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Copper(II) Complexes of a New Hydrazones with Aliphatic Groups: Synthesis, Characterisation and Nuclease Activity

Cansu TOPKAYA*[1]

Abstract

Two new copper(II) complexes of bidentate arylhydrazone ligands have prepared and studied. Elemental analysis, SEM-EDS, thermal gravimetric analysis (TGA), powder X-ray diffraction (XRD), and a number of spectroscopic techniques were used to determine the structures of the compounds (FTIR, UV-Vis, ^{1}H and ^{13}C-NMR). Using agarose gel electrophoresis, the DNA cleavage activities of the produced copper complexes were investigated in the absence and presence of hydrogen peroxide. It was also investigated the influence of compound dose on the DNA cleavage process. The results show that in the presence of an oxidant agent, all of the complexes break pBR322 DNA. At the concentration of 10 µM, $[Cu(L^1)_2]$ converted the supercoil DNA into two forms, while $[Cu(L^1)_2]$ formed only the nicked form. The mechanistic analyses show that the active oxidative species for DNA breakage is a species generated by hydrogen peroxide and the copper(II) metal. The compounds also cleaveged pBR322 DNA in the absence of H_2O_2, marginally.

Keywords: Arylhydrazone, copper(II) complex, DNA-cleavage, ROS

1. INTRODUCTION

Hydrazone derivatives' bioinorganic chemistry is fascinating and vital since they have exceptional biological activity. As a result, arylhydrazones and their transition metal complexes have been investigated for their biological features such as antibacterial, antifungal, antioxidant, and anticancer activities [1-9]. Furthermore, hydrazone derivatives are flexible ligands that may be used in a variety of ways to coordinate with transition metal ions found in biological systems. Arylhydrazone complexes have been discovered to serve as enzyme inhibitors, and their pharmacological uses make them valuable [10-13].

Since transition metal complexes play a critical role in anticancer treatment, the interaction between transition metal complexs and DNA has been widely explored in recent years. Cisplatin is a metal-based anticancer medicine that is extensively used in cancer treatment, although it has a number of drawbacks, including high toxicity, side effects, and a low administration dose. As a result, continuing efforts throughout the world are aimed at discovering new metal-based compounds with lower toxicity and unique methods of action. Copper ions are recognized as

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pharmacological agents and play a key function in biological systems. Also, copper is one of the essential components of biologically important metalloproteins. The use of a number of metal complexes to cleave DNA via hydrolytic or oxidative processes has been investigated [3, 14-18]. In the presence of oxidizing or reducing co-reactants, Cu(II) complexes frequently demonstrate DNA cleavage activity. A variety of copper complexes have been demonstrated to be active both *in vitro* and *in vivo* [19]. Copper(II) complexes have been reported to behave as possible anticancer and cancer suppressing agents. The synthesis and characterisation of novel Cu(II) complexes of arylhydrazones with long chain groups was detailed in this work, as well as the DNA cleavage activities of these complexes.

2. METHODOLOGY

2.1. Materials and Instrumentation

Solvents and chemicals used in the laboratory were obtained commercially from Merck and Sigma. Solvents used in synthesis and measurements were freshly distilled and dried according to the appropriate procedure. pBR322 DNA was obtained from Fermentas. On an LECO 932 CHNS analyzer, analysis of elemenets (C, N, and H) was done and On a DV 2000 Perkin Elber ICP-AES, also, copper values were measured using atomic absorption spectroscopy. A Sherwood Scientific MK1 Model Gouy Magnetic Susceptibility Balance was used to assess magnetic susceptibility on powdered materials at room temperature. NMR spectra were recorded on a Bruker 400 MHz spectrometer in DMSO_d_6 with TMS as the internal standard. Thermo-Scientific Nicolet iS10-ATR IR spectra were acquired using the ATR (attenuated total reflectance) method. A PG Instruments T80+ UV/Vis Spectrophotometer was used to record the electronic spectra of the ligands and complexes. The thermogravimetric analysis was performed at the Central Laboratory at METU with a Perkin Elmer Pyris 1 TGA thermal analyzer. The XRD and SEM-EDS experiments were carried out at Mugla Sıtkı Koçman University's Research Centre Laboratory. A JEOL SEM 7700F was used for scanning electron microscopy (SEM) and Energy-dispersive X-ray spectroscopy (EDS). Powder X-ray diffraction (XRD) patterns were acquired on a Rigaku-SmartLab diffractometer.

2.2. Synthesis

4-Hydroxy-N'-[(1E)-1-(4-methylphenyl) ethylidene] benzohydrazide [III] and 4-butoxy-N'-[(1E)-1-(4-methylphenyl)ethylidene] benzohydrazide (HL^1) were synthesized as described previously [9, 20].

2.2.1. Synthesis of 4-hexyloxy-N'-[(1E)-1-(4-methylphenyl)ethylidene]benzohydrazide (HL^2)

A mixture of compound III (1 mmol), 1-bromohexane (1 mmol) and dry K_2CO_3 in 30 mL acetone (1 mmol) were stirred for 24 hours under reflux and ice water (150 mL) was added to the mixture. The white precipitate was filtered and washed with water several times and dried. The product crystallized in ethanol.

Yield 69 %; Mp 147 °C; UV (EtOH, nm) 297; IR (KBr) (ν, cm^{-1}) 3283 (NH), 2871-2956 (CH), 1650 (C=O), 1610 (C=N), 1397 (C-N), 1263 (C-O); ^{1}H NMR ($CDCl_3$, ppm) δ 0.92 (t, 3H J=7.03, ^{f}*CH3*), 1.33-153 (sex, 4H, ^{e}*CH*$_2$^{d}*CH*$_2$), 1.68 (p, 2H, J= 7, ^{c}*CH*$_2$), 1.84 (p, 2H, ^{b}*CH*$_2$), 2.30 (s, 3H, H_3C^h-Ar), 2.43 (s, 3H, N=C-^{g}*CH*$_3$), 3.02 (t, 2H, J= 6,6, ^{a}CH_2), 6.98 and 7.52 (dd, 4H, *J*=9.0, Ar-H), 7.81-8.13 (m, 4H, Ar-H),8.94 (s, 1H, NH); ^{13}C NMR (DMSO_d_6, ppm) 162.0 (C8), 154.8 (C6), 140.1 (C12), 133.8 (C5), 131.1 (C2), 129.3 (C9), 129.1 (C4), 128.7 (C10), 125.2 (C3), 114.0, (C11), 66.3 (C13), 32.4 (C14), 21.2 (C7), 20.3 (C1), 19.8 (C15), 18.2 (17), 19.4 (C18) and 14.1 (C16). Analysis (%Calculated/found) for $C_{22}H_{28}N_2O_2$; C: 74.89/74.56, H: 7.94/8.10, N: 7.94/8.30.

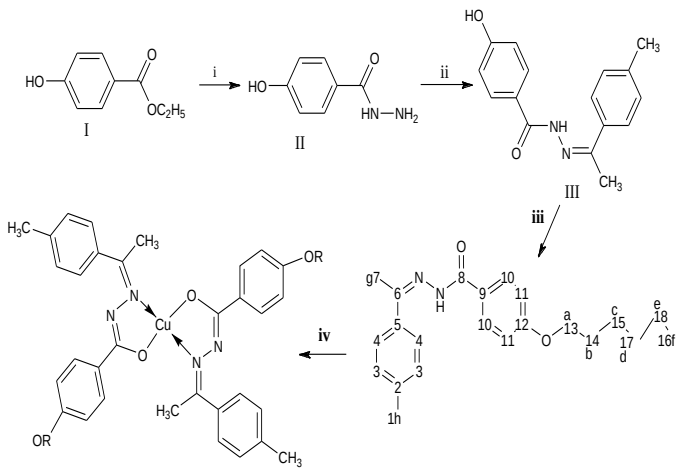
2.2.2. Preparation of copper(II) hydrazone complexes

After adding equivalent amounts of triethylamine to the solutions of HL^1 and HL^2 ligands (2 mmol) in ethanol (20 mL) separately, $(CH_3COO)_2Cu.H_2O$ (1 mmol) dissolved in

ethanol (10 mL) was added dropwise. two separate mixtures. The reaction was run under reflux for 3 hours. After standing overnight, the copper(II) complexes separated and were collected by filtering. Water was used to wash the complexes.

For $[Cu(L^1)_2]$: Dark brown complex; yield: 78%; m.p.: 160 ºC. μ_{eff} = 1.86 B.M.; UV (DMF, nm) 385, 299; FT-IR (KBr, cm^{-1}): 2871-2957 (CH), 1608 s (C=N−N=C), 1372 m (C-N), 1244 s and 1169 m (C−O−C). Analysis (%Calculated/found) for $C_{40}H_{46}CuN_4O_4$; C: 67.63/67.84, H: 6.53/6.39, N: 7.89/8.05, Cu: 8.95/9.69.

For $[Cu(L^2)_2]$: Brown complex; yield: 71%; m.p.: 137 ºC. μ_{eff} = 1.65 B.M.; UV (DMF, nm) 382.5, 299.0; FT-IR (KBr, cm^{-1}): 2857-2926 (CH), 1608 s (C=N−N=C), 1373 s (C-N), 1246 s and 1169 m (C−O−C). Analysis (%Calculated/found) for $C_{44}H_{54}CuN_4O_4$; C: 68.95/69.18, H: 7.10/6.99, N: 7.31/7.58, Cu: 8.29/8.15.



Scheme 1 Synthesis of ligands and complexes

2.3. DNA Cleavage

DNA cleavage tests were monitored by agarose gel electrophoresis. DMF was used to dissolve different amounts of complexes (10-100 M) were combined with 5 µl pBR322 (50 ng/l). After adding 5 µl H_2O_2 (5 mM) to the reaction mixture, it was diluted with tris buffer (100 mM Tris, pH: 8) to a total volume of 30 µl. After incubation at 37 ºC for two hours, samples (20 µl) were loaded onto a 1% agarose gel according to the procedure described above [8- 9]. The gel was operated at 100 V for 3 hours and imaged under UV light. Reactive oxygen scavengers (ROS) (100 µM), such as DMSO, KI, NaN_3, and EDTA, were added alternatively to the reaction mixture to test for the existence of reactive oxygen species formed during strand scission. The samples were processed in the same way as stated previously.

3. RESULT AND DISCUSSION

Compound III and HL^1 were synthesized as described previously [9, 20] while the arylhydrazones derivative HL^2 bearing hexyl chain group was reported for the first time. The reactions of III with 1-bromobutane and 1-bromohexane stoichiometrically in the presence of dry K_2CO_3 in dry acetone gave HL^1 and HL^2. The reactions performed successfully, yielding excellent yields of the relevant arylhydrazones (Scheme 1). They are water insoluble but soluble in typical organic solvents. The structure of the hydrazine ligands were determined using a variety of spectroscopic approaches. The complexes were created by reacting Arylhydrazones with copper(II) acetate at a 2:1 molar ratio. It was unable to extract crystals appropriate for single X-ray diffraction. As a result, analytical and spectroscopic findings for the hydrazone and copper(II) complexes correspond well with the chemical formula provided.

In the solid state, arylhydrazones like the ones generated in this work can be found in keto or enol tautomeric forms (Figure. 1-a). In the infrared spectra of the HL^2, the strong absorption band v(C = O) suggests that the ligand is in the solid state in the keto form, as in arylhydrazones [9, 21]. Furthermore, in solutions, these arylhydrazones, such as those produced in our study, may leave in an *E* or *Z* configuration (Figure. 1-b). The ^1H and ^{13}C-NMR spectra of HL^2 revealed just one signal for each hydrogen and carbon, indicating that arylhydrazones are in the *E* configuration [9, 22-23].

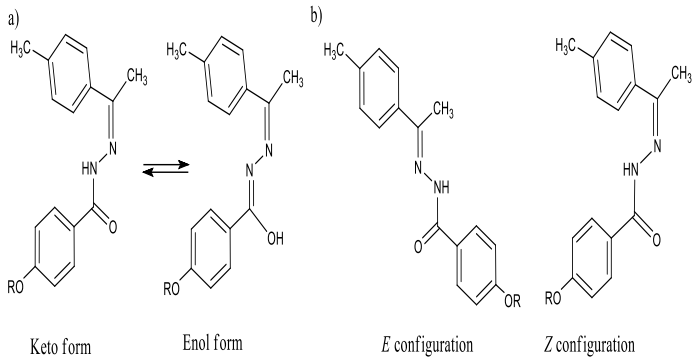


Figure 1 a) Keto-enol arylhydrazone tautomeric forms b) Structural configuration

3.1. NMR spectroscopy

The ^{1}H NMR spectra of the HL^2 was recorded at 25 ^{o}C in DMSO_d_6. The removal of the OH proton signal verified the development of the novel arylhydrazone (HL^2), present in the starting material of **III** and observed at 10.18 ppm. The NH proton is ascribed to the D_2O exchangeable singlet resonance that emerged at 8.94 ppm for the HL^2 ligand. The ^{1}H NMR spectra of the HL^2 exhibits two singlet peaks at 2.30 and 2.43 ppm due to *CH_3* protons. The first is made up of methyl protons (^{h}CH3) attached to the aromatic ring, whereas the second is made up of ^{g}CH3 protons near the imine group. On the other hands, the resonance observed as triplet at 0.92 ppm in the ^{1}H NMR spectra of HL^2 ligand is assigned to aliphatic methyl protons (^{f}*CH_3*). The spectrum also exhibits signals corresponding to protons of methylene groups in the ligand framework. These findings are consistent with earlier research on comparable substances [9, 21-27].

The structural characterisation of the hydrazone compounds is further supported by the ^{13}C-NMR spectra. The amide carbonyl (C8) and azomethine (C6) groups of the HL^2 arylhydrazones have unique chemical shifts of 162.5 ppm and 154.8 ppm, respectively. The ^{13}C NMR spectrum of the ligand exhibits signals between 140.1 and 114.0 ppm due to the carbon atoms of the aromatic rings. The signals corresponding to the carbon atoms in aliphatic rings are viewed between 14.1 and 68.1 ppm. These evaluated data show good agreement with previously published similar compounds. [9, 21-27]. The results obtained strongly indicate that the title arylhydrazone ligand is formed [28- 29].

3.2. IR spectroscopy

The free ligands' IR spectra and their copper complexes' IR spectra, which are important for identifying the ligands' mode of coordination. The starting material III has a large OH stretching vibration at 3296 cm^{-1} that is gone in the IR spectra of the HL^2, suggested that total condensation has occurred. The novel HL^2 arylhydrazones have an amide NH stretching band at 3283 cm^{-1}. The amide I band emerges at 1650 cm^{-1} for HL^2, whereas the other distinctive band owing to the azomethine group appears at 1610 cm^{-1}. The experimental section lists the additional peaks seen in the IR spectra of the arylhydrazones ligand produced in this study [21, 22, 24-27, 30-33].

C=O, C=N imine, and N-H bound bands were not detected in the IR spectra of both copper(II) complexes. Instead, around 1608 cm^{-1}, a new band arises, perhaps due to the newly created moiety of C=N-N=C. This showed that the azomethine nitrogen and enolic oxygen were coordinated with copper [21, 22, 28-30, 33]. According to the results obtained from the FTIR spectrum, the arylhydrazones, which are the subject of the study, are in coordination with the nitrogen atom of azomethine through the dehydrogenation of the tautomeric enolic form, and they are classified as mono anionic bidentate ligands.

3.3. X-ray diffraction analysis

Powder X-ray diffraction recorded at 2θ = 0–70° was used to get more information about the structure of the copper(II) complexes. The copper(II) complexes exhibit well-defined crystalline structures, as demonstrated in Figure 2's X-ray diffraction pattern. But very less number of peaks were observed in the case of $[Cu(L^1)_2]$ complexes. The sharp peaks are indicative of complex $[Cu(L^2)_2]$ having higher crystallinity than complex $[Cu(L^1)_2]$.

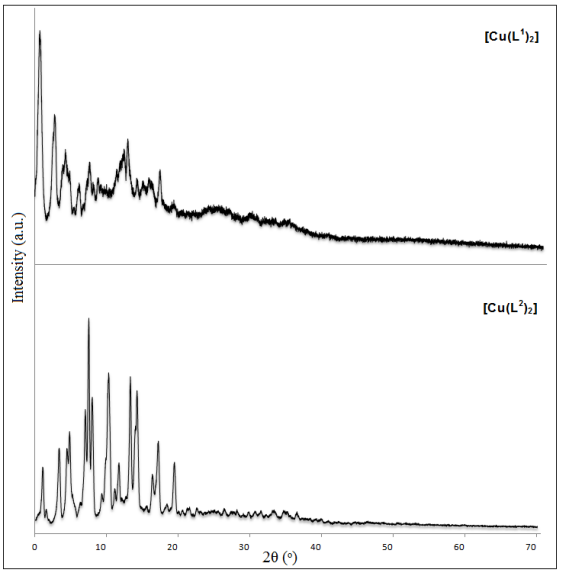


Figure 2 Copper(II) complexes X-ray diffraction patterns.

3.4. Analyses of SEM and EDS

SEM images taken to reveal the surface morphology of the ligand and its complex were obtained at 15 kV acceleration voltage and x500 magnification (Figure 3). Figure 4 shows the SEM image of the HL^2 ligand with a pyramidal structure and rod-shaped blocks vertically and horizontally, while the copper(II) complex shows non-uniformly sharp crystalline species (Figure 3). The HL^2 and its Cu(II) complex have average particle diameters of ~300 µm and 100 µm, respectively. The morphological structure of the hydrazone ligand following association with the copper ion, as observed in SEM pictures, has undergone a significant shift.

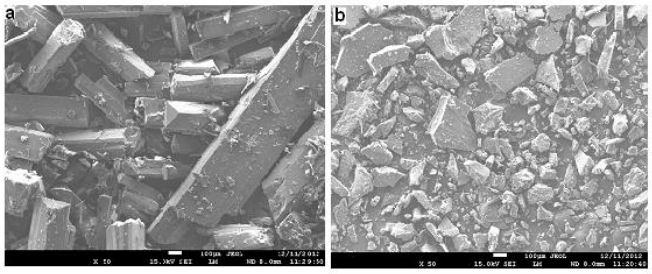


Figure 3 Comparison of SEM images of complex and ligand (a) HL^2; (b) $[Cu(L^2)_2]$

The composition of the $[Cu(L^2)_2]$ complex was defined by Energy dispersive spectroscopy (EDS) analysis (Figure 4). The EDS corroborates the presence of copper in the Arylhydrazones-copper derivative which confirms the formation of the copper complex. The C, N, O and Cu peaks were only observed in the Figure 4. indicating that the $[Cu(L^2)_2]$ complex powder has high purity.

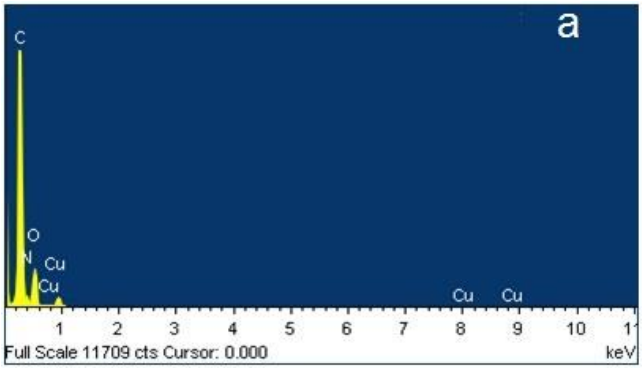


Figure 4 Energy dispersive spectrometer of the $[Cu(L^2)_2]$

3.5. Thermal analyses

Figure 5 shows the copper complexes' thermal gravimetric analysis (TGA) curves. Thermal decomposition of copper complexes happened in one step, according to TGA curves, and they are stable below 280 °C. When the temperature increases to 280 °C, both complexes decompose. The deterioration observed in the curves of the complexes as the temperature rises to the range of 280-530 °C corresponds to the detachment of arylhydrazone copper(II) complexes from the organic structure and the formation of a copper deposit as a residue.

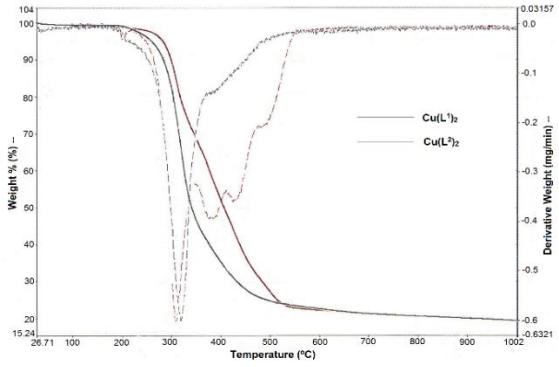


Figure 5 TGA curves of Cu(II) complexes.

3.6. Electronic absorption spectra

Two absorbance peaks corresponding to n→π* electronic transitions were observed for HL^1 and HL^2 arylhydrazones at 298 nm and 297 nm, respectively. In DMF solution, the complexes' electronic absorption spectra were obtained. When the absorption spectrums of arylhydrazone ligands and their copper(II) complexes were compared, it was discovered the metal free ligands' maximum bands had a red shift, which might be due to the arylhydrazone ligands' enol form as well as coordination of the copper(II) ion. Moreover, there is a band in the visible range of the electronic spectrums of copper complexes, 385 nm for $[Cu(L^1)_2]$ and 383 nm for $[Cu(L^2)_2]$, which can be ascribed to the LMCT transition. Interestingly, even in concentrated solution, the predicted weak d-d transition in the visible range for the one-electron paramagnetic (μ_{eff} = 1.86 and 1.65 BM) complexes cannot be identified. It might be lost in the charge transfer transition's low-energy tail [21, 22, 34- 35].

3.7. Nuclease activity

The DNA cleavage studies of the compounds were determined using agarose gel electrophoresis under physiological circumstances with/without H_2O_2 as an oxidant agent. The capacity of the complex to convert the supercoiled form (Form I) of DNA to its open circular form (Form II) and linear form (Form III) was used to assess its DNA cleavage activities [36-38].

The complex-mediated cleavage reactions were carried out at 37 ºC with various concentrations of complex (10-100 μM). In the presence of hydrogen peroxide, the copper complexes of the HL^1 and HL^2 ligands showed efficient DNA cleavage activity, transforming the plasmid supercoiled DNA into its cleavage forms (Form II and III), as illustrated in Figure 6. ($[Cu(L^1)_2]$ lane 1-5 and $[Cu(L^1)_2]$ lane 13-17.) It is apparent that when the concentration of all complexes rises, the quantity of supercoiled DNA (Form I) decreases.

The cleavage reactions meditated by the complexes were performed under different concentration of complex (10-100 μM) at 37 ºC. Figure 6 represents the gel electrophoretic separations of pBR322 DNA with increasing concentrations of complexes (10-100 μM) at 37 ºC in their optimum incubation times in the presence and absence of H_2O_2. The copper(II) complexes of the HL^1 and HL^2 ligands exhibit effective DNA cleavage activity converting the plasmid supercoiled DNA into its cleavage forms (Form II and III) in the presence of hydrogen peroxide as shown in Figure 6. (Lane 1-5 for $[Cu(L^1)_2]$ and lane 13-17 for $[Cu(L^1)_2]$). It is apparent that when the concentration of all complexes rises, the quantity of supercoiled DNA (Form I) decreases. Interestingly, the complex $[Cu(L^2)_2]$ can only formed of nickeded DNA, while the copper complex $[Cu(L^1)_2]$ can simultaneously separate supercoiled DNA into Form (II) and Form (III).

At a concentration of 10 μM, lane 1, the plasmid DNA was slightly cleaved by the complex $[Cu(L^1)_2]$ and all three forms of DNA are observed. The percentage of the nicked DNA molecules is higher than those of form I and form III. On the other hand, when the concentration of the complex was greater than 10 μM, the supercoiled DNA is disappeared completely and form II and form III are present for complex $[Cu(L^1)_2]$, lane 2-4, as a result of double-strand breaks over the plasmid molecule. Finally, at the concentration of 100 μM (lane 5), the supercoiled DNA degrades completely into small pieces as no bands is observed.

In the presence of $[Cu(L^2)_2]$ at 10 μM, it scarcely catalyzed the cleavage of plasmid DNA. However, when the concentration of the complex was increased to 25 μM, the cleavage of DNA was clearly observed. It was found that with the increase of the complex concentration, the density of supercoil DNA on the gel decreased, whereas the density of the nicked DNA increased significantly (lanes 14- 16), indicating that the DNA was fragmented by the complex by a single-stranded cleavage. Finally when the complex concentration is at 100 μM, lane 17, the supercoiled DNA is completely cleaved to nicked form.

Both copper complexes exhibit mild nuclease activity as an oxidative agent even under hydrolytic conditions. However, it can be concluded from the data in Figure 6. that $[Cu(L^1)_2]$ is much more effective artificial nuclease than that of $[Cu(L^2)_2]$ in the absence of an oxidative agent. It was observed that the percentage of nicked DNA increased, with increasing concentration of [Cu $(L^1)_2$]. On the other hand, in the case of the DNA cleavage by $[Cu(L^2)_2]$, a small amount of supercoiled DNA is converted to open circular DNA only when the concentration of the complex is up to 75 μM (Lane 21-22).

In the absence of an oxidative material in the medium, DNA cleavage is predicted to occur hydrolytically. In addition, in DNA cleavage mediated by certain copper complexes, the shearing activity has been found to be oxidative, even without the use of a reducing agent. The oxidative DNA cleavage in this scenario might be ligand-based, presumably due to the presence of a non-diffusible organic radical mechanism that induces oxidative DNA cleavage. An alternative approach is to produce dihydrogen peroxide when hydrogen is removed from the deoxyribose sugar in the presence of dioxygen. In a Fenton-type reaction, dihydrogen peroxide reacts with Cu(II) to form reactive oxygen species, making DNA cleavage oxidative or catalytic.

The reason why the $[Cu(L^1)_2]$ complex shows higher DNA cleavage activity than the $[Cu(L^2)_2]$ complex may be the inability of the radicals to take effect as a result of the steric effect of the long aliphatic chain of the ligand forming the $[Cu(L^2)_2]$ complex [18, 39- 40].

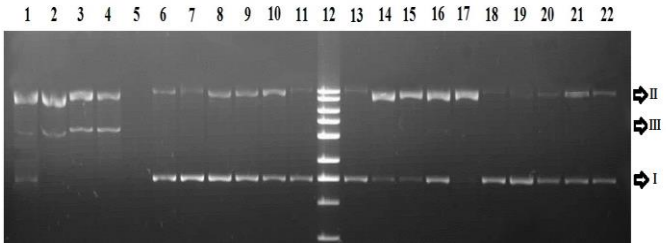


Figure 6 pBR322 plasmid DNA electrophoresis on an agarose gel with various amounts of copper(II) complexes. L1: $Cu(L^1)_2$ (10 μM) + DNA + H_2O_2, L2: $Cu(L^1)_2$ (25 μM) + DNA + H_2O_2, L3: $Cu(L^1)_2$ (50 μM) + DNA + H_2O_2, L4: $Cu(L^1)_2$ (75 μM) + DNA + H_2O_2, L5: $Cu(L^1)_2$ (100 μM) + DNA + H_2O_2, L6: $Cu(L^1)_2$ (10 μM) + DNA, L7: $Cu(L^1)_2$ (25 μM) + DNA, L8: $Cu(L^1)_2$ (50 μM) + DNA, L9: $Cu(L^1)_2$ (75 μM) + DNA, L10: $Cu(L^1)_2$ (100 μM) + DNA, L11: DNA control, L12: 1 kb marker, L13: $Cu(L^2)_2$ (10 μM) + DNA + H_2O_2, L14: $Cu(L^2)_2$ (25 μM) + DNA + H_2O_2, L15: $Cu(L^2)_2$ (50 μM) + DNA + H_2O_2, L16: $Cu(L^2)_2$ (75 μM) + DNA + H_2O_2, L17: $Cu(L^2)_2$ (100 μM) + DNA + H_2O_2, L18: $Cu(L^2)_2$ (10 μM) + DNA, L19: $Cu(L^2)_2$ (25 μM) + DNA, L20: $Cu(L^2)_2$ (50 μM) + DNA, L21: $Cu(L^2)_2$ (75 μM) + DNA, L22: $Cu(L^2)_2$ (100 μM) + DNA. *Lane:L

3.8. Mechanistic pathway of DNA cleavage

Mechanistic pathway of pBR322 cleavage induced by the complex using different scavenging agents was investigated and revealed. To investigate the involvement of the metal center in cleavage, we utilized DMSO as a hydroxyl radical scavenger, NaN_3 as a singlet oxygen quencher, KI as a superoxide scavenger, catalase as a hydrogen peroxide scavenger, and EDTA as a chelating agent [39- 41]. (Figure 7). The potential molecular mechanism of the cleavage of pBR322 plasmid DNA produced by complexes $[Cu(L^1)_2]$ and $[Cu(L^2)_2]$ has also been identified.

There was no suppression of DNA cleavage for either complex when NaN_3 was added (lanes 3 and 7). This shows that the solitary oxygen radical is not the active oxidative species that causes the complexes to cleave DNA. In addition to presence of hydrogen peroxide scavenger and hydroxyl radical scavenger block the DNA strand breakage for the $[Cu(L^1)_2]$ complex (lanes 5 and 6), suggesting that these species are involved in the DNA cleavage reactions while DMSO and KI are ineffective in the cleavage reaction for the $[Cu(L^2)_2]$ complex (lanes 9 and 10). With the addition of EDTA, which has the ability to form stable complexes by showing a chelating effect with metals, a noticeable decrease was observed in the DNA cleavage activities of complex compounds. This reveals that Cu(II) ions in the structure of the complex contribute to the cleavage activity of the complex.

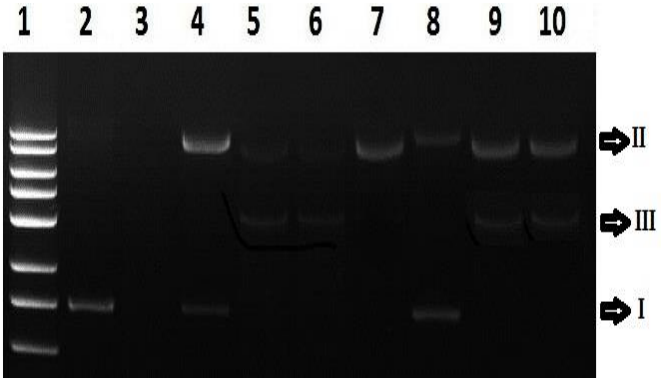


Figure 7 pBR322 plasmid DNA electrophoresis on an agarose gel after treatment with copper(II) complexes (100 μM) and possible inhibitors. L1: 1 kb marker, L2: DNA control, L3: $Cu(L^1)_2$ + DNA + H_2O_2 + NaN_3 , L4: $Cu(L^1)_2$ + DNA + H_2O_2 + EDTA, L5: $Cu(L^1)_2$ + DNA + H_2O_2+ KI, L6: $Cu(L^1)_2$ + DNA + H_2O_2 + DMSO, L7: $Cu(L^2)_2$ + DNA + H_2O_2 + NaN_3, L8: $Cu(L^2)_2$ + DNA + H_2O_2 + EDTA, L9: $Cu(L^2)_2$ + DNA + H_2O + KI, L10: $Cu(L^2)_2$ + DNA + H_2O_2 + DMSO. *Lane:L

4. CONCLUSION

Using distinct spectroscopic approaches, two novel copper(II) complexes containing arylhydrazones have been produced and studied. The arylhydrazones operate as monobasic bidentate ligands containing azomethine nitrogen and enolic oxygen, according to the elemental analyses, stoichiometric, and spectroscopic investigations detailed above. The copper(II) complexes' DNA cleavage capabilities in the presence of H_2O_2 indicated that the $[Cu(L^1)_2]$ complex cleaves circular supercoiled DNA into nicked DNA and linear DNA under aerobic conditions, whereas the $[Cu(L^2)_2]$ complex breaks circular supercoiled DNA into solely nicked DNA. In the absence of H_2O_2, however, they demonstrated decreased nuclease activity. In the absence of a reducing agent, both copper complexes have some nuclease activity. For both copper(II) complexes, increasing the concentration of the complexes resulted in more significant DNA scission. It was also shown that at concentrations of up to 100 μM, $[Cu(L^1)_2]$ fully destroys circular supercoiled DNA. Because it displays substantial DNA cleavage activity even in the absence of hydrogen peroxide, $[Cu(L^1)_2]$ has superior nuclease activity than $[Cu(L^2)_2]$. In the presence of reducing agents, both complexes are capable of performing effective oxidative cleavage of DNA, and copper(II) ion plays a crucial part in the cleavage process.

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Conflict of interest
There is no conflict of interest to be declared.

The Declaration of Ethics Committee Approval
This study does not require ethics committee approval or any special permission.

The Declaration of Research and Publication Ethics
The author of the paper declares that he complies with the scientific, ethical and quotation rules of SAUJS in all processes of the article and that he does not make any falsification on the data collected. In addition, he declares that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science

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