



Original article

Synthesis, characterisation and evaluation of N-mannich bases of 2-substituted Benzimidazole derivatives

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ABSTRACT

Rationale: Benzimidazoles and its derivatives represent one of the mainly biological active classes of literature.

Aim: In this present study aimed to synthesize N-mannich bases derivatives compounds bearing of 2-substituted benzimidazole moiety, in order to investigate their possible biological activity.

Method: Benzimidazole compounds were prepared from the condensation reaction between *ortho* phenylene diamine and various acids. Mannich base of newly synthesized Benzimidazole derivatives were synthesized from 2-substituted Benzimidazoles by reacting with secondary amines. The purity of the compounds was ascertained by melting point (m.p) and thin layer chromatography (TLC). Structures of the synthesized compounds were elucidated by spectral data. Antimicrobial assay was performed by microbroth dilution method. Bacterial genomic DNA cleavage was assessed by Agarose gel electrophoresis. Toxicity of the most effective compounds was studied by Brine-shrimp lethality assay.

Result: Among the synthesized compounds, compound 5E (a) and (b) was establish to be the most potent against all tested microorganisms. This two compounds exhibited complete bacterial DNA cleavage and non-toxic.

Conclusion: These results suggest that it an interesting compound compared to the current therapeutic agents and are considered to investigate further for the same.

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1. Introduction

Benzimidazole derivatives are eminent biologically active N-containing heterocycles,¹ it is well known that benzimidazole derivatives attain antimicrobial analgesic and anti-inflammatory activities, as well as proved to have activities against HIV and cancer. Heterocyclic nucleus and substituted amino group at 1-position of the benzimidazole were reported to be associated with potent anti-inflammatory activity.² Therefore it was thought that preparing Mannich base derivatives from 2-substituted benzimidazoles would probably result in compounds of having high biological activities toward many diseases.

The extensively used as drugs such as proton pump inhibitor³ (Omeprazole), Antihelmenthetics⁴ (Albendazole), antidopaminergic⁵ (Domperidone), specifically, the 2-substituted analogs of

benzimidazoles are known to be potent biologically active compounds against inflammation, viral and microbial infection. Furthermore, benzimidazole derivatives are structural isosteres of naturally occurring nucleotides, which allows them to interact easily with the biopolymers of the living systems.⁶

Mannich reaction is a condensation between a compound containing atleast one active hydrogen atom, formaldehyde and ammonia, secondary amine have been used as a synthetic tool in the preparation of various therapeutic agents like, fluoxetine as antidepressant agent, ethacrynic acid a high ceiling loop diuretic, benzoquinamide, a high psychotic agent, Ranitidine, Triprolidine an H-receptor antagonist, and Trihexylphenidyl hydrochloride, an antispasmodic.⁷ Mannich bases are physiologically reactive because of the basic function rendering the molecule soluble in aqueous solvent when it is transformed into ammonium salt.⁸ Over the past few decades, Mannich bases of heterocyclic molecules have been grabbing the attention of the synthetic chemists for their wide gamut of biological activities ranging from antibacterial,⁹ anticancer, antiparkinson to anticonvulsant, analgesic, antispasmodic, anti-HIV, anti-malarial as well as intermediates in drug synthesis.¹⁰ 2-Substituted benzimidazole derivatives, one of the

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most important derivatives of benzimidazole are known to possess varied biological activities. In SAR, the biological activities of benzimidazole compounds depend upon the substitution at the N-1 or C-2 position.^{11,12} These observations have been guiding for the development of new mannich bases of 2-substituted benzimidazole derivatives and evaluated for antibacterial and anthelmintic activity.

In this present study, we aim to synthesis of a number of Mannich bases derived from substituted benzimidazole, then these compounds were characterized by FT-IR, ¹H NMR spectra and their most probable antimicrobial and lethality test.

2. Materials and methods

The melting points of the synthesized compounds were determined using a melting point apparatus and are uncorrected. Completion of the reaction and the purity of the synthesized compounds were ascertained by TLC using the solvent system Chloroform and Methanol (9:1) and the spots were detect using UV-Chamber. The synthesized compounds were characterized using MB 3000 series FT-IR Spectrophotometer by KBr-pellet method. ¹H NMR spectra was recorded on AMX-400 NMR spectrophotometer at 400 MHz using DMSO-d₆ as the solvent and tetra methyl silane (TMS) as an internal standard. The chemical shifts are articulated in δ ppm. Synthesis of the intermediate and target compounds was accomplished according to the steps depicted in Scheme 1.

2.1. Synthesis of mannich bases

2.1.1. General procedure for the synthesis of 2-substituted benzimidazole derivatives

A solution of substituted acid (0.01 mol) and *o*-phenylene diamine (0.01 mol) in 20 ml acetic acid was refluxed for 4 h, the precipitate obtained after cooling was recrystallized from ethanol.¹³

2.1.2. General procedure for the synthesis of mannich bases

Mannich Bases were prepared by a solution of 2-substituted benzimidazole (0.005 mol) in 10 ml ethanol, 0.005 mol of secondary amine and 0.005 mol of formaldehyde and then the reaction mixture was refluxed for 8 h. On cooling, the product formed was filtered, dried and recrystallised from Di methyl foramide (DMF). Specific details given to each compounds.¹³

2.2. Biological activity

2.2.1. Antibacterial and antifungal assays

Minimal inhibitory concentration (MIC, $\mu\text{g/ml}$) is defined as the lowest concentration of target compounds that completely inhibit the growth of bacteria, by means of standard two-fold serial

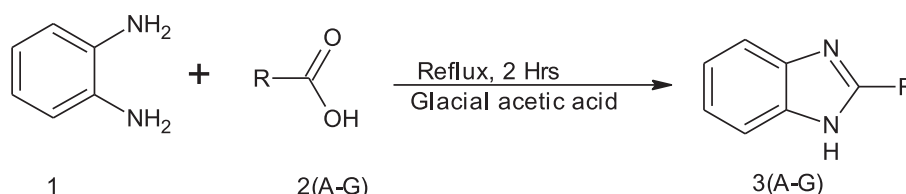
dilution method in 96-well micro-titer plates according to the National Committee for Clinical Laboratory Standards (NCCLS). Ampicillin, and Fluconazole, was used as control drugs for bacteria and fungi respectively. DMSO with inoculation bacterial not medicine was used as positive control to ensure that the solvent had no effect on bacteria growth. All the bacteria and fungi growth was monitored visually and UV-spectrophotometrically and the experiments were performed in triplicate.¹⁴

2.2.1.1. Preparation of inocula. The microorganisms were sub-cultured on Mueller Hinton broth (MHA) or Sabouraud dextrose broth (SDA) for bacteria and fungi, respectively, follow by incubation for 24 h at 37 °C. Inocula were prepared by transferring several colonies of microorganisms to sterile distilled water (5 ml). The suspensions were diluted in sterile distilled water were made to obtain the required working suspensions ($1-5 \times 10^4$ CFU/ml).

2.2.1.2. Preparation of plates. The test was performed in 96-well sterile microplates. All the wells received 100 μl of MHA (for bacteria) or Sabouraud broth (for fungus) supplemented with 10% glucose and 0.2% resazurin. The 100 μl of the working solution (1024, 512, 256, 128, 64, 32, 16, 8, 4, 2, 1 $\mu\text{g/ml}$) of 5A (a)-5G (b) were added into the wells in rows A to H in column 1. By using a multichannel pipette, 100 μl medium was transferred from column 1 to column 2, and the contents of the wells be mixed glowing. Identical serial 1:2 dilutions were continued through column 10 and 100 μl of excess medium was discarded from the wells in column 10. The 100 μl of the inoculums suspension was added to the wells in rows A to H in columns 1 to 11. Two wells column served as drug free controls. Another two-fold serial dilution of Ampicillin or Fluconazole was used as a positive control against bacteria and fungus, respectively. Each microplate was covered and incubated for 24 h at 37 °C. Any color changes from purple to pink or colorless were recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value. The average of three values was calculated and that was the MIC for the test material and bacterial or fungal strain. To confirm MIC s and to establish Minimum Bactericidal Concentration (MBC), 20 μl of each culture medium with no visible growth was removed from each well and inoculated in MHA or SDA agar plates. After 16–20 h of aerobic incubation at 37 °C, the number of surviving organisms was determined. MBC was defined as the lowest extract concentration at which 99.9% of the bacteria were killed. Each experiment was repeated twice.

2.2.2. DNA Cleavage study

2.2.2.1. Isolation of DNA. The 24 h old *Escherichia coli* culture (1.5 ml) is centrifuged to obtain the pellet, which is then dissolved in 0.5 mL of lysis buffer (100 mM tris pH 8.0, 50 mM EDTA, 10% SDS). To this 0.5 ml of saturated phenol was added and incubated at 55 °C



3A= R=H, 3B= R= -CH₃, 3C= R= -C₆H₅, 3D= R= -C₆H₄ (OH), 3E= R= -C₆H₃ (OH) SO₂OH
 3F= R= -COOH, 3G= R= -C₆H₄COOH

Scheme 1. Preparation of Mannich Base of 2-Substituted Benzimidazole derivatives.

for 10 min, then centrifuged at 10,000 rpm for 10 min and to the supernatant, equal volume of chloroform: isoamyl alcohol (24:1) and 1/20th volume of 3 M sodium acetate (pH 4.8) was added. Again centrifuging at 10,000 rpm for 10 min and to the supernatant, 3 volumes of cold absolute alcohol were added. The precipitated DNA was separated by centrifugation and the pellet was dried and dissolved in TAE buffer (10 mM tris pH 8.0, 1 mM EDTA) and stored in cold condition.

2.2.2.2. Agarose gel electrophoresis. Cleavage products were analyzed by agarose gel electrophoresis method. Test samples (1 mg/ml) were prepared in DMSO. The samples were added to the isolated DNA of *E. coli*. The samples were incubated for 2 h at 37 °C and then 20 mL of DNA sample (mixed with bromo phenol blue dye at 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g tris base, pH 8.0, 0.5 M EDTA/1 L) and finally loaded on agarose gel and passed the constant 50 V of electricity for around 30 min. Removing the gel and being stained with 30.0 mg/ml Acridine orange for 30 min. The stain was removed from the pan by hot running tap water for 5–10 min. Agarose gels was sufficiently de-stained after 1hr, the bands were observed under UV Transilluminator λ Max 254 nm. Photograph was taken by cannon digital camera the photograph used to determine the extent of DNA cleavage. Henceforth the results were compared with standard DNA marker.¹⁵

2.2.3. Brine-shrimp lethality assay

Brine-shrimp toxicity assay was used to determine cytotoxicity levels of the most active compounds 5E (a & b). Each test compound was dissolved in DMSO to obtain the stock concentration of 1000 μ g/ml and then stock solution was diluted to various concentrations (1000–1.953 μ g/ml). In order to prevent the toxicity results from possible false effect originated from DMSO's toxicity, stock solutions of the compounds were prepared according to suggested volume range by dissolving 1 mg of test compound in 10 μ L DMSO and completing to 1000 μ L with artificial seawater. Pure DMSO was used as a positive control for the toxicity assay. Fresh eggs of *Artemia salina* were hatched in a conical flask containing 300 ml artificial seawater made by dissolving a commercial marine salt in deionized water. The flasks were well aerated with the aid of an air pump, and kept in a water bath at 25–30 °C. The larvae were hatched with in 48 h. Ten larvae were transferred with pipette into each vial containing test compound and artificial seawater. A check count was performed after 24 h of exposure at room temperature and the number of dead larvae, exhibiting no internal or external movement during several seconds of observation, was noted. Three independent experiments were performed for each concentration of compounds.¹⁶

3. Results and discussion

3.1. Chemistry

The mannich bases of benzimidazole derivatives were synthesized by using the method described in earlier literature.¹³ All the compounds were obtained in good quantities. The condensation of the *ortho*-phenylene diamine (OPDA) and acids such as formic acid, acetic acid, benzoic acid, salicylic acid, sulpho salicylic acid, oxalic acid and pthalic acid yielded benzimidazole derivatives **3 (A–G)**. Targeted mannich bases compound [**5(A–G)a,b**] was obtained by a solution of 2-substituted benzimidazole (0.005 mol) in 10 ml ethanol, 0.005 mol of secondary amine and 0.005 mol of formaldehyde and then the reaction mixture was refluxed for 8 h. The completion of the reaction was confirmed by TLC. The melting point of the synthesized compounds was measured by using open

capillary tube method. All the synthesized compounds gave satisfactory IR and ¹H NMR spectra were consistent with the assigned structures. Synthesis procedure of the benzimidazole derivatives was outlined in Schemes 1 and 2. The physicochemical data of the compounds are presented in Table 1.

3.2. Physical and spectral data of synthesized compounds

3.2.1. 1-(1H-Benzimidazol-1-yl)-N,N-dimethylmethanamine 5A(a)

C₁₀H₁₃N₃, 66% yield, m.p. 170–173 °C, IR (KBr, ν cm⁻¹), 1201 {C–N str., (alkyl.)}, 1272, 1364 {C–N str., (aryl.)}, 1477{CH₂ (bend.)}, 1770 {C=N}, 1587{C=C}, 751{C–H (alkyl.)}, ¹H NMR (400 MHz, DMSO-d₆, δ ppm), 2.27(s, 6H, –CH₃), 4.80(s, 2H, –CH₂–), 7.26–8.08(m, 5H, Ar-H).

3.2.2. N-(1H-Benzimidazol-1-ylmethyl)-N-ethylethanamine 5A(b)

C₁₂H₁₇N₃, 67.5% yield, m.p. 178–180 °C, IR (KBr, ν cm⁻¹), 1201 {C–N str., (alkyl.)}, 1477{CH₂ (bend.)}, 1770{C=N}, 1587{C=C}, 751{C–H (alkyl.)}, ¹H NMR (400 MHz, DMSO-d₆, δ ppm), 1.00(t, 6H, –CH₃), 2.40(m, 4H, –CH₂–), 4.82(s, 2H, –CH₂–), 7.26–8.08 (m, 5H, Ar-H).

3.2.3. N,N-Dimethyl-1-(2-methyl-1H-benzimidazol-1-yl)methanamine 5B(a)

C₁₁H₁₅N₃, 68% yield, m.p. 262–264 °C, IR (KBr, ν cm⁻¹), 1012{C–N str., (alkyl.)}, 1286, 1157{C–N str., (aryl.)}, 1454{CH₂ (bend.)}, 1403 {CH₃(str.)}, 1644{C=N}, 1556, 1573{C=C}, 745{C–H (alkyl.)}, ¹H NMR (400 MHz, DMSO-d₆, δ ppm) 2.27–2.42(s, 9H, –CH₃), 4.78(s, 2H, –CH₂–), 7.26–8.08(m, 4H, Ar-H).

3.2.4. 1-N-[(2-Methyl-1H-benzimidazol-1-yl)methyl]ethanamine 5B(b)

C₁₃H₁₉N₃, 67% yield, m.p. 230–233 °C, IR (KBr, ν cm⁻¹), 1236{C–N str., (alkyl.)}, 1287{C–N str., (aryl.)}, 1404{CH₂ (bend.)}, 1352{CH₃ (str.)}, 1647{C=N}, 1522, 1544{C=C}, 745{C–H str., (alkyl.)}, ¹H NMR (400 MHz, DMSO-d₆, δ ppm), 1.00(t, 6H, CH₃), 2.40(m, 4H, –CH₂–), 2.42(s, 3H, –CH₃), 4.80(s, 2H, –CH₂–), 7.26–8.08(m, 4H, Ar-H).

3.2.5. N,N-Dimethyl-1-(2-phenyl-1H-benzimidazol-1-yl)methanamine 5C(a)

C₁₆H₁₇N₃, 65% yield, m.p. 326–328 °C, IR (KBr, ν cm⁻¹), 1271{C–N str., (aryl.)}, 1436{CH₂ (bend.)}, 1601{C=N}, 711{C–H (alkyl.)}, ¹H NMR (400 MHz, DMSO-d₆, δ ppm), 2.27(s, 6H, –CH₃), 4.80(s, 2H, –CH₂–), 7.26–7.70(m, 9H, Ar-H).

3.2.6. N-Ethyl-N-[(2-phenyl-1H-benzimidazol-1-yl)methyl]ethanamine 5C(b)

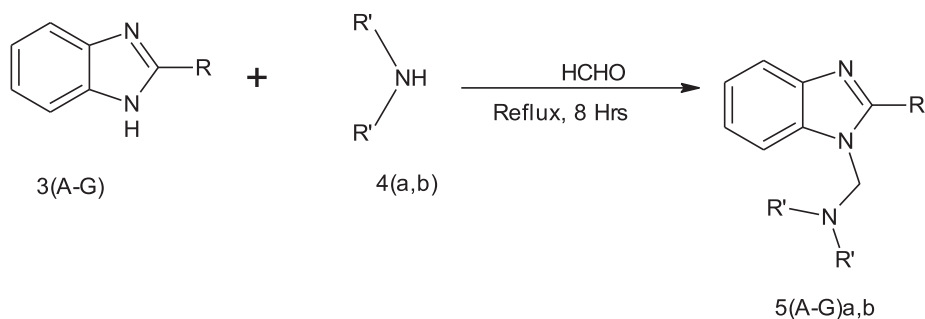
C₁₈H₂₁N₃, 65.5% yield, m.p. 315–318 °C, IR (KBr, ν cm⁻¹), 1011 {C–N str., (alkyl.)}, 1272{C–N str., (aryl.)}, 1434{CH₂ (bend.)}, 1600 {C=N}, 1555{C=C}, 712.28{C–H (alkyl.)}, ¹H NMR (400 MHz, DMSO-d₆, δ ppm), 1.00(t, 6H, –CH₃), 2.40(m, 4H, –CH₂–), 4.80(s, 2H, –CH₂–), 7.26–7.70(m, 9H, Ar-H).

3.2.7. 2-{1-[(Dimethylamino)methyl]-1H-benzimidazol-2-yl}phenol 5D(a)

C₁₆H₁₇ON₃, 63.7% yield, m.p. 256–259 °C, IR (KBr, ν cm⁻¹), 1345 {C–N str., (aryl.)}, 1413{CH₂ (bend.)}, 1627{C=N}, 1485{C=C}, 751{C–H (alkyl.)}, ¹H NMR (400 MHz, DMSO-d₆, δ ppm), 2.27(s, 6H, –CH₃), 4.80(s, 2H, –CH₂–), 5.00(s, 1H, –OH), 6.88–7.70(m, 8H, Ar-H).

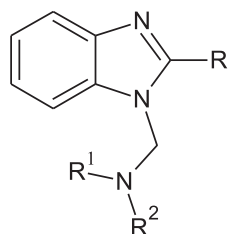
3.2.8. 2-{1-[(Diethylamino)methyl]-1H-benzimidazol-2-yl}phenol 5D(b)

C₁₈H₂₁N₃O, 64% yield, m.p. 260–262 °C, IR (KBr, ν cm⁻¹), 1217 {C–N Str., (alkyl.)}, 1270, 1345{C–N str., (aryl.)}, 1460{CH₂ (bend.)},



R' = a = -CH₃, R' = b = -C₂H₅

Synthesized compounds



| Compound s | R | R ¹ | R ² |
|------------|---|--------------------------------|--------------------------------|
| 5A(a) | -H | -CH ₃ | -CH ₃ |
| 5A(b) | -H | -C ₂ H ₅ | -C ₂ H ₅ |
| 5B(a) | -CH ₃ | -CH ₃ | -CH ₃ |
| 5B(b) | -CH ₃ | -C ₂ H ₅ | -C ₂ H ₅ |
| 5 C(a) | -C ₆ H ₅ | -CH ₃ | -CH ₃ |
| 5 C(b) | -C ₆ H ₅ | -C ₂ H ₅ | -C ₂ H ₅ |
| 5D(a) | -C ₆ H ₄ (2-OH) | -CH ₃ | -CH ₃ |
| 5D(b) | -C ₆ H ₄ (2-OH) | -C ₂ H ₅ | -C ₂ H ₅ |
| 5E(a) | -C ₆ H ₃ (2-OH)(5-SO ₂ OH) | -CH ₃ | -CH ₃ |
| 5E(b) | -C ₆ H ₃ (2-OH)(5-SO ₂ OH) | -C ₂ H ₅ | -C ₂ H ₅ |
| 5F(a) | -COOH | -CH ₃ | -CH ₃ |
| 5F(b) | -COOH | -C ₂ H ₅ | -C ₂ H ₅ |
| 5G(a) | -C ₆ H ₄ (2-COOH) | -CH ₃ | -CH ₃ |
| 5G(b) | -C ₆ H ₄ (2-COOH) | -C ₂ H ₅ | -C ₂ H ₅ |

Scheme 2. Preparation of 2- Substituted Benzimidazole derivatives from Ortho phenylene diamine.

1626{C=N}, 1484, 1556{C=C}, 702{C-O (str.)}, 752{C-H (alkyl.)}, ¹H NMR (400 MHz, DMSO-d₆, δ ppm), 1.00(t, 6H, -CH₃), 2.40(m, 4H, -CH₂-), 4.80(s, 2H, -CH₂-), 5.00(s, 1H, -OH), 6.88–7.70(m, 8H, Ar-H).

Table 1

Physicochemical parameters of 2-substituted Benzimidazole derivatives.

| Compound name | Molecular formula | Molecular weight (gms) | Percentage yield | R _f value | Melting point (°C) |
|---------------|---|------------------------|------------------|----------------------|--------------------|
| 5A(a) | C ₁₀ H ₁₃ N ₃ | 175.23 | 66% | 0.766 | 170–173 |
| 5A(b) | C ₁₂ H ₁₇ N ₃ | 203.283 | 67.5% | 0.865 | 178–180 |
| 5B(a) | C ₁₁ H ₁₅ N ₃ | 189.26 | 68% | 0.903 | 262–264 |
| 5B(b) | C ₁₃ H ₁₉ N ₃ | 217.31 | 67% | 0.838 | 230–233 |
| 5C(a) | C ₁₆ H ₁₇ N ₃ | 251.33 | 65% | 0.856 | 326–328 |
| 5C(b) | C ₁₈ H ₂₁ N ₃ | 279.38 | 65.5% | 0.677 | 315–318 |
| 5D(a) | C ₁₆ H ₁₇ ON ₃ | 267.33 | 63.7% | 0.806 | 256–259 |
| 5D(b) | C ₁₈ H ₂₁ N ₃ O | 295.38 | 64% | 0.75 | 260–262 |
| 5E(a) | C ₁₆ H ₁₇ N ₃ O ₄ S | 347.39 | 66.3% | 0.766 | 225–227 |
| 5E(b) | C ₁₈ H ₂₁ N ₃ O ₄ S | 375.44 | 67% | 0.838 | 230–234 |
| 5F(a) | C ₁₁ H ₁₃ N ₃ O ₂ | 219.24 | 66% | 0.863 | 245–247 |
| 5F(b) | C ₁₃ H ₁₇ N ₃ O ₂ | 247.29 | 65.5% | 0.872 | 258–261 |
| 5G(a) | C ₁₇ H ₁₇ N ₃ O ₂ | 295.33 | 67.5% | 0.834 | 276–279 |
| 5G(b) | C ₁₉ H ₂₁ N ₃ O ₂ | 323.38 | 66.5% | 0.829 | 298–301 |

3.2.9. 3-[1-[(Dimethylamino) methyl]-1H-benzimidazol-2-yl]-4-hydroxy benzene sulfonic acid 5E(a)

C₁₆H₁₇N₃O₄S, 66.3%, m.p. 225–227 °C, IR (KBr, ν cm⁻¹), 1041, 1157{C–N str., (alkyl.)}, 1220, 1261{C–N str., (aryl.)}, 1415{CH₂ (bend.)}, 1647{C=N}, 1480{C=C}, 1346{S=O}, 1296{C–O (str.)}, 734{C–H (alkyl.)}, ¹H NMR (400 MHz, DMSO-d₆, δ ppm), 2.00(s, 1H, -SO₃H), 2.27(s, 6H, -CH₃), 4.80(s, 2H, -CH₂-), 5.00(s, 1H, -SO₃H), 7.07–7.98(m, 7H, Ar-H).

3.2.10. 3-[1-[(Diethylamino) methyl]-1H-benzimidazol-2-yl]-4-hydroxy benzene sulfonic acid 5E(b)

C₁₈H₂₁N₃O₄S, 67%, m.p. 230–234 °C, IR (KBr, ν cm⁻¹), 1040, 1157{C–N str., (alkyl.)}, 1220, 1261{C–N str., (aryl.)}, 1437{CH₂ (bend.)}, 1647{C=N}, 1480{C=C}, 1296{S=O}, 674{C–H (alkyl.)}, ¹H NMR (400 MHz, DMSO-d₆, δ ppm), 1.00(t, 6H, -CH₃), 2.00(s, 1H, -SO₃H), 2.40(m, 4H, -CH₂-), 4.80(s, 2H, -CH₂-), 5.00(s, 1H, -SO₃H), 7.07–7.98(m, 7H, Ar-H).

3.2.11. 1-[(Dimethylamino)methyl]-1H-Benzimidazole-2-carboxylic acid 5F(a)

C₁₁H₁₃N₃O₂, 66% yield, m.p. 245–247 °C, IR (KBr, ν cm⁻¹), 1120{C–N str., (alkyl.)}, 1383{C–N str., (aryl.)}, 724{CH₂ (bend.)}, 3036

{O–H}, 1766{C–O (str.)}, 1544{N–H}, 2874{C–H (str.)}, ¹H NMR (400 MHz, DMSO-d₆, δ ppm), 2.27(s, 6H, –CH₃), 4.80(s, 2H, –CH₂–), 7.26–7.70(m, 4H, Ar–H), 11(s, 1H, –COOH).

3.2.12. 1-[(Diethylamino)methyl]-1H-Benzimidazole-2-carboxylic acid 5F(b)

C₁₃H₁₇N₃O₂, 65.5% yield, m.p. 258–261 °C, IR (KBr, ν cm⁻¹), 1121 {C–N str., (alkyl.)}, 1383{C–N str., (aryl.)}, 728{CH₂ (bend.)}, 1548 {N–H(bend.)}, 3043{O–H}, 1666{C=O}, 2875{C–H (alkyl.)}, ¹H NMR (400 MHz, DMSO-d₆, δ ppm), 1.00(t, 6H, –CH₃), 2.40(m, 4H, –CH₂–), 4.80(s, 2H, –CH₂–), 7.26–7.70(m, 4H, Ar–H), 11(s, 1H, –COOH).

3.2.13. 2-[1-[(Dimethylamino)methyl]-1H-benzimidazol-2-yl]benzoic acid 5G(a)

C₁₇H₁₇N₃O₂, 67.5% yield, m.p. 276–279 °C, IR (KBr, ν cm⁻¹), 1131 {C–N str., (alkyl.)}, 1401{C–N str., (aryl.)}, 742{CH₂ (bend.)}, 3340 {O–H}, 1736{C–O (str.)}, 1645{N–H}, ¹H NMR (400 MHz, DMSO-d₆, δ ppm), 2.27(s, 6H, –CH₃), 4.80(s, 2H, –CH₂–), 7.26–8.19(m, 8H, Ar–H), 11(s, 1H, –COOH).

3.2.14. 2-[1-[(Diethylamino)methyl]-1H-benzimidazol-2-yl]benzoic acid 5G(b)

C₁₉H₂₁N₃O₂, 66.5% yield, m.p. 298–301 °C, IR (KBr, ν cm⁻¹), 1081 {C–N str., (alkyl.)}, 1401{C–N (str.)}, 741{CH₂ (bend.)}, 3333{O–H}, 1548{C=C (str.)}, ¹H NMR (400 MHz, DMSO-d₆, δ ppm), 1.00(t, 6H, –CH₃), 2.40(m, 4H, –CH₂–), 4.80(s, 2H, –CH₂–), 7.26–8.19(m, 8H, Ar–H), 11(s, 1H, –COOH).

3.3. Biological activity

The title compounds [5(A–G) a,b] were evaluated for their *in vitro* antimicrobial activity against the following six human pathogenic microorganism *Bacillus subtilis* (NCIM 2458), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 15830), *Salmonella typhi* (ATCC 8713) *Candida albicans* (ATCC 76615), and *Aspergillus niger* (NCIM 1207) were tested for the antimicrobial efficiency of synthesized compounds. Ampicillin and fluconazole used as a standard drug for antibacterial and fungal study respectively. The observed antimicrobial data (MIC, MBC and MFC) of the compounds and the reference drugs are given in Tables 2 and 3.

When compared with the reference drug Ampicillin, most of the compounds in the series exhibited considerable antibacterial activity against gram-negative bacteria such as *E. coli* and *S. Typhi*

Table 2
In vitro Anti microbial Manich Base of 2-substituted Benzimidazole derivatives.

| Compound | A | B | C | D | E | F |
|-------------|------|------|------|------|------|------|
| 5A(a) | 128 | 128 | >64 | >128 | 512 | 512 |
| 5A(b) | 256 | 128 | 128 | 128 | >512 | 512 |
| 5B(a) | >256 | >512 | 128 | 256 | 128 | >256 |
| 5B(b) | 512 | >256 | 128 | >128 | 128 | >256 |
| 5C(a) | >128 | 256 | 256 | 512 | 512 | 512 |
| 5C(b) | 128 | 256 | >256 | >256 | 256 | >256 |
| 5D(a) | 256 | 512 | 256 | 256 | 512 | 256 |
| 5D(b) | 512 | >256 | 512 | >128 | >256 | 256 |
| 5E(a) | 16 | 16 | 8 | 16 | 32 | 64 |
| 5E(b) | 16 | >8 | 4 | >8 | >16 | >32 |
| 5F(a) | >64 | >32 | 64 | 32 | 128 | 64 |
| 5F(b) | 64 | >64 | 64 | 32 | >128 | >64 |
| 5G(a) | >256 | 256 | >128 | >256 | >512 | >256 |
| 5G(b) | 512 | 256 | 256 | 256 | 512 | 512 |
| Ampicillin | 2 | 2 | 1 | 1 | – | – |
| Fluconazole | – | – | – | – | 1 | 2 |

Note: **A:** *Bacillus subtilis* (NCIM 2458), **B:** *Staphylococcus aureus* (ATCC 25923), **C:** *Escherichia coli* (ATCC 15830), **D:** *Salmonella typhi* (ATCC 8713), **E:** *Candida albicans* (ATCC 76615), **F:** *Aspergillus niger* (NCIM 1207), **E:** *Candida albicans* (ATCC 76615), **F:** *Aspergillus niger* (NCIM 1207).

Table 3

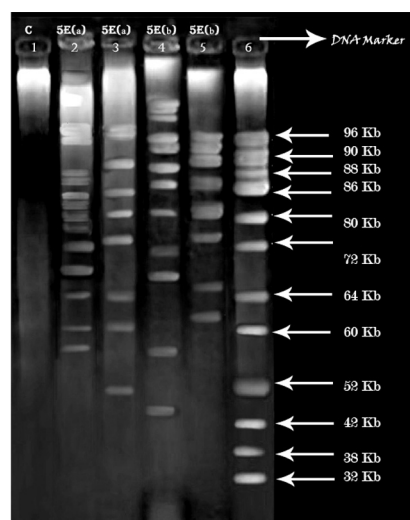
The Minimum bacterial concentration (MBC) and Minimum fungal concentration (MFC) Manich Base of 2-substituted Benzimidazole derivatives.

| Compound | A | B | C | D | E | F |
|-------------|------|------|------|------|------|------|
| 5A(a) | 256 | 256 | 128 | 256 | 1024 | 1024 |
| 5A(b) | 512 | 256 | 256 | 256 | 1024 | 1024 |
| 5B(a) | 512 | 1024 | 256 | 512 | 256 | 512 |
| 5B(b) | 1024 | 512 | 256 | 256 | 256 | 512 |
| 5C(a) | 256 | 512 | 512 | 1024 | 1024 | 1024 |
| 5C(b) | 256 | 512 | 512 | 512 | 512 | 512 |
| 5D(a) | 512 | 1024 | 512 | 512 | 1024 | 512 |
| 5D(b) | 1024 | 512 | 1024 | 256 | 512 | 512 |
| 5E(a) | 32 | 128 | 16 | 32 | 64 | 128 |
| 5E(b) | 32 | 16 | 8 | 16 | 32 | 64 |
| 5F(a) | 128 | 64 | 128 | 64 | 256 | 128 |
| 5F(b) | 128 | 128 | 128 | 64 | 256 | 128 |
| 5G(a) | 512 | 512 | 256 | 512 | 1024 | 512 |
| 5G(b) | 1024 | 512 | 512 | 512 | 1024 | 1024 |
| Ampicillin | 4 | 4 | 2 | 2 | – | – |
| Fluconazole | – | – | – | – | 2 | 4 |

Note: **A:** *Bacillus subtilis* (NCIM 2458), **B:** *Staphylococcus aureus* (ATCC 25923), **C:** *Escherichia coli* (ATCC 15830), **D:** *Salmonella typhi* (ATCC 8713), **E:** *Candida albicans* (ATCC 76615), **F:** *Aspergillus niger* (NCIM 1207).

(Table 2). The compounds 5E (a) and (b) were more potent than reference against gram-negative bacteria. The MIC values of compound 5E (a) against *E. coli* 8 µg/ml, *S. Typhi* 16 µg/ml, 5E (b) against *E. coli* 4 µg/ml, and *S. Typhi* 8 µg/ml. Among the compounds 5E (a) and (b) exhibited significant MBC values 16, 32 and 8, 16 µg/ml respectively against the gram-negative bacterial species. Antifungal activity of the tested mannich base benzimidazole derivatives was observed as significant growth inhibition against both funguses. Among the compounds 5E (a) and (b) were found to be higher MIC (32, 64 and 16, 32 µg/ml) against *C. albicans* and *A. niger* respectively. The MFC were found highest in the compound 5E (b) (33, 64 µg/ml) against the two tested fungal species respectively (Table 3).

From the results of antibacterial and antifungal screening, it was observed that of sulfo salicyl group of benzimidazole derivative 5E (a) and (b) exhibited dominating activity over the series. This may be attributed to their enhanced electronic character due to the presence of sulfonyl group at the benzimidazole residue favoring greater penetration through microbial membrane.



Note: The lane one serves as a control (DMSO) treated with DNA, there no cleavage induced by control. Lane 2 and 3 showed the DNA cleavage of compound 5E (a), the slicing occurring on the range from 96–52 kbs. The compound 5E (b) strongly cleavage the DNA without traces from >96–43 kbs in the lane 4 and 5. The marker DNA placed on the lane no. 6.

Fig. 1. DNA cleavage study for the compounds 5E (a & b).

Table 4
Brine-shrimp toxicity results of the compounds 5E (a & b).

| Concentration $\mu\text{g/ml}$ | Mortality | |
|--------------------------------|-------------|-------------|
| | 5E (a) | 5E (b) |
| 1000 | 4 | 4 |
| 500 | 3 | 4 |
| 250 | 3 | 3 |
| 125 | 2 | 2 |
| 62.5 | 1 | 1 |
| 31.25 | 1 | 1 |
| 15.625 | 1 | 1 |
| 07.812 | 1 | 1 |
| 03.906 | 1 | 1 |
| 01.953 | 1 | 1 |
| Control | 1 | 1 |
| LC ₅₀ | >1000 | >1000 |
| Toxicity level | No toxicity | No toxicity |

The results of DNA cleavage (Fig. 1) for mannich base benzimidazole compounds studied by agarose gel electrophoresis method. The gel after electrophoresis clearly revealed that, compounds 5E (a) and (b) did cleave the DNA completely, as no traces of DNA were found. The most impressive cleavage feature observed for 5E (b). This indicates that the compound 5E (b) is capable of performing direct double-strand scission, as a consequence, this compound serve better antimicrobial applications.

A chemical agent is valuable in medicinal field if only it possesses low toxicity with significant activity. Thus, toxicity of the compounds 5E (a) and (b) which have the highest antibacterial efficacy needs to be revealed. For this purpose Brine-Shrimp (*Artemia salina*) lethality assay was performed. This assay is regarded as a helpful method for preliminary screening of toxicity, and it has been used for establishing of microbial toxins, plant extract toxicity, heavy metals, and cytotoxicity testing of, natural and synthetic organic compounds. Moreover, *A. salina* toxicity test results show a good correlation with animals and human acute oral toxicity data. Likewise, the prognostic screening potential of the aquatic invertebrate tests for acute oral toxicity in man, including *A. salina* toxicity test, was slightly better than the rodent tests for test compounds. Toxicity test results calculate LC₅₀ values and 95% confidence intervals. The mannich base benzimidazole compounds 5E (a) and (b) give LC₅₀ values of the compounds and 95% confidence intervals because number of dead larvae did not exceed 50% of total larvae. This was a significant result demonstrating that the tested compounds are non-toxic in the tested concentration range. Toxicity test results were presented in (Table 4).

4. Conclusion

A series of mannich bases of 2-substituted benzimidazole derivatives were synthesized and their structures were elucidated by spectral data. The preliminary *in vitro* antibacterial and, antifungal

toxicological screening results of novel benzimidazole derivatives [5(A–G) a,b] reported good to moderate antimicrobial activity. The compound 5E (a) and (b) exhibited broad spectrum of antibacterial activity and antifungal activity. Most effective compounds were found to be non-toxic *A. salina* toxicity test and cleave the *E. coli* genomic DNA completely, as no traces of DNA were found. With the suitable molecular modification, these compounds can prove as potent antimicrobial agents in future.

Conflicts of interest

All authors have none to declare.

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