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Palladium complexes containing dithiocycloheptanespiro-5'-hydantoin ligand. Synthesis, characterization, theoretical analysis and cytotoxic activity

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A new palladium complexes with dithiocycloheptanespiro-5'-hydantoin as ligand were synthesized and characterized. Elemental analyses, IR and NMR spectral measurements were used to verify the structures of the ligand and its complexes. The results of spectroscopic characterization confirm the S-coordination modes of the ligand. The coordination geometries around the palladium atoms and vibration frequencies were evaluated by DFT method. The metal complexes were screened *in vitro* for their anticancer activity

Keywords*: palladium complexes, anticancer activity, DFT calculations*

INTRODUCTION

Antitumor drugs like cisplatin, carboplatin, oxaliplatin and other platinum complexes play a significant role in the treatment of solid tumors [1,2]. However, the above mention platinum-based antitumor drugs have several serious disadvantages such as nephrotoxicity, renal and cervical problems, allergy, elevated blood pressure and others [3]. Because of side effects, scientists have used another strategy to design new cancer drugs similar to cisplatin, changing the nature of a metal ion. Palladium ion is often selected because of its structural similarity to platinum ion and its coordination pattern, i. e. it also forms square planar complexes [4-6]. There are a numerous cases in which palladium complexes have demonstrated higher anticancer activity *in vitro* than their platinum counterparts [7,8]. On the other hand the appropriate choice of ligand plays a crucial role in modifying biological properties [1]. Sulfur-containing ligands could improve the cytotoxicity *in vitro* and *in vivo* of the metal complexes [3].

Herein we describe the synthesis and spectral investigation of new palladium complexes with dithiocycloheptanespiro-5'-hydantoin as ligand. In order to get more information about their molecular structures, the newly synthezed compounds were studied by theoretical methods

EXPERIMENTAL

General information

All chemicals were purchased from Fluka (UK) and Sigma-Aldrich. The newly synthesized Pd (II) and Pd (IV) complexes were characterized by elemental analyses, melting points, IR and NMR spectra. The elemental analyses were carried out on a "EuroEA 3000 – Single", EuroVectorSpA apparatus (Milan, Italy). Corrected melting points were determined, using a Buchi 535 apparatus (BuchiLabortechnik AG, Flawil, Switzerland). The IR spectra were recorded on Thermo Scientific Nicolet iS10 spectrophotometer (Thermo Scientific, USA) in the range of 4000-400 cm^{-1} as Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR). The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM 500 (500 MHz) spectrometer. All theoretical calculations were performed using the Gaussian 09 package [9] of programs. Optimization of the structures of the ligand and Pd complexes were carried out by DFT calculations, employing the B3LYP (Becke's three-parameter non-local exchange [10]) and Lee et al. correlation [11] hybrid functional and 6-311++G** set for all non-metal atoms and LANL2DZ basis set for the palladium atom.

Synthesis of 1,4-Dithiepan-6-one

The starting ketone was synthesized according to procedure described by Cook and Bergesen [12] with small modifications.

A solution prepared from sodium (6 g) and ethane-1,2-dithiol (11.3 mL) in absolute ethanol (100 mL), were added dropwise to a solution of 1,3-dichloroacetone (15.2 g) in anhydrous diethyl ether (100 mL). The mixture was stirred for 3 hours at room temperature, poured with stirring into a mixture of diethyl ether (l00 ml), 3% NaOH

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(250 mL) and powdered ice (100 g). The organic layer was separated and the aqueous layer extracted with diethyl ether (3 x 100 mL). The organic solutions were combined, filtrated, dried ($MgSO_4$) and the ether was evaporated with rotary evaporator. The crude ketone (7 g, 53%) was distilled at 94-98°C at 0.4 mmHg.

Synthesis of 3,6-dithiocycloheptanespiro-5'-hydantoin

1,4-dithiacycloheptan-6-one (2.96 g, 20 mmol) was dissolved in 60 mL aqueous ethanol. To the solution was added 2 g (40 mmol) NaCN and 6 g (60 mmol) $(NH_4)_2CO_3$. The mixture was stirred and heated at 65°C for 24 hours. After that the solution was acidified with conc. HCl in a strong ventilation hood to pH = 5. The precipitate was filtered off and recrystallized from aqueous ethanol. Yield 2.22 g (51%), m.p. 288-289°C.

Elemental analyses: $C_7H_{10}N_2O_2S_2$ (%), Calcd.: C 38.53; H 4.59; N 12.84; S 29.36. Found: C 38.71; H 4.71; N 12.35; S 28.98. ^{1}H NMR (250 MHz, DMSO-d_6): 10.63 (s, 1H, NH-3'); 8.23 (s, 1H, NH-1'); 3.27, 2.98 (AB quartet, 4H, J = 15 Hz, CH_2-5 + CH_2-7); 2.96-2.88 (m, 4H, CH_2-4 + CH_2-5). ^{13}C NMR (62.5 MHz, DMSO-d_6): 177.1 (C=O - 4'); 156.5 (C=O - 2'); 67.8 (C - 5'); 40.3 (C-5 + C-7); 39.1 (C-2 + C-3).

Synthesis of Pd(II) complex with 3,6-dithiacycloheptanespiro-5'-hydantoin

An aqueous ethanol solution of the ligand (**4**) (0.10 g, 0.45 mmol) was added to the aqueous solution of K_2PdCl_4 (0.10 g, 0.30 mmol) and stirred for 6 hours at ambient temperature. The yellow-brown crystals were filtered off and dried under KOH and P_2O_5. The new Pd(II) complex was dissolved in DMSO. The purity was checked by thin-layer chromatography with the eluent $CH_3COOC_2H_5/C_2H_5OH$ - 2:1, and elemental analyses.

Yield: 43%; mp. 307°C.

Elemental analyses: $C_{14}H_{20}N_4O_4S_4Cl_2Pd$ (%). Calcd.: C 27.39; H 3.26; N 9.13. Found: C 27.08; H 2.97; N 8.87. ^{1}H NMR (500 MHz, DMSO-d_6): 10.23 (s, 1H, NH-3'); 8.25 (s, 1H, NH-1'); 3,44 (m, 1H); 3.25 (m, 1H); 3.19 (m, 1H), 3.12 (m, 1H); (CH_2-5 + CH_2-7); 3.08, 2.96, 2.94, 2.83 (four multiplets, 4H, CH_2-2 + CH_2-4).

^{13}C NMR (125 MHz, DMSO-d_6): 177.2 (C=O - 4'); 156.2 (C=O - 2'); 80.1 (C-5 + C-7); 67.8 (C - 5'); 39.1 (C-4 + C-5).

Synthesis of Pd(IV) complex with 3,6-dithiacycloheptanespiro-5'-hydantoin

An aqueous ethanol solution of the ligand (**4**) (0.10 g, 0.46 mmol) and aqueous solution of K_2PdCl_6 (0.09 g, 0.23 mmol) were mixed and stirred for 6 hours at ambient temperature. The obtained yellow-brown crystals were filtered off and dried under KOH and P_2O_5. The new Pd(IV) complex was dissolved in DMSO. The purity was checked by thin-layer chromatography with the eluent $CH_3COOC_2H_5/C_2H_5OH$ - 2:1, and elemental analyses.

Yield: 31%; mp. 312°C (dec.).

Elemental analyses: $C_{14}H_{20}N_4O_4S_4Cl_4Pd$ (%), Calcd.: C 24.55; H 2.92; N 8.18. Found: C 24.46; H 2.65; N 7.89. ^{1}H NMR (500 MHz, DMSO-d6): 10,25 (s, 1H, NH-3'); 8,27 (s, 1H, NH-1'); 3,52 (m, 1H); 3,27 (m, 1H); 3,25 (m, 1H), 3,12 (m, 1H), (CH_2-5 + CH_2-7); 3,10, 2,98, 2,90, 2,82 (four multiplets, 4H, CH_2-2 + CH_2-4).

^{13}C NMR (125 MHz, DMSO-d6): 177,6 (C=O - 4'); 160,1 (C=O - 2'); 84,1 (C-5 + C-7); 68,1 (C - 5'); 41,1 (C-4 + C-5).

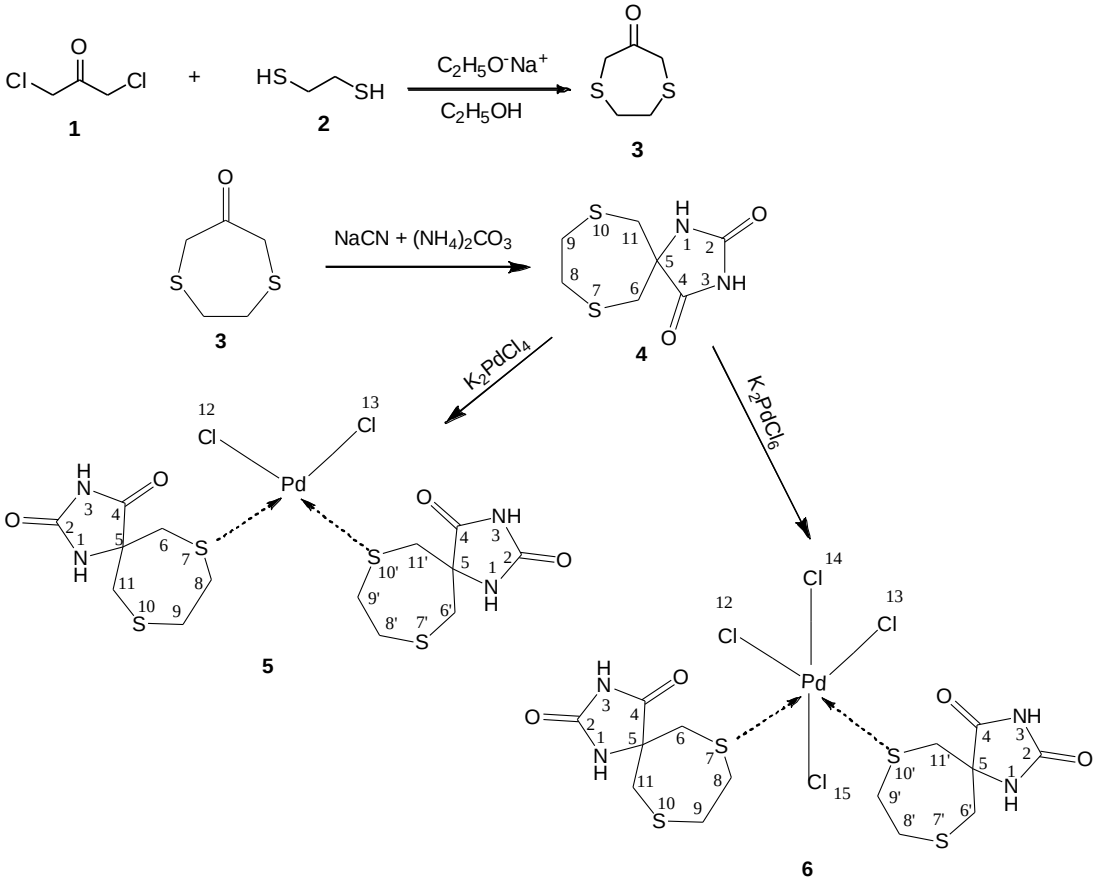
Cytotoxicity assessment

Cytotoxicity of the compounds was assessed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] dye reduction assay as described by Mossman [13] with some modifications [14]. Exponentially growing cells were seeded in 96-well microplates (100 μL/well at a density of 3.5 x 10^5 cells/mL for the adherent and 1 x 10^5 cells/mL for the suspension cell lines) and allowed to grow for 24 h prior the exposure to the studied compounds. Stock solutions of the investigated Pd(II) and Pd(IV) complexes were freshly dissolved in DMSO and then promptly diluted in RMPI-1640 growth medium, immediately before treatment of cells. At the final dilutions the solvent concentration never exceeded 0.5 %. Cells were exposed to the tested compounds for 72 h, whereby for each concentration a set of 8 separate wells was used. Every test was run in triplicate, i.e. in three separate microplates. After incubation with the tested compounds MTT solution (10 mg/mL in PBS) aliquots were added to each well. The plates were further incubated for 4 h at 37ºC and the formazan crystals formed were dissolved by adding 110 μL of 5 % HCOOH in 2-propanol. Absorption of the samples was measured by an ELISA reader (UniscanTitertec) at 580 nm. Survival fraction was calculated as percentage of the untreated control. In addition IC_{50} values were calculated from the concentration-response curves. The experimental data was processed using GraphPadPrizm software and was fitted to sigmoidal concentration/response curves *via* non-linear regression.

The structures of the synthesized compounds were confirmed by IR, NMR spectra and elemental analyses. The results were consistent with the assigned structures.

RESULTS AND DISCUSSION

Synthetic pathway to obtain the new ligand and its Pd complexes are illustrated on the Scheme1.



Scheme 1. Synthesis of 3,6-dithiocycloheptanespiro-5'-hydantoin (**4**) and its Pd complexes (**5,6**)

Vibrational analysis

The most important vibrational frequencies for the investigated ligand and its Pd complexes are stretching vibrations of secondary amino groups, $\nu_{C=O}$ and C-S bonds. Broad bands for N-H stretching vibration are observed at 3237 cm^{-1} for the ligand and 3232 cm^{-1}, 3229 cm^{-1} for the complexes. The vibrations of the C=O bonds appears as a two very strong bands at 1754 cm^{-1} and 1673 cm^{-1} for the ligand. In the complexes carbonyl stretching modes are observed around 1746 cm^{-1} and 1660 cm^{-1}. The bands for stretching vibrations of C-S bond of Pd complexes are shifted to lower frequency in comparison with ligand. They are measured at 644 cm^{-1} (theor.: 651 cm^{-1}) in the ligand and 623 cm^{-1} (theor.: 625 cm^{-1}) and 616 cm^{-1} (theor.: 625 cm^{-1}) in the complexes. The shifting of the frequencies characteristic for C-S bonds of 21 and 28 cm^{-1} in the complexes shows that the sulfur atom is coordinated with palladium ion.

NMR analysis

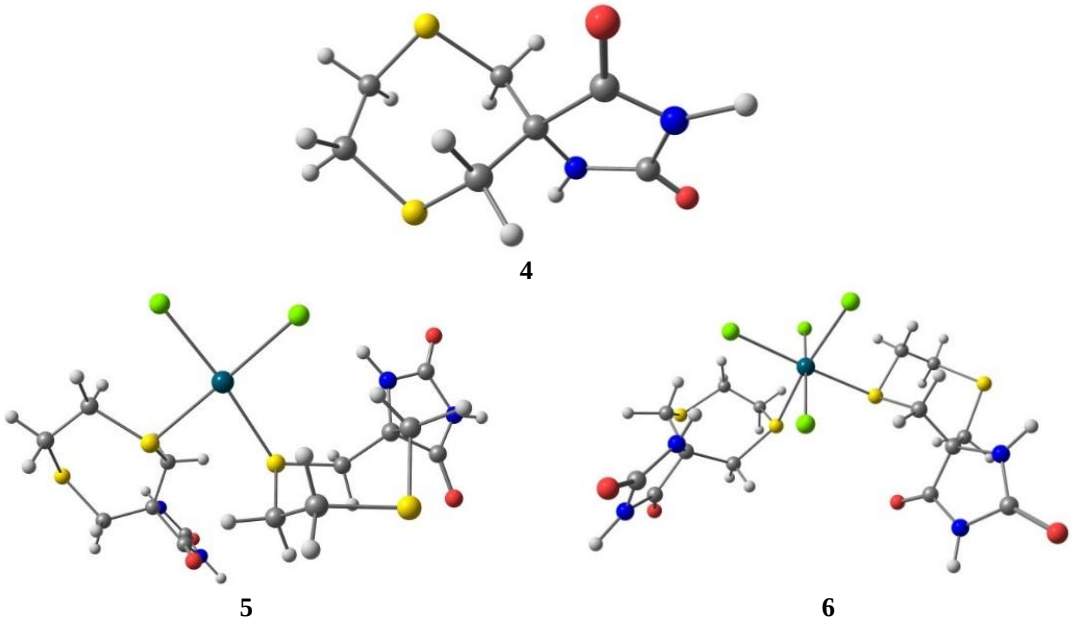
We assume that in the complexes only one sulfur atom is bonded. In the ^{1}H NMR spectrum of the Pd(II) complex, there is a splitting of two AB quartets for the two methylene groups at C-5 and C-7. The chemical shifts for the four protons of two methylene groups are 3.44 (axial) and 3.21 ppm (equatorial) of CH_2-7 and at 3.25(a) and 3.17(e) ppm of CH_2-5 while in the free ligand these values are 3.27(a) and 2.98(e) for CH_2-7 (increasing with 0.17 and 0.23 ppm), 2.95(a) and 2.88(e) for CH_2-5 (increasing with 0.30 and 0.29 ppm). This indicates the bonding between the metal ion and one of the sulfurs atoms in the ring. The shifting of the C-2 and C-4 methylene groups are not influenced in the Pd(II) complex. All signals are simplified in comparison of the CH_2 signals in the free ligand, due to the fact, that cycloheptane ring has fixed conformation in the metal complex.

In the ^{1}H NMR spectrum of Pd(IV) complex, the same differences of the chemical shifts of the CH_2-5 and CH_2-7 are observed: 0.25(a) and 0.27 (e) for CH_2-7, respectively 0.29(a) and 0.25(e) for CH_2-5.

Computational analysis

Due to the difficulties to obtain the crystals suitable for X-ray analysis we studied the structure of the ligand and it's complexes mainly by theoretical methods. All the structures were

optimized at the DFT level with the B3LYP functional and the 6-311++G** basis set for all non-metal atoms and LANL2DZ basis set for the palladium atom. The calculations were used to obtain the important information about coordination modes, geometrical parameters and spectroscopic properties of ligand (**4)** and complexes **(5, 6**). The optimized structures of the compounds **(4,5,6)** are presented in Figure 1.



4 **5** **6**

Figure 1. Optimized structures of ligand (**4**) and palladium complexes (**5, 6**)

Table 1. Selected calculated geometry parameters

Parameters	**Ligand**	**[PdL_2Cl_2]**	**[PdL_2Cl_4]**
μ(D)	3.3	10.18	9.74
Bond lengths (Å)			
Pd-Cl_{12}	-	2.36	2.39
Pd-Cl_{13}	-	2.37	2.40
Pd-Cl_{14}	-	-	2.42
Pd-Cl_{15}	-	-	2.42
Pd-S_7	-	2.50	2.53
Pd-S_{10}		2.51	2.54
C_6-S_7	1.83	1.90	1.91
C_8-S_7	1.83	1.91	1.91
Angles (º)			
C_6-S_7-C_8	100.4	99.9	99.2
$C_{9'}$-$S_{10'}$-$C_{11'}$	-	104.2	106.1
C_6-S_7-Pd	-	127.5	105.5
C_8-S_7-Pd	-	108.1	105.6
$C_{9'}$-$S_{10'}$-Pd		110.7	119.5
$C_{11'}$-$S_{10'}$-Pd		106.4	113.3
S_7-Pd-Cl_{12}	-	97.4	95.9
$S_{10'}$-Pd-Cl_{13}	-	90.2	87.6

The S-C bonds length becomes slightly longer than those in the ligand. There is small deviation in the calculated C-S-C bond angles in the complexes than those in the ligand by 3.8° and 0.5° in the complex **(5)** and 5.7° and 1.2° in the complex (**6)**.

The Pd-S bond distance in the PdL_2Cl_2 complex is slightly shorter than that in the PdL_2Cl_4 complex by 0.03 Å. The Pd-S bond lengths are similar to those found in the literature for other optimized Pd complexes [15,16].

Table 2. *In vitro* evaluation of cytotoxicity of the ligand **(4)** and its palladium complexes **(5,6)** in comparison with referent drug cisplatin in three human tumour cell lines.

Compound	IC_{50}(μM)		
	HL-60[a]	REH[b]	LAMA-84[c]
Ligand	132.5±6.6	n.d.[d]	142.2±6.9
Complex 5	98.6±6.8	48.7±5.1	60.0±6.2
Complex 6	123.1±11.5	29.4±3.7	33.1±4.1
Cisplatin	8.7	1.07	16.9

[a]Acute myeloid leukemia; [b]Acute lymphoblastic leukemia
[c]Human chronic myeloid leukemia; [d] n.d. - not detected

In vitro cytotoxicity

The complexes **(5,6)** were tested for cytotoxic activity on a panel of human tumor cell lines - acute myeloid leukemia - HL-60, acute lymphoblastic leukemia - REH and Human chronic myeloid leukemia - LAMA-84. The results are summarized in Table 2.

In all cellular test systems, the organic compound (**4)** and complexes (**5)** and **(6)** showed dose-dependent anticancer activity in the tested concentration range following 72 h exposure time, whereby chemosensitivity of the different cell lines varied within a wide range. As evident from the presented data, complex (**6)**, proved to be more active analogue then the complex (**5)** on the leukemic models REH and LAMA-84.

CONCLUSIONS

The structures of the synthesized compounds are confirmed by IR, NMR spectra and elemental analyses. Spectral analyses confirm that the ligand coordinates with metal ions through its S-atom indicating the monodentate nature of the ligand. The geometry of the ligand and its palladium complexes are optimized, using the DFT method. A good correlation between theoretical calculations and experimental results is observed.

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