglycosides may cause teratogenicity and ototoxicity to the foetus and it should not be used in pregnant women.

¹⁶⁷

Tuberculosis in Renal failure:

Since, INH, pyrazinamide and ethambutol are eliminated largely by the kidney therefore these drugs should be used with great precaution in severe renal failure. In such cases the

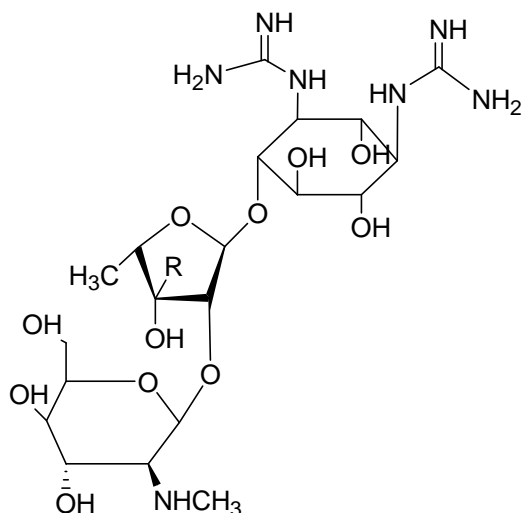
daily dosage of INH and pyrazinamide should be reduced, although rifampicin dosage may be same. Patients undergoing haemodialysis should be given drugs after dialysis.

Tuberculosis in Children:

Children are treated essentially the same way as adults using appropriately adjusted doses. Because of assessing their visual acuity and colour vision ethambutol should not be given to children.

ANTITUBERCULAR AGENTS EITHER IN USE OR UNDER DEVELOPMENT:

Streptomycin: It 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. Streptomycin was the first really effective drug against tuberculosis and derivatives of dihydrostreptomycin have also anti T. B. activities. It has an MIC value of 1µg/mL. It has 50-60 % plasma protein bound with plasma half-life 5-7 hours. It penetrates the inner membrane of *M. tuberculosis* and binds to the 30S subunit of the ribosome.¹⁶⁸ Different synthetic derivatives of streptomycin have been synthesized and evaluated against *M. tuberculosis*.¹⁶⁹

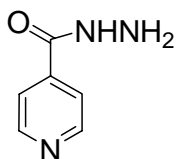


$R_1 = \text{CHO}$, Streptomycin

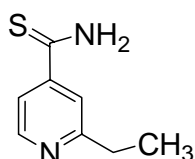
$R_1 = \text{CH}_2\text{OH}$, DihydroStreptomycin

Because of many toxic manifestations on peripheral, central nervous system at higher doses and hypersensitivity reactions it 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.

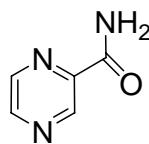
Isoniazid (1952): It is a prodrug that requires activation by the mycobacterial catalase peroxidase enzyme (kat G), which confers sensitivity in *M. tuberculosis* to INH. It is orally active and exhibits bacteriostatic action on the resting bacilli and is highly active against the *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum* and *M. Microti*). It has very low MICs (0.02-0.06 $\mu\text{g/ml}$) against these pathogens.¹⁷⁰ INH enters the organism by diffusion and oxygen-dependent active transport and it has been reported to have effect on almost every aspect of mycobacterial metabolism.¹⁷¹ INH inhibits the mycolic acid biosynthesis in mycobacterium tuberculosis by affecting an enzyme *mycolate synthetase*, unique for mycobacteria.¹⁷²⁻¹⁷³



Isoniazid



Ethionamide

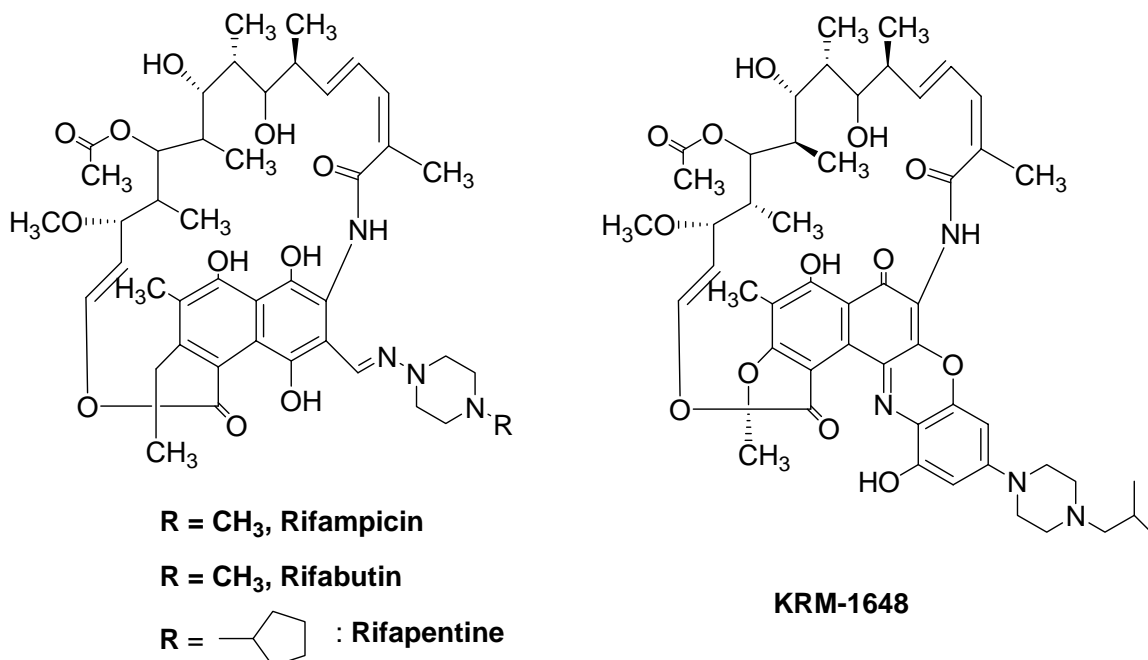


Pyrazinamide

A mutation within mycobacterial *inhA* gene was shown to confer resistance to both INH and ethionamide in *M. smegmatis* and in *M. bovis* suggesting that *inhA* is likely target of this drug and related agents.¹⁷⁴⁻¹⁷⁵ A large number of compounds belonging to INH have been synthesized and evaluated against *M. tuberculosis* H37Rv. Among them ethionamide and pyrazinamide are in clinics.

Rifamycins : These are a group of semisynthetic antibiotics of rifamycin B, isolated from *Streptomyces mediterrani* with characteristic ansa structure (chromophoric naphthaquinone group spanned by a long aliphatic bridge) which itself has very poor antimicrobial activity.¹⁷⁶ They inhibit prokaryotic DNA-dependent RNA polymerase, an enzyme necessary for RNA synthesis.¹⁷⁷ Rifampin acts on B subunit of this enzyme resulting into formation of stable complex causing 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 its penetration across the mycobacterial cell wall. To avoid rapid development of bacterial resistance rifampicin is recommended in combination with other first line agents either isoniazid or ethambutol. However, combination of INH and rifampicin may increase risk of hepatotoxicity. Rifampicin is effective against *M. tuberculosis* with MIC ranging from 0.1 to 0.2 µg/ml.

Rifampicin was modified and several analogs were prepared and out of them one compound KRM-1648, a benzoxazinorifamycin was found to be more potent than RF.¹⁷⁸ Inclusion of KRM-1648 in standard combination therapy led to significant shortening of duration of the treatment.¹⁷⁹⁻¹⁸³ Since drug resistance has a very close relation with the pharmacokinetics properties of the molecule, 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 render least exposure to mycobacteria bacilli for evoking mutation necessary for drug resistance.



Ethambutol: It is a synthetic amino alcohol (ethylene diamino-di-1-butanol), orally effective bacteriostatic agent and active against most strains of Mycobacterium.¹⁸⁴⁻¹⁸⁶

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 biosynthesis both in arabinogalactan 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 biosynthesis inhibits the formation of this complex and this may lead to increased permeability of the cell wall. It has been suggested that this drug inhibits the enzyme arabinosyl transferase and thereby disrupts the biosynthesis of AG and LAM. Arabinosyl transferase III is responsible for the polymerization of arabinose into arabinan of arabinogalactan during cell wall biosynthesis.¹⁸⁷

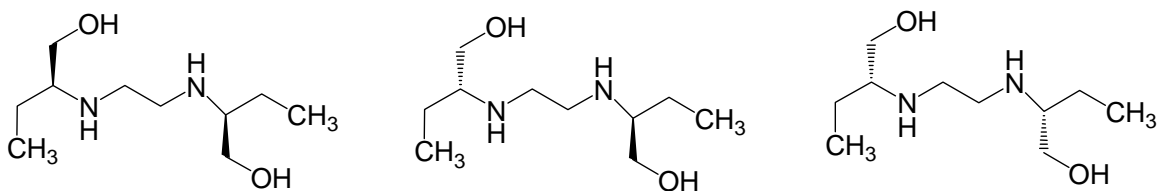
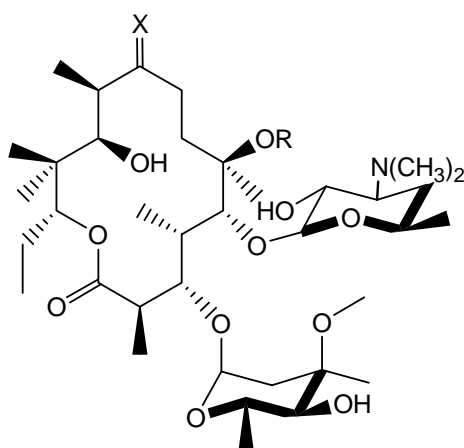
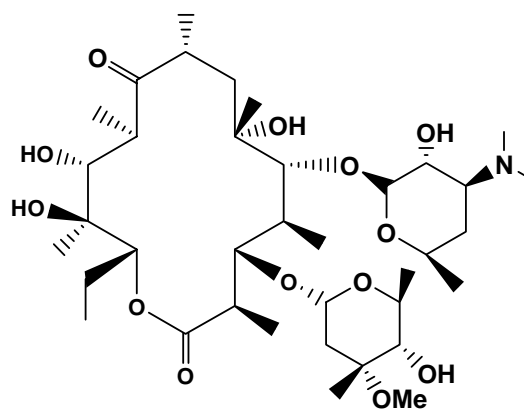


Figure : different isomers of Ethambutol

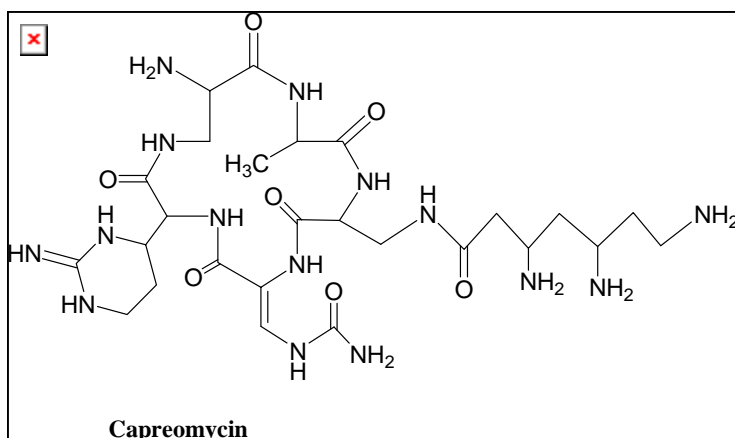
Macrolides: The macrolides comprise a family of antibiotics ranging from erythromycin (1952) to analogs synthesized more recently. Erythromycin is 14 membered macrolide consisting of a macrocyclic lactone ring attached to two sugar residues. Newer derivatives differ from the parent erythromycin in the size and/or substitution pattern of the lactose ring and include; roxithromycin, clarithromycin, azithromycin, rokitamycin and spiramycin¹⁸⁸⁻¹⁹² Although some of the macrolides display poor antimicrobial activity against enterobacteria, they can generally be regarded as broad-spectrum agents inhibiting mycobacterial growth also. However, none of them displayed interesting antitubercular activity. A number of semisynthetic derivatives with improved pharmacokinetic properties appear to be promising in the treatment of mycobacterial infections particularly those caused by non-tuberculosis species.¹⁹³



Roxithromycin X = NOCH₂OCH₂CH₂OCH₃, R= Me;
Clarithromycin X = O, R = Me



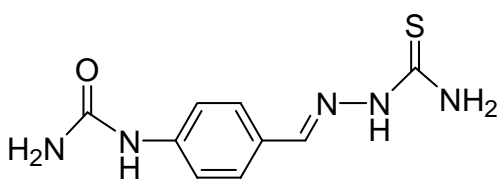
Erythromycin



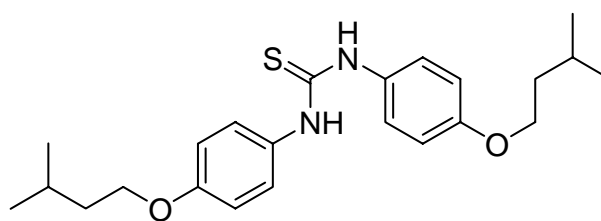
These antibiotics bind to high affinity site in the peptidyl *t*-RNA binding region of the bacterial 50S ribosome subunit, causing dissociation of peptidyl-tRNA from ribosomes and inhibition of bacterial protein synthesis.¹⁹⁴

Isoxyl (Thiocarlide):

A number of diacyl thioureas have shown activity in experimental tuberculosis. One of such agent 4,4'-diisooamyloxydiphenylthiourea (ISO) (4,4'-diisooamyloxydiphenyl thiocarbamide, isoxyl, thiocarlide)¹⁹⁵⁻¹⁹⁷ has proved clinically useful. The mode of action this drug has been shown to be the inhibition of mycolic acid biosynthesis in *M. bovis* during a 6h exposure to 10 µg/ml.



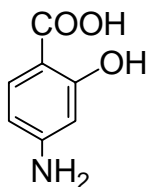
Thioacetazone



Isoxyl

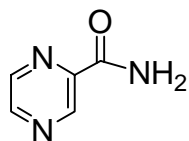
p-Aminosalicylic acid (PAS): The antimycobacterial activity of PAS was reported only in 1946 although it was synthesized a long back.¹⁹⁸ It has no effect against other bacteria and it is highly effective against *M. tuberculosis*.¹⁹⁹ Following DOTS it is rarely used today. However, it is occasionally used in the regimens for the treatment of tuberculosis caused by MDR T.B. The mode of action of this 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.



p- amino salicylic acid

Pyrazinamide (PZA): Pyrazinamide, a structural analog of nicotinamide, is first line drug of short course tuberculosis therapy. It is also active against semidormnant bacilli not affected by any other drug and has strong synergy with INH and rifampicin and shortens the therapy period to 6 months.²⁰⁰⁻²⁰¹ The drug has no significant bactericidal effect and is thought to 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*. Mutation in the *pncA* gene responsible for the production of pyrazinamidase has been shown to be the reason for resistance against this drug.²⁰²⁻²⁰³ Some pyrazinoic esters have also been reported to possess good antitubercular activities.²⁰⁴

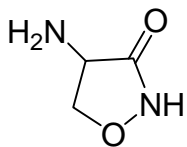


Pyrazinamide

Cycloserine

D-Cycloserine, a structural analogue of amino acid D-alanine, possesses activity against a wide range of bacteria²⁰⁵ and inhibits *M. tuberculosis* at concentrations of 5-20 µg/mL. It blocks peptidoglycan biosynthesis by inhibiting the enzyme D-alanine racemase and D-alanyl alanine synthetase.¹⁷⁷ Microorganisms treated with cycloserine accumulate a muramic-uridine-nucleotide-peptide, which differs from that produced by mycobacteria

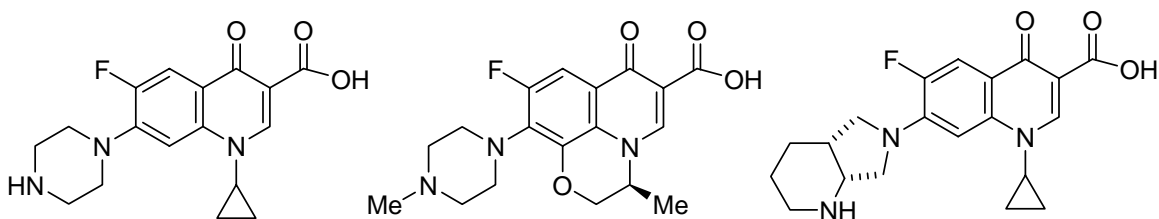
in the absence of terminal D-alanine dipeptide.²⁰⁶⁻²⁰⁷ Cycloserine results in the central nervous system toxicity and can also generate psychotic states with suicidal tendencies and epileptic convulsion.



D-Cycloserine

Fluoroquinolones

These are synthetic derivatives of nalidixic acid and display broad-spectrum antimycobacterial activity²⁰⁸⁻²¹⁰ as ciprofloxacin and ofloxacin used as a part of multi drug regimens, resulted in clinical and microbiological cure of patients infected with *M. tuberculosis* and *M. avium*. Structural modification of FQ to optimize antimycobacterial activity have been extensively carried out to produce candidates which are more efficacious than earlier FQ.²⁰⁹ Their bactericidal effects involve an interaction of the drugs with DNA-gyrase and DNA – topoisomerase IV.²¹¹



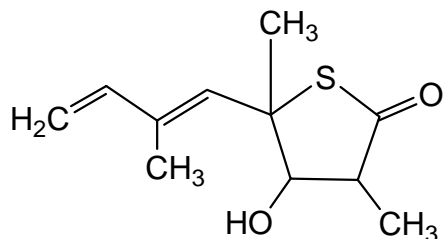
Ciprofloxacin

Levofloxacin

Moxifloxacin

Thiolactomycin: Thiolactomycin (TLM) [(4*R*) (2*E*, 5*E*) 2,4,6-trimethyl-3-hydroxy-2,5,7-octatriene-4-thiolide]²¹² belonging to small group of thioteronic acid antibacterials is an unique thiolactone exhibiting anti T.B. activity by inhibiting mycolic acid biosynthesis. It inhibits FAS-II of plant and bacterial origin but not of mammalian or yeast type FAS-I.²¹³ and in this way it is a reactive target to develop new drugs. TLM targets two β -keto acyl carrier protein synthases, *KasA* and *KasB* consistent to the fact that both enzymes belong to FAS-II involved in fatty acid and mycolic acid biosynthesis.

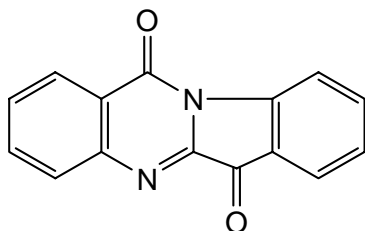
¹⁸⁵ It has high MIC of 5 μ g/ml but in the absence of *in vivo* toxicology and *in vitro* cytotoxicity data it is difficult to judge whether these concentrations are far below the toxic concentrations.²¹⁴



Thiolactomycin

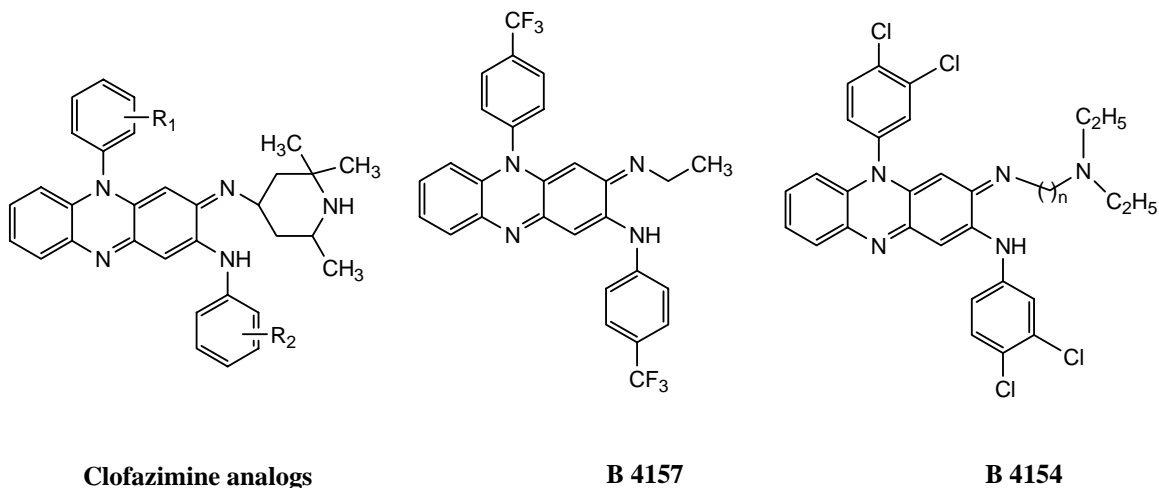
Tryptanthrin:

It is a potent structurally novel indoloquinazolinone alkaloid, active against MDR TB with an MIC of 0.5-1.0 μ g/ml. But *in vivo* data and *in vitro* toxicity are needed before this structural prototype is applied in MDR TB.²¹⁵

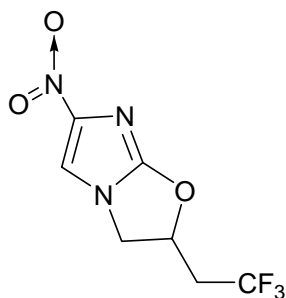


Tryptanthrin

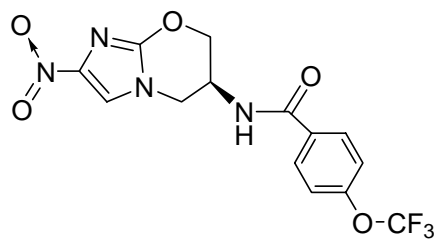
Clofazimine Analogs: Few of the tetramethyl piperidine substituted phenazines (TMP phenazines) were found to possess significantly more activity against *M. tuberculosis*, including MDR clinical strains than clofazimines²¹⁶ and the intracellular accumulation in mononuclear phagocytic cells, antiinflammatory activity, a low incidence of drug resistance and slow metabolic elimination rate, make them attractive candidate for the treatment of mycobacterial infections. The compounds of this series are active *in vivo* also.²¹⁶⁻²¹⁸



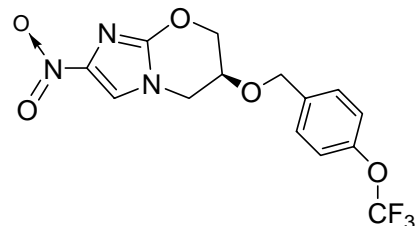
Nitroimidazofurans and Nitroimidazopyrans: Nitroimidazofurans originally used as radio sensitizer in cancer chemotherapy have been reported to possess in vivo antitubercular activities. However, because of mutagenic side effects this series of compounds could not enter into clinics for the treatment of tuberculosis.²¹⁹⁻²²⁰ Bicyclic nitroimidazopyrans (NAP) a narrow spectrum antimicrobials have recently been reported to possess antitubercular activity and one of the compounds PA -824 has emerged as a lead molecule as it was effective both in the replicating and latent *M. tuberculosis* cells with MIC from 0.015 to 0.25 $\mu\text{g}/\text{ml}$. Poly and multi drug resistant strains of this bacterium were susceptible to PA-824, indicating that there is no cross- resistance with current drugs.²²¹ The mode of action of this class of compound has been inhibition of protein biosynthesis by a mechanism dependent on *M. tuberculosis* F420 cofactor, and inhibition of biosynthesis of cell wall lipid with no effect on nucleic acid biosynthesis. Another orally active analog of NAP (PA 1343) has been developed and is in preclinical studies with MIC of 0.015 $\mu\text{g}/\text{ml}$.²²²



CGI-17341



PA-1343

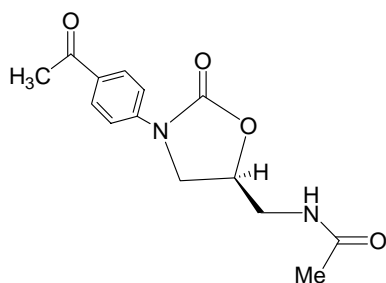


PA-824

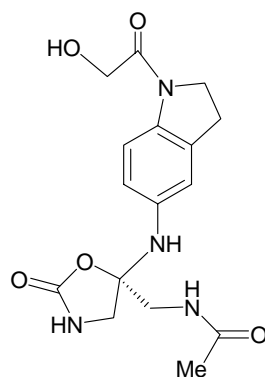
Comparable susceptibility of MDR strains of *M. tuberculosis* and PA-824 indicates that there is no cross resistance with current drugs. Effects on cell wall lipids accounts for an accumulation of hydroxy mycolic acid with a concomitant reduction in keto mycolate.

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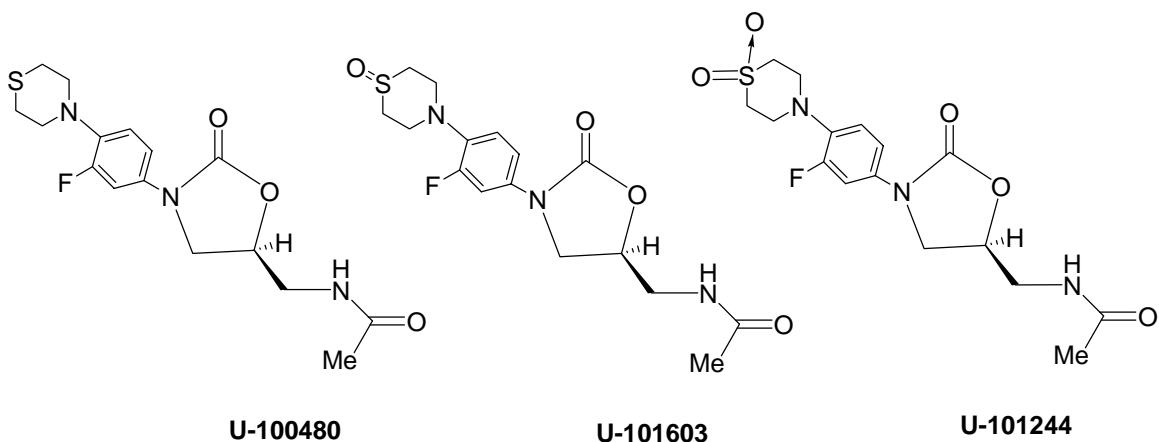
Oxazolidinones: Oxazolidinones, ²²⁴⁻²³⁰ exemplified by Dup 721, are totally synthetic, orally active antibacterial agents discovered by DuPont. They are bacterial protein synthesis inhibitors, with inhibition uniquely in the initiation phase of protein synthesis. Thiomorpholine analogues of U-100480 with the biphenyl methyl group replacing the acetamido methyl oxazolidinone moiety showed potent *in vitro* activity against *M. tuberculosis*. Some of such compounds have been shown below. Because of toxicity in rat model several modifications have been carried out. But unfortunately none of the compound of this series is in advanced stage of clinical trial.



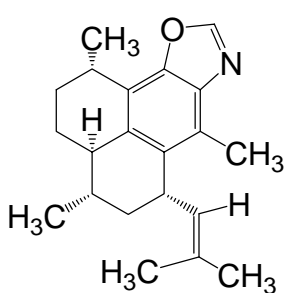
DUP-721



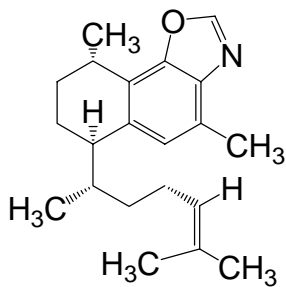
U-100480



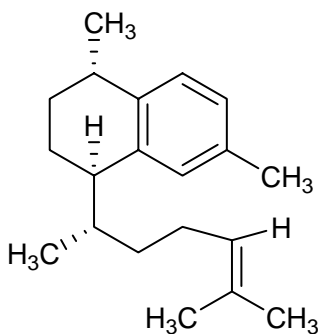
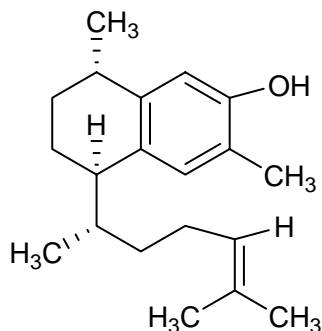
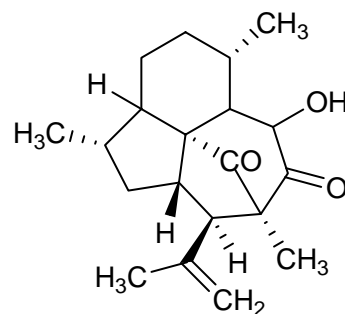
Diterpenoids: These compounds known for various medicinal values have recently been screened for antituberculosis activities against *M. tuberculosis*. Benzooxazole alkaloids isolated from Indian sea whip *Pseudopterogorgia elisabethae* were tested against the bacterium and it was found that pseudo-teroxazole has potent inhibitory activity (97 %) at 12.5 µg/ml in *M. tuberculosis* H37 Rv strain.²³¹⁻²³³ Many other analogs have also shown potent antimycobacterial 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.²³⁴⁻²³⁵



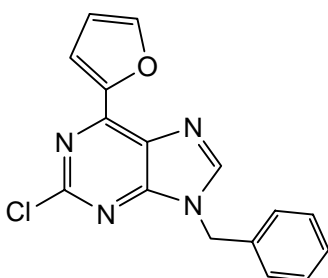
Pseudo-teroxazole



Seco-pseudo-teroxazole

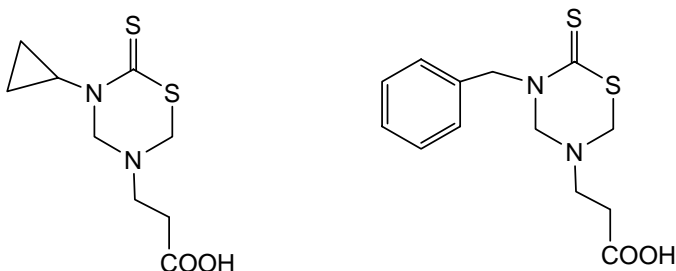
**Erogorgiaene****7-Hydroxy erogorgiaene****Elisapterosin B**

Purines: 9-Benzyl purines 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 $\mu\text{g/ml}$ in vitro.²³⁶ Antimycobacterial Activity of 6-Aryl purines²³⁷ and 9-Sulphonylated or Sulphenylated -6-Mercaptopurines are also known in literature.²³⁸

**2-Chloro-4-(2-furanyl)-9-benzylpurine**

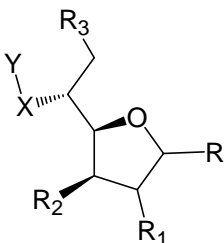
Thiazidine thiones: Thiazidine thiones a derivative of dithiocarbamic acids have been screened against *M. tuberculosis* because of their well-known biological activities including antifungal and antibacterial activities.^{239, 240} We have very recently reinvestigated the antitubercular activities in this class of molecule and one of the compounds have shown potent *in vitro* antitubercular activity against *M. tuberculosis*

H37Rv even in resistant strains.²⁴¹ and also protected the mice marginally in experimental tuberculosis.



Thiazidine thione derivatives

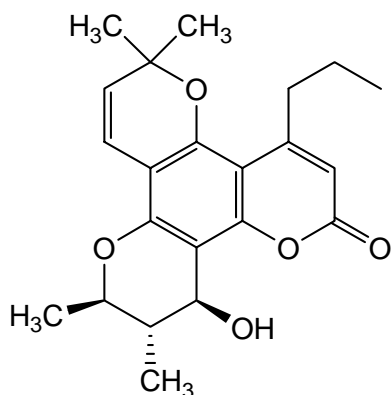
Simple Carbohydrate derivatives: Based on the observation that simple sugar derivatives possess inhibitory activity against the enzymes involved in cell wall biosynthesis, many simple monosachharide derivatives have been tested against *M. tuberculosis* H37 Rv and have shown potent activity *in vitro*.²⁴² Based on the DNA topoisomerase-II inhibitory potency of certain glycosyl amino acid based glycoconjugates²⁴³ we have also synthesized a number of derivatives from monosachharides and few of them possess antitubercular activity against *M. tuberculosis* H37Rv including the resistant strain.²⁴⁴ Few of the compounds have shown potent activity *in vitro* even in many clinical MDR of *M. tuberculosis* strains. However, many compounds displayed toxicity in the animals and an effort in this direction is continued.



Simple Sugars as antimycobacterials

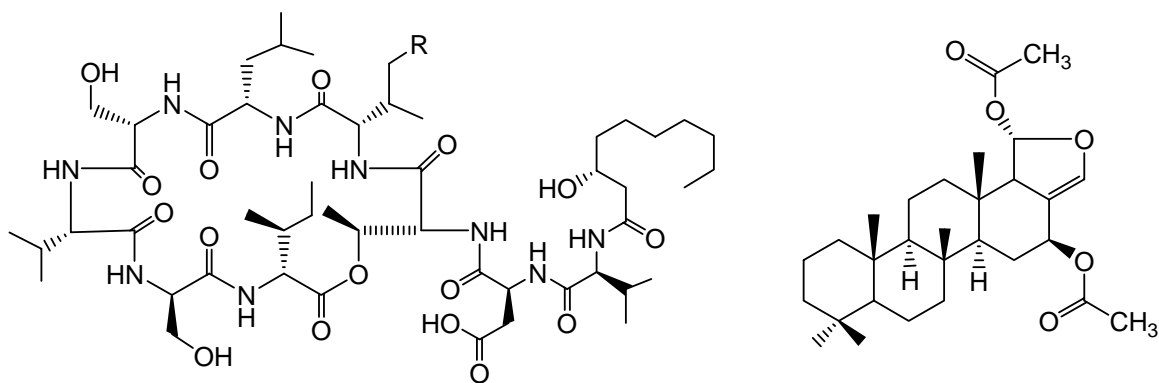
Benzopyran-2-ones: Antitubercular activity in this class of molecule was reported while investigating the anti HIV activity in natural products (+) calanolide and (-) clanolide A,

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 $\mu\text{g/ml}$.²⁴⁵



Calanolide A

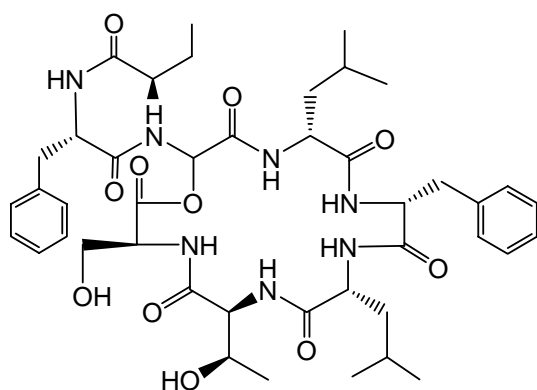
Marine natural products : Massetolide A and Viscosin B, cyclic depsipeptides isolated from cultures of two pseudomonans, a marine alga and tube worm were tested against *M. avium-intracellulare* having MIC values of 2.5-5 and 5-10 $\mu\text{g/ml}$ respectively.^{246a} Kahalalides A, isolated from the Sacoglossan mollusk *Elysia rufescens*,^{246b,c} inhibited the growth of *M. tuberculosis* H₃₇ Rv (83 %) at 12 $\mu\text{g/ml}$. Similarly, Litosterol and Nephasterol C, the C19 hydroxy steroids isolated from a red sea Nephtheasp;^{246d} had 90 and 96% inhibitory growth of *M. tuberculosis* H₃₇ Rv with MIC 3.13 and 12.5 $\mu\text{g/ml}$ respectively. Heteronemin a Sesquiterpene isolated from a red sea sponge;^{246e} displayed antitubercular activity against *M. tuberculosis* H₃₇ Rv with an MIC 6.25 $\mu\text{g/ml}$ and IC₅₀ 1.3 $\mu\text{g/ml}$



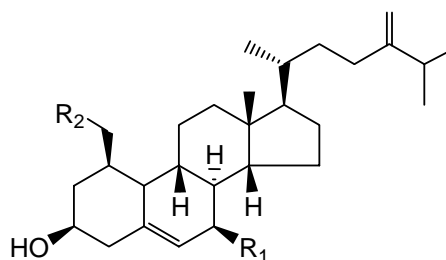
R = CH₃, **Massetolide A**

R = H, **Viscosin**

Heteronemin



Kahalalide A



R₁ = H, R₂ = OH; **Litosterol**

R₁ = OAc, R₂ = OH; **Nephasterol**

Structures Antitubercular Marine Natural products

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,²⁴⁷ 3-hydrazono-1 H-2-indolinones,²⁴⁸ quinoxaline-1, 4-di-N-oxides,²⁴⁹ cyclohexadiene²⁵⁰ N- (2-naphthyl) glycin hydrazide analogous,²⁵¹ isoxazoles and cynopyridines,²⁵² 4-phenyl-1, 8-naphthyridine,²⁵³ aminolupinanes,²⁵⁴ benzoyl thiozole-2-carbamates,²⁵⁵ thiosemicarbazones,²⁵⁶ dithiocarbamates,²⁵⁷ thiazolidinones,²⁵⁸⁻²⁶⁰ succinamides,²⁶¹ diarylsuccinamides,²⁶² glutaconyl-thiosemicarbazides,²⁶³ 2, 2'-dithio-bis (benzamides),²⁶⁴ 1,3,5-triazines,²⁶⁵ trimethyl silyl-3-(carboxy carbamoyl) propionates²⁶⁶ pyrrolnitrin and pyrrole derivatives²⁶⁷⁻²⁷⁰ diguanidino and “reversed”

diamidino 2,5- diaryl- furans, ²⁷¹ 1,2,4 triazoles, ²⁷²⁻²⁷³ thiazole, ²⁷⁴ 1,3,4 thiadiazole, ²⁷⁵ quinazolines, ²⁷⁶ quinolines, ²⁷⁷ azasugars, ²⁷⁸ quinoxaline 1,4 dioxides, ²⁷⁹ quinoxaline-2-carboxamide 1,4-di-N-oxide, ²⁸⁰ nitroquinolones, ²⁸¹ 2,4- dihydroxy quinolines ²⁸² 3-nitro and 3-bromo 4-hydroxy-2 quinooines, ²⁸³ quinazolinones, ²⁸⁴ 6-nitro quinazolones, ²⁸⁵ benzimidazole, ²⁸⁶ benzazole ²⁸⁷ substituted 2-(4-amino phenyl sulphonamido)-benzothiazoles, ²⁸⁸ 3-aryl substituted -2-[(1H(2H) benzotriazol-1(2)-yl)-acrylonitrile, ²⁸⁹ 3- aryl, 3-cyclohexyl and 3-heteroaryl- substituted -2-[1H (2H)-Benzotriazol-1(2)-yl) prop-2-enenitriles, ²⁹⁰ 2-azetidinones, ²⁹¹ pyrimidines, ²⁹²⁻²⁹⁵ pyrazine carboxylic acid, ²⁹⁶ pyrazines, α - oxo- ketene dithioacetals, ²⁹⁷ dihydropyridine, ²⁹⁸⁻²⁹⁹ spirothiazolidinones, ³⁰⁰ isonicotinoyl hydrazones, ³⁰¹ S-alkylisothiosemicarbazone, ³⁰² thiosemicarbazone, ³⁰³ hydrazinecarboxamides, ³⁰⁴ pyrazolines, ³⁰⁵⁻³⁰⁶ 4-aryl hydrazono-2-pyrazoline-5-ones, ³⁰⁷ N-alkyl-1,2- dihydro-2-thioxo- 3-pyridine carothioamides, ³⁰⁸ 2- benzylthiopyridine-4-carbothioamides, ³⁰⁹ 2-substituted pyrazines, ³¹⁰ 2,5- disubstituted 1,3,4-oxadiazoles, ³¹¹ 6-chloro cinnolinothiazoles, ³¹² imidazo[2,1-b]-thiazoles, ³¹³ sydnones, ³¹⁴ pyrazinoyl heterocycles, ³¹⁵⁻³¹⁶ pyrazinoic acid hydrazide, ³¹⁷ pyrazinamie derivatives, ³¹⁸ azetidinone, ³¹⁹⁻³²⁰ pyrazine carboxylic Acids isosteres, ³²¹⁻³²³ ureas and thioureas, ³²⁴ thiosemicarbazones, ³²⁵ hydantoins ³²⁶ and hydrazones. ³²⁷⁻³²⁹

7. The future perspectives ³³⁰⁻³³²

The present knowledge in tuberculosis has established that MDR TB, HIV-TB co-infection, and concern about transmission of drug-resistant strains represent a threat to TB control programmes. In the last few years there has been considerable progress in the biology, biochemistry of Mycobacterium tuberculosis and understanding the disease process, the mechanism of drug resistance and in establishing the value of DOTS in preventing the treatment failure. However, only a limited effort has been made to develop new active chemical entities or rapid diagnostic tests, and their relevance to the global tuberculosis control has been questioned. This decade has seen dramatic advances in understanding the biology, intracellular lifestyle and detailed biochemistry of the mycobacterium generating a wealth of information to undertake different targets for new drug development even against MDR TB. The new validated and selective target compatible to high throughput assays against all forms of tuberculosis should be

developed for drug susceptibility testing, baseline screening to identify new chemical entities and drug resistance early and to guide re-treatment efforts. Development of combinatorial and virtual libraries and in silico screening of the compounds should be prioritized to get an early lead for developing new drugs. For this enormous funding from the developed countries and voluntary organizations is needed.

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 countries with the introduction of clean water supplies, sewage disposal and hygiene education would certainly have a dramatic impact in the control of the disease in the population. It is well known that industrial smoke, as also smoke from active or passive smoking, weaken the lungs and make them susceptible to bacterial infection. Improvement in the literacy rate in females of developing countries particularly India would certainly lead to a better picture in curtailing this disease as it influences acceptance of hygienic measures in the entire family, incidentally, it is ironic that young females, in general, are more susceptible to infection by *M. tuberculosis* than young males of the same age.

Antimycobacterial agents should be developed with strict quality controls to assure adequate bioavailability. Un-interrupted supply of affordable drugs to the every corner of the world where need arises are critical for the care of patients with drug susceptibility and MDR TB. DOTS programme should be implemented properly for the control of all forms of tuberculosis.

The use of a vaccine to prevent the onset of the disease appears to offer the best prospect for controlling TB in developing countries would certainly lead to reduction in the burden of this disease. Application of vaccine to effectively treat cases of TB with drugs, and also because of the toxicity of the available drugs in certain cases should also be emphasized world-wide. 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.

The recommended chemotherapy should 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. Hopes for ending this pathetic state of affairs related to tuberculosis control rest on the development of a vaccine and the adherence by physicians and patients to a scientific attitude.

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