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SYNTHESIS, CHARACTERIZATION AND ANTI-TUBERCULAR SCREENING OF SOME SUBSTITUTED 5-ETHOXY BENZIMIDAZOLE DERIVATIVES

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ABSTRACT

Benzimidazole derivatives are very useful compound with well known biological activity. Notable among these are antimicrobial, anti-tubercular, antimalarial, anti-inflammatory, anticancer, antiviral, antiprotozoal, antihistaminic, antioxidant and anthelmintic actions.. In the current research work, the title compounds 5-ethoxy-2-substituted benzimidazole, were synthesized by nitration of phenacetin with concentrated nitric acid it gives N-(2-nitro-5-ethoxyphenyl) acetamide (I). Compound (I) on reduction with alcohol gives 5-ethoxy-2-nitroaniline (II). Reaction of compound (II) with hydrazine hydrate produced 5-ethoxy ortho phenylene diamine(III)**.** The reaction of compounds (III) with substituted acids yielded the corresponding 5 ethoxy-2-substituted benzimidazole (IV). The identification and characterization of the synthesized compounds were carried out by Elemental analysis, melting point, Thin Layer Chromatography, FT-IR, NMR and Mass data to ascertain that all synthesized compounds were of different chemical nature than the respective parent compound. The compounds were screened out for anti-tubercular activity. The anti-tubercular activity of compounds were done by using Microplate Alamar Blue Assay (MABA). The test compounds IVa, IVb and IVc showed significant anti-tubercular activity against $H_{37}R_V$ strain of *Mycobacterium tuberculosis*. The minimum inhibitory concentration (MIC) values were found in the range of 0.8 to 12.5 µg/ml compared with the standard drugs Pyrazinamide, Streptomycin and Ciprofloxacin.

KEYWORDS: Anti-tubercular activity, *Mycobacterium tuberculosis*, Benzimidazole, Pyrazinamide, Streptomycin, Ciprofloxacin.

INTRODUCTION

Benzimidazole ring is one of the most common heterocycle in medicinal chemistry and it possesses most remarkable and a wide range of biological activities. The substituted benzimidazole have been shown to exhibit antimicrobial, anti-tubercular, antimalarial, anti-inflammatory, anticancer, antiviral, antiprotozoal, antihistaminic, antioxidant and anthelmintic actions.

Literature survey showed that benzimidazole ring is a main pharmacophore for anti-tubercular activity.

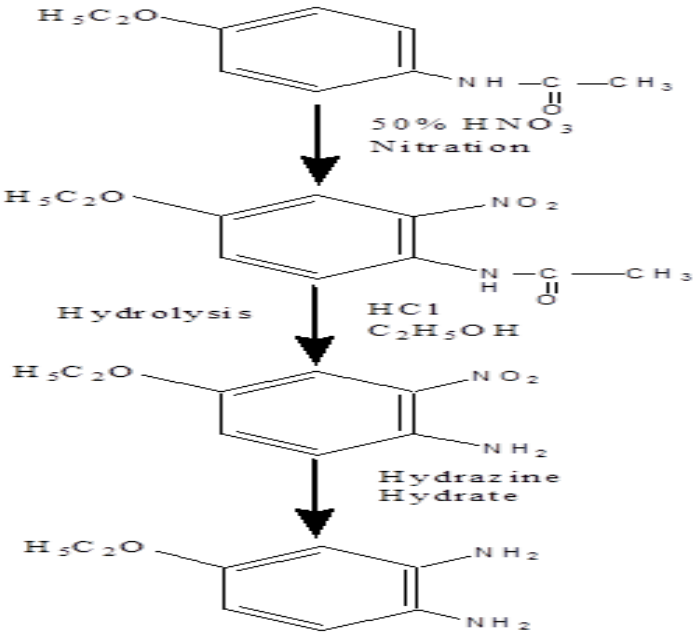
Tuberculosis(TB), the world's leading infectious disease, caused by *Mycobacterium tuberculosis* represents a major global health problem. Current epidemiological evidence suggests that one third of the world's population is infected with *Mycobacterium tuberculosis.* Eight million cases emerge annually and three million deaths per year are directly attributable to infection with this bacillus (WHO, 2002). Resistance to the current antituberculosis therapy is another threatening problem. Emergence of multi drug resistant tuberculosis (MDR-TB) makes the conditions most alarming. Up to 4% of all TB cases worldwide are resistant to more than one antitubercular drug because of incomplete or partial therapy.

MATERIALS AND METHODS

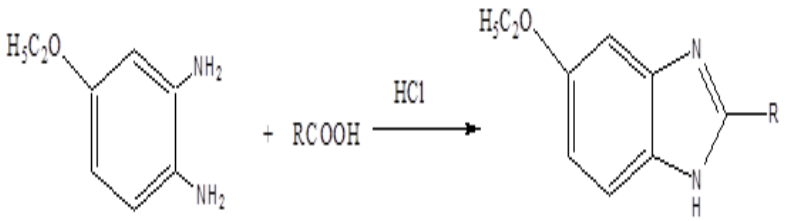
All the reagents and solvents used were of laboratory grade. The melting points of synthesized compounds were determined by open capillary method and were uncorrected. The purity and homogeneity of compounds were checked using TLC technique. IR spectra of compounds were recorded using KBr pellets on Perkin Elmer 337 spectrophotometer, ^{1}H-NMR spectra were recorded on Bruker Avance-300 MHz Spectrophotometer using dimethyl sulfoxamide as solvent and Mass Spectra of the synthesized compounds were recorded on Liquid Chromatography Mass Spectrometer at Diya Laboratory, Mumbai. The compounds were also subjected to C, H, N analysis at Diya Laboratory, Mumbai.

Scheme of synthesis

Scheme I



Scheme II



(IV)

Where R=substituted alkyl or aryl.

Anti-TB activity

1. The anti mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA).
2. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method.
3. Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.
4. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.
5. The final drug concentrations tested were 100 to 0.2 µg/ml.
6. Plates were covered and sealed with parafilm and incubated at 37ºC for five days.

7. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.
8. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.
9. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

RESULT AND DISCUSSION

Characterization of Synthesized Compounds

Compound IVa: 5-Ethoxy-2-phenyl-1H-benzimidazole

Percentage Yield- 62%, **M.P.** 90-93^0**,** $\mathbf{R_f}$-0.72 (Chloroform: Methanol).

IR (KBr) cm^{-1}: 3324 cm^{-1} (-NH-), 1250 cm^{-1} (C=N), 3020 cm^{-1} (Ar–H); 1043 cm^{-1} (ether group in ring).

^{1}H NMR: ($CDCl_3$) δ 7.21, δ 6.77, δ 7.59, (Ar-H), δ 3.9 (>CH_2), δ 1.33(-CH_3), 7.29 and δ 7.03(phenyl).

FAB-MS: (m/z, 100%): 237 ([M^+], 100%).

Compound IVb: Aceticacid3-(5-ethoxy-1H-benzimidazole-2yl)-phenyl ester:

Percentage Yield- 57.7%, **M.P.** 110-115^0**,** $\mathbf{R_f}$-0.75 (Chloroform: Methanol).

IR (KBr) cm^{-1}: 3320 cm^{-1} (-NH-), 1240 cm^{-1} (C=N), 3010 cm^{-1} (Ar–H); 1047 cm^{-1} (ether group in ring), 1730 cm^{-1} (C=O).

^{1}H NMR: ($CDCl_3$) δ 7.21, δ 6.77, δ 7.59, (Ar-H), δ 3.9 (>CH_2), δ 1.33 and 2.08(-CH_3), δ 7.29 and δ 7.03(substituted phenyl).

FAB-MS: (m/z, 100%): 296 ([M^+], 100%).

Compound IVc: 1-(5-Ethoxy-1H-benzimidazole-2yl)-ethanol

Percentage Yield- 56.4%, **M.P.** 90-95^0**,** $\mathbf{R_f}$-0.6 (Chloroform: Methanol).

IR (KBr) cm^{-1}: 3330 cm^{-1} (-NH-), 1245 cm^{-1} (C=N), 3010 cm^{-1} (Ar–H); 1044 cm^{-1} (ether group in ring), 3634 cm^{-1}(C-OH).

^{1}H NMR: ($CDCl_3$) δ 7.21, δ 6.77, δ 7.59, (Ar-H), δ 3.9 (>CH_2), δ 1.33 and 1.49(-CH_3), δ 5.0(-NH-),δ 2.0(-OH).

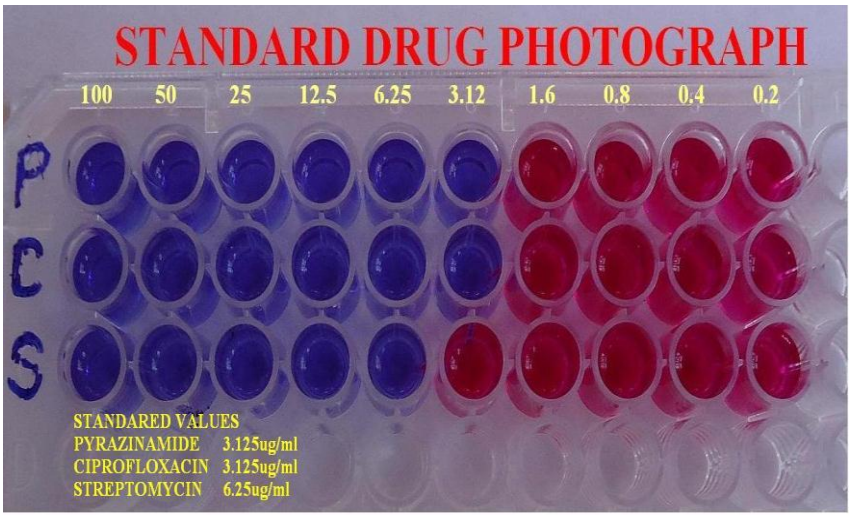
FAB-MS: (m/z, 100%): 205 ([M^+], 100%).

Elemental Analysis

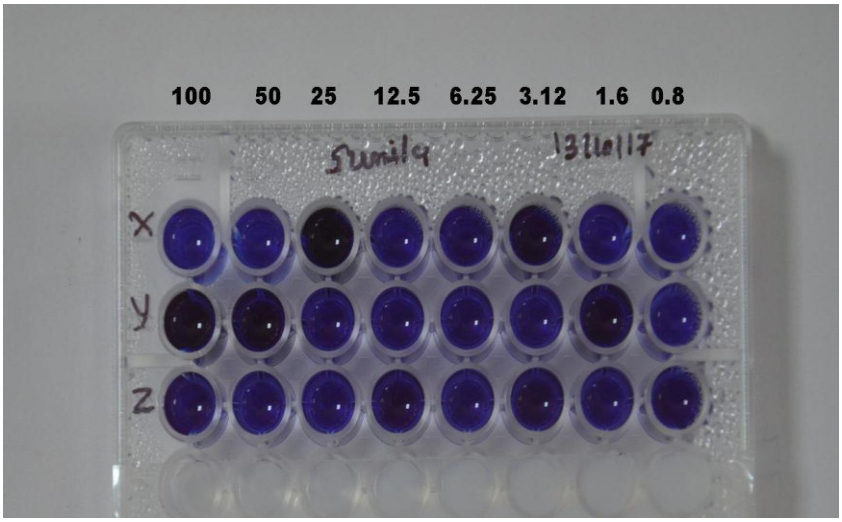
Comp. No.	Elemental Analysis (%)					
	Calculated			Found		
	C	H	N	C	H	N
IVa	75.94	5.77	21.32	75.98	5.44	11.85
IVb	68.91	5.40	9.45	68.87	5.44	9.49
IVc	64.39	6.34	13.65	64.41	6.38	13.61

Anti-TB Results

Standard



Test



Sr. No.	Comp.	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
01	IV_a	S	S	S	S	S	S	S	S
02	IV_b	S	S	S	S	S	S	S	S
03	IV_c	S	S	S	S	S	S	S	S

S - Sensitive

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

CONCLUSION

The test compounds IVa, IVb and IVc showed significant anti-tubercular activity against $H_{37}R_V$ strain of *Mycobacterium tuberculosis*. The minimum inhibitory concentration (MIC) values were found in the range of 0.8 to 12.5 µg/ml compared with the standard drugs Pyrazinamide, Streptomycin and Ciprofloxacin.

Hence, the presented work has another humble effort in the field of medicinal chemistry and sincerely contribute to a healthier and happier human life.

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