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# Synthesis and X-ray structure of platinum(II), palladium(II) and copper(II) complexes with pyridine–pyrazole ligands: Influence of ligands' structure on cytotoxic activity

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ABSTRACT

The new pyrazole ligand 5-(2-hydroxyphenyl)-3-methyl-1-(2-pyridylo)-1*H*-pyrazole-4-phosphonic acid dimethyl ester (**2a**) has been used to obtain a series of platinum(II), palladium(II) and copper(II) complexes (**3a**–**7a**) as potential anticancer compounds. The molecular structures of the platinum(II) and copper(II) complexes **3a** and **6a** have been determined by X-ray crystallography. The cytotoxicity of the phosphonic ligand **2a** and its carboxylic analog **2b** as well as their complexes has been evaluated on leukemia and melanoma cell lines. Copper(II) complexes were found to be more efficient in the induction of melanoma cell death than the platinum(II) or palladium(II) complexes. Cytotoxic effectiveness of compound **7b** against melanoma WM-115 cells was two times better than that of cisplatin. The reaction of compound **5b** with 9-methylguanine has been studied.



## 1. Introduction

New, efficient therapy for cancer is the challenge for medicine of the XXI century. The clinical success of cisplatin and carboplatin as antitumor agents constitutes the most impressive contribution to the use of metals in medicine [1]. The severe problems associated with these anticancer metal-based drugs include serious toxicity and side-effects, and major difficulties concerning tumor cells resistance. New potent and selective antitumor drugs are urgently needed.

A new series of antitumor *cis*- and *trans*-platinum(II) complexes that have nonplanar heterocyclic amine ligands such as piperidine, piperazine and 4-picoline have been described [2,3]. Some of these complexes, especially the *trans* analogs, could overcome multifactorial cisplatin resistance in human ovarian cell lines. The possibility to overcome cisplatin resistance could be related to the ability of these compounds to form DNA lesions that are different from those formed by cisplatin. While cisplatin interacts with double-helical DNA forming preferentially 1,2-GG or AG intrastrand cross-links, the trans complexes cannot form in double-helical DNA intrastrand cross-links between adjacent purines. Trans complexes can cross-link two bases on the same strand only if they are separated by at least one intervening base, forming mostly 1,3-GNG cross-links, where N is adenine, cytosine or thymine. Importantly, these cross-links are stable in single-stranded DNA, while in double-stranded DNA some of these cross-links readily isomerize to the interstrand cross-links [4–7].

Novel metal-based complexes containing metal such as palladium, copper, ruthenium, gold and rhodium have been reported with promising chemotherapeutic potential and different mechanisms of action in comparison with the platinum based drugs [8–10].

Synthesis and structures of palladium(II) complexes containing pyrazole and thiocyanate ligands have been documented in the literature [11]. Thiocyanate complexes of palladium(II) have been applied in analytical determinations [12] and in chemotherapy [13].

Copper(II) plays an important biological role in all living systems as an essential trace element [14]. The copper(II) complexes with organic ligands have been used as analgesic, antipyretic, antiinflammatory and platelet antiaggregating agents. Recently, several reports have appeared in the literature describing the anticancer activity of copper(II) derivatives of many classes of nitrogen donors including thiosemicarbazone and imidazole [15]. There are also examples of copper complexes of ligands containing

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α-diimino (N=C–C=N) moiety such as phenanthroline that can induce apoptosis [16] and 2-(4′-thiazolyl)benzimidazole that have antimicrobial activity [17]. Various anticancer copper(II) complexes occurred to alter the cell cycle and decrease the telomerase activity [18–21]. A wide variety of biological activities of copper(II) complexes can also be exemplified by recent finding of their antiamoebic activity against *Entamoeba histolytica* [22].

The interest in dinuclear copper(II) complexes with nitrogen-containing ligands is caused by their resemblance to the active sites of several copper-containing proteins. Besides that, the investigation of the magnetic properties of such complexes provides more insight into the relationship between their structural features and the strength of magnetic exchange interactions between the metal ions [23–29].

The introduction of the aromatic *N*-containing ligands as pyrazole, pyridine, imidazole and 1,10-phenanthroline and their derivatives whose donor properties are similar to the purine and pyrimidine bases to antitumor agents is drawing attention [30]. Complexes of pyrazole-type ligands have been used in catalysis [31,32], chemotherapy [33,34], electrochemical reduction of carbon dioxide [35], as metallomesogens [36,37] and as models of active sites in metalloenzymes [38].

In our previous papers we described the synthesis of 5-(2-hydroxybenzoyl)-3-methyl-1-(2-pyridinyl)pyrazol-4-carboxylic acid methyl ester **2b** and its complexes with platinum(II) (**3b**), palladium(II) (**4b**) and copper(II) (**5b** and **6b**) [39,40]. The cytotoxicity assay of the ligand **2b** and its complexes **3b**–**5b** were performed on L1210 and K562 leukemia cell lines. The complexes showed lower cytotoxicity than cisplatin and the platinum(II) (**3b**) and copper(II) (**5b**) complexes were found to be more efficient in the induction of leukemia cell death than the palladium(II) complex **4b**. Our investigation indicated that the antiproliferating activity of the platinum(II) (**3b**) and copper(II) (**5b**) complexes was partly due to the modulation of cellular differentiation. However, all these complexes **3b**–**5b** were not strong cellular differentiation inducers in comparison to cisplatin [40].

Here, we present the synthesis of new chelating ligand 5-(2-hydroxybenzoyl)-3-methyl-1-(2-pyridinyl)pyrazol-4-phosphonic acid dimethyl ester **2a** and its solid complexes with platinum(II), palladium (II) and copper(II) (**3a**–**7a**), the new copper(II) complex **7b** with carboxylic ligand **2b** as well as X-ray structures of the phosphonic platinum(II) and copper(II) complexes **3a** and **6a**, cytotoxic studies of both ligands **2a** and **2b** and their complexes performed on leukemia and melanoma cell lines and the reaction of copper(II) complex **5b** with 9-methylguanine (9MeG).

Synthesis of dimethyl 2-methyl-4-oxo-4*H*-chromen-3-yl-phosphonate (**1a**) [41], 2-methyl-4-oxo-4*H*-chromene-3-carboxylic acid methyl ester (**1b**) [42], 5-(2-hydroxyphenyl)-3-methyl-1-(2-pyridylo)-1*H*-pyrazole-4-carboxylic acid methyl ester (**2b**) [39] and its platinum(II) (**3b**), palladium(II) (**4b**) [40] and copper(II) (**5b** and **6b**) [39,40] complexes were described elsewhere.

## 2. Experimental

### 2.1. Materials

All substances were used without further purification. Potassium tetrachloridoplatinate(II), potassium tetrachloridopalladate(II), copper(II) chloride dihydrate and copper(II) 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 (acetone, diethyl ether, dimethylformamide, ethanol, ethyl acetate and methanol) were reagent grade or better and were dried according to standard protocols [43]. The melting points were determined using an Electrothermal 1A9100 apparatus and they are uncorrected. The IR spectra were recorded on a Pye-Unicam 200G Spectrophotometer in KBr and CsI. 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.

Dimethyl 2-methyl-4-oxo-4*H*-chromen-3-yl-phosphonate (**1a**) [41], 2-methyl-4-oxo-4*H*-chromene-3-carboxylic acid methyl ester (**1b**) [42], 5-(2-hydroxyphenyl)-3-methyl-1-(2-pyridylo)-1*H*-pyrazole-4-carboxylic acid methyl ester (**2b**) [39] and its platinum(II) (**3b**), palladium(II) (**4b**) [40] and copper(II) (**5b** and **6b**) [39,40] complexes were prepared as described elsewhere.

### 2.2. Synthesis of the ligand

#### 2.2.1. 5-(2-Hydroxybenzoyl)-3-methyl-1-(2-pyridinyl)-1H-pyrazol-4-phosphonic acid dimethyl ester (*2a*)

2-Hydrazinopyridin (109.1 mg, 1 mmol) in EtOH (5 ml) was added at room temperature to a solution of dimethyl 2-methyl-4-oxo-4*H*-chromen-3-yl-phosphonate **1a** (268.0 mg, 1 mmol) in EtOH (5 ml). The mixture was stirred for 24 h in room temperature. EtOH was evaporated under reduced pressure. Ethyl acetate was added to the oil product and it was left in 4 °C for 24 h. The solid white product was filtered off and dried. Yield: 179.0 mg (49.72%), mp: 101–105 °C. IR: $\nu_{max}/cm^{-1}$ 3170 (OH); 2940 (C–$CH_3$); 1607 (C=C, phenyl); 1228 (P=O); 1031 (O–$CH_3$); 807 (P–O) $cm^{-1}$. $^1$H NMR ($CDCl_3$): $\delta$ = 6.88–7.53 (m, 8H, aromat.); 2.56 (s, 3H, $CH_3$); 3.52–3.63 (dd, 6H, O–$CH_3$). $^{13}$C NMR ($CDCl_3$): $\delta$ = 14 (C–$CH_3$); 52.5 (O–$CH_3$); 107.3 (C–P); 154 (C–$CH_3$); 146 (C=C, pyrazole); 147.5 (C=N, pyridin); 151 (C–N); 155.5 (C–OH). $^{31}$P NMR ($CDCl_3$): $\delta$ = 17.0. MS *m/z*: 358.0. *Anal.* Calc. for $C_{17}H_{18}N_3O_4P$ (359.322): C, 56.82; H, 5.05; N, 11.69. Found: C, 56.59; H, 4.95; N, 11.32%.

### 2.3. Synthesis of the complexes

#### 2.3.1. Pt complex *3a*

The aqueous solution of $K_2[PtCl_4]$ (41.5 mg, 0.1 mmol, in 1 ml) was slowly added drop-wise to the methanolic solution of the ligand **2a** (36 mg, 0.1 mmol, in 5 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 **3a**. Yield: 24.0 mg (38.4%), td: 120 °C. IR: $\nu_{max}/cm^{-1}$ 3401 (OH); 2955 (C–$CH_3$); 1612 (C=C, phenyl); 1226 (P=O); 808 (P–O); 1028 (O–$CH_3$); 493 (Pt–N) $cm^{-1}$. $^1$H NMR (DMSO–$d_6$): $\delta$ = 6.67–7.53 (m, 8H, aromat.); 2.56 (s, 3H, $CH_3$); 3.38–3.50 (dd, 6H, O–$CH_3$). ESI MS(−): 624.1. *Anal.* Calc. for $C_{17}H_{18}N_3O_4PPtCl_2$ (625.306): C, 32.65; H, 2.90; N, 6.72. Found: C, 32.01; H, 2.43; N, 6.48%.

#### 2.3.2. Pd complex *4a*

An aqueous solution of $K_2[PdCl_4]$ (33.0 mg, 0.1 mmol, in 1 ml) was slowly added drop-wise to a methanolic solution of ligand **2a** (36.0 mg, 0.1 mmol, in 5 ml). The mixture was left with stirring at room temperature for 1 h. After 24 h the resulting yellow crystals were filtered off, washed with water, diethyl ether and dried. Yield: 41.0 mg (77.6%), td: 345 °C. IR: $\nu_{max}/cm^{-1}$ 3399 (OH); 2955 (C–$CH_3$); 1611 (C=C, phenyl); 1226 (P=O); 1028 (O–$CH_3$); 811 (P–O); 495 (Pd–N) $cm^{-1}$. $^1$H NMR (DMSO–$d_6$): $\delta$ = 6.60–7.60 (m, 8H, aromat.); 2.82 (s, 3H, $CH_3$); 3.44–3.53 (dd, 6H, O–$CH_3$). ESI MS(−): 535.9. *Anal.* Calc. for $C_{17}H_{18}N_3O_4PPdCl_2$ (536.648): C, 38.05; H, 3.38; N, 7.83. Found: C, 37.39; H, 3.57; N, 7.56%.

*2.3.3. Cu complex **5a***

A methanolic solution of $CuCl_2 \times 2H_2O$ (18.0 mg, 0.1 mmol, in 1 ml) was slowly added drop-wise to a solution of ligand **2a** in ethyl acetate (36.0 mg, 0.1 mmol, in 5 ml). The mixture was left with stirring at room temperature for 0.5 h. After 24 h the resulting green crystals were filtered off, washed with diethyl ether and dried. Yield: 39.0 mg (79.6%), td: 163.8 °C. IR: $\nu_{max}/cm^{-1}$ 3482 (OH); 2959 (C–$CH_3$); 1605 (C=C, phenyl); 1546 (C=N); 1239 (P=O); 1038 (O–$CH_3$); 799 (P–O); 508 (Cu–N) $cm^{-1}$. ESI MS(+): 457.0. *Anal.* Calc. for $C_{17}H_{18}N_3O_4PCuCl_2$ (493.774): C, 37.14; H, 3.42; N, 7.50; Cl, 15.99. Found: C, 40.75; H, 3.59; N, 8.09; Cl, 14.36%.

*2.3.4. Cu complex **6a***

Recrystallization done by the diffusion of diethyl ether (5 ml) into a DMF solution of **5a** (20.0 mg, 0.04 mmol, in 1 ml) gave a complex **6a**. Yield: 21.0 mg (91.3%), mp: 185 °C. IR: $\nu_{max}/cm^{-1}$ 3410, 3061 (OH); 2953 (C–$CH_3$); 1644 (C=O); 1600 (C=C, phenyl); 1233 (P=O); 1045 (O–$CH_3$); 797 (P–O); 419 (Cu–N) $cm^{-1}$. *Anal.* Calc. for $C_{20}H_{25}N_4O_5PCuCl_2$ (566.869): C, 42.38; H, 4.95; N, 9.88. Found: C, 41.59; H, 4.61; N, 9.74%.

*2.3.5. Cu complex **7a***

A methanolic solution of $Cu(ClO_4)_2 \times 6H_2O$ (26.5 mg, 0.07 mmol, in 2 ml) was slowly added drop-wise to a solution of ligand **2a** in ethyl acetate (51.3 mg, 0.14 mmol, in 5 ml). The mixture was refluxed 24 h. The resulting green crystals were filtered off, washed with diethyl ether and dried. Yield: 59.4 mg (84.9%), td: 285.4 °C. IR: $\nu_{max}/cm^{-1}$ 3371 (OH); 2956 (C–$CH_3$); 1614 (C=C, phenyl); 1230 (P=O); 1102 ($ClO_4^-$); 1033 (O–$CH_3$); 800 (P–O); 622 ($ClO_4^-$); 569 (Cu–N) $cm^{-1}$. ESI MS(+): 781.2. *Anal.* Calc. for $C_{34}H_{36}N_6O_{16}P_2CuCl_2$ (981.088): C, 41.62; H, 3.70; N, 8.57; P, 6.31; Cl, 7.23. Found: C, 41.32; H, 3.48; N, 8.24; P, 6.19; Cl, 7.21%.

*2.3.6. Cu complex **7b***

A methanolic solution of $Cu(ClO_4)_2 \times 6H_2O$ (30.9 mg, 0.08 mmol, in 2 ml) was slowly added drop-wise to a solution of ligand **2a** in ethyl acetate (51.5 mg, 0.16 mmol, in 5 ml). The mixture was refluxed 24 h. The resulting green crystals were filtered off, washed with diethyl ether and dried. Yield: 41.2 mg (56.1%), td: 268.8 °C. IR: $\nu_{max}/cm^{-1}$ 3383 (OH); 2951 (C–$CH_3$); 1723 (C=O); 1617 (C=C, phenyl); 1109 ($ClO_4^-$); 1048 (O–$CH_3$); 622 ($ClO_4^-$); 569 (Cu–N) $cm^{-1}$. ESI MS(+): 681.2. *Anal.* Calc. for $C_{34}H_{30}N_6O_{14}CuCl_2$ (881.094): C, 46.34; H, 3.43; N, 9.54; Cl, 8.05. Found: C, 46.01; H, 3.32; N, 8.88; Cl, 6.65%.

*2.3.7. Cu complex **8b***

A methanolic solution of 9-methylguanine (11.6 mg, 0.07 mmol, in 10 ml) was added to a methanolic solution of **5b** (31.0 mg, 0.07 mmol, in 5 ml). The mixture was refluxed 24 h. The resulting green solid was filtered off, washed with diethyl ether and dried. Yield: 19.9 mg (46.1%), mp: 202.9–203.4 °C. IR: $\nu_{max}/cm^{-1}$ 3493 (C–$NH_2$); 3125 (OH); 2924 (C–$CH_3$); 2849 (N–$CH_3$); 1713 (C=O); 1601 (C=C, phenyl); 1547 (C=N); 1011 (O–$CH_3$); 695 (Cu–N) $cm^{-1}$. UV–Vis: $\lambda_{max}$ = 285.5 nm. MS-FAB (*m*/*z*): 686 (20%), $[LCu9MeGCl_2+DMSO+H]^+$; 681 (100%), $[LCu9MeGCl_2+4H_2O]$; 644 (10%) $[LCu9MeGCl_2+2H_2O]$; 623 (5%), $[LCu9MeGCl_2+H_2O]$; 537 (10%), $[LCu9MeG]^{2+}$; 443 (10%), $[LCuCl_2]$; 408 (10%), $[LCuCl]^+$; 372 (40%), $[LCu]^{2+}$. *Anal.* Calc. for $C_{23}H_{22}N_8O_4CuCl_2 \times H_2O$ (626.93): C, 44.06; H, 3.53; N, 17.87. Found: C, 44.29; H, 3.84; N, 17.36%.

*2.4. Cell and cytotoxicity assay*

*2.4.1. 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 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 in 37 °C in a humidified atmosphere of 5% $CO_2$ in air.

*2.4.2. Cytotoxicity assay by MTT*

Cytotoxicity of ligands **2a**, **b**, their complexes **3a**, **b**–**7a**, **b**, carboplatin and cisplatin was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma, St. Louis, MO] assay as described [44]. Briefly, after 46 h of incubation with drugs, the cells were 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 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 cellular sensitivity to a given treatment. Ligands, complexes, carboplatin and cisplatin were tested for their cytotoxicity in a final concentration $10^{-7}$–$10^{-3}$ M. As a control, cultured cells were grown in the absence of drugs. Data points represent means of at least 12 repeats ± S.D.

*2.5. X-ray measurements*

A crystal of **3a** suitable for X-ray crystallography was selected by means of a polarization microscope, mounted on the tip of a glass fiber, and investigated on a Nonius KappaCCD diffractometer (Mo Kα radiation, λ = 0.71073 Å). A multi-scan absorption correction of the reflection intensities was performed with SADABS [45]. The structure was solved by direct methods with SHELXS-97 [46] and refined by full-matrix least-squares calculations based on $F^2$ (SHELXL-97) [47]. All non-hydrogen atoms are refined anisotropically. The C-bound hydrogen atoms are riding on their parent atoms while the O-bound hydrogen atoms were located from the electron density map.

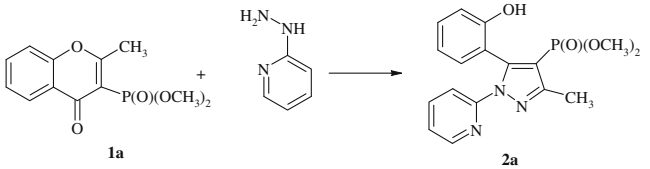
A crystal of **6a** suitable for X-ray crystallography was selected by means of a polarization microscope, mounted on the tip of a glass fiber, and investigated on a Nonius KappaCCD diffractometer (Mo Kα radiation, λ = 0.71073 Å). The structure was solved by direct methods with SIR-97 [48] and refined by full-matrix least-squares calculations based on $F^2$ (SHELXL-97) [47]. All non-hydrogen atoms are refined anisotropically. The hydrogen atoms are riding on their parent atoms.

## 3. Results and discussion

*3.1. Preparation of the ligands and their complexes*

In the reaction of dimethyl 2-methyl-4-oxo-4*H*-chromen-3-ylphosphonate [41] with 2-hydrazinopyridine in methanolic solution we have obtained the new ligand **2a** (Scheme 1).

First we have investigated this reaction with the phosphonic derivative **1a** in an NMR tube. The progress of the conversion of **1a** to **2a** was monitored by $^{31}P$ NMR spectroscopy and by thin layer




**Scheme 1.** The synthesis of new highly substituted pyrazole ligand **2a**.

chromatography. In the $^{31}P$ NMR spectrum (Fig. 1) obtained within one hour after starting the reaction, the signal of one new product at 17.20 ppm was observed, in addition to the signal of the substrate **1a** at 17.91 ppm. The intensity of this signal at 17.20 ppm gradually increased until only traces of substrate were left and we observed only one signal in the reaction mixture, what corresponds to only one product formed during this reaction. According to literature data hydrazinopyridine has only one dissociation constant $K_1 = 7.24$ for N2 and this nitrogen atom is responsible for nucleophilic attack [49]. The N1 does not have nucleophilic activity. Therefore, the first step of reaction is nucleophilic attack of hydrazinopyridine N2 atom at the chromone derivative **1a** C2 atom. The second step of the reaction is the opening of the γ-pyrone ring and cyclization to pyrazole derivative (Scheme 2). This reaction proceeds according to the general mechanism described for the reaction of chromones with nitrogen nucleophiles [50,51] such as hydrazines [52–54]. A multimilligram reaction of **1a** and **1b** with one equivalent of 2-hydrazinopyridine gave the same products as in the NMR tube.

The substituted pyrazoles **2a** and **2b** (Fig. 2) were used as the ligands L in the formation of the $MLCl_2$ complexes with platinum(II), palladium(II) and copper(II) ions (M) (Scheme 3 and 4). Complexes **3b**, **4b** and **5b** were obtained to compare the influence of the functional group on cytotoxic activity. The synthesis of the carboxylic ligand **2b** and its complexes **3b**, **4b**, **5b** and **6b** are described elsewhere [39,40].

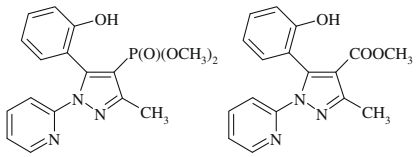



**Fig. 2.** Structures of ligands **2a** and **2b**.

Complexes **3a** and **4a** were obtained by adding aqueous solution of $K_2PtCl_4$ or $K_2PdCl_4$, respectively, to the methanolic solution of the ligand **2a** (Scheme 3). The copper(II) complexes were obtained in molar ratio L:Cu 1:1 (**5a** and **5b**) by adding methanolic solution of $CuCl_2 \times 2H_2O$ or 2:1 (**7a** and **7b**) by adding methanolic solution of $Cu(ClO_4)_2 \times 6H_2O$ to the solution of ligands **2a** or **2b** in

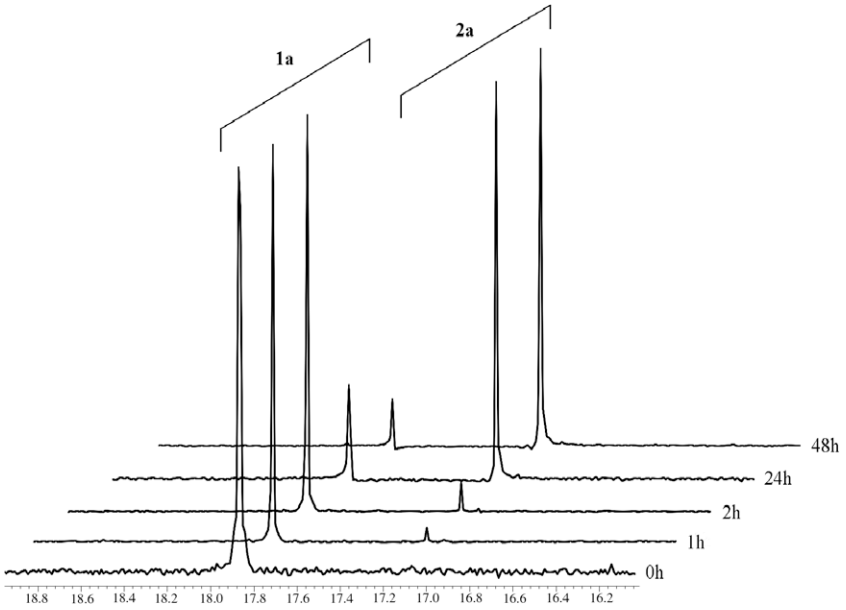
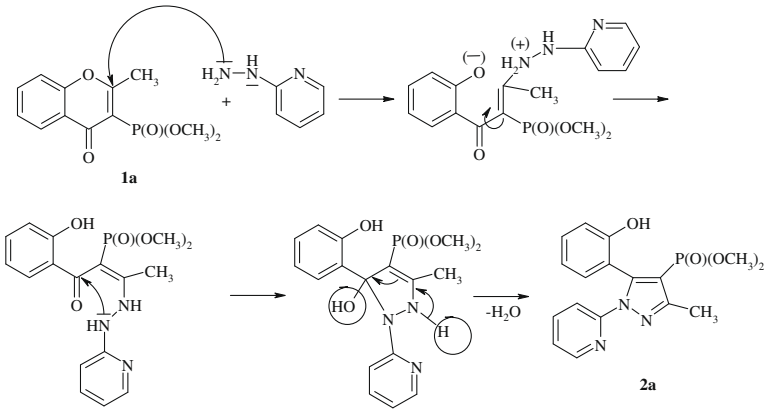
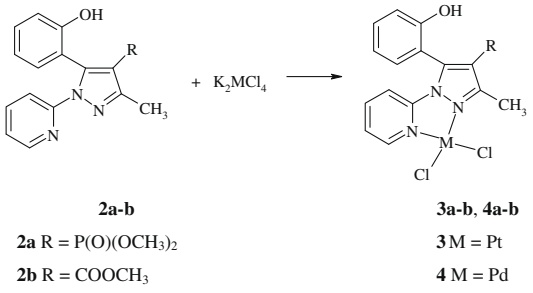


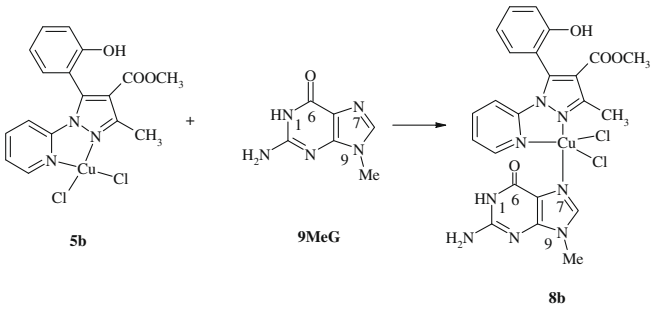

**Fig. 1.** $^{31}P$ NMR spectra of the reaction of transformation **1a** to **2a**.



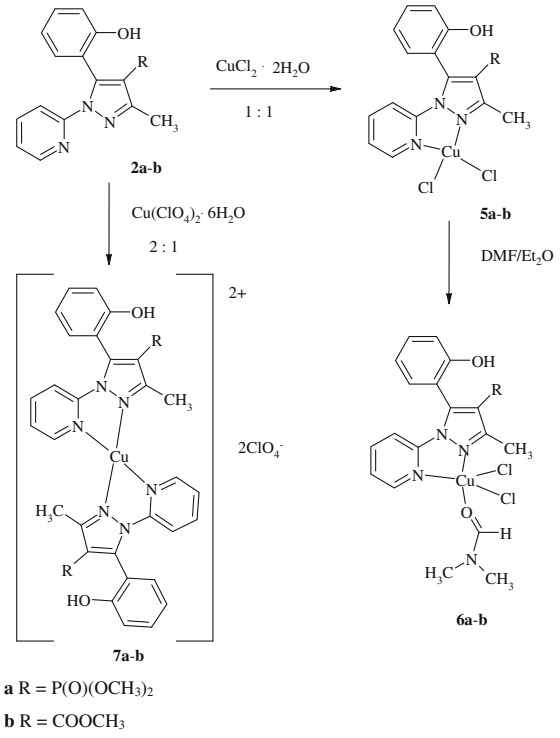

**Scheme 2.** Mechanism of formation of the ligand **2a**.




**Scheme 3.** Synthesis of the Pt(II) and Pd(II) complexes.




**Scheme 5.** Synthesis and proposed structure of complex **8b**.




**Scheme 4.** Synthesis of copper(II) complexes.

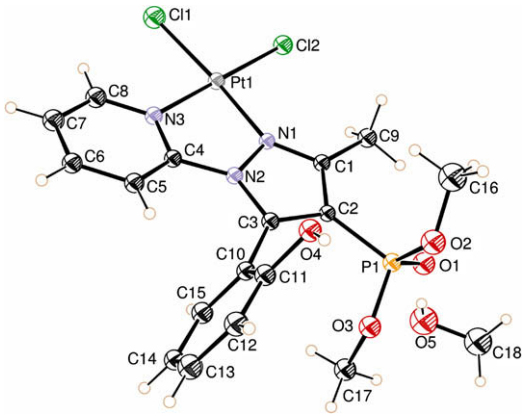



**Fig. 3.** The molecular structure of platinum(II) complex **3a**.

ethyl acetate (for complex **5a**) or methanol (for complexes **5b**, **7a** and **7b**). In one of our earlier works [39] we postulated that the interaction of ligand **2b** with copper(II) ions in solution might be related to interaction of the phenol 2-hydroxy group with metal ion. The ionisation of this group introduces an additional negative charge and enhances the donor properties of the potential nitrogen functions. As a result copper(II) complexes in two different L:Cu molar ratios, 1:1 or 2:1, could be formed (Scheme 4). Complexes **5a** and **5b** (L:Cu molar ratio: 1:1) occured to be neutral and complexes **7a** and **7b** (L:Cu molar ratio: 2:1) occured to be ionic.

Recrystallization of compounds **5a** and **5b** by dissolving in DMF and the diffusion of diethyl ether into the solution gave complexes **6a** and **6b**, respectively. All complexes were recrystallized but, unfortunately only compound **3a**, **6a** and **6b** yielded crystals suitable for X-ray diffraction. **6b** structure was described in our recent paper [40]. The structures **3a** and **6a** are presented here.

Knowledge of the type of interactions and the structural environment of metal complexes in relation to potential targets and other biomolecules has helped to establish binding patterns and can assist in the rational design of drugs. In this respect, interactions with DNA bases in particular have been investigated in detail resulting in a growing body of structural data on metal-nucleobase adducts [55]. Here, we have studied the reaction of complex **5b** with 9-methylguanine model DNA bases and we have obtained a complex **8b**. The compound **5b** was chosen basing on the fact that it was one of the most active among studied compounds. In this reaction we have observed the change of colors from yellow (complex **5b**) to green (complex **8b**), what can suggest occurring the pentacoordinated compound. Scheme 5 shows a probable structure of complex **8b** that has been proposed on the basis of complex **6a** structure (Fig. 4). It is possible that 9-methylguanine could coordinate to complex **5b** similar to DMF molecule.

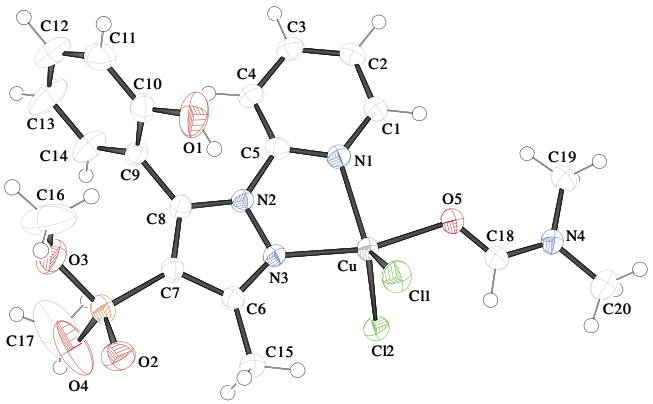



**Fig. 4.** Molecular structure of Cu(II)–complex **6a**.

**Table 1**
IR data ($\nu$, $cm^{-1}$) for ligand **2a**, its complexes **3a–7a** and complex **7b**.

| $\nu$ ($cm^{-1}$) | C–OH | C=C, phenyl | P=O | C=O | P–O | O–$CH_3$ | C–$CH_3$ | M–N |
|---|---|---|---|---|---|---|---|---|
| **2a** | 3170 | 1607 | 1228 | | 807 | 1031 | 2940 | |
| **3a** | 3401 | 1612 | 1226 | | 808 | 1028 | 2955 | 493 |
| **4a** | 3399 | 1611 | 1226 | | 811 | 1028 | 2955 | 495 |
| **5a** | 3482 | 1605 | 1239 | | 799 | 1038 | 2959 | 508 |
| **6a** | 3410 | 1600 | 1233 | 1644 | 797 | 1045 | 2953 | 419 |
| | 3061 DMF | | | DMF | | | | |
| **7a** | 3371 | 1614 | 1230 | | 800 | 1033 | 2956 | 569 |
| **7b** | 3383 | 1617 | | 1723 | | 1048 | 2951 | 569 |

### 3.2. Structural studies. Spectroscopic characterization of ligands **2a** and **2b** and their complexes

Spectroscopic characterization of ligand **2b** and its complexes with platinum(II) (**3b**), palladium(II) (**4b**) and copper(II) (**5b** and **6b**) have been described in our previous papers [39,40].

The assignments of the most important bands in the IR spectra for ligand **2a** and the platinum(II), palladium(II) and copper(II) complexes **3a–7a** and **7b** are listed in Table 1.

The IR-band at 3170 $cm^{-1}$ of the free ligand is assigned to hydroxy group of the phenyl ring. Shift of this band to lower energy may indicate the presence of hydrogen bond OH···O in the ligand's structure. This band shifts to higher energy for the complexes. The IR spectra of ligand **2a** and complexes show a large number of absorptions in the 797–1723 $cm^{-1}$ range, which can be assigned to the different vibration modes of phosphonic and carboxylate function. The characteristic band at 2940 $cm^{-1}$ of the methyl group of the free ligand which is assigned to the C–H vibration shifts to higher energy for the complexes. The new bands at about 493–569 $cm^{-1}$ in complexes may correspond to the metal–nitrogen vibrations involving the N-atoms of the pyrazole ring [56]. The new bands at 3061 and 1644 $cm^{-1}$ in **6a** complex may correspond to the C–OH and C=O vibration, respectively, involving the atoms of DMF. The bands observed at 1102 and 622 $cm^{-1}$ in the spectrum of **7a** and 1109 and 622 $cm^{-1}$ in the spectrum of **7b** are characteristic for non-coordinated perchlorate ions [57].

The selected chemical shifts assigned in the $^1H$ NMR spectra of the ligand **2a** and its platinum(II) and palladium(II) complexes **3a** and **4a** are shown in Table 2. The spectra are quite similar.

The ligand **2a** and its complexes **3a** and **4a** show signals in the range $\delta$ 2.56–2.82 ppm for the methyl groups of pyrazole ring, 3.38–3.63 for O–$CH_3$ of phosphonic groups and 6.60–7.60 for phenyl groups. The $^1H$ NMR spectra of copper(II) complexes could not be measured due to their paramagnetism.

The main peaks in ESI MS spectra of copper(II) complexes were observed for cations without chlorido or perchlorido ligands.

Occurring of the complex **8b** has been confirmed by IR, UV–Vis, FAB-MS and elemental analysis. Some characteristic IR vibrations for the complex **8b** are given in experimental section. Significant differences in the IR spectra of complex **8b** have been observed in comparison with that of complex **5b**. The latter shows a strong band at 1717 $cm^{-1}$ due to $\nu$(C=O). This band is shifted by 4 $cm^{-1}$ to lower frequencies in the spectra of complex **8b** [57]. Beside this we have observed the new bands characteristic for 9-methylguanine. In UV–Vis spectrum we have observed the change of $\lambda_{max}$ from 287 nm for complex **5b** to 285.5 for complex **8b**.

The FAB mass spectrum of **8b** recorded in positive ion mode contains a peak at $m/z$ 686 due to $[LCu9MeGCl_2+DMSO+H]^+$ ion and a strong peak at $m/z$ 681(100%) attributed to $[LCu9MeGCl_2+4H_2O]$. The peaks at $m/z$ 537 and 443, have been assigned to $[LCu9MeG]^{2+}$ and $[LCuCl_2]$, correspondingly. The purity of complex **8b** has been confirmed by elemental analysis.

**Table 2**
$^1H$ NMR spectra of ligand **2a** and the corresponding complexes with platinum(II) (**3a**) and palladium(II) (**4a**) metal ions (chemical shifts are given in ppm).

| | C–$CH_3$ | O–$CH_3$ | Aromaticity |
|---|---|---|---|
| **2a** | 2.56 | 3.52–3.63 | 6.88–7.53 |
| **3a** | 2.56 | 3.38–3.50 | 6.67–7.53 |
| **4a** | 2.82 | 3.44–3.53 | 6.60–7.60 |

#### 3.2.1. X-ray structure of platinum(II) complex **3a**

The molecular structure of complex **3a** is shown in Fig. 3, and selected bond lengths and angles are given in Table 1S. The monomeric complex **3a** contains the bidentate pyridine–pyrazole ligand and two chlorido ligands in *cis*-position. **3a** exhibits a distorted tetragonal planar configuration at the Pt(II) centre; the sum of the angles around Pt1 is exactly 360.00°. While the two Pt–Cl bonds are equal (Pt1–Cl1 2.2875(12), Pt1–Cl2 2.2871(13) Å), both Pt–N bond lengths differ slightly (Pt1–N1 2.020(3), Pt1–N3 2.030(4) Å). The angle N1–Pt1–N3 (80.11(13)°) of the chelate ligand is the smallest, then follows the angle Cl1–Pt1–Cl2 (86.38(5)°), whereas the two angles Cl1–Pt1–N3 = 94.00(9)° and Cl2–Pt–N1 = 99.51(11)° are significantly larger than 90°, because of the small angle of the *N*,*N*′-chelate system.

The five- and six-membered rings of pyridine–pyrazole lie in the same plane together with the substituents (C9, C10 and P1) at the pyrazole.

The phenolic substituent at atom C3 is bent out from this molecular plane, because of steric hindrance and the OH group at C11, which forms the hydrogen bridge O4–H4–O5′ (2.603(6) Å and 174°) with the O atom of a $CH_3OH$ molecule associated with **3a**.

The phosphite substituent at C2 shows a distorted tetrahedral configuration at the P(III) centre with two similar P–O distances (P1–O2 1.571(4), P1–O3 1.557(4) Å) but a short P1–O1 distance (1.459(4) Å) indicating the P=O double bond of the P(III) ester function.

#### 3.2.2. X-ray structure of copper(II) complex **6a**

The molecular structure of complex **6a** is shown in Fig. 4, and selected bond lengths and angles are given in Table 2S. The monomeric Cu(II) complex **6a** with the coordination number 5 for the central Cu(II) contains the bidentate pyrazole ligand, two chlorido ligands and an O-coordinated DMF solvent molecule. **6a** exhibits a distorted trigonal bipyramidal configuration at the Cu centre, whereby the two chlorido ligands Cl1 and Cl2 as well as the N1 atom of the chelate system form the equatorial plane, whereas the second N atom N3 together with the O5 atom of the DMF molecule lie in the axial positions. The Cu–Cl bonds differ more (Cu–Cl1 2.3677(9), Cu–Cl2 2.2982(9) Å) than in a similar complex published earlier by us [40], and the equatorial Cu–N bond (Cu–N1 2.101(2) Å) is surprisingly longer than the axial Cu–N3 bond (2.006(2) Å).

The Cu–O5 bond length (1.962(2) Å) is the shortest observed in **6a** around Cu, but is similar with those found in related Cu(II) complexes. The distortion of the trigonal bipyramide results from the three different angles within the trigonal plane (Cl1–Cu–Cl2 113.98(3), Cl1–Cu–N1 114.15(7), Cl2–Cu–N1 131.87(7)° and from the non-linear angle N3–Cu–O5 164.91(9)°).

The angle N1–Cu–N3 of 77.85(8)° is the smallest one observed in the trigonal bipyramide, and the other are larger than 90°C (Cl1–Cu–N3 96.83(7), Cl2–Cu–N3 96.02(6), Cl1–Cu–O5 92.38(7), Cl2–Cu–O5 91.09(6)°) with the only exception of N1–Cu–O5 angle (87.49(9)°).

The phosphite substituent at C7 shows a distorted tetrahedral configuration at the P(III) centre with two similar P–O distances (P–O3 1.551(3), P–O4 1.545(4) Å) but a short P–O2 distance (1.464(3) Å) indicating the P═O double bond of the P(III) ester function.

The bidentate heterocycle together with the connected atoms C9 and P of the substituents is nearly planar, with a maximum deviation from planarity of 1° (at C4).

The only exception is the phenyl substituent which shows the distortion angle N2–C8–C9–C10 of 70.2(4)°.

A view to the crystal packing of **3a** shows that the associated MeOH molecule forms two short and almost linear O–H–O bridges (O4–H4–O5′ 2.603(6) Å/173(7)°; O5–H5–O1′ 2.689(6) Å/174(6)° resulting in a polymeric chain shown in Fig. 5. Compound **6a**, however, forms a dimmer because of the hydrogen bonds (O1–H1–O2′ 3.009(2) Å/137.5(2)°) which are longer and significantly bent (Table 3).

**Table 3**
Crystallographic data of Pt(II) complex **3a** and Cu(II) complex **6a**.

| | | |
|---|---|---|
| Net formula | $C_{18}H_{22}Cl_2N_3O_5PPt$ | $C_{20}H_{25}Cl_2CuN_4O_5P$ |
| $M_r$ (g mol$^{-1}$) | 657.342 | 566.862 |
| Crystal size (mm) | 0.20 × 0.12 × 0.02 | 0.13 × 0.10 × 0.08 |
| $T$ (K) | 200(2) | 200(2) |
| Crystal system | Triclinic | Triclinic |
| Space group | $P\bar{1}$ | $P\bar{1}$ |
| $a$ (Å) | 8.5306(3) | 9.6019(3) |
| $b$ (Å) | 9.1983(3) | 10.3758(3) |
| $c$ (Å) | 15.4352(5) | 12.8774(3) |
| α (°) | 73.1170(19) | 74.7244(17) |
| β (°) | 82.9815(19) | 77.1466(16) |
| γ (°) | 69.927(2) | 87.7010(17) |
| $V$ (Å$^3$) | 1088.21(6) | 1206.37(6) |
| $Z$ | 2 | 2 |
| Calculated density (g cm$^{-3}$) | 2.00615(11) | 1.56056(8) |
| $\mu$ (mm$^{-1}$) | 6.802 | 1.232 |
| Transmission factor range | 0.448–0.873 | – |
| Reflections measured | 20466 | 10477 |
| $R_{int}$ | 0.0441 | 0.0316 |
| Mean $\sigma(I)/I$ | 0.0371 | 0.0539 |
| $\theta$ range | 3.16–27.20 | 3.36–27.48 |
| Observed reflections | 4348 | 4144 |
| $x$, $y$ (weighting scheme) | 0.0346, 1.4247 | 0.0584, 0.7208 |
| Reflections in refinement | 4792 | 5518 |
| Parameters | 283 | 303 |
| Restraints | 2 | 0 |
| $R(F_{obs})$ | 0.0293 | 0.0446 |
| $R_w(F^2)$ | 0.0700 | 0.1240 |
| $S$ | 1.069 | 1.051 |
| Shift/error$_{max}$ | 0.001 | 0.001 |
| Minimum/maximum electron density (e Å$^{-3}$) | −1.056, 2.142 | −0.767, 1.112 |
| CCDC | 679540 | 679541 |

### 3.3. Biological activity

In our recent publication [40] we presented the cytotoxicity assays of the ligand **2b** and its complexes **3b**–**5b** performed on L1210 and K562 leukemia cell lines. Here we present biological studies of these compounds as well as newly synthesized ligand **2a** and its complexes on human acute leukemia HL-60 and NALM-6 and melanoma WM-115 cell lines in order to compare the influence of the functional group on cytotoxic activity (Table 4). Compounds **6a** and **6b** were not tested for biological activity, as they were obtained only to establish X-ray structures. It can be supposed that their cytotoxicity would be strictly related to the presence of DMF molecule in their structure and DMF is highly cytotoxic by itself. That is why the test of biological activity of the clear complexes **5a** and **5b** without DMF molecule was only provided. Cisplatin and carboplatin were used as the reference compounds. Ligands **2a** and **2b** have very low cytotoxic activity. Most phosphonic compounds exhibited lower cytotoxicity than carboxylic analogs. The cytotoxic effect of platinum(II) and palladium(II) complexes are rather low. All copper(II) complexes exhibited relatively high cytotoxic activity with the $IC_{50}$ values in micromolar range against leukemia cell

**Table 4**
$IC_{50}$ values (in μM) of the ligands **2a** and **2b** and their complexes.

| Compound | HL-60 | NALM-6 $IC_{50}$[a] | WM-115 |
|---|---|---|---|
| **2a** | 716.5 ± 28.9 | 875.1 ± 119.9 | >1000 |
| **2b** | 649.4 ± 74.4 | >1000 | >1000 |
| **3a** | 613.1 ± 22.2 | 766 ± 40.4 | 549.0 ± 68.5 |
| **3b** | 58.1 ± 2.2 | 55.1 ± 3.8 | 76.7 ± 13.4 |
| **4a** | 610.2 ± 40.8 | 804.2 ± 57.2 | 591.3 ± 51.9 |
| **4b** | 133.9 ± 28.8 | 413.7 ± 40.4 | 483.6 ± 22.3 |
| **5a** | 56.96 ± 4.97 | 49.88 ± 2.23 | 90.5 ± 11.69 |
| **5b** | 48.9 ± 1.7 | 54.1 ± 2.4 | 93.4 ± 5.0 |
| **7a** | 43.9 ± 3.6 | 47.6 ± ± 4.6 | 81.7 ± 3.2 |
| **7b** | 6.5 ± ± 0.3 | 40.7 ± 5.5 | 8.0 ± 0.3 |
| *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 four experiments are presented.

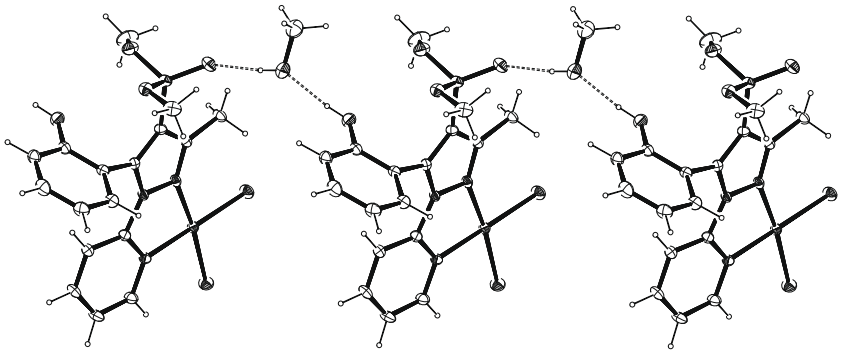


**Fig. 5.** Part of the crystal packing of **3a** with the intermolecular hydrogen bridges with MeOH.

lines although their cytotoxic effectiveness was lower in comparison to cisplatin and carboplatin. The copper(II) complex **7b** exhibited the highest cytotoxic activity with $IC_{50}$ values in the range of 6.5 μM for HL-60 and 8.0 μM for WM-115 cell lines. So, cytotoxic effectiveness of compound **7b** against melanoma WM-115 cells was two times better than that of cisplatin. The result strongly support the view that the observed cytotoxicity can be confidently attributed to the presence of the metal centres.

## 4. Conclusions

In this paper, we presented the synthesis and investigation of highly substituted phosphonic pyrazole ligand and its complexes with platinum(II), palladium(II) and copper(II). Cytotoxic activity of these new compounds was compared with activity of their carboxylic analogs in order to assess the influence of chemical groups on cytotoxic activity of the compounds.

The new phosphonic ligand **2a** was obtained in the reaction of dimethyl 2-methyl-4-oxo-4*H*-chromen-3-yl-phosphonate with 2-hydrazinopyridine in methanolic solution.

By monitoring the reaction by $^{31}P$ NMR spectroscopy we observed only one product after the opening of the γ-pyrone ring and cyclization to pyrazole derivative. This is in accordance to the fact that 2-hydrazinopyridine has only one dissociation constant for N2 and this nitrogen atom is responsible for nucleophilic attack at the chromone derivative C2 atom.

The highly substituted phosphonic pyrazole **2a** was used as ligand for the synthesis of novel neutral platinum(II), palladium(II) and copper(II) complexes **3a–7a**. Phosphonic compounds were compared with previously synthesized carboxylic analogs **2b**–**7b**, whose cytotoxic assay had been performed on L1210 and K562 leukemia cell lines. Copper(II) complexes with both phosphonic and carboxylic ligands were synthesized in 1:1 and 2:1 molar ratio. The structure of the ligands and their metal complexes was confirmed by spectral and elemental analyses. The molecular structures of phosphonic complexes of platinum(II) **3a** and copper(II) **6a** were confirmed by X-ray analysis. The complexes exhibit distorted tetragonal planar configuration and trigonal bipyramidal configuration at the platinum(II) and copper(II) centres, respectively.

Studies on the cytotoxicity evaluated on leukemia and melanoma cell lines showed that in most cases carboxylic compounds have higher activity than that of phosphonic analogs. Cytotoxic effectiveness of compound **7b** against melanoma WM-115 cells was two times better than that of cisplatin. Copper(II) complexes were more active in the induction of melanoma cell death than the platinum(II) and palladium(II) complexes. Higher cytotoxic activity of investigated copper(II) complexes suggests that the mechanism of actions of these compounds may differ from the mechanism of action of platinum(II) or palladium(II) complexes. The reaction of complex **5b** with 9-methylguanine has been investigated and we have obtained a new compound **8b** of the formula $LCu9MeGCl_2$.

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## Appendix A. Supplementary data

CCDC 679540 and 679541 contains the supplementary crystallographic data for **3a** and **6a**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.poly.2008.12.013.

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