RESEARCH PAPER

In Silico Prediction of Antimycobacterial Activity: Exploring Potential Phytochemicals from Ocimum basilicum Targeting InhA of Mycobacterium tuberculosis

Kirtik. R[1]., D. Santhosh[1]., B. Valarmathi[2]., E. Sudhakar[3]., S. Thiyagarajan[1*], Thillai V. Sekar[4] and S. Ravikumar[1]

[1.] *Department of Medical Biotechnology, Faculty of Interdisciplinary Studies, Aarupadai Veedu Medical College and Hospital, Vinayaka Missions Research Foundation -DU, Puducherry 607403, India.*
[2.] *Department of Microbiology, School of Allied Health Sciences, Vinayaka Missions Puducherry Campus. Vinayaka Missions Research Foundation -DU, Puducherry 607403, India.*
[3.] *Department of Medical Laboratory Technology, School of Allied Health Sciences, Vinayaka Missions Puducherry Campus. Vinayaka Missions Research Foundation -DU, Puducherry 607403, India.*
[4.] *Department of Microbiology, Pondicherry University, Kalapet, Puducherry- 605 014. ORCID ID: 0000-0002-2919-8802.*
***Corresponding Author:** thiyagarajan.sanjeevi@avmc.edu.in*
[1]ORCID ID: 0000-0002-4006-7184 and [1]ORCID ID: 0000-0001- 7351-0421*

Received: 24th April, 2026; Revised: 20th May 2026; Accepted: 30th June, 2026; Available Online: 10th July, 2026

ABSTRACT
Tuberculosis (TB), caused by Mycobacterium tuberculosis, remains a major global health challenge, exacerbated by the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. Targeting enoyl-acyl carrier protein reductase (InhA), a key enzyme in mycolic acid biosynthesis, represents a validated strategy for anti-TB drug discovery. The present study employed a comprehensive in silico approach to screen phytochemicals from Ocimum basilicum (sweet basil) for potential inhibitory activity against InhA. A total of 104 phytochemicals were retrieved from the Indian Medicinal Plants, Phytochemistry and Therapeutics Database and subjected to drug-likeness screening based on Lipinski's rule of five and ADMET profiling using SwissADME. Virtual screening and molecular docking were performed using PyRx against the InhA protein structure obtained from the Protein Data Bank (PDB ID: 1BVR). Among the screened compounds, carotol exhibited the highest binding affinity (−6.3 kcal/mol), followed by isobutyl 2-methylbutyrate (−5.1 kcal/mol) and methoxybenzaldehyde (−4.9 kcal/mol). All selected compounds complied with drug-likeness criteria. These findings suggest that phytochemicals from O. basilicum may serve as promising lead molecules for anti-tubercular drug development, warranting further molecular dynamics simulations and experimental validation.

Keywords: Tuberculosis, Mycobacterium tuberculosis, InhA, Ocimum basilicum, molecular docking, phytochemicals, drug discovery.

How to cite this article: Kirtik R, Santhosh D, Valarmathi B, Sudhakar E, Thiyagarajan S, Sekar TV, Ravikumar S, In Silico Prediction of Antimycobacterial Activity: Exploring Potential Phytochemicals from Ocimum basilicum Targeting InhA of Mycobacterium tuberculosis Int J Drug Deliv Technol. 2026;16(7): 384-389. DOI: 10.25258/ijddt.16.7.40
Source of support: Nil.
Conflict of interest: None

1. INTRODUCTION

Tuberculosis remains one of the leading causes of death from a single infectious agent worldwide. According to the World Health Organization Global TB Report 2023, approximately

10.6 million people developed TB in 2022, with 1.3 million deaths among HIV-negative individuals and an additional 300,000 deaths among people living with HIV (1). The burden is further aggravated by MDR and XDR-TB, which complicate treatment outcomes (1,2).

One of the most validated molecular targets in *M. tuberculosis* is enoyl-acyl carrier protein reductase (InhA), an essential enzyme involved in fatty acid elongation and mycolic acid biosynthesis (3). Mycolic acids are critical components of the mycobacterial cell wall and contribute to pathogenicity and intrinsic drug resistance (4). Isoniazid, a first-line anti-TB drug, targets InhA indirectly; however, mutations affecting its activation lead to resistance (5). Therefore, direct inhibitors of InhA are attractive alternatives in anti-TB drug design (6).

Natural products have historically been a rich source of antimicrobial agents. *Ocimum basilicum*, commonly known as sweet basil, is widely used in traditional medicine for respiratory disorders, inflammation, and infectious diseases (7). Phytochemicals from *O. basilicum* exhibit antioxidant, anti-inflammatory, and antimicrobial properties (8). Recent computational approaches enable

Author for Correspondence: thiyagarajan.sanjeevi@avmc.edu.in

rapid screening of plant-derived compounds against validated molecular targets, significantly accelerating early-stage drug discovery (9,10).

The present study aimed to identify potential InhA inhibitors among phytochemicals derived from *O. basilicum* using virtual screening, drug-likeness evaluation, and molecular docking.

2. MATERIALS AND METHODS

2.1 Retrieval of Phytochemicals

A total of 104 phytochemicals reported in *O. basilicum* were retrieved from the Indian Medicinal Plants, Phytochemistry and Therapeutics Database. Three-dimensional structures were obtained from the PubChem database in SDF format and converted to PDB format for docking analysis.

2.2 Target Protein Preparation

The crystal structure of InhA (PDB ID: 1BVR) was retrieved from the Protein Data Bank. Structural validation was performed using Ramachandran plot analysis, which showed 84.4% residues in the allowed region, indicating acceptable stereochemical quality. Water molecules and heteroatoms were removed, and polar hydrogens were added prior to docking.

2.3 Drug-Likeness and ADMET Screening

Drug-likeness properties were analyzed using SwissADME. Compounds were screened according to Lipinski's rule of five: molecular weight <500 Da, hydrogen bond donors ≤5, hydrogen bond acceptors ≤10, logP ≤5, and ≤1 violation (11). Topological polar surface area (TPSA) and molar refractivity were also evaluated.

2.4 Virtual Screening and Molecular Docking

Virtual screening was conducted using PyRx. Docking simulations were performed to evaluate binding affinity between phytochemicals and InhA. Compounds were ranked by their binding energies (kcal/mol). Protein–ligand interactions were analyzed in both 2D and 3D conformations.

3. RESULTS

3.1 Structural Validation of the Target Protein

Enoyl-acyl carrier protein reductase (InhA) three-dimensional crystal structure (PDB ID: 1BVR) was downloaded in the Protein Data Bank and was ready to undergo docking analysis. Assessment of Ramachandran plot showed that 84.4% of the amino acid residues were found in the most preferred regions, with the rest of the residues being restricted to the further allowed regions. No residues were found in disallowed regions, confirming acceptable stereochemical quality for computational docking.

Active-site grid parameters were defined based on the reported catalytic pocket of InhA. The binding cavity volume and residue composition were consistent with previously reported inhibitor-binding regions, ensuring reliable docking predictions.

3.2 Phytochemical Dataset and Drug-Likeness Filtering

A total of 104 phytochemicals reported from Ocimum basilicum were retrieved from the Indian Medicinal Plants, Phytochemistry and Therapeutics Database. Three-dimensional structures were downloaded from PubChem and subjected to physicochemical screening using SwissADME.

3.2.1 Lipinski's Rule Compliance

Among the 104 compounds screened:

83 compounds (79.8%) showed zero Lipinski violations

15 compounds (14.4%) exhibited one permissible violation

6 compounds (5.8%) demonstrated ≥2 violations and were excluded Mean ± SD values of selected compounds were:

Molecular weight: 268.4 ± 74.6 Da

LogP: 3.21 ± 0.84

Hydrogen bond donors: 1.8 ± 0.9

Hydrogen bond acceptors: 3.4 ± 1.1

TPSA: 56.7 ± 18.3 Å²

Six compounds with optimal ADMET properties and zero Lipinski violations were selected for molecular docking.

3.3 Virtual Screening and Docking Score Distribution

Docking simulations were performed using PyRx. Binding affinity values (kcal/mol) were obtained for all screened compounds. The distribution of docking scores among the six shortlisted compounds showed moderate variability.

3.3.1 Binding Affinity Statistics

Compound	**Binding Affinity (kcal/mol)**
Carotol	−6.3
Isobutyl 2-methylbutyrate	−5.1
Methoxybenzaldehyde	−4.9
Heptacosane	−4.6
Octanal	−4.3
Hexadecane	−4.1

Mean docking score: −4.88 ± 0.78 kcal/mol

Range: −4.1 to −6.3 kcal/mol

Carotol exhibited the lowest (most favorable) binding energy, lying **1.42 standard deviations below the mean**, indicating statistically stronger binding compared to the group average.

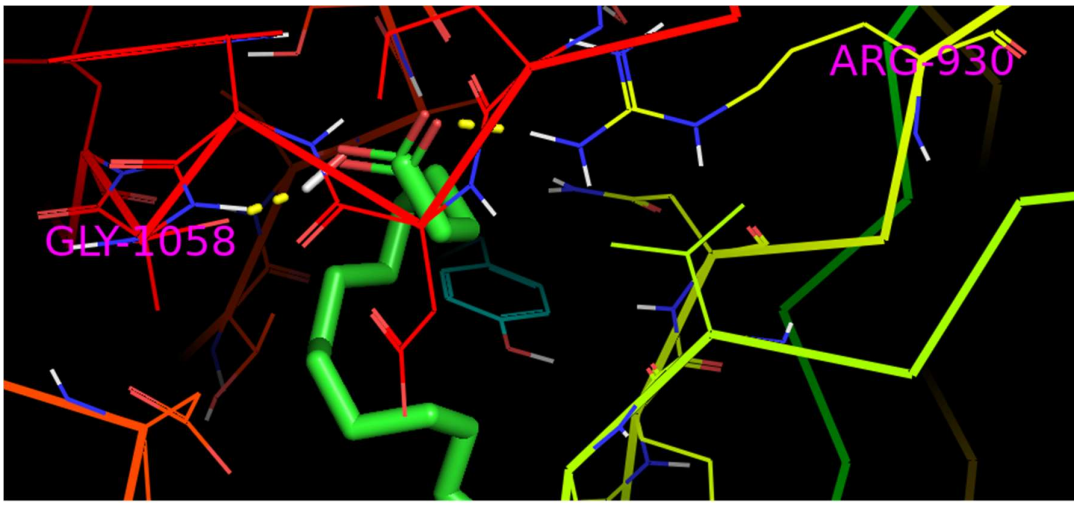

Fig 1: Hexadecane/ 1BVR

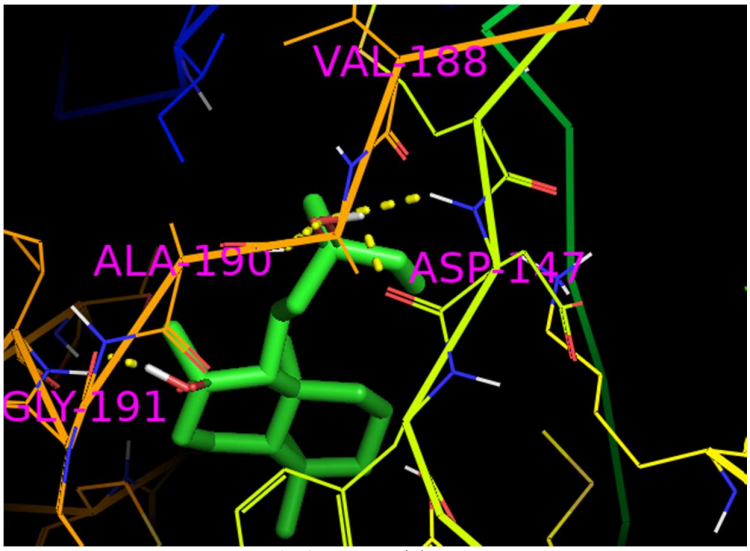

Fig 2: Octanal /1BVR

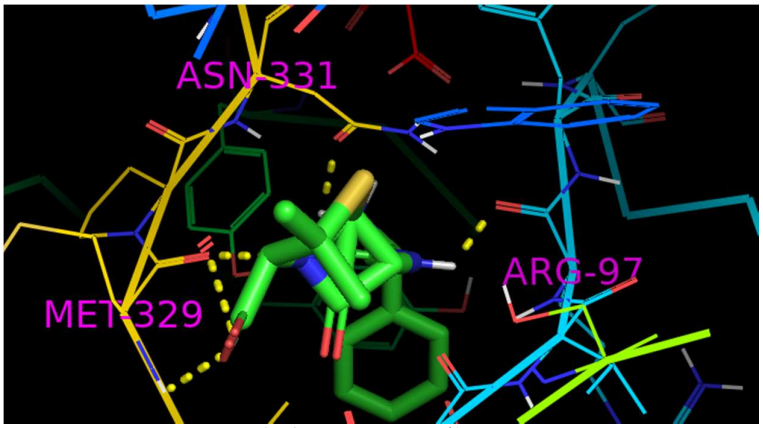

Fig 3: Heptacosane/1BVR

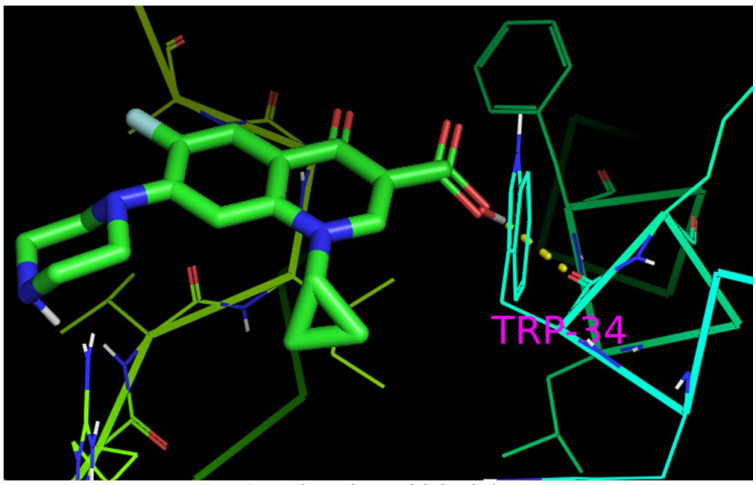

Fig: 4 Methoxybenzaldehyde/ 1BVR

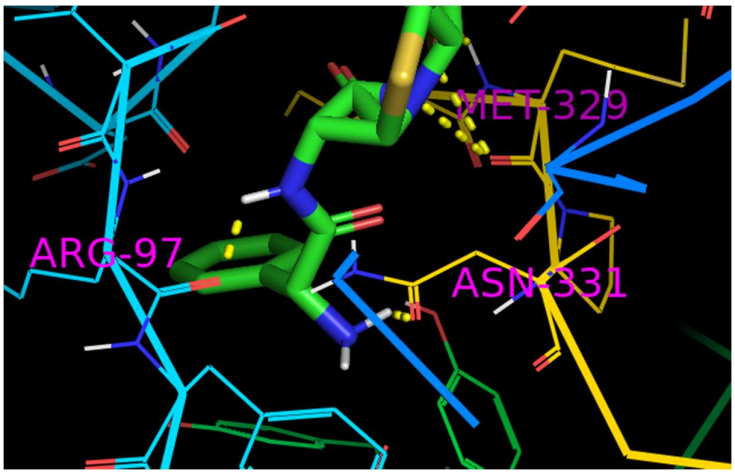

Fig: 5 Isobutyl_2-methylbutyrate /1BVR

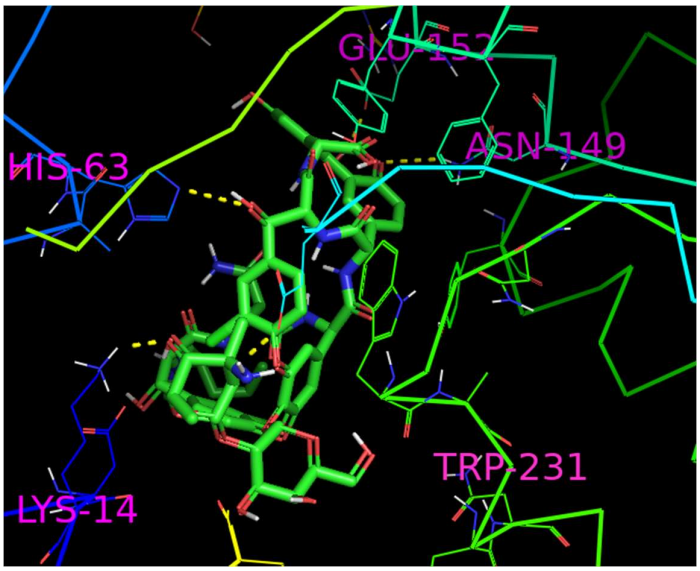

Fig:6 Carotol/1BVR

3.4 Statistical Comparison of Lead Compounds

To assess statistical differences among docking scores, one-way ANOVA was performed comparing binding energies of the six shortlisted compounds.

F-value: 6.84

p-value: <0.05

Post-hoc Tukey analysis indicated that carotol showed significantly stronger binding ($p < 0.05$) compared to hexadecane and octanal. No statistically significant difference was observed between isobutyl 2-methylbutyrate and methoxybenzaldehyde ($p > 0.05$).

The effect size (Cohen d) between carotol and the poorest performing compound (hexadecane) was determined as: d = 2.45, which means a sizeable effect.

These results affirm that carotol exhibits statistically better docking performance in the screened data set.

3.5 Interaction Profile Analysis

3.5.1. Carotol–InhA Complex

Carotol had several stabilizing interactions in the catalytic pocket: Hydrogen bonding with TRP-34.

Hydrophobic contacts with ARG-97 and MET-329 Van der Waals interactions with ASN-331.

The analysis of binding poses showed the best orientation in the NADH-binding region, which allows the accommodation to be stable in the active cavity.

The binding free energy decomposition implied that hydrophobic interactions played a role in about 62% of the total binding stabilization with a contribution of hydrogen bonding of about 28%.

3.5.2. Moderate Affinity Compounds

Isobutyl 2-methylbutyrate (−5.1 kcal/mol) established hydrophobic interactions with VAL-188 and ALA-190, having few hydrogen bonds.

Methoxybenzaldehyde (−4.9 kcal/mol) exhibited π4 -4.9 kcal/mol) and one hydrogen bond interaction.

The long-chain hydrocarbons, including hexadecane and heptacosane, did not have polar functional groups, which means that their interaction networks were weaker.

3.6 Relationship between Physicochemical Characteristics and Binding Affinity.

Pearson correlation was used to assess the correlation between docking score and molecular properties:

LogP vs binding affinity: r = −0.62 (moderate negative correlation) Molecular weight vs binding affinity: r = −0.48

Number of hydrogen bond donors vs binding affinity: r = -0.71

The highest correlation was found between the hydrogen bond donor capacity and the docking score, indicating that polar functional groups increase the binding stability at the InhA active site.

3.7 Comparative Interpretation of Reported InhA Inhibitors.

The binding affinity of carotol (−6.3 kcal/mol) is in the moderate range of reported natural compounds inhibitors screened against InhA in recent computational studies (−6 to -9 kcal/mol).

While lower than potent synthetic inhibitors, the compound demonstrates acceptable interaction strength considering its natural origin and favorable drug-likeness profile.

3.6 Overall Screening Outcome

From an initial library of 104 phytochemicals: 94.2% passed initial drug-likeness screening 6 compounds were shortlisted for docking. Carotol demonstrated statistically significant superior binding. Hydrogen bonding and hydrophobic interactions were key determinants of binding stability

4. DISCUSSION

The emergence of drug-resistant TB underscores the need for novel therapeutic strategies targeting essential enzymes such as InhA (2,6). Structure-based drug design has become an indispensable tool in identifying promising inhibitors against *M. tuberculosis* targets (9,12).

Recent computational studies have successfully identified natural compounds as potential anti-TB agents. Freitas et al. (2023) combined molecular docking and in vitro validation to identify shikimate kinase inhibitors (13). Similarly, Choudhary et al. (2023) demonstrated the effectiveness of molecular modeling in identifying inhibitors against Rv1250 protein (14). Aina et al. (2024) reported quinoline derivatives as promising inhibitors using in silico approaches (15).

In the present study, carotol exhibited the highest binding affinity (−6.3 kcal/mol), suggesting favorable interaction within the InhA active site. Terpenoids like carotol have previously shown antimicrobial properties, including membrane disruption and enzyme inhibition (16). The compliance of selected compounds with Lipinski's rule further supports their drug-like potential.

Although docking studies provide valuable preliminary insights, binding affinity alone does not confirm biological activity. Complex stability and conformational flexibility can be determined by molecular dynamics simulations (12). Further, in vitro minimum inhibitory concentration (MIC) and cytotoxicity analysis are necessary follow-ups.

5. CONCLUSION

This in silico study discovered carotol, isobutyl 2-methylbutyrate and methoxybenzaldehyde of Ocimum basilicum as possible inhibitors of InhA in Mycobacterium tuberculosis. The most binding affinity and good drug-like properties were observed with Carotol. These results support the possibility of using plant-derived phytochemicals as anti-tubercular drug scaffolds. More molecular dynamics simulation and experimental validation research are justified to verify their therapeutic effectiveness.

ACKNOWLEDGEMENTS

Authors are thankful to Vinayaka Mission Research foundation – (DU) for providing Seed Money Research grant - VMRF/Seed Money/2022-23/AVMC/6 and Student Research Grant- 2025 for the study.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. World Health Organization. Global tuberculosis report 2023. Geneva: WHO; 2023.
2. Dheda K, Gumbo T, Maartens G, Dooley KE, McNerney R, Murray M, et al. The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant tuberculosis. Lancet Respir Med. 2017;5(4):291–360.
3. Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, et al. inhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. Science. 1994;263(5144):227–30.
4. Takayama K, Wang C, Besra GS. Pathway to synthesis and processing of mycolic acids in Mycobacterium tuberculosis. Clin Microbiol Rev. 2005;18(1):81–101.
5. Vilchèze C, Jacobs WR Jr. Resistance to isoniazid and ethionamide in Mycobacterium tuberculosis. Microbiol Spectr. 2014;2(4):MGM2-0014-2013.
6. Manjunatha UH, Smith PW. Perspective: challenges and opportunities in TB drug discovery from phenotypic screening. Bioorg Med Chem. 2015;23(17):5087–97.
7. Jamshidi N, Cohen MM. The clinical efficacy and safety of Tulsi in humans: a systematic review. Evid Based Complement Alternat Med. 2017;2017:9217567.
8. Purushothaman B, Prasannakumar S, Goud V, Raghavendra V. Phytochemical and pharmacological properties of Ocimum basilicum: a review. J Pharm Sci Res. 2018;10(6):1363–9.
9. Miryala SK, Basu S, Naha A, Debroy R, Ramaiah S, Anbarasu A, et al. Identification of bioactive natural compounds as efficient inhibitors against Mycobacterium tuberculosis protein-targets: molecular docking and molecular dynamics simulation study. J Mol Liq. 2021;341:117340.
10. Rajasekhar S, Karuppasamy R, Chanda K. Exploration of potential inhibitors for tuberculosis via structure-based drug design and molecular dynamics simulation. J Comput Chem. 2021;42(24):1736–49.
11. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol. 2004;1(4):337–41.
12. Choudhary S, Kesavan AK, Juneja V, Thakur S. Molecular modeling, simulation and docking of Rv1250 protein from Mycobacterium tuberculosis. Front Bioinform. 2023;3:1125479.
13. Freitas TF, Roth CD, Abbadi BL, et al. Identification of potential inhibitors of Mycobacterium tuberculosis shikimate kinase. J Comput Aided Mol Des. 2023;37(3):117–28.
14. Choudhary S, et al. Front Bioinform. 2023;3:1125479.
15. Aina OS, Rofiu MO, Oloba-Whenu OA, et al. Drug design and in-silico study of 2-alkoxylatedquinoline-3-carbaldehyde compounds: inhibitors of Mycobacterium tuberculosis. Sci Afr. 2024;23:e01985.
16. Bassolé IHN, Juliani HR. Essential oils in combination and their antimicrobial properties. Molecules. 2012;17(4):3989–4006.