Chem. Biol. Lett. 2018, 5(2), 55-62 Article

Chemical Biology
LETTERS

Antimycobacterial activity of Schiff's Bases synthesized from substituted Benzaldehyde active against Mycobacterium

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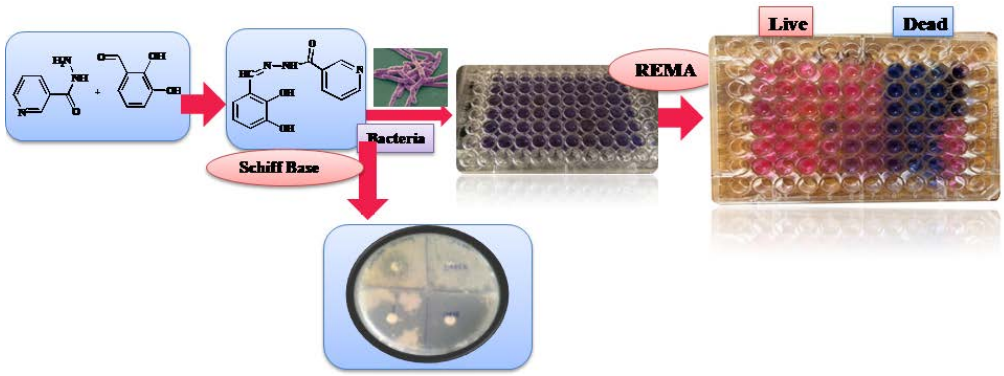
Submitted on: 1-Juky-2018, Accepted and Published on: 14-Aug-2018

ABSTRACT



In a preliminary screening we found that few substituted benzaldehyde have growth inhibitory effect against Mycobacterium. Study was planned to convert these substituted benzaldehyde to antimycobacterial Schiff's bases. Schiff's bases were synthesized by condensation of 2-Amino pyridine/ its derivative or Thiophene/ Furoic/ Nicotinic acid hydrazide with these substituted benzaldehydes. Compounds were purified and characterized by IR, ^{1}H and ^{13}C-NMR and were subjected to Antimycobacterial testing in two of the Mycobacterial strains; fast growing *Mycobacterium smegmatis* by disc diffusion method and further in to slow growing *Mycobacterium bovis* BCG strain using quantitative resazurin microplate assay. Schiff's bases 3c, 3f and 8a have shown 60, 40 and 50 mm zone of inhibition in *M. smegmatis,* compared to control, Isoniazid showed 40 mm zone of inhibition. In *Mycobacterium bovis,* compounds 3c, 3f and 8a showed 53.69, 49.41, and 46.49 % of cell killing at 500 µM, the activity was concentration dependent and reduced viability of 29.73, 37.75 and 36.15% were reported at 125 µM. Isoniazid has shown 60% cell killing at 40 µg/ml. Schiff's bases prepared from 4-hydroxy or 4-Nitro benzaldehyde with 2-amino pyridine (3c and 3f) and 2, 3 Dihydroxy benzaldehyde with nicotinic acid hydrazide (8a) have shown promising antimycobacterial potential.

Keywords: Mycobacterium smegmatis,Mycobacterium bovis, Schiff's bases, Resazurin Microtitre plate Assay (REMA)

INTRODUCTION

Tuberculosis (TB) is fast emerging as a threat and a cause of global concern that needs both surveillance and control.[1] Drug therapy is the cornerstone of TB management. However, the prolonged duration of the current therapy, the non-compliance of patients, the occurrence of multidrug-resistant (MDR; resistant to Isoniazid and rifampicin) and extensively drug-resistant (XDR; resistant to Isoniazid, rifampicin, quinolones and any one of kanamycin, capreomycin or amikacin) strains along with the increased co-incidence of HIV cases, high relapse rate, latent infection have all made the effective control and management of the disease difficult.[2] The current status of the problem clearly manifests the need to develop new potent inhibitory molecules that could help not only shorten the duration of the current therapy but also provide effective treatment of MDR, XDR and latent TB. But, unfortunately,

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Cite as: *Chem. Biol. Lett., 2018, 5(2), 55-62.*

 ISSN: 2347–9825 http://pubs.iscience.in/cbl

there has been no new drug molecule in the recent past and this is largely attributed to the cell wall of *Mycobacterium tuberculosis*, which is mainly composed of lipids that render the bacteria impermeable to most antibiotics/drugs. Various molecules, such as derivatives of fluoroquinolones (e.g. gatifloxacin and moxifloxacin), rifamycins (rifalazil), oxazolidinones (linezolid), diarylquinolines (TMC207), antifungal azoles, pyrrole (LL3858), nitroimidazopyran (PA824), nitroimidazole (OPC67683) and diamine (SQ109), are currently being projected as promising drug candidates. Bedaquline has been latest TB drug that has been approved for use, however it has been found to show side effect.[3,4]

Schiff bases are the compounds carrying imines or azomethine (–C=N–) functional group. These are the condensation products of primary amines with carbonyl compounds.[5] It is a chemical compound containing R_2C=N- functionality with numerous modifications, a celebrated drug, reported to have considerable bioactivities.[6] They are important compounds owing to their wide range of biological activities and industrial application; they have been found to exhibit superfluity of the pharmacological activities such as antitubercular,[7,8] anticancer,[9,10] antibacterial[11] and antifungal[12] analgesic,[13] CNS depressant,[14,15] anticonvulsant,[16,17] anti-inflammatory,[18] insecticidal,[19] plant growth inhibitors,[20] anti-mouse hepatitis virus (MHV),[21] inhibition of herpes simplex virus type 1 (HSV-1) and adenovirus type-5,[22] anti-mosquito larvae[23] and herbicidal activities.[24] They are also reported to be used as protective agent in natural rubber. [24]

In our laboratory, we found that few substituted benzaldehyde were active against Mycobacterial screening strain (*M. smegmatis* unpublished data). Aldehydes are associated with poor drug likeness and moderate toxicity. As discussed above, Schiff's bases have wide medicinal and pharmacological applications; therefore study was planned to prepare Schiff's bases from these biologically active benzaldehyde. These Schiff's bases were tested in two of the *Mycobacterial* strains (*Mycobacterium smegmatis* and *Mycobacterium bovis*) for their antimycobacterial activity. The aim of the study was to transform these substituted benzaldehyde to candidate drug molecules which may have clinical utility against tuberculosis.

MATERIAL AND METHODS

Materials

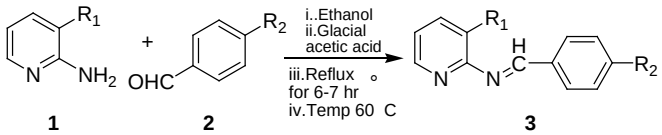
The starting materials such as 2-amino pyridine, 2-amino, 3-nitro pyridine, 2-amino, 3-bromo pyridine, Nicotinic or Furoic or Thiophene carboxylic hydrazide, trimethylamine, monochloroacetylchloride and various substituted benzaldehyde were purchased from Sigma-Aldrich. The solvents used were of analytical reagent grade purchase from Spectrochem. Culture medium 7H9 broth and 7H11 agar were purchased from Difco (BD and Company sparks, USA).

All melting points were expressed in degree centigrade and recorded in digital melting point apparatus (Lab India Analytical Instrument Pvt. Ltd. New Delhi). The infrared spectra were recorded on Perkin-Elmer Spectrum ES version 10.5.1 and ν_{max} are expressed in cm^{-1}. ^1H NMR and ^{13}C NMR were recorded on Jeol-delta-400 spectrometer using tetramethylsilane (TMS) as an internal standard and chemical shifts (δ) are expressed in ppm.

Microorganism: Mycobacterium species i.e., *Mycobacterium smegmatis* (*M. smeg.*)and *Mycobacterium bovis* (*M. bovis*)were obtained from collaborator Professor Jaya S. Tyagi laboratory at Department of Biotechnology AIIMS, New Delhi, INDIA. Strains were characterized by acid fast staining.

Synthesis of Schiff base:

Different analogue of Schiff's bases (3a-3f, 6a-6b, 8a) were synthesized by little modification of literature method[25,27] The compounds were synthesized by refluxing equimolar solutions of 2-amino pyridine and various substituted benzaldehyde in ethanol with vigorous stirring for 6-7 hours, few drops of glacial acetic acid was used as catalyst. Reaction progress was monitored by thin layer chromatography (TLC). After completion of reaction, the reaction mixture was cooled to room temperature and poured in crushed ice and product was filtered. Product was washed with cold water and diethyl ether. Product was recrystallized in ethanol.



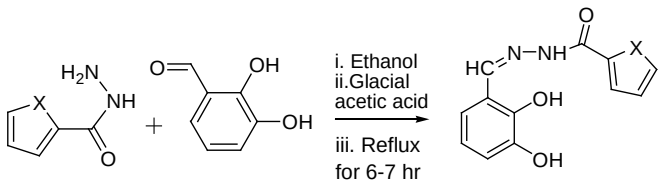
1 2 3

1a. R_1= H; 1b. R_1= H; 1c. R_1= H; 1d. R_1= 3-NO_2; 1e. R_1= 3-Br; 1f. R_1= H

2a. R_2= H; 2b. R_2= p-Cl; 2c. R_2= p-NO_2; 2d. R_2= p-NO_2; 2e. R_2= p-NO_2; 2f. R_2= p-OH

3a. R_1= H; R_2= H; 3b. R_1= H; R_2= p-Cl; 3c. R_1= H; R_2= p-NO_2; 3d. R_1= 3-NO_2; R_2= p-NO_2; 3e. R_1= 3-Br; R_2= p-NO_2; 3f. R_1= H; R_2= p-OH

Scheme 1: Synthetic representation and reaction scheme for synthesis of Schiff's base from 2-amino pyridine and its derivatives

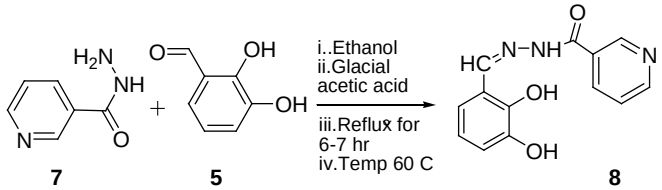


4 5 6

4a. X= O; 4b. X= S

6a. X=O; 6b. X= S

Scheme 2: Synthetic representation and reaction scheme for synthesis of Schiff's base from hetero atom five membered Thiophene and Furoic acid hydrazide



7 5 8

Scheme 3: Synthetic representation and reaction scheme for Synthesis of Schiff's base from Nicotinic acid hydrazide.

N-(benzylidene) pyridine-2-amine (3a): 2-amino pyridine (0.05mol) was dissolved in 20 ml of ethanol in a round bottom flask. In another clean and dry beaker (0.05mol) of benzaldehyde was dissolved in 20 ml of ethanol. Solution of benzaldehyde was added slowly to the solution of 2-amino pyridine and mixed properly. Few drops of glacial acetic acid were added to the mixture and then reaction mixture was allowed to reflux for 6-7 hours at 60 ºC. Progress of reaction was monitored by TLC. After completion of reaction, the reaction mixture was cooled to room temperature and poured in crushed ice) and product was filtered and recrystallized in ethanol: hexane (1:3). Yield: 45%. Mp: 94-97°C.[25] ^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.36 (m, 2H), 7.42 (m, 2H), 7.85(s, 1H), 8.15 (m, 4H).^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 114.63, 116.27, 122.49, 129.21, 129.43 129.63, 131.13, 131.89, 143.69, 163.22.

2-(4-chloro-benzylidene)-Pyridinyl-amine (3b): Synthesized by above procedure using 4-Chloro benzaldehyde and 2-amino pyridine. Yield: 50%. Mp: 85-88°C[25], ^{1}H NMR (400 MHz, $CDCl_3$) δ= 6.84 (d, 2H, J= 8.8 Hz), 7.25 (d, 2H, J = 8.8), 7.70 (m, 4H), 8.27 (s, 1H).^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 114.63, 116.27, 122.49, 129.21, 129.43 129.63, 131.13, 131.89, 143.69, 163.22

2-(4-nitro-benzylidene)-Pyridinyl-amine (3c): Synthesized by above procedure using 4-NO_2-benzaldehyde and 2-amino pyridine. Yield: 67%. Mp: 122-124°C,[251]^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.55 (d, 2H, J=9.2 Hz), 7.66 (d, 2H, J = 9.2), 7.96 (3, 4H), 8.76 (s, 1H).^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 116.87, 120.25, 121.22, 126.66, 127.39, 128.14, 137.43, 147.23.

(4-nitrobenzylidene)-3-nitropyridin-2-yl-amine (3d): Synthesized by above procedure using 4-NO_2 benzaldehyde and 2-amino, 3-nitro Pyridine**.** Yield 58%. Mp: 105-108°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ=6.84 (d, 2H, J= 8.8 Hz), 7.35 (d, 2H, J = 5.7 Hz), 7.70 (m, 1H), 7.95 (d, 1H, J= 8.8), 8.49 (s, 1H), 8.87 (d, 2H, J = 9.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 116.87, 120.25, 121.22, 126.66, 127.39, 128.14, 137.43, 147.23, 167.25, 166.12.

(3-bromopyridin-2-yl)-4-nitro-benzylidene amine (3e): Synthesized by above procedure using 4-NO_2-benzaldehyde and 2-amino 3-bromo pyridine**.** Yield 45%. Mp: 168-170°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ= 6.69 (d, 2H, *J*= 8.0 Hz), 7.16 (d, 2H, *J* = 8.0 Hz), 7.51 (m, 4H), 8.19 (s, 1H).^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 112.15, 116.07, 120.44, 129.01, 129.28 129.43, 130.13, 131.39, 137.69, 161.52.

4-(pyridine-2-yliminomethyl)-phenol (3f): Synthesized by above procedure using 4-Hydroxy-benzaldehyde and 2-amino pyridine. Yield 57%. Mp: 67-69°C,[25] ^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.35 (d, 2H, J=9.2 Hz), 7.69 (d, 2H, J = 9.2 Hz), 7.96 (m, 2H), 8.10 (m, 2H), 8.51 (s, 1H).^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 116.87, 120.25, 121.22, 126.66, 127.39, 128.14, 137.43, 147.23.

Furan-2-carboxylic acid (2,3-dihydroxy benzylidene) hydrazide (6a): Synthesized by above procedure using 2,3-dihydroxy benzaldehyde and furan-2-carboxylic hydrazide. Yield: 68%. Mp: 80-84ºC, IR (KBr, cm^{-1}): $_{C=N}$1644.55 cm^{-1}**,** $\nu_{AR\text{-}C\text{-}H}$ 3076.37 cm^{-1}, $\nu_{C\text{-}OH}$ 3246.77. ^{1}H NMR (300 MHz, DMSO-d_6) δ= 12.18 (s, NH), 11.03 (s, 2OH), 9.96 (s, CH-NH-), 8.61 (d, 1H, *J*= 6 Hz, -CH-O-), 6.86 (m, 2H), 6.63 (m, 3H).^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 154.44, 149.44, 146.68, 146.48, 146.06, 120.41, 119.68, 119.28, 117.86, 115.82, 112.70.(ESI-MS) for $C_{12}H_{10}N_2O_4$ $[M]^+$: calcd, 246.2251; found,246.8289.

Thiophene-2-carboxylic acid (2,3-dihydroxy benzylidene) hydrazide (6b): Synthesized by above procedure using 2,3-dihydroxy benzaldehyde and thiophene-2-carboxylic hydrazide Yield: 72%. Mp: 150-152 °C, IR (KBr, cm^{-1}): $_{C=N}$1643.61cm^{-1}**,** $\nu_{AR\text{-}C\text{-}H}$ 3074 cm^{-1}, $\nu_{C\text{-}OH}$ 3250.18 cm^{-1}, $\nu_{C\text{-}S}$ 723.50. ^{1}H NMR (300 MHz, DMSO-d_6) δ= 12.16 (s, NH), 10.97 (s, 2OH), 9.36 (s, CH-NH-), 8.16 (m, 3H, furan), 6.74 (m, 3H).^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 157.99, 148.95, 146.46, 146.07, 138.03, 132.65, 129.70, 128.73, 120.29, 119.69, 119.34, 117.85. (ESI-MS) for $C_{12}H_{10}N_2O_4$ $[M]^+$: calcd, 262.2869; found, 262.7404.

Nicotinic acid (2,3-dihydroxy benzylidene) hydrazide (8a): Synthesized by above procedure using 2,3-dihydroxy benzaldehyde and Nicotinic acid hydrazide. Yield 63%. Mp: 170-172°C, IR (KBr, cm^{-1}): $_{C=N}$1642.28cm^{-1}**,** $\nu_{AR\text{-}C\text{-}H}$ 3118.48cm^{-1}, $\nu_{C\text{-}OH}$ 3249.57cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ= 12.30 (s, NH), 11.30 (s, 2OH), 9.31 (s, CH-NH-), 7.94 (m, 3H, furan), 6.76 (m, 3H).^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 161.92, 152.90, 149.89, 149.08, 146.62, 146.08, 135.97, 129.10, 124.13, 120.44, 119.72, 119.21, 118.03.(ESI-MS) for $C_{12}H_{10}N_2O_4$ $[M]^+$: calcd, 257.2461; found, 257.7529.

Antimycobacterial Activity of Schiff's bases

Preparation of culture: Stock culture of *M. smeg.* or *M. bovis* was inoculated into Middle Brook 7H9 medium (containing 10% ADC [Albumin Dextrose Complex], 0.2% glycerol and 0.1% Tween-80) and incubated at 37ºC, 200 rpm in shaker incubator (Daihan Labtech India Pvt. Ltd) for 2-3 days for *M. smeg.* and 3-5 days for *M. bovis.* OD_{595} of cultures were measured and culture of OD – 0.3 to 0.4 were further sub cultured in the same media to prepare secondary culture of 0.3 to 0.4 OD. Secondary culture was serially diluted to a final OD of 0.0004 (2 x 10^4 cells) for *M. smeg*. to prepare bacterial lawn. Bacterial lawn were prepared in 7H11 medium agar containing 10% ADC and 0.5% glycerol. Lawn were dried for 30 minutes in biosafety cabinet (-ve pressure/BSL-2 level, Atlantis India Ltd.). Plates were marked in to quartet and four sterile were placed in each quartet. Each disc was loaded with 10 µl of test compound dilutions/standard drug Isoniazid/vector DMSO as control. Plates were closed and incubated at 37^0C for 48 hrs *M. smeg.* Zone of inhibition were located on the surface of medium, the diameter (in millimetre) of non-growth were determined and compared with standard drug Isoniazid, culture and vector controls (DMSO only).

Resazurin Microplate Assay (REMA) for *M. bovis*

As described by Taneja NK and Tyagi, 2007,[26]*M. bovis* stocks were grown in 7H11 medium, at 37ºC until logarithmic phase (O. D_{595} ~ 0.4, described above) is obtained. Cultures were further diluted to an OD_{595} of 0.050 (4 × 10^7 Cells). Two fold serial dilutions of test compounds or standard drug Isoniazid were prepared into 100µl of 7H11medium in clear

bottom 96-well plate followed by addition of 100µl actively growing aerobic test cultures of OD_{595} of 0.050 (final OD_{595} is 0.025, 2 X 10^7 Cells). Vector Controls wells containing DMSO as solvent (VC), Medium only (M) and drug only (to detect auto-fluorescence of drug) and were used to calculate percentage inhibition of viability. Plates were incubated at 37°C for 5-7 days. Thereafter, Resazurin (30 µl of 0.02%) and Tween-80 (12.5 µl of 20%) were added to each well. Wells will be observed after 24 and 48 hr for a color change from blue to pink. Fluorescence was measured with excitation at 530 nm and emission at 590 nm using Gemini XS spectroflourimeter (Spectra MAX) in bottom reading mode. Visual MIC was defined as the lowest concentration of drug that prevented the color change. For flourometric MIC, background fluorescence from medium (M) and drug wells were subtracted. Percentage inhibition of the viability is defined as: 1-(mean florescence of triplicate wells containing test compound/mean florescence of triplicate VC wells) x 100.

RESULTS AND DISCUSSION

Purity of compounds was confirmed by single spot on TLC and Compounds have shown sharp melting points.The structure of the synthesized compounds was confirmed by IR, ^{1}H NMR. The IR spectra of Schiff's bases have shown a strong band at 1614 -1650, characteristic peaks of -CN group. Compounds of 3 series (3a, 3b, 3c, 3d, 3e, 3f) were reported earlier [25] therefore characterized by reported data [25]. Compounds 6a, 6b and 8a are novel and were characterized by IR, ^{1}H NMR ^{13}C NMR & Mass Spectrometry, % yield of synthesized compounds, their Melting points, IR peaks, ^{1}H and ^{13}C & Mass data are summarized in experimental section. The spectrums of compounds are shown in supplementary data (Figure S1 to S12).

Antimycobacterial activity of Schiff's bases

Preliminary screening of compounds was carried out in fast growing mycobacterial strain *M. smegmatis* using simple disc diffusion assay. 10 µl of highest concentration 500 µg/ml (in

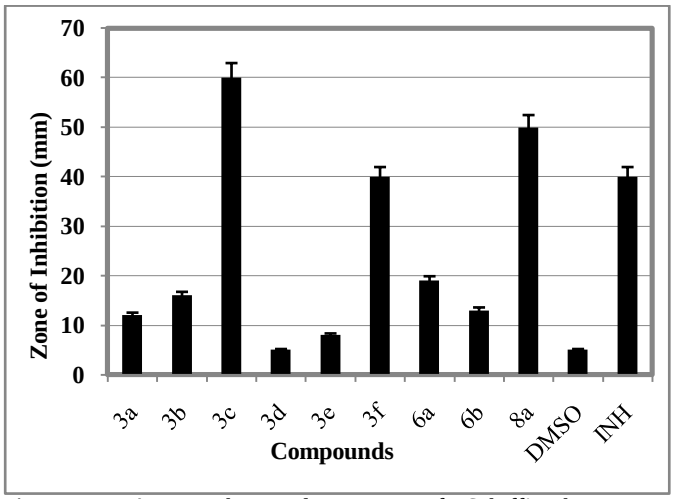


Figure 1: Antimycobacterial activity of Schiff's bases in *Mycobacterium smegmatis* using disc diffusion assay; growth inhibition activity of compounds are shown by zone of inhibition (mm).

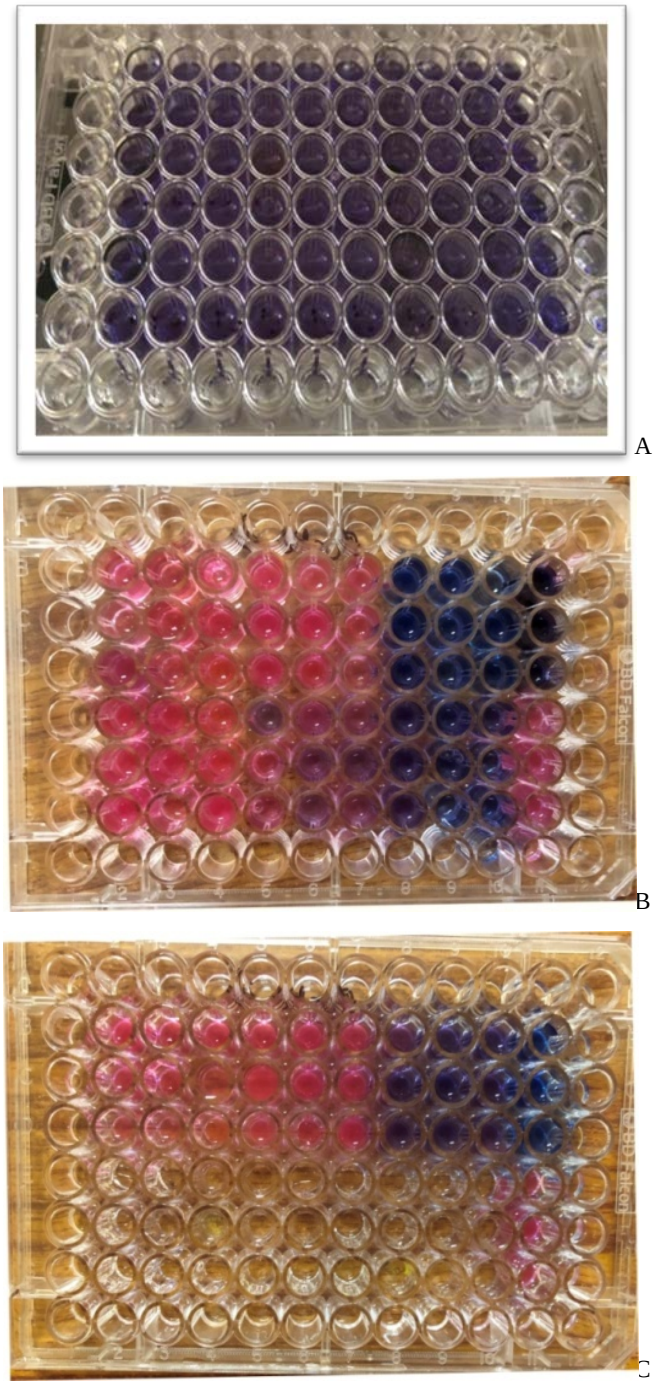


Figure 2 Antimycobacterial activity of Schiff's bases in *Mycobacterium bovis* usingResazurin Microplate Assay (REMA).

A. Plates just after adding Alamar Blue (Resazurin).

B. After 24 hours of incubation:Compound-3a: Triplicate Wells B2 to D2; 500 µM, B3 to D3; 250 µM, B4 to D4: 125 µM, Compound-3b: Triplicate Wells B5 to D5; 500 µM, B6 to D6; 250 µM, B7 to D7: 125 µM, Compound-3c: Triplicate Wells B8 to D8; 500 µM, B9 to D9; 250 µM, B10 to D10: 125 µM, Compound-3d: Triplicate Wells E2 to G2; 500 µM, E3 to G3; 250 µM, E4 to G4: 125 µM, Compound-3e: Triplicate Wells E5 to G5; 500 µM, E6 to G6; 250 µM, E7 to G7: 125 µM, Compound-3f: Triplicate Wells E8 to G8; 500 µM, E9 to G9; 250 µM, E10 to G10: 125 µM, Isoniazid (INH):

Wells B11-D11 40 µg/ml, DMSO (Vector Control): Wells E11-G11 500 µM, 250µM, 125µM respectively.

C. Compound-6a: Triplicate Wells B2 to D2; 500 µM, B3 to D3; 250 µM, B4 to D4: 125 µM, Compound-6b: Triplicate Wells B5 to D5; 500 µM, B6 to D6; 250 µM, B7 to D7: 125 µM, Compound-8a: Triplicate Wells B8 to D8; 500 µM, B9 to D9; 250 µM, B10 to D10: 125 µM, Blank (media+ dye): Wells B11-D11, Culture control (media+ dye+ culture): Wells E11-G11. All border wells are filled with 300µL of double distilled autoclaved water to reduce evaporation of culture medium.

DMSO) of each Schiff's base were tested. The zone of inhibition produced by each compound was determined by subtracting the inhibition zone produced by the vector control. Compounds 3c, 3f, 8a have shown 60 mm, 40 mm, and 50 mm zone of inhibition with respect to vector control. Compounds 3a, 3b, 3d, 3e and 6b have shown poor antimycobacterial activity (5 to 15 mm inhibition zone), compound 6a has shown 19 mm of inhibition zone. The standard drug INH has shown 40 mm zone of inhibition at 40µg/ml concentration, results were shown in the Figure 1. Schiff's bases 3c, 3f and 8a have shown promising antimycobacterial activity against *M. smegmatis*. antimycobacterial activity of synthesized compounds were reported at higher concentration i.e. 500 µg/ml with respect to standard INH drug (40µg/ml), because there was no earlier report or MIC data of synthesized compounds in Mycobacterium strains.

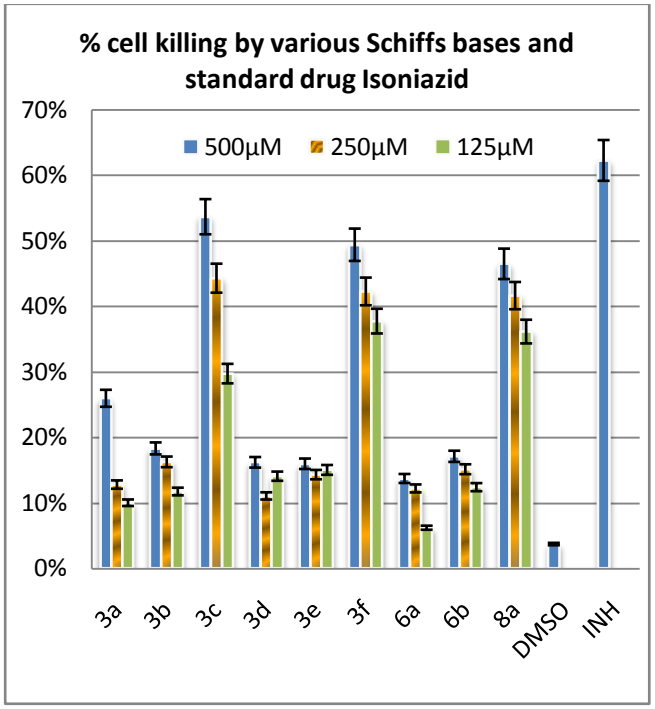


Figure 3: Antimycobacterial activity of Schiff's bases in *Mycobacterium bovis* usingResazurin Microplate Assay (REMA). % cell killing were calculated by fluorescence estimation. Mean of triplicate value is plotted in the graph, DMSO has not shown any inhibition at 250µM and lower concentration).

Compounds were further tested in *Mycobacterium bovis* BCG strain using quantitative and colorimetric Resazurin Microtitre Assay(REMA). Two fold dilutions of the compounds (in triplicate) were tested in 96 well plate format viz 500 µM, 250 µM and 125 µM. The growth inhibition was noticed by visual change in color of Alamar blue dye, from blue to pink by the vivel cells and no change in blue color by dead cells (Figure 2). The quantitative % cell killing was determined by estimation of fluorescence. Schiff's bases 3c, 3f and 8a have shown 53.69%, 49.41% and 46.49 % cell killing at 500 µM, the activity was concentration dependent and 44.30%, 42.28% and 41.64% of cell killing were noted at 250 µM, similarly 29.73%, 37.75% and 36.15% of cell killing was reported at 125 µM, respectively. Standard drug Isoniazid has shown 60% inhibition at 40 µg/ml concentration. Results are shown in Figure 3.

It was noticed that the antimycobacterial activity of benzaldehyde depends on position and type of substations present at benzene nucleus. Benzaldehyde carrying Nitro or hydroxyl substitutions showed antimycobacterial potential. On the other hand benzaldehyde and halogenated benzaldehydes were inactive. Preparation of Schiff's bases from these benzaldehydes may elicit one of the ways to modify their physical state, biological potential, bioavailability and toxicity. They may be turned in to potential candidate drug like compounds.

In our studywe found that simple benzaldehyde was inactive but 4-nitro benzaldehyde had goodantimycobacterialpotential, indicating that introduction of NO_2 group at para position turns the benzaldehyde to antimycobacterial molecule. We have tested all the benzaldehyde derivatives present in our laboratory (liquid and solids) and noted varied antimycobacterial activity. It was found that p-$NO_2$and p-OHbenzaldehydes weremore potent than ortho-meta Dihydroxy benzaldehyde, halogenated benzaldehyde like 4-Cl benzaldehydehad shown poor activity. Further we prepared variousSchiff's bases from these benzaldehydes and studied their antimycobacterial activity. Schiff's bases were prepared by usingamines viz 2-NH_2 pyridine or 3-substituted 2-amino pyridine (3-NO_2 and 3-Br_2, 2-amino pyridine) and Thiophene or Furoic or Nicotinic acid hydrazide.

It was observed that Schiff's bases prepared from either p-NO_2 or p-OH benzaldehyde with2-NH_2pyridine posses good antimycobacterial activity (they are compound 3c and 3f respectively). On the other hand Schiff's bases prepared from simple benzaldehyde or4-Cl benzaldehyde with 2-NH_2 pyridine were poor in antimycobacterial activity (compounds 3a and 3b respectively). Further we have used 3-substituted 2-amino pyridine (3-NO_2 or 3-Br_2)with 4-NO_2benzaldehyde to ensure whether activity can be modified by substitution at amine nucleus, these substitution results in reduced antimycobacterial activity.

Another series of compounds were prepared by using 2, 3 Dihydroxy benzaldehyde with Thiophene or Furoic of nicotinic acid hydrazide. It was noted that compounds with Thiophene or Furoic acid hydrazide were poor in antimycobacterial activity

while Schiff's bases with nicotinic hydrazide (compound 8a) posses good antimycobacterial activity.

CONCLUSION

In the present Study Schiff's bases were prepared from substituted benzaldehydes, which have shown antimycobacterial potential in preliminary screening. The substituted benzaldehydes were condensed with various amines like 2-amino pyridine/its derivatives or Thiophene or Furoic or Nicotinic acid hydrazide. Schiff's bases prepared from 4-NO_2 and 4-OH substituted benzaldehydes with 2-amino pyridine, have shown promising antimycobacterial activity compared to aldehyde alone. In another approach combining 2,3 Dihydroxy benzaldehyde with nicotinic acid hydrazide resulted in enhanced antimycobacterial potential compared to its corresponding benzaldehyde alone, against two of Mycobacterial test strains (*M. smegmatis and M. bovis*). Combining 2,3 Dihydroxy benzaldehyde with Thiophene or Furoicacid hydrazide resulted in reduced antimycobacterial activity (17 and 14% respectively at 500 µM). Therefore 2-amino pyridine and Nicotinic acid hydrazide are good amine partner for restoration and enhancement of antimycobacterial activity of substituted benzaldehydes.

We have used simple disc diffusion assay in *M. smegmatis* as screening assay. Compounds were further tested in more comprehensive REMA assay in *M. bovis* strain. The colorimetric resazurin microtiter assay is an inexpensive, rapid, and simple to perform,[30]Since resazurin has been recently identified as the main component of Alamar blue and recently resazurin microtiter assay (REMA) is used for detecting MDR-TB and demonstrated a very good correlation between results by this method and the proportion method (PM).[31, 32]

Synthesized schiffs bases have shown antimycobacterial potential in two of the mycobacterial test strains. Activity shown by compounds 3c, 3f and 8aare promising and the molecules may act as a potential hits, which can be transformed into potential leads through hit to lead optimization.Leads may be further tested in *Mycobacterium tuberculosis*and MDR strains totransformed into potential candidate drugs molecule against tuberculosis. Studies on mechanism of action of these compounds may lead to identification of new drug target for future drug discovery.

ACKNOWLEDGEMENT

RKG is thankful to Department of Biotechnology, Govt. of India, New Delhi, India for extramural research grants and DS is thankful to Netaji Subhas Institute of Technology, University of Delhi, New Delhi, India for research fellowship.

SUPPORTING INFORMATION

The IR and NMR spectra of the synthesized compounds is provided as supplementary file and can be downloaded from journal site free of charge.

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SUPPLEMENTARY INFORMATION

The spectral data for the synthesized have been provided as Supplementary file. Readers may download the data file from journal site free of charge.

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