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Research paper

Synthesis and Evaluation of Anti-Bacterial Activity of Benzimidazole Derivatives

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Benzimidazole is a heterocyclic aromatic organic compound. This bicyclic compound consists of the fusion of benzene and imidazole. It is a colorless solid. The benzimidazoles contain a phenyl ring fused to an imidazole ring as indicated in the structure. This important group of substances has found practical applications in a number of fields. Benzimidazole, which is a heterocyclic nucleus, plays an important role in various medicines. A number of therapeutic agents such as H_1 antihistaminic agent clemizole, a potent opioid analgesic etonitazene, non-nucleoside antiviral compound enviroxime, for promotion of excretion of uric acid irtemazole, non sedating antihistaminic agent astemizole, anti ulcer drugs omeprazole and pentoprazole, antihelmintic thiabendazole, antinematodal nocodazole etc. are based on benzimidazole heterocyclic nucleus.

Key words:- Benzimidazole, heterocyclic,aromatic, imidazole, antihistaminic agent

INTRODUCTION

Medicinal or pharmaceutical chemistry is a discipline at the intersection of chemistry and pharmacology involved with designing, synthesizing and developing pharmaceuticaldrugs. Medicinal chemistry involves the identification, synthesis and development of new chemical entities suitable for therapeutic use. It also includes the study of existing drugs, their biological properties and their quantitative structure-activity relationships (QSAR). Pharmaceutical chemistry is focused on quality aspects of medicines and aim to assure fitness for the purpose of medicinal products.

Application: Benzimidazoles are often bioactive. Many anthelmintic drugs (albendazole, meben - dazole, triclabendazole etc.) belong to the benzimidazole class of compounds.

Benzimidazole fungicides are commercialized. They act by binding to the fungal microtubules and stopping hyphal growth. It also binds to the spindle microtubules and blocks nuclear division.

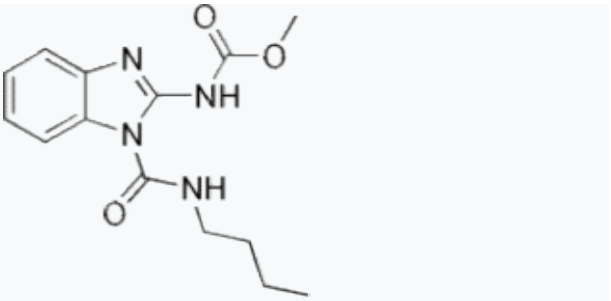


Fig. 1: Benomyl (Benlate) is a fungicide with a benzimidazole core

Importance of Proposed Investigations :

Medicinal chemistry and pharmaceutical chemistry are disciplines at the intersection of chemistry, especially synthetic organic

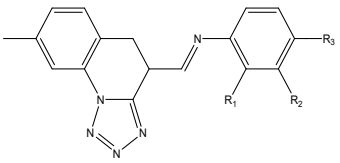
chemistry, and pharmacology and various other biological specialties, where they are involved with design, chemical synthesis and development for market of pharmaceutical agents, or bio-active molecules (drugs) At the biological interface, medicinal chemistry combines to form a set of highly interdisciplinary sciences, setting its organic, physical, and computational emphases alongside biological areas such as biochemistry, molecular biology, pharmacognosy and pharmacology, toxicology and veterinary and human medicine; these, with project management, statistics, and pharmaceutical business practices, systematically oversee altering identified chemical agents such that after pharmaceutical formulation, they are safe and efficacious, and therefore suitable for use in treatment of disease.

Scope of Proposed Study :

Total synthesis is the complete chemical synthesis of a complex molecule, often a natural product, from simple, commercially available precursors. It usually refers to a process not involving the aid of biological processes, which distinguishes it from semisynthesis. The target molecules can be natural products, medicinally important active ingredients, or organic compounds of theoretical interest. Often the aim is to discover new route of synthesis for a target molecule for which there already exist known routes. Sometimes no route exists and the chemist wishes to find a viable route for the first time. One important purpose of total synthesis is the discovery of new chemical reactions and new chemical reagents.

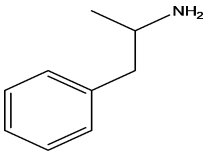
Review of work already done on the subject :

Bhargava S., ***et.al.*** (2010, 2008) have synthesized synthesis of schiff's bases of 8-methyl – tetrazolo(1,5-a) quinoline as potential anti-inflammatory and antimicrobial agents.



R_1= OCH_3
R_2= OCH_3
R_3 = OCH_3

Andrew A.,*et.al.*(2010) Worked on synthetic reductions in clandestine amphetamine and methamphetamine laboratories. This review summarizes the synthetic methods, reactions and biological applications of 2-chloroquinoline-3-carbaldehydes during the period from 1999 to 2011. The reactions are subdivided in groups that cover reactions at the chloro or aldehyde substituent and reactions which involve both groups. Most reaction types have been successfully applied and used in the production of biological active compounds.



Objectives:

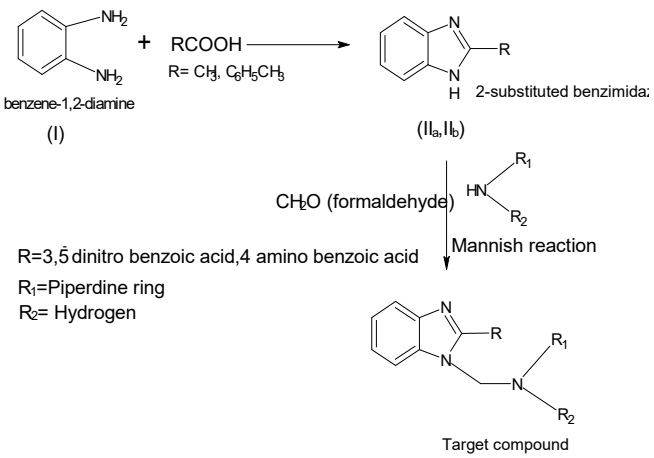
The objective of present work was synthesized some newer mannich bases of benzimidazoles. These derivatives were characterized by physico-chemical properties such as TLC, melting point &

spectral studies (I.R., N.M.R. and Mass spectroscopy) and finally the synthesized compounds were subjected to biological evaluation. The project work was comprised of following steps:-

1. Synthesis of mannich bases of benzimidazole derivatives.
2. Physicochemical characterization of synthesized compounds (I.R., N.M.R. and Mass Spectroscopy)
3. Evaluation of biological activities (Anti-Bacterial) of Synthesize compounds.

SCHEME OF WORK

Synthetic Scheme:



Step A: Synthesis of benzimidazole

Step B: Synthesis of 2-methylbenzimidazole

Step C: Synthesis of 2-benzylbenzimidazole

Step B1: Synthesis of 3-(2-methyl-1H-benzimi - dazol-1-yl)-1-phenylpropan-1-one

Step B2: Synthesis of 4-(2-methyl-1H-benzimi - dazol-1-yl) butan-2-one

Materials and Method

The synthetic studies of the compound were carried out using laboratory grade and analytical grade reagent as the case may be standard procedure or reported methods were followed with or without modification appropriately as and when required.

Synthetic Procedure

Step A: Synthesis of benzimidazole

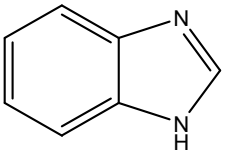
o-pheylenediamine (13.5 gm, 0.12 mol) was placed in a 250 ml round-bottomed flask and 85 % formic acid (9.2 gm, 8.4 ml, 0.17 mol) was added to it. The mixture was heated on a water bath for 100°C for 3 hours. The mixture was cooled, 10 percent sodium hydroxide solution was added slowly, with constant rotation of the flask, until the mixture was just alkaline to litmus. The crude product was filtered washed with ice cold water and dried. The product was dissolved in 200 ml of boiling water and about 1 gm decolorizing carbon was added and digested for 15 minutes. Then it was filtered, the filtrate was cooled to about 10°C, benzimidazole was filtered and washed with water and dry at 100°C. The completion of reaction was monitored by running TLC.

Solvent system: Ethylacetate: chloroform - (8:2)

Melting Point: 178-180°C

Yield: (8.25 gm) 56%

R_f value: 0.19



Step B: Synthesis of 2-methylbenzimidazole

The mixture of o-pheylenediamine dihydrochloride (5.43 gm, 0.03 mol), 20 ml of water, acetic acid (5.4 gm, 5.67ml, 0.09 mol) was refluxed for 4 hours. The reaction mixture was cooled and basified with gradual addition of concentrated ammonia solution, the precipitate was filtered, dried and recrystallised from 10

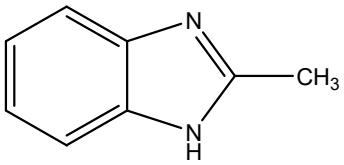
% aqueous ethanol. The completion of reaction was monitored by running TLC.

Solvent system: Ethylacetate : chloroform - (8:2)

Melting Point: 184-186ºC.

Yield: (2.3 gm) 58%

R_f value: 0.22



Step C: Synthesis of 2-benzylbenzimidazole

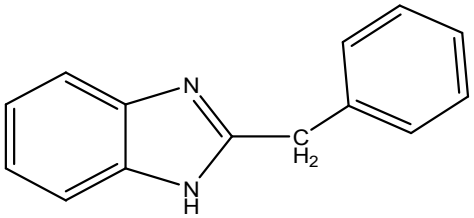
The mixture of o-pheylenediamine dihydrochloride (5.43 gm, 0.03 mol), 20 ml of water, phenyl acetic acid (12.3 gm, 0.09 mol) was refluxed for 6 hours. The reaction mixture was cooled and basified with gradual addition of concentrated ammonia solution, the precipitate was filtered, dried and recrystallised from 40% aqueous ethanol. The completion of reaction was monitored by running TLC.

Solvent system: Ethylacetate: chloroform - (8:2)

Melting Point: 194-196ºC.

Yield: (3.9 gm) 63%

R_f value: 0.79



Step B1: Synthesis of 3-(2-methyl-1H-benzimidazol-1-yl)-1-phenylpropan-1-one

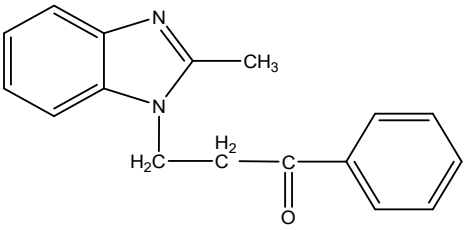
A solution of 2-methylbenzimidazole (2.15 gm, 0.0163 mol) formaldehyde (1.9 ml, 0.0163mol) and acetophenone (1.9 ml, 0.0163mol) in 3 ml of 95% alcohol and 0.4 ml of conc. hydrochloric acid were refluxed the mixture on water bath for 5 hours, the reaction mixture were filtered and about 15 ml of acetone was added to it and allowed to cool at room temperature and kept in refrigerator overnight. The crude product so obtained was filtered, dried and recrystallised with methanol.

Solvent system: Ethylacetate: chloroform - (8:2)

Melting Point: = 300 ºC.

Yield: (1.4 gm) 33%

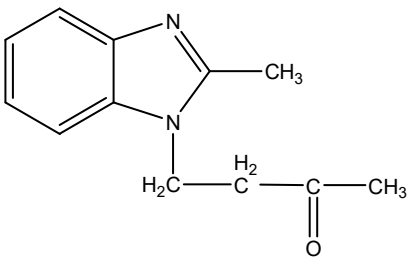
R_f value: 0.21



Step B2: Synthesis of 4-(2-methyl-1H-benzimidazol-1-yl) butan-2-one

A solution of 2-methylbenzimidazole (2.15 gm, 0.0163mol) formaldehyde (1.9 ml, 0.0163mol) and acetone (1.2 ml, 0.0163mol) in 3 ml of 95% alcohol and 0.4 ml of conc. hydrochloric acid were refluxed the mixture on water bath for 6 hours, the reaction mixture was then filtered and about 15 ml of acetone was added to it and allowed to cool at room temperature and kept in refrigerator overnight. The crude product so obtained was filtered, dried and recrystallised with methanol.

Solvent system: Ethylacetate: chloroform - (8:2)



Melting Point: 98-102 °C.

Yield: (0.7 gm) 21%

R_f value: 0.20

BIOLOGICAL EVALUATION

Anti-Bacterial Activity

All the synthesized compounds 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* (MTCC 96), and *Escherchia coli* (MTCC 443) which were representative types of gram +ve and gram -ve organisms respectively. The antibacterial activities of the compounds were assessing by disc-diffusion method.

Preparation of nutrient agar media

Media Composition and Procedure (pH 7.2 ± 2)

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

Table 1: Composition of nutrient agar media

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

Weigh quantities of peptone and beef extract were dissolve 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 were adjust to7.2 to7.4 by adding the sodium chloride and the volume of the final solution were made up to 1000 ml with distilled water. Then it was transferred in to a conical flask, plugged with non-adsorbent cotton and the media were sterilized by in autoclave at 121°C for 20 minutes at 15 lbs pressure.

Preparation of test solutions

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

Preparation of Standard Antibiotic Solution:

Amoxicillin was use as standard antibiotics for comparison and solutions were prepared by using sterile water, as they were water-soluble. The solutions were dilute 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 punch from No: 41 Whatmann filter paper with sterile cork borer of same size. These discs were sterilized by keeping in oven at 140°C for 60 minutes. Then standard and test solutions were added to each disc and discs was air-dried.

Method of Testing:

The sterilize media was cool to 45°C with gentle shaking to bring about uniform cooling and then inoculated with 18-24 hrs old culture under aseptic conditions, mix well by gentle shaking. This is pour into sterile Petri dishes (properly labeled) and allows the medium to set. After solidification all the

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Petri dishes were transferred to laminar air flow unit. Then the discs which were previously prepare and 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°C for 24 hours in an incubator. The extent diameter of inhibition after 24 hours was measured as the zone of inhibition in millimeters.

RESULTS AND DISCUSSION

Physical characteristics

All the synthesized compounds were light cream to brown colored crystalline solids. Most of the compounds were freely soluble in chloroform and other solvents like methanol, ethanol. The melting points of the compounds were in the range of 50ºC to 134ºC.

Spectral characteristics

IR spectra

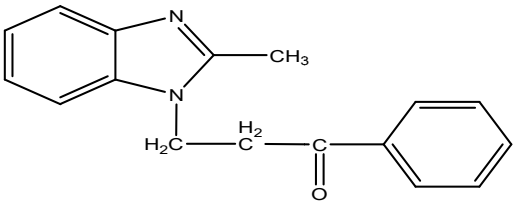
IR spectra of all compounds were recorded on FT-IR 8400S Shimadzu Spectrophotometer using KBr disc. All the synthesized compounds have shown characteristic stretching and bending in desired range.

Synthesized Compounds:

Physical and spectral characteristics Synthesis of 3-(2-methyl-1H-benzimidazol-1-yl)-1-phenyl propan-1-one (B1)

Table 1: Physical Characteristics (B1)

Molecular formula	% yield	Melting point Range (ºC)	R_f value
$C_{17}H_{16}ON_2$	33%	>300	0.21



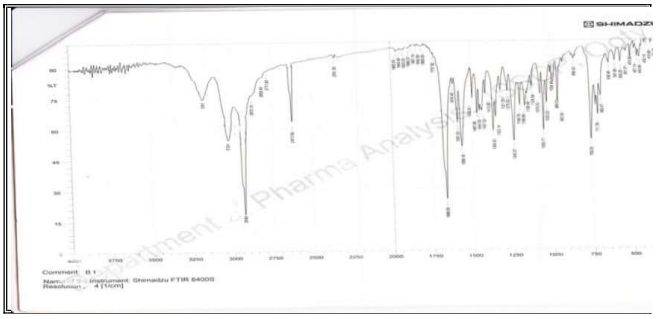


Fig. 1: IR Spectra of compound B1

Table 2:Spectral Characteristics

Compound code	IR (cm^{-1})
Comp-B1	Stretch 2989(Ar. C-H), Stretch 2613.55(-CH3), Stretch 1616.40(C=O), Stretch 1454.38(C=N), Stretch 1369.50(tert. N)

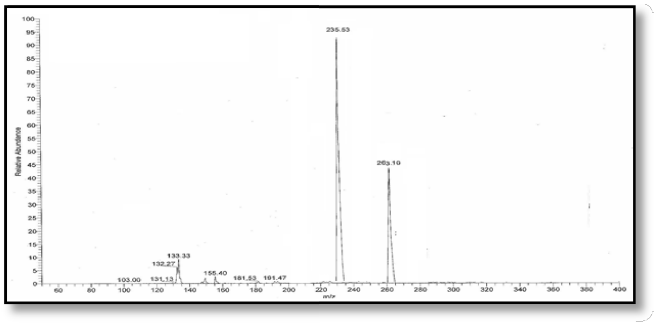


Fig. 2: Mass Spectra of compound B1

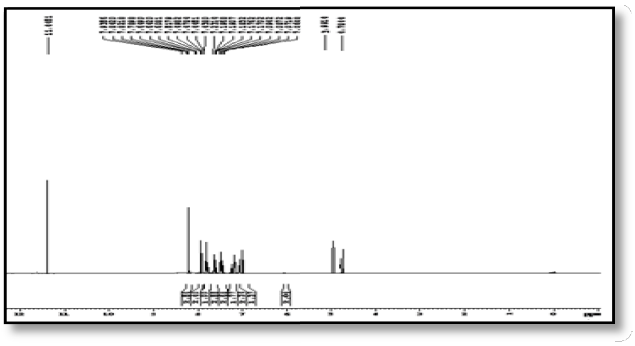


Fig. 3:^{1}H-NMR SPECTRA OF COMPOUND B1

Table 3: Spectral Characteristics

Sr. No.	Chemical shift (δ ppm)	Signal	Intensity	Type of proton
1	7.2627	*s*	1.000	-NH
2	7.01	*d*	1.000	-CH_2 Aromatic
3	6.5045	*d*	0.9987	-CH- Aromatic
4	5.005	*t*	1.0772	-CH- Aliphatic
5	3.5953	*s*	1.8885	-CH_3
6	3.4377	*s*	1.7470	-CH_3
7	2.2322	*s*	2.4763	-CH_3 Aromatic

Compound code	IR (cm^{-1})	Mass (m/e)	^{1}H-NMR δ ppm
Comp-3b	3034.68(NH), 2827.69(Ar-CH), 1648.89(C=O), 1543.64(C=N)	M^+ 281.35 Base peak: 251.35	4.7(s,1H, NH), 7.2-7.8(m, 9H, Ar-H), 3.70(s, 2H, CH_2), 1.6(s,1H,CH) 2.3(s, 3H, CH_3), 2.4(s, 3H, CH_3)

Physical and spectral characteristics Synthesis of 4-(2-methyl-1H-benzimidazol-1-yl) butan-2-one (B2)

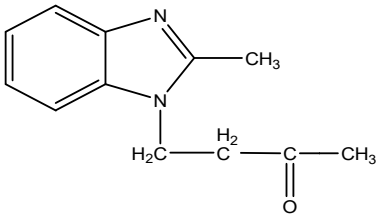


Table 4: Physical Characteristics

Molecular formula	% yield	Melting point Range (°C)	R_f value
$C_{12}H_{14}ON_2$	21%	98-102	0.20

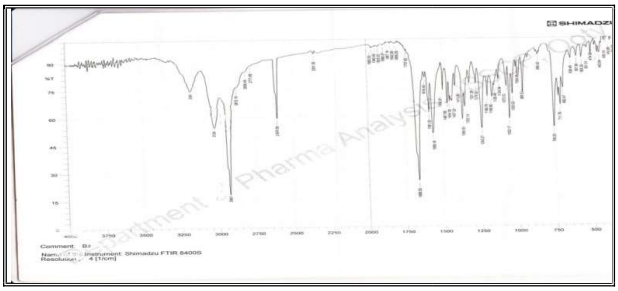


Fig. 4: IR Spectra of compound B2

Table 5: Spectral Characteristics

Compound code	IR (cm^{-1})
Comp-B2	Stretch 2961(Ar. C-H), Stretch 2624.55(-CH3), Stretch 1737.92(C=O), Stretch 1454.38(C=N), Stretch 1321.28(tert. N)

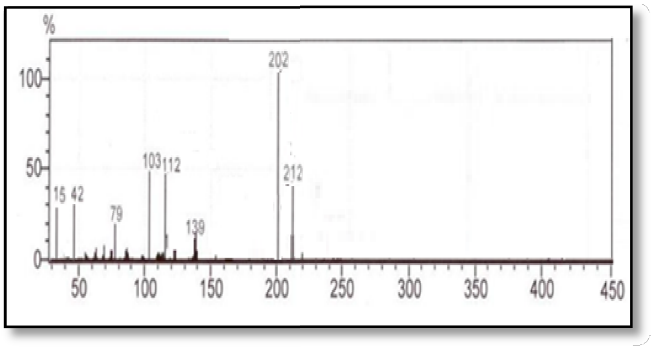


Fig. 5: Mass Spectra of compound B2

SUMMARY AND CONCLUSION

The present work, which had undertaken were bonafied, and novel for the synthesis of various Benzimidazole derivatives. In this view we had

made an attempt in reviewing the literature on substituted Benzimidazole derivatives for their medicinal significance with help of chemical abstract, journals and internet sites. All synthesized compounds were tested for the preliminary tests, physical constants and TLC. All structures of final compound were confirmed by IR.

REFERENCE

1. http://en.wikipedia.org/wiki/Medicinal_chemistry.

2. Preston P.N., "Synthesis, reaction and spectroscopic properties of benzimidazoles". *Chem. Rev.*1974, 74(3), 279.

3. Humaira A., "Synthesis and characterization of potentially bioactive nucleosides bearing different heterocyclic moieties", Department of Chemistry, Quaid-i-Azam University,Islambad, 2006.

4. Singh J., Goyal A. and Pathak D. P., "Benzimidazole: heterocyclic nucleus".*Indian J. Pharm. Educ. Res.*2006, 40(3), 147-52.

5. Mills K. and Joule J. A., "Heterocyclic Chemistry. Black well Publishing", 4th ed, 2000, 454-517.

6. Thomas L. G., "Heterocyclic Chemistry. Pearson Education". 3rd ed., 2007, 83, 311-17,341.

7. Bansan R. K., "Heterocyclic chemistry, synthesis, reaction & mechanism". Willey Estern Limited, 1990, 320.

8. Oztekin A., Kaessler A., Apcin Y., Yilmaz A. and Joachim J., "Comparative studies on conventional and microwave synthesis of some benzimidazole, benzothiazole and indole derivatives and testing on inhibition of hyaluronidase".*Molecules.* 2008, 13, 736-48.

9. Kumar J. R., Jawahar L. J. and Pathak D. P., "Synthesis of benzimidazole derivatives: as anti-hypertensive agents". *E-Journal of Chemistry*, 2006, 3, 278-85.

10. Mukhopadhyay C. and Tapaswi P. K., "PEG-mediated catalyst-free expeditious synthesis of 2-substituted benzimidazoles and bis-benzimidazoles under solvent-less conditions". *Tetr. Lett.*2008, 49, 6237–40.