Full Paper

Platinum Complexes with 5-Methyl-5(4-pyridyl)hydantoin and Its 3-Methyl Derivatives: Synthesis and Cytotoxic Activity – Quantitative Structure-Activity Relationships

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3,5-Dimethyl-5-(4-pyridyl)hydantoin (L) and its platinum(II) and platinum(IV) complexes with the general formula *cis*-$[PtL_2X_2] \cdot n\ H_2O$ and $[PtL_2Cl_4]$, where X = Cl, I and n = 2-4 were synthesized. A new Pt(IV) complex with 5-methyl-5-(4-pyridyl)hydantoin (L′) with the formula *cis*-$[Pt(L')_2Cl_2(OH)_2] \cdot 5\ H_2O$ was also synthesized. The novel compounds were characterized by elemental analysis, IR, ^{1}H-, ^{13}C-, ^{195}Pt-NMR spectra and molar conductivity. The cytotoxic effects of these complexes were examined on three human tumor cell lines by MTT-dye reduction assay. These four new Pt(II) and Pt(IV) complexes and a set of another twelve Pt(II), Pt(IV), and Pd(II) complexes previously synthesized and tested were compiled and a QSAR model was derived in order to direct the further rational synthesis.

Keywords: Cytotoxic activity / Hydantoins / Pt(II) and Pt(IV) complexes / QSAR

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Introduction

Cisplatin is one of the most successful anticancer drugs used for the therapeutic management of different solid tumors such as testicular, ovarian, bladder, and head and neck cancers [1]. Nevertheless its clinical use is limited by severe side effects such as nephrotoxicity, ototoxicity, neurotoxicity, debilitating nausea and vomiting, etc. [2, 3]. In order to improve the safety profile of the prototype drug a plethora of cisplatin analogs have been synthesized and tested, but only carboplatin (*cis*-diamine-(1,1-cyclobutanedicarboxylato)platinum(II) and oxaliplatin (*trans*-R,R-cyclohexane-1,2-diamine)oxalatoplatinum(II) have found wide application worldwide. To increase the efficacy and ameliorate the side effects many scientists are working in the field of platinum based anticancer drug design, whereby different amine ligands as carrier groups and various halogen ligands as leaving groups have been utilizing.

In our previous investigations we synthesized and characterized series of Pt(II), Pd(II), and Pt(IV) complexes with 5-methyl-5-(4-pyridyl)hydantoin and 3-amino-5-methyl-5-(4-pyridyl)hydantoin with the general formula *cis*-$[ML_2X_2] \cdot n\ H_2O$ and *cis*-$[PtL_2Cl_4]$, where M = Pt and Pd, L is the organic ligand, and X = Cl, Br and I. Data from the elemental analysis, IR, NMR spectral analysis and molar conductivity demonstrated the coordination mode of the ligands with metal ions. In all complexes the ligand was coordinated monodentatelly with M^{2+} and Pt^{4+} *via* N-atom from the pyridine ring [4–7].

The present study represents the synthesis and physicochemical evaluation of four new Pt(II) and Pt(IV) complexes with 3,5-dimethyl-5-(4-pyridyl)hydantoin (L) and 5-methyl-5-(4-pyridyl)hydantoin (L^1). Their *in-vitro* cytotoxic activity was assessed and compared to the clinically applied drug cisplatin. Additionally, these new Pt(II) and Pt(IV) complexes and a set of another twelve Pt(II), Pt(IV), and Pd(II) complexes, previously synthesized and tested by us, were compiled into one set and a QSAR model was derived in order to direct the further rational synthesis.

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Results and discussion

Chemistry

The new organic ligand 3,5-dimethyl-5-(4-pyridyl)hydantoin was synthesized from 5-methyl-5-(4-pyridyl)hydantoin according to [8] with some modifications. The metal complexes were prepared by using reported procedures [9–12] with minor revisions.

The elemental analysis for the new ligand 3,5-dimethyl-5-(4-pyridyl)hydantoin, its Pt(II) and Pt(IV) complexes, and the new Pt(IV) complex with 5-methyl-5-(4-pyridyl)-hydantoin were in agreement with the following formulae: $C_{10}H_{11}N_3O_2$, $Pt(C_{10}H_{11}N_3O_2)_2Cl_2 \cdot 2\,H_2O$ (**1**), $Pt(C_{10}H_{11}N_3O_2)_2I_2$ (**2**), $Pt(C_{10}H_{11}N_3O_2)_2Cl_4 \cdot 4\,H_2O$ (**3**) and $Pt(C_9H_9N_3O_2)_2Cl_2(OH)_2 \cdot 5\,H_2O$ (**4**). The determination of crystal water content in the complexes **1**, **3**, and **4** was defined by Karl Fisher titration. There was good correlation between theoretical and experimental results. In order to evaluate the mode of coordination of the ligands to the metal ions the IR, ^{1}H-, ^{13}C-, ^{195}Pt-NMR spectra of the new ligand 3,5-dimethyl-5-(4-pyridyl)hydantoin, its Pt(II) and Pt(IV) complexes, and the new Pt(IV) complex with 5-methyl-5-(4-pyridyl)hydantoin were recorded.

Chemical formulae of the new organic ligand (L) and the complexes (**1–4**) are given in Fig. 1. The structures are confirmed by the IR and NMR spectra of the new compounds and are in accordance with previously synthesized by us complexes [4–7].

IR spectra

The comparative analysis of the infrared spectra of the complexes **1–3** and of the metal-free ligand L revealed that the absorption bands characteristic for the stretching vibrations of pyridine -C=N- group were blue shifted from 1597.6 cm^{-1} for L to 1618.7, 1614.1, and 1619.1 cm^{-1} for **1–3**, respectively. Absorption band characteristic for the stretching vibrations of pyridine -C=N- group was blue shifted from 1605.3 cm^{-1} for the ligand L^1 to 1620.8 cm^{-1} for the complex **4**. This indicates that the pyridine nitrogen atom participates in the coordination to the platinum ion in all four complexes.

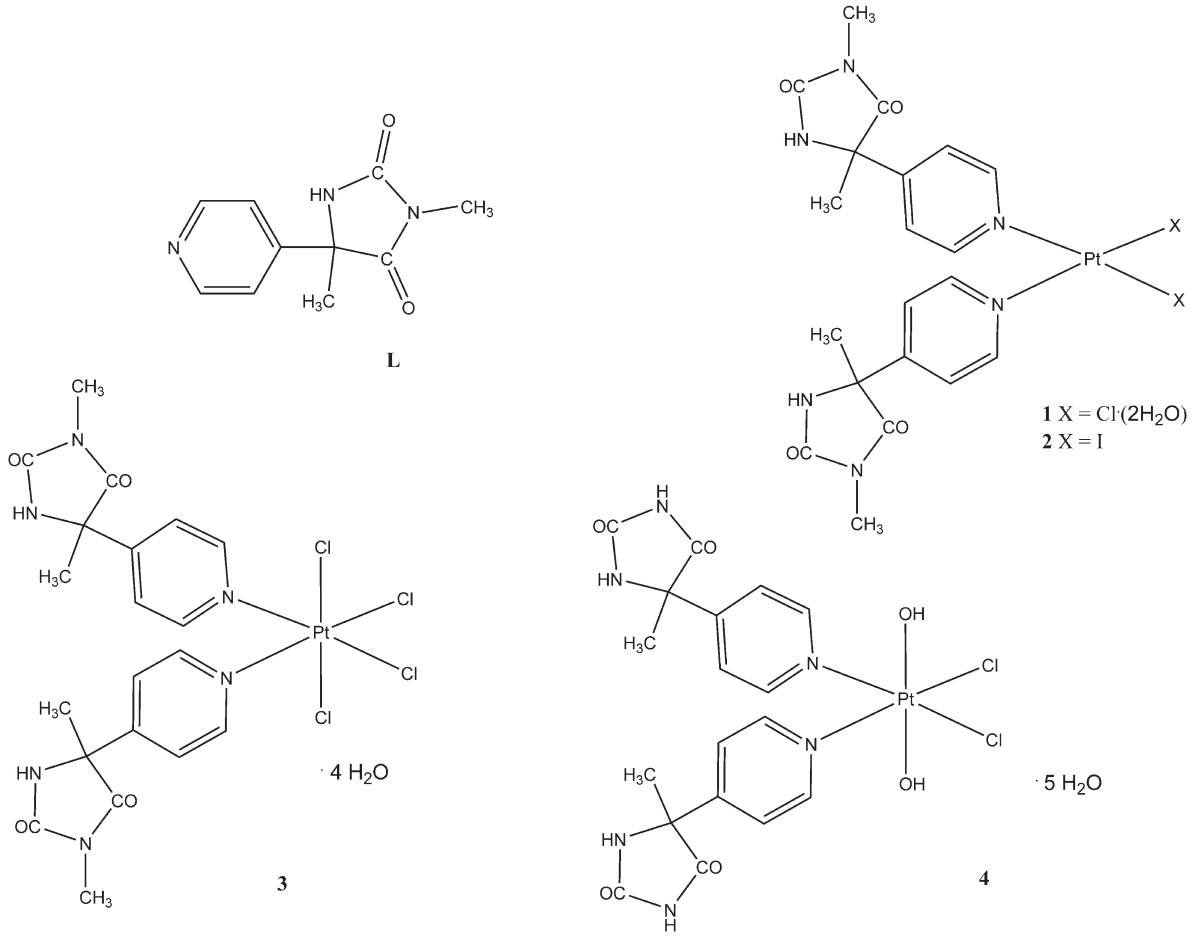

Figure 1. Molecular structures of the investigated Pt(II) and Pt(IV) complexes.

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In the IR spectra of the complex **4** absorption band characteristic for the stretching vibrations of Pt–OH at 533.4 cm^{-1} was observed.

The other characteristic bands of the pyridine ring of the metal-free ligand are blue-shifted upon complexation as well, providing further evidence for the coordination through the pyridine N atom. New bands at 350–180 cm^{-1} were assigned to the Pt–X stretching vibrations. In the IR spectra of **1–4** two bands for Pt–X stretching vibrations were observed, implying *cis*-location of halogenide ligands. [13]

The bands related to the stretching vibrations of the two carbonyl groups at 1786.7 cm^{-1} and 1716.1 cm^{-1} in the metal-free ligand did not shift upon coordination of L to Pt(II) and Pt(IV) indicating that the C=O groups were not involved in bounding to the metal.

NMR spectra

In the ^{1}H-NMR spectra of the complexes **1–3** the signals of the protons for H-2 and H-6 from the pyridine ring were shifted from 8.59 ppm in the spectrum of the free ligand (L) to 8.85, 8.73, and 8.76 ppm in the spectra of the complexes **1–3**, respectively. The differences between the chemical shifts of the protons of the ligand and those of the corresponding complexes are 0.26, 0.14, and 0.17 ppm. The signals of the H-3 and H-5 protons from the pyridine ring were also shifted from 7.49 ppm in the ligand to 7.65, 7.68, and 7.86 ppm in the complexes. The differences in the chemical shifts are 0.16, 0.19, and 0.37 ppm for the complexes **1–3** compared with the free ligand.

The signals of the protons for H-2 and H-6 from the pyridine ring were shifted from 8.56 ppm in the free ligand (L^1) through 8.83 ppm in its Pt(II) complex *cis*-[$PtL^1{}_2Cl_2$] [4] to 9.07 ppm in the new oxidized Pt(IV) complex *cis*-[$PtL^1{}_2Cl_2(OH)_2$]. The signals of the H-3 and H-5 protons from the pyridine ring were also shifted from 7.49 ppm in L to 7.65, 7.68, and 7.86 ppm in complexes **1**, **2**, and **3**, respectively.

In the ^{1}H-NMR spectra of all new complexes the signals of the protons in the pyridine ring were shifted, which is due to the coordination of platinum ion to the nitrogen atom from the pyridine ring. While the signals for the protons in the hydantoin ring and from the methyl groups were not shifted.

This indicates that the bounding of the ligands (L and L^1) to the platinum ion in complexes **1–4** occurs through the pyridine nitrogen atom.

In the ^{13}C-NMR spectra of the new complexes noticeable changes of the chemical shifts of all the carbon atoms in the pyridine ring, compared to the signals in the free ligands can be observed. Most significant are the differences for C-4: 3.8 ppm in complex **1**, 7.8 ppm in complex **3**, 8.1 ppm in complex **4**, and 5.3 ppm in complex **2** for C-2 and C-6. These results proved that platinum ion in all new complexes coordinates with the organic ligand through the pyridine nitrogen atom.

The signals of the two C=O groups of the hydantoin ring in L and L^1 were not shifted in complexes **1–4**. This is an indication that these groups are not involved in the binding to the metal ion.

The coordination of platinum ion through the pyridine nitrogen is also confirmed from the platinum satellites, which can be well seen in the ^{1}H- and ^{13}C-NMR of the Pt(IV) complexes.

The ligand environment around the platinum center can be best judged by the chemical shift of the ^{195}Pt nucleus, which spans a wide range of several thousand ppm, depending on identity of the coordinated atoms and the oxidation state of the metal center.

In the ^{195}Pt-NMR spectra of **1**, **2**, **3**, and **4** signals at −349, −1541, 1664, and 2443 ppm, respectively, were detected, referenced to $K_2[PtCl_4]$. Previously we published [6] that nearly the same signals were observed for the analogues of **1–3** with 3-amino-5-methyl-5(4-pyridyl)hydantoin. These data show that the changes in the hydantoin ring do not affect the chemical shifts in the ^{195}Pt-NMR, because the coordination is realized through the pyridine nitrogen.

A characteristic ''oxidation shift'' of approximately 2013 ppm (downfield, when comparing **3** with **1**) is very close to that reported in the literature (*e.g.* for *cis*-[Pt^{II}(bipy)Cl_2] and *cis*-[Pt^{IV}(bipy)Cl_4] where the δ^{195}Pt value increases with 2004 ppm) [14, 15].

The value of δ^{195}Pt chemical shift for **4** is indicative for *cis*,*cis*,*trans*-$Pt^{IV}N_2Cl_2O_2$ ligand sphere and correlated very good with the literature data for Pt(IV) complexes, which have axial Pt-O bonded ligands [16].

Coordination of the racematic 3,5-dimethyl-5-(4-pyridyl)hydantoin, which has a chiral center on C(5) should result in complexes, represented as mixtures of three different stereoisomers (R/R, S/S, S/R) in a ratio of 1:1:2. Nevertheless, in the ^{1}H- and ^{13}C-NMR spectras only one set of signals could be clearly detected. The same was observed for the complexes with 5-methyl-5-(4-pyridyl)hydantoin and 3-amino-5-methyl-5-(4-pyridyl)hydantoin, which have the same chiral centre. It can be proposed, that because of steric reasons one of the diasteromers is predominantly formed. In addition the chiral centre is far away from the coordination atom and most probably is not essential for the cytotoxic activity of the complexes.

Molar conductivity

Conductivity measurements for the metal-free ligand and the Pt(II) and Pt(IV) complexes were carried out by using DMSO as a solvent. For the ligand L a λ_M value of 1.32 S cm^2 mol^{-1} was obtained. For the complexes **1** and **2** the λ_M values were 5.27 and 6.39 S cm^2 mol^{-1} correspondingly, while a λ_M value for

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Table 1. Cytotoxic activity of the tested new platinum complexes vs. Cisplatin in a panel of human malignant cell lines as determined by MTT-dye reduction assay following 72 h continuous exposure.

	IC_{50} value (μM)		
	SKW-3	HL-60	HL-60/DOX
1	64.7	84.7	150.9
2	200.1	210.8	362.5
3	243.3	131.4	187.5
4	282.5	>400	>400
Cisplatin	11.4	8.3	21.2

the $K_2[PtCl_4]$ was 61.03 S cm^2 mol^{-1}. For the complex **3** a λ_M value was 3.03 S cm^2 mol^{-1}, but the value for the starting salt $PtCl_4$ was 3.77 S cm^2 mol^{-1}. For the ligand L^1 a λ_M value of 0.92 S cm^2 mol^{-1} was obtained and for the complex **4** the λ_M value was 2.37 S cm^2 mol^{-1}. According to the literature data all studied compounds can be regarded as non-electrolytes [17].

Pharmacology

In-vitro toxicity

The newly synthesized Pt(II) and Pt(IV) coordination compounds were evaluated for cytotoxicity against three human tumor cell lines, after 72 h continuous exposure, by means of the standard MTT-dye reduction assay. The platinum complexes exerted concentration-dependent cytotoxic effects. The corresponding IC_{50} values are summarized in Table 1.

Evident from the results obtained the dichloro Pt(II) compound **1** proved to be the most active cytotoxic agent among the series of novel complexes. The diiodo-analogue inhibited the proliferation of malignant cells at substantially higher concentrations. Among the higher oxidation state complexes the tetrachloro Pt(IV) compound **3** exhibited superior activity, whereas the compound with dihydroxo axial ligands exerted only marginal activity. These findings and especially the superior activity of chloro ligands-bearing complexes are in accordance with the structure activity rules established for similar series of compounds with hydantoin ligands presented in our previous studies [4, 6].

QSAR Study

Each compound was presented as a binary string of length 9 substituents (Table 2). A term is equal to 1 when a substituent presents at a particular position and 0 when it is absent. Descriptor Pt takes 1 when Pt presents in the complex and 0 when Pd presents. The dependent variable was $pIC_{50} = \log(1/IC_{50})$, measured on SKW-3 cell line. Free-Wilson model was derived by multiple linear regression (MLR) using partial least squares (PLS) method. The model is given by the following equation:

$$\begin{aligned} pIC_{50} = {} & 15.879 + 0.271 \times 1H - 0.263 \times 1NH_2 - 0.018 \\ & \times 1CH_3 + 0.363 \times 2Cl - 0.272 \times 2Br - 0.159 \\ & \times 2I + 0.139 \times Pt - 0.749 \times Pt(IV)\text{-}Cl - 0.670 \\ & \times Pt(IV) - OH \end{aligned}$$

$$n = 16,\ r^2 = 0.778,\ PC = 3$$

Three principal components explain 78% of the variance in the set. Substituents 2Cl and 1H have positive contribution in the cytotoxic activity of the investigated compounds. Substituents Pt(IV)-Cl^- and Pt(IV)-OH^- possess the highest negative contributions followed by $2Br^-$, $1NH_2$, $2I^-$, and $1CH_3$. Compounds with platinum are more active then those containing palladium.

Table 2. Compounds used in the QSAR study. Cytotoxic activity (IC_{50}) were tested on SKW-3 cell line.

Name	Structure	1H	$1NH_2$	$1CH_3$	2Cl	2Br	2I	Pt[a]	Pt(IV)-Cl	Pt(IV)-OH	IC_{50} μM	pIC_{50}	References
A1	$Pt(MPH)_2Cl_2$	1	0	0	1	0	0	1	0	0	39.7	4.401	
A2	$Pt(MPH)_2Br_2$	1	0	0	0	1	0	1	0	0	146.7	3.834	
A3	$Pt(MPH)_2I_2$	1	0	0	0	0	1	1	0	0	89.6	4.048	
A4	$Pt(MPH)_2Cl_4$	1	0	0	1	0	0	1	1	0	243.3	3.614	comp. **3** this paper
A5	$Pt(MPH)_2Cl_2(OH)_2$	1	0	0	1	0	0	1	0	1	282.5	3.549	comp. **4** this paper
A6	$Pd(MPH)_2Cl_2$	1	0	0	1	0	0	0	0	0	89.0	4.051	
A7	$Pd(MPH)_2Br_2$	1	0	0	0	1	0	0	0	0	160.2	3.795	
A8	$Pt(AMPH)_2Cl_2$	0	1	0	1	0	0	1	0	0	159.2	3.798	
A9	$Pt(AMPH)_2Br_2$	0	1	0	0	1	0	1	0	0	245.8	3.609	
A10	$Pt(AMPH)_2I_2$	0	1	0	0	0	1	1	0	0	191.4	3.718	
A11	$Pt(AMPH)_2Cl_4$	0	1	0	1	0	0	1	1	0	246.5	3.608	
A12	$Pd(AMPH)_2Cl_2$	0	1	0	1	0	0	0	0	0	180.8	3.743	
A13	$Pd(AMPH)_2Br_2$	0	1	0	0	1	0	0	0	0	182.6	3.738	
A14	$Pt(DMPH)_2Cl_2$	0	0	1	1	0	0	1	0	0	64.7	4.189	comp. **1** this paper
A15	$Pt(DMPH)_2I_2$	0	0	1	0	0	1	1	0	0	200.1	3.699	comp. **2** this paper
A16	$Pt(DMPH)_2Cl_4$	0	0	1	1	0	0	1	1	0	243.3	3.614	

a Descriptor Pt takes 1 when Pt presents in the complex and 0 when Pd presents.

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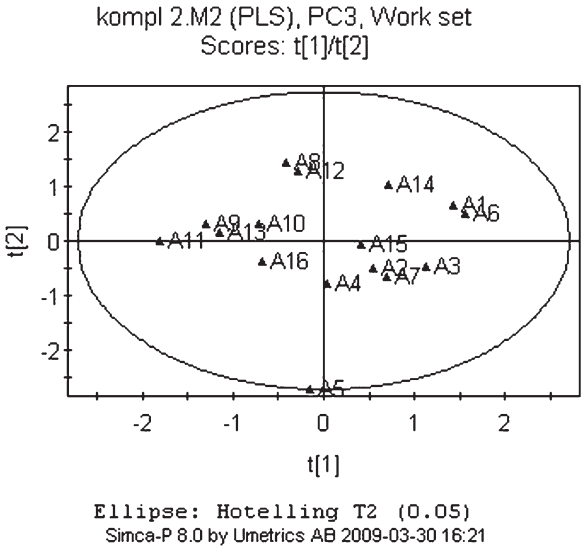

Figure 2. PLS score plot of the first two PCs explaining 74% of the total variance. The ellipse shows 95% confidence region of the model. Compound A5 is an outlier.

The score plot of the model is given in Fig. 2. Compounds with the same ligands but different metal ions (Pt or Pd) cluster together. Such pairs are A9 and A13, A8 and A12, A2 and A7, A1 and A6. Compound A5 is an outlier as it is the only compound in the set carrying Pt(IV)-OH. Excluding A5 there is no significant improvement of the model.

The loadings plots are given in Fig. 3a and 3b. PC1 is defined by the substituents at position 1 – 1H and $1NH_2$. PC2 relates to substituents at position 2 – $2Cl^-$, $2Br^-$ and $2I^-$ as well as to substituents Pt(IV)-Cl^- and Pt(IV)-OH^-. PC3 accounts for the presence of Pt or Pd in the complexes. Variables 1H and $2Cl^-$ are the closest to pIC_{50} and they possess the highest positive contributions in activity, while the variables Pt(IV)-Cl^- and Pt(IV)-OH^- are the outer most from pIC_{50} and they are deleterious for the cytotoxic activity of the investigated complexes.

Conclusion

Three new Pt(II) and Pt(IV) complexes with 3,5-dimethyl-5-(4-pyridyl)hydantoin as carrier ligand and various halogen anions as leaving groups were synthesized. One new Pt(IV) complex with 5-methyl-5-(4-pyridyl)hydantoin is also synthesized. The molecular formulae of the complexes were confirmed by elemental and spectral analysis as IR, ^{1}H-, ^{13}C-, ^{195}Pt-spectra and molar conductivity. The biological evaluation thereof unambiguously indicates that the dichloro-analogue is the most active compound, whereas the substitution of the leaving groups as well as the change in the oxidation state are associated with decrease in cytotoxicity. The QSAR study pointed out the positive contribution in cytotoxic activity of non-substituted ligands. Compounds with platinum are more active then those containing palladium. These conclusions will be utilized in further rational synthesis of new platinum complexes.

Experimental

Chemistry

Materials

Potassium tetrachloroplatinate(II) utilized for the synthetic procedures was purchased from Degussa and platinum(IV) chloride

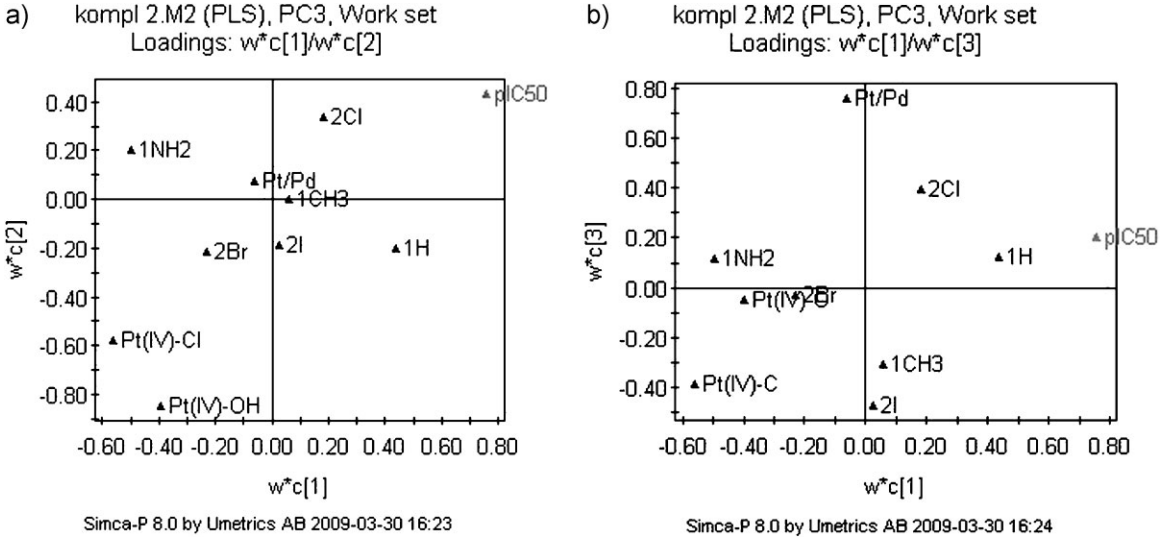

Figure 3. Loading plots of the three PCs explaining 78% of the total variance. a) PC1 *vs.* PC2; b) PC1 *vs.* PC3.

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from Heraeus GmbH. All of the other chemicals were of analytical grade.

The newly synthesized ligand 3,5-dimethyl-5-(4-pyridyl) hydantoin, its Pt(II) and Pt(IV) complexes, and the new Pt(IV) complex with 5-methyl-5-(4-pyridyl)hydantoin were characterized by elemental analysis, IR, ^{1}H-, ^{13}C-, ^{195}Pt-NMR spectra and molar conductivity. The carbon, nitrogen and hydrogen content of the compounds were determined by elemental analysis. The elemental analysis was carried out on a Carlo Erba apparatus.

General methods

The IR spectra were recorded on IFS 113 v Bruker FTIR spectrophotometer in the range of 4000–400 cm^{-1} as pellets KBr and 400–150 as polyethylene. The ^{1}H-NMR and ^{13}C-NMR spectra were registered on a Bruker WM 250 (250 MHz) and on Bruker Avance III 500 MHz NMR spectrometers in DMSO-d_6. ^{195}Pt-spectra were recorded in DMSO-d_6 or DMF-d_7 with Bruker Avance III 500 MHz NMR spectrometer operating at frequencies 107.55 MHz. Chemical shifts are reported relative to external standard $K_2[PtCl_4]$ in D_2O ($\delta = -1628$ ppm) *vs.* $K_2[PtCl_6]$ ($\delta = 0$ ppm). Corrected melting points were determined using a Bushi 535 apparatus. The determination of crystal water of some of the complexes was defined by Karl Fischer Titrator METTLER TOLEDO DL 31. The molar conductivity of 1×10^{-3} mol/L solutions of the complexes in DMSO was measured by means of a Metrohm conductometer 660 (cell constant -0.82 cm^{-1}).

Synthesis of the ligand and of the complexes

Preparation of the ligand 3,5-dimethyl-5-(4-pyridyl) hydantoin

9.55 g (0.05 mol) of 5-methyl-5(4-pyridyl)hydantoin was suspended in 50 mL of distilled water. 3.00 g sodium hydroxide dissolved in 50 mL C_2H_5OH was added to the mixture at constant stirring. The obtained composite was heated in the presence of a reflux condenser to homogeneity and added dropwise 4.7 mL (0.05 mol) dimethyl sulfate from dropping funnel. The mixture was boiled for 4 h and cooled at room temperature. After that was evaporated on rotary evaporator and obtained paste was extracted 3 times with 30 mL ethyl acetate. The obtained solution was washed twice with 10% sodium carbonate solution and dried under calcium dichloride. The dehydrate solution was evaporated and the rest was dissolved at boiling in 20 mL ethanol. The obtained solution was cooled and white product was obtained, which was filtered off and dried at 100°C for several hours. The purity is checked by TLC. The substance is soluble in DMSO and weakly soluble in water and ethanol. Yield: ca. 54%, m.p. = 135–136°C. $\lambda_M = 1.32$ S cm^2 mol^{-1}. IR (pellets KBr): 3314.0, 3276.0, 1786.7, 1716.1, 1597.6, 1412.2; ^{1}H-NMR (DMSO-d_6, δ, ppm): 9.00 (s, 1H, N(1)-H), 8.59 (d, 2H, $^{3}J_{H,H} = 7$ Hz, H-2, H-6), 7.49 (d, 2H, $^{3}J_{H,H} = 7$ Hz, H-3, H-5), 2.85 (s, 3H, N–CH_3), 1.68 (s, 3H, C–CH_3); ^{13}C-NMR (DMSO-d_6, δ, ppm): 174.4 (C=O-4'), 155.8 (C=O-2'), 149.9 (C-2, C-6), 148.3 (C-4), 120.6 (C-3, C-5), 62.5 (C-5'), 39.5 (N-CH_3), 24.6 (C-CH_3). Anal. calcd. (%) for $C_{10}H_{11}N_3O_2$: C, 58.03; H, 5.40; N, 20.48. Found (%): C, 57.58; H, 5.33; N, 19.97.

Preparation of cis-dichloro-bis(3,5-dimethyl-5-(4-pyridyl) hydantoin)platinum(II) dihydrate-cis-$[PtL_2Cl_2] \cdot 2\ H_2O$

0.1987 g (0.9693 mmol) of the ligand was dissolved in 10 mL distilled water. The obtained solution is filtered off and added dropwise to the water solution of $K_2[PtCl_4]$ (0.1997 g, 0.4812 mmol) at constant stirring and 40°C temperature for about 30 min. After the addition of the ligand the homogenous solution was stirred for 2 h. The solution was cooled to 0°C for about 24 h. A bright-yellow product was obtained, which was filtered off, washed several times with glacial water and dried in a vacuum desiccator. The substance is soluble in DMSO, DMF and weakly soluble in water and ethanol. Yield: ca. 75%, m.p.: >215°C (dec.). $\lambda_M = 5.27$ S cm^2 mol^{-1}; IR (pellets KBr and polyethylene): 3318.5, 3285.7, 1783.2, 1710.4, 1618.7, 1427.2, 333, 306; ^{1}H-NMR (DMSO-d_6, δ, ppm): 9.03 (s, 1H, N(1)-H), 8.85 (d, 2H, $^{3}J_{H,H} = 7$ Hz, H-2, H-6), 7.65 (d, 2H, $^{3}J_{H,H} = 7$ Hz, H-3, H-5), 2.82 (s, 3H, N-CH_3), 1.66 (s, 3H, C-CH_3); ^{13}C-NMR (DMSO-d_6, δ, ppm): 173.6 (C=O-4'), 155.8 (C=O-2'), 153.4 (C-2, C-6), 152.5 (C-4), 122.8 (C-3, C-5), 62.5 (C-5'), 39.5 (N-CH_3), 24.6 (C-CH_3); ^{195}Pt-NMR (DMF-d_7, δ, ppm): −349 (adjusted to K_2PtCl_6: −1977). Anal. calcd. (%) for $[Pt(C_{10}H_{11}N_3O_2)_2Cl_2] \cdot 2\ H_2O$: C, 33.71; H, 3.65; N, 11.80; H_2O, 5.55. Found (%): C, 33.33; H, 3.73; N, 11.71; H_2O, 5.05.

Preparation of cis-diiodo-bis(3,5-dimethyl-5-(4-pyridyl) hydantoin) platinum(II-)cis-$[PtL_2I_2]$

The complex *cis*-$[PtL_2I_2]$ (**2**) was prepared according to a reported procedure with some revisions [14]. 0.0997 g $K_2[PtCl_4]$ (0.2402 mmol) was mixed with a saturated solution of potassium iodide (in excess) (0.1987 g) and heated in water bath for 5 min. $K_2[PtCl_4]$ was quantitatively converted into a solution of $K_2[PtI_4]$. To this mixture 0.0978 g (0.4771 mmol) of L was added. The solution was stirred for 2 h at 40°C. After that the solution was cooled to 0°C for about 24 h. Yellow crystals were obtained, filtered off and washed several times with glacial water and dried in a vacuum desiccator. The complex is soluble in DMSO and DMF. Yield. ca. 78%, m.p.: >271°C (dec.). $\lambda_M = 6.39$ S cm^2 mol^{-1}; IR (pellets KBr and polyethylene): 3315.7, 3292.9, 1781.3, 1715.4, 1614.1, 1422.1, 189, 136; ^{1}H-NMR (DMSO-d_6, δ, ppm): 9.03 (s, 1H, N(1)-H), 8.73 (d, 2H, $^{3}J_{H,H} = 7$ Hz, H-2, H-6), 7.68 (d, 2H, $^{3}J_{H,H} = 7$ Hz, H-3, H-5), 2.86 (s, 3H, N-CH_3), 1.68 (s, 3H, C-CH_3); ^{13}C-NMR (DMSO-d_6 δ, ppm): 173.5 (C=O-4'), 155.6 (C=O-2'), 155.2 (C-2, C-6), 150.8 (C-4), 122.7 (C-3, C-5), 62.4 (C-5'), 39.5 (N-CH_3), 24.6 (C-CH_3); ^{195}Pt-NMR (DMF-d_7, δ, ppm): −1541 (adjusted to K_2PtCl_6: −3169). Anal. calcd. (%) for $[Pt(C_{10}H_{11}N_3O_2)_2I_2]$: C, 27.95; H, 2.56; N, 9.78. Found (%): C, 27.66; H, 2.77; N, 9.38.

Praparation of cis-tetrachloro-bis(3,5-dimethyl-5(4-pyridyl) hydantoin) platinum (IV)tetrahydrate-$[PtL_2Cl_4] \cdot 4\ H_2O$

The complex *cis*-$[PtL_2Cl_4] \cdot 4\ H_2O$ (**3**) was prepared by a method given in the literature [15]. Two solutions of the $PtCl_4$ and of the 3,5-dimethyl-5(4-pyridyl)-2,4-imidazolidenedione were prepared. The water/ethanol solution of the ligand (0.1822 g, 0.8888 mmol) was added dropwise to the ethanol/water solution of $PtCl_4$ (0.1529 g, 0.4537 mmol) at constant stirring for 5–6 h at 50°C. The solution was concentrated and cooled to 0°C. A bright-yellow product was obtained and filtered off, washed several times with cold water, cold ethyl ether and dried in a vacuum desiccator. The compound is soluble in DMSO and DMF and weakly soluble in water and ethanol. Yield: 58%, m.p.: >252°C (dec.). $\lambda_M = 3.03$ S cm^2 mol^{-1}; IR (pellets KBr and polyethylene): 3369.8, 3234.7, 1781.1, 1716.4, 1619.1, 1431.0, 354.0, and 326.0; ^{1}H-NMR (DMSO-d_6, δ, ppm): 9.13 (s, 1H, N(1)-H), 8.76 (d, 2H, $^{3}J_{H,H} = 7$ Hz, H-2, H-6), 7.86 (d, 2H, $^{3}J_{H,H} = 7$ Hz, H-3, H-5), 2.85 (s, 3H, N-CH_3), 1.73 (s, 3H, C-CH_3); ^{13}C-NMR (DMSO-d_6 δ, ppm): 173.6 (C=O-4'), 156.1 (C-4), 155.2 (C=O-2'), 152.0 (C-2, C-6), 124.5 (C-

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3, C-5, $^{3}J_{C,Pt} = 26.96$), 63.1 (C-5'), 40.2 (N-CH_3), 25.2 (C-CH_3); ^{195}Pt-NMR (DMSO-d_6, δ, ppm): 1664 (adjusted to K_2PtCl_6: 36). Calcd. for $[Pt(C_{10}H_{11}N_3O_2)_2Cl_4] \cdot 4\ H_2O$: C, 29.32; H, 3.69; N, 10.26; H_2O, 8.79%. Found: C, 29.79; H, 3.72; N, 10.51; H_2O, 9.20.

*Preparation of trans-cis-cis-dihydroxo-dichloro-bis (5-methyl-5(4-pyridyl)hydantoin) platinum(IV) pentahydrate-[PtL$^1{}_2$Cl$_2$(OH)$_2$] · 5 H$_2$O (**4**)*

0.1705 g (0.26 mmol) of the *cis*-[PtL$^1{}_2$Cl$_2$] complex (synthesized and described in [4]) was suspended in 15 mL of distilled water and 2.4 mL (23.50 mmol) of 30% H_2O_2 was added. The suspense was stirred at heating at 70°C for 5 h to obtain yellow solution. The obtained solution was concentrated to 2 mL on the rotary evaporator and leaved to stay in refrigerator at 0°C for a day. The separated bright yellow product was filtered off and dried in vacuum desiccator under P_2O_5/KOH. The compound is soluble in water, DMSO, DMF and insoluble in acetone and ethanol. Yield: 36%, m.p. >263°C (dec.). $\lambda_M = 2.37$ S cm^2 mol^{-1}; IR (pellets KBr and polyethylene) cm^{-1}: 3327.8, 3277.7, 1773.4, 1722.4, 1620.8, 1430.0, 533.4, 349.0, and 327.0; ^{1}H-NMR (DMSO-d_6, δ, ppm): 11.07 (s, 1H, N(3)-H), 9.07 (d, 2H, $^{3}J_{H,H} = 7$ Hz, H-2, H-6), 8.75 (s, 1H, N(1)-H), 7.85 (d, 2H, $^{3}J_{H,H} = 7$ Hz, H-3, H-5), 1.69 (s, 3H, C-CH_3); ^{13}C-NMR (DMSO-d_6 δ, ppm): 175.3 (C=O-4'), 156.4 (C-4), 154.4 (C=O-2'), 147.0 (C-2, C-6), 123.6 (C-3, C-5, $^{3}J_{C,Pt} = 26.98$), 64.0 (C-5'), 25.2 (C-CH_3); ^{195}Pt-NMR (DMSO-d_6, δ, ppm): 2443 (adjusted to K_2PtCl_6: 815). Calcd. for $[Pt(C_9H_9N_3O_2)_2Cl_2(OH)_2] \cdot 5\ H_2O$: C, 28.01; H, 3.91; N, 10.89; H_2O, 11.66%. Found: C, 28.60; H, 3.76; N, 11.22; H_2O, 11.32.

Pharmacology

The present study describes a comparative evaluation of the cytotoxic effects of the newly synthesized platinum complexes vs. the referent antineoplastic agent cisplatin on a panel of human tumor cell lines, using the standard MTT-dye reduction assay for cell viability.

Cell culture conditions

The following cell lines were used for the experiments: SKW-3 (a KE-37 derivative) (human T-cell leukemia, established from peripheral blood of a 61-year-old man with T-cell lymphocytic leukemia); HL-60 (human acute promyelocyte leukemia, established from the peripheral blood of a 35-year-old woman with acute myeloid leukemia (AML FAB M2) in 1976); HL-60/DOX (a HL-60 multi-drug resistant subline, established *via* cultivation in doxorubibin-containing medium; the cell line is characterized by overexpression of MRP-1 (ABCC-1) transporter, conditioning its pleiotropic drug resistance).

Cytotoxicity assessment (MTT-dye reduction assay)

The cell viability was assessed using the standard MTT-dye reduction assay as described by Mosmann [18] with minor modifications [19]. The method is based on the reduction of the yellow tetrazolium salt MTT to a violet formazan product via the mitochondrial succinate dehydrogenase in viable cells. Aliquots of 100 μL/well cellular suspension (at a density of 1×10^5 exponentially growing cells/ml) were seeded in 96-well flat-bottomed microplates and after 24 h incubation at 37°C were exposed to various concentrations of the tested compounds for 72 h. For each concentration at least 8 wells were used. After the incubation with the test compounds 10 μL MTT solution (10 mg/mL in PBS) were added to each well and the microplates were further incubated for 4 h at 37°C. Thereafter the formazan crystals formed were dissolved through addition of 100 μL/well 5% formic acid solution in 2-propanol. The MTT-formazan absorption was measured using an microprocessor-controlled ELISA reader (Labexim LMR-1) at 580 nm. Cell survival fractions were calculated as percentage of the untreated control. In addition IC_{50} values were calculated from the concentration-response curves. The experimental data were processed by means of GraphPad Prism software and were fitted to sigmoidal concentration-response curves via non-linear regression.

QSAR Methods

QSAR

The newly four synthesized and tested complexes were added to 12 previously evaluated compounds [4–7] and a set of 16 similar compounds was compiled. Free-Wilson model was derived by PLS and the contribution of each substituent was assessed quantitatively.

Free-Wilson QSAR model is based on the additively concept whereby each substituent makes an additive and constant contribution to the biological activity regardless of substituent variation in the rest of the molecule [20]. Each compound was presented as a binary string of length 9 substituents (Table 2). A term is equal to 1 when a substituent presents at a particular position and 0 when it is absent. The dependent variable was $pIC_{50} = \log(1/IC_{50})$. The contributions of each substituent were calculated by the multiple linear regression (MLR) method using the partial least squares (PLS) method.

PLS is a projection method [21], which can handle matrices with more variables than observations and with noisy and highly collinear data. PLS forms new variables, named principal components (PC), as linear combinations of the initial variables and then uses them to predict the dependent variable. The PLS linear regression, as implemented in SIMCA-P 8.0 [22], was used in the present study.

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