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Synthesis, characterization and *in vitro* antitumor activity of new palladium(II) complexes with (*S*,*S*)-R_2edda-type esters

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Dedicated to Professor Vukadin Leovac on the occasion of his 70th birthday.

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ABSTRACT

Six palladium(II) complexes with (*S*,*S*)-R_2edda-type esters ((*S*,*S*)-R_2edda-type: (*S*,*S*)-eddch = (*S*,*S*)-ethylenediamine-*N*,*N*′-di-2-(3-cyclohexyl)propanoate, R = Me, Et, *n*-Pr, **1**–**3**; (*S*,*S*)-pddch = (*S*,*S*)-propylenediamine-*N*,*N*′-di-2-(3-cyclohexyl)propanoate, R = Et, *n*-Pr, **4**, **5**; and (*S*,*S*)-eddip = (*S*,*S*)-ethylenediamine-*N*,*N*′-di-2-propanoate, R = *i*-Am, **6**) were synthesized, characterized by IR, NMR spectroscopy, ESI-MS and elemental analysis. DFT calculations indicate that in case of **1**–**4**, the most stable isomers are with (*S*,*S*)- and (*R*,*S*)-configuration of nitrogen atoms, but for complex **6** (*R*,*R*)- and (*R*,*S*)-*N*,*N*′-configured isomers. Furthermore, complex **5** was obtained as (*S*,*S*)-*N*,*N*′ configured isomer. Cytotoxicity study was performed against human cervical adenocarcinoma (HeLa), human alveolar basal adenocarcinoma (A549) and non-cancerous human fetal lung fibroblast (MRC-5) cell lines using colorimetric MTT assay. From the investigated palladium(II) complexes **2**, **3** and **5** exhibited highest cytotoxic potential against HeLa (IC_{50}: 28.5 ± 3.9, 29.5 ± 1.3 and 34.3 ± 3.2, respectively).

1. Introduction

The use of cisplatin and its derivatives has led to a major advancement in the treatment of cancer, with a high cytotoxic effect upon a variety of tumor types [1–3]. The limitations due to toxicity and resistance [4,5] has stimulated major research efforts into the discovery of non-platinum based chemotherapeutic agents [6,7]. All cytotoxic (cell-killing) drugs used today confront with lack of selectivity, because cancer cells are just subtly mutated forms of normal human cells that multiply without control [8]. Disappointingly, from over 500,000 compounds tested only about 20 new drugs have entered clinical trials [9–12]. Discovery of new, more selective compounds with improved therapeutic properties is an urgent issue.

Platinum complexes with various ligands have proven their anticancer activity [13–16]. On the basis of the structural analogy between platinum(II), gold(III) and palladium(II) complexes, there is much interest in studying palladium(II) derivatives as potential anticancer drugs [17]. However, the ligand-exchange kinetics of palladium complexes is 10^5 times faster than for platinum analogues. They dissociate readily in solution leading to very reactive species that are unable to reach their biological targets. Therefore, palladium(II) complexes are frequently used as model complexes to study the interaction of platinum(II) with DNA and to mimic the binding properties of various platinum(II) species [18]. Anticancer activity of metallodrugs depends on ligands, as they can modify reactivity, and lipophilicity [19]. Chelating ligands may reduce the reactivity of the palladium center. In the past few years, some palladium complexes, polynuclear derivatives with selected thiosemicarbazones [20], certain palladacycles [21,22] and palladated complexes derived from phenylacetaldehyde thiosemicarbazone [23] have been reported to be quite stable and good candidates for antitumoral drugs.

The presence of bidentate amines has been an almost constant feature of new palladium(II) agents [24–26], from amine neutral ligands such as ethylenediaminoalkanes [27,28], and diaminocyclohexane (DACH) [29] to alkylaminophosphine oxides [30], mercaptoimidazoles, and pyridine and pyrimidine derivatives [31–33].

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O,O′-dialkyl esters of (*S,S*)-ethylenediamine-*N,N′*-di-2-propanoic, (*S,S*)-ethylenediamine-*N,N′*-di-2-(3-cyclohexyl)propanoic and (*S,S*)-propylenediamine-*N,N′*-di-2-(3-cyclohexyl)propanoic acids belong to a "chiral subgroup" of the class of R_2edda-type ligands. They are bidentate chelating *NN* ligands with chiral C-atoms between –NH and –COO moieties [34–36]. Lately, a large number of their metal complexes was synthesized and screened for antitumor activity [37–40] (Fig. 1.). In case of the most simple chiral (*S,S*)-R_2eddip ligands (Fig. 1A) the highest antitumor activity was observed for corresponding platinum(IV) complexes [34], moderate cytotoxic action of palladium(II) complexes [41]. When (*S,S*)-R_2eddl ((*S,S*)-ethylenediamine-*N,N′*-di-2-(4-methyl)pentanoate) coordinates to a metal centre, the most significant antitumor activity is shown by platinum(II) complexes against chronic lymphocytic leukemia cells (Fig. 1B) [35]. In the most cases high cytotoxic action is accomplished upon coordination of ligands to metal ions, but some of the ligand precursors, in fact cyclohexyl-functionalized dihydrochlorides, also showed serious activity [36]. (Fig. 1C).

Because nitrogen becomes chiral by coordination to a metal ion, it is theoretically possible to obtain three stereoisomers: enantiomers (*R,R*) and (*S,S*) and diastereoisomer (*R,S*)≡(*S,R*). These isomers can be similar in energy and two or all three could be obtained. NMR spectra of palladium(II) and platinum(II) complexes with analogue ligands (Fig. 1A, B) showed formation of two isomers [34,38], (*R,R*)-*N,N′* and (*R,S*)-*N,N′*. This observation was confirmed by DFT calculations [41]. Platinum(IV) complexes with bulky ligands (Fig. 1C), prefer (*S,S*)-*N,N′* configuration [36], but with (*S,S*)-R_2edda-type ligands shown in Fig. 1A, B only (*R,R*)-*N,N′* isomers [34] were obtained.

Herein, we report synthesis of six novel palladium(II) complexes, **1**–**6**, with R_2edda-type esters ((S,S)-R_2edda-type: (*S,S*)-eddch = (*S,S*)-ethylenediamine-*N,N′*-di-2-(3-cyclohexyl)propanoate, R = Me, Et, *n*-Pr, **1**–**3**; (*S,S*)-pddch = (*S,S*)-propylenediamine-*N,N′*-di-2-(3-cyclohexyl)propanoate, R = Et, *n*-Pr, **4**, **5**; and (*S,S*)-eddip = (*S,S*)-ethylenediamne-*N,N′*-di-2-propanoate, R = *i*-Am, **6**). Complexes were characterized by IR, NMR spectroscopy, ESI-MS and elemental analysis. DFT calculations were performed to support experimental findings. In addition, cytotoxicity study was performed for complexes **1**–**6** against human cervical adenocarcinoma (HeLa), human alveolar basal adenocarcinoma (A549) and non-cancerous human fetal lung fibroblast (MRC-5) cell lines.

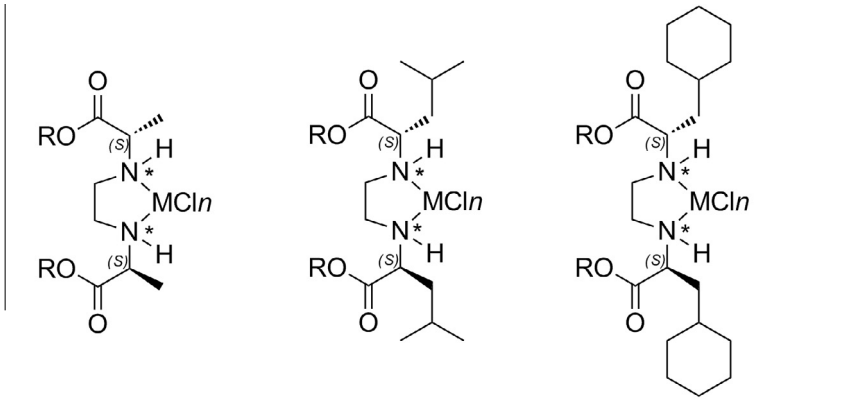
2. Experimental

2.1. Materials and measurements

$K_2[PdCl_4]$ is synthesized according to the literature method [42]. (*S*)-2-amino-3-cyclohexyl-propanoic acid hydrochloride was purchased from Senn Chemicals (Dielsdorf, Switzerland). (*S,S*)-ethylenediamine-*N,N′*-di-2-(3-cyclohexyl)propanoic acid dihydrochloride, (*S,S*)-propylenediamine-*N,N′*-di-2-(3-cyclohexyl)propanoic acid dihydrochloride, (*S,S*)-ethylenediamine-*N,N′*-di-2-propanoic acid and corresponding esters from this work, were obtained as described in literature [34,36]. Solvents were obtained commercially and used without further purification. Elemental analysis was carried out with Elemental Vario EL III microanalyzer. Infrared spectra were recorded on a Nicolet 6700 FT-IR spectrometer using ATR technique. The NMR spectra were recorded on a Varian Gemini 200 instrument. Chemical shifts for ^{1}H and ^{13}C spectra were referenced to residual ^{1}H and ^{13}C present in deuterated chloroform and DMSO. ESI–MS were carried out with a 6210 Time-of-Flight LC-MS instrument (G1969A, Agilent Technologies) in acetonitrile with addition formic acid for **2**, **3** and **5** and in methanol for **6**. Mass spectra of **1** and **4** were carried out with a Orbitrap LTQ XL instrument (Thermo Scientific, Bremen, Germany) in 95% acetonitrile in water, with addition formic acid.

2.2. Synthesis of dichlorido(*O,O′*-dialkyl-(*S,S*)-ethylenediamine-*N,N′*-di-2-(3-cyclohexyl)propanoate)palladium(II) complexes, *1*–*3*

Suspensions containing 0.31 mmol of each ligand precursor [(*S,S*)-H_2R_2eddch]Cl_2, R = Me (0.144 g), or Et (0.153 g), or *n*-Pr (0.161 g), respectively, were added in 10 mL of methanol and 0.62 mmol of $LiOH{\cdot}H_2O$ (0.026 g) was added. Suspension was stirred on ultrasound bath for a few minutes, until it became a pellucid solution. The solutions of ligands were slowly added to water solutions (10 mL) of the starting palladium complex (0.31 mmol, 0.10 g). After addition of full quantity of the ligand solutions,



[MCl_n{(*S,S*)-R_2eddip}]

M	Pd(II)	Pt(II)	Pt(IV)
n	2	2	4
R	*n*-Pr, *n*-Bu, *n*-Pe, *i*-Pr, *i*-Bu, *c*-Pe, Cy[a,b]		

A

[MCl_n{(*S,S*)-R_2eddl}]

M	Pd(II)	Pt(II)	Pt(IV)
n	2	2	4
R	Et, *n*-Pr, *n*-Bu, *n*-Pe, *i*-Pr[a,b], *i*-Bu[a,b]		

B

[MCl_n{(*S,S*)-R_2eddch}]

M	Pt(IV)	Au(III)
n	4	2
R	Me, Et, *n*-Pr, *n*-Bu, *i*-Bu[b], *i*-Am[b]	

C

Fig. 1. Complexes of metal ions with (*S,S*)-R_2edda-type ligands (Synthesized metal complexes with exception of: [a]platinum(II), [b]platinum(IV)).