Serbian Biochemical Society
Eighth Conference

with international participation

University of Novi Sad – Rectorate Hall
16.11.2018. Novi Sad, Serbia

"***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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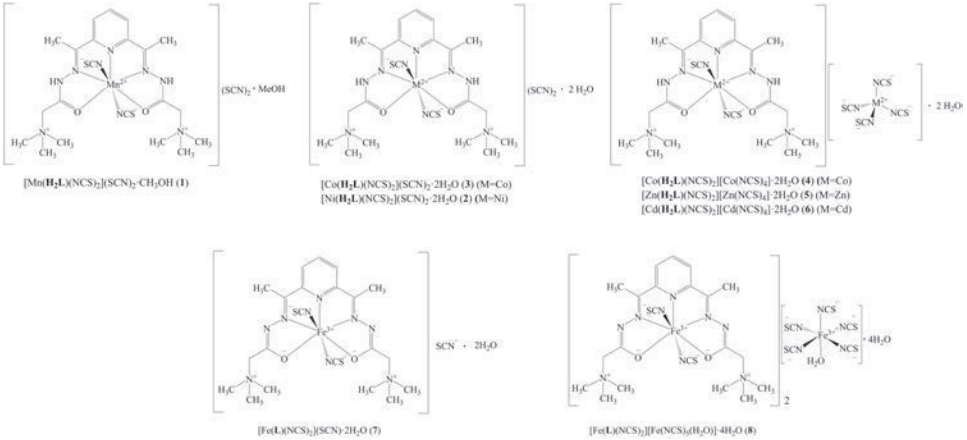
Pentagonal-bipyramidal complexes of 2,6-diacetylpyridine bis(acylhydrazone) ligands are attractive field of research not only in structural inorganic chemistry and magnetochemistry, but also in bioinorganic chemistry since they exhibit cytotoxic, antimicrobial, SOD mimetic, DNA/RNA binding and nuclease activity. In this work we investigated antitumor and antimicrobial activity of pentagonal-bipyramidal isothiocyanato complexes of Mn(II) ($[Mn(H_2L)(NCS)_2](SCN)_2 \cdot CH_3OH$) (1), Ni(II) ($[Ni(H_2L)(NCS)_2](SCN)_2 \cdot 2H_2O$) (2), Co(II) ($[Co(H_2L)(NCS)_2](SCN)_2 \cdot 2H_2O$ (3) and $[Co(H_2L)(NCS)_2][Co(NCS)_4] \cdot 2H_2O$) (4), Zn(II) ($[Zn(H_2L)(NCS)_2][Zn(NCS)_4] \cdot 2H_2O$) (5), Cd(II) ($[Cd(H_2L)(NCS)_2][Cd(NCS)_4] \cdot 2H_2O$) (6) and Fe(III) ($[Fe(L)(NCS)_2](SCN) \cdot 2H_2O$ (7) and $[Fe(L)(NCS)_2][Fe(NCS)_5(H_2O)] \cdot 4H_2O$) (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 properties [1–3]. The 2,6-diacetylpyridine bis(acylhydrazone) ligands possess at least five donor atoms (N_3O_2) 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 form [4]. Pentagonal-bipyramidal complexes of 2,6-diacetylpyridine bis(acylhydrazone) ligands have a wide spectrum of biological activities: cytotoxic [5], antimicrobial [6–8], SOD mimetic [9–11], DNA/RNA binding and nuclease activity [12–14]. 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 mixtures [15]. 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 activity [16]. Thiocyanate is an ambidentate pseudohalide ligand, which can be coordinated through nitrogen or sulfur donor atom as monodentate or as a bridge between metal centers [17]. In biological systems free SCN^- can be oxidized to hypothiocyanite by H_2O_2 produced in oxidative metabolism. Hypothiocyanite ($OSCN^-$) plays a role of an antimicrobial agent due to to its reactions with sulfhydryl groups of glycolytic enzymes and thiol-based antioxidants [18]. Here we report antitumor and antimicrobial activity of pentagonal-bipyramidal isothiocyanato complexes of Mn(II) ($[Mn(\mathbf{H_2L})(NCS)_2](SCN)_2\cdot CH_3OH$) (**1**), Ni(II) ($[Ni(\mathbf{H_2L})(NCS)_2](SCN)_2\cdot 2H_2O$) (**2**), Co(II) ($[Co(\mathbf{H_2L})(NCS)_2](SCN)_2\cdot 2H_2O$ (**3**) and $[Co(\mathbf{H_2L})(NCS)_2][Co(NCS)_4]\cdot 2H_2O$) (**4**), Zn(II) ($[Zn(\mathbf{H_2L})(NCS)_2][Zn(NCS)_4]\cdot 2H_2O$) (**5**), Cd(II) ($[Cd(\mathbf{H_2L})(NCS)_2][Cd(NCS)_4]\cdot 2H_2O$) (**6**) and Fe(III) ($[Fe(\mathbf{L})(NCS)_2](SCN)\cdot 2H_2O$) (**7**) and $[Fe(\mathbf{L})(NCS)_2][Fe(NCS)_5(H_2O)]\cdot 4H_2O$) (**8**) with the condensation product of 2,6-diacetylpyridine and Girard's T reagent (H_2LCl_2).

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 ($\mathbf{H_2L}Cl_2$) (Scheme 1) were obtained in the reactions of dihydrazone ligand, NH_4SCN 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, $FeCl_3\cdot 6H_2O$ and NH_4SCN. The same pentagonal-bipyramidal complex cation is present in both Fe(III) complexes, while the nature of their anions depends on mole ratio of NH_4SCN and $FeCl_3\cdot 6H_2O$ used in reaction [22].



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 ($\mathbf{H_2L}Cl_2$) was tested against five human cancer cell lines. The results of cytotoxic activity determined by MTT assay [23] 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* ± *S.D.*						
1	187.28±18.91	183.33±23.57	190.93±15.71	>200	187.66±21.38	198.35±2.86
2	186.85±22.77	187.85±17.18	194.51±7.76	170.82±33.19	144.42±7.69	46.79±1.52
3	96.75±11.82	135.01±1.55	76.99±10.54	176.90±11.44	129.77±28.30	92.08±7.82
4	70.46±13.59	76.51±17.69	38.66±3.49	115.25±28.19	79.53±14.47	54.57±14.24
5	190.10±17.15	111.37±4.74	122.80±0.30	170.72±41.42	130.85±5.77	121.78±14.49
6	71.06±5.95	59.40±14.21	72.98±6.10	160.46±16.44	84.76±3.08	86.89±13.94
7	110.98±24.45	115.69±7.86	75.94±5.20	171.84±34.78	98.72±1.55	80.42±0.06
8	106.61±15.92	87.27±4.06	48.33±6.87	116.11±31.00	70.45±7.60	40.19±0.73
H_2LCl_2	>200	>200	>200	>200	>200	>200
$MnCl_2 \cdot 4H_2O$	156.84±38.49	138.40±30.35	85.07±21.12	192.67±12.70	189.62±7.17	143.62±23.04
$NiCl_2 \cdot 6H_2O$	76.31±1.09	111.59±2.08	77.76±2.91	136.61±28.97	127.68±27.82	78.75±15.03
$CoCl_2 \cdot 6H_2O$	95.37±9.56	92.01±12.11	45.73±3.57	136.79±0.45	97.98±12.81	57.58±1.98

$ZnCl_2 \cdot 2H_2O$	>200	191.32±12.27	169.87±28.50	>200	200.00±0.00	105.61±25.82
$Cd(NO_3)_2 \cdot 4H_2O$	79.16±1.62	56.40±11.08	49.16±13.70	112.57±22.16	126.80±15.25	57.27±14.68
$FeCl_3 \cdot 6H_2O$	>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 IC_{50} and $2IC_{50}$ 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.

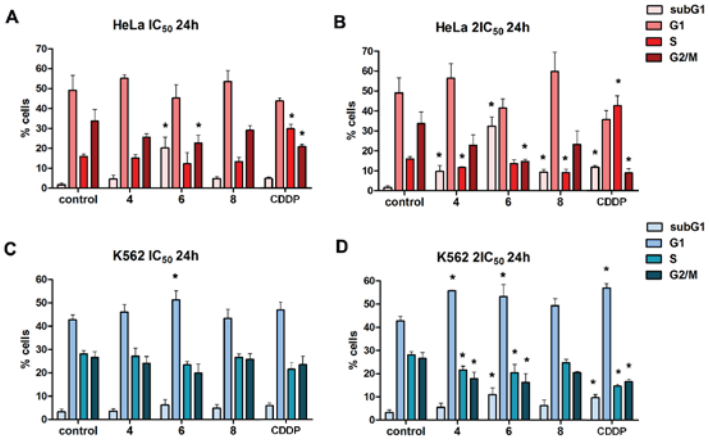


Figure 1. Changes in the cell cycle phase distribution of HeLa (A, B) and K562 cells (C, D) treated with IC_{50} and $2IC_{50}$ concentrations of complexes 4, 6 and 8, and cisplatin (CDDP).

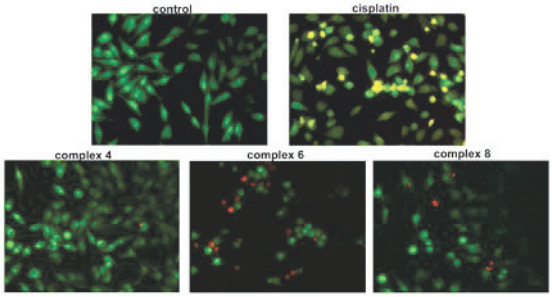


Figure 2. Photomicrographs of acridine orange/ethidium bromide-stained control HeLa cells and HeLa cells exposed to $2IC_{50}$ concentrations of the cisplatin and complexes 4, 6 and 8 for 24 h.

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.

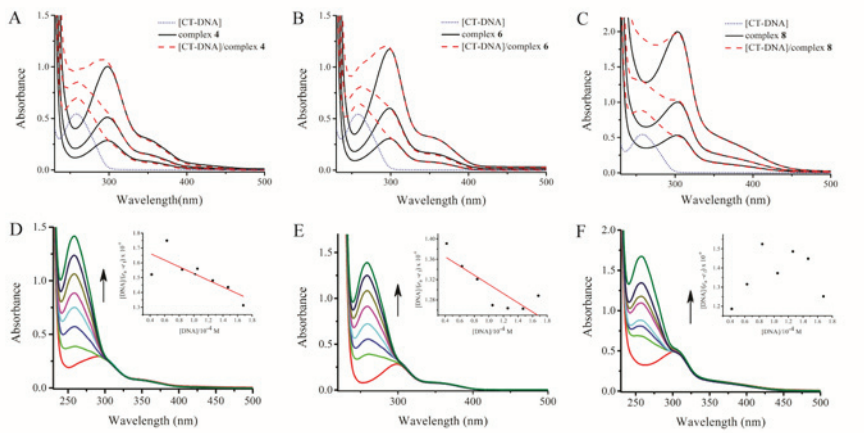


Figure 3. Changes in electronic absorption spectra of the complexes 4 (A), 6 (B) and 8 (C) (1, 2 and 4×10^{-5} M) after interactions with CT-DNA (8.4×10^{-5} M) and determination of binding constants by absorption titration of 4 (D), 6 (E) and 8 (F) at fixed concentration (1×10^{-5} M) with increasing 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 [DNA]/(ε_a–ε_f) *vs.* [DNA] and the binding constant (K_b) was calculated using eqn. $[DNA] \times (\varepsilon_a - \varepsilon_f)^{-1} = [DNA] \times (\varepsilon_b - \varepsilon_f)^{-1} + K_b^{-1} \times (\varepsilon_b - \varepsilon_f)^{-1}$ [25].

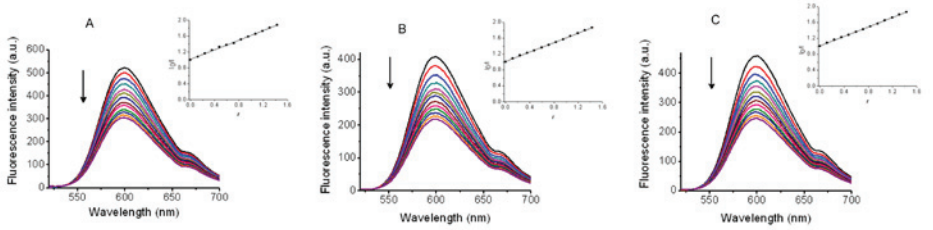


Figure 4. Emission spectra of ethidium bromide (EB) (2.5×10^{-5} M) bound to CT-DNA (8.4×10^{-5} M) and quenching of EB−CT-DNA system by 4 (A), 6 (B) and 8 (C) at increasing concentrations (1, 2, 3, 4, 5, 6×10^{-5} M). The arrows show that fluorescence intensity decreased with increasing

concentration of the complex. The insets show fluorescence quenching curves of EB bound to DNA at λ_{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*]/[CT-DNA],where I_0 and *I* represent the fluorescence intensities of EB−CT-DNA in absence and presence of the complex, and r = [complex]/[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).

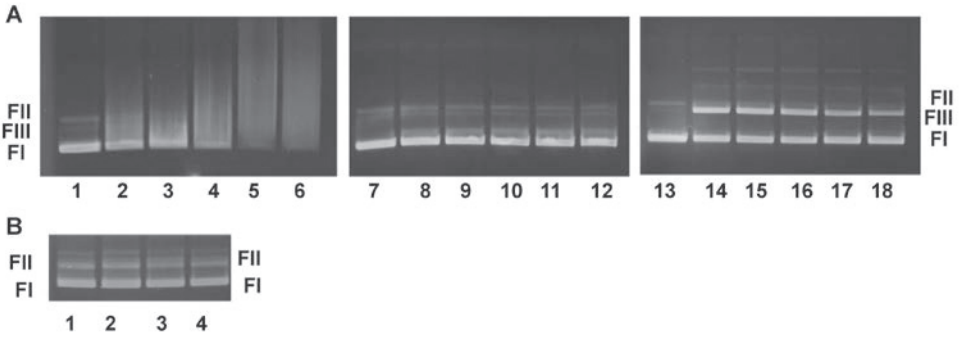


Figure 5. Agarose gel electrophoretic analysis of supercoiled forms FI and the open circular forms FII of plasmid pUC19 (2.6×10^{-9} M) (lanes 1, 7 and 13, (A); lane 1, (B)) after incubation (1.5 h at 37°C) with 0.25, 0.5, 1, 1.5 and 2 mM of complex 4, (lanes 2–6, respectively, panel A); with 0.25, 0.5,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

Acknowledgements

This work was supported by the Ministry of Education, Science and Technological development of the Republic of Serbia (Grant OI 172055 and Grant OI 175011).

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