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Eighth Conference
with international participation

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“Coordination in Biochemistry and Life”

Foreword

Dear Colleagues

Welcome to the 8th Conference of the Serbian Biochemical Society, entitled "*Coordination in Biochemistry and Life*".

The title of this year's Conference refers to an important place of coordination chemistry in biochemistry and biomedicine, but also to a need to coordinate the efforts towards new knowledge with fellow scientists from other fields in order to reach more. The collaboration within FEBS3+ (Croatia, Hungary, Slovenia, and Serbia) Meeting Programme continues with the invited lecture of our dear colleague Tantos Ágnes from Research Center for Natural Sciences, Budapest, Hungary. For the first time we have 'Diaspora Lecture' that will be delivered by Miloš Filipović, a top 'product' of Serbian biochemistry who is now affiliated at the Université de Bordeaux. We have more than forty PhD students from Serbia, Hungary, and Belarus with poster presentations, and for the first time the Conference is held outside the capital. It believe that we are getting better each year, and that we are prepared for future challenges.

I would like to express my gratitude to the members of the Scientific Board who suggested lecturers, to all respected colleagues who accepted the invitation, and to our dear hosts from the University of Novi Sad.

Editor of the Proceedings
Ivan Spasojević

Antitumor and antimicrobial properties of isothiocyanato pentagonal-bipyramidal d metal complexes with dihydrazone of 2,6-diacetylpyridine and Girard's T reagent

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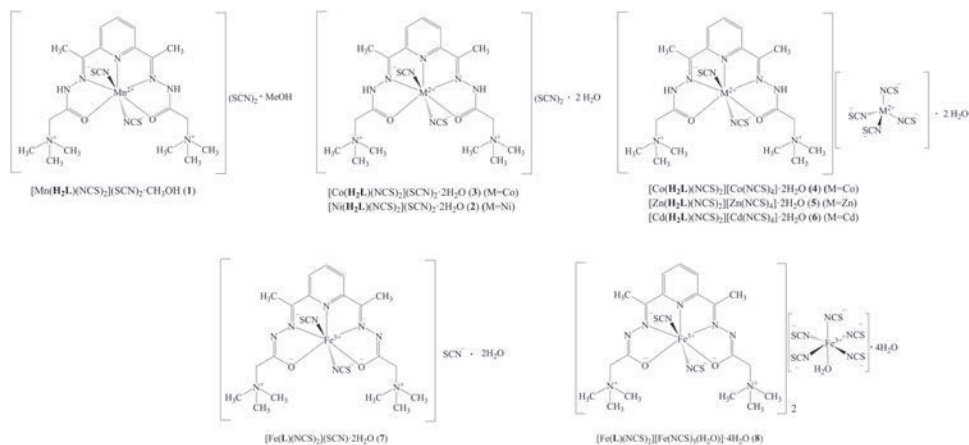
Pentagonal-bipyramidal complexes of 2,6-diacetylpyridine bis(acylhydrazone) ligands are attractive field of research not only in structural inorganic chemistry and magnetochemistry, but also in bioinorganic chemistry since they exhibit cytotoxic, antimicrobial, SOD mimetic, DNA/RNA binding and nuclease activity. In this work we investigated antitumor and antimicrobial activity of pentagonal-bipyramidal isothiocyanato complexes of Mn(II) ($[\text{Mn}(\text{H}_2\text{L})(\text{NCS})_2](\text{SCN})_2 \cdot \text{CH}_3\text{OH}$) (1), Ni(II) ($[\text{Ni}(\text{H}_2\text{L})(\text{NCS})_2](\text{SCN})_2 \cdot 2\text{H}_2\text{O}$) (2), Co(II) ($[\text{Co}(\text{H}_2\text{L})(\text{NCS})_2](\text{SCN})_2 \cdot 2\text{H}_2\text{O}$) (3) and $[\text{Co}(\text{H}_2\text{L})(\text{NCS})_2][\text{Co}(\text{NCS})_4] \cdot 2\text{H}_2\text{O}$) (4), Zn(II) ($[\text{Zn}(\text{H}_2\text{L})(\text{NCS})_2][\text{Zn}(\text{NCS})_4] \cdot 2\text{H}_2\text{O}$) (5), Cd(II) ($[\text{Cd}(\text{H}_2\text{L})(\text{NCS})_2][\text{Cd}(\text{NCS})_4] \cdot 2\text{H}_2\text{O}$) (6) and Fe(III) ($[\text{Fe}(\text{L})(\text{NCS})_2](\text{SCN}) \cdot 2\text{H}_2\text{O}$) (7) and $[\text{Fe}(\text{L})(\text{NCS})_2][\text{Fe}(\text{NCS})_5(\text{H}_2\text{O})] \cdot 4\text{H}_2\text{O}$) (8), with the condensation product of 2,6-diacetylpyridine and Girard's T reagent (H_2LCl_2). The complexes showed moderate to low cytotoxic activities towards five tested human cancer cell lines (HeLa, MDA-MB-453, K562, LS174 and A549), while the ligand was inactive. The best activity was observed in the case of complexes 8, 4 and 6. The potential of the most active complexes to induce HeLa and K562 cell cycle perturbations was also studied. Cd(II) complex (6) caused significant increase of apoptotic subG1 cells in both cell lines. Fe(III) complex (8) induced significant changes in cell cycle phase distribution only in HeLa cells. Morphological changes in HeLa cells treated with complexes 8, 4 and 6 were also indicative of apoptosis, with complex 6 having again the most pronounced effect. Complexes 8, 4 and 6 bind to DNA, most probably by electrostatic interactions, and perturb DNA structure. Complexes 4 and 8 cause cleavage of plasmid DNA in vitro. Iron (III) complexes showed better antimicrobial activity than complexes of other metals with this ligand, but lower than activity of standard antimicrobial drugs.

Introduction

Complexes of 2,6-diacetylpyridine bis(acylhydrazones) have been intensively studied over the years due to their interesting structural and magnetic properties¹⁻³. The 2,6-diacetylpyridine bis(acylhydrazone) ligands possess at least five donor atoms (N₃O₂) in spatial arrangement which supports formation of seven-coordinated complexes with pentagonal-bipyramidal (PBPY-7) geometry. The acidity of hydrazone function in 2,6-diacetylpyridine bis(acylhydrazone) ligands contributes to structural versatility of their complexes due to possibility of coordination of ligand in non-deprotonated, partially deprotonated and fully deprotonated form⁴. Pentagonal-bipyramidal complexes of 2,6-diacetylpyridine bis(acylhydrazone) ligands have a wide spectrum of biological activities: cytotoxic⁵, antimicrobial⁶⁻⁸, SOD mimetic⁹⁻¹¹, DNA/RNA binding and nuclease activity¹²⁻¹⁴. Girard's reagents (Girard's T (trimethylacetylhydrazide ammonium chloride), Girard's D (N,N-dimethylglycine hydrazide hydrochloride), Girard's P (pyridinioacetohydrazide chloride)) are N-substituted glycine hydrazides, which are mostly used in analytical chemistry for separation of carbonyl compounds from complex organic mixtures¹⁵. The presence of the quaternary ammonium group in the metal complexes of Girard's T reagent hydrazones increases their water solubility and has effect on their biological activity¹⁶. Thiocyanate is an ambidentate pseudohalide ligand, which can be coordinated through nitrogen or sulfur donor atom as monodentate or as a bridge between metal centers¹⁷. In biological systems free SCN⁻ can be oxidized to hypothiocyanite by H₂O₂ produced in oxidative metabolism. Hypothiocyanite (OSCN⁻) plays a role of an antimicrobial agent due to its reactions with sulfhydryl groups of glycolytic enzymes and thiol-based antioxidants¹⁸. Here we report antitumor and antimicrobial activity of pentagonal-bipyramidal isothiocyanato complexes of Mn(II) ([Mn(H₂L)(NCS)₂](SCN)₂·CH₃OH) (**1**), Ni(II) ([Ni(H₂L)(NCS)₂](SCN)₂·2H₂O) (**2**), Co(II) ([Co(H₂L)(NCS)₂](SCN)₂·2H₂O) (**3**) and [Co(H₂L)(NCS)₂][Co(NCS)₄]·2H₂O (**4**), Zn(II) ([Zn(H₂L)(NCS)₂][Zn(NCS)₄]·2H₂O) (**5**), Cd(II) ([Cd(H₂L)(NCS)₂][Cd(NCS)₄]·2H₂O) (**6**) and Fe(III) ([Fe(L)(NCS)₂](SCN)·2H₂O) (**7**) and [Fe(L)(NCS)₂][Fe(NCS)₅(H₂O)]·4H₂O (**8**) with the condensation product of 2,6-diacetylpyridine and Girard's T reagent (H₂LCI₂).

Chemistry

Isothiocyanato complexes of Mn(II) (**1**), Ni(II) (**2**), Co(II) (**3** and **4**), Zn(II) (**5**), Cd(II) (**6**) and Fe(III) (**7** and **8**) with the condensation product of 2,6-diacetylpyridine and Girard's T reagent (H₂LCI₂) (Scheme 1) were obtained in the reactions of dihydrazone ligand, NH₄SCN and corresponding metal(II) salts (chloride in the case of Mn(II) (**1**)¹⁹, Ni(II) (**2**), Co(II) (**3** and **4**)²⁰, and Zn(II) (**5**) complexes or nitrate in the case of Cd(II) complex (**6**)²¹. Iron(III) complexes **7** and **8** were obtained in the reaction of dihydrazone ligand, FeCl₃·6H₂O and NH₄SCN. The same pentagonal-bipyramidal complex cation is present in both Fe(III) complexes, while the nature of their anions depends on mole ratio of NH₄SCN and FeCl₃·6H₂O used in reaction²².



Scheme 1. Pentagonal-bipyramidal isothiocyanato complexes of Mn(II) (1), Ni(II) (2), Co(II) (3 and 4), Zn(II) (5), Cd(II) (6) and Fe(III) (7 and 8) with H_2LCl_2 ligand.

Cytotoxic activity

Cytotoxic activity of pentagonal-bipyramidal isothiocyanato complexes of Mn(II) (1), Ni(II) (2), Co(II) (3 and 4), Zn(II) (5), Cd(II) (6) and Fe(III) (7 and 8) with the condensation product of 2,6-diacetylpyridine and Girard's T reagent (H_2LCl_2) was tested against five human cancer cell lines. The results of cytotoxic activity determined by MTT assay²³ are given in Table 1.

Table 1. The cytotoxic activity of the investigated complexes and their precursors.

	HeLa	MDA-MB-453	K562	LS174	A549	MRC-5
IC_{50} [μM] mean \pm S.D.						
1	187.28 \pm 18.91	183.33 \pm 23.57	190.93 \pm 15.71	>200	187.66 \pm 21.38	198.35 \pm 2.86
2	186.85 \pm 22.77	187.85 \pm 17.18	194.51 \pm 7.76	170.82 \pm 33.19	144.42 \pm 7.69	46.79 \pm 1.52
3	96.75 \pm 11.82	135.01 \pm 1.55	76.99 \pm 10.54	176.90 \pm 11.44	129.77 \pm 28.30	92.08 \pm 7.82
4	70.46 \pm 13.59	76.51 \pm 17.69	38.66 \pm 3.49	115.25 \pm 28.19	79.53 \pm 14.47	54.57 \pm 14.24
5	190.10 \pm 17.15	111.37 \pm 4.74	122.80 \pm 0.30	170.72 \pm 41.42	130.85 \pm 5.77	121.78 \pm 14.49
6	71.06 \pm 5.95	59.40 \pm 14.21	72.98 \pm 6.10	160.46 \pm 16.44	84.76 \pm 3.08	86.89 \pm 13.94
7	110.98 \pm 24.45	115.69 \pm 7.86	75.94 \pm 5.20	171.84 \pm 34.78	98.72 \pm 1.55	80.42 \pm 0.06
8	106.61 \pm 15.92	87.27 \pm 4.06	48.33 \pm 6.87	116.11 \pm 31.00	70.45 \pm 7.60	40.19 \pm 0.73
H_2LCl_2	>200	>200	>200	>200	>200	>200
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	156.84 \pm 38.49	138.40 \pm 30.35	85.07 \pm 21.12	192.67 \pm 12.70	189.62 \pm 7.17	143.62 \pm 23.04
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	76.31 \pm 1.09	111.59 \pm 2.08	77.76 \pm 2.91	136.61 \pm 28.97	127.68 \pm 27.82	78.75 \pm 15.03
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	95.37 \pm 9.56	92.01 \pm 12.11	45.73 \pm 3.57	136.79 \pm 0.45	97.98 \pm 12.81	57.58 \pm 1.98

ZnCl ₂ ·2H ₂ O	>200	191.32±12.27	169.87±28.50	>200	200.00±0.00	105.61±25.82
Cd(NO ₃) ₂ ·4H ₂ O	79.16±1.62	56.40±11.08	49.16±13.70	112.57±22.16	126.80±15.25	57.27±14.68
FeCl ₃ ·6H ₂ O	>200	>200	>200	>200	>200	>200
cisplatin	4.73±0.88	6.05±1.12	5.63±0.21	24.86±3.41	9.43±0.60	8.56±1.58

Changes in the cell cycle phase distribution

Cell cycle analysis of HeLa and K562 cells treated with IC₅₀ and 2IC₅₀ concentrations of the most active complexes **4**, **6** and **8** for 24 h was performed (Figure 1)^{23,24}. Complexes **4** and **6** induced alterations in cell cycle phase distribution of HeLa and K562 cells. Complex **6** caused significant increase of apoptotic subG1 cells in both cell lines. Complex **8** induced significant changes in cell cycle phase distribution only in HeLa cells.

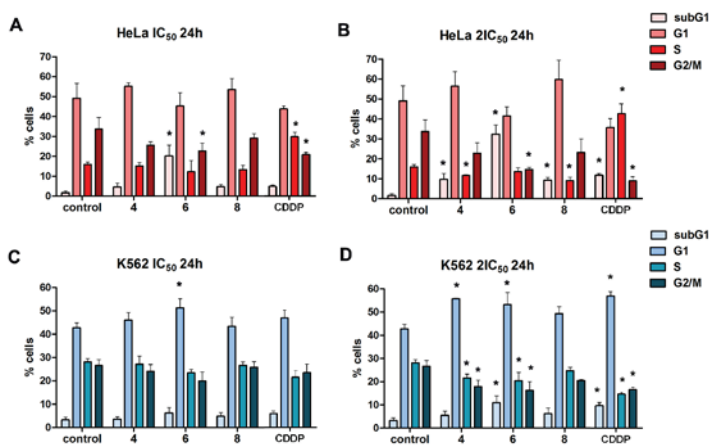


Figure 1. Changes in the cell cycle phase distribution of HeLa (A, B) and K562 cells (C, D) treated with IC₅₀ and 2IC₅₀ concentrations of complexes **4**, **6** and **8**, and cisplatin (CDDP).

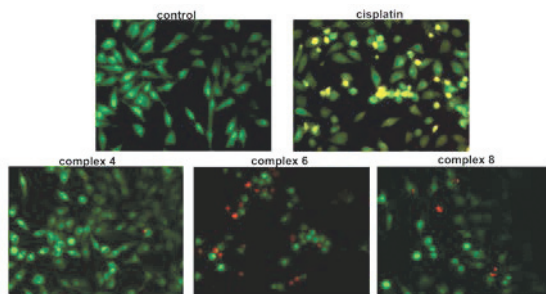


Figure 2. Photomicrographs of acridine orange/ethidium bromide-stained control HeLa cells and HeLa cells exposed to 2IC₅₀ concentrations of the cisplatin and complexes **4**, **6** and **8** for 24 h.

Morphological evaluation of HeLa cell death mode

Morphological changes in HeLa cells were also indicative of apoptosis, with complex 6 showed the most pronounced effect (Figure 2).

DNA binding study

Spectrophotometric methods were employed to ascertain the interaction modes of 4, 6 and 8 with CT DNA (Figure 3 and 4). Complexes 4, 6 and 8 bind to DNA, most probably by electrostatic interactions, and perturb DNA structure, causing displacement of both ethidium bromide and Hoechst 33 258.

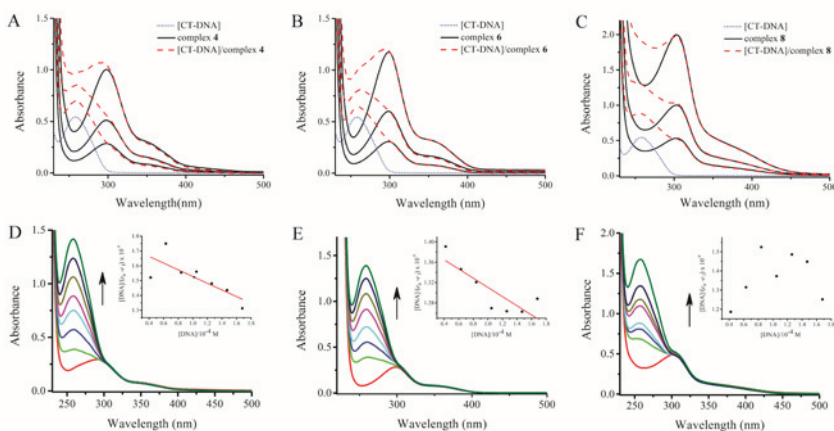


Figure 3. Changes in electronic absorption spectra of the complexes 4 (A), 6 (B) and 8 (C) ($1, 2$ and 4×10^{-5} M) after interactions with CT-DNA (8.4×10^{-5} M) and determination of binding constants by absorption titration of 4 (D), 6 (E) and 8 (F) at fixed concentration (1×10^{-5} M) with increasing concentrations of CT-DNA ($2.1, 4.2, 6.3, 8.4, 10.5, 12.6, 14.7$ and 16.8×10^{-5} M). The arrows show the changes in absorbance upon increasing amounts of CT-DNA. The insets show the linear fit of $[\text{DNA}]/(\epsilon_a - \epsilon_f)$ vs. $[\text{DNA}]$ and the binding constant (K_b) was calculated using eqn. $[\text{DNA}] \times (\epsilon_a - \epsilon_f)^{-1} = [\text{DNA}] \times (\epsilon_b - \epsilon_f)^{-1} + K_b^{-1} \times (\epsilon_b - \epsilon_f)^{-1}$ 25.

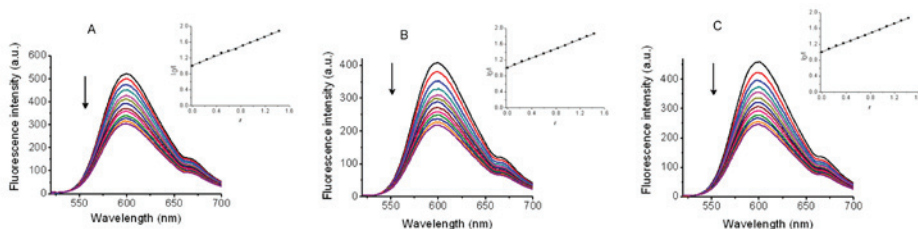


Figure 4. Emission spectra of ethidium bromide (EB) (2.5×10^{-5} M) bound to CT-DNA (8.4×10^{-5} M) and quenching of EB-CT-DNA system by 4 (A), 6 (B) and 8 (C) at increasing concentrations ($1, 2, 3, 4, 5, 6 \times 10^{-5}$ M). The arrows show that fluorescence intensity decreased with increasing

concentration of the complex. The insets show fluorescence quenching curves of EB bound to DNA at $\lambda_{\text{max}}=600$ nm by 4 (A), 6 (B) and 8 (C). The quenching constants K were calculated using eqn. $I_0/I = 1 + Kr$ by linear regression of the plot I_0/I against $[r]/[\text{CT-DNA}]$, where I_0 and I represent the fluorescence intensities of EB-CT-DNA in absence and presence of the complex, and $r = [\text{complex}]/[\text{CT-DNA}]$ ²⁶.

DNA cleavage

The ability of complexes 4, 6 and 8 to damage circular DNA were investigated using an agarose electrophoretic assay (Figure 5).

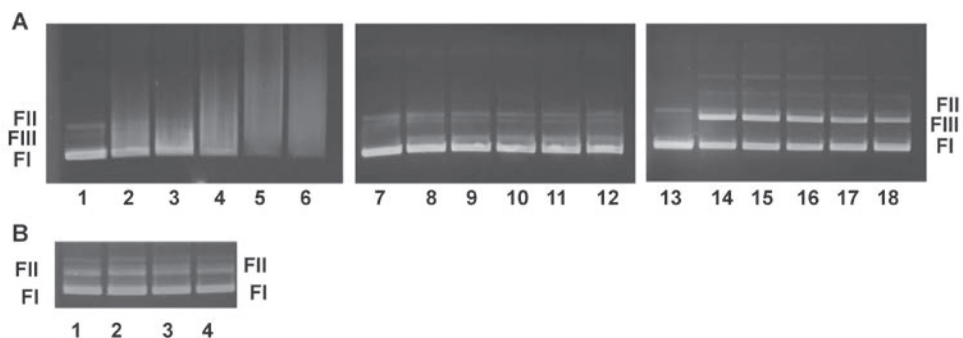


Figure 5. Agarose gel electrophoretic analysis of supercoiled forms FI and the open circular forms FII of plasmid pUC19 (2.6×10^{-9} M) (lanes 1, 7 and 13, (A); lane 1, (B)) after incubation (1.5 h at 37°C) with 0.25, 0.5, 1, 1.5 and 2 mM of complex 4, (lanes 2–6, respectively, panel A); with 0.25, 0.5, 1, 1.5 and 2 mM of complex 6, (lanes 8–12, respectively, panel A); with 0.25, 0.5, 1, 1.5 and 2 mM of complex 8, (lanes 14–18, respectively, (A)) and with 0.25, 1 and 2 mM of ligand H_2LCl_2 (lanes 2–4, respectively, (B)).

Antimicrobial activity

Fe(III) complexes 7 and 8 showed better antimicrobial activity than Mn(II) (1), Ni(II) (2), Co(II) (3) and (4), Zn(II) (5) and Cd(II) (6) complexes. The investigated complexes showed lower activity than standard antimicrobial drugs.

Conclusion

From the obtained results it can be concluded that factors affecting biological activity of studied compounds are very complex. They include type of metal ion in pentagonal-bipyramidal complex cation, structure of anion (thiocyanate or isothiocyanato metal complex), stability of complex cations and anions in solution as well as their redox activity. Further investigations are needed to better understand all these processes.

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References

1. Popov LD, Morozov AN, Shcherbakov IN, Tupolova YP, Lukov VV, Kogan VA. Metal complexes with polyfunctional ligands based of bis(hydrazones) of dicarbonyl compounds. *Russ Chem Rev* 2009;78:643–58.
2. Neto BAD, et al. Condensed, solution and gas phase behaviour of mono- and dinuclear 2,6-diacetylpyridine (dap) hydrazone copper complexes probed by X-ray, mass spectrometry and theoretical calculations. *Dalton Trans* 2013;42:11497–506.
3. Batchelor LJ, et al. Pentanuclear cyanide-bridged complexes based on highly anisotropic CoII seven-coordinate building blocks: synthesis, structure, and magnetic behavior. *Inorg Chem* 2011;50:12045–52.
4. Ivanović-Burmazović I, Andjelković K. Transition metal complexes with bis(hydrazone) ligands of 2,6-diacetylpyridine. Hepta-coordination of 3d metals. *Adv Inorg Chem* 2004;55:315–60.
5. Ferraz KSO, et al. Investigation on the pharmacological profile of 2,6-diacetylpyridine bis(benzoylhydrazone) derivatives and their antimony(III) and bismuth(III) complexes. *Eur J Med Chem* 2012;53:98–106.
6. Nomiya K, et al. Syntheses, crystal structures and antimicrobial activities of monomeric 8-coordinate, and dimeric and monomeric 7-coordinate bismuth(III) complexes with tridentate and pentadentate thiosemicarbazones and pentadentate semicarbazone ligands. *J Inorg Biochem* 2004;98:601–15.
7. Mazza P, Orcesi M, Pelizzi C, Pelizzi G, Predieri G, Zani F. Synthesis, structure, antimicrobial, and genotoxic activities of organotin compounds with 2,6-diacetylpyridine nicotinoyl and Isonicotinoylhydrazones. *J Inorg Biochem* 1992;48:251–70.
8. Kasuga NC, et al. Synthesis, structural characterization and antimicrobial activities of 12 zinc(II) complexes with four thiosemicarbazone and two semicarbazone ligands. *J Inorg Biochem* 2003;96:298–310.
9. Liu GF, Filipović M, Heinemann FW, Ivanović-Burmazović I. Seven-coordinate iron and manganese complexes with acyclic and rigid pentadentate chelates and their superoxide dismutase activity. *Inorg Chem* 2007;46:8825–35.
10. Gutman CT, Brunold TC. Spectroscopic and computational studies of a smallmolecule functional mimic of iron superoxide dismutase, iron 2,6-diacetylpyridinebis(semioxamazide). *Inorg Chem* 2012;51:12729–37.
11. Gutman CT, Guzei IA, Brunold TC. Structural, spectroscopic, and computational characterization of the azide adduct of FeIII(2,6-diacetylpyridinebis(semioxamazide)), a functional analogue of iron superoxide dismutase. *Inorg Chem* 2013;52:8909–18.
12. Kozłowski M, Kierzek R, Kubicki M, Radecka-Paryzek W. Metal-promoted synthesis, characterization, crystal structure and RNA cleavage ability of 2,6-diacetylpyridine bis(2-aminobenzoylhydrazone) lanthanide complexes. *J Inorg Biochem* 2013;126:38–45.
13. Gökçe C, Dilek N, Gup R. Seven coordinated cobalt(II) complexes with 2,6-diacetylpyridine bis(4-acylhydrazone) ligands: synthesis, characterization, DNAbinding and nuclease activity. *Inorg Chim Acta* 2015;432:213–20.
14. Gup R, Gökçe C, Dilek N. Seven-coordinated cobalt(II) complexes with 2,6-diacetylpyridine bis(4-hydroxybenzoylhydrazone): synthesis, characterisation, DNA binding and cleavage properties. *Supramol Chem* 2015;27:629–41.
15. Vojinović-Ješić LS, Novaković SB, Leovac VM, Češljević VI. Transition metal complexes with Girard reagents and their hydrazones. *J Serb Chem Soc* 2012;77:1129–55.

16. Čobeljić B, et al. Investigation of antitumor potential of Ni(II) complexes with tridentate PNO acylhydrazones of 2-(diphenylphosphino)benzaldehyde and monodentate pseudohalides. *J Biol Inorg Chem* 2016;21:145–62.
17. Milenković MR, Čobeljić B, Anđelković K, Turel I. Molecular Structures and Spin-States of Pseudohalide Metal Complexes with Hydrazones of Girard's T Reagent. *Eur J Inorg Chem* 2018;838–46.
18. Chandler JD, Day BJ. Thiocyanate: a potentially useful therapeutic agent with host defense and antioxidant properties. *Biochem Pharmacol* 2012;84:1381–7.
19. Vojinović-Ješić LS, et al. Transition metal complexes with Girard reagent-based ligands. Part V. Synthesis, characterization and crystal structure of pentagonal-bipyramidal manganese(II) complex with 2,6-diacetylpyridine bis (Girard-T hydrazone). *Inorg Chem Commun* 2010;13:1085–8.
20. Brađan G, et al. Synthesis, characterization and antimicrobial activity of pentagonal-bipyramidal isothiocyanato Co(II) and Ni(II) complexes with 2,6-diacetylpyridine bis(trimethylammoniumacetohydrazone). *J Coord Chem* 2016;69:801–11.
21. Brađan G, et al. Synthesis, characterization, DFT calculations and antimicrobial activity of pentagonal-bipyramidal Zn(II) and Cd(II) complexes with 2,6-diacetylpyridine-bis(trimethylammoniumacetohydrazone). *JCoord Chem* 2016;69: 2754–65.
22. Anđelković K, et al. Synthesis, characterization and crystal structures of two pentagonal-bipyramidal Fe(III) complexes with dihydrazone of 2,6-diacetylpyridine and Girard's T reagent. Anticancer properties of various metal complexes of the same ligand. *J Inorg Biochem* 2017;174:137–49.
23. Matić I.Z, et al. In vitro antitumor actions of extracts from endemic plant *Helichrysum zivojinii*. *BMC Complement Altern Med* 2013;13:36–48.
24. Ormerod MG. *Flow Cytometry. A Practical Approach*. Oxford University Press, Oxford, UK, 2000. 2.
25. Vijayalakshmi R, Kanthimathi M, Subramanian V, Unni Nair B. DNA cleavage by a chromium(III) complex. *Biochem Biophys Res Commun* 2000;271:731–4.
26. Lakowicz JR, Weber G. Quenching of fluorescence by oxygen. A probe for structural fluctuations in macromolecules. *Biochemistry* 1973;12:4161–70.