



**DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF
NOVEL BENZIMIDAZOLE, PYRIDINE AND OXADIAZOLE DERIVATIVES AS
ANTITUBERCULAR AGENTS AGAINST MYCOBACTERIAL TARGET
ENZYME INH A**

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ABSTRACT

Tuberculosis is the communicable disease. InhA the enoyl-ACP reductase in *Mycobacterium tuberculosis* is an attractive target. Benzimidazole, Oxadiazoles and Pyridine derivatives exhibit pharmacological activities such as antimicrobial, antiviral, anticancer, anti-inflammatory, analgesic, etc. Hence decided to develop novel Benzimidazole, Oxadiazoles and Pyridine derivatives as potent anti-tubercular agents against InhA enzyme (Enoyl acyl carrier protein reductase). Compounds were designed and docked against inh A enzyme by using online docking Software Argus lab 4.0.1[®]. The molecules screened for best docking score and protein interaction. The selected molecules were synthesized in good yield by simple reflux condensation. Synthesized compounds were purified and characterized and biologically evaluated for their Anti tubercular activity by Microplate Alamar Blue Assay (MABA technique). The research work concludes that the synthesized anti-tubercular molecules effectively inhibit target enzyme *Enoyl acyl carrier protein reductase* (inh A). The benzimidazole derivatives were active at concentrations of 1.6mcg/ml "b" and 3.125mcg/ml "a". The synthesized pyridine derivatives "c" and "d" showed good anti tubercular activity at 1.6mcg/ml. whereas oxadiazole derivatives "e" and "f" were active at 1.6mcg/ml. Further structural modifications of the synthesized compounds will aid in the development of potential anti tubercular molecule. The cytotoxic evaluation of all the synthesized compounds was performed. The IC₅₀ for Rifampicin is 113 µg/ml on vero cell line. Two compounds "a" and "b" shows decreased IC₅₀ values of 37.63 and 111.2 µg/ml. Compounds "c" and "d" showed increased IC₅₀ values 389.7 and 319.3. Compounds "e" and "f" also showed increased IC₅₀ values of 299.3 and 520.

KEYWORDS: Mycobacterium tuberculosis; MABA; Oxadiazole; Benzimidazole; Rifampicin.

INTRODUCTION

Tuberculosis a common communicable disease caused by the bacterium *Mycobacterium tuberculosis* is a major public health problem globally. The *Mycobacterium tuberculosis* complex includes strains of five species- *Mycobacterium tuberculosis*, *Mycobacterium canettii*, *Mycobacterium africanum*, *Mycobacterium microti* and *Mycobacterium bovis* and two sub species - *Mycobacterium caprae* and *Mycobacterium pinnipedii*. The most notable member of the complex is *Mycobacterium tuberculosis* the causative agent of human tuberculosis which has an exclusive tropism for this host.^[1-4]

Emergence of resistant strains have resulted in MDR TB, XDR TB and TDR TB. Strains of *Mycobacterium tuberculosis*, that are resistant against Isoniazid and rifampicin, the most effective drugs against tuberculosis

are defined as multi drug resistant (MDR-TB). In 2006, the WHO released the first data on extensively drug resistant strains (XDR-TB). There are strains that are resistant to any fluoroquinolones and to atleast one of the injectable drugs kanamycin, capreomycin and amikacin in addition to isoniazid and rifampicin. Patients infected with XDR-TB, are virtually untreatable with current drugs.^[5] The drug resistant strains can then spread from person to person. The recent rise in TB cases and especially the increase of drug resistant mycobacteria indicate an urgent need to develop new anti TB drugs.^[5] Inh A the enoyl Acyl Carrier Protein reductase in *Mycobacterium tuberculosis*, is one of the key enzyme involved in the mycobacterial fatty acid elongation cycle and has been considered as a promising target to design novel anti tubercular agents.^[12-14]

The substituted benzimidazoles exhibit pharmacological activities such as antimicrobial, antiviral, anti cancer, anti anti-inflammatory, analgesic etc. Benzimidazole nucleus is one of the bioactive heterocyclic nuclei that exhibit a range of biological activities. The pharmacological activities of the benzimidazoles containing moiety have been well documented. Albendazole, Mebendazole, and Thiabendazole are widely used as anthelmintic drugs.^[6,7]

Pyridine is a basic heterocyclic organic compound. Pyridine derivatives continues to attract great interest due to the wide variety of interesting biological activities observed for these compounds, such as anti cancer, analgesic, anti microbial and anti depressant, activities.

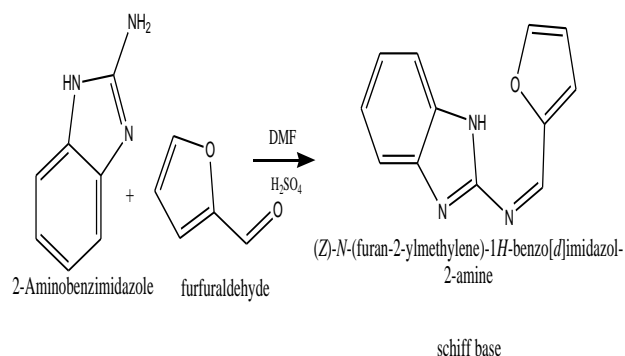
Oxadiazoles are a class of heterocyclic aromatic chemical compounds of the azole family.^[8,9,10] Compounds containing 1,3,4 – oxadiazole have been reported for their wide range of biological activities such as Antibacterial, Analgesic, Anticonvulsant. In this current work we have synthesized some novel Benzimidazole, Pyridine and Oxadiazole derivatives as antitubercular agents against mycobacterial target enzyme Inh A.

MATERIALS AND METHODS

In silico prediction for toxicity: Osiris Property Explorer is the online software of Thomas Sander, Actelion Pharmaceuticals Ltd., Gewerbestrasse 16, and 4123 Allschwil, Switzerland. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red, where as green color indicates drug conform behavior.^[11]

Experimental Procedures

Synthesis of Benzimidazole derivatives (Samples a and b): 2 – Aminobenzimidazole (0.01M) was suspended in DMF and Aromatic aldehyde (0.05M) was added along with 2-3 drops of concentrated H₂SO₄. The reaction mixture was then refluxed for 6-7 h. the resultant contents was poured into crushed ice. The crude product was filtered, washed with water until it was free from acidic catalyst, dried and re crystallized with methanol.

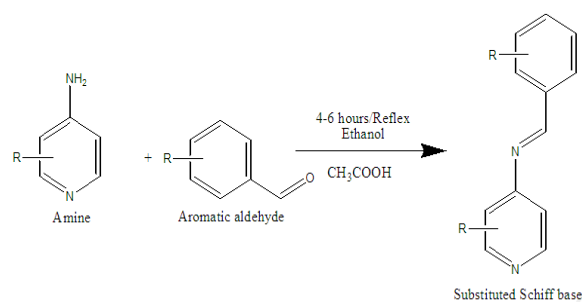


Scheme 1.

The aromatic aldehydes used were a) Para chloro benzaldehyde b) 3-Nitrobenzaldehyde.

Synthesis of Pyridine derivatives: (Samples c and d)

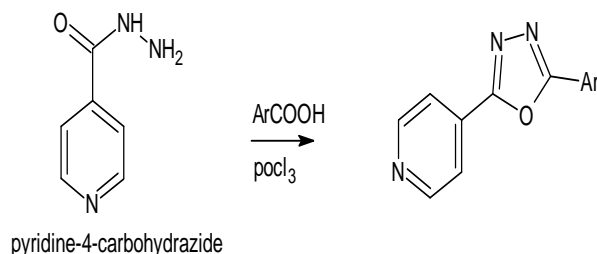
An equimolar quantity of 3, 5-dichloro 4-amino pyridine (0.01mole) was added to the aldehydes (0.01mole) and dissolved in absolute ethanol. Few drops of glacial acetic acid were added as a catalyst and the reaction mixture was refluxed for 4-6 hours. On the completion of the reaction was monitored by TLC, cooled to room temperature and the reaction poured to in to crushed ice. The mixture that was formed was filtered, dried and re crystallized using ethanol.



Scheme 2.

Synthesis of oxadiazole derivatives: (Samples e and f)

A mixture of 0.01mole pyridine -4-carbohydrazide and 0.01 mole of aromatic acid was dissolved in phosphorus oxychloride and refluxed for 18-22 hrs; the reaction mixture was slowly poured over crushed ice and kept overnight. The solid mass thus separated was filtered, dried and purified by re crystallization from ethanol.



Scheme 3.

The aromatic aldehydes used were a) 3-Nitrobenzaldehyde and b) 4-hydroxy benzaldehyde

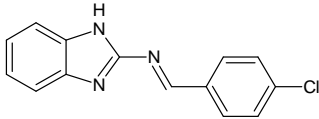
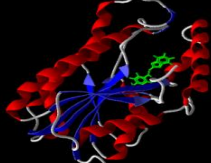
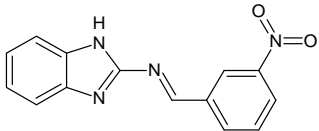

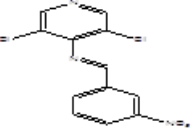
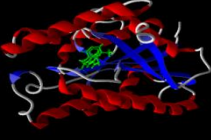
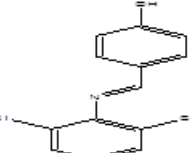
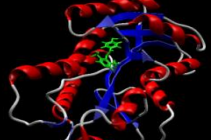
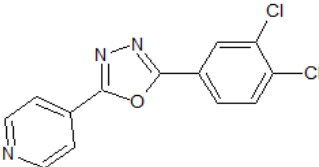
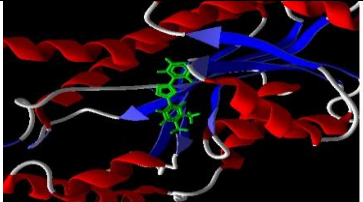
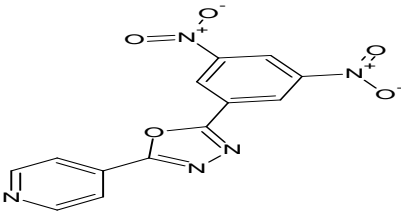
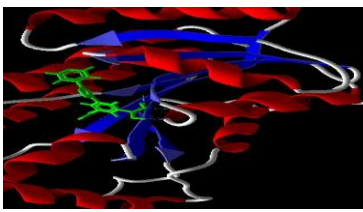
Biological Evaluation: The anti tubercular activity of synthesized compounds was evaluated against mycobacterium tuberculosis by Micro plate Alamar Blue Assay (MABA Method).^[15-17] Cell line study of synthesized compounds was performed on Vero (African green monkey kidney cells). Acute toxicity of synthesized compounds was studied on mice according to OECD guidelines 423.

RESULTS AND DISCUSSIONS

Table 1: Physical properties of synthesized compounds.

S. No	Sample code	Molecular weight In g/mole	Molecular formula	% Yield	Melting point	Solubility	Color
1	a	255.70	C ₁₄ H ₁₀ Cl N ₃	80%	180°C	Methanol, Ethyl acetate	yellow
2	b	266.25	C ₁₄ H ₁₀ N ₄ O ₂	75%	138°C	Methanol, Ethyl acetate	Yellowish Orange
3	c	295.06	C ₁₂ H ₇ Cl ₂ N ₃ O ₂	76%	78°C	Methanol, Ethyl acetate	Pale yellow
4	d	266.91	C ₁₂ H ₈ Cl ₂ N ₂ O	62%	104°C	Methanol, Ethyl acetate	Light brown
5	e	292.12	C ₁₃ H ₇ Cl ₂ N ₃ O	81%	195°C	Ethanol/ DMSO	Pale yellow
6	f	313	C ₁₃ H ₇ N ₅ O ₅	73%	205°C	Ethanol/ DMSO	Brown

Table 2: The docking score and docking view of compounds against the MTB enzyme Inh A (Enoyl Acyl Carrier Protein), (2h9i) by using Argus lab 4.0.1®

Compound Code	Structure	Docking Score	Docking View
a		-7.83 Kcal/mol	
b		-8.52 Kcal/mol	
c		-5.58 Kcal/mol	
d		-6.53 Kcal/mol	
e		-7.43 Kcal/mol	
f		-7.12 Kcal/mol	

Snapshot of the results of *in silico* toxicity results is shown in Fig: 1 to 6.

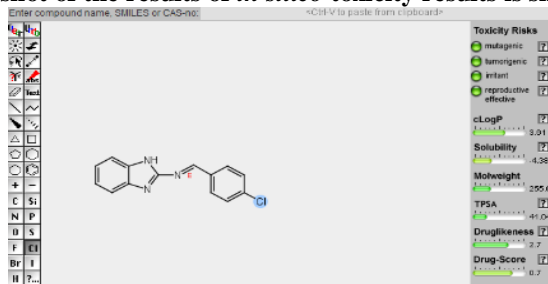


Fig. 1: Sample: a.

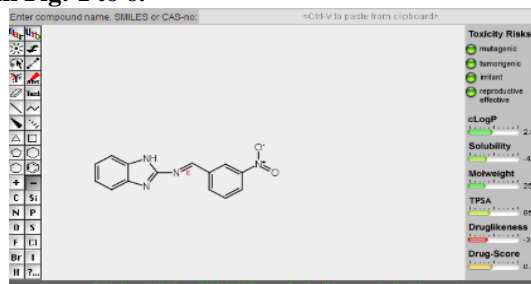


Fig. 2: Sample: b.

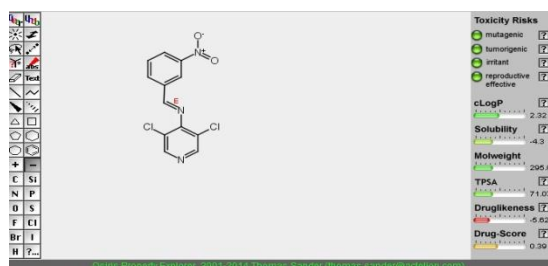


Fig. 3: Sample: c.

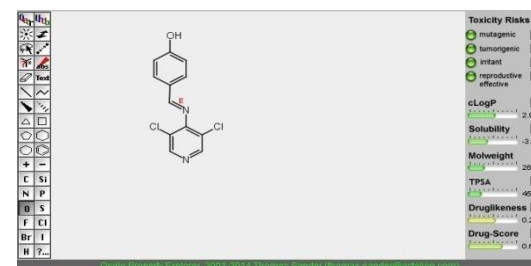


Fig. 4: Sample: d.

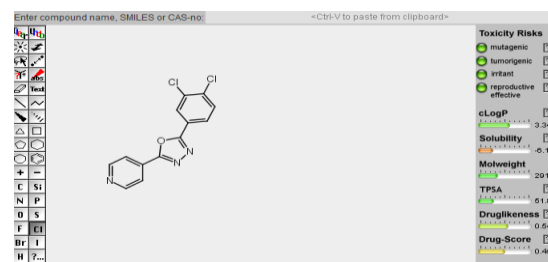


Fig. 5: Sample: e

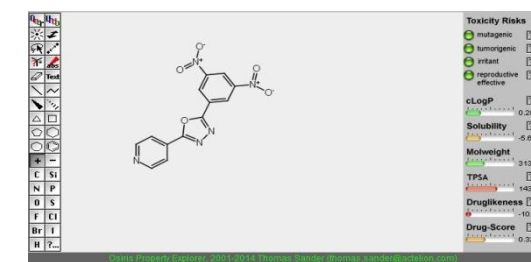


Fig. 6: Sample: f.

Spectral analysis: The structure of the synthesized compounds was confirmed by spectroscopic methods like IR, NMR and LC-MS.

Sample a): N-(1H-benzimidazole-2-yl)-1-(chlorophenyl)methanimine: IR: $\nu_{\max}/\text{cm}^{-1}$ 2684(-C=N). $^1\text{H NMR}$: δ 8.3-8.6 (4H, aryl protons). MS: m/z $^{+}255.99$.

Sample b): N-(1H-benzimidazole-2-yl)-1-(3-nitrophenyl)methanimine: IR: $\nu_{\max}/\text{cm}^{-1}$ 2746(-C=N). $^1\text{H NMR}$: δ 7.1-7.7 (4H, aryl protons). MS: m/z $^{+}267.03$.

Sample c): N-(3,5-dichloropyridine-4-yl)-1-(3nitrophenyl) methanimine: showed strong absorption bands at Ar C-H Str (3099.59 cm^{-1}), -C-Cl Str (732 cm^{-1}), -NO Str (1504.37 cm^{-1}), C=N Str (1629.09 cm^{-1}). NMR: $^1\text{H NMR}$ δ PPM: c 10.2 singlet, δ 6-8 multiplet. m/z $^{+}295.06$.

Sample d): 4- ((E)-[3, 5-dichloropyridin-4-yl]imino)methylphenol showed strong absorption bands at Ar C-H Str (3109.02 cm^{-1}), C-Cl Str (732.90 cm^{-1}), -OH Str (3448.47 cm^{-1}), C-N Str (1550.65 cm^{-1}). NMR: $^1\text{H NMR}$ δ ppm: δ 6-8 doublet; Mass: 266.91 g/mole. Ar C-H Str (3109.02 cm^{-1}), -C-Cl Str (732.90 cm^{-1}), -OH Str (3448.47 cm^{-1}), C=N Str (1550.65 cm^{-1}). NMR: $^1\text{H NMR}$ δ PPM: δ 6-8 doublet.

Sample e): 4-[5-(3, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl] pyridine. IR (cm^{-1}): 763 (C-Cl Str), 1002 (C-N Str), 1049 (C-O Str), 1681 (C=C)Str, 2823 (C-H Str); $^1\text{H NMR}$: 2.508 (solvent peak), 7-8 (Aromatic CH proton). Actual Mass: 292.12.

Sample f): 4-[5-(3, 5-dinitrophenyl)-1, 3, 4-oxadiazol-2-yl] pyridine. IR (cm^{-1}): 1072 (C-O Str), 1353 (C-N Secondary amine), 2877 (C-H)Str, 3101 (C-H Str); $^1\text{H NMR}$: 9.3- 9.4 (singlet), 9.2-9.3 (doublet), 9.0-9.1 (Multiplet), 8.9-9.0 (Singlet). Actual Mass: 313.

Biological evaluation

Sl. No.	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml	1.6 µg/ml	0.8 µg/ml
1.	a	S	S	S	S	S	S	R	R
2.	b	S	S	S	S	S	S	S	R
3	c	S	S	S	S	S	S	S	R
4	d	S	S	S	S	S	S	S	R
5	e	S	S	S	S	S	S	S	R
6	f	S	S	S	S	S	S	S	R

Note: S - Sensitive R - Resistant

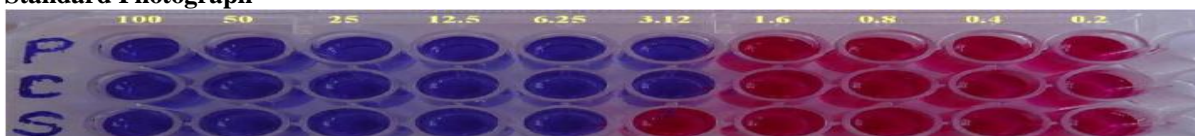
Strain used: *M.tuberculosis*(H37 RV strain): ATCC No- 27294.

Here are the *standard values* for the Anti-Tb test which was performed.

Pyrazinamide- 3.125µg/ml

Streptomycin- 6.25µg/ml

Ciprofloxacin-3.125µg/ml

Standard Photograph**Sample Photograph**

Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml	1.6 µg/ml	0.8 µg/ml
a								
b								
c								
d								
e								
f								

Acute toxicity evaluation: Animals were observed for behavioral signs of toxicity like motor activity. Tremor etc. and no significant toxic signs were observed during 14 days. The results of the acute toxicological studies revealed that the administration of the molecules by oral route up to 2000mg/kg/b.w did not produce any mortality.

Cytotoxicity evaluation

Concentration (µg/ml)	a	b	c	d	e	f
500	98.14	96.96	52.15	53.59	73.99	45.78
250	97.99	91.42	43.68	47.38	38.56	9.64
125	92.51	49.09	34.30	41.26	23.55	25.13
64.5	72.92	26.22	34.83	27.67	6.21	18.10
31.25	40.99	9.99	12.86	16.11	17.56	21.28
IC₅₀ from Prism	37.63	111.2	389.7	319.3	299.3	520

The drug Rifampicin was used as standard drug and the values of the synthesized compounds were compared. The IC₅₀ for Rifampicin is 113 µg/ml on vero cell line. Two compounds “a” and “b” shows decreased IC₅₀ values of 37.63 and 111.2 µg/ml. Compounds “c” and “d” showed increased IC₅₀ values 389.7 and 319.3. Compounds “e” and “f” also showed increased IC₅₀ values of 299.3 and 520.

CONCLUSION

The research work concludes that the synthesized anti-tubercular molecules effectively inhibit the specific target *Enoyl acyl carrier protein reductase* which is essential for the fatty acid biosynthesis of *Mycobacterial tuberculosis*. The benzimidazole derivatives were active at concentrations of 1.6mcg/ml (b) and 3.125mcg/ml (a). The synthesized pyridine derivatives c and d showed good anti tubercular activity at 1.6mcg/ml, whereas oxadiazole derivatives e and f were active at 1.6mcg/ml. Further structural modifications of the synthesized compounds will aid in the development of potential anti tubercular molecule.

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