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The Potential of Isoniazid Derivatives as Anti-Tuberculosis Drugs Targeting 6MA8: In Silico Study

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ABSTRACT.

Tuberculosis (TB) is a contagious disease caused by *Mycobacterium tuberculosis*. Isoniazid, a synthetic antimicrobial agent, remains one of the most crucial first-line medications in TB therapy. Enhancing TB treatment strategies can be achieved through structural modifications of existing drugs. This study investigates the potential of isoniazid derivatives as anti-tuberculosis agents targeting the CYP3A4 protein complexed with a small-molecule inhibitor (PDB ID: 6MA8) and evaluates their toxicity profiles using in silico methods. The ligands analyzed include isoniazid derivatives 1–5, with isoniazid as a reference compound and protoporphyrin as the native ligand. The structure of ligands was prepared using Avogadro software and optimized with ORCA software. The crystal structures of 6MA8 were retrieved from the PDB database and further validated using YASARA. In silico methods such as molecular docking and ProTox prediction were employed to evaluate the potential of these isoniazid derivatives as anti-TB drugs. The interactions were visualized using Biovia Discovery to assess the interaction between the isoniazid derivatives and the receptor. The results showed derivative 4 exhibited the lowest binding affinity (-71.56 kcal/mol) compared to isoniazid (-65.90 kcal/mol), derivative 1 (-63.65 kcal/mol), derivative 2 (-67.01 kcal/mol), derivative 3 (-67.37 kcal/mol), derivative 5 (-69.02 kcal/mol), and native ligand (-182.68 kcal/mol). Biovia Discovery Studio simulations indicated that the isoniazid derivatives interacted with 6MA8 via conventional hydrogen bonds, carbon-hydrogen bonds, and other interactions. The toxicity analysis showed that the derivatives had safe LD50 values, supporting their safety profiles. These results suggest that isoniazid derivatives have promising potential as anti-tuberculosis agents targeting 6MA8.

Keywords: 6MA8, Isoniazid derivative, in silico, molecular docking

1. INTRODUCTION

Tuberculosis (TB) remains one of the deadliest infectious diseases worldwide, caused by the bacterium *Mycobacterium tuberculosis* [1]. Despite the availability of effective treatments, the emergence of drug-resistant TB strains poses a significant challenge to global health. TB continues to be a global health concern, with over 10 million new cases reported annually and a high burden in low- and middle-income countries [2]. The World Health Organization (WHO) recommends a standard 6-month treatment for active, pulmonary, and pharmacoresistant TB, using different combinations of four first-line medications: isoniazid, rifampin, ethambutol, and pyrazinamide. Unfortunately, the complex long-term administrative duration leads to non-compliance with treatment, leading to failure and relapses, causing the bacterium to become resistant to anti-TB medications. Primary and secondary resistance contribute to the transfer of resistant strains to newer hosts and the development of drug resistance to two or more drugs. Resistance encompasses overexpressed drug targets resulting from mutation, alterations in drug targets, and drug efflux processes [3]. The primary challenges in TB treatment include lengthy therapy regimens, patient non-compliance, and the rapid emergence of multidrug-

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resistant (MDR) and extensively drug-resistant (XDR) TB strains [4]. These issues necessitate the development of novel drugs that target critical bacterial pathways and ensure effectiveness against resistant strains [5]. Developing innovative therapeutic strategies, particularly by modifying existing drugs, is essential to address these challenges.

Isoniazid is a widely used first-line drug in TB therapy due to its potent antimicrobial activity and relatively low cost [6]. It acts by inhibiting the synthesis of mycolic acids, essential components of the *Mycobacterium tuberculosis* cell wall [7]. However, its efficacy is increasingly threatened by the rise of isoniazid-resistant strains. Structural modifications of isoniazid can yield derivatives with enhanced pharmacological properties, including better specificity, reduced resistance, and improved pharmacokinetics [8]. These derivatives hold promise as next-generation TB therapeutics [9]. Several studies have synthesized and carried out activity tests as an anti-tuberculosis agent, including isoniazid derivatives with bulky side chains [10]. So, recently, we have designed a computational study of some isoniazid derivatives with simple side chain products.

The discovery and development of new, efficacious anti-TB drugs that target novel or existing cellular pathways in different ways is of paramount importance. On the other hand, besides structural differential analysis of the ligand-protein complex, quantitative structure–activity relationship (QSAR) studies have been employed to validate the understanding [11]. Cytochrome P450 (CYP) enzymes are widely distributed and function in the oxidation of diverse endogenous and exogenous substances, contributing significantly to cellular metabolism and homeostasis. Among these, CYP3A4 represents the most abundant and catalytically versatile isoform in humans, being responsible for metabolizing the majority of therapeutic drugs. The CYP3A4 protein complexed with a small-molecule inhibitor (PDB ID: 6MA8) has therefore gained attention as a potential molecular target in the search for novel anti-tuberculosis agents [12]. Its critical role and unique structural properties make it an ideal candidate for targeted drug design. Computational studies, including molecular docking, enable the exploration of isoniazid derivatives' interactions with 6MA8, offering valuable insights into their potential efficacy and stability [13]. This study aims to evaluate the binding interactions and pharmacological potential of isoniazid derivatives against the CYP3A4 protein using in silico techniques, paving the way for their development as effective anti-TB agents.

2. MATERIALS AND METHODS

2.1 Ligand and Target Protein Preparation

The crystal structure of the target protein, 6MA8, was retrieved from the PDB database [14], with specific criteria applied during the selection process. Firstly, the chosen structure needed to represent a target protein bound to a native ligand or small-molecule inhibitor. Secondly, the binding or catalytic site had to remain mutation-free. The three-dimensional configurations of the isoniazid derivative compounds were constructed using Avogadro software [15] and subsequently optimized using ORCA software. The optimization employed the DFT method with the B3LYP 6-31G(D) OPT FREQ function to ensure accurate molecular geometries.

2.2 Molecular Docking and Interaction Visualization

Docking validation was performed using YASARA software [16]. Firstly, water molecules were removed from the protein structures. Then, unwanted atoms and molecules were removed from the proteins. Involving the redocking of the native ligand or inhibitor into the binding site of the target protein. The validation yielded an RMSD value of less than 2 Å, confirming the suitability of the docking method for use with the selected crystal structure. The docking simulations were conducted on the target protein with isoniazid derivatives, isoniazid, and the native ligand as test ligands. This process utilized the PLANTS program [17]. The interactions between the ligands and the target protein were analyzed and visualized using Biovia Discovery Studio software.

2.3 Toxicity Prediction

Toxicity prediction was performed using the ProTox website, including parameters AMES toxicity, toxicity class, and LD50 values [18].

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3. RESULTS AND DISCUSSION

This study investigated the potential of isoniazid derivatives 1–5 as anti-tuberculosis agents targeting the 6MA8 protein using an in silico molecular docking approach. Alongside these test compounds, isoniazid served as a reference drug and a native ligand for comparison. While several previous studies have synthesized isoniazid derivatives with bulky side chains to improve target binding and pharmacological properties, our research focuses on short-chain isoniazid derivatives. Short side chains often provide greater molecular flexibility, allowing the compound to better adapt to the shape and contour of the target's active site. In contrast, bulky substituents may cause steric hindrance. Designing and synthesizing short-chain derivatives is generally less complex and more cost-effective compared to larger, sterically hindered molecules. This can be advantageous for scaling up drug development and facilitating early-stage screening. Therefore, the development of isoniazid derivatives with simple, short-chain substitutions represents a rational strategy to optimize both target engagement and drug-like properties, making them attractive candidates for further development as anti-tuberculosis agents. The structures of the isoniazid derivatives and comparator compounds are presented in Figure 1. The geometry optimization of all ligands was successfully performed using the DFT/B3LYP method with the 6-31G(D) basis set in ORCA. Subsequently, the optimized ligands were subjected to molecular docking studies against the target protein to evaluate their binding affinities and interaction profiles.

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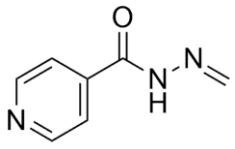

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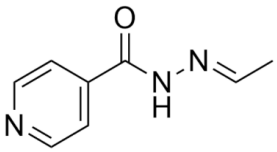

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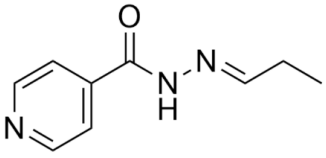

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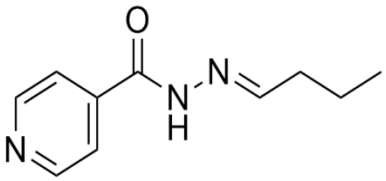

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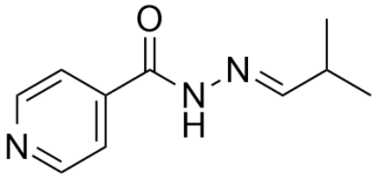

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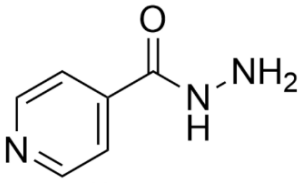

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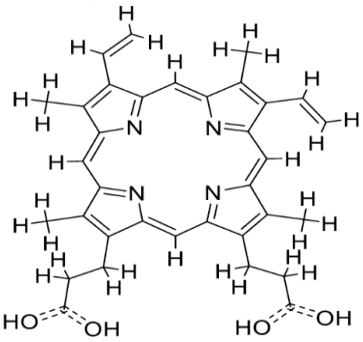

Figure 1. Molecular structure: (a) isoniazid derivative 1, (b) isoniazid derivative 2, (c) isoniazid derivative 3, (d) isoniazid derivative 4, (e) isoniazid derivative 5, (f) isoniazid, (g) native ligand.

A molecular docking simulation was performed to evaluate the interaction potential of isoniazid derivative compounds with 6MA8 at its active site. The docking process involved positioning the ligand within the receptor's active site to calculate the binding affinity for each tested compound. The molecular docking results are summarized in Table 1. The simulation produced docking scores [17], which reflect the strength of the interaction between ligands and the target receptor. The binding affinity values for isoniazid derivatives 1–5, isoniazid, and native ligand were -63.65 kcal/mol, -67.01 kcal/mol, -67.37 kcal/mol, -71.56 kcal/mol, -69.02 kcal/mol, -65.90 kcal/mol, and -182.68 kcal/mol, respectively. These results indicate that isoniazid derivative four exhibits a stronger binding interaction compared to the commercial drug isoniazid. Still, it is weaker than that of the native ligand, suggesting its potential as an anti-tuberculosis agent. The native ligand naturally binds to

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the active site with a stronger affinity. However, synthetic ligands showing comparable or moderately lower affinity may still be considered potent if they offer improved pharmacokinetic or toxicity profiles.

Table 1. Molecular Docking Analysis Results

Compound	Docking Score (kcal/mol)
Isoniazid derivate 1	-63.65
Isoniazid derivate 2	-67.01
Isoniazid derivate 3	-67.37
Isoniazid derivate 4	-71.56
Isoniazid derivate 5	-69.02
Isoniazid	-65.90
Native ligand	-182.68

The docking results were visualized using Biovia Discovery Studio to obtain position and interaction type data. The visualization results for the ligand compound and the 6MA8 receptor are presented in Figure 2, which shows various types of interactions, including conventional hydrogen bonds, carbon-hydrogen bonds, alkyl interactions, and π-alkyl interactions. The stability of the complex will increase with the number of interactions formed [19]. Based on our docking results, isoniazid derivative 4 showed interactions with several key amino acid residues. Isoniazid derivative 4 has two conventional hydrogen bond interactions with 6MA8 at the residues Ser312 and Arg212, both of which are polar residues commonly located near the substrate-binding region. Hydrogen bonds are considered one of the most critical forces in drug–receptor binding because they provide directional and specific interactions that enhance ligand affinity and stability within the binding site. It also interacts with Glu308 and Leu483 through two carbon-hydrogen bonds. While weaker than conventional hydrogen bonds, these interactions contribute additional polar stabilization, supporting proper ligand orientation. It forms a Pi-alkyl interaction with Ile369, Leu482, and Phe215. These hydrophobic contacts between the aromatic ring or π-system of the ligand and the nonpolar side chains of amino acids enhance van der Waals interactions, helping to anchor the ligand through non-specific hydrophobic stabilization. The cumulative contribution of these interactions increases the thermodynamic stability of the ligand–receptor complex, which is essential for potent inhibition. Thus, Derivative four is selected as the most promising compound, supported by both quantitative binding energy and qualitative interaction patterns.

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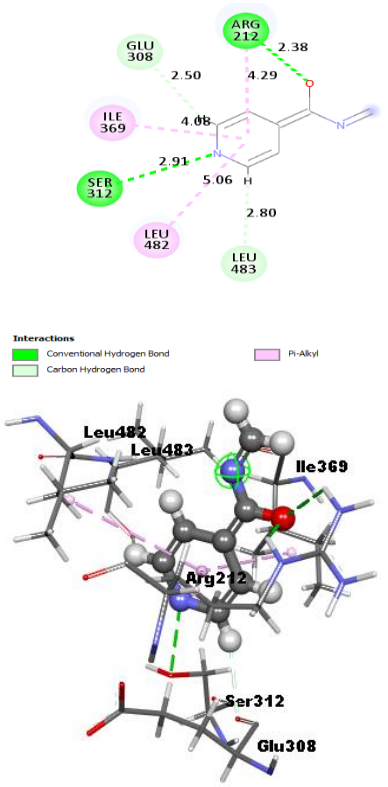

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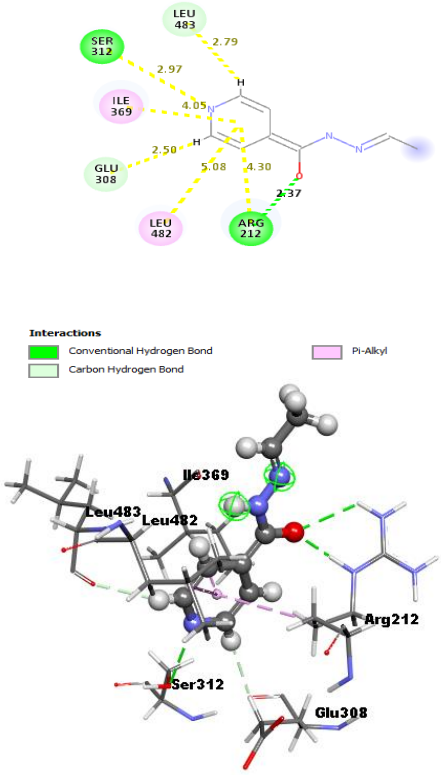

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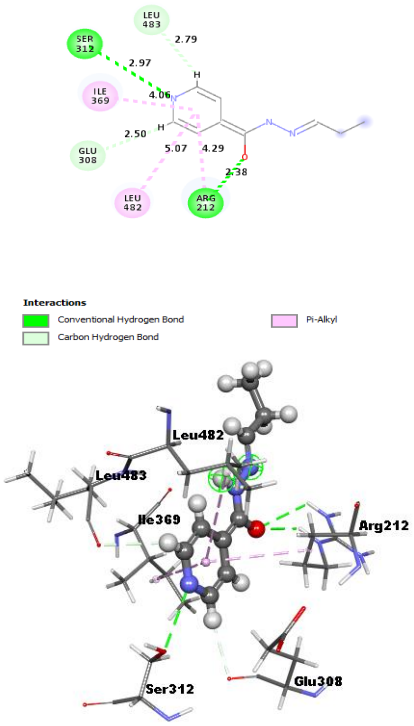

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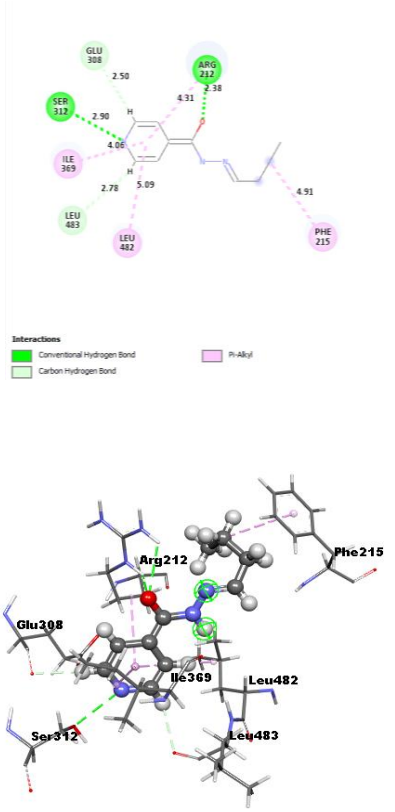

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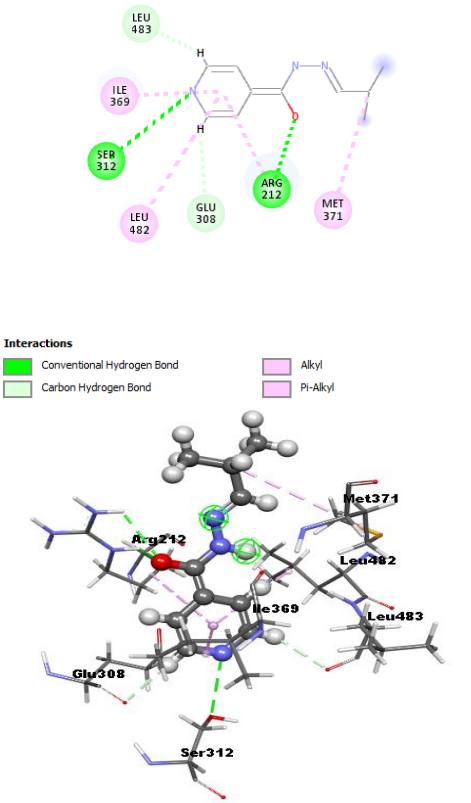

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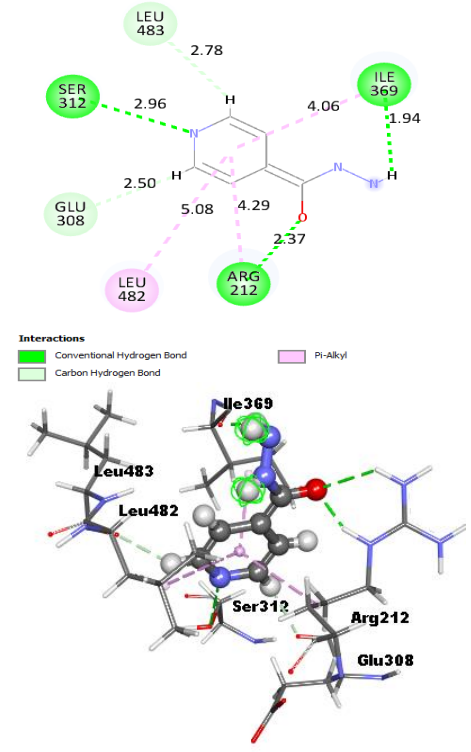

Figure 2. Visualization of Docking Results of Testing Compounds with 6MA8: (a) isoniazid derivative 1, (b) isoniazid derivative 2, (c) isoniazid derivative 3, (d) isoniazid derivative 4, (e) isoniazid derivative 5, (f) isoniazid.

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Toxicity prediction is done to determine the toxicity profile of isoniazid derivatives. Toxicity is the degree of damage caused by a compound when it enters the organism [20]. The parameters evaluated in this study included AMES toxicity, toxicity class, and LD50 values. The analysis results, summarized in Table 2, indicate that isoniazid derivative compounds are not predicted to exhibit AMES toxicity properties and possess toxicity class and LD50 values that suggest they are relatively less toxic than isoniazid. Derivative 4, the most promising compound based on docking results, falls into toxicity class 4 (LD_{50} = approx. 370 mg/kg), which is considered low toxicity and acceptable for early drug development. Compared to the parent isoniazid molecule, several derivatives demonstrated reduced predicted toxicity in at least one endpoint, indicating potential for improved safety. Toxicity prediction using ProTox revealed that the selected derivatives have acceptable toxicity classes. These findings suggest a favorable safety profile for further development of this pharmacological approach.

Table 2. Toxicity Prediction Result

Compound	AMES Toxicity	Toxicity class	LD50 (mg/kg)
Isoniazid	No	3	133
Isoniazid derivate 1	No	4	750
Isoniazid derivate 2	No	4	1000
Isoniazid derivate 3	No	4	370
Isoniazid derivate 4	No	4	370
Isoniazid derivate 5	No	4	370

4. CONCLUSION

Isoniazid derivative compounds exhibit a good interaction with 6MA8, as indicated by isoniazid derivative 4, which has more negative values than isoniazid. Isoniazid derivatives 4 are potent anti-tuberculosis agents with a good toxicity profile and a safe LD50. Further studies are needed to further uncover its potential with in vitro and in vivo tests.

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