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New mixed-ligand Ni(II) and Zn(II) macrocyclic complexes with bridged (*endo*,*endo*)-bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate: Synthesis, characterization, antimicrobial and cytotoxic activity

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Abstract: New carboxylate complexes of the tetraazamacrocyclic ligand *N*,*N'*,*N''*,*N'''*-tetrakis(2-pyridilmethyl)-1,4,8,11-tetraazacyclotetradecane (tpmc) with Ni(II) and Zn(II) as central ions were prepared. In mixed-ligand complexes (*endo*,*endo*)-bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate dianion ($C_9H_8O_4^{2-}$) is also coordinated to metal ions. The complexes were characterized by elemental analysis (C, H, N), FTIR and UV–Vis spectroscopy, molar conductivity determination and magnetic susceptibility measurement at room temperature. The analytical data of the complexes show the formation of binuclear $[Ni_2(C_9H_8O_4)tpmc](ClO_4)_2\cdot 4H_2O$ and tetranuclear $[Zn_4(C_9H_8O_4)(tpmc)_2](ClO_4)_6\cdot \cdot CH_3CN\cdot KClO_4\cdot 4H_2O$ complexes. In tetranuclear Zn(II) complex bicyclic dicarboxylate ligand is most likely to be bridge coordinated, and in binuclear Ni(II) complex it is coordinated in a combined bridged manner with chelate rings formation. In both complexes macrocyclic ligand was *exo* coordinated, out of cyclam ring and adopts a boat conformation. The Zn(II) complex is one of the rare tetranuclear Zn(II)-tpmc complexes with carboxylate ion bridging two Zn_2tpmc units. The complexes were tested for antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6633), Gram-negative bacterium *Escherichia coli* (ATCC 25922), and yeast *Candida albicans* (ATCC 10231), and were screened for antiproliferative activity against human cervix adenocarcinoma (HeLa) and human myelogenous leukemia (K562) cell lines.

Keywords: Ni(II) and Zn(II) complexes; tpmc; bicyclodicarboxylate; microbiological and antiproliferative activity.

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INTRODUCTION

Macrocyclic ligands are attractive because they offer a wide variety of donor atoms, ionic charges, coordination numbers and different geometries of their transition metal complexes.[1] Macrocyclic complexes have important implications for a range of chemical and biochemical applications.[2] Many of them have been examined because of their potential as dyestuffs or pigments.[3] Some of them are involved in a number of biological processes such as photosynthesis and dioxygen transport,[4] and also have high potential in antitumor therapy.[5] The formation of macrocyclic complexes depends significantly on the dimension of the internal cavity, on the rigidity of the macrocycles, on the nature of its donor atoms and on the complexing properties of the anion involved in the coordination.

Zinc is an important transition metal in the biological intracellular environment of living organisms[6] which plays critical roles in important physiological process. Zinc complexes can adopt diverse geometries with different coordination numbers. Zinc-containing carboxylate-bridged complexes have varied structural motifs in hydrolytic metalloenzymes.[7] Moreover, they have good pharmacological profiles[8] as radio-protective agents and tumor photosensitizers.[9,10] Zinc complexes have kindled interest, as they are less toxic than complexes of non-essential metals and have been used as drugs for the treatment of Alzheimer's disease, showing bactericidal, antimicrobial and cytotoxic activity.[11a] Nickel(II) complexes of macrocyclic ligands are well known to be biologically important and interesting because of their anticarcinogenic, antibacterial and antifungal properties. Also, they have been screened for their medicinal properties because they posses some degree of cytotoxic activity.[11b]

Nickel Schiff base complexes have a strong role in bioinorganic chemistry and redox enzyme systems.[7]

The bicyclic dicarboxylate ligand ((*endo*,*endo*)-bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate, Fig 1a) was used as additional ligand in our previously described Co(II) and Co(III) mono- and binuclear mixed-ligand complexes[12,13] and Cu(II) tetranuclear complex with tpmc (tpmc = *N*,*N*′,*N*″,*N*‴-tetrakis(2-pyridylmethyl)-1,4,8,11-tetraazacyclotetradecane, Fig 1b).[14]

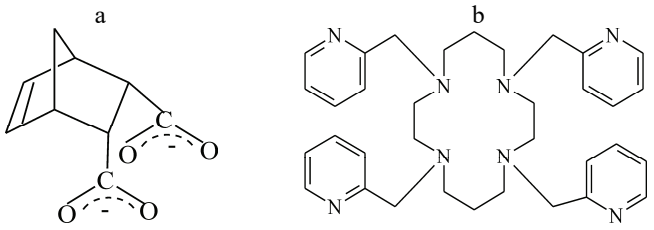

Fig. 1. Ligands. a) (*endo*,*endo*)-bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate dianion ($C_9H_8O_4^{2-}$); b) *N*,*N*′,*N*″,*N*‴-tetrakis(2-pyridilmethyl)-1,4,8,11-tetraazacyclotetradecane (tpmc).

This paper reports the synthesis, characterization, antimicrobial and cytotoxic activity of binuclear $[Ni_2(C_9H_8O_4)tpmc](ClO_4)_2 \cdot 4H_2O$ and tetranuclear $[Zn_4(C_9H_8O_4)(tpmc)_2](ClO_4)_6 \cdot CH_3CN \cdot KClO_4 \cdot 4H_2O$ complexes.

EXPERIMENTAL

Chemicals and materials

All chemicals and solvents used for synthesis and examination were of reagent grade. 1,4,8,11-tetraazacyclotetradecane 98 % as starting compound was used as obtained from Aldrich Chemical Company. Macrocyclic ligand tpmc[15] and complex $[Ni_2tpmc](ClO_4)_4 \cdot 3H_2O$[16] were obtained and purified according to literature procedures. All other chemicals were of p.a. grade and used as supplied.

Preparation

Caution! Although no problems were encountered in this work, perchlorate salts are potentially explosive. They should be prepared in small quantities and handled with care.

*$[Ni_2(C_9H_8O_4)tpmc](ClO_4)_2 \cdot 4H_2O$ (**1**)*. The complex **1** was prepared by mixing 10 mL solution of the complex $[Ni_2tpmc](ClO_4)_4 \cdot 3H_2O$ (126 mg; 0.11 mmol) in CH_3CN with 4 mL aqua solution of the $K_2C_9H_8O_4 \cdot H_2O$ (57 mg; 0.22 mmol). The obtained green mixture was refluxed (80 °C) by stirring for 3 h. The solution was filtered, evaporated to 1/2 initial volume and left in the refrigerator. After three days, the substance was crystallized in the form of microcrystalline solid with light blue color. Mole ratio is 1:2. Yield 90 mg (72 %). Anal. Calcd. for $Ni_2C_{43}H_{60}N_8O_{16}Cl_2$ (FW = 1133.39): C 45.57, H 5.33, N 9.89 %; found: C 45.82, H 5.46, N 9.98 %. Solubility: well soluble in DMSO and CH_3CN, insoluble in H_2O, CH_3OH, CH_3CH_2OH, DMF, $(CH_3)_2CO$.

*$[Zn_4(C_9H_8O_4)(tpmc)_2](ClO_4)_6 \cdot CH_3CN \cdot KClO_4 \cdot 4H_2O$ (**2**)*. Solution of $Zn(ClO_4)_2 \cdot 6H_2O$ (129 mg; 0.35 mmol) in water (5 mL), suspension of tpmc (97 mg; 0.17 mmol) in CH_3CN (6 mL) and aqueous solution of $K_2C_9H_8O_4 \cdot H_2O$ (33 mg; 0.13 mmol) were mixed and refluxed with stirring for 2 h in a water bath (80 ºC). After that, the reaction mixture was vacuum filtered, covered with needle-pierced parafilm and left in a refrigerator overnight. The white microcrystals that appeared after a few days were removed by vacuum filtration. Yield 137 mg (65 %). Anal. Calcd. for $Zn_4C_{79}H_{107}N_{17}O_{36}Cl_7K$ (FW = 2419.99): C 39.20, H 4.46, N 9.84 %; found C 38.99, H 4.38, N 9,99 %. Solubility: well soluble in CH_3CN and DMSO, insoluble in H_2O, CH_3OH, CH_3CH_2OH, DMF, $(CH_3)_2CO$.

Analytical, spectral and other physical measurements

Elemental analysis (C, H, N) was performed by standard micromethods at the ICTM, Center for Chemistry, University of Belgrade. The conductivity of the complex solutions in CH_3CN (1×10^{-3} M) was measured at room temperature using Thermo Orion Star A212 conductometer. Electronic absorption spectra of the complexes and ligands in CH_3CN (1×10^{-3} M) were recorded on spectrophotometer GBC UV–Vis Cintra 20 in the range 200–900 nm. The diffuse reflectance spectra were recorded by Labsphere RSA-PE-20, diffuse reflectance and transmittance accessory, which fits into the sample compartment of PerkinElmer UV–Vis spectrometer Lambda 35 in the wavelength range 190-1100 nm. $BaSO_4$ was used as referent standard.

FTIR spectra (ATR technique) were recorded using Nicolet 6700 FT-IR spectrophotometer in the range 400–4000 cm^{-1}. Magnetic susceptibility measurements were taken at room temperature (20±2 °C) using a MSB-MKI balance (Sherwood Scientific Ltd., England). Data were corrected for diamagnetic susceptibilities using Pascal's constants.[17]

In vitro *antimicrobial assay*

The antimicrobial activity of complexes, $[Ni_2(C_9H_8O_4)tpmc](ClO_4)_2{\cdot}4H_2O$ (**1**) and $[Zn_4(C_9H_8O_4)(tpmc)_2](ClO_4)_6{\cdot}CH_3CN{\cdot}KClO_4{\cdot}4H_2O$ (**2**) as well as ligands (tpmc and $K_2C_9H_8O_4{\cdot}H_2O$), salts ($Zn(ClO_4)_2{\cdot}6H_2O$ and $Ni(ClO_4)_2{\cdot}6H_2O$), was tested against the Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6633), Gram-negative bacteria *Escherichia coli* (ATCC 25922), and yeast *Candida albicans* (ATCC 10231). Broth microdilution method was used in order to determine minimal inhibitory concentrations (MICs). The assays were done in accordance with the procedures outlined by Clinical and Standards Institute (CLSI).[18]

The well containing minimum concentration of the compound in which there is no visual growth is considered as MIC. Each species was maintained on Mueller–Hinton agar (MHA) (Torlak, Belgrade), which was also used to confirm the absence of contamination and the validity of the inocula. Before testing, each species was recovered by subculturing in Mueller–Hinton broth (MHB) (Torlak, Belgrade), aerobically, for 24 h, at 37 °C. For *C. albicans* Sabourad agar (SA) (Torlak, Belgrade) and Sabourad dextrose broth (SB) (Torlak, Belgrade) were used. The suspensions were further diluted with MHB to obtain a final inoculum of 5×10^5 CFU/mL for bacteria, and 1×10^5 CFU/mL for *C. albicans*.

Compounds were dissolved in DMSO (2 %) to prepare stock solutions sterilized by filtration through a 0.22-mm membrane filter (Sartorius AG, Germany) and further diluted with MHB to working solutions. DMSO was chosen as a non-toxic solvent. Ampicillin and nystatin were used as a positive control.

Test bacterial culture (100 μL) in a MHB was added to the wells of a sterile 96-well microtiter plate (Sarstedt, Numbrecht, Germany) already containing 100 μL of twofold serially diluted compound in MHB. Also, 100 μL *C. albicans* in SB was added to the wells 96-well microtiter plate already containing 100 μL of twofold serially diluted compounds in SB. The final volume in each well was 200 μL. Microbial growth was determined after incubation at 37 °C for 24 h for bacteria, and after incubation for 48 h at 26 °C for fungi.

Wells with MHB were used for sterility control, while negative controls were wells with tested compound in 100 μL of MHB, but void of bacteria. Positive controls were wells with 100 μL suspension of microorganisms in 100 μL of MHB (or SB for *C. albicans*) and wells with a bacterial suspension in a MHB with DMSO (or SB for *C. albicans*), in amounts corresponding to the highest quantity present in the broth microdilution assay (to prove that DMSO had no inhibition effect on the microorganism's growth).

To indicate cellular respiration 2,3,5-triphenyltetrazolium chloride (TTC) (Aldrich Chemical Company Inc., Sigma-Aldrich, St. Louis, MO, USA) was added to the culture medium. The final concentration of TTC after inoculation was 0.05 %. Viable microorganisms enzymatically changed white TTC to a pink TPF (1,3,5-triphenylformazan). The *MIC* was defined as the lowest concentration of the investigated compound at which the microorganism does not demonstrate visible growth.[19]

In vitro *antiproliferative assay*

Stock solutions of the test compounds **1** and **2** were made up in DMSO at 10 mM concentration, filtered through Millipore filters (0.22 μm), and diluted to a relevant working concentrations for the use in the nutrient medium. For all the cells used, the nutrient medium was RPMI 1640 without phenol red, supplemented with L-glutamine (3 mM), streptomycin (100 μg mL^{-1}), and penicillin (100 IU mL^{-1}), fetal bovine serum (10 %; FBS; 56 °C heat-inactivated) and HEPES (25 mM), adjusted to pH 7.2 (bicarbonate solution). RPMI-1640, FBS, Hepes and L-glutamine were products of Sigma Chemical Co., St. Louis, MO, USA.

Human cervix adenocarcinoma (HeLa) cells were cultured as monolayers in the nutrient medium (see above), while human myelogenous leukemia (K562) cells were maintained as suspension cultures in the same nutrient medium. All these cells were grown at 37 °C in 5 % CO_2 and a humidified air atmosphere.

The metabolic activity was assessed using the CellTiter 96H Aqueous One Solution Cell Proliferation Assay (Promega, Madison/WI, USA), in accordance with the manufacturer's instructions.

The HeLa cells were seeded (5×10^4 mL^{-1}) into 96-well microtiter plates, and 20 h later, after cell adherence, five different concentrations of the test compounds were added to the wells. The final test compound concentrations were from 12.5 to 200 μM. In addition, the activity of the starting compounds (tpmc, $K_2C_9H_8O_4\cdot H_2O$, $Zn(ClO_4)_2\cdot 6H_2O$, $Ni(ClO_4)_2\cdot 6H_2O$, $NaClO_4$) were tested in the same concentration. Only nutrient medium was added to the cells in the control wells. Cisplatin was used as a positive control. For the K562 cells the test compounds were added to cell suspensions (5×10^4 mL^{-1}) 2 h after cell seeding to the same final concentrations applied to the HeLa cells. All experiments were carried out in triplicate. Nutrient medium, with the corresponding concentrations of the test compounds only, without cells, was used as the blank.

After the indicated period of time (48 h), each 100 μL of cell suspension was incubated with 10 μL of the supplied MTS tetrazolium compound. After 3 h of incubation under standard conditions, the absorbance was measured at 492 nm. To achieve cell survival (%), absorbance at 492 nm, of a sample with cells grown in the presence of various concentrations of compounds tested, was divided with absorbance of control sample (the absorbance of cells grown in nutrient medium only). Absorbance of blank was always subtracted from absorbance of corresponding sample with cells.[20]

IC_{50} was defined as the concentration of the compound inhibiting cell survival by 50 %, compared to a vehicle-treated control cells. All experimentally obtained IC_{50} data were means of three measurements done in triplicate.[19]

RESULTS AND DISCUSSION

The Ni(II) complex was prepared starting with $[Ni_2tpmc](ClO_4)_2\cdot 3H_2O$ and $C_9H_8O_4K_2\cdot H_2O$ in mole ratio 1:2 while Zn(II) complex was prepared by direct reaction of $Zn(ClO_4)_2\cdot 6H_2O$ with tpmc and $C_9H_8O_4K_2\cdot H_2O$ in mole ratio 4:2:1.5. The formulas $[Ni_2(C_9H_8O_4)tpmc](ClO_4)_2\cdot 4H_2O$ (**1**) and $[Zn_4(C_9H_8O_4)(tpmc)_2](ClO_4)_6\cdot CH_3CN\cdot KClO_4\cdot 4H_2O$ (**2**) were proposed. The elemental analyses data correspond well to the proposed formulae. Both complexes are stable on the air.

The molar conductivity values in acetonitrile are 310 and 890 S cm^2 mol^{-1} respectively for Ni(II) and Zn(II) complexes. The molar conductance measurements of the Ni(II) complex in acetonitrile correspond to 1:2 electrolyte.[21] The molar conductivity value is very high for the Zn(II) complex. It indicates that the assumption of about 1:6 Zn(II) complex electrolyte type is most likely correct. In the literature data for similar complexes, electrolyte type 1:6 molar conductivity are in the range 628–994 S cm^2 mol^{-1}.[13,14,22] The conductivity of a $NaClO_4$ solution in CH_3CN (4×10^{-3} M) of 540 S cm^2 mol^{-1} refers to the important contribution of conductivity from the $NaClO_4$ incorporated in the crystal lattice.[14]

Magnetic measurments

Magnetic moments are of great use in establishing the geometry of nickel(II) complexes. Octahedral nickel(II) show spin only value of magnetic moment (2.83 μ_B/Ni(II)), however, the magnetic moment is between 2.8–3.4 μ_B/Ni(II) for the complexes. For tetrahedral nickel(II) a large orbital contribution is expected and as the result the observed values of magnetic moment range between 3.5 and 4.2 μ_B/Ni(II). Square planar complexes are usually diamagnetic because the ground state in this case is a spin singlet state.[23]

The present Ni(II) complex has a magnetic moment of 3.4 μ_B/Ni(II). The magnetic measurements showed two unpaired electrons per Ni(II) ion suggesting also an octahedral geometry for the Ni(II) complexes.[23]

The Zn(II) complex is diamagnetic.

Spectral properties

UV–Vis spectra. Nickel(II) complexes are known to exhibit complicated equilibrium between coordination numbers six (octahedral) and four (square planar/tetrahedral).[24] Nickel(II) forms many complexes with octahedral, square-planar, and tetrahedral geometries and a smaller number of five-coordinate compounds with other stereochemical arrangements. It is generally considered that a combination of steric and electronic factors determines which of the three common geometries is assumed by a given compound.

The electronic spectrum of the Ni(II) complex in CH_3CN shows an absorption bands at the 402, 606 and 877 nm attributed to d–d transitions. These are assigned to the spin-allowed transitions $^3A_{2g}(F)\rightarrow{}^3T_{1g}(P)$, $^3A_{2g}(F)\rightarrow{}^3T_{1g}(F)$ and $^3A_{2g}(F)\rightarrow{}^3T_{2g}(F)$ respectively, consistent with their well-defined octahedral configuration. The reflectance spectrum in the visible region shows bands at 410, 610 and 870 nm. Reflectance and solution spectra of the Ni(II) complex corresponded to five- or six-coordinated nickel (II) and its magnetic moments (3.4 μ_B/Ni(II)) suggests a high-spin nickel state. A characteristic feature of the spectra of octahedral nickel(II) complexes is that the molar absorbances are low, for the Ni(II) complex are in the range from 35–74 $dm^3\,mol^{-1}\,cm^{-1}$. In UV part several sharp unresolved bands in the range from 221 to 260 nm (ε = 4400-4800 $dm^3\,mol^{-1}\,cm^{-1}$) were assigned to intraligand transitions bands. Intraligand transitions were found in the spectra of ligands in the range 220–280 nm. Based on all the applied characterization methods it was assumed that the geometry of the complex is most likely octahedral.

No d–d transitions are expected for d^{10} Zn(II) complexes. The Zn(II) complex is white and electronic spectra show only the intraligand transitions with very strong intensity bands at 207, 214 and 245 nm (ε = 4265–4652 $dm^3\,mol^{-1}\,cm^{-1}$).[25] The intraligand transitions in both complexes are slightly shifted during complexation.

FTIR spectra. The infrared spectra (Table I) of the complexes are very much consistent with the proposed formula and with data obtained by other methods. Peaks at 3600–3400 cm^{-1} for complexes are attributable to O–H stretching vibrations of water molecules. Both complexes display peak at 1609 cm^{-1} which is assigned to the skeletal vibration of pyridine from coordinated tpmc ligand. In the spectrum of both complexes there is the strongest band at 1100 cm^{-1} belonging to the $\nu(ClO_4^-)$ and sharp, medium intensity band at ~620 cm^{-1} assigned to $\delta(ClO_4^-)$. Broad intense bands at 1100 cm^{-1} show no splitting, indicating the absence of coordinated ClO_4^- in the complexes.[26] A weak broad band observed for the complexes and dicarboxylate salt in the range 2936-2955 cm^{-1} is likely to appear due to stretching vibration of CH, and two medium bands about 1438 and 1490 cm^{-1} from CH_2 bending vibrations.[14] Bands appearing at 479, 419, 478 and 412 cm^{-1} correspond to ν(Ni–N), ν(Ni–O), ν(Zn-N) and ν(Zn–O), respectively. It is an additional confirmation that ligands (macrocycle and bicyclodicarboxylate) are coordinated to metal ions.[27]

TABLE I. Selected FTIR absorption bands of the ligands and complexes

Compound	ν / cm^{-1}							
	O–H	C=N	ClO_4^-	ClO_4^{-a}	C–H	CH_2^a	M–N	M–O
$C_9H_8O_4K_2 \cdot H_2O$	354	–	–	–	2939	1440, 1490	–	–
tpmc	–	1588	–	–	2955	1438, 1469	–	–
1	3559	1609	1079	621	2936	1442, 1486	479	419
2	3456	1609	1090	625	2945	1442, 1463	478	412

[a]δ

The important FTIR absorption frequencies of the OCO group for compounds are shown in Table II together with the difference, $\Delta\nu$ between the two carboxylate bands $\nu_{as}(OCO)$ and $\nu_s(OCO)$, $\Delta\nu = \nu_{as}(OCO) - \nu_s(OCO)$. Analysis of the FTIR data for the $\nu_{as}(OCO)$ and $\nu_s(OCO)$ should provide useful information about the different binding modes of the carboxylate ligand.[27,28]

The relationship between $\Delta\nu$ and the types of coordination of the OCO^- group to metal ions indicates:

(1) The carboxylate group is bidentate chelated when the bands of $\nu_{as}(OCO)$ and ν_s (OCO) in the complex are shifted to lower and higher wavenumbers, compared to those for alkaline salt; or $\Delta\nu$ of complex << $\Delta\nu$ of alkaline salt.

(2) Bridge bonding exists when bands of $\nu_{as}(OCO)$ and $\nu_s(OCO)$ in complex are shifted to higher wavenumbers, compared to those for alkaline salt; or $\Delta\nu$ of complex ≈ $\Delta\nu$ of alkaline salt.

(3) For monodentate coordination of carboxylate group the bands of $\nu_{as}(OCO)$ and $\nu_s(OCO)$ in the complex are shifted to higher and lower wavenumbers, respectively, compared to those for alkaline salt; or $\Delta\nu$ of complex >> $\Delta\nu$ of alkaline salt.[28]

The asymmetric and symmetric stretching vibrations of the carboxylate groups appear at 1521 and 1360 cm^{-1}, respectively, for Ni(II) complex. These bands have been shifted relative to $C_9H_8O_4K_2$ which indicates the coordination of the carboxylate ligand for Ni(II).[27] The difference ($\Delta\nu$ = 161 cm^{-1}) between ν_{as}(OCO) and ν_s(OCO), which is lower than 181 cm^{-1} observed in ionic bicyclodicarboxylate, reflects the chelate coordination modes of the OCO groups (Table II) or μ-*O*,*O*′ coordination of each OCO group (Fig. 2a and b).[27,28] Because of the steric interference caused by the structure of the carboxylate ligand, it is unlikely that the ligand is coordinated by engaging both OCO^- groups in μ-*O*,*O*′ mode (Fig 2b).

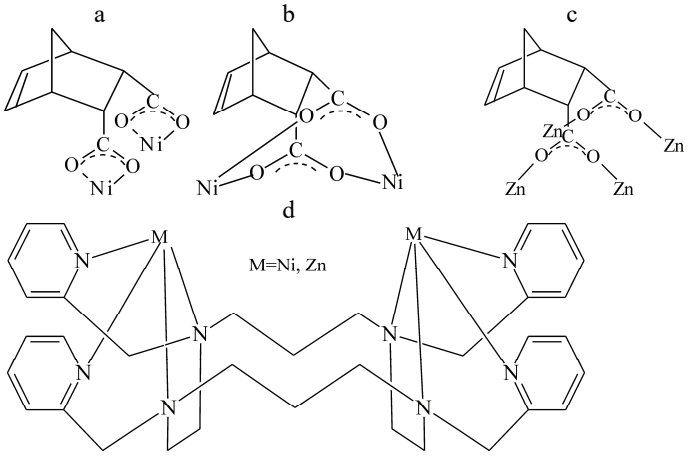

Fig. 2. Possible coordination modes of bicyclodicarboxylate ligand in complex cations of **1** (a and b) and **2** (c); *exo* coordination mode in binuclear units $[M_2tpmc]^{4+}$ (M = Ni, Zn) of complexes with a boat conformation (d).

The monodentate way of dicarboxylate coordination can be excluded (then coordination number would be 5). In the case of monodentate coordination, $\Delta\nu$ in the complex is significantly larger than the ionic value $\Delta\nu$, which is not the case here. Based on the applied characterization methods, it can be assumed that the combined bridge chelating mode of coordination of dicarboxylates is most likely in the Ni(II) complex (Fig 2a).

The asymmetric and symmetric stretching vibrations of the carboxylate group appear at 1573 and 1384 cm^{-1}, respectively, for Zn(II) complex. The value $\Delta\nu$ (Table II) for the complex **2** ($\Delta\nu$ = 189 cm^{-1}) is very close to the value for the ionic $C_9H_8O_4K_2$ ($\Delta\nu$ = 181 cm^{-1}). These results together with shift of ν_{asym} and ν_{sym} are consistent with the bridge binding mode for both carboxylate groups of the bicyclodicarboxylate dianion for Zn(II) centers.[27,28] Each of the four oxygen atoms are most likely coordinated with one of each Zn(II) in two tpmc units. It is

possible that one carboxylate group bridges two Zn(II) from the same or from two different tpmc units. Because of steric constrains, the first case is more probable (Fig. 2c).

TABLE II. Selected FTIR data of OCO (asymmetrical, ν_{as}, symmetrical vibrations, ν_s and $\Delta\nu = \nu_{as} - \nu_s$ in cm^{-1}) values for alkaline bicyclodicarboxylate salt, Ni(II) and Zn(II) complexes

Compound	$\nu_{as}(OCO^-)$	$\nu_s(OCO^-)$	$\Delta\nu$
$C_9H_8O_4K_2 \cdot H_2O$	1575	1394	181
$[Ni_2(C_9H_8O_4)tpmc](ClO_4)_2 \cdot 4H_2O$	1521	1360	161
$[Zn_4(C_9H_8O_4)(tpmc)_2](ClO_4)_6 \cdot CH_3CN \cdot KClO_4 \cdot 4H_2O$	1573	1384	189

In both complexes, other ways of dicarboxylate bridge bonding cannot be excluded completely. Macrocycle ligand tpmc is *exo* coordinated to Ni(II) or Zn(II), probably in the boat conformation (Fig. 2d).[13,14,16,19,22]

In vitro *antimicrobial activity*

After the revolution in "golden era", when almost all groups of important antibiotics were discovered and the main problems of chemotherapy were solved in the 1960s, the history repeats itself nowadays and these compounds are in danger of losing their efficacy because of the increase in microbial resistance.[29] Antibiotic resistance is rising to dangerously high levels in all parts of the world. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases. The most commonly reported resistant bacteria are *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Salmonella* spp. The control of many of the organisms mentioned above is key to health sustainability. For this reasons, discovery of new compounds with antimicrobial activity is a very important objective.[30]

Studies with antimicrobial compounds have often shown unexpected nonantibiotic effects that indicate a variety of other biological activities such as antiviral or antitumor.[31] Experimental results obtained from the study of antimicrobial activity (Table III) demonstrate that complexes $[Ni_2(C_9H_8O_4)tpmc](ClO_4)_2 \cdot \cdot 4H_2O$ (**1**) and $[Zn_4(C_9H_8O_4)(tpmc)_2](ClO_4)_6 \cdot CH_3CN \cdot KClO_4 \cdot 4H_2O$ (**2**) display bacteriostatic activity in the concentration range from 25–100 μg mL^{-1} towards both Gram-positive and Gram-negative bacteria.

The data concerning the study of antimycotic properties of compounds **1** and **2** show that they also display this activity in the concentration of 100 μg mL^{-1} towards examined fungi strain. The starting compounds have not exibited antimicrobial effect (data not shown).

The literature data state that metal chelates have greater antibacterial activity than the free ligand. The lipid membrane of the cell favors the passage of lipid soluble materials only and it is known that liposolubility is an important factor that controls antimicrobial activity.[32]

TABLE III. Antimicrobial activity of **1** and **2** and expressed as *MIC* in μg mL^{-1}, determined by the broth microdilution methods; Nt – not tested

Microbial strain	Compound			
	1	**2**	Ampicillin[19]	Nystatin[19]
Staphylococcus aureus ATCC 25923	57 (50*)	60 (25*)	1	Nt
Bacillus subtilis ATCC 6633	113(100*)	242 (100*)	8	Nt
Escherichia coli ATCC 25922	113 (>100*)	60 (25*)	2	Nt
Candida albicans ATCC 10231	113 (100*)	242 (100*)	Nt	4

The *in vitro* antimicrobial activity of the Ni(II) complexes with unsymmetrical Schiff-base ligands derived from 5-bromosalicylaldehyde and *o*-phenylenediamine have higher antimicrobial activity than the free ligands.[33] Also, similar to the presented results**,** Ni(II) and Zn(II) complexes with isoniazid-derived compound have shown antimicrobial activity against *S. aureus*, *K. pnemuniae*, *E. coli* and *C. albicans*[34] and Zn(II) and Ni(II) complexes of Schiff bases derived from 2-hydroxynaphtaldehyde with glycine and phenylalanine exhibited moderate to good activity against Gram-positive bacteria and fungi.[35a] Complex Ni(II) with pyridine showed antibacterial and antimicrobial activity.[35b]

In vitro *cytotoxicity*

Pahontu *et al.* demonstrated that Zn(II) complex with isoniazid-derived compound exhibited anti-proliferative effect against SKBR-3 (human breast cancer), A375 (human melanoma) and NCI-H1573 (lung adenocarcinoma) cells. Also, Ni(II) complex with isoniazid-derived compound have shown anti-proliferative effect towards SKBR-3 cells.[34] The Ni (II)-complexes with thiosemicarbazones derived from natural aldehydes induced an antiproliferative effect on U937, a human histiocytic lymphoma cell line, via programmed cell death and down--regulation of Bcl-2, alteration of mitochondrial membrane potential and caspase--3 activity, regardless of p53 function.[35c]

The cytotoxicity of the obtained complexes and starting compounds were tested. The cytotoxic properties of target compounds are presented in Table IV.

Table IV. Concentrations of compounds **1** and **2** that induced 50 % decrease in cell survival (IC_{50}±*SD* / μM)

Compound	Cell	
	HeLa	K 562
$[Ni_2(C_9H_8O_4)tpmc](ClO_4)_2·4H_2O$ (**1**)	124.06±9.35	92.25±7.18
$[Zn_4(C_9H_8O_4)(tpmc)_2](ClO_4)_6·CH_3CN·KClO_4·4H_2O$ (**2**)	95.10±7.76	80.35±8.81
tpmc, $K_2C_9H_8O_4·H_2O$, $Ni(ClO_4)_2·6H_2O$, $Zn(ClO_4)_2·6H_2O$, $NaClO_4$	>200	>200
Cisplatin	11.3±4.20	8.3±1.75

Both compounds have promoted decrease in the metabolic activity of the HeLa and K562 cells, which occurred in a dose-dependent fashion. Moderate

activity comes from the complexes because under the same conditions ligands and free salts were inactive.

CONCLUSION

The newly synthesized mixed-ligand complexes of Ni(II) and Zn(II) using the *N*,*N*′,*N*″,*N*‴-tetrakis(2-pyridilmethyl)-1,4,8,11-tetraazacyclotetradecane (tpmc) and (*endo*,*endo*)-bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate were characterized by spectral, conductometric and magnetic studies. For the binuclear Ni(II) complex, octahedral structure has been proposed with *exo* coordinated four nitrogen atoms from macrocycles and two oxygen atoms from carboxylate where combined bridge chelating mode of dicarboxylate is suggested. The Zn(II) complex is rare tetranuclear one with the bridge coordination mode for both carboxylate groups of the bicyclodicarboxylate dianion. It is proposed that each carboxylic group bridges two Zn(II) within one tpmc unit and that two Zn(II) ions are *exo* five-coordinated (four nitrogen atoms from macrocycles and one oxygen atom from carboxylate).

The complexes have shown *in vitro* bacteriostatic activity in the concentration range 25–100 mM towards both Gram-positive and Gram-negative bacteria and displayed antimycotic properties in the concentration of 100 mM towards examined fungi. Both compounds have promoted decrease in metabolic activity of the HeLa and K562 cells, depending on dose.

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ИЗВОД

НОВИ МЕШОВИТОЛИГАНДНИ МАКРОЦИКЛИЧНИ КОМПЛЕКСИ Ni(II) И Zn(II) СА МОСТОВНИМ (*endo*,*endo*)-БИЦИКЛО-[2.2.1]-ХЕПТ-5-ЕН-2,3-ДИКАРБОКСИЛАТОМ: СИНТЕЗА, КАРАКТЕРИЗАЦИЈА, АНТИМИКРОБНА И ЦИТОТОКСИЧНА АКТИВНОСТ

МИРЈАНА АНТОНИЈЕВИЋ НИКОЛИЋ[1], БРАНКА ДРАЖИЋ[2], ЈЕЛЕНА АНТИЋ СТАНКОВИЋ[2] и СЛАЂАНА ТАНАСКОВИЋ[2]

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Синтетисани су нови карбоксилато комплекси са тетраазамакроцикличним лигандом *N*,*N*′,*N*″,*N*‴-тетракис(2-пиридилметил)-1,4,8,11-тетраазациклотетрадеканом (tpmc) и Ni(II) и Zn(II) као централним јонима. У мешовитолигандним комплексима (*endo*,*endo*)-бицикло-[2.2.1]-хепт-5-ен-2,3-дикарбоксилато дианјон ($C_9H_8O_4^{2-}$) је такође везан за метални јон. Комплекси су окарактерисани елементалном анализом (C, H, N), FTIR и UV/Vis спектроскопијом, као и мерењем моларне проводљивости и магнетне сусцептибилности на собној температури. На основу добијених резултата претпостављена је бинуклеарна структура $[Ni_2(C_9H_8O_4)tpmc](ClO_4)_2{\cdot}4H_2O$ комплекса, односно, тетрануклеарна $[Zn_4(C_9H_8O_4)(tpmc)_2](ClO_4)_6{\cdot}CH_3CN{\cdot}KClO_4{\cdot}4H_2O$ комплекса. У тетрануклеарном Zn(II) комплексу, бициклични дикарбоксилато лиганд је највероватније мостно везан, док је у бинуклеарном Ni(II) комплексу мешовити мостно-хелатни начин координације. Други начини мостног везивања дикарбоксилата не могу бити у потпу-

ности искључени. У оба комплекса макроциклични лиганд је егзо координован, изван цикламовог прстена и заузима конформацију лађе. Добијени Zn(II) комплекс је један од ретких тетрануклеарних Zn(II)–tpmc комплекса са карбоксилато лигандом у мосту између две Zn_2tpmc јединице. Добијени комплекси су тестирани на антибактеријску активност према Грам-позитивним бактеријама *Staphylococcus aureus* (ATCC 25923) и *Bacillus subtilis* (ATCC 6633), Грам-негативној бактерији *Escherichia coli* (ATCC 25922) и квасцу *Candida albicans* (ATCC 10231). Испитивана је њихова антипролиферативна активност на хуманим малигним ћелијским линијама: цервикалног аденокарцинома (HeLa) и мијелогене леукемије (K562).

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