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Research Article

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Antimicrobial Screening of some Novel Substituted Benzimidazole Derivatives

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ABSTRACT

Heterocyclic chemistry comprises at least half of all organic chemistry research worldwide. In particular, heterocyclic structures form the basis of many pharmaceutical, agrochemical and veterinary products. The benzimidazole contains a phenyl ring fused to an imidazole ring, as indicated in the structure of benzimidazole. Diversity of biological response profile has attracted considerable interest of several researchers across the globe to explore this skeleton for its assorted therapeutic significance. By using novel synthetic methods new benzimidazole derivatives were synthesized and further Melting points were determined by using Precision melting point apparatus in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel G plates using n-Hexane, ethyl acetate (1:3) and methanol: chloroform (1:9) solvent system. The synthesized benzimidazole derivatives were characterized by IR, ^{1}H NMR spectral analysis. Benzimidazole is a lead nucleus for future developments to get effective compounds.

Key words: Benzimidazole, Acetohydrazide**,** Imidazole, n-Hexane, Ethyl acetate, Chloroform, Methanol

INTRODUCTION

Substituted benzimidazole have received considerable attention during last few decades as they are endowed with variety of biological activities and have wide range of therapeutic properties. A literature survey indicates that benzimidazole derivatives possess different pharmacological and biological activities, of which the most potent is, anti-microbial activity, anti-ulcer. We thought to synthesize some novel benzimidazole moiety incorporating with different aromatic and hetero cyclic aldehyde moiety. The conventional methodology was adopted to synthesize the titled compounds[1].

Heterocyclic chemistry comprises at least half of all organic chemistry research worldwide. In particular, heterocyclic structures form the basis of many pharmaceutical, agrochemical and veterinary products. The benzimidazole contains a phenyl ring fused to an imidazole ring, as indicated in the structure of benzimidazole. The important group of substances has found practical application in a number of fields. Recently in benzimidazole chemistry has been revived somewhat by the discovery that the 5, 6-dimethyl benzimidazole moiety is a part of the chemical structure of vitamin B12[2].

MATERIALS AND METHODS

Biological Evaluation

Bacteria

During the ninethteeth century, the French scientist Louis Paster and German thiysiciaan demonstrated the roll of bacteria as pathogens the discovery of compound produce by bacteria and fungi have shown their the lethal effect to other bacteria led to development of antibiotics[3].

Type of Bacteria

Scientist use various system for classifying bacteria based on different, shapes, dependence on oxygen and by staining techniques[4].

A. Classification by shapes

Table .1 Classifications of bacteria

Shapes	Description	Examples
Cocci	Cocci (berry) are spherical or oval shapes	Staphylococcus Aureus
Bacilli	Bacilli are rod shapes cells.	Clostridium tetani
Vibrios	Vibrius are comma shaped curved rods.	Vibrios cholera

1. Aerobic and anaerobic Bacteria

Bacteria can be classified according to need of the oxygen to survive. As aerobic bacteria require oxygen and anaerobic do not requires oxygen to survive

Eg. Bacillus Anthrax aerobics, Clostridium titani anarobics[5].

2. Autotrophic and Heterotrophic Bacteria

All bacteria require carbon for growth and reproduction bacteria called autotrophes get their carbon from carbon dioxide and heterotrophes from organic nutrient[6].

3. Gram Positive and Gram Negative

Bacteria can be classify by the Gram staining techniques which is identified as Gram positive and Gram Negative after staining they stains purple and pink respectively[7].

Eg. S. Aureus(Gram + ve), E.coli(gram -ve).

B. Disease caused by pathogenic Bacteria

Table.2 Disease by pathogenic Bacteria

S.no	Disease	Causative microorganism
1	Diphtheria	Corynebacterium diphtheriae
2	Tuberculosis	Mycobacterium Tuberculasis
3	Leprosy	Mycobacterium leprae
4	Tetanus	Clostridium Tetanus
5	Diarrhea	E.coli
6	Typhoid fever	Salemonella Typhi
7	Gonorrhea	Neisseria gonorrhoeae

C. Antibacterial Agents

Anti microbial agents can be divided according to their mechanism of action.

a. Agents that inhibit bacterial cell wall synthesis.
b. Agents that interfere with DNA-RNA synthesis.
c. Anti metabolites.
d. Agents that interfere with protein synthesis.

D. Agents that inhibit bacterial cell wall synthesis

This includes B-lactamase antibiotics, like Ampicilin and Cephalosporines B-lactamase inhibits D-alanyl-D-alanine transpeptidase activity by acylation,forming stable esters with opened lactum ring attached to hdroxyl group of the enzymes active site[7].

E. Agents that interfere with protein synthesis

This class includes, Tetra cyclones, which block and binds aminoacyl receptor site of tRNA, Chloramphinicol, and Erythromycines, binds p-sites of the 50S ribosomal subunit and inhibit translation[8].

F. Agents that interfere with DNA-RNA synthesis.

Quinolones, are bactericidals and they inhibit DNA gyrase synthesis, Sulphonamides inhibit microbial growth by inhibiting P-aminobenzoic acid(PABA) invoved in folic acid synthesis[9].
Eg: Ciprofloxacine, norfloxacine, sparfloxacine.

Fungi

Fungi are heterotropic organism, they don't form embryos. Fungi are eukaryotic chemoorganiotropic organism that has no chlorophyll. fungi posses rigid cell wall containing chitin, manoproteins, glucans, and polysaccharides.They divide asexually and sexually or by both process. they may be uni or multi cellular, fungi are generally aerobic[10].

A. Classes of Fungi

- Yeast
- Yeasts like fungi
- Moulds
- Dimorphic fungi

Table .3 Classes of Fungi

S.no	Disease	Causative organism
1	Aspergillosis	Aspergillus niger
2	Candidasis	Candida albicans
3	Tinea niger	Tina nigera
4	Mycetomas	Acremoniumfaliciforme

B. Anti Fungal Agents

Fungi cause a range of illnesses (mycoses) ranging from the chronic to the serious. These mycoses can manifest themselves in a variety of ways. Infections can be superficial, that is situated at or close to the surface of the skin or systemic which means they can affect the body as a whole, rather than individual parts or organs.

Diseases such as athlete's foot (Tinea pedis), 'jock' itch (Tinea cruris), Tinea manus (infection of the hand), thrush (oral and vaginal), and onchomycosis (affecting the nails)are examples of superficial infections caused by the dermatophytes from the Trichyphyton microsporum, C. albicans and Epidermophyton species. 'Ringworm' (Tinea corporis) is used as a general term for a fungal infection of the skin, in particular those of the scalp and feet. These infections are contagious, and cause intense itching. One or more of these organisms causes them[11].

An important aspect to consider when developing treatments for mycoses is that fungi are eukaryotic. That is to say they have a nucleus within the cell containing the all-important nucleic acids. In very simplistic terms this means that some of the biochemistry regulating fungi turns out to be very similar to animal cells. They are therefore unlike the prokaryotic bacteria, which do not have a cell nucleus. This can in turn pose potential problems with toxicity. For many enzymes in a fungus there are related enzymes performing the same transformations in the human cell. If we want to target one of these enzymes with drug then absolute potency may not be as important as the difference in potency of our drug towards the different forms of the enzyme[12].

Table.4 Classification of Antifungal agents

ANTIFUNGAL AGENTS		
1	Agents acting against the fungal cell nucleus.	Griseofulvin, 5-Fluorocytosine
2.	Steroidal antifungal agent.	Ergosterol, Lanosterol
3.	Agents acting against the fungal cell wall Polyene antibiotics: macrocyclic lactones,	Amphotericin, Nystatin. Abduction.
4.	Agents affecting ergosterol biosynthesis. Thiocarbamates, Allylamine derivatives. Morpholine derivatives.	Tolnaftate, Tolciclate. Naftifine, Terbinafine. Fenpropimorph, Amorolfine.
5.	Azole derivatives. First generation azoles: imidazoles. Secondgeneration: imidazoles.	Clotrimazole, Miconazole. Ketoconazole.
6.	Triazole derivatives.	Fluconazole, Itraconazole,

1. Anti –Bacterial activity

All the compounds synthesized in the present investigation were screened for their anti-bacterial activity by Cup plate Method. Antibacterial activities were tested on nutrient medium against, Staphylococcus aureus, and Escherchia coli which are representative types of gram positive and gram negative organisms respectively. The antibacterial activity of the compounds was assessed by disc-diffusion method.

Preparation of Nutrient Agar Media:

Media Composition and Procedure:

The nutrient agar media was prepared by using the following ingredients.

1) Peptone (Bacteriological) 20 gm
2) Beef extract (Bacteriological) 5 gm
3) Sodium chloride 5 gm
4) Agar Agar 20 gm
5) Distilled water up to 1000 ml.

Weighed quantities of peptone and beef extract were dissolved in distilled water by gentle warming and then specified amount of agar was dissolved by heating on water bath. Then the pH of the solution was adjusted to 7.2 - 7.4 by adding the sodium chloride and the volume of the final solution was made up to 1000 ml with distilled water. Then it was transferred in to a suitable container, plugged with non-adsorbent cotton and the media was sterilized by in autoclave at 121^0C for 20 minutes at 15 lbs pressure[10].

Preparation of Test Solutions:

10 mg of the compound was dissolved in 10 ml of DMF. From this 1 ml of solution was taken and diluted up to 10 ml with DMF. Now the concentration of the test solution was 100 µg/ml. From the stock solution 1ml of solution was taken and diluted with 1ml of DMF now the concentration is 50µg/ml.

Preparation Of Standard Antibiotic Solution:

Ampicillin was used as standard antibiotics for comparison and solutions were prepared by using sterile water, as they were water-soluble. The solutions are diluted by using sterile water so that the concentrations of the solutions were 100 µg/ml and 50 µg/ml[11].

Method of Testing

The sterilized media was cooled to 45^oC with gentle shaking to bring about uniform cooling and then inoculated with 18-24 hrs old culture under aseptic conditions, mixed well by gentle shaking. This was poured in to sterile Petri dishes (properly labeled) and allowed the medium to set. After solidification all the Petri dishes were transferred to laminar flow unit. Then the discs which were previously prepared were carefully kept on the solidified media by using sterilized forceps. These Petri dishes were kept as it is for one-hour diffusion at room temperature and then for incubation at 37^oC for 24 hours in an incubator.The extent diameter of inhibition after 24 hours was measured as the zone of inhibition in millimeters[12]

2. Anti-Fungal Activity

Broth dilution method is used for screening Antifungal activity as described below.

Broth double dilution method:

The broth double dilution method was used to evaluate the minimal inhibitory concentration (MIC) of the test compounds the classical method yields accurate, precise and quantitative results for the amount of antimicrobial agent that is needed to inhibit growth of microorganisms[13].

Determination of minimum inhibitory concentration (MIC)by broth double dilution method.

A. MIC of the entire test (synthesized compounds) was determined using the said method.
   - Drug control- Ketoconazole as reference standard was used.
   - Solvent control – DMF and DMSO were used as solvent controls.
B. Sabourauds Dextrose Broth (SDB) and Malt extract Glucose Yeast extract peptone broth (MGYP) was used as nutrient medium for growth of micro organism and MIC determination for C.albicans.
C. All the compounds were dissolved in DMF and standard dissolved in DMSO.

D. All the compounds were serially diluted.
E. Test compounds were dissolved in sterile DMF and Ketoconazole was dissolved in the sterile DMSO.
F. The test compounds and standard drug solution were diluted using Sabourauds Dextrose Broth (SDB) and Malt extract Glucose Yeast extract peptone broth (MGYP) so as to get required concentration.
G. To serially diluted solution, test organisms were added using saline solutions or broth.
H. Then the plates were incubated at 37^{o}C for 48 hrs.
I. The growth of micro organism in the test compound solutions and control drug was seen after incubation.

Methods Used For Screening

In screening the test compounds were dissolved in DMF, so as to give 8000µg/ml which was then serially diluted. Ketoconazole used as standard, was dissolved in sterile DMSO.

RESULTS & DISCUSSIONS

Benzimidazoles have been reported for number of pharmacological activities and some molecules have shown significant activities and some compounds shows moderate and good activities.Here we have synthesized novel benzimidazole analogues and screened them for their anti-bacterial activities and the results are as follows. The synthesized all benzimidazole derivatives were screened for anti bacterial activity using DMF as a solvent against the organisms, S.aureus and E.coli. Antifungal activity using Candida albicans. By disc diffusion method on nutrient agar media. The standard drug used was Ampicillin for antibacterial and Ketoconazole as standard for antifungal activity.The antimicrobial screening results presented on above table reveals that compounds **COM-3, COM -5,** exhibited poor activity at 50 µg/ml, but at 100 µg/ml they have shown moderate activity against S.aureus, and moderate activity against E.coli. The compounds **COM -1, COM -2, COM -4,** have shown the poor activity against E. Coli and S.Aureus at 50mg. but the same compounds at 100 µg/ml against same organism have shown moderate activity. **COM-5** has shown the very good activity against S.aureus at 100 µg/ml when compared with the standard drug Ampicillin. The Compounds were screened for the anti-fungal activity against Candida albicans the compounds **COM -2, COM -3, COM -5,** Showed highest degree of inhibition at 250 µg/ml and 500 µg/ml against C.albicans when compared with the standard drug Ketoconazole. However the activities shown by all the compounds tested were less than that of the standard. The compounds **COM -1, COM -3, COM -5,** have shown good anti bacterial activity due to the presence of electron donating group **OCH3, N(CH3)2 , CH3** group which is attached at 4 fourth position of the phenyl ring system.The anti fungal activity shown that **HC-2, HC-3, HC-5,** have shown good antifungal activity it also may be due to the presence of electron donating group **OCH3, N(CH3)2 , CH3** group which is attached at 4 fourth position of the phenyl ring system. However the activities shown by all the compounds tested were less than that of the standard.

Table. 5 Anti-bacterial activity data of synthesized benzimidazole

S.no	compound	Conc µg/ml	E.coli	S.Aureus
1	COM -1	50	7mm	8 mm
		100	10 mm	10 mm
2	COM -2	50	7 mm	7 mm
		100	7 mm	10 mm
3	COM -3	50	8 mm	7 mm
		100	11 mm	9 mm
4	COM -4	50	6 mm	7 mm
		100	7 mm	11 mm
5	COM -5	50	8 mm	12 mm
		100	14 mm	14 mm
6	Ampicillin	50	24 mm	25 mm
		100	25 mm	25 mm

Zone of inhibition of synthesized compounds:
Note: 6-8 mm poor activity, 9-11 mm moderate activity, 12-15 above good.

Table. 6 Anti-fungal activity data of synthesized benzimidazole

S.no	Compound	Conc µg/ml	Candida albicans
1	COM -1	250	+
		500	-
2	COM -2	250	-
		500	-
3	COM -3	250	-
		500	-
4	COM -4	250	+
		500	-
5	COM -5	250	-
		500	-
6	Keto	250	-
		500	-

CONCLUSION

From the data of the Table no.5 & 6 of antibacterial and anti-fungal activity it is clearly concluded that the synthesized compounds are promisingly significant, good antimicrobial and anti-fungal agents. As per the results of screening it is clearly indicated that all the synthesized novel benzimidazole derivatives have shown good antibacterial and antifungal activity equipotent with the standard drugs. This is because of the presence of groups like **OCH3, CH3, -N-(CH3)$_2$**, at the different positions of phenyl nucleus and heterocyclic system attached to benzimidazole nucleus which is attached to benzimidazole molecule.

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