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Synthesis, Structure, and Antiproliferative Activity of *trans*-Palladium(II) Complexes with Tetrazol-2-ylacetic Acid Derivatives

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Abstract—A series of *trans*-palladium(II) complexes (*trans*-$[PdCl_2L_2]$, L = ethyl 5-R-2*H*-tetrazol-2-ylacetate, 5-R-2*H*-tetrazol-2-ylacetamides, R = Me, Ph) has been synthesized, and their structure has been proved by ^1H and $^{13}C–\{^1H\}$ NMR and high-resolution mass spectra and X-ray analysis. Antiproliferative activity of the synthesized complexes has been estimated, and the mechanism of their interaction with DNA has been studied by UV and CD spectroscopy.

Keywords: tetrazole-containing Pd(II) complexes, tetrazol-2-ylacetic acid derivatives, interaction with DNA, antiproliferative activity

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Antitumor agents based on platinum metal complexes are extensively used in cancer therapy [1, 2]. A promising way in the design of new drugs of this type with improved efficiency and reduced side effects is the use of various heterocycles as ligands (phenanthroline, imidazole, pyrimidine, purine, etc.) [3]. Tetrazole derivatives are among such ligands [4, 5]. Some heterocyclic compounds of the tetrazole series exhibit high physiological activity in combination with a relatively low toxicity. In particular, tetrazole-containing coordination compounds of platinum group metals were shown to be efficient cytostatic agents [6].

We previously synthesized palladium(II) and platinum(II) complexes with tetrazol-1-yl- and tetrazol-5-ylacetic acid derivatives [7–9]. In continuation of these studies, herein we report the synthesis and antiproliferative activity of palladium(II) complexes with tetrazol-2-ylacetic acid derivatives (Scheme 1). The structure of complexes **2a**–**2f** was determined by high-resolution mass spectrometry (ESI^+), ^1H and $^{13}C\{^1H\}$ NMR, and X-ray diffraction {for *trans*-$[PdCl_2L_2]$ (**2a**, L = ethyl 5-methyl-2*H*-tetrazol-2-ylacetate)}, and a probable mechanism of their interaction with DNA was studied by UV and CD spectroscopy.

Methylene protons of **2a**–**2e** resonated in the ^1H NMR spectra in the region δ 5.07–5.75 ppm, and the tetrazole C^5 signal was located at δ_C 162.2–164.1 ppm in the $^{13}C–\{^1H\}$ NMR spectra. According to the X-ray diffraction data for complex **2a**, the 2,5-disubstituted tetrazole ring is coordinated to palladium(II) ion in a unidentate mode through the N^4 atom (Fig. 1). Complex **2a** crystallized in the monoclinic crystal

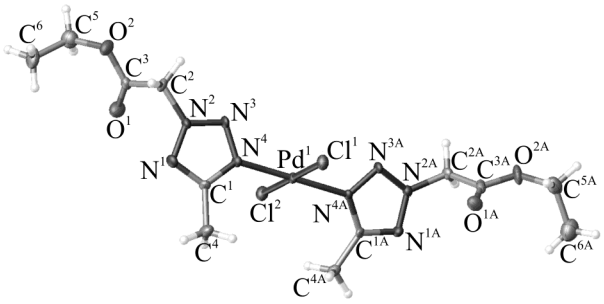
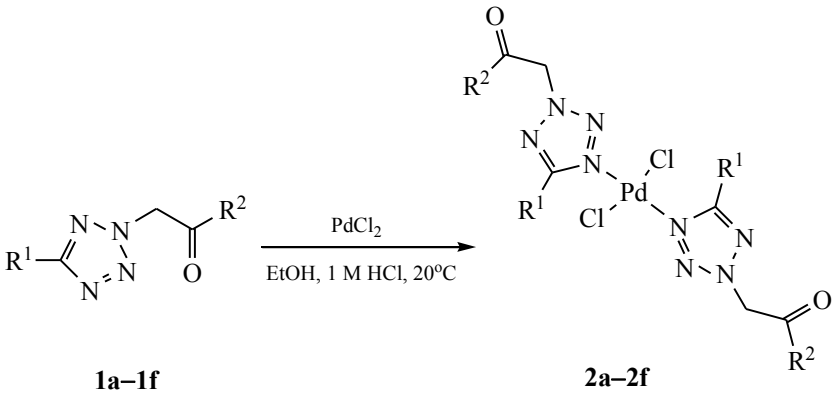


Fig. 1. Structure of palladium(II) complex **2a** in crystal according to the X-ray diffraction data.

Scheme 1.



1a–1f **2a–2f**

R^1 = Me, R^2 = COOEt (**a**), NH_2 (**b**), N-*sec*Bu (**c**), N-*cyclo*Pr (**d**); R^1 = Ph, R^2 = NH_2 (**e**), N-*sec*Bu (**f**).

system. The coordination entity of **2a** has a slightly distorted square planar configuration. Some bond lengths and bond angles are given in the table. The endocyclic bond lengths differ insignificantly: the shortest bond is N^3–N^4 1.309(8) Å, and the longest bond is N^1–N^2 1.342(8) Å.

Taking into account that complexes **2a**–**2e** are potential cytostatic agents, we examined the efficiency of their interaction with DNA as the main target of antitumor agents of this type [10]. Depending on the structure, charge, and ligand nature, metal complexes are capable of interacting with nucleic acids according to different mechanisms, [10]. Transition metal complexes can bind to DNA through both non-covalent interactions such as intercalation, electrostatic interactions, or groove binding and covalent bonding via replacement of a labile ligand by a nucleic acid base, e.g., guanine (N^7) [11].

We studied the interaction between tetrazole-containing complexes and calf thymus (CT) DNA by UV and CD spectroscopy. Interactions of metal complexes with DNA in aqueous solutions are generally accompanied by characteristic changes in the electronic absorption spectra [10], which essentially depend on the interaction mode. For example, intercalation is characterized by decrease of the absorbance, whereas groove binding gives rise to a significant hyperchromic effect at the CT absorption maximum at λ 260±3 nm [9, 10]. Figure 2 showed the electronic absorption spectra of CT DNA in a buffer solution (50 mM NaCl/50 mM Tris–HCl, pH = 7.2) at a constant DNA concentration and varied concentration of complex **2e**.

Changes in the absorption spectra are seen most clearly in the calculated normalized spectra. In particular, we observed an appreciable red shift of the DNA absorption maximum with rise in the concentration of **2e**. A similar pattern was observed for complex **2d**. The observed variations can be attributed to association of the metal complexes with DNA, excluding intercalation [9].

The circular dichroism spectrum of a solution of DNA is represented by a positive band at λ 275 nm due to stacking interaction of nucleotides and a negative band at λ 246 nm which is related to the chirality of the right-handed B-DNA helix. Circular dichroism spectroscopy can also be used to determined the mode of interaction between metal complexes and DNA [12–14].

Selected bond lengths and bond angles in complex **2a**

Bond	*d*, Å	Angle	ω, deg
Pd^1–N^4	2.002(6)	$N^1C^1N^4$	125.5(7)
Pd^1–Cl^1	2.2866(18)	$N^3N^2N^1$	114.5(6)
C^2–C^3	1.525(10)	$N^4N^3N^2$	104.6(6)
C^3–O^2	1.323(9)	$O^1C^3C^2$	126.2(7)
N^1–C^1	1.327(9)	$O^2C^3O^1$	127.0(7)
N^1–N^2	1.342(8)	$C^1N^4Pd^1$	130.3(5)
N^2–N^3	1.314(8)	$N^3N^4C^1$	108.7(6)
N^3–N^4	1.309(8)	$N^3N^4Pd^1$	120.7(4)
N^4–C^1	1.357(9)	$C^1N^1N^2$	102.1(6)
O^1–C^3	1.189(9)	N^4PdCl^1	91.25(17)

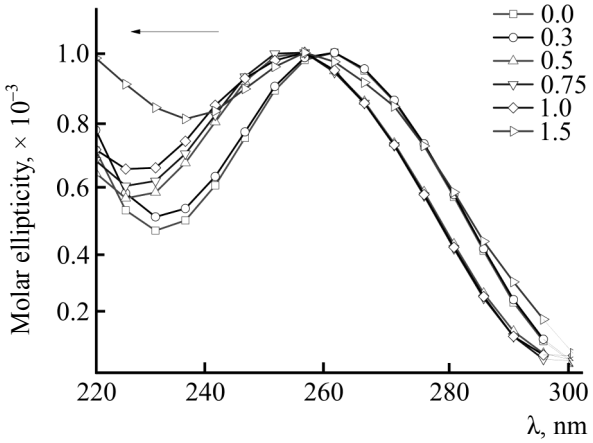


Fig. 2. Calculated normalized electronic absorption spectra of calf thymus DNA in the presence of complex **2e**. $A_{norm} = [A_{obs} - A_{2e}] [A_{max}]^{-1}$.

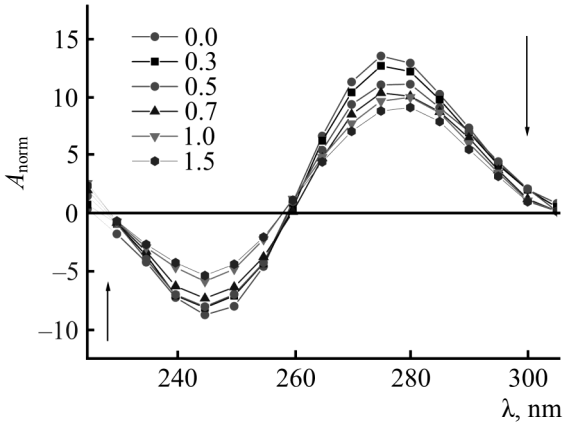


Fig. 3. Circular dichroism spectra of solutions of calf thymus DNA in 5 mM NaCl in the presence of complex **2d** at the following [**2d**]/[DNA] ratios: 0.3, 0.5, 0.7, 1.0, 1.5.

The CD spectra were recorded at a constant CT DNA concentration (3.0 μM) and different concentrations of metal complexes. The CD spectra of CT DNA solutions in the presence of complexes **2d** and **2e** showed similar variations in both negative and positive regions. As seen from Fig. 3, addition of complex **2d** to CT DNA leads to decrease of the intensity of both positive and negative absorption bands. The observed variations of the CD spectra suggest that tetrazole-containing complexes are capable of breaking stacking interactions between nucleotides in the DNA duplex (positive band), as well as changing secondary DNA structure (negative band) [10]. Similar variations were described previously for compounds interacting with DNA via groove binding mode [12–14].

The antiproliferative activity of palladium(II) complexes **2a**, **2e**, and **2f** against HL-60 cancer cell line *in vitro* was estimated by MTT assay. Among the tested compounds, complex **2a** showed the highest cytostatic effect (IC_{50} 42.44±0.81 μM). Complexes **2e** and **2f** with 5-phenyl-2*H*-tetrazol-2-ylacetamides exhibited insignificant activity (IC_{50} 125.84± 0.79 and 103.7±0.69 μM, respectively).

Thus, the examined compounds are capable of effectively interacting with DNA molecules according to the groove-binding mechanism. However, the cytostatic activity of compounds of this type requires further study. It should be noted that palladium(II) complexes like **2** can undergo fast hydrolysis in aqueous medium to give the corresponding aqua complexes *trans*-$[Pd(H_2O)_2L_2]$, and just the latter can be involved in interactions with biological targets.

EXPERIMENTAL

The ^1H and $^{13}C–\{^1H\}$ NMR spectra were recorded on Bruker DPX-300 (300.13 and 75.47 MHz, respectively) and Bruker Avance III 400 spectrometers (400.13 and 100.61 MHz, respectively) from solutions in DMSO-d_6 at 25°C. The high-resolution mass spectra were recorded on Bruker MicroTOF and Bruker MaXis instruments. The X-ray diffraction data were obtained using SuperNova and Xcalibur diffractometers; the complete set of crystallographic data for complex **2a** was deposited to the Cambridge Crystallographic Data Centre (CCDC entry no. 1815708). The UV spectra were measured in the range λ 220–320 nm on Shimadzu UV 2401 PC and Shimadzu UV-1800 spectrophotometers using quartz cells with a cell path of 1 cm. The circular dichroism spectra were recorded on a Jasco J-810 spectropolarimeter (cell path length 0.2 cm). Commercially available calf thymus DNA (Sigma) was used. The fulfillment of Beer–Lambert law was checked for solutions of all complexes and working DNA solutions in the concentration ranges 4.55–36.4 and 1.36–27.3 μM, respectively. The spectral measurements were performed in a 50 mM Tris–HCl/50 mM NaCl buffer (pH = 7.2).

General procedure for the synthesis of complexes 2a–2f. A solution of 0.07 mmol of tetrazol-2-ylacetic acid derivative **1a**–**1f** in 7 mL of ethanol was added to a solution of 0.035 mmol of palladium(II) chloride in 5 mL of 0.5 M aqueous HCl. The mixture was kept for 4–5 weeks at room temperature, and the crystalline solid was filtered off, washed with ethanol, and dried.

***trans*-Dichlorobis(ethyl 5-methyl-2*H*-tetrazol-2-ylacetate)palladium(II) (2a).** Yield 15.3 mg (76%), yellow crystals. ^{1}H NMR spectrum, δ, ppm: 1.21 m (6H), 2.48 s (6H), 4.18 m (4H), 5.72 s (4H). ^{13}C–{^{1}H} NMR spectrum, δ_C, ppm: 10.33, 13.91, 52.99, 61.87, 162.61 (C^5), 166.14 (C=O). Mass spectrum: *m*/*z*: 538.9927 [M + Na]$^+$ (calculated for $C_{12}H_{20}Cl_2N_8NaO_4Pd$: 538.9912). X-Ray diffraction data: $C_{12}H_{20}N_8O_4Cl_2Pd$, M = 517.66; monoclinic crystal system, space group $P2_1/c$; unit cell parameters: a = 8.6892(3), b = 22.6561 (8), c = 10.7915(5) Å; β = 110.396(5)°; V = 1991.26 (14) Å^3; Z = 4; total number of reflections 17293, including 4542 independent reflections (R_{int} = 0.0372).

***trans*-Dichlorobis(5-methyl-2*H*-tetrazol-2-ylacetamide)palladium(II) (2b).** Yield 11.7 mg (73%), yellow crystals. ^{1}H NMR spectrum, δ, ppm: 2.45 s (6H), 5.75 s (4H), 7.86 br.s (4H). ^{13}C–{^{1}H} NMR spectrum, δ_C, ppm: 10.42, 53.76, 163.23 (C^5), 166.32 (C=O). Mass spectrum: *m*/*z* 423.0117 [M – Cl]$^+$ (calculated for $C_8H_{14}ClN_{10}O_2Pd$: 423.0025).

***trans*-Bis[*N*-(butan-2-yl)-5-methyl-2*H*-tetrazol-2-ylacetamide]dichloropalladium(II) (2c).** Yield 15.6 mg (78%), yellow crystals. ^{1}H NMR spectrum, δ, ppm: 0.84 m (6H), 1.07 d (6H, J = 8.0 Hz), 1.41 m (4H), 2.46 s (6H), 3.67 m (4H), 4.21 m (2H), 5.11 s (4H), 8.27 br.s (2H). ^{13}C–{^{1}H} NMR spectrum, δ_C, ppm: 8.29, 10.29, 19.98, 28.73, 46.35, 48.61, 153.17 (C^5), 163.66 (C=O). Mass spectrum: *m*/*z*: 593.0859 [M + Na]$^+$ (calculated for $C_{16}H_{30}Cl_2N_{10}NaO_2Pd$: 593.0858).

***trans*-Dichlorobis(*N*-cyclopropyl-5-methyl-2*H*-tetrazol-2-ylacetamide)palladium(II) (2d).** Yield 14.4 mg (76%), yellow crystals. ^{1}H NMR spectrum, δ, ppm: 0.45 m (4H), 0.66 m (4H), 2.45 s (6H), 2.66 m (2H), 5.07 s (4H), 8.52 br.s (2H). ^{13}C–{^{1}H} NMR spectrum, δ_C, ppm: 5.56, 7.30, 10.38, 22.41, 54.22, 162.24 (C^5), 165.14 (C=O). Mass spectrum: *m*/*z* 561.0228 [M + Na]$^+$ (calculated for $C_{14}H_{22}Cl_2N_{10}O_2PdNa$: 561.0226).

***trans*-Dichlorobis(5-phenyl-2*H*-tetrazol-2-ylacetamide)palladium(II) (2e).** Yield 5.9 mg (29%), yellow crystals. ^{1}H NMR spectrum, δ, ppm: 5.48 s (4H), 7.54 m (6H), 7.87 s (4H), 8.08 m (4H). ^{13}C–{^{1}H} NMR spectrum, δ_C, ppm: 54.62, 126.33, 126.88, 129.33, 130.69, 164.08 (C^5), 166.04 (C=O). Mass spectrum: *m*/*z* 604.9991 [M + Na]$^+$ (calculated for $C_{18}H_{18}Cl_2N_{10}NaO_2Pd$: 604.9924).

***trans*-Bis[*N*-(butan-2-yl)-5-phenyl-2*H*-tetrazol-2-ylacetamide]dichloropalladium(II) (2e).** Yield 7.1 mg (29%), yellow crystals. ^{1}H NMR spectrum, δ, ppm: 0.88 t (6H, J = 6.0 Hz), 1.07 d (6H, J = 6.6 Hz), 1.59 m (4H), 3.64 m (2H), 5.47 s (4H), 7.56 m (6H), 7.72 s (2H), 8.06 m (4H). ^{13}C–{^{1}H} NMR spectrum, δ_C, ppm: 10.32, 20.02, 28.76, 48.12, 54.85, 126.87, 129.30, 130.59, 163.27 (C^5), 164.08 (C=O). Mass spectrum: *m*/*z* 717.1165 [M + Na]$^+$ (calculated for $C_{26}H_{34}Cl_2N_{10}NaO_2Pd$: 717.1170).

Antiproliferative activity of complexes 2a, 2e, and 2f *in vitro*. Human leukemia HL-60 cells were obtained from the Cell Culture Collection (Institute of Cytology, Russian Academy of Sciences) and were cultured in RPMI-1640 medium (Gibco, USA) containing 10% of inactivated embryo serum (Gibco, USA), penicillin (100 units/mL), and streptomycin (100 μg/mL; *Biolot*, Russia) at 37°C in a 5% CO_2 atmosphere using a CO_2 incubator (Sanyo, Japan). The cells were inoculated at a concentration of 5×10^5 cell/mL into the wells of a 96-well microplate, solutions of complexes **2a**, **2e**, and **2f** at different concentrations were added, and the microplate was incubated for 24 h. Control cells were incubated with addition of 1% of Triton X-100. A solution of MTT (5 mg/mL) was then added, the microplates were incubated for 2 h, propan-2-ol containing 0.04 N aqueous HCl was added to the wells, and the well content was stirred until dissolution of the formazan precipitate. The optical density of the resulting solutions was measured at λ 570 and 630 nm using a Fluorofot microplate reader (Russia). The cell viability was expressed in percent relative to the control cells. Each experiment was carried out in triplicate.

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CONFLICT OF INTERESTS

No conflict of interests was declared by the authors.

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