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Structure and DNA/BSA binding study of zinc(II) complex with 4-ethynyl-2,2'-bipyridine

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Abstract: In the present study, a zinc(II) complex with 4-ethynyl-2,2'-bipyridine (ebpy), [Zn(ebpy)Cl_2], was synthesized and characterized by spectroscopic (^{1}H-NMR, IR and UV–Vis) methods and molar conductivity measurement. The crystal structure of the [Zn(ebpy)Cl_2] complex was determined by single-crystal X-ray diffraction analysis, confirming the bidentate coordination of the ebpy ligand through its two nitrogen atoms, while the remaining two coordination sites are occupied by two chloride ions. With the aim to investigate the reactivity of the synthesized zinc(II) complex toward biologically important molecules, its binding affinity to calf thymus DNA (ct-DNA) and bovine serum albumin (BSA) was studied by fluorescence emission spectroscopy. From the obtained results, it can be concluded that [Zn(ebpy)Cl_2] complex binds to bovine serum albumin reversibly, while the combination of ethidium bromide (EthBr) and Hoechst 33258 (2'-(4-hydroxyphenyl)-5-[5-(4-methylpiperazine-1-yl)benzimidazo-2-yl]-benzimidazole) competitive binding study suggests that this complex interacts with ct-DNA through the minor groove binding, which is in agreement with molecular docking study.

Keywords: zinc(II); bipyridine; structural characterization; protein interactions; DNA interactions.

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INTRODUCTION

Zinc represents an essential trace element for all forms of life.[1] In living systems, the role of zinc can be catalytic or structural, but in some DNA interacting metalloproteins, this metal plays both roles.[2] In the human body, zinc is required for the normal function of immune system, for proper wound healing, the sense of taste and smell, and for the normal growth and development.[3] Besides that, this metal has an important role for the normal function of the brain and can modulate its excitability, and it also influences the learning process.[4] It is also important to mention that zinc supplementation is proposed for application in cancer prevention due to its beneficial effects, such as inhibition of angiogenesis and induction of inflammatory cytokines, leading to the apoptosis of cancer cells.[5]

The great importance of zinc has spawned numerous model complexes and significantly enriched knowledge of its coordination chemistry.[2] In living organisms, zinc exists only in +2 oxidation state, whereby Zn(II) ion has a filled d subshell (d^{10} electronic configuration). In complexes, this metal ion can adopt different coordination number and geometry, including tetrahedrally four-coordinated, trigonal-bipyramidal or square-pyramidal five-coordinated, and octahedrally to trigonal prismatic six-coordinated; all these geometries can also be distorted to different extent.[6] Vahrenkamp proposed that Zn(II) ion acts as both soft and hard Lewis acid, what can be seen from the dominance of the ZnN_2S_2 motif (N represents the donor atom of histidine, while S is from cysteine) in enzymes and zinc fingers.[6] Zinc(II) ion forms tetrahedral complexes with halides and oxygen-donor ligands. Different stable zinc(II) complexes are also known with nitrogen-donor ligands, including amines and azaheterocycles, while some complexes with sulfur-donors have unusual structural properties.[2]

In the last few decades, zinc(II) complexes have attracted great attention for the generation of 1D, 2D and 3D infinite structures and frameworks.[2] Beside its significance in coordination and supramolecular chemistry, numerous zinc(II) complexes have been found to be interesting from the point of view of medicinal chemistry, since these compounds have shown antimicrobial,[7] anticancer,[8] antidiabetic,[9] antioxidant[10] and anti-inflammatory[11] activities. Considering this, we have recently synthesized two mononuclear zinc(II) complexes, $[Zn(py\text{-}2py)X_2]$, X is Cl^- or Br^-, with a 2,2'-bipyridine derivative, namely dimethyl 2,2'-bipyridine-4,5-dicarboxylate (py-2py), and investigated their *in vitro* antimicrobial activity and cytotoxicity on the normal human lung fibroblast cells (MRC-5) and the model organism *Caenorhabditis elegans*.[12] It was found that of the two synthesized zinc(II) complexes, the one with chlorido ligands has shown a higher antifungal activity against the studied *Candida* strains, better ability in preventing *C. albicans* ATCC 10231 biofilm formation, and higher quorum sensing inhibitory potential on the two investigated biosensor strains (*Chromobacterium violaceum*

CV026 and *Serratia marcescens* ATCC 27117).[12] Leading by this study, herein, another 2,2'-bipyridine derivative, namely 4-ethynyl-2,2'-bipyridine (ebpy), reacted with $ZnCl_2$ to yield a mononuclear zinc(II) complex, [Zn(ebpy)Cl_2]. This ligand was previously used for synthesis of platinum(II), ruthenium(II) and rhenium(II) complexes conjugated to vitamin B12 as the formulations of anticancer prodrugs against MCF-7 breast cancer cells.[13] Moreover, a *fac*-$[Mn(CO)_3]^+$ complex bearing ebpy conjugated to vitamin B12, has shown unusual dark and light--induced cytotoxicity.[14] The presently synthesized [Zn(ebpy)Cl_2] complex was characterized by spectroscopy (^{1}H-NMR, IR and UV–Vis) and molar conductivity measurement, while its structure was determined by single-crystal X-ray diffraction analysis. The interactions of this complex with bovine serum albumin (BSA) and calf thymus DNA (ct-DNA) were investigated to check its binding affinity toward these biologically important molecules.

EXPERIMENTAL

Reagents

Zinc(II) chloride, ethanol, acetonitrile, dimethyl sulfoxide (DMSO), deuterated dimethyl sulfoxide (DMSO-d_6), phosphate buffer saline (PBS), bovine serum albumin (BSA), calf thymus DNA (ct-DNA), ethidium bromide (EthBr), Hoechst 33258 (2'-(4-hydroxyphenyl)-5--[5-(4-methylpiperazine-1-yl)benzimidazo-2-yl]-benzimidazole) and *n*-octanol were purchased from the Sigma–Aldrich (St. Louis, MO, USA). All these chemicals were of analytical reagent grade and used without further purification.

Measurements

The elemental analysis of ebpy and [Zn(ebpy)Cl_2] for carbon, hydrogen and nitrogen were performed by the Institute for Information Technologies Kragujevac, University of Kragujevac. The ^{1}H-NMR spectra of ebpy and [Zn(ebpy)Cl_2] were recorded at ambient temperature in DMSO-d_6 on a Varian Gemini 2000 spectrometer at 200 MHz. 5.0 mg of both compounds were dissolved in 0.6 mL of DMSO-d_6 solvent, and the obtained solutions were transferred in 5 mm NMR tube. Chemical shifts, δ, were calibrated relative to those of DMSO-d_6 and are given in ppm, while scalar couplings (J) are reported in Hz. The abbreviations for the peak multiplicities are the follows: *s* (singlet), *d* (doublet), *dd* (doublet of doublets), *t* (triplet) and *m* (multiplet). To follow the solution behaviour of [Zn(ebpy)Cl_2], its ^{1}H-NMR spectrum was recorded right after dissolution of the complex in DMSO-d_6 and after 1 and 2 days standing at ambient temperature. The UV–Vis spectra of ebpy and [Zn(ebpy)Cl_2] were recorded on a Shimadzu UV-1800 spectrophotometer over the wavelength range of 1100–200 nm at ambient temperature. The concentration of solution of these compounds in DMSO used for UV––Vis measurements was 7.41×10^{-5} and 8.40×10^{-5} M, respectively. The measurement of UV––Vis spectrum of [Zn(ebpy)Cl_2] was repeated after 24 and 48 h after its dissolution in DMSO. The IR spectra of ebpy and its zinc(II) complex were recorded on a Perkin Elmer Spectrum 2 spectrometer using KBr pellet technique over the range of 4000–450 cm^{-1} (the used abbreviations: *vs* for very strong, *s* for strong, *m* for medium, *w* for weak). The molar conductivity of [Zn(ebpy)Cl_2] complex was determined on a digital conductivity-meter Crison Multimeter MM 41 after its dissolution in DMSO at ambient temperature at concentration of 1.0×10^{-3} M. Jasco FP-6600 spectrophotometer was used for the measurement of the emission spectra for DNA/BSA interactions of [Zn(ebpy)Cl_2] complex.

Analytical and spectral data, as well as additional details, are given in the Supplementary material to this paper.

Synthesis of ebpy

4-Ethynyl-2,2'-bipyridine (ebpy) was synthesized in accordance with the method previously reported.[15] The purity and constitution of the synthesized compound were confirmed by elemental analysis and ^{1}H-NMR spectroscopy (data given in Supplementary material). The obtained data agree with those for the same compound previously reported.[15]

Synthesis of [Zn(ebpy)Cl$_2$] complex

5.0 mL of ethanolic solution of ebpy (1.0 mmol, 180.1 mg) was added dropwise to a solution of an equimolar amount of $ZnCl_2$ (1.0 mmol, 136.3 mg) in 5.0 mL of ethanol under stirring at ambient temperature. The stirring continued for 3 h at ambient temperature, and after that time, the resulted white precipitate was filtered and recrystallized in acetonitrile. The obtained solution was left to slowly evaporate at ambient temperature. Colorless crystals of [Zn(ebpy)Cl$_2$] complex suitable for single-crystal X-ray analysis were formed after 5 days. Yield: 68 % (215.2 mg).

Crystallographic data collection and refinement of the structure

Crystal data and details of the structure determination for [Zn(ebpy)Cl$_2$] are given in Table S-I of the Supplementary material. A suitable crystal was selected and mounted on a loop in oil on a STOE IPDS 2 diffractometer. The crystal of the complex was kept at 250(2) K during data collection. Using Olex2,[16] the structure was solved with the ShelXT[17] structure solution program using intrinsic phasing and refined with the ShelXL[18] refinement package using least squares minimization.

BSA binding experiment

The protein binding study was performed through fluorescence quenching experiment using BSA (20 μM) in phosphate buffer (PBS, pH 7.4). The quenching of the fluorescence emission intensity of BSA at 366 nm was monitored using [Zn(ebpy)Cl$_2$] as a quencher with the increasing amount (up to 50 μM). The fluorescence emission spectra were recorded in the range of 285–500 nm with an excitation wavelength of 280 nm. It is important to note that the fluorescence spectrum of the [Zn(ebpy)Cl$_2$] in PBS was recorded under the same experimental conditions, and no fluorescence emission was observed.

The Stern–Volmer constant (K_{sv}) for BSA interaction of the [Zn(ebpy)Cl$_2$] was calculated using the following equation:

$$F_0/F = 1 + K_q\tau_0 c(\text{complex}) = 1 + K_{sv}c(\text{complex}) \quad (1)$$

In this equation, F_0 and F represent the emission intensities in the absence and presence of the examined quencher, respectively, K_q is the quenching constant, while τ_0, which amounts 10^{-8} s, is the fluorophore lifetime without the quencher.[19]

The data obtained from the fluorescence measurements were also used to determine the number of the binding sites for the complex to BSA (n) and the equilibrium binding constant, K_A, based on the Scatchard equation:[20]

$$\log (F_0 - F)/F = \log K_A + n\log c(\text{complex}) \quad (2)$$

Lipophilicity assay

The flask-shaking method[21] was used for determination of the partition coefficient (log P) as a measure of lipophilicity for [Zn(ebpy)Cl$_2$]. This complex was dissolved in DMSO and added to water/*n*-octanol system. The obtained solution was vortexed for 1 h at ambient tem-

perature and, after that, left to stand for additional 24 h until the separation of the two phases was achieved. The absorbance value was determined from the calibration curve and used for calculation of the concentration of the complex in *n*-octanol phase (c_o) and water phase (c_w). The following equation was applied for calculation of the log *P* value:

$$\log P = \log (c_O/c_W) \quad (3)$$

DNA binding experiment

PBS buffer was used for the preparation of a stock solution of ct-DNA (1.43×10^{-2} M), which concentration was determined from the UV absorbance value at 260 nm and the molar extinction coefficient, $\varepsilon = 6.6\times10^3$ M^{-1} cm^{-1}.[22] Stock solutions of EthBr (10.1 mM), Hoechst 33258 (10 mM) and zinc(II) complex (10 mM) were prepared in DMSO.

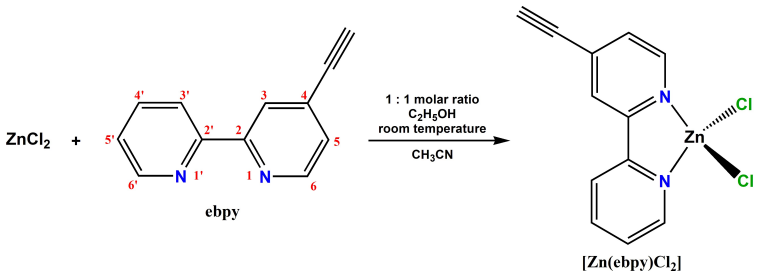
The competitive binding studies were performed at pH 7.4 in PBS, whereas the ratio [ct-DNA]:[EthBr/Hoechst 33258] was 10:1, while the concentration of the complex gradually increased (up to 100 μM). The spectra for the competitive interaction between EthBr and the complex toward ct-DNA were measured in the range of 525–750 nm, with the excitation wavelength of 520 nm, while the range for Hoechst 33258 competitive binding study was 351–750 nm, with the excitation wavelength of 346 nm. The K_{sv}, K_q and K_A constants, and the *n* number were calculated as described above for BSA binding experiment.[19,20]

Docking study

Docking calculations were performed as previously described.[23] The DNA target for the investigated zinc(II) complex was selected from Protein Data Bank (pdb code: 1BNA), as the double stranded (d(CpGpCpGpApApTpTpCpGpCpG) dodecamer with two G≡C rich regions flanking one A=T rich region.[24] Molecular docking calculations were carried out with the AutoDock Vina (version 1.2.0)[25] and the AutoDock4 (version 4.2.6)[26] software packages. The Biovia Discovery Studio Visualizer software (version 21.1.0.20298) was employed to analyze possible interactions between the complex and the DNA target.

RESULTS AND DISCUSSION

The ligand 4-ethynyl-2,2'-bipyridine (ebpy) was prepared in accordance with the method previously described[15] and its purity was confirmed by elemental analysis and ^{1}H-NMR spectroscopy. Zinc(II) complex, [Zn(ebpy)Cl_2], was synthesized by mixing equimolar amounts of $ZnCl_2$ and ebpy in ethanol at ambient temperature (Scheme 1).



Scheme 1. Schematic presentation of the synthesis of [Zn(ebpy)Cl_2]. Numeration of atoms in ebpy was done in accordance with IUPAC recommendations and does not match that applied in the X-ray analysis of zinc(II) complex.

The obtained white precipitate was filtered and recrystallized in acetonitrile to yield the colourless crystals of [Zn(ebpy)Cl_2] suitable for single-crystal X-ray diffraction analysis. In addition, ^{1}H-NMR, IR and UV–Vis spectroscopy and molar conductivity measurement were applied for characterization of the synthesized complex.

Solid state studies

The [Zn(ebpy)Cl_2] complex crystallizes in the monoclinic space group $P2_1/m$. The molecular structure of this complex with the anisotropic displacement ellipsoids and the atom numbering scheme is shown in Fig. 1, while its selected bond lengths (Å) and bond angles (°) with the estimated standard deviations are presented in Table S-II of the Supplementary material.

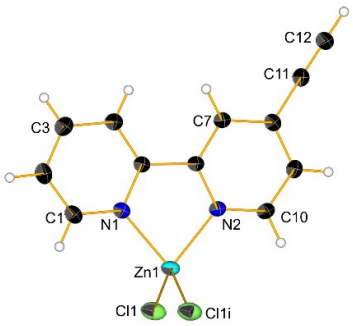


Fig. 1. Molecular structure of [Zn(ebpy)Cl_2] complex. Displacement ellipsoids are drawn at 50 % probability level and H atoms are represented by spheres of arbitrary size. Symmetry code: (*i*) *x*, 1/2–*y*, *z*.

As can be seen in Fig. 1, [Zn(ebpy)Cl_2] complex is a mononuclear species, in which the Zn(II) ion is coordinated by the ebpy acting as a bidentate ligand through its two nitrogen atoms and two chlorido ligands resulting in N_2Cl_2 coordination environment around the metal ion. The complex adopts a slightly distorted tetrahedral geometry, with the value of tetrahedral index τ_4[27] of 0.92. This tetrahedral index can be calculated as:

$$\tau_4 = (360° - (\beta + \alpha))/141° \quad (4)$$

where β and α are the largest angles around the Zn(II) ion (β = Cl1^i–Zn1–Cl1= = 116.77(4)° and α = N1–Zn1–Cl1^i = 113.93(3)°), while τ_4 value for the ideal tetrahedral geometry amounts 1.[27] This distortion from the ideal tetrahedral geometry can be attributed to the relatively low bite angle of ebpy of 80.46(10)°. The bond lengths and angles presented in Table S-II for [Zn(ebpy)Cl_2] are in accordance with those reported in the Cambridge Crystallographic Database (version 5.44, April 2023)[28] for the structurally similar zinc(II) complexes with bidentately coordinated 2,2'-bipyridine and its substituted derivatives. In the crystal structure of the [Zn(ebpy)Cl_2] complex, stacking interactions (π–π and weak C–H···X) are present and can contribute to the stabilization of the structure.

IR spectroscopy is a useful tool for identification of functional groups in a molecule, since each chemical bond has a specific energy absorption band. The IR spectrum of [Zn(ebpy)Cl_2] complex in the range of 4000–450 cm^{-1} agrees with its structure determined by single-crystal X-ray diffraction analysis. In this spectrum, the bands attributed to the characteristic vibrations of ebpy coordinated to the Zn(II) ion can be detected. Thus, the bands at 3215, 2109 and 623 cm^{-1} in the IR spectrum of the synthesized zinc(II) complex can be ascribed to $\nu(C_{alkyne}–H)$, $\nu(C\equiv C)$ and $\gamma(C_{alkyne}–H)$ vibrations, respectively, suggesting the presence of terminal alkyne (*i.e.*, monosubstituted) in its structure. The other most important bands are those which originate from the vibrations of heteroaromatic rings (analytical data are given in the Supplementary material).

Solution studies

^{1}H-NMR spectra of ebpy and [Zn(ebpy)Cl_2] were recorded in DMSO-d_6 to verify the coordination mode of this ligand to the Zn(II) ion in solution (Fig. S--1). As can be seen from Fig. S-1, the number of signals due to the ebpy in the zinc(II) complex was the same as that of the free ligand. Four resonances at δ 7.67, 8.12, 8.57 and 8.72 ppm are observed in the aromatic region of the ^{1}H--NMR spectrum of [Zn(ebpy)Cl_2] complex, due to C5H/C5'H, C4'H, C3H/C3'H and C6H/C6'H protons, respectively. In most cases, the chemical shifts of these protons are downfield shifted from those of the corresponding protons of uncoordinated ebpy. It is interesting to mention that the $\Delta(^1H)_{coord}$ coordination shift (determined in respect to uncoordinated ebpy) for C3H/C3'H protons (+0.18 ppm) is larger than those for C6H/C6'H protons (+0.00 ppm), which are adjacent to the pyridine nitrogen binding atoms (Fig. S-1). These protons appear at δ 8.39 and 8.72 ppm in the ^{1}H-NMR spectrum of the uncoordinated ebpy ligand, respectively. In the aliphatic region of the ^{1}H-NMR spectrum of [Zn(ebpy)Cl_2], a signal due to the acetylene proton is present at 4.84 ppm and it is shifted downfield after ebpy ligand coordination to the Zn(II) ion ($\Delta(^1H)_{coord}$ = +0.15 ppm). It is important to note that there are no visible changes in ^{1}H-NMR spectrum of [Zn(ebpy)Cl_2] recorded 2 days after its dissolution in DMSO-d_6 in respect to that recorded immediately (Fig. S-2), confirming the bidentate coordination of ebpy to the Zn(II) ion over this time period.

Non-electrolytic nature of [Zn(ebpy)Cl_2] in DMSO solution was confirmed by molar conductivity measurement.[29] Furthermore, the value of the molar conductance of this complex determined immediately after its dissolution (4.82 Ω^{-1} cm^2 mol^{-1}) remains almost the same for 2 days.

The UV–Vis spectrum of [Zn(ebpy)Cl_2] was recorded in DMSO at ambient temperature and compared with the corresponding spectrum of ebpy ligand. By comparison of these two spectra, it can be concluded that the absorbance peak at 283 nm and a shoulder at 308 nm in the UV–Vis spectrum of [Zn(ebpy)Cl_2] are

due to the intra-ligand charge transfer transitions.[30] With the aim to additionally confirm the stability of [Zn(ebpy)Cl_2] complex in DMSO, the measurement of its UV–Vis spectrum was repeated 24 and 48 h after its dissolution (Fig. S-3). As can be seen, the shape of spectra, position, and intensity of the absorption maxima of [Zn(ebpy)Cl_2] did not change for the investigated period, additionally confirming its solution stability.

BSA binding study

Serum albumin, the most abundant protein in the bloodstream, has a significant role in the transport of various compounds to the target organs and tissues. In the present study, fluorescence emission spectroscopy was used for studying the interaction of the [Zn(ebpy)Cl_2] with bovine serum albumin, BSA. After addition of this complex to BSA solution (20 μM), a decrease of the BSA fluorescence intensity was observed (33 % of its initial intensity), suggesting that [Zn(ebpy)Cl_2] complex interacts with the studied protein (Fig. 2). As a result of this interaction, a blue shift of 3 nm in the emission maximum was observed, being in accordance with the binding of zinc(II) complex with the hydrophobic active site of BSA.[31]

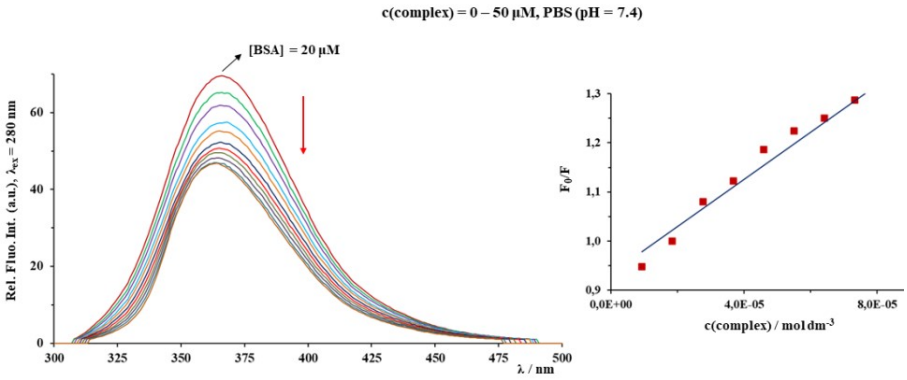


Fig. 2. Fluorescence emission spectra of BSA in the presence of an increasing amount of [Zn(ebpy)Cl_2]. The red arrow shows the changes of fluorescence intensity after the addition of this complex. Stern–Volmer plot of F_0/F *vs.* *c*(complex) is inserted.

The fluorescence quenching data for [Zn(ebpy)Cl_2] was analyzed using the Stern–Volmer and Scatchard equations given in the Experimental section. The determined values of K_{sv}, K_q and K_A constants, and *n* number of binding sites of the zinc(II) complex per BSA are given in Table I. These values indicate that [Zn(ebpy)Cl_2] has a moderate affinity toward BSA, whereas one site per protein is available for its binding. The BSA binding constants of the recently synthesized [Zn(py-2py)X_2] complexes, X is Cl^- or Br^-, py-2py is dimethyl 2,2'-bipyr-

idine-4,5-dicarboxylate,[12] are 3.5 – 100 fold higher than those calculated for [Zn(ebpy)Cl_2] complex. Considering that the value of K_q constant for [Zn(ebpy)Cl_2] complex is higher than the limiting diffusion rate constant of the biomolecule (2×10^{10} M^{-1} s^{-1}),[32] it can be concluded that the mechanism of BSA fluorescence quenching is static. Although the value of K_A constant for the investigated complex shows its affinity for BSA, it is significantly lower than 10^{15} M^{-1}.[33] This indicates that upon arrival to the target site, [Zn(ebpy)Cl_2] will be released from the serum protein.

TABLE I. Values of binding constants of [Zn(ebpy)Cl_2] with BSA

K_{sv} / M^{-1}	Hypochromism, %	K_q / M^{-1} s^{-1}	K_A / M^{-1}	n
$(5.13\pm0.02)\times10^3$	33.5	5.13×10^{11}	2.35×10^3	0.90

Lipophilicity assay

The lipophilicity of a compound, expressed as a partition coefficient (log P) between the hydrophobic octanol phase and the hydrophilic water phase, indicates its ability to be transported through the lipid structures.[21] The log P value for [Zn(ebpy)Cl_2] is 1.35, being in the range of −0.4 to 5.6, reported previously for different therapeutic agents.[34] Besides that, the molecular weight of this complex is lower than 500, and follows the Lipinski's rule of five for orally used therapeutic agents.[35]

DNA competitive binding study

To further investigate the reactivity of [Zn(ebpy)Cl_2] towards DNA as a potential biological target, its interaction with ct-DNA was followed using the fluorescence quenching method in a competitive study with an intercalator ethidium bromide (EthBr) and with a minor groove binder Hoechst 33258 (2'-(4-hydroxyphenyl)-5-[5-(4-methylpiperazine-1-yl)benzimidazo-2-yl]-benzimidazole; Hoe). The emission spectra of ct-DNA–EthBr and ct-DNA–Hoe solutions, in which the ratio [ct-DNA]:[EthBr/Hoe] is 10:1, were recorded after the adding the increasing concentration of the [Zn(ebpy)Cl_2] (0–100 μM). The fluorescence titration spectra are presented in Fig. 3, while the corresponding numerical data calculated by the Stern–Volmer and Scatchard equations are given in Table II.

As it was mentioned above, EthBr intercalates between adjacent base pairs in the ct-DNA double helix, leading to the fluorescence enhancement.[19] After addition of an investigated compound, a decrease in the fluorescence emission intensity of the ct-DNA–EthBr system will be observed if it substitutes EthBr and intercalates into ct-DNA or if it binds to this system and forms a non-fluorescent species.[19] As can be seen from Table II, the value of K_A constant for binding of [Zn(ebpy)Cl_2] to the ct-DNA–EthBr system is significantly lower than that for EthBr ($K_A = 2\times10^6$ M^{-1}),[32] being in accordance with its non-intercalative nature.

This is also in accordance with the very low percentage of hypochromism of only 6% (Table II). On the other hand, the value of K_A constant for binding of [Zn(ebpy)Cl$_2$] to the ct-DNA–Hoe system, with the order being 10^4, shows the possibility of its interaction with ct-DNA *via* minor groove binding. In both cases, the values of K_q constants indicate that the mechanism of fluorescence quenching is static.[32]

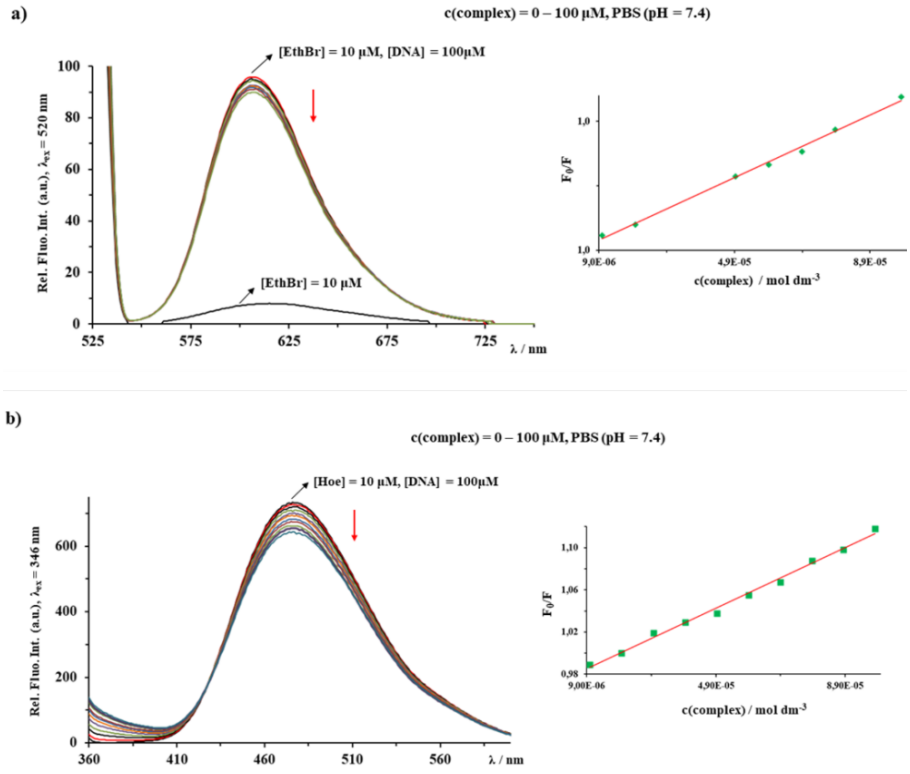


Fig. 3. Fluorescence emission spectra of: a) ct-DNA–EthBr and b) ct-DNA–Hoe systems in the presence of an increasing concentration of [Zn(ebpy)Cl$_2$]. The red arrows show the changes of fluorescence intensity after the adding the zinc(II) complex. Stern–Volmer plots of F_0/F *vs.* *c*(complex) are also inserted.

The non-intercalative nature and minor groove binding of [Zn(ebpy)Cl$_2$] were confirmed by docking calculations using a double stranded d(CpGpCpGpApApTpTpCpGpCpG) dodecamer (Fig. 4). Molecular docking results indicate that minor groove binding is the most stable binding mode for [Zn(ebpy)Cl$_2$]. The binding energy (or docking affinity) of the complex for the

model dodecamer is –6.7 kcal* mol^{-1}. The [Zn(ebpy)Cl$_2$] does not intercalate into base pairs. Its docking mode is not stabilized by π–π stacking interactions, but only by long distance, rather weak coulombic interactions between the Zn(II) ion and the negatively charged phosphates in the DNA backbone.

TABLE II. Values of binding constants of [Zn(ebpy)Cl$_2$] with ct-DNA in the presence of EthBr and Hoe

Binding structure	K_{sv} / 10^2 M^{-1}	Hypochromism, %	K_q / 10^{11} M^{-1} s^{-1}	K_A / M^{-1}	n
ct-DNA–EthBr	5.87±0.01	6.1	0.587	67.6	0.77
ct-DNA–Hoe	14.8±0.1	12.2	1.48	1.09×10^4	1.22

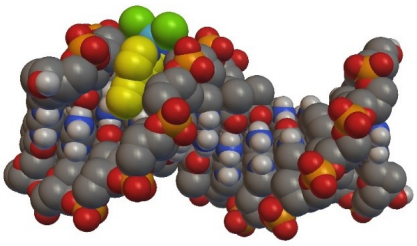


Fig. 4. Space-filling model of computer-generated lowest energy binding pose of [Zn(ebpy)Cl$_2$] with DNA (d(CpGpCpGpApApTpTpCpGpCpG) dodecamer sequence).

CONCLUSION

We have shown that the reaction between equimolar amounts of 4-ethynyl-2,2'-bipyridine (ebpy) and ZnCl$_2$ in ethanol leads to the formation of mononuclear zinc(II) complex, [Zn(ebpy)Cl$_2$]. The crystallographic results revealed that this complex has a slightly distorted tetrahedral geometry with ebpy being bidentately coordinated to the Zn(II) ion, while the remaining two sites are occupied by chlorido ligands. The synthesized [Zn(ebpy)Cl$_2$] complex has an affinity to bind to BSA reversibly, while toward ct-DNA, it behaves as a minor groove binder. Considering the biological importance of zinc(II) complexes and their potential application as metal-based therapeutics, further studies will be aimed on the investigation of antimicrobial and antiproliferative activities of the presently synthesized zinc(II) complex and its structural analogues.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/12428, or from the corresponding author on request. CCDC 2266337 contains the supplementary crystallographic data for this article. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via https://www.ccdc.cam.ac.uk/structures/.

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* 1 kcal = 4184 J

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ИЗВОД

СТРУКТУРА И ИСПИТИВАЊЕ DNA/BSA ИНТЕРАКЦИЈА КОМПЛЕКСА ЦИНКА(II) СА 4-ЕТИНИЛ-2,2'-БИПИРИДИНОМ

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У овом раду, синтетисан је и применом спектроскопских (^{1}H-NMR, IR и UV–Vis) метода и мерењем моларне проводљивости окарактерисан комплекс цинка(II) са 4-етинил-2,2'-бипиридином (ebpy), [Zn(ebpy)Cl_2]. Кристална структура [Zn(ebpy)Cl_2] комплекса је одређена применом дифракције X-зрака са монокристала, при чему су добијени кристалографски подаци потврдили да се ebpy лиганд бидентатно координује за јон метала преко два атома азота, док преостала два координациона места заузимају два хлоридо лиганда. У циљу одређивања реактивности синтетисаног комплекса цинка(II) са биолошки значајним молекулима, испитиване су његове интеракције са ДНК молекулом тимуса телета (ct-DNA) и албумином говеђег серума (BSA) применом флуоресцентне емисионе спектроскопије. На основу добијених спектроскопских резултата, може се закључити да се [Zn(ebpy)Cl_2] комплекс реверзибилно везује за BSA, док компетитивно испитивање везивања етидијум-бромида (EthBr) и Hoechst 33258 (2'-(4-хидроксифенил)-5-[5-(4-метилпиперазин-1-ил)бензимидазо-2-ил]-бензимидазол) указује да се цинк(II) комплекс везује за ct-DNA преко малог жлеба, што је у складу са резултатима молекулског доковања.

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