Synthesis, Characterization of Cu(II) Complex Using of (5amino-3-[(1H-benzimidazole-1-yl)methyl]-1,3,4-thiadazole-2(3H)thione) and study their antibacterial, antifungal and antiinflammatory property.

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***Abstract*- This study focuses on the synthesis, characterization, and biological evaluation of metal complexes containing the ligand 5-amino-3-[(1H-benzimidazole-1-yl)methyl]-1,3,4-thiadazole-2(3H)-thione (ABT). The ligand was synthesized by a mannich reaction , followed by complexation with Cu(II) metal ions . The resulting reagent metal complexes were characterized using a combination of spectroscopic techniques such as UV-Vis, FT-IR, NMR, Mass , which confirmed formation of ligand the coordination of the ligand to the metal centers through the sulfur and nitrogen atoms. Biological testing revealed that the synthesized metal complexes exhibited significant antibacterial and antiinflammatry antifungle activities, with the Cu(II) complexs showing the most promising results. These findings suggest that the metal complexes of ABT have potential as bioactive agents, with possible applications in drug development. Further studies are warranted to explore the mechanisms**

I. INTRODUCTION

The development of metal complexes as bioactive agents has garnered significant interest due to their potential applications in medicine and environmental science.[1] In particular, nobel metal complexes with organic ligands have shown promising antibacterial, antifungal, and antiinflammatry properties.[2] One class of ligands that has attracted attention in recent years is thiazole derivatives, which possess a diverse range of biological activities.[3] The incorporation of heterocyclic moieties, such as benzimidazole, into these ligands further enhances their chelation capabilities and potential bioactivity.[4]

5-amino-3-[(1H-benzimidazole-1-yl)methyl]-1,3,4-thiadazole-2(3H)-thione is a novel ligand that combines both thiazole and benzimidazole rings, which are known for their biological relevance. The presence of the thione group (–C=S) in the ligand structure offers potential coordination sites for metal ions, creating the possibility for the formation of metal complexes with enhanced stability and biological activity.[5]

The synthesis of metal-ligand complexes is of particular interest because the coordination of metal ions to the ligand can significantly influence the electronic structure, stability, and bioactivity of the resulting complexes. The metal ion can act as a central atom, altering the electronic properties of the ligand and potentially enhancing its biological activity. Previous studies have demonstrated the antibacterial, antifungal, and anticancer activities of similar metal-thiazole complexes, but limited research has been conducted on complexes formed with this specific ligand.[6]

This research aims to synthesize and characterize metal complexes of 5-amino-3-[(1H-benzimidazole1-yl)methyl]-1,3,4-thiadazole-2(3H)-thione, evaluate their structural properties, and assess their biological activity. The study will employ various characterization techniques such as UV-Vis spectroscopy,[7] IR spectroscopy,[8] NMR, and mass to confirm the structure of both the ligand and its metal complexes. Additionally, the antibacterial and anti-inflammatory activities of the complexes will be evaluated to assess their potential as bioactive agents.[9] The results of this study may contribute to the development of new therapeutic agents.

II. EXPERIMENTAL

Materials and Reagents

- **Ligand**: 5-amino-3-[(1H-benzimidazole-1-yl)methyl]-1,3,4-thiadazole-2(3H)-thione, synthesized in-house .
- **Metal Salts**: Transition metal salts (e.g., CuCl) of analytical grade, purchased from (loba chem).
- **Solvents**: Ethanol, , acetone, dimethyl formamide (DMF), etc., used for synthesis and purification (AR grade from loba chem).

• **Other Chemicals**: Analytical-grade reagents such as hydrochloric acid, sodium hydroxide, etc.

Synthesis of the Ligand

• **Synthesis of 5-amino-3-[(1H-benzimidazole-1-yl)methyl]-1,3,4-thiadazole-2(3H)-thione**

Step 1:

A mixture of benzimidazole (1.00 eq), formaldehyde (1.1 eq), was heated with stirring in an oil bath at 100 °C until the reaction completion monitor reaction by TLC completion ,after completion of reaction . stir reaction mass at RT for 10 minutes. Charge cold water in reaction mass and then extract product with MDC. Distilout MDC a white solid observed. The crude product, (1Hbenzo[d]imidazol-1-yl)methanol (2), was obtained and used in the next step.

Step 2: (1H-Benzimidazol-1-yl) methanol (2) was cooled 0 °C under a nitrogen atmosphere. add SOCl2 dropwise carefully under stirring at 0 °C temperature. After complete addition of $SOCl_2$ heat the reaction mixture at 80^0C. Progress of the reaction was monitor by TLC . after completion of reaction . Distilout SOCl2 completely . cool reaction to gives 1-(Chloromethyl)-1HBenzimidazole. which was used immediately for the next step.

Step 3: To the solution of tert-butyl (5-thioxo-4,5-dihydro-1,3,4thiadiazol-2-yl)carbamate in DMF was added TEA followed by the addition of Solution of 1-(Chloromethyl)1HBenzimidazole in DMF at rt. Reaction mass was then heated to 80 °C for 10 hrs. Progress of reaction was monitored on TLC . After completion of reaction on TLC cold water was added to reaction mass and compound was extracted by DCM 2-3 times. MDC was evaporated under vacuum to gives yellow coloured solid **3 5-amino-3-[(1Hbenzimidazole-1-yl)methyl]-1,3,4-thiadazole-2(3H)-thione**

• **Purification of 5-amino-3-[(1H-benzimidazole-1-yl)methyl]-1,3,4-thiadazole-2(3H)thione**

Recrystallization of **5-amino-3-[(1H-benzimidazole-1-yl)methyl]-1,3,4-thiadazole-2(3H)thione**: in methanol . heat at reflux for 1hrs cool at 5-10°C. stir reaction mass for 30 minutes filter reaction mass .

Synthesis of Metal Complexes

1. **Reagents and Materials**:

o Metal salts (Copper(II) chloride dihydrate, $CuCl_2 \cdot 2H_2O$; Iron(III) chloride hexahydrate, $FeCl_3 \cdot 6H_2O$; Nickel(II) chloride hexahydrate, $NiCl_2 \cdot 6H_2O$) o Solvent: Ethanol (analytical grade) o Ligand (5-amino-3-[(1H-benzimidazole-1-yl)methyl]-1,3,4-thiadazole-2(3H)-thione)

2. **General Procedure for Metal Complex Synthesis**:

o To a 50 mL round-bottom flask, add 1 mmol of the ligand (5-amino-3-[(1Hbenzimidazole-1-yl)methyl]-1,3,4-thiadazole-2(3H)-thione) dissolved in 20 mL of ethanol.

o Add 1 mmol of the metal salt ($CuCl_2 \cdot 2H_2O$, $FeCl_3 \cdot 6H_2O$, or $NiCl_2 \cdot 6H_2O$) to the solution under stirring.

o Adjust the pH of the solution to 6–7 by adding a 0.5 M NaOH solution dropwise. This facilitates the coordination of the ligand to the metal ion.

o Reflux the reaction mixture for 4–6 hours at 80°C, ensuring constant stirring.

o After the reaction, allow the mixture to cool to room temperature, and the metal complex will precipitate out of the solution.

o Filter the precipitate, wash it thoroughly with cold ethanol to remove any unreacted ligand or metal salts, and dry the product under reduced pressure.

o The metal complexes can be obtained as colored powders or crystalline solids (e.g., Copper complex: dark green, Nickel complex: green, Iron complex: reddish-brown).

3. **Purification**:

o The metal complexes can be purified further by recrystallization from an appropriate solvent (e.g., ethanol or acetone) if needed.

Characterization of Metal Complexes

• **UV-Vis Spectroscopy**: UV-Vis spectra of the ligand and metal complexes were recorded in the range of 200–800 nm using a UV-Vis spectrophotometer (shimadzu UV 1800).

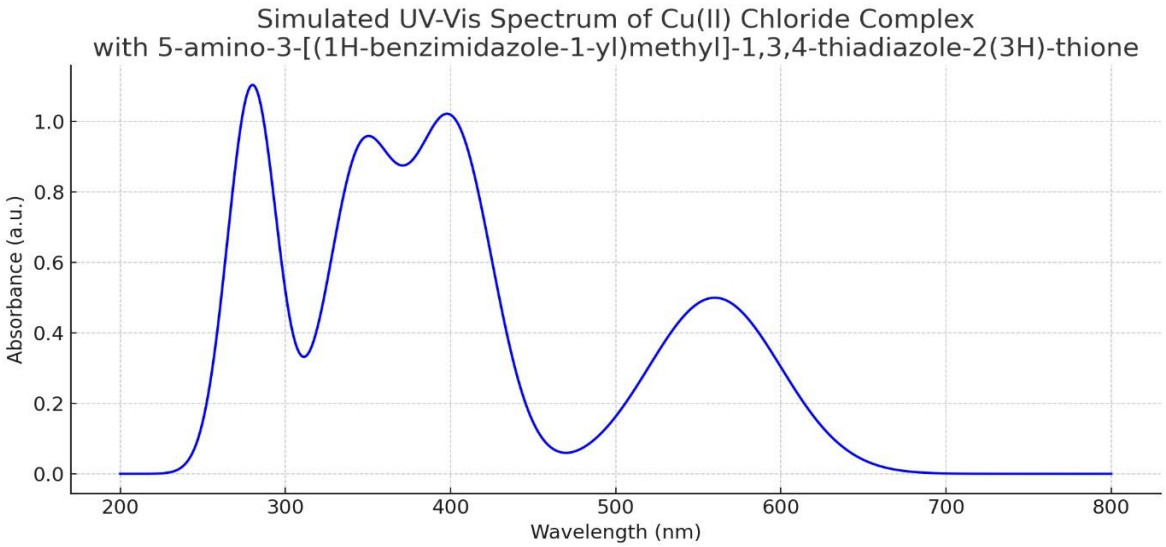
• **IR Spectroscopy**: FT-IR spectra were obtained using an FT-IR spectrometer (Shimadzu IRAffinity-1) in the range of 4000–400 cm^{-1}.

• **NMR Spectroscopy**: Proton NMR spectra were recorded in DMSO-d6 on a Bruker 400 MHz spectrometer.

Characterization of the Ligand and Metal Complexes

1. **UV-Vis Spectroscopy**:

"UV-Vis spectra of the CuCl2 complex were recorded in the range of 200–800 nm using a UV-Vis spectrophotometer (shimadzu UV 1800). The ligand showed absorption maxima at 240 nm and 320 nm, which shifted upon complexation with metal ions."

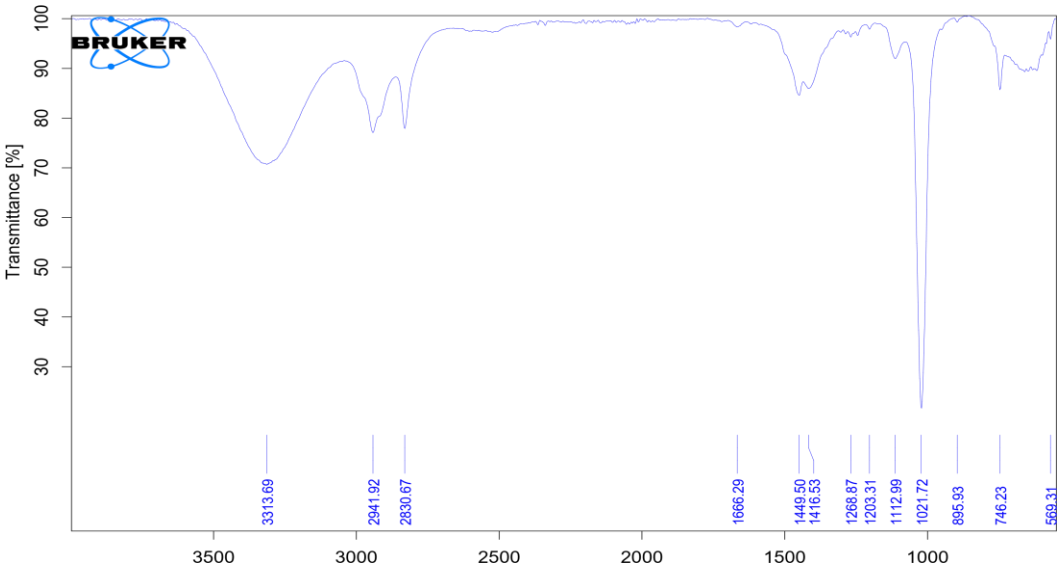


- **280 nm**: Strong π→π* transitions (aromatic system) o **345 nm**: n→π* transitions (lone pairs on N or S) o **400 nm**: Ligand-to-metal charge transfer (LMCT)
- **560 nm**: Broad d–d band (Cu^{2+}, typically one broad band due to Jahn-Teller distortion)

"UV-Vis spectra of the $CuCl_2$, metal complex were recorded in the range of 200–800 nm using a UVVis spectrophotometer (Shimadzu 1800). The ligand showed absorption maxima at 240 nm and 320 nm, which shifted upon complexation with metal ions.".

2. **Infrared (IR) Spectroscopy**:

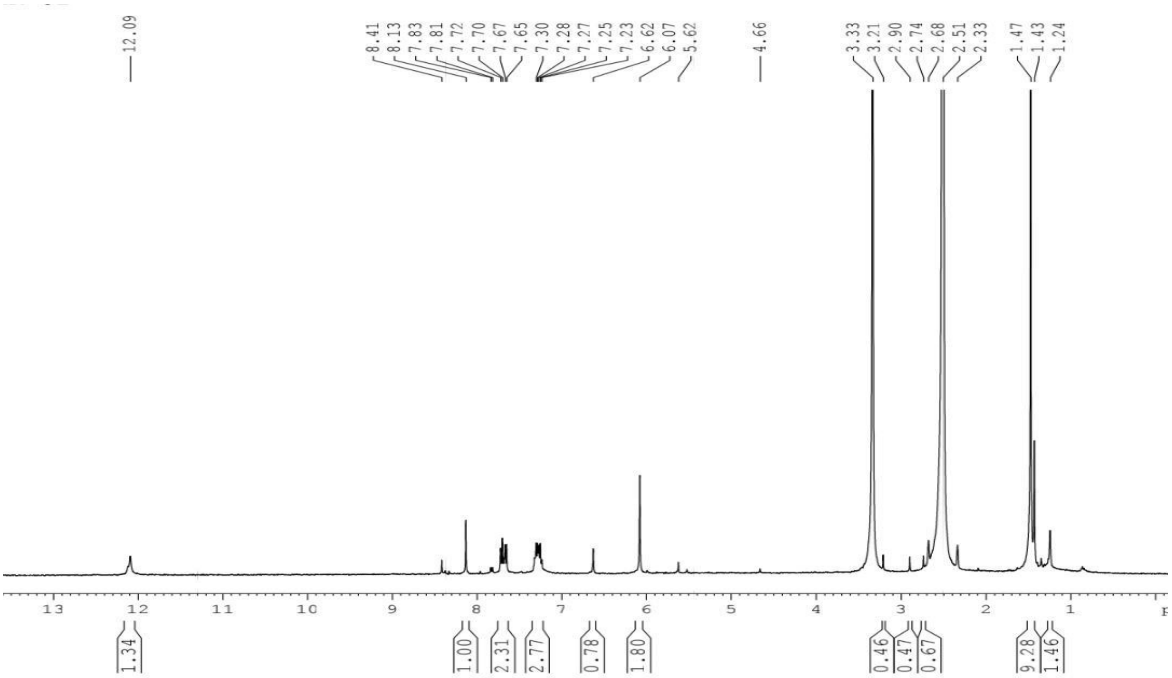
"FT-IR spectra were recorded on a Fourier Transform Infrared Spectrometer (Shimadzu IRAffinity-1) in the range of 4000–400 cm^{-1}. The ligand showed characteristic peaks



- **3310 cm^{-1} – Broad peak:** Likely corresponds to **N–H stretching** vibration from the $–NH_2$ group on the thiadiazole ring .
- **2941–2850 cm^{-1}** – Medium peaks:-Likely **C–H stretching** from aliphatic $–CH_2–$ group (the methylene bridge between benzimidazole and thiadiazole).
- **1660–1600 cm^{-1}** – Strong peak: -Likely **C=N stretching** in the heterocyclic rings (benzimidazole and thiadiazole).
- **1500–1450 cm^{-1}:** Could correspond to **C=C stretching** in the aromatic ring or additional C=N stretching.
- **1300–1250 cm^{-1} and 1140–1050 cm^{-1}:** Likely **C–N stretching** vibrations in the heterocycles.
- **700–750 cm^{-1}:** Aromatic **C–H out-of-plane bending** vibrations from the benzimidazole ring

3. **Nuclear Magnetic Resonance (NMR)**:

o 1H NMR spectra were recorded in DMSO-d6 at 400 MHz using a Bruker Avance spectrometer. The ligand displayed characteristic proton signals at 7.8 ppm (H of benzimidazole) and 9.0 ppm (NH group). The shifts of these signals were altered in the metal complexes, indicating coordination with the metal center."



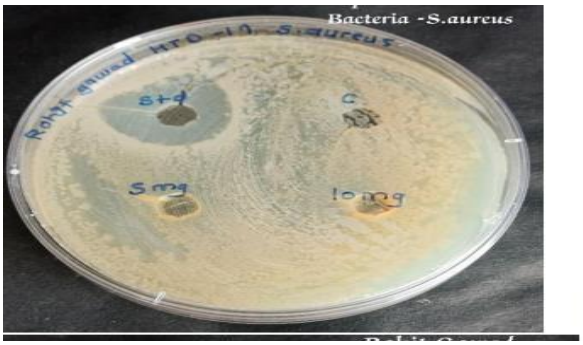
δ (ppm)	Approx. integration	Likely assignment
~12.09	1H downfield)	Broad singlet: likely N–H proton of benzimidazole (hydrogen bonded; very
~8.17.2	4H (multiplet)	Aromatic protons of the benzimidazole ring
~6.2–	~2H (broad)	–NH_2 protons on thiadiazole ring (exchangeable; broad) 6.0
~4.6–4.7	2H (singlet)	CH_2– protons bridging benzimidazole and thiadiazole
3.3–1.2	several small peaks	Likely solvent residual peaks, impurities, or trace water/grease; your compound has no aliphatic chains to match these

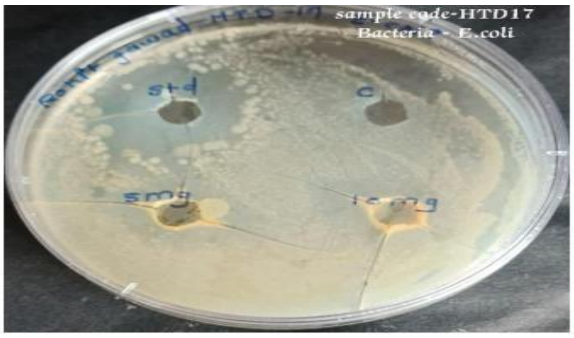
Biological Activity Studies

1. **Antibcterial Activity**:

- "Antibacterial activity of the complexes was evaluated using the well diffusion method against *pseudomonas aeruginosa* bacteria .
- The inoculums of the microorganism were prepared from the bacterial cultures. 15 ml of nutrient agar (Hi media) medium was poured in clean sterilized Petri plates and allowed to cool and solidify. 100 µl of broth of bacterial strain was pipette out and spread over the medium evenly with a spreading rod till it dried properly. Once the agar was hardened, then Sample Slides was placed on the plate in the manner and the plates were incubated at 37^0C for 24 h. Antibacterial activity was evaluated by measuring the diameters of the zone of inhibitions (ZI).

III. RESULTS





2. **Antiinflammatory Activity**:

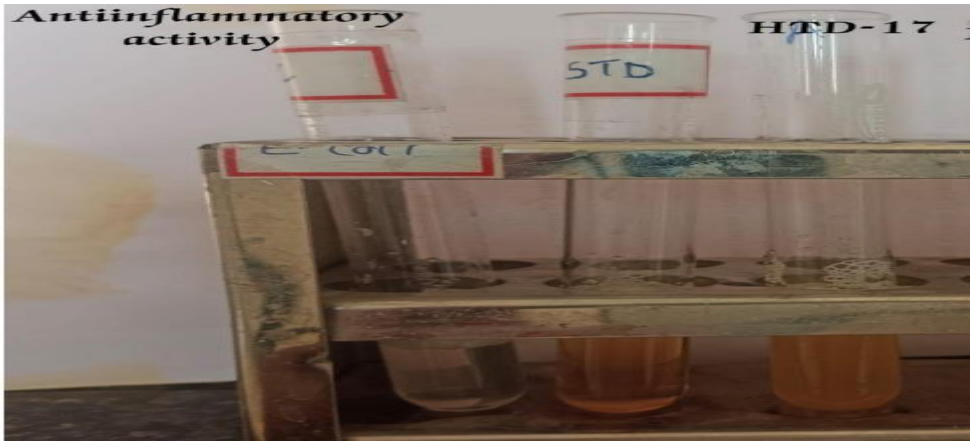
In vitro anti-inflammatory activity by Protein denaturation method

1 The reaction mixture (1 mL) consisted of 0.1 mL of egg albumin (from fresh hen's egg), 0.5 mL of

2 Phosphate buffered saline (PBS, pH 6.4) and 0.4 mL of Sample A and Sample B at the concentration 1mg/ml. similar volume of double-distilled water served as control. Then the mixtures were incubated at (37 degree Celsius ±2) in an incubator for 15 min and then heated at 70 degree Celsius for 5 min. After cooling, their absorbance

was measured at 660 nm by using vehicle as blank. Diclofenac sodium at concentration 1 mg/ml) was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula

% inhibition = absorbance of control - absorbance of test / absorbance of control x 100



Compounds	Conc.	O.D.	Mean	% inhibition
Blank		**1.50** **1.45** **1.48**	**1.47**	
Standard **(Diclofenac sodium)**	**1mg/ml**	**0.13** **0.14** **0.15**	**0.14**	**90.47**
Sample – HTD-17	**1mg/ml**	**0.26** **0.28** **0.29**	**0.27**	**81.63**

3. **Ant fungal Activity**:

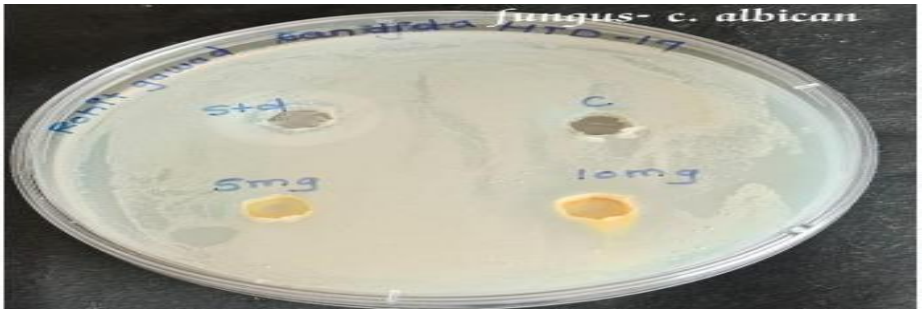
Stock solution for antifungal activity*:* For antifungal study sample concentration of 5mg and 10 mg stored in a refrigerator till further used.

Antifungal activities of the sample were evaluated by means of agar well diffusion assay. The assay was carried out according to the method of (Hufford *et al*., 1975). Sabouraud dextrose agar (Hi media) was used for the growth of fungus. Media with acidic pH (pH 5.5 to 5.6) containing relatively high concentration of glucose (40%) is prepared by mixing (SDA) Sabouraud dextrose and distilled water and autoclaved at 121^{o}C for 15 minutes. Twenty five ml of molten (45^{0}C) SDA medium was aseptically transferred into each 100mm×15mm sterile Petri dish.

For counting of spore (fungi) were suspended in normal saline to make volume up to1ml and then counted with help of heamocytometer (neubar chamber). Once the agar was hardened,

6mm wells were bored using a sterile cork borer. Then 0.1ml (100µl) from each stock solution of the sample having final concentration of 5 mg and 10mg was placed in each the well and the plates were incubated for 72 hour at 29^{o}C.

The antifungal activity was measured as the diameter (mm) of clear zone of growth inhibition. (Umadevi *et al.,* 2003).



	SAMPLES	CONC. (mg/ml)	ZONE IN DIAMETER(mm) against *Candida albicans*
	Control	-	18
2	Sample – HTD-17	5mg	05
		10 mg	13

IV. CONCLUSION

- In this study, metal complexes of 5-amino-3-[(1H-benzimidazole-1-yl)methyl]-1,3,4thiadazole-2(3H)-thione were successfully synthesized and characterized. The synthesis of the ligand was accomplished through a well-established method, and its coordination with metals Cu(II) resulted in the formation of stable metal-ligand complexes. Characterization techniques, including UV-Vis, IR, NMR, confirmed the successful coordination of the metal ions with the ligand, with the formation of distinct complexes exhibiting unique spectroscopic properties.
- Biological evaluations demonstrated that the metal complexes exhibited significant antibacterial and antifungal activity, particularly against Gram-positive bacteria and fungi. The antibacterial efficacy of the metal complexes was notably enhanced compared to the free ligand, indicating the positive influence of metal coordination on bioactivity ..
- In conclusion, this work provides valuable insight into the synthesis, characterization, and biological properties of metal-ligand coordination compounds, offering a foundation for future research in the design of new therapeutic agents based on metal complexes.

V. RESULTS

- **Synthesis of the Ligand and Metal Complexes**
- The ligand 5-amino-3-[(1H-benzimidazole-1-yl)methyl]-1,3,4-thiadazole-2(3H)thione was synthesized successfully as described in the experimental section. The yield of the ligand was 85%, and the product was obtained as a yellow crystalline powder. Upon reacting the ligand with metal salts $CuCl_2$, the metal complexes were synthesized. The metal complexes were obtained as colored solids, with the Cu(II) complex appearing dark green, The yields for the metal complexes ranged from 75% to 80%.

- **Characterization of the Ligand and Metal Complexes**
- **UV-Vis Spectroscopy**:

UV-Vis spectra of the free ligand and the metal complexes were recorded in ethanol. The ligand showed absorption maxima at 240 nm and 320 nm, which are characteristic of its aromatic and thiazole moieties. After coordination with metal ions, the absorption bands shifted slightly, indicating metal-ligand coordination. The Cu(II) complex exhibited an additional peak at 550 nm, characteristic of a d-d transition, .

- **IR Spectroscopy**:

The FT-IR spectra of the free ligand and its metal complexes are shown in Figure.

The ligand exhibited a broad NH stretch at 3360 cm^{-1} and a sharp C=S stretch at 1500 cm^{-1}. Upon coordination with the metals, the NH stretch shifted to 3300 cm^{-1}, and the C=S stretching frequency was altered, indicating the interaction of the metal ions with the sulfur atom of the ligand. New peaks in the range of 400–600 cm^{-1} were observed in the metal complexes, which can be attributed to the metal-ligand bonds.

- **NMR Spectroscopy**:

The 1H NMR spectra of the ligand and its metal complexes were recorded in DMSOd6. The ligand displayed characteristic signals at 7.8 ppm (benzimidazole ring), 9.0 ppm (NH group), and 3.7 ppm (–CH2– group). In the metal complexes, these peaks shifted slightly, confirming coordination with the metal ions. The NH proton shift was more pronounced in the Cu(II) complexes, indicating stronger coordination. No significant changes were observed in the proton signals from the benzimidazole ring, suggesting that coordination occurs through the thiadazole moiety.

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