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Synthesis and Cytotoxic Activity of Platinum(II) and Platinum(IV) Complexes with 2-Hydroxymethylbenzimidazole or 5(6)-Chloro-2-hydroxymethylbenzimidazole Ligands against MCF-7 and HeLa Cell Lines

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Four platinum(II) and 4 platinum(IV) complexes with the structures $[PtL_2Cl_2]$, $[PtL_2I_2]$, $[PtL_2Cl_4]$, and $[PtL_2Cl_2(OH)_2]$ (L = 5(6)-non/orchloro-substituted-2-hydroxymethylbenzimidazole ligands as "non-leaving groups"), respectively, were synthesized and characterized by their elemental analyses, and IR and ^{1}H-NMR spectra. In vitro cytotoxic activities of the platinum(II) and platinum(IV) complexes were tested against the human MCF-7 (breast cancer) and HeLa (cervix cancer) cell lines using the cell culture method. In general, the platinum(II) complexes were more active than the corresponding platinum(IV) complexes. The complexes, which were found to be less active than cisplatin, exhibited cyctotoxicity comparable to carboplatin on the human MCF-7 and HeLa cell lines.

Key Words: Benzimidazole, cytotoxic activity, HeLa cell line, MCF-7 cell line, platinum(IV) complexes, platinum(II) complexes.

Introduction

Cisplatin [*cis*-diamminedichloroplatinum(II)], and other platinum-based drugs, such as carboplatin [*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II)] and oxaliplatin [*trans-R, R*-cyclohexane-1,2-diamine

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oxalatoplatinum(II)], are used to treat testicular tumors as well as a variety of other human solid tumors, but many are intrinsically resistant and acquired resistance commonly develops during treatment.[1]

DNA is the principal cellular target of these compounds.[2] The major adducts are 1,2-intrastrand and 1,3-intrastrand cross-links.[3]

Platinum drug resistance can occur by several mechanisms, including drug efflux, drug inactivation, alterations in drug target, processing of drug-induced damage, decreased drug penetration, and evasion apoptosis.[4]

Cells deficient in DNA repair enzymes are hypersensitive to cisplatin, indicating that repair plays an important role in the molecular mechanism of the drug.[5]

Nucleotide excision repair is a major cellular defense mechanism against the toxic effects of the anticancer drug cisplatin and other platinum-based chemotherapeutic agents.[6]

The need for cisplatin analogs that are less toxic and have a broader spectrum of activity led to the synthesis of a large number of platinum complexes over the past 3 decades.[7]

The different nature of the DNA adduct formed by some targeted platinum complexes may overcome resistance based on improved nucleotide excision repair of cisplatin DNA adducts.[8]

The replacement of the leaving chloride groups affects mainly tissue and intracellular distribution of the cisplatin analogues. On the other hand, the replacement of ammine groups can result in different structural and conformational alterations in target DNA, which may affect the character of biological effects of the analogues.[4]Using more hydrophobic cisplatin analogues was expected to enhance affinity of the damage-recognition proteins to the platinated site, which could more effectively protect it from excision repair. It has been shown that increasing cytotoxicity of cisplatin analogues, in which NH_3 groups were replaced by more hydrophobic amine ligands, correlated with growing hydrophobicity of these analogues.[9]

There is growing interest in 6-coordinated platinum(IV) complexes because of their anticancer activity, especially since these complexes are toxic to tumors that are resistant to cisplatin.[10]

Some platinum(IV) complexes have shown potential as powerful anticancer drugs. It is widely thought that reduction to platinum(II) is essential for the anticancer activity of platinum(IV) complexes to be effected. 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.[11]

On the other hand, there are a few papers reporting that platinum(IV) complexes can bind to DNA and RNA fragments without being reduced.[12]

Although some platinum(IV) compounds, including 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, there are still no platinum(IV)-based therapeutics in routine clinical use.[7,11−13]

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.[14] The DNA-binding properties of these 2 platinum(II) complexes were also examined and it was determined that the DNA platinated with these compounds was specifically recognized by high mobility group (HMG) domain protein, HMG 1.[15] 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),[16] MCF-7, and

HeLa cell lines.[17,18]

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 complexes, a series of platinum(II) and platinum(IV) complexes with 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 were synthesized and evaluated for their preliminary in vitro cytotoxic activities on the human MCF-7 and HeLa cell lines.

Experimental

Chemistry

Materials

All chemicals and solvents used in the synthesis were purchased from Merck and Aldrich. The cisplatin and carboplatin used in the cytotoxicity test were purchased from Sigma.

Melting points were measured on an Electrothermal 9200 melting point apparatus and are uncorrected. Elemental analyses were performed with a LECO CHNS 932 analyzer at TÜBİTAK Instrumental Analyses Center (Ankara, Turkey). IR spectra of the compounds were recorded in KBr pellets and in Nujol mulls on a Bruker Vector 22 IR spectrophotometer. ^{1}H-NMR spectra were recorded in DMSO-d_6 on a Bruker 400 AC NMR spectrometer. Thin-layer chromatography (TLC) was performed on pre-coated aluminum plates Merck Silica Gel 60 F_{254}. Plates were visualized by UV light, Dragendorff reagent, or iodine vapor.

Biological test

Preliminary cytotoxicity test

Cell lines and growth conditions

The human MCF-7 and HeLa cell lines used in this study were obtained from the Cell Culture Collection (HUKUK No: 00092502 and 90061901, respectively) of the Institute for Foot and Mouth Disease (IFMD, Turkey).

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 on the human MCF-7 and HeLa cancer cell lines

The preliminary in vitro testing of the platinum complexes on antitumor activity was carried out on human MCF-7 and HeLa cells according to a previously published microtiter test.[21] Briefly, the cells were seeded into 96-well plates (Greiner GmbH, Germany) in a volume of 100 μL so as to be 18-22 cells per microscopic area. After attachment to the culture surface, the cells were incubated in an atmosphere containing 5% CO_2 at 37 °C for 24 h. After 48 h, the growth medium was carefully removed by suction and 200 μL of

fresh medium was added to 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 (0.5, 1, 5, 10, 20, and 40 μM, solvent: DMSO, the complexes tested were added to the culture medium such that the final DMSO was 0.1% (v/v)). Sixteen wells were used for each compound (**C1-C8,** cisplatin, and carboplatin) tested in individual concentrations, while 16 wells were reserved for the cell culture control, which contained the corresponding amount of DMSO. After 72 h of incubation at 37 °C, the medium was removed and the cells were fixed with 100 μL of 1% glutardialdehyde in phosphate-buffered saline (PBS) per well for 25 min. The fixative was replaced by 150 μL of PBS per well and the plates were stored in the refrigerator (4 °C). Cell biomass was determined by a crystal violet stained technique.[22]

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

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

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 578 nm using a Titertek Multiscan plus MKII Autoreader. The results correspond to 3 independent experiments.

Results and Discussion

Results

Synthesis of the ligands

The ligands, 2-hydroxymethylbenzimidazole ($\mathbf{L}^1$) and 5(6)-chloro-2-hydroxymethyl benzimidazole ($\mathbf{L}^2$), were prepared according to the Phillips method[19,20] as shown in the Figure.

2-Hydroxymethylbenzimidazole **($\mathbf{L}^1$)**: Yield 59%. ^{1}H-NMR (DMSO-d_6): δ (ppm) 12.17 (s, 1H, N-H, exchangeable with D_2O), 7.45-7.47 (m, 2H, ArH), 7.07-7.16 (m, 2H, ArH), 5.53 (broad s, 1H, -OH exchangeable with D_2O), 4.68 (s, 2H,-CH_2-).

5(6)-Chloro-2-hydroxymethylbenzimidazole ($\mathbf{L}^2$)**:** Yield 34%. ^{1}H-NMR (DMSO-d_6): δ (ppm) 12.50 (broad s, 1H, N-H, exchangeable with D_2O), 7.53-7.48 (m, 2H, ArH), 7.17-7.15 (m, 1H, ArH), 5.75 (broad t, J = 5.2 Hz, 1H, -OH exchangeable with D_2O), 4.69 (d, J = 4.8 Hz, 2H, -CH_2-).

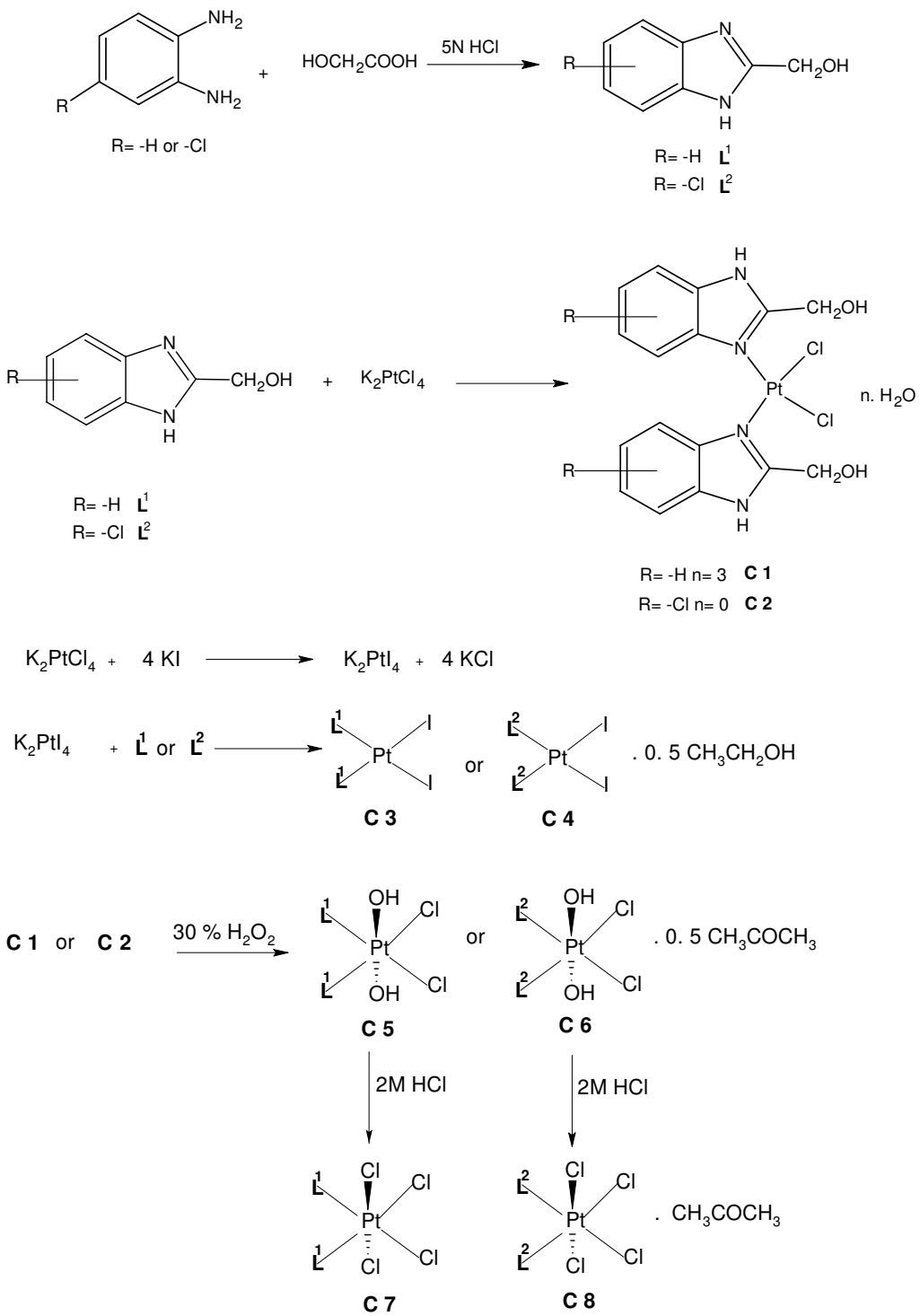

Figure. Synthesis of the carrier ligands, and platinum(II) and platinum(IV) complexes.

Synthesis of the platinum(II) complexes

Complexes *cis*-[dichloro-di(2-hydroxymethylbenzimidazole)platinum(II)] (**C1**) and *cis*-[dichloro-di(5(6)-chloro-2-hydroxymethylbenzimidazole)platinum(II)] (**C2**) were synthesized as described previously[14] by the reac-

tion of $\mathbf{L}^1$ or $\mathbf{L}^2$ and K_2PtCl_4 in ethanol/water solution as presented in the Figure.

***cis*-[Dichloro-di(2-hydroxymethylbenzimidazole)platinum(II)].3H_2O [$PtL^1_2Cl_2$].3H_2O (C1)**

Yield 25%. ^{1}H-NMR (DMSO-d_6): δ (ppm) 13.41 (s, 2H, 2x N-H, exchangeable with D_2O), 8.14 (d, J= 8.97 Hz, 2H, ArH), 7.57-7.37 (m, 6H, ArH), 6.28 (s, 2H, 2x O-H, exchangeable with D_2O), 5.48 (dd, J = 4.0 and 16.4 Hz, 2H, -CH_2-), 5.07-4.92 (m, 2H, -CH_2-). IR (KBr): υ 330, 318 (Pt-Cl) cm^{-1}. Anal. Calcd. for $C_{16}H_{16}Cl_2N_4O_2Pt.3H_2O$: C, 31.18; H, 3.60; N, 9.09. Found: C, 31.12; H, 3.42; N, 9.30.

***cis*-[Dichloro-di(5(6)-chloro-2-hydroxymethylbenzimidazole)platinum(II)] [$PtL^2_2Cl_2$] (C2)**

Yield 42%. ^{1}H-NMR (DMSO-d_6): δ (ppm) 13.29 (broad s, 2H, 2x N-H, exchangeable with D_2O), 8.16-7.79 (m, 2H, ArH), 7.36-7.17 (m, 4H, ArH), 6.12 (broad s, 2H, 2x O-H, exchangeable with D_2O), 5.42-4.69 (m, 4H, 2x -CH_2-). IR (KBr): υ 330 and 310 (Pt-Cl) cm^{-1}. Anal. Calcd. for $C_{16}H_{14}Cl_4N_4O_2Pt$: C, 30.44; H, 2.23; N, 8.87. Found: C, 30.09; H, 2.07; N, 8.80.

***cis*-[Diiodo-di(2-hydroxymethylbenzimidazole)platinum(II)] [$PtL^1_2I_2$] (C3)**

K_2PtCl_4 (0.422 g, 1.02 mmol) and KI (0.678 g, 4.08 mmol) were dissolved in water (15 mL) and stirred at 60 °C for 45 min. Then $\mathbf{L}^1$ (0.300 g, 2.03 mmol) in a 2/8 ethanol/water mixture (10 mL) was added dropwise to the resulting K_2PtI_4. The reaction mixture, protected from light, was heated at 60 °C for 2 days. The resulting yellowish precipitate was filtered off, washed several times with small portions of water, ethanol, and diethylether, and dried in vacuo. Yield 36%. ^{1}H-NMR (DMSO-d_6): δ (ppm) 13.18 (s, 2H, 2x N-H, exchangeable with D_2O), 8.13-8.00 (m, 2H, ArH), 7.36-7.12 (m, 6H, ArH), 6.18 (t, J = 5.4 Hz, 1H, O-H, exchangeable with D_2O), 6.10 (t, J = 5.6 Hz, 1H, O-H, exchangeable with D_2O), 5.27-5.21 (m, 2H, -CH_2-), 5.00 (dd, J =5.3 Hz, and 16.00 Hz, 1H, -CH_2-), 4.54 (dd, J =5.7 Hz, and 16.00 Hz, 1H, -CH_2-). Anal. Calcd. for $C_{16}H_{16}I_2N_4O_2Pt$: C, 25.79; H, 2.16; N, 7.52. Found: C, 26.19; H, 2.03; N, 7.38.

***cis*-[Diiodo-di(5(6)-chloro-2-hydroxymethylbenzimidazole)platinum(II)].0.5 CH_3CH_2OH, [$PtL^2_2I_2$].0.5 CH_3CH_2OH (C4)**

A procedure similar to that described for **C3** was carried out using $\mathbf{L}^2$ (0.370 g, 2.03 mmol) and K_2PtCl_4 (1.000 g, 2.40 mmol) at 60 °C for 3 days. Yield 60%. ^{1}H-NMR (DMSO-d_6): (δ ppm) 13.47 (t, J =32.3 Hz, 2H, 2x N-H, exchangeable with D_2O), 8.28-8.11 (m, 2H, ArH), 7.53-7.29 (m, 4H, ArH), 6.30-6.15 (m, 2.5H, 2x O-H and 0.5 CH_3CH_2OH exchangeable with D_2O), 5.27–5.34 (m, 2H, -CH_2-), 4.97–5.08 (m, 1H, -CH_2-), 4.47–4.51 (m, 1H, -CH_2-), 3.45 (q, J = 1.7 Hz, 1H, 0.5 CH_3CH_2OH), 1.06 (t, J =7.0 Hz, 1.5H, 0.5 CH_3CH_2OH). Anal. Calcd. for $C_{16}H_{14}Cl_2I_2N_4O_2Pt$. 0.5 CH_3CH_2OH: C, 24.39; H, 2.04; N, 6.69. Found: C, 24.86; H, 2.14; N, 6.52.

Synthesis of the platinum(IV) complexes

***cis, trans, cis*-[Dichloro-dihydroxy-di(2-hydroxymethylbenzimidazole)platinum (IV)] [$PtL^1_2Cl_2(OH)_2$] (C5)**

To a suspension of **C1** (0.140 g, 0.23 mmol) in water (15 mL) was added an aqueous solution of 30% of H_2O_2 (1 mL), and the solution was stirred at 60 °C for 5 days. The resulting pale yellow precipitate was filtered off, washed several times with small portions of water, ethanol, acetone, and diethylether, and

dried in vacuo. Yield 46%. ^{1}H-NMR (DMSO-d_6): δ (ppm) 13.50 (s, 2H, 2x N-H, exchangeable with D_2O), 8.03-7.96 (m, 2H, ArH), 7.49-7.23 (m, 6H, ArH), 6.22 (broad s, 2H, 2x –OH, exchangeable with D_2O), 5.36 (d, J =47.2 Hz, 2H, -CH_2-), 4.83 (dd, J =20.0 and 47.2 Hz, 2H, -CH_2-), 2.89 (s, 1H, Pt-OH, exchangeable with D_2O), 2.74 (s, 1H, Pt-OH, exchangeable with D_2O). IR (KBr): υ 540 (Pt-O) and 340 (Pt-Cl) cm^{-1}. Anal. Calcd. for $C_{16}H_{18}Cl_2N_4O_4Pt$: C, 32.22; H, 3.04; N, 9.39. Found: C, 32.03; H, 3.40; N, 8.99.

***cis, trans, cis*-[Dichloro-dihydroxy-di(5(6)-chloro-2-hydroxymethylbenzimidazole) platinum(IV)].0.5 CH_3COCH_3, [$PtL^2_2Cl_2(OH)_2$].0.5 CH_3COCH_3 (C6)**

A procedure similar to that described for **C5** was carried out using **C2** (0.150 g, 0.23 mmol) at 60 °C for 5 days. Yield 46%. ^{1}H-NMR (DMSO-d_6): δ (ppm) 13.27 (broad s, 2H, 2x N-H, exchangeable with D_2O), 8.40-6.90 (m, 6H, ArH), 6.12 (broad s, 2H, 2x O-H, exchangeable with D_2O), 5.40-4.45 (m, 4H, 2x -CH_2-), 2.80 (s, 1H, Pt-OH, exchangeable with D_2O), 2.64 (s, 1H, Pt-OH, exchangeable with D_2O), 1.80 (s, 3H, 0.5 CH_3COCH_3). IR (KBr): υ 1654 (C=O), 545 (Pt-O) and 335 (Pt-Cl) cm^{-1}. Anal. Calcd. for $C_{16}H_{16}Cl_4N_4O_4Pt$.0.5 CH_3COCH_3: C, 30.28; H, 2.76; N, 8.07 Found: C, 30.60; H, 2.85; N, 8.52.

***cis*-[Tetrachloro-di(2-hydroxymethylbenzimidazole)platinum(IV)] [$PtL^1_2Cl_4$] (C7)**

C5 (0.060 g, 0.10 mmol) was dissolved in aqueous 2 M HCl (5 mL) and heated at 60 °C for 1 day. The resulting precipitate was filtered off, washed several times with small portions of water, ethanol, acetone, and diethylether, and dried in vacuo. Yield 23%. ^{1}H-NMR (DMSO-d_6): δ (ppm) 13.73 (broad s, 2H, 2x N-H, exchangeable with D_2O), 8.04-7.16 (m, 8H, ArH), 6.13 (broad s, 2H, 2x O-H, exchangeable with D_2O), 5.49-4.38 (m, 4H, 2x -CH_2-). IR (KBr): υ 340 (Pt-Cl) cm^{-1}. Anal. Calcd. for $C_{16}H_{16}Cl_4N_4O_2Pt$: C, 30.34; H, 2.54; N, 8.84 Found: C, 29.90; H, 2.90; N, 8.93.

***cis*-[Tetrachloro-di(5(6)-chloro-2-hydroxymethylbenzimidazole)platinum(IV)] .CH_3 $COCH_3$, [$PtL^2_2Cl_4$].CH_3COCH_3(C8)**

A procedure similar to that described for **C7** was carried out using **C6** (0.150 g, 0.23 mmol) at 60 °C for 1 day. Yield 64%. ^{1}H-NMR (DMSO-d_6): δ (ppm) 13.70 (broad s, 2H, 2x N-H, exchangeable with D_2O), 8.10-7.01 (m, 6H, ArH), 6.61 (s, 1H, -OH, exchangeable with D_2O), 6.56 (s, 1H, -OH, exchangeable with D_2O), 5.40-4.38 (m, 4H, 2x -CH_2-), 1.77 (s, 6H, CH_3COCH_3). IR (KBr): υ 1697 (C=O) and 342 (Pt-Cl) cm^{-1}. Anal. Calcd. for $C_{16}H_{14}Cl_6N_4O_2Pt.CH_3COCH_3$: C, 30.02; H, 2.65; N, 7.37 Found: C, 30.53; H, 2.53; N, 7.94.

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 as "leaving ligands", and the platinum(IV) complexes, which were the oxidation products of the platinum(II) complexes, **C5-C8,** with axial chloro or hydroxo ligands were determined on the human MCF-7 and HeLa cell lines. MCF-7 and HeLa cells were incubated for 72 h with 40, 20, 10, 5, 1, 0.5 μM of the platinum(II) and platinum(IV) complexes and cisplatin and carboplatin were used as reference compounds. The antiproliferative activity values of the complexes and the reference compounds expressed as $T/C_{corr.}$ are presented in Tables 1 and 2.

Table 1. Cytotoxic activities of the platinum(II) and platinum(IV) complexes on the MCF-7 cell line.

	$[T/C]_{corr.}$ (%) (S.D.)[a]					
Compound	40 μM	20 μM	10 μM	5 μM	1 μM	0.5 μM
C1	12.11 ± 7.07	44.56 ± 5.66	68.15 ± 1.41	83.50 ± 6.36	> 100	> 100
C2	5.31 ± 1.46	44.58 ± 0.93	76.11 ± 0.36	92.21 ± 4.70	> 100	> 100
C3	-25.00 ± 9.01[b]	8.39 ± 11.87	30.39 ± 4.70	59.69 ± 10.84	> 100	> 100
C4	-27.01 ± 10.71[b]	19.52 ± 1.74	49.29 ± 2.93	73.64 ± 11.61	> 100	> 100
C5	32.78 ± 7.25	22.33 ± 4.72	62.89 ± 13.19	81.41 ± 1.22	> 100	> 100
C6	36.97 ± 5.93	84.26 ± 4.38	94.55 ± 2.46	> 100	> 100	> 100
C7	27.34 ± 4.55	70.30 ± 2.08	84.13 ± 4.76	> 100	> 100	> 100
C8	20.64 ± 4.04	66.27 ± 3.08	80.63 ± 8.85	> 100	> 100	> 100
Cisplatin	-49.23 ± 6.25[b]	-42.60 ± 9.23[b]	-22.30 ± 3.00[b]	10.22 ± 5.12	66.70 ± 9.10	82.50 ± 4.9
Carboplatin	-9.95 ± 9.25[b]	-9.10 ± 3.51[b]	87.22 ± 20.17	81.54 ± 1.32	> 100	> 100

[a] S.D. Standard deviation.
[b] Cytocidal effect.

Table 2. Cytotoxic activities of the platinum(II) and platinum(IV) complexes on the HeLa cell line.

	$[T/C]_{corr.}$ (%) (S.D.)[a]					
Compound	40 μM	20 μM	10 μM	5 μM	1 μM	0.5 μM
C1	6.67 ± 3.55	32.25 ± 5.65	67.63 ± 10.61	93.85 ± 2.13	> 100	> 100
C2	24.72 ± 3.39	54.25 ± 2.82	88.13 ± 4.24	> 100	> 100	> 100
C3	-40.31 ± 13.47[b]	37.65 ± 4.53	61.70 ± 1.19	74.24 ± 1.47	> 100	> 100
C4	-30.24 ± 7.09[b]	62.77 ± 3.53	86.45 ± 2.54	> 100	> 100	> 100
C5	34.73 ± 2.73	64.74 ± 3.65	74.34 ± 5.68	> 100	> 100	> 100
C6	72.55 ± 5.78	96.56 ± 5.58	> 100	> 100	> 100	> 100
C7	55.29 ± 1.67	82.93 ± 17.61	85.84 ± 1.78	> 100	> 100	> 100
C8	67.28 ± 3.23	80.90 ± 11.91	90.34 ± 7.14	90.54 ± 13.70	> 100	> 100
Cisplatin	-53.12 ± 1.59[b]	-55.26 ± 4.46[b]	-54.22 ± 4.25[b]	-53.18 ± 1.48[b]	12.50 ± 1.8	48.2 ± 8.34
Carboplatin	16.67 ± 9.87	56.75 ± 14.78	69.91 ± 12.28	85.23 ± 4.14	> 100	> 100

[a] S.D. Standard deviation.
[b] Cytocidal effect.

Discussion

Synthesis and characterization of the complexes

The ligands $\mathbf{L}^1$ and $\mathbf{L}^2$, used as "non-leaving ligands" in the structure of the platinum(II) and platinum(IV) complexes, were prepared according to the Phillips method as shown in the Figure. The ligands had been reported previously and their melting points and IR and ^{1}H-NMR spectral data were in accordance with the literature.[14]

The procedures of the synthesis of platinum(II) and platinum(IV) complexes are shown in the Figure. Platinum(II) complexes of the type $[Pt(\mathbf{L}^1 \text{or } \mathbf{L}^2)_2Cl_2]$ (**C1** or **C2**) were synthesized as described previously by the reaction of the corresponding ligands and K_2PtCl_4 in ethanol/water solution. IR and ^{1}H-NMR spectral data of the complexes **C1** and **C2** were in accordance with the data reported previously.[14]

Reaction of K_2PtCl_4 with an excess of KI produced K_2PtI_4 in solution. $K_2PtI_4$was then reacted with 2 equivalents of $\mathbf{L}^1$ or $\mathbf{L}^2$to obtain platinum(II) complexes of the type $[Pt(\mathbf{L}^1 \text{or } \mathbf{L}^2)_2I_2]$ (**C3** or **C4**).

The dichloro complexes $[Pt(\mathbf{L}^1 \text{ or } \mathbf{L}^2)_2Cl_2]$ were used as the precursor for platinum(IV) complexes **C5**-**C8**.

Platinum(II) complexes of the type [Pt($\mathbf{L}^1$or $\mathbf{L}^2$)$_2$Cl$_2$] were oxidized with 30% H_2O_2 to form corresponding axial dihydroxyplatinum(IV) complexes **C5** and **C6**. Complexes **C7** and **C8** were obtained by the reaction of the dihydroxyplatinum(IV) complexes with 2 M HCl.

The chemical structures of the platinum(II) and platinum(IV) complexes were characterized by their elemental analyses and ^{1}H-NMR and IR spectra compared with those of the ligands. The elemental analyses and spectroscopic data confirm the structure of the complexes.

For complexes **C1**, **C4**, **C6**, and **C8** molecules of water, ethanol, acetone, and acetone, respectively, were included as justified by the analytical results. With the exception of water molecules (for **C1**) these were also observed clearly in the ^{1}H-NMR spectra of the complexes. It is expected that **C2**, **C4**, **C6,** and **C8** bearing ligand $\mathbf{L}^2$ are probably a mixture of constitutional isomers since more than one isomer could be formed from $\mathbf{L}^2$ upon coordination to platinum. Similar IR and ^{1}H-NMR spectral data have been reported in our previous papers for platinum(II) complexes of differently substituted benzimidazoles. Most of the general points have been discussed in the previous papers.[14,16−18,23]

In their IR spectra the ligands $\mathbf{L}^1$ and $\mathbf{L}^2$ show very broad bands in the region 3500-2300 cm^{-1} due to the inter-molecular hydrogen bonded imidazole N-H. The platinum(II) complexes exhibited N-H stretching bands centered at 3230 cm^{-1} sharper than those of the free ligands due to breaking of tautomerism.[24,25]

In the IR spectra of the platinum(IV) complexes **C5**-**C8**, N-H stretching vibrations appeared at highest frequencies from 3443 to 3650 cm^{-1}. Being influenced by oxidation states of the central metal, the N-H vibrations in platinum(IV) compounds were broad in comparison with those in platinum(II) compounds.[26]

The platinum(II) complexes **C1** and **C2** showed in their IR spectra characteristic Pt-Cl stretching vibrations in the region 310-330 cm^{-1}. These bands were shifted (∼10 cm^{-1}) towards higher frequencies in the spectra of platinum(IV) **C5**-**C8** upon oxidation.[27] The complexes **C5**-**C8** exhibit only one υ (Pt-Cl) absorption; this is consistent with the literature.[28] (Pt-I) band of the *cis*-diiodo complexes should show 195-183 cm^{-1}in the far-IR region of the complexes spectra.[29] The υ (Pt-I) stretching bands for **C3** and **C4** could not be measured on the spectrophotometer used.

The hydroxyplatinum(IV) complexes **C5** and **C6**, which were obtained by the oxidation reactions of **C1** and **C2** with hydrogen peroxide, were readily identified by their characteristic PtO-H stretching bands at 3480 and 3445 cm^{-1} and Pt-O stretching bands at 540 and 545 cm^{-1}, respectively.[30,31]

The insolubility of the complexes in the other organic solvents made it necessary to record ^{1}H-NMR spectra in dimethylsulfoxide-d_6 (DMSO-d_6). All ^{1}H-NMR measurements were recorded immediately in order to avoid the ligand exchange reaction between the platinum complexes synthesized and DMSO-d_6. Almost all signals were shifted upon complexation as the result of the electric field effect caused by complexation.

The large downfield shifts in the imidazole N-H signal in the spectra of the complexes with respect to their ligands are a result of an increase in the N-H acid character after platinum binding.[32] That the 4 protons are not chemically equivalent is indicated by the split ^{1}H-NMR signals corresponding to the 2 methylene groups of the platinum complexes. The splittings are probably due to restricted rotation of the methylene groups. The ^{1}H-NMR signals of the platinum complexes tend to broaden, which is consistent with the literature.[33]

^{1}H-NMR spectra of **C5** and **C6** showed clearly 2 distinguished singlets for -OH ligands.

Preliminary cytotoxicity test

In the test on the human MCF-7 and HeLa cancer cell lines at 0.5 and 1 μM concentration $T/C_{corr.}$ values of all complexes synthesized and the reference compound carboplatin were >100. At these concentrations on the HeLa cell line the reference compound cisplatin possessed considerably higher activity.

At a dose of 5 and 10 μM (except for **C3** at 10 μM concentration on MCF-7 cell line) no significant cytotoxicity on MCF-7 and HeLa cell lines was observed for any of the complexes synthesized.

On the MCF-7 cell line at 10 μM concentration among the complexes synthesized, platinum(II) complexes bearing iodo ligands, **C3** and **C4**, were found to be the most active compounds, with $T/C_{corr.}$ values of around 30% and 49%, respectively.

Considerable $T/C_{corr.}$ values of all the complexes synthesized on the MCF-7 cell line were at 40 μM concentration. At this concentration cytocidal effects were obtained for **C3** and **C4**. A clear antiproliferative effect on the HeLa cell line was observed only for complexes **C1-C4** by increasing the concentration to 40 μM.

All complexes tested showed a concentration-dependent reduction in 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.

The overall potencies of the platinum(IV) complexes were less than those obtained for the platinum(II) complexes and the reference compounds cisplatin and carboplatin.

It is generally thought that since platinum(IV) complexes are inert on ligand substitution reactions relative to their platinum(II) analogues they must be reduced to platinum(II) species before binding to DNA. The reduction potentials of diam(m)ine platinum(IV) complexes are dependant on the nature of the axial and equatorial ligands, but the axial ligands generally exert the stronger influence.[11] On the other hand, Choi et al.[12] studied a range of complexes and suggested that the rate of reduction of platinum(IV) complexes depended on the bulkiness of the equatorial ligands. Although more factors are involved for the cytotoxicity of platinum(IV) complexes, including membrane transport, absorption, reduction, and possibly direct interaction with DNA, the steric hindrance of the "non-leaving ligands" of the platinum(IV) complexes (**C5-C8**) may be related to their lower cytotoxicity than the corresponding platinum(II) complexes.

Conclusion

In general, it was found that the platinum(II) complexes were more active than the platinum(IV) complexes. The complexes, which were found to be less active than cisplatin, exhibited moderate cyctotoxicity comparable to carboplatin on the MCF-7 and HeLa cell lines.

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