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Copper(II) complexes with different diamines as inhibitors of bacterial quorum sensing activity

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Abstract: Three copper(II) complexes, *trans*-[Cu(1,3-pd)$_2$Cl$_2$]·H$_2$O (**Cu1**; 1,3--pd is 1,3-propanediamine), *trans*-[Cu(2,2-diMe-1,3-pd)$_2$Cl$_2$] (**Cu2**; 2,2-diMe--1,3-pd is 2,2-dimethyl-1,3-propanediamine) and *trans*-[Cu(1,3-pnd)$_2$Cl$_2$]·H$_2$O (**Cu3**; 1,3-pnd is (±)-1,3-pentanediamine), were synthesized and structurally characterized by elemental microanalyses, IR, electronic absorption and reflectance spectroscopy and molar conductivity measurements. The antimicrobial efficiency of the complexes against four clinically relevant microorganisms and their antiproliferative effect on the normal human lung fibroblast cell line MRC-5 were evaluated. Since in many bacteria, pathogenicity is regulated by an intercellular communication process called quorum sensing (QS), the effect of the copper(II) complexes **Cu1**–**3** on bacterial QS was examined. The obtained results showed that these complexes inhibited violacein production in *Chromobacterium violaceum* CV026, indicating their anti-QS activity *via* the homoserine lactone (HSL) pathway. Two biosensor strains were used to determine which pathway, C4-HSL (*N*-butanoylhomoserine lactone) or 3OC12-HSL (*N*-(3-oxododecanoyl)homoserine lactone), was affected by the copper(II) complexes. The biological activities of the copper(II) complexes were compared with those for the nickel(II) complexes of the general formula *trans*--[Ni(L)$_2$(H$_2$O)$_2$]Cl$_2$ (L = 1,3-pd, 2,2-diMe-1,3-pd and 1,3-pnd).

Keywords: metal complexes; nitrogen-donor ligands; antimicrobial activity; cytotoxicity; interbacterial communication.

INTRODUCTION

Metal complexes occupy a prominent position in medicinal chemistry by offering different possibilities for the design of therapeutic agents not accessible

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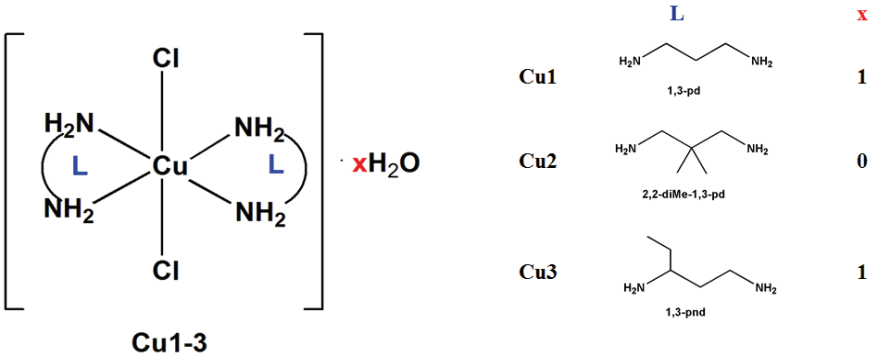
for organic compounds.[1] For instance, metal complexes have favourable features compared to organic compounds, such as enhanced stereochemistry and reactivity, lipophilicity, redox potential and high possibility of different modes of action.[2] Many studies revealed that metal complexes show significantly greater biological activity in respect to the organic compounds used as ligands for their synthesis.[3] Among metal complexes that have been assessed for different biological activities, copper(II) complexes have gained particular attention because copper is an essential element for humans and most other aerobic organisms.[4–6] This metal is important for the function of several enzymes and proteins involved in energy metabolism, respiration and DNA synthesis,[7] which depends mainly on the geometric arrangement of the ligands around the copper(II) ion.[8] In addition, the concentration of this metal is found to be elevated in cancerous tissues with respect to normal ones,[9–12] probably as a consequence of its central role in angiogenesis, a process controlling tumour growth, invasion and metastasis.[13,14]

Administration of copper(II) ions in the form of a complex could have the advantage of their selective delivery to diseased tissues.[15] Considering this, a large number of copper(II) complexes have been synthesized and assessed for their biological activities, including antibacterial, antifungal and antitumor.[4,16–20] Very recently, five copper(II) complexes with aromatic nitrogen-containing heterocycles (*N*-heterocycles), pyrimidine, pyrazine, quinazoline and phthalazine, were synthesized and their efficiency against three clinically relevant microorganisms, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*, and antiproliferative activity against a normal human fibroblast cell line MRC-5 evaluated.[21] Although none of the copper(II) complexes showed significant growth inhibiting activity, they were shown as effective inhibitors of bacterial quorum sensing (QS) by successful modulation of the production of signalling molecules that are part of the QS system.[21] QS refers to cell to cell communication between microorganisms that occurs *via* production and reception of signalling molecules and controls bacterial population-dependant gene expression.[22,23] These genes are involved in improving bacterial survival under various threats, such as virulence and pathogenicity, in secondary metabolite production, plasmid transfer, motility and biofilm formation.[22,24] The finding that copper(II) complexes with the above mentioned *N*-heterocycles represent a new class of quorum sensing inhibitors that attenuate virulence without a pronounced effect on the bacterial growth, thus offering a lower risk for resistance development, prompted the present synthesis of copper(II) complexes with diamines, 1,3-propanediamine (1,3-pd; **Cu1**), 2,2-dimethyl-1,3-propanediamine (2,2-diMe-1,3-pd, **Cu2**) and (±)-1,3-pentanediamine (1,3-pnd, **Cu3**), Scheme 1. These complexes were assessed for their *in vitro* antimicrobial and antiproliferative activities, and evaluated as modulators of bacterial QS. The biological activities of the copper(II) complexes were compared with those previously reported in the literature

for copper(II) complexes with *N*-heterocycles,[21] and for the nickel(II) complexes, *trans*-$[Ni(L)_2(H_2O)_2]Cl_2$, containing the same diamine ligands.[25]



Scheme 1. Schematic presentation of *trans*-$[Cu(L)_2Cl_2]\cdot xH_2O$ complexes (**Cu1–3**).

EXPERIMENTAL

Reagents

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

*Synthesis of the copper(II) complexes **Cu1–3***

The copper(II) complexes with diamine ligands were synthesized by modification of a previously described method for the preparation of the nickel(II) analogues.[25] The required diamine (0.02 mol) was added slowly under stirring to a solution containing $CuCl_2\cdot 2H_2O$ (1.71 g, 0.01 mol) in H_2O (15.0 mL). The resulting solution was stirred at 40 °C for 30 min. The formed copper(II) hydroxide was removed by filtration and the filtrate was left standing at room temperature to evaporate to a volume of 3.0 mL. The concentrated solution was stored in a refrigerator at 4 °C and a blue powder of the respective copper(II) complex formed during three days. This powder was filtered off and dried at room temperature. The yield was 74 % for *trans*-$[Cu(1,3\text{-pd})_2Cl_2]\cdot H_2O$ (**Cu1**; 2.23 g), 71 % for *trans*-$[Cu(2,2\text{-diMe-}1,3\text{-pd})_2Cl_2]$ (**Cu2**; 2.41 g) and 72 % for *trans*-$[Cu(1,3\text{-pnd})_2Cl_2]\cdot H_2O$ (**Cu3**; 2.57 g).

Measurements

Elemental microanalyses of the copper(II) complexes for carbon, hydrogen and nitrogen were performed by the Microanalytical Laboratory, Faculty of Chemistry, University of Belgrade. The IR spectra were recorded as KBr pellets on a Perkin Elmer Spectrum One spectrometer over the wavenumber range 4000–450 cm^{-1}. The far-IR spectra were measured on a Perkin Elmer 983 spectrophotometer using Nujol mull supported between CsI sheets. The electronic absorption spectra were recorded over the wavelength range of 1100–190 nm on a Perkin Elmer Lambda 35 double-beam spectrophotometer equipped with thermostated 1.00-cm quartz Suprasil cells after dissolving the corresponding copper(II) complex in water. For these measurements, 5×10^{-3} M solutions of the copper(II) complexes were used. The electronic reflectance spectra were run on a Shimadzu UV–Visible UV-2600 (Shimadzu Corporation, Tokyo, Japan) spectrophotometer equipped with an integrated sphere (ISR-2600 Plus). The

molar conductivities were measured at room temperature on a Crison EC-meter basic 30+ digital conductivity-meter. The concentration of the solutions of copper(II) complexes in DMF and water used for conductivity measurements was 1×10^{-3} M.

Determination of the biological activity

Antibacterial activity. Antibacterial properties of the copper(II) complexes **Cu1–3** and $CuCl_2\cdot2H_2O$ were tested against three bacteria (*Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* ATCC 379 and *Pseudomonas aeruginosa* PAO1 NCTC 10332) and one fungal strain (*Candida albicans* ATCC 10231). All compounds were dissolved in distilled water in stock solutions of 50 mg mL^{-1}. *MIC* concentrations (lowest concentration that inhibited the growth after 24 h at 37 °C) were determined according to the standard broth microdilution assays, recommended by the National Committee for Clinical Laboratory Standards (M07--A8) for bacteria and Standards of European Committee on Antimicrobial Susceptibility Testing (EDef7.1.). The highest concentration used was 500 μg mL^{-1} and the inoculums of test organisms were 10^5 colony forming units, CFU mL^{-1}, for bacteria and 10^4 CFU mL^{-1} for the *Candida* strain. Microbial growth was measured *via* optical density at 600 nm (OD_{600}) using a Tecan Infinite 200 Pro multiplate reader (Tecan Group Ltd., Männedorf, Switzerland).

Cytotoxicity. Cell viability was tested by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[26] The assay was realized using human lung fibroblasts (MRC5) after 48 h of cell incubation in the media, containing copper(II) complexes **Cu1**–**3** and $CuCl_2\cdot2H_2O$ at concentrations ranging from 0.1–500 μg mL^{-1}. MRC5 cells were plated in a 96-well flat bottom microtiter plate at a concentration of 1×10^4 cells per well, in RPMI-1640 medium, supplemented with 100 μg mL^{-1} streptomycin, 100 U mL^{-1} penicillin and 10 vol. % foetal bovine serum (FBS), all from Sigma, Munich, Germany, and grown in humidified atmosphere of 95 % air and 5 % CO_2 at 37 °C. The extent of MTT reduction was measured spectrophotometrically at 540 nm using a Tecan Infinite 200 Pro multiplate reader (Tecan Group Ltd., Männedorf, Switzerland), and the cell survival was expressed as percentage of the control (untreated cells) that was arbitrarily set to 100 %.

QS modulating activity. Biosensor strains used in this study included *Chromobacterium violaceum* CV026,[27] *Pseudomonas aeruginosa* PA14-(ΔlasI Prsal::lux)[28] and PAOJP2/pKD--rhlA (ΔrhlA PrhlA::lux).[29] The bacteria where grown in Lauria–Bertani (LB) medium (1 % NaCl, 1 % tryptone, 0.5 % yeast extract) with shaking (180 rpm) at 37 or 30 °C. When required, the antibiotic kanamycin (BioReagent, Sigma–Aldrich, Germany) was incorporated into growth medium at a concentration of 50 μg mL^{-1} for *C. violaceum* CV026 and 200 μg mL^{-1} for PAOJP2/pKD-rhlA (ΔrhlA PrhlA::lux).

C. violaceum CV026 is a mutated strain that cannot produce violacein without exogenous *N*-acyl homoserine lactones (AHLs), and hence, it can be used as an indicator organism for QS inhibition. An overnight culture of *C. violaceum* CV026 (50 μL) was added to 5 mL of semi-solid LB agar (0.3 %) in addition to *N*-hexanoyl-L-homoserine lactone (Sigma, Germany) in a final concentration of 5 μM and poured over an LB agar plate. When the agar had solidified, sterilized discs were placed on the top and compounds were added in a final concentration of 250 μg per disc. The Petri dishes were incubated for 12–16 h at 30 °C.

Biosensor strains where grown overnight in LB medium at 37 °C with shaking (180 rpm) and the addition of kanamycin (200 μg mL^{-1}) for the PAOJP2/pKD-rhlA strain. Cultures were diluted in LB medium and adjusted to an absorbance of 0.045 at 600 nm. The diluted culture was dispensed in a black clear-bottom 96-well microtitre plate (Greiner Bio One, Germany) in the presence of an autoinducer (3OC12-HSL for PA14-R3 and C4-HSL for PAOJP2/pKD-rhlA) in a concentration of 6 μM in a total volume of 200 μL. The plates were incubated for 4

h at 37 °C with shaking (70 rpm). The cell density (OD_{600}) and bioluminescence (light counts per second, LCPS) were measured using a Tecan Infinite 200 Pro multiplate reader (Tecan Group Ltd., Männedorf, Switzerland). The luminescence values were normalized per cell density.

RESULTS AND DISCUSSION

*Synthesis and structural properties of the copper(II) complexes **Cu1–3***

Three diamines, 1,3-propanediamine (1,3-pd), 2,2-dimethyl-1,3-propanediamine (2,2-diMe-1,3-pd) and (±)-1,3-pentanediamine (1,3-pnd), were used for the synthesis of copper(II) complexes **Cu1**–**3**, respectively. These diamines and $CuCl_2{\cdot}2H_2O$ were reacted in 2:1 mole ratio in water at 40 °C to yield *trans*-$[Cu(L)_2Cl_2]{\cdot}xH_2O$ complexes, x is 0 (**Cu2**) or 1 (**Cu1** and **Cu3**), in which the corresponding diamine is bidentately coordinated to the Cu(II) ion (Scheme 1). The synthesis of **Cu1** and **Cu2** complexes was previously reported,[30–33] while **Cu3** was synthesized for the first time in this study. Moreover, the crystal structure of **Cu2** complex was previously determined by single-crystal X-ray diffraction analysis.[32,33] In the present study, the stoichiometries of the **Cu1**–**3** complexes were confirmed by elemental microanalysis, and their structures emerge from IR, electronic absorption and reflectance spectra and molar conductivity measurements.

The electronic absorption spectra of the investigated complexes **Cu1**–**3** are presented in Fig. S-1 of the Supplementary material to this paper, while the wavelengths of the maximum absorption (λ_{max}/nm) and molar extinction coefficients (ε / M^{-1} cm^{-1}) determined immediately after their dissolution in water, are given in Table S-I. All blue complexes exhibit a single symmetric band in the expected region for *trans*-$[Cu(N\text{-}N)_2X_2]$-type complexes having D_{4h} symmetry.[34] These bands could be assigned to the d_{z^2}, d_{xy}, d_{xz}, $d_{yz} \rightarrow d_{x^2-y^2}$ transitions with a $d_{x^2-y^2}$ ground state.[35] The absorption maximum for **Cu2** was slightly shifted to the higher energy in respect to those for **Cu1** and **Cu3**. The absorption intensity for **Cu1** was lower than those found for complexes **Cu2** and **Cu3**. The higher absorption intensities for the latter two complexes could be attributed to the presence of two methyl (**Cu2**) and one ethyl (**Cu3**) groups in the six-membered 1,3-propanediamine ring. The reflectance spectra of these complexes (Table S-I of the Supplementary material), with respect to those taken from solution, retain nearly the same shape, indicating that the geometry of the complexes was the same in both solution and the solid state. It is worth noting that the shape and position of the band maxima in the reflectance spectra for the investigated *trans*-$[Cu(L)_2Cl_2]$ complexes are almost identical to that previously reported for the *trans*-$[Cu(2,2\text{-diMe-}1,3\text{-pd})_2Cl_2]{\cdot}4H_2O$ complex of known crystal structure.[33] However, the positions of the band maxima in the reflectance spectra were shifted toward lower energy with a difference within 19–33 nm compared to those in the absorption spectra; this indicates a substitution of the axial chlorides with water molecules in aqueous solutions, which is further sup-

ported by the molar conductivity measurements (see Supplementary material). Thus, **Cu1**–**3** complexes are found to be non-electrolyte in DMF solution, implying the coordination of the chloride anions to Cu(II).[36] However, the Λ_M values measured in water indicated that a substitution of axial chloride ions with water molecules had occurred, being in agreement with the 1:2 electrolytic natures of the complexes in aqueous solution.[37]

In the absence of X-ray crystallographic data, IR spectra have proven to be the most suitable technique to give sufficient information to elucidate the manner in which ligands bond to metal ions.[38] The main stretching frequencies of the IR spectra of complexes **Cu1**–**3** are listed in Table S-II of the Supplementary material. The IR spectra of these complexes recorded in the range of 4000–450 cm^{-1} showed the expected bands attributable to bidentately coordinated diamine ligands and crystalline water molecule. The complexes exhibited two very strong and sharp bands at approximately 3200 and 3100 cm^{-1}, which were assigned to the asymmetric and symmetric stretching vibration of the coordinated amino group, respectively.[39] In addition, the sharp bands at 1587, 1586 and 1588 cm^{-1} are due to N–H deformation in the **Cu1**–**3** complexes, respectively. In the far-IR region, the most obvious features are the bands at 367, 359 and 365 cm^{-1} for **Cu1**–**3**, respectively, which could be assigned to the stretching vibration of the Cu–Cl bond. This is in accord with a previous IR study of *trans*-$[ML_4X_2]$-type compounds with D_{4h} symmetry, in which it was found that this type of compounds show only one M–X stretching (X is halogen).[40] Moreover, the presence of strong bands at 411, 418 and 420 cm^{-1} for **Cu1**–**3**, respectively, due to the stretching vibration of Cu–N bond,[31] additionally support the proposed structures for these complexes (Scheme 1).

*Antimicrobial and antiproliferative activity of the copper(II) complexes **Cu1**–**3***

In vitro antimicrobial activity assays of copper(II) complexes **Cu1**–**3** revealed no significant activity against any of the tested microorganisms with *MIC* concentrations higher than 500 μg mL^{-1} (Table I). The inorganic salt $CuCl_2 \cdot 2H_2O$ showed slightly better antimicrobial activity than the copper(II) complexes, with *MIC* concentrations against *M. luteus* and *P. aeruginosa* about two times lower. When the antimicrobial activity of copper(II) complexes **Cu1**–**3** is compared with the activity of the nickel(II) complexes with the same diamine ligands,[25] some differences could be observed. Similarly to **Cu1–3**, there was also no significant antibacterial activity, however, the nickel(II) complexes with the same diamine ligands showed a certain level of selectivity towards fungal strains, with the nickel(II) complex with 2,2-diMe-1,3-pd ligand exhibiting the best anti-*Candida* activity.[25] Notably, none of the diamine ligands showed either antibacterial or antifungal activity (Table I).

TABLE I. Minimal inhibitory concentrations (*MIC*, μg mL^{-1}) against different microbial strains and IC_{50} values against MRC5 cells (concentration that inhibits 50 % of cell growth after treatment with the tested compounds, μg mL^{-1}); the results are from three independent experiments, each performed in triplicate. Standard deviations were within 1–3 %; **Ni1**: *trans*-[Ni(1,3-pd)$_2$(H$_2$O)$_2$]Cl$_2$; **Ni2**: *trans*-[Ni(2,2-diMe-1,3-pd)$_2$(H$_2$O)$_2$]Cl$_2$; **Ni3**: *trans*-[Ni(1,3-pnd)$_2$(H$_2$O)$_2$]Cl$_2$

Compound	*S. aureus* ATCC 25923	*M. luteus* ATCC 379	*P. aeruginosa* PAO1	*C. albicans* ATCC 10231	MRC-5
Cu1	>500	>500	>500	>500	120
Cu2	>500	>500	>500	>500	65
Cu3	>500	>500	>500	>500	80
$CuCl_2 \cdot 2H_2O$	500	250	250	500	40
Ni1[25]	> 500	> 500	> 500	31.2	500
Ni2[25]	> 500	> 500	> 500	31.2	80
Ni3[25]	> 500	> 500	> 500	31.2	500
$NiCl_2 \cdot 6H_2O$[25]	500	500	500	250	100
1,3-pd[25]	> 500	> 500	> 500	> 500	> 500
2,2-diMe-1,3-pd[25]	> 500	> 500	> 500	> 500	100
1,3-pnd[25]	> 500	> 500	> 500	> 500	50

In parallel with the antimicrobial activity, *in vitro* cytotoxicity of copper(II) complexes **Cu1**–**3** against healthy human lung fibroblasts (MRC-5) were examined (Table I). The investigated copper(II) complexes exhibited considerably lower negative effects on the viability of the healthy human lung fibroblast cell line MRC-5 in comparison to the inorganic salt $CuCl_2 \cdot 2H_2O$ and the corresponding diamine ligands. Nevertheless, the cytotoxicity of the copper(II) complexes **Cu1** and **Cu3** with IC_{50} concentration values of 120 and 80 μg mL^{-1} was 4 and 6 times higher in comparison to the corresponding 1,3-pd- and 1,3-pnd-nickel(II) complexes, respectively, while **Cu2** and *trans*-[Ni(2,2-diMe-1,3-pd)$_2$(H$_2$O)$_2$]Cl$_2$ exhibited similar cytotoxicity.

In line with previous observations, mixed-ligand copper(II) complexes, [Cu(dipn)(N-N)]Br$_2$ (dipn = dipropylenetriamine, N-N = ethylenediamine and 1,3-propanediamine), showed weak antimicrobial properties and antiproliferative activity against the human keratinocyte cell line with IC_{50} values of 155 and 152 μM, respectively.[41]

*Effect of the copper(II) complexes **Cu1**–**3** on bacterial quorum sensing (QS)*

Prompted by studies that showed a negative effect of $CuSO_4$ on the virulence of pathogenic *Edwardsiella tarda* and *Vibrio harveyi*[42,43] and anti-QS activity of copper(II) complexes with pyrimidine, pyrazine, quinazoline and phthalazine,[21] it was decided to examine whether the copper(II) compounds utilized within this study exerted an effect on bacterial QS. Violacein production in *Chromobacterium violaceum* CV026 is dependent on exogenous acyl homoserine lactones (HSL) and it was used in this study to test the effect of **Cu1**–**3** on QS *via*

the HSL pathway. The clear zone around the complex-loaded discs indicated inhibition of violacein production and anti-QS activity of the investigated complexes (Fig. 1a). A similar effect was detected for all three complexes, while the corresponding diamine ligands did not inhibit violacein production under the tested conditions. It was recently shown a similar effect on violacein production in this strain using copper(II) complexes with aromatic *N*-heterocycles, pyrimidine, pyrazine, quinazoline and phtalazine.[21] On the other hand, *trans*-$[Ni(L)_2(H_2O)_2]Cl_2$ complexes with the same diamine ligands showed no such activity (data nor shown).

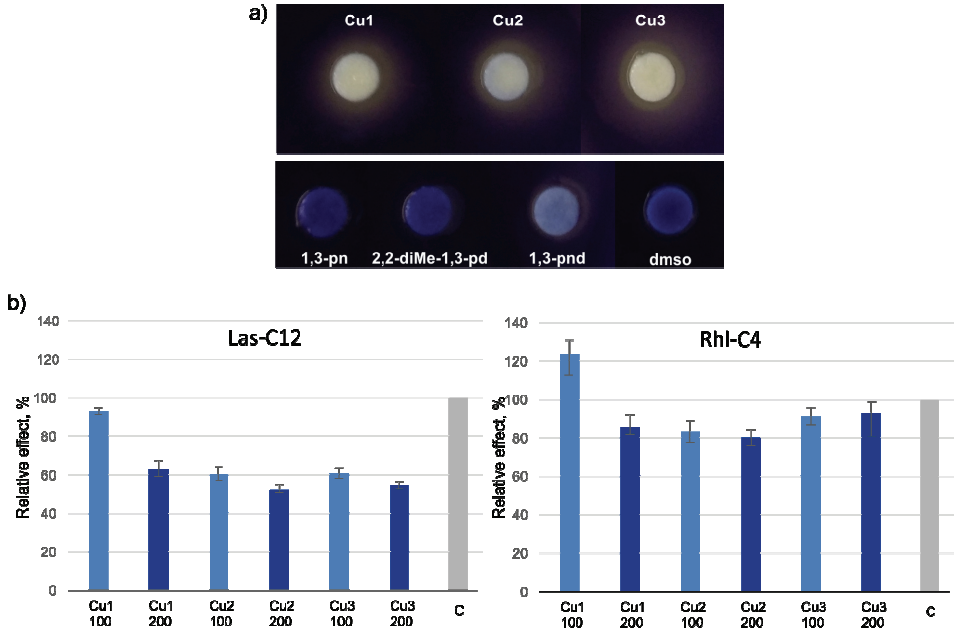


Fig. 1. Effect of the copper(II) complexes **Cu1**–**3** on bacterial QS. a) Violacein production inhibition in *Chromobacterium violaceum* CV026 caused by 250 μg of the complexes applied on the cellulose disc. b) **Cu1**–**3** complexes (100 and 200 μg mL^{-1}) in competition with exogenous autoinducers for a specific receptor of the biosensor strain (autoinducer 3OC12-HSL/biosensor strain PA14-R3 (Las-C12 histogram), and autoinducer C4-HSL/biosensor strain PAOJP2/pKD-rhlA (Rhl-C4 histogram).

This finding led to the determination of which of the HSL pathways was affected by **Cu1**–**3** in the direct competition for the receptor assays (Fig. 1b). It was found that the competition for the receptor between the exogenously added 3OC12-HSL and **Cu1**–**3** was greater than between the exogenously added C4-HSL and these copper(II) complexes. The tested complexes in the concentration

of 200 μg mL^{-1} blocked the 3OC12-HSL pathway by around 40 %, while the C4-HSL pathway at the same concentration of the complexes was affected by between 10 and 20 %. A previous study showed that the polynuclear copper(II) complex containing pyrimidine had a significant effect on the long chain 3OC12--HSL production in *P. aeruginosa* (≈40 %), as well as on the short chain C4-HSL production (≈30 %).[21]

CONCLUSIONS

The reaction of $CuCl_2 \cdot 2H_2O$ with three diamines (L), 1,3-pd, 2,2-diMe-1,3--pd and 1,3-pnd, led to the formation of octahedral *trans*-$[Cu(L)_2Cl_2]$ complexes. The *in vitro* antimicrobial activity of these copper(II) complexes was investigated and the obtained results were compared to those previously reported for the *trans*-$[Ni(L)_2(H_2O)_2]Cl_2$ complexes containing the same diamine ligands.[25] No significant antibacterial activity was observed for the investigated copper(II) and nickel(II) complexes against the tested bacterial strains. However, in respect to the presently investigated copper(II) complexes, the nickel(II) analogues showed a certain level of selectivity towards the fungal strain.[25] Contrary to this, *in vitro* cytotoxicity of copper(II) complexes with 1,3-pd and 1,3-pnd diamines against healthy human lung fibroblast cell line MRC-5 was higher in comparison to the corresponding nickel(II) complexes, while copper(II) complex with 2,2-diMe--1,3-pd exhibited a cytotoxicity similar to that of nickel(II) analogue. Although the investigated copper(II) complexes were not efficient growth inhibitors of the investigated bacterial strains, they were able to inhibit violacein production in *C. violaceum* CV026, indicating their anti-QS activity *via* the homoserine lactone (HSL) pathway. No such activity was observed for the nickel(II) complexes with the same diamines. These latest results could serve as a basis for further design of copper(II) complexes as a new class of quorum sensing inhibitors that attenuate virulence without a pronounced effect on bacterial growth, thus offering a lower risk for resistance development.

SUPPLEMENTARY MATERIAL

Optical, spectral and analytical data for complexes **Cu1**–**3** are available electronically at the pages of journal website: http:// //www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

КОМПЛЕКСИ БАКРА(II) СА РАЗЛИЧИТИМ ДИАМИНИМА КАО ИНХИБИТОРИ ИНТЕРБАКТЕРИЈСКЕ КОМУНИКАЦИЈЕ

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Синтетисана су три комплекса бакра(II), *trans*-[Cu(1,3-pd)$_2$Cl$_2$]•H$_2$O (**Cu1**; 1,3-pd је 1,3-пропандиамин), *trans*-[Cu(2,2-diMe-1,3-pd)$_2$Cl$_2$] (**Cu2**; 2,2-diMe-1,3-pd је 2,2-диметил-1,3-пропандиамин) и *trans*-[Cu(1,3-pnd)$_2$Cl$_2$]•H$_2$O (**Cu3**; 1,3-pnd је (±)-1,3-пентандиамин). Комплекси су окарактерисани помоћу елементалне микроанализе, инфрацрвених, електронских апсорпционих и рефлексионих спектара, као и на основу мерења моларне проводљивости. Испитивана је антимикробна активност комплекса према четири клинички важна микроорганизма, као и њихово антипролиферативно дејство према здравој MRC-5 ћелијској линији фибробласта плућа. Пошто се код многих бактеријских врста патогено дејство одвија кроз интерцелуларну ћелијску комуникацију, познату под називом "quorum sensing" (QS), испитиван је утицај комплекса бакра(II) **Cu1**–**3** на бактеријски QS. Добијени резултати су показали да ови комплекси инхибирају настајање виолацеина у *Chromobacterium violaceum* CV026, што указује на њихову анти-QS активност преко хомосеринлактонског (HSL) механизма. У циљу одређивања механизма деловања комплекса бакра(II) коришћена су два биосензора, C4-HSL (*N*-бутаноилхомосерин лактон) и 3OC12-HSL (*N*-(3-оксододеканоил)хомосерин лактон). Биолошка активност комплекса бакра(II) је поређена са одговарајућом активношћу комплекса никла(II) опште формуле *trans*-[Ni(L)$_2$(H$_2$O)$_2$]Cl$_2$ (L = 1,3-pd, 2,2-diMe-1,3-pd и 1,3-pnd).

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