

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDY OF NEW DINUCLEAR ZINC(II) AND NICKEL(II) OCTAAZA MACROCYCLIC COMPLEXES

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Two new nitrate complexes of zinc and nickel with 1,4,8,11-tetrakis(2-pyridylmethyl)-1,4,8,11-tetraazacyclotetradecane (tpmc), have been synthesized and characterized. The IR spectral peaks showed that the coordinated and ionic nitrate ions are in agreement with the formula proposed by elemental analysis. Conductometric titrations predicted methanol to be a convenient solvent for synthesis and revealed the stoichiometry of the complexes, while molar electrical conductivities indicated a 1 : 3 complex electrolyte type for the zinc complex, and a 1 : 2 complex electrolyte type for the nickel complex. The optimized complex structure was obtained by molecular modeling and density functional theory calculations. The biological activity of the novel complexes was examined by screening eight different bacterial strains and two cancer cell lines. The zinc complex showed better antimicrobial activity against the bacterial strains, while the complexes did not show significance antiproliferative activity toward cancer cells MCF-7 and MDA-MB-231.

Keywords: zinc; nickel; tpmc; complex; biological activity

СИНТЕЗА, КАРАКТЕРИЗАЦИЈА И БИОЛОШКО ИСПИТУВАЊЕ НА НОВИ ДИНУКЛЕАРНИ ЦИНК(II) И НИКЕЛ(II) ОКТААЗА МАКРОЦИКЛИЧНИ КОМПЛЕКСИ

Синтетизирани и карактеризирани се два нови комплекса со 1,4,8,11-тетракис(2-пиридилметил)-1,4,8,11-тетраазациклотетрадекан (tpmc) како лиганд. Спектралните IR пикови покажуваат дека координираните и јонските нитратни јони се во согласност со формулата што произлегува од елементната анализа. Кондуктометриските титрации предвидуваат дека метанолот е соодветен растворувач за синтеза и ја даваат стехиометријата на комплексите, додека моларните електрични кондуктивности укажуваат на 1 : 3 електролитен тип на комплекс за комплексот на цинк и 1 : 2 електролитен тип на комплекс за комплексот на никел. Оптимизираната комплексна структура е добиена со молекулско моделирање и теоретски пресметки на функционалот на густина. Биолошката активност на новите комплекси беше испитана со анализа на осум различни бактериски видови и со размножување на два вида канцерогени клетки. Комплексот на цинк покажува подобра антимикуробна активност кон бактериските видови, додека комплексите немаат значајна антиразмножувачка активност кон клетките на рак MCF-7 и MDA-MB-231.

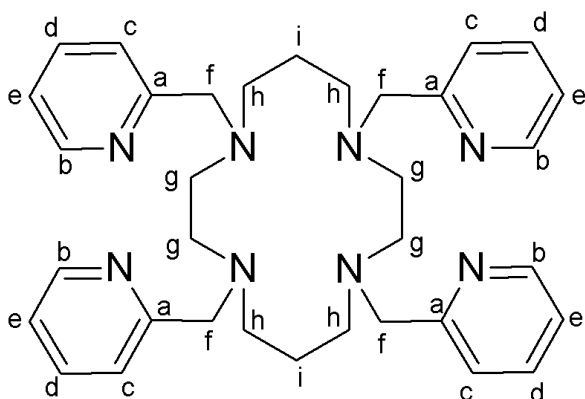
Клучни зборови: цинк; никел; tpmc; комплекс; биолошка активност

1. INTRODUCTION

In recent years, interest in macrocyclic polyamines has been on the increase as a result of their attractive structures and their possible applications in many different fields [1–3]. The reason for that is their possibility to react or coordinate with many different metal ions as well as organic molecules *via* by nitrogen atoms [4–7].

Cyclam (1,4,8,11-tetraazacyclotetradecane) is one of the most widely-used macrocyclic ligands [8–10]. Some of its derivatives block the entry of HIV into cells and can be highly potent anti-HIV drugs [11, 12], but they also show antimicrobial activities [13]. As a molecule, cyclam has four nitrogen atoms; in derivatives, these atoms are often substituted with different groups which can increase the number of coordination sites.

When the amine protons of cyclam are substituted with 2-pyridylmethyl groups, the derivative is known as tpmc (1,4,8,11-tetrakis-(2-pyridylmethyl)-1,4,8,11-tetraazacyclotetradecane) with eight possible coordination sites (Scheme 1). Transition metals can form mono- or dinuclear complexes with tpmc, while it can be coordinated using two, three or four nitrogen atoms from pyridyl groups and cyclam [14–17]. Until now, only a few metal complexes with coordinated tpmc have been synthesized [15–21], mostly with copper(II) ions, in dinuclear complexes [22–24]. These complexes have shown different electrochemical properties [25, 26], as well as antibacterial [4, 17] and cytotoxic activity [19, 27, 28].



Scheme 1. The structure of tpmc with C-atoms assigned

According to our knowledge, this is the first report of the synthesis, characterization, and electrochemical and biological study of a dinuclear zinc complex with tpmc containing nitrate ion as a bridging ligand, as well as a dinuclear nickel–nitrate complex with tpmc ligand.

2. EXPERIMENTAL

Elemental analysis (C, H, N) was carried out by standard micro methods at the Center of Instrumental Analysis, University of Belgrade. IR spectra (ATR technique) were recorded on a Perkin-Elmer FTIR 31725 X spectrophotometer, while ^1H NMR and ^{13}C NMR spectra were run on a Bruker Avance III spectrometer (500 and 50 MHz, respectively) in DMSO-d_6 at room temperature. Molar electrical conductivities of the complex ($c = 1 \times 10^{-3}$ M) solutions in acetonitrile were measured at room temperature using a Jenway 4010 conductometer.

2.1. Synthesis of compounds

1,4,8,11-Tetrakis(2-pyridylmethyl)-1,4,8,11-tetraazacyclotetradecane (tpmc): This compound was prepared using a previously described method [29].

$[\text{Zn}_2(\text{tpmc})(\mu\text{-NO}_3)](\text{NO}_3)_3 \cdot \text{CH}_3\text{OH}$ (complex 1): The ligand (tpmc) (143.7 mg, 0.254 mmol) was suspended in a 1.5 : 1 methanol/water mixture (5 ml) at 50 °C. To this solution a methanol solution (4 ml) of zinc(II) nitrate hexahydrate (147.7 mg, 0.496 mmol) was added. The mixture was stirred for 1.5 h at 65 °C and then cooled to room temperature. The white crystals formed were filtered off and washed with ethanol (5 ml). The precipitate was recrystallized from a mixture of water and ethanol (1:1 v/v). The yield of complex **1** was 129 mg (53%). Anal. Calc. for $\text{C}_{35}\text{H}_{48}\text{N}_{12}\text{O}_{13}\text{Zn}_2$: C 43.09, H 4.96, N 17.23. Found: C 42.81, H 4.83, N 17.08%. IR (ATR, cm^{-1}): 2868.9 $\nu(\text{CH}_2)$, 1610.1 $\nu(\text{CN})$, 1573 $\nu(\text{C}=\text{C})$, 1477.9 $\nu(\text{NO}_3)$, 1361.4 $\nu(\text{NO}_3)$, 1021.3 $\nu(\text{NO}_3)$, 826.4 $\nu(\text{NO}_3)$, 761.6 $\nu(\text{Py})$. ^1H NMR (ppm) (DMSO-d_6 , 500 MHz) δ : 1.80–2.05 (m, 4H, $\text{CH}_2\text{CH}_2\text{CH}_2$); 2.38–2.51 (m, 4H, $\text{CH}_2\text{CH}_2\text{CH}_2$); 2.94 (m, 4H, $\text{CH}_2\text{CH}_2\text{CH}_2$); 3.12–3.19 (m, 8H, CH_2CH_2 , 1H, CH_3OH); 4.17 (m, 8H, $\text{N}_{\text{py}}\text{-CH}_2$, 3H, CH_3OH); 7.45–7.86 (m, 8H, pyridine ring); 8.17–8.42 (m, 4H, pyridine ring); 8.60 (m, 4H, pyridine ring). ^{13}C NMR (ppm) (DMSO-d_6 , 50 MHz) δ : 38.45, 38.87(C_i); 50.26 (CH_3OH); 53.62 (C_g), 56.32(C_h); 58.02 (C_f); 123.79, 124.69 (C_c); 125.37, 125.51(C_e); 138.03, 141.82 (C_d); 148.25, 149.47 (C_a); 154.65, 155.38 (C_b).

$[\text{Ni}_2(\text{tpmc})(\text{NO}_3)_2](\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (complex 2): To a warm solution of tpmc (144.3 mg, 0.255 mmol) in methanol (9 ml), a methanol solution (4 ml) of nickel nitrate hexahydrate (146.5 mg, 0.503 mmol) was added. The mixture was stirred for 1 h at 75 °C and then cooled to room temperature. The

violet precipitate was filtered off and washed with ethanol (5 ml). The compound was recrystallized from a mixture of water and ethanol (1:1 v/v). The final yield was 175 mg (69.7 %). Anal. Calc. for $C_{34}H_{50}N_{12}O_{15}Ni_2$: C 41.49, H 5.12, N 17.08. Found: C 41.10, H 5.32, N 17.07 %. IR (ATR, cm^{-1}): 3457.7 $\nu(OH)$, 2890.7 $\nu(CH_2)$, 1610.3 $\nu(CN)$, 1572.6 $\nu(C=C)$, 1503.3 $\nu(NO_3)$, 1360.8 $\nu(NO_3)$, 1027.4 cm^{-1} $\nu(NO_3)$, 852.9 cm^{-1} $\nu(NO_3)$, 827.5 cm^{-1} $\nu(NO_3)$, 778.4 $\nu(Py)$.

2.2. Electrochemical measurements

The conductance measurements were performed on a WTW Model 330i digital conductometer (Germany). The experimental procedure for conductometric titrations was reported in our previous work when complexation of different metal ions with tpmc in acetonitrile and aqueous solutions was investigated [22]. The conductance of the solution was measured in thermostated titration cell with methanol or ethanol solution of 1.0×10^{-4} M nitrate salt of Zn^{2+} and Ni^{2+} ions, while the tpmc concentration was increased by adding the ligand solution (5×10^{-3} M) in aliquots of 0.05 ml. The molar conductance of synthesized dinuclear Zn/tpmc nitrate complexes was measured in acetonitrile solution at a concentration of 1 mM [30].

2.3. Computational details

Geometry of the $[Zn_2(tpmc)NO_3]^{3+}$ complex ion was fully optimized with the DFT (Density Functional Theory) method, more specifically the hybrid B3LYP functional using standard Pople's basis set $-6-31G(d,p)$ for nonmetallic atoms (O, N, C and H) and LanL2DZ basis set with Effective Core Potential (ECP) for inner core electrons for the zinc atoms.

The global minimum in the gas phase was found and nature of the minimum was confirmed by frequency calculation. All DFT calculations were performed with Gaussian09 software package [31].

2.4. Antibacterial screening

Antimicrobial activity of complexes and tpmc was performed using *Staphylococcus pseudintermedius* (clinical isolate), *Staphylococcus aureus* ATCC 29213, *Staphylococcus saprophyticus* ATCC 15305, *Staphylococcus xylosus* ATCC 29991, *Staphylococcus pseudintermedius* ATCC 49444, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922. The isolation of *S. pseudintermedius* was from clin-

ical material delivered to the Department of Microbiology of the Faculty of Veterinary Medicine, University of Belgrade. The clinical isolate was identified with a BBL Crystal Gram-Positive ID kit (Becton Dickinson).

Minimal inhibitory concentration (MIC) values were determined by broth microdilution in accordance with the CLSI recommendations (Clinical and Laboratory Standards Institute 2006) [32]. Cation-adjusted Mueller Hinton II broth (CAMHB, Becton Dickinson) was used and method was performed in microtiter plates (Sarstedt) with "U" bottom. Investigated concentrations of compounds were 1280; 640; 320; 160; 80; 40; 20; 10; 5; 2.5; 1.25; 0.625 and $0.3175 \mu g ml^{-1}$. The compounds complex **1**, tpmc and its nickel complex **2**, were first dissolved in DMSO and the two-fold serial dilutions down to the lowest tested concentration were prepared in CAMHB. The desired inoculum density of 5×10^5 colony forming units (CFU) ml^{-1} was achieved by preparing suspensions of bacteria of approximately $1-2 \times 10^8$ CFU ml^{-1} , which was the density equal to McFarland standard. Volumes of 0.5 and 50 μl of this suspension were added to 100 μl CAMHB, after which the number of bacteria in the media was approximately $5 \times 10^5 ml^{-1}$. The media were incubated at 37 °C for 24 h. All investigations were carried out in triplicate.

2.5. Cytotoxic activity

Cell culture: HS5 (ATCC® CRL11882™), MCF-7 (ATCC® HTB22™) and MDA-MB-231 (ATCC® HTB-26™) were cultured in HAM's F12: DMEM (1:1) (DMEM, Sigma-Aldrich, St Louis, MO, USA) supplemented with 10% fetal serum plus 100 units ml^{-1} penicillin/streptomycin (both from PAA Laboratories) in a humidified atmosphere at 37 °C with 5% CO_2 .

Proliferation assay: The cell proliferation cytotoxic effects of the compounds were analyzed by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium (MTT) assay. Briefly, 10×10^3 cells/well were seeded into 96-well plates. Stock solutions of investigated compounds were prepared in sterile PBS at concentrations of 20 mM. The following day, cells were treated with the indicated compounds diluted in culture medium ranging in concentration from 25 to 400 μM for an additional three days. Then, 10-times concentrated MTT (Sigma-Aldrich) was added to each well to a final concentration of 0.5 $mg ml^{-1}$, followed by an additional 2 h of incubation. Cell supernatants were then removed and 100 μl of isopropa-

nol/dimethyl sulfoxide (3 : 2) was added to solubilize the formazan crystals. The absorbance was read at 630 nm, and IC_{50} values (defined as the concentration at which growth is inhibited by 50 %) were calculated by using Excel software.

3. RESULTS AND DISCUSSION

Conductometric titration study. In order to evaluate the possibility of the formation of complexes of Ni and Zn with tpmc ligand, their stoichiometry and a prediction of the most convenient solvent for synthesis, conductometric titrations of $Zn(NO_3)_2$ and $Ni(NO_3)_2$ solutions with tpmc solution at 20 °C were performed. As seen in Figure 1, the addition of tpmc to Zn^{2+} cation in pure methanol and ethanol resulted first in a decrease in molar conductivity until the molar ratio of metal:ligand reached 2 : 1 and then to an increase. The equivalent point of titration was sharp in methanol solution, and the formation of dinuclear Zn/tpmc complex was evident. The shape of conductometric titration curves (when conductance increases after

complex formation) is characteristic of processes where very unstable ion pairs (or sandwich structures) are formed [33, 34]. In the conductometric curve for titration of Ni^{2+} with tpmc in methanol and ethanol, molar conductance increased continuously and an equivalent point of titration could not be specified at any ratio (figure not presented). Titration of Ni^{2+} in acetonitrile and water [26], in a similar way to the titration in methanol and ethanol in this work, also gave undefined titration curves. Previous conductometric titration studies of Zn^{2+} in acetonitrile and aqueous solution indicated the formation of mono- and dinuclear Zn/tpmc complexes, and even the formation of a 1 : 2 (Zn/tpmc) complex in acetonitrile [26]. Based on our previous conductometric results, we assumed that synthesis of Ni^{2+} and Zn^{2+} /tpmc complexes in acetonitrile and aqueous media could be very difficult, but synthesis in methanol, especially for Zn, could be promising. We assumed that methanol as a solvent favors the coordination environment for the successful synthesis of dinuclear Zn and maybe Ni/tpmc complexes.

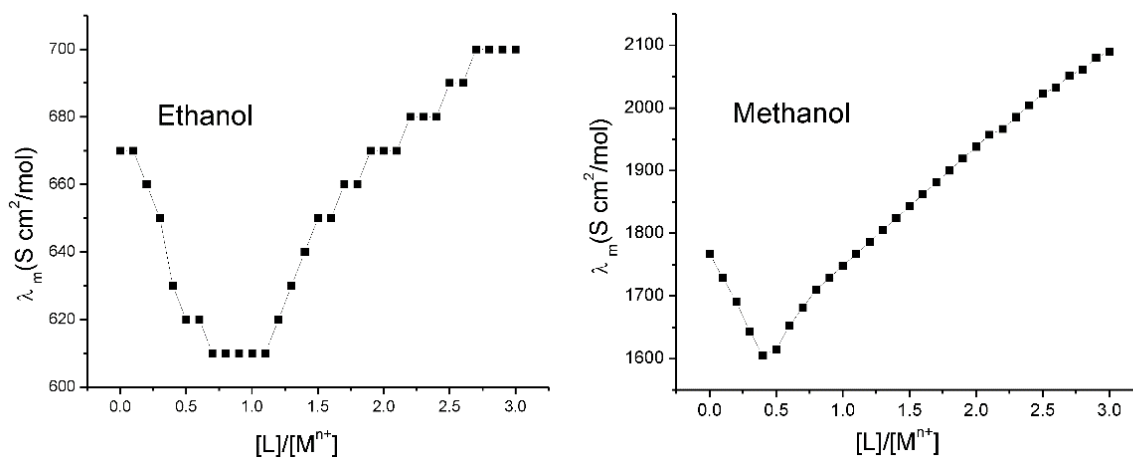


Fig. 1. A plot of molar conductance, λ , vs. V (ml of added ligand solution) for the binding of Zn^{2+} by tpmc in ethanol and methanol at 20 °C

Spectral characterization. The synthesis of Zn and Ni/tpmc complexes was carried out according to the synthesis procedure given in Section 2.1. Based on elemental analysis, dinuclear structures of the obtained complexes $[Zn_2(tpmc)NO_3](NO_3)_3 \cdot CH_3OH$ (complex 1) and $[Ni_2(tpmc)(NO_3)_2](NO_3)_2 \cdot 3H_2O$ (complex 2) are proposed. The complexes were prepared by the reaction of zinc(II) nitrate hexahydrate or nickel nitrate hexahydrate and tpmc in a 2 : 1 molar ratio. The compounds are stable microcrystalline solids, soluble in acetonitrile and dimethyl sulfox-

ide. The molar conductivity (λ_M) value for complex 1 of 420 S cm² mol⁻¹ indicate a 1:3 complex electrolyte type and points to a boat conformation of the tpmc ligand (Fig. 2b). In the same conditions, the molar conductivity (λ_M) value for complex 2 of 304 S cm² mol⁻¹ indicate a 1 : 2 complex electrolyte type and suggest a chair conformation of the tpmc ligand (Fig. 2a) and possible distorted octahedral geometry of the complex. Both values in accordance with literature data [30].

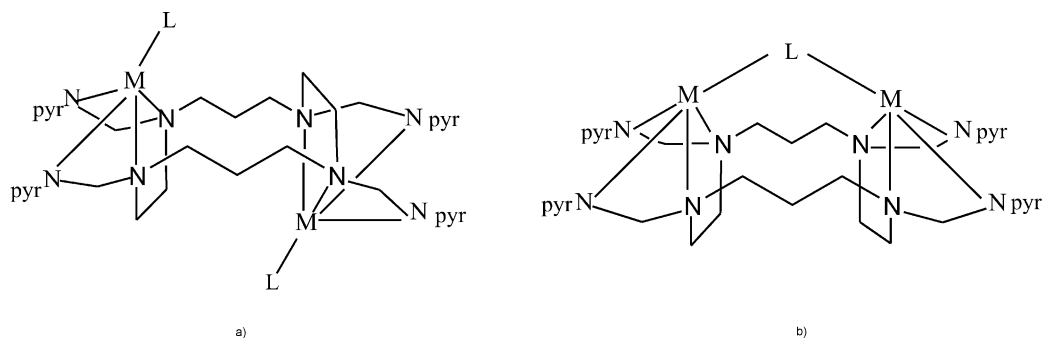
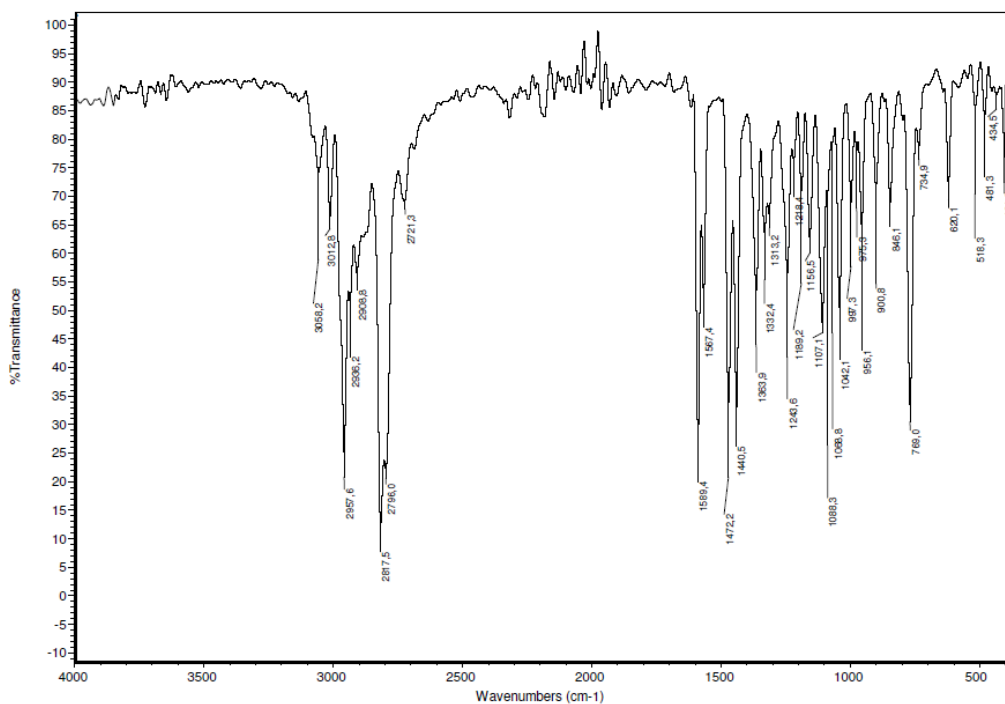


Fig. 2. Proposal structure of the complex cation in the: a) Ni(II) complex and b) Zn(II) complex; L = NO₃⁻.

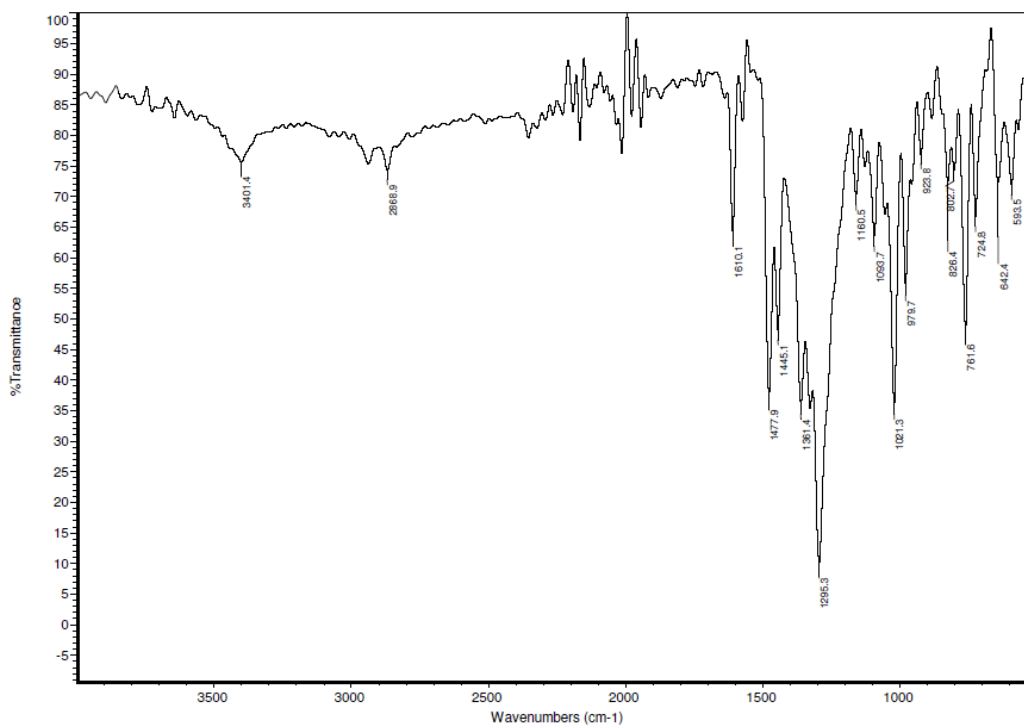
In the IR spectrum of complex **1** (Fig 3b) a weak broad band at 2868.9 cm⁻¹ originates from the stretching vibrations of CH₂ group, and a band at 1573 cm⁻¹ from aromatic ν(C=C) bending vibrations. A sharp band at 1589.4 cm⁻¹, ascribed to pyridine ring skeletal vibration in the spectrum of free tpmc (Fig. 3a) is displaced to 1610 cm⁻¹ upon coordination [4]. Strong broad bands at 1361.4–1295.3 cm⁻¹ and 1021.3 cm⁻¹ are assigned to coordinated nitrate [35]. Complex **2** (Fig. 3c) showed a broad band due to hydrogen bonds at 3457.7 cm⁻¹ from bonded water crystal molecules. Also, the strong asymmetric and symmetric vibrations of CH₂ group at 2900–2700 cm⁻¹ observed in free tpmc were weak and displaced in the spectra of both complexes, which confirms the coordination of tpmc. A weak band in the IR spectrum of complex **2** at 2890.7 cm⁻¹ (m, CH₂) were shifted to

higher energy when compared to that of free tpmc as well as complex **1**, thus indicating a different conformation of the tpmc ligand. The band at 1610.3 cm⁻¹, the (CN) vibration of pyridine, was displaced to a higher-energy region compared to free ligand [16], as in complex **1**. The strong and broad bands at 1506.3 cm⁻¹ and 1360.8 cm⁻¹ indicated vibration of symmetrically coordinated nitrate ions, besides the bands at 1274.3 cm⁻¹ and 1027.4 cm⁻¹, which corresponded to coordinated nitrate [16, 21]. In IR spectra of both complexes showed bands at 761.6 cm⁻¹ (**1**) and 778.4 cm⁻¹ (**2**) due to vibration of 2-substituted pyridine [21]. Comparing the band seen in the spectrum of the free tpmc ligand at 769 cm⁻¹, the complexes showed shifts toward lower and higher energies, which coincides with the acquirement of a different conformation.

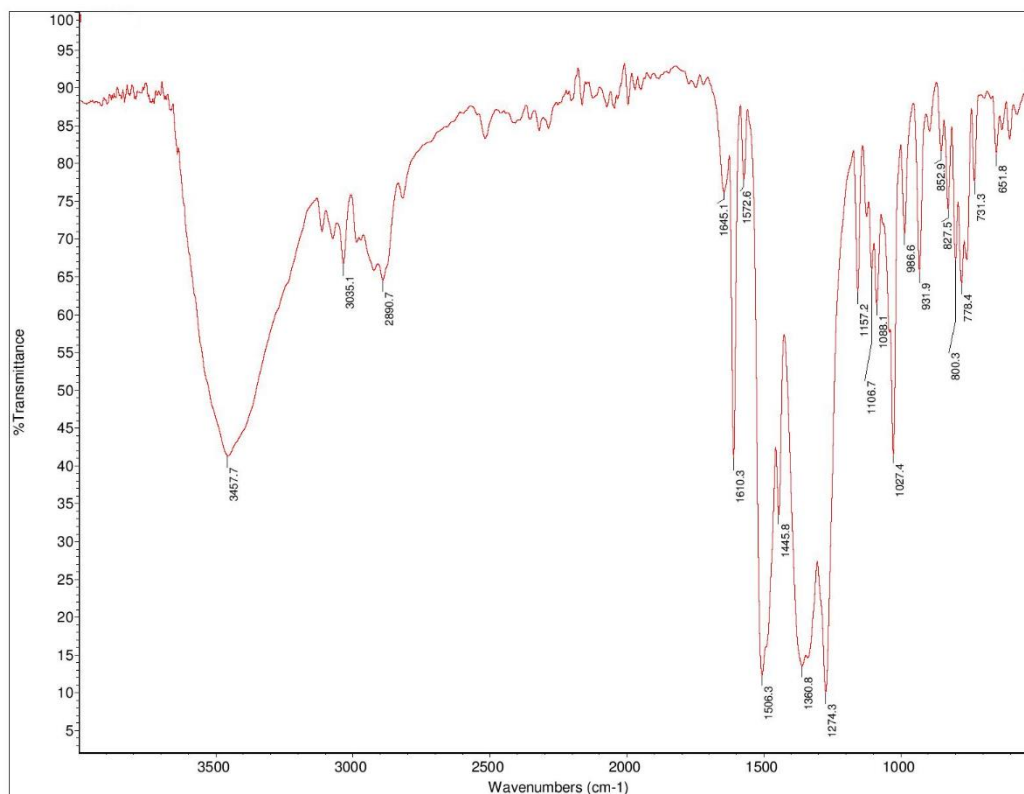
Figure 3



a)



b)



c)

Fig. 3. IR spectra of a) tpmc, b) complex 1 and c) complex 2

The NMR spectra of complex **1** were recorded in DMSO- d_6 at 500 MHz for ^1H and 50 MHz for ^{13}C , as described in the experimental section. The ^1H NMR spectrum of complex **1** showed characteristic pyridine proton peaks at 7.45–8.60 ppm. Protons of aliphatic methylene groups appeared below 3 ppm, while linked CH_2 protons between pyridine and nitrogen belonging to cyclotetradecane showed peaks at 4.17 ppm, as previously re-

ported [29]. Chemical shifts in the ^{13}C NMR spectrum of aliphatic carbon atoms from the cyclam ring were at 38–56 ppm, while near 58 ppm were methylene carbon atoms in groups connecting cyclam and pyridine. Meanwhile, the chemical shifts of pyridine carbon atoms were above 120 ppm, but the most intensive chemical shifts (154.65 and 155.38 (C_b)) were seen for carbons near the pyridine nitrogen (Fig.4).

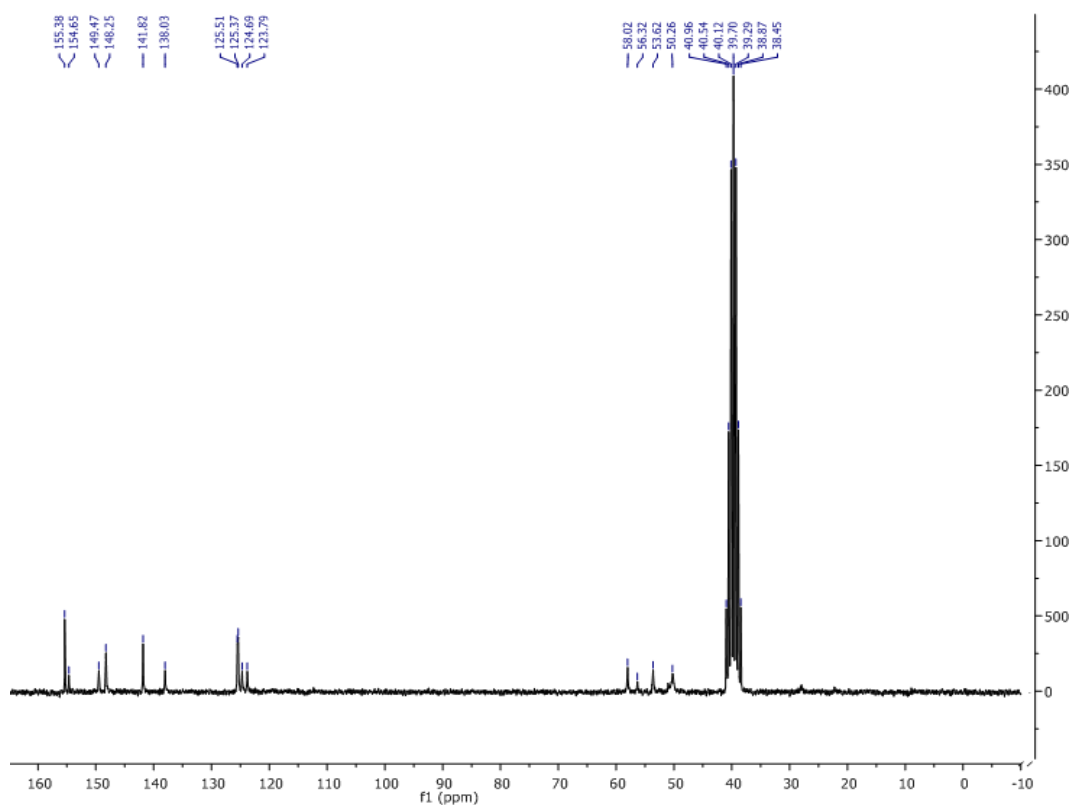


Fig. 4. ^{13}C NMR spectrum of complex **1**

To elucidate the preferred geometry of the $[\text{Zn}_2(\text{tpmc})\text{NO}_3]^{3+}$ complex, different model systems were used for ion DFT calculations. The geometry of the model system with the lowest calculated energy is shown in Figure 5. In this structure, the 14-membered tpmc ring adopts a boat conformation and the whole structure belongs to symmetric B set [26] with short metal–metal distance (4.8 Å). The coordination geometry around both Zn^{2+} centers is close to trigonal-bipyramidal (τ parameters are 0.92 and 0.86 for Zn1 and Zn2 centers). Both metal center at equatorial positions are occupied by one oxygen atom from the NO_3^- bridging ligand, nitrogen atom from 2-pyridylmethyl group and nitrogen atom from tpmc. Apical positions are occupied by two nitrogen atoms, one from tpmc and the second from a 2- pyridylmethyl group.

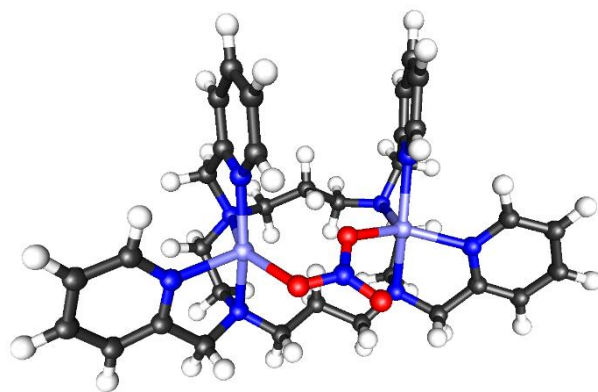


Fig. 5. Optimized structure of the $[\text{Zn}_2(\text{tpmc})\text{NO}_3]^{3+}$ complex ion. Colors: violet – zinc; red – oxygen; blue – nitrogen; gray – carbon

Antibacterial activity. *In vitro* bacterial activity of free ligand, tpmc and its complexes were investigated on six Gram-positive and two Gram-negative bacterial strains. The MIC values of the tested compounds are shown in Table 1.

All compounds showed weak antibacterial activity against Gram-negative strains *E. coli* and *Klebsiella*, compared to their activities on Gram-positive strains. These results are in agreement with previous research into tpmc complexes [19, 28]. The free ligand was almost inactive against selected bacterial strains and showed only low activity against an isolated strain of *S. pseudintermedius*. Complexes **1** and **2** displayed different antibacterial activity compared to tpmc.

Noticeably, all tested compounds showed similar antibacterial activities toward almost all investigated *Staphylococcus* strains. Actually, Cu(II)–tpmc dianionic complexes displayed similar antibacterial activity [28] against *S. aureus*. Never-

theless, the exception was complex **1**, which exhibited a higher antibacterial activity against *S. pseudintermedius* ($80 \mu\text{g ml}^{-1}$). Meanwhile, the MIC values of complexes **1** and **2** against *Klebsiella* were 320 and $640 \mu\text{g ml}^{-1}$; the free ligand possessed lower activity toward the same bacterial strain. This confirms previous studies into cyclam derivative complexes [9]. On the other hand, complex **1** exhibited moderate activities against all Gram-positive bacterial strains ($80\text{--}640 \mu\text{g ml}^{-1}$), while the analogous nickel complex showed weak effects ($640\text{--}1280 \mu\text{g ml}^{-1}$). Similar results have been seen for zinc and nickel complexes with macrocyclic tetraaza ligands [36]. Different antibacterial activities of complexes and tpmc could be explained by chelation effects of the macrocyclic ligand, which reduce the polarity of the metal ions and may result in increasing uptake of compounds through the bacterial cell membrane.

Table 1

Antimicrobial activity of investigated compounds

Bacterial strain	MIC ($\mu\text{g ml}^{-1}$)				
	tpmc	Complex 1	Complex 2	O	A
<i>Staphylococcus aureus</i> ATCC 29213	>1280	>640	>1280	0.25	–
<i>Staphylococcus saprophyticus</i> ATCC 15305	1280	>640	1280	0.125	–
<i>Staphylococcus pseudintermedius</i> isolat	640	640	640	>256	–
<i>Staphylococcus xylosus</i> ATCC 29991	>1280	>640	>1280	0.66	–
<i>Staphylococcus pseudintermedius</i> ATCC 49444	1280	80	640	0.25	–
<i>Enterococcus faecalis</i> ATCC 29212	1280	320	640	–	≤ 0.125
<i>Escherichia coli</i> ATTC 25922	>1280	>640	>1280	–	4
<i>Klebsiella pneumoniae</i> ATTC 700603	>1280	>640	>1280	–	>128

O – Oxacillin, **A** – Ampicillin

Cytotoxicity. Cytotoxicity of investigated compounds was determined by MTT assay in normal human HS-5 cells and MCF-7 and MDA-MB-231 breast cancer cell lines (Fig. 6).

Interestingly, experimental results indicate that both complexes, after three days incubation, displayed lower cytotoxic activities on cancer cell lines and normal human cell ($\text{IC}_{50} > 200 \mu\text{M}$) than did tpmc. In fact, under the same conditions, tpmc

showed an inhibition of both MCF-7 and MDA-MB-231 cell viability ($\text{IC}_{50} 122 \pm 14 \mu\text{M}$ and $168 \pm 17 \mu\text{M}$, respectively). Even though several metal complexes have shown antiproliferative activities on different cancer cell lines, all of them have a coordinated sphere of other organic ligands besides tpmc; as a consequence, this may increase the cytotoxic effects of metal complexes [19, 27, 28].

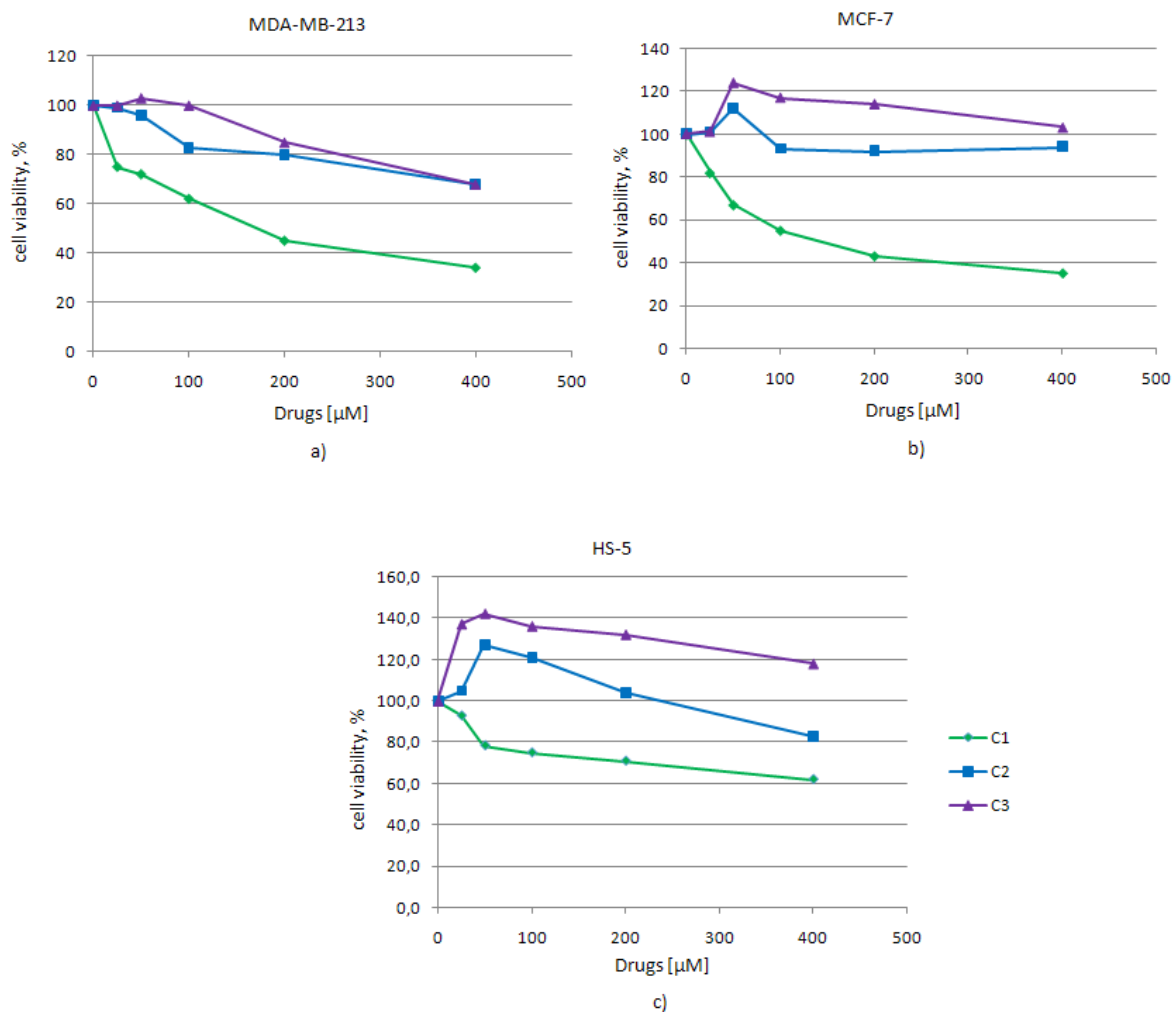


Fig. 6. Cytotoxicity curves of complexes on a) MDA-MB-231, b) MCF-7 and c) HS-5. C₁ – tpmc, C₂ – complex 1, C₃ – complex 2

4. CONCLUSIONS

This study reported the synthesis and characterization of two new dinuclear complexes, $[\text{Zn}_2(\text{tpmc})(\mu\text{-NO}_3)](\text{NO}_3)_3 \cdot \text{CH}_3\text{OH}$ (**1**) and $[\text{Ni}_2(\text{tpmc})(\text{NO}_3)_2](\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (**2**) by different techniques. Based on elemental analysis, molar conductivity and spectroscopy, in complex **1** Zn(II) ions are five-coordinated with trigonal-bipyramidal geometry, while in complex **2** the Ni(II) ions are six-coordinated with a distorted octahedral geometry. Also, these complexes displayed different conformations of the tpmc ligand molecule, which in complex **1** revealed a boat conformation, but a chair conformation in complex **2**. The complexes showed lower cytotoxicity activities toward breast cancer cell lines compared to the ligand-free tpmc molecule. Furthermore, complex **1** demonstrated better antimicrobial activity than did complex **2**, which may be related to the structural conformation of the molecules. Finally, both complexes

can be used as starting compounds for the synthesis of new mixed complexes, such as the already used analogous complexes with tpmc as ligand.

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