SYNTHESIS, CHARACTERIZATION AND ANTI-MICROBIAL ACTIVITY OF SOME NOVEL BENZIMIDAZOLE DERIVATIVES
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ABSTRACT
In the present research O-Phenylenediamine used as a starting material and reacted with five different carboxylic acid derivatives. The product thus formed reacted with P-aminooacetophenone leading to the formation of five benzimidazole derivatives (BD-1 to BD-5). The structures of the synthesized compounds were confirmed by IR & Mass spectral analysis and they were evaluated for their antibacterial and antifungal activities. The antibacterial activities of synthesized compounds were screened against both gram positive S. aureus and gram negative E. coli by agar cup-plate method using ciprofloxacin as standard drug. The antifungal activities of synthesized compounds were screened against Candida albicans by agar cup-plate method using ketoconazole as standard drug. Results revealed that BD-4 and BD-5 showed good antibacterial & antifungal activity.

INTRODUCTION
Antibiotics are the substances that destroys or inhibits the growth of microorganisms and is used in the treatment of external or internal infections. While some antibiotics are produced by microorganisms ,most are now manufactured synthetically.

Benzimidazole:
The benzimidazole ring system is an important pharmacophore in medicinal chemistry and modern drug discovery. Compound bearing benzimidazole nucleus has been of great interest to synthetic and medicinal chemists from a long time due to their unique chemical and biological properties mainly related to traditional anthelmintics like Albendazole and Oxibendazole. Benzimidazole, benzimidazole carbamate (methyl-5-propythio-1Hbenzimidazole-2-yl carbamate) with extensive clinical use as an anthelmintic drug can also inhibit hepatocellular carcinoma cell proliferation under both in vitro and in vivo experimental conditions.

Benzimidazoles are very useful intermediates/subunits for the development of molecules of pharmaceutical or biological interest. The benzimidazole contains a phenyl ring fused to an imidazole ring. Imidazole nucleus was first discovered by Debus in the year 1859. The first benzimidazoles was prepared in 1872 by Hoebrecker and these are being explored in Pharmaceutical industries and substituted benzimidazole derivatives have also been found in the diverse therapeutic applications such as in anti-ulcers,anti-hypertensives,anti-virals,anti-fungals,anti-cancers,anti-histaminics.

Need of newer Antibiotics:
A prolonged and extensive clinical uses of classical antibiotics has lead to wide spread increase in resistant pathogenic bacteria. Therefore it is of considerable interest to find newer antibiotic which can potentially evade the emergence of resistance. Hence the identification of new potential...
antimicrobial agents is significantly in the present scenario. Keeping all these rational points we plan to focus our research on Benimidazole derivatives. [9]

EXPERIMENTAL SECTION

Materials:
O-phenylenediamine, P-aminoacetophenone, P- amino benzoic acid, 4N HCl, 10% NaOH, Phenoxo acetic acid, 4-methoxy benzoic acid, 3-nitro benzoic acid, 4-nitro benzoic acid, formaldehyde, Ethanol.

General procedure for synthesis of benimidazole derivatives:-

STEP-1
A mixture of o-phenylenediamine (0.05 mol) and carboxylic acid derivatives (0.05 mol) was refluxed in 4 N HCl for 4 hours on a heating mantle. After completion of reaction, solution was poured onto crushed ice,10% NaOH solution was added drop wise to neutralize and the resulting solid was filtered, washed with cold water, dried and recrystallized. By using this method synthesized 1A, 1B, 1C, 1D, 1E products. [9]

STEP-2
Synthesized 1A,1B, 1C, 1D, 1E products (0.01 mol) reacted with p-aminoacetophenone (0.01 mol) was dissolved in 40 ml of ethanol. To the above solution, formaldehyde (0.01 mol) was added and stirred magnetically at room temperature for 3 hours. Then, the resulting solution was refluxed on a water bath for 1 hour and cooled in an ice bath. [7] The product thus separated was filtered, dried and crystallized using ethanol. By using this method synthesized 2A, 2B, 2C, 2D, 2E products.

SYNTHESIS

Synthesis of 4-(3H-benzo[d]imidazol-2-yl) aniline:-

A mixture of o-phenylenediamine (0.05 mol; 5.40 g) and p-amino benzoic acid (0.05 mol; 6.85 g) was refluxed in 4 N HCl for 4 h on a heating mantle. After completion of reaction, solution was poured onto crushed ice,10% NaOH solution was added drop wise to neutralize and the resulting solid was filtered, washed with cold water, dried and recrystallized. [5]

Molecular Formula: C_{13}H_{13}N_{3} Molecular Weight: 209.25, solid, M.P. (°C) -93, % Yield - 75.35

Synthesis of 1-(4-((2-(4-amino phenyl) -1H-benzo[d]imidazol-1-yl) methyl) amino) phenyl) ethanolone:- (BD-1)
1-(4H-benzo[d]imidazol-2-yl) aniline (1.18 g, 0.01 mol) and p-aminoacetophenone (1.35 g, 0.01 mol) was dissolved in 40 mL of ethanol. To the above solution, formaldehyde (0.3 g, 0.01 mol) was added and stirred magnetically at room temperature for 3 h. Then, the resulting solution was refluxed on a water bath for 1 h and cooled in an ice bath. The product thus separated was filtered, dried and crystallized from ethanol.
Molecular Formula: C_{16}H_{14}N_{2}O Molecular Weight: 357, solid, M.P. (°C) -96, % Yield - 65.30

IR (KBr) cm$^{-1}$: 1652 (N-H), 3509 (NH$_2$), 1423 (Phenyl C=C), 1682 (C=O).

Mass spectroscopy: The m/z values of possible fragments 92.05, 119.05, 156.06, 284.5720, 316.581

Synthesis of 2-(phenoxy methyl)-1H-benzo[d]imidazole :- (2a)
A mixture of o-phenylenediamine 1 (0.05 mol; 5.40 g) and phenoxycetic acid 2 (0.05 mol; 7.60 g) in round bottom flask and refluxed in 4 N HCl for 3 h on water bath. After completion of the reaction, the solution was poured onto crushed ice,10% NaOH solution was added drop wise to neutralize and the resulting solid was filtered, washed with cold water, dried and recrystallized. [9]

Molecular Formula:C_{18}H_{17}N_{3}O, Molecular Weight: 224.26, solid, M.P. (°C) - 98, % Yield - 79.29

Synthesis of 1-(4-((2-ethyl-1H-benzo[d]imidazol-1-yl) methyl) amino) phenyl) ethanolone:- (BD-2)
2-(phenoxy methyl)-1H-benzo[d]imidazole (1.18 g, 0.01 mol) and p-aminoacetophenone (1.35 g, 0.01 mol) was dissolved in 40 mL of ethanol. To the above solution, formaldehyde (0.3 g, 0.01 mol) was added and stirred magnetically at room temperature for 3 h. Then, the resulting solution was refluxed on a water bath for 1 h and cooled in an ice bath. The product thus separated was filtered, dried and crystallized from ethanol.
Molecular Formula: C_{16}H_{14}N_{2}O Molecular Weight: 358, solid, M.P. (°C) -92, % Yield - 63.75

IR (KBr) cm$^{-1}$: 3362 (NH$_2$), 2243 (C=O), 1568 (C=C) Mass spectroscopy: The m/z values of possible fragments 91.8838, 190.7308, 347.5536

Synthesis of 2-(4-methoxy phenyl)-1H-benzo[d]imidazole :- (3a)
A mixture of o-phenylenediamine 1 (0.05 mol; 5.40 g) and 4-methoxy benzoic acid 2 (0.05 mol; 7.60 g) was refluxed in 4 N HCl for 4 h on a heating mantle. After completion of reaction, solution was poured onto crushed ice,10% NaOH solution was added drop wise to neutralize and the resulting solid was filtered, washed with cold water, dried and recrystallized.
Molecular Formula: C_{16}H_{14}N_{2}O Molecular Weight: 357, solid, M.P. (°C) -96, % Yield - 65.30

Mass spectroscopy: The m/z values of possible fragments 91.8838, 190.7308, 347.5536

Synthesis of 1-(4-((2-(4-methoxy phenyl)-1H-benzo[d]imidazol-1-yl) methyl) amino) phenyl) ethanolone:- (BD-3)
2-(4-methoxyphenyl)-1H-benzo[d]imidazole (1.18 g, 0.01 mol) and p-aminoacetophenone (1.35 g, 0.01 mol) was dissolved in 40 mL of ethanol. To the above solution, formaldehyde (0.3 g, 0.01 mol) was...
added and stirred magnetically at room temperature for 3 h. Then, the resulting solution was refluxed on a water bath for 1 h and cooled in an ice bath. The product thus separated 3 was filtered, dried and recrystallized from ethanol.

Molecular Formula: C_{10}H_{11}N_2O_2, Molecular Weight: 239.23, solid, M.P (°C) -95, % Yield - 72.69

Synthesis of 1-[(2-(3-nitrophenyl)-1H-benzol[d]imidazol-1-yl)methyl] amino phenyl ethanone:-

(1a) A mixture of o-phenylenediamine 1 (0.05 mol; 5.40 g) and 3-nitrobenzoic acid 2 (0.05 mol; 8.35 g) was dissolved in 40 mL of ethanol. To the above solution, formaldehyde (0.3 g, 0.01 mol) was added and stirred magnetically at room temperature for 3 h. Then, the resulting solution was refluxed on a water bath for 1 h and cooled in an ice bath. The product thus separated 3 was filtered, dried and recrystallized from ethanol.

Molecular Formula: C_{10}H_{11}N_2O_2, Molecular Weight: 239.23, solid, M.P (°C) -95, % Yield - 72.69

Synthesis of 2-(4-nitrophenyl)-1H-benzol[d]imidazole - (5a)

A mixture of o-phenylenediamine 1 (0.05 mol; 5.40 g) and 3-nitrobenzoic acid 2 (0.05 mol; 8.35 g) was refluxed in 4 N HCl for 4 h on a heating mantle. After completion of reaction, solution was poured onto crushed ice.10% NaOH solution was added drop wise to neutralize and the resulting solid was filtered, washed with cold water, dried and recrystallized. Molecular Formula: C_{10}H_{11}N_2O_2, Molecular Weight: 239.23, solid, M.P (°C) -95, % Yield - 72.69

Synthesis of 2-(3-nitrophenyl)-1H-benzol[d]imidazole - (4a)

A mixture of o-phenylenediamine 1 (0.05 mol; 5.40 g) and 3-nitrobenzoic acid 2 (0.05 mol; 8.35 g) was refluxed in 4 N HCl for 4 h on a heating mantle. After completion of reaction, solution was poured onto crushed ice.10% NaOH solution was added drop wise to neutralize and the resulting solid was filtered, washed with cold water, dried and recrystallized. Molecular Formula: C_{10}H_{11}N_2O_2, Molecular Weight: 239.23, solid, M.P (°C) -95, % Yield - 72.69

Synthesis of 1-[(2-(4-nitrophenyl)-1H-benzol[d]imidazol-1-yl)methyl] amino phenyl ethanone:-

(1b) A mixture of o-phenylenediamine 1 (0.05 mol; 5.40 g) and 3-nitrobenzoic acid 2 (0.05 mol; 8.35 g) was dissolved in 40 mL of ethanol. To the above solution, formaldehyde (0.3 g, 0.01 mol) was added and stirred magnetically at room temperature for 3 h. Then, the resulting solution was refluxed on a water bath for 1 h and cooled in an ice bath. The product thus separated 3 was filtered, dried and recrystallized from ethanol.

Molecular Formula: C_{10}H_{11}N_2O_2, Molecular Weight: 239.23, solid, M.P (°C) -92, % Yield - 70.85

Mass spectroscopy: The m/z values of possible fragments 118.7888, 134.7885, 187.7582, 224.7054, 346.5789, 254.6568

Inhibition (in mm)

<table>
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<th>S.No</th>
<th>Compounds</th>
<th>Zone of Inhibition (in mm)</th>
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<td></td>
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<td>C.albicans</td>
</tr>
<tr>
<td>1</td>
<td>BD-1</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>BD-2</td>
<td>21</td>
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<tr>
<td>3</td>
<td>BD-3</td>
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<tr>
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<tr>
<td>6</td>
<td>Ciprofloxacin (50 µg)</td>
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**RESULTS AND DISCUSSION**

With reference to the scheme, parent benzimidazoles were synthesized by using O-phenylenediamine and different types of carboxylic acids. The synthesized parent benzimidazoles were further reacted with p-aminoacetoephene. The satisfactory yield was obtained for the reactions. The completion of the work was checked by thin layer chromatography, silica gel was used as stationary phase and chloroform: methanol was used as mobile phase. The iodine vapour was used as detecting agent. The compounds were purified by recrystallization with ethanol. The structures of the synthesized compounds were consistent with IR, and mass spectra. The antibacterial activities of synthesized compounds were screened against both gram positive *S.aureus* and gram negative *E.coli* by agar cup plate method using ciprofloxacin as standard drug. The results are given below.

**Screening of Antimicrobial Activity**

i) **Antibacterial Activity**

The antibacterial activities of synthesized compounds were screened against both gram positive *S.aureus* and gram negative *E.coli* by agar cup-plate method using ciprofloxacin as standard drug. The results are given below.

<table>
<thead>
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<th>S.No</th>
<th>Compounds</th>
<th>Zone of Inhibition (in mm)</th>
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<tr>
<td></td>
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<td>C.albicans</td>
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<tr>
<td>1</td>
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<tr>
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<td>Ketonazole</td>
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</tbody>
</table>

The antibacterial activities of synthesized benzimidazoles where screened against gram positive staphlococcus aureus and gram negative equally using DMF as control and Ciprofloxacin as standard drug. All the synthesised benzimidazoles showed significant activity and the activity increases as the concentration increases.

Among five benzimidazole derivatives BD1 to BD5, BD1, BD2, BD3 and BD4 shown moderate activity against both gram positive and gram negative organisms. BD5 exhibited more activity against streptococcus aureus and BD4 exhibits better activity on E.Coli.
The reason for better activity of BD5 may be presence of electron with drawing group i.e., -NO2. Similarly better activity for BD4 against gram negative may be presence of NO2 group at meta positions. Remaining benzimidazoles derivatives showing moderate activity because of may be presence of electron donating group like –OCH3,-NH3.

The spectrum of activity is almost similar to both bacterial and fungal organisms but it may not be accurate statement since both BD4, BD5 have equal activity to that of BD1.

REFERENCES
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