DESIGN, SYNTHESIS AND EVALUATION OF SOME PYRAZOLE DERIVATIES AS ANTITUBERCULAR AGENTS

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Abstract

Tuberculosis is an infectious disease caused by bacillus *Mycobacterium tuberculosis* (MTB). New drugs with different mechanisms of action against tuberculosis must be discovered and developed in response to the rising prevalence of multidrug-resistant MTB. Several pyrazole derivatives have been synthesized and tested for anti-tubercular activity in past years. Enoyl acyl carrier protein reductase (InhA) and Decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE1), is a clinically proven target for tuberculosis treatment. In our research work we have designed, synthesized and evaluated pyrzole moiety containing compounds. All the synthesized compounds were characterized by various spectroscopic techniques (such as IR, NMR and mass spectroscopy). Synthesized compounds InhA Inhibitors **(5a-o)** and DprE1 inhibitors**(6a-6o)** and were subjected to *In- vitro* anti-mycobacterial activity against the *M. tuberculosis* (H37Rv) strain via the microplate alamar blue assay method and antioxidant activity using the DPPH assay. The cytotoxicity study was performed for most active antitubercular compounds. Furthermore, *in-silico* ADME predictions indicated advantageous oral bioavailability and drug-like characteristics across the series. To gain mechanistic insight, molecular docking studies were conducted against the mycobacterial enoyl acyl carrier protein reductase(InhA) and Decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE1)enzyme, assisted by molecular dynamics simulations to evaluate the key molecular interaction, conformational changes and stability of docked complexes.

Keywords: Mycobacterium tuberculosis, Enoyl acyl carrier protein reductase (InhA), Decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE1), Docking, MD simulation, Cytotoxicity, Antitubercular activity

Brief description on the state of the art of the research topic

- *Mycobacterium tuberculosis* is an infectious agent that causes tuberculosis (TB). One of the leading causes of morbidity and mortality worldwide is *M. tuberculosis* (Mtb), transmitted through airborne droplets. Tuberculosis(TB) is largely preventable and curable disease. As per the "Global Tuberculosis Report 2024," there were 10.8 million new tuberculosis cases worldwide in 2023, with an incidence of 134 cases per 100,000 people. In these new cases, 6,62,000(6.1%) were HIV-co-infected and 400,000 (3.7%) were patients with multidrug-resistant tuberculosis(MDR) or rifampicin-resistant tuberculosis[1]. A growing number of MTB strains are becoming resistant to one or more of the widely used anti-TB drugs, which complicates treatment and contributing to the rise in drug-resistant and latent TB infections. The emergence of MDR and XDR MTB infections accelerated efforts to find new anti-TB drugs with their mechanism of action[2][3]. Mycolic acids are a significant and essential element of the mycobacterial cell membrane. The enoyl-ACP reductase encoded by the mycobacterium gene InhA is an essential enzyme in the biosynthesis of mycolic acid, the single most abundant component of the Mycobacterium tuberculosis cell wall. InhA enzyme is required by this organism for cell wall synthesis, and inhibition of this enzyme results in death of the bacterial cell[4]. DprE1 plays a crucial part in the biosynthesis of lipoarabinomannan and arabinogalactan for the cell wall of Mtb and represents a promising target for anti-TB drug development[5]. Numerous biological activities have been discovered for the pyrazole skeleton, which is one of the most significant scaffolds in pharmacologically active substances[6]. Pyrazole derivatives possesses a wide spectrum of activities including anticancer, anti-inflammatory, antimicrobial, antiviral and antitubercular etc.Wide range of biological activity, pyrazole scaffold offers tremendous potential for medicinal chemistry and is of interest to researchers. It has been discovered that pyrazole heterocycle have interesting antitubercular activity.

  Based on literature, in our research work we have designed, synthesized and evaluated pyrazole heterocyclic ring containing compounds which are the inhibitors of InhA (enoyl acyl carrier protein reductase and DprE1 (Decaprenylphosphoryl-β-D-ribose 2'-epimerase).

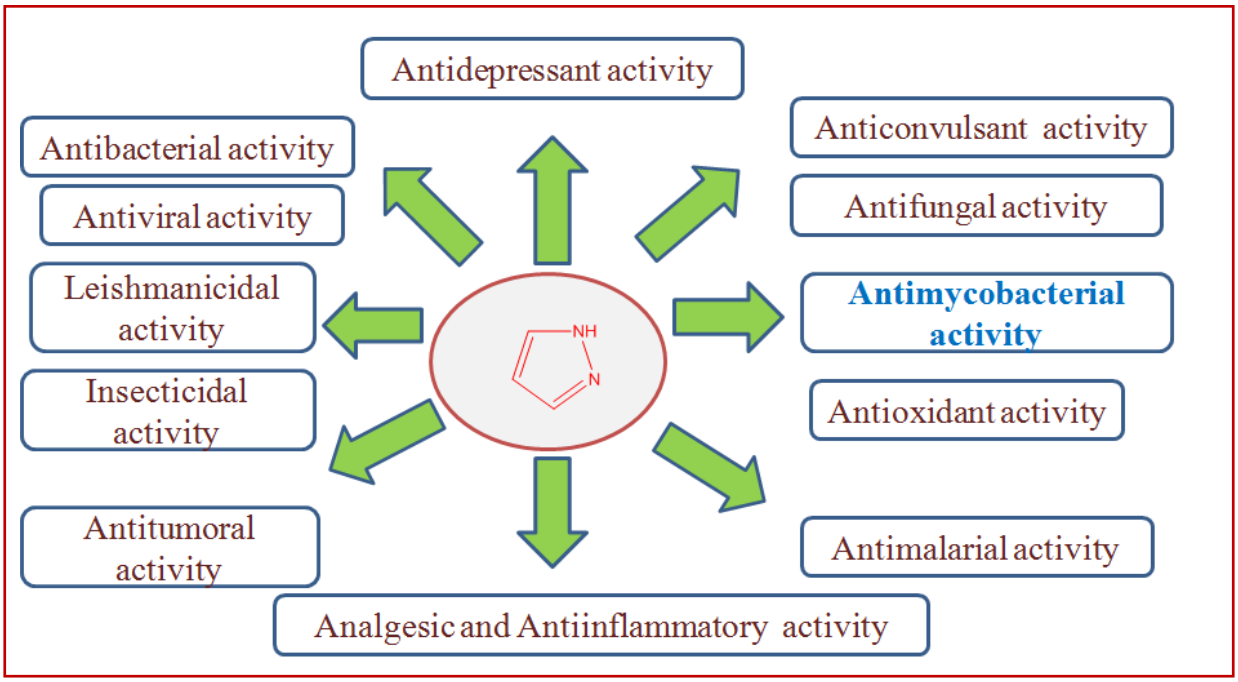
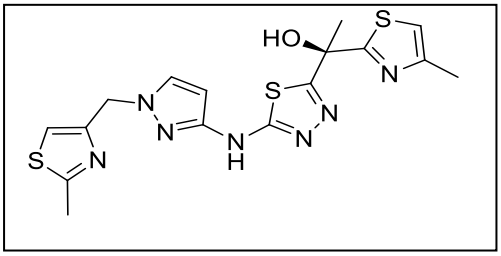


Figure1: Various Biological activities of Pyrazole[7]

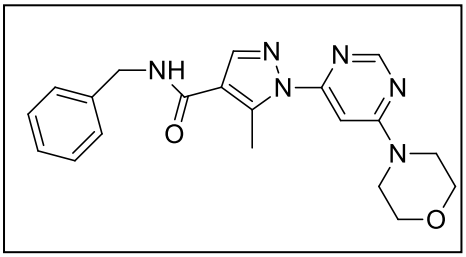
Literature Review

Pyrazole as InhA (Enoyl acyl carrier protein reductase) inhibitors

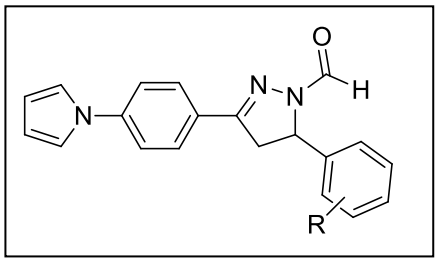
- **María Martínez-Hoyos** ***et al*** reported GSK693 is a promising InhA direct inhibitor having MIC- 0.2 µM [8].



- **Mayur K. Vekariya** ***et al*** performed docking study, synthesis and evaluation of morpholinopyrimidine based pyrazole derivatives. The molecular docking studies also revealed the good interaction of the active tubercular compounds with the active site of the target enzymes InhA having MIC 6.25 (µg/mL)[9] .

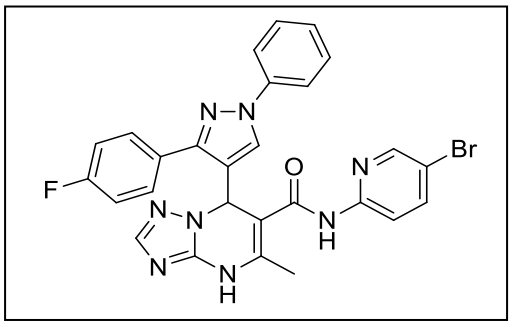


- **Sheshagiri R. Dixit *et al*** synthesized novel compounds 3-(4-(1*H*-pyrrol-1-yl)phenyl)-5-substituted phenyl-4,5-dihydro-1*H*-pyrazole-1-carbaldehydes as potent InhA inhibitors[4].



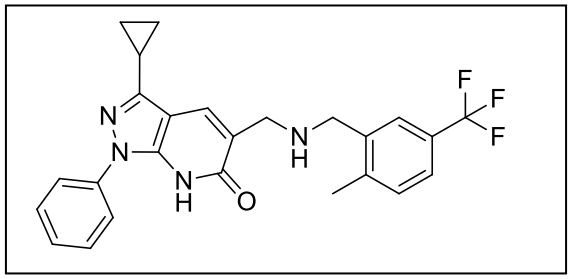
R=3-OCH_3 MIC= 6.25 µg/mL, 2,4-di –OCH_3 MIC =3.125µg/mL

- **Jaimin D. Bhatt *et al*** synthesized a series of novel pyrazole linked triazolo-pyrimidine hybrids and evaluated for their anti-tubercular activity against M.tb H37Rv strain. Some of the screened entities rendered promising anti-TB activity having MIC 0.39µg/mL[10].



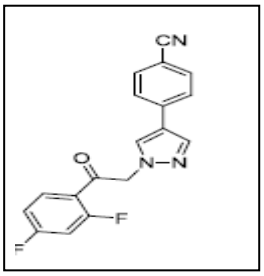
Pyrazole as Decaprenylphosphoryl-β-D-ribose 2′-epimerase (DprE1) Inhibitors

- **Panda *et al*** reported pyrazolopyridone class of inhibitors active against DprE1 enzyme and MIC was MIC= 0.1µM. [11].

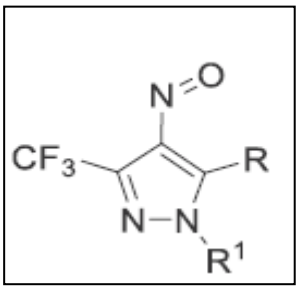


- **Maciej K. Rogacki *et al*** reported the discovery and profiling of a novel antimycobacterial inhibitors of the decaprenylphospho-β-D-ribofuranose 2-oxidase (DprE1). The series is characterized by high enzymatic and whole-cell activity, low

cytotoxicity, and physicochemical profile DprE1 pIC50=<4.0, Mtb MIC= >125 μM [12].



- **Yanina V. Burgart *et al*** synthesized pyrazole derivatives which is active against DprE1. MIC is 0.36, 3.1,6.2 μg/mL[13].



Definition of the Problem:

Today the difficulties faced in managing TB are due to the long duration treatment regimens, emergence of drug resistant Mtb strains, coinfection with HIV/AIDS. The *M. tuberculosis* cell wall has an intricate and unique structure composed of a thick peptidoglycan layer and an outer membrane made up mostly of various lipopolysaccharides and fatty acids with imbedded glycolipids and wax esters. Most of the antitubercular drugs are unable to cross cell wall due to thick layer of mycolic acid. The combination therapy for tuberculosis developed over 40 years ago includes four drugs: Isoniazid, Pyrazinamide, Rifampicin, and Ethambutol. These therapies possess drawbacks including extended treatment durations, drug interactions, unwanted adverse reactions and poor patient compliance. Various side effects are exist to the administration of first line antitubercular drugs, including hepatitis, kidney failure, skin reactions, blood disorders, and gastrointestinal discomfort[14]. These observations gave motivation to search for potential pharmacologically active leads carrying pyrazole substituents.

Objectives

- Emergence of multi-drug resistant tuberculosis (MDR) and extensive drug resistant tuberculosis (XDR) have made controlling of this infectious disease more complicated and challenging.
- The azole groups containing heterocyclic compounds possess significant pharmacokinetic property, lipophilicity that influence the ability of drug to reach the target by transmembrane diffusion and show promising activity against resistant TB by inhibiting the biosynthesis of Fatty acid.
- Pyrazole derivatives have been reported to inhibit the enoyl ACP reductase and Decaprenylphosphoryl-β-D-ribose 2′-epimerase (DprE1) which is the key enzyme responsible for the cell wall of mycobacteria.
- Enoyl ACP reductase (inhA) responsible for the mycolic acid biosynthesis and Decaprenylphosphoryl-β-D-ribose 2′-epimerase (DprE1) responsible for the preparation of arabinogalactan both are essential components in cell wall of mycobacteria.
- The *M. tuberculosis* cell wall has an intricate and unique structure composed of a thick peptidoglycan layer and an outer membrane made up mostly of various lipopolysaccharides and fatty acids with imbedded glycolipids and wax esters. Most of the antitubercular drugs are unable to cross cell wall due to thick layer of mycolic acid. It is plan to synthesize pyrazole derivatives using molecular docking study.

Scope of Work

Pyrazole moiety has been successfully coupled with another promising heterocycles. The substances examined in this research work are enoy lacyl carrier protein reductase (InhA) and Decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE1) inhibitors and their antitubercular and antioxidant activity.

This research supports to the idea that a direct inhibitor of InhA and DprE1 are the practical way to produce novel compounds for use in anti-tubercular medications.

Original contribution by the thesis

The entire work in this synopsis, as well as thesis is original. Extensive literature review was

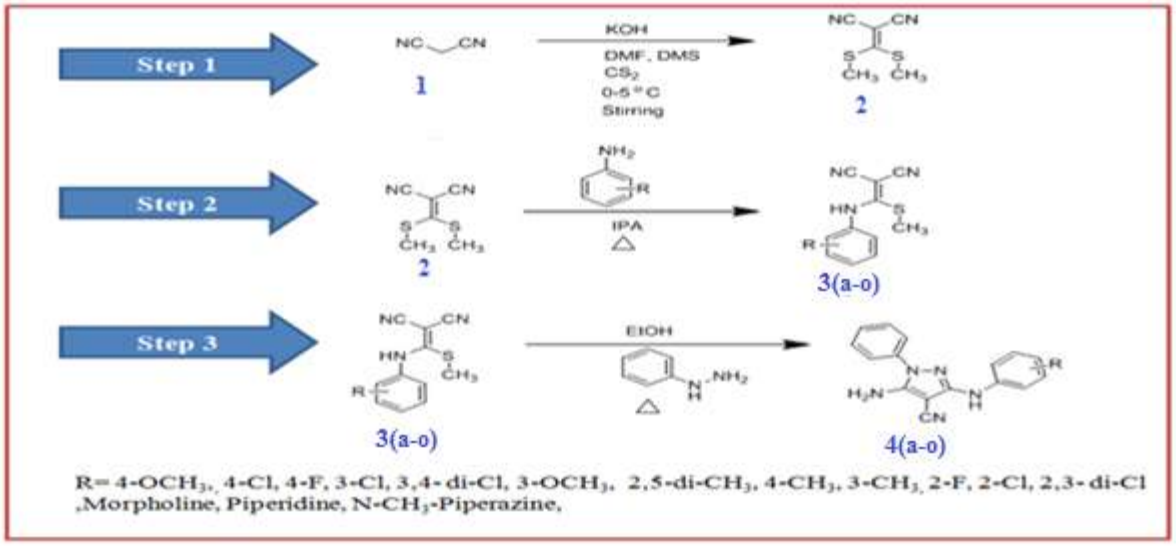
carried out on the inhibitors of InhA and DprE1.

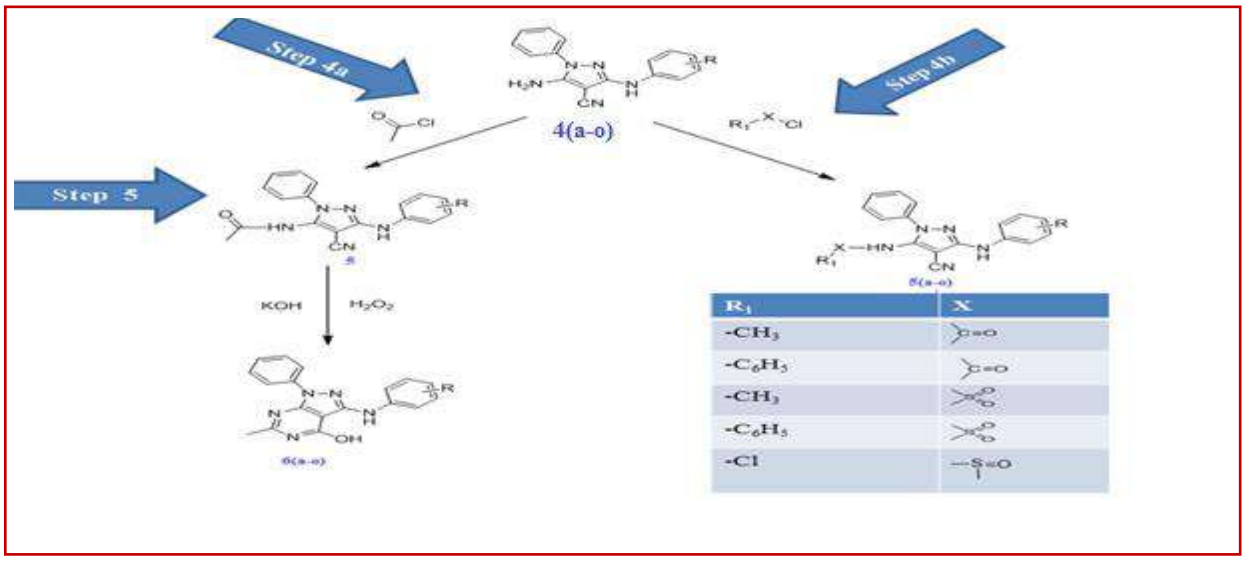
Synthesized pyrazole containing InhA and DprE1 inhibitors have shown potential antitubercular and antioxidant activity. Cytotoxicity of most potent antitubercular compound was checked and it was safe. All the synthesized compounds shown good ADMET properties.

It is our aim that this research work would encourage researchers to focus on InhA and DprE1 inhibitors and develop novel potential lead antitubercular compounds by drug discovery.

Methodology of Research and Results

1. Proposed reaction scheme





2. DockingStructure Preparation for docking:

enoyl ACP reductase (InhA)

- Crystal structure of 4U0J with bound inhibitor having resolution 1.62 Å were retrieved from Protein Data Bank. Docking performed in Autodock vina.

Decaprenylphosphoryl-β-D-ribose 2′-epimerase (DprE1)

- Crystal structure of 4NCR with bound inhibitor having resolution 1.88 Å were retrieved from Protein Data Bank. Docking score of **5(a-o)**and **6(a-o)** mentioned in table

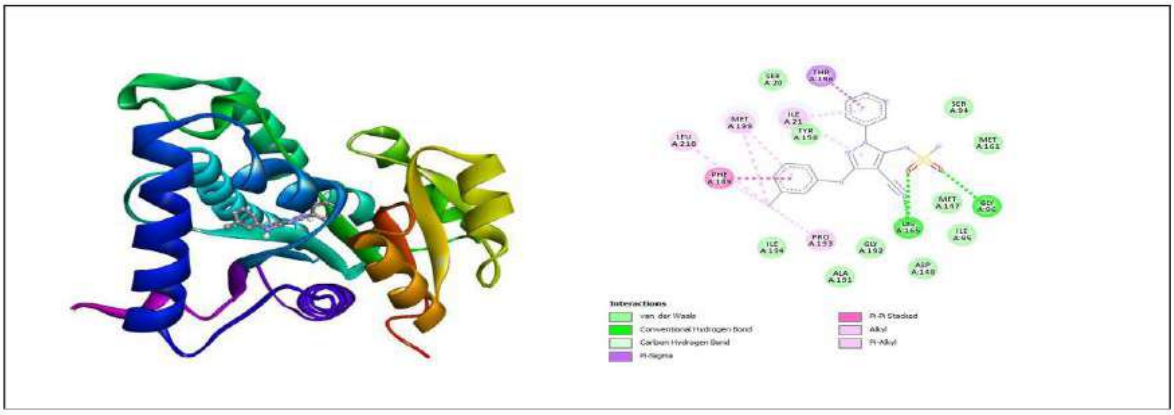


Figure 2: 3D and 2D structure of interaction of compound **5m** with protein 4U0J

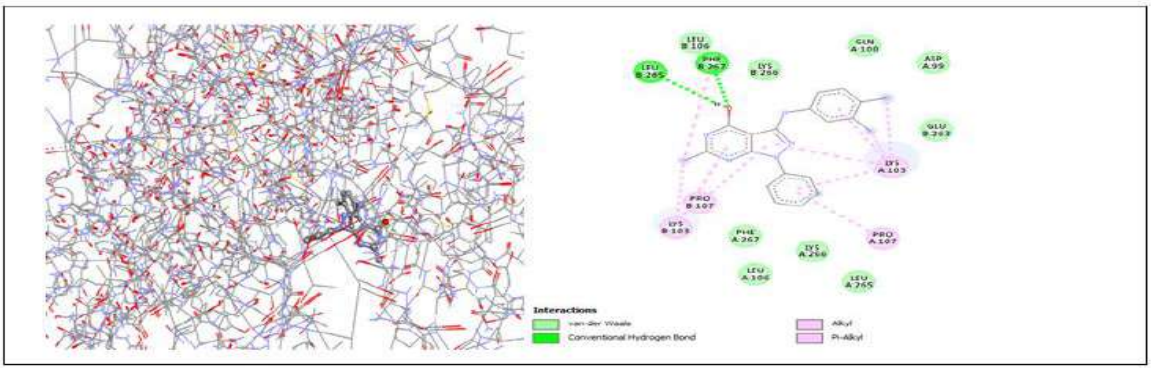


Figure 3: 3D and 2D structure of interaction of compound **6g** with protein 4NCR

3. Synthetic procedure

Step-1: Synthesis of 2-(bis(methylthio)methylene)malononitrile (S,S- acetal) (2)

Potassium hydroxide(0.2 mol) dissolved in 10 mL of water and stirred for 15 min. To this 15mL dimethylformamide was added with cooling and stirring. After 20 min. malononitrile(0.1mol) was added in the reaction mixture. Reaction mixture was stirred for 30 min. In cool condition.To this reaction mixture carbon disulfide(0.1mol) was added dropwise and cooled for 30 min. Dimethyl sulphate(0.2mol) was added dropwise with cooling and stirring. Reaction progress was monitored by TLC. Reaction mixture was diluted with in an ice-water mixture, solid obtained was filtered, washed with water and dried to afford product 2-(bis(methylthio)methylene)malononitrile as colorless crystalline compound.

Step-2: Synthesis of 2-((methylthio)(aminosubstituted)methylene)malononitrile (3a-o)

0.017mol of 2-(bis(methylthio)methylene)malononitrile **(2)** was dissolved in 20 mL of isopropyl alcohol. To this aryl amine (0.017mol) was added and refluxed for 6-7 hrs. Progress of reaction mixture was monitored by TLC. After completion of reaction, it was cooled and poured in ice-water mixture. Solid obtained was filtered, washed with water and dried. Recrystallized with methanol**.**

Step-3: Synthesis of 5-amino-3-(aminesubstituted)-1-phenyl-1*H*-pyrazole-4-carbonitrile (4a-o)

2-((methylthio)(aminosubstituted)methylene)malononitrile **(3a-o)** (0.01mol) and 0.01 mol phenyl hydrazine mixed and heat to 70-80 ^{0}C for 1 hr. After that addition of ethanol and reflux the mixture for 2-3 hrs. Progress of reaction was monitored by TLC. After completion, reaction mixture was cooled; Crude product was filtered and purified by recrystallization using ethanol.

Synthesis of acid chloride substituted pyrazole derivatives 5 & 5(a-o)

Step-4a

A mixture of compounds**(4a-o)** (0.01mol), pyridine and acetyl chloride(0.02mol) was refluxed in ethanol for 4-5 hrs. at 50-60 ^{0}C. Reaction mixture was monitored by TLC. Reaction mixture was cooled to room temperature. The precipitated solid was filtered and re-crystallized from ethanol.

Step-4b:

Procedure-1: A mixture of compound **4(a-o)** (0.01mol), pyridine and acetyl

chloride(0.02mol) Was refluxed in ethanol for 4-5 hrs. at 50-60 ^{0}C. Reaction mixture was monitored by TLC. Reaction mixture was cooled to room temperature. The precipitated solid was filtered and recrystallized from ethanol.

Procedure-2: A mixture of compound **4(a-o)** (0.01mol) ethanol (15mL), substituted acid chloride(0.04mol) and NaoH was stirred and reflux for 6-8 hrs. Reaction mixture was monitored by TLC. After completion of reaction, it was cooled and poured in ice-water mixture. Solid obtained was filtered, washed with water and dried. The precipitated solid was filtered and recrystallized from ethanol.

Step-5: synthesis of amino substituted 6-methyl-1-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-ol **(6-o)**

Compound **5(a-o)** (0.5 g.) was dissolved in a solution of potassium hydroxide, hydrogen peroxide (4 ml) and ethanol. The mixture was reflux at 70-75^0 C for 2-3 hrs. It was then acidified with glacial acetic acid to give a precipitate. Washed the product with water. The product was recrystallized from ethanol.

4. Antitubercular Activity

Microplate alamar blue assay(MABA) method was used to evaluate the antitubercular activity of the synthesized compounds **(5a-o)** and **(6a-o)** against *M.tuberculosis.* This method is employs a thermally stable reagent, non- toxic and has good correlation with both proportional and BACTEC radiometric techniques. Standard reference drugs used for comparison included Isoniazid, Ethambutol, Pyrazinamide, Rifampicin, Streptomycin, and Moxifloxacin. Briefly, 200 µl of deionized sterile water is added to the outside of all 96 well sterile plates in order to minimize evaporation from the test wells during incubation. Each test well received 100 µl of Middlebrook 7H9 broth, and serial dilutions of the compounds were prepared directly in the plate. The final concentrations tested ranged from 100 to 0.2 µg/ml. The plates were covered, sealed with parafilm and incubated for five days at 37˚C. After incubation, 25 µl of the freshly prepared 1:1 mixture of alamar blue reagent and 10%of the Tween 80 were added to each well and then continued to incubate for further 24 hours. Wells retaining a blue color were interpreted as no bacterial growth, whereas a pink color indicated growth. The minimum inhibitory concentration (MIC) was defined as the lowest compound concentration that prevented the color change from blue to pink[15].

Among the screened derivatives, compounds **5i** and **5m**, bearing an electron- withdrawing substituted amino group on one side and a sulfonyl group on the other side of the pyrazole ring, exhibited excellent activity against *M. tuberculosis* H37Rv, with MIC values of 6.25 µg/mL and 3.12 µg/mL respectively. Notably, compound **5m** demonstrated MIC of 3.12 µg/mL, which is similar to the standard drug Pyrazinamide. However, compounds with electron-donating substitutions (5g, 5h, and 5j) showed decrease potency, exhibiting MIC values of 12.5 µg/mL.

Anti-tubercular activity results of the synthesized compounds **5(a-o)** and **6(a-o)** are displayed in Table 1 and Table 2. Among the synthesized compounds, **6e**, **6j**, and **6k** exhibited good anti-tubercular activity against the *M. tuberculosis* H37Rv strain with MIC 12.5µg/mL. Compound **6g** demonstrated excellent activity with MIC of 6.25 µg/mL.

5. MD simulation

MD simulations provide a dynamic perspective of protein-ligand interactions, offering insights into binding mechanism, conformational changes, and thermodynamic properties. These simulations provide in- depth understanding of binding affinities and optimizing drug design by offering detailed insights into the atomistic-level behaviour of the complex. In this study the lead **5m** and **6g** was simulated with the *M.tuberculosis* enoyl reductase protein(InhA) and Decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE1) for 100ns to understand the binding stability. The strucutural changes occurred during the course of simulations were analysed via RMSD (Root Mean Square Deviation), RMSF (Root Mean Square Fluctuation), SASA (Solvent Accessible Surface Area), radius of gyration (Rg) and number of hydrogen bonds.

Molecular dynamics (MD) simulation of compound 5m was performed for 100 ns within a physiological pH environment to estimate its binding stability with the target protein. The system files necessary for performing simulations, such as .gro, .psf, and related files, were generated using the CHARMM-GUI server[16]. The TIP3P water model was used to solvate the water box, then counter ions were added to neutralize it. The system was first minimized for 50,000 steps using the steepest descent algorithm, followed by equilibration steps: 100 ps of NVT and NPT ensembles, each at constant temperature (using the Nos´e-Hoover thermostat) and pressure (using the Parrinello-Rahman barostat). The equilibrated system was then subjected to a 100 ns production run. All the MD simulations were performed using GROMACS [17] on HPC server provided by Param-Shivaye at IIT-BHU Varanasi.

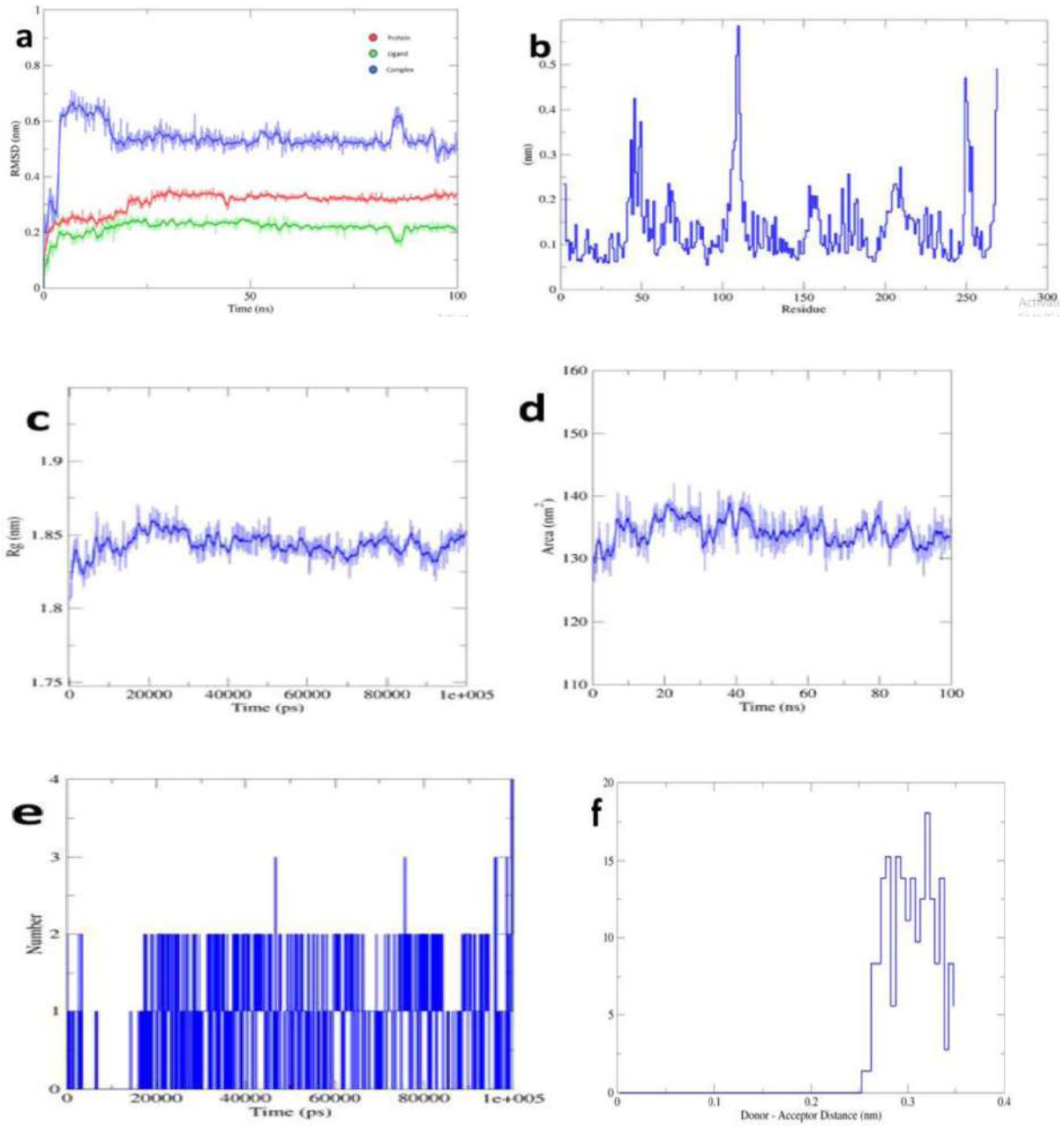


Figure 4: MD analysis, a) RMSD, b)RMSF, c).Radius of gyration, d) SASA, e) Hydrogen bonding number and f) hydrogen bonding distance of compound **5m**

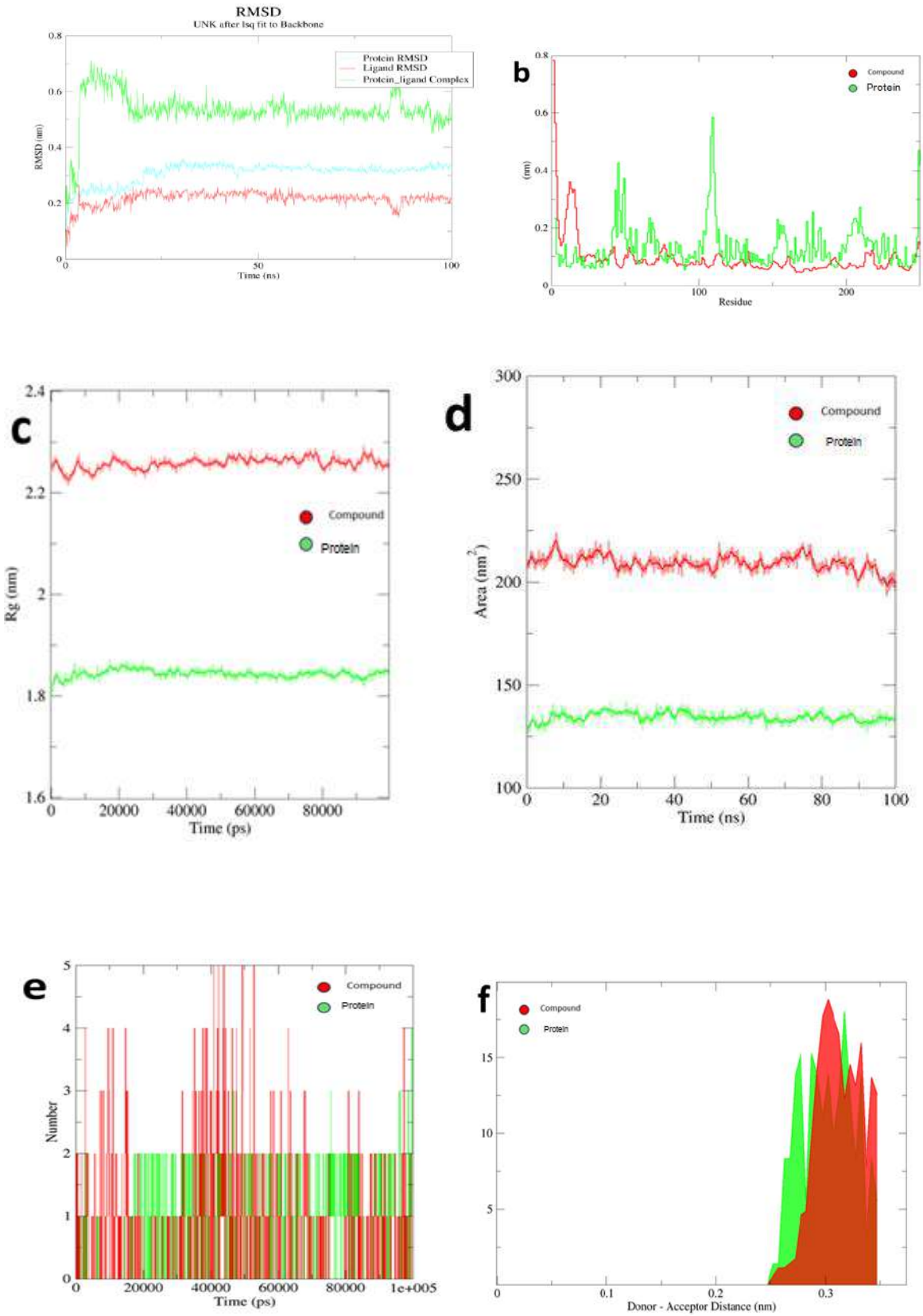


Figure 5: Illustration of post MD analysis, **a**. RMSD, **b**. RMSF, **c.** Radius of gyration, **d**. SASA, **e**. Hydrogen bonding number and **f**. hydrogen bonding distance of compound **6g**

Table -1 Docking score, antitubercular activity and antioxidant activity of the synthesized compounds.

Compounds	**R**	**R1**	**X**	**Docking Score**	**Antitubercular activity (µg/mL)**	**Antioxidant activity (%RSA)**
5a	4-Cl	$-CH_3$	-C=O	-8.5	25	1.61
5b	4-OCH_3	$-CH_3$	-C=O	-8.4	25	25.90
5c	Morpholine	$-CH_3$	-C=O	-8.0	25	1.37
5d	4-CH_3	$-CH_3$	-C=O	-8.6	25	15.73
5e	4-F	$-C_6H_5$	-C=O	-10.1	25	0.9
5f	Morpholine	$-C_6H_5$	-C=O	-9.5	50	3.47
5g	N-methyl Piperazine	$-C_6H_5$	-C=O	-9.1	12.5	1.61
5h	4-CH_3	$-C_6H_5$	**-C=O**	-10.2	12.5	1.20
5i	3,4-di-Cl	$-C_6H_5$	O=S=O	-9.9	6.25	9.90
5j	2,5-di-CH_3	$-C_6H_5$	O=S=O	-9.5	12.5	1.60
5k	2,3-di-Cl	$-C_6H_5$	O=S=O	-9.7	25	1.50
5l	3-CH3	$-CH_3$	O=S=O	-9.2	25	1.60
5m	3-Cl	$-CH_3$	O=S=O	-9.0	3.12	43.01
5n	2-Cl	$-CH_3$	O=S=O	-8.7	25	5.89
5o	Piperidine	-Cl	-S=O	-8.0	100	1.61
Co-crystalline ligand 4U0J				-5.8		
Isoniazid				-8.6	1.6	
Ethambutol					1.6	
Pyrazinamide					3.12	
Rifampicin					0.8	
Streptomycin					0.8	
Moxifloxacin					0.8	
Ascorbic acid						90.15
Curcumin						80.95

Table -2 Docking score, antitubercular activity and antioxidant activity of the synthesized compounds.

Compounds	**R**	**Docking Score**	**Antitubercular activity (µg/mL)**	**Antioxidant activity (%RSA)**
6a	2-Cl	-8.2	25	12.69
6b	4-Cl	-8.2	25	19.37
6c	4-OCH_3	-8.1	25	4.01
6d	4-CH_3	-8.3	25	20.48
6e	4-F	-8.2	12.5	15.59
6f	Morpholine	-7.4	25	7.57
6g	3,4-di-Cl	-8.5	6.25	6.90
6h	2,5-di-CH_3	-8.6	50	7.34
6i	2,3-di-Cl	-8.4	100	12.91
6j	3-CH_3	-8.3	12.5	10.69
6k	3-Cl	-8.2	12.5	7.10
6l	3-OCH_3	-8.0	25	4.67
6m	Piperidine	-7.7	100	2.00
6n	2-F	-8.3	100	2.00
6o	N-methyl Piperazine	-7.7	100	2.00
Co-crystalline ligand 4NCR		-8.1		
Isoniazid			1.6	
Ethambutol			1.6	
Pyrazinamide			3.12	
Rifampicin			0.8	
Streptomycin			0.8	
Moxifloxacin			0.8	
Ascorbic acid				90.15
Curcumin				80.95

6. Cytotoxicity study

The most potent molecules **5i**, **5m**, **6g** and **6j** were selected based on their anti-tubercular activity and further evaluated for its cytotoxicity using the MTT assay against HepG2 cell line. The results, summarised in Table 3, showed that compounds **5i, 5m**, **6g** and **6g** exhibited cytotoxicity values of 125 ± 0.16 μM , 273.8 ± 0.05 μM, 71.63 ± 0.10μM and 9.281 ± 0.20μM respectively. Both compounds were found to be relatively non-toxic toward the HepG2 cell line.

Table-3 Ctytotoxicity of the most potent antitubercular compounds

Compounds	**Antitubercular activity MIC (μg/mL)**	**Cytotoxicity IC_{50} μM**	**SI Index IC_{50}/MIC**
5i	6.25	125 ± 0.16	20
5m	3.12	273.8 ± 0.05	87.75
6g	6.25	71.63 ± 0.10μM	11.46
6j	12.5	9.281 ± 0.20μM	0.742

7. Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay method was used to assessed the antioxidant potential of the synthesized series of compounds **(5a-o)** and **(6a-o).** This method is based on the reduction of the stable DPPH free radical, which possesses a deep violet coloration and exhibits a characteristic absorbance at 517 nm in the visible region of the spectrum. Upon reaction with an antioxidant compound capable of donating an electron or hydrogen atom. The DPPH radical is reduced, resulting in a measurable decrease in absorbance intensity. For the assay, each test compound was prepared at concentration of 100 μM in methanol. A methanolic solution of DPPH (0.1mM) was used as the radical source. An equal volume (1:1) of DPPH solution was mixed with the compound solutions in a 96-well microplate format, resulting in a total reaction volume of 200 μL per well. Methanol was used as the solvent control (vehicle), and ascorbic acid and curcumin were included as reference antioxidant standards, also at a concentration of 100 μM[18].

The DPPH radical scavenging activity of each compounds were calculated using the following formula:

$$\%RSA=\frac{(A(control) - A(sample))}{(A(control))} * 100$$

Where:

- A(control) is the absorbance of the control (DPPH solution without the test compound).
- A(sample) is the absorbance of the test sample (DPPH solution with the test compound).

The DPPH radical scavenging antioxidant aactivity (%RSA) of compounds **(5a-o)** and **(6a-o)** and the standards ascorbic acid and cucumin are shown in Table 1 and 2

8. **ADMET study**

The pkCSM and Swiss ADME web tools were utilized to understand the pharmacokinetic characteristics of the synthesized compounds **(5a-o)** and **(6a-o)**. Table 4, 5, 6 and 7 illustrate the pharmacokinetic properties of the synthesized compounds.

Table-4 Molecular properties descriptors and lead-likeness of compounds **(5a-o)**

Sr. no.	Molecular Formula	Mol. wt. gm/mol	HBA	HBD	mlogP	Synthetic accessibility	Bioavailability score	Lipinski violation	Drug likeness
5a	$C_{18}H_{14}ClN_5O$	351.79	3	2	2.89	2.99	0.55	0	YES
5b	$C_{19}H_{17}N_5O_2$	347.37	4	2	1.69	3.08	0.55	0	YES
5c	$C_{16}H_{17}N_5O_2$	311.34	4	1	1.07	2.91	0.55	0	YES
5d	$C_{19}H_{17}N_5O$	331.37	3	2	2.62	3.05	0.55	0	YES
5e	$C_{23}H_{16}FN_5O$	397.40	4	2	3.94	3.18	0.55	0	YES
5f	$C_{21}H_{19}N_5O_2$	373.41	4	1	2.28	3.12	0.55	0	YES
5g	$C_{22}H_{22}N_6O$	386.45	4	1	2.49	3.23	0.55	0	YES
5h	$C_{24}H_{19}N_5O$	393.44	3	2	3.78	3.30	0.55	0	YES
5i	$C_{22}H_{15}Cl_2N_5O_2S$	484.36	4	2	3.85	3.60	0.55	0	YES
5j	$C_{24}H_{21}N_5O_2S$	443.52	4	2	2.91	3.79	0.55	0	YES
5k	$C_{22}H_{15}Cl_2N_5O_2S$	484.36	4	2	3.85	3.63	0.55	0	YES
5l	$C_{18}H_{17}N_5O_2S$	367.42	4	2	1.52	3.32	0.55	0	YES
5m	$C_{17}H_{14}ClN_5O_2S$	387.84	4	2	1.79	3.27	0.55	0	YES
5n	$C_{17}H_{14}ClN_5O_2S$	387.84	4	2	1.79	3.29	0.55	0	YES
5o	$C_{15}H_{16}ClN_5OS$	349.84	3	1	1.87	3.53	0.55	0	YES

Table-5 Molecular properties descriptors and lead-likeness of compounds **(6a-o)**

Compounds	Molecular Formula	Molecular weight gm/mol	HBA	HBD	mlogP	Synthetic accessibility	Bioavailability score	Lipinski violation	Drug likeness
6a	$C_{18}H_{14}ClN_5O$	351.79	4	2	3.39	3.02	0.55	0	YES
6b	$C_{18}H_{14}ClN_5O$	351.79	4	2	3.53	2.97	0.55	0	YES

6c	$C_{19}H_{17}N_5O_2$	347.37	5	2	2.59	3.05	0.55	0	YES
6d	$C_{19}H_{17}N_5O$	331.37	4	2	3.26	3.01	0.55	0	YES
6e	$C_{18}H_{14}FN_5O$	335.34	5	2	3.28	2.94	0.55	0	YES
6f	$C_{16}H_{17}N_5O_2$	311.34	5	1	1.97	2.91	0.55	0	YES
6g	$C_{18}H_{13}Cl_2N_5O$	386.23	4	2	4.03	3.02	0.55	0	YES
6h	$C_{20}H_{19}N_5O$	345.40	4	2	3.76	3.17	0.55	0	YES
6i	$C_{18}H_{13}Cl_2N_5O$	386.23	4	2	4.29	3.06	0.55	1	YES
6j	$C_{19}H_{17}N_5O$	331.37	4	2	3.53	3.01	0.55	0	YES
6k	$C_{18}H_{14}ClN_5O$	351.79	4	2	3.80	3.00	0.55	0	YES
6l	$C_{19}H_{17}N_5O_2$	347.37	5	2	3.00	3.08	0.55	0	YES
6m	$C_{17}H_{19}N_5O$	309.37	4	1	3.01	2.88	0.55	0	YES
6n	$C_{18}H_{14}FN_5O$	335.34	5	2	3.68	3.02	0.55	0	YES
6o	$C_{17}H_{20}N_6O$	324.38	5	1	2.22	3.03	0.55	0	YES

Table-6 ADME Properties of Compounds **(5a-5o)**

Sr. no.	Absorption	Distribution		Metabolism CYP Inhibitor					Excretion	Toxicity		
	Intestinal absorption (human)	log BB	logPS	CYP 1A2	CYP 2C19	CYP 2C9	CYP 2D6	CYP 3A4	Total Clearance	AMES	LD50	Hepatotoxicity
5a	90.015	-0.272	-1.905	YES	YES	YES	NO	YES	-0.129	NO	2.447	YES
5b	87.673	-0.126	-2.165	YES	YES	YES	NO	YES	0.185	NO	2.891	YES
5c	95.612	-0.526	-2.531	NO	YES	NO	NO	NO	0.344	YES	2.602	YES
5d	91.473	-0.097	-1.945	YES	YES	YES	NO	YES	0.149	NO	2.44	YES
5e	92.275	-0.114	-1.751	YES	YES	YES	YES	YES	-0.107	YES	2.586	YES
5f	95.883	-0.498	-2.247	YES	YES	YES	NO	YES	0.265	NO	2.32	YES
5g	93.75	-0.268	-2.186	YES	NO	NO	NO	YES	0.358	NO	2.433	YES
5h	92.88	0.094	-1.605	YES	YES	YES	NO	YES	0.064	YES	2.515	YES
5i	100	-0.398	-1.762	YES	YES	YES	NO	YES	-0.018	YES	2.753	YES
5j	95.735	-0.023	-1.736	YES	YES	YES	NO	YES	0.252	YES	2.325	YES
5k	100	-0.523	-1.761	YES	YES	YES	NO	YES	0.12	NO	2.766	YES
5l	91.009	-0.312	-2.215	YES	YES	YES	NO	YES	0.246	NO	2.796	YES
5m	91.717	-0.487	-2.175	YES	YES	YES	NO	YES	0.027	NO	2.762	YES

5n	91.674	-0.489	-2.168	YES	YES	YES	NO	YES	0.237	YES	2.745	YES
5o	92.031	-0.64	-2.273	YES	YES	NO	NO	NO	0.006	YES	2.969	YES

Table-7 ADME Properties of Compounds **(6a-o)**

Compounds.	Absorption	Distribution		Metabolism Inhibitors CYP					Excretion	Toxicity		
	Intestinal absorption (human)	log BB	logPS	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	Total Clearance	AMES	LD50	Hepato toxicity
6a	92.691	0.042	-2.146	YES	YES	YES	NO	NO	0.227	YES	3.282	YES
6b	91.797	0.151	-1.922	YES	YES	YES	NO	YES	-0.026	YES	2.824	YES
6c	95.567	-0.035	-2.475	YES	YES	NO	NO	YES	0.168	NO	3.05	YES
6d	93.256	-0.394	-1.962	YES	YES	NO	NO	YES	0.153	YES	2.828	YES
6e	94.073	-0.019	-2.343	YES	YES	NO	NO	NO	0.022	NO	3.054	YES
6f	96.954	-0.832	-2.661	YES	YES	NO	NO	NO	0.483	NO	2.335	YES
6g	90.956	-0.567	-1.847	YES	YES	YES	NO	YES	0.158	NO	3.07	YES
6h	92.997	-0.26	-1.976	YES	YES	NO	NO	YES	0.173	NO	3.406	YES
6i	90.09	-0.594	-1.895	YES	YES	NO	NO	YES	0.279	YES	3.347	YES
6j	93.433	-0.261	-2.071	YES	YES	NO	NO	NO	0.161	YES	3.461	YES
6k	91.974	-0.437	-2.03	YES	YES	NO	NO	YES	0.014	YES	3.424	YES
6l	93.709	-0.464	-2.349	YES	NO	NO	NO	YES	0.187	NO	3.36	YES
6m	95.215	0.07	-2.297	YES	YES	NO	NO	NO	0.519	YES	2.381	YES
6n	91.96	-0.47	-2.209	YES	NO	NO	NO	YES	-0.073	YES	3.385	YES
6o	94.751	-0.746	-2.6	YES	NO	NO	NO	NO	0.389	YES	2.47	YES

Achievements with respect to objectives

- All the designed compounds **(5a-o)** and **(6a-6o)** were successfully synthesized and characterized by various spectroscopic methods.
- Compound **5i** and **5m** showed promising anti-tubercular activity with MIC 6.25μg/mL and 3.12 μg/mL.
- Compound **5m** showed good antioxidant activity.
- Compounds **5i** and **5m** were less cytotoxic to HepG2 cell line.

- Docking interaction revealed higher affinity against InhA.
- Designed, synthesized and evaluated new series of new amino substituted 6-methyl-1-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-ol derivatives as DprE1 Inhibitor.
- Four compounds have shown better DprE1 inhibitory activity. Compound **6g** was most potent with MIC 6.25 μg/mL. Compound **6g** was less cytotoxic to HepG2 cell line.
- Compound **6b** and **6d** showed good antioxidant activity.
- Docking interaction revealed higher affinity against DprE1

Conclusion

- A brief introduction to tuberculosis has been described.
- A brief review on therapeutic importance of pyrazole as InhA and DprE1 inhibitor has been given.
- brief of docking studies including docking scores has been given.
- Synthesized docked compounds having good score.
- Total 30 nos. of compounds has been synthesized.
- All the synthesized compounds has been characterized by IR, Mass, ^{1}H-NMR and ^{13}C-NMR spectroscopy.
- All the synthesized compounds were evaluated for their antimycobacterial activity by microplate alamar blue assay against *M. tuberculosis* H37Rv strain.
- Amongst all the synthesized pyrazole derivatives **5g**, **5h**, **5i**, **5j**, **5m**, **6e**, **6g**, **6j** and **6k** were showed good inhibitory activity against *M. tuberculosis* H37Rv strain.
- MD simulation study performed for most potent compounds **5m** and **6g**.
- Cytotoxicity study performed for most potent compounds **5i**, **5m**, **6g** and **6j**.
- Checked the ADMET properties of all synthesized compounds.
- Antioxidant activity checked by DPPH assay.
- Series of novel pyrazole derivatives as InhA and DprE1 inhibitors has potential to be developed as antitubercular agents.

List of Publications

1. Nayee Kinjal, Been Kumar Prajapati, and Hansal Kumar. "Design, synthesis, molecular dynamic simulation, ADMET studies, antioxidant and antitubercular screening of amine and acid chloride substituted pyrazole derivatives as an enoyl acyl carrier protein reductase (InhA) inhibitors." *Journal of Molecular Structure* (2025): 144928. (Scopus Index) – Research Paper

2. Kinjal Nayee, Beenkumar Prajapati, Haribhai Rabari; "Pyrazole derivatives as an enoyl acyl carrier protein reductase(InhA) Inhibitors with promising antitubercular activity.*" World Journal of Pharmaceutical Research*, Volume 14, Issue 5, 1108-1134.- Review paper

3. Kinjal Nayee, BeenKumar Prajapati; "Design, Synthesis and Antitubercular Activity of Novel Pyrazole Derivatves as InhA Inhibitors" NTP043, pg. No. 102 (Abstract Published)

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