WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL RESEARCH

Research Article

2025
Volume: 02
Issue: 04
Page: 38-58

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL BENZOTHIAZOLE DERIVATIVES

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Received: 22 February 2025 **Revised: 11 March 2025** **Accepted: 31 March 2025**

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ABSTRACT

In human body, liver serves a number of important roles i.e., those related to metabolism, immunity, digestion, detoxification, and vitamin storage, to mention a few. The present research was based on synthesis and evaluation of novel benzothiazole derivatives for its hepatoprotective activity. Derivatives were identified and characterized based on physicochemical properties i.e., percentage yield, physical appearance, melting point, Rf value through TLC, FTIR analysis, NMR analysis, Mass spectroscopy. And evaluated the hepatoprotective potential of synthesized novel derivatives. Thin layer chromatography, or TLC for short, is a technique in synthetic chemistry that uses a compound's variable Rf value to deduce the molecule's synthesis. The Rf values for C1, C2, C3, C4, C5, and C6 were found to be0.64,0.69,0.71,0.67,0.67, and 0.70, respectively. The physicochemical characteristics of each of the synthesized benzothiazole derivatives, such as melting point and % yield, were examined. In determination of SOD level, distilled water exhibited SOD level 58.34± 0.35 U/mg of protein in control group. However, Derivatives (C1-C6) exhibited SOD level as 3.42±0.5U/mg of protein, 3.96±0.36 U/mg of protein, 4.62±0.29 U/mg of protein, respectively. Benzothiazole showed the decreased SOD level. In conclusion, the antioxidant

activity and hepatocyte (liver cell) healing properties of synthetic benzothiazole derivatives may account for their hepatoprotective effects. Its mechanism of action must be established by molecular research in order to determine which receptor subtypes it operates on and how binding efficiency might be raised.

KEYWORDS: Benzothiazole, hepatoprotective, SOD, docking study, Rf value.

INTRODUCTION

In human body, liver serves a number of important roles i.e., those related to metabolism, immunity, digestion, detoxification, and vitamin storage, to mention a few. It makes up roughly 2% of a person's total body weight. The liver is a special organ because it receives blood from hepatic artery & portal vein, around a 75 percent split (approx. 25 percent).[1] A member of the Carduus marianum family of medicinal plants, Silybum marianum (Milk Thistle), has been used for thousands of years to cure a wide range of ailments, including liver and gallbladder diseases, protecting the liver from snakebites and bug stings, mushroom poisoning, and alcoholism.[2] The primary silybin (A & B), iso-silybin (A & B) and other flavono-lignants including silychristin, neosilyhermin, silyhermin, and silydianin which are found in larger concentrations in the fruit and seeds than in other sections, make up silymarin, the active component of this herb.[3]

Benzothiazole

A ring containing nitrogen and sulfur atoms is joined to a benzene atom to form benzothiazoles, which are heterocyclic dicyclic compounds.[4] It can be used medicinally as an anti-inflammatory,[5] anti-diabetic,[6] analgesic,[7] and a cancer preventive.[8] Benzothiazoles are found in a wide variety of marine and terrestrial chemicals that have beneficial biological properties. Numerous illnesses, such as cancer, central muscle relaxants, and neurological diseases, are treated with benzothiazole.[9]

The biological properties of benzothiazoles are easily obtained, which makes the development of novel benzothiazoles easier.[10] Benzothiazole is utilized as a building block to create more complicated compounds with desirable biological characteristics because it is a heterocyclic molecule. Its aromaticity makes it rather stable, but because it is a heterocycle with reactive sites, it can be functionalized. Benzothiazole has a melting and boiling points of 2ºC and 227–228ºC, respectively. Benzothiazole has a density of 1.24 g/ml and a molecular

mass of 135.19 g/mol. Benzothiazole has no use in the house. Both academic and practical uses exist for it.[11]

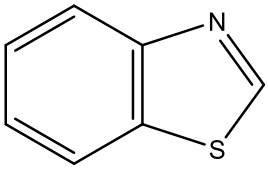


Fig. 1. Structure of Benzothiazole.

IUPAC name: 1,3-Benzothiazole

Molecular formula: C_7H_5NS

MATERIALS AND METHODS

Experimental Requirements

Benzothiazole, water, ethanol, weight balance, RBM, condenser, thermometer, plethysmograph, & pH meter.

Synthesis of novel derivatives of benzothiazole

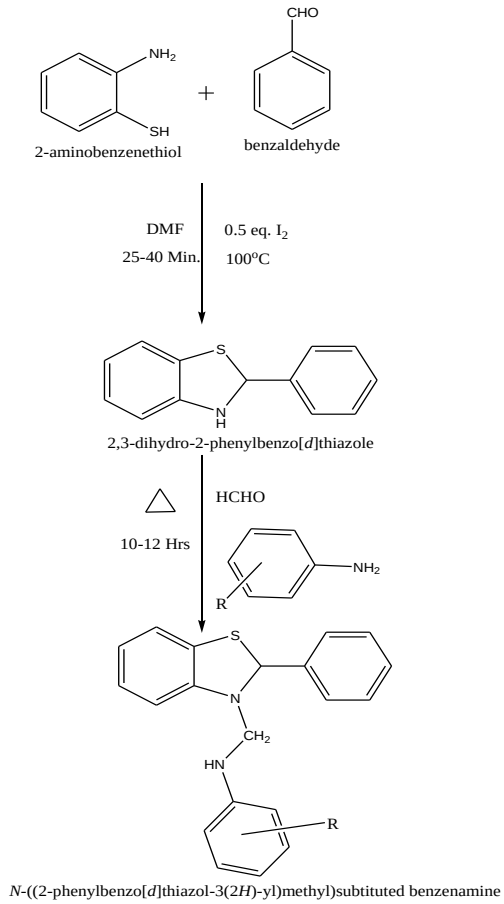
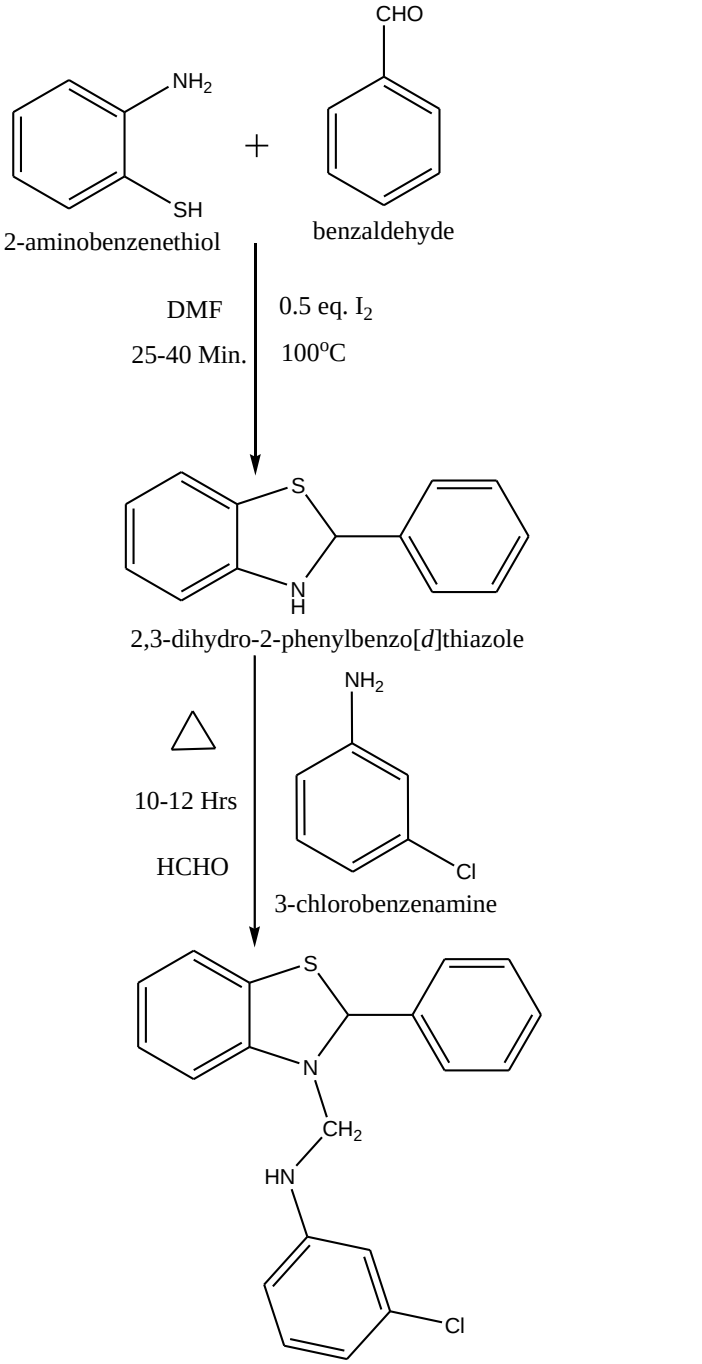


Fig. 2. Scheme for synthesis of benzothiazole derivatives.

All the 6 novel synthesized derivatives will be evaluated for physiochemical parameters then pharmacological (hepatoprotective) activity as follows.

Synthesis of C1



3-chloro-*N*-((2-phenylbenzo[*d*]thiazol-3(2*H*)-yl)methyl)benzenamine

Fig. 3. Synthesis of C1.

Synthesis of C2

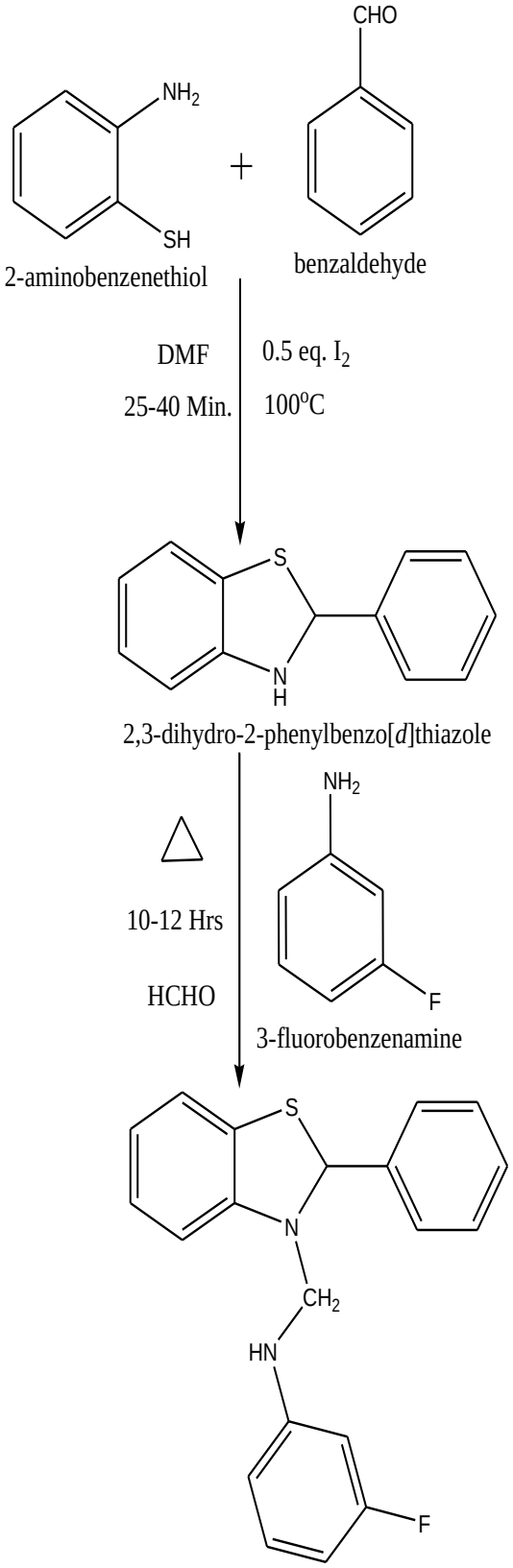


Fig. 4. Synthesis of C2.

Synthesis of C3

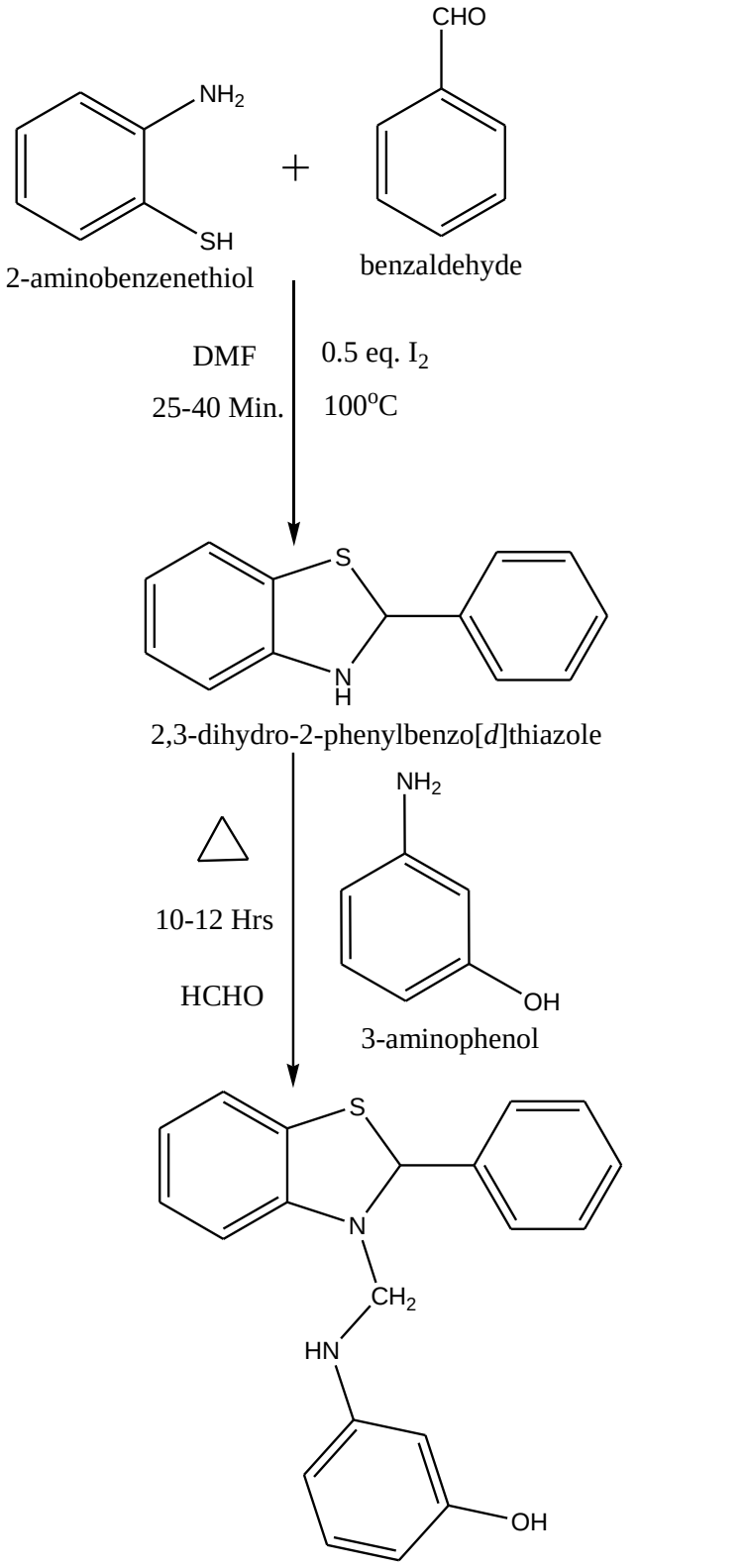
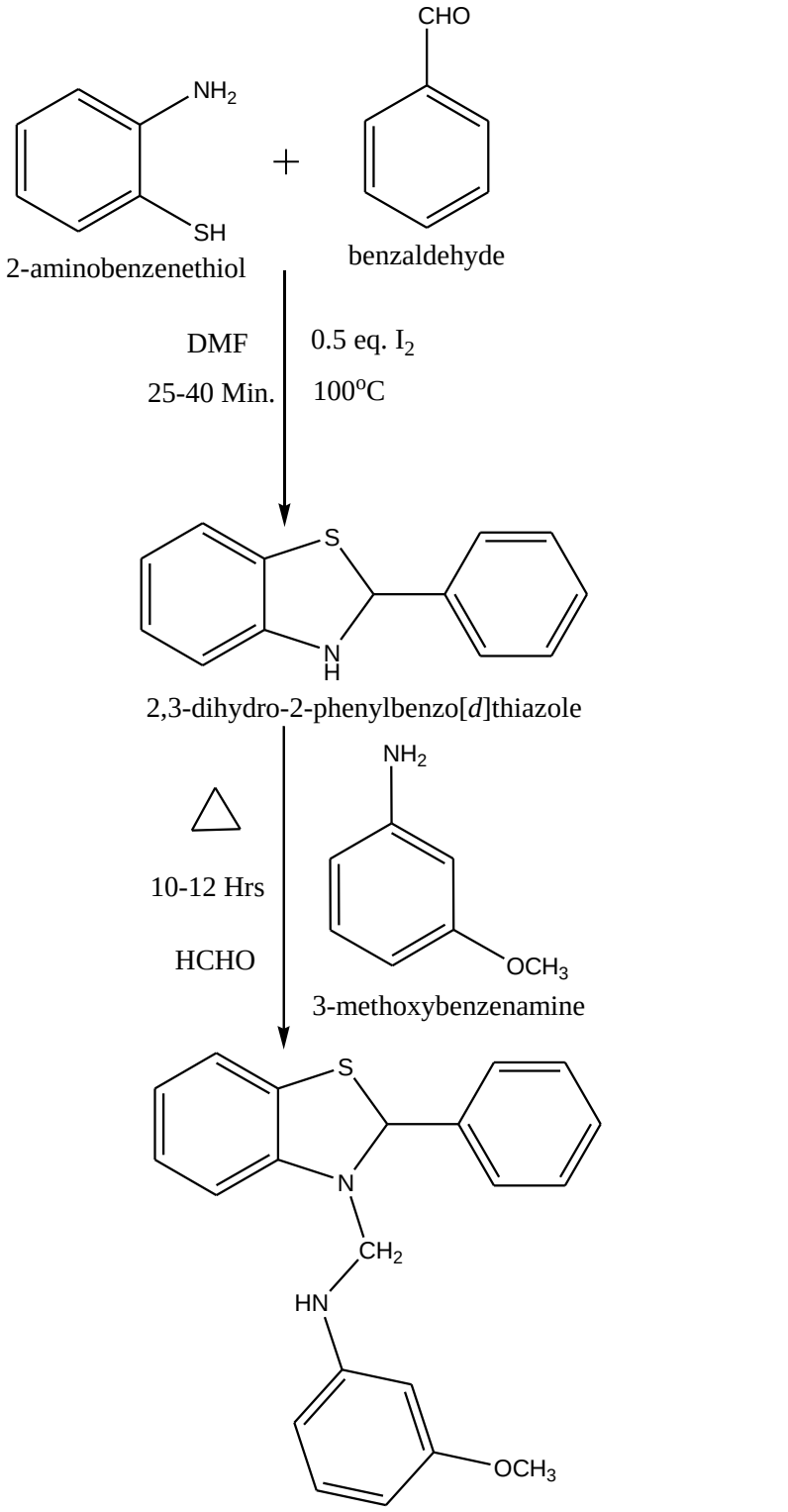


Fig. 5. Synthesis of C3.

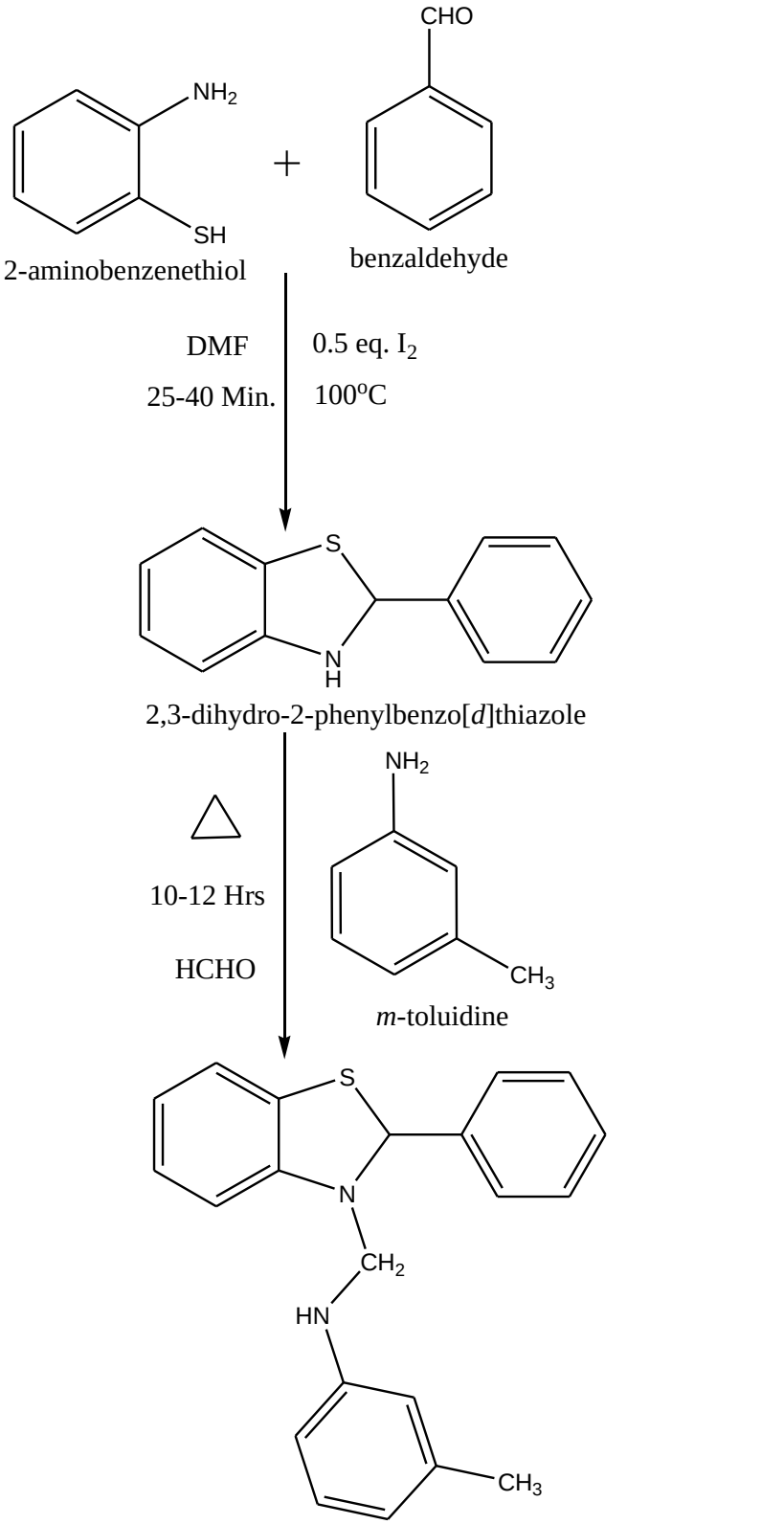
Synthesis of C4



3-methoxy-*N*-((2-phenylbenzo[*d*]thiazol-3(2*H*)-yl)methyl)benzenamine

Fig. 6. Synthesis of C4.

Synthesis of C5



3-methyl-*N*-((2-phenylbenzo[*d*]thiazol-3(2*H*)-yl)methyl)benzenamine

Fig. 7. Synthesis of C5.

Synthesis of C6

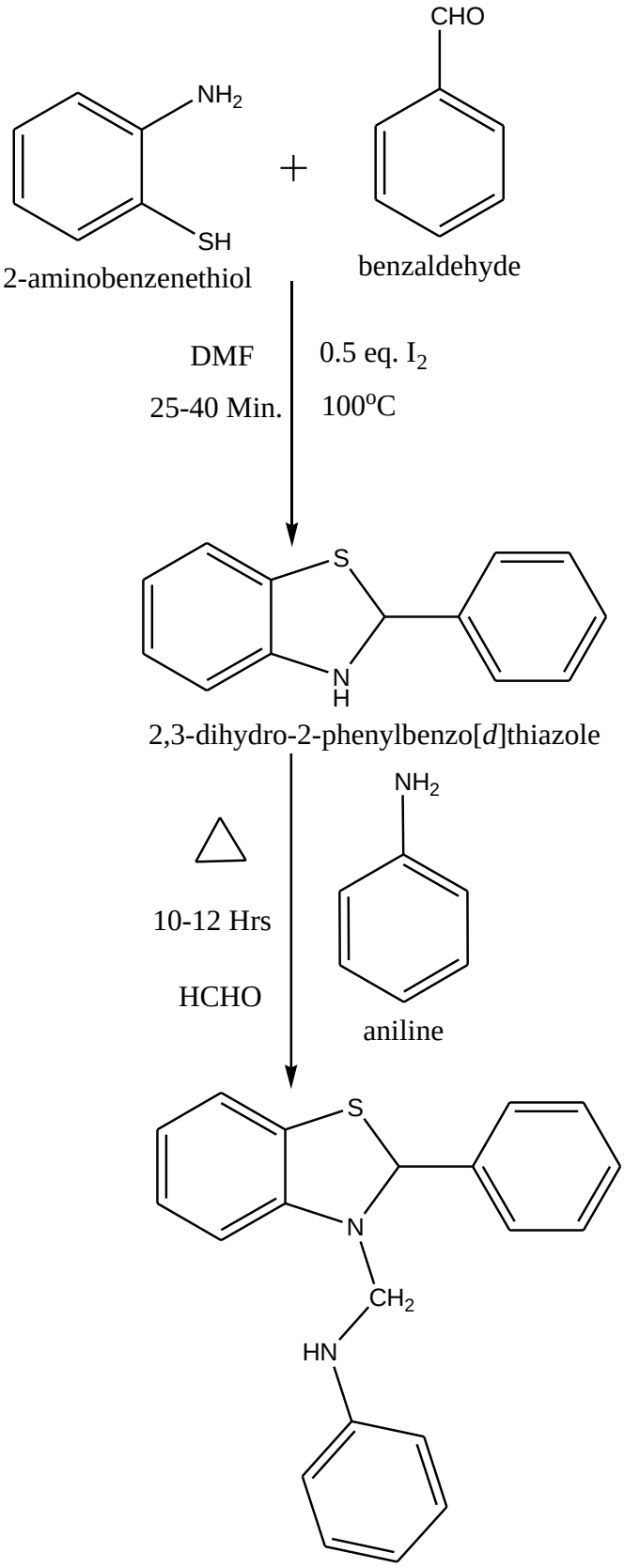


Fig. 8. Synthesis of C6.

A. Identification of physicochemical properties

➢ Melting point

The melting point of an organic compound was ascertained using Thiel's melting point tube. Finding a compound's melting point is the most crucial and direct way to differentiate one from another.[12]

➢ Rf value

Thin layer chromatography, or TLC for short, is a technique in synthetic chemistry that uses a compound's variable Rf value to deduce the molecule's synthesis. It also helps to validate the reaction's advancement.[13]

➢ Infrared Spectroscopy

One classifies the infrared spectrum as a vibrational-rotational spectrum. For solid compounds, the KBr pellet technique is utilized; for liquid compounds, the Nujol mull method is employed. It is a very useful document that provides details about the functional groups found in organic molecules. When electromagnetic radiation with a wavelength spanning from 500 cm-1 to 4000 cm-1 passes through a sample, the mechanism of bond stretching and bending occurs.[14]

➢ NMR Spectroscopy

Proton NMR is the most widely utilized NMR method due to its high sensitivity and extensive characteristic information. The chemical shift (δ) range is 0–14 ppm. The test unknown compound's chemical shift was compared to TMS protons, which had an attribution of 0 ppm. However, the shift extends to the component.[15] for the organic compound range δ 0–14.

B. Evaluation of Pharmacological activity

➢ Estimation of total antioxidant activity

The fractions' total antioxidant activity was determined using the Prieto et al. technique. A few of the ingredients are 0.3 ml sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate.[16] The reaction mixture was incubated in a water bath at 95°F for ninety minutes. The absorbance of each sample combination was calculated at 695 nm. Total antioxidant activity was calculated using the ascorbic acid equivalents in mg/g of extract.

Determination of SOD activity

In a cuvette, 0.5 mL carbonate buffer, 0.1 mL EDTA, and 1.0 mL epinephrine were mixed together. The optical density of produced adrenochrome was measured at 480 nm for three minutes at 30 second intervals. Standard solutions of 0.01 U/ml, 0.1 U/ml, 1 U/ml, and 10 U/ml were used to create the SOD calibration curve. The enzyme activity was measured in units per minute per milligramme of tissue.[17]

Assay of lipid peroxidation

1ml of material was combined with 0.2 mL sodium dodecyl sulphate, 1.5 mL acetic acid in hydrochloric acid, and 1.5 mL thiobarbituric acid in hydrochloric acid. The resulting mixture was heated for 1 hour in a hot water bath at 85°C. At 532 nm, the intensity of pink colour generated was measured against a blank.[18]

RESULTS AND DISCUSSION

Determinations of physical properties

Melting point

Melting point was determined as 126ºC, 107ºC, 85ºC, 89ºC, 76ºC and 104ºC for the benzothiazole derivatives C1, C2, C3, C4, C5 and C6, respectively.

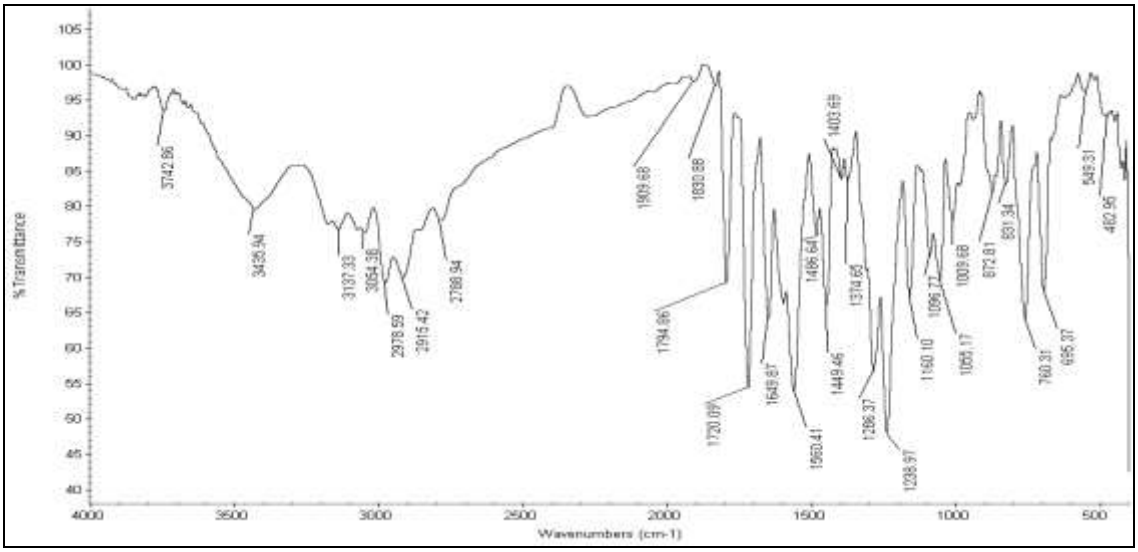
Rf value

- Thin layer chromatography, or TLC for short, is a technique in synthetic chemistry that uses a compound's variable Rf value to deduce the molecule's synthesis. The Rf values for C1, C2, C3, C4, C5, and C6 were found to be0.64,0.69,0.71,0.67,0.67, and 0.70, respectively. The physicochemical characteristics of each of the synthesized benzothiazole derivatives, such as melting point and % yield, were examined.
- The highest yielding crops, C4 and C6, were shown to be 71.46% and 69.30%, respectively.
- C2 had the lowest yield percentage, 64.48%. The compounds C2 and C5 were found to have the highest melting points, at 7ºC and 8ºC. The compound with the highest melting point has the strongest density.
- The maximum Rf of 0.72 was observed in C5. The physical characteristics of every chemical were compiled in the table that follows.

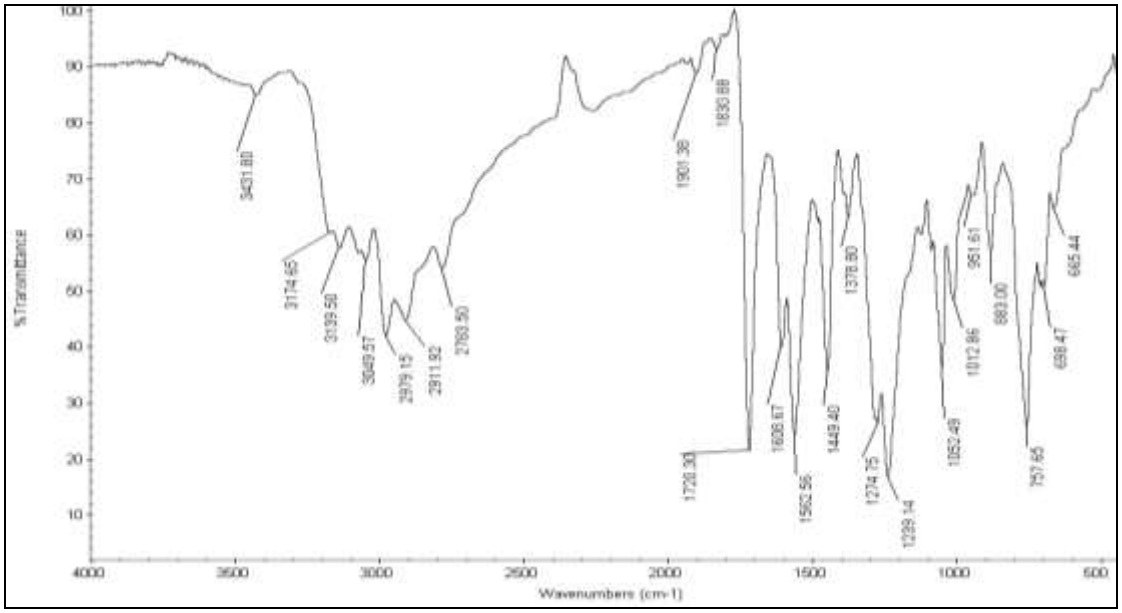
Table 1: Physical properties of synthesized benzothiazole derivatives.

Compound	Yield (%)	Rf Value	Melting point
C1	68.42	0.64	126ºC
C2	64.48	0.69	107ºC
C3	70.12	0.71	85ºC
C4	69.30	0.67	89ºC
C5	66.20	0.72	76ºC
C6	71.46	0.70	104ºC

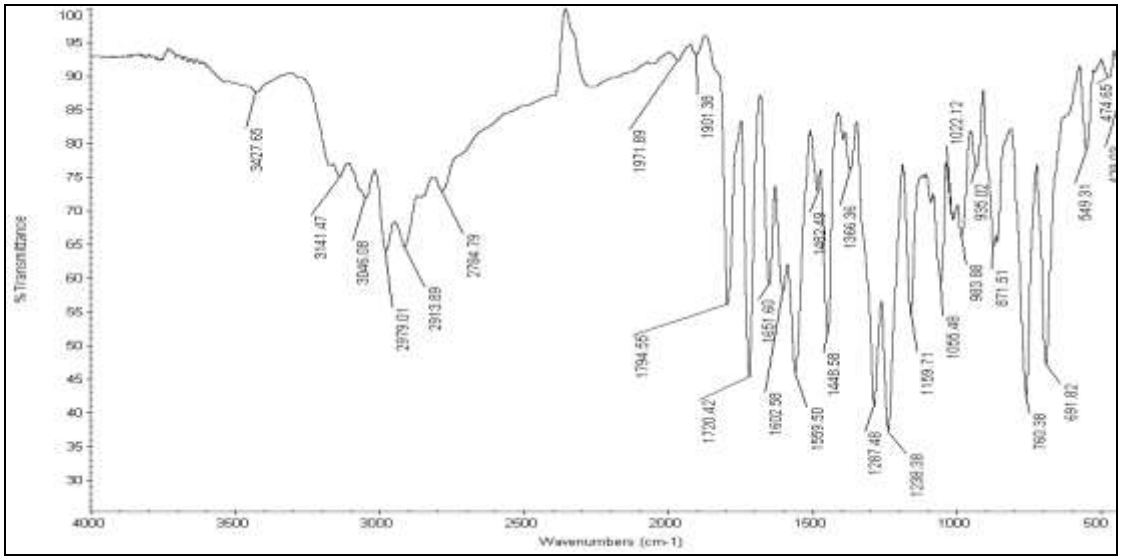
Infrared Spectroscopy



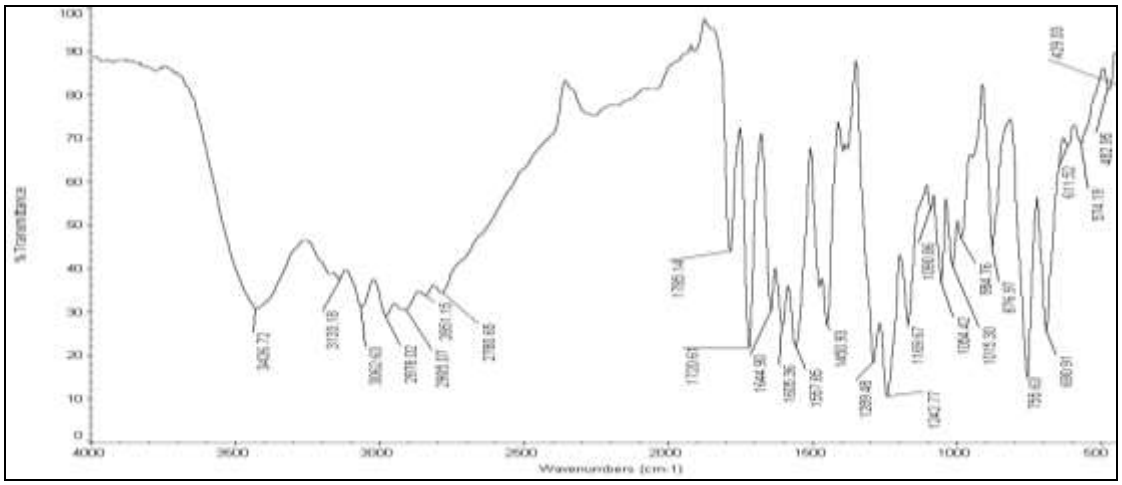
IR Spectra of C1



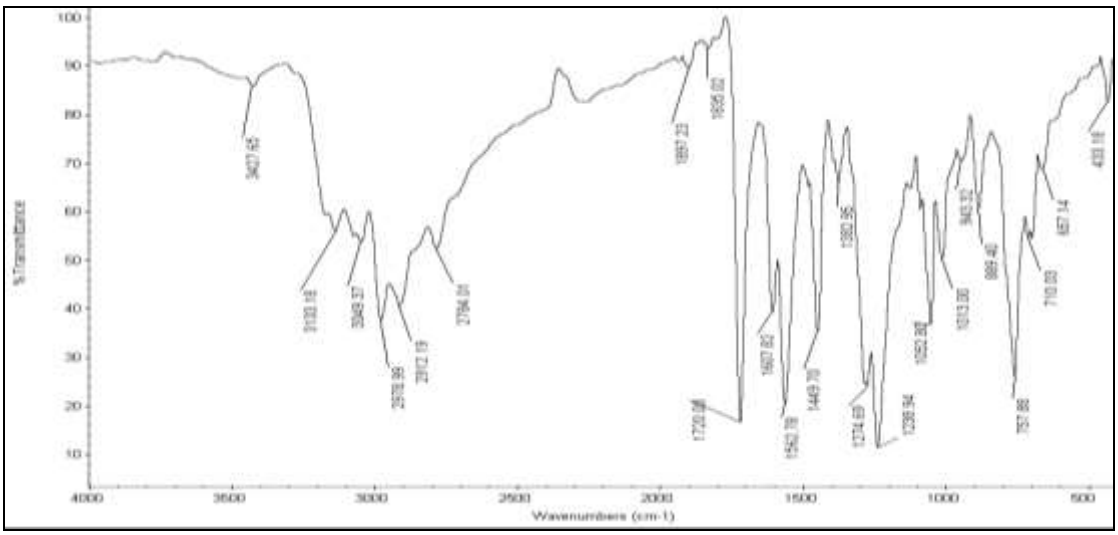
IR Spectrum of C2



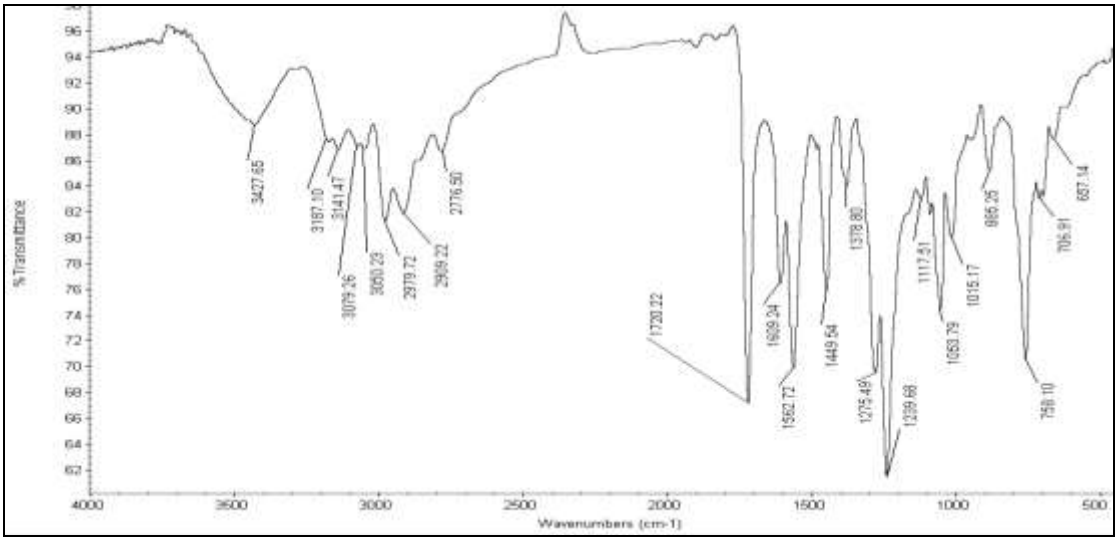
IR Spectrum of C3



IR Spectrum of C4

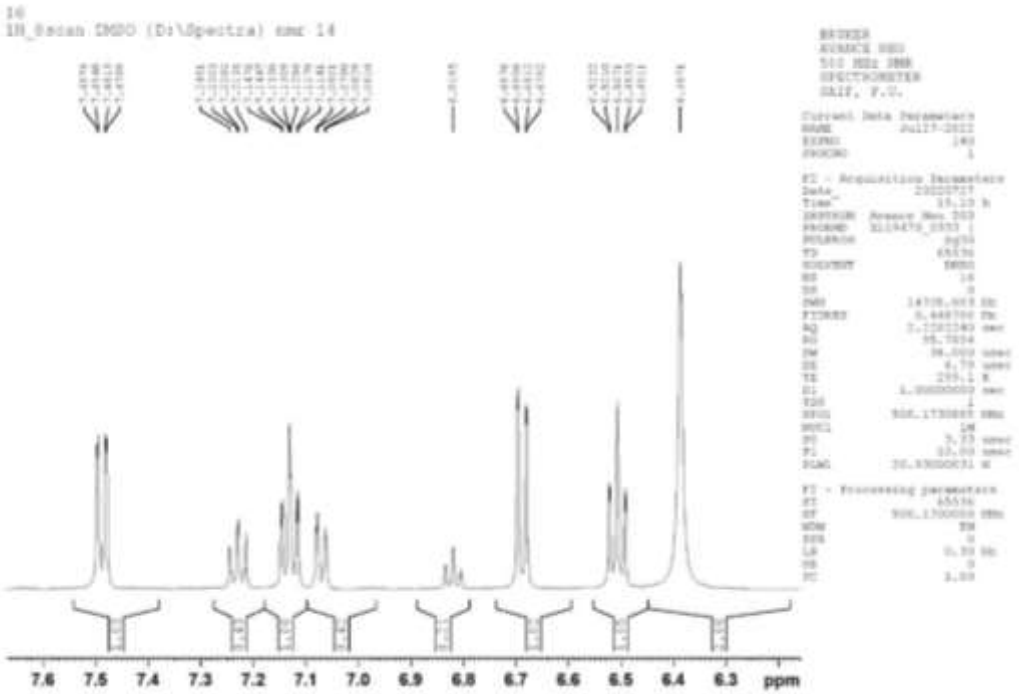


IR Spectrum of C5

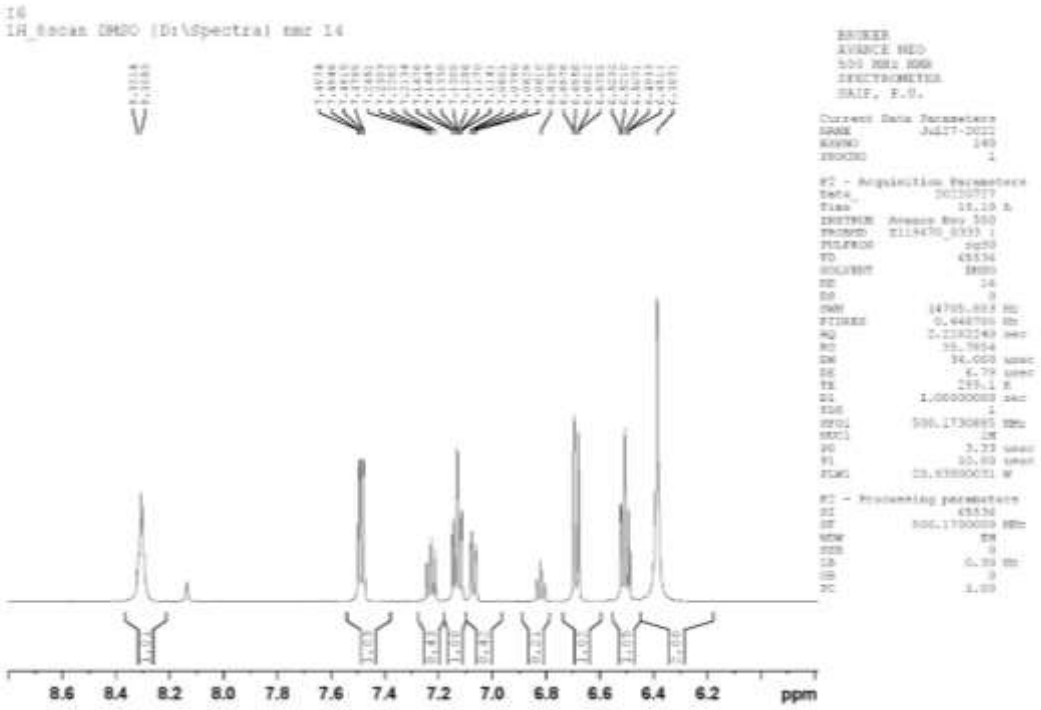


IR Spectrum of C6

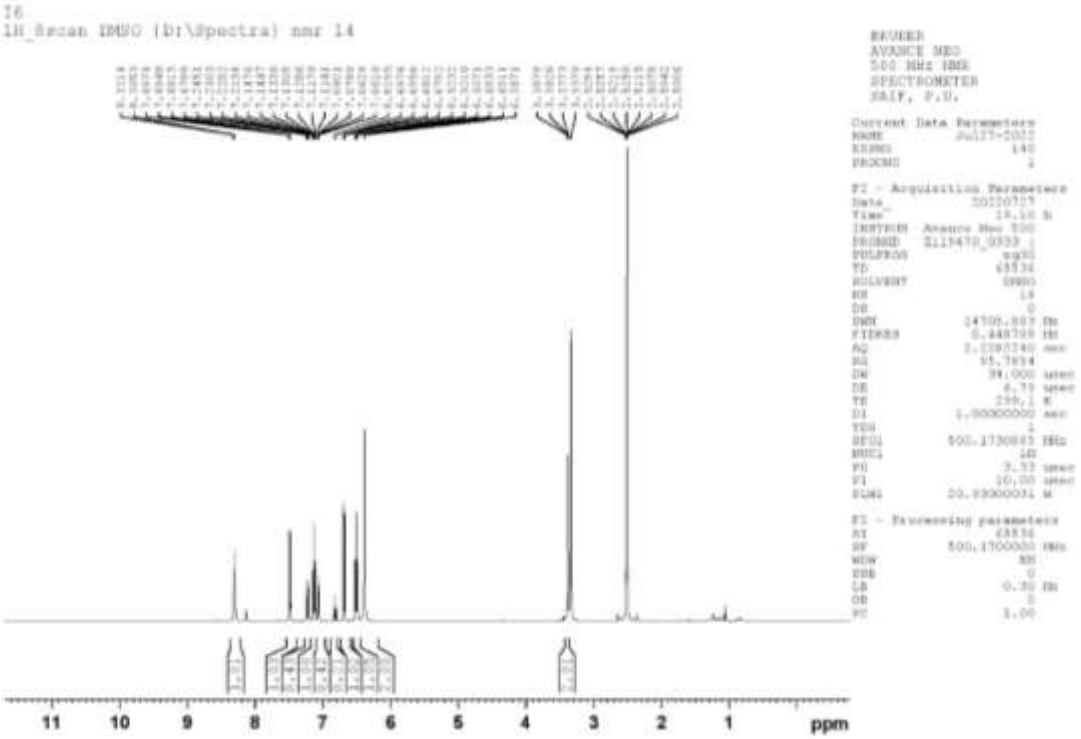
NMR spectroscopy



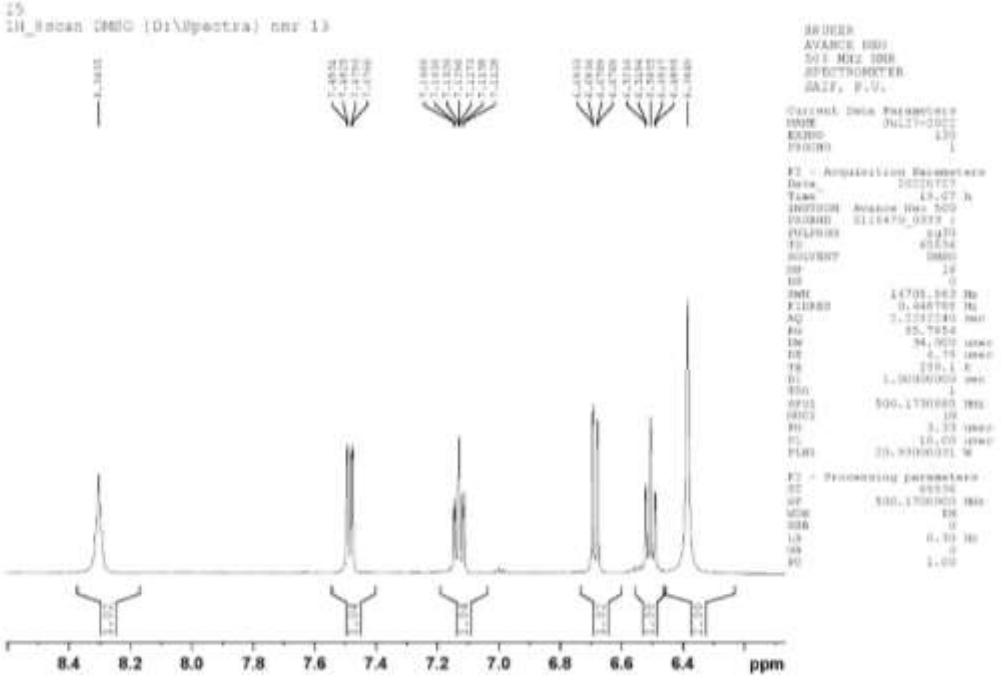
NMR Spectrum of C1



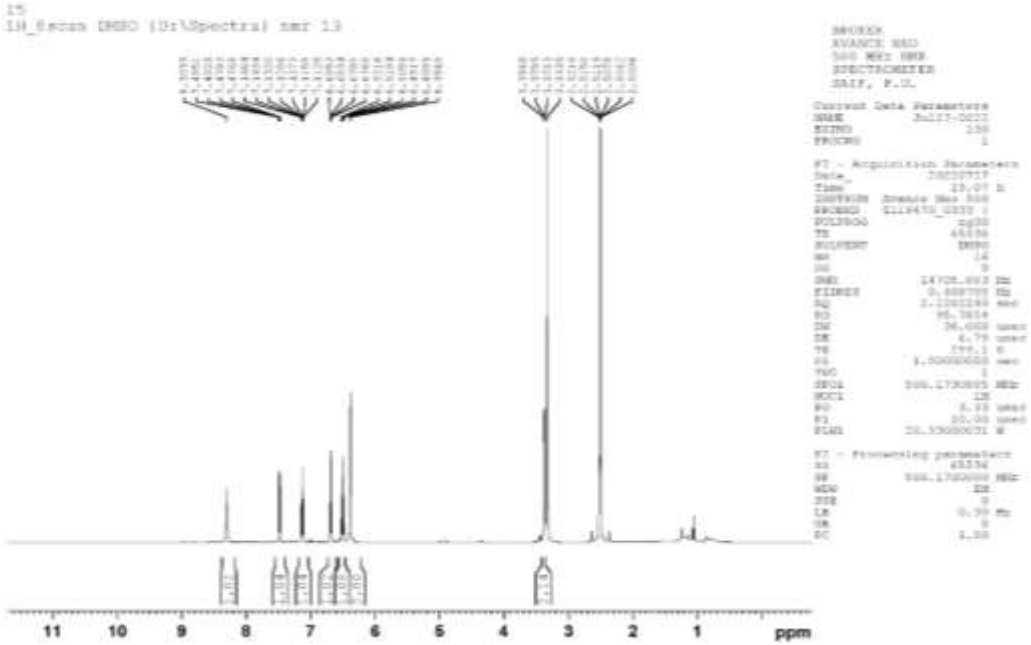
NMR Spectrum of C2



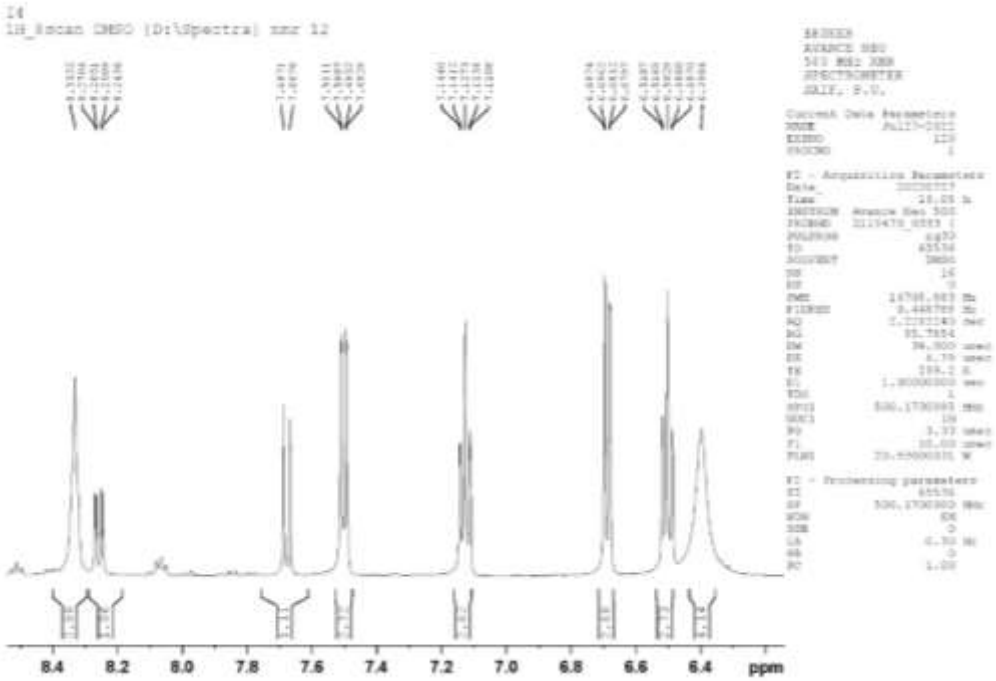
NMR Spectrum of C3



NMR Spectrum of C4



NMR Spectrum of C5



NMR Spectrum of C6

Estimation of total antioxidant activity

In estimation of total antioxidant activity, Derivatives (C1-C6) exhibited total antioxidant level as 59.43± 0.83µg/ml, 61.21± 0.31 µg/ml, 63.54± 0.23 µg/ml, 58.12± 0.39µg/ml, 62.34± 0.31 µg/ml and 62.21± 0.29 µg/ml, respectively. So, it significantly demonstrated anti-oxidant potential that indicates for its hepatoprotective effect when compared with positive control.

The following table shows the total anti-oxidant potential-

Table 2: Estimation of total anti-oxidant activity of benzothiazole derivatives.

Treatment	Total anti-oxidant activity (Conc. µg/ml)
Normal saline	34.28± 0.29
C1 (200µg/ml)	59.43± 0.83
C2 (200µg/ml)	61.21± 0.31
C3 (200µg/ml)	63.54± 0.23
C4 (200µg/ml)	58.12± 0.39
C5 (200µg/ml)	62.34± 0.31
C6 (200µg/ml)	62.21± 0.29

Estimation of TBARS level

Similarly, highest inhibition of TBARS was observed as 192.37±0.27nM/mg of protein in C3 (200µg/ml). The effect was observed in ascending order with increase in the dose. Minimum TBARS level was estimated in control group.

Table 3. Estimation of TBARS level.

Group	TBARS level± S.D.
Distilled water	139.34±0.24
C1 (200µg/ml)	171.29±0.38
C2 (200µg/ml)	178.45±0.19
C3 (200µg/ml)	192.37±0.27
C4 (200µg/ml)	189.37±0.16
C5 (200µg/ml)	191.37±0.20
C6 (200µg/ml)	187.37±0.12

Determination of SOD level

In determination of SOD level, distilled water exhibited SOD level 58.34± 0.35 U/mg of protein in control group. However, Derivatives (C1-C6) exhibited SOD level as 3.42±0.5U/mg of protein, 3.96±0.36 U/mg of protein, 4.62±0.29 U/mg of protein, respectively. Benzothiazole showed the decreased SOD level.

The following table shows the SOD level-

Table 4: Determination of SOD level of benzothiazole derivatives.

Treatment	SOD level (U/mg of protein)
Distilled water	58.34± 0.35
C1 (200µg/ml)	3.42±0.57
C2 (200µg/ml)	3.96±0.36
C3 (200µg/ml)	4.62±0.29
C4 (200µg/ml)	4.12±0.25
C5 (200µg/ml)	4.72±0.31
C6 (200µg/ml)	4.63±0.16

Phthalimide is a synthetic heterocycle with benzene fused nitrogen that has extensive biological activity, including antiviral, analgesic, anti-inflammatory, anti-convulsant, and anticancer effects. As a result, various phthalimide hybrids were identified as powerful anticancer agents, leading to the discovery of new, powerful hybrids. The use of benzothiazole-phthalimide hybrids as powerful anticancer agents has been reported by Philoppes et al.[19] Thiazolidinone was well-known for having a wide range of

pharmacological properties, including antiviral, antibacterial, antidiabetic, and anticancer effects.

When compared to the control, benzothiazole derivatives showed hepatoprotective effects in all the criteria. It proved to be more successful than the control group. Lipid peroxidation was reduced, indicating its antioxidant activity. Benzothiazole was shown to have an elevated SOD level, suggesting a hepatoprotective action. As a result, benzothiazole may help treat liver conditions like hepatotoxicity and provide dose-dependent liver nutrition.

CONCLUSION

In conclusion, the antioxidant activity and hepatocyte (liver cell) healing properties of synthetic benzothiazole derivatives may account for their hepatoprotective effects. Its mechanism of action must be established by molecular research in order to determine which receptor subtypes it operates on and how binding efficiency might be raised.

Research suggests that benzothiazole derivatives are effective as hepatoprotective but it's mode of action is still unknown. So further research may carried-out to confirm its mode of action and optimum doses required in healing of hepatotoxicity.

It also suggests to identify the mechanism of action that how it is effective against hepatotoxicity. After, suitable dosage form will develop for better patient compliance and bioavailability.

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