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Synthesis and Biological Evaluation of Novel Benzimidazole Derivative with Aspirin as Potent Antimicrobial & Antifungal Agents

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ABSTRACT

Benzimidazole is a heterocyclic aromatic organic compound. It is an important pharmacophore and a privileged structure in medicinal chemistry. This compound is bicyclic in nature which consists of the fusion of benzene and imidazole. Nowadays is a moiety of choice which possesses many pharmacological properties. Benzimidazole and its derivatives have a long history as antimicrobial agents. Several thousands of benzimidazole analogs have been synthesized and screened for pharmacological activity. They are of wide interest because of their diverse biological activity and clinical applications. These heterocyclic systems have different activities as they can act as bacteriostats or bactericides, as well as fungicides and they are present in numerous antiparasitic, antitumoral and antiviral drugs. Also, some of them exhibit appreciable antiprotozoal activity. It was shown that they have a moderate *in vitro* anti-HIV activity.

In the present work, the novel Benzimidazole derivative was synthesized with Aspirin and evaluated for their antimicrobial & antifungal activities. The newly synthesized compound was subjected to antibacterial activity against *Staphylococcus aureus* ATCC 250, *Pseudomonas aeruginosa* ATCC 25619, *Escherichia coli* RSKK 313 & antifungal activity against *Candida albicans* RSKK 628. The antibacterial & antifungal activities were compared with the respective standard drugs. The MIC of Benzimidazole derivative was found to be in the range 20-200 mg/ml on all the tested microorganisms.

KEYWORDS: Benzimidazole, Synthesis, Aspirin, Antimicrobial, Antifungal.

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INTRODUCTION

Benzimidazoles have a long and distinguished history extending from the days of their discovery as important heterocycle to their current use in the chemotherapy of AIDS. During the last two decades, several Benzimidazole derivatives have been developed as chemotherapeutic agents and have found wide clinical applications. The benzimidazole derivatives have different pharmacological activities like antimicrobial, antifungal, neuroleptic, anti-HIV, anthelmintic, antihistaminic, antiulcer, cardio tonic & antihypertensives[1, 13, 22].

In order to obtain more effective chemotherapeutic agents, a variety of reports have been presented on the synthesis and biological evaluation of novel benzimidazole derivatives. The literature survey revealed the importance of the substitutions at 1, 2 and 5 positions of the benzimidazole ring for their pharmacological activities. 2-(phenyl substituted) benzimidazoles with different biological activities like antimicrobial, antibacterial, antitumor, antiviral, and anti-inflammatory have been reported[5, 8, 9].

Aspirin (Acetyl Salicylic acid) have analgesic and anti-inflammatory activities. Aspirin undergoes reaction with O-phenylenediamine and replace the -OOH group in the presence of dilute (10%) hydrochloric acid. These newly synthesized Benzimidazole derivatives with Aspirin & the corresponding O-phenylenediamine were evaluated for their biological activities.

MATERIAL & METHODS

All melting points were determined using an Omega melting point apparatus and were uncorrected. The newly synthesized derivative was characterized by thin layer chromatography which was shown in **Fig.1**. Silica gel plates (Merck F254) were used for analytical chromatography[6, 7]. IR spectra were recorded on a Jasco FT/IR 420 spectrophotometer as potassium bromide disks. An IR spectrum of the derivative was shown in **Fig. 2** & IR spectral data of the same was shown in **Table1.**

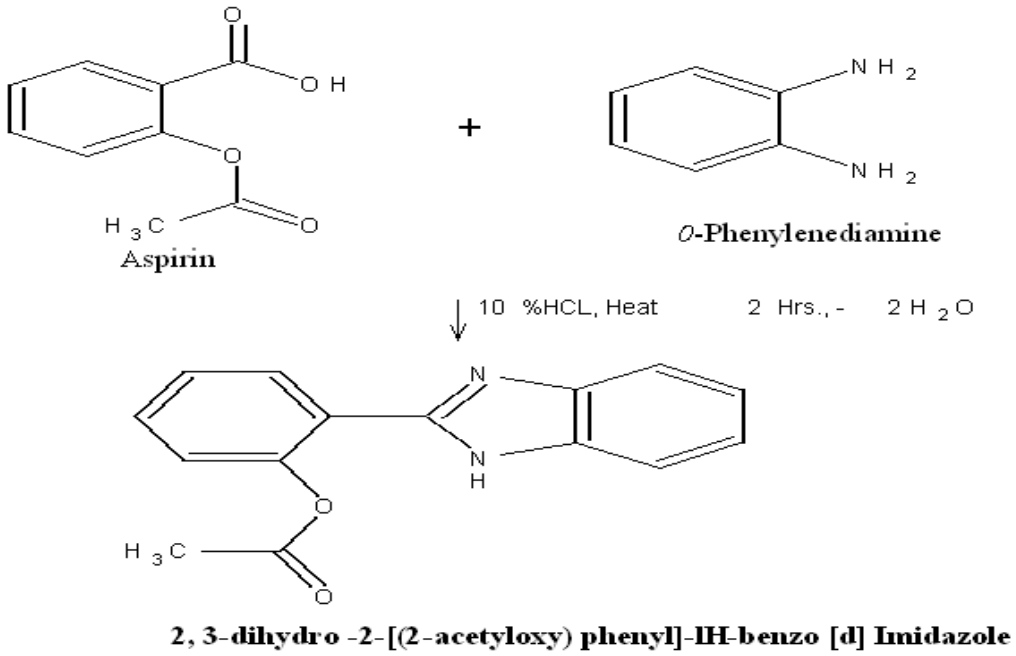
^{1}H NMR analyses were performed with a Varian NMR mercury 300 spectroscopy. ^{1}H NMR spectra of the derivative was shown in **Fig. 3** & ^{1}H NMR spectral data of the same was shown in **Table 2.**

The Aspirin, *0*-Phenylenediamine, Hydrochloric acid, Sodium hydroxide and Ethanol used in this study were purchased from Milton chemicals.

Synthesis of 2, 3-dihydro -2-[(2-acetyloxy) phenyl]-lH-benzo [d] Imidazole:

A mixture of 1.08 gm (0.0l mole) of *0*-Phenylenediamine, 1.8 gm (0.01 mole) of aspirin, 5ml of dilute hydrochloric acid were taken into a 250ml round bottomed flask & the mixture was heated on a water bath at 100°C for two hours. The reaction mixture was cooled and 10% sodium hydroxide solution was added slowly with constant stirring of the flask, until the mixture was just alkaline to

litmus paper. The brown colored solid obtained was filtered on a Buchner funnel, washed with ice cold water, drained well and washed again with ice cold water, air dried & recrystallized from Ethanol[10, 11, 12, 2]. The yield of the pure product, m. p. 160-164°C, was 2.2 gm (87.3%). The scheme of synthesis of the derivative was shown by **Scheme 1.**



Scheme 1: Synthesis of 2, 3-dihydro -2-[(2-acetyloxy) phenyl]-lH-benzo [d] Imidazole

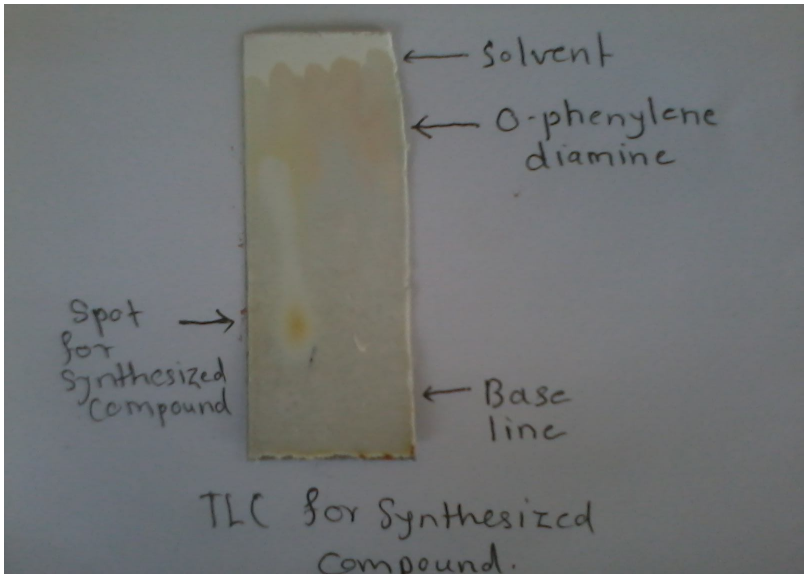


Fig. 1: Characterization of the newly synthesized derivative by TLC

RESULTS & DISCUSSIONS

2, 3-dihydro -2-[(2-acetyloxy) phenyl]-lH-benzo [d] Imidazole (Scheme 1) was synthesized from aspirin and *0*-phenylenediamine by hydrolysis[3]. Aspirin was coupled with the amine group of *0*-phenylenediamine. In this reaction, two water molecules were released in the presence of hydrochloric acid on heating[13, 14]. IR spectral data of synthesized compound has been shown in the **Table 1** and protocol of the ^{1}H NMR prediction in **Table 2**.

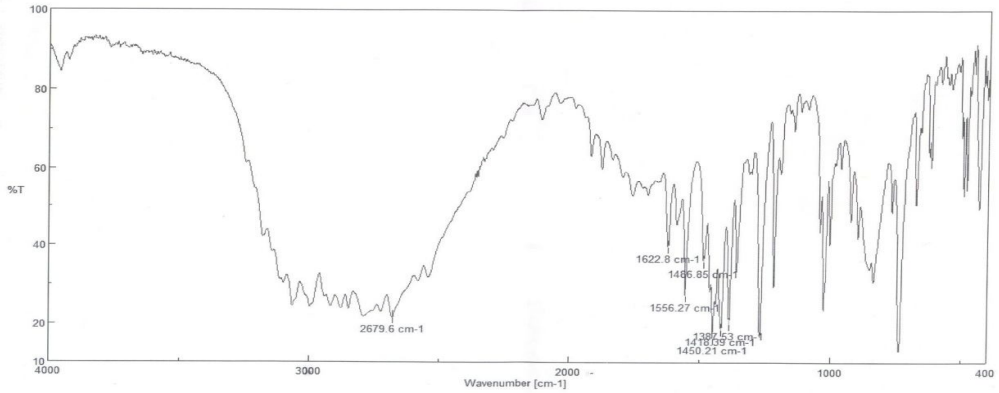


Fig 2: IR spectra of 2, 3-dihydro -2-[(2-acetyloxy) phenyl]-lH-benzo [d] Imidazole

Table 1: IR spectral data of the synthesized compound

Functional Group	Type of Vibration	Characteristic Absorptions (cm^{-1})	Intensity
O-H	stretch	2679.6	strong, very broad
C=C	stretch	1622.8	variable
N-H	bending	1556.27	-
C=C	stretch	1486.85	medium-weak, multiple bands
-C-H	bending	1450.21	variable
C=C	stretch	1418.39	medium-weak, multiple bands
-C-H	bending	1418.39	variable
-C-H	bending	1387.53	variable

The compound 2, 3-dihydro -2-[(2-acetyloxy) phenyl]-lH-benzo [d] Imidazole was evaluated for its antimicrobial activity against *S. aureus, P. aeruginosa, E. coli* & antifungal activity against *C. albicans* by means of tube dilution technique[15, 16].

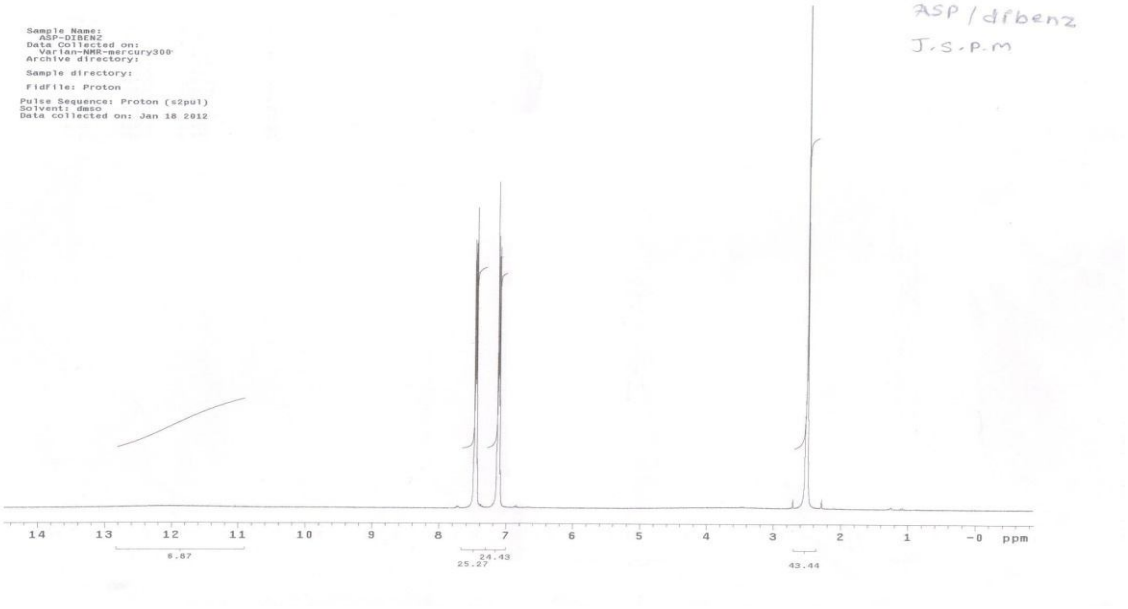


Fig 3: ^{1}H- NMR spectra of 2, 3-dihydro -2-[(2-acetyloxy) phenyl]-lH-benzo [d] Imidazole

Table 2: Protocol of the ^{1}H- NMR prediction

Index	Frequency	ppm	Height
1.	2244.1	7.479	90.1
2.	2241.2	7.469	85.4
3.	2238.4	7.460	88.4
4.	2235.0	7.449	100.8
5.	2231.7	7.437	16.7
6.	2144.0	7.145	21.6
7.	2140.6	7.134	109.1
8.	2137.3	7.123	91.3
9.	2134.4	7.113	84.6
10.	2131.6	7.104	87.1
11.	2128.2	7.093	12.9
12.	757.3	2.524	35.6
13.	750.2	2.500	549.7

Table 3 shows the results of in vitro activity determination by tube dilution technique. The synthesized compound shows excellent antibacterial activity than Ampicillin & antifungal activity than fluconazole.

BIOLOGICAL ACTIVITY

1. Antimicrobial activity:

The in vitro antimicrobial activity of the synthesized compound was tested by the tube dilution technique. Test and reference compounds (Amikacin & Ampicillin trihydrate) were dissolved in 12.5% DMSO, at concentrations of 200 mg/ml, further dilutions of the compounds and standards in the test medium were prepared at the required concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 mg/ml.

The final inoculum size was 10 CFU/ml. The minimum inhibitory concentrations (MIC) were defined as the lowest concentrations of the compounds that prevented visible growth[4, 21]. It was determined that the solvent had no antimicrobial activity against any of the test microorganism.

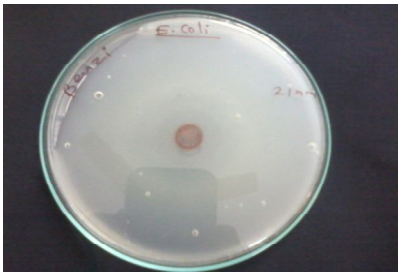
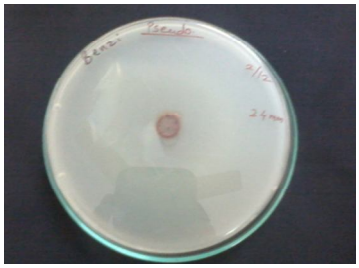
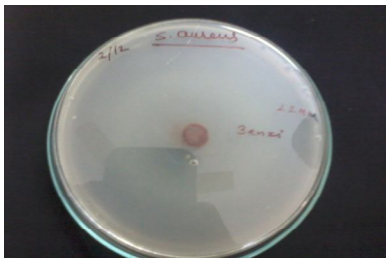
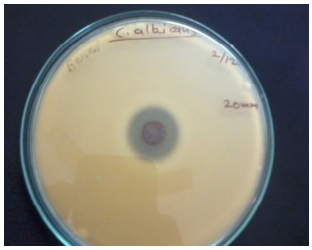
	
Antimicrobial activity against E. Coli.	Antimicrobial activity against Pseudomonas aeruginosa.
	
Antimicrobial activity against Staphylococcus aureus.	Antifungal activity against Candida albicans.

Fig. 4: Antimicrobial & antifungal activity of 2, 3-dihydro -2-[(2-acetyloxy) phenyl]-lH-benzo [d] Imidazole.

All the compounds were tested for their in vitro growth inhibitory activity against *Staphylococcus aureus* ATCC 250, *Pseudomonas aeruginosa* ATCC 25619 as Gram positive and *Escherichia coli* RSKK 313 as Gram negative bacteria. The antimicrobial & antifungal activities of the derivative was shown in **Fig. 4 & Table 3.**

Table 3: Antimicrobial & antifungal activity of synthesized compound in terms of zone of inhibition in mm

Compound	S. aureus	P. aeruginosa	E. coli	C. albicans
2, 3-dihydro -2-[(2-acetyloxy) phenyl]-lH-benzo [d] Imidazole	22	24	21	20
Amikacin	23	28	32	Not tested
Ampicillin	29	28	19	Not tested
Fluconazole	Not tested	Not tested	Not tested	19

2. Antifungal activity:

The yeast *C. albicans* was maintained in Sabouraud Dextrose Broth (Difco) after incubation for 48 h at 25.91^{o}C. Testing was performed in Sabouraud Dextrose Broth at pH 7.4 and the two-fold dilution technique was applied[17, 18].

A set of tubes containing only inoculated broth was kept as controls. After incubation for 48 h at 25.91^{o}C, the last tube with no growth of yeast was recorded to represent MIC expressed in mg/ml[19, 20].

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