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BIOAVAILABILITY ENHANCEMENT TECHNIQUES OF ANTI-TUBERCULOSIS DRUGS - A REVIEW

Richa Srivastava, *Neha Mathur, Nikhat Fatima.

Amity Institute of Pharmacy, Amity University, Uttar Pradesh, Lucknow, India

***Correspondence Author** E-mail ID: nmathur1@amity.edu

ABSTRACT

Tuberculosis (TB) is one of the widespread, fatal diseases caused by Mycobacterium tuberculosis complex affecting human population. However, infection by the organism does not necessarily lead to disease and only 5-10% of these individuals will progress to active disease each year (WHO 2007). 10% people infected with TB bacteria have a lifetime risk of falling ill with TB. The World Health Organization (WHO) estimates that globally there were 9.4 million cases of active TB leading to 1.3 million deaths. However, lives can also be saved with effective diagnosis and treatment. This review focuses firstly on the occurrence and prevalence of this disease, secondly, on ways of its diagnosis and treatment, thirdly on the new tuberculosis drugs under development and lastly on the various bioavailability enhancement approaches which are under process so that the problem of poor/variable bioavailability of drugs, in particular, in fixed dose combinations (FDC's) or due to their enhanced decomposition in stomach acidic conditions etc can be minimized.

KEYWORDS: Bioavailability enhancement, Anti tuberculosis drugs, Bioenhancement techniques, Anti TB bioavailability enhancement.

INTRODUCTION

Tuberculosis, is a widespread, and in many cases fatal, infectious disease caused by a group of five closely related species, which forms the *Mycobacterium tuberculosis* complex: *M. tuberculosis, M. bovis, M. africanum, M. microti,* and *M. canettii.* The main characteristic of the genus *Mycobacterium* is the property called acid-fastness, which causes it to withstand decolorization with an acid-alcohol mixture after staining with carbol fuchsin or auramine-rhodamine. Mycobacteria are primarily intracellular pathogens, have slow growth rates, are obligate aerobes, and produce a granulomatous reaction in normal hosts. In cultures, *M. tuberculosis* does not produce significant amounts of pigment, has a buff-colored, smooth surface appearance, and biochemically produces niacin. These characteristics make them useful in differentiating *M. tuberculosis* from nontuberculous mycobacteria. It is estimated that one third of the world's population (approximately 2 billion people) are infected with the tubercle bacilli. However persons with compromised immune systems, such as people living with HIV, malnutrition or diabetes, or people who use tobacco, have a much higher risk of falling ill. The remaining 90% of infected individuals will initially be asymptomatic and experience latent infection, later from which reactivation may occur. In 2008, The World Health Organization (WHO) estimate that globally there were 9.4 million cases of active TB leading to 1.3 million deaths. [1] When a person develops active TB (disease), the symptoms (cough, fever, night sweats, weight loss etc.) may be mild for many months. This can lead to delays in seeking care, and results in transmission of the bacteria to others. People ill with TB can infect up to 10-15 other people in close contact over the course of one year. Without proper treatment up to two thirds of people ill with TB can even die.

Since 2000 more than 37 million lives have been saved through effective diagnosis and treatment. Active, drug-sensitive TB disease is treated with a standard 6-month course of four antimicrobial drugs

that are provided with information, supervision and support to the patient by a health worker or trained volunteer. The vast majority of TB cases can be cured when medicines are provided and taken properly.

The World Health Organization (WHO) has developed the directly observed therapy short course (DOTS) strategy to optimize response and adherence to TB treatment. DOTS is however, labor-intensive and expensive. It causes a high burden on public health programs, especially in developing countries with limited human resources.[2] Also, TB diagnosis in the DOTS strategy is based on sputum microscopy, rather than sputum culture.[3] Detection by sputum microscopy requires qualified microscopists and also it can detect only advanced pulmonary TB.[4] Consequently, TB detection rates are suboptimal and resistant *M. tuberculosis* strains are not detected by DOTS strategy. [4, 5] Clearly, there is an urgent need to improve treatment by either enhancing the application of existing agents or introducing new drugs.

OCCURRENCE AND PREVALENCE OF THE DISEASE

An increasing morbidity and mortality from tuberculosis (TB) in the near future is forecast for the world at large, with the number of newly occurring cases predicted to increase from 7.5 million a year in 1990 to 8.8, 10.2 and 11.9 million in the years 1995, 2002 and 2005 respectively; an increase amounting to 58.6 per cent over a 15-yr period.[6] These estimates were subsequently found to be appropriate for the year 2000 using a new method.[7] The proportion of tuberculosis cases co-infected with human immuno deficiency virus (HIV) was also found to be rising, being 2-10 times greater for the 1997 estimates, than for1990. The association with HIV and increasing multi drug resistant tuberculosis (MDRTB) appears to be a serious issue, especially for the developing nations. In 2007 there were an estimated 13.7 million chronic active cases,[8] and in 2010 there were 8.8 million new cases, and 1.45 million deaths, mostly in developing countries.[9] China has achieved particularly dramatic progress, with an 80 percent decline in its TB mortality rate.[10] The distribution of tuberculosis is not uniform across the globe; about 80% of the population in many Asian and African countries test positive in tuberculin tests, while only 5–10% of the U.S. population test positive.[11] In 2007, the country with the highest estimated incidence rate of TB was Swaziland, with 1200 cases per 100,000 people. As of 2014, India has the largest total incidence, with an estimated 2.2 million new cases. India has more than 0.3 million deaths and economic losses of $23 billion (Rs. 143123 crore) every year.[12, 8] Tuberculosis caused the second highest number of deaths in India with 63265 casualties in 2011, 61887 in 2012 and 57095 in 2013.[13]

The incidence of TB varies with age. In Africa, TB primarily affects adolescents and young adults.[14] However, in countries where TB has gone from high to low incidence, such as the United States, TB is mainly a disease of older people, or of the immunocompromised.[15]

SYMPTOMS, DIAGNOSIS AND TREATMENT OF TUBERCULOSIS

Symptoms of TB disease depend on the site of growth of the TB. TB bacteria usually grow in the lungs (pulmonary TB). TB disease in the lungs may cause symptoms such as a bad cough that lasts 3 weeks or longer, pain in the chest or coughing up blood or sputum (phlegm from deep inside the lungs). Other symptoms of TB disease are weakness or fatigue, weight loss, no appetite, chills, fever, sweating at night.

People who have latent TB infection do not feel sick, do not have any symptoms, and cannot spread TB to others.[16, 17] People suspected of having TB disease should be referred for a medical evaluation, which will include:

1. ***Medical history.***
2. ***Physical examination:*** during the physical exam, your doctor will check your lymph nodes for swelling and use a stethoscope to listen carefully to the sounds your lungs make while you breathe.
3. ***Test for TB infection (TB skin test or TB blood test):***
   a) ***Skin test:*** A small amount of a substance called PPD tuberculin is injected just below the skin of your inside forearm. Within 48 to 72 hours, a health care professional will check your arm for swelling at the injection site. A hard, raised red bump means you're likely to have TB infection. The size of the bump determines whether the test results are significant.
   b) ***Blood tests****:* Blood tests may be used to confirm or rule out latent or active tuberculosis. These tests use sophisticated technology to measure your immune system's reaction to TB bacteria. Quantiferon-TB Gold in-Tube test and T-Spot TB test are two examples of TB blood tests.
4. ***Chest radiograph (X-ray):*** If you've had a positive skin test, your doctor is likely to order a chest X-ray or a CT scan. This may

show white spots in your lungs where your immune system has walled off TB bacteria, or it may reveal changes in your lungs caused by active tuberculosis. CT scans provide more-detailed images than do X-rays.

5. ***Sputum tests****:* If your chest X-ray shows signs of tuberculosis, your doctor may take samples of your sputum — the mucus that comes up when you cough. The samples are tested for TB bacteria.

Sputum samples can also be used to test for drug-resistant strains of TB. This helps your doctor choose the medications that are most likely to work. These tests can take four to eight weeks to be completed. [17]
The aim of TB treatment is to cure the patient and restore quality of life and productivity; to prevent relapse of TB; to reduce the transmission of TB to others; to prevent the development and transmission of drug resistant TB.

First line agents: These drugs have high antitubercular efficacy as well as low toxicity; and are used routinely namely Ethambutol. Isoniazid. Pyrazinamide. Rifampicin. Streptomycin. For new patients with presumed drug susceptible pulmonary TB, the World Health Organisation (WHO) recommends that they should have six months of TB treatment. This consists of a two month intensive treatment phase followed by a four month continuation phase. For the two month intensive TB treatment phase they should receive: Isoniazid plus rifampicin plus pyrazinamide plus ethambutol followed by Isoniazid plus rifampicin for the continuation TB treatment phase.

Table 1: first-line tuberculosis drugs

Treatment of resistant TB, especially MDRTB (Multiple Drug Resistant Tuberculosis), is frequently unsuccessful, requiring the use of more toxic, expensive drugs, surgery, or both. Thus, emphasis should be on strategies developed to avoid the emergence of drug resistance. Primary resistance occurs in patients with active TB who have never received antituberculous drugs. Secondary (or acquired) resistance is the occurrence of resistance after a mutant's selection or facilitation in the presence of various antituberculous drugs. MDRTB is defined as the presence of at least 1% of *Mycobacterium* strains in a bacterial population or culture that are resistant to at least INH and RIF. The drugs that are used for the treatment of drug resistant TB are grouped according to how effective they are, how much experience there is of their use and the drug class. All the “first line” anti TB drugs are in Group 1, apart from streptomcycin which is classified with the other injectable agents in Group 2. [18]

Group 1: First Line Oral Agents
pyrazinamide
ethambutol
capreomycin

Group 2 : Injectable Agents
kanamycin
amikacin
streptomycin, rifabutin

Group 3 : Fluoroquinolones
Levofloxacin
Moxifloxacin
Ofloxacin

Group 4: Oral Bacteriostatic Second Line Agents
para–aminosalicylic acid
cycloserine
terizidone
thionamide, protionamide

Group 5: Agents with an unclear role in the treatment of drug resistant TB
clofazimine
linezolid
amoxicillin/clavulanate
thioacetazone
imipenem/cilastatin
high dose isoniazid
clarithromycin

NEW TUBERCULOSIS DRUGS UNDER DEVELOPMENT

New TB drugs are needed because of the complexity and toxicity of the current TB drug regimens and the major problem of TB drug resistance. This together with the problem of the interactions of the current TB drugs with the antiretroviral drugs taken by HIV positive people, means that there is an urgent need for newer TB drugs.
However, new TB drugs needs to provide:

- Shorter and simpler, but still affordable, multi drug regimens for drug sensitive TB
- Shorter, more effective , less toxic, and less expensive regimes for drug resistant TB
- Short, simple, easily tolerable and safe regimes for latent TB
- Drugs with few drug-drug interactions, so they can be safely provided to people with HIV .[19]

There are currently at least ten compounds in various stages of clinical development for TB. Four of these are existing drugs that are either being redeveloped or

repurposed for the treatment of TB, and there are six new chemical compounds that are being specifically developed as TB drugs. [20, 21] A number of known drugs are being currently investigated for their contribution in simplification or improvement of the current TB drug regimen. These include rifamycins and fluoroquinolones.

Rifamycins (rifampin and rifapentine)

Rifampin, used at 10mg/kg, is the cornerstone of first-line therapy against TB. Higher doses have recently been shown to have higher bactericidal activity, [22] and clinical trials assessing the potential use of high-dose rifampin to shorten TB treatment will begin soon. Because of its greater potency against Mycobacterium tuberculosis and its longer half-life compared with rifampin, rifapentine is an attractive candidate for shortening or simplifying therapy. [23] Recent studies [24, 25] in the mouse model suggested that a regimen of daily rifapentine, pyrazinamide, and either isoniazid or moxifloxacin could dramatically shorten the duration of treatment. Phase II trials are underway to evaluate the ability of rifapentine given 5 or 7 days a week to shorten the treatment.

Fluoroquinolones

Fluoroquinolones are broad-spectrum antimicrobial agents that have shown potent activity against M. tuberculosis *in vitro* and *in vivo.* They are used as second-line drugs in MDR-TB treatment.[26, 27] Two newer methoxy fluoroquinolones, gatifloxacin and moxifloxacin, have demonstrated more potent *in-vitro* activity against M. tuberculosis than the older compounds, ofloxacin and ciprofloxacin.[28-31]

Nitroimidazopyran is the center of attention in the world today as a most potent novel drug candidate for TB. Its leading compound PA-824 is being developed at the stage of the first clinical trial phase I. PA-824 possesses two types of mechanism; inhibitions of the biosynthesis of protein and cell wall lipid of M. tuberculosis. PA-824 exhibits bactericidal activity

against both replicating and static M. tuberculosis. It also shows potent bactericidal activity against Multiple drug resistant (MDR) tuberculosis.

Caprazamycin-B (CPZ-B) is the promising novel antibiotic recently developed in Japan, which was isolated from Streptomyces species. In contrast to current anti-TB drugs, CPZ-B with a novel chemical structure possesses specific bactericidal activity only against Mycobacterial species especially M. tuberculosis including MDR strains. CPZ-B inhibits the biosynthesis of the cell wall of Mycobacteria, and exhibits moderate therapeutic efficacy that is dose size dependent in pulmonary tuberculosis model induced in mice. Any cytotoxicity is not observed in the preceding animal experiments.

Figure 1: Compounds that are currently in preclinical or clinical development for the treatment of active tuberculosis

VARIOUS BIOAVAILABILITY ENHANCEMENT APPROACHES

Pharmacokinetics (PK) is defined as the study of the kinetics of drug absorption, distribution, metabolism and elimination. Pharmacodynamics (PD) is defined as the time course for the drug effect and the relationship between the drug concentration and the observed therapeutic effects.[32] These two are the most important considerations in drug development and therapy. Another important factor is the bioavailability of a drug, which is the fraction of administered dose that reaches the systemic circulation. The route of administration is a major contributing factor. For instance, a drug administered via the intravenous route is 100% bioavailable since it is administered directly into the systemic circulation. Absorption of a drug determines the amount of drug which becomes available at the site of action and is not equal to the amount of drug administered since the metabolic pathway of drugs are affected by the contents in the stomach and 'first pass' through the liver. The fraction of the drug eventually reaching the systemic circulation determines the bioavailability of the drug . [32]

The problem of poor/variable bioavailability of rifampicin, which is shown, in particular when the drugs are present in anti-tubercular fixed-dose combination (FDC) products, is a matter of serious concern. There is a potential of failure of therapy in patients with an active disease. It perhaps also is a contributory factor towards the increasing resistance to anti-tubercular drugs. The enhanced decomposition of rifampicin in the presence of isoniazid *in situ* in stomach acidic conditions after ingestion is indicated to be the key factor behind the problem.[33] The decomposition of rifampicin has varied from 8.5 to50% in the acidic environment of the stomach in the time range corresponding to the gastric residence time for most dosage forms in humans (≈15 minutes to 105 ± 45 minutes).[3, 4] However, the gastric-emptying time for some single-unit dosage forms may reach 6 hours.[34] The use of substandard FDC will ultimately result in drug resistant TB and treatment failure. [35] . Shishoo *et al* [36] have indicated that RIF in the presence of INH as a FDC may undergo greater decomposition in the acidic conditions of the stomach, as compared to when RIF is administered (orally) alone. Thus, less RIF will be available for absorption from FDC's as

compared to RIF administered as a separate formulation. This will be reflected in the poor bioavailability from the former formulation. There is thus an urgent need to modify or segregate the FDC formulation in such a way that RIF and INH are not released simultaneously in the stomach. Alternatively both drugs need to be administered separately after an interval corresponding to average gastric residence time, which is somewhat unpredictable due to high intra- and inter-subject variability.[33, 35, 37]

Pandey and colleagues [38] demonstrated that the nanoparticles provided sustained release of the anti-TB drugs, this also considerably enhanced their efficacy as compared to orally administered drugs when observed at 9 to 11 d interval. In contrast, free (unbound) drugs were cleared from the plasma within 12 to 24 h after administration. Three frontline drugs, rifampin (RMP), isoniazid (INH), and pyrazinamide (PZA) were coencapsulated in poly (lactide-co-glycolide) (PLG) nanoparticles. After a single oral administration of this formulation to mice, the drugs could be detected in the circulation for 4 d (RMP) and 9 d (INH and PZA); therapeutic concentrations in the tissues was the prime efficacy of the nanoparticle treatment of *M. tuberculosis* infected mice with the nanoparticle-bound drugs (five oral doses every 10th day) which resulted in complete bacterial clearance from the organs. Free drugs were able to produce bacterial clearance only after daily administration of 46 doses. Similar efficacy of the nanoparticle-bound drugs was also observed in guinea pigs. [39]

In an another study , Poly (DL-lactide-co-glycolide) (PLG) nanoparticles were encapsulated by three front-line antitubercular drugs, i.e. rifampicin, isoniazid and pyrazinamide. Absolute bioavailability was increased several-fold as compared with unencapsulated drugs. Further, drug-loaded PLG nanoparticles resulted in undetectable bacterial counts in the lungs and spleen of Mycobacterium tuberculosis-infected individual, thereby demonstrating a better chemotherapeutic efficacy, as compared with daily free drug treatment. Hence, injectable PLG nanoparticles hold promise for increasing drug bioavailability and reducing dosing frequency for better management of tuberculosis. [37]

Study was undertaken to evaluate the effect of a herbal bioenhancer, *Carum carvi* on pharmacokinetics of rifampicin, isoniazid, and pyrazinamide in fixed dose combination (FDC). The volunteers were administered a single dose of FDC containing rifampicin (450 mg), isoniazid (300 mg), and pyrazinamide (1000 mg) and after 10 days washout period the same FDC along with *C. carvi* extract (100 mg) was administered. Blood samples were collected at different time-points and analyzed by high-performance liquid chromatography (HPLC). Additions of *C. carvi* extract lead to increase in plasma levels of rifampicin, isoniazid, and pyrazinamide and hence *C. carvi* acts as a bio enhancer and modifies the kinetics of antitubercular treatment favorably. [40]

The pharmacokinetic interaction of some herbal products and a pure molecule isolated from *Cuminum cyminum* with Rifampicin is shown. An aqueous extract derived from cumin seeds produced a significant enhancement of Rifampicin's levels in rat plasma. This activity was found to be due to a flavonoid glycoside, 3',5-dihydroxyflavone 7-O-beta-D-galacturonide 4'-O-beta-D-glucopyranoside (CC-I). CC-I enhanced the Cmax by 35% and AUC by 53% of Rifampicin. The permeation enhancing effect of this glycoside could be the possible reason for the altered bioavailability profile of RIF. [41]

Alginate micro particles were developed as oral sustained delivery carriers for antitubercular drugs in order to improve patient compliance. Pharmacokinetics and therapeutic effects of alginate micro particle encapsulated antitubercular drugs, i.e. isoniazide, rifampicin and pyrazinamide was examined. The encapsulation of drug in alginate micro particles resulted in up to a nine-fold increase in relative bioavailability compared with free drugs.[42]

M.M Mehanna *et al* [43] reported the use of respirable drug loaded nanocarriers as a potential way of local and passive delivery of antituberculosis therapy. Common examples of natural polymers suitable for pulmonary delivery are chitosan, alginate and gelatin. On the other hand, poly lactic-co-glycolic acid, poly lactic acid, poly anhydride and poly acrylate are examples of synthetic polymers. [44] Polymeric nanoparticles have considerable advantages in the treatment of pulmonary diseases as they provide a controlled and targeted pulmonary drug deposition resulting in enhanced drug bioavailability and so improving patient compliance with a possible dose frequency reduction. Trehalose, another saccharide was used to formulate inhalable nanocomposite particles by Tomoda *et al.* [45] aiming at deep lung deposition followed by decomposition into nanoparticles to overcome the disadvantages of nanoparticle aerosolization performance. Among the naturally occurring polymers, numerous studies reported that alginates and chitosan are the most popular polymers used in pulmonary drug delivery systems. Chitosan and alginate polymers possess attractive features, namely biocompatibility, low toxicity, biodegradability, and being approved by the FDA for oral use. [46, 47] Additionally, as a result of chitosan cationic nature, it has mucoadhesive properties and considered to be a permeation enhancer. [48] Microencapsulation of chitosan

nanoparticles has been widely used for pulmonary drug delivery. [49]

Pandey and Khuller [50] investigated the encapsulation of the first line anti TB drugs (rifampicin, isoniazid and pyrazinamide) within solid-lipid nanoparticles. Solid-lipid nanoparticles (SLN) are colloidal lipidic nanocarriers ranging in size from 40 to 1000 nm consisting of a solid core made of biodegradable physiological lipids coated with surfactants for stabilization [51] prepared via emulsion solvent diffusion method and assessed their chemotherapeutic potentials in the guinea pig tuberculosis model. The entrapment efficiency was 51, 45 and 41% for RIF, INH and PZA, respectively. *In vitro* release studies in simulated gastric fluid during 72 h showed that about 12% was released from the drugs. This can be explained as the more lipophilic the drug was, the slower the drug was released.

Polymeric nanosytems are another effective means to fight against mycobacterial infections. For example, poly-isobutylcyanoacrylate (PIBCA) nanoparticles increased the intracellular accumulation (cell-association) of all the three tested drugs: isoniazid, rifampin and streptomycin, and enhanced the antimicrobial activity of isoniazid and streptomycin against M. tuberculosis residing in human monocyte derived macrophages. [52]

S.Agrawal *et al* conducted eight bioequivalence studies at National Institute of Pharmaceutical and research (NIPER) to study all the intrinsic and extrinsic factors affecting rifampicin absorption. They found out that out of eight fixed dose combination (FDC) formulations, six formulations were bioequivalent for rifampicin whereas one formulation was below and one was above the limits of bioequivalence. It was observed that more variability in rifampicin blood levels is associated with FDC formulations when compared to rifampicin only formulations. Further, one of the rifampicin-only capsule showed unexpectedly lesser plasma levels indicating possible role of physical characteristics of rifampicin bulk material. It was also seen that rifampicin shows dose-dependent pharmacokinetics even at the modest increase in dose due to saturation of efflux system at absorption site and metabolizing enzymes for elimination. Other components of FDC formulations such as isoniazid and pyrazinamide due to high solubility and permeability have shown very less variability and were bioequivalent to separate formulations even for formulations those were failed for rifampicin. [53]

CONCLUSION

We conclude that though the fixed dose combinations (FDC's) have better patient compliance than the individual drug therapy; they have their own disadvantages, like drug –drug interaction, increased decomposition of one drug in the presence of other, poor bioavailability of one drug etc. In this review we have tried to cover the problems faced during the administration of FDC's in a tuberculosis patient and what are the various techniques by which the bioavailability can be enhanced for the future dose management and optimization in a tuberculosis patient.

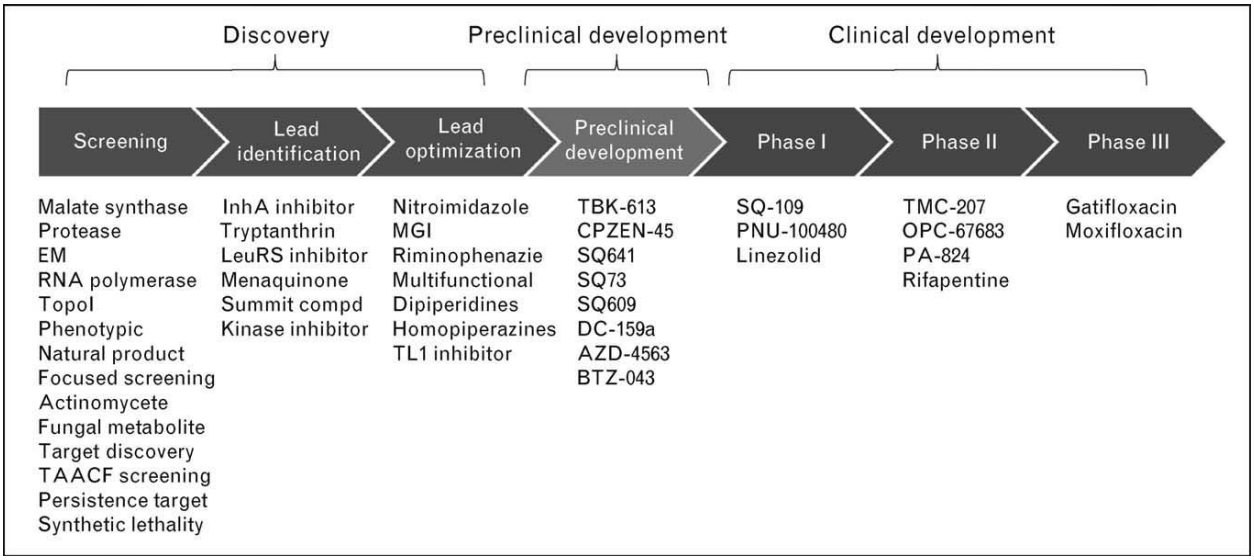

Figure 1: compounds that are currently in preclinical or clinical development for the treatment of active tuberculosis. Produced by the working group on new drugs of the stop TB partnership.

Table 1: first-line tuberculosis drugs

Drug	Side Effects	Daily	Dosage Regimen (mg/kg) Two or Three Times/wk
Isoniazid	Hepatitis, peripheral neuropathy, lupus-like syndrome, drug interactions	5 (max, 300 mg)	15 (max, 900 mg)
Rifampin	Drug interactions, orange discoloration of body fluids, gastrointestinal upset, hepatitis, fever, hypersensitivity, acute renal failure, hemolytic anemia	10 (max, 600 mg)	10 (max, 600 mg)
Pyrazinamide	Hyperuricemia, gouty arthritis, rarely hepatitis	15-30 (max, 2 g)	50-70 (max, 4 g)
Ethambutol	Optic neuritis, exfoliative rash	15-25	25-30
Streptomycin	Cochleo- and vestibulotoxicity, nephrotoxicity	15	25-30
Amikacin	Cochleo- and vestibulotoxicity, nephrotoxicity	7.5-10	

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