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Design of Furfuraldehyde Formazans as Direct Inhibitors of Enoyl Acyl Carrier Protein Reductase for the Treatment of Tuberculosis

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ABSTRACT

Mycobacterial enoyl acyl carrier protein reductase (InhA) is a clinically validated target for the treatment of tuberculosis infection, a disease that still causes the death of at least a million people annually. The current study aims to design and explore the possible mechanism of action of various furfuraldehyde formazans as direct inhibitors of InhA through molecular docking studies. A series of furfuraldehyde formazans were computationally designed and energy minimized. These compounds were docked into the active site of InhA (PDB code, 2NSD) using software *Glide* from the suite of Schrödinger. These compounds were identified as potential inhibitors of InhA on the basis of Glide Score (G-Score), hydrogen bonding (H-bond) and van der Waals (vdw) interaction between ligand and the receptor. Ten furfuraldehyde formazans displayed G-Scores (-7.351 to -9.148) higher than that of isoniazid (-7.250). These compounds have good hydrogen bonding and hence good affinity for the enzyme, when compared with isoniazid. Further, we plan to synthesize these compounds and screen them for antimycobacterial and enzyme inhibitory activity.

Keywords: Tuberculosis, InhA, Furfuraldehyde, Formazans, Glide.

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INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*). It is the leading cause of morbidity and mortality among the infectious diseases. The World Health Organization (WHO) has estimated that one-third of the world's population, nearly 2 billion people, mostly in the developing countries[1] has been infected with *M. tuberculosis*. Among the infected individuals, 8 million develop active TB and nearly 2 million people die from the disease annually[2]. In recent years, the pandemic of AIDS had a major impact on the worldwide TB problem. On one hand, HIV infection is the most potent risk factor for converting latent TB into the active, transmissible form, thus fueling the spread of TB; on the other hand, TB bacteria can accelerate the progress of AIDS infection. One-third of the increase in the incidence of TB in the past 5 years can be attributed to coinfection with HIV[2]. This situation has been further exacerbated by the emergence of multidrug-resistant tuberculosis (MDR-TB) strains that are resistant to some or most current anti-TB drugs[3]. Over the decade, it is estimated that as many as 50 million people worldwide have been infected with MDR-TB strains. Despite the increasing worldwide incidence of TB and its alarming threat to the public health, no novel antitubercular drugs have been introduced into clinical practice over the past 4 decades. The HIV coinfection with tuberculosis, multidrug resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) have brought tuberculosis into the failure of current standard treatment regimens[1]. This fact prompts the research to develop novel and more potent drug candidates to treat *M. tuberculosis* strains resistant to the existing drugs. The enoyl acyl carrier protein reductase (InhA) of *M. tuberculosis* catalyzing the NADH-specific reduction of 2-trans-enoyl-ACP[4] is an attractive target for designing novel antibacterial agents[5-10]. The enzyme InhA has been identified as the primary target of isoniazid (INH), one of the most effective first-line anti-TB drugs[11-16]. The enzyme InhA is inhibited by the active adduct of INH (INH-NAD)[17-18], which is covalently formed between NAD^+ and the reactive acyl radical of INH, generated by the activation of catalase-peroxidase (KatG)[19-25]. The major mechanism of INH resistance arises from mutations in KatG[26-27]. To overcome the INH resistance associated with mutations in KatG, compounds which directly inhibit the InhA enzyme without requiring activation of KatG, called direct InhA inhibitors, are new promising agents against tuberculosis. Because of the remarkable properties of direct InhA inhibitors, many research groups have been attempting to develop direct InhA inhibitors, e.g. triclosan[28], diphenyl ethers[29-31], pyrrolidine carboxamide[32] and arylamide derivatives[33-34]. Therefore, inhibitors targeting InhA directly, without a requirement for activation, would be the

promising candidates for the development of agents against the ever increasing threat from drug-resistant *M. tuberculosis* strains. The aim of the present study is to conduct the molecular docking studies on a series of novel furfuraldehyde formazans. The current study also further aims to explore the possible mechanism of action of various furfuraldehyde formazans as direct inhibitors of InhA through molecular docking studies.

MATERIALS AND METHOD

Computational Tools

Maestro 9.10 – the Molecular Modeling Software from Schrodinger Inc., USA, was used to perform all the computational studies. *Maestro 9.10* provides a graphical user interface for all the Schrödinger computational programs like *LigPrep, QikProp, Strike, Glide* etc. 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 following steps were undertaken for Molecular docking studies

Ligand preparation:

Structures of the ligands were sketched using *build* panel on *Maestro* and refined for docking by the program *LigPrep* using OPLS-2005 force field, which gave the corresponding low energy 3D conformers of the ligands, energy minimized using MMFF force field.

Protein preparation:

The X-ray crystal structure of InhA complexed with a direct InhA inhibitor, N-(4-methylbenzoyl)-4-benzylpiperidine, was employed for molecular docking. The InhA enzyme was downloaded from Protein Data Bank (PDB code 2NSD). The selected enzyme was processed by using *protein preparation wizard.* All hydrogens were added, unwanted chain ‘B’ from the protein was removed as the protein selected was the homodimer. Water molecules were removed from the protein and the energy minimization was carried out using “OPLS-2005” force field (Figure 1).

Grid generation:

Glide grid generation wizard was used to define the docking space. Receptor was defined and the cocrystallized ligand was differentiated from the active site of receptor ‘A’ chain. Grid generation was done with selection of flexible docking. By keeping remaining data unchanged, the grid was prepared (Figure 2). Docking was performed using the Standard Precision (SP) mode.

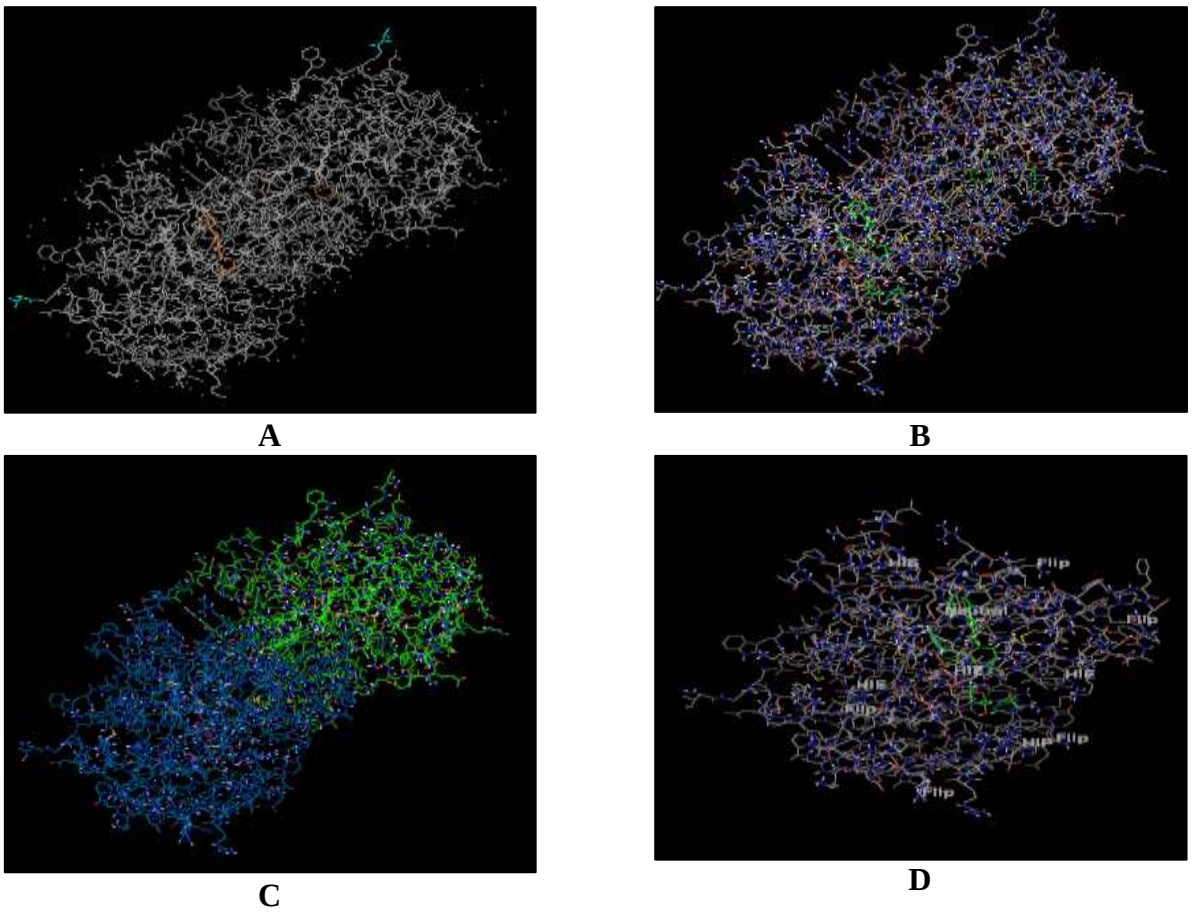


Figure 1: Protein preparation of InhA (PDB ID: 2NSD) (A) Imported InhA B) Preprocessed InhA (C) Chain A and B (D) Optimized InhA

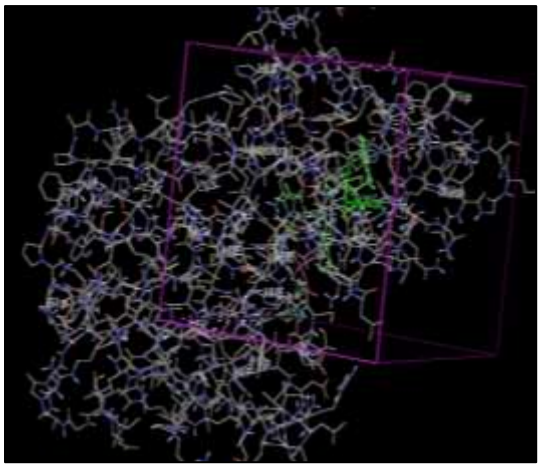
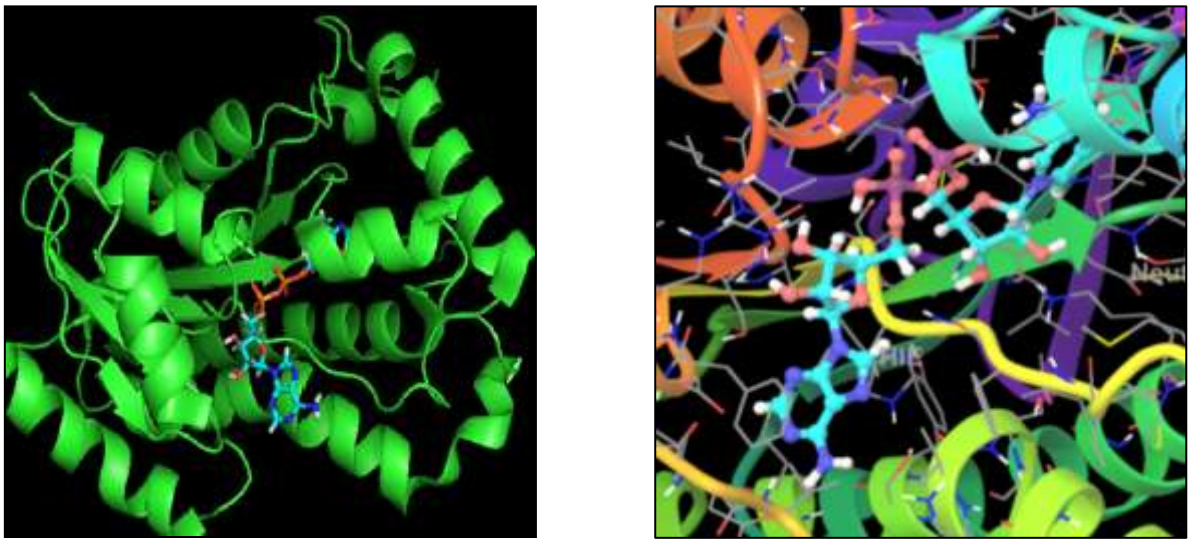


Figure 2: Grid Generation for docking of compounds (C1 – C18) with 2NSD

Docking:

Molecular docking studies were performed using the program *Glide* to understand the interaction of ligands and the enzyme. During docking procedure, ligand was flexible, whereas the receptor was held rigid. The best docked pose was saved. The root mean square deviation (RMSD) between

the crystal structure and the docked pose was 0.52 A^0, thereby validating the docking protocol. The compounds were docked in the active site of InhA (Figure 3). The docking results were expressed in terms of G-Score (Glide Score). Ligand binding to its receptor was evaluated, based on the following aspects, G score, hydrogen bonding (H-bond) and Contacts [van der Waals (vdw) interaction]. The comparative analysis of the docking parameters was carried out with the standard, isoniazid.



A **B**

Figure 3: (A) Isoniazid docked in the active site of the enzyme 2NSD (B) Compound 4 docked in the active site of the enzyme 2NSD

RESULTS AND DISSCUSSION

The structures of 18 synthetic formazans have been shown in Table 1. Table 2 shows G-scores and number of H-bonds and bad and ugly vdw contacts of synthetic formazans and isoniazid.

G-Score is a scoring function, designed to estimate the free energy of binding for a protein-ligand complex. Hydrogen bonds provide the stability to the protein-ligand complex. If the ligand has more hydrogen bonds with its binding pocket, then it is more likely to stay bound or docked with the receptor, thereby providing stability to the complex.

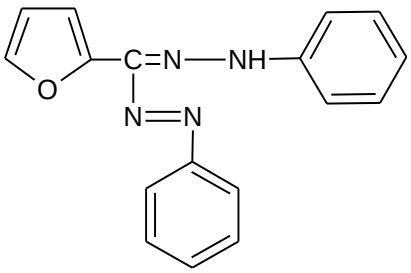
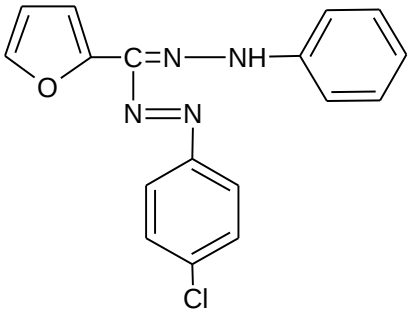
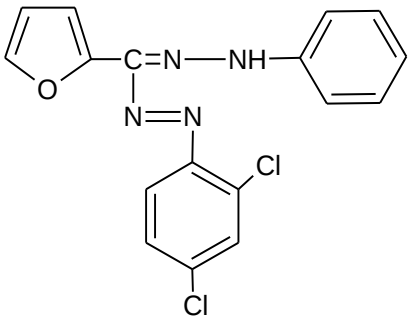
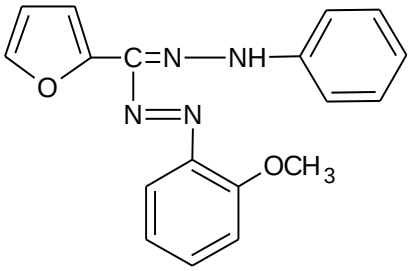
Compounds from **C10 – C18** have higher G-Scores **(G = -7.351 to -9.148)** as compared to compounds from **C1 – C9 (G = -5.693 to -7.351) (Table 2)**. The 5-nitrofurfuladehyde series **(C10 – C18)** displayed higher activity due to the electron withdrawing nature of the nitro substituent than the furfuraldehyde series **(C1 – C9)** (Table 2).

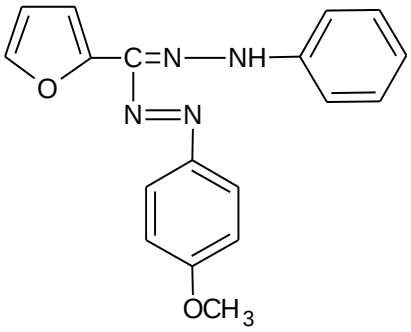
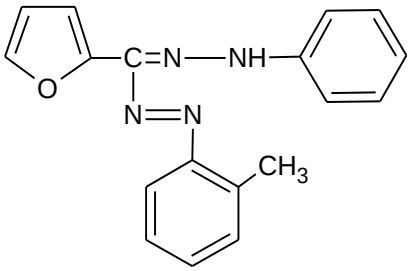
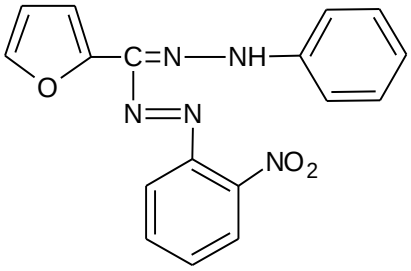
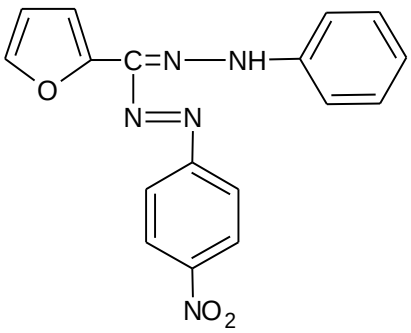
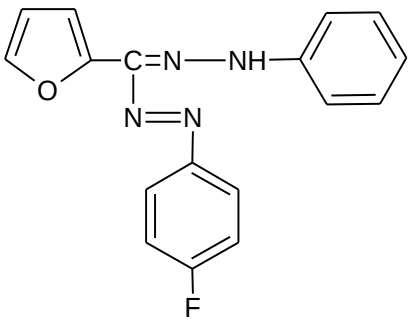
Moreover, the electron withdrawing groups like chloro **(C11 & C12, G = -8.954 & -8.542, respectively)** and methoxy **(C13 & C14, G = -8.351 & -8.377, respectively)** displayed relatively higher G-Scores as compared to methyl **(C15, G = -7.863)** and phenyl **(C10, G = -7.693)**

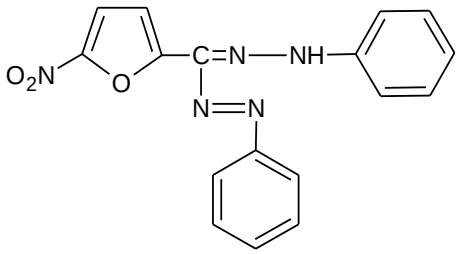
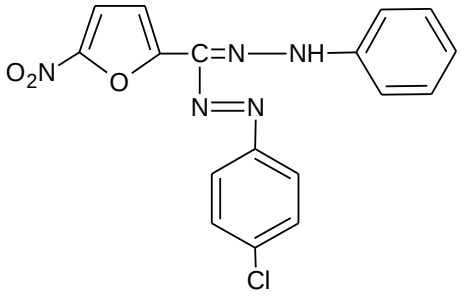
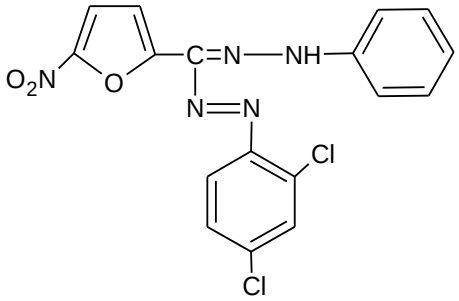
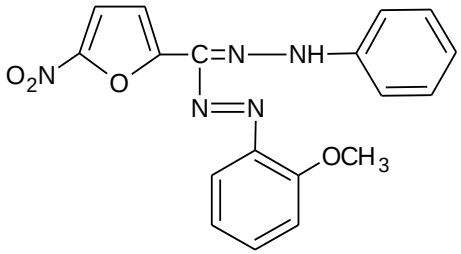
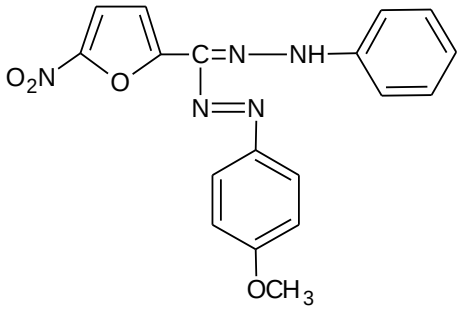
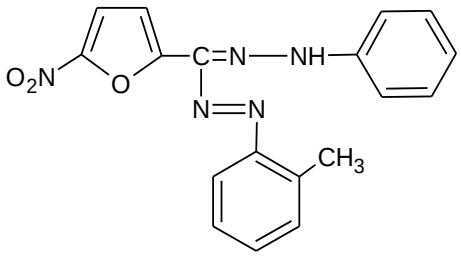
substituents. The compounds **C16** and **C17** displayed higher G-Scores **(G = -9.033 & -9.148, respectively)** as compared to other compounds. Ten compounds displayed higher G-Scores **(-7.351 to -9.148)** as compared to isoniazid **(-7.250)**.

All the designed compounds formed 1-3 hydrogen bonds with the receptor, thus, predicting good affinity towards InhA, resulting in better efficacy. For all the designed compounds, there was an absence of ugly vdw contacts, and the bad vdw contacts were in the range of 2-3. Thus, these can be predicted to form a stable complex with InhA enzyme and predicted to possess good antitubercular activity.

Table 1: Structures of synthetic furfuraldehyde formazans

Compound No	Structure of compound	Name of the compound
C1		1-[Furan-2-yl(2-phenylhydrazinylidene)methyl]-2-phenyldiazene
C2		1-(4-Chlorophenyl)-2-[furan-2-yl(2-phenylhydrazinylidene)methyl]diazene
C3		1-(2,4-Dichlorophenyl)-2-[furan-2-yl(2-phenylhydrazinylidene)methyl]diazene
C4		1-[Furan-2-yl(2-phenylhydrazinylidene)methyl]-2-(2-methoxyphenyl)diazene

C5		1-[Furan-2-yl(2-phenylhydrazinylidene)methyl]-2-(4-methoxyphenyl)diazene
C6		1-[Furan-2-yl(2-phenylhydrazinylidene)methyl]-2-(2-methylphenyl)diazene
C7		1-[Furan-2-yl(2-phenylhydrazinylidene)methyl]-2-(2-nitrophenyl)diazene
C8		1-[Furan-2-yl(2-phenylhydrazinylidene)methyl]-2-(4-nitrophenyl)diazene
C9		1-(4-Fluorophenyl)-2-[furan-2-yl(2-phenylhydrazinylidene)methyl]diazene

C10		1-[(5-Nitrofuran-2-yl)(2-phenylhydrazinylidene)methyl]-2-phenyldiazene
C11		1-(4-Chlorophenyl)-2-[(5-nitrofuran-2-yl)(2-phenylhydrazinylidene)methyl]diazene
C12		1-(2,4-Dichlorophenyl)-2-[(5-nitrofuran-2-yl)(2-phenylhydrazinylidene)methyl]diazene
C13		1-(2-Methoxyphenyl)-2-[(5-nitrofuran-2-yl)(2-phenylhydrazinylidene)methyl]diazene
C14		1-(4-Methoxyphenyl)-2-[(5-nitrofuran-2-yl)(2-phenylhydrazinylidene)methyl]diazene
C15		1-(2-Methylphenyl)-2-[(5-nitrofuran-2-yl)(2-phenylhydrazinylidene)methyl]diazene

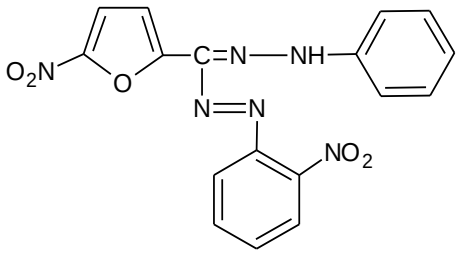
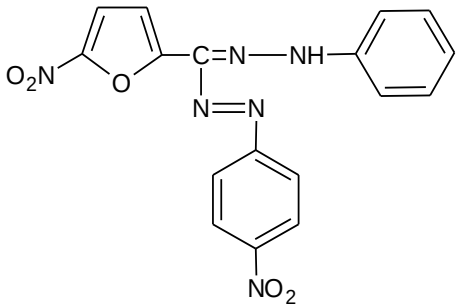
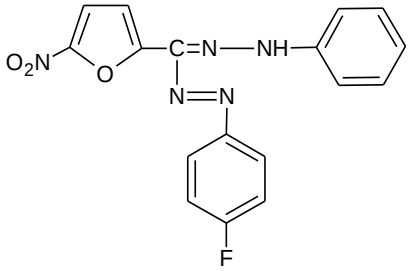
C16		1-[(5-Nitrofuran-2-yl)(2-phenylhydrazinylidene)methyl]-2-(2-nitrophenyl)diazene
C17		1-[(5-Nitrofuran-2-yl)(2-phenylhydrazinylidene)methyl]-2-(4-nitrophenyl)diazene
C18		1-(4-Fluorophenyl)-2-[(5-nitrofuran-2-yl)(2-phenylhydrazinylidene)methyl]diazene

Table 2: The G-score, number of H bonds, number of bad and ugly vdw contacts of all the synthesized compounds and the standard isoniazid

Compound code	G-Score	No of H bonds	No of Bad vdw contacts	No of ugly vdw contacts
C1	-5.693	1	2	0
C2	-6.954	2	2	0
C3	-6.542	1	2	0
C4	-6.351	1	1	0
C5	-6.377	0	2	0
C6	-6.863	2	2	0
C7	-6.033	2	2	0
C8	-6.148	2	2	0
C9	-7.351	1	2	0
C10	-7.693	2	2	0
C11	-8.954	2	2	0
C12	-8.542	1	2	0
C13	-8.351	2	0	0
C14	-8.377	3	2	0
C15	-7.863	2	2	0
C16	-9.033	2	0	0
C17	-9.148	1	2	0
C18	-7.546	1	2	0
Isoniazid	-7.250	0	2	0

CONCLUSION

The InhA is one of the key enzymes involved in the type II fatty acid biosynthesis pathway of *M. tuberculosis*. In this study, molecular docking studies were carried out on a series of furfuraldehyde formazans using the software *Glide*. The compounds were identified as potential direct inhibitors of InhA on the basis of G-Scores obtained, H-bonds and vdw contacts between ligand and the receptor. Compounds C9-C18 have G-Scores more than that of isoniazid. Also, these compounds have good hydrogen bonding than isoniazid. Hence, it can be concluded that the designed compounds can be potent antimycobacterial agents. In future research work, we plan to synthesize these compounds and screen them for their *in vitro* antimycobacterial activity. Moreover, the enzymatic activity will also be performed to further confirm the mechanism of action of these compounds.

REFERENCES

1. World Health Organization. Multidrug and extensively drug-resistant TB (M/XDR-TB): 2014 global report on surveillance and response.
2. Toossi Z. *Short communication*: Induction of HIV type 1 expression correlates with T-Cell responsiveness to mycobacteria in patients co infected with HIV type 1 and *Mycobacterium tuberculosis.* Int J Infect Dis 2003; 188: 1146-1153.
3. Bostrom J. Reproducing the conformations of protein-bound ligands: a critical evaluation of several popular conformational searching tools. J Comput Aided Mol Des 2001; 15: 1137-1152.
4. Quemard A, Sacchettini JC, Dessen A, Vilcheze C, Bittman R, Jacobs Jr WR, Blanchard JS. Enzymatic characterization of the target for isoniazid in *Mycobacterium tuberculosis*. Biochemistry 1995; 34: 8235-8241.
5. Campbell JW, Cronan Jr JE. Bacterial fatty acid biosynthesis: Targets for antibacterial drug discovery. Annu Rev Microbiol 2001; 55: 305-332.
6. Heath RJ, Rock CO. Fatty acid biosynthesis as a target for novel anti-bacterials. Curr Opin Invest Drugs 2004; 5: 146-153.
7. White SW, Zheng J, Zhang YM, Rock CO. The structural biology of type II fatty acid biosynthesis. Annu Rev Biochem 2005; 74: 791-831.
8. Zhang YM, Lu YJ, Rock CO. The reductase steps of the type II fatty acid synthase as antimicrobial targets. Lipids 2004; 39: 1055-1060.

9. Wen L, Chmielowski JN, Bohn KC, Huang JK, Timsina YN, Kodali P, Pathak AK. Functional expression of Francisella tularensis FabH and FabI, potential antibacterial targets. Protein Expr Purif 2009; 65: 83-91.
10. Wright HT, Reynolds KA. Antibacterial targets in fatty acid biosynthesis. Curr Opin Microbiol 2007; 10: 447-453.
11. Rozwarski DA, Grant GA, Barton DH, Jacobs Jr WR, Sacchettini JC. Modification of the NADH of the isoniazid target (InhA) from *Mycobacterium tuberculosis*. Science 1998; 279: 98–102.
12. Vilcheze C, Wang F, Arai M, Hazbon MH, Colangeli R, Kremer L, Weisbrod TR, Alland D, Sacchettini JC, Jacobs Jr WR. Transfer of a point mutation in *Mycobacterium tuberculosis* InhA resolves the target of isoniazid. Nat Med 2006; 12: 1027–1029.
13. Dessen A, Quemard A, Blanchard JS, Jacobs Jr WR, Sacchettini JC. Crystal structure and function of the isoniazid target of *Mycobacterium tuberculosis*. Science 1995; 267: 1638–1641.
14. Lei B, Wei CJ, Tu SC. Action mechanism of antitubercular isoniazid: Activation by *Mycobacterium tuberculosis* KatG, isolation, and characterization of InhA inhibitor. J Biol Chem 2000; 275: 2520–2526.
15. Johnsson K, King DS, Schultz PG. Studies on the mechanism of action of isoniazid and ethionamide in the chemotherapy of tuberculosis. J Am Chem Soc 1995; 117: 5009–5010.
16. Quemard A, Dessen A, Sugantino M, Jacobs Jr WR, Sacchetini JC, Blanchard JS. Binding of catalase-peroxidase-activated isoniazid to wild-type and mutant *Mycobacterium tuberculosis* enoyl-ACP reductases. J Am Chem Soc 1996; 118: 1561-1562.
17. Timmins GS, Deretic V. Mechanisms of action of isoniazid. Mol Microbiol 2006; 62: 1220-1227.
18. Johnsson K, Froland WA, Schultz PG. Overexpression, purification, and characterization of the catalase-peroxidase KatG from *Mycobacterium tuberculosis*. J Biol Chem 1997; 272: 2834-2840.
19. Saint-Joanis B, Souchon H, Wilming M, Johnsson K, Alzari PM, Cole ST. Use of site-directed mutagenesis to probe the structure, function and isoniazid activation of the catalase/peroxidase, KatG, from *Mycobacterium tuberculosis*. Biochem J 1999; 338: 753-760.
20. Zhao X, Yu H, Yu S, Wang F, Sacchettini JC, Magliozzo RS. Hydrogen peroxide-mediated isoniazid activation catalyzed by *Mycobacterium tuberculosis* catalase-peroxidase (KatG) and its S315T mutant. Biochemistry 2006; 45: 4131-4140.

21. Metcalfe C, Macdonald IK, Murphy EJ, Brown KA, Raven EL, Moody PC. The tuberculosis prodrug isoniazid bound to activating peroxidase. J Biol Chem 2008; 283: 6193-6200.
22. Sinha BK. Enzymatic activation of hydrazine derivatives. J Biol Chem 1983; 258: 796-801.
23. Nguyen M, Claparols C, Bernadou J, Meunier B. A fast and efficient metal-mediated oxidation of isoniazid and identification of isoniazid-NAD(H) adducts. Chembio Chem 2001; 2: 877-883.
24. Heym B, Zhang Y, Poulet S, Young D, Cole ST. Characterization of the katG gene encoding a catalase-peroxidase required for the isoniazid susceptibility of *Mycobacterium tuberculosis*. J Bacteriol 1993; 175: 4255-4259.
25. Johnsson K, Schultz PG. Mechanistic studies of the oxidation of isoniazid by the catalase peroxidase from *Mycobacterium tuberculosis*. J Am Chem Soc 1994; 116: 7425–7426.
26. De La Iglesia AI, Morbidoni HR. Mechanisms of action of and resistance to rifampicin and isoniazid in *Mycobacterium tuberculosis*: new information on old friends. Rev Argent Microbiol 2006; 38: 97-109.
27. Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, Collins D, de Lisle G, Jacobs Jr WR. InhA, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. Science 1994; 263: 227–230.
28. Freundlich JS, Wang F, Vilcheze C, Gulten G, Langley R, Schiehser GA, Jacobus DP, Jacobs Jr WR, Sacchettini JC. Triclosan derivatives: Towards potent inhibitors of drug-sensitive and drug-resistant *Mycobacterium tuberculosis*. ChemMedChem 2009; 4: 241-248.
29. Ende CW, Knudson SE, Liu N, Childs J, Sullivan TJ, Boyne M, Xu H, Gegina Y, Knudson DL, Johnson F, Peloquin CA, Slayden RA, Tonge PJ. Synthesis and *in vitro* antimycobacterial activity of B-ring modified diaryl ether InhA inhibitors. Bioorg Med Chem Lett 2008; 18: 3029-3033.
30. Boyne ME, Sullivan TJ, Ende CW, Lu H, Gruppo V, Heaslip D, Amin AG, Chatterjee D, Lenaerts A, Tonge PJ, Slayden RA. Targeting fatty acid biosynthesis for the development of novel chemotherapeutics against *Mycobacterium tuberculosis*: evaluation of A-ring-modified diphenyl ethers as high-affinity InhA inhibitors. Antimicrob Agents Chemother 2007; 51: 3562-3567.
31. Sullivan TJ, Truglio JJ, Boyne ME, Novichenok P, Zhang X, Stratton CF, Li HJ, Kaur T, Amin A, Johnson F, Slayden RA, Kisker C, Tonge PJ. High affinity InhA inhibitors with activity against drug-resistant strains of *Mycobacterium tuberculosis*. Chem Biol 2006; 17: 43-53.

32. He X, Alian A, Stroud R, Ortiz de Montellano PR. Pyrrolidine carboxamides as a novel class of inhibitors of enoyl acyl carrier protein reductase from *Mycobacterium tuberculosis*. J Med Chem 2006; 49: 6308-6323.
33. He X, Alian A, Ortiz de Montellano PR, Inhibition of the *Mycobacterium tuberculosis* enoyl acyl carrier protein reductase InhA by arylamides. Bioorg Med Chem 2007; 15: 6649–6658.
34. 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, Sacchettini JC, Fidock DA. Targeting tuberculosis and malaria through inhibition of Enoyl reductase: compound activity and structural data. J Biol Chem 2003; 278: 20851-20859.