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Original article

Synthesis, characterization and cytotoxic activity of novel platinum(II) iodido complexes

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ABSTRACT

Novel Pt(II) complexes of general formula $[PtI_2(L^{1-3})]$, (**C1**–**C3**): where L^{1-3} are isobutyl, *n*-pentyl and isopentyl esters of (*S,S*)-1,3-propanediamine-*N,N'*-di-2-(3-cyclohexyl)propanoic acid has been synthesized and characterized by elemental analysis, UV/Vis, IR, (^{1}H, ^{13}C and HSQC, Pt) NMR spectroscopy and ESI mass spectrometry. Spectroscopic data and computational studies have shown the usual square planar coordination geometry of synthesized complexes, with coordination of ligands *via* nitrogen donor atoms. The cytotoxic activity of novel ligands and corresponding complexes were investigated on a palette of different cells line. Complexes **C1**–**C3** exhibited activity comparable to *cisplatin*, with IC_{50} values (μM) ranging from 4.6 ± 0.6 to 17.2 ± 2, and showed the highest potential in HeLa, LS-174 and EA.hy.926 cells. Ligands **L1**–**L3** exhibited two- to four-times less activity than corresponding complexes. Analysis of the mode of action in HeLa cells, by ICP-MS study, showed markedly higher intracellular accumulation and DNA binding affinity of **C1**–**C3** versus *cisplatin*, after 4 h and 20 h post-treatment. Annexin-V-FITC assay, flow cytometry and fluorescence microscopy study demonstrated occurrence of cell death through both apoptotic and necrotic changes. Tested complexes, at corresponding IC_{50} concentrations, caused considerable "sub-G1" peak, without other substantial alterations of cell cycle, while only **C1** induced higher level of phosphatidylserine externalization (11.7%), comparing to ligand **L1** (4.9%) and *cisplatin* (8.4%). Structure-activity comparison indicated variations of **C1**–**C3** cytotoxicity, related to the drug/ligand lipophilicity (C log P value), while intracellular platinum content and DNA platination increased on increase of length and branching of ester chain, in sequence: **C1** (isobutyl) < **C2** (*n*-pentyl) < **C3** (isopentyl).

1. Introduction

Metal complexes ensure a very diverse platform for drug design in the field of anticancer therapy. In addition to various oxidation states of metal ions, complexes of metals have different geometries and coordination numbers that allow the fine-tuning of their chemical reactivity [1–3]. In the last decades complexes with metal ions such as: platinum, ruthenium, gallium, gold, osmium and titanium have been investigated for their anticancer properties [4–6]. *Cisplatin* is still the most widely used metal-based drug in the treatment of different human tumors [7]. Among the second-generation platinum drugs, *carboplatin* and *oxaliplatin* has led to improvement in cancer therapy. Due to the many side effects of these drugs and cellular resistance, thousands of platinum complexes were synthesized, in order to find a more suitable antitumor drug [8,9]. The design of third generation platinum complexes was intended to overcome this problem. Diaminocyclohexane platinum complexes and their anticancer properties were intensively studied in recent years. *cis*-Dichlorido(1,2-aminocycloxexane)platinum(II) (DACHPt) is a *cisplatin* derivative with cyclohexane ring attached to the amino groups and this molecule has outstanding biological properties, much broader spectrum of activity and a lack of cross-resistance with *cisplatin* [10,11]. Unfortunately, this compound is completely insoluble in water and most other solvents, which is a problem in the application of this substance in biological systems. By substituting chlorides with oxalato ligand, in the mentioned compound, *oxaliplatin* was obtained and achieved worldwide clinical approval [12,13].

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Platinum(II) compounds, of the type *cis*-$PtX_2(amine)_2$, where X is labile ligand (leaving anionic group) and an amine represents inert ligand (any primary or secondary amine or chelate amine ligand) exhibit significant anticancer activity against a variety of cell lines [14,15]. The most complexes of this type exhibit cytotoxicity by binding to DNA (in particular to the N7 atom of adjacent guanines), giving rise to a similar biological mode of action [16]. Also, platinum(II) complexes have shown reactivity toward different model proteins as well as to extra- and intracellular sulfur containing biomolecules [17–19]. Of the great interest as unconventional metal based drugs are the iodide analogs of *cisplatin*. These complexes were not interesting in medicinal chemistry because of their higher stability and lower reactivity of Pt–I bonds versus Pt–Cl bonds in aqueous solution [20]. A few papers can be found in the literature in which were described a special interactions of iodido platinum compounds with some important biomolecules. The significant difference in the reactivity of *cis/trans* iodido complexes was observed. In many cases it has been shown that the *trans* isomers may release their iodide ligands upon biomolecule binding, while the *cis* isomers preferentially release the amine ligands with retention of iodides [17,21,22]. Investigation of binding of these complexes to DNA models showed that both isomers bind with retention of amine ligand [17]. Pt(II) and Pt(IV) with ethylenediamine-*N,N′*-diacetate (edda)-type of ligand and their derivatives exhibit structure–activity relationships based on *in vitro* testing against of numerous cancer cell lines [23–25]. Esters of (*S,S*)-ethylenediamine-*N,N′*-di-2-(3-cyclohexyl)propanoic acid and corresponding platinum complexes showed enhanced biological activity in comparison to molecules with similar structure [26,27].

In an attempt to combine Pt(II) iododo complexes with derivatives of ethylenediamine-*N,N′*-diacetate (edda)-type of ligands, i.e., esters of (*S,S*)-1,3-propanediamine-*N,N′*-di-2-(3-cyclohexyl) propanoic acid to obtain new potent anticancer drugs, in this study we describe the synthesis, spectroscopic characterization, computational studies and *in vitro* biological evaluation of three new ligands and corresponding complexes. Promising biological activity and potential in *cisplatin* resistant cell lines provide useful information for further biological evaluation (interaction with small model proteins and oligonucleotides) and future drug design strategies.

2. Experimental

2.1. Chemistry

2.1.1. Starting agents

(*S*)-2-amino-3-cyclohexyl-propanoic acid hydrochloride was purchased from Senn Chemicals (Dielsdorf, Switzerland). (*S,S*)-1,3-propanediamine-*N,N′*-di-2-(3-cyclohexyl)propanoic acid dihydrochloride were prepared according to new procedure [28], while corresponding esters were obtained as described in this paper. $K_2[PtCl_4]$ was prepared by reduction of the $K_2[PtCl_6]$ with hydrazine [29]. Solvents were obtained commercially and used without further purification.

2.1.2. Measurements

Elemental analysis was carried out with Elemental Vario EL III microanalyzer. Infrared spectra were recorded on a Nicolet 6700 FT-IR spectrometer using the ATR technique. The NMR spectra (^1H, ^{13}C, HSQC and Pt) were recorded on a Bruker Avance III 500 spectrometer. Mass spectra of ligands were carried out with a 6210 Time-of-Flight LC-MS instrument (G1969A, Agilent Technologies) in methanol with addition formic acid. Mass spectra of complexes were carried out with an Orbitrap LTQ XL instrument (Thermo Scientific, Bremen, Germany) in 95% acetonitrile in water, with addition formic acid. Electronic spectra of 1×10^{-4} M solutions of platinum complexes were recorded in acetone, using a GBC UV/Vis Cintra 6 spectrophotometer. Melting points were determined on an Electrothermal melting point apparatus.

The lipophilicity parameters of the compounds were computed by employing the program available in the Internet (Alog PS 2.1-vcclab: C log P, Alog Ps, AC log P, milog P, KOWWIN, Xlog P2, Xlog P3 [30]). Prediction of lipophilicity by the Alog PS 2.1 program is described in two articles [31,32]. Clog *P* values were calculated by the ChemDraw Ultra 9.0 software [33].

2.1.3. Computational details

All calculations have been performed using the Amsterdam Density Functional (ADF) program package [34] version 2013.01, with general gradient approximation consisting of OPTX [35] for the exchange and PBE [36] functional for correlation (OPBE [37]). Molecular orbitals were expanded in an uncontracted set of Slater type orbitals (STOs) [38], of triple-ζ quality containing diffuse functions plus one set of polarization functions (TZP). Due to the presence of platinum, scalar relativistic corrections have been included self-consistently by using the zeroth-order regular approximation (ZORA) [39]. Default integration and gradient convergence criterions were used. Analytical harmonic frequencies [40] were calculated in order to ascertain that the optimized structures correspond to the minima on the potential energy surface. In order to check the possible influence of an environment, we also performed additional calculations with a dielectric continuum model (COSMO) [41] (using water as a solvent) as implemented in ADF [42].

2.2. Biology

2.2.1. Cell culture

Human cervical adenocarcinoma (HeLa), human alveolar basal adenocarcinoma (A549), human breast carcinoma (MDA-MB-231), human colorectal adenocarcinoma (LS-174), and human fetal lung fibroblast cell line (MRC-5), were maintained in the RPMI 1640 medium (Sigma Aldrich). The endothelial permanent human cell line derived by fusing human umbilical vein endothelial cells-HUVEC with human lung cells-A549 (EA.hy 926) was maintained in the nutrient medium, Dulbecco's Modified Eagle Medium (DMEM) (Sigma Aldrich). Both RPMI and DMEM media were supplemented with 10% heat inactivated fetal calf serum (FCS) (Sigma Aldrich), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) (25 mM), penicillin (100 units/mL), and streptomycin (100 mg/mL), while DMEM was additionally supplemented with D-Glucose (4.5 g/l). Cells were maintained as a monolayer culture in tissue culture flasks (Thermo Scientific Nunc™) in an incubator at 37 °C, in a humidified atmosphere composed of 5% CO_2.

2.2.2. MTT assay

Antiproliferative activity of tested complexes was determined using 3-(4,5-dymethylthiazol-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma–Aldrich) assay [43]. Cells were seeded into 96-well cell culture plates (Thermo Scientific Nunc™), at a cell density of 4000 c/w (HeLa), 8000 c/w (A549), 7000 c/w (LS-174, MDA-MD-231, MRC-5), 3000 c/w (EA.hy 926), in 100 μl of culture medium. After 24 h of growth, cells were exposed to the serial dilutions of the tested complexes. The complexes were dissolved in DMSO at a concentration of 10 mM as stock solution, immediately prior the use. Serial dilutions were made in culture medium so that final concentration of DMSO per well was less than 0.1% (v/v), in all experiments. Final concentrations achieved per wells were: 6.25 μM, 12.5 μM, 25 μM, 50 μM and 100 μM. Each concentration