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Pd(II) and Pd(IV) complexes with new hydantoin based ligand. Synthesis, characterization, computational and pharmacological studies

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New Pd(II) and Pd(IV) complexes with 3-amino-5-methyl-5-benzylhydantoin have been synthesized and characterized using different spectroscopic techniques, such as FTIR, NMR and elemental analysis. DFT calculations have been employed to investigate the structure of the ligand and its complexes. The free ligand and the metal complexes have been tested *in vitro* for their cytotoxic activity against HL-60, REH and HT-29 human tumor cell lines.

Keywords: Pd complexes;hydantoins; IR spectra; DFT calculations; cytotoxicity.

INTRODUCTION

Transition metal complexes play a crucial role in medical and pharmaceutical chemistry due to their pharmacological effects based on the antitumor activity [1–3]. Given the similar structure, chemical properties and coordination modes between platinum and palladium, palladium complexes [4–8] are of great interest. It is supposed that they will have the same cytotoxic activity like platinum complexes. The similarity and side effects of the platinum complexes lead to the study of Pd complexes as antitumor drugs [3, 9, 10]. In several cases the palladium complexes show significant cytotoxic activity in normal tumor cells and lower resistance of tumor cells to clinical treatments as well as lower side effects than their platinum counterparts [4]. From a thermodynamic and kinetic point of view, the palladium compounds are more labile than the corresponding platinum compounds [11].

Herein is presented the synthesis, spectral investigation and cytotoxic activity of Pd(II) and Pd(IV) complexes with 3-amino-5-methyl-5-benzylhydantoin. In order to get more information about their molecular structure, the new synthezed compounds were studied by computational methods.

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EXPERIMENTAL

General: All chemicals were purchased from Fluka (UK) and Sigma-Aldrich. The new 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 Bushi 535 apparatus (Bushi Labortechnik 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 registered on Bruker WM 500 (500 MHz) spectrometers in DMSO-d_6. The mass spectrum of the ligand was recorded on LC-MS (Thermo Scientific q Exactive Plus – Dionex 3000RSLC).

Synthesis

*Preparation of the 5-benzyl-5-methyl hydantoin (**2**)*

The organic compound 5-benzyl-5-methyl hydantoin was obtained by different synthetic pathway from this described by Herbst et al. [12]. Phenylacetone (**1**) (4.02 g, 30 mmol) was dissolved in an aqueous ethanol. 2.45 g (50 mmol) NaCN and 5.3 g (60 mmol) $(NH_4)_2CO_3$ were added. The

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resulting mixture was stirred, heated at 65 °C for 24 hours and acidified with conc. HCl to pH = 5. The precipitate was filtered off, recrystallized from aqueous ethanol and dried. Yield: 4.05 g (66%), m.p. 227–228 °C (lit. m.p. 228–229 °C). ^{1}H NMR (500 MHz, DMSO-d_6): 10.45 (s, 1H, NH-3); 8.58(s, 1H, NH-1); 7.21–7.18 (m, 5H, C_6H_5); 2.94; 2.74(dd, AB quartet, 2H, CH_2); 1.33 (s, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 174.7 (C-4); 155.7 (C-2); 135.6; 130.4; 128.4; 127.3(C_6H_5); 60.8 (C-5); 43.3 (CH_2); 24.5 (CH_3). Mass-spectrum – M+ = 204.

*Preparation of 3-amino-5-benzyl-5-methyl hydantoin (**3**)*

5-benzyl-5-methyl hydantoin (2.043 g, 10 mmol) and 20 ml hydrazine hydrate were heated with reflux condenser for 3 hours. The solution was diluted with 50 ml water and placed in refrigerator for 12 hours. The forming precipitate was filtered off and recrystallized from aqueous ethanol. Yield: 1.05 g (43%), m.p. 232–233°C. IR (ATR, cm^{-1}): 3323, 3284, 3202, 1763, 1722, 1626. ^{1}H NMR (500 MHz, DMSO-d_6): 8.58(s, 1H, NH-1); 7.21–7.18 (m, 5H, C_6H_5); 4.51 (s, 2H, NH_2); 2.95; 2.75(dd, AB quartet, 2H, CH_2); 1.34 (s, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 174.9 (C-4); 155.6 (C-2); 135.7; 130.4; 128.4; 127.3(C_6H_5); 60.9 (C-5); 43.3 (CH_2); 24.5 (CH_3). Mass-spectrum – M+ = 219.

*Preparation of 3-amino-5-benzyl-5-methyl hydantoin dichlorido palladium(II) – [PdLCl$_2$] (complex **4**)*

An aqueous ethanol solution of (**3**) (0.0996 g, 0.45 mmol) was added dropwise to an aqueous solution of $K_2[PdCl_4]$ (0.0903 g, 0.22 mmol) with constant stirring. The homogenous solution obtained was stirred for 9–10 h at ambient temperature. Subsequently, the reaction mixture was concentrated and cooled to 4 °C. The light yellow precipitate obtained was filtered off, washed several times with Et_2O and dried in a vacuum desiccator. The product was recrystallized from EtOH. The substance is soluble in DMSO and slightly soluble in water. Yield: 73%, m.p. 213 °C (dec.). IR (ATR, cm^{-1}): 3191, 1772, 1712, 1607. ^{1}H NMR (500 MHz, DMSO-d_6): 8.20(s, 1H, NH-1); 7.35–7.27 (m, 5H, C_6H_5); 7.15 (s, 2H, NH_2); 2.99; 2.81(dd, AB quartet, 2H, CH_2); 1.35(s, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 177.7(C-4); 158.8 (C-2); 138.7; 133.5; 131.5; 130.3(C_6H_5); 63.9 (C-5); 46.4 (CH_2); 27.5 (CH_3).

*Preparation of 3-amino-5-benzyl-5-methyl hydantoin dichlorido palladium(IV) – [PdLCl$_4$] (complex **5**)*

An aqueous solution of $K_2[PdCl_6]$ (0.0990 g, 0.3033 mmol) was added dropwise to an aqueous ethanol solution of (**3**) (0.1305 g, 0.4826 mmol) at constant stirring. The homogenous solution obtained was stirred for 8–9 h at ambient temperature. Then, the reaction mixture was concentrated and cooled to 4 °C. The yellow precipitate obtained was filtered off, washed several times with Et_2O and dried in a vacuum desiccator. The product was recrystallized from EtOH. The substance is soluble in DMSO and slightly soluble in water. Yield: 82%, m.p. 227 °C (dec.). IR (ATR, cm^{-1}):3199, 1771, 1708, 1608. ^{1}H NMR (500 MHz, DMSO-d_6): 8.20(s, 1H, NH-1); 7.35–7.27(m, 5H, C_6H_5); 7.14 (s, 2H, NH_2); 2.99; 2.81(dd, AB quartet, 2H, CH_2); 1.34(s, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 177.6(C-4); 158.9 (C-2); 138.7; 133.5; 131.3; 130.4(C_6H_5); 63.9 (C-5); 46.4 (CH_2); 27.4 (CH_3).

Computational details

The molecular structures and vibrational spectra of the ligand (**3**) and its complexes (**4**, **5**) were studied by computational methods. All theoretical calculations were performed using the Gaussian 03 package of programs [13]. Optimization of the structures of the ligand and its complexes was carried out by hybrid DFT calculations, employing the B3LYP (Becke's three-parameter non-local exchange) [14, 15] correlation functional and 6-311++G** basis set for all non-metal atoms and LANL2DZ basis set for metal center.

Cytotoxic activity

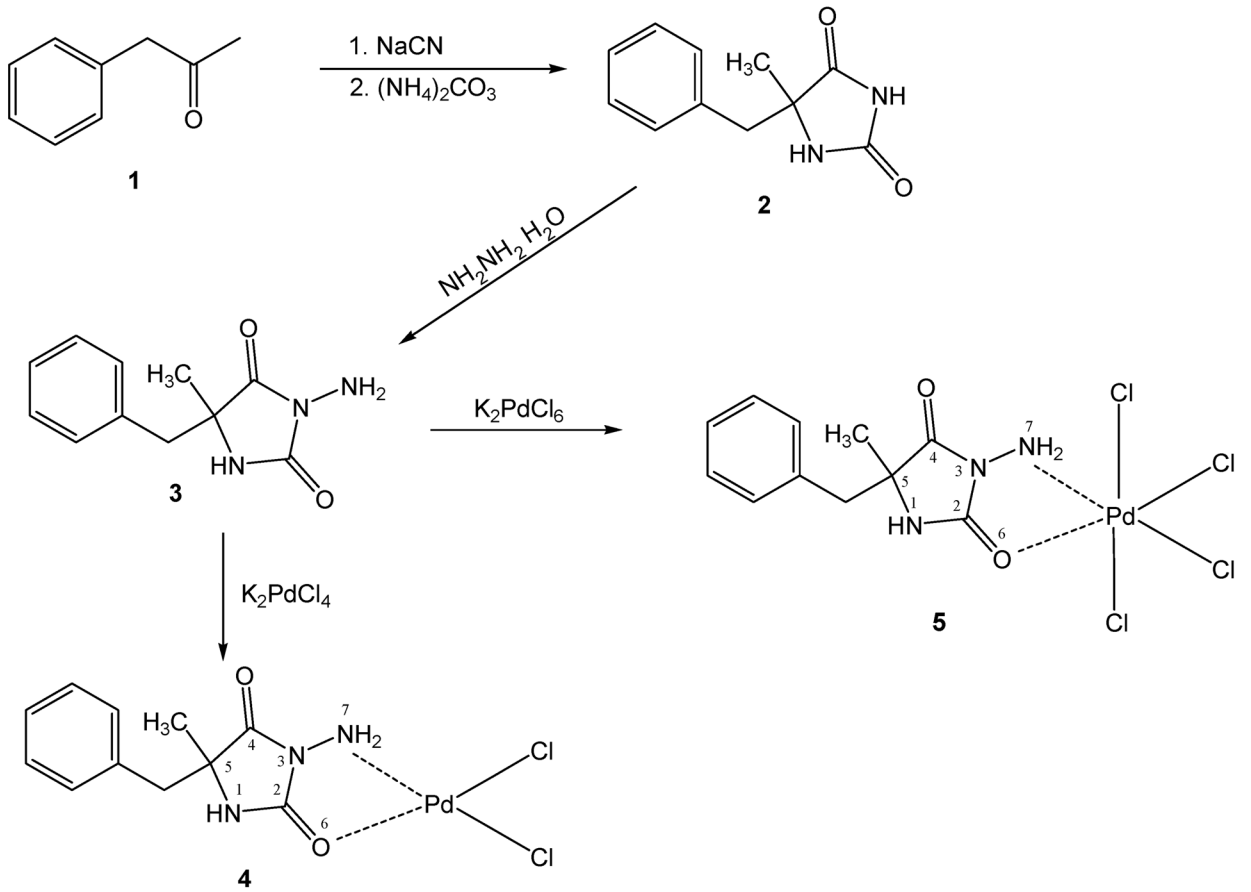
Cytotoxicity of the ligand (**3**) and complexes (**4**, **5**) was evaluated *in vitro* against three human tumor cell lines (Table 1). The cell lines used for the experiments were: (i) HL-60 (acute myeloid leukemia, established from the peripheral blood of a patient with acute promyelocyte leukemia), (ii) REH (acute lymphoblastic leukemia, established from the peripheral blood of a 15-year-old North African girl with acute lymphoblastic leukemia in 1973), (iii) HT-29 (colon adenocarcinoma, established from the primary tumor of a 44-year-old Caucasian woman with colon adenocarcinoma in 1964). The cell lines were obtained from DSMZ German Collection of Microorganisms and Cell Cultures and were well validated in our laboratory as a proper test system for metal complexes. Their DSMZ catalogue numbers are as follows: HL-60 (ACC 3), REH (ACC 22) and HT-29 (ACC 299). 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 [16] with some modifications [17]. Exponentially growing cells were seeded in 96-well microplates (100 μL/well at a density of 3.5×10^5 cells/mL for the ad-

herent and 1×10^5 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 37 °C 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.

RESULTS AND DISCUSSION

The method of obtaining of the organic compound 5-benzyl-5-methyl hydantoin (**2**) described by Herbst et al [12] was improved and shortened. Synthetic pathway to obtain the new ligand and its complexes is illustrated on the Scheme 1. The structure of the ligand (**3**) and its metal complexes (**4**, **5**) were confirmed by various spectroscopic techniques such as IR, ^{1}H NMR, ^{13}C NMR spectra and elemental analyses. The results were consistent with the assigned structures.

The complexes (**4**, **5**) were stable in solid state at room temperature and their melting points were over 200 °C. As a result of elemental analyses, metal complexes were supposed to have the following general formulas $[PdLCl_2]$ and $[PdLCl_4]$.



Scheme 1. Synthesis of 5-benzyl-5-methyl hydantoin (**2**), ligand (**3**), Pd(II) and Pd(IV) complexes (**4**, **5**).

The structural characterization

Infrared spectra

The accurate assignment of the main experimental frequencies of ligand and complexes (**4**, **5**) to the corresponding normal modes was supported by DFT method employing B3LYP functional, 6-311++G** basis set was selected for C, N, H and O atoms and LANL2DZ was applied for the Pd atom. All calculated frequencies are positive. This is confirmed that the structures are correctly optimized.

The IR spectrum of the free ligand shows three characteristic bands in the region 3323–3202 cm^{-1} (theor. 3520–3372 cm^{-1}) associated with the stretching modes of the NH and NH_2 groups, which is typical for hydrogen bonded systems. While in the spectra of the complexes (**4**, **5**), they are shifted to lower frequencies (exp. 3205–3110 cm^{-1}/theor. 3554–3344 cm^{-1}).

The bands observed at 1764 and 1723 cm^{-1} corresponding to υ(C=O) vibrations in the ligand are slightly shifted to higher frequencies in the complexes: 1772, 1712 cm^{-1} in Pd(II) and 1772, 1708 cm^{-1} in Pd(IV) complex. The band at 1626 cm^{-1} attributed to the δ(NH_2) vibration in the spectrum of the ligand is slightly shifted to lower wavenumbers by 18 cm^{-1} in the complexes.

NMR spectra

In the ^{1}H NMR spectra of the ligand the signal of NH(1) is at 8.59 ppm, the signals of two diastereotopic CH_2 protons are in the 2.75–2.95 ppm. N-NH_2 protons are singlet at 4.51 ppm. The comparative analysis of the ^{1}H NMR spectra of the complexes (**4**, **5**) and the ligand (**3**) showed that there was a significant shifting of the signal of N-NH_2 group (from 4.51 in the ligand to 7.15–7.20 ppm in the complexes). The signals of the other protons were not shifted extraordinary. This shows that the coordination of the ligand with metal ions was realized by nitrogen atom of the amino group.

In the ^{13}C NMR spectra, as well as in the ^{1}H spectra of the complexes, the signals of two carbonyl groups are slightly influenced. But the shifting of the C-2 carbonyl carbon (C=O-2) is relatively great (from 155.6 to 158.9). It revealed that ligand coordinates with palladium cation bidentate, through C-2 carbonyl oxygen and the amine group. All other carbon atoms do not shift significantly after coordination.

Theoretical analysis

The theoretical calculations were used to obtain the important information about structural characteristics due to the difficulties to obtain crystals suitable for X-ray analysis. The geometries of the ligand and complexes were optimized at the B3LYP level, 6-311++G ** and LANL2DZ basis sets (Fig. 1).

The palladium(II) ion has square planar environments with the ligand, bonded through the nitrogen atom from amine group and carbonyl oxygen atom

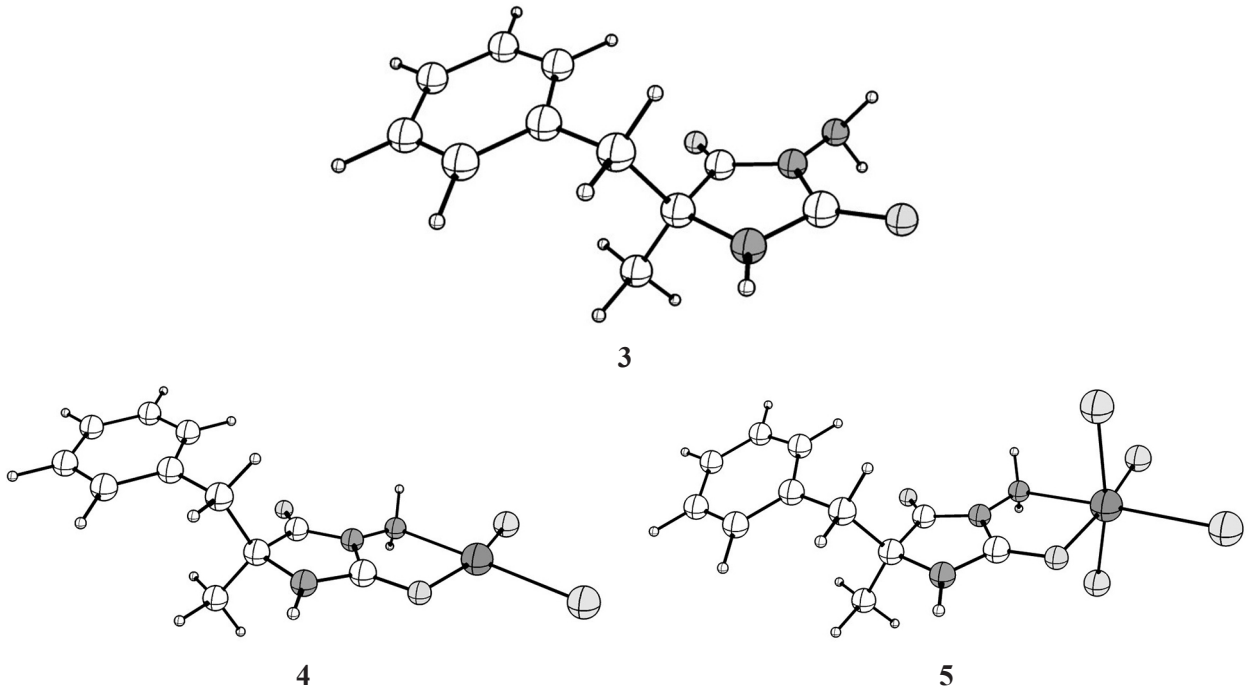


Fig. 1. Optimized structures of the ligand (**3**) and its complexes (**4**, **5**).

Table 1. *In vitro* evaluation of cytotoxicity of the ligand (**3**) and complexes (**4**, **5**) in comparison with referent drug cisplatin in three human tumour cell lines

Compounds	IC_{50}(μM)		
	HL-60	REH	HT-29
Ligand	167 ± 10	> 200	> 200
Complex Pd(II)	127 ± 5	> 200	> 200
Complex Pd(IV)	133 ± 5	> 200	> 200
Cisplatin	8.7	1.07	170

(C=O-2). The last one is more stable by 0.33 kJ/mol compared to coordination of metal ion with C=O group on fourth position. In case of complex (**5**) the metal adopts a distorted octahedral coordination.

Theoretical analysis showed that the bond lengths in the complexes are slightly longer than the ligand by 0.03–0.05 Å. The Pd–N and Pd–O bond lengths are 2.16 Å and 2.21 Å, respectively, consistent with those found in other palladium compounds [18, 19]. The angle, $N_5N_3C_2$ is slightly affected upon complexation by 2.9–3.9°. The computed values of angles N_5PdO_6 in complexes are 79.84° and 80.81° respectively.

In vitro cytotoxicity

Cytotoxicity of the ligand (**3**) and complexes (**4**, **5**) was evaluated *in vitro* against three human tumor cell lines (Table 1). Pd(II) and Pd(IV) complexes showed higher cytotoxic activity than the ligand on HL-60 cell line. This cell line maybe is more sensitive to palladium complexes than the other two lines. The new complexes are less active than the referent cisplatin.

CONCLUSIONS

The paper describes synthesis, characterization and theoretical analysis of 3-amino-5-methyl-5-benzyl hydantoin as a ligand and its palladium complexes. Their structures were determined by several spectroscopic methods. The DFT calculations show that palladium ion exhibit a square planar geometrical arrangement in Pd(II) complex and distorted octahedral coordination in Pd(IV) complex. Theoretical analysis confirmed the experimental data for bidentate coordination of the ligand with metal ions. Pd(II) and Pd(IV) complexes showed higher cytotoxic activity than the ligand on HL-60 cell line.

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КОМПЛЕКСИ НА Pd (II) И Pd (IV) С НОВ ХИДАНТОИНОВ ЛИГАНД: СИНТЕЗ, ОХАРАКТЕРИЗИРАНЕ, ТЕОРЕТИЧНИ И ФАРМАКОЛОГИЧНИ ИЗСЛЕДВАНИЯ

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(Резюме)

Нови комплекси на Pd(II) и Pd(IV) с 3-амино-5-метил-5-бензилхидантоин бяха синтезирани и охарактеризирани, използвайки различни спектроскопски методи като ИЧ, ЯМР и елементен анализ. Квантово-химични изчисления бяха приложени за изследване на структурата на лиганда и неговите комплекси. Свободният лиганд и комплексите бяха тествани *in vitro* за цитотоксична активност върху три човешки туморни клетъчни линии: HL-60, REH и HT-29.