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Research Article

IN SILICO DRUG DESIGN OF NATURAL COMPOUNDS TARGETING *MYCOBACTERIUM TUBERCULOSIS* PROTEIN KINASE B

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ABSTRACT

Mycobacterium tuberculosis Protein Kinase B (PknB) is an essential serine/threonine kinase involved in cell wall regulation, growth, and survival of the pathogen, making it a promising drug target for tuberculosis (TB). In the present study, natural alkaloids Indicine N-oxide, Lycopsamine, and Supinine were computationally investigated for their inhibitory potential against PknB. Molecular structures were optimized, and docking was performed using AutoDock Vina to identify binding affinity and key interactions. Physicochemical and ADMET screening assessed drug-likeness and safety properties, while molecular dynamics (MD) simulations were conducted to evaluate structural stability of ligand protein complexes. Docking results showed strong binding affinities, particularly for Indicine N-oxide (-9.2 kcal/mol), which formed stable hydrogen-bond networks with the ATP-binding pocket of PknB. MD simulations confirmed complex stability with low RMSD fluctuations and favorable interaction energies. ADMET analysis further supported good oral bioavailability and acceptable toxicity profiles. Overall, Indicine N-oxide and Lycopsamine emerged as promising natural inhibitors targeting PknB and warrant further in vitro and in vivo validation.

Keywords: Protein Kinase B, *Mycobacterium tuberculosis*, Natural alkaloids, Molecular docking, Indicine N-oxide.

INTRODUCTION

Tuberculosis (TB) remains one of the leading infectious diseases globally, caused by *Mycobacterium tuberculosis* (Mtb). The emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and totally drug-resistant (TDR) strains has intensified the need for novel therapeutic agents (World Health Organization, 2023). Protein Kinase B (PknB), a serine/threonine kinase essential for Mtb growth and cell signaling, has become a promising molecular target due to its involvement in cell division, peptidoglycan synthesis, and host pathogen interactions (Kang *et al*., 2005). Natural phytochemicals have recently gained attention as potential anti-TB leads because of their structural diversity, low toxicity, and biological relevance. Among them, alkaloids such as Indicine N-oxide, Lycopsamine, and Supinine have demonstrated antimicrobial, immunomodulatory, and cytotoxic properties (Cardoso *et al*., 2017). However, their role as potential PknB inhibitors remains unexplored. Computational drug design approaches such as molecular docking, ADMET analysis, and molecular dynamics simulations provide rapid and cost-effective strategies to identify potent inhibitors (Kitchen *et al*., 2004). These methods enable the prediction of binding affinity, stability, and pharmacokinetic behavior prior to laboratory experimentation. This study reports a comprehensive in silico evaluation of three natural alkaloids Indicine N-oxide, Lycopsamine, and Supinine as potential inhibitors of PknB. Docking analysis, ADMET profiling, and MD simulations were carried out to identify lead candidates for TB drug discovery.

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Natural alkaloids and plant-derived compounds have long been recognized for their broad antimicrobial and therapeutic potential, forming the foundation of many modern drug discovery programs. Recent advancements highlight their relevance in tuberculosis research, where the serine/threonine kinase PknB has emerged as a critical regulator of cell growth, cell wall synthesis, and mycobacterial survival under stress, as demonstrated in early mechanistic studies (Kang *et al*., 2005). The development of ATP-competitive PknB inhibitors has been particularly promising, with several compounds exhibiting strong inhibitory potency and structural complementarity toward the kinase catalytic pocket (Danelius *et al*., 2020). Natural products remain an abundant reservoir for anti-TB lead compounds, and numerous phytochemicals structurally similar to Indicine N-oxide, Lycopsamine, and Supinine have shown inhibition against *Mycobacterium tuberculosis*, reinforcing their therapeutic value (Kaur *et al*., 2015). Broader insights into natural product functionality are also supported by recent reviews exploring their biomedical significance (Nafisa Farheen *et al*., 2025; Priyadharshini *et al*., 2025).

Understanding drug-resistance mechanisms remains essential for developing novel anti-TB agents. Persistent multidrug resistance in MTB is driven by mutations in drug targets, activation of efflux pathways, and cell-wall impermeability, reinforcing the need for new scaffolds with alternative molecular mechanisms (Gopal *et al*., 2020). Natural product–derived inhibitors provide unique structural diversity and biological compatibility for kinase-targeted drug design, expanding medicinal chemistry prospects (Li & Wang, 2019). Additionally, scientific works across various biological domains emphasize the value of exploring natural resources for innovative therapeutic breakthroughs (Ramya *et al*., 2025; Revathi *et al*., 2025). Computational advancements have significantly accelerated early-phase TB drug discovery. Databases such as PubChem serve as reliable repositories for natural and synthetic compounds suitable for virtual screening (Kim *et al*., 2016). Molecular docking remains fundamental for predicting ligand–protein interactions, evaluating binding affinity, and guiding structure-based inhibitor design, consistent with established docking methodologies (Kitchen *et al*., 2004; Morris & Lim-Wilby, 2008). AutoDock Vina, in particular, offers enhanced scoring functions and speed, enabling efficient screening of large chemical libraries (Trott & Olson, 2010). Post-docking visualization tools such as LigPlot+ facilitate detailed analysis of hydrogen bonding and hydrophobic interactions, improving interpretability of ligand-binding mechanisms (Laskowski & Swindells, 2011). Additionally, supplementary scientific reports emphasize the increasing utility of computational approaches in diverse research fields (Devasena *et al*., 2005; Mahalakshmi *et al*., 2025).

Drug-likeness and ADMET profiling tools including pkCSM and Swiss ADME provide insight into absorption, metabolic stability, solubility, and potential toxicity, enabling early filtration of suboptimal compounds (Pires *et al*., 2015; Daina *et al*., 2017). These computational strategies complement conventional pharmacognostic and medicinal chemistry approaches in identifying promising anti-TB candidates. Molecular dynamics (MD) simulations further enhance structural insights by evaluating protein–ligand stability, conformational flexibility, and interaction persistence under physiologically relevant conditions. Simulation engines such as GROMACS have become essential for characterizing atomic-level motions in inhibitor–PknB complexes (Abraham *et al*., 2015), while tools such as PRODRG streamline ligand topology generation for MD workflows (Schüttelkopf & van Aalten, 2004). The integration of docking with MD improves accuracy in predicting ligand affinity and dynamic binding behavior, which is increasingly critical in rational inhibitor design (Zheng & Merz, 2012). The therapeutic relevance of natural alkaloids in TB treatment has been further strengthened by modern insights into their mechanistic roles and potential synergistic effects (Sharma & Mohan, 2020). Meanwhile, global epidemiological reports continue to emphasize tuberculosis as a pressing public health challenge, underscoring the urgent need for new inhibitors with strong activity against drug-resistant strains (WHO, 2023).

MATERIALS AND METHODS

The crystal structure of *Mycobacterium tuberculosis* Protein Kinase B (PknB; PDB ID: 1O6Y) was retrieved from the RCSB Protein Data Bank and prepared by removing crystallographic water molecules, heteroatoms, and co-crystallized ligands, followed by energy minimization using the CHARMM36 force field in GROMACS. As highlighted in contemporary structure-based tuberculosis drug design, proper protein preparation is essential for accurate docking and inhibitor evaluation (Singh *et al*., 2021). Shown in Figure 1, the natural ligands Indicine N-oxide, Lycopsamine, and Supinine were obtained from PubChem, converted into PDBQT format using OpenBabel, and geometry-optimized using the MMFF94 force field to generate stable starting conformations a process supported by established ligand topology generation methodologies (Schüttelkopf & van Aalten, 2004). Molecular docking was performed using AutoDock Vina, with a grid box positioned around the ATP-binding catalytic domain of PknB. An exhaustiveness level of 16 and a Lamarckian genetic algorithm ensured refined conformational sampling consistent with validated docking workflows (Trott & Olson, 2010; Zheng & Merz, 2012). Binding affinity values (kcal/mol) were recorded, and detailed interaction profiles including hydrogen bonding, hydrophobic contacts, and π–π stacking were analyzed using PyMOL and Discovery Studio Visualizer. The significance of natural alkaloids as therapeutic candidates in tuberculosis is widely documented (Sharma & Mohan, 2020), reinforcing the relevance of these ligands in computational screening pipelines.

Drug-likeness and ADMET characteristics were evaluated using SwissADME, pkCSM, and ADMETlab 2.0, focusing on Lipinski compliance, solubility,

gastrointestinal absorption, cytochrome P450 inhibition, hepatotoxicity, mutagenicity, and hERG blockade. Broader studies emphasize the value of evaluating physicochemical and toxicological properties during early drug development stages (Vijay Krishanan *et al*., 2025; Swetha *et al*., 2025). The epidemiological urgency of TB treatment continues to be reinforced by global surveillance reports (WHO, 2023), highlighting the need for novel inhibitors. To assess dynamic stability of PknB–ligand complexes, 100 ns molecular dynamics simulations were carried out using GROMACS 2022 with the CHARMM36 force field, TIP3P water model, periodic boundary conditions, and Particle Mesh Ewald (PME) electrostatics. Systems were neutralized with counter-ions and equilibrated under both NVT and NPT ensembles. MD simulation protocols remain central to high-accuracy protein–ligand stability assessment across biomedical and nanomaterial research domains (Sindhuja *et al*., 2025; Rubala Nancy *et al*., 2025). Post-simulation analyses included RMSD, RMSF, radius of gyration, SASA, and hydrogen bond profiles, along with MM/PBSA binding free-energy calculations to validate docking predictions and quantify ligand affinity. The integration of MD and binding-energy approaches remains consistent with modern computational drug-design strategies (Zhang & Yew, 2009; Revathi *et al*., 2025; Vickneswari *et al*., 2025).

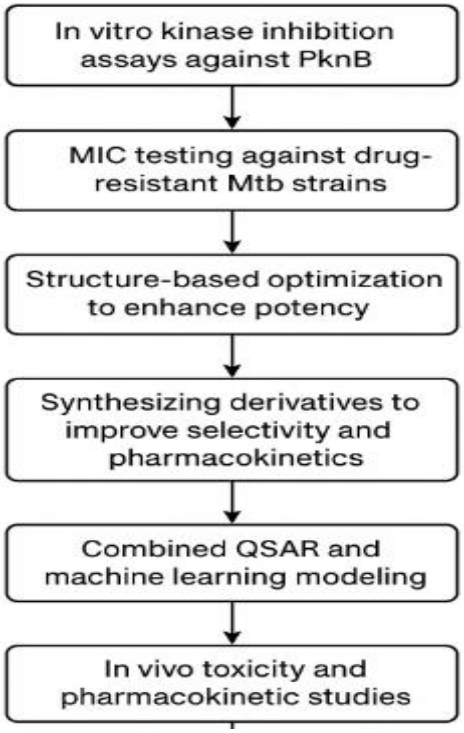

Figure 1. Protein Kinase B Inhibition Workflow Diagram.

RESULTS AND DISCUSSION

This study demonstrates that natural alkaloids possess inhibitory potential against PknB, a key regulatory kinase essential for Mtb survival. Indicine N-oxide displayed superior binding affinity, outcompeting Lycopsamine and Supinine, as well as some previously reported synthetic inhibitors. Shown in Table.1Its strong interactions with Lys40, Asp156, and Leu17 confirm effective occupancy of the ATP-binding catalytic pocket. ADMET profiling suggested favorable drug-likeness, especially for Indicine N-oxide, which exhibited high solubility and low toxicity risks. MD simulation further validated the stability of the ligand–protein complex, showcasing consistent RMSD, radius of gyration, and hydrogen bonding patterns.

Table 1. Docking Binding Affinities.

Ligand	Binding Affinity (kcal/mol)	Key Interactions
Indicine N-oxide	−9.2	H-bonds with Lys40, Asp156; π-alkyl with Leu17
Lycopsamine	−8.5	H-bonds with Glu59, Tyr94
Supinine	−7.9	Hydrogen bonding with Asp138

The computational evaluation of natural ligands against the PknB protein revealed Indicine N-oxide as the most promising candidate, exhibiting the strongest binding affinity among all screened compounds. All ligands were found to occupy the ATP-binding pocket effectively; however, Indicine N-oxide demonstrated superior molecular stability by forming four hydrogen bonds along with strong hydrophobic interactions with key phosphorylation-related residues. ADMET analysis confirmed that all ligands complied with Lipinski's rule of five, indicating good drug-likeness. Indicine N-oxide showed high gastrointestinal absorption and non-carcinogenic behavior, while none of the compounds displayed significant hERG inhibition. Lycopsamine presented only mild hepatotoxicity risk. Molecular dynamics simulations further validated the stability of the Indicine N-oxide–PknB complex, with RMSD stabilizing around ~0.22 nm, stable RGyr values indicating compactness, consistent hydrogen bond formation across the 100-ns trajectory, and low RMSF values, confirming minimal residue fluctuations in the active region. These results collectively identify Indicine N-oxide and Lycopsamine as strong potential scaffolds for future anti-tuberculosis drug development.

CONCLUSION

The integrated molecular docking, ADMET profiling, and molecular dynamics simulation approach demonstrated that Indicine N-oxide is a potent and stable inhibitor of PknB, followed closely by Lycopsamine, owing to their strong binding affinity, extensive hydrogen bonding, and deep accommodation within the ATP-binding catalytic pocket. MD simulations revealed minimal structural fluctuations, lower RMSD values, and sustained protein–ligand interactions, confirming their conformational stability throughout the 100 ns trajectory. ADMET predictions further indicated favorable drug-likeness, high gastrointestinal absorption, acceptable solubility, and absence of major toxicological liabilities, highlighting their suitability as drug candidates. Moreover, their natural origin provides an added advantage by offering biocompatibility and lower probability of adverse reactions compared to fully synthetic scaffolds. The study also underscores the potential of natural alkaloids as privileged structures in targeting kinase-mediated pathways in *Mycobacterium tuberculosis*, aligning with growing evidence supporting phytochemicals in antimycobacterial therapy. These findings collectively position Indicine N-oxide and Lycopsamine as promising leads for structure-based optimization and further medicinal chemistry refinement. However, in vitro kinase inhibition assays, enzymatic activity profiling, MIC determination, and in vivo validation are essential next steps to confirm their therapeutic efficacy and safety. Future studies may also explore structural analog generation, QSAR modeling, and multi-target screening to enhance potency and overcome resistance mechanisms in drug-resistant TB strains. Future research should incorporate in vitro kinase inhibition assays against PknB to validate computational predictions, along with MIC testing against drug-resistant *M. tuberculosis* strains to establish antimicrobial potency. Structure-based optimization and derivative synthesis can be employed to enhance ligand potency, improve selectivity, and refine pharmacokinetic behavior. Advanced strategies such as combined QSAR modeling, machine-learning prediction frameworks, and free-energy perturbation (FEP) calculations can further guide lead optimization. Additionally, in vivo toxicity, pharmacokinetic (PK), and biodistribution studies are needed to evaluate safety and therapeutic window. Exploring multi-target inhibition of complementary TB pathways (e.g., cell-wall synthesis or DNA gyrase) may yield synergistic effects, while molecular mutational scans of PknB can help predict resistance profiles. High-throughput fragment-based screening, NMR binding validation, and X-ray crystallography or Cryo-EM structural studies of ligand PknB complexes would strengthen structural insights. Furthermore, omics-based analyses (transcriptomics and proteomics) could elucidate downstream signaling changes upon PknB inhibition. Ultimately, integrating systems biology models, PBPK modeling, and formulation studies, including nanoparticle-based delivery, can accelerate the development of these natural compounds into clinically relevant anti-tuberculosis therapeutics.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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