

Recent advances in research on antituberculars

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Alarming rise in the incidence of tuberculosis cases worldwide has made an infectious disease like tuberculosis, one of the top priorities on World Health Organization's agenda. Although, a variety of new antibacterial compounds are becoming available for fighting a number of infectious diseases, antitubercular drugs do not form a part of it, perhaps owing to the perception of it being not-so-lucrative market opportunity, coupled with complexity of the research involved in finding new anti-TB drugs. The present article is intended to highlight the current therapy available to combat TB and to focus on research that is being carried out at various laboratories for finding out potent new therapeutics and to highlight the lead compounds emerging from these efforts.

Introduction

Tuberculosis (TB), an airborne communicable disease and one of the three World Health Organization (WHO) priority infectious diseases, is caused by transmission of aerosolized droplets of *Mycobacterium tuberculosis* organism. TB kills about 2 million people every year and the epidemic, which is spreading globally, is assuming alarming proportions. Around 8 million people become infected with TB every year. The WHO 'fact sheet' on TB¹ estimates that between 2000 and 2020, nearly one billion people will be newly infected, 200 million people will get sick, and 35 million will die from TB, if effort to control is not further intensified. In India alone, one person dies of TB every minute¹. Approximately 50% of the India's population is reported to be tuberculin test positive. Every year about 0.4 million deaths and one million new cases of tuberculosis are reported². In short, tuberculosis remains a major health problem in India.

Each untreated person with active TB will infect on an average between 10 and 15 people every year, but people infected with TB will not necessarily succumb to the disease. The immune system 'walls off' the TB bacilli which, protected by a thick waxy coat, can lie dormant for years and when the immune system is weakened, the chances of succumbing to the disease become higher. The WHO 'fact sheet'¹ on TB highlights the spread of the disease :

- Someone in the world is newly infected with TB every second.
- Overall, 1/3rd of the world's population is currently infected with the TB bacillus.

- Nearly 1% of the world's population is newly infected with TB each year.
- 5–10% of people who are infected with TB become sick or infectious at some time during their life.

The reasons for the increase in the number of tuberculosis cases are probably due to growing epidemic of HIV infection, malnutrition leading to reduced immunity, use of indiscriminate/inadequate chemotherapy and multi-drug resistance due to partial adherence to chemotherapy.

Twin threats to global tuberculosis control are the continued explosion of HIV related TB and the increasing prevalence of drug resistance. HIV appears to be a potent facilitator of TB. By destroying the cells most important to the containment of tubercle bacilli (macrophages and CD4-receptor-bearing lymphocytes), HIV vigorously promotes progression of recent or remotely acquired TB infection to active disease. With the spread of HIV infection the number of TB cases has increased; it is a leading cause of death among people who are HIV-positive. It accounts for about 15% of AIDS deaths worldwide.

Until about 50 years ago, there were no drugs to cure TB. Since then with the available chemotherapy, unfortunately resistant strains to all major anti-TB drugs have emerged. Drug-resistant TB is mainly caused by inconsistent or partial treatment/patient in compliance to the dosage regimen. Multidrug-resistant TB (MDR-TB), which is defined as the disease due to TB bacilli resistant to at least isoniazid and rifampicin, the two most powerful anti-TB drugs, is the most dangerous form of drug-resistant TB.

The biology of *Mycobacterium tuberculosis*

Mycobacterium is a genus of bacteria, which are slow growing, aerobic and distinguished by acid-fast staining. Typically, the mycobacteria are obligate aerobes, characterized as gram-positive. The genus mycobacterium includes the highly pathogenic organisms that cause tuberculosis, *Mycobacterium tuberculosis* (*M. tuberculosis*) and sometimes *M. bovis* and leprosy (*M. leprae*).

Tuberculosis (TB) is an infectious disease caused by the bacterium *M. tuberculosis*, which is spread almost exclusively by airborne transmission. Although the disease can affect any site in the body, it most often affects the lungs. When persons with pulmonary TB cough, they produce tiny droplet nuclei that contain TB bacteria, which can remain suspended in the air for prolonged periods of time. Anyone who breathes air that contains these droplet nuclei can become infected with TB.

The cell wall of *Mycobacterium* species in its full structural and functional integrity, is essential for its growth and survival in the infected host. In fact, some of the most effective antimycobacterial drugs including isoniazid and ethambutol are known to inhibit the biogenesis of cell wall components. *M. tuberculosis* possesses a cell wall dominated by covalently linked mycolic acids, arabinogalactan and peptidoglycan (AGP), the mycolic acids of which are complemented by glycolipids such as α,α -trehalose monomycolate (TMM)³. This mycolic acid based permeability barrier shields the organism from environmental stress and contributes to disease persistence and the refractoriness of *M. tuberculosis* to many antibiotics.

One of the most prominent macromolecular entities of mycobacterial cell wall is arabinan, a common constituent of both arabinogalactan (AG) and lipoarabinomannan (LAM)³. In the chemical setting of the mycolylarabinogalactan-peptidoglycan complex, AG forms an integral part of cell wall proper, whereas LAM, based on a phosphatidylinositol anchor, apparently exists in a state of flux. LAM is an essential part of the cell envelope, which lacks covalent association with the cell wall core. Anchored in the cell membrane and transversing the cell wall, as well as appearing as an excretory product, LAM has been implicated as a key surface molecule in host-pathogen interactions. The biosynthetic pathways leading to formation of the key mycobacterial cell wall components AG and mycolic acids, are the targets for the rational design of new antitubercular agents.

The complete genome sequence of the best-characterized strain of *M. tuberculosis*, H37Rv has been deter-

mined⁴. It could provide a number of new targets for novel antitubercular drugs.

Multidrug-resistant tuberculosis (MDRTB) :

Tuberculosis therapy involves an initial intensive two-month regime comprising multiple antibiotics – isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB) or streptomycin (SM). For next four months, only INH and RIF are administered to eliminate any persisting tubercle bacilli. These two drugs kill more than 99% of tubercle bacilli within two months of initiation of therapy. Along with these two drugs, PZA, with a high sterilizing effect, appears to act on semi-dormant bacilli not affected by any other antitubercular drugs⁵. This multidrug regimen is to ensure that mutants resistant to a single drug do not emerge. These drugs in conjunction with each other shorten the duration of therapy from 18 to 6 months. Hence, emergence of any strains resistant to either of these drugs poses grave risk, as other drugs are not as effective, possess toxic side-effects which may result in higher mortality.

Multidrug-resistant tuberculosis (MDRTB) refers to simultaneous resistance to at least INH and RIF (with or without resistance to other drugs). Multidrug resistance arises from the sharing of genes between different species or genera, generally mediated by small pieces of extra-chromosomal DNA known as transposons or plasmids⁶. Some antibiotics can actually induce the transfer of these resistance genes⁷. Alternatively, as with the problematic multidrug-resistant *M. tuberculosis* (MDRTB) strains, accumulation of multiple point mutations in the chromosomal DNA can take place⁸. Contamination of some commercial antibiotic preparations with the DNA (containing the inherent resistance genes) of the organisms that produce the antibiotic has been implicated as a source of drug resistance genes. The presence of DNA encoding drug resistance in antibiotic preparations has been proposed as a factor in the rapid development of multiple drug resistance in bacteria⁹.

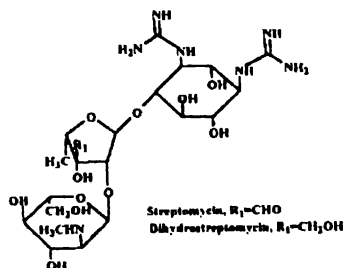
Current therapy

The drugs that have been used to fight tuberculosis include isoniazid, rifampicin, pyrazinamide, ethambutol, streptomycin, *p*-aminosalicylic acid, ethionamide, cycloserine, capreomycin, kanamycin, thioacetazone etc. The important first-line antituberculosis drugs, which include streptomycin, isoniazid, rifampicin, ethambutol and pyrazinamide and their mechanism of action is discussed below in brief.

Streptomycin :

Streptomycin, an aminoglycoside antibiotic derived from *Streptomyces griseus* made up of three components,

streptidine, streptose and *N*-methyl-L-glucosamine, poorly absorbed from gastrointestinal tract, is administered intramuscularly. It was the first really effective drug for tuberculosis. The chemical derivative dihydrostreptomycin has almost the same antibacterial activity as the parent compound. Concentrations of streptomycin of the order of 1 µg/ml inhibit the growth of *M. tuberculosis* H37Rv. The drug exerts its effect by interfering with bacterial protein synthesis. It penetrates the inner membrane of *M. tuberculosis* and binds to the 30S subunit of the ribosome¹⁰.

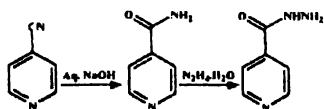


Mutations in the *rpsL* gene of ribosomal S12 protein of mycobacteria or base substitutions in the 16S rRNA region confers resistance to streptomycin¹¹.

Due to the toxic effects on peripheral, central nervous system at higher dosage and hypersensitivity reaction, streptomycin is not a popular choice for treating tuberculosis. Although dihydrostreptomycin, now discarded, was thought to be less toxic; it caused severe damage to eighth cranial nerve, sometimes inducing irreversible impairment of auditory function¹⁰.

Isoniazid (INH) :

Isoniazid has been commercially prepared from 4-pyridinecarboxylic acid or its esters and hydrazine hydrate or from 4-cyanopyridine and hydrazine hydrate. 4-Cyanopyridine was converted to corresponding amide, followed by reaction with hydrazine hydrate to synthesize isoniazid¹² (Scheme 1).



Scheme 1. Synthesis of isoniazid.

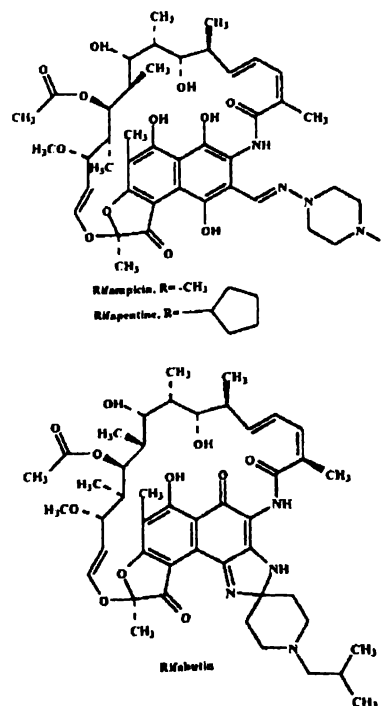
The *M. tuberculosis* *katG* gene encodes a dual-function enzyme called catalase peroxidase, which confers sensitivity in *M. tuberculosis* to INH. Isoniazid is a prodrug that requires activation by the mycobacterial catalase peroxidase enzyme (*katG*) to an active form, which then exerts a lethal effect on intracellular targets. INH, highly active against the MTB complex (*M. tuberculosis*, *M. bovis*,

M. africanum and *M. microti*) has very low MICs (0.02 to 0.06 µg/ml)¹³ against these pathogens. INH enters the organism by diffusion and by oxygen-dependent active transport¹⁴. The drug has been reported to affect virtually every aspect of mycobacterial metabolism. Many components of *M. tuberculosis* have been proposed as possible targets of INH. It inhibits the synthesis of mycolic acids (long chain α -branched β -hydroxylated fatty acids) in *M. tuberculosis* by affecting an enzyme mycolase synthase, which is unique for mycobacteria¹⁵.

Different biological effects that INH exerts on *M. tuberculosis* make its mode of action multifactorial. The resistance mechanism of INH is being elucidated as new facts come to light. The absence of a functional catalase peroxidase enzyme or its altered form leads to resistance. This theory is supported by the observation that after transferring *katG* gene into INH-resistant *M. tuberculosis*, INH-susceptibility is restored¹⁶. A mutation within the mycobacterial *inhA* gene was shown to confer resistance to both INH and ethionamide (ETH) in *M. smegmatis* and in *M. bovis* suggesting that *InhA* is likely target of action for INH and ethionamide¹⁷.

Rifampicin (RIF) :

Rifamycins (e.g. rifampicin, rifabutin and rifapentine), group of antibiotics characterized by a natural ansa structure (chromophoric naphthohydroquinone group spanned by a long aliphatic bridge), are potent inhibitors of prokaryotic DNA-dependent RNA polymerase¹⁸. This

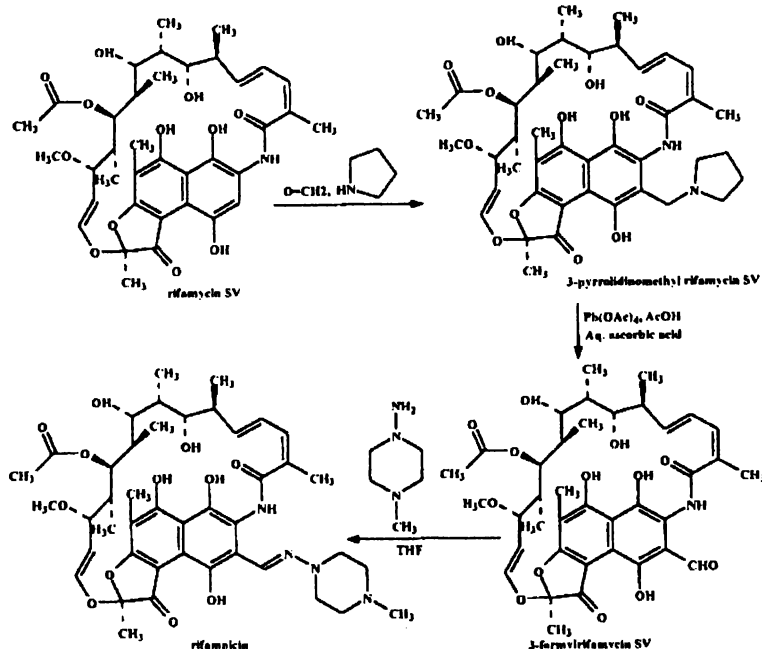


group of antimicrobial agents are compounds composed of aromatic rings linked by an aliphatic bridge. The lipophilic properties of the molecule are important for the binding of the drug to the polymerase and aid in the penetration of the drug across the mycobacterial cell wall.

RIF is extremely effective against MTB; it has MICs of 0.1 to 0.2 μg ¹⁹. RIF had long been believed to target the mycobacterial RNA polymerase and thereby kill the organism by interfering in the transcription process. RNA polymerase is the target for rifampicin, and RIF specifically

hydroxyl groups of D-arabinofuranose residues of arabinogalactan forming the complex. Disruption of the arabinogalactan synthesis inhibits the formation of this complex and may lead to increased permeability of the cell wall. It was demonstrated that EMB specifically inhibits arabinogalactan synthesis²¹.

Ethambutol is commercially produced in good yield and high purity by the reaction of (S)-2-amino-1-butanol and ethylene dichloride. One such synthesis employing butene-1 as a starting material is shown in Scheme 3²².



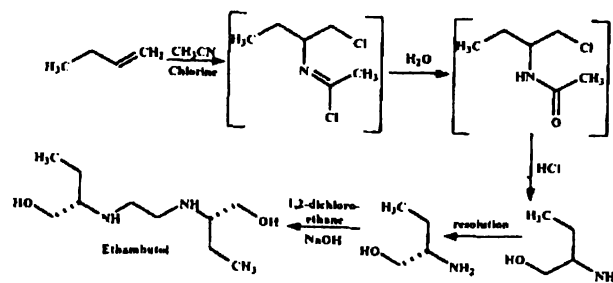
Scheme 2. Synthesis of rifampicin.

inhibits the transition from synthesis of short oligoribonucleotides to full-length transcripts. Isolating RNA polymerase from *M. smegmatis*, it has been demonstrated that RIF specifically inhibited the elongation of full-length transcripts and had virtually no effect on the initiation of transcription¹⁸.

Scheme 2 outlines rifampicin synthesis. Rifampicin is prepared by mild oxidation of a Mannich base of rifamycin SV (obtained by fermentation), followed by mild reduction to obtain 3-formylrifamycin SV, which is then reacted with 1-amino-4-methylpiperazine to form rifampicin²⁰.

Ethambutol (EMB) :

Ethambutol, a synthetic compound with profound antimycobacterial activity, is a first-line anti-MTB drug. The core of the mycobacterial cell wall is the complex mycolylarabinogalactanpeptidoglycan formed by three covalently attached macromolecules, viz. mycolic acid, peptidoglycan and arabinogalactan. Mycolic acids are linked through their carboxy groups to the end-terminal 5'-



Scheme 3. Synthesis of ethambutol.

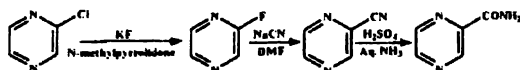
The target of ethambutol lies in the pathway for the biosynthesis of cell wall arabinogalactan. EMB specifically inhibits arabinosyl transfer, suggesting that arabinosyl transferase is the primary cellular target for EMB. In the cell wall biosynthesis arabinosyl transferase III is responsible for the polymerization of arabinose into arabinan of arabinogalactan. The genes *embAB* of *M. avium* encode the drug target for ethambutol, the arabinosyl transferase

responsible for the polymerization of arabinose into the arabinan of arabinogalactan and overproduction of this ethambutol-sensitive target leads to ethambutol resistance²³.

Pyrazinamide (PZA) :

Pyrazinamide (PZA), a structural analog of nicotinamide, is a first-line drug for short-course tuberculosis therapy. It is active against semidormant bacilli not affected by any other drug. It has strong synergy with INH and RIF and shortens the therapy period for tuberculosis treatment to 6 months. It shows no significant bactericidal effect and is primarily considered a 'sterilizing drug'⁵.

A pyrazinamide synthesis is outlined in Scheme 4. 2-Chloropyrazine is reacted with an alkali metal fluoride to form 2-fluoropyrazine, by reaction with alkali metal cyanide it is converted to 2-cyanopyrazine, which on reaction with concentrated H₂SO₄ gives pyrazinamide²⁴.



Scheme 4. Synthesis of pyrazinamide.

The activity of PZA depends on the presence of a bacterial amidase which converts PZA to pyrazinoic acid, the active antimycobacterial molecule and this activity of PZA is highly specific for *M. tuberculosis*. Resistance to PZA is usually accompanied by loss of pyrazinamidase activity in *M. tuberculosis*. The gene *pncA* encoding the *M. tuberculosis* Pyrazinamidase has been sequenced, and mutations in *pncA* were found in small number of PZA-resistant *M. tuberculosis* strains²⁵. Mutations in *pncA* have been identified in PZA-resistant strains, and transformation of these strains with a functional *pncA* gene restored pyrazinamidase activity and PZA susceptibility²⁶.

These first-line antituberculosis drugs INH, RIF, EMB and PZA can be given as single-drug formulations or as fixed-dose combination (FDC) formulations where two or more drugs are present in fixed proportions in the same formulation.

The need for new anti-TB drugs

The recommended treatment regimen stated above is highly effective and rates of severe adverse reactions are low. However, unpleasant side-effects and relatively long course of treatment are the drawbacks, which increase the rate of non-compliance to treatment regimen. Such non-adherence with the course of treatment leads to treatment failure and the development of drug resistance. The second-line drugs used for MDRTB are more expensive, less effective and more toxic than the four-drug standard regimen.

MDR appeared to result from the stepwise acquisition of new mutations in the genes for different drug targets. Thus, the origin of MDRTB is due more to treatment difficulties, including non-compliance and administration of inadequate treatment regime, which provides the selection mechanism needed for the development of resistance in those mycobacteria not destroyed by the drugs. Awareness of widespread tuberculosis epidemic and the emergence of MDRTB have stressed the urgent need for new, effective antimycobacterial drugs. To achieve this goal, development of new antimycobacterial drugs having significant structural changes, which may exert their effect via different mode/mechanism of action, is the order of the day. Favorable pharmacokinetic properties, low incidence of side-effects and cost effectiveness are other characteristics that would make a new drug suitable for extensive use.

The goal is to develop bactericidal drugs in a cost-effective manner, which efficaciously treats infections of MDR strains of *M. tuberculosis* and latent infection, having shortened treatment period or reduced frequency of doses.

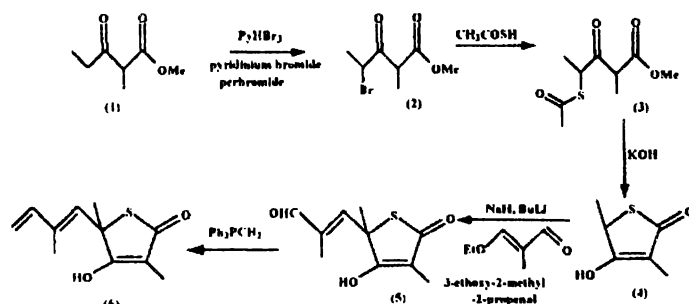
Emerging trends in TB therapy

Thiolactomycin :

Thiolactomycin (TLM) is a unique thiolactone that has been shown to exhibit antimycobacterial activity by specifically inhibiting fatty acid and mycolic acid biosynthesis. It is of relevance that TLM inhibits bacterial and plant type II fatty acid synthase (FAS-II) but not mammalian or yeast type I fatty acid synthase (FAS-I)²⁷. TLM targets two beta-ketoacyl-acyl-carrier protein synthases, KasA and KasB, consistent with the fact that both enzymes belong to the fatty-acid synthase type II system involved in fatty acid and mycolic acid biosynthesis. A multidrug-resistant clinical isolate was also found to be highly sensitive to TLM, indicative of a promise in counteracting multidrug-resistant strains of *M. tuberculosis*. The design and synthesis of several TLM derivatives have led to compounds more potent both *in vitro* against fatty acid and mycolic acid biosynthesis and *in vivo* against *M. tuberculosis*²⁸.

TLM[(4*R*)(2*E*,5*E*)-2,4,6-trimethyl-3-hydroxy-2,5,7-octatriene-4-thiolide] belongs to a small group of antibacterial compounds which have been collectively termed the thiotetronic acids²⁹. Total synthesis of racemic TLM (6) is a five-step procedure²⁹ (Scheme 5) starting from methyl α -propionylpropionate (1).

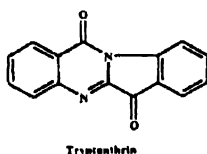
The synthesis of TLM and analogs is feasible, its mechanism of action is known and *in vivo* activity has been demonstrated. The *in vivo* inhibitory concentration (25



Scheme 5. Synthesis of thiolactomycin.

$\mu\text{g/ml}$) and *in vitro* IC_{50} ($5 \mu\text{g/ml}$) of TLM against *M. tuberculosis* are quite high²⁹ 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.

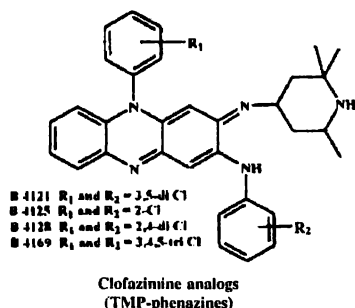
Tryptanthrin :



Tryptanthrin is a potent and structurally novel indoloquinazolinone alkaloid, which was tested against MDR strains of *M. tuberculosis*. The MIC of tryptanthrin was $0.5\text{--}1.0 \mu\text{g/ml}$. Many analogs of this lead structure have been synthesized and evaluated³⁰. Because of its novel structure, risk of cross-resistance with existing drug may be avoided but *in vivo* toxicology and *in vitro* cytotoxicity data need to be generated.

Clofazimine analogs :

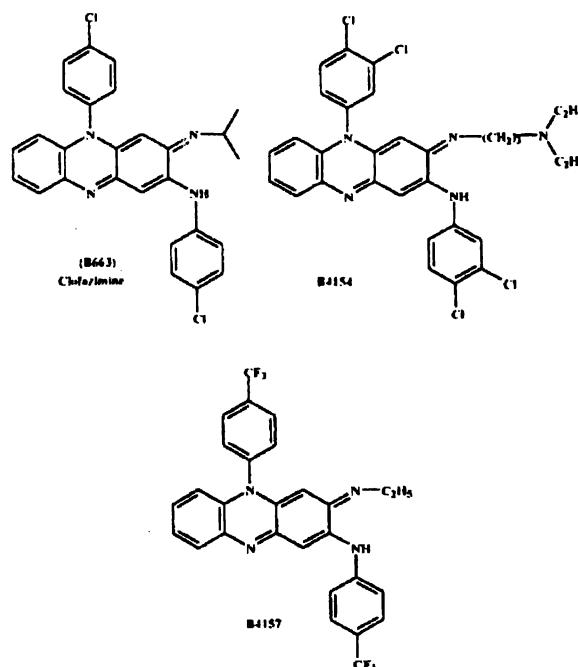
Tetramethylpiperidine-substituted phenazines (TMP-phenazines)



The intra- and extracellular activities of 5 novel tetramethylpiperidine (TMP)-substituted phenazines against *M. tuberculosis* H37Rv (ATCC 27294) were determined and compared with those of clofazimine and rifampicin³¹. Two of these agents, together with clofazimine, were also tested for their activities against drug-resistant strains of *M. tuberculosis*. Three of the TMP-substituted phenazine com-

pounds were significantly more active than clofazimine against *M. tuberculosis*, including multidrug-resistant clinical strains, demonstrating a lack of cross-resistance between the riminophenazines and standard antituberculous drugs³¹. The most important virtues of riminophenazines, such as intracellular accumulation in mononuclear phagocytic cells, anti-inflammatory activity, a low incidence of drug resistance and slow metabolic elimination, make them attractive candidates for the treatment of mycobacterial infections³².

Although the mode of action is not clearly known, the MIC values of the TMP-phenazines are low and they are active against MDRTB strains, extra- and intracellularly.

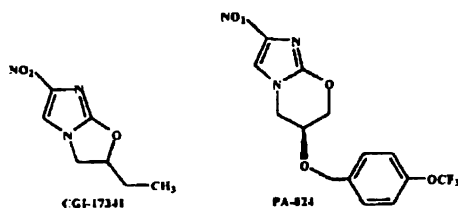


Antituberculosis activities of clofazimine and its analogs B4154 and B4157 have been investigated. *In vitro* these compounds have been tested against 20 *M. tuberculosis* strains including 16 drug-resistant strains (strains resistant to one or more antituberculosis drugs) for their

susceptibilities to these compounds³³. All of the strains were found to be susceptible to B4154 and B4157, one strain showing moderate resistance to clofazimine. The MICs of B4154, B4157 and clofazimine at which 90% of population growth was inhibited were 0.25, 0.12 and ≤ 1.0 $\mu\text{g/ml}$, respectively. The chemotherapeutic activity was evaluated *in vivo* in mice at a dose of 20 mg/kg. Because of its poor *in vivo* activity B4154 was not investigated further. The other two compounds were found to be effective and prevented mortality. The animals treated with B4157 showed less pigmentation than those receiving clofazimine³³.

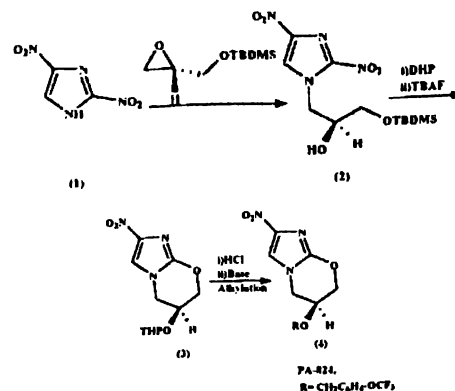
Nitroimidazoles :

A series of bicyclic nitroimidazofurans possessing antimycobacterial activity have been investigated earlier. However, the lead compound in this series, CGI-17341 was mutagenic discouraging further investigation. These studies suggested that the bicyclic nitroimidazoles might be potential antitubercular agents. From a series of 3-substituted nitroimidazopyrans (NAPs) studied, the lead compound established is PA-824³⁴. The NAPs are a narrow spectrum, synthetic small molecule antibiotics possessing a novel bactericidal mechanism of action. In contrast to conventional antitubercular drugs, NAPs exhibited bactericidal activity against both replicating and stationary *M. tuberculosis* cells³⁴.



The MIC values of PA-824 against a panel of *M. tuberculosis* pan-sensitive and rifampin mono-resistant clinical isolates ranged from 0.015 to 0.25 $\mu\text{g/ml}$. Poly- and multi-drug-resistant strains of *M. tuberculosis* exhibited comparable susceptibility to PA-824, indicating that there is no cross-resistance with current drugs. After activation by a mechanism dependant on *M. tuberculosis* F420 cofactor, NAPs inhibited the synthesis of protein and cell wall lipid³⁴.

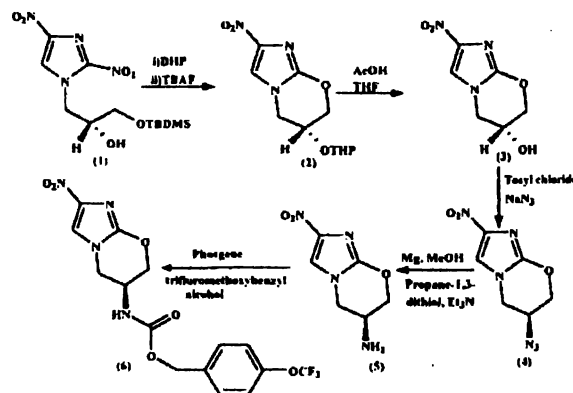
A synthetic scheme for ether analogs like PA-824 is outlined in Scheme 6. Reaction of 2,4-dinitroimidazole (1) with (*R*)-glycidol *tert*-butyl dimethylsilyl (TBDMS) ether gave hydroxyimidazole (2). Compound 2 was transformed into bicyclic nitroimidazole THP ether (3), followed by deprotection and alkylation to produce ether analogs (4)³⁵.



Scheme 6. Synthesis of PA-824.

Studies on macromolecular effects of PA-824 on *M. tuberculosis* revealed that both protein and lipid synthesis were substantially inhibited at drug concentrations and time intervals when nucleic acid synthesis was unaffected. Analysis of the effects on cell wall lipids indicated that PA-824 produced an accumulation of hydroxymycolic acid with a concomitant reduction in ketomycolate. As hydroxymycolate is a known biosynthetic precursor of cell wall ketomycolate, PA-824 may inhibit an enzyme or deplete a cofactor responsible for the oxidation of hydroxymycolate to ketomycolate³⁴.

Another NAP of interest is PA 1343, which is orally active, whereas PA 824 is not, due to low water solubility resulting in poor oral bioavailability. In preclinical studies, PA 1343 is more active than PA 824 against *M. Tuberculosis in vitro* with a MIC of 0.015 $\mu\text{g/ml}$ ³⁴. Scheme 7³⁶ details the synthesis of PA-1343.

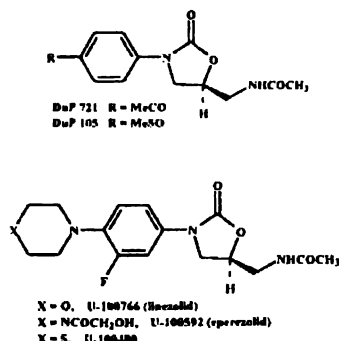


Scheme. 7 Synthesis PA-1343.

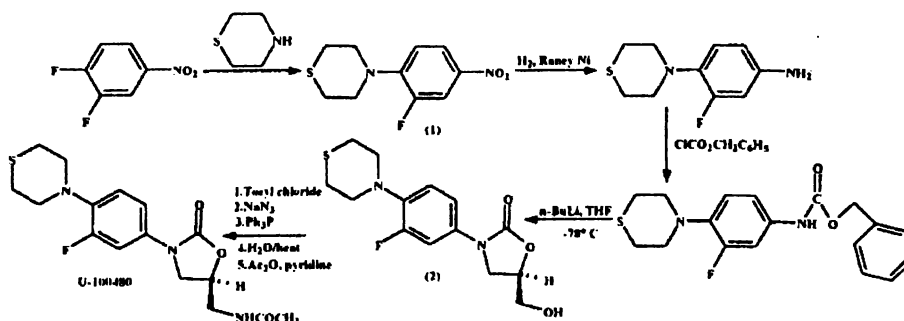
Oxazolidinones :

The oxazolidinones represent a unique family of antimicrobial agents. In mid-1980s, DuPont scientists developed a series of oxazolidinone derivatives, two of which (DuP 721 and DuP 105) were active orally and parenterally and showed an effective *in vitro* spectrum of activity

against various gram-positive bacteria, including staphylococci, streptococci, and enterococci, as well as anaerobes and *M. tuberculosis*. They were not developed for clinical use because DuP 721 was shown to have lethal toxicity in the rat³⁷.



Only the oxazolidinone enantiomer with a (5*S*)-acetamidomethyl configuration possesses antibacterial activity³⁸. Further chemical modification of the basic oxazolidinone nucleus by scientists at the Upjohn Company resulted in the discovery of two (*S*)-5-acetamidomethyl-2-derivatives (U-100592 and U-100766; eperezolid and linezolid, respectively) that had *in vitro* activity similar to that of DuP721 but without acute animal toxicity³⁷. Structure-activity relationship studies on polysubstituted *N*-phenyloxazolidinone showed that activity declines rapidly with increased steric bulk in 3-substituent. In general, there is a requirement for coplanarity of the arene and oxazolidinone rings and with limited space available on one side of the aromatic ring away from the main axis of the phenyloxazolidinone pharmacophore³⁹. Other chemical substitutions (thiomorpholine side-chain) have given this class of drug even greater activity against mycobacteria, viz. compound U-100480⁴⁰.



Scheme 8. Synthesis U-100480.

Compound U-100480 (Scheme 8) was synthesized from thiomorpholine and 3,4-difluoronitrobenzene adduct (1), which was converted to the (*R*)-5-(hydroxymethyl)-oxazolidinone (2) in 82% overall yield, which was further modified to obtain U-100480⁴⁰.

Oxazolidinone U-100480 and its metabolites (the cor-

responding sulfoxide and sulfone) exhibited potent *in vitro* activity against *M. tuberculosis*. When *in vitro* activity was tested against *M. tuberculosis* H37Rv, MICs of U-100480 and its corresponding sulfoxide metabolite (prepared by sodium metaperiodate oxidation of U-100480) was demonstrated to be $\leq 0.125 \mu\text{g/ml}$ ⁴⁰.

The oxazolidinones inhibit bacterial protein synthesis⁴¹ at a very early step in the initiation complex formation involved in the process of translating mRNA into protein, and are not cross-resistant with any known antibiotic because of this unique mechanism. In general, the oxazolidinones are not active against Gram-negative organisms. DuP 721 demonstrated potent activity against Gram-positive pathogens⁴², Gram-negative anaerobes and *M. tuberculosis*⁴³.

AZD 2563 is an oxazolidinone antibiotic in preclinical development in Europe for the treatment of bacterial infections. No report of its anti-TB property lies in public domain.

DNA gyrase inhibitors :

DNA gyrase is a topoisomerase which catalyzes the topological changes in DNA during important cellular processes such as replication and transcription. All topoisomerases have the ability to relax the supercoiled DNA but only DNA gyrase can introduce negative supercoils into DNA following the replication process⁴⁴. Fluoroquinolones (FQ) have primarily been used as therapeutic alternatives in MDRTB. DNA gyrase, a member of the type II DNA topoisomerases, is the primary target for FQ action.

In the past, the primary mode of action of quinolones

was believed to be to interfere with the activity of DNA gyrase, an essential type II topoisomerase that exists only in bacteria⁴⁴. The A and B subunits of DNA gyrase are encoded by the *gyrA* and *gyrB* genes. The DNA gyrase functions within the viable bacterial cell include introduction of negative supercoils and separation of interlocked replicated

daughter chromosomes. It is essential for DNA recombination and repair. The main effects of fluoroquinolones are the inhibition of DNA supercoiling and damage to DNA, whose synthesis is rapidly interrupted.

DNA gyrase catalyzes the cutting of DNA and strand separation. The quinolone drugs bind with a greater affinity to single-stranded DNA than double-stranded DNA. Consequently, by binding to the single-stranded DNA, the quinolones may inhibit religation, thereby imposing an effective transcriptional block resulting in cellular death⁴⁵.

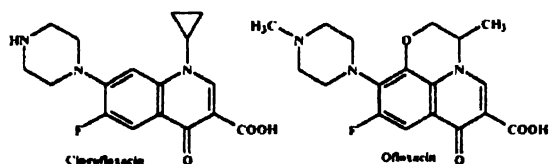
The action of quinolones on gyrase was determined from enzymatic and binding assays employing the purified *E. coli* enzyme as well as from the examination of the sites in the genome that had undergone mutation when strains of *E. coli* became resistant to fluoroquinolones. The discovery of topoisomerase IV, an essential enzyme that unlinks daughter DNA catenates that are produced through chromosome replication, led to the finding that, in both Gram-positive and Gram-negative bacteria, topoisomerase IV enzyme is also a target for the action of quinolones⁴⁶.

The fluoroquinolones such as ciprofloxacin are totally synthetic antibacterial agents. Derivatives recognized with activity against mycobacteria are ofloxacin, ciprofloxacin, sparfloxacin, levofloxacin, lomefloxacin etc. MICs of levo-

floxacin to be one of the most active quinolones against bacteria with resistance to penicillins and macrolides, including *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *M. tuberculosis*. Additionally, moxifloxacin exhibits activity against methicillin-resistant *Staphylococcus aureus*⁴⁸.

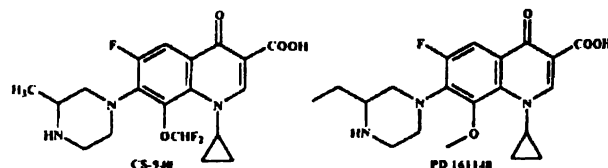
The compound of formula 4 is reacted with octahydropyrrolo[3,4-*b*]pyridine (3) to synthesize the compounds of formula 5 (moxifloxacin $R_1 = H$, $R_2 = F$, $R_3 = OCH_3$) in the presence of bases such as alkali metal hydroxides, alkali metal carbonates or organic amines etc. Compound 3 is prepared by hydrogenation of pyridine-2,3-dicarboxylic acid-*N*-benzylimide (1) as represented in Scheme 9⁴⁹.

A recent study in mice has indicated that moxifloxacin is highly effective in reducing *M. tuberculosis* infection and has high activity comparable to that of isoniazid. Combination therapy with moxifloxacin plus isoniazid was found to be superior to that with either of the drugs given alone, in reducing bacterial counts, suggesting that moxifloxacin may be useful in multiple-drug regimens for treatment of tuberculosis in humans⁴⁷. Moxifloxacin was tested for activity in mice against *M. tuberculosis* CSU93, a highly virulent, recently isolated clinical strain. The MIC was found to be 0.25 $\mu\text{g/ml}$ ⁴⁷. While the drug has been approved in the US (Avelox, Bayer) for treatment of certain common respiratory tract infections, it could have a useful role in treating tuberculosis as well.

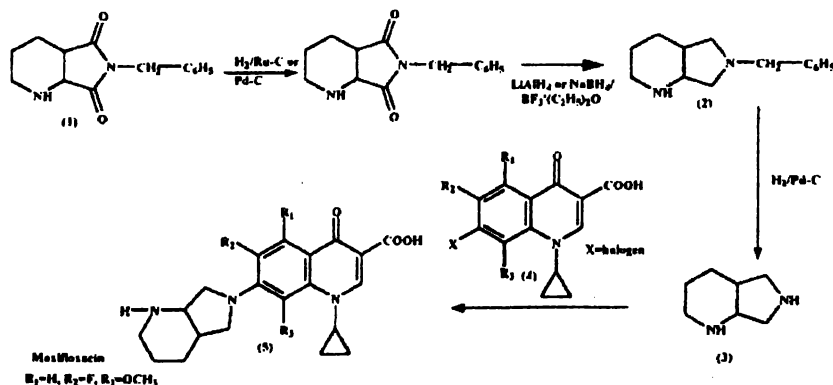


floxacin, ofloxacin and ciprofloxacin of about 1 $\mu\text{g/ml}$ for *M. tuberculosis* have been reported⁴⁷.

Moxifloxacin (BAY 12-8039) is an 8-methoxyquinolone with *in vitro* activity against aerobic and anaerobic Gram-positive and Gram-negative organisms, as well as atypical organisms. *In-vitro* testing has confirmed moxi-



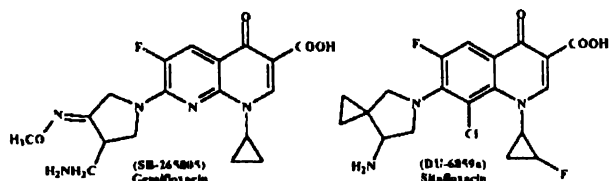
The MICs to the fluoroquinolones ofloxacin, ciprofloxacin, sparfloxacin, norfloxacin, balofloxacin and CS-940, were determined in 100 clinical isolates of *M. tuber-*



Scheme 9. Synthesis of moxifloxacin.

*culosis*⁵⁰. Among the six fluoroquinolones, CS-940 and sparfloxacin showed the greatest antimycobacterial activities with inhibition of 50% of all the isolates at the concentrations 0.25–0.5 µg/ml. Ofloxacin, ciprofloxacin and balofloxacin followed in potency at 0.5–2.0 µg/ml. Norfloxacin was less potent requiring 8–16 µg/ml to inhibit 50% of the isolates. *In vitro* antimicrobial activity of CS-940 was tested against 761 clinical isolates⁵¹.

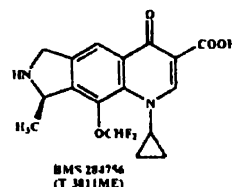
PD 161148 is a third generation fluoroquinolone selected from analysis of structure activity relationship (SAR), having potent activity against *M. tuberculosis*. It was observed that a C-8 methoxyl group enhances the bactericidal activity of quinolones with N1-cyclopropyl substitution⁵². However, there is no information on the frequent side-effects of fluoroquinolones such as complexation with metallic ions, phototoxicity and P450 inhibition nor on its rarer but life-threatening side-effects such as renal, hepatic and cardiac toxicity.



Sitafloxacin (DU-6859a) is a potent, well-tolerated antibacterial agent⁵³. It was found to be more potent *in vitro* than sparfloxacin and ofloxacin⁵⁴. However, it does not give great advantage over existing regimens with other fluoroquinolones due to its short half-life. Gemifloxacin

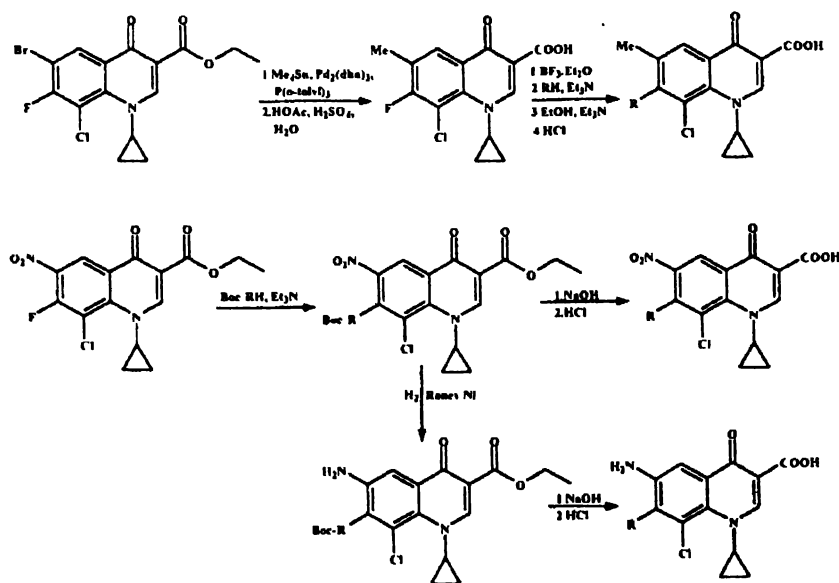
(SB-265805) is a safe broad-spectrum fluoroquinolone antibiotic⁵⁵. However, its MIC90 value against *M. tuberculosis* is rather high⁵⁶.

Desfluoroquinolones :



Desfluoroquinolones (des-F(6)-quinolone) lack the 6-position fluorine substituent of the existing fluoroquinolones. BMS 284756 (T-3811ME), an 8-alkoxy-1-cyclopropyl-des-F(6)-quinolone showed activity against *M. tuberculosis* (MIC90, 0.0625 µg/ml) that was potent and superior to that of trovafloxacin⁵⁷. The compound is an excellent antibiotic, which is potent towards Gram-negative and -positive cocci, quinolone resistant bacteria and against anaerobic bacteria.

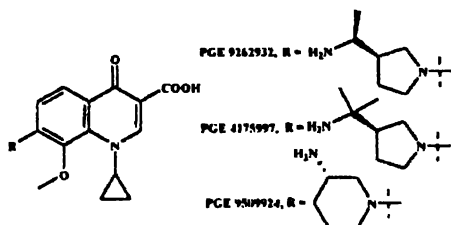
A study on the influence of the C6 position in non-fluorinated quinolones⁵⁸ showed that increased steric bulk at C6 had a negative impact on the activity of the quinolone derivatives in combination with electronic factors. A series of new non-fluorinated quinolones were synthesized (Scheme 10) by maintaining a generally consistent substitution pattern at N1 (cyclopropyl), C8 (chloro or methoxy) and C7 (aminopyrrolidine or aminoethylpyrrolidine) while varying the C6 moiety. Although it was possible to achieve activity comparable to marketed quinolones against some strains as evident from biological testing, groups larger than



Scheme 10. Synthesis of non-fluorinated quinolones

chlorine were disfavoured as compared to their direct 6-H or 6-fluoro analogs. The compounds were found to be active against Gram-positive bacteria including quinolone resistant *S. aureus*.

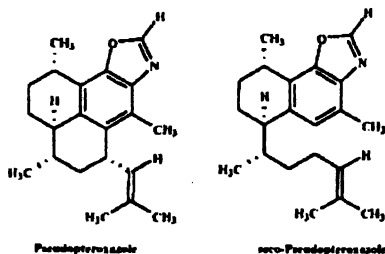
In vitro activity of three non-fluorinated quinolones PGE 9262932, PGE 4175997 and PGE 9509924 was tested against *M. pneumoniae*, *C. pneumoniae* and *Legionella pneumophila*⁵⁹. The result indicated potent activity of these three compounds against the isolates of atypical respiratory tract pathogens, with their comparative potency ranked as



PGE 9262932 > PGE 4175997 > PGE 9509924.

Diterpenoids :

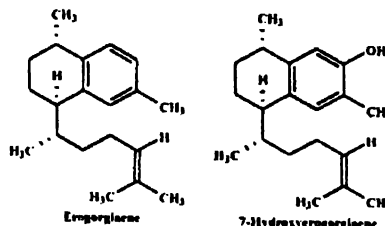
Diterpenes continue to be the target of investigations world over for potential biomedical uses as they are one of the valuable natural sources of unique metabolites because of the interesting biological activities, like analgesic, anti-inflammatory, antibacterial etc. associated with the compounds isolated from them.



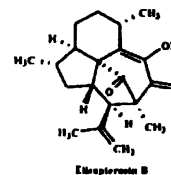
A search for new antituberculosis agents from the West Indian sea whip *Pseudopterogorgia elisabethae* led to the discovery of two benzoxazole alkaloids, pseudopterogoxazole and *seco*-pseudopterogoxazole⁶⁰. Pseudopterogoxazole was

found to effect potent inhibitory activity (97%) against *M. tuberculosis* H37Rv at a concentration of 12.5 µg/ml and *seco*-pseudopterogoxazole inhibited 66% of mycobacterial growth. The potent activity was attributed, at least in part, to the benzoxazole functionality.

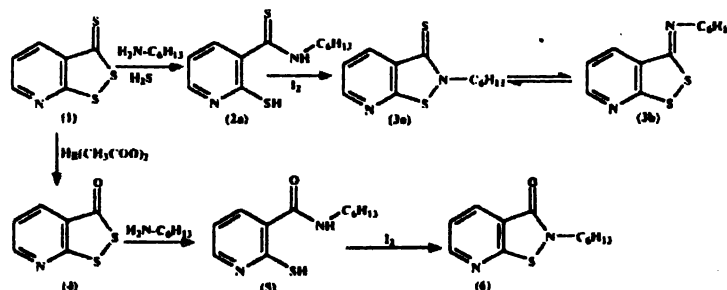
Two novel serrultane diterpenes having antimycobacterial activity have also been isolated from the West Indian sea whip coral, *Pseudopterogorgia elisabethae*, namely, erogorgiaene and 7-hydroxyerogorgiaene⁶¹. Erogorgiaene induced 96% growth inhibition for *M. tuberculosis* H37Rv at a concentration of 12.5 µg/ml and 7-hydroxyerogorgiaene inhibited 77% of mycobacterial growth at a concentration of 6.25 µg/ml, indicating that C-7 hydroxylation



does not reduce the activity. Since *seco*-pseudopterogoxazole, erogorgiaene, pseudopterogoxazole induced 66, 96, and 97% inhibition of *M. tuberculosis* growth *in vitro* at 12.5 µg/ml, respectively, the benzoxazole moiety is not essential for activity.



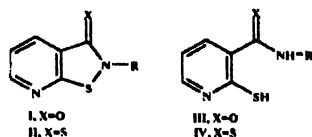
Novel diterpene, elisapterosin B, possessing cage-like elisapterane carbon skeleton, isolated from the hexane solubles of West Indian sea whip *Pseudopterogorgia elisabethae* Bayer displays strong *in vitro* antituberculosis activity. The tetracyclic carbon skeleton of the elisapterosins constitutes a new class of C₂₀ rearranged diterpenes. *In vitro* studies on elisapterosin B showed strong inhibitory activity (79%) against *M. tuberculosis* H37Rv at a concentration of 12.5 µg/ml⁶².



Scheme 11. Synthesis of *N*-alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamides.

Diterpenoids having antimycobacterial properties have been isolated from roots of *Salvia multicaulis*⁶³ and from petroleum ether extract of *Azorella madrepurica* Clos.⁶⁴.

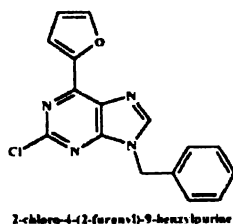
N-Alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamides :



N-Alkylisothiazole[5,4-*b*]pyridin-3(2*H*)-ones (I) and thiones (II) and their intermediates of synthesis (III, IV) have been synthesized and evaluated for their antimycobacterial properties⁶⁵. For the most active structure IV, several *N*-alkyl derivatives were prepared. Synthesis of *N*-hexyl derivative is represented in Scheme 11.

Reaction of 3*H*-1,2-dithiolo[3,4-*b*]pyridine-3-thione (1) and 3*H*-1,2-dithiolo[3,4-*b*]pyridin-3-one (4) with *n*-hexylamine, followed by cyclization resulted in desired compounds (Scheme 11). Compound 2a, *N*-hexyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide with MIC 1–2 µg/ml displayed best activity *in vitro*. The preliminary microbiological data indicated that *N*-alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamides IV, are the most active compounds⁶⁵.

9-Benzylpurines :



9-Benzylpurines with a variety of substituents in the 2-, 6- and/or 8-position have been prepared and screened for antimycobacterial effects. High inhibitory activity against *M. tuberculosis* was found for 9-benzylpurines carrying a phenylethynyl, *trans*-styryl or aryl substituents in the 6-position and generally chlorine in the 2-position tends to increase activity. The above compound was shown to potently inhibit *M. tuberculosis* H37Rv with a MIC value of 0.78 µg/ml⁶⁶.

Imidazo[4,5-*c*]pyridines :

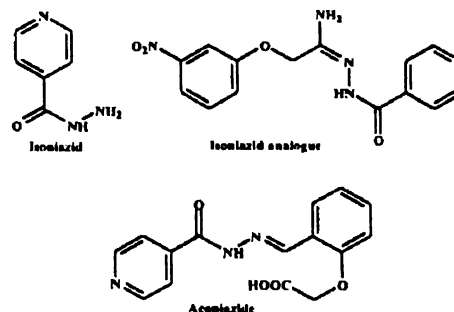


These compounds were originally prepared as antimitotic agents for cancer chemotherapy but some less cytotoxic agents were found to have anti-TB activity⁶⁷. From a series of imidazo[4,5-*c*]pyridines, the compound shown above inhibited *M. tuberculosis* H37Rv by 99% at 6.25 µg/ml.

Isocitrate lyase inhibitors :

A recent report indicates that the persistence of *M. tuberculosis* in mice is facilitated by isocitrate lyase (ICL), an enzyme essential for the metabolism of fatty acids⁶⁸. ICL plays a pivotal role in the persistence of *M. tuberculosis* in mice by sustaining intracellular infection in inflammatory macrophages. The prototype inhibitors-3-bromopyruvate and 3-nitropropionate have been identified from the three-dimensional structure of *M. tuberculosis* ICL. These compounds were shown to bind with ICL⁶⁹.

Isoniazid derivatives/analogs :



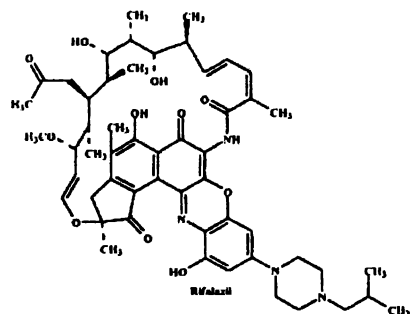
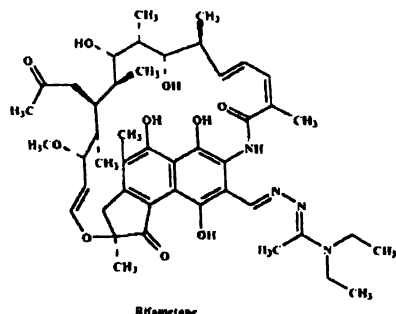
Various analogs/derivatives of isoniazid have been synthesized as possible antimycobacterial agents⁷⁰. Because of structural similarities to INH cross-resistance may develop rapidly.

Aconiazide is a prodrug of isoniazid, and it is hydrolyzed in the body to isoniazid. A pharmacokinetic evaluation of aconiazide on healthy volunteers was carried out. They received aconiazide tablets (650 mg containing 300 mg isoniazid) and isoniazid tablets (300 mg). The relative bioavailability of aconiazide was found to be 50.7% compared with isoniazid⁷¹. Although the toxicity of aconiazide is less than that of isoniazid, it is a prodrug of isoniazid and its anti-TB activity will depend upon its conversion to isoniazid. Consequently there will be cross-resistance with isoniazid.

New rifamycins :

Rifametane : Rifametane is a new semisynthetic 3-azinomethyl rifamycin derivative with a bactericidal spectrum and potency similar to that of rifampicin, but with much better pharmacokinetic properties⁷². An open randomized cross-over study was conducted in 8 healthy male

volunteers to study the pharmacokinetic pattern and the safety of a 300 mg single oral dose of rifameter compared with 300 mg of conventional rifampicin. Although it is apparent that rifameter will develop cross-resistance with rifampicin, the pharmacokinetic profiles of rifameter were significantly more favourable than rifampicin and it also showed a good safety profile after 300 mg single oral dose⁷³.



Rifalazil (KRM 1648) : Rifalazil, 3'-hydroxy-5'-(4-isobutyl-1-piperazinyl)benzoxazinorifamycin, is more potent than rifampicin in animal model. Its efficacy was tested *in vivo* in animals infected with *M. avium*. Rifalazil

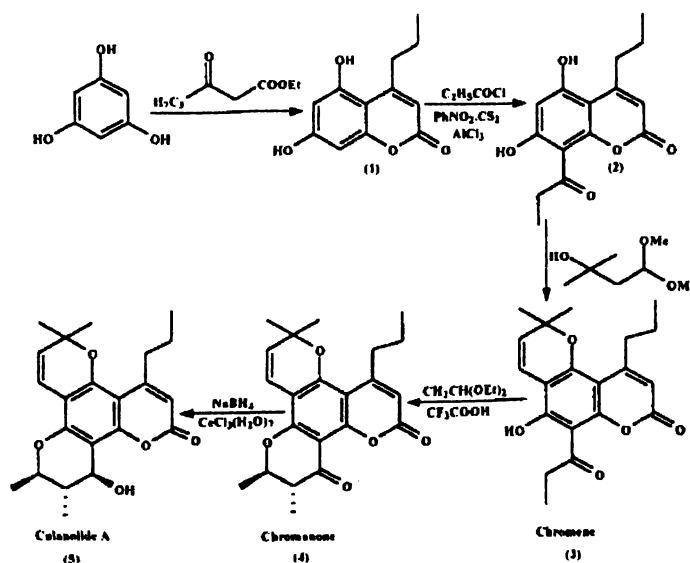
treated rabbits survived longer and had no granulomatous lesions in lungs, liver, spleen and kidneys and restored histopathologic features of healthy tissue in the visceral organs⁷⁴. Its *in vitro* and *in vivo* activities against *M. tuberculosis* H37Rv were compared with those of rifampicin. MIC of KRM 1648 was reported to be 0.035 µg/ml and that of rifampicin 2.5 µg/ml⁷⁵. But due to severe side-effects identified in the 4-day phase II trial, development of rifalazil was stopped.

Calanolide A :

The natural product, calanolide A, a novel dipyrano coumarin has dual activity against TB and HIV infections. (+)-Calanolide A and (-)-calanolide A demonstrated inhibitory antimycobacterial activity against *M. tuberculosis* H37Rv with 96 and 98% growth inhibition, respectively⁷⁶. The actual MIC for (+)-calanolide A was found to be 3.13 µg/ml.

A synthetic process (Scheme 12) has been developed for large-scale preparation of calanolide A starting from phloroglucinol and ethyl butyrylacetate and forming chromene (3) and chromanone (4) as key intermediates. Calanolide A can be resolved into optically active forms using a preparative HPLC chiral separating system⁷⁷.

In addition, listed below are some other classes of compounds, which have been evaluated for their antitubercular properties, such as 4-(3-coumarinyl)-4-thiazolin-2-one benzylidenehydrazones⁷⁸, ethanolic extract of *Galipea of ficinalis* bark⁷⁹, 3-hydrazono-1H-2-indolinones⁸⁰, quinoxaline-1,4-di-*N*-oxides⁸¹, cyclohexadienes⁸², *N*-(2-naphthyl)glycin hydrazide analogs⁸³, isoxazoles⁸⁴, cyano-



Scheme 12. Synthesis of calanolide A.

pyridines⁸⁴, 4-phenyl-1,8-naphthyridine⁸⁵, (*E*)-phytol and derivatives⁸⁶, pyridinecarboxamidrazone derivatives⁸⁷, quinolizidine derivatives⁸⁸, aminolupinanes⁸⁹, benzoyl-thiazole-2-carbamates⁹⁰, thiosemicarbazones⁹¹, dithiocarbamates⁹², thiazolidinones⁹³, succinamides⁹⁴, diarylsuccinamide⁹⁵, glutaconylthiosemicarbazides⁹⁶, 2,2'-dithiobis-(benzamide) derivatives⁹⁷, 1,3,5-triazines⁹⁸ and trimethylsilyl 3-(carbethoxycarbamoyl)propiolates⁹⁹. Some of these compounds show moderate activity, some are evaluated only *in vitro* and lack of toxicology data etc. makes it difficult to assess their potential as antitubercular agents.

Conclusion

It is to be pointed out that this article assembles information on compounds with reported antimycobacterial activity, some showing good *in vitro* activity, some others with good *in vivo* activity but not yet developed into drugs.

In comparison to research in other areas of anti-infectives where practically every year, a host of new molecules are coming out to fight diseases caused by microbes, unfortunately, for tuberculosis, essentially a disease of developing countries, the scene is dismal. The first line drugs used in current therapy are as old as about 50 years, supposedly, because of not-so-lucrative market for TB drugs, approximately, about US \$600 million per annum, coupled with the complexity and slow pace of progress in research in anti-TB area. Because of spread of HIV infection, the menace of TB has reached alarming proportions worldwide. Many international donor agencies are taking initiative in funding and encouraging research and development in TB therapy.

Since the problem is already acute in India, it is imperative that Indian academic research institutions and industrial organizations collaborate without any further delay in carrying out joint research projects for developing novel and cost-effective chemical entities for fighting tuberculosis. This should be one of our national research perspectives. Fortunately, steps in this direction have been initiated under the leadership of C.S.I.R., New Delhi.

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