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RESEARCH ARTICLE

Design and One-Pot Synthesis of (1*H*, 3*H*) Imidazo[4,5-*b*] Pyridines: Novel Therapeutic Agents Against *M. Tuberculosis*

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Abstract: Prevalence to the various biological activities of imidazo[4,5-b] pyridine nucleus has promoted us for an attempt of one pot synthesis of (1*H*, 3*H*) imidazo [4,5-*b*] pyridines (desazopurines) as inhibitors of Lumazine synthase in *M. tuberculosis* (PDB 2C92). Major advantage of Lumazine synthase enzyme behind consideration as target protein in docking study is its absence in mammalian cell but presence in various reported microorganism. All synthesized compounds were characterized by spectral studies (FT-IR, ^{1}H NMR, ^{13}C NMR and HR-MS), elemental analysis (C, H and N). In docking analysis best fit in the active pocket of target protein found were 4-(3*H*-imidazo[4,5-b]pyridin-2-yl)-2-methoxyphenol **(1f)** and 4-(2-methyl-2,3-dihydro-1*H*-imidazo[4,5-b]pyridin-2-yl)benzene-1,2,3-triol **(2b)** studied further for binding interactions. Microplate Alamar Blue Assay **(MABA)** method used for *in vitro* anti-tubercular testing has revealed 1-(3*H*-imidazo[4,5-b]pyridin-2-yl)-butane-1,2,3,4-tetraol **(1a)**, 1-(3*H*-imidazo[4,5-b]pyridin-2-yl)pentane-1,2,3,4,5-pentol **(1b)**, 1-(3*H*-imidazo[4,5-b]pyridin-2-yl) butane-1,2,3,4-tetrol **(1c)**, 4-(3*H*-imidazo[4,5-b]pyridin-2-yl)benzene-1,2-diol **(1j)**, 4-chloro-2-(2-methyl-2,3-dihydro-1*H*-imidazo[4,5-b]pyridin-2-yl)phenol **(2a)**, 3-(aminomethyl)-4-(2-methyl-2,3-dihydro-1*H*-imidazo[4,5-b]pyridin-2-l)benzene-1,2-diol **(2c),** 4-(2-methyl-2,3-dihydro-1*H*-imidazo[4,5-b]pyridin-2-yl)benzene-1,3-diol **(2f)** were found potentially significant anti-tubercular agents (MIC=3.12 µg/mL). These compounds could be considered as promising leads over the Pyrazinamide (MIC= 3.125 µg/mL) and Streptomycin (MIC= 6.25 µg/mL). Present study could be a unique source for development of newer therapeutic agents in the treatment of infection by *M. tuberculosis.*

Keywords: Imidazo[4,5-*b*]pyridine, Lumazine synthase, *M. tuberculosis*, MABA, Structure activity relationship

Introduction

Mycobacterium tuberculosis is one of the human pathogens responsible for causing eight million cases of new infections and two million human deaths every year in both developing and industrialized countries[1]. Treatment of the active forms of the disease has become increasingly difficult because of the growing antibiotic resistance of *Mycobacterium tuberculosis*. The elucidation of the complete genomes of *Mycobacterium tuberculosis* and the related *Mycobacterium leprae* has provided powerful tools for the development of novel drugs that are urgently required[2-4]. Both *Mycobacterium tuberculosis* and *Mycobacterium*

leprae comprise complete sets of genes required for the biosynthesis of riboflavin (vitamin B2) involving catalytic step of Lumazine synthase. As the genome of *Mycobacterium leprae* has undergone a dramatic process of gene fragmentation, the fact that all riboflavin biosynthesis genes were retained in apparently functional form indicates that the biosynthetic pathway is of vital importance for the intracellular lifestyle of the pathogen. By extrapolation of this argument, it appears likely that the riboflavin pathway genes are also essential for *Mycobacterium tuberculosis*.

In particular, *M. tuberculosis* is reported for different inhibitors of Lumazine synthase by considering structural and thermodynamic insights into the binding mode[5]. Riboflavin biosynthesis path involving Lumazine synthase catalysis is reported for list of other micro-organism like *Bacillus subtilis*[6], *Aquifex aeolicus*[7] and *Spinacia oleracea*[8], as an icosahedral capsid formed from 60 identical subunits (12 pentamers). In *Saccharomyces cerevisiae*[8], *Schizosaccharomyces pombe*[9], *Brucella abortus*[10] and *Magnaporthe grisea*[11] presence of homopentameric enzymes. Riboflavin (Vitamin B_2) is biosynthesized by plants and numerous microorganisms but not by animals, whereas animals obtain riboflavin from dietary sources. A rational approach to therapeutically useful antibiotics would be to selectively inhibit an enzyme present in a parasite but absent in the host. Inhibition of the bio-synthesis of riboflavin provides such a strategy, since pathogenic microorganisms synthesize their own riboflavin, whereas mammals obtain this vitamin through dietary sources.

Riboflavin biosynthesis is therefore would be an attractive target for the design and synthesis of new antibiotics against *M. tuberculosis*, which are urgently needed because pathogens are becoming drug resistant at an alarming rate. The imidazopyridine moiety is an important pharmacophore that has proven to be useful for a number of biologically relevant targets[12]. Imidazo[4,5-*b*]pyridine, known as 1-desasapurine, is a common structural motif found in numerous molecules that display antiviral, antifungal, antibacterial activities[13]. The potent biological activity and the prevalence of 1-desazapurines in both natural products and pharmaceuticals have inspired significant interest in the synthesis of these heterocycles. Compounds that belong to the imidazo[4,5-*b*]pyridin-2-one class have been shown to be nonsteroidal anti-inflammatory and analgesic agents[14-17] and to possess antidepressant[15-18], antiphlogistic[7-19], cardiotonic[17-20], hypotensive and anti-arrhythmic activity[18-21]. In addition certain members of this class had been reported to be a potent inhibitor of Aurora-A[22], adenosine deaminase (ADA) inhibitors[23], potent inhibitors of inosine 5'-monophosphate dehydrogenase (IMPDH)[24]. Recent studies from many laboratories, implicate the role of these scaffolds in the treatment of many of the most common human diseases, including diabetes[25], cancers[26] and an array of neurological syndromes[27].

In the present study, some possible derivatives of 1-desazopurines as (1*H*,3*H*) imidazo[4,5-*b*]pyridines were proposed and docked against Lumazine synthase enzyme from *Mycobacterium tuberculosis* (PDB 2C92). Different sets of interactions were measured in between title compounds and target protein Lumazine synthase. In-silico method is a huge breakthrough in the expensive and lengthy process of drug design and development. With the aim to move for anti-tubercular activity of selected scaffolds, we next arrive at *in vitro* anti-tubercular screening of test compounds by Microplate Alamar Blue Assay (MABA) method. Anti-tubercular standard compounds used in the assay were Pyrazinamide and Streptomycin. The result was measured and reported in the form of minimum inhibitory concentration (MIC), can be defined as lowest concentration of test compound required to prevent color change from blue to pink.

Structures of all synthesized compounds were confirmed using ^{1}H NMR, ^{13}C NMR, FT-IR, mass spectroscopy and CHN analysis. Both the reactions reported (Scheme 1 and Scheme 2) are single step and one pot methods explaining reduction of energy, time and ultimately cost. MIC of tested compounds was stated in μg/ml demonstrated that number of the tested compounds showed good anti-tubercular activity as compared to standard drugs Pyrazinamide and Streptomycin. Comparison of output data of docking and MIC findings of in-vitro assay testing showed good results. Our aim is to propose (1*H*,3*H*) imidazo[4,5-*b*] pyridines as inhibitors of Lumazine synthase in *M. tuberculosis* could be potential lead compounds for the design of therapeutically useful antibiotics to eradicate multi drug resistant (MDR) and extensively drug resistant (XDR) strain of *M. tuberculosis*.

Materials and methods

All chemicals used were of research grade quality and purchased from S.D. Fine Chemicals Ltd., Mumbai, Loba Chemie, Bangalore. Melting points (°C) were determined using a Fischer-Jones melting point apparatus and are uncorrected. Microanalyses (CHNO and X=halogen) were performed at the microanalytical center, Pune University using Rapid analyser. Fourier Transform Infrared spectra (FT-IR, KBr cm^{-1}), were run on JASCO 401 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on BRUKER AVANCE II FT-NMR (400 MHz) using TMS as an internal standard (chemical shifts in δ, ppm), s=singlet, d=doublet, m=multiplet, bs=broad singlet. The relative integrals of peak areas agreed with those expected for the assigned structures. Mass spectra were recorded on WATERS Q-TOF Micromass (LC-MS), performed at SAIF, Punjab University, Chandigarh. TLC analysis was carried out on silica gel-protected aluminum sheets (Type 60 F 254, Merck) and the spots were detected under UV-Lamp at λ 254 nm.

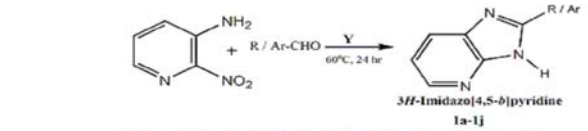
Experimental

The crystal structures of the target protein was obtained from Protein Data Bank and saved in standard 3D coordinate format. The protein Data Bank (PDB) is a repository for 3-D structural data of proteins and nucleic acids. This database provides the 3-D structure of all the proteins by NMR or by X-ray crystallography. After conducting adequate literature review lumazine synthase of *M. tuberculosis* (PDB entry code 2C92), was selected as the target for the present study. Ligand preparation was done by drawing the structures using ChemSketch 12 and Chem Draw Ultra 7 in 2D and saved as MDL Molfile format. Further conversion of ligands to 3D format using VLife Engine tools of Vlifemds 4.3. Protein visualization was done by loading the structure in SWISS PDB Viewer. Further the energy minimization was performed by Vlifemds 4.3 software. Docking score for all test ligand was reported in Table 1. Docking simulations were performed using Biopredicta tool in the grip docking mode. The number of docking run was set to 10. Different types of interactions were studied between docked 3D ligands and 3D macromolecule target. Standard compound Pyrazinamide (PYZ) was used for comparison of docking score and different types of interaction with the test ligands (Figure 5-9).

*General experimental procedure for the synthesis of compounds **1a-1k (Procedure A)***

Mixture of 2 nitro 3 amino pyridine (1.0 mmol) and various aldehydes (1.0 mmol) was prepared in DMF (4 mL). It was further treated with 1 M aqueous $Na_2S_2O_4$ (3.0 mmol, 3 mL) subjected for heating the reaction mixture at 60 ^{o}C for 24 h. The reaction mixture was filtered to remove unreacted $Na_2S_2O_4$ and filterate was cooled to room temperature. Excess solvent was removed by high vacuum distillation. The concentrated residue formed was

washed with water (2×15 mL) and dried under reduced pressure to afford the desired product in satisfactory purity. Further recrystallization was carried using ethanol. Purified compounds were subjected for melting point and reaction progress was monitored with TLC and respective chemical test.

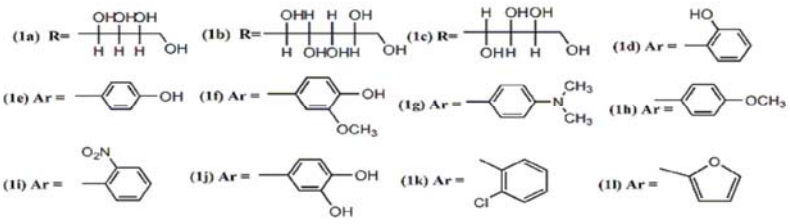


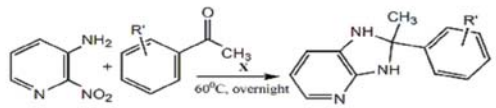
3*H*-Imidazo[4,5-*b*]pyridine

1a-1j

Scheme 1. Synthesis of substituted 3*H* imidazo[4,5-*b*]pyridines

Reagents and conditions: Y=3 equi. $Na_2S_2O_4$ (1 M in H_2O)/DMF





2-Methyl-2-phenyl-2,3-dihydro-1*H*-imidazo[4,5-*b*]pyridine

2a -2j

Scheme 2. Synthesis of substituted 1*H* imidazo[4,5-*b*]pyridines

Reagents and conditions: X=$SnCl_2.2H_2O$/EtOH/HCOOH

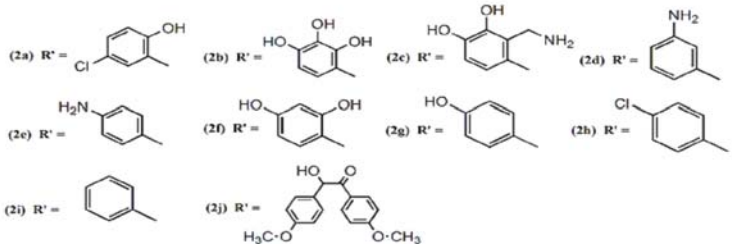


Figure 1. Scheme of synthesis of title compounds

Table 1. MolDock scores of various 3*H*-imidazo[4,5-*b*]pyridines (**1a-1k**) and 1*H*-imidazo[4,5-*b*]pyridines (**2a-2j**)

Ligand	Minimum Docking Score (**1a-1k**)	Ligand	Minimum Docking Score (**2a-2j**)
1a	-38.985672	**2a**	-37.808265
1b	-39.087022	**2b**	-37.596247
1c	-39.279918	**2c**	-38.243998
1d	-37.647217	**2d**	-36.600264
1e	-38.648783	**2e**	-37.390861
1f	-42.341387	**2f**	-37.593597
1g	-37.234782	**2g**	-38.955099
1h	-39.206669	**2h**	-37.595832
1i	-37.546072	**2i**	-37.190620
1j	-39.290139	**2j**	-42.708385
1k	-38.926303	**-**	**-**

1-(3H-Imidazo[4,5-b]pyridin-2-yl)-butane-1,2,3,4-tetraol ***(1a)***

This compound (**1a**) was obtained as pale yellow powder (3.5 g, 79.65%) according to the procedure **A**. M.p. 160-165 ^{o}C; FT-IR (KBr, cm^{-1}): 3600 (O-H$_{ali}$), 3350 (NH), 3250 (CH$_{arom}$), 2900 (CH$_{aliph}$), 1650 (C=N$_{arom}$), 1500 (C=C$_{arom}$), 1350 (C-C$_{ali}$), 1275 (C-N$_{arom}$), 1100 (C-O$_{aliphalco}$), 800 (bend CH$_{aliph}$), 770 (bend CH$_{arom}$). ^{1}H NMR (400MHz, CDCl$_3$): 13.5 (s, 1H), 7.89 (t, *J*=6.8 Hz, 3H), 3.78 (s, 4H), 2.0 (s, 4H).^{13}C NMR (400 MHz, CDCl$_3$): 154, 149, 138, 128, 116, 33. HRMS (ESI): calcd. for $C_{10}H_{13}N_3O_4$ $[M+H]^+$: 240.097; found 240.227. Analysis Calcd. for $C_{10}H_{13}N_3O_4$ (239.23); C, 49.06; H, 5.57; N, 15.59; O, 29.71%. Found: C, 49.06; H, 5.55; N, 15.59; O, 29.79%.

1-(3H-Imidazo[4,5-b]pyridin-2-yl)pentane-1,2,3,4,5-pentol ***(1b)***

This compound was obtained as gray solid (2.7 g, 89.58%) according to the procedure **A**. M. p. 145-150 ^{o}C. FT-IR (KBr, cm^{-1}): 3600 (O-H$_{ali}$), 3350 (N-H), 3250 (CH$_{arom}$), 2900 (CH $_{aliph}$), 1650 (C=N$_{arom}$), 1500 (C=C$_{arom}$), 1350 (C-C$_{aliph}$), 1275 (C-N$_{arom}$), 1100 (C-O$_{aliph\ alcoholic}$), 800 (bend CH$_{aliph}$), 770 (bend CH$_{arom}$). ^{1}HNMR (400MHz, CDCl$_3$): 13.5 (s, 1H), 7.87 (s, 3H), 3.79 (t, *J*=4.6Hz, 5H), 2 (t, *J*=3.8Hz, 5H). HRMS (ESI): calcd. for $C_{11}H_{15}N_3O_5$ $[M+H]^+$: 270.108447; found 270.118349. Analysis Calcd. for $C_{11}H_{15}N_3O_5$ (269.253); C, 50.20; H, 5.43; N, 17.55; O, 26.75%. Found: C, 50.22; H, 5.33; N, 17.54; O, 26.65%.

1-(3H-Imidazo[4,5-b]pyridin-2-yl)butane-1,2,3,4-tetrol ***(1c)***

This compound was obtained as gray solid (2.7 g, 79.65%) according to the procedure **A**. M. p. 150-154 ^{o}C. FT-IR (KBr, cm^{-1}): 3600 (O-H$_{aliph}$), 3350 (N-H), 3250 (C-H$_{arom}$), 2900 (C-H$_{aliph}$), 1650 (C=N$_{arom}$), 1500 (C=C$_{arom}$), 1350 (C-C$_{aliph}$), 1275 (C-N$_{arom}$), 1100 (C-O$_{aliph\ alcoholic}$), 800 (bend CH$_{aliph}$), 770 (bend CH$_{arom}$). ^{1}H NMR (400MHz, CDCl$_3$): 13.5 (s, 1H), 7.89 (t, *J*=6.8 Hz, 3H), 3.78 (s, 4H), 2.0 (s, 4H).^{13}C NMR (400 MHz, CDCl$_3$): 154, 149, 138, 128, 116, 33. HRMS (ESI): calcd. for $C_{10}H_{13}N_3O_4$ $[M+H]^+$: 240.097; found 239.227. Analysis Calcd.for $C_{10}H_{13}N_3O_4$(239.23); C, 49.06; H, 5.57; N, 15.59; O, 29.71%. Found: C, 49.06; H, 5.55; N, 15.59; O, 29.79%.

2-(3H-Imidazo[4,5-b]pyridin-2-yl)phenol ***(1d)***

This compound was obtained as faint yellow solid (1.8 g, 68.00%) according to the procedure **A,** M. p. 135-140 ^{o}C. IR (KBr, cm^{-1}): 3600 (OH), 3350 (NH), 3250 (CH$_{arom}$), 1650 (C=N$_{arom}$), 1500 (C=C$_{arom}$), 1275 (C-N$_{arom}$), 1250 (C-O$_{phenolic}$), 770 (bend CH$_{arom}$). ^{1}HNMR (400MHz, CDCl$_3$): 13.4 (s,1H), 7.8 (s,3H), 7.3 (s, 4H), 5.1 (s,1H). ^{13}C NMR (400 MHz, CDCl$_3$): 154, 149, 138, 128, 116, 33. HRMS (ESI): calcd. for $C_{12}H_9N_3O$ $[M+H]^+$: 212.08183; found 212.07346. Analysis Calcd.for $C_{12}H_9N_3O$ (211.21); C, 68.17; H, 4.26; N, 19.89; O, 5.57%. Found: C, 68.17; H, 4.24; N, 19.89; O, 5.56%.

4-(3H-Imidazo[4,5-b]pyridin-2-yl)phenol ***(1e)***

This compound was obtained as gray solid (2.0 g, 78.00%) according to the procedure **A**, M. p. 150-155 ^{o}C. IR (KBr, cm^{-1}): 3600 (OH), 3350 (NH), 3250 (CH$_{arom}$), 1650 (C=N$_{arom}$), 1500 (C=C$_{arom}$), 1275 (C-N$_{arom}$), 1250 (C-O$_{phenolic}$), 770 (bend CH$_{arom}$). ^{1}HNMR (400MHz, CDCl$_3$, in ppm): 13.4 (s,1H), 7.8 (s,3H), 7.3 (s, 4H), 5.1 (s,1H). ^{13}C NMR (400 MHz, CDCl$_3$): 154, 149, 138, 128, 116, 33. HRMS (ESI): calcd. for $C_{12}H_9N_3O$ $[M+H]^+$: 212.08183; found 212.07346. Analysis Calcd.for $C_{12}H_9N_3O$ (211.21); C, 68.17; H, 4.26; N, 19.89; O, 5.57%. Found: C, 68.17; H, 4.24; N, 19.89; O, 5.56%.

4-(3H-Imidazo[4,5-b]pyridin-2-yl)-2-methoxyphenol ***(1f)***

This compound was obtained as yellow solid (1.89 g, 70.65%) according to the procedure **A,** M. p. 148-150 ^{o}C. FT-IR (KBr, cm^{-1}): 3600 (OH), 3350 (NH), 3250 (CH_{arom}), 1650 ($C=N_{arom}$), 1500 ($C=C_{arom}$), 1275 ($C-N_{arom}$), 1250 ($C-O_{phenolic}$), 770 (bend CH_{arom}). ^{1}HNMR (400MHz, $CDCl_3$): 13.4 (s,1H), 7.8 (s,3H), 7.3 (s, 4H), 5.1 (s,1H). ^{13}C NMR (400 MHz, $CDCl_3$): 154, 149, 138, 128, 116, 33. HRMS (ESI): calcd. for $C_{12}H_9N_3O$ $[M+H]^+$: 212.08183; found 212.07346. Analysis Calcd.for $C_{12}H_9N_3O$ (211.21); C, 68.17; H, 4.26; N, 19.89; O, 5.57%. Found: C, 68.17; H, 4.24; N, 19.89; O, 5.56%.

4-(3H-Imidazo[4,5-b]pyridin-2-yl)-N,N-dimethylaniline ***(1g)***

This compound was obtained as yellow solid (1.5 g, 68.65%) according to the procedure **A,** M. p. 150-153 ^{o}C. FT-IR (KBr, cm^{-1}): 3600 (OH), 3350 (NH), 3250 (CH_{arom}), 1650 ($C=N_{arom}$), 1500 ($C=C_{arom}$), 1275 ($C-N_{arom}$), 1250 ($C-O_{phenolic}$), 770 (bend CH_{arom}). ^{1}HNMR (400MHz, $CDCl_3$, in ppm): 13.4 (s,1H), 7.8 (s,3H), 7.3 (s, 4H), 5.1 (s,1H). ^{13}C NMR (400 MHz, $CDCl_3$): 154, 149, 138, 128, 116, 33. HRMS (ESI): calcd. for $C_{12}H_9N_3O$ $[M+H]^+$: 212.08183; found 212.07346. Analysis Calcd.for $C_{12}H_9N_3O$ (211.21); C, 68.17; H, 4.26; N, 19.89; O, 5.57%. Found: C, 68.17; H, 4.24; N, 19.89; O, 5.56%.

2-(4-Methoxyphenyl)-3H-imidazo[4,5-b]pyridine ***(1h)***

This compound was obtained as gray solid (2.5 g, 82.5%) according to the procedure **A**, M. p. 189-193 ^{o}C. FT-IR (KBr, cm^{-1}): 3350 (NH), 3250 (CH_{arom}), 2850 (CH_3), 1500 ($C=C_{arom}$), 1650 ($C=N_{arom}$), 1375 (bend CH_3), 1275 ($C-N_{arom}$), 770 (bend CH_{arom}). ^{1}HNMR (400MHz, $CDCl_3$): 13.4 (s, 1H). 7.8 (s, 3H), 7.5 (s, 4H).^{13}C NMR (400 MHz, $CDCl_3$): 149.8, 123.6, 122.3, 138, 128, 56. HRMS (ESI): calcd. for $C_{13}H_{11}N_3O$ $[M+H]^+$: 226.097488; found 226.096565. Analysis Calcd. for $C_{13}H_{11}N_3O$ (225.24); C, 69.20; H, 4.9; N, 18.64; O, 7.10%. Found: C, 69.20; H, 4.91; N, 18.59; O, 7.11%.

2-(2-Nitrophenyl)-3H-imidazo[4,5-b]pyridine ***(1i)***

This compound was obtained as gray solid (1.25 g, 63.03%) according to the procedure **A,** M. p. 175-180 ^{o}C. FT-IR (KBr, cm^{-1}): 3600 (OH_{arom}), 3350 (NH), 3250 (CH_{arom}), 2850 (CH_3), 1650 ($C=N_{arom}$), 1500 ($C=C_{arom}$), 1475 (N=O), 1375 (bend CH_3), 1275 ($C-N_{arom}$), 1250 ($C-O_{phenolic}$), 770 (bend CH_{arom}). ^{1}H NMR (400MHz, $CDCl_3$): 13.4 (s, 1H) 7.7(s, 3H), 7.3 (s, 4H). ^{13}C NMR (400 MHz, $CDCl_3$): 149, 145, 135, 130, 117, 121.3. HRMS (ESI): calcd. for $C_{13}H_{11}N_3O$ $[M+H]^+$: 226.097488; found 226.096565. Analysis Calcd. for $C_{13}H_{11}N_3O$ (225.24); C, 69.20; H, 4.9; N, 18.64; O, 7.10%. Found: C, 69.20; H, 4.91; N, 18.59; O, 7.11%.

4-(3H-Imidazo[4,5-b]pyridin-2-yl)benzene-1,2-diol ***(1j)***

This compound was obtained as greenish brown solid (1.9 g, 85.55%) according to the procedure **A,** M. p. 180-185 ^{o}C. FT-IR (KBr, cm^{-1}): 3600 ($O-H_{phenol}$), 3350 (N-H), 3250 (CH_{arom}), 1650 ($C=N_{arom}$), 1500($C=C_{arom}$), 1350 ($C-C_{aliph}$), 1275 ($C-N_{arom}$), 1250 ($C-O_{phenol}$), 770 (bend CH_{arom}).^{1}H NMR (400MHz, $CDCl_3$): 13.4 (s,1H), 7.5 (t, 3H), 6.5 (t, 3H), 5.0 (s, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): 149.8, 123.6, 122.3, 138, 128, 56. HRMS (ESI): calcd. for $C_{12}H_9N_3O_2$ $[M+H]^+$: 228.076753; found 228.108912. Analysis Calcd. for $C_{12}H_9N_3O_2$ (227.21); C, 63.37; H, 3.96; N, 18.47; O, 14.08%. Found: C, 63.37; H, 3.96; N, 18.47; O, 14.08%.

2-(2-Chlorophenyl)-3H-imidazo[4,5-b]pyridine ***(1k)***

This compound was obtained as off-white solid (3.0 g, 98.22%) according to the procedure **A,** M. p. 169-172 ^{o}C. FT-IR (KBr, cm^{-1}): 3350 (N-H), 3250 (CH_{arom}), 1650 ($C=N_{arom}$), 1500

(C=C_{arom}), (C-NAr) 1275, 750 (C-Cl), 770 (bend CH_{arom}). ^{1}H NMR (400MHz, $CDCl_3$): 13.4 (s,1H), 7.7 (t, *J*=6.8Hz, 3H), 7.2 (m, 4H). ^{13}C NMR (400 MHz, $CDCl_3$): 149.8, 136.3, 135.7, 129.6, 128.3, 122.4. HRMS (ESI): calcd. for $C_{12}H_8ClN_3$ $[M+H]^+$: 230.66502; found 230.57092. Analysis Calcd. for $C_{12}H_8ClN_3$ (229.66); C, 62.88; H, 3.49; N, 18.34; O, 15.28%. Found: C, 62.88; H, 3.49; N, 18.34; O, 15.28%.

*General experimental procedure for the synthesis of compounds **2a-2j (Procedure B)***

In the present case, treatment of the *o*-nitroaniline and substituted acetophenones with $SnCl_2.H_2O$ in EtOH at 60 ^oC overnight produced exclusively 2,3 dihydrobenzimidazoles and further suggested for imidazo pyridines. This type of product has been reported previously by Tumelty *et al.*,[28] and is presumably formed via formylation of the aniline nitrogen, nitro reduction and cyclization. Formylation of the aniline nitrogen is believed to assist nitro reduction. This approach has provided the shortest solid phase synthesis of imidazo pyridines to date. Since a wide range of amines and aldehydes are commercially available, a large number of pyridoimidazoles can be easily prepared using this method.

*4-Chloro-2-(2-methyl-2,3-dihydro-1H-imidazo[4,5-b]pyridin-2-yl)phenol **(2a)***

This compound was obtained as gray solid (2.15 g, 78.11%) according to the procedure **B,** M. p. 219-223 ^oC. FT-IR (KBr, cm^{-1}): 3500 (O-H_{phenol}), 3350 (N-H), 3250 (CH_{arom}), 2850 (CH_3), 1650 (C=N_{arom}), 1456 (C=C_{arom}), 1275 (C-N_{arom}), 1250 (C-O_{phenol}), 800 (bend CH_{aliph}), 770 (bend CH_{arom}), 750 (C-Cl). ^{1}HNMR (400MHz, $CDCl_3$): 7.7 (m, 4H), 7.0 (m, 3H), 5 (s, 1H, OH), 4 (s, 2H, NH), 1.5 (s, 3H, CH_3), ^{13}C NMR (400 MHz, $CDCl_3$): 150, 135, 130, 123, 65, 35. HRMS (ESI): calcd. for $C_{13}H_{12}ClN_3O$ $[M+H]^+$: 262.074166; found 262.04697. Analysis Calcd. for $C_{13}H_{12}ClN_3O$ (261.70); C, 59.61; H, 4.58; N, 16.04; O, 6.11; Cl, 13.37%. Found: C, 59.61; H, 4.58; N, 16.04; O, 6.11; Cl, 13.37%.

*4-(2-Methyl-2,3-dihydro-1H-imidazo[4,5-b]pyridin-2-yl)benzene-1,2,3-triol **(2b)***

This compound was obtained as gray solid (1.25 g, 67.89%) according to the procedure **B**, M. p. 167-170 ^oC. FT-IR (KBr, cm^{-1}): 3600 (O-H_{aliph}), 3350 (N-H), 3250 (CH_{arom}), 2900 (CH_{aliph}), 1650 (C=N_{arom}), 1500 (C=C_{arom}), 1350 (C-C_{aliph}), 1275 (C-N_{arom}), 1100 (C-$O_{aliph\ alcoholic}$), 800 (bend CH_{aliph}), 770 (bend CH_{arom}). ^{1}H NMR (400MHz, $CDCl_3$): 13.5 (s, 1H), 7.87 (s, 3H), 3.79 (t, 5H), 2 (t, *J*=3.8Hz, 5H).^{13}C NMR (400 MHz, $CDsCl_3$): 149, 144, 139, 130, 123, 113, 65, 33. HRMS (ESI): calcd. for $C_{13}H_{13}N_3O_3$ $[M+H]^+$: 260.102968; found 262.14397. Analysis Calcd.for $C_{13}H_{13}N_3O_3$ (259.26); C, 60.17; H, 6.17; N, 16.19; O, 18.51%. Found: C, 60.17; H, 6.18; N, 16.20; O, 18.52%.

*3-(aminomethyl)-4-(2-methyl-2,3-dihydro-1H-imidazo[4,5-b]pyridin-2-l)benzene-1,2-diol **(2c)***

This compound was obtained as pale yellow solid (1.89 g, 73.00%) according to the procedure **B**, M. p. 211-214 ^oC. FT-IR (KBr, cm^{-1}): 3500 (O-$H_{phenolic}$), 3350 (N-H_{arom}), 3300 (N-H_{aliph}), 3250 (CH_{arom}), 2850 (CH_3), 2700 (CH_2), 1650 (C=N_{arom}), 1456 (C=C_{arom}), 1275 (C-N_{arom}), 1250 (C-$O_{phenolic}$), 1100 (C-N_{aliph}), 800 (bend CH_{aliph}), 770 (bend CH_{arom}). ^{1}HNMR (400MHz, $CDCl_3$): 7.5 (m, 3H), 6.5 (m, 4H), 4.0 (s, 2H, NH), 1.5 (s, 3H, CH3). ^{13}C NMR (400 MHz, $CDsCl_3$): 149, 142, 138, 135, 130, 123, 114, 33, 31. HRMS (ESI): calcd. for $C_{14}H_{16}N_4O_2$ $[M+H]^+$: 273.134602; found 273.144312. Analysis Calcd. for $C_{14}H_{16}N_4O_2$ (272.30); C, 61.69; H, 5.87; N, 20.56; O, 11.75%. Found: C, 61.69; H, 5.87; N, 20.55; O, 11.72%.

3-(2-Methyl-2,3-dihydro-1H-imidazo[4,5-b]pyridin-2-yl)aniline ***(2d)***

This compound was obtained as yellow solid (1.50 g, 77.00%) according to the procedure **B**, M. p. 224-228 ^{o}C. FT-IR (KBr, cm^{-1}): 3450 (N-H), 3350 (N-H$_{arom}$), 3250 (CH$_{arom}$), 2850 (CH$_3$), 1650 C=N$_{arom}$), 1456 (C=C$_{arom}$), 1275 (C-N$_{arom}$), 770 (bend CH$_{arom}$). ^{1}H NMR (400MHz, CDCl$_3$): 7.5 (m, 3H), 6.5 (m, 4H), 4.0 (s, 2H, NH), 1.5 (s, 3H, CH3). ^{13}C NMR (400 MHz, CDCl$_3$): 149, 143, 138, 135, 123, 113, 117, 74, 32. HRMS (ESI): calcd. for $C_{13}H_{14}N_4$ $[M+H]^+$: 227.129123; found 227.11813. Analysis Calcd. for $C_{13}H_{14}N_{42}$ (226.27); C, 68.4; H, 6.18; N, 24.74%. Found: C, 68.4; H, 6.18; N, 24.74%.

4-(2-Methyl-2,3-dihydro-1H-imidazo[4,5-b]pyridin-2-yl)aniline ***(2e)***

This compound was obtained as gray solid (1.25 g, 67.89%) according to the procedure **B**, M. p. 220-223 ^{o}C. FT-IR (KBr, cm^{-1}): 3450 (N-H), 3350 (N-H$_{arom}$), 3250 (CH$_{arom}$), 2850 (CH$_3$), 1650 C=N$_{arom}$), 1456 (C=C$_{arom}$), 1275 (C-N$_{arom}$), 770 (bend CH$_{arom}$). ^{1}H NMR (400MHz, CDCl$_3$): 7.5 (m, 3H), 6.5 (m, 4H), 4.0 (s, 2H, NH), 1.5 (s, 3H, CH3). ^{13}C NMR (400 MHz, CDCl$_3$): 149, 143, 138, 135, 123, 113, 117, 74, 32. HRMS (ESI): calcd. for $C_{13}H_{14}N_4$ $[M+H]^+$: 227.129123; found 227.11813. Analysis Calcd.for $C_{13}H_{14}N_{42}$ (226.27); C, 68.4; H, 6.18; N, 24.74%. Found: C, 68.4; H, 6.18; N, 24.74%.

4-(2-Methyl-2,3-dihydro-1H-imidazo[4,5-b]pyridin-2-yl)benzene-1,3-diol ***(2f)***

This compound was obtained as pale yellow solid (1.39 g, 77.00%) according to the procedure **B**, M. p. 238-240 ^{o}C. FT-IR (KBr, cm^{-1}): 3500 (OH$_{phenol}$), 3350 (N-H), 3250 (CH$_{arom}$), 2850 (CH$_3$), 1650 (C=N$_{arom}$), 1456 (C=C$_{arom}$), 1275 (C-N$_{arom}$), 1250 (C-O$_{phenol}$), 800 (bend CH$_{aliph}$), 770 (bend CH$_{arom}$). ^{1}HNMR (400MHz, CDCl$_3$): 7.5 (m, 3H), 6.9 (m, 3H), 5.0 (s, 1H, OH), 4.0 (s, 2H, NH), 1.5 (s, 3H, CH$_3$). ^{13}C NMR (400 MHz, CDCl$_3$): 157, 149, 138, 130, 122, 108, 102, 64, 31. HRMS (ESI): calcd. for $C_{13}H_{13}N_3O_2$ $[M+H]^+$: 244.108053; found 244.117458. Analysis Calcd. for $C_{13}H_{13}N_3O_2$ (243.26); C, 64.12; H, 5.34; N, 17.26; O, 13.15%. Found: C, 64.12; H, 5.33; N, 17.24; O, 13.16%.

4-(2-Methyl-2,3-dihydro-1H-imidazo[4,5-b]pyridin-2-yl)phenol ***(2g)***

This compound was obtained as yellow solid (1.89 g, 82.56%) according to the procedure **B**, M. p. 230-234^{0}C. FT-IR (KBr, cm^{-1}): 3500 (O-H$_{phenol}$), 3350 (N-H), 3250 (CH$_{arom}$), 1650 (C=N$_{arom}$), 1456 (C=C$_{arom}$), 1275 (C-N$_{arom}$), 1250 (C-O$_{phenol}$), 800 (bend CH$_{aliph}$), 770 (bend CH$_{arom}$), ^{1}HNMR (400MHz, CDCl$_3$): 7.5(m,3H), 6.9 (m, 4H), 5.0 (s, 1H, OH), 4.0 (s, 2H, NH), 1.5(s,3H,CH3). ^{13}C NMR (400 MHz, CDCl$_3$): 155, 149, 138, 135, 130, 123, 115, 74, 32. HRMS (ESI): calcd. for $C_{13}H_{13}N_3O$ $[M+H]^+$: 228.113138; found 228.113458. Analysis Calcd. for $C_{13}H_{13}N_3O$ (227.26); C, 68.64; H, 5.72; N, 18.48; O, 7.04%. Found: C, 68.64; H, 5.72; N, 18.48; O, 7.04%.

2-(4-Chlorophenyl)-2-methyl-2,3-dihydro-1H-imidazo[4,5-b]pyridine ***(2h)***

This compound was obtained as fine yellow solid (1.69 g, 89.89%) according to the procedure **B**, M. p. 215-218^{0}C. FT-IR (KBr, cm^{-1}): 3350 (N-H), 3250 (CH$_{arom}$), 2850 (CH$_3$), 1650 (C=N$_{arom}$), 1456 (C=C$_{arom}$), C-N Ar 1275, 800 (bend, CH$_{aliph}$), 770 (bend CH$_{arom}$), 750 (C-Cl). ^{1}HNMR (400MHz, CDCl$_3$): 7.25 (m, 3H), 6.9 (m, 3H), 4.0 (s, 2H, NH), 1.5 (s, 3H, CH3). ^{13}C NMR (400 MHz, CDCl$_3$): 149, 140, 138, 130, 123, 128, 113, 74, 32. HRMS (ESI): calcd. for $C_{13}H_{12}ClN_3$ $[M+H]^+$: 246.079252; found 246.085552. Analysis Calcd. for $C_{13}H_{12}ClN_3$ (245.70); C, 63.49; H, 4.88; N, 17.09; Cl, 14.24%. Found: C, 63.49; H, 4.88; N, 17.09; Cl, 14.24%.

2-Methyl-2-phenyl-2,3-dihydro-1H-imidazo[4,5-b]pyridine ***(2i)***

This compound was obtained as yellow solid (1.35 g, 75.99%) according to the procedure **B**, M. p. 119-124 ^{o}C. FT-IR (KBr, cm^{-1}): 3350 (N-H), 3250 (CH_{arom}), 2850 (CH_3), 1650 ($C=N_{arom}$), 1456 ($C=C_{arom}$), 1275 ($C-N_{arom}$), 800 (bend CH_{aliph}), 770 (bend CH_{arom}). ^{1}HNMR (400MHz, $CDCl_3$): 7.2 (m, 5H), 7.0 (m, 3H), 4.0 (s, 2H, NH), 1.5 (s, 3H, CH3). ^{13}C NMR (400 MHz, $CDCl_3$): 149, 142, 138, 130, 128, 123, 74, 32. HRMS (ESI): calcd. for $C_{13}H_{13}N_3$ $[M+H]^+$: 212.118224; found 212.109232. Analysis Calcd. for $C_{13}H_{13}N_3$ (211.26); C, 73.84; H, 6.15; N, 19.88%. Found: C, 73.84; H, 6.15; N, 19.88%.

(4-Methoxyphenyl)[2-(4-methoxyphenyl)-2,3-dihydro-1H-imidazo[4,5-b]pyridine-2-yl]methanol ***(2j)***

This compound was obtained as white solid (1.39 g, 69.89%) according to the procedure **B**, M. p. 250-254 ^{o}C. FT-IR (KBr, cm^{-1}): 3600 (O-H), 3350 (N-H), 3250 (CH_{arom}), 2850 (CH_3), 1650 ($C=N_{arom}$), 1456 ($C=C_{arom}$), 1290 (C-O), 1275 (C-Narom), 800 (bend CH_{aliph}), 770 (bend CH_{arom}). ^{1}H NMR (400MHz, $CDCl_3$): 7.2 (m, 3H), 6.9 (m, 4H), 5.3 (s, 1H, CH_{aliph}), 4.0 (s, 2H, NH), 2.0 (s, 1H, OH), 3.5 (s, 6H). ^{13}C NMR (400 MHz, $CDCl_3$): 160, 128, 123, 115, 90, 87, 57. HRMS (ESI): calcd. for $C_{21}H_{21}N_3O_3$ $[M+H]^+$: 364.165568; found 364.155549. Analysis Calcd. for $C_{21}H_{21}N_3O_3$ (363.40); C, 69.34; H, 5.77; N, 11.55; O, 13.20%. Found: C, 69.34; H, 5.77; N, 11.55; O, 13.20%.

***In vitro* anti-tubercular screening studies**

Microplate alamar blue assay method

The anti-tubercular activity of the newly synthesized compounds was evaluated by micro plate Alamar Blue assay method (MABA)[29]. on bacterial strain *M. tuberculosis* H37 Rv ATCC (American Type Culture Collection), inoculums was grown on 100 mL of Middle brook 7H9 broth (Difco, Detroit Mich.) supplemented with 0.2% (v/v) glycerol, 10% (v/v) OADC (Oleic acid, albumin, dextrose, catalase, Difco) and 0.5% (v/v) Tween 80. The complete medium referred to as 7H9GC-T80. The Anti-TB susceptibility testing was performed in black, clear bottomed, 96-well microplates in order to minimize background fluorescence. Initial drug dilution was prepared in dimethyl sulfoxide and subsequent two fold dilutions were performed in 0.1 mL of 7H12 media in the micro plates.

The H37RV was diluted in 7H9 media to reach approximately $2x10^5$cfu/mL and 0.1 mL was added to wells. Wells containing compounds only were used to detect auto fluorescence of the compounds and were incubated at 37 ^{o}C. At day 7 of incubation, 20 µL of Alamar blue solution and 12.5 mL of 20% Tween 80 were added to all the wells and the plates were re-incubated at 37 ^{o}C for 24 h. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. The pyrazinamide and streptomycin were employed as standard drugs for the comparison of antitubercular activity and the results were tabulated in the Table 2, 3.

Results and Discussion

In-silico docking experiment

An attempt for in silico design of title compounds aiming to inhibit lumazine synthase. Binding interaction of test motifs studied using crystal protein lumazine synthase from *Mycobacterium tuberculosis* (PDB 2C92) in complex with reference ligand Pyrazinamide (PYZ). With the aim of rationalizing the anti-tubercular activity, docking study was performed

for the test ligand imidazo[4,5-*b*] pyridine **1a-1k** and **2a-2j**. Docking score for individual test ligand was reported in Table 1. Possible binding interactions with target protein were studied (Figure 5-9) and compared with the reference ligand PYZ. Best fit obtained from the group of test ligand was further studied for different types of binding interaction with the target protein.

Binding interactions of reference ligand PYZ were showed in Figure 2-4 for validation of docking protocol and confirmation of the biological data. PYZ showing *H*-bonding with Asp95C (2.499A^0) along with various Vander Waal's interaction. Types of interaction between the enzyme's active site and test compounds **1f** and **2b** showed in Figure 5-9. Test ligand (**1f**) showed Hydrophobic interaction with ASP91B (4.777A^0) along with Vander Waal's interaction, whereas test ligand (**2b**) showed *H*-bonding with GLN99B (2.331A^0) and ASP95E (2.216A^0) along with Vander Waal's interaction. Different types of interactions studied for the test ligands provide some important SAR points supporting possible interactions observed in the docking protocol suggested in Figure 10.

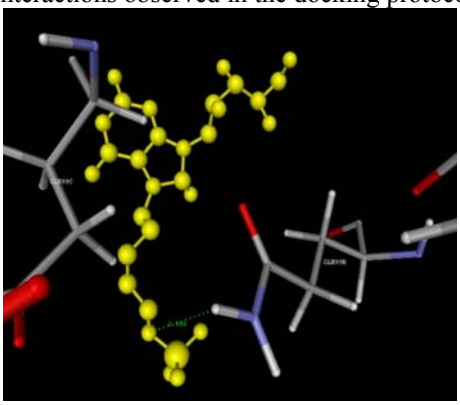


Figure 2. PYZ showing H-bond with GLN99B (2.586A^0)

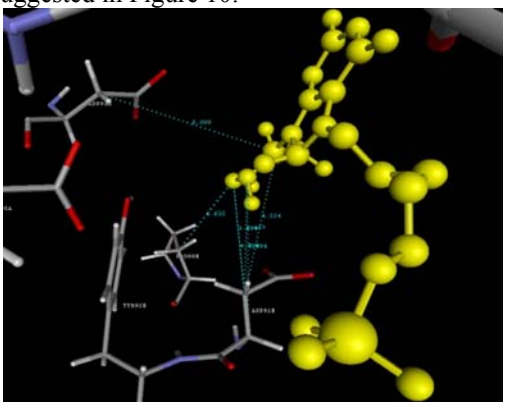


Figure 3. PYZ showing hydrophobic bond with ASP91A, PRO88E, ASP91E, ASP91E, ASP91E, ASP91E, ASP91E

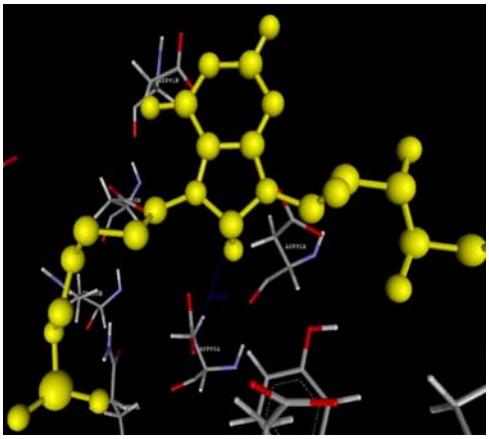


Figure 4. PYZ showing charge interaction with ASP95A (4.420A^0)

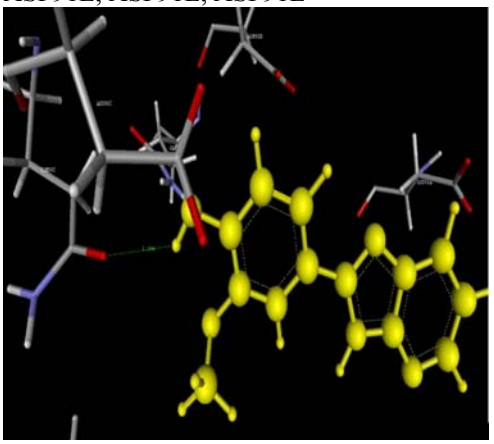


Figure 5. Ligand 4-(3*H*-imidazo[4,5-b] pyridin-2-yl)-2-methoxyphenol **(1f)** shows H-bond with GLN99C (2.304 A^0)

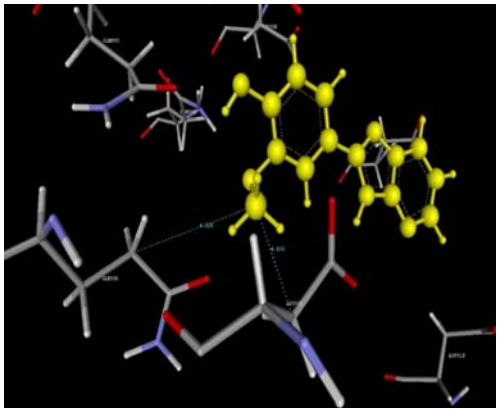


Figure 6. Ligand 4-(3*H*-imidazo[4,5-b]pyridin-2-yl)-2-methoxyphenol **(1f)** showing hydrophobic interaction with ASP95D, GLN99D

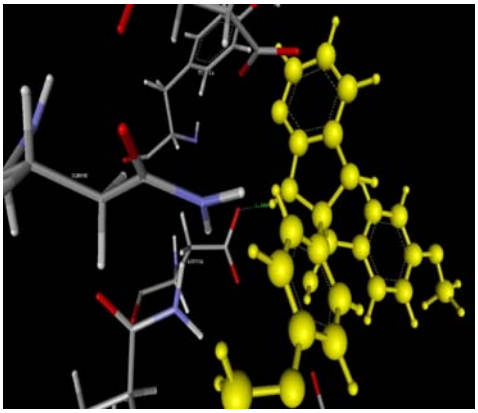


Figure 7. Ligand 4-methoxyphenyl)[2-(4-methoxyphenyl)-2,3-dihydro-1*H*-imidazo [4,5-b]pyridine-2yl]methanol **(2j)** showing H-bond with ASP95A (1.560 A^0)

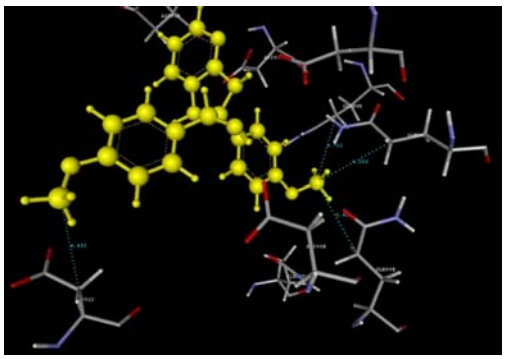


Figure 8. Ligand 4-methoxyphenyl)[2-(4-methoxyphenyl)-2,3-dihydro-1*H*-imidazo [4,5-b]pyridine-2yl]methanol **(2j)** showing hydrophobic binding with GLN99A, GLN99B, ASP91D, GLN99E

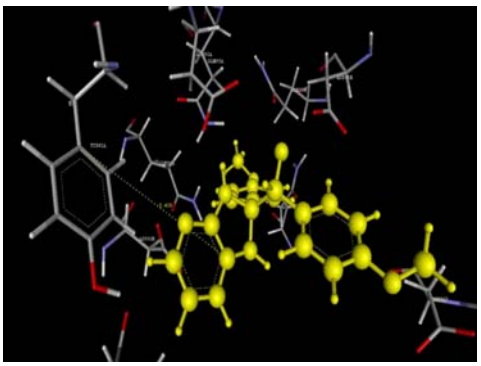


Figure 9. Ligand 4-methoxyphenyl)[2-(4-methoxyphenyl)-2,3-dihydro-1*H*-imidazo [4,5-b]pyridine-2yl]methanol **(2j)** showing Pi-stacking bonding with TYR92A (5.438A^0)

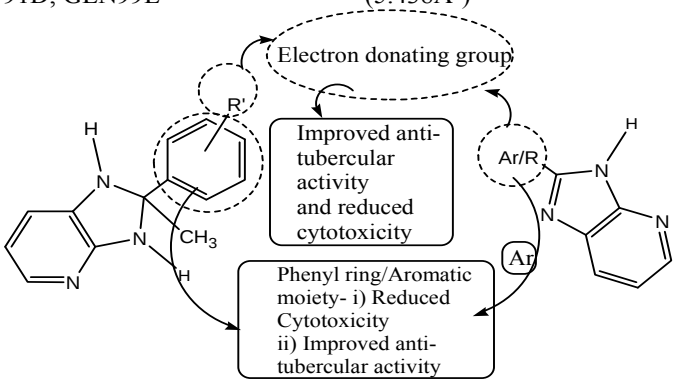


Figure 10. Possible structural features for anti-TB activity

Chemistry

Previously one step method was reported for benzimidazole synthesis from o-nitro aniline and substituted aldehydes[30]. In the present one step synthesis of 3*H* imidazo[4,5-*b*] pyridines **(1a-1k)** successful attempt was made from different substituted aldehydes and 2-nitro-3-amino pyridine via reductive cyclisation using $Na_2S_2O_4$. Aqueous paste of $Na_2S_2O_4$ (1 M) was prepared in H_2O and added in 3 equivalent proportion to the reaction mixture (*cf*. Figure 1, Scheme 1).

Second reaction for one step synthesis of 1*H* imidazo[4,5-*b*] pyridine **(2a-2j)** was obtained from different substituted ketones and 2-nitro-3-amino pyridine via reductive cyclization using $SnCl_2.2H_2O$ as reductive catalyst **(***cf*. Figure 1, Scheme 2). This type of approach has been reported previously for obtaining benzimidazole motifs[31]. Imidazo pyridine scaffolds were visited after treatment of the substituted acetophenones and 2-nitro-3-amino pyridine with addition of $SnCl_2.H_2O$ in presence of formic acid. It is presumably formed via formylation of the aniline nitrogen, nitro reduction and cyclization. Formylation of the aniline nitrogen is believed to assist nitro reduction, In summary, we have demonstrated that imidazo pyridines can be efficiently prepared from a support-bound 2 nitro 3 amino pyridine as like the same way benzimidazoles reported using a 'one-pot' reduction–cyclisation method. Both reactions were clear and without any form of side products or byproducts as impurities.

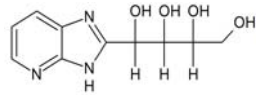
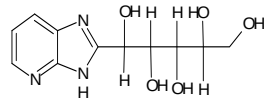
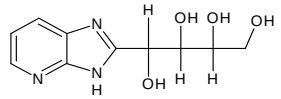
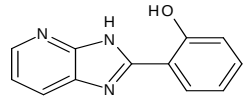
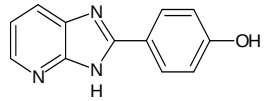
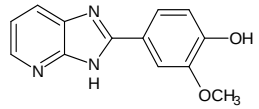
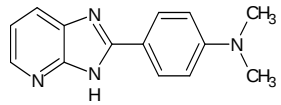
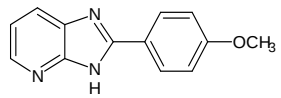
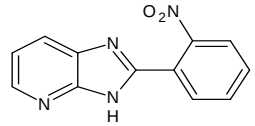
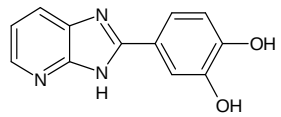
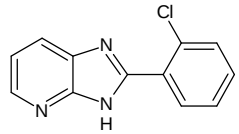
In vitro anti-tubercular screening

Alamar blue is an oxidation reduction dye used for screening of anti tubercular activity. The original oxidized form of alamar blue turns in to pink color upon reduction. Since *M. tuberculosis* is an aerobic organism during its growth turns alamar blue to pink. This technique has been used to predict the growth rise or inhibition of *M. tuberculosis* for testing anti-mycobacterial agents. Pink color indicates the presence of growth and blue color indicates the absence of growth[32-34].

In vitro anti-mycobacterial testing was carried against *M. tuberculosis* (H37Rv). Results of anti-tubercular testing (MIC) were given in Table 2 and 3 indicated that varying degree of anti-mycobacterial activity was observed well interconnected with the structural variations of the synthesized compounds. Pyrazinamide and Streptomycin were the standard compounds used for *in vitro* testing of activity. However all the title compounds showed positive activity against *M. tuberculosis* (H37Rv) ranging from 3.125 to 25 µg/mL and predicted in terms of µM/mL x 10^{-3}. Effect of various substituents of phenyl ring attached at 2^{nd} position of 1*H* imidazo[4,5-b]pyridine nucleus and nature of aliphatic and aromatic substituents attached at 2^{nd} position of 3*H* imidazo[4,5-b]pyridine nucleus have showed influence on activity. Descending order of activity was found to be as **1a, 1b, 1c, 1j, 2a, 2c** and **2f** (MIC=3.12 µg/mL) > **1d, 1e, 1g, 2a, 2e** and **2j** (MIC=6.25 µg/mL) > **1f, 1h, 2d, 2g** and **2i** (MIC=12.50 µg/mL) > **1i, 1k** and **2h** (MIC=25.00 µg/mL). Whereas MIC of Pyrazinamide observed was 3.125 µg/mL and for Streptomycin was 6.25 µg/mL. From the MIC output data of the anti-mycobacterial activity it was observed that compounds **1a, 1b, 1c, 1j, 2a, 2c** and **2f** were extremely active with observed MIC (3.125 µg/mL) equivalent to the standard drug Pyrazinamide(PYZ). These compounds could be selected as lead compounds and explored further to develop anti-mycobacterial agents.

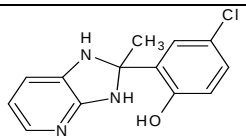
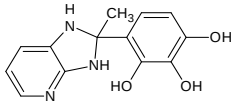
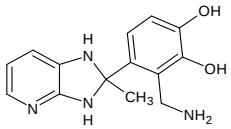
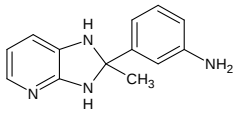
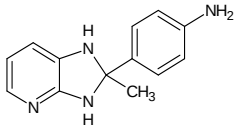
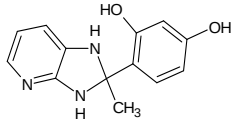
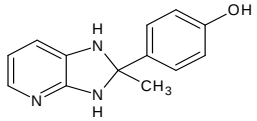
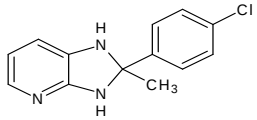
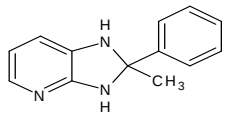
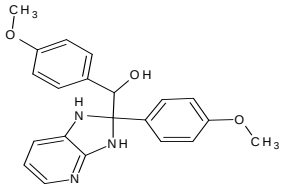
Structural requirements for producing anti-mycobacterial activity is illustrated in Figure 10. From the observed anti-tubercular activity of synthesized compounds 1*H* imidazo[4,5-b]pyridines and 3*H* imidazo[4,5-b]pyridines following SAR could be explained.

Table 2. Minimum inhibitory concentration (μg/mL)[a] of title compounds (**1a-1k**).

Compound Code	Compound	Molecular Formula (Molecular Weight)	*Mycobacterium tuberculosis* (MIC)[a]	
			μg/mL	μM/mLx10^{-3}
1a		$C_{10}H_{13}N_3O_4$ (239.23)	3.125	13.06x10^{-3}
1b		$C_{11}H_{15}N_3O_5$ (269.253)	3.125	11.60x10^{-3}
1c		$C_{10}H_{13}N_3O_4$ (239.227)	3.125	13.06x10^{-3}
1d		$C_{12}H_9N_3O$ (211.219)	6.25	29.59x10^{-3}
1e		$C_{12}H_9N_3O$ (211.219)	6.25	29.59x10^{-3}
1f		$C_{13}H_{11}N_3O_2$ (241.243)	12.50	51.80x10^{-3}
1g		$C_{14}H_{14}N_4$ (238.287)	6.25	26.29x10^{-3}
1h		$C_{13}H_{11}N_3O$ (225.245)	12.50	55.49x10^{-3}
1i		$C_{12}H_8N_4O_2$ (240.217)	25.00	10.4x10^{-3}
1j		$C_{12}H_9N_3O_2$ (227.218)	3.125	13.75x10^{-3}
1k		$C_{12}H_8N_3Cl$ (229.66)	25.00	10.8x10^{-3}
G	Pyrazinamide (PYZ)	-	3.125	-
H	Streptomycin (STC)	-	6.25	-

[a]*The lowest concentration of compound required to prevent color change from blue to pink*

Table 3. Minimum inhibitory concentration (μg/mL)[a] of title compounds **2a-2j**

Compound Code	Compound	Molecular Formula (Molecular Weight)	*Mycobacterium tuberculosis* (MIC)[a]	
			μg/mL	μM/mLx10^{-3}
2a		$C_{13}H_{12}ClN_3O$ 261.706	6.25	23.88x10^{-3}
2b		$C_{13}H_{13}N_3O_3$ 259.260	3.125	12.05x10^{-3}
2c		$C_{14}H_{16}N_4O_2$ 272.302	3.125	11.47x10^{-3}
2d		$C_{13}H_{14}N_4$ 226.277	12.50	55.24x10^{-3}
2e		$C_{13}H_{14}N_4$ 226.277	6.25	27.62x10^{-3}
2f		$C_{13}H_{13}N_3O_2$ 243.261	3.125	12.84x10^{-3}
2g		$C_{13}H_{13}N_3O$ 227.261	12.50	55.00x10^{-3}
2h		$C_{13}H_{12}ClN_3$ 245.707	25.00	10.70x10^{-3}
2i		$C_{13}H_{13}N_3$ 211.262	12.50	59.16x10^{-3}
2j		$C_{21}H_{21}N_3O_3$ 363.409	6.25	17.19x10^{-3}
G	Pyrazinamide (PYZ)	-	3.125	-
H	Streptomycin (STC)	-	6.25	-

[a]*The lowest concentration of test compound required to prevent color change from blue to pink*

Structure activity relationship for Anti-TB activity

Compounds with electron donating groups like OH, NH_2 showed potent activity. However such compounds with reduced lipophilicty, more polarizibility and greater electron donating power has reduced cytotoxicity and improved anti-tubercular activity. Compounds having to possess electron withdrawing groups like halogen (Cl) attached to the phenyl ring substituted at 2nd position of imidazo[4,5-b]pyridine nucleus has comparative reduced activity.

Conclusion

In conclusion, we have synthesized series of 1*H* imidazo [4,5-b]pyridine and 3*H* imidazo[4,5-b]pyridine analogues with aim to screen *in vitro* anti-tubercular activity against *M. tuberculosis* (H37RV). Pyrazinamide and streptomycin were the standard drugs used in the activity. All the compounds tested showed positive response for anti-tubercular activity. Among the compounds tested **1a, 1b, 1c, 1j, 2a, 2c** and **2f** were found equally active to that of standard drug Pyrazinamide (MIC=3.12 µg/mL) and greater active than standard drug Streptomycin (MIC=6.25 µg/mL). Activity of these compounds may be because electron donating functional groups in their structure. Such compounds could be selected as promising leads to the standard drugs available against *M. tuberculosis*. Hopefully, series of synthesized title compounds would be a unique source for development of newer drugs in the treatment of multi drug resistant (MDR) and extensively drug resistant infection caused by *M. tuberculosis.*

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