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Synthesis, DFT calculations and characterisation of new mixed Pt(II) complexes with 3-thiolanespiro-5′-hydantoin and 4-thio-1*H*-tetrahydropyranspiro-5′-hydantoin‡

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Cisplatin is an anticancer drug widely used in the treatment of a wide range of solid tumours (head and neck, lung, bladder etc.), testicular and ovarian cancers. Because of its severe toxicity profile and spontaneous development of drug resistance in tumours, a number of Pt(II) complexes have been synthesised and tested for anti-tumour activity. Some of the investigations have focused on using ligands bearing donor atoms other than N (e.g., S, P, O). Two new mixed Pt(II) complexes of the general formula *cis*-[$Pt(NH_3)LCl_2$] where L is 3-thiolanespiro-5′-hydantoin and 4-thio-1*H*-tetrahydropyranspiro-5′-hydantoin were synthesised. The complexes were studied by elemental analysis, melting points, IR and ^{1}H NMR spectra. The hybrid DFT calculations were used for optimisation of the structure geometries of the ligands *III*, *IV* and their Pt(II) complexes *V* and *VI*. The structural parameters so calculated, such as bond lengths and angles, are in good agreement with the experimental data for similar hydantoins and their platinum complexes. The results showed that the geometries of complexes *V* and *VI* are plane square and the bounding of ligands *III* and *IV* with platinum ions is effected by the sulphur atom from the cyclic ring. The complexes thus obtained were chemically examined in comparison with previously synthesised and published complexes of the general formula *cis*-[PtL_2Cl_2] (*VII* and *VIII*) with the same ligands. The new compounds *V* and *VI*, as well as the previously investigated complexes (*VII* and *VIII*), were analysed for cytotoxicity in vitro on SKW-3 and HL-60 human tumour cell lines. The results showed that all the complexes exerted concentration-dependent anti-proliferative activity.

Keywords: Pt(II) complexes, S-heterocyclic organic ligands, DFT calculations, cytotoxicity

Introduction

Since the discovery of the activity of one of the most extensively used anticancer drugs, the complex *cis*-diamminedichloroplatinum(II) (*cis*-[$Pt(NH_3)_2Cl_2$, Fig. 1), known clinically as cisplatin (Rosenberg et al., 1969), thousands of platinum complexes have been synthesised and evaluated for anticancer activity. A necessary requirement for an active Pt-drug was for it to consist of two am(m)ine groups (or at least one NH group) or bidentate amine ligand and two leaving groups with an intermediate binding strength (e.g. Cl^-, SO_4^{2-}, citrate or oxalate) in *cis* configuration (Abu-Surrah & Kettunen, 2006). Recently, the conception has gradually been adjusted towards different directions. One is connected with utilising the carrier ligands containing donor atoms other than nitrogen, such as sulphur, phosphorus

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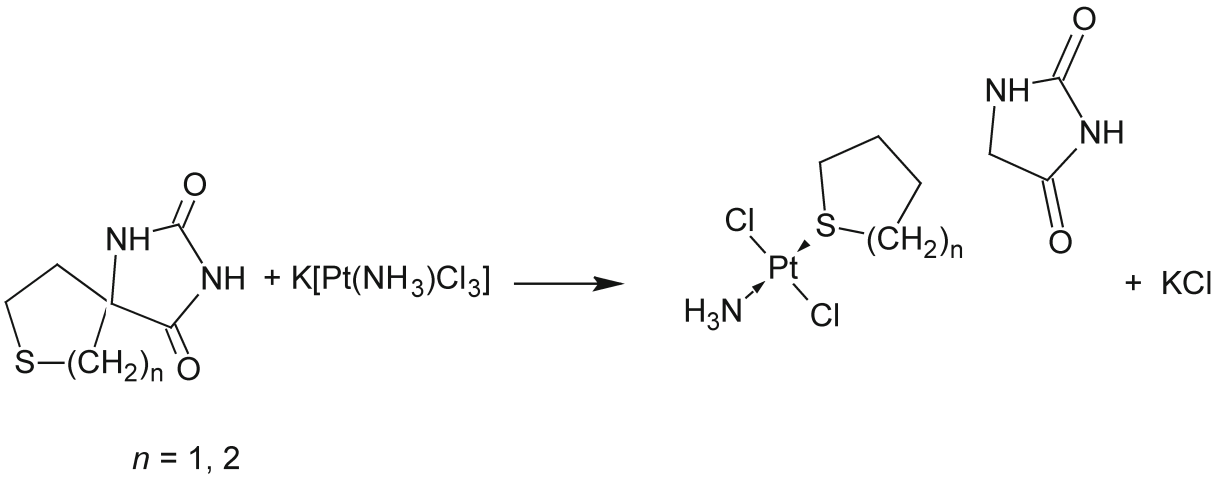

Fig. 1. General scheme of synthesis of new platinum complexes *V* and *VI*.

and oxygen. In this relation, the modification of *cis*-$[Pt(NH_3)_2Cl_2]$ to *cis*-$[Pt(NH_3)LCl_2]$, where L is the S-heterocyclic organic ligand leads to obtaining the novel "non-classical" Pt(II) complexes where one ammine ligand is replaced by a S-heterocyclic organic ligand. Some derivatives of a recently described S-containing anti-tumour platinum complex, bis(*O*-ethyldithiocarbonato)platinum(II), denoted as thioplatin, have been studied and analysed. All the reported platinum complexes displayed a significantly higher cytotoxic activity than thioplatin and cisplatin (Friebolin et al., 2004). Pt(II) has a high affinity to sulphur (Bugarčić et al., 2012). In particular, S-containing biomolecules have a high affinity to Pt(II) complexes, e.g. thiols and thioethers. Namely, Pt(II) as a "soft" acid forms very stable compounds with sulphur donors ("soft" bases). Two cisplatin complexes analogous with S-containing carrier ligands, *cis*-$[Pt(CH_3SCH_2CH_2SCH_3)Cl_2]$ and *cis*-$[Pt(dmso)Cl_2]$ (dmso is dimethylsulphoxide) were studied in vitro against a human breast cancer cell line (Bogdanović et al., 2002). The results showed that the complexes strongly inhibited the growth of MCF7 cells in a dose- and time-dependent manner.

Hydantoin derivatives possess a variety of biochemical and pharmacological properties such as fungicidal, herbicidal, anti-tumour, anti-inflammatory, anti-HIV, hypolipidemic, antiarrhythmic and antihypertensive activities (Dylag et al., 2004; Kruger et al., 2006). They are biologically active molecules widely used in medicine as antischistosomal, antiepileptic, antiarythmic, antibacterial and tuberculostatic drugs (Thenmozhiyal et al., 2004; Kruger et al., 2006). Hydantoin derivatives exhibit various bioactivities which for decades have induced scientists to conduct research involving these ring systems (Abdulrahman et al., 2013). With the accessibility of such studies, scientists have been able to synthesise and develop novel derivatives which extend the therapeutic activities of such heteroatoms. Recently, the cytotoxic activity of spirohydantoin derivatives has been tested in ovarian and breast cancer cells (Kavitha et al., 2009). It has been shown that spirohydantoin derivatives induce growth inhibition and apoptosis in leukemic cells (Bakalova et al., 2013).

This study sought to prepare novel "non-standard" Pt(II) complexes with one ammine group and one S-containing heterocyclic organic ligand and two Cl^- ions as leaving groups in *cis*-configuration. The study represents the synthesis, physicochemical evaluation, DFT calculations and pharmacological investigation of two new cisplatin analogous with sulphur- containing carrier ligands (3-thiolanespiro-5′-hydantoin and 4-thio-1*H*-tetrahydropyranspiro-5′-hydantoin) of the general formula *cis*-$[Pt(NH_3)LCl_2]$. The platinum complexes so obtained were chemically and pharmacologically examined against previously synthesised Pt(II) complexes with the same ligands and general formula *cis*-$[PtL_2Cl_2]$ and the clinically applied drug cisplatin (Bakalova et al., 2015).

Experimental

Tetrahydrothiophene-3-one (*I*) and tetrahydro-1*H*-thiopyran-4-one (*II*) used for the preparation of two new organic compounds, 3-thiolanespiro-5′-hydantoin (*III*) and 4-thio-1*H*-tetrahydropyranspiro-5′-hydantoin (*IV*), were purchased from Sigma–Aldrich (USA). $K[Pt(NH_3)Cl_3]$ was a commercial product and was purchased from Sigma–Aldrich. All other chemicals were of analytical grade and purchased from Fluka (UK) and Sigma–Aldrich.

The newly synthesised Pt(II) complexes with 3-thiolanespiro-5′-hydantoin (*V*) and 4-thio-1*H*-tetrahydropyranspiro-5′-hydantoin (*VI*) were characterised by elemental analysis, melting points, IR and ^{1}H NMR spectra. The carbon, nitrogen and hydrogen contents of the compounds were determined by elemental analysis. The elemental analysis was carried out on a "EuroEA 3000 – Single", EuroVector SpA (Italy) ap-

paratus. Corrected melting points were determined using a Bushi 535 apparatus (Bushi Labortechnik AG, Switzerland). The IR spectra were recorded on a Thermo Scientific Nicolet iS10 spectrophotometer (Thermo Scientific, USA) in the range of 4000–400 cm^{-1} as ATR and IFS 113 v Bruker FTIR spectrophotometer (Bruker, Germany) in the range of 400–150 cm^{-1} as polyethylene. The ^1H NMR spectra were recorded on a Bruker WM 250 (250 MHz; Bruker) spectrometer using DMSO-d_6 as solvent. TMS was used as an internal standard and chemical shifts are given in δ relative to TMS.

The synthesis and investigation of the organic compounds, *III* and *IV* were published in a previous work (Bakalova et al., 2015). These compounds were used as carrier ligands to prepare new platinum(II) complexes, *VII* and *VIII*, of the general formula *cis*-$[PtL_2Cl_2]$. The acquisition and study of the newly synthesised platinum complexes, *VII* and *VIII*, were published in the same article (Bakalova et al., 2015).

The new complexes *V* and *VI* of the general formula *cis*-$[Pt(NH_3)LCl_2]$ were prepared by using procedure reported by Bierbach et al., 1999 with minor revisions.

Synthesis of new Pt(II) complexes

Compound *V* was synthesised in accordance with the following procedure.

Water/ethanol solution (4 mL; $\varphi_r = 1:1$) of *III* (0.0488 g, 0.2837 mmol) was added dropwise to water solution (3 mL) of $K[Pt(NH_3)Cl_3]$ (0.1019 g, 0.2849 mmol) under constant stirring at ambient temperature. The homogenous solution was stirred for 5–6 h, concentrated and cooled to 4 °C. A light yellow product was obtained, which was separated by filtration and dried in a vacuum desiccator. The substance is soluble in dimethylsulphoxide (DMSO). The purity was confirmed by thin layer chromatography with the eluent $CH_3COOC_2H_5/C_2H_5OH$ ($\varphi_r = 2:1$) and elemental analysis.

Compound *VI* was synthesised in accordance with the following procedure. Ethanol solution (6 mL) of *IV* (0.0516 g, 0.2774 mmol) was added dropwise to the water solution (3 mL) of $K[Pt(NH_3)Cl_3]$ (0.1 g, 0.2796 mmol) under constant stirring at ambient temperature. The homogenous solution was stirred for 14 h, concentrated and cooled to 4 °C. A lemon yellow product was obtained, which was separated by filtration and dried in a vacuum desiccator. The substance is soluble in DMSO. The purity was confirmed by thin layer chromatography with the eluent $CH_3COOC_2H_5/C_2H_5OH$ ($\varphi_r = 2:1$) and elemental analysis.

All theoretical calculations were performed using the Gaussian 09 package (Frisch et al., 2009) of programs. Optimisation of the structures of ligands *III* and *IV* and two possible conformers of their Pt(II) complexes, *V* and *VI*, was carried out by hybrid DFT calculations, employing the B3LYP (Becke's three-parameter non-local exchange (Stephens et al., 1994) and Lee et al. (1988) correlation functional and 6-31++G* basis set for all non-metal atoms and LANL2DZ basis set for platinum.

The present study describes a comparative evaluation of the cytotoxic effects of the two newly synthesised Pt(II) complexes with the general formula *cis*-$[Pt(NH_3)LCl_2]$ and the two previously synthesised and studied Pt(II) complexes with the same ligands and a different general formula *cis*-$[PtL_2Cl_2]$. The cytotoxicity of all Pt(II) complexes was compared with the cytotoxic effects of metal-free ligands *III* and *IV* and the referent antineoplastic agent cisplatin.

The following cell lines were used for the experiments: (i) SKW-3 or a KE-37 derivative (human T-cell leukaemia, established from the peripheral blood of a 61-year-old man with T-cell lymphocytic leukaemia); (ii) HL-60 (acute myeloid leukaemia, established from the peripheral blood of a patient with acute promyelocyte leukaemia). The cell lines were obtained from the DSMZ German Collection of Microorganisms and Cell Cultures (Germany) and were duly validated in our laboratory as a proper test system for platinum agents. Their DSMZ catalogue numbers are as follows: HL-60 (ACC 3) and SKW-3 (ACC 53).

The cytotoxicity of the compounds was assessed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Merck) dye reduction assay as described by (Mosmann, 1983) with some modifications by (Konstantinov et al., 1999). Exponentially growing cells were seeded in 96-well microplates (100 μL per well at a density of 3.5×10^5 cells per mL for the adherent and 1×10^5 cells per mL for the suspension cell lines) and allowed to grow for 24 h prior to exposure to the studied compounds. Stock solutions of the Pt(II) complexes investigated were freshly dissolved in DMSO and then promptly diluted in RMPI-1640 growth medium, (Sigma–Aldrich) immediately prior to treatment of cells. Previous experience with water-insoluble platinum agents, including cisplatin, indicated that the dose-response curves following dissolution in water or a stock solution in DMSO (which is then promptly diluted in aqueous phase) overlap and there is no significant modulation of the individual cell lines' chemosensitivity. At the final dilutions, the solvent concentration never exceeded 0.5 mass %. Cells were exposed to the tested compounds for 72 h, whereby a set of 8 separate wells was used for each concentration. Each test was run in triplicate, i.e. in three separate microplates. After incubation with the tested compounds, MTT solution (10 mg mL^{-1} in PBS) aliquots were added to each well. The plates were further incubated for 4 h at 37 °C and the formazan crystals formed were dissolved by adding 110 μL of 5 mass % HCOOH in 2-propanol. Absorption of the samples was measured using an Elisa reader (Uniscan

Table 1. Characterisation data of newly prepared compounds

Compound	Formula	M_r	w_i(calc.)/% w_i(found)/%			Yield	M.p
			C	H	N	%	°C
V	$C_6H_{11}N_3O_2SCl_2Pt$	454.90	15.82	2.42	9.23	56	267[a]
			15.58	2.64	9.15		
VI	$C_7H_{13}N_3O_2SCl_2Pt$	468.90	17.91	2.77	8.96	53	282[a]
			18.07	2.95	8.68		

a) Decomposes.

Titertec) (Germany) at 580 nm. The survival fraction was calculated as a percentage of the untreated control. In addition, IC_{50} values were calculated from the concentration-response curves. The experimental data were processed using GraphPad Prizm software package for PC (Germany) and fitted to sigmoidal concentration/response curves via non-linear regression.

Results and discussion

Chemistry

The organic ligands *III*, *IV* were prepared by a mixture of water/ethanol solutions of *I* and *II* with NaCN or $(NH_4)_2CO_3$ under constant stirring and heating for 30 h. A detailed description of the method for preparation of these organic ligands was reported previously (Bakalova et al., 2015). The Pt(II) complexes *V* and *VI* were obtained following the reported procedure with minor revisions (Bierbach et al., 1999). The method consisted of the interaction of water/ethanol solutions of the ligands *III* and *IV* with an aqueous solution of $K[Pt(NH_3)Cl_3]$ under constant stirring. A scheme of the synthesis of the new mixed Pt(II) complexes is shown in Fig. 1.

The elemental analysis of the new Pt(II) complexes *V* and *VI* was in good agreement with the corresponding chemical formulas. The data from elemental analysis and some physical properties are summarised in Table 1.

In order to evaluate the mode of coordination of the ligands to the metal ions, IR, NMR spectra and DFT calculations of the metal-free ligands as well as their Pt(II) complexes were recorded.

Spectral characterisation

In comparing the IR spectra of the newly synthesised Pt(II) complexes, *V*, *VI* with those of the metal-free ligands, *III*, *IV*, a shifting of the band corresponding to the C—S bond is observed: ν(C—S) in a metal-free ligand *III* is at 1052 cm^{-1}, while in the complex *V* is at 1091 cm^{-1}; in the ligand *IV* the band corresponding to the C—S bond is at 1013 cm^{-1}, whereas in the complex *VI* it is at 1040 cm^{-1}. This shifting of the bands towards the higher frequencies indicated that probably the coordination of the ligands to platinum ions was effected by the sulphur atom from the cyclic ring. In the IR spectra of the complexes (*V* and *VI*), the new bands at 421 cm^{-1} and 419 cm^{-1} appeared. The stretching vibrations can be attributed to the ν(Pt—S) coordination bonds. In the FIR range, the new bands at 332 cm^{-1}, 313 cm^{-1} and 318 cm^{-1}, 309 cm^{-1} were assigned to the ν(Pt—Cl) stretching vibrations for the complexes *V* and *VI,* respectively. The two bands for ν(Pt—Cl) stretching vibrations in both the complexes were observed, implying *cis*-location of chloride ligands in accordance with the literature data (Nakamoto, 1978).

The bands related to the stretching vibrations of the two carbonyl groups in the metal-free ligands did not shift upon the coordination of *III* and *IV* to Pt(II) ions, indicating that the C═O groups were not involved in binding to the metals.

All these conclusions conformed to the previously obtained and studied Pt(II) complexes with the same ligands (Bakalova et al., 2015). In this earlier reported research, using DFT method, it was calculated that the coordination mode of the ligand *III* with the platinum ion in *cis*-$[PtL_2Cl_2]$ *VII* was effected by the sulphur atom from the cyclic ring. The results confirmed the square-planar geometry of the complex *VII* with the valence angles S—Pt—Cl close to 90° or 180°, depending on their position.

In the ^{1}H NMR spectrum of a freshly prepared DMSO-d_6 solution of complex *V*, the signals of the protons for CH_2 (C-2) and CH_2 (C-5) (axial and equatorial) were between δ 3.08 and 2.84. This could be explained by the S-containing ring being fixed and the sulphur atom bonding with the platinum ion (axial and equatorial hydrogens are easily discerned).

In the ^{1}H NMR spectrum of complex *VI* the signals of the protons for CH_2 (C-2) and CH_2 (C-6) (axial and equatorial) are between δ 2.81 and 2.50. In this case, the ring is fixed because the sulphur is bonded to platinum ions (Table 2).

In the ^{1}H NMR spectra of complexes *V*, *VI*, new broad signals appeared at δ 4.23 and 4.21, respectively, which could be assigned to the NH_3 molecules included in the complexes.

Table 2. Spectral data of newly prepared compounds *V* and *VI* and previously studied ligands *III* and *IV*

Compound	Spectral data
III[a]	IR, $\tilde{\nu}$/cm^{-1}: 1052 (C—S), 1738, 1780 (C═O), 3199, 3253 (NH) ^{1}H NMR (DMSO-d_6), δ: 2.17–2.28 (m, 2H, CH_2 (C-4)), 2.93 (d, 1H, J = 12.0 Hz, CH_2 (C-2(e))), 3.06 (d, 1H, J = 7.0 Hz, CH_2 (C-5(e))), 3.11 (d, 1H, J = 7.0 Hz, CH_2 (C-5(a))), 3,22 (d, 1H, J = 12.0 Hz, CH_2 (C-2(a))), 8.38 (s, NH (N-1)), 10.78 (s, NH (N-3))
IV[a]	IR, $\tilde{\nu}$/cm^{-1}: 1013 (C—S), 1735, 1772 (C═O), 3068, 3189 (NH) ^{1}H NMR (DMSO-d_6), δ: 1.74–1.84 (m, 2H, CH_2 (C-3), CH_2 (C-5(e))), 1.87–1.94 (m, 2H, CH_2 (C-3), CH_2 (C-5(a))), 2.55–2.63 (m, 2H, CH_2 (C-2), CH_2 (C-6(e))), 2.74–2.85 (m, 2H, CH_2 (C-2), CH_2 (C-6(a))), 8.47 (s, NH (N-1)), 10.65 (s, NH (N-3)),
V	IR, $\tilde{\nu}$/cm^{-1}: 322, 313 (Pt—Cl), 421 (Pt—S), 1091 (C—S), 1716, 1770 (C═O), 3197, 3262 (NH) ^{1}H NMR (DMSO-d_6), δ: 1.94–2.16 (m, 2H, CH_2 (C-4)), 2.84 (d, 1H, J = 12.0 Hz, CH_2 (C-2(e))), 2.99 (m, 1H, CH_2 (C-5(e))), 3.03 (m, 1H, CH_2 (C-5(a))), 3.08 (d, 1H, CH_2 (C-2(a)), J = 12.0 Hz), 4.23 (brs, 3H, NH_3), 8.39 (s, NH (N-1)), 10.78 (s, NH (N-3))
VI	IR, $\tilde{\nu}$/cm^{-1}: 318, 309 (Pt—Cl), 419 (Pt—S), 1064 (C—S), 1704, 1767 (C═O), 3073, 3191 (NH) ^{1}H NMR (DMSO-d_6), δ: 1.68–1.79 (m, 2H, CH_2 (C-3), CH_2 (C-5(e))), 1.82–1.89 (m, 2H, CH_2 (C-3), CH_2 (C-5(a))), 2.50–2.58 (m, 2H, CH_2 (C-2), CH_2 (C-6(e))), 2.71–2.81 (m, 2H, CH_2 (C-2), CH_2 (C-6(a))),4.21 (brs, NH_3), 8.41 (s, NH (N-1)), 10.60 (s, NH (N-3))

a) Data were published in a previous article Bakalova et al. (2015).

The signals for NH (N-1) and NH (N-3) in both the complexes were not influenced, indicating that the NH groups from the hydantoin ring were not involved in binding to the metals.

All the spectral data of the newly prepared complexes *V* and *VI* in comparison with the metal-free ligands *III* and *IV* previously studied are presented in Table 2.

Geometry optimisation

The structures of two possible *cis*-conformations of the complexes, namely boat and chair, are fully optimised. For the boat conformer, the platinum atom is at the same side as the C-5 atom, while for the chair conformer the platinum atom is located on the opposite side to the C-5 atom. The calculations revealed the boat conformer to be more stable than the chair conformer by 21 kJ mol^{-1}.

The theoretically predicted geometry of the ligands showed the hydantoin fragment to be essentially flat with an N_1—C_2—N_3—C_4 dihedral angle of –4.64° for ligand *III* and 2.97° for ligand *IV*. The remaining part of the molecule is oriented perpendicularly to the hydantoin fragment.

The calculations for the complexes showed the platinum(II) centre to be bonded to the sulphur atom from the 3-thiolanespiro-5′-hydantoin and the 4-thio-1*H*-tetrahydropyranspiro-5′-hydantoin and the nitrogen atom from NH_3. The coordination around the platinum atom has square planar geometry with valence angles S—Pt—Cl and S—Pt—N_3 close to 90° and 180°.

Selected geometrical parameters for ligands *III*, *IV* and their Pt(II) complexes *V*, *VI*, obtained at the B3LYP level of theory with 6-31++G* basis set for *III*, *IV* and LANL2DZ for their platinum complexes *V*, *VI* are summarised in Tables 3 and 4.

Table 3. Selected calculated parameters of ligand *III* and its Pt(II) complex *V*

Parameters	Ligand *III*	Complex *V*
Dipole moment, μ/D	1.94	10.31
bond lengths/Å		
Pt-S_7	–	2.44
Pt-$Cl_{1'}$	–	2.41
Pt-$Cl_{2'}$	–	2.40
Pt-$N_{3'}$	–	2.09
angles/°		
N_1-C_2-N_3	105.50	–
N_1-C_5-C_4	101.50	–
C_2-N_3-C_4	113.30	–
C_6-S_7-C_8	93.40	–
S_7-Pt-Cl_1	–	92.10
S_7-Pt-N	–	91.50
Cl_1-Pt-Cl_2	–	92.90
N-Pt-Cl_2	–	83.30
dihedral angles/°		
C_5-N_1-C_2-N_3	7.00	–
C_H5-C_4-N_3-C_2	0.50	–
C_5-C_6-S_7-C_8	22.80	
C_5-C_9-C_8-S_7	–30.70	–

The calculated geometrical parameters for ligands, *III* and *IV* and their Pt(II) complexes, *V* and *VI* are in good agreement with the experimental values reported for similar hydantoins and their platinum complexes (Ghani & Mansour, 2011; Bakalova et al., 2010, 2015).

The calculated IR vibrational frequencies of the

Table 4. Selected calculated parameters of ligand *IV* and its Pt(II) complex *VI*

Parameters	Ligand *IV*	Complex *VI*
Dipole moment, μ/D	1.36	10.72
bond lengths/Å		
Pt-S_8	–	2.45
Pt-$Cl_{1'}$	–	2.41
Pt-$Cl_{2'}$	–	2.40
Pt-$N_{3'}$	–	2.09
angles/°		
N_1-C_2-N_3	105.30	–
N_1-C_5-C_4	100.60	–
C_2-N_3-C_4	113.20	–
C_7-S_8-C_9	99.20	–
S_8-Pt-$Cl_{1'}$	–	93.50
S_8-Pt-N	–	90.80
$Cl_{1'}$-Pt-$Cl_{2'}$	–	92.30
$N_{3'}$-Pt-$Cl_{2'}$	–	83.10
dihedral angles/°		
C_5-N_1-C_2-N_3	5.90	–
C_5-C_4-N_3-C_2	–0.80	–
C_5-C_6-C_7-S_8	–68.30	–
C_5-C_{10}-C_9-S_8	–68.90	–
C_6-C_7-S_8-C_9	33.10	–

ligands and the most stable conformers of their platinum complexes were compared with the experimental values. Good correlations were found between the theoretical and experimental data.

The computed vibrational frequencies were used to determine the types of molecular motion associated with each of the experimental bands observed. The majority of the experimental frequencies are very well reproduced by the theoretical method.

Table 5. Cytotoxicity of ligands *III*, *IV*, Pt(II) complexes *V*, *VI*, *VII*, *VIII* and cisplatin in two human tumour cell lines

Compound	IC_{50} values/μM	
	SKW-3[a]	HL-60[b]
III[c]	114.0	202.8
IV[c]	92.6	180.9
V	18.2	93.2
VI	112.5	89.6
VII[c]	142.3	147.2
VIII[c]	109.4	127.7
cisplatin	11.4	8.7

a) T-cell leukaemia; *b*) acute myeloid leukaemia; *c*) data reported previously Bakalova et al. (2015).

From the data obtained from the spectroscopic analysis and DFT calculations for ligands *III*, *IV* and complexes *V*, *VI* it can be concluded that the most probable bonding of the organic ligands with the metal ions in the reported complexes was effected through the sulphur atom from the cyclic ring. The IR spectra of ligands *III*, *IV* and their platinum complexes *V*, *VI* were calculated using the DFT method and compared with the experimental IR spectra of the same compounds. The data showed a very good correlation between the experimental and theoretical data of the IR spectra. The optimised structures of *III*, *IV* and the most stable conformers of their Pt(II) complexes, *V*, *VI* are presented in Figs. 2 and 3.

On the basis of all the results obtained, such as elemental analysis, melting points, IR, NMR spectra and DFT calculations, the following chemical formulas of the newly synthesised Pt(II) complexes could be proposed (Figs. 4 and 5).

All the platinum(II) complexes and metal-free ligands tested exerted cytotoxic effects after 72 h of continuous exposure, whereby the individual chemosensitivity varied among the different cell lines, as indicated

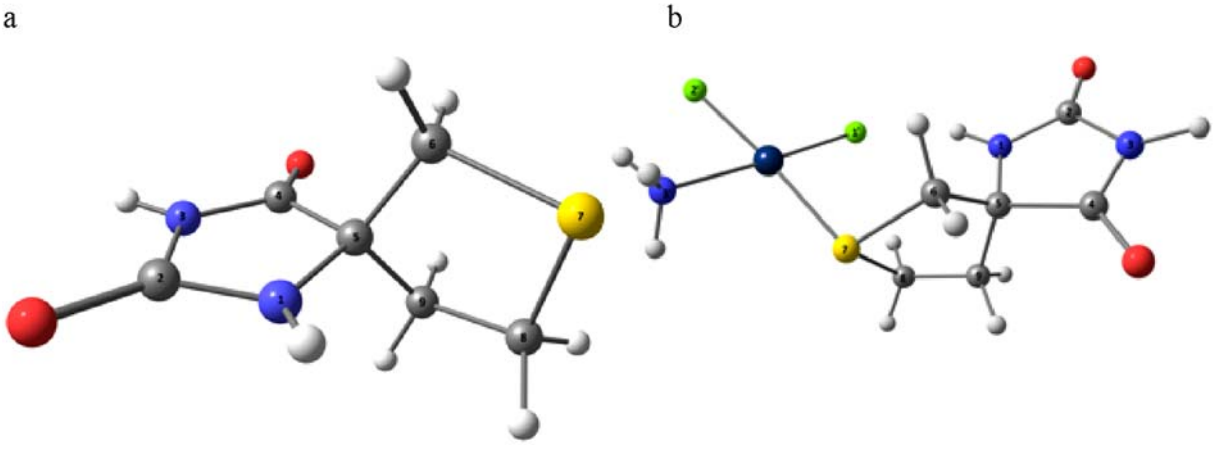

Fig. 2. Optimised geometries of *III* (a) and its platinum complex *V* (b).

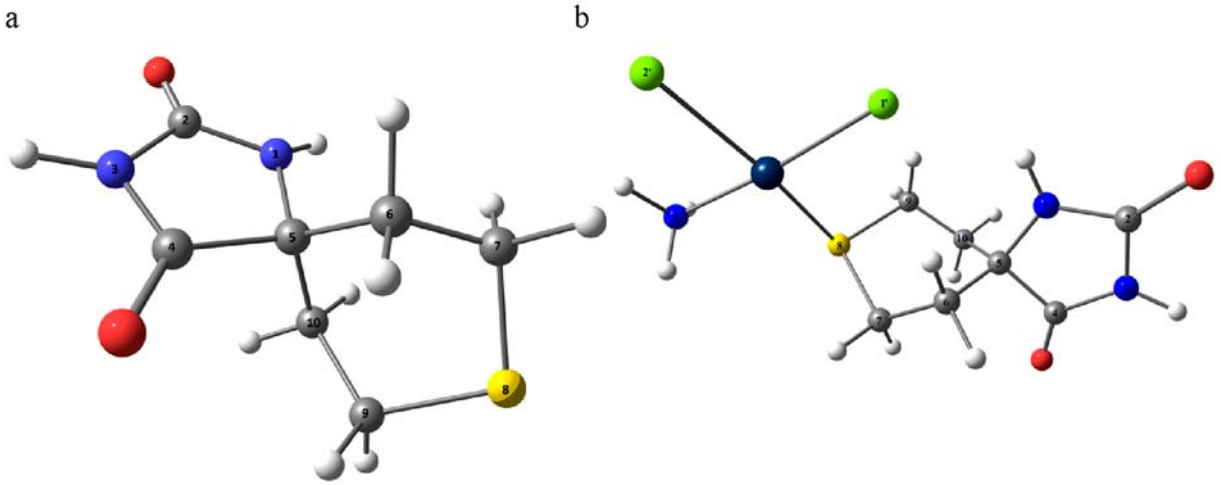

Fig. 3. Optimised geometries of *IV* (a) and its Pt(II) complex *VI* (b).

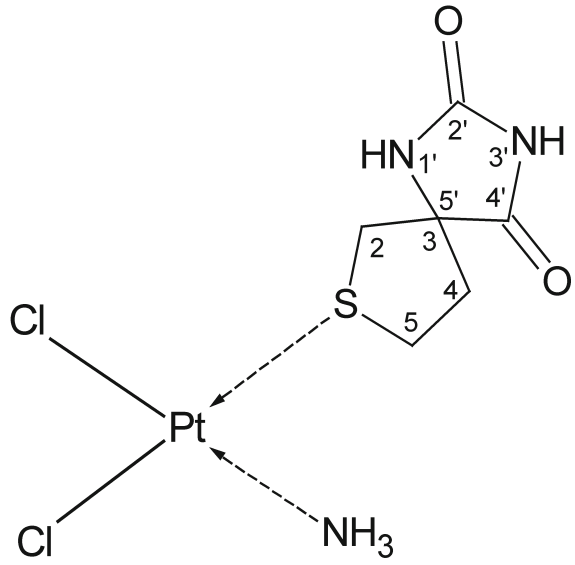

Fig. 4. Chemical formula of complex *V* as reported in the article.

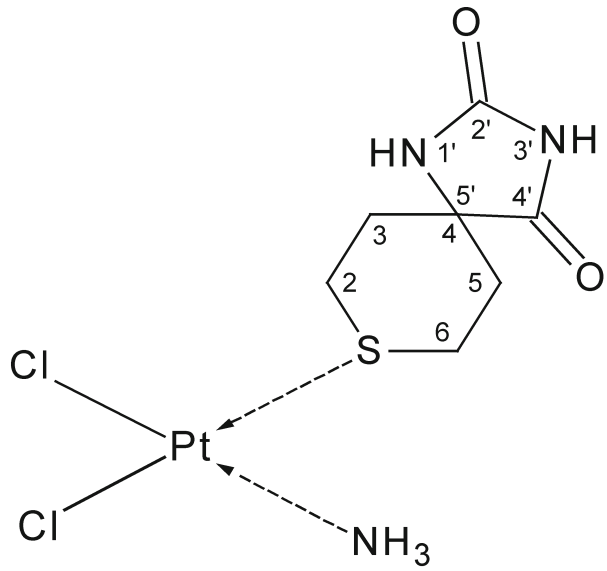

Fig. 5. Molecular formula of complex *VI*.

by the IC_{50} values summarised in Table 5.

When comparing the activities of the new Pt(II) complexes *V*, *VI* with the previously reported Pt(II) complexes *VII*, *VIII* and metal-free ligands *III*, *IV* it could be seen that complex *V* exhibited an activity similar to cisplatin on the SKW-3 cell line. This could be explained by the fact that complex *V* is cisplatin's analogue. On the other hand, complex *V* belongs to the "non-classical" platinum complexes, because it contains the S-containing organic ligand instead of the ammine ligand. The same complex is more active than all the other complexes on the HL-60 cell line. The cytotoxicity of complex *VI* was comparable with that of ligand *IV* and complex *VIII* on the SKW-3 cell line while complex *VI* was more active than complexes *IV* and *VIII* on the HL-60 cell line. On the same cell line, complex *V* was more active than ligand *III* and complex *VII*. The platinum compounds studied displayed cytotoxic effects in a concentration-dependent manner. In conclusion, the cytotoxicity of complexes *V* and *VI* where one ammine ligand was replaced by an S-containing organic ligand was higher than the cytotoxicity of the other complexes *VII* and *VIII*.

Conclusions

Two novel Pt(II) complexes of the general formula *cis*-$[Pt(NH_3)LCl_2]$, where L is 3-thiolanespiro-5′-hydantoin and 4-thio-1*H*-tetrahydropyranspiro-5′-hydantoin were synthesised and investigated. The molecular formulas of the complexes were confirmed by elemental analysis, melting points, IR and ^{1}H NMR spectroscopy. The coordination mode of the ligands

with metal ions was confirmed by using hybrid DFT calculations of ligands *III*, *IV* and the most stable conformers of their platinum complexes *V*, *VI*. The compounds tested exerted concentration-dependent cytotoxicity on a SKW-3 and HL-60 human tumour cell lines. The most significant cytotoxicity was observed for complex *V*, which inhibited the viability of cells tested at low micromolar concentrations and the IC_{50} value was comparable with that of cisplatin. The new Pt(II) complexes reported of the general formula *cis*-$[Pt(NH_3)LCl_2]$ *V*, *VI* exhibited higher cytotoxic activity than the previously studied Pt(II) complexes with the general formula *cis*-$[PtL_2Cl_2]$ *VII*, *VIII* and metal-free ligands *III*, *IV*.

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