Development of Benzimidazole Derivatives: Synthesis, Characterization, and Evaluation of Antimicrobial Properties

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***Abstract*— Benzimidazole derivatives are known for their diverse pharmacological properties, including antimicrobial potential. In this study, novel benzimidazole compounds were synthesized starting from o-phenylenediamine and benzoic acid to form Intermediate 1, which was sulfonated with chlorosulfonic acid to yield Intermediate 2. This intermediate was then reacted with aniline under microwave irradiation to produce the final compound. The synthesized compounds were evaluated for antimicrobial activity using the agar diffusion method at concentrations of 10, 20, 30, and 40 µg/ml. The test organisms included Staphylococcus aureus (Gram-positive), Escherichia coli and Pseudomonas aeruginosa (Gram-negative), and Candida albicans (fungus). Amoxicillin served as the reference standard. IR spectroscopy confirmed the structure of compound (1), showing N-H stretching at 1598.17 cm^{-1}. Among the synthesized compounds, Intermediate 1, Intermediate 2, and compound 3a were spectroscopically characterized. The results demonstrated that the synthesized compounds exhibited significant antimicrobial activity, comparable to the standard drug, suggesting their potential as antimicrobial agents.**

***Index Terms*— Antimicrobials, Benzimidazole, Escherichia coli, Staphylococcus aureus**

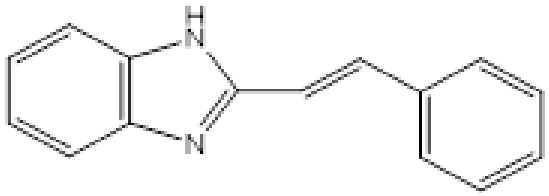
I.INTRODUCTION

Antimicrobials are compounds that are used medicinally to cure or prevent infections. They include antibiotics, antivirals, antifungals, antiparasitic, and antiseptics. Antimicrobial substances called disinfectants are used on inanimate surfaces. By focusing on essential cellular metabolic processes, such as the production of biological macromolecules, the activity of cellular enzymes, or cellular structures like the cell wall and cell membranes, antimicrobials can eradicate germs and/or prevent their development. Whether an ecosystem is naturally occurring or intentionally produced, the presence of antimicrobial compounds always has an ecological impact.

Aquatic ecosystems are especially impacted by the thousands of tons of antimicrobials and their byproducts that are released into the environment each year. Additionally, certain antimicrobials are highly persistent in the environment, which facilitates their accumulation and diffusion throughout different compartments. This type of xenobiotic has ecological effects in both natural and manmade contexts, such as methane fermentation sewage sludge treatment plants and wastewater treatment facilities. One of the main causes of antibiotic resistance in soils and aquatic systems is the continuous exposure of microbial populations to high and sub-inhibitory concentrations of antibiotics. Antibiotic-resistant microorganisms and the spread of genetic resistance features are encouraged by this circumstance. In this regard, environmental biofilms and the varoom that goes along with them act as antimicrobial resistance reservoirs. One strategy for fighting germs that are resistant to different antibiotics is the creation of nanoparticles with antibacterial qualities.

Drinking water is frequently produced and distributed using chlorine-based disinfection techniques. However, increased human exposure to waterborne pathogens may result from the survival and regrowth of bacteria after chlorination, which is ascribed to microbial chlorine resistance and tolerance. While tolerance is associated with temporary phenotypic changes, such as the production of extracellular

polymeric molecules and the development of biofilms, resistance is genetically based and may be inherited.



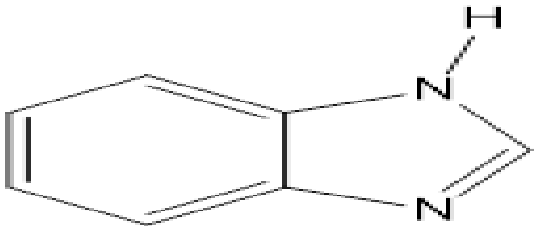
II. BENZIMIDAZOLE

The fundamental structure of benzoimidazole, a kind of heterocyclic aromatic organic molecule, consists of a six-membered benzene ring joined to the fourth and fifth places of a five-membered imidazole ring system. Easy tautomerization of the hydrogen atom bound to the nitrogen at the benzimidazole nucleus's 1-position results in isomerization in the final products. Both modest basicity and somewhat significant acidity are displayed by the NH group in benzimidazole. With ionization constants (pKa) of 12.8 for benzimidazole and 5.6 for its conjugate acid, respectively, it is categorized as an amphoteric molecule.

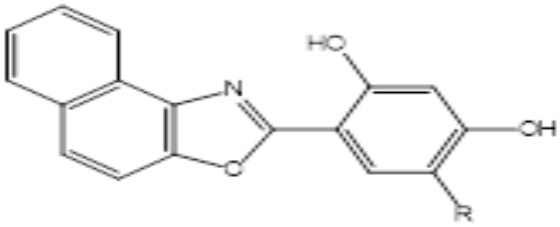
According to published research, several substituents at positions 1, 2, 5, and 6 of the benzimidazole nucleus have been found to have strong analgesic and anti-inflammatory properties. The nucleus's locations four and seven, however, cannot be changed. The 1-position benzimidazole may be unsubstituted (as in TRPV-1 antagonists) or contain cycloalkanes, polyhydroxy sugars, methyl or phenylsulfonyl groups, or aryl/heteroaryl moieties that have been appropriately substituted with electronic, heterocyclic, or alkyl groups. Bulky lipophilic aryl/heteroaryl or alkyl moieties that are further substituted with electronic, heterocyclic, or alkyl groups can likewise take the role of the 2-position. Functional groups such halogens, nitro, amino, methyl, trifluoromethyl, hydroxyl, alkoxy, sulfonyl, or N-sulfonamide, as well as substituted aryl/heteroaryl groups, can be substituted or left unsubstituted at positions 5 or 6 of the nucleus.[6]]

Structure and Properties

Benzimidazole is a heterocyclic aromatic compound composed of a fused ring system, where a six-membered benzene ring is connected to a five-membered imidazole ring at the 4th and 5th positions. A key feature of this structure is the NH group at position 1, which allows tautomerization of the hydrogen atom, potentially resulting in isomerization of derivatives. This NH group shows amphoteric behavior, meaning it can act as both a weak base and a weak acid. The ionization constants reflect this, with a pKa of approximately 12.8 for the neutral molecule and 5.6 for its conjugate acid. Various functional groups can be introduced at positions 1, 2, 5, and 6 to modify the molecule's biological activity, while positions 4 and 7 are typically unreactive and cannot be substituted. Such modifications can enhance its analgesic, anti-inflammatory, and antimicrobial properties



(A)



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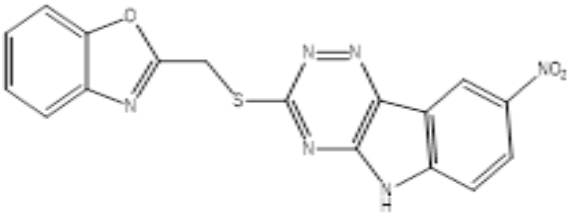

(C)

Reactivity

The benzimidazole nucleus is chemically versatile. The NH group at position 1 can participate in hydrogen bonding and tautomerism, making it reactive toward substitution. The nitrogen at position 1 can be substituted with various moieties such as cycloalkyl groups, polyhydroxy sugars, or phenylsulfonyl derivatives. The C-2 position is also reactive and often substituted with bulky lipophilic groups like aryl or heteroaryl rings, which may be further modified with

electron-donating or withdrawing groups to improve activity. Positions 5 and 6 commonly undergo electrophilic substitution reactions, allowing the introduction of groups like halogens, nitro, sulfonyl, amino, or alkyl groups. These chemical modifications influence the molecule's electron density and overall pharmacological performance.

Physical Properties Benzimidazole has the molecular formula $C_7H_6N_2$ and a molar mass of approximately 118.14 g/mol. It typically appears as a white to off-white crystalline solid and has a melting point in the range of 170–174 °C. It is slightly soluble in water but readily dissolves in organic solvents such as ethanol and DMSO. Benzimidazole is odorless and exhibits strong UV absorption due to its aromatic structure, which is useful in analytical techniques like UV-visible spectroscopy. These physical characteristics, combined with its chemical flexibility, make benzimidazole a valuable scaffold in drug development and research. Benzimidazole ($C_7H_6N_2$) is a heterocyclic aromatic organic compound with a molar mass of approximately 118.14 g/mol. It appears as a white to off-white crystalline solid under standard conditions and is odorless. The compound has a melting point ranging between 170–174 °C and a boiling point around 360 °C, indicating good thermal stability. It is only slightly soluble in water due to its aromatic and relatively non-polar structure but is readily soluble in polar organic solvents such as ethanol, methanol, chloroform, acetone, and dimethyl sulfoxide (DMSO). This solubility profile enhances its compatibility with various chemical reactions and formulations in medicinal chemistry. Benzimidazole exhibits strong ultraviolet (UV) absorption, primarily due to its conjugated aromatic system, making it useful in analytical techniques like UV-Vi's spectroscopy. It is chemically stable under normal conditions and possesses both basic and slightly acidic properties due to the imidazole nitrogen atoms, allowing it to participate in diverse chemical reactions. These attributes make benzimidazole a versatile and valuable core structure in pharmaceutical research and drug design.

III. MATERIAL AND METHOD

Materials

All chemicals necessary for the synthesis of the new test molecule are of standard quality, sourced from LOBACHEME, while ethanol and benzoyl chloride are obtained from MOLYCHEM. All synthesized compounds underwent purification through recrystallization and were characterized using physicochemical and spectral analysis.

1.1 Reaction scheme

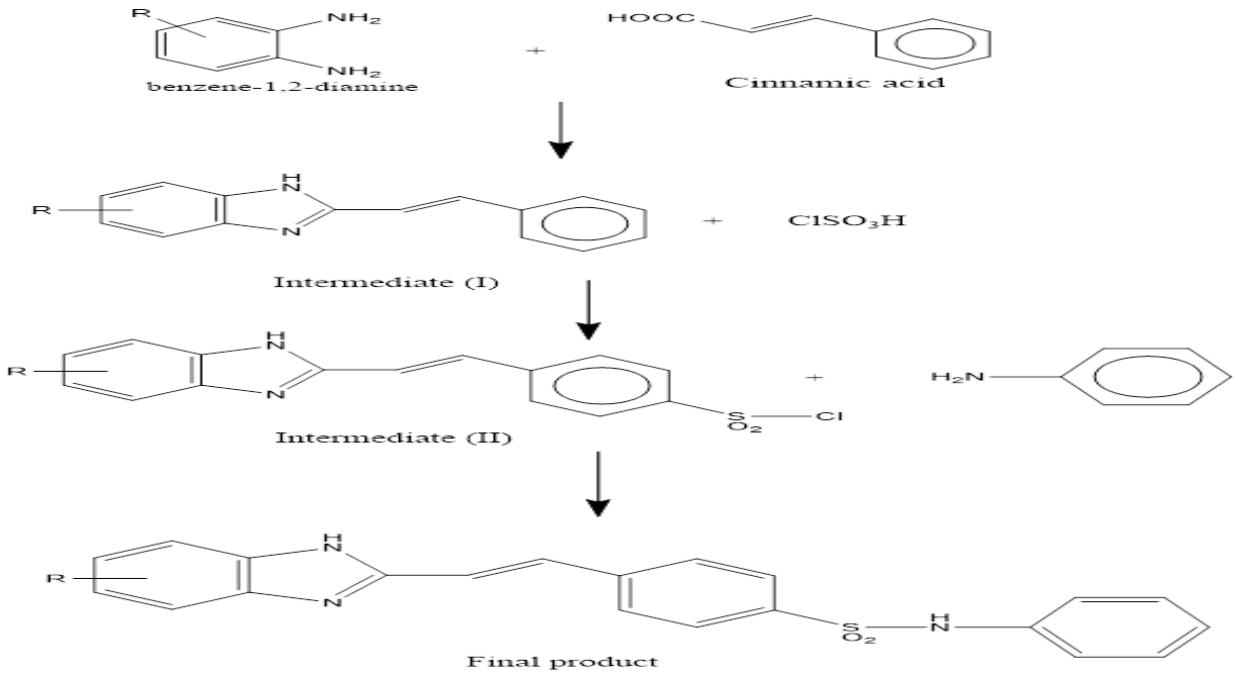

Table-1: Physical data of substituts

S. No.	Compound code	R	Molecular formula	Molecular weight	Colour	Melting point
1	3a	4-CH_3	$C_{22}H_{21}N_3O_2S$	391.49	White	825
2	3b	4-OCH_3	$C_{22}H_{21}N_3O_3S$	407.13	Pale yellow	830
3	3c	4-Cl	$C_{21}H_{18}ClN_3O_2S$	411.90	Pale yellow	826
4	3d	4-Br	$C_{21}H_{18}BrN_3O_2S$	456.36	White	833
5	3e	4-NO_2	$C_{21}H_{18}N_4O_4S$	422.46	White	810

Table-2: Reagent and Reaction condition

Code	Reagent	Reaction. condition
a	4.5 gm Benzoic acid	Reflux for 2 hours. at ~110ºC
b	15 ml. Chlorosulphonic. acid	Reflux for 2 hours. at ~110oC
c	5 gm Aniline	Reflux for 10 minutes at 80-100ºC

1.2 Procedure

1.2.1 Synthesis of (E)-2-styryl-1H-benzo[d]imidazole [32]

In a 250 ml of round-bottom flask, take 5 gm of o-phenylenediamine (O.P.D) and 4.5 gm of Benzoic acid (90%). Subsequently, the reaction mixture was subjected to reflux on a hot water bath for 2 hours. Upon completion of reflux, the reaction mixture was cool at room temperature. Then 10% aqueous sodium hydroxide solution were then added dropwise under continuous stirring to initiate alkalizing the mixture. A pale-yellow precipitate formed immediately, indicating the formation of the desired compound. The solid product was collected by vacuum filtration and washed with cold water to remove any residual impurities. The crude product was then air-dried to yield a pale-yellow solid, referred to as Intermediate 1

1.2.2 Synthesis of (E)-4-(2-(1H-benzo[d]imidazol-2-yl) vinyl) benzenesulfonyl chloride [33]

Dissolved Intermediate 1 (3 mol) to a 15 ml chlorosulphonic acid with stirring while maintaining the temperature at 0–5 °C for 2 hours. Then continuously stirring at room temperature for 1-2 hours. Upon completion of sterring, the reaction mixture was poured into ice-cold distilled water with gentle stirring. A black precipitate formed immediately, indicating the formation of the desired compound. The solid product was collected by vacuum filtration and washed with cold water to remove any residual impurities. The crude product was then air-dried to yield a black solid, referred to as Intermediate 2.

1.2.3 Synthesis of final product (3a-3e)[34]

Dissolve Intermediate 2 to a 10 ml sodium hydroxide (10%) with stirring then add 5 gm of aniline with continuous stirring. Then the reaction mixture was allowed to come to room temperature and then refluxed for 5 minutes, in microwave recto at 80-100^0C (200 watt). Upon completion of reflux, the reaction mixture was poured into ice-cold distilled water with gentle stirring. A white precipitate formed immediately, indicating the formation of the desired compound. The solid product was collected by vacuum filtration and washed with cold water to remove any residual impurities. The crude product was then air-dried to yield a white solid, referred to as final product.

1.3 Identification And Characterization

5.3.1 Physical Characterization

1. Melting point determination [35]

The melting point of a solid compound refers to the temperature at which it transitions from a solid to a liquid state under atmospheric pressure. This property is primarily utilized for the identification of organic compounds. The melting points of synthesized compounds were determined using the open capillary tube method with a melting point apparatus, and the data was recorded in degrees Celsius.

1. Procedure

- First, a little capillary tube that was 5–6 cm long was made. As the tube rotated, one end was sealed by placing it horizontally into the edge of a tiny, steady Bunsen flame for a few seconds.
- On a permeable plate, a little amount of the compound. whose melting point is unknown. was put and ground with a spatula into a powder.

- After that, the open end of the capillary tube was inserted into the powder, and the compound was allowed to settle into the closed end by gently tapping it. Three or four times, this procedure was carried out.
- To maintain the heating temperature, the thermometer was placed with the capillary tube in the melting point device and turned on.
- The temperature at which the compound starts to melt and melts completely was finally noted.

2. Solubility studies

The solubility of compound shows the nature of organic compounds i.e. lipophilicity of compound. Solubility studies of new compounds were done by using various polar and non-polar solvents.

3. Determination of R_f value

To find the Rf value, the produced compounds were subjected to thin layer chromatography. The distance traveled by the solvent divided by the solute's distance traveled is known as the Rf value. Silica gel G was used as the stationary phase in this study, while iodine was used as the visualizing agent in a mobile phase made up of methanol and chloroform at a 7:3 ratio. Each chemical was represented by a single spot on the TLC plate, with different Rf values. The purity of the novel chemicals is shown by this single spot.

IV. SPECTRAL ANALYSIS

1) UV Spectroscopy for determination of λmax [36]

The Shimadzu UV-1790 (UV Spectrophotometer) was used to measure the maximum absorbance's (λmax) of each produced molecule. The wavelength in the absorption spectrum where the absorbance is highest is referred to as a "max" (absorbance maxima). The wavelength range that molecules typically absorb is centered at the absorbance maxima. It serves as a qualitative metric for comparing the range of absorption of various molecules.

1. Infrared spectroscopy [37]

a. IR spectroscopy is one of most powerful analytical method used for identification of functional group present in organic compound. The infrared spectrum of the synthesized compound was obtained at the National Institute of Technology Tiruchirappalli using a PerkinElmer Spectrum 2 FTIR spectrophotometer with the potassium bromide pellet method.

2. NMR (Nuclear Magnetic Resonance) Spectroscopy [38]

a. The foundation of NMR spectroscopy is the way atoms' nuclei absorb electromagnetic radiation in the 4-900 MHz radio frequency range. The difference in parts per million between the measured proton's resonance frequency and that of the hydrogen atom in tetramethyl silane (TMS) is known as a chemical shift. Using the appropriate solvent ethanol and tetramethyl silane (TMS) as an internal standard, the NMR spectra was recorded at the Indian Institute of Science Education and Research Berhampur (IISER), in Odisha, on Bruker Advance III 400 MHz and 700 MHz NMR spectrometers. The chemical shift data were presented as TMS-related delta values in parts per million.

4)Mass Spectroscopy [39]

By recording the mass spectrum, mass spectroscopy is a great way to ascertain the molecular weight, molecular formula, and fragmentation pattern of organic molecules. Plotting relative abundance versus the mass/charge ratio (m/e) is known as the mass spectrum. Using an ESI mass spectrometer, the mass spectra of the synthesized chemical were captured at the Indian Institute of Science Education and Research Berhampur (IISER), in Odisha.

5)Biological Evaluation

5.4.1 Evaluation of Antimicrobial Activity: [40]

The antimicrobial evaluation of newly developed compounds is performed through microbiological assays. This process demonstrates the therapeutic effectiveness of antimicrobial agents by measuring their ability to inhibit microbial growth under standardized conditions. The microbiological evaluation of these agents involves comparing the inhibition zone produced by microorganisms against a specified concentration of antibiotics known for their efficacy. Two main methods are employed for antibiotic evaluation:

1. Agar diffusion/cup plate/cylinder plate method

2. Turbid metric and tube assay method. Agar diffusion method:
This technique relies on the diffusion of an antibiotic-filled cup or cylinder into the agar layer that contains microorganisms. A zone is created around the cups. The inhibition zone is measured by its diameter and compared to a standard drug. This method indicates the extent of microorganism growth.

3 Turbid metric method:
This technique relies on preventing a microbiological culture in a stable antibiotic solution from growing in a fluid medium that promotes rapid growth in the antibiotic's absence.
Gram-positive bacteria like Staphylococcus aureus, Bacillus coagulans, and Streptococcus mutans, as well as gram-negative bacteria like Escherichia coli and the fungus Candida albicans, were tested for their antimicrobial qualities at different concentrations using the agar diffusion technique.

- Collection of test microorganisms:

All experimental bacteria and fungi were sourced from the Microbial Type Culture Collection and Gene Bank (MTCC) at the CSIR - Institute of Microbial Technology in Chandigarh. All test microorganisms were preserved in slants and kept in a refrigerator.

Table-3: Microbial Cultures

S. No.	Name of microorganisms	Microbial type
1.	Staphylococcus aureus	Gram positive bacteria
2.	Pseudomonas Aeruginosa	Gram negative bacteria
3.	Escherichia coli	Gram negative bacteria
4.	Candida albicans	Fungus

- Culture Media:[41]

Two types of culture media were utilized for the antimicrobial study. Nutrient broth media served for the preparation of bacterial inoculum, while nutrient agar media was employed for the antimicrobial screening of newly synthesized compounds
Preparation of nutrient broth media:

S. No.	Ingredient	Quantity
1.	Beef extract	10g
2.	Peptone	10g
3.	Sodium chloride	5g
4.	Distilled water	1000 ml (q. s.)
	pH	7.2-7.4

Table-4: Formula for preparation of nutrient broth media

Procedure
1. All ingredients were precisely measured and dissolved in distilled water.
2. The media is then heated in a water bath until all components are fully dissolved, resulting in a clear yellow liquid.
3. The conical flask containing the broth is sealed with a cotton swab and placed in an autoclave for media sterilization.
4. The culture media is sterilized at a temperature of 121°C under 15 lbs. of pressure for 20 minutes.

Preparation of nutrient agar media

S. No.	Ingredient	Quantity (g)
1.	Beef extract	10g
2.	Peptone	10g
3.	Sodium chloride	5g
4.	Distilled water	1000 ml (q. s.)
5.	Agar	20g
	pH	7.0-7.4

Table-5: Formula for preparation of nutrient agar media

Procedure:
1. Every component was precisely measured and dissolved in purified water.
2. A 1N HCl or 1N NaOH solution was used to alter the pH of the culture medium.
3. A clear yellow liquid was produced by heating the medium in a water bath until all of the constituents had dissolved.
4. A conical flask was filled with agar, sealed with a cotton swab, and put in an autoclave to sterilize the medium.
5. The medium was sterilized for 20 minutes at 121°C with 15 pounds of pressure.

Preparation of stock culture:
Stock cultures were prepared by a loopful of test microorganisms were aseptically transferred to 100 ml of sterile broth and incubated for 24 hours at 37°C.

- Subculturing or aseptic transfer of microorganism

1. All the glasswares were sterilized during the same time of media preparation.
2. The working platform cleaned with disinfectant and flame the burner.
3. The culture media and all glasswares were placed to this sterilized aseptic area.
4. Test tube used for subculture was labeled with name of microorganism and date.
5. The inoculating loop was sterilized by incineration.
6. Hold the test tube in left hand, separate the test tube to form V shape and right hand was used for handling inoculating loop.
7. Remove the plug of test tube near the burner and flame the neck of test tube.
8. Then inserted the inoculating loop into stock culture to pick up small number of microbes.
9. This loop dipped into broth cultured tube and shaken to dislodge the microorganisms.
10. Reflame the neck of test tube and reseal it with cotton plug.
11. Also, reflame the inoculating loop before keeping it aside.
12. All the subculture test tubes were incubated at 37ºC for 24 to 48 hours for growth of a pure culture.

❖ Preparation of drug dilutions [42]

The dilutions of test compounds and standard drug were prepared in ethanol was used for their antimicrobial screening.

- Preparation of stock solution: Stock solution of test compounds having concentration 1000µg/ml prepared by dissolving 10mg of synthesized compound in up to 10ml ethanol.
- Preparation of working solution From above stock solution of synthesized compound different concentration such as 10 µg/ml, 20µg/ml, 30µg/ml, and 40µg/ml were prepared and amoxicillin same concertation was used as standard antibiotic for comparison and it was prepared by using ethanol.

❖ Antimicrobial screening by cup plate/ Agar diffusion method

1. All the petri plates were washed thoroughly and sterilized in hot air oven at 160ºC for one hour.
2. Nutrient agar media was prepared and sterilized in autoclave at 121ºC and 15 lbs. pressure for 20 minute and temperature maintained at 50-55ºC.
3. Petri plates were prepared by pouring 30 ml of above agar media into petridish and allow the medium to solidify for few minutes.
4. The test microorganisms seeded on the surface of Petri plates by spread plate technique using sterile cotton swabs.
5. By using flame sterilized corn borer four to five cups in each plate keeping adequate distance from each other was prepared.
6. The different dilutions such as 10 µg/ml, 20µg/ml, 30µg/ml and 40µg/ml were prepared using ethanol and amoxicillin 10 µg/ml, 20µg/ml, 30µg/ml and 40µg/ml was used as standard antibiotic for comparison and it was prepared by using ethanol.
7. Mark each cup or cavity as per dilutions. Then standard and test antibiotic dilutions were added in respective labelled cavity of plates.
8. All the petri plates were transferred in incubator and incubated at 37ºC for 48 hours.

❖ Result And Discussion

In the present project work a series of benzene ring containing benzimidazole derivatives (3a-3e) respectively were synthesized by reaction of substituted (E)-4-(2-(1H-benzo[d]imidazol-2-yl) vinyl) benzenesulfonyl chloride with aniline using microwave assisted method. The newly synthesized compounds were purified by recrystallization method using suitable solvent and characterized by physicochemical and spectral analysis (UV, IR, ^{1}HNMR, ^{13}CNMR, Mass spectroscopy and elemental analysis)

❖ Chemistry:

The synthesis of a series of sulphonyl urea containing benzimidazole derivatives (3a-3e) involves multiple steps. Various intermediates and substituted were synthesized to achieve target molecule. According to reaction scheme the first step involves synthesis of (E)-2-styryl-1H-benzo[d]imidazole (1), was prepared by reaction of commercially available o-phenylenediamine (O.P.D) with benzoic acid (90%) in `10% NaOH solution. These resulting (E)-2-styryl-

1H-benzo[d]imidazole (1) was then used in second step for preparation of (E)-4-(2-(1H-benzo[d]imidazol-2-yl) vinyl) benzenesulfonyl chloride (2) by refluxing in hot water bath with chlorosulphonic acid. In final step a series of target molecules (E)-4-(2-(1H-benzo[d]imidazol-2-yl) vinyl)-N-phenylbenzene sulfonamide (3a-3e) were prepared with better yield by treatment of (E)-4-(2-(1H-benzo[d]imidazol-2-yl) vinyl) benzene sulfonyl chloride (2) with series of aniline respectively.

The determination of melting point of synthesized compounds mostly used physical properties and was done by open capillary tube method using melting point apparatus and data recorded in ^{o}C. Melting points of all the synthesized compounds was found to be sharp which indicated the purity of the synthesized compounds and the observed melting point of synthesized compound was tabulated in table-6.

Table-6: Physical properties of synthesized compound (3a-3e)

S. No.	Compound code	R	Molecular formula	Molecular weight	Colour	Melting point
1	3a	4-CH_3	$C_{22}H_{21}N_3O_2S$	391.49	White	825
2	3b	4-OCH_3	$C_{22}H_{21}N_3O_3S$	407.13	Pale yellow	830
3	3c	4-Cl	$C_{21}H_{18}ClN_3O_2S$	411.90	Pale yellow	826
4	3d	4-Br	$C_{21}H_{18}BrN_3O_2S$	456.36	White	833
5	3e	4-NO_2	$C_{21}H_{18}N_4O_4S$	422.46	White	810

The solubility studies of target molecules were done by using various polar and non-polar solvents. The results of solubility studies indicated that all synthesized compounds were soluble in organic solvent. This confirmed the lipophilic nature of the synthesized compound.

Table-7:- Solubility profile of synthesized compound (3a-3e)

S. No.	Solvent	3a	3b	3c	3d	4a	4b	4c	4d
1.	Water	-	-	-	-	-	-	-	-
2.	Ethanol	++	++	++	++	++	++	++	++
3.	Methanol	++	++	++	+	++	+	++	+
4.	Butanol	++	+	++	+	++	++	+	++
5.	Isopropyl alcohol	++	++	-	++	-	+	+	++
6.	Ethyl acetate	++	-	+	++	-	++	+	++
7.	Chloroform	++	++	++	++	++	+	++	++
8.	Diethyl ether	++	+	++	++	+	-	++	++
9.	Toluene	++	+	-	++	+	++	-	+
10.	Benzene	++	++	++	+	++	++	+	++
11.	n-Hexane	++	+	++	++	+	++	++	+
12.	1,4-Dioxane	++	+	-	++	+	-	-	++

Where: (++) Soluble, (+) slightly soluble, (-) Insoluble

The R_f values of synthesized compounds was determined by thin layer chromatography (TLC) using silica gel- G as stationary phase and ethanol: water (7:3) as mobile phase. A single and clear spot of compounds have been found and values of R_f revealed that synthesized compounds are pure and contain little impurities

Table-8: R_f values of synthesized compounds

S.No.	Compound code	Solvent. system	Rf. Value
1.	3a	Ethanol: Water (7:3)	0.89
2.	3b	Ethanol: Water (7:3)	0.79
3.	3c	Ethanol: Water (7:3)	0.82
4.	3d	Ethanol: Water (7:3)	0.83
5.	3e	Ethanol: Water (7:3)	0.78

The structure of synthesized compounds was characterized by spectroscopic analysis. The determination of UV spectra of synthesized compounds was done by using Shimadzu UV-1800 Spector photometer. Ethanol is used as the solvent because all synthesized compounds are soluble in ethanol and λmax of synthesized compounds was determined and result was shown in table- 14.

Table-9:λmax of synthesized compounds

S.No.	Compound Code	λ max (nm)
1.	3a	268
2.	3b	265
3.	3c	265
4.	3d	270
5.	3e	260

The infrared spectra of the synthesized compounds were acquired using a PerkinElmer Spectrum 2 FTIR spectrophotometer at the National Institute of Technology Tiruchirappalli. Each IR spectrum of the synthesized compounds exhibits distinctive absorption patterns that correspond to their structural functional groups.

Only three derivatives i.e. intermediate (1), intermediate (2), 3a were taken for spectral studies. The results showed the presence of derivatives which were predicted in the reaction scheme.

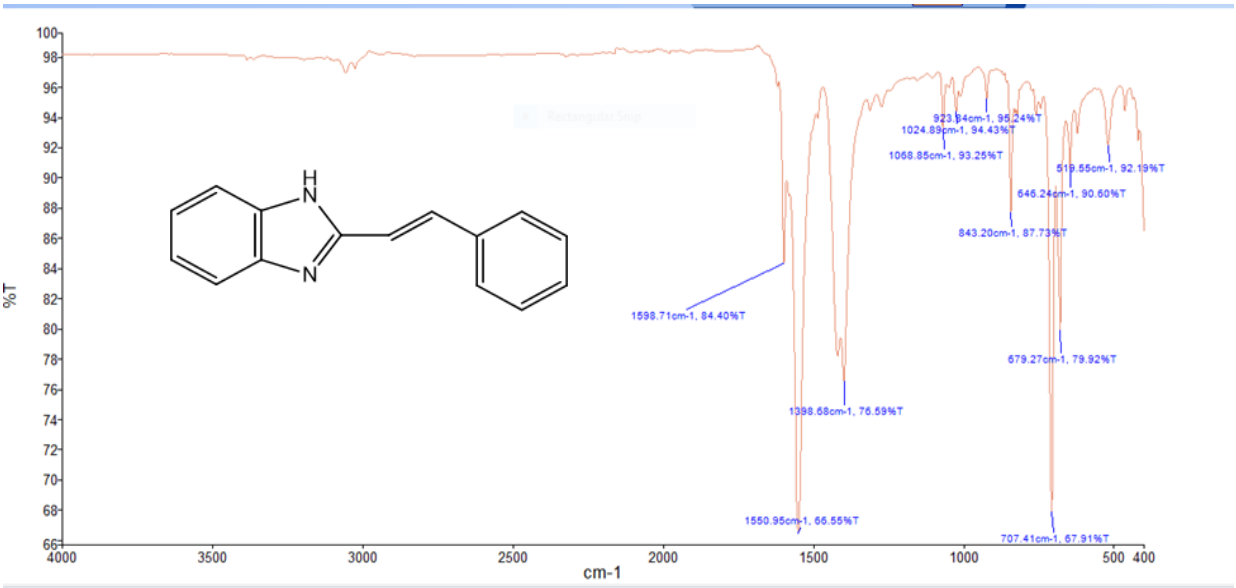

Figure-1: IR spectrum of compound (1)

Table-10: IR data for Compound (1)

S. No.	Wave number (cm-1)	Function group interpretation
1.	1598.17	N-H
2.	1398.68	C-H
3.	1068.85	C-NH_3

The IR data of compound (1) show presence of N-H stretching frequency at 1598.17 cm^{-1} revealed that formation of (E)-2-styryl-1H-benzo[d]imidazole (1) by reaction of 4-amino benzoic acid with benzaldehyde.

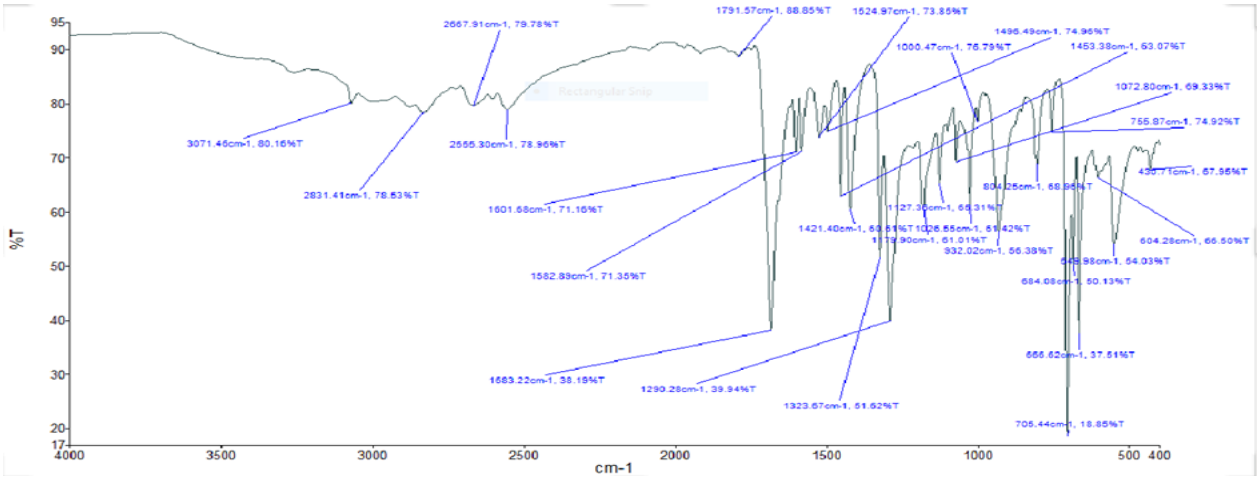

Figure-2:IR spectrum of compound (2)

Table-11: IR data of compound (2)

S. No.	Wave number (cm-1)	Function group interpretation
1.	3071.46	C-H (aromatic)
2.	2831.41	C-H (aldehyde)
3.	1323.07	C-N

The above IR data of (E)-4-(2-(1H-benzo[d]imidazol-2-yl) vinyl) benzenesulfonyl chloride (2) reported the presence of C-H stretching band at 3071.46, C-N stretching band at 1323.07. All this IR data confirmed that cyclization of compound (1) into (E)-4-(2-(1H-benzo[d]imidazol-2-yl) vinyl) benzenesulfonyl chloride (2).

Figure-3: IR data of compound (3a)

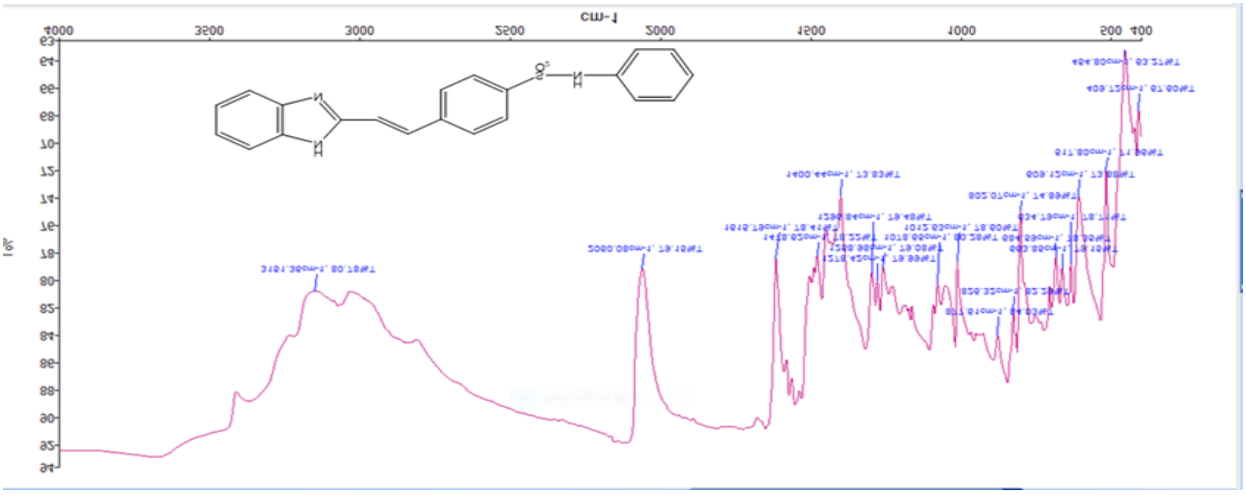

Table-12: IR data of compound (3a)

S. No.	Wave number (cm-1)	Function group interpretation
1.	3151.36	C-H
2.	2060.08	C=C
3.	1615.79	C-C
4.	1296.84	C-N
5.	1615.79	N-H

IR spectrum of compound (3a) represents broad spectrum of band. The presence of N-H stretching at 1615.79 cm^{-1}, AR C=C stretching at 2060.08 cm^{-1} and C=H stretching at 3151.36 cm^{-1} revealed that reaction of Thio semi carbazide with compound (2) and confirmed the formation of compound (3a)

NMR spectra were recorded at the Indian Institute of Education and Science Research (IISER) in Berhampur, Odisha, utilizing Bruker Avance III 400 MHz and 700 MHz NMR spectrometers with an appropriate solvent (DMSO), and ethanol was used as the internal standard. The chemical shift data were represented as delta values in relation to TMS in ppm. Only one compound (3a) was selected for 1H NMR spectroscopy and 13C NMR spectroscopy.

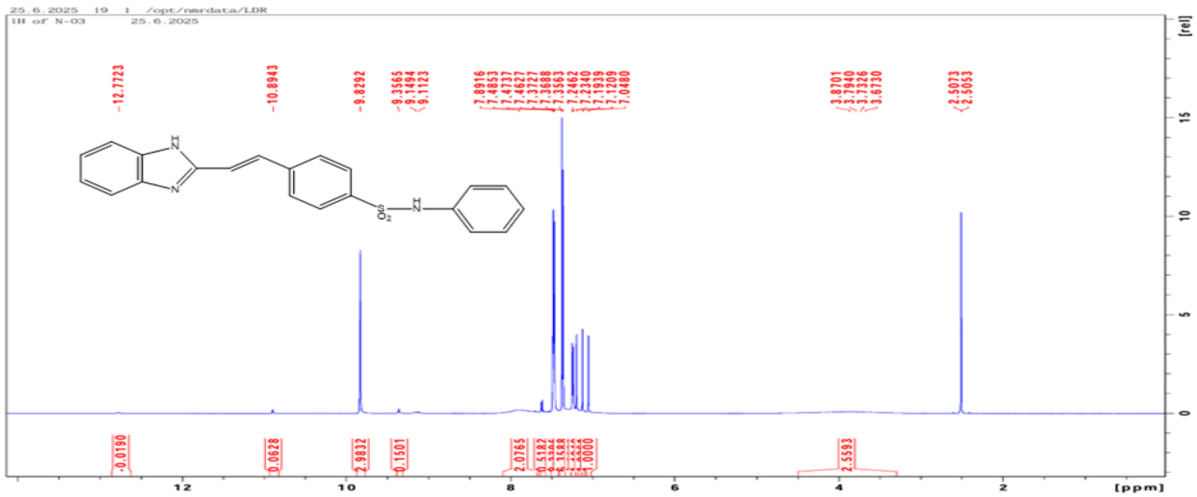

Figure.-4:[1]HNMR spectrum of compound (3a)

Table-13: [1]HNMR Data of compound (3a)

S. No.	δ ppm value	Interpretation	J value
1.	7.59 - 7.68	m, AR,-CH, 4H	2.0765
2.	7.25 - 7.60	m, 4H, AR-CH	0.5182
3.	12.7723	m, 1H, Benzimidazole NH	0.0190
4.	10.8943	s, 1H, Benzine NH	0.0628

In [1]HNMR spectrum of compound (3a) shows the sharp chemical shiftand data reported confirms the structure of target molecule (3a)

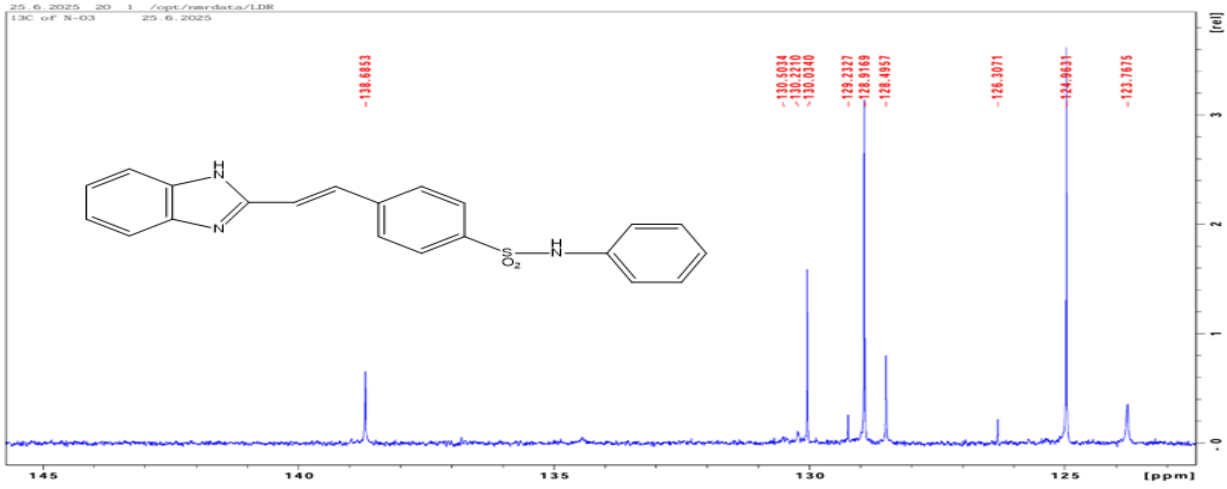

Figure. -5:[13]CNMR Spectrum of compound (3a)

Table-14: ^{13}CNMR data of compound (3a)

S. No.	δvalue ppm	Interpretation
1.	123.76	m, Ar,-CH, 4H
2.	128 - 129	s, 1H, Benzene NH
3.	126.30 – 138.68	m, 4H, Ar-CH

The above ^{13}CNMR data of compound (3a) represent sharp chemical shift for carbon and data reported confirmed the structure of synthesized compound. The mass spectra of synthesized compound were recorded at Indian Institute of Education and Science Research (IISER), Berhampur Odisha, using ESI mass spectrometer. Only one compound (3a) was taken for mass spectra.

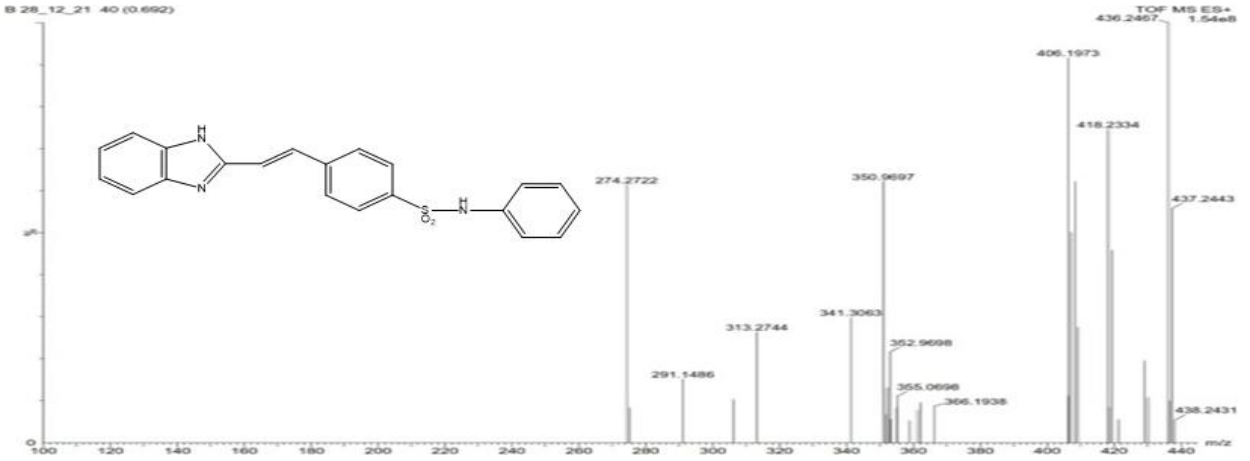

Figure-6: Mass spectrum of compound (3a)

Table-15: Mass spectroscopy data of compound 3(a)

S. No.	Mass spectrum	Molecular formula	Molecular ion peak
1.	3a	$C_{22}H_{21}N_3O_2S$	406.1 m/z

The above mass spectrum data confirmed the molecular weight of compound (3a)

V. BIOLOGICAL ACTIVITY

2.1 Antimicrobial activity:

The in vitro antimicrobial screening of a series of newly synthesized compounds was conducted using the agar diffusion method at various concentration levels, with amoxicillin serving as the standard for antibacterial activity. All synthesized compounds were evaluated against different microbial strains, including one gram-positive bacterium (Staphylococcus aureus), two gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa), and one fungus (Candida albicans).

S. No.	Compound code	Concentration (µg/ml)	Zone of inhibition (nm)			
			Gram positive bacteria	Gram negative bacteria		Fungus
			S. aureus	P. aeruginosa	E. coli	C. albicans
1.	Amoxicillin	10	8	10	10	11
		20	10	13	11	13
		30	14	14	13	15
		40	15	16	15	16
2.	3a	10	10	14	12	13
		20	12	15	13	14
		30	13	17	15	15

		40	16	20	16	16
3.	3b	10	12	12	10	10
		20	13	13	12	11
		30	14	16	14	13
		40	15	18	15	15
4.	3c	10	15	15	10	10
		20	16	16	12	11
		30	17	18	14	12
		40	18	20	15	13
5.	3d	10	10	10	12	13
		20	20	12	13	14
		30	30	13	16	15
		40	40	16	18	16
6.	3e	10	10	12	10	12
		20	20	13	11	13
		30	30	14	13	14
		40	40	15	15	15

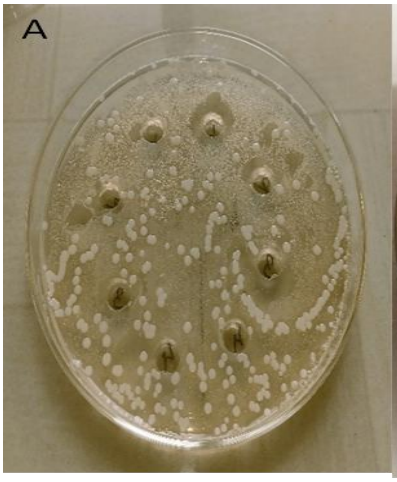

Fig-7: Zone of inhibition of test compound against (A)

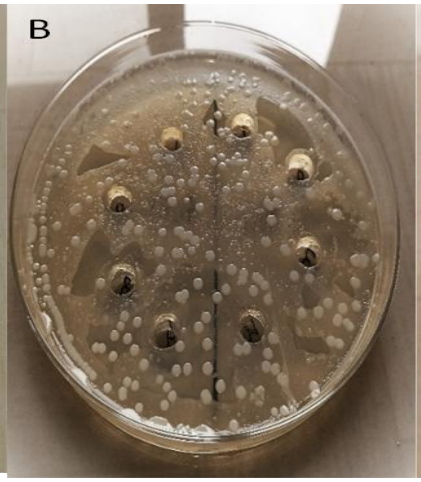

Fig-8: Zone of inhibition of test compound against (B 9B(B)

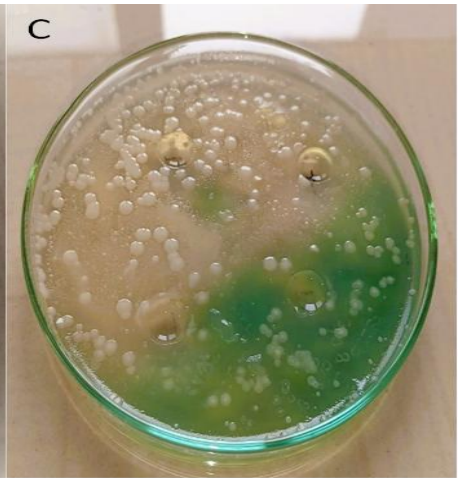

Fig-9: Zone of inhibition of test compound against (C)

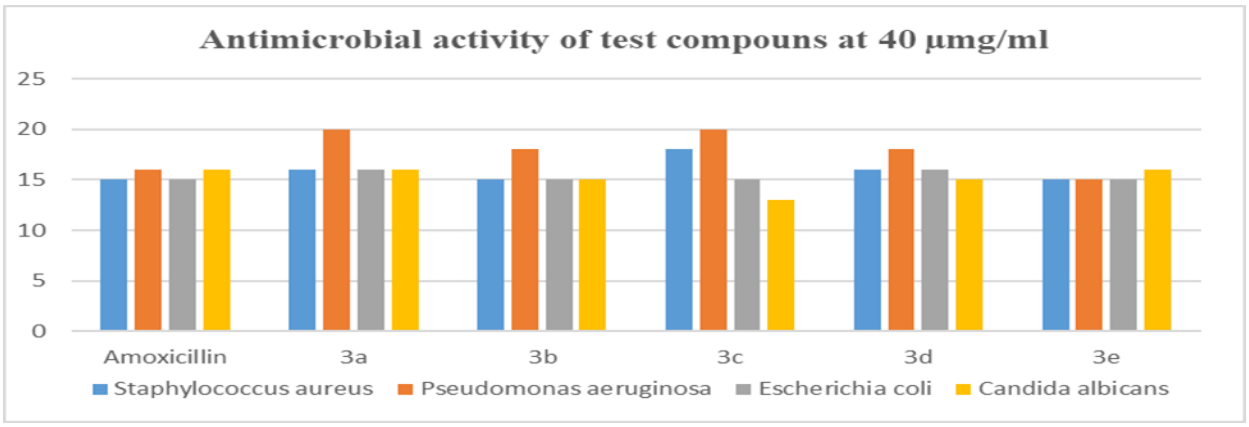

Figure-10: Bar diagram for zone of inhibition of test compounds against different microbial strains.

The antimicrobial screening of synthesized compounds was performed by agar diffusion method at concentrations 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, against different microbial strains like one-gram positive bacteria (Staphylococcus aureus), two-gram negative bacteria (Escherichia coli, Pseudomonas aeruginosa) and one fungus (Candida albicans) and their antimicrobial activity was compared to standard antibiotic, amoxicillin.

The data obtained indicated that all the synthesized compounds (3a-3e) exhibited variable antimicrobial activity against all tested microbial strains. It was observed that majority of synthesized compounds showed zone of inhibition against all microbial strains. All compounds are active against gram positive bacteria, gram negative bacteria, and fungi. Rest of compounds 3a, 3b, 3c, 3d were found to be effective against all gram positive and gram-negative bacterial strains and exhibited good antibacterial activity when compared with standard drug (amoxicillin) at concentration 40 µg/ml.

The result of antimicrobial activity revealed that compound 3b was exhibited narrow spectrum antimicrobial activity and rest of compounds exhibited broad spectrum antimicrobial activity.
The comparative study of antimicrobial activity of drugs (3a-3e) the data obtained from antimicrobial screening indicated that compounds (3a-3e) show antimicrobial activity

VI. CONCLUSION

The present study successfully synthesized and characterized benzimidazole derivatives using a simple and efficient method. Spectral analysis confirmed the structure of the synthesized compounds, particularly compound (1) as (E)-2-styryl-1H-benzo[d]imidazole. Antimicrobial evaluation using the agar diffusion method demonstrated that the synthesized compounds, especially Intermediate 1, Intermediate 2, and compound 3a, exhibited significant activity against both Gram-positive and Gram-negative bacteria, as well as fungal strains. Their activity was found to be comparable to the standard drug, amoxicillin. These findings highlight the potential of benzimidazole-based compounds as promising candidates for the development of new antimicrobial agents. Further structural modifications and in-depth biological studies are recommended to enhance their efficacy and spectrum of action.

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