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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SOME NEW 2-SUBSTITUTED BENZIMIDAZOLES

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ABSTRACT

2-substituted benzimidazole was prepared from O-phenylenediamine and carboxylic acid in presence of 4N HCl, and reacting with 1-benzyl 2-substituted benzimidazoles the yield of different derivatives of benzimidazole. It is noteworthy that such a procedure for rapid preparation of various benzimidazoles affords advantages of short reaction time, moderate yield and simple workup. As expected, benzimidazole derivatives exhibited significant antimicrobial activity when compared with standard drugs. There is no such a thing as completely safe drug. Drugs are powerful tools, which alter physiological processes for the better or for the worse. A society that wishes to benefit from them will not achieve all the benefits open to it, if it ignores the fact and seeks for impossible standards or harmlessness.

KEYWORDS: As expected, benzimidazole derivatives exhibited significant antimicrobial activity when compared with standard drugs.

INTRODUCTION

Many important biochemical compounds and drugs of natural origin contain heterocyclic ring structures. Among these e.g. Carbohydrates, Proteins, essential amino acids, vitamins, alkaloids, glycosides etc. the presence of heterocyclic structures in such diverse types of

compounds is strongly indicative of the diverse types of the pharmacological activity and recognition of this is reflected in efforts to find useful synthetic drugs.

An important feature of modern pharmaceutical chemistry is the introduction of more refined and sensitive methods of physicochemical analysis such as Spectroscopy and chromatography that enable one to assay the quality and quantity of the drugs more accurately with the smallest consumption of the analyze, reagent and time.

Pharmaceutical chemistry is a science that makes use of general laws of chemistry to study drugs i.e. their preparation, chemical nature, composition, structure, influence on an organism and studies of the physical and chemical properties of drugs, the methods of quality control and the conditions of their storage. Pharmaceutical chemistry occupies the most important place among the related sciences e.g. Drug technology, Toxicological chemistry, Pharmacognosy, and the organization of the pharmacy.

At the same time pharmaceutical chemistry being a specialized science depends on their chemical (inorganic, organic, analytical, physical and colloidal chemistry) and also on medico biological (pharmacology, physiology, biological chemistry) disciplines.

Pharmaceutical chemistry began in 16th century and gave birth to Medicinal chemistry in the second half of the 17th century. Pharmacists played a major role in the birth and development of pharmaceutical chemistry. Medicinal chemistry, according to Berger, "tried to be based on the ever increasing hope that biochemical rationale for drug discovery may be found". The first use of synthetic molecules for interference with the life process was probably, when chloroform and ether were introduced for Anesthesia in the first half of 19th century. Phenacetin probably the first drug to be designed as results of knowledge of biochemical transformations.

Chemotherapeutic agents be considered, combine with receptor areas of the cells by ordinary chemical reactions, although modified to include more types of bond formations. Ehrlich concluded that drug resistance developed, when the drug was no longer absorbed by the parasite. His ideas were thus supported experimental facts. Chemical modifications of drug molecules to locate the number of series having optimal effects, and will probably continued to be a factors necessary to drug discovery to establish the effect of drug molecules the new

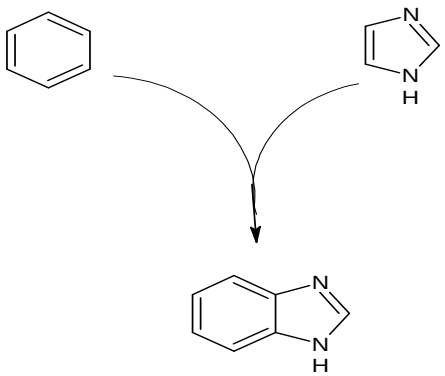
invention in physicochemical directions such as X-ray analysis, UV, IR, and ^{1}H NMR are immensely helpful for medicinal chemist.

In the biochemical view the knowledge of drug receptor interactions, pharmacokinetic advancements in Enzymology, have immensely helped medicinal chemist in hypothesizing the correct mechanism of action of drug molecule.

The approach to practice medicinal chemistry has developed from an empirical one involving organic synthesis of new compounds, based largely on modification of structures of known activity. According to Manfred Wolf, present development of medicinal chemistry has resistance, stating that "underlying the new age in foundation that includes explosive development of molecular biology since 1960, the advances in physical chemistry and physical organic chemistry has made possible by high speed computers and new powerful analytical methods.

Numerous heterocyclic compounds, cyclic anhydrates, cyclic imides, cyclic acetals of dihydroxy alcohols, the solvents, dioxanes and tetrahydrofuran, in all of these, the chemistry is essentially that of their open chain analogues. Heterocyclic intermediates are being used more and more in synthesis as protecting groups, readily generated, and readily removed.

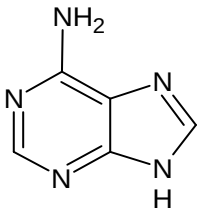
Benzimidazoles are a class of heterocyclic, aromatic chemical compounds which share a fundamental structural characteristic of six membered benzene fused to five membered imidazole.



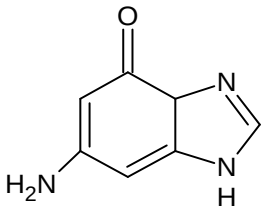
The benzimidazole skeleton is the fusion of benzene (top left) and imidazole (top right)

The basic ‘6+5' heterocyclic structure is shaired by another class of chemical compounds, like purines. Among the members of this group are several very well known and important

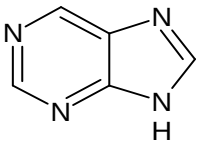
biomolecules, such as Adenine and Guanine, two of the four nucleic acid, bases, uric acid, and caffeine.[1]



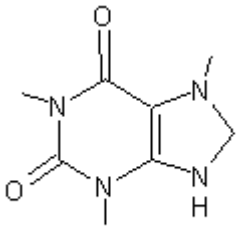
Adenine



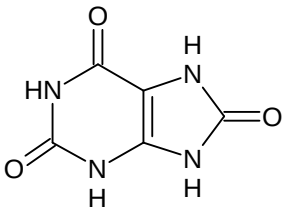
Guanine



Purine



Caffeine



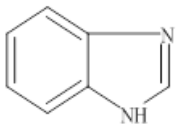
Uric acid

Purines which include some of the most well known biomolecules, Share the he '6+5' heterocyclic structure of benzimidazole.

BENZIMIDAZOLES

Benzimidazole[2]

The benzimidazole contains a phenyl ring fused to an imidazole ring, as indicated in the structure of benzimidazole (I).

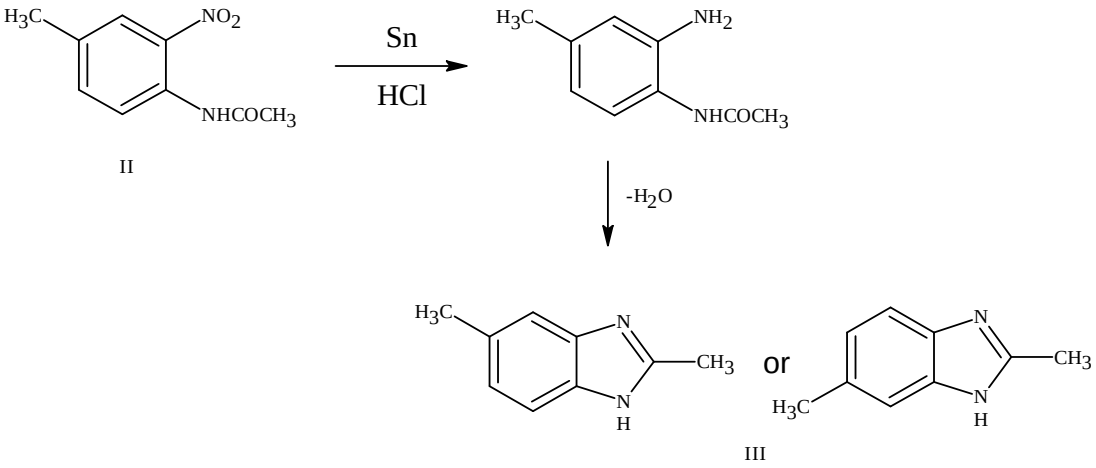


I

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.

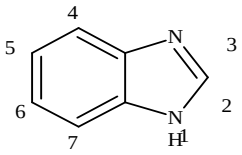
structure of vitamin B_{12}.

Historically, the first benzimidazole was prepared in 1872 by Hoebrecker, who obtained 2, 5 or 2, 6-dimethyl benzimidazole (III) by the reduction of 2-nitro-4-methyl acetanilide (II).

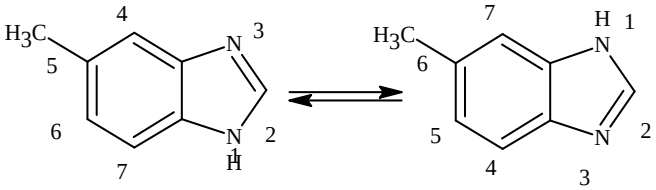


II III

The numbering system for the benzimidazole is as follows.



The benzimidazoles are also known as benziminazolones or benzoglyoxalines. They have been named also as derivatives of o-phenylene diamines. Benzimidazole which contain a hydrogen atom attached to nitrogen in the 1-position readily tautomerise, this may be depicted as follows.[3]



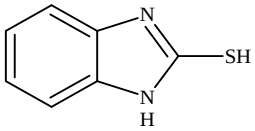
IV V

The benzimidazoles in fact, may be considered as a cyclic analogue of the amidines. Because of this tautomerism in benzimidazoles certain derivatives which appear at first to be isomers are in reality tautomers, although two non equivalent structures can be written, only one compound is known. This may be illustrated with 5 or 6-methyl benzimidazole. Thus, 5-methyl benzimidazole (IV) is a tautomer of 6-methyl benzimidazole (V) and both compounds represents the same compound.

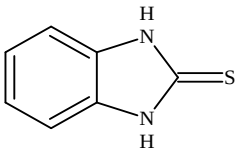
Benzimidazoline-2-thiones[4]

A number of benzimidazoline 2-thiones have been synthesized by the general method described by Van allan and Deacon. The 2-mercapto benzimidazole (VI) and benzimidazole -

2-thione (VII) are depicted as under.

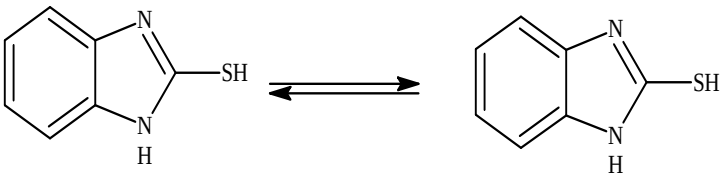


VI



VII

2-mercapto-benzimidazole which contain a hydrogen atom attached to nitrogen in the 1-position readily tautomerise. This may be depicted as follows.



Physical Properties of Benzimidazoles

i) Benzimidazoles have high melting points. The introduction of substituentat 1 position lowers the melting point.

ii) Benzimidazoles are usually soluble in polar solvents and sparingly soluble in non polar solvents.

iii) Benzimidazoles are weakly basic, being somewhat less basic than imidazole.

iv) Benzimidazoles are also sufficiently acidic to be generally soluble in aqueous alkali and form N-metallic compounds. The acidic properties of benzimidazoles, like those of imidazole, seem to be due to stabilization of the ion by resonance.

v) The pKa value of benzimidazoles, pKa = 5.30 for 2-methyl benzimidazole and pKa = 12.33 for 2-amino benzimidazole.

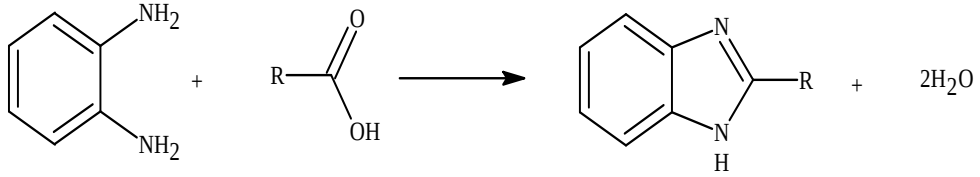
A) Synthesis of benzimidazole[2]

1) Reaction with carboxylic acids

a) Monobasic acids

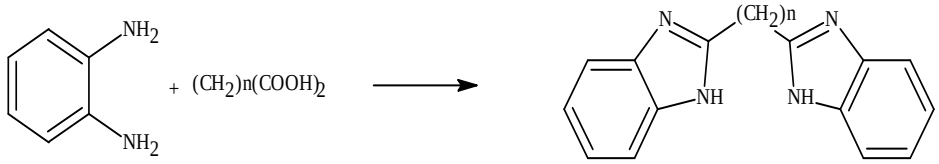
O-phenylenediamines react readily with most carboxylic acids to give 2-substituted benzimidazoles, usually with very good yields. The reaction is carried out usually by heating the reactants together on a steam bath, by heating together under reflux or at an elevated temperature or by heating in a sealed tube.

Benzimidazole may be prepared in 83-85 % yield by using 90 % formic acid.[5]



b) Dibasic acids

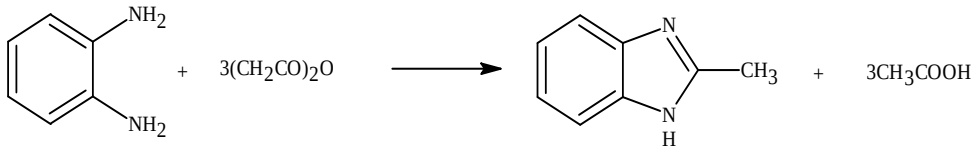
When dibasic acids are caused to react with o-phenylene- diamines the product formed depend on the mole ratio of the reactant and the experimental conditions. When two or more moles of o-phenylenediamines are heated with one mole of the dibasic acid, the products in most cases are bis benzimidazoles.[6]



2) By reaction with acid Anhydride

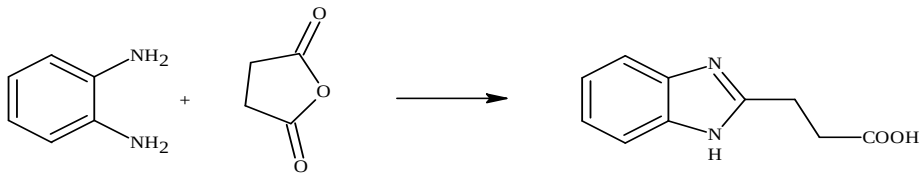
a) Anhydrides of monobasic acids

The reaction of acid anhydrides and o-phenylenediamines will lead to benzimidazole or N,N-diacylphenylenediamines depending on the condition employed. It was formerly thought that o-phenylenediamines yield benzimidazole with acids and diacyl derivatives with acid anhydrides. Practically the acid anhydride that has been used in the preparation of benzimidazole has been acetic anhydride. However mixed formic-acetic anhydride and benzoic anhydride have also been used successfully.[7] And the example is as under, o-phenylenediamine when heated under reflux for several hours with acetic anhydride are completely converted to 2-methyl benzimidazole.



b) Anhydrides of dibasic acid

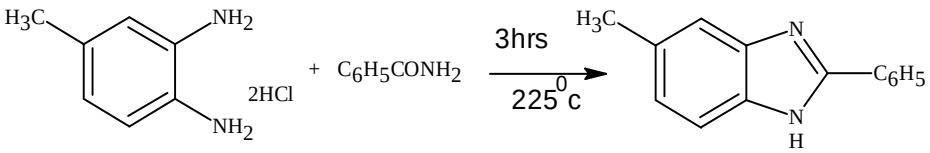
The anhydrides of dibasic acids reacts as monobasic acids for example succinic anhydride with o-phenylenediamine gives (2-benzimidazole) propionic acid and with phthalic anhydride gives *o*-(2-benzimidazole) benzoic acid.



3-(1*H*-benzimidazol-2-yl)propanoic acid

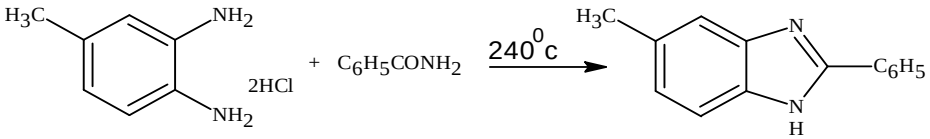
3) Reaction with esters

Von Neimentowski first investigated the reaction of esters and o-phenylenediamines to give benzimidazole. Equimolar amounts of 3, 4-diamino toluene dihydrochloride and ethylformate when heated in a sealed tube for 3 hrs, at 225^0 give 84% of 5 or 6-methyl benzimidazole hydrochloride.



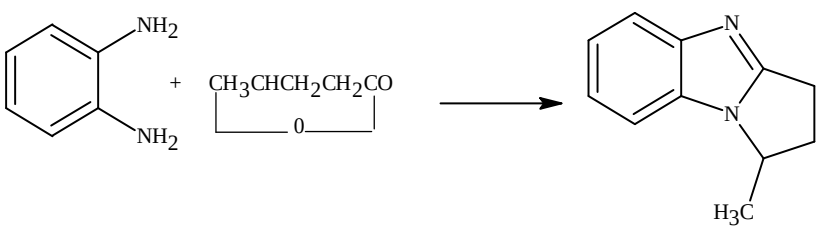
4) Reaction with amides

Relatively few amides have been used for the synthesis of benzimidazole. However good yields have been obtained in most cases. Equimolar amounts of 3, 4-diamino toluene dihydrochloride and benzamide when heated to 240-250^0C give an almost quantitative yield of 2-phenyl, 5-methyl benzimidazole.



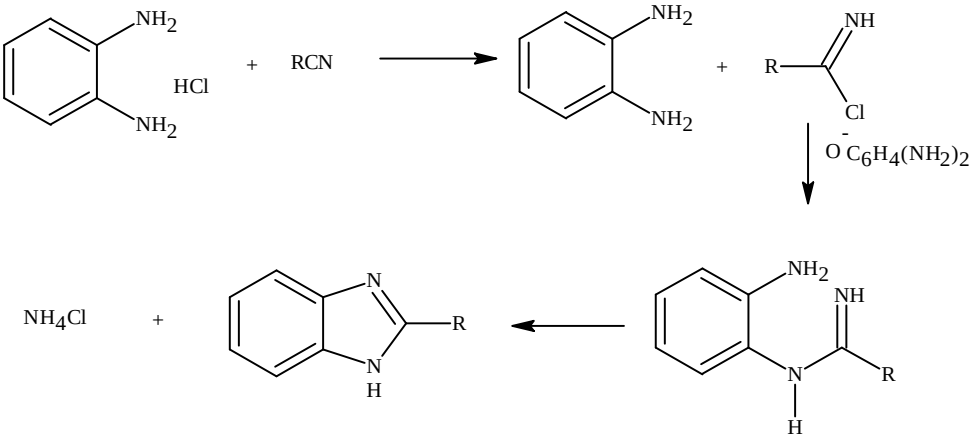
5) By reaction with lactones

The reaction of lactones with o-phenylenediamines was first studied by Bistrzycki and Schmutz, who investigated several lactones of alcohol acids and Phenol acids. Velerolactone when refluxed with o-phenylenediamine gives only a small yield of 1, 2-(1'- methyl tri methylene) benzimidazole.



6) By reaction with nitriles

Nitriles when heated with *o*-phenylenediamine hydrochloride give 2-substituted benzimidazoles. This reaction has been studied by Holljes and Wagner[8], who find that the reaction proceeds under acid condition and probably involves hydrogen ion catalysis. The mechanism of the reaction is as under.

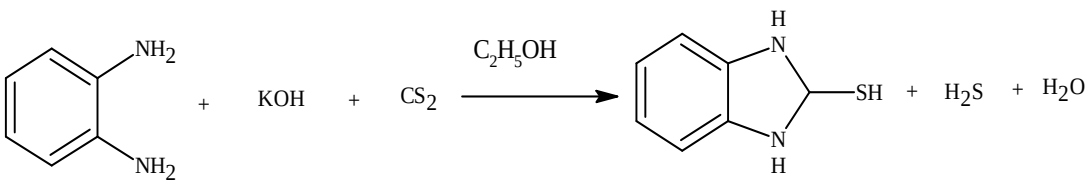


The reaction is carried out usually at about 200^{0}C at this temperature ammonium chloride will undergo decomposition to regenerate additional hydrogen chloride and cause the reaction to proceed further. The reaction proceeds further under anhydrous condition and is not due to the generation of acid or amide in situation. The first step in the reaction appears to be the rate determining step.

B) Synthesis of benzimidazoline 2-thione[9]

Preparation of 2-mercapto benzimidazole

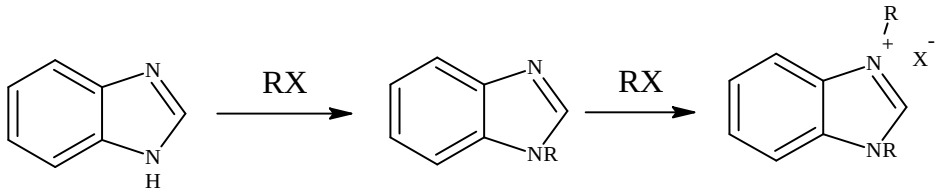
A mixture of o-phenylenediamine, potassium hydroxide and carbon disulfide reacted in presence of 95% ethanol and water in a round bottom flask heated under reflux for three hours. After 3 hrs Charcoal is added cautiously and the mixture is heated at the reflux for 10 minutes the charcoal is removed by filtration. The filtrate is heated to 60-70^{0}C, warm water is added, and then acidified with acetic acid. The product separated as glistening white crystals, and the mixture is placed in a refrigerator for three hours to complete the crystallization. The product is recrystallised from ethanol. m.p. is 300-305°C.



I) Reactions of Benzimidazoles

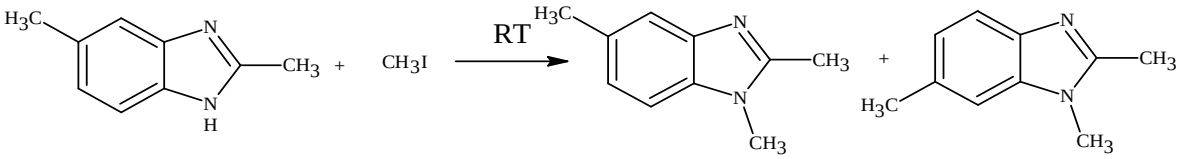
1) Alkylation

Benzimidazoles upon alkylation with alkyl halides yields 1-alkyl benzimidazoles and under more vigorous conditions 1, 3-diallylbenzimidazolium halides.



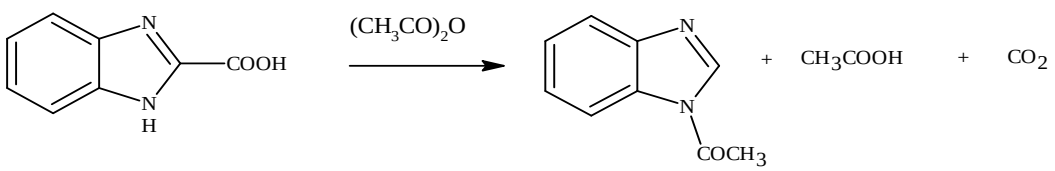
The alkylation of benzimidazoles has been extensively studied especially by O. Fisher the alkylation is carried out by various alkyl and aryl alkyl groups the reaction is carried out usually by heating the benzimidazole with an excess of alkyl halide in methanol under pressure at a temperature of about 110-150^{0}C. Alkyl iodides are used usually and in some cases periodides are obtained as by product or as exclusive product.

When alkylation reaction is carried out at lower temperature with one equivalent of alkyl halide 1-alkyl benzimidazole may be the main product. Thus equimolar amounts of benzimidazole and methyl iodide in methanol at 90-100^{0}C to give 1-methyl benzimidazole.



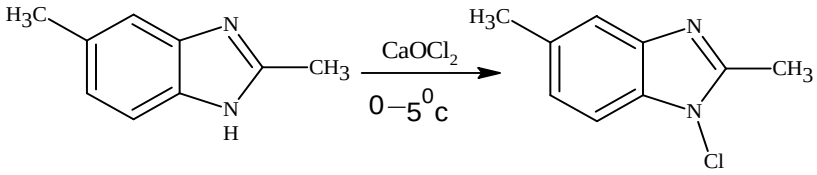
2) Acylation

N-acyl benzimidazoles may be prepared by the action of acid chlorides or anhydrides on benzimidazoles. The reaction is usually carried out in the absence of water. In the presence of water and especially in the alkaline solution cleavage of imidazole ring may occur. 1-acetyl benzimidazole has been prepared by heating 2-benzimidazole carboxylic acid with acetic anhydride decarboxylation occurs and forms the product.

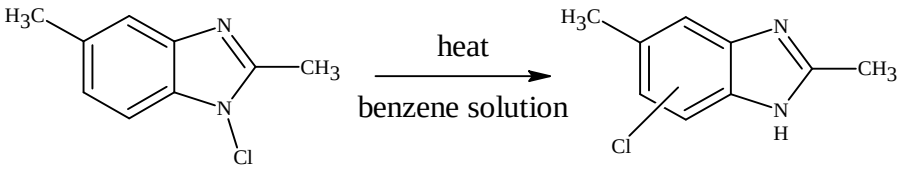


3) Halogenations

When 2, 5 or 2, 6-dimethyl benzimidazole in an aqueous acid solution is treated with the saturated solution of bleaching powder at 0-5^{0}C 1-chloro 2, 5- or 2, 6-dimethyl benzimidazole is obtained.



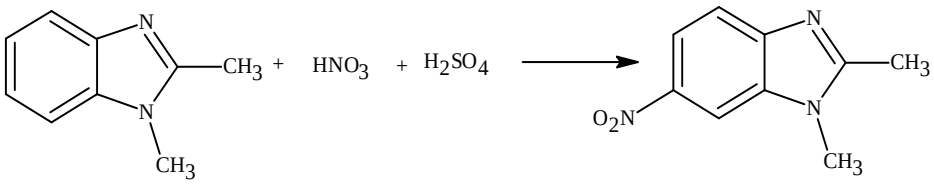
The N-chloro compound loses chlorine quite readily, even at relatively low temperature. When heated under reflux in benzene solution a rearrangement of the chlorine atom to the benzene ring takes place. The N-chloro derivative of this compound may be prepared by treatment again with bleaching powder.



Treatment of the later compound by refluxing in benzene solution rearranges the N-chlorine atom again into the ring. This process may be repeated until the totally chlorinated compound (1, 4, 5, 6-tetrachloro-2, 5-dimethyl benzimidazole) is obtained.

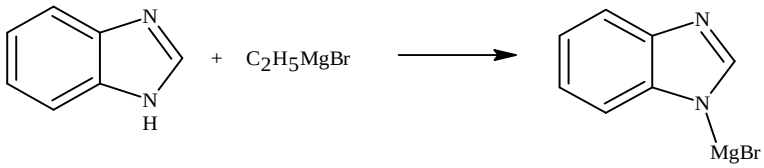
4) Nitration

The nitration of benzimidazole proceeds readily. In most cases nitration appears to take place preferentially in the 5 or 6 places. However the nitro group may also enter 4 or 7-position, especially if 5 or 6 positions are Nitrobenzimidazoles that have been obtained by the nitration of benzimidazoles.

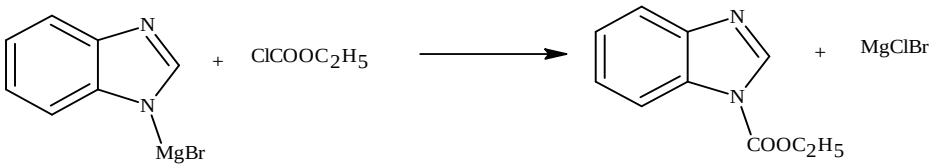


5) The action of Grignard reagents on benzimidazoles

Grignard reagent reacts with the active hydrogen in the 1-position of benzimidazole.

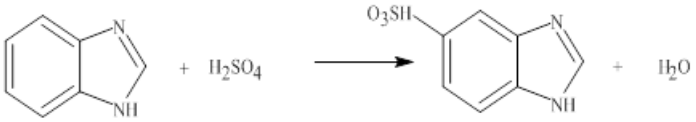


Benzimidazole-1-magnesium bromide reacts with aliphatic acid chloride or anhydrides to yield 1-acyl benzimidazole with ethylchloroformate, 1-carbeothoxy benzimidazole is obtained.



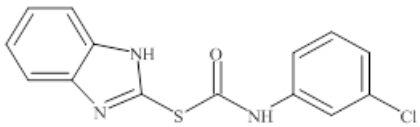
6) Miscellaneous reaction

Sulfonated benzimidazoles are obtained by the sulfonation of benzimidazoles with either sulfuric acid or chlorosulfonic acid. Treatment of benzimidazole with concentrated sulfuric acid gives 5-benzimidazole sulfonic acid.



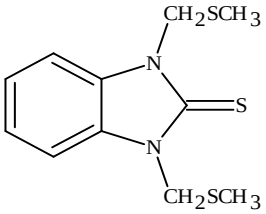
II) Reactions of benzimidazoline 2- thione

The majority of data on benzimidazoline 2- thione relates to S-alkylation and closely related processes are the synthesis of 2-thiocyanatobenzimidazoles from the reaction of benzimidazoline 2-thione with cyanogen chloride or bromide and 2-benzimidazolyl thiocarbamates (VIII) from addition of the 2-thione to aryl isocyanates.

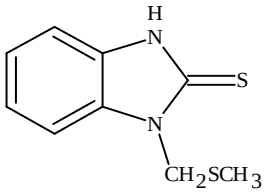


VIII

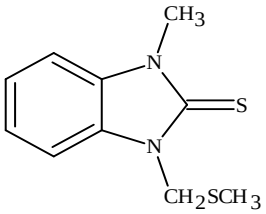
Other routine procedures are the oxidation of 2-thiones to bis benzimidazolyl- disulfidestand benzimidazole 2-sulfonic acids by hydrogenperoxide. The varieties of compounds are obtained IX, X, XI, XII.



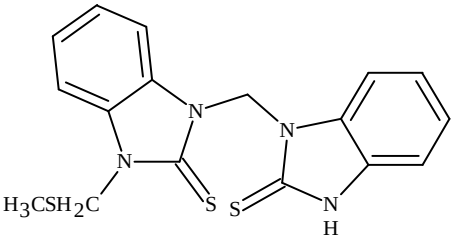
IX



X

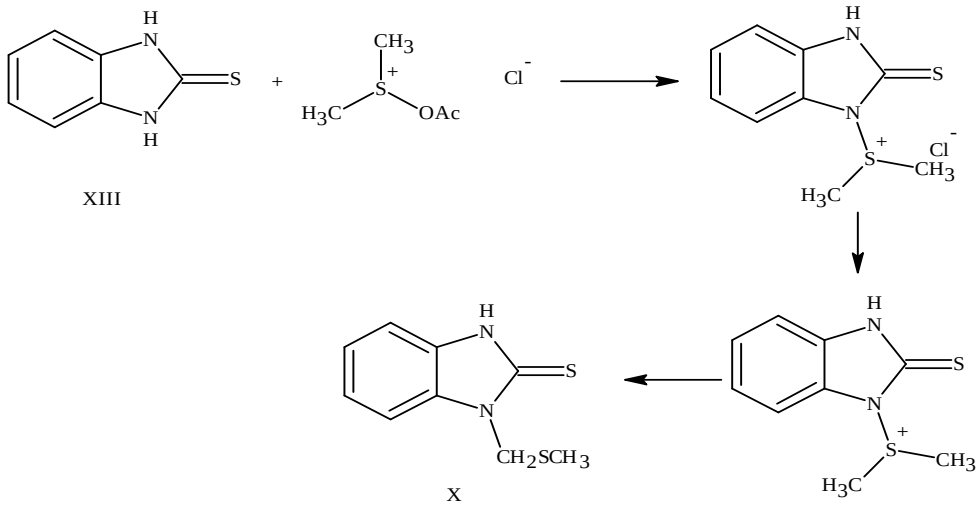


XI



XII

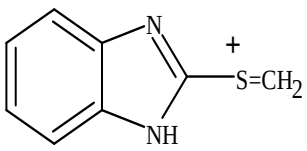
When benzimidazoline 2-thione (X) is allowed to react with a mixture of dimethylsulfoxide and acetyl chloride at 50-60^{0}C the formation of these products can be satisfactorily rationalized in term of displacement reactions by the thione (XIII) intermediate sulfonium acetate.



XIII

X

Interestingly if this reaction is carried out below 30^{0}C the reaction product includes the compound and also the novel 2-(methylene sulfonium) benzimidazolide in 20% yield (XIV).



Reported Pharmacological Activities of Benzimidazole

Benzimidazole and various derivatives of benzimidazoles possess a variety of pharmacological activities. Benzimidazole, 2-methyl benzimidazole, benzimidazole-2- thione studied pharmacologically by Aurmann. The few selected activity reported as under.

1. Large number of benzimidazoles are reported to antimicrobial activity of some novel substituted benzimidazole derivatives having potent activity against MRSA.[10]
2. The number of benzimidazoles related to *in vitro* antiprotozoal activity of benzimidazole-pentamidin hybrids.[11]
3. Benzimidazole shows antibacterial and antifungal activities of electrone-rich olefins derived benzimidazole compounds.[12]
4. Benzimidazole shows anti-inflammatory, analgesic and kinase (CDK-1,CDK-5 and GSK-3) inhibition activity evaluation of benzimidazole/benzoxazole derivatives and some Schiff's bases.[13]
5. Clubbed [1,2,3] triazoles by fluorine benzimidazole: a novel approach to H37Rv inhibitors as a potential treatment for tuberculosis.[14]
6. Discovery and sar of 2-(1-propylpiperidin-4-yl)- 1H- benzimidazole- 4 - carboxamide a potent inhibitor of poly (ADP-ribose) polymerase (PARP) for the treatment of cancer.[15]

2. OBJECTIVES

The discovery and development of pharmacologically active molecules has been guided not only by classical medicinal chemistry but also by the use of sophisticated mechanistic approaches and biochemical assay. The reviews clearly emphasize the importance of Heterocycles in naturally occurring as well as synthetic agents and does an important class itself possess diversified pharmacological actions such as antimicrobial, antiprotozoal, antimalarial, and antiallergic etc. This point encouraged further investigation in the field. The logic supporting the work presented in this dissertation was formulated, bearing in mind that the biological activities of known moieties and attempting certain structural modification or adaptation in light of the recent trends in drug research incorporating newly emerged pharmacophores on existing moiety.

Exclusive literature survey of benzimidazole revealed that it has diversified activities such as potent activity against HIV-1[16], respiratory syncytial virus fusion inhibitors[17], treatment of ischemic injuries[18], antineoplastic activity[19], HIV-1 integrase inhibitors.[20] The development of resistance to current antibacterial therapy continues to stimulate the search

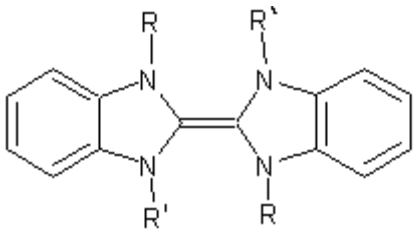
for more effective agents. The increasing clinical importance of drug resistant and bacterial pathogens has lent additional urgency to microbiological research and development of novel biologically active compounds. Hence in the present study we plan synthesize some novel benzimidazoles with good activity and less toxic effects.

Hence we planned in the present objectives of the study will be,

1. Development of the synthetic method for the synthesis of the titled 2-Substituted Benzimidazoles compounds.
2. Chemical characterization of the newly synthesized compound by IR, ^{1}H NMR and Mass spectral data.
3. Screening for Antimicrobial activity.

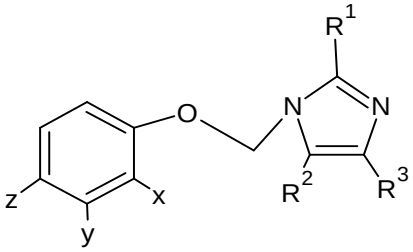
4. REVIEW OF LITERATURE

1. Hasan Kucukbay et.al.,[21] synthesis, antibacterial and antifungal activities of electron-rich olefins derived benzimidazole compounds.



R, R'= Me, Et, CH_2Ph

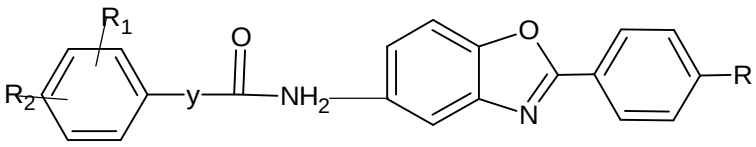
2. A.Khalafi Nezhad et.al.,[22] design, synthesis, antibacterial and QSAR studies of benzimidazole & imidazole chloroaryloxyalkyl Derivative.



X, Y, Z = Me, Cl, H, Ph

R^1,R^2,R^3 = Me, NO_2, H

3. Ilkay Oren et.al.,[23] synthesis and antimicrobial activity of some novel 2, 5 and /or 6-substituted benzoxazole and benzimidazole derivative.



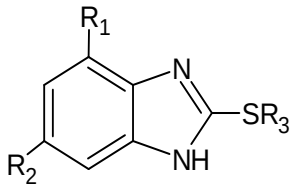
Y = - CH_2 –

R = -C_2H_5

R_1= -H, -F, -Cl, -Br

$R_{2=}$ -H, -Cl, -OCH_3, -CH_3

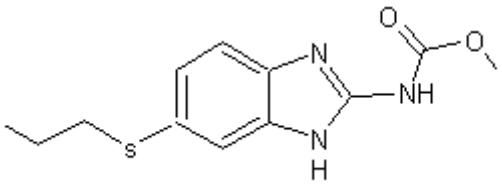
4. Mariola Andrzejewska et.al.,[24] synthesis and antiprotozoal and antibacterial activity of 5-substituted 4, 6 dibromo and 4, 6 dichloromercapto benzimidazole.



R_1, R_2 = Cl

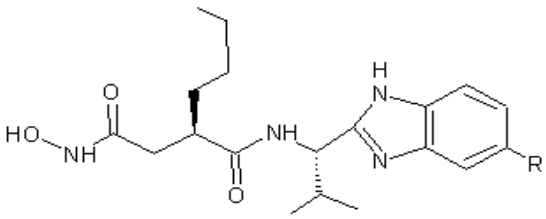
R_3 = CH_2CH_2OH

5. Ramanatham Vinodkumar et.al.,[25] synthesis, anti-bacterial, anti-asthmatic and anti-diabetic activities of novel N-substituted-2-(4-phenylethynyl-phenyl-1H benzimidazoles and N-substituted2[4-(4,4-Dimethyl thiochroman-6-ylethynyl)-phenyl]-1H-benzimidazole.

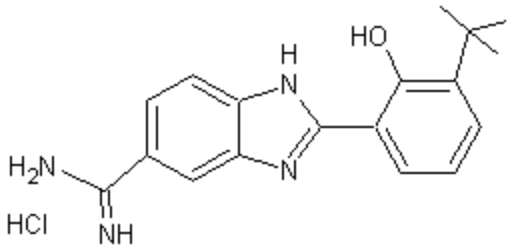


Albendazole

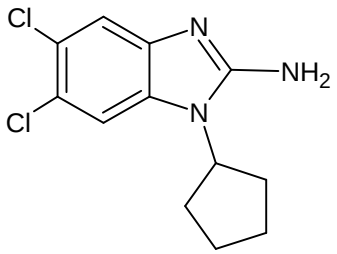
6. Datong Zhang et.al.,[26] design, the synthesis and antibacterial activity of novel actinonine derivatives containing benzimidazole heterocycles.



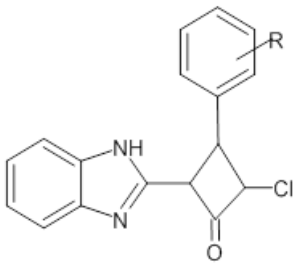
7. Hakan Goker et.al.,[27] synthesis and potent antibacterial activity against MRSA of some novel 1,2-disubstituted-1-H-benzimidazole-N- alkylated-5-carboxamidines.



8. Meral Tuncbilek et.al.,[28] synthesis and in vitro antimicrobial activity of some novel substituted benzimidazole derivatives having potent activity.



9. Malleshappa Noolvi et.al.,[29] synthesis, antimicrobial and cytotoxic activity of novel azetidine-2-one derivatives of 1H-benzimidazole.



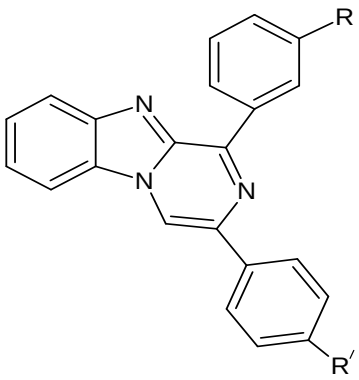
R = 4-Chloro

2-Nitro

3-Nitro

4-Nitro

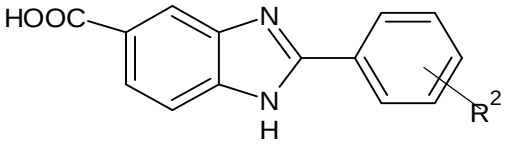
10. Seref Demirayak et.al.,[30] microwave supported synthesis of some novel 1, 3-diarylpyrazino [1,2a] benzimidazole derivatives and investigation of their anticancer activities.



R= H, OCH_3

R'= H, CH_3

11. Chao cong et.al.,[31] synthesis and antibacterial activity of novel 4-O-benzimidazolyl clarithromycin derivatives.

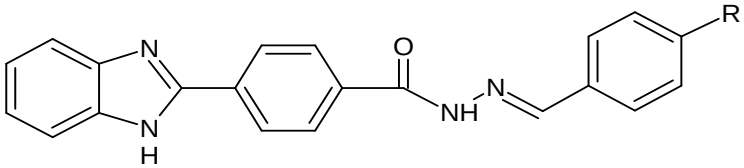


R^2 = Hydrogen

4-Methyl

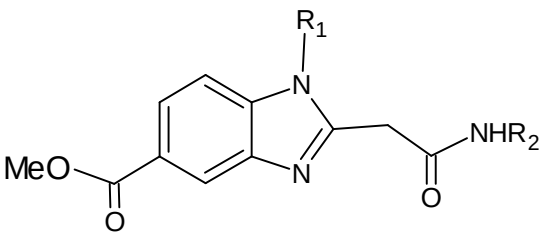
2-Methoxyl

12. Yusuf Ozkay et.al.,[32] antimicrobial activity and a SAR study of some novel benzimidazole derivatives bearing hydrazone moiety.



R = -H, -OH, N $(CH_3)_2$, -Cl, -Br, -F, $-CH_3$

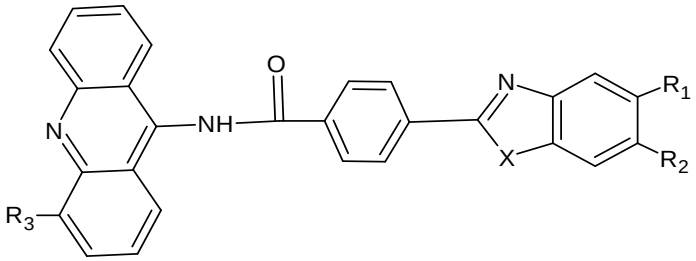
13. Seckin Ozden et.al.,[33] synthesis and potent antimicrobial activity of some novel methyl or ethyl 1H-benzimidazole-5-carboxylates derivatives carrying amide or amidine groups.



R_1 = H, Ethyl, Methyl, Cyclopropyl, Benzyl

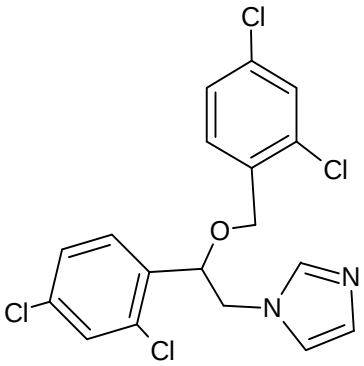
R_2 = Isopropyl, n-butyl, 3-diethyl aminopropyl,
3-pyridilmethyl, 3-dimethyl aminopropyl.

14. Sham M. Sondhi et.al.,[34] synthesis, anti-inflammatory, analgesic and kinase (CDK-1, CDK-5, and GSK-3) inhibition activity evaluation of benzimidazole /benzoxazole derivatives and some schiffs bases.

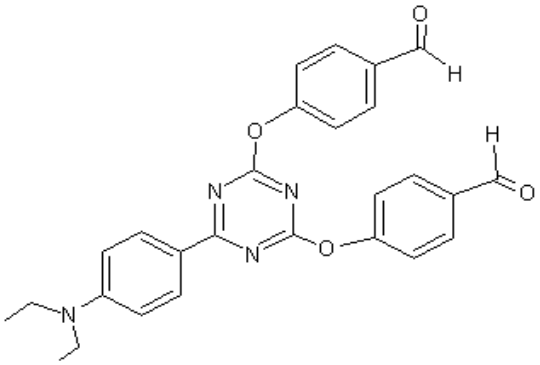


R_1	R_2	R_3	X
Cl	H	H	NH
NO_2	H	H	NH
NO_2	H	OCH_3	NH

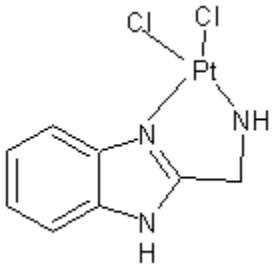
15. Ozden Ozel Guven et.al.,[35] synthesis and antimicrobial activity of some novel phenyl and benzimidazole substituted benzyl ethers.



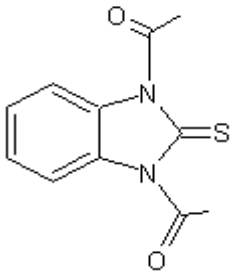
16. Vikas S. Padalkar et.al.,[36] synthesis of novel dipodal-benzimidazole, benzoxazole and benzothiazole from cyanuric chloride: structural, photophysical and antimicrobial studies.



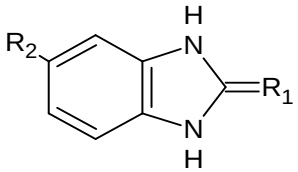
17. Fatma Gumus et.al.,[37] synthesis, cytotoxic activity on MCF-7 cell line and mutagenic activity of platinum (II) complexes with 2-substituted benzimidazole ligands.



18. Stephan Braun et.al.,[38] design of benzimidazole and benzoxazole-2-thione derivatives as inhibitors of bacterial hyaluronan lyase.

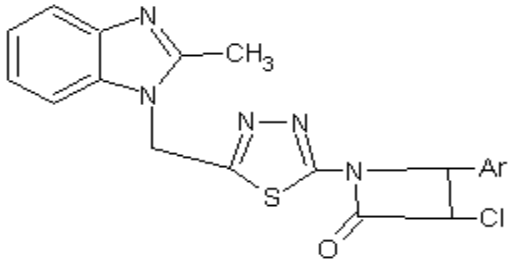


19. Jaime perez-villanueva et.al.,[39] comparative molecular field analysis (CoMFA) comparative molecular similarity indices analysis (CoMSIA) of some benzimidazole derivatives with trichomonicidal activity.

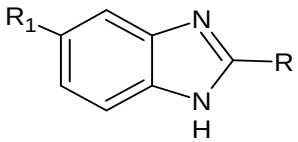


R_1 = SH, NH_2

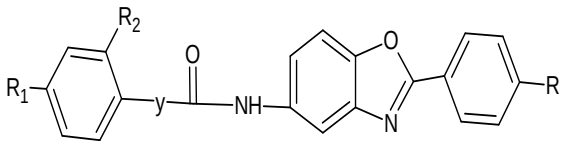
20. K.F.Ansari et.al.,[40] synthesis and evaluation of some new benzimidazole derivatives as potential antimicrobial agents.



21. Shweta sharma et.al.,[41] convenient one-pot synthesis of novel 2-substituted benzimidazoles tetrahydrobenzimidazoles and imidazoles and evaluation of their in vitro antibacterial and antifungal activities.



22. Ozlem Temiz Arpaci et.al.,[42] synthesis and antimicrobial activity of some novel N-[2-(p-substitutedphenyl)–5-benzoxazolyl]–cyclohexylcarboxamide, cyclo- hexyl acetamide and cyclohexyl propionamide derivatives.



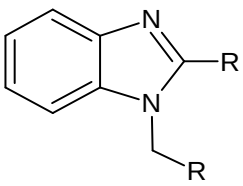
Y= -CH_2, -SCH_2-, OCH_2-,

R= -H, -C_2H_5, F

R_1= -H, -F, -Cl, -Br

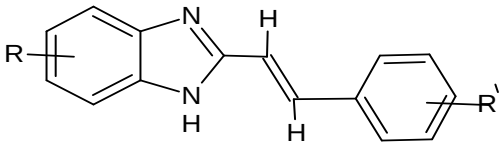
R_2= -H, -Cl, -OCH_3

23. M.P.Kaushik et.al.,[43] exploration of in vitro time point quantitative evaluation of newly synthesized benzimidazole and benzothiazole derivatives as potential antibacterial agents.



R = C_6H_5, 4-MeC_6H_4, 4-$(Me)_2NC_6H_4$

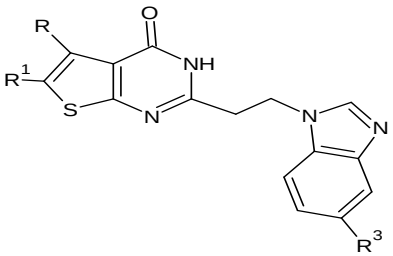
24. Kallappa M. Hosamani et.al.,[44] synthesis and evaluaton of invitro anti-microbial and anti-tubercular activity of 2-styryl benzimidazoles.



R = -NO_2

R' = H

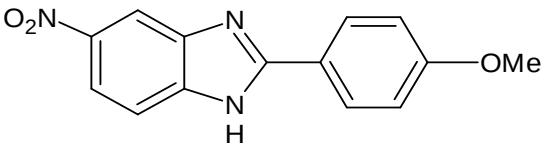
25. Anelia Ts. Mavrova et.al.,[45] synthesis, antitrichinnellosis and antiprotozoal activity of some novel thieno [2, 3-d] pyrimidine-4(3H)-ones containing benzimidazole ring.



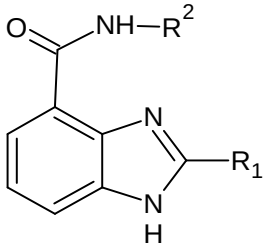
R= R^1 = $(CH_2)_4$

R^3 = H

26. Gabriel Navarrete-Vazquez et.al.,[46] relaxant activity of 2-(substituted phenyl)-1H-benzimidazoles on isolated rat aortic rings design and synthesis of 5-nitro derivatives.



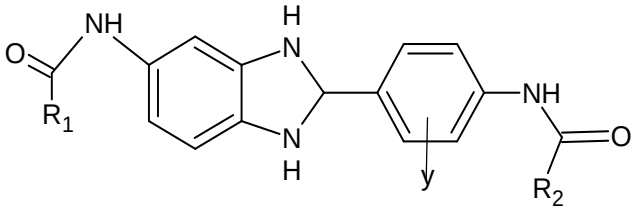
27. Yue Wang et.al.,[47] inhibitory properties of 2-substituent-1H-benzimidazole-4-carboxamide derivatives against enteroviruses.



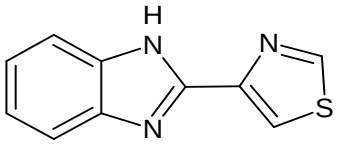
R_1 = Aryl and heteroaryl

R_2 = Alkyl, aryl and heteroaryl

28. Mark L. Richards et.al.,[48] substituted 2-phenyl-benzimidazole derivatives: novel compounds that suppress key markers of allergy.



29. Ramchandra Bhimrao Mane et.al.,[49] synthesis and antibacterial activity of some novel 2-(6-fluorochroman-2-yl)-1-alkyl/acyl/aroyl-1H-benzimidazoles.



4. METHODOLOGY

Chemicals and Reagents

The chemicals and reagents used in the present project were of AR grade and LR grade, purchased from Lancaster, sigma, Qualigens NR Chem., Rolex, S.D.Fine Chem. Ltd., Merck, Loba and Hi-media.

Table No: 4.0.

List of Chemicals.

O-phenylenediamine	4-Methoxy benzaldehyde
Salicylic acid	P-Chlorobenzaldehyde
Benzoic acid	M-Chlorobenzaldehyde
Cinnamic acid	4-Methyl benzaldehyde
Pthalic acid	4-Nitro-o-phenylenediamine
Hydrochloric acid	Benzaldehyde
Sodium hydroxide	Ethyl acetate
Ethanol	Ammonia
Chloroform	

Analytical Techniques

1. Physical data

Melting point of the synthesized compounds were determined using Thiel's melting point apparatus and were found uncorrected.

2. Thin Layer Chromatography (TLC)

Purity of the compounds was checked by thin layer chromatography using silica gel G as

stationary phase and various combinations of chloroform and methanol are used as mobile phase. The spot resolved were visualized as brown coloured spots by using iodine chamber.

Instrumentation

The techniques employed for the characterization of the synthesized compounds were IR spectra, ^{1}H NMR, and Mass spectra.

1. Infrared spectra

The IR spectra of the synthesized compounds were recorded using dry KBr pellets in range of 4000-400 cm^{-1} on a SHIMADZU α-Fourier transform IR spectrometer at Poona College of pharmacy, Pune and frequencies were recorded in wave numbers.

2. ^{1}H NMR magnetic resonance spectra

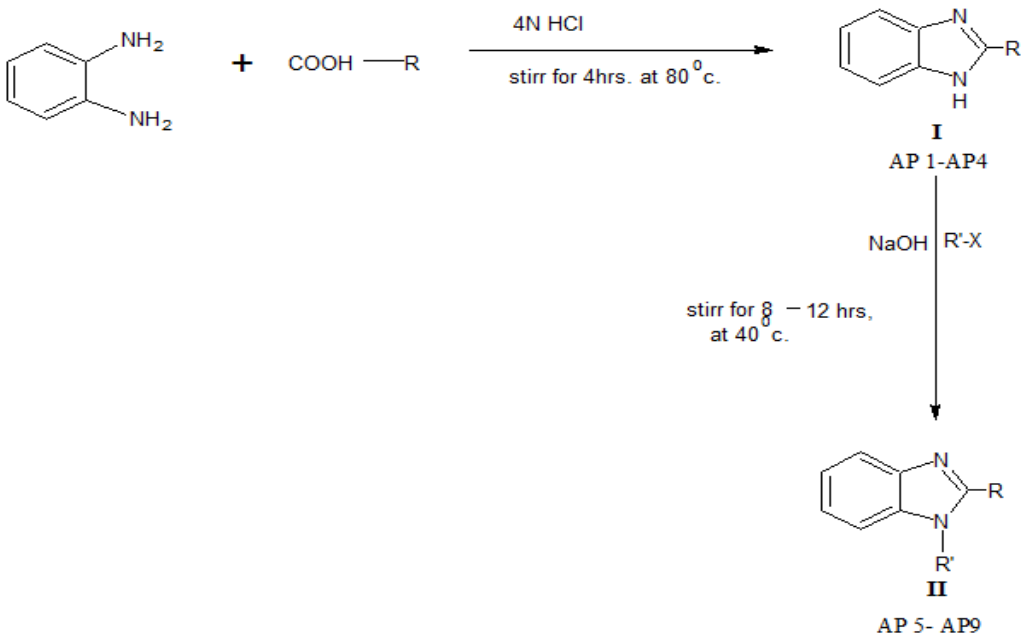
^{1}H NMR spectra were recorded in Varian-NMR-mercury 300 using cdc13 as solvent at Poona College of pharmacy, pune.

3. Mass spectra

Mass spectra were done by LC-MS technique at Oxygen healthcare research Pvt-Ltd Ahmedabad.

Experimental Part

SCHEME



R= $C_7H_6O_3$, $C_7H_6O_2$, $C_9H_8O_2$, $C_8H_6O_4$

R' = $C_8H_8O_2$, C_7H_5ClO, C_7H_5ClO, C_8H_8O, $C_6H_7N_3O_2$

PLAN OF WORK

A. Synthetic studies

I) Preparation of 2-substituted-benzimidazole-

1. Preparation of 2-(1*H*-benzimidazol-2-yl)phenol (AP-1)
2. Preparation of 2-phenyl-1*H*-benzimidazole (AP-2)
3. Preparation of 2-[(*E*)-2-phenylethenyl]-1*H*-benzimidazole (AP-3)
4. Preparation of 2-(1*H*-benzimidazol-2-yl)benzoic acid (AP-4)

II) Preparation of 1-benzyl 2-substituted benzimidazoles

5. 2-[1-(4-methoxybenzyl)-1*H*-benzimidazol-2-yl]phenol (AP-5)
6. 2-[1-(4-chlorobenzyl)-1*H*-benzimidazol-2-yl]phenol (AP-6)
7. 1-(3-chlorobenzyl)-2-phenyl-1*H*-benzimidazole (AP-7)
8. 1-(4-methylbenzyl)-2-phenyl-1*H*-benzimidazole (AP-8)
9. N^4-hydroxy-N^4-{2-[(*E*)-2-phenylethenyl]-1*H*-benzimidazol-1-yl}benzene-1,2,4-triamine (AP-9)

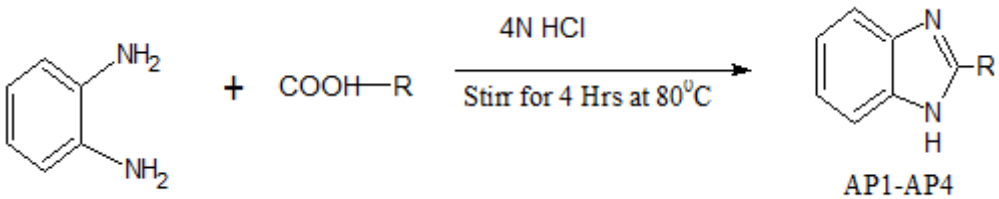
B. Physicochemical studies

1. Physical constant (Melting/Boiling point)
2. Thin layer chromatography
3. IR- Spectra (FT-IR spectral studies)
4. ^{1}H NMR studies
5. Mass spectroscopy

C. Biological studies

1. Antimicrobial activity

I. Synthesis of 2-substituted benzimidazole (AP1-AP4)

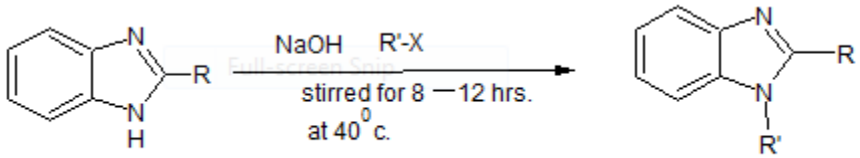


AP1-AP4

O-Phenylenediamine (4 gm, 0.04 mole) was condensed with carboxylic acids (0.03 mole)

like salicylic acid, Benzoic acid, Cinnamic acids, Pthalic acid in 50ml 4 N HCl. The reaction mixture was stirred for about 4 hr. with magnetic stirrer at 80^0c. The compounds were precipitated by adding concentrated ammonia solution filtered through suction pump and washed with cold water. Compounds were recrystalised from water and ethanol.

II. Synthesis of 1-benzyl 2-substituted benzimidazoles (AP5-AP9)



AP5-AP9

2-substituted benzimidazoles (0.02 mole) were treated with 4-methoxy benzaldehyde (2.5 gm, 0.02 mole) in the presence of little quantity of sodium hydroxide (2 gm) in 4N HCl. The reaction mixture was stirred for 8-12 hr. at 400 c. Excess solvent was removed by distillation and crude product was washed with water, extracted with ethyl acetate and finally recrystalized from water and ethanol.

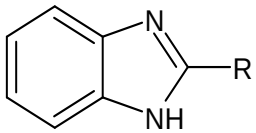
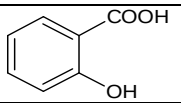
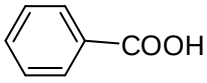
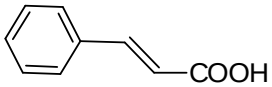
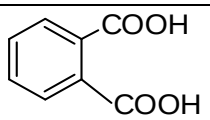


Table no.-4.1: Physical Characterization Data of 2-Substituted Benzimidazole.

Sr.No	Compound Code	R	Molecular formula	Molecular weight	% yield	M.P. (^{0}C)
1.	AP-1		$C_{13}H_{10}N_2O$	210.23	70	210-215
2.	AP-2		$C_{13}H_{10}N_2$	194.23	72	230-235
3.	AP-3		$C_{15}H_{12}N_2$	220.26	69	215-220
4.	AP-4		$C_{14}H_{10}N_20_2$	238.24	73	225-230

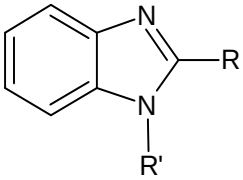
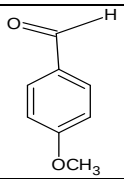
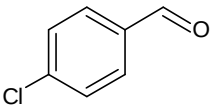
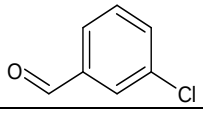
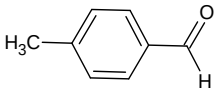
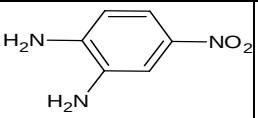
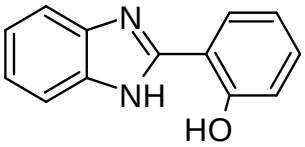


Table No.-4.2: Physicochemical Parameters of Benzimidazole Derivatives.

Sr.No	Compound Code	R'	Molecular formula	Molecular weight	% yield	M.P. (^{0}C)
1	AP-5		$C_{22}H_{21}N_2O_2$	345.41	62	210-215
2	AP-6		$C_{20}H_{15}ClN_2O$	334.79	75	205-210
3	AP-7		$C_{20}H_{15}ClN_2$	318.79	73	195-200
4	AP-8		$C_{21}H_{18}N_2$	298.38	62	200-205
5	AP-9		$C_{21}H_{19}N_5O$	357.40	72	190-195

SPECTRAL CHARACTERIZATION

Compound AP-1



2-(1*H*-benzimidazol-2-yl) phenol

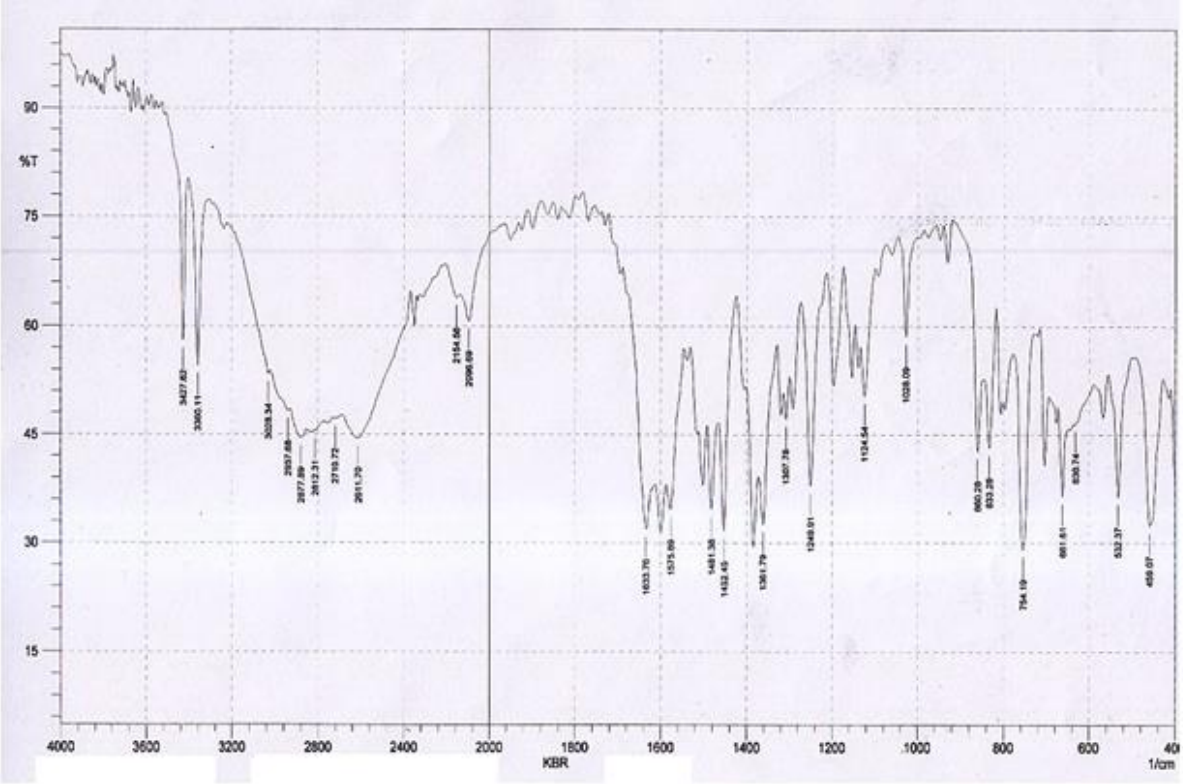


Fig 4.1: IR Spectra of AP-1.

Table No.-4.3.

Functional group assigned	Group frequency in Wave number (cm^{-1})
N-H stretching (Benzimidazole)	3427
O-H stretching (Phenolic)	3360
C-H stretching (Aromatic)	3028
C=N (Imines)	1633
C=C stretching (Aromatic)	1481
C-N (Amine)	1361

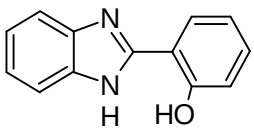
IR (KBr cm^{-1}): N-H stretching (Benzimidazole) 3427, O-H stretching (Phenolic) 3360, C-H stretching (Aromatic) 3028, C=N (Imines) 1633, C=C stretching (Aromatic) 1481, C-N (Amine) 1361. ^{1}H NMR (cdc 13 δ ppm): 1.3 Singlet (1H, NH), 6.8 Singlet (1H, OH), 6.9-7.89 Multiplet (8H, Ar-CH).

Mass spectra (m/z): Molecular ion peak appear at 209 as (M-1).

Molecular Formula: $C_{13}H_{10}N_2O$

Molecular Weight: 210.23

Melting Point: 210-215^{0}C.



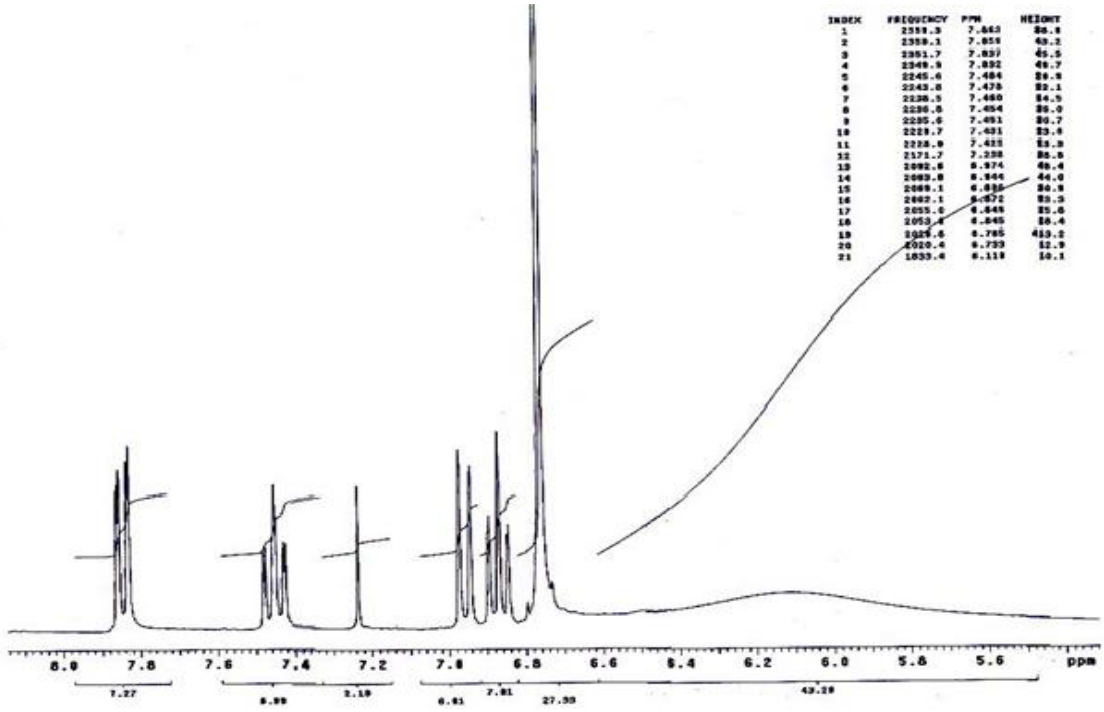
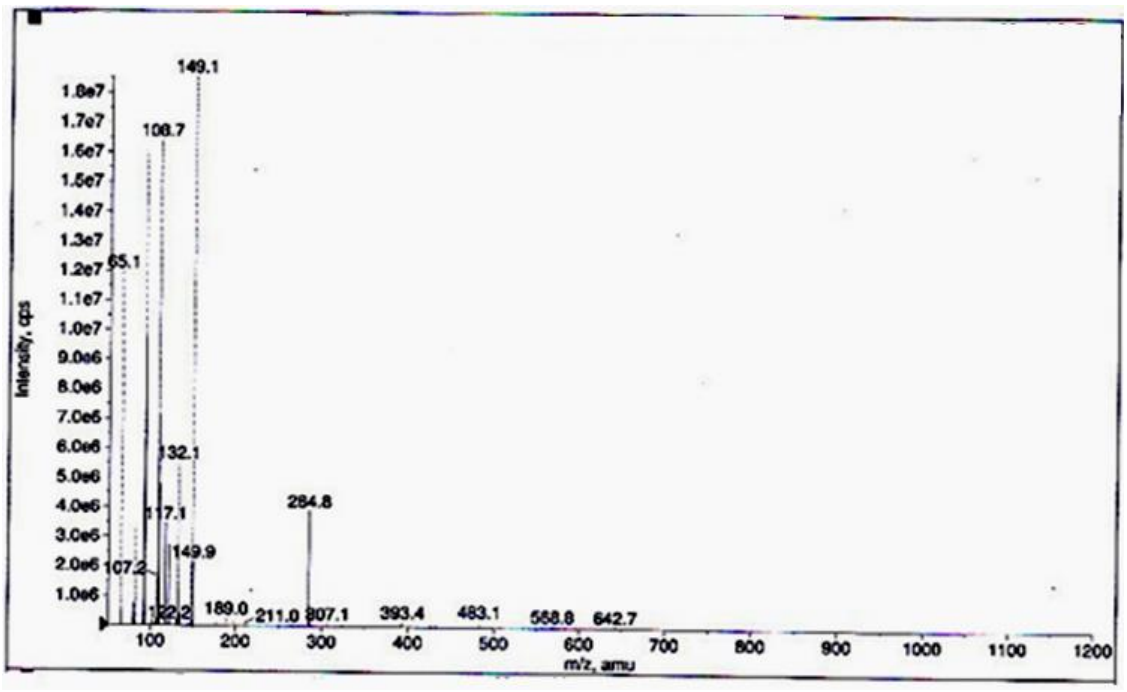


Fig 4.2: ^{1}H NMR Spectra of AP-1.

Table No.-4.4.

Sr.No.	Value (δ)	Nature of segment	Type
1	1.3	Singlet	1H, NH
2	6.8	Singlet	1H, OH
3	6.9-7.89	Multiplet	8H, Ar-CH



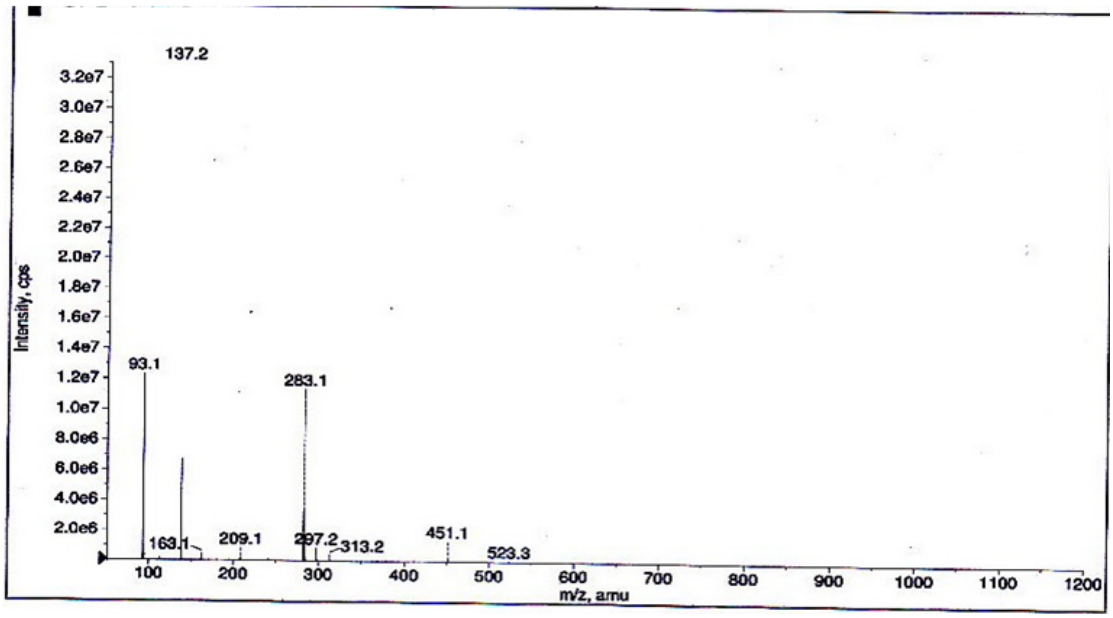
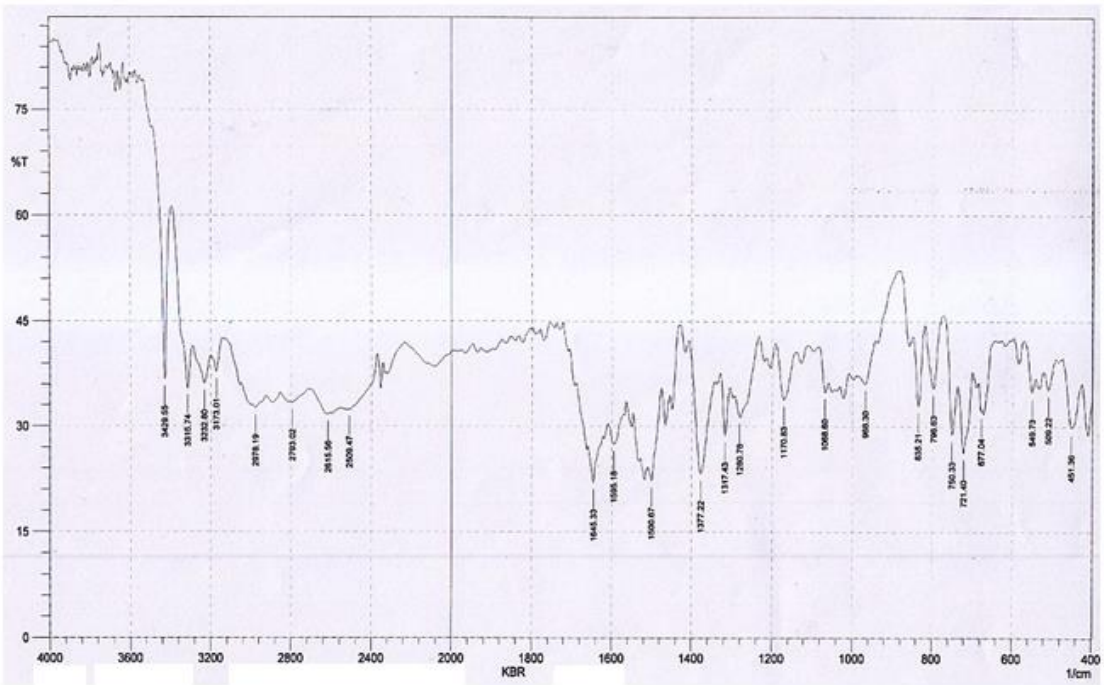


Fig 4.3: Mass Spectra of AP-1

Mass spectral data of compound (AP-1)

The molecular weight of the compound is 210 and the mass spectral data matching the same as 209 m/z it shows that the m-1 peak.



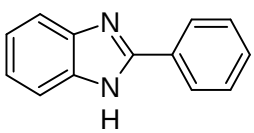
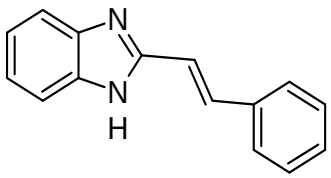


Fig 4.4: IR Spectra of AP-2.

TABLE No.-4.5.

Functional group assigned	Group frequency in Wave number (cm^{-1})
N-H stretching (Benzimidazole)	3429
C-H stretching (Aromatic)	3173
C=N stretching (Imines)	1645
C=C stretching (Aromatic)	1595
C-N stretching (Amine)	1377

Compound AP-3



2-[(*E*)-2-phenylethenyl]-1*H*-benzimidazole

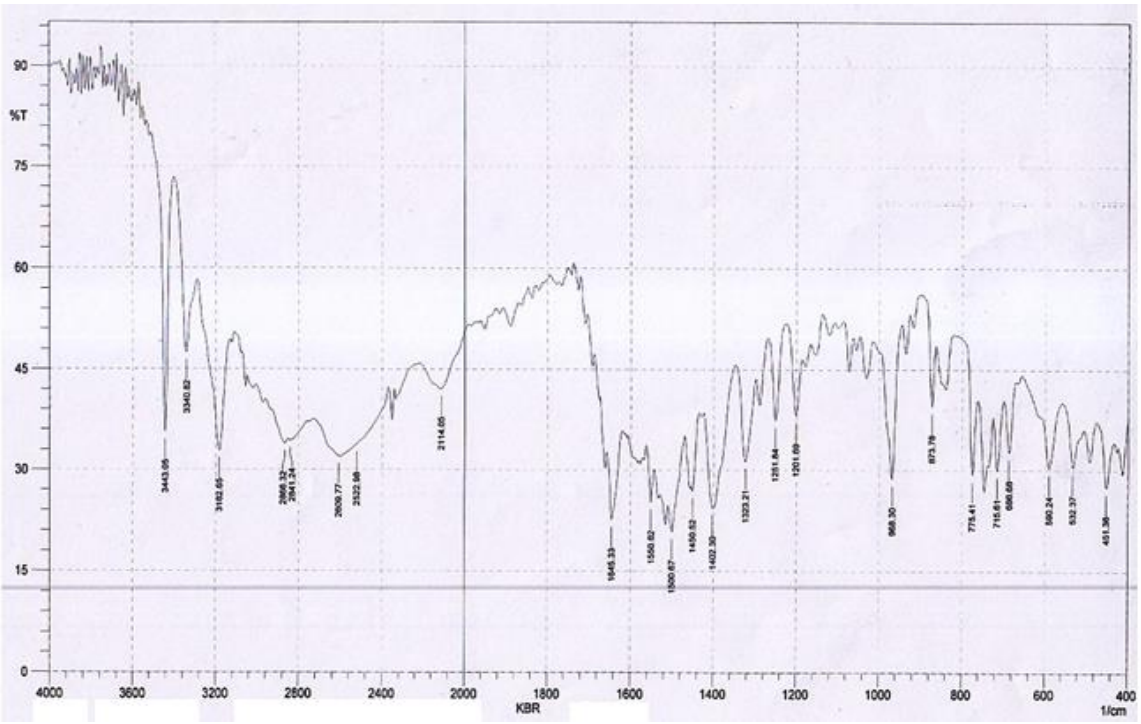
IR (KBr cm^{-1}): N-H stretching (Benzimidazole) 3443, C-H stretching (Aromatic) 3182, C=C stretching (Aromatic) 1645, C=N stretching (Imine) 1645, C-N stretching (Amine) 1323.

^{1}H NMR (cdc 13 δ ppm): 5.1 Singlet (1H, NH), 6.4 Doublet (1H, CH), 6.65-7.6 Multiplet (9h, Ar-CH), 7.8 Doublet (1H, CH).

Molecular Formula: $C_{15}H_{12}N_2$

Molecular Weight: 220.26

Melting Point: 215-220



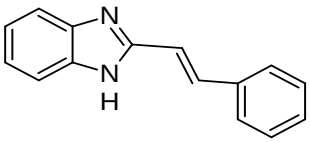
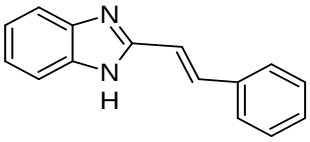
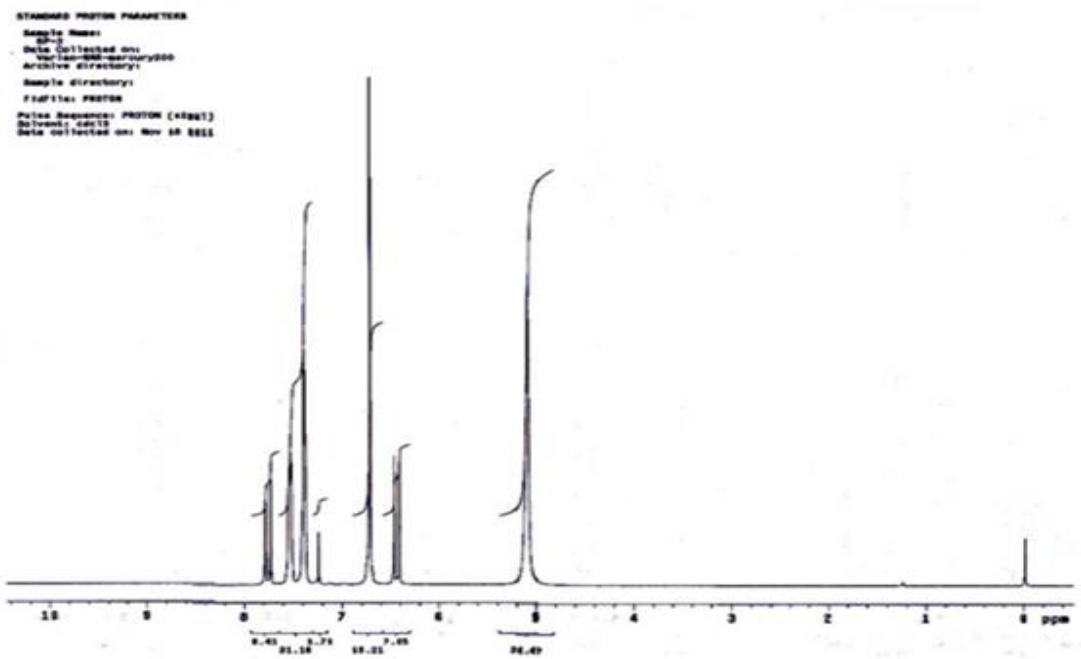


Fig 4.5: IR Spectra of AP- 3.

TABLE No.-4.6.

Functional group assigned	Group frequency in Wave number (cm^{-1})
N-H stretching (Benzimidazole)	3443
C-H stretching (Aromatic)	3182
C=C stretching (Aromatic)	1645
C=N stretching (Imine)	1645
C-N stretching (Amine)	1323





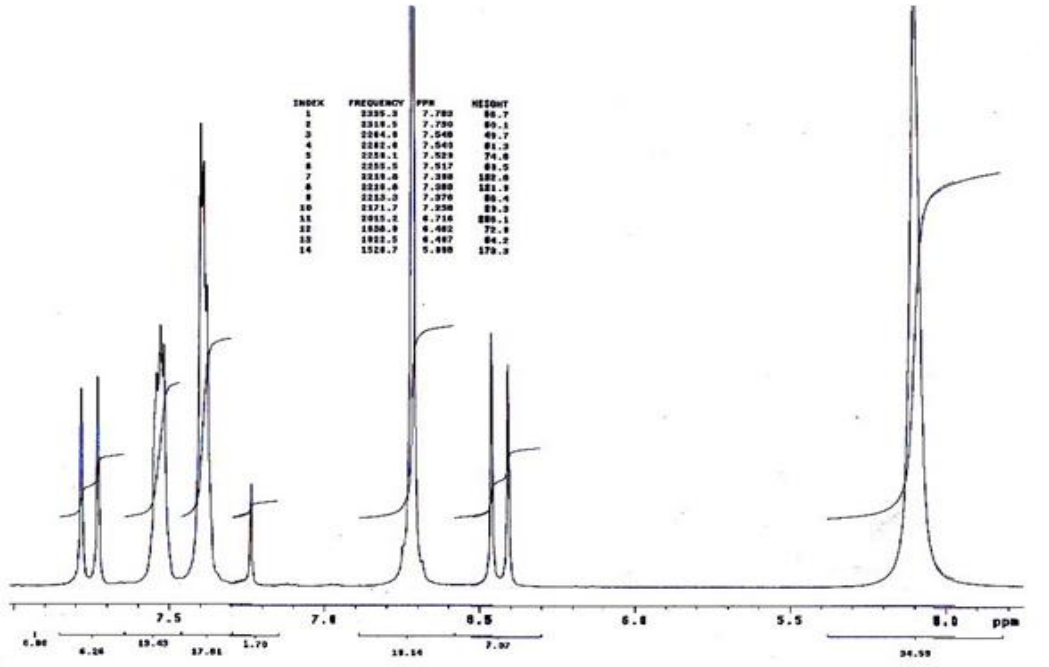
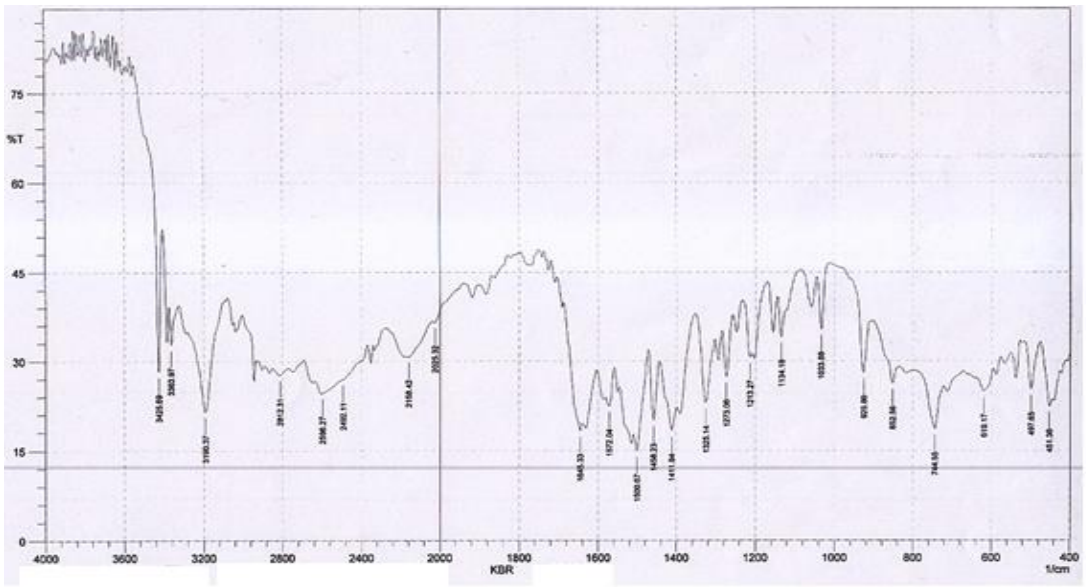


Fig 4.6: ^{1}H NMR Spectra of AP- 3.

Table No.-4.7.

Sr.No.	Value (δ)	Nature of segment	Type
1	5.1	Singlet	1H, NH
2	6.4	Doublet	1H, CH
3	6.65-7.6	Multiplet	9H, Ar-CH
4	7.8	Doublet	1H, CH



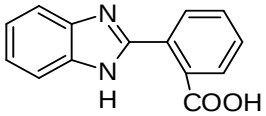
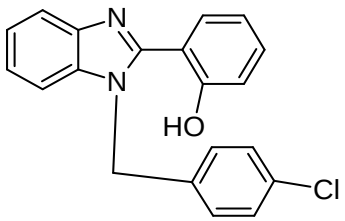


Fig 4.7: IR Spectra of AP- 4.

Table No.-4.8.

Functional group assigned	Group frequency in Wave number (cm^{-1})
N-H stretching (Benzimidazole)	3425
O-H stretching (Carboxylic acid)	3363
C-H stretching (aromatic)	3190
C=N stretching (Imine)	1645
C=C stretching (aromatic)	1572
C-N stretching (Amine)	1325

Compound AP-6



2-[1-(4-chlorobenzyl)-1*H*-benzimidazol-2-yl] phenol

The IR spectrum of AP-6 compound exhibited O-H absorption peak at 3373 cm^{-1} which is the normal place of absorption of O-H of phenolic moiety. The aromatic C-H peak is noticed at 3171 cm^{-1}. The C=N of the Imine appeared at 1616 cm^{-1}. The aromatic C=C at 1599 cm^{-1}. The amine C-N at 1269 cm^{-1}. And the halide C-Cl at 761 cm^{-1}. These data are in confirmative with the structure of the synthesized compound.

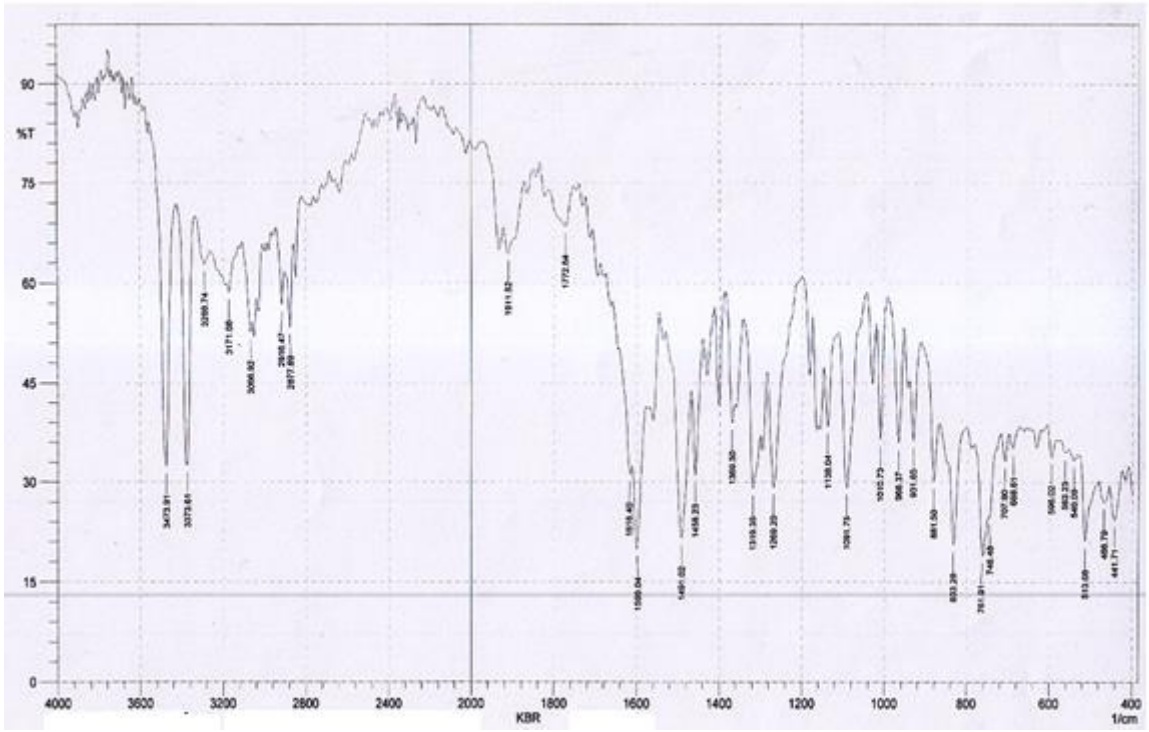
The ^{1}H NMR of this compound when recorded in cdc 13 δ ppm. The 2H of C-H is seen from 3.8-4.2δ as Singlet. The 1H of O-H is seen from 5.4 δ as Singlet. The 12H of aromatic C-H is seen from 6.65-8.6 δ as Multiplet. These ^{1}H NMR data are in confirmative with the structure of the molecule proposed under investigation.

Mass spectra (m/z): Molecular ion peak appear at 334 as (M+).

Molecular Formula: $C_{20}H_{15}ClN_2O$

Molecular Weight: 334.79

Melting Point: 205-210



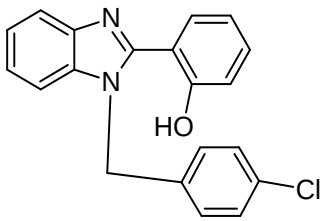
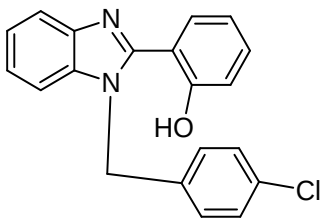


Fig 4.8: IR Spectra of AP-6.

Table No.-4.9.

Functional group assigned	Group frequency in Wave number (cm^{-1})
O-H stretching (Phenolic)	3373
C-H stretching (aromatic)	3171
C=N stretching (Imine)	1616
C=C stretching (Aromatic)	1599
C-N stretching (Amine)	1269
C-Cl stretching (Halide)	761



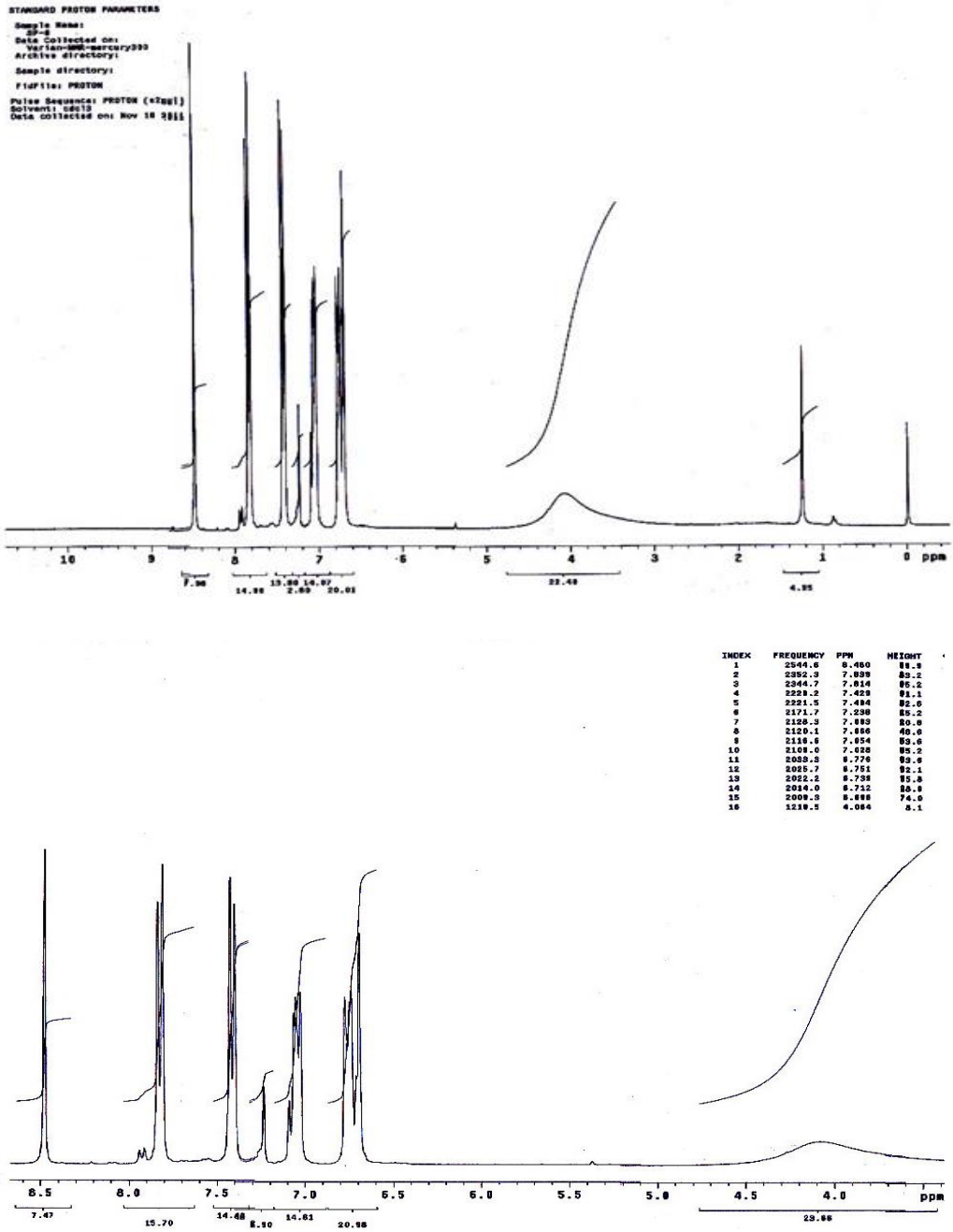


Fig 4.9: ^{1}H NMR Spectra of AP-6.

Table No.-4.10.

Sr.No.	Value (δ)	Nature of segment	Type
1	3.8-4.2	Singlet	2H, CH
2	5.4	Singlet	1H, OH
3	6.65-8.6	Multiplet	12H, Ar-CH

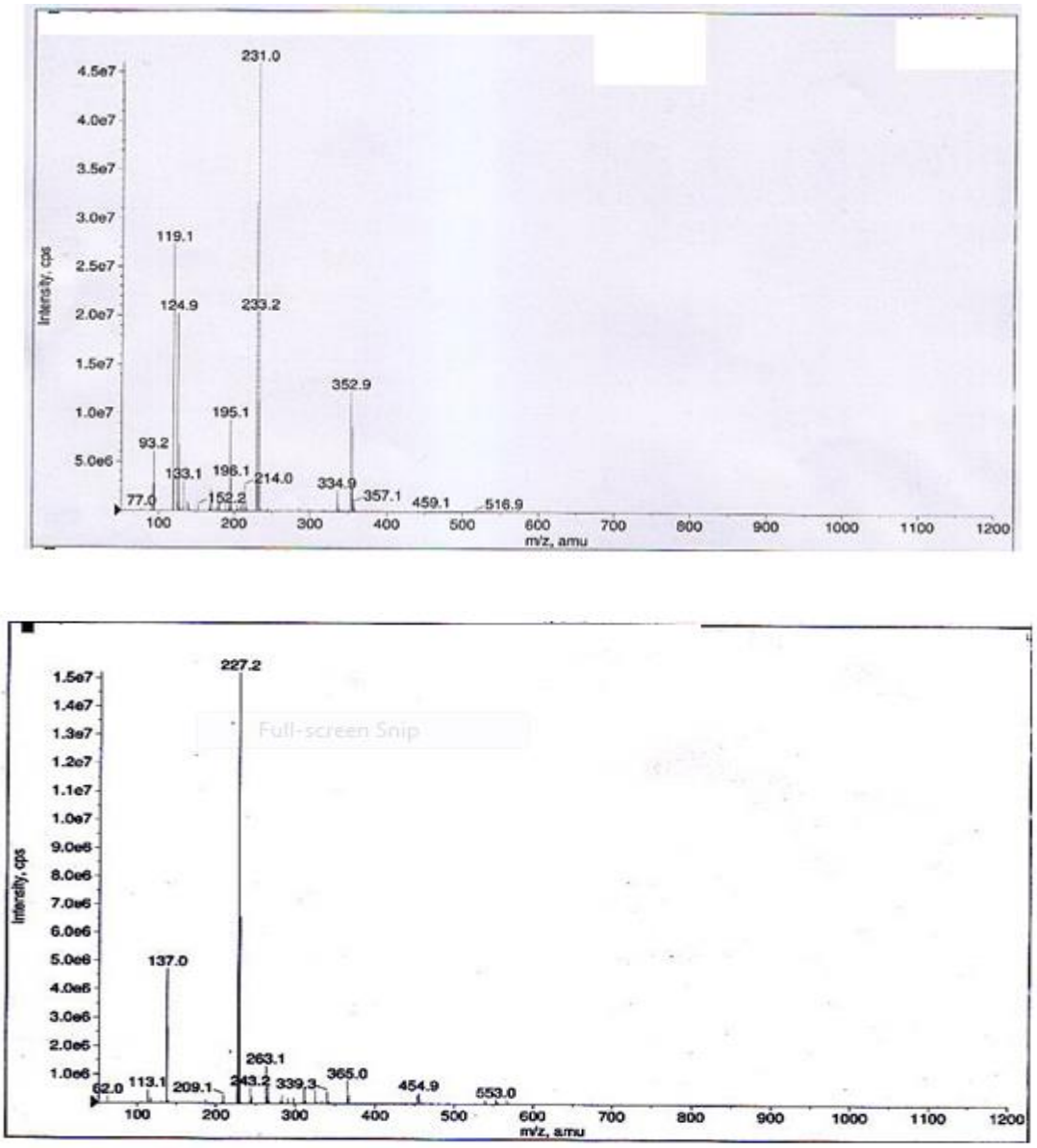
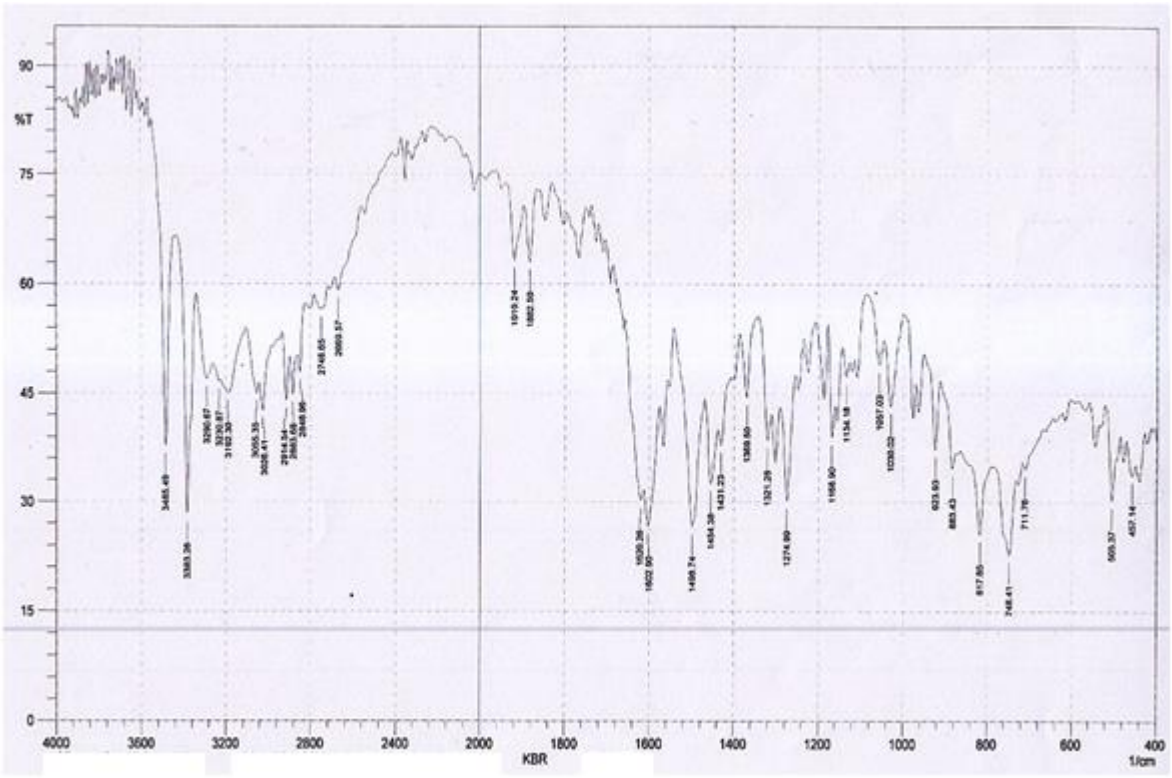


Fig 4.10: Mass spectra of AP -6.

Mass spectral data of compound (AP-6)

Mass Data: The molecular weight of the compound is 334 and the mass spectral data matching the same as 334 m/z it shows that the m+ peak.



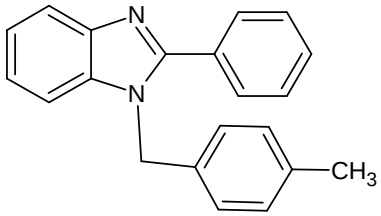
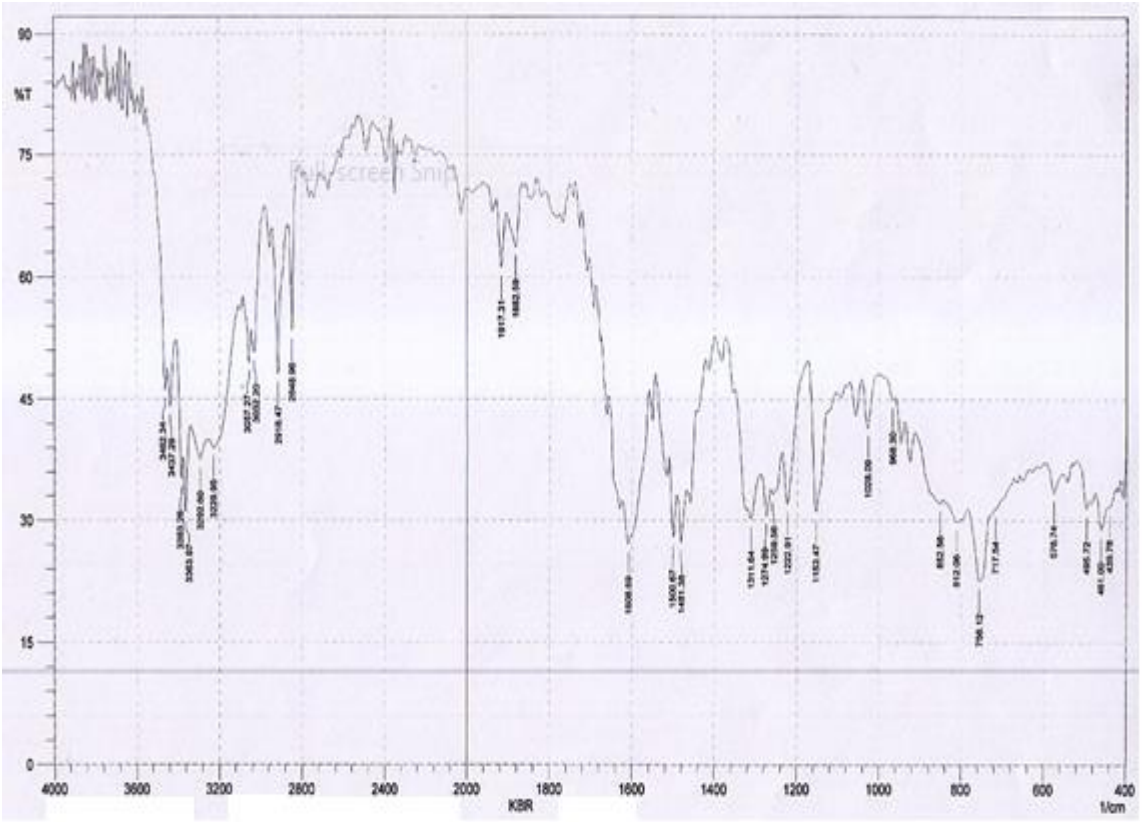


Fig 4.11: IR Spectra of AP-8.

Table No.-4.11.

Functional group assigned	Group frequency in Wave number (cm^{-1})
C-H stretching (Aromatic)	3192
C-H stretching (Alkane)	2914
C=N stretching (Imines)	1620
C=C stretching (Aromatic)	1602
C-N stretching (Amine)	1321



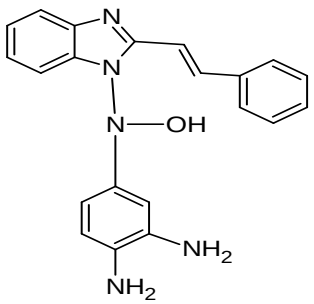


Fig 4.12: IR Spectra of AP-9.

Table No.-4.12.

Functional group assigned	Group frequency in Wave number (cm^{-1})
O-H stretching (Alcoholic)	3437
N-H stretching (Primary amine)	3363
C-H stretching (aromatic)	3057
C=C stretching (aromatic)	1608
C=N stretching (Imines)	1608
C-N stretching (Amine)	1311

5. BIOLOGICAL ACTIVITY

Antimicrobial Activity

All the compounds synthesized in the present investigation were screened for their antibacterial activity by Cup plate Method. Antibacterial activities were tested on nutrient medium against, *Staphylococcus aureus*, and *Escherichia 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 20 gm
5. Distilled water up to 1000 ml.

Weighed quantities of peptone and beef extract was 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 to 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 in autoclave at 121^0C for 20 minutes at 15 lbs pressure.

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 50 µg/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.

PREPARATION OF DISCS

Discs of 6-7 mm in diameter were punched from NO: 1 Whattmann filter paper with sterile cork borer of same size. These discs were sterilized by keeping in oven at 140^0C for 60 minutes. Then standard and test solutions were added to each disc and discs were air dried.

METHOD OF TESTING

The sterilized media was cooled to 45^0C 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 37^0C for 24 hours in an incubator. The extent diameter of inhibition after 24 hours was measured as the zone of inhibition in millimeters and the results were shown in Table-No- 5.0.

Table No. 5.0: Anti-Microbial activity of synthesized compounds (AP5-AP9).

Sr.No.	Compound Code	Concentration µg/ml	*E. coli* (mm)	*S. Aureus* (mm)
1.	AP-5	50	15	17
		100	16	17
2.	AP-6	50	**22**	**23**
		100	**24**	**24**
3.	AP-7	50	16	15
		100	15	15
4.	AP-8	50	18	19
		100	19	20
5.	AP-9	50	**21**	**22**
		100	20	19
6.	Ampicillin	50	**23**	**25**
		100	**25**	**25**
7.	DMF	-	-	-

Standard: Ampicilin

Note: 15-17 mm poor activity, 18-21mm moderate activity, 22-25mm good activity.

6. RESULTS AND DISCUSSION

The conventional methodology was adopted to synthesize the titled compounds. The synthesis of titled compounds from starting material i.e 2-substituted benzimidazole was prepared from O-phenylenediamine and Carboxylic acid like salicylic acid, benzoic acid,

Cinnamic acid, Pthalic acid in presence of 4N HCl. The reaction of 2-substituted benzimidazole on reacting with 1-benzyl 2-substituted benzimidazoles yield 60-77 % of different derivatives of benzimidazole. It is noteworthy that such a procedure for rapid preparation of various benzimidazole affords advantages of short reaction time, moderate yields and simple workup. The IR spectrum of AP-6 compound exhibited O-H absorption peak at 3373 cm^{-1} which is the normal place of absorption of O-H of phenolic moiety. The aromatic C-H peak is noticed at 3171 cm^{-1}. The C=N of the Imine appeared at 1616 cm^{-1}. The aromatic C=C at 1599 cm^{-1}. The amine C-N at 1269 cm^{-1}. And the halide C-Cl at 761 cm^{-1}. These data are in confirmative with the structure of the synthesized compound.

The ^{1}H NMR of this compound when recorded in cdc 13. The 2H of C-H is seen from 3.8-4.2δ as Singlet. The 1H of O-H is seen from 5.4 δ as Singlet. The 12H of aromatic C-H is seen from 6.65-8.6 δ as Multiplet. These ^{1}H NMR data are in confirmative with the structure of the molecule proposed under investigation. The IR spectrum of AP-8 also gives aromatic C-H peak is seen at 3192 cm^{-1}, alkane C-H peak is seen at 2914 cm^{-1}, imines C=N at 1620 cm^{-1}, aromatic C=C at1602 cm^{-1}, and amine C-N at 1321 cm^{-1}. These are the expected concurrent data for proposed molecule. The next analogue of the compound AP-9. In this case N-H of primary amine at 3363 cm^{-1}. The aromatic C-H peak found to be absorbed at 3057cm^{-1}. The C=C of aromatic group gave absorption peak at 1608 cm^{-1}. The C=N of imines group Gave absorption peak at 1608 cm^{-1}.The C-N of amine group gave absorption peak at 1311 cm^{-1}. These are the expected IR data for the molecule under investigation. The synthesized all benzimidazole derivatives were screened for antimicrobial activity using DMF as a solvent against the organisms, *S.aureus* and *E.coli.* By disc diffusion method on nutrient agar media. The Ampicillin was used as standard drug for antimicrobial activity. AP-5 & AP-7 showed poor activity at concentration 50 µg/ml and at concentration 100 µg/ml. AP-8 showed moderate activity at concentration 50 µg/ml and concentration 100 µg/ml. AP-6 and AP-9 showed good activity at concentration 50 µg/ml and concentration 100 µg/ml they possess good activity against *E.Coli* and *S .Aureus.* However the activities shown by all the compounds tested were less than that of the standard.

CONCLUSION

In this work, the reaction of 2-substituted benzimidazole and 1-benzyl 2-substituted benzimidazoles in the synthesis of benzimidazole derivatives is successfully carried out. The data obtained from IR, ^{1}H NMR and Mass data resembled with expected data. The data

obtained are in confirmative with the structural propose of the synthesized compound or molecule. From the above data of antimicrobial activity it is clearly concluded that the synthesized compounds are significant antimicrobial agents. The substituted benzimidazole moieties are already known for different biological activities. As per the results of screening it is clearly indicated that the compounds of the scheme have shown good antibacterial activity equipotent with that of standard drugs. This is because of the presence of groups like $-NO_2$, -Cl**,** at the different positions of phenyl ring attached to benzimidazole nucleus. Since some of the present new benzimidazole derivatives exhibit moderate activity compared with the standard employed, it is desirable to determine their toxicity to decide on whether to go for further screening or not.