Journal of Molecular Structure 1171 (2018) 155–167

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Picoplatin-based complexes with the bioactive orotate and 5-fluoroorotate ligands: Synthesis, DFT calculations, structure, spectroscopic characterization and *in vitro* cytotoxicity

Magdalena Malik-Gajewska [a], Justyna Trynda [b], Wiktor Zierkiewicz [a], Katarzyna Helios [a], Rafał Latajka [a], Joanna Wietrzyk [b], Danuta Michalska [a, *]

[a] Faculty of Chemistry, Wrocław University of Science and Technology, Smoluchowskiego 23, 50-370, Wrocław, Poland
[b] Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114, Wrocław, Poland

ARTICLE INFO

Article history:
Received 12 March 2018
Received in revised form
1 June 2018
Accepted 1 June 2018
Available online 4 June 2018

Keywords:
Platinum(II) complexes
Picoplatin
Orotates
Vibrational spectra
DFT calculations
Anticancer activity

ABSTRACT

Two novel, sterically hindered platinum(II) complexes Pt(Oro)(NH_3)(2-pic), **1**, and Pt(5-FOro)(NH_3)(2-pic), **2**, were synthesized by connecting the *cis*-[Pt(NH_3)(2-picoline)]$^{2+}$ fragment of picoplatin (new anticancer agent, being currently under clinical trials) with the chelating orotate or 5-fluoroorotate bioactive ligands. The complexes were characterized in depth using IR, Raman and multinuclear (^{1}H, ^{13}C, ^{19}F, ^{195}Pt) NMR spectroscopy, high resolution mass spectrometry (ESI-TOF), elemental analysis and thermogravimetry. The experimental vibrational spectroscopic studies combined with the theoretical (DFT) calculations were indispensable to elucidate the molecular structures of these complexes. A complete assignment of the IR and Raman spectra was made on the basis of the calculated potential energy distribution (PED). The spectroscopic (FT-IR and NMR) data have shown that of two possible isomers of each complex, just one isomer (A) exists in the solid state and in DMSO solution. Interestingly, the DFT calculations with the PBE0 and B3LYP methods have revealed that the other isomer (B) with the intramolecular N–H···O hydrogen bond is more stable than A, in the gas phase. It is concluded that during the synthesis the steric and kinetic effects of the 2-picoline ligand play the dominant role, therefore, the isomer A is formed. The antiproliferative activity of **1**, **2** and 5-fluoroorotic acid (**3**) was evaluated *in vitro* against several human cancer cell lines. The studied compounds exhibited larger IC_{50} values, in comparison to cisplatin, however **1**, **2** and picoplatin showed much lower toxicity against the normal cell lines as compared with cisplatin. Complex **2** was significantly more potent than **1**, **3** and picoplatin against HT-29 (human colorectal adenocarcinoma).

1. Introduction

Platinum complexes are among leading drugs in the fight against cancer [1–6]. Cisplatin and carboplatin are particularly effective in the treatment of testicular, ovarian, cervical, bladder, head and neck tumors. Oxaliplatin exhibits high efficacy in the colorectal cancer. However, the medical success of these drugs is limited by severe side effects, such as neurotoxicity, ototoxicity and nephrotoxicity (cisplatin), myelosuppression (carboplatin) and peripheral neuropathy (oxaliplatin) [2]. Moreover, many tumors display the inherent resistance to the known platinum drugs, while others develop acquired resistance after initial treatment [7]. Thus, there is a continuous search for new platinum-based drugs. Several new platinum agents have been approved in the anticancer therapy in the specific countries. Among them are: nedaplatin in Japan [8], lobaplatin in China [9,10], heptaplatin in South Korea [11], and miriplatin, the most recently approved platinum drug in Japan [12].

All the above compounds are cisplatin analogs, which have been synthesized by using different leaving and non-leaving ligands. The leaving groups (monodentate anionic or chelating dianionic fragments) are replaced by water molecules and leave the platinum coordination sphere during the hydrolysis (aquation) process. Unfortunately, when hydrolysis is too fast, the reactive aqua-platinum(II) species can easily bind to the S-donor biomolecules (glutathione, metallothioneins or S-containing proteins) causing toxic side-effects [13,14].

* Corresponding author.
E-mail address: danuta.michalska@pwr.edu.pl (D. Michalska).

https://doi.org/10.1016/j.molstruc.2018.06.006
0022-2860/

The nonleaving ligands "carry" the platinum cation to the cellular DNA. The substitution of one or two ammine carrier ligands in cisplatin for a bulky ligand(s) modifies the structure and properties of the adduct of Pt complex with DNA [3].

Several drugs are currently at various stages of clinical trials [2]. One of them is the sterically hindered picoplatin, *cis*-[$PtCl_2(NH_3)$(2-picoline)], also called AMD473 or ZD0473, [15–17]. The important structural feature of this complex is the presence of the sterically demanding 2-picoline nonleaving ligand, which prevents an axial attack from biological nucleophiles (e.g. glutathione) at the platinum center and leads to a much slower hydrolysis, in comparison to cisplatin [17]. This plays an important role in a greatly reduced toxic side effects of picoplatin, in comparison to other platinum drugs [18]. It is remarkable that this new platinum complex shows activity against a wide range of cancer cells with intrinsic of acquired resistance to cisplatin and oxaliplatin [19,20]. Sadler et al. [21] performed NMR studies on the reaction of picoplatin with the 14-mer DNA duplex and demonstrated that the binding to DNA is highly stereoselective.

Currently, picoplatin undergoes clinical trials in the treatment of colorectal cancer [2,22] and the hormone-refractory prostate cancer [2,23]. Brown et al. [24] combined picoplatin with another anticancer agent, BBR3464, a trinuclear platinum complex, which was recently in clinical trials. They synthesized a family of sterically hindered platinum complexes as potential anticancer agents. Osella and co-workers [25] reported the synthesis and characterization of a series of octahedral bis-(carboxylato)Pt(IV) complexes based on picoplatin. These Pt(IV) analogs revealed promising results in the treatment of malignant pleural mesothelioma (MPM). To increase the water solubility of picoplatin, Zhang et al. [26] prepared the inclusion complex with γ-cyclodextrin (γ-CD) and showed that the obtained picoplatin/(γ-CD) complex maintained the anticancer properties.

The aim of this study was to synthesize and characterize new sterically hindered platinum complexes, which would be less toxic and more selective in action than cisplatin. Thus, we have combined the *cis*-$[Pt(NH_3)$(2-picoline)$]^{2+}$ fragment of picoplatin with the chelating orotate or 5-fluoroorotate ligands. Both the ligands exhibit biological activity. Orotic acid (6-carboxyuracil) is a key precursor of all pyrimidine bases, which are required for the synthesis of nucleic acids and for all pyrimidine nucleotide-dependent biosynthetic processes [27], while 5-fluoroorotic acid can be considered as a prodrug of 5-fluorouracil, which is used in the treatment of colon cancer [28,29]. Recently, Nath et al. [30] reported the anticancer activity of tri- and di-organotin(IV) orotate complexes.

We expected that the replacement of the chloride ions in picoplatin with the orotate or 5-fluoroorotate ligands should improve the selectivity of these new complexes, because rapidly dividing cancer cells need the pyrimidine bases for *de novo* biosynthesis of nucleic acids. The obtained complexes have been characterized in depth using IR, Raman and multinuclear (^{1}H, ^{13}C, ^{19}F, ^{195}Pt) NMR spectroscopy, high resolution mass spectrometry (ESI-TOF), elemental analysis and thermogravimetry. Unfortunately no single crystals suitable for X-ray analysis could be obtained. Thus, in order to elucidate the molecular structures of these complexes we have performed thorough experimental vibrational spectroscopic study combined with the density functional theory (DFT) calculations. In our earlier works [31–35] we have shown that two DFT methods, mPW1PW91 and PBE0 (PBE1PBE), are superior to the *ab initio* MP2 method and to other DFT functionals (including B3LYP) in a simultaneous prediction of the molecular structure and vibrational spectra of cisplatin, carboplatin, picoplatin and other platinum complexes.

The antiproliferative activity of the new complexes was tested *in vitro* in several human cancer cell lines. For comparison, the same cytotoxicity tests have been performed for 5-fluoroorotic acid, picoplatin and cisplatin.

2. Experimental

2.1. Synthesis and characterization of Pt(II) complexes, **1** and **2**

Potassium aminetrichloroplatinate(II), orotic acid sodium salt and 5-fluoroorotic acid hydrate were obtained from Aldrich. 2-Methylpyridine (2-picoline) was purchased from Merck. Silver nitrate, potassium iodide and potassium chloride were supplied by POCH. All compounds were of reagent grade purity and were used without further purification. Picoplatin was synthesized as described in the literature [35,36]. All reactions were carried out under protection from light.

The new complexes were obtained in two steps. In the first step, $K[PtCl_3(NH_3)]$ (0.50 g, 1.4 mmol) and KCl (0.11 g, 1.5 mmol) were dissolved in water (10 ml) and stirred during the addition of KI (0.70 g, 4.2 mmol) in water (3 ml). Next, 2-picoline (0.15 ml, 1.5 mmol) was added and the mixture was stirred for about 20 h. The yellow precipitate of *cis*-$[PtI_2(NH_3)$(2-pic)] was collected by filtration, washed with water and methanol and dried in a desiccator. In the second step, a solution of $AgNO_3$ (0.37 g, 2.2 mmol) in water (10 ml) was added dropwise to a stirred suspension of *cis*-$[PtI_2(NH_3)$(2-pic)] (0.60 g, 1.1 mmol) in water (50 ml) at room temperature, and stirring was continued for 8 h. The silver iodide that precipitated from solution was removed by filtration. Next, to the filtrate containing *cis*-$[Pt(NH_3)$(2-pic)$]^{2+}$ cation, 1.1 mmol of sodium orotate (for complex **1**) or 5-fluoroorotic acid hydrate (for complex **2**) in water (30 ml) was added slowly with stirring. Prior to the complexation, both the orotate ligands were dissolved in hot water (70 °C) and then cooled to room temperature. The synthesis of complexes **1** and **2** was conducted for 3 days. The pH of the final solutions was less than 3. The resulting pale yellow precipitate of Pt(Oro)(NH_3)(2-pic) (**1**) and the off-white precipitate of Pt(5-FOro)(2-pic) (**2**) were collected by filtration, washed with cold water and acetone and dried in a desiccator.

Pt(Oro)(NH_3)(2-pic) (**1**) Yield: 0.15 g, 0.33 mmol (25% based on $K[PtCl_3(NH_3)]$). Anal. calcd: C 28.8; N 12.2; H 2.6. Found: C 28.6; N 12.1; H 2.3%. HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd for Pt($C_5H_2N_2O_4$)(NH_3)(2-pic): 460.0585, found: 460.0569.

The NMR spectra were measured in DMSO-d6. The spectra are shown in the Electronic Supplementary Information (ESI, Figs. S1, S2 and S3).

^{1}H NMR: 3.04 ppm CH_3, 3H, s (19–21); 5.20 ppm NH_3, 3H, s (11–13); 5.65 ppm CH, 1H, d, $J = 2.27$ Hz (R); 7.44 ppm Ar, 1H, t, $J = 6.75$ Hz (15); 7.62 ppm Ar, 1H, d, $J = 7.82$ Hz (17); 7.97 ppm Ar, 1H, t, $J = 7.76$ Hz (16); 8.92 ppm Ar, 1H, d, $J = 5.91$ Hz (14); 10.82 ppm NH, 1H, d, $J = 2.0$ Hz (30). ^{13}C NMR: 25.2 ppm CH_3 (18); 102.6 ppm (28); 123.3 ppm (7); 126.4 ppm (9); 139.2 ppm (8); 153.7 ppm (6 and 24), 159.9 ppm (25); 161.5 ppm (10); 164.1 ppm (27); 174.8 ppm (22). ^{195}Pt NMR: −2103 ppm.

Thermal analysis: decomposition occurs in one step, which begins at ~320 °C; two endothermic DTA peaks are at 335 °C (the loss of 0.5 molecule of NH_3) and 344 °C (the loss of 0.5 molecule of NH_3 and one molecule of 2-picoline); further whole decomposition of the orotate ligand ($C_5H_2N_2O_4$) occurs above 600 °C.

Pt(5-FOro)(NH_3)(2-pic) (**2**) Yield: 0.17 g, 0.35 mmol (25% based on $K[PtCl_3(NH_3)]$). Anal. Calcd: C 27.7; N 11.7; H 2.3. Found: C 27.6; N 11.8; H 2.3%. HRMS (ESI-TOF): *m/z* $[M+H]^+$, calcd for Pt(5-$FC_5H_1N_2O_4$)(NH_3)(2-pic): 478.0491, found: 478.0483.

The NMR spectra were measured in DMSO-d6. The spectra are shown in the Electronic Supplementary Information (ESI, Figs S4-S7).