Scientific paper

Synthesis, Characterization and DNA Cleaving Studies of New Organocobaloxime Derivatives

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Abstract

Dioxime ligand (H_2L) was synthesized by condensation reaction between 4-biphenylchloroglyoxime and 4-chloroaniline. The metal complexes of the types, $[Co(HL)_2(i\text{-}Pr)Py]$, $[CoL_2(i\text{-}Pr)PyB_2F_4]$ and $[CoL_2(i\text{-}Pr)Py(Cu(phen))_2](ClO_4)_2$ [H_2L = 4-(4-chlorophenylamino)biphenylglyoxime; phen = 1,10-phenanthroline; *i*-Pr = isopropyl; Py = pyridine] were synthesized and characterized by elemental analysis, FT-IR, ^1H NMR and magnetic susceptibility, conductivity measurements. The results of elemental analyses, IR and NMR confirmed the stoichiometry of the complexes and the formation of ligand frameworks around the metal ions. The magnetic moment measurements of the complexes indicated that the complexes are diamagnetic (low-spin d^6 octahedral) except trinuclear complex. Furthermore the interaction between the dioxime ligand and its complexes with DNA has also been investigated by agarose gel electrophoresis. The trinuclear Cu_2Co complex with H_2O_2 as a cooxidant exhibited the strongest DNA cleaving activity.

Keywords: Organocobaloxime; BF_2^+ bridged, trinuclear, DNA cleavage, by thermal

1. Introduction

Vitamin B_{12} or cyanocobalamin is a diamagnetic six-coordinate cobalt(III) complex containing a macrocyclic corrin ring and it is the first natural product found to contain a metal. Coenzyme form of vitamin B_{12} is also a natural organometallic complex and contain a metal-carbon bond.[1] Studies on cobalt(III) complexes of dioxime ligands (cobaloximes) have received considerable attention in view of as model compounds for the coenzyme vitamin B_{12}[2–4] as well as to their usefulness as catalysts in many chemical processes.[5–7] However, at present, a few articles about DNA-cleavage studies of cobaloximes are reported.

Deoxyribonucleic acid (DNA) offers chemists a very powerful tool. The detection of specific DNA sequences provides the fundamental basis for monitoring a wide variety of genetic diseases, viral infections and infectious diseases. Moreover, an understanding of how small molecules interact with DNA is potentially useful in the design of new drugs and diagnostic reagents. DNA biosensors based on nucleic acid recognition processes have received considerable attention in rapid and inexpensive DNA assays.[8–10] In order to find anticarcinogens that can recognize and cleave DNA, people synthesized and developed many kinds of complexes. Among these complexes, metals or ligands can be varied in an easily controlled way to facilitate the individual applications.[11–14] The interactions between DNA and octahedral complexes with rigid bidentate ligands, such as 1,10-phenanthroline or 2,2'-bipyridyl, have been widely investigated subject due to their potential application in the molecular recognition of nucleic acids.[15–17] 1,10-Phenanthroline copper complexes and their derivatives have been attracted great attention due to their high nucleolytic efficiencies,[18–23] which are able to break the DNA chain in the presence of H_2O_2 and reducing agents. These complexes have also been broadly used as foot printing agents of both proteins[24] and DNA[25] probes of the dimensions of the minor groove of duplex structures, and identifiers of transcription starting sites.[13,26] Zhang group[27] also studied the interaction mechanism between 1,10-phenanthroline cobalt(II) complex $[Co(phen)_2ClH_2O]Cl$ and salmon sperm DNA. Electrochemical and spectroscopic studies on the

interaction between tetracoordinate macrocyclic cobalt(III), copper(II) and nickel(II) complexes and DNA were also performed by Zhang et al. to find highly efficient ligands for hepatic asialoglycoprotein receptor (ASGPR).[9,28] On the other hand, only a few studies on the organometallic species have been previously reported.[29–31] But we have not found in the literature an example of the interaction of DNA with organocobaloximes.

Therefore, we thought it worth to synthesize new organocobaloxime derivatives and to investigate their interactions with plasmid DNA (pBR322 DNA) employing gel electrophoresis. In this paper, we report the synthesis and structural assignment of a series of new cobalt(III) glyoximato complexes using 4-(4-chlorophenylamino)biphenylglyoxime, pyridine as axial base and isopropyl as alkyl. Additionally we have prepared BF_2^+-bridge containing complex by replacing of the bridging protons of the cobalt(III)-dioxime complex with BF_2 group and trinuclear Cu_2Co complex using 1,10-phenanthroline.

2. Experimental

Materials and methods: All chemicals used in this work were commercially pure compounds and used as received. Acetonitrile (ACN) used as a solvent was dried before use.[66] 4-Biphenylchloroglyoxime and 4-(4-chlorophenylamino)biphenylglyoxime were prepared according to Karipcin *et al.*[32–33]

Physical measurements: The elemental analyses and metal contents were performed by using on a LECO 932 CHNS analyzer and a Perkin Elmer Optima 5300 DV ICP-OES Spectrometer. The IR spectra (4000–400 cm^{-1}) were recorded as KBr discs using a Schimadzu IRPrestige-21 FT-IR Spectrophotometer. ^1H NMR spectra were recorded in $CDCl_3$ using a Bruker Avance 400 NMR spectrometer with Me_4Si as an internal standard. Magnetic susceptibility measurements were carried out using a Sherwood Scientific Magnetic Susceptibility Balance (Model MX1) at room temperature. The electrical conductivities were obtained on an Optic Ivymen System conductivity meter. Melting points were determined using an Electrothermal model IA 9100.

DNA cleavage: For the agarose gel electrophoresis experiments, 0.25 μg/mm^3 supercoiled pBR322 DNA (0.5 mm^3) was treated with 1.5 mm^3 of 1 mM the tested the ligand and its complexes in DMF and 2 mm^3 of 0.1M Tris–HCl (pH 8.0) buffer in the absence and presence of 4 mm^3 of 5.0 mM hydrogen peroxide as a co-oxidant reagent. After incubation at 37 °C for 2 h, 1 mm^3 of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol in H_2O) was added to each tube and the mixed solution was loaded on 1% agarose gel. The electrophoresis was carried out for 1.5 h at 100 V in TBE buffer (89 mM Tris–borate, pH 8.3, 2.5 mmol/dm^3 EDTA). Gels were stained with ethidium bromide (1 mg/cm^3) for 10 min prior to being photographed under UV light. The efficiency of the DNA cleavage was measured by determining the ability of the complex to form linked circular (LC) or nicked circular (NC) DNA from its supercoiled (SC) form by quantitatively estimating the intensities of the bands using the DNR Minibis Pro Gel Documentation System. The fraction of each form of DNA was calculated by dividing the intensity of each band by the total intensities of all the bands in the lane.

Synthesis: *Synthesis of the ligand (H_2L)* **(1):** 4-(4-Chlorophenylamino)biphenylglyoxime ligand was prepared according to Karipcin *et al.*[32–33] The dioxime ligand has been obtained by the reaction of 4-chloroaniline (2.2 mmol; 0.281 g) with 4-biphenylchloroglyoxime (2 mmol, 0.55 g) in the presence triethylamine (309 μL, 2.2 mmol). 4-Chloroaniline and triethylamine dissolved in 10 mL methanol were slowly added to a suspension of 4-biphenylchloroglyoxime in 50 mL methanol over 15 min. The reaction mixture was stirred further for 5-6 h, then diluted 100 mL water. The resulting precipitate was filtered and then recrystallized from ethanol-water (1:4). The product was filtered, washed several times with water and dried. Yellow powder, mp. 85°C, was isolated in 90% yield; IR (KBr disc, cm^{-1}): 3357(N-H), 3202(O-H), 3030(C-$H_{(arom)}$), 2894(C-$H_{(aliph)}$), 1596,1633(C=N), 1493(C=C), 949 (N-O); ^1H-NMR (δ, ppm): ^1H-NMR (DMSO-d_6, δ, ppm): 10.64 s (1H, O-H), 11.80 s (1H, O-H), 6.98–8.18 m (13H, Ar-H), 6.84 s (1H, NH); Anal. Calc. for $C_{20}H_{16}N_3O_2Cl$ (%): C, 65.66; H, 4.38; N, 11.49, Found (%): C, 66.17; H, 4.59; N, 11.15.[32]

Synthesis of organocobaloxime [Co(HL)$_2$(i-Pr)Py] **(2):** A modification of the method used by Yamazaki *et. al.* was employed for the preparation of the complexes.[34] $CoCl_2 \cdot 6H_2O$ (0.95 g, 4 mmol) and the ligand (8 mmol; 2.93 g H_2L) were stirred in methanol (35 mL), and dry nitrogen was passed through the mixture for 0.5 h. An aqueous solution of sodium hydroxide (0.32 g, 8 mmol, 2 mL) was added to the mixture, followed by pyridine (323 μL, 4 mmol). The mixture was cooled to 0 °C and aqueous solution of sodium borohydride (0.38 g, 10 mmol, 2 mL) was added. After 10 min., 2-bromopropane (376 μL, 4 mmol in 2 mL of diethyl ether) was added dropwise to the reaction mixture. The reaction mixture was stirred for 5 h in a nitrogen atmosphere and in the dark. Then the mixture poured into 100 mL of ice-cold water containing a few drops of pyridine. The precipitate was filtered, washed with water and dried over P_2O_5. Brown complex, mp. 285 °C, was isolated in 84% yield; IR (KBr disc, cm^{-1}): 3446(N-H), 3368(O-H), 3056(C-$H_{(arom)}$), 2933(C-$H_{(aliph)}$), 1575, 1593(C=N), 1489(C=C), 1091(N-O), 500(Co-N); ^1H-NMR ($CDCl_3$, δ, ppm): 9.72 s (2H, O-H…H), 8.45 d (J = 5 Hz, αH-Py, 2H), 8.03 t (J = 4 Hz, γH-Py, 1H), 7.60 t (J = 4 Hz, βH-Py, 2H), 7.10-7.58 m (26H, Ar-H), 7.03 s (2H, NH), 1.98 m (1H, Pr), 1.26 d (J = 5 Hz, 6H, Pr); molar conductivity, Λm (DMF solution, Ω^{-1} cm^2 mol^{-1}): 20; diamagnetic; Anal. Calc. for $C_{48}H_{42}N_7O_4CoCl_2$ (%): C,

63.26; H, 4.61; N, 10.76, Co, 6.48, Found (%): C, 63.30; H, 4.51; N, 10.54; Co, 6.14.

Synthesis of BF_2^+ bridged complex [Co(L)$_2$(i-Pr)Py-B$_2$F$_4$] **(3):** A modification of the method used by Moore *et. al.* was employed for the preparation of the complexes.[35] A large excess of $C_2H_6O{\cdot}BF_3$ (280 μL, 3 mmol) was added to [Co(HL)$_2$(*i*-Pr)Py] (0.5 mmol; 0.455 g) that was sealed in a flask under N_2. After the suspension was stirred for 5 min, 100 mL ACN and Et_3N (0.5 mL in 20 mL ACN) were added in succession. The suspension, sonicated for 10 min to break up large particles, was stirred overnight in the dark and N_2. Then the solution was allowed to stand at –18 °C overnight. After evaporation most of ACN under a reduced pressure and was added excess of *iso*-propyl alcohol. The precipitate was filtered and dried over P_2O_5. Dark brown complex, mp. 260 °C, was isolated in 46% yield; IR (KBr disc. ν, cm^{-1}): 3366(N-H), 3056 (C-H$_{(arom)}$), 2929(C-H$_{(aliph)}$), 1593(C=N), 1489(C=C), 1010(N-O), 520(Co-N), 1092(B-O), 1036(B-F); ^{1}H-NMR (δ, ppm): 7.68–7.08 m (31H, Ar-H), 7.04 s (2H, NH), 1.98 m (1H, Pr), 1.54 d (J = 6 Hz, 6H, Pr); molar conductivity, Λm (DMF solution, Ω^{-1} cm^2 mol^{-1}): 30; diamagnetic; Anal. Calc. for $C_{48}H_{40}N_7O_4B_2F_4CoCl_2$ (%): C, 57.29; H, 4.01; N, 9.74, Co, 5.86, B, 2.15, Found (%): C, 57.33; H, 4.39; N, 9.90; Co, 5.60, B, 2.08.

Synthesis of trinuclear complex [Co(L)$_2$(i-Pr)Py (Cu-Phen)$_2$](ClO$_4$)$_2$ **(4)**: A modification of the method used by Kılıç *et. al.* was employed for the preparation of the complexes.[36] Et_3N (20 μL, 0.125 mmol) in 50 mL ethanol was added to [Co(HL)$_2$(*i*-Pr)Py] (0.255 mmol; 0.233 g) where was in a flask and the mixture was stirred for 1.5 h. The solution of $Cu(ClO_4)_2{\cdot}6H_2O$ (0.21 g, 0.5 mmol) in ethanol and 1,10-phenanthroline monohydrate (0.122 g, 0.5 mmol) was successively added to the mixture, then it was boiled under reflux in the dark for 5.5 h. The precipitate was filtered, washed several times ethanol and dried over P_2O_5. Brown complex, mp. 260 °C, was isolated in 16% yield; IR (KBr disc, cm^{-1}): 3447(N-H), 3063(C-H$_{(arom)}$), 2937(C-H$_{(aliph)}$), 1560,1587(C=N), 1488(C=C), 1009(N-O), 511(Co-N), 624(ClO_4); molar conductivity, Λm (DMF solution, Ω^{-1} cm^2 mol^{-1}): 151; μ_{eff} = 0.74 B.M; Anal. Calc. for $C_{72}H_{56}N_{11}O_{12}Cu_2CoCl_4$ (%): C, 54.16; H, 3.51; N, 9.65, Co, 3.69, Cu, 7.96, Found (%): C, 54.28; H, 3.38; N, 9.56; Co, 3.64, Cu, 7.85.

3. Results and Discussion

The dioxime ligand was prepared by reaction between 4-biphenylchloroglyoxime and 4-chloroaniline in the presence triethylamine.[32–33] The complexes of the types, [Co(HL)$_2$(*i*-Pr)Py], [CoL$_2$(*i*-Pr)PyB$_2$F$_4$] and [CoL$_2$(*i*-Pr)Py(Cu(phen))$_2$](ClO$_4$)$_2$ were synthesized by reacting with the ligand in the presence of appropriate metal salts and reagents. The formation of the ligand and its complexes, was deduced on the basis of results of elemental analyses, characteristic bands in the FT-IR, resonance signals in the ^{1}H NMR spectra, conductance and magnetic measurements as well as the thermal analysis (TG/DTG). The analytical data of the isolated solid complexes are in good agreement with the proposed structure. The solid complexes are stable in air and insoluble in common organic solvents but soluble in DMF and DMSO. The molar conductance data of the mononuclear complexes **(2** and **3)** in DMF are 20 and 30 $\Omega^{-1}cm^2mol^{-1}$, respectively, which indicated their non electrolyte nature.[37–38] Complex **4** behaves as ionic compound and its molar conductance values is 151 $\Omega^{-1}cm^2mol^{-1}$. This value indicated that the trinuclear complex containing perchlorate ions behaves as 1:2 electrolyte,[22] consistent with the formulae from elemental analysis. From all of the above observations, the structures of the complexes (**2–4**) are given as Figures 1–3. Various attempts to develop the crystals suitable for X-ray diffraction studies such as slow diffusion and crystallization using different solvent mixtures were unsuccessful.

Infrared spectra: The IR spectra (4000–400 cm^{-1}) of the ligand and its metal complexes absorption bands characteristics of various functional groups of macrocyclic moiety providing information regarding the formation of macrocyclic ligands and their coordination mode in the complexes. The IR spectrum of ligand, the C=N stretching frequencies are in the 1596–1598 and 1635–1636 cm^{-1} region and N–O stretching frequencies are in the 949–953 cm^{-1} region as reported for similar ligands.[32–34] The ap-

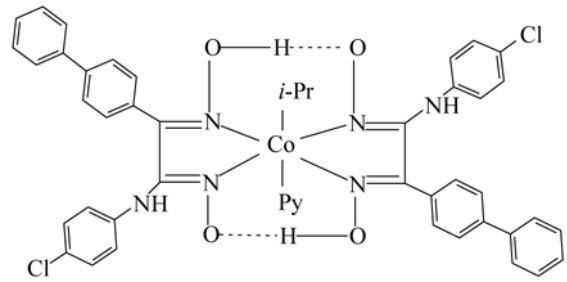

Figure 1. The mononuclear complex with the dioxime ligands

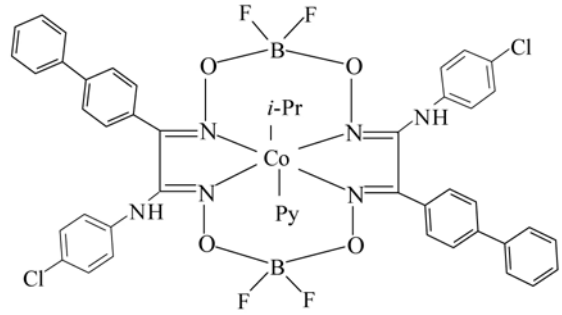

Figure 2. The mononuclear BF_2^+ bridged complex with the dioxime ligands

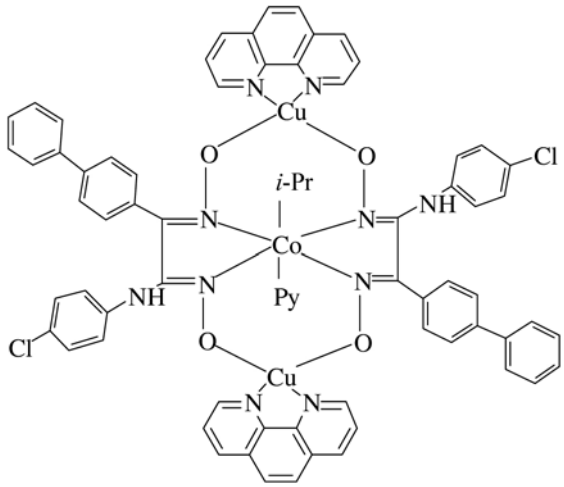

Figure 3. The trinuclear complex with the dioxime and 1,10-phenanthroline ligands

pearance of two bands for the C=N groups indicate the asymmetrical nature of the free ligands.

The bands are assigned to the ν(C=N) stretching frequency shift to 1560–1575 and 1587–1593 cm^{-1} in the complexes. Burger *et al.* reported on the basis of the frequency shift of the C=N vibration that the lower the C=N vibration frequency, the stronger the metal → N=C donor π-bond.[39] The results suggest that the increase in electron density on the cobalt causes the increase of back donation from cobalt to nitrogen atoms of the dioxime ligands, resulting in the increase in the conjugation of the five membered chelate rings. Similar trends have been reported in the literature for the cobaloxime derivatives.[34,39–40] This is further supported by the appearance of a new medium intensity band in the region 500–520 cm^{-1} assignable to Co–N stretching frequency. The ν(N–O) stretching frequencies shift to 1009–1091 cm^{-1} in the complexes. The coordination of axial electron donating base to Co atom causes the increase in electron density in Co atom. This facilitates the back donation from Co to the nitrogen atoms of dioximato ligands, resulting in the increase in electron densities in C=N and N–O bonds. The increase in electron density in N–O bonds causes the stronger hydrogen bridges of O–H···O and the higher frequency shifts of N–O stretching vibrations. Most of the bands appear as medium to strong sharp bands. The occurrence of well-defined sharp bands indicates that there is coordination between the metal and the lone pairs of electrons on the nitrogen.[34,41–44]

In the mononuclear complex **(2)**, the ν(O–H) band due to O–H···O hydrogen bridges in the ligand is assigned at 3368 cm^{-1} and it appears as a very broad band. In complex **3**, this broad band disappeared upon insertion of BF_2 groups with the simultaneous appearance of peaks 1092 and 1035 cm^{-1} for the B–O and B–F resonances, respectively.[45–47] The IR spectrum of the trinuclear complex **(4)** did not show the ν(O–H) bands. Trinuclear complex **(4)** shows a strong band at 624, which is typical for perchlorate groups.[22,48]

^{1}H NMR spectra: The ^{1}H NMR data for the ligand and its mononuclear Co(III) complexes **(2–3)** were recorded in DMSO-d6 and used as important evidence for the assigned structures. The ^{1}H NMR spectra of the complexes show a singlet at 9.72 (only complex **2**) and 7.03–7.04 ppm range assigned for hydrogen bonded OH and NH protons, respectively. A multiplet observed in the range 7.08–7.68 ppm may be attributed to the aromatic ring protons.[49–51]

In the ^{1}H NMR spectrum of complex **2**, the protons of the attached pyridine may readily be identified in the range δ = 7.60–8.45 ppm (α, β and γH of pyridyl group). But the BF_2^+ bridged complex **3**, pyridine protons appear as multiplet signal with the hydrogen of the aromatic rings in the same region (7.68–7.08 ppm). In both complexes **(2, 3)**, the propyl protons appear as the expected a doublet (1.98 ppm) and a multiplet (1.26–1.54 ppm). However, the disappearence of oxime OH signals in the complexes indicates the coordination of ligands to Co(III) ion. These data are in agreement with previously reported for similar compounds and confirmed the suggested formulation of the compounds.[50–53]

Magnetic properties: The room temperature magnetic moments of the complexes showed that mononuclear cobalt complexes **(2,3)** are diamagnetic, which corresponds to the +3 oxidation state of cobalt (low-spin octahedral d^6-system, S = 0). The measured magnetic moment of the trinuclear complex **(4)** is 0.74 μ_B. This value is lower than the spin-only value (2.83 BM), implying the operation of an antiferromagnetic spin-exchange interaction. Because the central cobalt(III) ion with an octahedral environment is diamagnetic, the two trinuclear complexes can be considered as a homodinuclear copper(II)-copper(II) system. Some oximate ligands mediate very strong antiferromagnetic exchange interactions between d^9 Cu(II) centers as reported previously for trinuclear copper complexes with oximate bridge ligands.[36,54–55] Because of the symmetry properties of δ interaction, the $d_{x^2-y^2}$ orbitals in the Cu(II) ions and δ orbitals of the bridging oxygen atoms are involved in the exchange pathway for the unpaired spin density.[54]

Cleavage of plasmid pBR322 DNA: The cleavage of the supercoiled form of pBR322 DNA with the ligand **1**, its mononuclear cobalt(III) **2**, BF_2^+ bridged cobalt(III) complex **3** and heterotrinuclear Cu_2Co **4** complexes was studied in the absence or presence of H_2O_2 as a cooxidant. In fact, we recently published some preliminary results[22,56–57] which demonstrated that some multinuclear copper(II), cobalt(II) and other transition metal complexes were able to promote DNA cleavage under physiological pH conditions (6.1 and 8.0). DNA cleavage was analyzed by monitoring the conversion of supercoiled DNA (Form I) to nicked circular DNA (Form II) and linear DNA (Form III) in aerobic condition. When circular plasmid

DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact supercoil form (form I). If scission occurs on one strand (nicking), the supercoil 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 form I and form II will be generated.[57–59] The results of gel electrophoresis separations of plasmid pBR322 DNA by the ligand (**1**) and its complexes (**2-4**) in the absence or presence of H_2O_2 are depicted in the Fig. 4. Control experiments are applied using only DNA and DNA+H_2O_2. As shown in Fig. 4, incubation of the pBR322 DNA at 37 °C for 2 h with 1.5 μg of the compounds cause the conversion of form I to form II and form III. The cleavage efficiency after incubation for 2 h in the absence of H_2O_2, follows the order: **4 > 3 > 2 > 1**. The cleavage percentages are listed in Table 2. These results indicate that the examined complexes induces very similar conformational changes in supercoiled DNA as conversion of supercoiled form to nicked form than a linear form in a sequential manner. But mononuclear complexes (**2, 3**) are less effective than trinuclear complex (**4**). On the other hand, the pBR322 DNA treated with the ligand (**1**) showed less change in the form levels compared with the complexes. Namely, the ligand alone is less effective. The different DNA cleavage efficiency of the ligand and the complexes may be due to the different binding affinity of the complexes to DNA.[59–61]

Fig. 4 shows agarose gel electrophoresis patterns for the cleavage of plasmid pBR322 DNA after treatment with H_2O_2 as a cooxidant (line 6–10). The degradation of pBR322 DNA is also dependent on cooxidant used. The pBR322 DNA treated with the ligand **1**+H_2O_2 shows only insignificant changes in the form levels compared with the DNA+H_2O_2. Namely, the ligand **1** alone is cleavage-inactive. In the mononuclear complexes (**2**,**3**), the intensities of the circular supercoiled DNA (Form I) bands are found decrease, while that of nicked DNA bands (Form II) and linear DNA bands (Form III) increase apparently (Lane 8, 7, respectively) in the presence of H_2O_2. In the trinuclear Cu_2Co complex (**4**), the cleavage is found to be much more efficient, the supercoiled DNA (Form I) completely disappeared and the linear DNA (form III) apparently appeared in lane 6. These observations suggest that the metal ions, the structure of the complexes and cooxidant play important role in the cleavage. Copper complexes induce efficient cleavage of DNA, because of their high nucleobase affinity and the relatively strong Lewis acidity of Cu(II) ions.[62–63] Recently, the 1,l0-phenanthroline-copper(II) complex has been shown to cleave DNA in the presence of oxygen. However, for an efficient DNA cleavage the metal cation needs to be positioned in the close proximity of the DNA backbone.[62,64] In this study, the presence of H_2O_2 all the complexes (**2**–**6**) are remarkably degrading the pBR322 DNA. This indicates the necessity of oxygen in the cleavage reactions and oxygen playing a role in the cleavage chemistry.[58–59] Trinuclear Cu_2Co complex (**4**) showed better chemical nuclease activity. These results are similar to that observed for some copper and cobalt complexes as chemical nuclease.[57,60–62,66–67] Further studies are undergoing to clarify the cleavage mechanism.

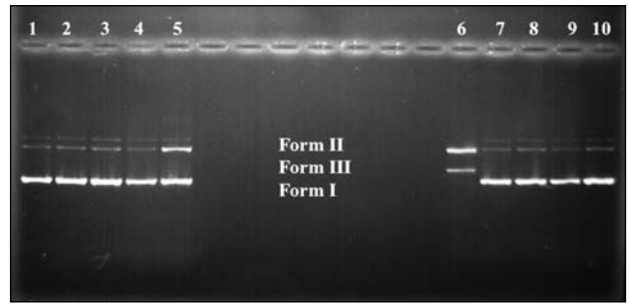

Figure 4. Gel electrophoresis diagram showing the cleavage data of pBR322 plasmid DNA by the ligand and its complexes in DMF-Tris buffer medium (pH 8.0) in air after incubation at 37°C for 2 h. Lane 2–5, pBR322 plasmid DNA + the compounds (**1**, **2**, **3**, **4,** respectively) ; lane 6–9, pBR322 plasmid DNA + the compounds (**4**, **3**, **2**, **1,** respectively) + H_2O_2; lane 1, untreated pBR322 plasmid DNA; lane 10, pBR322 plasmid DNA + H_2O_2.

Table 1. DNA cleavage data of pBR322 plasmid DNA by **1**–**6**

Lane no	Reaction conditions	Form I %SC	Form II %NC	Form III %LC
1	DNA	91.33	2.35	6.32
2	DNA + **1**	85.84	5.04	9.12
3	DNA + **2**	79.53	7.75	12.72
4	DNA + **3**	76.51	9.52	13.97
5	DNA + **4**	60.50	8.26	31.24
6	DNA + **4** + H_2O_2	ND	59.87	40.13
7	DNA + **3** + H_2O_2	64.39	11.09	24.52
8	DNA + **2** + H_2O_2	68.23	10.81	20.96
9	DNA + **1** + H_2O_2	69.38	9.00	21.62
10	DNA + H_2O_2	72.86	9.58	17.56

SC, NC, LC are supercoiled, nicked circular and linked circular, smaller forms of DNA, respectively. ND: not detected.

4. Conclusions

New organocobaloxime derivatives of the type $[Co(HL)_2(i\text{-}Pr)Py]$, $[CoL_2(i\text{-}Pr)PyB_2F_4]$ and $[CoL_2(i\text{-}Pr)Py(Cu(phen))_2](ClO_4)_2$ [H_2L = 4-(4-chlorophenylamino)biphenylglyoxime; phen = 1,10-phenanthroline; *i*-Pr = isopropyl; Py = pyridine] were synthesized and characterized by elemental analysis, ICP-OES, magnetic susceptibility, conductivity measurements, ^1H NMR and FT-IR. The spectral and magnetic susceptibility data conform to the octahedral geometry expected for the mononuclear complexes. There were not much variation of magnetic properties, metal: ligand ratio and geometry of these complexes due to replacement of BF_2 groups by the bridging protons of the dioxime complexes. The conductance data

indicate that these complexes are non-electrolytes and the trinuclear complexes containing perchlorate ions behave as 1:2 electrolytes. In addition we have tested the DNA cleavage activity of the ligand and its complexes. The DNA cleavage results showed that the trinuclear Cu_2Co complex **4** could effectively cleave supercoiled DNA to form nicked and linear DNA. The cleavage in the complexes was found to be much more efficient in the presence of hydrogen peroxide as co-oxidant.

5. Acknowledgments

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Povzetek

Dioksimski ligand (H_2L) smo pripravili z reakcijo kondenzacije med 4-bifenilkloroglioksimom in 4-kloroanilinom. Z dobljenim ligandom smo sintetizirali kovinske komplekse $[Co(HL)_2(i\text{-Pr})Py]$, $[CoL_2(i\text{-Pr})PyB_2F_4]$ in $[CoL_2(i\text{-Pr})Py(Cu(phen))_2](ClO_4)_2$ [H_2L = 4-(4-klorofenilamino)bifenilglioksim; phen = 1,10-fenantrolin; *i*-Pr = izopropil; Py = piridin]. Sintetizirane komplekse smo okarakterizirali z elementno analizo, FT-IR in ^{1}H NMR spektroskopijo ter merjenjem magnetne susceptibilnosti in prevodnosti. Rezultati elementne analize in obeh spektroskopskih metod potrjujejo stehiometrijo kompleksov in obliko ogrodja ligandov okrog kovinskih ionov. Merjenje efektivnega magnetnega momenta kaže, da sta z izjemo trijedrnega kompleksa ostala dva diamagnetna (nizkospinska d^6 oktaedrična oblika). Z elektroforezo na agaroznem gelu smo raziskali interakcije med ligandom in njegovimi kompleksi z DNA. Trijedrni Cu_2Co kompleks s H_2O_2 kot kooksidantom kaže najmočnejšo aktivnost cepitve DNA.