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ORIGINAL ARTICLE

Cytotoxic effects of palladium(II) and platinum(II) complexes with *O,O'*-dialkyl esters of (*S,S*)-ethylenediamine-*N,N'*-di-2-(4-methyl) pentanoic acid on human colon cancer cell lines

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Summary

Purpose: As novel therapeutic agents relevant to colon cancer therapy are explored continuously, we tested 4 R_2edda-type ligand precursors *O,O'*-dialkyl esters of (*S,S*)-ethylenediamine-*N,N'*-di-2-(4-methyl)pentanoic acid (L1·2HCl–L4·2HCl) and corresponding palladium(II) and platinum(II) complexes against the human colon cancer cell lines CaCo-2, SW480 and HCT116.

Methods: The effects of the tested compounds on cell viability were determined using MTT colorimetric technique.

Results: Analysis of cancer cell viability showed that all tested ligand precursors, palladium(II) and platinum(II) complexes were cytotoxic on human colon cancer cells in dose-dependent manner. The cytotoxic activity of all palladium(II) and platinum(II) complexes toward selected cancer cells was significantly higher in comparison to cisplatin. Among the tested platinum(II) and palladium(II) complexes the lowest activity was observed for the compounds with the shortest ester chain and the highest activity was noted for palladium(II) complex No.2 with the n-Pr group in ester chain and for platinum(II) complex No.7 with the n-Bu group in ester chain.

Conclusion: Palladium(II) complex No.2 and platinum(II) complex No.7 seem to be good candidates for future pharmacological evaluation in the field of colon cancer research and treatment.

Key words: cytotoxic effects, human colon cancer cell lines, palladium(II) complexes, platinum(II) complexes

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Introduction

Colorectal cancer is the 3rd most common cancer in women and the 4th most common cancer in men worldwide [1]. Although novel molecular pathways relevant to colon cancer biology and colon cancer therapy are explored continuously [2-4], it is expected that a whole array of new agents should be tested in combination or in sequence to standard chemotherapy with the aim to improve the outcome of colon cancer patients.

Cisplatin is used against diverse tumor types including testicular, ovarian, head and neck, bladder, esophageal, and small lung cancer cells [5]. However, cisplatin exhibits only limited activity against tumors like colon and breast cancer, and in time resistance frequently occurs [6]. The next generation of platinum(II)-based drugs used in the clinical treatments includes carboplatin, with similar cytotoxicity but less side effects than cisplatin, and oxaliplatin, with antiproliferative effects even in cancers insensitive to cisplatin (for example, advanced colorectal tumors [7]). Because of the proven cytotoxicity of these platinum (II) compounds, a great number of new platinum complexes are continuously being prepared and tested for antitumor activity [8,9].

Recently, we and others reported the synthesis and characterization of the palladium(II) and platinum(II/IV) complexes with R_2edda-type esters of (*S,S*)-ethylenediamine-*N,N*'-di-2-propanoic acid dihydrochloride, (*S,S*)-R_2eddip·2HCl [10-13]. An antiproliferative activity of these complexes was determined *in vitro* against several tumor cell lines: human adenocarcinoma (HeLa) cells, human malignant melanoma (Fem-x) cells, human myelogenous leukemia (K562) cells and normal immunocompetent cells, such as human peripheral blood mononuclear cells (PBMC) [10-13].

As continuation of our work, we report herein the *in vitro* cytotoxic activity of *O,O*'-diethyl-(*S,S*)-ethylenediamine-*N,N*'-di-2-(4-methyl)-pentanoate dihydrochloride ligand precursors (aklyl = diethyl, L1·2HCl; propyl, L2·2HCl; dibutyl, L3·2HCl; dipentyl, L4·2HCl), their corresponding palladium(II) and platinum(II) complexes against human cancer colon cell lines CaCo-2, SW480 and HCT116.

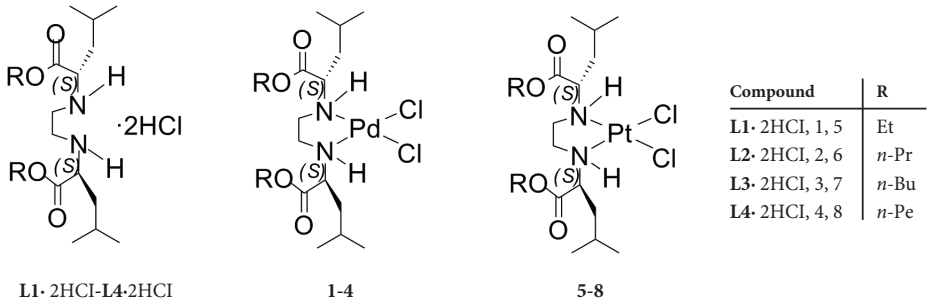
Methods

Chemicals and ligands

Dialkyl esters of (*S,S*)-ethylenediamine-*N,N*'-di-2-(4-methyl)pentanoic acid dihydrochloride, (L1·2HCl–L4·2HCl), corresponding palladium(II) (labelled as No.1–4) and platinum(II) complexes (labeled as No.5–8) (Figure 1) were prepared as previously described [14,15]. The structure and purity of the samples was confirmed by ^{1}H and ^{13}C NMR spectroscopy; ^{1}H and ^{13}C NMR spectra were recorded by a Varian "Gemini 2000" (200 MHz) spectrometer in $CDCl_3$ using tetramethylsilane as internal standard.

Cell culture

CaCo-2 and SW480 cells were purchased from the American Type Culture Collection (ATCC, Manassas, USA). HCT-116 cells were kindly provided by Dr Danijela Vignjevic (Institute Curie, Paris, France). All cell lines were maintained in RPMI 1640 (Sigma Aldrich, Munich, Germany) supplemented with 10% fetal bovine serum (FBS, Sigma), penicillin (100 IU/mL), streptomycin (100 μg/mL), and in a humidified



L1· 2HCl-L4·2HCl 1-4 5-8

Compound	R
L1· 2HCl, 1, 5	Et
L2· 2HCl, 2, 6	*n*-Pr
L3· 2HCl, 3, 7	*n*-Bu
L4· 2HCl, 4, 8	*n*-Pe

Figure 1. Synthesized esters L1.2HCl–L4.2HCl, palladium(II) (1–4) and platinum(II) complexes (5–8).

Table 1. IC_{50} (μM)* for the 72 h of action of the investigated compounds, ligand precursors, palladium (II) and platinum (II) complexes on CaCo-2, SW480 and HCT116 cell lines, as determined by MTT assay. The cytotoxic activity of all tested palladium (II) and platinum (II) complexes were significantly higher ($p<0.05$) in comparison to cisplatin

Compound	*CaCo-2*	*HCT116*	*SW480*
L1·2HCl	70.02 ± 13.15	113.47 ± 22.34	120.37 ± 5.38
L2·2HCl	42.68 ± 4.37	72.49 ± 15.48	107.97 ± 0.80
L3·2HCl	37.01 ± 2.97	7.00 ± 5.17	42.66 ± 9.77
L4·2HCl	28.23 ± 9.24	8.75 ± 2.05	25.83 ± 4.57
1	5.94 ± 0.50	33.01 ± 8.92	121.16 ± 35.75
2	5.10 ± 1.31	5.10 ± 0.06	4.69 ± 2.32
3	6.01 ± 0.91	9.15 ± 4.20	80.33 ± 16.32
4	1.50 ± 0.05	10.57 ± 3.55	6.57 ± 4.29
5	54.27 ± 13.07	21.29 ± 7.69	20.70 ± 3.47
6	19.57 ± 8.65	8.20 ± 7.22	23.11 ± 9.16
7	11.23 ± 2.47	5.09 ± 2.06	4.02 ± 1.53
8	17.62 ± 0.40	8.42 ± 2.91	21.59 ± 2.74
Cisplatin	161.25 ± 12.61	51.64 ± 23.29	64.74 ± 5.31

*mean values ± standard deviation from experiments

atmosphere of 95% air/5% CO_2 at 37°C. Cell number and viability were determined by trypan blue staining.

Cytotoxicity assays

The effects of the tested compounds on cell viability were determined using MTT colorimetric technique [16].

CaCo-2, SW480 and HCT116 cells were diluted with RPMI medium to $5´10^4$ cells/ml and aliquots ($5x10^3$ cells/100 ml) were placed in individual wells in 96-multiplates. The next day the medium was exchanged with 100μL of different compounds, which had been serially diluted 2-fold in the medium to concentrations ranging from 500 μM to 3.9 μM in RPMI 1640 medium. Each compound was tested in triplicate. Cells were incubated at 37°C in a 5% CO_2 for 72 h. After incubation the supernatant was removed and MTT solution (5 mg/mL in PBS, 10 μL) was added to each well. After an additional 4 h of incubation at 37°C in a 5% CO_2, the medium with MTT was removed and DMSO (150 μL) with glycine buffer (20 μL) was added to dissolve the crystals. The plates were shaken for 10 min. The optical density of each well was determined at 595 nm. The percentage of cytotoxicity was calculated using the formula: % cytotoxicity = 100-((TS-BG0) - E/(TS-BG0)´100), where "BG0" stands for background of medium alone, "TS" for total viability/spontaneous death of untreated target cells, and "E" for experimental well.

Results

Analysis of cancer cell viability showed that all tested platinum(II) and palladium(II) complexes were cytotoxic to human colon carcinoma cells CaCo-2, SW480 and HCT-116 (Figures 2-4). The cytotoxic effect was dose-dependent: the decrease of concentration of the tested complexes was followed by markedly increase of tumor cell viability.

Analysis of IC_{50} values showed that palladium (II) complex No.2 and platinum complex No.7 were the most cytotoxic towards CaCo-2, SW480 and HCT-116 cells (Table 1). Among all complexes, the best cytotoxic effects on HCT-116 cells were achieved

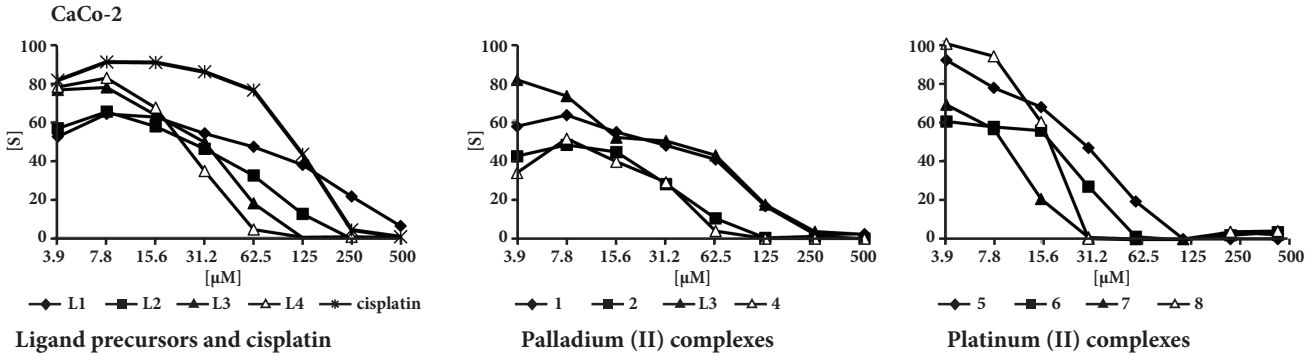


Figure 2. Representative graphs and IC_{50} values of CaCo-2 cell survival after 72 h cell growth in the presence of palladium(II), platinum(II) complexes, ligand precursors and cisplatin.

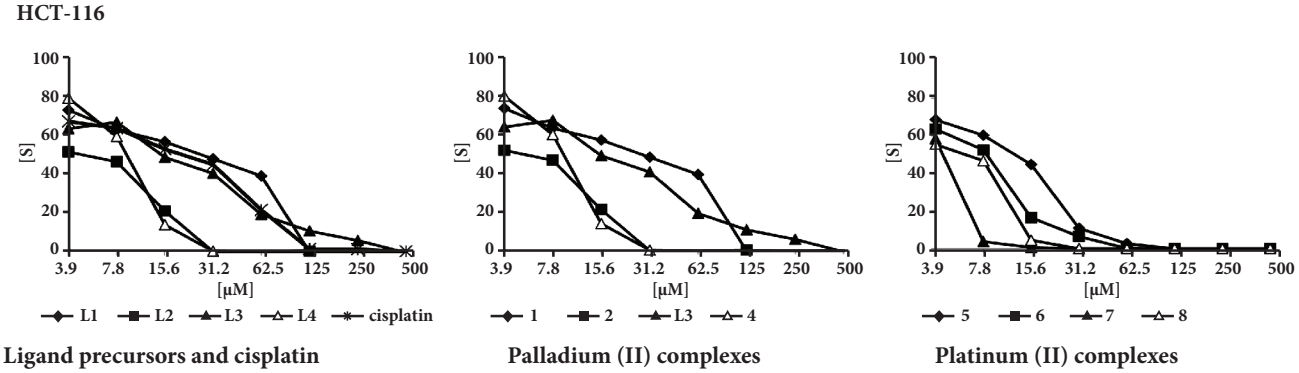


Figure 3. Representative graphs and IC_{50} values of HCT-116 cell survival after 72 h cell growth in the presence of palladium(II), platinum(II) complexes, ligand precursors and cisplatin.

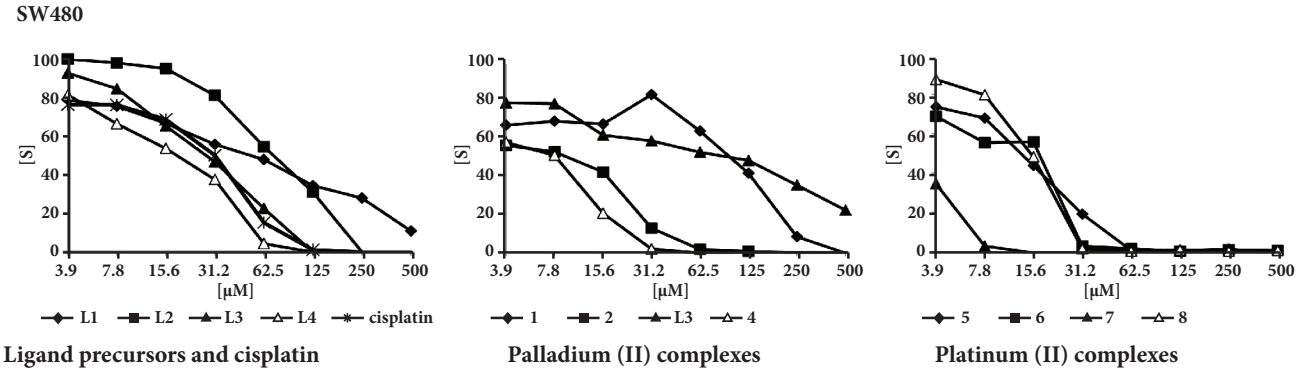


Figure 4. Representative graphs and IC_{50} values of SW-480 cell survival after 72 h cell growth in the presence of palladium(II), platinum(II) complexes, ligand precursors and cisplatin.

by the palladium(II) complex No.2 (IC_{50} = 1.94) and platinum(II) complex No.7 (IC_{50} = 5.09). In comparison with other platinum(II) complexes, platinum(II) complex No.7 showed similar cytotoxicity on SW480 and CaCo-2 cells at concentrations ranging from 500 μM to 250 μM (Figures 2 and 4). Nevertheless, at concentrations from 31.25 μM to 3.9 μM, platinum(II) complex No.7 was significantly

more cytotoxic on SW480 (IC_{50}=4.02) and CaCo-2 cells (IC_{50}=11.23) than other tested platinum(II) complexes. The palladium(II) complexes were cytotoxic on SW480 cells at concentrations ranging from 500 μM to 250 μM (Figure 4). Concerning SW480 cells, the palladium(II) complexes No.2 and 4 were more cytotoxic than complexes No.1 and 3 at concentrations ranging from 125 μM to 31.25 μM, while palladium(II) complex No.3 was the most cytotoxic at concentrations from 31.25 μM to 7.8 μM (Figure 4). All platinum(II) and palladium(II) complexes were more cytotoxic than cisplatin on all target human colon carcinoma cell lines (Figures 2-4).

All of the tested ligand precursors were cytotoxic on CaCo-2, SW480 and HCT-116 cells at concentrations ranging from 500 μM to 62.5 μM (Figures 2-4). However, concentration decrease resulted in significant decrease of the cytotoxic effect of these ligands. In comparison with other ligand precursors, L4·2HCl was significantly more cytotoxic on CaCo-2 and SW480 cells than other tested ligand precursors (Figures 2 and 4), while similar cytotoxic effects were noticed on HCT-116 cells after treatment with L3·2HCl and L4·2HCl (Figure 3).

Discussion

This study demonstrated for the first time that 4 newly synthesized R_2edda-type ligand precursors *O,O'*-dialkyl esters of (*S,S*)-ethylenediamine-*N,N'*-di-2-(4-methyl)pentanoic acid (L1·2HCl–L4·2HCl), their corresponding palladium(II) and platinum(II) complexes exhibit relevant cytotoxic properties on 3 different human cancer colon cell lines: CaCo-2, SW480 and HCT116. The CaCo-2 cell line is a continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells [17], HCT-116 cell line is an adherent epithelial cell line originating from human colorectal carcinoma [18], while SW480 cell line was established from a primary adenocarcinoma of the colon [19]. Importantly, all tested palladium(II) and platinum(II) complexes were more cytotoxic than cisplatin on target tumor cells (Figures 2-4). The cytotoxicity was dose-dependent: decrease of concentration was followed by markedly increase of tumor cell viability.

From the investigated platinum(II) complexes the lowest activity was observed for the compound with the shortest ester chain (platinum(II) complex No.5). Changing Et (platinum(II) complex No.5) with n-Pr (platinum(II) complex No.6), n-Bu group (platinum(II) complex No.7) and n-Pe group (platinum(II) complex No.8) in the ester chain of the platinum(II) complexes significantly increased cytotoxic activity against all tested colon cancer cell lines (Figures 2-4). The platinum(II) complex No.8, having n-Pe group in the ester chain, showed similar activity as platinum(II) complex No.6 but it was significantly less cytotoxic than platinum(II) complex No.7. Thus, the highest activity was noted for platinum(II) complex No.7 with the n-Bu group in the ester chain. It may be possible that the highest activity of this complex could be associated with the higher intercellular accumulation which was found and recently reported [20]. It is interesting to observe that the increased length of the ligands alkyl side chain is apparently associated with the higher activity of platinum(II) complexes No.6,7 and 8 relative to the complex No.5. Thus, it seems that the impact of larger (S,S)-R2eddl ligand coordinated to dichloroplatinum(II) moiety has positive influence on the *in vitro* anti-tumor activity of these complexes against selected human cancer colon cell lines.

From the tested palladium(II) complexes the lowest activity was observed for the compound with the shortest ester chain (palladium(II) complex No.1). Changing Et (palladium(II) complex No.1) with n-Pr (palladium(II) complex No.2), n-Bu group (palladium(II) complex No.3) and n-Pe group (palladium (II) complex No.4) in the ester chain of the palladium(II) complexes significantly increased the cytotoxic activity against all of the tested colon cancer cell lines (Figures 2-4). Among all palladium(II) complexes, the highest activity was noted for complex No.2 with the n-Pr group in the ester chain.

By coordination of ligands to dichloropalladium(II) moiety, an increase in cytotoxicity has been observed. When platinum(II) is exchanged with palladium(II) ion, higher cytotoxic activity is achieved against CaCo-2, SW480 and HCT116 cells. The IC_{50} value of palladium(II) complexes is 2-4 times higher than

that of the corresponding platinum(II) complexes (Table 1). The coordination mode of palladium(II) and platinum(II) is analogous, and due to the similar coordination modes and chemical properties of palladium(II) and platinum(II) compounds, both complexes showed similar, moderate or high, cytotoxic activity against several cancer cell lines [13-15,21].

Some structural relationships for the cytotoxicity of palladium(II) and platinum(II) complexes could be observed: the *in vitro* cytotoxic activity is increasing in the following order L·2HCl ≤ $[PtCl_2L]$ ≤ $[PdCl_2L]$ (L = L1–L4). These results are in accordance with our previous published study which investigated the cytotoxic activity of the ligand precursors L1·2HCl–L4·2HCl and the corresponding palladium(II) and platinum(II) complexes against chronic lymphocytic leukemia (CLL) cells [14,15].

At the end, it should be emphasized that the activity of all tested platinum(II) and palladium(II) complexes toward selected human cancer colon cells were significantly higher in comparison to cisplatin (Figures 2-4). The low activity of cisplatin against colon carcinoma cells is in agreement with earlier reports [6].

In conclusion, the cytotoxic activity of 4 R_2edda-type ligand precursors *O,O'*-dialkyl esters of (*S,S*)-ethylenediamine-*N,N'*-di-2-(4-methyl)pentanoic acid (L1·2HCl–L4·2HCl), palladium(II) and platinum(II) complexes against the human cancer colon cell lines CaCo-2, SW480 and HCT116, palladium(II) complex No.2 and platinum(II) complex No.7 showed the best cytotoxic effects and their cytotoxicity was significantly higher than cisplatin. In line with the obtained results, these complexes seem to be good candidates for future pharmacological evaluation in the field of colon cancer research and treatment.

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