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RESEARCH ARTICLE

DISCOVERY OF INHA INHIBITORS AS ANTI-TB AGENTS: DESIGN, MOLECULAR DOCKING AND ADMET PREDICTION

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ABSTRACT

As one of the top 10 causes of death, tuberculosis (TB) is a very fatal infectious illness that affects millions of people worldwide. TB still poses a serious threat to world health, particularly in areas with little resources, despite considerable advancements in detection and treatment. Numerous challenges face the current pharmacological therapy for TB, impeding successful outcomes. The emergence of antibiotic resistance within the bacterial population is one of the most urgent problems. This resistance is especially concerning when it progresses to extensively drug-resistant TB (XDR-TB) and multidrug-resistant TB (MDR-TB), which render conventional treatments ineffective or completely ineffective. Drug-resistant TB has a wide range of effects, including extended treatment regimens, higher medical costs, and occasionally unfavorable patient outcomes. Additionally, the currently accessible medications may come with significant. In the course of our research, computational techniques like molecular docking and ADMET predictions were used to find novel and powerful InhA inhibitors as anti-TB medicines. Using Autodock version 1.5.7, the designed compounds were docked on the InhA enzyme. Additionally, ADMET predictions were made using the OSIRS Property Explorer. It was determined that compounds 2, 4, and 6 may be used as effective and bioactive anti-TB medicines based on the results of molecular docking and ADMET predictions. These compounds will be produced, and their biological activity will be evaluated. The results of this research identified compounds 2, 4, and 6 as good candidates for more study, suggesting a potential advancement in the fight against tuberculosis.

Key words: Tuberculosis, InhA inhibitors, Molecular Docking, ADMET

INTRODUCTION

The only lethal infectious agent that causes tuberculosis (TB), which infects around one-third of the world's population each year, is Mycobacterium tuberculosis (Mtb). One of the top 10 killers in the world is tuberculosis. It remains a substantial public health concern despite great advancements in detection and treatment, especially in low- and middle-income nations. The World Health Organization (WHO) estimates that 10 million individuals contracted TB in 2019 and 1.4 million died as a result of the illness. Though TB can be found anywhere in the world, some places have higher rates of the disease. Sub-Saharan Africa has the highest TB burden, followed by nations in South and Southeast Asia. When the TB-causing bacteria develop a resistance to the drugs, it results in drug-resistant TB. Extensively drug-resistant TB (XDR-TB) and multidrug-resistant TB (MDR-TB) necessitate longer and more involved treatment regimens [1] [2]. Inadequate care and incorrect use of antibiotics can lead to the development of drug resistance. Additionally, the condition is common in places with a dense population, extreme poverty, and poor access to medical care. The most significant is multidrug resistance, which is developed during conventional therapy and involves an intensive phase with three or four distinct drugs, including isoniazid (INH) [3], rifampin (RFP), pyrazinamide (PZA), and ethambutol (EB).

MATERIAL AND METHODS

Design of Molecules

Intense literature review was done to find out best acting compound on target InhA. Ethambutol was selected as the reference compound. The selected molecules were drawn in Chem Draw Pro 8.0. The .cdx format of these molecules were converted into .pdb and. pdbqt for docking. As all the molecules exhibit excellent InhA inhibitory activity, the best among all was found by docking. The structures of all compounds are given in Table-1 [4] to [10].

Table 1: Structure of Selected Molecules for Docking

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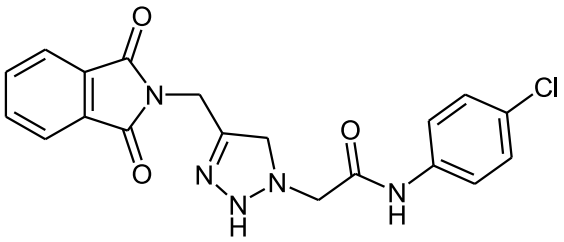
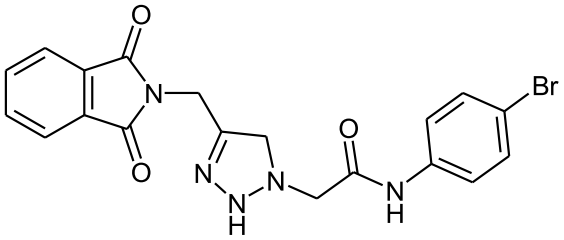
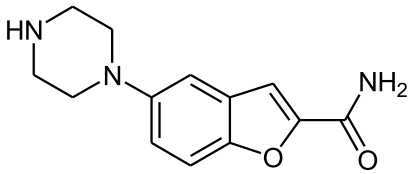
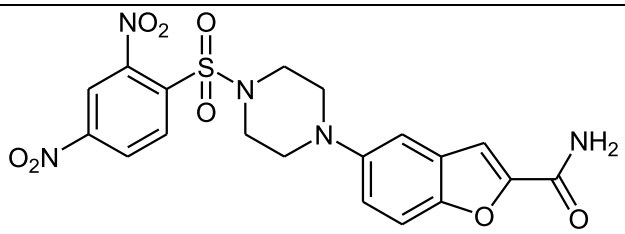
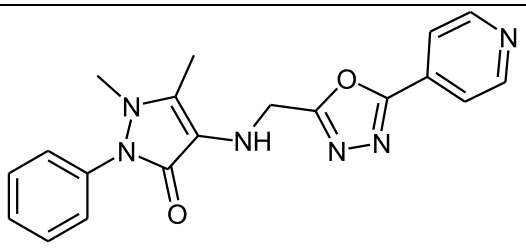
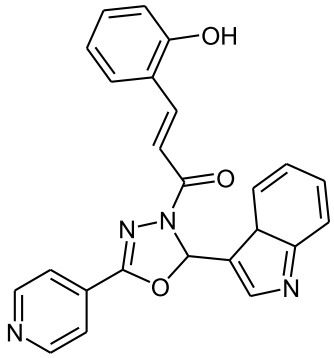
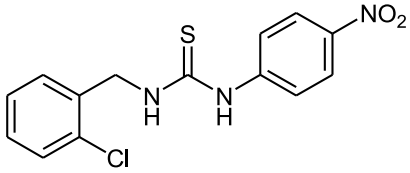
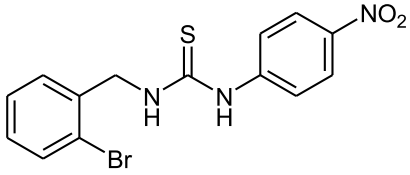
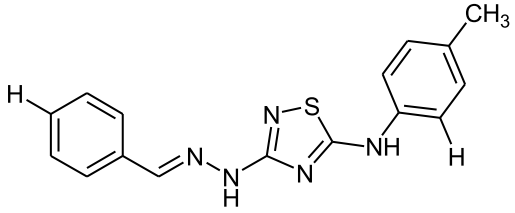
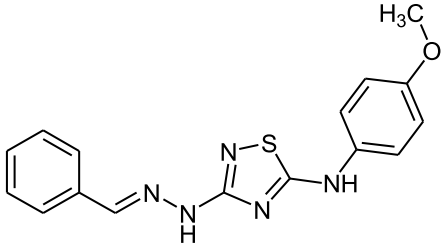
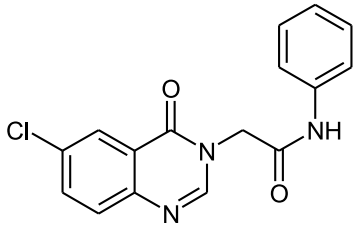
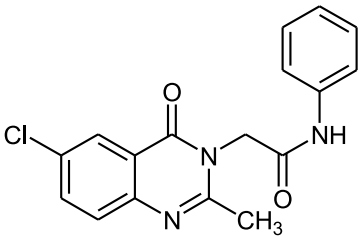
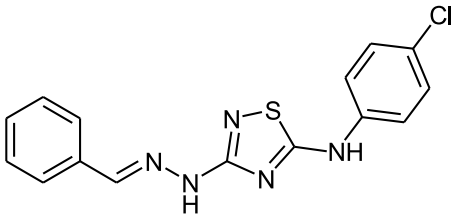
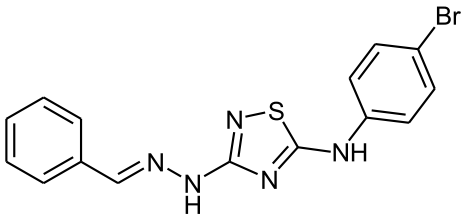
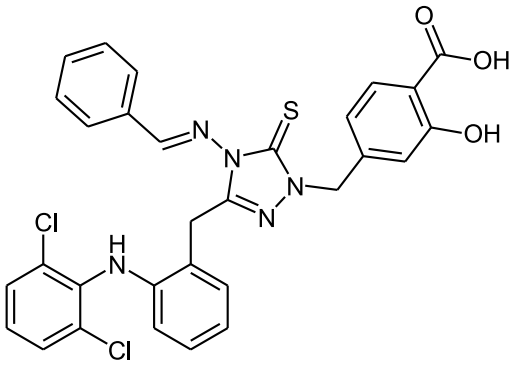
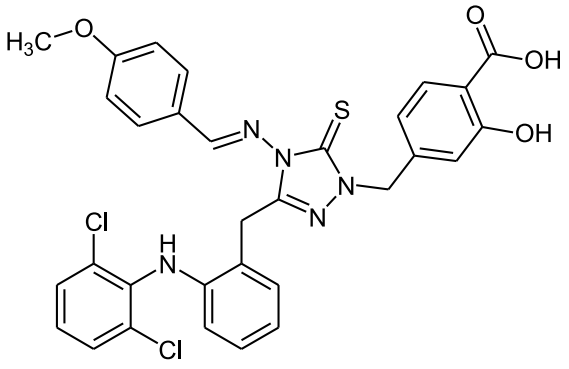
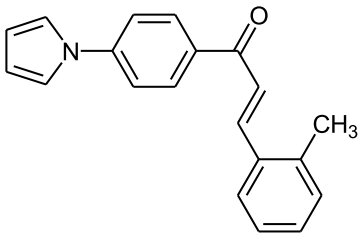
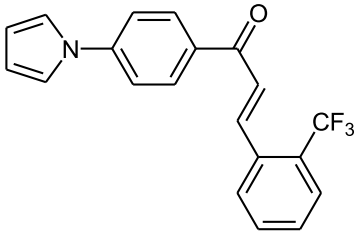
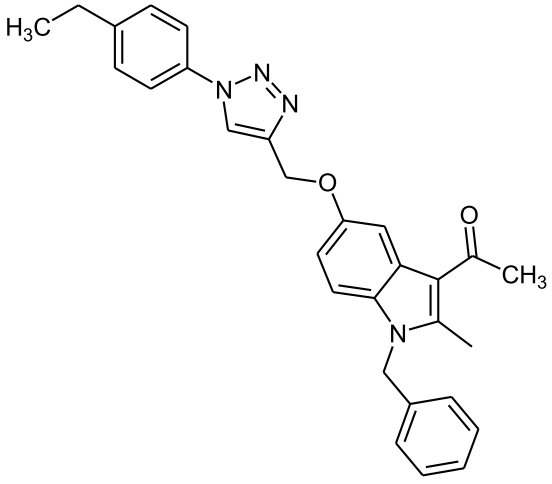
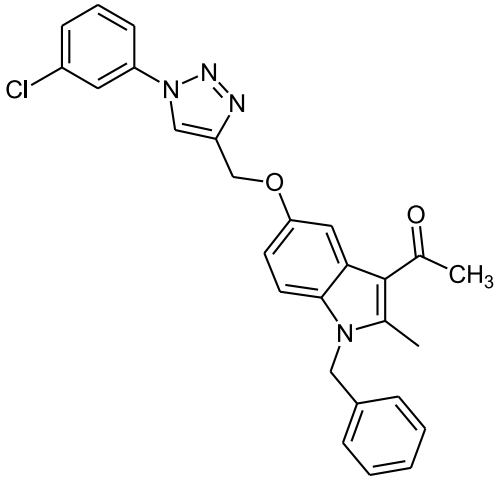
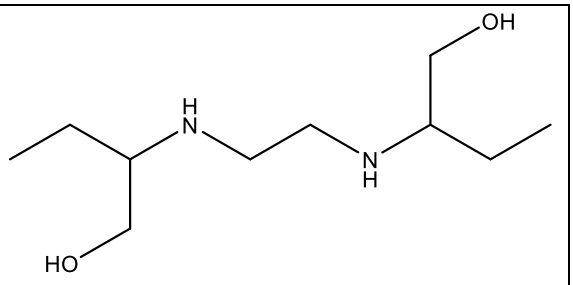
1	
2	
3	
4	
5	

6	
7	
8	
9	
10	
11	

12	
13	
14	
15	
16	

17	
18	
19	
20	

Reference: Ethambutol	

Docking

After designing of molecules, co-crystallized structure of InhA was downloaded from https://www.rcsb.org/. The code of the protein is 6SQ7. Crystal structure of M. tuberculosis InhA in complex with NAD+ and 2-(4-chloro-3-nitrobenzoyl)benzoic acid. This co-crystallized structure was opened in Autodock version 1.5.7. Autodock is a free docking software suite which predicts the binding of small molecules like substrates or drug candidates to a receptor with a known 3D structure. It is widely used in the research community for various purposes such as virtual screening (HTS), protein-protein docking, structure-based drug design, lead optimization and combinatorial library design [11] [12]. To perform docking study in Autodock, following steps are the prerequisites:

Protein preparation

The protein was prepared by removing water molecules that interfere with ligand binding. NAD+ and 2-(4-chloro-3-nitrobenzoyl)benzoic acid, the ligands attached with the InhA protein were also deleted. The heteroatoms were also deleted to avoid steric hindrance. Missing Polar hydrogens were added and missing atom search was carried out. At the end, Kollman charges were added and prepared file was saved in pdbqt format.

Ligand preparation

The pdb file of the selected molecule was added in Autodock software to prepare it for the protein ligand interaction [13]. First, the Gasteiger charges were computed. This method is based on the partial equalization of orbital electronegativity. Then, the root of the structure was detected and number of torsions was set. The prepared file was saved in pdbqt format.

Grid formation

Autogrid is used to create maps that show the energy of different atoms in the ligand being docked. These maps help speed up the docking calculations. Each point on the map represents the energy of an atom or group of atoms in the ligand due to their interactions with the atoms in the macromolecule. Grid box was formed upon prepared protein. The grid box dimension was set in such a way that it

covers specific area for docking. This file was saved as gpf format. Launching of the autogrid generates map files which is the indication of successful grid run.

This is followed by docking of prepared ligand on the protein. Here, protein was kept as a rigid file and rotation of ligand was allowed around its root axis. The docking command was given based on genetic algorhythm and the Lamarckian output followed dpf file format. This dpf file was run to generate dlg format. This contains histogram which shows the binding energy of the ligand with the protein. The binding energy of the best pose of all the ligands are mentioned in Table-2.

ADMET

The molecules with lowest binding energy were further considered for ADMET profiling [14] [15]. Drug-likeness model score was calculated using Molsoft. (https://molsoft.com/mprop/). The toxicity profiling of the filtered molecule was done using Osiris property explorer (http://www.cheminfo.org/flavor/cheminformatics/Utility/Property_explorer/index.html).

Results and Discussion

All in all, 20 compounds having best activity on InhA receptor were identified and docking was done to find out the most active compound among them. To compare the energy score with the reference molecule, Ethambutol was also docked. Low binding energy indicates stable ligand-target complex which is given in Table-2. Bioavailability score, Toxicity data, bioactivity prediction data are collected which can be utilized for comparison with the reference molecules. The ligand showing more stable interaction with receptor (lowest binding energy) is preferred for synthesis. The binding interaction of compound-2, 4 and 6 are given in Fig.1, 2 and 3 respectively. But at the same time, virtual screening of the selected ligands for the toxicity gives clear indication about which compound should be given priority for synthesis. Docking score of compound number 15 found to be lowest among all the compounds but during toxicity prediction it shows mutagenicity and effect on reproduction. Hence, priority for synthesis will be given to safe compounds (2, 4 and 6). The binding affinity of both of the compounds(-11.18 and -13.31 respectively) found to be better than reference ligand Ethambutol (-9.13). Table-3 represents the top three most active InhA inhibitors among all docked compounds. Selected molecules with drug-likeness model score and toxicity data are mention in Table-4.

Table 2: Binding Energy of Selected Molecules

Sr. No	Binding Energy
1	-10.17
2	-11.18
3	-8.67
4	-13.31
5	-10.45
6	-12.74
7	-9.21
8	-9.47
9	-9.52
10	-9.14
11	-9.50
12	-9.36
13	-9.27
14	-9.93
15	-13.10
16	-12.43
17	-9.52
18	-9.46
19	-12.13
20	-12.43
Reference: Ethambutol	-9.13

Table 3: Most Active InhA Inhibitors

Sr. No.	Binding energy	Drug likeliness score	Toxicity
2	-11.18	0.29	Safe
4	-13.31	0.29	Safe
6	-12.74	-0.17	Safe

The interaction of above-mentioned ligands with InhA protein is shown below.

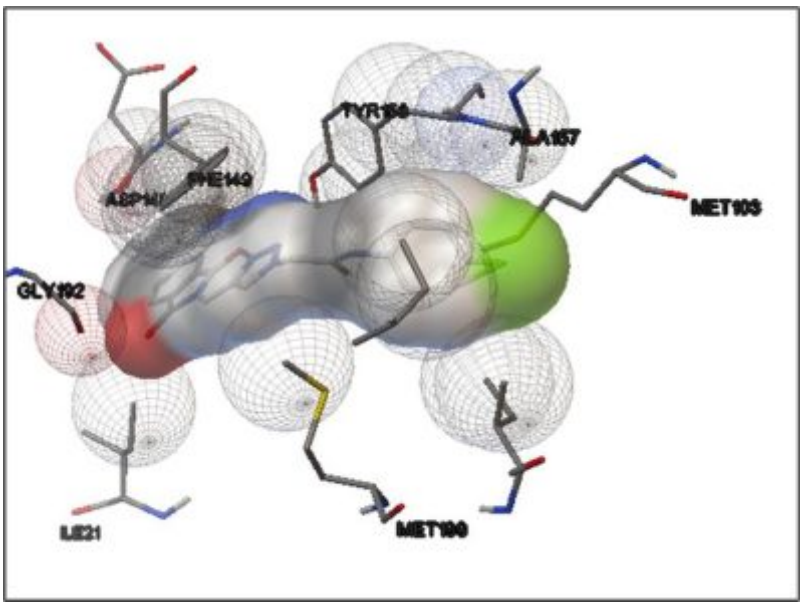


Figure 1: Protein Ligand Complex for Compound 2

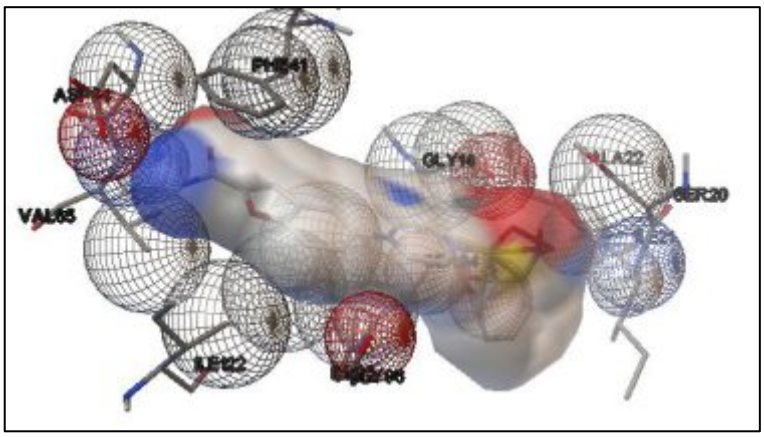


Figure 2: Protein Ligand Complex for Compound 4

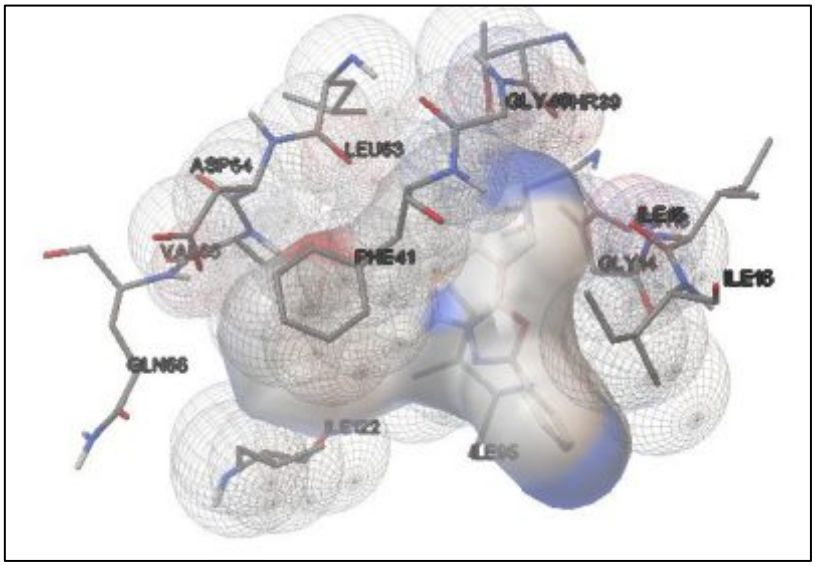


Figure 3: Protein Ligand Complex for Compound 6

Table 4: Drug-Likeness Model Score and Toxicity of Selected Ligands

Sr. No.	Binding energy	Drug likeliness score	Toxicity
2	-11.18	0.29	Safe
4	-13.31	0.29	Safe
6	-12.74	-0.17	Safe
15	-13.1	0.29	Mutagenic and effect on reproduction
20	-12.43	-0.05	Effect on reproduction

Conclusion

Based on the docking score, energy of various conformers and toxicity data, 3 lead compounds namely, compound number 2, compound number 4 and compound number 6 were selected as the final outcome of the entire experiment. Compound number 15 has shown the lowest energy and thereby the most stability with protein. But the toxicity analysis concludes the mutagenicity and reproductive effect of the compound. Besides, binding energy score was found to be low for compound 2, compound 4 and compound 6 as compared to compound 15 and reference ligand Ethambutol. Hence, the synthesis preference will be given to compound 2, compound 4 and compound 6.

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