

# Combating Tuberculosis Infection: A Forbidding Challenge

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Rawal and Butani: Combatting Tuberculosis Infection: A Forbidding Challenge

After 50 years drought, several drugs are looming from the pipeline to combat tuberculosis. They will serve as a boon to the field that has been burdened with primitive, inadequate treatments and drug-resistant bacterial strains. From the decades, due to lack of interest and resources, the field has suffered a lot. Learning from the flaws, scientists have renovated their approaches to the finding of new antitubercular drugs. The first line drugs take about six months or more for the entire treatment. The second line remedy for resistant-tuberculosis requires daily injections which carry severe side effects. Drug resistance remains a constant menace because patients stop the medication once they start feeling better. So new drugs are required to be explored which are effective against tuberculosis especially drug resistant tuberculosis. These drugs need to work well with other drugs as well as with antivirals used for the treatment of human immunodeficiency virus. It is also very important to be considered that the treatments need to be cheap, as tuberculosis primarily affects people more in the developing countries. Further, new drugs must cure the disease in short span of time than the current six to nine month regimen. Recently a few new and potent drugs such as bedaquiline, delamanid, teixobactin have been evolved which may serve as a nice step forward, with a better outcome. Teixobactin, a new antibiotic has been found to have promising action against resistant strains, is also under consideration.

Key words: Tuberculosis, latent, bedaquiline, teixobactin

Several potent drugs have been available for the treatment of tuberculosis (TB) since past 70 years. However, poor management of tuberculosis continues to take a heavy count of human lives<sup>[1,2]</sup>. The disease has become prevalent in countries like Africa, India, China and Russia<sup>[3-5]</sup>. In India nearly 500,000 people die every year due to this dreadful disease<sup>[6-10]</sup>. In the early times, in the absence of any cure, infected patients faced slow and painful death. Many patients died unattended in infirmaries<sup>[11-13]</sup>.

There are about 10 million new tuberculosis cases added every year worldwide<sup>[14]</sup>. About 2 million people succumb to this life threatening disease<sup>[15]</sup>. Some of the main factors which has contributed to this death dealing disease are the absence of basic sanitary facilities and the emigration of people from rural to urban crowded slums<sup>[16-18]</sup>.

Tuberculosis is caused by rod shaped, aerobic, acid fast, gram positive bacteria named *Mycobacterium*

*tuberculosis* which was first identified by a German Physician, Robert Koch, in 1882 (Table 1)<sup>[6,7]</sup>. The bacterium exists in both latent and active forms.

## LATENT TUBERCULOSIS (DORMANT FORM)

About 2 billion people are carrying *M. tuberculosis* in the dormant form worldwide. Out of this, only 1% will develop active form of the disease during their life time. The development of the disease takes place whenever the immunity goes down due to various factors like old age, HIV/AIDS. It becomes

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difficult to understand how the bacteria survive for months and years without multiplying or showing any signs of the disease. However the bacteria remains noninfectious during its dormant form but this form continues to be an unlimited pool of infection. Several diagnostic tests have been available to detect latent infection. The antiquated tuberculin test for latent TB is not authentic as it gives false positive results. A small amount of TB proteins is injected under the top layer of the skin of the inner forearm. Appearance of red bumps within 2 days after injection indicates the presence of tuberculosis infection. IFN $\gamma$  release assays (IGRAs) can be used as an alternative to this age old tuberculin test. These are based on the fact that T-lymphocytes when exposed to specific antigens will release IFN $\gamma$ <sup>[19-25]</sup>.

## CURRENT TREATMENT REGIME

WHO in 1970 recommended a drug regime consisting of four first line drugs- isoniazid (INH), rifampicin (RFM), pyrazinamide (PZ) and ethambutol (ETB), detailed in Table 2<sup>[13]</sup>. This regime can be used for the effective treatment of sensitive, non resistant strains of *M. tuberculosis*. There are two phases of drug administration. Initially during the intensive or rigorous phase of the first two months of the treatment, all the above four drugs are administered on alternate days followed by the continuous phase of next 4-6 months in which only two drugs, rifampicin and isoniazid are administered (Table 3). The above four drugs are effective for the following sub populations of bacteria (a) active growers that are killed by isoniazid (b) periodic metabolic bursts killed by rifampicin (c) low metabolic activity state-susceptible to pyrazinamide. However no drug is effective for dormant bacilli<sup>[26-28]</sup>.

### Hindered bioavailability of rifampicin:

Rifampicin and isoniazid interact in the acidic medium. Rifampicin degrades to form 3-formylrifampicin which reacts with isoniazid to form inactive hydrazone of rifampicin. The interaction results in about 20-30% reduction in the bioavailability of rifampicin. To overcome this issue, many research studies are going on<sup>[29-32]</sup>.

### Poor patient complaisance:

The above drug regime has many flaws which have led to the poor patient complaisance. The patient

**TABLE 1: MILESTONES IN TUBERCULOSIS**

Years	Milestones
1882	Robert Koch isolated and cultivated the bacteria responsible for TB
1920	French Microbiologist A. Calmette and his veterinarian student, C. Guerin developed BCG vaccine
1943	Dr. Selman Waksman developed streptomycin as a drug cure for TB
1944	J. Lehman isolated para amino salicylic acid and later discovered isoniazid
1959	Dr. Melly Ellen Avery discovered the role of surfactant in keeping the air sacs of the lungs open
1966	Italian Pietro Sensi isolated rifamycin S from a fungus
1970	TB treatment course was introduced employing 4 drugs for 6-9 months
1989	HIV was identified and people became more vulnerable to TB
2005	USFDA recognized improved diagnostic tests based on the DNA of <i>Mycobacterium tuberculosis</i>

BCG: Bacillus Calmette-Guérin, TB: tuberculosis, USFDA: United States Food and Drug Administrative

**TABLE 2: INVENTION OF FIRST LINE DRUGS AND THEIR MODE OF ACTION**

Drug	Year of invention	Mode of action
Isoniazid	1952	Inhibits cell wall synthesis
Rifampicin	1968	Nucleic acid synthesis inhibitor with weak effect
Pyrazinamide	1952	Inhibits membrane energy metabolism with weak effect
Ethambutol	1961	Inhibits cell wall synthesis

**TABLE 3: DOSE REGIME**

Phase	Drugs	Time
Intensive or rigorous	INH + RIF + PZ + ETB	First 2 months
Continuous	INH + RIF	4-6 months

INH: Isoniazid, RFM: rifampicin, PZ: pyrazinamide, ETB: ethambutol

has to face severe side effects such as nausea and hepatotoxicity. For this reason the patients do not remain stick to the regime. Only a highly disciplined patient can stick to the regime till the end of the treatment. For this purpose WHO has launched DOTS (Directly observed treatment short course) programme. A patient has to visit a TB clinic on alternate days for about 6-9 months and the formulation is put into the mouth and visually confirmed that patient has swallowed the drug. However, in India DOTS has not been very successful. India has the burden of having maximum number of TB patients of about 2 million out of which total number of deaths is 5 lakh per year. About 70% of TB patients are aged between 15-54 years. The poor patient compliance is a major factor which results in the development of resistance to first line drugs. The development of drug resistance is a constant threat as patients stop taking their medicines once they feel better<sup>[33-35]</sup>.

**Persistent form of *Mycobacterium tuberculosis*:**

All the TB drugs are effective as long as the *M. tuberculosis* remains in the multiplying phase. However, isoniazid kills over 90% of the mycobacteria in the first two days of the treatment. But still the combination of two drugs rifampicin and isoniazid takes about 6-9 months to kill the persistent form of the microorganism. Soon after the multiplying phase, the bacteria enters a persistent phase in which it stops multiplying and undergoes hibernation. As in case of latent phase, there is no diagnostic test for the detection of persistent form of bacteria. Rifampicin is the only drug which is effective against the persistent *M. tuberculosis* but has weak activity which elongates the duration of the treatment for several months<sup>[36,37]</sup>.

**Disease recurrence (relapse of the disease):**

After a few months of cessation of the drug combination treatment, there are chances of relapse of the persistent form into active form. The disease may even develop in more nasty form i.e., drug-resistant tuberculosis (DR-TB). There is no biomarker available to confirm the complete absence of persistent form in the host<sup>[38]</sup>.

**Drug-resistant tuberculosis:**

Mismanagement of the antitubercular drugs may lead to the development of drug resistance. The time duration for treatment is 6-9 months and symptoms of disease usually disappear after 2-3 months of the treatment. Hence patient may discontinue the medication which is major cause for the drug resistance development. Sometimes it also occurs in the patients who delay the regular administration of the drugs or do not take all the four prescribed drugs. Patients may also develop tuberculosis again after the treatment in the past. DR-TB is communicable through air from one person to another. DR-TB occurs in many forms such as multi-drug resistant tuberculosis, extensively drug resistant tuberculosis and totally drug resistant tuberculosis<sup>[38]</sup>.

**Multidrug-resistant tuberculosis:**

Multidrug-resistant tuberculosis (MDR-TB) is the disease in which the strains of *M. tuberculosis* become non responsive to the two most effective first line drugs. Several injectables like streptomycin, amikacin, kanamycin and oral fluoroquinolones like moxifloxacin, gatifloxacin can also be given as an alternate therapy but these are highly toxic, less efficacious and very expensive. Apart from these, the duration of the treatment may extend upto

18-24 months instead of 6-9 months. The faulty treatment regimen and unreliable diagnostic tests are also the major factors which may lead to the development of resistance. Only 1% of the cases are on proper treatment but still have poor outcomes<sup>[39-41]</sup>.

**Extensively drug-resistant tuberculosis:**

Patients with extensively drug-resistant tuberculosis (XDR-TB) are resistant to rifampicin, isoniazid, and fluoroquinolones and at least one injectable antibiotic. It is mainly widespread in the countries like Africa, Asia and Soviet Union having majority of cases of HIV/AIDS. XDR-TB has the highest mortality and morbidity rates if co-infected with HIV/AIDS.

**Totally drug-resistant tuberculosis:**

It is the worst form of tuberculosis. It is a condition in which the patients become resistant to all the first and second line drugs ultimately leading to death<sup>[42]</sup>.

**Tuberculosis as an obstreperous disease:**

TB is very challenging essentially due to several reasons (a) poor detection rate as in India it takes about 6 months or more to detect the resistant strains, (b) co-infection with HIV/AIDS (c) enhanced transmission in overcrowded slums, hospitals and prisons, (d) malnutrition, poverty and poor healthcare system, (e) increasing cases of diabetes due to foetal malnutrition, (f) poor patient complaisance and (g) absence of biomarkers to monitor the success of the treatment and complete eradication of the disease. The above reasons presuppose the need of new drugs for the complete and successful treatment of DR-TB<sup>[43,44]</sup>.

**Prevalence of tuberculosis in HIV/AIDS patients:**

Outburst of majority of the cases of TB has been reported among the immunocompromised patients. In immunocompromised patients, the progress of the disease is rapid and fiery leading to high death rates. In this critical situation, there is an urge for new potent agents which must tackle both the dreadful diseases. However, from practical point of view, immunocompromised patients are not considered suitable for controlled trial studies<sup>[45-48]</sup>.

**EVOLVING NEW DRUGS FOR TUBERCULOSIS**

Several antitubercular drugs have been in the use for five to six decades. Eventually, *M. tuberculosis* has

developed resistance to rifampicin and isoniazid. The incidence of TB is high in countries like India, China, former USSR states, while in some African states it is the highest due to the prevalence of HIV/AIDS. Second line drugs can be administered but they are very expensive and their duration of treatment is about 2 long years. These drugs are required to be administered in very high doses which lead to the development of toxicity<sup>[49-56]</sup>.

### Barriers in the discovery of new drug for tuberculosis:

The barriers in the discovery of new drugs for TB are numerous like (a) limited knowledge about the basic biology of *M. tuberculosis*, (b) unavailability of animal models for the screening of new drugs against latent and persistent forms, (c) the compatibility of the drug molecule with rifampicin and isoniazid, (d) unavailability of biomarkers to evaluate the efficacy of new drugs, (e) a new drug is always tested in combination and not as a single drug, (f) the drug must not induce or inhibit CYP-450 enzyme<sup>[57,58]</sup>.

### Basic biology of *Mycobacterium tuberculosis*:

The drug uptake by cell wall of *M. tuberculosis* is very slow as the microorganism is a clever adversary. Its ability to hunker down and survive under stress is very trickier. The bacterium has an outer lipid bilayer which provides protection to the organism and is hard to penetrate. This outer bilayer is the thickest biological membrane known and has very low permeability. Additionally the fatty acid cell wall acts as a forbidding barrier towards diffusion of hydrophilic and hydrophobic compounds thus enhancing the chances of survival of the organism<sup>[59]</sup>.

## NEW DRUG MOLECULES UNDERGOING CLINICAL TRIALS

There is an urge to develop novel and effective drugs against TB. Several drugs have been discovered which are under clinical trials (Table 4)<sup>[60-65]</sup>.

### Bedaquiline (TMC 207):

Chemically it is a diarylquinoline compound (fig. 1). It is currently in Phase II but soon to enter Phase III. It has a long terminal half-life. The drug has excellent activity against susceptible TB. It does not show any cross resistance to the present drugs. It exhibits a potent sterilization effect and is active against both

**TABLE 4: CURRENT STATUS OF NEW DRUGS IN CLINICAL TRIALS**

Drug	Class	Status	Mechanism
Gatifloxacin	Fluoroquinolones	Currently in Phase-III	Inhibits DNA gyrase, DNA replication
Moxifloxacin	Fluoroquinolones	Currently in Phase-III	Inhibits DNA replication and transcription
Bedaquiline	Diarylquinoline	Phase-II	Inhibits ATP synthesis
Delamanid	Nitroimidazole	Phase-III	Inhibits protein and mycolic acid synthesis
PA-824	Nitroimidazole	Currently in Phase-II	Inhibits cell wall synthesis by releasing nitrous oxide
LL-3858	Pyrrrole	Currently in Phase-I	Information related to molecular mechanism currently in Phase-I trials
Rifalazil	Rifamycin derivative	Currently in Phase-II	Exhibits long acting oral activity against bacteria
Teixobactin	A new class antibiotic	Yet to begin the trials	Act by targeting lipid molecules which serve as building blocks of cell wall

ATP: Adenosine triphosphate

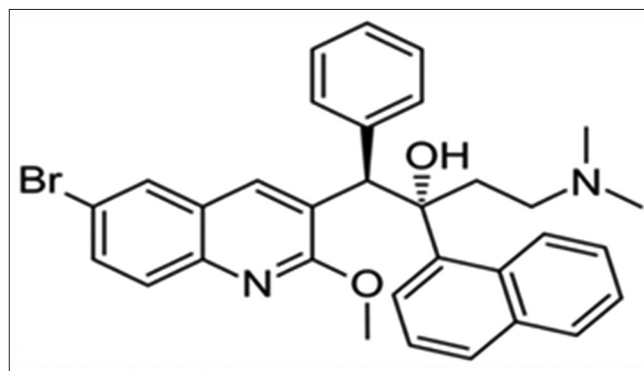


Fig. 1: Chemical structure of bedaquiline.

the fast and slow growing *M. tuberculosis* with a novel mechanism of action of inhibiting bacterial ATP synthase. It is highly potent as compared to isoniazid and rifampicin. Further in the fed conditions, the serum concentration of the drug increases by two fold when compared to fasting condition. It gets metabolized by Cytochrome P450 3A4 (CYP3A4) enzyme. Thus the serum concentration of the drug gets reduced upto 50% in presence of rifampicin which is a CYP3A4 inducer<sup>[65-67]</sup>.

### Nitroimidazoles derivatives:

Nitroimidazole derivatives were synthesized in 1970s. Amongst several nitroimidazoles, some were found to have good anti mycobacterial properties. Of these, CG-17341 was developed as the lead compound. But later it was found to be mutagenic. Two nitroimidazole drugs, PA-824 and OPC-67683 (Delamanid, figs. 2 and 3) have been further developed and are under clinical studies.



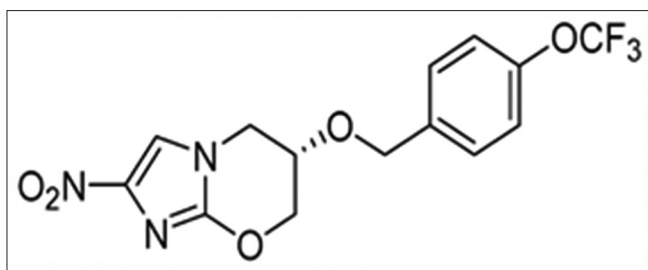


Fig. 2: Chemical structure of PA-824.

**PA-824, nitroimidazoloxazine:**

The drug will soon complete Phase II clinical trials. It is effective against normal as well as resistant strains of bacteria. It has high *in vitro* and *in vivo* activity and does not exhibit cross resistance to any other TB drugs in use. It is basically a prodrug which has been found to show good activity against dormant form along with rifampicin, isoniazid and pyrazinamide. The compound also has a long half-life. It acts by two mechanisms, the cell wall synthesis inhibition and respiratory poisoning in the bacterium. However, replacement of isoniazid or pyrazinamide with PA-824 may lead to the relapse of the disease after 6 months<sup>[68-71]</sup>.

**Delamanid (OPC-67683):**

The drug has recently entered Phase III clinical study. The drug exhibited excellent *in vitro* activity with no cross resistance with any first line anti-TB drug. It does not get metabolized by any of the CYP enzymes. The drug also has a long half-life. It also exhibits powerful activity which may be due to the release of reactive nitrogen species<sup>[71]</sup>.

**Fluoroquinolones:**

The fluoroquinolones are also found to have powerful activity against resistant strains. They mainly act by inhibiting DNA gyrase. Currently, two fluoroquinolones gatifloxacin and moxifloxacin are presently entering Phase III study<sup>[72-75]</sup>.

**LL-3858:**

The drug is currently in Phase I. It has a pyrrole alkaloid nucleus. It is also an analogue of Isoniazid. It also exhibited a potential activity against MDR-TB in mice<sup>[75]</sup>.

**Oxazolidinones:**

They serve as synthetic antibacterial compounds which are active orally. Linezolid, an oxazolidinone compound got approved in 2000 for the treatment

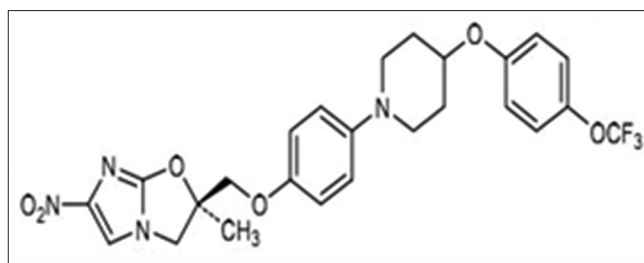


Fig. 3: Chemical structure of OPC-67683 (delamanid).

of drug resistant gram positive bacteria. Linezolid is a potent oxazolidinone which can be used for MDR-TB. It acts by inhibiting protein synthesis. It mainly interferes with the formation of the initiation complex. It does not exhibit any cross resistance with the first line drugs. The main target of linezolid is bacterial ribosome (23S RNA in 50S ribosomal subunit). It also exhibits a highly potent activity. Sutezolid (PNU-100480) and AZD-5847 are also chemically related to linezolid. In the recent studies sutezolid is found to be more active than linezolid in recent studies. AZD-5847 has also been recognized for its anti-TB properties. The main concern is that it may lead to hematological issues<sup>[76,77]</sup>.

**Ethylenediamine derivatives:**

SQ-109, an ethylenediamine derivative is also a potent anti-TB compound with the similar structure to the first line drug ethambutol. The mode of action is not completely clear, but it has been found to inhibit lipid/cell wall synthesis<sup>[78]</sup>.

**Rifalazil (KRM-1648):**

It is a rifamycin derivative which is under Phase II clinical trials. It has been found to be a promising candidate which exhibits long acting oral activity against the bacteria in both animal and human models<sup>[79,80]</sup>.

**Caprazamycin-B:**

A promising liponucleoside antibiotic which has been developed in Japan is Caprazamycin-B. The drug exhibited bactericidal activity specifically against Mycobacterium species including resistant strains. It mainly inhibits the synthesis of mycobacterial cell wall. The therapeutic efficacy was found to be moderate in mice models<sup>[81,82]</sup>.

**Teixobactin:**

Teixobactin, A new class powerful antibiotic that kills drug resistant bacteria, which has been reported

in January 2015 as a promising compound for its activity against drug resistant Gram positive pathogens (fig. 4). It exhibits a unique mechanism of action by targeting the lipid molecules which are required by the bacteria to build their cell walls. It has been reported that teixobactin has such a rapid activity that it does not allow the resistance development in bacteria. Teixobactin has been commended as the first new antibiotic drug against resistant bacteria for the last 30 years. It hasn't been tested in human beings yet but has been found to overcome the development of resistance with the present antibiotic drugs. It has also been found to strike multiple targets in the bacteria. It also showed be potent activity against resistant tuberculosis in rat models. Lipid II is the precursor of peptidoglycan synthesized in the cytoplasm and lipid III is the precursor of wall teichoic acids (WTA). Teixobactin has the ability to bind to both these lipids to form stoichiometric complexes. These complexes serve as an obstruction in the formation of functional cell envelope<sup>[83-86]</sup>.

## NEW DRUG DELIVERY STRATEGIES FOR ANTITUBERCULAR DRUGS

The techniques of drug delivery also determine the efficacy of a treatment. New drug delivery systems have been evolved to reduce the degradation and loss of the drug, to enhance the bioavailability and to reduce the toxic effects. Pulmonary drug delivery systems are gaining utter importance currently due to their numerous advantages like large surface area for absorption, high permeability and good blood supply. New strategies for drug targeting to alveoli are achieving a great attention as the alveolar epithelium

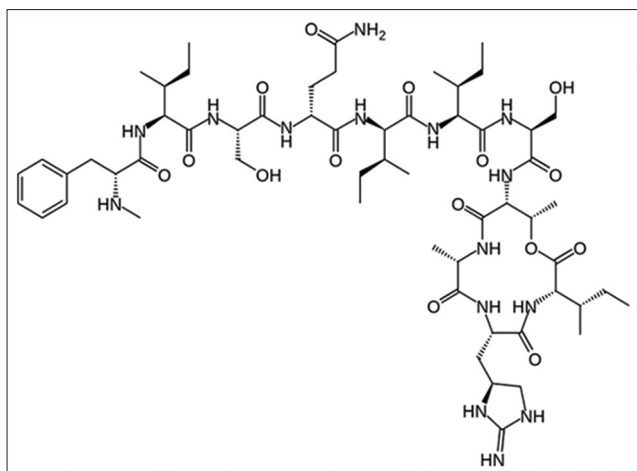


Fig. 4: Chemical structure of teixobactin.

has been shown to serve as a prominent site for the absorption of various therapeutics. The formulations such as liposomes, solid lipid nanoparticles, nanoemulsions, polymeric micelles are gaining utmost importance due to their potential advantages to target the drug to specific site<sup>[87-93]</sup>.

## NANOTECHNOLOGY AND TUBERCULOSIS TREATMENT

Upgraded research has paved the way for the development of novel drug delivery systems based on nanotechnology. Reduction of the particle size to nanoscale improves the solubility of the poorly soluble drugs<sup>[94-100]</sup>.

### Nanoemulsions and nanosuspensions:

The drop size of nanoemulsions lies between 10 and 100 nm. These are thermodynamically stable oil-in-water dispersions which provide the advantages of ease of large scale production and spontaneous generation. Nanosuspensions are colloidal dispersions of drugs which have been stabilized with surfactants. Ahmed *et al.* developed nanoemulsions of rifampicin which indicated a great potential of intravenous delivery of this antitubercular drug<sup>[101]</sup>.

### Liposomes/niosomes:

Liposomes are lipidic bimembrane structures produced by cholesterol hydration, phospholipids while niosomes additionally contain nonionic surfactants. Niosomal encapsulation of pyrazinamide has been done by El-Ridy *et al.* in order to achieve sufficient macrophage targeting and to overcome drug resistance<sup>[102]</sup>.

### Polymeric and nonpolymeric nanoparticles:

The polymeric and nonpolymeric nanoparticles have been evolved as modes for targeting and stabilization. Extensive studies of antitubercular drugs like rifampicin, isoniazid, pyrazinamide entrapped in the poly DL-lactide coglycolide or chitosan nanoparticles have been done<sup>[103-105]</sup>.

### Polymeric micelles:

Polymeric micelles contain polymers and surfactants which form spherical structure above the critical micelle concentration. The self-assembling of amphiphilic polymers lead to the generation of these polymeric micelles<sup>[106]</sup>.

Nanotechnology has lead to the development of inhaled drug delivery system with potential merits such as direct drug delivery to the site of infection, avoiding the first pass metabolism, reducing the dose of the drug and hence reducing the toxicity of the drug. The nanoparticles get swamped by the alveolar macrophages, thus leading to the direct release of antiTB drugs into the alveolar macrophages. This alveoli targeting strategy is playing a prominent role in attacking the TB bacilli. The various nanoformulations can be converted into dry powder to potentiate their merits<sup>[107]</sup>.

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### Conflicts of interest:

There are no conflicts of interest.

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