Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 82 (4) 389–398 (2017)
JSCS–4974
UDC 546.742+547.415.1:615.28–188:576+615.9
Original scientific paper

In vitro antimicrobial activity and cytotoxicity of nickel(II) complexes with different diamine ligands

NENAD S. DRAŠKOVIĆ[1#], BILJANA Đ. GLIŠIĆ[2*#], SANDRA VOJNOVIC[3], JASMINA NIKODINOVIC-RUNIC[3**] and MILOŠ I. DJURAN[2#]

[1]*University of Priština, Faculty of Agriculture, Kopaonička bb, 38228 Lešak, Serbia,* [2]*Department of Chemistry, Faculty of Science, University of Kragujevac, R. Domanovića 12, 34000 Kragujevac, Serbia and* [3]*Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, 11000 Belgrade, Serbia*

(Received 13 January, revised 30 January, accepted 13 February 2017)

Abstract: Three diamines, 1,3-propanediamine (1,3-pd), 2,2-dimethyl-1,3-propanediamine (2,2-diMe-1,3-pd) and (±)-1,3-pentanediamine (1,3-pnd), were used for the synthesis of nickel(II) complexes **1**–**3**, respectively, of the general formula $[Ni(L)_2(H_2O)_2]Cl_2$. The stoichiometries of the complexes were confirmed by elemental microanalysis, and their structures were elucidated by spectroscopic (UV–Vis and IR) and molar conductivity measurements. The complexes **1**–**3**, along with $NiCl_2{\cdot}6H_2O$ and the diamine ligands, were evaluated against a panel of microbial strains that are associated with skin, wound, urinary tract and nosocomial infections. The obtained results revealed no significant activity of **1**–**3** against the investigated bacterial strains. On the other hand, they showed good antifungal activity against pathogenic *Candida* strains, with minimum inhibitory concentration (*MIC*) values in the range from 15.6 to 62.5 μg mL^{-1}. The best anti-*Candida* activity was observed for complex **2** against *C. parapsilosis*, while the least susceptible to the effect of the complexes was *C. krusei*. The antiproliferative effect on normal human lung fibroblast cell line MRC-5 was also evaluated in order to determine the therapeutic potential of nickel(II) complexes **1**–**3**. These complexes showed lower negative effects on the viability of the MRC-5 cell line than the clinically used nystatin and comparable selectivity indexes to that of this antifungal drug.

Keywords: nickel(II) complexes; diamines; antimicrobial activity; *Candida*; cytotoxicity.

INTRODUCTION

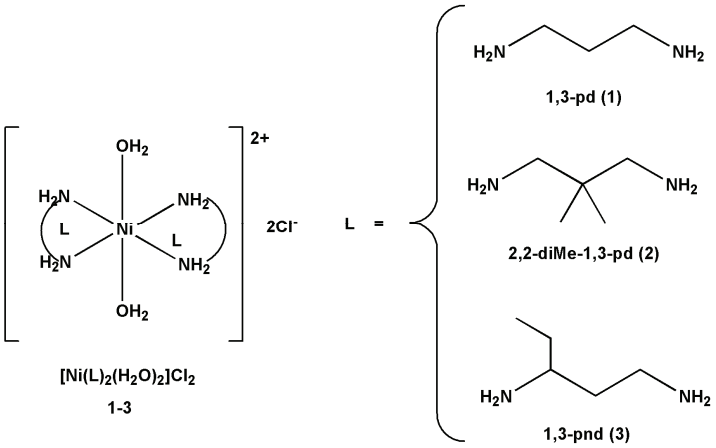
The field of medicinal application of metal-based compounds has attracted widespread attention of researchers over the decades.[1] This broad interest is due

*,** Corresponding authors. E-mail: (*)bglisic@kg.ac.rs; (**)jasmina.nikodinovic@gmail.com
Serbian Chemical Society member.
https://doi.org/10.2298/JSC170113026D

to the constant demand of medicinal chemistry for innovation and input of novel metal-based compounds to cope with very important challenges, such as broader spectrum of activity, selectivity, reduced toxicity and emerging resistance. The development of a new therapeutic agent is a multi-stage process involving synthesis, characterization, evaluation of biological activity, and pre-clinical and clinical testing. Considering the fact that this process becomes very expensive in the later stage, it is important to use the best possible models in the identification of those compounds that have the desired biological activity. Furthermore, it was found previously that microorganisms and infections play major roles in carcinogenesis, as well as in antitumor response.[2] Of the 12.7 million new cancer cases that occurred in 2008, around 2 million could be attributed to infections. Bacteremia is a major cause of life-threatening complications in patients with cancer, who are at extremely high risk for infections caused by antibiotic-resistant Gram-negative bacteria. Invasive candidiasis is the fourth most common bloodstream infection (surpassing many bacterial pathogens) with mortality rates remaining disturbingly high at 40 %.[3] More than 17 different *Candida* species are known to be etiological agents of human infection, however, more than 90 % of invasive infections are caused by *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. krusei*.[4] Considering this and in the view of the global problem of multi-drug resistant microbial strains, the search for new antibacterial and antifungal therapeutics is of paramount importance.

Nickel was considered for many years as an element without important biological significance, until its existence in the active center of the enzyme urease was established in 1975.[5,6] Since then, Ni^{2+} was confirmed to be competitive antagonists with both Mg^{2+} and Ca^{2+} and the presence of nickel was established in the active sites of different metallo-enzymes, and hence, interest for the evaluation of its biological properties has rapidly expanded.[7,8] It was found that chronic exposure to nickel could be connected with increased risk of lung cancer, cardiovascular disease, neurological deficits, developmental deficits in childhood and high blood pressure.[9] Nickel is also considered as a potential allergen, which may cause contact dermatitis.[10] Nevertheless, a broad spectrum of beneficial biological activities of various nickel(II) complexes has hitherto been reported. Nickel(II) complexes were reported to act as anticonvulsant,[11] antiepileptic,[12] antibacterial,[13] antifungal,[14] antileishmanial,[15] antioxidant[16,17] and antiproliferative agents.[18] Considering this, in the present study, three diamines, 1,3-propanediamine (1,3-pd), 2,2-dimethyl-1,3-propanediamine (2,2-diMe-1,3-pd) and (±)-1,3-pentanediamine (1,3-pnd) were used for the synthesis of nickel(II) complexes of the general formula $[Ni(L)_2(H_2O)_2]Cl_2$ (Scheme 1). Although the synthesis of these complexes was reported previously,[19–22] their antimicrobial effects have not been investigated. In order to determine the therapeutic potential

of these complexes, their antiproliferative effect on the normal human lung fibroblast cell line MRC-5 was also evaluated.



Scheme 1. Structural representation of $[Ni(L)_2(H_2O)_2]Cl_2$ complexes **1**–**3** (L = 1,3-pd (**1**), 2,2-diMe-1,3-pd (**2**) and 1,3-pnd (**3**)).

EXPERIMENTAL

Reagents

Distilled water was demineralized and purified to a resistance of greater than 10 MΩ cm. Nickel(II) chloride hexahydrate, 1,3-propanediamine (1,3-pd), 2,2-dimethyl-1,3-propanediamine (2,2-diMe-1,3-pd) and (±)-1,3-pentanediamine (1,3-pnd) were purchased from Sigma–Aldrich. All the employed chemicals were of analytical reagent grade.

*Synthesis of the nickel(II) complexes **1**–**3***

The nickel(II) complexes with the above-mentioned diamine ligands were synthesized by modification of a previously described method.[19] The corresponding diamine (0.02 mol; 1.7 mL of 97 % 1,3-pd, ρ = 0.887 g mL^{-1}; 2.4 mL of 99 % 2,2-diMe-1,3-pd, ρ = 0.851 g mL^{-1} and 2.4 mL of 98 % 1,3-pnd, ρ = 0.855 g mL^{-1}) was added slowly under stirring to a solution containing 0.01 mol of $NiCl_2 \cdot 6H_2O$ (2.38 g) in 10.0 mL of water. The formed nickel(II) hydroxide was removed by filtration and the filtrate was stirred at 40 °C for 15 min, and then left standing at ambient temperature to evaporate slowly to a volume of 3.0 mL. The concentrated solution was then stored in refrigerator and purple crystals of the nickel(II) complexes had formed after two days. These crystals were filtered off and dried at ambient temperature. The yield was 83 % for $[Ni(1,3\text{-pd})_2(H_2O)_2]Cl_2$ (**1**; 2.61 g), 77 % for $[Ni(2,2\text{-diMe-}1,3\text{-pd})_2(H_2O)_2]Cl_2$ (**2**; 2.85 g) and 79 % for $[Ni(1,3\text{-pnd})_2(H_2O)_2]Cl_2$ (**3**; 2.92 g).

Measurements

Elemental microanalyses of the nickel(II) complexes for carbon, hydrogen and nitrogen were performed by the Microanalytical Laboratory, Faculty of Chemistry, University of Belgrade. The IR spectra were recorded as KBr pellets on a Perkin Elmer Spectrum One spectrometer over the wavenumber range 4000–450 cm^{-1}. The UV–Vis spectra were recorded over the wavelength range of 1100–190 nm on a Shimadzu UV-1800 spectrophotometer after dissolving the corresponding nickel(II) complex in water. The concentration of the nickel(II)

complexes was 5×10^{-2} M. The molar conductivities were measured at room temperature on a Crison multimeter MM 41 digital conductivity-meter. The concentration of the aqueous solutions of nickel(II) complexes used for conductivity measurements was 1×10^{-3} M.

Analytical and spectral data of the synthesized compounds are given in the Supplementary material to this paper.

Determination of the biological activity

Nickel(II) complexes **1**–**3**, $NiCl_2\cdot6H_2O$ and the diamine ligands were dissolved in distilled water to give stock solutions of 50 mg mL^{-1}, which were used immediately for biological assessment of their activities. The *MIC* concentrations (concentration value corresponding to the lowest concentration that inhibited the growth after 24 h at 37 °C) were determined according to the standard broth microdilution assays, recommended by the National Committee for Clinical Laboratory Standards (M07-A8) for bacteria and Standards of the European Committee on Antimicrobial Susceptibility Testing (EDef 7.1.). The highest concentration used was 500 μg mL^{-1}. The test organisms included *Pseudomonas aeruginosa* PAO1 (NCTC 10332), *Staphylococcus aureus* (NCTC 6571), *Candida albicans* (ATCC 10231), *C. glabrata* (ATCC 2001), *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 14243). The inoculums were 10^5 colony-forming units, *CFU*, per mL for the bacteria and 10^4 CFU mL^{-1} for the *Candida* strains.

Cell viability was tested by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[23] The assay was performed using human lung fibroblasts (MRC-5) after 48 h of cell incubation in the media containing the compounds at concentrations ranging from 0.1–500 μg mL^{-1}. The MRC-5 cell line was maintained in RPMI-1640 medium supplemented with 100 μg mL^{-1} streptomycin, 100 U mL^{-1} penicillin and 10 vol.% fetal bovine serum (FBS), all from Sigma, as a monolayer (1×10^4 cells per well) and grown in a humidified atmosphere of 95 % air and 5 % CO_2 at 37 °C. The extent of MTT reduction was measured spectrophotometrically at 540 nm using a Tecan Infinite 200 Pro multiplate reader (Tecan Group, Männedorf, Switzerland), and the cell survival was expressed as percentage of the control (untreated cells).

RESULTS AND DISCUSSION

*Synthesis and structural features of the nickel(II) complexes **1**–**3***

Three nickel(II) complexes of the general formula $[Ni(L)_2(H_2O)_2]Cl_2$, where L stands for bidentately coordinated 1,3-pd (**1**), 2,2-diMe-1,3-pd (**2**) or 1,3-pnd (**3**), were prepared in high yields (≈80 %) by reacting $NiCl_2\cdot6H_2O$ with the corresponding diamine in 1:2 mole ratio in water using a modified procedure reported previously.[19] The crystal structures of the $[Ni(L)_2(H_2O)_2]^{2+}$ complexes were previously determined by single-crystal X-ray diffraction analysis.[19–22] In this study, spectroscopic (UV–Vis and IR) and conductivity measurements were used for structural characterization of the $[Ni(L)_2(H_2O)_2]Cl_2$ complexes.

The UV–Vis spectra of the investigated complexes **1**–**3** are presented in Fig. 1, while the wavelengths of the maximum absorption (λ_{max} / nm) and molar extinction coefficients (ε / M^{-1} cm^{-1}), determined immediately after dissolution of the complexes, are listed in Table I. The shape of the UV–Vis spectra for the investigated complexes was similar to that of the octahedral $[Ni(en)_2(H_2O)_2]CO_3$

complex (en is bidentately coordinated ethylenediamine) with the same N_4O_2 coordination environment.[24] In accordance with the previously established results for the $[Ni(en)_2(H_2O)_2]CO_3$ complex,[24] the interpretation of UV–Vis spectra of the presently investigated complexes **1**–**3** was realized using an octahedral model (O_h): $^3A_{2g} \rightarrow {}^3T_{2g}(F)$ (band I), $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ (band III) and $^3A_{2g} \rightarrow {}^3T_{1g}(P)$ (band IV). In addition, in each spectrum, there was a shoulder at approximately 735 nm (band II, Table I), which occurs on the higher-energy side of the spin allowed band I. As was previously found, this shoulder arises from a spin forbidden triplet-to-singlet transition, $^3A_{2g} \rightarrow {}^1E_g(D)$.[24,25] As could be seen from Fig. 1, the absorption maxima of the bands I and III for the investigated nickel(II) complexes were slightly shifted to higher energy in the following order **1** > **3** > **2**. Moreover, the molar absorptivity of the absorption maxima for these bands increased in the same order. These differences in the spectra could be attributed to the presence of the substituent in the six-membered 1,3-propanediamine ring of the corresponding nickel(II) complex, *i.e.*, two methyls for **2** and an ethyl for **3**. It could be assumed that these substituents affect some changes in the strain of the six-membered 1,3-propandiamine ring. Moreover, all absorption maxima of the investigated complexes **1**–**3** were shifted to lower energies with respect to those for the $[Ni(en)_2(H_2O)_2]^{2+}$ complex.[24] This shifting results from the presence of a six-membered 1,3-propanediamine ring in **1**–**3**, which is less strained than the five-membered ethylenediamine ring in the $[Ni(en)_2(H_2O)_2]^{2+}$ complex.

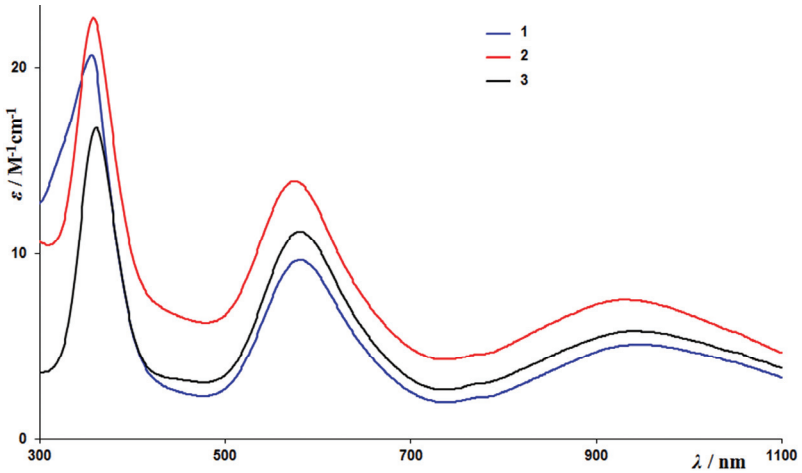


Fig. 1. Electronic absorption spectra of the investigated nickel(II) complexes **1**–**3** measured in water ($c = 5\times10^{-2}$ M).

The IR spectroscopic data for the nickel(II) complexes are listed in the Supplementary material to this paper and are consistent with the structural formula presented in Scheme 1. The IR spectra of these complexes recorded in the range

of 4000–450 cm^{-1} showed the expected peaks attributable to the coordinated diamine and water ligands. Thus, a broad absorption in the 3400–3300 cm^{-1} region attributed to the stretching vibration of OH confirmed the presence of a coordinated water molecule.[26] Moreover, the complexes exhibited two very strong and sharp bands at approximately 3300 and 3200 cm^{-1}, which were assigned to the asymmetric and symmetric stretching vibration of the coordinated amino group, respectively.[22]

TABLE I. Electronic absorption data for the nickel(II) complexes **1**–**3**. For comparison the corresponding data for the previously reported $[Ni(en)_2(H_2O)_2]CO_3$ complex is given[24]

Complex		Absorption		Assignments
		λ / nm	ε / M^{-1} cm^{-1}	
$[Ni(en)_2(H_2O)_2]CO_3$	I	905	–	$^3A_{2g} \rightarrow {}^3T_{2g}(F)$
	II	690	–	$\rightarrow {}^1E_g(D)$
	III	555	–	$\rightarrow {}^3T_{1g}(F)$
	IV	349	–	$\rightarrow {}^3T_{1g}(P)$
$[Ni(1,3\text{-pd})_2(H_2O)_2]Cl_2$ (**1**)	I	946	5.1	
	II	738	2.0	
	III	581	9.7	
	IV	356	20.7	
$[Ni(2,2\text{-diMe-}1,3\text{-pd})_2(H_2O)_2]Cl_2$ (**2**)	I	931	7.5	
	II	734	4.3	
	III	575	13.9	
	IV	358	22.7	
$[Ni(1,3\text{-pnd})_2(H_2O)_2]Cl_2$ (**3**)	I	941	5.8	
	II	734	2.6	
	III	580	11.2	
	IV	361	16.8	

Molar conductivity values for the nickel(II) complexes **1**–**3**, being approximately 250 Ω^{-1} cm^2 mol^{-1} (see Supplementary material), are sufficiently high to assess the non-coordinated nature of the two chloride anions, *i.e.*, these values in water solvent are in agreement with 1:2 electrolytic nature of the synthesized complexes.[27]

*Biological activity of the nickel(II) complexes **1**–**3***

In vitro antimicrobial activity assays of nickel(II) complexes **1**–**3**, $NiCl_2 \cdot 6H_2O$ and the corresponding diamine ligands revealed no significant activity against two bacterial strains (*P. aeruginosa* PAO1 and *S. aureus*) even at 500 μg mL^{-1} (data not shown), while *MIC* values against the pathogenic *Candida* strains were between 15.6–62.5 μg mL^{-1} for complexes **1**–**3** and 250 μg mL^{-1} for the inorganic salt (Table II). Therefore, a certain level of selectivity of **1**–**3** towards fungal strains could be concluded. The best anti-*Candida* activity was that of complex **2** against *C. parapsilosis*, while *C. krusei* was the least susceptible to the effects of

the complexes. In contrast, nickel(II) thiohydrazide and thiodiamine complexes exhibited significant activity towards *P. aeruginosa* and *Escherichia coli*, and comparable activity against a selection of fungal *Aspergillus* strains.[28] Antifungal activity of $NiCl_2 \cdot 6H_2O$ and nickel(II) complexes derived from amino sugars against *C. albicans* was reported by Yano *et al*. with *MIC* values in the 200–250 μM range, which is 2.5–5-fold higher in comparison to the *MIC* values of **1**–**3**.[29] Furthermore, the complexes from the present study showed better anti-*Candida* activities in comparison to nickel(II) complexes with pyrazoline-based ligand, which had *MIC* values ranging from 100 – 1000 μg mL^{-1}.[30] It was shown that certain selectivity against *Candida* strains by nickel(II) compounds was due to the competitive inhibition of fungal chitinase (chitin-degradation enzyme).[29]

TABLE II. Minimal inhibitory concentrations (*MIC* / μg mL^{-1}) against *Candida* strains and IC_{50} values against MRC-5 cells (concentration that inhibits 50 % of cell growth after treatment with the tested compounds, μg mL^{-1}); the results are from three independent experiments, each performed in triplicate. Standard deviations were within 1–3 %

Compound	*C. albicans* ATCC 10231	*C. glabrata* ATCC 2001	*C. parapsilosis* ATCC 22019	*C. krusei* ATCC 14243	MRC-5
1	31.2	31.2	31.2	62.5	500
2	31.2	31.2	15.6	62.5	80
3	31.2	31.2	31.2	62.5	500
$NiCl_2 \cdot 6H_2O$	250	250	250	250	100
1,3-pd	>500	>500	>500	>500	>500
2,2-diMe-1,3-pd	>500	>500	>500	>500	100
1,3-pnd	>500	>500	>500	>500	50
Nystatin	4	2	2	8	40

In parallel, to determine the applicability of complexes **1**–**3** as potential antifungals, their *in vitro* cytotoxicity against healthy human lung fibroblasts was examined (Table II, Fig. 2). While the ligands exerted no activity against *Candida* strains at 500 μg mL^{-1}, 2,2-diMe-1,3-pd and 1,3-pnd had IC_{50} values of 100 and 50 μg mL^{-1}, respectively. 1,3-Propanediamine was not cytotoxic even at 500 μg mL^{-1} (Table II). Accordingly, **2** was the most cytotoxic of the nickel(II) complexes, while **1** and **3** had IC_{50} values of 500 μg mL^{-1}. The inorganic salt $NiCl_2 \cdot 6H_2O$ had a toxic effect on the cells in a dose-dependent manner (IC_{50} value of 100 μg mL^{-1}), while the cytotoxicity of **1**–**3** did not follow this trend (Fig. 2). This may be due to differing dissociation dynamics of the complexes and different toxicity of the ligands. Thus, the selectivity index for the complexes was between 1.3 and 16, while the antiproliferative effect of the inorganic salt was higher than its antifungal effect (Table II). Although the *MIC* values of **1**–**3** were 8–16-fold higher in comparison to that of the clinically used nystatin, the selectivity indexes were comparable. This finding is encouraging for further development of nickel(II)-based complexes for antifungal treatment.

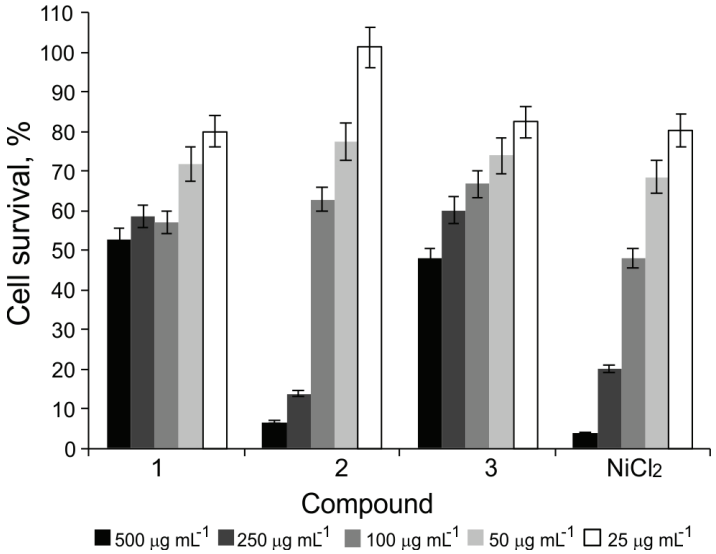


Fig. 2. *In vitro* cytotoxic effect on healthy human fibroblasts (MRC-5) of various concentrations of nickel(II) compounds upon 48 h treatment.

CONCLUSIONS

This work presents a modified procedure for the preparation of $[Ni(L)_2(H_2O)_2]Cl_2$ complexes **1**–**3** in high yields. The octahedral geometry of these complexes was confirmed by spectroscopic and conductivity measurements. *In vitro* antimicrobial activity assays of these complexes showed their good selectivity towards the investigated *Candida* strains. The best anti-*Candida* activity was observed for complex **2** against *C. parapsilosis*, while the least susceptible to the effect of complexes was *C. krusei*. Moreover, an *in vitro* cytotoxicity study showed that complex **2** was the most cytotoxic against healthy human lung fibroblasts. This arises from the presence of two methyl substituents in the six-membered 1,3-propanediamine ring of **2**, indicating that better antimicrobial and cytotoxic activities of bis(diamine)nickel(II) complexes could be achieved by structural modification of the chelated diamine ligand. The obtained results are encouraging for further development of nickel(II) complexes with diamine ligands as antifungal agents. A study in this sense is in progress.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of the synthesized compounds are available electronically at the pages of the journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgement. This work was funded in part by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project Nos. 172036 and 173048).

И З В О Д

IN VITRO АНТИМИКРОБНА АКТИВНОСТ И ЦИТОТОКСИЧНОСТ КОМПЛЕКСА НИКЛА(II) СА РАЗЛИЧИТИМ ДИАМИНСКИМ ЛИГАНДИМА

НЕНАД С. ДРАШКОВИЋ[1], БИЉАНА Ђ. ГЛИШИЋ[2], САНДРА ВОЈНОВИЋ[3], ЈАСМИНА НИКОДИНОВИЋ-РУНИЋ[3] И МИЛОШ И. ЂУРАН[2]

[1]Пољопривредни факултет, Универзитет у Приштини, Копаоничка бб, 38228 Лешак, [2]Институт за хемију, Природно–математички факултет, Универзитет у Крагујевцу, Р. Домановића 12, 34000 Крагујевац и [3]Институт за молекуларну генетику и генетичко инжењерство, Универзитет у Београду, Војводе Степе 444а, 11000 Београд

Три диамина, 1,3-пропандиамин (1,3-pd), 2,2-диметил-1,3-пропандиамин (2,2-diMe-1,3-pd) и (±)-1,3-пентандиамин (1,3-pnd), коришћена су за синтезу никал(II) комплекса **1**–**3** опште формуле $[Ni(L)_2(H_2O)_2]Cl_2$. Комплекси су окарактерисани применом елементалне микроанализе, UV–Vis и IR спектроскопије и мерењем моларне проводљивости. Никал(II) комплекси **1**–**3**, $NiCl_2{\cdot}6H_2O$ и одговарајући диамини су испитивани као потенцијални антимикробни агенси према различитим сојевима бактерија и гљива, који могу узроковати инфекције коже и рана, као и уринарне и интрахоспиталне инфекције. Добијени резултати су показали да комплекси **1**–**3** немају значајну активност према испитиваним сојевима бактерија. Насупрот томе, ови комплекси показују добру активност према испитиваним патогеним сојевима гљива, при чему су вредности минималне инхибиторне концентрације (*MIC*) у опсегу од 15,6 до 62,5 μg mL^{-1}. Највећу антифунгалну активност према *C. parapsilosis* показује комплекс **2**, док је активност комплекса најмања према *C. krusei*. У циљу одређивања терапеутског потенцијала ових комплекса, испитивана је њихова антипролиферативна активност према нормалној ћелијској линији фибробласта плућа. Добијени резултати су показали да су комплекси никла(II) мање токсични на MRC-5 ћелијској линији у односу на нистатин и да имају индексе селективности сличне овом антифунгалном агенсу.

(Примљено 13. јануара, ревидирано 30. јануара, прихваћено 13. фебруара 2017)

REFERENCES

1. E. Alessio, *Bioinorganic Medicinal Chemistry*, Wiley–VCH, Weinheim, Germany, 2011
2. N. D. Savić, D. R. Milivojevic, B. Đ. Glišić, T. Ilic-Tomic, J. Veselinovic, A. Pavic, B. Vasiljevic, J. Nikodinovic-Runic, M. I. Djuran, *RSC Adv.* **6** (2016) 13193
3. M. A. Pfaller, S. A. Messer, G. J. Moet, R. N. Jones, M. Castanheira, *Int. J. Antimicrob. Agents* **38** (2011) 65
4. J. C. Sardi, L. Scorzoni, T. Bernardi, A. M. Fusco-Almeida, M. J. Mendes Giannini, *J. Med. Microbiol.* **62** (2013) 10
5. N. E. Dixon, C. Gazzola, R. L. Blakeley, B. Zerner, *J. Am. Chem. Soc*. **97** (1975) 4131
6. C. Tserkezidou, A. G. Hatzidimitriou, G. Psomas, *Polyhedron* **117** (2016) 184
7. R. R. Crichton, *Biological Inorganic Chemistry: An Introduction*, Elsevier, Amsterdam, 2003
8. D. M. Di Toro, H. E. Allen, H. L. Bergman, J. S. Meyer, P. R. Paquin, R. C. Santore, *Environ. Toxicol. Chem.* **20** (2001) 2383
9. Y. Chervona, A. Arita, M. Costa, *Metallomics* **4** (2012) 619
10. J. P. Thyssen, A. Linneberg, T. Menné, J. D. Johansen, *Contact Dermatitis* **57** (2007) 287
11. G. Morgant, N. Bouhmaida, L. Balde, N. E. Ghermani, J. d'Angelo, *Polyhedron* **25** (2006) 2229
12. P. Bombicz, E. Forizs, J. Madarasz, A. Deak, A. Kalman, *Inorg. Chim. Acta* **315** (2001) 229

13. K. C. Skyrianou, E. K. Efthimiadou, V. Psycharis, A. Terzis, D. P. Kessissoglou, G. Psomas, *J. Inorg. Biochem.* **103** (2009) 1617
14. K. Alomar, A. Landreau, M. Allain, G. Bouet, G. Larcher, *J. Inorg. Biochem.* **126** (2013) 76
15. I. Ramírez-Macías, C. R. Maldonado, C. Marín, F. Olmo, R. Gutiérrez-Sánchez, M. J. Rosales, M. Quirós, J. M. Salas, M. Sánchez-Moreno, *J. Inorg. Biochem.* **112** (2012) 1
16. K. C. Skyrianou, F. Perdih, A. N. Papadopoulos, I. Turel, D. P. Kessissoglou, G. Psomas, *J. Inorg. Biochem*. **105** (2011) 1273
17. P. Sathyadevi, P. Krishnamoorthy, E. Jayanthi, R. R. Butorac, A. H. Cowley, N. Dharmaraj, *Inorg. Chim. Acta* **384** (2012) 83
18. F. Bisceglie, S. Pinelli, R. Alinovi, M. Goldoni, A. Mutti, A. Camerini, L. Piola, P. Tarasconi, G. Pelosi, *J. Inorg. Biochem.* **140** (2014) 111
19. S. Koner, A. Ghosh, C. Pariya, D. Das, H. Kikuchi, K.-I. Okamoto, R. Ikeda, *J. Mol. Struct.* **345** (1995) 265
20. C.-H. Kim, S.-G. Lee, *Acta Crystallogr., C: Cryst. Struct. Commun*. **58** (2002) 421
21. I. O. Fritsky, J. Świątek-Kozłowska, A. Dobosz, T. Yu. Sliva, N. M. Dudarenko, *Inorg. Chim. Acta* **357** (2004) 3746
22. S. Chattopadhyay, P. Chakraborty, M. G. B. Drew, A. Ghosh, *Inorg. Chim. Acta* **362** (2009) 502
23. M. B. Hansen, S. E. Nielsen, K. Berg, *J. Immunol. Methods* **119** (1989) 203
24. R. Olar, G. V. Scaeteanu, I. D. Vlaicu, L. Marutescu, M. Badea, *J. Therm. Anal. Calorim.* **118** (2014) 1195
25. D. D. Radanović, U. Rychlewska, B. Warżajtis, M. S. Cvijović, M. Dj. Dimitrijević, M. I. Djuran, *Polyhedron* **26** (2007) 4799
26. L. K. Thompson, T. C. Woon, D. B. Murphy, E. J. Gabe, F. L. Lee, Y. Le Page, *Inorg. Chem*. **24** (1985) 4719
27. M. Sneed, J. Maynard, *General Inorganic Chemistry*, New York, 1942
28. A. K. Mishra, N. K. Kaushik, *Spectrochim. Acta, A* **69** (2008) 842
29. S. Yano, S. Inoue, R. Nouchi, K. Mogami, Y. Shinohara, Y. Yasuda, M. Kato, T. Tanase, T. Kakuchi, Y. Mikata, T. Suzuki, Y. Yamamoto, *J. Inorg. Biochem.* **69** (1998) 15
30. I. Ali, W. A. Wani, A. Khan, A. Haque, A. Ahmad, K. Saleem, N. Manzoor, *Microb. Pathog.* **53** (2012) 66.