

## Review article

**Elucidating the molecular mechanisms underlying mutations in *Mycobacterium tuberculosis* RNA polymerase that confer resistance to rifampicin and its structural analogues**

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Corresponding author: **Varalakshmi Vummidi**. Email: vvummidi@gitam.edu**ABSTRACT**

Tuberculosis (TB) is a major global health problem caused by *Mycobacterium tuberculosis* (Mtb), and is responsible for significant morbidity and mortality worldwide. Rifampicin and its structural analogues are essential first-line anti-TB drugs that inhibit RNA synthesis by binding to the beta-subunit of Mtb RNA polymerase (RNAP). However, the emergence of rifampicin resistant Mtb strains poses a major challenge for TB control efforts. Mutations in the *rpoB* gene encoding the beta-subunit of RNAP are the most common cause of rifampicin resistance in Mtb. Understanding the molecular mechanisms underlying these mutations and their effects on RNAP function is crucial for developing new drugs and combination therapies to overcome rifampicin resistance in Mtb. This review discusses the molecular mechanisms underlying rifampicin resistance in Mtb RNAP, including the genetic basis and identification of mutations. It can be hypothesized that rifampicin resistance in Mtb RNAP is a multifactorial phenomenon involving structural, biochemical, and genetic factors. The review highlights strategies for developing new drugs and combination therapies to overcome rifampicin resistance in Mtb and future directions for research on the molecular mechanisms underlying rifampicin resistance in Mtb RNAP.

**Keywords:** Rifampicin; analogs; *Mycobacterium tuberculosis*; drug resistance; RNA polymerase.

**INTRODUCTION**

**R**ifampicin is a potent antibiotic that is commonly used to treat tuberculosis (TB). It is frequently combined with other drugs, such as isoniazid, pyrazinamide, and ethambutol, as the primary treatment for tuberculosis. *Mycobacterium tuberculosis* is a bacterium that can infiltrate the respiratory system and other parts of the body, causing tuberculosis. Rifampicin is effective against *M. tuberculosis* because it targets the enzyme RNA polymerase, which is essential for the bacteria's development and replication. By inhibiting RNA polymerase, rifampicin prevents bacteria from producing proteins, resulting in their eventual demise. Rifampicin is essential for the treatment of tuberculosis because it inhibits the pathogenesis of drug-resistant strains. To ensure that all pathogens are eradicated, TB therapy frequently involves a drug combination administered over a period of several months. Rifampicin has been shown to be exceedingly advantageous in the management of tuberculosis, with treatment efficacy of 90% or higher in drug-sensitive TB. Despite its efficacy, rifampicin can cause additional side effects such as nausea, vomiting, diarrhea, and gastrointestinal pain. Because rifampicin may induce liver injury, regular liver function tests are required during treatment. In addition, rifampicin can interact with other medications, including certain HIV-treatment antiretrovirals. Overall, rifampicin is an essential drug for the treatment of tuberculosis, and its use in combination with other medications has been instrumental in reducing the global TB burden (1).

**Rifampicin as primary medication for TB**

The main drug used to treat TB, a potentially fatal infection brought on by the bacteria *Mycobacterium tuberculosis*, is rifampicin. When bacteria's RNA polymerase is inhibited, transcription of their DNA is prevented, which results in bacterial death. Rifampicin is a powerful bactericidal drug. Rifampicin is particularly successful when used in combination therapy with other anti-tuberculosis medications because it has a broad spectrum of action against mycobacteria, including drug-resistant forms (1).

Rifampicin works by attaching to the bacterial RNA polymerase beta subunit and preventing the transcription of bacterial DNA. Due to the occurrence of mutations in the *rpoB* gene, which encodes the beta subunit of RNA polymerase, rifampicin-resistant *M. tuberculosis* strains are often seen. Rifampicin resistance provides a significant obstacle to the treatment of TB because it reduces the efficacy of rifampicin-based combination therapy. Despite rifampicin's clinical efficacy in the treatment of TB, there are several drawbacks to using it. Drug interactions with other drugs, the emergence of drug resistance, adverse drug responses, and pharmacokinetic heterogeneity among patient groups are a few of these. Furthermore, poor patient compliance, treatment failure, and recurrence may result from the lengthy (6 to 9 month) course of therapy needed to cure TB (2).

Several approaches are being sought to create new and improved rifampicin analogues with improved pharmacological characteristics and lower toxicity to

overcome these limitations. One method is the synthesis of rifampicin derivatives with alterations to the basic structure of rifamycin, such as the insertion of new heterocyclic rings or the substitution of the benzoxazole moiety. These changes can preserve the rifampicin analogues' antibacterial effectiveness while enhancing their solubility, stability, and bioavailability as pharmacokinetic features. The creation of combination therapy regimens containing rifampicin and additional anti-tuberculosis medications with complementary modes of action, such as isoniazid, pyrazinamide, and ethambutol, is another tactic. These combination medications can help TB patients have better treatment results and slow the spread of drug-resistant *M. tuberculosis* strains. Using fixed-dose combination formulations, which combine many anti-tuberculosis medications into a single pill, can also streamline treatment plans, boost patient compliance, and lower the risk of treatment failure and recurrence (2).

New paths for studying the molecular processes behind rifampicin resistance in Mtb and developing innovative anti-tuberculosis medications have been opened by recent developments in molecular and computational biology. The development of novel therapeutic targets and possible lead compounds for the treatment of TB has been hastened using high-throughput screening techniques, structural biology, and systems biology approaches. Rapid testing of vast libraries of compounds is made possible by high-throughput screening techniques, offering a time- and money-efficient method of finding new drugs. The structure and operation of rifampicin and its analogs, as well as the processes underlying their interactions with *M. tuberculosis* RNA polymerase, have also been illuminated by structural biology. This knowledge has made it easier to develop novel substances with better pharmacokinetic and pharmacodynamic features. The intricate connections between *M. tuberculosis* and its host, as well as the pathways and networks involved in treatment resistance, are also being thoroughly understood using systems biology techniques.

Despite the drawbacks of its application, rifampicin is still the major drug used to treat TB. It is possible to generate new and better anti-tuberculosis medications thanks to developments in drug development, combination therapy regimens, and the use of creative research techniques. These initiatives are essential to tackling the worldwide impact of TB and realizing the elimination of this terrible illness (1).

### **Mechanism of action of rifampicin on RNA polymerase**

The RNA polymerase enzyme, which is essential for the growth and replication of bacteria, including the germs that cause TB, is where rifampicin attaches. The contact prevents the enzyme from producing RNA, which eventually causes the bacterium to perish. The process of creating RNA from DNA templates is

carried out by an enzyme known as RNA polymerase. A large subunit of the bacterial RNA polymerase, which is responsible for synthesizing RNA, is one of the many subunits that make up this enzyme. Rifampicin slows the movement of the RNA polymerase enzyme along the DNA template by binding to its beta subunit. This stops the enzyme from making RNA, which ultimately causes the bacterium to die. Rifampicin binds to RNA polymerase with a high degree of specificity, and it has no impact on the function of other cell-based enzymes. This specificity is crucial since it lessens the possibility of off-target effects and lowers the possibility of the medication developing resistance. Rifampicin is a vital drug to treat TB and other bacterial diseases because of its therapeutic impact on RNA polymerase, which is incredibly helpful in destroying bacteria (2).

### **Genetic involvement of rifampicin resistance in *M. tuberculosis***

The genetic basis of *M. tuberculosis* rifampicin resistance is well-established and has been intensively researched. Rifampicin resilience is most usually caused by mutations in the *rpoB* gene, which produces the beta subunit of the RNA polymerase enzyme. The *rpoB* gene is a good target for antimicrobials like rifampicin since it is essential for transcription in bacteria and is highly conserved among species. Mutations in the *rpoB* gene prevent rifampicin from binding to its target site, which also obstructs RNA production. The "Rifampicin Resistance-Determining Region," (RRDR), a particular region of the *rpoB* gene, has changes in most *M. tuberculosis* strains that are rifampicin-resistant. The highly polymorphic RRDR of the *rpoB* gene carries the codons linked to rifampicin resistance. In *M. tuberculosis*, mutations at codons 531 (encoding the amino acid substitution Ser531Leu) and 526 (encoding the amino acid alteration His526Tyr) account for 75–80% of all rifampicin resistance mutations. Additional, less common variants of the *rpoB* gene that cause rifampicin resistance have also been found outside of the RRDR region. These include alterations to other genes linked to DNA maintenance and repair as well as the upstream promoter region of the *rpoB* gene. However, the emergence of TB strains that are resistant to drugs continues to be a significant challenge, demanding continued efforts to discover innovative therapies and combat the development of drug-resistant strains (3).

### **Mechanism of rifampicin resistance in *M. tuberculosis***

Mutations in the *rpoB* gene can affect the structure of the RNA polymerase enzyme, preventing rifampicin from binding to its target site and reducing RNA production. These mutations have the potential to provide rifampicin resistance via several different pathways. The RNA polymerase mutation can alter the structure of the enzyme in a way that prevents

rifampicin from attaching to its target location. This is known as steric hindrance. Another way that the mutation affects rifampicin's ability to attach to its target location is through diminished affinity (3). The RRDR region of the *rpoB* gene is where *M. tuberculosis* most frequently exhibits rifampicin resistance-related mutations. The *rpoB* gene's RRDR, which is very variable, contains the codons linked to rifampicin resistance. About 75–80% of all rifampicin resistance mutations in *M. tuberculosis* are mutations at codons 531 (encoding amino acid substitution Ser531Leu) and 526 (encoding amino acid change His526Tyr). Outside of the RRDR region of the *rpoB* gene, other uncommon mutations that give rifampicin resistance have also been discovered. These include changes to the *rpoB* gene's upstream promoter region and changes to other genes involved in DNA maintenance and repair. In general, mutations in the *rpoB* gene change the structure or affinities of the RNA polymerase enzyme, preventing rifampicin from binding to its target site and blocking RNA production. This results in rifampicin resistance in *M. tuberculosis*. It's essential to comprehend these pathways to create potent TB medication resistance defenses (4).

#### Identification of mutations in the *rpoB* gene of *M. tuberculosis*

Several molecular methods may be used to find mutations in the *M. tuberculosis* *rpoB* gene that result

in rifampicin resistance. DNA sequencing is one of the most used techniques for identifying rifampicin resistance variants. The *rpoB* gene's DNA may be sequenced using either Next-Generation Sequencing (NGS) technologies, which enable more fast and high-throughput sequencing, or Sanger sequencing, a more conventional sequencing technique. Codons 531 and 526, which are the most altered codons in rifampicin-resistant *M. tuberculosis* strains, are two examples of codons related with rifampicin resistance that may be identified by sequencing. Molecular diagnostic assays like the Xpert MTB/RIF assay are another way to find rifampicin resistance variants. By focusing on the *rpoB* gene, this quick molecular diagnostic test may identify both *M. tuberculosis* and rifampicin resistance. The assay uses real-time PCR technology to identify certain mutations, such as those at codons 531 and 526, in the RRDR of the *rpoB* gene. Other molecular techniques for finding rifampicin resistance mutations include DNA microarrays, high-resolution melting analysis, and PCR-based assays like the amplification refractory mutation system (ARMS), which are shown in Table 1. Rifampicin resistance mutations can be used to inform treatment choices and track the establishment and spread of drug-resistant organisms after they have been discovered (5).

**Table 1:** Types of mutations in the *rpoB* gene and their association with different levels of rifampicin resistance

Codon Position	Amino Acid Change	Nucleotide Change	Frequency (%)
531	Ser -> Leu	AGC -> CTC	60-70
526	His -> Tyr	CAC -> TAC	10-20
516	Leu -> Pro	CTG -> CCG	1-3
533	Asp -> Val	GAC -> GTC	1-3
512	His -> Asn	CAC -> AAC	1-2
526	His -> Asp	CAC -> GAC	1-2
526	His -> Asn	CAC -> AAC	1-2
531	Ser -> Trp	AGC -> TGG	<1
522	Asp -> Gly	GAC -> GGC	<1

#### Rifampicin analogs and their effectiveness against rifampicin-resistant strains of *M. tuberculosis*

Rifampicin is a crucial part of TB control programs all over the world since it is extremely efficient against Mtb and has a short (6 month) course of therapy. The development of rifampicin resistant Mtb strains, however, has significantly complicated attempts to treat and eradicate TB. Mutations in the *rpoB* gene, which codes for the Mtb -subunit of RNA polymerase (RNAP), which give resistance to rifampicin and its analogues, are one method by which rifampicin resistance might develop. Rifampicin analogs are drugs that have been created to combat *M. tuberculosis*'s rifampicin resistance (6). Many other rifampicin analogues have been created and studied for their ability to combat Mtb. Rifabutin, rifapentine,

and rifalazil are a few of the most popular rifampicin analogues. These analogues differ from rifampicin in their pharmacokinetic properties, such as their half-life, and against Mtb activity (7). Rifampicin analogs can be classified based on their chemical structure and mode of action. Structurally, rifampicin analogs can be categorized as:

#### Ansamycins

Ansamycins are antibiotics with a distinct chemical structure comprised of a macrocyclic ring. Rifamycin, the most well-known member of this family, is an essential part of first-line treatment for Mtb. Alternative medications with unique modes of action have been created to address the problem of rifampicin resistance. Rifabutin and rifalazil are examples of the

ansamycin chemical class, which is one of these substances. The action of the ansamycins against rifampicin resistant Mtb strains is a result of their shared macrocyclic ansa bridge structure (6).

The Hsp90 chaperone, which is necessary for the correct folding and stability of several signaling proteins and transcription factors in Mtb, is inhibited by ansamycins as part of their mode of action. The breakdown of misfolded proteins and proteotoxic stress brought on by Hsp90 ATPase inhibition cause bacterial mortality. The connection between the Hsp90 chaperone and its co-chaperone, which is necessary for the correct folding of RNAP and its binding to DNA, has also been demonstrated to be disrupted by ansamycins. As a result of this interference with RNAP's functionality, transcription and bacterial growth are inhibited. Rifampicin-resistant tuberculosis has been successfully treated with ansamycin rifabutin, both alone and in combination with other anti-TB medications. In a short-course regimen for the treatment of TB, rifapentine is combined with other medications and has been demonstrated to be effective against rifampicin-resistant TB. On the other hand, rifalazil is a new ansamycin that is currently being researched and has produced encouraging outcomes in preclinical investigations (7).

Antibiotics containing ansamycin do have certain restrictions despite these benefits. They, for example, have low oral bioavailability, which reduces their use as oral medicines. They may also interact with other drugs, creating the possibility of drug-drug interactions. Given the widespread usage of these antibiotics, the rise of bacterial strains that are resistant to ansamycin raises some possible safety concerns (7).

#### Aminopiperidine analogs

The family of rifampicin analogs known as amino piperidine analogs consists of compounds with amino piperidine substituents linked to the main rifampicin scaffold. The aminopiperidine substituent offers a special interaction with the target site of *M. tuberculosis* strains that are resistant to rifampicin, increasing its effectiveness against these strains. An amino group substituted piperidine ring connected to the rifampicin scaffold defines the chemical structure of aminopiperidine analogs. The kind of the substituent at the C-3 position of the ansa chain distinguishes these analogs from rifampicin (7).

Like how rifampicin works against *M. tuberculosis* strains that are resistant to it, aminopiperidine analogs also work against such bacteria. They bind to the DNA-dependent RNA polymerase and block transcription initiation by targeting the beta-subunit of *M. tuberculosis* RNA polymerase. Studies have demonstrated that rifampicin-resistant strains of *M. tuberculosis*, including those with mutations in the *rpoB* gene, which encodes the beta-subunit of RNA polymerase, are effectively neutralized by aminopiperidine analogs. The aminopiperidine analogs exhibit a greater binding affinity for the mutant RNA polymerase than rifampicin and bind to the RNA polymerase active site (8).

#### Spirocyclic analogs

Spirocyclic analogs are a subclass of rifampicin analogs that have a spirocyclic structure. These analogs have distinctive pharmacological characteristics because of the spirocyclic moiety. Spirocyclic analogs were created to combat rifampicin resistance in *M. tuberculosis*, and preclinical research has proven encouraging. Spirocyclic analogs have a bicyclic or tricyclic system with a spirocyclic bridge between two rings, which distinguishes their chemical structure. The cyclopropane, cyclobutane, or cyclopentane ring that serves as the spirocyclic bridge often gives conformational stiffness and increases the binding affinity of the analogs to the target enzyme (6).

Like rifampicin, spirocyclic analogs suppress bacterial RNA production by binding to the -subunit of RNA polymerase, which is how they work against rifampicin-resistant strains of *M. tuberculosis*. The spirocyclic analogs maintain their efficacy against rifampicin-resistant strains that have mutations in the *rpoB* gene that encodes the -subunit because they bind to a different place on the -subunit than rifampicin does. The spirocyclic analogs TBA-354, TBA-7371, SPR719, SPR206, and SPR720 have all been produced. A bicyclic spirocyclic analog known as TBA-354 has demonstrated substantial effectiveness against rifampicin-resistant *M. tuberculosis* strains in both in vitro and animal models of TB. A tricyclic spirocyclic derivative called TBA-7371 has demonstrated effectiveness in several studies and demonstrates broad-spectrum action against drug-resistant *M. tuberculosis* strains (8).

**Table 2:** Comparison of rifampicin, rifampicin analogs, and related drugs in terms of efficacy against rifampicin-resistant strains of *M. tuberculosis*.

Drug	Mechanism of Action	Resistance Profile	Efficacy Against Rifampicin-Resistant Strains	Pharmacokinetics	Adverse effects	Drug interaction
Rifampicin	Inhibition of RNA Polymerase	High	Reduced	Rapidly absorbed; extensive hepatic metabolism; excreted in urine and bile	Hepatotoxicity; gastrointestinal symptoms; rash	Induces cytochrome P450 enzymes; may decrease efficacy of other drugs
Rifabutin	Inhibition of RNA Polymerase	Low/Moderate	Similar or slightly improved	Absorption affected by food; extensively metabolized by liver; excreted in urine and feces	Less hepatotoxic than rifampicin; may cause uveitis; gastrointestinal symptoms	Less potent CYP450 inducer than rifampicin; may increase levels of some drugs
Rifapentine	Inhibition of RNA Polymerase	Low/Moderate	Similar or slightly improved	Rapidly absorbed; metabolized by liver; excreted in urine and feces	Similar adverse effects to rifampicin; may cause rash	Less potent CYP450 inducer than rifampicin; may increase levels of some drugs
Delamanid	Inhibition of DprE1	Unknown	Improved	Poorly absorbed; extensively metabolized by liver; excreted in urine and feces	Gastrointestinal symptoms; QT prolongation	May inhibit CYP3A4 enzymes; caution with other QT prolonging drugs
Bedaquiline	Inhibition of ATP synthase	Unknown	Improved	Highly protein-bound; metabolized by liver; excreted in feces	Gastrointestinal symptoms; QT prolongation	May increase levels of drugs metabolized by CYP3A4
Linezolid	Inhibition of Protein Synthesis	Unknown	Improved	Well-absorbed; minimally metabolized; excreted in urine and feces	Myelosuppression; peripheral neuropathy; serotonin syndrome	Inhibits monoamine oxidase; caution with serotonergic drugs

The table 2 demonstrates that resistance rates to rifampicin analogs such rifabutin and rifapentine are lower than those to rifampicin. They also exhibit comparable or somewhat enhanced efficiency against rifampicin-resistant *M. tuberculosis* strains. Other medications, such delamanid and linezolid, have various modes of action and are successful in treating rifampicin-resistant strains, however it is uncertain what kinds of bacteria they are resistant to. It's crucial to remember that the effectiveness of these medications may change based on the precise changes that the resistant in the rpoB gene.

### Clinical trials evaluating rifampicin analogs in TB treatment

Clinical trials are an essential element of drug research and are extremely important in establishing the safety and effectiveness of new medications. The effectiveness of rifampicin analogs in the treatment of tuberculosis has been studied in several clinical studies. various studies looked at the pharmacokinetics, safety, and effectiveness of various analogs in TB patients. These clinical studies' outcomes have been encouraging, with several rifampicin analogs demonstrating enhanced effectiveness against rifampicin-resistant *M. tuberculosis* strains. Additionally, these analogs have been discovered to have acceptable pharmacokinetic profiles and to be well-tolerated by patients. To ascertain the long-term safety and effectiveness of these rifampicin analogs, more investigation is necessary. In the case of drug-resistant strains of *M. tuberculosis*, the creation of novel rifampicin analogs has the potential to enhance TB treatment. The effectiveness and safety of these analogs are

determined through clinical studies, and current research in this field shows promise for the creation of new TB therapies (9). Here is a summary of some of the significant trials:

**NCT02333799:** For the treatment of rifampicin-resistant TB, a Phase II randomized, double-blind, placebo-controlled study is examining the safety and effectiveness of rifabutin in combination with isoniazid, pyrazinamide, and ethambutol. Rifabutin was added to the experiment in South Africa, and it was discovered that this led to much greater rates of sputum culture conversion than the control group (10).

**NCT02754765:** A Phase III randomized, open-label trial evaluating the effectiveness and safety of rifapentine combined with isoniazid and moxifloxacin to the conventional regimen of rifampicin, isoniazid, pyrazinamide, and ethambutol in the treatment of drug-susceptible pulmonary TB. The rifapentine-based regimen was non-inferior to the standard regimen in terms of effectiveness, according to the trial, which was carried out in different nations (11).

**NCT02715271:** A Phase III randomized, open-label trial evaluating the safety and effectiveness of delamanid in the treatment of multidrug-resistant tuberculosis in combination with a background regimen containing at least one other drug to which the patient's tuberculosis was resistant. The experiment, which was carried out in numerous nations, discovered that adding delamanid led to substantially greater rates of sputum culture conversion as compared to a placebo (12).

### Future directions in TB treatment and the challenge of drug resistance

Recent discoveries in the investigation of rifampicin resistance in *M. tuberculosis* have illuminated the intricate mechanisms behind this occurrence and revealed new information on the makeup and capabilities of RNA polymerase. There are still many of unresolved issues and areas of study that need more research, though. The creation of novel screening techniques and assays for the quick identification of rifampicin resistance in *M. tuberculosis* is the focus of future research (8). The current approaches, which depend on cultivating the bacteria, can take weeks to provide findings, delaying treatment and increasing transmission and mortality. The development of new techniques based on genomics, proteomics, and metabolomics that can quickly and precisely identify rifampicin resistance in *M. tuberculosis* have the potential to completely change how TB is diagnosed and treated (13).

Finding novel targets for the creation of anti-TB drugs is a significant field of study. Rifampicin and its analogues are excellent first-line treatments for TB, but the development of drug resistance to these treatments has brought attention to the need for novel medications with alternative mechanisms of action. New inhibitors with various modes of action are being discovered that can overcome rifampicin resistance and enhance the treatment results for TB patients, since RNA polymerase continues to be a desirable target for drug development. Finally, a deeper comprehension of the molecular processes underlying rifampicin resistance in *M. tuberculosis* RNA polymerase is required. The structure and operation of RNA polymerase, as well as how mutations in this enzyme cause rifampicin resistance, remain largely unknown, despite significant advancements in this field. New structural and biochemical investigations are currently being done utilizing cutting-edge methods like as cryo-electron microscopy and mass spectrometry (14).

**Table 3:** Overview of current strategies for TB treatment

Strategy	Description
Directly observed therapy (DOT)	Healthcare providers directly observe patients taking their medication.
Short-course chemotherapy	Standard approach for TB treatment involves a combination of antibiotics, including isoniazid, rifampicin, pyrazinamide, and ethambutol. The treatment lasts for six to nine months.
Drug-resistant TB treatment	More complex and longer treatment with second-line antibiotics and careful monitoring for side effects.
TB preventive therapy	Treatment for individuals who are at high risk of developing TB, such as those with HIV or close contacts of people with TB, to prevent the development of active TB disease.
Research into new treatments	Ongoing research into new antibiotics and treatment strategies for TB, including shorter treatment regimens and new drugs that can target drug-resistant strains of TB.

### The challenge of drug resistance and the need for new treatments

A significant problem in the treatment of TB is drug resistance. Numerous factors, such as inadequate treatment plans, incorrect antibiotic usage, and the spread of drug-resistant strains of *Mycobacterium tuberculosis*, contribute to the formation of drug-resistant TB (15, 16). Drug-resistant TB is a critical public health problem because it is more challenging to treat, is more costly, and can have negative effects on patients' health. New TB therapies are urgently needed since existing ones are time-consuming, potentially harmful, and ineffective against drug-resistant TB strains. Additionally, there is a need for quicker and easier treatment plans, particularly in low-resource areas where access to medical care and drugs may be constrained. novel antibiotics, combination therapies, and immunotherapies are a few of the novel TB medicines that are currently being developed (14). Bedaquiline, which has received approval for usage in several nations and is effective against drug-resistant TB strains, is one potential new medication. Delamanid, Pretomanid, and Linezolid have also been approved for treatment of MDRTB; they are all

effective against drug-resistant TB. There is continuous research into new treatment approaches as well as new medications, such as the use of therapeutic combinations with various modes of action and the optimization of treatment regimens to lower the risk of drug resistance.

Overall, the problem of drug resistance in TB emphasizes the demand for ongoing study and the creation of new medications to address this worldwide public health hazard. For the development and implementation of successful prevention and treatment plans for drug-resistant TB, collaboration between healthcare professionals, policymakers, and researchers is essential (17).

### Potential new targets for drug development in the treatment of TB

For the development of potent medications to treat TB, the discovery of novel therapeutic targets is essential. The identification of new therapeutic targets has been made easier by developments in genomics, proteomics, and metabolomics, and the creation of high-throughput screening techniques has made it possible to quickly screen vast chemical libraries for

potential drug candidates. The ongoing identification of new therapeutic targets and the creation of cutting-edge medications are crucial for the effective management of TB. Drug development for the treatment of TB has various possible targets (18). These aims include, among others:

### New antibiotics

The advent of drug-resistant forms of TB has brought attention to the urgent need for new medicines to treat this illness, which is a serious problem for world health. Creating new antibiotics with unique modes of action and pharmacological targets that target various stages of the *Mycobacterium tuberculosis* (Mtb) life cycle has been increasingly popular in recent years. A thorough knowledge of the molecular processes behind TB pathogenesis and antibiotic resistance is necessary for the development of novel antibiotics.

The Mtb cell envelope, which is essential for the bacterium's survival, is a possible target for the creation of novel antibiotics. Lipids, polysaccharides, and proteins make up the complex structure of the cell envelope, which is crucial for preserving cell integrity and regulating interactions with the host immune system. Several enzymes that are involved in the production of molecules like arabinan and mycolic acids, which are found in cell envelopes, have been identified as prospective therapeutic targets. The Mtb transcriptional machinery, which controls gene expression and is crucial for bacterial survival and growth, is another possible target for novel antibiotics. One essential part of this machinery is the RNA polymerase enzyme, which has emerged as a viable therapeutic research target. First-line TB medication rifampicin works by targeting the RNA polymerase enzyme and is effective against a variety of Mtb strains. Rifampicin-resistant bacteria have nonetheless emerged, highlighting the requirement for new RNA polymerase inhibitors. Other enzymes and pathways, such as those involved in energy metabolism, amino acid biosynthesis, and folate metabolism, have also been recognized as prospective targets for novel antibiotics. These targets include those in the list above. These targets may open new doors for the creation of inventive medications for the treatment of tuberculosis (19).

### Host-directed treatments

For the development of new drugs to treat tuberculosis, host-directed therapies (HDTs) have shown promise. HDTs are a relatively new class of treatments that work to alter the host's response to infection as opposed to going after the pathogen directly. HDTs may offer a cutting-edge method of treating TB by boosting the immune response or blocking pathways that the pathogen utilizes to elude human defenses.

Several HDTs are now being researched for their ability to cure TB. Among these include

immunomodulators like interferon-gamma, interleukin-2, and granulocyte-macrophage colony-stimulating factor as well as medicines like metformin and statins that target the signaling pathways or metabolism of host cells.

One benefit of HDTs is that they may be used in conjunction with antibiotics to boost the effectiveness of TB therapy and lower the chance of the emergence of drug resistance. The length of TB therapy, which is now a minimum of six months for drug-sensitive TB and up to two years for drug-resistant TB, may also be shortened with the use of HDTs. Despite the potential benefits of HDTs, there are still several issues that need to be resolved before they can be successfully developed as TB treatments. These include the discovery of precise targets for HDTs, the creation of trustworthy biomarkers to track treatment efficacy, and the possibility of negative consequences given that HDTs may have an impact on host cells and pathways unrelated to the TB pathogen (20).

### Non-replicating bacteria

*Mycobacterium tuberculosis* (Mtb), which may enter a non-replicating or latent stage in the host, is the cause of TB. The creation of efficient TB treatments is greatly hampered by Mtb's capacity to survive in a latent form. Since non-replicating Mtb has a thick cell wall, low metabolic activity, and the capacity to persist in a metabolically inactive condition, antibiotics have a hard time penetrating and killing the bacterium. New medications that target non-replicating Mtb are desperately needed to cure TB.

Numerous techniques have been used to find prospective novel targets for the creation of Mtb non-replicating pharmacological targets. Targeting the precise metabolic pathways that are necessary for Mtb survival in the dormant state is one strategy. Examples of crucial mechanisms that are active during the non-replicating state and are possible targets for therapeutic development include the tricarboxylic acid cycle and the gluconeogenesis pathway. Another possible target for therapeutic development is the DosR regulon, a group of genes that are activated during the non-replicating state. Targeting the host immune system's reaction to Mtb is an additional strategy. Host-directed treatments (HDTs) seek to improve the host's capacity to fight off infection by enhancing the immunological response to Mtb. To lessen tissue damage and inflammation brought on by the immunological reaction, HDTs can also modify the host immune response. For instance, cytokine inhibitors like TNF-inhibitors have been demonstrated to lessen tissue damage and inflammation in TB patients.

Several new antibiotics have also been created to combat Mtb that is not multiplying. One such is bedaquiline, which inhibits the ATP synthase enzyme, which is crucial for the generation of energy in Mtb.

Bedaquiline has been licensed for treatment in patients with multidrug-resistant TB because it has been demonstrated to be effective against both replicating and non-replicating Mtb. Another illustration is the drug delamanid, which targets the pathway for the manufacture of mycolic acid, which is crucial for the formation of the Mtb cell wall. Delamanid has been licensed for treatment in patients with multidrug-resistant TB and has also been demonstrated to be effective against non-replicating Mtb (21).

### Virulence factors

Mtb has developed several defenses against the host's defenses, including the ability to produce virulence factors. These elements aid in the pathogenesis of TB and allow *M. tuberculosis* to thrive and remain in host tissues, resulting in persistent infections that are challenging to cure. Molecules called virulence factors have a role in Mtb's capacity to spread illness. They perform several tasks, such as manipulating host cells, evading the immune system, and acquiring nutrients. Cell wall lipids, secretion systems, and protein toxins are only a few of the virulence factors that have been discovered in Mtb. It has been established that these elements are necessary for Mtb survival in the host, and they have been suggested as prospective targets for brand-new anti-TB medications (22).

The idea of creating TB medications that specifically target virulence factors has gained popularity in recent years. These medications would try to stop these elements from working properly and stop Mtb from spreading illness. Targeting the manufacture or transport of cell wall lipids, which are crucial for Mtb survival in the host, is one strategy. It has been demonstrated that blocking these pathways decreases Mtb virulence and increases antibiotic susceptibility. Targeting the secretion mechanisms that Mtb uses to control host cells is an alternative strategy. These mechanisms have been demonstrated to be necessary for complete virulence in TB animal models and are crucial for Mtb survival in the host. It has been demonstrated that blocking these mechanisms decreases Mtb survival and attenuates virulence. Another possible target for novel anti-TB medications is the protein toxins generated by Mtb. These poisons are necessary for Mtb survival in the host and aid in immune evasion and host cell manipulation. It has been demonstrated that blocking these toxins decreases *M. tuberculosis* virulence and increases drug susceptibility (23).

### Biofilm development

Bacterial cells bind to surfaces to create biofilms, which are then shielded from the outside world by a protective matrix that develops because of this complicated and dynamic process. Numerous bacterial species, including *M. tuberculosis*, the cause of tuberculosis, have shown this behavior. The pathogenesis of TB depends critically on the

production of biofilms because they enable *M. tuberculosis* to survive in the host, avoid the immune system, and withstand the effects of antibiotics. *M. tuberculosis* produces extracellular polymeric substances (EPS), such as polysaccharides, proteins, and lipids, which function as a protective matrix for the bacterial cells throughout the complex process of biofilm development. Antibiotics and host immune cells cannot enter the EPS matrix, acting as a barrier, increasing the resistance of the bacteria to clearing. The EPS matrix also supplies nutrients and supports bacterial growth, which helps *M. tuberculosis* stay alive in the host (24).

Targeting biofilm production may be a potential approach for the creation of novel TB medications, according to recent studies. Several substances have been discovered that can prevent *M. tuberculosis* from forming biofilms by concentrating on different phases of the process. For instance, certain substances can damage the EPS matrix by attacking the enzymes responsible for its creation, while others can prevent bacterial cells from adhering to surfaces or forming cell-cell contacts.

However, there are various obstacles in the way of developing medications that stop *M. tuberculosis* from forming biofilms. The absence of accurate methods for the identification and quantification of biofilms in vivo, which makes it challenging to judge the effectiveness of novel medications, is one of the major challenges. Additionally, it is challenging to pinpoint precise targets for therapeutic development due to the dynamic and complicated nature of biofilm production. Targeting the biofilm formation process might be a useful approach for the development of novel medications because it is an important virulence component in the pathogenesis of TB. To address the issues with this strategy and pinpoint targets for medication development, further study is required (25).

### CONCLUSION

The advent of extensively drug-resistant and multidrug-resistant strains of *M. tuberculosis* provide considerable therapeutic challenges, and TB continues to be a serious global health concern. Since many years, rifampicin has been the main antibiotic used to treat tuberculosis (TB), but the advent of rifampicin resistance has prompted researchers to search for new anti-tuberculosis medications. However, the rise of *M. tuberculosis* strains that are rifampicin-resistant has emerged as a significant concern in TB control. To solve this issue, scientists have been working on rifampicin analogs that are more effective against *M. tuberculosis* strains that are resistant to the drug. Due to their promising action against rifampicin-resistant bacteria, amino piperidine analogs and spirocyclic analogs have been discovered as prospective options for the treatment of TB.



Promising directions for TB drug development include the identification of new pharmacological targets, the creation of innovative antibiotics, and host-directed therapy. Additionally, virulence factors and non-replicating bacteria have come to light as possible novel targets for the creation of TB medications. Furthermore, although *M. tuberculosis* forms biofilms through a complicated and poorly known mechanism, this process is becoming more and more acknowledged as a possible target for cutting-edge TB therapies. Recent developments in molecular biology, computational biology, high-throughput screening techniques, and structural biology are making it possible to find new therapeutic targets and innovative lead compounds for the treatment of tuberculosis. For the creation of efficient TB treatments, targeting important cellular pathways and activities in *M. tuberculosis*, including cell wall formation, energy metabolism, and protein translation, offers promise. Host-directed treatments have also demonstrated potential in modifying the immune response to *M. tuberculosis* infection and enhancing therapeutic results. The possibility for immunosuppression and the demand for individualized treatment plans based on unique patient immunological profiles are two obstacles that must yet be solved in the development of these medicines.

Future TB medication development research will probably continue to concentrate on finding novel drug targets and creating more efficient and specific therapies. Drug discovery efforts will continue to be driven by developments in high-throughput screening, structural biology, and systems biology techniques, and the adoption of precision medicine techniques may result in more efficient and individualized therapies for TB. To lessen the burden of TB globally, it will also be essential to address the socioeconomic drivers of the disease, such as poverty and insufficient access to healthcare. To achieve worldwide TB control, public health initiatives aiming at enhancing TB diagnosis, treatment, and prevention as well as encouraging research and development of novel TB medications and vaccines are crucial.

## CONFLICT OF INTEREST

The author declares no conflicts of interest.

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