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CYTOTOXIC ACTIVITY OF PLATINUM(II) AND PLATINUM(IV) COMPLEXES BEARING 5(6)-NON/CHLOROSUBSTITUTED-2-HYDROXYMETHYL BENZIMIDAZOLE LIGANDS AGAINST HEp-2 CELL LINE

5(6)-NON/KLOROSUBSTİTÜE-2-HİDROKSİMETİLBENZİMİDAZOL LİGANDI TAŞIYAN PLATİN(II) VE PLATİN(IV) KOMPLEKSLERİNİN HEp-2 HÜCRELERİNE KARŞI SİTOTOKSİK AKTİVİTESİ

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ABSTRACT

In vitro cytotoxic activities of four platinum(II) and four platinum(IV) complexes with the structures $[PtL_2Cl_2]$, $[PtL_2I_2]$ and $[PtL_2Cl_4]$, $[PtL_2Cl_2(OH)_2]$ (L= 5(6)-non/chloro-substituted-2-hydroxymethyl benzimidazole ligands as "non-leaving groups") respectively were tested on the human HEp-2 (larynx carcinoma) cell line using Cell Culture Method. In general the complexes, which were found to be more active than carboplatin exhibited moderate cyctotoxicity comparable to cisplatin on the human HEp-2 cell line.

***Keywords**: Benzimidazole, , Cytotoxic activity, HEp-2 cell line, , Platinum(II) complexes, Platinum(IV) complexes*

ÖZET

Yapıları sırasıyla $[PtL_2Cl_2]$, $[PtL_2I_2]$ ve $[PtL_2Cl_4]$, $[PtL_2Cl_2(OH)_2]$ (L= 5(6)-non/kloro-substitüe-2-hidroksimetilbenzimidazol "taşıyıcı ligand") olan dört platin(II) ve dört platin(IV) kompleksi in vitro sitotoksik aktivitleri Hücre Kültür Metodu kullanılarak insan HEp-2 (larinks kanseri) hücre hattında test edildi. Genel olarak insan HEp-2 hücre hattına karşı, karboplatinden daha aktif bulunan kompleksler sisplatin ile karşılaştırılabilir sitotoksik etki gösterdi.

Anahtar Kelimeler: *Benzimidazol, Sitotoksik aktivite, HEp-2 hücre hattı, Platin(II) kompleksi, Platin(IV) kompleksi*

INTRODUCTION

Cisplatin [*cis*-diamminedichloroplatinum(II)] has first been synthesized in 1844 by Michael Peyrone (1). More than a century later, the anticancer properties of cisplatin were reported in 1969 by Barnett Rosenberg as result of his investigations about the influence of an electric field on bacterial growth (2).

Today, cisplatin, is one of the most successful drugs currently used in clinical cancer therapy and widely applied in the treatment of a various types of cancer such as testicular, ovarian and bladder carcinomas (3). Although cisplatin can induce apoptosis selectively in cancer cells through binding to DNA, the drug undergoes many non-selective reactions with a variety of biomolecules, such as proteins and phospholipids (4).

Furthermore, the drug is rapidly distributed throughout the whole body upon administration, interacting with both healty and cancer tissue (5). This interaction gives rise to intrinsic and acquired drug-resistance, cumulative and irreversible toxicities particularly nephrotoxicity, peripheral neuropathy, ototoxicity, nausea and vomiting (6,7).

In order to overcome these problems a great deal of efforts have been develop to find innovative platinum complexes that broader spectrum of activity, improved clinical efficacy and reduced toxicity, better then cisplatin (8,9)

In addition to square-planar platinum(II) complexes, an attempt has also been made more recently to octahedral platinum(IV) complexes. Therefore, platinum(IV) complexes have enormous potential as anticancer agents in terms of both high activity and low toxicity probably because they are reduced too readily in the bloodstream (10).

It is widely believed that reduction to platinum(II) is essential for the anticancer activity of platinum(IV) complexes to be effected (11). The reduction potentials of diam(m)ine platinum(IV) complexes are dependent on the nature of the axial and equatorial ligands, but the axial ligands generally exert the stronger influence (12). Numerous experimental results support that platinum(IV) complexes are reduced by both extracellular and intracellular reducing agent such as cysteine, the sulfhydryl protein, glutathione and ascorbic acid (13). On the other hand there are a few papers reporting that platinum(IV) complexes can bind to DNA and RNA fragments without being reduced (14).

Some platinum(IV) complexes have shown sufficient promise to enter clinical trials: Iproplatin, [*cis*-dichloro-*trans*-dihidroxybis(isopropylamine)platinum(IV)], tetraplatin, [tetrachloro [(1,2-diaminocyclohexane)platinum(IV)]], and satraplatin, [*trans*,*cis*-bis(acetato) amminedichloro (cyclohexylamine)platinum(IV)cyclohexylamine], have been tested in clinical trials (15). One of them, satraplatin which is currently awating approval by the US Food and Drug Adminstration, significantly decreased the risk for disease progression in a phase III trial in hormone-refractory prostate cancer (HRPC) (16).

In a previous paper, we reported the synthesis and characterization of the platinum complexes of the structure, *cis*-$[Pt(L_2)Cl_2].H_2O$ where L is 5(6)-non/or chloro-substituted-2-hydroxymethylbenzimidazole and the determination of their preliminary *in vitro* cytotoxic effects by "Rec-Assay" test (17). The DNA-binding properties of these two platinum(II) complexes were also examined and it was determined that the DNA platinated with these compounds were specifically recognized by high mobility group (HMG) domain protein, HMG 1 (18). It was also determined that some of the new 2-substituted benzimidazoleplatinum(II) complexes we synthesized have *in vitro* cytotoxic activities on the human RD (Rhabdomyosarcoma) (19), MCF-7 and HeLa cell lines (20,21).

In the present study, as an extension of the investigation on the probable antitumor activity of platinum complexes of benzimidazole ligands, to determine the effect of axial and equatorial ligand variation on the cytotoxic activities of the platinum(II) and platinum(IV) complexes, with the structures $[PtL_2Cl_2]$, $[PtL_2I_2]$, $[PtL_2Cl_4]$ and $[PtL_2Cl_2(OH)_2]$, bearing 2-hydroxymethylbenzimidazole ($\mathbf{L^1}$) or 5(6)-chloro-2-hydroxymethylbenzimidazole ($\mathbf{L^2}$) as non-leaving amine ligands and chloro, iodo, hydroxo ligands as leaving groups (**Figure 1**) which were synthesized previously by us (22) were evaluated for their preliminary *in vitro* cytotoxic activities on the human HEp-2 (larynx carcinoma) cell line.

MATERIALS AND METHODS

Preliminary cytotoxicity test

Cell line and growth conditions

Human HEp-2 (Human larynx epidermidis carcinoma) cell line used in this study was 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.

In vitro chemosensitivity assay HEp-2 cell line

The preliminary *in vitro* testing of the platinum complexes on antitumour activity was carried out on HEp-2 cells according to a previously published microtiter test (23). Briefly, in 96-well plates, 100 μL of a cell suspension at $1x10^6$ cells/mL culture medium were plated into each well and incubated at 37 °C for 24 h in a humidified atmosphere (5% CO_2). 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 and 80 μM, solvent: dimethylformamide (DMF), the complexes tested were added to the culture medium such that the final DMF was 0.1% (v/v)). Sixteen wells were used for each complex (**C1-C8**, 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 incubation for 72 h 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 30 min. The fixative was replaced by 100 μL PBS/well and the plates were stored in the refrigerator (4 °C). Cell biomass was determined by a crystal violet stained technique (24).

The effect of the platinum complexes were expressed as corrected T/C values according to the following equations:

$$T/C_{corr.}[\%] = [(T - C_o)/(C - C_o)] \times 100$$

where T is the mean absorbance of the treated cells, C the mean absorbance of the controls, and C_o the mean absorbance of the cells at the time (t=0) when the drug was added.

When the absorbance of treated cells was less than that of the culture at t=0 (C_o), the extent of cell killing was calculated as:

Cytocidal effect [%] = $[(C_o - T)/ C_o] \times 100$

Absorbance was measured at 492 nm using a Titertek Multiscan plus MKII Autoreader. The results correspond to two independent experiments.

RESULTS AND DISCUSSION

Chemistry

Synthesis and detailed structural analyses of platinum(II) (**C1-C4**) and platinum(IV) (**C5-C8**) complexes were reported in our a previous study (22). Chemical structures of the **C1-C8** are given in **Figure 1**.

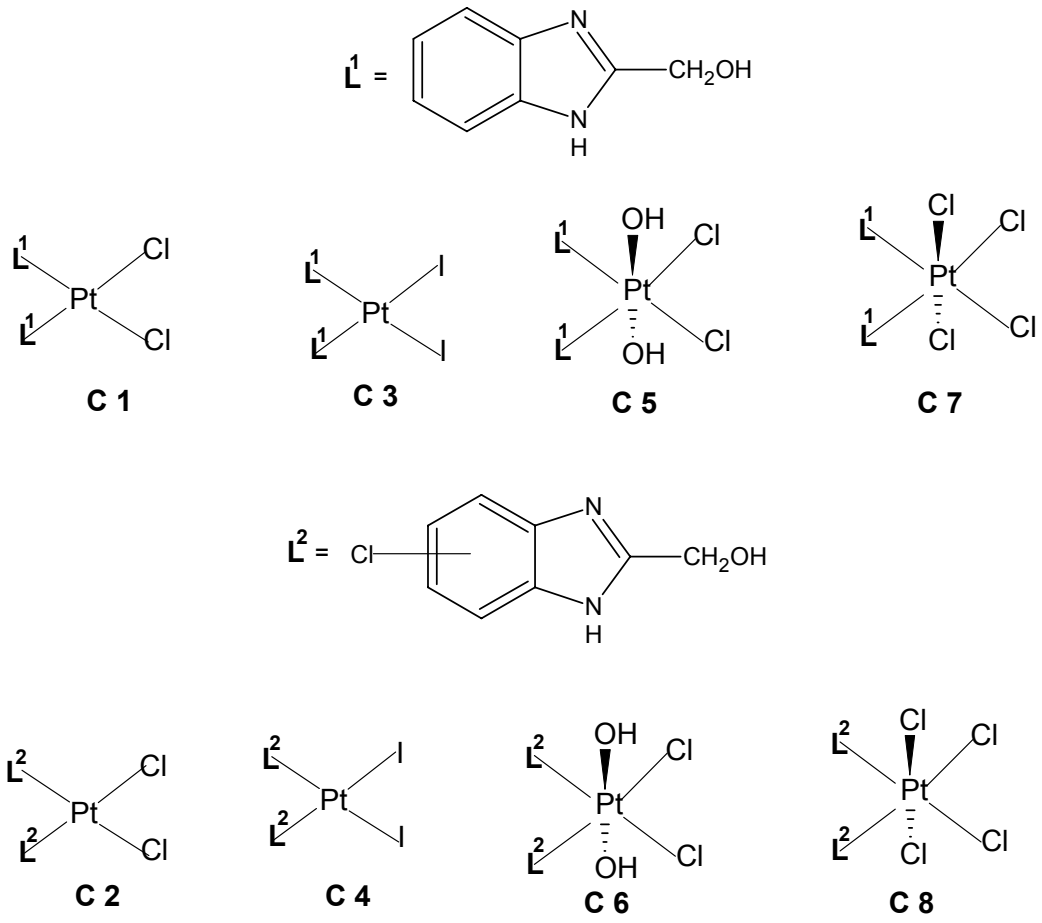

Figure 1. Chemical structrures of the platinum(II) and platinum(IV) complexes.

Preliminary cytotoxicity test

The preliminary antiproliferative activities of the platinum(II) complexes bearing $\mathbf{L^1}$ or $\mathbf{L^2}$ as "non-leaving ligands" and chloro or iodo atoms (**C1-C4**) as "leaving ligands", and the platinum(IV) complexes, which the oxidation products of the platinum(II) complexes, **C5-C8** with axial chloro or hydroxo ligands were determined on the human HEp-2 cell line. HEp-2 cell was incubated for 72 h with 80, 40, 20, 10, 5 and 1 μM of the platinum(II) (**C1-C4**) and platinum(IV) (**C5-C8**) complexes and cisplatin and carboplatin used as reference compounds. The antiproliferative activity values of the complexes and the reference compounds expressed as $T/C_{corr.}$ are presented in **Table 1**.

Table 1. Cytotoxic activities of the platinum(II) and platinum(IV) complexes on the HEp-2 cell

	$T/C_{corr.}$ [%] ± (S.D.)[a]					
Compound	**80μM**	**40μM**	**20μM**	**10μM**	**5μM**	**1μM**
C1	-5.45 ± 1.38 [b]	>100	>100	>100	>100	>100
C2	-66.62 ± 4.29 [b]	12.39 ± 1.91	>100	>100	>100	>100
C3	-62.40 ± 0.67 [b]	-43.19 ± 5.17 [b]	-15.48 ± 1.09 [b]	88.40 ± 0.66	>100	>100
C4	-66.56 ± 5.32 [b]	-55.57 ± 9.99 [b]	76.72 ± 4.80	>100	>100	>100
C5	-77.18 ± 3.36 [b]	-49.38 ± 9.01 [b]	-27.30 ± 4.81 [b]	>100	>100	>100
C6	-75.41 ± 1.79 [b]	18.46 ± 3.61	>100	>100	>100	>100
C7	-52.09 ± 3.20 [b]	29.00 ± 0.71	91.13 ± 8.96	>100	>100	>100
C8	-60.18 ± 5.23 [b]	44.78 ± 2.77	92.96 ± 7.40	>100	>100	>100
Cisplatin	-42.21 ± 3.03 [b]	-42.09 ± 1.82 [b]	-8.43 ± 1.63 [b]	76.61 ± 9.33	>100	>100
Carboplatin	27.03 ± 3.24 [b]	>100	>100	>100	>100	>100

[a] S.D. Standard deviation.

[b] Cytocidal effect.

In the test on the human HEp-2 cancer cell line at 1, 5 and 10 μM concentration $T/C_{corr.}$ values of all the complexes tested except for **C3** and cisplatin at 10 μM concentration were >100.

At 20 μM concentration, cytotoxic effects of the complexes **C4**, **C7** and **C8** amounted to $T/C_{corr.}$ values of ca. (76.72%, 91.13% and 92.96% respectively). While at the same concentration cytocidal effects were obtained for the complexes **C3** and **C5** and reference compound cisplatin (-

15.48% and -27.30% and –8.43% respectively). For the other complexes tested (**C1**, **C2**, **C6** and reference compound carboplatin) $T/C_{corr.}$ values were >100 at 20 μM concentration.

A clear antiproliferative effect was observed for the **C2**, **C6**, **C7** and **C8** complexes by increasing the concentration to 40 μM concentration. At these dosage $T/C_{corr.}$ values of complexes **C2**, **C6**, **C7** and **C8** were around (12.39%, 18.46%, 29.00% and 44.78% respectively). At this concentration cytocidal effects were observed for **C3**, **C4** and **C5** and reference compound cisplatin (-43.19%, -55.57%,-49.38% and -42.09% respectively). At the same concentration $T/C_{corr.}$ values of **C1** and carboplatin were >100.

At the concentration of 80 μM, cytocidal effects were observed for the complexes **C1-C8** and reference compound cisplatin except for carboplatin. At this concentration, the reference compound carboplatin had $T/C_{corr.}$ values of 27%.

All complexes tested showed a concentration dependent reduction of cell proliferation. The test results show that replacing the chloro ligands with the iodo ligands has significant effects on the antiproliferative activities of the platinum(II) complexes. In general, it was found that platinum(II) and platinum(IV) complexes were more active than the reference compound carboplatin, exhibited slightly more cytotoxicity than the reference compound cisplatin. Although the complexes bearing 5(6)-chloro-2-hydroxymethylbenzimidazole as “carring-ligands” were found to be slightly more active than the complexes bearing 2-hydroxymethylbenzimidazole ligands against HEp-2 cancer cell line, no significant differences between the platinum(II) and platinum(IV) complexes were observable.

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