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New copper(II) cyclam complexes with aminocarboxylate co-ligands: synthesis, characterization, and *in vitro* antiproliferative and antibacterial studies

BRANKA DRAŽIĆ[1]*, MIRJANA ANTONIJEVIĆ-NIKOLIĆ[2], MILENA MARINOVIĆ-CINCOVIĆ[3], VUKOSAVA ŽIVKOVIĆ-RADOVANOVIĆ[4], BRANKA BOROVIĆ[5] and SLAĐANA B. TANASKOVIĆ[1]

[1]*Faculty of Pharmacy, Vojvode Stepe 450, 11000 Belgrade, Serbia;* [2]*Academy of Applied Studies Šabac, Department for Medical, Business and Technological Studies, Hajduk Veljkova 10, 15000 Šabac, Serbia;* [3]*Institute of Nuclear Sciences Vinča, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Vinča, Belgrade, Serbia;* [4]*Faculty of Chemistry, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia and* [5]*Institute of Meat Hygiene and Technology, Kaćanskog 13, 1100 Belgrade, Serbia*

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Abstract: Two new cationic Cu(II) complexes of cyclam (1,4,8,11-tetraazacyclotetradecane) and aminocarboxylate coligands glycine or alanine have been synthesized. The complexes were characterized by elemental analysis (C, H, and N), molar electrical conductivity, magnetic susceptibility measurement at room temperature, spectral methods (UV/vis and FTIR), as well as TG and DTA. The analytical data of the complexes show the formation of mononuclear complexes with general formula $[Cu(L)cyc](ClO_4)_2 \cdot nH_2O$, (**A**): L = glycine, n = 1.5 and (**B**): L = alanine, n = 2.5. The tetradentate ligand cyclam was coordinated to metals through four N donors. The spectroscopic data suggested that the amino carboxylate ligands coordinated via their carboxylate ion moieties. The six-coordinate octahedral geometry around Cu(II) in both complexes was proposed. TG-DTA analysis indicates that complex **B** decomposes exothermally in a single step in the range of 310 -400 °C . The cytotoxic activity of Cu(II) complexes and the starting ligands were tested against human cervix adenocarcinoma cell line (HeLa), human melanoma (FemX) and human colon carcinoma (LS174). The *IC*50 values for the Cu(II) complexes were from 48.35-82.25 μM. Both complexes were tested for their antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and the yeast *Candida albicans*.

Keywords: copper(II) complexes, cyclam, glycine, alanine, antimicrobial and cytotoxic activity.

*Corresponding author: bdrazic@pharmacy.bg.ac.rs
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INTRODUCTION

The synthesis of transition metal complexes containing O- and/or N-donor ligands has become very important and attractive in the field of medical research due to the discovery of the antimicrobial and antiproliferative properties of these compounds.[1]

Cyclam (1,4,8,11-tetraazacyclotetradecane) (Fig. 1), as 14-membered tetra-amine macrocycle, is the one of the simplest N4-macrocycles which can strongly bind a wide range of metal ions. Some of these complexes have been used for: catalyzing different reactions, pharmacology and therapy, medical and radiopharmaceutical applications, as enzyme mimics, chemical sensors, selective metal ion recovery as well.[2] Medical interest has centered on clinical trials of a bicyclam for the treatment of AIDS and for stem cell mobilization, and on adducts with Tc and Cu radionuclides for diagnosis and therapy.[3,4]

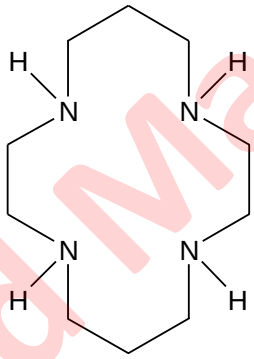

Fig. 1. 1,4,8,11-tetraazacyclotetradecane (cyclam)

Cyclam bonds metal ions through four donor nitrogen atoms. By substituting one or more hydrogen from nitrogen, with groups containing ligator atoms, numerous ligands have been made that have the properties of both macrocyclic and non-cyclic ligands. This modification of the basic macrocyclic ring changes its flexibility during the coordination of metal ions, which is a consequence of the participation of ligators from pendant groups in the coordination.

Metal cyclam complexes have a potentially rich configurational chemistry[4]. Each of the coordinated N atoms is chiral. The metal ion can commonly be 4-, 5-, or 6-coordinate. The macrocyclic 14-membered cyclam derivatives have moderately flexible structures and commonly adopt the six configurations, both trans- and cis-isomers.[3]

Copper is an essential trace mineral and plays a key role in such functions within the body as red blood cell production, iron absorption, the regulation of heart rate and blood pressure, and the development and maintenance of connective tissue, bones and organs. Another reason why copper is especially important for

human health in pandemic conditions like the present, is the part it plays in immune system maintenance and activation. Copper helps to ensure a healthy supply of white blood cells, many of which are phagocytes that protect the body by engulfing bacteria, foreign particles and dying cells. Copper enzymes usually contain metal ions bound to a specific amino acid residue or directly to the amide group – carbonyl or nitrogen – in the peptide backbone, offering different coordination environments.[5-7]

In the last two decades numerous complexes of transition metals with cyclam were synthesized. Different ways of coordination of macrocycle and its derivatives for different metal cations allows their application as antitumor, antiviral, antibacterial, antifungal or antimalarial agents.[8-12]

The goal of the article was to define structure of the mixed Cu(II) complexes with cyclam and amino acids, glycine and alanine, as additional ligand, to screen the *in vitro* cytotoxicity of the compounds compared to the effect of commonly used drug, cis-platin against human tumor cell lines.

EXPERIMENTAL

Chemicals and materials: The chemicals: L-glycine, L-alanine, CH_3CN was used as p.a. commercial products by Merck, Germany; 1,4,8,11-tetraazacyclotetradecane (cyclam) and copper(II) perchlorate hexahydrate by Aldrich, USA.

Preparation

(Caution! Perchlorate compounds are potentially explosive, which should be prepared in small amounts and handled with care)

$[Cu(gly)cyc](ClO_4)_2 \cdot 1.5H_2O$ and $[Cu(ala)cyc](ClO_4)_2 \cdot 2.5H_2O$

General procedure. Complexes with general formula $[Cu(L)cyc](ClO_4)_2 \cdot nH_2O$, (**A**): L=glycine (gly, $^+H_3N\text{-}CH_2\text{-}COO^-$), n = 1.5 and (**B**): L = alanine (ala, $^+H_3N\text{-}CH(CH_3)\text{-}COO^-$), n = 2.5 were obtained by reaction $Cu(ClO_4)_2 \cdot 6H_2O$ and cyclam with L-glycine or L-alanine.

Aqueous solution $Cu(ClO_4)_2 \cdot 6H_2O$ (111 mg, 0.30 mmol in 5 mL H_2O) was added in suspension of cyclam (61 mg, 0.30 mmol) in acetonitrile (10 mL). The violet reaction mixture was stirred at 80°C for 30 min. Aqueous solution of glycine/alanine (34 mg/40 mg; 4,5 mmol) was added in this solution and continuously stirred at 80°C for 90 min. The resulting reaction mixture was cooled to room temperature, filtered and left in the fridge a few days. A pink solid production was separated by filtration, and was dried an air. The product recrystallized from mixture CH_3CN:H_2O (1:1; v/v).

$[Cu(gly)cyc](ClO_4)_2 \cdot 1.5\ H_2O$ (**A**), Yield 87 %. Anal. Calcd. for $C_{12}H_{28}O_{11.5}N_5CuCl_2$ (FW = 560.89) C 25.92; H 5.30; N 12.06. Found C 25.71; H 5.03; N 12.49. At temperature 20 ± 2 °C the complexes are well soluble in CH_3CN, DMSO, $(CH_3)_2CO$ and DMF, and insoluble in EtOH, CH_3OH and H_2O.

$[Cu(ala)cyc](ClO_4)_2 \cdot 2.5H_2O$ (**B**), Yield 79 %. Anal. Calcd. for $C_{13}H_{32}O_{12.5}N_5CuCl_2$ (FW = 592.93) C 26.35; H 5.43; N 12.17. Found C 26.34; H 5.44; N 11.82. At temperature 20 ± 2 °C the complexes in well soluble in CH_3CN, DMSO, $(CH_3)_2CO$ and DMF, and insoluble in EtOH, CH_3OH and H_2O.

Measurements: The Elemental analyses were performed by standard methods in the Centre for Instrumental Analyses, ICTM in Belgrade. The electronic absorption spectra of the

complexes and ligands in CH_3CN (c = 10^{-3} M) were recorded on spectrophotometer GBC UV-Vis Cintra 20 in the range 200–900 nm. FTIR spectra were recorded on NICOLET 6700 FTIR (ATR technique) in the range 400-4000 cm^{-1}. The molar conductivities were measured on conductometer Therma orion star A212 (at 20 ± 2 ºC) in CH_3CN (c = 10^{-3} M). The magnetic susceptibility measurements were taken at room temperature (20 ± 2 °C) using a MSB-MKI balance (Sherwood Scientific Ltd., England). The data were corrected for diamagnetic susceptibilities using Pascal's constants.[13]

The thermal stability of the sample **B** was investigated by simultaneous non-isothermal thermo-gravimetric analysis (TG) and differential thermal analysis (DTA) using a SETARAM SETSYS Evolution 1750 instrument. The measurements were conducted at a heating rate of 10 °C/min in a dynamic argon atmosphere (flow rate was 20 mL/min) in the temperature range of 30 – 400 °C. The mass of the sample was about 2mg.

In vitro antiproliferative evaluation

Cell Culture: HeLa (human adenocarcinoma), FemX (human melanoma) and LS174 (human colon carcinoma) cell lines were obtained from the American Type Culture Collection 8 (Manassas, VA, USA).

Culture medium: All cancer cell lines were maintained in RPMI-1640 (Sigma-Aldrich, USA) medium supplemented with 10 % heat-inactivated (56 °C) fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 mg/mL streptomycin and 25 mM HEPES and adjusted to pH 7.2 using bicarbonate solution. Cells were cultured in a humidified chamber (37 °C, 5 % CO_2, 95 % air).

Other chemicals: dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA), sodium dodecyl-sulfate (SDS) (Sigma-Aldrich, USA), 3(-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), (Sigma-Aldrich, USA), fetal bovine serum (FCS), (Sigma-Aldrich, USA), cis-platin (EBEWE, Pharma, Austria).

MTT assay: In vitro cytotoxicity of the free ligands (cyclam, glycine, alanine), complexes **A, B**, and cis-platin were evaluated against three cancer cell lines: human cervix adenocarcinoma (HeLa), human melanoma (FemX) and human colon carcinoma (LS174). All cells were cultured in RPMI-1640 supplemented with 10 % heat-inactivated (56 °C) fetal bovine serum, 1 % penicillin (100 IU/mL), streptomycin (100 mg/mL), l-glutamine (3 mM) and 25 mM HEPES and adjusted to pH 7.2 by bicarbonate solution. Cell lines were maintained at 37 ºC in a 5 % CO_2 atmosphere with 95 % humidity. The compounds and cis-platin were dissolved in dimethyl sulfoxide (10 mM) and diluted to the required concentration with culture medium before use. Neoplastic HeLa cells (2000 cells per well), FemX cells (5000 cells per well) and LS174 cells (7000 cells per well) were seeded into 96-well microtiter plates, and 24 h later, after the cell adherence, five different, double diluted concentrations of the investigated compounds, were added to the wells. Final concentrations applied to target cells were 200, 100, 50, 25 and 12.5 µM. Control wells were prepared by the addition of culture medium without cells. The plates were incubated at 37 ºC for 72 h in a 5% CO_2. The effect of compounds on cancer cell survival was determined by the MTT test according to Mosmann,[14] modification by Ohno and Abe,[15] 72 h upon addition of the compounds. After the completion of the incubation, 20 µL of MTT solution (5 mg MTT/mL PBS) was added to each well. Samples were incubated for additional 4 h at 37ºC in 5 % CO_2 in humidified air atmosphere. Subsequently, 100 µL of 10 % SDS was added to extract the insoluble formazan formed from the conversion of the MTT dye by viable cells. The number of viable cells in each well was proportional to the intensity of the absorbance of light, which was then read in an ELISA plate reader at 570 nm. Absorbance (A) at 570 nm was measured 24 h later. To get the cell survival (%), A of a sample with cells grown in the presence of various concentrations of the

investigated extracts was divided by control optical density (the A_c of the control cells grown only in nutrient medium), and multiplied by 100. A_s of the blank was always subtracted from A of the corresponding sample with target cells.

The cell survival (S) was calculated by the equation (1):

$$S = \frac{(A_t - A_s)}{(A_c - A_s)} 100 \quad (1)$$

where A_c is the absorbance of the control, A_t is the absorbance of the treated cells, A_s the absorbance of the blank.

All experiments were performed in triplicate. The IC_{50} value was determined as the concentration of the complex that is required to reduce the absorbance by half of the control. Cis-platin was used as standard cytotoxic agent.

Antimicrobial activity: The antimicrobial activity of the new Cu(II) complexes (**A** and **B**) 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 ligands was 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) was 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.

RESULTS AND DISCUSSION

In the reaction $Cu(ClO_4)_2 \cdot 6H_2O$ with cyclam, glycine or alanine formed a mononuclear complexes, with general formula $[Cu(L)cyc](ClO_4)_2\ nH_2O$ (**A**): L = glycine, n = 1.5 and (**B**): L = alanine, n = 2.5.

The analytical data correspond to a metal– cyclam –amino acid ratio of 1:1:1 and 1.5 or 2.5 moles of water per mole of metal for complexes **A** or **B**. Data suggested a mononuclear structure for the Cu(II) complexes obtained as microcrystalline powder. The conductivity values in CH_3CN are 330 and 340 S cm^2 mol^{-1}, respectively for (**A**) and (**B**) and correspond to 1:2 electrolytes for both complexes.[16] The μ_{eff} values for the new complexes were determined at room temperature by the Gouy method. The values for A (1.98 BM) and B (2.15 BM) in agreement with the values reported for several Cu(II) octahedral complexes (1.9+-2.2 BM).[17]

Spectral studies

UV-visible spectroscopy: The absorption spectra in the UV and visible regions were recorded for new complexes in CH_3CN solutions. Both complexes are pink. Broad absorption bands in the visible region at 503 (ε=172 dm^3 mol^{-1} cm^{-1}) for **A** and 508 nm (ε=170 dm^3 mol^{-1} cm^{-1}) for **B**, corresponding to Cu(II) d-d transitions. Bands at 503 and 508 nm, can be attributed to the transition $^2E_g \rightarrow {}^2T_{2g}$ and this is expected for an octahedral d^9 configuration.[18] The positions of the maxima in the apsorption spectra of complexes indicate the presence of the CuN_4O_2 chromophore, being coordinated by the four N atoms of cyclam (Figure 2a) and two O. In the visible part of the spectra of complexes Cu(II) with cyclam and various carboxylate ligands with octahedral geometry, one maximum is observed for d-d transitions at a wavelength of 505 to 546 nm.[17, 19] These spectral features are consistent with six coordinate geometry for Cu(II) complexes. Besides octahedral geometry Cu(II) complexes of square pyramid or distorted square pyramid geometries exhibit a band in the 550-660 nm range, whereas the corresponding trigonal bipyramidal complexes usually show a maximum at λ_{max} 800 nm with a higher energy shoulder.[20] It is difficult to infer the geometry of Cu(II) complexes from electronic spectra alone, as they vary with the distortion within a given coordination number, due to the plasticity of the Cu(II) coordination environment.[21]

Based on all the applied characterization methods it was assumed that the geometry of the both complexes is most likely octahedral.

The electronic spectra of complexes exhibit an intense intraligand transitions in the UV region: 212–279 nm (ε = 4937- 5727 $dm^3mol^{-1}cm^{-1}$) for **A** and 223-288 nm (ε = 4903- 5730 dm^3 mol^{-1} cm^{-1}) for **B**[19], alongside a comparatively weaker absorption band in the visible region.[21] Intraligand transitions were found in the spectra of ligands in the range 220–250 nm. The intraligand transitions in both complexes are slightly shifted during complexation. The value of ε for complexes **A** and **B** (172 and 170 dm^3 mol^{-1} cm^{-1}, respectively), is in the interval obtained for mononuclear Cu(II) cyclam complexes.[22]

FTIR spectra: In the FTIR spectra of the of the new synthesized complexes (**A**, **B**), the following bands were present: a strong bands of ν_s (ClO_4^-) and ν_{as} (ClO_4^-) which was not included in the coordination at 930 and 1063 cm^{-1}, as well as a

medium sharp band from $\delta(ClO_4^-)$ at 621 cm^{-1}.[23] The infrared spectra of these complexes in comparison with free cyclam and the respective free amino acids show characteristic band positions, shifts and intensities, which can be correlated to cyclam binding and coordination amino acid. The IR spectra of two new synthesized complexes are compared in Table I with spectra of ligands.

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

	ν / cm^{-1}					δ/ cm^{-1}	
Compound	N-H and O-H)	ClO_4^-	C-H	M–N	M–O	ClO_4^-	CH_2
glycine	-	-	2902	-	-	-	-
alanine	-	-	2937	-	-	-	-
cyclam	3268	-	2867			-	1462
A	3241	1063	2878	528	436	621	1448 & 1467
B	3242	1063	2880	528	436	621	1453 &1467

A stretching O–H band is observed at 3241 and 3242 cm^{-1} for the complexes **A** and **B**, respectively, together with ν(N-H). This band as well as the one located at 1606 cm^{-1} indicate the presence of water in both compounds.[23] In the spectra of ligand cyc ν(N-H) was found at 3268 cm^{-1}. In both complexes ν(N-H) are slightly shifted during coordination cyclam to the Cu(II). Weak broad band at 2878 (**A**) and 2880 (**B**) cm^{-1} likely showing due to stretching vibration of CH, and two medium bands about 1440 and 1470 cm^{-1} from CH_2 bending vibrations.

Comparing the FTIR spectra of the complexes and amino acids in the region characteristic for $\nu(NH_3^+)$, $\delta_{as}(NH_3^+)$ and $\delta_s(NH_3^+)$, no shift was observed (Table II). It was assumed that - NH_3^+ zwitter ions do not participate in coordination. However, by comparing the FTIR spectra in the region characteristic for OCO^-, a shift of $\nu_{as}(OCO^-)$ and $\nu_s(OCO^-)$ was observed. It was assumed that a carboxylate group participates in the coordination.[24-26]

TABLE II. FTIR spectral data for the free amino acids and their complexes.

Compound	$\nu(NH_3^+)$ / cm^{-1}	$\delta_{as}(NH_3^+)$ / cm^{-1}	$\delta_s(NH_3^+)$ / cm^{-1}	$\nu_{as}(OCO^-)$ / cm^{-1}	$\nu_s(OCO^-)$ / cm^{-1}
glycine	2902	1606	1524	1594	1413
A	2939	1605	-	1485	1388
alanine	2937	1618	1515	1590	1412
B	2939	1606	-	1485	1362

The asymmetric stretching mode of the carboxylate group of amino carboxylates ligands occurs at about 1590 cm^{-1} and the symmetric stretching mode occurs about 1410 cm^{-1} (Table II). In unidentate coordination the redistribution of electron density takes place, which shifts the asymmetric carboxylate stretch to higher wavenumbers in comparison to uncoordinated OCO^- group. Consequently,

the $\Delta\nu$ value for unidentate carboxylate coordination is higher than ionic. On the contrary, bidentate coordination shifts the position of the asymmetric carboxylate stretch to lower wavenumber in comparison to uncoordinated group and thus lowers the value of $\Delta\nu$.

In these complexes the $\nu_{as}(OCO^-)$ and $\nu_s(OCO^-)$ shows negative shifts, which confirm the coordination of the carboxylate group, which is the case when OCO^- participates in coordination through both oxygens or when OCO participates in H-bonds formation.[23,24] For the complexes **A** and **B**, the difference between $\Delta\nu(OCO^-)$ is 97 or 123 cm^{-1} respectively, which is much lower than $\Delta\nu$ gly or ala (181 cm^{-1} for gly or 178 cm^{-1} for ala).

Complexes with a "pseudo-bridging" binding method have been described in the literature where one oxygen of the carboxylate group is coordinated to the metal and the other is hydrogen bonded to another ligand even though the $\Delta\nu$ of complex is less than the $\Delta\nu$ of carboxylato ligand.[24]

In the spectra of both complexes there are bands at 528 cm^{-1} which originating from ν(C–N) and bands at 436 cm^{-1} from ν(C–O). These vibrations confirm the coordination of ligands to the central metal ions and the involvement of nitrogen atoms (from cyclam) and oxygen atoms (from OCO^- group of gly/ala) in the coordination.[23,24]

Considering the structure of cyclam and the data obtained from the FTIR spectrum, the coordination mode of the aminocarboxylate ligand cannot be reliably determined.

Thermal analysis

The thermal decomposition of the complex **B** is shown in the Figure 2. The complex is thermally decomposed in the range 310–400 °C at one step. The complex is stable up to 310 °C which may indicate that the water present in the complex is coordinated for Cu (II) or that it participates in the strong hydrogen bonds.[17]

DTA curve of this complex reveals that decomposes at one step showing exothermic peak at 310 °C.

Although TG indicates high stability of the complex up to 310 ° C and possible stronger binding of the water present in the complex (in coordination with Cu (II) or building strong hydrogen bonds), based on the applied methods the coordination mode of aminocarboxylate cannot be assumed.

It can be concluded that: the obtained complexes are mononuclear in which cyclam is coordinated for Cu (II); the amino acid is coordinated as a zwitter ion, via the OCO group; the coordination number of metals is 6 and that water molecules are present in both complexes. Based on the applied methods, it is difficult to determine the method of coordination of the carboxyl group for metal as well as the participation of water in the coordination or crystal lattice. In the

further work, obtaining monocrystalline of complexes and their analysis will give answers to these questions.

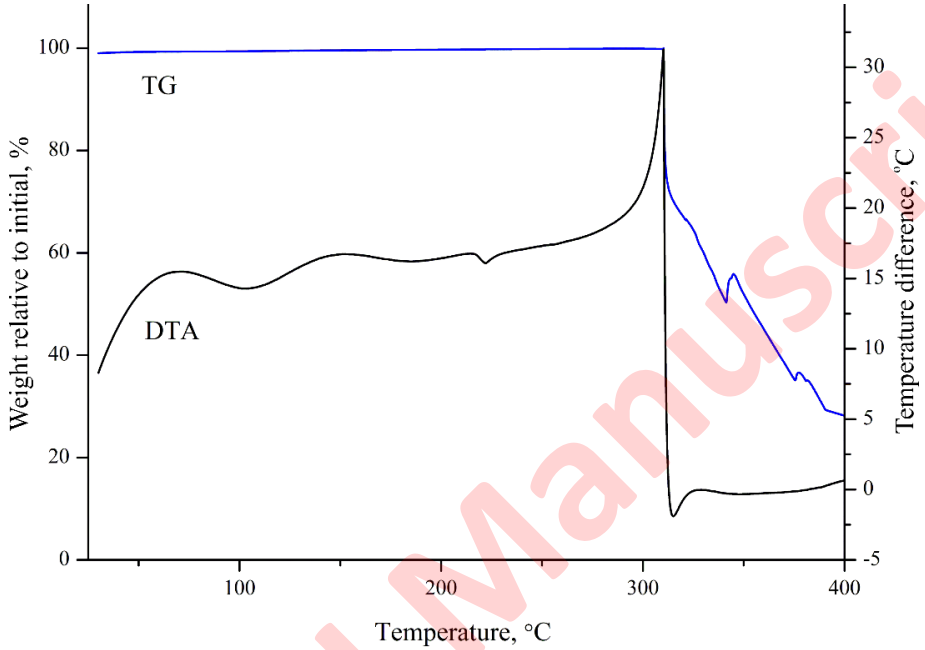

Fig. 2. Simultaneous TG-DTA of complex **B**.

Antibacterial and antiproliferative activity

Antiproliferative activity

The *in vitro* antiproliferative activities of compounds **A**, **B**, cyclam and co-ligands were evaluated against cell lines HeLa (human adenocarcinoma), FemX (human melanoma) and LS174 (human colon carcinoma) 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 III together with the activity of cisplatin as the referent cytostatic drug. The IC_{50} values of the complexes were in the range of 48.35-82.25μM against the three tested cell lines, while for cisplatin, they were in the range 8.35–10.92 μM (Table III).

TABLE III. IC_{50}±SD values after 72 h of action of the investigated complexes **A** and **B**, ligands and cisplatin on the tested cell lines, determined by the MTT test.

Compounds	IC_{50} ± SD /μM		
	HeLa	K 562	LS 174
A	74.06±6.35	82.25±7.18	60.35±6.81
B	53.10±4.36	50.35±4.21	48.35±8.81
cyclam, glycine, alanine	>200	>200	>200
Cisplatin	10.9±23.50	8.35±1.84	9.47±1.74

Compounds A and B showed moderate activity against all three cell lines. On the contrary, ligands did not show cytotoxic activity (IC_{50} > 200 μM) under the same conditions. Both compounds have promoted decrease in the metabolic activity of the HeLa, FemX and LS174 cells, which occurred in a dose-dependent fashion.

A number of Cu(II) chelate complexes that exhibit cytotoxic activity through cell apoptosis or enzyme inhibition have been reviewed.[27] Such complexes containing different ligands are effective in reducing tumor size, delaying of metastasis, and significantly increasing the survival of the hosts. Chelates of curcuminoids show significant reduction of solid tumor volume in mice (P < 0.001), while complexes of pyridine- 2-carbohidrazide derivatives inhibit the expression of c-Src, a nonreceptor tyrosine kinase, which plays a significant role in growth-mediated signaling pathway, thus showing cytotoxicity against colon cancer cell lines. Similarly, Cu(II) chelates of salclaldoxime and resorcylal-doxime[28] are potent antiproliferative agents, exhibiting strong cytotoxic effects by inducing cell cycle arrest and apoptosis. Their action may involve the inhibition of the enzyme topoisomerase II activity, by preventing dimer formation of the enzyme and its interaction with DNA.[29] Though copper is an essential cofactor for tumor angiogenesis processes, several Cu(II) binary complexes have been reported to function as proteasome inhibitors, inducing apoptosis in various types of human cancer cells. In such complexes, described as “organic copper compounds, the metal is coordinated either to neutral heteroatomic molecules such as phenan-throline or to anionic organic ligands such as 8-hydroxyquinolinate, pyrrolidine dithiocarbamate, or (pyridine-2-ylmethylamino)methyl phenolate). It is noticeable that the free ligands themselves are not efficient inhibitors, and complex formation is necessary for the transportation of copper ions through the cell membrane, in order to achieve proteasome inhibition.[30] This seems to be the result of the increasing lipophilicity of the metal upon ligand coordination. Detailed molecular mechanisms for tumour-associated copper elevation are not completely elucidated.[31]

Antimicrobial activity

The growing resistance of microorganisms to drugs is becoming a serious threat for the suppression of microbial infections. New, more effective substances, such as coordination compounds, are still being sought to treat infections with highly resistant strains. This is also important due to the fact that cancer patients are more susceptible to bacterial infections. The Cu (II) complexes did not show activity against the Gram-(–) bacteria and the yeast *C. albicans*. Both tested complexes showed activity against Gram-(+) bacteria, *S. aureus* and *B. subtilis.* it is a consequence of the difference in the permeability of the membrane of Gram-(+) bacteria in comparison to the Gram-(–) one due to the difference in their structure. Gram-(+) bacteria are known to be more susceptible to amino acids complexes.[32]

From the experimental results of the in vitro antimicrobial activities, it has been concluded that none of the preliminary tested compounds showed antifungal activity, so further investigation was focused only on bacteria. The applied method was the same as the one used in the study of antibiotics. Under the same condition controls were inactive at concentrations up to 400 mg/ml. This indicates that the activity of the complexes, where it was found, originated from themselves. For both complexes MIC values against *S. aureus* and *B. subtilis* were 200 μg mL^{-1}. Classification antimicrobial activity of new compounds, based on MIC results 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} is given in the literature.[33] In this way, it was possible to evaluate the antimicrobial activity of the examined metal complexes as moderate. The examined compounds showed moderate bactericidal effect on *S. aureus* and *B. subtilis* to the other Cu(II) carboxylato complexes.[34]

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 biological activity of the complex is also influenced by: the chelating effect, the total charge of the complex ion, the nature of the counter ion, the nature of the donor ligands, the nuclear properties of metal centers in the complex, the nature of the central metal ion and many other factors such as the solubility, the type and length of the bond between metal and ligand, *etc*.[34,35]

CONCLUSION

The newly synthesized mononuclear mixed-ligand complexes of Cu(II) using the 1,4,8,11-tetraazacyclotetradecane (cyclam) and aminoacids glycine/alanine were characterized by spectral, conductometric and magnetic studies, and TG-DTA analysis. For the complexes, octahedral structure has been proposed with coordinated four nitrogen atoms from cyclam and two oxygen atoms. TG-DTA analysis indicates that complex decomposes exothermally in a single step in the range of 310 -400 °C. The Cu (II)complexes did not show activity against the Gram-(–) bacteria and the yeast *C. albicans*, while have moderate bactericidal effect on Gram-(+) bacteria: *S. aureus* and *B. subtilis*. Both compounds have promoted decrease in metabolic activity of the HeLa (human adenocarcinoma), FemX (human melanoma) and LS174 (human colon carcinoma), which occurred in a dose-dependent fashion.

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И З В О Д

НОВИ КОМПЛЕКСИ БАКАР(II) ЦИКЛАМА СА АМИНОКАРБОКСИЛАТНИМ КОЛИГАНДИМА: СИНТЕЗА, КАРАКТЕРИЗАЦИЈА И *In vitro* АНТИПРОЛИФЕРАТИВНА И АНТИБАКТЕРИЈСКА АНАЛИЗА

БРАНКА ДРАЖИЋ[1] , МИРЈАНА АНТОНИЈЕВИЋ-НИКОЛИЋ2, МИЛЕНА МАРИНОВИЋ-ЦИНЦОВИЋ 3, ВУКОСАВА ЖИВКОВИЋ-РАДОВАНОВИЋ4, БРАНКА БОРОВИЋ5 И СЛАЂАНА Б. ТАНАСКОВИЋ1

[1]Фармацеутски факултет, Универзитет у Београду, Војводе Степе 450, 11000 Београд, Србија; [2]Академија примењених студија Шабац, Одсек за медицинске, пословно - техничке студије, Хајдук Вељкова 10, 15000 Шабац, Србија; [3]Институт за нуклеарне науке Винча, Универзитет у Београду, Мике Петровића Аласа 12-14, 11351 Винча, Београд, Србија; [4]Хемијски факултет, Универзитет у Београду, Студентски трг 16, 11000 Београд, Србија и [5]Институт за хигијену и технологију меса, Каћанског 13, 11000 Београд, Србија

Синтетисана су два нова катјонска Cu(II) комплекса са цикламом (1,4,8,11-тетра-азациклотетрадекан) и аминокарбоксилатним колигандима: глицином или аланином. Комплекси су окарактерисани елементалном анализом (C, H, N), моларном електричном проводљивошћу, мерењем магнетног момента на собној температури, спектралним методама (UV/Vis и FTIR) као и TG и DTA анализом. Аналитички подаци комплекса показују формирање мононуклеарних комплекса опште формуле $[Cu(L)cyc](ClO_4)_2 \cdot nH_2O$, (**A**): L = глицин, n = 1.5 and (**B**): L = аланин, n= 2.5. Тетрадентатни лиганд циклам је координован са металима преко четири N донора. Спектроскопски подаци сугеришу да су аминокарбоксилатни лиганди координовани преко својих карбоксилатних јона. У оба комплекса претпостављена је октаедарска геометрија око Cu(II). TG-DTA анализа показује да се комплекс **B** егзотермно разлаже у једном кораку у опсегу од 310 -400 °C. Цитотоксична активност Cu(II) комплекса и почетних лиганада је тестирана на ћелијским линијама хуманог аденокарцинома грлића материце (HeLa), хуманог меланома (FemiX) и хуманог карцинома дебелог црева (LS174). Вредности IC_{50} за комплексе Cu(II) биле су од 48,35-82,25 μM. Антимикробна активност оба комплекса је тестирана према *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* и *Candida albicans*.

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