Co(III), Ni(II), and Cu(II) complexes with tetradentate Schiff base ligand: Synthesis, Characterization, Electrochemical Behavior, Binding assessment and *In vitro* cytotoxicity activity

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A B S T R A C T

Two new Schiff base cobalt(III) ([Co(LH)Cl_2], **1**) and nickel(II) ([Ni(LH)ClO_4], **2**) complexes with a diimine-dioxime ligand, (4,9-diaza-3,10-diethyl-3,9-dodecadiene-2,11-dione bis oxime (**LH_2**)), were synthesized and characterized. The compounds were obtained in MeOH from corresponding metal salts and **LH_2** in molar ratio 1:1 and further characterized by mass spectrometry, IR spectroscopy, electrochemistry, and elemental analysis. Previously reported copper(II) analog, ([$Cu_2(LH)_2$]·$(ClO_4)_2$, **3**) was joined to **1** and **2**, and the three metal analogs, **1-3**, were further investigated in terms of their electrochemical behavior. The binding studies of the complexes with deoxyribonucleic acid (DNA) and human serum albumin (HSA) were carried out using both spectrophotometric and electrochemical methods. All three complexes exhibit binding affinity towards the DNA chain through intercalative interaction. The binding reaction with HSA showed for **1** and **3** complexes decrease in the peak current obtained in the case of complexes before the addition of HSA, while resulted compound obtained from Ni complex – HSA possesses the same electroactivity as starting complex. Furthermore, the cytotoxicity of **LH_2** as well as its metal complexes, and cisplatin were evaluated on CT-26 mouse colon carcinoma and human LS174T cancer cell lines employing MTT assay. The copper(II) complex exhibited very promising anticancer activity compared with cisplatin.

Keywords: Schiff base ligand, Metal complex, Electrochemical behavior, DNA interaction, HSA binding, Anticancer activity

1. Introduction

Schiff bases are a well-known class of organic compounds having imine or azomethine (–C=N–) functional group. Commonly formed by the condensation of amine NH_2 group with active –C=O carbonyl group with the general structure $R^1R^2C=NR^3$ (with $R^3 \neq H$) [1]. They were first reported in 1864 by Hugo Schiff [2]. Schiff base ligands can easily form stable complexes with different metal ions due to their rich coordination chemistry regarding the presence of oxygen and/or nitrogen donor groups. Metal complexes of Schiff bases have been tested and used for a variety of applications in analytical, medicinal, and industrial areas, as well as for essential biological activities including antibacterial, anticancer, antifungal, etc.[3–6].

The use of classical cisplatin-type drugs for the therapy of cancers with the aim to induce tumor cell death causes numerous side effects. Hence, it is desirable to consider improving the efficiency of anticancer drugs that are currently in use [7]. Nowadays, different metal complexes, such as copper, ruthenium, and, lanthanum complexes, are examined as the most suitable alternates for platinum complexes [8,9,18,10–17].

Copper, as an essential trace element, is necessary for many life processes and has a key role in many physiological cellular processes. Its high redox activity makes the free copper ions highly cytotoxic, therefore the intracellular level of copper need to be strongly controlled. The tumor and normal cell can often give a different response when copper complexes with various sets of ligands are used as anticancer agents. A large number of copper complexes prepared for this reason displayed significant *in vitro* cytotoxicities [11,19–24], while a much smaller number was tested preclinically for *in vivo* use [22–24].

Co, Cu, and Ni complexes of Schiff base have attracted curiosity also due to DNA binding and cleavage properties under physiological conditions. It is known that DNA, as a primary anti-tumor target, could form DNA adducts with therapeutically metal complexes which cause DNA damage and result in apoptosis or other mechanisms of cell death [25,26]. Human serum albumin (HSA) is the most represented and important carrier protein in the blood. The binding activities of metal complexes to HSA have a significant impact on the absorption, distribution, metabolism, toxicity, and stability of anticancer drugs [27,28].

The interactions between metal complexes and DNA or HSA have been widely investigated using various spectroscopic [29,30], calorimetric [31], and electrochemical methods

[32,33]. Electrochemical studies of these interactions are increasingly represented due to their low cost and the use of simpler and smaller devices in comparison to the spectroscopic methods.

The present investigation describes the synthesis, characterization, electrochemical behavior, and *in vitro* cytotoxic activity of Cu(II), Ni(II), and Co(III) complexes of tetradentate Schiff base ligand. The structures of the complexes are characterized using elemental analysis, Fourier transform infrared spectroscopy (FT-IR), mass spectrometry, and cyclic voltammetry. DNA and HSA binding affinity of the complexes were also investigated. In addition, the anticancer activity of diimine-dioxime ligand, its metal complexes, and cisplatin was examined on two different cancer cell lines (i.e., CT-26 and LS174T cells) and evaluated by MTT assay.

2. Experimental

2.1. Materials and methods

All reagents obtained from commercial sources were of analytical grade and used without further purification. The synthesis and characterization of the ligand, $\mathbf{LH_2}$ in ethanol was earlier described [34]. Elemental analysis (C, H, N) of the samples was performed using a VARIO EL III Elemental analyzer, elementar Analysensysteme GmbH, Germany. The infrared spectra were obtained using a Nicolet Summit FTIR Spectrometer, Thermo Fisher Scientific, USA. The samples were analyzed in the form of KBr pellets after removal of the liquid under a high vacuum at low temperature. Signal intensities were reported according to their relative intensities as very strong (vs), strong (s), medium (m), and weak (w). The melting points (uncorrected) were determined on Mel-Temp melting point apparatus (Laboratory Devices Inc., USA). UV-Vis absorption spectra were carried out at room temperature on a Perkin Elmer Lambda 950 spectrophotometer, USA. LTQ Orbitrap XL (MS/ 250A) Mass Spectrometer (Heated ESI) was used for recording mass spectra in a positive mode in acetonitrile (**1**) and methanol (**2**). The synthesis and characterization of corresponding Cu complex, $[Cu_2(LH)_2]\cdot(ClO_4)_2$ (**3**) was recently reported [35] and **3** was further investigated alongside with **1** and **2**.

2.2. Synthesis of the complexes

The general synthetic procedure included mixing metal precursors, M(II) chloride hexahydrate (M= Co, Ni) primarily dissolved in MeOH, and hot MeOH ligand suspension in a

molar ratio 1:1 followed by the addition of a counter ion for **2**. The crude products of **1** and **2** were isolated in moderate yields.

2.2.1. Synhesis of $[Co(LH)Cl_2]$ (1)

Cobalt(II) chloride hexahydrate (148 mg, 0.625 mmol), dissolved in 10 ml MeOH, was added to a hot MeOH suspension (10 ml) of ligand LH_2 (175 mg, 0.625 mmol). The reaction mixture was then refluxed for 2 h, and the solvent was allowed to evaporate slowly to produce a crystalline green product. The obtained precipitate was collected by filtration, washed with cold MeOH and diethyl ether, and dried under vacuum. Yield 63%, mp: >220 °C. Anal. Calcd. For $C_{14}H_{25}Cl_2CoN_4O_2$: C, 40.89; H, 6.13; N, 13.62. Found: C, 40.62; H, 6.48; N, 13.55%. IR (cm^{-1}): 2932.6 (s), 2901.7 (w), 2870.5 (w), 1603.3 (w), 1560.3 (w), 1518.8 (vs), 1448.1 (s), 1367.8 (w), 1339.2 (w), 1319.7 (w), 1284.9 (w), 1231.4 (m), 1194.7 (w), 1134.2 (s), 1097.3 (m), 1061.8 (m), 1051.1 (m), 1011.9 (w), 989.2 (m), 907.6 (m), 821.1 (m), 693.2 (m), 630.1 (m), 516.4 (w), 482.6 (w), 457.8 (w). ESI-MS (m/z, (relative abundance, %)): 375.099 $[M - Cl, 100]^{2+}$, 340.130 $[M - 2Cl, 50]^{+}$.

2.2.2. Synhesis of $[Ni(LH)]ClO_4]$ (2)

Nickel(II) chloride hexahydrate (148 mg, 0.625 mmol), dissolved in 10 ml of MeOH, was added to a hot MeOH suspension (10 ml) of ligand LH_2 (175 mg, 0.625 mmol). The reaction mixture was then refluxed for 3 h. Orange-red product was isolated as the perchlorate salt by the addition of a $NaClO_4$ (77 mg, 0.625 mmol). The reaction mixture was allowed to stand at room temperature for 3 days, and orange-red crystals were formed, collected, and dried under vacuum. Yield 47%, mp: 215 °C. Anal. Calcd. For $C_{14}H_{25}ClN_4NiO_6$: C, 38.26; H, 5.73; N, 12.75%. Found: C, 37.98; H, 5.95; N, 12.53%. IR (cm^{-1}): 2985.5 (w), 2942.2 (m), 2920.5 (w), 2855.3 (w), 1604.5 (w), 1511.3 (m), 1451.4 (m), 1373.4 (w), 1353.7 (w), 1338.9 (w), 1289.7 (w), 1251.4 (w), 1230.0 (w), 1191.5 (w), 1137.8 (m), 1087.2 (vs), 1001.1 (w), 943.0 (w), 911.9 (w), 848.2 (w), 776.6 (w), 752.7 (w), 708.6 (w), 653.3 (w), 621.2 (s), 524.2 (w), 488.5 (w), 439.2 (w). ESI-MS (m/z, (relative abundance, %)): 339.132 $[M - ClO_4, 100]^{+}$.

2.2.3. Synthesis of $[Cu_2(LH)_2](ClO_4)_2$ (3)

Synthesis and single-crystal X-ray diffraction analysis of $[Cu_2(LH)_2](ClO_4)_2$ (**3**) were previously reported by our group following identical synthetic route of **2** [35]. Yield 68%; mp: >225 °C explosive. Anal. Calcd. for $C_{28}H_{50}Cl_2Cu_2N_8O_{12}$: C, 37.84; H, 5.67; N, 12.61%. Found: C, 37.67; H, 5.99; N, 12.38%. IR (cm^{-1}): 2989.5 (w), 2943.1 (w), 2918.5 (w), 1663.4 (w), 1625.4 (m), 1520.1 (s), 1449.2 (m), 1424.8 (w), 1368.5 (m), 1336.7 (m), 1301.0 (w), 1241.8 (w), 1206.9 (w), 1183.3 (w), 1117.4 (m), 1087.1 (vs), 953.7 (m), 934.2 (m), 789.0 (w), 704.9 (w), 653.1 (w), 621.7 (s), 519.9 (w), 478.1 (m), 448.4 (w).

2.3. DNA binding interactions and complexes' electrochemistry

Reagent grade Calf-thymus DNA (DNA) was supplied by Sigma Aldrich Chemical Co. Two approaches were used for the investigation of the DNA binding interactions: spectrophotometric and electrochemical. Experiments were designed to keep the concentration of the DNA constant, complexes were added gradually to the DNA solution, in the range of 0-150 mM. Before starting measurements, solutions were incubated for 5 min and adsorption (200 to 800 nm) and electrochemical spectra (0-1.5 V) were recorded. Electrochemical measurements were done in the three-electrode system where glassy carbon electrode was used as working electrode, Ag/AgCl (3M) as a reference, and platinum wire as counter electrode, controlled by Autolab 302 N (The Netherland). Before each measurements, GC electrode was polished with 0.3 and 0.05 alumina and rinsed with ultrapure water.

2.4. HSA binding study

For the investigation of interactions of complexes with HSA, two different approaches were used - electrochemical and spectrophotometric titration. Increased concentration of the HSA standard solution (0–150 mM) were added to the constant amount of the metal complex solution (20 mM). The electrochemical response (using cyclic voltammetry) and adsorption spectra were recorded using the same experimental conditions as for DNA - complexes interactions.

2.5. MTT cytotoxicity assay

The antiproliferative activity of the complexes **1**, **2** and **3** as well as $\mathbf{LH_2}$ and cisplatin was determined on CT-26 mouse colon carcinoma and human LS174T cancer cell lines using 3-(4,5-

dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [7]. Cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA). Cells were cultured in tissue culture flasks using RPMI medium with the addition of 10% fetal bovine serum (FBS), 100 IU/ml penicillin, and 100 ug/ml streptomycin. Incubation was done at 37 °C in a 5% CO_2-95% air humidified incubator. Cells were harvested using trypsin/ethylenediaminetetraacetic acid (EDTA) solution. After resuspension, 1×10^4 cells/well were placed into wells of 96-well plates. The attached cells were exposed to serial dilutions up to 100 µM of cisplatin, and 1000 µM of **LH_2**, complexes **1**, **2** and **3** for 24 h in the incubator. After exposure, 20 µl of MTT solution were added to each well. The samples were incubated at 37 °C for 4 h with 5% CO_2 in a humidified atmosphere. In order to dissolve Formazan crystals, 100 µl of 10% sodium dodecyl sulfate (SDS) was added. The absorbance was recorded using microplate reader (Molecular Devices Spectramax 250) at 540 nm. The concentration of tested compounds which produce 50% inhibition of cell survival (IC_{50}) values were determined using "Quest Graph™ IC_{50} Calculator." AAT Bioquest, Inc, 18 Jan. 2021, https://www.aatbio.com/tools/ic50-calculator.

3. Results and discussion

3.1. Preparation and characterization of complexes 1 and 2

New nickel and cobalt complexes of diimine-dioxime ligand were obtained via reaction of methanolic solutions of the metal precursor and ligand **LH_2** refluxed for 3 h. The crystalline green product of **1** was separated from the mother solution by filtration while **2** was precipitated by adding a proper amount of counter ion. The synthetic route and proposed structures for the obtained orange nickel and green cobalt complexes are presented in Scheme 1. Proposed structures of **1** and **2** are in a good agreement with the literature data [36, 37]. In **1**, cobalt atom display Co(III) oxidation states while in **2** the nickel atom show Ni(II) oxidation states. Isolated crystalline precipitates were air-stable, partially soluble in water, soluble in polar (e.g. DMSO, $CHCl_3$, CH_2Cl_2, CH_3CN), and insoluble in non-polar solvents (e.g. toluene, benzene).

Scheme 1.

IR spectra of the **LH_2**, **1**, **2**, and **3** are given within the Supplementary Material (Figs S1-4) and summarized in Table 1. The absorption broad band located at app. 3230 cm^{-1} in the

spectra of the free ligand originates from OH oxime group. Its noticeable disappearance within the same region in the spectra of **1-3**, indicates potential (O–H...O) band formation [38]. The characteristic imine C=N bond observed at 1621.7 cm^{-1} in the ligand spectra demonstrated considerably stronger intensity in comparison to the shifted, less intense vibration of **1** and **2** located at app. 1600 cm^{-1}. Moreover, the azomethine oxime stretching band appeared in the spectra of newly synthesized complexes in the region from 1520 cm^{-1} to 1510 cm^{-1} pointing out the involvement of oxime nitrogens. The proposed binding mode is further supported by obvious intensity increasing of N – O band and minor negative shifts of **1-3** in comparison to the $\mathbf{LH_2}$ [30]. Finally, in the area below 550 cm^{-1}, a novel set of three bands was observed for all three complexes, which were attributed to M – N, and M – O vibrations [39]. Thus, assigned IR spectra to propose coordination mode of metal center(s) via four nitrogen atoms of the imine-oxime type of the ligand, $\mathbf{LH_2}$.

Table 1. IR spectral data of $\mathbf{LH_2}$, **1, 2** and **3**

	ν/ cm^{-1}					
Compound	OH	-C-H (arom.)	C = N (imine)	C = N (oxime)	N-O (oxime)	M – N and M – O
$\mathbf{LH_2}$	3229.6 (oxime)	2942.2	1621.7	not observed	1133.5	-
1	2932.6 (water)	2901.7	1603.3	1518.8	1134.2	516.4, 482.6, 457.8
2	2942.2 (water)	2920.5	1604.5	1511.3	1087.2	524.2, 488.5, 439.2
3	2943.1 (water)	2918.5	1625.4	1520.1	1087.1	519.9, 478.1, 448.4

ESI–MS spectra of **1** clearly detected two fragmentation ions (fig. S5). The first specie is observed at m/z = 375.099 and assigned to $[M – Cl]^{2+}$ fragment with a distinctive isotope pattern of Co^{3+} complex. The positive match between the expected isotope pattern of **1** and the experimentally observed signal provided a clear evidence for peak assignment. The second, single-charged peak is located at m/z = 340.130 originating from $[M − 2Cl]^{+}$ fragment. The same

fragmentation trend was noticed for **2** with a $[M - ClO_4]^+$ fragment at m/z = 339.132 also displaying the isotope pattern match (fig. S6).

3.2. Electrochemical behavior of complexes 1-3

The electrochemical behavior of the tested complexes was investigated in the water-based solution using sodium chloride as a supporting electrolyte at the concentration of 0.1 M, while complexes were at a concentration of 1 mM. Obtained voltammograms are shown in Fig. 1. As can be seen, in the anodic scan all complexes showed similar behavior. In the case of the copper complex, four oxidation peaks were observed. Peaks at potential higher than 0 V belongs to the oxidation of the first C = N group – higher oxidation potential around 1.4 V (peaks visible in the Cu – and Co – complexes), that in the region 0.6 to 0.8 V is attributed to oxidation of second C = N group (peaks visible in the Cu – and Ni – complexes). In the case of Co – complex oxidative wave does not have its counterparts in the return scan, while in the case of Ni – and Cu – complexes, oxidation of second C = N group at lower potential is followed with corresponding reduction peaks. However, the ratios I_{pa}/I_{pc} is much lower than 1, indicating quasi-reversible electrochemical behavior for this group.

At lower potential (below 0 V) obtained peaks belong to the metal-centered electron transfer reactions. In the case of the Cu complex, one reduction peak was observed at a potential of around – 0.35 V which belongs to the reduction of Cu^{2+} to Cu^{1+}, with a corresponding oxidation wave. The second oxidation peak that appears at a potential of around 0 V is the oxidation of Cu^{2+} to Cu^{3+}. In the case of Ni – complex, only one reduction peak was observed, which is attributed to the Ni^{2+}/Ni^{1+} redox reaction. Contrary to this, Co – complex showed two irreversible reductive waves, where the first one is attributed to the Co^{3+} reduction to the Co^{2+} and the second, at a lower potential, to the Co^{2+} reduction to Co^{1+}.

Figure 1.

3.3. DNA and HSA binding studies using spectrophotometric and electrochemical tests

For investigation of DNA binding interaction of **1**, **2**, and **3**, two experimental approaches were used: spectrophotometric and electrochemical studies. Results are given in Fig. 2 A – F. Increasing amounts of all three studied complexes the spectra of DNA showed similar behavior.

In all cases, spectrums exhibit hyporchromism followed by bathochromic shift. Comparing complexes incidents in hypochromisms and red shifts, it can be observed that there are no significant differences in their behavior and that all complexes bind to the DNA chain through intercalative interaction. An increase in the amount of added complexes is followed by a corresponding decrease in DNA adsorption. Obviously, the highest rate of binding was obtained with Cu – complex, where absorbance decrease was fastest. Additional peaks that occur after the addition of complexes belong to their absorbance spectra.

Similar to these studies, electrochemical spectra confirm the binding of all three complexes to DNA (Fig. 2A, 2C, and 2D). The constant decrease in the peak's currents, obtained from adenine and guanine from the DNA chain, was recorded after the addition of all three testing complexes in the DNA solution. The occurrence of additional peaks in the voltammograms are followed by the increase in the amount of added complexes, and, based on their electrochemical behavior, are attributed to the C = N group's oxidation. Positive shifts in the peak potential in the DNA solution with the addition of each amount of complexes evidences intercalative nature of their binding properties, and these results strongly acknowledged with absorption studies.

Figure 2.

The absorbance of a solution at certain wavelength is a combination of absorbances of each species. Taking DNA as a host (H), metal complex as a guest (G) and assuming 1:1 complexation ratio between them the stability constant could be derived from the following equation:

$$A = A_H + A_G + A_{HG}$$

$$A = \varepsilon_H[H] + \varepsilon_G[G] + \varepsilon_{HG}[HG] \quad (1)$$

Molar absorptivities of H (ε_H) and (ε_G) are experimental constants and could be calculated from the absorption spectra of pure DNA and metal complex, respectively. Equilibrium concentrations of H and G could be calculated as:

$$[H] = [H]_{tot} - [HG]$$

$$[G] = [G]_{tot} - [HG]$$

where $[H]_{tot}$ and $[G]_{tot}$ are the initial, known concentrations of DNA and metal complex. Therefore, to solve equation (1) molar absorptivity of HG complex (ε_{HG}) and equilibrium concentration of HG complex, [*HG*] should be calculated.

The stability constant was calculated from UV-Vis spectroscopy data by using the absorbances at 260 nm for each compound and fitting the equation (1) into the experimental absorbances using Solver function in Microsoft Excel. The ε_{HG} and [*HG*] were iterated until the difference between experimental and predicted *A* reached zero. The formation constant of HG complex (*K*) was then calculated from:

$$K = \frac{[HG]}{[H][G]}$$

The stability constants and molar absorptivity coefficients for 1:1 DNA-ligand complexes are given in Table 2.

Table 2. Stability constants and molar absorptivities of DNA-ligand (HG) complexes

Compound	*K*, M^{-1}	ε_{HG}, $M^{-1}cm^{-1}$
Co-complex (1)	9.82×10^{4}	32700
Ni-complex (2)	4.23×10^{3}	8970
Cu-complex (3)	1.42×10^{4}	16500

Electrochemical and spectrophotometric methods were used to study interactions of the complexes with the human serum albumin. Cyclic voltammetry study was based on redox behavior of the constant amount of the complexes, in the absence and presence of the HSA. The same approach was used for spectrophotometric tests. Results for these measurements are given in Fig. 3 A-F. As it can be seen, in the case of the cobalt and copper complexes there are decreases in the peak current obtained in the case of complexes before the addition of HSA. This is followed by the slight shifts in the peak potentials for both complexes. This can be attributed to the formation of the non-electrically active supra-molecular compound between protein and complex, which reduces free complex concentration. Obtained structure results in the lowering

of the electrochemical activity of the complexes. The same results were obtained with spectrophotometric tests. Interestingly, in the case of the nickel complexes, no differences were observed before and after HSA addition, suggesting no supra-molecular structure formation. However, spectrophotometric studies indicate that there were changes in the spectra before and after HSA addition, confirming the formation of the new structure. It is concludable that resulted compound obtained from Ni complex – HSA possesses the same electroactivity as starting complex.

Figure 3.

3.4. Cytotoxicity study

Figure 4.

The cytotoxic activity of Co(III), Ni(II), Cu(II) complexes of diimine-dioxime ligand, ligand, and cisplatin as positive control are presented in Fig. 4. Cytotoxicity was determined on cultured CT-26 mouse colon carcinoma and human LS174T cancer cell lines for 24 h to the medium containing the respective complexes and ligand in dilution range from 3.90 to 1000.00 μM as well as cisplatin in dilution range from 0.40 to 100.00 μM as a positive control. Ligand and complex **1** did not show cytotoxic activity against CT26 and LS174T cell lines in the examined concentration range (Fig. 4A, and 4B). Complex **2** showed significantly higher cytotoxic activity against CT26 compared to LS174T cell line (Fig. 4C) and IC_{50} value on CT26 cell line was 116.24 μM, and for LS174T was 208.21 μM (Fig. 5). In the present study complex **3** have shown the best cytotoxic activity (Fig. 4D) and IC_{50} value on CT26 cell line was 33.79 μM and on LS174T 65.29 μM (Fig. 5). Cisplatin served as a positive control in this study and IC_{50} of 2.48 μM for CT26 cell line and 14.73 μM for LS174T was found (Fig. 5).

Figure 5.

Conclusion

From the elemental analyses, FT-IR spectroscopy, and mass spectrometry characterizations discussed above, the diimine-dioxime ligand has been shown to act as a tetradentate which coordinates via the nitrogen atoms of the oxime and imine groups with metal ions and to form, except with copper, new mononuclear complexes of nickel and cobalt, $[Ni(LH)]ClO_4$] and $[Co(LH)Cl_2]$. The binding reaction of all complexes with DNA showed that all complexes bind to the DNA chain over intercalative interaction. After testing the binding of all complexes with HSA, it can be concluded that only resulted in a compound obtained from Ni (II) complex – HSA possesses the same electroactivity as starting complex. In MTT assay studies, the Cu(II) complex of diimine-dioxime ligand clearly demonstrates the cytotoxicity effects against CT-26 and LS174T cancer cells and the potential to become a novel antitumor agent for humans.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Figure captions

Scheme 1. Synthetic route for **1** and **2**.

Figure 1. Electrochemical behavior of **1**, **2** and **3** at glassy carbon working electrode

Figure 2. A) Cyclic voltammograms for pure DNA and after addition of Co – complex; B) Absorption spectra for pure DNA and after addition of Co – complex; C) Cyclic voltammograms for pure DNA and after addition of Ni – complex; D) Absorption spectra for pure DNA and after addition of Ni – complex; E) Cyclic voltammograms for pure DNA and after addition of Cu – complex; D) Absorption spectra for pure DNA and after addition of Cu – complex;

Figure 3. Cyclic voltammetry and spectrophotometric spectra of the Co-, Cu- and Ni-complexes before and after HSA addition.

Figure 4. The cytotoxic activity of LH_2, Co(III), Ni(II), Cu(II) complexes, and cisplatin as a positive control.

Figure 5. IC_{50} values for Ni(II), Cu(II) complexes, and cisplatin as a positive control.