EJPMR, 2026, 13(4), 367-373 **Research Article** **Impact Factor: 8.158**

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

ISSN (O): 2394-3211
ISSN (P): 3051-2573
Coden USA: EJPMAG

A NEW DISCOVERY TOWARDS NOVEL SKELETON OF BENZIMIDAZOLE AS UNIQUE EFFECTIVE ANTIMICROBIAL AGENTS

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How to cite this Article: [1]*Dr. Ojas Patel, [2]Dr. Mona Patel, [3]Ms. Sanjana Patel, [4]Ms. Suhani Patel, [5]Mr. Priyank Patel, [6]Ms. Neha Patel, [7]Mr. Pratik Patel. (2026). A New Discovery Towards Novel Skeleton of Benzimidazole As Unique Effective Antimicrobial AgentsJournal of Pharmaceutical and Medical Research, 13(4), 367–373.
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Article Received on 05/03/2026 **Article Revised on 25/03/2026** **Article Published on 04/04/2026**

ABSTRACT
This study has been successfully synthesized two novel benzimidazole derivatives via a two-step reaction process in which o-phenylenediamine was reacted with acetic acid and formic acid to obtain 2-methylbenzimidazole and benzimidazole, respectively, followed by N-alkylation with benzyl chloride to produce N-benzyl-2-methylbenzimidazole and 1-benzylbenzimidazole. The synthesized compounds were purified and characterized by standard methods and further evaluated for antimicrobial activity using the agar well diffusion method. The biological results were compared against the standard drug tetracycline and showed moderate antimicrobial activity against selected microbial strains.

KEYWORDS: Benzimidazole, N-alkylation, Antimicrobial, Agar well diffusion method, Tetracyclines a strong case for adding BCM to standard dialysis care worldwide, especially in regions where healthcare resources are limited.

1. INTRODUCTION

Medicinal Chemistry is the science that deals with the discovery or design of new therapeutic chemicals and their development into useful medicines. It may involve synthesis of new compounds, investigations of their relationships between the structure of natural or synthetic compounds and their biological activities, elucidations of their interactions with receptors of various kinds, including enzymes and DNA, the determination of their absorption, transport, and distribution properties, and studies of the metabolic transformations of these chemicals into other chemicals.

Benzimidazole derivatives are a class of organic compounds derived from the parent structure benzimidazole, which is a fused heterocyclic ring system made up of a benzene ring joined with an imidazole ring**.**

Antibiotics are medicines that fight bacterial infections in people and animals. They work by killing the bacteria or by making it hard for the bacteria to grow and multiply.

The successful outcome of antimicrobial therapy with antibacterial compounds depends on several factors. These include host defense mechanisms, the location of infection, and the pharmacokinetic and pharmacodynamic properties of the antibacterial.

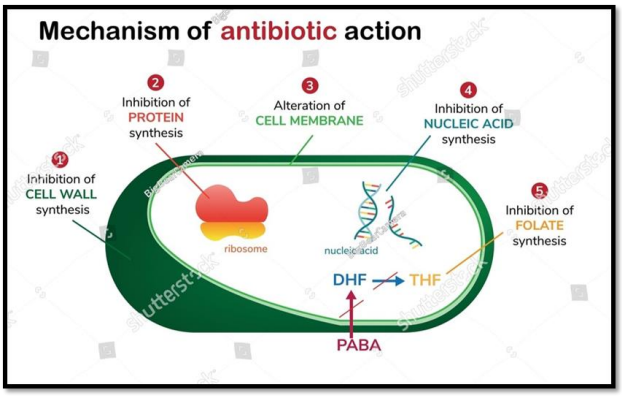


Figure No. 1: Mechanism of action of Antibiotic.

2. EXPERIMENTAL AND CHARACTERISATION

1. Synthetic scheme: 1

Synthetic procedure of new 1 benzyl- benzimidazole derivative compound

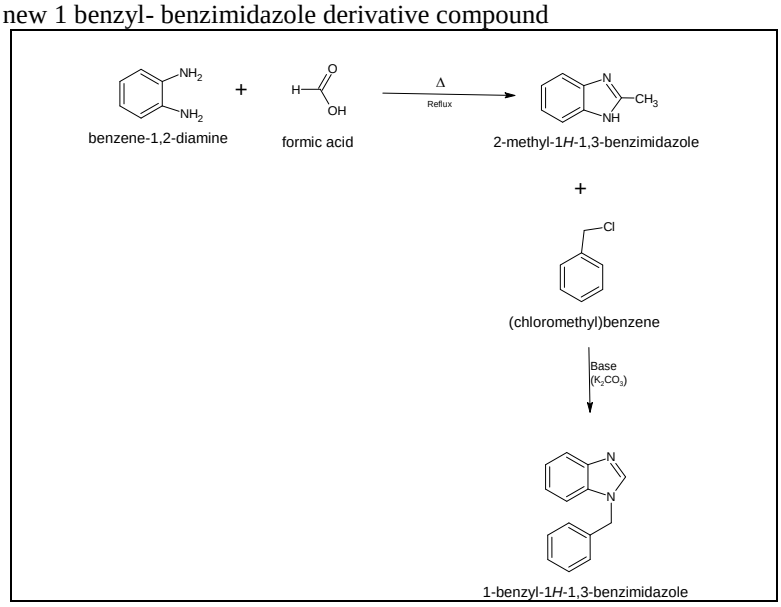


Figure No. 2: Synthetic scheme of compound 1.

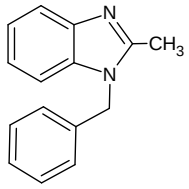
PROCEDURE

Step: 1

o-Phenylenediamine is taken in a round-bottom flask and mixed with glacial acetic acid, which acts as both solvent and reagent. The reaction mixture is heated under reflux for about 1–2 hours using a water bath or heating mantle, leading to cyclization and formation of 2-methylbenzimidazole. After completion, the mixture is allowed to cool to room temperature, and then it is made slightly alkaline by adding sodium hydroxide solution or ammonia slowly, resulting in precipitation of the crude product. The solid is filtered, washed with cold water, and purified by recrystallization from hot water or ethanol to obtain pure 2-methylbenzimidazole, which is then dried.

Step: 2

2-Methylbenzimidazole is taken and dissolved in a suitable solvent, and sodium bicarbonate or sodium carbonate solution is added to make the medium basic. To this mixture, benzyl chloride is added slowly with continuous stirring. The reaction mixture is then shaken or stirred vigorously for a sufficient time to allow N-benzylation to occur, forming 2-methyl-1-benzylbenzimidazole. After completion of the reaction, the mixture is acidified with dilute hydrochloric acid to neutralize excess base and remove impurities. The product is then separated, washed with water, and purified by recrystallization from a suitable solvent such as ethanol to obtain pure 2-methyl-1-benzylbenzimidazole, which is finally dried.



1-benzyl-2-methyl-1*H*-1,3-benzimidazole

Structure of compound:1.

TLC Plate

Table of physical data of compound:1.

IUPAC Name	**N-benzyl-2-methylbenzylimidazole**
Molecular formula	$C_{15}H_{14}N_2$
Molecular wight	222.29 g/mol
Melting point	163-183 °C
TLC	R_f Value of TLC Plate : 1.67
	Solvent system : chloroform : Ethanol: DMSO (6:3:1)
	Detecting Agent : Iodine Chamber

IR Graph of compound 1

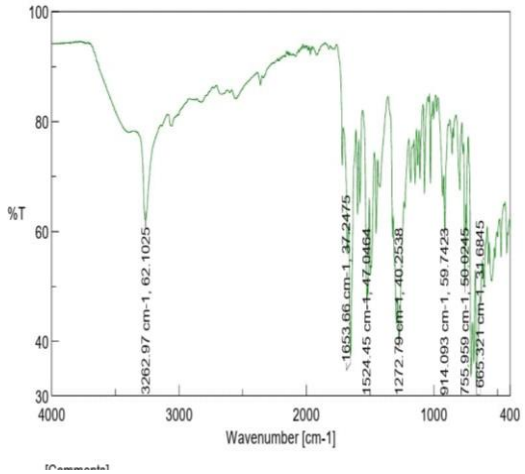


Table of interpretation of compound 1 IR Data.

Functional group	**Standard peak (cm-1)**	**Observed peak (cm-1)**
Aliphatic - CH_3	1470-1450	1488
3^O Amine- N	3500-3300	3266
Aromatic- CH	1600-1400	1524

2. Synthetic scheme: 2

Synthetic procedure of new 2-methyl-1-benzyl benzimidazole

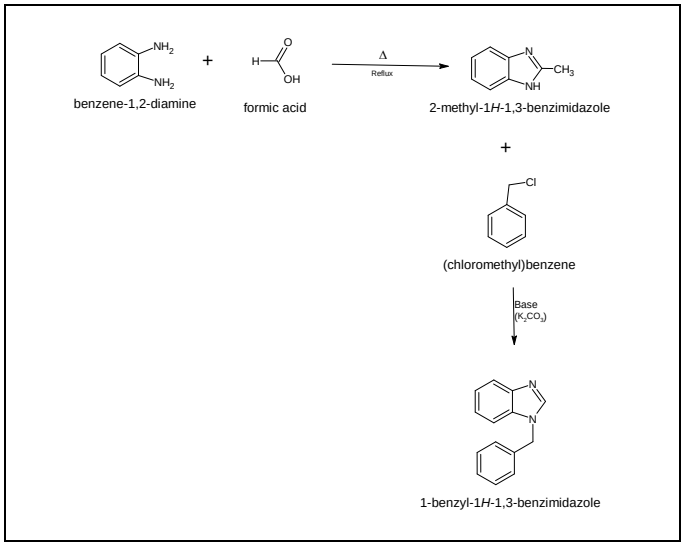


Figure No. 3: Synthetic scheme of compound: 2.

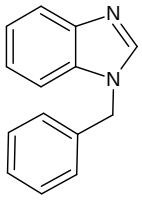
PROCEDURE

Step:1

Benzimidazole is synthesized by condensation of o-phenylenediamine with formic acid under acidic and heated conditions. In this procedure, o-phenylenediamine is taken in a round-bottom flask and an appropriate amount of formic acid is added slowly with continuous stirring. The reaction mixture is then heated on a water bath or under reflux for about 1–2 hours. During heating, cyclization occurs leading to the formation of the benzimidazole ring system. After completion of the reaction, the mixture is allowed to cool to room temperature and then poured into cold water. The solution is neutralized by the gradual addition of sodium hydroxide solution until it becomes slightly alkaline. This results in the precipitation of crude benzimidazole. The solid product is then filtered, washed with cold water to remove impurities, and dried. Further purification is carried out by recrystallization from hot water or suitable solvent to obtain pure white crystals of benzimidazole.

Step:2

Benzimidazole was dissolved in a suitable solvent such as dimethylformamide (DMF), and an appropriate base (e.g., potassium carbonate) was added to facilitate the reaction. Benzyl chloride was then added gradually to the reaction mixture, and the contents were stirred under controlled temperature conditions to promote N-alkylation, yielding 1-benzyl benzimidazole. Upon completion of the reaction, the mixture was poured into ice-cold water to precipitate the product. The precipitated solid was collected by filtration, washed to remove impurities, and further purified by recrystallization from ethanol to obtain the desired compound in pure form.



1-benzyl-1*H*-1,3-benzimidazole

Structure of compound 2

TLC Plate

Table of Physical Data of compound: 2.

Functional group	Standard peak (cm-1)	Observed peak (cm-1)
3°Amine Error! Filename not specified.	3500-3300	3262
Arometic CH	1600-1400	1524

IR Graph of compound: 2.

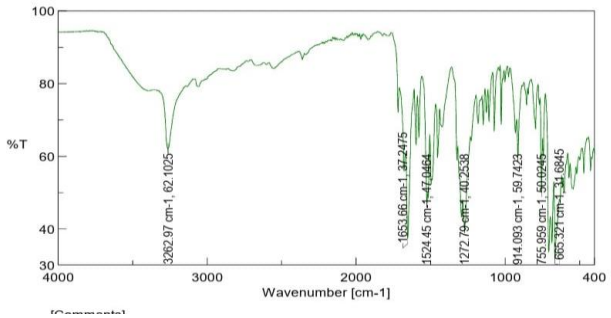


Table of interpretation of compound 2 IR Data.

IUPAC Name	1- Benzyl-1H-Benzimidazole
Molecular Formula	$C_{14}H_{12}N_2$
Molecular weight	208.26 g/mol
Melting point	137-140 °C
TLC	R_f Value of TLC Plate : 1
	Solvent system : Chloroform: Ethanol: DMSO (6:3:1)
	Detecting Agent : Iodine Chamber

3. MICROBIAL SCREENING

All above synthesized compounds were evaluated and tested for their antibacterial activity against Bacillus substillis as Gram positive bacteria.

Agar disc diffusion method was widely used for determination of the preliminary antibacterial. tetracycline used as a standard drug.

The results were recorded for each tested compound as the average diameter of zone of inhibition of bacterial growth around the discs in mm.

Agar well Diffusion Method

Composition of medium

- Beef extract
- Peptone
- Agar
- Dist. Water- 250ml
- pH 7.0-7.2.

Microorganism used

- Bacillus subtiles.

Standard drug used

- Tetracycline.

PROCEDURE

Step:1 Preparation of Agar medium

Prepare MHA from the dehydrated medium according to the manufacturer's instructions. Media should be prepared using distilled water or deionized water.

Heat with frequent agitation and boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 min.

- Check the pH of each preparation after it is sterilized, which should be between 7.2 and 7.4 at room temperature.
- Cool the agar medium to 40-50°C. Pour the agar into sterile glass or plastic petri dish on a flat surface to a uniform depth of 4 mm.
- Allow to solidify.
- Prior to use, dry plates at 30-37°C in an incubator, with lids partly ajar, for not more than 30 minutes or until excess surface moisture has evaporated. Media must be moist but free of water droplets on the surface. Presence of water droplets may result to swarming bacterial growth, which could give inaccurate results. They are also easily contaminated.

Step: 2 Inoculum

- From a pure bacterial culture (not more than 48 hours, old except for slow growing organisms), take four or five colonies with a wire loop.
- Inoculate the agar by streaking with the swab containing the inoculum.
- Rotate the plate by 60° and repeat the rubbing procedure. Repeat two times. This will ensure an even distribution of the inoculum.
- Allow the surface of the medium to dry for 3-5 minutes but not longer than 15 minutes to allow for absorption of excess moisture.

Step: 3 Agar well diffusion

- The inoculated agar was poured into the assay plate (9 cm in diameter), and allowed to cool down on a leveled surface.
- Once the medium had solidified, four wells, each 4 mm in diameter, were cut out of the agar, and 20 l of the anti-fungal agent were placed into each well. A total of four antibacterial were placed into each plate and incubated at 35 ◦C for 24 hours.
- Because of disagreement over the criteria to determine the end point of the diameters of the clear zone of inhibition of growth, it was measured both at 50% and 80%.

Step: 4 Incubation

- Incubate plates in an inverted position at 30°C or at an optimum growth temperature.
- Observe for the zone of inhibition after 16 to 18 hours. Slow growing organisms may require longer incubation period.

Step: 5 Reading and Measurement of Zone of Inhibition

- The zone of inhibition is the point at which no growth is visible to the unaided eye.
- Record the presence of individual colonies within zones of inhibition.

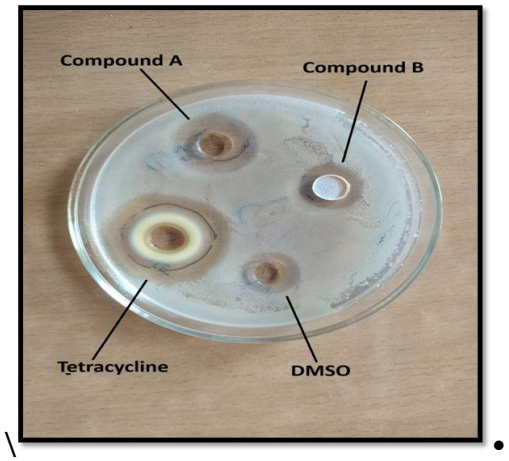


Figure No. 4: Biological Activity.

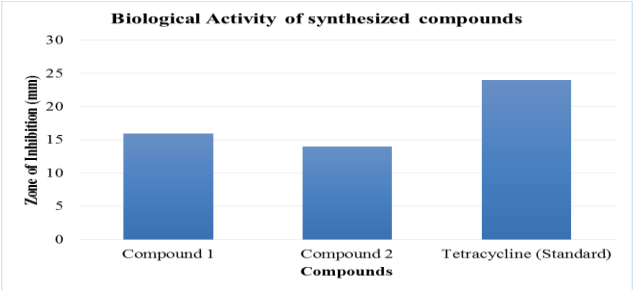
Read and record the diameter of the zones of inhibition using a ruler graduated to 0.5mm

OBSERVATION

Biological activity Results Table.

Synthesized Compound	Zone of Inhibition (mm)
Compound 1	16
Compound 2	14
Tetracycline (Standard)	24

Graph of Results



4. CONCLUSION

In this study Benzimidazole derivative compound been successfully synthesized by condensation with benzyl chloride in the basic conditions. Chemical structures of all the synthesized compounds were confirmed by IR Spectroscopy analysis, also it is verified by TLC and melting point method. Microbial Assay of benzimidazole derivatives showed Antibacterial Activity. Among these

two synthesised compounds, compound no. 1 having highest Antibacterial activity than compound 2, its indicate that substituted benzimidazole with methyl group is gives better antibacterial activity as compared to non substituted benzimidazole.

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