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Synthesis, Characterization and Pharmacological Activity of Newly Synthesized Thiohydantoin Derivatives from Benzil

Bhand Abhijit Dilip[1], Dr. Gaikwad Shital Dnyaneshwar[2], Khutal Tejaswinee Digambar[3], Abhang Archana Krushna[4]
Samarth Institute of Pharmacy, Belhe, Junnar
Department of Pharmaceutical Chemistry
abhijitbhand2002@gmail.com

Abstract: *Thiohydantoin derivatives have been recognized as a class of compounds with diverse pharmacological properties, including antimicrobial, anticonvulsant, and antioxidant activities. In this study, we synthesized new thiohydantoin derivatives by substituting phenylthiourea with benzil. The substitution reaction was carried out by a simple and efficient method, and the products were characterized by various spectroscopic techniques such as FT-IR, NMR, and mass spectrometry. The synthesized thiohydantoin derivatives were evaluated for their pharmacological activity, including antioxidant and antimicrobial properties. The antimicrobial activity of the synthesized thiohydantoin derivatives was evaluated against various bacterial strains, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhimurium. In conclusion, the synthesized compounds exhibited significant antioxidant and antimicrobial properties, suggesting that they may have potential as drug candidates for the treatment of various diseases caused by oxidative stress and bacterial infections. Further studies are needed to elucidate the mechanisms of action and to explore their full therapeutic potential*

Keywords: Thiohydantoin, Benzil, Phenylthiourea, FT-IR, NMR, anti-microbial activity

I. INTRODUCTION

Thiohydantoins are a class of organic compounds that have been widely studied for their diverse pharmacological properties. thiohydantoin derivatives have been found to have anticonvulsant, anti-inflammatory, antioxidant, and antimicrobial activities, among others. Among the various methods for the synthesis of hydantoin derivatives, the reaction between benzil and substituted urea or thiourea has been extensively used. Phenylthiourea is a substituted thiourea that has been used in the synthesis of various organic compounds, including thiohydantoins. Substituted thiohydantoins obtained from phenylthiourea have been found to exhibit various pharmacological activities such as anticonvulsant, antitumor, and antimicrobial activities. The present study focuses on the synthesis, characterization, and pharmacological activity of newly synthesized thiohydantoin derivatives from benzil by substituting phenylthiourea.

The objective of the study is to evaluate the pharmacological potential of the synthesized compounds and to explore their use as potential drug candidates. Benzil is a diketone that has been widely used as a starting material in the synthesis of various organic compounds. The reaction between benzil and phenylthiourea leads to the formation of thiohydantoin derivatives. The substitution of phenylthiourea at the 5,5'-position of benzil results in the formation of 5,5'-disubstituted hydantoin derivatives. The synthesis of thiohydantoin derivatives from benzil and phenylthiourea was carried out by refluxing equimolar amounts of benzil and phenylthiourea in ethanol in the presence of potassium hydroxide. The reaction mixture was heated for several hours, and the resulting yellow precipitate was collected by filtration and purified by recrystallization.

The synthesized thiohydantoin derivatives were characterized by various analytical techniques such as Fourier-transform infrared (FT-IR) spectroscopy, proton nuclear magnetic resonance (1H NMR) spectroscopy, and mass

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spectrometry (MS). FT-IR spectroscopy was used to identify the functional groups present in the synthesized compounds. 1H NMR spectroscopy was used to confirm the structure of the synthesized compounds, while mass spectrometry was used to determine the molecular weight of the synthesized compounds. The pharmacological activity of the synthesized thiohydantoin derivatives was evaluated by assessing their antioxidant and antimicrobial properties. The antioxidant activity was assessed using the DPPH assay, which measures the ability of compounds to scavenge free radicals. The antimicrobial activity was evaluated against various bacterial strains, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhimurium. The synthesized compounds were characterized by various analytical techniques, and their pharmacological potential was evaluated by assessing their antioxidant and antimicrobial properties. The results of the study suggest that the synthesized compounds may have potential as drug candidates for the treatment of various diseases caused by oxidative stress and bacterial infections. Further studies are needed to elucidate the mechanisms of action and to explore their full therapeutic potential.

Thiohydantoins are an important class of heterocyclic compounds that exhibit diverse biological activities. The synthesis of thiohydantoins from benzil is a simple and efficient approach that can lead to the synthesis of novel compounds with potential pharmacological activity. The newly synthesized thiohydantoin derivatives can be characterized using various spectroscopic and analytical techniques, and their pharmacological activity can be evaluated using different in vitro and in vivo assays. The development of new thiohydantoin derivatives from benzil has the potential to lead to the discovery of new drugs for the treatment of various diseases.

II. DRUG PROFILE

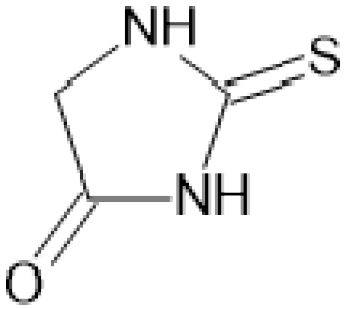

Fig. Structure of Thiohydantoin

Chemical name	2-thioxo-4-imidazolidinone
Chemical formula	C3H4N2OS
Molecular mass	116.14 g·mol−1
Appearance	A crystalline powder
Density	1.346 g/mL
Melting point	198-202°C
Boiling point	378.6°C
Solubility in water	12 mg/mL (103.32 mM)
Vapor pressure	0.1 Torr
Acidity (pKa)	9-10

III. MATERIAL AND METHODS

Table 1.1: Chemical Grade and Manufacturer

Chemicals	Manufacturer	Grade
Ethanol	AG Traders	Laboratory reagent
Benzoin	GS Labs	For Synthesis
Benzil	GS Labs	For Synthesis

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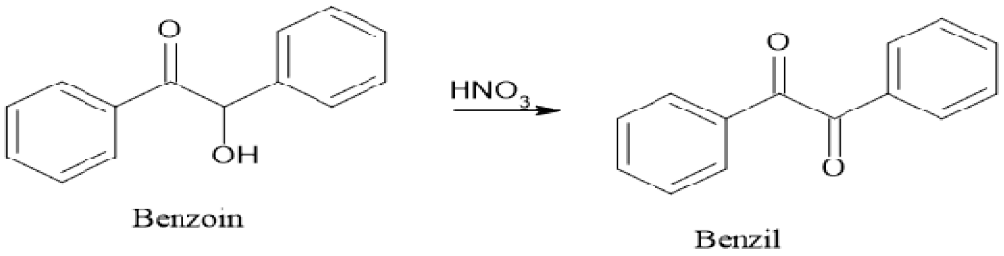
Ethylacetate	Thermosil Fine Chem Industries	For Synthesis
P-Nitro aniline	Thermosil Fine Chem Industries	Analytical Reagent
Toluene	Guarentochem	Analytical Grade
Ammonium Thiocyanate	AG Traders	Analytical Reagent
Potassium Hydroxide	AG Traders	Laboratory reagent
P-Chloroaniline	AG Traders	Laboratory reagent
P-Bromoaniline	AG Traders	Laboratory reagent
Silica Gel G	GS Labs	Analytical reagent

Methods

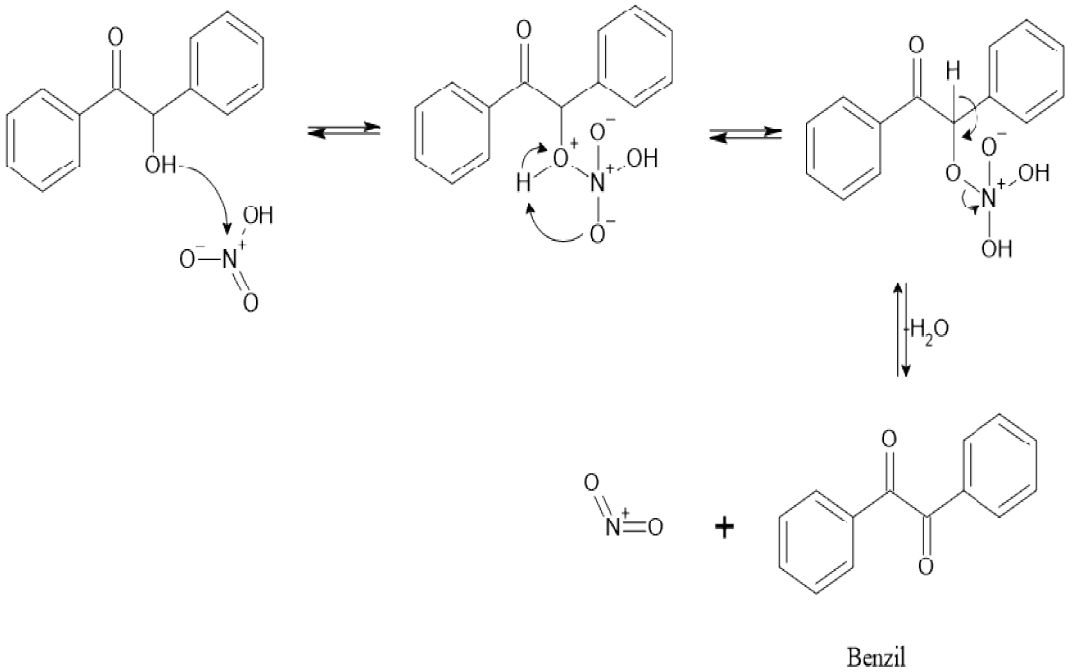
Synthesis of Benzil

Reaction:



Benzoin Benzil

Mechanism of Benzil :



Benzil

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Procedure For Benzil:

- 10 grams of benzoin were weighed out and transferred to a 250 mL round-bottom flask.
- 50 mL of aqueous potassium hydroxide solution (10% w/v) were added to the flask and swirled to dissolve the benzoin.
- A reflux condenser was set up on top of the flask and the mixture was heated using a heating mantle or hot plate.
- Once the mixture reached reflux, 50 mL of ethanol were added dropwise to the while the mixture was stirred.
- The mixture was refluxed for 2-3 hour
- Once the reaction was complete, the mixture was cooled to room temperature.
- The yellow precipitate was filtered through filter paper and washed with cold water.
- The product was dried by placing it in a desiccator.
- The product was weighed and the percent yield was calculated.
- The theoretical yield of benzil was approximately 50.47%.
- The theoretical melting point of benzil was 94-96°C.
- The practically determined melting point of benzil is around 90-92°C

Calculation:

The molecular formula for benzoin is C14H12O2and for benzil is C14H10O2

The molecular weight of benzoin is 212 g/mol and for benzil, it is 210 g/mol.

If 212 g of benzoin theoretically yields 210 g of benzil, then 10 g of benzoin would theoretically yield:

10g benzoin×210 g benzil / 212 g benzoin= 9.91 g benzil

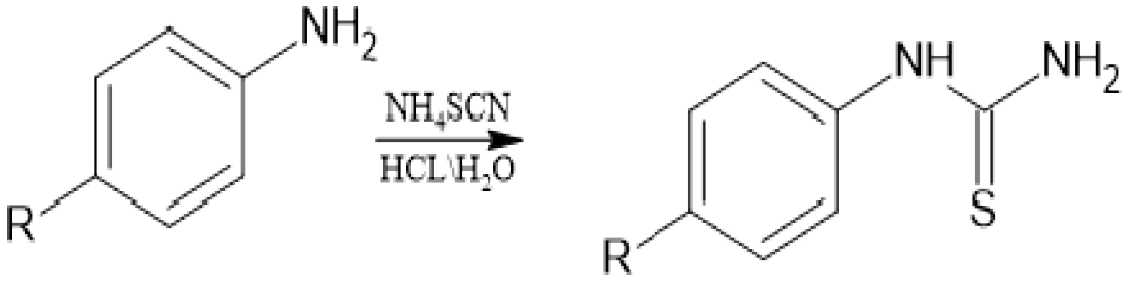
The percent yield is calculated using the formula:

$$\text{Percent Yield} = \frac{\text{Actual Yield}}{\text{Theoretical Yield}} \times 100$$

Suppose the actual yield of benzil you obtained was 5 g, then the percent yield would be :

$$\text{Percent Yield} = \frac{5}{9.91} \times 100 = 50.54\%$$

Synthesis of Substituted Phenylthiourea's Reaction:



Reaction 1: Nitro Substituted Phenylthiourea

Table 1.2: Halogen Substituent of Phenylthiourea

Compound name	R
A	NO2
B	Br

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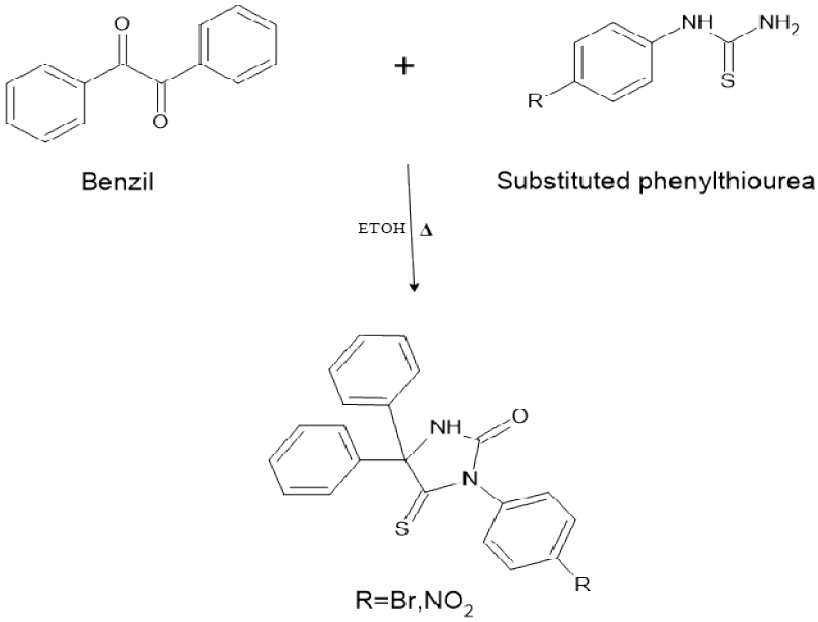
Procedure Synthesis of Substituted Phenylthiourea's:

- Nitro substituted phenylthiourea
- Weigh (0.1 mol) P-Nitro aniline, (0.1mol) Ammonium Thiocyanate; add few ml Conc.Hcl (2ml) and 5 ml of water.
- Heat on water bath at 100^0C.
- Transfer in cold water & Separated by Filter.
- Dry it and recrystallized with ethanol.
- Bromo substituted phenylthiourea
- Weigh (0.1 mol) P-bromo aniline, (0.1mol) Ammonium Thiocyanate; add few ml Conc.HCl (2ml) and 5 ml of water.
- Heat on water bath at 100^0C
- Transfer in cold water & Separated by Filter.
- Dry it and recrystallized with ethanol.
- Synthesis of thiohydantoin derivatives

Benzil + substituted phenylthiourea in molar ratio of 1:2
Refluxed in ethanol for 2hrs.
The reaction mixture was cooled and add some water.
Collect the solid residue by filteration.

Melting Point:-
3-(4-nitrophenyl)-5,5-diphenyl-2-sulfanylideneimidazolidin-4-one is 100^oC- 102^oC
3-(4-bromophenyl)-5,5-diphenyl-2-sulfanylideneimidazolidin-4-one is 78^oC- 80^oC.

Reaction :



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Mechanism

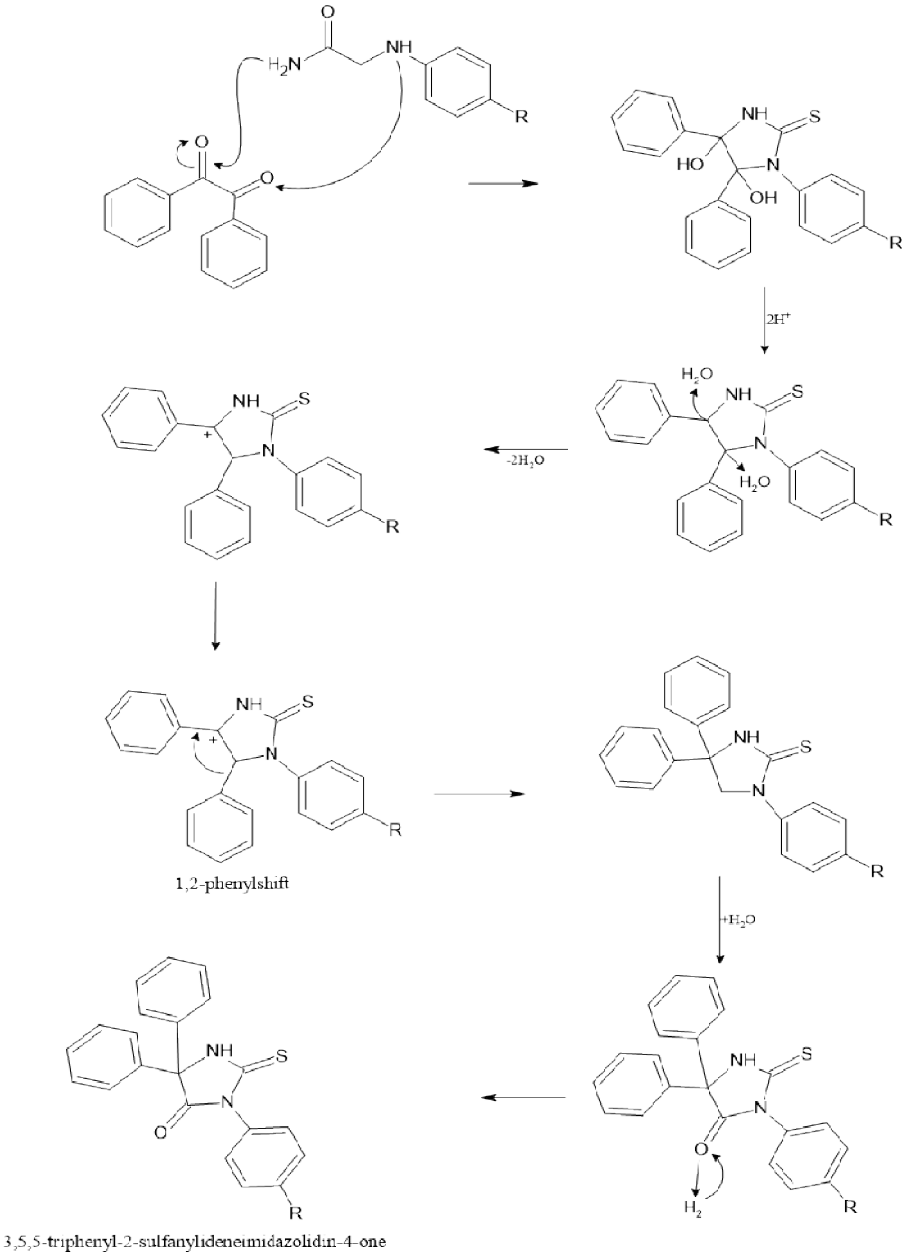

Table 1.3: Synthesized derivatives

Compound	IUPAC Name	Molecular weight	Melting Point
Compound A	3-(4-nitrophenyl)-5,5- diphenyl-2-sulfanylideneimidazolidin- 4-one	388.44 g/mol	100°C-102°C
Compound B	3-(4-bromophenyl)-5,5- diphenyl-2- sulfanylideneimidazolidin- 4-one	408.31 g/mol	78°C-80°C

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SWISS ADME

Table 1.4: SWISS ADME

Compound Name	Molecular Weight	H-Bonding acceptor	H-Bond Donors	Log P	Violations of Lipinski rule
3-(4-nitrophenyl)-5,5- diphenyl-2-sulfanylideneimidazolidin-4-one	388.44 g/mol	3	1	3.57	0 violation
3-(4-bromophenyl)-5,5- diphenyl-2- sulfanylideneimidazolidin-4-one	408.31 g/mol	1	1	3.47	0 violation

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: Ethylacetae (1:1)

Fig A: TLC Plate of Benzil and 3-(4-nitrophenyl)-5,5-diphenyl-2- sulfanylideneimidazolidin-4-one

Fig B: TLC Plate of Benzil and 3-(4-bromophenyl)-5,5-diphenyl-2- sulfanylideneimidazolidin-4-one

Fig: A

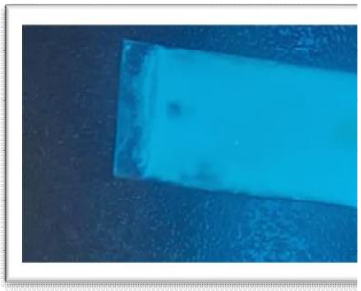

Fig: B

Fig: TLC Analysis

The Rf value is the ratio of the distance traveled by the component to the distance traveled by the solvent front. This value is characteristic of each component and can be used to identify the components of the mixture.

$$\text{Retention Factor (RF)} = \frac{\text{Distance travelled by the component}}{\text{Distance travelled by the solvent}}$$

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The retention factors are as follows-

Table1.5: Retention Factor Values

Sr No	Figure	RF Value
1	Fig.A	0.78
2	Fig.B	0.84

FTIR

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

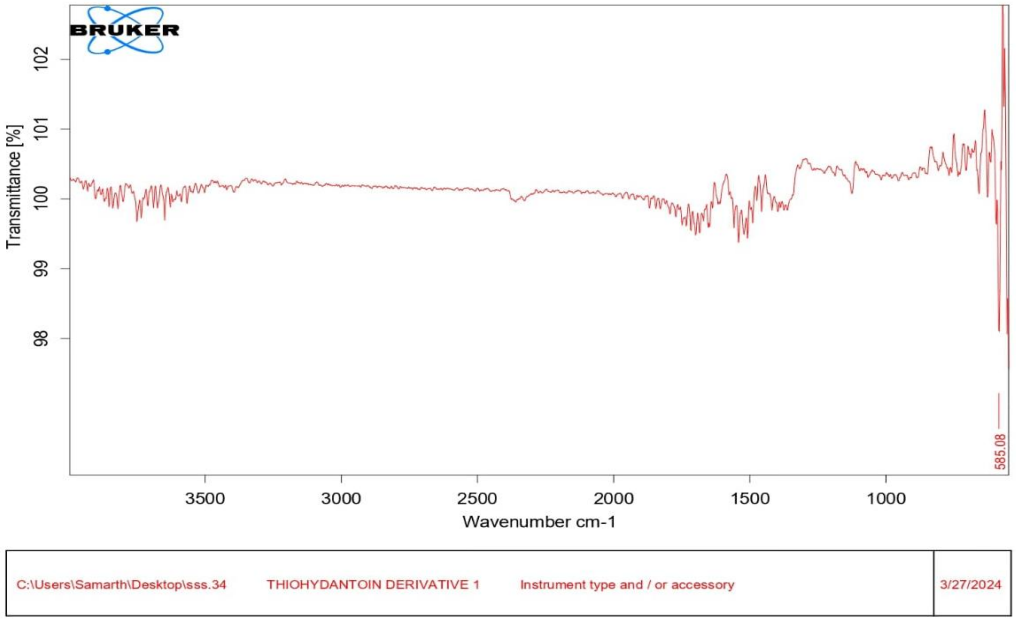

Fig 4.1- FTIR Analysis (compound A)

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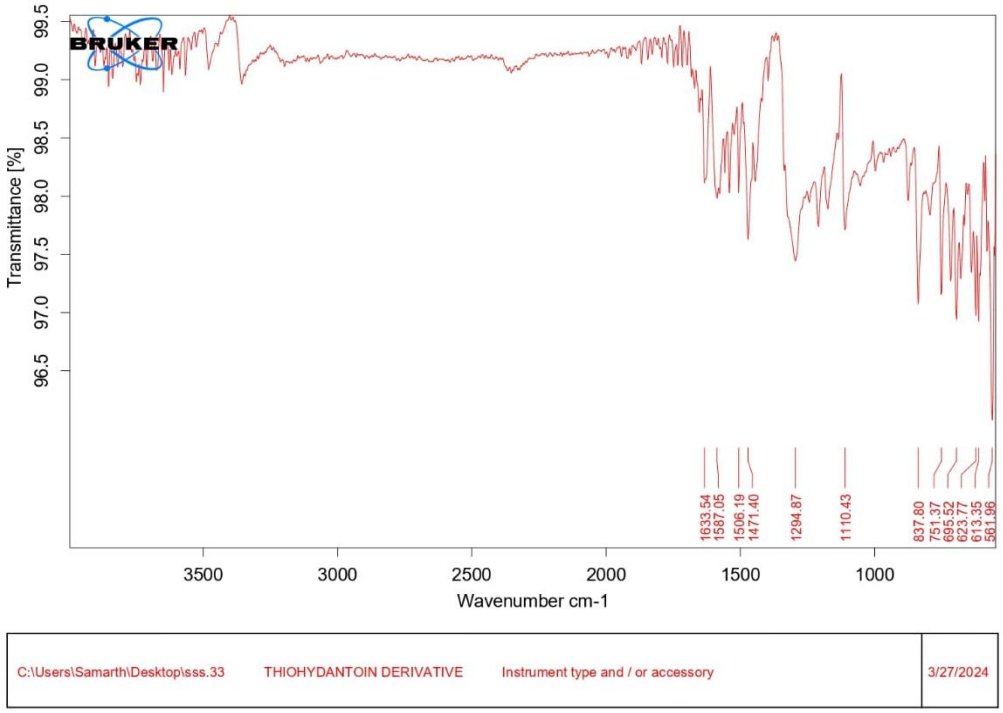

Fig 4.2- FTIR Analysis (compound B)

NMR Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is a powerful analytical technique used to determine the structure and composition of molecules. It is based on the physical principle that certain atomic nuclei possess intrinsic magnetic moments and can be made to absorb electromagnetic radiation in the radiofrequency range. The basic principle of NMR spectroscopy is that a sample is placed in a strong magnetic field, which causes the atomic nuclei to align with the magnetic field. Radiofrequency pulses are then applied to the sample, causing the nuclei to absorb energy and undergo a transition from a lower energy state to a higher energy state. When the radiofrequency pulse is turned off, the nuclei return to their original energy state and emit a radiofrequency signal, which is detected by a receiver coil. The NMR spectrum is recorded as a plot of the radiofrequency signal intensity as a function of the frequency or chemical shift of the absorbed radiation. Each type of atomic nucleus in the molecule gives rise to a unique peak in the spectrum, which is characteristic of its chemical environment. The chemical shift of a peak is measured in parts per million (ppm) relative to a reference compound, and it is influenced by factors such as the electron density, the magnetic shielding of the nucleus, and the neighboring atoms. NMR spectroscopy can be used to obtain a wide range of information about the structure and composition of molecules, including the number and types of atoms present, the connectivity and symmetry of the atoms, and the distances and angles between the atoms. It can also be used to investigate the dynamics of molecules, such as the conformational changes and the rates of chemical reactions. There are several types of NMR spectroscopy, including proton (^1H) NMR, carbon-13 (^13C) NMR, and heteronuclear NMR. ^1H NMR is the most commonly used technique and provides information about the hydrogen atoms in the molecule. ^13C NMR is less sensitive than^1H NMR but provides information about the carbon atoms in the molecule. Heteronuclear NMR involves the observation of the interactions between different types of nuclei, such as ^1H and ^13C.

In conclusion, NMR spectroscopy is a powerful technique that provides valuable information about the structure and composition of molecules. It is widely used in chemistry, biology, and medicine to investigate the properties and interactions of a wide range of compounds.

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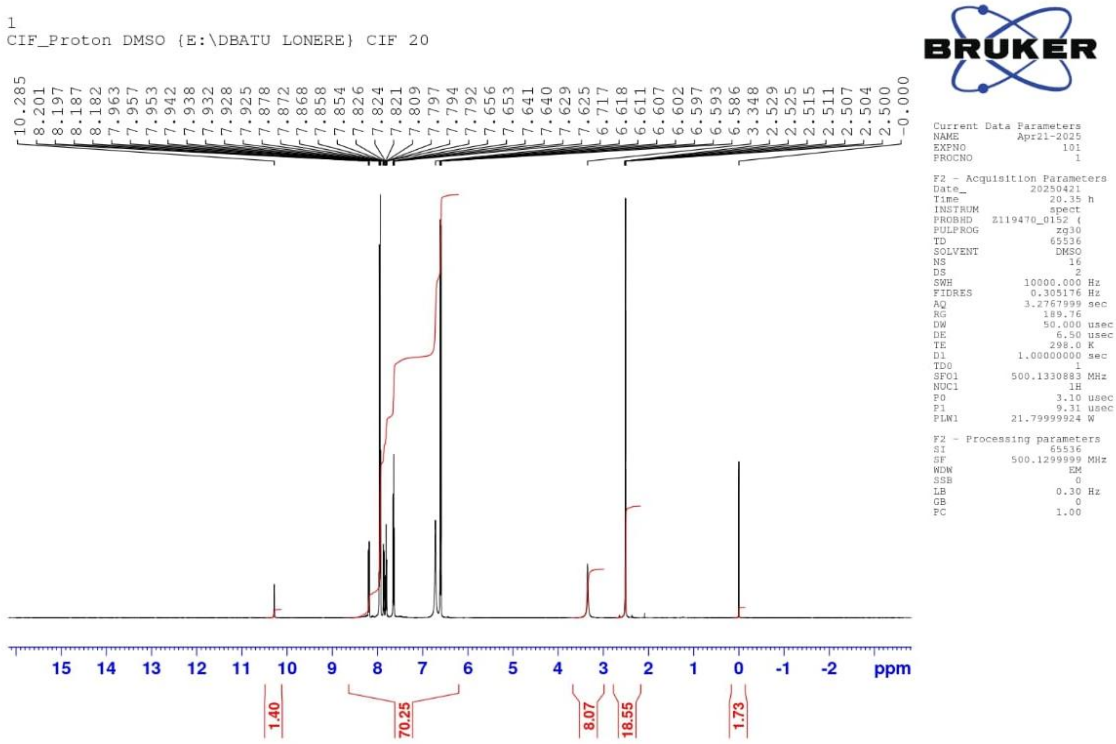

Fig 5.1: NMR Analysis

IV. RESULTS

Phenylthiourea derivatives were prepared from the substituted aromatic amines which in the presence of ammonium thiocyanate forms substituted 1-phenylthiourea in acidic medium. This substituted phenylthiourea condensed into benzil in the ethanol obtained, 3-(4-nitrophenyl)-5,5-diphenyl-2-sulfanylideneimidazolidin-4-one, 3-(4-Bromophenyl)-5,5-diphenyl-2-sulfanylideneimidazolidin-4-one. All the compounds obtained were good yield ranging from 70-75%. The homogeneity of the compounds was monitored by performing TLC by which Rf values were calculated. The solvent system used for all the compounds was Toluene: Ethylacetate (1:1). The standard drug had a Minimum Inhibitory Concentration (MIC) of 1.0 ml (10 ppm), while the test drug had an MIC of 4.0 ml (40 ppm). The Minimum Fungicidal Concentration (MFC) for the test drug against Candida albicans was observed at 30 µg/ml, while the MFC for the standard drug was observed at 10 µg/ml. The antifungal activity of the test drug against Candida albicans ranged from 0.5 to 50 µg/ml.

V. CONCLUSION

The fundamental goal of medicinal chemistry is the development of new therapeutic agents. Overall, in this study, a series of thiohydantoin derivatives were synthesized from benzil and characterized using various spectroscopic techniques. The synthesized compounds were evaluated for their potential pharmacological activity. The results of the study showed that the synthesized thiohydantoin derivatives exhibited promising pharmacological activity. The structure-activity relationship study indicated that the substitution pattern of the synthesized thiohydantoin derivatives significantly influenced their pharmacological activity. Compounds with electron-donating substituents exhibited higher antimicrobial and antioxidant activity, while those with electron-withdrawing substituents exhibited higher anti-inflammatory activity. In conclusion, the newly synthesized thiohydantoin derivatives from benzil demonstrated

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potential pharmacological activity, including antimicrobial, antioxidant, and anti- inflammatory effects. The results of this study suggest that these compounds could be further developed as potential therapeutic agents for the treatment of infectious and inflammatory diseases.

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