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Evaluation of antitumor potential of Cu(II) complex with hydrazone of 2-acetylthiazole and Girard's T reagent

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Abstract: In this paper, the previously synthesized Cu(II) complex ($[CuL^1(N_3)(CH_3OH)]BF_4$) with *N*,*N*,*N*-trimethyl-2-oxo-2-(2-(1-(thiazol-2-yl)ethylidene)-hydrazinyl)ethan-1-aminium chloride, has been characterized and its biological activity has been studied in detail. The Cu(II) complex consists of ligand coordinated in a deprotonated, formally neutral zwitter-ionic form, *via* NNO atoms, one azido ligand and one methanol molecule. The Cu(II) complex was selected due to results of the cytotoxic activity, the brine shrimp test and DPPH radical scavenging activity, which were previously performed. The effects of Cu(II) complex on cell cycle phase distribution of cervical adenocarcinoma HeLa cells were investigated in order to examine the mechanisms of its anticancer activity. The measurement of intracellular ROS levels in HeLa and HaCaT cell lines were evaluated in order to explore their possible generation and the role in cytotoxic activity. The possible anti-invasive and anti-angiogenic properties of Cu(II) complex were evaluated. DNA binding experiments, including fluorescence displacement study and DNA cleavage experiments, were performed in order to obtain information on the type of DNA–metal complex interactions.

Keywords: *N*-acylhydrazone; cell cycle analysis; gene expression analyses; UV–Vis measurements.

INTRODUCTION

In recent years, Schiff base complexes, especially hydrazone based ones, represent an interesting group of complexes, due to its easy preparation and

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structural diversity of ligands and wide spectrum of biological activities. *N*-Acylhydrazones contain a carbonyl group that enables the electron delocalization and can act as an additional coordination site. Many hydrazones show different biological activities, but in many cases their metal complexes show higher activity then the free ligand.[1,2]

Copper (II) complexes have d^9 geometries (Jahn–Teller effect) and therefore show distorted octahedral and tetrahedral symmetries. The distortion is observed as axial elongation so that Cu(II) complexes can have square planar or square pyramidal geometries, but some can also possess trigonal bipyramidal geometry.[3]

Most biologically active *N*-acylhydrazone copper complexes synthesized so far possess tridentate ligands, with ONO[4–8] and NNO[9–12] sets of donor atoms. Considering the structure of the complex, there are no recorded structures of copper complexes with *N*-acylhydrazones containing a five-membered ring in the part of the molecule that originate from aldehyde/ketone, however the analogous structures of thiosemicarbazone Cu(II) complexes have been reported.[13,14] In all reported complexes, the geometry around copper(II) ion is square pyramidal. Biological studies of these complexes showed moderate antibacterial[13] and cytotoxic activities.[14]

By biological investigation of complexes of copper with *N*-acylhydrazone ligands it was observed that they can show wide spectra of biological activity, such as cytotoxic,[4,6,7,12] antibacterial[5,8–11] and antifungal,[5,8] with higher activity than referent compounds.[3]

In continuation of our previous investigations of synthesis and characterrization of Cu(II) complexes with Girard's reagent T-based hydrazones,[15–17] biological properties of a previously synthesized Cu(II) complex were investigated in this paper. In previous biological studies Cu(II) complex with *N*,*N*,*N*-trimethyl-2-oxo-2-(2-(1-(thiazol-2-yl)ethylidene)hydrazinyl)ethan-1-aminium chloride showed moderate antibacterial activity against all tested bacterial strains (best activity towards *Escherichia coli* strain and very weak selectivity towards Gram-negative bacteria) and moderate antifungal activity.[19] Also, this complex exhibited high radical-scavenging activity which is not surprising given the existence of a redox-active Cu^{2+} in structure. Another significant feature is the pronounced cytotoxic activity against tested human cancer cell lines.

Bearing that in mind, in this paper the previously synthesized Cu(II) complex has been subjected to further biological examination that includes: investigation of mechanisms of anticancer activity, ROS generation in mediating cytotoxic effects, *in vitro* anti-angiogenic activity, effects on gene expression levels and DNA interaction studies.

EXPERIMENTAL

Chemistry

2-Acetylthiazole (99 %) was obtained from Acros, and Girard's T reagent (99 %) from Aldrich. IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer using the ATR technique in the region 4000−400 cm^{-1} (*vs* – very strong, *s* – strong, *m* – medium, *w* – weak). Elemental analyses (C, H and N) were performed by standard micro-methods using the Elementar Vario ELIII C.H.N.S.O analyzer.

*General procedure for the synthesis of the ligand and Cu(II) complex (**1**).* The ligand **HL**Cl was synthesized by the reaction of Girard's T reagent and 2-acetylthiazole according to the previously described method.[18]

The Cu(II) complex **1** was synthesized according to the previously described method,[19] equimolar amounts of ligand **HL**Cl and $Cu(BF_4)_2 \cdot 6H_2O$ were briefly dissolved in methanol. After complete dissolution of $Cu(BF_4)_2 \cdot 6H_2O$ in the reaction mixture, 4 eq. of NaN_3 were added and mixture was refluxed.

Biology

Cell cycle analysis. The changes in cell cycle distribution of HeLa cells treated with Cu(II) complex were analyzed by flow cytometry. HeLa cells were exposed to IC_{50} and $2IC_{50}$ concentrations of Cu(II) complex for 24 h. Afterwards, the cells were collected by trypsinization, washed with phosphate buffered saline (PBS) and fixed in 70 % ethanol on ice, according to standard protocol.[20] The cell samples were stored at –20 °C for at least one week before analysis. At the day of analysis, the cell samples were washed, resuspended in PBS containing RNase A and incubated for 30 min at 37 °C. Subsequently, the propidium iodide solution was added to cell samples. Percentages of cells within subG1, G1, S, and G2/M phases of the cell cycle were assessed using a BD FACS Calibur flow cytometer. The analyses of acquired data (10000 events collected for each gated cell sample) were performed by BD CellQuest software. Cell cycle data are presented as mean ± *S.D.* of three independent experiments.

Endothelial cell tube formation assay. The possible *in vitro* anti-angiogenic activity of tested Cu(II) complex was investigated on human umbilical vein EA.hy926 cells using endothelial cell tube formation assay.[21,22] The EA.hy926 cells were seeded on the surface of Corning® Matrigel® basement membrane matrix and incubated with subtoxic IC_{20} concentration of the complex for 24 h (the applied concentration was 20 μM, as determined by MTT test for 24 h treatment). After the incubation of 20 h, the photomicrographs of control and treated EA.hy926 cells were captured under the inverted phase-contrast microscope.

Gene expression analyses. HeLa cells were seeded into 25 cm^2 cell culture flasks (1.5×10^6 cells per flask). After 24 h, the cells were treated with subtoxic IC_{20} concentration of Cu(II) complex (the applied concentration was 15 μM, as determined by MTT test for 24 h treatment). Control cell sample was incubated in nutrient medium only. After 24 h treatment, HeLa cells were collected by trypsinization, washed with PBS, and stored at –80 °C. Total RNA was isolated from HeLa cells using TRI Reagent® (Sigma Aldrich) according to the manufacturer's protocol. The concentration and quality of isolated RNA for each sample was determined spectrophotometrically (BioSpec-nano, Shimadzu). High-capacity cDNA reverse transcription kit (Thermo Fisher Scientific) was used for reverse transcription of 1 μg of total RNA into single-stranded cDNA. Measurement of mRNA expression levels of selected genes was done by real-time quantitative PCR (RT-qPCR) and TaqMan®gene expression assays (MMP2-Hs01548727_m1, MMP9-Hs00957562_m1 and VEGFA-Hs00900055_m1) using LightCycler® 480 II system (Roche Diagnostics GmbH, Mannheim, Germany). Gene expres-

sion values were normalized to *GAPDH* (Hs02758991_g1) and were obtained by comparative ΔΔCt method, analyzed with LightCycler® 480 software.

UV–Vis measurements experiments

For DNA binding experiments, calf thymus DNA (CT-DNA, lyophilized, highly polymerized, obtained from Serva, Heidelberg, Germany) was dissolved in 40 mM bicarbonate buffer, pH 8.4) and left overnight at 4 °C. This stock solution was stored at 4 °C and was stable for several days. A solution of CT-DNA in water gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280} of 1.89–2.01, indicating that DNA was sufficiently free of protein. The concentration of DNA (3 mg/mL, 9.98 mM) was determined from the UV absorbance at 260 nm using the extinction coefficient $\varepsilon_{260} = 6600$ M^{-1} cm^{-1}.[23] The stock solution of complex $[Cu\mathbf{L}^1(N_3)(CH_3OH)]BF_4$ (**1**) was freshly prepared by dissolving the compound in dimethyl sulfoxide at concentrations of 10 mM (5.28 mg/mL).

Reaction mixtures (1 mL in 40 mM bicarbonate buffer, pH 8.4) consisting of different concentrations of the complex **1** (1, 5 and 10 μM) and 49.9 μM of CT-DNA (calculated per phosphate) were incubated at 37 °C for 60 min with occasional vortexing. The absorbance titration was performed at a fixed concentration of the compound (10 μM) with gradual increase of the concentration of double stranded CT-DNA (4.99, 5.98, 6.98, 7.98, 8.98, 9.98, 10.98, 11.97, 12.97, 13.97 and 14.97×10^{-5} M). The absorbance at 258 nm was monitored for each concentration of DNA. The binding constant K_b was determined using:[24]

$$c_{DNA}(\varepsilon_a-\varepsilon_f)^{-1} = c_{DNA}(\varepsilon_b-\varepsilon_f)^{-1} + K_b^{-1}(\varepsilon_b-\varepsilon_f)^{-1} \quad (1)$$

where ε_a, ε_f, ε_b are absorbance/[compound], extinction coefficient of the free compound and extinction coefficient of the bound compound, respectively.

Fluorescence measurements

The competitive interactions of **1** and the fluorescence probe, either ethidium bromide (EB) or Hoechst 33258 (H), with CT-DNA were studied by measuring the change of fluorescence intensity of the probe-DNA solution after addition of the complex. Reaction mixtures containing 50 μM of CT-DNA (calculated per phosphate) in 1 mL of 40 mM bicarbonate solution (pH 8.4) were pretreated with 1.5 μL of 1 % H probe solution (28 μM final concentration) or 1 μL of 1 % EB solution (25 μM final concentration, in separate experiments) for 20 min and the mixture was analyzed by fluorescence measurement. Then the increasing concentrations of the complexes (0.5, 1, 2, 3, 5, 7, 9, 11, 13 and 15×10^{-5} M for displacement of EB and 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4×10^{-5} M for displacement of H) were successively added and the changes in the fluorescence intensity were measured using a Thermo Scientific Lumina Fluorescence spectrometer (Finland) equipped with a 150 W Xenon lamp. The slits on the excitation and emission beams were fixed at 10 nm. All measurements were performed by the excitation at 350 nm for Hoechst 33258, and by the excitation at 500 nm for EB in the range of 390−600 nm. The control was probe-CT-DNA solution. The obtained fluorescence quenching data were analyzed according to the Stern–Volmer Equation:

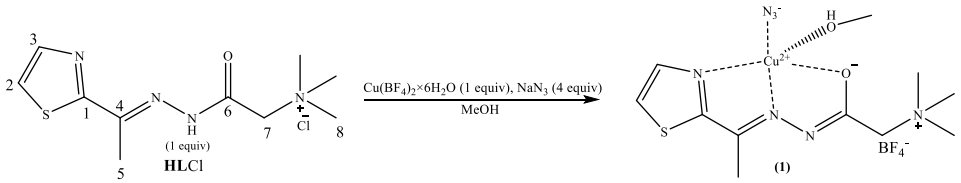
$$I_0/I = 1 + Kc_{\mathbf{1}}/c_{CT\text{-}DNA} \quad (2)$$

where I_0 and I represent the fluorescence intensities of probe-CT-DNA in absence and presence of **1**, respectively, where K is the quenching constant. The K value was calculated from the ratio of the slope to the intercept from the plot of I_0/I *versus* $c_{\mathbf{1}}/c_{CT\text{-}DNA}$.

The primary spectra of all spectrometric measurements were imported into Origin 2018.

RESULTS AND DISCUSSION

The reaction of 2-acetylthiazole and Girard's T reagent was performed according to the previously reported method[18] and yielded the ligand *N*,*N*,*N*-trimethyl-2-oxo-2-(2-(1-(thiazol-2-yl)ethylidene)hydrazinyl)ethan-1-aminium chloride (**HL**Cl), which was used for the synthesis of Cu(II) complex (Scheme 1). The reaction of the ligand **HL**Cl with metal salt $Cu(BF_4)\cdot 6H_2O$ and NaN_3 in mole ratio 1:1:4 in methanol resulted in formation of mononuclear Cu(II) complex (**1**) with the composition $[Cu\mathbf{L}^1(N_3)(CH_3OH)]BF_4$ (Scheme 1). The ligand is coordinated in a deprotonated formally neutral zwitter-ionic form *via* thiazole nitrogen, azomethine nitrogen and carbonyl oxygen atoms. Ligand and complex **1** were characterized by IR spectroscopy (Figs. S-3 and S-4 of the Supplementary material to this paper) and elemental analysis.



Scheme 1. Synthesis of Cu(II) complex (**1**).

Effects of Cu(II) complex on cell cycle phase distribution

Since the Cu(II) complex **1** exerted a strong cytotoxic activity against cancer cell lines further examination of mechanisms of its anticancer activity was undertaken. The effects of **1** on cell cycle phase distribution of cervical adenocarcinoma HeLa cells were investigated after incubation with IC_{50} and $2IC_{50}$ concentrations of the compound for 24 h. These results are presented in Fig. 1. Cu(II) complex **1** applied at IC_{50} concentration caused a small increase in the percentages of HeLa cells in subG1 and S phases of the cell cycle in comparison with untreated HeLa cell sample as a control. In addition, the pronounced increase in the percentage of cells in G2/M cell cycle phase was observed after the treatment of HeLa cells with IC_{50} concentration of Cu(II) complex **1**, when compared with control cells. These changes were accompanied with a decrease of percentage of HeLa cells in G1 phase. Similar changes in the cell cycle phase distribution were found in HeLa cells exposed to higher ($2IC_{50}$) concentration of complex – a small increase of the percentage of cells in subG1 phase and a prominent increase of the percentage of cells in G2/M phase. The only exception was the absence of changes in the percentage of cells in S phase of the cell cycle. These results suggest that the antiproliferative activity of Cu(II) complex **1** might be attributed to a pronounced arrest of cells in G2/M cell cycle phase. The G2/M cell cycle arrest induced by the complex in HeLa cells might be explained by the

effects on cell cycle progression from G2 phase to mitosis at G2/M checkpoint, or by the effects on cell progression through mitosis – M phase arrest. The observed G2/M phase arrest might be associated with possible DNA damage effects of the copper complex.

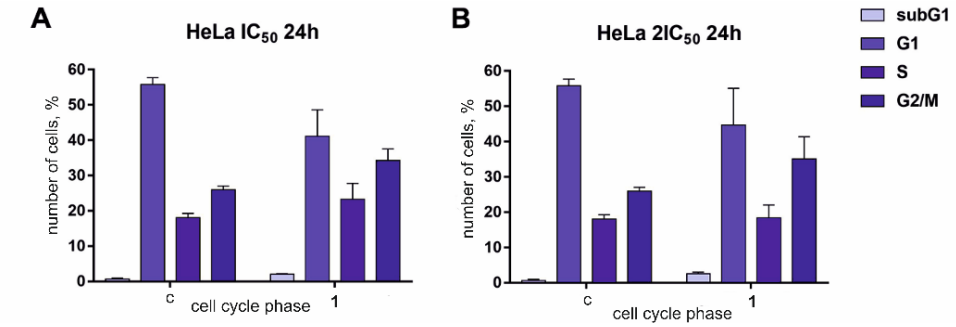


Fig. 1. Changes in the cell cycle phase distribution of HeLa cells treated with IC_{50} (A) and $2IC_{50}$ concentrations (B) of Cu(II) complex for 24 h.

Effect of Cu(II) complex on in vitro angiogenesis

The possible *in vitro* anti-angiogenic activity of the novel Cu(II) complex **1** on human umbilical vein endothelial EA.hy926 cells was investigated using tube formation assay. As it could be seen in Fig. 2 the incubation of EA.hy926 cells with subtoxic IC_{20} concentration of Cu(II) complex **1** for 24 h notably decreased angiogenesis *in vitro*. The complex suppressed the extensive sprouting of elongated EA.hy926 cells and the formation of capillary-like tubes, in comparison with the untreated EA.hy926 cells as control. The *in vitro* anti-angiogenic activity of investigated **1** is in agreement with the findings about inhibitory effects of other Cu(II) complexes on angiogenesis *in vitro*.[25,26]

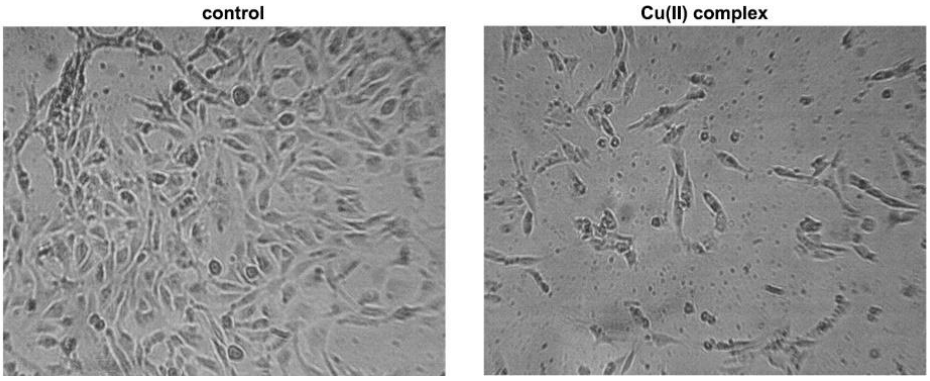


Fig. 2. Photomicrographs of control EA.hy926 cells and EA.hy926 cells treated with subtoxic IC_{20} concentration of Cu(II) complex for 20 h.

Effects of Cu(II) complex on gene expression levels

In order to further explore the possible anti-invasive and anti-angiogenic properties of Cu(II) complex (**1**), its effects in HeLa cells on the expression levels of genes whose protein products are implicated in cancer cell invasion and angiogenesis: matrix metalloproteinase-2 (MMP2), matrix metalloproteinase-9 (MMP9) and vascular endothelial growth factor A (VEGFA) were evaluated.

A slight decrease in MMP9 gene expression level was detected in HeLa cells exposed for 24 h to subtoxic IC_{20} concentration of the complex in comparison with this level in control HeLa cells (Fig. 3). However, the increase in the MMP2 gene expression level was observed in HeLa cells exposed to the tested complex. The Cu(II) complex **1** notably downregulated expression level of VEGFA in HeLa cells, when compared with this level in control cell sample. Since VEGFA gene encodes growth factor, which is involved in activating angiogenesis, lowering its level in cancer cells confirms the anti-angiogenic effect of this complex *in vitro*. The ability of copper complex to suppress angiogenesis *in vitro* represents an important anticancer property of this novel compound because targeting angiogenesis, which is essential for the expansion of malignant tumor growth, can inhibit further cancer progression.[27]

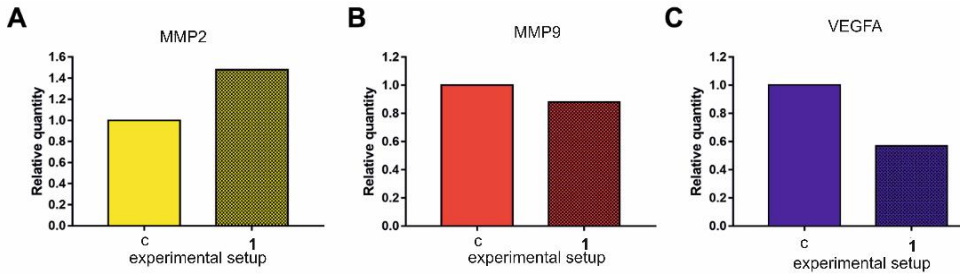


Fig. 3. Changes in gene expression levels of MMP2 (A), MMP9 (B), and VEGFA (C), in HeLa cells treated with subtoxic IC_{20} concentration of Cu(II) complex for 24 h.

DNA interaction studies

DNA is one of the medicinal therapeutic targets of anticancer agents. Transition metal complexes interact with DNA non-covalently or by covalent binding. Non-covalent binding usually occurs either in the minor groove by combination of hydrogen bonding, van der Waals contacts and electrostatic interactions, or by intercalation of a planar aromatic moiety between two base pairs of DNA *via* π-stacking take place.[28,29] Covalent modifications of DNA usually occur at N7--positions on easily accessible purines in the major groove of the helix.[30] A combination of these interactions is possible and the consequences of such interactions might be reversible or irreversible.[31] In this work, spectroscopic methods were employed to investigate the interaction modes of complex **1**.

The electronic absorption spectra of **1**, recorded at different concentrations with fixed concentration of CT-DNA or without it, are shown in Fig. S-1A of the Supplementary material. It was found that the absorption maximum of the complex was centered at 358 nm. After the interaction with CT-DNA, the changes in spectra were detected. Analysis of the absorption changes induced upon binding of **1** to CT-DNA is shown in the inset in Fig. S-1A. By comparison of the sum of absorbances at 258 nm of free complex **1** and free CT-DNA and the observed absorbance of the **1**-CT-DNA, it can be concluded that there was a concentration-dependent difference between them. The bands which correspond to CT-DNA showed the broadening of the peak at 258 nm in concentration-dependent way, and the hypochromism of 1.87, 6.67 and 8.58 % for 1, 5 and 10 μM of the complex, respectively (Fig. S-1A, inset). Significant changes in spectra of **1** after the interaction with CT-DNA were observed for bands at 358 nm, as evidenced by hypochromism of 42.31, 23.26 and 18.79 % (for 1, 5 and 10 μM of the complex, respectively). Hypochromism and red shift are common evidence for intercalation. Given that no shifts in the spectrum were detected and considering the positive charge on molecule of the complex, the obtained results indicate that the electrostatic interaction is the probable mode of binding.

In order to obtain more information on interaction of the complex with DNA, spectroscopic titration of the solution of the complex the concentration of which was kept constant (10 μM) with the solutions of increasing concentrations of CT-DNA was performed. The results are shown in Fig. S-1B. A quantitative determination of the binding strength of the compound to DNA is made by calculation of binding constants K_B of the compound from Eq. (1).[24] The intrinsic binding constant K_B of **1** (inset in Fig. S-1B) was calculated as 2.9×10^3 M^{-1}. The obtained value was lower than the value reported for a classical intercalator (ranged 10^6 to 10^7 M^{-1})[32]. The value of K_B was comparable to those for metal complexes binding in the minor groove of DNA.[33] However, non-linearity of the curve showed that at least two types of binding sites are available for the interaction of complex **1** with DNA, suggesting different binding modes.

In order to further investigate the binding mode, fluorescence spectroscopy was performed by the fluorescence displacement experiments carried out with two different dyes: ethidium bromide (EB), a typical intercalator, and Hoechst 33258 (H), a minor groove binder. While EB fluoresces weakly, its fluorescence yield increases significantly in the presence of CT-DNA (Fig S-2A). Binding of EB to CT-DNA was followed by the excitation at 500 nm with fluorescence maximum at 600 nm. The addition of increasing concentrations of **1** to EB–CT-DNA system caused reduction in the fluorescence intensity of the band at 600 nm by 32 % with the maximal applied concentration of the complex. The criterion for fluorescence quenching by a typical strong intercalator is the fluorescence value reduction of more than 50 %.[34–36] This means that that the mech-

anism of fluorescence of EB quenching by complex **1** is different from intercalation. The extent of this quenching of EB by **1** was estimated using Stern–Volmer Equation (2). The fluorescence quenching constant (K) was calculated from the ratio of the slope to the intercept from the plot I_0/I *versus* $c_{\mathbf{1}}$ as shown in the inset in Fig. S 2A. The plot is linear and gives a K of 3.07×10^3 M^{-1}.

Hoechst 33258 recognizes at least four AT base pairs and binds strongly and selectively with high affinity to double-stranded B-DNA structure by the combination of hydrogen bonding, van der Waals contacts with the walls of the minor groove, and the electrostatic interactions between its cationic structure and the DNA.[37] As the emission spectra showed (Fig. S-2B), the fluorescence of H increased significantly upon the interaction with CT-DNA. A successive addition of **1** to H-CT-DNA solution caused appreciable reduction in the fluorescence intensity of the band at 443 nm (about 60 % with maximal applied concentration of **1**), accompanied by a small blue shift of emission maximum to 441 nm. The quenching constant calculated by the linear regression of a plot I_0/I against $c_{\mathbf{1}}$ (inset in Fig. S-2B) was $K = 4.16\times10^4$ M^{-1}. The plot displayed a good linear relationship for the investigated concentration ranges of **1**, indicating the displacement of H33258 from H−CT-DNA. By comparing the results, it can be concluded that the competition of **1** with H 33258 was more efficient than in case of ethidium bromide in binding to DNA.

In view of fluorescence and UV–Vis absorption spectral results, it can be deduced that the interaction of the complex **1** with DNA occurred *via* minor groove binding and electrostatic interactions between a positively charged quarternary ammonium group and negatively charged phosphate groups of DNA backbone.

For effects of Cu(II) complex on intracellular ROS levels, BSA fluorescence measurements and DNA cleavage experiments, see Supplementary Material.

CONCLUSIONS

The ligand **HL**Cl and Cu(II) complex **1** have been characterized by elemental analysis and IR spectroscopy. The five-coordination geometry of the Cu(II) ion (mononuclear complex **1**) can be described as distorted square-based pyramidal. Cu(II) ion is coordinated through NNO set of donor atoms, of formally neutral zwitter-ionic form, of **HL**Cl ligand, one nitrogen atom of the azide ligand, while fifth remaining coordination site is occupied by the oxygen atom from methanol. The effects of Cu(II) complex on cell cycle phase distribution showed a small increase in the percentages of HeLa cells in subG1 and S phases and also a pronounced increase in the percentage of cells in G2/M phase of the cell cycle. The results of effects of Cu(II) complex on ROS generation suggested the antioxidant properties of the tested complex at lower concentration. The results obtained *via* DNA interaction studies indicate that minor groove binding and electrostatic interactions are the probable mode of binding for complex **1**. The

lower value of intrinsic binding constant K_B of **1** and nonlinearity of the curve indicates that at least two types of binding sites are responsible for the interaction of the complex with DNA. Of a particular interest is anti-angiogenetic activity of the complex, since the formation of blood vessels is crucial for tumor growth.

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/11447, or from the corresponding author on request.

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ИЗВОД

ИСПИТИВАЊЕ АНТИТУМОРСКОГ ПОТЕНЦИЈАЛА КОМПЛЕКСА CU(II) СА ХИДРАЗОНОМ 2-АЦЕТИЛТИАЗОЛА И ЖИРАРОВОГ Т-РЕАГЕНСА

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У овом раду урађена је карактеризација и детаљно испитивање биолошке активности претходно синтетисаног комплекса Cu(II) са *N*,*N*,*N*-триметил-2-оксо-2-(2-(1-(тиазол-2-ил)етилиден)хидразинил)етан-1-аминијум-хлоридом. Комплекс Cu(II) састоји се из депротонованог, формално неутралног, лиганда координованог преко NNO атома у цвитер-јонском облику, једног азидо лиганда и једног молекула метанола. Комплекс Cu(II) одабран је на основу резултата претходно испитиване цитотоксичне активности, урађених тестова на рачићима *Artemia salina* и DPPH теста. У циљу одређивања механизма антитуморског дејства, урађена је анализа ћелијског циклуса аденокарцинома грлића материце. Мерењем продукције реактивних оксидативних врста проучаван је њихов утицај и улога у цитотоксичној активности. Испитивана су и потенцијална антиинвазивна и анти-ангиогена својства комплекса Cu(II). Ради одређивања типа интеракција између комплекса метала и ДНК урађена су испитивања: интеракција са ДНК, флуоресценције и цепања ДНК ланца.

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