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# Synthesis, Characterization and Docking study of Fused Benzimidazole and Pyrazole Derivatives as Antitubercular Agents

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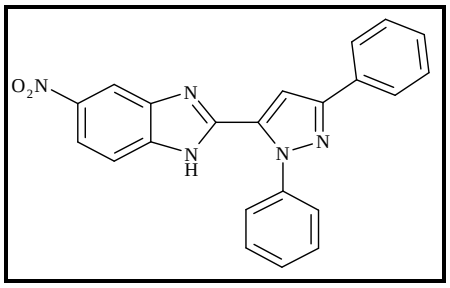
## ABSTRACT

*A series of fused benzimidazole with pyrazole derivatives (4A-D) were synthesized from 5-Nitro-1H-benzo[d]imidazol-2-yl)-3-phenylpropan-1-one on the basis of docking score which has been taken by Schrodinger. In docking study all the designed compounds were targeted with active site of M. Tuberculosis enoyl ACP reductase enzyme encoded with the gene InhA. (5 JFO), which is an important to mycobacteria for the synthesis of mycolic acid. Synthesized compounds were characterized by IR, H'NMR, Mass spectroscopy and evaluated for in-vitro anti-TB activity against Mycobacterium Tuberculosis H37Rv strain by Alamar-Blue assay. The results expressed as MIC (minimum inhibitory concentration) in mg $mL^{-1}$. Antitubercular assay has been carried out at Micropharm Diagnosis Center, Gandhi nagar. Among the four compounds 4A, 4C, 4D shown same docking and gliding score and activity at (MIC 6.25 mg $mL^{-1}$) followed by 4B( MIC 12.5 mg $mL^{-1}$).*

## Graphical Abstract




Pharmacophoric pattern required for Anti-tubercular activity

**Keywords:** Benzimidazole, Pyrazole, Alamar-blue assay, Antitubercular, MIC.

## INTRODUCTION

Tuberculosis is a chronic granulomatous disease caused by *Mycobacterium Tuberculosis*, which mainly affects the Lung and other organs and tissues, According to latest statistical data provided by World Health Organization, in India 10.4 million of peoples are affected by TB and more than 1.4 million people die in 2016. Tuberculosis is called as a primary killer of the people across the world. Main hurdles are increasing population, overcrowding, malnutrition, ignorance, discontinuation of the treatment and important one is the increasing resistance of *Mycobacterium* over the existed antitubercular therapy [1]. So there is a need to introduce a new drug which can target to an enzyme which is necessary for the survival of *Mycobacterium.*

Enoyl acyl carrier protein reductase is the enzyme encoded with InhA gene, which is essential for the survival of mycobacterium through the biosynthesis of mycolic acid. In the biosynthesis InhA plays an important role of chain elongation by catalyzing the hydride transfer to long-chain enoyl thioester substrates that is final essential enzymatic step in fatty acid elongation in the FAS-II pathway, converting 2-trans-enoyl-ACP to acyl-ACP via a hydride transfer from the 4S hydrogen of NADH to the C3 position of the 2-trans-enoyl-CoA(ACP) substrate. MtInhA is a member of the mycobacterial type II dissociated fatty acid biosynthesis system, and is the bona fide target for ionized, the most prescribed drug for tuberculosis treatment [2]. Here, a series of Benzimidazole fused Pyrazole derivatives were synthesized and screened as MtInhA inhibitors with pharmacophoric pattern as per shown in figure 1.

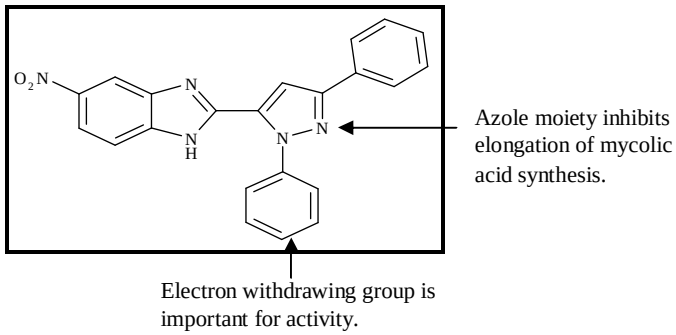



**Figure 1.** Pharmacophoric pattern required for Anti-tubercular activity.

## MATERIALS AND METHODS

Melting points were determined using a VEEGO make microprocessor based melting point apparatus having silicone oil bath and are uncorrected. IR spectra (wave numbers in $cm^{-1}$) were recorded on a BRUKER ALPHA FT-IR spectrophotometer using Potassium bromide discs. NMR spectra were recorded on BRUKER AVANCE II 400 MHz instrument in $CDCl_3$ with TMS as internal standard for $^1H$ NMR. Chemical shift values are mentioned in δ, ppm. Mass spectra were recorded on Advion Expression, CMS, USA at Syn Zeal Research Solutions, Gandhi nagar. Chromatographic separations were performed on columns using silica gel 100–200 mesh. The progress of all reactions was monitored by TLC on 2 cm X 5 cm pre-coated silica gel 60 $F_{254}$ (Merck) plates of thickness of 0.25 mm. The chromatograms were visualized under UV 254 nm and/or exposure to iodine vapours. All reagents used were of analytical reagent grade, obtained from LOBA chemicals, SDFCL and Spectrochem. Chemicals and solvents were purified by general laboratory techniques before use. All moisture free operations were performed in oven dried glass wares and under nitrogen atmosphere.

### Synthesis and Spectral Data

**(5-Nitro-1*H*-benzo[*d*]imidazol-2-yl) ethanol (1):** Substitute to phenylenediamine (0.1 mol, 0.5 g.) was mixed with lactic acid (0.1 mol, 0.6 g.) in a RBF and reflux in water bath for 6-8 h. The reaction

mixture was cooled to room temperature and added with 10% NaOH until basic to litmus paper. The product obtained was thoroughly washed with water until it free from the base in the product. The product obtained was dried over a hot air oven and recrystallized with hot water [3, 4]. Yield 0.42 g. (79.2 %), m.p.194-196 ºC, $R_f$ 0.53 (Hexane: Ethyl Acetate, 6:4), IR (KBr, $cm^{-1}$) 3570, 3340, 3108, 1517, 1346.

**(5-Nitro-1*H*-benzo[*d*]imidazol-2-yl) ethanone (2):** To a solution of1-(5-Nitro-1*H*-benzo[*d*]imidazol-2-yl) ethanol, (10 mmol, 0.42 g.) in aqueous acetic acid (5% v/v, 10 mL) was added at room temperature a solution of potassium dichromate (10 mmol, 0.54 g.) in aqueous acetic acid (5% v/v, 10 mL) and the mixture stirred for over a period of 15 min by using a magnetic stirrer. The separated product 2-acetyl benzimidazole was filtered, washed with water, dried and purified by recrystallization with ethanol [3, 4]. Yield 0.45 g. (67.1 %), m.p.198-200 ºC, $R_f$ 0.45 (Hexane: Ethyl Acetate, 6:4), IR (KBr, $cm^{-1}$) 3384, 3000, 1590, 1335.

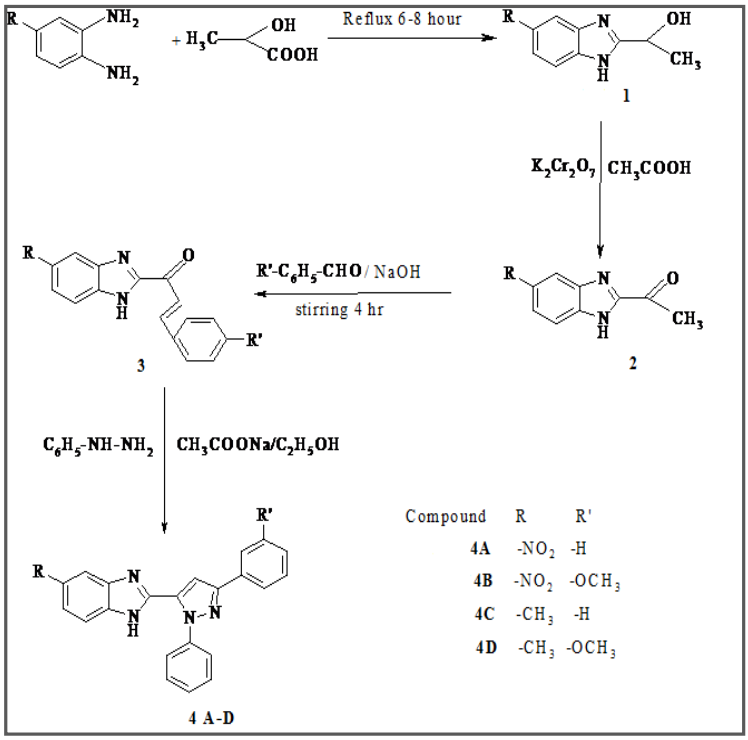



**Figure 2.** Scheme for the synthesis of Benzimidazole fused Pyrazole Derivatives.

**(5-Nitro-1*H*-benzo[d]imidazol-2-yl)-3-phenylpropan-1-one(3):** 1-(5-Nitro-1*H*-benzo[*d*] imidazol-2-yl) ethanone (0.01 mol, 0.45 g.) was taken in aq. NaOH (10%, 30 mL) and added with the benzaldehyde (0.01 mol, 0.51 mL) at room temperature. The reaction mixture was stirred for over a period of 4 h by using a magnetic stirrer. The separated solid the chalcone derivative of benzimidazole was filtered, washed with water, dried and purified by recrystallization with ethanol [3, 4]. Yield 0.48 gm. (70.3 %) m.p. 214-216ºC, $R_f$ : 0.56 (Benzene : Acetone, 9:1), IR (KBr, $cm^{-1}$) 3234, 2925, 1642, 1573,1511.

**(1, 3-Diphenyl-1*H*-pyrazol-5-yl)-5-nitro-1H-benzo[d]imidazole(4A):** To an equimolar amount of **(**1-(5-Nitro-1*H*-benzo[d]imidazol-2-yl)-3-phenylpropan-1-one (0.03 mol, 0.4 g.) and phenyl hydrazine (0.03 mol, 0.6 mL) were mixed in ethanolic sodium acetate (25 mL) and refluxed for 6-7 h. The reactions were monitored by TLC. The mixture was concentrated on water bath and poured into ice-cold water. The precipitate obtained was filtered, washed with water, dried and purified by

recrystallization from ethanol. Yield 0.45 gm. (67.2 %) m.p. 230- 232 ºC $R_f$0.49 (Benzene: Acetone, 9:1) IR (KBr, cm-1) 3323, 3058, 1569, 1497, 1330, 883, NMR (δ ppm) 5.0 (s, NH), 7.22-7.62 (m, Ar C-H), 8.05 ( m, C-H)MASS (*m/z*)Molecular ion peak (M-1) 380.39.

**(3-(4-Methoxy phenyl)-1-phenyl-1*H*-pyrazol-5-yl)-5-nitro-1*H*-benzo[d] imidazole (4B):** To an equimolar amount of mixture of 3-(4-Methoxyphenyl)-1-(5-nitro-1*H*-benzo[d]imidazol-2-yl)propan-1-one (0.03 mol, 0.5 g.) and phenyl hydrazine (0.03 mol, 0.63 mL) were mixed in ethanolic sodium acetate (25 mL) and refluxed for 6-7 h. The reactions were monitored by TLC. The mixture was concentrated on water bath and poured into ice-cold water. The precipitate obtained was filtered, washed with water, dried and purified by recrystallization from ethanol [**3**]. Yield 0.47 gm. (69.3 %), m.p. 250-252ºC, $R_f$ : 0.51 (Benzene : Acetone, 9:1), IR (KBr, $cm^{-1}$) 3319, 2922, 1597, 1495, 1332,1067, 916, NMR (δ ppm) 3.73 (t,$OCH_3$), 5.0 (s, NH), 7.24-7.52 (m, Ar C-H), 8.03( m, C-H), MASS (*m/z*) Molecular ion peak (M+1) 412.41.

**(1, 3-Diphenyl-1*H*-pyrazol-5-yl)5-methyl-*1H*-benzo[d]imidazole (4C):** To an equimolar amounts of mixture of 1-(5-Methyl-1*H*-benzo[d]imidazol-2-yl)-3-phenylpropan-1-one (0.03 mol, 0.6 g.) and phenyl hydrazine(0.03 mol, 0.72 mL) were mixed in ethanolic sodium acetate (25 mL) and refluxed for 6-7 h. The reactions were monitored by TLC. The mixture was concentrated on water bath and poured into ice-cold water. The precipitate obtained was filtered, washed with water, dried and purified by recrystallization with ethanol. Yield 0.52 g. (77.4 %), m.p.226-228ºC, $R_f$ 0.53 (Benzene: Acetone, 9:1), IR (KBr, $cm^{-1}$) 3345, 2920, 1607, 831. NMR (δ ppm) 2.33 (t, CH3), 5.2 (s, NH), 7.24-7.66 (m, Ar C-H), 8.03( m, C-H).MASS (*m/z*) Molecular ion peak (M-1) 349.42.

**(3-(4-Methoxy phenyl)-1-phenyl-1*H*-pyrazol-5-yl)-5-methyl-1*H*-benzo[d]imidazole (4D):** To an equimolar amount of mixture of 3-(4-Methoxy phenyl)-1-(5-methyl-1*H*-benzo[d]imidazol-2-yl) propan-1-one (0.03 mol, 0.48 g.) and phenyl hydrazine (0.03 mol, 0.6 mL) were mixed in ethanolic sodium acetate (25 mL) and refluxed for 6-7 h. The reactions were monitored by TLC. The mixture was concentrated on water bath and poured into ice-cold water. The precipitate obtained was filtered, washed with water, dried and purified by recrystallization with ethanol [**3, 4**]. Yield 0.45 gm. (67.12 %), m.p. 244-246°C, $R_f$ 0.55 (Benzene : Acetone, 9:1) , IR (KBr, cm-1) 3328, 2925, 1597, 1072, 916, NMR (δ ppm)3.84 (t, $OCH_3$), 2.53 (t, $CH_3$), 5.0 (s, NH), 7.22-7.62 (m, Ar C-H), 8.02( m, C-H), MASS (*m/z*) Molecular ion peak (M+1) 381.44.

**Molecular Docking:** Scrodinger software was used to perform all docking simulations. A set of new benzimidazole fused Pyrazole derivatives were subjected to docking with *Enoyl acyl carrier protein Reductase* (PDB ID 5JFO) From the Protein Data Bank (RCSB) (http://www.rcsb.org/pdb). To carry out in docking studies, the 2D structures of the synthesized ligands 4(A-D**)** were drawn and converted to energy minimized 3D, By removing the hetero atoms, water molecule and cofactors, the target protein file was prepared by leaving the associated residue with protein by using Auto Dock 4.2 (MGL tools-1.5.6). Preparation of target protein file Auto Dock 4.2 (MGL tools-1.5.6) tool has been done, which involves the assign of Gasteiger charges for all the atoms of molecules converting into AD4 type (Figure 3-6). Docking simulations for the compounds 4 (A-D) were performed against InhA the active site of *Enoyl acp reductase*. [**5, 6, 7**] Docking results tabulated in table 1.

**Table 1.** Result of the Docking study

| S.No. | Conformer Code | Docking Score | Glide Score |
|---|---|---|---|
| 1 | 4A | -6.039 | -6.170 |
| 2 | 4B | -5.488 | -5.618 |
| 3 | 4C | -6.227 | -6.583 |
| 4 | 4D | -6.207 | -6.562 |
| 5 | Streptomycin | -6.857 | -7.029 |

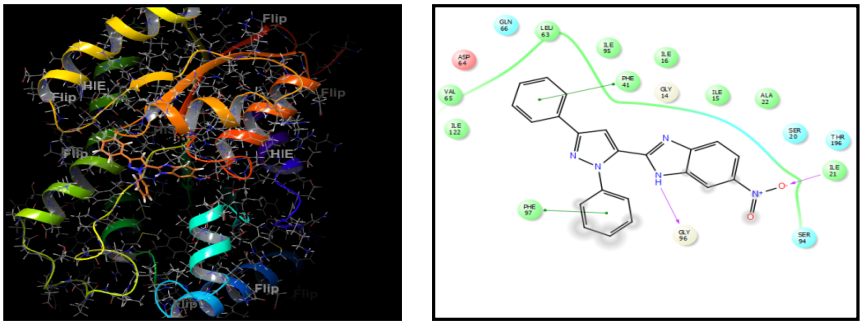


**Figure 3.** Docking and 2D interactions of compound 4A with the active amino acids of 5JFO.

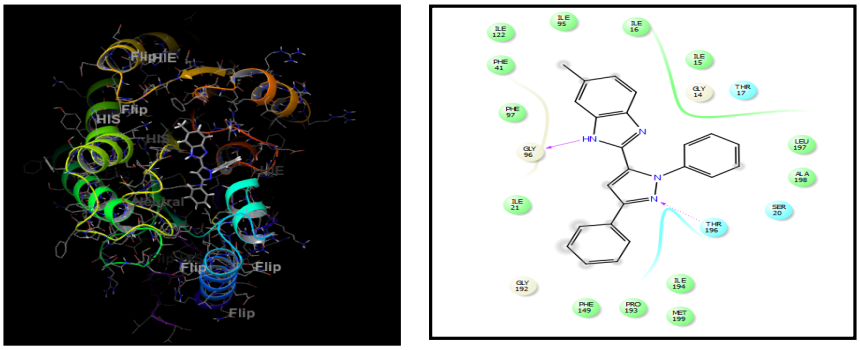


**Figure 4.** Docking and 2D interactions of compound 4C with the active amino acids of 5JFO.

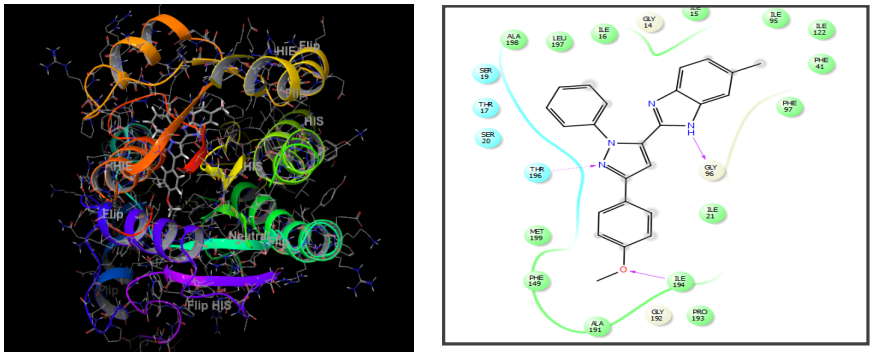


**Figure 5.** Docking and 2D interactions of compound 4D with the active amino acids of 5JFO.

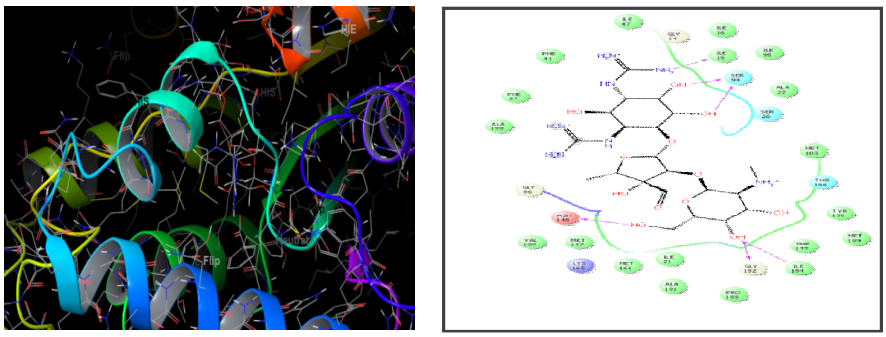


**Figure 6.** Docking and 2D interactions of Streptomycin with the active amino acids of 5JFO.

**Biological Activity:** The antimycobacterial activity of compounds was assessed against mycobacterium strain H37Rv using the microplate Alamar blue assay (MABA) (Table 2). The 96 well plates received 100 µL of the Middle brook 7H9 broth (having loopful inoculum of bacteria-108 CFU). Different dilutions of the respective compounds were made directly on the plate. The maximum concentration of the compounds tested was 50 mg $mL^{-1}$. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25 µL of a freshly prepared 1:1 mixture of Alamar blue reagent and 10% Tween-80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth (antimycobacterial activity) and a pink color was scored as growth susceptibility tests of the compounds and standard was carried out by well diffusion method. The sensitivity of the strains to the compounds was determined by measuring the diameter of the zone of inhibition surrounding the well by using millimeter scale. The compounds which were found to be satisfactory at a maximum concentration of 50 mg $mL^{-1}$ were diluted further to concentrations viz. 25, 12.5, 6.25, 3.125 and 1.56 mg $mL^{-1}$ respectively. Similarly, streptomycin was further diluted to 25 mg $mL^{-1}$ in order to check the antitubercular activity. The MIC of the potent compounds was performed in micro titer plates by Alamar blue assay (Table 3). MIC is defined as the lowest drug concentration which prevented a color change from blue to pink [**8, 9, 10**].

**Table 2.** Results of Antitubercular activity by Alamar Blue Assay

| Concentration mg $mL^{-1}$ | 25 | 12.5 | 6.25 | 3.125 | 1.56 |
|---|---|---|---|---|---|
| 4A | S | S | S | R | R |
| 4B | S | S | R | R | R |
| 4C | S | S | S | R | R |
| 4D | S | S | S | R | R |
| Streptomycin | S | S | S | S | R |

**Table 3.** MIC and MLC of synthesized compounds against M. tuberculosis H37Rv

| Code | Diameter of zone of inhibition (mm) | MIC in mg $mL^{-1}$ | MLC in mg $mL^{-1}$ |
|---|---|---|---|
| 4A | >20 | 6.25 | 12.5 |
| 4B | <15 | 12.5 | 25 |
| 4C | > 20 | 6.25 | 12.5 |
| 4D | >20 | 6.25 | 12.5 |
| Streptomycin | >25 | 3.12 | 6.25 |

## RESULTS AND DISCUSSION

All the benzimidazole fused pyrazole derivatives were synthesized as per the designed scheme which has been started by substituted o-Phenylenediamine and lactic acid after getting a docking score and gliding score. Molecular docking was performed by Scrodinger software. All the designed compounds and standard drug streptomycin were docked against Enoyl acyl carrier protein reductase enzyme encoded with InhA gene on PDB Id 5JFO. Compound 4C has shown the highest docking score when compared with standard and other compounds have shown almost equal interaction with active site of InhA. All the compounds were synthesized with satisfied yield, and characterized by IR, MASS and $H^1$NMR.

Antitubercular activity of the entire compound was taken by microplate Alamar blue assay, at Micropharm Diagnosis Center, Gandhi nagar, and result were expressed as MIC in mg $mL^{-1}$. In the study resistance of the *Mycobacterium* for the standard streptomycin was found at a Minimum concentration of 3.12 mg $mL^{-1}$ and the resistance of *mycobacterium* for compounds 4A, 4C, 4D was found to be at same concentration i.e. 6.25 mg $mL^{-1}$ and for the compound 4B *mycobacterium* resistance was found at 12.5 mg $mL^{-1}$.

## APPLICATION

The azoles which are prepared in this study are useful in the treatment of Tuberculosis.

## CONCLUSION

A new series of 2-(aryl)-1-phenyl-1*H*-pyrazol-5-yl)-5-methyl-1*H*-benzo[*d*]Imidazole (4A-D) were synthesized as potential antitubercular agents. Compounds 4A, 4C, and 4D were found to be the most potent in this study (MIC=6.25 mg $mL^{-1}$), the reference drug used in this study was Streptomycin. The docking study had shown that all the docked compounds exhibited good interaction with targeted enzyme of *M. Tuberculosis*.

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**Conflict of interest statement:** The authors report no conflict of interest.

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