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RESEARCH PAPER

Molecular Docking Study of Modified Isoniazid Compounds on Mycolic Acid Synthase in the Cell Wall of Mycobacterium Tuberculosis

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Abstract: Using the isoniazid in antituberculosis therapy can lead to mutations in the KatG and inhA genes of Mycobacterium tuberculosis, resulting in the development of resistance and necessitating modifications to the isoniazid compound. This study aims to assess the potential and level of toxicity of modified compounds, namely 4-pyridine carboxylic acid, pyridine aldehyde, and methyl pyridine, on the mycolic acid receptor through a molecular docking approach. PyRx was employed for the docking process using a protocol with an exhaustiveness of 106 and a center grid box at X=42.424, Y=22.4321, and Z=46.6391. Additionally, the ProTox-II website was used to determine the toxicity level of the test compounds. The results obtained from this research consist of the respective affinity values of the test compounds: -6, -5.4, and -5.2 kcal/mol. The toxicity levels of the test compounds are as follows: class 5, class 4, and class 4. All test compounds interact with amino acids on the target protein, specifically with residue numbers Histidine (HIS A:8), Phenylalanine (PHE A:142) through hydrogen bonding, Leucine (LEU A:95) through pi-Sigma (π) bonding, and Valine (VAL A:12) through pi-Alkyl (π) bonding. In conclusion, the 4-pyridine carboxylic acid compound exhibits potential as a promising drug candidate but comes with a high level of toxicity.

Keywords: Molecular Docking, Antituberculosis, Modified Isoniazid Compounds, Autodock VINA, ProTox-II5.

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INTRODUCTION

Tuberculosis (TB) is a disease that affects the lungs caused by Mycobacterium tuberculosis. Isoniazid is a selective antibiotic with a spectrum of activity against mycobacteria, primarily inhibiting the synthesis of mycolic acid, which disrupts the formation of the cell wall of Mycobacterium tuberculosis [1–4]. Mycolic acid is an α-alkyl-β-hydroxy long-chain compound found in the cell wall of mycobacteria, playing a vital role in mycolic acid biosynthesis and serving as a target for the development of efficient antibiotics [5, 6]. Mycolic acid synthesis involves two types of fatty acid-synthase systems (FAS): FAS-I and FAS-II. FAS-II comprises a series of enzymes responsible for elongating fatty acid chains synthesized by FAS-I. Inactivation or deficiency of any of these enzymes can hinder mycolic acid biosynthesis, making them potential targets for OAT development [7, 8].

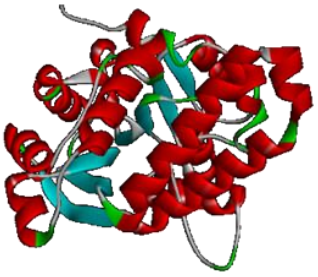


Figure 1. Structure of the cyclopropane synthase MmaA2 from Mycobacterium tuberculosis

This research was conducted using in silico molecular docking methods [9, 10]. This study aims to predict the affinity between ligands and proteins (mycolic acid), obtain interaction profiles of the compound, and assess the toxicity level of the test compounds..

MATERIALS AND METHODS

Instrument

The instrument used in this study are hardware with The device used in this research includes a Notebook Asus X441U, Intel Core i3 6006U, and SSD 320GB. The applications used in this research are the Protein Data Bank (PDB) (https://www.rcsb.org/), Discovery Studio 2021, Autodock Vina (Version 4.2, updated for version 4.2.6), ChemDraw 2D (Version 19.1), and ChemDraw 3D (Version 19.1) for generating 3D structures.

Material

The materials used in this research are the three-dimensional structures of 4-pyridine carboxylic acid ligand, pyridine aldehyde, and pyridine methyl from ChemDraw (Version 19.1) in pdb format. The receptor used in this study is mycolic acid, with the natural receptor being S-Adenosyl-L-Homocysteine with the code 1TPY, which can be downloaded from the Protein Data Bank (PDB) (https://www.rcsb.org/).

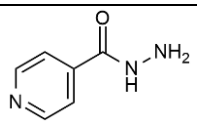
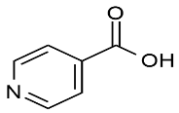
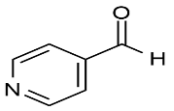
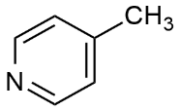
The mycolic acid protein used was downloaded from the Protein Data Bank (PDB) and sub-sequently prepared using the Discovery Studio 2016 application. This preparation involved the removal of unwanted ligands, resulting in the retention of the natural protein 1TPY, which was saved in .pdb format. Following this, geometric optimization and steric energy minimization were performed and saved in .pdbqt format within a single file [9].

Methods

Ligand Preparation

The two-dimensional structures of 4-pyridine carboxylic acid, pyridine aldehyde, and pyridine methyl are obtained using the computer program ChemOffice (Version 19.0). Attention is required when creating stereochemistry, as the presence of symmetric carbon atoms can influence the interaction process with the drug receptor, thus impacting the compound's activity. The ligands test are converted using Chem3D (Version 19.0) to examine and understand them to the most stable form for optimal interaction with the receptor. This process ensures that the compounds' three-dimensional structures are well-suited for their interactions with the stereochemistry of the receptor [11].

Table 1. 2-D structure of the test compound

Compound	Structure
Isoniazid	
4-pyridine carboxylic acid	
Pyridine Aldehyde	
Methyl palmitolenat	

Protein Preparation

The mycolic acid synthase, the target receptor, can be downloaded from the Protein Data Bank (PDB) at https://www.rcsb.org/ with the structure code 1TPY in .pdb format. Compound preparation is carried out using AutoDock Tool (Version 1.5.6). The receptor target is prepared to eliminate water residues and remove non-polar atom chain residues from the protein data. An initial optimization of geometry and energy minimization is performed. Afterward, the protein data format is changed from .pdb to .pdbqt following the addition of hydrogen molecules to the protein compound. Then, the coordinates for ligand binding to the protein target are determined [9].

Native Ligand Preparation

The Native ligand preparation is performed using Discovery Studio 2016 for the native ligand from Mycobacterium Tuberculosis protein, downloaded from the Protein Data Bank (PDB). The native ligand separates the receptor and all unused molecules. Subsequently, it is used for method validation [12].

The natural ligand from the Mycobacterium Tuberculosis protein can be downloaded from the Protein Data Bank (PDB) website at https://www.rcsb.org/ with the structure code 1TPY, and it is named S-Adenosyl-L-Homocysteine in .pdb format.

Validation

The next step in the validation process involves looking at the Root Mean Square Deviation (RMSD) values, where a good result is indicated by an RMSD value of < 2 Å, using the PyMol program. The testing of the RMSD values is determined by examining the interactions of the natural ligand S-Adenosyl-L-Homocysteine and the native ligand 1TPY with mycolic acid (Juni E, 2018). An RMSD value < 2 Å indicates that the receptor is valid and can be used for further docking studies [9, 10, 13].

Molecular Docking with AutoDock Vina

The molecular docking process uses the Vina Wizard program to analyze ligands with Mycobacterium Tuberculosis proteins integrated within the PyRx software. The docking protocol sets the ligand grid on the PyRx application according to the XYZ grid obtained. The grid on the protein with the code 7RCW shows X=42.424, Y=22.4321, and Z=46.6391. The XYZ grid represents the position where the ligand compound will act on the target protein. The results obtained from this program include affinity values and the binding of ligands to the target receptor protein. The complex compounds produced by the program are subsequently analyzed to visualize the mechanism of interaction between the compound molecules and the receptor [10].

Data analysis

Data analysis is conducted to determine physical-chemical properties (LogP and BM) in compounds such as 4-pyridine carboxylic acid, pyridine aldehyde, and methyl pyridine. This analysis is carried out per Lipinski's Rule of Five, which is used to predict compounds' absorption and permeability properties. The assessment is based on the strength of the interaction between the drug and its receptor. The more negative the affinity values formed during the docking process between the drug and its receptor, the more stable it is, and it is predicted to have a higher biological affinity [14].

Toxicity analysis

Toxicity analysis is carried out using the ProTox-II application based on the Random Forest (RF) machine learning algorithm. This program is used to construct classification and prediction models for the tested chemical compounds' hepatotoxicity, cytotoxicity, mutagenicity, and carcinogenicity [15, 16].

After determining the affinity values, it is necessary to check the level of toxicity of the test compounds using ProTox-II. The result allows us to assess the safety level of the test compounds when consumed, referencing research [17].

RESULTS AND DISCUSSION

Physical-chemical analysis

The analysis of the physicochemical properties of a compound is necessary to determine whether the ligand compound to be used complies with Lipinski's Rule before proceeding with the docking process. The test compound is analyzed using the pkCSM website, a modern in silico approach to high-throughput drug prediction, providing a faster way to predict pharmacokinetics. The result allows determining values such as molecular weight (BM), LogP, the number of H-donors, and the number of H-acceptors for the tested compounds.

The use of the pkCSM website is complemented by the PubChem website, which helps determine the SMILES string or structural bonding information for the test compounds. This facilitates the retrieval of the desired data.

Physical-chemical property analysis is conducted using the pkCSM and PubChem websites, based on in silico screening methods using pharmacokinetic predictions to help reduce the risk of failure [18]. The results of the determination of the physical-chemical properties of the test compounds can be seen in the following Table 2.The validation results show that the RMSD value for the natural ligand S-Adenosyl-L-Homocysteine ligand in the receptor with the code 1TPY is 0.102 Å. A visualization of the validation results can be seen in Figure 2. The validation results show that the positions of the atoms are not much different and even overlap between the test ligand and the native ligand S-Adenosyl-L-Homocysteine on the 1TPY receptor.

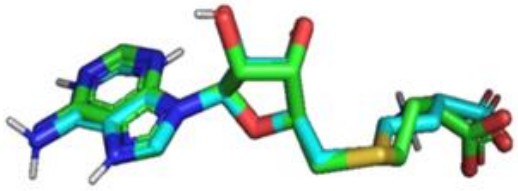


Figure 2. Visualization of 1TPY validation results

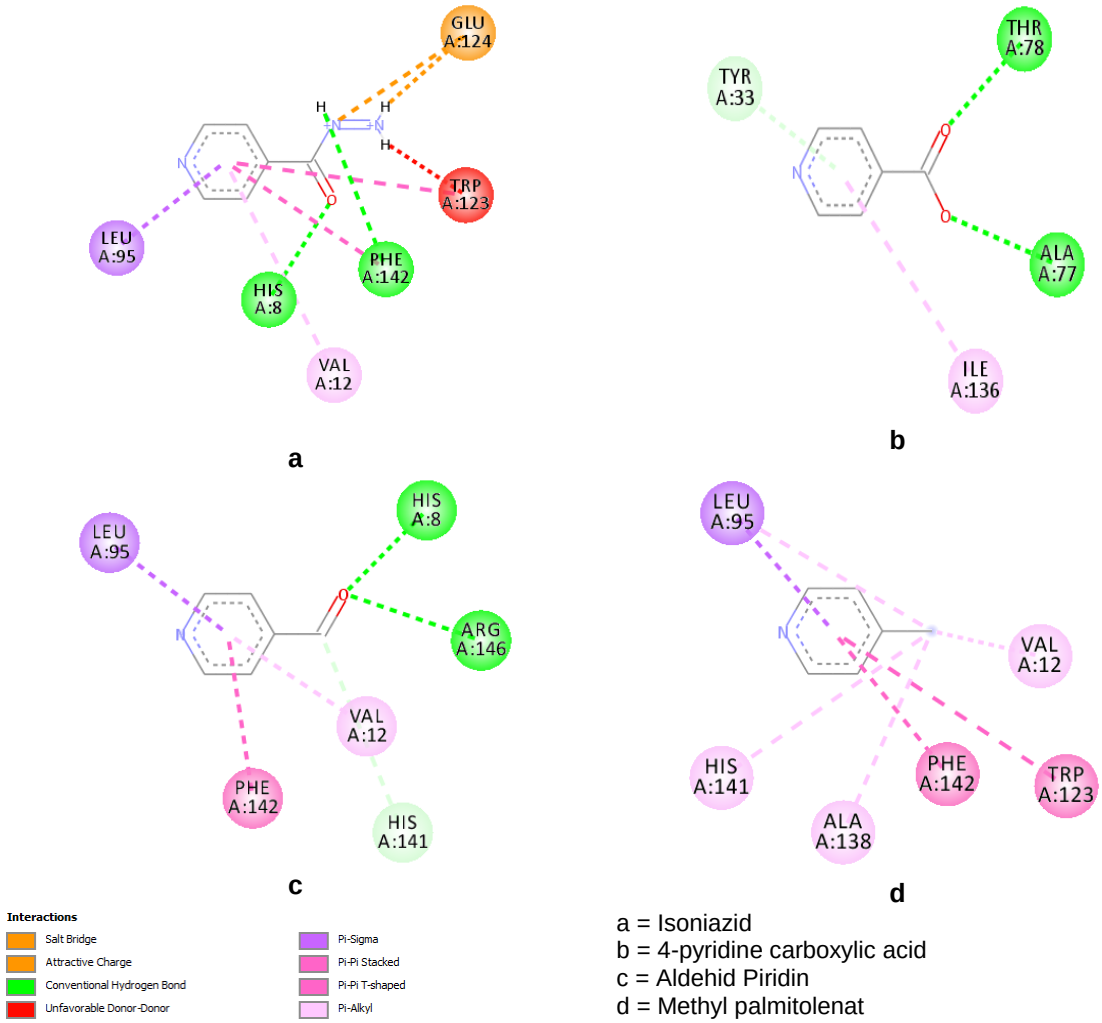
Molecular Docking Result

The docking process was conducted by importing the test compound data intro the marco-molecular protein data format(.pdbqt) using the PyRx program. Subsequently, steric energy minimization was performed on all test ligands, aimed at achieving a mor stable structure of the test compounds [19].

The docking process conducted for the test compounds is similar to that for protein compounds, using the same protocol with an exhaustiveness value of 116. The grid box values are determined as follows: X=41.806420, Y=25.489360, Z=48.855680. Docking proses occurred between the test ligand compounds and the mycolic acid receptro (MA) coded 1TPA, which had been prepared and polarized. The results of the docking simulation conducted using the PyRx program were evaluated based on parameters such as free receptor and amino acid residue binding energy, as observed in Figure 3 and Table 3 below [20, 21].

The docking simulation results performed using the PyRx program are assessed with parameters including the receptor's free binding energy and amino acid residues, as shown in the following Figure 4 and Table 3.

Based on the results of the molecular docking analysis conducted between the test compounds and the mycolic acid (MA) receptor with code 1TPY under clean and polar conditions, the obtained values in Table 3 indicate the affinity or Gibbs free binding energy (ΔG) for the test compounds as follows. The pyridine carboxylic acid test compound has a ΔG value of -6.1 kcal/mol; the pyridine aldehyde test compound has a ΔG value of -5.2 kcal/mol, the pyridine methyl test compound has a ΔG value of -5 kcal/mol, the natural ligand S-Adenosyl-L-Homocysteine has a ΔG value of -8.4 kcal/mol. It is worth noting that the control drug used in treating tuberculosis, which isoniazid, has a ΔG value of -6 kcal/mol.



a = Isoniazid
b = 4-pyridine carboxylic acid
c = Aldehid Piridin
d = Methyl palmitolenat

Figure 3. 2D-Visualization of docking result against 1TPY reseptor

Table 3. Results of docking analysis against 1TPY receptor

Receptor Type	Compound Name	Receptor-Ligand Binding Energy (kcal/mol)	Amino Acid Residues Involved in Hydrogen Bonds	Amino Acid Residues from Other Bonds, Alkyl Bonds, Sigma Pi Bonds, Sigma Alkyl Bonds, and Amide Pi Bonds
Mycolic Acid	Isoniazid	- 6	2.33 (HIS A:8), 2.87 (PHE A:142)	3.13 (GLU A:124), 1.71 (TRP A:123), 3.36 (LEU A:95), 5. 45 (VAL A:12)
	4-pyridine carboxylic acid	- 6.1	2.31 (ALA A:77), 2.24 (THR A:78)	2.86 (TYR A:33), 5.46(ILE A:136)
	Pyridine Aldehyde	- 5.2	2.17 (HIS A:8), 2.83 (ARG A:146), 3.60 (HIS A:141)	3.36 (LEU A:95), 4.67 (PHE A:142), 5.36 (VAL A:12)
	methyl palmitolenat	- 5		3.38 (LEU A:95), 4.38 (VAL A:12), 5.19 (TRP A:123), 4.68 (PHE A:142), 4.70 (ALA A:138), 4.23 (HIS A:141)

Out of the four test compounds used in this study, it is found that isoniazid, pyridine aldehyde, and pyridine methyl interact with six amino acid residues. In contrast,pyridine carboxylic acid interacts with four amino acid residues in the active site of mycolic acid. Based on the i*n-silico* assay results, it is concluded that the modified compound, pyridine aldehyde, shows good potential and can fulfill pharmacodynamic requirements compared to pyridine carboxylic acid and pyridine methyl. However, it is less effective than the positive control, isoniazid.

The information provided suggests that the interactions observed in the positive control, as indicated in Table 3, involve the mycolic acid (MA) binding to hydrogen-donor residues of the amino acid histidine (HIS A:8) and phenylalanine (PHE A:142). This interaction also includes pi-sigma bonding with the amino acid residue leucine (LEU A:95) and pi-alkyl bonding with the amino acid residue valine (VAL A:12). The determination of suitable candidate compounds in molecular docking requires increasingly negative Gibbs free binding energy (ΔG) values and the presence of hydrogen-bond interactions with amino acids that exhibit similar interactions, indicating their affinity [14].

Toxicity Testing of Ligand Compounds

The test compounds' toxicity levels are determined using the ProTox-II website (https://tox-new.charite.de/protox_II/). The PubChem website https://pubchem.ncbi.nlm.nih.gov/) is used to obtain the SMILES (Simplified Molecular-Input Line-Entry System) notation [22, 23]. Subsequently, the chemical structure of the test compounds is input, and the 'Organ Toxicity' menu with the 'Hepatotoxicity' tool is selected to obtain the predicted toxicity levels.

The ProTox Web Server is a virtual laboratory website that can predict the toxicity level of a test compound. Predicting the toxicity of test compounds is necessary for predicting and designing new drug compounds. The ProTox website offers cross-validation features through clusters based on the similarity of fragments, encompassing 33 predictive models for toxicity points [24]. The appearance of the toxicity prediction results for test compounds is shown in figure 5. After obtaining the toxicity prediction results from the ProTox-II website for the test compounds, they are placed into a table to analyze the toxicity levels of these test compounds. This simplifies the process of data analysis. The results of the test compound predictions can be observed in Table 4.

The toxicity level for isoniazid is indicated by an LD50 value of 133 mg/kg and is predicted to be in toxicity class 3, meaning it is toxic if ingested. The toxicity level for pyridine carboxylic acid is indicated by an LD50 value of 3123 mg/kg and is predicted to be in toxicity class 5, signifying that it might be hazardous if ingested. The toxicity level for pyridine aldehyde is represented by an LD50 value of 350 mg/kg and is predicted to be in toxicity class 4, which means it is dangerous if ingested. The toxicity level for pyridine methyl is indicated by an LD50 value of 350 mg/kg and is also predicted to be in toxicity class 4, signifying it is hazardous if ingested. The toxicity classes for the test compounds are determined according to the globally harmonized chemical classification and labeling system known as the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). Pyridine carboxylic acid is predicted to be hepatotoxic. In contrast, pyridine aldehyde and pyridine methyl are classified as toxic (class 4) but are not predicted to be hepatotoxic based on the results of toxicity and organ toxicity predictions using the ProTox-II website.

Table 4. Results of Predicted Toxicity Levels of Ligand Compounds

Compound	LD50 (Lethal Dose 50) in mg/Kg	Predicted Toxicity Class	Predicted Hepatotoxicity
Isoniazid	133	3	Active
4-pyridine carboxylic acid	3123	5	Active
Pyridine Aldehyde	350	4	Non active
methyl palmitolenat	350	4	Non active

CONCLUSION

This research concludes that the compound pyridine carboxylic acid has the best affinity with a value of -6.1 kcal/mol compared to the other test compounds. The compound pyridine aldehyde exhibits better interactions in the right pose with interactions involving leucine (LEU A:95) and valine (VAL A:12) compared to the other test compounds. The toxicity levels for pyridine aldehyde and pyridine methyl are in class 4, while pyridine carboxylic acid is in class 5. In this context, higher class numbers indicate lower toxicity.

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