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# PART XVI – STEREOSPECIFIC LIGANDS AND THEIR COMPLEXES

## SYNTHESIS, CHARACTERIZATION AND *IN VITRO* ANTIPROLIFERATIVE ACTIVITY OF NEW PLATINUM(IV) COMPLEXES WITH SOME *O,O'*-DIALKYL ESTERS OF (*S,S*)-ETHYLENEDIAMINE-*N,N'*-DI-2-PROPANOIC ACID AGAINST BREAST CANCER (MDA-MB-231) AND COLON CANCER (HCT-116 AND SW-480) CELL LINES

**Verica V. Jevtić[1], Gordana P. Radić[1], Dragana S. Šeklić[2], Marko N. Živanović[2], Snežana D. Marković[2], Srećko R. Trifunović[1*]**

[1]*Department of Chemistry, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Republic of Serbia*
[2]*Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Republic of Serbia*
srecko@kg.ac.rs

Synthesis of four new platinum(IV) complexes (C1–C4) with bidentate *N,N'*-ligand precursors, *O,O'*-dialkyl esters (L1–L4) (alkyl = ethyl, propyl, butyl and pentyl) of (*S,S*)-ethylenediamine-*N,N'*-di-2-propanoic acid, $H_2$-*S,S*-eddp were reported. The platinum(IV) complexes were characterized by elemental analysis and their structures determined on the basis of infra-red, $^1$H and $^{13}$C NMR spectroscopy. Cytotoxicity against human breast carcinoma MDA-MB-231, human colon carcinoma HCT-116 and SW-480 tumour cell lines was determined using the MTT assay, which indicated the larger the size of the complex or ligand, the greater the cytotoxicity.

**Keywords***:* platinum(IV) complexes; *O,O'*-dialkyl esters; antiproliferative activity; MTT test

# XVI ДЕЛ – СТЕРЕОСПЕЦИФИЧНИ ЛИГАНДИ И НИВНИ КОМПЛЕКСИ

## СИНТЕЗА, КАРАКТЕРИЗАЦИЈА И *IN VITRO* АНТИПРОЛИФЕРАТИВНА АКТИВНОСТ НА НОВИ КОМПЛЕКСИ НА ПЛАТИНА(IV) СО НЕКОИ *O,O'*-ДИАЛКИЛ ЕСТЕРИ НА (*S,S*)-ЕТИЛЕНДИАМИН-*N,N'*-ДИ-2-ПРОПАНСКА КИСЕЛИНА ПРОТИВ КЛЕТОЧНИ КУЛТУРИ НА РАК НА ДОЈКА (MDA-MB-231) И РАК НА ДЕБЕЛОТО ЦРЕВО (HCT-116 И SW-480)

Синтетизирани се четири нови комплекси на платина(IV) (**C1–C4**) со бидентатни *N,N'*-лиганди прекурзори, *O,O'*-диалкилестери (**L1–L4**) (алкил = етил, пропил, бутил и пентил) на (*S,S*)-етилендиамин-*N,N'*-ди-2-пропанска киселина, $H_2$-*S,S*-eddp. Комплексите на платина(IV) се карактеризирани со помош на инфрацрвена, $^1$H и $^{13}$C NMR спектроскопија. Цитотоксичноста против култури на туморни клетки на хуман карцином на дојка MDA-MB-231, хуман карцином на дебелото црево HCT-116 и SW-480 се определени со тестот MTT, кој покажа дека колку се поголеми димензиите на лигандот толку е поголема цитотоксичноста.

**Клучни зборови**: комплекси на платина(IV); *O,O'*-диалкилестери; антипролиферативна активност; тест MTT

## 1. INTRODUCTION

The impressive impact of cisplatin on cancer cells [1–8] had a direct effect on the development of new derivatives with improved pharmaceutical properties. Cisplatin has become the prototype for a unique class of antineoplastic agents [8] although its usage is limited due to nephrotoxicity, emetogenic properties and neurotoxicity [9, 10].

There is a growing interest in platinum(IV) complexes due to their high activity and lower toxicity than platinum(II) complexes. Also, platinum(IV) complexes are kinetically more inert than platinum(II) complexes, which allows them to be administered orally, reducing toxicity during platinum-based chemotherapy. Recently, several platinum(IV) complexes containing alkyl esters of edda-type ligands (edda = ion of ethylenediamine-*N*,*N'*-diacetic acid) have been tested against different cancer cells [11–18], which indicated these complexes exhibited significantly greater cytotoxicity than cisplatin. These studies also brought to the forefront the possibility for the synthesis of platinum(IV)/alkyl ester complexes.

In this study, the synthesis, characterization and antiproliferative effects of tetrabromido(*O*,*O'*-diethyl-(*S*,*S*)-ethylenediamine-*N*,*N'*-di-2-propanoate)-platinum(IV)(**C1**), tetrabromido(*O*,*O'*-dipropyl-(*S*,*S*)-ethylenediamine-*N*,*N'*-di-2-propanoate)platinum(IV) (**C2**), tetrabromido(*O*,*O'*-dibutyl-(*S*,*S*)-ethylenediamine-*N*,*N'*-di-2-propanoate)platinum(IV)(**C3**), tetrabromido(*O*,*O'*-dipentyl-(*S*,*S*)-ethylenediamine-*N*,*N'*-di-2-propanoate)platinum(IV) (**C4**) complexes were explored.

## 2. EXPERIMENTAL

### 2.1. *Reagents and measurements*

Potassium hexabromidoplatinate(IV) was obtained from Merck and used without further purification. (*S*,*S*)-ethylenediamine-*N*,*N'*-di-2-propanoic acid ($H_2$-*S*,*S*-eddp) was prepared [19] and esterified as previously described [20, 21]. Where required, distilled water was used.

Elemental analyses for C, H and N were recorded on a Vario *III* CHNS Elemental Analyser and water content determined thermogravimetrically. Infrared spectra were recorded by a Perkin-Elmer Spectrum One FTIR spectrophotometer using the KBr pellet technique (4000–400 $cm^{-1}$), and $^1H$ and $^{13}C$ NMR spectra recorded by a Varian "Gemini 2000" (200 MHz) spectrometer in $CDCl_3$ using tetramethylsilane as the internal standard.

#### 2.1.1. *Preparation of tetrabromido*(*O*,*O'*-*diethyl*-(*S*,*S*)-*ethylenediamine-N*,*N'*-*di-2-propanoate*)*platinum*(*IV*), [*$PtBr_4$*(*det*-(*S*,*S*)-*eddp*)] (***C1***)

$K_2[PtBr_6]$ (0.100 g, 0.133 mmol) was dissolved in 10 mL of water in a steam bath and *O*,*O'*-diethyl-(*S*,*S*)-ethylenediamine-*N*,*N'*-di-2-propanoate dihydrochloride, det-*S*,*S*-eddp·2HCl, (0.0443 g, 0.133 mmol) was added. The mixture was stirred for 6 h and an aqueous solution of LiOH (0.0064 g, 0.266 mmol in 10 mL of water) was introduced. The solution was then filtered and evaporated in a steam bath until a precipitate appeared. After cooling, [$PtBr_4$(det-*S*,*S*-eddp)] (**C1**) as a yellow precipitate, was filtered, washed with ethanol and ether and air-dried. Yield: 0.0599 g (58.14%). *Anal.* Calcd. for $C_{12}H_{24}Br_4N_2O_4Pt$ ($M_r$ = 775.032) (%): C, 18.60; H, 3.12; N, 3.62; $H_2O$, 0.00. Found: C, 19.00; H, 3.22; N, 3.69; $H_2O$, 0.11%. $^1H$ NMR (200 MHz, $CDCl_3$, $\delta$ ppm): 1.34 (t, 6H, $CH_3$-Et), 1.65 (d, 6H, $CH_3$), 2.89 (m, 4H, $CH_2$), 4.35 (q, 2H, CH), 4.92 (q, 4H, $CH_2$-Et). 6.75-6.90 (broad s, 2H, NH). $^{13}C$ NMR (50 MHz, $CDCl_3$, $\delta$ ppm): 13.94 ($CH_3$-Et), 15.83 ($CH_3$), 50.08 ($CH_2$), 63.50 (CH), 71.24 ($CH_2$-Et) 172.21 (COO-Et). IR ($cm^{-1}$): 3128 (s), 2994 (w), 1730 (s), 1451 (w), 1322 (m), 1255 (s), 1205 (m), 1106 (m), 1014 (m), 745 (w).

#### 2.1.2. *Preparation of tetrabromido*-(*O*,*O'*-*dipropyl*-(*S*,*S*)-*ethylenediamine-N*,*N'*-*di-2-propanoate*)*platinum*(*IV*), [*$PtBr_4$*(*dpr-S*,*S-eddp*)]

$PtBr_4$(dpr-*S*,*S*-eddp) (**C2**) was prepared as for **C1**, using *O*,*O'*-dipropyl-(*S*,*S*)-ethylenediamine-*N*,*N'*-di-2-propanoate dihydrochloride trihydrate, dpr-*S*,*S*-eddp·2HCl·3$H_2O$, (0.0553 g, 0.133 mmol). Yield: 0.0640 g (59.93%). *Anal.* Calcd. for $C_{14}H_{28}Br_4N_2O_4Pt$ ($M_r$ = 803.084): C, 20.94; H, 3.51; N, 3.49; $H_2O$, 0.00. Found: C, 21.26; H, 3.52; N, 3.56; $H_2O$, 0.10%. $^1H$ NMR (200 MHz, $CDCl_3$, $\delta$ ppm): 0.97 (t, 6H, $CH_3$-*n*--Pr), 1.23 (d, 6H, $CH_3$), 1.71 (m, 4H, $CH_2$-*n*-Pr), 3.44 (m, 4H**,** $CH_2$), 4.23 (q, 2H, CH), 4.93 (t, 4H, $CH_2$-*n*-Pr), 6.70–6.95 (broad s, 2H, NH). $^{13}C$ NMR (50 MHz, $CDCl_3$, $\delta$ ppm): 10.31 ($CH_3$-*n*-Pr), 15.94 ($CH_3$), 21.68 ($CH_2$-*n*-Pr), 50.12 ($CH_2$), 59.86 (CH), 68.97 ($CH_2$-*n*-Pr), 172.36 (COO-*n*-Pr). IR ($cm^{-1}$): 3125 (m), 2966 (w), 1733 (s), 1454 (w), 1396 (m), 1317 (m), 1255 (s), 1202 (m), 1107 (s), 1048 (m), 942 (w), 745 (w).

#### 2.1.3. *Preparation of tetrabromido*-(*O*,*O'*-*dibutyl*-(*S*,*S*)-*ethylenediamine-N*,*N'*-*di-2-propanoate) platinum*(*IV*), [*$PtBr_4$*(*dbu-S*,*S-eddp*)]

$PtBr_4$(dbu-*S*,*S*-eddp) (**C3**) was prepared as for **C1**, using *O*,*O'*-dibutyl-(*S*,*S*)-ethylenediamine-

*N,N'*-di-2-propanoate dihydrochloride trihydrate, dbu-*S,S*-eddp·2HCl·3$H_2O$, (0.0589 g, 0.133 mmol). Yield: 0.0646 g (58.48%). *Anal.* Calcd. for $C_{16}H_{32}Br_4N_2O_4Pt$ ($M_r$ = 831.136): C, 23.12; H, 3.88; N, 3.37; $H_2O$, 0.00. Found: C, 23.41; H, 3.86; N, 3.41; $H_2O$, 0.09%. $^1$H NMR (200 MHz, $CDCl_3$, $\delta$ ppm): 0.94 (t, 6H, $CH_3$-*n*-Bu), 1.21 (d, 6H, $CH_3$), 1.31 (m, 4H, $CH_2$-*n*-Bu), 1.68 (m, 4H**,** $CH_2$-*n*-Bu), 2.87 (m, 4H, $CH_2$), 4.25 (q, 2H, CH), 4.93 (t, 4H, $CH_2$-*n*-Bu), 6.75-6.90 (broad s, 2H, NH). $^{13}$C NMR (50 MHz, $CDCl_3$, $\delta$ ppm): 13.61 ($CH_3$-*n*-Bu), 15.84 ($CH_3$), 19.03 ($CH_2$-*n*-Bu), 30.25 ($CH_2$), 50.02 ($CH_2$-*n*-Bu), 59.81 (CH), 67.32 ($CH_2$-*n*-Bu), 172.37 (COO-*n*-Bu). IR ($cm^{-1}$): 3119 (m), 2961 (m), 2872 (w), 1733 (s), 1455 (m), 1396 (m), 1316 (m), 1255 (m), 1201 (m), 1107 (m), 1048 (m), 943 (w), 745 (w).

### 2.1.4. *Preparation of tetrabromido-*(*O,O'-dipentyl-*(*S,S*)*-ethylenediamine-N,N'-di-2-propanoate*) *platinum*(*IV*), [*$PtBr_4$*(*dpe-S,S-eddp*)]

$PtBr_4$(dpe-*S,S*-eddp) (**C4**) was prepared as for **C1**, using *O,O'*-dipentyl-(*S,S*)-ethylenediamine-*N,N'*-di-2-propanoate dihydrochloride dihydrate, dpe-*S,S*-eddp·2HCl·2$H_2O$, (0.0603 g, 0.133 mmol). Yield: 0.0595 g (52.11%). *Anal.* Calcd. for $C_{18}H_{36}Br_4N_2O_4Pt$ ($M_r$ = 859.188): C, 26.16; H, 4.22; N, 3.26; $H_2O$, 0.00. Found: C, 26.39; H, 4.29; N, 3.18; $H_2O$, 0.08%. $^1$H NMR (200 MHz, $CDCl_3$, $\delta$ ppm): 0.91 (t, 6H, $CH_3$-*n*-Pe), 1.19 (d, 6H, $CH_3$), 1.29 (m, 4H, $CH_2$-*n*-Pe), 1.41 (m, 4H, $CH_2$-*n*-Pe), 1.59 (m, 4H, $CH_2$-*n*-Pe), 2.87 (m, 4H, $CH_2$), 3.74 (q, 2H, CH), 4.24 (t, 4H, $CH_2$-*n*-Pe), 6.90–7.25 (broad s, 2H, NH). $^{13}$C NMR (50 MHz, $CDCl_3$, $\delta$ ppm): 13.94 ($CH_3$-*n*-Pe), 15.80 ($CH_3$), 22.23 ($CH_2$-*n*-Pe), 27.91 ($CH_2$-*n*-Pe), 40.5 ($CH_2$), 59.5 ($CH_2$-*n*-Pe), 60.1 (CH), 67.3 ($CH_2$-*n*-Pe), 172.1 (COO-*n*-Pe). IR ($cm^{-1}$): 3130 (m), 2958 (s), 2931 (s), 2865 (m), 1735 (s), 1461 (m), 1389 (w), 1315 (m), 1255 (s), 1196 (s), 1110 (m), 1055 (m), 966 (w), 748 (w).

## 2.2. *In vitro studies*

### 2.2.1. *Cell preparation and culturing*

Cancer cell lines were propagated and maintained in DMEM (Dulbecco's Modified Eagle Medium; Gibco, Invitrogen, Carlsbad, CA, USA) supplemented with 10% foetal bovine serum (PAA, Pasching, Austria) and antibiotics (100 IU/ml penicillin and 100 μg/ml streptomycin) at 5% $CO_2$ and 37°C in a humidified atmosphere. MDA-MB-231 (breast cancer isolated from metastatic tissue), HCT-116 and SW-480 (colon cancer cell lines isolated from primary tumour tissue) were obtained from the American Tissue Culture Collection (Manassas, VA, USA). Cells were grown in 75 $cm^2$ culture flasks and after five passages, all tested cell lines were seeded into 96-well plate.

### 2.2.2. *Cell viability assay* (*MTT assay*)

After reaching the cell confluence of about 70 to 80% all cell viability studies were performed simultaneously. MDA-MB-231, HCT-116 and SW-480 cells were seeded in a 96-well plate ($10^4$ cells per well). Cells were allowed to adhere 24 h and then, cells were treated with 100 μl of each concentration (0.1, 1, 10, 50, 100 and 500 μM) for each compound. Cells untreated with investigated compounds served as the control, and have only been supplemented by replacement of growing medium. After 24 and 72 h of treatment, cell viability was determined by MTT assay according to the manufacturers' protocol. Briefly, medium with tested substances and control medium were replaced with 100 μl DMEM and 25 μl of 5 mg/ml MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) dissolved in PBS and the 96-well plates incubated at 37°C in 5% $CO_2$ for 2 h. After incubation, MTT medium was replaced with 150 μl of DMSO, and absorbance of coloured solution of formazan product was measured at 550 nm. Cell proliferation was calculated as the absorbance of the treated group divided by the absorbance of control group multiplied by 100 to yield percent proliferation [22].

## 2.3. *Statistical analysis*

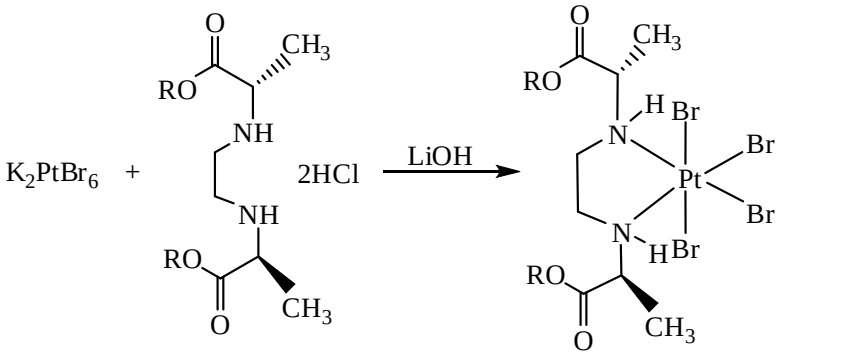
The data were expressed as the mean ± standard error (SE). Biological activity was the result of one individual experiment performed in triplicate for each dose. The magnitude of correlation between variables was calculated using the SPSS statistical software package (SPSS for Windows, ver. 17, 2008, Chicago, IL, USA). The effect of each extract was expressed as the $IC_{50}$ (inhibitory dose which inhibit 50% growth cells) and by the maximal effect magnitude for exposed cells. The $IC_{50}$ values were calculated from the dose curves using CalcuSyn software for Windows, ver. 2.0 (Biosoft, Cambridge, UK).

# 3. RESULTS AND DISCUSSION

## 3.1. *Synthesis and characterization*

$PtBr_4$($R_2$-*S,S*-eddp) complexes were synthesized by mixing an aqueous solution of $K_2[PtBr_6]$ and the corresponding esters (Scheme 1). The resulting

complexes were soluble in chloroform and dimethylsulfoxide, but not in water. The results of microanalysis confirmed the predicted content of the isolated complexes.




**Scheme 1.** The preparation of the $[PtBr_4(R_2\text{-}S,S\text{-eddp})]$ complexes

The IR spectra showed specific ν(C=O) absorption bands at 1730, 1733, 1733, 1734 $cm^{-1}$ (strong), (typical absorption for aliphatic esters), ν(C–O) bands at 1205, 1202, 1201, 1201 $cm^{-1}$ and ν($CH_3$) bands at 2968, 2967, 2961, 2961 $cm^{-1}$. The infrared spectra of the complexes confirmed the expected *N-N* coordination of the ligands to the platinum(IV) ion. Coordination via nitrogen ligand atoms could be proven by the presence of bands for secondary amino groups (3128 $cm^{-1}$ for **C1**, 3125 $cm^{-1}$ for **C2**, 3119 $cm^{-1}$ for **C3**, and 3118 for **C4**) and absence of the protonated secondary ammonium group, found in the IR spectra of the $R_2$-*S,S*-eddp precursors (3437 $cm^{-1}$ for **C1**, 3461 $cm^{-1}$ for **C2**, 3461 $cm^{-1}$ for **C3** and 3462 $cm^{-1}$ for **C4**) [20, 21].

The position and number of signals in the $^1H$ and $^{13}C$ NMR spectra for the isolated complexes confirmed the coordination of the esters to the platinum(IV) ion. In the $^1H$ NMR spectra of **C1**–**C4**, hydrogen atoms of secondary amino groups showed broad signals at 6.70-7.25 ppm. Coordination-induced shifts in the **C1–C4** spectra (of up to 0.9 ppm) were attributed to the signals of the $CH_2$ protons of the ethylenediamine bridge which implied nitrogen coordination. In the $^{13}C$ NMR spectra, ester carbon atom resonances were found at the expected position (~170 ppm) for all compounds, which indicated that oxygen was not a ligating atom.

### 3.2. *Antiproliferative activity*

After cell seeding in standard DMEM medium, cells were exposed to different drug concentrations for 24 and 72 h at 37°C and MTT results are presented by $IC_{50}$ values shown in Table 1. By comparing the ligand antiproliferative effect especially on MDA-MB-231 cells (as well as on HCT-116 and SW-480 cells), the $IC_{50}$ values and MTT values indicated that cytotoxicity increased as the size of the ligand increased (ethyl, **L1** < propyl, **L2** < < buthyl, **L3** < pentyl, **L4**) though the sensitivity differed (SW-480 < HCT-116 < MDA-MB-231). The $IC_{50}$ values indicated that MDA-MB-231 cells were more sensitive to investigated ligands than HCT-116 and SW-480 cells because $IC_{50}$ for MDA-MB-231 for propyl and butyl ligands indicated values lower than 500 μM, while $IC_{50}$ for investigated colon cancer cell lines were greater than 500 μM for all ligands with exception of impact of L4 on HCT-116.

The complexes showed a similar trend for antiproliferative activity, i.e. the larger the complex, the greater the antiproliferative effect (**C1** < **C2** < **C3** < **C4)**. These data suggested activity was related to the R substituent in the (*S,S*)-$R_2$eddip, which was supported by published data indicating that increasing the number of C atoms in the chain increases cytotoxic activity [23]. By comparing the effects, the data indicated the complexes were not significantly more cytotoxic than the corresponding ligands, with the exception of **C4** complex activity on MDA-MB-231 cells (where complex was more cytotoxic), and **C3** and **C4** complexes on SW-480 cells (Table 1).

T a b l e 1

*$IC_{50}$ values (μM) for ligands and complexes on MDA-MB-231, HCT-116 and SW-480 cells after 24 and 72 h of exposure*

| MDA-MB-231 | 24 h | 72 h | HCT-116 | 24 h | 72 h | SW-480 | 24 h | 72 h |
|---|---|---|---|---|---|---|---|---|
| **L1** | > 500 | > 500 | **L1** | > 500 | > 500 | **L1** | > 500 | > 500 |
| **L2** | 272 | > 500 | **L2** | > 500 | > 500 | **L2** | > 500 | 200 |
| **L3** | > 500 | 158 | **L3** | > 500 | > 500 | **L3** | > 500 | > 500 |
| **L4** | 355 | 235 | **L4** | 225 | 74 | **L4** | > 500 | > 500 |
| **C1** | > 500 | > 500 | **C1** | > 500 | > 500 | **C1** | > 500 | > 500 |
| **C2** | > 500 | > 500 | **C2** | > 500 | > 500 | **C2** | > 500 | > 500 |
| **C3** | > 500 | > 500 | **C3** | > 500 | > 500 | **C3** | 344 | 58 |
| **C4** | 218 | 192 | **C4** | 225 | 363 | **C4** | 57 | 3 |

## 4. CONCLUSIONS

Four novel platinum(IV) complexes (**C1**–**C4)** were synthesized and characterized by IR, $^{1}$H NMR, $^{13}$C NMR spectroscopy, and elemental analysis. Antiproliferative effect of the investigated substances on cell lines of human colon (HCT-116 and SW-480) and breast (MDA-MB-231) cancer showed higher antiproliferative effect compared to the appropriate ligands, what is in agreement with our expectations that these complexes have moderate to high antiproliferative impact. As with ligands also with complexes, with increasing the size of investigated drug, i.e. with increasing the number of C-atoms in ligand side chains, the antiproliferative effect increases. By comparing of the obtained results we notice that the effect of tested substances is usually not acute, i.e. a significantly higher antiproliferative effect is observed after 72 h than after 24 h from treatment. Regarding the moderate antiproliferative activity of ligands, we conclude that the highest effect is achieved on MDA-MB-231 cells, while complexes showed the highest impact on SW-480 cells. As we discussed above, this effect is particularly obvious with complex **C4** (with pentyl ligand) with $IC_{50}$ values of 57 μM for 24 h and even of 3 μM for 72 h from treatment on SW-480 cell line. Such a significant anti-tumor *in vitro* activity shows once again that the Pt(IV) complexes are suitable for the synthesis of novel chemotherapeutic products with high antiproliferative effects on tumor tissue providing a low toxicity for healthy cells.

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