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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES OF MANNICH BASES OF BENZIMIDAZOLE DERIVATIVES

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KEYWORDS

Benzimidazole derivatives, Mannich bases, antimicrobial activity, QSAR, PASS prediction, FT-IR, ^{1}H-NMR.

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Abstract

Benzimidazole derivatives are an important class of heterocyclic compounds exhibiting a wide range of pharmacological activities. In the present study, a novel series of Mannich bases derived from substituted benzimidazoles were synthesized and evaluated for their biological potential. The synthesized compounds were prepared via condensation of *o-phenylenediamine* with various amino acid derivatives followed by Mannich reaction using formaldehyde and secondary amines. The synthesized molecules were characterized by physicochemical parameters, Fourier Transform Infrared (FT-IR) spectroscopy and proton Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy. Drug-likeness properties and preliminary QSAR parameters were evaluated using Molinspiration software, and biological activity spectra were predicted using PASS prediction tools. Antibacterial activity of the synthesized compounds was assessed using the disc diffusion method against Bacillus subtilis, Pseudomonas aeruginosa, and Staphylococcus aureus. Most of the compounds showed moderate antibacterial activity when compared with the standard drug tetracycline. The computational predictions indicated potential activities such as antischistosomal, cachexia treatment, taurine dehydrogenase inhibition and gluconate-2-dehydrogenase inhibition. The results suggest that the synthesized benzimidazole Mannich bases could serve as promising scaffolds for further development of antimicrobial agents.

INTRODUCTION

Medicinal chemistry plays a crucial role in the discovery and development of new therapeutic agents. It integrates principles from organic chemistry, pharmacology, biochemistry and molecular biology to design and synthesize biologically active compounds that can be used for the treatment and prevention of various diseases. Drug discovery involves identification of potential lead molecules, optimization of their physicochemical properties and evaluation of their biological activities through various experimental and computational approaches (1–3).

Nitrogen-containing heterocyclic compounds are widely distributed in many biologically active natural products and synthetic drugs. Among these, benzimidazole derivatives have attracted considerable attention due to their diverse pharmacological activities including antimicrobial, anti-inflammatory, antiviral,

anticancer and antiparasitic effects (4,5). Benzimidazole derivatives have also been reported to possess antioxidant, antihypertensive and antifungal properties, making them valuable scaffolds in medicinal chemistry research (6).

The benzimidazole nucleus consists of a fused benzene and imidazole ring system and is considered an important pharmacophore in drug design. Several clinically important drugs such as proton pump inhibitors (e.g., omeprazole) and anthelmintic agents (e.g., albendazole and mebendazole) contain the benzimidazole scaffold, highlighting its therapeutic importance (7,8). Because of its structural versatility and ability to interact with multiple biological targets, extensive research has been conducted on the synthesis and biological evaluation of substituted benzimidazole derivatives (9).

The Mannich reaction is one of the most important synthetic methods for introducing aminomethyl groups into organic molecules. This reaction involves the condensation of an active hydrogen compound with formaldehyde and an amine to form β-aminocarbonyl compounds known as Mannich bases (10). Mannich bases are well known for their wide range of pharmacological activities including antimicrobial, analgesic, anti-inflammatory and anticancer properties (11). Incorporation of Mannich base functionality into heterocyclic systems such as benzimidazole often enhances biological activity and improves pharmacokinetic properties of the molecules (12).

In recent years, computational drug design approaches such as quantitative structure–activity relationship (QSAR) analysis and prediction of activity spectra for substances (PASS) have become important tools in medicinal chemistry. These computational methods help predict biological activity profiles, drug-likeness and pharmacological potential of newly synthesized compounds prior to biological screening (13). Such strategies significantly reduce the time, cost and chemical resources involved in traditional drug discovery processes.

Considering the biological significance of benzimidazole derivatives and the pharmacological potential of Mannich bases, the present study focuses on the synthesis, characterization and biological evaluation of novel Mannich bases derived from substituted benzimidazoles. The synthesized compounds were characterized using FT-IR and ^{1}H-NMR spectroscopy and evaluated for their antimicrobial activity against selected bacterial strains.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade. The major reagents included:

o-Phenylenediamine, Formaldehyde, Dimethylamine, Diethylamine, Formic acid, Glacial acetic acid, Ethanol, Phenylalanine, Tyrosine, Glycine, Sulfosalicylic acid, Methanol, Chloroform, Dimethylformamide (DMF), Dimethyl sulfoxide (DMSO)

Synthesis of Benzimidazole Derivatives

The synthesis involved two major steps:

Step 1: Synthesis of 2-substituted benzimidazole

A mixture of o-phenylenediamine (0.01 mol) and appropriate carboxylic acid derivative in glacial acetic acid (20 ml) was refluxed for 2–4 h. The reaction mixture was cooled and the precipitated product was filtered, washed and recrystallized from ethanol to obtain substituted benzimidazole derivatives.

Step 2: Synthesis of Mannich Bases

The synthesized benzimidazole derivative (0.005 mol) was dissolved in ethanol (10 ml). To this solution, formaldehyde (0.005 mol) and secondary amine (dimethylamine or diethylamine) were added. The reaction mixture was refluxed for 8 h. After completion of the reaction, the mixture was cooled and the precipitated product was filtered, dried and recrystallized using DMF.

Characterization of Synthesized Compounds

Melting Point

Melting points of the synthesized compounds were determined using a melting point apparatus and were uncorrected.

Thin Layer Chromatography (TLC)

Reaction completion and purity of compounds were confirmed by TLC using chloroform:methanol (9:1) solvent system. Spots were visualized under UV light.

FT-IR Spectroscopy

Infrared spectra were recorded using an FT-IR spectrophotometer using the KBr pellet method.

^{1}H-NMR Spectroscopy

Proton NMR spectra were recorded to confirm the structural features of the synthesized compounds.

IN SILICO STUDIES

Preliminary QSAR Analysis

QSAR parameters including:

LogP

Topological Polar Surface Area (TPSA)

Molecular Volume

Number of Rotatable Bonds were calculated using Molinspiration software.

PASS Prediction

The biological activity spectrum of synthesized molecules was predicted using PASS software. Predicted activities included:

- Antischistosomal activity
- Cachexia treatment
- Taurine dehydrogenase inhibition
- Omptin inhibition
- Gluconate-2-dehydrogenase inhibition

ANTIBACTERIAL ACTIVITY

Test Organisms

The antibacterial activity of the synthesized compounds was evaluated against:

- Bacillus subtilis
- Pseudomonas aeruginosa
- Staphylococcus aureus

Tetracycline was used as the standard drug and DMSO served as the control.

Method

Antibacterial activity was determined using the disc diffusion method.

Nutrient agar medium was prepared and sterilized. The test microorganisms were inoculated into sterile Petri plates containing Mueller–Hinton agar. Sterile filter paper discs impregnated with synthesized compounds (200 µg/ml) were placed on the agar surface and incubated at 37°C for 24 h. Zones of inhibition were measured and compared with the standard drug.

RESULTS & DISCUSSION

Infrared Spectral Analysis of Synthesized Compounds

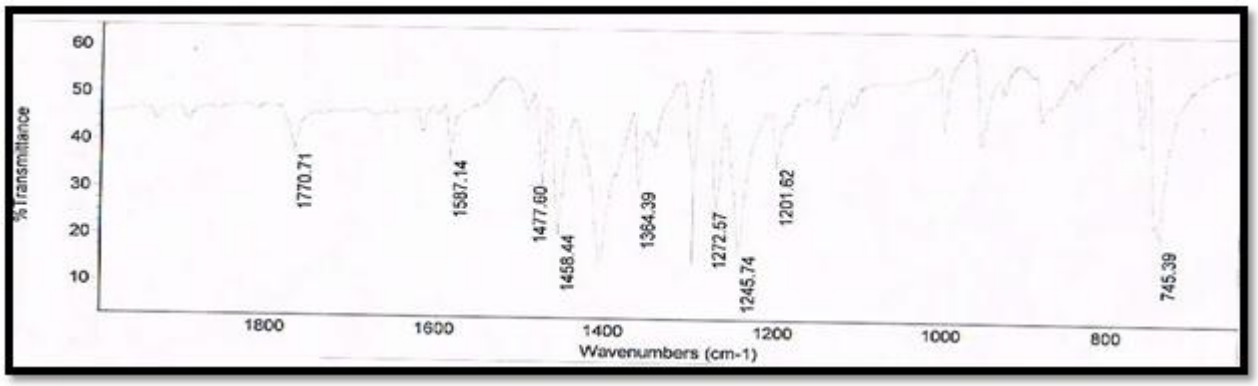

Fig.1: IR Spectrum of Compound

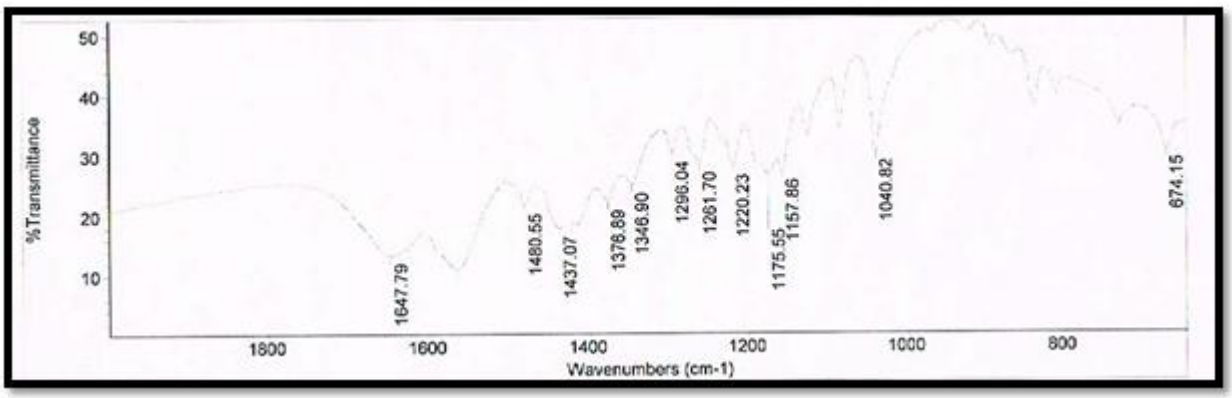

Fig.2: IR Spectrum of Compound

NMR spectral analysis of synthesized compounds:

Table.1: NMR Spectra

Compounds	Chemical shift:-
5a(i)	7.26 – 8.08 (5H, -CH Aryl protons) 2.27 – (6H, -N(CH_3)$_2$ protons) 4.80 – (2H, -CH_2 protons)
5a(ii)	7.26 – 8.08 (5H, -CH Aryl protons) 1.00 - 2.40 – (10H, -N(C_2H_5)$_2$ protons) 4.80 – (2H, -CH_2 protons)
5b(i)	7.08 – 7.70 (9H, -CH Aryl protons) 2.27 – (6H, -N(CH_3)$_2$ protons) 3.22 - 4.80 – (5H, alkyl protons) 2.0 – (2H, -NH_2 protons)
5b(ii)	7.08 – 7.70 (9H, -CH Aryl protons) 1.00 - 2.40 – (10H, -N(C_2H_5)$_2$ protons) 3.22 - 4.80 – (5H, alkyl protons) 2.0 – (2H, -NH_2 protons)
5c(i)	6.68 – 7.70 (8H, -CH Aryl protons) 2.27 – (6H, -N(CH_3)$_2$ protons) 3.22 - 4.80 – (5H, alkyl protons) 2.0 – (2H, -NH_2 protons) 5.0 – (1H, -OH proton)
5c(ii)	6.68 – 7.70 (8H, -CH Aryl protons) 1.00 - 2.40 – (10H, -N(C_2H_5)$_2$ protons) 3.22 - 4.80 – (5H, alkyl protons) 2.0 – (2H, -NH_2 protons) 5.0 – (1H, -OH proton)
5d(i)	7.26 – 7.70 (4H, -CH Aryl protons) 2.27 – (6H, -N(CH_3)$_2$ protons) 1.38 – (3H, -CH_3 protons) 2.0 – (2H, -NH_2 protons) 4.80 – (2H, -CH_2- proton)
5d(ii)	7.26 – 7.70 (4H, -CH Aryl protons) 1.00 - 2.40 – (10H, -N(C_2H_5)$_2$ protons) 1.38 – (3H, -CH_3 protons) 2.0 – (2H, -NH_2 protons) 4.80 – (2H, -CH_2- proton)
5e(i)	**Chemical shift:-** 7.07 – 7.98 (7H, -CH Aryl protons) 2.27 – (6H, -N(CH_3)$_2$ protons) 5.0 – (1H, -OH proton) 2.0 – (1H, -HSO_3 proton) 4.80 – (2H, -CH_2- proton)
5e(ii)	**Chemical shift:-** 7.07 – 7.98 (7H, -CH Aryl protons) 1.00 - 2.40 – (10H, -N(C_2H_5)$_2$ protons) 5.0 – (1H, -OH proton)

	2.0 – (1H, -HSO_3 proton) 4.80 – (2H, -CH_2- proton)

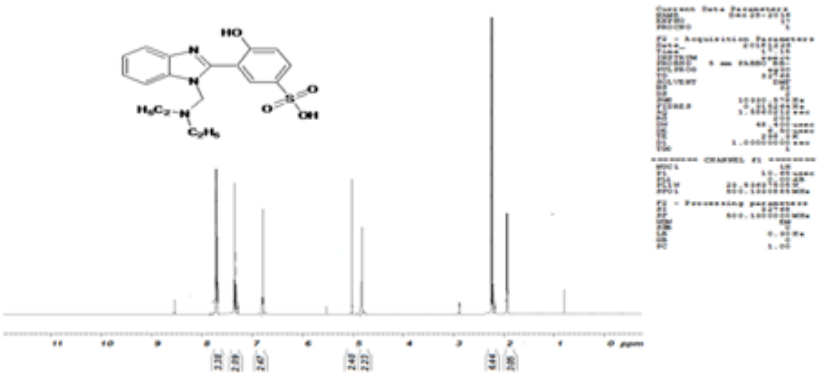

Fig.3: NMR spectrum of compound 5e(i)

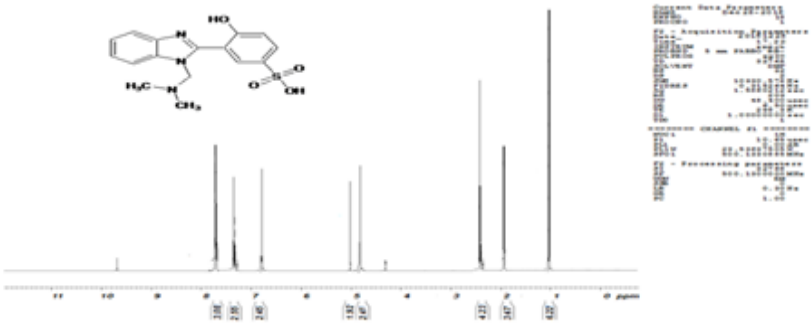

Fig.3: NMR spectrum of compound 5e(ii)

DISCUSSION

The synthesized benzimidazole Mannich bases were obtained in moderate to good yields (56–68%). Reaction completion was confirmed by TLC analysis.

FT-IR spectra confirmed the presence of characteristic functional groups including C=N, C–N, C=C and CH_2 stretching vibrations. The ^{1}H-NMR spectra showed signals corresponding to aromatic protons, aminomethyl groups and alkyl substituents, confirming the proposed structures.

QSAR analysis indicated that all synthesized compounds followed Lipinski's rule of five, suggesting good drug-likeness and potential oral bioavailability.

PASS prediction studies suggested several possible biological activities, particularly antischistosomal, enzyme inhibition and antimicrobial activity.

The antibacterial screening results indicated that several compounds exhibited moderate antibacterial activity against the tested microorganisms. Compounds 5b(i), 5c(i), and

5a(ii) demonstrated relatively better activity compared to other synthesized derivatives.

CONCLUSION

A series of novel benzimidazole-based Mannich bases were successfully synthesized and characterized using FT-IR and ^{1}H-NMR spectroscopy. Computational studies indicated favorable drug-likeness properties and predicted several potential biological activities. Biological evaluation revealed that the synthesized compounds exhibited mild to moderate antibacterial activity against *Bacillus subtilis, Pseudomonas aeruginosa,* and *Staphylococcus aureus*. These findings suggest that benzimidazole Mannich bases may serve as promising lead molecules for further development of antimicrobial agents.

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CONFLICT OF INTERESTS

The authors declare no conflict-of-interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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