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SYNTHESIS AND EVALUATION OF ANTI-BACTERIAL ACTIVITY OF BENZIMIDAZOLE DERIVATIVES

Sivakumar Peta*[1], Pallepati Kavitha[2]

[1]Associate Professor, Department of Pharmaceutical Chemistry, Gokula Krishna College of Pharmacy,Sullurupeta, Andhra Pradesh 524121.

[2]Associate Professor, Department of Pharmaceutics, Gokula Krishna College of Pharmacy,Sullurupeta, Andhra Pradesh 524121.

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***Corresponding author**
Sivakumar Peta

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Abstract

Synthesis of molecules and materials are important aspects of the development of science. In the past, synthesis is based on some of the name reactions in organic and inorganic chemistry. The attempts to synthesis new molecule and materials have always depended on the molecules. The synthesis of heterocyclic compounds has always drawn the attention of chemist over the years mainly because of their important biological properties. To synthesize substituted benzimidazoles from o-phenylene diamine using various acids and amines. Heterocyclic compound previously reported benzimidazole was synthesized by treating o-phenylene diamine with benzoic acid (1a) and o-phenylene diamine with formic acid (1b) in presence of ethanol undergoes condensation to form required 2-substituted benzimidazoles. Latter compound 1 was treated with acid chloride i.e chloro acetyl chloride in presence of base 10% NaOH to yield the respective 1-substituted benzimidazole- chloro derivatives (2a-2b). Finally the formed chloro derivative benzimidazoles were refluxed with different amines (methylamine, diethylamine) at certain time period to yield the title compounds in good yields. The completion of the reactions was monitored by TLC. The synthesized compounds were made pure by means of recrystallization method using different solvent systems like ethanol, chloroform. Substituted benzimidazoles derivatives were synthesized utilizing o-phenylene diamine and substituted acids with amines results in good yields.

***Keywords*:** Benzimidazoles, TLC, Heterocyclic compound, recrystallization method, good yields.

Introduction

The research conducted with in this area combines several fields of synthetic chemistry. Synthetic organic chemistry is focused largely on compounds of pharmaceutical importance and covers novel molecule design natural product analogous and derivatives of natural products [1-2]. One of things that make chemistry unique among the science is the synthesis. Chemists make things, new pharmaceuticals, food additives, materials, agricultural chemicals, coating adhesives, and all sorts of useful new molecules. The chemists prepare them from simpler more readily available starting material; there are two aspects to organic synthesis first development of a synthetic strategy or plan of action and the second the actual implantation of that plan in a chemical laboratory. Synthesis of molecules and materials are important aspects of the development of science. In the past, synthesis is based on some of the name reactions in organic and inorganic chemistry. The attempts to synthesis new molecule and materials have always depended on the molecules. However, in the last two decades, the synthesis has become a well-established science going beyond the recollections and adoption of the existing procedures. These procedures have been satisfying the normal curiosity the chemicals for new molecules. However today, chemistry is the study of molecules, materials of functionalities and hence they have to be synthesizes, built, and architecture and designed. Today the molecules and materials are for many applications. Every sector of life requires

molecules/materials with functionalities, stability, as well as durability under adverse conditions. Molecules of highly active functionalities with stability, durability and appropriate stress-strain relationship (global properties of dissolution, digestion, attachment to species and others) are the demand of today. Most often materials required have to be in the Meta stable but should be able to be controlled by size, shape, orientation, and morphology. Over the last thirty years, synthetic chemists have developed and assortment of routes to obtain optically pure compounds. Many strategies include the use of pure starting material with choral centers. In addition, the utilization of choral auxiliary groups have been implanted to achieve an increase in stereo-selectivity and to simplify the purification process. Lastly, asymmetric catalysis using enzymes or inorganic catalysis has also been used to affordthe desired stereochemistry. Heterocyclic chemistry comprisesat least half of all organic chemistry research worldwide. In particular, heterocyclic structures from the basis of many pharmaceutical products. Among a wide variety of nitrogen heterocyclic that have been explored for developing pharmaceutically important role in medicinal chemistry and subsequentlyhave emerges as a pharmacophore [3-6].

Benzimidazole

Benzimidazole is a heterocyclic aromatic organic compound. This bicyclic compound consists of the fusion of benzene and imidazole. The molecular formula of benzimidazole is $C_7H_6N_2$. The benzimidazole as the name implies is a bicyclic ring system in which benzene has been fused at 4th and 5th position of the heterocycle (Imidazole). The NHCs are usually used as ligands for transition metal complexes. They are often prepared by deprotonating an N,N1-disubstituted benzimidazolium salt at the 2-position with a base. The first benzimidazole was prepared by 'Hoebrecker' who obtain 2, 5-dimethyl benzimidazole by the reduction and dehydration of 2-nitro-4-methylacetanilide. The benzimidazole ring system as a nucleus from which to develop potential chemotherapeutic agents was established in the 1950's when it was found that 5,6-dimethyl-1-(alpha-D-ribofuranosyl) benzimidazole was an integral part of the structure of the vitamin B12.

Chemistry of benzimidazoles

Benzimidazole is a slightly white powder, melting at 172°c, boils at 360°c, slightly soluble in water and ethanol. Benzimidazole had been synthesized to figure out the potentialpharmacophore against various disorders. Benzimidazole and its derivatives are used in organic synthesis of vermicides or fungicides as they inhibit the action of certain micro-organisms. Examples of benzimidazole class fungicides include benomyl, carbendazim, cypendazole, debacarb, fuberidazole, mercabinzid, thiabendazole, thiophanate. Benzimidazole structure is the nucleus in some drugs such as proton pump inhibitors and anthlemintic agents.

Benzimidazole from natural sources:

The most prominent benzimidazole compound in nature is N-ribosyl-dimethyl benzimidazole, which serves as an axial ligand for cobalt in vitamin B_{12}.

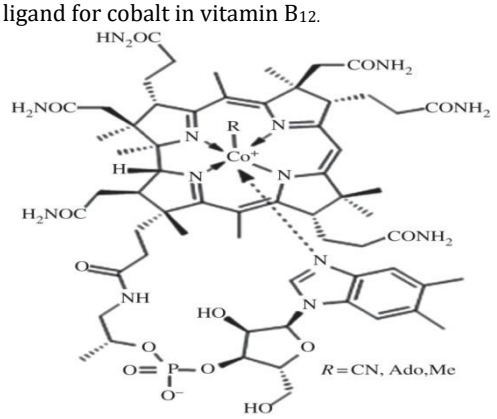
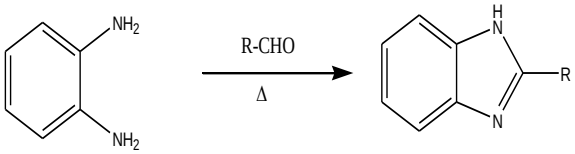


Fig 01: Vitamin B12

Preparation of benzimidazole

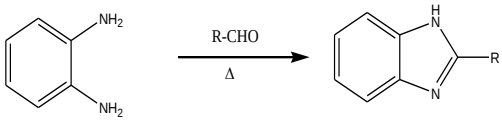
The synthetic pathway to the various benzimidazoles usually proceeds through two steps:

Step 1: Benzimidazole is produced by condensation of O-phenylene diamine with formic acid or the equivalent trimethyl orthoformate.



O-phenylene diamineBenzimidazole

Step 2: Benzimidazole is produced by condensation of O-phenylene diamine with carboxylic acid used,this method is generally able to afford 2- substituted benzimidazoles.

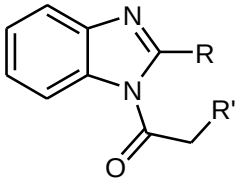


o-phenylene diamine 2-substituted benzimidazole

Generally, the synthesis of benzimidazoles involves the treatment of 1,2-phenylene diamine either with formic acid under strongly acidic conditions or with aldehydes under oxidative conditions.

Objectives

- The synthesis of heterocyclic compounds has always drawn the attention of chemist over the years mainly because of their important biological properties.
- To synthesize substituted benzimidazoles from o-phenylene diamine using various acids and amines.



- To characterize the synthesized compounds by physical (melting point, Rf values) and spectral data (IR spectra).
- Benzimidazole ring system is of biological and chemical interest since long. The literature survey reveals that the benzimidazoles are associated with a wide range of therapeutic activities such as anti-bacterial, anti-fungal, anti-helminthic, anti-tumour and proton pump inhibitors.
- Keeping in view of the above observations, our aim is to synthesise new series of substituted benzimidazoles and screening them for anti-microbial activity.

Experimental Procedure

Synthesis of compounds (1a-1b):

Chemicals and Reagents:

O-phenylene diamine, substituted acids i.e.; benzoic acid and formic acid, ethanol, Sodium hydroxide, ice cubes, distilled water.

Method

Take 27g(0.25 mole) of o-phenylene diamine and 17.5g(16ml,0.34 mole), 4grams of substituted acids i.e.; Benzoic acid and formic acid in round bottom flask and reflux condenser is attached to it for condensation for 2-4Hr. Now hot solution is poured into a beaker and is made alkali by stirring until a clean white precipitate gets formed. Then filtrate off and dried. The dried product is subjected in to digestion into hot water for removal of colour. Now it is filtered, the filtrate is collected then add ice cubes and stir well. A white precipitate of 2-substituted benzimidazole gets formed. It is filtered off and dried and weighed. The product was recrystallised from distilled water.

Synthesis of compound (2a-2b)

Chemicals and reagents

10% NaOH, chloro acetyl chloride,water, chloroform.

Method

In a conical flask 10% NaOH solution (0.01m) was taken. To this add 1gm of 2-substituted benzimidazole (1a-1b) with continuous stirring. The conical flask was cooled on ice bath in fuming hood and 1.5ml of chloro acetyl chloride was added dropwise by dropping funnel.The addition of chloro acetyl chloride was stopped till the fumes stops completely. After complete addition of chloro acetyl chloride the reaction mixture was cooled for sometime. The product is separated out by filteration, washed with water filtered and dried. The product was rerecrystallised from chloroform.

Synthesis of compound (3a-3b)

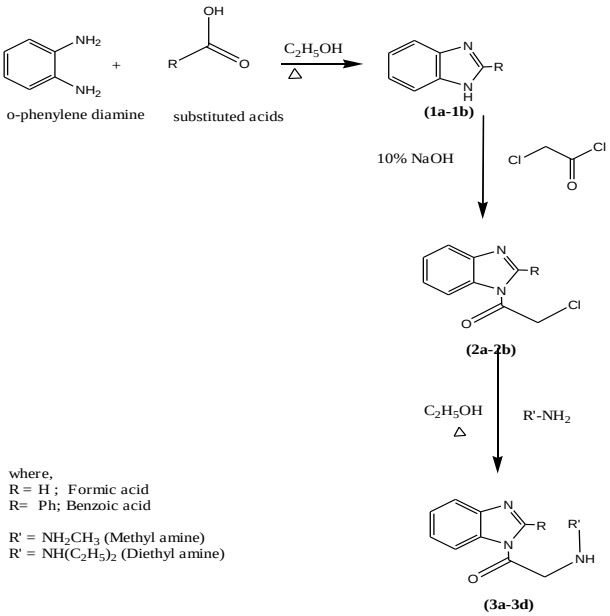
Chemicals and reagents

Ethanol, substituted amines (methyl amine and diethyl amine), ice cold water.

Method

In a solution of 0.01 mole of compound (2a-2b) and ethanol add 1 mole of substituted amines (methyl amine and diethyl amine) were mixed in round bottom flask. The reaction mixture was refluxed for 5hours. The excess ethanol was removed under vaccum. And residue mixture was allowed to cool on ice bath and diluted with ice coldwater. The precipitate were filtered and washed with ice cold water. The product was recrystallised from ethanol [7-9].

Scheme



Physicochemical Data of Compounds

Compound (1a)

TLC Studies

- Solvent system: Petroleum ether : Ethyl acetate (3:1)
- Rf value: 0.2

Physical Properties

- Molecular formula: $C_{13}H_{10}N_2$
- Molecular weight: 194.08 g/mol
- Melting point: 296 °C
- Recrystallization solvent: Ethanol
- Percentage yield: 94%

Spectral Data

IR (KBr, cm^{-1}):

3313.98 (N–H stretch), 1320.03, 1273.09 (C–N stretch), 1504.68 (C–C stretch),
3062.54 (aromatic C–H stretch), 1775.54, 1653.89 (C=C stretch)

Compound (1b)

TLC Studies

- Solvent system: Petroleum ether : Ethyl acetate (3:1)
- Rf value: 0.4

Physical Properties

- Molecular formula: $C_7H_6N_2$
- Molecular weight: 118.05 g/mol
- Melting point: 171 °C
- Recrystallization solvent: Ethanol
- Percentage yield: 85%

Spectral Data

IR (KBr, cm^{-1}):

3285.67 (N–H stretch), 1327.80, 1266.24 (C–N stretch), 3033.01 (aromatic C–H stretch), 1633.33 (C=N stretch)

Compound (2a): 2-Chloro-1-(2-phenyl-benzimidazol-1-yl)ethanone

TLC Studies

- Solvent system: Petroleum ether : Ethyl acetate (3:1)
- Rf value: 0.5

Physical Properties

- Molecular formula: $C_{15}H_{11}ClN_2O$
- Molecular weight: 270.06 g/mol
- Melting point: 195 °C
- Recrystallization solvent: Ethanol
- Percentage yield: 85%

Spectral Data

IR (KBr, cm^{-1}):

1328.35 (aromatic C–N stretch), 1658.19, 1373.17 (C=O stretch),
2852.39 (CH_2 methylene stretch), 691.15 (C–Cl stretch),
1558.10 (C–C stretch)

Compound (2b)

TLC Studies

- Solvent system: Petroleum ether : Ethyl acetate (3:1)
- Rf value: 0.3

Physical Properties

- Molecular formula: $C_9H_7ClN_2O$
- Molecular weight: 194.02 g/mol
- Melting point: 280 °C
- Recrystallization solvent: Ethanol
- Percentage yield: 88%

Spectral Data

IR (KBr, cm^{-1}):

1335.07 (aromatic C–N stretch), 1664.75 (C=O stretch),
2851.97 (CH_2 methylene stretch), 770.77 (C–Cl stretch)

Compound (3a)

TLC Studies

- Solvent system: Petroleum ether : Ethyl acetate (3:1)
- Rf value: 0.5

Physical Properties

- Molecular formula: $C_{16}H_{15}N_3O$
- Molecular weight: 265.12 g/mol
- Melting point: 298 °C
- Recrystallization solvent: Ethanol
- Percentage yield: 87%

Spectral Data

IR (KBr, cm^{-1}):

3325.08, 1248.90, 1170.69 (aliphatic N–H),
1654.56, 1612.32 (C=O stretch),
2854.64 (CH_2 methylene), 2921.74 (CH_3 stretch),
1509.17 (C–C stretch)

Compound (3b): 2-Diethylamino-1-(2-phenyl-benzimidazol-1-yl)ethanone

TLC Studies

- Solvent system: Petroleum ether : Ethyl acetate (3:1)
- Rf value: 0.7

Physical Properties

- Molecular formula: $C_{19}H_{21}N_3O$
- Molecular weight: 307.17 g/mol
- Melting point: 205 °C
- Recrystallization solvent: Ethanol
- Percentage yield: 86%

Spectral Data

IR (KBr, cm^{-1})

1367.95, 1325.35 (aromatic C–N stretch),
1657.11 (C=O stretch),
2962.25, 2923.87 (CH_2 methylene),
1450.01 (CH_2 stretch), 1589.91 (C–C stretch)

Compound (3c): 1-Benzimidazol-1-yl-2-methylamino-ethanone

TLC Studies

- Solvent system: Petroleum ether : Ethyl acetate (3:1)
- Rf value: 0.8

Physical Properties

- Molecular formula: $C_{10}H_{11}N_3O$
- Molecular weight: 189.09 g/mol
- Melting point: 292 °C
- Recrystallization solvent: Ethanol
- Percentage yield: 80%

Spectral Data

IR (KBr, cm^{-1}):

3323.85, 1023.26, 1108.33 (aliphatic N–H),
1654.88, 1612.10 (C=O stretch),
2855.15 (CH_2 methylene), 2922.43 (CH_3 stretch)

Compound (3d): 1-Benzimidazol-1-yl-2-diethylamino-ethanone TLC Studies

- Solvent system: Petroleum ether : Ethyl acetate (3:1)
- Rf value: 0.7

Physical Properties

- Molecular formula: $C_{13}H_{17}N_3O$
- Molecular weight: 231.14 g/mol
- Melting point: 299 °C
- Recrystallization solvent: Ethanol
- Percentage yield: 87%

Spectral Data

IR (KBr, cm^{-1}):

1374.63, 1322.84 (aromatic C–N stretch),
1657.82 (C=O stretch),
2968.97, 2922.48 (CH_2 methylene),
1444.36 (CH_2 stretch)

Biological Evaluation

Bacterial infections may result in severe tissue damage and life-threatening diseases such as food poisoning, rheumatic fever, and diarrhoea, particularly in developing countries. More than 50 million people worldwide are affected, with approximately 110,000 deaths reported annually. The rapid increase in bacterial resistance has created an urgent need for the discovery and development of new antibacterial agents.

Antibacterial Activity

The synthesized compounds were evaluated for antibacterial activity against **Escherichia coli** (Gram-

negative, NCIM 2068) and **Staphylococcus aureus** (Gram-positive, NCIM 2076) using the **agar diffusion (cup-plate) method**.

The antibacterial activity was assessed by measuring the **zone of inhibition**, which reflects the ability of the compounds to inhibit bacterial growth. The test compounds were prepared at concentrations of **50 µg/ml and 100 µg/ml**, while **amikacin (500 µg/ml)** was used as the standard drug. Dimethyl sulfoxide was employed as the control solvent.

Preparation of Nutrient Agar Medium

Composition (per 1000 ml)

- Peptone: 5.0 g
- Sodium chloride: 5.0 g
- Beef extract: 1.5 g
- Yeast extract: 1.5 g
- Agar: 15.0 g
- Distilled water: up to 1000 ml
- pH: 7.0

The medium was sterilized by autoclaving at 15 lb/in^2 for 20 minutes. After cooling to 40 °C, bacterial inoculum was added and poured into Petri plates under aseptic conditions.

Results and Discussion

- Benzimidazole derivatives (1a and 1b) were synthesized by condensation of *o-phenylenediamine* with benzoic acid and formic acid, respectively.
- These compounds were further reacted with chloroacetyl chloride in the presence of 10% NaOH to yield 1-substituted benzimidazole chloro derivatives (2a–2b).
- The chloro derivatives were refluxed with various amines (methylamine and diethylamine) to obtain the final compounds (3a–3d) in good yields.
- Reaction progress was monitored using TLC.
- Purification was achieved by recrystallization using ethanol or chloroform.
- All synthesized compounds were characterized by physical and IR spectral data.
- Antibacterial screening revealed that several compounds exhibited significant activity against both **Gram-positive (S. aureus)** and **Gram-negative (E. coli)** bacteria at concentrations of **50 µg/ml and 100 µg/ml [15]**.

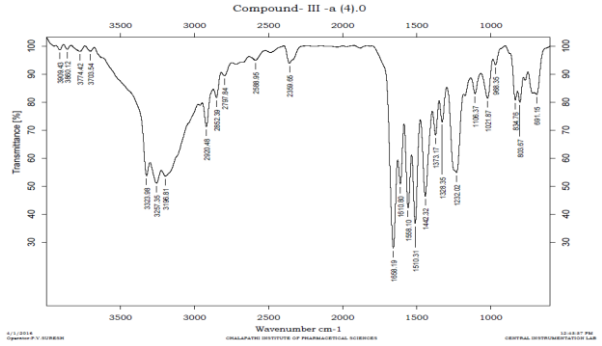


Fig 01

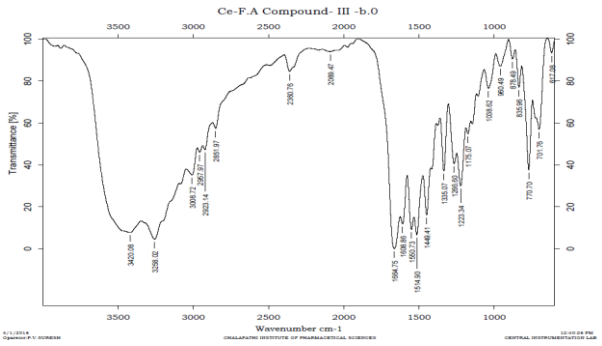


Fig 02

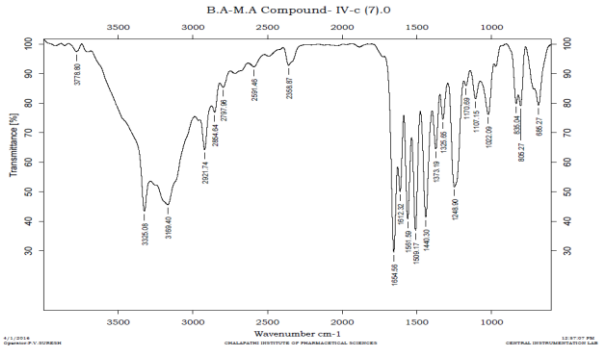


Fig 03

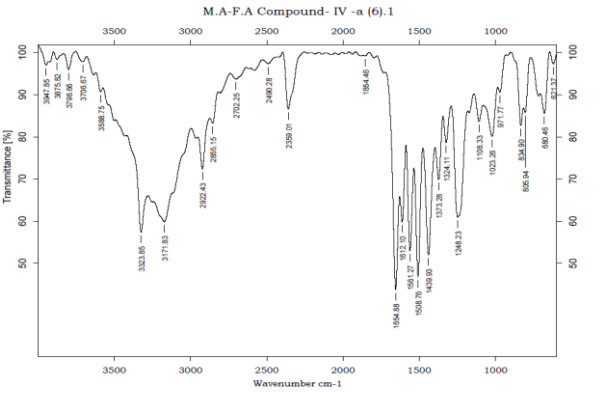


Fig 04

Conclusion

- Substituted benzimidazoles derivatives were synthesized utilizing o-phenylene diamine and substituted acids with amines results in good yields.
- All synthesized compounds were characterized by physical (Rf value, M.P) and spectral data (IR data).
- Later all the compounds were screened for antibacterial activity.

- Further spectral studies (NMR and Mass spectroscopy) are necessary to characterize the compounds completely and planned to evaluate for anti-helminthic and anti-inflammatory activity.

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None

Conflict of Interest
The authors declare no conflict of interest.

Informed Consent
No confirming informed consent required

Ethical Statement
No ethical approval required

Author Contribution
Both authors are contributed equally.

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