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An In Silico and In Vitro Approach to Develop Novel Iminopyridines as EmbB Arabinosyl Transferase Targets to Combat H37Rv Strains of Mycobacterium Tuberculosis

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ABSTRACT: Tuberculosis is a virulent infection caused by Mycobacterium tuberculosis and inclined as a major global health concern. Development of multidrug-resistance and extensively drug-resistance by strains, drug toxicity and co-infection with HIV are the significant reasons for limiting the success of existing therapies. Therefore, it is a challenge for medicinal chemists to develop safer, more effective, novel anti-TB drugs with new mechanisms of action which are able to eliminate existing drug related issues. The current study involved the design, synthesis, characterization, and in vitro anti-tubercular activity evaluation of new iminopyridine derivatives (Compounds S1-S10) Using the Schrödinger Maestro software, the compounds were subjected for molecular docking investigations against the EmbB protein of arabinosyl transferase enzyme (PDB ID: 6X0O) with which electron-withdrawing substituted derivatives engage favourably, according to docking studies. Hence all the designed compounds implied Lipinski's Rule of Five and exhibited acceptable pharmacokinetic characteristics, according to ADME prediction studies conducted using ADMET Lab 3.0 web server, the tailored compounds were synthesized and characterised by spectral methods.

Anti-tubercular activity was carried out by micro dilution broth method to measure minimum inhibitory concentration (MIC) of all compounds. Rifampicin, standard drug has MIC at 0.25 µg/mL. Compounds S1, S2 and S3 with electron withdrawing groups like nitro and halo groups showed the greatest efficacy among the produced derivatives at the 6.25, 42 and 50 µg/mL respectively. Compounds S5, S9 and S10 demonstrated poor activity event at 100 µg/mL and above, but Compound S6 shown moderate activity 90 µg/mL).

INTRODUCTION

Imines are highly reactive bases possessing azomethine or imine bond (–CH=N–) moieties and distinguished as schiff bases [1], a significant class of chemical molecules. Schiff bases have a wide range of usage in synthetic chemistry. Hence the imino group serves as pharmacophore, Schiff bases contribute many medicinal benefits [2]. A number of imines have demonstrated antibacterial [3], anticancer [4] and antimitotic activities.

In the present study, the author has designed novel iminopyridines [5] using 3-aminopyridine and aromatic aldehydes. An approach has been made to assess the molecular properties, drug likeliness [6].

EmbB protein of *arabinosyl transferase* [7] (PDB ID 6X0O) was selected as the drug target to assess the docking score of designed molecules. This enzyme plays a vital role in polymerization of arabinose residues during the production of arabinogalactan [8] which is essential for cell wall synthesis and the survival of bacteria and utilises decaprenylphosphoryl-D-arabinose (DPA) as the arabinose donor substrate [9]. Crucially, ethambutol, and many first line anti TB drugs target cell wall assembly and act by interfering with arabinan production, which reduces *arabinosyltransferase* activity [10]. However, prolonged clinical use of ethambutol and other first line drugs develop resistance hence they cause mutations in the EmbB gene [11,12].These mutations modify the structural form of binding pockets and reduce the binding affinity[13] of the drugs to the enzyme .This reduction in binding affinity may lead to develop therapeutic resistance [14].Therefore, the present study made an effort to design and develop structurally diverse compounds capable of inhibiting both wild-type and mutant EmbB proteins of *arabinosyltransferase* enzyme.

The designed molecules with considerable docking score were selected for synthesis and evaluated for *in vitro* anti tubercular activity by micro dilution broth [15] on H37Rv strain of *Mycobacterium tuberculosis* (Mtb) [16]. Rifampicin was used as reference standard [17]. *In silico* properties, reaction mechanism, characterization of compounds by spectral studies, results of anti -TB activity were tabulated and described in the manuscript.

MATERIAL AND METHODS

In silico studies:

Chemical structures of proposed molecules were drawn using ACD/ChemSketch software. Swiss ADME online drug design software was used to predict molecular properties such as log P values, solubility, total polar surface area (TPSA), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), rotatable bonds (RB),Lipinski rule violations, and synthetic accessibility and blood-brain barrier (BBB) permeability index and depicted in the results part of the manuscript . Schrödinger Glide software was used to perform molecular docking studies of designed molecules.

All the chemicals used in this research were purchased from Himedia and Sigma-Aldrich. Melting points of all synthesized compounds were determined using a digital capillary melting point apparatus.^{1}H NMR and Mass spectra were received from Sophisticated Analytical Instrumentation Facility of Punjab University, Chandigarh. .^{1}H NMR spectra was recorded in the specified solvent on a Bruker Advance Neo 500 MHz spectrophotometer with TMS as an internal standard. Mass spectra was recorded on MALDI SYNAPT XS HD Mass spectrophotometer.

Molecular Docking:

The proposed structures of Iminopyridine bases docking studies were analyzed by Schrodinger Glide software version 2024_1.

The Maestro interface of the Schrödinger Molecular Modeling Suite version 12.8 was used for molecular docking studies to assess the binding affinity of molecules against arabinosyltransferase of EmbB protein [18]. The crystal structure of EmbB was retrieved from the Protein Data Bank (PDB ID: 6X0O) as shown in Figure 1 and employed as the target protein for docking studies. Ethambutol was used as the reference ligand for comparative analysis [19]. The Epik module was used to optimize the protonation states of amino acid residues at pH (7.0 ± 2.0). After refining hydrogen-bond networks, restricted energy reduction was carried out using the OPLS4 force field until the heavy atoms root mean square deviation (RMSD) [20] met a convergence criterion of 0.30 Å. The prepared protein structure was then utilized for receptor grid generation.

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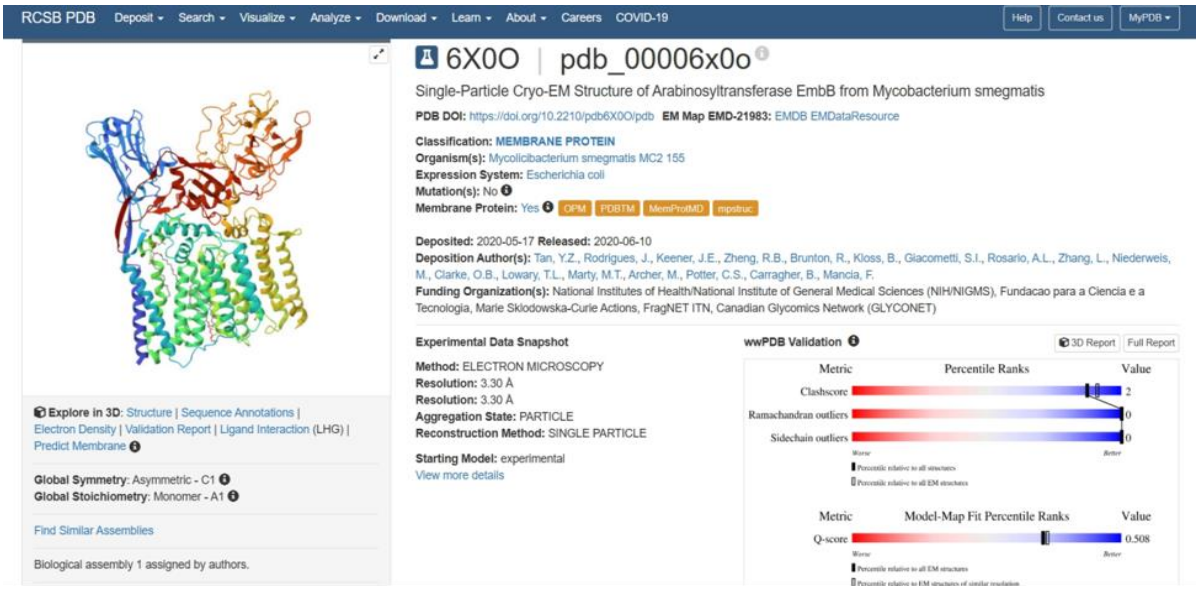


Figure. 1: Protein structure of EmbB (6x0O**)**

Protein preparation:

The Protein Data Bank provided the three-dimensional crystal structure of EmbB (PDB ID: 6X0O), which was then imported into Maestro's Protein Preparation Wizard [21]. In order to pre-process the protein structure, bond orders were assigned, missing hydrogen atoms were added, disulfide bonds were made when necessary, and crystallographic water molecules unrelated to ligand binding were eliminated [22]. The Prime module was used to fix any missing side chains and loops. The Epik module was used to optimize the protonation states of amino acid residues at physiological pH (7.0 ± 2.0).

Receptor grid generation in 6X0O protein:

The active-site region of the EmbB enzyme was identified based on the co-crystallized ligand-binding cavity and previously reported active-site residues. The Glide Grid Generation panel in Maestro was used to generate receptor grids [23]. The grid box was centered on the active-site region of the protein to ensure complete coverage of the ligand-binding pocket .

The receptor grid's measurements were established as X-axis: 22 Å; Y-axis: 33 Å

The active-site residues involved in ligand recognition and binding were sufficiently covered by the grid parameters chosen. The generated receptor grid was subsequently employed for molecular docking calculations.

Ligand Preparation:

Maestro was used to sketch the chemical structures of the intended compounds and the reference medication ethambutol (CID: 14052), which were then processed using the LigPrep module. The OPLS4 force field was used to optimize the geometry of the generated structures. Using Epik, potential ionization states, tautomeric forms, and stereochemical variants were produced at physiological pH (7.0 ± 2.0). Docking calculations were then performed using energy-minimized ligand conformations.

Ligand docking:

The Glide Extra Precision (XP) module of Schrödinger Maestro 12.8 [24] was used for molecular docking. The prepared ligands, including the designed compounds and the reference drug ethambutol (CID: 14052), were docked into the active-site grid generated for the EmbB protein (PDB ID: 6X0O). The receptor was kept in a rigid conformation while flexible ligand docking was performed [25]. A number of docking positions were produced, and the most negative Glide XP score and Glide energy were used to determine which stance was optimal for each ligand [26]. Protein–ligand interactions inside the active site were further examined in the chosen complexes. Observations of above studies were described and tabulated in results and discussion part.

Synthetic procedure:

The proposed molecules were prepared by acid catalysed Schiff base condensation reaction using 3-aminopyridine as primary amine and different aromatic aldehydes to form imines. Schiff base condensation reaction follows two main steps as described in Figure 2: nucleophilic addition and dehydration [27] and scheme of the proposed reaction is described in Figure 3.

1. Nucleophilic Addition: The lone pair electrons of nitrogen on the primary amine's nitrogen (R_1-NH_2) attacks the electrophilic carbonyl carbon of the aldehyde(C=O) to form an unstable carbinolamine. Then loss of a proton occurs from nitrogen to form neutral amino alcohol. In mild acidic condition, alcohol will be protonated to form an unstable oxonium ion .

2. Dehydration (Elimination): In the second step, protonate alcohol loose a molecule of water and forms iminium ion. On further loss of proton from nitrogen of iminium ion it forms stable Schiff base [28] with double bond.

Reaction Mechanism:

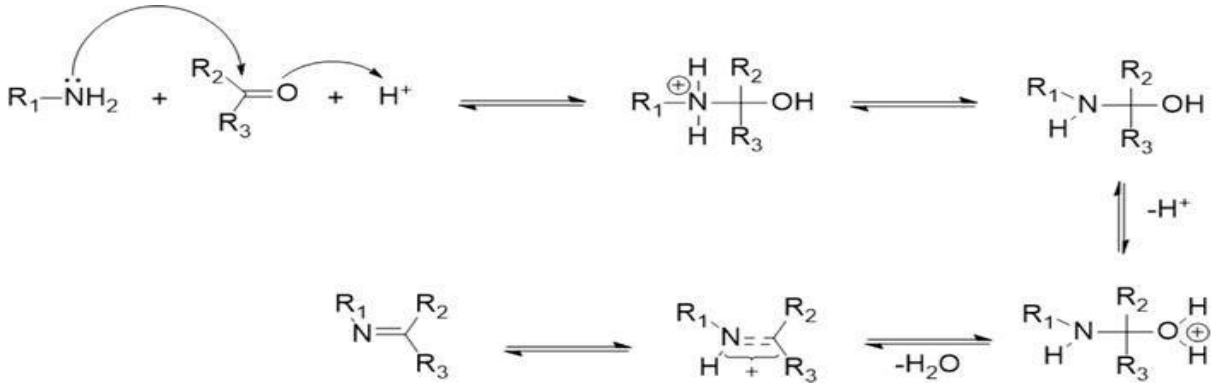


Figure. 2: Reaction mechanism of Schiff base Condensation

General method of Synthesis of Imino Pyridine Bases (S1-S10)

Equimolar (0.01 moles) quantities of 3-aminopyridine equivalent to 0.941 g, and substituted benzaldehydes (e.g., 4-nitrobenzaldehyde, 1.51 g) were taken in a thoroughly desiccated 100 ml round bottom flask. This mixture was dissolved in 25 ml of absolute ethanol. Three to five drops of glacial acetic acid and 1 ml of concentrated nitric acid were added to this homogenous solution to serve as acid catalyst. The reaction mixture was vigorously stirred on a magnetic stir bar and refluxed ne mobile phase at 45-minutes. The reaction vessel was cooled to room temperature, filtered and recrystallized from ethanol [29].

TLC [30], 1H−NMR [31] and mass spectral studies [32] were used to verify structural purity and validate the molecular structures. Results obtained were explained and tabulated in the manuscript.

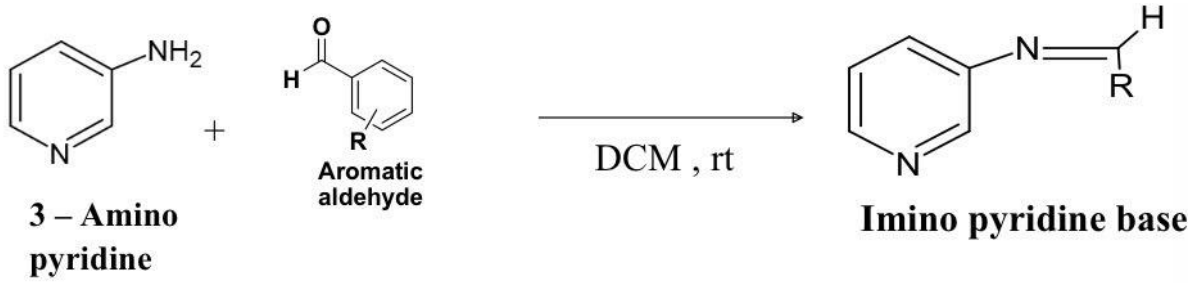


Figure 3: General reaction of Iminopyridines synthesis

R=2,4 di(-NO_2) in S1, R=P-(-NO_2) in S2, R=P(-cl) in S3, R=P-(CN) in S4, R= 3,4- di(-CHO) in S5, R=2-[(CH_3)2-N-] in S6,R=P(-OH) in S7,R= 2-amino in S8,R=2(-OH) in S9,R=3-(-OCH_3),R=4(-OH) in S10

Antitubercular activity:

Determination of Minimum Inhibitory Concentration (MIC) by broth dilution method on *M. tuberculosis* H37Rv.

MIC of the given compounds determined at the concentration ranging from 100-0.78 µg/ml against *M. tuberculosis* H37Rv [33]. The dilution made by using the 7H9 medium [34] without altering the medium composition. The culture suspension was adjusted to 1 McFarland standard and diluted to a 1:10 ratio so that each well contained 1×10^5 cells. Rifampicin at the critical concentration of 0.25 µg/mL taken as a reference compound. Culture control and solvent control (DMSO) [35] included in the assay. The plates observed under the microscope after five days incubating at 37°C for determining gradation based on the serpentine cord formation [36, 37] of M tb culture growth. Screening performed in duplicate in a microtiter plate and the minimal concentration of the compounds that completely inhibited the growth of the Mtb cultures considered as MIC.

RESULTS AND DISCUSSION

ADME studies:

Swiss ADME studies of all the synthesized iminopyridines viz (S1-S10) shown in Table. 1 The ADME profiling of the designed compounds demonstrated considerable drug-like properties for majority of compounds. Most of the compounds possess acceptable molecular weights (ranging ~110–450 Da) and Log P values within drug-like ranges, suggesting good membrane permeability and bioavailability. All compounds obey Lipinski's Rule of Five, indicating a lower likelihood of oral bioavailability issues. High gastrointestinal (GI) absorption was predicted for all compounds, with several also showing blood–brain barrier (BBB) permeability, particularly S4, S5, S9 and S10 molecules. Solubility varied across the series: many compounds were categorized as moderately soluble based on Log S values, which is favourable for oral formulations. S9 and S10 are poorly soluble importantly, all designed molecules demonstrated reasonable synthetic accessibility scores (ranging from 2.4–3.4), indicating they can be synthesized without significant synthetic challenges with promising drug-like characteristics, assuring further optimization focusing particularly on improving solubility for some of new imino pyridines.

Table 1: *In silico* properties of synthesized molecules

Code	LogP	Log S	TPSA (Å²)	HBD	HBA	RB	GI absorption&BBB Permeability	Lipinski Violations	Synthetic accessibility
S1	2.65	-3.88	111.53	0.0	8.0	4	High &No	0	3.27
S2	2.15	-2.68	45.3	1	3	6	High & No	0	2.85
S3	3.10	-3.27	25.4	0	2	4	High &Yes	0	2.78
S4	1.876	-2.45	49.04	0	3	2	Medium &Yes	0	2.76
S5	2.40	-1.08	34.6	0	3	4	High &Yes	0	2.88
S6	2.50	-3.02	25.4	0	2	1	Very High &No	0	3.15
S7	2.85	-2.21	71.1	0	4	1	Moderate & No	0	2.93
S8	1.998	-2.94	51.27	2	3	2	Medium &Yes	0	3.35
S9	1.85	-3.65	46.2	1	3	2	Poor & Yes	0	2.76
S10	1.683	-3.85	66.45	1	3	3	Medium &Yes	0	3.64

Molecular Docking Studies:

Docking scores of the designed molecules with the active site of the enzyme 6X0O were obtained using the Schrödinger Glide software and are presented in Table 2. After protein preparation, the binding site was selected by generating a receptor grid surrounding the active-site region of the protein. The receptor grid dimensions were established as X-axis: 22 Å, Y-axis: 33 Å, and Z-axis: 22 Å.

The structural quality of the 6X0O protein was assessed using a Ramachandran plot (to evaluate the backbone dihedral angles (φ and ψ) of its amino acid residues. As shown in **Figure 4**, the majority of residues were located within the most favored regions, indicating that the protein possesses a stable and stereochemically reliable conformation. Only a small proportion of residues were present in the additionally allowed regions, while very few residues were observed in the disallowed regions. This distribution demonstrates that the 6X0O protein structure is geometrically sound and suitable for downstream computational analyses such as molecular docking and molecular dynamics simulations. The high percentage of residues in favoured regions reflects proper folding and minimal steric hindrance within the protein backbone, which is crucial for maintaining the structural integrity of the active site and ensuring accurate prediction of ligand-binding interactions.

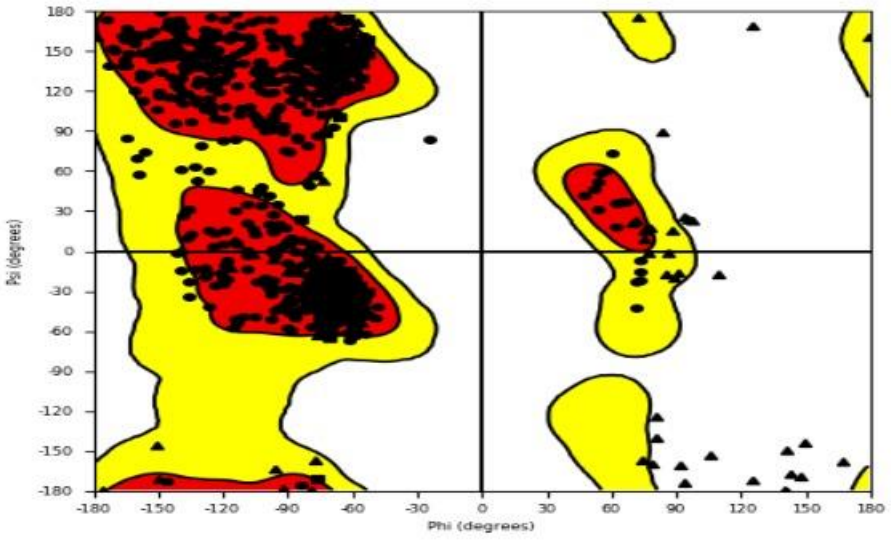
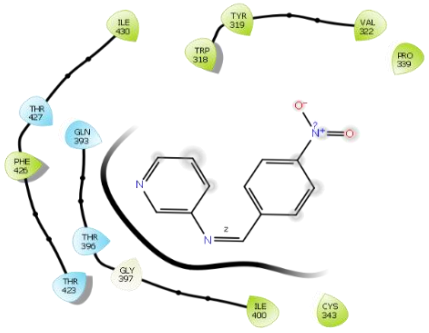


Fig. 4. Ramachandran plot of the EmbB protein structure showing the distribution of backbone dihedral angles (φ, ψ).

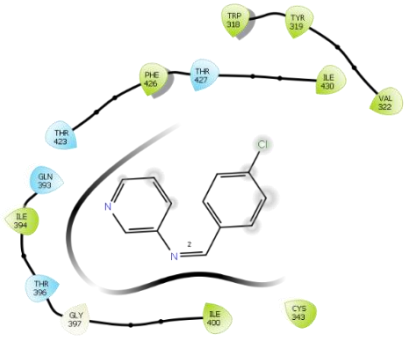
The molecular docking study of the synthesized imino pyridine derivatives against the EmbB protein, an essential enzyme involved in the biosynthesis and mycobacterial cell wall formation, revealed favorable binding affinities for several compounds when compared with the standard anti-tubercular drug ethambutol. The docking scores of the synthesized compounds ranged from −4.66 to −6.47 as listed in **Table.2**, while the corresponding Glide energies varied from −26.60 to −31.52 , indicating stable ligand–protein interactions within the active site of the enzyme. Compound S1 exhibited the highest binding affinity with a docking score of −6.47 and a Glide energy of −31.52 , forming interactions with key amino acid residues TYR319, THR427, and ASP428 as depicted in **Fig. 5**. Compound S4 also demonstrated significant binding potential with a docking score and interacted with TRP318 and THR396 residues. Similarly, compounds S4 and S8 showed favorable docking scores of −6.26 and −6.25 , respectively, and established interactions with important residues including GLY397, THR396, THR423, and VAL322.Compounds S3 and S9 exhibited moderate binding affinities with docking scores of −5.96 and −5.92 , respectively, interacting primarily with GLN393, THR427, TRP316, and THR396. Meanwhile, compounds S2, S5,S6 and S7 demonstrated comparatively lower docking scores ranging from −5.66 to −5.04 but maintained interactions with several catalytically relevant residues such as ASP482, ILE342, LEU325, PHE426, GLY397, and TYR319.

Table 2. Docking scores, Glide energies, and key binding interactions of compounds with the aminoacids of EmbB enzyme.

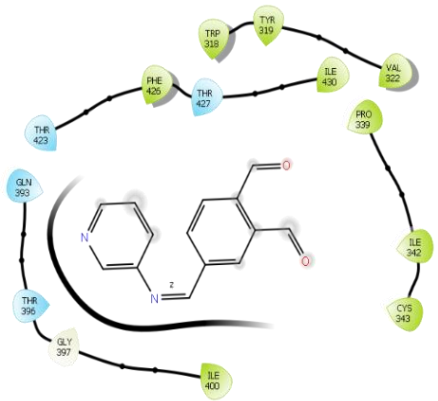
Compound Code	Docking score *(kcal/mol)*	Glide energy *(kcal/mol)*	Interactions
S1	-6.47	-31.52	TYR319,THR427,ASP428
S2	-5.15	-27.97	THR396,GLY397,TYR319,THR423,PHE426
S3	-5.96	-30.38	GLN393
S4	-6.38	-31.05	TRP318,THR396
S5	-4.66	-27.76	ASP482,THR427,ILE342
S6	-5.04	-26.60	GLN393,PHE426,THR427
S7	-5.51	-27.97	LEU325,ASP482,ASP314
S8	-6.25	-30.72	GLY397,THR423,VAL322
S9	-5.02	-27.97	THR427,GLN393,TRP316,THR396
S10	-4.95	-30.81	GLY397,TRP318,THR396



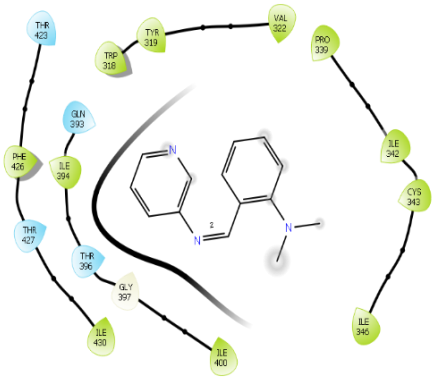
S2



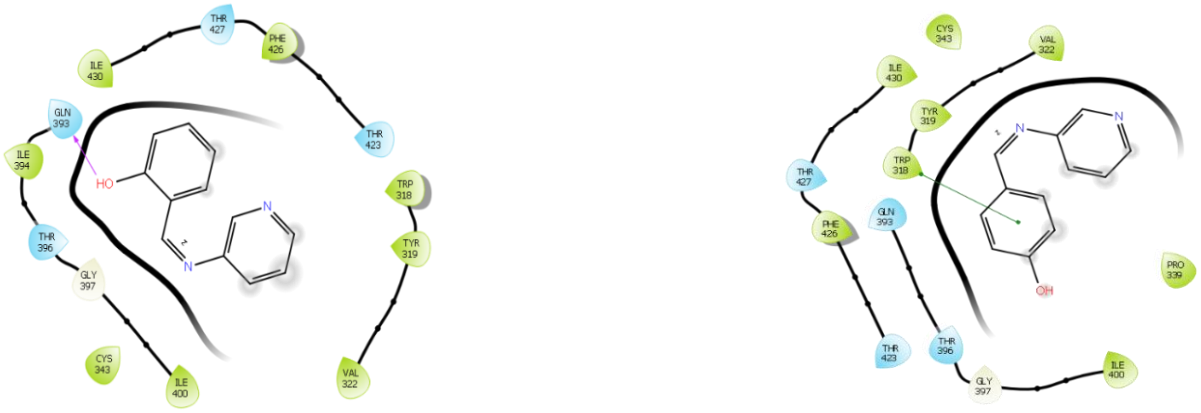
S3



S5



S6



S7 **S9**

Fig. 5. Docking interactions of ligands with the EmbB Enzyme.

Spectral characters of compounds (S1-S10)

Figure 6.a to 6.c represents IR,NMR & Mass Spectra of S3 compound respectively.

(*E*)-1-(2,4-dinitrophenyl)-*N*-(pyridin-3-yl)methanimine (S1)

Formula: $C_{12}H_8N_4O_4$; IR 3059 (ArC–H), 1617 (C=N)**,**1538 (NO_2),1351(NO_2)**,**1557 (pyridine ring),810 **(** C–H out-of-plane); ^{1}H NMR -Total no. of protons 8H: **Pyridine ring**- 1T-J= 7.82 4.55 0.50Hz, 2d-J=7.82 1.89 1.45 Hz, 4.55 1.89 1.85 Hz, 1dd-J=1.85 1.45 0.50 Hz, **Aromatic ring-** 2d-J= 8.69 0.52 Hz, 8.69 1.83 Hz, 1dd-J= 1.83 0.52 Hz**, Imine group-**1 Singlet; MS m/z = 272.22g/mol.

1-(4-nitrophenyl)-*N*-(pyridin-3-yl)methanimine (S2)

Formula: $C_{12}H_9N_3O_2$; IR 1616(C=N azomethine), 1526 (NO_2),1594(C=C/C=N),842(C-H); ^{1}H NMR -Total no. of protons 9H: **Pyridine ring**- 1T-J= 8.0 2.3 1.2Hz ,2d – J =7.8 3.2 0.8Hz, 1Singlet, **Aromatic ring - ** 2d- J= 8.2 2.4 0.5Hz, 8.2 1.8 0.5Hz, **Imine group-**1 Singlet; MS m/z =261.22g/mol.

(*E*)-1-(4-chlorophenyl)-*N*-(pyridin-3-yl)methanimine (S3)

Formula: $C_{12}H_9N_2Cl$; IR 1612(C=C),1560 (C=N),1291(C-N),772(C-Cl),811(C-H); ^{1}H NMR -Total no. of protons 9H: **Pyridine ring**- 1T-J=7.6 4.7 0.6Hz, 2d –J=7.6 1.9 1.7Hz,4.7 1.9 1.8Hz ,1Singlet, **Aromatic ring**- 2d-J=8.6 1.6 0.5Hz **,** 8.6 1.2 0.5Hz, **Imino group-**1Singlet; MS m/z =251.66 g/mol.

4-{(*E*)-[(pyridin-3-yl)imino]methyl}benzonitrile (S4)

Formula: $C_{13}H_9N_3$; IR 3052 (Ar- C–H), 2218 (C≡N), 1625 (C=N) stretching ,1577 (pyridine ring),1278 **(**C–N stretching) ,774 **(**Ar C–H) out-of-plane bending; ^{1}H NMR -Total no. of protons 9H: **Pyridine ring**- 1T-J=7.81 4.78 0.54 Hz, 2d-J=4.78 1.89 1.84 Hz, 7.81 1.89 1.63 Hz, 1dd-J=1.84 1.63 0.54 Hz, **Aromatic ring**- 4d-J=8.81 1.89 0.49 Hz, 8.81 1.67 0.49 Hz, **Imino group-** 1Singlet; MS m/z =208.23g/mol.

4-{[(pyridin-3-yl)imino]methyl}benzene-1,2-dicarbaldehyde (S5)

Formula: $C_{15}H_{16}N_2O_2$; IR 3358(-OH), 3058 (Ar-H), 1624 (CH=N), 1496 (Ar-Pyridine), 1268(C-O), 1123(C-N); ^{1}H NMR -Total no. of protons 10H: **Pyridine ring**- 1T-J=7.8 4.8 0.5Hz, 2d- J=7.8 1.9 1.6Hz, 4.8 1.9 1.9Hz, 1Singlet, **Aromatic ring-** 2d-J=1.8 0.5Hz, 8.4 0.5Hz , 1Singlet **, Imino group-** 1Singlet; MS m/z =200.30 g/mol.

_N,N_-dimethyl-3-{[(pyridin-3-yl)imino]methyl}aniline (S6)

Formula: $C_{14}H_{15}N_2$; IR 2931(C-H), 1656 (C=N), 1591(C=C), 1253(C-N), 1459(-CH_3), 862(C-H)); ^{1}H NMR -Total no. of protons 10H: **Pyridine ring**-1T – J= 8.0 4.7 0.5Hz, 2d-J= 1.8 1.8 0.5Hz 8.0 1.9 1.8Hz 1Singlet, **Aromatic ring-**1T– J=8.1 7.4 1.3Hz, 2d- J=8.1 1.3 0.5Hz, 7.7 7.4 1.3Hz, **Imino group-** 1Singlet ; MS m/z = 212.28g/mol.

4-{[(pyridin-3-yl)imino]methyl}phenol (S7)

Formula: $C_{12}H_{10}N_2O$; IR 3413 (phenolic –OH), 1629 (C=N), 1572 (C=C), 1239(C-O);^{1}H NMR -Total no. of protons 10H: **Pyridine ring**-1T – J=8.2 4.7 0.5Hz 2d - J=8.2 1.9 1.7Hz 4.7 1.9 1.8Hz ,1Singlet , **Aromatic ring-** 2d – J= 8.2 1.0 0.4Hz, 8.2 1.8 0.4Hz, 1Singlet , **Imino group-** 1Singlet ; MS m/z =223.23 g/mol.

2-{(*E*)-[(pyridin-3-yl)imino]methyl}aniline (S8)

Formula: $C_{12}H_{11}N_3$; IR 3471 (NH_2), 1623 (C=N) , 1560 (pyridine), 1324 **(**Ar C–N), 844 (Ar- C–H) out-of-plane;^{1}H NMR -Total no. of protons 10H: **Pyridine ring**-1T – J=7.35 4.75 0.55,2d-J= 4.75 1.88 1.81, 7.35 1.88 1.76, 1dd-J=1.81 1.76 0.55,**Aromatic ring**- 1T-J=7.70 7.42 1.22, 2d-J=8.06 7.42 1.32, 7.70 1.32 0.48 ,1dd-J=8.06 1.22 0.48,**Imino group-** 1Singlet ; MS m/z = 197.31g/mol.

2-{(*Z*)-[(pyridin-3-yl)imino]methyl}phenol (S9)

Formula: $C_{12}H_{10}N_2O$; IR 1714(Ar-C=O), 1617 (C=N), 2782 (ald-OH), 3063(Ar-CH),1575(Pyridine);^{1}H NMR -Total no. of protons 10H: **Pyridine ring**-1T – J=8.2,4.7,0.5Hz, 2d-J=8.2 1.9 1.7Hz, 4.7 1.9 1.8Hz,1Singlet,Aromatic ring-1T- J=8.0 0.75 1.4Hz, 8.0 1.2 0.4Hz,**Imino group-** 1Singlet ; MS m/z =199.22 g/mol.

2-methoxy-4-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol (S10)

Formula: $C_{13}H_{12}N_2O_2$; IR 3410 (Ar O–H), 1619 azomethine (C=N) ,1514 (pyridine ring), 1052 **(**C–O–C) methoxy group ,762 (C–H out-of-plane);^{1}H NMR -Total no. of protons 10H: **Pyridine ring**-1T – J=6.17 4.79 0.55Hz, 2d-J=1.93 1.68 0.55 Hz, 6.17 1.90 1.68Hz ,1dd-J=4.79 1.93 1.90Hz, **Aromatic ring-** 2d-J=8.44 1.79Hz , 8.44 0.47Hz,1dd-J=1.79 0.47Hz,1Singlet, **Imino group-** 1Singlet ; MS m/z = 228.25g/mol.

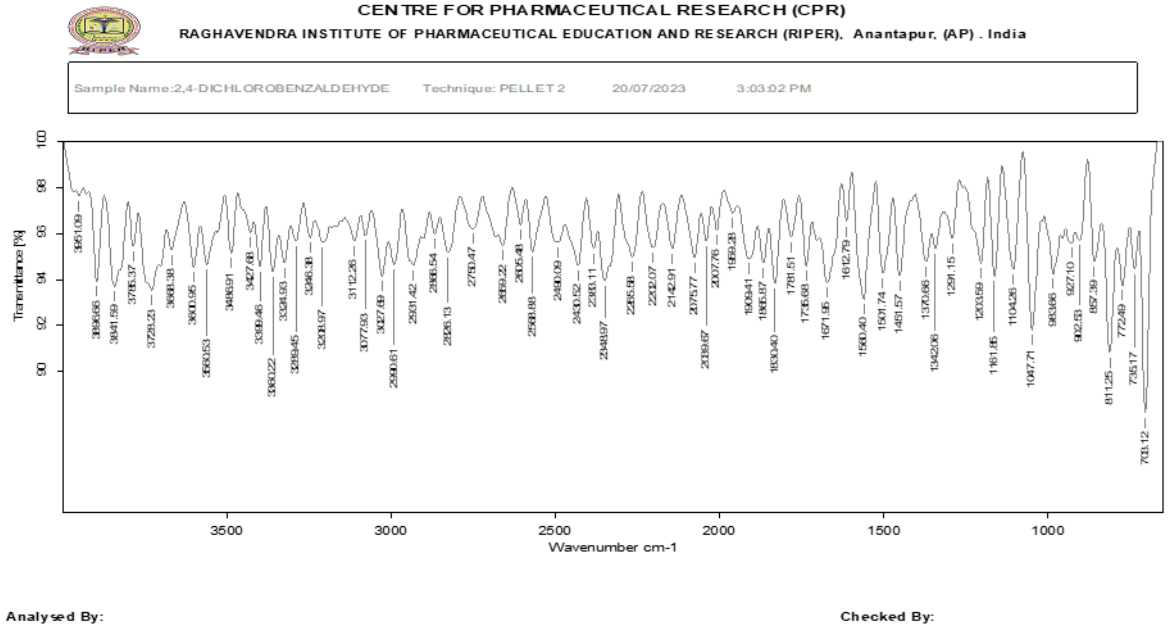


Fig. 6.a: IR spectrum of (S3)- (*E*)-1-(4-chlorophenyl)-*N*-(pyridin-3-yl)methanimine

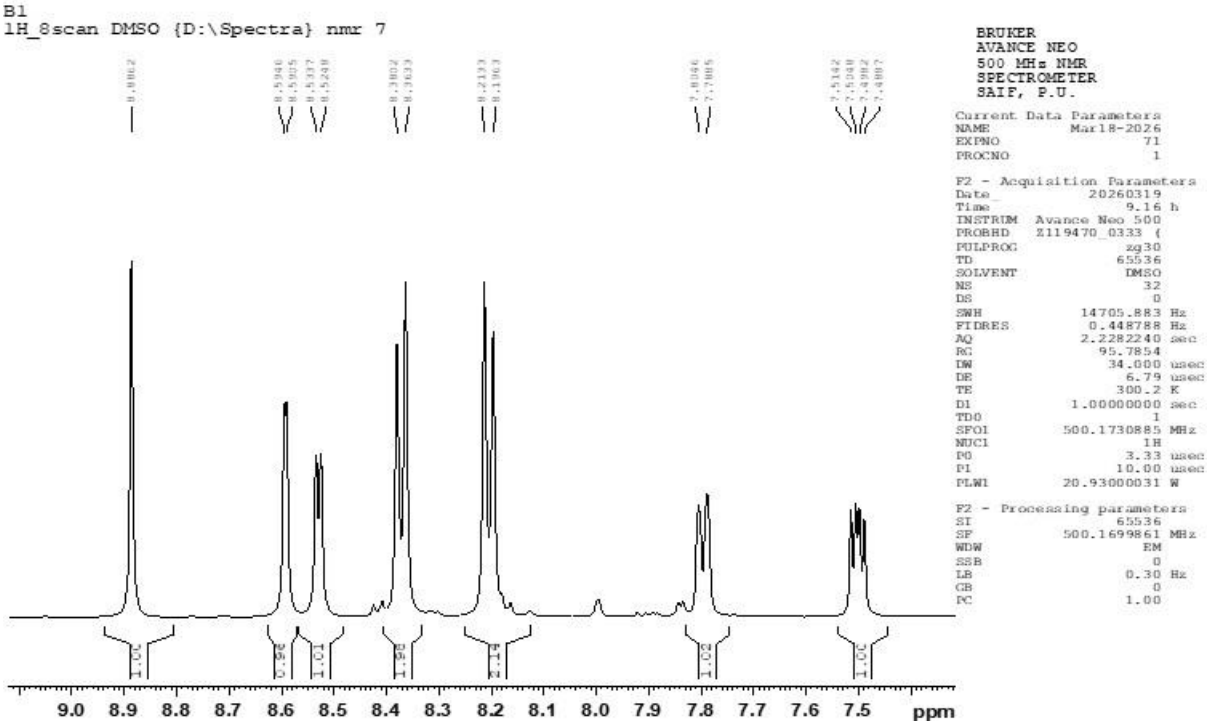


Fig. 6.b: NMR spectrum of (S3)- (*E*)-1-(4-chlorophenyl)-*N*-(pyridin-3-yl)methanimine

Fig. 6.c: Mass spectrum of (S3)- (*E*)-1-(4-chlorophenyl)-*N*-(pyridin-3-yl)methanimine

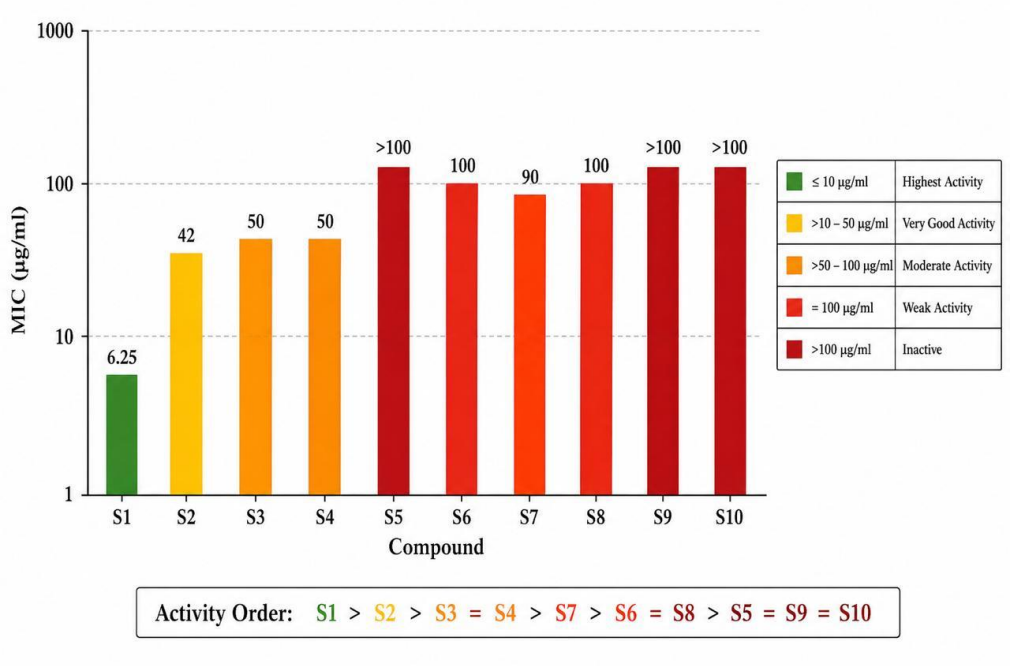
Anti-tubercular activity by Micro dilution broth method

Micro dilution broth method which had been performed to evaluate *in vitro* anti-tubercular activity by observing Minimum Inhibitory Concentration of synthesized molecules against drug resistant *M. tuberculosis* H37Rv strain. This study indicated that several designed compounds exhibit strong considerable anti- TB activity by inhibiting the bacterial growth at different concentrations as shown in **Table.3** and **Fig. 7.** S1,S2,S3,S4 which possess electron withdrawing groups viz dinitro, P-nitro, P-chloro, P-cyano have demonstrated excellent anti-tubercular potential at 6.25 µg/ml for S1, 42 µg/ml for S2 and 50 µg/ml for S3 and S4.Other promising candidates like S6,S7 and S8 also showed moderate inhibition levels at less than 100 µg/ml as

they have electron releasing groups. On the other hand S5, S9 and S10 which have strong electron releasing groups like 3,4-dimethoxy,2-hydroxy and 3-methoxy 4-hydroxy have shown lower inhibitory activity (>100 µg/ml)indicating limited anti-TB effectiveness. Particularly, S1, S2 compounds may emerge as highly promising candidates for future optimization and evaluation hence these compounds show mere activity as Rifampicin standard (0.25 µg/mL).

Table .3: Minimum inhibitory Concentrations of synthesized imino pyridines

Code	Substituent on benzaldehyde	MIC µg/ml
S1	Di nitro	6.25
S2	p-nitro	42
S3	p-chloro	50
S4	p-cyano	50
S5	3,4-di methoxy	>100
S6	2-dimethylamino	100
S7	p-hydroxy	90
S8	2-amino	100
S9	2-hydroxy	>100
S10	3-methoxy,4-hydroxy	>100



Fig, 7. Anti TB activity of Iminopyridines : Micro dilution broth method

CONCLUSION

This study utilises an integrated computational and experimental approach to evaluate the anti-tubercular potential of novel iminopyridines derived from 3-aminopyridine and substituted aldehydes. Molecular docking studies against the 6X0O enzyme revealed that novel imino pyridine with electron withdrawing groups have strong binding affinities and favourable interactions

with key binding pockets of 6X0O to inhibit enzymatic activity remarkably. *In silico* ADME studies indicate that most of designed compounds possess favourable pharmacokinetic properties such as gastrointestinal absorption, BBB permeability, drug-likeness, and synthetic accessibility etc. for further lead development. Importantly, a clear inverse correlation between docking scores and MIC was observed for all synthesized compounds, affirming that efficient inhibitors suppress the growth of *M.tb* H37Rv strain. This study emphasizes the value of combining *in silico* modelling to accentuate anti-TB drug discovery and novel therapeutics against multidrug-resistant and extensively drug-resistant tuberculosis.

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AUTHOR CONTRIBUTIONS

Kanala Somasekhar Reddy**:** Conceptualization

Sugali Gayatri**:** Laboratory Analysis.

Saluvadi Vedavathi **:** Data Analysis.

Palem Ranjitha : Methadology

Mallela Vijaya Jyothi**:** Supervision, Writing, Review & Editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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