Research Article

Novel Thiadiazole Derivative as a Schiff Base and Its Metal Complexes: Synthesis, Structural Characterization and Cytotoxic Activity

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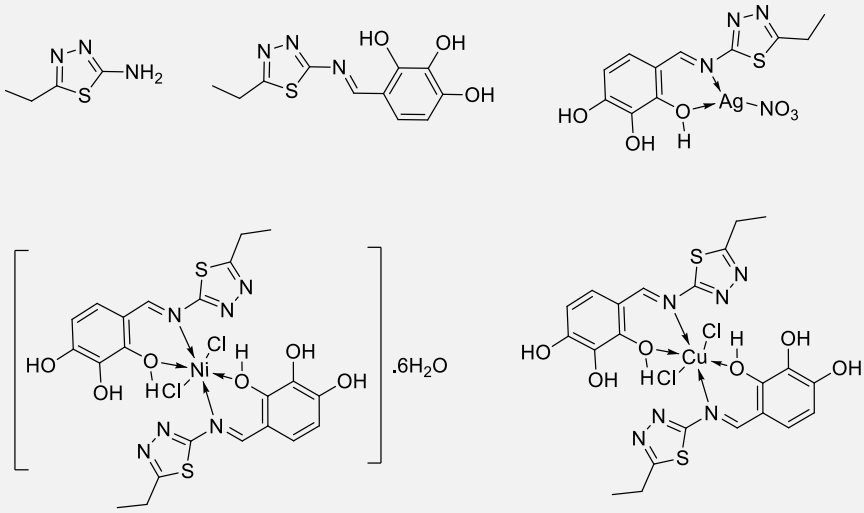
ABSTRACT

This study presents the synthesis and characterization of a Schiff base ligand, [(E)-4-(((5-ethyl-1,3,4-thiadiazol-2-yl)imino)methyl)benzene-1,2,3-triol] (ETIMBT), achieved by condensing equimolar amounts of 2-amino-5-ethyl-1,3,4-thiadiazole and 2,3,4-trihydroxybenzaldehyde. The ligand was subsequently reacted with Ag(I), Ni(II), and Cu(II) in the ratios of 1:1, 2:1, and 2:1, respectively, to form the corresponding metal complexes. These complexes were characterized using CHN analysis, FT-IR, electronic spectra, ^{1}H NMR spectra, and mass spectrometry. The in vitro anticancer activity of the ligand and its metal complexes was evaluated against HeLa and PC3 cell lines. Notably, the ETIMBT-Cu(II) complex demonstrated significant cytotoxic activity against HeLa cells, highlighting its potential as an anticancer agent.



Keywords: Schiff base; Complexes; Thiadiazole; Cytotoxic activity.

INTRODUCTION

Thiadiazoles are known for their wide range of therapeutic properties, including anti-inflammatory[1], antifungal[2], antibacterial[3], analgesic[4], antitubercular[5], antiviral[6], and anticancer effects[7,8]. Among the various azoles, 1,3,4-thiadiazoles are particularly noteworthy due to their biological significance[9]. Schiff bases and their metal complexes have been extensively studied due to their numerous biological applications, with the azomethine group found in Schiff bases playing a significant role in co-ordination chemistry[10, 11]. The therapeutic effects of Schiff bases are commonly attributed to this azomethine group. Schiff base compounds with Thiadiazole as a basic group[12] have attracted the attention of researchers in the fields of antitubercular and anticancer research[13, 14]. In this study, we prepared and characterized a Schiff base ligand and its complexes with various metal salts, focusing on their spectral properties and anticancer activity.

MATERIALS AND METHODS

The chemicals and solvents used in this experiment include: 2-amino-5-ethyl-1,3,4-thiadiazole, silver(I) nitrate ($AgNO_3$), nickel(II) chloride hexahydrate ($NiCl_2{\cdot}6H_2O$), copper(II) chloride ($CuCl_2$), p-toluenesulfonic acid, methanol (CH_3OH), and ethanol (CH_3CH_2OH), all of analytical grade. They were sourced from Aldrich Chemical Company and used without further purification. The melting points of all prepared compounds were measured with an electrothermal melting

point apparatus. Fourier-transform infrared spectra were obtained. Elemental analyses (C, H, N) were conducted using an Exeter CE-440 elemental analyzer, and the results were in agreement with the proposed structures within ±0.04% of the calculated values. UV-Vis absorption spectra were recorded using a Pye-Unicam 8800 UV-visible spectrophotometer equipped with a dip-type cell. The ligand and its complexes were analyzed using a Varian Gemini-200 spectrometer (300 MHz) to obtain proton NMR (^{1}H NMR) spectra. Mass spectra were obtained using a Shimadzu QP-2010 Plus Mass Spectrometer. The cytotoxicity assay was conducted at the Regional Center for Mycology and Biotechnology, Al-Azhar University in Cairo, Egypt.

Synthesis of Schiff base ligand; [(E)-4-(((5-ethyl-1,3,4-thiadiazol-2-yl)imino)methyl)benzene-1,2,3-triol] (ETIMBT):

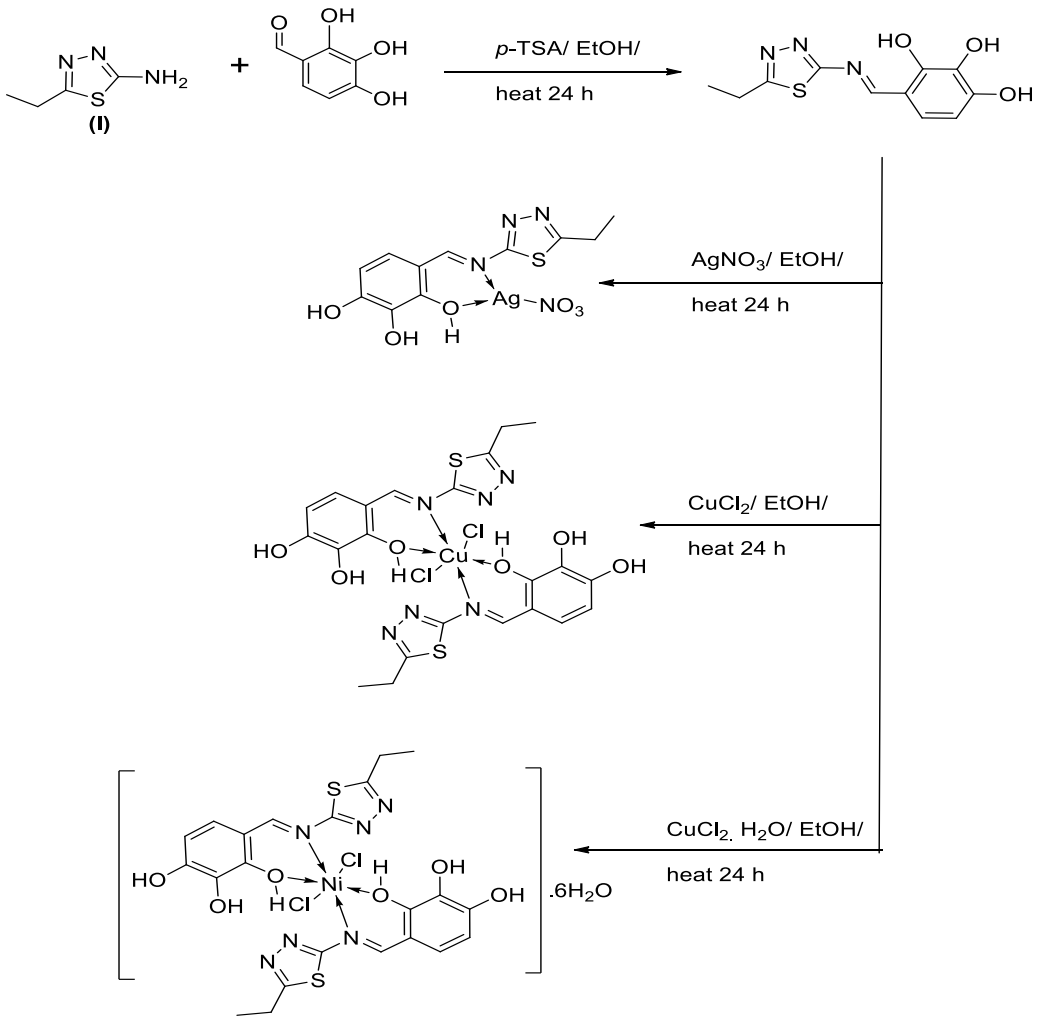
In this experiment, 0.001 moles of ethyl thiadiazole amine and 0.001 moles of trihydroxybenzaldehyde were dissolved individually in absolute ethanol before being combined. A small amount of p-toluenesulfonic acid was added, and the resulting mixture was heated under reflux for 24 hours. The yellow powder that formed as the Schiff base ligand (ETIMBT) was filtered and washed with ether and ethanol before being air-dried for 24 hours.

Synthesis of ETIMBT-Ag Complex (1:1)(L:M):

To synthesize this complex, 0.0003 moles of the Schiff base ligand (ETIMBT) were dissolved in 10 ml of hot ethanol. Separately, 0.0003 moles of $AgNO_3$ were dissolved in 10 ml of ethanol. The $AgNO_3$ solution was then added to the Schiff base (ETIMBT), and the resulting mixture was stirred under reflux conditions for 24 hours. The ETIMBT-Ag complex was subsequently precipitated, filtered, and washed with methanol before being dried in air.

Synthesis of ETIMBT-Cu or ETIMBT-Ni Complexes (2:1) (L:M):

The metal chloride (Cu or Ni) (0.001 mol) was reacted with the Schiff base ligand (ETIMBT) (0.002 mol) in a hot methanolic solvent. The resulting mixture was refluxed for 24 hours. The resulting brown and yellow solids were filtered to obtain the ETIMBT-Cu or ETIMBT-Ni complexes, respectively.



Scheme I. Synthesis of ETIMBT ligand and its metal complexes

Cytotoxicity Evaluation

The cytotoxicity of the compounds was evaluated using a viability assay. Cancer cell lines were seeded into Corning 96-well tissue culture plates at a concentration of 5 × 10^4 cells/well. After incubation for 24 hours, the number of viable cells was determined using the MTT assay. The culture medium was then removed, and 100 µl of freshly prepared RPMI 1640 medium without phenol red, along with 10 µl of a 12 mM MTT stock solution (5 mg of MTT in 1 ml of PBS) per well, was added to each well, including untreated controls. A dose-response curve was plotted for each tumor cell line after treatment with the selected compound to determine the relationship between surviving cells and drug concentration. The inhibitory concentration of 50% (IC_{50}), which causes toxic effects in 50% of healthy cells, was estimated by graphing the dose-response curve for each concentration.

RESULTS AND DISCUSSION

Synthesis and Characterization

The synthesis of the target ligand and its metal complexes is outlined in Scheme I. The condensation of 2-amino-5-ethyl-1,3,4-thiadiazole with 2,3,4-trihydroxybenzaldehyde, refluxed in ethanol containing a catalytic amount of p-toluenesulfonic acid for an extended period, yielded the Schiff base ligand [(E)-4-(((5-ethyl-1,3,4-thiadiazol-2-yl)imino)methyl)benzene-1,2,3-triol] (ETIMBT) in good yield. The Schiff base complex of Ag^+ (ETIMBT-Ag) was synthesized by heating the ETIMBT ligand with an equimolar quantity of silver nitrate in ethanol for 24 hours. In a similar manner, Schiff base complexes of Cu^{2+} and Ni^{2+} were synthesized by refluxing two molar ratios of ETIMBT with one molar ratio of the corresponding metal chloride in ethanol for 24 hours. Their structural formulas were elucidated and confirmed by IR, electronic absorption, and ^1H NMR spectral data, as well as mass analysis, which showed that the actual mass values matched the theoretically calculated molecular weight values (see Figures 1-4). Table 1 summarizes the physical properties of all synthesized compounds.

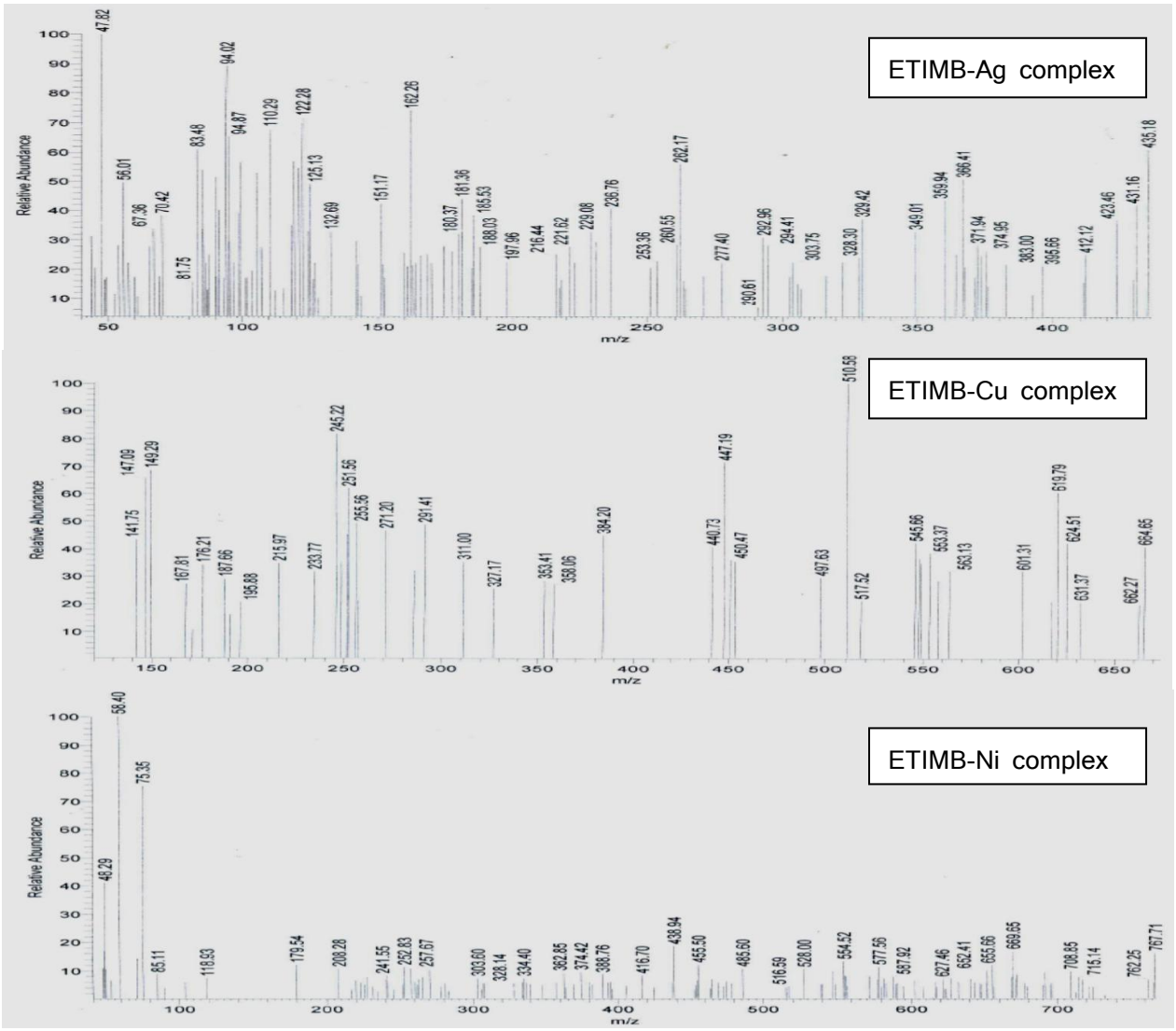


Figure 1: Mass spectra of the ETIMBT Metal complexes.

Table 1: Physical properties of ETIMBT and its complexes.

Compound	F. wt.	M^+	Color	Yield	M.P. °C
ETIMBT	265.05	-	yellow	90%	84-86
ETIMBT-Ag complex	435.16	435.18	Dark brown	60%	231-233
ETIMBT-Cu complex	664.55	664.65	Brown	65%	168-170
ETIMBT-Ni complex	767.69	767.71	yellow	50%	>300

Table 2: Main IR absorption bands of ETIMBT and its complexes.

Compound	C=N imine	CH aliphatic	OH	C= N $_{Ring}$
ETIMBT	1645	2924	3323	1595
ETIMBT-Ag complex	1618	2923	3338,3261	1535
ETIMBT-Cu complex	1602	2924	3368	1580
ETIMBT-Ni complex	1601	2926	3329,3316	1529

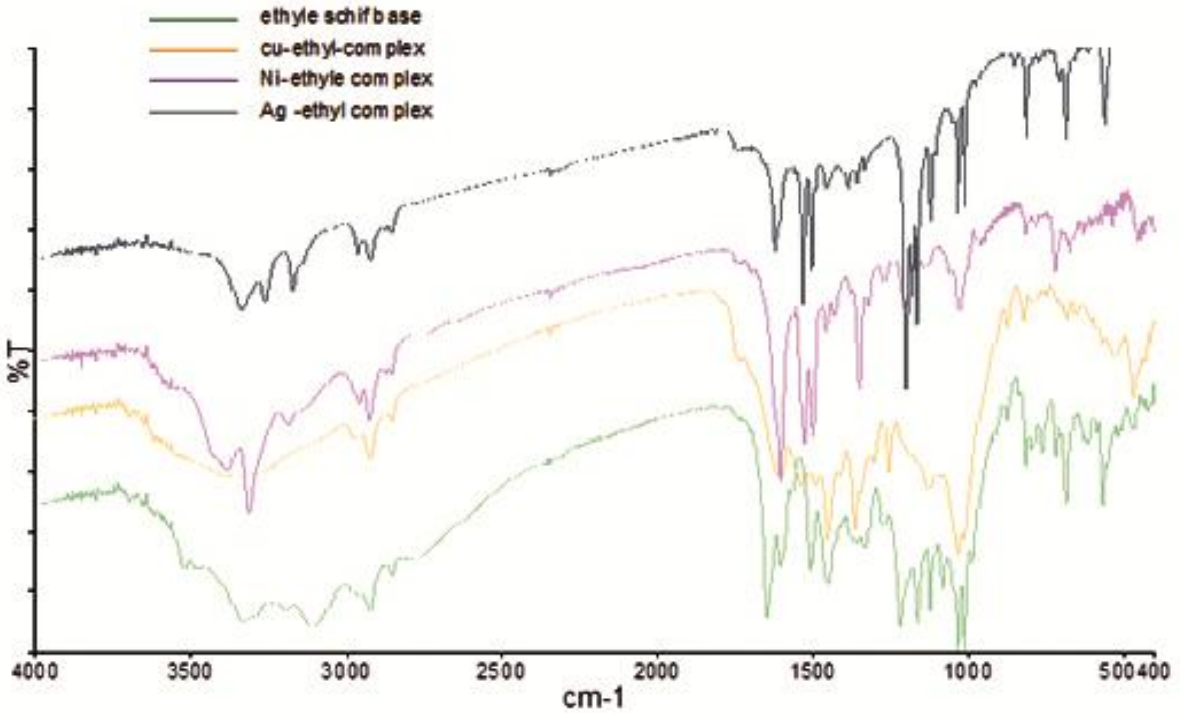


Figure 2: IR spectra for the ETIMBT and its complexes.

IR Spectra

Table 2 summarizes the main IR spectral data of the compounds, which are also presented in Figure 2. The IR spectra of the ETIMBT ligand and its complexes showed a strong absorption band in the range of approximately 3323 cm^{-1}, corresponding to the O–H stretching vibrations of the hydroxyl groups in the ligand and its complexes. The ETIMBT-Ag, ETIMBT-Cu, and ETIMBT-Ni complexes exhibited frequency shifts and intensity changes in the C=N group upon complexation, suggesting the involvement of the C=N group in coordination. The strong band at 1645 cm^{-1} assigned to the C=N group in the free ligand (ETIMBT) was shifted to lower wavenumbers in the complexes (1618, 1602, and 1601 cm^{-1}, respectively), indicating the participation of the C=N group in coordination and confirming complexation.

Electronic spectra

The UV-Vis spectra of ETIMBT and its Ag, Cu, and Ni complexes were measured in the range of 200–800 nm in DMF as the solvent. The spectra showed two π–π* (K-band) λ_{max} at 270–340 nm for the ligand and its complexes. In addition, a charge transfer transition band n–π* (R-band) at λ_{max} 360–400 nm was observed due to intra-ligand transitions of the Schiff base and its complexes. The complexes also exhibited d–d electronic transitions with λ_{max} ranging from 440 to 510 nm. The main electronic spectra of the ligand and its complexes are summarized in Table 3 and illustrated in Figure 3.

Table 3: Main Electronic absorption bands of ETIMBT and its complexes.

Compound	π-π* nm, benzene	π-π* nm, imine	n- π* nm	d-d nm
ETIMBT	270	310	370	
ETIMBT-Ag complex	270	340		510
ETIMBT-Cu complex	270	310	360	510
ETIMBT-Ni complex	270		400	440

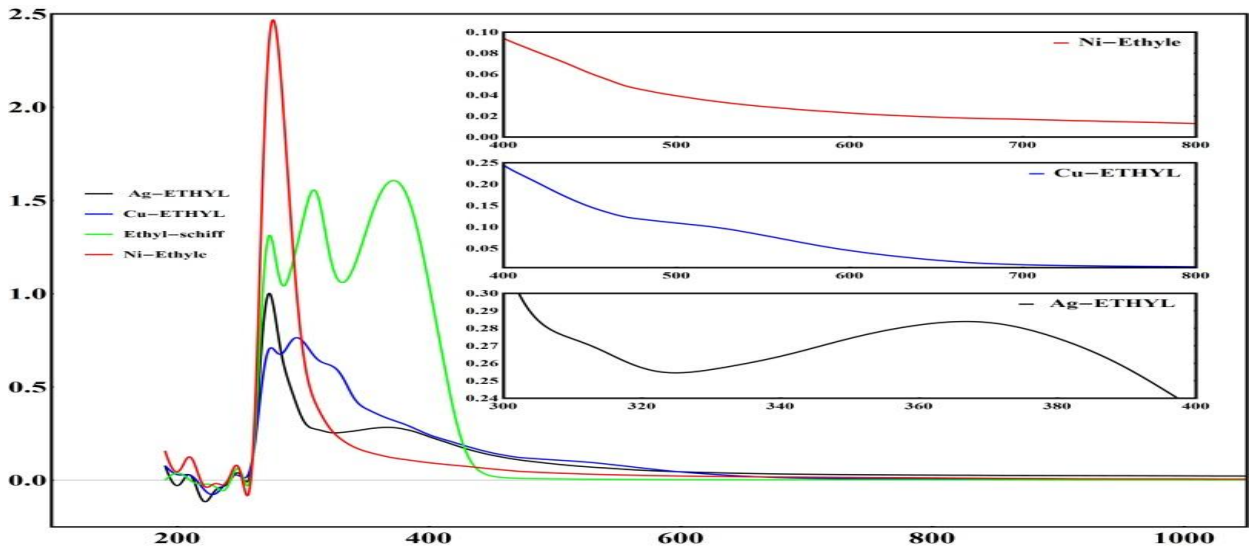


Figure 3: Electronic Spectra UV-vis spectra of ETIMBT and its metal complexes

^{1}H NMR Studies of ETIMBT

The Schiff base compound (ETIMBT) was characterized by ^{1}H NMR spectroscopy. The spectrum displayed a peak at 2.51 ppm (t, 3H) corresponding to the CH_3 group, and a peak at 2.82 ppm (q, 2H) corresponding to the CH_2 group. The azomethine group was observed at 8.95 ppm (s, 1H), while the signals at 7.50–7.52 ppm (m, 2H) were attributed to the aromatic protons. Additionally, signals were observed at 8.59, 9.80, and 8.10 ppm (s, 3H) due to the OH groups, as illustrated in Figure 4.

^{1}H NMR Studies of ETIMBT Metal Complexes

The electron density shift from the ligand to the metal in the ETIMBT complexes (Ag(I), Cu(II), and Ni(II)) was demonstrated in their ^{1}H NMR spectra. The azomethine proton peaks of the ligand appeared at 8.59 ppm (s, 1H), whereas in the ETIMBT-Ag, ETIMBT-Cu, and ETIMBT-Ni complexes, they appeared at 7.68 ppm, 8.69 ppm, and 7.56 ppm, respectively. This shift in peaks confirmed the formation of the complexes.

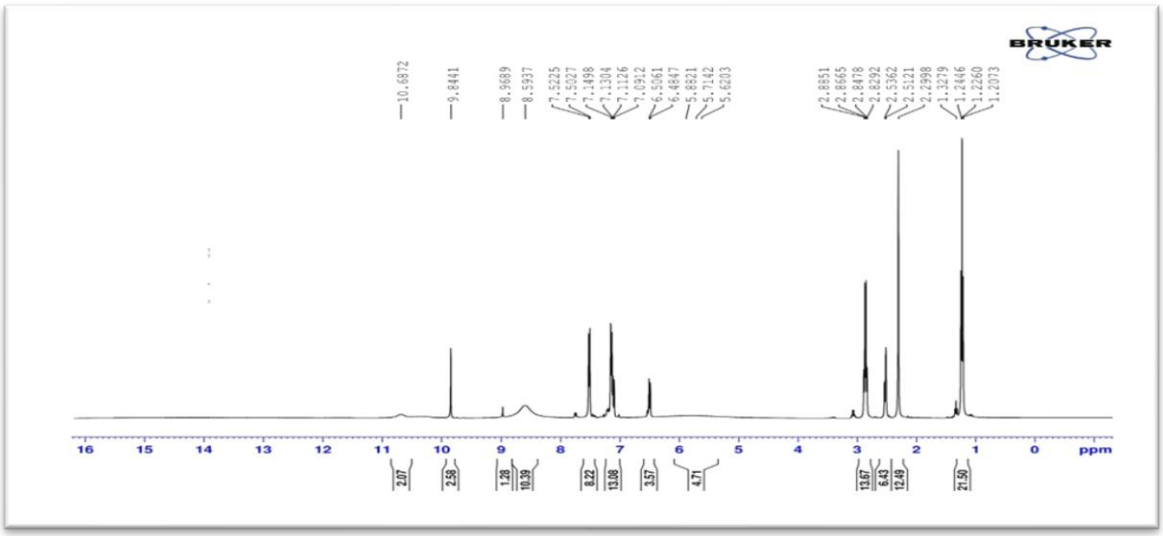


Figure 4: ^{1}H NMR spectrum of ETIMBT

Anticancer Activity

The cytotoxicity of the ligand and its complexes was evaluated in vitro against human PC3 and HeLa cells and compared to cisplatin as a positive control. Among the tested compounds, the ETIMBT-Cu complex exhibited the highest cytotoxic activity against both cell lines compared to the other complexes and the ligand. The results of the cytotoxic activity of the tested compounds are summarized in Table 4.

Table 4: IC_{50} values (µg/ml) of ETIMBT and its complexes tested against Hela cells and PC3 cells.

Compound No.	PC3	HeLa
	IC_{50} values ±SD (µg/ml)	IC_{50} values ±SD (µg/ml)
ETIMBT	160±9.1	242±11.4
ETIMBT-Ag complex	10.8±0.62	23.1±13
ETIMBT-Cu complex	5.12 ±0.39	11±0.84
ETIMBT-Ni complex	36.6±1.8	62.1±2.7
Cisplatin as control	5.09 ±0.031	7.61±0.49

CONCLUSION

In conclusion, we successfully synthesized the Schiff base and its complexes and evaluated their cytotoxicity against HeLa and PC3 cells. The complexes exhibited higher cytotoxic activity compared to the free ligand toward both cell lines. Among the tested complexes, the copper complex demonstrated the most promising anticancer activity and showed comparable efficacy to cisplatin against PC3 cells. Therefore, the Schiff base copper complex can be considered a promising candidate for further development as an anticancer drug.

DECLARATIONS

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

AUTHOR CONTRIBUTIONS

Sama A. Al-Aghbari, Abeer Obeid, Elham A. Al-Taifi, and Etify A. Bakhite conceived the original idea, supervised the project, and prepared the manuscript. Ebrahim Al-Kabodi, Zianab Ragih, and Esraa Khamies carried out experiments. Omar M. Al-Shuja'a and Omaima F. Ibrahim contributed to the interpretation of the results and writing the manuscript. All authors discussed the results and contributed to the final version of the manuscript.

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