Polyhedron 28 (2009) 3459–3466

Contents lists available at ScienceDirect

Polyhedron

journal homepage: www.elsevier.com/locate/poly

Physical characterization and biological studies of a (streptidine)($Pt^{II}Cl_4$) compound

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ARTICLE INFO

Article history:
Received 3 June 2009
Accepted 13 July 2009
Available online 18 July 2009

Keywords:
Streptidine
Platinum compound
XAS
Cytotoxic assays
Antimicrobial studies
Fluorescence studies

ABSTRACT

The synthesis, characterization and biological properties of a novel platinum compound, (H_2Std)$PtCl_4(H_2O)_x$ (**1**, where x = 1–4; H_2Std stands for streptidine, the cationic sugar component of streptomycin), are reported in the solid state and in solution. Reaction of streptidine sulfate ((H_2Std)SO_4) with K_2PtCl_4, yields the sparingly soluble hydrated compound (H_2Std)$PtCl_4$. Structural information has been attained by a variety of spectroscopic techniques, as well as by using Pt L_3- and Cl K-edge X-ray absorption spectroscopy (XAS). The analyses of the data were completed by performing extended X-ray structural characterization of the free ligand as streptidine sulfate, which was found crucial to interpret the nature and structural arrangement of complex **1**.

In addition, this work also presents the biological activity of complex **1**, i.e. cytotoxic assays, antimicrobial studies, DNA titration and fluorescence *in vitro* experiments. The studies presented in this paper contribute to our understanding of the synergy between platinum and pharmaceutically relevant compounds.

1. Introduction

Over the last several decades an extensive family of antibiotics has been reported which exhibit highly cytotoxic activity against a variety of tumor cells [1–10]. In addition there are a number of platinum compounds of diverse architectures which are highly potent anti-tumoral agents applicable in several cancers [11–15]. However, much less is known about the symbiosis between antibiotics and platinum, where the range of activity could be improved by incorporating the antibiotic as a ligand to Pt.

Concerning this matter, streptomycin (St) arises as part of a class of drugs called aminoglycosides and is used as a treatment in developing countries against tuberculosis and other infectious diseases [16,17]. Soon after the discovery, Carter and Peck described for the first time streptidine (H_2Std), a fundamental constituent of streptomycin [18–20]. Later, some of us reported the crystallographic structure of H_2Std and studied the reactivity of this organic moiety with 3d metals [21]. Over the years, several biological studies have pointed to the relevance of streptidine in the final activity of streptomycin, as well as the toxic effects of this molecule [22] and inherent properties as fluorescence [23].

From a chemical point of view, streptidine is an attractive molecule, formed by a bulky, flexible backbone that contains two guanidines (organic groups commonly used as denaturants of proteins) [24] and a number of hydroxides that may play a crucial role providing coordination and/or intermolecular interactions. Fig. 1 depicts the molecular structure of this sugar moiety. Unfortunately, limited information on the reactivity and coordination of this organic component is currently available.

On the other hand, several platinum compounds are known worldwide as anticancer agents: cisplatin [25], carboplatin [26] and oxaliplatin [14] among a few others are the most relevant metal-based drugs in clinical use [14]. Nevertheless, their severe side-effects, narrow anti-tumor activity and the development of resistance to these drugs necessitated the search for improved platinum–anticancer compounds [27–30]. New platinum-based agents containing moieties that are present in organic, anticancer drugs or antibiotics (e.g.: doxorubicin and daunorubicin) [31,32] display noticeable anti-tumor properties, provided by the symbiosis of both, the organic and metallic centers [33].

Starting from this idea, our strategy was to combine two effective drugs; i.e. a platinum source and streptidine with the aim of accomplish a dual-action agent and to study its potential biomedical applications. This conjugate system would contain two well-

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doi:10.1016/j.poly.2009.07.022

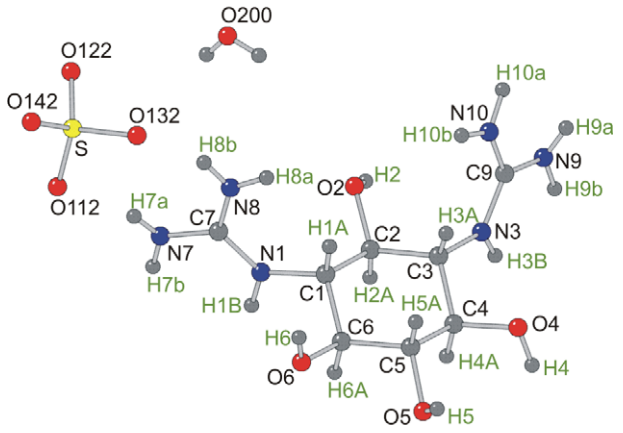

Fig. 1. Atomic numbering and ball and stick representation of the structure $(H_2Std)(SO_4)(H_2O)$, as taken from its crystal structure (*vide infra*). Hydrogen atoms are labeled in green for the sake of clarity. Nitrogen atoms are in dark blue, oxygen in red, sulfur in yellow and carbon and hydrogen in grey colours. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

defined parts, both of them with expected bioactivity: (i) as a platinum drug, the anticancer properties of the final complex would be tested; (ii) by utilizing the activity and fluorescence properties of the ligand (H_2Std), *in vitro* studies using digital fluorescence microscopy could be performed and additional antibacterial studies can also be carried.

This paper describes the synthesis and detailed characterization (including Pt L_3- and Cl K-edge XAS) of the first solid platinum–streptidine compound (**1**, $(H_2Std)PtCl_4$). An accurate X-ray diffraction study of the hydrated H_2Std sulfate was performed at room temperature, to assist in the characterization of compound **1**. However, during the preparation for publication of this work, a structural report of H_2Std sulfate at low *T* was published [34], so in this manuscript we will only elude to some of the most relevant features of the former to assist the structural refinement of $(H_2Std)PtCl_4$.

Finally, in this manuscript the results of biological *in vitro* studies of compound **1** (cytotoxicity, UV–Vis titration, fluorescence and antibacterial experiments) will be discussed.

2. Experimental

2.1. Chemistry

K_2PtCl_4 was obtained from Johnson and Matthey and 3-acetylpyridine was used without further purification as purchased from Aldrich. Synthesis was performed in the absence of light, and the final product was kept away from light to avoid any photochemical decomposition.

Infrared spectra were recorded from 4000 to 300 cm^{-1} on Perkin–Elmer Paragon 1000 FTIR spectrometer equipped with a Golden Gate ATR device, using the reflectance technique. ^1H, ^{13}C and ^{195}Pt NMR experiments were performed on a Bruker DPX 300 spectrometer, with measurements carried out at room temperature in dmso-d_6. Elemental analysis of C, H, N were carried out on a Perkin–Elmer 2400 series II analyzer. Pt analysis was carried out by AAS and ICP. Electrospray ionization-mass spectra (ESI-MS) were achieved from dmso (dimethyl sulfoxide) and recorded on a Thermo Finnigan AQA apparatus. XAS and EXAFS experiments were performed at the Stanford Synchrotron Radiation Lightsource (SSRL), as described below.

2.2. X-ray measurements

Diffraction data for the H_2Std sulfate monohydrate were collected at room temperature on a Siemens P4 diffractometer using standard procedures [35] (see Table S1 and the deposited CIF file for details). Handedness of the enantiomorphic space group was arbitrarily chosen and the structure refined in $P3_1$ on the basis of non-corrected data. The relative stereochemistry of chiral centers was assigned after refining a Flack parameter, which corresponds to that expected for a Std derivative. The high resolution of the diffraction data allowed for accurate determination of the H atom positions [36]. The sulfate ion appeared to be disordered over two positions. Sulfate O atoms were found distributed over two sites, and their respective occupancies refined with their sum constrained to 1. Site occupation factors converged to 0.775(7) and 0.225(7). Final least-squares cycles were performed without restraints or constraints for the geometry.

2.3. Cytotoxicity studies

The A2780 and A2780R cells were generously provided by Dr. J.M. Perez (Universidad Autónoma de Madrid, Spain). The cells were grown as monolayers in Dulbecco's modified Eagle's Medium supplemented with 10% fetal calf serum (Gibco, Paisley, Scotland), penicillin (100 units/mL: Dufecha, the Netherlands) and streptomycin (100 μg/mL: Dufecha, the Netherlands). The L1210 and L1210R cell lines were cultured in McCoy's 5a medium supplemented with 10% fetal calf serum (Gibco, Paisley, Scotland), penicillin (100 units/mL: Dufecha, the Netherlands) and streptomycin (100 μg/mL: Dufecha, the Netherlands).

For the cell growth assay, cells were pre-cultured in 96 multiwell plates for 48 h at 37 °C in a 5% CO_2 containing incubator and subsequently treated with 45 μL of compound in triplicate. After 48 h incubation, 50 μL of a 5 mg/mL MTT solution in PBS was added to each well, and allowed to develop at 37 °C for 2 h. After this, the medium was removed carefully and 100 μL of dmso was added to each well. The absorbance of the resulting purple solutions were measured at 590 nm using a Biorad 550 microplate reader. The cytotoxicity is determined by the IC_{50} values of each compound. The IC_{50} values are drug concentrations that inhibit cell growth for 50% with respect to control. The lower the IC_{50} value the better anti-tumor activity can be expected. The numbers were determined graphically using Graphpad Prism analysis software.

2.4. UV–Vis titration

UV–Vis titration experiments were performed in order to clearly discern the behavior of the Pt complex and CT-DNA at different ratios and also to support previous CD analysis. Tris buffer pH 7.2 was used in all the solutions and variations on the amount of platinum compound versus a constant concentration of DNA were accomplished (*R* ratio from 5 to 0.3). Data were collected on Varian Cary 50 UV–Vis Spectrophotometer with new Fiber Optic Dip Probe accessory. The Cary 50 utilizes a very long-life Xenon source lamp with wavelength range between 190 and 1100 nm as well as photometric range of +/−3.3 Å.

2.5. XAS

All data were measured at the Stanford Synchrotron Radiation Lightsource (SSRL) under ring conditions of 3.0 GeV and 60–100 mA.

Pt L_3-edge XAS data were measured on focused 16-pole wiggler beam line 9-3. A Si(2 2 0) monochromator was utilized for energy selection. A harmonic rejection mirror was (set to a 15 keV cutoff) was used to minimize higher harmonic components in the X-ray