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Square-pyramidal mononuclear, dinuclear and polymeric copper(II) complexes with (2-pyridinylmethyl)amino derivatives

STEFAN RICHTER[1], PETER LÖNNECKE[1], DIJANA BOVAN[2], SANJA MIJATOVIĆ[2], DANIJELA MAKSIMOVIĆ-IVANIĆ[2], GORAN N. KALUĐEROVIĆ[3] AND EVAMARIE HEY-HAWKINS[1]*

[1]Universität Leipzig, Faculty of Chemistry and Mineralogy, Institute of Inorganic Chemistry, Johannisallee 29, 04103 Leipzig, Germany, [2]Department of Immunology, Institute for Biological Research "Siniša Stanković" National Institute of Republic of Serbia, University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia, [3]Department of Engineering and Natural Sciences, University of Applied Sciences Merseburg, Eberhard-Leibnitz-Str. 2, 06217 Merseburg, Germany

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Abstract: The coordination behavior of three ligand precursors 2-[(2-pyridinylmethyl)amino]acetic acid hydrochloride, 4-[(2-pyridinylmethyl)amino]benzoic acid hydrochloride and 4-{[2-(pyridin-2-ylmethylamino)ethylamino]methyl}benzoic acid hydrochloride, **HL1**·HCl–**HL3**·HCl, respectively, in copper(II) complexes is described. The complexes were characterized by elemental analysis, ESI mass spectrometry and IR spectroscopy, as well as X-ray structural analysis. The reaction of copper(II) with **HL1**·HCl in methanol afforded the polymeric complex [{Cu(μ-Cl)$_2$(**MeL1**-κ^2*N*,*N*')}$_n$] (**1**) featuring the methyl ester of **L1** (**MeL1**). With **HL2**·HCl or **HL3**·HCl, the dimeric complex [{CuCl(μ-Cl)(**HL2**-κ^2*N*,*N*')}$_2$] (**2**) or the mononuclear complex [CuCl$_2$(**HL3**-κ^3*N*,*N*',*N*'')] (**3**) were obtained. All complexes exhibited square-pyramidal geometries. In **1**, polymeric chains are formed through bridging chlorido ligands without typical hydrogen bonding interaction. Contrarily, the COOH group in **2** is participating in the formation of intermolecular hydrogen bonding forming a supramolecular structure. In **3**, intermolecular hydrogen bonding (Cl···O) leads to a 1-D polymeric structure. The copper(II) complex **2** diminished viability of human 8505C, MCF-7, 518A2 and SW480 cell lines. The tumoricidal effect of **2** was realized mainly through caspase-mediated apoptosis.

Keywords: copper(II); pyridine; X-ray structure; *in vitro* anticancer activity.

*Corresponding author E-mail: hey@uni-leipzig.de
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INTRODUCTION

The Jahn-Teller active copper(II) ion exhibits remarkable flexibility in its coordination sphere, allowing it to adopt a diverse range of coordination geometries, spanning from four-coordinate (tetrahedral or square planar) to six-coordinate elongated octahedral structures.[1] In copper(II) complexes containing halido ligands enhanced flexibility is observed by facilitating halide bridging between copper(II) centers, leading to the formation of extended oligomeric or polymeric complexes.

Pyridine-based multidentate ligands are a highly adaptable group of compounds, primarily because they can be easily be functionalized using efficient synthetic methods.[2] The flexibility in their synthesis allows for the creation of custom-made ligands.[3,4] When such molecules are coordinated to metal ions, they enable the manipulation of both the electronic and structural characteristics of the resulting complexes.

Transition metal complexes with *N,N'*-bidentate pyridylmethylamine ligands remain a subject of ongoing interest, primarily due to the high coordination versatility achievable through the introduction of diverse substituents on the amine and/or pyridyl unit.[5–8] Numerous structural variations in pyridylmethylamines and their corresponding complexes have been documented highlighting the significance of their steric and electronic properties in various targeted chemical applications.[9–11] Previous studies have utilized *N*-(2-hydroxybenzyl)-amino acids and *N*-(2-pyridylmethyl)-amino acids as useful polydentate ligands to form multidimensional and oligomeric structures.[12–19]

Here we report the investigation of the influence of the amino *N* substituent in (2-pyridinylmethyl)amino ligands on the solid-state structure of copper(II) complexes. Depending on the substituent polymeric polynuclear [{Cu(μ-Cl)$_2$(**MeL1**-κ^2*N,N'*)}$_n$], dinuclear, [{CuCl(μ-Cl)(**HL2**-κ^2*N,N'*)}$_2$] or mononuclear [CuCl$_2$(**HL3**-κ^2*N,N',N''*)] complexes were obtained. The complexes were characterized by elemental analysis, ESI-MS and IR spectroscopy and single crystal structure analysis.

EXPERIMENTAL

Materials and methods

Chemicals and instruments

Methanol, $CuCl_2$, RPMI 1640 medium, fetal bovine serum, propidium iodide (PI), acridine orange (AO), and sulforhodamine B (SRB), were ordered from Sigma-Aldrich (St. Louis, MO). AnnexinV-FITC (AnnV), was obtained from Biotium (Hayward, CA) while Apostat was from R&D (R&D Systems, Minneapolis, MN USA). For ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) spectroscopy (Fourier-300, Bruker) tetramethylsilane (TMS) was used as an internal standard and deuterated solvent as reference. The melting points were determined in a capillary using a Gallenkamp instrument. Elemental analysis was performed on Thermo Scientific Flash Smart CHNS (Italy). The IR spectra were recorded with an FT-IR spectrometer Spectrum 2000

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(PerkinElmer) using KBr pellets in the range of 4000 to 400 cm^{-1}. The ESI mass spectra in a positive mode were recorded with an FT-ICR mass spectrometer (Bruker Daltonics). The isotope distribution was determined with the program Molecular Weight Calculator 6.45.[20] The specification of the molecular ion peak refers to the highest signal in the observed isotope pattern. The absorbance for the SRB assay was measured at 540 nm with the reference wavelength at 670 nm. Fluorescence activated cell sorting (FACS) experiments were conducted on CyFlow® Space Partec by using the Partec FloMax® software.

Synthesis of the ligand precursors HL1·HCl–HL3·HCl

*Synthesis of **HL1**·HCl and **HL2**·HCl*

Ligand precursors **HL1**·HCl (2-[(2-pyridinylmethyl)amino]acetic acid hydrochloride) and **HL2**·HCl (4-[(2-pyridinylmethyl)amino]benzoic acid hydrochloride) were obtained as described in the literature.[13,21]

*Synthesis of 4-{[2-(pyridin-2-ylmethylamino)ethylamino]methyl}benzoic acid hydrochloride **HL3**·HCl*

The compound 4-[(2-aminoethylamino)methyl]benzoic acid (synthetic procedure was described earlier)[22] (1.32 g, 0.007 mol) was reacted with KOH (0.45 g, 0.007 mol) in water (10 mL). The resulting clear light yellow solution was cooled to 0 °C. Pyridine-2-carbaldehyde (0.73 g, 0.007 mol) was then added within 30 min, followed by stirring at 0 °C for 1 h. $NaBH_4$ (0.25 g, 0.007 mol) dissolved in water (5 mL) was slowly added to the reaction solution The resulting pale yellow suspension was stirred overnight at room temperature. Hydrochloric acid (2 M, 8 mL) was added to the clear yellow solution, foaming vigorously and changing color to orange; the pH of the solution was adjusted to 3. The solvent was then completely removed in vacuum. The crude yellow product was recrystallized from methanol (150 mL). The resulting light yellow solid was filtered off, washed with diethyl ether (50 mL) and dried in vacuum. For atoms numeration see Fig. 1.

Properties: yellowish powder; soluble in chloroform, moderately soluble in methanol, DMSO; insoluble in diethyl ether.

Yield: 1.05 g (40%). ^{1}H NMR (300 MHz, DMSO-d_6, in ppm): δ 3.35−3.41 (m, 4H, CH_2^aCH_2^b), 4.23 (s, 2H, CH_2^c), 4.33 (s, 2H, CH_2^d), 7.41 (‘t’, 1H, CH^f), 7.56 (‘d’, 1H, CH^e), 7.70 (d, $^3J_{H,H}$ = 8.0 Hz, 2H, CH^g), 7.87 (‘t’, 1H, CH^l), 7.95 (d, $^3J_{H,H}$ = 8.0 Hz, 2H, CH^h), 8.59 (‘d’, 1H, CH^m). ^{13}C{^{1}H} NMR (75 MHz, DMSO-d_6, in ppm): δ 43.2 (s, CH_2^a), 43.4 (s, CH_2^b), 49.9 (s, CH_2^c), 50.9 (s, CH_2^d), 123.3 (s, CH^e), 123.7 (s, CH^f), 129.5 (s, CH^g), 130.1 (s, CH^h), 130.2 (s, C^j), 131.2 (s, C^k), 137.4 (s, CH^l), 149.1 (s, CH^m), 152.4 (s, C^n), 167.1 (s, CO). IR: $\tilde{\nu}$ (cm^{-1}) 3435 (s, br), 2930 (s), 2757 (s), 2696 (s), 2587 (m), 2418 (m), 1683 (s), 1615 (m), 1432 (m), 1320 (m), 1277 (m), 1232 (m), 1106(s), 809 (s), 760 (s).

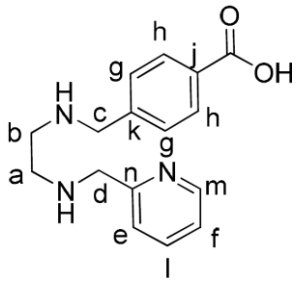

Fig 1. Atom numbering scheme of **HL3**.

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Synthesis of the copper(II) complexes 1–3

*Synthesis of **1***

Solid **L1**·HCl (300 mg, 1.5 mmol) was added to a solution of $CuCl_2$ (200 mg, 1.5 mmol) in methanol (15 mL). The resulting clear green reaction solution was stirred for three days at room temperature. Afterwards, a light blue precipitate had formed from the bright green reaction solution. This crude product was filtered off and washed with diethyl ether (40 mL) and dried in air.

Properties: green solid; soluble in DMF, DMSO, methanol; moderately soluble in ethanol; insoluble in diethyl ether.

Yield: 260 mg (58%). Decomposition temperature: 212 °C (green to black). Elemental analysis for $C_9H_{12}Cl_2CuN_2O_2$ (314.65), Calculated: C, 34.35; H, 3.84; N, 8.90 %; Found: C, 34.75, 3.91; N, 9.11 %. ESI-MS (CH_3OH), positive mode: *m*/*z* 278.2 $[M–Cl]^+$. IR: $\tilde{\nu}$ (cm^{-1}) 3419 (br, w), 3209 (s), 3033 (m), 2949 (m), 1751 (s), 1608 (m), 1452 (m), 1440 (m), 1419 (w), 1357 (m), 1240 (s), 1204 (m), 1159 (w), 1131 (w), 1112 (w), 1028 (m), 1008 (m), 931 (w), 772 (s).

*Synthesis of **2***

A suspension of **L2**·HCl (396 mg, 1.5 mmol) in methanol (10 mL) was added dropwise within 5 min at room temperature to a bright green solution of $CuCl_2$ (200 mg, 1.5 mmol) in methanol (10 mL). The color of the reaction solution changed from light green to dark green. After stirring overnight, the green precipitate was filtered off and washed with diethyl ether (8 mL). The product was dried in vacuum.

Properties: dark green powder; soluble in water, DMSO; moderately soluble in methanol; insoluble in diethyl ether.

Yield: 377 mg (70%). Decomposition temperature: 211 °C (dark green to black). Elemental analysis for $C_{26}H_{24}Cl_4Cu_2N_4O_4$ (725.37), Calculated: C, 43.05; H, 3.34; N, 7.72 %; Found: C, 42.83, 3.30; N, 7.72 %. ESI-MS (CH_3OH), positive mode: *m*/*z* 688.9 $[M–Cl]^+$. IR: $\tilde{\nu}$ (cm^{-1}) 3421 (br, s), 3210 (m), 2963 (m), 2360 (w), 1717 (m), 1699 (m), 1684 (w), 1608 (s), 1558 (w), 1541 (w), 1507 (w), 1261 (m), 1177 (m), 1104 (m).

*Synthesis of **3***

$CuCl_2$ (100 mg, 0.75 mmol) was dissolved in methanol (10 mL) at room temperature. A solution of **HL3**·HCl (280 mg, 0.75 mmol) was added to this bright green solution. After 12 h stirring the light blue precipitate was filtered off and washed with methanol (5 mL) and diethyl ether (5 mL). The product was dried in vacuum.

Properties: light blue solid; soluble in water, DMSO; moderately soluble in methanol; insoluble in diethyl ether.

Yield: 240 mg (70%). Decomposition temperature: 190 °C (light blue to black). ESI-MS (CH_3OH), positive mode: *m*/*z* 383.0 $[M–Cl]^+$. IR: $\tilde{\nu}$ (cm^{-1}) 3417 (br, s), 3208 (m), 3157 (m), 2925 (s), 1712 (s), 1670 (m), 1611 (m), 1452 (m), 1319 (w), 1262 (s), 1112 (s), 1023 (s), 801 (m), 769 (m).

Single crystal X-ray structural analysis

The data for the single crystal X-ray structure analyses of **1**–**3** were collected on a Gemini diffractometer Rigaku Oxford Diffraction using Mo-Kα radiation and ω-scan rotation. Data reduction was performed with CrysAlis Pro[23] including the program SCALE3 ABSPACK for empirical absorption correction and an analytical numeric absorption correction using a multifaceted crystal model based on expressions derived by Clark and Reid.[24] The structures were solved by direct methods using the program SHELXS-2013 and refined with SHELXL-

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2018.[25] Crystallographic parameters are collected in Table I. The structural images were generated and processed with Diamond.[26] Supplementary information of the crystallographic data can be accessed through https://www.ccdc.cam.ac.uk/structures/ CCDC 2285917 (**1**), CCDC 2285918 (**2**), CCDC 2287497 (**3**).

TABLE I. Crystallographic parameters of copper(II) complexes **1**–**3**

Name	**1** †	**2**	**3**
Formula	$C_9H_{12}Cl_2CuN_2O_2$	$C_{26}H_{24}Cl_4Cu_2N_4O_4$	$C_{16}H_{19}Cl_2CuN_3O_2$
Fw (g mol^{-1})	314.65	725.37	419.78
Temperature (K)	130(2)	130(2)	130(2)
crystal color	green	green	blue
crystal size (mm)	0.29 × 0.03 × 0.02	0.15 × 0.03 × 0.01	0.30 × 0.10 × 0.02
crystal system	monoclinic	orthorhombic	monoclinic
space group	$P2_1/c$	*Pccn*	$P2_1/c$
a (Å)	19.195(1)	8.8843(5)	13.2615(4)
b (Å)	8.7089(4)	20.018(1)	10.3590(3)
c (Å)	6.9006(4)	15.899(1)	14.5507(6)
α (°)	90	90	90
β (°)	96.350(5)	90	113.210(4)
γ (°)	90	90	90
V (nm^3)	1.1465(1)	2.8276(3)	1.8371(1)
Z	4	4	4
calcd density (g cm^{-3})	1.823	1.704	1.518
F(000)	636	1464	860
no. of collected reflns	2629	16673	15658
no. of independent reflns	2629	2501	4563
R_{int}	0.0868	0.2079	0.0563
no. of reflns observed	1602	1290	3443
restrains/parameters	0 / 150	2 / 189	0 / 293
R [$I > 2\sigma(I)$]	$R_1 = 0.0629$, $wR_2 = 0.1359$	$R_1 = 0.0542$, $wR_2 = 0.0869$	$R_1 = 0.0406$, $wR_2 = 0.0758$
R (all data)	$R_1 = 0,1039$, $wR_2 = 0,1435$	$R_1 = 0,1427$, $wR_2 = 0,1108$	$R_1 = 0.0635$, $wR_2 = 0.0844$
Goof, *S*	1.006	1.000	1.032
$\Delta\rho_{max}/\Delta\rho_{min}$ (e Å^{-3})	1.234 /−0.625	0.629/−0.497	0.478/−0.377

†: twinned crystal needle.

In vitro studies

Complex **2** was dissolved in DMF at 20 mM (stock solution) and diluted to working concentrations up to 100 µM with completed RPMI 1640 nutrient medium. The tumor cell lines were seeded at 1000 (518A2), 1500 (8505C and SW-480) and 2000 cells/well (MCF-7) and the SRB assay was performed, in triplicate, as described in the literature.[27,28] The assays AnnV/PI, apostat and AO were conducted as described recently.[29,30] For flow cytometry experiments MCF-7 cells were seeded in 6-well plates at $1{\cdot}10^5$ cells/well and concentration applied in treatments was IC_{50} of complex **2**.

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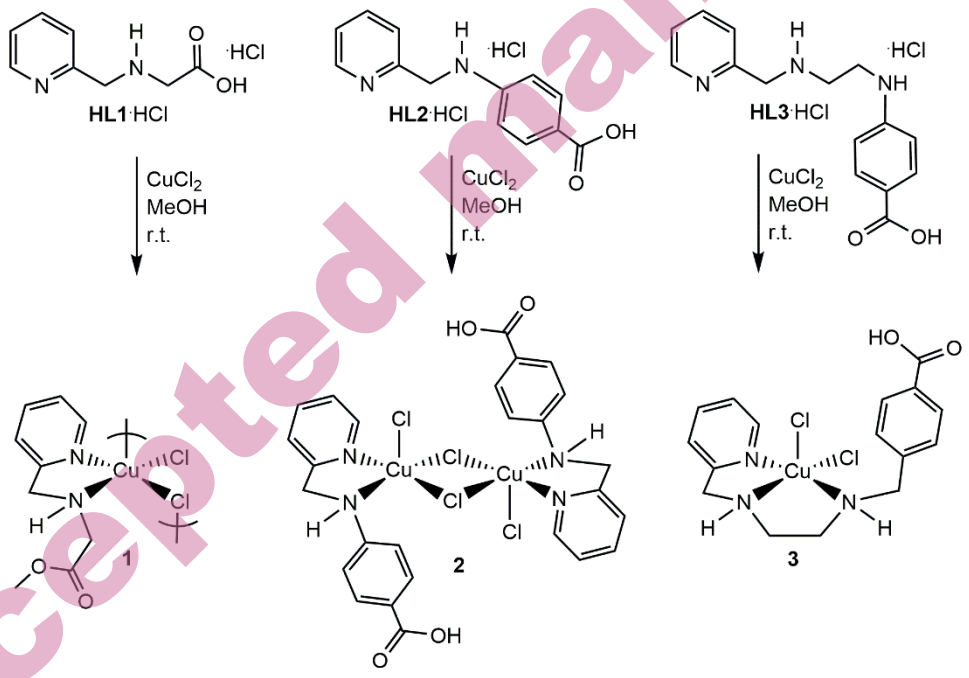
RESULTS AND DISCUSSION

Synthesis of copper(II) complexes 1–3

In the present work, two bidentate (**HL1**·HCl and **HL2**·HCl) and one tridentate (**HL3**·HCl) 2-pyridinylmethyl)amino derivatives were reacted with copper(II) chloride in methanol (Scheme 1). The corresponding copper(II) complexes **1**–**3** were obtained in moderate yields. Consistent with our recent findings whereas a ruthenium dimer was used,[21] copper(II) reacts with **HL1**·HCl in methanol with esterification of the carboxylic acid group (complex **1**). The characterization of the complexes was carried out by mass spectrometry, IR spectroscopy as well as by X-ray single crystal structure analysis.



Scheme 1. Synthesis of copper(II) complexes **1**–**3**.

Characterization

The purity of copper(II) complexes **1**–**3** was confirmed by elemental analysis, verifying their composition. Furthermore, ESI mass spectrometry is particularly informative for copper(II) complexes with chlorido ligands. Considering the natural abundance of only two copper (^{63}Cu 69.17% and ^{65}Cu 30.83%)[31] and chlorine isotopes (^{35}Cl 75.77, ^{37}Cl 24.23%),[32] the isotopic patterns of the copper(II) complexes are significantly more line-poor than those of the comparable palladium or platinum complexes.[33,34] Thus, relatively effortless conclusive molecular composition of the respective copper(II) complexes from the isotopic pattern can

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be obtained. The shape of the isotopic pattern of **1** at 278.2 *m*/*z* suggests only one coordinated chlorido ligand, which makes the corresponding molecular fragment [**1**–Cl]$^+$ being positively charged. The shape and isotopic ratio of the simulated molecular ion peak agree well for all three copper(II) complexes with those of the measured molecular ion peaks. Similarly, for **2** and **3** under the same conditions an [M–Cl]$^+$ ion was observed at *m*/*z* 688.9 and 383.0, respectively. IR spectra of copper(II) complexes **1**–**3** show a characteristic strong absorption band at 1751 and 1608 or 1611 cm^{-1}, which indicates a C=O stretching vibration of the ester in **1** or protonated carboxylic groups in **2** and **3**, respectively.[12,21,35,36] Appropriate asymmetric COO vibrations were found at 1357 (**1**), 1261 (**2**) and 1262 cm^{-1} (**3**). For copper(II) complex **1**, N–H vibrations were observed at 3209 cm^{-1}, while **2** and **3** exhibited O–H and N–H vibrations at ca 3420 or 3417 and 3210 or 3208 cm^{-1}, respectively.

Molecular structure of 1

Light blue needles of **1** appropriate for X-ray structural analysis were obtained by slow evaporation of a methanol solution. Complex **1** crystallizes in the monoclinic space group $P2_1/c$. The molecular structure is shown in Fig. 2.a. Selected bond lengths and angles are summarized in Table II.

A similar copper(II) complex, with a carboxylic acid in the ligand backbone, instead of its methyl ester reported herein (**1**), has already been described.[12] Thus in that report, coordination in a κ*N,N*',κ*O* fashion to copper(II) occurs along with formation a second five-membered ring. The five-membered ring in complex **1** (Cu–N1–C5–C6–N2) has an envelope conformation. Through the index of trigonality τ_5 (0.12), calculated with equation $\tau_5 = (\beta-\alpha)/60$ (α and β largest bond angles around copper(II) ion),[37] the coordination polyhedron around copper(II) is described as a square-pyramid with minor distortion. In the crystal lattice, zig-zag chains typical for copper(II) halido complexes along the *c*-axis are formed, where the copper(II) atoms are bridged *via* one chlorido ligand forming a 1D polymer (Fig. 2.b).

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TABLE II. Selected bond lengths (Å) and angles (°) in **1**–**3**

bond lengths (Å)		bond angles (°)	
		1	
Cu−N1	2.017(8)	Cl1−Cu-Cl2	92.49(9)
Cu−N2	2.062(8)	Cl1−Cu-N1	92.9(2)
Cu−Cl1	2.265(2)	Cl1−Cu-N2	167.5(2)
Cu−Cl2	2.297(3)	Cl2−Cu-N2	93.9(2)
Cu'−Cl1	2.699(3)	Cl2−Cu-N1	174.5(2)
		N1−Cu-N2	81.1(3)
		Cu−N1−C1	124.9(7)
symmetry code ' = *x*, 0.5–*y*, 0.5+*z*		Cu−N1−C5	115.2(6)
		2	
Cu−N1	2.005(5)	Cl1−Cu−Cl2	99.10(6)
Cu−N2	2.078(5)	Cl1−Cu−N1	94.1(2)
Cu−Cl1	2.266(2)	Cl1'−Cu−Cl2	93.80(7)
Cu−Cl2	2.248(2)	Cl1'−Cu−N1	94.1(2)
Cu−Cl1'	2.783(2)	Cl1'−Cu−N2	174.7(2)
Cu'−Cl1	2.783(2)	N1−Cu−Cl2	166.6(2)
O1···Cl2	3.024(5)	N1−Cu−N2	81.0(2)
O2···N2	2.832(7)	Cu−N1−C1	114.2(4)
symmetry code ' = –*x* , 1–*y*, 2–*z*		Cu−N1−C5	126.7(4)
		3	
Cu−Cl1	2.2486(7)	Cl1−Cu−Cl2	98.29(2)
Cu−Cl2	2.7100(7)	Cl1−Cu−N1	96.34(6)
Cu−N1	2.015(2)	N1−Cu−N2	81.66(8)
Cu−N2	2.006(2)	N1−Cu−N3	160.07(9)
Cu−N3	2.035(2)	N1−Cu−Cl2	97.26(6)
Cl2···O2'	3.032(2)	N2−Cu−Cl1	175.99(7)
		N2−Cu−N3	84.23(8)
		N3−Cu−Cl1	96.84(6)
symmetry code ' = –*x* , 1–*y*, 2–*z*		N3−Cu−Cl2	95.57(6)

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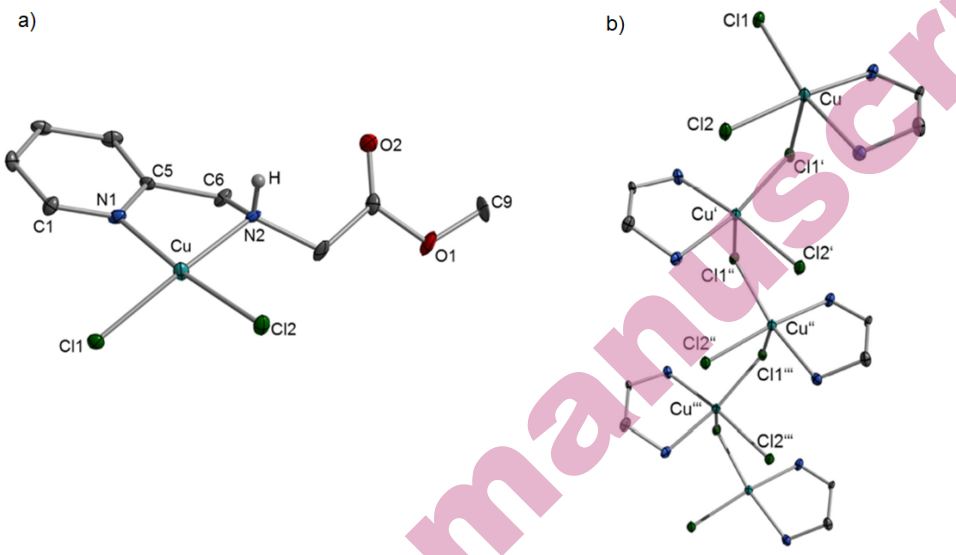

Fig. 2. a) Molecular structure of **1** and b) zig-zag chain along [001]. Only N1, N2, C5 and C6 of the bidentate ligand are shown. The ellipsoids shown correspond to a residence probability of 30%. For reasons of clarity, only the N–H atom is shown.

Molecular structure of 2

Slow continuous evaporation of a methanol solution of **2** yielded blue needles suitable for single-crystal X-ray structure analysis. The complex crystallizes in the orthorhombic space group *Pccn*. The molecular structure is shown in Fig. 3. Selected bond lengths and angles are summarized in Table II. Complex **2** forms typical chlorido-bridged dimers, with a crystallographic inversion center located in the center of the four-membered Cu–Cl1–Cu'–Cl1' ring. The Cu–Cl1 (2.266(2) Å) and Cu–Cl2 (2.248(2) Å) bond lengths are significantly shorter than the one for Cu'-Cl1 (2.783(2) Å). Coordination of the chelating ligand **HL2** to the copper atom results in a five-membered ring (Cu–N1–C5–C6–N2) in an envelope conformation with λ- or δ-configuration. The bond angles Cu–N1–C1 (114.2(4)°) and Cu–N1–C5 (126.7(4)°) deviate considerably from the ideal angle (120°), but are similar to comparable copper(II) complexes, i.e. [$CuCl_2$(py-2-CH_2NH_2)] and others.[12,38,39] Contrary to general expectation, no carboxylic acid dimers are found in the solid state of **2**. Instead, moderately strong hydrogen bonding occurs between (O1)H···Cl2 (3.024(5) Å and O2···H(N2) (2.832(7) Å) (Fig. 3.) building (100) oriented layers.[40] The angular structural parameter τ_5 (0.13), a general descriptor of five-coordinate molecules, indicates a square-pyramidal copper(II) complex.

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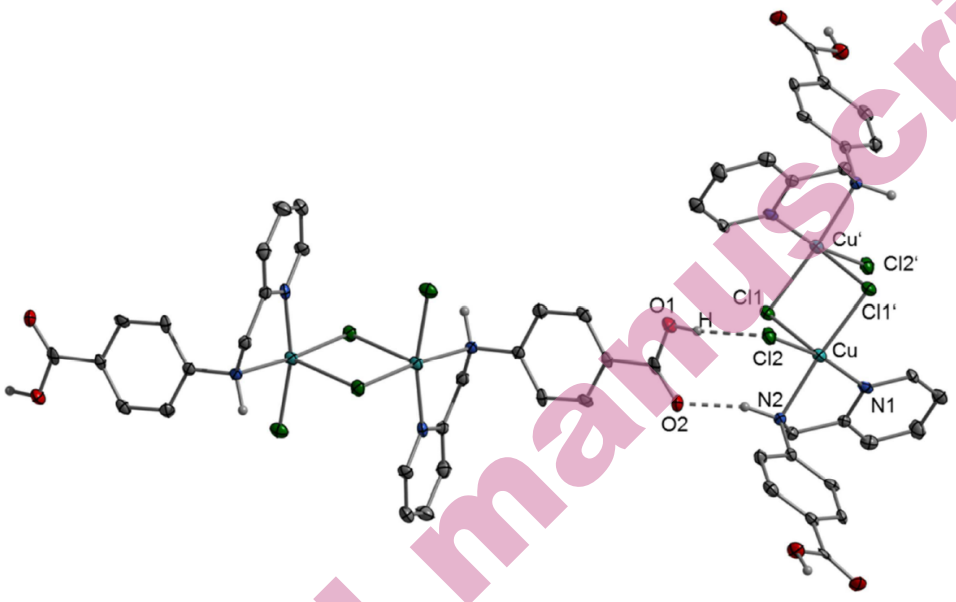

Fig. 3. Intermolecular hydrogen donor-acceptor bonds between two dimers in the solid state of **2.** The ellipsoids shown correspond to a residence probability of 30%. For clarity, only the N–H and O–H hydrogen atoms are shown.

Molecular structure of 3

Deep blue plates of complex **3** suitable for X-ray single crystal structure analysis were obtained by slow cooling a boiling aqueous solution of **3** to room temperature and subsequent storage at 4 °C for 24 h. Complex **3** crystallizes in the monoclinic space group $P2_1/c$. The molecular structure is shown in Fig. 4.a. Selected bond lengths and angles are summarized in Table II. **HL3** is coordinated at the copper(II) ion in an κ^3*N,N',N''* bonding mode generating two five-membered rings which differ in their conformation. Accordingly, ring 1 (Cu–N1–C5–C7–N2) has an envelope conformation and ring 2 (Cu–N2–C7–C8–N3) *δ*- or *λ*-conformation. Hydrogen bonds occur between the atoms (O2')H and Cl2 (Fig 4.b.), resulting in a 1D chain along [201]. The shortest distance Cl2···O2' is 3.031(2) Å and is in a comparable range to the O1···Cl2 distance of complex **2** (3.024(5) Å). In **3**, the copper(II) ion has a distorted square-pyramidal geometry with an angular structural parameter $\tau_5 = 0.26$.

In vitro study of 2

To assess the *in vitro* potential of copper(II) complexes, as a representative compound complex **2** was selected and tested against four cell lines 8505C, MCF-7, 518A2 and SW-480. The viability was determined using an SRB assay (Fig. 5). Complex **2** was found to be active on all investigated cell lines with IC_{50} values ranging from 12.5 to 22.5 µM. Its activity was found to be lower on the same cell

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lines (SRB assay, 96 h of treatment) in comparison to the clinically used drug cisplatin.[41–43]

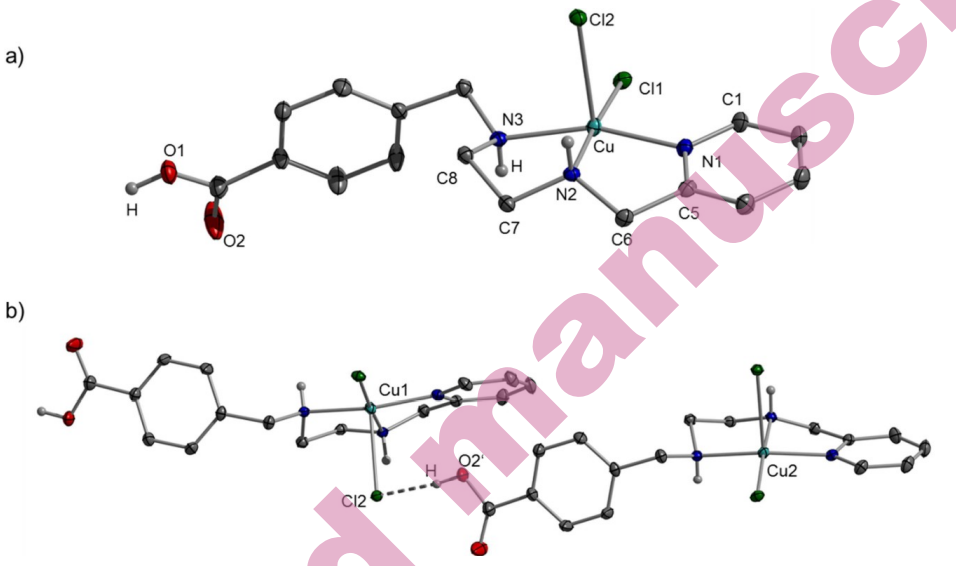

Fig. 4. a) Molecular structure of complex **3** and b) hydrogen bonding in the solid state of **3**. The ellipsoids shown correspond to a residence probability of 30%. For clarity, only the N–H and O–H hydrogen atoms are shown.

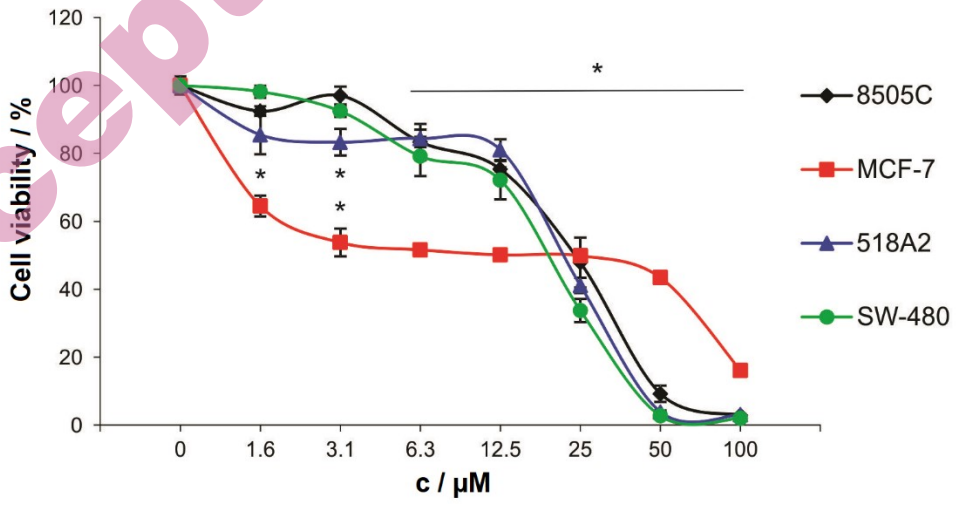

Fig. 5. Dose-dependent response of selected tumor cell lines treated with copper(II) complex **2** (SRB assay, 96 h). IC_{50} concentrations: 8505C, 22.35 ± 2.33; MCF-7, 12.5 ± 0.92; 518A2, 19.95 ± 3.19; SW-480, 22.35 ± 3.75 µM. *p<0.05 in comparison to control.

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MCF-7 cells were chosen for further experiments as the most sensitive cell line displaying a specific plateau effect in response to treatment (Fig. 5). Upon exposure to an IC_{50} dose of copper(II) complex **2**, the MCF-7 cells underwent massive apoptosis (Fig. 6.a). This effect was synchronized with intensive total caspase activation despite the fact that these cells are caspase 3 deficient (Fig. 6.b).[44,45] Also, complex **2** did not elevate the number of autophagic vesicles in comparison to untreated cells. Even lower in activity than cisplatin, copper(II) complex **2** deserves attention since copper, as essential element, is involved in main cellular functions.[46] Since it was found that tumor cells have a higher demand for copper compared to normal cells, it can be speculated that compounds based on copper(II) will be more efficiently internalized by neoplastic cells thus representing a form of targeted therapy.[47]

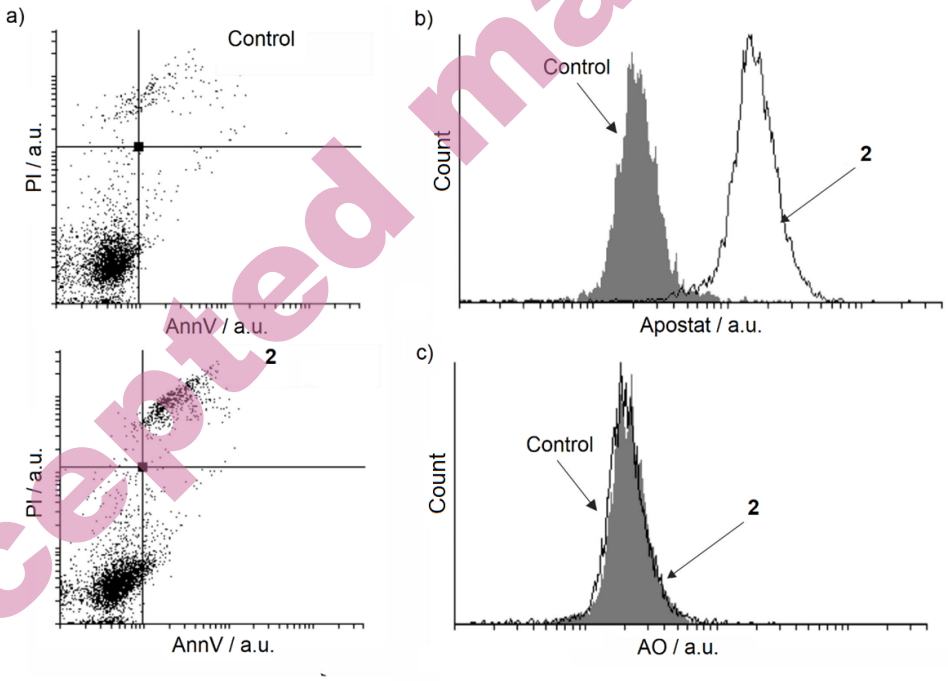

Fig. 6. Triggering of a) apoptosis, b) caspases and c) autophagy in MCF-7 cells with complex **2**.

CONCLUSION

Preparation and characterization of three copper(II) complexes containing 2-[(2-pyridinylmethyl)amino](methyl acetate) (**1**), 4-[(2-pyridinylmethyl)amino]benzoic acid (**2**) and 4-{[2-(pyridin-2-ylmethylamino)ethylamino]methyl}benzoic acid (**3**) is described. Elemental

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analyses verify the purity of the complexes. Furthermore, ESI mass spectrometry and infrared spectroscopy confirm complex formation. In the solid state, complexes form polymeric (**1**), dimeric (**2**) or mononuclear (**3**) structures with copper(II) in a square-pyramidal environment. Complex **1** forms 1D chains *via* bridging chlorido ligands, while dimers of **2** are constructing a supramolecular structure through intermolecular hydrogen bonding interactions, and complex **3** forms polymeric chains by intermolecular hydrogen bonding. Complex **2** efficiently suppresses the growth of 8505C, MCF-7, 518A2 and SW480 tumor cell lines. Induction of caspase-dependent apoptosis was found to be a leading cause of the tumoricidal activity of complex **2**.

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И З В О Д

КВАДРАТНО-ПИРАМИДАЛНИ МОНОНУКЛЕАРНИ, ДИНУКЛЕАРНИ И ПОЛИНУКЛЕАРНИ КОМПЛЕКСИ БАКРА(II) СА (2-ПИРИДИНИЛМЕТИЛ)АМИНО ДЕРИВАТИМА

STEFAN RICHTER[1], PETER LÖNNECKE[1], ДИЈАНА БОВАН[2], САЊА МИЈАТОВИЋ[2], ДАНИЈЕЛА МАКСИМОВИЋ-ИВАНИЋ[2], ГОРАН Н. КАЛУЂЕРОВИЋ[3] И EVAMARIE HEY-HAWKINS[1]*

[1]Universität Leipzig, Faculty of Chemistry and Mineralogy, Institute of Inorganic Chemistry, Johannisallee 29, 04103 Leipzig, Germany; [2]Odeljenje za imunologiju, Institut za biološka istraživanja „Siniša Stanković", Institut od nacionalnog značaja za Republiku Srbiju, Univerzitet u Beogradu, 11000 Beograd, Srbija and [3]Department of Engineering and Natural Sciences, University of Applied Sciences Merseburg, Eberhard-Leibnitz-Str. 2, 06217 Merseburg, Germany

Описана је координација три лиганд прекурсора 2-[(2-пиридинилметил)амино]сирћетне киселине хидрохлорида, 4-[(2-пиридинилметил)амино]бензоеве киселине хидрохлорида и 4-{[2-(пиридин-2-илметиламино)етиламино]метил}бензоеве киселине хидрохлорида, **HL1**·HCl–**HL3**·HCl, са бакром(II). Награђени комплекси су окарактерисани елементалном анализом, ЕСИ масеном спектрометријом и ИР спектроскопијом, као и рендгенском структурном анализом. У реакцији бакра(II) са **HL1**·HCl у метанолу формиран је комплекс формуле $[\{Cu(\mu\text{-}Cl)_2(\mathbf{MeL1}\text{-}\kappa^2N,N')\}_n]$ уз естерификацију **L1** (**MeL1**). Са **HL2**·HCl, односно **HL3**·HCl бакар(II) је наградио динуклеарни комплекс $[\{CuCl(\mu\text{-}Cl)(\mathbf{HL2}\text{-}\kappa^2N,N')\}_2]$ (**2**), односно мононуклеарни комплекс $[CuCl_2(\mathbf{HL3}\text{-}\kappa^3N,N',N'')]$ (**3**). У сва три комплекса централни јон је у квадратно-пирамидалном окружењу. Код комплекса **1** формирана је полимерна структура преко мостовних хлоридо лиганада, а без типичних водоничних веза. Супротно томе, COOH група у комплексу **2** учествује у грађењу интермолекулске водоничне везе дајући супрамолекуларну структуру. Код комплекса **3** интермолекулсе водоничне везе (Cl···O) образују 1Д полимерну структуру. Комплекс **2** је показао значајну активност на тестираним ћелијама 8505C, MCF-7, 518A2 и SW-480 хуманог порекла. Основни механизам путем кога је реализована туморицидна активност је апоптоза зависна од активације каспаза.

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