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Synthesis And Evaluation Of Benzimidazole For Their Antimicrobial Activity

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Abstract

A library of 53 benzimidazole derivatives, with substituents at positions 1, 2 and 5, were synthesized and screened against a series of reference strains of bacteria and fungi of medical relevance. The SAR analyses of the most promising results showed that the antimicrobial activity of the compounds depended on the substituents attached to the bicyclic heterocycle. In particular, some compounds displayed antibacterial activity against two methicillin-resistant Staphylococcus aureus (MRSA) strains with minimum inhibitory concentrations (MICs) comparable to the widely-used drug ciprofloxacin. The compounds have some common features; three possess 5-halo substituents; two are derivatives of (S)-2-ethanaminebenzimidazole; and the others are derivatives of one 2-(chloromethyl)-1H-benzo[d]imidazole and (1H-benzo[d]imidazol-2-yl)methanethiol. The results from the antifungal screening were also very interesting: 23 compounds exhibited potent fungicidal activity against the selected fungal strains. They displayed equivalent or greater potency in their MIC values than amphotericin B. The 5-halobenzimidazole derivatives could be considered promising broad-spectrum antimicrobial candidates that deserve further study for potential therapeutic applications.

Keywords: Gram-negative; Gram-positive; antibacterial activity; antifungal activity; benzimidazole; heterocycle; resistance.

Introduction:

Microbial drug resistance is a serious issue, especially as increasing numbers of strains are becoming resistant to multiple antimicrobial agents, with some bacteria now being resistant to all available antibiotics. There is thus a critical need to develop new drugs with novel mechanisms of action. However, the investment available for such development is frequently lower than the required level. The development of new drug entities is hampered by several issues, notably the high cost and length of time required, as well as the logistical and regulatory challenges of performing the necessary clinical evaluations across multiple geographical areas. Therefore, a few new classes of antimicrobials have been developed since the late 1980s [1–3], and much research has focused only on the chemical modification of existing drugs to
improve their potency and/or ability to overcome antibiotic resistance mechanisms. Even if this approach does not improve antimicrobial activity directly, it may lead to derivatives that can usefully inhibit virulence mechanisms [4]. Compounds having benzimidazole as a structural motif have been widely used in medicinal chemistry and drug development, and researchers are actively seeking new uses and applications of this heterocycle [5].
Benzimidazole-containing compounds have numerous medical and biological activities, such as
antitumor [6] antibacterial [7–10], antifungal [11], antiviral [12–16], anticonvulsant [17], antidepressant [18], analgesic [19], anti-inflammatory[20] and antidiabetic properties [21]. For example, derivatives, such as thiabendazole, cambendazole, parbendazole, mebendazole, albendazole and flubendazole, are widely-used anti-helminth drugs, used to treat people and animals with gastrointestinal worm infections [22]. Two groups of benzimidazole derivatives, namely 5,6-dinitro- and 2-triflurомethyl derivatives, are particularly well known for their use as antihelminth drugs [23]. 2-Methoxycarbonylamino derivatives have shown good antiprotozoal activities against some protozoan parasites, such as *Giardia lamblia* and *Entamoeba histolytica*, by inhibiting tubulin polymerization, and hence, making these better antiprotozoal agents than

metronidazole and albendazole [24]. Nitrogen-containing heterocyclic systems have a diverse spectrum of pharmacological properties. Different heterocyclic motifs can be incorporated to produce molecules with enhanced biological properties. Recent reports include benzimidazoles bearing the 1,3,4-oxadiazole moiety, which have broad spectrum antimicrobial properties [25], and molecules containing both the benzimidazole and indole heterocycles, which exhibit selective antibacterial activity [26]. A review of the literature thus suggests that there is the scope for the design of additional benzimidazole derivatives with antimicrobial activity, by examining the effect of a number of different functional groups. In this paper, we report on the synthesis of a series of benzimidazole derivatives and their antimicrobial activity. A detailed study of the structure-activity relationship of these derivatives will pave the road to designing more potent compounds. The compounds were tested for their ability to inhibit isolates amongst a reference panel of 26 bacterial and 10 fungal strains, and key results are presented in Tables 1–3.

Aim and Objectives

Literature review revealed that benzimidazole and its derivatives show different pharmacological activities like antibacterial, anthelmintic, antioxidant, antiviral, antidiabetic activities etc.

Objectives:

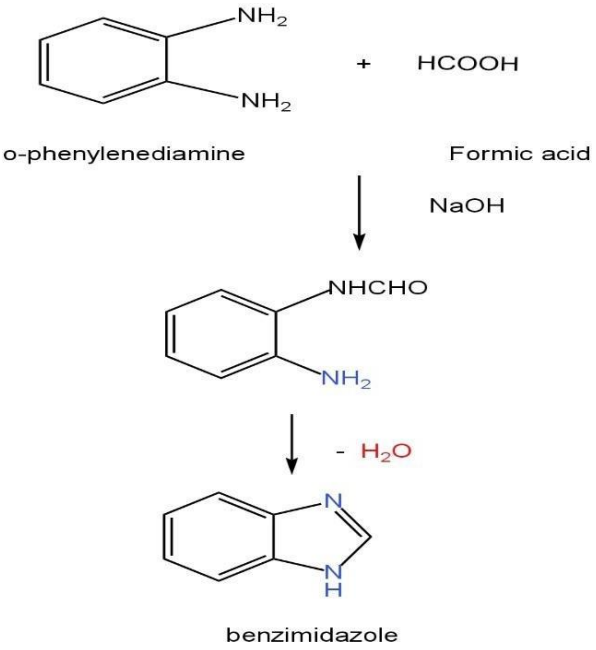
- In the present study we planned to synthesize benzimidazole derivatives associated with antibacterial, antioxidant properties
- To synthesis new series of various compounds benzimidazole derivatives and to purity the final compounds by appropriate recrystallization and chromatographic methods
- To characterize all the new compounds by analytical and spectral analysis

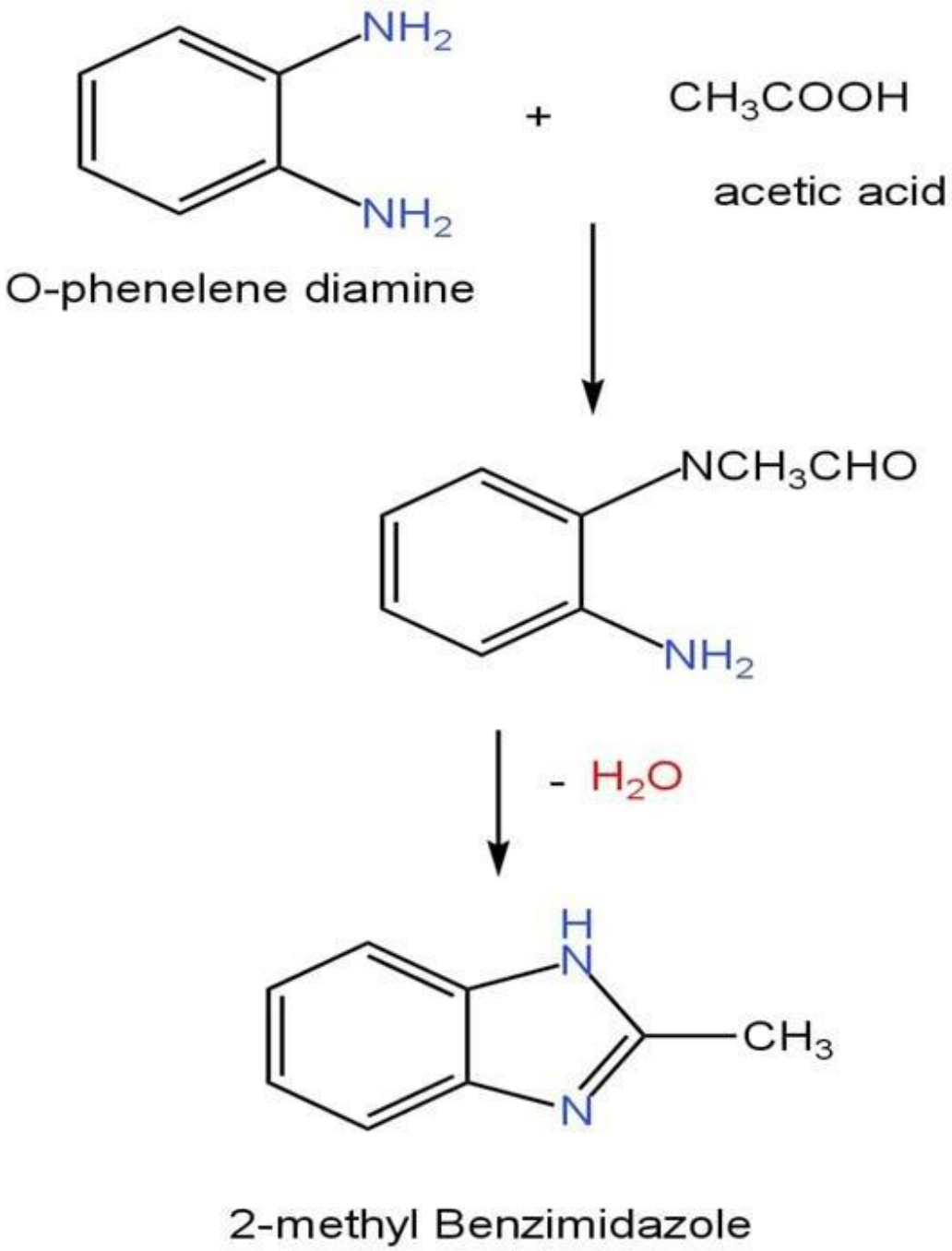
Materials And Methods

Chemicals Used:
O-phenylenediamine, formic acid (90%), acetic acid, Sodium hydroxide, silica gel(G), iodine, Benzene, Acetone etc.
Apparatus Used:
Round bottomed flask (250ml), Beaker, Buchner funnel, measuring cylinder, stirrer, ignitiontubes, capillary tube, TLC plates, Filter paper, weighing machine.
Analytical Work

- Reactions were monitored by thin layer chromatographic (TLC) on a coated silica gelusing benzene and acetone in the ration of 7:3.
- NMR and MASS SPECTR were recorded on PLANTEX from Lila implex, Vijayawadausing TMS as standard
- IR spectra were also recorded from of Hindu College of pharmacy, Guntur
- Melting points were determined by using open capillary method.

Scheme:





SCHEME

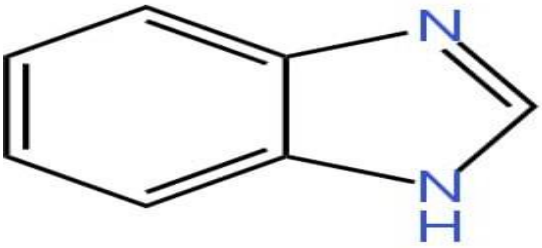
Experimental Method:

General procedure for the synthesis of (1H-1,3benzimidazole) substituted 2methyl -1H-1,3-benzimidazole

Method-1: The molar concentrations of ortho-phenylenediamine (27gm, 0.25mol) inround bottom flask and add formic acid (17.5gm, 16ml, 0.34mol) heat the mixture at 100^0cfor2 hours, cool and add 10% sodium hydroxide solution was Slowly with constant rotation of the flask. Until the mixture is just alkaline to litmus the completion of the reaction (progress of thereaction checked by TLC). Then it was filtered, with water, dried and recrystallized from water

Method-2: In a 500ml RBF 12.5gm of o-phenylenediamine was treated with 11.25gm of90% acetic acid. The mixture was heated in a water bath at 100°C for 2 hours. After cooling, 10% sodium hydroxide solution was added slowly with through mixing by rotation of the flask until the mixture was just alkaline to litmus. The crude Benzimidazole was collected with suction in a 75mm Buchner funnels. Ice cold water is used to rinse all solid out of the reaction flask. The crude precipitate is pressed thoroughly on the filter washed with ice cold water andthen purified.

Experimental Work
Compound: 1



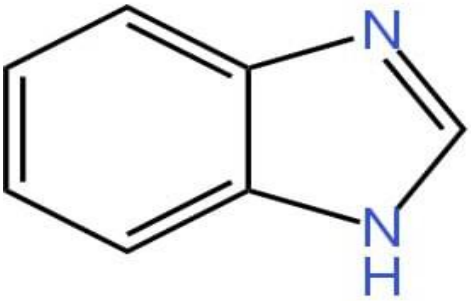
Chemical name :1H- 1,3-Benzdiazole

Molecular formula :$C_7H_6N_2$

TLC :Rf (7:3 benzene: acetone) : 0.904
^{1}H NMR ($CDCl_3$) : δ 7.251-7.258 (d.2H Ar-H)
: δ 7.609-7.615 (d.2H Ar-H)
: δ 8.109-8131 (d.1H Ar-H)
FTIR (KBr) cm^{-1} : 1453.50 Cm^{-1} (C-H) ,1649.14 Cm^{-1} (C=C) 1683.35 Cm^{-1} (C-C), 1237.39 Cm^{-1} (C-N)
3646.35 Cm^{-1} (N-H), 1552.46 Cm^{-1}(C=N)
MASS (m/z) : 117.1 M^+

Experimental Method:
General procedure for the synthesis of (1H-1,3benzimidazole) substituted 2methyl -1H-1,3-benzimidazole
Method-1: The molar concentrations of ortho-phenylenediamine (27gm, 0.25mol) inround bottom flask and add formic acid (17.5gm, 16ml, 0.34mol) heat the mixture at 100^0cfor2 hours, cool and add 10% sodium hydroxide solution was Slowly with constant rotation of the flask. Until the mixture is just alkaline to litmus the completion of the reaction (progress of thereaction checked by TLC). Then it was filtered, with water, dried and recrystallized from water
Method-2: In a 500ml RBF 12.5gm of o-phenylenediamine was treated with 11.25gm of90% acetic acid. The mixture was heated in a water bath at 100°C for 2 hours. After cooling, 10% sodium hydroxide solution was added slowly with through mixing by rotation of the flask until the mixture was just alkaline to litmus. The crude Benzimidazole was collected with suction in a 75mm Buchner funnels. Ice cold water is used to rinse all solid out of the reaction flask. The crude precipitate is pressed thoroughly on the filter washed with ice cold water andthen purified.

EXPERIMENTAL WORK



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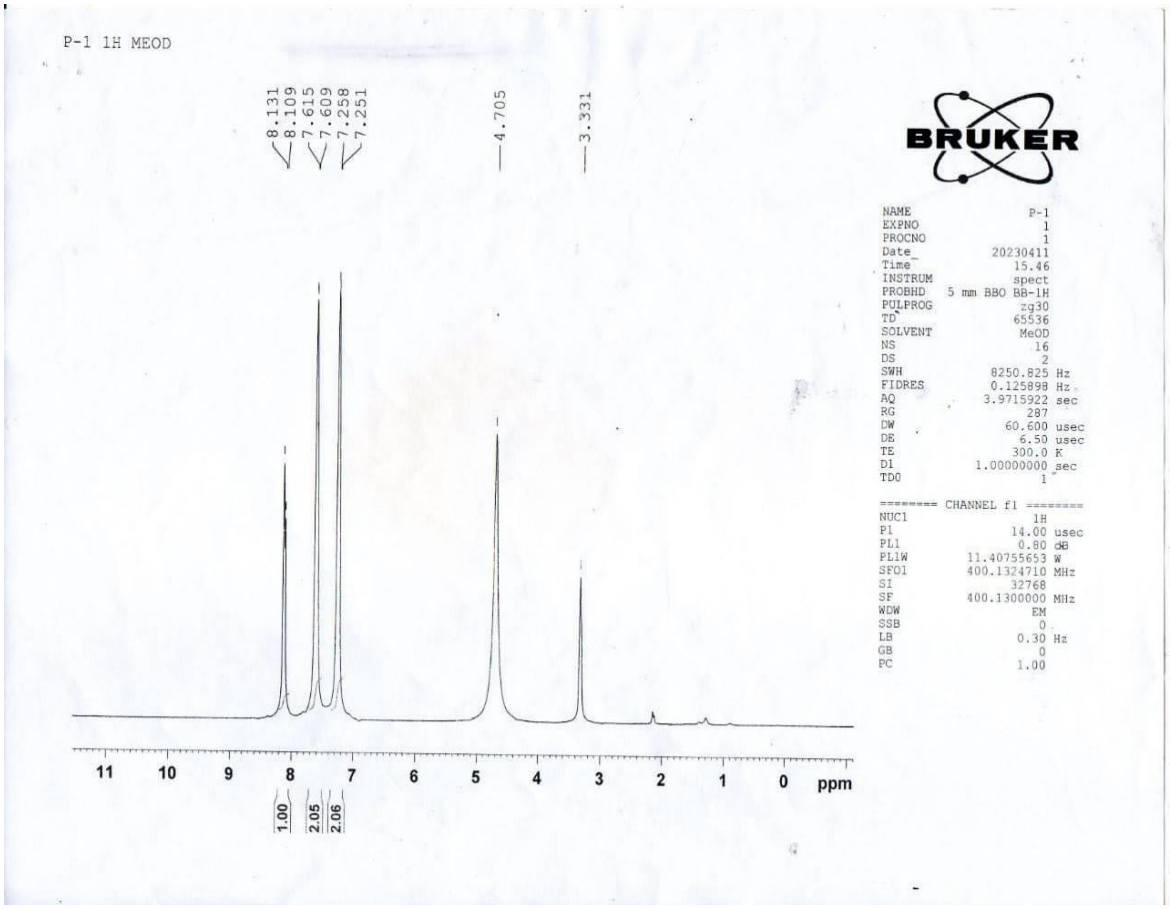
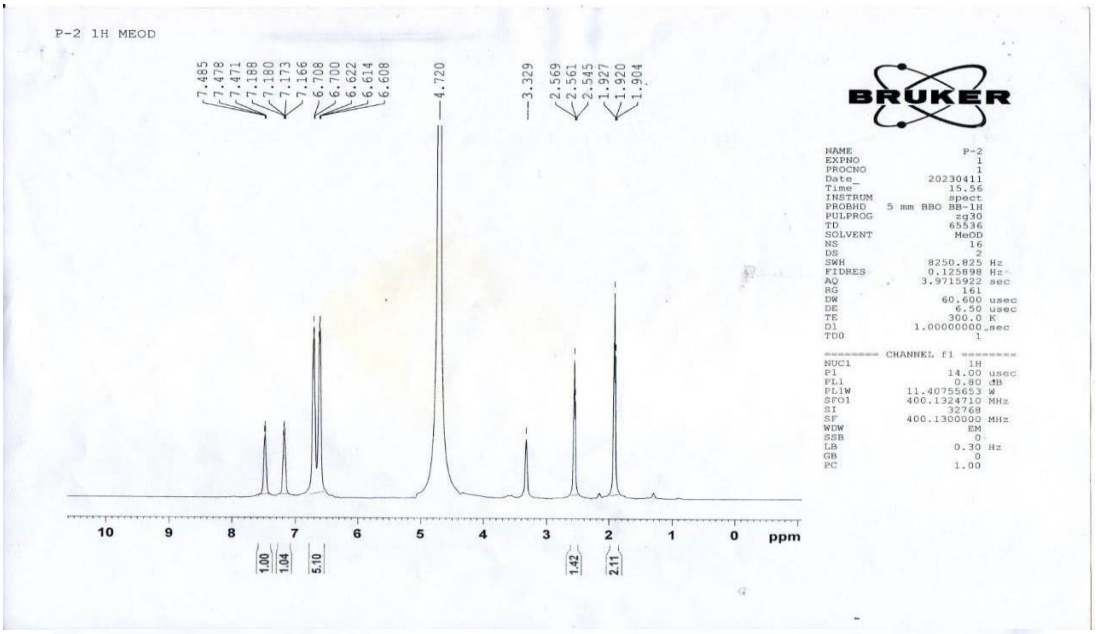
3646.35 Cm^{-1} (N-H), 1552.46 Cm^{-1}(C=N)
MASS (m/z) : 117.1 M^+

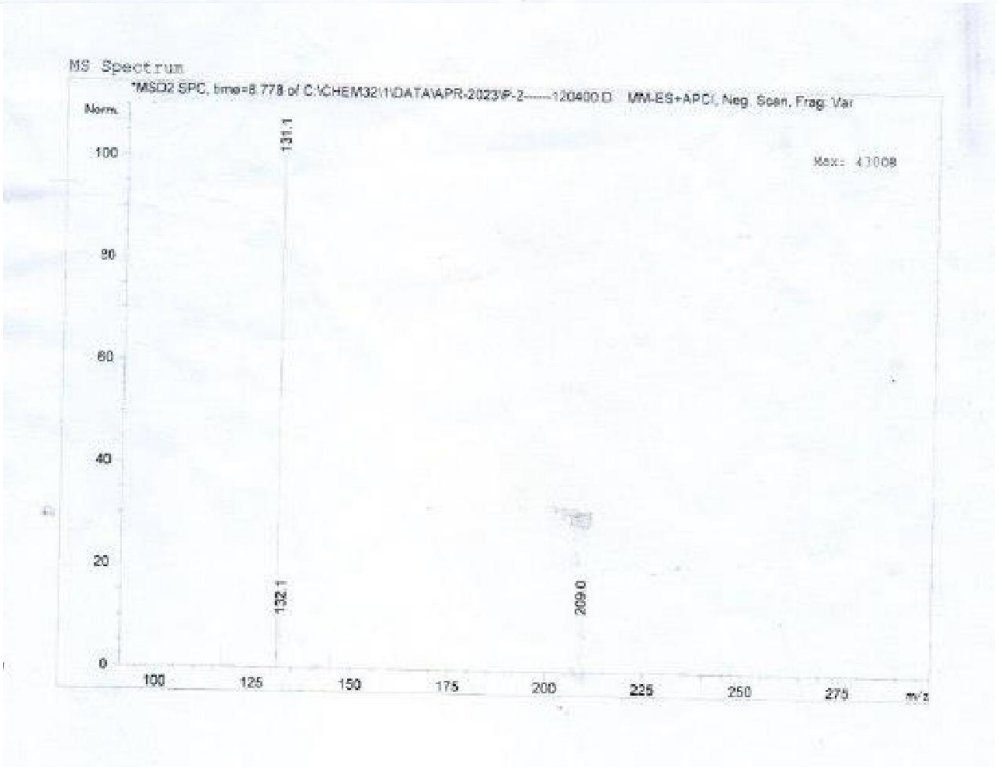
Compound :2
Chemical name : 2- Methyl -H -1,3 -Benzimidazole
Molecular formula : $C_8H_8N_2$
TLC : R_f (7:3 benzene : acetone)
^{1}H NMR ($CDCl_3$) : δ 7.166 -7.188 (d.1H Ar-H)
: δ 7.471 -7.485 (d.1H Ar-H)
FTIR (KBr)Cm^{-1} : 3268.78 Cm^{-1} (C-H), 1640.24 Cm^{-1} (C=C)
: 1548.56 Cm^{-1} (C-C), 3358.89 Cm^{-1}(N-H)
: 1548.56 Cm^{-1}(C=N), 1267.59 Cm^{-1} (C-N),1407.62 (CH_3)
MASS (m/z) : 131.1 (M^+)

Experimental Work
Table 1: Physical and yield substituted -1(1H- 1,3 Benzimidazole) 2 methyl 1H-1,3 benzimidazole

COMPOUNNO.	R	M.P.^{0} C	YEILD%	MOL. FORMULA
1	H	158	94	$C_8H_6N_2$
2	CH_3	175	98	$C_8H_8N_2$







Anti Bacterial Activity

The reported Benzimidazoles possess anti-microbial activity. So the Benzimidazoles prepared during the course of the present work were tested for anti-bacterial activity.

Apparatus and chemicals required:

Non-absorbent cotton	:	Rama surgical cotton Ltd.
Conical flask	:	Borosil

Test tubes	;	Borosil
Petri Dishes	:	SD fine chem. Ltd
Hot air oven	:	Techinco equipments
Autoclave	:	Universal autoclave
Laminar flow unit	:	Clean air Instruments Inc.
Incubator	:	Techinco incubator

The anti-bacterial activity screening was carried out in the pharmaceutical Biotechnology laboratory, A.M.Reddy Memorial College of Pharmacy, Narasaraopet, Andhra Pradesh, India.

Nutrient Agar Medium

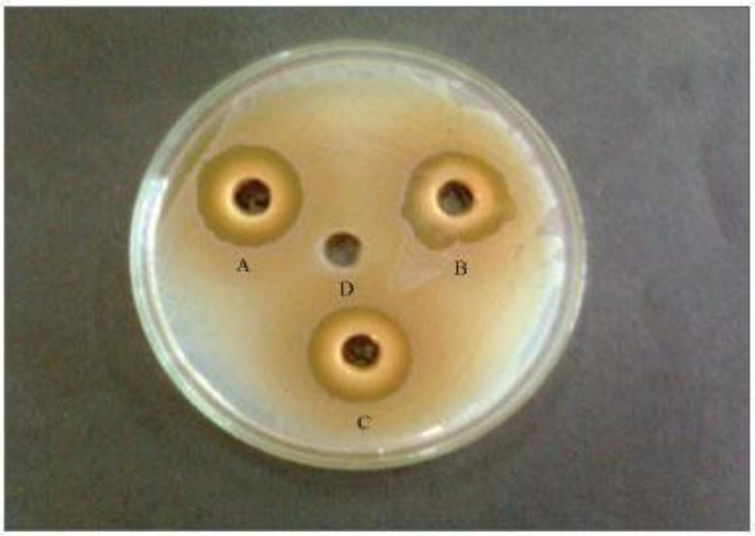
Beef extract : 10gms
Peptone : 10gmsSodium chloride : 5gms Agar-agar : 15gms
Distilled water : 1000ml
P^H : 7.2-7.4

Experimental Procedure:
Nutrient agar was dissolved and distributed in 25 ml quantities in 100ml conical flask and were sterilized in an autoclave at 121^0c (151lbs/sq. in) for 20 minutes. The medium was inoculated at one percent level using 18hrs old culture of the test organism mentioned above aseptically into sterile Petri dishes and allowed to set at room temperature for about 30 minutes.In a size of 4 inches Petri dishes, four cups of 8mm diameter at equal distance were made in each plate. In each plate, one cup was used for control i.e. DMSO (Dimethyl sulphoxide), other for standard ciprofloxacin with 100mg/ml. Other two cups with concentration of test compound i.e. 50µl and 100 µl solutions[72]. The plates thus prepared were left for 90 minutes in refrigerator for diffusion. After incubation for 24 hours at 37^0c± 1^0c, the plates were examinedfor inhibitions zones. The experiments were performed in duplicate and the average diameter of the zones of inhibition measured were recorded. There is no zoneof inhibition for control.

Antibacterial Activity:

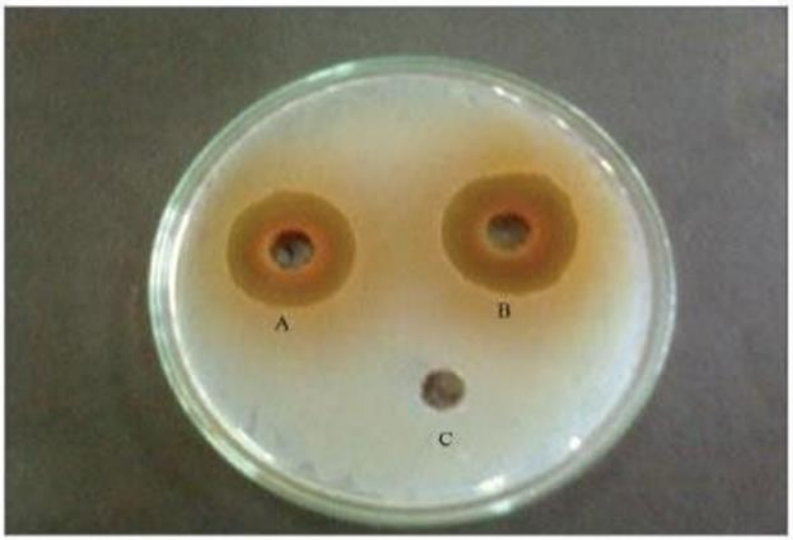
The antimicrobial activity was tested by *cup-plate* method. The antibacterial activity of Benzimidazoles were tested and compared with standard(ciprofloxacin) solution at concentration of 100mg/ml. DMSO (dimethyl sulphoxide) was used as a solvent and control .Benzimidazoles on organisms. So, the final compound that is more active against organisms is Benzimidazoles
The following organisms are used.
Test organisms: Gram positive bacteria: *Staphylococus aureus* Gram negitive bacteria :E.coli

S.No	**ZONE OF INHIBITION (in cm)**			
	Staphylococcus aureus		**E.coli**	
	0.1%	**0.2%**	**0.1%**	**0.2%**
Standard	3.9	7.5	3.5	6.9
Control	-	-	-	-
1	2.5	4.7	2.3	4.5
2	3.7	7.1	3.3	6.5



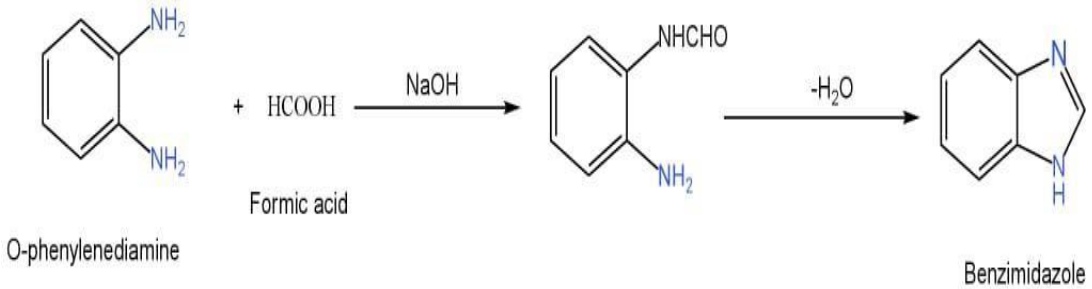
Activity Against Gram –ve Bacteria

Activity Against Gram +ve Bacteria



Chemistry:

There are several methods reported for synthesis of 2- substituted benzimidazole derivatives. Mainly two methods are widely employed, which are coupling of O-phenylenediamine with formic acid and acetic acid in the presence of strong acidic medium and the coupling of O-phenylene diamine with different substituted aldehyde.



In the present study, ring substituted benzimidazole were synthesized by condensation using1H-1,3 Bendiazole and substituted aromatic aldehydes.

The benzimidazole prepared were:

1.1H-1,3 benzdiazole

2. 2 methyl 1H-1,3 benzdiazole
The physical data such as melting points and yields are given in the table
The IR spectra compounds (1,2) displayed bands at 3060.20Cm^{-1}-3268.78Cm^{-1}due to
stretching, 1552.46Cm^{-1}-1640Cm^{-1} due to C=N stretching ,1237.39Cm^{-1}-1548.56 Cm^{-1}C-C stretching ,1649.14 Cm^{-1}-1640.24Cm^{-1}-due to stretching.
^{1}H NMR spectra were taken for compounds (1,2) which also supported the structures assigned. These compounds displayed a doublet at δ 7.251-7.258 (d. 2H Ar-H) of the nitrogen group and singlet in the ring at δ 8.109- 8.131 (d.1H Ar.-H) and doublet in the rangeof δ 7.609- 7.615 (d.2H Ar-H) due to aromatic hydrogen (Ar-H). Compounds displayed a doublet at due to methoxy substitution on the ring.
The structure of the compounds was also assigned by MASS spectral analysis which showed($^{+}$M) peaks of the compounds. The mass spectra of the compound1 showed a characteristic of molecular ion peaks (M+) at 117.1 M^{+} and the compound -2 showed acharacteristic molecular ion peaks (M^{+}) at 131.1.

Summary

A great deal of interest has been developed in the synthesis of benzimidazoles and their derivatives. In the experiment, benzimidazoles were synthesized from o- phenylenediamine , formic acid & acetic acid.The synthesized compounds werecharacterized by antimicrobial activity. In the experiment, benzimidazole derivatives were synthesized using a two-component reaction under extreme conditions. The reaction involved the condensation of o-phenylenediamine, carboxylic acid derivatives in the presence of NaOH.All the compounds were purified by recrystalization using water and methanol as solvents. . TLC was done to check the purity of the compound and to check different components in mixture. The synthesized derivatives were characterized using various spectroscopic techniques such as UV-Vis, IR, and NMR. The biological activity ofthe synthesized compounds was also evaluated. The two compounds were synthesized withthe yields generally ranging from (65-72%) ,Benzimidazole (72%) yields more than 2- methyl benzimidazole(85%).Synthesized compounds were evaluated for antimicrobial activities. Among these compounds 2-methyl benzimidazoles showed grater activity against gram positive and gram-bacteria. Where as bezimidazole showed moderate activityagainst gram positive and gram negative bacteria

Conclusion:

Substituted Benzimidazoles were prepared by reacting o-phenylenediamine, formic acid & acetic acid, by using reflux condenser temperature at 100^{0}C in the presence of aqueous NaOH
.among the synthesized compounds 2-methyl benzimidazole showed greater activity at 0.2% concentration and Benzimidazole showed moderate antib acterial activity at 0.2% concentration .these compounds may show more anti bacteria activity at higher concentration

Acknowledgement:

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