International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:3, No:7, 2009

In vitro Anti-tubercular Screening of Newly Synthesized Benzimidazole Derivatives

M. Shahar Yar, M. Mustaqeem Abdullah, Jaseela Majeed

Abstract**—**A series of 1-(1*H*-benzimidazol-2-yl)-3-(substituted phenyl)-2-propen-1-one were allowed to react with hydrazine hydrate and phenyl hydrazine in submitted reactions to get pyrazoline and phenyl pyrazoline derivatives. All the compounds entered for screening at the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) for their *in vitro* antibacterial activity against Mycobacterium tuberculosis H37Rv strain (ATCC 27294) using Microplate Alamar Blue Assay (MABA) susceptibility test. The results expressed as MIC (minimum inhibitory concentration) in µg/mL. Among the fifteen compounds, eight compounds were found to have MIC values less than 10 µg/mL. These were subjected for cytotoxicity assay in VERO cells to determine CC_{50} (cytotoxic concentration 50%) values and finally SI (Selectivity Index) were calculated. Compound (XV) 2-[5-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1H-3-pyrazolyl]-1H-benzimidazole was considered the best candidate of the series that could be a good starting point to develop new lead compounds in the fight against tuberculosis.

Keywords**—**anti-tubercular activity, benzimidazole, pyrazoline.

I. Introduction

TUBERCULOSIS is still the greatest infectious cause of mortality world wide. It is the only disease which does not require any vector for transportation from one person to another or to cross the physical boundary of the countries. Being air born disease with no vaccine, it is the single largest disease encountered by both developing and developed countries. Two of the common problems associated with treatment, one is serious and life-threatening adverse effects of existing antitubercular drugs such as hepatotoxicity, neuritis, depression, asthenia, anorexia etc. which many a time forces to withdraw the treatment temporarily or change of treatment. Other one is the development of resistance due to non completion of treatment regime by patients and hence gene mutation by organisms made its management more difficult[1-2]. Another major concern is that tuberculosis is the most common HIV-related opportunistic infection, and caring for patients with both the disease is a major public health challenge[3]. Such circumstances forced the scientists across the globe to search newer molecules that can be used as lead compounds for the development of newer antitubercular drugs with better and safer therapeutic effects. To put some efforts in this field we reviewed the literature[4-11] and found worth to synthesize some benzimidazole derivatives with presumed antitubercular activity as extension of our earlier reported work[12-13].

Authors are with the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi, 110062. e-mail: jessfriend4u@yahoo.com

II. Materials and Methods

All the chemicals used were laboratory grade and provided by E. Merck (Germany) and S.D. Fine Chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Thin layer chromatography (TLC) plates prepared by silica gel G were used to monitor the reactions as well as to confirm the purity of the compounds synthesized & to verify the purity of commercial reagents. For the said purpose two different solvent systems; toluene: ethyl acetate: formic acid (5:4:1) and petroleum ether: toluene: acetic acid (5:4:1), were used to run the TLC. The spots were visualized under iodine vapors/UV light. IR spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer using KBr Pellets. ^{1}H NMR spectra were recorded on Bruker AC 400 MHz using TMS as internal standard in DMSO-d_6. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer. Antimycobacterial activities were performed by Microplate Alamar blue Assay method. (MABA) against Mycobacterium tuberculosis H37Rv strain.

General procedure for synthesis of chalcones (I-V)

According to Claisen-Schmidt condensation, 2-acetyl benzimidazole[14] was allowed to react with substituted aromatic aldehydes in 10% ethanolic NaOH solution to obtain the desired chalcone derivatives.

1-(1H-Benzimidazol-2-yl)-3-phenyl-2-propen-1-one (I)

IR (KBr, cm^{-1}): 3251 (N-H), 1661 (C=O), 1594 (C=N), 1329 (C-N). ^{1}H-NMR (DMSO-d_6): 6.68 (1H, t, J = 7.6, 7.6 Hz, Ar-H), 6.92 (1H, d, J = 16.8 Hz, H_α), 7.11 (1H, d, J = 16.4Hz, H_β), 7.20-7.32 (6H, m, Ar-H), 7.42 (1H, d, J = 8.0Hz, Ar-H), 7.50 (1H, d, J = 8.0Hz, Ar-H), 11.92 (1H, s, NH benzimidazole). Anal. Calcd. for $C_{16}H_{12}N_2O$: C, 77.40; H, 4.87; N, 11.28. Found: C, 77.12; H, 4.88; N,11.25%.

1-(1H-Benzimidazol-2-yl)-3-(4-methoxyphenyl)-2-propen-1-one (II)

IR (KBr, cm^{-1}): 3261 (N-H), 1653 (C=O), 1576 (C=N), 1330 (C-N). ^{1}H-NMR (DMSO-d_6): 3.63 (3H, s, OCH_3), 7.01(2H, d, J = 7.6 Hz, Ar-H), 7.32 (4H, m, Ar-H), 7.46 (1H, d, J = 16.4Hz, H_α), 7.54 (1H, d, J = 16.0 Hz, H_β), 7.81 (1H, d, J = 8.0 Hz, Ar-H), 7.85 (1H, d, J = 7.6 Hz, Ar-H), 13.11 (1H, s,

NH benzimidazole). Anal. Calcd. for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.52; H, 5.06; N, 10.08%.

1-(1H-Benzimidazol-2-yl)-3-(4-chlorophenyl)-2-propen-1-one (III)

IR (KBr, cm^{-1}): 3266 (N-H), 1653 (C=O), 1578 (C=N), 1332 (C-N), 771 (C-Cl). ^{1}H-NMR (DMSO-d_6): 7.28 (2H, d, J = 7.6 Hz, Ar-H), 7.48 (4H, m, Ar-H), 7.62 (1H, d, J = 16.0Hz, H_α), 7.68 (1H, d, J = 16.0 Hz, H_β), 7.72 (1H, d, J = 8.0 Hz, Ar-H), 7.75 (1H, d, J = 7.6 Hz, Ar-H), 11.80 (1H, s, NH benzimidazole). FAB MASS m/e: 283(M^{+1}). Anal. Calcd. for $C_{16}H_{11}N_2OCl$: C, 67.97; H, 3.92; N, 9.91. Found: C, 68.12; H, 3.91; N, 9.90%.

1-(1H-Benzimidazol-2-yl)-3-(4-bromophenyl)-2-propen-1-one (IV)

IR (KBr, cm^{-1}): 3261 (N-H), 1653 (C=O), 1576 (C=N), 1378 (C-N). ^{1}H-NMR (DMSO-d_6): 7.03-7.12 (4H, m, Ar-H), 7.32 (2H, d, J = 8.0 Hz, Ar-H), 7.36 (1H, d, J = 8.0 Hz, Ar-H), 7.39 (1H, d, J = 8.0 Hz, Ar-H), 7.51 (1H, d, J = 16.0 Hz, H_α), 7.55 (1H, d, J = 16.4 Hz, H_β), 13.11 (1H, s, NH benzimidazole). Calcd. for $C_{16}H_{11}N_2OBr$: C, 58.74; H, 3.39; N, 8.56. Found: C,58.75; H, 3.39; N, 8.56%.

1-(1H-Benzimidazol-2-yl)-3-(4-fluorophenyl)-2-propen-1-one (V)

IR (KBr, cm^{-1}): 3260 (N-H), 1650 (C=O), 1577 (C=N), 1335 (C-N). ^{1}H-NMR (DMSO-d_6): 6.89-7.20 (7H, m, Ar-H), 7.28 (1H, d, J = 15.6 Hz, H_α), 7.32 (1H, d, J = 15.6 Hz, H_β), 7.38 (1H, d, J = 7.6 Hz, Ar-H), 7.41 (1H, d, J = 8.0 Hz, Ar-H), 12.80 (1H, s, NH benzimidazole). Anal. Calcd. for $C_{16}H_{11}N_2OF$: C, 72.17; H, 4.16; N, 10.52. Found: C, 71.98; H, 4.15; N, 10.51%.

General procedure for synthesis of compounds (VI - X)

To an ethanolic solution of compound (I-V), hydrazine hydrate was added dropwise. The reaction mixture was heated under reflux for 6h and then cooled and poured onto crushed ice. The solid pyrazoline product was filtered and recrystallized from ethanol.

2-(5-Phenyl-4,5-dihydro-1H-3-pyrazolyl)-1H-benzimidazole (VI)

IR (KBr, cm^{-1}): 3103 (N-H), 1638 (C=N), 1330 (C-N). ^{1}H-NMR (DMSO-d_6): 3.21 (1H, dd, J = 8.4, 8.4 Hz, H_a), 4.02 (1H, dd, J = 13.2, 13.2 Hz, H_b), 5.81 (1H, dd, J = 8.0, 8.0 Hz, H_x), 6.82 (4H, m, Ar-H), 6.98 (1H, d, J = 8.0 Hz, Ar-H), 7.31 (1H, d, J = 8.0 Hz, Ar-H), 7.35 (3H, m, Ar-H), 11.01 (2H, s, 2 x NH). Anal. Calcd. for $C_{16}H_{14}N_4$: C, 73.26; H, 5.38; N, 21.36. Found: C, 73.51; H, 5.38; N, 21.40%.

2-[5-(4-Methoxyphenyl)-4,5-dihydro-1H-3-pyrazolyl]-1H-benzimidazole (VII)

IR (KBr, cm^{-1}): 3047 (N-H), 1608 (C=N), 1332 (C-N). ^{1}H-NMR (DMSO-d_6): 3.52 (1H, dd, J = 8.8, 8.8 Hz, H_a), 3.58 (3H, s, OCH_3), 4.09 (1H, dd, J = 13.6, 13.2 Hz, H_b), 6.13 (1H, dd, J = 8.4, 8.4 Hz, H_x), 6.73 (2H, d, J = 8.4 Hz, Ar-H), 7.13 (4H, m, Ar-H), 7.63 (1H, d, J = 8.0 Hz, Ar-H), 7.68 (1H, d, J = 8.0 Hz, Ar-H), 11.87 (2H, s, 2 x NH). FAB MASS m/e: 290(M^{-2}). Anal. Calcd. for $C_{17}H_{16}N_4O$: C, 69.85; H, 5.52; N, 19.17. Found: C,70.08; H, 5.50; N, 19.21%.

2-[5-(4-Chlorophenyl)-4,5-dihydro-1H-3-pyrazolyl]-1H-benzimidazole (VIII)

IR (KBr, cm^{-1}): 3003 (N-H), 1610 (C=N), 1330 (C-N), 766 (C-Cl).^{1}H-NMR (DMSO-d_6): 3.42 (1H, dd, J = 8.4, 8.4 Hz, H_a), 3.63 (1H, dd, J = 13.2, 13.2 Hz, H_b), 4.27 (1H, dd, J = 7.6, 8.0 Hz, H_x), 6.68 (6H, m, Ar-H), 6.81 (1H, d, J = 7.6 Hz, Ar-H), 6.92 (1H, d, J = 8.0 Hz, Ar-H), 7.32 (1H, d, J = 8.0 Hz, Ar-H), 11.18 (2H, s, 2 x NH). Anal. Calcd. for $C_{16}H_{13}N_4Cl$: C, 64.76; H, 4.42; N, 18.88. Found: C, 65.01; H, 4.43; N, 18.84%.

2-[5-(4-Bromophenyl)-4,5-dihydro-1H-3-pyrazolyl]-1H-benzimidazole (IX)

IR (KBr, cm^{-1}): 3112 (N-H), 1622 (C=N), 1333 (C-N). ^{1}H-NMR (DMSO-d_6): 3.01 (1H, dd, J = 8.4, 8.0 Hz, H_a), 3.76 (1H, dd, J = 13.2, 13.2 Hz, H_b), 4.41 (1H, dd, J = 8.0, 8.0 Hz, H_x), 6.73 (2H, d, J = 7.6 Hz, Ar-H), 7.28 (5H, m, Ar-H), 7.38 (1H, d, J = 8.0 Hz, Ar-H), 11.21 (2H, s, 2 x NH). Anal. Calcd. for $C_{16}H_{13}N_4Br$: C, 56.32; H, 3.84; N, 16.42. Found: C, 56.51; H, 3.99; N, 16.46%.

2-[5-(4-Fluorophenyl)-4,5-dihydro-1H-3-pyrazolyl]-1H-benzimidazole (X)

IR (KBr, cm^{-1}): 3008 (N-H), 1622 (C=N), 1330 (C-N). ^{1}H-NMR (DMSO-d_6): 3.12 (1H, dd, J = 8.0, 8.0 Hz, H_a), 3.92 (1H, dd, J = 13.6, 13.6 Hz, H_b), 4.23 (1H, dd, J = 7.6, 8.0 Hz, H_x), 6.67 (2H, d, J = 8.0 Hz, Ar-H), 6.85 (2H, d, J = 8.0 Hz, Ar-H), 6.91 (3H, m, Ar-H), 7.32 (1H, d, J = 7.6, Hz, Ar-H) 12.02 (2H, s, 2 x NH). Anal. Calcd. for $C_{16}H_{13}N_4F$: C, 68.56; H, 4.67; N, 19.99. Found: C, 68.67; H, 4.66; N, 19.98%.

General procedure for synthesis of compounds (XI-XV)

To a solution of compound (I-V) in ethyl alcohol, phenyl hydrazine was added dropwise. The reaction mixture was heated under reflux for 12h and then cooled and poured onto crushed ice. The solid phenyl pyrazoline product (XI-XV) was filtered and recrystallized from ethanol.

2-(1,5-diphenyl-4,5-dihydro-1H-3-pyrazolyl)-1H-benzimidazole (XI)

IR (KBr, cm^{-1}): 2832 (N-H), 1608 (C=N), 1374 (C-N). ^{1}H-NMR (DMSO-d_6): 3.11 (1H, dd, J = 9.2, 9.2 Hz, H_a), 3.75 (1H, dd, J = 14.0, 14.0 Hz, H_b), 5.82 (1H, dd, J = 9.2, 9.2 Hz, H_x), 6.62-7.81 (14H, m, Ar-H), 10.52 (1H, s, NH benzimidazole). Anal. Calcd. for $C_{22}H_{18}N_4$: C, 78.08; H, 5.36; N, 16.56. Found: C, 78.01; H, 5.53; N, 16.55%.

2-[5-(4-Methoxyphenyl)-1-phenyl-4,5-dihydro-1H-3-pyrazolyl]-1H-benzimidazole (XII)

IR (KBr, cm^{-1}): 3047 (N-H), 1582 (C=N), 1374 (C-N). ^{1}H-NMR (DMSO-d_6): 3.02 (1H, dd, J = 9.6, 9.6 Hz, H_a), 3.56 (3H, s, OCH_3), 3.72 (1H, dd, J = 14.0, 14.0 Hz, H_b), 5.91 (1H, dd, J = 9.6, 9.2 Hz, H_x), 6.62 (3H, m, Ar-H), 6.92 (2H, d, J = 8.4 Hz, Ar-H), 7.21 (1H, d, J = 8.0 Hz, Ar-H), 7.29 (4H, m, Ar-H), 7.33 (1H, d, J = 8.0 Hz, Ar-H), 7.45 (2H, m, Ar-H), 11.21 (1H, s, NH benzimidazole). Anal. Calcd. for $C_{23}H_{20}N_4O$: C, 74.98; H, 5.47; N, 15.21. Found: C, 75.01; H, 5.47; N, 15.22%.

2-[5-(4-Chlorophenyl)-1-phenyl-4,5-dihydro-1H-3-pyrazolyl]-1H-benzimidazole (XIII)

IR (KBr, cm^{-1}): 3003 (N-H), 1582 (C=N), 1332 (C-N), 738 (C-Cl).^{1}H-NMR (DMSO-d_6): 3.41 (1H, dd, J = 9.6, 9.2 Hz, H_a), 3.58 (1H, dd, J = 13.6, 13.6 Hz, H_b), 5.81 (1H, dd, J = 9.2, 9.2 Hz, H_x), 6.66 (5H, m, Ar-H), 6.72 (1H, d, J = 8.4 Hz, Ar-H), 6.78 (3H, m, Ar-H), 6.82 (1H, d, J = 8.4 Hz, Ar-H), 6.91-7.21 (3H, m, Ar-H), 9.82 (1H, s, NH benzimidazole). Anal. Calcd. for $C_{22}H_{17}N_4Cl$: C, 70.87; H, 4.60; N, 15.03. Found: C, 70.88; H, 4.61; N, 15.03%.

2-[5-(4-Bromophenyl)-1-phenyl-4,5-dihydro-1H-3-pyrazolyl]-1H-benzimidazole (XIV)

IR (KBr, cm^{-1}): 3014 (N-H), 1608 (C=N), 1328 (C-N). ^{1}H-NMR (DMSO-d_6): 3.26 (1H, dd, J = 9.2, 9.2 Hz, H_a), 3.82 (1H, dd, J = 14.0, 13.6 Hz, H_b), 6.11 (1H, dd, J = 9.2, 9.2 Hz, H_x), 6.67 (1H, d, J = 8.0 Hz, Ar-H), 6.78 (2H, d, J = 8.0 Hz, Ar-H), 6.81 (3H, m, Ar-H), 6.88 (1H, d, J = 8.0 Hz, Ar-H), 6.92-7.28 (6H, m, Ar-H), 11.01 (1H, s, NH benzimidazole). Anal. Calcd. for $C_{22}H_{17}N_4Br$: C, 63.32; H, 4.11; N, 13.43. Found: C, 63.33; H, 4.11; N, 13.44%.

2-[5-(4-Fluorophenyl)-1-phenyl-4,5-dihydro-1H-3-pyrazolyl]-1H-benzimidazole (XV)

IR (KBr, cm^{-1}): 3004 (N-H), 1608 (C=N), 1305 (C-N). ^{1}H-NMR (DMSO-d_6): 3.30 (1H, dd, J = 9.6, 9.6 Hz, H_a), 3.56 (1H, dd, J = 14.0, 14.0 Hz, H_b), 4.92 (1H, dd, J = 9.6, 9.6 Hz, H_x), 6.78 (1H, t, J = 7.6, 7.6 Hz, Ar-H), 6.81 (2H, d, J = 7.6 Hz, Ar-H), 6.88 (4H, m, Ar-H), 6.91 (2H, d, J = 8.0 Hz, Ar-H), 7.01 (1H, d, J = 7.6 Hz, Ar-H), 7.21(1H, d, J = 7.6 Hz, Ar-H), 7.28 (2H, m, Ar-H), 9.88 (1H, s, NH benzimidazole). Anal. Calcd. for $C_{22}H_{17}N_4F$: C, 74.14; H, 4.81; N, 15.72. Found: C, 74.18; H, 4.80; N, 15.72%.

Biology

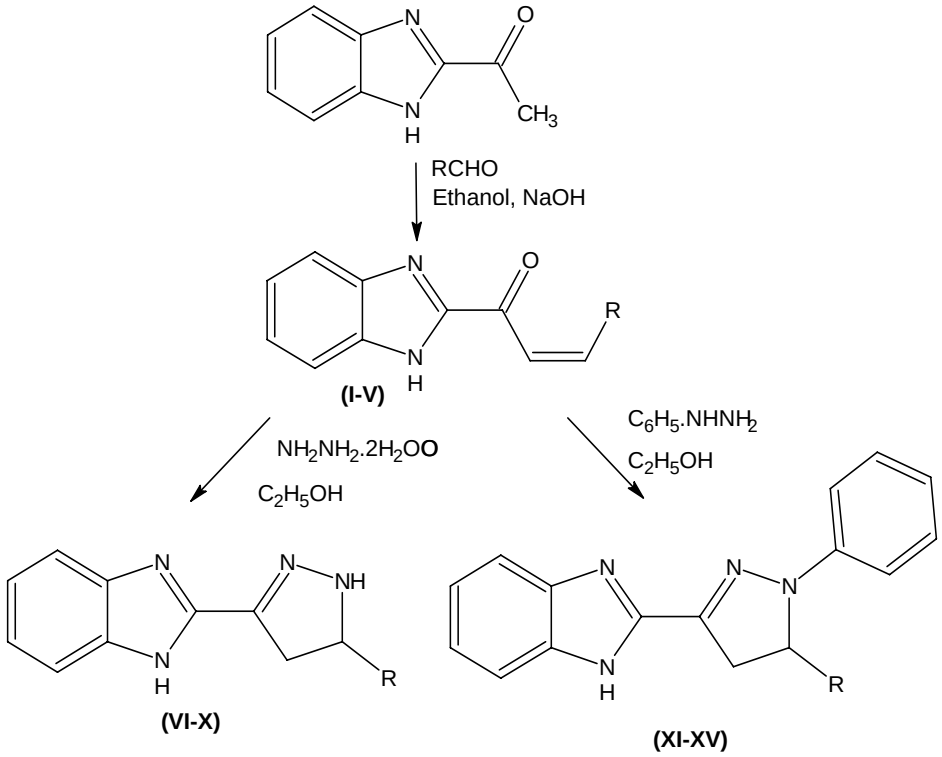
Antimycobacterial activity by Alamar blue susceptibility test (MABA)[15]: Antimicrobial susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument Company, Meriden, Conn.) in order to minimize background fluorescence. Outer perimeter wells were filled with sterile water to prevent dehydration in experimental wells. Initial drug dilutions were prepared in either dimethyl sulfoxide or distilled deionized water, and subsequent twofold dilutions were performed in 0.1 ml of 7H9GC (no Tween 80) in the microplates. BACTEC 12B-passaged inocula were initially diluted 1 : 2 in 7H9GC, and 0.1 ml was added to wells. Subsequent determination of bacterial titers yielded 1×10^6, 2.5×10^6 and 3.5×10^5 CFU/ml in plate wells for *M. tuberculosis* $H_{37}Rv$. Frozen inocula were initially diluted 1 : 20 in BACTEC 12B medium followed by a 1 : 50 dilution in 7H9GC. Addition of 1/10 ml to wells resulted in final bacterial titers of 2.0×10^5 and 5×10^4 CFU/ml for *M. tuberculosis* $H_{37}Rv$. Wells containing drugs only were used to detect autofluorescence of compounds. Additional control wells consisted of bacteria only (B) and medium only (M). Plates were incubated at 37°C. Starting at day 4 of incubation, 20 µl of 10× alamar Blue solution (Alamar Biosciences/Accumed, Westlake, Ohio) and 12.5 µl of 20% Tween 80 were added to one B well and one M well, and plates were reincubated at 37°C. Wells were observed at 12 and 24 h for a color change from blue to pink and for a reading of ≥50,000 fluorescence units (FU). Fluorescence was measured in a Cytofluor II microplate fluorometer (PerSeptive Biosystems, Framingham, Mass.) in bottom-reading mode with excitation at 530 nm and emission at 590 nm. If the B wells became pink by 24 h, reagent was added to the entire plate. If the well remained blue or ≤50,000 FU was measured, additional M and B wells were tested daily until a color change occurred, at which time reagents were added to all remaining wells. Plates were then incubated at 37°C, and results were recorded at 24 h post-reagent addition. Visual minimum inhibitory concentration (MIC) was defined as the lowest concentration of drug that prevented a color change. For fluorometric MICs, a background subtraction was performed on all wells with a mean of triplicate M wells. Percent inhibition was defined as 1-(test well FU/mean FU of triplicate B wells) × 100. The lowest drug concentration effecting an inhibition of ≥90% was considered the MIC.

III. Results and Discussion

1-(1H-Benzimidazol-2-yl)-3-(substituted phenyl)-2-propen-1-one (I-V), 2-[5-(Substituted phenyl)-4,5-dihydro-1H-3-pyrazolyl]-1H-benzimidazole (VI-X) and 2-[5-(4-Substituted phenyl)-1-phenyl-4,5-dihydro-1H-3-pyrazolyl]-1H-benzimidazole (XI-LV) described in this study were synthesizes according to outlined Scheme-I and their physical data is presented in Table-I. The chalcones (I-V) were prepared by reacting 2-acetyl benzimidazole with appropriate aldehydes in presence of a base by well established Claisen-Schmidt condensation. The reaction between newly synthesized chalcones with hydrazine hydrate and phenyl hydrazine separately in ethanol led to synthesis of novel pyrazoline derivatives bearing benzimidazole. The purity of the compounds was checked by TLC and elemental analysis. Both analytical and spectral data (IR, & ^{1}H NMR) of all the synthesized compounds were in full agreement with the proposed structures. In general all the compound in the infrared (IR) spectra revealed N-H, C=N, C-N peaks at 3000, 1600 and 1330 cm^{-1} respectively. In the proton nuclear magnetic resonance (^{1}H NMR) spectra the signals of the respective protons of the prepared titled compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed three distinct and characteristic double doublets (dd), in the aliphatic region, each with integration equal to one proton. Among the three double doublets, two exhibited nearly identical *J* values were assigned one for each of the 2 protons of pyrazoline at C-4 (methylene) where as third double doublet with different *J* value assigned to one proton at C-5 of pyrazoline, for the same reason, two protons at C-4 of pyrazoline are indicated as Ha and Hb whereas one proton at C-5 is indicated by Hx. The N-H protons were found deuterium exchangeable. The elemental analysis results were within ±0.4% of the theoretical values.

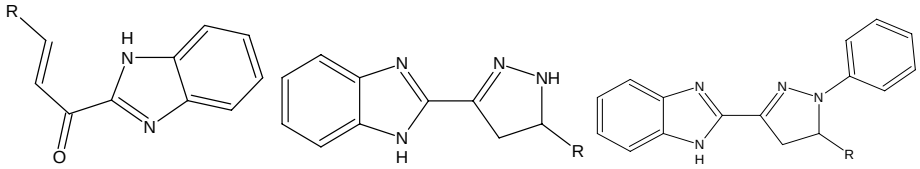
Antimycobacterial activity

All the compounds of the series (I-LX) entered for screening at the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) and evaluated for their *in vitro* antibacterial activity against Mycobacterium tuberculosis H37Rv strain (ATCC 27294) using Microplate Alamar Blue Assay (MABA) susceptibility test. The results expressed as MIC (minimum inhibitory concentration) in µg/mL. Among fifteen compounds, eight compounds were found to have MIC values less than 10µg/mL. These were subjected for cytotoxicity assay in VERO cells to determine CC_{50} (cytotoxic concentration 50%) values and finally SI (Selectivity Index) were calculated. Compound (XV) 2-[5-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1H-3-pyrazolyl]-1H-benzimidazole, exhibited 90% inhibition at a concentration of 5.855 µg/mL with selectivity index of more than 3, was considered the best candidate of the series that could be a good starting point to develop new lead compounds in the fight against tuberculosis.



Scheme -I

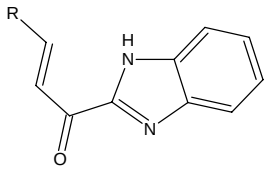
TABLE I PHYSICAL CONSTANT



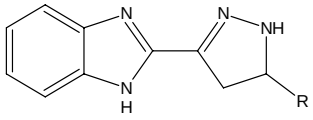
(I-V) (VI-X) (XI -XV)

Compound code	R	Molecular Formula	Molecular Weight	Melting Point ºC
I	Phenyl	$C_{16}H_{12}N_2O$	248	194-196
II	4-Methoxyphenyl	$C_{17}H_{14}N_2O_2$	278	184-188
III	4-Chlorophenyl	$C_{16}H_{11}N_2OCl$	282	202-204
IV	4-Bromophenyl	$C_{16}H_{11}N_2OBr$	327	224-226
V	4-Fluorophenyl	$C_{16}H_{11}N_2OF$	266	182-184
VI	Phenyl	$C_{16}H_{14}N_4$	262	140-142
VII	4-Methoxyphenyl	$C_{17}H_{16}N_4O$	292	234-236
VIII	4-Chlorophenyl	$C_{16}H_{13}N_4Cl$	296	168-170
IX	4-Bromophenyl	$C_{16}H_{13}N_4Br$	341	160-162
X	4-Fluorophenyl	$C_{16}H_{13}N_4F$	280	152-154
XI	Phenyl	$C_{22}H_{18}N_4$	338	136-137
XII	4-Methoxyphenyl	$C_{23}H_{20}N_4O$	368	120-124
XIII	4-Chlorophenyl	$C_{22}H_{17}N_4Cl$	372	138-140
XIV	4-Bromophenyl	$C_{22}H_{17}N_4Br$	417	142-144
XV	4-Fluorophenyl	$C_{22}H_{17}N_4F$	356	128-130

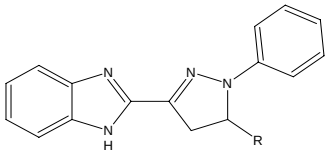
TABLE II ANTI-MYCOBACTERIAL ACTIVITY AGAINST H37RV STRAIN OF MYCOBACTERIUM TUBERCULOSIS



(I-V)



(VI-X)



(XI -XV)

Sample code	R (Substitutions)	TAACF Sample I.D.	MABA: H37Rv Data		CTG: Vero Cell	SI (CC_{50}/IC_{90})
			IC_{50} (µg/mL)	IC_{90} (µg/mL)	CC_{50} (µg/mL)	
I	Phenyl	424217	1.255	1.83	2.446	1.336
II	4-methoxyphenyl	424218	5.302	6.588	3.78	0.573
III	4-chlorophenyl	424219	3.552	13.178	-	-
IV	4-bromophenyl	424220	49.838	>50	-	-
V	4-fluorophenyl	424221	1.538	1.751	2.967	1.694
VI	Phenyl	424222	8.562	10.864	-	-
VII	4-methoxyphenyl	424223	13.431	20.812	-	-
VIII	4-chlorophenyl	424224	10.413	11.387	-	-
IX	4-bromophenyl	424225	5.264	19.454	-	-
X	4-fluorophenyl	424226	15.037	21.356	-	-
XI	Phenyl	424227	3.336	6.352	9.156	1.441
XII	4-methoxyphenyl	424228	4.109	6.536	11.546	1.766
XIII	4-chlorophenyl	424229	2.696	5.8	8.321	1.434-
XIV	4-bromophenyl	424230	2.759	7.047	17.164	2.435
XV	4-fluorophenyl	424231	3.424	5.855	18.964	3.238

MABA Microplate Alamar Blue Assay.
H37Rv Strain of micobacterium tuberculosis used in the study.
IC_{50}, IC_{90} Concentration that causes 50% & 90% microbial growth inhibition respectively.
CTG: Cytotoxicity in Vero Cell Culture to determine 50% Cytotoxic concentration (CC_{50}).
SI Selectivity Index, should be more than or equal to 10 for further studies.
- Not applicable

REFERENCES

[1] Brenwald, N.P., Gill, M.J., and Wise, R .(1998) Prevalence of a putative efflux mechanism among fluoroquinolone -resistant clinical isolates of streptococcus pneumoniae.Antimiarob.Agents.Chemother 42,2032-2035.

[2] Klemens,S.P., De Stefano,M.S., and Cynamon,M.H (1993.Therapy of multidrug resistant tuberculosis:lessons from studies with mice. Antimiarob.Agents.Chemother 37, 2344-2347.

[3] Corbett,E.L., Watt,C.J., Walker,N.,Maher,D., Williams,B.G., Raviglione,M.C., and Dye,C.(2003) The growing burdens of tuberculosis:global trends and interactions with HIV epidemic. Arch Intern Med 163,1009-1021

[4] Aridoss, G., Amirthaganesan, S., Kumar, N.A., Kim, J.T., Lim, K.T., Kabilan, S., Jeong, Y.T. A facile synthesis, antibacterial, and antitubercular studies of some piperidin-4-one and tetrahydropyridine derivatives. Bioorg Med Chem Lett. 2008, 18(24):6542-8. Epub 2008 Oct 14.

[5] Gill, C., Jadhav, G., Shaikh, M., Kale, R., Ghawalkar, A., Nagargoje, D., Shiradkar. Clubbed [1, 2, 3] triazoles by fluorine benzimidazole: a novel approach to H37Rv inhibitors as a potential treatment for tuberculosis M. Bioorg Med Chem Lett. 2008, 18(23):6244-7. Epub 2008 Oct 2

[6] Mohamed, B.G., Hussein, M.A., Abdel-Alim, A.A., Hashem, M. Synthesis and antimicrobial activity of some new 1-alkyl-2-alkylthio-1, 2, 4-triazolobenzimidazole derivatives. Arch Pharm Res. 2006, 29(1):26-33.

[7] Gasparová, R., Lácová, M., el-Shaaer, H.M., Odlerová, Z. Synthesis and antimycobacterial activity of some new 3-heterocyclic substituted chromones. Farmaco. 1997, 52(4):251-3.

[8] Geban, O., Ertepinar, H., Ozden, S. QSAR analysis of a set of benzimidazole derivatives based on their tuberculostatic activities. Pharmazie. 1996, 51(1):34-6.

[9] Alunni Bistocchi, G., De Meo, G., Pedini, M., Ricci, A., Bastianini, L., Sposini, T., Sbaraglia, G., Jacquignon, P. [New heterocyclic derivatives of benzimidazole with germicidal activity. III. Synthesis and activity of derivatives of (formyl-5'-furyl-2')-2-benzimidazole with different substitutions at position 5]. Farmaco [Sci]. 1986 41(12):970-83.
[10] Bell, S.C., Wei, P.H. Syntheses of heterocylic fused thiazole acetic acids. 2. Bell SC, Wei PH. J Med Chem. 1976, 19(4):524-30.
[11] Sawlewicz J, Milczarska B, Manowska W. Reactions of cyanomethylbenzimidazoles. Part II. Reaction of cyanomethylbenzimidazoles with aldehydes methylketones and nitroso compounds. Pol J Pharmacol Pharm. 1975, 27(2):187-201.
[12] Synthesis, structure activity relationship and anti-tubercular activity of novel pyrazoline derivatives. M.A. Ali, M. Shaharyar, A. A. Siddiqui, Eur. J. Med. Chem. 42 (2007) 268-275.
[13] Synthesis and in vitro antimycobacterial activity of N1-nicotinoyl-3-(4'-hydroxy-3'-methyl phenyl)-5-[(sub)phenyl]-2-pyrazoline. M. Shaharyar, A.A.Siddiqui, M.A. Ali, D. Shriram, P.Yogeeshwari, Bioorg. Med. Chem. Lett.16 (2006) 3947-3949.
[14] G.W.H. Cheeseman, J. Chem. Soc. (1964) 4645-46.
[15] Collins, L. and Franzblau, S. G. (1997) Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium. Antimicrob. Agents Chemother., 41, 1004–1009.