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Platinum(II) complexes with thiourea derivatives containing oxygen, sulfur or selenium in a heterocyclic ring: computational studies and cytotoxic properties

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Abstract Platinum(II) complexes with 1-(2-oxolanylmethyl)-2-thiourea, 1-(2-thiolanylmethyl)-2-thiourea and 1-(2-selenolanylmethyl)-2-thiourea were synthesized in order to compare their cytotoxic activities with those of the free thioureas. Their equilibrium geometries and bonding energies in the gas phase and in solution were calculated using density functional theory at the MPW1PW/LanL2DZ level. The IR spectra of the complexes and their free ligands were compared. Stability of the complexes in 0.9% saline aqueous solution was tested by means of HPLC. The cytotoxic activities of the species against five human cell lines were evaluated, as well as the antimicrobial activities against twelve bacterial strains.

Introduction

Platinum-based drugs are among the most active anticancer agents available. They are used for the treatment of a variety of human solid tumors and form the largest class of anticancer drugs. They destroy malignant cells by interfering with the DNA, via inter- and intrastrand crosslinks, where platinum binds primarily to the adjacent guanine-N7 sites [1–5], thereby preventing cell division and growth. The most frequently used platinum-containing drugs are *cis*platin and carboplatin (first-generation drugs) or oxaliplatin, proplatin and spiroplatin (second generation). Although they are extremely effective, cancer cells quickly become resistant to them. Therefore, the search for new platinum drugs has focused on identifying compounds with improved tolerability profiles and, in particular, on those that may overcome the mechanisms of resistance. Recently, it has been shown that when irradiated even in small nuclear reactors like the scientific reactor TRIGA, platinum(II) compounds contain detectable amounts of the ^{193m}Pt and ^{195m}Pt radionuclides [6]. Both radionuclides decay by electron capture, so compounds containing these radionuclides may also be useful for the Auger Electron Therapy (AET) of cancers.

In the last two decades, interest in platinum(II) complexes with chelating ligands containing both nitrogen and sulfur donor atoms has increased, because these complexes can exhibit either higher anticancer activity or reduced toxicity in comparison with the known metal-containing drugs [7–11]. As a result of these studies, a number of novel Pt(II) complexes sufficiently interesting for clinical trials have been synthesized. However, only a few of them are superior to the parent drug in their efficacy [12]. Here, mention should be made of platinum(II) complexes with certain thiourea derivatives (in particular containing an acridine fragment, called ACRAMTUs [13, 14]), which are potential pharmaceutical agents. Their therapeutic action differs, however, from that established already for *cis*platin because they interact with DNA in the intercalative mode. In the same vein, the main objective of this study was to synthesize and characterize new platinum complexes as possible chemotherapeutic agents.

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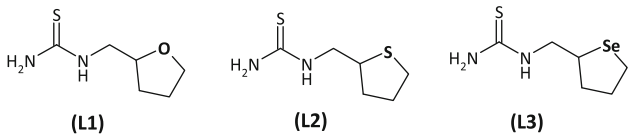
In living cells, there is competition for Pt between binding to guanine-N7 and to S-donor ligands. The toxicity of the Pt complexes is thought to be related to protein binding, in particular via reactions with thiol groups [15]. Thus, preventing these thiol-Pt interactions should reduce side effects and so increase the share of the drug that may react with the guanine-N7 target. One effective way of perturbing the Pt-amino acid interactions is additional complexation of the Pt-atom with S-donor ligands, which are relatively weakly bound to Pt, for example thioethers. Previous studies of competition for nucleobases and such weak Pt-containing compounds with S-donor ligands have shown that easy transfer of Pt from a thioether ligand (but not from thiolates) to guanine-N7 occurs [15]. Recent theoretical calculations and experimental competitive studies showed that platinum forms a weaker bond with the thioether group of methionine than with the thiol group of cysteine [16]. These findings resulted in combined chemotherapeutic procedures consisting of the application of various Pt(II) complexes together with compounds containing S-donor groups (in this case called chemoprotectants or rescue agents), which have been tested with the aim of reducing the platinum-based side effects. It can be hypothesized that the higher anticancer activity or reduced toxicity of platinum(II) complexes with chelating ligands containing both nitrogen and sulfur donor atoms is due to the above-mentioned mechanism.

A question arises as to whether substitution of the S-donor groups in the Pt-containing complexes by Se-donor groups could result in better (or worse) therapeutic effects. We were especially interested in answering the question whether substitution of an ether O atom by S or Se in a chelating ligand could result in greater cytotoxicity. To this end, we synthesized the compounds shown in Scheme 1: 1-(2-oxolanylmethyl)-2-thiourea (**L1**), 1-(2-thiolanylmethyl)-2-thiourea (**L2**) and 1-(2-selenolanylmethyl)-2-thiourea (**L3**), and their Pt complexes. The equilibrium geometries of the complexes were investigated by quantum theoretical methods, and their cytotoxicities, the main objective of the present study, were tested on five cell lines.

As mentioned above, it would be interesting to compare biological features of a series of compounds containing one of the three consecutive chalcogens in the five-membered ring of the ligand, i.e. oxygen, sulfur or selenium. The biological properties of the oxygen-containing compounds and their thio analogs have already been compared in several papers. For example, it was found that three thio analogs of Cl-IB-MECA, an agonist at the human A3 adenosine receptor, exhibited higher binding affinities than Cl-IB-MECA itself [17]. It has also been reported that 2-acetyl-S-octadecyl-rac-1-thioglycero-3-phosphocholine, a thio analog of a known platelet-activating factor, showed lower platelet-aggregating potency compared with the parent ether compound [18]. However, to our knowledge, no series of compounds containing three consecutive chalcogens has been investigated to date, and the present study is thus devoted to this aim.



Scheme 1 Thiourea ligands used in this study

Experimental

All chemicals and solvents were purchased from Sigma-Aldrich and used without further purification unless otherwise noted. 1-(2-Oxolanylmethyl)-2-thiourea (**L1**), 1-(2-thiolanylmethyl)-2-thiourea (**L2**), and 1-(2-selenolanylmethyl)-2-thiourea (**L3**) were prepared by the procedures given below.

Preparation of **L1**

1-(2-Oxolanylmethyl)-2-thiourea was synthesized according to the procedure described in Ref. [19]. A mixture of tetrahydrofurfurylamine (2-oxolanylmethylamine) (7 g, 69 mmol), triethylamine (10 mL, 7.3 g, 72 mmol) and dioxane (10 mL, 117 mmol) was cooled to −10 °C and treated with 4.2 mL (70 mmol) of carbon disulfide. The solution was allowed to warm to room temperature, chilled again to −10 °C and treated dropwise with 7.5 mL of ethylchloroformate. After filtering out the precipitated triethylamine hydrochloride, a solution of 10 mL of triethylamine in 20 mL of chloroform was added to the filtrate. When the copious production of carbon oxysulfide had ceased (after about 5 min at room temperature), the solution was distilled *in vacuo* (159–162 °C/32 mm Hg) to furnish 4 g of the product, tetrahydrofurfuryl isothiocyanate. The compound was stirred with 20 mL of concentrated NH_3 overnight. The precipitate of 1-(2-oxolanylmethyl)-2-thiourea was recrystallized from water. Colorless crystals of the product were obtained, yield 4 g (25 mmol, 36%).

Preparation of **L2**

2-Thiolanylmethylamine was synthesized as described in Ref. [20]. The final product was prepared by a modified method applied for the preparation of *n*-butyl isothiocyanate [19]: A mixture of 2-thiolanylmethylamine (1.23 g, 10.5 mmol), triethylamine (1 mL, 0.73 g, 7.2 mmol) and 2 mL of dioxane was chilled to −10 °C and treated with

carbon disulfide (0.5 mL, 0.63 g, 8.3 mmol). The solution was allowed to warm to room temperature, chilled and treated dropwise with ethyl chlorocarbonate (0.75 mL, 0.85 g, 8 mmol). Then, a solution of triethylamine (1 mL, 7.2 mmol) in chloroform (4 mL) was added at room temperature. After filtering off the precipitate of triethylamine hydrochloride, the solution was distilled *in vacuo* (170 °C / 302 mm Hg) to furnish 1 g (6.3 mmol) of the isothiocyanate derivative (yield 60%). This was stirred with 5 mL of concentrated NH_3 overnight. The product was recrystallized from water: the colorless crystals (about 240 mg) had a m.p. of 72 °C. The filtrate was evaporated to dryness and chromatographed by means of TLC. An additional 340 mg of the product (**L2**) was obtained as colorless crystals. Total yield: 580 mg (3.3 mmol, 54%).

Preparation of **L3**

The compound was synthesized in a similar way to **L2** from 2-selenolanylphenylamine. Synthesis of the amine has not previously been described. It was obtained in a similar way to 2-methylselenolane [21]: $NaBH_4$ (4.6 g, 121 mmol) was gradually added (under N_2 flux) to 150 mL of an ethanolic suspension of selenium powder (4.8 g, 61 mmol). After Se dissolution, 2,5-dichloropentylamine hydrochloride (11.6 g, 60 mmol) was added, and the solution was boiled for 3 h. Then, ethanol was evaporated off, 200 mL of water was added and the product was extracted with two 50-mL aliquots of diethyl ether. The ether solution was dried with K_2CO_3. The product, after evaporation of the ether, was distilled under vacuum (b.p. 110–120 °C / 302 mm Hg). The distillate containing the amine was used further without purification. Colorless crystals of **L3** were obtained. Yield 12.0 g (54 mmol, 90%).

The purity of the compounds **L1**–**L3** was monitored by HPLC analysis under gradient conditions.

Preparation of the platinum(II) complexes

The title complexes were prepared by the following general synthetic procedure [22]:

A solution of the appropriate ligand (0.5 mM) in MeOH (1 mL) was added dropwise at room temperature to an aqueous solution of K_2PtCl_4 (0.5 mM; 1 mL) until a molar ratio of about 1:1 was achieved. The yellow or salmon pink solid products, which started to precipitate immediately after adding the first drop, were filtered off, washed several times with fresh aliquots of warm water and dried in a vacuum desiccator until a constant mass was reached. The molecular weights of the Pt complexes with ligands **L1** and **L2** were determined by mass spectrometry, courtesy of the Institute of Inorganic Chemistry, Zurich University, on a Merck Hitachi M-8000 HPLC/MS spectrometer with electrospray ionization. All spectra were recorded in the positive ion mode. The platinum complexes obtained are labeled Pt(**LX**)Cl_2, (where X stands for **1**, **2** or **3**, respectively). Numerous attempts to obtain crystals of the complexes suitable for X-ray diffraction studies have failed. The complexes were insoluble in the organic solvents commonly used in crystallography, except DMSO. Once dissolved in the latter, the complexes could be further diluted in water.

Elemental analyses for C, H, N, S and Cl were performed in the Laboratory of Analytical Chemistry, Chemical Department (Warsaw Technical University). The platinum content was established by atomic absorption spectroscopy (AAS) in the Analytical Department of the Institute of Nuclear Chemistry and Technology. Analytical data are presented in Table 1.

Quantum calculations

All calculations were carried out using Gaussian 03 revision B04 [22]. The ground state of the neutral complexes was a closed-shell singlet because large ligand field values lead to low-spin d^8 complexes of platinum(II) having tetracoordinated planar geometry [23]. The geometry optimizations of the ligands and their complexes were performed with the MPW1PW91 functional and LanL2DZ basis set, taking into account relativistic effects [24]. It has already been shown that calculations performed at the DFT level give a reasonable description of the structural features of platinum(II) complexes with thiourea [25]. In addition, when structures of the Pt(II) and Pt(IV) complexes with histamine were simulated by using over 20 DFT functionals, the X-ray and theoretical results were sufficiently consistent for three of them, including the MPW1PW91 functional applied in this study [26].

The bonding energy of the complexes was obtained as the difference between the energy of a given complex and the energies of the appropriate free ligand and $PtCl_2$. The energies in aqueous solution were calculated using the dielectric polarizable continuum model (DPCM), belonging to the self-consistent reaction field (SCRF) models, together with the united atom topological model for Hartree–Fock (UAHF) atomic radii [27], since this method worked relatively well in calculations of acidic dissociation constants of a set of molecules [28].

HPLC analysis and spectroscopy

The chromatography was performed using a Merck L7100 chromatograph equipped with an analytical Supelcosil LC-C_{18} column and a Shimadzu SPD-10AVP UV-Vis detector. The purity of the free ligands was checked by the following

Table 1 Chemical composition, molecular mass and formulae of the compounds

Compounds/formulae of the ligands	Chemical composition[a]							Molecular mass	Color
	C	H	N	S	Cl	Pt	Cl:Pt		
L1 / $C_6H_{12}N_2OS$	45.0	7.5	17.5		–	–	–	160	White
	(45.0)	*(7.6)*	*(17.5)*		–	–		*(160.2)*	
Pt(**L1**)Cl_2	17.5	2.6	6.6		12.8	34.8	2.02	427	Yellow
	(16.9)	*(2.8)*	*(6.6)*		*(12.7)*	*(34.7)*		*(426.3)*	
L2 / $C_6H_{12}N_2S_2$	41.0	6.8	15.6	36.0	–	–	–	176	White
	(40.9)	*(6.9)*	*(15.9)*	*(36.4)*	–	–		*(176.3)*	
Pt(**L2**)Cl_2	16.9	2.9	6.3	15.3	15.9	43.9	1.99	442	Pink
	(16.3)	*(2.7)*	*(6.3)*	*(14.5)*	*(16.1)*	*(44.1)*		*(442.4)*	
L3 / $C_6H_{12}N_2SSe$	32.8	5.5	12.6	14.1	–	–	–	224	White
	(32.3)	*(5.4)*	*(12.5)*	*(14.4)*	–	–		*(223.2)*	
Pt(**L3**)Cl_2	15.4	2.8	5.8	6.9	14.3	39.8	2.03	489	Pink
	(14.7)	*(2.5)*	*(5.7)*	*(6.5)*	*(14.5)*	*(39.9)*		*(489.2)*	

[a] Found—normal text; calculated—in italics

gradient elution: 0–20 min: 20 to 80% A; 20–30 min: 80% A (where solvent A consisted of acetonitrile with 0.1% trifluoroacetic acid and solvent B of water with 0.1% trifluoroacetic acid). The flow rate was 1 mL/min. UV detection was carried out at 250 nm. Studies of the stability of the complexes as a function of time were performed by isocratic elution with a mixture of water and acetonitrile, vol/vol = 80:20. The UV detector was set at 230 nm.

Infrared spectra were recorded with 1-cm^{-1} resolution in the region of 4,000–400 cm^{-1} on a BRUKER Equinox 55® Fourier transform spectrophotometer. The spectra were recorded as KBr pellets.

Biological studies

The cytotoxicities of the complexes were estimated in vitro by the MTT[1] assay. The complexes were dissolved in DMSO and diluted in minimum essential medium (MEM) to the required concentrations; the final DMSO concentration did not exceed 1%. Five human cell lines: normal (WS1) and neoplastic (ME18, ME18/R, HeLa, KB-V1), sensitive (ME18, HeLa) and resistant to cytostatics (ME18/R, KB-V1), were tested. The IC_{50} values were determined in three independent experiments as the concentration of the complexes required for 50% inhibition of cell growth.

Cell lines: WS1, HeLa and KB-V1 were obtained from the American Type Culture Collection (ATCC), ME18 cell line was a gift from the Institute of Oncology, Warsaw, and ME18/R line was obtained experimentally by the National Medicines Institute. Cells were grown in MEM supplemented with 10% fetal calf serum and antibiotics.

The following microorganisms were obtained from the ATCC: *Enterococcus hirae* ATCC 10541, *Stenotrophomonas maltophilia* ATCC 12714, *Escherichia coli* ATCC 10538 and ATCC 8739, *Bordetella bronchiseptica* ATCC 4616, *Bacillus pumilus* ATCC 14884, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 6538P, *Pseudomonas aeruginosa* ATCC 9027 and ATCC 15442, *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* 16404. Wild-type *Staphylococcus aureus* strains were isolated from clinical material: MRSA 11 from blood and MRSA 2 from nasal fluid.

Amounts of the complexes equivalent to 12.5 25, 50 or 100 μM were successively dissolved in 0.25 mL of DMSO and mixed with 4.75 mL of a Mueller-Hinton 2 Agar at 45 °C to obtain solutions with a concentration of 2.5, 5, 10 or 20 mM/mL. Then, 2 μL of a particular cell suspension of optical density 0.5 unit on the McFarland scale were applied to the surface of the agar. The lowest concentration of a tested compound that totally inhibited the growth of the examined strain was chosen as the MIC value.

Results and discussion

Structures of the free ligands and complexes and ligand-bonding energies

As expected, the main differences in the structures of the free ligands occur within the five-membered ring that includes the oxygen, sulfur, or selenium heterocyclic atoms. Both theoretical [29] and experimental data [30] prove that the conformation of the free tetrahydrofuran ring

[1] Test for measuring cell growth with colorimetric assay using yellow colorimetric indicator: MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide.

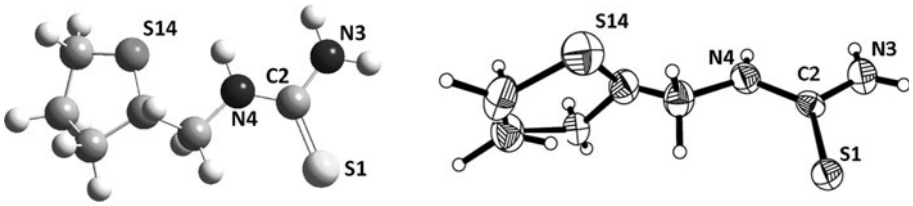


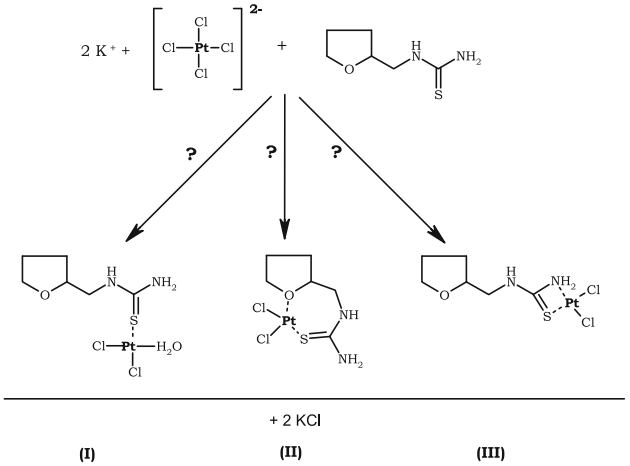
Fig. 1 Structure of minimum energy calculated for **L2** (*left*) compared with that found experimentally for **L2** (*right*; thermal ellipsoids are drawn at the 30% probability level

Table 2 Calculated bond lengths and angles in the free ligands (Scheme 1) at the MPW1PW91/LanL2DZ level

	(**L1**), X=O	(**L2**), X=S	(**L3**), X=Se
Bond lengths (Å)			
C(2)–S(1)	1.732	1.731	1.730
C(2)–N(3)	1.370	1.368	1.368
C(L2)–N(4)	1.357	1.363	1.364
C(11)–X(14)	1.478	1.907	2.024
C(18)–X(14)	1.466	1.895	2.013
C(13)–C(11)	1.537	1.536	1.536
C(13)–C(17)	1.543	1.539	1.539
C(17)–C(18)	1.533	1.534	1.534
Angles (°)			
N(3)–C(2)–N(4)	115.62	115.36	115.47
N(3)–C(2)–S(1)	120.46	120.36	120.47
C(13)–C(11)–X(14)	105.78	105.01	104.78
C(11)–X(14)–C(18)	109.82	93.03	89.43
Dihedral C11–C13–C17–C18	35.05	48.84	52.07

is an envelope structure with a dihedral angle φ (calculated for the four carbon atoms) of 52.5°. In the tetrahydrofuran ring, which is a fragment of **L1**, the ring structure is modified. Namely, it takes on a twisted conformation characterized by the coplanarity of three adjacent ring atoms (C11–O–C18) and the midpoint between the opposite (C13–C17) bond. The conformation of the five-membered rings in **L2** and **L3** is similar to that in **L1**. The calculated structure of **L2** when compared with the experimental structure obtained by X-ray diffraction is shown in Fig. 1. The corresponding bond lengths and angles in free ligands are shown in Table 2.

Given that the analytically determined Pt : Cl molar ratio in the platinum(II) complexes is close to 1:2 (Table 1), we searched for the most probable structure of these complexes. Three tentative configurations were proposed as starting points for the calculations. Scheme 2 presents the configurations for the first compound in the series, namely for the platinum(II) complex with 1-(2-oxolanylmethyl)-2-thiourea. The acyclic species with **L1** bound monodently to the $PtCl_2$ synthon is denoted below as Pt-(**L1-I**). The two cyclic structures are denoted as Pt-(**L1-II**) and Pt-(**L1-III**), respectively. The structure of Pt-(**L1-II**) contains a seven-membered ring, whereas that of Pt-(**L1-III**) has a four-membered ring. Similar structures



Scheme 2 Postulated coordination modes of 1-(2-oxolanylmethyl)-2-thiourea (**L1**) with the $PtCl_2$ synthon

were taken into consideration for complexes containing ligands **L2** and **L3**, respectively. The corresponding complexes with L**2** are denoted as Pt-(**L2-I**), Pt-(**L2-II**), and Pt-(**L2-III**), while those with L**3** are denoted as Pt-(**L3-I**), Pt-(**L3-II**), and Pt-(**L3-III**).

The structures shown in Scheme 2 assume that the ligands remain in the thione tautomers. This is most probably true in the gas phase as well as in the solid state structures of the complexes. This hypothesis is supported by the calculations of the relative energies of the thiol and thione forms of the ligands and of three forms of the complexes. The thiol tautomers are higher in energy by tens of kcal/mol. Bonding energies calculated in the gas phase for the thione tautomers of the complexes are shown in Table 3, and their geometric parameters are displayed in Table 4.

The data shown in Table 3 show that in the gas phase, structure **II**, containing a 7-membered ring, is the most stable for all three complexes. On that basis, we may assume that the complexes mainly, if not exclusively, take the form of structure **II** (Scheme 2) in the solid state as well. The higher energy of structures **III** seems to be consistent with the lower stability of four-membered rings caused by the relatively large ring strains and angle deformations. However, this structure has been experimentally found in thioplatin—a drug proposed for cancer therapy in 2001 [31]. The energy differences between structures **II** and **I** or **III** are significantly greater for the

Table 3 Calculated bonding energies and relative bonding energies (kcal/mol) of three structures of Pt(**L1**)Cl_2, Pt(**L2**)Cl_2 and Pt(**L3**)Cl_2 in the gas phase

	Gas phase	
	E_{bond}	E_{bond} (rel)
Pt(**L1**)Cl_2		
Pt-(**L1-I**)	−50.05	6.33
Pt-(**L1-II**)	−56.38	0
Pt-(**L1-III**)	−49.01	7.37
Pt(**L2**)Cl_2		
Pt-(**L2-I**)	−51.35	18.0
Pt-(**L2-II**)	−69.34	0
Pt-(**L2-III**)	−43.15	26.2
Pt(**L3**)Cl_2		
Pt-(**L3-I**)	−51.83	22.6
Pt-(**L3-II**)	−74.42	0
Pt-(**L3-III**)	−41.66	32.8

complexes containing sulfur and selenium than the oxygen heteroatom. Figure 2 presents the structures of Pt-(**L1-II**) (complex containing a seven-membered ring) and Pt-(**L1-III**) (containing a four-membered ring) optimized at the MPW1PW91/LanL2DZ level.

The data shown in Table 4, as well as the structures shown in Fig. 2, refer to the lowest energy conformers that were found in the process of energy minimization. Two intramolecular hydrogen bonds were found for these most stable conformers of structures **II** and **III**. For example, for Pt-(**L1-II**), the bonds are N–H···Cl (2.644 Å) and C–H···Cl (2.557 Å) (Table 4). For Pt-(**L1-III**), they are N–H···O

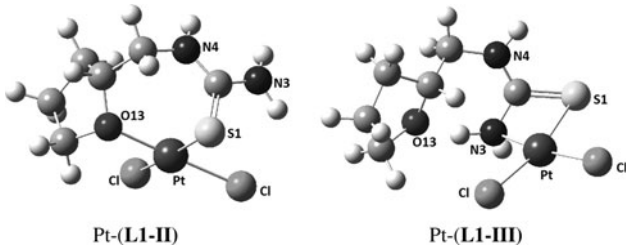


Fig. 2 The lowest energy structures of the platinum(II) complexes with 1-(2-oxolanylmethyl)-2-thiourea, optimized at the MPW1PW91/LanL2DZ level

(1.594 Å) and C–H···Cl (2.932 Å). However, for each of the structures, a set of conformers of higher energy was found. Hydrogen bonds in the higher energy conformers were longer, which suggests weaker energy stabilization due to these H-bonds.

In aqueous solutions, however, the energy considerations lead to different results. Here, the energy difference between the thiol and thione tautomers is sufficiently high to make the existence of the unionized thiol forms unlikely. Nevertheless, after proton dissociation, the energy of the system composed of the anionic form of the complex (thiolate) and the solvated proton significantly decreases relative to the energy of unionized thiol and thione structures (the aqueous solvation energy of the proton, of −265.9 kcal/mol, was taken from ref. [32]). However, for conformers **III**, the energies of the thione structures remain the lowest, whereas for the conformers **I**, **II**, and for the free ligands, the energy differences between the thiolates and thiones and between thiolates and thiols are strongly negative (of the order of −30 to −100 kcal/mol).

Table 4 Calculated bond lengths (Å) and angles (°) in the platinum complexes having the lowest energy conformation

	Pt-(**L1-II**), *X=O*	Pt-(**L2-II**), *X=S*	Pt-(**L3-II**), *X=Se*
Bond lengths (Å)			
Pt–S	2.444	2.448	2.450
Pt–Cl(1)	2.359	2.389	2.403
Pt–Cl(2)	2.396	2.389	2.387
Pt–C	2.656	2.819	2.829
C–NH_2	1.347	1.343	1.342
C–NH	1.357	1.352	1.351
H(1)···Cl(1)	2.644	2.492	2.423
H(2)···Cl(2)	2.557	2.503	2.409
Pt–X	2.145	2.408	2.480
Angles (°)			
Cl(1)–Pt–Cl(2)	90.07	90.66	90.33
S–C–NH_2	119.24	118.73	118.79
NH–C–NH_2	119.36	119.73	119.91
X–Pt–S	88.79	86.25	86.71
C–X–C	102.95	89.35	87.54
C–C–C–C dihedral in 5-membered ring	17.62	31.72	48.49

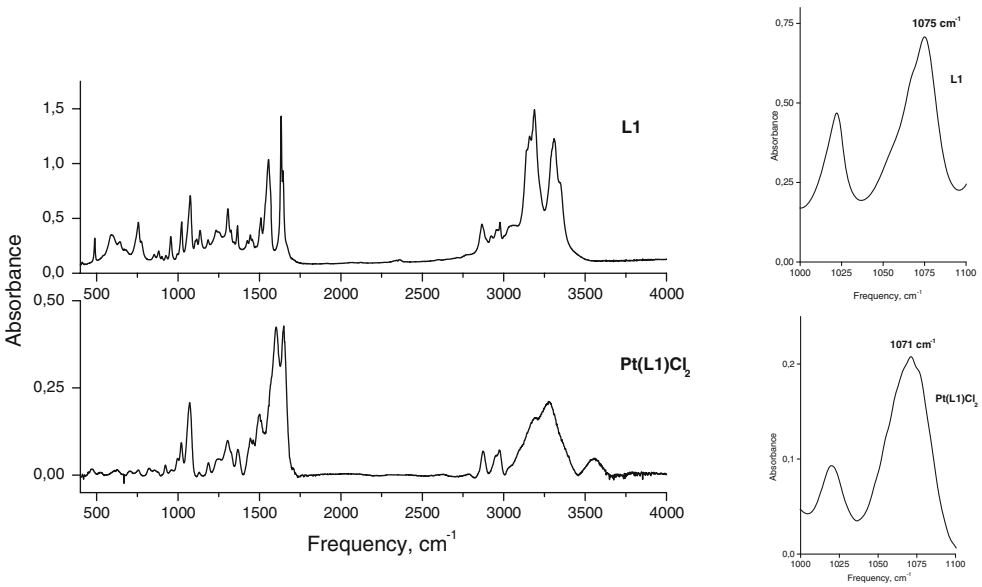


Fig. 3 Spectra of **L1** (*upper*) and Pt(**L1**)Cl_2 (*bottom*); KBr pellet

Therefore, it can be assumed that in aqueous solutions, the complexes might exist in the form of thiolates and take also the linear conformations.

Vibrational spectra

Representative IR spectra, namely of **L1** and Pt(**L1**)Cl_2, are shown in Fig. 3. The spectra of **L2** and Pt(**L2**)Cl_2 as well as of **L3** and Pt(**L3**)Cl_2 appeared not to differ significantly from these of the oxygen ones.

The absence of any band in the 2,450–2,650 cm^{-1} region, which might be assigned to the ν(S–H) vibrations of the ligands [33], suggests the absence of any thiol tautomer in the solid state. On the other hand, both ν(C=S) bands at ca. 1,350 cm^{-1} and different NH-stretching vibrational bands at above 3,150 cm^{-1} [33] can be observed. This indicates that the species remain mostly as the thione tautomers.

The ν(C=S) bands are weakened and shifted to higher wavenumbers in all the complexes when compared to the corresponding free ligands (60, 20 and 30 cm^{-1} for the Pt(**L1**)Cl_2, Pt(**L2**)Cl_2 and Pt(**L3**)Cl_2, respectively). On the other hand, bands in the region of 3,440–3,270 cm^{-1}, attributed to different stretching modes of both amine groups in the spectra of the free ligands, show appreciable differences to the spectra of the complexes. All these differences in the spectra may be a consequence of the metal coordination *via* the sulfur donor atom from the thiourea moiety, as reported earlier [22]. Of special interest are the bands related to the C-**X**-C ring vibrations of the ligands (in the region 1,500–750 cm^{-1}). Comparison of the spectra registered for Pt(**L1**)Cl_2, Pt(**L2**)Cl_2 and Pt(**L3**)Cl_2 with those of the appropriate free ligands reveals that the relevant bands change their position and intensity. Namely they move from 1,075 (**L1**) to 1,071 cm^{-1} (Pt(**L1**)Cl_2), from 935 (**L2**) to 928 cm^{-1} (Pt(**L2**)Cl_2) and from 922 cm^{-1} (**L3**) to 913 cm^{-1} (Pt(**L3**)Cl_2). This suggests that in the solid phase, the oxygen/sulfur/selenium heteroatoms of the five-membered rings are also involved in the metal complexation (as in the structure **II** shown in Scheme 2).

Stabilities of the complexes

The stabilities of Pt(**L1**)Cl_2, Pt(**L2**)Cl_2 and Pt(**L3**)Cl_2 in standard aqueous physiological solution (0.9% NaCl; pH 4.5) were checked by recording their UV–Vis spectra (200–900 nm) vs. time. No changes in the spectra were observed within about 1 week. The HPLC chromatograms of samples withdrawn occasionally from the saline solutions of the complexes did not exhibit noticeable changes either. The same was observed for the aqueous solutions of the complexes to which glutathione (GSH), a tripeptide being one of the main donors of the SH groups in the cytoplasmic environment, was added.

Cytotoxicities of the complexes

Results obtained for the cytotoxicities of complexes, measured by the half-maximal inhibitory concentration (IC_{50}), are shown in Table 5. It can be concluded that the cytotoxicities of Pt(**L1**)Cl_2 and Pt(**L2**)Cl_2 are similar for all types of cells examined. However, for Pt(**L3**)Cl_2, the IC_{50} was lower than those for Pt(**L1**)Cl_2 and Pt(**L2**)Cl_2 for three cell lines. The effectiveness of Pt(**L3**)Cl_2 in inhibiting the growth of HeLa cancer cells was comparable to that of carboplatin (IC_{50} 48 vs. 23 μg/mL [34]).

Table 5 IC_{50} values of the investigated platinum(II) complexes

Cell line	Pt(**L1**)Cl_2		Pt(**L2**)Cl_2		Pt(**L3**)Cl_2	
	IC_{50} ± SD (μg/mL)	IC_{50} (mM)	IC_{50} ± SD (μg/mL)	IC_{50} (mM)	IC_{50} ± SD (μg/mL)	IC_{50} (mM)
WS1 (human embryonic skin fibroblasts)	64.6 ± 18.8	0.152	42.3 ± 14.6	0.096	78.4 ± 23.3	0.160
ME18 (human melanoma cells)	81.0 ± 27.3	0.191	100.0 ± 21.0	0.226	75.1 ± 6.8	0.154
ME18/R (human melanoma subline resistant to doxorubicin)	102.0 ± 20.8	0.239	83.6 ± 9.0	0.189	48.5 ± 13.7	0.099
HeLa (human cervix carcinoma cells)	173.0 ± 27.0	0.406	163.6 ± 20.0	0.370	56.5 ± 3.6	0.116
KB-V1 (HeLa subline resistant to vinblastine)	114.0 ± 20.0	0.268	110.0 ± 10.5	0.249	52.2 ± 12.7	0.107

Antimicrobial activities of the complexes

The antimicrobial activities of Pt(**L1**)Cl_2 and Pt(**L2**)Cl_2 were expressed as their minimal inhibitory concentrations (MIC). The results of the MIC measurements are presented in Table 6.

It can be seen that both complexes were particularly efficient against *Staphylococcus epidermidis*; Pt(**L1**)Cl_2 inhibited growth of the strain at a concentration of 5 mM, whereas Pt(**L2**)Cl_2 was active at 2.5 mM. The latter was also active against *Bacillus pumilus* at a concentration of 5 mM. All other bacterial strains were inhibited by Pt(**L1**)Cl_2 at a concentration of 10 mM, and by Pt(**L2**)Cl_2 at a concentration of 20 mM. In addition to antimicrobial activities, the activities of Pt(**L1**)Cl_2 and Pt(**L2**)Cl_2 against two fungal strains were also tested (Table 6). However, the two strains were resistant to the Pt complexes up to a concentration of 20 mM.

Conclusions

The results of the structure modeling suggest the formation of Pt complexes with the thiourea derivatives possessing a seven-membered ring in the solid state. Based on the thermodynamic stabilities of the complexes, one would expect platinum(**II**) to interact most strongly with **L3**. In consequence, Pt(**L3**)Cl_2 could be expected to be a better anticancer agent than Pt(**L1**)Cl_2 and Pt(**L2**)Cl_2, at least with respect to some cell lines. Of the five cancer cell lines studied, three displayed enhanced susceptibility to the complex containing selenium, according to the diminished IC_{50} value. The best result was an IC_{50} of 48 μg/mL for ME18/R (human melanoma subline resistant to doxorubicin). The results indicate that studies concerning Pt complexes containing a selenium atom in the ligand are worthy of continuation.

Table 6 MIC values of the investigated platinum(II) complexes

Strains	MIC (mM)	
	Pt(**L1**)Cl_2	Pt(**L2**)Cl_2
Bacteria		
Enterococcus hirae ATCC 10541	10	20
Stenotrophomonas maltophilia ATCC 12714	10	20
Escherichia coli ATCC 10538	10	20
Escherichia coli ATCC 8739	10	20
Bordetella bronchiseptica ATCC 4616	10	20
Bacillus pumilus ATCC 14884	10	5
Staphylococcus epidermidis ATCC 12228	5	2.5
Staphylococcus aureus MRSA 11 from the blood	10	20
Staphylococcus aureus MRSA 2 from nose	10	20
Staphylococcus aureus ATCC 6538P	10	20
Pseudomonas aeruginosa ATCC 9027	10	20
Pseudomonas aeruginosa ATCC 15442	10	20
Fungi		
Candida albicans ATCC 10231	>20	>20
Aspergillus brasiliensis ATCC 16404	>20	>20

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