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# Cinnamate-amine hybrids: Antituberculosis activity and molecular docking

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**ABSTRACT**

Antituberculosis activity and molecular docking experiments were performed on 14 cinnamate-amine hybrids. Cinnamamides **1a,b,c,d,i,j** effectively inhibited the growth of *Mycobacterium tuberculosis* $H_{37}Rv$, with cinnamamide **1c** having the highest inhibition effects with a minimum inhibitory concentration value of 3.13 µg/ml. Based on the docking results, the molecule of cinnamamide **1c** (binding energy of −4.13 kcal/mol) was found to be stretched from the edge of the active site to its sub-binding pocket, referred to as an extended conformation when binding to the InhA active site. Cinnamamide **1c** turned out to be an inhibitor whose potency is unaffected by the conserved interaction network with the catalytic residue Tyr158. These findings suggest these cinnamate-amine hybrids as potential lead compounds in developing new antituberculosis drugs.

## INTRODUCTION

Tuberculosis (TB) is a disease caused by *Mycobacterium tuberculosis* that spreads through the air and increases the risk of catching the Human Immunodeficiency Virus [1,2]. It ranks among the top 10 worldwide causes of death [3]. The death toll from TB reached 1.5 million people in 2020, equal to the COVID-19 death toll of 1.8 million people [4].

Drug resistance is the principal obstacle in TB treatment. It significantly impacts patients' clinical and financial aspects [5]. The two common types of drug-resistant TB are rifampicin-resistant TB (RR-TB) and multidrug-resistant TB (MDR-TB), of which the latter refers to TB that is resistant to at least the two most effective first-line TB drugs, namely rifampicin and isoniazid (INH) [6]. An estimated 500,000 new cases of RR/MDR-TB are reported globally each year, requiring treatments with second-line TB drugs, which are less effective, pricier, and more toxic than first-line TB drugs [7]. Overcoming drug resistance in TB is an urgent matter in the global fight against antimicrobial resistance [8]. Bedaquiline was the first antituberculosis drug with a novel mode of action against mycobacteria to be approved for DR-TB treatment by the Food and Drug Administration in over four decades [9]. However, this drug has been shown to cause several adverse effects, including gastrointestinal problems, otovestibular dysfunction, vomiting, dizziness, headaches, arthralgia, and prolonged QT interval, which can lead to a fatal cardiac rhythm [10]. These circumstances highlight the importance of developing new antituberculosis drugs to improve and complement existing treatment regimens [11].

INH is one of the most effective first-line TB drugs, possessing a minimum inhibitory concentration (MIC) value of 0.02–0.1 µg/ml [12]. However, its usage has been limited due to resistance. INH primarily inhibits the InhA enzyme, which produces long-chain fatty acids, particularly mycolic acids essential for *M. tuberculosis* survival [13]. It is worth noting that molecular hybridization of experimentally proven antituberculosis molecules with pharmacophores of several bioactive compounds to create new hybrid molecules that

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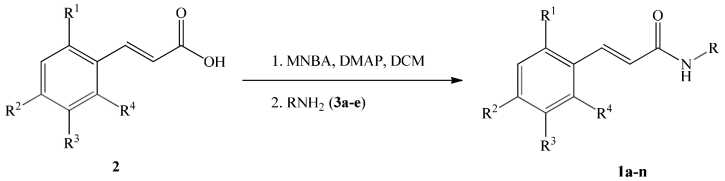
are expected to have greater affinity and efficacy against *M. tuberculosis* can have the potential to delay the emergence of drug resistance. Several INH-hybrid compounds with antituberculosis activity have been reported in numerous studies [14–17].

On the other hand, cinnamic acid and its derivatives are compounds that are commonly present in plant food and have been reported to exhibit a variety of biological activities, including antituberculosis [18,19], α-glucosidase inhibitor [20,21], and anti-inflammatory [22,23]. Cinnamic acid has been shown to have low toxicity in humans [24]. Furthermore, it has also been reported that hydrazide-hydrazones are a powerful and non-toxic antituberculosis agent with InhA inhibitory activity [25]. In our previous published work, we applied a hybridization strategy to embed the hydrazide-hydrazone structural motif into cinnamic acid to generate cinnamate-amine hybrid molecules [26]. Meanwhile, in this study, we present the antituberculosis

**Table 1.** Synthesis of cinnamamides **1a-n**.




**2** **1a-n**

**1a** $R^1$ = H $R^2$ = H $R^3$ = H $R^4$ = H

**1b** $R^1$ = H $R^2$ = $N(Me)_2$ $R^3$ = H $R^4$ = H

$R^2$ = Br $R^3$ = H

**1d** $R^1$ = H $R^2$ = F $R^3$ = H $R^4$ = H

**1e** $R^1$ = Cl $R^2$ = F $R^3$ = Cl $R^4$ = H

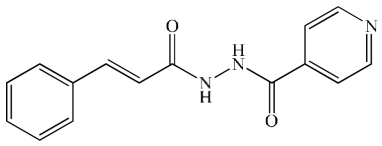
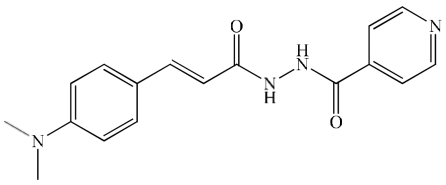
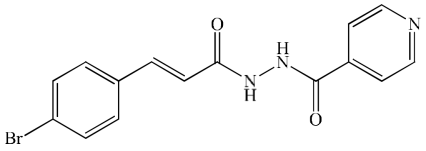
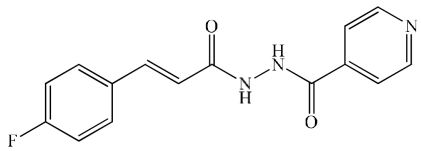
**1f** $R^1$ = F $R^2$ = H $R^3$ = H $R^4$ = F

**1g** $R^1$ = Me $R^2$ = H $R^3$ = H $R^4$ = H

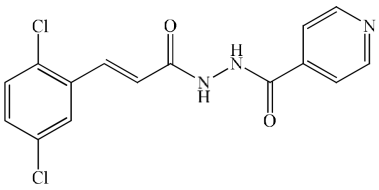
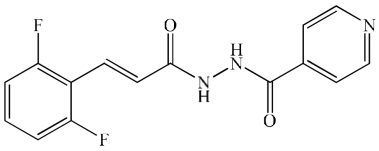
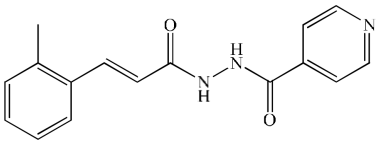
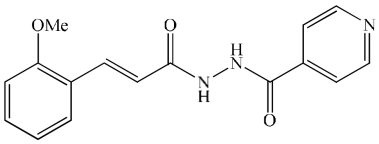
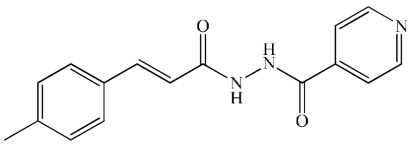
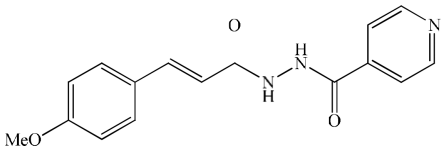
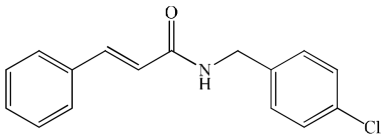
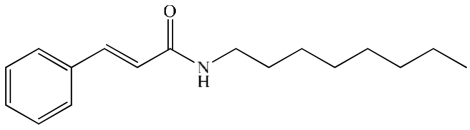
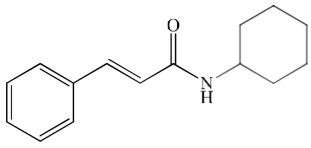
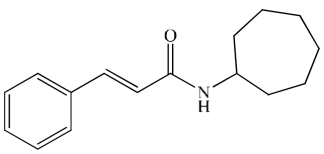
**1h** $R^1$ = OMe $R^2$ = H $R^3$ = H $R^4$ = H

**1i** $R^1$ = H $R^2$ = Me $R^3$ = H $R^4$ = H

**1j** $R^1$ = H $R^2$ = OMe $R^3$ = H $R^4$ = H

| Entry | Amine | $R^1$ | $R^2$ | $R^3$ | $R^4$ | Cinnamamides |
|---|---|---|---|---|---|---|
| 1 | **Isoniazid (3a)** | H | H | H | H |  |
| 2 | **INH (3a)** | H | N(Me)2 | H | H |  |
| 3 | **INH (3a)** | H | Br | H | H |  |
| 4 | **INH (3a)** | H | F | H | H |  |

***Continued***

| Entry | Amine | $R^1$ | $R^2$ | $R^3$ | $R^4$ | Cinnamamides |
|---|---|---|---|---|---|---|
| 5 | **INH (3a)** | Cl | H | Cl | H |  |
| 6 | **INH (3a)** | F | H | H | F |  |
| 7 | **INH (3a)** | Me | H | H | H |  |
| 8 | **INH (3a)** | OMe | H | H | H |  |
| 9 | **INH (3a)** | H | Me | H | H |  |
| 10 | **INH (3a)** | H | OMe | H | H |  |
| 11 | **4-Chloro-benzylamin e (3b)** | H | H | H | H |  |
| 12 | **Octylamine (3c)** | H | H | H | H |  |
| 13 | **Cyclohexyl-amine (3d)** | H | H | H | H |  |
| 14 | **1n** | H | H | H | H | |
| 15 | INH | - | - | - | - | |
| 16 | Cyclohexyl-amine (3d) | - | - | - | - |  |

activity of cinnamamides based on *in vitro* and molecular docking experiments.

## MATERIAL AND METHODS

### Antituberculosis study

The antimycobacterial activity of the synthesized compounds **1a-n** was investigated by adapting the antimycobacterial activity test method of pyrazinamide [27]. Compounds **1a-n** were first prepared as 1,000 µg/ml stock solutions in DMSO 20%. The solutions were then serially diluted to produce solutions with concentrations of 25, 12.5, 6.25, 3.13, 1.56, and 0.78 µg/ml. Each 100 µl of the test solution was put into a 96-well plate and further added with 100 µl of *M. tuberculosis* $H_{37}$Rv suspension. The plate was then covered, sealed with plastic clips, and incubated at 37°C for 7 days. Subsequently, 30 µl of 0.01% resazurin was added to the plate and incubated at 37°C for 1 day, after which the color change was observed. Resazurin reduction is indicated by a shift in hue from blue to pink, indicating bacteria growth. Each test compound was subjected to a triple measurement (*triplo*). INH was used as a standard drug and tested at concentrations of 1.0, 0.5, and 0.25 µg/ml using the same procedure as the test compounds **1a-n**.

### Molecular docking studies

The molecular docking of cinnamamides on the crystallographic structure of the enoyl-acyl carrier protein reductase (InhA) of *M. tuberculosis* was performed using Autodock 4.2.6 [28]. The 3D structure of InhA protein (PDB ID: 3FNG) was downloaded from the Protein Data Bank (www.rcsb.org) and then prepared using MGLTools 1.5.6 by eliminating the water and non-protein molecules, adding polar hydrogens, and adding Kollman charges. The 3D structure of cinnamamides was created and minimized using MMFF94 in MarvinSketch 20.18. The docking pocket is located in the center of the active site and is large enough to cover the entire active site. The docking simulation was run using the Lamarckian genetic algorithm. The docking outcomes were visualized using Biovia Discovery Studio 2020. Validation of the docking parameter in this study was carried out by redocking the native ligand, namely 5-(cyclohexylmethyl)-2-(2,4-dichlorophenoxy) phenol (JPL).

## RESULTS AND DISCUSSION

### Antituberculosis activity

Synthesis compounds **1a-n** have previously been detailed in prior work [26]. The synthesis of compounds **1a-n** is shown in Table 1. Compounds **1a-n** were examined for their antimycobacterial activity against *M. tuberculosis* $H_{37}$Rv using a redox indicator-based colorimetric method, namely Resazurin Microtiter Assay [29]. A color change identifies drug resistance due to the reduction of resazurin that is directly proportional to the amount of *M. tuberculosis* living in the medium so that it can be used to determine the MIC of a drug [30]. The obtained MIC values of cinnamamides **1a-n** ranged from 3.13 to more than 25 µg/ml, as shown in Table 2. INH was the control standard, with a MIC value of 0.25 µg/ml [31].

Different inhibitory effects were seen on *M. tuberculosis* by the cinnamamides **1c** and **1d** with an electron-withdrawing group substituent (F, Br) at the *para* position in the phenyl ring of the cinnamic skeleton. Cinnamamide **1c** with the bromo group inhibited *M. tuberculosis* at a 3.13 µg/ml concentration. However, cinnamamide **1d** with the fluoro group exhibited the same growth-inhibitory effects on *M. tuberculosis* as cinnamamide **1a** (MIC >3.13 µg/ml), which has no substituent group in its phenyl ring. It was discovered

**Table 2.** The antituberculosis activity of cinnamamides **1a-n**.

| Entry | Cinnamamides | R | $R^1$ | $R^2$ | $R^3$ | $R^4$ | MIC (µg/ml) | MIC (µM) |
|---|---|---|---|---|---|---|---|---|
| 1 | **1a** | Isonicotinohydrazide | H | H | H | H | >3.13 | >0.012 |
| 2 | **1b** | Isonicotinohydrazide | H | N(Me)2 | H | H | >3.13 | >0.010 |
| 3 | **1c** | Isonicotinohydrazide | H | Br | H | H | 3.13 | 0.0090 |
| 4 | **1d** | Isonicotinohydrazide | H | F | H | H | >3.13 | >0.011 |
| 5 | **1e** | Isonicotinohydrazide | Cl | H | Cl | H | 25 | 0.074 |
| 6 | **1f** | Isonicotinohydrazide | F | H | H | F | >25 | >0.082 |
| 7 | **1g** | Isonicotinohydrazide | Me | H | H | H | >25 | >0.089 |
| 8 | **1h** | Isonicotinohydrazide | OMe | H | H | H | 25 | 0.084 |
| 9 | **1i** | Isonicotinohydrazide | H | Me | H | H | >3.13 | >0.011 |
| 10 | **1j** | Isonicotinohydrazide | H | OMe | H | H | >3.13 | >0.011 |
| 11 | **1k** | 4-Chlorobenzylamine | H | H | H | H | >25 | >0.092 |
| 12 | **1l** | *n*-Octylamine | H | H | H | H | >25 | >0.096 |
| 13 | **1m** | Cyclohexylamine | H | H | H | H | >25 | >0.11 |
| 14 | **1n** | Cyclohepthylamine | H | H | H | H | >25 | >0.10 |
| 15 | INH | - | - | - | - | - | 0.25 | 0.0018 |
| 16 | Pyrazinamide [33] | - | - | - | - | - | 100 | 0.81 |

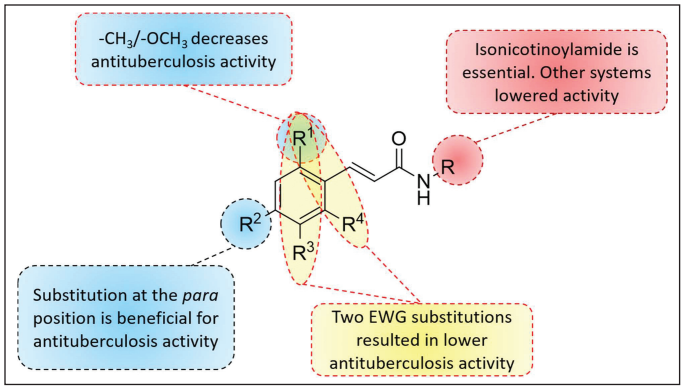



**Figure 1.** Structure-activity relationship analysis for the antituberculosis activity of cinnamamides.

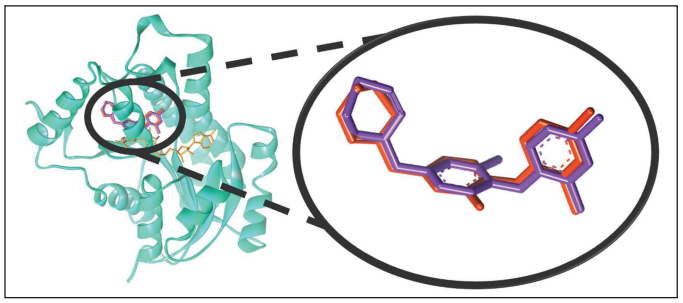


**Figure 2.** Binding pose of redocked JPL (red) with an RMSD value of 0.78 Å when superimposed on native JPL (purple) in the active site of InhA protein (PDB ID: 3FNG). Orange color represents the NAD cofactor.

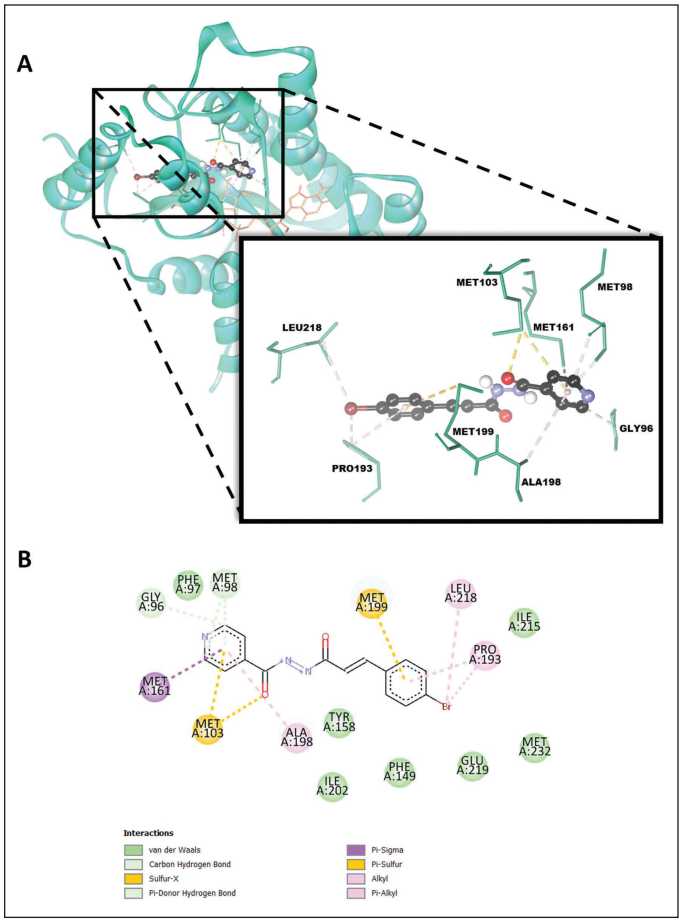



**Figure 3.** The binding mode of cinnamamide 1e is shown in (A) a three-dimensional view (note that NAD is intentionally not shown in magnification to clarify visualization) and (B) a two- dimensional view in the active site of the InhA protein (PDB ID: 3FNG).

that introducing two electron-withdrawing groups (F or Cl) at the *ortho* or *meta* positions in cinnamamides **1e** and **1f** reduced the inhibitory effects. Cinnamamide **1e** was able to inhibit *M. tuberculosis* growth more potently than cinnamamide **1f** with a MIC value of 25 µg/ml.

Introducing the electron-donor group (Me or OMe) at the *para* position (compounds **1i** and **1j**) in the phenyl ring of the cinnamic skeleton was found to be more advantageous for activity than at the *ortho* position (compounds **1g** and **1h**), with a MIC value of >3.13 µg/ml, which was the same as cinnamamide **1b**. Cinnamamide **1h** with substitution of the methoxy group at the *ortho* position was able to inhibit *M. tuberculosis* growth with a MIC value of 25 µg/ml. However, cinnamamide **1g** with substitution of the methyl group could not inhibit *M. tuberculosis* growth.

According to the research findings, replacing the isonicotinohydrazide moiety with an amine group in a cyclic, acyclic, or aromatic group diminishes the inhibitory effect of cinnamamide. Cinnamamides **1k,l,m,n** exhibited inhibitory activity four times lower than cinnamamide **1a**, with MIC values greater than 25 µg/ml. The structure-activity relationship of cinnamamides is depicted in Figure 1.

### Molecular docking studies

Molecular docking studies were conducted to estimate the binding energy of cinnamamide **1c** as it binds to the active site of the InhA protein (PDB ID: 3FNG) and to comprehend its antituberculosis mode of action (Fig. 2) Redocking of JPL at the active site of the InhA protein produces an RMSD value of 0.78 Å (Fig. 3), which is less than 2 Å, meaning that the parameters employed can replicate the native binding pose and interaction of JPL.

The binding energy of the cinnamamide **1c** was −4.13 kcal/mol, as shown in Table 3. This compound is bound to InhA in an extended conformation, where its molecules stretch from the edge of the binding pocket to the sub-binding pocket of the InhA active site, as seen in Figure 3. Most renowned InhA direct inhibitors also bind to the protein in the extended conformation [31]. The phenyl ring of the cinnamoyl skeleton occupied the sub-binding pocket of the InhA active site. It formed hydrophobic π-alkyl and π-sulfur interactions with hydrophobic residues on the substrate binding loop (SBL) region, namely Pro193 and Met199. At the same time, the bromo group at the cinnamoyl skeleton formed hydrophobic alkyl interactions with Leu218 at the sub-binding pocket and Pro193. The isonicotinic moiety of cinnamamide **1c** occupied the edge of the binding pocket. The carbonyl group bound to the pyridinyl ring formed a sulfur-X interaction with Met103 at the edge of the binding pocket. The pyridinyl ring formed

**Table 3.** Binding energy and residue interactions of cinnamamide **1c** on the active site of InhA protein (PDB ID: 3FNG).

| Compound | Binding energy (kcal/mol) | Binding site residue | Interacting units of compound | Interaction type |
|---|---|---|---|---|
| **1c** | −4.13 | Leu218 | bromo | Hydrophobic alkyl |
| | | Met161 | pyridinyl | Hydrophobic π-σ |
| | | Met103 | CO isonicotinoyl | Sulfur-X |
| | | | pyridinyl | π-sulfur |
| | | Ala198 | pyridinyl | Hydrophobic π-alkyl |
| | | Gly96 | pyridinyl | CH-bond |
| | | Met98 | pyridinyl | CH-bond |
| | | Pro193 | bromo | Hydrophobic alkyl |
| | | | phenyl | Hydrophobic π-alkyl |
| INH | -5.25 | Tyr158 | NH hydrazide | H-bond |
| | | | NH hydrazide | H-bond |
| | | Met199 | pyridinyl | Hydrophobic π-σ |
| | | Phe149 | pyridinyl | Hydrophobic π-π stacked |
| JPL | -10.98 | NAD | A-ring hydroxy | Hydrophobic π-alkyl |
| (redocked) | (0.78Å) | | -O- | H-bond |
| | | | Chlorine | CH-bond |
| | | Leu218 | cyclohexyl | Hydrophobic alkyl |
| | | Tyr158 | cyclohexyl | Hydrophobic π-alkyl |
| | | Phe149 | cyclohexyl | Hydrophobic π-alkyl |
| | | | A-ring hydroxy | Hydrophobic π-π T-shaped |
| | | Met161 | B-ring chlorines | Hydrophobic π-σ |
| | | Phe97 | chlorine | Hydrophobic π-alkyl |
| | | Met103 | B-ring chlorines | π-sulfur |
| | | | chlorine | Hydrophobic alkyl |
| | | Ala198 | B-ring chlorines | Hydrophobic π-σ |
| | | | chlorine | Hydrophobic π-alkyl |
| | | Met199 | A-ring hydroxy | Hydrophobic π-alkyl |
| | | | B-ring chlorines | Hydrophobic π-alkyl |
| | | | cyclohexyl | Hydrophobic alkyl |

interactions with several residues in different areas. This ring formed a hydrophobic π-σ with Ala198 on the SBL and a hydrophobic π-alkyl with Met161 at the sub-binding pocket. It also formed two carbon-hydrogen and one π-donor hydrogen bonds with Gly96 and Met98 at the edge of the binding pocket. Notably, cinnamamide **1c** did not form any hydrogen bonds with the catalytic residue Tyr158 and only interacted via van der Waals interactions, indicating that its potency does not rely on the conserved interaction network with Tyr158 [32]. Van der Waals contacts were also formed between cinnamamide **1c** and the side chain residues Phe97, Phe149, Ile202, Ile215, Glu219, and Met232.

## CONCLUSION

Studies on the antituberculosis activity of cinnamamide derivatives have been carried out *in vitro* and *in silico*. *In vitro* antituberculosis studies showed that introducing a substituent at the *para* position of the cinnamoyl moiety in a cinnamate-INH hybrid is more advantageous than substituting at other positions. Cinnamamide **1c** with a bromo group at the *para* position of the cinnamoyl moiety has the greatest inhibitory effect on *M. tuberculosis* growth, with a MIC value of 3.13 µg/ml. Docking experiments revealed that cinnamamide **1c** inhibits the activity of InhA (3FNG) through an extended binding confirmation whose potency is independent of the conserved interaction network with Tyr158. These findings pave the way for developing an InhA inhibitor to help improve TB treatment regimens.

## ACKNOWLEDGMENT

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## AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

## CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

## DATA AVAILABILITY

All data generated and analyzed are included within this research article.

## USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

## PUBLISHER'S NOTE

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