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ORIGINAL ARTICLE

Synthesis and antiproliferative activity of new carboplatin analogues

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Summary

Purpose: *The aim of this study was to present the synthesis and characterization of two carboplatin analogues and to investigate their antiproliferative activity against human tumor cell lines.*

Materials and methods: *The carboplatin analogues cis-1,2-propylendiammine (cyclobutane-1,1-dicarboxylato) platinum (II) (MD2), and cis- izobutylendiammine (cyclobutane -1,1-dicarboxylato)platinum (II) (MD3) were characterized by elemental analysis and ^{1}H-NMR-measurements. The compounds were tested for antiproliferative activity against the following human tumor cell lines: myelogenous leukemia K562, colon adenocarcinoma HT- 29, breast adenocarcinoma MCF-7, and human lung fetal fibroblast cell line MRC-5. The active substance of carboplatin (MD1) was used as reference compound. Cells were exposed to complexes for 24 h at concentrations ranging from 10^{-3} to 10^{-8}M. Growth inhibition was evaluated by the colorimetric SRB assay. The IC_{50} value of each carboplatin compound was determined by median effects analysis.*

Results: *Both carboplatin analogues induced dose-dependent growth inhibition of human tumor cell lines after 24 h of treatment. The MD3 analogue was 60-fold and the MD2 was 2-fold more active against K562 cell line compared to the referent compound. The activity of both analogues was comparable to the referent compound against MCF-7 cell line. Colon adenocarcinoma cell line HT-29 was found to be 4-fold less sensitive to MD2 but equally sensitive to MD3 with respect to carboplatin referent compound. Both carboplatin and its analogues induced moderate cytotoxicity on MRC-5 cell line ranging from 25% (10^{-7}M) to 46% (10^{-3}M).*

Conclusion: *This study showed that the two novel carboplatin analogues inhibited human cell lines in a different manner depending on cell line. Carboplatin analogues were more active against human tumor cell lines than against human lung fibroblast cell line MRC-5.*

Key words: antiproliferative activity, cancer cell lines, carboplatin analogues, cell growth, platinum complexes, synthesis

Introduction

Cisplatin and its analogues have made a significant impact on the treatment of a variety of solid tumors for nearly 30 years [1]. Despite the unique activity of the first-generation platinum complexes, significant side effects were observed with cisplatin in early clinical trials. High nephrotoxicity, neurotoxicity, and ototoxicity and primary or acquired cell resistance to cisplatin gave rise to the development of structural analogues that are less toxic and more active. Substitution of the leaving groups of cisplatin by a cyclobutanedicarboxylate moiety resulted in a new compound, carboplatin, with reduced nephrotoxicity but equivalent activity. At effective doses, carboplatin produces less adverse effects than cisplatin but myelosuppression is the dose-limiting toxicity of carboplatin [1,2].

Carboplatin has shown substantial anticancer activity in clinical settings – it is indistinguishable from cisplatin in its clinical activity in a wide variety

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of tumors and is the most frequently used platinum (II) compound [1].

Despite this progress, the search for novel platinum-based chemotherapeutic agents is continuing, since both carboplatin and other second-generation platinum (II) complexes, although less toxic than cisplatin, appear to be highly cross-resistant with cisplatin [2-6].

Platinum (II) complexes investigated in this study were synthesized and characterized in the laboratories of the Department of Chemistry, Faculty of Sciences, University of Kragujevac.

Novel carboplatin analogues contain structural changes in the diammine compartment of Pt (II) complex with different number of CH3-groups. It could be expected that the number of CH3-groups and diammine chelate ring sterically affected the activity of these complexes.

The aim of this study was to investigate the antiproliferative activity of carboplatin (active substance) and two carboplatin analogues against the following human tumor cell lines: myelogenous leukemia, colon adenocarcinoma, breast adenocarcinoma, and normal fetal lung fibroblast. The biological activity of the novel carboplatin analogues was compared with the activity of the referent substance - commercial preparation of carboplatin.

Materials and methods

Materials

Distilled water was demineralized and purified to a resistance greater than 10 $M\Omega cm^{-1}$. The compounds $K_2[PtCl_4]$ and D_2O were obtained from Aldrich Chemical Co. All common chemicals were of reagent grade. Diammine, 1,2-propylenediammine (1,2-pn) and isobutylenediammine (ibn) were obtained from Sigma Chemical Co.

Preparation of platinum(II) complexes

a) *Preparation of carboplatin complex [Pt (CBDCA-O,O')$(NH_3)_2$]*

This complex was prepared according to the methods described in the relevant literature [7-10].

b) *Preparation of [Pt(CBDCA-O,O')(1,2-pn)] and [Pt(CBDCA-O,O')(ibn)] complexes*

These two complexes were prepared in accordance to the methods described in the literature for preparation of carboplatin complex [Pt(CBDCA-*O,O'*)$(NH_3)_2$] [7-10].

$K_2[PtCl_4]$ (0.2075 g, 0.0005 mol) was dissolved in water (5 cm^3), and to this solution KI (0.3652 g, 0.002 mol in 10 cm^3 of water) was added. The mixture was stirred at room temperature for 5 min. To the resulting dark-brown solution an equivalent amount, calculated in relation to $K_2[PtCl_4]$ complex of the corresponding diammine ligand (42.6 µl of 1,2-propylenediammine, 1,2-pn; or 53.5 µl of isobutylenediammine, ibn) was added and the mixture was stirred at room temperature for 30 min. The dark-yellow platinum(II) complex, $[PtI_2(1,2\text{-pn})]$ or $[PtI_2(ibn)]$, was filtered off, washed with water, and dried in air. Yield: 0.2348 g (90%) for the $[PtI_2(1,2\text{-pn})]$ and 0.2518 (94%) for the $[PtI_2(ibn)]$ complex.

The above obtained platinum(II) complexes (0.2348 g, 0.00045 mol of $[PtI_2(1,2\text{-pn})]$ and 0.2518 g, 0.00047 mol of $[PtI_2(ibn)]$) were treated separately, with 2 equivalents of $AgNO_3$ in water solution, and the mixture was stirred at room temperature in the dark for up to 24 h. The precipitated AgI was removed and to the filtrate an equivalent amount of cyclobutane-1,1-dicarboxylic acid (H_2CBDCA), calculated in relation to the corresponding platinum(II)-aqua complex (0.0646 g, 0.00045 mol of H_2CBDCA for solution containing $[Pt(1,2\text{-pn})(H_2O)_2]^{2+}$, and 0.0676 g, 0.00047 mol of H_2CBDCA for solution containing $[Pt(ibn)(H_2O)_2]^{2+}$) was added. The pH of the solution was adjusted to *ca.* 4 by the addition of 1 M KOH and the mixture was then stirred with heating at 55° C for up to 2 h. The volume of the solution was reduced to *ca.* 5 cm^3, and after cooling at room temperature, the resulting pale-yellow solution was left overnight in a refrigerator. The crystals were removed by filtration, washed with a small amount of ethanol, and air-dried. Yield: 0.1100 g (60%) for [Pt(CBDCA-*O,O'*)(1,2-pn)], and 0.1380 g (65%) for [Pt(CBDCA-*O,O'*)(ibn)] complex.

Abbreviations used for platinum (II) complexes in this study are as follows:

MD1=[Pt(CBDCA-*O,O'*)$(NH_3)_2$] = *cis*-diammine (cyclobutane-1,1-dicarboxylato) platinum (II)

MD2=[Pt(CBDCA-*O,O'*)(1,2-pn)] = *cis*-1,2-propylendiammine (cyclobutane-1,1-dicarboxylato) platinum (II)

MD3=[Pt(CBDCA-*O,O'*)(ibn)] = *cis*-izobutylendiammine(cyclobutane -1,1-dicarboxylato) platinum (II)

Proton NMR (^1H NMR) measurements

^1H NMR measurements of D_2(deuterium)O solutions containing TSP (3-trimethylsilylpropane-1-sulfonate) as internal reference were recorded with a Varian Gemini 200 MHz spectrometer. The platinum

(II) complex was dissolved in D_2O and for these measurements about 20 mM concentration of each complex was used.

pH measurements

All pH measurements were made at 298 K. The pH meter (Iskra MA 5704) was calibrated with Fisher certified buffer solutions of pH 4.00 and 7.00. The results were not corrected for the deuterium isotope effect.

Elemental microanalyses

Elemental microanalyses for carbon, hydrogen and nitrogen were done by the Microanalytical Laboratory, Faculty of Chemistry, University of Belgrade, Serbia and Montenegro.

Cell lines

The cell lines used in this study were: MCF-7 (human breast adenocarcinoma, estrogen receptor positive, ER+), K562 (myelogenous leukemia), HT-29 (colon adenocarcinoma), and MRC-5 (human fetal lung fibroblasts). K562 cells were grown in RPMI 1640 medium and MCF-7, HT-29 and were grown in Dulbecco's modified Eagle's medium (DMEM) with 4.5% of glucose. Both media were supplemented with 10% of fetal calf serum (FCS, NIVNS) and antibiotics: 100 IU/ml of penicillin and 100 μg/ml of streptomycin (ICN Galenika). All cell lines were cultured in flasks (Costar, 25 cm^2) at 37° C in 100% humidity atmosphere and 5% of CO_2. Only viable cells were used in the assays [11].

Platinum (II) complexes

Platinum complexes MD1, MD2 and MD3 were dissolved in 1 ml of distilled water (stock solution) and further diluted with culture medium (Sigma) to set an array of working solutions. Dissolved substances of proper concentration were added in volume of 10 μl/well in microtiter plates. Stock and working solutions were prepared fresh for each series of the experiment, and stored in well-sealed plastic tubes at 4°C and protected from light.

The complexes were tested at equimolar concentrations ranging from 10^{-3} M to 10^{-7}M.

Commercially available solutions for intravenous (i.v.) administration of carboplatin (MD1; Ebewe, Austria) were used in the experiment at concentrations of 10^{-2} as stock solution. Working solutions of concentrations ranging from 10^{-3} M to 10^{-7} M were prepared by dilution of stock solution with DMEM without FCS.

SRB assay

Growth inhibition was evaluated by the colorimetric SRB assay [12]. Briefly, single cell suspension was plated into 96-well microtiter plates (Spektar, flat bottom) [10^4 K562 and $5 \cdot 10^3$ (MCF-7, HT-29, and MRC-5) cells per 180 ml of medium] and preincubated for 24 h, at 37° C, and 5% CO_2. The tested substances were added in growth medium to all wells except the control and microplates were incubated for 24 h. Two hours after the incubation period, the medium was replaced with a fresh one. After 24 h the SRB assay was carried out. Optical density was measured on a microplate reader (Multiscan MCC340, Labsystems, at 540/690 nm). Growth inhibition was calculated according to the formula:

$$(1\text{-}A_{TEST} / A_{CONTROL}) \times 100$$

and expressed as a percent of cytotoxicity (CI %).

Data analysis

The IC_{50} of platinum complexes was determined by the median effect analysis [13]. IC_{50} values represented the dose of the compound that inhibits the cell growth by 50% and were interpolated from concentration response curves following 24 h exposure time.

Data in Tables and Figures represent the mean of the quadruplicate wells.

Results

Synthesis and characterization of carboplatin analogues

The platinum(II) complexes used in this study are shown in Figure 1.

The carboplatin analogues were prepared in accordance to the method described in the literature for preparation of carboplatin complex [Pt(CBDCA-*O,O'*)$(NH_3)_2$](see materials and methods). Schematic representation of the preparation of [Pt(CBDCA-*O,O'*)(1,2-pn)] and [Pt(CBDCA-*O,O'*)(ibn)] complexes is given in Figure 2.

Analytical calculation for [Pt(CBDCA-*O,O'*)(1,2-pn)] = $C_9H_{16}N_2O_4Pt$ (FW = 411.31): C, 26.28; H, 3.92; N, 6.81(%). Found: C, 26.37; H, 3.81; N, 6.69(%).

Analytical calculation for [Pt(CBDCA-*O,O'*)(ibn)] = $C_{10}H_{18}N_2O_4Pt$ (FW = 425.34): C, 28.24;

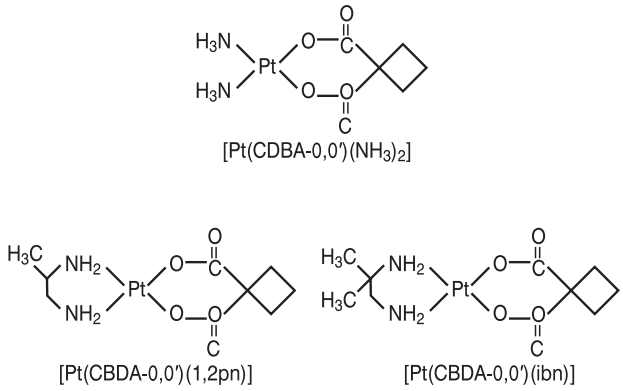

Figure 1. Chemical structure of platinum (II) complexes used in this study.

H, 4.27; N, 6.58(%). Found: C, 28.33; H, 4.41; N, 6.69(%).

The obtained platinum(II) complexes were characterized by ^{1}H NMR spectroscopy (Figure 3a and 3b).

^{1}H NMR resonance (δ, ppm) of [Pt(CBDCA-*O,O'*)(1,2-pn)] complex (200 MHz, D_2O): 1.29 (*s*, $2CH_3$ of ibn); 1.89 (*q*, CH_2 of CBDCA); 2.51 (*m*, CH_2 of 1,2-pn); 2.88 [*m* (2*t* overlapped), CH of 1,2-pn and 2 terminal CH_2 of CBDCA] (Figure 3a).

^{1}H NMR resonance (δ, ppm) of [Pt(CBDCA-*O,O'*)(ibn)] complex (200 MHz, D_2O): 1.42 (*d*, CH_3 of 1,2-pn); 1.89 (*q*, CH_2 of CBDCA); 2.51 (*s*, CH_2 of ibn); 2.88 (*t*, 2 terminal CH_2 of CBDCA) (Figure 3b).

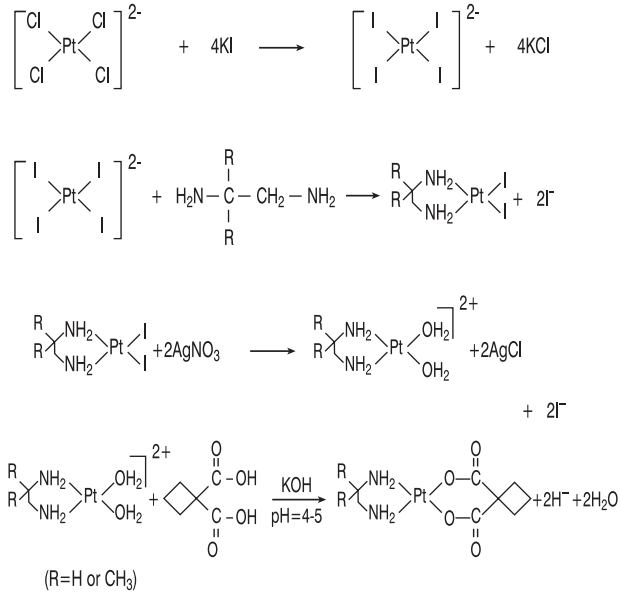

Figure 2. Schematic representation of the preparation of platinum(II) complexes [Pt(CBDCA-*O,O'*)(1,2-pn)] and [Pt(CBDCA-*O,O'*)(ibn).

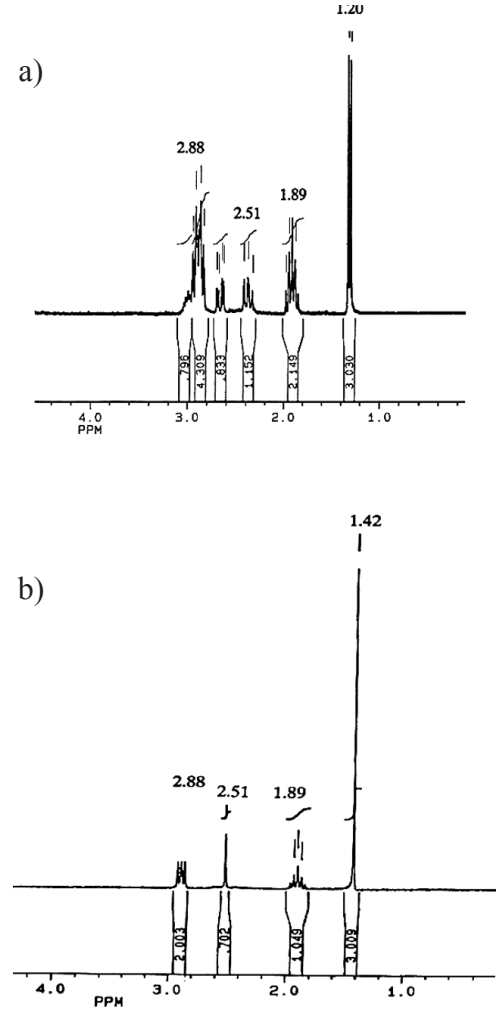

Figure 3. Proton NMR (^{1}H NMR) spectra of [Pt(CBDCA-*O,O'*)(1,2-pn)] (*a*) and [Pt(CBDCA-*O,O'*)(ibn)] (*b*).

Antiproliferative activity of carboplatin analogues

The antiproliferative activity of the two novel carboplatin analogues was studied against various human cancer cell line. Cells were continuously exposed to platinum complexes for 24 h. The results are shown in Figure 4 and Table 1.

Both MD2 and MD3 analogues induced dose-dependent growth inhibition of human tumor cell lines after 24 h treatment (Figure 4).

MCF-7 cells were the most sensitive, and equally sensitive to all investigated compounds. There was no significant difference in action between carboplatin and its analogues.

MD3 derivative was much more active than MD2 on K562 cells, and approximately 60-fold more effective than carboplatin.

MD1 exerted almost the same activity as the original compound used for cancer treatment.

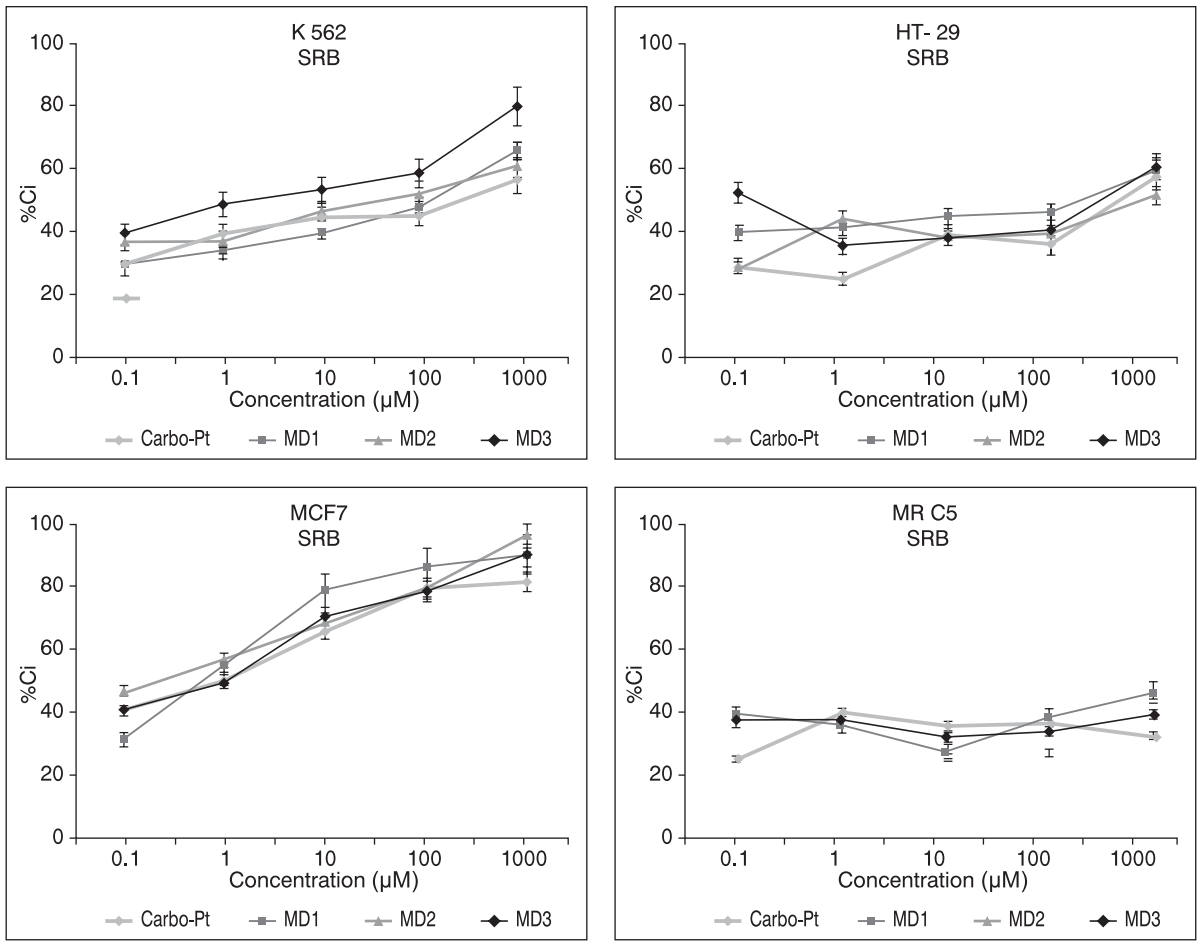

Figure 4. Cytotoxic effect of the new carboplatin analogues on K562, HT-29, MCF-7 , and MRC-5 cell lines. Cells were exposed to analogues for 24h after plating. Cytotoxicity was evaluated by the SRB assay. Data are the means ± SD of quadruplicate wells. MD1: [Pt(CBDCA-*O,O'*)$(NH_3)_2$]; MD2: [Pt(CBDCA-*O,O'*)(1,2-pn)]; MD3: [Pt(CBDCA-*O,O'*)(ibn)].

The MD3 analogue was 60-fold and the MD2 was 2-fold more active against the K562 cell line compared to the referent compound. The activity of both analogues was comparable to the referent compound against the MCF-7 cell line. The colon adenocarcinoma cell line HT-29 was found to be 4-fold less sensitive to MD2 but equally sensitive to MD3 with respect to carboplatin referent compound. Both carboplatin and its analogues induced moderate cytotoxicity on MRC-5 cell line ranging from 25% (10^{-8}M) to 46% (10^{-3}M) (Table 1).

Table 1. IC_{50}* values of carboplatin and its analogues

		IC_{50} (μM)		
Cell line	*Carboplatin*	*MD1*	*MD2*	*MD3*
K562	14.61	13.24	6.34	0.22
HT-29	17.38	16.25	83.49	20.98
MCF-7	0.55	0.62	0.42	0.65
MRC-5	>1000	>1000	>1000	>1000

*The IC_{50} was determined by Median Effect Analysis
MD1: [Pt(CBDCA-*O,O'*)($NH_3)_2$]; MD2: [Pt(CBDCA-*O,O'*)(1,2-pn)]; MD3: [Pt(CBDCA-*O,O'*)(ibn)]

Discussion

In this paper we described the synthesis and characterization of the two carboplatin structural analogues and the study of their antiproliferative activity against various human tumor cell lines. Growth inhibition was evaluated by the colorimetric SRB assay after 24 h of cell treatment. The activity of the new carboplatin analogues was compared with the activity of the parent compound and commercial preparation of carboplatin. This is the first report on growth-inhibitory activity of the described carboplatin analogues.

There are numerous literature data on growth-inhibitory activity of carboplatin and its analogues against solid tumors and leukemic cells [2,5,6,14-16].

In order to improve the biological activities of carboplatin, a large number of its derivatives and analogues have been synthesized, and many modifications of the carboplatin molecule have been made. Generally, modification of the leaving group of platinum (II) complex results in compounds with different pharmacokinetics, while modification of the carrier ligands alters the activity of the resulting complex [1].

The carboplatin analogues used in this study had structural changes in the diammine compartment of carboplatin, containing one (MD2) or two (MD3) CH3-group. It was expected that the number of CH3-groups and the diammine chelate ring sterically affect the activity of these complexes.

We found that the cytotoxicity profiles of these carboplatin analogues were different, but each of them influenced the cell growth equally or more effectively as compared to carboplatin at equimolar concentrations. Cell survival rate was found to be concentration -and cell line- dependent. MCF-7 cells were found to be equally sensitive to all of the investigated compounds and the most sensitive among cell lines. The MD3 analogue that contained two CH3 groups was much more active on K562 and HT-29 cells than MD2. The MD3 analogue was approximately 60-fold more active than carboplatin against the leukemia cell line.

The newly synthesized carboplatin analogues exerted almost the same activity as the original compound used for cancer treatment. The different growth inhibition activity of the new carboplatin analogues can be attributed to their structure - the number of methyl groups correlated with the antiproliferative activity.

Our study showed that these two novel carboplatin analogues inhibited the growth of cell lines but the growth inhibition was cell line-and compound-dependent. Both analogues were more active against human tumor cell lines than against human fibroblast. The significant difference in the activity of analogues deserves further biological investigation due to their selective inhibition of cancer cells.

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