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Understanding the key challenges in tuberculosis drug discovery: what does the future hold?

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Abstract:

Introduction: Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), remains a major global health concern. It spreads through airborne droplets and has a high mortality rate, particularly without treatment. Drug resistance is rising, with treatments against multidrug-resistant TB (MDR-TB) showing poor treatment success rates. The thick, lipid-rich wall of *Mtb* and its slow growth reduce antibiotic effectiveness, requiring long treatment courses of 4–6 months. Current therapies often fail against drug-resistant strains, highlighting the urgent need for new, short-course treatment, affordable, and combination-friendly drugs.

Areas covered: The following topics regarding *Mtb* resistance emergence and treatment strategies were reviewed and addressed: i) Existing treatment ii) Resistance evolution in *Mtb*; iii) Key challenges in drug discovery targeting *Mtb*; iv) Emerging strategies and recent advances in *Mtb* drug discovery, and v) Next-generation approaches.

Expert opinion: The authors focused on advancing anti-evolution strategies and integrating AI in drug discovery to tackle drug-resistant tuberculosis. AI accelerates the discovery of bioavailable and safe preclinical drug candidates, though data limitations and biological complexity remain challenging. Future progress requires multi-modal models, open-access datasets, and interdisciplinary collaboration.

Keywords:

Anti-microbial resistance (AMR), Anti-evolution drugs, Drug-resistance mechanism, *Mycobacterium tuberculosis*, AI-Driven drug discovery.

Article highlights:

- Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains a major global health threat, causing 1.25 million deaths in 2023, with rising concerns about drug-resistant strains.
- The standard treatment for drug-sensitive TB involves a 4-6 month regimen of multiple antibiotics, though long treatment durations and side effects hinder patient adherence.
- The emergence of multidrug-resistant TB (MDR-TB) and more resistant forms like XDR-TB presents significant treatment challenges, with less effective and more toxic regimens required.
- *Mtb* develops resistance through genetic mutations, compensatory mutations, and metabolic adaptations, including a thick cell wall and efflux pumps that limit drug efficacy.
- TB treatment is complicated by the bacterium's ability to reside within macrophages and granulomas, limiting drug access, and the host's immune responses further complicate treatment.
- Developing new TB drugs is time-consuming, costly, and high-risk, with a focus on creating combination therapies that are shorter, less toxic, and effective against drug-resistant strains.
- New strategies in TB drug discovery target the bacterium's cell wall, genetic machinery, and energy metabolism, with promising compounds in clinical development.
- Host-Directed Therapy (HDT) strategies aim to enhance the host's immune response, limit damage, and aid in *Mtb* clearance, providing complementary treatment alongside antibiotics.
- Future approaches focus on anti-evolution strategies, CRISPR-based target identification, and AI-driven drug discovery to overcome resistance and accelerate treatment development.
- With advancements in crystallography, cryo-electron microscopy (cryo-EM), and more recently, AI-based protein structure prediction, virtual screening has become an integral component of modern anti-TB drug discovery.
- Artificial intelligence such as predictive modelling and molecular simulations, is revolutionizing TB drug discovery by streamlining the identification of safe drug candidates, though challenges remain in translating AI discoveries into approved treatments.

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), remains a significant global health issue, particularly in regions with limited healthcare resources [1]. TB is a highly contagious bacterial infection that spreads to healthy individuals through aerosols released by an infected person. In 2023, TB likely regained its position as the world's leading cause of death from a single infectious agent, surpassing coronavirus disease (COVID-19) after three years. TB caused an estimated 1.25 million deaths in 2023, including 1.09 million among HIV-negative people and 161 000 among people with HIV [2].

Without treatment, TB has a high mortality rate, nearing 50% [3]. However, with the WHO recommended treatment a 4-6 month course of anti-TB drugs around 85% of drug-susceptible TB cases can be successfully cured. The global impact of this disease is intensified by the emergence of drug resistance which contributes to long-term morbidity, with approximately 0.4 million deaths annually linked to antimicrobial resistance (AMR) [2]. The prevalence of these resistant strains is alarmingly high, with an estimated 410,000 MDR-TB cases worldwide. In 2023, only 63% of MDR-TB cases were successfully treated [4]. This ongoing challenge highlights the urgent need for the discovery of new anti-tubercular drugs to enhance and complement existing therapies [5]. Unlike most other bacteria, mycobacteria possess a uniquely thick, lipid-rich cell wall that makes them inherently resistant to many conventional antibiotics [6]. They also exhibit a slow growth rate, which further complicates timely treatment and drug efficacy. These distinguishing features of mycobacteria necessitate a tailored approach in drug design. Various considerations should

direct the development of new anti-tubercular agents. These compounds ideally need to be affordable, compatible in combination therapies, reduce the treatment duration (currently 6 months minimum), and have a low mutation frequency of resistance [7]. This review provides an in-depth examination of recent progress in the discovery and development of anti-TB drugs. It explores the challenges associated with creating novel TB treatments, and highlights key advancements in drug development. Additionally, the review proposes strategic solutions to overcome existing barriers, ultimately aiming to facilitate the creation of more effective therapeutic options.

2. Existing TB drugs

The standard treatment for drug-sensitive tuberculosis (DS-TB) consists of an initial two-month regimen of isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and pyrazinamide (PZA), followed by an additional four months of INH and RIF [4]. These antibiotics, discovered nearly six decades ago [8] target *Mtb* through distinct mechanisms (**Table 1**). INH, a prodrug, is activated within the bacterial cell and inhibits enoyl-acyl carrier protein reductase (InhA), a key enzyme in mycolic acid biosynthesis [9]. RIF, a bactericidal agent, binds to the β -subunit of bacterial RNA polymerase, disrupting early gene transcription [10-11]. Like INH, PZA is a prodrug that converts to pyrazinoic acid (POA) via the pyrazinamidase enzyme upon diffusion into TB granulomas, leading to bacterial death, although its exact mode of action remains unclear [12]. EMB, a bacteriostatic drug, interferes with the synthesis of arabinogalactan and lipoarabinomannan (essential cell wall components) by targeting the arabinosyltransferases EmbA, EmbB, and EmbC [13]. Despite its effectiveness, the standard anti-DS-TB regimen can cause hepatotoxicity, neuropathy, ocular toxicity, gastrointestinal disturbances, and skin reactions [14,15]. Moreover, the lengthy treatment duration, high pill burden, and these undesirable side effects can lead to poor patient adherence.

Table 1: Mechanisms of action and resistance of current tuberculosis drugs.

Drug Class	Drug name and discovery date	Mechanism of action	Resistance mechanism and target	Molecular Weight (g/mol)	LogP ^a	Notes
First-line drugs	Isoniazid (INH) 1952	Inhibits mycolic acid synthesis, disrupting cell wall	Molecular: Mutations in <i>katG</i> or <i>inhA</i> promoter	137.14	-0.64	Bactericidal, highly soluble in water
	Rifampicin (RIF) 1965	Inhibits RNA synthesis by binding to RNA polymerase	Molecular: Mutations in <i>rpoB</i> gene	822.94	3.7	Bactericidal, poorly soluble in water
	Pyrazinamide (PZA) 1952	Disrupts energy metabolism and membrane transport	Molecular: Mutations in <i>pncA</i> gene	123.11	-0.37	Bacteriostatic or bactericidal, soluble in water

	Ethambutol (EMB) 1961	Inhibits arabinogalactan synthesis in cell wall	Molecular: Mutations in <i>embB</i> gene (Arabinogalactase)	204.31	-0.15	Bacteriostatic, soluble in water
Second-line drugs	Levofloxacin/Moxifloxacin (Fluoroquinolones) 1982s	Inhibits DNA gyrase, blocking DNA replication	Molecular: Mutations in <i>gyrA/gyrB</i> genes (DNA gyrase)	361.37/401.43	-0.41/-0.43	Bacteriostatic and Bactericidal, soluble in water
	Amikacin/Kanamycin (Aminoglycosides) 1972/1957	Inhibits protein synthesis by binding 30S ribosome	Molecular: Mutations in <i>rrs</i> gene (16S rRNA) (Ribosome)	585.6/484.5	-5.8/-4.3	Bactericidal injectable drug, Highly soluble in water
	Linezolid (Oxazolidinones) 1996	Inhibits protein synthesis by blocking ribosomal assembly	Molecular: Mutations in <i>23S rRNA</i> (Ribosome)	337.35	0.55	Modest early bactericidal activity, moderately soluble
New and repurposed drugs	Bedaquiline 2005 (FDA approved 2012)	Inhibits ATP synthase, disrupting energy production	Molecular: Mutations in <i>atpE</i> gene (ATP synthase) Intrinsic (efflux pumps): overexpression	555.5	7.3	Bacteriostatic during the first 7–14 days, and bactericidal activity only begins subsequently, poorly soluble in water
	Delamanid/Pretomanid 2007 (Delamanid, FDA approved 2014), 2000 (Pretomanid, FDA approved 2019)	Inhibits mycolic acid synthesis, causing cell death	Molecular and Intrinsic: Mutations in <i>ddn</i> gene or increased nitroreductase activity (Deazaflavin-dependent nitroreductase)	534.5/359.3	5.6/1.9	Bactericidal, poorly soluble in water

a: LogP (partition coefficient) indicates a compound's lipophilicity. Negative values suggest hydrophilicity, while positive values indicate lipophilicity.

The misuse and overuse of antibiotics have further driven the emergence of drug resistance, resulting in various strains with differing levels of resistance to available anti-TB drugs [14]. Initially, TB was largely drug-susceptible and could be effectively treated with first-line anti-TB medications. However by the 1990s, cases of multidrug-resistant TB (MDR-TB) emerged, exhibiting resistance to the first-line drugs RIF and INH [16]. The WHO's 2024 guidelines for the MDR-TB treatment recommend two main regimens: a 6-month BPaLM/BPaL regimen (Bedaquiline (BDQ), Pretomanid, Linezolid, with or without Moxifloxacin) for patients aged 14 and above without extensive TB or prior exposure to these drugs, excluding pregnant women and those with CNS/disseminated TB; and a 9-month all-oral regimen (BDQ, Levofloxacin/Moxifloxacin, Linezolid, Clofazimine, Pyrazinamide, EMB, and INH) for cases without fluoroquinolone resistance, suitable for all ages and pregnant women with modifications. The cure rates for MDR-TB are typically significantly lower than DS-TB (WHO, 2024). Notably, BDQ recently added to the second-line TB regimen, is the first anti-TB drug with a novel mechanism of action to be approved in over 50 years [17]. It works by inhibiting mycobacterial ATP synthase, an essential enzyme for energy production in *Mtb* leading to cell death, making it effective against both replicating and dormant TB bacteria [18,19].

In 2006, pre-extensively drug-resistant TB (Pre-XDR-TB) was identified, referring to strains resistant not only to first-line drugs but also to either fluoroquinolones or an injectable second line agent [20]. By 2009, reports of extensive drug-resistant TB (XDR-TB) surfaced, describing strains impervious to first line drugs with additional resistance to at least one fluoroquinolone (such as moxifloxacin or levofloxacin) and any of the injectable second-line TB drugs (such as amikacin) [21] (**Figure 1**). Treatment options for Pre-XDR-TB and XDR-TB are severely limited, resulting in exceptionally high mortality rates and raising concerns about a potential return to the pre-antibiotic era [22]. Typically, treatment lasts between 14 and 24 months and consists of both intensive and continuation phases, utilizing a combination of second-line agents to which the *Mtb* strain remains susceptible [23].

Humans can develop MDR-TB or XDR-TB in two main ways: either through a primary infection involving person-to-person transmission of drug-resistant bacteria, or through the development of resistance caused by the improper use or mismanagement of anti-TB medications in individuals already undergoing treatment. The treatment of these resistant strains typically requires a significantly longer duration (up to two years) compared to the standard regimen for drug-sensitive TB. Second-line anti-TB drugs, recommended for these resistant forms, are generally more toxic, more expensive, and less effective than first-line therapies. These factors contribute to poor patient adherence and increased transmission within communities, reinforcing TB as a persistent global health challenge.

Evolution of Drug Resistance in TB

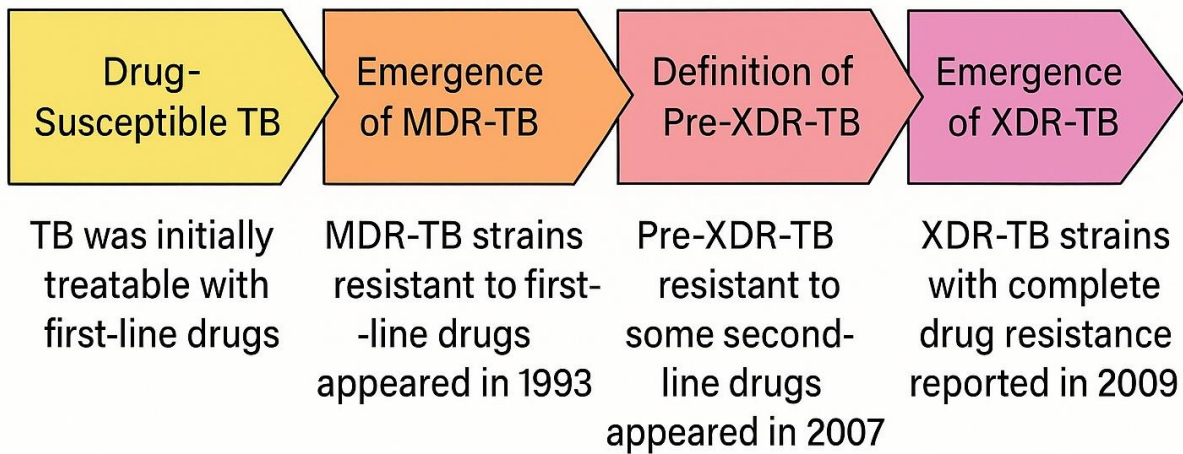


Figure 1: Timeline of the progressive development of drug resistance in *Mtb*. Initially, TB was treatable with first-line drugs, but the emergence of multidrug-resistant TB (MDR-TB) in the 1990s marked resistance to rifampicin and isoniazid. By 2006, pre-extensively drug-resistant TB (Pre-XDR-TB) was identified, showing additional resistance to fluoroquinolones and injectable second-line drugs. In 2009, extensively drug-resistant TB (XDR-TB) strains were reported, exhibiting complete resistance to both first and second line treatments highlighting the escalating challenge of TB drug resistance and the need for novel therapeutic strategies.

3. Key Challenges in Drug Discovery in *Mtb*

Drug discovery is a complex and systematic process aimed at identifying new therapeutic compounds. This process involves extensive research, screening, and optimization to ensure efficacy and safety. However, developing new drugs is highly time-consuming, often taking years before reaching commercialization. It also demands substantial financial investment and carries a high risk of failure, making it one of the most challenging aspects of pharmaceutical research [24,25]. The primary objective of developing new TB therapeutics is to create a short-course treatment, less toxic, and widely accepted treatment regimen that effectively targets drug-resistant TB and ensures a rapid cure. The following paragraph will explore the various challenges associated with drug discovery in *Mtb*.

3.1. Resistance Emergence

AMR occurs when a population of pathogens infecting a patient possesses biochemical mechanisms that enable them to survive and multiply despite exposure to an antimicrobial agent that would normally eliminate them [26,27]. These mechanisms usually come at the cost of fitness in an environment deprived of antimicrobial agent [28]. Thus AMR is ultimately a biological process subject to evolutionary forces [29,30]. Here we explain different mechanisms of resistance in *Mtb*.

Molecular mechanisms

In *Mtb*, drug resistance primarily results from mutations in genes encoding drug targets or drug-activating enzymes [31,32]. These mutations predominantly consist of single nucleotide polymorphisms (SNPs), insertions, or deletions (indels), while large deletions are relatively rare [4]. Classical *in vitro* studies of drug resistance in *Mtb* involve exposing bacterial cultures to

increasing antibiotic concentrations and selecting resistant mutants, followed by whole-genome sequencing to identify causative mutations. These approaches have historically revealed key resistance-conferring mutations, such as *rpoB* mutations responsible for rifampicin resistance [33], *katG* and *inhA* mutations responsible for isoniazid resistance [34,35], and *gyrA/gyrB* mutations for fluoroquinolone resistance [36] (**Table 1**).

More recently, genome-wide association studies (GWAS) have offered a high-throughput and unbiased approach to identifying resistance mutations at the population level. For instance, a study applied GWAS methods to a large, global dataset of *Mtb* isolates, successfully confirming known resistance mutations and uncovering novel loci associated with drug resistance [37]. This study highlighted the power of GWAS to detect genetic determinants beyond those identified by traditional candidate gene approaches.

In addition to primary resistance mutations, compensatory mutations play a crucial role in restoring fitness costs associated with drug resistance. These mutations frequently occur in genes that modulate the structural or functional consequences of resistance-conferring changes. For example, compensatory mutations in *rpoC* and *rpoA* can mitigate the fitness cost of *rpoB* mutations conferring rifampicin resistance [38]. Similarly, compensatory mutations have been reported in *gidB* for aminoglycoside resistance, as well as in *prpR* for isoniazid resistance [39]. The emergence of compensatory mutations may facilitate the long-term persistence and transmission of drug-resistant *Mtb* strains, contributing to the global challenge of tuberculosis control.

Impact of mutations regulating mutation rate on evolution

Unlike many bacteria, *Mtb* does not acquire resistance through horizontal gene transfer [40]. Instead, genetic diversity arises from DNA replication errors or mutagenesis induced by DNA repair mechanisms, collectively influencing the mutation rate [41,42]. Accordingly, higher bacterial mutation rates have been linked to increased AMR *in vitro* and in natural bacterial populations [43–46].

In *Mtb*, missense mutations in putative antimutator (*mut*) genes have been identified in the Beijing strain family and linked to increased mutation rates [47,48]. Additionally, studies have shown that mutations in the *nucS* gene, which encodes a putative endonuclease, result in a hypermutator phenotype in *Msm*, a non-pathogenic model for studying MTBC [49]. Similar effects have been observed in two other Actinobacteria: *Streptomyces coelicolor* [49] and the industrially significant *Corynebacterium glutamicum* [50,51]. Furthermore, recent research identified 18 SNPs associated in *Mtb* clinical samples with genotypic drug resistance in DNA repair genes, with mutation frequency tests in *Msm* confirming the hypermutability of mutants in four genes: *nucS*, *mutM*, *xthA*, and *fpg2* [52]. Yet, this hypermutability seems to be tightly limited in *Mtb* as no strain with a large accumulation of mutations could be identified, in contrast to findings in *M. leprae* [46,52].

Another mechanism favoring mutations emergence involves the impact of suboptimal drug concentrations on mutation rates. In the case of fluoroquinolones, low concentration can induce transcriptional changes in DNA repair genes, activating pathways such as the SOS response [53]. This again illustrates that adaptive evolution in the organism is closely tied to DNA repair processes [54]. Further research into these DNA repair mechanisms is essential to fully understand their role in resistance development.

Contribution of tolerance mechanisms

In addition to molecular resistance mechanisms, tolerance mechanisms play a fundamental role in *Mtb* drug resistance. The first mechanism of tolerance is intrinsic resistance which refers to

inherent bacterial traits that reduce susceptibility to antimicrobials, independent of acquired genetic mutations. These mechanisms include limited cell wall permeability and active drug efflux, both of which contribute to *Mtb*'s ability to withstand antibiotic treatment.

Mtb possesses a thick, hydrophobic cell wall that provides resistance to low pH, high temperatures, desiccation, and disinfectants, while also significantly restricting antimicrobial uptake by limiting passage through porins [55,56]. Compared to bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, drug diffusion across the mycobacterial cell wall occurs at a much slower rate, reducing antibiotic efficacy and contributing to intrinsic resistance [57,58].

Additionally, *Mtb* exhibits intrinsic resistance to most β -lactam antibiotics due to the presence of the *blaC* gene, which encodes an extended-spectrum β -lactamase that deactivates these drugs (Hugonnet and Blanchard 2007; Tremblay, Fan, and Blanchard 2010). As a result, the combination of a highly impermeable cell wall and β -lactamase activity significantly limits treatment options and complicates drug discovery efforts [57,58].

Beyond cell wall permeability, efflux pump systems further contribute to tolerance resistance by actively expelling antimicrobial compounds from the bacterial cell. *Mtb* encodes one of the largest known sets of efflux pumps, with 148 genes dedicated to membrane transport proteins within its 4.4 *Mtb* genome. These efflux systems have been shown to facilitate multidrug resistance (MDR) by reducing intracellular drug accumulation, thereby allowing bacteria to survive sublethal drug concentrations and acquire resistance mutations more readily [58]. Overexpression of efflux pumps is believed to promote the emergence of high-level resistance, enabling *Mtb* to persist even at clinically relevant drug concentrations (Frimodt-Møller et al., 2019). Moreover, the broad substrate specificity of these pumps allows them to expel multiple antibiotics simultaneously, further driving the development of the MDR phenotype [59].

Together, limited cell wall permeability and active drug efflux create a formidable intrinsic resistance barrier in *Mtb*, complicating treatment strategies and highlighting the need for novel therapeutic approaches.

In addition, *Mtb* exhibits metabolic adaptability under stress, enabling survival through alternative pathways [60]. Additionally, *Mtb* enters a dormant state under low oxygen and nutrient depletion, evading immune responses and reducing antibiotic efficacy [61,62]. These adaptations create significant obstacles in drug discovery, requiring novel strategies to overcome *Mtb* persistence.

3.2. Host-related factors influencing resistance

Host factors play a crucial role in TB infection and significantly impact drug efficacy, bacterial persistence, immune responses, and treatment outcomes. One major challenge for drug discovery is that *Mtb* primarily resides within macrophages, usually gathered in granulomas i.e. in acidic or hypoxic environments, making it difficult for many drugs to effectively penetrate intracellular compartments. Macrophages, as key immune cells, are responsible for controlling *Mtb* infection by internalizing and eliminating the bacteria. However, in patients with TB, antigen presentation within macrophages is often impaired and bacteria escape lysosomes, reducing their ability to eliminate *Mtb*. A critical process for bacterial destruction is the fusion of lysosomes with *Mtb*-containing phagosomes; when this fusion is evaded, the bactericidal function of macrophages is compromised [63]. In addition, studies document the escape of bacteria from lysosomes, allowing them to multiply directly in the cytosol [64]. Moreover, studies suggest that granulomas act as a protective niche for both the pathogen and the host, maintaining homeostasis through the actions

of phagocytes such as macrophages, dendritic cells, and neutrophils [65]. As a result, *Mtb* remains largely confined within the host environment, limiting its accessibility to antibiotics. Beyond this physical restriction, *Mtb* infection induces chronic inflammation, which can significantly impact drug metabolism and clearance [66]. Since the effectiveness of any treatment depends on the lifespan of the drug within the body, the host's detoxification systems play a crucial role in determining therapeutic success [67]. This highlights the potential need for immune-modulating therapies alongside antibiotics to enhance treatment outcomes. Moreover, latent TB infection presents an additional challenge, as *Mtb* can transition into a non-replicative, drug-tolerant state influenced by the host environment [66]. Given that most current TB drugs target actively dividing bacteria, eliminating persistent *Mtb* remains a significant hurdle in TB treatment.

4. Emerging strategies and recent advances in targeting *Mtb* drug discovery

The discovery of new anti-tubercular treatment strategies requires collaboration across multiple scientific disciplines, such as biochemistry, microbiology, structural biology, chemistry, computational biology, and informatics. Within this multidisciplinary approach, drug discovery must continuously advance by incorporating cutting-edge technologies while building upon established methodologies to develop more effective therapies.

Target identification, though not always feasible, plays a crucial role in determining a compound's potential as a preclinical candidate [30]. Drug discovery mainly follows two approaches: target-to-drug, where compounds are designed for known targets [68], and drug-to-target, where active compounds are identified before their targets [69]. While target-based methods offer specificity, they often suffer from high attrition. Drug-to-target strategies confirm cellular activity early but require later target identification.

4.1. Promising new molecules targeting *Mtb* Mechanisms

In recent years, the two approaches have yielded approximately a dozen candidate molecules with novel mechanisms of action and demonstrated proof-of-concept efficacy against *Mtb*, many of which are now in advanced preclinical or clinical development [70,71]. Briefly these candidates act through three primary pathways: inhibition of cell wall synthesis, interference with genetic information processing, and disruption of energy metabolism.

The structurally unique and lipid-rich *Mtb* cell wall is essential for bacterial survival and immune evasion, making it an attractive drug target (**Figure 2a**). Inhibitors such as GSK693, which blocks InhA (encoding enoyl-ACP reductase), and DG167, targeting kasA (β -ketoacyl-ACP synthase), interfere with mycolic acid biosynthesis (Ghollet et al., 2018; Kumar et al., 2018). Other compounds target arabinogalactan assembly by inhibiting dprE1 (decaprenylphosphoryl- β -D-ribose 2'-oxidase), as seen with BTZ043, PBT169, and OPC-167831 [72], or lipid transport via mmpL3 inhibition, using agents like SQ109, AU1235, and BM212 [73,74].

In parallel, disrupting *Mtb*'s central genetic processes including DNA replication, transcription, and translation has provided effective strategies, especially for drug-resistant strains (**Figure 2b**). Novel DNA gyrase inhibitors such as SPR719/720 act on the *gyrB* subunit and show activity against both tuberculous and non-tuberculous mycobacteria [75]. Transcription is targeted through inhibition of RNA polymerase, encoded by *rpoB*, using drugs such as rifampicin and rifapentine, while novel modulators like piperine are being explored for their synergistic effects [76,77]. Protein synthesis inhibitors target several pathways: aminoglycosides bind to the 30S ribosomal subunit, oxazolidinones interfere with the 50S subunit, and leucyl-tRNA synthetase is blocked by specialized inhibitors—altogether disrupting genes such as *rrs*, *rplC*, and *leuS* [74,78].

Energy metabolism and bioenergetics present another promising frontier, particularly for targeting persistent or non-replicating *Mtb* (**Figure 2c**). NDH-2 (menaquinone oxidoreductase type II) is essential for ATP generation and redox balance and is inhibited by phenothiazines and modified derivatives like polyhalogenated PTZs and phenoselenazines [79,80]. Inhibitors of MenA (menaquinone biosynthesis) exhibit bactericidal activity under both replicating and dormant states [81,82]. The cytochrome bc1:aa3 complex, with QcR_B as a key component, is blocked by Q203 and thienopyrimidines, leading to ATP depletion [83,84]. F₁F₀ ATP synthase remains a cornerstone target, inhibited by bedaquiline and next-generation compounds such as GaMF1 and TBAJ-series agents [85,86]. Additionally, isocitrate lyase (*icl1/icl2*), vital for *Mtb* persistence in latent states, is targeted by salicylanilide, fluoroquinolone derivatives, and 3-nitropropionate, all of which bind to the ICL active site [87,88].

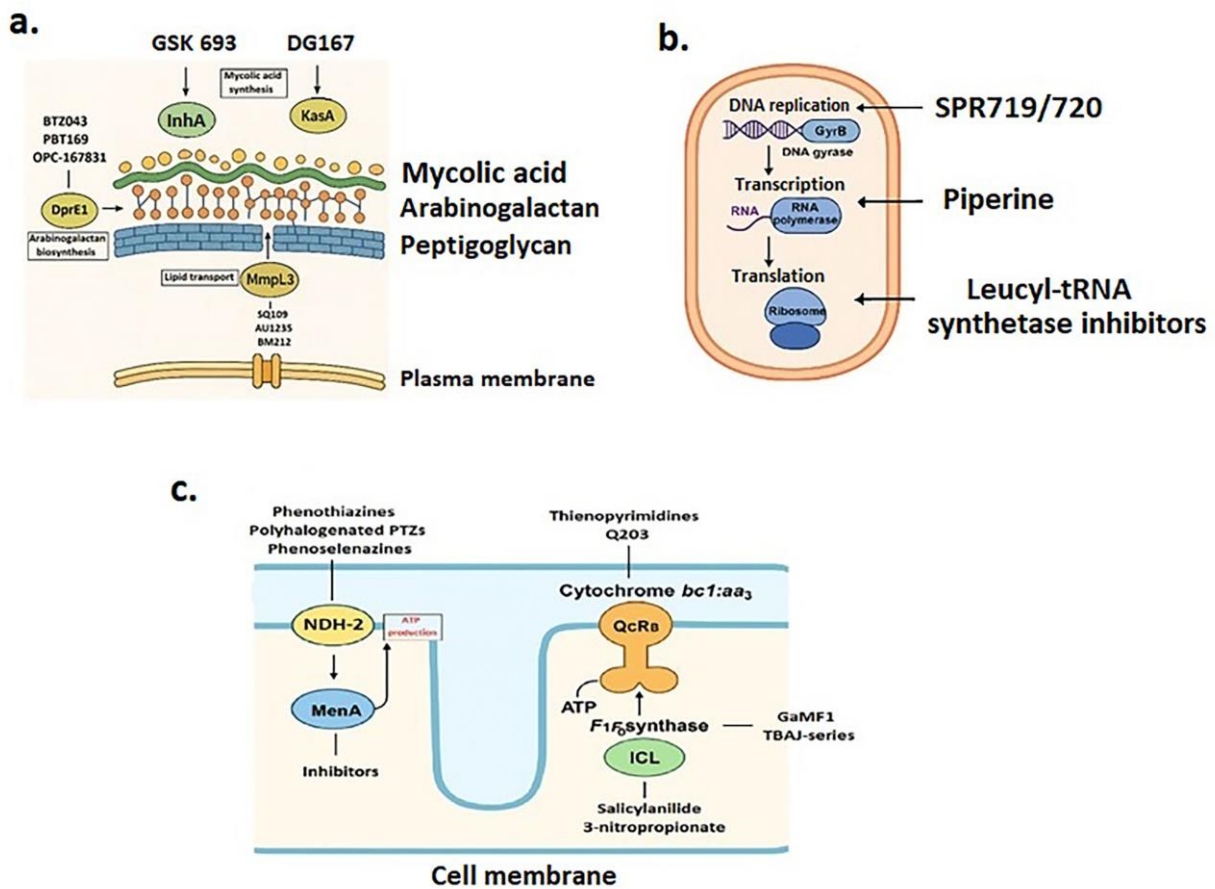


Figure 2. Schematic representation of three major pathways targeted by recently identified anti-tubercular compounds. **a.** Inhibition of cell wall synthesis, including disruption of mycolic acid biosynthesis, arabinogalactan assembly, and lipid transport. **b.** Interference with genetic information processing, involving inhibition of DNA replication, RNA transcription, and protein synthesis. **c.** Disruption of energy metabolism, targeting pathways such as ATP production, cytochrome bc1:aa3 complex, F₁F₀ ATP synthase, and isocitrate lyase (ICL).

These advances are reflected in the expanding global drug development pipeline for tuberculosis, which is comprehensively detailed in the latest WHO report [2]. Several candidates are currently progressing through clinical trials at various stages. Phase I trials include six compounds focused on early safety and pharmacokinetic profiling. Phase II trials encompass twelve drugs, including Delpazolid (an oxazolidinone with improved pharmacokinetics) and TBA-7371, a dprE1 inhibitor with no cross-resistance to existing therapies. Phase III trials feature one

candidate, Sudapyridine, a derivative of bedaquiline that targets atpE and offers an improved safety profile [70]. Collectively, these efforts underscore the growing momentum toward developing more effective, selective, and safer treatment options for tuberculosis, particularly in addressing drug-resistant and persistent infections.

4.2. Promising targets for host-directed therapy (HDT)

Host-Directed Therapy (HDT) is an emerging approach that enhances host immune functions or mitigates tissue damage by targeting the host's biological pathways rather than directly acting on *Mtb* [89]. HDT strategies modulate immune signaling, metabolism, and cellular responses to improve infection control. In this section, we briefly introduce several promising host-targeted pathways currently under investigation. Receptor Tyrosine Kinases (RTKs), such as EGFR, are hijacked by *Mtb* to support survival; their inhibition by repurposed drugs like gefitinib, AT9283, and dovitinib suppresses *Mtb* replication and modulates cytokine responses [89,90]. Purinergic receptors, particularly P2X7, mediate immune activation through ATP signaling; their modulation enhances macrophage apoptosis and host defense [91,92]. Tumor Necrosis Factor- α (TNF- α) is essential for infection control but can also trigger macrophage necrosis and bacterial dissemination via ROS-driven pathways; this can be counteracted by metabolic modulators like metformin, which inhibit reverse electron transport (RET) and reduce inflammation [93,94]. The Glucocorticoid Receptor (GCR) axis is disrupted in TB, with altered GR α /GR β expression linked to severe disease; GCR targeting may help rebalance immune responses [95]. The mTOR signaling pathway, which regulates autophagy, is subverted by *Mtb* to avoid immune clearance; inhibition by agents like CC214-2 promotes bacterial elimination and enhances the efficacy of standard regimens [96]. Lastly, CD8⁺ T cells are critical for immune-mediated control of *Mtb*, and their activation through IL-15 signaling offers another promising HDT strategy [97]. Collectively, these host-directed pathways provide novel therapeutic avenues to complement antimicrobial treatment and combat persistent or drug-resistant TB.

5. Next-Generation Approaches: what does the future hold?

Current TB treatments, despite their high efficacy in clinical trials, face significant challenges in real-world applications. Long treatment durations, severe side effects, and high patient dropout rates undermine their effectiveness. Many TB patients struggle with physical and mental endurance due to drug toxicity, leading to incomplete treatments, bacterial resistance, and relapse. Additionally, *Mtb* exists in multiple physiological states within the host, including dormant forms within granulomas that are difficult to eliminate with existing drugs. These factors contribute to treatment failure and the persistent global TB burden [98,99].

To overcome these limitations, future TB drug discovery must focus on developing more effective and patient-friendly therapies. One approach is modifying existing drugs or designing novel compounds with improved efficacy and reduced toxicity. Natural products, such as plant-derived compounds with antimicrobial and immune-boosting properties, offer promising alternatives with fewer side effects [100]. Adjunctive therapies, including hepatoprotective agents and antioxidants, can help mitigate drug-induced toxicities, while pharmacogenomic profiling could enable personalized treatment plans based on genetic factors [100]. Host-directed therapies that enhance immune responses may also improve TB outcomes when combined with traditional antibiotics.

The future of TB drug discovery relies on a multidisciplinary strategy integrating novel pharmacological interventions, immune-based therapies, and precision medicine to develop safer, more effective, and shorter treatments. Building on the pathways discussed earlier, the following

sections explore emerging approaches that will shape the next phase of *Mtb* drug discovery (Figure 3).

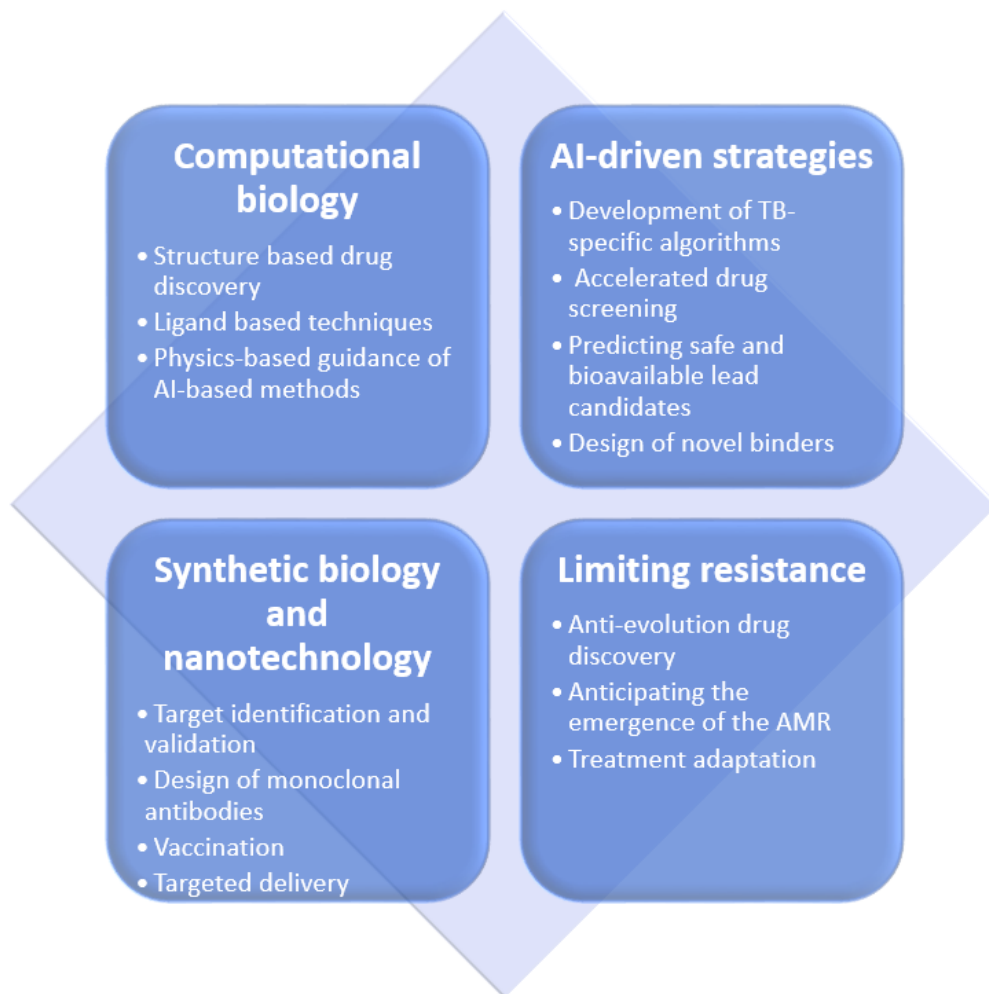


Figure 3. Emerging interdisciplinary strategies for tuberculosis drug discovery and resistance management. The diagram highlights four key domains: computational biology, AI-driven methods, synthetic biology and nanotechnology, and approaches aimed at limiting antimicrobial resistance. Each domain encompasses specific tools and techniques contributing to the development of effective and targeted TB therapies.

5.1. Breaking the cycle: Anti-evolution strategies to overcome drug resistance

A promising way to fight antibiotic resistance is through anti-evolution drugs, compounds that slow or block the development of resistance [101,102]. These drugs act by targeting stress responses, DNA repair systems, or enzymes that drive mutations. When used with antibiotics, they may extend drug lifespan and reduce treatment failure. One approach is to target nonessential pathways that do not affect bacterial fitness, lowering resistance pressure. For example, error-prone DNA polymerases like DnaE2, which promote mutations under stress, can be targeted to cut resistance to drugs like rifampicin in TB [103,104]. Another target is the SOS response, regulated by LexA and RecA, which drives mutagenesis and biofilm formation under stress [105,106]. Inhibiting LexA or RecA reduces mutation rates and boosts antibiotic sensitivity, with LexA being especially attractive due to its lack of human equivalents [107,108]. A third key target is Mfd, a

DNA translocase that increases mutation rates without harming fitness [109,110]. Mfd promotes mutagenesis across species and has multiple druggable domains [111,112]. Although more research is needed, anti-evolution strategies could be a game-changer in TB therapy and beyond.

5.2. CRISPR-Based Genetic Screens for Novel Targets

CRISPR-based genetic screens have transformed the search for novel drug targets in *Mtb* by enabling precise, high-throughput functional genomics. These approaches use CRISPR interference (CRISPRi) and CRISPR knockout (CRISPR-KO) to systematically analyze gene function, revealing vulnerabilities that can be exploited for new treatments. A genome-wide CRISPRi screen combined with transcriptional and metabolomic analysis has mapped druggable weaknesses in an isoniazid-resistant *katG* mutant of *Mtb*, providing crucial insights into drug-resistant strains [113]. A genome-scale functional genomic platform using *Streptococcus thermophilus* Cas9 (Cas9Sth1) has been developed to investigate genetic pathways affecting bedaquiline efficacy and identify chemical-genetic interactions that could optimize TB treatment (Yan et al., 2020). The endogenous CRISPR/Cas10 system in *Mtb* has been utilized for gene editing and RNA interference (RNAi), allowing genome-wide functional screening to uncover genes regulating intracellular bacterial growth, facilitating the discovery of new drugs and vaccines [114]. Additionally, a codon-optimized *dCas9* in mycobacteria has been shown to enable stable and non-toxic CRISPRi applications, expanding the potential for antibiotic target discovery [115,116]. These advancements highlight the power of CRISPR-based genetic screens in accelerating TB drug discovery.

5.3. AI-driven strategies: from diagnostic to active compound prediction

AI is revolutionizing TB diagnosis, treatment, and research by addressing critical gaps in disease control and drug discovery. In diagnostics, AI-powered tools, such as computer-aided detection (CAD) algorithms, have significantly improved TB detection by achieving expert-level sensitivity (95.5%) [117], and enabling cost-effective screening, particularly in resource-limited settings [118] where human experts are scarce. These advancements leverage large-scale radiographic imaging datasets, though challenges remain in the need for expert-labeled training data [118]. AI is also enhancing treatment strategies by predicting patient outcomes, identifying those at risk of relapse or adverse effects, and enabling personalized treatment plans. While promising machine learning (ML) models incorporating clinical and genomic data exist to predict treatment failure [119,120] their integration into clinical practice is hindered by validation challenges and dataset heterogeneity [121]. On the other hand, AI's role in TB drug discovery is equally transformative, with ML accelerating compound screening [122], prioritization, and the identification of novel drug targets, addressing the complexity of *Mtb* and the growing issue of drug resistance. Furthermore, AI-driven analysis of high-dimensional molecular datasets, such as single-cell multi-omics, is uncovering new insights into host-pathogen interactions, immune responses, and potential therapeutic targets [123]. Despite these advances, concerns over AI's interpretability, potential biases, and healthcare professionals' trust in AI-driven decision-making must be addressed to ensure effective implementation. Collaborative efforts in data sharing, model validation, and explainable AI are essential for bridging the gap between AI innovations and clinical application, ultimately improving TB control and drug discovery.

5.4. Monoclonal Antibodies: A Novel Approach for Infection Therapies

Monoclonal antibody (mAb) therapy is emerging as a promising strategy for infectious disease treatment, offering targeted approaches beyond traditional antibiotics [124]. While bacterial exotoxins have been a primary focus, the discovery of bacterial surface proteins has revealed new

antibody-mediated mechanisms [125]. Additionally, studies on polysaccharide serotype prevalence in antibiotic-resistant populations highlight opportunities to exploit human selective pressures for therapeutic gain [126]. Several mAb candidates are progressing through clinical trials, with structural modifications enhancing their efficacy. Although some trials have faced setbacks, these challenges provide valuable insights into target vulnerabilities, guiding future development. However, bridging the gap between laboratory findings and clinical success requires establishing relevant *in vitro* correlates and improving preclinical models. Antigen heterogeneity and the emergence of new bacterial clones further complicate pharmaceutical investment. Despite these obstacles, recent advancements suggest that mAbs could become primary treatments against multidrug-resistant (MDR) pathogens, with antibiotics serving as complementary agents in combination therapies [127]. With continued innovation and investment, mAb-based treatments have the potential to revolutionize infectious disease management and overcome the limitations of conventional antibiotics.

5.5. Anti-TB drug development using *in silico* approaches

The application of *in silico* screening has become an integral component of modern anti-*Mtb* drug discovery, enabling the rapid identification and optimization of novel inhibitors targeting *Mtb* [128,129]. Many of the examples highlighted above were either discovered through virtual screening or employed computational modeling as a basis for further rationalization and optimization. For instance, several recent studies have successfully employed structure-based virtual screening to identify novel inhibitors of DprE1, a key enzyme in cell wall biosynthesis [130,131]. A particularly notable study by Hu et al. (2022) reported the discovery of a compound with a minimum inhibitory concentration (MIC) of 1.25 μM against *Mtb* [131]. Another example involves the identification of small molecules and fragment-like inhibitors targeting ThyX [132,133], an essential flavin-dependent thymidylate synthase, using a combination of experimental screening [134,135] and structure-based modeling approaches [136,137].

6. Expert Opinion

Future strategies to combat resistance demand greater societal investment in research, innovation, and policy. Efforts should focus on slowing resistance spread, preserving antibiotic efficacy, developing novel antimicrobial approaches that minimize resistance, and modifying host-microbe interactions to manage disease without directly targeting pathogens.

A particularly underexplored but promising approach is the anti-evolution strategy. Mutation plays a key role in the evolution of drug resistance. Unlike many other bacteria, *Mtb* relies almost entirely on spontaneous mutations rather than gene transfer [31,138]. For a drug-sensitive strain of *Mtb* to survive standard treatment, it typically needs to acquire at least four precise mutations. Moreover, resistant strains often become more robust by accumulating additional, compensatory mutations. Accordingly, several hypermutator strains due to altered 3R genes were found to be associated with extreme drug resistance [52,138], highlighting the central role of mutation rate regulation in resistance evolution. Therefore, blocking the ability to mutate not only prevents appearance of resistance but also avoids the emergence of highly fit, multi-resistant strains. One avenue involves targeting the key 3R system proteins involved in DNA replication and repair, such as NucS, DnaE1, and DnaE2. Although enhancing these proteins' efficiency is difficult, it may be more feasible to reduce their activity by blocking their active sites. The consequence would be the accumulation of mutations, which in turn would facilitate the emergence of resistance, in seeming contradiction with our goal. Yet, an excessive mutation rate generates deleterious mutations and

an instability of the phenotype, preventing the bacteria from adapting and thus thriving in its environment [139]. Exploring molecules that interact with the 3R system's key components could thus be of interest, either because they may be compatible with the anti-evolution strategy, or because they would induce uncontrolled, deleterious evolution that is harmful for the bacterium. Targets with no homolog in the host (including mitochondria), such as NucS, and/or with controlled delivery should be favored to avoid interaction with host cell metabolism.

Another promising avenue for future progress is the extended use of Artificial intelligence (AI) methodology which provide powerful tools to accelerate the discovery process. AI can predict which molecules are likely to be effective before they are synthesized, streamlining the traditionally slow and expensive drug development pipeline. Classical physics-based approaches such as molecular docking and molecular dynamics simulations remain vital for understanding molecular interactions, particularly with the support of enhanced force fields like OpenFF and CGenFF that improve accuracy in modeling small-molecule behaviour [140,141]. These methods have been significantly accelerated through high-performance computing platforms like OpenMM [142]. The integration of AI has taken this further by enabling predictive modeling even without knowing the 3D structure of target. Crucially, AlphaFold2 and AlphaFold3 have revolutionized structural biology by predicting protein structures with high confidence, including those of previously undruggable or uncharacterized targets [143,144]. In tuberculosis research, this has been instrumental in identifying potential inhibitors for challenging *Mtb* targets [145].

Also, AI models like Chemprop and SyntheMol exemplify the predictive power of AI in antibiotic discovery [146]. Chemprop, a graph neural network, predicts antimicrobial activity based on molecular structure [147], while SyntheMol generates chemically valid and synthesizable molecules using a Monte Carlo tree search approach guided by desired traits like potency or synthesis feasibility [148]. Advances in AI, including these two models, have led to the discovery of novel antibiotics such as halicin and abaucin [146]. More recently, graph-based models have emerged as a next-generation approach. These models simulate a reverse-noising process to generate realistic 3D molecular structures, enabling structure-based drug design without relying on crystallography [149]. They can predict not only drug-like properties such as synthesizability, toxicity, and Lipinski compliance but also protein-ligand docking poses and folding behavior. While these models are more complex and less interpretable, their performance often outweighs the need for transparency in high-throughput settings.

In parallel, normalizing flow models have emerged as highly promising architectures for the generation of molecules with therapeutic potential. Recent work, such as DrugFlow [150], demonstrates the power of continuous flow matching combined with discrete Markov bridges to capture complex chemical, geometric, and physical features of protein-ligand interactions, while also offering uncertainty estimates to guide sampling. At the same time, addressing the critical challenge of synthetic accessibility, SynFlowNet [151] introduces a GFlowNet-based approach that sequentially constructs molecules using chemically validated reactions, ensuring that the generated candidates are not only diverse and high-utility but also synthetically feasible. Together, these developments represent two of the most promising current directions in deep learning-based drug discovery, tackling both the quality and practicality of computationally designed molecules.

Despite notable progress, significant challenges remain in therapeutic innovation. While AI-driven drug discovery shows 80-90% success range in Phase I clinical trials compared to historical averages around 40-65%, this data predominantly pertains to therapeutic drugs for example in the fields of oncology and immunology [152]. Success in antibiotic discovery is yet limited, as most

candidates are still in the preclinical evaluation. Although AI-driven efforts have identified promising antibiotic candidates, none have yet been approved for clinical use, with most remaining in preclinical stages. This slow progress is partly due to the substantial human effort still required for validation, synthesis, and navigating regulatory pathways. Furthermore, TB-specific pharmacological and resistance data is relatively scarce since AI models require large and diverse datasets in order to generalise to unseen data. Datasets are often also biased towards high-income regions of the world, despite TB being most prevalent in low- and middle-income countries [153]. Lack of open-access data on TB biology (genomes and resistance phenotypes) constrains model generalisation and performance. Additionally, the unique physiology of TB makes it ineffectual to use models trained on other microbes such as *E. Coli* [154]. Furthermore, the same general drug discovery limitations mentioned before still apply, such as validation and synthesisability.

To address these gaps, future work should prioritize multidisciplinary pipelines and the creation of standardized, open-access datasets [152]. Multi-modal AI models that combine diverse data types graphs, sequences, and images can support human-in-the-loop systems that guide model output more reliably. One promising direction includes models that pair structural information from AlphaFold with sequence-based predictions of AMR genes [155]. By combining models that predict antibiotic function (e.g., MycoBCP), resistance likelihood, and candidate generation in a unified workflow, it becomes possible to design antibiotics that are not only effective but also robust against the evolution of resistance (**Figure 4**).

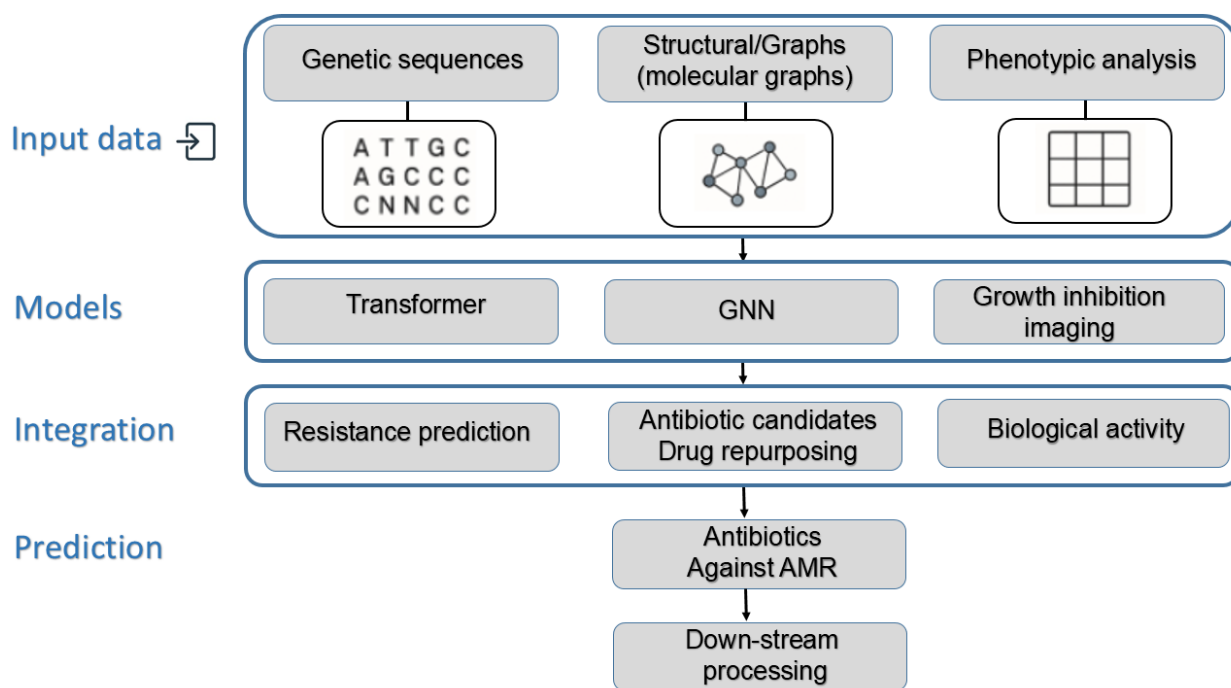


Figure 4. Integrated AI-driven drug discovery pipeline combining structural data, sequence analysis, and predictive models. This multi-modal, in-the-loop framework enables the design of effective antibiotics with reduced resistance potential.

By combining anti-evolution strategies and AI-assisted drug discovery, we can build a more effective and sustainable approaches to tackling drug-resistant tuberculosis.

7. Conclusion

Tuberculosis remains one of the most pressing infectious disease challenges globally, particularly with the rise of drug-resistant strains. Despite the success of standard regimens for drug-susceptible TB, treatment for MDR and XDR-TB remains prolonged, toxic, and less effective. Drug discovery in *Mtb* is hindered by the pathogen's unique biology, resistance mechanisms, and host-related factors. Recent innovations in both pathogen-targeted and host-directed therapies show promise in addressing these challenges. Anti-evolution strategies offer a novel route to limit resistance development. AI and structure prediction tools are accelerating the discovery of new therapeutic candidates. However, clinical translation remains slow, and very few AI-predicted drugs have reached regulatory approval. Building standardized, TB-specific datasets and interdisciplinary collaboration are essential to future success. Emphasis must be placed on equitable access and context-specific research, particularly for low-resource settings. A concerted global effort is needed to overcome the multifaceted challenge of TB and antimicrobial resistance.

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Declaration of interest

The authors declare no competing interests

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References

Papers of special note have been highlighted as: • of interest •• of considerable interest

1. Marais BJ, Hesselning AC, Cotton MF. Poverty and tuberculosis: is it truly a simple inverse linear correlation? *Eur Respir J*. 2009;33:943–944. doi: 10.1183/09031936.00173608.
2. WHO. Global Tuberculosis Report [Internet]. Geneva; 2024. Available from: <https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022>.
- **A review highlighting the urgent need for new anti-tuberculosis drugs due to rising drug resistance and mycobacterial challenges.**
3. Tiemersma EW, Van Der Werf MJ, Borgdorff MW, Williams BG, Nagelkerke NJD. Natural History of Tuberculosis: Duration and Fatality of Untreated Pulmonary Tuberculosis in HIV Negative Patients: A Systematic Review. Pai M, editor. *PLoS ONE*. 2011;6:e17601. doi: 10.1371/journal.pone.0017601.
4. WHO. Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance, 2nd ed [Internet]. 2023. Available from: <https://iris.who.int/handle/10665/374061>.
5. Migliori GB, Tiberi S, Zumla A, Petersen E, Chakaya JM, Wejse C, Muñoz Torrico M, Duarte R, Alffenaar JW, Schaaf HS, et al. MDR/XDR-TB management of patients and contacts: Challenges facing the new decade. The 2020 clinical update by the Global Tuberculosis Network. *International Journal of Infectious Diseases*. 2020;92:S15–S25. doi: 10.1016/j.ijid.2020.01.042.
6. Daffé M, Draper P. The Envelope Layers of Mycobacteria with Reference to their Pathogenicity. *Advances in Microbial Physiology* [Internet]. Elsevier; 1997 [cited 2025 Apr 9]. p. 131–203. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0065291108600168>.
7. Sotgiu G, Centis R, D’ambrosio L, Migliori GB. Tuberculosis Treatment and Drug Regimens. *Cold Spring Harbor Perspectives in Medicine*. 2015;5:a017822–a017822. doi: 10.1101/cshperspect.a017822.
- **A comprehensive overview of existing TB treatments, highlighting the evolution from first-line therapies to complex, less effective regimens for drug-resistant strains.**
8. Zumla A, Nahid P, Cole ST. Advances in the development of new tuberculosis drugs and treatment regimens. *Nat Rev Drug Discov*. 2013;12:388–404. doi: 10.1038/nrd4001.
9. Rawat R, Whitty A, Tonge PJ. The isoniazid-NAD adduct is a slow, tight-binding inhibitor of InhA, the *Mycobacterium tuberculosis* enoyl reductase: Adduct affinity and drug resistance. *Proc Natl Acad Sci USA*. 2003;100:13881–13886. doi: 10.1073/pnas.2235848100.
10. Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, Darst SA. Structural Mechanism for Rifampicin Inhibition of Bacterial RNA Polymerase. *Cell*. 2001;104:901–912. doi: 10.1016/S0092-8674(01)00286-0.
11. Louw GE, Warren RM, Gey Van Pittius NC, Leon R, Jimenez A, Hernandez-Pando R, McEvoy CRE, Grobbelaar M, Murray M, Van Helden PD, et al. Rifampicin Reduces Susceptibility to Ofloxacin in Rifampicin-resistant *Mycobacterium tuberculosis* through Efflux. *Am J Respir Crit Care Med*. 2011;184:269–276. doi: 10.1164/rccm.201011-1924OC.
12. Zhang Y, Mitchison D. The curious characteristics of pyrazinamide: a review. 2003;7(1):6–21.
13. Goude R, Amin AG, Chatterjee D, Parish T. The Arabinosyltransferase EmbC Is Inhibited by Ethambutol in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2009;53:4138–4146. doi: 10.1128/AAC.00162-09.

14. Chiang C, Centis R, Migliori GB. Drug-resistant tuberculosis: Past, present, future. *Respirology*. 2010;15:413–432. doi: 10.1111/j.1440-1843.2010.01738.x.
15. Yee D, Valiquette C, Pelletier M, Parisien I, Rocher I, Menzies D. Incidence of Serious Side Effects from First-Line Antituberculosis Drugs among Patients Treated for Active Tuberculosis. *Am J Respir Crit Care Med*. 2003;167:1472–1477. doi: 10.1164/rccm.200206-626OC.
16. Frieden TR, Sterling T, Pablos-Mendez A, Kilburn JO, Cauthen GM, Dooley SW. The Emergence of Drug-Resistant Tuberculosis in New York City. *N Engl J Med*. 1993;328:521–526. doi: 10.1056/NEJM199302253280801.
17. Zumla A, Chakaya J, Centis R, D'Ambrosio L, Mwaba P, Bates M, Kapata N, Nyirenda T, Chanda D, Mfinanga S, et al. Tuberculosis treatment and management—an update on treatment regimens, trials, new drugs, and adjunct therapies. *The Lancet Respiratory Medicine*. 2015;3:220–234. doi: 10.1016/S2213-2600(15)00063-6.
18. Tran SL, Cook GM. The F₁ F₀ -ATP Synthase of *Mycobacterium smegmatis* Is Essential for Growth. *J Bacteriol*. 2005;187:5023–5028. doi: 10.1128/JB.187.14.5023-5028.2005.
19. Koul A, Vranckx L, Dendouga N, Balemans W, Van Den Wyngaert I, Vergauwen K, Göhlmann HWH, Willebrords R, Poncelet A, Guillemont J, et al. Diarylquinolines Are Bactericidal for Dormant Mycobacteria as a Result of Disturbed ATP Homeostasis. *Journal of Biological Chemistry*. 2008;283:25273–25280. doi: 10.1074/jbc.M803899200.
20. Alexander PE, De P. The Emergence of Extensively Drug-Resistant Tuberculosis (TB): TB/HIV Coinfection, Multidrug-Resistant TB and the Resulting Public Health Threat from Extensively Drug-Resistant TB, Globally and in Canada. *Canadian Journal of Infectious Diseases and Medical Microbiology*. 2007;18:289–291. doi: 10.1155/2007/986794.
21. Velayati AA, Masjedi MR, Farnia P, Tabarsi P, Ghanavi J, ZiaZarifi AH, Hoffner SE. Emergence of New Forms of Totally Drug-Resistant Tuberculosis Bacilli. *Chest*. 2009;136:420–425. doi: 10.1378/chest.08-2427.
22. Koul A, Arnoult E, Lounis N, Guillemont J, Andries K. The challenge of new drug discovery for tuberculosis. *Nature*. 2011;469:483–490. doi: 10.1038/nature09657.
23. Nahid P, Mase SR, Migliori GB, Sotgiu G, Bothamley GH, Brozek JL, Cattamanchi A, Cegielski JP, Chen L, Daley CL, et al. Treatment of Drug-Resistant Tuberculosis. An Official ATS/CDC/ERS/IDSA Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2019;200:e93–e142. doi: 10.1164/rccm.201909-1874ST.
- **An overview of the diverse genetic and physiological mechanisms behind *Mtb* drug resistance and tolerance.**
24. Shukla R, Tripathi T. Molecular Dynamics Simulation in Drug Discovery: Opportunities and Challenges. In: Singh SK, editor. *Innovations and Implementations of Computer Aided Drug Discovery Strategies in Rational Drug Design* [Internet]. Singapore: Springer Singapore; 2021 [cited 2025 Feb 19]. p. 295–316. Available from: http://link.springer.com/10.1007/978-981-15-8936-2_12.
25. Aghila Rani KG, Hamad MA, Zaher DM, Sieburth SM, Madani N, Al-Tel TH. Drug development post COVID-19 pandemic: toward a better system to meet current and future global health challenges. *Expert Opinion on Drug Discovery*. 2021;16:365–371. doi: 10.1080/17460441.2021.1854221.
26. Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV. Molecular mechanisms of antibiotic resistance. *Nat Rev Microbiol*. 2015;13:42–51. doi: 10.1038/nrmicro3380.
27. Munita JM, Arias CA. Mechanisms of Antibiotic Resistance. Kudva IT, Zhang Q, editors. *Microbiol Spectr*. 2016;4:4.2.15. doi: 10.1128/microbiolspec.VMBF-0016-2015.
28. Andersson DI. The biological cost of mutational antibiotic resistance: any practical conclusions? *Current Opinion in Microbiology*. 2006;9:461–465. doi: 10.1016/j.mib.2006.07.002.

29. Zur Wiesch PA, Kouyos R, Engelstädter J, Regoes RR, Bonhoeffer S. Population biological principles of drug-resistance evolution in infectious diseases. *The Lancet Infectious Diseases*. 2011;11:236–247. doi: 10.1016/S1473-3099(10)70264-4.
30. Hughes D, Andersson DI. Evolutionary Trajectories to Antibiotic Resistance. *Annu Rev Microbiol*. 2017;71:579–596. doi: 10.1146/annurev-micro-090816-093813.
31. Wirth T, Hildebrand F, Allix-Béguec C, Wölbeling F, Kubica T, Kremer K, van Soolingen D, Rüsche-Gerdes S, Loch C, Brisse S, et al. Origin, spread and demography of the *Mycobacterium tuberculosis* complex. *PLoS Pathog*. 2008;4:e1000160. doi: 10.1371/journal.ppat.1000160. Cited in: : PMID: 18802459.
32. Palomino J, Martin A. Drug Resistance Mechanisms in *Mycobacterium tuberculosis*. *Antibiotics*. 2014;3:317–340. doi: 10.3390/antibiotics3030317.
33. Telenti A, Imboden P, Marchesi F, Matter L, Schopfer K, Bodmer T, Lowrie D, Colston MJ, Cole S. Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. *The Lancet*. 1993;341:647–651. doi: 10.1016/0140-6736(93)90417-F.
34. Zhang Y, Heym B, Allen B, Young D, Cole S. The catalase—peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. *Nature*. 1992;358:591–593. doi: 10.1038/358591a0.
35. Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, Collins D, De Lisle G, Jacobs WR. *inhA*, a Gene Encoding a Target for Isoniazid and Ethionamide in *Mycobacterium tuberculosis*. *Science*. 1994;263:227–230. doi: 10.1126/science.8284673.
36. Takiff HE, Salazar L, Guerrero C, Philipp W, Huang WM, Kreiswirth B, Cole ST, Jacobs WR, Telenti A. Cloning and nucleotide sequence of *Mycobacterium tuberculosis gyrA* and *gyrB* genes and detection of quinolone resistance mutations. *Antimicrob Agents Chemother*. 1994;38:773–780. doi: 10.1128/AAC.38.4.773.
37. Coll F, Phelan J, Hill-Cawthorne GA, Nair MB, Mallard K, Ali S, Abdallah AM, Alghamdi S, Alsomali M, Ahmed AO, et al. Genome-wide analysis of multi- and extensively drug-resistant *Mycobacterium tuberculosis*. *Nat Genet*. 2018;50:307–316. doi: 10.1038/s41588-017-0029-0.
38. Comas I, Borrell S, Roetzer A, Rose G, Malla B, Kato-Maeda M, Galagan J, Niemann S, Gagneux S. Whole-genome sequencing of rifampicin-resistant *Mycobacterium tuberculosis* strains identifies compensatory mutations in RNA polymerase genes. *Nat Genet*. 2012;44:106–110. doi: 10.1038/ng.1038.
39. Merker M, Barbier M, Cox H, Rasigade J-P, Feuerriegel S, Kohl TA, Diel R, Borrell S, Gagneux S, Nikolayevskyy V, et al. Compensatory evolution drives multidrug-resistant tuberculosis in Central Asia. *eLife*. 2018;7:e38200. doi: 10.7554/eLife.38200.
40. Gillespie SH. Evolution of Drug Resistance in *Mycobacterium tuberculosis*: Clinical and Molecular Perspective. *Antimicrob Agents Chemother*. 2002;46:267–274. doi: 10.1128/AAC.46.2.267-274.2002.
41. Denamur E, Matic I. Evolution of mutation rates in bacteria. *Molecular Microbiology*. 2006;60:820–827. doi: 10.1111/j.1365-2958.2006.05150.x.
42. Singh A. Guardians of the mycobacterial genome: A review on DNA repair systems in *Mycobacterium tuberculosis*. *Microbiology*. 2017;163:1740–1758. doi: 10.1099/mic.0.000578.
43. Chopra I, O’Neill AJ, Miller K. The role of mutators in the emergence of antibiotic-resistant bacteria. *Drug Resistance Updates*. 2003;6:137–145. doi: 10.1016/S1368-7646(03)00041-4.
44. Oliver A, Mena A. Bacterial hypermutation in cystic fibrosis, not only for antibiotic resistance. *Clinical Microbiology and Infection*. 2010;16:798–808. doi: 10.1111/j.1469-0691.2010.03250.x.
45. Couce A, Rodríguez-Rojas A, Blázquez J. Bypass of genetic constraints during mutator evolution to antibiotic resistance. *Proc R Soc B*. 2015;282:20142698. doi: 10.1098/rspb.2014.2698.

46. Benjak A, Avanzi C, Singh P, Loiseau C, Girma S, Busso P, Fontes ANB, Miyamoto Y, Namisato M, Bobosha K, et al. Phylogenomics and antimicrobial resistance of the leprosy bacillus *Mycobacterium leprae*. *Nat Commun*. 2018;9:352. doi: 10.1038/s41467-017-02576-z. Cited: in : PMID: 29367657.
47. Ebrahimi-Rad M, Bifani P, Martin C, Kremer K, Samper S, Rauzier J, Kreiswirth B, Blazquez J, Jouan M, van Soolingen D, et al. Mutations in putative mutator genes of *Mycobacterium tuberculosis* strains of the W-Beijing family. *Emerg Infect Dis*. 2003;9:838–845. doi: 10.3201/eid0907.020803. Cited: in : PMID: 12890325.
48. Stagg HR, Cohen T, Becerra MC, Anderson LF, Abubakar I. A tale of two settings: the role of the Beijing genotype in the epidemiology of multidrug-resistant tuberculosis. *Eur Respir J*. 2014;43:632–635. doi: 10.1183/09031936.00140513.
49. Castañeda-García A, Prieto AI, Rodríguez-Beltrán J, Alonso N, Cantillon D, Costas C, Pérez-Lago L, Zegeye ED, Herranz M, Płociński P, et al. A non-canonical mismatch repair pathway in prokaryotes. *Nat Commun*. 2017;8:14246. doi: 10.1038/ncomms14246. Cited: in : PMID: 28128207.
50. Ishino S, Skouloubris S, Kudo H, l'Hermitte-Stead C, Es-Sadik A, Lambry J-C, Ishino Y, Myllykallio H. Activation of the mismatch-specific endonuclease EndoMS/NucS by the replication clamp is required for high fidelity DNA replication. *Nucleic Acids Research*. 2018;46:6206–6217. doi: 10.1093/nar/gky460.
51. Takemoto N, Numata I, Su'etsugu M, Miyoshi-Akiyama T. Bacterial EndoMS/NucS acts as a clamp-mediated mismatch endonuclease to prevent asymmetric accumulation of replication errors. *Nucleic Acids Research*. 2018;46:6152–6165. doi: 10.1093/nar/gky481.
52. Zein-Eddine R, Le Meur A, Skouloubris S, Jelsbak L, Refrégier G, Myllykallio H. Genome wide analyses reveal the role of mutator phenotypes in the emergence of drug resistance in *Mycobacterium tuberculosis* [Internet]. 2025 [cited 2025 Apr 28]. Available from: <http://biorxiv.org/lookup/doi/10.1101/2025.01.17.633521>.
53. Gillespie SH, Basu S, Dickens AL, O'Sullivan DM, McHugh TD. Effect of subinhibitory concentrations of ciprofloxacin on *Mycobacterium fortuitum* mutation rates. *Journal of Antimicrobial Chemotherapy*. 2005;56:344–348. doi: 10.1093/jac/dki191.
54. Fonseca JD, Knight GM, McHugh TD. The complex evolution of antibiotic resistance in *Mycobacterium tuberculosis*. *International Journal of Infectious Diseases*. 2015;32:94–100. doi: 10.1016/j.ijid.2015.01.014.
55. Jankute M, Cox JAG, Harrison J, Besra GS. Assembly of the Mycobacterial Cell Wall. *Annu Rev Microbiol*. 2015;69:405–423. doi: 10.1146/annurev-micro-091014-104121.
56. Dulberger CL, Rubin EJ, Boutte CC. The mycobacterial cell envelope — a moving target. *Nat Rev Microbiol*. 2020;18:47–59. doi: 10.1038/s41579-019-0273-7.
57. Jarlier V, Nikaido H. Mycobacterial cell wall: Structure and role in natural resistance to antibiotics. *FEMS Microbiology Letters*. 1994;123:11–18. doi: 10.1111/j.1574-6968.1994.tb07194.x.
58. Gygli SM, Borrell S, Trauner A, Gagneux S. Antimicrobial resistance in *Mycobacterium tuberculosis*: mechanistic and evolutionary perspectives. *FEMS Microbiology Reviews*. 2017;41:354–373. doi: 10.1093/femsre/fux011.
59. Te Brake LHM, De Knecht GJ, De Steenwinkel JE, Van Dam TJP, Burger DM, Russel FGM, Van Crevel R, Koenderink JB, Aarnoutse RE. The Role of Efflux Pumps in Tuberculosis Treatment and Their Promise as a Target in Drug Development: Unraveling the Black Box. *Annu Rev Pharmacol Toxicol*. 2018;58:271–291. doi: 10.1146/annurev-pharmtox-010617-052438.
60. Esaulova E, Das S, Singh DK, Choreño-Parra JA, Swain A, Arthur L, Rangel-Moreno J, Ahmed M, Singh B, Gupta A, et al. The immune landscape in tuberculosis reveals populations linked to disease and latency. *Cell Host & Microbe*. 2021;29:165-178.e8. doi: 10.1016/j.chom.2020.11.013.

61. Esteban J, García-Coca M. Mycobacterium Biofilms. *Front Microbiol.* 2018;8:2651. doi: 10.3389/fmicb.2017.02651.
62. Tizzano B, Dallenga TK, Utpatel C, Behrends J, Homolka S, Kohl TA, Niemann S. Survival of hypoxia-induced dormancy is not a common feature of all strains of the Mycobacterium tuberculosis complex. *Sci Rep.* 2021;11:2628. doi: 10.1038/s41598-021-81223-6.
- **A study highlighting the impact of host-related factors on TB drug efficacy and the need for host-directed and personalized treatment strategies.**

63. Restrepo BI, Khan A, Singh VK, Erica de-Leon, Aguillón-Durán GP, Ledezma-Campos E, Canaday DH, Jagannath C. Human monocyte-derived macrophage responses to M. tuberculosis differ by the host's tuberculosis, diabetes or obesity status, and are enhanced by rapamycin. *Tuberculosis.* 2021;126:102047. doi: 10.1016/j.tube.2020.102047.
64. Simeone R, Bobard A, Lippmann J, Bitter W, Majlessi L, Brosch R, Enninga J. Phagosomal Rupture by Mycobacterium tuberculosis Results in Toxicity and Host Cell Death. Ehrt S, editor. *PLoS Pathog.* 2012;8:e1002507. doi: 10.1371/journal.ppat.1002507.
65. Cambier CJ, Falkow S, Ramakrishnan L. Host Evasion and Exploitation Schemes of Mycobacterium tuberculosis. *Cell.* 2014;159:1497–1509. doi: 10.1016/j.cell.2014.11.024.
66. Chandra P, Grigsby SJ, Philips JA. Immune evasion and provocation by Mycobacterium tuberculosis. *Nat Rev Microbiol.* 2022;20:750–766. doi: 10.1038/s41579-022-00763-4.
- **A study showing emerging anti-TB compounds with novel mechanisms**
67. Roden DM, George Jr AL. The genetic basis of variability in drug responses. *Nat Rev Drug Discov.* 2002;1:37–44. doi: 10.1038/nrd705.
68. Kana BD, Karakousis PC, Parish T, Dick T. Future target-based drug discovery for tuberculosis? *Tuberculosis.* 2014;94:551–556. doi: 10.1016/j.tube.2014.10.003.
69. Grzelak EM, Choules MP, Gao W, Cai G, Wan B, Wang Y, McAlpine JB, Cheng J, Jin Y, Lee H, et al. Strategies in anti-Mycobacterium tuberculosis drug discovery based on phenotypic screening. *J Antibiot.* 2019;72:719–728. doi: 10.1038/s41429-019-0205-9.
70. Dartois V, Dick T. Therapeutic developments for tuberculosis and nontuberculous mycobacterial lung disease. *Nat Rev Drug Discov.* 2024;23:381–403. doi: 10.1038/s41573-024-00897-5.
71. Agnivesh PK, Roy A, Sau S, Kumar S, Kalia NP. Advancements and challenges in tuberculosis drug discovery: A comprehensive overview. *Microbial Pathogenesis.* 2025;198:107074. doi: 10.1016/j.micpath.2024.107074.
72. Zhang G, Guo S, Cui H, Qi J. Virtual Screening of Small Molecular Inhibitors against DprE1. *Molecules.* 2018;23:524. doi: 10.3390/molecules23030524.
73. Delmar JA, Chou T-H, Wright CC, Licon MH, Doh JK, Radhakrishnan A, Kumar N, Lei H-T, Bolla JR, Rajashankar KR, et al. Structural Basis for the Regulation of the MmpL Transporters of Mycobacterium tuberculosis. *Journal of Biological Chemistry.* 2015;290:28559–28574. doi: 10.1074/jbc.M115.683797.
74. Kumar N, Sharma S, Kaushal PS. Protein synthesis in Mycobacterium tuberculosis as a potential target for therapeutic interventions. *Molecular Aspects of Medicine.* 2021;81:101002. doi: 10.1016/j.mam.2021.101002.
75. Kashyap A, Singh PK, Silakari O. In silico designing of domain B selective gyrase inhibitors for effective treatment of resistant tuberculosis. *Tuberculosis.* 2018;112:83–88. doi: 10.1016/j.tube.2018.08.005.
76. Mosaei H, Zenkin N. Inhibition of RNA Polymerase by Rifampicin and Rifamycin-Like Molecules. Lovett ST, Hinton D, editors. *EcoSal Plus.* 2020;9:10.1128/ecosalplus.ESP-0017–2019. doi: 10.1128/ecosalplus.esp-0017-2019.
77. Murase LS, Perez De Souza JV, Meneguello JE, Seixas FAV, Hegeto LA, Scodro RBDL, Siqueira VLD, Caleffi Ferracioli KR, Cardoso RF. Possible Binding of Piperine in

- Mycobacterium tuberculosis RNA Polymerase and Rifampin Synergism. *Antimicrob Agents Chemother.* 2019;63:e02520-18. doi: 10.1128/AAC.02520-18.
78. Palencia A, Li X, Bu W, Choi W, Ding CZ, Easom EE, Feng L, Hernandez V, Houston P, Liu L, et al. Discovery of Novel Oral Protein Synthesis Inhibitors of *Mycobacterium tuberculosis* That Target Leucyl-tRNA Synthetase. *Antimicrob Agents Chemother.* 2016;60:6271–6280. doi: 10.1128/AAC.01339-16.
 79. Beites T, O'Brien K, Tiwari D, Engelhart CA, Walters S, Andrews J, Yang H-J, Sutphen ML, Weiner DM, Dayao EK, et al. Plasticity of the *Mycobacterium tuberculosis* respiratory chain and its impact on tuberculosis drug development. *Nat Commun.* 2019;10:4970. doi: 10.1038/s41467-019-12956-2.
 80. Nizi MG, Desantis J, Nakatani Y, Massari S, Mazzarella MA, Shetye G, Sabatini S, Barreca ML, Manfroni G, Felicetti T, et al. Antitubercular polyhalogenated phenothiazines and phenoselenazine with reduced binding to CNS receptors. *European Journal of Medicinal Chemistry.* 2020;201:112420. doi: 10.1016/j.ejmech.2020.112420.
 81. Dhiman RK, Pujari V, Kincaid JM, Ikeh MA, Parish T, Crick DC. Characterization of MenA (isoprenyl diphosphate:1,4-dihydroxy-2-naphthoate isoprenyltransferase) from *Mycobacterium tuberculosis*. Manganeli R, editor. *PLoS ONE.* 2019;14:e0214958. doi: 10.1371/journal.pone.0214958.
 82. Berg K, Hegde P, Pujari V, Brinkmann M, Wilkins DZ, Parish T, Crick DC, Aldrich CC. SAR study of piperidine derivatives as inhibitors of 1,4-dihydroxy-2-naphthoate isoprenyltransferase (MenA) from *Mycobacterium tuberculosis*. *European Journal of Medicinal Chemistry.* 2023;249:115125. doi: 10.1016/j.ejmech.2023.115125.
 83. Kalia NP, Shi Lee B, Ab Rahman NB, Moraski GC, Miller MJ, Pethe K. Carbon metabolism modulates the efficacy of drugs targeting the cytochrome bc1:aa3 in *Mycobacterium tuberculosis*. *Sci Rep.* 2019;9:8608. doi: 10.1038/s41598-019-44887-9.
 84. Harrison GA, Mayer Bridwell AE, Singh M, Jayaraman K, Weiss LA, Kinsella RL, Aneke JS, Flentie K, Schene ME, Gaggioli M, et al. Identification of 4-Amino-Thieno[2,3-*d*]Pyrimidines as QcrB Inhibitors in *Mycobacterium tuberculosis*. Dunman P, editor. *mSphere.* 2019;4:e00606-19. doi: 10.1128/mSphere.00606-19.
 85. Bogdanović N, Sundararaman L, Kamariah N, Tyagi A, Bhushan S, Raganathan P, Shin J, Dick T, Grüber G. Structure and function of *Mycobacterium*-specific components of F-ATP synthase subunits α and ϵ . *Journal of Structural Biology.* 2018;204:420–434. doi: 10.1016/j.jsb.2018.10.006.
 86. Kumar P, Capodagli GC, Awasthi D, Shrestha R, Maharaja K, Sukheja P, Li S-G, Inoyama D, Zimmerman M, Ho Liang HP, et al. Synergistic Lethality of a Binary Inhibitor of *Mycobacterium tuberculosis* KasA. Projan SJ, editor. *mBio.* 2018;9:e02101-17. doi: 10.1128/mBio.02101-17.
 87. Shukla H, Kumar V, Singh AK, Rastogi S, Khan SR, Siddiqi MI, Krishnan MY, Akhtar MdS. Isocitrate lyase of *Mycobacterium tuberculosis* is inhibited by quercetin through binding at N-terminus. *International Journal of Biological Macromolecules.* 2015;78:137–141. doi: 10.1016/j.ijbiomac.2015.04.005.
 88. Pham TV, Mellott DM, Moghadamchargari Z, Chen K, Krieger I, Laganowsky A, Sacchettini JC, Meek TD. Covalent Inactivation of *Mycobacterium tuberculosis* Isocitrate Lyase by *cis*-2,3-Epoxy-Succinic Acid. *ACS Chem Biol.* 2021;16:463–470. doi: 10.1021/acscchembio.0c00740.
 - **A study that shows promising host pathways for enhancing immune control and combating drug-resistant TB.**
 89. Korbee CJ, Heemskerk MT, Kocev D, Van Strijen E, Rabiee O, Franken KLMC, Wilson L, Savage ND, Džeroski S, Haks MC, et al. Combined chemical genetics and data-driven bioinformatics approach identifies receptor tyrosine kinase inhibitors as host-directed antimicrobials. *Nat Commun.* 2018;9:358. doi: 10.1038/s41467-017-02777-6.

90. Sogi KM, Lien KA, Johnson JR, Krogan NJ, Stanley SA. The Tyrosine Kinase Inhibitor Gefitinib Restricts *Mycobacterium tuberculosis* Growth through Increased Lysosomal Biogenesis and Modulation of Cytokine Signaling. *ACS Infect Dis*. 2017;3:564–574. doi: 10.1021/acsinfecdis.7b00046.
91. Petit-Jentreau L, Tailleux L, Coombes JL. Purinergic Signaling: A Common Path in the Macrophage Response against *Mycobacterium tuberculosis* and *Toxoplasma gondii*. *Front Cell Infect Microbiol*. 2017;7:347. doi: 10.3389/fcimb.2017.00347.
92. Eberhardt N, Bergero G, Mazzocco Mariotta YL, Aoki MP. Purinergic modulation of the immune response to infections. *Purinergic Signalling*. 2022;18:93–113. doi: 10.1007/s11302-021-09838-y.
93. Arbués A, Brees D, Chibout S-D, Fox T, Kammüller M, Portevin D. TNF- α antagonists differentially induce TGF- β 1-dependent resuscitation of dormant-like *Mycobacterium tuberculosis*. Hawn TR, editor. *PLoS Pathog*. 2020;16:e1008312. doi: 10.1371/journal.ppat.1008312.
94. Acharya MP, Pradeep SP, Murthy VS, Chikkannaiah P, Kamar V, Narayanashetty S, Burugina Nagaraja S, Gangadhar N, Yoganand R, Satchidanandam V. CD38+CD27–TNF- α + on *Mtb* -specific CD4+ T Cells Is a Robust Biomarker for Tuberculosis Diagnosis. *Clinical Infectious Diseases*. 2021;73:793–801. doi: 10.1093/cid/ciab144.
95. Gallucci S, Matzinger P. Danger signals: SOS to the immune system. *Current Opinion in Immunology*. 2001;13:114–119. doi: 10.1016/S0952-7915(00)00191-6.
96. Liu Q, Liu H, Shi L, Gan M, Zhao X, Lyu L-D, Takiff HE, Wan K, Gao Q. Local adaptation of *Mycobacterium tuberculosis* on the Tibetan Plateau. *Proc Natl Acad Sci USA*. 2021;118:e2017831118. doi: 10.1073/pnas.2017831118.
97. Winchell CG, Nyquist SK, Chao MC, Maiello P, Myers AJ, Hopkins F, Chase M, Gideon HP, Patel KV, Bromley JD, et al. CD8+ lymphocytes are critical for early control of tuberculosis in macaques. *Journal of Experimental Medicine*. 2023;220:e20230707. doi: 10.1084/jem.20230707.
98. Rao SPS, Alonso S, Rand L, Dick T, Pethe K. The protonmotive force is required for maintaining ATP homeostasis and viability of hypoxic, nonreplicating *Mycobacterium tuberculosis*. *Proc Natl Acad Sci USA*. 2008;105:11945–11950. doi: 10.1073/pnas.0711697105.
99. Nathan C, Barry CE. TB drug development: immunology at the table. *Immunological Reviews*. 2015;264:308–318. doi: 10.1111/imr.12275.
100. Gautam S, Qureshi KA, Jameel Pasha SB, Dhanasekaran S, Aspatwar A, Parkkila S, Alanazi S, Atiya A, Khan MMU, Venugopal D. Medicinal Plants as Therapeutic Alternatives to Combat *Mycobacterium tuberculosis*: A Comprehensive Review. *Antibiotics*. 2023;12:541. doi: 10.3390/antibiotics12030541.
- **A study showing anti-evolution strategies to overcome drug resistance**
101. Cirz RT, Romesberg FE. Controlling Mutation: Intervening in Evolution as a Therapeutic Strategy. *Critical Reviews in Biochemistry and Molecular Biology*. 2007;42:341–354. doi: 10.1080/10409230701597741.
102. Gessner S. Investigating the composition and recruitment of the mycobacterial ImuA'–ImuB–DnaE2 mutasome. *eLIFE*. 2023;12.
103. Boshoff HIM, Reed MB, Barry CE, Mizrahi V. DnaE2 Polymerase Contributes to In Vivo Survival and the Emergence of Drug Resistance in *Mycobacterium tuberculosis*. *Cell*. 2003;113:183–193. doi: 10.1016/S0092-8674(03)00270-8.
104. Yang W. An Overview of Y-Family DNA Polymerases and a Case Study of Human DNA Polymerase η . *Biochemistry*. 2014;53:2793–2803. doi: 10.1021/bi500019s.
105. Smollett KL, Smith KM, Kahramanoglou C, Arnvig KB, Buxton RS, Davis EO. Global Analysis of the Regulon of the Transcriptional Repressor LexA, a Key Component of SOS Response in *Mycobacterium tuberculosis*. *Journal of Biological Chemistry*. 2012;287:22004–22014. doi: 10.1074/jbc.M112.357715.

106. Recacha E, Machuca J, Díaz De Alba P, Ramos-Güelfo M, Docobo-Pérez F, Rodríguez-Beltrán J, Blázquez J, Pascual A, Rodríguez-Martínez JM. Quinolone Resistance Reversion by Targeting the SOS Response. Bonomo RA, editor. *mBio*. 2017;8:e00971-17. doi: 10.1128/mBio.00971-17.
107. Luo Y, Pfuetzner RA, Mosimann S, Paetzel M, Frey EA, Cherney M, Kim B, Little JW, Strynadka NCJ. Crystal Structure of LexA. *Cell*. 2001;106:585–594. doi: 10.1016/S0092-8674(01)00479-2.
108. Chen Z, Yang H, Pavletich NP. Mechanism of homologous recombination from the RecA–ssDNA/dsDNA structures. *Nature*. 2008;453:489–494. doi: 10.1038/nature06971.
109. Million-Weaver S, Samadpour AN, Moreno-Habel DA, Nugent P, Brittnacher MJ, Weiss E, Hayden HS, Miller SI, Liachko I, Merrih H. An underlying mechanism for the increased mutagenesis of lagging-strand genes in *Bacillus subtilis*. *Proc Natl Acad Sci USA* [Internet]. 2015 [cited 2025 Mar 20];112. doi: 10.1073/pnas.1416651112.
110. Ragheb MN, Thomason MK, Hsu C, Nugent P, Gage J, Samadpour AN, Kariisa A, Merrih CN, Miller SI, Sherman DR, et al. Inhibiting the Evolution of Antibiotic Resistance. *Molecular Cell*. 2019;73:157-165.e5. doi: 10.1016/j.molcel.2018.10.015.
111. Park J-S, Marr MT, Roberts JW. E. coli Transcription Repair Coupling Factor (Mfd Protein) Rescues Arrested Complexes by Promoting Forward Translocation. *Cell*. 2002;109:757–767. doi: 10.1016/S0092-8674(02)00769-9.
- **High-throughput CRISPR tools reveal genetic vulnerabilities in Mtb, accelerating drug and vaccine target discovery.**
112. Gregersen LH, Svejstrup JQ. The Cellular Response to Transcription-Blocking DNA Damage. *Trends in Biochemical Sciences*. 2018;43:327–341. doi: 10.1016/j.tibs.2018.02.010.
113. Wang X, Jowsey WJ, Cheung C-Y, Smart CJ, Klaus HR, Seeto NE, Waller NJ, Chrisp MT, Peterson AL, Ofori-Anyinam B, et al. Whole genome CRISPRi screening identifies druggable vulnerabilities in an isoniazid resistant strain of *Mycobacterium tuberculosis*. *Nat Commun*. 2024;15:9791. doi: 10.1038/s41467-024-54072-w.
114. Rahman K, Jamal M, Chen X, Zhou W, Yang B, Zou Y, Xu W, Lei Y, Wu C, Cao X, et al. Reprogramming *Mycobacterium Tuberculosis* CRISPR System for Gene Editing and Genome-Wide RNA Interference Screening. *Genomics, Proteomics & Bioinformatics*. 2022;20:1180–1196. doi: 10.1016/j.gpb.2021.01.008.
115. Zein-Eddine R, Refrégier G, Cervantes J, Yokobori NK. The future of CRISPR in *Mycobacterium tuberculosis* infection. *J Biomed Sci*. 2023;30:34. doi: 10.1186/s12929-023-00932-4.
116. Rock J. Tuberculosis drug discovery in the CRISPR era. Kline KA, editor. *PLoS Pathog*. 2019;15:e1007975. doi: 10.1371/journal.ppat.1007975.
- **A study showing AI-driven strategies: from diagnostic to active compound prediction**
117. Codlin AJ, Dao TP, Vo LNQ, Forse RJ, Van Truong V, Dang HM, Nguyen LH, Nguyen HB, Nguyen NV, Sidney-Annerstedt K, et al. Independent evaluation of 12 artificial intelligence solutions for the detection of tuberculosis. *Sci Rep*. 2021;11:23895. doi: 10.1038/s41598-021-03265-0.
118. Santosh K, Allu S, Rajaraman S, Antani S. Advances in Deep Learning for Tuberculosis Screening using Chest X-rays: The Last 5 Years Review. *J Med Syst*. 2022;46:82. doi: 10.1007/s10916-022-01870-8.
119. Heyckendorf J, Marwitz S, Reimann M, Avsar K, DiNardo AR, Günther G, Hoelscher M, Ibrahim E, Kalsdorf B, Kaufmann SHE, et al. Prediction of anti-tuberculosis treatment duration based on a 22-gene transcriptomic model. *Eur Respir J*. 2021;58:2003492. doi: 10.1183/13993003.03492-2020.
120. Hamada Y, Penn-Nicholson A, Krishnan S, Cirillo DM, Matteelli A, Wyss R, Denking CM, Rangaka MX, Ruhwald M, Schumacher SG. Are mRNA based transcriptomic

signatures ready for diagnosing tuberculosis in the clinic? - A review of evidence and the technological landscape. *eBioMedicine*. 2022;82:104174. doi: 10.1016/j.ebiom.2022.104174.

121. Peetluk LS, Rebeiro PF, Ridolfi FM, Andrade BB, Cordeiro-Santos M, Kritski A, Durovni B, Calvacante S, Figueiredo MC, Haas DW, et al. A Clinical Prediction Model for Unsuccessful Pulmonary Tuberculosis Treatment Outcomes. *Clinical Infectious Diseases*. 2022;74:973–982. doi: 10.1093/cid/ciab598.
122. Radchenko EV, Antonyan GV, Ignatov SK, Palyulin VA. Machine Learning Prediction of Mycobacterial Cell Wall Permeability of Drugs and Drug-like Compounds. *Molecules*. 2023;28:633. doi: 10.3390/molecules28020633.
123. Rood JE, Maartens A, Hupalowska A, Teichmann SA, Regev A. Impact of the Human Cell Atlas on medicine. *Nat Med*. 2022;28:2486–2496. doi: 10.1038/s41591-022-02104-7.
- **Targeted mAb treatments offer a promising alternative to antibiotics for combating multidrug-resistant pathogens.**
124. Casadevall A, Dadachova E, Pirofski L. Passive antibody therapy for infectious diseases. *Nat Rev Microbiol*. 2004;2:695–703. doi: 10.1038/nrmicro974.
125. Kobayashi SD, Porter AR, Freedman B, Pandey R, Chen L, Kreiswirth BN, DeLeo FR. Antibody-Mediated Killing of Carbapenem-Resistant ST258 *Klebsiella pneumoniae* by Human Neutrophils. Keim P, editor. *mBio*. 2018;9:e00297-18. doi: 10.1128/mBio.00297-18.
126. Doyle CR, Moon J-Y, Daily JP, Wang T, Pirofski L. A Capsular Polysaccharide-Specific Antibody Alters *Streptococcus pneumoniae* Gene Expression during Nasopharyngeal Colonization of Mice. Freitag NE, editor. *Infect Immun*. 2018;86:e00300-18. doi: 10.1128/IAI.00300-18.
127. Motley MP, Banerjee K, Fries BC. Monoclonal antibody-based therapies for bacterial infections. *Current Opinion in Infectious Diseases*. 2019;32:210–216. doi: 10.1097/QCO.0000000000000539.
- **A study showing In silico approaches for anti-TB drug development**
128. Timo GO, Reis RSSVD, Melo AFD, Costa TVL, Magalhães PDO, Homem-de-Mello M. Predictive Power of In Silico Approach to Evaluate Chemicals against *M. tuberculosis*: A Systematic Review. *Pharmaceuticals*. 2019;12:135. doi: 10.3390/ph12030135.
129. Aleksandrov A, Myllykallio H. Advances and challenges in drug design against tuberculosis: application of in silico approaches. *Expert Opinion on Drug Discovery*. 2019;14:35–46. doi: 10.1080/17460441.2019.1550482.
130. Gao Y, Xie J, Tang R, Yang K, Zhang Y, Chen L, Li H. Identification of a pyrimidinetrione derivative as the potent DprE1 inhibitor by structure-based virtual ligand screening. *Bioorganic Chemistry*. 2019;85:168–178. doi: 10.1016/j.bioorg.2018.12.018.
131. Hu X, Yang L, Chai X, Lei Y, Alam MS, Liu L, Shen C, Jiang D, Wang Z, Liu Z, et al. Discovery of novel DprE1 inhibitors via computational bioactivity fingerprints and structure-based virtual screening. *Acta Pharmacol Sin*. 2022;43:1605–1615. doi: 10.1038/s41401-021-00779-1.
132. Myllykallio H, Lipowski G, Leduc D, Filee J, Forterre P, Liebl U. An Alternative Flavin-Dependent Mechanism for Thymidylate Synthesis. *Science*. 2002;297:105–107. doi: 10.1126/science.1072113.
- **Integrating anti-evolution strategies with AI-driven drug discovery offers a promising path to combat TB resistance and develop evolution-resilient therapies.**
133. Sengupta P, Satpati P. Selective Inhibitor Design against Thymidylate Synthase of *Mycobacterium tuberculosis* Using Alchemical Simulations. *ACS Omega*. 2025;10:13966–13976. doi: 10.1021/acsomega.4c10518.
134. Basta T, Boum Y, Briffotiaux J, Becker HF, Lamarre-Jouenne I, Lambry J-C, Skouloubris S, Liebl U, Graille M, Van Tilbeurgh H, et al. Mechanistic and structural basis for inhibition of thymidylate synthase ThyX. *Open Biol*. 2012;2:120120. doi: 10.1098/rsob.120120.

135. Djaout K, Singh V, Boum Y, Katawera V, Becker HF, Bush NG, Hearnshaw SJ, Pritchard JE, Bourbon P, Madrid PB, et al. Predictive modeling targets thymidylate synthase ThyX in *Mycobacterium tuberculosis*. *Sci Rep*. 2016;6:27792. doi: 10.1038/srep27792.
136. Azaldegui CA, Vecchiarelli AG, Biteen JS. The emergence of phase separation as an organizing principle in bacteria. *Biophysical Journal*. 2021;120:1123–1138. doi: 10.1016/j.bpj.2020.09.023.
137. Myllykallio H, Becker HF, Aleksandrov A. Mechanism of Naphthoquinone Selectivity of Thymidylate Synthase ThyX. *Biophysical Journal*. 2020;119:2508–2516. doi: 10.1016/j.bpj.2020.10.042.
138. Naz S, Paritosh K, Sanyal P, Khan S, Singh Y, Varshney U, Nandicoori VK. GWAS and functional studies suggest a role for altered DNA repair in the evolution of drug resistance in *Mycobacterium tuberculosis*. *Elife*. 2023;12:e75860. doi: 10.7554/eLife.75860. Cited in : PMID: 36695572.
139. Sprouffske K, Aguilar-Rodríguez J, Sniegowski P, Wagner A. High mutation rates limit evolutionary adaptation in *Escherichia coli*. Matic I, editor. *PLoS Genet*. 2018;14:e1007324. doi: 10.1371/journal.pgen.1007324.
140. Qiu Y, Smith DGA, Boothroyd S, Jang H, Hahn DF, Wagner J, Bannan CC, Gokey T, Lim VT, Stern CD, et al. Development and Benchmarking of Open Force Field v1.0.0—the Parsley Small-Molecule Force Field. *J Chem Theory Comput*. 2021;17:6262–6280. doi: 10.1021/acs.jctc.1c00571.
141. Croitoru A, Kumar A, Lambry J-C, Lee J, Sharif S, Yu W, MacKerell AD, Aleksandrov A. Increasing the Accuracy and Robustness of the CHARMM General Force Field with an Expanded Training Set. *J Chem Theory Comput*. 2025;21:3044–3065. doi: 10.1021/acs.jctc.5c00046.
142. Eastman P, Galvelis R, Peláez RP, Abreu CRA, Farr SE, Gallicchio E, Gorenko A, Henry MM, Hu F, Huang J, et al. OpenMM 8: Molecular Dynamics Simulation with Machine Learning Potentials. *J Phys Chem B*. 2024;128:109–116. doi: 10.1021/acs.jpcc.3c06662.
143. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Židek A, Potapenko A, et al. Highly accurate protein structure prediction with AlphaFold. *Nature*. 2021;596:583–589. doi: 10.1038/s41586-021-03819-2.
144. Abramson J, Adler J, Dunger J, Evans R, Green T, Pritzel A, Ronneberger O, Willmore L, Ballard AJ, Bambrick J, et al. Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature*. 2024;630:493–500. doi: 10.1038/s41586-024-07487-w.
145. Sadybekov AV, Katritch V. Computational approaches streamlining drug discovery. *Nature*. 2023;616:673–685. doi: 10.1038/s41586-023-05905-z.
146. Stokes JM, Yang K, Swanson K, Jin W, Cubillos-Ruiz A, Donghia NM, MacNair CR, French S, Carfrae LA, Bloom-Ackermann Z, et al. A Deep Learning Approach to Antibiotic Discovery. *Cell*. 2020;180:688–702.e13. doi: 10.1016/j.cell.2020.01.021.
147. Heid E, Greenman KP, Chung Y, Li S-C, Graff DE, Vermeire FH, Wu H, Green WH, McGill CJ. Chemprop: A Machine Learning Package for Chemical Property Prediction. *J Chem Inf Model*. 2024;64:9–17. doi: 10.1021/acs.jcim.3c01250.
148. Rajasekar AA, Raman K, Ravindran B. Goal directed molecule generation using Monte Carlo Tree Search [Internet]. arXiv; 2020 [cited 2025 Apr 29]. Available from: <https://arxiv.org/abs/2010.16399>.
149. Corso G, Stärk H, Jing B, Barzilay R, Jaakkola T. DiffDock: Diffusion Steps, Twists, and Turns for Molecular Docking [Internet]. arXiv; 2022 [cited 2025 Apr 17]. Available from: <https://arxiv.org/abs/2210.01776>.
150. Schneuing A, Harris C, Du Y, Didi K, Jamasb A, Igashov I, Du W, Gomes C, Blundell TL, Lio P, et al. Structure-based drug design with equivariant diffusion models. *Nat Comput Sci*. 2024;4:899–909. doi: 10.1038/s43588-024-00737-x.

151. Cretu M, Harris C, Igashov I, Schneuing A, Segler M, Correia B, Roy J, Bengio E, Liò P. SynFlowNet: Design of Diverse and Novel Molecules with Synthesis Constraints [Internet]. arXiv; 2025 [cited 2025 Apr 28]. Available from: <http://arxiv.org/abs/2405.01155>.
152. Kp Jayatunga M, Ayers M, Bruens L, Jayanth D, Meier C. How successful are AI-discovered drugs in clinical trials? A first analysis and emerging lessons. *Drug Discovery Today*. 2024;29:104009. doi: 10.1016/j.drudis.2024.104009.
153. Nsubuga M, Galiwango R, Jjinga D, Mboowa G. Generalizability of machine learning in predicting antimicrobial resistance in *E. coli*: a multi-country case study in Africa. *BMC Genomics*. 2024;25:287. doi: 10.1186/s12864-024-10214-4.
154. Smith T, Wolff KA, Nguyen L. Molecular Biology of Drug Resistance in *Mycobacterium tuberculosis*. In: Pieters J, McKinney JD, editors. *Pathogenesis of Mycobacterium tuberculosis and its Interaction with the Host Organism* [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012 [cited 2025 Apr 29]. p. 53–80. Available from: http://link.springer.com/10.1007/82_2012_279.
155. Qabel A, Ennadir S, Nikolentzos G, Lutzeyer JF, Chatzianastasis M, Bostrom H, Vazirgiannis M. Advancing Antibiotic Resistance Classification with Deep Learning Using Protein Sequence and Structure [Internet]. 2022 [cited 2025 Apr 29]. Available from: <http://biorxiv.org/lookup/doi/10.1101/2022.10.06.511103>.