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Synthesis, characterization, and biological evaluation of new tetrazole-based platinum(II) and palladium(II) chlorido complexes — Potent cisplatin analogues and their *trans* isomers

Tatiyana V. Serebryanskaya [a,*], Tatiana Yung [b], Alexey A. Bogdanov [b], Andrei Shchebet [c], Steven A. Johnsen [c,d], Alexander S. Lyakhov [a], Ludmila S. Ivashkevich [a], Zhanna A. Ibrahimava [e], Tatiyana S. Garbuzenco [e], Tatiyana S. Kolesnikova [e], Natalya I. Melnova [e], Pavel N. Gaponik [a], Oleg A. Ivashkevich [a]

[a] *Research Institute for Physical Chemical Problems, Belarusian State University, Leningradskaya 14, 220030, Minsk, Belarus*
[b] *Department of Molecular Biophysics, Faculty of Physics, St. Petersburg State University, Ulyanovskaya 1, 198504 St. Petersburg, Russian Federation*
[c] *Department of Molecular Oncology, Göttingen Center for Molecular Biosciences, University of Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany*
[d] *Center of Experimental Medicine, Department of Tumor Biology, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany*
[e] *Scientific and Production Republican Unitary Enterprise "LOTIOS", Z. Byaduli 10, 220034 Minsk, Belarus*

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ABSTRACT

Two series of tetrazole-containing platinum(II) and palladium(II) chlorido complexes, *trans*-$[ML_2Cl_2]$ (M = Pt, Pd) and *cis*-$[PtL_2Cl_2]\cdot nH_2O$ ($n = 0, 1$), where L is 1- or 2-substituted 5-aminotetrazole, have been synthesized and thoroughly characterized. Configuration of platinum(II) complexes obtained from the reaction of 5-aminotetrazoles with K_2PtCl_4 has been found to vary depending on the nature of tetrazole derivatives and reaction conditions. According to *in vitro* cytotoxic evaluation, only platinum complexes display noticeable antiproliferative effect, and their cytotoxicity depends strongly on their geometry and hydrophobicity of the carrier ligands. The most promising complexes are *cis*-$[Pt(1\text{-apt})_2Cl_2]\cdot H_2O$ and *cis*-$[Pt(2\text{-abt})_2Cl_2]\cdot H_2O$, where 1-apt is 5-amino-1-phenyltetrazole and 2-abt is 5-amino-2-*tert*-butyltetrazole. In comparison with cisplatin, they show comparable cytotoxic potency against cisplatin-sensitive human cancer cell lines, *cis*-$[Pt(2\text{-abt})_2Cl_2]\cdot H_2O$ performing substantially higher activity against cisplatin-resistant cell lines. Cell cycle studies in H1299 cell line indicated that *cis*-$[Pt(2\text{-abt})_2Cl_2]\cdot H_2O$ induced apoptosis launched from G2 accumulations. The DNA interaction with *cis*-$[Pt(1\text{-apt})_2Cl_2]\cdot H_2O$ was followed by UV spectroscopy, circular dichroism, hydrodynamic and electrophoretic mobility studies. Both *cis*-$[Pt(1\text{-apt})_2Cl_2]\cdot H_2O$ and *cis*-$[Pt(2\text{-abt})_2Cl_2]\cdot H_2O$ complexes appeared to be significantly less toxic than cisplatin in mice, while only compound *cis*-$[Pt(1\text{-apt})_2Cl_2]\cdot H_2O$ displayed noticeable efficacy *in vivo*.

1. Introduction

Platinum complexes with nitrogen-containing heterocycles are of great interest as potent antitumor agents. The spatial hindrance caused by these ligands has been shown to enhance activity of both *cis* and *trans* platinum complexes [1–4]. Moreover, an application of *N*-containing heterocycles appeared to be efficient in designing active palladium-based compounds [5].

A great number of *cis* platinum complexes as well as their *trans* analogues with different heterocycles have been investigated up to date. Most of the studies in this area were concerned with the complexes of pyridine or its derivatives [6–15]. Among other considered heterocycles were imidazoles [15–19], thiazole [6], pyrazoles [20–24], quinoline and isoquinoline [6]. Biological activity of platinum complexes with polynitrogen heterocycles, particularly tetrazoles and triazoles, is considerably less studied. For example, Bekhit et al. reported the antitumor activity of platinum(II) complexes of tetrazolo[1,5-a] quinolines [25]. More recently, two tetrazolato-bridged binuclear platinum(II) complexes were shown to be considerably more active than their triazolato- and pyrazolato-analogues when studied against H460 human NSCLC cell line, and one of them appeared to be non-cross-resistant with cisplatin *in vitro* and low-toxic *in vivo* [26]. It should be noted that the ability of tetrazole moiety to decrease toxicity of modified medicines is well known and, owing to this fact, it is widely used as bioisoster of different biologically active groups [27,28]. Due to variety of possible substituents, *N*-mono- and *C,N*-disubstituted tetrazoles are promising carrier ligands for designing new potential antitumor agents that are able to overcome the most common drawbacks of convenient platinum drugs such as high toxicity, low solubility

* Corresponding author. Tel.: +375 17 2095198; fax: +375 17 2264696.
E-mail address: serebryanskaya.t@gmail.com (T.V. Serebryanskaya).

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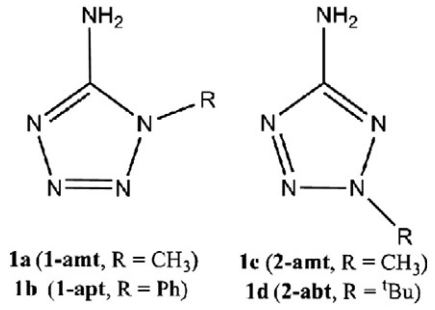

Fig. 1. Structures of 1- and 2-substituted 5-aminotetrazoles employed as ligands.

and tumor resistance. Despite that, at the beginning of our study, there were no examples in the literature dealing with antitumor activity of cisplatin analogues containing N-substituted tetrazoles as carrier ligands. Moreover, synthesis and structure of platinum complexes with these ligands were insufficiently explored to carry out their cytotoxic studies.

Recently, *trans* palladium(II) and platinum(II) chlorido complexes with 5-amino-2-*tert*-butyltetrazole have been synthesized and structurally characterized [29]. The platinum complex has been shown to possess promising cytotoxic activity against HeLa tumor cells, the IC_{50} for this compound being average between cisplatin and carboplatin. The present study was initiated to extend the knowledge on the reactivity of tetrazole-containing ligands towards platinum and palladium salts and biological activity of the tetrazole-based cisplatin analogues and their *trans* isomers. In the present paper, we report the synthesis of twelve platinum(II) and palladium(II) chlorido complexes with 1- and 2-substituted 5-aminotetrazoles **1a**–**1d** (Fig. 1). Physico-chemical properties of the complexes were thoroughly investigated, and their antitumor activity *in vitro* and *in vivo* was characterized.

2. Results and discussion

2.1. Synthesis and characterization

The complexes *trans*-$[PdL_2Cl_2]$ were prepared by the reaction of tetrazoles **1a**–**1d** with palladium(II) chloride ($PdCl_2$) dissolved in 1 M HCl as described previously [29].

General procedures for the synthesis of the *cis* and *trans* platinum(II) chlorido complexes with 5-aminotetrazoles **1a**–**1d** are depicted in Fig. 2. The reaction of potassium tetrachloridoplatinate (K_2PtCl_4) with

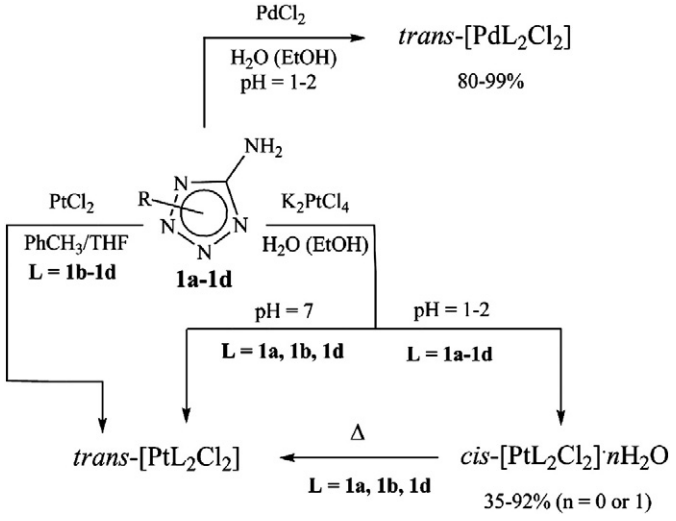

Fig. 2. Synthesis of platinum(II) and palladium(II) chlorido complexes with 5-aminotetrazoles **1a**–**1d**.

2 equiv of the nitrogen-containing nucleophiles is considered to be a common method to prepare cisplatin analogues [30]. In spite of this fact, potassium tetrachloridoplatinate was found to react with 2 equiv of 5-aminotetrazoles **1a**, **1b**, and **1d** to give *trans*-isomeric products or inseparable mixtures of both isomers, and only for tetrazole **1c** this method afforded to obtain *cis*-isomeric product. Therefore, *cis* platinum(II) chlorido complexes with substituted 5-aminotetrazoles were prepared by direct reaction of 2 equiv of the tetrazole derivative (**1a**–**1d**) with K_2PtCl_4 in 1 M aqueous solution of HCl. *Cis* platinum complexes of 5-aminotetrazoles **1a**, **1b**, and **1d** were isolated as monohydrates. It is noteworthy that formation of *trans* platinum complexes or mixtures of *cis* and *trans* isomers under the reaction of K_2PtCl_4 with nitrogen-containing nucleophiles has been previously described for pyrazoles [31–38], 2-nitroimidazoles [39,40], 3- and 4-acetylpyridines [41], 1- and 2-adamantylamines [42], anilines [43] *etc*. Addition of HCl to the reaction mixture was earlier used to improve the yield and purity of pyrazole-based cisplatin analogues [31–35]. Although no explanation was suggested in those papers to clarify how the presence of HCl in solution could promote formation of *cis*-isomeric products, it is likely that an excess of chloride ions in the reaction mixture leads to a decrease in solubility of *cis* platinum complexes, so that it accelerates their precipitation and prevents the process of isomerization in solution.

Platinum(II) complexes *trans*-$[PtL_2Cl_2]$ (L = **1b**–**1d**) were synthesized by reaction of tetrazole L with platinum(II) chloride ($PtCl_2$) in toluene/THF mixture. Alternatively, complexes *trans*-$[PtL_2Cl_2]$ (L = **1a**, **1d**) were obtained by reaction of K_2PtCl_4 with free 5-aminotetrazoles in neutral aqueous media, as mentioned above, or by *cis*/*trans* isomerization occurring under recrystallization of corresponding *cis* forms from hot acetonitrile. It is noteworthy that *cis*/*trans* isomerization under similar conditions, *e.g.* under heating in organic solvents, has been previously reported for *cis* platinum(II) chlorido complexes of 5-nitroimidazoles [40,44], pyrazoles [31–35], and cyclobutylamine [45]. The similarities in solution behavior of platinum(II) complexes of pyrazoles, nitroimidazoles and *N*-substituted tetrazoles may be caused by low basicity of these heterocycles, as suggested earlier [39], or by spatial hindrance they induce in the structure of *cis*-isomeric compound [40].

All complexes were characterized by elemental analysis, APCI mass spectrometry, and IR spectroscopy in the range 4000–50 cm^{-1}. Both *cis* and *trans* platinum(II) complexes were also studied using ^1H and ^{13}C NMR spectroscopy, and their purity was ascertained by HPLC. Under APCI conditions, the fragmentation of palladium and platinum complexes, dissolved in CH_3CN, proceeded through the formation of ionic species $[ML_2(CH_3CN)Cl]^+$, where M = Pd, Pt and L = 5-aminotetrazole **1a**–**1d**, in agreement with previous results for other platinum complexes with *N*-heterocyclic ligands [9,46,47].

The assignment of the bands in the IR spectra was based on previous works on vibrational spectra of *N*-substituted 5-aminotetrazoles [48] and their silver(I) [49], platinum(II), and palladium(II) complexes [29] as well as platinum(II) complexes with other *N*-containing heterocycles [50]. The spectra of *cis* platinum compounds showed more bands in comparison with their *trans* isomers (Table S1). Noteworthy, not only the bands assigned to coordination unit's stretching vibrations ν(Pt–N) {250–300 cm^{-1}} and ν(Pt–Cl) {320–345 cm^{-1}}, but also the bands corresponding to stretching-deformation $\nu(C–NH_2)+\delta(NH_2)$, deformation δ(N–H), rocking $\rho(NH_2)$ {1100–1150 cm^{-1}} and mixed ν(Pt–N) + γ_{tz} {450–500 cm^{-1}} vibrations were found to split or broaden in the spectra of *cis* forms. The appearance of additional bands in the *mid*-IR spectra of *cis* isomers can be caused by their lower symmetry, in agreement with previous data on splitting of the δ(N–H) bands in the vibrational spectra of cisplatin [51] and *cis* isomeric cobalt(III) ethylendiamine complexes [52–54].

In the ^1H NMR spectra of platinum complexes, the most characteristic NH_2 peaks undergo large downfield shifts with respect to those of the free 5-aminotetrazoles **1a**–**1d** (Table 1). This can be explained by an electron density withdraw after coordination binding resulting