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Original article

Synthesis, X-ray structures and cytotoxic activity of platinum(II), palladium(II) and copper(II) complexes with chelating ligands

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ABSTRACT

Here we present the synthesis of the new chelating ligands 1-benzothiazol-2-yl-5-(2-hydroxyphenyl)-3-methyl-1*H*-pyrazole-4-carboxylic acid methyl ester (**2a**) and 1-(6-chloropyridazin-3-yl)-5-(2-hydroxyphenyl)-3-methyl-1*H*-pyrazole-4-carboxylic acid methyl ester (**2b**), obtained in the reaction of 2-methyl-4-oxo-4*H*-chromene-3-carboxylic acid methyl ester (**1**) with hydrazine derivatives. These ligands **2a** and **2b** create solid complexes with Pt(II) (**4a**, **4b**), Pd(II) (**5a**, **5b**) and Cu(II) (**6a**, **7a**, **8a**, **8b** and **9b**) metal ions or can be cyclized to 1-benzothiazol-2-yl-3-methyl-1*H*-chromeno[4,3-*c*]pyrazol-4-one (**3a**) or 1-(6-chloropyridazin-3-yl)-3-methyl-1*H*-chromeno[4,3-*c*]pyrazol-4-one (**3b**). The crystal and molecular structures of ligand **2a**, its Cu(II) complexes **6a** and **7a** were determined by X-ray diffraction method. Cytotoxic activity of the ligands **2a** and **2b** and their complexes **4a**, **4b**, **5a**, **5a**, **6a**, **8a**, **8b** and **9b**, and modulation of expression of *BAX* and *P53* genes are also shown.

1. Introduction

During the last decades, a growing interest in transition metal complexes has been stimulated by their potential applications as anti-neoplastic agents. We have been investigating the synthesis, X-ray structures, cytotoxic effect and structures-activity relationship of metal ions complexes with different ligands [1–3]. Special attention has been paid to pyrazoles and related *N*-containing heterocyclic derivatives, which play an important role in many biological processes due to their coordination ability with metal ions. Platinum–sulfur interactions in the human body influence uptake, excretion, DNA binding, the toxicity of platinum compounds and drug resistance of cancer cells. Knowledge of these interactions is very helpful for the synthesis of new platinum anticancer drugs [4]. A modification of cisplatin structure can be connected with the displacement of ammonia with more stable *N*- and *S*- donor ligands. The best known of these *N*,*S*-donor ligands are: thiosemicarbazones [5], thiohydrazides [6], aminoacids (cysteine, methionine) [7] and thiazole [8]. Beside them, there are many heterocyclic compounds that can act as *N*-donor ligands, such as pyrazoles, pyridazine, pyridines [9,10], piperidine, piperazine, picoline [11] and imidazoles [12]. Pyrazole-derived ligands have attracted scientists' attention over the last decades because of their interesting coordination chemistry, unusual structural features and remarkable physical and chemical properties [13]. Pyrazoles are potential imidazole mimics and therefore can serve in the development of ligand systems that resemble active sites of metalloenzymes [14]. Although a large number of complexes containing pyrazole ligands have been synthesized, the design and synthesis of novel pyrazole-containing complexes by varying the nature of the reactants and synthetic conditions are still under investigation [15].

The anticancer activity of many drugs involving cisplatin is often related to the modulation of expression of some genes engaged in programmed cell death – apoptosis. Among them is *BAX* – a proapoptotic member of the *Bcl*-2 family of genes. The Bax (Bcl-2-associated protein) protein forms a heterodimer with Bcl-2 and functions as an apoptotic activator. Overexpressed *BAX* accelerates apoptotic death induced by cytokine deprivation in an IL-3-dependent cell line. Overexpressed *BAX* also counters the death-repressor activity of *Bcl-2* [16]. The expression of the *BAX* gene is regulated by the tumor suppressor *P53* and has been shown to be involved in *P53*-induced apoptosis. *P53* is central to many of

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the cell's anticancer mechanisms. It can induce growth arrest, apoptosis and is responsible for genetic stability and inhibition of angiogenesis [17,18]. Pharmacological manipulation of *BAX* and *P53* expression, which enhances their proapoptotic effect, has implications for many diseases involving apoptosis such as cancer.

Novel metal-based complexes containing metals such as palladium(II), copper(II), ruthenium(III), gold(III) and rhodium(III) have been reported with promising chemotherapeutic potential and varied mechanisms of action in comparison with the platinum-based drugs [19]. In our previous papers we described the synthesis of 5-(2-hydroxyphenyl)-3-methyl-1-(2-pyridinyl)-1*H*-pyrazol-4-carboxylic acid methyl ester and its complexes with platinum(II), palladium(II) and copper(II) ions [20,21]. These complexes showed lower cytotoxicity than cisplatin and the platinum(II) and copper (II) complexes were found to be more efficient in the induction of leukemia cell death than the palladium(II) complex.

As a part of our systematic investigation here we present the synthesis of new chelating ligands 1-benzothiazol-2-yl-5-(2-hydroxyphenyl)-3-methyl-1*H*-pyrazole-4-carboxylic acid methyl ester (**2a**) and 1-(6-chloropyridazin-3-yl)-5-(2-hydroxyphenyl)-3-methyl-1*H*-pyrazole-4-carboxylic acid methyl ester (**2b**). In comparison with the ligand's structure described in our previous paper [2] the pyridinyl group was replaced by a benzothiazoyl group in the ligand's **2a** structure to check if a sulfur atom can take part in the coordination of the metals' ions or alter the cytotoxic activity. In this paper we also present the cyclization of ligands **2a** and **2b** to very low soluble 1-benzothiazol-2-yl-3-methyl-1*H*-chromeno[4,3-*c*]pyrazol-4-one (**3a**) and 1-(6-chloropyridazin-3-yl)-3-methyl-1*H*-chromeno[4,3-*c*]pyrazol-4-one (**3b**), synthesis of ligands **2a** or **2b** solid complexes with platinum(II) (**4a**, **4b**), palladium (II) (**5a**, **5b**) and copper(II) (**6a**, **7a**, **8a**, **8b** and **9b**), X-ray structures of ligand **2a** and its copper(II) complexes **6a** and **7a**, cytotoxic activity of ligands **2a** and **2b** and their complexes performed on leukemia and melanoma cell lines, and the modulation of expression of *BAX* and *P53* genes by two chosen complexes: Pt(II) **4a** and Cu(II) **6a**.

2. Results and discussion

2.1. Preparation of the ligands and their complexes

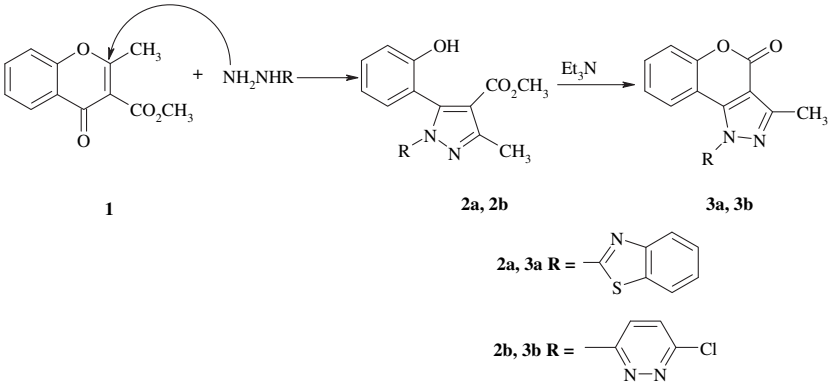
In the reaction of 2-methyl-4-oxo-4*H*-chromene-3-carboxylic acid methyl ester (**1**) with 2-hydrazinobenzothiazole or 3-chloro-6-hydrazino-pyridazine in ethanolic solution we have obtained the new ligands **2a** and **2b**, respectively (Scheme 1). This reaction proceeds according to the general mechanism described for the reaction of chromones with nitrogen nucleophiles [22] such as hydrazines [23]. Compounds **2a** and **2b** easily cyclize under basic conditions such as triethylamine or in high temperature to form 1-benzothiazol-2-yl-3-methyl-1*H*-chromeno[4,3-*c*]pyrazol-4-one (**3a**) and 1-(6-chloropyridazin-3-yl)-3-methyl-1*H*-chromeno[4,3-*c*]pyrazol-4-one (**3b**), respectively. Thermogravimetric analysis of the copper(II) complex described in our recent paper [21] confirmed detachment of the methanol molecule and DMF. The detachment of the methanol molecule could suggest the cyclization of the pyrazole ligand to the coumarin derivative. According to these data, we successfully performed the thermal cyclization at 150 °C of the pyrazole ligands **2a** and **2b** to their coumarin derivatives **3a** and **3b**, in order to compare the cytotoxic activity of pyrazole derivatives **2a** and **2b** and coumarin derivatives **3a** and **3b**. Unfortunately, compound **3a**, with its very poor solubility in any solvents, could not be used to synthesize the complexes. Despite the fact that the ligand **3b** has better solubility, it does not react with metal salts and does not produce complexes.

The ligands **2a** and **2b** in the reactions with the metals' salts (potassium tetrachloridoplatinate(II), bis(benzonitrile)palladium (II) chloride, copper(II) chloride dihydrate, copper(II) perchlorate hexahydrate) create solid complexes **4a**, **b**, **5a**, **b**, **6a**, **7a**, **8a**, **8b** and **9b** (Schemes 2 and 3). The metals' ions are coordinated by the two nitrogen atoms of the ligands **2a** and **2b**. The sulfur atom does not take part in the coordination. Recrystallization of the copper(II) complex **6a** by the slow diffusion of diethyl ether to DMF gave the pentacoordinated copper(II) complex **7a**.

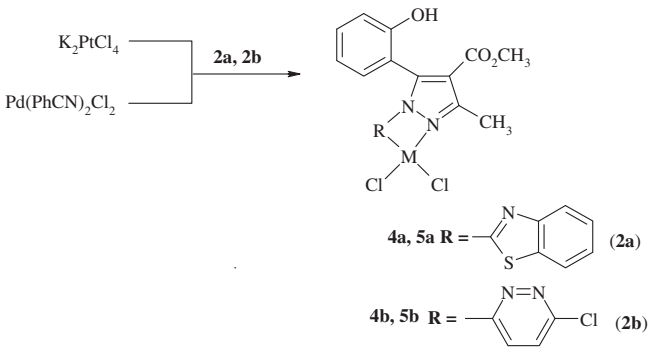
2.2. Structural studies

2.2.1. Spectroscopic characterization of ligands and complexes

The IR-bands at 3416–3056 cm^{-1} in the spectra of the ligands **2a** and **2b** and their complexes **4a**, **4b**, **5a**, **5b**, **6a**, **7a**, **8a**, **8b**, **9b** are assigned to the hydroxy group of the phenyl ring. We could not observe this band in the spectra of compounds **3a** and **3b**, because they do not have the hydroxy group in their structures. The bands at 1617–1476 cm^{-1} in the spectra of the ligands **2a** and **2b** are assigned to the aromatic group of the phenyl ring. In the spectrum of ligand **3a** the bands assigned to the aromatic group of the chromene are in wider range of frequencies (1609–1566 cm^{-1}) and in the spectrum of ligand **3b**, in lower frequencies (1562–1450 cm^{-1}). The characteristic bands at 3103–2915 cm^{-1} of the methyl group of the ligands **2a**, **2b**, **3a**, **3b** and complexes **4a**, **4b**, **5a**, **5b**, **6a**, **7a**, **8a**, **8b**, **9b** are assigned to C–H vibration. The bands at 1718 cm^{-1} and 1706 cm^{-1} in the spectra of ligands **2a** and **2b**, respectively, which were assigned to the C=O vibrations, shift to higher energies for the complexes **4a**, **4b**, **5a**, **5b**, **6a**, **7a**, **8a**, **8b**, **9b** (1727–1715 cm^{-1}) and especially for the compounds **3a** (1736 cm^{-1}) and **3b** (1742 cm^{-1}). The bands at 1109 cm^{-1} and 1094 cm^{-1} in the spectra of the ligands **2a** and **2b**,



Scheme 1. Synthesis of the ligands **2a**, **2b** and **3a**, **3b**.



Scheme 2. Synthesis of the pyrazole derivatives Pt(II) and Pd(II) complexes **4a**, **4b** – **5a**, **5b**.

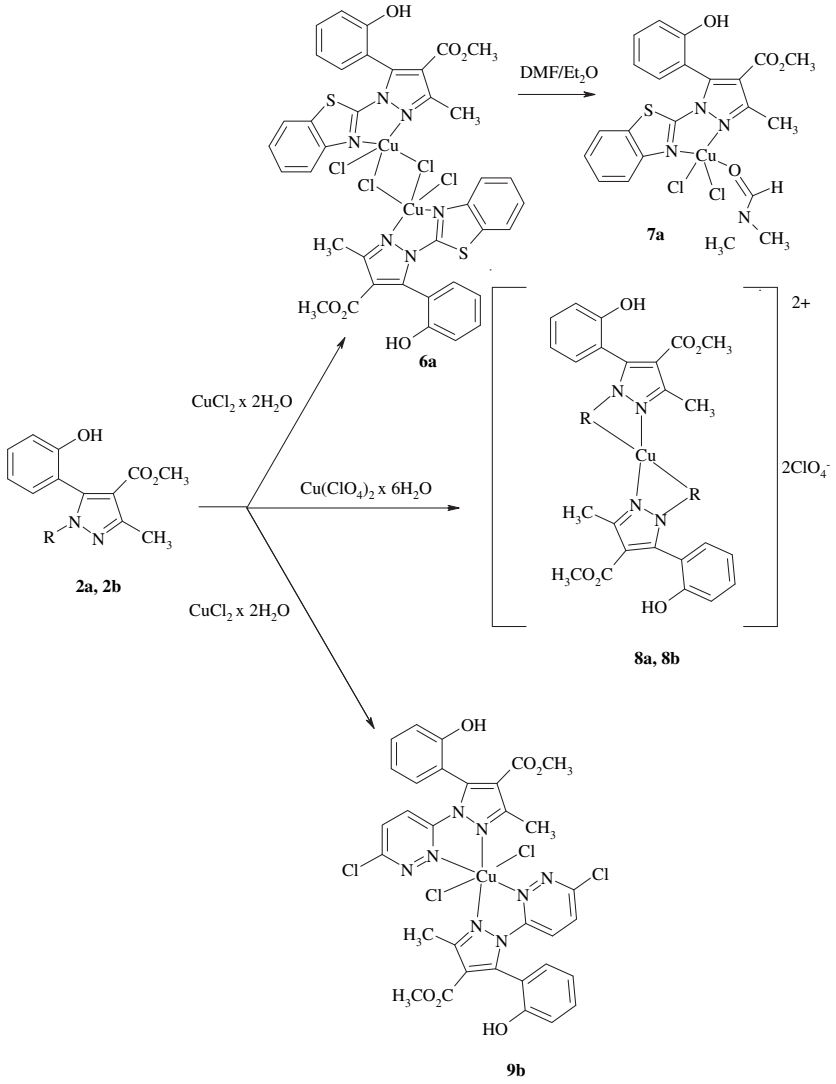
respectively, are assigned to the methoxy group. This band shifts to higher energy for the compound **3a** (1134 cm^{-1}) and to lower energy for the compound **3b** (1053 cm^{-1}). The new bands at 550–426 cm^{-1} in the spectra of complexes **4a**, **5a**, **5b**, **6a**, **7a**, **9b** correspond to the metal–nitrogen vibration involving the nitrogen atoms of the pyrazole ring. The bands observed at 1117, 1106, 623 and 621 cm^{-1} in the spectra of **8a** and **8b** are characteristic for non-coordinated perchlorate ions [24].

The ^{1}H NMR spectra of ligands **2a**, **2b**, **3b** and complexes **4a** and **5a** show signals in the range of δ 2.50–2.60 ppm for the methyl groups of pyrazole ring, 3.59–3.70 ppm for the methoxy groups and 6.82–8.05 ppm for phenyl groups. In the spectrum of complex **4a** we observe the signal at δ 9.64 ppm for the hydroxy group. In the spectrum of ligand **2a** the signal for the hydroxy group was observed in the aromatic region. The ^{1}H NMR spectra of copper(II) complexes could not be measured due to their paramagnetism [25].

In the ^{13}C NMR spectra of ligand **2a** and **2b**, signals for carbon atoms of sp^3 hybridization are the most characteristic. Ligand **2a** shows a signal at δ 14.65 ppm, ligand **2b** – at δ 14.68 ppm for the methyl group of pyrazole ring and ligand **2a** – at δ 51.85 ppm, ligand **2b** – at δ 51.93 ppm for the methoxy group [26].

2.2.2. Conductivity measurements

The conductivity data of the studied copper(II) complexes in ethanol at 298 K indicate that the complexes **6a**, **7a** and **9b** are non-electrolyte compounds, but complexes **8a** and **8b** possess ionic structure. Therefore chloride is bonded to the copper(II) ion, while the perchlorate ion is outside the metal coordination sphere.



Scheme 3. Synthesis of the pyrazole derivatives Cu(II) complexes **6a**, **7a**, **8a**, **8b** and **9b**.

2.2.3. X-ray structure of ligand **2a** and its copper(II) complexes **6a** and **7a**

The crystal data and details of structure refinement of ligand **2a** and its complexes **6a** and **7a** are given in Table 1. The basic framework of ligand **2a** (Fig. 1) is almost planar. The torsion angle between the benzothiazole and pyrazole ring comes to only 6°. Even the substituents CH_3 and $CH_3OC(O)$ at C9 and C10 lie within the pyrazole plane, their dihedral angles are less than 2°. Only the phenyl substituent is turned out of the plane at 81° in an almost perpendicular orientation. This might be due to sterical reasons of the hydroxy group at C13 and its formation of a hydrogen bridge between O13 of **2a** and N3 of an adjoining **2a** (O3···H83···N3i 3.008 (9) Å, 149.00°). All bond lengths and angles of **2a** lie in the normal range. This applies also for the second molecule **2a** found within the cell. It is noteworthy that in both cases N1 (N4) of the pyrazole ring and N3 (N6) of the benzothiazole system lie on the same side of **2a** indicating their favourable bidentate ligand function.

In contrast to the monomeric complex **7a** (Fig. 3), complex **6a** (Fig. 2) is a dimer exhibiting a distorted trigonal bipyramidal configuration at both Cu(II) centers connected by two asymmetrical chlorido bridges. The chlorido ligands Cl1 (terminal) and Cl2a (bridging) and the N1 of benzothiazole form the trigonal plane and the N3 of pyrazole together with the second chlorido bridge Cl2 lie in axial positions. Both μ_2-Cl bridges are asymmetrical with two different Cu–Cl distances (Cu–Cl_2 2.2714(13) and Cu–Cl2a 2.5010 (13)Å); the distance to the terminal Cl1 atom is the shortest (2.2341 (13) Å). The Cu–N distances are also quite different, Cu–N1 2.170(3) and Cu–N3 1.989(3) Å, where the last one in axial position is again shorter as in the Cu-complex **7a** because of the better π-acceptor character of the pyrazole part. As in **7a**, the trigonal plane is rather undistorted with a sum of angles of 358°, but different angles (Cl1–Cu–N1 128.85(10), Cl1–Cu–Cl2a 131.24(5) and N1–Cu–Cl2a 98.28(9)°). The smallest angles around Cu(II) are the bite angle N1–Cu–N3 77.25(13)° and the angle between both chlorido bridges (Cl2–Cu–Cl2a 87.34(4)°). Due to this bonding situation, the trans-apical angle Cl2–Cu–N3 is somewhat bent (175.61(10)°).

The geometry at Cu(II) in **7a** is a distorted trigonal bipyramidal with two chlorido ligands and N3 of the pyrazole part of **2a** in equatorial positions. The N1-atom of the benzothiazole part and O4 of the coordinated solvent molecule DMF occupy the apical positions. Both bond lengths Cu–Cl differ somewhat (Cu–Cl1 2.369(2), Cu–Cl2 2.291(2) Å), whereas those of Cu–N differ substantially (Cu–N1 2.011(5), Cu–N3 2.198(5)Å). This is noteworthy because N1 lies in the apical position with normally longer bonds than in the equatorial one. This may be due to the better π-acceptor character of N1 in benzothiazole compared to N3 of substituted pyrazole. The distance Cu–O4 1.953(5) is found in the normal range of DMF complexes of Cu(II). The trigonal plane with the sum of angles = 360° around Cu possesses two very similar small-bond angles (Cl1–Cu–Cl2 115.99(7), Cl1–Cu–N3 116.49(16)°) and a rather enlarged angle Cl2–Cu–N3 (127.50(15)°). The bite angle N1–Cu–N3 of the chelate ligand **2a** is naturally the smallest around Cu and found to be 78.36(19)°. The further angles between the equatorial and axial atoms are found more or less at 90° (N3–Cu–O4 88.901(19), N1–Cu–Cl1 93.25(16)°) except the angle N1–Cu–Cl2 97.49(16)°. This results from the bent axis through N1, Cu, O4 which is bent with the angle 166.77(19)° towards the ligand **2a** because of the steric demand of ligand DMF.

All the other bonds and angles within the ligand in **7a** are the same as found in free **2a**. The dihedral angle, however, between the planes of the pyrazole and the phenyl ring has changed to 72.18°. The triazametallacycle shows a slight envelope conformation with an angle of 5° along the N1/N3 axis.

Table 1
Crystal data and details of structure refinement of ligand **2a** and complexes **6a** and **7a–f.**

Compound	**2a**	**6a**	**7a**
Formula	$C_{19}H_{15}N_3O_3S$	$C_{44}H_{42}Cl_4Cu_2N_6O_8S_2$	$C_{22}H_{22}Cl_2CuN_4O_4S$
M_r/g mol^{-1}	365.407	1115.875	572.952
Crystal size/mm	0.16 × 0.12 × 0.03	0.20 × 0.09 × 0.02	0.19 × 0.16 × 0.04
Crystal system	Monoclinic	Monoclinic	Triclinic
Space group	$P2_1$	$P2_1/n$	$P\bar{1}$
a/Å	11.7434(5)	8.42230(10)	9.6420(3)
b/Å	6.9352(3)	30.0030(6)	11.1059(3)
c/Å	21.0770(10)	9.7527(2)	11.8270(4)
α/°	90	90	94.9177(18)
β/°	103.001(2)	106.0611(11)	106.0409(19)
γ/°	90	90	94.3708(17)
V/Å^3	1672.57(13)	2368.26(7)	1206.06(6)
Z	4	2	2
calc. density/g cm^{-3}	1.45113(11)	1.56485(5)	1.57774(8)
μ/mm^{-1}	0.219	1.271	1.251
Refls. measured	10874	14380	7241
R_{int}	0.0533	0.0300	0.0270
Mean $\sigma(I)/I$	0.0917	0.0320	0.0394
θ range	3.44–25.36	3.14–25.34	3.23–24.00
Observed refls.	4840	3646	3316
x, y (weighting scheme)	0.1117, 3.2270	0.0219, 7.4375	0.0238, 10.5702
Refls in refinement	6056	4326	3746
Parameters	473	302	311
Restraints	1	0	0
R (F_{obs})	0.0892	0.0509	0.0598
R_w (F^2)	0.2402	0.1159	0.1566
S	1.101	1.124	1.137
Shift/error$_{max}$	0.001	0.001	0.001
Max electron density/e Å^{-3}	0.875	1.155	1.357
Min electron density/e Å^{-3}	−0.412	−0.664	−0.467

2.3. Biological assays

2.3.1. Cytotoxicity studies

The cytotoxicity of ligands **2a** and **2b** and their metal complexes **4a**,**b**, **5a**,**b**, **6a**, **8a**,**b**, **9b** was assayed against melanoma WM-115 cells as well as leukemia promyelocytic HL-60 and lymphoblastic NALM-6 cells. Cisplatin and carboplatin were used as the reference compounds. Cells were exposed to a broad range of drug concentrations (10^{-7} to 10^{-3} M) for 48 h and cell viability was analyzed by MTT assay. IC_{50} values are presented in Table 2. Metal complexes **4a**, **b**, **5a**,**b**, **6a**, **8a**,**b**, **9b** exhibited rather moderate cytotoxicity to both acute leukemia HL-60 and NALM-6 cell lines although complex **8a** was more active. Relatively high cytotoxic activity was observed for complexes **6a** and **8a** in the case of skin melanoma WM-115 cells. The cytotoxic effectiveness of these compounds with an IC_{50} of 17.4 μM (**6a**) and 8.2 μM (**8a**) was comparable or higher than that of cisplatin (18.2 μM) and was much higher than that of carboplatin (422.2 μM). It should be noted that melanomas often characterize themselves by low susceptibility to many chemotherapeutics.

2.3.2. The effect of complexes **4a** and **6a** on BAX and P53 gene expression

In this study, using real-time PCR we evaluated the effect of complexes **4a** and **6a** on *BAX* and *P53* gene expression in WM-115 melanoma cells after 5 and 48 h exposure. The Cu(II) complex **6a** was chosen for this study because of the best cytotoxic activity on WM-115 cell lines, and Pt(II) complex **4a** was chosen as a structural analog of complex **6a**. It was observed that the platinum(II) complex **4a** caused distinct overexpression of *BAX* after a relatively short exposure time (5 h) (Fig. 4A). Overexpression of the *P53* gene was less pronounced. The expression of *BAX* and *P53* genes was relatively decreased after 48 h. Various patterns of *BAX* and *P53*

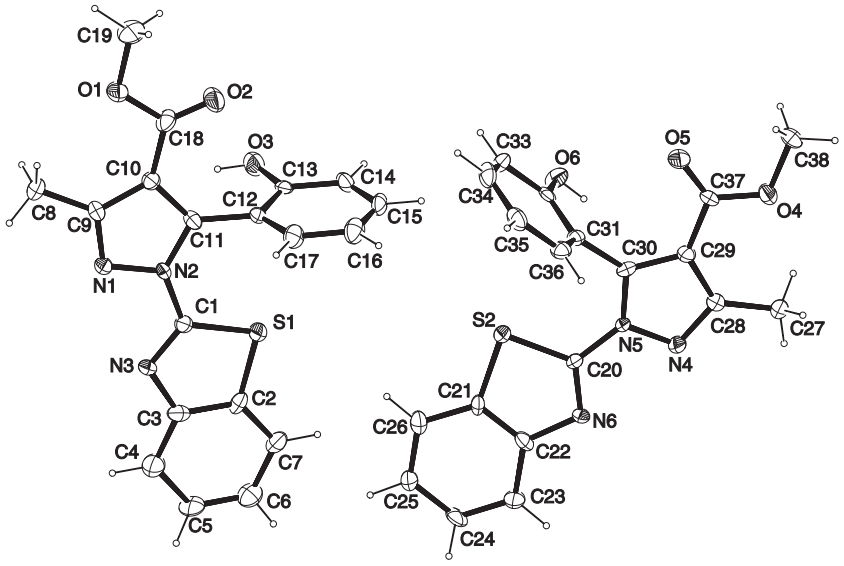


Fig. 1. Molecular structure of ligand **2a**.

gene expression were observed in melanoma cells exposed to the copper(II) complex **6a**. Complex **6a** did not induce the expression of both genes although a weak growing tendency for *P53* after 48 h was seen (Fig. 4B). Obtained results may suggest that proapoptotic, antiproliferative and cytotoxic pathways triggered by complexes **4a** and **6a** are different.

3. Conclusion

The highly substituted pyrazoles **2a** and **2b** were synthesized and used as ligands for the synthesis of novel platinum(II), palladium(II) and copper(II) complexes (**4a**, **4b**, **5a**, **5a**, **6a**, **8a**, **8b** and **9b**). The cyclized derivatives **3a** and **3b** of the ligands **2a** and **2b** did not create complexes. Ligand **2a** formed two copper(II) complexes: non-electrolyte copper(II) complex **6a** in molar ratio 1:1, which after recrystallization by slow diffusion of diethyl ether to DMF, gave the pentacoordinated complex **7a**, and the ionic complex **8a** in a molar ratio of 2:1. Ligand **2b** formed two copper(II) complexes in a molar ratio of 2:1: the non-electrolyte complex **9b** in and ionic complex **8b**. The structures of the ligands and their metal complexes were confirmed by spectral and elemental analyses. Additionally, the molecular structures of the ligand **2a** and its Cu(II) complexes **6a** and **7a** were confirmed by X-ray analysis. Cytotoxicity studies revealed the high effectiveness of Cu(II) complexes **6a** and **8a** for skin melanoma WM-115 cells at the level comparable to that of cisplatin for complex **6a** and two times higher for complex **8a**. Therefore, compound **6a** and its structural analog – Pt(II) complex **4a** – were chosen for *BAX* and *P53* gene expression study.

4. Experimental

4.1. Chemistry

All substances were used without further purification. Potassium tetrachloridoplatinate(II), bis(benzonitrile)palladium (II) chloride, copper(II) chloride dihydrate and copper(II)

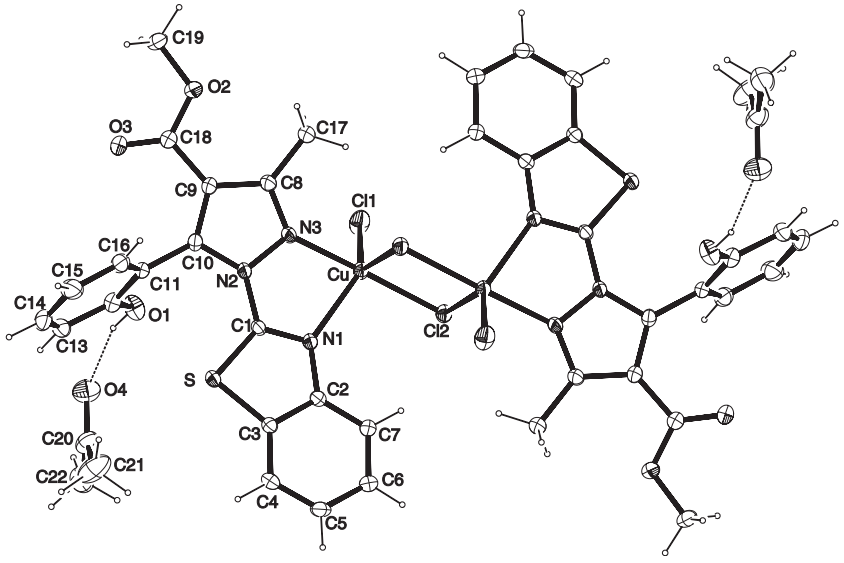


Fig. 2. Molecular structure of complex **6a**.

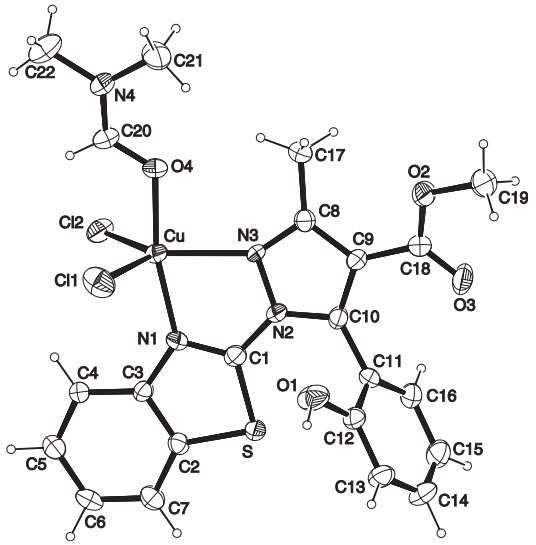


Fig. 3. Molecular structure of complex **7a**.

perchlorate hexahydrate were purchased from Aldrich. Chloroform-d and DMSO-d_6 solvents for NMR spectroscopy were obtained from Dr. Glaser AG, Basel. Solvents for synthesis (chloroform, diethyl ether, methanol) were reagent grade or better and were dried according to standard protocols [27]. The melting points were determined using a Buchi B-540 apparatus and are uncorrected. The IR spectra were recorded on a Pye-Unicam 200G Spectrophotometer in KBr. The ^{1}H NMR spectra were registered at 300 MHz on a Varian Mercury spectrometer. The MS data were obtained on a LKB 2091 mass spectrometer (70 eV ionisation energy). The MS-FAB data were determined on Finnigan Matt 95 mass spectrometer (NBA, Cs^+ gun operating at 13 keV). For the new compounds, satisfactory elemental analyses were obtained using a Perkin Elmer PE 2400 CHNS analyser. 2-Methyl-4-oxo-4*H*-chromene-3-carboxylic acid methyl ester (**1**) was prepared as described elsewhere [28]. Conductivity experiments with the complexes studied (in 1 mmol dm^{-3} ethanolic solution) were carried out in an Elmetron CC-41 (specific conductivity = 21μS/cm at 298 K).

Table 2
Cytotoxic activity of ligands **2** and **2a** and their metal complexes **4a**, **b**, **5a**, **b**, **6a**, **8a**, **b**, **9b**, cisplatin and carboplatin to HL-60, NALM-6 and WM-115 cells from at least 3 experiments.

Compound	Cytotoxicity (μM)[a]		
	HL-60	NALM-6	WM-115
2a	> 1000	74.0 ± 12.9	55.1 ± 8.2
2b	228.6 ± 46.9	183.4 ± 33.2	439.8 ± 50.9
4a	332.2 ± 30.0	49.7 ± 5.4	66.8 ± 2.3
4b	52.5 ± 2.8	59.8 ± 4.8	63.8 ± 4.8
5a	230.3 ± 35.7	77.2 ± 6.2	72.7 ± 3.9
5b	71.9 ± 6.6	117.3 ± 7.3	148.0 ± 37.1
6a	51.9 ± 3.4	32.6 ± 2.9	**17.4 ± 3.0**
8a	8.31 ± 0.67	12.04 ± 2.35	**8.2 ± 0.2**
8b	47.6 ± 5.5	55.2 ± 4.5	50.0 ± 7.6
9b	52.2 ± 2.8	57.4 ± 7.3	54.8 ± 4.6
Cisplatin	0.8 ± 0.1	0.7 ± 0.3	18.2 ± 4.3
Carboplatin	4.3 ± 1.3	0.7 ± 0.2	422.2 ± 50.2

[a] IC_{50}-concentration of a tested compound required to reduce the fraction of surviving cells to 50% of that observed in the control, non-treated cells. Mean values of IC_{50} (in μM) ±S.D. from 2 to 4 experiments, each performed five times, are presented.

4.1.1. Synthesis of the ligands

*4.1.1.1. 1-Benzothiazol-2-yl-5-(2-hydroxyphenyl)-3-methyl-1H-pyrazole-4-carboxylic acid methyl ester (**2a**).* 2-Hydrazinobenzothiazole (826 mg, 5 mmol) and *p*-toluensulfonic acid as catalyst were added at room temperature to a solution of 2-methyl-4-oxo-4*H*-chromene-3-carboxylic acid methyl ester **1** (1.09 g, 5 mmol) in EtOH (20 mL). The mixture was refluxed for 4 h. The solid white product was filtered off, washed with ethanol (2 mL) and dried. Yield: 1.61 g (50%), mp: 235–237 °C. IR(KBr) ν (cm^{-1}): 3333(C–OH), 3063(C–CH_3), 1718(C=O), 1617, 1570, 1522(C=C, phenyl), 1109 (O–CH_3). ^{1}H NMR($CDCl_3$) δ (ppm): 2.59(s, 3H, –CH_3), 3.69(s, 3H, –OCH_3), 6.98–7.79(m, 8H, ArH). ^{13}C NMR($CDCl_3$) δ (ppm): 14.65 (–CH_3), 51.85(O–CH_3), 115.83, 118.44; 119.34; 121.15; 121.46; 123.20; 126.77; 131.99; 132.44; 133.55; 144.57; 149.66; 154.09; 154.98; 159.48; 163.93 (C=O). MS-EI (*m*/*z*): 365 (65%); 333 (100%). Anal. found: C, 62.27; H, 3.80; N, 11.38. Calc for $C_{19}H_{15}N_3O_3S$ (365.397): C, 62.45; H, 4.14; N, 11.50.

*4.1.1.2. 1-(6-Chloropyridazin-3-yl)-5-(2-hydroxyphenyl)-3-methyl-1H-pyrazole-4-carboxylic acid methyl ester (**2b**).* 3-Chloro-6-hydrazinopyridazine (360 mg, 2.5 mmol) and *p*-toluensulfonic acid as catalyst were added at room temperature to a solution of 2-methyl-4-oxo-4*H*-chromene-3-carboxylic acid methyl ester **1** (550 mg, 2.5 mmol) in methanol (20 mL). The mixture was refluxed for 2 h. The solid white product was filtered off, washed with methanol (0.5 mL) and dried. Yield: 554 mg (64%), mp: 147–148 °C. IR(KBr) ν (cm^{-1}): 3416 (C–OH), 1706 (C=O), 1690 (C=N), 1613, 1577, 1476 (C=C, phenyl), 1094 (C–O–C). ^{1}H NMR($CDCl_3$), δ (ppm): 2.59(s, 3H, –CH_3), 3.70 (s, 3H, –OCH_3), 6.89–7.36 (m, 4H, ArH), 7.58 (d, 1H, ≡CH, $^3J_{HH}$ = 9.125 Hz), 7.80 (d, 1H, ≡CH, $^3J_{HH}$ = 9.125 Hz). ^{13}C NMR ($CDCl_3$), δ (ppm): 14.68(–CH_3), 51.93(O–CH_3), 115.48, 118.61; 118.96; 121.01; 125.31; 130.67; 131.71; 144.64; 153.88; 154.54; 154.59; 155.76; 164.31 (C=O). MS-EI (*m*/*z*): 344 (3.54%); 327 (100%); 311 (35.39%). Anal. found: C, 55.17; H, 3.28; N, 16.19. Calc for $C_{16}H_{13}N_4O_3Cl$ (344.745): C, 55.74; H, 3.80; N, 16.25.

*4.1.1.3. 1-Benzothiazol-2-yl-3-methyl-1H-chromeno[4,3-c]pyrazol-4-one (**3a**).* Triethylamine (100 mg, 0.14 mL, 1 mmol) was added at room temperature to a suspension of 1-benzothiazol-2-yl-5-(2-hydroxyphenyl)-3-methyl-1*H*-pyrazole-4-carboxylic acid methyl ester (**2a**) (365 mg, 1 mmol) in EtOH (10 ml). The mixture was refluxed for 1.5 h and cooled at 4 °C for 24 h. The solid white product was filtered off and dried. Yield: 250.0 mg (75%), mp: 277–279 °C. IR(KBr) ν (cm^{-1}): 3069(C–CH_3), 1736(C=O), 1609, 1580, 1556(C=C, chromene), 1134(C–O–C). MS-DEI (*m*/*z*): 333 (100%). Anal. found: C, 64.89; H, 3.37; N, 12.59. Calc for $C_{18}H_{11}N_3O_3S$ (333.355): C, 64.85; H, 3.33; N, 12.61.

*4.1.1.4. 1-(6-Chloropyridazin-3-yl)-3-methyl-1H-chromeno[4,3-c]pyrazol-4-one (**3b**).* Triethylamine (100 mg, 0.14 mL, 1 mmol) was added at room temperature to a suspension of 1-(6-chloropyridazin-3-yl)-5-(2-hydroxyphenyl)-3-methyl-1*H*-pyrazole-4-carboxylic acid methyl ester (**2b**) (344.7 mg, 1 mmol) in EtOH (10 ml). The mixture was refluxed for 1 h. The solid white product was filtered off and dried. Yield: 200.0 mg (64%), mp: 246–247 °C. IR (KBr) ν (cm^{-1}): 3069 (C–CH_3), 1742 (C=O), 1612 (C=N), 1562, 1511, 1450 (C=C, phenyl), 1053 (C–O–C). ^{1}H NMR (DMSO), δ (ppm): 2.60 (s, 3H, C–CH_3); 7.26–7.75 (m, 4H, ArH); 8.34 (d, 2H, –CH=CH–). MS-EI (*m*/*z*): 311 (100%). Anal. found: C, 57.20; H, 2.39; N, 17.92. Calc for $C_{15}H_9N_4O_2Cl$ (312.716): C, 57.61; H, 2.90; N, 17.92.

4.1.2. Synthesis of the complexes

*4.1.2.1. Pt(II) complex **4a**.* The aqueous solution of $K_2[PtCl_4]$ (82.9 mg, 0.1997 mmol, in 2 mL) was slowly added dropwise to the

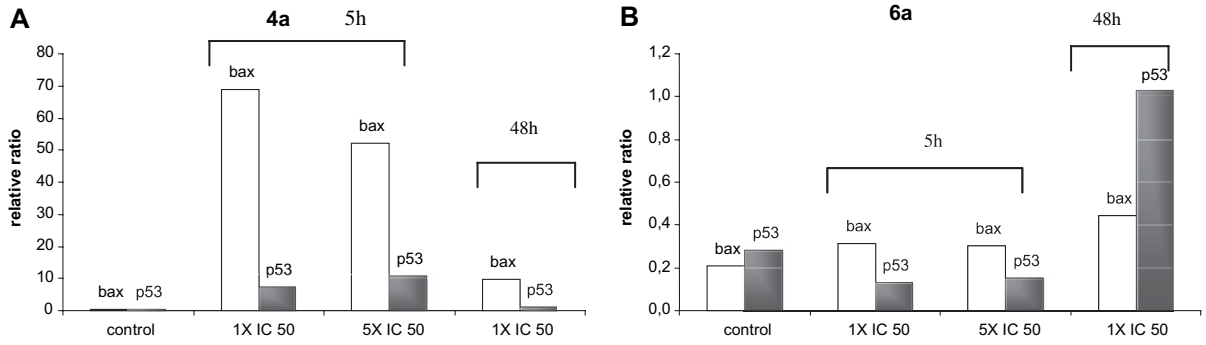


Fig. 4. Effect of **4a** complex (A) and **6a** complex (B) on *BAX* (white box) and *P53* (black box) gene expression at the level of mRNA in WM-115 melanoma cells as measured by real-time PCR. Melanoma cells were exposed to **4a** and **6a** at concentration equal to IC_{50} and $5 \times IC_{50}$ for 5 and 48 h.

methanolic solution of the ligand **2a** (73 mg, 0.1997 mmol, in 10 mL). The mixture was stirred at room temperature for 1 h. The yellow solid that precipitated after 24 h was filtered off, washed with water and diethyl ether and dried to yield complex **4a**. Yield: 68.11 mg (54%), dec.: 380 °C. IR (KBr) ν (cm^{-1}): 3397 (C—OH), 2951 (C—CH_3), 1720 (C═O), 1617, 1571, 1521 (C═C, phenyl), 1108 (O—CH_3), 462 (Pt—N). ^{1}HNMR (DMSO-d_6), δ (ppm): 2.50 (s, 3H, —CH_3), 3.59 (s, 3H, —OCH_3), 6.82—8.05 (m, 8H, ArH), 9.64 (s,1H,OH). MS-FAB (*m*/*z*): 631 (0.13%), Pt(L)Cl_2; 595 (0.7%), $[Pt(L)Cl]^+$; 559 (0.1%), $[Pt(L)]^{2+}$; 366 (40%), L. Anal. found: C, 36.54; H, 2.70; N, 6.60. Calc. for $C_{19}H_{15}N_3O_3SPtCl_2$ (631.37): C, 36.14; H, 2.39; N, 6.65.

4.1.2.2. Pt(II) complex ***4b***. The aqueous solution of $K_2[PtCl_4]$ (62.3 mg, 0.15 mmol, in 2 mL) was slowly added dropwise to the methanolic solution of the ligand **2b** (51.7 mg, 0.15 mmol, in 10 mL). The mixture was stirred at room temperature for 24 h. The orange solid that precipitated after 24 h was filtered off, washed with water and diethyl ether and dried to yield complex **4b**. Yield: 68 mg (54%). IR (KBr) ν (cm^{-1}): 3402(C—OH), 2952 (C—CH_3), 1722 (C═O), 1613, 1573, 1439 (C═N), 1101 (C—O—C). ^{1}HNMR (DMSO-d_6), δ (ppm): 2.95 (s, 3H, —CH_3), 3.57 (s, 3H, —OCH_3), 6.70—8.12(m, 8H, ArH), 9.54 (s, 1H, OH), 8.37 (d, 1H, ≡CH, $^3J_{HH} = 9$ Hz), 8.47 (d, 1H, ≡CH, $^3J_{HH} = 9$ Hz). MS-FAB (*m*/*z*): 609 (85%), Pt(L)Cl_2; 573 (100%), $[Pt(L)Cl]^+$. Anal. found: C, 31.95; H, 1.97; N, 9.50. Calc. for $C_{16}H_{13}N_4O_3Cl_3Pt$ (610.731): C, 31.46; H, 2.15; N, 9.17.

4.1.2.3. Pd(II) complex ***5a***. A solution of $(C_6H_5CN)_2PdCl_2$ (76.6 mg, 0.1997 mmol, in 3 mL chloroform + 3 mL methanol) was slowly added dropwise to a chloroform solution of ligand **2a** (73.0 mg, 0.1997 mmol, in 5 mL). The mixture was stirred at room temperature. After 24 h the resulting yellow crystals were filtered off, washed with water and diethyl ether and dried. Yield: 57.5 mg (53%), dec.: 370 °C. IR (KBr) ν (cm^{-1}): 3406 (C—OH), 2957 (C—CH_3), 1723 (C═O), 1618, 1516, 1491 (C═C, phenyl), 1103 (C—O—C), 426 (Pd—N). ^{1}HNMR (DMSO-d_6), δ (ppm): 2.50 (s, 3H, —CH_3), 3.59 (s, 3H, —OCH_3), 6.82—8.02(m, 8H, ArH), 9.64 (s, 1H, OH). MS-FAB (*m*/*z*): 542 (0.05%), Pd(L)Cl_2; 507 (0.18%), $[Pd(L)Cl]^+$; 470 (0.26%), $[Pd(L)]^{2+}$. Anal. found: C, 40.27; H, 2.37; N, 7.20. Calc. for $C_{19}H_{15}N_3O_3SPdCl_2 + H_2O$ (560.74): C, 40.69; H, 3.05; N, 7.49.

4.1.2.4. Pd(II) complex ***5b***. A solution of $(C_6H_5CN)_2PdCl_2$ (57.5 mg, 0.15 mmol, in 3 mL chloroform + 3 mL methanol) was slowly added dropwise to a solution of ligand **2b** (51.7 mg, 0.15 mmol, in 10 mL chloroform + 10 mL methanol). The mixture was stirred at room temperature. After 24 h the resulting brown solid was filtered off, washed with water and diethyl ether and dried. Yield: 44.9 mg (55%). IR (KBr) ν (cm^{-1}): 3387 (C—OH), 2923 (C—CH_3), 1727 (C═O), 1617, 1574, 1447 (C═N), 1100 (C—O—C), 550 (Pd—N). MS-FAB (*m*/*z*): 450 (0.05%), $[Pd(L)]^{2+}$; 345 (50%), L. Anal. found: C, 35.84; H, 2.24; N, 10.25. Calc. for $C_{16}H_{13}N_4O_3\ Cl_3Pd + H_2O$ (522.071): C, 35.58; H, 2.43; N, 10.37.

4.1.2.5. Cu(II) complex ***6a***. A methanolic solution of $CuCl_2 \times 2H_2O$ (34.0 mg, 0.1997 mmol, in 1 mL) was slowly added dropwise to a solution of ligand **2a** in ethyl acetate (73.0 mg, 0.1997 mmol, in 10 mL). The mixture was stirred at room temperature for 24 h. The resulting yellow solid was filtered off and dried. Yield: 84.0 mg (84%), dec.: 230 °C. IR (KBr) ν (cm^{-1}): 3271 (C—OH), 2976 (C—CH_3), 1719 (C═O), 1612, 1591, 1561 (C═C, phenyl), 1110 (O—CH_3), 536 (Cu—N). MS-FAB (*m*/*z*): 463 (3.4%), $[Cu(L)Cl]^+$; 428 (5.0%), $[Cu(L)]^{2+}$. Anal. found: C, 45.24; H, 3.13; N, 8.22;. Calc. for $C_{19}H_{15}N_3O_3SCuCl_2$ (499.84): C, 45.65; H, 3.02; N, 8.41.

4.1.2.6. Cu(II) complex ***7a***. Recrystallization done by the diffusion of diethyl ether into a DMF solution of 6a gave complex 7. dec.: 260 °C. IR (KBr) ν (cm^{-1}): 3204 (C—OH), 2953 (C—CH_3), 1719 (C═O), 1643 (C═O, DMF), 1576, 1519 (C═C, phenyl), 1189 (C—O—C), 1096 (O—CH_3), 537 (Cu—N). MS-FAB (*m*/*z*): 590 (3%), $CuLCl_2 + DMF + H_2O$; 428 (100%), $[Cu(L)]^{2+}$. Anal. found: C, 44.63; H, 3.56; N, 9.47;. Calc. for $C_{22}H_{122}N_4O_4SCuCl_2 + H_2O$(590.962): C, 44.70; H, 4.09; N, 9.48.

4.1.2.7. Cu(II) complex ***8a***. A methanolic solution of Cu$(ClO_4)_2 \times 6H_2O$ (37 mg, 0.1 mmol, in 2 mL) was slowly added dropwise to a solution of ligand **2a** in ethyl acetate (73 mg, 0.2 mmol, in 10 mL). The mixture was stirred at room temperature for 24 h. The resulting green solid was obtained by the diffusion of diethyl ether into the mixture, then filtered off, washed with diethyl ether and dried. Yield: 57 mg (57%), mp: 235—239 °C. IR (KBr) ν (cm^{-1}): 3371 (C—OH), 2956 (C—CH_3), 1715 (C═O), 1615 (C═N), 1593, 1568, 1518 (C═C), 1117 (ClO_4^-), 1052 (C—O—C), 621 (ClO_4^-). MS-FAB (*m*/*z*): 794 (45%), $[Cu(L)_2]^{2+}$; 429 (100%), $[Cu(L)]^{2+}$. Anal. found: C, 44.04; H, 3.23; N, 7.73; Calc. for $C_{38}H_{30}N_6O_{14}\ S_2Cl_2Cu$ (993.238): C, 45.39; H, 3.04; N, 8.46.

4.1.2.8. Cu(II) complex ***8b***. A methanolic solution of Cu$(ClO_4)_2 \times 6H_2O$ (27.8 mg, 0.075 mmol, in 1 mL) was slowly added dropwise to a solution of ligand **2b** in ethyl acetate (51.7 mg, 0.15 mmol, in 10 mL). The mixture was stirred at room temperature for 48 h. The resulting green solid was filtered off and dried. Yield: 26.3 mg (48%), dec.: 221 °C. IR (KBr) ν (cm^{-1}): 3407 (C—OH), 2957 (C—CH_3), 1726 (C═O), 1696 (C═N), 1613, 1575, 1474 (C═C), 1106 (ClO_4^-), 1052 (C—O—C), 623 (ClO_4^-). MS-FAB (*m*/*z*): 753 (45%), $[Cu(L)_2]^{2+}$; 407 (100%), $[Cu(L)]^{2+}$. Anal. found: C, 40.03; H, 2.76; N, 11.28;. Calc. for $C_{32}H_{26}N_8O_{14}Cl_4Cu$ (951.939): C, 40.37; H, 2.75; N, 11.77.

*4.1.2.9. Cu(II) complex **9b**.* A methanolic solution of $CuCl_2 \times 2H_2O$ (25.6 mg, 0.15 mmol, in 3 mL) was slowly added dropwise to a solution of ligand **2b** in ethyl acetate (51.7 mg, 0.15 mmol, in 10 mL). The mixture was stirred at room temperature for 1.5 h. The resulting yellow solid was filtered off and dried. Yield: 43 mg (69%), dec.: 212 °C. IR (KBr) ν (cm^{-1}): 3056 (C–OH), 2915 (C–CH_3) 1717 (C=O), 1591 (C=N), 1569, 1476 (C=C), 1104 (C–O–C), 546 (Cu–N). MS-FAB (*m*/*z*): 753 (45%), $[Cu(L)_2]^{2+}$; 407 (100%), $[Cu(L)]^{2+}$. Anal. found: C, 46.26; H, 2.59; N, 13.55;. Calc. for $C_{32}H_{26}N_8O_6Cl_4Cu$ (823.939): C, 46.64; H, 3.18; N, 13.60.

4.2. Biological assays

4.2.1. Cell cultures

Human skin melanoma WM-115 cells as well as human leukemia promyelocytic HL-60 and lymphoblastic NALM-6 cell lines were used. Leukemia cells were cultured in RPMI 1640 medium (Invitrogen, U.K.) supplemented with 10% fetal bovine serum and antibiotics (100 μg/ml streptomycin and 100 U/ml penicillin). For melanoma WM-115 cells Dulbecco's minimal essential medium (DMEM) instead of RPMI 1640 was used. Cells were grown at 37 °C in a humidified atmosphere of 5% CO_2 in air.

4.2.2. Cytotoxicity assay by MTT

Cytotoxicity of ligands **2a** and **2b**, their complexes **4a**, **b**, **5a**, **b**, **6a**, **8a**, **b**, **9b**, carboplatin and cisplatin was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma, USA] assay as described [29]. After 46 h of incubation with drugs, the cells were briefly treated with the MTT reagent and incubation was continued for 2 h. MTT – formazan crystals were dissolved in 20% SDS and 50% DMF at pH 4.7 and absorbance was read at 562 and 630 nm on an ELISA – plate reader (ELX 800, Bio-Tek, USA). The values of IC_{50} (the concentration of the test compound required to reduce the cells survival fraction to 50% of the control) were calculated from concentration-response curves and used as a measure of cellar sensitivity to a given treatment. Ligands, metal complexes, carboplatin and cisplatin were tested for their cytotoxicity in a final concentration of 10^{-7}–10^{-3} M. As a control, cultured cells were grown in the absence of drugs. Data points represent means from at least 3 experiments each performed at 5 repeats ± S.D.

4.2.3. Analysis of BAX and P53 gene expression in melanoma WM 115 cells exposed to drugs

Melanoma WM-115 cells treated with **4a** and **6a** (in two concentrations: 1 × IC_{50} and 5 × IC_{50}) were used for analysis of *BAX* and *P53* gene expression by real-time PCR. After 5 and 48 h of incubation with the tested compound, the cells were spun down, washed twice with cold 0.01 M phosphate buffer (pH 7.0) containing 0.9% NaCl. Cell pellets were than used for RNA extraction.

4.2.3.1. RNA extraction and cDNA synthesis. Total RNA was isolated by Total RNA prep plus Minicolumn Kit (A&A Biotechnology, Poland) based on a method described earlier [30]. RNA concentration and its quality were determined by the measurement of absorbance at 260 and 280 nm.

To obtain cDNA, reverse transcription reaction was done using Enhanced Avian HS RT-PCR-100 Kit (Sigma, Germany) according to the manufacturer's instruction using 400 ng of total RNA per reaction.

4.2.3.2. Real-time PCR analysis of BAX and P53 genes. In all cases, before the quantitative analysis of gene expression by real-time PCR reaction, the parameters were checked using qualitative PCR. PCR reaction was conducted according to protocol "AccuTaq™ LA DNA Polymerase Kit" (Sigma, Germany). The reaction mixture for PCR amplification consisted of a cDNA template, 0.5 μM of each primer, 10 × AccuTaq Buffer, 0.5 U of AccuTaq LA DNA Polymerase Mix, 0.2 mM each dNTP, water to a final volume of 20 μl. Negative control was included in each experiment (sample without a cDNA template). The primers sequences for both genes were planned by using software Primer3: WWW primer tool (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). The *BAX* primer set 5′-CAGCTCTGAGCAGATCATGAAGACA-3′ (forward) and 5′-GCCCAT-CTTCTTCCAGATGGTGAG-3′ (reverse), and the *P53* primer set 5′-CACATGACGGAGGTTGTGAG-3′ (forward) and 5′- CATAGGGCAC-CACCACACTA-3′ (reverse) were used.

Real-time PCR reactions were done using MX3005P™ System (STRATAGEN). The *BAX* gene and a housekeeping gene, β-actin (ACTB) were amplified in parallel for each sample during the same PCR. β-Actin was utilized as an internal positive control and as a normalizer for expression data correction. For the *BAX* and *P53* the same primer sets as above were used and for the *β-actin* 5′-GTGGGGCGCCCCAGGCACCA-3′ (forward) and 5′-CTCCTTAATGT-CACGCACGATTTC-3′ (reverse). For each PCR run, a reaction mixture was prepared consisting of 12.5 μl SYBR® Green JumpStart™ Taq ReadyMix™ (Sigma), 0.5 μl forward primer (final concentration 0.2 μM), 0.5 μl reverse primer, 9 μl nuclease-free water and 2.5 μl template cDNA. The thermal cycling conditions comprised an initial denaturation step at 95 °C for 3 min, 35 cycles at 94 °C for 15 s, 59 °C(*BAX*)/57 °C(*P53*) for 15 s and 72 °C for 45 s and a final extension step at 72 °C for 3 min. After reaction, a melting curve was performed to confirm reaction specificity. Experiments for all samples were performed in triplicate. The relative level of *BAX* and *P53* expression was calculated as described previously [31].

4.3. X-ray structure determinations of **2a**, **6a** and **7a**

X-ray data were collected at 200 K with MoKα radiation ($\lambda = 0.71073$ Å) with a Nonius KappaCCD diffractometer equipped with a rotating anode. The structures were solved by direct methods [32] and refined with SHELXL-97 by full-matrix least-squares on F^2 [33]. All non-hydrogen atoms were refined anisotropically. The crystal data and X-ray details are given in Table 1. Further details are available from the Crystallographic Data Centre under the depository numbers CCDC 727843 (**2a**), 727845 (**6a**) and 727844 (**7a**). Copies of the data can be obtained free of charge upon application to CCDC, 12, Union Road, Cambridge CB2 1EZ, U.K., E-mail: deposit@ccdc.cam.ac.uk.

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References

[1] E. Budzisz, I.-P. Lorenz, P. Mayer, P. Paneth, L. Szatkowski, U. Krajewska, M. Rozalski, M. Miernicka, N J. Chem. 32 (2008) 2238–2244.
[2] E. Budzisz, M. Miernicka, I.-P. Lorenz, P. Mayer, U. Krajewska, M. Rozalski, Polyhedron 28 (2009) 637–645.
[3] M. Grazul, E. Budzisz, Coord. Chem. Rev. 253 (2009) 2588–2598.
[4] X. Wang, Z. Guo, Anti-Cancer Agents Med. Chem. 7 (2007) 19–34.
[5] A.E. Liberta, D.X. West, BioMetals 5 (1992) 121–126.
[6] N.K. Singh, S.T. Singh, N. Singh, A. Shriviastav, BioMetals 16 (2003) 471–477.
[7] C. Rothenburger, M. Galanski, V.B. Arion, H. Görls, W. Weigand, B.K. Keppler, Eur. J. Inorg. Chem. 18 (2006) 3746–3752.
[8] J. Kasparkova, O. Novakova, V. Marini, Y. Najajreh, D. Gibson, J.M. Perez, V. Brabec, J. Biol. Chem. 278 (2003) 47516–47525.

[9] D. Kovala-Demertzi, A. Papageorgiou, L. Papathanasis, A. Alexandratos, P. Dalezis, J.R. Miller, M.A. Demertzis, Eur. J. Med. Chem. 44 (2009) 1296–1302.
[10] D. Kovala-Demertzi, M.A. Demertzis, E. Filiou, A.A. Pantazaki, P.N. Yaday, J. R. Miller, Y. Zheng, D.A. Kariakidis, Biometals 16 (2003) 411–418.
[11] J. Kasparkova, V. Marini, Y. Najajreh, D. Gibson, V. Brabec, Biochemistry 42 (2003) 6321–6332.
[12] F. Saczewski, E. Dziemidowicz-Borys, P.J. Bednarski, M. Gdaniec, Arch. Pharm. Chem. Life Sci. 340 (2007) 333–338.
[13] R. Mukherjee, Coord. Chem. Rev. 203 (2000) 151–218.
[14] T.N. Sorrell, Tetrahedron 45 (1989) 3–68.
[15] M. Viciano-Chumillas, S. Tanase, G. Aromí, J.M.M. Smits, R. de Gelder, X. Solans, E. Bouwman, J. Reedijk, Eur. J. Inorg. Chem. 18 (2007) 2635–2640.
[16] G.Z. Rassidakis, L.J. Medeiros, T.J. McDonnell, S. Viviani, V. Bonfante, G. Nadali, T.P. Vassilakopoulos, R. Giardini, M. Chilosi, C. Kittas, A.M. Gianni, G. Bonadonna, G. Pizzolo, G.A. Pangalis, F. Cabanillas, A.H. Sarris, Clin. Cancer Res. 8 (2002) 488–493.
[17] S. Haupt, M. Berger, Z. Goldberg, Y. Haupt, J. Cell Sci. 116 (2003) 4077–4085.
[18] F. Kayaselcuk, T.Z. Nursal, A. Polat, T. Noyan, S. Yildirim, A. Tarim, G. Seydioglu, J. Exp. Clin. Cancer Res. 23 (2004) 105–112.
[19] M. Gielen, E.R.T. Tiekink, in: Metallotherapeutic Drugs and Metal-based Diagnostic Agents. The Use of Metals in Medicine, Wiley, Chichester, UK, 2005, p. 39.
[20] A. Kufelnicki, M. Wozniczka, L. Checinska, M. Miernicka, B. Budzisz, Polyhedron 26 (2007) 2589–2596.
[21] M. Miernicka, A. Szulawska, M. Czyz, I.-P. Lorenz, P. Mayer, B. Karwowski, E. Budzisz, J. Inorg. Biochem. 102 (2008) 157–165.
[22] K. Kostka, Chem. Anal. 14 (1969) 1145–1160.
[23] S. Kerrison, P.J. Sadler, Chem. Commun. 23 (1977) 861–863.
[24] B. Stuart, Infrared Spectroscopy – Fundamentals and Applications. Wiley, Chichester, UK, 2004, pp. 45–111.
[25] R.S. Macomber, A Complete Introduction to Modern NMR Spectroscopy. Wiley, New York, USA, 1998, pp. 68–87.
[26] R.S. Macomber, A Complete Introduction to Modern NMR Spectroscopy. Wiley, New York, USA, 1998, pp. 88–103.
[27] J.T. Wrobel, Preparatyka i elementy syntezy organicznej. PWN, Warsaw, Poland, 1983, 9–52.
[28] G.M. Coppola, R.W. Dodsworth, Synthesis 75 (1981) 523–524.
[29] E. Budzisz, U. Krajewska, M. Rozalski, Pol. J. Pharmacol. 56 (2004) 473–478.
[30] P. Chomczyński, N. Sacchi, Anal. Biochem. 162 (1987) 156–159.
[31] K.J. Livak, T.D. Schmittgen, Methods 25 (2001) 402–408.
[32] A. Altomare, M.C. Burla, M. Camalli, G.L. Cascarano, C. Giacovazzo, A. Guagliardi, A.G.G. Moliterni, G. Polidori, R. Spagna, J. Appl. Crystallogr. 32 (1999) 115–119.
[33] G.M. Sheldrick. SHELXL-97 97-2 Version. University of Göttingen.