JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF NOVEL BENZIMIDAZOLE DERIVATIVES

Sakshi Santosh Sonwane*, Shaikh Risal, Dr. Jeevan Ghormade, Isha Chouhan
Thakur Shiv kumar Singh Memorial Pharmacy College, Burhanpur, M.P. India

Abstract : Benzimidazole is an aromatic heterocyclic compound composed of a fused ring system that integrates a benzene ring with an imidazole ring. Derivatives of benzimidazole, which are nitrogen-containing heterocycles, have attracted significant attention due to their broad spectrum of biological and pharmacological activities. These include antibacterial, anti-inflammatory, anti-ulcer, anti-diabetic, and other therapeutic effects. Researchers have identified that the benzimidazole scaffold offers modifiable sites, particularly at the 2nd and 5th positions, which can be functionalized to enhance biological efficacy. This study focuses on the synthesis of various phenyl-substituted benzimidazole derivatives and evaluates their antibacterial potential.

***IndexTerms*- Heterocyclic compounds, Aromatic systems, Benzimidazole derivatives, Antibacterial activity, Anti-inflammatory, Anti-ulcer.**

I. Introduction

Benzimidazole is a heterocyclic aromatic organic compound. This bicyclic compound may be viewed as fused rings of the aromatic compound benzene and imidazole. It is a white solid that appears in form of tabular crystals.[1]
Benzimidazole was discovered during research on vitamin B_{12}. The benzimidazole nucleus was found to be a stable platform on which drugs could be developed.[2] Benzimidazole is produced by condensation of o-phenylenediamine with formic acid,[3] or the equivalent trimethyl orthoformate:

$C_6H_4(NH_2)_2 + HC(OCH_3)_3 \rightarrow C_6H_4N(NH)CH + 3\ CH_3OH$

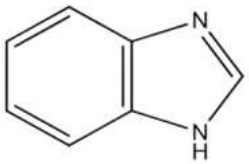

Fig. 1 Benzimidazole

2-Substituted derivatives are obtained when the condensation is conducted with aldehydes in place of formic acid, followed by oxidation .[4]
Benzimidazole are fused heterocyclic ring systems containing two nitrogen atoms. They have vital therapeutic significance in drug discovery. Many clinically approved drugs have been developed from benzimidazole, and these include liarozole and Parecinostat (anticancer), omeprazole (proton pump inhibitors), oxfendazole (Anthelmintic), enviroxine (antiviral), ilaprazole (antiulcer), ridinilazole (antibacterial), flubendazole (antiparasitic), bilastine (antihistaminic), and many more. The vast therapeutic applications of benzimidazole and its derivatives have propelled many researchers to develop more biologically active compounds bearing benzimidazole, thus broadening the scope of finding a remedy for other diseases; as a result, many new pharmaceutical drugs containing benzimidazole are expected to be available within the next decade. In this review, we describe bioactive benzimidazole hybrids in the recent year, from 2020 to 2022, to accentuate the pros of using benzimidazole in drug development.
Benzimidazole derivatives are among the most frequently used ring systems for small molecule drugs listed by the United States Food and Drug Administration .[5] Many pharmaceutical agents belong to the benzimidazole class of compounds. For example:

- Angiotensin II receptor blokers such as azilsartan candesartan and telmisartan.

- Anthelmintic agents such as albendazole, ciclobendazole,fenbendazole,fkubendazole, mebendazole oxfendazole, oxibendazole, triclabendazole, and thiabendazole.These drugs work by binding tubulin, a vital part of the cytoskeleton and mitotic spindle. Benzimidazoles are selectively toxic towards parasitic nematodes, selectively binding and depolymerising their tubulins.[6]
- Antihistamines such as astermizole, bilastine, clemizole, emedastine, mizolastine and oxatomide.
- Benzimidazole fungicides suc as benomyl, carbendazim, fuberidazole and thiabendazole. These drugs selectively bind to and depolymerise fungal tubulin.[6]
- Benzimidazole opioids such as bezitramide, brorphine, clonitazene, etodesnitazene, etonitazene, etonitazenepipne, etonitazepyne, isotonitazene, metodenitazene and metonitazene.
- Proton pump inihibitores such as dexlansoprazole, esomeprazole, ilaprazole, lansoprazole, omeprazole, pantoprazole, rabeprazole, and tenatoprazole.
- Typical antipsychotics such as benperidol, clopimozide, droperidole, clopimozide droperidole, neflumozide, and oxiperomide ans pimozide.
- Other notable pharmaceutical agents which contain a benzimidazole group include abemaciclib, bendamustine, dabigatran daridorexant, and glasdegib.

II. SIGNIFICANCE OF RESEARCH

Antibiotic-resistant bacteria have become a major issue as their occurrence has skyrocketed in recent years, and health-care-associated infections have emerged as a major cause of morbidity and mortality in reality, the increased prevalence of MDR pathogens, which pose a significant threat to antimicrobial therapy, is making the management of bacterial infections more difficult.

The enormous potentiality of benzimidazole-based compounds in medicinal chemistry has led to a lot of work being directed towards the feasible prolific applications of benzimidazole derivatives in diverse areas

Hence it was envisioned to synthesize some novel benzimidazole derivatives using method as far as possible and assess them for antimicrobial activity

III. PLAN OF WORK

The entire research work is phased as under

1. Literature Survey
2. Procurement of chemicals
3. Design of synthetic Scheme
4. Synthesis of benzimidazole compounds according to the optimized scheme
5. Characterization of the synthesized compounds.
   - ❑ Colour, yield, melting point
   - ❑ Solubility analysis
   - ❑ Rf value (by TLC)
6. Antimicrobial evaluation of the synthesized compounds by Cup and plate method to determine zone of inhibition

IV. RESEARCH METHADOLOGY

4.1 Method for synthesis

The scheme for the synthesis of the benzimidazoles was designed for reported methods and modified for synthesis of derivatives. The entire synthetic scheme was performed in two distinct steps:

1. Synthesis of 2-(4-nitrophenyl) benzomidazole.
2. Synthesis of 5-nitro-2-(4-nitrophenyl)-1H-benzomidazole

4.2 Synthesis of benzimidazole with formic acid

Weight accurate 30gm of O-phenylenediamine add 20 ml of formic acid and pour it on mix it mixture well in RBF (round bottom flask) then put the mixture on water bath /heating mentle at 100^0C for 2 hour, Preapare the 10% solution of NaOH (10 gm of Na Now allow to cool it after 2 hour, Add the solution in mixture drop by drop, then PPT particals are formed the dissolve the particals in ethanol because the sodium are react in this and formed salt then salt are dissolve in ethanol and evaporate with ethanol then recrystalised and benzimidazole crystal are formed

4.3 Synthesis of benzimidazole with benzoic acid

Weight accurate 30gm of O-phenylenediamine add 20gm of benzoic acid add 20 ml of water and pour it on mix it mixture well in RBF (round bottom flask) then put the mixture on water bath /heating mentle at 100^0C for 2 hour, Preapare the 10% solution of NaOH (10 gm of Na Now allow to cool it after 2 hour, Add the solution in mixture drop by drop, then PPT particals are formed the dissolve the particals in ethanol because the sodium are react in this and formed salt then salt are dissolve in ethanol and evaporate with ethanol then recrystalised and benzimidazole crystal are formed

PHYSIOCHEMICAL CHERACTERIZATION OF SYNTHESIZED COMPOUND

- Organoleptic properties
- Determination of Solubility
- TLC Method (RF Value)
- Determination of Melting point
- Antibacterial Activity

V. EVALUATION

5.1 Organoleptic evaluation:- Organoleptic evaluation refers to the evaluation of formulation by colour, odour, taste and texture. Organoleptic Techniques: How Testing is Performed. Organoleptic testing is not merely tasting or smelling a product; it's an analytical process that quantifies the sensory experience to provide objective assessments. How exactly is this intricate procedure conducted? Let's take a detailed walk-through.

Step 1: Select a Trained Expert

The first step is to choose trained experts with superior sensory acuity and the ability to articulate their sensory experiences. Organoleptic experts undergo rigorous training to identify taste profiles, textures, aromas, and visual aspects of food and beverages. To conduct seafood decomposition analysis for FDA imports, analysts must undergo training provided by the FDA.

Step 2: Sample Preparation

Next, samples are prepared under controlled conditions to maintain consistency. This may involve serving the food at a specific temperature or using standardized containers to eliminate visual bias. It's important to preserve the product's inherent organoleptic properties during the preparation process. Step 3: Blind Analysis Sessions

In blind analysis sessions, the analyst evaluates samples without any branding or packaging that could influence their judgment. They rely solely on their senses to evaluate the product.

Step 4: Evaluation and Scoring

The analyst assesses the products based on specific criteria like taste, aroma, texture, and appearance. They use a standardized vocabulary to describe their sensory experiences accurately. The analyst scores the product for an overall assessment of its organoleptic properties.

Step 5: Analysis and Interpretation

Finally, a sensory scientist analyzes and interprets the scores and feedback. They use statistical methods to understand the data and draw conclusions about the product's organoleptic quality. This step-by-step procedure ensures a thorough, objective, and standardized assessment of a product's organoleptic properties. It goes beyond taste and smell, delving into the intricacies of sensory perception and its impact on consumer behavior.

5.2 Melting point :-Melting points are often used to characterize organic and inorganic crystalline compounds and to ascertain their purity. Pure substances melt at a sharp, highly-defined temperature (very small temperature range of 0.5 – 1 °C) whereas impure, contaminated substances generally exhibit a large melting interval. The temperature at which all material of a contaminated substance is molten is usually lower than that of a pure substance. This behavior is known as melting point depression and can be used to obtain qualitative information about the purity of a substance. In general, melting point determination is used in the lab in research and development as well as in quality control in various industry segments to identify and check the purity of different substances.

Melting point is a characteristic property of solid crystalline substance. It is the temperature at which the solid phase changes to the liquid phase. This phenomenon occurs when the substance is heated. During the melting process, all of the energy added to the substance is consumed as heat of fusion, and the temperature remains constant (see diagram below). During the phase transition, the two physical phases of the material exist side-by-side.

Crystalline materials consist of fine particles that for a regular, 3-dimensional arrangement – a crystalline lattice. The particles within the lattice are held together by lattice forces. When the solid crystalline material is heated, the particles become more energetic and start to move more strongly, until finally the forces of attraction between them are no longer strong enough to hold them together. The Crystalline structure is destroyed and the solid material melts. The stronger the forces of attraction between the particles, the more energy is needed to overcome them. The more energy is needed, the higher the melting point. The melting temperature of a crystalline solid is thus an indicator for the stability of its lattice. At the melting point not only the aggregate state changes; quite a lot of other physical characteristics also change significantly. Amongst these are the thermodynamic values, specific heat capacity, enthalpy, and rheological properties such as volume or viscosity. Last but not least, the optical properties birefringence reflection and light transmission change. Compared to other physical values the change in light transmission can easily be determined and can therefore be used for melting point detection.

5.2.1Melting Point Determination Principle

At the melting point, there is a change in light transmission. Compared to other physical values the change in light transmission can easily be determined and can therefore be used for melting point detection. Powdered crystalline

materials are opaque in the crystalline state and transparent in the liquid state. This distinct difference in optical properties can be measured in order to determine the melting point by recording the percentage of light intensity shining through the substance in the capillary, the transmittance, in relation to the measured furnace temperature.
There are different stages of the melting point process of a solid crystalline substance: At the collapse point, the substance is mostly solid and comprises only a small amount of molten material. At the meniscus point, most of the substance has melted but some solid material is still present. At the clear point, the substance has completely melted.

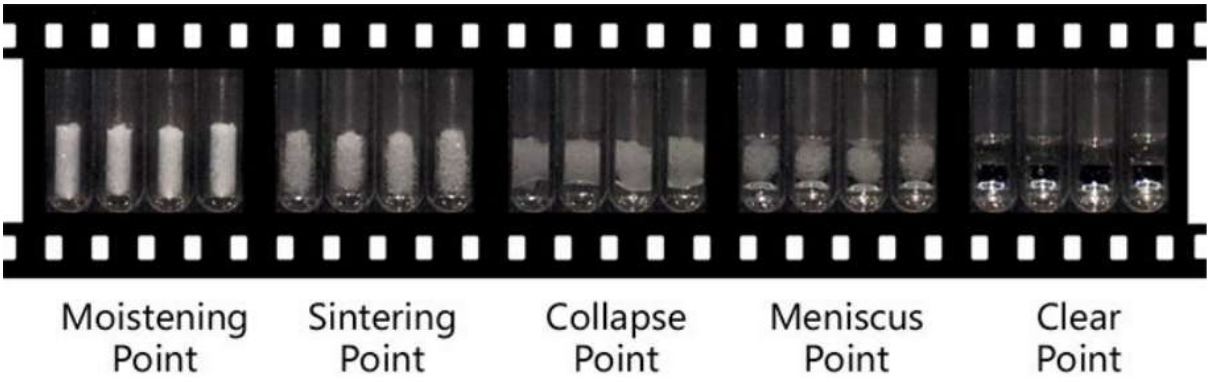

Figure no.2 Principle or melting point determination

5.2.2 The Capillary Method

The melting point measurement is usually performed in thin glass capillary tubes with an internal diameter of 1 mm and a wall thickness of 0.1 – 0.2 mm. A finely-ground sample is placed in the capillary tube to a filling level of 2 – 3 mm and introduced in a heated stand (liquid bath or metal block) in close proximity to a high accuracy thermometer. The temperature in the heating stand is ramped at a user-programmable fixed rate. The melting process is visually inspected to determine the melting point of the sample. Modern instruments, like the melting point excellence instrument by METTLER TOLEDO, enable automated detection of the melting point and melting range and visual inspection by a video camera. The capillary method is required in many local pharmacopeias as the standard technique for melting point determination. With the Melting Point Excellence instruments by METTLER TOLEDO up to 6 capillaries can be measured at the same time.

5.3.3 Instrument Setup

Along with proper sample preparation, the settings on the instrument are as well essential for the exact determination of the melting point. Correct selection of the start temperature, the end stop temperature and the heating ramp rate are necessary to prevent inaccuracies due to a heat increase in the sample that is incorrect of too fast:

a) Start Temperature

Melting point determination starts at a predefined temperature close to the expected melting point. Up to the start temperature, the heating stand is rapidly preheated. At the start temperature the capillaries are introduced into the furnace, and the temperature starts to rise at the defined heating ramp rate.
Common formula to calculate the start temperature: Start Temperature = expected MP – (5 min * heating rate)

b) Heating Ramp Rate

The heating ramp rate is the fixed rate of temperature rise between the start and stop temperatures for the heating ramp. Results depend strongly on the heating rate - the higher the heating rate the higher the observed melting point temperature. Pharmacopeias apply a constant heating rate of 1 °C/min. For highest accuracy and non-decomposing samples use 0.2 °C/min. With substances that decompose, a heating rate of 5 °C/min should be applied. For exploratory measurements a heating rate of 10 °C/min may be used.

c) Stop Temperature

The maximum temperature to be reached in the determination. Common formula to calculate the stop temperature. Stop Temperature = expected MP + (3 min * heating rate).

Figure no.3 Melting point apparatus

5.3 Solubility :-The **solubility**, which is also known as the **solubility limit**, of a solute corresponds to the maximum amount of that chemical that can dissolve in a given amount of solvent to check that benzimidazole is soluble in which solvent.

Because, based on this definition, a solubility limit is a ratio of the amount of solute that can dissolve, relative to the quantity of solvent that is utilized to prepare the corresponding solution, solubilities are typically expressed as proportions, as shown below.

Maximum Amount of Solute
Given Amount of Solvent

A solution is a homogeneous mixture of one or more solutes in a solvent. Sugar cubes added to a cup of tea or coffee are a common example of a solution. The property which helps sugar molecules to dissolve is known as ***solubility***. Hence, the term solubility can be defined as a property of a substance (solute) to dissolve in a given ***solvent***. A ***solute*** is any substance which can be either solid or liquid or gas dissolved in a solvent. On the basis of solubility, the factors affecting solubility vary on the state of the solute:

A. Liquids In Liquids:- Solubility is the new bond formation between the solute molecules and solvent molecules. In terms of quantity, solubility is the maximum concentration of solute that dissolves in a known concentration of solvent at a given temperature. Based on the concentration of solute dissolves in a solvent, solutes are categorized into highly soluble, sparingly soluble or insoluble. If a concentration of 0.1 g or more of a solute can be dissolved in a 100ml solvent, it is said to be soluble. While a concentration below 0.1 g is dissolved in the solvent it is said to be sparingly soluble. Thus, it is said that solubility is a quantitative expression and expressed by the unit gram/litre (g/L).

B. Solids In Liquids:- It has been observed that solid solubility depends on the nature of the solute as well as the solvent. We often see that substances like sugar, common salt (NaCl), etc readily dissolve in water while substances like naphthalene do not dissolve in water. From the various observations and experimental results, it has been seen that only polar solutes tend to dissolve in the polar solvent and non-polar solvents dissolve only non-polar solutes. Hence, the nature of the solvent can be seen as one of the prominent factors affecting solubility. The above observation led to the statement that like dissolves like, that is polar solvents will dissolve polar solutes and non-polar solvents dissolve non-polar solutes.

C. Gases In Liquids:- Gas solubility in liquids deals with the concept of gas dissolving in a solvent. Let us first define solubility. For any substance, solubility is the maximum amount of solute that can be dissolved in a given solvent at a particular temperature. Now our concern is gas solubility in liquids. The gas solubility in liquids is greatly affected by temperature and pressure as well as the nature of the solute and the solvent.

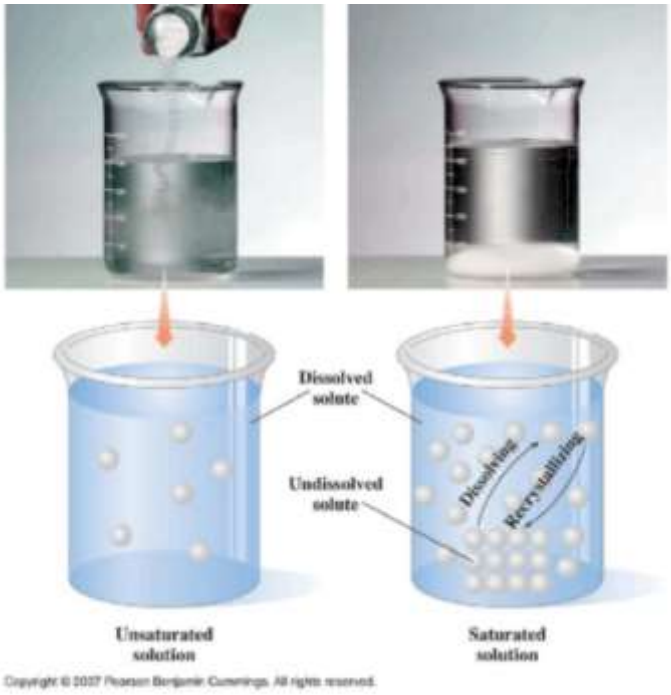

Figure no.4 solubility tests

5.4 TLC (RF Value):- The qualitative evaluation is slowly based on the determination of Rf values of unknown spots vis-a-vis Rf values of standard substances. The Rf value (Retention Factor) various separated solutes is determined accurately. Thin layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound. TLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation. TLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. The goal of TLC is to obtain well defined, well separated spots.[59]

5.4.1 Retention Factor

After a separation is complete, individual compounds appear as spots separated vertically. Each spot has a retention factor (Rf) which is equal to the distance migrated over the total distance covered by the solvent. The Rf formula is

$$Rf = \frac{\text{distance traveled by sample}}{\text{distance traveled by solvent}}$$

The Rf value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions, the compound with the larger Rf value is less polar because it does not stick to the stationary phase as long as the polar compound, which would have a lower Rf value.

5.4.2 Apparatus

Plates (Stationary Phase)

As stated earlier, TLC plates (also known as chromate plates) can be prepared in the lab, but are most commonly purchased. Silica gel and alumina are among the most common stationary phases, but others are available as well. Many plates incorporate a compound which fluoresces under short-wave UV (254 nm). The backing of TLC plates is often composed of glass, aluminum, or plastic. Glass plates are chemically inert and best withstand reactive stains and heat, but are brittle and can be difficult to cut. Aluminum and plastic plates can be cut with scissors, but aluminum may not withstand strongly acidic or oxidizing stains, and plastic does not withstand the high heat required to develop many stains. Aluminum and plastic plates are also flexible, which may result in flaking of the stationary phase. Never under any circumstances touch the face of a TLC plate with your fingers as contamination from skin oils or residues on gloves can obscure results. Instead, always handle them by the edges, or with forceps.

The properties of your sample should be considered when selecting the stationary phase, silica gel can be exclusively used for amino acids and hydrocarbons. It is also important to note that silica gel is acidic. Therefore, silica gel offers poor separation of basic samples and can cause a deterioration of acid-labile molecules. This would be true for alumina plates in acidic solutions as well. It is important to note that there are differences between silica gel and alumina. Alumina is basic and it will not separate sample sizes as large as silica gel would at a given layer thickness. Also, alumina is more

chemically reactive than silica gel and as a result, would require more care of compounds and compound classes. This care would avoid decomposition and rearrangement of the sample.[60]

Figure no. 5 TLC Method

Table no: 1 Stationary phase and mode of separation

Stationary Phase	Chromatographic Mechanism	Typical Application
Silica Gel	adsorption	steroids, amino acids, alcohols, hydrocarbons, lipids, aflaxtoxin, bile, acids, vitamins, alkaloids
Silica Gel RP	reversed phase	fatty acids, vitamins, steroids, hormones, carotenoids
Cellulose, kieselguhr	partition	carbohydrates, sugars, alcohols, amino acids, carboxylic acids, fatty acids
Aluminum oxide	adsorption	amines, alcohols, steroids, lipids, aflatoxins, bile acids, vitamins, alkaloids
PEI cellulose	ion exchange	nucleic acids, nucleotides, nucelosides, purines, pyrimidines
Magnesium silicate	adsorption	steroids, pesticides, lipids, alkaloids

5.4.3 Solvent (Mobile Phase)

Proper solvent selection is perhaps the most important aspect of TLC, and determining the best solvent may require a degree of trial and error. As with plate selection, keep in mind the chemical properties of the analytes. A common starting solvent is 1:1 hexane:ethyl acetate. Varying the ratio can have a pronounced effect of Rf values range from 0 to 1 with 0 indicating that the solvent polarity is very low and 1 indicating that the solvent polarity is very high. When performing your experiment, you do not want your values to be 0 or 1 because your components that you are separating have different polarities. If the value is 0, you need to increase your solvent polarity because the sample is not moving and sticking to the stationary phase. If the value is 1, you need to decrease your solvent polarity because the compound was not able to separate.

If you know that one component of a mixture is insoluble in a given solvent, but another component is freely soluble in it, it often gives good separations. How fast the compounds travel up the plate depends on two things:

- If the compound is soluble in the solvent, it will travel further up the TLC plate
- How well the compound likes the stationary phase. If the compound likes the stationary phase, it will stick to it, which will cause it to not move very far on the chromatogram.

5.4.4 Pipettes

- Spots are applied to the plate using very thin glass pipettes. The capillary should be thin enough to apply a neat spot, but not so thin as to prevent the uptake of an adequate quantity of analyte. Here is a popular method of producing TLC pipettes.
- Heat a glass capillary in the very tip of a Bunsen burner flame just until it becomes pliable and then pull the ends apart until the center of the capillary is significantly narrower. Snap this in half and use the thin end to apply spots.

5.4.5 Spotting and Developing

Developing a TLC plate requires a developing chamber or vessel. This can be as simple as a wide-mouth jar, but more specialized pieces of glassware to accommodate large plates are available. The chamber should contain enough solvent to just cover the bottom. It should also contain a piece of filter paper, or other absorbent material to saturate the atmosphere with solvent vapors. Finally, it should have a lid or other covering to minimize evaporation.

1. Cut the plate to the correct size and using a pencil (never ever use a pen), gently draw a straight line across the plate approximately 1 cm from the bottom. Do not use excessive forces when writing on a TLC plate as this will remove the stationary phase. It is important to use a pencil rather than a pen because inks commonly travel up the plate with the solvent. An example of how black ink separates is shown in the section labeled "examples".
2. Using TLC pipettes, apply spots of analyte to the line. Make sure enough sample is spotted on the plate. This can be done by using the short-wave UV. A purple spot should be seen. If the spot is not visible, more sample needs to be applied to the plate. If a standard of the target compound is available, it is good practice to produce a co-spot by spotting the standard onto a spot of the unknown mixture. This ensures the identity of the target compound.
3. Place the plate into the chamber as evenly as possible and lean it against the side. Never allow the bulk solvent to rise above the line you drew. Allow capillary action to draw the solvent up the plate until it is approximately 1 cm from the end. Never allow the solvent to migrate all the way to the end of the plate.
4. Remove the plate and immediately draw a pencil line across the solvent front.
5. Use a short-wave UV light and circle the components shown with a pencil.

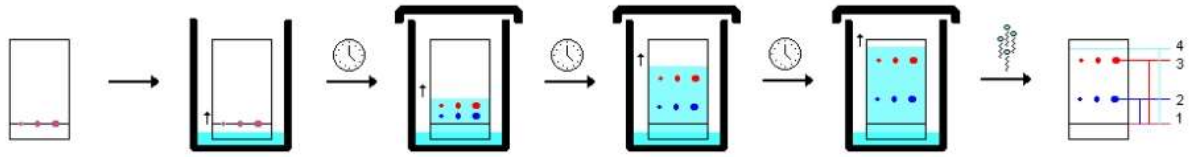

Figure no.6 TLC Spotting and Developing

5.4.6 Visualizing

If fluorescent plates are used, a number of compounds can be seen by illuminating the plate with short-wave UV. Quenching causes dark spots on the surface of the plate. These dark patches should be circled with a pencil. For compounds which are not UV active, a number of chemical stains can be used. These can be very general, or they can be specific for a particular molecule or functional group.

Iodine is among the most common stains. Plates are placed in a jar containing iodine crystals, or covered in silica gel with iodine dispersed throughout, for approximately one minute. Most organic compounds will be temporarily stained brown. Some popular general use stains are Permanganate, ceric ammonium molybdate (CAM), and p-anisaldehyde. These can be kept in jars which plates are dipped into, or in spray bottles.

To develop a plate with permanganate, spray or dip the plate and heat it with a heat-gun. Hold the plate face up 10 to 20 cm above the heat gun until the bulk water evaporates. Then move the plate to 5 to 10 cm above the heat gun and heat it until white/yellow/brown spots appear. Overheating will turn the entire plate brown, obscuring the spots. If glass plates are used it is often easier to see spots through the backing because it is harder to overheat. CAM and p-anisaldehyde stained plates are developed similarly. Overheating CAM stained plates turns everything blue.[61]

Methods to evaluate antimicrobial activity

The relentless quest for novel and highly effective antimicrobial compounds remains an ongoing pursuit driven by the pressing challenge of antimicrobial resistance, which diminishes the effectiveness of traditional antibiotics. To assess potential antimicrobial compounds, whether recently discovered or well-known, researchers employ a wide array of antimicrobial assay techniques. Among these, the following antimicrobial assay methods are extensively used for the identification and evaluation of antimicrobial activity.

The Cup and Plate Method is a microbiological technique used primarily for the quantitative and qualitative assessment of antimicrobial activity or for the enumeration of microorganisms. It is commonly applied in pharmaceutical, food, and clinical microbiology settings. Below is a clear description:

5.5 Procedure of antibacterial testing

The sterilized media (nutrient agar) was cooled to 45^0c with gentle shaking for uniform cooling and then inoculated with 18 -24 h old bacterial subculture under aseptic conditions in a laminar air flow bench and mixed well by gentle shaking. This was poured in to sterile petri dishes and allowed to set. After solidification all the petri dishes were transferred to laminar flow bench and mixed well by gentle shaking. This was poured in to petri dishes and allowed to set. After solidification all the petri dishes were transferred to laminar flow unit and the test sample discs were carefully kept on the solidified media by using sterilized forceps. These petri dishes were kept in the laminar air flow unit undisturbed for one hour diffusion at room temperature and then for incubation at 37^0c for 24 h an incubator. The extent diameter of inhibition after 24 h was measured as the zone of inhibition in millimeters (mm).

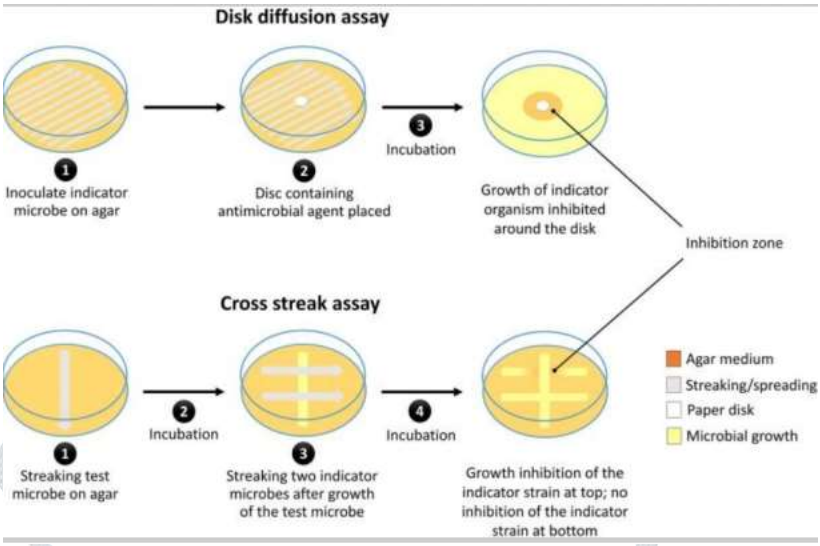

Figure no. 7 Anti-microbial evaluation by Cup and Plate Method

VI. RESULT AND DISCUSSION

Table no. 2:- organoleptic property of benzimidazole

Compound no.	Color	Picture
Benzimidazole 01	Orange	
Benzimidazole 02	Transparent	
Benzimidazole 03	Brown	
Benzimidazole 04	Transparent	

Benzimidazole 05	Yellowish orange	

Table no. 3:- Percent yield of benzimidazole

Compound no.	% yield
Benzimidazole 01	93.23%
Benzimidazole 02	63.68%
Benzimidazole 03	83.34%
Benzimidazole 04	77.08%
Benzimidazole 05	59.10%

Table no. 4:- Solubility test of benzimidazole

s. no	compound name (solute)	Chemical name (solvent)	Soluble/insoluble
1	Benzimidazole	Ethanol	Soluble
2	Benzimidazole	Benzene	Mild soluble
3	Benzimidazole	Ethylacetoaceta	Soluble
4	Benzimidazole	NaOH	Insoluble
5	Benzimidazole	Dil. HCL	Insoluble
6	Benzimidazole	Water	Insoluble
7	Benzimidazole	Dis. Water	Insoluble
8	Benzimidazole	Nitrobenzene	Soluble

Table no.5 :- crystal shapes of benzimidazole

Compound no.	Type of crystal	Picture
Benzimidazole 01	Tabular (plate like)	
Benzimidazole 02	Needle	
Benzimidazole 03	Acicular	
Benzimidazole 04	Needle	
Benzimidazole 05	Tabular (Thin plate like)	

Table no. 6:- Melting point of benzimidazole

Compound no.	Melting point (◦C)
Benzimidazole 01	293-295
Benzimidazole 02	268-271
Benzimidazole 03	276-278
Benzimidazole 04	284-287
Benzimidazole 05	290-293

Table no. 7:- Rf value of benzimidazole

Compound no.	Rf value
Benzimidazole 01	0.56
Benzimidazole 02	0.67
Benzimidazole 03	0.51
Benzimidazole 04	0.72
Benzimidazole 05	0.75

Table no. 8:- Antimicrobial evaluation

The result revealed that the antibacterial action of the synthesized compound was dose dependent. The compounds were mild to moderately antibacterial. The presence of electron withdrawing group in the compound fovourd antibacterial activity against gram positive (BZI_2, BZI_4, & BZI_5) wheras elctron donating group favourd activity against gram positive (BZI_3). Compound 5a did not exibit significance antibacterial action as compared to the control (DMF). The lack of significant activity in BZI_1 signifies the important of tge substitution of ring attached to 1-position of benzimidazole nucleus for antibacterial action. The positional effect of the substitution on this ring was though not studied.

Antibacterial action

The antibacterial activity of the synthesized benzimidazoles was determined measuring the zone of inhibition in the agar plate. Three concentrations of synthesized compounds were tested for antibacterial action against ciprofloxacin as the standard drug for antibacterial action. The zone of inhibition of the test compounds is presented in table:-

Table no. 8 :- Antibacterial activity of the compounds

Compound Code	Zone Of Inhibition (Mm)*											
	B. Subtilis			S. auerus			E. Coli			Salmonella		
	25 µ g	50 µ G	100 µ g	25 µ g	50 µ G	100 µ g	25 µ g	50 µ g	100 µ g	25 µ g	50 µ g	100 µ g
BZI_1	4	6	7	4	7	10	6	8	12	7	10	13
BZI_2	6	9	13	5	9	14	10	15	**23**	9	15	**24**
BZI_3	9	15	**24**	8	16	**25**	6	9	13	6	8	13
BZI_4	6	9	13	5	9	14	10	16	**25**	9	16	**24**
BZI_5	6	8	12	6	10	13	11	14	**23**	10	15	**23**
Ciprofloxaci n	15	**21**	**33**	13	**22**	**27**	15	**23**	**35**	16	**22**	**36**

*Below 12mm- Poor activity; 13-18mm – moderate activity & above 18mm – good activity

VII. CONCLUSION

From the literature review, it is observed that functional group present on molecule plays important role in physicochemical properties showing by molecule. To discover better medicinal agent, researcher should understand the relative contributions of each functional group. Benzimidazole molecule played an important role in medicinal chemistry as it is bioactive and structurally simple heterocyclic compound. It can become a part of development and discovery of new drugs with potential biological activity. During last decade efforts has been taken to synthesize medicinally important benzimidazole derivatives and researchers discovered many benzimidazole derivatives showing promising biological activity. In present review efforts are taken to summarize synthesis of different derivative of benzimidazole along with their biological activity. It is hope that, this review will benefit to budding researchers in the field of benzimidazole based drug design.

VIII. REFERENCES

1. Benzimidazole | CAMEO Chemicals | NOAA". cameochemicals.noaa.gov. Retrieved 2023-01-11.

2. *Bennet-Jenkins, E.; Bryant, C. (1996).* "Novel sources of anthelmintics". *International Journal for Parasitology.* ***26*** *(8–9): 937–947*

3. E. C. Wagner, W. H. Millett (1939). "Benzimidazole". Organic Syntheses.

4. *Smiley, Robert A. (2000), "Phenylene- and Toluenediamines", Ullmann's Encyclopedia of Industrial Chemistry.*

5.Taylor, R. D.; MacCoss, M.; Lawson, A. D. G. J Med Chem 2014, 57, 5845.>

6.^ Jump up to:*[a] [b] Wang, C. C. (January 1984).* "Parasite enzymes as potential targets for antiparasitic chemotherapy". *Journal of Medicinal Chemistry.*

7. *Berneth, Horst (2008), "Methine Dyes and Pigments", Ullmann's Encyclopedia of Industrial Chemistry,*

8. E. Vitaku, D.T. Smith, J.T. Njardarson Analysis of the structural diversity, substitution patterns, and frequency of nitrogen heterocycles among U.S. FDA approved pharmaceuticals J. Med. Chem., 57 (2014), pp. 10257-10274,

9. J.B. Wright The chemistry of the benzimidazoles Chem. Rev., 48 (1951), pp. 397-541,

10. U.A. Çevik, I. Celik, A. Işık, R.R. Pillai, T.E. Tallei, R. Yadav, Y. Özkay, Z.A. Kaplancıklı Synthesis, molecular modeling, quantum mechanical calculations and ADME estimation studies of benzimidazole-oxadiazole derivatives as potent antifungal agents J. Mol. Struct., 1252 (2022), Article 132095,
11. V. Rep, R. Štulić, S. Koštrun, B. Kuridža, I. Crnolatac, M.R. Stojković, H.Č. Paljetak, M. Perić, M. Matijašić, S. Raić-Malić Novel tetrahydropyrimidinyl-substituted benzimidazoles and benzothiazoles: synthesis, antibacterial activity, DNA interactions and ADME profiling RSC Med. Chem., 13 (2022), pp. 1504-1525
12. J. Chen, L. Xu, B. Wang, D. Zhang, L. Zhao, Z. Bei, Y. Song Design, synthesis, and biological evaluation of benzimidazole derivatives as potential lassa virus inhibitors *Molecules*, 28 (2023), p. 1579
13. R. Radhamanalan, M. Alagumuthu, N. Nagaraju Synthesis and drug efficacy validations of racemic-substituted benzimidazoles as antiulcer/anti-gastric secretion agents Future Med. Chem., 10 (2018), pp. 1805-1820,
14. E. Mulugeta, Y. Samuel, S.A. Mohamed Synthesis of benzimidazole-sulfonyl derivatives and their biological activities Biochem. Res. Int., 2022 (2022), pp. 1-13
15. M. Ashfaq, S.S. Shah, T. Najam, M.M. Ahmad, R. Tabassum, G. Rivera Synthetic thioamide, benzimidazole, quinolone and derivatives with carboxylic acid and ester moieties: a strategy in the design of antituberculosis agents Curr. Med. Chem., 21 (2014), pp. 911-931,
16. B. Dik, D. Coskun, E. Bahcivan, K. Uney Potential antidiabetic activity of benzimidazole derivative albendazole and lansoprazole drugs in different doses in experimental type 2 diabetic rats Turk. J. Med. Sci., 51 (2021), pp. 1579-1586
17. B. Adalat, F. Rahim, M. Taha, F.J. Alshamrani, E.H. Anouar, N. Uddin, S.A.A. Shah, Z. Ali, Z.A. Zakaria Synthesis of benzimidazole-based analogues as anti-Alzheimer's disease compounds and their molecular docking studies *Molecules*, 25 (2020),
18. S. Kapil, P.K. Singh, A. Kashyap, O. Silakari Structure based designing of benzimidazole/benzoxazole derivatives as anti-leishmanial agents SAR QSAR Environ. Res., 30 (2019), pp. 919-933
19. A.B. Popov, L. Krstulovic, S. Kostrun, D. Jelic, A. Bokulic, M.R. Stojkovic, I. Zonjic, M.C. Taylor, J.M. Kelly, M. Bajic, *et al.* Design, synthesis, anti-trypanosomal activity, DNA/RNA binding and in vitro ADME profiling of novel imidazoline-substituted 2-arylbenzimidazoles Eur. J. Med. Chem., 207(2020), Article 112802,
20. P. Jain, P.K. Sharma, H. Rajak, R.S. Pawar, U.K. Patil, P.K. Singour Design, synthesis and biological evaluation of some novel benzimidazole derivatives for their potential anticonvulsant activity Arch. Pharm. Res., 33 (2010), pp. 971-980,