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Review

Structure-Based Virtual Screening in Tuberculosis Drug Discovery Pharmacological Constraints Failure Modes and Translational Lessons

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Abstract

Structure-based strategies are widely used in tuberculosis drug discovery; however, their translational impact remains limited. This review examines how structure-based virtual screening (SBVS) is applied in practice to *Mycobacterium tuberculosis* targets and explores why docking-derived predictions frequently fail to translate into measurable biological activity. Rather than treating docking scores as quantitative predictors of potency, representative case studies are analyzed to demonstrate that SBVS is most effective when employed as a prioritization framework integrated with appropriate target preparation, physicochemical filtering, and early experimental validation. Across diverse targets, molecular dynamics simulations emerge as a critical discriminator, enabling the identification of binding instability and false-positive hits that persist after static docking. Tuberculosis-specific constraints—including cofactor-dependent catalysis, resistance-associated mutations, membrane-rich environments, and permeability barriers—are discussed as key factors decoupling *in silico* affinity from whole-cell efficacy. Collectively, these observations support a workflow-oriented view of computational drug discovery in tuberculosis, in which iterative integration of structural modeling and experimental validation is required for meaningful lead identification.

Keywords: tuberculosis; multidrug-resistant tuberculosis; *in silico* drug discovery; virtual screening; molecular docking; machine learning in drug discovery

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1. Introduction

Tuberculosis (TB) remains a major global health problem and continues to rank among the leading causes of death from infectious diseases worldwide. The disease is caused by *Mycobacterium tuberculosis* (MTB) and imposes a persistent public health burden despite decades of therapeutic availability [1–4]. According to recent estimates from the World Health Organization (WHO), more than 10 million individuals develop TB annually, and approximately 1.3 million deaths are reported each year at the global level. The sustained incidence of TB, together with its disproportionate impact on vulnerable populations,

including people living with HIV, reflects the limited effectiveness of current control strategies and the slow pace of progress achieved with existing treatment regimens [1,5].

The global expansion of tuberculosis strains resistant to first- and second-line drugs has substantially reduced the effectiveness of standardized treatment regimens. As resistance accumulates and spreads, particularly in regions with high TB incidence, the clinical utility of conventional therapies has been progressively compromised [6–8]. Resistance in TB encompasses a broad spectrum, ranging from mono-resistance to individual first-line drugs to poly-resistance that excludes combined resistance to isoniazid (INH) and rifampicin. Multidrug-resistant TB is defined by concurrent resistance to both isoniazid and rifampicin, whereas extensively drug-resistant TB involves additional resistance to fluoroquinolones and at least one second-line injectable agent, such as amikacin, kanamycin, or capreomycin [9]. These resistance profiles substantially complicate treatment decisions and limit therapeutic options.

According to the Global TB Report 2025, an estimated ~390,000 people worldwide developed multidrug resistance or rifampicin-resistant tuberculosis (MDR/RR-TB) in 2024. Resistance was identified in approximately 3.2% of newly diagnosed TB cases and around 16% of previously treated patients, proportions that have remained largely stable in recent years. MDR/RR-TB continues to account for a substantial fraction of TB-related mortality, reflecting persistent gaps in timely diagnosis and access to effective treatment [10]. Despite the magnitude of this burden, access to appropriate treatment remains inadequate, with fewer than half of affected individuals receiving effective therapy. Delays in diagnosis and treatment initiation continue to contribute to high mortality rates, particularly in resource-limited settings where health systems are often under-resourced [1,11,12].

Compared with drug-susceptible tuberculosis, the management of multidrug- and extensively drug-resistant TB involves prolonged multidrug regimens that increase treatment costs by up to twenty-fold and are associated with greater toxicity, poorer tolerability, and reduced adherence, frequently leading to treatment interruption. These challenges are most pronounced in low- and middle-income countries, where the infrastructure required to support prolonged and complex multidrug regimens is often insufficient. As a result, the growing prevalence of resistant TB strains continues to erode the effectiveness of established treatment strategies and underscores the urgent need for alternative therapeutic approaches and more efficient drug discovery pipelines [5,13–16].

Transmission dynamics further exacerbate the global TB burden. Socioeconomic factors such as poverty, overcrowding, and limited access to healthcare facilitate ongoing transmission, while the nonspecific nature of early TB symptoms frequently leads to delayed diagnosis and prolonged infectiousness [11,12,17]. The long-standing reliance on a restricted repertoire of antimicrobial agents, combined with inconsistent treatment adherence, has played a central role in the emergence and persistence of drug-resistant TB over time [18–20].

In this context, the application of computational approaches to antitubercular drug discovery has gained increasing attention. This review provides a critical overview of *in silico* methodologies used in the identification and development of novel antitubercular agents. It encompasses structure-based and ligand-based drug design strategies, pharmacophore modeling, virtual screening workflows, molecular dynamics (MD) simulations, and ADMET prediction, as well as recent advances in artificial intelligence (AI) and machine learning (ML). Representative case studies are discussed alongside methodological limitations and practical factors that influence experimental validation and translational applicability. The objective is to provide a balanced and technically grounded assessment of how computational strategies can be effectively integrated into contemporary anti-TB drug discovery efforts.

2. Constraints in TB Drug Development and the Role of *In Silico* Drug Discovery

Pathogen-specific biological and pharmacological features of MTB limit the transferability of conventional antibacterial development strategies, while the global rise in MDR and extensively drug-resistant (XDR) tuberculosis has progressively reduced the effectiveness of first-line therapies and narrowed available treatment options [4,21–23]. Current WHO-recommended regimens reflect this reality, relying on prolonged, multi-drug combinations that must balance efficacy, toxicity, and pharmacokinetic compatibility rather than single-agent potency (Figure 1).

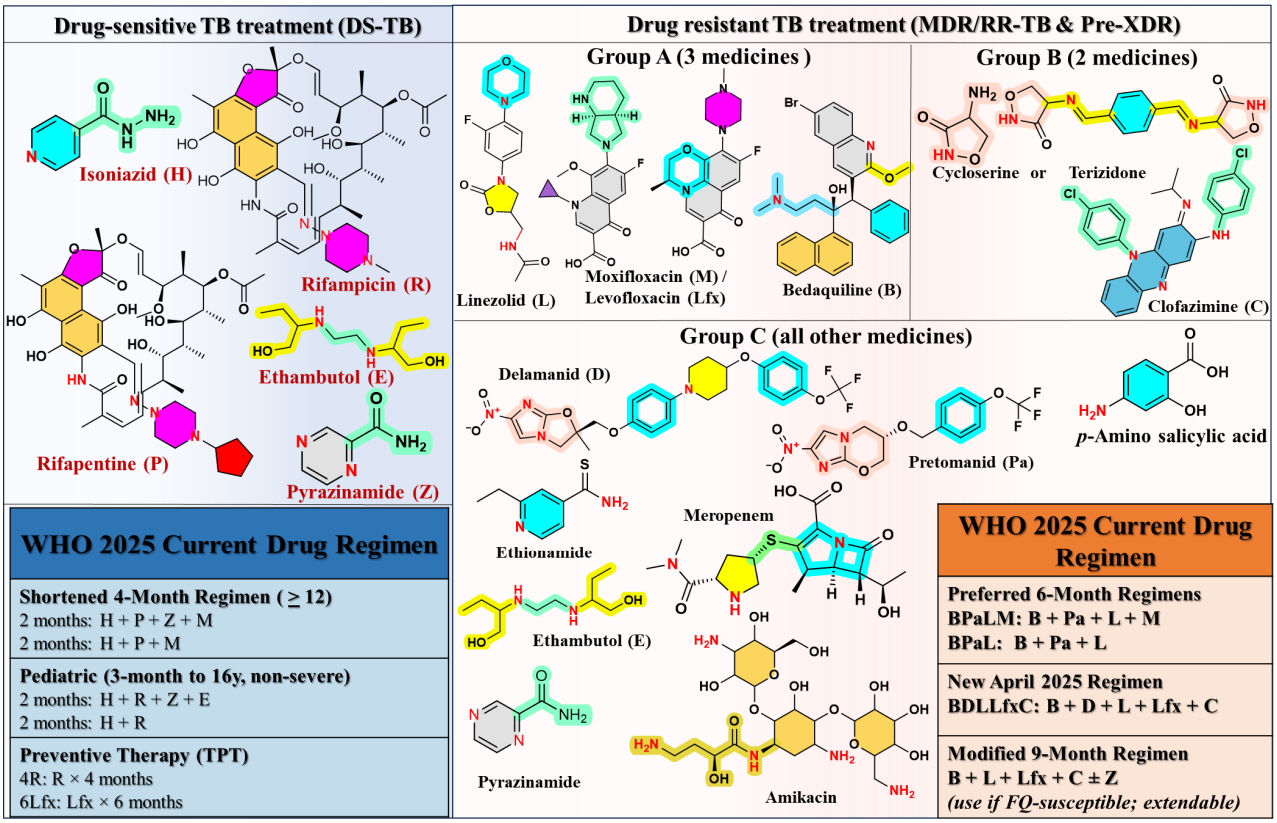


Figure 1. Overview of WHO 2025 drug regimens for tuberculosis (TB), categorized by drug-sensitive TB (DS-TB) and drug-resistant TB (MDR/RR-TB and Pre-XDR). The figure illustrates the classification of anti-TB drugs into Group A, B and C based on their use in treatment combination, with chemical structures (**right side**). First-line drugs for drug-sensitive TB (DS-TB) are shown with associated short-course, pediatric, and preventive regimens (**Left side**). Inspired from [24], MDPI, 2020, and WHO, 2025.

From a biological perspective, MTB presents multiple barriers that limit the predictive value of standard experimental screening. The lipid-rich, mycolic acid-containing cell envelope restricts drug penetration and excludes many compounds that appear active in simplified in vitro assays [15,18]. In addition, the intracellular localization of MTB within macrophages reduces antibiotic exposure, while the ability of the pathogen to enter non-replicating or dormant states confers tolerance to drugs targeting actively dividing cells [19,21,25–29]. These features contribute to the frequent discrepancy between in vitro activity and in vivo efficacy observed in antitubercular drug candidates.

Conventional antibacterial discovery pipelines were developed for pathogens with relatively simple growth dynamics and extracellular lifestyles, which limits their applicability to the biological and pharmacological complexity of MTB. Experimental screening strate-

gies are typically labor-intensive, time-consuming, and costly, often requiring 15–20 years to progress from target identification to clinical approval and exhibiting high attrition rates during late-stage development [30]. In tuberculosis, these challenges are compounded by the restricted chemical space of existing antitubercular agents and by the need to simultaneously optimize antibacterial potency, intracellular activity, safety, and resistance mitigation. A schematic comparison between traditional discovery pipelines and computational or repurposing-based strategies is provided in Figure 2.

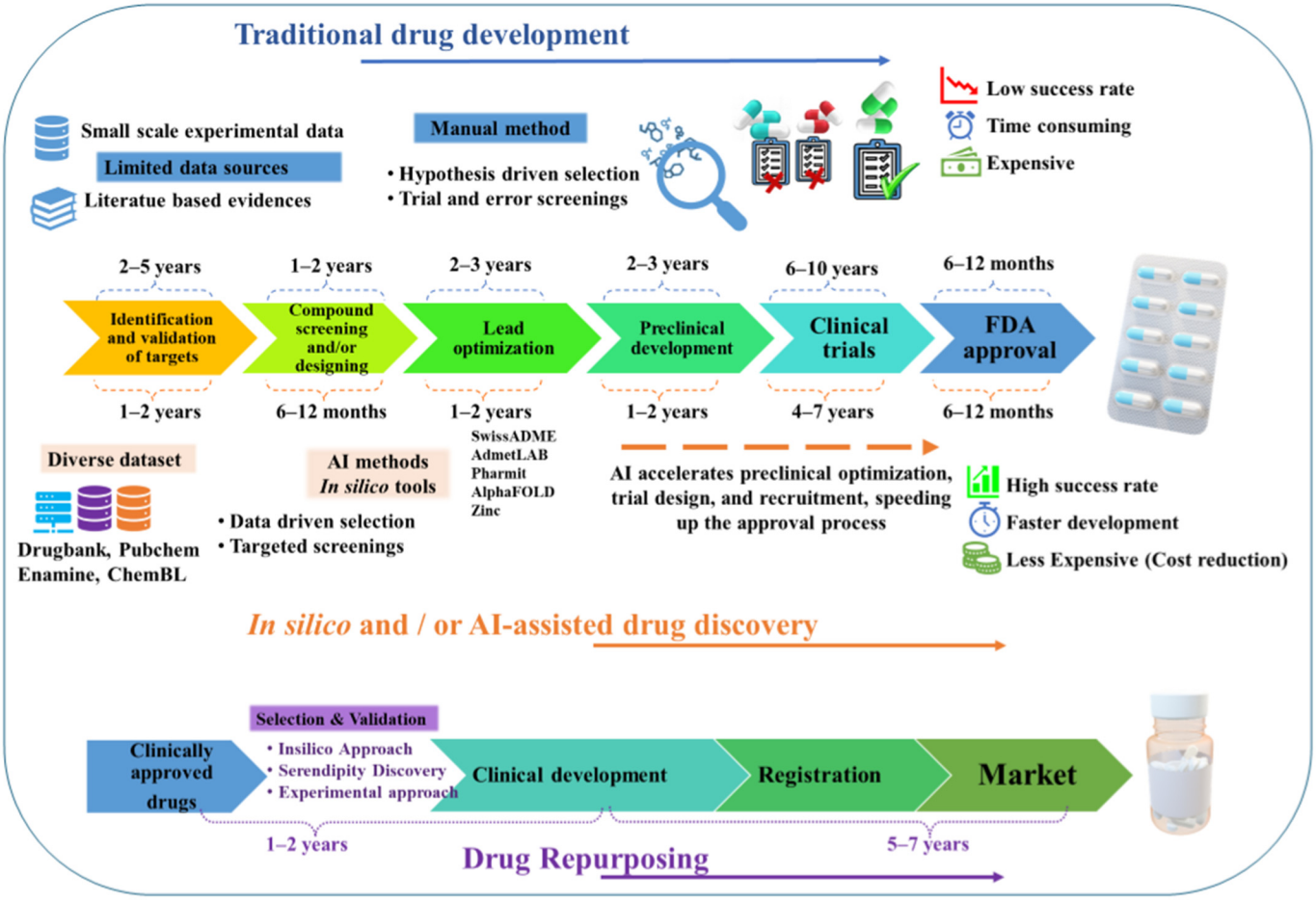


Figure 2. Comparison of traditional, *in silico*/AI-assisted, and drug repurposing discovery pipelines. Traditional drug discovery relies on sequential experimental screening, limited data, and longer timelines, often leading to high late-stage attrition. In contrast, *in silico*/AI-assisted methods utilize diverse datasets and computational tools for early-stage filtering, prioritization, and optimization, significantly reducing development time. Drug repurposing accelerates the process by leveraging clinically approved compounds, enabling the identification of new antitubercular uses within a shorter 5–7 year timeline. Based on the conceptual framework described in [31], MDPI 2025.

Computational drug discovery methods provide a framework for addressing several of these constraints at early stages of development. *In silico* approaches enable the integration of target essentiality, structural accessibility, and resistance-associated features that are specific to tuberculosis. Techniques such as virtual screening, molecular docking, and molecular dynamics simulations facilitate the prioritization of compounds with a higher probability of intracellular target engagement, while ADMET prediction tools support the early exclusion of candidates with unfavorable pharmacokinetic or toxicity profiles prior to experimental validation. ML-based models further expand these capabilities by supporting the prediction of resistance-associated mutations, the optimization of chemical scaffolds to

circumvent known resistance mechanisms, and the identification of potentially synergistic drug combinations relevant to TB therapy [32,33].

The applicability of these approaches is supported by multiple TB-focused case studies. Structure-based modeling has contributed to the identification of inhibitors targeting InhA and EthR, followed by experimental validation [34]. Virtual screening efforts directed at the ATP synthase complex have yielded novel chemical scaffolds that are currently undergoing preclinical evaluation [35]. In addition, *in silico*-guided drug repurposing strategies have identified antitubercular activity in approved compounds, substantially shortening development timelines compared with de novo discovery [35]. Together, these examples illustrate how computational strategies can be integrated into TB drug discovery to address limitations inherent to traditional experimental pipelines.

In response to these substantial limitations, *in silico* drug discovery has emerged as a powerful solution, offering the potential to accelerate the early stages of drug development. *In silico* drug discovery enables the coordinated evaluation of biological target selection, chemical space exploration, and early safety-related parameters before experimental testing. In TB research, this integration is particularly relevant in light of the high attrition rates observed in conventional pipelines and the pathogen-specific constraints of MTB, including intracellular persistence, restricted drug permeability, and resistance-associated mutations. Computer-aided drug design (CADD) enables the systematic prioritization of targets and compounds, allowing critical decisions to be made before resource-intensive preclinical studies are initiated (Figure 3) [36–39].

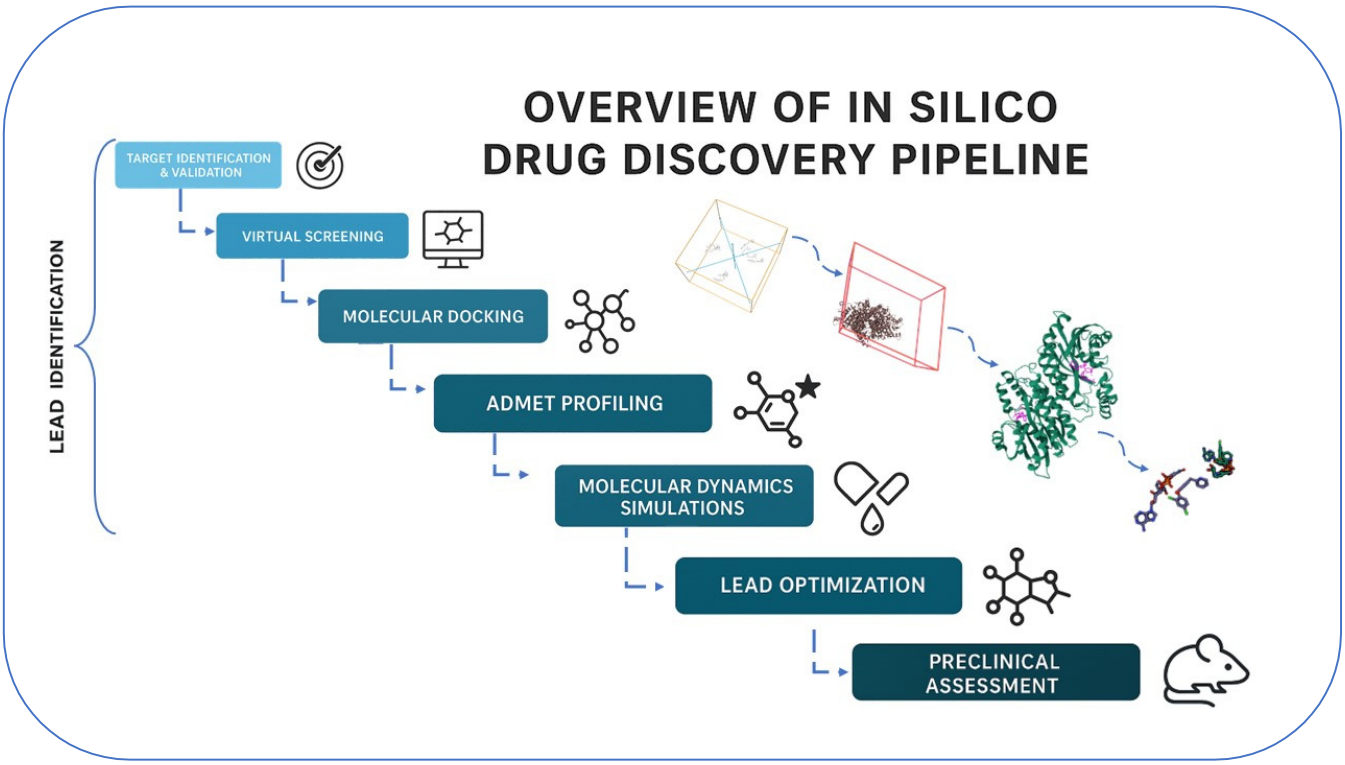


Figure 3. Overview of *in silico* drug discovery pipeline: highlights key computational steps include target identification, virtual screening, docking, ADMET profiling, molecular dynamics, hit optimization, and preclinical evaluation. Based on the conceptual framework described in [40]. MDPI, 2023.

The *in silico* pipeline begins with target identification and validation, a critical step for defining biological and chemical constraints prior to compound screening. Omics-based approaches, including genomics, transcriptomics, and proteomics, are routinely applied to

analyze MTB biology and to identify proteins essential for survival or pathogenicity [41,42]. Curated resources such as Tuberculist and KEGG are routinely used to inform target selection in TB drug discovery by integrating information on gene essentiality, functional annotation, and documented resistance mechanisms. Within this framework, enzymes involved in mycobacterial cell wall biosynthesis, including DprE1, InhA, and KasA, are frequently selected because of their indispensable biological roles and their demonstrated vulnerability to small-molecule inhibition. Once targets are defined, hit identification is typically carried out through the screening of large chemical libraries, such as ZINC, ChEMBL, and Enamine, using virtual screening and molecular docking protocols to identify compounds with plausible target affinity [43–46]. Increasingly, these workflows are complemented by ML–based models that assist in filtering false positives and in identifying early signals of toxicity or unfavorable physicochemical behavior [47].

Lead optimization proceeds through an iterative refinement process in which potency, selectivity, and safety-related parameters are systematically adjusted prior to experimental validation. At this stage, interaction fingerprints derived from docking studies and ligand efficiency metrics are often used to prioritize hits with favorable binding characteristics before chemical modification. Computational pipelines commonly combine quantitative structure–activity relationship (QSAR) analysis, pharmacophore modeling, molecular docking, and MD simulations implemented in platforms such as GROMACS [48], AMBER [49], Desmond [50], or Discovery Studio [51,52]. Pharmacophore models delineate the spatial and physicochemical features required for biological activity, whereas docking calculations provide predicted binding modes and interaction patterns within the target site [53,54].

MD simulations further extend these analyses by accounting for conformational flexibility, binding stability, solvent effects, and transient interactions, features that are particularly relevant for MTB targets embedded in lipid-rich or dynamically reorganizing environments. Free-energy estimation methods, including MM/GBSA and metadynamics, are frequently applied to rank candidate compounds and to guide rational structural refinement during optimization [37,47,55–59]. In parallel, ADMET prediction is incorporated early in the optimization phase to identify liabilities that may compromise intracellular exposure, metabolic stability, or long-term tolerability.

ADMET prediction is integrated early during lead optimization to identify liabilities that may compromise intracellular exposure, metabolic stability, or long-term tolerability. Computational tools such as SwissADME [60], pkCSM [61], and ADMETlab 3.0 [62] are routinely employed to assess drug-likeness, solubility, blood–brain barrier permeability, and cytochrome P450 interactions. Predictive toxicology tools such as ProTox-II [63], ToxPred, and PreADMET [64] are commonly applied to flag mutagenic, hepatotoxic, or cardiotoxic liabilities during early stages of compound selection, thereby reducing the probability of late-stage attrition [65]. Candidates that meet these computational criteria are subsequently advanced to experimental assessment, including in vitro and in vivo evaluation [66]. In TB drug discovery, compounds such as BTZ-043 and Q203 (telacebec) illustrate how *in silico* optimization contributed to refining interactions with DprE1 and the respiratory cytochrome bc1 complex, respectively, supporting improvements in efficacy and metabolic stability prior to clinical progression [24,67–69].

During the transition to preclinical development, computational methods remain integrated into decision-making through pharmacokinetic modeling and predictive toxicology. *In silico* pharmacokinetic simulations are used to estimate tissue distribution and exposure profiles, while complementary toxicology models identify metabolic or safety liabilities that may not be evident in early cell-based assays. Tools such as GLORY, developed by de Bruyn Kops et al. for predicting cytochrome P450-mediated metabolism, together with transporter inhibition models described by Montanari and colleagues, are applied to assess metabolic

stability and potential drug–drug interaction risks [66,70–74]. These approaches support the selection of candidates with more favorable pharmacological and safety profiles prior to in vivo testing.

Computational predictions are further refined through iterative incorporation of experimental data. Outputs from docking and QSAR analyses are re–calibrated using molecular dynamics simulations and available structural information, while ligand-based models are updated as high-throughput screening data become available [75]. Within this workflow, structure-based drug design (SBDD) constitutes the stage at which molecular hypotheses are formulated and evaluated against defined biological targets. In tuberculosis research, the growing availability of high-resolution structural data for enzymes involved in cell wall biosynthesis, energy metabolism, and intracellular survival has enabled the systematic application of SBDD to rational compound design. The following section outlines the principles, tools, and tuberculosis-specific considerations relevant to structure-based antitubercular drug discovery.

3. Structure-Based Drug Design

SBDD relies on the explicit use of three-dimensional structural information to analyze drug–target interactions at atomic resolution. By focusing on defined binding sites rather than empirical screening alone, SBDD allows ligand binding modes to be examined, interaction networks to be rationalized, and molecular modifications to be guided on the basis of structural constraints [76]. In tuberculosis research, this approach has become increasingly applicable due to both the accumulation of drug-resistant MTB strains and the expanding availability of structural data for enzymes involved in cell wall biosynthesis, energy metabolism, and intracellular survival [74]. High-resolution structures of MTB targets have supported the design of inhibitors with improved selectivity and mechanistic specificity, providing a complementary route to traditional phenotypic screening strategies [74,77–79].

At its core, SBDD is based on molecular recognition, whereby ligand binding is rationalized through shape complementarity, hydrogen bonding, electrostatic interactions, hydrophobic effects, desolvation, and entropic contributions [76]. Structural inputs are primarily derived from X-ray crystallography and nuclear magnetic resonance (NMR), with cryo-electron microscopy increasingly contributing high-resolution structures of membrane proteins and large macromolecular assemblies relevant to TB drug discovery [80–83]. Recent advances in AI-based protein structure prediction have substantially expanded structural coverage of the MTB proteome. However, predicted models often require refinement, particularly with respect to side-chain positioning, protonation states, and induced-fit effects, prior to docking and interaction analysis [84–86].

The increasing availability of high-resolution three-dimensional structures for therapeutically relevant MTB proteins has substantially expanded the applicability of SBDD. Rather than operating in isolation, SBDD is now routinely integrated with ligand-based drug design approaches to improve hit identification and lead optimization, particularly when structural or activity data are incomplete [78,87,88]. The combined use of SBDD and ligand-based drug design (LBDD) enables systematic interrogation of catalytic pockets, rational ligand design, and iterative optimization of potency and selectivity prior to experimental validation. In TB research, this integrative strategy has been further supported by the growing availability of multi-omics datasets and structural biodata, which facilitate target prioritization, cheminformatics analyses, and homology-based modeling [24,74,87–89].

Structure-based virtual screening (SBVS) represents a central application of SBDD, allowing large chemical libraries comprising millions of drug-like compounds to be docked against defined target sites. These approaches enable rapid prioritization of candidate ligands for tuberculosis-relevant targets and support structure–activity analyses that in-

form both mechanism of action and early ADMET considerations [90–92]. Information derived from both successful and unsuccessful screening campaigns contributes to iterative refinement of screening strategies and scoring functions. Publicly accessible platforms, such as the Click2Drug resource hosted by the Swiss Institute of Bioinformatics, aggregate a wide range of commercial and open-access CADD tools that support structure-based and ligand-based workflows across different stages of drug discovery [93].

When experimentally determined structures are unavailable, SBDD workflows rely on computational structure prediction to generate target models suitable for docking and interaction analysis. Homology modeling, also referred to as comparative modeling, remains the most widely applied approach, using structurally resolved homologues as templates to predict three-dimensional protein structures with acceptable accuracy [94,95]. In cases of low sequence similarity, threading (fold recognition) or ab initio modeling strategies may be employed as alternatives [96]. Regardless of the modeling strategy, structural validation is essential prior to downstream applications. Model quality is commonly assessed using stereochemical and energetic criteria, including Ramachandran plot analysis of backbone dihedral angles, together with complementary validation metrics [77,97,98].

Following structure preparation, identification of ligand-binding sites constitutes a critical step in SBDD. Binding-site prediction is complicated by protein flexibility and conformational dynamics, particularly for enzymes and membrane-associated targets relevant to tuberculosis. Computational approaches therefore combine interaction energy calculations with probe-based mapping strategies to identify regions that favorably interact with small molecules. Tools such as Q-SiteFinder [99], FTSite [100], LISE [101], and SITEHOUND [102] employ van der Waals or force-field-based energy evaluations to cluster favorable probe positions and rank potential binding pockets, enabling the prioritization of sites with the highest likelihood of supporting productive ligand binding [103].

3.1. Structure-Based Virtual Screening for Lead Identification

SBVS prioritizes candidate molecules by explicitly modeling their fit within a defined binding site of an MTB target [89]. The method starts from a curated 3D structure (crystal, cryo-EM, or a carefully validated model) and converts the biological question into a tractable physical problem: which chemical scaffolds can satisfy the site's steric constraints and interaction pattern under realistic protonation, solvation, and cofactor states. In TB drug discovery, SBVS is used both for hit finding against essential enzymes and for target-focused repurposing, where the chemical space is smaller but the translational constraints (exposure, safety, formulation) are clearer [104,105].

A practical SBVS workflow is defined by three technical choices that dominate outcome quality. First, target preparation must reflect biology rather than the PDB file alone: protonation/tautomer states, metal coordination, conserved waters, and alternate side-chain conformers can shift rank ordering more than changing docking software. Second, library design should match the goal: very large vendor sets (for scaffold novelty) versus focused sets (for chemotype exploration or repurposing), with early physicochemical filtering to avoid "hydrophobic collapse" artifacts. Third, pose generation and rescoring should be treated as hypothesis generation, not as a surrogate for affinity. This is where multi-stage docking, consensus scoring, and post-docking refinement (e.g., MM/GBSA and short MD stability checks) become useful—not as decoration, but as a way to remove unstable or chemically implausible poses before synthesis or purchase [104,106].

Real TB case studies show what SBVS can do when it is anchored to experimental follow-through. One example used an energy-based pharmacophore derived from an MTB enzyme complex and then screened an in-house set, followed by docking-based prioritization and stepwise analog synthesis, yielding measurable enzyme inhibition and

further optimization around two scaffolds [107]. This illustrates a common TB pattern: SBVS provides the initial chemotypes, while activity gains come from iterative chemical series expansion guided by site interactions rather than single-score ranking.

DprE1-focused SBVS provides another instructive template because the target is essential and structurally well characterized [108,109]. In a repurposing-oriented workflow, a structure-based screen of approved-drug space was followed by higher-precision docking and MM/GBSA estimation plus ADMET triage to prioritize candidates with both binding plausibility and a more realistic development path. The paper is valuable less for claiming a "winner" from docking and more for demonstrating a defensible down-selection logic that reduces false positives before wet-lab testing. In parallel, other DprE1 programs used SBVS to define a focused benzothiazole series built explicitly from a screened binding hypothesis, then validated activity experimentally—an example of SBVS functioning as a series generator rather than a one-off ranking exercise.

SBVS has also been used for membrane-associated TB targets such as MmpL3, where the binding site environment and ligand lipophilicity can easily bias docking. A recent hybrid virtual screening strategy combined structure-based prioritization with subsequent validation steps to arrive at experimentally supported leads, illustrating why TB SBVS needs stricter compound-property control and post-docking refinement than many soluble enzymes [110]. Similarly, SBVS campaigns for InhA continue to be reported, typically using docking-driven selection followed by biochemical and/or whole-cell follow-up; these studies reinforce a practical point: docking scores alone rarely correlate with potency unless the workflow explicitly controls for assay context and target state [107].

Across these examples, the technical failure modes are consistent and should be stated explicitly because they shape what SBVS results mean. False positives cluster into (i) scoring-function bias toward large hydrophobic ligands, (ii) mis-specified target microstates (wrong protonation/tautomer/metal handling), and (iii) overconfidence in a single pose without stability checks [89]. In TB, an additional layer is cell entry and efflux, which can decouple enzyme affinity from whole-cell activity; therefore, SBVS outputs should be framed as binding hypotheses that require early property triage and rapid experimental falsification [108]. With this SBVS foundation, the next step is to detail the SBDD modules that improve reliability—receptor modeling and validation, binding-site detection, docking protocols (including ensemble and induced-fit strategies), and rescoring/MD refinement—because these components determine whether a virtual "hit" becomes a chemically actionable lead in TB.

3.2. Reframing Molecular Docking in TB Drug Discovery

Molecular docking remains the central analytical engine of structure-based drug discovery for MTB, yet its application to TB targets presents challenges that are substantially more complex than those encountered in typical Gram-negative or eukaryotic systems [109]. This increased complexity stems from the physicochemical idiosyncrasies of mycobacterial proteins, the lipid-rich cellular environment, and the frequent involvement of cofactors, redox chemistry, and slow-binding mechanisms that are poorly represented by standard docking formalisms. At its core, docking attempts to approximate the binding free energy of a ligand–protein complex through a combination of sampling algorithms and scoring functions [111]. Sampling explores translational, rotational, and conformational degrees of freedom of the ligand within a predefined binding region, while scoring functions estimate interaction energies using force-field, empirical, or knowledge-based terms [112]. However, in TB drug discovery, this approximation is frequently strained by hydrophobic cavities, buried active sites, and conformationally adaptive enzymes, which collectively undermine the reliability of static docking predictions [113,114].

In practice, these limitations become evident when docking is applied to key MTB drug targets without contextual refinement [114,115]. InhA, an NADH-dependent enoyl-ACP reductase, undergoes cofactor-induced conformational rearrangements that modulate the geometry and electrostatics of the binding site [113,115]. Docking campaigns that treat the enzyme as a rigid structure routinely fail to discriminate between weak binders and nanomolar inhibitors, as docking scores do not capture cofactor dynamics, entropic penalties, or slow-binding kinetics that dominate experimental inhibition constants [113–115]. A related but mechanistically distinct challenge is illustrated by DprE1, which represents a particularly demanding docking target due to its flavin-dependent redox mechanism and the prevalence of covalent or semi-covalent inhibitors [116,117]. Standard scoring functions are intrinsically incapable of modeling covalent bond formation, electron transfer, or redox cycling, leading to systematic misranking of ligands unless covalent docking or post hoc mechanistic filters are applied [118]. As a consequence, many reported DprE1 docking studies generate inflated hit rates that collapse during biochemical validation [109,119].

An additional layer of complexity arises for membrane-associated targets, such as MmpL3 and QcrB, as these proteins operate at lipid–protein interfaces where ligand recognition is governed not only by amino acid residues but also by membrane insertion depth, lipid composition, and structured interfacial water networks [120,121]. Docking studies that ignore membrane context often predict binding poses that are sterically or energetically incompatible with the native environment, a limitation that has been repeatedly demonstrated for MmpL3 inhibitors when docking is performed in aqueous-only models [110,122].

The performance of docking in TB drug discovery is further constrained by scoring functions that were parameterized primarily on soluble, drug-like protein–ligand complexes [123]. Hydrophobic over-stabilization, poor treatment of desolvation penalties, and inaccurate modeling of conserved water molecules are recurrent issues [123,124]. In MTB targets with deeply buried hydrophobic pockets, such as KasA, scoring functions frequently overestimate binding affinity by favoring nonspecific hydrophobic contacts while underweighting geometric strain and entropic costs [125–127]. Accordingly, comparative benchmarking studies have shown that absolute docking scores rarely correlate with MIC or IC_{50} values for TB targets [127–129]. Instead, docking performs best as a filtering tool within chemically coherent series, where relative ranking can guide structure–activity exploration rather than predict absolute potency. This distinction is critical but often ignored in the TB literature, leading to overinterpretation of docking scores as quantitative predictors of biological activity [123,130].

To overcome these intrinsic limitations of static docking, MD simulations have become an essential second-stage filter in TB-focused docking workflows [108,119]. MD simulations allow explicit treatment of protein flexibility, solvent reorganization, and time-dependent stability of ligand binding modes. As a result, many docking poses that appear favorable under static conditions rapidly destabilize during MD equilibration, revealing false positives that would otherwise advance to synthesis [110,131,132].

Consistent with this role, MD refinement has proven particularly informative for targets characterized by conformational plasticity, lipid exposure, or cofactor-dependent catalysis, such as EthR, MmpL3, InhA, and DprE1 [34,133–135]. In these systems, ligand recognition is governed not only by static complementarity but also by time-dependent stability of interaction networks, which explains why compounds maintaining persistent contacts over extended simulations—typically 100–200 ns—are more likely to display measurable biochemical activity than candidates selected solely on docking score [110,119].

This conceptual shift becomes especially apparent in membrane-associated TB targets, where binding occurs at lipid–protein interfaces rather than within well-defined aqueous pockets [136,137]. In mycolic acid-rich membrane models that recapitulate key features of

the MTB outer envelope, peptide analogs exhibiting comparable docking affinities against membrane-associated proteins can display markedly divergent biological profiles once their dynamic behavior is resolved. Molecular dynamics simulations reveal that descriptors such as conformational persistence, residue-level fluctuation, and center-of-mass stability relative to the lipid interface, rather than docking score or insertion depth alone, govern functional engagement [138]. Peptides maintaining low RMSD and restricted RMSF preserve compact amphipathic conformations and sustain productive interfacial binding, whereas highly dynamic analogs undergo deep but unstable insertion events that fail to translate into antimicrobial efficacy. These observations demonstrate that MD provides mechanistic discrimination inaccessible to docking, particularly for membrane-active ligands operating within the complex lipid–protein environments characteristic of MTB [138–140].

Building on this dynamic framework, end-point free energy methods such as MM/GBSA and MM/PBSA are commonly applied to MD trajectories to refine ligand ranking within chemically coherent series [141,142]. While these approaches do not deliver absolute binding free energies, they consistently outperform raw docking scores in reproducing experimental trends when force-field selection, protonation states, and cofactor treatment are carefully parameterized for TB-specific systems [142–144].

Despite the advances provided by molecular dynamics simulations, these approaches remain computational approximations of conformational behavior and cannot directly resolve experimentally populated protein states. To bridge this gap, time-resolved synchrotron-based structural techniques are being employed, providing experimental validation of ligand-induced conformational changes predicted *in silico* [145,146]. High-brilliance X-ray sources available at facilities such as PETRA III and the next-generation PETRA IV enable the interrogation of protein–ligand complexes with spatial and temporal resolution that is inaccessible to static crystallography [147,148].

In the context of TB drug discovery, these techniques are particularly relevant for targets characterized by slow-binding kinetics, redox-dependent catalysis, or conformational gating [113,149,150]. Time-resolved crystallography and serial data collection approaches allow direct observation of ligand binding pathways, intermediate conformational states, and active-site rearrangements, offering an experimental framework to validate or refute docking- and MD-derived binding hypotheses [151,152].

For mycobacterial targets such as InhA, DprE1, and membrane-associated systems including MmpL3 and QcrB, access to experimentally resolved dynamic states is critical, as ligand recognition is frequently coupled to cofactor positioning, redox transitions, or membrane engagement [113,120,121,149,150]. Synchrotron-based approaches provide direct structural evidence for binding modes that may only be transiently populated during catalysis or transport, clarifying discrepancies between docking predictions and biochemical activity. In this framework, experimental dynamics do not replace computational workflows but function as an orthogonal validation layer, anchoring *in silico* predictions to physically observed conformational landscapes and strengthening structure–activity interpretations in TB drug discovery [145,147,152].

Resistance-associated mutations in MTB frequently remodel binding-site geometry while preserving enzymatic function, decoupling structural integrity from catalytic competence. Under these conditions, docking protocols restricted to wild-type structures systematically underestimate clinically relevant binding modes. Ensemble docking frameworks that integrate MD-derived conformational states or resistance-associated variants enable ligands to be evaluated across mutation-accessible binding landscapes rather than against a single static snapshot. In TB drug discovery, where resistance evolution and structural plasticity are intrinsic properties of therapeutic targets, this approach improves

prioritization of chemotypes capable of retaining binding robustness across both conformational and mutational space.

3.3. De Novo and Fragment-Based Drug Design

De novo drug design focuses on the generation of novel molecular scaffolds by assembling small chemical building blocks directly within the structural constraints of a defined target site. Rather than relying on pre-existing compounds, this approach constructs candidate molecules with predicted affinity for the target active site, which are subsequently subjected to experimental validation [153]. Because the chemical search space explored during de novo design is inherently large, algorithmic strategies incorporate prior knowledge of the target environment to guide exploration and reduce combinatorial complexity [154].

In practice, de novo design frameworks often combine positive and negative design principles. Positive design restricts exploration to regions of chemical space that are compatible with target binding requirements, thereby increasing the probability of identifying molecules with favorable interaction profiles. In contrast, negative design applies exclusion criteria to avoid chemical features associated with poor binding, instability, or false-positive predictions. Although computational scoring functions are effective for ranking and filtering candidate structures, they remain complementary to experimental organic synthesis and cannot substitute for chemical feasibility or synthetic validation [155,156].

Evaluation of candidate molecules is therefore integrated throughout the design process. Scoring functions represent a central component of this evaluation and are commonly implemented in multi-objective optimization frameworks that simultaneously consider binding affinity, physicochemical properties, and synthetic constraints [153,157]. These scoring systems generate multiple descriptors in parallel, supporting prioritization rather than definitive selection of lead candidates.

De novo design strategies can be broadly divided into ligand-based and receptor- or enzyme-based approaches. In structure-guided applications, receptor-based design is more frequently employed, as it explicitly exploits structural information from the target protein. Successful implementation requires high-quality protein structures and accurate characterization of the active site geometry and key interaction features [158]. Candidate molecules are generated by positioning and orienting fragment moieties within the binding pocket to satisfy spatial and physicochemical constraints, using computational algorithms or, in some cases, structural data derived from ligand–receptor co-crystallization.

Fragment-based de novo design typically proceeds through either fragment linking or fragment growing strategies. Fragment linking identifies discrete interaction hotspots within the active site and connects complementary fragment moieties to form a complete molecular scaffold. In contrast, fragment growing initiates from a core fragment anchored in the binding pocket and incrementally expands the molecule by adding functional groups under the guidance of search algorithms optimized for structural compatibility and interaction strength [153,154,159,160].

4. Ligand-Based Drug Design

The effective application of SBDD depends on the availability of reliable, high-resolution three-dimensional structures of biological targets. When such structural information is incomplete or unavailable, LBDD provides a complementary strategy for lead identification and optimization [161]. LBDD is particularly relevant in situations where biological activity cannot be readily rationalized from target structures alone, including cases influenced by membrane permeability, prodrug activation, intracellular accumulation, or toxicity-related effects [162]. In addition to known active ligands, ligand-based approaches

may also exploit natural products or substrate analogs that interact with the target and elicit the desired pharmacological response. Among ligand-based methodologies, QSAR analysis and pharmacophore modeling remain the most widely applied techniques [163–165].

Classical QSAR approaches establish statistical relationships between molecular descriptors and biological activity across a series of compounds, enabling the optimization of potency by correlating hydrophobic, steric, and electronic features with experimental response data [163,166]. In traditional QSAR frameworks, substituent contributions are quantified individually, and the activity of a modified compound is approximated as a linear combination of these effects relative to a parent scaffold [164]. More recent QSAR models extend this paradigm by incorporating three-dimensional descriptors, including geometric parameters such as hydrogen bond donor and acceptor orientations, as well as physicochemical and quantum-chemical properties [164]. Because these descriptors are inherently conformation-dependent, careful selection of ligand geometries is essential, particularly for flexible molecules whose bioactive conformations may differ from those used during model construction. To address this limitation, advanced methods such as CSP-SAR explicitly account for multiple ligand conformations within the modeling framework, thereby improving robustness and predictive performance [161].

Pharmacophore modeling represents a complementary ligand-based strategy that captures the spatial arrangement of molecular features required for biological activity. These features typically include hydrogen bond donors and acceptors, hydrophobic regions, aromatic moieties, and charged functional groups, abstracted into a three-dimensional interaction pattern shared among active compounds [167,168]. In TB drug discovery, pharmacophore modeling is particularly valuable when structurally diverse ligands exhibit similar activity profiles or when target structural data are limited. Pharmacophore models are widely applied in virtual screening workflows to identify novel scaffolds from large chemical libraries and to enable scaffold hopping while preserving essential interaction features. In practice, pharmacophore-based screening is frequently combined with QSAR analysis, molecular docking, and experimental validation to enhance hit prioritization and lead optimization [169,170].

Ligand-based methodologies have been implemented across a broad range of molecular modeling and cheminformatics platforms, including commercial software such as Discovery Studio, Phase [171], and MOE [172], as well as open-source toolkits including RDKit [173], scikit-learn [174], XGBoost [175], LightGBM [176], and DeepChem [177]. Workflow-oriented platforms such as KNIME [178] further enable end-to-end QSAR and pharmacophore modeling pipelines. Beyond target interaction profiling, ligand-based approaches are also applied to assess drug permeability and intracellular exposure, considerations that are particularly critical given the complex and lipid-rich cell envelope of MTB [73,179,180].

The increasing availability of large and chemically diverse virtual libraries has substantially strengthened ligand-based and hybrid computational pipelines in tuberculosis research. These libraries support efficient hit identification, scaffold hopping, and lead optimization by providing access to extensive collections of synthetically accessible, drug-like molecules that can be prioritized through computational screening prior to experimental validation [181–183]. Commercial resources such as Enamine REAL Space [184], WuXi AppTec's GalaXi, and ZINC22 [185] offer access to vast chemical spaces suitable for pharmacophore-based screening, similarity searches, and docking workflows, while maintaining feasible synthetic pathways [185,186]. These platforms are complemented by open-access databases including GDBMedChem, PubChem [187], CHIPMUNK, SCUBIDOO, and ChEMBL [188], which provide curated chemical collections enriched with medicinal chemistry-relevant scaffolds, bioactivity annotations, and filters related to synthesizabil-

ity and developability [187,189–192]. Collectively, these resources facilitate systematic exploration of diverse chemical space and support the identification of inhibitors targeting both classical enzymatic targets and more complex systems, including protein–protein interactions in MTB.

An illustrative example of ligand-based virtual screening (LBVS) in TB drug discovery is provided by the work of Pakamwong and colleagues, who investigated novel inhibitors of the ATPase domain of MTB DNA gyrase [193]. Using a ligand-based screening strategy applied to the Specs compound library, the authors identified indole-derived scaffolds, including the seed molecule G24. Following filtering based on Lipinski's rule of five and PAINS criteria, fourteen compounds comprising carbazole, benzoindole, and indole derivatives were selected for further evaluation. These candidates were subsequently tested for inhibition of MTB growth and DNA gyrase ATPase activity. Among them, compounds **8**, **11**, and **14** exhibited IC_{50} values of 0.26, 0.56, and 0.08 µM, respectively, outperforming the reference inhibitor novobiocin (IC_{50} = 1.27 µM), and demonstrated corresponding activity in MIC assays. Notably, carbazole derivative 8 emerged as a previously unreported scaffold for DNA gyrase ATPase inhibition, displaying the strongest activity in both enzymatic and whole-cell assays. Molecular dynamics simulations identified hydrogen bond interactions with Asp79 as critical for GyrB ATPase binding, with compounds **8**, **11**, and **14** showing the most stable interaction profiles (Figure 4). Subsequent structure–activity relationship analysis indicated that hydroxyalkyl amine substituents enhance the activity of indole and benzoindole derivatives [193].

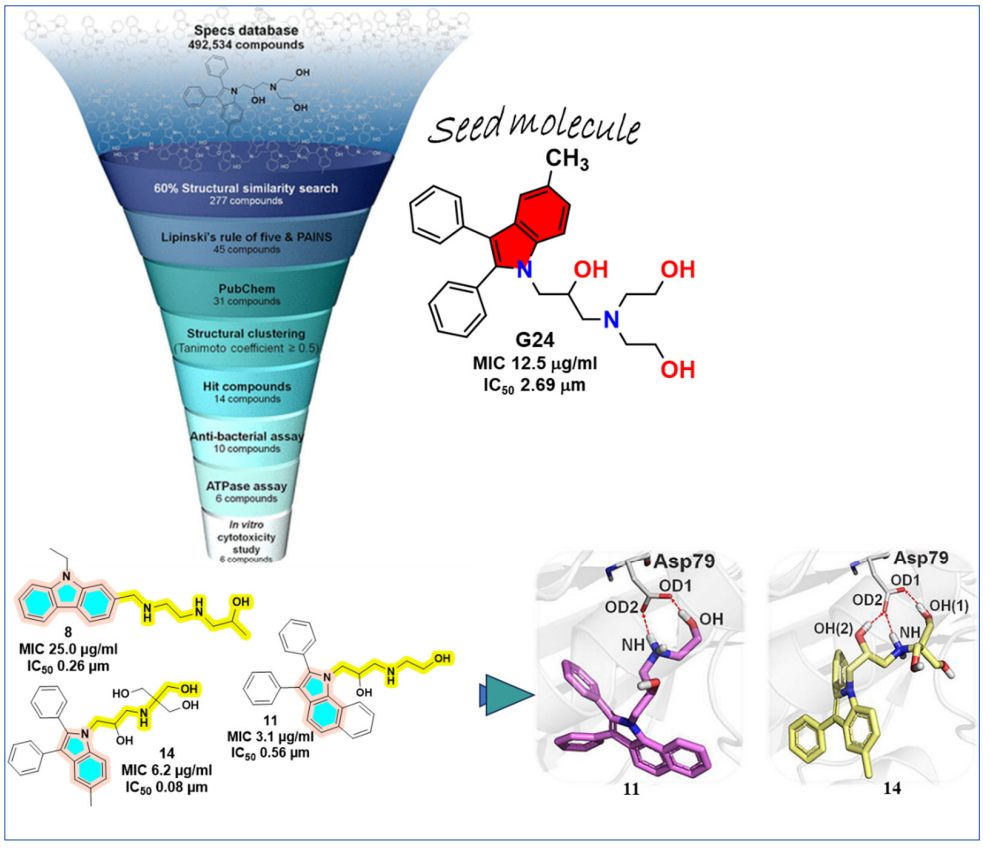


Figure 4. Ligand-based discovery and optimization of DNA gyrase ATPase inhibitors in *Mycobacterium tuberculosis* (MTB) are presented. The workflow begins with a seed molecule (G24) and results in prioritized hits (**8**, **11**, **14**). Chemical structures, antimycobacterial activity, and ATPase inhibition are represented. Docking and molecular dynamics analyses demonstrate conserved binding within the GyrB ATPase pocket, with stabilizing interactions observed at Asp79. Reprinted and adapted from [193], American Chemical Society, 2024.

5. ADMET and Drug-Likeness Prediction

In the current landscape of drug discovery, evaluating a compound's ADMET properties has become a cornerstone of early-phase development. These properties are essential for determining whether a compound will be bioavailable, safe, and pharmacologically effective in vivo [194,195]. Computational approaches to ADMET and drug-likeness profiling have become increasingly integrated into the *in silico* drug discovery pipeline, enhancing efficiency by enabling high-throughput, cost-effective assessments prior to experimental validation. This is particularly important in diseases like TB, where drug resistance and treatment complexity necessitate multi-target, multi-drug strategies. ADMET modeling is essential for prioritizing and optimizing potential therapeutics during the early stages of drug discovery [60,66].

Several web-based platforms and ML models now dominate the ADMET prediction landscape. SwissADME [60] and pkCSM [61], both among the most commonly used tools, provide a comprehensive set of physicochemical descriptors, such as lipophilicity (LogP), topological polar surface area (TPSA), and hydrogen bond donors and acceptors. They also predict gastrointestinal absorption and blood–brain barrier permeability and apply medicinal chemistry filters, including Lipinski's Rule of Five, to assess drug-likeness and oral bioavailability [60,61]. In the context of TB drug discovery, where intracellular penetration is vital for targeting MTB within macrophages, such early predictions streamline the selection of lead compounds [24,68,70–72].

Another powerful resource is ADMETlab 3.0, which integrates a diverse range of *in silico* models, including those based on deep learning and ensemble learning, to evaluate over 200 ADMET-related endpoints [196,197]. It offers both qualitative (binary classification) and quantitative (regression) predictions and includes advanced features such as property space visualization and toxicity profiling. ADMET lab's ability to identify compounds with hidden liabilities, such as hepatotoxicity or poor metabolic stability, makes it particularly useful for filtering out unsuitable candidates early in the TB drug discovery process.

For early-stage screening, Molinspiration also supports early filtering by calculating molecular descriptors that correlate with oral bioavailability and bioactivity [196,197]. In addition to traditional properties like LogP and molecular weight, it estimates the likelihood that a compound acts on specific protein families GPCRs, kinases, and proteases, which are increasingly relevant in TB for host-directed therapy strategies. Its simplicity and batch-processing capabilities make it a common choice during large-scale virtual screening campaigns [62,198,199].

From a toxicity standpoint, several specialized platforms have emerged to provide detailed predictions. ProTox-II [63] uses a hybrid model combining ML and similarity analysis to classify compounds into six toxicity classes, predict LD_{50} values, and flag risks such as hepatotoxicity, carcinogenicity, immunotoxicity, and cytotoxicity [63]. It supports compound prioritization based on systemic toxicity and organ-specific effects, a vital aspect of TB drug development where hepatotoxicity remains a primary concern. Similarly, eToxPred focuses on peptide toxicity classification, an increasingly relevant area as interest grows in peptide-based TB therapies [200]; PreADMET offers predictions for Ames mutagenicity, skin sensitization, plasma protein binding, and blood–brain barrier permeability, making it valuable for evaluating metabolic liabilities in both synthetic and repurposed drugs for TB treatment [201,202]. Innovative tools like GLORY, described by de Bruyn Kops et al., further enhance ADMET profiling by predicting cytochrome P450mediated metabolism [203,204], and Montanari et al. developed predictive models for liver transporter inhibition, especially BSEP and OATP transporters, which are crucial for screening compounds for potential cholestatic effects, a critical safety concern in long-duration TB treatment regimens [205].

In recent years, advances in ADMET prediction have extended beyond single-agent compounds to drug combinations, helping researchers predict pharmacokinetic compatibility and optimize multi-drug regimens [34,193,206–210]. Overall, the integration of ADMET and drug-likeness prediction tools in early drug discovery has transformed the way compounds are screened, optimized, and prioritized. These tools not only serve as filters but also act as engines of design, allowing medicinal chemists to tailor compounds with favorable pharmacokinetic profiles [34,108,211]. As computational models evolve with the integration of AI, transcriptomic data, and systems pharmacology, the accuracy and scope of ADMET predictions will continue to expand, which will be particularly impactful in TB drug discovery, where minimizing toxicity and maximizing efficacy are paramount to combating one of the world's deadliest infectious diseases.

6. Machine Learning and Artificial Intelligence in TB Drug Discovery

The increasing use of *in silico* tools for ADMET and drug-likeness assessment reflects a gradual incorporation of data-driven methodologies into TB drug discovery. Unlike earlier computational strategies that relied mainly on structure-based or ligand-based modeling in isolation, ML-driven frameworks enable the combined analysis of chemical, biological, pharmacokinetic, and toxicological data within integrated workflows (Figure 5) [212]. This multidimensional approach is particularly relevant in tuberculosis research, where prolonged treatment regimens, intracellular infection, and the emergence of multidrug- and extensively drug-resistant strains impose strict constraints on efficacy, selectivity, and safety.

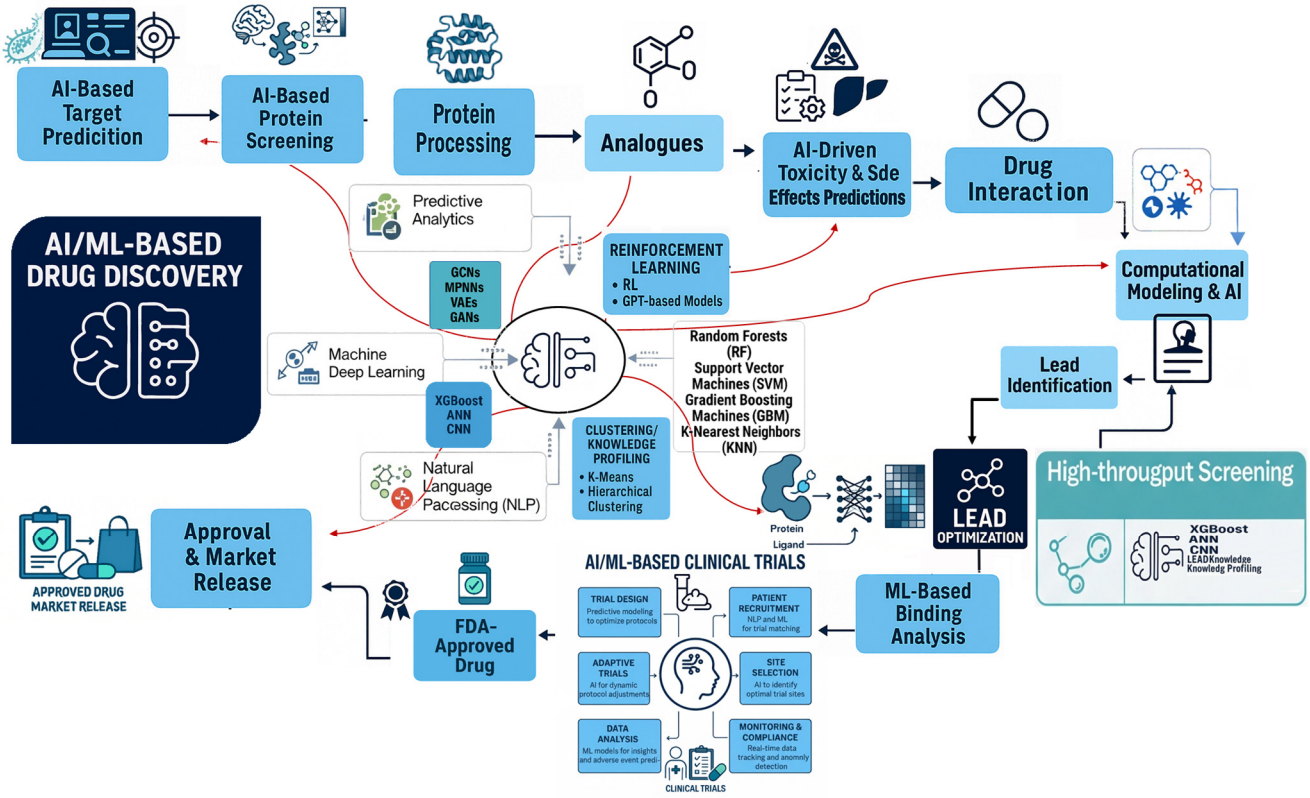


Figure 5. The role of artificial intelligence in the design of anti-TB drugs—the figure illustrates the enhancement of traditional drug development methods with the incorporation of artificial intelligence and ML. Certain preliminary visual elements were generated with the assistance of ChatGPT4 (OpenAI) during the conceptual drafting stage. The final figure was critically revised, scientifically validated, and fully edited by the authors to ensure accuracy, coherence, and compliance with journal standards. Any institutional or regulatory symbols shown in the schematic are purely illustrative and do not represent official logos or imply endorsement by regulatory agencies.

Compound prioritization represents one of the most established applications of ML in tuberculosis research. Predictive models trained on experimentally derived bioactivity datasets are used to rank or classify small molecules according to their likelihood of anti-TB activity, enabling large virtual or experimental libraries to be reduced to focused subsets suitable for biological evaluation. This strategy reduces experimental burden and accelerates downstream validation. High predictive performance has been reported for several tuberculosis-relevant targets, including InhA, with consistent correlations observed between predicted and experimental activity values across multiple studies [213,214].

In parallel, ML-assisted automation has been applied to high-throughput screening workflows, supporting the rapid evaluation of extensive compound collections. These approaches improve hit identification by minimizing experimental redundancy and optimizing resource allocation during early discovery phases. By integrating predictive modeling with screening data, ML-driven systems enable more efficient exploration of chemical space while maintaining alignment with experimentally measurable endpoints [215].

Beyond compound screening, ML and AI-based methods have been applied to target identification through the analysis of large-scale genomic, transcriptomic, and proteomic datasets from MTB. These approaches facilitate the prioritization of proteins and metabolic pathways essential for bacterial survival and virulence, expanding the target landscape beyond extensively characterized enzymatic systems. Feature-based and sequence-driven models, including ensemble learning techniques such as extreme gradient boosting (XGBoost), have been used to support rational target selection. In parallel, ML-driven strategies have gained relevance in drug repurposing, where approved or clinically characterized compounds are evaluated for previously unrecognized antitubercular activity by identifying latent relationships between drugs, targets, and disease-associated pathways [216].

ML-based models are increasingly integrated into toxicity prediction and lead optimization workflows. These approaches complement conventional ADMET tools by enabling early identification of compounds with unfavorable safety profiles, a critical consideration given the hepatotoxicity risks and long-term exposure associated with tuberculosis therapy [217,218]. During lead optimization, predictive models are applied to estimate the impact of structural modifications on potency, selectivity, and pharmacokinetic behavior, thereby guiding medicinal chemistry decisions and reducing late-stage attrition. When combined with structure-based design, ligand-based modeling, and ADMET prediction, ML-driven methodologies form a coherent computational framework that supports systematic progression of candidates through TB drug discovery pipelines [216].

6.1. Deep Learning in QSAR Modeling

ML and deep learning have become transformative approaches to QSAR modeling, a key method for drug discovery and chemical property prediction. They are recognized as data-driven strategies and play an important role in the development of QSAR models [219]. Whereas conventional QSAR workflows depend on predefined molecular descriptors and statistical modeling, recent deep learning methods enable QSAR models to be trained directly from molecular representations. In particular, long short-term memory (LSTM) neural networks trained on SMILES strings or alternative linear molecular notations can learn relevant structural features without relying on precomputed descriptors, making them suitable for large and diverse chemical datasets [220].

Using state-of-the-art natural language processing models of bidirectional encoder architectures, including bidirectional encoder representations from transformers, this approach embedded SMILES strings into a high-dimensional space using a two-stage training process. The model was first pre-trained with a masked SMILES token prediction task and subsequently fine-tuned for QSAR-based property prediction. This framework is flexible,

requires minimal domain-specific knowledge, and can be readily used to predict a range of pharmacokinetics and toxicity parameters, enabling rapid and accurate evaluation of ADMET [221].

Deep learning-based methodologies have been proposed to perform QSAR predictions by integrating an end-to-end encoder–decoder architecture with a convolutional neural network (CNN). The encoder–decoder structure primarily functions to create latent, fixed-dimensional representations of chemical compounds; these representations are subsequently incorporated into the CNN model to develop a robust and reliable system, the ultimate goal of which is to predict biologically active substances [222]. Furthermore, GNNs have been used alongside traditional descriptors commonly used in QSAR, combining the advantages of both methods. This combined strategy consistently outperforms traditional descriptors or GNN methods used separately on nine well-selected high-throughput screening datasets for various therapeutic targets [223].

6.2. AI-Assisted Virtual Screening and Feature Extraction

Virtual screening and feature extraction with AI are transforming drug discovery by leveraging AI, especially ML and deep learning. Scientists can efficiently explore vast chemical fields, predict compound properties, and accelerate the discovery process in pharmaceutical science. Feature extraction refers to the process of identifying and selecting the most relevant information from molecular data or docking results so that AI algorithms can analyze them and make more accurate decisions [224].

AI improves SBVS and LBVS, improving the accuracy of predicting binding positions and affinities. Tools such as RosettaVS and PyRMD have been developed to automate and streamline these processes [225,226]. On the other hand, generative AI can create new candidate molecules, while predictive models evaluate properties such as absorption, distribution, metabolism, excretion, and toxicity (ADMET), further refining candidate selection [227–229].

In this context, deep learning models can automatically learn which features are important from basic data such as atom types, residues, and positions in protein–ligand complexes, without the need for manual intervention. For example, DeepVS uses vector representations of atomic contexts to extract relevant information directly from docking results [230,231]. On the other hand, in large databases, techniques are applied to select only the most useful features, eliminating redundant or irrelevant information, which improves the accuracy and efficiency of virtual projection models [232,233]. In addition, molecular scriptors (physicochemical properties), interaction energies, binding patterns, or graphical representations of the molecules can be included [234].

6.3. Predictive Modeling of Resistance and Drug Synergy

The field of predictive modeling related to drug resistance is rapidly evolving and leverages sophisticated computational methodologies to forecast resistance trends in various pathogens, including MTB. By combining genomic information and ML techniques, these models aim to improve therapeutic efficacy and guide clinical judgment. The following sections explain the fundamental elements of predictive modeling related to drug resistance [235]. The models and their applications in predicting drug resistance involved in the treatment of TB are described in Table 1.

Table 1. Methods and tools used in predictive modeling of drug resistance.

Methods/Tools	Covered Medications	Sensitivity/Specificity (Range)	Notable Features/Findings	Ref.
Extreme Gradient Boosting (XGBC)	Ethambutol, Isoniazid, Rifampicin	Sensitivity: 0.90–0.97, Specificity: 0.96–0.99	Outperformed other ML models; binary mutation representation effective	[236]
Deep Learning (TB-DROP, HGAT-AMR)	Multiple first-line drugs	Sensitivity: up to 0.95, Specificity: up to 0.96	Handles incomplete data, identifies key mutations	[237,238]
Whole-Genome Sequencing (WGS)	6–11 drugs	Sensitivity: 75–97%, Specificity: 64–100%	Rapid, comprehensive, but limited by unknown mechanisms	[239]
Mykrobe, DrPRG, TB-Profiler	Multiple drugs	Sensitivity/Specificity: 82–100%/82–100%	Graph-based, supports various sequencing platforms	[240,241]
Statistical Learning (L2 regression, Random Forest)	10 drugs	AUC: 0.88–0.98, Sensitivity: 82–88%, Specificity: 90–93%	Including rare variants improves accuracy	[242]

On the other hand, the domain of predictive modeling for drug synergy represents a burgeoning area that leverages sophisticated computational methodologies to determine potent drug combinations. Various models, including graph neural networks and deep learning architectures, have been formulated to improve the accuracy and efficiency of predictive results. These models integrate a multitude of data sources, spanning drug chemical composition and gene expression profiles, to predict synergistic interactions, ultimately accelerating the drug discovery process and improving therapeutic outcomes (Table 2) [243,244].

Table 2. Methods and tools used in predictive modeling of drug synergy.

Model/Method	Focus and Characteristics	Result	Ref.
INDIGO-MTB	Uses transcriptomic profiles from single-drug treatments to predict synergy	Screened > 1 million combinations; predictions correlated with clinical efficacy; identified key regulators	[245]
DRonA and ML Synergy	Transcriptome-based algorithms for rapid drug response and synergy prediction	Detects MTB killing and predicts multidrug interactions in various contexts, including patient samples	[246]
DiaMOND	Efficient geometric sampling and scoring of high-order drug interactions	Systematically identifies and factorizes synergistic/ antagonistic combinations, including 3+ drugs	[247]
Systematic in vitro + Classifiers	Large-scale measurement of 2–and 3–drug combinations in diverse conditions; ML classifiers	Predicts in vivo outcomes in mouse models; identifies signatures distinguishing superior regimens	[248]

7. Applications of Structure-Guided *In Silico* Approaches in MTB

SBVS has been widely applied to MTB to interrogate essential targets involved in lipid metabolism, DNA replication, redox homeostasis, signal transduction, and cell wall biosynthesis. Across these studies, SBVS is consistently used as a prioritization and hypothesis-generation strategy rather than as a definitive predictor of anti-TB efficacy, with its reliability strongly dependent on subsequent biochemical and cellular validation. SBVS campaigns addressing resistance-associated targets, including InhA, FabD, and AhpC, demonstrate that computational modeling can rationalize drug resistance at the molecular level by capturing binding losses caused by target mutations and identifying alternative chemotypes capable of stabilizing mutant active sites [208].

These studies highlight the value of mutant-specific docking and pocket analysis for resistance-aware design, while simultaneously emphasizing that such predictions remain interaction-based and insufficient to infer functional efficacy in the absence of experimental confirmation. For ATP-dependent enzymes such as DNA gyrase and protein kinases, SBVS pipelines integrating docking with molecular dynamics and free-energy refinement provide improved resolution of binding stability and interaction persistence [210,249] (Figure 6). In these cases, dynamic simulations enable discrimination between compounds that engage the intended target and those whose apparent activity arises from off-target or phenotypic effects. Nevertheless, multiple studies converge on the observation that strong computational binding and enzymatic inhibition do not necessarily translate into robust whole-cell activity, largely due to physicochemical and permeability constraints intrinsic to the mycobacterial envelope [249–251].

Similar limitations are evident in SBVS efforts targeting enzymes involved in cell wall and nucleotide biosynthesis, including DapB, MurB, and thymidylate kinase [251–253]. While docking and molecular dynamics efficiently identify stable ligand–protein complexes and prioritize chemically tractable scaffolds, the absence of consistent cellular potency underscores the biological gap between enzymatic inhibition and anti-TB efficacy. These findings reinforce the necessity of interpreting SBVS outputs within the broader context of intracellular accessibility and metabolic redundancy. SBVS-based drug repurposing approaches, exemplified by screening of approved-drug libraries against targets such as trehalose sulfotransferase, offer a complementary strategy by reducing chemical risk and accelerating translational assessment [254]. However, these studies also illustrate that favorable binding stability and pharmacokinetic predictions alone are insufficient to establish therapeutic relevance in tuberculosis, confirming the exploratory nature of repurposing-driven SBVS.

To provide a structured overview of how these structure-guided computational strategies have been applied in practice, Table 3 summarizes representative SBVS-driven studies targeting essential MTB proteins. The table collates the biological targets addressed, the computational strategies employed, the nature of the screened libraries, and the level of experimental validation achieved, with an emphasis on what each study demonstrates conceptually rather than on quantitative performance metrics. This comparative framework highlights both the versatility of SBVS across diverse mycobacterial pathways and the recurring limitations that constrain translational impact, thereby contextualizing the case studies discussed above within a unified analytical perspective.

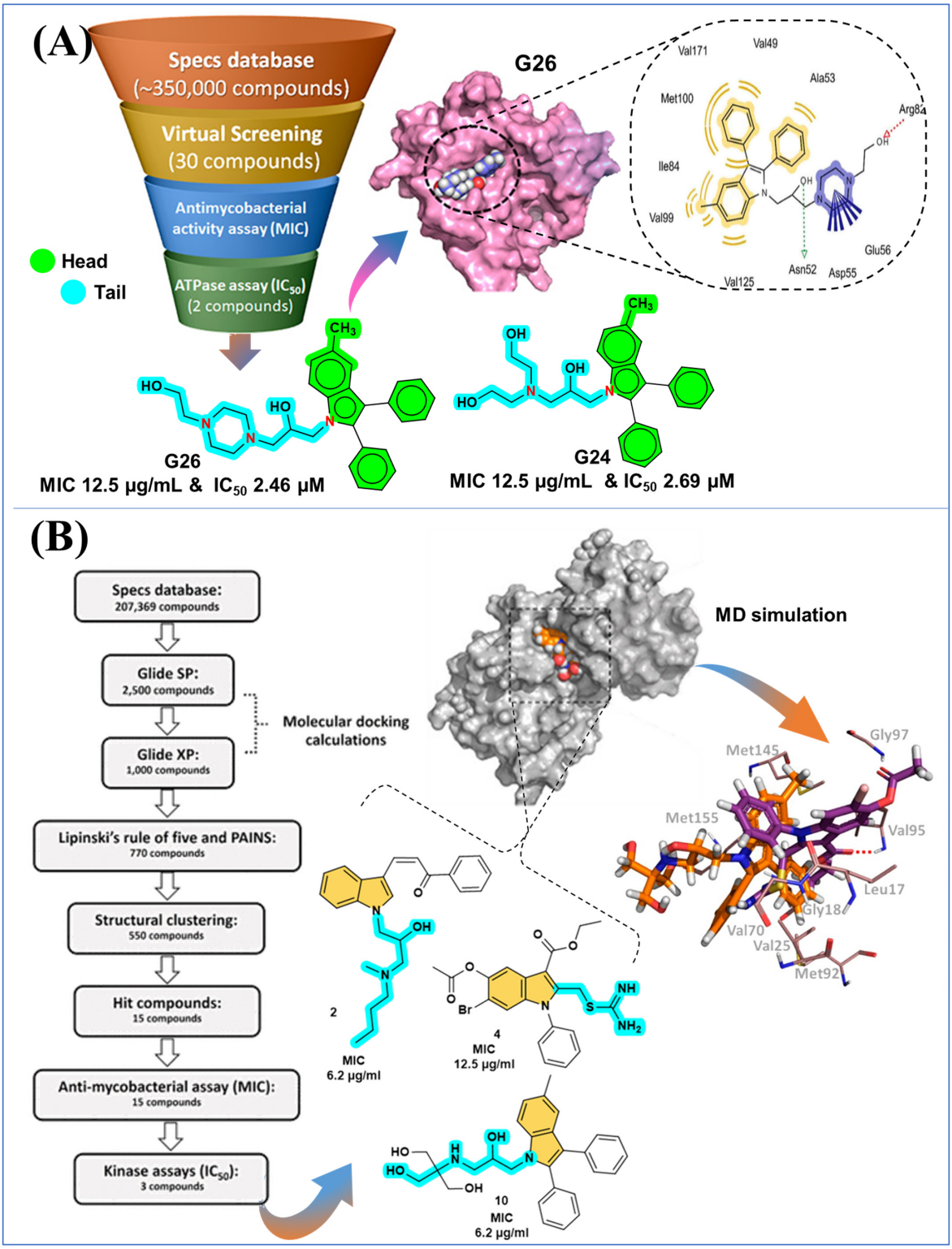


Figure 6. Representative applications of structure-based *in silico* virtual screening against MTB targets. (**A**) Workflow targeting the ATPase domain of DNA gyrase (GyrB), showing library reduction, lead identification (G24, G26), and key binding interactions in the ATP-binding pocket from molecular dynamics simulations. Adapted with permission from [249], American Chemical Society, 2022. (**B**) virtual screening pipeline applied to the serine/threonine kinase PknB inhibitor, using hierarchical docking, filtering, hit selection, MD analyses and experimental validation. Active compounds and their stabilized binding modes in the PknB active site are shown, integrating both computational and experimental approaches. Reprinted and adapted with permission [210], American Chemical Society, 2022.

Table 3. Representative structure-based virtual screening studies applied to *Mycobacterium tuberculosis* targets.

Target (Biological Role)	SBVS Strategy	Library	Computational Workflow	Experimental Outcome	Conceptual Takeaway	What the Study Demonstrates	Ref.
InhA, FabD, AhpC (INH resistance, fatty acid biosynthesis, oxidative stress)	Docking against mutant targets (GOLD)	ChEMBL (826 compounds)	Mutant modeling → CASTp pocket detection → GOLD docking → ADMET	3 leads showed improved binding vs. INH in mutant targets	SBVS can rationalize resistance-associated binding loss, but requires accurate mutant modeling	SBVS can rationalize resistance-induced loss of INH binding, but predictive power remains interaction-based without functional validation	[208]
PknB (Ser/Thr kinase, signaling)	Glide HTVS → filtered SBVS	Specs (>207,000)	Glide docking → Lipinski + PAINS filtering → MD → MM/GBSA	Compounds **2** and **10** active (MIC 6.2 µg/mL)	SBVS effective when coupled to early biochemical validation	Integration of SBVS with enzymatic assays distinguishes on-target activity from phenotypic activity	[210]
DNA gyrase ATPase (fluoroquinolone resistance)	Bioisosteric design + docking	Bioisosteric series	SwissBioisostere → docking → MD	Lead IC_{50} = 0.42 µM, low cytotoxicity	Bioisosteric design + SBVS improves drug-likeness beyond docking hits	SBVS combined with scaffold optimization improves drug-likeness beyond docking-ranked hits	[250]
DapB (lysine/cell wall biosynthesis)	Molecular Docking (AutoDock4)	NCI (95,000)	AutoDock → ADMET → enzyme assays	29 hits > 80% inhibition; best IC_{50} = 4.4 µg/mL	SBVS identifies enzymatic inhibitors, whole-cell activity remains limiting	Enzymatic inhibition does not translate directly to whole-cell potency, highlighting permeability constraints	[251]
MurB (cell wall synthesis)	Multi-database SBVS	ChemSpider/ DrugBank/ZINC	Docking → ADMET → MD	4 stable complexes identified	Stability ≠ potency; cellular context remains unresolved	Docking + MD identifies stable binders but lacks biological resolution without cellular assays	[252]
Rv0295c (trehalose sulfotransferase)	Repurposing drug SBVS	FDA-approved drugs (1800)	Docking → ADMET → 100 ns MD	Dihydroergotamine stable binder	Repurposing narrows chemical risk but does not guarantee TB efficacy	SBVS repurposing narrows chemical risk but remains hypothesis-generating without functional validation	[254]
Thymidylate kinase	HTVS + SBVS	Kinase-focused library	HTVS → docking → MD → FEL	TM1–TM4 stable binders	Free-energy landscapes improve confidence beyond docking	Free-energy landscapes improve confidence in binding stability beyond static docking	[253]
Multiple (QSAR-driven)	Descriptor-guided SBVS/QSAR	Triazole derivatives (40)	QSAR + docking + ADMET	Compounds X–Z favorable	Descriptor-driven design complements SBVS	Predictive models assist prioritization but do not replace target-specific validation	[90]
Pks13, PknG (lipid metabolism, signaling)	Natural-product SBVS	Natural products	Molecular networking → docking → MD → DFT	Vazabitide A optimized	Multimodal workflows outperform single-method screens	Multimodal workflows outperform docking alone, but attribution of activity to single compounds remains indirect	[255]

8. Challenges and Limitations

Despite significant advancements in *in silico* drug discovery, several challenges and limitations remain, particularly in the context of TB treatment. A major concern is the precision and reliability of computational predictions, which heavily depend on the quality of input data. Incomplete or inaccurate data can compromise the efficacy of the virtual screening process and molecular modeling outcomes, especially when addressing dynamic biological targets like MTB. Although *in silico* strategies can rapidly identify promising drug-like molecules, these predictions must be followed by experimental validation, typically in vitro or in vivo, to confirm their efficacy. This need for experimental confirmation highlights the gap between computational predictions and real-world results. The availability of high-quality molecular and clinical data remains another challenge, limiting the effectiveness of computational strategies. Furthermore, predicting drug–target interactions, particularly in complex conditions like latent TB, remains difficult, and existing models fail to adequately account for changes that lead to drug resistance. The computational approaches required for high-throughput virtual screening and MD simulations also face scalability challenges, particularly in resource-limited environments. With the growing reliance on AI/ML, concerns about model bias and generalization arise, as poorly trained models may yield distorted predictions. Addressing these challenges is crucial for enhancing the efficacy of *in silico* techniques in TB drug discovery and ensuring their clinical relevance.

9. Future Perspectives

The continued emergence of multidrug-resistant and extensively drug-resistant tuberculosis underscores the limitations of conventional drug discovery paradigms when applied to a pathogen characterized by high genetic plasticity and prolonged treatment exposure. In this context, computational drug discovery approaches, including structure-based modeling, ligand-based methods, and data-driven analyses, are increasingly integrated into tuberculosis research as tools to address challenges related to target identification, resistance, and candidate prioritization. The combined use of sequence, structural, and protein–ligand interaction data enables systematic exploration of chemical space and supports the identification of drug-like molecules that would be difficult to access through empirical screening alone.

Looking ahead, the refinement of ML-based methodologies is expected to improve the predictive accuracy of resistance-associated mutations, pharmacokinetic behavior, and combination effects relevant to tuberculosis therapy. Active learning frameworks and iterative model updating based on experimental feedback may contribute to more efficient progression from hit identification to preclinical validation. In parallel, advances in predictive modeling are likely to enhance drug repurposing efforts by facilitating the identification of existing compounds with previously unrecognized antitubercular activity, potentially shortening development timelines. These computational strategies are not meant to replace experimental methods. Instead, they serve as helpful tools that support better decision-making throughout the discovery and development process, aligning drug discovery efforts with the biological and clinical complexity of tuberculosis.

10. Conclusions

The study highlights the revolutionary potential of *in silico* drug discovery in revolutionizing TB research and treatment development. By using computation methods such as molecular docking, virtual screening, AI/ML, researchers can uncover novel drug-like molecules more efficiently and cost-effectively. These methods significantly decrease the time and resources usually necessary for traditional drug discovery, enabling faster screen-

ing and optimization without the need for wet lab experiments. Furthermore, AI-enhanced predictive models provide essential insights into drug resistance, optimizing drug combinations and early evaluation of compounds toxicity. As these methods advances, they hold the promise of enhancing the development of safe, effective drugs with reduced timeframes and improved clinical outcomes. Ultimately, *in silico* methods will be very important for dealing with the challenges caused by drug-resistant TB, contributing significantly to a more robust global response and better control of the TB crisis.

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