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IDENTIFICATION OF NOVEL InhA INHIBITORS USING IN-SILICO SCREENING APPROACH

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ABSTRACT

Introduction: Mycobacterium tuberculosis, bacillus tuberculosis, is one of the infectious diseases that can be fatal. Feeling unwell, losing weight, having a temperature, and having night sweats are some of the symptoms. Coughing, chest pain, and blood in the cough are signs of lung disease. DOTS therapy, which contains the medications Isoniazid, Rifampin, Pyrazinamide, and Ethambutol, can be used to treat it.

Method: Using Open Eye Scientific Software (Cadence Molecular Sciences), very effective therapeutic compounds were found. Zinc, Molport, PubChem, and ChemSpider databases were used for *in-silico* similarity searching. With NITD-916, an oral active InhA inhibitor, having a similar structure, 84 top-ranked compounds were identified. OMEGA was used to generate conformers. Using the FRED software, docking was performed on 84 comparable structures to learn more about how they interacted with the InhA active site.

Results: When target molecules were docked into the putative binding site of the InhA enzyme, one or more hydrogen bonds were established with the amino acid residues. The top five scoring compounds were chosen for additional research.

Conclusion:

From the entire study, it was determined that the essential amino acids that interact through hydrogen bonds are ILE194 and LYS165.

Keywords: Tuberculosis, NITD-916, Open Eye Scientific Software, ChemSpider, Molport, OMEGA, FRED

INTRODUCTION

The infectious disease tuberculosis is caused by the Mycobacterium tuberculosis bacteria. It is a deadly disease that mostly affects the lungs and can potentially cause death. Robert Koch, a German microbiologist, made the original discovery of the tubercle bacillus in 1882. Numerous studies suggest that TB is a terrible illness that has existed since ancient times. The illness used to be known as "consumption" because of the way it would devour anyone who contracted it. A few inhaled droplets containing M. tuberculosis bacilli can cause tuberculosis infection through exposure **[1]**.

There are two phases to the pathogenesis of M. tuberculosis after infection. Latent tuberculosis infection is a stage that typically follows primary infection. There are no symptoms at this point. The active stage of tuberculosis is the second stage. When an infection becomes out of control, the body's immune system fails to fight it. At this stage, TB disease signs and symptoms are visible **[2, 3]**.

The World Health Organization declared tuberculosis to be a worldwide public health emergency. More than two billion people on the planet are estimated to have TB infection. Symptoms of tuberculosis include fever, nocturnal sweats, weight loss, and a sick or weak sensation. Chest pain, coughing up blood, and coughing up mucus are signs of lung disease[4]. When a TB infected sick person coughs, sneezes, or sings, tuberculosis can be transmitted. This will release microscopic germ-filled droplets into the atmosphere. It is possible for another individual to breathe in this droplet-filled environment, which will cause bacteria to enter their lungs.

A total of five categories into which drugs used to treat tuberculosis can be divided. First-line oral medications belong to Group I. They consist of Ethambutol, Pyrazinamide, Rifampin, and Isoniazid. Injectable medicines belong to Group II. Amikacin, Capreomycin, Kanamycin, and Streptomycin are a few of these. Drugs classified as Group III are fluoroquinolones, such as Ciprofloxacin, Ofloxacin, Levofloxacin, and Moxifloxacin. Oral medications classified as group IV. Ethionamide, Prothionamide, Teridone, Cycloserine, Para-aminosalicylic acid, Rifabutin, and Rifapentine are a few of them. Drugs in Group V have unknown effectiveness. The medications in question are Bedaquiline, Clarithromycin, Clofazimine, Linezolid, Coamoxiclav, and Imipenem **(Figure 1) [5, 6]**.

Classification of Anti-tubercular Drugs

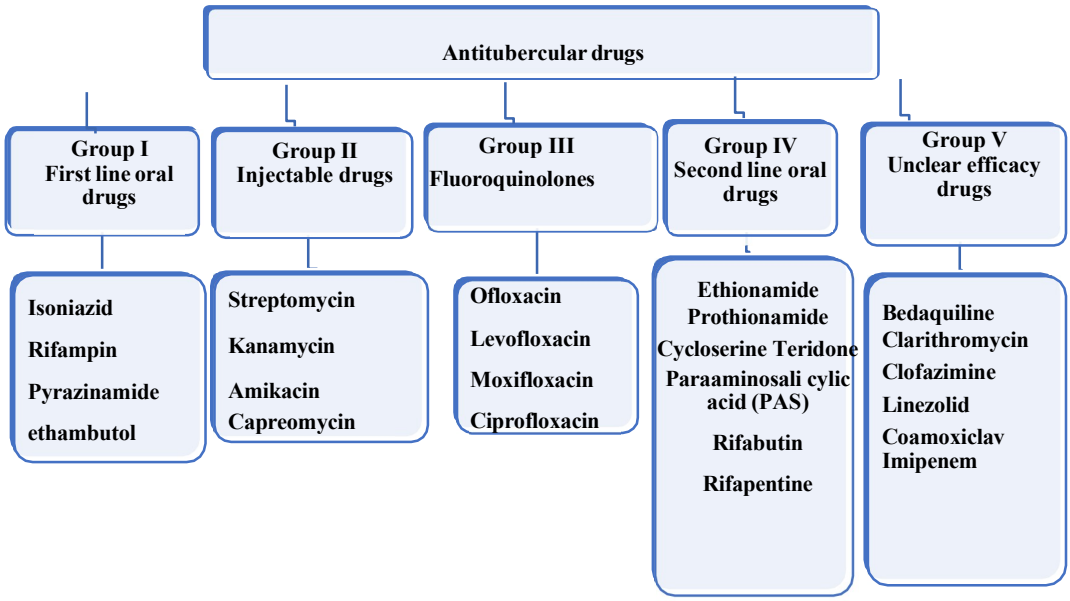

Figure 1: Classification of antitubercular drugs

Directly Observed Therapy, Short Course (DOTS Therapy)

For individuals with HIV infection and drug-resistant tuberculosis, DOTS is a very important treatment. A qualified healthcare professional administers the recommended TB medications and observes the patient as they swallow each dosage in DOTS therapy. Ethambutol, Pyrazinamide, Rifampicin, and Isoniazid are among the medications used in the DOTS therapy **[7]**.

Isoniazid works as pro-drug and as a drug target product by inhibiting the synthesis of mycolic acid. Rifampicin works as a drug target by inhibiting the synthesis of bacterial RNA polymerase, thus the synthesis of nucleic acid will be inhibited. Ethambutol works as drug target by inhibiting bacterium arabinogalactan, hence cell wall synthesis gets inhibited. Pyrazinamide works as a drug target compound as well as pro-drug conversion by depleting and inhibiting the membrane energy **[8]**.

The inhibition of the cell wall, cell wall acids, peptidoglycan (WecA) synthesis, DNA gyrase and topoisomerases, DNA replication, protein synthesis, ATP synthase, Lipid synthesis, DprE1, InhA, QcrB, LeuRS, MmpL3 protein, and L,D-transpeptidase is the mode of action of both established and newly developed drugs against mycobacteria.

Among the novel anti-MTB agents are: i) derivatives of quinolone such as Fluoroquinolones (e.g., DC-159a, Gatifloxacin, and Ofloxacin) ii) Derivatives of dialrylquinoline (e.g., Bedaquiline, TBAJ-587, TBAJ-876) iii) Derivatives of

nitroimidazoles, such as TBA-354, Delamanid, and Pretomanid iv) Derivatives of Oxazolidinone (e.g., Sutezolid and Linezolid); v) Derivatives of Ethylenediamine (e.g., SQ-109). vi) Inhibitors of DprE1 (e.g., TBA-7371, BTZ-043, Macozinone (PBTZ-169) vii) Caprazamycins, such as Caprezene-4-butylanilide (CPZEN-45), viii) Derivatives of pyrrole (such as BM212 and LL-3858) ix) Derivatives of pyridine (e.g., NITD-916) x) Oxoborates (e.g., GSK-3036656 (GSK-070) **[9-11]**.

Attractive Molecular Targets for Drug Therapy against Tuberculosis

The presented molecular targets have not been targeted by any clinically used first-line anti-TB drugs, making them suitable for targeting MDR or XDR Mycobacterium tuberculosis strains. Here, **Table 1** shows targeted antitubercular drugs with their respective targeted sites **[12, 13]**.

Table 1: Numerous Targeted Drugs with their Targeted Sites

Sr. No.	Targeted sites	Targeted Drugs
1	GyrA/B	Moxifloxacin, gatifloxacin
2	QcrB	Arylvinylpiperazine amides
3	DnaN	griselymicin
	PptT	Amidino-urea compound (8918)
5	FadD32	Benzimidazole, thiopyrimidine, pyrrozole, quinolone and iso-quinolone
6	Pks13	Benzofurans and coumestan derivatives
7	MmpL3	Bortezomib derivatives
8	DprE1	Benzothiazinones, Macozinone
9	InhA	Oxoborates (NITD-916)
10	EfpA	Efflux pump inhibitors

AIM AND OBJECTIVE

In the field of discovery of novel InhA inhibitors the basic challenge is to design selective and potent inhibitors. The study's objective was to investigate different computational methods for identifying effective InhA inhibitors. NITD-916 (4-hydroxy-2- pyridone drrivative) was first identified by Novartis Institute for Tropical Diseases[14]. One of the main components of TB chemotherapy is isoniazid, which mainly targets InhA[15]. Numerous teams have discovered structurally-diverse direct InhA inhibitors in light of the common occurrence of KatG-mediated isoniazid resistance in patients **[16-22]**. Nevertheless, a major obstacle still lies in converting the strong enzyme inhibitors into substances with anti-Mtb activity and the physicochemical characteristics necessary to achieve ideal bioavailability and in vivo effectiveness. Our structural data provide a path for further optimization of 4-hydroxy-2- pyridones through rational design.

METHODOLOGY

Using Open Eye Scientific Software, structure-based drug design software was used to identify highly effective therapeutic compounds. Chemspider, Molport, Pubchem, Chemexpress, and Zinc databases were searched for *in-silico* similarities. The OMEGA program was used to create

conformers. FRED software was used for docking. The highest scoring compounds were ultimately chosen **(Figure 2)**.

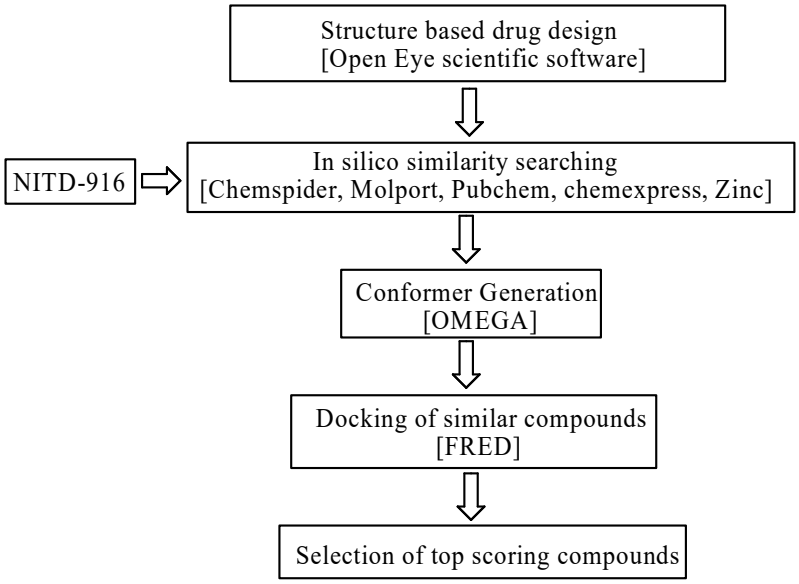

Figure 2: Flowchart of the selection of top scoring compounds

IN-SILICO DESIGN OF NOVEL INHA inhibitors

Preparation of the Receptor Structure

Protein Database provided the target protein InhA's three-dimensional structure (PDB ID: 2X22). To prepare the receptor file, Make Receptor 3.0.0 was utilized. To prepare the receptors, chain A was chosen. The receptor grid generation panel determined a receptor's structure. The dimensions of the docking box measured 20.00 Å 17.67 Å 17.33 Å, and its volume, centred on the receptor, was 6124 Å **[3]**.

Molecular docking of NITD-916

Using FRED 2.2.5, a docking analysis for NITD-916 was performed. The program creates a set of low-energy conformers independently using the multi-conformer docking algorithm, and then rigidly docks to each conformer. **Figure 3** illustrates the important H-bond interactions with Thr-196 within the InhA active site.

Molecular Docking of Screened Database

The important amino acid interactions were revealed by the docking of NITD-916 inside the InhA active region. The InhA crystal structure, PDB ID 2X22, was since it has been shown to be the most thoroughly researched crystal structure for structure-based drug design. **Figure 4 and 5** shows the proposed binding mode of compounds I, II, and III along with other important interactions. Every structure has established one or more hydrogen bonds with the essential InhA amino acid residues. **Table 2** shows Chemgauss score of NITD-916 as well as compounds I, II, and III.

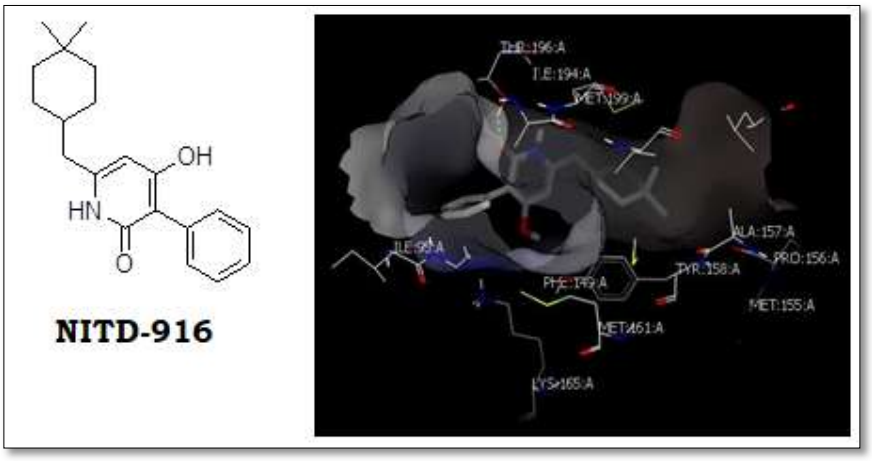

Figure 3: Docked conformation and H-bond interaction of NITD-916 in the active site of InhA (PDB ID: 2X22)

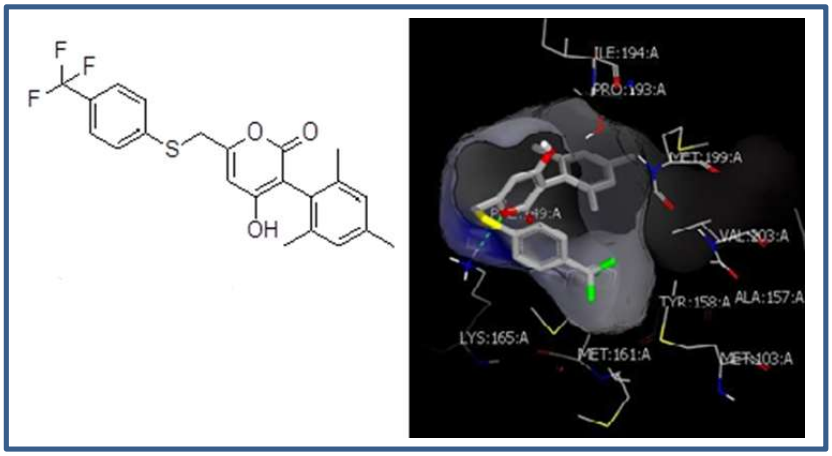

Figure 4: Docked conformation top Scoring compound I from Pubchem database and H-bond interaction within active site of InhA

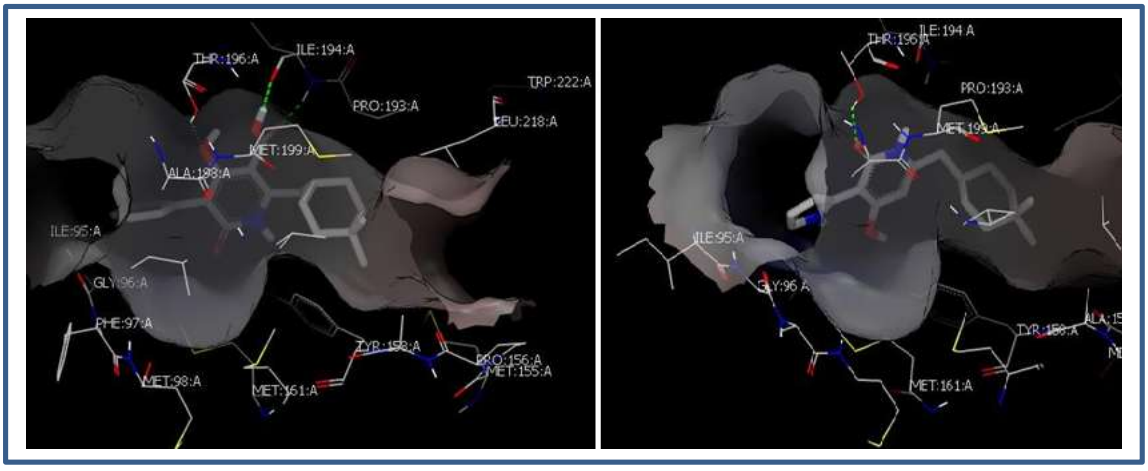

Figure 5: Docked conformation Top Scoring compounds II and III and H-bond interaction of with active site of InhA

Table 2: In Silico docking studies of compound

Sr. no	Compounds	Chemguass Score (kcal/mol)	H-bond Interaction
1	NITD-916	-10.2	THR-196A
2	I	-11.1	LYS-165A
3	II	-11.7	ILE-194A
4	III	-11.5	THR-196A

RESULTS AND DISCUSSION

Docking of target compounds similar to NITD-916 formed one or more hydrogen bonds with amino acid residues in the putative binding site of InhA enzyme (As shown in **Table 2**). This study was performed to predict potential binding sites and interaction of amino acid to ligands with hydrogen and oxygen bond.

CONCLUSION

FRED v2.2.5 of OpenEye Scientific software was used to identify putative binding sites. ILE194 and LYS165 are the key amino acids which interact via hydrogen bond with compound. The identified compounds I, II, and III showed the Chemgauss scores between -11.1 to -11.5 kcal/mol which are comparable with NITD-916 Chemgauss score (-10.2 kcal/mol). From the entire research study it can be concluded that the identified compounds can surely become potential drug candidates as anti-TB agents.

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