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Synthesis and determination of antibacterial activity of Benzimidazole derivatives

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Abstract
The synthesized benzimidazoles compounds were prepared from the condensation reaction between oPhenylenediamine and various carbonyl compounds. The present paper deals with series of benzimidazole derivatives synthesized by a single step process by reacting o- phenylenediamine and benzoic acid. The structures of all the synthesized compounds were elucidated by using IR, ^{1}H-NMR, ^{13}C-NMR. The synthesized target compounds were evaluated in vitro antibacterial activity against three bacterial strains by employing the disc diffusion method using Ciprofloxacin as a standard drug.

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I. Introduction

Benzimidazole derivatives possess a wide range of bioactivities including antimicrobial, anthelmintic, antiviral, anticancer, and antihypertensive activities. From time immemorial, heterocyclic compounds have continued to attract the interest of medicinal chemists, because of their numerous therapeutic applications and effective druglike property among heterocyclic compounds benzimidazole derivatives occupied a major part in the field of pharmaceutical chemistry [1] and very important field of heterocyclic chemistry [2]. These substructures are often called 'privileged' due to their wide recurrence in bioactive compounds [3]. The Benzimidazole structure is part of the nucleotide portion of vitamin B12 and the nucleus of some drugs [4]. Benzimidazole, versatile pharmacophore, have received considerable attention due to their association with diverse biological activities, The Benzimidazole nucleus is of significant importance in medicinal chemistry research, and many Benzimidazole containing compounds exhibit important biological activities such as antibacterial[5-9], antitubercular[10], Anti- inflammatory, antiviral. anticancer antitumor [11] activities. Various potent drugs that are now being currently practiced in the market, like albendazole, omeprazole, mebendazole, etc. contain benzimidazole ring [12].

The prepared compounds were subjected to physiochemical studies like melting point determination, TLC and percentage yield. The structures of synthesized compounds were characterized by IR and NMR spectroscopy. The biological evaluation of newly synthesized compounds was carried out against three bacterial strains by employing the disc diffusion method using Ciprofloxacin as a standard drug.

II. Experimental

Melting point were determined in open capillary tubes and are uncorrected**.** The time required for the completion of the reaction was monitored by TLC using Silica gel G plates and spots were exposed in Iodine chamber.IR spectra were recorded on Perkin Elmer 1800(FTIR) spectrophotometer. Nuclear Magnetic Resonance (1HNMR) spectra were recorded on a Bruker using $CDCl_3$. The Chemical shift values are reported in parts per million (ppm) relative to Tetra methyl silane as internal reference. Infra-red (IR) spectra were recorded with a Bruker spectrophotometer. Purity of the compound and progress of the reaction were monitored by thin layer chromatography (TLC), with detection by Ultra-violet (UV) light and / or spots were visualized by exposure to iodine vapors [13].

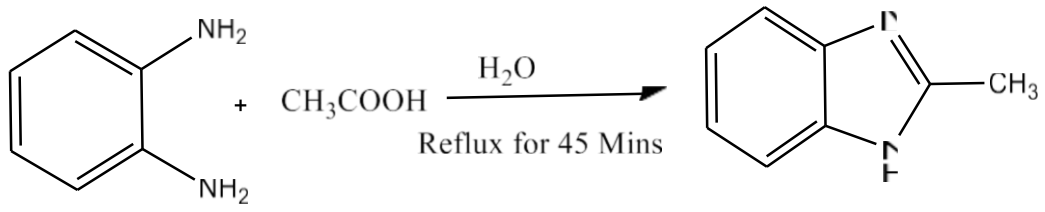
III. Methodology

Materials and Methods.

Synthesis

Synthesis: The compounds were synthesized by following synthesis pathway.

Benzene 1,2 diamine (A) and acetic acid undergoes reaction in the presence of H_2O. Reflux the reaction mixture for 45 minutes to get the product 2 methyl-1H- benzo(d) Imidazole.



(A)
Benzene-1,2-diamine

(B)
2 methyl-1H-benzo[d] imidazole

Benzimidazole compounds were synthesized starting from o-phenylenediamine and acidic moieties.

General Procedure for Synthesis of Compounds
O-phenylenediamine was refluxed with formic acid, benzoic acid and its derivatives in the presence of 4N hydrochloric acid (4N HCl) for 5 - 7 hrs at temperature 60 - 110 °C, rpm 380 on reflux condenser. The completion of the reaction was checked by TLC. On completion, 8 % NaOH (w/v) was gradually added until the reaction becomes alkaline. Reaction mixture was cooled in ice bath, allowed to stand for 25 - 30 mins to obtain clear precipitate. The product was filtered many times between the folds of filter papers, dried and recrystallized from EtOH.

Compound 1
2- Methyl H- Benzimidazole
It is a white or colorless solid.
Appearance: Crystalline powder
Solubility: Highly soluble in polar organic solvents and water.
Yield : 46%
M.P : 169 – 171^{0}C
Chemical Formula: $C_8H_8N_2$
Molecular weight: 132
Density: 1.1083
Pka: 6.19 (at 25 ^{0}C)
Refractive index: 1.6313
Mass Fragmentation: 132[M]$^+$, 133 [M+H] $^+$
The IR spectrum of this compound shows an absorption pack at 3178 cm-1 refers to (N- H) group and an absorption pack at 2916 cm^{-1} refers to (C-H) aliphatic.
^{1}HNMR (300 MHz) δ(ppm) Benz: (7.26t 2H and 7.71t 2H), N-H (2.45), CH3 (2.20s).

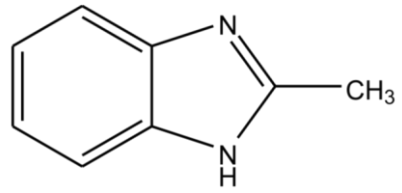
Compound 2
2-Phenyl-1H-Benzimidazole
Appearance: Pale grey to beige solid
Solubility: Slightly soluble in water and freely soluble in alcohol
Yield : 48%
M.P. : 293-296 0C
Chemical Formula: $C_{13}H_{10}N_2$
Molecular weight :194.24
Density: 1.02g/cm^3
Pka Value: 11.91 at 25^{0}C
Refractive index: 1.6360
Mass Fragmentation: 195 [M+H] ,194[M] $^+$
IR : N-H (3529.43cm -1), C = C (1443.83, 1409.39 cm-1), C = N (1569.57 cm -1).

[1] HNMR (DMSO, 300MHz): δ5.00 (s, 1H NH), 7.24 (dd, 2H, Ar-H), 7.54-7.46 (m, 3H, Ar-H), 7.66 (dd, 2H), 8.39-8.31 (m, 2H, Ar-H.

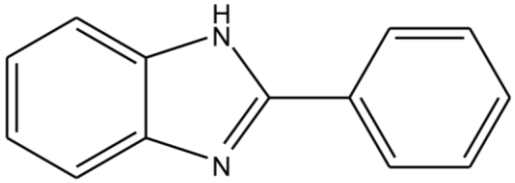
Compound 3
2- (1H- 1, 3- benzodiazol- 2- yl) aniline
Appearance: A crystalline solid with a white to pale yellow color
Solubility: Moderately soluble in organic solvents not highly soluble in water.
Yield: 57%
M.P.: 224 - 226 °C
Chemical Formula: $C_{13}H_{11}N_3$
Molecular weight: 209.25
Density: 209.25gm/mol
Pka: 4.63 at 25^0C
Mass Fragmentation: 209$[M]^+$, 210 $[M+H]^+$
IR N-H (3354.70 cm^{-1}), C = C (1289.25, 1351.07 cm^{-1}), C = N (1701.08 cm^{-1}).
[1]HNMR (DMSO, 300MHz): δ 6.72 (1H, ddd, ArH), 7.15-7.25 (3H, 7.15 (ddd, Ar-H Benz), 7.23 (ddd, Ar-H Benz), 7.39 (ddd, Ar-H)), 7.49 (1H, ddd, Ar-H), 7.57-7.58 (2H, 7.57 (ddd, Ar-H),
7.58 (ddd, Ar-H Benz)).

Compound – 4
2- (3, 4- dimethoxyphenyl)- 1H- 1, 3- benzodiazole
Appearance: White to off white crystalline solid
Solubility: Sparingly soluble in water but more soluble in organic solvents.
Yield: 70%
M.P.: 234 - 237 °C
Chemical Formula: $C_{15}H_{14}N_2O_2$
Molecular weight: 254
Density: 254.28 g/mol
Pka: 5.6 at 25^0C
Mass Fragmentation: 254$[M]^+$, 255 $[M+H]^+$
IR: N-H (3547.86 cm-1), C = C (1514.94, 1465.30 cm-1), C = N (1675.17 cm-1), Asymmetric
C-O-C (1265.64 cm-1), Symmetric C-O-C (1134.19 cm -1), Aromatic C-H stretch (3360.64 cm-1), Out of plane ring C-O blend (627.52 cm-1), Out of plane CH bend (723.04, 761.76 cm-1)

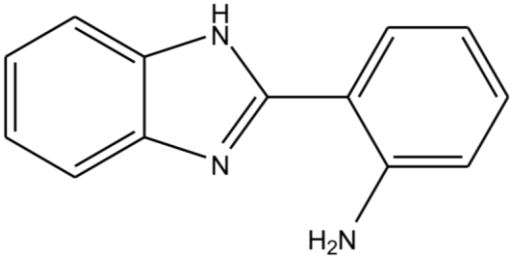
Structures of synthesized compounds



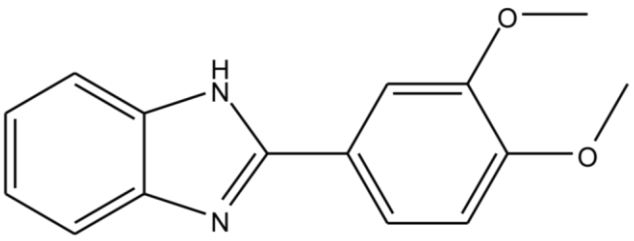
2-methyl-1*H*-benzo[*d*]imidazole



2-Phenyl-1H-Benzimidazole :



2-(1*H*-benzo[*d*]imidazol-2-yl)aniline



2- (3, 4- dimethoxyphenyl)- 1H- 1, 3- benzodiazole

[1] HNMR (DMSO, 300MHz): δ 3.83 (3H, 3.83 (s), 3.83 (s), 3.83 (s)), 3.83 (3H, 3.83 (s), 3.83 (s), 3.83(s)), 6.99 (1H, dd, Ar-H), 7.22 (1H, ddd, Ar-H), 7.25-7.28 (2H, 7.25 (dd, Ar-H), 7.28 (ddd, Ar-H)), 7.54 (1H, dd, Ar-H), 7.59 (1H, ddd, Ar-H), 7.60 (1H, ddd, Ar-H).

Antibacterial Activity of the Synthesized Compounds

The antimicrobial activity of newly synthesized compounds was evaluated against three bacterial strains, *Staphylococcus auraes (S. auraes), Escherichia coli (E. coli) and Klebsiella pneumonia (K. pneumonia)* using Ciprofloxacin (5µg/disc) as a standard drug.

Culture Media and Disc Preparation.

Nutrient agar, Muller Hinton agar and Nutrient broth were prepared according to the manufacturer instruction in which the prepared media was autoclaved at 116^0C for 25 minutes. Then the prepare culture media was checked for the sterility for 24 hours at 47^0C. Quality control stains of *Staphylococcus auraes (S. auraes), Escherichia coli (E. coli) and Klebsiella pneumonia (K. pneumonia)* was used to perform the antibacterial activities of the agents. Whatman filter paper was used to prepare a disc of 8 mm diameter using manual paper punching. Solution was prepared according to the standard protocol [14]. Analytical balance was used to measure a 40µg, 30µg, 20µg and 10µg of each chemical powder and added to 150µl dimethylsulfoxide (DMSO) and mixed to form a homogenous solution A 10µl of solution was added to the sterile disc prepared before using sterile micropipette. The plate was incubated for 24 hours. at 47^0C. A 3-5 colonies was picked and suspended in 5ml nutrient broth to form 0.5 McFarland standards. From the suspension by using sterile swab deepen and swab on Muller Hinton agar plate in three directions to form uniform inoculum. Then a disc with a control gentamicin and solution impregnated disc was placed on the plate and incubated for 24 hours at 47^0C. Each disc was labeled with its unique ID number on the back of the Petri-dish. Antibacterial activity was considered if there is zone of inhibition around the disc. The synthesized compounds were evaluated in vitro for antimicrobial activity against three bacterial strains, Staphylococcus auraes (S. auraes), Escherichia coli (E. coli) and Klebsiella pneumonia (K. pneumonia) using Ciprofloxacin as a standard. Gentamicin was used as a control in this study and DMSO used as a solvent. Disc diffusion method was used for the sensitivity testing and the diameter of zones of inhibition (ZOI) was documented in millimeter (Table-1).

Table-1: Antimicrobial activity of compounds with zones of inhibition in millimeter

Zone of Inhibition (mm)

Compound code/Conc.	Bacteria		
	S. auraes	*E. coli*	*K. pneumonia*
Compound 1 10µg	10	6	8
Compound 2 20µg	7	8	6
Compound 3 30µg	9	7	6
Compound 4 40µg	6	7	8
Standard	24	32	6

IV. Conclusion

In the present study, biologically active benzimidazole derivatives were synthesized and evaluated for antimicrobial activity. All the newly synthesized compounds were evaluated in vitro for antimicrobial activity by the disc diffusion method and its zone of inhibition was determined against three different bacterial strains. Among the synthesized compounds, compounds and 2 showed better antimicrobial activity against S. auras bacterial strain. Compound 3 and 4 exhibited moderate activity against all the bacterial strains.

Acknowledgement

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