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*Original scientific paper*

# Synthesis and characterization of copper(II) octaazamacrocyclic complexes with glycine derivatives. *In vitro* antiproliferative and antimicrobial evaluation of the Cu(II) and Co(II) analogous

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*Abstract*: Two new complexes with the general formula $[Cu_2(L)tpmc](ClO_4)_3 \cdot nH_2O$ (tpmc = *N*,*N*′,*N*″,*N*‴-tetrakis(2-pyridylmethyl)--1,4,8,11-tetraazacyclotetradecane, L = *N*-methylglycine, *n* = 3; L = *N*,*N*--dimethylglycine, *n* = 2) were isolated and their composition, some physical and chemical properties and geometries were proposed based on elemental analysis (C, H, N), conductometric and magnetic measurements and spectroscopic data (UV–Vis, FTIR). It is evident that the complexes are binuclear and an *exo* coordination mode of the macrocyclic ligand in the boat conformation was proposed. The co-ligands are coordinated as a bridge using both oxygen atoms of the $OCO^-$ group. The cytotoxic activity of Cu(II) complexes as well as their Co(II) analogs, the starting ligands and the free salts were tested against human cervix adenocarcinoma cell line (HeLa), human chronic myelogenous leukemia cells (K562), human breast cancer cell line (MDA-MB-453), and a non-cancerous cell line, human embryonic lung fibroblast (MRC-5). The $IC_{50}$ values for the Cu(II) complexes were from 21.6±0.04 to 66.1±0.8, and for the Co(II) analogs were within the range from 8.8±0.74 to 15.40±1.52. All four complexes were tested for their antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and the yeast *Candida albicans*.

*Keywords*: Cu(II) and Co(II) complexes; octaazamacrocycle; antiproliferative activity; antimicrobial activity.

## INTRODUCTION

The serious medical problem of bacterial and fungal resistance and the rate at which it develops have led to increasing levels of resistance to classical antibiotics. An urgent task for infectious diseases research programs have become

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the discovery and development of effective antibacterial and antifungal drugs with novel mechanism of action.[1] Currently, cancer rates continue to dramatically increase and pose a serious threat to public health. Although many antitumor agents have been developed in the recent years, the survival rate of patients is not satisfactory.[2] There is a strong relationship between metals or their complexes, and their antibacterial, antitumor, and anticancer activities. A number of *in vivo* studies have indicated that biologically active compounds become more bacteriostatic and carcinostatic upon chelation.[3] A number of metal complexes of amino acid with transition metals possess anticarcinogenic activity. Amino acids have been effectively used to direct nitrogen mustards into cancer cells.[4] The design and synthesis of mixed ligand coordination complexes of Cu(II) and Co(II) have received considerable attention.[5] Macrocyclic structures are extremely favorable for metal complexation,[6] especially polyazamacrocyclic chelating ligand cyclams and cyclam-derived such as tpmc (*N,N′,N″,N‴*-tetrakis(2-pyridylmethyl)-1,4,8,11-tetraazacyclotetradecane). Numerous mixed-ligand Cu(II) and Co(II) complexes containing pendant macrocycle tpmc and one or two additional ligands of various type are applied as antitumor,[7,8] antiviral, anti-HIV,[9] antibacterial, antifungal or antimalarial agents.[10–13] Depending on the reaction conditions, and structure and number of the co-ligands, the macrocycles have the possibility to form mono-, bi- and tetranuclear transition metal complexes. On the other hand, it is a fact that amino acids or their derivates are attractive ligands due to their biological activity (acids are the building blocks of proteins and participate in all major processes in organisms). Therefore, they are often used as ligands in the synthesis of coordination compounds. Amino acids, like other aminocarboxylato ligands, are bonded in one of many modes, *i.e.*, as *N*-monodentate; *N*,*O*-bonded as chelate in mononuclear complexes or in binuclear ones as bridging ligands between the two metallic centers in the *N*,*O*,*O′*--mode. In addition, one or both oxygen atoms may be included in the coordination and the $–NH_2$ group remains uncoordinated. There are various possibilities for bonding. They can coordinate non-symmetrically, symmetrically or in a combined chelate-bridged manner.[14] Sarcosine (*N*-methylglycine) is an amino acid occurring in various living organisms as an intermediate in amino acid metabolism and as a component of peptides. Additionally, it can potentially serve as a liposome cryoprotectant and as a drug for treatment of schizophrenia.[4] In previous papers, the synthesis and the properties of mixed-ligand Co(II) complexes with the general formula $[Co_2(Y)tpmc]Z$ (HY= glycine or *N*-methylglycine/*N*,*N*--dimethylglycine, Z = $BF_4^-$ or $ClO_4^-$) were described.[15] They crystallized with different amounts of crystal solvents ($H_2O$/$CH_3CN$). In continuation of this research, two new Cu(II) complexes with mixed ligands of macrocycle tpmc and the same amino acid derivate co-ligands were synthesized. The structures of the compounds were proposed based on spectral (UV–Vis and IR) and elemental

analysis (C, H, N) data. The complexes were characterized by conductometric and magnetic measurements. The antimicrobial and antiproliferative activities of the new Cu(II) and the previously prepared Co(II) complexes with the same ligands[15] were tested and the relationship between the biological activities of all complexes and their structures are discussed.

## EXPERIMENTAL

*Chemicals and materials*. Ligand tpmc and complexes $[Cu_2tpmc](ClO_4)_4$, $[Co_2(N\text{-mgly})tpmc](BF_4)_3\cdot 4H_2O$ (**3**) and $[Co_2(N,N\text{-dmgly})tpmc](BF_4)_3\cdot 3H_2O$ (**4**), were prepared and purified as described in the literature.[15-17] The other chemicals: *N*-methylglycine (*N*-mgly) and *N,N*-dimethylglycine (*N*,*N*-dmgly), $CH_3OH$ and NaOH as *p.a.* commercial products were provided by Merck, while 1,4,8,11-tetraazacyclotetradecane (cyclam), 2-picolyl chloride hydrochloride, penicillin, streptomycin, nutrient medium RPMI-1640, DMSO and 0.05 % triphenyltetrazolium chloride (TTC) were obtained from Sigma–Aldrich and the fetal bovine serum (FBS) was obtained from Biochrom AG (Berlin, Germany).

*Preparation*

**Caution!** Perchlorate complexes are potentially explosive.

*General procedure*. The complex $[Cu_2tpmc](ClO_4)_4$ (0.050 g/0.50 mmol) was dissolved in 5 mL of $CH_3OH$ under continuous stirring and refluxed in a water bath (80 °C). The solution of the co-ligand *N*-methylglycine or *N,N*-dimethylglycine (0.0688 g/0.0788 g; 0.75 mmol) in $CH_3OH$ was added to the hot solution of $[Cu_2tpmc](ClO_4)_4$. The pH of the solution was adjusted to 6 using 0.1 M NaOH. The reaction mixture was continuously stirred and refluxed for the following 5 h. Finally, the solvent was evaporated to 1/4 of the initial volume. The solution was left in a refrigerator overnight, until precipitation of the solid blue microcrystalline product occurred. The precipitate was separated by suction, washed properly with small portions of cold water and dried at room temperature. The product was purified by recrystallization from methanol.

*$[Cu_2(N\text{-mgly})tpmc](ClO_4)_3\cdot 3H_2O$ (**1**)*. Yield: 77 mg (68 %); Anal. Calcd. for $C_{37}H_{56}N_9O_{17}Cu_2Cl_3$ (*FW* = 1132.50): C, 39.23; H, 4.41; N, 11.13 %. Found: C, 39.02; H, 4.17; N, 10.66 %.

*$[Cu_2(N,N\text{-dmgly})tpmc](ClO_4)_3\cdot 2H_2O$ (**2**)*. Yield: 82 mg (73 %); Anal. Calcd. for $C_{38}H_{56}N_9O_{16}\ Cu_2Cl_3$ (*FW* = 1128.43): C, 40.44; H, 4.90; N, 11.16 %. Found: C, 40.70; H, 4.46; N, 10.78 %.

At room temperature, the complexes are soluble in $CH_3OH$ and insoluble in $H_2O$.

*Analytical spectral and other physical measurements*. The elemental analyses were performed by standard methods. The electronic absorption spectra of the complexes in $CH_3OH$ solution ($c = 1.0\times10^{-3}$ M) were recorded on a GBC UV–Vis spectrophotometer Cintra 20. The FTIR spectra were recorded on a Nicolet 6700 FTIR (ATR technique) in the range of 400––4000 $cm^{-1}$. The molar conductivities were measured using HANNA instruments HI 8820N conductometer (at 20±2 °C) in $CH_3OH$ ($c = 1.0\times10^{-3}$ M). The magnetic susceptibilities were measured on an MSB-MKI magnetic balance, Sherwood Scientific Ltd., England, at room temperature (20±2 °C). The data were corrected for diamagnetism using Pascal's constants.[18]

In vitro *evaluation of antimicrobial and antiproliferative activity*

*Antimicrobial activity.* The antimicrobial activity of the new complexes Cu(II) (**1** and **2**) and the Co(II) analogs $[Co_2(N\text{-mgly})tpmc](BF_4)_3\cdot 4H_2O$ (**3**) and $[Co_2(N,N\text{-dmgly})tpmc](BF_4)_3\cdot 3H_2O$ (**4**), were assayed using the broth-microdilution method against the following laboratory

strains obtained from the American Type Culture Collection (ATCC): Gram-positive bacteria *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633, Gram-negative bacteria *Escherichia coli* ATCC 25922 and one strain of the yeast *Candida albicans* ATCC 10231. Stock solutions (10 mM) of the compounds were prepared in dimethyl sulfoxide (DMSO), and diluted to the working concentrations in fresh Müller–Hinton broth for bacteria and Sabouraud broth for *C. albicans*. Bacterial and yeast suspensions were prepared by the direct colony method. The colonies were taken directly from the plate and suspended in 5 mL of sterile 0.85 % saline. The turbidity of the initial suspension was adjusted by comparing it to 0.5 McFarland's standard. When adjusted to the turbidity of the 0.5 McFarland's standard, the bacterial suspension contained about 108 colony forming units, CFU $mL^{-1}$, and the suspension of yeast contained 106 CFU $mL^{-1}$. Ten-fold dilutions of the initial suspension were additionally prepared into Müller–Hinton broth for the bacteria and Sabouraud broth for *C. albicans*. Each dilution of complexes was poured in triplicates into a 96-well microtiter plate and inoculated with previously prepared bacterial suspension. For a negative control for each plate, only the medium was used. As a positive control of growth, wells containing only the microorganisms in the broth were used. In addition, the activity of the starting compounds: $[Cu_2tpmc](ClO_4)_4$, tpmc, *N*-methylglycine and *N,N*-dimethylglycine were also tested. The *MIC*s of ampicillin, and nystatin were determined in parallel experiments. In the tests, 0.05 % TTC was also added to the culture medium as a growth indicator. TTC is a redox indicator used for differentiation between metabolically active and non-active cells. The colorless compound is enzymatically reduced to red 1,3,5-triphenylformazan by cell dehydrogenases, indicating metabolic activity (red color of the medium in microtiter plate well). The bacteria growth was determined after 24 h, while the growth of *C. albicans* was determined after 48 h of incubation at 37 °C. The lowest concentration of the extract at which the microorganism does not demonstrate visible growth (*MIC*) and the minimal bactericidal or fungicidal concentration (*MBC*) were determined in broths from each well (10 mL) and inoculated in Müller–Hinton agar for 24 h at 37 °C for bacterial strains, and in Sabouraud dextrose agar for 48 h at 26 °C for the fungi. All determinations were performed in triplicate.

*Antiproliferative activity*

*Preparation of stock solutions of the test compounds.* The solutions of the investigated compounds (**1**–**4**), the starting ligands and the free salts were prepared in dimethyl sulfoxide at concentrations of 10 mM, and diluted by nutrient medium to working concentrations. The complete nutrient medium RPMI-1640 was supplemented with 10 % fetal bovine serum, 2 mM L-glutamine and 1 % penicillin/streptomycin.

*Cell lines.* Human cervix adenocarcinoma cell line (HeLa), human chronic myelogenous leukemia cells (K562), human breast cancer cell line (MDA-MB-453), and the non-cancerous cell line, human embryonic lung fibroblast (MRC-5) were grown in complete RPMI-1640 medium.

*Determination of cell survival.* Targeted adherent cells HeLa (2,500 cells/well), MDA-MB-453 (3,000 cells/well) and MRC-5 (5,000 cells/well) were seeded into the wells of a 96-well flat-bottomed microtiter plate. Twenty-four hours later, after cell adhesion, different concentrations of examined compounds were added to the wells, except for the controls, where only the complete medium was added. For non-adherent K562 cells (6,000 cells/well), the extracts were applied 2 h after the cell seeding. Culture medium with corresponding concentrations of the investigated compounds, but without the cells, was used as blank. The cultures were incubated for 72 h, and the effects of the investigated compounds on cancer and normal cell survival were determined using the microculture tetrazolium test (MTT), accord-

ing to Mosmann[19] with modification by Ohno and Abe,[20] 72 h after the addition of the investigated compounds. Briefly, 20 μL of MTT dye solution (5 mg $mL^{-1}$ of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide in phosphate-buffered saline) was added to each well. Samples were incubated for an additional 4 h at 37 °C in a humidified atmosphere of 5 vol.% $CO_2$. Afterward, 100 mL of 10 % sodium dodecyl sulfate (SDS) were added in order to extract the insoluble formazan, which represents the product of the conversion of the MTT dye by viable cells. The number of viable cells in each well is proportional to the intensity of the absorbance (*A*) of light, which was measured in microtiter plate reader at 570 nm, 24 h later. To determine cell survival (*S%*), the *A* of a sample with cells grown in the presence of various concentrations of the investigated compounds was divided by the control optical density (the *A* of control cells grown only in nutrient medium) and multiplied by 100. The *A* of the blank was always subtracted from the *A* of the corresponding sample incubated with the target cells. All experiments were performed in triplicates.

## RESULTS AND DISCUSSION

The reaction conditions were carefully adjusted by controlling the pH and temperature. Under these conditions the mixture of the solutions of $[Cu_2(tpmc)](ClO_4)_3$ and *N*-methyl/*N*,*N*-dimethylglycine in $CH_3OH$ in a molar ratio 1:1.5 resulted in blue microcrystalline products presented by general formula $[Cu_2(L)tpmc]\ (ClO_4)_3 \cdot nH_2O$. With L = *N*-methylglycine, the water content was $n = 3$ (**1**), while with L = *N,N*-dimethylglycine $n = 2$ (**2**). The yields were 68 and 73 %, respectively. The results from the elemental analyses are in accordance with the proposed dinuclear structure. The physical characteristics of the obtained compounds and related Co(II) complexes are presented in Table I. The molar conductivity values 350 and 395 S $cm^2$ $mol^{-1}$ for **1** and **2**, respectively in $CH_3OH$ ($1.0\times10^{-3}$ M) are in agreement with the values for 1:3 type electrolytes.[21]

TABLE I. Magnetic moment at room temperature, electronic spectral data and molar conductivity in $CH_3OH$ ($c = 1.0\times10^{-3}$ M) for new Cu(II) complexes compared with some Co(II) analogues

| Complex | $\lambda_{max}$ / nm ($\varepsilon$ / $dm^3$ $mol^{-1}$ $cm^{-1}$) | $\mu_{eff}$ / $\mu_B$ per Cu(II) | $\Lambda_m$ S $cm^2$ $mol^{-1}$ |
|---|---|---|---|
| $[Cu_2$(*N*-mgly)tpmc$](ClO_{4)3} \cdot 3H_2O$ (**1**) | 640 (316) | 1.86 | 350 |
| $[Cu_2$(*N*,*N*-dmgly)tpmc$](ClO_4)_3 \cdot 2H_2O$ (**2**) | 683 (558) | 1,80 | 395 |
| $[Co_2$(*N*-mgly)tpmc$](BF_4)_3 \cdot 4H_2O$ (**3**)[15] | 455(30) 508(53) 544(35) | 4.77 | 360 |
| $[Co_2$(*N*,*N*-dmgly)tpmc$](BF_4)_3 \cdot 3H_2O$ (**4**)[15] | 487(38) 510(42) 546(28) | 4.70 | 366 |

*Magnetic properties.* The magnetic moment values for Cu(II) complexes are 1.86 (for **1**) and 1.80 $\mu_B$ (for **2**) at room temperature (see Table I). These data are consistent with one unpaired electron in paramagnetic pentacoordinate Cu(II) complexes.[22] In similar 5-coordinated Cu(II) complexes with carboxylate co-ligands the values of the magnetic moments are in the range of 1.75–2.20 $\mu_B$/Cu(II).[14,23–25] However, similar values are reported for binuclear Cu(II) complexes with no magnetic interaction between the copper(II) ions as well.[23–25]

*Spectroscopic properties*

*UV–Vis.* The electronic absorption spectra in methanol of **1** and **2** show a broad maximum at 640 and 683 nm and molar absorption coefficients ($\varepsilon$) of 316 and 558 $M^{-1}$ $cm^{-1}$ (Table I). The large width of the spectral bands, the position of $\lambda_{max}$, and the shapes correspond to d–d transitions of the Cu(II) complexes.[26] The similarity of these spectra to the spectra of the related aminocarboxylate complex with *S*-phenylalanine and with numerous carboxylate Cu(II) complexes[14,23–25] additionally supports the proposed five-coordinate geometry of Cu(II). There is a bathochromic shift of the absorption maximum in the case of complex **2** in comparison to complex **1**. This could be ascribed to the fact that the $–CH_3$ group/s at the N atom affects the strength of the ligand field in the aminocarboxylate derivatives. Based on these data, it was concluded that in both complexes the co-ligands are coordinated in the same way. The chromophore indicates that the co-ligands are coordinated *via* the $OCO^–$ group. The value of the molar coefficient of absorptivity, $\varepsilon$, which decreases with increasing symmetry of the molecules, was found in similar complexes.[26] The lower value of $\varepsilon$ in complex **1** indicates its higher symmetry compared to complex **2** (see Table I). In the UV part of the electronic spectra, very intensive multiple bands, ascribed to CT appeared in the 205–330 nm range ($\varepsilon$ was in the range 5000–5700 $M^{-1}$ $cm^{-1}$) for the *N*-mgly and *N*,*N*-dmgly complexes.

*FTIR spectra.* In the infrared spectra of the newly synthesized complexes (**1** and **2**), containing *N*-methylglycinato/*N*,*N*-dimethylglycinato anions, the following bands were found: a broad multiple band of 3586/3590 $cm^{-1}$ arising from ν(O–H), indicating the presence of a water molecule; a band at 3439 $cm^{-1}$ of ν(NH) for the secondary amino group excluded from coordination in complex **1**; bands at 3090/3084 $cm^{-1}$ ν(C–H) from pyridine rings; a weak broad band in the range of 2963–2894 $cm^{-1}$ likely showing stretching vibration of CH; and two medium bands about 1440 and 1490 $cm^{-1}$ due to $CH_2$ bending vibrations; a sharp strong band at 1611/1613 $cm^{-1}$ from the skeletal stretching valence vibration of the tpmc pyridine included in coordination that was found at 1588 $cm^{-1}$ in the spectrum of free tpmc; a broad intensive band at 1096/1094 $cm^{-1}$ from ν($ClO_4$) and a medium sharp band at around 623/622 $cm^{-1}$ from δ($ClO_4$) for both complexes. In the low-frequency region, in the spectra of both complexes, the bands are in the range 462/466 $cm^{-1}$ and in the range 418/419 $cm^{-1}$, which are attributed to the existence of Cu–O and Cu–N bonds, respectively, with the copper(II) ions.[27] These vibrations confirm the coordination of ligands to the central metal ions and the involvement of nitrogen (from tpmc) and oxygen atom ($OCO^–$ from *N*-mgly/*N*,*N*-dmgly) in the coordination. The aminocarboxylic acid ligands feature multiple coordination sites that combine the characteristics of amine and carboxylic groups and are able to exhibit different coordination modes depending on the nature of the reaction system. The FTIR spectra of complexes and ligands

show strong evidence in support of the involvement of carboxylate group from *N*-mgly/*N*,*N*-dmgly in the coordination. In comparison to the free amino acids derivatives, $\nu_{as}(OCO^{-})$ and $\nu_{s}(OCO^{-})$ bands were shifted, which confirm the coordination of the carboxylate group. The difference in the FTIR stretching frequencies of the bound carboxylate complexes, $\Delta\nu(OCO^{-})$ between the observed asymmetric, $\nu_{as}(OCO^{-})$ and the symmetric, $\nu_{s}(OCO^{-})$ bands provide useful information about the different binding modes of the coordination carboxylate ligands. The absorption band due to $\nu_{as}(OCO^{-})$ and $\nu_{s}(OCO^{-})$ appear at 1574 and 1375 $cm^{-1}$ for (**1**) and 1573 and 1376 $cm^{-1}$ for (**2**), respectively. The band assigned to the carboxyl group is red shifted compare to the free ligand thus indicating coordination to the metal. The changes of the $\Delta\nu = \nu_{as} - \nu_{s}$ values for the complexes compared with those found for their corresponding aminocarboxylate derivatives showed that $OCO^{-}$ group is also coordinated to Cu(II).[28] The observed range is in accordance with the values reported for coordinated $OCO^{-}$ stretching bands in amino–carboxylate Cu(II) tpmc complexes.[25] A comparison of the difference $\Delta\nu = 199\ cm^{-1}$ (for **1**) and $\Delta\nu = 197\ cm^{-1}$ (for **2)** with the "ionic" value for Na-*N*-mgly ($\Delta\nu = 190\ cm^{-1}$) and Na-*N*,*N*-dmgly ($\Delta\nu = 218\ cm^{-1}$) suggests bridge coordination modes of the $OCO^{-}$ group.[27,28] It could be concluded that in both complexes, the ligands are coordinated as a bridge using both oxygen atoms $COO^{-}$ group. The most probable way of coordination of the carboxyl group is $\mu$-*O*,*O*′ as in Cu(II) complexes with bridge *S*-phenylalanine as well as with other carboxylate co-ligands (benzoate and phthalate).[14,23,25] Monodentate coordination of the $OCO^{-}$ group could be excluded (in this case, $\Delta\nu$ would be much larger in the complex compared to the ligand) as well as the chelate-bridged binding ($\Delta\nu$ was much less in the complex compared to the ligand).[27,28] The participation of the amino nitrogen in the coordination for Cu(II) is excluded in both complexes. The substitution of the amino group of glycine with $–CH_3$ group reduces the probability of coordination of atom N even though its donor properties increase. However, due to steric disturbances, this is highly unlikely. The existence of the zwitter ion of aminocarboxylate derivatives and its coordination for Cu(II) cannot be excluded using the applied methods. In the Co(II) analogs, the anions of *N*-methyl derivatives are also coordinated through $OCO^{-}$, in a combined chelate-bridged manner ($\Delta\nu$ values in Co(II) complexes were significantly lower than in the spectra of the respective free co-ligand). From all the data presented, it is presumed that the two newly synthesized complexes are binuclear with an *exo* coordination mode of the macrocyclic pendant ligand in the boat conformation. Each Cu(II) ion is coordinated by five donor atoms using the two pyridyl and two cyclam nitrogens of tpmc and the oxygen atom of the co-ligand. The participation of the co-ligand nitrogen atom is excluded. Probably both oxygens of $OCO^{-}$ are engaged in the coordination thus forming a bridge between two metal ions from the same tpmc unit, Fig. 1.

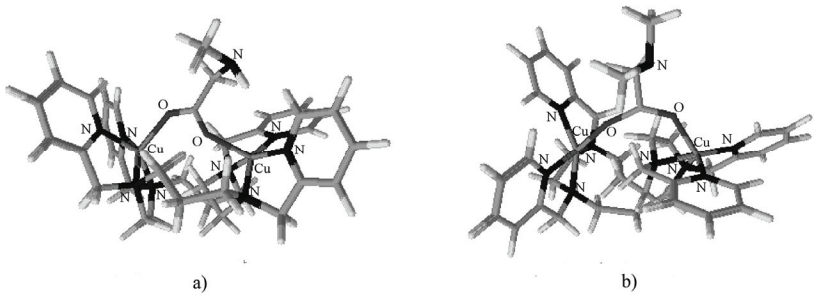


Fig. 1. Proposed structure of complex cations: a) $[Cu_2(N\text{-mgly})tpmc]^{3+}$of **1a** and b) $[Cu_2(N,N\text{-dmgly})tpmc]^{3+}$of **2b**.

*Antibacterial and antiproliferative activity*

*Antimicrobial activity*. The increasing drug resistance of microorganisms is becoming a serious threat for countering microbial infections. New, more effective therapies and alternative substances, such as coordination complexes, that are effective against highly resistant strains are still being sought.[29] The results for the *in vitro* antimicrobial activity of the new complexes and previously described Co(II) analogs are given in Table II.

TABLE II. Antimicrobial activity of the tested complexes (**1**–**4**) and referent antibiotics, expressed as *MIC* values (μg $mL^{-1}$), determined by broth microdilution methods; n.t. – not tested

| Microbial strain | Compound | | | | | |
|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | Ampicillin | Nystatin |
| *S. aureus* | 275.75 | 274.75 | 141.56 | 141.00 | 2.4 | n.t. |
| *B. subtilis* | 275.75 | 274.75 | 283.13 | 282.00 | 4.8 | n.t. |
| *E. coli* | >1000 | >1000 | >1000 | >1000 | 4.8 | n.t. |
| *C. albicans* | >1000 | >1000 | >1000 | >1000 | n.t. | 3.125 |

The Cu (II) and Co (II) complexes with amino acid derivatives did not show activity against the Gram-(–) bacteria and the yeast *C. albicans*. All four tested complexes showed activity against Gram-(+) bacteria. This could be explained by the difference in the permeability of the membrane of Gram-(+) bacteria in comparison to the Gram-(–) one due to the difference in their structure.[30] Gram-(+) bacteria are known to be more susceptible to amino acids complexes.[31] Literature data show that bimetallic complexes of sarcosine with Zn(II) and Sn(IV) are more active against Gram-(+) bacterial strain than against Gram-(–) bacteria.[34] Several studies have used a classification based on the *MIC* results to evaluate the antimicrobial activity of new compounds as: good, *MIC* less than 100 μg $mL^{-1}$; moderate: *MIC* between 100 and 500 μg $mL^{-1}$; weak: *MIC* between 500 and 1000 μg $mL^{-1}$; and inactive when the *MIC* value is more than 1000 μg $mL^{-1}$.[32] In this way, it was possible to evaluate the antimicrobial acti-

vity of the examined metal complexes as moderate (Table III). Furthermore, when the *MBC*/*MIC* ratio is less than or equal to 4.0, the investigated agent would be considered as bactericidal, and when this ratio is more than 4.0 should be considered as bacteriostatic. The examined compounds showed bactericidal effect on *S. aureus* and *B. subtilis*.

TABLE III. Minimal bactericidal concentration (MBC / μg $mL^{-1}$) and *MBC*/*MIC* ratio (in parentheses) for the tested complexes **1**–**4**

| Bacterium | Complex | | | |
|---|---|---|---|---|
| | **1** | **2** | **3** | **4** |
| *S. aureus* | 275.75 (1) | 549.50 (2) | 283.13 (2) | 141.00 (1) |
| *B. subtilis* | 551.50 (2) | 1099.0 (4) | 283.13 (1) | 282.00 (2) |

*Antiproliferative activity.* The *in vitro* antiproliferative activities of compounds **1**–**4**, tpmc and co-ligands were evaluated against cell lines Human cervix adenocarcinoma (HeLa), human chronic myelogenous leukemia (K562), human breast cancer (MDA-MB-453), and a non-cancerous cell line, human embryonic lung fibroblast (MRC-5) by the MTT colorimetric assay method. The obtained $IC_{50}$ values (concentration of compounds that induced a 50 % decrease in cell survival) are given in Table IV together with the activity of cisplatin as the referent cytostatic drug. All four compounds promoted a significant decrease in the metabolic activity of the HeLa, K562, MDA-MB-453 and MRC-5 cells, which occurred in a dose-dependent manner (cell survival, *S vs*. concentration of compounds, Fig. 2). The $IC_{50}$ values of the complexes were in the range of 8.80–66.1 μM against the four tested cell lines, while for cisplatin, they were in the range 5.82–8.63 μM (Table IV). On the other hand, compounds **1** and **2** showed moderate activity, regarding all four cell lines. However, compounds **3** and **4** showed excellent antiproliferative activity against the investigated cells. On the contrary, compounds: tpmc, starting salts and free ligands did not show cytotoxic activity ($IC_{50}$ > 200 μM) under the same conditions. The obtained results are in agreement with data indicating that cobalt-containing complexes are very promising as

TABLE IV. $IC_{50}$±*SD* values (μM) after 72 h of action of the investigated complexes **1**–**4**, ligands and cisplatin on the tested cell lines, determined by the MTT test

| Complex | Cell line | | | |
|---|---|---|---|---|
| | HeLa | K562 | MDA-MB-453 | MRC-5 |
| $[Cu_2$(*N*-mgly)tpmc$](ClO_{4)3}\cdot 3H_2O$ (**1**) | 66.1±0.8 | 64.2±1.2 | 44.9±2.1 | 45.6±0.1 |
| $[Cu_2$(*N*,*N*-dmgly)tpmc$](ClO_4)_3\cdot 2H_2O$ (**2**) | 40.6±1.7 | 38.0±1.54 | 37.8±0.3 | 21.60±0.04 |
| $[Co_2$(*N*-mgly)tpmc$](BF_4)_3\cdot 4H_2O$ (**3**) | 15.40±1.52 | 9.70±0.28 | 12.68±1.02 | 9.51±1.00 |
| $[Co_2$(*N*,*N*-dmgly)tpmc$](BF_4)_3\cdot 3H_2O$ (**4**) | 11.88±0.40 | 10.66±0.88 | 13.43±1.25 | 8.80±0.74 |
| Tpmc, *N*-mgly, *N*,*N*-dmgly | >200 | >200 | >200 | >200 |
| Cisplatin | 6.90±1.71 | 5.82±0.17 | 6.73±0.48 | 8.63±1.38 |

potential antitumor agents.[32] It was observed that the Co(II) complexes have a higher cytotoxic activity than the Cu(II) complexes. This is also the case with complexes **1**–**4**. The data presented in the Table IV show that the $IC_{50}$ values for the MRC-5 cell line are similar to the ones obtained for cisplatin.

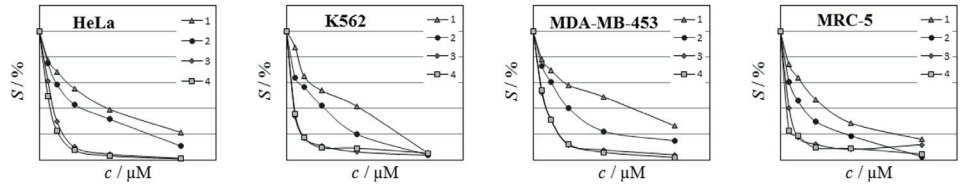


Fig. 2. Dose-response curves for the cytotoxicity of complexes **1**–**4** toward HeLa, K562, MDA-MB-453 and MRC-5 cells.

Due to the similarity of the structures of the Co(II) and Cu(II) complexes, the different antimicrobial activity could be explained by electronic and steric factors. The distribution of the molecular force in the molecule depends on the co-ligand and reduces the antiproliferative activity. In addition, the bridging of two metal centers by one co-ligand modifies the geometry of the whole molecule. A particular geometric shape could facilitate the contact with microorganisms and rapidly inhibit their growth if there are no steric disturbances by the ligands. The strength of the M–O bonds in the above described complexes is a consequence of various factors, such as, steric repulsions between the alkyl groups from the aminocarboxylates/derivatives and the pyridyl groups from tpmc, changes the inductive effects of the introduced $–CH_2–/–CH_3$ groups and their positions, the size of the alkyl group in relation to the size of the macro-cyclic cavity, non-covalent interactions, *etc*. It is difficult to determine the contribution of each factor, as they are all responsible for the overall structure. The influence of the described factors on cytotoxicity is significant, but nevertheless the most significant influence is that of the central ion.

## CONCLUSIONS

In this article, the synthesis, spectroscopic, magnetic properties of new Cu(II) mixed-ligand complexes with octaazamacrocyclic ligand tpmc and glycine derivatives, *N*-methylglycine and *N*,*N*-dimethylglycine, were reported. From all the obtained data, it could be concluded that two new synthesized complexes are binuclear with an *exo* coordination mode of the macrocyclic pendant ligand in the boat conformation. Each Cu(II) ion is coordinated by five donor atoms using two pyridyl and two cyclam nitrogen atoms of tpmc and the oxygen atom of the co-ligand. The participation of the nitrogen of co-ligand atom was excluded. Probably, both oxygens of $OCO^-$ are engaged in coordination thus forming a bridge between two metal ions from the same tpmc unit. The results for the antibacterial

activities for the new Cu(II) complexes and Co(II) analogous show that they have bacteriostatic activity, while the antiproliferative activity is moderate for Cu(II) complexes, whereas Co(II) complexes showed excellent cytotoxic activity against the four tested human cell lines.

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ИЗВОД

## СИНТЕЗА И КАРАКТЕРИЗАЦИЈА БАКАР(II) КОМПЛЕКСА СА ДЕРИВАТИМА ГЛИЦИНА. *in vitro* АНТИПРОЛИФЕРАТИВНА И АНТИМИКРОБНА ЕВАЛУАЦИЈА Cu(II) И Co(II) АНАЛОГА

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Синтетисана су два нова комплекса опште формуле $[Cu_2(L)tpmc](ClO_4)_3 \cdot nH_2O$ (tpmc = *N*,*N*′,*N*″,*N*‴-тетракис(2-пиридилметил)-1,4,8,11-тетраазациклотетрадекан, L = *N*-метилглицин, $n = 3$; L = *N*,*N*-диметилглицин, $n = 2$) а њихов састав, неке физичке и хемијске особине и геометрија су предложени на основу елементалне анализе (C, H, N), кондуктометријских и магнетних мерења и спектроскопских података (UV–Vis, FTIR). Утврђено је да су комплекси бинуклеарни и предложена је егзо координација макроцикличког лиганда у конформацији лађе. Ко-лиганди су координовани као мост, користећи оба атома кисеоника $OCO^-$-групе. Цитотоксична активност Cu(II) комплекса и њихових Co(II) аналога, полазних лиганада и стартних соли тестирана је на ћелијским линијама аденокарцинома цервикса (HeLa), хроничне мијелогене леукемије (K562), карцинома дојке (MDA-MB-453) и неканцерозној ћелијској линији, хуманог ембриоланог плућног фибробласта (МРЦ-5). $IC_{50}$ вредности за Cu(II) комплексе су од 21,60±0,04 до 66,1±0,8, а за Co(II) аналоге су у опсегу од 8,8±0,74 до 15,40±1,52. Сва четири комплекса су тестирана на антимикробну активност према *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* и *Candida albicans*.

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