Research Article

Novel Pyrimidine Scaffolds as Anti-Tubercular Agents Targeting Enoyl Acyl Carrier Protein Reductase: A Molecular Docking Study

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ABSTRACT

In recent years, tuberculosis (TB) has continued to be a global public health concern. The screening of novel bioactive compounds with novel targets and distinct mechanisms of action is desperately needed. Pyrimidine-cored heterocyclic molecules have become increasingly significant in medical chemistry. The 3,4 dihydropyrimidine scaffold is crucial to the development of anti-tuberculosis drugs since its derivatives have demonstrated exceptional efficacy. We created a new series of 3,4 dihydropyrimidine derivatives in this article and used Autodock Vina to perform docking studies on them. Isoniazid was the standard drug used, and all drugs had good binding affinities with the enoyl acyl carrier protein reductase.

Keywords: Tuberculosis, 3,4 dihydropyrimidine, Enoyl acyl carrier protein reductase, Antitubercular drugs, Autodock Vina.

INTRODUCTION

One of the most important infectious illnesses in the world today is tuberculosis (TB).[1] The bacteria that causes tuberculosis (TB) is called *Mycobacterium tuberculosis*. While TB primarily affects the lungs, it can also impact other parts of the body, including the brain, spine, and kidneys. TB can be fatal if treatment is not received.[2] Throughout history, tuberculosis has been known by a number of names, including the white plague, scrofula, consumption, and Pott's sickness.[3] When someone with lung or throat TB coughs, sneezes, talks, or sings, the bacteria that causes TB are released into the air. Nearby individuals may inhale the microorganisms and develop the infection.[2] In January, 19.88 lakh TB cases were declared in India, according to the WHO Global TB Report 2024, India notified 19.88 lakh TB cases between January and September, a 4.2 percentage rise from 19.08 lakhs during the same time in 2023.[4] The unique FAS-II system, which includes the enoyl-ACP reductase (InhA) from *Mycobacterium tuberculosis*, uses longer-chain fatty acyl substrates to create mycolic acids, which are essential for the formation of the mycobacterial cell wall. Compared to other enoyl-ACP reductases, InhA has a longer substrate binding loop, creating a deeper binding crevice that enables it to identify longer chain fatty acyl substrates.[5] *Mycobacterium tuberculosis* produces the oxidoreductase enzyme indicated by the protein structure with PDB ID 4TRJ. Enoyl-[acyl-carrier-protein] reductase (NADH) is an enzyme involved in the production of fatty acids. The structure is made up of a single chain, called Chain A, that is 269 amino acids long and weighs 29.58 kDa. It is linked to the INHA or MT1531 genes and has two ligands.[6] The bacterium's metabolic processes depend on the enzyme's operation, which makes it a possible target for TB medication development. In medicinal chemistry, pyrimidine analogues are well known for their wide range of therapeutic applications.[7] The chemistry and biological activity of pyrimidine derivatives have received a lot of attention since they were discovered. The synthesis of pyrimidine derivatives through a variety of techniques that allow for the production of multifunctionalized pyrimidine compounds has garnered increasing attention in recent years. By specifically targeting the enzymes involved in Mtb's metabolic activities, a pyrimidine derivative has shown antitubercular efficacy and inhibited Mtb development.[8]

In this work, we developed a novel series of 3,4 dihydropyrimidine compounds and conducted docking investigations on them using Autodock Vina. All of the medications demonstrated good binding affinities with the enoyl acyl carrier protein reductase, with isoniazid being the most commonly utilized.

MATERIALS AND METHODS

Materials

Numerous biological databases, bioinformatics tools, and software were used in the current investigation. Table 1 lists the utilized software programs together with associated utilities.

Methods

Protein preparation

The Protein Data Bank (www.pdb.org) provided the protein (PDB) ID 4TRJ [fig 1] in PDB format. Enoyl-[acyl-carrier-protein] reductase [NADH], the 4TRJ protein, has 269 residues and one chain. NADH-dependent enoyl-ACP reductase is another name for this enzyme. The Active Site Prediction Server, SCFBio, which may be accessed at http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp, was used to forecast the active site. The protein was subsequently energy-minimized by using MOE software.

Table 1. List of softwares and their applications

S.No	Softwares	Applications
1.	ChemSketch	Software to create and modify chemical structures in 2D and 3D
2.	Chem 3D Pro 8.0	Software used for generating 3D models and performing energy minimization of ligands.
3.	MOE (Molecular Operating Environment)	Software used for energy minimization of proteins by selecting the active chain.
4.	PyRx -Virtual Screening Tool	Autodock Vina software
5.	Discovery Studio	Identifying the active site of the protein and analyzing the docking results.

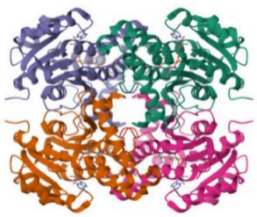


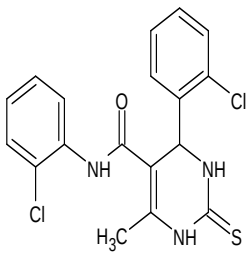
Figure 1: 3D Structure of 4TRJ

Structural assessment of the protein

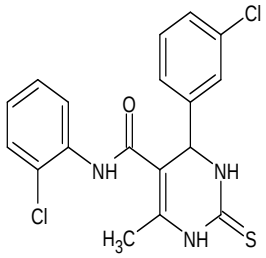
By creating Ramachandran plots and examining parameters like residue types, Chi1-Chi2 plots, main-chain and side-chain properties, bond lengths, bond angles, RMS distances from planarity, and geometric distortions for input atoms only, the PDBsum database was used to assess protein structure.

Preparation of ligands

The protein with PDB ID 4TRJ was docked with roughly 20 pyrimidine derivatives. The ligands' 2D structures were created in ChemSketch, transformed into 3D structures, and their energy minimization was completed in Chem 3D Pro 8.0 before being saved in PDB format.



1a



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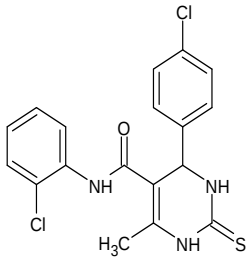

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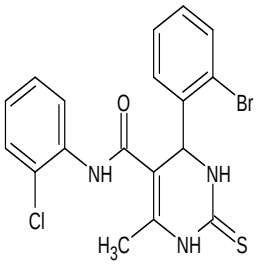

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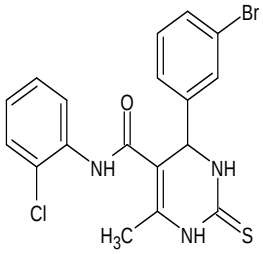

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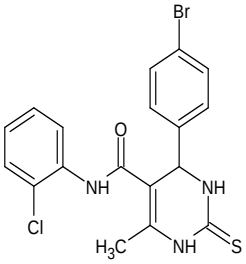
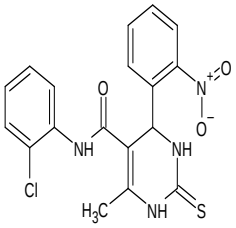


1f



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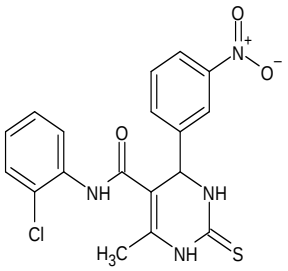
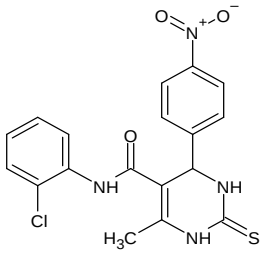


1h



1i

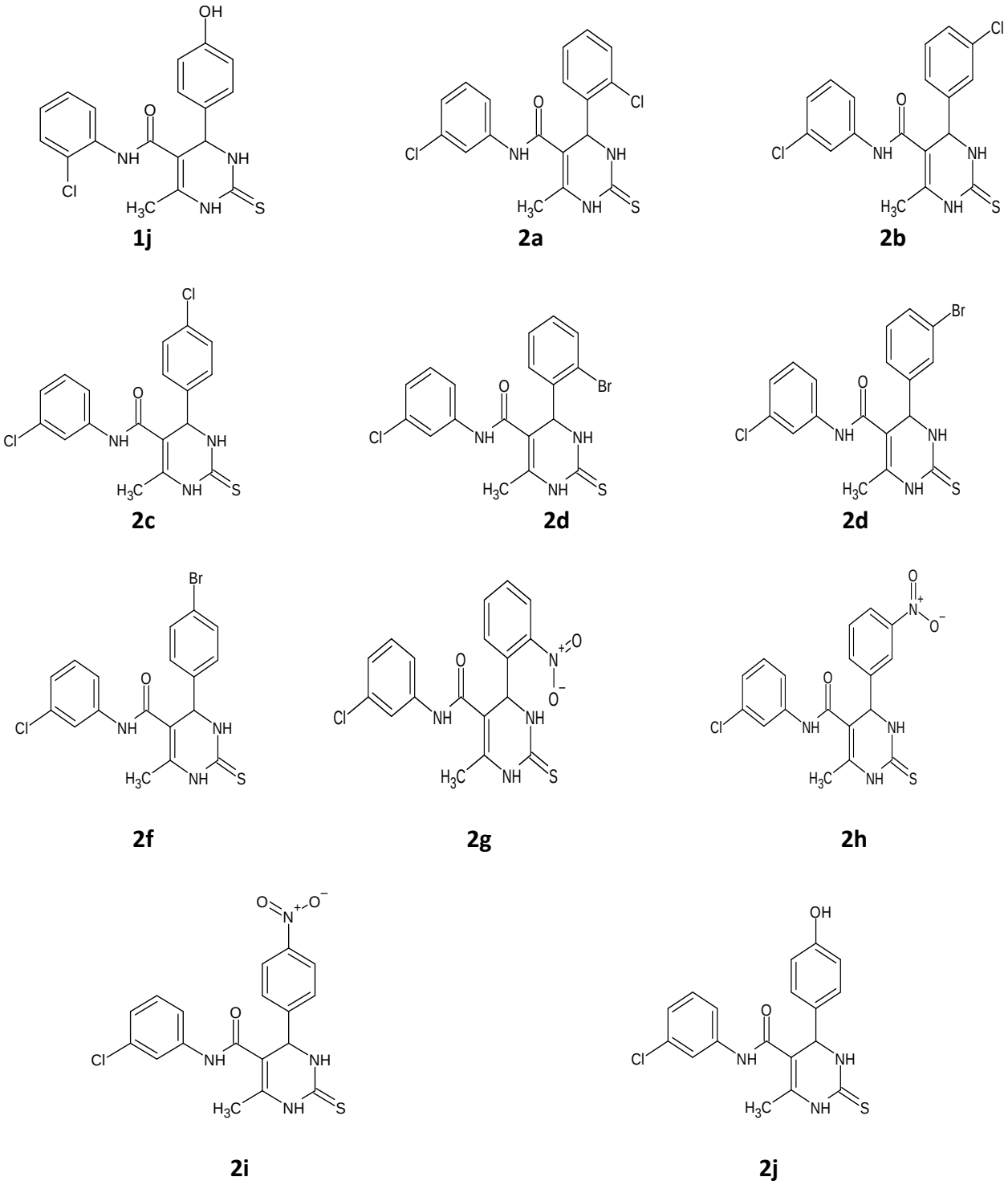


Figure 2: 2D structures of the ligands

Lipinski's rule of five (Ro5)

In drug discovery, Lipinski's Rule of Five (Ro5) is a guideline that uses a compound's chemical properties to predict its permeability, solubility, and pharmacological activity. According to Lipinski's criteria, the majority of chemicals that work well when taken orally are tiny and have a moderate lipophilic. To find successful hits in screening and improve lead compounds for potency, specificity, and selectivity, drug development uses Ro5 compliance.[9]

Docking studies

By estimating and evaluating molecules' binding affinities using a variety of scoring systems, docking makes it possible to screen a compound database. Based on how well the binders interact with the target protein, this method aids in identifying the strongest binders. By attaching to receptors, docking investigates how compounds, such as medications and enzymes, fit together and may alter their function. Docking is used to examine the drug-receptor complex, and then molecular dynamics is used to determine stability and free energy simulations to determine binding affinity.[10-14]

Pyrimidine was used as the nucleus and enoyl-[acyl-carrier-protein] reductase [NADH] (4TRJ) as the receptor in this investigation. Using AutoDock Vina via PyRx, a docking analysis between the receptor and ligands was carried out. One of the main targets for creating new pyrimidine-based antitubercular medications is the structure of 4TRJ (Fig.1). In order to prepare the protein for docking investigations, all ligands and water molecules were eliminated. Using Biovia Discovery Studio 2020 Client, the proposed compounds with 4TRJ were visualized and subjected to docking analysis.

RESULT AND DISCUSSION

Structural assessment of protein

Figure 3 displays the study of the Ramchandran plot. Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 2.0 a good quality model would be expected to have over 90% in the most favoured regions [A,B,L]. The plot shows that 91.6% is most favoured region with 206 residues. 7.1% is additional allowed region [a,b,l,p] with 16 residues. 0.0% is generously allowed regions [~a,~b,~l,~p] with 0 residues and 1.3% is Disallowed regions [XX] with 3 residues.

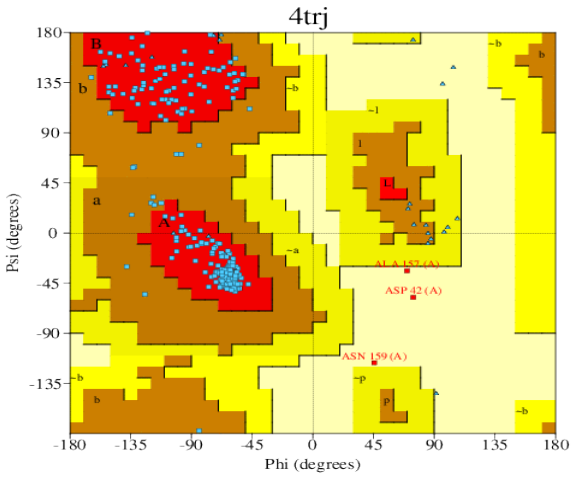


Figure 3: The Ramchandran plot analysis of protein 4TRJ

Lipinski's Rule of five (Ro5)

When compared to the reference medication isoniazid, the designed pyrimidine derivatives met Lipinski's rule, which includes the following criteria: molecular mass less than 500 Dalton, logP less than 5, hydrogen bond donors less than 5, hydrogen bond acceptors less than 10, and molar refractivity between 40 and 130 (Table.2).

Table 2: Lipinski's properties of the compounds

S.No	Compound code	Mol.wt [g/mol] <500	LOG P <5	H-Donor <5	H-Acceptor <10	MR 40-140
1	1a	392.3	5.04+/- 0.65	3.0	5.0	113.1
2	1b	392.3	5.04+/- 0.65	3.0	5.0	113.1
3	1c	392.3	5.04+/- 0.65	3.0	5.0	113.1
4	1d	436.7	5.22+/- 0.67	3.0	5.0	115.8
5	1e	436.7	5.22+/- 0.67	3.0	5.0	115.8
6	1f	436.7	5.22+/- 0.67	3.0	5.0	115.8
7	1g	370.8	4.18+/- 0.65	3.0	5.0	109.9
8	1h	402.8	4.18+/- 0.65	3.0	6.0	105.6
9	1i	402.8	4.18+/- 0.65	3.0	6.0	105.6
10	1j	373.8	3.71+/- 0.65	4.0	6.0	110.1
11	2a	392.3	5.44+/- 0.73	3.0	5.0	113.1
12	2b	392.3	5.44+/- 0.73	3.0	5.0	113.1
13	2c	392.3	5.44+/- 0.73	3.0	5.0	113.1
14	2d	436.7	5.62+/- 0.77	3.0	5.0	115.8
15	2e	436.7	5.62+/- 0.77	3.0	5.0	115.8
16	2f	436.7	5.62+/- 0.77	3.0	5.0	115.8
17	2g	402.8	4.58+/- 0.73	3.0	6.0	105.6
18	2h	402.8	4.58+/- 0.73	3.0	6.0	105.6
19	2i	402.8	4.58+/- 0.73	3.0	6.0	105.6
20	2j	373.8	4.11+/- 0.73	4.0	6.0	110.1
STD	Isoniazid	137.1	0.7763	2.0	4.0	35.1

DOCKING

The scoring function for the docking run is the binding energy, or E bind, between the ligands and the protein. Overall, there was no link between the calculated E bound values and the experimentally determined IC50 and Ki values from earlier research. A thorough examination of the binding interactions was carried out in order to have a better understanding of this experimental observation.[15]

Autodock Vina was used to conduct molecular interaction experiments with bioactive chemicals and PyRx. An important consideration in the drug development process is how a natural chemical interacts with the target protein.[16] Based on the root-mean-square deviation (RMSD), the software chose the optimal docked conformation. The 20 compounds' energy levels were discovered to be between -5.8 and -8.3 Kcal.mol-1. Four derivatives with the highest binding affinities, ranging from -7.8 to -8.3 Kcal.mol-1, were found by docking 20 molecules with the 4TRJ protein. The remaining derivatives exhibited moderate activity, while one molecule had the lowest binding affinity at -5.8Kcal.mol-1. Table 3 lists the receptor-ligand interactions for 20 of the derivatives along with the names of the amino acids that interact with the ligand.

When 20 derivatives' Autodock results are compared to the standard isoniazid, 2f has the highest binding affinity. The binding affinity of 2f is -8.3Kcal.mol-1. GLY A:96, SER A:94, GLY A:14 by conventional H-bond, PHE A:41, THR A:196, MET A:199, MET A:103, PHE A:97, MET A:161, LYS A:165, PHE A:149, ILE A:21, SER A:20 by wander Vaal forces, ILE A:16, ILE A:95 by alkyl bond, and MET A:147, ALA A:198 by Pi-alkyl bonds highlight the compounds' competitive inhibitory properties.

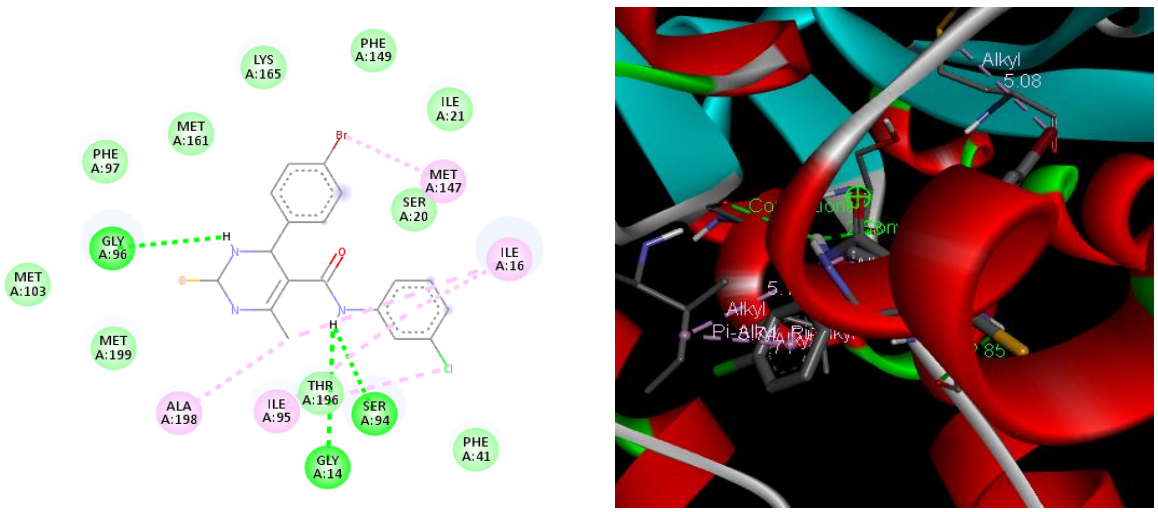
Table 3: Interaction and binding affinities of designed pyrimidine derivatives

Code	Binding affinity	H-Bond	Vander waals forces	Pi-Sigma	Alkyl	Pi-Alkyl	Pi-Sulphur	Pi-Pi Stacked	Carbon-hydrogen Bond	RMSD
1a	-5.8		LYS A :118 ARG A :43 GLN A :66 PHE A :41 ASP A :64 ILE A :122 ILE A :95 GLY A :96	PHE A :97				PHE A :97		0.0
1b	-7.3		ILE A :202 ALA A :198 PHE A :97 MET A :161 PRO A :193 GLY A :14 SER A :20 GLY A :96 SER A :94 THR A :196 ILE A :21 ILE A :194	ILE A :16		ILE A :95 MET A :199				0.0
1c	-7.5	GLY A :96 GLY A :14	MET A :161 TYR A :158 PHE A :149 PRO A :193 ILE A :194 ILE A :21 THR A :196 ILE A :16 SER A :20 SER A :94 ILE A :95		ILE A :202 MET A :199	PHE A :97 ALA A:198				0.0
1d	-7.3	LYS A :165 SER A :94	SER A :20 THR A :196 ILE A :194 MET A :199 ASP A :148 GLY A :192 PHE A :149 PRO A :193		ILE A :21	ALA A :191				0.0

			MET A :147 TYR A :158							
1e	-6.4	ASP A :261 GLY A :262	THR A :241 GLY A :263 VAL A :238		TRP A :230 ALA A:260	PRO A :237 ILE A :228				0.0
1f	-7.4	ILE A :194	PRO A :193 THR A :196 MET A :161 ILE A :202 SER A :20 GLY A :14 GLY A :96 SER A :94	ILE A :16	ILE A :21 ILE A :95	ALA A :198 MET A :103 PHE A :97	MET A :199			0.0
1g	-7.3	GLY A :14 GLY A :96	MET A :161 MET A :199 THR A :196 ILE A :194 PRO A :193 PHE A :149 ALA A :191 MET A :147 ALA A :22 SER A :20 SER A :94 ILE A :16 ILE A :96 LYS A :165	ILE A :21						0.0
1h	-8.1	ASP A :148 GLY A :192 ALA A :191 ALA A :22 SER A :94 SER A :20 ILE A :21	PHE A :97 GLY A :96 MET A :161 MET A :199 TYR A :158 PRO A :193 MET A :147 GLY A :14 THR A :196 LYS A :165	ILE A :21			PHE A :149 MET A :103			0.0
1i	-7.5	ILE A :194	GLY A :14 GLY A :96 SER A :94 ILE A :21 PRO A :193 THR A :196 ILE A :202 MET A :161 MET A :103 MET A :98 SER A :20	ILE A :16		ILE A :95 ALA A :198	MET A :199		PHE A :97	0.0
1j	-7.5	ILE A :194 SER A :94 SER A :20	MET A :161 TYR A :158 MET A :199 PRO A :193 THR A :196 ILE A :95 SER A :13 GLY A :14 ALA A :22 GLY A :96							0.0
2a	-7.5	GLY A :96 ILE A :194	MET A :161 GLY A :14 SER A :94	ILE A :36	ILE A :95	ALA A :198	MET A :199			0.0

			SER A :20 LYS A :165 MET A :147 ILE A :21 PRO A :193 THR A :196 ILE A :202 PHE A :97							
2b	-7.8	GLY A :96	ILE A :122 VAL A :65 ALA A:198			ILE A :95 ILE A :16		PHE A :97 PHE A :41		0.0
2c	-7.6	ASP A :148	LEU A :218 THR A :196 SER A :20 ILE A :16 GLY A :14 SER A :94 ILE A :95 GLY A :96 MET A :161 ILE A :21 MET A :147 ALA A :191 TYR A :158 ILE A :194	MET A :199	ILE A :215	PRO A :193		PHE A :149		0.0
2d	-7.4	ILE A :194 GLY A :96	PHE A :97 MET A :161 THR A :196 PRO A :193 ILE A:21 MET A :147 LYS A :165 SER A :94 SER A :20 GLY A :14	ILE A :16	ILE A :95	ALA A :198	MET A :199			0.0
2e	-7.5	ILE A :194 SER A :94 LYS A :165 THR A :196	GLY A :96 MET A :161 PHE A :97 ALA A :198		ILE A :21 ILE A :16	PHE A :149 TYR A :158 PRO A :193	MET A :199			0.0
2f	-8.3	GLY A :96 SER A :94 GLY A :14	PHE A :41 THR A :196 MET A :199 MET A :103 PHE A :97 MET A :161 LYS A :165 PHE A :149 ILE A :21 SER A :20		ILE A :16 ILE A :95	MET A :147 ALA A :198				0.0
2g	-7.4	MET A :199 SER A :20 ILE A :21 SER A :94	MET A :130 LYS A :165 MET A :147 ALA A :22 ILE A :95 GLY A :14 GLY A :96 ILE A :16 THR A :196		ILE A :202	ALA A :198 PHE A :94	MET A :161			0.0
2h	-7.4	GLY A :96 LYS A :165	ILE A :194 SER A :20	ILE A :21	MET A :199		PHE A :97		GLY A :96	0.0

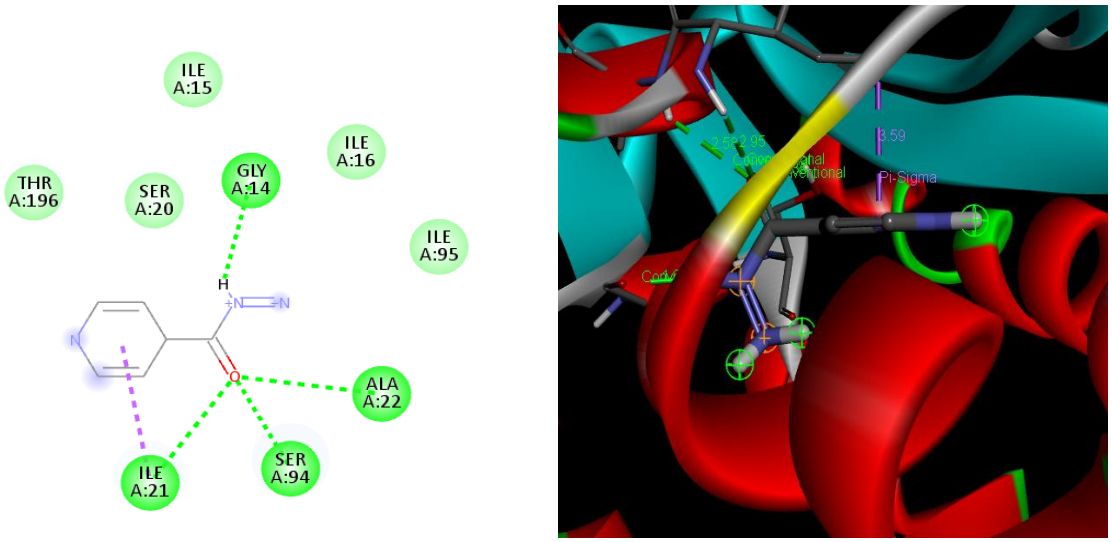
			ILE A :16 ALA A :22 THR A :196 ALA A :198 ILE A :202 SER A :123 MET A :147 MET A :161 ILE A :95 SER A :94 GLY A :14 TYR A :158							
2i	-7.8	SER A :20 ILE A :194	ILE A :202 MET A :103 MET A :98 MET A :161 GLY A :14 THR A :196 GLY A :96 SER A :94 ILE A :21 PRO A :193	ILE A :16	ILE A :95	ALA A :198	MET A :199		PHE A :97	0.0
2j	-7.5	LYS A :165	ALA A:22 MET A :147 SER A :94 MET A :161 GLY A :96 ILE A :95 PHE A :149 PRO A :193 ILE A :194 THR A :196 GLY A :14 ILE A :16 SER A :20			ILE A :21				0.0
Isoniazid	-5.0	GLY A :14 ILE A :21 SER A :94 ALA A :22	ILE A :15 ILE A :16 THR A :196 SER A :20 ILE A :95	ILE A :21						0.0



a) b)

a) 2D structure of ligand 2f interaction with binding site of protein 4TRJ. b) 3D structure of ligand 2f interaction with binding site of protein 4TRJ

Figure 4: Binding interaction between the ligand and amino acids at the binding



c) d)

c) 2D structure of Isoniazid interaction with binding site of protein 4TRJ. d) 3D structure of Isoniazid interaction with binding site of protein 4TRJ

Figure 5: Binding interaction of standard drug Isoniazid at the binding sites

CONCLUSION

In conclusion, using Autodock vina PyRx tools and protein pdb id 4TRJ, a novel series of twenty 3,4 dihydropyrimidine derivatives were developed and their docking experiments were conducted. These 20 compounds' binding affinities were compared to that of the standard drug isoniazid. Out of these 20 compounds, 1h and 2f have the highest binding affinities (8.1 and 8.3 kcal/mol, respectively), while 1a has the lowest affinity (5.0 kcal/mol) in comparison to the standard drug isoniazid. In summary, every derivative demonstrates a high affinity for binding the protein. Compared to derivatives with an electron-donating group substituted, compounds with an electron-withdrawing group substituted exhibit the highest binding affinity.

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