Exploring the Anticancer Activity of Gold Complex with Newly Ligand (DDIBM): Synthesis, Spectral Identification and Magnetic Susceptibility of Its Metallic Complexes

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Received: October 19, 2023
Accepted: January 29, 2024
DOI: 10.22146/ijc.89954

Abstract: The new heterocyclic ligand, 5-(dimethylamino)-2-(((2-((E)-(4,5-diphenyl-1H-imidazol-2-yl)diazenyl)benzyl)imino)methyl)phenol (DDIBM), was synthesized via the condensation of p-aminobenzylamine with 4,5-diphenyl imidazole, and the resultant compound was condensed with 4-(dimethylamino)-2-hydroxybenzaldehyde. Various instrumental techniques such as mass, 1H-NMR, IR, C.H.N elemental analysis, and UV-vis spectroscopy were used to analyze a newly synthesized ligand. A novel series of complexes was prepared by complexing the ligand with Ni(II), Cu(II), Co(II), and Au(III) and characterized using some of the mentioned techniques. Flame atomic absorption spectroscopy was used to measure the metal ion percentages in the complexes. The magnetic susceptibility and molar conductivity were studied. The electronic spectral data and the magnetic measurement predict the octahedral structure of the complexes except Au(III) complex which has square planer geometry. All complexes showed electrolyte properties. This study aimed to conduct an in vitro cytotoxicity comparative study of DDIBM and its Au(III) complex on human breast cancer cells (MCF-7) and other normal cells. The Au(III) complex was found to be highly selective in targeting cancer cells without affecting normal healthy cells, compared to the ligand. Thus, this complex can be considered as a new drug for treating breast cancer cells (MCF-7), and an attempt in the future to study its effect on other types of cancer.

Keywords: azo-imine ligand; breast cancer; metallic complexes; ortho-amino benzylamine derivatives

INTRODUCTION

Azo-imine ligands containing imidazole groups and their metal complexes have established remarkable attention in the past few years, not only because of their spectroscopic characters and applications but also their pharmacological as well as the electrochemical, stereochemical [1-2], and biological [3] effects. The properties of these type of heterogeneous compounds are due to their containing of imine (C=N) group that resulted from the reaction between primary amines with carbonyl compounds by releasing H₂O [4-5]. As far as the azo compounds containing ¬N=N¬ link in their composition, these compounds are used as bright synthetic colorants and applied in diverse fields such as drug and rust inhibitors and anti-corrosion, such as coating metal surfaces and spoons [6-7].

Azo-imine derivatives are chemical combinations that have received great attention in scientific research compared with imine compounds and azo dyes [8]. Because of the existence of two reactive ¬N=CH¬ and ¬N=N¬ groups [9], azo-imines are important due to their structural, electronic, flexibility and selectivity properties to metal ions [10-11]. These compounds are types of ligands that could be coordinated by various means. They could be coordinated via nitrogen atoms of azo and imine groups [12], or by nitrogen of azo group alone, and finally can coordinate via nitrogen atoms belonging to azo imine [13].
Currently, azo-Schiff compounds find special applications in all stages of life [14] as well as in industrial, analytical, and biological fields [15] as they are used for corrosion inhibition in manufacturing, antioxidants [16-17], nuclear waste disposal [18], and the manufacture of plastics, leather, and textiles [19]. As cancer tumors arise that are incredibly resistant to the effects of conventional chemotherapy, it becomes interesting to explore various treatment approaches, including the development of new energy drugs for drug-resistant cancers [20-22]. Azo-Schiff bases complexes have proven their worth as antifungals, anticancer [23], antibacterial [24], and herbicides [25].

Cancer is a complex, difficult-to-treat disease that begins when the growth of cells gets out of control. Cancer has different types, depending on the type of organ it affects, as it has the ability to grow anywhere in the body, therefore, it is called cancer depending on the affected part. MCF-7 is a breast cancer cell line that was isolated from Caucasian woman at the age of 69 in 1970 [26-27]. MCF-7 referred to the institute in Detroit where Herbert Soule and coworkers [28] in 1973 established the cell line and this is also the acronym of the Michigan Cancer Foundation-7. The MCF-7 contains estrogen receptors, providing an alternative experimental system for studying hormone-regulated genes. In response to estrogen, certain proteins are induced [29].

In this paper, Co(II), Ni(II), Cu(II), and Au(III) complexes with new azo-imine containing imidazole group ligand were synthesized and characterized. The activity of the ligand and its Au(III) complex against MCF-7 and normal cells was investigated.

**EXPERIMENTAL SECTION**

**Materials**

The materials, 2-amino benzylamine, hydrochloric acid, and silver nitrate, were purchased from Sigma Aldrich Chemical Company in England. Benzil and 4-(dimethylamino)-2-hydroxybenzaldehyde were from Fluka Company. Cobalt(II) chloride hexahydrate, nickel(II) chloride hexahydrate, copper(II) chloride dihydrate, ethanol, methanol, and sodium carbonate were from B.D.H Company. Benzene was from G.C.C. Company while hydrogen tetrachloroaurate(III) trihydrate was from Glentham Life Sciences. Absolute ethanol was from Sharlut Company. Dimethyl sulfoxide (DMSO) was from A.C.S. Company. Glacial acetic acid was from Merck Chemical Company. All these chemicals used were utilized as received without any further purification.

**Instrumentation**

Melting points were measured using Type 9300 for ligands and their complexes. 1H-NMR spectra were acquired as solutions in DMSO-<i>d</i><sub>6</sub> solvent applying a Varian 500 MHz spectrophotometer while the mass spectra were recorded on a Shimadzu Agilent Technologies 5975C. The Shimadzu dual-band model 1700 spectrophotometer was used for recording UV-vis spectra. Magnetic susceptibility measurements were performed using the Faraday method with a balanced magnet MSB-MKI. Diamagnetic correction using Pascal’s constant. Infrared (IR) spectra were measured using a Shimadzu FTIR 8400 spectrometer utilizing KBr in the 4000–400 cm<sup>−1</sup> wavelength range. C.H.N elemental analysis was studied using C.H.N elemental analyzer (EURO 2012EA 300). The devices manufacture’s name/state and type of equipment used for toxicological studies are biohazard safety cabinet class II BGenex, USA; autoclave Arnold Sons, USA; centrifuge Hermle, Germany; cell culture incubator, Memmert, Germany; deep freezer (−80 °C) Marubeni, Japan; cooling centrifuge, Beckman model J2-21, USA; distillatory Ogawa Seiki, Japan; drying and sterilizing oven, Hermle, Germany; incubator, Memmert, Germany; ELISA reader by Organon Teknika; inverted microscope, Leica, Germany; multiwall plate; microtiter plate; 96-well plate, USA; pH-meter; LKB, Sweden; millipore filter (0.22 μm), Sartorius, Germany; sterile tissue culture flasks (25 and 75 cm<sup>2</sup>), Nunc, Denmark; water bath, Memmert, Germany; and vacuum pump, Leitz, Germany.

**Procedure**

**Synthesis of new azo-imine ligand (DDIBM)**

A new Schiff base ligand (DDIBM) was synthesized by reacting diazonium salt and a suitable amount of imidazole derivatives in an alkaline medium. The diazo
solution was prepared by dissolving 1.222 g of o-amino benzylamine (0.01 mol) in 30 mL deionized water and 8 mL conc. HCl with shaking continuously. The solution of NaNO₂ (0.01 mol, 0.7 g) was prepared in 5 mL of deionized water. It was added to the diazo solution dropwise with stirring and shaking to complete the nitridation process at a temperature range of 0–5 °C, and then it was left to stand for 30 min and let sit longer. This diazo solution was dropwise added to 2.202 g of 4,5-diphenyl imidazole (0.01 mol) dissolved in 50 mL absolute ethanol and 50 mL of 40% Na₂CO₃ solution at 0–5 °C. The color slowly changes to orange-red which indicates the coupling process occurs between the two solutions. The synthesis of azo compounds that were neutralized subsequently by adding dilute HCl until pH reached ~7.5. The mixture formed was left overnight, filtered, and washed carefully with deionized water. Then crystallized twice in hot ethanol and oven-dried at 40 °C for 1 h [30].

In the second step, new Schiff base ligand (5-(dimethylamino)-2-(((2-(((E)-(4,5-diphenyl-1H-imidazole-2-yl)(diazeylnylbenzyl))imino)methyl)phenol (DDIBM)) was prepared by dissolving 1.651 g of 4-(dimethylamino)-2-hydroxybenzaldehyde (0.01 mol) in 10 mL absolute ethanol, stirring for 2 min, then adding 3 drops of glacial acetic acid, and then let stand at laboratory temperature for 5 min. Then, 3.534 g of azo dye (0.01 mol) was dissolved in 10 mL anhydrous solution, ethanol was slowly added, and the solution was heated to the temperature of azo dye Schiff (78 °C) for 12 h to obtain basic ligands. The reaction was carefully monitored by applying TLC technique using 1 mL benzene and 4 mL ethanol. The product obtained was cooled, dried, and recrystallized through hot absolute ethanol [31]. The physical properties are listed in Table 1. Scheme 1 shows the steps for preparing the DDIBM ligand.

Scheme 1. Synthesis of new azo-Schiff base ligand (DDIBM)
Table 1. Physical properties of DDIBM ligand and its metal complexes

<table>
<thead>
<tr>
<th>Chemical formula</th>
<th>Color</th>
<th>M.wt (g/mol)</th>
<th>m.p. (°C)</th>
<th>Yield (%)</th>
<th>R f</th>
</tr>
</thead>
<tbody>
<tr>
<td>L = C₃₁H₂₈N₆O</td>
<td>Reddish Orange</td>
<td>500.61</td>
<td>115–117</td>
<td>81</td>
<td>0.68</td>
</tr>
<tr>
<td>[CoLCl(H₂O)]Cl</td>
<td>Olive</td>
<td>648.45</td>
<td>282–285, Decompose</td>
<td>85</td>
<td>0.58</td>
</tr>
<tr>
<td>[NiLCl(H₂O)]Cl</td>
<td>Reddish Brown</td>
<td>648.21</td>
<td>262–265</td>
<td>88</td>
<td>0.67</td>
</tr>
<tr>
<td>[CuLCl(H₂O)]Cl</td>
<td>Brown</td>
<td>653.07</td>
<td>218–220</td>
<td>84</td>
<td>0.57</td>
</tr>
<tr>
<td>[AuLCl]Cl₂·2H₂O</td>
<td>Reddish Brown</td>
<td>839.95</td>
<td>242–246</td>
<td>89</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Synthesis of metal complexes

Various metal complexes were synthesized by mixing 0.0002 mol of each of NiCl₂·6H₂O, CoCl₂·6H₂O, CuCl₂·2H₂O, and AuCl₃·3H₂O in 10 mL ethanol solution with 10 mL absolute ethanol solution of 1.167 g of ligand (0.0002 mol) for new DDIBM ligand in 1:1 ratio (metal:ligand). The resulted mixture was then refluxed for 1 h. After evaporation, the complexes were filtered off and vacuum dried. The physical properties of synthesized complexes are summarized in Table 1. Scheme 2 illustrates the steps of preparing the metal complexes with the DDIBM ligand.

Cell cytotoxicity (in vitro cytotoxicity) and viability assays

Cell line. The MCF-7, which was obtained from Pastor Institute - Iran, was used in this study, and cancer cells were sustained and developed. The tests were performed on them at the University of Tehran.

Development of breast cancer cell line. The method of Freshney was followed to grow the cells MCF-7. The MCF-7 cells were carefully thawed in a water bath at 37 °C and then transferred to a container. A culture of cells with 25 cm diameter containing the culture medium (RBMI-1640) and 10% cow calf blood serum was incubated at 37 °C for 24 h with 5% CO₂. After 24 h, when the growth in the cell culture and decontamination was confirmed, these cells were studied through an inverted microscope in order to ensure their viability, free from contaminants, and the cells growth to the required number (about 500–800 thousand cell/mL). These cells were then shifted to the growth cabin and the utilized culture medium was wasted by washing the cells with PBS. Then trypsin was added in sufficient amount to the cells and incubated at 37 °C for 30–60 s. It was regularly monitored until a mono-cell layer transformed into a single cell, and then
the enzyme was stopped by adding a new growth medium containing bovine calf serum. The cells were carefully separated via centrifugation for 10 min at 2,000 rpm at room temperature to precipitate the cells and discard the utilized culture medium and trypsin. The separated cells were carefully suspended in a fresh culture medium, which contained 10% bovine calf serum. The number of cells was studied by adding a certain cell suspension volume to the Trepan Blue dye having the same amount for determining the cells number and their vitality using a Hem cytometer slide and applying Eq. (1) [32];

\[
C = N \times 10^4 \times F/\text{mL}
\]

whereas \( C \) represents the number of cells in 1 mL of solution, \( N \) shows the cells number in the slide, \( F \) is the dilution factor, and \( 10^4 \) is for slice dimensions.

The cell viability in the sample was measured using a Hemacytometer chip. Live cell viability ratio is calculated using Eq. (2). The suspension of cells was then distributed into new containers and incubated at 37 °C for 24 h in 5% \( \text{CO}_2 \).

\[
\text{Live cell viability ratio} = \frac{\text{number of living cells}}{\text{number of dead cells}} \times 100\%
\]

**MTT staining test for breast cancer cell viability.**

The cytotoxic effect of the complex of Au(III) with DDIBM ligand on MCF-7 cells was determined in this test, for the purpose of demonstrating its efficacy as a cancer drug [3]. The cancer cells were prepared using the steps before [33], and the cell suspension formed was then placed in a plate having flat-bottom holes and then incubated at 37 °C for 24 h in 5% \( \text{CO}_2 \), then with a volume of 200 \( \mu \text{L} \) per hole was added. Gold complex at 6.25, 12.5, 25, 50, and 100 \( \mu \text{g/mL} \) concentration was added to the cell suspension from each hole for each concentration. The plate was then incubated at a temperature of 37 °C for 24 h. Then to each hole, 10 mL of MTT solution was carefully added at 0.5 mg/mL concentration. The plate was again incubated for an additional 4 h at 37 °C then 100 \( \mu \text{L} \) of DMSO solution was added via each hole for dissolving the formazan crystals. The sample absorbance was monitored at a 570 nm wavelength using an ELASIS device.

**RESULTS AND DISCUSSION**

The effect of dissolution on both the DDIBM as well as its solid complexes was evaluated in DMSO and DMF. It was used to determine the ability to dissolve in different solvents and must be added to the chemicals used methanol and ethanol. Also, all compounds were stable against humidity and temperature factors. Elemental analysis was studied for all compounds. The complexes analytical data are well matched with the obtained experimental results data. The value shows a 1:1 ratio of the metal to ligand, as presented in Table 2. The magnetic susceptibility of the chelate complexes of Ni(II), Co(II), and Cu(II) were consistent at normal temperature with octahedral geometry, while Au(III) complex was square planer. All the synthesized chelate complexes displayed higher conductivity values, proving the electronic nature of complexes.

**Mass Spectrum**

At room temperature, the mass spectrum of DDIBM ligand was recorded. Fig. 1 and Scheme 3 represent the mass spectrum of ligand and the proposed mass fractionation pathway. The peaks obtained confirm the formula which were proposed for the prepared compound. The mass spectrum displayed the molecular ion peak at \( m/z \) 500.23 (\( C_{31}H_{28}N_6O \)) which confirmed the formula proposed for the compound. This small abundance (3%) was due to the large molecular weight.

<table>
<thead>
<tr>
<th>Table 2. The DDIBM ligand and its complexes element analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formula</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>L</td>
</tr>
<tr>
<td>[CoLCl(H2O)Cl]</td>
</tr>
<tr>
<td>[NiLCl(H2O)Cl]</td>
</tr>
<tr>
<td>[CuLCl(H2O)Cl]</td>
</tr>
<tr>
<td>[AuLCl]Cl2·2H2O</td>
</tr>
</tbody>
</table>
Scheme 3. Mass fractionation pathways of DDIBM
high bombardment energy, and the large number of heterogeneous atoms in its chemical structure [34-35].

**1H-NMR Spectra**

The spectrum of DDIBM ligand provided acceptable data and their molecular structure was obtained on the basis of 1H-NMR chemical shift using DMSO-d$_6$ as a solvent with internal reference (TMS). The 1H-NMR spectrum of the DDIBM was presented in Fig. 2. The ligand 1H-NMR spectrum displayed clear signals of singlet at 2.5 ppm which belong to the protons of DMSO, while multiplet signals at 6.34–9.07 ppm were allocated to the aromatic protons of the phenyl ring of benzilidenimin and imidazole. Singlet at 1.88 ppm represents the protons of methyl (–N(CH$_3$)$_2$). Singlet at 6.05 ppm shows the proton of methylene (CH$_2$). Singlet
at 9.62 ppm shows the proton of azomethine (–CH=N). The singlet at 11.27 ppm represents the proton of OH group, and the singlet at 13.66 ppm shows the proton of the imidazole ring (–C–NH) [30].

**IR Spectra Studies of the DDIBM Ligand and Its Complexes**

The IR spectra of the metal complexes and free ligand are compared to determine the changes created during the complexation process [36-37]. The IR spectra of the metal complexes and free ligand were presented in Fig. S1. Table 3 gives a summary of IR data of the DDIBM ligand and its metal complexes in cm\(^{-1}\). The IR spectra give the important and the characteristic bands in the complexes chemical structure which confirm the complexes formation. The bands in the ligand spectrum such as the –OH, –NH–, and C=N in the imidazole ring, C=N of azo-imine group, and the azo group appear at 3400, 3300, 1517, 1631 and 1425 cm\(^{-1}\) respectively. In all complexes, these bands were shifted or changed in their intensities or positions which suggested the complexes formation. For example, the O–H group is shifted to 3410–3377 cm\(^{-1}\) in complexes which suggested the coordination between the metal and the hydroxyl group. The –NH– group in the imidazole ring is slightly shifted to 3377–3132 cm\(^{-1}\) in complexes. While the C=N of the imidazole is changed in its intensity and that suggests the coordination with the metals by this group as this stimulated from the previously published articles [17,25,31]. The azo-imine group which appears at 1631 cm\(^{-1}\) in the free ligand was shifted to 1629, 1624, 1627, and 1595 cm\(^{-1}\) in Co(II), Ni(II), Cu(II), and Au(II) complexes, respectively, which suggests the coordination between the metal and the ligand azo-imine group. Finally, the azo group which appears at 1425 cm\(^{-1}\) in the free ligand was shifted to 1444, 1436, 1442, and 1361 cm\(^{-1}\) in Co(II), Ni(II), Cu(II), and Au(II) complexes, respectively, which suggests the coordination between the metal and the ligand azo group. Other new bands in the range 400–700 cm\(^{-1}\) refer to the formation of M–N and M–O bond types.

**Magnetic Susceptibility**

The results of the magnetic susceptibility measurements are consolidated in Table 4. The magnetic moment values of Ni(II), Cu(II), and Co(II) complexes reach 4.79, 2.95, and 1.76 B.M respectively, indicating paramagnetic characteristic [38]. As for the complex of Au(III) it has shown diamagnetic properties due to electron cover saturation (nd) in the electrons [39].

**Measurement of Molar Conductivity**

It is clear from the results of molar electrical conductivity measurements for solutions of chelate complexes at a concentration of 0.001 M in DMSO solvent at room temperature, these compounds displayed ionic properties as listed in Table 4. These results are well-matched with the reported literature [40]. The metal complexes derived from DDIBM gave a molar electrical conductivity close to the conductivity values of the metal complexes that have ionic properties (1:1), which confirms the validity of the proposed structures.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>(\mu_{\text{eff}}) (B.M)</th>
<th>(\Lambda_{M}) (S cm(^2) mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>[CoLCl(H(_2)O)]Cl</td>
<td>34.30</td>
<td>4.79</td>
</tr>
<tr>
<td>[NiLCl(H(_2)O)]Cl</td>
<td>49.00</td>
<td>2.95</td>
</tr>
<tr>
<td>[CuLCl(H(_2)O)]Cl</td>
<td>32.30</td>
<td>1.76</td>
</tr>
<tr>
<td>[AuLCl]Cl(_2)-2H(_2)O</td>
<td>79.00</td>
<td>Dia*</td>
</tr>
</tbody>
</table>

*Diamagnetic (magnetic value is 0)

**Table 3.** IR spectra frequencies for DDIBM ligand and its metal complexes in cm\(^{-1}\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>(v(\text{OH}))</th>
<th>(v(\text{NH}))</th>
<th>(v(\text{C=N}))</th>
<th>(v(\text{C=N}))</th>
<th>(v(\text{N=N}))</th>
<th>(v(\text{M–N}))</th>
<th>(v(\text{M–O}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>3400</td>
<td>3300</td>
<td>1517</td>
<td>1631</td>
<td>1425</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[CoLCl(H(_2)O)]Cl</td>
<td>3410</td>
<td>3178</td>
<td>1606</td>
<td>1629</td>
<td>1444</td>
<td>584</td>
<td>567</td>
</tr>
<tr>
<td>[NiLCl(H(_2)O)]Cl</td>
<td>3377</td>
<td>3377</td>
<td>1595</td>
<td>1624</td>
<td>1436</td>
<td>561</td>
<td>553</td>
</tr>
<tr>
<td>[CuLCl(H(_2)O)]Cl</td>
<td>3398</td>
<td>3392</td>
<td>1585</td>
<td>1627</td>
<td>1442</td>
<td>642</td>
<td>551</td>
</tr>
<tr>
<td>[AuLCl]Cl(_2)-2H(_2)O</td>
<td>3408</td>
<td>3132</td>
<td>1502</td>
<td>1595</td>
<td>1361</td>
<td>700</td>
<td>636</td>
</tr>
</tbody>
</table>
**Electronic Spectra**

The UV-vis spectra of DDIBM and its complexes are represented in Table 5 and Fig. S2. The spectrum of DDIBM in ethanol showed three absorption peaks, two peaks at 204 (49019.607 cm⁻¹) and 291 nm (34364.261 cm⁻¹) due to π→π* electron transition. While the third peak was credited at 344 nm (29069.767 cm⁻¹) due to n→π* electron transition, due to the ligand having double bonds with atoms having unshared electron pairs.

The ligand spectrum of the ligand was compared with the Co(II) complex spectrum, which showed two absorption peaks, the first at 502 nm (19920.318 cm⁻¹) due to the electron transition 4T₁g(F)→4T₁g(P)=(ν₃) and an absorption peak at 688 nm (14534.883 cm⁻¹) has been attributed to the electron transition 4T₁g(F)→1A₂g(F)=(ν²). This fact is consistent with the literature on the appearance of this band in octahedral Co(II) complexes [41]. The UV-vis spectrum of Ni(II) complex solution recorded an absorption peak at 496 nm (20161.290 cm⁻¹) due to the electron transition 3A₂g→3T₁g(F)=(ν²) and this is consistent with what was mentioned in the literature regarding octahedral Ni(II) complexes [41]. The UV-vis spectrum of Cu(II) complex solution showed a broad absorption peak at 471 nm (21231.422 cm⁻¹) due to the electron transition (2E₉g→2T₂g), and this is consistent with what was mentioned in the literature [42].

As for the electronic spectrum, the Au(III) complex with DDIBM ligand. This complex exhibited one band at 459 nm (21786.492 cm⁻¹) which was assigned to 1A₁g→1B₁g transition [43]. The Au(III) complex has diamagnetic moment and has square planar geometry [44]. The proposed compound structures of the metallic complexes are represented in Fig. 3.

Recently, complexes of Au(III) ions have been used in medical fields to treat cancer. Its importance has significantly increased in this field. The reason is because Au(III) is electronically identical to Pt(II), and the Au(III) complexes, which are tetra-symmetric and have a square planar shape, are similar to cisplatin in electronic arrangement and geometric shape, or both.

### Table 5. The electronic spectra of the DDIBM ligand and its complexes with metals in ethanol solvent

<table>
<thead>
<tr>
<th>Compounds</th>
<th>λ_max (nm)</th>
<th>Absorption bands (cm⁻¹)</th>
<th>Transitions</th>
<th>Geometry</th>
<th>Hybridization</th>
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<tbody>
<tr>
<td>L</td>
<td>204</td>
<td>49019.607</td>
<td>π→π*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>291</td>
<td>34364.261</td>
<td>π→π*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>344</td>
<td>29069.767</td>
<td>n→π*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>[CoLCl(H₂O)]Cl</td>
<td>502</td>
<td>19920.318</td>
<td>⁴T₁g(F)→⁴T₁g(P)=(ν³)</td>
<td>Octahedral</td>
<td>sp¹d²</td>
</tr>
<tr>
<td></td>
<td>688</td>
<td>14534.883</td>
<td>⁴T₁g(F)→¹A₂g(F)=(ν²)</td>
<td>Octahedral</td>
<td>sp¹d²</td>
</tr>
<tr>
<td>[NiLCl(H₂O)]Cl</td>
<td>496</td>
<td>20161.290</td>
<td>³A₂g→⁴T₁g(F)=(ν²)</td>
<td>Octahedral</td>
<td>sp¹d²</td>
</tr>
<tr>
<td>[CuLCl(H₂O)]Cl</td>
<td>471</td>
<td>21231.422</td>
<td>³E₉g→⁷T₃g</td>
<td>Octahedral</td>
<td>sp¹d²</td>
</tr>
<tr>
<td>[AuLCl₂·2H₂O]</td>
<td>459</td>
<td>21786.492</td>
<td>¹A₁g→¹B₁g</td>
<td>Square planar</td>
<td>dsp²</td>
</tr>
</tbody>
</table>

**Fig 3.** Proposed structure of the metallic complexes
These complexes possess possible anti-cancer properties for three decades. Au(III) decomposes quickly and is reduced to Au(I), and this property is necessary to provide biological properties. Therefore, it was chosen to study its effectiveness against MCF-7 cancer cells.

**Anticancer Screening (In Vitro Cytotoxicity)**

*Effect of [AuLCl]Cl₂·2H₂O on growth of MCF-7 and healthy cells (MCF-10A)*

The effect of the complex [AuLCl]Cl₂·2H₂O on the growth of MCF-7 cells and on the growth of healthy cells (MCF-10A) was studied. Fig. 4 shows the half inhibitory concentration of MCF-7 and MCF-10A cells [3,45]. Fig. S3 and S4 show MCF-7 and MCF-10A cells treated with [AuLCl]Cl₂·2H₂O at different concentrations after adding MTT. The highest percentage of inhibition of the complex was 80.79% at 100 μg/mL concentration, while the lowest percentage of inhibition of the complex of healthy cell line was 8.39% at concentration of 6.25 μg/mL. It was observed that the half-inhibitory concentration of the complex [AuLCl]Cl₂·2H₂O in the cells of the MCF-7 equals 33.92 μg/mL, which is very low compared to the half-inhibitory concentration of the cells of the healthy line, which is equal to 133.04 μg/mL. It is an excellent result because we need a very high concentration to kill half of the healthy cells, and this result shows the possibility of utilizing the complex [AuLCl]Cl₂·2H₂O as a novel treatment for such type of cancer.

**CONCLUSION**

The spectroscopic studies and analytical data of the metal complexes demonstrated a tetradentate chelating agent coordination via the nitrogen atoms of the imidazole ring, azo group, azomethine group, and the oxygen atom of the hydroxyl group. Except for the Au(III) complex, the ligand acts as a tridentate chelating agent. This complex is coordinated through the nitrogen atoms of the imidazole ring, azo, and azomethine groups. The results show the highest anti-cancer activity of Au(III) complex compared to the effectiveness of the ligand Au(III) complex was found highly selective in targeting cancer cells without affecting normal healthy cells, as compared with the ligand. In conclusion, this complex can be considered as a new drug for the treatment of breast cancer cells (MCF-7) and an attempt in the future to study its effect as an antidote to other types of cancer, such as colon and lung cancers.

**ACKNOWLEDGMENTS**

The authors would like to express our deep gratitude to the technical staff at Central Laboratory of the College of Pharmacy, Kufa University for providing the necessary technical assistance and support in the experiment. We would like to thank the Department of Chemistry at the Faculty of Education for Girls, Kufa University for the help to finish this work.

**CONFLICT OF INTEREST**

The authors do not have conflict of interest.

**AUTHOR CONTRIBUTIONS**

Siham Sami Noor conducted the experiment, and Ibtihal Kadhim Kareem wrote and revised the
manuscript. All authors agreed to the final version of this manuscript.

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