

Synthesis, Structure, Protolytic Properties, Alkylating and Cytotoxic Activity of Novel Platinum(II) and Palladium(II) Complexes with Pyrazole-Derived Ligands

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Novel platinum(II) and palladium(II) complexes [PtCl₂(HL¹)] (**3a**), [NH₂(CH₃)₂][PtCl₂(L¹)] (**3b**), [PdCl₂(HL¹)] (**4a**), [NH₂(CH₃)₂][PdCl₂(L¹)] (**4b**) and [PdCl(CH₃CN)(L¹)] (**4c**), where HL¹ = 4-(2-hydroxybenzoyl)-2-(pyridin-2-yl)-1H-pyrazol-3-ol, have been synthesised as potential anticancer compounds. Protonation constants of the ligand were determined by pH-metric titration (log K₁ = 7.74 ± 0.03; log K₂ = 2.85 ± 0.05). The solid-state structures of complexes **3b** and **4c** have been determined by X-ray diffraction, showing square-planar coordination geometry of Pt^{II} and Pd^{II} ions. In

addition, the compounds have been characterised by IR, ¹H NMR and FAB mass spectrometry. The results of preliminary studies on the cytotoxic activity in vitro against HL-60 and NALM-6 leukaemia cell lines are also reported and the IC₅₀ values compared with those of the metal-free ligand and cisplatin. Cytotoxic evaluation revealed that Pt^{II} and Pd^{II} complexes were active in the micromolar concentration range.

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Introduction

Cisplatin [diamminedichloroplatinum, *cis*-PtCl₂(NH₃)₂] is known as a DNA-modifying agent with strong anticancer potency.^[1–3] Despite its wide clinical application, cisplatin causes many serious side effects, such as nephrotoxicity, ototoxicity, peripheral neuropathy and allergy.^[4] In addition, the therapeutic efficacy of cisplatin is limited by inherent or treatment-induced resistance of tumour cell subpopulations.^[5] Therefore, various platinum(II) and palladium(II) complexes with nitrogen-containing ligands were evaluated in the search for less toxic and more selective an-

ticancer drugs.^[6] Simple structural analogues of cisplatin were found to possess no advantages because of similar mechanism of action, structure of DNA adducts, toxicity profile, adverse effects and tumour cross-resistance.^[7] Recently, attention has been focused on inert, nonleaving ligands, whose steric and electronic features may affect the mechanism of substitution at the metal centre, DNA binding and drug metabolism. It is believed that complexes containing bulky ligands are kinetically and thermodynamically more stable than simple cisplatin analogues.^[8] Taking into account the structural analogy between Pt^{II} and Pd^{II} complexes, a variety of studies on Pd^{II} complexes have been performed, including on their cytotoxicity^[9] and antitumour activity.^[10–12] Palladium complexes with β-carboline alkaloids,^[13–15] pyrazoles^[16] and DMSO^[17] were tested against solid tumour cell lines, and in some cases exhibited remarkable activity. It was suggested that their biological activity depends on the nature of the ligand, the type of counterion used and the configuration of the complex.^[18]

For many years the research in our laboratory has been directed towards the synthesis of chromone (benzo-γ-pyrone) and coumarin (benzo-α-pyrone) derivatives and the study of their reactivity towards nucleophilic reagents containing nitrogen, oxygen, sulfur and carbon.^[19] In particular, in protolytic solvents at room temperature chromones have been converted efficiently into enamine-type compounds with a typical lemon-yellow colour.^[20,21] The chromones and coumarins react with hydrazines to afford pyr-

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azole derivatives.^[22] A class of pyrazole-containing palladium(II) complexes has been reported to possess antibacterial,^[23] antidepressant,^[24] monoamine oxidase inhibiting^[25] and antitumour activity.^[26,27]

In addition, both type of compounds (chromones and coumarins) exhibit interesting biological properties,^[28,29] including anticonvulsant,^[30] antimicrobial,^[31] spasmolytic,^[32] antioxidant^[33] and antitumour activities.^[34] The metal complexes with coumarin as a ligand reveal anticoagulant action^[35,36] and possess antiproliferative activity.^[37,38] Notably, complexes with cerium(III), zirconium(IV), copper(II), zinc(II), bismuth(III) and cadmium(II) show pronounced cytotoxic activity in vitro.^[39,40] The rare earth metal complexes of coumarin derivatives endowed with anticoagulant and anti-HIV activity were also documented.^[41–43] Current efforts are focused on developing metal-based drugs with improved clinical effectiveness, reduced general toxicity and a broader spectrum of activity.

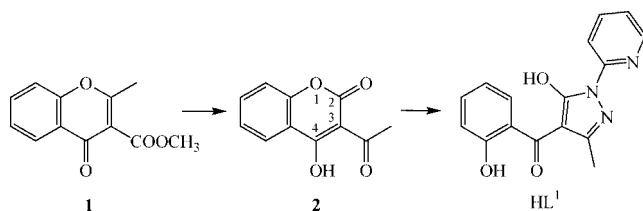
Herein we report the synthesis of a novel pyrazole ligand HL¹ by the reaction of coumarin with 2-hydrazinopyridine. Protonation constants of the ligand were determined by pH-metric titration. This ligand, as will be shown below, forms *cis* complexes with platinum(II) and palladium(II), which are often more cytotoxic than the *trans* isomers.^[44] The structures of two Pt^{II} and Pd^{II} complexes determined by X-ray diffraction are reported. The results of preliminary studies on the alkylating and in vitro cytotoxic activity against HL-60 and NALM-6 leukaemia cell lines are also discussed.

Results and Discussion

Preparation of the Ligands

Pyrazole-based chelating ligands have attracted our attention, because of their ability to form *cis*-configured complexes. The anticancer activity of a metal complex depends not only on the metal itself, but also on its oxidation state, on the number and type of bound ligands, and the coordination geometry of the complex.^[45]

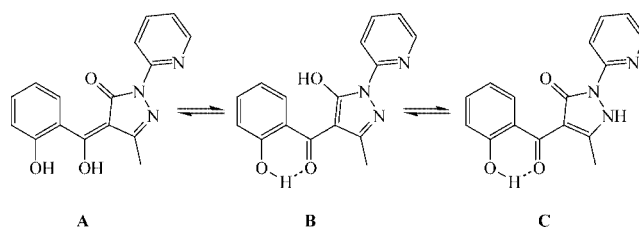
By reaction of 3-acetyl-4-hydroxycoumarin (**2**), which was obtained from methyl ester **1**, with 2-hydrazinopyridine in a 1:1 molar ratio in refluxing methanol, we obtained 4-(2-hydroxybenzoyl)-2-(pyridin-2-yl)-1*H*-pyrazol-3-ol (HL¹) in 62% yield (Scheme 1).



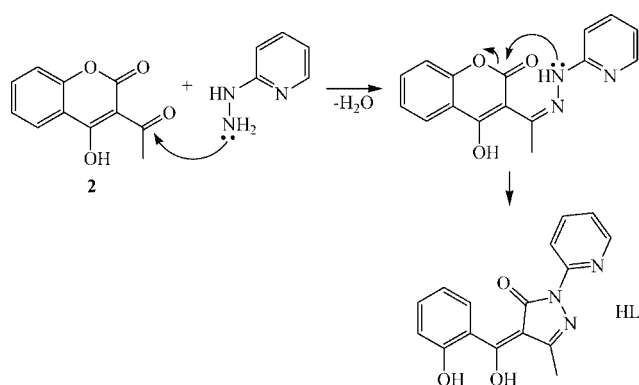
Scheme 1. Synthesis of the ligand HL¹.

Three different tautomeric forms (A, B and C) can be envisaged for HL¹ (Scheme 2). Of these, B and C are stabilised by intramolecular hydrogen bonding. The formation

of HL¹ presumably proceeds by an attack of 2-hydrazinopyridine on the carbonyl group (C-3 position in coumarin), followed by the nucleophilic attack of the hydrazine nitrogen on the carbonyl atom at C-2 position (Scheme 3).



Scheme 2. Possible tautomeric forms of the ligand HL¹.



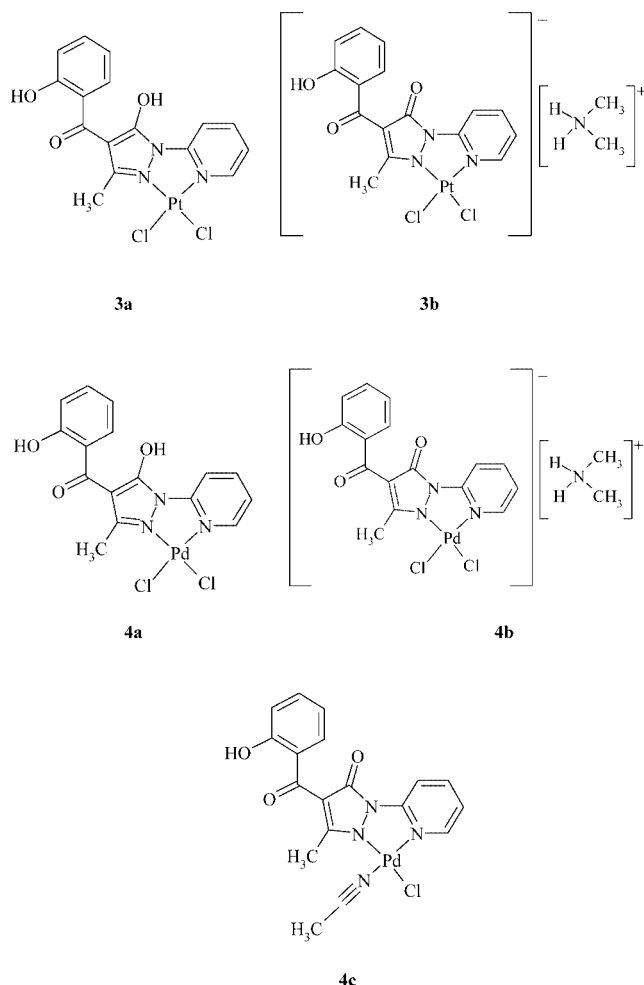
Scheme 3. Stepwise formation of HL¹.

The ligand has been characterised by elemental analysis, IR spectroscopy, ¹H and ¹³C NMR spectroscopy and mass spectrometry (see Experimental Section).

Synthesis of Complexes

The synthesised ligand HL¹ behaves as a strong bidentate chelating agent forming a five-membered metallocycle through coordination of the pyrazole and the pyridine nitrogen atoms to the corresponding metal ion. A review of the coordination chemistry of acylpyrazolone derivatives has been recently published.^[46]

Complexes [M^{II}Cl₂(HL¹)], where M = Pt (**3a**) and Pd (**4a**) (Scheme 4), were prepared at room temperature by mixing equimolar amounts of HL¹ and potassium tetrachloroplatinate(II) or potassium tetrachloropalladate(II), respectively, in water/methanol. Reactions of K₂[PtCl₄] or K₂[PdCl₄] with HL¹ in 1:1 molar ratio in dimethylformamide (DMF) produced (after two weeks) the complexes [NH₂(CH₃)₂][MCl₂(L¹)], where M = Pt (**3b**) and Pd (**4b**). The cation [NH₂(CH₃)₂]⁺ has been presumably formed by light-induced decomposition of DMF and protonation of the arising dimethylamine.^[47] The complex [PdCl(CH₃CN)(L¹)] (**4c**) resulted from the reaction of [PdCl₂(C₆H₅CN)₂] with HL¹ in 1:1 molar ratio in chloroform/methanol (2:1), followed by recrystallisation from acetonitrile/diethyl ether.



Scheme 4. The library of novel complexes prepared.

The synthesised complexes **3a** and **4a** are sparingly soluble in water, acetone and ethanol, but well soluble in DMSO and DMF.

The composition and the structure of the complexes prepared were confirmed by elemental analyses, IR spectra, ^1H NMR spectra, mass spectrometry measurements and X-ray diffraction for compounds **3b** and **4c**. Some characteristic IR vibrations for these complexes are given in Table S1 (see Supporting Information). Significant differences in the IR spectra of complexes **3** and **4** were observed in comparison with that of ligand HL^1 . The latter shows a strong band at 1660 cm^{-1} due to $\nu(\text{C}=\text{O})$. This band is shifted by $14\text{--}32\text{ cm}^{-1}$ to lower frequencies in the spectra of metal complexes. Moderately strong bands at 355 , 334 and 328 cm^{-1} can be attributed to $\nu(\text{M}-\text{Cl})$ of the complexes. Stretching vibrations of the $\text{M}-\text{N}$ bonds were observed at 420 cm^{-1} . The FAB mass spectrum of **3a** recorded in positive-ion mode contains a peak at m/z 563 due to the $[\text{M} + \text{H}]^+$ ion and a strong peak at m/z 528 attributed to $[\text{M} - \text{Cl}]^+$. Analogously, the spectrum of **4a** shows two peaks at m/z 473 and 438, which were assigned to $[\text{M} + \text{H}]^+$ and $[\text{M} - \text{Cl}]^+$, correspondingly. For complex **4c** we have observed a peak at m/z 478 due to $[\text{M} + \text{H}]^+$. The purity of complexes **3b** and **4b** was confirmed by elemental analysis.

The Protonation Constants of the Ligand HL^1

The potentiometric measurements performed within the pH range 2.5–11.0 showed that under titration with KOH, the ligand HL^1 acts as a weak acid with a protonation constant of about 10^8 . These acidic properties can be attributed to the hydroxy group attached to the aromatic ring. Its acidity, slightly higher than that observed for phenols, is probably enhanced by the vicinity of the carbonyl group. In the presence of a strong acid the ligand HL^1 acts as a weak base. However, its basicity is small and, within accessible range of concentrations, it was possible to determine potentiometrically only one additional protonation constant. Supposedly, it refers to the equilibrium of protonation of the pyridine nitrogen atom, which is expected to have a higher affinity for the proton than the pyrazole nitrogen atom.

Thus, the stepwise protonation constants of $\log K_1 = 7.74 \pm 0.03$ and $\log K_2 = 2.85 \pm 0.05$ were calculated from titration curves. This implies that, under physiological conditions, the nitrogen atoms of the ligand present in solution are unprotonated.

X-ray Crystallography

Single crystal X-ray diffraction studies of compound **3b** were undertaken to elucidate the coordination sphere of platinum(II) and the tautomeric form of the ligand. The molecular structure of **3b** with the atom numbering scheme is shown in Figure 1. Selected bond lengths and angles are given in Table 1. The asymmetric unit consists of one $[\text{PtCl}_2(\text{L})]^-$ anion and one $[\text{NH}_2(\text{CH}_3)_2]^+$ cation. The coordination environment of Pt^{II} atom is planar, formed by two nitrogen atoms and two chloride ligands. The PtN_2Cl_2 “square” adopts a *cis* configuration. The geometry about the Pt atom deviates from the ideal square plane: the atoms N1, N3 and Cl1, Cl2 form angles smaller than 90° with the Pt atom, and consequently the $\text{N3}-\text{Pt}-\text{Cl2}$ and $\text{N1}-\text{Pt}-\text{Cl1}$ angles are larger than 90° . The distribution of electron density over the ligand backbone and the presence of a counteranion indicate that HL^1 acts as a monodeprotonated ligand and adopts the tautomeric form C (Scheme 2).

The crystal lattice is stabilised by a net of intermolecular and intramolecular hydrogen bonds. The atom O1 participates in two $\text{O}-\text{H}\cdots\text{O}$ hydrogen bonds: intramolecular $\text{O1}-\text{H5}\cdots\text{O2}$, which closes an extra six-membered ring, and intermolecular $\text{O5}-\text{H5}\cdots\text{O1}^{(i)}$. In addition, $\text{N4}-\text{H16}\cdots\text{O3}$ interaction is of note. The atom N4 is also involved in hydrogen bonds to the Cl atoms (see Table S2).

The packing of the molecules in the crystal lattice is stabilised by $\text{C}-\text{H}\cdots\pi$ interactions. Atom C11 is involved in a weak $\text{C}-\text{H}\cdots\pi$ intermolecular interaction with the centroid of the ring 3 (N3/C12/C13/C14/C15) [symmetry code (ii): $2 - x, 1 - y, -z$] and C18 interacts with Cg4, where Cg4 is the centroid of ring C1–C6 (more details in Table S3). A number of $\pi-\pi$ stacking interactions are observed in the crystal lattice (see Figure S2).

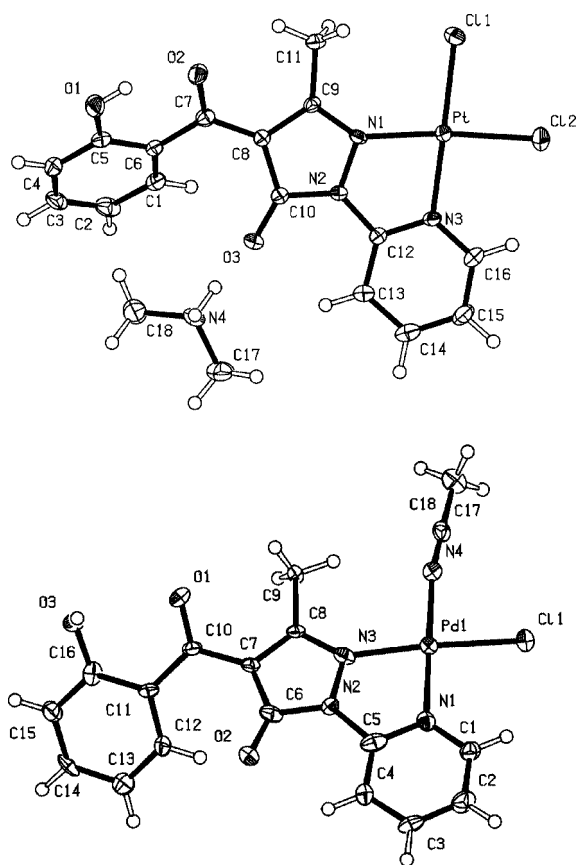


Figure 1. ORTEP drawing of **3b** (top) and of the first independent molecule of **4c** (bottom) with thermal ellipsoids depicted at 50% probability level.

Compound **4c** crystallised in the orthorhombic space group $Pca2_1$ with four crystallographically independent molecules of $[PdCl(CH_3CN)(L^1)]$ in the unit cell. In all four molecules the organic species acts as a monodeprotonated bidentate ligand coordinating to the Pd ion through two nitrogen atoms with formation of a five-membered metallo-cycle. The square-planar coordination geometry of each Pd^{II} ion is completed by one chlorido ligand and one CH₃CN molecule. The bond lengths in the coordination polyhedron of all four independent molecules are given for comparison in Table 1. The bond lengths C6–O2 of 1.243(13), C10–O1 of 1.240(12) and C16–O3 of 1.343(13) Å clearly indicate the stabilisation of tautomer C in the complex (Scheme 2). The same tendency in bond length distribution is observed in three other crystallographically independent molecules (Table 1).

Table 1. Selected bond lengths [Å] for compounds **3b** and **4c**.

Structure 3b				Structure 4c					
Molecule 1A				Molecule 1B		Molecule 1C		Molecule 1D	
Pt–N1	2.019(4)	Pd1–N1	2.022(9)	Pd2–N5	2.001(10)	Pd3–N9	2.019(10)	Pd4–N13	2.003(10)
Pt–N3	2.033(3)	Pd1–N3	2.022(9)	Pd2–N7	2.007(10)	Pd3–N11	2.024(9)	Pd4–N15	2.007(10)
Pt–C12	2.302(2)	Pd1–N4	2.014(10)	Pd2–N8	1.989(10)	Pd3–N12	1.972(11)	Pd4–N16	1.979(10)
Pt–Cl1	2.303(2)	Pd1–Cl1	2.297(3)	Pd2–Cl2	2.286(3)	Pd3–Cl3	2.285(3)	Pd4–Cl4	2.294(3)

Biological Characterisation

Alkylating Activity

Alkylating agents belong to the first class of cytostatics used for chemotherapy.^[48] Under in vivo conditions they alkylate nucleophilic centres of nucleobases and amino acids, resulting either in cleavage or cross-linking of double-stranded DNA molecules or proteins. Such cleavage destroys DNA, while covalent cross-links prevent unwinding of nucleic acids, which is functionally important in replication and transcription processes.^[49] Cisplatin and carboplatin are the nonclassical alkylating agents. These drugs interact with cellular DNA according to the same mechanism^[50,51] by metallation of nucleobases of both DNA strands.

An effective drug concentration for carboplatin is 4–7-fold higher as compared to cisplatin, and the former is also less toxic.^[52] The “alkylating” activities of the ligand HL¹ and of the complexes **3a** and **4a** were determined by the Preussmann test.^[53] The level of “alkylation” of 4-(4-nitrobenzyl)pyridine (NBP) was quantified spectrophotometrically at 560 nm. The molar absorbances (*A*) for complexes **3a** and **4a** were 0.303 and 0.069, respectively, compared with 0.047 for HL¹ (Table 2). Interestingly, cisplatin in this test shows *A* = 0.300^[54] and carboplatin alkylates NBP giving *A* = 0.231. According to the Preussmann scale (see footnote to Table 2), the complex **3a** and both reference compounds cisplatin and carboplatin exhibit moderate alkylating activity in this test.

Table 2. Alkylating activity of substituted pyrazoles and their Pt^{II} and Pd^{II} complexes.

Compound	ϵ [M ^{−1} cm ^{−1}]	Absorbance ^[a] $\lambda_{\max}$ = 560 nm	Alkylation activity ^[b]
HL ¹	47.3	0.0473	–
3a	303.4	0.3034	++
4a	69.0	0.0690	+
Cisplatin ^[c]	300.0	0.3000	++
Carboplatin	231.2	0.2312	++

[a] Means from 3 determinations. [b] According to ref.^[53]: (–) *A* < 0.05, (+) *A* = 0.05–0.1, (++) *A* = 0.1–0.5, (+++) *A* > 0.5. [c] According to ref.^[54].

Cytotoxicity Assay

Cytotoxicity assays for complexes **3a** and **4a** were performed on human acute leukaemia HL-60 and NALM-6 cell lines (Figure 2). Cisplatin and carboplatin were used as

the reference compounds. Both metal complexes exhibited relatively high cytotoxic activity with the IC_{50} values in the micromolar range. Their cytotoxic effectiveness was nearly the same as that for carboplatin and only 5 (**3a**) or 10 (**4a**) times lower in comparison to cisplatin, a well-established therapeutic agent used for the treatment of leukaemia (Table 3). The most potent was the platinum(II) complex **3a**, with an IC_{50} of 4.7 and 3.9 μM for HL-60 and NALM-6 cells, respectively, in line with alkylating activity towards NBP.

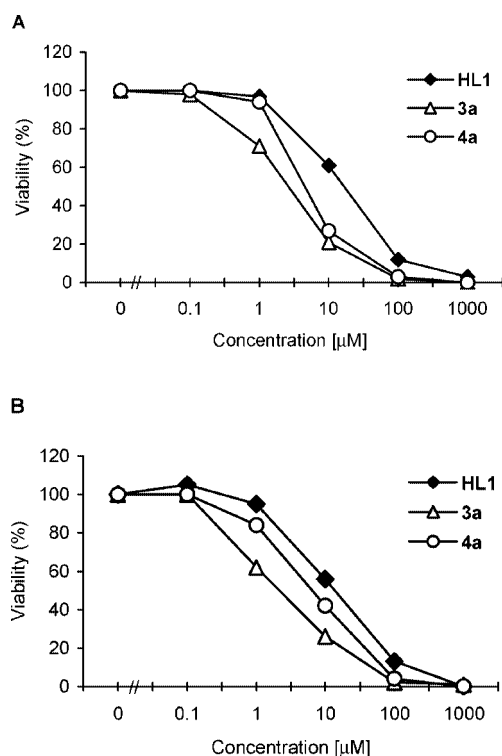


Figure 2. A: Survival curves for HL-60 and B: NALM-6 leukaemia cells exposed for 48 h to tested compounds **HL**¹, **3a** and **4a**. Each point represents the mean of four independent determinations. Standard deviations are excluded for clarity and do not exceed 20% of the mean value for each point.

Table 3. IC_{50} values [μM] for **HL**¹ and complexes **3a** and **4a**.

Compound	IC_{50} ^[a]	
	HL-60	NALM-6
HL ¹	29.9 ± 8.3	30.0 ± 5.3
3a	4.7 ± 0.6	3.9 ± 0.6
4a	7.0 ± 0.5	8.3 ± 0.3
Cisplatin	0.8 ± 0.1	0.7 ± 0.3
Carboplatin	4.3 ± 1.3	0.7 ± 0.2

[a] IC_{50} – concentration of a tested compound required to reduce the fraction of surviving cells to 50% of that observed in the control, nontreated cells. Mean values of IC_{50} (in μM) $\pm$ S.D. from four experiments are presented.

Comparison of the data in Table 3 clearly shows that the in vitro antiproliferative activity of complexes **3a** and **4a** exceeds that of uncomplexed ligand **HL**¹. This strongly supports the view that cytotoxicity can be quite safely ascribed to the presence of the metal centre.

Conclusions

In this paper we have described the synthesis of a new chelating pyrazole ligand **HL**¹, which is characterised by two protonation constants determined by pH-metric titration ($\log K_1 = 7.74 \pm 0.03$; $\log K_2 = 2.85 \pm 0.05$). The ligand forms neutral complexes of the type $[MCl_2(HL^1)]$, where $M = Pt$ (**3a**) and Pd (**4a**) in aqueous methanol. In DMF solutions the ionic species $[NH_2(CH_3)_2][PtCl_2(L^1)]$ with $M = Pt$ (**3b**) and Pd (**4b**) have been isolated. Starting from $[PdCl_2(C_6H_5CN)_2]$ and **HL**¹, and using acetonitrile for recrystallisation, the complex $[PdCl(CH_3CN)(L^1)]$ (**4c**) has been prepared. X-ray diffraction studies of **3b** and **4c** showed that the ligand in both square-planar complexes acts as a monodeprotonated one, adopting the tautomeric form C.

Complexes **3a** and **4a** exhibit in vitro antiproliferative activity in HL-60 and NALM-6 leukaemia cells, with the cytotoxicity IC_{50} values similar to those for carboplatin. The sequence in activity against HL-60 and NALM-6 leukaemia cells is as follows **3a** > **4a** > **HL**¹. The alkylating activity of the platinum compound **3a** was comparable with those of cisplatin and carboplatin.

Experimental Section

Materials: All substances were used without further purification. Potassium tetrachloroplatinate(II) and potassium tetrachloropalladate(II) were purchased from Aldrich. $CDCl_3$ and $[D_6]DMSO$ solvents for NMR spectroscopy were obtained from Dr. Glaser AG, Basel, Switzerland. Solvents for synthesis (toluene, methanol, dimethylformamide, acetone) were reagent-grade or better and were dried according to standard protocols.^[55] The melting points were determined using an Electrothermal 1A9100 apparatus and they are uncorrected. The IR spectra were recorded with a Pye-Unicam 200G Spectrophotometer in KBr or CsI pellets. The ¹H 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 FAB mass spectra were recorded with a Finnigan Matt 95 mass spectrometer (NBA, Cs⁺ gun operating at 13 keV). For the new compounds satisfactory elemental analyses ($\pm 0.3\%$ of the calculated values) were obtained using a Perkin-Elmer PE 2400 CHNS analyser. Methyl 2-methyl-4-oxo-4H-chromene-3-carboxylate (**1**)^[56] and 3-acetyl-4-hydroxychromen-2-one (**2**)^[57,58] were prepared as described in the literature.

4-(2-Hydroxybenzoyl)-2-pyridin-2-yl-1H-pyrazol-3-ol (HL**¹):** 2-Hydrazinopyridine (0.13 g, 1.15 mmol) in methanol (5 mL) was added to a solution of **2** (0.24 g, 1.15 mmol) in methanol (15 mL) and the mixture was refluxed for 1 h. The solid crude product formed was filtered off, dried in air and recrystallised from ethanol as a white solid. Yield 0.21 g (62%); m.p. 197–198 °C, $R_f = 0.78$ (methanol/chloroform, v/v 1:1). IR (KBr): $\tilde{\nu} = 3428$ (OH), 1735 (C=O), 1630, 1589 (C=N), 1458 (C=C) cm^{-1} . ¹H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 2.47$ (s, 3 H, CH_3), 6.88 (t, $J = 6.15$ Hz, 1 H), 6.99 (d, $J = 2$ Hz, 1 H), 7.22–7.26 (m, 2 H), 7.46–7.49 (m, 1 H), 7.86 (t, $J = 6.35$ Hz, 1 H), 7.96 (d, $J = 3.4$ Hz, 1 H), 8.26 (dt, 1 H, Ar) ppm (see Figure S1a, Supporting Information). ¹³C NMR (75.4 MHz, $[D_6]DMSO$, 25 °C): $\delta = 14.45$, 103.18, 112.22, 116.25, 117.89, 119.79, 123.23, 131.33, 133.17, 141.71, 143.67, 153.66, 158.77, 160.91, 190.92 ppm (Figure S1b). EI-MS: m/z (%) = 295 (51) [M^+],

175 (100%), 79 (7%). C₁₆H₁₃N₃O₃·1/3H₂O (301.29): calcd. C 63.78, H 4.57, N 13.94; found C 63.93, H 4.33, N 13.60.

Pt Complex 3a: A solution of K₂PtCl₄ (0.04 g, 0.10 mmol) in water (5 mL) was added dropwise to a solution of HL¹ (0.03 g, 0.10 mmol) in methanol (5 mL). The reaction mixture was stirred at room temperature for 48 h. The solvent was removed under reduced pressure at room temperature to half of the initial volume. After 3 h the precipitated yellow solid was filtered off, washed with methanol/water, 1:1 and dried under reduced pressure over CaCl₂. Yield 38.2 mg (68%); m.p. 315–317 °C. IR spectrum in CsI, selected bands: $\tilde{\nu}$ = 3428 ν (OH); 1631 (C=O), 1593 (C=N), 1460 (C=C), 420 (Pt–N), 384 (Pt–Cl) cm^{−1}. ¹H NMR (300 MHz, [D₆]-DMSO, 25 °C): δ = 2.71 (s, 3 H, CH₃), 6.79–6.86 (m, 2 H), 7.22 (t, 1 H), 7.34 (t, 1 H), 7.82 (d, 1 H), 8.02 (t, 1 H), 8.42 (d, 1 H), 8.78 (d, 1 H) ppm. FAB-MS: *m/z* (%) = 563. C₁₆H₁₃Cl₂N₃O₃Pt (561.26): calcd. C 34.24, H 2.33, N 7.48; found C 34.43, H 2.16, N 7.46.

Pt Complex 3b: A solution of K₂[PtCl₄] (0.04 g, 0.10 mmol) in DMF (10 mL) was added dropwise to a solution of HL¹ (0.03 g, 0.10 mmol) in DMF (10 mL). The mixture was stirred at room temperature for 24 h. The KCl formed was removed by filtration and the diethyl ether was allowed to diffuse into a DMF solution of the complex. After two weeks the yellow solid was filtered off, washed with diethyl ether and dried in air. The single crystals suitable for X-ray analyses were obtained directly from the synthesis. Yield 27.28 mg (45%); m.p. 251–253 °C. C₁₈H₂₀Cl₂N₄O₃Pt (606.34): calcd. C 35.65, H 3.32, N 9.24; found C 35.72, H 3.41, N 9.23.

Pd Complex 4a: A solution of K₂[PdCl₄] (0.09 g, 0.27 mmol) in water (3 mL) was added dropwise to a stirred solution of HL¹ (0.08 g, 0.27 mmol) in methanol (15 mL). A yellow solid formed immediately; the suspension was stirred for a further 30 min, then the solid was filtered off, washed with cold water, cold methanol and diethyl ether, and dried in air. Yield 93.15 mg (73%); m.p. 284–285 °C. IR spectrum in CsI, selected bands: $\tilde{\nu}$ = 3457 (OH); 1654 (C=O), 1593 (C=N), 1475 (C=C), 420 (Pd–N), 338 (Pd–Cl) cm^{−1}. ¹H NMR (300 MHz, [D₆]-DMSO, 25 °C): δ = 2.41 (s, 3 H, CH₃), 6.77–6.87 (m, 2 H), 7.20 (t, 1 H), 7.34 (t, 1 H), 7.81 (d, 1 H), 8.02 (t, 1 H), 8.42 (d, 1 H), 8.73 (d, 1 H) ppm (Figure S1c). FAB-MS: *m/z* (%) 473. C₁₆H₁₃Cl₂N₃O₃Pd·H₂O (490.626): calcd. C 39.16, H 3.08, N 8.56; found C 38.96, H 3.11, N 8.63.

Pd Complex 4b: A solution of K₂[PdCl₄] (0.03 g, 0.10 mmol) in DMF (10 mL) was added dropwise to a solution of HL¹ (0.03 g, 0.10 mmol) in DMF (10 mL). The mixture was stirred at room temperature for 48 h. The KCl formed was removed by filtration and the diethyl ether was allowed to diffuse into a DMF solution of the complex. After two weeks the yellow solid was filtered off, washed with diethyl ether and dried in air. Yield 27.3 mg (45%); m.p. 253–254 °C. C₁₈H₂₀Cl₂N₄O₃Pd (606.34) calcd. C 35.65, H 3.32, N 9.24; found C 35.72, H 3.41, N 9.23.

Pd Complex 4c: A suspension of [Pd(C₆H₅CN)₂Cl₂] (0.10 g, 0.25 mmol) in chloroform (5 mL) was added to a stirred solution of HL¹ (0.074 g, 0.25 mmol) in methanol/chloroform (1:1) (5 mL). The product precipitated immediately from the reaction mixture. Stirring was continued for 30 min and the complex was filtered off, washed with cold water, cold methanol and diethyl ether, and dried in air. Recrystallisation from acetonitrile/diethyl ether (1:1) afforded a red crystalline product **4c**. Yield 53.7 mg (45%); m.p. 276.4–278.0 °C. FAB-MS: *m/z* (%) 478. C₁₈H₁₅ClN₄O₃Pd (477.198): calcd. C 45.30, H 3.17, N 11.74; found C 45.35, H 2.98, N 11.83.

Potentiometric Measurements: The protonation constants of HL¹ were determined by pH-metric titrations of five identical 10-mL

samples at (25.0 ± 0.1) °C. The total ligand concentration in each sample was 2.859 mmol dm^{−3}. The samples contained also 3 mmol dm^{−3} HClO₄ and 0.05 mol dm^{−3} NaClO₄. Owing to very low solubility in pure water, a mixed 70% (v/v) ethanol/water solvent was used. The titrations were carried out with carbonate-free KOH solutions of known concentration (1 mol dm^{−3}) using an autoburette. The drop volume during these titrations was 1 μ L. The experimental value of p*K*_w = 15.50 ± 0.03 was obtained from our acid–base calibration in the mixed solvent. The pH was measured with a combined glass electrode ESAgP-301 WM (Eurosens) connected to computer-aided universal electrochemical meter EMU0,^[59] produced by the Technical University of Wrocław, Poland. The same equipment was also used to control the autoburette, as well as for the data acquisition and their introductory treatment. The electrode was calibrated by titration of 0.01 M HCl (in 70% ethanol) with 1 M KOH at (25 ± 0.1) °C. Stepwise protonation constants were calculated with our own program based on algorithm PKAS.^[60,61]

X-ray Crystallographic Data: X-ray diffraction data for yellow and red single crystals of **3b** and **4c**, respectively, were collected at 193 K on a STOE IPDS diffractometer and at 120 K on a Nonius Kappa CCD diffractometer. The unit cell parameters were determined from all reflection data in θ ranges 2.26–28.08° and 2.71–24.73°. 11713 and 11440 intensities were collected using graphite-monochromated Mo-*K*_α radiation. 4316 and 11440 independent reflections were measured in the ranges $-9 \leq h \leq 9$, $-13 \leq k \leq 13$, $-18 \leq l \leq 18$, and $-51 \leq h \leq 51$, $-8 \leq k \leq 8$, $-24 \leq l \leq 24$, respectively for **3b** and **4c**. In the data-reduction step, intensities were corrected for Lorentz and polarisation effect^[62] for **3b** and using Denzo-SMN software^[63] for **4c**. The structure was solved by direct methods using the SHELXS86^[64] program and refined by full-matrix least-squares calculation on *F*² using SHELXL97.^[65] Non-hydrogen atoms were refined with anisotropic displacement parameters. The H atoms were placed in calculated positions and allowed to ride. The molecular geometry was calculated by PLATON.^[66] The drawings were made by PLATON and are presented in Figure 1. The crystal data and details of data collection for both compounds are given in Table 4.

CCDC-622068 (for **3b**) and -622067 (for **4c**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Biological Measurements

Determination of Alkylating Properties: The test compound (0.005 mmol) was dissolved in 2-methoxyethanol (1 mL) and a solution of 4-(4-nitrobenzyl)pyridine (NBP) in 2-methoxyethanol (5% solution, 1 mL) was added. The sample was heated at (100 ± 0.5) °C for 1 h and then quickly cooled to 20 °C. 2-Methoxyethanol (2.5 mL) and piperidine (0.5 mL) were added to the sample to give a total volume of 5 mL. The final concentration of the test compound was 1 × 10^{−3} M. After 90 s the absorbance was measured at λ_{560} nm in a glass cell (1 cm). 2-Methoxyethanol was used as a reference solvent.

Cell and Cytotoxicity Assay: The cytotoxicity was determined in the human leukaemia promyelocytic HL-60 and lymphoblastic NALM-6 cell lines. Cells were cultured in RPMI 1640 medium supplemented with 10% foetal calf serum and antibiotics (penicillin (100 U/mL) and streptomycin (100 μ g/mL) in 5% CO₂/95% air. Exponentially growing cells were seeded at 3 × 10⁵ per well of a 24-well plate (Nunc), and cells were then exposed to the compounds for 48 h. Stock solutions were prepared freshly in DMSO, then dilutions from 10^{−3} to 10^{−7} M in complete culture medium were

Table 4. Crystal data and details of data collection for **3b** and **4c**.

	C ₁₆ H ₁₂ Cl ₂ N ₃ O ₃ Pt·C ₂ H ₆ NH ₂ (3b)	C ₁₈ H ₁₅ ClN ₄ PdO ₃ (4c)
Formula mass	606.37	477.19
Space group	<i>P</i> $\bar{1}$	<i>Pca</i> 2 ₁
<i>a</i> [Å]	7.633(2)	44.112(9)
<i>b</i> [Å]	10.431(2)	7.519(2)
<i>c</i> [Å]	13.763(2)	21.226(4)
<i>a</i>	105.90(2)	
<i>β</i>	93.17(2)	
<i>γ</i>	110.98(2)	
<i>V</i> [Å ³]	969.6(2)	7040(3)
<i>Z</i>	2	16
<i>λ</i> [Å]	0.71073	0.71073
<i>ρ</i> _{calcd.} [g cm ^{−3}]	2.077	1.801
Crystal size [mm ³]	0.50 × 0.25 × 0.06	0.10 × 0.08 × 0.04
<i>μ</i> [cm ^{−1}]	7.539	12.34
<i>R</i> ₁ ^[a]	0.0391	0.0699
<i>wR</i> ₂ ^[b]	0.0808	0.1667
Gof ^[c]	0.981	1.128

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$. [c] Gof = $\{\Sigma[w(F_o^2 - F_c^2)^2]/(n - p)\}^{1/2}$, where *n* is the number of reflections and *p* is the total number of parameters refined.

made. The number of viable cells was counted in a Bürker haemocytometer using trypan-blue exclusion assay.^[67] The values of IC₅₀ (the concentration of test compounds required to reduce the cell survival fraction to 50% of the control) were calculated from concentration–response curves (as illustrated in Figure 2) and used as a measure of cellular sensitivity to a given treatment. All data were expressed as means ± SD.

Supporting Information (see also the footnote on the first page of this article): IR data (Table S1), ¹H NMR, ¹³C NMR spectra (Figure S1, a–c), crystallographic data (Tables S2 and S3, and Figure S2).

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