A Review on Antitubercular Drugs: Mechanism of Actions, Resistance, SARs & Synthesis

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelors in Pharmacy (B.Pharm.)

School of Pharmacy
Brac University
September 2024

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
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Approval

The thesis titled "A Review on Antitubercular Drugs: Mechanism of Actions, Resistance, SARs & Synthesis" submitted by Lamisa Nur (20146080), of Spring, 2020 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

The study does not involve any animal or human clinical trials.

Abstract

Mycobacterium tuberculosis (M.tb) is considered the second leading cause of death after COVID-19. The rate of mortality and morbidity is increasing day by day due to its resistance to drugs. People in developing countries tend to ignore the symptoms of tuberculosis which leads to not taking proper treatments and more transmission. Researchers from different countries are focusing on the discovery and development of anti-tubercular drugs. The main focus is to eradicate tuberculosis completely from the world through a specific therapy. Ensuring early diagnosis and appropriate treatment can help stop the transmission of M.tb. This paper reviews the comprehensive details regarding the diagnoses and treatments of M.tb as well as the mechanism of actions and synthetic strategies of current anti-tubercular drugs. This review focusses on both convention and latest information so that it can help researchers to know and understand about the tuberculosis and aid development of new anti-tubercular drugs.

Keywords: Mycobacterium tuberculosis; anti-mycobacterial activity; mechanisms; resistances; synthetic approaches; SARs.

Dedication

I want to dedicate this to my parents, my grandparents, and my family who supported and believed in me throughout this journey. I also want to thank my friends for motivating me whenever I thought I wouldn't be able to finish it. Your unwavering friendship kept me going. Finally, thanks to my sanity for coping with this project's stress and late nights.

Acknowledgement

Firstly, I want to express my gratitude to the Almighty for the countless blessings that have supported me on this journey. I would like express my sincere gratitude to my supervisor, Dr. Humair Bin Md Omer, Assistant Professor, School of Pharmacy, BRAC University for his unwavering guidance and support throughout this project. Also, I am grateful to all the faculty members and seniors of the School of Pharmacy for their guidance, motivation, and love.

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List of Acronyms

AIDS	Acquired Immune Deficiency Syndrome
Anti-TB	Anti-Tubercular
ART	Antiretroviral Therapy
ATBI-HIV	Active Tuberculosis Infection- Human Immunodeficiency Virus
BDQ	Bedaquiline
CDI	1,1′ Carbonyldiimidazoles
CFX	Clofazimine
COPD	Chronic Obstructive Pulmonary Disease
DDIs	Drug-Drug Interactions
DIPEA	*N*,*N*-Diisopropylethylamine
DLM	Delamanid
DM	Diabetes Mellitus
DMF	Dimethyl Formamide
DMP	Dess–Martin Periodinane
DMSO	Dimethyl Sulfoxide
DM-TB	Diabetes Mellitus-Tuberculosis
DS-PTB	Drug-Susceptible Pulmonary Tuberculosi
DS-TB	Drug Susceptible-Tuberculosis

ELISA	Enzyme-linked Immunosorbent Assay
EMB	Ethambutol
EPTB	Extrapulmonary Tuberculosis
GMP	Good Manufacturing Practices
HIV	Human immunodeficiency Virus
IFNγ	Interferon-γ
IGRA	Interferon-gamma Release Assay
ISN	Isoniazid
LAMP	Loop-mediated Isothermal Amplification
LTBI-HIV	Latent Tuberculosis Infection-Human Immunodeficiency Virus
M.tb	Mycobacteria Tuberculosis
MDR-TB	Multidrug-Resistant Tuberculosis
miRNA	MicroRNA
MMPs	Matrix Metalloproteinases
MOX	Moxifloxacin
murA	UDP-N-acetylglucosamine enolpyruvyl transferase
NGS	Next Gene Sequencing
NK cells	Natural Killer Cells
PMD	Pretomanid

Pre-XDR-TB	Pre-extensively Drug-Resistant Tuberculosis
PTB	Pulmonary tuberculosis
PZA	Pyrazinamide
RIF	Rifampicin
RMP	Rifampicin
RRDR	RIF Resistance-Determining Region
RR-TB	Rifampicin Resistance-Tuberculosis
TB	Tuberculosis
TDM	Trehalose Dimycolate
TEA	Triethylammonium Carboxylate
THF	Tetrahydrofuran
TST	Tuberculin Skin Tests
WHO	World Health Organization
XDR-TB	Extensively Drug-Resistant Tuberculosis

Chapter 1

1.1 Introduction to Tuberculosis (TB)

Global morbidity and mortality rates are increasing due to tuberculosis, which originates from Mycobacterium tuberculosis. Infection affects approximately one-third of the population worldwide, comprising 85% of pulmonary tuberculosis cases [1] and 15% of extrapulmonary tuberculosis cases.[2] As per the World Health Organization, this infectious disease have affected around 10.6 million individuals by 2023[3], with developing countries accounting for over 90% of both mortality and morbidity rates.[4] TB, considered an airborne disease, spreads through coughing or sneezing. It's frequently seen in individuals who have COPD, HIV, and diabetes.[5] Hemoptysis, pneumonia, bronchial stenosis, airway blockage, and liver damage are potential contraindications.[5] Regrettably, there is no identified treatment yet capable of fully eliminating latent TB. Physicians frequently face difficulties in promptly diagnosing and treating tuberculosis patients due to the disease's complexity. Despite the increasing prevalence of resistance to antimicrobial drugs in Mycobacterium tuberculosis, it is critical to strengthen and augment the World Health Organization's END-TB approach to more effectively combat tuberculosis. Hence, a crucial objective in eradicating tuberculosis is to disrupt the spread of the disease. Researchers are trying to develop and repurpose synthetic methods of anti-tubercular drugs to find a permanent treatment to eradicate tuberculosis. In this paper, synthetic methods of existing anti-tubercular drugs along with their repurposed methods have been discussed. Ongoing synthetic methods of few anti-tubercular drugs have been mentioned here too.

1.1.1 Prevalence

Globally, tuberculosis detection and therapy progressed significantly in the year 2022, but it continued to be among the top two causes of mortality as worldwide targets remained unmet.

Global TB cases increased to 10.6 million in 2022 from 10.3 million in 2021 and 10.0 million in 2020.[4] However, the decline in TB-related mortality from 2015 to 2022 was a mere 19%, falling short of the 75% reduction target set by the End TB planning of WHO for 2025.[4] Comparably, the anticipated global incidence rate of tuberculosis was 95% in 2022, down 8.7% from 2015, but still well short of the WHO's target of a 50% decrease by 2025.[4] In terms of demographics, in 2022, 33% of TB cases were in women, 12% were children under the age of 14, and 55% of cases involved men.[4] 192 nations, including all those with a significant TB burden, submitted data on TB cases in 2023.[4] The countries with the highest percentages of tuberculosis cases worldwide are seen in **Figure 1**.[4] The National Tuberculosis Control Programme showed Bangladesh diagnosed 301,564 TB patients in 2023, but 20% cases continue unreported.[6] Reducing the spread of tuberculosis in South Asia is quite a challenge due to factors like economic inequality, inadequate healthcare systems, low literacy levels, and insufficient funding.[2]

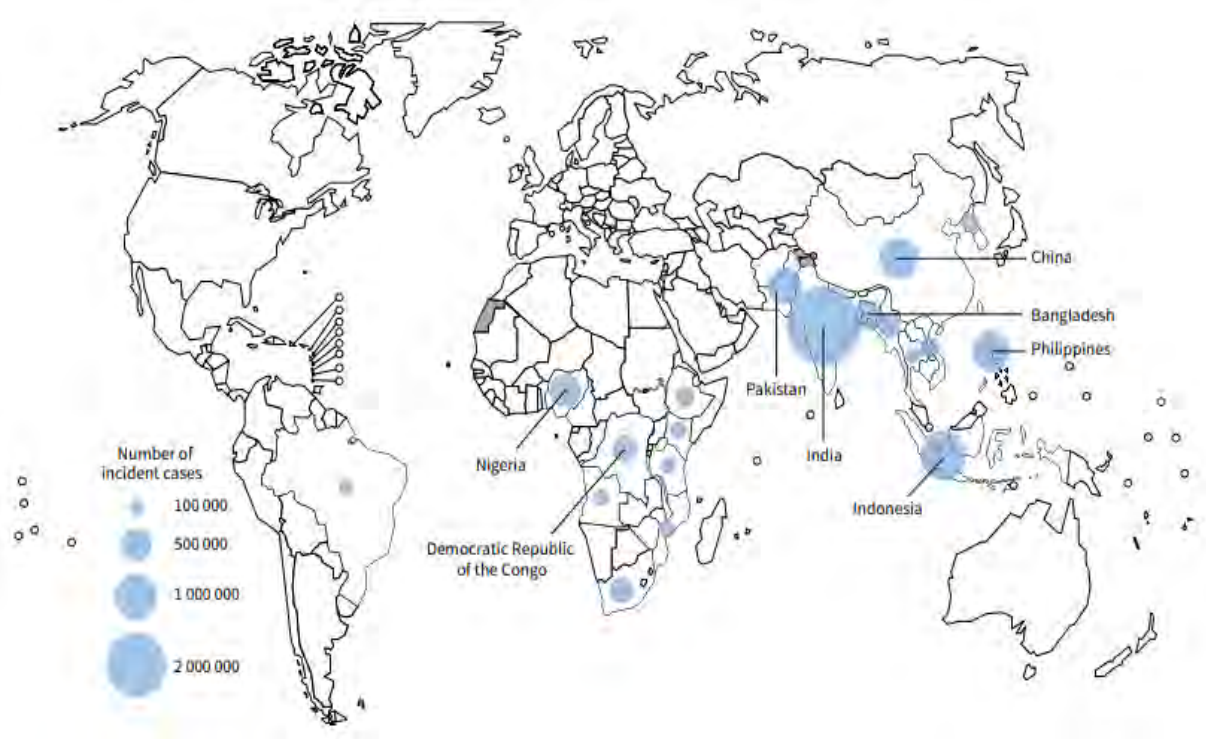

Figure 1: Predicted prevalence of incident TB cases in 2022 for nations having a minimum of 100,000 reported cases (Global Tuberculosis Report 2023, 2023).

1.1.2 Disease Mechanism

The germs are disseminated through the atmosphere and invade the lungs when TB patients cough, sneeze, or speak. The immune system is triggered by tuberculosis infection, but if it is unable to eradicate the germs, they grow within alveolar macrophages.[7] As the germs are discharged and ingested by further macrophages, the cycle continues. A cell-mediated immune response is triggered when lymphocytes are drawn to the infection site. The course of action tries to preserve the germs, which might then be destroyed or have latency inside the granuloma (**Figure 2**).[7]

It is difficult to treat TB because the bacteria can grow within granulomas, wherein immune system cells change shape.[7] The bacteria produce foam cells and caseum, which provide the bacteria a place to reside as a result of their disruption of lipid metabolism.[7] Foam cells are formed predominantly by the mycolic acids present in the bacterial cell wall. The bacteria can stay latent within the caseous lesions, but they may eventually become active and spread to other hosts (**Figure 2**).[7] To restrict bacterial reproduction and stop the spread of illness, the host's immune system must function properly.

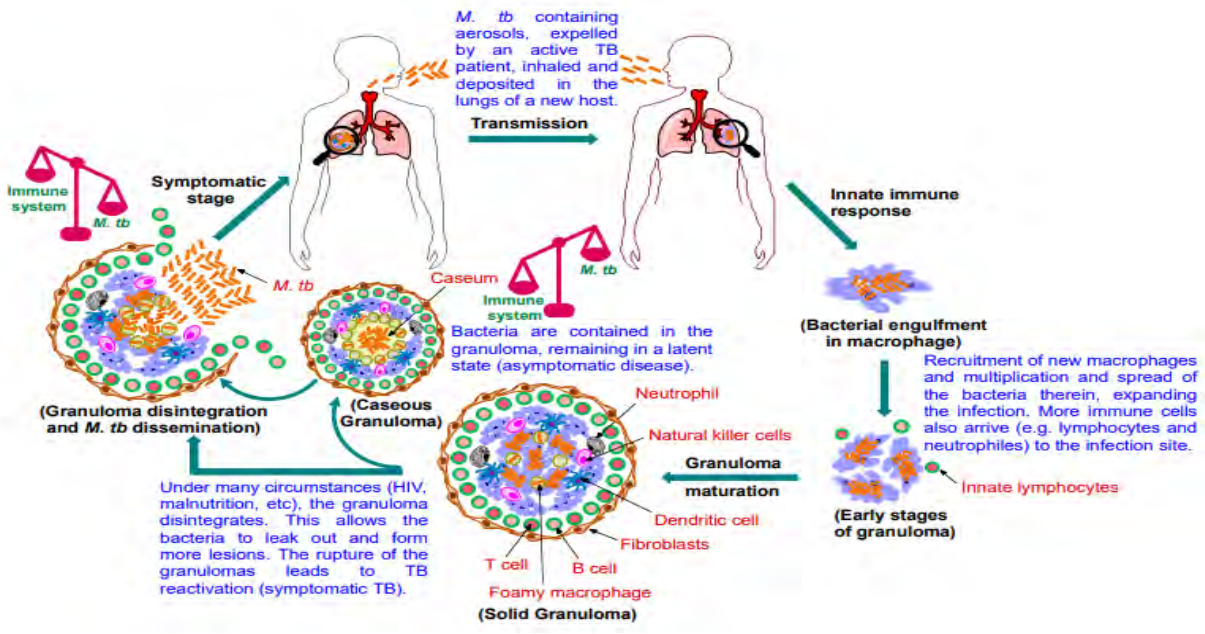

Figure 2: Disease mechanism of pulmonary Tuberculosis (Alsayed & Gunosewoyo, 2023).

Reactivation of TB can be caused by HIV co-infection, but other variables including chemotherapy, immune-suppressive drugs, smoking, and malnourishment may trigger it. [7] Dormant TB bacteria revive when the immune system is compromised. This results in the liquefaction of the granuloma structure and the release of infectious bacteria and damage to the lungs.[7] The infection grows with the nutrients from the caseous material, leading to highly contagious active tuberculosis (**Figure 2**). Reactivation of tuberculosis is thought to be associated with the advancement of granuloma.[7]

1.1.3 Resistance Mechanism

The global therapeutic control of tuberculosis is challenged by drug resistance resulting from prescription abuse. Along with other problems, it concerns patients who stop therapy before the recommended course of action is finished and who receive treatment for longer than what is recommended by doctors. Three recognized types of drug resistance in tuberculosis are multidrug-resistant tuberculosis, pre-extensively drug-resistant tuberculosis, and extensively drug-resistant tuberculosis.[8]

Individuals with **XDR-TB** have few treatment options because their tuberculosis is resistant to second-line injectable medications such as amikacin, kanamycin, capreomycin, and fluoroquinolones.[8] This is particularly alarming because it raises the chance of developing tuberculosis and death in people with weakened immune systems, like those living with HIV. A phase among **MDR-TB** and **XDR-TB** known as **Pre-XDR-TB** is characterized by resistance to an injectable second-line medication or fluoroquinolone.[8] For pre-extensively drug-resistant tuberculosis (**Pre-XDR-TB**) and extensively drug-resistant tuberculosis (**XDR-TB**), a range of second-line drugs may be administered during the 14–24-month course of treatment.[9] **MDR-TB** is the term for a tuberculosis infection that is resistant to both isoniazid and rifampicin.[8] Infractions, ineffective national programs, and inadequate patient care are the

primary factors. It's a new global health problem that requires expensive medications to be taken for at least 18 months.[8] Diagnostics for MDR-TB include genotypic assays, microscopic-observation drug susceptibility, and the nitrate reductase assay.[8]

Ethambutol, Pyrazinamide, Rifampicin, and Isoniazid are the main medications used to treat TB.[10] These drugs work by inhibiting mycolic acid production, suppressing transcription, preventing cell wall construction, and interfering with energy metabolism.[10] Despite the global increase in drug-resistant strains of tuberculosis, combining these medications reduces the risk of recurrence.[10] Mycobacterium tuberculosis evades treatment through various mechanisms, including target mimicry, drug modification and degradation, efflux pumps, and cell wall impermeability, which can be classified as acquired and intrinsic resistance mechanisms.[10]

1.1.3.1 Intrinsic Resistance Mechanism

Mycobacterium tuberculosis bacteria are naturally resistant to various treatments because of their thick, hydrophobic cell wall, which prevents medication penetration.[10] Arabinogalactan in the mycomembrane functions as a barrier, while β-lactamase enzymes can limit antibiotic efficiency.[10] Some bacteria have impermeable cell walls, which prevent antibiotic entrance and promote drug resistance.

M. tuberculosis's resistance to fosfomycin is due to TDM synthesis by ag85 gene-encoded proteins, which create an impenetrable cell wall.[10] Deactivation of the ag85 gene restores drug sensitivity. Porins in the outer cell wall allow for limited material penetration due to a lipid-based structure with fewer porins.[10] Despite this, the outer membrane protein CpnT allows antibacterial drugs to be effective against microorganisms.[10] M.tb efflux pumps eliminate anti-TB drugs such as isoniazid, streptomycin, rifampicin, bedaquiline, ethambutol, clofazimine, and fluoroquinolones, which contribute to drug resistance.[10]Antibiotics are

modified by enzymes such as β-lactamases and MfpA, and medicine binding is prevented through target modification. Overexpression of efflux pump genes like drrA, MMR, jefA, etc. can lead to resistance against isoniazid, streptomycin, and ethambutol.[10] The molecular mechanism of intrinsic drug resistance is shown in **Figure 3**.[11]

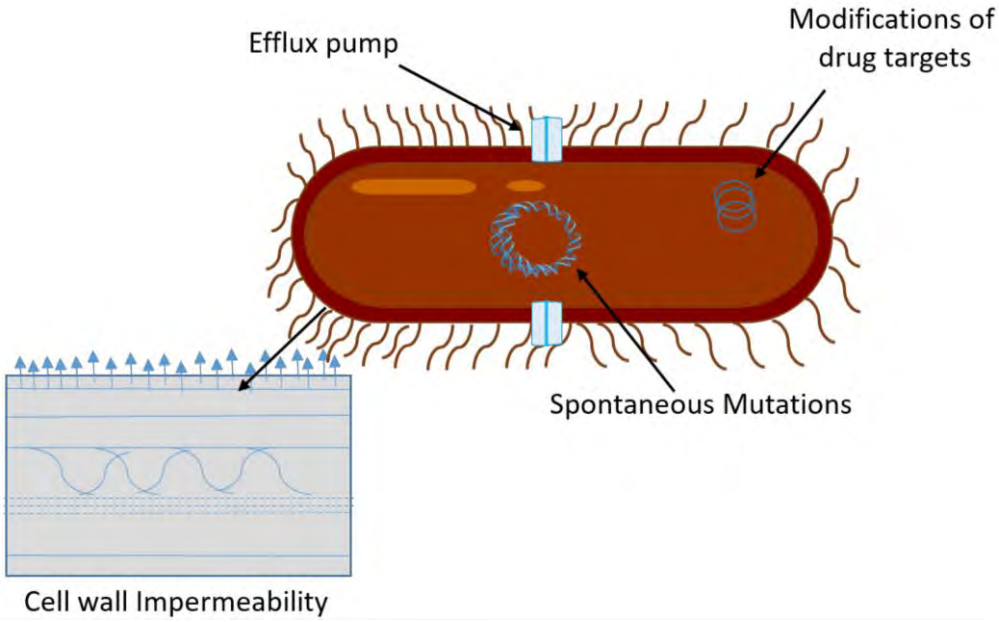

Figure 3: Graphical representation of the intrinsic mechanism by which M.tb generates resistance to drugs (Rabaan et al., 2022a).

1.1.3.2 Acquired Resistance Mechanism

M.tb drug resistance can be caused by a variety of causes, including chromosomal mutations that cause prodrugs to be inactivated, drug target alteration, or drug-specific target overexpression.[10] The most prevalent cause of acquired resistance is the alteration of medication targets. M. tb is frequently resistant to a variety of medications, including aminoglycosides, cyclic peptides, rifampicin, para-aminosalicylic acid, isoniazid, oxazolidinone, and fluoroquinolone, due to nucleotide substitutions in the operon generating rRNA or changes in the genes responsible for the drugs.[10] Drug resistance can be caused by prodrug inactivation, point mutations, or insertions/deletions in mycobacteria's chromosomes.

The probability of getting multidrug-resistant TB increases with the number of gene alterations. These mutations are typically found within an 81-base pair area of the rpoB gene's RRDR.[10] When a nonsynonymous mutation in the RRDR replaces an amino acid with a shortened side chain with one with a longer side chain, impacting the RNA polymerase active site, the binding is inactivated and rifampicin resistance is conferred.[10] The molecular mechanism of intrinsic drug resistance is shown in **Figure 4**.[11]

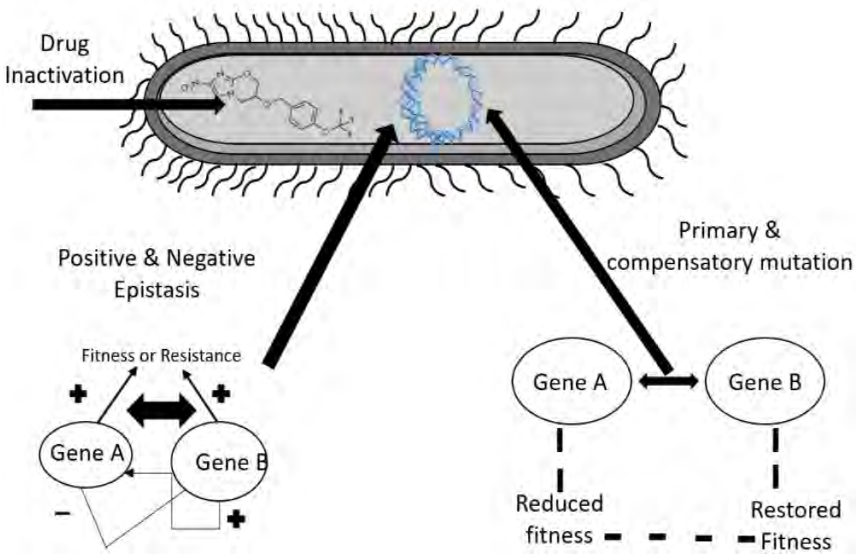

Figure 4: Graphical representation of the acquired mechanism by which M.tb generates resistance to drugs (Rabaan et al., 2022b).

Chapter 2

Material & Methodology

This review paper examined various sources including Google Scholar, PubMed, MDPI, Wiley Online Library, BMC Infectious Diseases, ScienceDirect, Biomedicine, Royal Society of Chemistry, ACS Publications, International Journal of Molecular Sciences, Cellular, Molecular and Biomedical Reports, and Frontiers for literature on anti-tubercular drug mechanisms and synthetic methods. The statistical analysis in this review also considered reports from WHO's Global Tuberculosis Reports, the National Tuberculosis Control Programme, the US Centers for Disease Control and Prevention, and the Morbidity and Mortality Weekly Report. The keywords used in the search included terms such as general TB information, global TB prevalence, TB diagnosis, TB therapy and challenges, TB medication resistance, pharmacological effects, and synthetic methods for anti-TB pharmaceuticals. A total of 221 papers from 2018-2024 were collected and grouped into different topics. 31 papers were selected and organized under general introduction, diagnosis, treatment, vaccine, and synthetic methods. Additional papers were collected during the writing process, focusing on synthetic methods for specific drugs like Isoniazid and Rifampicin.

Chapter 3

3.1 Diagnosis of Tuberculosis

Tuberculosis is difficult to diagnose since its indications resemble those of other respiratory ailments.[9] Effective intervention with a precise diagnosis was essential for the United Nations to reach its 2022 treatment goal of 40 million drug-sensitive tuberculosis patients and 1.5 million drug-resistant TB patients.[12] Numerous diagnostic techniques have been developed for detecting tuberculosis (TB), including conventional approaches such as acid-fast bacillus smear microscopy and microbial culture, and advanced techniques such as CRISPR Cas, Gene Xpert, and LAMP. [9] Conventional methods are still widely employed in high TB burden nations regardless of their lagging and lengthy nature because of financial and accessibility problems.[12] Nanotechnology and photonics are being used to screen and treat tuberculosis, offering precise capabilities to determine suitable medications and therapies.[12] A graphical depiction of the techniques of diagnosis for variant TB infections is shown in **Figure 5**.

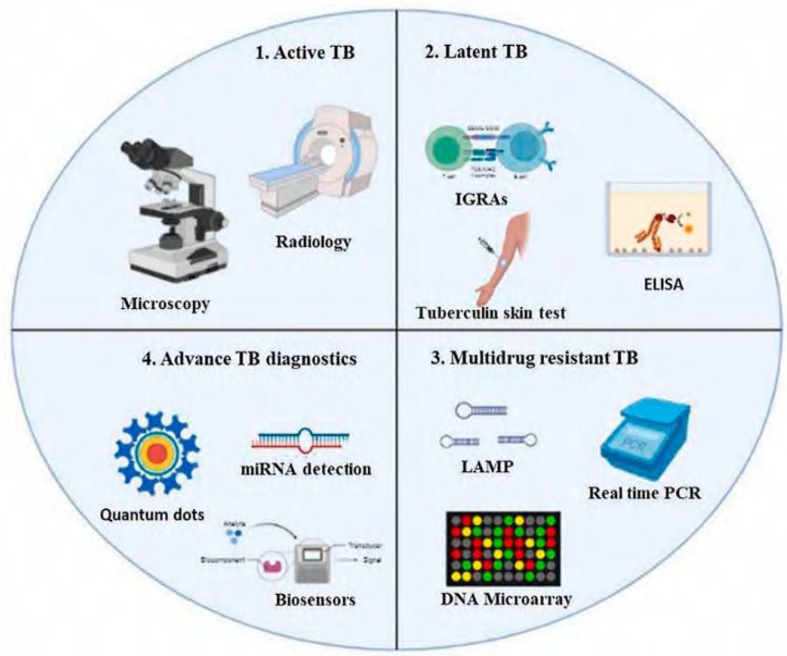

Figure 5: Different techniques of diagnosis for variant TB infections (Mukherjee et al., 2023a).

3.1.1 Conventional Diagnostic Methods

Active tuberculosis is frequently diagnosed by a microscopical investigation by collecting samples of infected individuals to detect the bacteria using the microscope.[12] The usual recommendation is light-emitting diode fluorescence microscopy.[12]

Radiological imaging such as CT scans, PET-CT scans, chest X-rays, and MRI scans is essential when diagnosing active pulmonary TB.[12] [13] Chest X-rays assess the lungs and pleural membrane, CT scans differentiate TB from pneumonia and show disease progression, MRI scans provide detailed lung anomalies without radiation, and PET-CT scans detect TB granulomas and examine specific body parts. The radiological report is provided on the same day. [12]

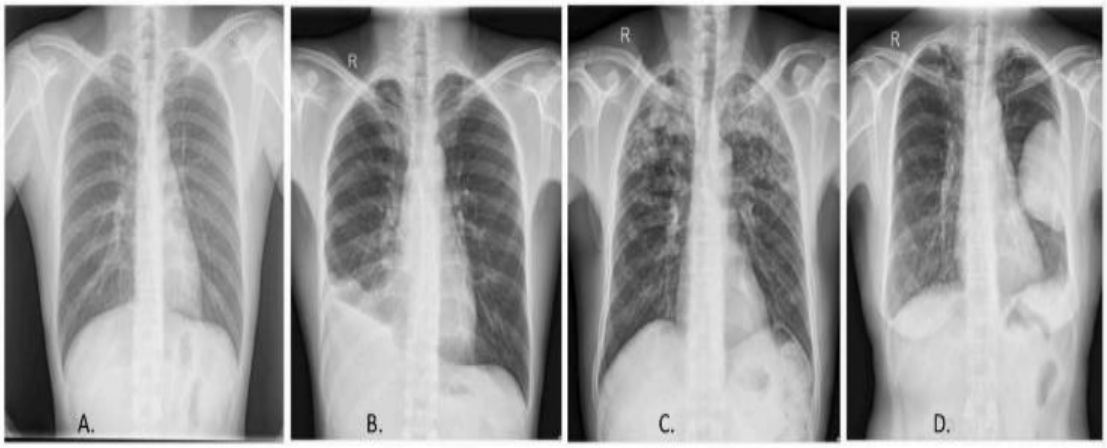

Figure 6: Chest X-ray in (A) indicates normal X-ray, whereas (B), (C), & (D) X-rays expose pleural effusion, infiltrates, and cavity lung lesions (Mukherjee et al., 2023b).

T-cell immunity to the mycobacterial antigen is detected using the Tuberculin skin test.[12] A protein-derived derivative is injected into the forearm, leading to swelling and redness in the area 48–72 hours later.[12] However, the test can produce inaccurate results due to its inability to differentiate between tuberculosis infection and other factors like BCG vaccination.[12]

Despite its limitations, the Tuberculin skin test remains the primary method for detecting Mycobacterium tuberculosis.

Other conventional methods to diagnose TB are IGRAs, ELISA, Drug susceptibility testing, Line probe assay, and LAMP.[12] Above all, the conventional methods have drawbacks for its limited sensitivity, false negatives, limited efficiency, tedious findings, difficulty in distinguishing amongst strains, and lack of viable bacteria testing.[12]

3.1.2 Advanced Diagnostic Methods

Better TB diagnosis tests need technological advances. Drug-sensitive and drug-resistant microorganisms can be effectively identified for precise treatment using sophisticated tests such as CRISPR, mass spectrometry, whole genome sequencing, nanodiagnostics, and digital PCR (**Figure 7**).[14][15]

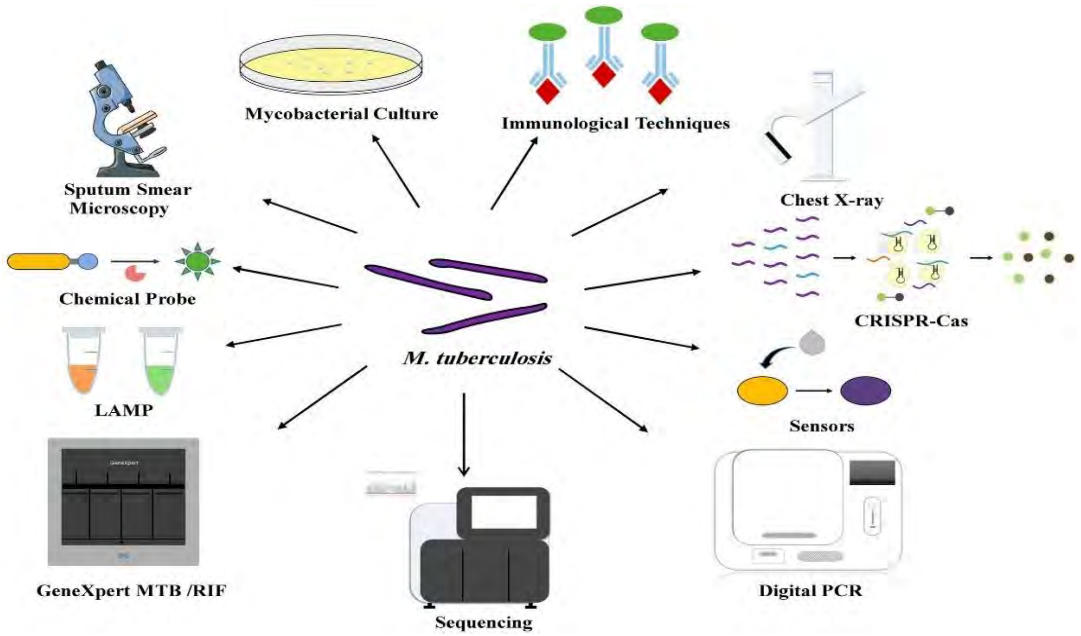

Figure 7: Different advanced techniques to detect TB infection (Dong et al., 2022).

Diagnoses like **Xpert MTB/RIF** test may be made more precise and consistent with **digital PCR**.[12] It can detect tuberculosis outside the lungs even in cases where DNA levels are low.[12]

With merely a few copies, the technique can magnify and yield precise findings.[12] Five hours pass between results and conventional **DST** testing, which takes four days.[12] The lower spread of TB is the result of this improved detection of complex diseases.

The cutting-edge method known as **NGS** amplifies the genes linked to treatment resistance in tuberculosis infections to identify antibiotic resistance.[12] Its great sensitivity allows it to detect several genes at once by giving precise details.[12] Duration and expense have been lowered by the latest technologies in NGS, including vast parallel gene sequencing methods.[12] The precise identification is hampered, though, because it is rarely accessible in nations with minimal incomes.[12]

CRISPR Cas technology is considered the easiest method to detect the TB. Recombinase polymerase amplification and CRISPR Cas 12a are used to provide a 100% specificity rate and 99.29% sensitivity rate.[12] This technique may be used with existing methods to boost efficiency.[12] Drug resistance for first- and second-line medications can be genetically revealed and many drug response sites can be concurrently detected via **CRISPR**.[12] Regarding susceptibility and turnaround time, it outperforms culture and **Xpert MTB/RIF**. [12]

MiRNA identification contributes to knowledge of the infectious phases of Mtb in an infected individual.[12] Initial detection to avert tuberculosis spread and resistance to RNA breakdown are two benefits of miRNA as a biomarker. The part that miRNA plays in drug resistance has to be investigated more.[12] TB expression measurement and treatment require an integrated miRNA and proteome strategy.[12]

Rapid and effective point-of-care testing for viral diseases and TB is made possible by **nanotechnology**.[12] Because of their special optical and magnetic characteristics, potential nanomaterials are quantum dots, nano-shells, gold nanoparticles, and nanotubes.[15] The most recent advances in nanomedicine are microfluidic or lab-on-a-chip systems, including several

DNA analysis methods on a silicon and glass platform with fluorescence detectors, electrophoresis chambers, heaters, and temperature sensors.[15] Therefore, advanced methods like digital PCR, CRISPR-Cas, and nano diagnostics in conjunction with standard techniques would enable a prompt diagnosis and effective therapeutic strategy.[12]

3.2 Advanced TB Vaccination Approaches

The only approved TB vaccine is **BCG**, suitable for most people except those with HIV or other immunological disorders.[16] BCG provides up to 10 years of protection against meningeal and disseminated tuberculosis.[16] Currently, 21 vaccines are in clinical evaluation for tuberculosis, including **live whole-cell**, **inactivated whole-cell**, **mRNA**, **subunit**, and **viral vector vaccines** as shown in **Table 1**.[16]

Vaccination trials face challenges in setting quantifiable criteria for diagnosis of M. tuberculosis due to difficulties in diagnosing asymptomatic cases. Current microbiology methods can be confusing and inaccurate. **TSTs** are inexpensive but cannot differentiate between BCG vaccination and M.tb infection.[16] The **Interferon-Gamma Release Assay** (**IGRA**) is the gold standard for tuberculosis diagnosis, reliably identifying M. tuberculosis infection even in those immunized with BCG.[16]

Although it can't distinguish between a cleared and a chronic infection, the T-cell response analysis does show the presence of M. tuberculosis.[16] A few vaccine concepts moved past Phase I testing.[16] For newborns immunized with BCG, the MVA85A vaccine offered no further defense against tuberculosis.[16] The research did demonstrate, meanwhile, that massive effectiveness investigations in high-TB incidence areas are feasible.[16] Recent studies have significantly advanced TB control, with the M72/AS01E vaccination resulting in 49.7% fewer incidences of active tuberculosis in a trial with HIV-negative adults.[16] In another trial, H4:IC31 was found to have only 30.5% effectiveness in preventing M.tb infection in vulnerable

teenagers and was stopped. On the other hand, BCG revaccination demonstrated 45.4% effectiveness in avoiding prolonged IGRA transformation.[16]

The results of BCG revaccination show promising outcomes, including increased production of IFNγ by NK cells, and increased expression of Th1 and IL-22 by CD4 T-cells.[16] A larger study involving 1800 South African teenagers is being sought to confirm its effectiveness, and results should be available by early 2026.[16] Developing TB vaccines would require a measurable CoP in vaccines, and the positive results from M72/AS01 and BCG revaccination support the feasibility of efficient TB vaccine development.[16] However, preclinical approaches are essential for identifying effective vaccine concepts. Establishing an immunological CoP would justify cost-effective investigations into preventative diseases.

Table 1: Classes of Tuberculosis vaccines in clinical or preclinical trials (Lai et al., 2023)

Types of Vaccines	Functions	Drawbacks
Whole-cell live vaccines (BCGs like VPM1002 & revaccination, IV BCG)	• Uses live organisms as delivery • Stimulates immune responses, mirroring those produced via pathogen • Contain antigens in large amounts	• Not beneficial for the immunocompromised individuals
Viral vectored vaccines (AdHu85A, AdHu35, MVA85A)	• Produce immune response against certain pathogens • Designed to produce genes for M.tb proteins	• Complications for pre-existed immunity
mRNA vaccines (ID91)	• No pre-existed immunity • Varieties of immune responses	• Provides immune response to a determinate number of antigens

Protein subunit & adjuvant (M72/AS01, H4:IC31)	• Beneficial for immunocompromised patients • Provide immunological responses to certain antigens via pathogens.	• Necessitates booster and adjuvant
Inactivated wholes cell vaccines (RUTI, V7, DAR901)	• Influences to increase the larger amount of immune responses • Safer for the immunocompromised individuals	• Have side effects such as injection site reactions • Necessitates booster

Chapter 4

4.1 Standard Treatment for Tuberculosis

The treatment for TB patients involves a combination of medications. The duration of therapy (4, 6, or 9 months) is recommended based on the patient's condition. The most effective **first-line therapeutic drugs** include isoniazid, pyrazinamide, rifampin, ethambutol, and streptomycin.[17] The **second-line therapeutic drugs** include ethionamide, kanamycin, amikacin, viomycin, ciproflaxin, etc.[17] The **third-line therapeutic agents** have unverified efficacy including rifabutin, microlides, linezolid, bedaquiline, thioacetazone, arginine, etc.[17]

The **4-month** regimen is given including rifapentine, moxifloxacin, isoniazid, and pyrazinamide.[18] This 4-month regimen showed a similar therapeutic benefit as the **6-month** regimen and is mainly provided for the treatment of patients with DS-PTB.[18] It consists of the **intensive phase** (**8 weeks**) and the **continuation phase** (**9 weeks**). The intensive phase includes rifapentine, moxifloxacin, isoniazid, and pyrazinamide, followed by the continuation phase which includes rifapentine, moxifloxacin, and isoniazid.[18] This therapy is also helpful for patients with HIV and ≥ 100 cells of CD4.[19] The standard **6-** and **9-month** regimens include isoniazid, rifampicin, pyrazinamide, and ethambutol, suggested for DS-TB. Both therapies include rifampin, isoniazid, pyrazinamide, and ethambutol.[18] If severity or recurrence is seen in the treatment duration, the treatments can take **12** or **20 months**.[17] The main goal is to provide convenient, brief, and safe treatment for TB patients.

4.1.1 Anti-Tubercular Drugs: Mechanism of Actions

The two major medications used to treat TB are isoniazid and rifampin which can successfully treat drug-sensitive TB for nine months.[13] **Isoniazid (INH)** is activated by the KatG, directing the enoyl-AcpM reductase InhA in M.tb that leads to the inhibition of metabolic pathways of mycolic acids in the cell wall.[20] This inhibition is generated by the isonicotinic acyl–NADH

complex. This, ultimately, results in the inhibition of the InhA's active site (2-trans-enoyl-ACP reductase) that kills the M.tb (**Figure 8**).[20] The **Rifampin** focuses on reversibly blocking the DNA-dependent RNA polymerase to constrain bacteria's transcription and protein synthesis.[21] **Pyrazinamide (PZA)** is converted to its active form pyrazinoic acid and exerts its effect by inhibiting trans-translation and possibly coenzyme A synthesis needed for the bacteria to survive. A bacteriostatic drug, **Ethambutol (EMB)**, targets the EmbA, EmbB, and EmbC by impeding the components of the mycobacterial cell wall, arabinogalactan & lipoarabinomannan syntheses.[7]

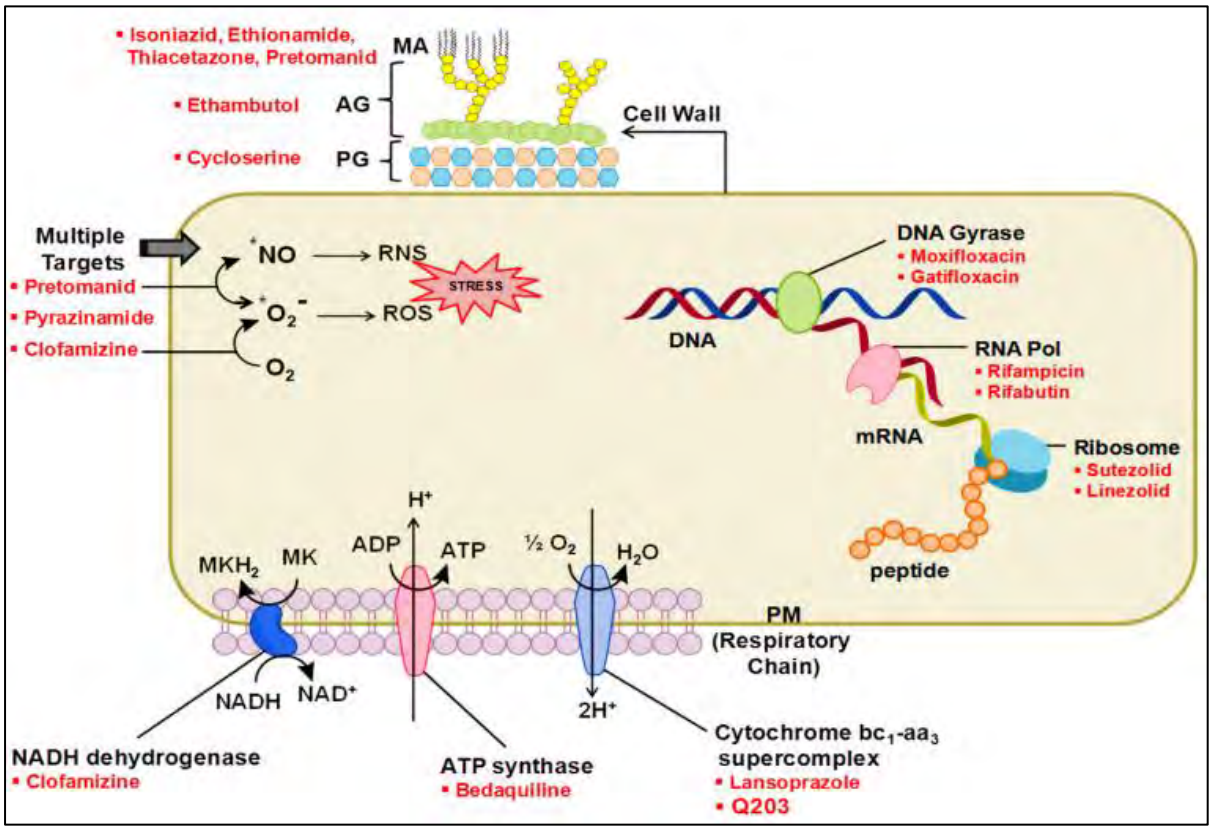

Figure 8: Mechanism of actions of anti-tubercular drugs (Bendre et al., 2021).[22]

Other drugs such as rifapentine, rifabutin, moxifloxacin, levofloxacin, kanamycin, amikacin, and pyridoxine are used as alternatives when the first-line drugs show less effectiveness for the patients.[23] **Rifapentine** and **rifabutin** are substrates of the RMP with somehow similar activity. The rifapentine shows less drug-drug interaction whereas the rifabutin shows a longer

half-life.[23] Both **kanamycin** and **amikacin** target the 30S ribosomal unit to hinder the synthesis of proteins.[24] **Moxifloxacin** and **levofloxacin** are also considered fluoroquinolones that inhibit the biosynthesis of mycolic acid and supercoiling DNA by targeting the InhA and DNA gyrase.[24]

Bedaquiline (BDQ), **pretomanid (PMD)**, and **delamanid (DLM)** are the new anti-TB drugs that showed promising treatment outcomes against MDR and XDR-TB. **BDQ** can inhibit the mitochondrial ATP synthase, whereas PMD inhibits the cell wall synthesis associated with respiratory poisoning.[24] The CDC suggested taking a low dose of **linezolid** along with the **BDQ** and **PMD** as it showed greater effectiveness.[25] Many other drugs are still undergoing clinical trials to make treating Tuberculosis easier, safer, and more effective.

4.1.2 Treatment Challenges in Tuberculosis

Variations in disease development, host response, and medicament resistance complicate treatments and lead to the discovery of novel drugs. Treatments may vary for elderly, pregnant, pediatric, and immunocompromised patients, as well as based on socioeconomic factors.

4.1.2.1 Treatment Challenges for Pregnant Patients

During pregnancy, maternal TB can pose risks for both the mother and the baby. The likelihood of developing tuberculosis is higher during this period, which can lead to premature birth, intrauterine growth restriction, and infant TB.[26] Additionally, there is a high chance of developing HIV in mothers with TB.[27] Thus, Diagnosis can be challenging and there is an increased risk of mortality during pregnancy.

Simpson et al. found that most patients received DS-TB treatment for maternal TB or within six months postpartum. The suggested therapy involves sustained medication exposure. The 9-month regimen is not recommended considering its incorporation of prothionamide.[28] BDQ,

linezolid, and fluoroquinolone effectively manage MDR-TB and RR-TB but have various side effects.[28]

4.1.2.2 Treatment Challenges for Pediatric Patients

Treating pediatric patients poses unique challenges due to ambiguous clinical presentations, the need for precise specimen collection, microbiological confirmation, and interpretation of radiological findings.[29] Diagnosing conditions in children can be particularly challenging due to difficulty differentiating symptoms. Most families also try to conceal it due to social stigma, depriving children of treatment.[29]

The use of **BDQ**, **PLM**, **DLM**, **MFX**, and **CFX** as second-line drugs has shown great potential in treating pediatric DR-TB.[29] The World Health Organization advocates using BDQ and DLM in children of all ages. These newly developed and repurposed drugs present a valuable opportunity to shorten the treatment duration, thereby reducing the occurrence of severe adverse effects and drug interactions.[29]

4.1.2.3 Treatment Challenges for Immunocompromised Patients

Immunocompromised individuals, such as those with HIV/AIDS, severe fungal infections, renal disease, cancer, TNF-α antagonist medication, diabetes, or organ transplantation, are at higher risk of tuberculosis.[30] EPTB may be more common than PTB in these patients, and the infection frequently causes abnormal and persistent symptoms, resulting in prolonged detection and management.[31]

The initial treatment regimens for **LTBI-HIV** patients with no drug-drug interactions are once weekly in **12 weeks** of combination therapy of **ISN**, **rifapentine**, and **antiretroviral therapy** (**ART**).[32] If DDIs are seen with RMP then rifabutin is suggested as alternative therapy and if seen interaction of ARTs with RMP and rifapentin, daily dose of ISN is recommended for **6-9 months**.[32] Moreover, the initial treatment regimens for **ATBI-HIV** patients are either **6**

months of **RIPE** or **4 months** of **rifapentine**, **MOX,** and **efavirenz**. However, CD4+ counts needed to be observed to treat **ATBI-HIV** patients. If the CD4+ count level is >50 cells/mm^3, ART should be started by 2 weeks and <50 cells/mm^3 ART should be started by 8 weeks before TB treatment. [32] In treating patients with renal tuberculosis, the **RIPE** regimens are suggested for **4 months** along with a **4-month** continuation phase of **RMP** and **ISN**.[33] Sometimes, a longer period of treatment is suggested for immunosuppressed patients. In contrast, the treatment strategies differ for **DM-TB** patients. The treatment strategies for TB have been shown to worsen when administered to **DM** patients. For DM-TB patients associated with peripheral neuropathy, ISN along with pyridoxine is recommended.[34] Rifampicin should be avoided because it can interact with other drugs, leading to high blood sugar when taken with hypoglycemic drugs.[34] It can also cause high blood sugar and high insulin levels in non-diabetic patients. Thus, the treatment strategies are quite challenging for immunocompromised patients.

4.1.3 Advancement in the Therapeutic Strategies of Anti-TB Drugs

Several new approaches are being developed to combat tuberculosis infections. Many anti-TB drugs are being repurposed to discover new therapeutic indications and uses. Recently, some of the classes of Cephalosporin drugs (**Cefdinir**, **Cefazolin** & **Cefpodoxime**) and Carbapenem (**Imipenem**, **Ertapenem**, **Feropenem** & **Meropenem**) showed more promising treatment outcomes than the byproducts of **Penicillin**.[35] **Cefdinir** and **Cefpodoxime** both can inhibit the synthesis of the cell wall.[35] The clinical trial of **ISN** and **Co-trimoxazole** has completed phase III, demonstrating a reduction in infection among HIV-TB pediatric patients; however, the results are yet to be published.[35] Recently, **Doxycycline** has been an efficacious adjunctive HDT as it can control the degradation of tissue degradation triggered by MMPs in the clinical trial of Phase II. **Gatifloxacin**, **metronidazole**, **doxycycline,** and **nitazoxanide** also showed their effectiveness against the M.tb which is going through clinical trials. Many other drugs

have passed in different phases of clinical trials like **Auranofin** (anti-rheumatic agent), **Rimonabant**, **Pranlukast**, **Fusidic Acid,** and many more.[35] **Vitamin D** has shown its effectiveness in promoting autophagy in the infected monocytes of M.tb.[35] Also, anti-diabetic drugs like **metformin** and **acarbose** can be used as anti-tubercular drugs as they can reduce the growth of TB infection.[35] Also, **triazolothiadiazine derivatives** (triazolo[3,4-b]thiadiazines, triazolo[1,5-c]thiadiazines etc.) showed beneficial for the treatment of Tuberculosis.[36] **Chalcones** have also shown antiviral activity including HIV as it has O-benzyl constituents.[37]

Besides all these, flavonoids, terpenoids, and flavonoids are compounds found in medicinal plants that have the potential to cure tuberculosis (TB). Flavonoids can reduce bacterial pathogenicity and biofilm formation by inhibiting ATP synthase, while terpenoids rupture cell walls.[38] Many other plants like *Aframomum melegueta, Merwilla plumbea, Artemisia sativa* L., *Citrus lemon, Cannabis sativa* L., *Carica papaya* are in the clinical trial phases now.[38]

Chapter 5

5.1 Synthetic Methods for Anti-TB Drugs

Many anti-tubercular drugs have been synthesized to develop an effective drug with the potential to fight against TB infection. Modifying the existing anti-tubercular drugs or developing new drugs for TB is every researcher and pharmaceutical company's primary goal. However, the synthesis of drugs depends on their target site of the Mycobacterium tuberculosis. For instance, drugs like isoniazid, ethambutol, cycloserine, ethionamide, pretomanid, prothionamide, and delamanid act as mycobacterial cell wall inhibitors; moxifloxacin, levofloxacin, and analogs of rifamycin act as the inhibitors of the nucleic acid synthesis; kanamycin, amikacin, linezolid, and streptomycin act as the inhibitors of the protein synthesis; bediquiline, *p*-aminosalycylic and pyrazinamide are the inhibitors of the membrane energy metabolism.[39] The synthetic approaches of each drug are described in the following sections.

5.1.1 Isoniazid (ISN)

The chemical name of ISN is isonicotinic acid hydrazide and the molecular formula is $C_6H_7N_3O$. The chemical synthesis approach for the preparation of the ISN involves the 4-cyanopyridine and hydrazine hydrate.[40]

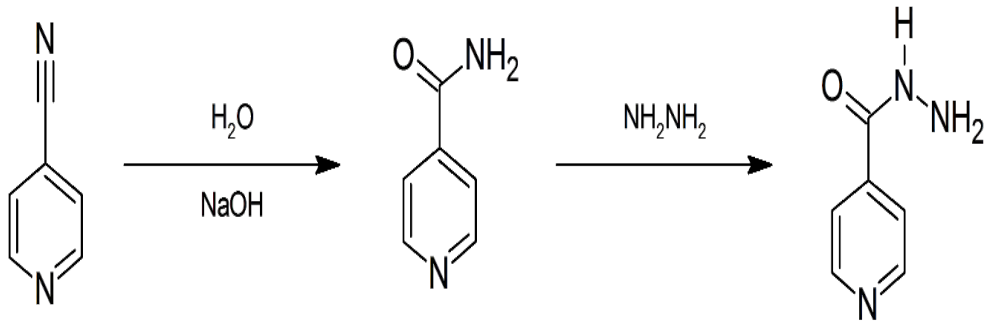

Figure 9: Preparation of ISN from 4-cyanopyridine and NH_2NH_2 (Rode et al., 2019a).

It can also be synthesized enzymatically using lipases which include ethyl isonicotinate and hydrazine.[40]

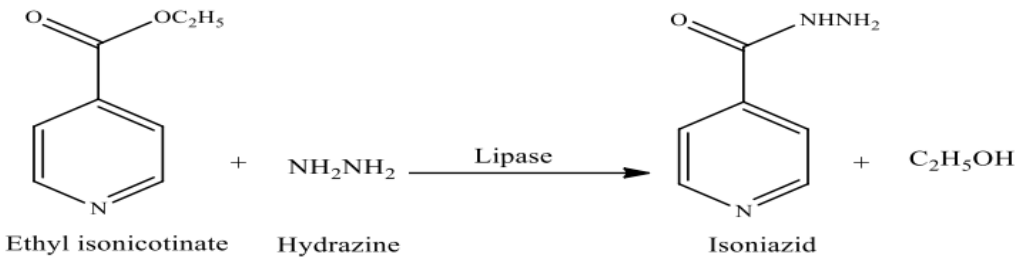

Figure 10: Preparation of ISN from ethyl isonicotinate and hydrazine hydrate (Badawy et al., 2023a).

It is noticed that the starting compounds were changed but the hydrazine hydrate is present always. Hydrazine hydrate (NH_2NH_2) plays an important role in isoniazid. Range of active and inactive compounds is produced when the hydrazine hydrate of the ISN is substituted with the alkyl group.[40]

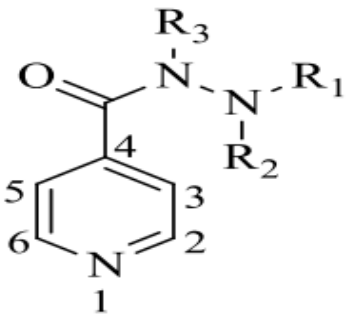

Figure 11: The structure-activity relationship (SAR) of ISN (Badawy et al., 2023b).

Terminal nitrogen replaced with alkyl (R1 & R2) and hydrogen (R3) will produce active products.[41] In addition, the methyl group at position 2 can be swapped since it possesses an antitubercular effect when contrasted with ISN. Again, ISN along with quinoxaline 1,4-di-N-oxide derivatives can inhibit the growth of the infection.[40]

5.1.2 Analogues of Rifamycin

The analogs of rifamycin include rifampicin, rifapentine, rifabutin, and rifalazil. Rifampicin is used as the first-line therapeutic drug along with Isoniazid. Rode et al. showed that Rifampicin has 0.4 μg mL^{-1} MIC whereas rifapentine, rifabutin, and rifalazil have 0.031 μg mL^{-1}, <0.015 μg $mL^{-1,}$ and <0.015 μg mL^{-1} MIC against M.tb. Rifapentine, rifabutin, and rifalazil are recommended when rifampicin fails to fight against M.tb.

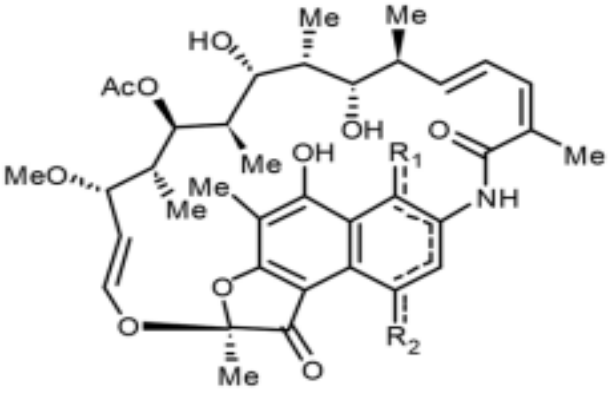

Table 2: Analogs of Rifamycin (Rode et al., 2019b)

Analogs	R_1	R_2
Rifamycin B	-OH	$-OCH_2COOH$
Rifamycin O	=O	=(1,3-dioxolan-4-on)-2-yl
Rifamycin S	=O	=O
Rifamycin SV	-OH	-OH

The process of producing rifampicin involves a semi-synthesis from rifamycin S, which results in 3-Pyrrolidinomethyl-rifamycin SV.[41] This compound then undergoes an oxidation process using lead tetraacetate to produce 3-formyl-rifamycin SV. The next step involves a condensation reaction with an aldehyde via amino piperazine, resulting in the formation of rifampicin.[39] There are also other ways of the synthesis approach for producing Rifampicin

like forming rifamycin S from N-bishydroxymethyl-amine or using a microchannel reaction device.[39] Researchers have been working on modifying the synthesis of rifampicin to achieve the best results.

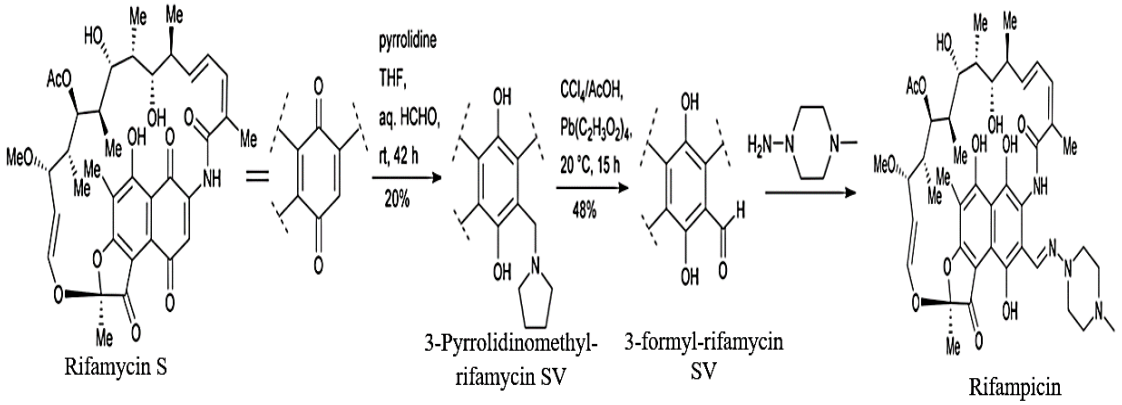

Figure 12: Synthesis approach of Rifampicin from Rifamycin S (Rode et al., 2019c).

Synthesis approaches for rifapentine, rifabutin, and rifalazil are similar. Rifapentine, a cyclopentyl-substituted analog of rifampicin, has improved duration of action, bioavailability, longer half-life, lower liver toxicity, and reduced gastrointestinal irritation.[41][42] Rifalazil is a benzoxazinorifamycin analog of rifamycin, formed through a reaction between 3'-tert-butyldimethyl-silyloxybenzoxazinorifamycin and piperazine in oxidative environments.[39]

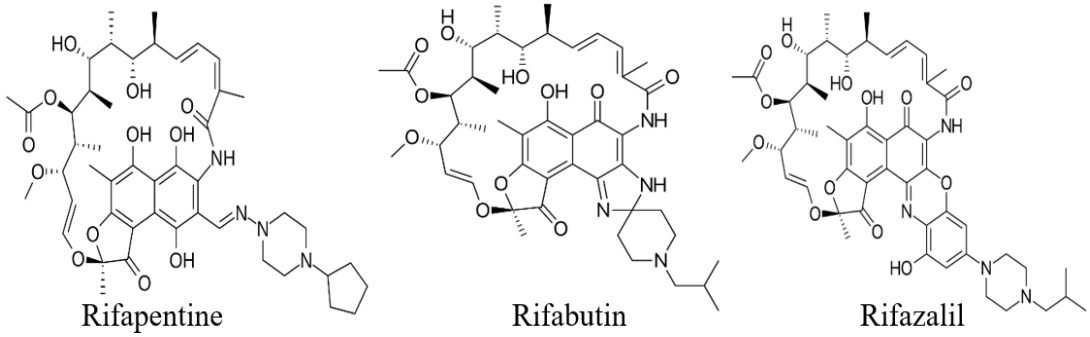

Figure 13: Chemical structures of Rifapentine, Rifabutin and Rifalazil (Rode et al., 2019d).

5.1.3 Ethambutol

The ethambutol contains the most antimycobacterial agent through the inhibition of the lipoarabinomannan and arabinogalactan synthesizes.[39] It can be synthesized using various

starting materials like butadiene monoepoxide, L-methionine, and n-butyraldehyde.[39] Starting with butadiene monoepoxide first reacts with phthalimide using palladium asymmetric allylic alkylation catalyst to produce alcohol that further converts into oxalamide.[39] The oxalamide is then reduced using Red-Al to produce deamine, which later undergoes hydrogenolysis and saturation to create ethambutol with 42% yielding value.[39]

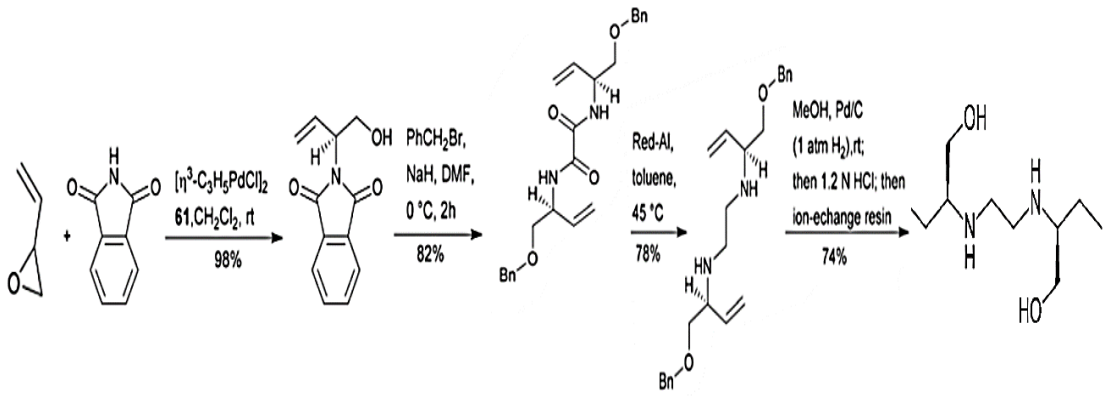

Figure 14: Synthesis of the ethambutol from butadiene monoepoxide (Rode et al., 2019e).

Starting with L-methionine, it undergoes two steps to produce an intermediate, as shown in **Figure 15**. The intermediate's thiomethyl group then undergoes a desulfurization reaction using raney nickel, followed by a reduction reaction, resulting in the production of ethambutol as a white solid with a 37% yield.[39]

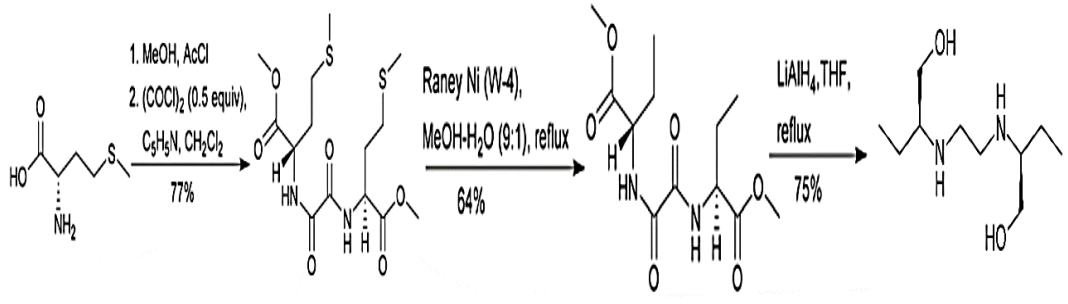

Figure 15: Synthesis of the ethambutol from the material L-methionine (Rode et al., 2019f).

N-butyraldehyde reacts with nitrosobenzene in the presence of L-proline to produce aminooxy aldehyde.[39] This aminooxy aldehyde is then reduced in situ using sodium tetrahydridoborate

to produce alcohol. The alcohol can then be converted to azide in four steps, and then further transformed into ethambutol in three additional steps.[39]

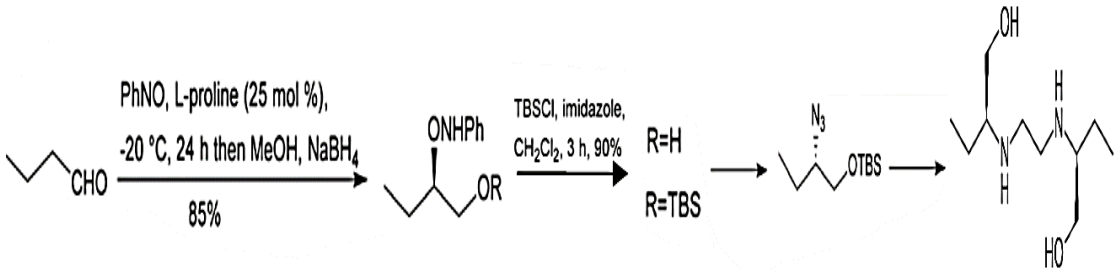

Figure 16: Synthesis of the ethambutol using N-butyraldehyde (Rode et al., 2019g).

5.1.4 Pyrazinamide

A 2-carboxamidopyrazine, pyrazinamide, is activated by the pyrazine-2-carboxylic acid, cyanuric trichloride, biotransformation of pterin, hydration of nitrile, AgHAP, hydrogen peroxide, ammonolysis of methyl pyrazinoate, etc.[43]

At first, pyrazine-2-carboxylic acid or 6-chloro-pyrazine-2-carboxylic acid is deprotonated resulting in TEA carboxylate salt.[43] This is then reacted with 2,4,6-trichlorobenzoyl chloride to form anhydride that further reacts with 4-dimethylaminopyridine to form dimethylaminopyridine-substituted acyl. Lastly, the dimethylaminopyridine-substituted acyl reacts with aniline or amine to produce the pyrazinamide analogs.[43] The substitution of 6-chloro on the pyrazine ring of the pyrazinamide analogs showed better effectiveness. Substituting the N atom of the pyrazinamide with adamantyl, ethylphenyl, octyl, cycloheptyl, and 4-chlorobenzyl can be produced under thionyl chloride.[43]

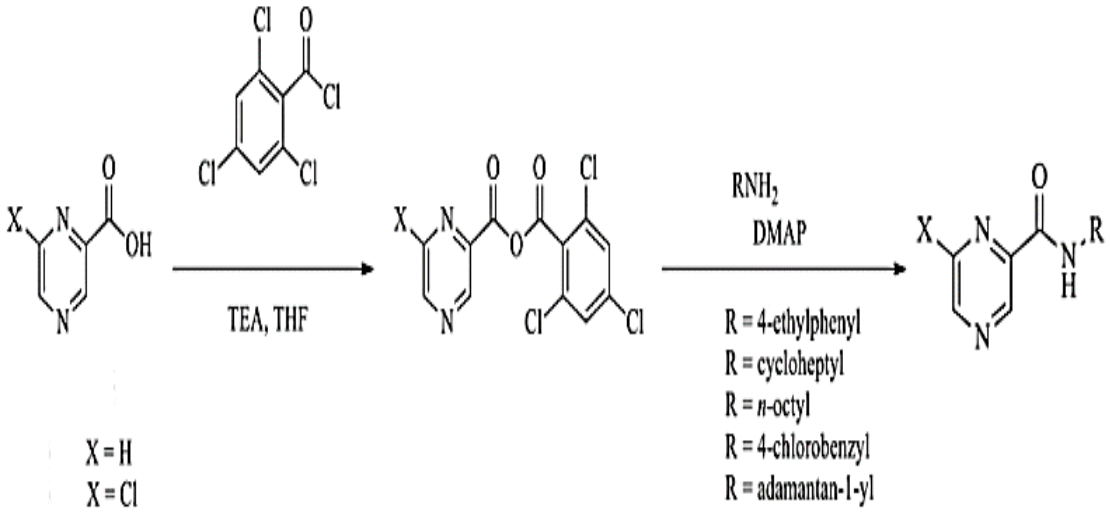

Figure 17: Synthesis of the pyrazinamide and its analogs (Zulqurnain et al., 2023).

5.1.5 Streptomycin

The production of streptomycin involves dihydrostreptomycin, which involves two steps: (i) treatment with benzyl chloroformate and (ii) treatment with 20% acetic acid in methanol, which generates the mono-isopropylidene compound.[39]

The mono-isopropylidene molecule is then acetylated with acetic anhydride to get an acetylated product.[39] This acetylated molecule is then hydrolyzed in aqueous acetic acid, yielding a compound containing free tertiary and primary hydroxy groups as part of the dihydrostreptose.[39] Finally, the derivatives undergo Pfitzner-Moffatt oxidation, and streptomycin is generated via catalytic hydrogenolysis.[39]

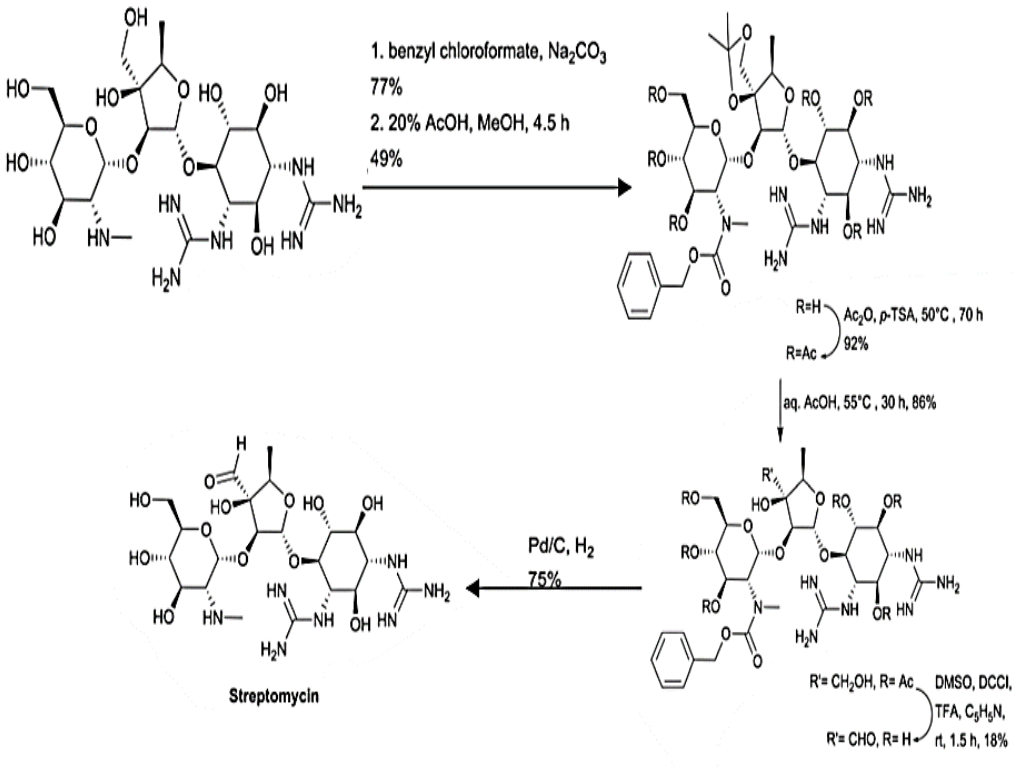

Figure 18: Synthesis strategy of the streptomycin (Rode et al., 2019h).

5.1.6 Bedaquiline

Bedaquiline (BDQ) can be synthesized through the catalyzes including enantioselective proton migration (12 steps), sharpless asymmetric epoxidation (12 steps), chiral base in the deprotonation phase, and sulfur ylide-mediated asymmetric epoxidation (9-steps).[44]

BDQ has recently been generated with trisubstituted or disubstituted epoxides and an asymmetric sulfur ylide-mediated epoxidation catalyst.[45] The trisubstituted epoxide provided a straightforward approach for combining sulfonium salt and ketone; nevertheless, it lacked oversight of diastereoselectivity and enantioselectivity formation.[44] Bashir et al. used a disubstituted epoxide to increase its efficiency, although a diaryl epoxide requires a regioselective ring opening. A disubstituted epoxide is formed by combining a sulfonium salt with an aldehyde. Bashir et al. investigated the regioselectivity of the diaryl epoxide's ring opening by reacting 2-methoxyquinoline-3-carboxaldehyde with a sulfonium salt. They

achieved a high yield (88%) and a trans: cis ratio of 98:2 for the 1,2-disubstituted epoxide when treating the sulfonium salt and aldehyde with KOH in a 9:1 MeCN/H_2O solvent.[44]

Bashir et al. demonstrated a nine-step synthesis of bedaquiline by isothiocineole sulfur ylide asymmetric epoxidation. They began with 6-bromo-2-chloroquinoline-3-formaldehyde and ended with 6-bromo-2-methoxyquinoline-3-formaldehyde. Then, using LiHMDS and THF, they reacted 6-bromo-2-methoxyquinoline-3-formaldehyde with a sulfonium salt to form a trans epoxide.[44] This epoxide underwent regioselective ring opening with PhMgBr and CuCN to produce alcohol, which was then oxidized to a ketone with DMP and CH_2Cl_2. The ketone interacted with allylzinc bromide, resulting in a 1:1 alcohol combination. Aldehyde was produced by oxidative cleavage of an alkene with $RuCl_3$ and $NaIO_4$, followed by in situ elimination with $NaBH_4$ to yield a diol. The diol underwent two more processes to produce (+)-BDQ (+)-1 and its epimer.[44] The same synthesis approach is again repeated using isothiocineole where (1R,2S) -(−)-bedaquiline (−)-1 is formed.[44]

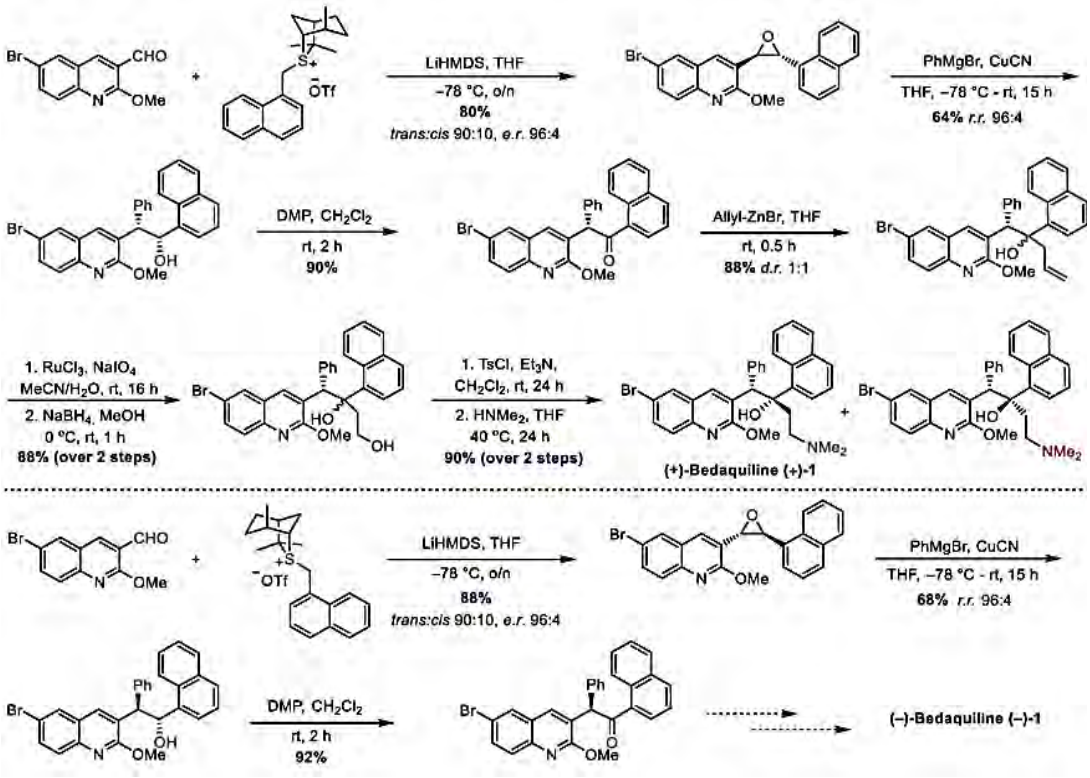

Figure 19: Synthesis strategy of bedaquiline (Bashir et al.,2023).

5.1.7 Pretomanid

A variety of starting materials are used to produce pretomanid including 2,4-dinitroimidazole, 2-bromo-4-nitroimidazole, 2-chloro-4-nitroimidazole and 2-bromo-4-nitroimidazoline. To start the reaction, Read and Fairlamb used the 2-bromo-4-nitroimidazole which is nucleophilically substituted with the TBS-protected glycidol.[45] The aryl moiety is then added the protecting group is removed and pretozamide is formed through cyclization.

Zhai et al. utilized 2-chloro-4-nitroimidazole as the main component in combination with (S)-epichlorohydrin.[45] The diol is created by hydrolyzing the N-alkylated imidazole. The alcohol is formed by benzylation of the secondary OH, made possible by selectively protecting the primary alcohol with TBS. Ultimately, pretomanid is produced by removing the silyl-protecting group and initiating cyclization.

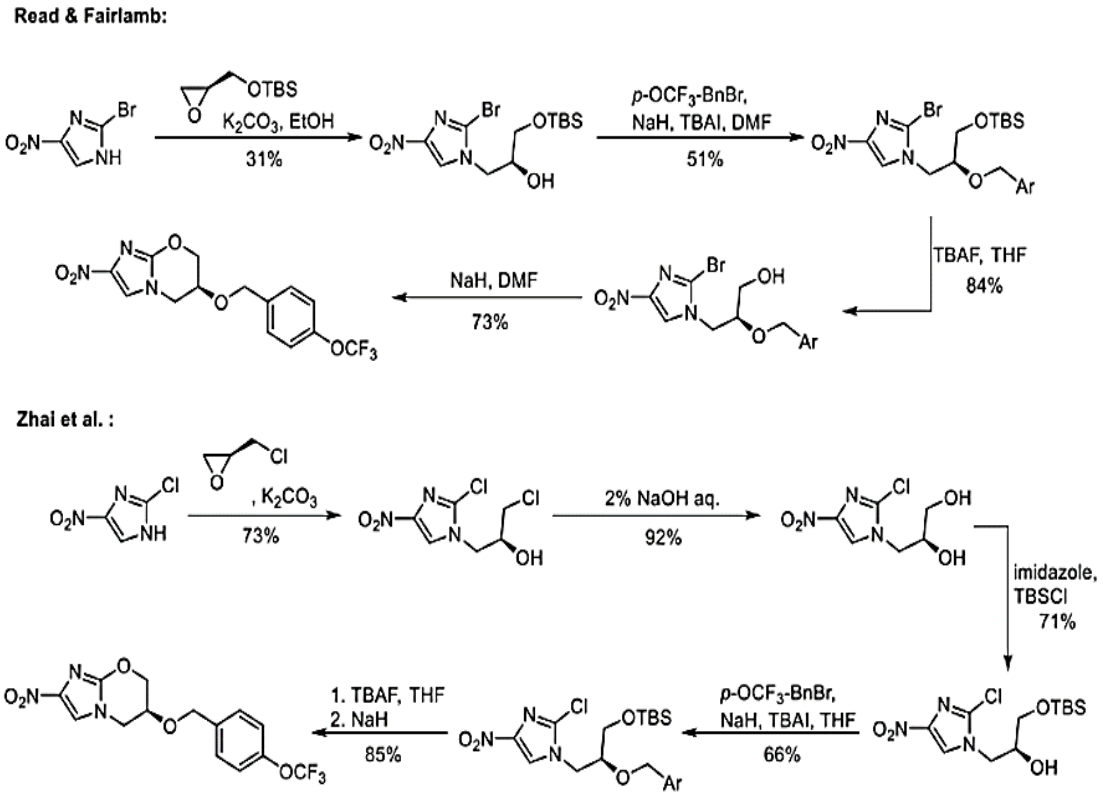

Figure 20: Synthesis strategy of pretomanid by Read & Fairlamb and Zhai et al (Lucas et al., 2023a).

In 2023, Lucas et al. showcased a synthetic method for manufacturing pretomanid. They used 2-bromo-4-nitroimidazoline and (R)-glycidols as the primary materials for the process. The reaction involved the use of various O-protecting groups to prevent product loss and eliminate the need for intermediate purifications.[45]

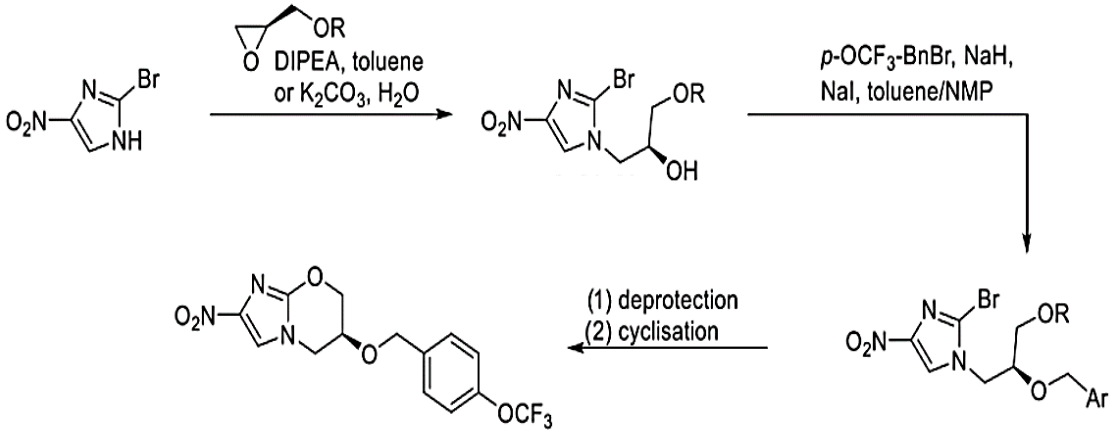

Figure 21: Synthesis strategy of pretomanid by Lucas et al (Lucas et al., 2023b).

5.1.8 Delamanid

The previous synthetic method of Delamanid included sharpless epoxidation of 2-methyl allyl alcohol, following an opening of the ring with 4-bromophenol, and finally coupling via 4(4-trifluoromethoxyphenyl)piperidine fragments using palladium catalysis.

Recently, Sharma et al. used the starting material, 2-methylallyl chloride along with 4-iodophenol under potassium carbonate and DMF at a temperature of 60˚C resulting in 1-iodo-4-((2-methylallyl)oxy)benzene. This compound then forms a C–N bond formation with 4-hydroxypiperidine under DMF at a temperature of 80˚C leading to the generation of N-arylated or 4-phenylpiperidin-4-ol and O-arylated products. The 4-phenylpiperidin-4-ol then formed ether bond formation with the 4-tri-fluoromethoxyphenol under mesyl chloride and Et_3N producing O-mesylated product.[46] This O-mesylated product is combined with 4-tri-fluoromethoxyphenol under triphenyl phosphine, tetrahydrofuran and diethyl azodicarboxylate

to produce the 1-(4-((2-methylallyl)oxy)phenyl)-4-(4-trifluoromethoxy) phenoxy)piperidine. This intermediate undergoes sharpless asymmetric dihydroxylation through AD mix-α and AD mix-β to produce R-(-)- diol. Then, the conversion of R-(-)- diol to epoxide under mesylation and ring formation is formed. Lastly, the epoxide and 2-bromo-4- nitroimidazole coupled under DIPEA where the produced product undergoes further reaction under Cs_2CO_3 resulting in 63% yield of Delamanid.[46]

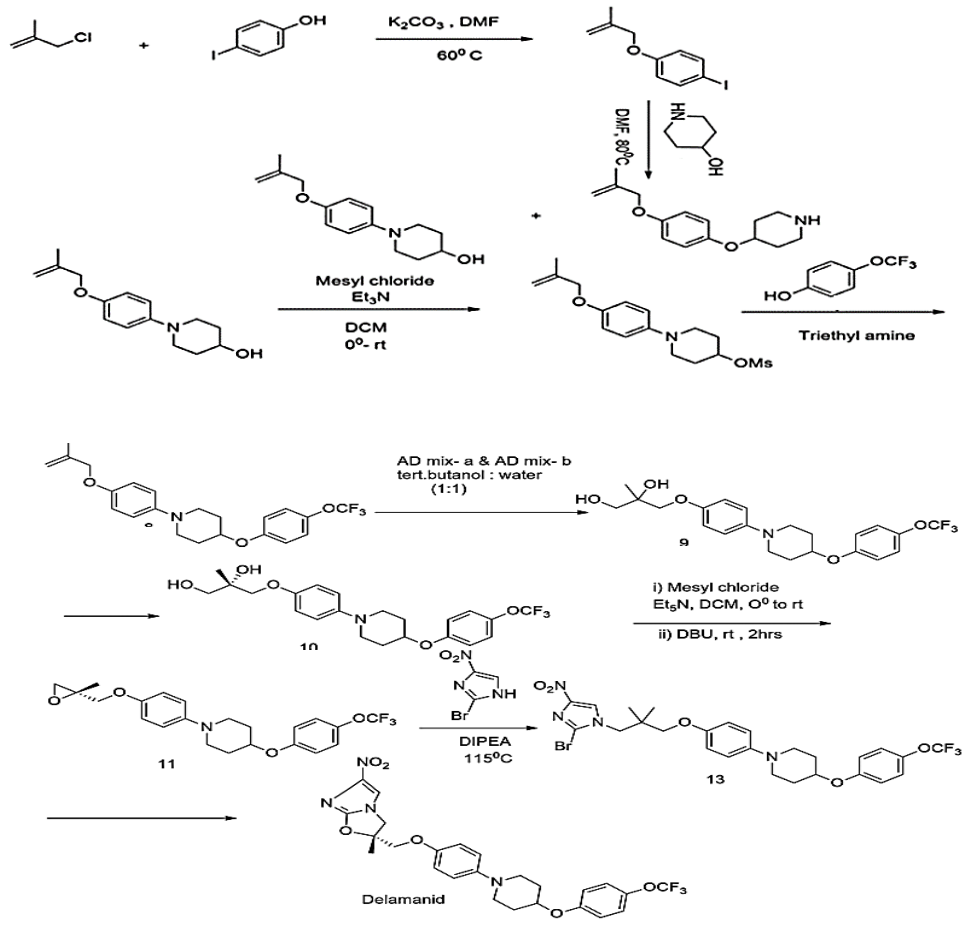

Figure 22: Synthesis of Delamanid (Sharma et al., 2020).

5.1.9 Linezolid

Russell et al. demonstrated the seven steps of the synthesis of the linezolid with the starting material, (+)-epichlorohydrin and acetonitrile along with boron trifluoride etherate to undergo a Ritter-type reaction. This reaction produced nitrilium intermediate which underwent a

reaction with 2-propanol to produce an intermediate. This intermediate then produced epoxide intermediate under lithium *tert*-butoxide in a 1:1 THF:1,2-dichloroethane (DCE) mixture.[47] Aside from this, a nucleophilic aromatic substitution occurs between morpholine and 3,4-difluoronitrobenzene. Next, a mass flow controller is used for hydrogenation to produce 3-fluoro-4-morpholinoaniline.[47] The reaction takes place in a combination of 1,4-dioxane and N,N-dimethylformamide solvents. The middle stage comprises treatment with N,N-carbonyldiimidazole before offline crystallization to obtain the final product, linezolid.[47]

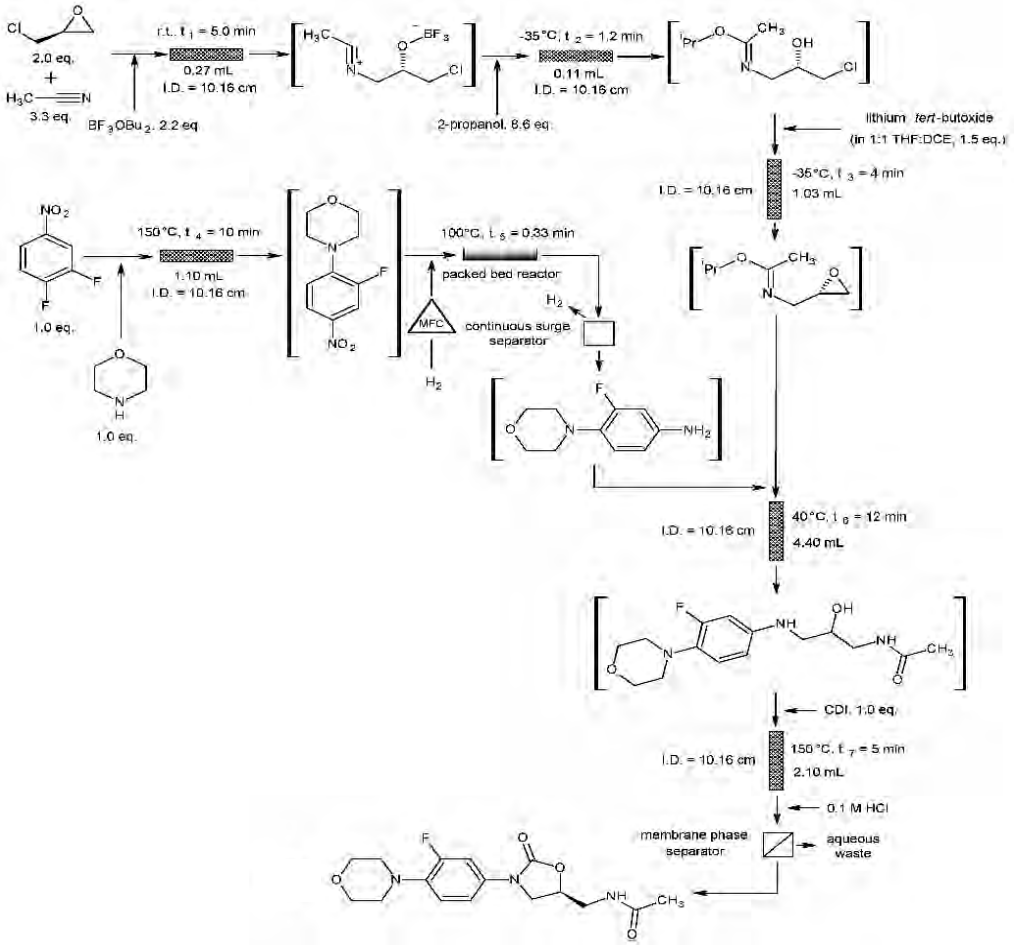

Figure 23: Synthesis of Linezolid (Russell et al.,2019)

5.1.10 Kanamycin & Amikacin

Kanamycin comes in three forms: Kanamycin A, Kanamycin B, and Kanamycin C.[39] The process starts with paromamine, which undergoes two stages. The first step involves combining

it with carbobenzoxy chloride and sodium carbonate in aqueous acetone at -10 °C, followed by treatment with p-TSA and DMF at 110°C, resulting in the formation of an intermediate product. This compound goes through benzylation and deacetonation to produce a different chemical. The synthesis of Kanamycin C involves mercuric cyanide in a condensation procedure. Kanamycin A starts with 6-O-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine and 6-amino-6-deoxy-D-glucose. Kanamycin B starts with the α-glycoside of neamine and 3-amino-3-deoxy-D-glucose.[39]

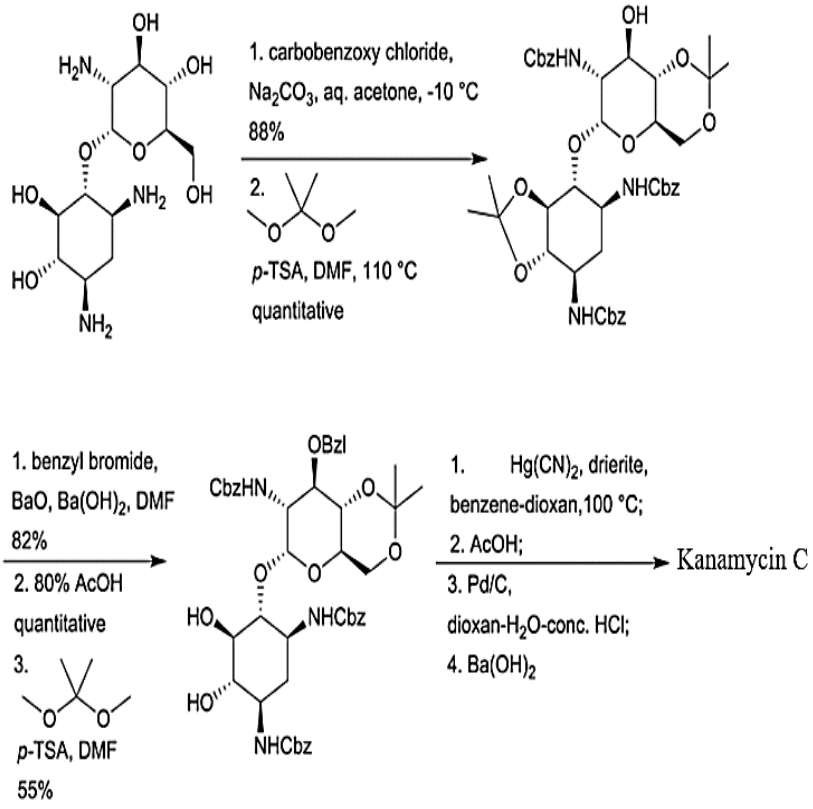

Figure 24: Synthesis of Kanamycin C (Rode et al., 2019i).

Through the modification of the Kanamycin A, Amikacin can be produced.[42] Amikacin is formed by initially treating Kanamycin A with N-(benzyloxycarbonyl)succinimide for N1-carbobenzoxylation, subsequently via the N-hydroxysuccinimide ester of L-(−)-γ-benzyloxycarbonylamino-α-hydroxybutyric acid for N2-acylation, and finally hydrogenolysis.[39]

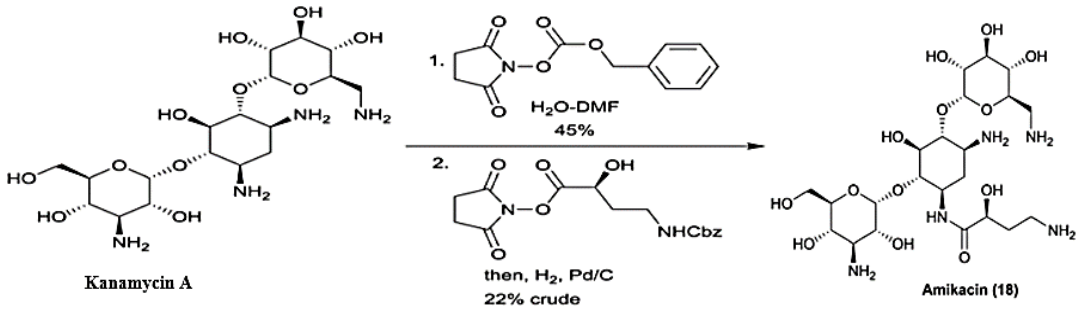

Figure 25: Synthesis of Amikacin from Kanamycin A (Rode et al., 2019j).

5.1.11 Moxifloxacin

The synthetic method of the MOX involves the 4-quinolinone which undergoes aromatic nucleophilic substitution at its 7-halo position along with the chiral amine.[39] Chava et al. demonstrated the synthesis of the MOX using 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinic acid ethyl ester that underwent through boric acid and acetic anhydride to form a borane chelate. This borane chelate further reacted with Et_3N, CH_3CN and (4aS, 7aS)-octahydro-1H-pyrrole3,4-bipyridine producing an intermediate.[39] The intermediate reacted further with MeOH, HCl and EtOH to produce the Moxifloxacin.

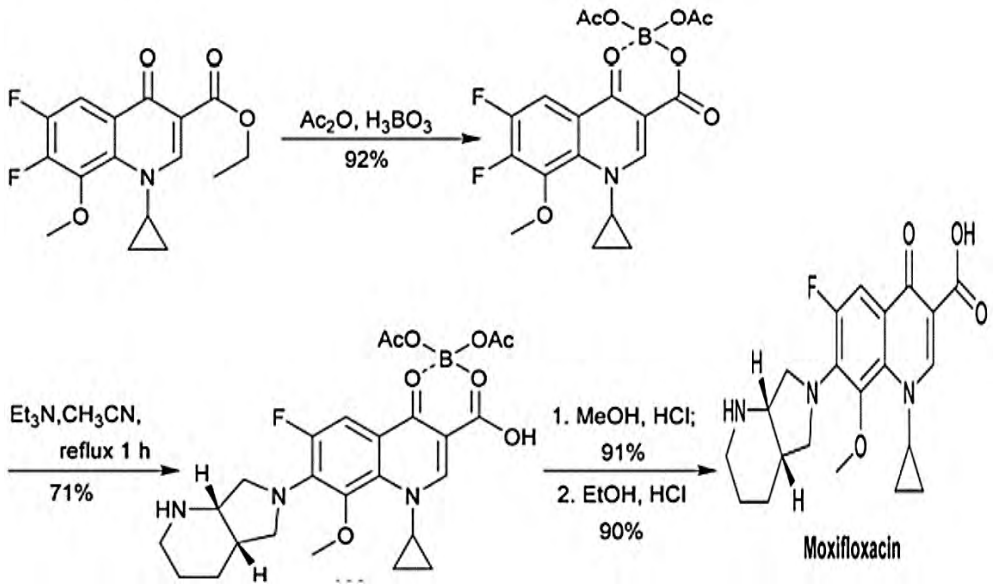

Figure 26: Synthesis of Moxifloxacin by Chava et al. (Rode et al., 2019k)

5.1.12 Levofloxacin

Levofloxacin can be prepared from several compounds like tetrafluoro benzoic acid derivatives, 4-chloro-5-fluoro-2-chloro-3 nitrobenzoic acid derivative, piperazine, 3-oxo-3-(2,3,4,5- tetrafluorophenyl)propanoate, methyl (S)-3-(4-methyloxazolidin-3- yl)acrylate, etc.[39]

Through the Grohe–Heitzer Mitscher reaction, Kouji et al., Ye et al., Zhang et al., Rao et al., Kim et al. and Mitscher et al. demonstrated the synthetic strategy for the preparation of Levofloxacin.[39] Here, tetrafluoro benzoic acid derivative was used along with L-alanilol where the intermediate was found followed by two steps. The cyclization of the intermediate along with the sodium hydride under DMSO where the produced product converted into Levofloxacin under aqueous potassium hydroxide, THF and C_5H_5N.[39]

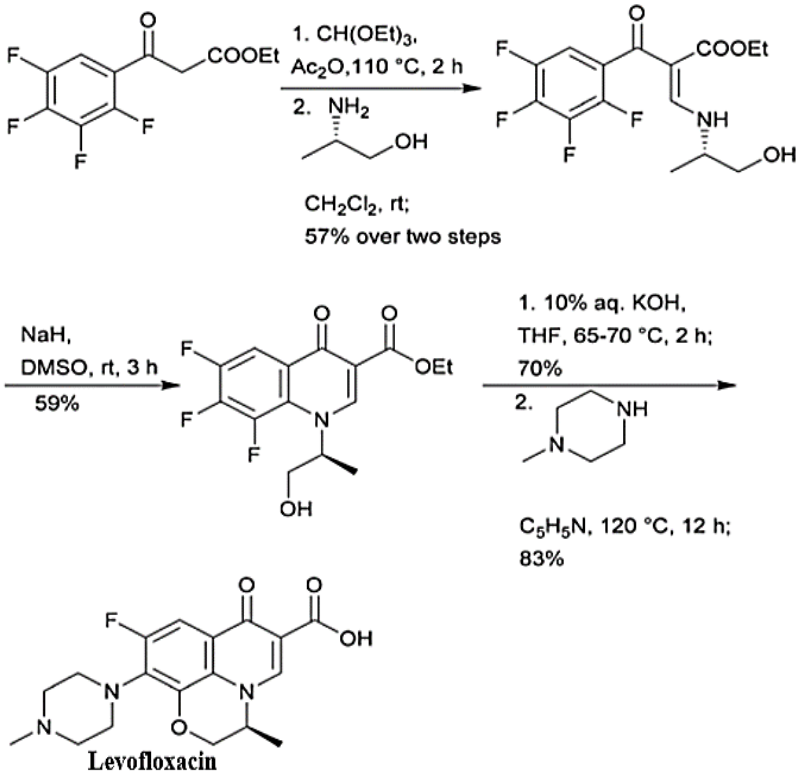

Figure 27: Synthetic approach for the preparation of Levofloxacin (Rode et al., 2019l).

The highest yielded (75%) Levofloxacin was found from the synthetic approach of Ghorai et al. The starting compound was the activated aziridine (Lewis acid-catalysed S_N2-type ring opened) which underwent two steps along with substituted phenol. The intermediate was produced first through NaH, $LiClO_4$, CH_3CN and then CuI, DMF, K_2CO_3. Lastly, the conversion of the intermediate produced the 75% of the Levofloxacin.[39]

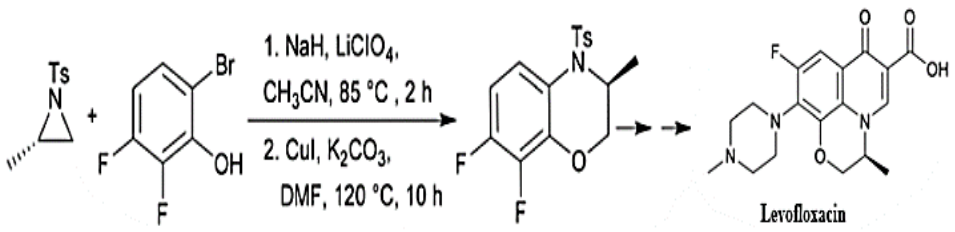

Figure 28: Synthetic approach for the preparation of Levofloxacin by Ghorai et al. (Rode et al., 2019m)

5.1.13 Chalcone

A type of flavonoid, Chalcone, has an α,β-unsaturated ketone with a diphenyl substitution that showed benefit against M.tb. Chalcones are derived naturally from *Desmodium gangeticum, Piper hispidum, Neoraputia magnifica, Humulus lupulus, Butea monosperma, Angelica keiskei,* etc.[37]

The chemical synthetic approach for preparing the chalcone includes the Claisen–Schmidt condensation reaction and the greener approaches like microwave-assisted process, grinding system, ultrasound-irritated synthesis, and coupling reactions.[37]

Aldehyde and acetophenone are used under different bases (NaOH, Ba(OH)2, etc.) and organic bases (piperidine, pyridine, etc.) for the synthesis of chalcones through Claisen–Schmidt condensation reaction. Different kind of heterogeneous catalysts (chitosan, BF_3-etherate, activated carbons, etc.) used to increase the yield of chalcones.[37]

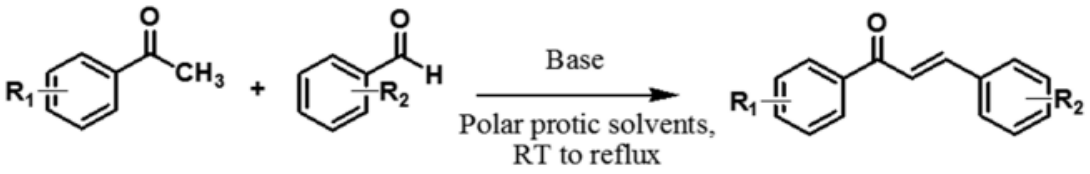

Figure 29: Synthetic strategy for the preparation of chalcone through Claisen–Schmidt condensation reaction (Rajendran et al., 2022a).

Through the microwave-assisted method, the synthesis of chalcones under base catalyst (K_2CO_3) and acidic base along with the pyrazoline derivatives showed higher yielded value.[37] The ultrasound-irradiated method is beneficial compared to a microwave-assisted method as it takes less time (10 seconds) under a zeolite-based catalyst to give higher yielded chalcones. Other than all these, coupling reactions like cross-dehydrogenative coupling, reductive (3+2) annulation, Julia–Kocienski olefination, a photo-Fries rearrangement, etc. used for the synthesis method for the preparation of chalcones showed in **Figure 30**.[37]

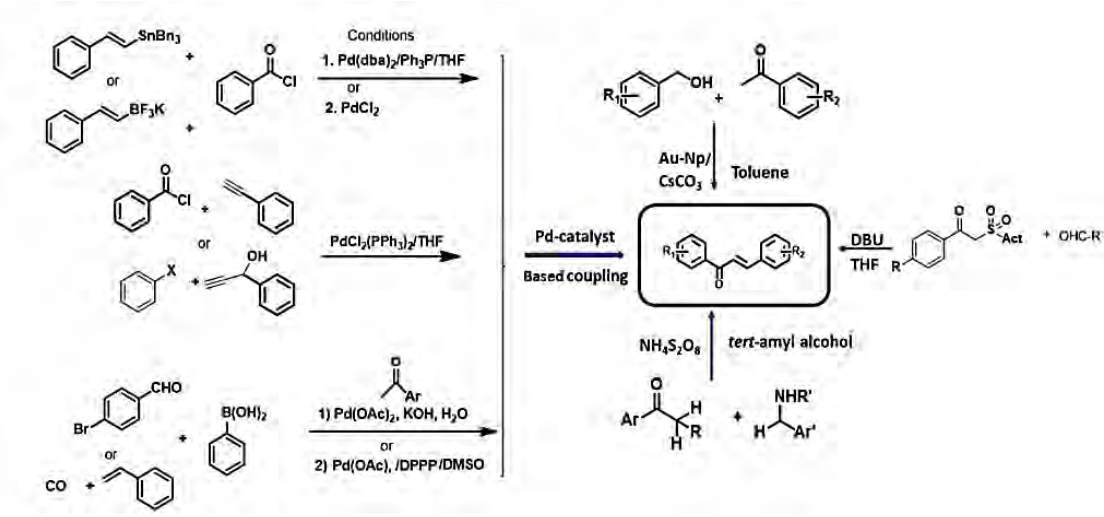

Figure 30: Different synthetic strategies for the preparation of chalcone through coupling reaction (Rajendran et al., 2022b).

Coupling reaction such as cross-dehydrogenative coupling uses catalyst like ammonium persulfate; Julia–Kocienski olefination gives the highest yield by using DBU and THF as the catalyst to reduce SO_2 from the product of the reaction among action involves β-ketosulfones;

and reductive (3+2) annulation uses the $P(NMe_2)_3$ catalyst along with pyrylium salts and benzil to synthesis the furanyl rings of *cis*-chalcones.[37] Moreover, chalcone analogs such as quinolines-based chalcones, extended phenyl skeletons-based chalcones, a class of acetylenic chalcone, spirochrome chalcones, seventeen C-dimethylated-based chalcones, thiazole-based chalcones and twelve triazole-based chalcone showed intense antitubercular activities.[37]

5.1.14 Quinoxaline

A six-membered ring analog of benzodiazine, quinoxaline, showed effectiveness against the M.tb. It can be synthesized from compounds like condensation between *o*-phenylenediamine and glyoxal, reduction of amino acid and 1,5-difluoro-2,4-dinitrobenzene, alkynes, etc. under varieties of catalysts such as $PdCl_2/PPh_3$, Gallium (III) triflate, *o*-iodoxybenzoic acid, sulfamic acid, etc.[48]

In 2018, J. Zi et al. used alkyne which underwent oxidative cyclization along with OPD under DMSO via a one-pot cascade to yield 70–90% of quinoxaline. In 2020, Wang et al. used 2-(1H-pyrrol-1-yl)aniline along with DMF under the TBHP and $FeCL_3$ catalysts in the presence of atmospheric air which gave two derivatives of quinoxaline which are indolo[1,2-a]quinoxaline and pyrrolo[1,2-a]quinoxaline.[48]

Figure 31: Synthetic strategies for the preparation of quinoxaline by J. Zi et al. (Suthar et al., 2022a)

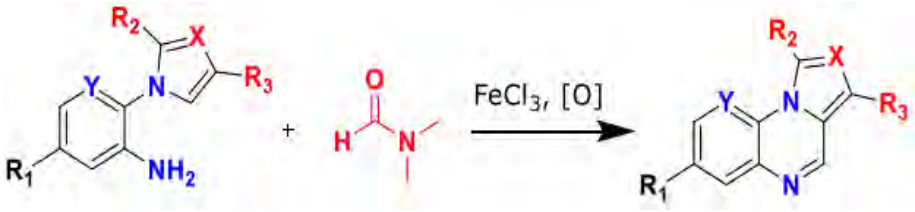

Figure 32: Synthetic strategies for the preparation of quinoxaline by Wang et al. (Suthar et al., 2022b)

Pan et al. showed anti-tubercular activity using aliphatic amine, amide, and thioether through N-oxide-quinoxaline, whereas, Wang et al. showed better results of antimycobacterial activity through employing pyrrolo[1,2-a]quinoxaline and Fernandes et al. showed it changing the C3 position with cyano-group and C2 position with aryl or hetero-ring of quinoxaline N-oxide.[48]

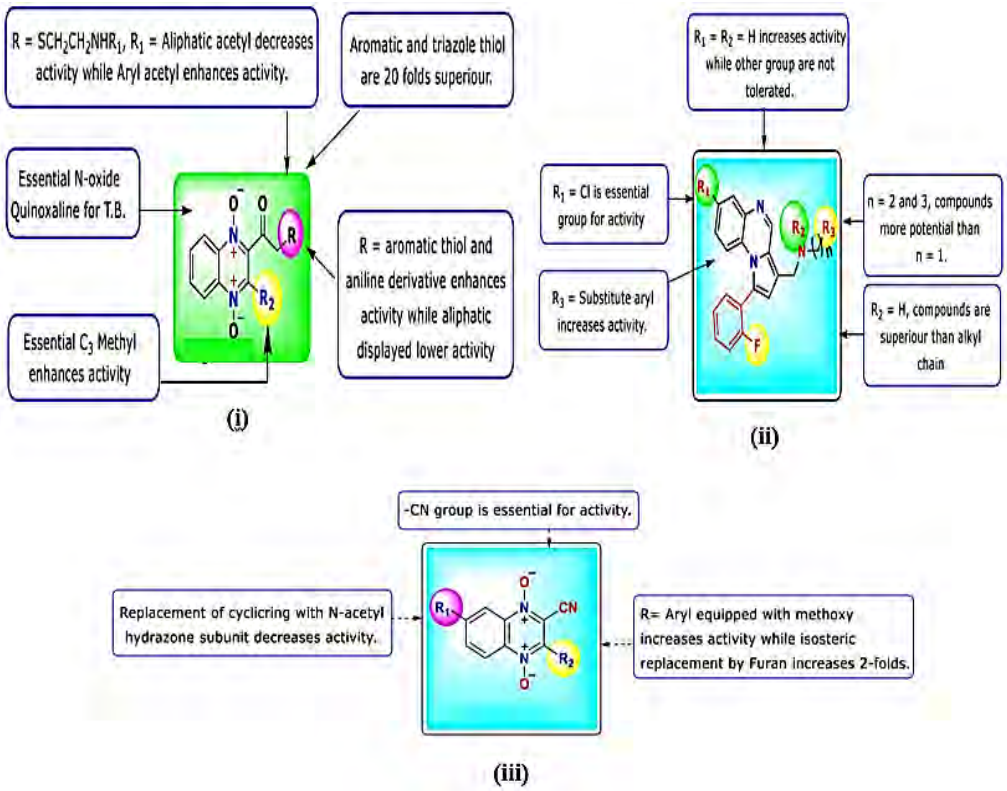

Figure 33: Antimycobacterial activities through quinoxaline derivatives showed by (i) Pang et al. (ii) Wang et al & (iii) Fernandes et al. (Suthar et al., 2022c)

5.1.15 Benzothiazinone

Benzothiazinone showed a promising outcome as an anti-tubercular drug. Several scientists have prepared benzothiazinone through several synthetic methods by keeping 2-chlorobenzoic acid derivatives as the starting material.[49] It underwent four types of pathways, such as acylisothiocyanate, dithiocarbamate, alkylxanthogenate, and alkylsulfanyl pathways. However, these pathways showed drawbacks. Hence, Richter et al. in 2022 demonstrated a pathway that has fewer toxicity materials and follows GMP.[49] Richter et al. used 2-chloro-3-nitro-5-(trifluoromethyl)benzoic acid as the starting compound in the presence of thionyl chloride which produced the acid chloride. This acid chloride further underwent a reaction with the *N, N*-dialkyl thiourea derivative to form a thiazinone ring system in only one step. This pathway showed no toxicities compared to other pathways. Through the thiourea pathway, 31 benzothiazinones were produced successfully.[49]

Figure 34: Synthesis approach of benzothiazinone (Richter et al., 2021)

5.1.16 Analogs of Benzothiazole

Benzothiazole and its analogs also proved to be an effective anti-tubercular drug for its anti-mycobacterial properties. Benzothiazole analogs such as carbanilide derivatives of benzothiazole, benzothiazole-based Schiff bases, azo-ester complexes of benzothiazoles, coumarin-based azo dye molecules, and pyrazole conjugates of benzothiazole derivatives showed the best outcomes for the treatment of tuberculosis.[50]

T. M. Dhamelia and co-workers used 2-aminothiophenol (R=H, Cl, Cl_3) under ethyl glyoxylate, water, and sodium dioctyl sulfosuccinate to produce ethylbenzo[d]thiazole-2-carboxylates (R=H, Cl, CF_3). This intermediate undergoes THF.H_2O and LiOH.H_2O to further produce benzo[d]thiazole-2-carboxylic acids. Lastly, benzo[d]thiazole-2-carbanilides are produced through direct CDI and THF-mediated reactions among benzo[d]thiazole-2-carboxylic acids and aromatic amines.[50]

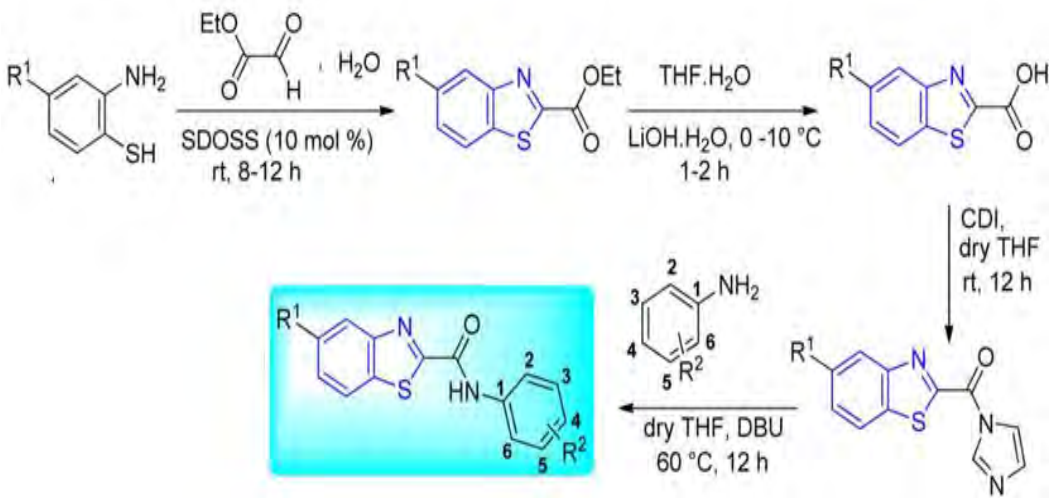

Figure 35: Dhamelia et al's synthesis approach of N-arylbenzothiazole-2-carbanilides (Yadav et al., 2023a).

To synthesize the benzothiazole-based Schiff bases, J. K. Suyambulingam and co-workers used amino benzothiazole derivative as the starting material which reacted with salicylaldehyde/ bromosalicylaldehyde which underwent straightforward condensation reactions in presence of ethanol to produce two Schiff bases 2-[6-methylbenzothiazol-2-ylimino] methyl phenol and 3-bromo-2-[6-methylbenzothiazol-2-ylimino] methyl phenol.[50] Lastly, both coumarin-based azo dye molecules and benzothiazole-based Schiff bases show the same outcomes (MIC of 0.8–1.6 µg mL^{-1} which is more beneficial than Streptomycin.[50]

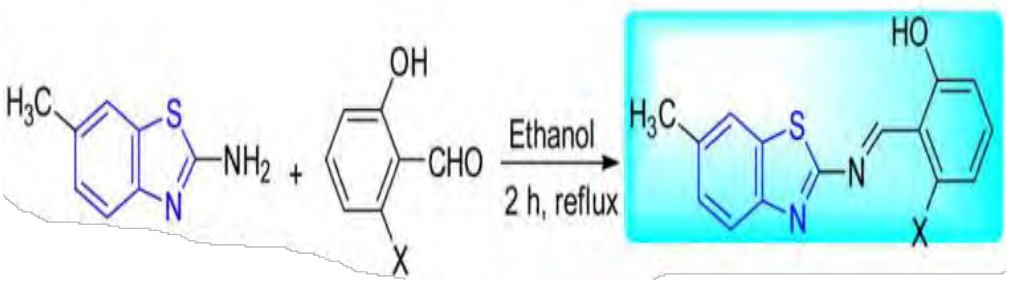

Figure 36: Synthesis approach of benzothiazole based Schiff bases by Suyambulingam et al. (Yadav et al., 2023b)

Bhat et al. synthesize a series of azo-ester derivatives of benzothiazole utilizing dicyclohexylcarbodiimide (coupling reagent) and 4-(dimethylamino)pyridine (nucleophile) through the reaction of Steglich esterification.[50] Firstly, 2-amino substituted benzothiazoles underwent diazotization to form the diazotized product.[50] The coupling among diazotized product and phenol under sodium hydroxide produces the azo-dye complex which further reacts under substituted carboxylic acid, dicyclohexylcarbodiimide, and 4-(dimethylamino)pyridine to produce benzothiazole azo-ester derivatives. Moreover, this analog showed more benefits than Pyrazinamide and Streptomycin.[50]

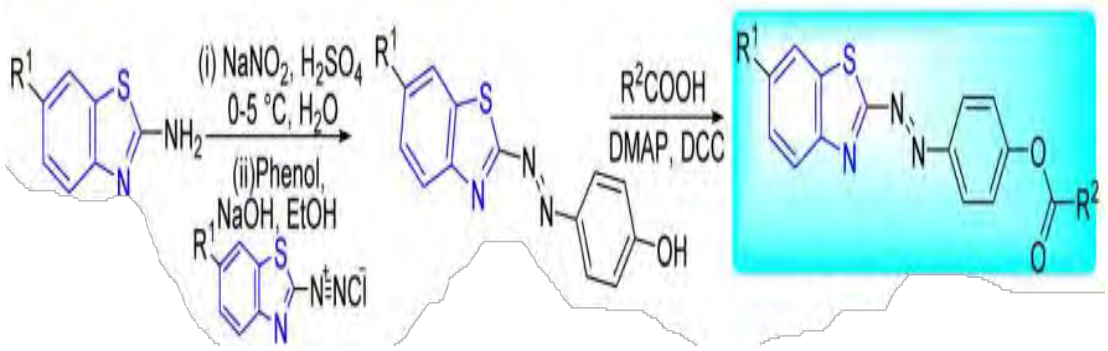

Figure 37: Synthesis approach of azo-ester derivatives of benzothiazole by Bhat et al. (Yadav et al., 2023c)

The pyrazole conjugates of benzothiazole derivatives show benefits against Streptomycin and Ciprofloxacin.[50] Employing various acetophenones and phenyl hydrazines, Bhat et al.

synthesized 1-phenyl-2-(1- phenylethylidene) hydrazines which further underwent reflux conditions and $POCL_3$ to form the pyrazole-conjugated benzothiazole analogs.[50] This analog further underwent 2-hydrazinyl benzothiazole and benzothiazole-2-carbohydrazide to produce two preferred compounds.[50]

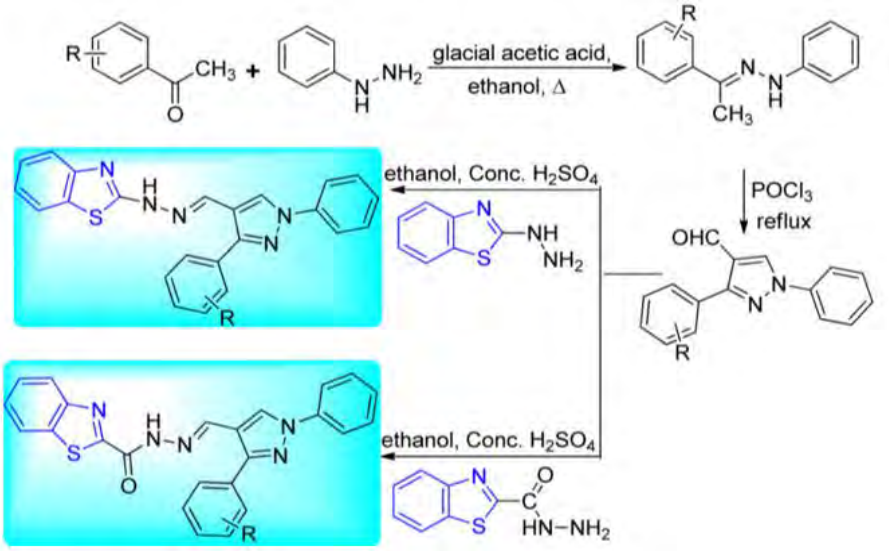

Figure 38: Synthesis approach of pyrazole-conjugated benzothiazole analogs by Bhat et al. (Yadav et al., 2023d)

5.1.17 Pyrazolylpyrazoline-Based Triazole and Tetrazole Derivatives

In 2023, Zala et al. showed an increase in antitubercular effectiveness through pyrazolylpyrazoline-based triazole and tetrazole derivatives. Among the derivatives, 3′-((4H-1,2,4-Triazol-4-yl)amino)-5′-methyl-1′-phenyl-5-(thiophen-2-yl)-3,4-dihydro-1′H,2H-[3,4′-bipyrazol]-2-yl)(pyridin-4-yl)methanone **(i)**; (3′-((1H-Tetrazol-5-yl)amino)-5′-methyl-1′-phenyl-5-(thiophen-2-yl)-3,4-dihydro-1′H,2H-[3,4′ bipyrazol]-2-yl)(pyridin-4-yl)methanone **(ii)**; (3′-((1H-Tetrazol-5-yl)amino)-5- (furan-2-yl)-5′-methyl-1′-phenyl-3,4-dihydro-1′H,2H-[3,4′-bipyrazol]-2-yl)(pyridin-4-yl)methanone **(iii)**; (3′-((1H-Tetrazol-5-yl)amino)-5′-methyl-1′-phenyl-5-(thiophen-2-yl)-3,4-dihydro-1′H,2H-[3,4′-bipyrazol]-2-yl)(pyridin-3-yl)methanone **(iv)**; (3′-((1H-Tetrazol-5-yl)amino)-5-(furan-2-yl)-5′-methyl-1′-phenyl-3,4-

dihydro-1′H,2H-[3,4′- bipyrazol]-2-yl)(pyridin-3-yl)methanone **(v)**showed the most effectiveness.[51]

All these compounds were synthesized from using 5-((4H-1,2,4-triazol-4-yl)amino)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde and 5-((1H-tetrazol-5-yl)amino)-3- methyl-1-phenyl-1H-pyrazole-4-carbaldehyde as the starting materials.[51] Both of these compounds underwent a reaction with 2-acetyl thiophene and 2-acetyl furan in the presence of hydrazide derivatives and 50% ethanolic NaOH. Here, compounds **(i)** and **(iv)** are more potent than rifampicin as they showed 98 % and 99% of inhibitions against M.tb.[51]

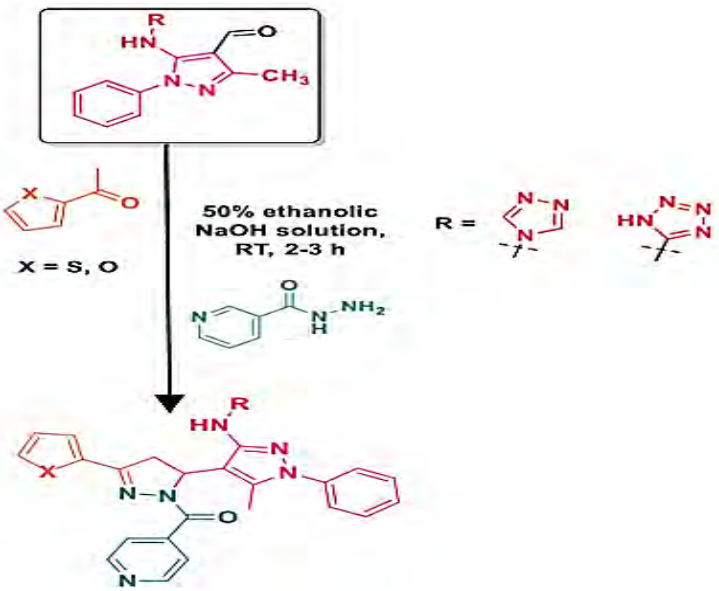

Figure 39: Synthesis approach of pyrazolylpyrazoline-based triazole and tetrazole derivatives by Zala et al.

Chapter 6

Impact of the Review Article

It is difficult to control tuberculosis because of its complex drug resistance. Current drugs are being repurposed to discover the most effective drug regimen to eradicate tuberculosis worldwide. This review article provides comprehensive details about anti-tubercular drugs and their synthetic methods. It covers different strategic approaches to old and existing drugs and drug targets identified by various researchers, which can be beneficial in developing new synthetic strategies and analogs.

Additionally, this review might be valuable for researchers exploring new biochemical pathways and therapeutic options to improve clinical results. The article also delves into the mechanisms of action of each drug, providing insight for future researchers on drug-drug interactions, pathways, and effectiveness. Additionally, the article discusses drugs such as Gatifloxacin, Doxycycline, and Nitazoxanide, undergoing trial phases to assess their effectiveness in eradicating tuberculosis infection.[35] Gatifloxacin has shown effectiveness by targeting DNA gyrase, while Nitazoxanide has bactericidal effects by disrupting the membrane potential.[35] Furthermore, more research is necessary on these new drugs to determine whether they can be included in the treatment strategy. Since the treatment strategy is challenging due to various factors, drug regimens also vary and may carry side effects that lead to various other health issues. Therefore, it is crucial to develop drugs with minimal to no side effects.

Chapter 7

Conclusion

To fight Mycobacterium tuberculosis infection, ongoing research aims to develop new and improve existing anti-tubercular drugs. This review offers comprehensive information on the diagnosis, treatment, challenges, pharmacological aspects, and synthetic strategies for up-to-date antitubercular drugs. It covers both conventional and advanced information on every aspect of M.tb. While a few potent compounds have been identified as anti-TB medications, only some have reached clinical phases. It's crucial to find effective, safe, and innovative treatments for each strain of TB.

Moreover, it's important to focus on cost-effective treatment and screening methods for developing countries like Bangladesh, India, Pakistan, and others with poor economic status, as many patients are unable to seek treatment due to financial constraints. Therefore, an affordable treatment strategy is necessary. To ensure cost-effective treatment programs, stakeholders such as researchers, medical professionals, and industry experts require enough financing and research opportunities, which should be navigated with the government. There is a widespread notion that expediting progress toward health benefits requires integrating new vaccine research methodologies, strengthening laboratory models, expediting clinical trials, and removing impediments to expanding successful programs. This review can be valuable for researchers and the general public, offering extensive knowledge about tuberculosis, its mechanism of resistance and synthetic methods towards novel antitubercular drugs. It's hoped that this information will assist researchers in developing new anti-tubercular drugs or modifying existing ones.

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