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ABSTRACT
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, anticancer, antiparkinson to anticonvulsant, anti-inflammatory, analgesic and anti-HIV. Using the knowledge of important features a novel series was designed to obtain improved antibacterial and antifungal activity. Therefore in the present research, we decided to synthesize 2-substituted benzimidazole derivatives by conventional method. Further the study will extended to introduce sulphanilamide and piperazine group substitution on N-1 position of 2-substituted benzimidazole by Mannich reaction and to screen the newly synthesized compounds for their antibacterial and antifungal activity.

INTRODUCTION:
Mannich Bases of wide variety of heterocyclic/nonheterocyclic nucleus have been revealed to posses cytotoxic, antibacterial, antifungal, anticonvulsant, anti-inflammatory and antimalarial activity. Benzimidazole derivatives were reported to possess antibacterial, antifungal, anti-inflammatory, antiviral, antitubercular, antioxidant, antiprotozoal, anthelmintic, , antihypertensive, antidiabetic and antinfluenza activity. The presence of basic mannich side chain in a drug may overcome the water insolubility problem through the formation of hydrochlorides.

The various strategies are available for the synthesis of benzimidazole and using a variety of starting material. In literature review, there are two general methods for the synthesis of 2-substituted benzimidazoles revealed that the condensation of Ophenylenediamine with carboxylic acid derivatives like anhydrides, ester, amides and acid chlorides to yield the corresponding the titled benzimidazole compound which often requires strong acidic conditions, and sometimes combines with very high temperatures or microwave irradiation. The other way involves a two-step procedure that includes the oxidative cyclo-dehydrogenation of schiff bases, which are often generated from the condensation of phenylenediamines and aldehydes.

In the present research, we decided to synthesize 2-substituted benzimidazole derivatives by conventional method. Further the study will extended to introduce sulphanilamide and piperazine group substitution on N-1 position of 2-substituted benzimidazole by Mannich reaction and to screen the newly synthesized compounds for their antibacterial and antifungal activity. Identification and characterization of synthesized compound was done by FT IR, 1H NMR, UV spectroscopy and Mass spectra method.
MATERIALS AND METHODS:

1. Experimental Section

The melting points were recorded in open capillaries on Elico melting point apparatus and were uncorrected. Spectral analysis were performed in the Sophisticated Analytical Instrumentation, using 1H NMR (Bruker – NMR 500MHz) Spectrometer, Mass (JEOL GC mate) spectrometer, and IR (Thermo Fischer – Nicolet iS5) and UV- Visible Spectrometer (Double beam UV Spectrometer – SHIMADZU -1700). Analytical samples were dried in vacuum and they were free of significant impurities on TLC. For antimicrobial activity by using two gram positive bacteria (Staphylococcus aureus MTCC 740 and Bacillus subtilis MTCC 121) and two gram negative bacteria (Escherichia coli MTCC 1302 and Pseudomonas aeruginosa MTCC 741) and two fungal organisms (Candida albicans ATCC 24433 and Trichophyton rubrum ATCC 2327).

• Procedure for synthesis of compounds SR1, SR2 and SR3:

13.5 g (0.125 mol) of O-phenylene diamine was placed in a 250 ml of round bottom flask and added 10.2 g (0.17 mol) of acetic acid for SR1, 14.96 g (0.17 mol) of propionic acid for SR2 and 17.34 g (0.17 mol) of butyric acid for SR3. The mixture was heated on a water bath at 100° C for 6-8 h, cooled and added 10% sodium hydroxide solution slowly with constant rotation of the flask, until the mixture was just alkaline to litmus. The crude benzimidazole derivative was filtered at the pump, and then washed with ice cold water, drained well and washed again with 25 ml of cold water. The crude product was dissolved in 200 ml of boiling water; 2 g of decolorizing carbon was added and digested for 15 min. The product was filtered rapidly at the pump through preheated buchner funnel and flask. The filtrate was cooled to about 10° C and the filtered product was again washed with 25 ml of cold water, dried at 100° C and weighed.

(a) Synthesis of 2-methyl-benzimidazole (SR1)

(b) Synthesis of 2-ethyl-benzimidazole (SR2)
Synthesis of 2-propyl-benzimidazole (SR₃)

- **Procedure for synthesis of compounds SR₄, SR₅ and SR₆**: To the 15 ml of methanolic solution, 0.66 g (0.005 mol) of 2-methyl benzimidazole for SR₄, 0.73 g (0.005 mol) of 2-ethyl benzimidazole for SR₅ and 0.80 g (0.005 mol) of 2-propyl benzimidazole for SR₆ was added to 0.86 g (0.005 mol) of sulphanilamide slowly with constant stirring under rigorous ice cooling. The reaction mixture was cooled well and 0.138 ml (0.005 mol) of formaldehyde solution (37% v/v) was added slowly with constant stirring. The reaction mixture was then adjusted to the pH of 3.5 with hydrochloric acid. The reaction mixture was kept in efficient ice cooling for half an hour to avoid losses of formaldehyde and then refluxed on water bath up to 3 h. The reflux time was dependent upon the sulphonamide chosen. After refluxing, the refluxed mixture was cooled at 0° C for 4 days, when crystallized product was obtained, which was recrystallized with dry distilled ethanol and DMF.

(d) Synthesis of 1-((sulphanilamido) methyl)-2-methyl-benzimidazole SR₄
(e) Synthesis of 1-((sulphanilamido) methyl)-2-ethyl-benzimidazole SRs

To a solution 1.32 g (0.01 mol) of 2-ethyl benzimidazole for SR7, 1.46 g (0.01 mol) of 2-propyl benzimidazole for SR9 in 15 ml of ethanol, 0.86 g (0.01 mol) of piperazine and 0.30 ml (0.01 mol) of formaldehyde solution (37% v/v) were added slowly with constant stirring under rigorous ice cold condition. The reaction mixture was kept in efficient ice cooling for half an hour to avoid losses of formaldehyde and then the reaction mixture was refluxed on water bath up to 30 min. The refluxed mixture was cooled at 0° C for 2-3 days in deep freeze. When crystallized product was obtained, filtered and dried. The product obtained was purified by recrystallized with dry distilled ethanol.

(f) Synthesis of 1-((sulphanilamido) methyl)-2-propyl-benzimidazole SRs
(g) Synthesis of 1-((piperazino) methyl)-2-methyl-benzimidazole (SR7)

\[
\text{2-methyl benzimidazole} + \text{HCHO} + \text{Piperazine} \rightarrow \text{N-Mannich base of 2-methyl benzimidazole}
\]

1 h stirring and reflux for 30 min.

(h) Synthesis of 1-((piperazino) methyl)-2-ethyl-benzimidazole (SR8)

\[
\text{2-ethyl benzimidazole} + \text{HCHO} + \text{Piperazine} \rightarrow \text{N-Mannich base of 2-ethyl benzimidazole}
\]

1 h stirring and reflux for 30 min.
(i) Synthesis of 1-((piperazino) methyl)-2-propyl-benzimidazole (SR₉)

Table - 1: Physical and Analytical data of the synthesized compounds

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Name</th>
<th>Nature</th>
<th>Solubility</th>
<th>Molecular weight (g)</th>
<th>Molecular formula</th>
<th>Melting point (°C)</th>
<th>Percentage yield (% w/w)</th>
<th>Rf values (CH₃OH:H₂O) 8:2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR₁</td>
<td>2-methyl-1-H-benzimidazole</td>
<td>Light yellow crystalline, solid</td>
<td>Freely soluble: Methanol. Soluble: Ethanol, benzene, acetone and chloroform. Insoluble: Water, hexane and ethyl acetate.</td>
<td>132.16</td>
<td>C₈H₈N₂</td>
<td>176</td>
<td>60.73</td>
<td>0.92</td>
</tr>
<tr>
<td>SR₂</td>
<td>2-ethyl-1-H-Benzimidazole</td>
<td>Yellowish white crystals</td>
<td>Freely soluble: Methanol and acetone. Soluble: Benzene, ethanol, ethyl acetate and chloroform.</td>
<td>146.18</td>
<td>C₉H₁₀N₂</td>
<td>211</td>
<td>38.89</td>
<td>0.72</td>
</tr>
<tr>
<td>Compound</td>
<td>Color and Form</td>
<td>Solubility</td>
<td>Molecular Weight</td>
<td>Molar Mass</td>
<td>Purity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
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<td></td>
</tr>
<tr>
<td>2-propyl-1-H-benzimidazole (SR3)</td>
<td>Light yellow crystals</td>
<td>Insoluble: Hexane and water. Freely soluble: Methanol. Soluble: Benzene, ethanol, ethyl acetate, chloroform and acetone. Insoluble: Hexane and water.</td>
<td>160.21</td>
<td>C_{10}H_{12}N_{2}</td>
<td>183</td>
<td>85.86 0.78</td>
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<td></td>
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<tr>
<td>1-((sulphanilamido) methyl)-2-methyl-benzimidazole (SR4)</td>
<td>Blackish red crystals</td>
<td>Soluble: Methanol. Slightly soluble: Acetone, benzene, ethanol, ethyl acetate and chloroform. Insoluble: Hexane and water.</td>
<td>316.37</td>
<td>C_{15}H_{16}N_{4}O_{2}S</td>
<td>142</td>
<td>45.58 0.93</td>
<td></td>
<td></td>
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<tr>
<td>1-((sulphanilamido) methyl)-2-ethyl-benzimidazole (SR5)</td>
<td>Light yellowish white powder</td>
<td>Soluble: Acetone. Slightly soluble: Methanol, ethyl acetate and chloroform. Insoluble: Ethanol, benzene, hexane and water.</td>
<td>330.40</td>
<td>C_{16}H_{18}N_{4}O_{2}S</td>
<td>123</td>
<td>31.61 0.81</td>
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<td></td>
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<tr>
<td>1-((sulphanilamido) methyl)-2-propyl-benzimidazole (SR6)</td>
<td>Black crystals</td>
<td>Soluble: Methanol, ethanol and acetone. Slightly soluble: Benzene, ethyl acetate and chloroform. Insoluble: Hexane and water.</td>
<td>344.43</td>
<td>C_{17}H_{20}N_{4}O_{2}S</td>
<td>149</td>
<td>28.45 0.82</td>
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</tr>
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</table>
### Spectral Data of synthesized Compounds:

(a) **2-methyl-benzimidazole (SR1):**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IR (KBr cm⁻¹)</th>
<th>UV (λmax nm, εmax)</th>
<th>¹H NMR</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-((piperazino)methyl)-2-methyl-benzimidazole (SR1)</td>
<td>3385 (s) N-H stretching, 3057 (w) Aromatic (=C-H) stretching, 2916 (m) Aliphatic C-H stretching in CH₂, 1624 (s) C=C stretching, 1591 (s) C=N stretching, 736 (s) Aromatic C-H out-of-plane bending.</td>
<td>λmax - 280.5 (εmax 0.2668), λmax - 243.0 (εmax 1.0140)</td>
<td>7.70 δ (2H, m, Ar-H of C₄ and C₇), 7.26 δ (2H, m, Ar-H of C₅ and C₆), 5.0 δ (1H, s, broad, NH), 2.42 δ (3H, s, -CH₃).</td>
<td></td>
</tr>
</tbody>
</table>

(b) **2-ethyl-benzimidazole (SR2):**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IR (KBr cm⁻¹)</th>
<th>UV (λmax nm, εmax)</th>
<th>¹H NMR</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-((piperazino)methyl)-2-ethyl-benzimidazole (SR2)</td>
<td>316(s) N-H stretching, 3052 (w) Aromatic (=C-H) stretching, 2973(m) Aliphatic C-H stretching in –CH₂, 1622 (s) C=C stretching, 1589 (s)C=N stretching, 1324 (s) CH3 bending, 1457 (m)CH2 bending, 794 (s) Aromatic C-H out-of-plane bending.</td>
<td>λmax - 243.0 (εmax 0.4058)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
\[ \lambda_{\text{max}} - 274.0 \ (\varepsilon_{\text{max}} 0.4591) \]

1H NMR: 7.70 \( \delta \) (2H, m, Ar-H of C₄ and C₇), 7.26 \( \delta \) (2H, m, Ar-H of C₅ and C₆), 5.0 \( \delta \) (1H, s, broad, NH), 2.59 \( \delta \) (2H, q, CH₂, broad), 1.24 \( \delta \) (3H, t, -CH₃).

(c) 2-propyl-benzimazole (SR₃):
IR (KBr cm⁻¹): 3385 (s)N-H stretching, 3055 (w) Aromatic (=C-H) stretching, 2957 (m) Aliphatic C-H stretching in –CH₂, 1632 (s) C=C stretching, 1591 (s) C=N stretching, 1320 (s) CH₃ bending, 1457 (w) CH₂ bending, 748 (s) Aromatic C-H bending.
UV: \[ \lambda_{\text{max}} - 239.0 \ (\varepsilon_{\text{max}} 0.5980) \]
\[ \lambda_{\text{max}} - 296.5 \ (\varepsilon_{\text{max}} 0.2899) \]
1H NMR: 7.70 \( \delta \) (2H, m, Ar-H of C₄ and C₇), 7.26 \( \delta \) (2H, m, Ar-H of C₅ and C₆), 5.0 \( \delta \) (1H, s, broad, NH), 2.55 \( \delta \) (2H, t, -CH₂), 1.66 \( \delta \) (2H, q, -CH₂), 0.96 \( \delta \) (3H, t, -CH₃).

(d) 1-((sulphonamido)-methyl)-2-methyl benzimazide (SR₄):
IR (KBr cm⁻¹): 3376 (s)1° N-H stretching in sulphonamide, 3263 (s) 2° N-H stretching in sulphonamide, 3063 (w) Aromatic (=C-H) stretching, 2921 (m)-H stretching in –CH₂, 1595 (s) C=N stretching, 1460 (s) C=C stretching, 1438 (m) CH₃ asymmetric bending, 1312 (s) SO₂ stretching, 1386 (s) C-N stretching (Aromatic tertiary amine), 746 (s) C-H out-of-planes bending (Aromatic C-H).
UV: \[ \lambda_{\text{max}} - 262.5 \ nm \ (\varepsilon_{\text{max}} - 1.4568) \]
1H NMR: 7.10 – 7.64 \( \delta \) (8H, m, Ar-H – C₄, C₅, C₆, C₇ and 4-phenyl protons), 6.06 \( \delta \) (2H,s, -SO₂NH₂), 4.57 \( \delta \) (2H, s, -SO₂NH₂), 5.82 \( \delta \) (1H, s, -NH), 2.53 \( \delta \) (3H, s, -CH₃).

(e) 1-((sulphonamido)-methyl)-2-ethyl benzimazide (SR₅):
IR (KBr cm⁻¹): 3378 (s)1° N-H stretching in sulphonamide, 3263 (s) 2° N-H stretching in sulphonamide, 3057 (w) Aromatic (=C-H) stretching, 2938 (m) C-H stretching in –CH₂, 1597 (s) C=N stretching, 1460 (s) C=C stretching, 1438 (m) Aliphatic CH₂ bending, 1313 (s) SO₂ stretching, 1387 (s) C-N stretching (Aromatic tertiary amine), 744 (s) C-H out-of-planes bending (Aromatic C-H)
UV: \[ \lambda_{\text{max}} - 240.0 \ nm \ (\varepsilon_{\text{max}} - 0.7504) \]
\[ \lambda_{\text{max}} - 293.0 \ nm \ (\varepsilon_{\text{max}} - 0.3855) \]
1H NMR: 7.12 – 7.64 \( \delta \) (8H, m, Ar-H – C₄, C₅, C₆, C₇ and 4-phenyl protons), 6.14 \( \delta \) (2H, s, -CH₂), 5.83 \( \delta \) (1H, s, -NH), 4.57 \( \delta \) (2H, s, -SO₂NH₂), 2.80 (2H, q, -CH₂), 1.28 \( \delta \) (3H, t, -CH₃).

(f) 1-((sulphonamido)-methyl)-2-propyl benzimazide (SR₆):
IR (KBr cm⁻¹): 3385 (s)1° N-H stretching in sulphonamide, 3289 (s) 2° N-H stretching in sulphonamide, 3026 (w) Aromatic (=C-H) stretching, 2931 (m) C-H stretching in –CH₂, 1561 (s) C=N stretching, 1458 (s) C=C stretching, 1421 (m) Aliphatic CH₂ bending, 1320 (s) SO₂ stretching, 1383 (s), C-N stretching (Aromatic tertiary amine), 748 (s) C-H out-of-planes bending (Aromatic C-H).
UV: \[ \lambda_{\text{max}} - 263.0 \ nm \ (\varepsilon_{\text{max}} - 0.7203) \]
1H NMR: 7.10 – 7.64 \( \delta \) (8H, m, Ar-H – C₄, C₅, C₆, C₇ and 4-phenyl protons), 6.18 \( \delta \) (2H, s, -CH₂), 5.76 \( \delta \) (1H, s, -NH), 4.60 \( \delta \) (2H, s, -SO₂NH₂), 2.95 \( \delta \) (2H, t, -CH₂), 1.78 – 1.85 \( \delta \) (2H, m, -CH₂), 0.95 \( \delta \) (3H, t, -CH₃).
(g) 1-(piperazino)-methyl)-2-methyl benzimidazole (SR7):
IR (KBr cm-1): 3409 (w) 2° N-H stretching in piperazine, 3063 (w) Aromatic (=C-H) stretching, 2933 (m) C-H stretching in CH2 group, 2874 (m) C-H stretching in piperazine, 1622 (s) C=N stretching, 1450 (s) C=C stretching, 1415 (s) CH2 asymmetric bending, 1346 (s) C-O stretching, 2934 (m) C-H stretching in piperazine and CH2 of ethyl group), 1.28 δ (3H, t, CH3).

UV: λmax 280.0 nm (εmax – 0.3619)  
λmax 273.5 nm (εmax – 0.2915)  
λmax 237.5 nm (εmax – 0.3762)

1H NMR: 7.15 – 7.64 δ (4H, m, Ar-H – C4, C5, C6 and C7), 5.47 δ (2H, s, -CH2), 2.53 δ (3H, s, -CH3), 2.37 – 2.93 δ (8H, m, CH2 of piperazine), 1.13 δ (1H, s, -NH).

(h) 1-(piperazino)-methyl)-2-ethyl benzimidazole (SR8):
IR (KBr cm-1): 3416 (w) 2° N-H stretching in piperazine, 3053 (w) Aromatic (=C-H) stretching, 2936 (m) C-H stretching in CH2 group, 2796 (m) C-H stretching in piperazine, 1622 (s) C=N stretching, 1429 (s) CH2 asymmetric bending, 1347 (s) C-N stretching (Aromatic tertiary amine), 741 (s) C-H out-of-plane bending (Aromatic C-H).

UV: λmax 280.5 nm (εmax – 0.2572)  
λmax 274.5 nm (εmax – 0.2415)

1H NMR: 7.15 – 7.64 δ (4H, m, Ar-H – C4, C5, C6, C7), 5.61 δ (2H, s, -CH2), 2.37 – 2.93 δ (10H, m, CH2 of piperazine and CH2 of ethyl group), 1.28 δ (3H, t, -CH3), 1.13 δ (1H, s, -NH).

(i) 1-(piperazino)-propyl)-2-ethyl benzimidazole (SR9):
IR (KBr cm-1): 3385 (w) 2° N-H stretching in piperazine, 3026 (w) Aromatic (=C-H) stretching, 2934 (m) C-H stretching in CH2 group, 2874 (m) C-H stretching in piperazine, 1631 (s) C=N stretching, 1430 (m) CH2 asymmetric bending, 1347 (s) C-N stretching (Aromatic tertiary amine), 749 (s) C-H out-of-plane bending (Aromatic C-H).

UV: λmax 280.5 nm (εmax – 0.2052)  
λmax 274.0 nm (εmax – 0.1995)

1H NMR: 7.15 – 7.64 δ (4H, m, Ar-H – C4, C5, C6 and C7), 5.47 δ (2H, s, -CH2), 2.64 – 2.93 δ (8H, m, CH2 of piperazine), 2.36 δ (2H, t, -CH2), 1.81 – 1.84 δ(2H, m, -CH2). 1.15 δ (1H, s, -NH), 0.94 δ (3H, t, -CH3).

2. Biological Screening:

a) Evaluation of in vitro antibacterial activity:

The in vitro anti bacterial study was carried out by using selected gastrointestinal tract infection (GIT) causing pathogens which includes two gram positive bacteria (Staphylococcus aureus MTCC 740 and Bacillus subtilis MTCC 121) and two gram negative bacteria (Escherichia coli MTCC 1302 and Pseudomonas aeruginosa MTCC 741). The synthesized compounds (SR4 – SR9) were tested for anti bacterial activity by disc diffusion method (Collin C.H., et al., 1995). They were dissolved in DMSO and sterilized by filtering through 0.45 μm millipore filter. Final inoculum of 10^8 CFU/ml of each bacterium was used. Nutrient agar medium was prepared and sterilized by an autoclave (121°C and 15 lbs for 20 min) and it transferred to previously sterilized petridishes (9 cm in diameter). After solidification, petriplates was inoculated with gram negative bacterial organisms
Escherichia coli MTCC 1302, Pseudomonas aeruginosa MTCC 741 and gram positive bacterial organisms Staphylococcus aureus MTCC 740 and Bacillus subtilis MTCC 121 in sterile nutrient agar medium at 45° C under aseptic condition. Sterile Whatmann filter paper discs (previously sterilized in U.V. lamp) were impregnated with synthesized compounds at concentrations of 25, 100 µg/ disc were placed in the organism-impregnated petri plates under sterile condition. The plates were left for 30 min to allow the diffusion of compounds at room temperature. Antibiotic disc of ciprofloxacin (100 µg/ disc) was used as positive control, while DMSO used as negative control. Then the plates were incubated for 24 h at 37 ± 1° C. The zone of inhibition Table 2 was calculated by measuring the minimum dimension of the zone of no microbial growth around each disc.

Table 2: *In vitro* antibacterial activity of synthesized compounds by disc diffusion method

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>SR4 (µg/disc)</th>
<th>SR5 (µg/disc)</th>
<th>SR6 (µg/disc)</th>
<th>SR7 (µg/disc)</th>
<th>SR8 (µg/disc)</th>
<th>SR9 (µg/disc)</th>
<th>Ciprofloxacin (µg/disc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>17</td>
<td>21</td>
<td>17</td>
<td>12</td>
<td>17.3</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>13</td>
<td>17</td>
<td>14</td>
<td>21</td>
<td>27</td>
<td>11</td>
<td>16.4</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>12</td>
<td>17</td>
<td>13</td>
<td>19</td>
<td>22.3</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>15</td>
<td>18</td>
<td>17</td>
<td>22</td>
<td>24</td>
<td>17</td>
<td>21.5</td>
</tr>
</tbody>
</table>

**Table 2: In vitro antibacterial activity of synthesized compounds by disc diffusion method**

b) Evaluation of *in vitro* antifungal activity:

The synthesized compounds (SR4 - SR9) were tested for anti-fungal activity by disc diffusion method. They were dissolved in DMSO and sterilized by filtering through 0.45 µm millipore filter. Final inoculum of 100 µl suspension containing 108 CFU / ml of each fungus was used. Sabouraud’s dextrose agar medium was prepared and sterilized by an autoclave (121° C and 15 lbs for 20 min) and transferred to previously sterilized petridishes (9 cm in diameter).
After solidification, petriplates were inoculated with fungal organisms Candida albicans ATCC 24433 and Trichophyton rubrum ATCC 2327 in sterile sabouraud’s dextrose agar medium at 45° C in aseptic condition. Sterile Whatmann filter paper discs (previously sterilized in U.V. lamp) were impregnated with synthesized compounds at a concentration of 25, 100 µg/ disc were placed in the organism-impregnated petri plates under sterile condition. The plates were left for 30 min to allow the diffusion of compounds at room temperature. Antibiotic disc of ketoconazole (100 µg/ disc) were used as positive control, while DMSO used as negative control. Then the plates were incubated for 48 h at 37±1° C for antifungal activity.

The zone of inhibition (Table-3) was calculated by measuring the minimum dimension of the zone of no microbial growth around each disc.

### Table 3: *In vitro* antifungal activity of synthesized compounds by disc diffusion method

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>SR4 (µg/disc)</th>
<th>SR5 (µg/disc)</th>
<th>SR6 (µg/disc)</th>
<th>SR7 (µg/disc)</th>
<th>SR8 (µg/disc)</th>
<th>SR9 (µg/disc)</th>
<th>Ketoconazole (µg/disc)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
<td>100</td>
<td>25</td>
<td>100</td>
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<tr>
<td><strong>Candida albicans</strong></td>
<td>16</td>
<td>21</td>
<td>17</td>
<td>21</td>
<td>14</td>
<td>17</td>
<td>11</td>
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<tr>
<td><strong>Trichophyton rubrum</strong></td>
<td>12</td>
<td>15</td>
<td>14</td>
<td>17.5</td>
<td>16</td>
<td>19.1</td>
<td>13</td>
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</tbody>
</table>

**RESULTS AND DISCUSSION:**

1. **Experimental Section**

The study involves the replacement of N-1 hydrogen of novel benzimidazole derivatives with different types of substitutions like sulphanilamide and piperazine to form N-methyl substituted benzimidazole derivatives by mannich reaction. The structure of the synthesized compounds were elucidated by physical and spectral (UV, IR, 1H NMR and Mass) analysis. The physical and analytical data of synthesized compounds were presented in Table 1. The NH band (3164-3385 cm⁻¹) and NH proton signal (δ 4.80 – 5.0 ppm) of 2-substituted benzimidazole in IR and ¹H NMR spectrum respectively in the synthesized compounds (SR1–SR3), confirmed the formation of benzimidazole nucleus.

In SR1, ¹H NMR spectrum showed a singlet for 3 protons at δ 2.42 confirmed the substitution of methyl group at C2 of benzimidazole nucleus. In SR2, gave quartet peak for 2 protons at δ 2.59 and a triplet peak
for 3 protons at δ 1.27 indicated the presence of ethyl group at C2 of benzimidazole. In SR3, a two triplet peak for 5 protons at δ 2.55 and δ 0.96 and a multiplet peak for 2 protons at δ 1.66 indicated the presence of propyl group at C2 of benzimidazole.

The IR spectrum of each N-mannich bases of SR4 to SR6 showed the characteristic IR absorption bands in the region of 3376-3385 cm\(^{-1}\), 3263-3289 cm\(^{-1}\) and 1312-1320 cm\(^{-1}\) due to the presence of primary amino, secondary amino and SO2 stretching of sulphonamide moiety. The structural confirmation of each N-mannich bases of SR4 to SR6 was further made using 1H NMR spectra. It showed signals at δ, ppm: 6.06 - 6.18 (2H, s, -CH2 proton), 4.57 – 4.60 (2H, s, SO2NH2 proton) and 5.76 – 5.83 (1H, s, NH of sulphonamide). Thus, confirmed the proposed structures for above N-mannich bases of corresponding 2-substituted benzimidazole derivatives.

The IR spectrum of each N-mannich bases of SR7 to SR9 showed the characteristic IR absorption bands in the region of 3385 - 3416 cm\(^{-1}\) due to the presence of 2° N-H (secondary amino) stretching of piperazine ring. The structural confirmation of each N-mannich bases of SR7 to SR9 was further made using 1H NMR spectra. The spectral data of compounds (SR1 to SR9) were presented in below. It showed signals at δ, ppm: 2.37 – 2.93 (8H, m, -CH2 of piperazine), 5.47 –5.61 (2H, s, CH2 proton) and 1.13 – 1.15 (1H, s, NH of piperazine).

Thus, confirmed the proposed structures for above N-mannich bases of corresponding 2-substituted benzimidazole derivatives. The structural confirmation of synthesized compounds of SR1 to SR9 was further made using Mass spectra. The molecular ion (M+) peaks such as 132.12, 146.21, 160.11, 316.37, 330.40, 344.43, 230.30, 244.33 and 258.36 for SR1, SR2, SR3, SR4, SR5, SR6, SR7, SR8 and SR9 respectively corresponded with their molecular weights. The predicted chemical structure of title compounds was further supported by the fragmentation peaks.

2. Biological Screening –

The compounds were screened for their antibacterial and antifungal activities. The activities reported by means of zone of inhibition in millimeter. All the compounds showed very good antibacterial and antifungal activities at the tested dose level.

a) Evaluation of \textit{in vitro} antibacterial activity:

The sulphanilamide group containing N-mannich bases were more superior for inhibiting the growth of \textit{Staphylococcus aureus} and \textit{Bacillus subtilis} than piperazine containing N-mannich bases. Among the compounds, SR1 to SR6, the compound SR5 was more active than the other compounds against the growth of \textit{Staphylococcus aureus}. Likewise, the compound SR6 was more active than SR4 and SRs to inhibit the growth of \textit{Bacillus subtilis}.

The piperazine group containing N-mannich bases were more superior for inhibiting the growth of \textit{Escherichia coli} and \textit{Pseudomonas aeruginosa} than sulphanilamide group containing N-mannich bases. Among the compounds SR7 to SR9, the compound SR8 was more active than the other compounds against the growth of \textit{Escherichia coli} and \textit{Pseudomonas aeruginosa}.

Among the tested compounds, piperazine derivatives were more superior to sulphanilamide derivatives against gram negative bacteria. But sulphanilamide derivatives were more active than piperazine derivatives against gram positive bacteria. The \textit{In vitro} antibacterial activity of synthesized compounds is presented in Table 2.
b) Evaluation of \textit{in vitro} antifungal activity:

The anti-fungal evaluation of compounds (SR\textsubscript{4} to SR\textsubscript{9}), the piperazine group containing N-mannich bases were more superior for inhibiting the growth of Candida albicans and Trichophyton rubrum than sulphanilamide group containing N-mannich bases. Among the compounds of SR\textsubscript{7} to SR\textsubscript{9}, the compound SR\textsubscript{9} was more active than the other compounds against the growth of Candida albicans and Trichophyton rubrum. The \textit{In vitro} antifungal activity of synthesized compounds is presented in Table \textit{3}. 

CONCLUSION:

The study suggests that the benzimidazole ring is an important pharmacophore in modern drug discovery and the tested derivatives of benzimidazoles have excellent scope for further development as commercial antimicrobial agents in the chemotherapeutic approach in humans. All the synthesized compounds showed very good antibacterial and antifungal activities at the tested dose level. This activity of synthesized compounds can be increased (or) equalized by altering the number of carbon atoms in side chain (or) introducing aromatic ring (or) substituted aromatic ring (or) heterocyclic ring (or) by introducing double bond in side chain in the 2nd position of benzimidazole nucleus.

REFERENCES: