DOI: 10.1002/ejic.201000322

In Vitro Cytotoxic-Active Platinum(II) Complexes Derived from Carboplatin and Involving Purine Derivatives

Lukáš Dvořák,[a] Igor Popa,[a] Pavel Štarha,[a] and Zdeněk Trávníček*[a]

Keywords: Platinum / Nucleobases / Cytotoxicity / Antitumor agents

Six platinum(II) complexes of the general formula [Pt(cbdc)(HL$_n$)$_2$] (**1**–**6**; cbdc = cyclobutane-1,1-dicarboxylate and HL$_1$–HL$_6$ = benzyl-substituted 6-benzylamino-2-chloro-9-isopropylpurine derivatives) have been synthesized by the reaction of [Pt(cbdc)(dmso)$_2$] with the corresponding HL$_n$ compound. The prepared complexes were characterized by elemental analysis and FTIR, Raman and NMR (^{1}H, ^{13}C, ^{15}N and ^{195}Pt) spectroscopy. Based on the results of these techniques, it can be concluded that the central Pt^{II} atom of the complexes **1**–**6** is coordinated to two oxygen atoms originating from the cyclobutane-1,1-dicarboxylate group and to two nitrogen atoms from two HL$_n$ molecules, that is, having a PtN$_2$O$_2$ donor set. Detailed multinuclear and two-dimensional NMR studies indicated the N-7 atom to be the coordination site of the purine derivatives. The coordination mode was proven by a single-crystal X-ray analysis of the [Pt(cbdc)(dmso)(HL$_7$)]·H$_2$O (**7a**·H$_2$O) intermediate [HL$_7$ = 2-chloro-6-(2-methoxybenzyl)amino-9-isopropylpurine]. The geometry is slightly distorted square-planar and the central Pt^{II} atom is coordinated to one bidentate cyclobutane-1,1-dicarboxylate dianion, one dmso molecule through the sulfur atom and one HL$_7$ molecule through the N-7 atom of the purine ring, that is, with a PtNO$_2$S donor set. The complexes **1**–**6** were tested for their in vitro cytotoxicity against K-562 (chronic myelogenous leukaemia) and MCF7 (breast adenocarcinoma) human cancer cell lines. Values of IC$_{50}$ (drug concentrations lethal for 50 % of the tumour cells) ranged from 4.5 to 14.1 μM for the K-562 cells and from 4.3 to 21.0 μM for the MCF7 cells. The in vitro cytotoxicities were in several cases comparable or even higher than those of therapeutically used platinum-based anticancer drugs, that is, cisplatin, carboplatin and oxaliplatin.

Introduction

One of the best known platinum-based complexes used in the treatment of cancer is *cis*-diamminedichloridoplatinum(II) complex (cisplatin).[1] Since 1978 it has been used in chemotherapy against testicular, ovarian, oesophageal, lung, head, neck and other human malignancies.[2] However, the therapy itself is accompanied by several unwanted side-effects (e.g., nephrotoxicity and ototoxicity) and drug resistance. These limitations led to the preparation of new platinum(II) complexes, that is, diamminecyclobutane-1,1-dicarboxylatoplatinum(II) (carboplatin), (1*R*,2*R*)-diaminocyclohexaneoxalatoplatinum(II) (oxaliplatin) and diammineglycolatoplatinum(II) (nedaplatin), which have also been approved as platinum-based anticancer drugs. In the case of carboplatin and nedaplatin, the carrier N-donor ligands (NH$_3$) are identical to those in cisplatin. However, the replacement of the chlorido-leaving ligands in cisplatin by the cyclobutane-1,1-dicarboxylate (carboplatin) or glycolate (nedaplatin) dianion led to the suppression of unwanted side-effects such as nephrotoxicity.[3]

The derivatives of 6-benzylamino-2-chloro-9-isopropylpurine (HL$_n$) used for the preparation of the platinum(II) complexes **1**–**6** were chemically derived from a 6-benzylaminopurine (N6-benzyladenine, bap) skeleton. The latter represents one of the groups of plant growth regulators called cytokinins.[4] 6-Benzylamino-2-chloro-9-isopropylpurine itself is an inactive precursor of cyclin-dependent kinase (CDK) inhibitors such as 6-benzylamino-2-(3-hydroxypropylamino)-9-isopropylpurine (bohemine) or 6-benzylamino-2-(2-hydroxymethyl-1-propylamino)-9-isopropylpurine (roscovitine, ros).[5] These types of organic compounds have formerly been used in the synthesis of metallocomplexes, including platinum(II) and platinum(IV) complexes. Compounds *cis*-[PtCl$_2$(HL)$_2$], *trans*-[PtCl$_2$(HL)$_2$], [Pt(ox)(HL)$_2$], [PtCl$_3$(H$^+$HL)], *cis*-[PtCl$_2$(H$^+$HL)$_2$]Cl$_2$ and [PtCl$_5$(H$^+$HL)] have been reported and their in vitro cytotoxic activity on selected human cancer cell lines have also been discussed (HL = variously substituted bap; H$^+$HL = protonated form of bap derivatives; see ref.[6] and references cited therein). The best results were obtained for *cis*-[PtCl$_2$(ros)$_2$], the IC$_{50}$ (concentration of the tested compound lethal for 50% of cells) values of which were equal to 1 μM for the K-562 (chronic myelogenous leukaemia), G-361 (malignant melanoma) and HOS (osteogenic sarcoma) human cancer cell lines and to 2 μM for the MCF7 (human breast adenocarcinoma) cells. The in vitro cytotoxicity of this complex exceeds that of cisplatin, the IC$_{50}$ values of

[a] Department of Inorganic Chemistry, Faculty of Science, Palacký University
Tř. 17. listopadu 12, 77146 Olomouc, Czech Republic
Fax: +420-585-634-357
E-mail: zdenek.travnicek@upol.cz

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejic.201000322.

which were determined to be 3, 3, 5 and 11 μM, respectively, for the above cell lines. With the exception of the above-mentioned compounds with 6-benzylaminopurine derivatives, complexes of the type [Pt(1,4dach)(L)$_2$]X involving different types of nucleobase or their derivatives (L = adenine, hypoxanthine, 9-methylguanine, cytosine and 1-methylcytosine) and 1,4-diaminocyclohexane (1,4dach) can be regarded as platinum(II) complexes with similar types of N-donor ligand; X = SO_4^{2-} or Cl_2^{-}).[7]

A total number of 308 platinum(II) complexes involving the [PtN$_2$(cbdc)] motif have been reported to date (SciFinder Scholar, 2004 edition). Moreover, 28 X-ray structures of platinum(II) square-planar complexes have been deposited at the Crystallographic Structural Database (CSD ver. 5.31, November 2009 update),[8] but only two of them, namely [Pt(cbdc)(2-mp)$_2$] and [Pt(cbdc)(hmi)$_2$]·H$_2$O, have two unidentate N-donor heterocyclic ligands (2-methylpyridine, 2-mp; hexamethyleneimine, hmi) coordinated to the Pt^{II} atom.[9] In relation to this, the complexes **1**–**6** represent the first ever prepared carboplatin-based complexes with two substituted purine molecules coordinated to the metal centre.

In this work we report the preparation and characterization of the platinum(II) complexes [Pt(cbdc)(HL$_n$)$_2$] **1**–**6** bearing N-donor carrier ligands derived from 6-benzylamino-2-chloro-9-isopropylpurine (HL$_n$) and the cyclobutane-1,1-dicarboxylate dianion (cbdc) as the leaving bidentate O-donor group. The complexes prepared were screened in an acetoxymethyl (AM) assay for their in vitro cytotoxicity against K-562 and MCF7 human cancer cell lines.

Results and Discussion

Synthesis

The 6-benzylamino-2-chloro-9-isopropylpurine derivatives (HL$_n$), depicted in Scheme S1 (see the Supporting Information), were synthesized from 2,6-dichloropurine, as shown in Scheme 1.[10]

A series of light-grey platinum(II) complexes **1**–**6** of the general formula [Pt(cbdc)(HL$_n$)$_2$], formally derived from carboplatin, were prepared by a general procedure with [Pt(cbdc)(dmso)$_2$] as a key intermediate, which was allowed to react with 2 mol equiv of the 6-benzylamino-2-chloro-9-isopropylpurine derivatives (HL$_1$–HL$_6$) to give the final products of general formula [Pt(cbdc)(HL$_n$)$_2$] (summarized in Scheme 1; dmso = dimethyl sulfoxide).[11] The reactions were performed in distilled water/isopropyl alcohol (1:1, v/v) at 90 °C. The substitution of the two dmso molecules proceeded in two steps, as reported for the reactions of [Pt(cbdc)(dmso)$_2$] with 1,2-diaminocyclohexane (dach), aminocyclohexane (ach) and *n*-propylamine (pa).[11] In the

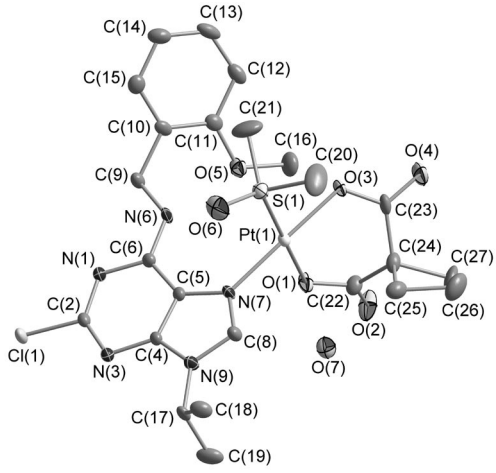
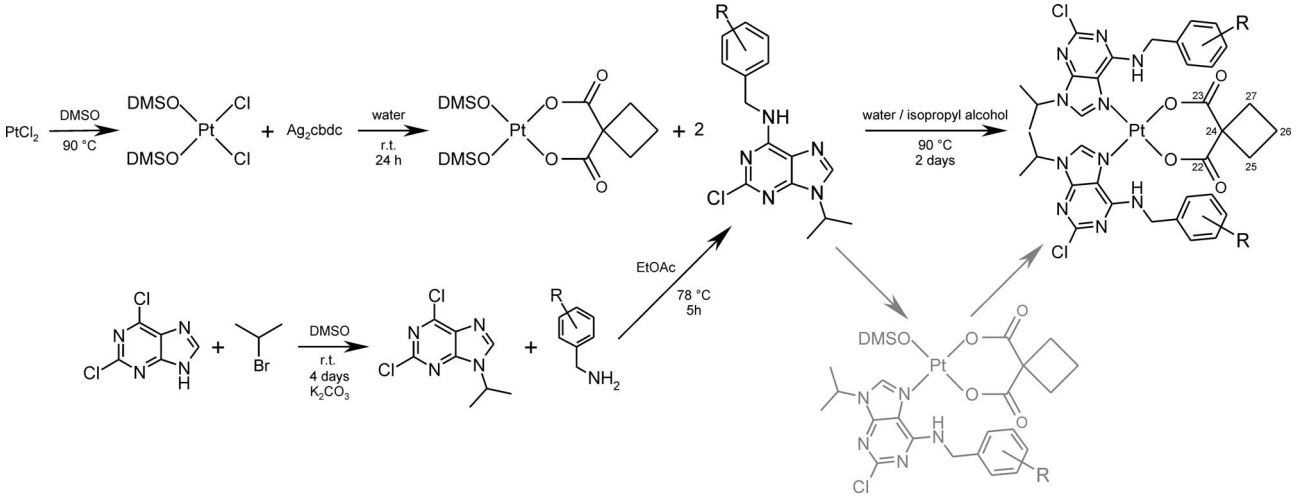


Figure 1. Molecular structure of [Pt(cbdc)(dmso)(HL$_7$)]·H$_2$O (**7a**·H$_2$O) with non-hydrogen atoms drawn as thermal ellipsoids at the 50% probability level. Hydrogen atoms have been omitted for clarity.



Scheme 1. Mechanism for the synthesis of 6-benzylamino-2-chloro-9-isopropylpurine derivatives (HL$_n$) and the [Pt(cbdc)(HL$_n$)$_2$] complexes **1**–**6** via the [Pt(cbdc)(dmso)(HL$_n$)] intermediates (shown in grey). R = 5-bromo-2-fluoro (HL$_1$; complex **1**), 3,4-dichloro (HL$_2$; **2**), 3-bromo (HL$_3$; **3**), 2-trifluoromethyl (HL$_4$; **4**), 3-trifluoromethyl (HL$_5$; **5**) and 4-trifluoromethyl (HL$_4$; **6**) derivatives of 6-benzylamino-2-chloro-9-isopropylpurine.

cases of the complexes **1**–**6**, the syntheses proceeded by a two-step reaction mechanism, the first stage involving the substitution of one dmso molecule in the starting [Pt(cbdc)(dmso)$_2$] complex by one HL$_n$ molecule to form [Pt(cbdc)(dmso)(HL$_n$)]. It is supposed that intermediates of this type are quite stable, probably due to the relative kinetic inertness of the latter complex and the intra- and intermolecular non-covalent interactions (e.g., hydrogen bonds) present both in the solid state and solution, which makes the substitution of the second dmso molecule quite difficult and the whole process longer. The described mechanism was proven by determining the molecular (Figure 1) and crystal structures of [Pt(cbdc)(dmso)(HL$_7$)]·H$_2$O (**7a**·H$_2$O). Subsequently, the second dmso ligand was substituted by another HL$_n$ molecule to form the final product [Pt(cbdc)(HL$_n$)$_2$] (see Scheme 1).

FTIR and Raman Spectroscopy

Most of the bands observed in the FTIR spectra of **1**–**6** between 640 and 900 cm^{-1} could be assigned to the purine skeletal vibrations of the coordinated HL$_n$ molecules.[12] The very strong bands detected in the 1609–1621 cm^{-1} region belong to ν(C=N)$_{ar}$ vibrations. The weak-to-medium bands observed between 3050 and 3142 cm^{-1} may be attributed to ν(C–H)$_{ar}$ vibrations, whereas the maxima of the ν(C–H)$_{ar}$ vibrations were detected in the 2873–2983 cm^{-1} region. The ν(C–Cl)$_{al}$ vibration is characterized by a band of medium or strong intensity with the maximum between 1163 and 1171 cm^{-1}. Three bands with maxima at around 1480, 1530 and 1580 cm^{-1} can be assigned to ν(C=C)$_{ar}$ vibrations. The ν_{as}(C=O) vibration can be attributed to the band observed at 1679–1680 cm^{-1}, which is the typical region for this vibration and it has previously been assigned to the carboxy groups of the cbdc dianion.[11,13] The bands observed for **1**–**6** in the 150–600 cm^{-1} region, with maxima at 540–545 and 554–563 cm^{-1}, could be assigned to the ν(Pt–N) and ν(Pt–O) stretching vibrations, respectively.[14] The presence of these two vibrations in the far-FTIR spectra indirectly confirmed the coordination of both types of organic ligands, that is, HL$_1$–HL$_6$ and cbdc, to the central Pt^{II} atom.

As can be seen from the data given in the Exp. Sect., only some of the above characteristic vibrations were detected in the Raman spectra of **2**, **3**, **5** and **6** (**1** and **4** were burnt under the laser beam). Nevertheless, it can be noted that the positions of the band maxima assignable to these vibrations for both the HL$_n$ and cbdc ligands correlate well in the FTIR and Raman spectra of the particular complexes **1**–**6**. The bands with maxima at 3063–3140 and 2870–2987 cm^{-1} can be assigned to ν(C–H)$_{ar}$ and ν(C–H)$_{al}$ vibrations, respectively.[15] The very strong skeletal vibration of the purine ring was detected in the range of 1338–1340 cm^{-1}.[16]

NMR Spectroscopy

All the signals of the free HL$_n$ molecules detected in the ^{1}H and ^{13}C NMR spectra of the appropriate starting compounds (HL$_n$) were also found in the spectra of the complexes **1**–**6**. However, most of these signals were shifted as a consequence of the coordination of HL$_n$ to the Pt^{II} atom and the formation of the final products **1**–**6**. The highest coordination shifts ($\Delta\delta = \delta_{complexes} - \delta_{ligand}$) were found for the 8-H and 6-H signals in the ^{1}H NMR spectra; these were shifted significantly more than for the other proton signals. Significant coordination shifts were also observed in the ^{13}C NMR spectra for the C-5 and C-8 atoms of the purine moieties of HL$_n$: the C-5 signals are shifted upfield and the C-8 signals downfield by more than 3.0 ppm. These findings indirectly support the conclusion that the organic molecules HL$_n$ are coordinated to the Pt^{II} atom through their N-7 atoms.

^{1}H–^{15}N gs-HMBC experiments were performed on **2**–**6** (unfortunately, signals from the nitrogen atoms in the structure of **1** were not detected) to confirm the above conclusion. Table 1 summarizes the chemical and coordination shifts obtained, the values of which correlate well for the individual nitrogen atoms for all the complexes. The largest values of $\Delta\delta$ were found for the N-7 atom of the purine ring with values of around –110 ppm (Table 1). The coordination shifts for the N-1, N-3, N-6 and N-9 atoms are much smaller. These NMR results clearly prove that 6-benzylamino-2-chloro-9-isopropylpurine derivatives (HL$_n$) are coordinated to the metal centre of the prepared platinum(II) complexes through the N-7 atom.

Table 1. Results of the ^{1}H–^{15}N gs-HMBC experiments given as chemical shifts with the coordination shifts ($\Delta\delta = \delta_{complexes} - \delta_{ligand}$) given in parentheses.

	δ [ppm]				
	N1	N3	N6	N7	N9
1[a]	–	–	–	–	–
2	232.7 (5.7)	225.3 (1.2)	96.3 (8.4)	129.3 (–109.4)	185.2 (7.1)
3	232.4 (7.1)	n.o.[b]	97.4 (7.7)	129.1 (–107.7)	184.9 (8.8)
4	230.8 (6.3)	n.o.[b]	92.4 (5.9)	128.0 (–109.3)	184.1 (7.2)
5	231.8 (4.0)	223.9 (–0.3)	97.3 (5.4)	129.0 (–111.6)	185.3 (6.2)
6	232.4 (4.4)	225.0 (–0.5)	96.5 (5.3)	129.0 (–112.0)	185.4 (6.4)

[a] Signals from the N-1, N-3, N-6, N-7 and N-9 atoms of **1** were not detected. [b] The N-3 signal was not observed for **3**, HL$_3$ or **4**.

Signals characterizing the cyclobutane-1,1-dicarboxylate dianion were detected in both the ^{1}H and ^{13}C NMR spectra of **1**–**6** (see the Exp. Sect.) and these signals were refined by ^{1}H–^{13}C gs-HMQC and gs-HMBC 2D NMR experiments. The most characteristic signal of the coordinated cbdc dianion, which belongs to the C-22 and C-23 atoms of the two carboxy groups, was found at around 177.5 ppm.

The ^{195}Pt NMR spectra of **1**–**6** exhibit signals between –1631 and –1620 ppm. Note that the ^{195}Pt NMR chemical shifts of platinum(II) complexes with the formula [Pt(cbdc)(L)], in which L symbolizes two monodentate or one bidentate N-donor ligand, namely amine, cyclopentylamine (cpa), 1,2-ethylenediamine (en), 1,2-diaminopropane (meen), *N*,*N*-dimethylethylenediamine (Me$_2$en) and 1,2-diaminocyclohexane (dach), range from –1968 to –1647 ppm.[17] Thus, it can be said that the shifts for **1**–**6** approach the upper limit of ^{195}Pt NMR chemical shifts of

the cited complexes. Moreover, these values are also similar to those obtained for oxalatoplatinum(II) complexes (ca. –1690 ppm) involving 6-benzylamino-2-chloro-9-isopropylpurine-based N-donor ligands.[6b]

Single-Crystal X-ray Analysis of [Pt(cbdc)(dmso)(HL_7)]·H_2O (**7a**·H_2O)

Attempts to prepare single crystals of platinum(II) complexes **1**–**6** suitable for single-crystal X-ray analysis were unsuccessful. Nevertheless, very important findings about the compositions and coordination modes of these complexes were obtained by analysis of the [Pt(cbdc)(dmso)(HL_7)]·H_2O (**7a**·H_2O) intermediate, the molecular (Figure 1) and crystal (Figure 2) structures of which were determined by this important method (Table 2). Selected bond lengths and angles are presented in Table 3 and non-bonding contacts are given as a footnote in Figure 2.

Table 2. Crystal data and structure refinement details for [Pt(cbdc)(dmso)(HL_7)]·H_2O (**7a**·H_2O).

Molecular formula	$C_{24}H_{32}ClN_5O_7PtS$
Formula weight	765.15
Temperature [K]	120(2)
Wavelength [Å]	0.71073
Crystal system	orthorhombic
Space group	*Pbca*
Unit cell dimensions	
a [Å]	13.8086(3)
b [Å]	14.4052(3)
c [Å]	28.3693(5)
α [°]	90
β [°]	90
γ [°]	90
V [Å^3]	5643.1(2)
Z, $D_{calcd.}$ [g cm^{-3}]	8, 1.801
Absorption coefficient [mm^{-1}]	5.193
Crystal size [mm]	0.40 × 0.35 × 0.30
F (000)	3024
θ range for data collection [°]	2.83 ≤ *θ* ≤ 25.00
Index ranges (*h, k, l*)	–16 ≤ *h* ≤ 16
	–17 ≤ *k* ≤ 12
	–32 ≤ *l* ≤ 33
Reflections collected/unique (R_{int})	44469/4960 (0.0393)
Max./min. transmission	0.3049/0.2305
Data/restraints/parameters	4960/0/363
Goodness-of-fit on F^2	1.256
Final *R* indices [$I > 2\sigma(I)$]	R_1 = 0.0285, wR_2 = 0.0616
R indices (all data)	R_1 = 0.0334, wR_2 = 0.0639
Largest peak/hole [e Å^{-3}]	0.822/–1.789

The [Pt(cbdc)(dmso)(HL_7)]·H_2O (**7a**·H_2O) complex has a distorted square-planar arrangement with a $PtNO_2S$ donor set and it contains one water molecule of crystallization (Figure 1). The donor atoms originate from 2-chloro-6-(2-methoxybenzyl)amino-9-isopropylpurine (HL_7), the bidentate-coordinated cyclobutane-1,1-dicarboxylate dianion and the *S*-coordinated dmso molecule. The coordination site of HL_7 is the N(7) atom of the purine moiety.

The HL_7 molecule consists of three aromatic systems, benzene (A), pyrimidine (B) and imidazole (C), with the biggest deviations from planarity being 0.003(5) Å for C(13), 0.018(4) Å for N(1) and 0.012(4) Å for C(5). The torsion angles C(6)–N(6)–C(9)–C(10), C(5)–C(6)–N(6)–C(9) and N(6)–C(9)–C(10)–C(15) are equal to 165.1(4), 176.6(4) and –120.6(5)°, respectively. The dihedral angle formed by the benzene and purine rings is 53.62(13)°. Moreover, the dihedral angle between the purine ring and the basal plane formed by the atoms of the $PtNO_2S$ donor set was determined to be 87.34(8)°. The cyclobutane-1,1-dicarboxylate dianion is coordinated to the metal centre through its O(1) and O(3) atoms. The Pt–O bond lengths determined for **7a**·H_2O (Table 3) correlate well with those of the cyclobutane-1,1-dicarboxylatoplatinum(II) complexes deposited in the CSD,[8] which range from 1.977 to 2.065 Å (mean of 2.016 Å). The dihedral angle, typical of platinum(II) complexes coordinated to the cbdc dianion, formed by the basal plane ($PtNO_2S$ donor set) and the cyclobutane ring, is equal to 79.5(2)°. The dimethyl sulfoxide molecule is coordinated to the Pt^{II} atom through its S(1) atom.

Table 3. Selected bond lengths and angles for **7a**·H_2O.

Bond lengths [Å]		Bond angles [°]	
Pt(1)–O(1)	2.018(3)	O(1)–Pt(1)–O(3)	89.89(13)
Pt(1)–O(3)	2.004(3)	O(1)–Pt(1)–N(7)	88.56(15)
Pt(1)–N(7)	2.011(4)	O(1)–Pt(1)–S(1)	178.00(11)
Pt(1)–S(1)	2.1819(12)	O(3)–Pt(1)–N(7)	178.45(15)
N(1)–C(2)	1.331(6)	O(3)–Pt(1)–S(1)	88.94(10)
N(1)–C(6)	1.346(6)	N(7)–Pt(1)–S(1)	92.60(12)
C(2)–N(3)	1.312(6)	Pt(1)–N(7)–C(5)	128.0(3)
N(3)–C(4)	1.353(6)	Pt(1)–N(7)–C(8)	125.3(3)
C(4)–C(5)	1.367(6)	Pt(1)–O(1)–C(22)	119.9(3)
C(4)–N(9)	1.372(6)	Pt(1)–O(3)–C(23)	119.8(3)
C(5)–C(6)	1.407(6)	Pt(1)–S(1)–C(20)	107.2(2)
C(5)–N(7)	1.380(6)	Pt(1)–S(1)–C(21)	108.7(2)
N(7)–C(8)	1.313(6)	Pt(1)–S(1)–O(6)	118.7(2)
C(8)–N(9)	1.350(6)	C(5)–N(7)–C(8)	106.6(4)
O(1)–C(22)	1.302(6)	N(7)–C(5)–C(4)	108.3(4)
O(3)–C(23)	1.308(6)	N(7)–C(5)–C(6)	134.3(4)
C(22)–O(2)	1.218(6)	N(7)–C(8)–C(9)	111.4(4)
C(22)–C(24)	1.520(7)	O(1)–C(22)–O(2)	121.3(5)
C(23)–O(4)	1.216(6)	O(1)–C(22)–C(24)	117.2(4)
C(23)–C(24)	1.525(7)	O(3)–C(23)–O(4)	121.1(5)
S(1)–C(20)	1.763(6)	O(3)–C(23)–C(24)	117.7(4)
S(1)–C(21)	1.745(6)		
S(1)–O(6)	1.455(4)		

A network of intra- and intermolecular hydrogen bonds and non-covalent contacts stabilize the crystal structure of **7a**·H_2O (Figure 2). As a consequence of the intramolecular N(6)–H(6)···O(5) hydrogen bond [*d*(D–H) = 0.8800 Å, *d*(H···A) = 2.435(4) Å, *d*(D···A) = 2.815(5) Å and <(DHA) = 106.5(3)°], the methoxy group is orientated towards the imidazole ring. The water molecule is involved in hydrogen bonds of the O–H···O type with the O(2) and O(4) atoms of two different **7a** molecules to form the following hydrogen bonds: O(7)–H(7W)···O(4)^{i} [*d*(D–H) = 0.89(7) Å, *d*(H···A) = 1.93(7) Å, *d*(D···A) = 2.767(6) Å and <(DHA) = 157(6)°] and O(7)–H(7V)···O(4)^{ii} [*d*(D–H) = 0.81(7) Å, *d*(H···A) = 2.00(7) Å, *d*(D···A) = 2.806(6) Å and <(DHA) = 168(6)°] [Figure 2; symmetry codes: (i) 1 – *x*, *y* + 0.5, 0.5 – *z*; (ii) *x* + 0.5, *y*, 0.5 – *z*]. Finally, C–H···O, C–H···Cl, C···Cl and Cl···O non-covalent contacts were detected in the crystal structure of the complex **7a**·H_2O and their parameters are given in Table S1 of the Supporting Information.

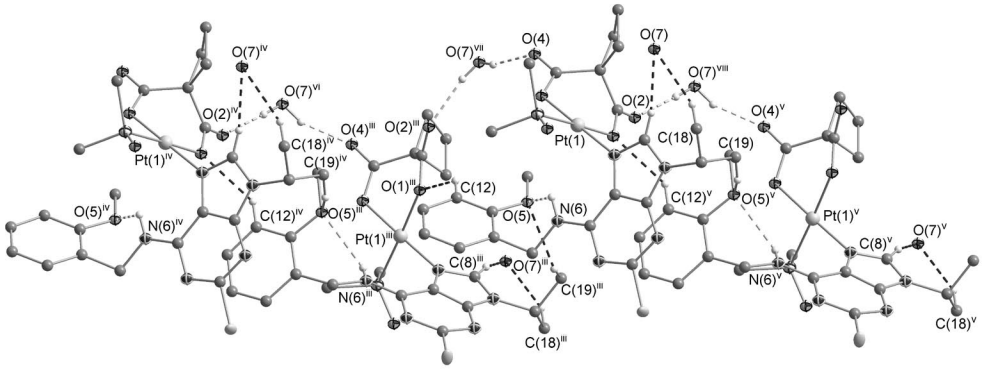


Figure 2. Part of the crystal structure of [Pt(cbdc)(dmso)(HL_7)]·H_2O (**7a**·H_2O) showing N–H···O and O–H···O hydrogen bonds (dashed light-grey lines) and C–H···O non-covalent contacts (dashed dark-grey lines). Hydrogen atoms not involved in hydrogen bonds have been omitted for clarity. Symmetry codes: (iii) 0.5 – *x*, *y* – 0.5, *z*; (iv) *x*, *y* – 1, *z*; (v) 0.5 – *x*, *y* + 0.5, *z*; (vi) *x* – 0.5, *y* – 1, 0.5 – *z*; (vii) 1 – *x*, *y* – 0.5, 0.5 – *z*; (viii) *x* – 0.5, *y*, 0.5 – *z*.

In Vitro Cytotoxic Activity

The prepared complexes **1**–**6** were tested for their in vitro cytotoxic activity by performing a calcein AM assay on the chronic myelogenous leukaemia (K-562) and breast adenocarcinoma (MCF7) human cancer cell lines (Table 4).

Table 4. IC_{50} values (μM) of the in vitro cytotoxicity of the complexes **1**–**6** and platinum-based anticancer drugs against K-562 (chronic myelogenous leukaemia) and MCF7 (breast adenocarcinoma) human cancer cell lines; the cells were exposed to the compounds for 72 h; experiments were repeated three-times.

Complex	IC_{50} [μM]		Ref.
	K-562	MCF7	
1	9.5 ± 2.5	9.0 ± 2.3	this work
2	4.8 ± 0.6	4.3 ± 0.2	this work
3	4.5 ± 1.0	5.0 ± 0.3	this work
4	7.0 ± 1.3	7.9 ± 2.3	this work
5	14.1 ± 3.3	21.0 ± 1.4	this work
6	7.5 ± 1.9	19.2 ± 8.6	this work
Cisplatin	4.7	10.9	[18a]
Carboplatin	60.0	250.0	[18b,18c]
Oxaliplatin	8.8	18.2	[18a]

The results reveal very promising cytotoxic activities with IC_{50} values in the range of 4.5–14.1 and 4.3–21.0 μM for the K-562 and MCF7 cell lines, respectively. In the case of the K-562 cells, complexes **2** and **3** have an in vitro cytotoxicity comparable to that of cisplatin. Moreover, these compounds, together with **4** and **6**, exceed the activity of oxaliplatin and all the tested substances are significantly more effective than carboplatin. As for the MCF7 human cancer cells, **1**–**6** are again more cytotoxic than carboplatin. The in vitro cytotoxic activities of the complexes **1**–**4** were evaluated as even higher than those of commercially used platinum-based drugs cisplatin and oxaliplatin.

Conclusions

The seven novel [Pt(cbdc)(HL_n)$_2$] (**1**–**6**) and [Pt(cbdc)(dmso)(HL_7)]·H_2O (**7a**·H_2O) cyclobutane-1,1-dicarboxylatoplatinum(II) complexes derived from carboplatin have been prepared by a synthetic strategy with [Pt(cbdc)(dmso)$_2$] as a key intermediate. The products obtained were fully characterized. Based on the results of various physicochemical techniques it can be concluded that the central Pt^{II} atom is tetracoordinated to two unidentate N-donor carrier ligands derived from 6-benzylamino-2-chloro-9-isopropylpurine (HL_1–HL_6) and the bidentate O-donor cyclobutane-1,1-dicarboxylate dianion (cbdc). Multinuclear and two-dimensional NMR studies proved the N-7 atom to be the coordination site of the HL_n ligands, which was also confirmed by single-crystal X-ray analysis of the [Pt(cbdc)(dmso)(HL_7)]·H_2O (**7a**·H_2O) intermediate. IC_{50} values for the in vitro cytotoxicity of the prepared platinum(II) complexes showed that several of the prepared complexes can be considered to have comparable or even higher in vitro cytotoxicity than the platinum-based anticancer drugs, that is, cisplatin, carboplatin and oxaliplatin.

Experimental Section

Materials: The chemicals and solvents were purchased from commercial sources, namely Sigma–Aldrich, Acros Organics, Lachema and Fluka, and they were used as received.

The 6-benzylamino-2-chloro-9-isopropylpurine derivatives (HL_n; see Scheme S1 in the Supporting Information) were synthesized from 2,6-dichloropurine according to previously reported procedures for the preparation of the 2,6,9-trisubstituted purine derivatives.[10] The synthetic strategy is given in Scheme 1. The molecules of HL_1–HL_6 were characterized by elemental analysis, melting-point determination and FTIR, Raman and NMR (^1H, ^{13}C and ^{15}N) spectroscopy. The results can be found in the Supporting Information. Yields ranged from 76–88%.

Methods: Chemical analysis (C, H, N) was performed with a Flash EA1112 Elementar Analyzer (ThermoFinnigan). The purities of the prepared organic compounds HL_1–HL_7 were determined with a Beckman HPLC. Melting points were determined with a Büchi B-540 melting-point apparatus with a temperature gradient of 2 °C min^{-1}. The FTIR spectra were obtained with KBr pellets (400–4000 cm^{-1}) and by using the Nujol technique (150–600 cm^{-1}) with a Nexus 670 FT–IR device (ThermoNicolet). Raman spectroscopy was performed on the complexes **2**, **3**, **5** and **6** (**1** and **4** burned

under the laser beam) in the 150–3750 cm^{-1} region with a FT–Raman Nicolet NXR 9650 spectrometer with a liquid-nitrogen-cooled NXE Genie germanium detector.

^1H, ^{13}C and ^{195}Pt NMR spectra as well as ^1H–^1H gs-COSY, ^1H–^{13}C gs-HMQC and ^1H–^{13}C gs-HMBC ([D_7]DMF solutions) data were collected with a Bruker Avance 300 spectrometer for HL_n and **1**–**6** {[Pt(cbdc)(dmso)$_2$] is insufficiently soluble in the used solvent}. ^1H–^{15}N gs-HMBC experiments were performed on complexes **2**–**6** (at natural abundance) using the same device. Spectra were calibrated against $SiMe_4$ (for ^1H and ^{13}C) and K_2PtCl_4 (D_2O; for ^{195}Pt, –1628 ppm) and against the residual signals of the solvent (for ^{15}N, 104.7 ppm). The multiplicities in the proton spectra are defined as: s = singlet, d = doublet, t = triplet, quint = quintet, sept = septet, dd = doublet of doublets and m = multiplet.

Synthesis of [Pt(cbdc)(dmso)$_2$]: The silver(I) salt of cyclobutane-1,1-dicarboxylic acid (Ag_2cbdc) and *cis*-[$PtCl_2$(dmso)$_2$] were synthesized according to previously reported methods with cyclobutane-1,1-dicarboxylic acid (cbdca) and $PtCl_2$ used as starting compounds.[17a,19] The prepared *cis*-[$PtCl_2$(dmso)$_2$] was dissolved in distilled water and an equimolar amount of Ag_2cbdc was added to the solution.[11] The mixture was stirred in darkness at room temperature for 24 h. The solvent was evaporated and white crystals of [Pt(cbdc)(dmso)$_2$] formed (Scheme 1). Yield 88%. IR (Nujol): $\tilde{\nu}$ = 567 (s, PtO) cm^{-1}. IR (KBr): $\tilde{\nu}$ = 1663 (CO) cm^{-1}. $C_{10}H_{18}O_6PtS_2$ (493.46): calcd. C 24.34, H 3.68; found C 24.42, H 3.96. M.p. 200–202 °C.

Synthesis of [Pt(cbdc)(HL$_n$)$_2$] 1–6: [Pt(cbdc)(dmso)$_2$] (0.2 mmol) was dissolved in distilled water (15 mL) and the appropriate 6-benzylamino-2-chloro-9-isopropylpurine derivative (HL_n; 0.4 mmol) suspended in isopropyl alcohol (20 mL) was added (Scheme 1).[11] The mixture was stirred at 90 °C and a light-grey precipitate formed in 2 days. The product was removed by filtration, washed with cold distilled water and dried in the desiccator over silica gel.

[Pt(cbdc)(HL$_1$)$_2$] (1): IR (Nujol): $\tilde{\nu}$ = 563 (vs, PtO), 541 (vs, PtN) cm^{-1}. IR (KBr): $\tilde{\nu}$ = 3142 (w), 3095 (w), 3053 (w, CH_{ar}), 2980 (m), 2933 (w), 2873 (w, CH_{al}), 1679 (m, CO), 1621 (vs, CN), 1583 (s), 1540 (m), 1484 (s, CC), 1171 (m, CCl) cm^{-1}. ^1H NMR ([D_7]-DMF): δ = 9.38 (t, J = 6.4 Hz, 1 H, 6-H), 9.08 (s, 1 H, 8-H), 7.59 (s, 1 H, 15-H), 7.49 (d, J = 8.4 Hz, 1 H, 13-H), 7.32 (d, J = 7.7 Hz, 1 H, 12-H), 4.96 (d, J = 6.0 Hz, 2 H, 9-H), 4.87 (sept, J = 6.7 Hz, 1 H, 17-H), 2.86 (m, 2 H, 25-H^a, 27-H^a), 1.80 (m, 1 H, 26-H^a), 1.67 (m, 2 H, 25-H^b, 27-H^b), 1.63 (d, J = 6.8 Hz, 6 H, 18-H, 19-H), 1.48 (m, 1 H, 26-H^b) ppm. ^{13}C NMR ([D_7]DMF): δ = 177.4 (C-22, C-23), 155.3 (C-6), 153.7 (C-2), 150.1 (C-4), 144.0 (C-8), 141.9 (C-10), 132.3 (C-11), 130.8 (C-14), 130.6 (C-12), 129.7 (C-13), 128.8 (C-15), 116.5 (C-5), 56.8 (C-24), 49.9 (C-17), 44.3 (C-9), 31.4 (C-25, C-27), 22.1 (C-18, C-19), 15.9 (C-26) ppm. ^{195}Pt NMR: δ = –1626 ppm. $C_{36}H_{34}Br_2Cl_2F_2N_{10}O_4Pt$ (1134.51): calcd. C 38.11, H 3.02, N 12.35; found C 37.74, H 2.82, N 12.57.

[Pt(cbdc)(HL$_2$)$_2$] (2): IR (Nujol): $\tilde{\nu}$ = 563 (v, PtO) cm^{-1}. IR (KBr): $\tilde{\nu}$ = 2980 (w, CH_{al}), 1633 (vs, CN), 1586 (m, CC), 1169 (m, CCl) cm^{-1}. Raman: $\tilde{\nu}$ = 3123 (w), 3064 (m, CH_{ar}), 2982 (s), 2942 (vs), 2873 (w, CH_{al}), 1587 (s), 1535 (w), 1483 (m, CC), 1164 (m, CCl), 565 (w, PtO) cm^{-1}. ^1H NMR ([D_7]DMF): δ = 9.44 (t, J = 6.4 Hz, 1 H, 6-H), 9.32 (s, 1 H, 8-H), 7.68 (d, J = 1.8 Hz, 1 H, 11-H), 7.58 (dd, J = 8.4, 1.8 Hz, 1 H, 14-H), 7.53 (d, J = 8.4 Hz, 1 H, 15-H), 4.96 (d, J = 6.2 Hz, 2 H, 9-H), 4.84 (sept, J = 6.8 Hz, 1 H, 17-H), 2.90 (m, 2 H, 25-H^a, 27-H^a), 1.80 (quint, J = 7.7 Hz, 1 H, 26-H^a), 1.67 (m, 2 H, 25-H^b, 27-H^b), 1.62 (d, J = 6.8 Hz, 6 H, 18-H, 19-H), 1.47 (m, 1 H, 26-H^b) ppm. ^{13}C NMR ([D_7]DMF): δ = 177.6 (C-22, C-23), 155.2 (C-6), 153.9 (C-2), 150.3 (C-4), 143.9 (C-8), 140.9 (C-10), 132.1 (C-12), 131.1 (C-15), 130.6 (C-13), 130.0 (C-11), 128.5 (C-14), 116.7 (C-5), 56.8 (C-24), 49.8 (C-17), 44.1 (C-9), 31.4 (C-25, C-27), 22.1 (C-18, C-19), 15.8 (C-26) ppm. ^{15}N NMR ([D_7]DMF): δ = 232.7 (N-1), 225.3 (N-3), 185.2 (N-9), 129.3 (N-7), 96.3 (N-6) ppm. ^{195}Pt NMR: δ = –1622 ppm. $C_{36}H_{34}Cl_6N_{10}O_4Pt$ (1078.51): calcd. C 40.09, H 3.18, N 12.99; found C 39.89, H 3.11, N 13.20.

[Pt(cbdc)(HL$_3$)$_2$] (3): IR (Nujol): $\tilde{\nu}$ = 554 (vs, PtO), 540 (vs, PtN) cm^{-1}. IR (KBr): $\tilde{\nu}$ = 3138 (w), 3092 (w), 3054 (w, CH_{ar}), 2978 (m), 2936 (m), 2878 (w, CH_{al}), 1679 (s, CO), 1620 (vs, CN), 1584 (vs), 1524 (m), 1477 (s, CC), 1166 (m, CCl) cm^{-1}. Raman: $\tilde{\nu}$ = 3140 (w), 3063 (m, CH_{ar}), 2983 (s), 2942 (s), 2870 (w, CH_{al}), 1679 (w, CO), 1583 (s), 1537 (w), 1484 (m, CC), 1166 (m, CCl), 540 (w, PtN) cm^{-1}. ^1H NMR ([D_7]DMF): δ = 9.42 (t, J = 6.6 Hz, 1 H, 6-H), 9.31 (s, 1 H, 8-H), 7.65 (t, J = 1.7 Hz, 1 H, 11-H), 7.58 (dd, J = 7.9, 1.3 Hz, 1 H, 15-H), 7.47 (dd, J = 7.9, 2.0 Hz, 1 H, 13-H), 7.28 (t, J = 7.9 Hz, 1 H, 14-H), 4.94 (d, J = 6.4 Hz, 2 H, 9-H), 4.85 (sept, J = 6.8 Hz, 1 H, 17-H), 2.89 (m, 2 H, 25-H^a, 27-H^a), 1.79 (m, 1 H, 26-H^a), 1.69 (m, 2 H, 25-H^b, 27-H^b), 1.62 (d, J = 6.7 Hz, 6 H, 18-H, 19-H), 1.48 (m, 1 H, 26-H^b) ppm. ^{13}C NMR ([D_7]-DMF): δ = 177.5 (C-22, C-23), 155.2 (C-6), 153.9 (C-2), 150.0 (C-4), 144.3 (C-8), 142.5 (C-10), 131.1 (C-13), 130.9 (C-14), 130.6 (C-15), 127.2 (C-11), 122.5 (C-12), 116.6 (C-5), 56.8 (C-24), 49.8 (C-17), 44.6 (C-9), 31.4 (C-25, C-27), 22.1 (C-18, C-19), 15.8 (C-26) ppm. ^{195}Pt NMR: δ = –1621 ppm. $C_{36}H_{36}Br_2Cl_2N_{10}O_4Pt$ (1098.53): calcd. C 39.36, H 3.30, N 12.75; found C 39.76, H 3.11, N 13.04.

[Pt(cbdc)(HL$_4$)$_2$] (4): IR (Nujol): $\tilde{\nu}$ = 562 (vs, PtO), 540 (vs, PtN) cm^{-1}. IR (KBr): $\tilde{\nu}$ = 3095 (w), 3050 (w, CH_{ar}), 2982 (m), 2939 (w), 2883 (w, CH_{al}), 1679 (m, CO), 1621 (vs, CN), 1583 (s), 1536 (w), 1482 (m, CC), 1163 (s, CCl) cm^{-1}. ^1H NMR ([D_7]DMF): δ = 9.44 (t, J = 6.4 Hz, 1 H, 6-H), 9.42 (s, 1 H, 8-H), 7.74 (m, 3 H, 13-H, C14-H, 15-H), 7.48 (m, 1 H, 12-H), 5.64 (d, J = 6.0 Hz, 2 H, 9-H), 4.88 (sept, J = 7.0 Hz, 1 H, 17-H), 2.90 (m, 2 H, 25-H^a, 27-H^a), 1.80 (m, 1 H, 26-H^a), 1.66 (d, J = 6.8 Hz, 6 H, 18-H, 19-H), 1.61 (m, 2 H, 25-H^b, 27-H^b), 1.43 (m, 1 H, 26-H^b) ppm. ^{13}C NMR ([D_7]DMF): δ = 177.7 (C-22, C-23), 155.2 (C-6), 154.1 (C-2), 150.1 (C-4), 143.7 (C-8), 138.1 (C-10), 133.1 (C-14), 128.2 (C-15), 127.7 (C-13), 127.4, 123.8 (C-16), 126.7 (C-12), 116.6 (C-5), 56.9 (C-24), 49.9 (C-17), 41.4 (C-9), 31.5 (C-25, C-27), 22.1 (C-18, C-19), 15.8 (C-26) ppm. ^{195}Pt NMR: δ = –1631 ppm. $C_{38}H_{36}Cl_2F_6N_{10}O_4Pt$ (1076.73): calcd. C 42.39, H 3.37, N 13.01; found C 42.33, H 3.28, N 13.19.

[Pt(cbdc)(HL$_5$)$_2$] (5): IR (Nujol): $\tilde{\nu}$ = 560 (m, PtO), 542 (s, PtN) cm^{-1}. IR (KBr): $\tilde{\nu}$ = 3093 (w), 3050 (w, CH_{ar}), 2982 (w), 2941 (w), 2884 (w, CH_{al}), 1680 (m, CO), 1609 (vs, CN), 1583 (s), 1537 (w), 1484 (m, CC), 1166 (s, CCl) cm^{-1}. Raman: $\tilde{\nu}$ = 3072 (s, CH_{ar}), 2987 (vs), 2946 (vs, CH_{al}), 1609 (w, CN), 1579 (s), 1538 (m), 1480 (m, CC), 1170 (w, CCl) cm^{-1}. ^1H NMR ([D_7]DMF): δ = 9.48 (t, J = 6.2 Hz, 1 H, 6-H), 9.31 (s, 1 H, 8-H), 7.88 (d, J = 7.9 Hz, 1 H, 15-H), 7.84 (s, 1 H, 11-H), 7.65 (d, J = 7.9 Hz, 1 H, 13-H), 7.55 (t, J = 7.9 Hz, 1 H, 14-H), 5.03 (d, J = 6.2 Hz, 2 H, 9-H), 4.83 (sept, J = 6.8 Hz, 1 H, 17-H), 2.89 (t, J = 8.0 Hz, 4 H, 25-H, 27-H), 1.78 (quint, J = 8.0 Hz, 2 H, 26-H), 1.61 (d, J = 6.8 Hz, 6 H, 18-H, 19-H) ppm. ^{13}C NMR ([D_7]DMF): δ = 177.6 (C-22, C-23), 155.1 (C-6), 154.0 (C-2), 150.0 (C-4), 144.8 (C-8), 141.2 (C-10), 132.3 (C-15), 130.8, 130.4, 130.0, 129.6 (C-12), 130.1 (C-14), 130.7, 127.1, 123.5, 119.9 (C-16), 125.1, 125.0 (C-11), 124.5, 126.4 (C-13), 116.6 (C-5), 56.8 (C-24), 49.8 (C-17), 44.8 (C-9), 31.4 (C-25, C-27), 22.1 (C-18, C-19), 15.8 (C-26) ppm. ^{195}Pt NMR: δ = –1620 ppm. $C_{38}H_{36}Cl_2F_6N_{10}O_4Pt$ (1076.73): calcd. C 42.39, H 3.37, N 13.01; found C 42.01, H 3.34, N 13.26.

[Pt(cbdc)(HL$_6$)$_2$] (6): IR (Nujol): $\tilde{\nu}$ = 560 (m, PtO), 545 (m, PtN) cm^{-1}. IR (KBr): $\tilde{\nu}$ = 3142 (w), 3097 (w), 3055 (w, CH_{ar}), 2983

(w), 2939 (w), 2883 (w, CH_{al}), 1679 (m, CO), 1618 (vs, CN), 1585 (s), 1530 (w), 1483 (m, CC), 1167 (m, CCl) cm^{-1}. Raman: $\tilde{\nu}$ = 3072 (s, CH_{ar}), 2984 (s), 2945 (vs), 2876 (w, CH_{al}), 1679 (w, CO), 1618 (m, CN), 1582 (s), 1534 (m), 1483 (m, CC), 1164 (m, CCl), 549 (w, PtN) cm^{-1}. ^1H NMR ([D$_7$]DMF): δ = 9.47 (t, J = 6.2 Hz, 1 H, 6-H), 9.35 (s, 1 H, 8-H), 7.75 (d, J = 8.2 Hz, 2 H, 11-H, 15-H), 7.65 (d, J = 8.2 Hz, 2 H, 12-H, 14-H), 5.02 (d, J = 6.2 Hz, 2 H, 9-H), 4.84 (sept, J = 6.8 Hz, 1 H, 17-H), 2.90 (t, J = 8.0 Hz, 4 H, 25-H, 27-H), 1.80 (quint, J = 8.0 Hz, 2 H, 26-H), 1.62 (d, J = 6.8 Hz, 6 H, 18-H, 19-H) ppm. ^{13}C NMR ([D$_7$]DMF): δ = 177.7 (C-22, C-23), 155.2 (C-6), 154.0 (C-2), 150.0 (C-4), 144.6 (C-10), 143.8 (C-8), 130.8, 127.2, 123.6 (C-16), 129.4, 129.0, 128.6, 128.1 (C-13), 128.7 (C-11, C-15), 125.8, 125.8 (C-12, C-14), 116.7 (C-5), 56.8 (C-24), 49.8 (C-17), 44.6 (C-9), 31.4 (C-25, C-27), 22.1 (C-18, C-19), 15.8 (C-26) ppm. ^{195}Pt NMR: δ = –1621 ppm. $C_{38}H_{36}Cl_2F_6N_{10}O_4Pt$ (1076.73): calcd. C 42.39, H 3.37, N 13.01; found C 41.93, H 3.27, N 13.36.

[Pt(cbdc)(dmso)(HL$_7$)]·H$_2$O (7a·H$_2$O): The complex was prepared using the same synthetic strategy as in the cases of **1**–**6**, but in a shorter reaction time. The powder product obtained after 24 h was removed by filtration. It was found that the product contains a mixture of [Pt(cbdc)(HL$_7$)$_2$] and [Pt(cbdc)(dmso)(HL$_7$)] (**7a**). Recrystallization of this mixture gave crystals of [Pt(cbdc)(dmso)(HL$_7$)]·H$_2$O (**7a**·H$_2$O) suitable for a single-crystal X-ray analysis.

Single-Crystal X-ray Analysis of [Pt(cbdc)(dmso)(HL$_7$)]·H$_2$O (7a·H$_2$O): Diffraction data were collected with an Xcalibur™ 2 diffractometer (Oxford Diffraction Ltd.) with Mo-K_α radiation (Monochromator Enhance, Oxford Diffraction Ltd.) and a Sapphire2 CCD detector at 100 K. Data collection and reduction were performed by using CrysAlis software.[20] The same software was used for data correction of the absorption effect by the empirical absorption correction using spherical harmonics as implemented in the SCALE3 ABSPACK scaling algorithm. Both structures were solved by direct methods using SHELXS-97 software and refined on F^2 using the full-matrix least-squares procedure (SHELXL-97).[21] Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located in a difference map and refined with the riding model [C–H: 0.95 and 0.99 Å, N–H: 0.88 Å and U_{iso}(H) = 1.2U_{eq}(CH, CH$_2$, NH) or 1.5U_{eq}(CH$_3$)]. The molecular and crystal structures were drawn using DIAMOND,[22] which was also used to interpret the additional structural parameters. The crystal data and structure refinement are summarized in Table 2.

CCDC-770266 contains 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.

In Vitro Cytotoxic Activity: Testing of the in vitro cytotoxicity was performed by a calcein acetoxymethyl (AM) assay on the breast adenocarcinoma (MCF7) and chronic myelogenous leukaemia (K-562) human cancer cell lines. Cell lines were kept in plastic tissue culture flasks and grown on Dulbecco's modified Eagle's cell culture medium (DMEM) under conditions of 37 °C, 5% CO_2 atmosphere and 100% humidity. The suspension (ca. 1.25×10^5 cells mL^{-1}) of cancer cells was distributed between 96-well microtitre plates (Nunc) and preincubated (12 h). Diluted DMF solutions of the complexes **1**–**6** (final DMF concentration of 0.6%) were added to the suspensions of the cancer cells in concentrations of between 0.2 and 25 μM. After 72 h of incubation, the cells were incubated for another hour with calcein AM. The fluorescence of the live cells was measured at 485/538 nm (excitation/emission) with Fluoroskan Ascent (Labsystems). Experiments were repeated three times and the IC_{50} values are given in Table 4 together with their standard deviations.

Supporting Information (see also the footnote on the first page of this article): The structural formulae of the 6-benzylamino-2-chloro-9-isopropylpurine derivatives (HL$_n$), the results of elemental analyses, melting-point determinations and FTIR, Raman and NMR data (^1H, ^{13}C and ^{15}N).

Acknowledgments

This research was supported by the The Ministry of Education, Youth and Sports of the Czech Republic (Grant No. MSM6198959218). The authors thank Dr. Miroslava Matiková-Maľarová for measurement of the FTIR and Raman spectra and Dr. Vladimír Kryštof and Mrs. Dita Parobková for in vitro cytotoxicity testing.

[1] B. Rosenberg, V. L. Camp, T. Krigas, *Nature* **1965**, *205*, 698–699.
[2] R. B. Weiss, M. C. Christian, *Drugs* **1993**, *46*, 360–365.
[3] M. J. Abrams, B. A. Murrer, *Science* **1993**, *261*, 725–730.
[4] F. Skoog, H. Q. Hamzi, A. M. Szweykowska, N. J. Leopard, K. L. Carraway, T. Fujii, J. P. Helgeson, R. N. Loeppky, *Phytochemistry* **1967**, *6*, 1169–1192.
[5] a) V. Brun, M. Legraverend, D. S. Grierson, *Tetrahedron Lett.* **2001**, *42*, 8161–8164; b) V. Brun, M. Legraverend, D. S. Grierson, *Tetrahedron Lett.* **2001**, *42*, 8165–8167.
[6] a) L. Szűčová, Z. Trávníček, I. Popa, J. Marek, *Polyhedron* **2008**, *27*, 2710–2720; b) P. Štarha, Z. Trávníček, I. Popa, *J. Inorg. Biochem.*, doi:10.1016/j.jinorgbio.2010.02.005, in press.
[7] S. Shamsuddin, M. S. Ali, K. H. Whitmire, A. R. Khokhar, *Polyhedron* **2007**, *26*, 637–644.
[8] F. A. Allen, *Acta Crystallogr., Sect. B: Struct. Sci.* **2002**, *58*, 380–388.
[9] a) M. J. Xie, Y. Yu, W. P. Liu, S. Q. Hou, Q. S. Ye, *Acta Crystallogr., Sect. E: Struct. Rep. Online* **2007**, *63*, m2589; b) M. S. Ali, J. H. Thurston, K. H. Whitmire, A. R. Khokhar, *Polyhedron* **2002**, *21*, 2659–2665.
[10] a) G. B. Brown, V. S. Weliky, *J. Org. Chem.* **1958**, *23*, 125–126; b) C. H. Oh, S. C. Lee, K. S. Lee, E. R. Woo, C. Y. Hong, B. S. Yang, D. J. Baek, J. H. Cho, *Arch. Pharm. Pharm. Med. Chem.* **1999**, *332*, 187–190.
[11] P. Bitha, G. O. Morton, T. S. Dunne, E. F. D. Santos, Y. Lin, S. R. Boone, R. C. Haltiwanger, C. G. Pierpoint, *Inorg. Chem.* **1990**, *29*, 645–652.
[12] C. J. Pouchert, *The Aldrich Library of Infrared Spectra*, Aldrich Chemical Company Press, Milwaukee, WI, **1981**.
[13] G. Bernhardt, H. Brunner, N. Gruber, C. Lottner, S. K. Pushpan, T. Tsuno, M. Zabel, *Inorg. Chim. Acta* **2004**, *357*, 4452–4466.
[14] K. Nakamoto *Infrared and Raman Spectra of Inorganic and Coordination Compounds Part B: Applications in Coordination, Organometallic and Bioinorganic Chemistry*, Wiley, New York, **1991**.
[15] a) H. Poel, G. Koten, K. Vrieze, *Inorg. Chem.* **1980**, *19*, 1145–1151; b) L. D. Via, O. Gia, S. M. Magno, A. Dolmella, D. Marton, V. Di Noto, *Inorg. Chim. Acta* **2006**, *359*, 4197–4206.
[16] Z. Dhaouadi, M. Ghomi, J. C. Austin, R. B. Girling, R. E. Hester, P. Mojzes, L. Chinsky, P. Y. Turpin, C. Coulombeau, H. Jobic, J. Tomkinson, *J. Phys. Chem.* **1993**, *97*, 1074–1084.
[17] a) F. D. Rochon, G. Massarweh, *Inorg. Chim. Acta* **2006**, *359*, 4095–4104; b) M. Becker, R. E. Port, H. J. Zabel, W. J. Zeller, P. Bachert, *J. Magn. Reson.* **1998**, *133*, 115–122.
[18] a) Z. Trávníček, I. Popa, M. Čajan, R. Zbořil, V. Kryštof, J. Mikulík, *J. Inorg. Biochem.* **2010**, *104*, 405–417; b) M. Carland, K. J. Tan, J. M. White, J. Stephenson, V. Murray, W. A. Denny, W. D. McFadyen, *J. Inorg. Biochem.* **2005**, *99*, 1738–1743; c)

A. R. Ghezzi, M. Aceto, C. Cassino, E. Gabano, D. Osella, *J. Inorg. Biochem.* **2004**, *98*, 73–78.
[19] J. H. Price, A. N. Williamson, R. F. Schramm, B. B. Wayland, *Inorg. Chem.* **1972**, *11*, 1280–1284.
[20] *CrysAlis CCD* and *CrysAlis RED*, version 1.171.33.52, Oxford Diffraction Ltd., Abingdon, England, **2009**.
[21] G. M. Sheldrick, *Acta Crystallogr., Sect. A* **2008**, *64*, 112–122.
[22] K. Brandenburg, *DIAMOND*, rel. 3.1f, Crystal Impact GbR, Bonn, **2006**.

Received: March 22, 2010
Published Online: June 15, 2010

