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IN SILICO DESIGN OF 1,3,5-TRIAZINE DERIVATIVES AS ENOYL ACYL CARRIER PROTEIN REDUCTASE INHIBITORS

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ABSTRACT

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. It affects millions of people worldwide. Isoniazid (INH) is a frontline antitubercular agent. It requires activation by the enzyme catalase-peroxidase (KatG) to convert to an active form, which forms an adduct with NADH, to inhibit the enzyme enoyl acyl carrier protein reductase (InhA). Mutations in the KatG enzyme has resulted in the development of isoniazid resistant strains of the organism. A series of 1,3,5-triazine derivatives were designed *in silico* and docked in the active site of the enzyme enoyl ACP reductase. These compounds were designed as direct inhibitors of the enzyme. Direct InhA inhibitors require no prior activation by any enzyme. The interaction of 24 compounds in the active site of the enzyme was studied. It was observed that some of the compounds exhibited hydrogen bond formation with the residue Tyr158, which is a conserved feature among the inhibitors of this enzyme. The potential to inhibit the enzyme was analyzed on the basis of the *Glide Score* (G-Score), hydrogen bonding and van der Waals interaction. Out of the 24 compounds, the ones with good G-score would be synthesized and their mechanism by inhibition of the enoyl ACP reductase would be confirmed subsequently.

KEYWORDS: Tuberculosis, InhA, direct inhibitors, triazine derivatives, *Glide score.*

Mycobacterium tuberculosis are aerobic bacteria, with a waxy cell wall, composed of mycolic acid.[1-2] It is the main cause of tuberculosis, an infectious disease, which is the ninth leading cause of death worldwide.[3] Tuberculosis mainly affects the lungs but can affect other sites as well. It spreads when people who are sick with pulmonary TB, expel bacteria into the air by coughing. A person infected with *Mycobacterium tuberculosis* may not develop clinically significant disease. The viable organism may remain dormant and only reactivate when the host's immune defenses are lowered, to take on an infectious and life-threatening form.[4]

Worldwide around 10 million people fall ill with TB and it is one of the top ten causes of death. There were 5,58,000 new cases with resistance to rifampicin (RRTB), of which 82% had multi-drug resistant TB (MDR-TB).[3] The statistics does not paint an encouraging picture of the current status of the disease. The factors that are responsible for tuberculosis to have such a strong hold as a feared infectious disease are the appearance of multi-drug resistant strains, non-compliance to the drug regimen and AIDS epidemic.[5] The emergence of MDR-TB and extensively drug-resistant TB (XDR-TB) has prompted researchers to explore different pathways existing in mycobacterium and trying to find potential lead molecules.

The cell wall of *Mycobacterium tuberculosis* is associated with its pathogenicity[5]. The main constituent of its cell wall is composed of mycolic acid. Enoyl acyl carrier protein reductase is a key fatty acid synthase type II enzyme. It catalyzes the final enzymatic step converting trans-2-enoyl-ACP to acyl-ACP in a NADH dependent reaction. Isoniazid (INH) is a front-line antitubercular drug. Activity of INH is dependent on its conversion to an acyl radical by catalase-peroxidase KatG enzyme. The acyl radical binds covalently to NADH, the co-substrate for InhA. This adduct functions as a potent inhibitor of InhA.[6-10] Clinical resistance to INH has been linked to mutations in KatG.[11-12] The novelty of direct InhA inhibitors lies in the fact that they do not have to be activated by an enzyme and hence, is less prone to develop resistance by the organism.[13]

The aim of the study is to find potential leads amongst the substituted 1,3,5-triazine derivatives as direct inhibitors of the InhA enzyme by performing molecular docking studies.

MATERIALS AND METHODS

Computational Tools

Docking studies were performed using *Maestro 11*, a Molecular Modeling Software from Schrödinger. *Maestro* is the graphical user interface for nearly all of

Schrödinger computational programs. It was installed on a computer system having Windows XP as an operating system and having a configuration of 3.4 GHz Pentium-4 processor with 1 GB RAM and 160 GB Hard Disk.

The steps involved in the docking studies were as follows:

Ligand preparation

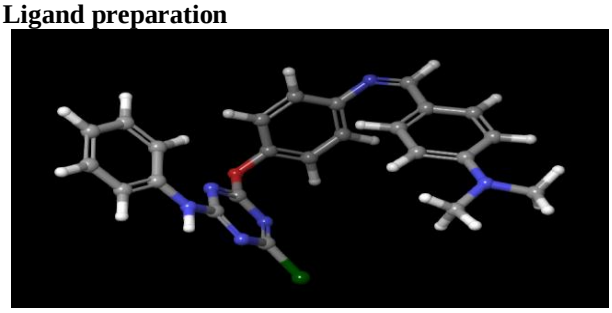
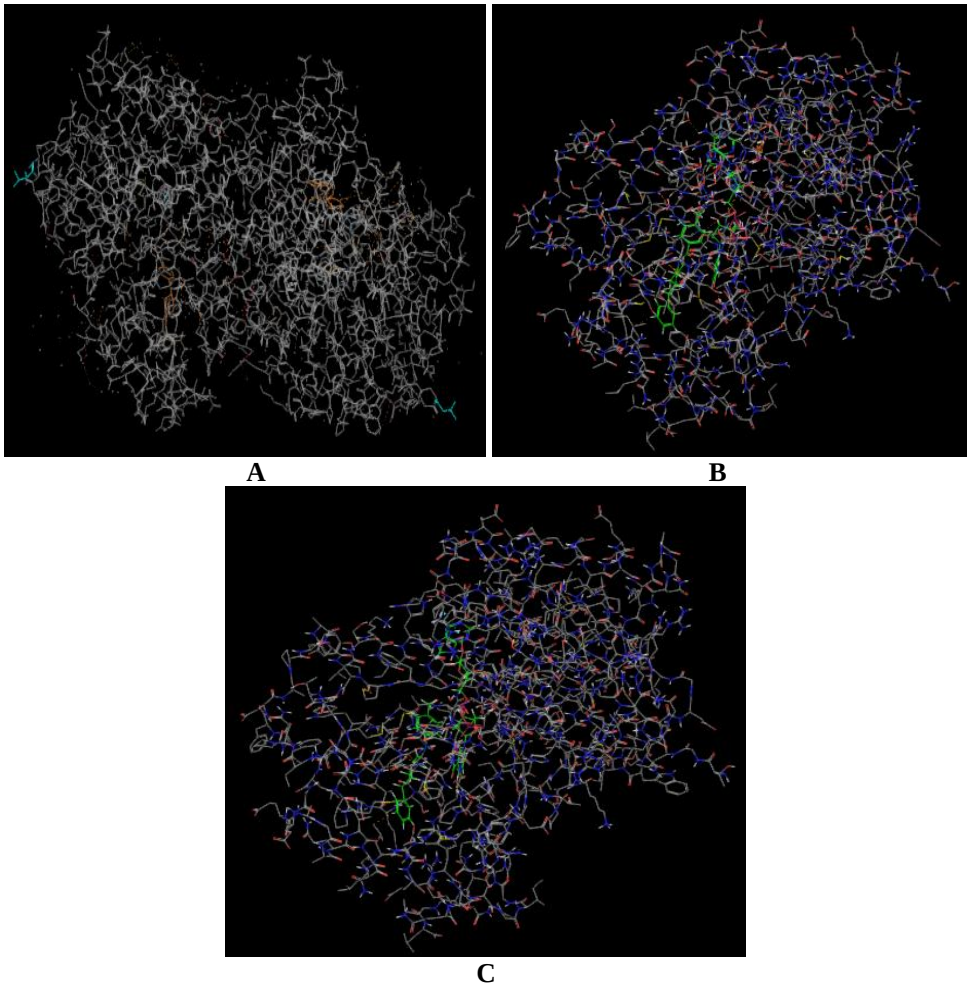


Figure 1: Ligand structure by *LigPrep*.

The ligands were sketched using the *build* panel on *Maestro* and optimized by using program *LigPrep*, using OPLS-2003 force field. *LigPrep*, which is a ligand preparation tool, provides high quality, all atom 3D structures.

Protein preparation



A **B**

C

Figure 2: Protein preparation of InhA (PDB ID: 2NSD) (A) Raw protein (B) Pre-processed protein (C) Optimized and minimized protein.

The X-ray crystallographic structure of InhA, the enoyl acyl carrier protein reductase from *Mycobacterium tuberculosis*, in complex with N-(4-methylbenzoyl)-4-benzylpiperidine (4PI), which is a direct inhibitor of the enzyme, was downloaded from Research Collaboratory for Structural Bioinformatics (RCSB) having PDB ID 2NSD and a resolution of 1.9 A°. The enzyme was processed using *Protein Preparation Wizard*.

Grid generation

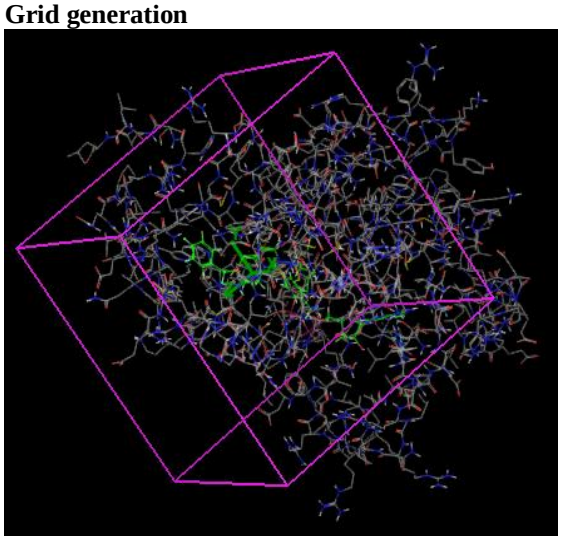


Figure 3: Receptor grid generation.

The receptor grid can be generated by using the *Glide Receptor Grid Generation Wizard*. The protein structure usually has a co-crystallized ligand. During receptor grid generation, the co-crystallized ligand around which a grid box is to be generated is picked up. The size of the grid can be adjusted. The co-crystallized ligand is excluded from the receptor grid generation calculations.

Validation

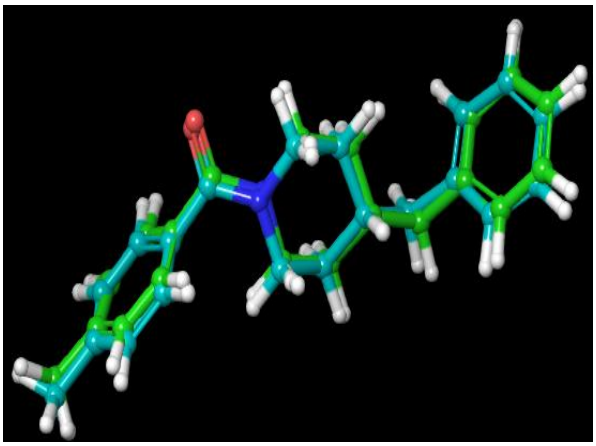
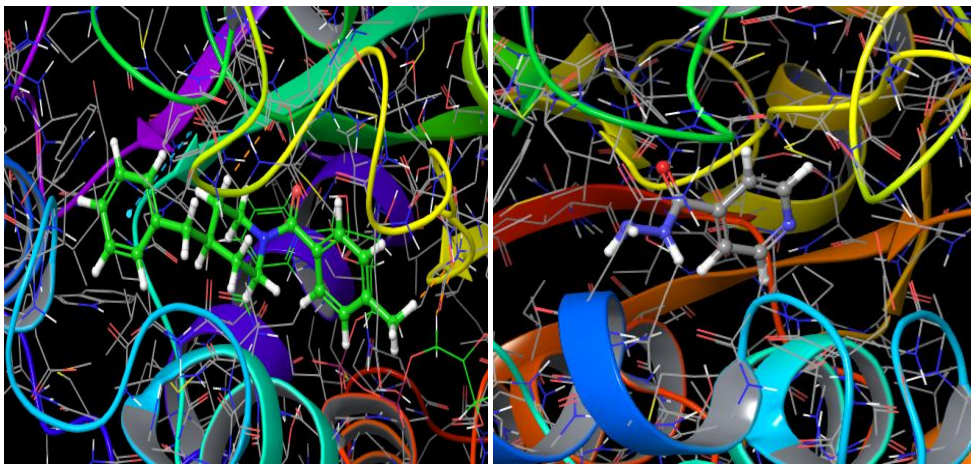


Figure 4: Super-positioning of de-docked and re-docked co-crystallized inhibitor 4PI.

The inhibitor, N-(4-methylbenzoyl)-4-benzylpiperidine (4PI), was split from the receptor (de-docked). It was re-docked onto the active site. The de-docked and re-docked inhibitor 4PI were superimposed. The root mean square deviation (RMSD) should be less than one.

Docking



A **B**

Figure 5: (A) 4PI docked in the active site of enzyme 2NSD (B) Isoniazid docked in the active site of enzyme 2NSD.

Docking studies were performed using the program *Glide.* During docking studies, ligand was flexible and the receptor was held rigid. The compounds were docked in the active site of the enzyme 2NSD. The docking results were expressed in terms of their G-scores.

RESULTS AND DISSCUSSION

Validation of the protocol

The RMSD value obtained when the de-docked and re-docked inhibitor 4PI were superimposed, was 0.4524, which is less than 1, showing that the docking protocol was validated.

Figure 4 shows the superimposed de-docked and re-docked co-crystallized inhibitor 4PI.

Docking studies

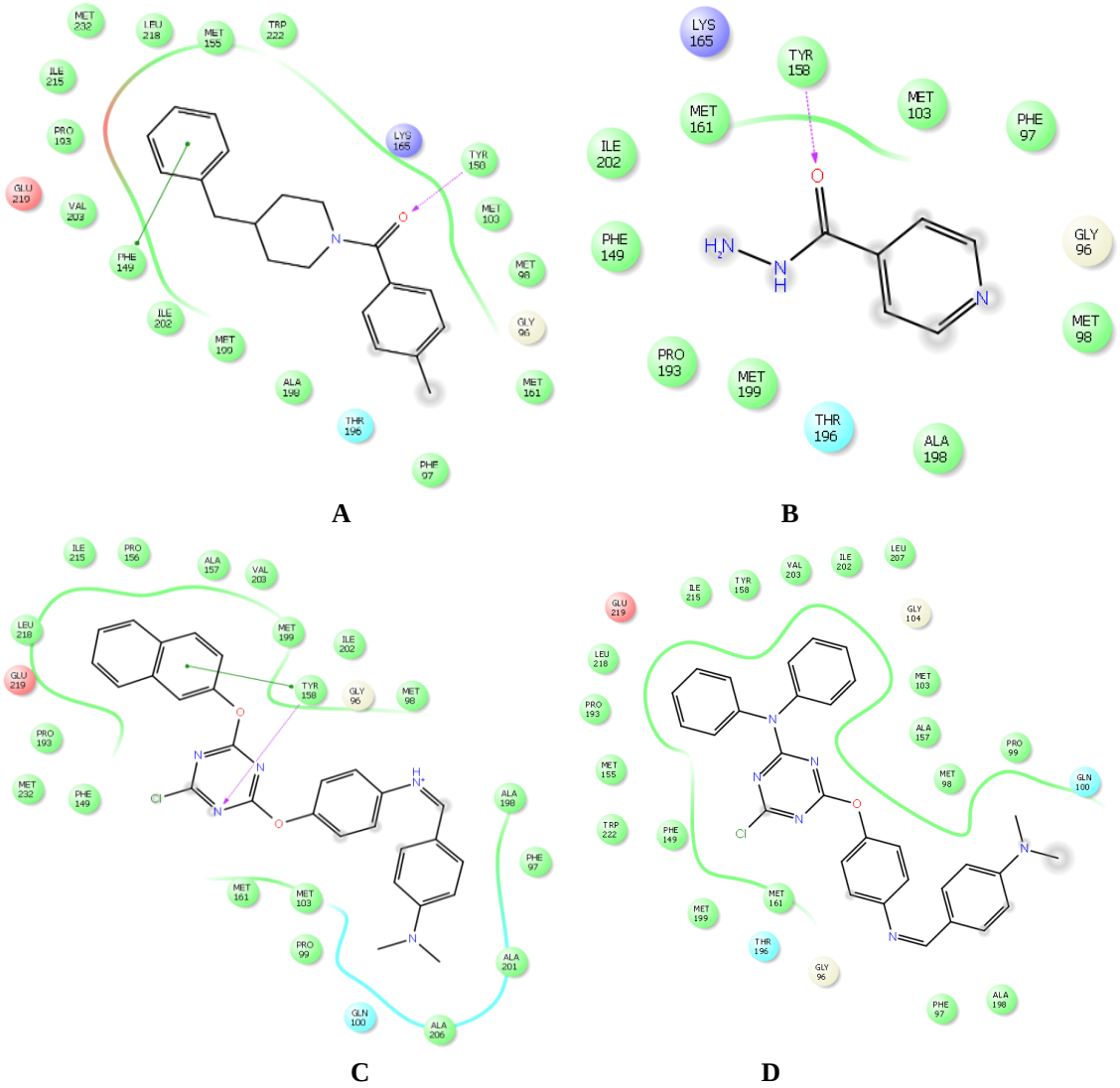


Figure 6: Ligand interaction of (A) Inhibitor 4PI (B) Isoniazid (C) & (D) Compounds C18 and C15 with enzyme 2NSD.

Glide Score is an empirical scoring function designed to maximize separation of compounds with strong binding affinity from those with little to no binding ability. Particularly beneficial to the binding is the formation of one or more protein-ligand hydrogen bonds, which helps the ligand to stay bound in the active site of the receptor thereby providing stability to the complex.

Figure 6A shows the interaction of the co-crystallized ligand 4PI in the active pocket of enzyme 2NSD. The unsubstituted aromatic ring shows pi-pi stacking interaction, shown by a green line, with the residue Phe149, and the oxygen of the carboxyl group shows hydrogen bond formation with the side chain of the residue Tyr158, shown by dashed pink line. In Fig. 6B, the oxygen of the carboxyl group of isoniazid is seen to form hydrogen bond with the side chain of the residue Tyr158.

The InhA inhibitors observed in the literature show a signature hydrogen bond with the residue Tyr158, which is one of the catalytic residues of the enzyme. This seems to be a conserved feature among these inhibitors.

In the compound C18 (Table 1), the nitrogen of the 1,3,5-triazine moiety forms hydrogen bond with the side chain of the residue Tyr158. It also shows pi-pi stacking interaction with the naphthalene ring of compound C18. These interactions may be the reason for the high G-score of -12.25 exhibited by this compound, higher than the co-crystallized ligand 4PI.

Compound C15 has a G-score similar to the co-crystallized ligand 4PI. The high G-score of -11.65 exhibited by this compound can be attributed to the presence of bulky substituent in the form of the diphenyl group, which helps in increasing the hydrophobic contact between the compound and the hydrophobic residues in the active site of the enzyme 2NSD.

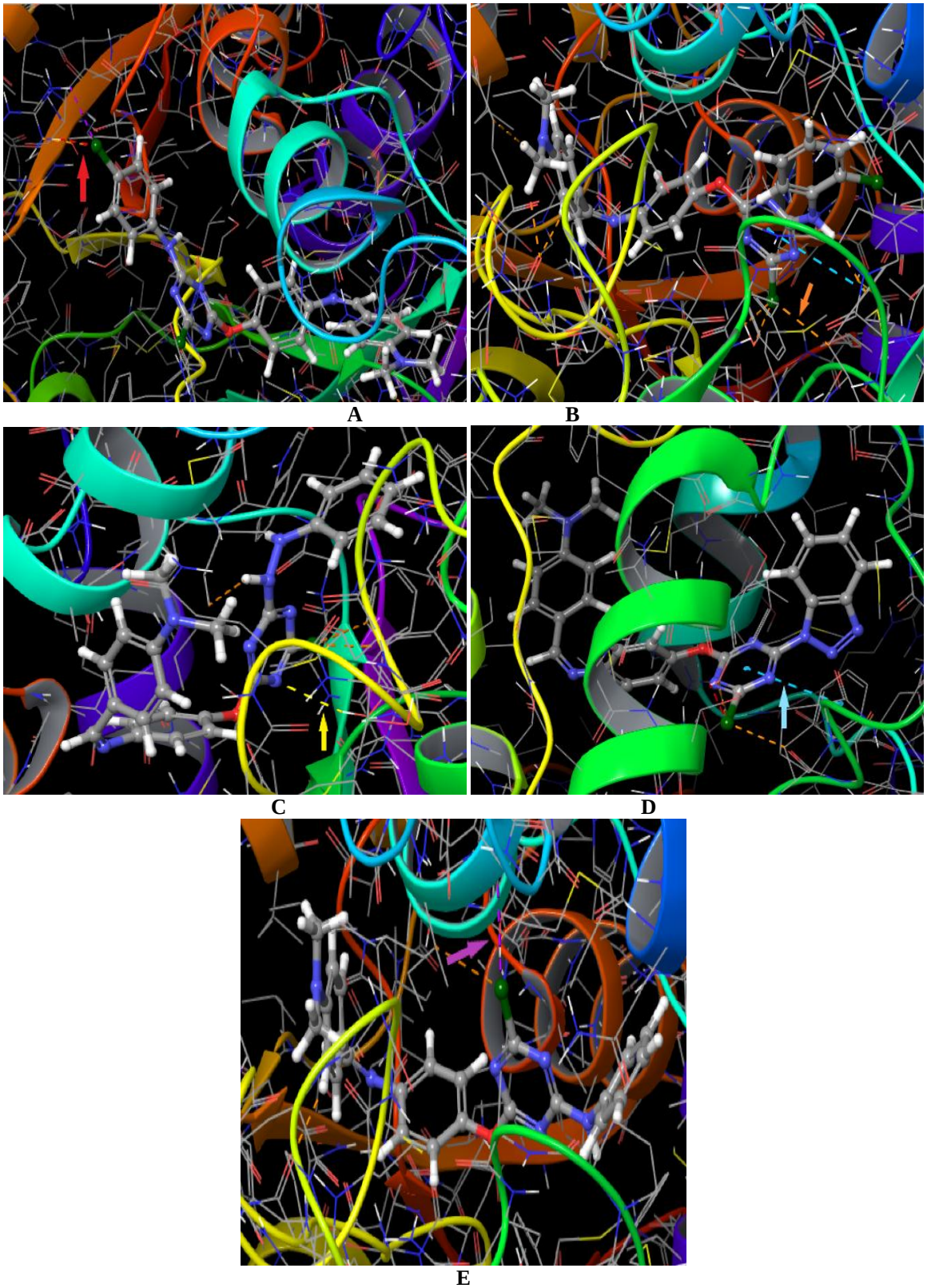


Figure 7: (A) Ugly bond (B) Bad bond (C) Hydrogen bond (D) pi-pi stacking (E) Halogen bond formed by the compounds with various residues.

Figure 7A show the ugly bond which can be seen as a red dashed line, the bad bond as an orange dashed line (Fig. 7B), hydrogen bond as a yellow dashed line (Fig. 7C), pi-pi stacking interaction as a cyan dashed line (Fig. 7D) and halogen bond as a purple dashed line (Fig. 7E).

The designed compounds showed 0-2 hydrogen bonds with not only the residue Tyr158, but also with residues such as Arg43 and Met98. They showed halogen bonds with residues Met199 and Arg43. Salt bridge formation was also seen with the residue Arg43. The number of

ugly vdw contacts ranged from 0-1 and the bad vdw contacts ranged from 0-8.

Compounds C1, C4, C5, C6, C9, C11, C13, C14, C16, C17, C18, C20 and C21 have G-scores ranging from -9.095 to -9.934. Compounds C2, C3, C7, C8, C10, C12, C19, C22, C23 and C24 have G-scores in the range of -10.076 to -10.844. All the compounds have G-scores higher than those of isoniazid and ethionamide. Table 1 shows various substituents and the G-scores of the compounds.

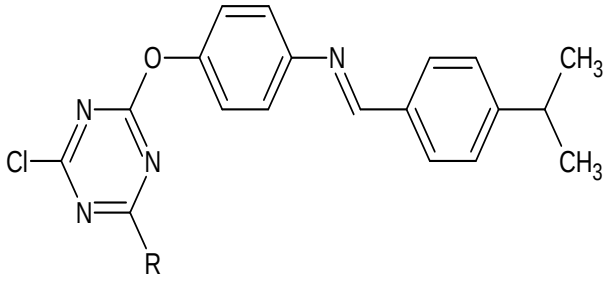
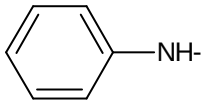
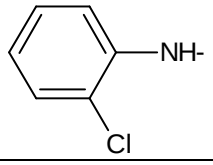
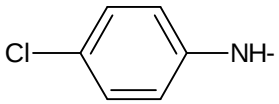
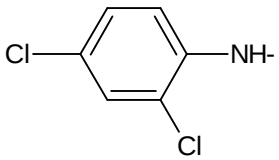
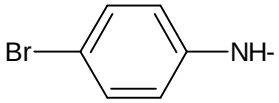
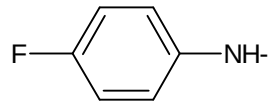
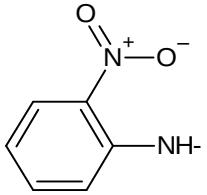
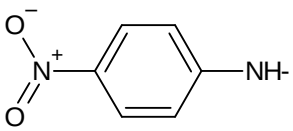
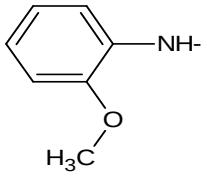
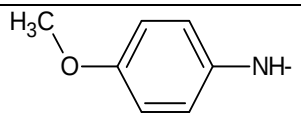
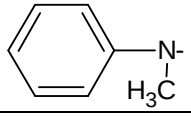
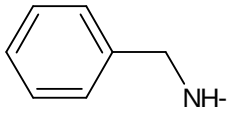
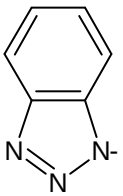
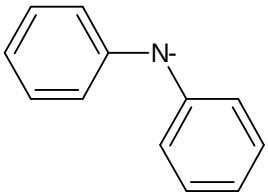
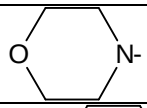
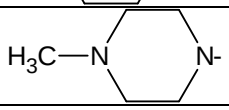
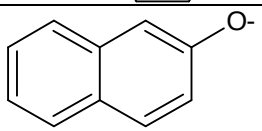
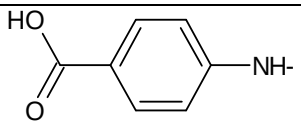
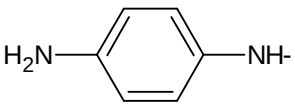
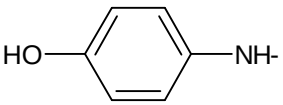
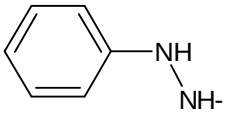
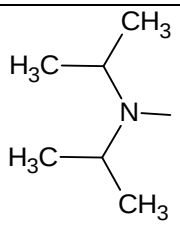
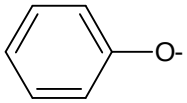


Figure 8: General structure of the 1,3,5-triazine derivatives.

Table 1: Various substituents and G-scores of the 1,3,5-triazine derivatives

Compound No.	R	G-score
C1	-Cl	-9.934
C2		-10.844
C3		-10.118
C4		-9.771
C5		-9.574
C6		-9.23
C7		-10.262
C8		-10.241
C9		-9.095
C10		-10.148

C11		-9.488
C12		-10.33
C13		-9.905
C14		-9.342
C15		-11.65
C16		-9.105
C17		-9.366
C18		-12.25
C19		-10.076
C20		-9.401
C21		-9.894
C22		-10.712
C23		-10.196

C24		-10.456
4PI	-	-11.62
Isoniazid	-	-6.915
Ethionamide	-	-7.541

CONCLUSION

The discovery of novel InhA inhibitors is essential since direct InhA inhibitors circumvent the need to be activated by enzymes to show activity. In this study, docking studies were carried out on a series of 1,3,5-triazine derivatives using the software *Glide*. The compounds showed good G-scores and also interaction with the residue Tyr158, which has been observed with other active InhA inhibitors as well. Aside from hydrogen bond with the residues Tyr158, Met98 and Arg43, the compounds also showed interactions like pi-pi stacking, halogen bonds and formation of salt bridges. Hence, it can be concluded that these compounds may be potent antimycobacterial agents. In future research work, synthesis of these compounds will be taken up and the synthesized compounds will be screened for *in vitro* antimycobacterial activity. The mechanism of action of these compounds by InhA inhibition will be confirmed by InhA enzyme inhibition studies.

REFERENCES

1. Pelczar MJ, Chan ECS, Krieg NR, Microbiology, 5th edition, Tata McGraw-Hill publication, New Delhi, 2006; 296.
2. Lone MY, Athar M, Gupta VK, Jha PC, Identification of *Mycobacterium tuberculosis* enoyl-acyl carrier protein reductase inhibitors: A combined *in silico* and *in vitro* analysis, Journal of Molecular Graphics and Modelling, 2017; 76: 172-180.
3. World Health Organization, Global Tuberculosis Report, 2018.
4. Kumar V, Abbas AK, Fausto N, Robbins and Cotran, Pathologic basis of disease, 7th edition, Elsevier, New Delhi, 2007; 381-386.
5. Takayama K, Wang C, Besra GS, Pathway to synthesis and processing of mycolic acids in *Mycobacterium tuberculosis*, Clinical Microbiology Reviews, 2005; 18(1): 81-101.
6. More UA, Joshi SD, Aminabhavi TM, Gadad AK, Nadagouda MN, Kulkarni VH, Design, synthesis, molecular docking and 3D-QSAR studies of potent inhibitors of enoyl acyl carrier protein reductase as potential antimycobacterial agents, European Journal of Medicinal Chemistry, 2014; 71: 199-218.
7. Saharan VD, Mahajan SS, Design of furfuraldehyde formazans as direct inhibitors of enoyl acyl carrier protein reductase for the treatment of tuberculosis, American Journal of Pharmtech Research, 2015; 5(4): 367-39.
8. Saharan VD, Mahajan SS, Development of gallic acid formazans as novel enoyl acyl carrier protein reductase inhibitor for the treatment of tuberculosis, Bioorganic & Medicinal Chemistry Letters, 2017; 27: 808-815.
9. Prati F, Zuccotto F, Fletcher D, Convery MA, Fernandez Menendez R, Bates R, Encinas L, Zeng J, Chung C, Anton PD, Mendoza-Losana A, Mackenzie C, Green SR, Huggett M, Barros D, Wyatt PG, Ray PC, Screening of a novel fragment library with functional complexity against *Mycobacterium tuberculosis* InhA, Chem Med Chem., 2018; 13: 672–677.
10. Heath RJ, Rock CO, Fatty acid biosynthesis as a target for novel antibacterials, Current Opinion in Investigational Drugs, 2004; 5(2): 146–153.
11. He X, Alian A, Stroud R, Ortiz de Montellano PR, Pyrrolidine carboxamides as a novel class of inhibitors of enoyl acyl carrier protein reductase (InhA) from *Mycobacterium tuberculosis*, Journal of Medicinal Chemistry, 2006; 49(21): 6308–6323.
12. Kuo MR, Morbidoni HR, Alland D, Sneddon SF, Gourlie BB, Staveski MM, Leonard M, Gregory JS, Janjigian AD, Yee C, Musser JM, Kreiswirth B, Iwamoto H, Perozzo R, Jacobs, Jr. WR, James C. Sacchettini JC, Fidock DA, Targeting tuberculosis and malaria through inhibition of enoyl reductase, The Journal of Biological Chemistry, 2003; 278(23): 20851–20859.
13. He X, Alian A, Ortiz de Montellano PR, Inhibition of *Mycobacterium tuberculosis* enoyl acyl carrier protein reductase InhA by arylamides, Bioorganic Medicinal Chemistry, 2007; 15(21): 6649-6658.