ORIGINAL RESEARCH ARTICLE
Antimicrobial Studies of Some New Benzimidazole Derivatives

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ABSTRACT
In this study 22 novel benzimidazole compounds bearing oxadiazole moiety were synthesized in order to investigate their possible antibacterial and antifungal activity. Structures of the synthesized compounds were elucidated by spectral data. Different gram-negative and gram-positive bacterial strains were used in antibacterial activity tests. Antifungal activity tests were also performed against fungal strains. The result of antimicrobial effect of all tested compounds were reported as zone of inhibition in mm and Minimum inhibitory concentration. All the synthesized compounds were screened for their antimicrobial activity against Escherichia coli representing Gram – negative bacteria, Bacillus subtilis and Staphylococcus aureus representing Gram - positive bacteria, Saccharomyces Cerevisiae representing Fungi. The result revealed that most of newly synthesised compounds exhibited promising antibacterial and antifungal activities. Generally the test compounds showed good activity against Gram – positive bacteria, Gram – positive bacteria, fungi. Other compounds showed moderate activity against Gram – positive bacteria, Gram – positive bacteria, fungi. The results showed that all of the compounds have exhibited antimicrobial activity.

Key words: Benzimidazole, antimicrobial activity, antifungal activity.

INTRODUCTION
Many of the currently available drugs are toxic, enable recurrence because they are bacteriostatic/fungistatic and not bactericidal/fungicidal or lead to the development of resistance due in part to the prolonged administration. Many observations suggest that benzimidazole molecules are effective against various strains of microorganisms [1-4]. This was confirmed by various spectral and pharmacological studies. Benzimidazoles are important class of heterocyclic compounds possessing a huge spectrum of biological activities. Mainly, this nucleus is a constituent of vitamin-B12 [5]. Benzimidazoles ring play an important role in biological fields such as antiparasitic [6,7], anthelmintics [8], anti-inflammatory [9], anticonvulsant [10], anti-HIV [11] activities. Literature reviews suggest that oxadiazole nucleus is also gives pharmacological actions. It shows antmycobacterial [12] antifungal [13] and anticonvulsant [14] activities. Observations suggest the importance of benzimidazole and oxadiazole nucleus, it was believed that it would be worthy to design and synthesize some noval benzimidazole derivatives bearing oxadiazole moiety and screen them for potential pharmacological activities. The present work comprises of in vitro antimicrobial activity of new benzimidazole derivatives.

MATERIALS AND METHODS
Chemistry
The present work comprises of synthesis of new antimicrobial agent, in which 4- chloro-ortho phenylene diamine are used as a starting material to which formic acid/acetic acid reacts and form 2-substituted 6-chloro-benzo[d]imidazole, this further heated with ethylbromoacetate and forms 2-substituted Ethyl 2-(6-chloro-benzo[d]imidazo[1-yl])acetate. The resulting intermediate on treatment with hydrazine hydrate yields Ethyl 2-(6-chloro-benzo[d]imidazol-1-yl)hydrazide which on further reaction with one equivalent of different substituted aromatic carboxylic acids/aldehydes in the presence of phosphorl chloride afforded the corresponding target compounds 6-chloro-2-substituted-1-[(5-
substituted aryl]-1,3,4-oxadiazol-2-yl] methyl]-
1H-benzimidazole and (E)-N'-(4- substituted
benzylidene)-2-(6-chloro-2-substituted-1H-
benzo[d]imidazol-1-yl) acetohydrazide
respectively as shown in (Fig 1).

Antibacterial and anti Fungal Activity (In-
vitro) [15-25]

Zone of Inhibition

Method:
Disc-plate method.

Organisms:
Escherichia coli (Gram negative).
Bacillus subtilis (Gram positive).
Staphylococcus aureus (Gram positive)
Saccharomyces Cerevisiae (Fungi).

Preparation of nutrient medium:
The definite volumes of peptone (0.65%), yeast
extract (0.15%), dipotssium dihydrogen phosphate
(0.36%) and potassium dihydrogen phosphate
(0.13%) were dissolved in distilled water and pH
was adjusted to 7.2. This solution was sterilized
by autoclaving at 15 lbs. for 20 min.

Preparation of sub-culture:
One day prior to this testing, inoculation of the
above bacteria cultures were made in the nutrient
agar and incubated at 37°C for 18-24 hr.

Preparation of test solutions:
Test compound (5.0 mg) was dissolved in
dimethylformamide (5.0 ml) to give a 1,000
µg/ml from this stock solution 100
µg/ml of this solution
was prepared and used for testing.

Method of testing:
Paper Discs, (3 mm diameter), were saturated with
the dilutions of and placed on the surface of the
seeded agar (each disc absorbs approximately 0.08
ml of solution).Two discs saturated with the
reference standard were placed on assay plate
opposite each other, and other discs of samples
were placed in the quadrants. All plates were
incubated for 24-48 hrs at 37°C. The diameter of
zone of inhibition of the reference standard discs
was measured by the use of millimeter scale.

MINIMUM INHIBITORY CONCENTRATION:
Minimum inhibitory concentration (MIC) is also
called bacteriostatic values, but first term is
preferable. The minimum inhibitory concentration
of an antimicrobial agent, for a particular
organism, is the lowest concentration that just
prevents growth of that organism.

Methods for determination of MIC:

Method: Tube Dilution Method
The tube dilution method test is the standard
method for determining levels of resistance to an
antimicrobial agent. Serial dilutions of the
antimicrobials are made in a liquid medium which
is inoculated with a standardized number of
organisms and incubated for a prescribed time.
The lowest concentration (highest dilution) of
antimicrobial agent preventing appearance of
 turbidity is considered to be the minimum
inhibitory concentration (MIC). At this dilution
the antimicrobial agent is bacteriostatic.
Graded concentrations of antimicrobial agents are
prepared in liquid broth and an accurate volume of
suspension of the organism is added to each. After
shaking to mix, the dilutions are incubated,
usually for 2-3 days at 37°C, and examined for
growth.

The MIC lies between the lowest concentration
inhibiting growth and the highest concentration
allowing growth. The determination can be
repeated, using a range of dilutions between these
two values, for a more precise result. The
dilutions are normally made in geometric series
but sometimes an arithmetic series seems to be
more suitable. Two tubes are taken as control one
of which contains no inhibitor and confirms that
the culture is viable. The second control tube
contains highest concentration of inhibitor but no
organism and is to ensure no precipitation caused
by interaction of broth constituents and inhibitor
because this can be confused with turbidity due to
microbial growth.

In expressing MIC values the conditions under
which it was obtained should be specified because
the result is influenced by many factors including
the strain, age and number of organisms, the
nature and pH of the culture medium and the
temperature and time of incubation.

Bacterial strain used: B. subtilis NCIM 2063

Culture media: Nutrient broth (liquid media)
procured by Hi-media, Mumbai.
Composition Quantity (g/litre)
Beef extract 1.5
Peptic digest of animal tissues 5.0
Sodium chloride 5.0
Yeast extract 1.5

*The media was prepared by dissolving 13 gram
of nutrient agar in 1000 ml of distilled water.
*Sterilization of media was done by autoclaving at
15 lbs at 121°C for 15 minutes.
Assessment of bacterial growth:
By visual comparison of turbidity with control tube.

PROCEDURE:
Subculturing Of Bacterial Strain: B. subtilis was subcultured in conical flasks by loop inoculation method in liquid broth using sterile technique. The conical flasks was then incubated at 37+2 °C for 24 hrs.

Preparation of Culture Media: 13 g of nutrient broth (Hi-media, Mumbai) was dissolved in 1000 ml of distilled water in a conical flask by stirring. Then the media was sterilized by autoclaving at 15 lbs at 121°C for 15 minutes.

Preparation of Test Solutions: Test solutions of 10 different concentrations (5, 10, 15, 20, 25, 30, 35, 40, 45, 50 µg/ml) of each compound were prepared in DMSO (dimethyl sulfoxide) from a stock solution of 1000 µg/ml.

Preparation of culture tubes: 5 ml of liquid broth was added to 11 culture tubes previously labeled for each compound. Then each tube was inoculated with bacterial culture using sterile loop technique. Then 1 ml of each test compound was added to the respectively labeled culture tubes. Tube numbered 11 was taken as control without any compound. After mixing the contents, the tubes were then incubated at 37°C for 24 hrs.

Observations: After 24 hrs culture tubes were examined for turbidity.

RESULTS AND DISCUSSIONS
The Compounds 5(a-f), 6(a-f), 7(a-e), 8(a-e) were evaluated for their antimicrobial activity against Escherichia coli representing Gram – negative bacteria, Bacillus subtilis and Staphylococcus aureus representing Gram - positive bacteria, Saccharomyces Cerevisiae representing Fungi.
The result of antimicrobial effect of all tested compounds were reported as zone of inhibition in mm and MIC are shown in (Table 1, 2 & 3). The result revealed that most of newly synthesised compounds exhibited promising antibacterial and antifungal activities. Generally the test compounds 5(e, f), 6(e, f), 7(d,e) and 8(d - e) showed good activity against Gram – positive bacteria as compared to ciprofloxacin. Other compounds showed moderate activity against gram – positive bacteria.
Compounds 5(b-f) showed good activity against Gram – negative bacteria as compared to ciprofloxacin. Compound 6(f), exhibited excellent activity against Gram – negative bacteria as compared to ciprofloxacin. Other compounds showed moderate activity against gram – negative bacteria.
Compounds 6e, 7e, and 8b showed good activity against Saccharomyces Cerevisiae. Other compounds showed moderate anti fungal activity.

<table>
<thead>
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<th>S. NO</th>
<th>COMPOUNDS</th>
<th>ZONE OF INHIBITION IN mm (after 24 hr)</th>
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</tr>
<tr>
<td></td>
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<td>S. aureus</td>
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<td>1</td>
<td>5(a)</td>
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Fig 1: Structures of benzimidazole derivatives
Table 2: Antimicrobial activity data at 100µg/ml (after 48 hr)

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<tr>
<td>2</td>
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<tr>
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<tr>
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Table 3: MIC of compounds in µg/ml (B. subtilis)

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<tr>
<td>6p</td>
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REFERENCE

18. Phair, J. P.; Watanakunakorn, C.; banister, T. In Vitro Susceptibility of Pseudomonas aeruginosa to Carbenicillin and the Combination of Carbenicillin and Gentamicin.In American Society for Microbiology; (1969), pp 303-306.
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