SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL ACTIVITY OF NEWLY SYNTHESIZED N-[2-(1H-BENZIMIDAZOL-2-YL) PHENYL]THIOUREA DERIVATIVES BY MICROWAVE ASSISTED TECHNIQUE

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ABSTRACT

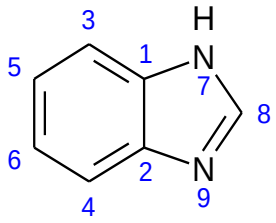
The microwave-assisted synthesis is a unique and simple technique which offers fast and efficient processing of materials with higher reproducibility. Benzimidazole ring is an important pharmacophore in modern drug discovery. The titled compund were synthesized from substituted phenylthiourea oxidation by bromine water in acidic medium. Thiourea are important sulphur and nitrogen containing compound. Some of them derivatives posses valuable antifungal and antimicrobial activity. The process of alignment or orientation of molecules by the external electrical field may result in the production of internal heat which is responsible for a reduction in processing time and energy required. It is especially due to the heating homogeneity of microwaves. Microwave assisted method has several advantages shorter reaction times compared with other methods, higher yields, or very good reproducibility.

KEYWORDS

Anti-Microbial, Anti-fungal, Microwave, phenylthiourea, pharmacophore.

INTRODUCTION

Benzimidazoles have only limited solubility in water; consequently, minor differences in solubility tend to have a major effect on absorption. Benzimidazole is a bicyclic heterocyclic aromatic compound in which benzene fused to imidazole moiety.[1-2]



N-phenylthiourea is a member of the class of thioureas that is thiourea in which one of the hydrogens is replaced by a phenyl group. Depending on their genetic makeup, humans find it either very bitter-tasting or tasteless. This unusual property resulted in N-phenylthiourea being used in paternity testing prior to the advent of DNA testing.

Thiourea and its derivatives are versatile ligand which coordinate to form stable compounds. These thiourea and their derivatives are not only important in their coordinating ability but are found to exhibit broad spectrum of biological activity. They show wide range of biological activities like analgesic, malaricidal, bactericidal, fungicidal, herbicidal, and insecticidal activities. It was also obserbed that this activity was enhanced by complexing with certain transition metal elements.[3]

N-Phenylthiourea has been used as a precursor in the synthesis of compounds with antibacterial and antifungal activities. It has also been used as a genetic marker for taste because only some people detect it as a bitter taste.

Thiourea can be synthesized in a reaction similar to that for urea by thermal decomposition of ammonium thiocyanate. Upon heating the main reaction of thiourea decomposition around 170°C is the formation of thiocyanic acid and ammonia in a reaction Thiourea can be synthesized in a reaction similar to that for urea by thermal decomposition of ammonium thiocyanate. Upon heating the main reaction of thiourea decomposition around 170°C is the formation of thiocyanic acid and ammonia in a reaction The substituted phenylthiourea is used as intermediate in different reactions because they play an important role in synthesizing the heterocyclic compounds.[4-5]

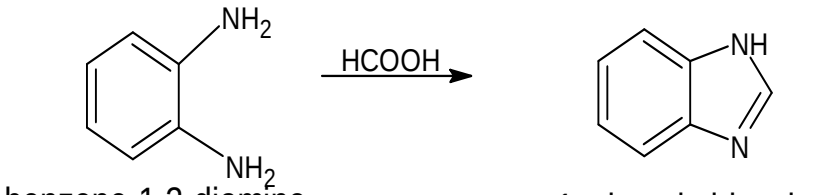
Material And Method

Table 1- Material

Chemicals –	Manufacturer	Grade
Ethanol	Otto Chemie Pvt. Ltd.	For HPLC
Benzaldehyde	Otto Chemie Pvt. Ltd.	Analytical Reagent
Ethylacetate	LOBA Chemie	For HPLC And UV Spectroscopy
P-Nitro aniline	Otto Chemie Pvt. Ltd.	Analytical Reagent
Hexane	Spectrum Chemical	HPLC Grade
Conc. Hydrochloric Acid	LOBA Chemie	Analytical Reagent
Ammonium Thiocyanate	Otto Chemie Pvt.Ltd.	Analytical Reagent
Water	Wako Pure Chemicals	HPLC Grade
Ethanolic Sodium Hydroxide	PanReac Applichem	Analytical Reagent
P-Chloroaniline	Otto Chemie Pvt. Ltd.	Analytical Reagent
P-Bromoaniline	Otto Chemie Pvt. Ltd.	Analytical Reagent
Silica Gel G	LOBA Chemie	For HPLC

Synthesis Of Benzimidazole[6-7]

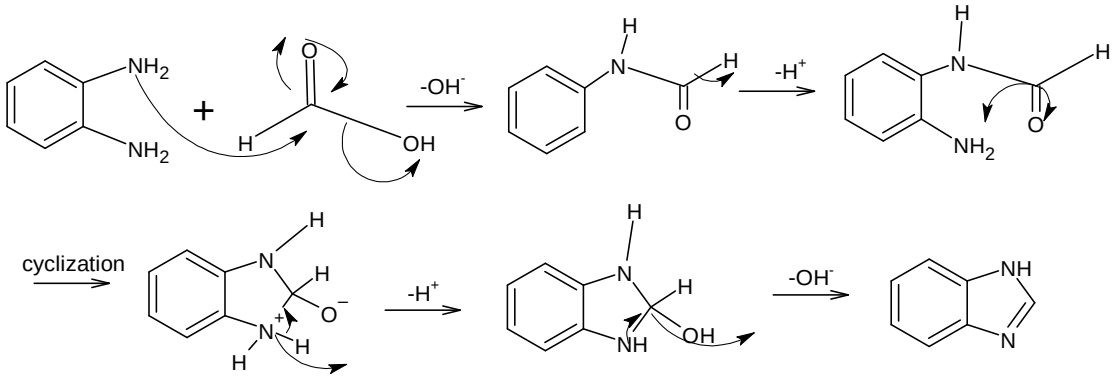
Reaction-



benzene-1,2-diamine 1*H*-benzimidazole

Mechanism-

Mechanism of Benzimidazole

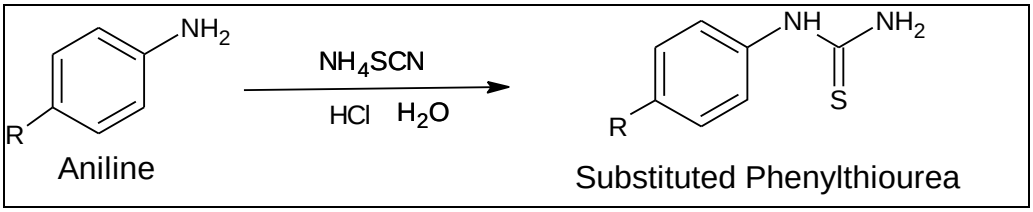


Procedure of synthesis of Benzimidazole-

1. 27 g of o-phenylenediamine was placed in a 250 ml round bottom flask and 16 ml of formic acid was added.
2. The mixture was heated on a water bath at 100°C for 2 hours.
3. 10 percent sodium hydroxide solution was cooled with constant shaking until the mixture is just alkaline to litmus.
4. Filter off the crude benzimidazole at the pump, was washed with ice-cold water, drain well and washed again with 25 ml of cold water.
5. It was Filter rapidly at the pump through a preheated Buchner funnel.
6. Flask. Cool the filtrate to about 10°C, filter off the benzimidazole, wash with 25 ml of cold water and dry at 100°C.

Synthesis of Substituted Phenylthiourea's: -[8]

Reaction:-



Procedure of Synthesis of Substituted Phenylthiourea's: -

Weighed (0.1 mol) P-Nitro aniline, (0.1mol) Ammonium Thiocyanate; added few ml Conc.Hcl (2ml) and 5 ml of water. Heat on water bath at 100°C. Transferred in cold water. Seperated By filtration .Dry it and recrystallized with ethanol. Melting Point; - 148°C

Microwave Assisted Synthesis Newly Synthesized of N-[2-(1H-benzimidazol-2-yl)phenyl]thiourea Derivatives:-[9-10]

1. Phenylthiourea (0.01mol) and Benzimidazole (0.01mol) was taken into the 100ml RBF.
2. The reaction was carried out in ethanol with catalytic amount of glacial acetic acid in microwave assisted assembly
3. After completion of the reaction, obtained solid product was filtered and purified by recrystallization from ethanol.

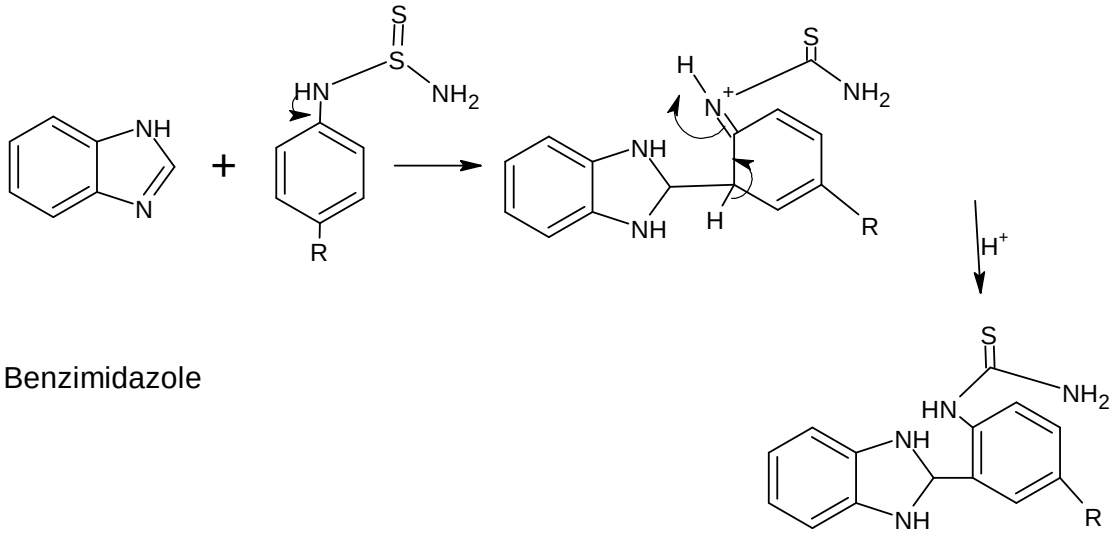
-

Melting Point –

Table 2- Melting Point

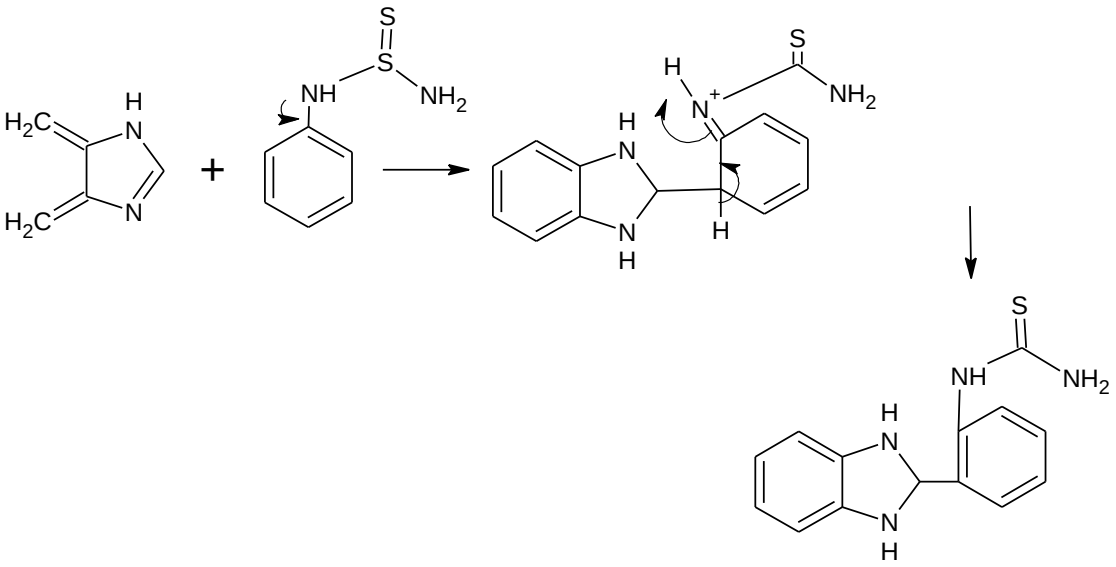
Compound Name	Melting Point
N-[2-(1H-benzimidazol-2-yl)-5-bromophenyl]thiourea	180 ℃
N-[2-(1H-benzimidazol-2-yl)-5-chorophenyl]thiourea	150℃
N-[2-(1H-benzimidazol-2-yl)-5-nitrophenyl]thiourea	166 ℃

Reaction Of Final Product-



Benzimidazole

Mechanism-[11]



Chromatographic Analysis

Thin Layer Chromatography:-

Thin layer chromatography (TLC) is a type of chromatography used to separate and identify different components of a mixture. It involves placing a small spot of the sample on a thin layer of adsorbent material, such as silica gel or alumina, which is coated on a glass or plastic plate. The plate is then placed in a solvent, which travels up the plate by capillary action, carrying the components of the sample along with it.

As the solvent moves up the plate, the different components of the sample are separated based on their affinity for the adsorbent material and the solvent. Components that have a strong affinity for the adsorbent material will move more slowly up the plate, while those with a weaker affinity will move faster. This results in the components of the sample separating out into distinct bands or spots on the plate.

Once the separation is complete, the plate is removed from the solvent and dried. The spots or bands can then be visualized using various techniques, such as staining with a chemical reagent or exposing the plate to UV light. The Rf value, or retardation factor, can be calculated for each component, which is the ratio of the distance traveled by the component to the distance traveled by the solvent. The Rf value is a characteristic property of a compound and can be used to identify unknown compounds by comparing their Rf values to those of known compounds. Silica gel G acted as stationary phase whereas the following solvent systems were used as mobile phase

- Toluene: Methanol (8:2)

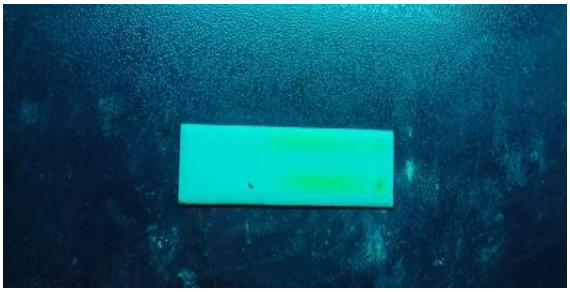


Fig : A

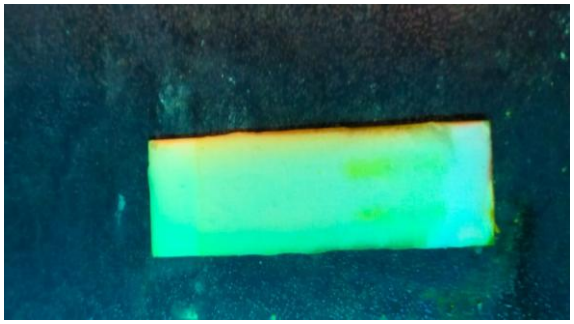


Fig : B

Fig : C

Fig-

(A) TLC Plate of Benzimidazole and N-[2-(1H-benzimidazol-2-yl)-5-bromophenyl]thiourea

(B) TLC Plate of Benzimidazole and N-[2-(1H-benzimidazol-2-yl)-5-chlorophenyl]thiourea

(C) TLC Plate of Benzimidazole and N-[2-(1H-benzimidazol-2-yl)-5-nitrophenyl]thiourea

$$\text{RF Value} = \frac{\textit{Distance Travelled by Solute}}{\textit{Distance Travelled by Solvent}}$$

Table 3-Retention Factor

Sr No	Figure	RF Value
1	Fig.A	0.79
2	Fig.B	0.85
3	Fig.C	0.86

SWISS ADME STUDY[12]

Table 4-Swiss ADME Study

Compound Name	Molecular Weight	H-Bonding acceptor	H-Bond Donors	Log P	Violations of Lipinski rule
N-[2-(1H-benzimidazol-2-yl)-5-bromophenyl]thiourea	347.23 g/mol	1	3	3.06	0 violation
N-[2-(1H-benzimidazol-2-yl)-5-chlorophenyl]thiourea	302.78 g/mol	1	3	2.97	0 violation
N-[2-(1H-benzimidazol-2-yl)-5-nitrophenyl]thiourea	313.33 g/mol	3	3	1.67	0 violation

Spectral Analysis

FTIR Spectroscopy[13]

Fourier Transform Infrared Spectroscopy (FTIR) is a widely used analytical technique that provides valuable information about the chemical composition and structure of materials. It is a non-destructive technique that works by measuring the interaction between a sample and infrared radiation. FTIR is commonly used in a variety of fields, including chemistry, materials science, pharmaceuticals, and forensic science.

FTIR works by shining an infrared beam of light through a sample, and measuring the intensity of the light that is absorbed by the sample at different wavelengths. This absorption spectrum provides information about the chemical bonds present in the sample, which can be used to identify functional groups and determine the chemical composition of the material.

The resulting spectrum is a plot of the intensity of light absorbed by the sample as a function of wavelength, which is referred to as an infrared spectrum.

FTIR can be used to analyze a wide range of samples, including gases, liquids, solids, and powders. It is a powerful tool for identifying unknown compounds, monitoring chemical reactions, and characterizing materials. The technique is relatively fast, non-destructive, and requires very small amounts of sample, making it an attractive option for many applications.

Overall, FTIR spectroscopy is a valuable analytical technique that provides important information about the chemical composition and structure of materials. Its wide range of applications makes it an essential tool in many fields, and its ease of use and reliability make it a popular choice among researchers and practitioners.

NMR Spectroscopy[14]

Nuclear Magnetic Resonance (NMR) is a powerful analytical technique used to investigate the structure, composition, and dynamics of molecules. NMR is based on the interaction between atomic nuclei and a strong magnetic field and radiofrequency radiation. It is widely used in various fields, including chemistry, biochemistry, materials science, and medicine.

In NMR, a sample is placed in a strong magnetic field and then subjected to radiofrequency radiation. The nuclei in the sample absorb and re-emit the radiation at a characteristic frequency, which depends on the local environment of the nucleus. By detecting the frequency of the emitted radiation, NMR can provide detailed information about the molecular structure and composition of the sample.

NMR can provide a wealth of information about a sample, including the number and types of atoms, the chemical environment of each atom, and the connectivity of atoms within a molecule. This information can be used to determine the identity of unknown compounds, monitor chemical reactions, and study molecular dynamics.

The NMR technique is often used in combination with other analytical techniques, such as mass spectroscopy and infrared spectroscopy, to provide a more complete picture of the sample. The most commonly used NMR technique is proton NMR, which is sensitive to the behavior of hydrogen atoms in the sample. Other nuclei, such as carbon-13 and nitrogen-15, can also be studied using NMR.

Overall, NMR spectroscopy is a versatile and powerful analytical technique that provides valuable information about the structure, composition, and dynamics of molecules. Its wide range of applications makes it an essential tool in many fields, and its sensitivity and precision make it a popular choice among researchers and practitioners.

Mass Spectroscopy [15]

Mass spectrometry (MS) is a powerful analytical technique used to identify and quantify molecules based on their mass-to-charge ratio (m/z). It is widely used in various fields, including chemistry, biology, pharmaceuticals, and forensics.

The basic principle of mass spectrometry involves ionizing a sample, separating the ions based on their

m/z values, and detecting and analyzing the resulting ion spectra. The ionization process can be achieved by various methods, such as electron impact ionization (EI), electrospray ionization (ESI), and matrix-assisted laser desorption/ionization (MALDI).

The ions are then separated using a mass analyzer, which can be a magnetic or electric field, a time-of-flight (TOF) analyzer, or an ion trap. The resulting ion spectrum is then detected and analyzed to determine the mass and abundance of each ion.

Mass spectrometry can be used to identify unknown compounds, determine the molecular weight and structural information of a compound, and quantify the amount of a particular compound in a sample. It is also used in proteomics and metabolomics to study the structure and function of proteins and metabolites, respectively.

Overall, mass spectrometry is a highly versatile technique that provides valuable information about the composition and structure of molecules, making it an essential tool in modern research and analysis.

Result and Discussion-

All synthesized derivatives of N-[2-(1H-benzimidazol-2-yl)phenyl]thiourea derivatives were monitored by thin layer chromatography and evaluated with physical and biological methods and all compounds were subjected to antimicrobial activity .i.e. antibacterial activity. in antibacterial activity, Compound A, B, C shows significant activity against E.Coli when compared to standard drug azithromycin. Compound was evaluated by Spectral Analysis. (IR,NMR,Mass Spectroscopy).

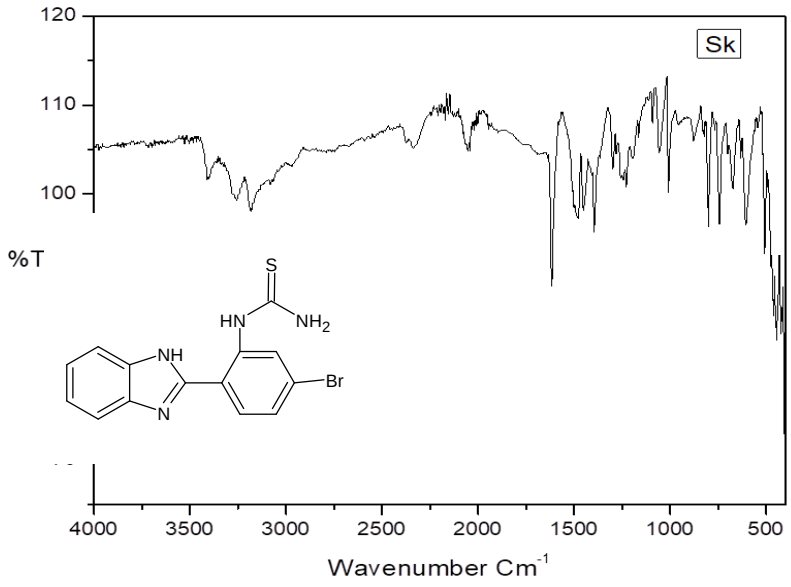
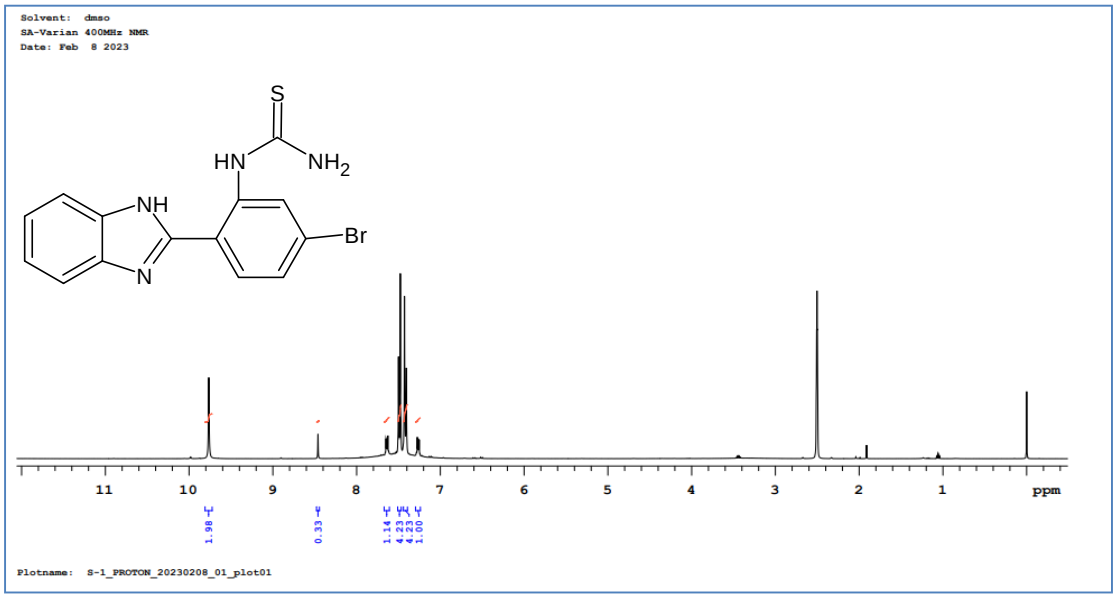


Fig.E-IR Spectrum

Table 5- IR Spectroscopic Result

Frequency Range cm-1	Absorption cm-1	Appearance	Group	Compound Class
4000-3000 cm-1	3200	Weak Broad	O-H stretching	alcohol
2000-1650 cm-1	1710-1680	Strong	C=O stretching	conjugated acid
	1710-1685	strong	C=O stretching	conjugated aldehyde
1400-1000 cm-1	1440-1395	medium	O-H bending	carboxylic acid
	1420-1330	Medium	O-H bending	alcohol
	1415-1380	Strong	S=O stretching	sulfate
	1070-1030	Strong	S=O stretching	sulfoxide

NMR Spectroscopy:-



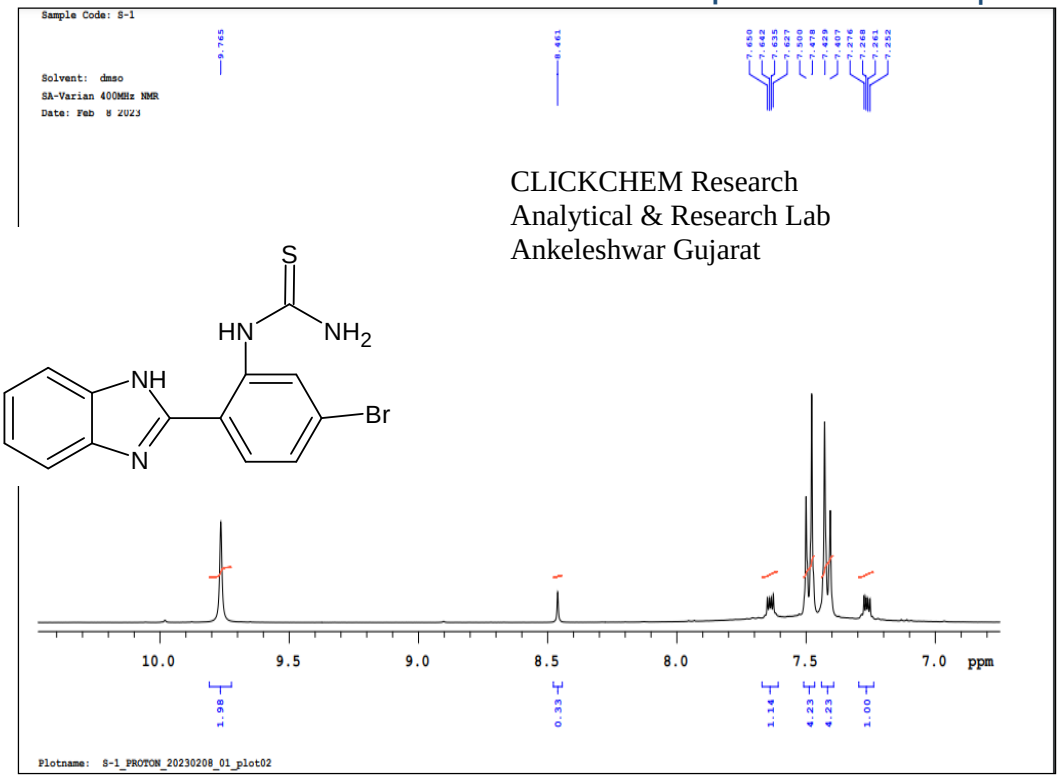


Fig.F-NMR Spectrum

Table 6- Interpretation of NMR

Delta (ppm)	Nb Atom	Multipliciy	Couplings
2.231	H1	-	7.38,7.67
2.231	H2	-	8.44
7.114	H1	Triplet	7.88,7.67,1.40
7.182	H1	Doublet	8.38,1.76
7.231	H1	Triplet	8.08,7.88,1.59
7.666	H1	Triplet	7.67,1.59,0.52
7.816	H1	Doublet	8.38,0.44
7.877	H1	Doublet	176,0.44
7.904	H1	Triplet	8.08,1.40,0.52
8.117	H1	-	8.08

Mass Spectroscopy: -

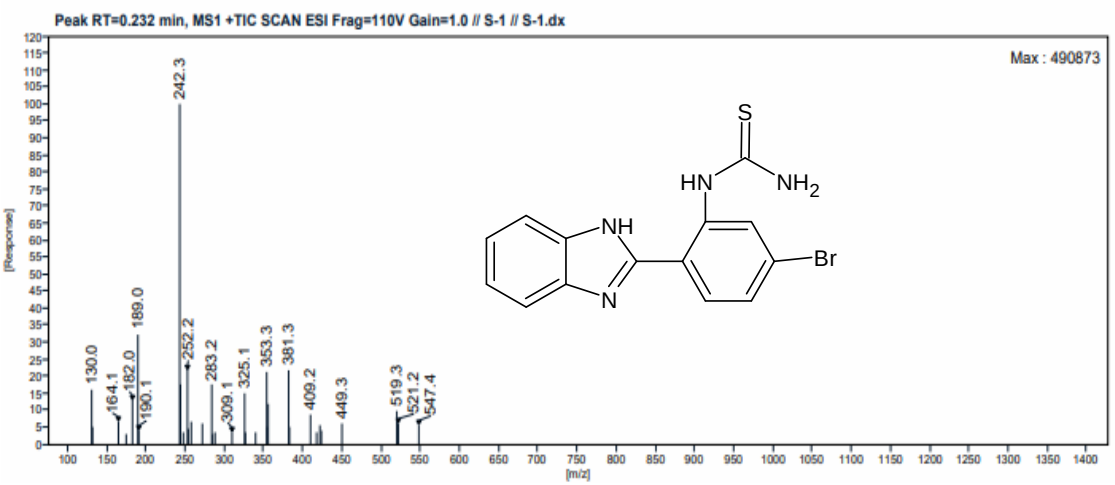
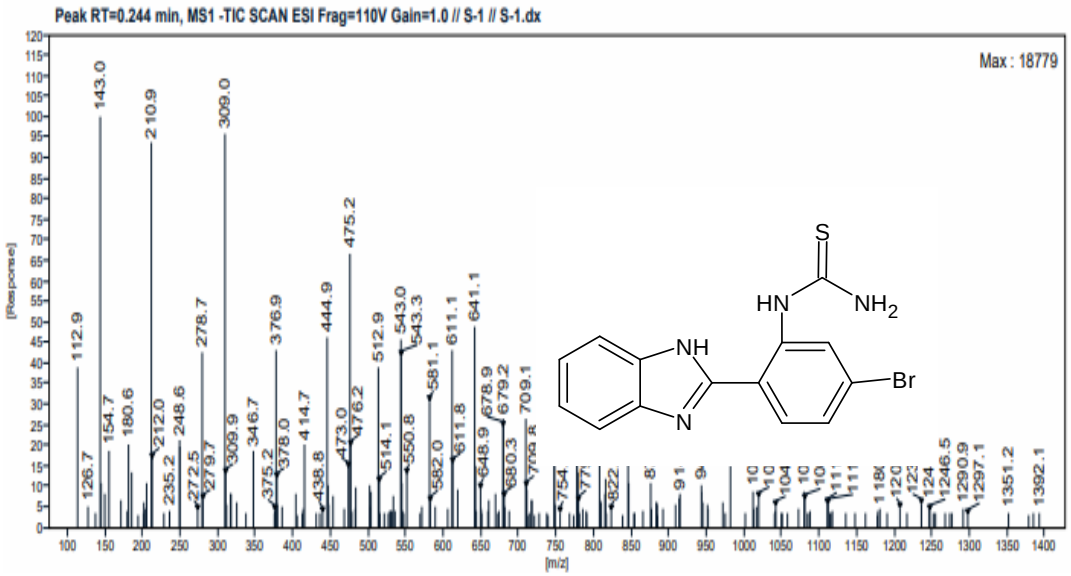
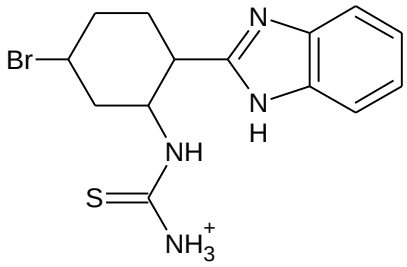
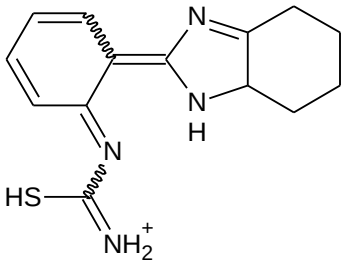
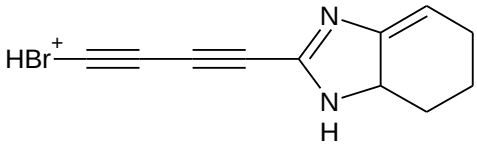
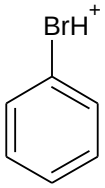
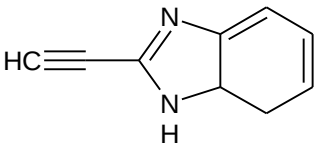
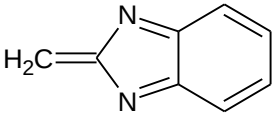


Fig.G-Mass Spectrum



Structure	Mass
	353.04
	273.11
	249.0
	156.96
	145.07
	131.06

Antimicrobial Screening[16]

Liquid Dilution Method or Tube Dilution Method-

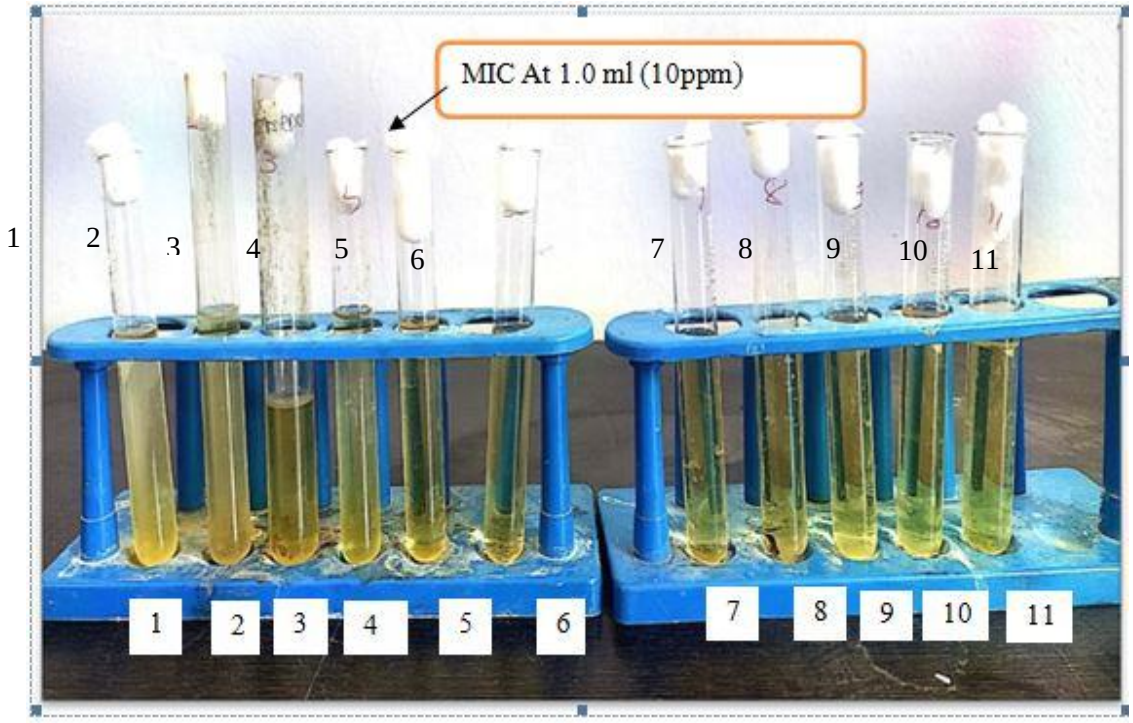
Use a series of test tubes which contain a double-strength medium and are labelled as shown in Table. In the first tube (un-inoculated), inoculum is not added which is used for checking the sterility of the medium. All other in a ten test tubes, inoculum (3 to 4 drops) is added to reach the final concentration of microorganisms is 105 to 106 cells/ml. In all test tubes, test chemical is added ranging from 0.5 to 5 ml except in the uninoculated tube. The final volume (10 ml) in all test tubes is adjusted by using sterile water. The contents of all test tubes ar properly mixed and incubated at 37°C for 2 to 3 days. After incubation, all test tubes are examined for the growth in the form of turbidity and the results are recorded and minimum inhibitory concentration is calculated. It is also necessary to conduct a experiment to determine the approximate range

(test solution) which would be suitable for preliminary the test.

Table 7- tube dilution method

Tube Number	Volume Of Double Strength Medium(ml)	Volume Of test Chemical (ml)	Volume of sterile Water(ml)
0(Uninoculated)	5	0	5.0
1	5	0.5	4.5
2	5	1.0	4.0
3	5	1.5	3.5
4	5	2.0	3.0
5	5	2.5	2.5
6	5	3.0	2.0
7	5	3.5	1.5
8	5	4.0	1.0
9	5	4.5	0.5
10	5	5.0	0.0

Fig.H-Test Drug Dilution



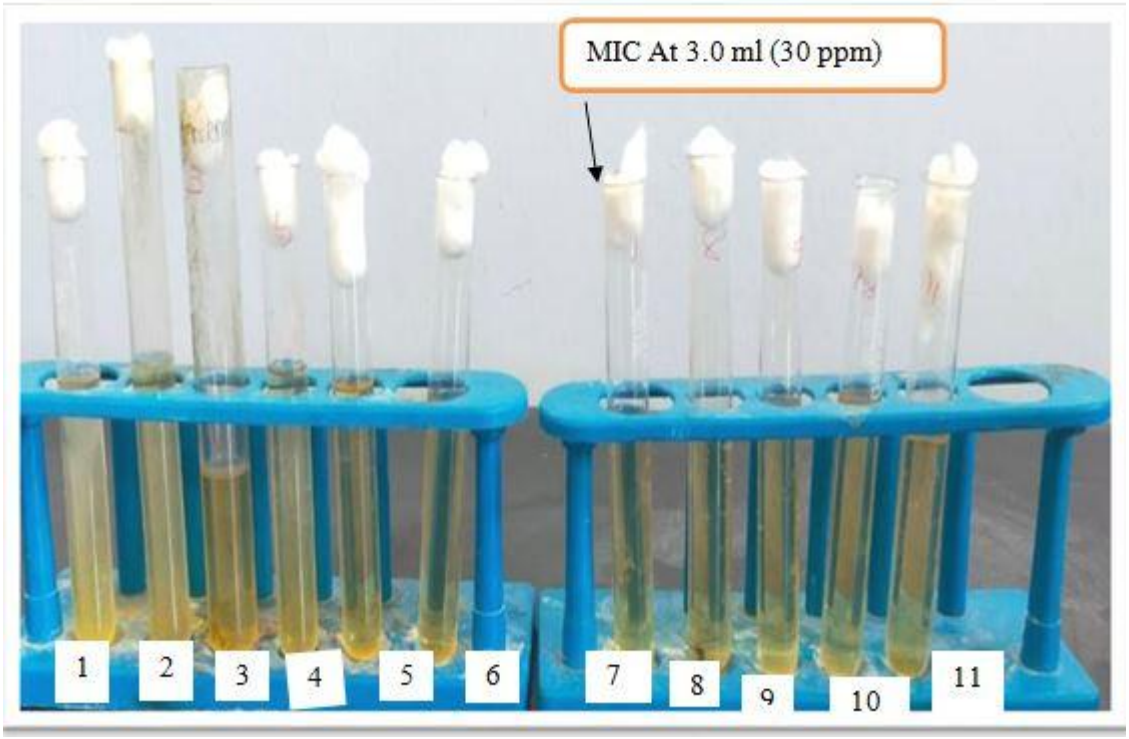


Fig.I-Standard Drug Dilution

Observation-

The Minimum Inhibitory Concentration Of Standard drug(Azithromycin) was found to be 1ml i.e.,10PPM and the minimum Inhibitory Concentration of test drug N-[2-(1H-benzimidazol-2-yl)phenyl]thiourea was found to be 3ml.i.e.,30PPM.So the test drug shows antibacterial activity.

Antifungal Activity[17-19]

1. Determine the specific fungus or fungi you want to test the antifungal activity against Common choices include Candida albicans.
2. Prepare a stock solution of the substance to be tested. Make sure the substance is soluble in an appropriate solvent (DMSO).
3. Prepare the appropriate culture medium for the fungus you are testing. Sabouraud dextrose agar (SDA) is commonly used for fungal cultures, but specific media may be required for certain fungi.
4. Using a sterile technique, transfer a small amount of the fungal culture to the prepared culture medium. Spread the culture evenly on the surface of the agar using a sterile loop or spreader.
5. Using sterile techniques, create wells or discs on the surface of the agar to accommodate the test

substance. Add a specific volume of the test substance solution or apply the substance directly to the wells/discs.

6. Place the prepared agar plates in an incubator set to the appropriate temperature for the test organism's growth conditions. Incubation times can vary depending on the fungus, but typically range from 24 to 72 hours.

7. Examine the agar plates for inhibition zones around the test wells/discs. Inhibition zones indicate the antifungal activity of the tested substance. Measure the diameter of the zones of inhibition using a ruler or calipers.

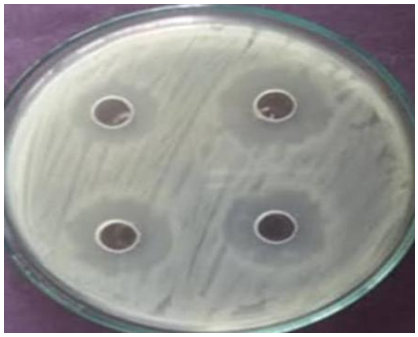


Fig.J- Petridish for Test drug

Fig.k- Petridish for Standard Drug

Observation-

Test drug screened against Standard drug Fluconazole(Antifungal) So, the test drug(10PPM and 20PPM)shows 5 mm and 11mm zone of inhibition and standard drug shows 11mm and 17mm of zone of inhibition.so the test drug shows zone of inhibition at conc.20PPM.

CONCLUSION

The fundamental goal of medicinal chemistry is the development of new antifungal therapeutic agents. In conclusion, microwave-assisted synthesis, characterization, and anti-microbial activity of newly synthesized derivatives of phenylthiourea are promising areas of research with a wide range of potential applications in the fields of organic chemistry and drug discovery. Microwave-assisted synthesis has been shown to be a rapid and efficient method for synthesizing new compounds, while characterization techniques such as FT-IR, 1H-NMR, and mass spectroscopy can provide valuable information on the molecular structure and purity of the synthesized compounds. The evaluation of antimicrobial activity is essential in the development of new drugs and antibiotics, and the discovery of new antimicrobial agents is critical in the fight against infectious diseases.The evaluation of antimicrobial activity showed that some of the synthesized compounds

had promising activity against a range of microorganisms. Overall, this research contributes to the growing body of literature on the use of microwave-assisted synthesis in the field of organic chemistry and provides valuable insights into the structure-activity relationship of newly synthesized derivatives of phenylthiourea. Further research is needed to explore the potential applications of these compounds in the development of new drugs and antibiotics, as well as to investigate other areas of research where microwave-assisted synthesis can be applied.

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