ISSN: 2694-166X
Scientific Journal
Biology & Life Sciences
DOI: 10.33552/SJBLS.2025.03.000580
Iris Publishers

Review Article

Copyright © All rights are reserved by Megha Saha

Advancing Drug Discovery for *Mycobacterium Tuberculosis*: Challenges and Strategies

Megha Saha*

Department of Biochemistry, University of California, Riverside, United States

***Corresponding author:** Megha Saha, Department of Biochemistry, University of California, Riverside, United States

Received Date: April 01, 2025

Published Date: April 16, 2025

Abstract

The emergence of drug-resistant *Mycobacterium tuberculosis* strains has underscored the urgent need for novel therapeutic approaches. Structure-based drug design (SBDD) specifically the pharmacophore-based drug design has become essential tools in this endeavour, enabling the identification of new inhibitors that target specific bacterial mechanisms, such as the tertiary structure of bacterial RNA polymerase. Computational techniques, including molecular docking, virtual screening, and machine learning, facilitate the rational design of compounds with enhanced potency and reduced resistance potential. This review article is highlighting the recent advancements in the development of new therapeutic approaches against this deadly infectious disease.

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), remains one of the major global health burdens even in the 21st century, responsible for millions of deaths annually [1]. According to the World Health Organization (WHO), tuberculosis (TB) caused 1.25 million deaths globally in 2023, including 161,000 among people with HIV [2]. TB has likely regained its position as the leading cause of death from a single infectious agent. The disease affects all countries and age groups. In 2023, an estimated 10.8 million people developed TB globally, comprising 6.0 million men, 3.6 million women, and 1.3 million children [3]. Despite advancements in diagnosis and treatment, TB remains a major global health challenge, emphasizing the urgent need for improved prevention, treatment strategies, and efforts to combat drug-resistant strains [4]. *M. tuberculosis*, also known as Koch's bacillus, is a highly adaptable pathogen that has evolved sophisticated mechanisms to evade host immune responses and persist within macrophages, one of the major immune cells in our system [5]. Its unique cell wall composition, slow replication rate, and ability to modulate host immune signalling contribute to its persistence and pathogenicity [6].

As one of the oldest known human diseases, TB predominantly affects the lungs (pulmonary TB) but can also disseminate to other organs, resulting in extrapulmonary manifestations [7]. The disease presents with a spectrum of clinical symptoms, including chronic cough, fever, night sweats, weight loss, and fatigue [8]. In cases of extrapulmonary TB, organ-specific complications arise, such as neurological impairments in TB meningitis and joint destruction in skeletal TB [9]. One of the greatest challenges in TB control is latent TB infection (LTBI), wherein individuals harbor the bacteria

This work is licensed under Creative Commons Attribution 4.0 License

without manifesting symptoms but remain at risk of reactivation, particularly under immunocompromised conditions [10]. This dormant state of infection serves as a reservoir for future outbreaks, complicating eradication efforts [10]. Furthermore, the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains has rendered conventional treatments increasingly ineffective, underscoring the urgent need for novel therapeutic approaches [11]. This review article highlighting the recent advancements in the development of new therapeutic approaches against this deadly infectious disease.

Pathogenesis of Tuberculosis

Onset and Early Infection (1-7 Days)

TB is primarily transmitted through inhalation of aerosolized respiratory droplets containing M. tuberculosis from an infected individual. Upon entering the alveolar spaces of the lungs, the bacteria encounter alveolar macrophages, the first line of immune defense. The ability of macrophages to effectively eliminate *Mycobacterium tuberculosis* is influenced by both bacterial virulence factors and host immune competence. In cases where the macrophages fail to eradicate the bacteria, M. tuberculosis survives intracellularly and begins to replicate. The infection progresses as the bacteria proliferate within macrophages until the host cells undergo lysis, releasing bacterial progeny. These bacteria are then engulfed by other alveolar macrophages, continuing the cycle of intracellular infection [12,13].

Symbiosis and Granuloma Formation (7-21 Days)

As the infection advances, additional macrophages are recruited from the bloodstream. These newly recruited macrophages internalize the bacteria, creating a microenvironment that supports bacterial persistence without immediate destruction. This phase resembles a symbiotic interaction, as the bacteria slow their replication while residing within host cells. However, rapid proliferation eventually exhausts the supply of non-activated macrophages, leading to localized oxygen depletion and a decrease in pH. These factors restrict further bacterial growth, leading to a state of dormancy [14,15]. The immune system responds by forming granulomas—organized cellular structures aimed at containing the infection. The core of the granuloma consists of infected macrophages surrounded by immune cells, including T lymphocytes, which recognize *M. tuberculosis* antigens. This immune response triggers the release of cytokines, particularly interferon-gamma (IFN-γ), which enhances macrophage activation and improves bacterial clearance [15,16]. However, the granuloma's central region becomes increasingly hypoxic and necrotic, leading to caseous necrosis, a hallmark of TB pathology.

Immune Response and Disease Progression (After 21 Days)

While granulomas serve to contain the infection, they also create a reservoir for persistent bacteria. Some macrophages within the granuloma remain inactive, providing a niche where M. tuberculosis can survive [17,18]. If the granuloma expands or ruptures, bacteria may disseminate into the bronchial tree, spreading within the lungs or gaining access to the bloodstream. Hematogenous dissemination results in extrapulmonary TB, affecting sites such as bones, lymph nodes, joints, and the genitourinary system, leading to miliary TB. In advanced stages, granulomas may undergo liquefaction, creating a highly favourable environment for bacterial growth. This phase accelerates disease progression and facilitates transmission as bacteria are expelled through respiratory secretions [19-21].

Latent TB and Reactivation

Following primary infection, *M. tuberculosis* can persist in a dormant state for years or even decades. Reactivation occurs when immune defences are compromised due to factors such as HIV/AIDS, malnutrition, aging, or chronic stress, leading to active disease recurrence [22].

Existing drugs to combat *Mycobacterium tuberculosis*

Mycobacterium tuberculosis is a highly adaptable pathogen capable of altering its metabolic pathways in response to drug exposure and environmental stresses. This adaptability enables Mtb to persist in a dormant state, complicating treatment strategies. TB treatment is divided into first line and second-line drugs based on their efficacy, safety, and use in drug-resistant TB cases [23].

First-Line Anti-TB Drugs

First-line drugs are the most effective and commonly used medications in standard TB treatment regimens. They are preferred due to their potent bactericidal or sterilizing effects and relatively lower toxicity [24]. These drugs include

a. **Isoniazid (INH)** was the first successful TB drug, introduced in the 1950s [25]. It functions as a prodrug that requires activation by the catalase-peroxidase enzyme in M. tuberculosis, generating highly reactive isonicotinoyl radicals. These radicals form a complex with NAD+ and NADP+, which inhibits key bacterial enzymes, including dihydrofolate reductase (DHFR), thereby interfering with nucleic acid synthesis [26].

b. **Clofazimine,** originally developed in 1957 for TB treatment, is a phenazine derivative that generates reactive oxygen species, disrupting the respiratory chain of M. tuberculosis [27]. However, its precise mechanism of action remains unclear. Although it demonstrated promising results in early screenings, clofazimine showed limited efficacy in non-human primate models [28].

c. **Rifampicin (RIF)** is a cornerstone of TB therapy due to its potent inhibition of bacterial transcription. It binds to the β subunit of RNA polymerase (encoded by the rpoB gene), preventing nucleotide incorporation and causing transcriptional stalling [29-31]. This mechanism is highly effective, making rifampicin a key component of standard TB treatment regimens.

d. **Ethambutol (EMB)** functions by disrupting mycobacterial cell wall synthesis. It inhibits arabinosyl transferases (EmbA and EmbB), preventing arabinogalactan polymerization, an essential component of the cell wall [32-34]. Additionally, ethambutol interferes with peptidoglycan biosynthesis, further

compromising bacterial cell wall integrity [35].

e. **Pyrazinamide (PZA),** a nicotinamide analog, was instrumental in shortening TB treatment regimens (Mitchison, 1985). It requires conversion to pyrazinoic acid (POA) by the enzyme pyrazinamidase (PZase) in M. tuberculosis. Mutations in the pncA gene, which encodes PZase, are a major cause of resistance to pyrazinamide [36].

Second-Line Anti-TB Drugs

Second-line drugs are used when first-line treatment fails, particularly in MDR-TB (resistant to at least isoniazid and rifampicin) and extensively drug-resistant TB (XDR-TB, resistant to isoniazid, rifampicin, fluoroquinolones, and at least one second-line injectable drug). Second-line drugs are generally less effective, more toxic, and require longer treatment durations [37].

a. **Fluoroquinolones** (Levofloxacin, Moxifloxacin, Gatifloxacin) inhibit DNA gyrase (encoded by gyrA and gyrB), an essential enzyme for bacterial DNA replication. They are highly effective against TB, particularly in MDR-TB treatment regimens. However, mutations in the gyrA or gyrB genes can lead to fluoroquinolone resistance, complicating treatment [38].

b. **Injectable Aminoglycosides** (Amikacin, Kanamycin, Capreomycin) inhibit bacterial protein synthesis by binding to the 30S ribosomal subunit. These drugs are reserved for MDR-TB and XDR-TB cases but are associated with nephrotoxicity and ototoxicity, making their use challenging [39].

c. **Bedaquiline** (BDQ) is a novel second-line drug that targets the ATP synthase enzyme, disrupting bacterial energy metabolism. It is highly effective against drug-resistant TB and is used in combination therapies. However, it carries a risk of QT prolongation, which requires careful monitoring during treatment [40].

d. **Linezolid** inhibits bacterial protein synthesis by targeting the 50S ribosomal subunit. It is an effective option for treating MDR-TB and XDR-TB but is associated with significant adverse effects, including bone marrow suppression and neuropathy [41].

e. **Pretomanid** is a new nitroimidazole drug used in combination regimens for drug-resistant TB. It disrupts mycolic acid biosynthesis and induces respiratory poisoning under hypoxic conditions. It is particularly effective when used with bedaquiline and linezolid for XDR-TB [42].

f. **Delamanid** is another nitroimidazole compound that inhibits mycolic acid synthesis, reducing bacterial cell wall integrity. It is used as part of MDR-TB and XDR-TB treatment regimens but requires careful monitoring due to potential cardiac side effects [43].

Mechanisms of drug resistance in *Mycobacterium tuberculosis*

The genus Mycobacterium is well known for its inherent resistance to a wide range of antibiotics, primarily due to its uniquely thick, lipid-rich cell envelope, which acts as a formidable barrier to drug penetration [44]. Even when antibiotics successfully traverse this barrier, Mtb employs multiple mechanisms to neutralize their effects, including enzymatic degradation or structural modification of the drug, ultimately rendering it ineffective [45-48]. Additionally, Mtb possesses efflux pump systems that contribute to reduced intracellular drug concentrations. These efflux systems are expressed under various conditions, and while their role in clinically significant resistance is still debated, they may serve as an initial adaptive response that facilitates the emergence of high-level resistance [49-50].

Unlike many bacterial species that acquire antimicrobial resistance through horizontal gene transfer, *M. tuberculosis* exhibits a striking lack of this mechanism. While horizontal gene transfer has been documented among other *Mycobacterium* species [51], it does not appear to play a significant role in Mtb's resistance acquisition [52-54]. Instead, drug resistance in Mtb primarily arises through chromosomal mutations, rather than through plasmids or other mobile genetic elements. These mutations typically occur in genes encoding drug targets, drug-activating enzymes, or transcriptional regulators, leading to reduced drug susceptibility [54]. A validated target for broad-spectrum antibiotics in mycobacteria is RNA polymerase (RNAP). However, the increasing prevalence of antibiotic-resistant strains has significantly diminished the effectiveness of existing drugs targeting this enzyme. Resistance to rifampicin, a cornerstone of TB therapy, is predominantly conferred by mutations in the rpoB gene, which encodes the β-subunit of RNAP. These mutations alter the rifampicin-binding site, reducing the drug's affinity for the enzyme and leading to treatment failure [55].

Structure Based Drug Design to Overcome *Mycobacterium tuberculosis* Drug Resistance

The rise of multidrug-resistant (*MDR-TB*) and extensively drug-resistant tuberculosis (*XDR-TB*) has necessitated the development of innovative drug discovery strategies that can overcome resistance mechanisms and enhance therapeutic efficacy. Structure-based drug design (*SBDD*) has emerged as a powerful approach, utilizing molecular insights to identify and optimize drug-target interactions [56]. By leveraging high-resolution structural data, SBDD enables the rational design of compounds that can bind effectively to essential *Mycobacterium tuberculosis* (*Mtb*) enzymes while avoiding resistance mutations. A key subcomponent of SBDD is pharmacophore-based drug design (PBDD), which focuses on defining and utilizing critical chemical features required for biological activity [57]. The integration of these two methods has significantly accelerated the discovery of novel anti-TB agents with improved efficacy and resistance-evading properties.

Structure-based drug design (SBDD) plays a pivotal role in the drug discovery process by integrating structural insights with computational methodologies to identify and optimize drug candidates. As an iterative process, SBDD progresses through multiple refinement cycles to enhance drug efficacy and specificity, ultimately leading to clinical trials [58]. The drug discovery pipeline encompasses four key phases: discovery, development, clinical trials, and regulatory approval [59]. The foundation of SBDD lies in the identification and validation of a target protein. This is achieved through

integrative structural biology techniques such as nuclear magnetic resonance (NMR), X-ray crystallography, and cryo-electron microscopy [60]. When experimental structures are unavailable, computational methods such as homology modelling, threading, and ab initio modelling are employed. Among these, homology modelling is particularly effective when the target shares over 40% sequence similarity with a homologous protein. Following structural prediction, model validation is performed using stereochemical assessment tools like the Ramachandran plot, ensuring structural accuracy and reliability [61].

Once the target protein structure is established, identifying the binding pocket is crucial. Binding site prediction methods leverage interaction energy calculations and van der Waals (vdW) forces to map potential ligand-binding sites. Energy-based approaches, such as Q-SiteFinder, evaluate vdW interaction energies with probes like methyl groups, clustering and ranking potential binding sites based on interaction energy. Functional annotation of interacting residues further aids in binding site determination [62]. Enzymes such as RNA polymerase (rpoB, targeted by rifampicin), ATP synthase (targeted by bedaquiline), and enoyl-ACP reductase (InhA, targeted by isoniazid) have been extensively studied using these techniques, allowing researchers to identify precise binding sites and mechanisms of action [63-65]. Molecular docking and molecular dynamics simulations further aid in predicting how potential drug candidates interact with these targets, optimizing their binding affinity, metabolic stability, and resistance evasion. This approach has been particularly useful in designing next-generation rifamycins that can overcome *rpoB* mutations, which confer resistance to rifampicin, a first-line anti-TB drug [65-67].

Structure based drug design (SBDD) Approaches

Virtual Screening for Lead Identification

Hit discovery is initiated by docking compound libraries into the binding cavity of the target protein. Virtual screening (VS), a robust computational approach for lead identification, screens large databases of drug-like compounds against well-characterized target proteins. VS is categorized into ligand-based (LBVS) and structure-based (SBVS) virtual screening. LBVS relies on biological activity data to differentiate active and inactive compounds, using pharmacophore modelling and similarity analysis to identify promising scaffolds. Top computational hits from VS are subsequently validated through in vitro assays [68,69].

De Novo Drug Design

De novo drug design generates novel chemical compounds tailored to fit target binding sites. This approach employs stochastic algorithms, optimizing chemical space exploration to identify lead candidates with desired properties. Two primary strategies exist: ligand-based and receptor-based de novo drug design, with the latter being more prevalent. Receptor-based design constructs suitable small molecules by assembling molecular fragments within the binding cavity, facilitated either computationally or through co-crystallization of ligand-receptor complexes [70-72].

Molecular Docking and Scoring Functions

Molecular docking simulates ligand-target interactions, predicting ligand conformations, binding modes, and affinities. This technique is instrumental in assessing ligand stability and binding efficacy. Molecular docking is categorized into flexible-ligand search docking and flexible-protein docking. Flexible-ligand docking employs systematic, stochastic, or simulation-based algorithms to model ligand conformational flexibility. Systematic algorithms analyse degrees of freedom using fragmentation methods, whereas stochastic approaches, including genetic algorithms and Monte Carlo simulations, introduce probabilistic modifications to ligand structures. Additionally, molecular dynamics (MD) simulations provide insights into ligand-induced conformational changes in proteins, improving docking accuracy [73,74].

SBDD in Drug Development:

SBDD has revolutionized drug discovery, leading to the successful development of FDA-approved therapeutics. Notable examples include HIV-1 protease inhibitors such as amprenavir, discovered using protein modelling and MD simulations, and raltitrexed, a thymidylate synthase inhibitor. Additionally, the antibiotic norfloxacin was identified through SBDD methodologies, underscoring the technique's broad applicability in antimicrobial drug discovery [75].

Pharmacophore-based drug design (PBDD): A new avenue for *Mycobacterium tuberculosis* drug development

A pharmacophore is the set of steric and electronic characteristics required to ensure better interactions with a particular biological target and able to generate its biological response [76]. Pharmacophore-based drug design is a method used in drug discovery to identify and optimize the essential structural features required for a molecule to interact with a specific biological target [77]. A pharmacophore represents a set of molecular features, such as hydrogen bond donors, acceptors, aromatic rings, and hydrophobic regions, that are necessary for the compound to bind effectively to the target receptor [78,79]. Unlike SBDD, which relies on direct structural insights, PBDD abstracts these key features from known inhibitors, allowing for a broader search of structurally diverse compounds that retain the necessary interactions [80].

This approach involves the creation of a 3D model of the pharmacophore based on known active compounds, followed by screening and virtual screening of compound libraries to identify potential drug candidates that fit the pharmacophore model [81]. By focusing on the interactions between the drug and the target, pharmacophore-based design enables the rational design of molecules with improved potency, selectivity, and reduced toxicity [82,83]. It is especially useful when there is limited structural information about the target, as it does not require the detailed 3D structure of the receptor, making it a powerful tool in modern drug discovery [84]. PBDD has played a crucial role in anti-TB drug discovery by enabling virtual screening of chemical libraries, rapidly identifying

lead compounds that match pharmacophore models of established TB drugs. The integration of pharmacophore modeling with SBDD ensures that potential drug candidates possess not only an optimal structural fit but also the necessary chemical features for effective inhibition [85,86].

Pharmacophore-based drug design (PBDD) approaches

Ligand-Based Pharmacophore Modelling

Ligand-based pharmacophore modelling (LBPM) is crucial for drug discovery when target structures are unknown. It identifies common chemical features from active ligands, requiring ligand conformation generation and alignment. Ligand flexibility is addressed through pre-enumeration, which is memory-intensive but efficient, or on-the-fly generation, which is computationally demanding. Advanced algorithms like MED-3DMC and Cyndi enhance conformation sampling and molecular alignment accuracy [87,88].

Structure-Based Pharmacophore Modelling

Structure-based pharmacophore modelling (SBPM) derives key interaction features from macromolecular structures. It includes macromolecule–ligand-complex-based and macromolecule-based approaches. The former extract features from ligand-bound structures but is limited when ligands are unknown, while the latter identifies pharmacophoric features directly from the protein's binding site. SBPM often generates excessive features, necessitating computational filtering methods such as machine learning for efficient selection in virtual screening [89-91].

Pharmacophore-Based Virtual Screening (PBVS)

PBVS identifies bioactive compounds by searching chemical databases based on pharmacophore models. It addresses protein flexibility and scoring challenges better than docking-based screening but struggles with molecular flexibility and pharmacophore pattern accuracy. Strategies like ligand pre-enumeration and graph-based searching enhance efficiency. Hybrid approaches integrating PBVS with docking-based screening and molecular dynamics simulations improve accuracy, aiding lead identification in drug discovery [92-94]. Pharmacophore-based drug design offers a rational and innovative approach for developing novel therapeutics against Mycobacterium tuberculosis (Mtb), specifically by targeting the secondary channel of Mtb RNA polymerase (RNAP) [95-98]. RNAP is a well-validated target for tuberculosis (TB) treatment, with rifampicin serving as a frontline antibiotic [99]. However, the rise of rifampicin-resistant strains necessitates alternative therapeutic strategies that circumvent existing resistance mechanisms [100]. The secondary channel of RNAP, which plays a crucial role in nucleotide entry and enzyme function, represents an attractive target for inhibition [101]. Small-molecule inhibitors designed to obstruct this channel can effectively impede transcription and bacterial survival, offering a promising avenue for drug discovery [102].

To identify potential inhibitors, a pharmacophore model can be constructed based on the structural and chemical features of known RNAP inhibitors, including rifampicin analogs, newly identified small molecules and lasso peptides like Microcin J25, that inhibits bacterial transcription by binding to and obstructing the nucleotide-uptake channel of bacterial RNA polymerase [103-105]. This model captures essential pharmacophoric elements—such as hydrogen bond donors and acceptors, hydrophobic regions, and aromatic interactions—that are critical for binding within the secondary channel [106]. Virtual screening of large chemical libraries using this pharmacophore model can facilitate the identification of structurally diverse scaffolds that align with key molecular features, enabling scaffold hopping and the discovery of novel chemical entities with enhanced potency and reduced susceptibility to resistance [107].

After pharmacophore modelling, and ligand selection, integrating molecular docking to further identify the correct inhibitor, refines the selection process by evaluating the binding interactions of screened compounds within the secondary channel of RNAP [108]. Molecular docking simulations help predict binding affinities, orientation, and key intermolecular interactions, ensuring that identified hits exhibit strong and stable interactions with the target site [109]. Incorporating induced-fit docking or molecular dynamics (MD) simulations enhances the predictive accuracy by accounting for protein flexibility, thereby improving the identification of compounds with optimal binding conformations [110]. Additionally, MD simulations allow for the assessment of ligand stability over time, ensuring the robustness of candidate inhibitors under physiological conditions [111].

To optimize lead compounds, cheminformatics approaches such as quantitative structure-activity relationship (QSAR) modeling and machine learning-based optimization can be employed to refine pharmacophore features and improve drug-like properties [112]. The combination of pharmacophore-based virtual screening, molecular docking, and computational optimization offers a comprehensive strategy for rational drug design [113]. By leveraging these computational techniques, novel inhibitors targeting the secondary channel of Mtb RNAP can be systematically identified, paving the way for the development of next-generation anti-tubercular agents capable of overcoming drug resistance and improving TB treatment outcomes [114,115].

Conclusion

Tuberculosis, a serious infectious disease, is going through an emergence of drug-resistant strains, has significantly hindered the effectiveness of standard treatments. This resistance is mainly due to mutations in key targets of existing antibiotics, making it increasingly difficult to treat TB effectively. Therefore, there is an urgent need for new therapeutic strategies to combat this global health threat. One promising approach is pharmacophore-based drug design, which aims to identify compounds that target specific sites within the bacterial machinery. The secondary channel of M. tuberculosis RNA polymerase (RNAP) has been identified as a potential target for novel inhibitors, as it plays an essential role in RNA synthesis and is less likely to develop resistance compared to the primary active site. By using pharmacophore modelling, one can design molecules that specifically bind to this secondary channel. Incorporating molecular docking into this approach helps

to further refine and optimize the binding interactions of potential drug candidates. This strategy allows for the development of more effective and targeted treatments, offering hope for overcoming drug resistance in TB.

References

1. Anderer S (2025) New Global Tuberculosis Cases Hit Record High. JAMA 333(1): 12-15.
2. Heidary M, M Shirani, Melika Moradi, Mehdi Goudarzi, Ramin Pouriran, et al. (2022) Tuberculosis challenges: Resistance, co-infection, diagnosis, and treatment. Eur J Microbiol Immunol 12(1): 1–17.
3. Litvinjenko S, Magwood O, Wu S, Wei X (2023) Burden of tuberculosis among vulnerable populations worldwide: an overview of systematic reviews. Lancet Infect Dis 23(12): 1395–1407.
4. Yayan J, Franke KJ, Berger M, Windisch W, Rasche K (2024) Early detection of tuberculosis: a systematic review. Pneumonia 16(1): 11-19.
5. Chai Q, Zhang Y, Liu CH (2018) *Mycobacterium tuberculosis*: An Adaptable Pathogen Associated With Multiple Human Diseases. Front Cell Infect Microbiol 8(2): 158-165.
6. Ghazaei C (2018) *Mycobacterium tuberculosis* and lipids: Insights into molecular mechanisms from persistence to virulence. J Res Med Sci Off J Isfahan Univ Med Sci 23(2): 63-69.
7. Moule MG, Cirillo JD (2020) *Mycobacterium tuberculosis* Dissemination Plays a Critical Role in Pathogenesis. Front Cell Infect Microbiol 10(8): 65-72.
8. Natarajan A, Beena PM, Devnikar AV, Mali S (2020) A systemic review on tuberculosis. Indian J Tuberc 67(3): 295–311.
9. Natali D, Georges Cloatre, Christian Brosset, Pierre Verdalle, Alain Fauvy, et al. (2020) What pulmonologists need to know about extrapulmonary tuberculosis. Breathe Sheff Engl 16(4): 200216-200225.
10. Kiazyk S, Ball TB (2017) Latent tuberculosis infection: An overview. Can Commun Dis Rep 43(3-4): 62–66.
11. Rastmanesh S, Zeinaly I, Alivirdiloo V, Mobed A, Darvishi M (2025) Biosensing for rapid detection of MDR, XDR and PDR bacteria. Clin Chim Acta 567(5): 120121-120128.
12. Alsayed SSR, Gunosewoyo H (2023) Tuberculosis: Pathogenesis, Current Treatment Regimens and New Drug Targets. Int J Mol Sci 24(6): 5202-5208.
13. Yang J, Zhang L, Qiao W, Luo Y (2023) *Mycobacterium tuberculosis*: Pathogenesis and therapeutic targets. Med Comm 4(5): e353-e360.
14. Ehlers S, Schaible UE (2012) The granuloma in tuberculosis: dynamics of a host-pathogen collusion. Front Immunol 3(1): 411-415.
15. Cronan MR, Rebecca W Beerman, Allison F Rosenberg, Joseph W Saelens, Matthew G Johnson, et al. (2016) Macrophage Epithelial Reprogramming Underlies Mycobacterial Granuloma Formation and Promotes Infection. Immunity 45(4): 861-876.
16. Tobin DM, Ramakrishnan L (2008) Comparative pathogenesis of Mycobacterium marinum and Mycobacterium tuberculosis. Cell Microbiol 10(5): 1027–1039.
17. Silva Miranda M, Breiman A, Allain S, Deknuydt F, Altare F (2012) The tuberculous granuloma: an unsuccessful host defence mechanism providing a safety shelter for the bacteria? Clin Dev Immunol 2012(3): 139127-139133.
18. Chandra P, Grigsby SJ, Philips JA (2022) Immune evasion and provocation by Mycobacterium tuberculosis. Nat Rev Microbiol 20(12): 750–766.
19. Lyu J, Drew E Narum, Susan L Baldwin, Sasha E Larsen, Xiyuan Bai, et al. (2024) Understanding the development of tuberculous granulomas: insights into host protection and pathogenesis, a review in humans and animals. Front Immunol 15(1): 1427559-1427568.
20. Bussi C, Gutierrez MG (2019) Mycobacterium tuberculosis infection of host cells in space and time. FEMS Microbiol Rev 43(4): 341–361.
21. Cardona PJ, Ruiz Manzano J (2004) On the nature of Mycobacterium tuberculosis -latent bacilli. Eur Respir J 24(1): 1044–1051.
22. Lin PL, Flynn JL (2010) Understanding Latent Tuberculosis: A Moving Target. J Immunol 185(1): 15-22.
23. Yang J, Zhang L, Qiao W, Luo Y (2023) Mycobacterium tuberculosis: Pathogenesis and therapeutic targets. Med Comm 4(5): e353-e360.
24. Bakare AA, Victoria Y Moses, Charles T Beckely, Toluwani I Oluyemi, Gift O Ogunfeitimi, et al. (2022) The first-line antituberculosis drugs, and their fixed-dose combination induced abnormal sperm morphology and histological lesions in the testicular cells of male mice. Front Cell Dev Biol 10(1): 1023413-1023420.
25. Farhat MR, Razvan Sultana, Oleg Iartchouk, Sam Bozeman, James Galagan, et al. (2016) Genetic Determinants of Drug Resistance in Mycobacterium tuberculosis and Their Diagnostic Value. Am J Respir Crit Care Med 194(2): 621–630.
26. Karakousis PC, Williams EP, Bishai WR (2008) Altered expression of isoniazid-regulated genes in drug-treated dormant Mycobacterium tuberculosis. J Antimicrob Chemother 61(2): 323–331.
27. Mirnejad R, Arezoo Asadi, Saeed Khoshnood, Habibollah Mirzaei, Mohsen Heidary, et al. (2018) Clofazimine: A useful antibiotic for drug-resistant tuberculosis. Biomed Pharmacother 105(1): 1353–1359.
28. O connor JA, O reilly B, Corcoran GD, O mahony J, Lucey B (2015) Mycobacterium diagnostics: from the primitive to the promising. Br J Biomed Sci 72(1): 32–41.
29. Taniguchi H, H Aramaki, Y Nikaido, Y Mizuguchi, M Nakamura, et al. (1996) Rifampicin resistance and mutation of the rpoB gene in Mycobacterium tuberculosis. FEMS Microbiol Lett 144(1): 103–108.
30. Vall Spinosa A, Lester W, Moulding T, Davidson PT, McClatchy JK (1970) Rifampin in the Treatment of Drug-Resistant Mycobacterium tuberculosis Infections. N Engl J Med 283(12): 616–621.
31. Singh R, SP Dwivedi, US Gaharwar, R Meena, P Rajamani, et al. (2020) Recent updates on drug resistance in Mycobacterium tuberculosis. J Appl Microbiol 128(6): 1547–1567.
32. Belanger AE, GS Besra, ME Ford, K Mikusová, JT Belisle, et al. (1996) The embAB genes of Mycobacterium avium encode an arabinosyl transferase involved in cell wall arabinan biosynthesis that is the target for the antimycobacterial drug ethambutol. Proc Natl Acad Sci 93(21): 11919–11924.
33. Amin AG, Renan Goude, Libin Shi, Jian Zhang, Delphi Chatterjee, et al. (2008) EmbA is an essential arabinosyltransferase in Mycobacterium tuberculosis. Microbiology 154(Pt 1): 240–248.
34. Angala SK, Belardinelli JM, Huc Claustre E, Wheat WH, Jackson M (2014) The cell envelope glycoconjugates of Mycobacterium tuberculosis. Crit Rev Biochem Mol Biol 49(5): 361–399.
35. Pawar A, Jha P, Chopra M, Chaudhry U, Saluja D (2020) Screening of natural compounds that targets glutamate racemase of Mycobacterium tuberculosis reveals the anti-tubercular potential of flavonoids. Sci Rep 10(1): 949-959.
36. Scorpio A, Zhang Y (1996) Mutations in pncA, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. Nat Med 2(6): 662–667.
37. Wang S, Lina Davies Forsman, Chunhua Xu, Haoyue Zhang, Yue Zhu, et al. (2024) Second-line antituberculosis drug exposure thresholds predictive of adverse events in multidrug-resistant tuberculosis treatment. Int J Infect Dis 140(5): 62–69.
38. Devasia RA, Amondrea Blackman, Tebeb Gebretsadik, Marie Griffin, Ayumi Shintani, et al. (2009) Fluoroquinolone Resistance in Mycobacterium tuberculosis: The Effect of Duration and Timing of Fluoroquinolone Exposure. Am J Respir Crit Care Med 180(4): 365–370.

39. Quenard F, Fournier PE, Drancourt M, Brouqui P (2017) Role of second-line injectable antituberculosis drugs in the treatment of MDR/XDR tuberculosis. Int J Antimicrob Agents 50(2): 252–254.

40. Khoshnood S, Mehdi Goudarzi, Elahe Taki, Atieh Darbandi, Ebrahim Kouhsari, et al. (2021) Bedaquiline: Current status and future perspectives. J Glob Antimicrob Resist 25(1): 48–59.

41. Hashemian SM, Farhadi T, Ganjparvar M (2018) Linezolid: a review of its properties, function, and use in critical care. Drug Des Devel Ther 12(2): 1759–1767.

42. Stancil SL, Mirzayev F, Abdel Rahman SM (2021) Profiling Pretomanid as a Therapeutic Option for TB Infection: Evidence to Date. Drug Des Devel Ther Volume 15(2): 2815–2830.

43. Xavier AS, Lakshmanan M (2014) Delamanid: A new armor in combating drug-resistant tuberculosis. J Pharmacol Pharmacother 5(3): 222–224.

44. Jarlier V, Nikaido H (1994) Mycobacterial cell wall: Structure and role in natural resistance to antibiotics. FEMS Microbiol Lett 123(1-2): 11–18.

45. Chambers MA, A Williams, D Gavier Widén, A Whelan, G Hall, et al. (2000) Identification of a Mycobacterium bovis BCG Auxotrophic Mutant That Protects Guinea Pigs against M. bovis and Hematogenous Spread of Mycobacterium tuberculosis without Sensitization to Tuberculin. Infect Immun 68(12): 7094–7099.

46. Nguyen L (2016) Antibiotic resistance mechanisms in M. tuberculosis: an update. Arch Toxicol 90(7): 1585–1604.

47. Wang C, Pascale Peyron, Olga Mestre, Gilla Kaplan, Dick van Soolingen, et al. (2010) Innate Immune Response to Mycobacterium tuberculosis Beijing and Other Genotypes. PLoS ONE 5(10): e13594-e13598.

48. Warrier T, Kanishk Kapilashrami, Argyrides Argyrou, Thomas R Ioerger, David Little, et al. (2016) N-methylation of a bactericidal compound as a resistance mechanism in Mycobacterium tuberculosis. Proc Natl Acad Sci 113(31): E4523-E4530.

49. Wang J, Bing Xi Li, Pu Pu Ge, Jie Li, Qi Wang, et al. (2015) Mycobacterium tuberculosis suppresses innate immunity by coopting the host ubiquitin system. Nat Immunol 16(3): 237–245.

50. Zhang Y, Yew WW (2009) Mechanisms of drug resistance in Mycobacterium tuberculosis. Int J Tuberc Lung Dis 13(11): 1320–1330.

51. Silva Junior GBD, Brito LDS, Rabelo STDO, Saboia ZMRMD (2016) Chronic kidney disease related to renal tuberculosis: a case report. Rev Soc Bras Med Trop 49(3): 386–388.

52. Cole ST, R Brosch, J Parkhill, T Garnier, C Churcher, et al. (1998) Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature 393(6685): 537-544.

53. Gagneux S, Small PM (2007) Global phylogeography of Mycobacterium tuberculosis and implications for tuberculosis product development. Lancet Infect Dis 7(5): 328–337.

54. Gygli SM, Borrell S, Trauner A, Gagneux S (2017) Antimicrobial resistance in Mycobacterium tuberculosis: mechanistic and evolutionary perspectives. FEMS Microbiol Rev 41(3): 354–373.

55. Sachan RSK, Vyoma Mistry, Mayuri Dholaria, Abhishek Rana, Inderpal Devgon, et al. (2023) Overcoming Mycobacterium tuberculosis Drug Resistance: Novel Medications and Repositioning Strategies. ACS Omega 8(36): 32244–32257.

56. Ejalonibu MA, Segun A Ogundare, Ahmed A Elrashedy, Morufat A Ejalonibu, Monsurat M Lawal, et al. (2021) Drug Discovery for Mycobacterium tuberculosis Using Structure-Based Computer-Aided Drug Design Approach. Int J Mol Sci 22(24): 13259-13265.

57. Ejalonibu MA, Segun A Ogundare, Ahmed A Elrashedy, Morufat A Ejalonibu, Monsurat M Lawal, et al. (2021) Drug Discovery for Mycobacterium tuberculosis Using Structure-Based Computer-Aided Drug Design Approach. Int J Mol Sci 22(24): 13259-13265.

58. Batool M, Ahmad B, Choi S (2019) A Structure-Based Drug Discovery Paradigm. Int J Mol Sci 20(11): 2783-2789.

59. Mdluli K, Kaneko T, Upton A (2015) The tuberculosis drug discovery and development pipeline and emerging drug targets. Cold Spring Harb. Perspect Med 5(6): a021154-a021159.

60. Cebi E, Joohyun Lee, Vinod Kumar Subramani, Nayeon Bak, Changsuk Oh, et al. (2024) Cryo-electron microscopy-based drug design. Front Mol Biosci 11(1): 1342179-1342186.

61. Venselaar H, Robbie P Joosten, Bas Vroling, Coos AB Baakman, Maarten L Hekkelman, et al. (2010) Homology modelling and spectroscopy, a never-ending love story. Eur Biophys J 39(4): 551–563.

62. Tsujikawa H, Kenta Sato, Cao Wei, Gul Saad, Kazuya Sumikoshi, et al. (2016) Development of a protein–ligand-binding site prediction method based on interaction energy and sequence conservation. J Struct Funct Genomics 17(2-3): 39–49.

63. Dookie N, Rambaran S, Padayatchi N, Mahomed S, Naidoo K (2018) Evolution of drug resistance in Mycobacterium tuberculosis: a review on the molecular determinants of resistance and implications for personalized care. J Antimicrob Chemother 73(5): 1138–1151.

64. Nguyen L (2016) Antibiotic resistance mechanisms in M. tuberculosis: an update. Arch Toxicol 90(7): 1585–1604.

65. Bhat ZS, Rather MA, Maqbool M, Ahmad Z (2018) Drug targets exploited in Mycobacterium tuberculosis: Pitfalls and promises on the horizon. Biomed Pharmacother 103(1): 1733–1747.

66. Santos LHS, Ferreira RS, Caffarena ER (2019) Integrating Molecular Docking and Molecular Dynamics Simulations. Docking Screens for Drug Discovery (ed. De Azevedo, WF) (Springer New York, New York, NY, 2019) 2053(1): 13–34.

67. Fatoki TH, Tosin Christianah Balogun, Adebayo Emmanuel Ojewuyi, Aduragbemi Christianah Omole, Oluwaseun Victor Olukayode, et al. (2024) In silico molecular targets, docking, dynamics simulation and physiologically based pharmacokinetics modeling of oritavancin. BMC Pharmacol Toxicol 25(1): 79-85.

68. Lionta E, Spyrou G, Vassilatis D, Cournia Z (2014) Structure-Based Virtual Screening for Drug Discovery: Principles, Applications and Recent Advances. Curr Top Med Chem 14(16): 1923–1938.

69. Batool M, Ahmad B, Choi SA (2019) Structure-Based Drug Discovery Paradigm. Int J Mol Sci 20(11): 2783-2786.

70. Mouchlis VD, Antreas Afantitis, Angela Serra, Michele Fratello, Anastasios G Papadiamantis, et al. (2021) Advances in De Novo Drug Design: From Conventional to Machine Learning Methods. Int J Mol Sci 22(4): 1676-1690.

71. Tang Y, Moretti R, Meiler J (2024) Recent Advances in Automated Structure-Based De Novo Drug Design. J Chem Inf Model 64(6): 1794–1805.

72. Sliwoski G, Kothiwale S, Meiler J, Lowe EW (2014) Computational Methods in Drug Discovery. Pharmacol Rev 66(1): 334–395.

73. Agu PC, CA Afiukwa, OU Orji, EM Ezeh, IH Ofoke, et al. (2023) Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Sci Rep 13(1): 13398-13411.

74. Meng XY, Zhang HX, Mezei M, Cui M (2011) Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery. Curr Comput Aided-Drug Des 7(2): 146–157.

75. Batool M, Ahmad B, Choi SA (2019) Structure-Based Drug Discovery Paradigm. Int J Mol Sci 20(11): 2783.

76. Giordano D, Biancaniello C, Argenio MA, Facchiano A (2022) Drug Design by Pharmacophore and Virtual Screening Approach. Pharmaceuticals 15(5): 646-658.

77. Seidel T, Wieder O, Garon A, Langer T (2020) Applications of the Pharmacophore Concept in Natural Product inspired Drug Design. Mol Inform 39(11): e2000059-e2000065.

78. Langer T, Hoffmann RD (2006) Pharmacophore modelling: applications in drug discovery. Expert Opin Drug Discov 1(1): 261–267.

79. Momin Y, Beloshe V (2025) Pharmacophore modeling in drug design. Advances in Pharmacology 103(1): 313-324.

80. Anderson AC (2012) Structure-Based Functional Design of Drugs: From Target to Lead Compound. Molecular Profiling (eds. Espina V, Liotta LA) 823(2): 359–366.

81. Giordano D, Biancaniello C, Argenio MA, Facchiano A (2022) Drug Design by Pharmacophore and Virtual Screening Approach. Pharm Basel Switz 15(5): 646-652.

82. Biala G, et al. (2023) Research in the Field of Drug Design and Development. Pharm Basel Switz 16: 1283.

83. Wang S, Dong G, Sheng C (2019) Structural simplification: an efficient strategy in lead optimization. Acta Pharm Sin B 9(5): 880–901.

84. Chang Y, Bryson A Hawkins, Jonathan J Du, Paul W Groundwater, David E Hibbs, et al. (2022) A Guide to In Silico Drug Design. Pharmaceutics 15(1): 49-60.

85. Ejalonibu MA, Segun A Ogundare, Ahmed A Elrashedy, Morufat A Ejalonibu, Monsurat M Lawal, et al. (2021) Drug Discovery for Mycobacterium tuberculosis Using Structure-Based Computer-Aided Drug Design Approach. Int J Mol Sci 22(24): 13259-13269.

86. Bhowmik R, Ravi Kant, Ajay Manaithiya, Daman Saluja, Bharti Vyas, et al. (2023) Navigating bioactivity space in anti-tubercular drug discovery through the deployment of advanced machine learning models and cheminformatics tools: a molecular modeling based retrospective study. Front Pharmacol 14(7): 1265573-1265585.

87. Chang C, Ekins S, Bahadduri P, Swaan PW (2006) Pharmacophore-based discovery of ligands for drug transporters. Adv Drug Deliv Rev 58(12-13): 1431–1450.

88. Molla M, Mohammed Othman Aljahdali, Md Afsar Ahmed Sumon, Amer H Asseri, Hisham N Altayb, et al. (2023) Integrative Ligand-Based Pharmacophore Modeling, Virtual Screening, and Molecular Docking Simulation Approaches Identified Potential Lead Compounds against Pancreatic Cancer by Targeting FAK1. Pharmaceuticals 16(1): 120-125.

89. Pirhadi S, Shiri F, Ghasemi JB (2013) Methods and Applications of Structure Based Pharmacophores in Drug Discovery. Curr Top Med Chem 13(9): 1036–1047.

90. Oktay L, Sayyah E, Durdağı S (2023) Dynamic Structure-based Pharmacophore Models for Virtual Screening of Small Molecule Libraries Targeting the YB-1. pp. 1-18.

91. Khandoker Minu M, Md Enamul Kabir Talukder, Ramzi A Mothana, Sk Injamamul Islam, Abdullah R Alanzi, et al. (2024) In-vitro and in-silico evaluation of rue herb for SARS-CoV-2 treatment. Int Immunopharmacol 143(Pt 1):113318-113328.

92. Chen, Z, Hong lin Li, Qi jun Zhang, Xiao guang Bao, Kun qian Yu, et al. (2009) Pharmacophore-based virtual screening versus docking-based virtual screening: a benchmark comparison against eight targets. Acta Pharmacol Sin 30(12): 1694–1708.

93. Sun H (2008) Pharmacophore-Based Virtual Screening. Curr Med Chem 15(10): 1018–1024.

94. Choudhury C, Bhardwaj A (2020) Hybrid Dynamic Pharmacophore Models as Effective Tools to Identify Novel Chemotypes for Anti-TB Inhibitor Design: A Case Study With Mtb-DapB. Front Chem 8(6): 596412-596419.

95. Rajeswaran W, Shireen R Ashkar, Pil H Lee, Larisa Yeomans, Yeonoh Shin, et al. (2022) Optimization of Benzoxazinorifamycins to Improve Mycobacterium tuberculosis RNA Polymerase Inhibition and Treatment of Tuberculosis. ACS Infect Dis 8(8): 1422–1438.

96. Zhang X, Ruixia Zhao, Yao Qi, Xiong Yan, Gaoxiu Qi, et al. (2024) The progress of *Mycobacterium tuberculosis* drug targets. Front Med 11(1): 1455715-1455725.

97. Stephanie F, Tambunan USF, Siahaan TJ (2022) *M. tuberculosis* Transcription Machinery: A Review on the Mycobacterial RNA Polymerase and Drug Discovery Efforts. Life 12(11): 1774-1778.

98. Aderemi AV, Snee M (2025) Targeting Mycobacterium tuberculosis F420-dependent dehydrogenases for new anti-tubercular drug discovery. Indian J Tuberc S0019570725000836.

99. Mosaei H, Zenkin N (2020) Inhibition of RNA Polymerase by Rifampicin and Rifamycin-Like Molecules. EcoSal Plus 9(1): 10.1128-10.1139.

100. Chinemerem Nwobodo D, Malachy Chigozie Ugwu, Clement Oliseloke Anie, Mushtak T S Al-Ouqaili, Joseph Chinedu Ikem, et al. (2022) Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. J Clin Lab Anal 36(9): e24655-e24666.

101. Nickels BE, Hochschild A (2004) Regulation of RNA Polymerase through the Secondary Channel. Cell 118(3): 281–284.

102. Lv C, Ying Li, Yuxia Wei, Jiayu Wang, Hui Yu, et al. (2022) Research Progress on Small Molecular Inhibitors of the Type 3 Secretion System. Molecules 27(23): 8348-8365.

103. Semenova E, Yuzenkova Y, Peduzzi J, Rebuffat S, Severinov K (2005) Structure-activity analysis of microcinJ25: distinct parts of the threaded lasso molecule are responsible for interaction with bacterial RNA polymerase. J Bacteriol 187(11): 3859–3863.

104. Bayro MJ, Jayanta Mukhopadhyay, GVT Swapna, Janet Y Huang, Li Chung Ma, et al. (2003) Structure of Antibacterial Peptide Microcin J25: A 21-Residue Lariat Protoknot. J Am Chem Soc 125(141): 12382–12383.

105. Braffman NR, Frank J Piscottab, Jesse Hauvera, Elizabeth A Campbella, A James Link, et al. (2019) Structural mechanism of transcription inhibition by lasso peptides microcin J25 and capistruin. Proc Natl Acad Sci 116(4): 1273–1278.

106. Islam S, Amin Md A, Rengasamy KRR, Mohiuddin AKM, Mahmud S (2024) Structure-based pharmacophore modeling for precision inhibition of mutant ESR2 in breast cancer: A systematic computational approach. Cancer Med 13(15): e70074–e70085.

107. Kaserer T, Beck KR, Akram M, Odermatt A, Schuster D (2015) Pharmacophore Models and Pharmacophore-Based Virtual Screening: Concepts and Applications Exemplified on Hydroxysteroid Dehydrogenases. Mol Basel Switz 20(12): 22799–22832.

108. Saravanan V, Chagaleti BK, Packiapalavesam SD, Kathiravan M (2024) Ligand based pharmacophore modelling and integrated computational approaches in the quest for small molecule inhibitors against hCA IX. RSC Adv 14(5): 3346–3358.

109. Meng XY, Zhang HX, Mezei M, Cui M (2011) Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery. Curr Comput Aided Drug Des 7(2): 146–157.

110. Lill MA (2011) Efficient incorporation of protein flexibility and dynamics into molecular docking simulations. Biochemistry 50(28): 6157–6169.

111. Hollingsworth SA, Dror RO (2018) Molecular Dynamics Simulation for All. Neuron 99(6): 1129–1143.

112. Mao J, Javed Akhtar, Xiao Zhang, Liang Sun, Shenghui Guan, et al. (2021) Comprehensive strategies of machine-learning-based quantitative structure-activity relationship models. iScience 24(9): 103052-103058.

113. Vuppala S, Kim J, Joo BS, Choi JM, Jang JA (2022) Combination of Pharmacophore-Based Virtual Screening, Structure-Based Lead Optimization, and DFT Study for the Identification of S. epidermidis TcaR Inhibitors. Pharmaceuticals 15(5): 635-640.

114. Sunita Singhvi N, Gupta V, Singh Y, Shukla P (2024) Computational Approaches for the Structure-Based Identification of Novel Inhibitors Targeting Nucleoid-Associated Proteins in Mycobacterium Tuberculosis. Mol Biotechnol 66(4): 814–823.

115. Stephanie F, Tambunan USF, Siahaan TJ (2022) M. tuberculosis Transcription Machinery: A Review on the Mycobacterial RNA Polymerase and Drug Discovery Efforts. Life 12(11): 1774-1779.