JOURNAL OF SCIENTIFIC RESEARCH IN ALLIED SCIENCES

Contents available at: www.jusres.com

SYNTHESIS, CHARACTERIZATION, AND ANTI-TUBERCULAR ACTIVITY OF BENZIMIDAZOLE DERIVATIVES

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ARTICLE INFO

Article History
Received: December 2022
Accepted: February 2023
Keywords:
Benzimidazole, Anti-tubercular, NMR, Mass, Schiff bases

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ABSTRACT

The most prevalent illness with a high contagiousness profile and the leading cause of death from infectious agents is tuberculosis. The emergence of Mycobacterium TB drug-resistant strains and the sharp rise in tuberculosis infection rates globally demonstrate the pressing need for the creation of novel, potent drugs for the treatment of tuberculosis. Given that several derivatives of benzimidazoles, such as those containing electron-withdrawing groups, have already been shown to have antimycobacterial action, they constitute one potential source of novel molecules. In the present work, a series of new benzimidazole derivatives were synthesized from Schiff's bases using different aromatic aldehydes. The structures of these compounds were confirmed by IR, NMR, Mass, and elemental analysis. The synthesized compounds (**BID_1-BID_5**) were screened for anti-tuberculosis activity against Mycobacterium tuberculosis and the results show that some of these derivatives possess good activity against Mycobacterium tuberculosis.

ORIGINAL RESEARCH ARTICLE

1. INTRODUCTION

The most common infectious disease that results in death worldwide is still tuberculosis (TB), a contagious infection brought on by the airborne transmission of Mycobacterium tuberculosis [1]. It is the most common sickness experienced by both developing and developed countries since it is an airborne illness for which there is no vaccination. Two common issues are related to treatment. The first is a serious and potentially fatal side effect of an anti-tubercular drug, such as hepatotoxicity, neuritis, depression, asthma, anorexia, etc., which frequently requires a temporary withdrawal from treatment or a change in the course of treatment. The other is resistance development brought on by a patient's failure to adhere to their treatment plan, which led to organisms' mutating their genes and making management more challenging [2–3].

Hence, it is a top priority to find new medications that are active against latent TB, extensively drug-resistant TB, and multidrug-resistant TB [4]. Three to four medications are currently used to treat TB for a duration of six to nine months [5]. Novel medications that can shorten this lengthy treatment period and target MDR, XRD, and latent strains of TB are therefore critically needed. Since microorganisms have outlasted all other forms of life in their ability to resist prophylactic or

treatment, the infectious microbial disease continues to be a serious issue on a global scale [6]. The discovery and creation of new classes of antimicrobial drugs have garnered a great deal of interest due to the rise in microbial resistance. Due to its presence in many natural and synthesized pharmaceutical substances, benzimidazole is the bioactive heterocycle that has been the most thoroughly researched. A variety of marketed drugs containing benzimidazole are thiabendazole, flubendazole (anthelmintic), astmizole (antihistaminic), lansoprazole and omeprazole (antiulcerative) [7-8]. Hence, with these observations, we examine the feasibility and efficiency of an approach to the synthesis of benzimidazole derivatives, which turns to exhibit significant antitubercular activities.

2. MATERIALS AND METHODS

Melting points were determined in open capillary tubes and were uncorrected. The IR spectra were recorded in KBr pellets on a Nicolet 400D spectrometer and ^{1}H NMR spectra were recorded in DMSO with TMS as the internal standard on a Bruker spectrometer at 400 MHz. LC-MS of selected samples taken on LC-MSD-Trap-SL-01046. The purity of compounds was checked by thin-layer chromatography on silica gel plate of 0.25 mm thickness using the different solvent systems. All the chemicals were laboratory grade and purchased from local markets and benzimidazole derivatives were prepared by the reported method [9-12].

2.1 The Experimental Work Comprises in three Steps.

2.1.1 Step-I: Preparation of o-phenylenediamine

4-chloro-2-nitro aniline was undergoes hydrogenation under atmospheric pressure and hydrogen was passed through a 25% aqueous ammonium hydroxide solution at 35^{O}C. After fitting the catalyst the solvent was distilled off from the filtrate until crystallization of o-phenylene diamine was observed. Cooling and filtration afford o-phenylene diamine with a yield off 86% catalyst that can be repeatedly used up.

2.1.2 Step-II: Synthesis of benzo[d]imidazol-2-amine

1, 2-diamine (o-phenylenediamine) 0.36 g (0.00072 mol) was dissolved in ethanol 10 ml. To the mixture CNBr 0.174 g(0.0003 mol) was added in the fuming hood. The reaction was stirred for 12 hr to obtain benzo[d]imidazol-2- amine.

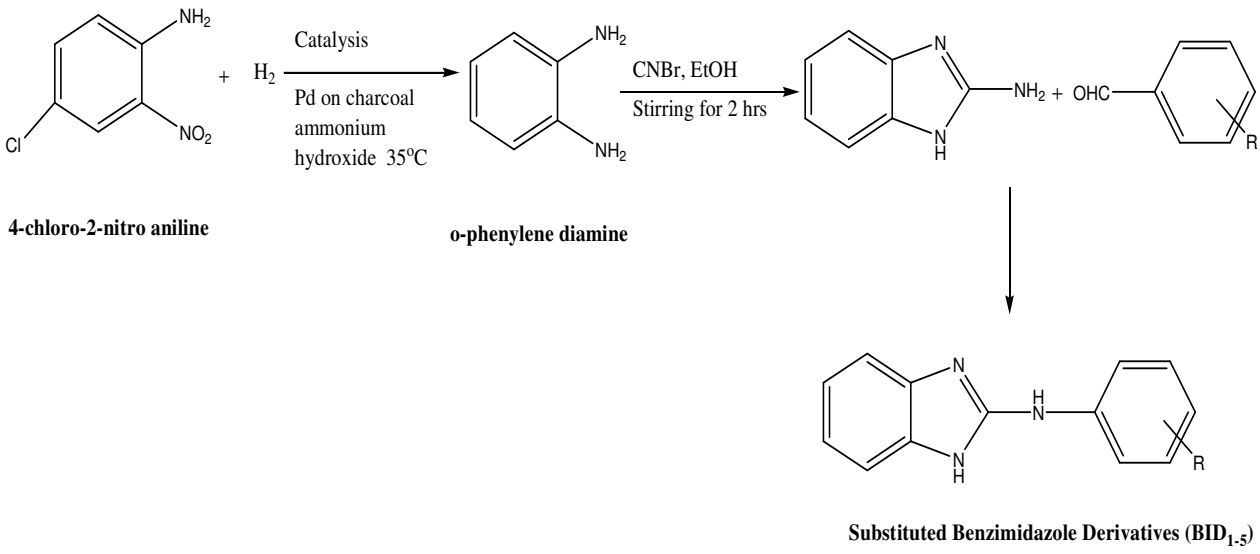
2.1.3 Step-III: Synthesis of Schiff's bases by using different aromatic aldehydes

The compound benzo[d]imidazol-2-amine 0.1g (0.0002 mol) was separately treated with various aromatic aldehyde (1eqivalent) and nickel nitrate (1eqivalent) in methanol stirring for 2hr at room temperature. After 2 hr the solution was poured into ice cold water. Then extracted with ethyl acetate. The organic layer was dried over Na_2SO_4.

Step-IV: Preparation of N-(4-chlorophenyl)-1H-benzo[d] imidazole-2-amine

Benzo[d]imidazol-2-amine 0.1g (0.0002 mol) was separately treated with para chloro phenyl benzaldehyde (1eqivalent) and nickel nitrate (1eqivalent) in methanol stirring for 2hr at room temperature. After 2 hr the solution was poured in to ice-cold water. Then extracted with ethyl acetate. The organic layer was dried over Na2SO4. The product was recrystallized using ethanol as a solvent. The Scheme shows how the reaction will proceed. (Figure-1)

SCHEME



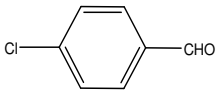
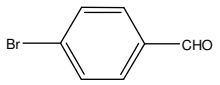
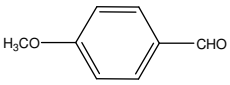
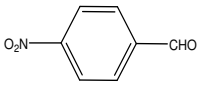
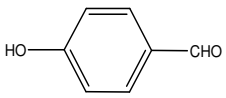
4-chloro-2-nitro aniline

o-phenylene diamine

Substituted Benzimidazole Derivatives (BID_{1-5})

Figure 1: Scheme: Synthetic route of targeted compounds

Table 1: List of Various Aromatic Aldehydes

S. No.	Compounds Code	Substituted Aromatic Aldehydes (R)	Structure of Aromatic Aldehydes (R)
1	BID_1	p-chloro benzaldeyde	
2	BID_2	p-bromo benzaldeyde	
3	BID_3	p-methoxy benzaldeyde	
4	BID_4	p-nitro benzaldeyde	
5	BID_5	p-hydroxy benzaldeyde	

3. Biological Activity

In-vitro study of the potency of a compound as an antitubercular agent can be measured in terms of a MIC and MIC_{50} (Minimum inhibitory Concentration)

The MIC value of a drug is the minimum concentration of the drug to prevent the growth of microorganisms. MIC_{50} value of a drug is the unit concentration of the drug to prevent the 50% growth of microorganisms

4. RESULTS AND DISCUSSION

4.1 Chemistry: All the novel benzimidazole derivatives were synthesized, purified and separated by using the recrystallization method. Synthesized compounds were characterized by using Elemental analysis, FT-IR, ^{1}HNMR and Mass Spectrometric studies. The integration curves fully support the orientation of protons in the analyzed compounds. Furthermore, all the compounds demonstrated the characteristic chemical shifts for the thiazine nucleus. Additionally, synthesized compounds were analyzed by mass spectra and indicated no difference in the fragmentation pattern among the set of synthesized series.

BID_1: N-(4-chlorophenyl)-1H-benzo[d]imidazol-2-amine

Yellowish Grey solid, Molecular Formula: $C_{13}H_{10}ClN_3$, Molecular weight: 243.69, Yield: 68.39%,M.P.: 193-195^{o}C, R_f value: 0.64, **FT-IR (KBr, cm^{-1}):** 3434. 67 (N-H Str.), 3117. 92 (=C-H Str.), 1608.26 (C=C Str.), 1629.41 (C=N Bend.), 1254.04 (C-N Bend.), 710.77 (Ar C-H Bend.), 743.35 (C-Cl Bend.), **^{1}H-NMR (400 MHz, DMSO, δ ppm):** 4.0 (s, 1H, NH), 5.0 s, 1H, NH (imidazole ring), 6.4-7.70 (m, 8H, Ar-H). **Mass Spectra:M^+** 245. 02, M^{+2} 162.03. **Elemental Analysis, % found (% required):** C, 64.03 (64.07); H, 4.11 (4.14); N, 17.25 (17.24); Cl, 14.54 (14.55).

BID_2: N-(4-bromophenyl)-1H-benzo[d]imidazol-2-amine

Greyish brown colored solid, Molecular Formula: $C_{13}H_{10}BrN_3$, Molecular weight: 288.26,Yield: 67.37%, M.P.: 202-204^{o}C, R_f value: 0.69i, **FT-IR (KBr, cm^{-1}):** 3434.65 (O-H Str.), 3068.65 (=C-H Str.), 1657.18 (C=C Str.), 1257.53 (C-N Bend.), 1623.02 (C=N Bend.), 676.84 (Ar C-H Bend.), 730.43 (C-Cl Bend.), 667.01 (C-Br Bend.), **^{1}H-NMR (400 MHz, DMSO, δ ppm):** 4.0 (s, 1H, NH), 5.0 s, 1H, NH (imidazole ring), 6.35-7.70 (m, 8H, Ar-H). **Mass Spectra:M^+** 290.21, M^{+2} 176.16 . **Elemental Analysis, % found (% required):** C, 54.17 (54.19); H, 3.48 (3.5); N, 14.54 (14.58); Br, 27.70 (27.73).

BID_3: N-(4-methoxyphenyl)-1H-benzo[d]imidazol-2-amine

Pale brown colored solid, **Molecular formula:** $C_{14}H_{13}N_3O$, **Molecular weight:** 238.97, **Yield:** 68.16%, **M.P.:** 231-233 ^{o}C, **R_f value:** 0.72, **FT-IR (KBr, cm^{-1}):** 3375.68 (O-H Str.), 3146.93 (=C-H Str.), 1617.67 (C=C Str.), 1278.82 (C-N Bend.), 1602.38 (C=N Bend.), 761.10 (Ar C-H Bend.), **^{1}H-NMR (400 MHz, DMSO, δ ppm):** 3.73(s, 3H, OCH_3), 4.0 (s, 1H, NH), 5.0 s, 1H, NH (imidazole ring), 6.35-7.70 (m, 8H, Ar-H). **Mass Spectra: M^+** 176.16, M^{+1} 239.03. **Elemental Analysis, % found (% required):** C, 70.27 (70.28); H, 5.45 (5.48); N, 17.53 (17.56); O, 6.71 (6.69).

BID_4: N-(4-nitrophenyl)-1H-benzo[d]imidazol-2-amine

Creamiest brown colored solid, **Molecular Formula:** $C_{13}H_{10}N_4O_2$, **Molecular weight:** 256.24,**Yield:** 73.44%, **M.P.:** 215-217 ^{o}C, **R_f value:** 0.73, **FT-IR (KBr, cm^{-1}):** 3369.95 (O-H Str.), 3117.92 (=C-H Str.), 1629.41 (C=C Str.), 1292.84 (C-N Bend.), 1600.54 (C=N Bend.), 710.77 (Ar C-H Bend.). **^{1}H-NMR (400 MHz, DMSO, δ ppm):** 4.0 (s, 1H, NH), 5.0 s, 1H, NH (imidazole ring), 6.72-7.94 (m, 8H, Ar-H). **Mass Spectra: M^+** 157.01, M^{+1} 257.18. **Elemental Analysis, % found (% required):** C, 61.37 (61.41); H, 3.94 (3.96); N, 22.01 (22.04); O, 12.62 (12.59).

BID_5: N-(4-hydroxyphenyl)-1H-benzo[d]imidazol-2-amine

Pale reddish orange colored solid, **Molecular Formula:** $C_{13}H_{11}N_3O$, **Molecular weight:** 225.17, **Yield:** 70.06%, **M.P.:** 207-209 ^{o}C, **R_f value:** 0.79, **FT-IR (KBr, cm^{-1}):** 3407.09 (O-H Str.), 3108.02 (=C-H Str.), 1609.91 (C=C Str.), 1296.02 (C-N Bend.), 1546.13 (C=N Bend.), 734.56 (Ar C-H Bend.). **^{1}H-NMR (400 MHz, DMSO, δ ppm):** 4.0 (s, 1H, NH), 5.0 s, 1H, NH (imidazole ring), 6.29-7.70 (m, 8H, Ar-H). **Mass Spectra: M^+** 162.03, M^{+1} 226.43. **Elemental Analysis, % found (% required):** C, 69.31 (69.32); H, 4.90 (4.92); N, 18.64 (18.66); O, 7.11 (7.1).

4.2 Biological Activity [13-14]

The novel synthesized compounds have shown moderate to strong activity against mycobacterium tuberculosis compared to standard drugs. The compound having chloro substituent on phenyl ring BID_1 was found to be most active against show good antitubercular activity against M. Tuberculosis with zone of inhibition 26mm. On further observation, it is analyzed that a minimum 08 mg weight of BID_1 is required to inhibit the growth of Mycobacterium tuberculosis (MIC-value) and 16 mg weight of compound is required to inhibit the 50% growth of microorganism r. (MIC_{50}).

Compound BID_2 show moderate antitubercular activity against M. Tuberculosis with zone of inhibition 23mm. On further observation it is analyzed that minimum 14 mg weight of BID_2 is required to inhibit the growth of Mycobacterium tuberculosis (MIC value) and 12 mg weight of the compound is required to inhibit the 50% growth of microorganism. (MIC_{50} value). Compound BID_3 show remarkable antitubercular activity against M. Tuberculosis with zone of inhibition 20 mm and minimum 12 mg weight is required to inhibit the growth of Mycobacterium tuberculosis (MIC value) and 10 mg weight of the compound required to inhibit the 50% growth of microorganism. Compound BID_4 show moderate antitubercular activity against M. Tuberculosis with zone of inhibition 16 mm. On further observation, it is analyzed that a minimum 17 mg weight of BID_4 is required to inhibit the growth of Mycobacterium tuberculosis (MIC value) and 08 mg weight of the compound is required to inhibit the 50% growth of microorganisms. (MIC_{50} value). Compound BID_5 show good antitubercular activity against M. Tuberculosis with zone of inhibition 17 mm and minimum 14 mg weight is required to inhibit the growth of Mycobacterium tuberculosis (MIC value) while 09 mg weight of the compound is required to inhibit the 50% growth of microorganism. (MIC_{50} value). (Table-2) (Figure-2)

Table 2: Zone of Inhibition (mm) & MIC Value of synthesized benzimidazole derivatives

S. No.	Compound Code	Z.I.(mm)	MIC	MIC_{50}
1.	BID_1	26	08	14
2.	BID_2	23	10	12
3.	BID_3	20	12	10
4.	BID_4	16	17	08
5.	BID_5	17	14	09
6.	Moxifloxacin	31	02	04

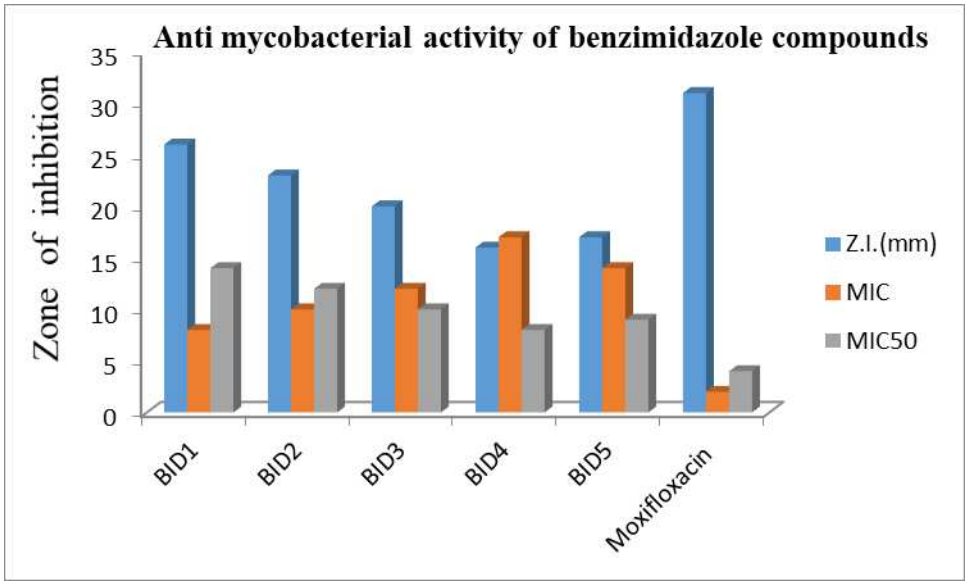


Figure 2: Anti-mycobacterial activity of synthesized benzimidazole derivatives

5. CONCLUSION

A series of benzimidazole derivatives had been synthesized and characterized by IR, NMR, mass and elemental analysis. The final compounds were screened for antitubercular activity against mycobacterium tuberculosis strains. The *in-vitro* anti-tubercular activities of all synthesized compounds against *M. Tuberculosis* by taking the Moxifloxacin (Z.I. = 24-31 mm) as standard drug and MIC and MIC_{50} value of each synthesized compound are evaluated. Such test results indicate that compounds BID_1 (Z.I. = 26 mm, MIC = 08 mg), BID_2 (Z.I. = 23 mm, MIC = 10 mg), BID_3 (Z.I. = 20 mm, MIC = 12 mg), BID_4 (Z.I. = 16 mm, MIC = 17 mg) and BID_5 (Z.I. = 17 mm MIC = 14 mg) are potential anti-tubercular agents and capable to inhibit grow. Results were significant to the standard drug Moxifloxacin.

ACKNOWLEDGMENT

The authors are thankful to Oriental University, Indore for providing the necessary facility to conduct the research and Punjab University, Chandigarh for providing spectral and analytical data of synthesized compounds.

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