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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 a important role in biological fields such as antiparasitic [6,7], antihelmintics [8], anti-inflammatory [9], anticonvulsant [10], anti-HIV [11] activities. Literature reviews suggest that oxadiazole nucleus is also gives pharmacological actions. It shows antimycobacterial [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

Chemisty

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]imidazol-1-yl)acetate. The resulting intermediate on treatment with hydrazine hydrate yields Ethyl 2-(6-chloro-2-substituted-1H-benzo[d]imidazol-1-yl)hydrazide which on further reaction with one equivalent of different substituted aromatic carboxylic acids/aldehydes in the presence of phosphoryl chloride afforded the corresponding target compounds 6-chloro-2-substituted-1-[{(5-

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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^{o}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 digests 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^{o}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 ^{o}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^{o}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^{o}C for 24 hrs.

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

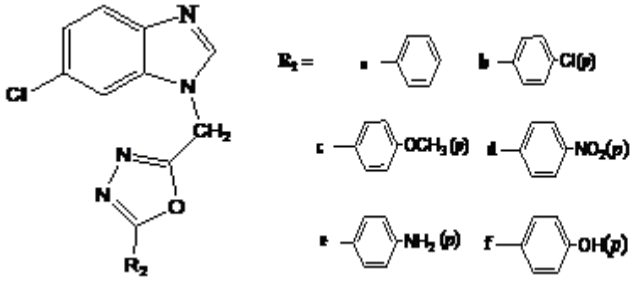
RESULTS AND DISCUSSIONS

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.

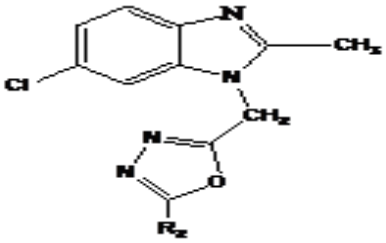
The result of antimicrobial effect of all tested compounds were reported as zone of inhibition in mm and MIC are shown in (**Table 1, 2 & 3**). The result revealed that most of newly synthesised compounds exhibited promising antibacterial and antifungal activities. Generally the test compounds **5(e, 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 agianst Gram – negative bacteria as compared to ciprofloxacin. Compound 6(**f**), exhibited excellent activity agianst Gram – negative bacteria as compared to ciprofloxacin. Other compounds showed moderate activity against gram – negative bacteria.

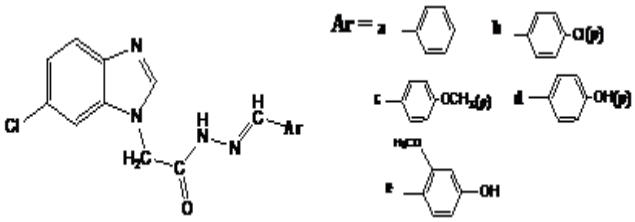
Compunds 6e, 7e, and 8b showed good activity agianst *Saccharomyces Cerevisiae*. Other compounds showed moderate anti fungal activity.



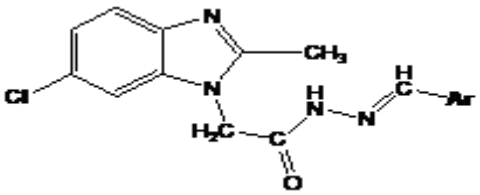
6-chloro-1-((5-(aryl)-1,3,4-oxadiazol-2-yl)methyl)-1H-benzo[d]imidazole. 5(a-f)



6-chloro-1-((5-(aryl)-1,3,4-oxadiazol-2-yl)methyl)-2-methyl-1H-benzo[d]imidazole 6(a - f)



(E)-N'-(substitued aryl benzylidene)-2-(6-chloro-1H-benzo[d]imidazol-1-yl) acetohydrazide 7(a-e)



(E)-N'-(substitued aryl benzylidene)-2-(6-chloro-2-methyl-1H-benzo[d]imidazol-1-yl) acetohydrazide 8(a-e)

Fig 1: Structures of benzimidazole derivatives

Table 1: Antimicrobial activity data at 100 µg/ml (after 24 hr)

S. NO	COMPOUNDS	ZONE OF INHIBITION IN mm (after 24 hr)			
		Antibacterial			Antifungal
		S. aureus	*B.subtilis*	*E.coli*	*S. Cerevisiae*
1	**5 (a)**	13	12	13	13

2	**5 (b)**	14	12	13	13
3	**5 (c)**	13	15	12	16
4	**5 (d)**	12	14	13	16
5	**5 (e)**	16	17	15	15
6	**5 (f)**	17	16	16	14
7	**6 (a)**	13	12	15	16
8	**6 (b)**	9	11	13	13
9	**6 (c)**	9	14	9	13
10	**6(d)**	11	13	9	12
11	**6 (e)**	13	12	11	16
12	**6 (f)**	7	16	13	17
13	**7(a)**	9	17	7	13
14	**7(b)**	14	13	9	9
15	**7(c)**	15	9	14	9
16	**7(d)**	7	9	15	11
17	**7(e)**	9	11	7	7
18	**8(a)**	16	13	9	9
19	**8(b)**	12	10	16	14
20	**8(c)**	12	9	12	13
21	**8(d)**	11	13	14	14
22	**8(e)**	9	16	13	18
11	**Ciprofloxacin**	30	29	30	27

Table 2: Antimicrobial activity data at 100µg/ml (after 48 hr)

S.NO	COMPOUNDS	ZONE OF INHIBITION IN mm (after 48 hr)			
		Antibacterial			Antifungal
		S. aureus	***B.subtilis***	***E.coli***	***S. Cerevisiae***
1	**5 (a)**	16	18	15	20
2	**5 (b)**	17	18	20	20
3	**5 (c)**	17	19	20	19
4	**5 (d)**	18	17	21	20
5	**5 (e)**	21	18	18	19
6	**5 (f)**	20	21	21	19
7	**6 (a)**	18	20	20	18
8	**6 (b)**	19	18	19	18
9	**6 (c)**	17	19	19	17
10	**6(d)**	21	17	18	18
11	**6 (e)**	22	17	21	21
12	**6 (f)**	20	18	22	20
13	**7(a)**	19	21	20	18
14	**7(b)**	18	20	17	19
15	**7(c)**	21	18	17	17
16	**7(d)**	22	19	18	20
17	**7(e)**	20	22	21	21
18	**8(a)**	17	20	20	18
19	**8(b)**	19	19	18	21
20	**8(c)**	17	18	19	20
21	**8(d)**	20	21	17	18
22	**8(e)**	22	22	18	18
11	**Ciprofloxacin**	30	29	30	27

Table 3: MIC of compounds in µg/ml (*B. subtilis*)

Compound	MIC µg/ml
5a	45
5b	25
5c	30
5d	25
5e	15
5f	35
6a	20
6b	15
6c	45
6d	10
6e	15
6f	15
7a	15
7b	40
7c	10
7d	30
7e	25
8a	45
8b	20
8c	10
8d	10
8e	25

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