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Proceeding Paper

Synthesis and Biological Evaluation of Some Substituted Benzimidazole Derivatives †

Sunila Patil [1,*], Parloop Bhatt [2], Hemant Suryawanshi [3,*], Javesh Patil [4] and Rajesh Chaudhari [5]

[1] Department of Pharmaceutical Chemistry, P.S.G.V.P.M's College of Pharmacy, Shahada 425409, Dist-Nandurbar, Maharashtra, India
[2] Department of Pharmacology, L. M. College of Pharmacy, Ahmedabad 380009, Gujrat, India; parloop72@gmail.com
[3] Department of Pharmacology, P.S.G.V.P.M's College of Pharmacy, Shahada 425409, Dist-Nandurbar, Maharashtra, India
[4] Department of Pharmacognosy and Phytochemistry, P.S.G.V.P.M's College of Pharmacy, Shahada 425409, Dist-Nandurbar, Maharashtra, India; javesh4u@gmail.com
[5] Department of Pharmaceutical Chemistry, Tapi Valley Education Society, College of Pharmacy, Faizpur, Maharashtra, India; rychaudhari@gmail.com
* Correspondence: sunila_patil22@rediffmail.com (S.P.); hemant.surya@gmail.com (H.S.); Tel.: +91-9423916682/+91-8668906443 (S.P.); +91-9923032073 (H.S.)
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Abstract: In the current research work, the title compounds 5-ethoxy-benzimidazole, were synthesized by nitration of phenacetin with concentrated nitric acid it gives N-(2-nitro-5-ethoxyphenyl) acetamide (I), which on reduction with alcohol gives 5-ethoxy-2-nitroaniline (II). Reaction of hydrazine hydrate with 5-ethoxy-2-nitroaniline produced 5-ethoxy ortho phenylene diamine (III). The substituted acids reacted with 5-ethoxy ortho phenylene diamine then yielded the corresponding 5-ethoxy-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. The synthesized compounds were evaluated for anti-tubercular activity. The test compounds were subjected to in vitro screening by the tube dilution technique employing the human virulent $H_{37}Rv$ strain of M. tuberculosis. The test compounds IVa, IVc and IVd showed significant anti-tubercular activity against $H_{37}Rv$ 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 Isoniazid.

Keywords: anti-tubercular activity; *Mycobacterium tuberculosis*; benzimidazole; isoniazid

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1. Introduction

Benzimidazole ring is most common heterocycle in medicinal chemistry and it possesses most remarkable and a extesiven range of biological activities. The substituted benzimidazoles were shown to exhibit antimicrobial [1,2], antiulcer [3], anthelmintic [4], anticancer [5–7], anti-inflammatory [8], anti-tubercular [9–11], antimalarial [12], antihistaminic [13] and antioxidant [14] actions. Literature survey showed that benzimidazole ring is a main pharmacophore for anti-tubercular activity [9–11].

Tuberculosis (TB), the world's most common infectious disease caused by the bacteria *Mycobacterium tuberculosis*, is a serious worldwide health issue. According to current epidemiological evidence, *Mycobacterium tuberculosis* infects one-third of the world's population. Each year, eight million illnesses and three million fatalities are directly linked to infection with this bacillus (WHO, 2002) [15]. Another serious issue is resistance to current anti-tuberculosis treatments. The emergence of multidrug-resistant tuberculosis (MDR-

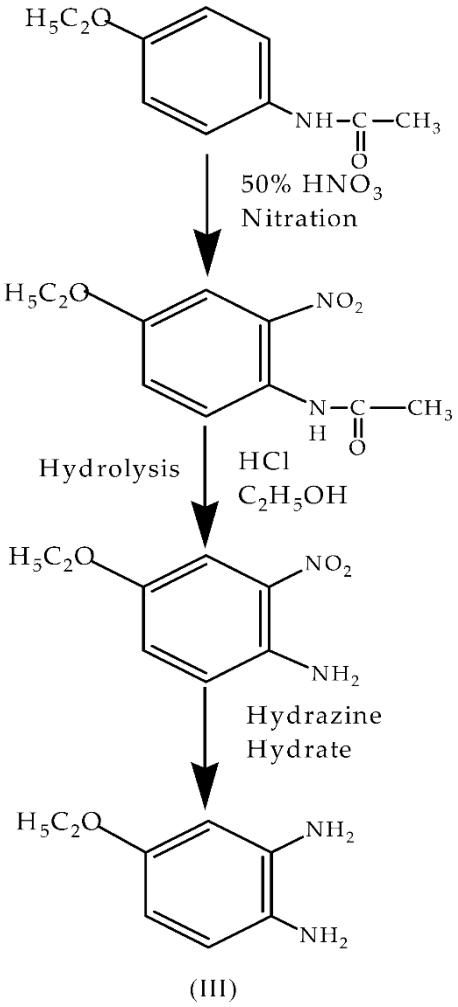
TB) has made the situation much worse. Because of incomplete or partial treatment resistance, up to 4% of all TB cases globally are resistant to more than one anti-tubercular therapy [16–19].

2. Materials and Methods

2.1. General

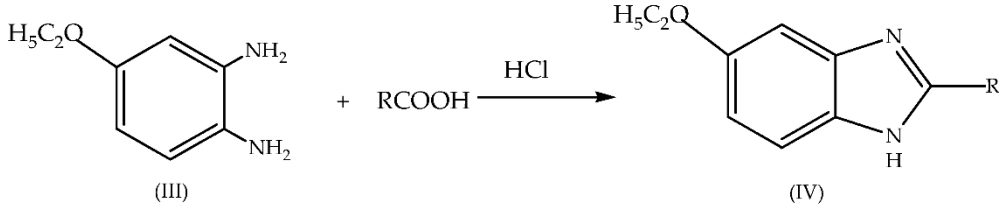
All of the reagents and solvents utilised were of the highest quality. The open capillary method was used to determine the melting points of synthesised compounds, and the results were uncorrected. TLC was used to verify the purity and homogeneity of the compounds. Compounds' IR spectra were recorded using KBr pellets on a Perkin Elmer 337 spectrophotometer, ^{1}H-NMR spectra were recorded using dimethyl sulfoxamide as solvent on a Bruker Avance-300 MHz Spectrophotometer, and mass spectra were recorded on a Liquid Chromatography Mass Spectrometer at Diya Laboratory in Mumbai. The compounds were also tested for C, H, and N at Mumbai, Diya Laboratory.

2.2. Scheme of Synthesis



(III)

Scheme 1. ethoxy Ortho Phenylene Diamine [OPD].



where R = Substituted alkyl or aryl

Scheme 2. Synthesis of substituted benzimidazole derivatives.

2.3. Synthesis of Compounds

2.3.1. Scheme 1: Preparation of 5-Ethoxy Ortho Phenylene Diamine [OPD]

I. N-(2-nitro-5-ethoxyphenyl) acetamide

To finely powdered phenacetin (3 g), nitric acid (50%, 3 mL), was added gradually with efficient stirring and the temperature was maintained below 25 °C [21]. Stirring was continued for 1 h, the solid was filtered at pump, washed with water till neutral to litmus. The dried product was recrystalised from ethanol, yield 2.5 g (66.5%), m.p. 82–84 °C.

II. 5-ethoxy-2-nitroaniline

To 2.0 g of compound I in ethanol (6 mL), concentrated hydrochloric acid was added, the reaction mixture was refluxed for 2 h. The reaction mixture was diluted with water (15 mL) and distilled to remove ethyl acetate formed during the reaction and excess of ethanol [22]. The hot solution was poured to crushed ice and basified with sodium hydroxide solution (20%). The separated solid was filtered at pump, washed with water till nutral to litmus, dried and recrystalised from ethanol, yield 1.7 g (87%), m.p. 90–92 °C.

III. 5-ethoxy Ortho Phenylene Diamine (OPD)

To the solution of compound II (1.5 g) in ethanol (20 mL), equimolar ratio of hydrazine hydrate (2.1 mL) was added. The solution was just warmed on a steam bath and little amount of freshly prepared raney nickel was added till the solution changed its colour from reddish brown to yellow. Still, more catalyst was added to decompose the excess hydrazine hydrate and the reactant solution was refluxed for 1 h to complete the reaction and drive off dissolve gas. The content were filtered hot to remove the catalyst, decolourised using charcoal, concentrated (2.5 mL) and poured to large excess of cold water, dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure [23]. The residue was recrystallized from ether, yield 1.2 g (76%), m.p. 92–94 °C.

2.3.2. Scheme 2: Compound IVa: 5-Ethoxy-2-Methyl-1H-Benzimidazole

To compound III (1 g) in 25 mL of round bottom flask 0.9 mL acetic acid was added. The mixture was heated on water bath at 100 °C for 2 h. After cooling the mixture sodium hydroxide solution (10%) was added slowly with constant shaking of flask, till the mixture was just alkaline to litmus. The crude 5-ethoxy-benzimidazoles were filtered at pump, washed it with ice-cold water, drained well and wash again with 25 mL cold water. The mixture was filtered by Buchner funnel. The residue was recrystalised from ethanol, yield 1.2 g (78%), m.p. 82–84 °C.

Remaining compounds were prepared by following the above procedure using different substituted alkyl and aromatic substituted carboxylic acids such as, salicylic acid, benzoic acid, aspirin, and propionic acid.

2.4. Anti-Tubercular Activity

The test compounds were screened in vitro using the tube dilution method with the human virulent $H_{37}R_V$ strain of *Mycobacterium tuberculosis*. In this method, Kirchner's medium containing Tween-80 was used [20].

3. Result and Discussion

3.1. Characterization of Compounds

5-ethoxy-2-methyl-1H-benzimidazole (IVa). Yield: 78%; m.p. 82–84 °C; R_f: 0.74; IR (KBr) cm^{-1}: 3342 cm^{-1} (-NH-), 1250 cm^{-1} (C=N), 3012 cm^{-1} (Ar–H), 1040 cm^{-1}(ether group in ring), 2950 cm^{-1} (CCH_3); ^1H NMR: ($CDCl_3$) δ 7.21, δ 6.77, δ 7.59 (Ar-H), δ 5.0 (-NH-), δ 3.9 (>CH_2), δ 3.98 and δ 2.42(-CH_3); FAB-MS: (*m*/*z*, 100%): 174 ([M^+], 100%).

5-ethoxy-2-ethyl-1H-benzimidazole (IVb). Yield: 75%; m.p. 86–88 °C; R_f: 0.75; IR (KBr) cm-1: 3327 cm^{-1} (-NH-), 1248 cm^{-1} (C=N), 3010 cm^{-1} (Ar–H); 1039 cm^{-1} (ether group in ring); 2990 cm^{-1} (CC_2H_5); ^1H NMR: ($CDCl_3$) δ 3.9 and 2.59 (>CH_2), δ 3.98 and δ 2.42 (-CH_3), δ 7.21, δ 6.77, δ 7.59, (Ar-H), δ 5.0 (-NH-); FAB-MS: (*m*/*z*, 100%): 189 ([M+], 100%).

5-ethoxy-2-phenyl-1H-benzimidazole (IVc). Yield: 62%; m.p. 90–92 °C; R_f: 0.72; IR (KBr) cm^{-1}: 3324 cm^{-1} (-NH-), 1250 cm^{-1} (C=N), 3020 cm^{-1} (Ar–H); 1043 cm^{-1}; ^1H NMR: ($CDCl_3$) δ 3.9 (>CH_2), δ 1.33(-CH_3), δ 7.21, δ 6.77, δ 7.59, (Ar-H), 7.29 and δ 7.03(phenyl); FAB-MS: (*m*/*z*, 100%): 237 ([M+], 100%).

Acetic acid3-(5-ethoxy-1H-benzimidazole-2yl)-phenyl ester (IVd). Yield: 60.7%; m.p. 110–112 °C; R_f :0.75; IR (KBr) cm^{-1}: 1240 cm^{-1} (C=N), 3010 cm^{-1} (Ar–H); 3320 cm^{-1} (-NH-), 1047 cm^{-1}, 1730 cm^{-1} (C=O); ^1H NMR: ($CDCl_3$) δ 3.9 (>CH_2), δ 1.34 and 2.08(-CH_3), δ 7.21, δ 6.77, δ 7.59, (Ar-H), δ 7.29 and δ 7.03(substituted phenyl); FAB-MS: (*m*/*z*, 100%): 296([M+], 100%).

2-(5-ethoxy-1H-benzimidazole-2yl)-phenol (IVe). Yield: 68%; m.p. 80–82 °C; R_f-0.67; IR (KBr) cm^{-1}: 1249 cm^{-1} (C=N), 3034 cm^{-1} (Ar–H), 3323 cm^{-1} (-NH-), 1039 cm^{-1} (ether group in ring), 3634 cm^{-1}(C-OH); ^1H NMR: ($CDCl_3$) δ 3.9 (>CH_2), δ 1.33 (-CH_3), δ 7.21, δ 6.77, δ 7.59, (Ar-H), δ 6.79, δ 7.05, δ 6.88 and δ 7.31(substituted phenyl), δ 5.0 (-OH).

1-(5-ethoxy-1H-benzimidazole-2yl)-ethanol (IVf). Yield- 56.4%, m.p. 92–94 °C, R_f: 0.6; 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). ^1H NMR: ($CDCl_3$) δ 3.9 (>CH_2), δ 1.33 and 1.49(-CH_3), δ 7.21, δ 6.77, δ 7.59, (Ar-H), δ 5.0(-NH-),δ 2.0(-OH); FAB-MS: (*m*/*z*, 100%): 205 ([M+], 100%).

3.2. Elemental Analysis of Compounds

Table 1. % Elemental analysis of synthesized compounds with calculated and found values**.**

Comp. No.	Elemental Analysis (%)					
	Calculated			Found		
	C	H	N	C	H	N
IVa	68.96	5.74	16.09	68.92	5.78	16.05
IVb	69.84	6.87	14.8	69.80	6.91	14.4
IVc	75.94	5.48	11.81	75.98	5.44	11.85
IVd	68.91	5.40	9.45	68.87	5.44	9.49
IVe	71.14	5.13	11.06	71.10	5.17	11.02
IVf	64.39	6.34	13.65	64.41	6.38	13.61

3.3. Anti-Tubercular Activity of Compounds

Table 2. The minimum inhibitory concentration (MIC) values of synthesized compounds and standard drug**.**

Comp. No.	R	Anti-Tubercular Activity
		H_{37}Rv Strain of *M. tuberculosis*
		21 days (μg/mL)
IVa	Methyl	12.5
IVb	Ethyl	25
IVc	Phenyl	1.6
IVd	2-subst-acetic acid phenyl ester	0.8

IVe	2-subst.phenol	25
IVf	2-subst.ethanol	25
Std.	Isoniazid	0.8

The literature survey on the benzimidazole ring revealed that substituted benzimidazole derivatives were tested for anti-tubercular activity. Benzimidazole possesses diversified pharmacological activity; among them the anti-tubercular activity is prominent. It has been reported that 2-pyridinyl substituted benzimidazole derivatives possess anti-tubercular activity [24]. The salicylic acid, acetyl salicylic acid and benzoic acid possess anti-tubercular activity [9–11]. Hence it was planned to synthesize benzimidazole derivatives by using these acids and other aliphatic carboxylic acids at 2nd position to give better anti-tubercular activity.

4.. Conclusions

The test compounds IVa, IVc and IVd 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 Isoniazid.

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

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