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Design, Synthesis and *In Vitro* Evaluation of Aryl Amides as Potent Inhibitors against *Mycobacterium Tuberculosis*

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Abstract:
A series of new aryl amides were synthesized in order to develop small molecules as new lead for anti-tubercular agents. The titled compounds synthesized were achieved by the reactions of aryl acid chlorides with appropriate aryl amines/cyclic secondary amines and characterized by IR, ^{1}HNMR, ^{13}CNMR and LCMS studies. *In vitro* antibacterial and anti-tubercular activities of the synthesized compounds were determined by two-fold serial dilution technique against *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922) and MABA method against *Mtb* H_{37}Rv strain respectively. The anti-tubercular activity data indicated that tested compounds exhibited moderate activity. Among them, compounds **4d** and **4e** have shown MIC of 6.25 µg/mL while compounds **3a**, **4b**, **4c** have showed a MIC value of 12.5 µg/mL against *Mtb* H_{37}Rv strain. Compounds **3a** and **3c** have shown potential antibacterial activity (MIC value of 6.25 µg/mL), while compounds **3d**, **3f** and **3i** have shown better activity with MIC value of 12.5 µg/mL against all the tested microorganisms. All the synthesized compounds showed good safety profiles against Vero and HepG2 cells. The docking studies on InhA enzyme revealed that the synthesized molecules have similar interactions as that of co-crystallized ligand with TYR-158 and NAD^{+}. Hence, further detailed investigation required on these molecules to develop a good lead molecule.

Keywords: Aryl Amides, InhA, MABA, MTT Assay, *Mycobacterium tuberculosis*.

INTRODUCTION:

Mycobacterium tuberculosis (*Mtb*) is a key microorganism responsible for tuberculosis (TB), a deadly disease affecting worldwide resurrection [1,2]. Several factors may be responsible for the elevation of infection rate like infection with Human Immunodeficiency virus (HIV) which are changing the socio-economic circumstances and wane the tuberculosis control programmes [3]. Despite the modern chemotherapy, globally TB remains the principal infectious disease, largely owing to the perseverance of the tubercle bacillus and the ineffectiveness of the current chemotherapy. According to the WHO report 2018, around 300,000 patients were died suffering from TB associated with HIV+ as compared to the patients suffering from TB associated with HIV-which is nearly double the HIV negative patients [4-7].

Many studies were reported on mycobacterium cell wall inhibition and in particular mycolic acid biosynthesis (FAS-II) which is the essential structural component of mycobacterium cell wall, as it itself generates its precursors which are the rich sources of antibacterial targets. *Mtb* has two types of fatty acid synthase (FAS) systems, FAS-I [8] is responsible for the *de-novo* synthesis of C_{16} - C_{26} fatty acids and FAS-II is extends the fatty acids up to C_{56} chains in order to make the precursors of mycolic acids. Enoyl-ACP reductase one of the enzymes involved in the synthesis of mycobacterium cell wall which, catalyses the NADH specific reduction of a *trans* carbon-carbon double bond to produce saturated acyl-ACP. Earlier studies as well as recent advancement validated and reported that, *InhA* is one of the key targets for both frontline and second line anti-tubercular drugs [9-14]. Therefore, the inhibition of *InhA* disrupts the biosynthesis of the mycolic acids.

INH as a prodrug, must be first activated by the mycobacterial catalase-peroxidase *KatG* into its active form of acyl radical which functions as a potent *InhA* inhibitor by forming a covalent bond between *InhA* co-substrate NADH, or its oxidation product NAD^{+}. Similarly, ETA is activated by a flavoprotein monooxyganase (*EtaA*), rather by *KatG* and it forms NAD^{+}-ETA adduct thereby it act as an effective *InhA* inhibitor. From the past 40 years INH has been widely applied in the treatment of tuberculosis and recently clinical studies showed, *KatG*- or *EtaA*- associated mutations are responsible for these prodrug (INH & ETA)-resistant clinical isolates [15,16]. As these reported *InhA* inhibitors required to be activated to show their inhibitory activity, therefore, there is a need of developing newer inhibitors without a prior activation towards the ever-increasing threat from drug resistant *Mtb* strains.

Amides refereed to the conjugate base of ammonia or of an organic amine, amide linkage referred to a defining molecular feature of proteins and in a biochemical context they are called as peptide bonds. Many drugs are amides including paracetamol, penicillin and *N*-alkylamides have shown wide range of biological activities [17-20] in particular antitubercular potentials [21]. To address the problems of ever-increasing resistance, serious side effects of some anti-TB drugs, long term treatment and incompatibility of antiretroviral therapies for current TB regimen made the researcher to develop novel anti-TB agents with stronger efficacy have become an utmost priority.

In view of above observations and continuation of our research in developing a new series of *InhA* inhibitors [22-24], herein we reported the design, synthesis and evaluation of aryl amide analogues as antitubercular candidates as shown in figure **1**. To sustain the typical *InhA* molecular interaction at the receptor binding site, structure-based drug design technique was used to explore the structural alternates of aryl amides.

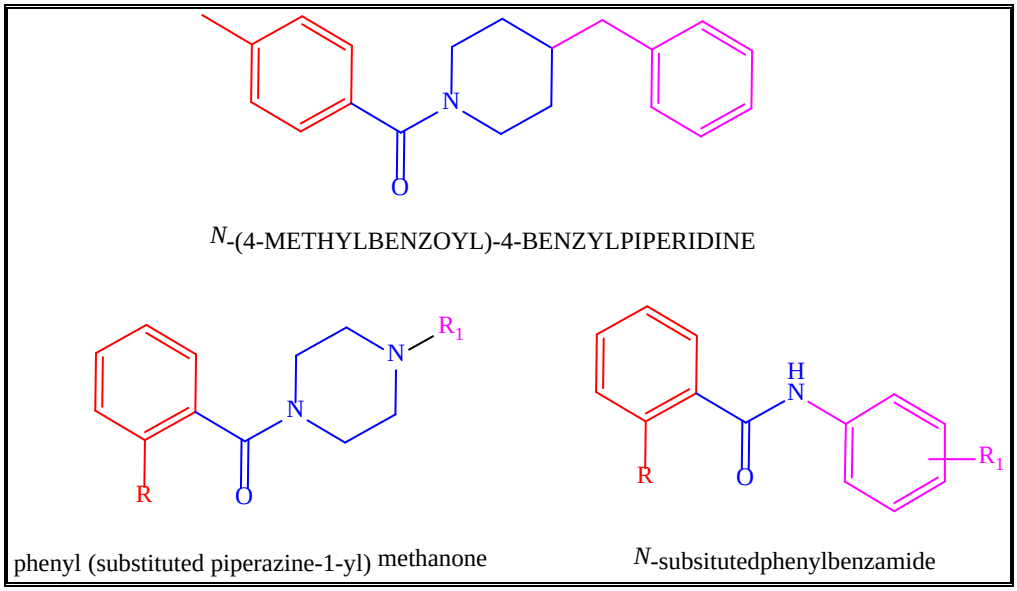


Figure **1**: Design concept for the synthesis of titled compounds

MATERIAL AND METHODS

Molecular docking study

A library prepared ligand was subjected to physicochemical properties screening to calculate descriptors like lipophilicity (logP), molecular weight, number of nitrogen and oxygen, hydrogen bond donor/ acceptor, solubility, number of rotors, polar surface area (PSA) were taken into consideration for the molecular docking. The X-ray crystal structure of *M. tuberculosis InhA* (PDB ID: 2NSD) was extracted from the Brookhaven Protein Database (PDB http://www.rcsb.org/pdb). In the present situation, biopolymer and each molecule in the data set was energetically minimized by employing MMFF94s force field. The *InhA* protein was optimized through protein preparation tool, protein was pre-processed by assigning bond order, adding hydrogens and treating disulphide. Unnecessary water molecules were removed from the binding site. Co-crystallized ligand was extracted, used as a reference ligand and Using default parameters receptor binding site was generated around the co-crystallized ligand (2NSD).

Synthesis

Chemicals used for the synthesis, were of laboratory grade and the solvents of analytical grade. The progress of the reactions was monitored periodically by TLC (Thin Layer Chromatography) using Petroleum ether: Ethyl acetate (2:1and 8:2) as a mobile phase. The melting points of the synthesized compounds were obtained by open capillary method, expressed in °C. IR spectra were recorded on Shimadzu FT-IR 8400-S spectrophotometer by potassium bromide pellet technique and were expressed in cm^{-1}. ^{1}HNMR spectra were recorded on BRUKER SPECTROSPIN-400MHz using TMS (trimethyl silane) and dimethyl sulphoxide (DMSO-d_6) as an internal standard and solvent respectively. The chemical shift data were expressed in terms of δ values relative to TMS. LCMS data were recorded on EIMS (Electron Ionization Mass Spectroscopy) instrument.

General synthesis of aryl acid chlorides (2a, b)

1 mol of an appropriate aryl acid was added to an ice-cold solution of pyridine placed in a three necked round bottom flask fitted with a dropping funnel and a guard tube. A solution of 1.1 mol of thionyl chloride in ice cold solution of pyridine was added drop wise with continuous stirring to the mixture of aryl acid through dropping funnel and the reaction mixture was stirred overnight at room temperature. The progress of the reaction was monitored by using TLC, after completion of reaction excess of thionyl chloride was removed using rotary flash evaporator and resulted product was dried [29].

Synthesis of *N*-(2-hydroxyphenyl) benzamide (3a)

To a solution of 0.001 mol of 2-hydroxy aniline in an appropriated quantity of dichloromethane, 0.001 benzoyl chloride (**2a, b**) was added slowly with continuous stirring and the reaction mixture was refluxed for 5h at 40-50 ^{o}C. Progress of the reaction was monitored by TLC. After completion of the reaction the obtained grey precipitate was filtered and poured into ice to get a crude precipitate and filtered, dried and recrystallised from ethanol to get a pure *N*-(2-hydroxyphenyl) benzamide **(3a).** Similar procedure was adopted to synthesize other *N*-substituted benzamides **(3b-i).**

R_f=0.95 (PE/EtOAc, 6:4); mp: 40-43°C; (FTIR) cm^{-1}: (NH) 3470, (OH) 3280, (C=O) 1645; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 12.19 (s, 1H; OH), 9.15 (s, 1H; NH), 7.86-8.03 (m, 2H; Ar-H), 7.77 (s, 1H; Ar-H), 7.31-7.62 (m, 3H; Ar-H), 7.11 (s, 1H; Ar-H), 6.87 ppm (s, 2H; Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ: 164.87, 149.72, 134.51, 132.20, 128.92, 127.58, 125.78, 123.10, 121.61, 116.20 ppm; LC/MS: purity 97.21%. (ESI): *m/z* calcd for $C_{13}H_{11}NO_2$: 213.08; found: 213.23.

N-(4-hydroxyphenyl) benzamide (3b)

R_f =0.85 (PE/EtOAc, 6:4); mp: 44 °C; (KBR) cm^{-1}: (NH) 3510, (OH) 3300, (C=O) 1645; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 12.19 (s, 1H; OH), 9.15 (s, 1H; NH), 7.86-8.03 (m, 2H; Ar-H), 7.77 (s, 1H; Ar-H), 7.31-7.62 (m, 3H; Ar-H), 7.11 (s, 1H; Ar-H), 6.87 ppm (s, 2H; Ar-H); ^{13}C

NMR (75 MHz, DMSO-d_6): δ: 163.87, 150.72, 134.23, 132.01, 128.32, 127.40, 121.61, 115.25 ppm; LC/MS: purity 96.71%. (ESI): *m/z* calcd for $C_{13}H_{11}NO_2$: 213.02; found: 213.23.

N-(5-chloro-2- hydroxyphenyl) benzamide (3c)

R_f =0.91(PE/EtOAc, 6:4); m.p:47-49 °C; (KBR) cm^{-1}: (NH) 3520, (OH) 3290, (C=O) 1649, (C-Cl) 712; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 12.10 (s, 1H; OH), 9.16 (s, 1H; NH), 7.86-7.95 (m, 2H; Ar-H), 7.62-7.77 (m, 2H; Ar-H), 7.51 (s, 2H; Ar-H), 6.87-7.11 ppm (m, 2H; Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ: 165.07, 145.72, 134.65, 132.41, 129.32, 126.40, 118.61, 113.25 ppm; LC/MS: purity 97.71%. (ESI): *m/z* calcd for $C_{13}H_{10}ClNO_2$: 247.04; found: 247.68.

N-(pyridine-2-yl) benzamide (3d)

R_f =0.90 (PE/EtOAc, 6:4); m.p:50-52 °C; (KBR) cm^{-1}: (NH) 3510, (C=O) 1645, (C-N) 1240; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 11.12 (s, 1H; NH), , 7.95-8.30 (m, 3H; Ar-H), 7.44-7.88 (m, 4H; Ar-H), 7.31 (s, 1H; Ar-H), 6.87-7.11 ppm (m, 1H; Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ: 164.07, 150.02, 140.22, 134.23, 132.01, 128.32, 127.23, 118.78, 113.02 ppm; LC/MS: purity 93.81%. (ESI): *m/z* calcd for $C_{12}H_{10}N_2O$: 198.08; found: 198.22.

2-hydroxy-N-(2- hydroxyphenyl) benzamide (3e)

R_f =0.88 (PE/EtOAc, 6:4); m.p:51-53 °C; (KBR) cm^{-1}: (NH) 3530, (OH) 3310, (C=O) 1650; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 12.19 (s, 2H; OH), 9.55 (s, 1H; NH), 7.86 (s, 1H; Ar-H), 7.31-7.62 (m, 3H; Ar-H), 6.87-7.11 ppm (m, 4H; Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ: 164.07, 155.02, 149.72, 134.23, 130.51, 121.61, 120.01, 116.20 ppm; LC/MS: purity 95.01%. (ESI): *m/z* calcd for $C_{13}H_{11}NO_3$: 229.07.

2-hydroxy-N-(4- hydroxyphenyl) benzamide (3f)

R_f =0.88 (PE/EtOAc, 6:4); m.p:53-55 °C; (KBR) cm^{-1}: (NH) 3570, (OH) 3320, (C=O) 1650; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 12.01 (s, 1H; OH), 9.45 (s, 1H; NH), 9.15 (s, 1H; OH of 4-hydroxyph), 7.86 (s, 1H; Ar-H), 7.11-7.45 (m, 3H; Ar-H), 7.01 (s, 2H; Ar-H), 6.87 ppm (s, 2H; Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ: 164.07, 155.02, 133.23, 130.07, 122.77, 121.61, 120.01, 116.20 ppm; LC/MS: purity 93.05%. (ESI): *m/z* calcd for $C_{13}H_{11}NO_3$: 229.07; found: 229.23.

N-(5-chloro-2-hydroxyphenyl)-2- hydroxy benzamide (3g)

R_f =0.78 (PE/EtOAc, 6:4); m.p:50-52 °C; (KBR) cm^{-1}: (NH) 3510, (OH) 3301, (C=O) 1670, (C-Cl) 745; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 12.19 (s, 1H; OH), 11.10 (s, 1H; OH of chloroph), 9.25 (s, 1H; NH), , 7.86-7.95 (m, 2H; Ar-H), 7.31 (s, 1H; Ar-H), 6.87-7.11 ppm (m, 4H; Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ: 163.70, 155.02, 146.72, 132.23, 130.07, 128.66, 121.00, 119.89, 116.20 ppm; LC/MS: purity 94.10 %. (ESI): *m/z* calcd for $C_{13}H_{10}ClNO_3$: 263.03.

4- [(2- hydroxybenzoyl)amino]benzoic acid (3h)

R_f =0.88 (PE/EtOAc, 6:4); m.p:51-53°C; (KBR) cm^{-1}: (NH) 3490, (OH) 3330, (COOH) 1720, ($CONH_2$) 1670; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 12.56 (s, 1H; acidic OH), 11.22 (s, 1H; OH), 9.45 (s, 1H; NH), 7.87-8.04 (m, 5H; Ar-H), 6.95-7.22 ppm (m, 3H; Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ: 170.01, 165.70, 155.40, 144.72, 132.55, 130.07, 128.66, 123.88, 121.00, 118.89, 116.90 ppm; LC/MS: purity 95%. (ESI): *m/z* calcd for $C_{14}H_{11}NO_4$: 257.07; found: 257.24.

2-hydroxy-N-(pyridine-2-yl) benzamide (3i)

R_f =0.61 (PE/EtOAc, 6:4); m.p:54-56 °C; (KBR) cm^{-1}: (NH) 3510, (OH) 3330, (C=O) 1680, (C=N) 1640; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 12.10 (s, 2H; OH and NH), 7.98 (s, 1H; Ar-H), 7.76 (s, 1H; Ar-H), 7.31-7.62 (m, 3H; Ar-H), 7.11 ppm (m, 1H; Ar-H), 6.85 ppm (m, 2H; Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ: 165.70, 155.80, 149.72, 140.55, 132.07, 128.90, 122.88, 118.89, 116.90, 113.89 ppm; LC/MS: purity 93%. (ESI): *m/z* calcd for $C_{12}H_{10}N_2O_2$: 214.07; found: 215.24.

Synthesis of phenyl (piperazine-1-yl) methanone (4a)

0.001 mol of Piperazine 0.001mol in an appropriate quantity of dichloromethane were taken in a 100ml round bottom flask. Benzoyl chloride (**2a, b**, 0.001mol) was added slowly to the above mixture with continuous stirring. The reaction mixture was refluxed for 4h at 50-60°C. The progress of the reaction was monitored using TLC. The white precipitate obtained was filtered and poured into ice [20]. The crude white product obtained was filtered, dried and recrystallized from ethanol to obtain phenyl (piperazine-1-yl) methanone **(4a)**. Similar procedure was adopted to synthesize other 4-substituted-piperazin-1-yl(phenyl) methanone **(4b-e)**.

R_f =0.86 (PE/EtOAc, 6:4); m.p:110-111°C; (KBR) cm^{-1}: (C-H) 3071, (C=O) 1720, (C-N) 1248; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 7.63-8.03 (m, 3H; Ar-H), 7.45 (m, 2H; Ar-H), 2.45-3.46 (m, 8H; piperazine-H),1.91 ppm (s, 1H; NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ: 169.70, 135.19, 129.90, 128.64, 127.18, 53.88, 48.52 ppm; LC/MS: purity 93%. (ESI): *m/z* calcd for $C_{11}H_{14}N_2O$: 190.11; found: 190.24.

(4-methylpiperazin-1- yl)(phenyl) methanone (4b)

R_f=0.80 (PE/EtOAc, 6:4); m.p:100-102 °C; (KBR) cm^{-1}: (C-H) 3035, (C=O) 1687, (C-N) 1293; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 7.80-7.99 (m, 2H; Ar-H), 7.44-7.64(m, 3H; Ar-H), 2.76-3.32 (m, 4H; piperazine-H), 2.26-2.66 (m, 4H; piperazine-H), 2.15 ppm (s, 3H; CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ: 168.70, 134.79, 129.60, 128.84, 126.48, 52.88, 47.52 ppm; LC/MS: purity 93%. (ESI): *m/z* calcd for $C_{12}H_{16}N_2O$: 204.13; found: 205.27.

[4-ethylpiperazin-1-yl](phenyl) methanone (4c)

R_f=0.81(PE/EtOAc, 6:4); m.p:104-106°C; (KBR) cm^{-1}: (N-H) 3346, (C=O) 1688; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 7.98 (s, 2H; Ar-H), 7.44-7.64 (m, 3H; Ar-H), 2.15-3.32 (m, 10H, piperazine-H & $2CH_2$), 1.28 (s, 3H, $-CH_3$); ^{13}C NMR (75 MHz, DMSO-d_6): δ: 168.70, 135.79, 130.05, 128.84, 58.88, 55.94, 49.88, 13.40 ppm; LC/MS: purity 91%. (ESI): *m/z* calcd for $C_{13}H_{18}N_2O$: 218.30; found: 218.55.

(2-hydroxyphenyl) (4- methylpiperazin-1-yl)methanone (4d)

R_f=0.62 (PE/EtOAc, 6:4); m.p:100-102 °C; (KBR) cm^{-1}: (OH) 3320, (C=O) 1650; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 10.22 (s, 1H; OH), 7.82 (s, 1H; Ar-H), 6.86-7.78 (m, 3H; Ar-H), 3.31 (s, 4H; piperazine-H), 2.17-2.67 (m, 4H; piperazine-H), 2.10 (s, 3H; CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ: 168.70, 160.10, 129.60, 121.48, 118.09,

52.88, 47.52 ppm; LC/MS: purity 96%. (ESI): *m/z* calcd for $C_{12}H_{16}N_2O_2$: 220.12; found: 220.27.

(2-hydroxyphenyl) [4-ethylpiperazin-1-yl] methanone (4e)

(KBR) cm^{-1}: (NH) 3530, (OH) 3320, (C=O) 1745; ^1H NMR (400 MHz, DMSO-d_6): δ: 10.12 (s, 1H; OH), 7.90 (s, 2H; Ar-H), 7.34-7.64 (m, 3H; Ar-H), 2.25-3.42 (m, 10H, piperazine-H & $2CH_2$), 1.30 (s, 3H; $-CH_3$); ; ^{13}C NMR (75 MHz, DMSO-d_6): δ: 168.70, 160.50, 131.05, 128.84, 121.84, 117.01, 59.08, 56.54, 50.28, 13.50 ppm; LC/MS: purity 92%. (ESI): m/z calcd for $C_{13}H_{18}N_2O_2$: 234.30; found: 235.31.

BIOLOGICAL ACTIVITY

In-vitro Anti-tubercular activity [30]

Synthesized titled compounds were evaluated for anti TB activity against *M. tuberculosis* $H_{37}Rv$ (ATCC-27294) from 100 to 3.125 µg/mL concentration using bifold dilutions in the initial screen. Log phase culture of *M. tuberculosis* $H_{37}Rv$ was diluted to give final OD_{550nm} of 0.05 in Middle brook 7H9 broth medium. 190µL of culture was disbursed into 96 well white plates. The final test concentration of 25µM was made by allotting the DMSO solution of test compound into the well plates. Then these plates were incubated at 37°C/5 % CO_2 for 5 days. 25µL of 1:1 mixture of Alamar Blue reagent and 10% tween 80 was freshly prepared on the 5th day and this was added to each well of the plates. Then these plates were again incubated under the same conditions as mentioned above. The fluorescence was read on BMG polar star with excitation frequency at 544nm and emission frequency at 590nm.

In-vitro Antibacterial activity [31, 32]

Evaluations of in vitro antibacterial activity of the synthesized compounds were done by using two-fold serial dilution technique. This involves a series of six assay tubes for each test compound against two bacterial strains. The entire test was done in duplicate. First assay tube consists of 1.8mL of seeded broth and 0.2mL of the test compound (1µM) and thoroughly mixed, the bi- fold serial dilution was done up to the last tube containing 1mL of the seeded broth. Aseptic condition was used for the addition of the drug solution and serial dilution. Solvent control, negative control (growth control) and drug control were maintained. The assay tubes were incubated for 24h at 37°C. The lowest concentration which apparently caused complete inhibition of growth of microorganisms was considered as the minimum inhibitory concentration (MIC).

Cytotoxicity Screening

MTT (Microculture Tetrazolium) based assay [33-35] was used for the selected compounds to check the cytotoxicity towards Vero and HepG2 cells. To achieve a concentration of 300, 250, 200, 150, 100 and 50µM, stock solution of the tested compounds were diluted in a 96 deep well plate aseptically with MEM (without FBS) and they were kept inverted on filter paper to remove the supernatant media and washed gently with PBS and decanted. 100µL of sterile water and each test compound dilutions were added to outer perimeter wells and DMSO was used as control. All the plates were incubated at 37°C, for 24h and 72h in incubator (5% CO_2) for Vero cells and HepG2 respectively. After the incubation, plates were inverted on filter paper to remove the supernatant media followed by PBS washing. To this, 50µL of MTT solution was added to each well in dark place and incubated for 3h. After the incubation, the MTT solution was removed from the well by inverting gently on filter paper and 50µL of DMSO was added to each well and kept in dark place for 1-2h. Then the optical density readings of the plates were taken using Elisa reader at 540nm.

Determination of safety profile (CC_{50})

% Cell Inhibition = 100 - % Cell Viability

CC_{50} was calculated by extrapolating a graph with % cell inhibition on Y-axis against concentration of test compound on X-axis.

RESULTS AND DISCUSSION

Computational study

Aryl amides have reported to possess *InhA* inhibitory activity and exhibited an IC_{50} value in the nanomolar range. These molecules have moderate anti-tubercular activity on whole cell assay against *Mtb*, showing a MIC >125 µg/mL, the low MIC value might be due to their low permeability or the activation of efflux pumps. Therefore, scaffold hopping of these *InhA* inhibiting derivatives was performed in order to improve potency. The aim of modifications is to improve interactions without affecting catalytic interactions and orientation at the binding site of the enzyme.

All the synthesized compounds satisfied Lipinski's parameter of drug likeness as shown in **Table-1** and were docked against *InhA*, results revealed that the designed compounds overlaid and making interactions at the *InhA* binding site as like reference ligand. Furthermore, studies suggested that there is an interaction between carbonyl oxygen of the designed compounds with amino acid residue Tyr-158 and co-factor NAD-300 at the binding pocket of the enzyme (**Fig-2**). All the designed compounds showed consistent hydrogen bonding network, and all the reported *InhA* inhibitor complexes also showed this type of hydrogen bonding which is essential. The results obtained from the docking studies were depicted in **Table-2.**

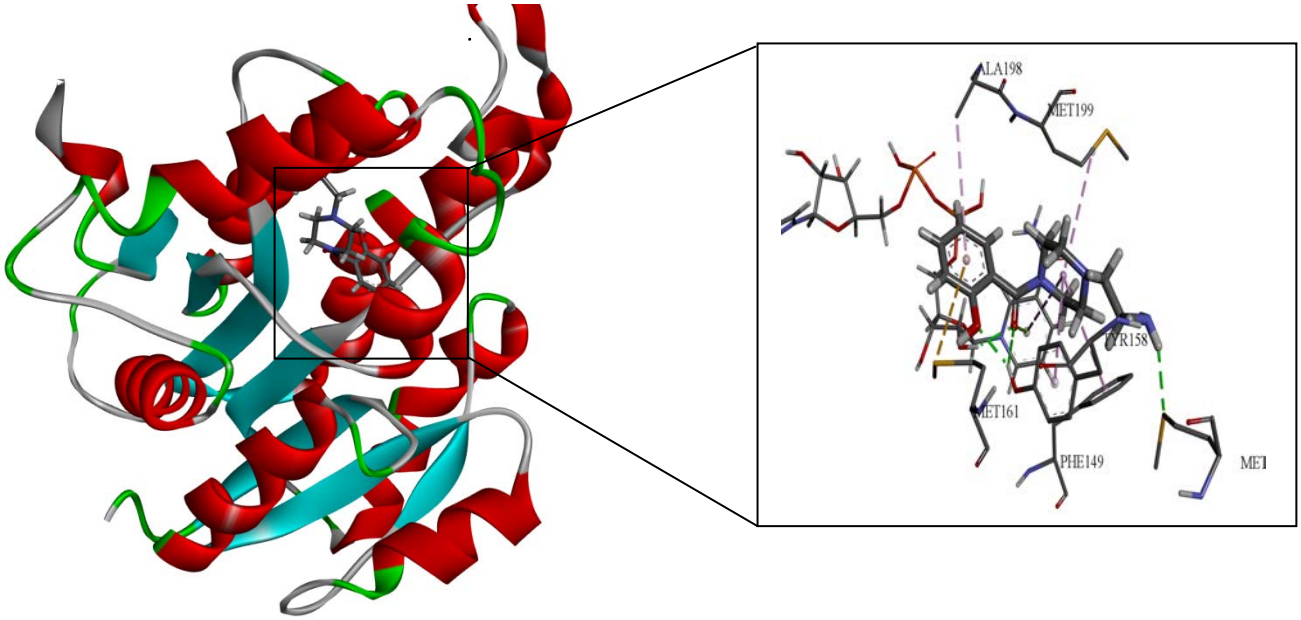


Figure 2: a) Docked view of compound **4e** at the active site of protein PDB ID: 2NSD; **b**) 2D representation of the compound **4e**

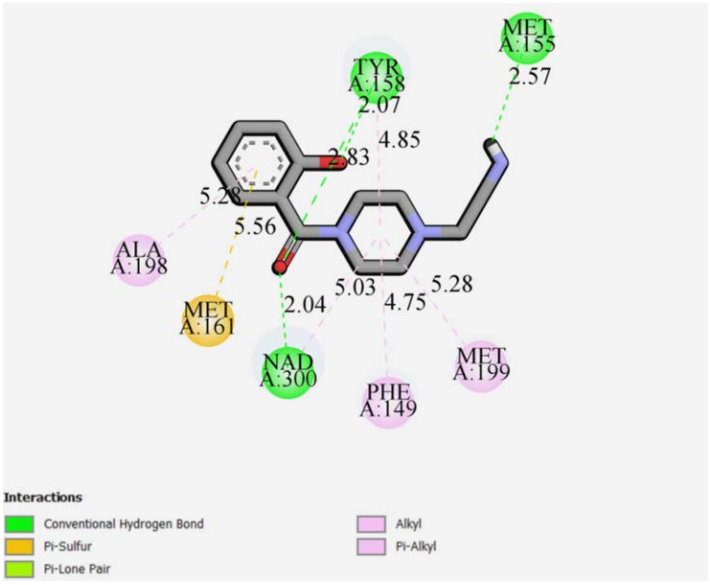


Table 1: Lipinski's rule of 5 Data of the synthesized compounds

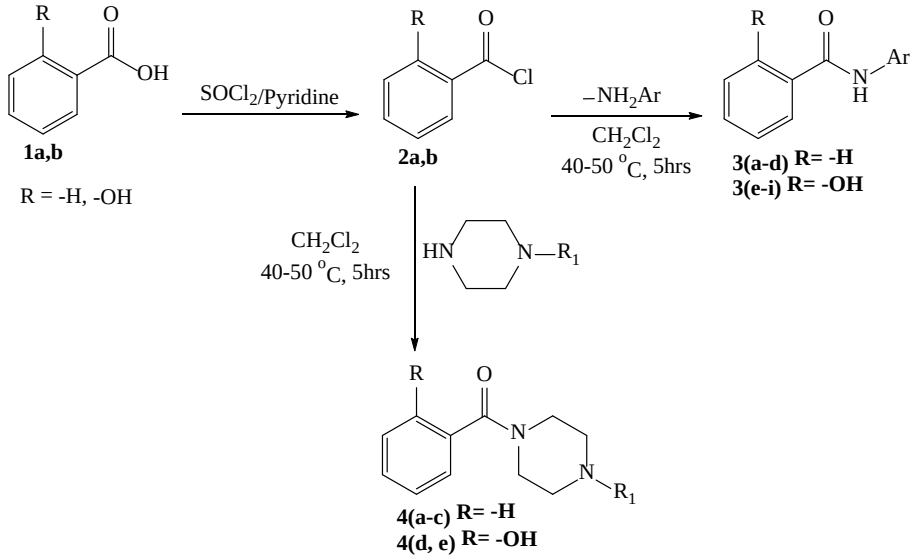
Compounds	cLogP	Acceptor	Donor	Lipinski violation
3a	2.213	3	2	0
3b	1.983	3	2	0
3c	3.300	3	2	0
3d	1.981	3	1	0
3e	2.835	4	3	0
3f	2.605	4	3	0
3g	3.922	4	3	0
3h	3.309	5	3	0
3i	2.022	6	3	0
4a	0.758	3	1	0
4b	1.333	3	0	0
4c	0.835	4	1	0
4d	1.206	4	1	0
4e	0.708	5	0	0

Table 2: Docking results of synthesized compound **3(a-i)** and **4(a-e)** at the active site of PDB ID: 2NSD

Comp.	C score[a]	Crash score[b]	Polar score[c]	D score[25]	PMF score[26]	G score[27]	Chem score[28]
ligand	6.48	-1.10	1.13	-261.03	-45.66	-211.28	-43.65
3a	4.99	-0.73	1.34	-235.14	-31.42	-144.90	-29.03
3b	4.38	-1.11	1.11	-274.63	-29.10	-138.88	-31.85
3c	3.64	-1.71	1.01	-293.92	-28.21	-147.36	-30.38
3d	3.91	-1.29	1.34	-173.14	-39.77	-157.25	-28.42
3e	4.62	-0.84	3.24	-328.00	-53.56	-118.28	-33.86
3f	4.35	-0.83	3.40	-366.66	-45.54	-114.32	-34.67
3g	3.78	-0.59	2.25	-353.20	-55.69	-126.02	-32.92
3h	4.54	-0.82	3.24	-281.43	-40.92	-145.53	-30.23
3i	4.42	-0.83	1.67	-338.59	-38.19	-141.40	-28.46
4a	4.81	-0.38	2.33	-272.96	-46.23	-137.67	-32.98
4b	5.33	-0.40	1.14	-347.60	-28.21	-149.11	-30.45
4c	5.37	-0.76	2.31	-366.93	-18.75	-152.21	-30.71
4d	5.19	-0.88	2.13	-364.26	-12.15	-148.79	-28.35
4e	**5.66**	-1.44	2.78	-578.27	-32.87	-162.44	-30.54

[a]CScore (Consensus Score) integrates a number of popular scoring functions for ranking the affinity of ligands bound to the active site of a receptor and reports the output of total score.
[b]Crash-score revealing the inappropriate penetration into the binding site. Crash scores close to 0 are favorable. Negative numbers indicate penetration.
[c]Polar indicating the contribution of polar interactions to the total score.

Scheme of synthesis:



3a-i = Ar; a) 2-OHC_6H_4, **b)** 4-OHC_6H_4, **c)** 2-OH-5-ClC_6H_3, **d)** 2-C_5H_5N, **e)** 2-OHC_6H_4, **f)** 4-OH-C_6H_4', **g)** 2-OH-5-Cl-C_6H_3, **h)** 4-$COOHC_6H_4$, **i)** 4-$COOHC_5H_4N$

4a-e = R_1; a) -H, **b)** -CH_3, **c)** -C_2H_5, **d)** -CH_3, **e)** -C_2H_5

Chemistry

The synthetic route used for the title compounds is depicted in the scheme**.** The synthetic route started with the appropriate key intermediate, aryl acid chloride **(2a,b)** by reacting with appropriate substituted benzoic acid (**1a,b)** with thionyl chloride using ice cold pyridine. The aryl acid chlorides **(2a, b)** were treated with aryl amines/ *N*-substituted piperazines to afford *N*-substituted benzamides **(3a-i)** and aryl(substituted piperazine-1-yl)methanones **(4a-e)** in good yields. Structures of all the synthesized compounds were confirmed by IR, ^{1}HNMR, ^{13}CNMR and LCMS studies. The physical data of the compounds were given Table 3. The IR spectrum of *N*-(2-hydroxyphenyl)benzamide **(3a)** shows the absorption peaks at 3700 and 3280 cm^{-1} due to the presence of amine and hydroxyl group. In ^{1}HNMR spectrum of compound **3a** shows a singlet peak for secondary amine at 9.15 δ ppm, a singlet at 5.35 δ ppm is assigned to hydroxyl group, and the aromatic protons resonated in the region of 6.90-8.03 δ ppm. The IR spectrum of (2-hydroxyphenyl)(4-methylpiperazin-1-yl)methanone **(4d)** shows the absorption peaks at 3282 and 1691 cm^{-1} indicates the presence of -OH and carbonyl groups respectively. The ^{1}H NMR spectrum of compound **4d** exhibits a complex

multiplet of protons between 2.32 and 3.46 δ ppm was due to the presence of aliphatic hydrogens of piperazine ring. Two singlets at 5.35 and 2.27 δ ppm were assigned to protons of -OH and $-CH_3$ respectively and a multiplet ranging from 6.95-7.86 δ ppm corresponding aromatic protons.

***In-vitro* Anti-tubercular activity**

Microplate Almar Blue Assay was used to determine *in vitro* anti-tubercular activity of the synthesized compounds against *Mtb*. Synthesized compounds were evaluated for their *in vitro* anti-tubercular activity at a concentration ranging from 100 - 6.25 µg/mL using Isoniazid as a standard and Dimethyl Sulfoxide (DMSO) served as the solvent control. Among the synthesized compounds, **4d** and **4e** have shown activity at 6.25µg/mL and compounds **3a**, **4b**, **4c** have shown a MIC value of 12.5µg/mL. The MIC values of the tested compounds are depicted in **Table 4.**

***In-vitro* antibacterial activity**

Two-fold serial dilution method was used to evaluate the synthesized compounds for *in vitro* antibacterial activity against *Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (ATCC 25922) using Ciprofloxacin and Norfloxacin as standard. The antibacterial data reveals that, among the amine derivatives, compounds 3a, and 3c have shown antibacterial activity at MIC value of 6.25 µg/mL, 3d, 3f and 3i have shown potential activity with a MIC value of 12.5 µg/mL against all the bacterial strains. Among the piperazine derivatives, **4a**, **4b** and **4c** have shown antibacterial activity with a MIC value of 6.25 µg/mL, remaining compounds showed moderate to low antibacterial activity against tested bacterial strains. The MIC data of the synthesized compounds are given in Table **4**.

Cytotoxicity Screening

Cytotoxicity studies was carried out for the selected compounds (**3f, 3g, 3i, 4b** and **4c**) using MTT based assay against Vero Cell Lines (African Green monkey kidney epithelial cells) and HepG2 (hepatic carcinoma cells). The compounds have showed a good safety profile for both the cells and it is evident from the results (**Table 4**) that the tested compounds are non-cytotoxic. Hence, the results of antitubercular activity of the synthesized compounds were not due to cytotoxicity.

Table 3: Physical data of the synthesized compounds

Comp.	R	R_1	Ar	Mol. formula	Mol. wt	% yield
3a	H	_	2-OH-C_6H_4	$C_{13}H_{11}NO_2$	213.23	92
3b	H	_	4-OH-C_6H_4	$C_{13}H_{11}NO_2$	213.23	95
3c	H	_	2-OH-5-Cl-C_6H_3	$C_{13}H_{10}ClNO_2$	247.68	92
3d	H	_	2-pyridyl	$C_{12}H_{10}N_2O$	198.22	88
3e	OH	_	2-OH-C_6H_4	$C_{12}H_{10}N_2O$	198.22	65
3f	OH	_	4-OH-C_6H_4	$C_{13}H_{11}NO_3$	229.23	63
3g	OH	_	2-OH-5-Cl-C_6H_3	$C_{13}H_{10}ClNO_3$	263.68	64
3h	OH	_	4-carboxyphenyl	$C_{14}H_{11}NO_4$	257.24	60
3i	OH	_	pyridine-4-carbonamide	$C_{13}H_{11}N_3O_3$	257.24	61
4a	H	-H	_	$C_{11}H_{14}N_2O$	190.24	86
4b	H	$-CH_3$	_	$C_{12}H_{16}N_2O$	204.27	80
4c	H	$-CH_2CH_3$	_	$C_{13}H_{19}N_3O$	233.31	81
4d	OH	$-CH_3$	_	$C_{12}H_{16}N_2O_2$	220.27	62
4e	OH	$- CH_2CH_3$	_	$C_{13}H_{19}N_3O_2$	249.31	61

Table 4. Biological activities data of the synthesized compounds

Compound Code	Minimum Inhibitory Concentration (in µg/mL)			Cytotoxicity (in µg/mL)	
	M. tuberculosis $H_{37}Rv$	***B. subtilis***	***E. coli***	**Vero**	**HepG2**
3a	12.5	6.25	6.25	>300	NC
3b	25	50	25	>300	NC
3c	50	6.25	6.25	>300	NC
3d	50	12.5	12.5	>300	NC
3e	50	25	25	>300	NC
3f	50	12.5	12.5	>300	280
3g	75	50	12.5	>300	278
3h	100	12.5	25	>300	NC
3i	75	12.5	12.5	>300	277
4a	25	6.25	12.5	>300	NC
4b	12.5	12.5	6.25	>300	298
4c	12.5	6.25	6.25	>300	297
4d	6.25	50	25	>300	270
4e	6.25	25	25	>300	NC
Ciprofloxacin	-	2	2	-	-
Norfloxacin	-	1	12	-	-
Isoniazid	0.25	-	-	-	-

*NC = not carried out

CONCLUSION

The titled compounds were synthesized in good yield as per scheme of synthesis. The spectral analyses were in consistent with the structure proposed within the range of theoretical values. Computational studies revelled that the synthesized compounds have shown the hydrogen bonding interaction at the active site of the enzyme and were similar to that of the co-crystallized ligand as we observed in case of arylamide derivatives, the carbonyl oxygen caters the formation of H-bonding with TYR- 158 and 2'-OH of the ribose sugar part of NAD^+. MABA method was used to determine *in vitro* antitubercular activity of the synthesized compounds against *Mtb*H_{37}Rv strain. The data revealed that aryl amides formed with cyclic secondary amines have shown better antitubercular activity than primary aryl amines. Among the tested compounds, only **4d** and **4e** have shown MIC value at 6.25µg/mL concentration and compound **3a, 4b & 4c** have shown the MIC value at a concentration of 12.5 µg/mL. The compounds have shown potential *in vitro* antibacterial activity against *Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (ATCC 25922) using Ciprofloxacin and Norfloxacin as standard. Compounds **3a**and **3c** have showed MIC of 6.25 µg/mL, compounds **3d**, **3f** and **3i** have showed potential activity with MIC of 12.5 µg/mL against all the bacterial strains. Among the piperazine derivatives **4a**, **4b** and **4c** have shown antibacterial activity at a MIC of 6.25 µg/mL. These compounds also showed good safety profiles against Vero and HepG2 cells. One of the reasons for better activity of aryl cyclic amines over aryl amides is due to the restriction of nitrogen atom flexibility.

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