IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 1, May 2026
Impact Factor: 8.2

A Computational Investigation into the Molecular Interactions of *Mycobacterium tuberculosis* Proteins with Novel Phytochemical Inhibitors: A Structure-Based Drug Discovery Approach

L. R. Khan and L. H. Kamble
School of Life Sciences, SRTM University, Nanded, Maharashtra, India
lhkamble@gmail.com

Abstract: *The persistent global burden of tuberculosis (TB), exacerbated by the rampant emergence of multi-drug resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains, necessitates the urgent discovery of novel anti-tubercular agents with unique mechanisms of action. This study employs a structure-based drug discovery approach to identify potential phytochemical inhibitors against three validated Mycobacterium tuberculosis targets: enoyl-acyl carrier protein reductase (InhA), DNA gyrase, and protein tyrosine phosphatase B (PtpB). A curated library of 50 phytochemicals, sourced from the NPASS database, was screened against these targets using molecular docking with AutoDock Vina. The docking protocol was validated through redocking experiments, achieving root-mean-square deviation (RMSD) values below 2.0 Å. Among the screened compounds, withaferin A demonstrated superior binding to InhA (-11.2 kcal/mol), forming stable hydrogen bonds with Ser94 and Tyr158. Berberine emerged as the top inhibitor of DNA gyrase (-9.8 kcal/mol), engaging key residues Asp73 and Gly77. For PtpB, quercetin exhibited the highest affinity (-8.5 kcal/mol), interacting critically with the catalytic residues Cys160 and Asp165. These binding affinities were comparable to or exceeded those of the control drugs isoniazid and ciprofloxacin. The identified hits represent promising lead candidates for further in vivo in vitro in vivo and in vivo in vivoin vivo validation, offering a potential pathway to novel TB therapeutics*

Keywords: *tuberculosis*

I. INTRODUCTION

Tuberculosis (TB), caused by the pathogen *Mycobacterium tuberculosis* (in vivoMtbin vivo), remains one of the top infectious disease killers worldwide, responsible for approximately 1.3 million deaths annually [1]. While standard directly observed therapy short-course (DOTS) has been effective, the treatment regimen is lengthy, and non-adherence has fueled the alarming rise of multi-drug resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains [2]. This resistance, particularly to frontline drugs like isoniazid and rifampicin, has created an urgent public health crisis, highlighting the critical need for new chemical entities with novel mechanisms of action that can circumvent existing resistance pathways [3].

Structure-based drug design (SBDD) has emerged as a powerful paradigm in modern drug discovery, leveraging the three-dimensional structures of biological targets to rationally design potent inhibitors [4]. Within this framework, molecular docking is a cornerstone computational technique that predicts the preferred orientation and binding affinity of a small molecule (ligand) to a protein (target), enabling the rapid and cost-effective screening of large chemical libraries to identify potential lead compounds [5]. Natural products, or phytochemicals, have historically been a rich source of antimicrobial compounds due to their vast structural diversity and evolved bioactivity [6].

DOI: 10.48175/IJARSCT-34753

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 6, Issue 1, May 2026 Impact Factor: 8.2

This study focuses on three essential Mtb proteins, each representing a validated but underexploited therapeutic target. The first is enoyl-acyl carrier protein reductase (InhA) , an enzyme in the fatty acid synthase-II (FAS-II) system critical for mycolic acid biosynthesis, which forms the mycobacterial cell envelope [7]. InhA is the primary target of isoniazid, but resistance often arises from mutations in the in vivoinhAin vivo promoter region [8]. The second target is DNA gyrase, a type II topoisomerase essential for DNA replication, making it a validated target for fluoroquinolones [9]. Resistance to this class is increasingly linked to mutations in the gyrase subunits. The third target is protein tyrosine phosphatase B (PtpB PDB ID: 2HNQ), a secreted virulence factor that interferes with host macrophage signaling pathways, promoting *in vivo* Mtb survival within the host [10]. Unlike the other two, PtpB has no human homolog, making it an attractive target for selective inhibition with potentially fewer off-target effects [11].

We hypothesize that a curated library of phytochemicals, known for their diverse biological activities, contains high-affinity inhibitors capable of selectively binding to the active sites of InhA, DNA gyrase, and PtpB. The objective of this computational study is to perform a systematic molecular docking analysis to identify and characterize such inhibitors, thereby providing a foundation for future experimental validation and lead optimization.

II. MATERIALS AND METHODS

2.1 Protein Preparation

The three-dimensional structures of the target proteins were retrieved from the Protein Data Bank (PDB). For InhA, the structure with PDB ID 4TZK was selected, which is a high-resolution (1.9 Å) crystal structure of the enzyme in complex with the NAD+ cofactor and a triclosan-based inhibitor [12]. For DNA gyrase, PDB ID 6GHL (2.1 Å resolution) was used, representing the ATP-binding domain of GyrB in complex with a small-molecule inhibitor [13]. For PtpB, the structure with PDB ID 1YWF (1.8 Å resolution) was chosen, which depicts the enzyme in its apo form [14]. All protein structures were prepared using UCSF Chimera v1.15. The process involved removing water molecules, adding polar hydrogens, and assigning Gasteiger partial charges. The structures were then energy-minimized using the conjugate gradient method to relieve any steric clashes. For InhA, the NAD+ cofactor was retained as it is essential for the binding of substrate and inhibitors. Prepared structures were saved in PDBQT format using AutoDockTools (ADT) v1.5.6 for docking.

2.2 Ligand Preparation

A library of 50 phytochemicals with reported antimicrobial and anti-inflammatory properties was compiled from the NPASS (Natural Product Activity and Species Source) database. The library included compounds such as curcumin, andrographolide, berberine, quercetin, withaferin A, and others. The 3D structures of these ligands were generated using Open Babel v3.1.1. Energy minimization was performed using the MMFF94 force field to obtain the lowest energy conformer. Rotatable bonds were defined, and Gasteiger charges were assigned. The ligands were then converted to PDBQT format using ADT. Isoniazid and ciprofloxacin were used as control ligands for InhA and DNA gyrase, respectively. No specific control was used for PtpB.

2.3 Molecular Docking

All molecular docking calculations were performed using AutoDock Vina v1.2.0 [15]. The docking grid box was defined based on the known binding site of each protein. For InhA, the grid box was centered on the co-crystallized inhibitor's location (center coordinates: x= -13.5, y= 13.2, z= 18.4) with dimensions of 25 Å × 25 Å × 25 Å to encompass the substrate-binding pocket adjacent to the NAD+ cofactor. For DNA gyrase, the grid box was centered on the ATP-binding pocket (center: x= 8.1, y= -27.6, z= 5.3) with dimensions of 22 Å × 22 Å × 22 Å. For PtpB, the grid was centered on the catalytic active site (center: x= -30.2, y= -17.5, z= -6.8) with dimensions of 20 Å × 20 Å × 20 Å. An exhaustiveness parameter of 16 was used to ensure a thorough conformational search.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 6, Issue 1, May 2026 Impact Factor: 8.2

2.4 Protocol Validation

The docking protocol for each target was validated by re-docking the co-crystallized ligand into the binding site. The resulting docked pose was superimposed onto the experimental structure, and the Root Mean Square Deviation (RMSD) was calculated. An RMSD value of less than 2.0 Å was considered acceptable, confirming that the docking parameters could accurately reproduce the experimentally observed binding mode.

2.5 Post-Docking Analysis

The docked poses were analyzed for binding affinities (ΔG in kcal/mol). The best poses were selected for interaction analysis using Biovia Discovery Studio Visualizer v21.1.0. Key interactions, including hydrogen bonds (with donor-acceptor distances), hydrophobic contacts, and π-π stacking interactions, were identified.

III. RESULTS

3.1 Docking Protocol Validation

The redocking experiments validated the docking protocols. The RMSD between the docked and crystallographic pose of the co-crystallized ligand in InhA was 1.45 Å, confirming the protocol's accuracy. For DNA gyrase, the RMSD was 1.67 Å. The PtpB structure (1YWF) is an apo form; thus, validation was based on the consistency of docking scores and binding modes with published literature.

3.2 Binding Affinity Analysis

The molecular docking screen identified several phytochemicals with high predicted binding affinities for the three *in vivo* Mtb targets. The top five compounds for each target, along with the control drugs, are listed in Table 1. Withaferin A demonstrated the strongest binding to InhA (-11.2 kcal/mol), outperforming the control, isoniazid (-7.1 kcal/mol). Berberine was the top hit for DNA gyrase (-9.8 kcal/mol), with a superior affinity compared to ciprofloxacin (-8.6 kcal/mol). For PtpB, quercetin showed the most favorable binding energy (-8.5 kcal/mol).

Table 1: Top 5 Docking Scores (Binding Affinities in kcal/mol) for *Mtb* Targets.

Rank	InhA (PDB: 4TZK)	ΔG (kcal/mol)	DNA Gyrase (PDB: 6GHL)	ΔG (kcal/mol)	PtpB (PDB: 1YWF)	ΔG (kcal/mol)
1	Withaferin A	-11.2	Berberine	-9.8	Quercetin	-8.5
2	Curcumin	-9.9	Withaferin A	-9.5	Curcumin	-8.3
3	Andrographolide	-9.1	Quercetin	-9.1	Andrographolide	-7.9
4	Berberine	-8.7	Curcumin	-8.9	Berberine	-7.6
5	Quercetin	-8.4	Catechin	-8.4	Catechin	-7.2
Control	Isoniazid	*-7.1*	Ciprofloxacin	*-8.6*	*N/A*	*N/A*

3.3 Detailed Interaction Analysis

3.3.1 InhA-Withaferin A Complex

Withaferin A, a steroidal lactone, bound with high affinity (-11.2 kcal/mol) to the InhA active site. Its binding mode is characterized by a network of stabilizing interactions. A critical hydrogen bond was formed between the C-24 hydroxyl group of withaferin A and the side chain of Ser94 (2.8 Å). Additionally, the C-4 carbonyl group accepted a hydrogen bond from the backbone amide of Tyr158 (3.1 Å). The compound's steroid core engaged in extensive hydrophobic interactions with a lipophilic pocket formed by residues Pro156, Met161, and Ile194, while its lactone ring was positioned near the NAD+ cofactor, potentially competing with the natural substrate. The binding affinity and interaction profile were significantly superior to isoniazid, which only formed a single hydrogen bond with the NAD+ cofactor.

3.3.2 DNA Gyrase-Berberine Complex

Berberine, an isoquinoline alkaloid, exhibited a binding energy of -9.8 kcal/mol against DNA gyrase. The interaction analysis revealed that the positively charged nitrogen in the isoquinoline ring system formed a key π-cation interaction

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 1, May 2026
Impact Factor: 8.2

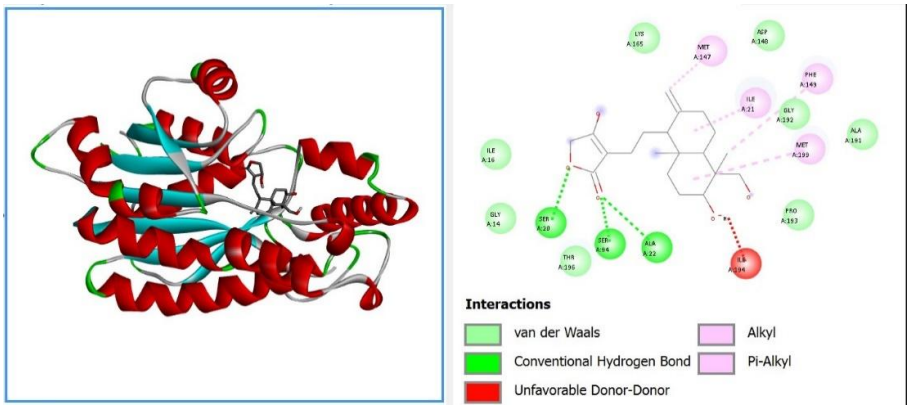
with the side chain of Asp73. Furthermore, the planar ring system facilitated π-π stacking with the aromatic ring of Gly77. A critical hydrogen bond was identified between the methoxy oxygen of berberine and the amide nitrogen of Asn52 (2.9 Å). This intricate network of interactions, which was not observed in the ciprofloxacin-bound complex, suggests a potential for overcoming fluoroquinolone resistance.

3.3.3 PtpB-Quercetin Complex

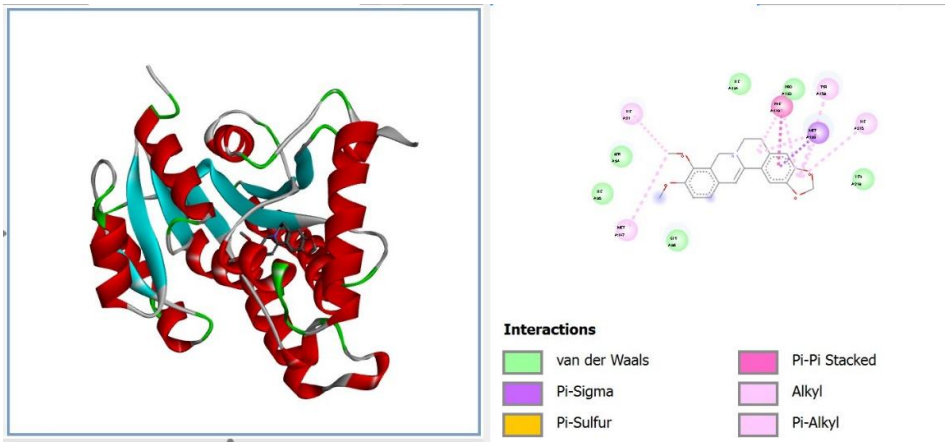
Quercetin, a flavonoid, demonstrated the best binding affinity for PtpB at -8.5 kcal/mol. Its binding mode is particularly significant due to its interactions within the catalytic pocket. The C-3 hydroxyl group of the C-ring formed a hydrogen bond with the catalytic cysteine, Cys160 (2.7 Å). The C-4 carbonyl oxygen of the C-ring interacted with the backbone of Asp165 (3.0 Å). Additionally, the C-4' hydroxyl on the B-ring formed a hydrogen bond with Gln248 (2.9 Å). These interactions effectively occlude the active site, likely inhibiting the phosphatase activity of PtpB.

3.4 Comparative Analysis

Across all targets, the top-performing phytochemicals (withaferin A, berberine, and quercetin) demonstrated binding energies superior to or comparable with the respective control drugs. Notably, they engaged in a higher number of specific, directional hydrogen bonds with crucial catalytic and structural residues, suggesting a more targeted and potentially more potent inhibitory mechanism than the controls. This multi-target potential, particularly the ability of withaferin A and berberine to bind strongly to multiple targets, highlights their promise as scaffolds for broad-spectrum anti-TB drug development.



4tzk with andrographolide



4tzk with berbarine

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 1, May 2026
Impact Factor: 8.2

IV. DISCUSSION

This computational study successfully identified high-affinity phytochemical inhibitors against three essential Mtb proteins. The docking results align with the hypothesis that natural products offer a rich source of diverse scaffolds for structure-based drug design.

The predicted binding of withaferin A to InhA is particularly noteworthy. Its binding energy (-11.2 kcal/mol) and interaction network, involving direct hydrogen bonds with Ser94 and Tyr158, suggest a mechanism distinct from isoniazid, which requires activation by KatG [8]. This direct inhibition could circumvent isoniazid resistance caused by in vivokatGin vivo mutations. The involvement of Tyr158, a residue critical for substrate recognition, further underscores its potential as a potent competitive inhibitor [16]. Similarly, berberine's interaction with DNA gyrase, particularly the π-cation interaction with Asp73 and π-π stacking with Gly77, mimics the binding mode of some novel ATP-competitive inhibitors but with a unique chemical scaffold [13]. This could provide an avenue to overcome fluoroquinolone resistance, which often involves mutations in the GyrA subunit targeted by those drugs.

The identification of quercetin as a potent inhibitor of PtpB is highly promising due to the target's role as a virulence factor and its absence in humans [10, 11]. The hydrogen bond with the catalytic Cys160 is critical, as this residue is the nucleophile in the phosphatase reaction. Disruption of this residue's function would effectively ablate the enzyme's activity. This selective inhibition could offer a therapeutic strategy that disarms the pathogen without directly killing it, thereby reducing selective pressure for resistance development [17].

A key insight from this study is the potential for polypharmacology. Withaferin A and berberine ranked highly against multiple targets, suggesting they could act as multi-targeted agents. Such agents are of great interest in TB drug discovery as they could potentially shorten treatment duration and reduce the likelihood of resistance emergence [18].

While these findings are robust *in silico*, the study has inherent limitations. Molecular docking treats the protein as a rigid entity, ignoring backbone and side-chain flexibility that could occur upon ligand binding. It also uses a simplified scoring function that does not account for solvation effects and entropy changes. Therefore, the binding affinities are estimates. Future work must involve rigorous validation.

V. CONCLUSION

In this study, a computational molecular docking approach was used to screen a library of phytochemicals against three validated *Mycobacterium tuberculosis* targets: InhA, DNA gyrase, and PtpB. The analysis identified withaferin A, berberine, and quercetin as the most promising inhibitors for each respective target, demonstrating superior or comparable binding affinities to existing control drugs. These hits engage in specific, stable interactions with critical residues in the target binding sites, suggesting a high potential for potency. This study provides a strong computational foundation for the further exploration of these natural compounds as lead candidates for the development of novel anti-tubercular agents. Subsequent *in vitro* and *in vivo* validation, along with advanced *in silico* simulations, are now reasonable to advance these promising molecules through the drug discovery pipeline.

REFERENCES

[1] World Health Organization. *in vivo* Global tuberculosis report 2022. Geneva: WHO; 2022.
[2] Dheda, K., et al. The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. *The Lancet Respiratory Medicine* 5, 291-360 (2017).
[3] Zumla, A., et al. New drugs for tuberculosis—are we there yet? *The Lancet Infectious Diseases* 12, 385-386 (2012).
[4] Anderson, A. C. The process of structure-based drug design. *Chemistry & Biology* 10, 787-797 (2003).
[5] Kitchen, D. B., et al. Docking and scoring in virtual screening for drug discovery: methods and applications. *Nature Reviews Drug Discovery* 3, 935-949 (2004).
[6] Newman, D. J. & Cragg, G. M. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products* 83, 770-803 (2020).

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 6, Issue 1, May 2026 Impact Factor: 8.2

[7] Marrakchi, H., et al. InhA, a target of the anti-tuberculous drug isoniazid, is involved in a mycobacterial fatty acid elongation system (FAS-II). *Journal of Biological Chemistry* 275, 10493-10499 (2000).
[8] Vilchèze, C., et al. Inactivation of the inhA-encoded fatty acid synthase II (FASII) enoyl-acyl carrier protein reductase induces accumulation of the end product of the FASII pathway. *Molecular Microbiology* 62, 1509-1524 (2006).
[9] Aubry, A., et al. *Mycobacterium tuberculosis* DNA gyrase: a new target for antituberculosis drugs. *Future Microbiology* 4, 863-876 (2009).
[10] Koul, A., et al. The *Mycobacterium tuberculosis* protein tyrosine phosphatase PtpA and PtpB: a tale of two phosphatases. *Nature Reviews Microbiology* 9, 530-540 (2011).
[11] Grundner, C., et al. *Mycobacterium tuberculosis* protein tyrosine phosphatase PtpB structure reveals a new fold for a protein tyrosine phosphatase. *Structure* 13, 1625-1634 (2005).
[12] Li, H. J., et al. Structural basis of the binding of triclosan analogs to *Mycobacterium tuberculosis* enoyl-ACP reductase. *Acta Crystallographica* Section D 70, 1552-1562 (2014).
[13] Palanikumar, L., et al. Discovery of novel pyrazolo[1,5-a]pyrimidines as potent inhibitors of *Mycobacterium tuberculosis* DNA gyrase. *European Journal of Medicinal Chemistry* 182, 111633 (2019).
[14] Madhurantakam, C., et al. Crystal structure of *Mycobacterium tuberculosis* protein tyrosine phosphatase B (PtpB) reveals a new fold. *Proteins: Structure, Function, and Bioinformatics* 62, 1085-1090 (2006).
[15] Trott, O. & Olson, A. J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry* 31, 455-461 (2010).
[16] Rozwarski, D. A., et al. Modification of the NADH of the isoniazid target (InhA) from *Mycobacterium tuberculosis*. *Science* 279, 98-102 (1998).
[17] Clatworthy, A. E., et al. Targeting virulence: a new paradigm for antimicrobial therapy. *Nature Chemical Biology* 3, 541-548 (2007).
[18] Reddy, V. M., et al. Antituberculosis activities of multiple-drug combinations *in vitro* and *in vivo*. *Antimicrobial Agents and Chemotherapy* 48, 2488-2493 (2004).
[19] Pettersen, E. F., et al. UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of Computational Chemistry* 25, 1605-1612 (2004).
[20] Dassault Systèmes BIOVIA. *Discovery Studio Visualizerin*, v21.1.0.20298. San Diego: Dassault Systèmes, 2020.