Bulgarian Chemical Communications, Volume 49, Number 4, (pp. 908 – 913) 2017

DNA binding by copper (II) complexes of semithiocarbazone containing ligands

B. Coban*[1], N. Eser[1], I. Babahan[2]

[1]*Bülent Ecevit University, Faculty of Arts and Sciences, Department of Chemistry, Zonguldak, 67100, Turkey.*
[2]*Adnan Menderes University, Faculty of Arts and Sciences, Department of Chemistry, Aydin, 09010, Turkey.*

Received December 23, 2016; Accepted May 30, 2017

DNA binding properties of two previously synthesized copper complexes of *vic*-dioximes bearing thiosemicarbazone units (2*E*)-2-[4-(dimethylamino)benzylidene]-*N*-[(1*Z*,2*E*)-*N*-hydroxy-2-(hydroxyimino)ethanimidoyl]hydrazine carbothioamide (**1**) and (2*E*)-2-[4-(diethylamino)benzylidene]-*N*-[(1*Z*,2*E*)-*N*-hydroxy-2-(hydroxyimino)ethanimidoyl] hydrazine carbothioamide (**2**), were investigated using absorption spectroscopy, fluorescence spectroscopy, and agarose gel electrophoresis methods. Experimental studies suggested that the complexes bind to DNA through intercalation. Their intrinsic binding constants (K_b) were calculated as **1**: 5.50 ± 0.25 × 10^4 M^{-1}; **2**: 2.10 ± 0.18 × 10^5 M^{-1}. These complexes also promote the cleavage of plasmid pBR322, both in the absence and presence of hydrogen peroxide.

Keywords: Cu(II) complex, thiosemicarbazone, *vic*-dioxime, DNA, intercalation

INTRODUCTION

Interaction between DNA and drug molecules is a very popular subject [1-3], especially for the designing of new DNA-targeted drugs and their *in vitro* screening. The biochemistry of thiosemicarbazones has received considerable attention because of their promising biological implications, and structural diversity. The chemistry of transition metal complexes of thiosemicarbazone ligands has also been of interest, primarily due to their bioinorganic relevance. The potential biological benefits of this class of complexes have been found to include antibacterial, antimalarial, antiviral, and antitumour activities [4-10]. Dioximes have also been applied to analytical, biological, pigment, and medicinal chemistry applications. In particular, they have been found to play an important role in coordination chemistry, mainly due to their planar structure stabilized by hydrogen bonding interactions [5, 11, 12].

Many techniques, such as UV–vis spectrophotometry, fluorescence spectroscopy, circular dichroism spectroscopy, mass spectrometry, and voltammetry have been used to study the binding between metal complexes and DNA [13-17].

In this work, the interactions of previously synthesized [5] thiosemicarbazone containing ligands and their Cu(II) complexes with calf thymus (CT)-DNA were comparatively investigated with the aid of the absorption and fluorescence spectroscopy and their DNAse activity was investigated against pBR322 plasmid DNA *via* agarose gel electrophoresis assays.

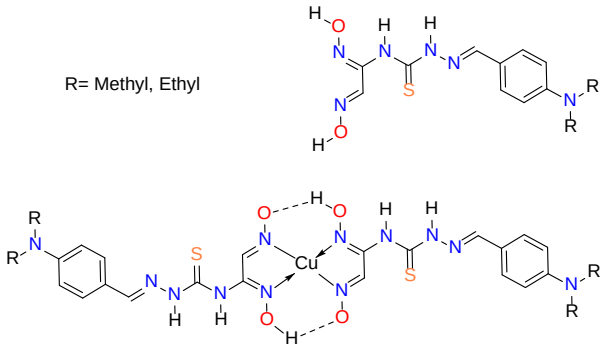

Fig. 1. Chemical structures of ligands **LH1** and **LH2** and complexes **1** (Cu**L1**$_2$) and **2** (Cu**L2**$_2$).

EXPERIMENTAL

The ligands and the complexes were prepared according to the literature [5]. All reagents and solvents were of commercial origin and used without further purification unless otherwise noted. Solutions of calf thymus DNA (CT-DNA purchased from Sigma) in 50 mM ammonium acetate (pH 7.5) had a UV–Vis absorbance ratio of 1.8–1.9: 1 at 260 and 280 nm (A_{260}/A_{280} = 1.9), indicating that the DNA was sufficiently free of protein [16]. The concentration of DNA was determined spectrophotometrically using a molar absorptivity of 6,600 M^{-1} cm^{-1} (260 nm) [16]. Double distilled water was used to prepare buffers. Stock solution of CT-DNA was stored at 4 °C and used within 4 days.

Physical measurements

UV–Vis spectra were recorded with a Varian Cary 100 spectrophotometer and emission spectra were recorded with a Perkin Elmer LS 55 fluorescence spectrometer at room temperature.

* To whom all correspondence should be sent:
E-mail: burakcoban@yahoo.com

Absorption and emission titrations

For the absorption and emission titrations, ligands and Cu(II) complexes (about 1 mmol) were dissolved in a minimum amount of DMSO (0.5 mL), and were then diluted in 5 mM ammonium acetate buffer, pH 7.5) to a final concentration of 20 μM. Titrations were performed in a 10-mm stoppered quartz cell by using a fixed concentration of the compound (20 μM), to which the CT-DNA stock solution was added in increments of 1 μL to a DNA-to-compound concentration ratio of 6:1. Analysis was performed by means of a UV–Vis or fluorescence spectrophotometer by recording the spectrum after each addition of DNA. Compound–DNA solutions were incubated for 10 min before the spectra were recorded. A control solution of 20 μM of the drug in the same buffer was treated in the same manner. Cell compartments were thermostated at 25 ± 0.1°C.

For emission intensity measurements, the excitation wavelength was fixed and the emission range was adjusted before the measurements. Ammonium acetate (5 mM), pH 7.5 buffer was used as a blank to make preliminary adjustments. All measurements were performed with a 5-nm entrance slit and a 5-nm exit slit. The complexes were excited at 383 and 400 nm, respectively; the emission spectra were monitored between 710 and 740 nm.

Competitive studies

The competitive behavior of each compound with ethidium bromide (EB) was investigated by fluorescence spectroscopy in order to examine whether the compound is able to displace EB from the DNA-EB complex.

DNA was pretreated with EB at a DNA-to-EB concentration ratio of 50:1 for 30 min at 27 °C to prepare the initial DNA-EB complex. The intercalating effect of the compounds with the DNA-EB complex was studied by adding a certain amount of a solution of the compounds in increments to the solution of the DNA-EB complex. The influence of each addition of compounds to the solution of the DNA-EB complex was estimated by recording the change in the fluorescence peak at 640 nm. To study the competitive binding of the compounds with EB, the latter was excited at 453 nm in the presence of DNA alone as well as in the presence of the compounds.

DNAse activity by gel electrophoresis

Gel electrophoresis experiments were performed using pBR322 negatively supercoiled plasmid DNA and 1 % agarose gel together with a tris(hydroxymethyl)aminomethane-borate-EDTA running buffer solution. Reaction mixtures (10 mL) containing 0.1 μg pBR322 together with different amounts of **1** and **2** (0, 5, 500 μM) in 50 mM ammonium acetate buffer, pH 7.5 were prepared at 0 °C, were then incubated at 36 °C for 1 h and 24 h in the dark and for 1 h with the addition of 2 μL of 30 % hydrogen peroxide solution. Prior to the samples being loaded onto the gel, 2.5 mL of 0.25 % bromophenol blue loaded buffer and sucrose in water (40 % w/v) was added to the reaction mixtures. Gels were obtained at room temperature by using a Thermo midi horizontal agarose gel electrophoresis system and applying a potential of 35 V for 4 h. The resulting gels were stained with EB solution (0.5 μg mL^{-1}) for 45 min, after which they were soaked in water for further 20 min. Gels were visualized under UV light and photographed.

RESULTS AND DISCUSSION

Both ligands have very similar UV spectra with an absorption peak around 280-360 nm wavelength. Addition of DNA caused very little or no change in the absorption spectra seen in Fig 5.

Very low hypochromic and bathochromic effects are seen in the electronic absorption spectra of the complexes upon addition of DNA (Fig 2). The hypochromicity and bathochromic shift increased when the complexes were titrated with DNA solution (Fig 2).

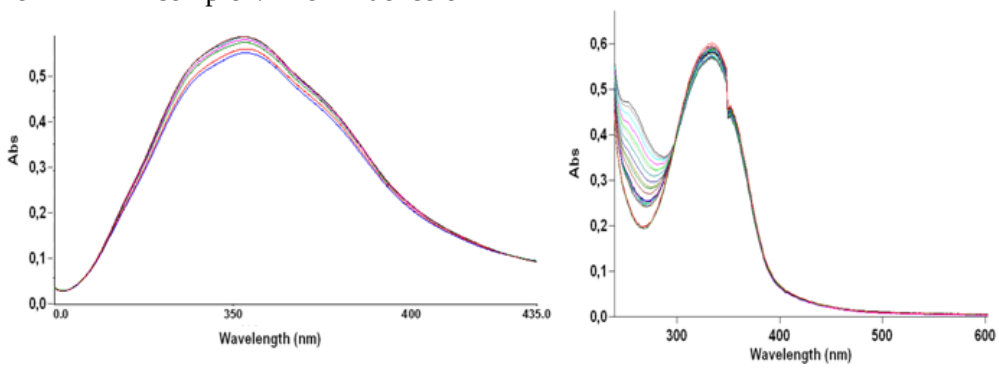

Fig. 2. Absorption spectra of the complexes in 50 mM ammonium acetate buffer on gradual additions of calf thymus DNA at pH 7.0 and 298 K. [complex] = 20 μM, [ct-DNA] = 0-40 μM. Arrows indicate the absorbance changes with increase in ct-DNA concentration.

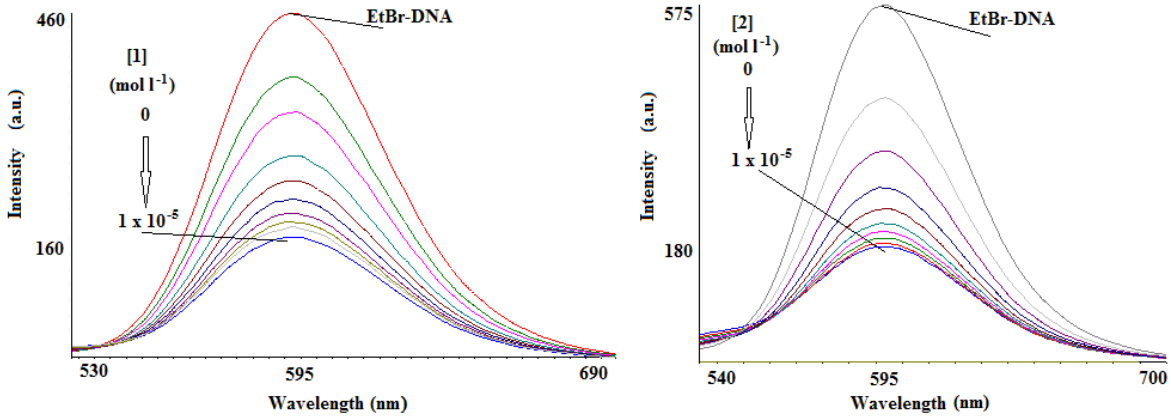

Fig. 3. Fluorescence spectra of the competition between complexes and ethidium bromide (EB) at 298 K. [EB] = 20.0 μM and [ct-DNA] = 100.0 μM at λ_{exc} = 363 nm. [Complex] = 0.5-10.0 μM.

In the absence of DNA, the complexes **1** and **2** emit weak luminescence in ammonium acetate buffer at ambient temperature, with a maximum at 383 and 455 nm, respectively. On addition of CT-DNA, the emission intensity slightly increases (Fig 6).

The competitive titrations of ethidium bromide (EB) dye and complexes in the presence of DNA are shown in Fig 3. According to the spectra, EB fluorescence intensity was decreased by the addition of complexes.

The potentials of the complexes to cleave DNA were studied by agarose gel electrophoresis using pBR322 plasmid DNA. When circular DNA is subjected to gel electrophoresis, relatively fast migration will be observed for the supercoiled form (form I). If scission occurs on one strand (nicked circular), the supercoiled form will relax to generate a slower moving open circular form (form II). If both strands are cleaved, a linear form (form III) that migrates between the two forms will be generated [15]. Fig 4a shows the separation of pBR322 by gel electrophoresis after 1 h incubation of lanes 1-6 and 24 h incubation of lanes 7-12 with the complexes. Lanes 1, 4, 7, 10 are controls, having only DNA. The plasmid DNA was cleaved by the complexes and a small portion of form II was produced (lanes 2-3 and 5-6) in 1 h but a larger portion of the plasmid DNA was converted to form II by 24 h incubation by the complexes (lanes 8-9 and 11-12). In Fig 4b, lane 1 is the control, having only DNA and lane 2 is the negative control DNA incubated with peroxide solution. Addition of complexes caused DNA cleavage in the lanes 3-8. A small portion of form II was produced by the complexes at 10 mM concentration. However, at the highest concentration of **2**, plasmid DNA was extensively damaged, multiply occurred in the DNA backbone and therefore small DNA fragments cannot be seen in the gel.

The complexes exhibit strong interaction compared with their corresponding ligands. There were very weak interactions of the ligands with DNA. The binding strengths of the ligands and complexes to CT DNA were determined by calculating the binding constant (K_b) using the equation:

$$[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/(\varepsilon_b-\varepsilon_f)+1/Kb\ (\varepsilon_b-\varepsilon_f)$$

The absorption coefficients ε_a, ε_f, and ε_b correspond to A_{obs}/[complex], the extinction coefficient for the free complex, and the extinction coefficient for complex in the fully bound form, respectively. The plot of [DNA]/(ε_a–ε_f) *versus* [DNA] gives a slope of 1/(ε_a–ε_f) and an intercept of 1/*K*b (ε_b–ε_f). The binding constants indicate that the complexes bind to CT-DNA. In addition, the complexes interacted with DNA in an intercalative manner because hypochromic and bathochromic effects were measured (a decrease in the absorption peak at ~400 nm wavelength with a small red shift ~1 nm) (Fig. 2). This may be explained by allowing the planar 4-diethylaminobenzene ring of the ligand binding to DNA by intercalation between the base pairs. Their intrinsic binding constants (K_b) were calculated as **1**: $5.50 \pm 0.25 \times 10^4$ M^{-1}; **2**: $2.10 \pm 0.18 \times 10^5$ M^{-1}. The binding values are comparable with the literature values [8]. The more hydrophobic complex **2** has a higher binding constant. The binding constants were much smaller than those of classical intercalators, thus, the interaction would be explained as partial intercalation. As a control experiment, the interaction between DNA and Cu(II) ions was studied (Fig 7) and the resulting hyperchromic effect and absence of bathochromic effect on the spectra indicates that Cu(II) ions bind DNA electrostatically with an external binding mode [18,19].

The aqueous solutions of the complexes emitted weak luminescence but addition of DNA increased the luminescence intensity indicating that the complexes interact with CT-DNA and are protected

by the hydrophobic environment inside the DNA helix reducing the accessibility of solvent water molecules to the complex and the mobility of the complex is restricted at the binding site, leading to a decrease in the vibrational modes of relaxation (Fig 6).

The intercalated EB was displaced from the EB–DNA complex by the complexes (Fig 3). The strength of the displacement can be summarized as **2** > **1**. This displacement was in accordance with the UV titration findings where the hydrophobicity of the N-ethyl compound would be higher than that of N-methyl.

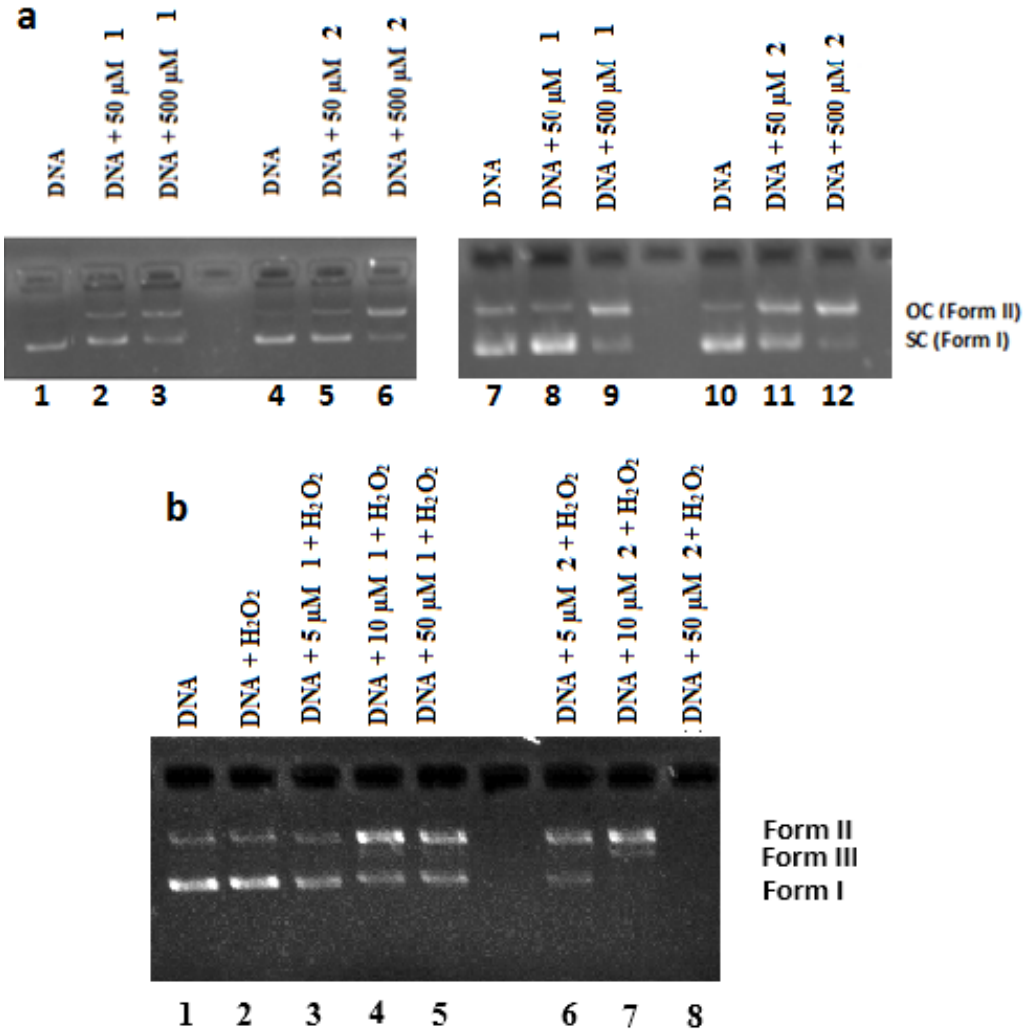

Fig. 4. Agarose gel electrophoresis pictogram showing the cleavage pattern of pBR322 plasmid DNA (50 µg) with 50 and 500 µM concentrations of the complexes at 37 °C **a.** Lanes 1-6 for 1h incubation and lanes 7-12 for 24 h incubation. **b.** Effect of additional peroxide: lane 1: DNA control, lane 2: peroxide, lanes 3-5 and 6-8: peroxide+5, 10, 50 µM of complexes **1** and **2**, respectively.

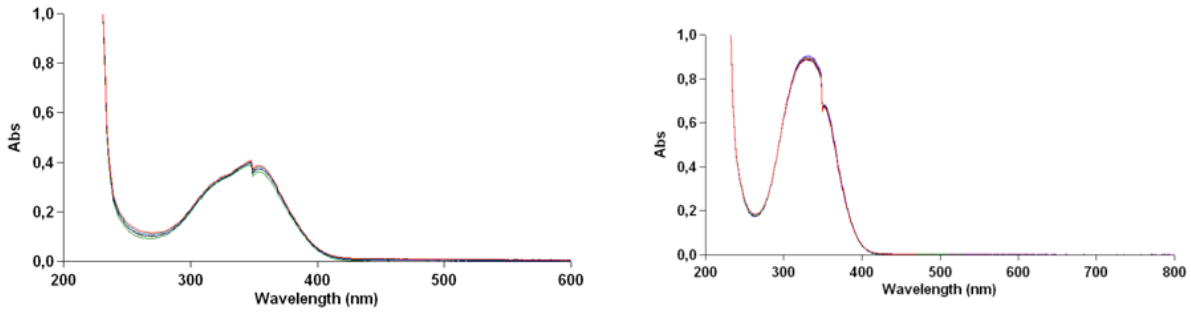

Fig 5. Absorption spectra of Ligands **L1H** (left) and **L2H** (right) in 50 mM ammonium acetate buffer on the gradual additions of calf thymus DNA at pH 7.0 at 298 °K. [ligand] = 20 µM, [ct-DNA] = 0-40 µM. Arrows indicate the absorbance changing with increase of ct-DNA concentration

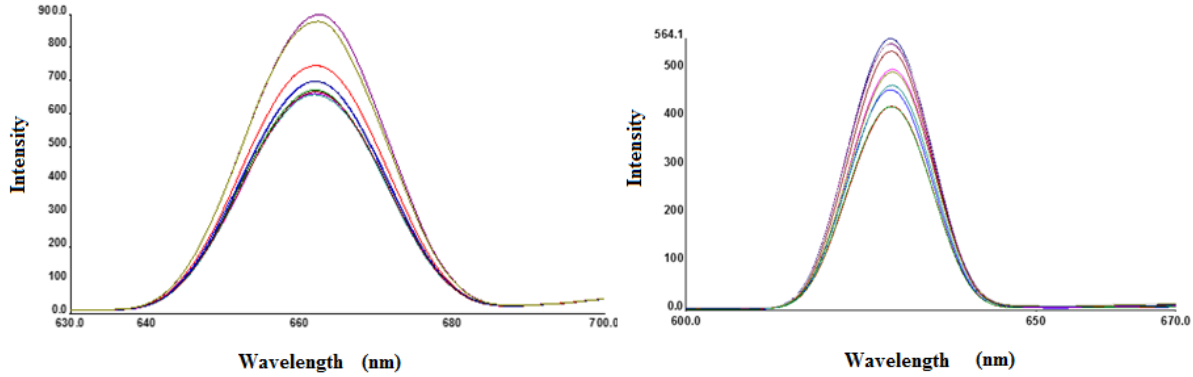

Fig 6. Emmision spectra of **1** (left) and **2** (right) (10 μM) in ammonium acetate buffer at 25 ◦C in the presence of 0–20 μM calfthymus-DNA. The intensity increases with increasing DNA concentration in both cases. The complexes were excited at 400 and 380nm, respectively; the emission spectra were monitored between 660 and 600 nm

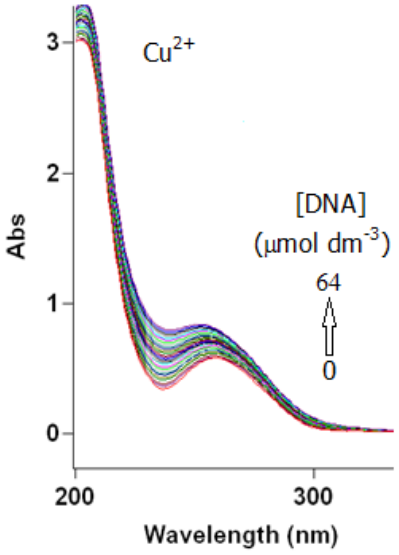

Fig. 7. Absorption spectra of calf thymus DNA in 50 mM ammonium acetate buffer on the gradual additions of Cu^{2+} at pH 7.0 at 298 ◦K. [ct-DNA] = 100.0 μM, [Cu^{2+}] = 6, 12, 18, 24, 30, 35, 41, 47, 53, 58, 64 μM. Arrows indicate the absorbance changing with increase of Cu^{2+} concentration

CONCLUSIONS

DNAse activities of the complexes were compared and the same displacement was found as **2** > 1. The complexes show strong DNAse activity in the presence of peroxide (Fig 4). This may be due to the advanced redox cycling activity of Cu(II) where *in situ* formed cuprous ions (Cu^{+}) can convert H_2O_2 to hydroxyl radicals and binding of the complex to DNA provides centers for generation of hydroxyl radicals close to sites susceptible to breakage [20]. Thus, the more hydrophobic complex gives a higher level of damage to plasmid DNA.

In conclusion, the absorption and fluorescence spectroscopy studies demonstrated that the complexes **1** and **2** interacted with DNA base pairs (binding constant, K_b = (1.82 ± 0.20) × 10^5 M^{-1}). A competitive reaction, monitored by fluorescence spectrophotometry, between fluorescent probe, ethidium bromide (EB) dye, DNA and complexes showed that the intercalated EB was displaced from the EB–DNA complex by **1** and **2**. According to both spectrophotometric studies, the complexes bind DNA stronger than the actual ligand. Very similar results were found by electrophoresis of the plasmid DNA in the presence of the complexes with and without peroxide addition. The complexes induced strong DNA damage in the presence of peroxide and **2** has better DNAse activity as expected from the spectrophotometric studies.

Acknowledgements: *We are grateful for the support of Bulent Ecevit University with grant #2015-72118496-07.*

REFERENCES

1. S. Grguric-Sipka, R. Vilaplana, J. Perez, M. Fuertes, C. Alonso, Y. Alvarez, T. Sabo, F. Gonzalez-Vilchez, *J. Inorg. Biochem.*, **97**, 215 (2003).
2. N. Ljubijankić, A. Zahirović, E. Turkušić, E. Kahrović, *Croat. Chem. Acta*, **86**, 215 (2013).
3. S. Rauf, J. Gooding, K. Akhtar, M. Ghauri, M. Rahman, M. Anwar, A. Khalid, *J. Pharm. Biomed. Anal.*, **37**, 205 (2005).
4. A.A. Al-Amiery, Y.K. Al-Majedy, H. Abdulreazak, H. Abood, *Bioinorg. Chem. Appl.*, Article ID 483101, 6 pages (2011).
5. İ. Babahan, A. Özmen, N. Orhan, D. Kazar, E.H. Değirmenci, *Bioorg. Chem.*, **53**, 92 (2014).

6. J.S. Casas, M.S. García-Tasende, J. Sordo, *Coord. Chem. Rev.*, **209**, 197 (2000).
7. X. Du, C. Guo, E. Hansell, P.S. Doyle, C.R. Caffrey, T.P. Holler, J.H. McKerrow, F.E. Cohen, *J. Med.Chem.*, **45**, 2695 (2002).
8. M.A. Hussein, T.S. Guan, R.A. Haque, M.B.K. Ahamed, A.M.S.A. Majid, *Polyhedron*, **85**, 93 (2015).
9. A.I. Matesanz, C. Hernández, P. Souza, *J. Inorg. Biochem.*, **138**, 16 (2014).
10. G. Pelosi, *Open Crystallogr. J.*, **3**, 16 (2010).
11. B. Dede, İ. Özmen, F. Karipcin, M. Cengiz, *Appl. Organometal. Chem.*, **23**, 512 (2009).
12. A. Sengul, H. Agac, B. Coban, E. Eroglu, *Turk. J. Chem.*, **35**, 25 (2011).
13. A. Arslantas, A.K. Devrim, M. Sözmen, H. Necefoglu, *Rasayan J. Chem.*, **3**, 201 (2010).
14. B. Coban, I.O. Tekin, A. Sengul, U. Yildiz, I. Kocak, N. Sevinc, *J. Biol. Inorg. Chem.*, **21**, 163 (2016).
15. B. Coban, U. Yildiz, *Appl. Biochem. Biotech.*, **172**, 248 (2014).
16. B. Coban, U. Yildiz, A. Sengul, *J. Biol. Inorg. Chem.*, **18**, 461 (2013).
17. I. Kocak, U. Yildiz, B. Coban, A. Sengul, *J. Solid State Electrochem.*, **19**, 2189 (2015).
18. R.F. Pasternack, E.J. Gibbs, J.J. Villafranca, *Biochem.*, **22,** 2406 (1983).
19. S.-P. Tang, L. Hou, Z.-W. Mao, L.-N. Ji, *Polyhedron*, **28**, 586 (2009).
20. A.S.H. Li, B. Bandy, S.S. Tsang, A.J. Davison, *Free Radic. Biol. Med.*, **30**, 943 (2001).

КОМПЛЕКСИ НА ДНК С МЕД (II) И ЛИГАНДИ ОТ СЕМИКАРБАЗОНИ

Б. Джобан *[1], Н. Есер[1], И. Бабахан[2]

[1]Университет „Бюлент Еджевит“, Факултет за изкуство и наука, Департамент по химия, Зонгулдак, Турция

[2] Университет „Аднан Мендерес“, Факултет за изкуство и наука, Департамент по химия, Айдин 09010, Турция

Постъпила на 23 декември, 2016 г.; коригирана на 30 май, 2017 г.

(Резюме)

Изследвани са свързващите свойства на ДНК на два предварително синезирани медни комплекса от *vic*-диоксими, носещи тиосемикарбазониюни единици (2*E*)-2-[4-(диметиламино)бензилиден]-*N*-[(1*Z*,2*E*)-*N*-хидрокси-2-(хидрокси-иино)етанимидоил]хидразин карбо-тиоамид (**1**) и (2*E*)-2-[4-(диетиламино)бензилиден]-*N*-[(1*Z*,2*E*)-*N*-хидрокси-2-(хидроксиимино)етанимидоил] хидразин карботиоамид (**2**). Изследванията са извършени с помощта на абсорбционна спектроскопия, флуоресцентна спектроскопия и агароза-гел-електрофореза. Експерименталните изследвания предполагат свързването на комплексите с ДНК чрез интеркалация. Техните присъщи константи на свързване (K_b) са изчислени на **1**: $5.50 \pm 0.25 \times 10^4$ M^{-1}; на **2**: $2.10 \pm 0.18 \times 10^5$ M^{-1}. Тези комплекси подпомагат разкъсването на плазмида pBR322, в присъствие и отсъствие на водороден пероксид.