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RESEARCH ARTICLE

In Vitro Cytotoxic Activities of Platinum(II) and Platinum(IV) Complexes Bearing Benzimidazole Ligands

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Summary

The in vitro cyotoxic activities of two platinum(II) and three platinum(IV) complexes with the structures $[PtL_2Cl_2]$, $[PtL_2I_2]$, $[PtL_2Cl_4]$, $[PtL_2Cl_2(OCOCH_3)_2]$ and $[PtL_2Cl_2(OH)_2]$ (L= benzimidazole ligands as "non-leaving groups") were investigated using Cell Culture Method on the HRT-18 cell line. In general the preliminary test results showed that the complexes tested were more active than cisplatin and carboplatin on the HRT-18 cell line. The platinum(IV) complex C4, bearing acetato ligands, exhibited significant in vitro cytotoxic activity against the cell line used.

***Key Words**: Benzimidazole, cytotoxic activity, HRT-18 cell line, platinum complexes*

Benzimidazol Ligandları Taşıyan Platin(II) ve Pt(IV) Komplekslerinin İn vitro Sitotoksik Aktiviteleri

Özet

*HRT-18 hücre hattı üzerinde Hücre Kültür Metodu kullanılarak yapıları $[PtL_2Cl_2]$, $[PtL_2I_2]$, $[PtL_2Cl_4]$, $[PtL_2Cl_2(OCOCH_3)_2]$ ve $[PtL_2Cl_2(OH)_2]$ (L= benzimidazol ligandları "taşıyıcı ligandlar") olan iki platin(II) ve üç platin(IV) kompleksinin in vitro sitotoksik aktivitleri araştırıldı. Genel olarak test sonuçları test edilen komplekslerin HRT-18 hücre hattı üzerinde sisplatin ve karboplatinden daha aktif olduğunu gösterdi. Asetat ligandları taşıyan platin(IV) kompleksi **C4**, kullanılan hücre hattına karşı önemli ölçüde in vitro sitotoksik aktivite sergiledi.*

***Anahtar Kelimeler:** Benzimidazol, sitotoksik aktivite, HRT-18 hücre hattı, platin kompleksleri*

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INTRODUCTION

Cisplatin [*cis*-diamminedichloroplatinum(II)], is used in nearly 50% of all tumor chemotherapies and it is one of the world's best selling anticancer drugs (1). Besides cisplatin, carboplatin [*cis*-diammine (1,1-cyclobutanedicarboxylato) platinum(II)] and oxaliplatin [*trans*-R, R-cyclohexane-1, 2-diamine) oxalatoplatinum(II)] (**Figure 1**) are widely used in the treatment of testicular, ovarian cancer and a variety of other human solid tumors (2), but many are intrinsically resistant and, even among initially sensitive tumors, acquired resistance commonly develops during treatment (3, 4) Acquired resistance is a particular problem, as tumors may become resistant not only to the drugs used to treat them,

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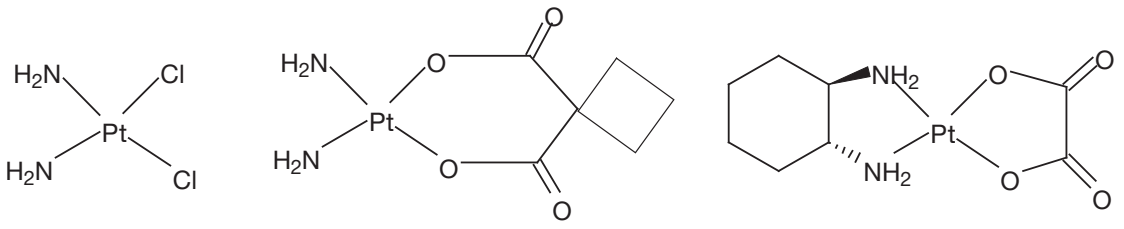


cisplatin carboplatin oxaliplatin

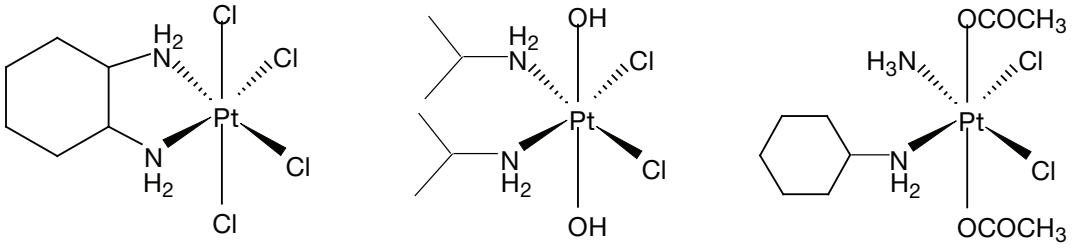
Figure 1. Chemical structures of platinum(II) complexes in the clinical uses.

but also to other drugs with different mechanisms of action (5). Elucidation of the molecular mechanisms that mediate cisplatin resistance holds promise for the design of pharmacological strategies for preventing, overcoming, or reversing this form of drug resistance (6).

Platinum(IV) complexes have attracted much interest recently as alternatives to the existing platinum(II) based clinical cisplatin, carboplatin and oxaliplatin, due to the toxic effects platinum(IV) complexes display (7) and the resistance that is exhibited to such treatment (8).

Octahedral platinum(IV) complexes undergo ligand substitution reactions that are slow relative to those of their platinum(II) analogues. They have been considered as the compounds which are unable to react directly with DNA. The antitumor activity of platinum(IV) compounds has been suggested to require in vivo reduction to the kinetically more labile, and therefore reactive, platinum(II) derivatives (9-11). A number of cellular reductants could achieve this, including cystein, the sulfhdryl protein, glutathione and ascorbic acid (12). On the other hand, the markedly higher activity in cisplatin-resistant cell lines has been observed for a platinum(IV) drug [JM149, *bis*-(hydroxy) amine-dichloro(cyclohexylamine)platinum(IV)] than for its platinum(II) analogue [JM118, *cis*-ammine(cyclohexylamine)dichloroplatinum(II)] (13). This result suggests that other mechanisms than reduction to active platinum(II) species are important for cytostatic efficiency of platinum(IV) drugs.

Consequently, kinetically more inert platinum(IV) complexes (**Figure 2**) like tetraplatin, [tetrachloro [(1,2-diaminocyclohexane) platinum(IV)]] and iproplatin, [dichlorodihidroxy bis(isopropylamine) platinum(IV)], were evaluated in phase I clinical trials. Surprisingly, their behavior *in vivo* was significantly different: tetraplatin was abandoned because of intense side effects (neurotoxicity), whereas iproplatin was abandoned because of lacking activity (14,15). The other platinum(IV) complexes satraplatin [formerly known as JM-216 bis-(acetato)amine-dichloro(cyclohexylamine)platinum(IV)] is a fourth-generation platinum analog that was rationally synthesized to be orally available. Satraplatin is not associated with the dose-limiting renal toxicity



tetraplatin iproplatin satraplatin

Figure 2. Chemical structures of anticancer platinum(IV) complexes in clinical evaluation.

seen with cisplatin, nor the neurotoxicity seen with oxaliplatin. In addition, the results of a multicentre Phase III double blind randomized trial involving 950 patients with hormone-refractory prostate cancer showed that satraplatin significantly reduced the risk of disease progression in these patients (16,17). In contrast to cisplatin, carboplatin, and oxaliplatin, which must all be given intravenously, oral administration of satraplatin is more convenient, eliminates the need and complications of venous devices, and may improve patient acceptability of the treatment (12).

In previous studies, taking into consideration the fact that variations in the chemical structure of the leaving and non leaving-groups of cisplatin can have significant effects on the cytotoxic activity and toxicity of platinum complexes, we synthesized some Pt(II) and also Pt(IV) complexes with the 2-non- or substituted benzimidazole ligands (18-26). It was determined that some of these platinum complexes had *in vitro* cytotoxic activities on *RD* (20), *HeLa* (21, 23, 25, 26), *HEp-2* (24, 26) and *MCF-7* (21-23, 25, 26) cell lines.

In a previous paper, with the aim to determine the effect of axial and equatorial ligand variation on the cytotoxic activities of the Pt(II) and Pt(IV) complexes, we synthesized some Pt(II) and Pt(IV) complexes with the structures of $[PtL_2Cl_2]$ (**C1)**, $[PtL_2I_2]$ (**C2)**, $[PtL_2Cl_2(OH)_2]$ (**C3)**, $[PtL_2Cl_2(OCOCH_3)_2]$ (**C4)**, and $[PtL_2Cl_4]$ (**C5**) (L= benzimidazole as carrier ligands) and tested for their preliminary *in vitro* cytotoxic activities against the human *MCF-7 breast*, *HeLa cervix*, and *HEp-2 larynx carcinoma* cell lines. The plasmid DNA interaction and the inhibition of *BamH1* restriction enzyme activity of the compounds **C1-C5** were also studied (26). In general, it was found that the compounds **C1-C5** were less active than cisplatin and carboplatin against *MCF-7* and *HeLa* cell lines and **C1** and **C3** were found to be significantly more active than cisplatin and carboplatin against *HEp-2* cell line.

In the present paper, to determine whether cytotoxic activities of the Pt(II) and Pt(IV) complexes **C1-C5** (**Figure 3**), which were synthesized and tested for their *in vitro* cytotoxic activities against *MCF-7, HeLa* and *HEp-2* cell lines previously by us as stated above (26), were cell-type dependent, and we tested their preliminary *in vitro* cytotoxic activities against the *HRT-18 rectal adenocarcinoma* cell line.

MATERIAL and METHODS

Preliminary Cytotoxicity Test

Cell Line and Growth Conditions

Cisplatin and carboplatin were obtained from Sigma with a purity of more than 99.9%. The *HRT-18 rectal adenocarcinoma* cancer cell lines used in this study were obtained from University of Ankara Faculty of Veterinary Medicine Department of Virology. The cells were grown in Dulbecco's (Seromed, Germany) minimal essential medium (DMEM) enriched with 10% fetal calf serum (FCS) (Biochrom, Germany), 100 mg mL^{-1} streptomycin and 100 IU mL^{-1} penicillin in a humidified atmosphere of 5% CO_2 at 37°C. The cells were harvested using Trypsin (Bibco Life Technologies, UK)/Versen (0.05%:0.02%) solution. Mycoplasma contamination was routinely monitored and only mycoplasma–free cultures were used.

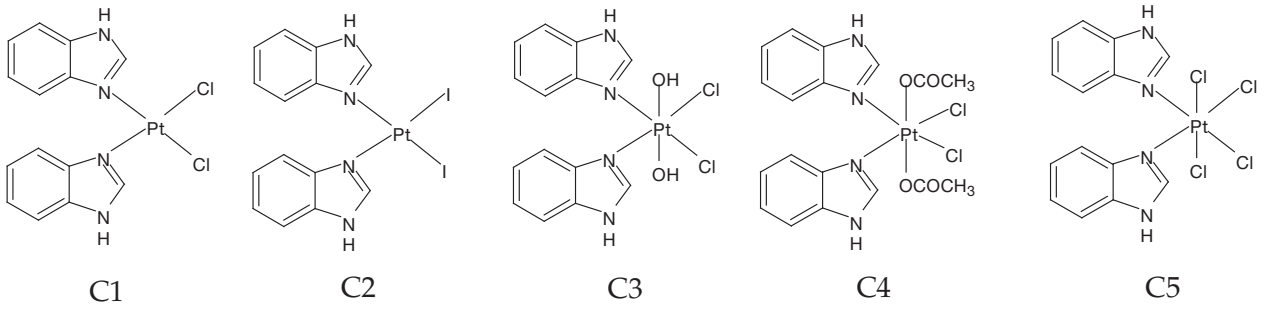


Figure 3. Chemical structrures of the platinum(II) (**C1-C2**) and platinum(IV) (**C3-C5**) complexes.

In Vitro Chemosensitivity Assay

The preliminary *in vitro* testing of the compound **C1-C5** on antitumor activity was carried out on the *HRT-18* cancer cells according to a previously published microtiter test (27). Briefly, the cells were seeded into 96-well plates (Greiner GmbH, Germany) in a volume of 100 μL as to be 18-22 cells/microcospic area (The cells were counted using automatic Vi-Cell (Beckman Coulter, USA) system when needed in the study. For this purpose, cells detached from culture vessel were re-suspended in DMEM and 0.5 mL of cell suspension was loaded into the counting chamber of Vi-Cell. The total numbers of viable cells were recorded after calculation by the machine.). After attachment to the culture surface, the cells were incubated in an atmosphere containing 5% CO_2 at 37 °C for 24 h. At the end of this period the growth medium was carefully removed by suction and 100 μL of fresh medium were added into each well. The medium used contained an adequate volume of a stock solution of the respective compound in order to obtain the desired test concentration (1, 5, 10, 20, 40, 80 and 100 μM, solvent: dimethylformamide (DMF)), the complexes tested were added to the culture medium in such a way that the final DMF was 0.1% (v/v)). Sixteen wells were used for each complex (**C1-C5**, reference compound cisplatin and carboplatin) tested were individual concentrations, while sixteen wells were reserved for the cell culture control, which contained the corresponding amount of DMF. After 72 h of incubation at 37 °C, the medium was removed and the cells were fixed with 100 μL 1% glutardialdehyde in phosphate-buffered saline (PBS) per well for 25 min. The fixative was replaced by 150 μL PBS/well and the plates were stored in the refrigerator (4 °C). Cell biomass was determined by a crystal violet stained technique (28). Absorbance was measured at 492 nm using a Titertek Multiscan plus MKII Autoreader. The results correspond to three independent experiments.

IC_{50} values of the complexes **C1-C5** and reference compounds cisplatin and carboplatin were calculated from the dose-survival curves for the growth inhibition of all cell lines. (In the calculations Prism4 GraphPad Software was used. The results are presented as mean value and ± standard deviation (SD)).

The complexes **C1-C5** and reference compounds cisplatin and carboplatin were tested in three independent experiments.

RESULTS AND DISCUSSION

Chemistry

Synthesis and detailed structural analyses of platinum(II) (**C1-C2**) and platinum(IV) (**C3-C5**) complexes were reported in our a previous paper (26). Briefly, Pt(II) complexes **C1** and **C2** were synthesized by the reaction of the benzimidazole with potassium tetrachloroplatinate and potassium tetraiodoplatinate respectively in ethanol-water. Platinum(II) complex **C1** was oxidized with 30% H_2O_2 to form the corresponding axial dihydroxyplatinum(IV) complex **C3**. In addition **C4** was synthesized by the reaction of the **C1** complex with acetic acid, acetic acid anhydride and hydrogen peroxide. The other Pt(IV) complex **C5** was synthesized by the reaction of **C4** with 15% HCl. Chemical structures of the **C1-C5** are given in **Figure 3.**

Preliminary Cytotoxicity Test

The preliminarily *in vitro* antiproliferative activities of the compounds **C1-C5** and cisplatin and carboplatin used as reference compounds were carried out on the *HRT-18* cell lines, according to a previously published microtiter assay (27, 28) and the results are shown in **Table**. *HRT-18* cell was incubated for 72 h with 1, 5, 10, 20, 40, 80 and 100 μM of the platinum(II) (**C1-C2**)

Table. IC_{50} (μM) values of the platinum(II) and platinum(IV) complexes on *HRT-18* cell line.

IC_{50} (μM)	
Compound	***HRT-18***
C1	6.05 ± 1.45
C2	>100*
C3	33.80 ± 2.05
C4	1.88 ± 0.23
C5	46.77 ± 2.00
Cisplatin	>100*
Carboplatin	>100*

*no *in vitro* cytotoxic activity was found in the concentrations tested (1-100 μM)

and platinum(IV) (**C3**-**C5**) complexes and cisplatin and carboplatin.

The Pt(II) complex **C1** bearing chloro ligands as leaving groups was found to be more active than **C2** bearing iodo ligands as leaving groups on the *HRT-18* cell line. This finding is consistent with the data reported in literature that the cisplatin analogues, containing chloro ligands, exhibit much stronger therapeutic features than the analogues in which the ligands are azides, cyanides, iodides or rhodanates (29). It is also reported that the binding to DNA of these drugs with halogeno leaving groups are almost certainly preceded by aquation, with loss of one or more of the leaving groups (30) and also iodo ligands is generally a poorer leaving group than chloro ligands in the structure of Pt(II) complexes (31).

In the case of Pt(IV) complexes, the compounds **C3, C4** and **C5** bearing hydroxo, acetato and chloro groups respectively as axial ligands were found to be more active than cisplatin and carboplatin (IC_{50} values of **C3, C4** and **C5** were 33.80, 1.88 and 46.77 µM respectively). The platinum(IV) complexes **C4** bearing acetato ligands was found to be the most active compound against *HRT-18* cancer cell line. This finding is consistent with the data reported in literature that at present, the most interesting candidate is satraplatin, with axial acetato groups and an intermediate reduction potential, which was rationally synthesized to be orally availed drug (16, 17, 32).

In general, it was found that platinum(II) and platinum(IV) complexes tested were significantly more active than the reference compounds cisplatin and carboplatin. Considering the IC_{50} values obtained in the previous study reported by us (26) and obtained in this study, it may be concluded that cytotoxicity of compounds **C3** and **C5** are cell-type dependent. (IC_{50} values of **C3** and **C5** for *HRT-18*, *MCF-7(26)*, *HeLa* (26) and *HEp-2* (26) respectively were ~33.8, >100*, >100* and 7.19 and 46.77, >100*, 20.70 and >100* µM respectively (*: no *in vitro* cytotoxic activity was found at the concentrations tested (1-100 µM)). It can also be concluded that the *in vitro* cytotoxicity of compounds **C1** and **C4** against *HRT-18* cell line is noteworthy and must be taken into consideration in the future studies.

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