Synthesis, Cytotoxicity, and DNA Interactions of New Cisplatin Analogues Containing Substituted Benzimidazole Ligands

Fatma Gümüş,*[,†] Gökçen Eren,[†] Leyla Açık,[‡] Ayten Çelebi,[§] Fatma Öztürk,[‡] Şükran Yılmaz,[∞] Rahşan Ilıkçı Sağkan,[#] Sibel Gür,[⊥,×] Aykut Özkul,[⊥] Ayhan Elmalı,[||] and Yalçın Elerman[||]

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey, Department of Biology, Faculty of Art and Science, Gazi University, 06500 Ankara, Turkey, Department of Biology, Faculty of Art and Science, Kirikkale University, 71450 Kirikkale, Turkey, Cell and Virus Bank Department, Foot and Mouth Disease Institute, Ankara, Turkey, Department of Immunology, Gulhane Military Medicinal Academia, Ankara, Turkey, Department of Virology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey, and Department of Engineering Physics, Faculty of Engineering, Ankara University, 06100 Ankara, Turkey

Received January 31, 2008

Six new platinum(II) complexes with 1-H or methyl-2-chloromethyl or acetoxymethyl or 2′-hydroxyethyl-benzimidazole carrier ligands were synthesized and evaluated for their reactivity against model nucleophile I^-, cellular uptake, and in vitro antiproliferative activities against the human MCF-7 breast and HeLa cervix cancer cell lines. The effect of the compounds on pBR322 plasmid DNA was studied by gel electrophoretic mobility measurements. Flow cytometric analysis was also carried out to study the effect of representative compounds **1** and **2**, bearing 2-chloromethyl or -acetoxymethylbenzimidazole carrier ligands, on the cell cycle distribution of MCF-7 and HeLa cells, respectively. In general, it was found that Pt(II) complexes were less cytotoxic than cisplatin and were comparable to carboplatin. The results of the plasmid DNA interaction and the restriction studies suggest that changing the chemical structure of the benzimidazole ligands may modulate DNA binding mode and the sequence selectivity. Compounds **1** and **2** had no significant effect on the cell cycle profile of the cells used. However, compound **2** induced a significant increase in the SubG1 cell population at a concentration of 20 μM.

Introduction

Cisplatin (*cis*-diamminedichloroplatinum(II)) is a widely used chemotherapeutic agent for the treatment of testicular cancer, and it is used in combination regimens for a variety of other tumors, including ovarian, cervical, bladder, lung, and those of the head and neck.[1] The clinical success of cisplatin is limited by significant side effects and acquired or intrinsic resistance. Therefore, much attention has focused on designing new platinum compounds with improved pharmacological properties and a broader range of antitumor activity.[2]

Although there is some evidence to suggest that other biological targets may be important in the mechanism, it is generally accepted that DNA is the primary biological target of the drug.[3] The platinum atom of cisplatin forms covalent bonds to the N7 positions of purine bases to afford primarily 1,2- or 1,3-intrastrand adducts and a lower number of interstrand cross-links.[4] It is generally believed that biological activity of cisplatin is associated with the recognition of its DNA adducts by cellular proteins such as repair enzymes, transcription factors, histones, and high mobility group (HMG) domain proteins.[5] It has been shown that major 1,2-intrastrand d(GpG) cross-link can create a hydrophobic notch at the site of the cross-link generated by the destacking of platinated guanines. This hydrophobic notch serves as a high affinity and specific binding site of damaged DNA recognition proteins, such as HMG domain proteins.[6] Binding of the HMG domain proteins to cisplatin−DNA lesions has been suggested to mediate the antitumor activity of the drug.[7,8] The specific binding of HMG domain proteins to the widened, hydrophobic minor groove can shield the major 1,2-intrastrand adducts from access of the nucleotide excision repair (NER) complex, thus preventing resistance.[9] An interesting concept for the design of new platinum antitumor drugs has been introduced on the basis of preventing resistance by enhancing this mechanism using more hydrophobic platinum compounds.[10]

There is evidence that other cellular repair mechanisms, such as mismatch repair (MMR), can affect antitumor efficacy of cisplatin and that dysfunction of this type of DNA repair may result in cisplatin resistance or tolerance.[5] Two models have been proposed to explain how MMR induces cell death. In the first model, MMR of mismatches that contain cisplatin−DNA guanine adducts leads to the removal and resynthesis of the paired cytidine base in the newly synthesized strand instead of removing the cisplatin−DNA lesions, which ultimately induces cell death. In the second model, recognition and binding of the MMR proteins to cisplatin adducts alone could be a signal that triggers cell cycle arrest or programmed cell death.[4,5]

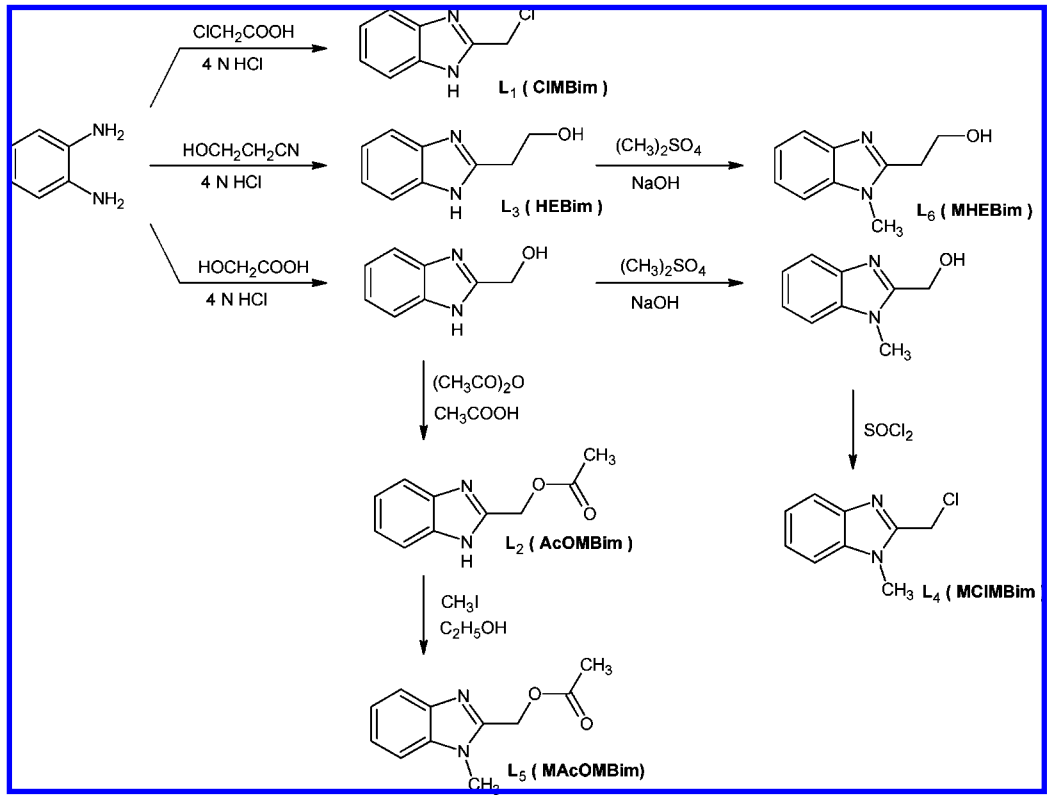
In metal−DNA binding the kinetics of the metal−ligand binding are more important than the thermodynamic binding.[11] In the amine part of cisplatin derivatives, substitutions allow the introduction of DNA binding or repelling side arms so that the kinetics of the DNA binding can be influenced by charge, hydrogen-bonding, or steric effects. The stabilization after platinum binding at guanines can be influenced in this way.[11]

The carrier ligand of the platinum DNA-damaging agents may affect the biodistribution, rates and type of DNA adduct formation, and recognition of damaged DNA by repair enzymes or regulatory/binding proteins.[12] The use of sterically demanding

* To whom correspondence should be addressed. Phone and Fax: +90-312-2120236. E-mail: fgumus@gazi.edu.tr or gumus53@gmail.com.
† Department of Pharmaceutical Chemistry, Gazi University.
‡ Department of Biology, Gazi University.
§ Kirikkale University.
∞ Foot and Mouth Disease Institute.
Gulhane Military Medicinal Academia.
⊥ Department of Virology, Ankara University.
× Current Address: Department of Virology, Faculty of Veterinary Medicine, Kocatepe University, Afyon, Turkey.
|| Department of Engineering Physics, Ankara University.

10.1021/jm8000983 CCC: $40.75
Published on Web 02/16/2009

Scheme 1



diamines as carrier ligands as an alternative compound to cisplatin can slow or block repair enzymes.[13] In addition, platinum complexes with DNA binding modes distinctively different from that of cisplatin may provide higher antitumor activity against cisplatin-resistant cancer cells.[14]

In previous studies, taking into consideration the fact that variations in the chemical structure of the ammine groups of the cisplatin can have significant effects on the cytotoxic activity and toxicity of platinum complexes, we synthesized some platinum(II) complexes with the 2-substituted benzimidazole ligands.[15–20] It was determined that some of these platinum complexes have in vitro cytotoxic activities on RD,[16] HeLa,[19] and MCF-7[17,19] cell lines. It was also determined that the DNA platinated with *cis*-$[Pt(L_2)Cl_2]\cdot H_2O$, where L is 5(6)-nonsubstituted or 5(6)-chloro-substituted 2-hydroxymethylbenzimidazole, was specifically recognized by the HMG domain protein HMG1.[21]

In the present study, six platinum(II) complexes having 1-H or methyl-2-chloromethyl or acetoxymethyl or 2′-hydroxyethylbenzimidazole carrier ligands were designed and synthesized as potential antitumor compounds on the basis of previous findings on some Pt(II) complexes of benzimidazole derivatives.[19–21] One of the major goals in the design of the new platinum(II) complexes was to produce possibly different types of adducts than those of cisplatin on the DNA, which may recognized and processed differently by cellular proteins. It was also aimed to investigate the role of the substituents on position 2 of the benzimidazole carrier ligands and the necessity of the free N1−H moiety of these ligands on the cytotoxic activity of platinum(II) complexes.

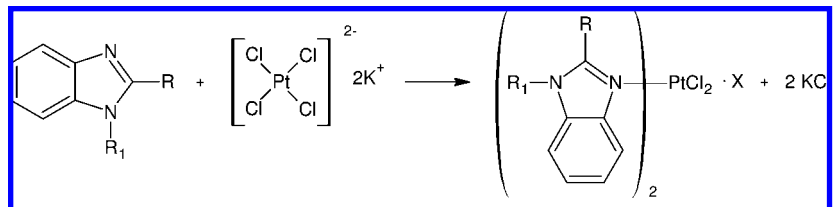
Benzimidazole ligands, chosen as the carrier ligands of the platinum(II) complexes, have four main features that could be important in the interaction of their platinum(II) complexes with DNA. (i) The benzimidazole nucleus is found in a variety of naturally occurring compounds such as vitamin B_{12} and its derivatives, and it is structurally similar to purin bases. (ii) The substituents on position 1 and/or position 2 of the benzimidazole ligands can induce notable changes in the electronic, steric, and hydrophobic properties of the compounds. (iii) The nonplaner benzimidazole ligands are flexible and bulky enough to affect the kinetics and cytotoxicities of the corresponding platinum(II) complexes. (iv) The benzimidazole ligands having acetoxy, hydroxyl, and/or free N1−H moiety which would have hydrogen-bond donor and/or acceptor properties, could facilitate novel types of lesions with cellular DNA, and might exhibit sequence selectivity.

In this paper, we report the synthesis and the initial results on the reactivity against model nucleophile I^-, the cellular uptake, in vitro testing of the preliminary antiproliferative activities on human MCF-7 breast and HeLa cervix cancer cell lines, and the plasmid DNA interactions of the new dichloro monosubstituted or disubstituted benzimidazole Pt(II) complexes. We also report the effect of the representative compounds on the cell cycle modification.

Results and Discussion

Chemistry. The ligands $\mathbf{L_1}-\mathbf{L_6}$, used as carrier ligands in the structure of the new Pt(II) complexes **1**−**6** were prepared according to the published procedures as shown in Scheme 1 and their melting points were in accordance with the literature.[22–27] The Pt(II) complexes **1**−**6** were synthesized (Scheme 2) and characterized by elemental analysis, fast atom bombardment mass spectrometry (FAB-MS), infrared (IR) spectroscopy, ^{1}H nuclear magnetic resonance (^{1}H NMR) spectrometry, and electrospray ionization liquid chromatography/mass spectrometry (ESI-LC/MS). For the characterization of the complexes by comparison with the data of the ligands, IR and ^{1}H NMR data of the ligands were also obtained. The structure of compound **2**, which was the only compound that could be

Scheme 2



Compound No.	R	R_1	X
1	$-CH_2Cl$	-H	$3H_2O$
2	$-CH_2OCOCH_3$	-H	2DMF
3	$-CH_2CH_2OH$	-H	H_2O
4	$-CH_2Cl$	$-CH_3$	-
5	$-CH_2OCOCH_3$	$-CH_3$	$6H_2O$
6	$-CH_2CH_2OH$	$-CH_3$	H_2O

crystallized among the compounds synthesized, was also determined by X-ray crystallography. Melting points and ^{1}H NMR data of ligands $\mathbf{L_1}$−$\mathbf{L_6}$, elemental analysis, IR, ^{1}H NMR, FAB-MS, and ESI-LC/MS data of compounds **1**−**6** and crystallographic data of compound **2** are presented in Supporting Information. For compounds **1**, **3**, **5**, **6**, and **2**, molecules of water and DMF were included as justified by the analytical results. All major species were identified by ESI-LC/MS. Both the retention times and the MS spectra of the peaks in samples are evidence of the purity and the expected structures of the compounds. From the IR data, the presence of hydration lattice water in the structure of these compounds except for compound **3** can be inferred.

The IR spectra of the compounds were measured in the region 4000−200 cm^{-1} and showed characteristic changes when compared to those of the free ligands. The Pt−N and Pt−Cl vibrations are considered characteristic for diamine−dichloro platinum complexes. However, the metal−nitrogen stretching bands could not be distinguished from other ring skeleton vibrations present in the spectra. According to the kinetic trans effect,[28] the synthesis method used is expected to yield complexes with cis geometry. In the far-IR region of the compounds' spectra a new broadband with a half-width of about 30 cm^{-1} appeared assigned to ν (Pt−Cl) centered at ∼325 cm^{-1}. It is well-known that *cis*-dichloro complexes should show two bands of medium intensity because the vibrations are additive, but in a lot of cases the second band is only a shoulder.[29] In some cases, *cis*-dichloro complexes show only one band due to low resolutions independent from cis or trans-configurations.[30] Although no shoulder was apparently observable at the Pt−Cl stretching band of the compounds **1**−**6**, the broad nature of the band suggested the presence of the bands overlapped in this domain. In addition, the position of the ν (Pt−Cl) bands of the complexes was at ∼325 cm^{-1} and the geometry can be assigned as cis.

The insolubility of the compounds in the other organic solvents made it necessary to record ^{1}H NMR spectra in dimethyl sulfoxide-d_6 (DMSO-d_6). All ^{1}H NMR measurements were recorded immediately in order to avoid ligand exchange reactions between the Pt(II) complexes and DMSO-d_6. The ^{1}H NMR spectrum of the Pt(II) complexes in DMSO-d_6 was indicative of complex formation and exhibited only one set of signals for the carrier ligands protons. The large downfield shifts in the imidazole N1−H signal in the spectra of the complexes with respect to their ligands are a result of an increase in the N1−H acid character after platinum binding.[31] The presence of the bulky ligands in the structures of compounds **1**−**6** has a large influence on the chemical shifts as evidenced by the multiplicity of some of the signals in the ^{1}H NMR spectra, since the rotation around the Pt−N bound is limited. The existence of only one set of signals in the ^{1}H NMR spectrum of compound **2**, which was recrystallized from DMF, suggests that the recrystallization procedure did not cause conversion to the trans isomer. For complexes having the N1-methyl group, two conformations, syn and anti, are believed to exist. For compounds **5** and **6** the integrated areas under these signals correspond to the ratios ∼1:2 and 2:1, respectively.

The synthesized compounds except **4** (compound **4** has limited solubility in the conditions of tests applied) were investigated for their biological activities.

Characterization of Compound 2 in the Solid State. The structure of compound **2** consists of discrete *cis*-$[Pt(L_2)_2Cl_2]$ molecules and two crystallization DMF molecules. An ORTEP view of the molecule with the DMF molecules is shown in Figure 1, and a selection of bond distances and angles are given in Table 1. The coordination geometry of the Pt center is square-planar, and Pt is surrounded by two N and two Cl atoms. The PtN_2Cl_2 square has a cis configuration, which is consistent with the preparation method. The most interesting feature of this structure is its head to head orientation exhibited by the two AcOMBim moieties. The dihedral angle formed by the two coordination planes of AcOMBim moieties is 73.55(9)°, and the whole molecule is not planar. The Pt−N and Pt−Cl bond lengths are comparable with the bond lengths reported in other analogous platinum complexes.[32,33]

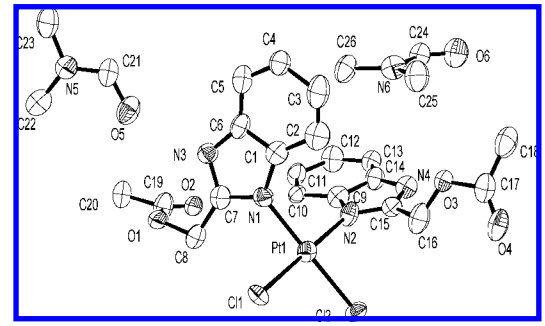


Figure 1. ORTEP view of compound **2**, *cis*-$[Pt(AcOMBim)_2Cl_2]\cdot$ 2DMF.

Table 1. Selected Bond Distances and Angles for Compound **2**, *cis*-[Pt(AcOMBim)$_2$Cl$_2$]·2DMF

	distance, Å
Pt−Cl1	2.2975(8)
Pt−Cl2	2.3150(10)
Pt−N1	2.077(3)
Pt−N1	2.099(3)
	angle, deg
N1−Pt1−N2	90.32(11)
N2−Pt1−Cl1	179.26(8)
N2−Pt1−Cl2	88.44(8)
N1−Pt1−Cl1	89.90(8)
N1−Pt1−Cl2	178.57(8)
Cl1−Pt1−Cl2	91.35(4)

Determination of the Reactivity of the Compounds against Model Nucleophile I$^-$. The antitumor platinum drugs are readily degraded into nonactive complexes by both glutathione and methionine.[34] This degradation occurs through the ability of the sulfur atoms to displace both the chloride and the monodentate amine ligands of some *cis*-Pt(II) complexes.[34,35] These interactions are generally believed to play a role in mechanisms underlying tumor resistance to platinum compounds, their inactivation, and side effects.

Recently, in a study on the reaction of Zn_7 metallothionein with *cis*- and *trans*-[Pt(N-donor)$_2$Cl$_2$] anticancer complexes, which bear less voluminous carrier ligands than those of the carrier ligands of our complexes, it has been reported that N-donor ligands of *cis*-[Pt(N-donor)$_2$Cl$_2$] compounds were replaced by cysteine thiolates but that the majority of *trans*-Pt(II) compounds retain their N-donor ligands.[35] In the study it has been determined that steric hindrance represents the major factor responsible for the different reactivities of Pt(II) drugs with Zn_7 metallothionein. It has also been reported that a lower reactivity of drugs could also be accomplished by modulating the electrophilicity of the Pt(II) center. It is expected that the steric hindrance and the electronic effect of the carrier ligands may influence the stability of the benzimidazole−platinum(II) complexes. Therefore, we have been particularly interested in examining whether the carrier ligands of *cis*-[PtL$_2$Cl$_2$] complexes **1**−**3**, **5**, and **6** are replaced by nucleophiles. For the reactivity studies we used iodide as nucleophile. In the literature, it was reported that from an experimental point of view, application of I$^-$ as nucleophile instead of bionucleophiles like nucleotides, oligonucleotides, and nucleophilic plasma components such as S-containing amino acids, peptides, or proteins was of considerable advantage, since it allows a fast and convenient quantitative determination of the products by HPLC.[36–42] It was also reported that in the stability studies of some antitumor active Pt(II) complexes, the use of iodide instead of the main plasma component albumin as nucleophile gave the same order of reactivity and allowed the assessment of the in vivo stability.[38]

In this study, the reactivity of compounds **1**−**3**, **5**, and **6** against attack of strong nucleophile I$^-$ was monitored by ESI-LC/MS spectroscopy (for experimental details, see Supporting Information). The reactions of the compounds with KI were carried out under pseudo-first-order conditions using excess potassium iodide. The starting and the reaction products were well separable and identified by ESI-LC/MS. Platinum containing ions were distinguishable by the *m*/*z* cluster value resulting from the presence of isotopes. The Pt content of the collected fractions was determined by inductively coupled plasma mass spectrometry (ICP-MS) under the conditions presented in Supporting Information.

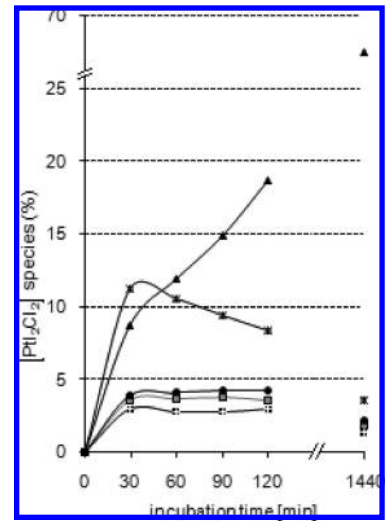


Figure 2. Time dependence of the formation of PtI_2Cl_2 after incubation of the compounds *cis*-[PtL$_2$Cl$_2$] (**1**−**3**, **5**, and **6**) (L = **L$_1$**−**L$_3$**, **L$_5$**, and **L$_6$**) with excess aqueous KI solution at 37 °C. For compounds: **1** (▲); **2** (grey box); **3** (grey star); **5** (black cross); **6** (●).

When the compounds were reacted with excess KI for 30, 60, 90, 120, and 1440 min, for all compounds tested two major peaks were observed that were due to the [PtI$_2$Cl$_2$] species ([M − 2L + 2I + Na]$^+$ and unchanged [PtL$_2$Cl$_2$]. For compounds **1**, **2**, and **3**, which are the N1−H free ligands, [M − 2Cl + 2I]$^+$, [M − 2Cl + 2I − 2OAc]$^+$, and [M − 2Cl + 2I]$^+$ species, respectively, could also be identified. Under the employed reaction conditions aquated intermediates are not detectable. Mass spectrometry also revealed the formation of other minor degradation species. The relative amounts of [PtI$_2$Cl$_2$] species, as a percentage of total Pt, are shown in Figure 2.

Although it was not in a significant amount, the initial reaction of the compounds tested with KI leads to loss of the carrier ligands and rapid formation of [PtI$_2$Cl$_2$] species. It is noteworthy that for all the compounds tested except for compound **1** the amount of the [PtI$_2$Cl$_2$] species did not change significantly after the incubation time of 30 min.

On the basis of the % amount of [PtI$_2$Cl$_2$], the relative order of the reactivity of the compounds tested assessed from the 24 h values is **1** ≫ **3** > **6** > **2** > **5**.

Compounds **5** and **6** having N1-methyl moiety in their structures were slightly more stable compounds against model nucleophile I$^-$ than their N1−H free analogues. This finding must be taken into consideration for the design of new benzimidazole−platinum(II) complexes in future studies. The less reactivity of compounds **5** and **6** may be partly related to electron releasing effect of their methyl groups.

The specific interactions between the two carrier ligands may also play a role in the stability of the complexes. Compound **1** lost its carrier ligands in a higher amount than the other compounds tested. The driving force for the dissociation of ClMBim carrier ligands from compound **1** in the test conditions used might be the steric and electronic repulsion between the two chloromethyl substituents.

Cytotoxicity. The preliminary in vitro antiproliferative activities of the new sterically hindered benzimidazole−platinum(II) complexes **1**−**3**, **5**, and **6** and cisplatin and carboplatin used as reference compounds were determined on the human MCF-7 breast and HeLa cervix cancer cell lines (for experimental procedure, see Supporting Information). The antiproliferative activity values of the compounds tested expressed as T/C_{corr} are presented in Figures 3 and 4. IC_{50} values of the platinum

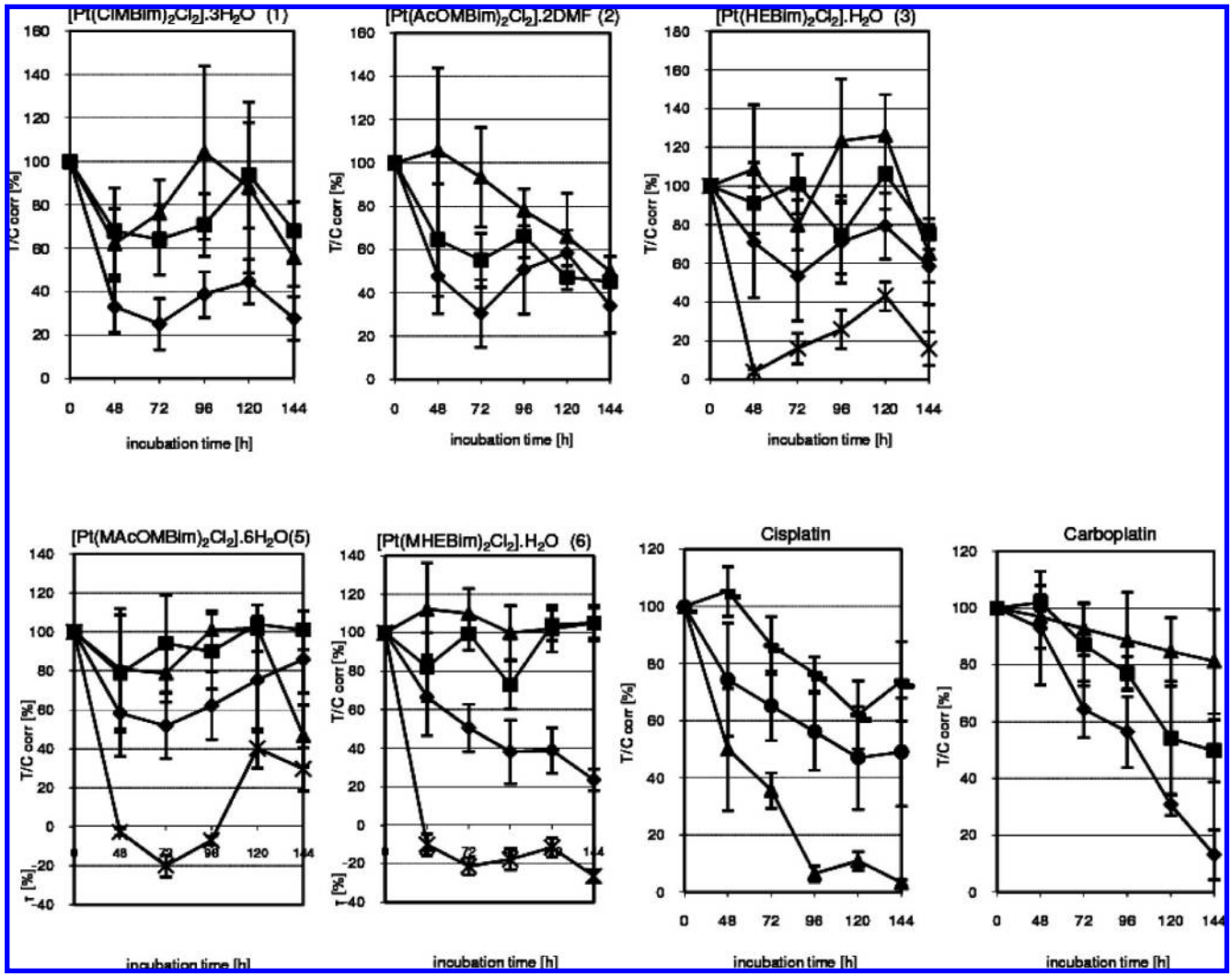


Figure 3. Antiproliferative effects of the benzimidazole−platinum(II) complexes (**1**−**3**, **5**, **6**), cisplatin, and carboplatin on the MCF-7 cell line (−, 0.5 μM; ●, 1 μM; ▲, 5 μM; ■, 10 μM; ◆, 20 μM; ×, 40 μM).

complexes tested, calculated (using Prism4, GraphPad Software) from the dose−survival curves for the growth inhibition of both cell lines used for each incubation time measured, are presented in Table 2. For comparison, in vitro antiproliferative activities of the ligands $\mathbf{L_1}$−$\mathbf{L_3}$, $\mathbf{L_5}$, and $\mathbf{L_6}$ against both cell lines used were also performed. IC_{50} values of the ligands are the averages of two independent determinants and are presented in Table 3.

In the test on the human MCF-7 cell line at 0.5, 1, 5, and 10 μM concentrations (except for compound **2** and carboplatin at 10 μM concentration) T/C_{corr} values of all the compounds synthesized and the reference compound carboplatin were >100 or not a considerable amount. Cisplatin possessed a considerably higher activity and almost completely blocked the growth of the MCF-7 cells at a concentration of 5 μM at the last measuring time point, 144 h.

Although compound **2** was moderately cytotoxic at 10 μM concentration at the last measuring time point (T/C_{corr} = 45.15% at 10 μM, t = 144 h), there seemed to be no great difference between the antiproliferative activity values of compounds **1**, **2**, and **6** at 20 μM concentration (T/C_{corr} = 27.20%, 34.03%, and 23.61%, respectively, at 20 μM, t = 144 h). For compounds **3** and **5** no significant cytotoxicity was observed over a period of 144 h at 20 μM. At 40 μM, they showed a significant reduction in cell growth (T/C_{corr} = 15.97% and 18.63%, respectively, t = 144 h).

In contrast to cisplatin and carboplatin, which reached their maximum effects at the last measuring time point (T/C_{corr} = 3.32% at 5 μM, t = 144 h, and T/C_{corr} = 13.20% at 20 μM, t = 144 h, respectively) compounds **1**, **2**, **3**, and **5** reached their maximum effects at an incubation time of 72 h (T/C_{corr} = 25.13%, 30.59%, 53.57%, and 51.79%, respectively, at 20 μM, t = 72 h). Compound **6** required up to 144 h to reach its maximum effect.

At a concentration of 40 μM, cytocidal effects were observed for compounds **1**, **2**, and **6** at all the time points measured against MCF-7 cell line (data not shown except for **6**).

In the test on the human HeLa cell line at dosages of 0.5, 1, 5, and 10 μM, T/C_{corr} values of all the compounds synthesized (except for compound **2** at 10 μM concentration (T/C_{corr} = 49.31%, t = 96 h)) were >100 or did not amount to considerable values.

Compound **2** was found to be more active than carboplatin and caused a significant reduction of cell growth at 20 μM concentration (T/C_{corr} = 44.07%, 23.16%, 4.98%, and 2.43%; t = 48, 72, 96, and 120 h, respectively). At 20 μM concentration, compound **6** resulted in an almost 2-fold increase in cytotoxicity (T/C_{corr} = 11.32%, 19.76%, 25.04%, and 24.74%; t = 48, 72, 96, and 120 h, respectively) relative to compounds **1** and **5** in the HeLa cell line.

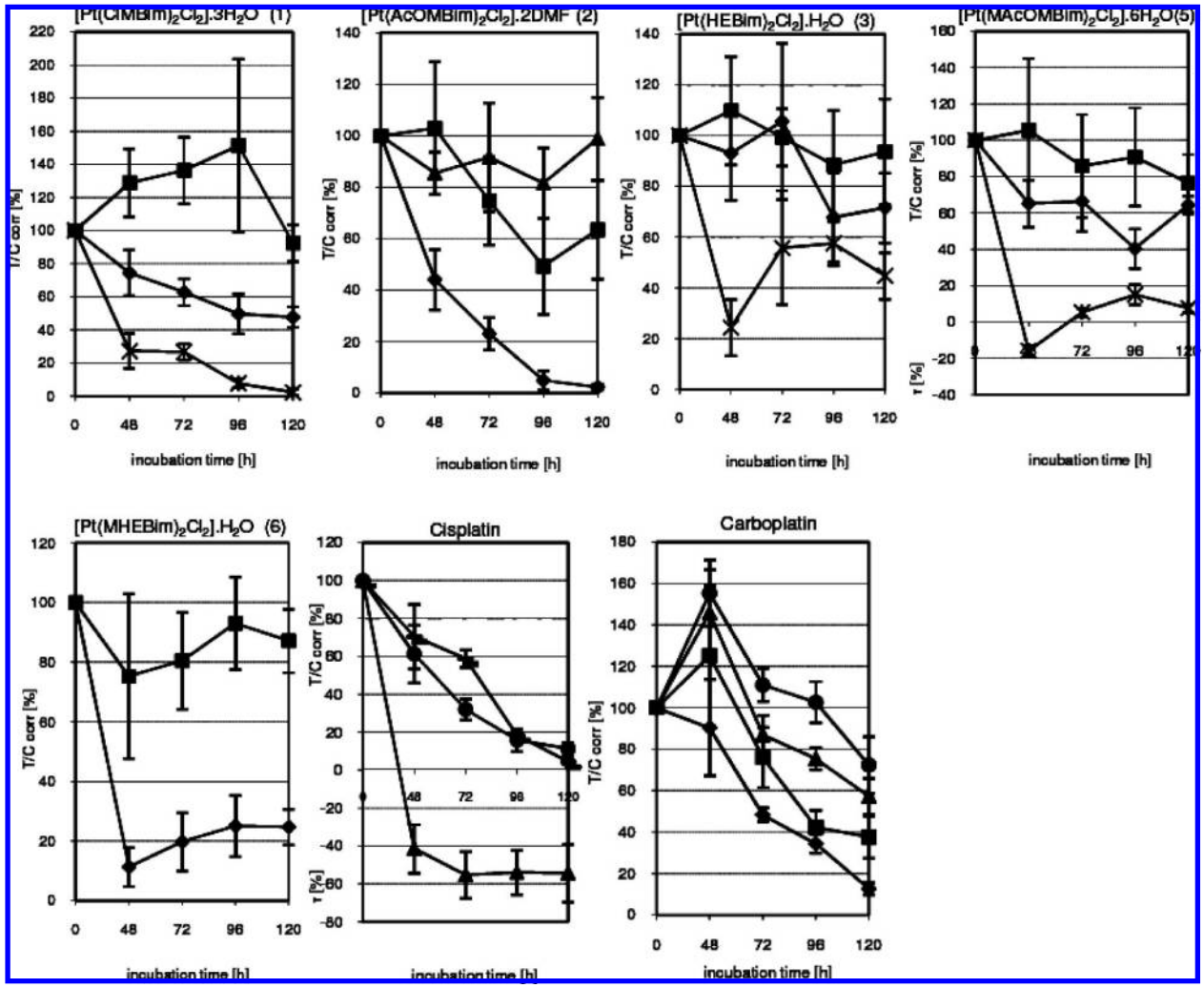


Figure 4. Antiproliferative effects of the benzimidazole−platinum(II) complexes (**1**−**3**, **5**, **6**), cisplatin, and carboplatin on the HeLa cell line (−, 0.5 μM; ●, 1 μM; ▲, 5 μM; ■, 10 μM; ◆, 20 μM; ×, 40 μM).

Table 2. IC_{50} Values Obtained for Compounds **1**−**3**, **5**, **6**, Cisplatin, and Carboplatin

	IC_{50} (μM)								
	MCF-7					HeLa			
compd	48 h	72 h	96 h	120 h	144 h	48 h	72 h	96 h	120 h
1	13.73 ± 2.20	12.96 ± 1.49	15.84 ± 4.15	19.88 ± 7.98	11.43 ± 4.79	29.39 ± 1.32	29.16 ± 3.09	18.90 ± 1.03	16.04 ± 1.22
2	16.68 ± 2.05	13.49 ± 3.42	19.25 ± 9.76	14.04 ± 6.49	7.10 ± 1.13	17.31 ± 2.27	11.29 ± 3.23	13.10 ± 1.29	11.25 ± 3.72
3	26.38 ± 11.37	20.27 ± 6.13	24.48 ± 9.84	36.36 ± 12.07	20.98 ± 9.60	27.32 ± 4.99	21.54 ± 1.38	10.04 ± 3.75	36.52 ± 12.64
5	20.21 ± 8.97	20.98 ± 6.18	23.52 ± 3.54	33.95 ± 9.85	34.14 ± 12.37	31.67 ± 9.23	20.76 ± 5.76	11.69 ± 1.25	18.68 ± 10.87
6	23.12 ± 6.13	23.71 ± 4.78	16.11 ± 3.79	19.84 ± 2.40	16.96 ± 3.23	11.42 ± 1.78	15.89 ± 6.79	12.54 ± 4.81	16.23 ± 4.53
cisplatin	5.06 ± 2.83	3.75 ± 1.23	1.47 ± 0.69	0.96 ± 0.34	1.06 ± 0.56	1.25 ± 0.54	0.75 ± 0.31	1.00 ± 0.31	1.22 ± 0.47
carboplatin	33.57 ± 8.17	22.53 ± 6.23	23.62 ± 6.73	12.39 ± 5.06	9.36 ± 3.15	33.57 ± 12.35	15.67 ± 4.47	10.39 ± 2.13	6.20 ± 1.98

Table 3. IC_{50} Values Obtained for Ligands L_1−L_3, L_5, and L_6 after 72 h of Incubation

	IC_{50} (μM)	
	MCF-7	HeLa
L_1	>40	>40
L_2	32.27	35.13
L_3	25.60	38.42
L_5	>40	>40
L_6	37.21	>40

The result of the preliminary antiproliferative activity studies indicates that, in general, on the basis of calculated IC_{50} values, the following order of relative in vitro antiproliferative activity of the compounds tested with MCF-7 and HeLa cell lines could be considered: cisplatin > **1**, **2**, **6** > carboplatin, **3**, **5** (for the incubation time of 96 h) and cisplatin > **2** > **6**, carboplatin > **1**, **3**, **5** (for the incubation time of 72 h), respectively.

At this stage of the study it is not possible to explain clearly why compound **3** was less cytotoxic in both cell lines than the other compounds tested. But it might be thought that hydroxyl groups of this compound may interact strongly with the membrane and/or intracellular proteins, causing the compound to be less active. Its higher reactivity against I^- compared to the other compounds tested except for **1** is in agreement with its less antiproliferative activity.

For the interpretation of the cytotoxic properties, if the relative order of reactivity (against KI, **1** ≫ **3** > **6** > **2** > **5**) and antiproliferative activity (**1**, **2**, **6** > **3**, **5** and **2**, **6** > **1**, **3**, **5** against MCF-7 and HeLa cell lines, respectively) of the compounds is

Table 4. In Vitro Drug Uptake for Compounds **1**−**3**, **5**, **6**, and Cisplatin

	Pt uptake (μmol of Pt per 1×10^6 cells)[a]		
compd	MCF-7	HeLa	log P[b]
1	0.300	0.198	2.02 ± 0.25
2	0.228	0.099	1.56 ± 0.28
3	3.877	1.245	0.87 ± 0.23
5	0.253	0.103	1.86 ± 0.59
6	0.823	0.572	1.13 ± 0.57
cisplatin	0.057	0.061	

[a] Cellular uptake of platinum in MCF-7 and HeLa cells treated with 30 μM concentrations of compounds **1**−**3**, **5**, **6**, and cisplatin for 4 h. Data represent the mean values of two independent experiments. [b] The partition coefficient of the carrier ligands of the corresponding complexes obtained by using the software ACD/LogP.

compared, although there is not a clear correlation, there seem to be an inverse correlation between the reactivity and antiproliferative activity of compounds **2**, **3**, and **6**.

In vitro antiproliferative activity studies of the ligands against MCF-7 and HeLa cell lines for an incubation time of 72 h demonstrated that ligands $\mathbf{L_1}$, $\mathbf{L_5}$, and $\mathbf{L_6}$ (except for $\mathbf{L_6}$ against the MCF-7 cell line) were inactive in the range of concentrations tested. Although their cytotoxicity was less than those of their corresponding Pt(II) complexes, $\mathbf{L_2}$ and $\mathbf{L_3}$ were found to be cytotoxic (Table 3). It might be thought that they may play a role in cytotoxicity of their corresponding Pt(II) complexes **2** and **3**.

Cellular Uptake Studies. To determine drug accumulation in MCF-7 and HeLa cells, the cells were incubated with compounds **1**−**3**, **5**, **6**, and cisplatin for 4 h. The intracellular platinum content was then measured using ICP-MS (for experimental details, see Supporting Information) and calculated as μmol of Pt per 1×10^6 cells (Table 4). The cellular uptake experiments used in this study cannot distinguish between cytosolic platinum and membrane bound platinum. For this reason, the results do not necessarily reflect the actual cytosolic concentration of the platinum(II) complexes. In general, the benzimidazole Pt(II) complexes tested showed higher cellular accumulation in both cell lines used than cisplatin. Comparison of the cellular concentrations of the compounds tested in both cell lines showed that the order of the platinum accumulation was **3** $\gg$ **6** $\gg$ **1** > **5** > **2** > cisplatin, while their relative cytotoxicities against MCF-7 and HeLa cell lines were cisplatin > **1**, **2**, **6** > **3**, **5**, and cisplatin > **2** > **6** > **1**, **3**, **5**. For compounds **3** and **6**, cellular accumulation was found to be about 68- and 14-fold (for MCF-7 cells) and 20- and 9-fold (for HeLa cells) higher than that of cisplatin. Interestingly, platinum accumulation of the benzimidazole Pt(II) complexes in MCF-7 cells, which is estrogen-receptor positive breast cancer cell type, was found to be higher than that in HeLa cells. This result must be taken into consideration and investigated in detail in future studies.

Interaction with pBR322 Plasmid DNA. In order to detect whether synthesized compounds **1**−**3**, **5**, and **6** induce conformational changes in the DNA helix and whether there is a relationship between the plasmid−DNA binding affinity and the cytotoxicity of the compounds, we investigated their capacity to remove and reverse the supercoiling of closed circular pBR322 plasmid DNA as assessed by electrophoretic mobility measurements on agarose gels (see Supporting Information). In the electrophoretograms the untreated pBR322 plasmid DNA, which is a mixture of mainly covalently closed circular form I and a small amount of open circular form II bands, was used as the control (Figures 5 and 6).

When pBR322 plasmid DNA was interacted with increasing concentrations of compounds **2**, **3**, and **6** and cisplatin, a decrease in mobility for the form I bands was observed (Figure 5). These compounds accelerated the mobility of the form II band of plasmid DNA similarly as did cisplatin, whose bifunctional binding shortens and condenses the DNA helix.[43] These compounds significantly modified electrophoretic mobility; a coalescence of the two forms I and II bands was observed, similar to cisplatin. The concentration required to achieve total unwinding of the pBR322 plasmid DNA for the compounds **2**, **3**, **6**, and cisplatin were 50, 25, 12.5, and 6.25 μM, respectively (for compounds **2**, **3**, **6**, and cisplatin, lines 11, 10, 9, and 8, respectively in Figure 5). The presence of a coalescence point indicates a strong unwinding of the supercoiled DNA.[44] Figure 5 also suggests that compounds **3**, **6**, and cisplatin changed conformations of plasmid DNA from negative supercoil to positive supercoil at higher concentrations than that of their coalescence points.

For Pt(II) complexes **1** and **5**, no comigration of the forms I and II bands of plasmid DNA was observed at the concentration range tested. However, in the range of concentrations from 12.5 to 100 μM the electrophoretic mobility of form I DNA decreased slightly and form II DNA increased slightly. The slight increase in the mobilities of form II and particularly form I plasmid DNA treated with compounds **1** and **5** with respect to untreated DNA was observed in a range of concentrations from 0.097 to 12.5 μM (Figure 5). In the literature, it has been reported that bifunctional DNA−cisplatin adducts, both intra- and interstrand cross-links, unwind DNA with a higher efficiency than monoadducts.[44] Small changes in the mobility of the form I band of pBR322 plasmid DNA treated with some of the Pt(II) and Pd(II) complexes containing sterically demanding carrier ligands were attributed to monofunctional binding.[45] Thus, the slight increase in the mobilities of form II and particularly form I plasmid DNA treated with compounds **1** and **5** may be interpreted that binding of these compounds to plasmid DNA below 12.5 μM is a monofunctional manner. The reason for the lower reactivity of compounds **1** and **5** to plasmid DNA compared with that of the other compounds tested is not clear. But it may be thought that the greater electron density on the chlorine atoms and acetoxy groups of compounds **1** and **5**, respectively, might influence their interactions with DNA negatively. The potential for the formation of hydrogen bonds with DNA by these compounds also seems to be very limited.

The increase in intensity of the form II band (compared to that in the untreated DNA) with the increase in concentrations of the compounds tested may be due to partial nicking of form I DNA to produce form II DNA as a result of covalent binding of the compounds.

To analyze the ability of the compounds to nick DNA, the gels were also run in the presence of ethidium bromide (data not shown except for representative compound **2**) (Figure 6). In the presence of ethidium bromide, the decrease in the electrophoretic migration of the form I bands of the pBR322 plasmid DNA−Pt(II) complexes adducts was eliminated. However, the increase in the electrophoretic migration of the form II bands was observed as it has been observed in the case of the gels that had been run without ethidium bromide. The data obtained suggest that after the nicking event the platinum complexes would remain attached to DNA, ensuring a three-dimensional shape change in form II that is not reverted in the presence of ethidium bromide.

As has been previously reported for cisplatin, the unwinding of the DNA produced by the Pt(II) complexes **2**, **3**, and **6**, which have the potential to form a hydrogen bond with DNA, may support the formation of DNA intrastrand cross-links.[46] Al-

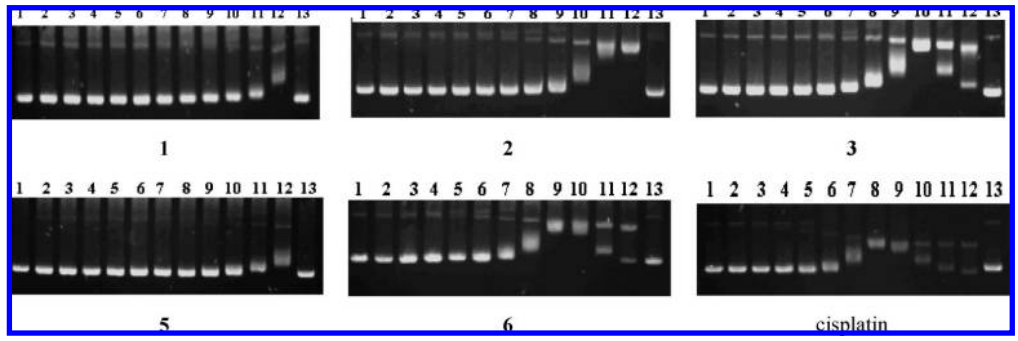


Figure 5. Modification of gel electrophoretic mobility of pBR322 plasmid DNA when incubated with various concentrations of compounds **1**−**3**, **5**, **6**, and cisplatin. Concentrations (in μM) are as follows: (lines 1 and 13) untreated pBR322 plasmid DNA; (lines 2) 0.097; (line 3) 0.195; (line 4) 0.39; (line 5) 0.78; (line 6) 1.56; (line 7) 3.125; (line 8) 6.25; (line 9) 12.5; (line 10) 25; (line 11) 50; (line 12) 100. The top and the bottom bands correspond to form II (open circular) and form I (covalently closed circular) plasmids, respectively.

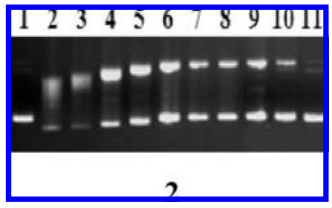


Figure 6. Electrophoresis in agarose gel containing ethidium bromide following incubation of pBR322 plasmid DNA with various concentrations of representative compound **2**. Concentrations (in μM) are as follows: (lines 1 and 11) untreated pBR322 plasmid DNA; (line 2) 100; (line 3) 50; (line 4) 25; (line 5) 12.5; (line 6) 6.25; (line 7) 3.125; (line 8) 1.56; (line 9) 0.78; (line 10) 0.39. The top and the bottom bands correspond to form II (open circular) and form I (covalently closed circular) plasmids, respectively.

though it is not possible to conclude that the cytotoxic activity of these compounds is directly correlated to their interactions with DNA, strong unwinding of the supercoiled DNA caused by compounds **2** and **6** and cisplatin was found to be in accordance with their in vitro antiproliferative activities.

Compounds **3** and **6** were accumulated in both cell lines used and/or interacted strongly with the membrane proteins of these cells in significantly higher amounts than cisplatin (Table 4). The interaction of these compounds, particularly **6** with the pBR322 plasmid DNA, was observed at an approximately similar effective concentration as with cisplatin. But these compounds were found to be less antiproliferative active than cisplatin against the cell lines used. This gives support to the idea that DNA platination is a necessary condition for cytotoxic activity of platinum complexes but may not be the sole mechanism that determines their cytotoxicity.[47] It is well-known that the role of additional hydrogen-bonding interactions both in the kinetics of the DNA binding process and in the stabilization of the adduct structure is very important.[11] Structural factors, i.e., steric and electronic constraints imposed by the 2′-hydroxyethyl moiety, may further stabilize the platinated-plasmid DNA adduct and may be responsible for the decreased antiproliferative activity and the increased reactivity presumably to bionucleophiles of the compound **3**. It is also very possible that in the test conditions the hydroxyl group could form an intramolecular hydrogen bond with the N1−H moiety of the benzimidazole ring, and this interaction may be responsible for the poor antiproliferative activity, since compound **6**, the N1-CH_3 analogue of compound **3**, was found to be more active than compound **3** in both cell lines used.

***Bam*HI and *Hind*III Digestion of Drug−pBR322 Plasmid DNA.** In order to assess whether the benzimidazole−Pt(II) complexes show affinity toward guanine−guanine (GG) and/or adenine−adenine (AA) regions, we carried out restriction endonuclease analysis of the compound−pBR322 plasmid DNA adducts digested by *BamH*I and *Hind*III enzymes (see Supporting Information). *BamH*I and *Hind*III enzymes bind at the recognition sequence 5′-G/GATCC-3′ and 5′-A/AGCTT-3′ and cleaves these sequences just after 5′-guanine and 5′-adenine sites, respectively, and, as a result, convert form I and form II DNA to linear form III DNA.[48]

As the concentration of the compounds tested was increased, it was seen that *BamH*I and *Hind*III (except for compound **2**) digestion was increasingly prevented (Figures 7 and 8). This is probably due to conformational change in the DNA brought about by the covalent binding of the compounds with the plasmid DNA. Arranged in the order of decreasing prevention of *BamH*I and *Hind*III digestion, the compounds were cisplatin > **3** > **1**, **6** > **2** > **5** and **5** > **1**, **6** > cisplatin > **3** > **2**, respectively.

Among the benzimidazole Pt(II) complexes tested, compounds **2** and **3**, with the ligands having a free N1−H moiety, inhibit *Bam*HI restriction enzyme activity to a greater extent than *Hind*III restriction enzyme activity, thus demonstrating their affinity for GG regions of the plasmid DNA. On the other hand, it is interesting to note the greater inhibition of *Hind*III over *Bam*HI restriction enzyme activity by compounds **5** and **6** (especially **5**) (those with N1-methylated ligands). It can be speculated that in modification of pBR322 plasmid DNA, MAcOMBim ligands in the structure of compound **5** may play a specific role, since compound **5** is not recognized by *Hind*III enzyme even at its 0.39 μM concentration. It is interesting to note that besides its low antiproliferative activity and reactivity (against KI), compound **5** is also the least reactive compound to plasmid DNA and nearly inactive for binding to GG regions. It is also noteworthy that compound **5** is highly effective at targeting adenine-rich sequence on DNA (Figures 7 and 8). The different cellular processing of DNA modified by this compound might be relevant to the low antiproliferative activity of this compound especially against MCF-7 cells. The formation of monofunctional adducts assumed may be sufficient to cause enough conformational change in pBR322 plasmid DNA treated with **1** and **5** such that *Hind*III digestion is prevented below their concentrations, 12.5 μM.

The results of the plasmid DNA interaction and the restriction studies suggest that changing the chemical structure of the benzimidazole ligands may modulate DNA binding mode and the sequence selectivity, and the presence of a hydrogen-bond donor, such as a hydroxyl group or N1−H moiety on the benzimidazole carrier ligands of the Pt(II) complexes, seems to have a significant effect on the DNA unwinding.

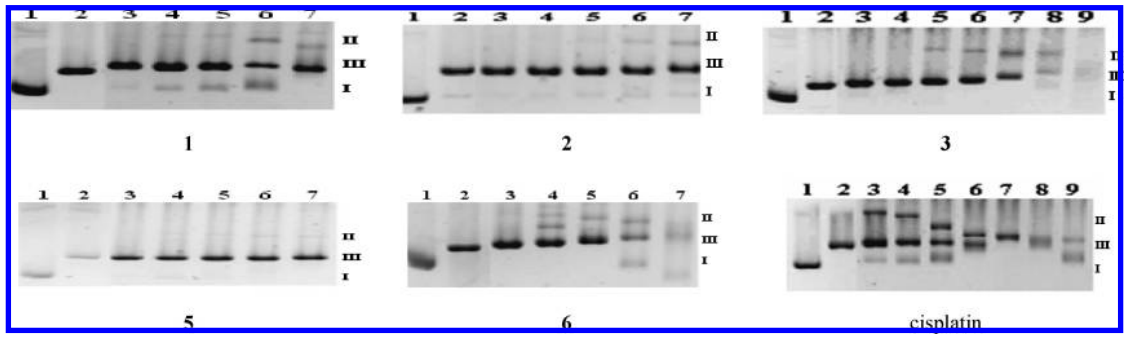


Figure 7. Electrophoretograms for the *BamH*I digested mixtures of pBR322 plasmid DNA after their treatment with various concentrations of compounds **1**−**3**, **5**, **6**, and cisplatin. Concentrations (in μM) are as follows: (lines 1 and 2) untreated pBR322 plasmid DNA and pBR322 DNA linearized by *BamH*I, respectively. For compounds **1**, **2**, **5**, and **6**: (line 3) 6.25; (line 4) 12.5; (line 5) 25; (line 6) 50; (line 7) 100. For compound **3**: (line 3) 1.56; (line 4) 3.125; (line 5) 6.25; (line 6) 12.5; (line 7) 25; (line 8) 50; (line 9) 100. For cisplatin: (line 3) 0.39; (line 4) 0.78; (line 5) 1.56; (line 6) 3.125; (line 7) 6.25; (line 8) 12.5; (line 9) 25. Roman numerals I, II, and III indicate form I (covalently closed circular), form II (open circular), and form III (linear) plasmids, respectively.

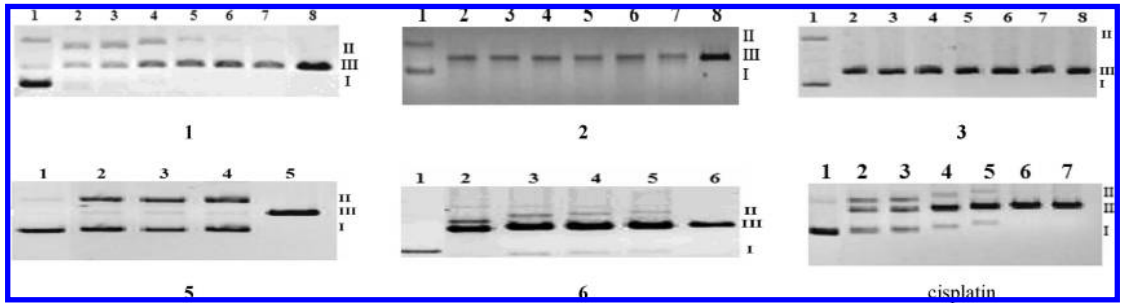


Figure 8. Electrophoretograms for the *Hind*III digested mixtures of pBR322 plasmid DNA after their treatment with various concentrations of compounds **1**−**3**, **5**, **6**, and cisplatin. Concentrations (in μM) are as follows: (first and the last lines) untreated pBR322 plasmid DNA and pBR322 DNA linearized by *Hind*III, respectively. For compound **1**: (line 2) 6.25; (line 3) 3.125; (line 4) 1.56; (line 5) 0.78; (line 6) 0.39; (line 7) 0.195. For compound **2** and **3**: (line 2) 100; (line 3) 50; (line 4) 25; (line 5) 12.5; (line 6) 6.25; (line 7) 3.125. For compound **5**: (line 2) 1.56; (line 3) 0.78; (line 4) 0.39. For compound **6**: (line 2) 3.125; (line 3) 1.56; (line 4) 0.78; (line 5) 0.39. For cisplatin: (line 2) 50; (line 3) 25; (line 4) 12.5; (line 5) 6.25; (line 6) 0.39. Roman numerals I, II, and III indicate form I (covalently closed circular), form II (open circular), and form III (linear) plasmids, respectively.

Cell Cycle Studies. The effects of the representative compounds **1** and **2**, which were found to be the most active compounds against the cell line tested, for initial studies on the cell cycle profile of the MCF-7 and HeLa cells, respectively, were examined by propidium iodide (PI) staining and flow cytometry (see Supporting Information). Cisplatin was used under the same experimental conditions as the control and for comparison. After treatment of MCF-7 and HeLa cells for 72 h with compounds **1** and **2**, respectively, at 20 μM concentrations equivalent to their T/C_{corr} values of ∼20% (drug concentration required to inhibit cell growth by ∼80%), it was observed that compounds **1** and **2** had no significant effect on the cell cycle profile of MCF-7 and HeLa cells, respectively, compared to untreated controls (Figure 9). On the other hand, compound **2** induced a significant increase in the SubG1 cell population (which is representative of cells with fragmented DNA) with no arrest in G2/M and G0/G1 phases under the test conditions applied in HeLa cells with respect to untreated control and cisplatin. This indicates that compound **2** induces sufficient levels of DNA damage. The results of the cytotoxicity and the plasmid DNA helix unwinding and the cell cycle modification studies on compound **2**, one of the two most stable compounds against model nucleophile I^- among the compounds tested, seem to be in accordance with each other. The result obtained in this study is in agreement with the results obtained in our ongoing studies using annexin V and PI double staining by flow cytometry in which compound **2** induced "early apoptotic" and "late apoptotic and necrotic" cells in HeLa cells by ∼2- and ∼5-fold, respectively, compared to untreated control (data not shown). However, the effect of antiproliferative compounds on apoptosis inductions and cell cycle modifications may depend on cell type and concentration of the test compounds; the treatment of MCF-7 cells over 72 h with compound **1** did not appear to produce any significant SubG1 population increase compared to the untreated control. This is consistent with its much weaker modification on pBR322 plasmid DNA compared to cisplatin.

Treatment of both MCF-7 and HeLa cells for 72 h with cisplatin at 20 and 1 μM concentrations, respectively, equivalent to T/C_{corr} values of ∼20%, resulted in a small increase in the SubG1 cell population and a significant increase in G2/M cell fractions and a reduction in G0/G1 cell fractions. These findings are consistent with previous reports that cisplatin, whose mode of cell death is concentration dependent,[49] induces G2/M arrest in MCF-7[50] and HeLa[51] cell lines at the same concentrations used in this study.

Most of the antineoplastic drugs in current use block the cell cycle in the S or G2/M phases. Many tumor cells are resistant to classical chemotherapeutic agents, since they are endowed with mutations that inactivate apoptotic machinery or activate antiapoptotic pathways. Therefore, the characterization of novel, apoptosis-independent anticancer drugs appears to be significantly relevant for possible therapeutic applications.[52] The different effects of compounds **1** and **2** on the cell cycle profiles of the MCF-7 and HeLa cells, respectively, can be due to multiple causes that may be related to DNA and/or non-DNA targets interactions of these compounds. Especially for compound **1**, which was found to be the most reactive compound

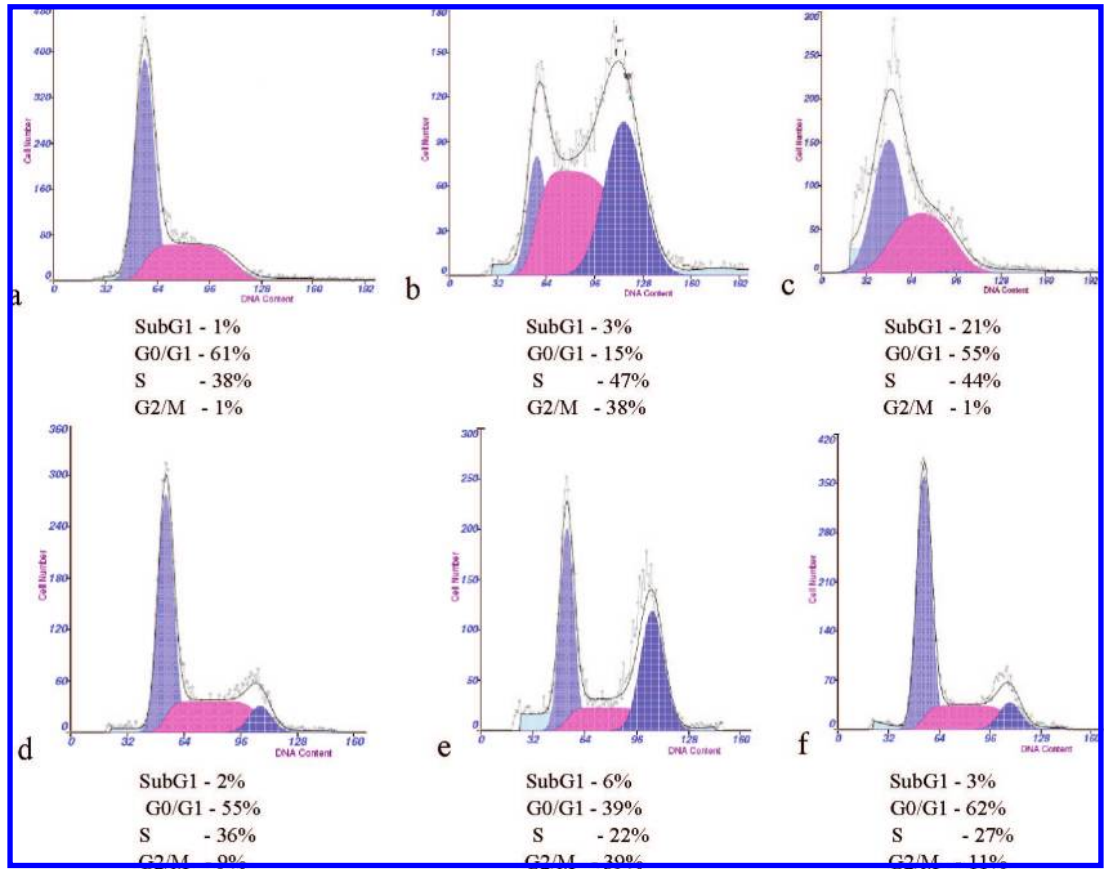


Figure 9. Cell cycle distribution and SubG1 peak demonstration. a: HeLa cell control consists of medium. b: HeLa cells were exposed to cisplatin. c: HeLa cells were exposed to compound **2**. d: MCF-7 cell control consists of medium. e: MCF-7 cells were exposed to cisplatin. f: MCF-7 cells were exposed to compound **1**. Data are representative of three separate experiments with similar results. The horizontal axis of each histogram represents DNA content; the vertical axis denotes cell number. ((light-blue) SubG1; (light-purple) G0/G1; (pink) S; (dark-purple) G2/M).

in "iodide assay" and less reactive compound against pBR322 plasmid DNA, non-DNA targets interaction may be important for its antiproliferative activity against MCF-7 cell line. To confirm all the assumptions, it is necessary to study the interaction of the Pt(II) complexes with DNA and the specific proteins in detail.

The biochemical mechanism of cisplatin cytotoxicity involves the binding of the drug to DNA and non-DNA targets and the subsequent induction of cell death through apoptosis, necrosis, or both.[46] It is known that most cisplatin molecules that enter the cell are bound to proteins rather than DNA, and there is experimental evidence showing that the former type of damage also plays a role in the initiation of apoptotic pathways.[53] For example, oxaliplatin, the *R*,*R* isomer of [Pt(oxalate)(diaminocyclohexan)], is currently in clinical use against metastatic colorectal cancer in which cisplatin is inactive[54] and shows a level of DNA binding lower than ciplatin. Although the overall conformational alterations induced in DNA by the major intrastrand adducts of cisplatin and oxaliplatin are similar,[55] oxaliplatin induces higher levels of apoptosis in cell lines endowed with resistance to cisplatin. The structures of these two compounds are distinct and differently recognized by mismatch-repair proteins and some damage-recognition proteins. It has been reported that in cancer cells the rate of protein binding is high for oxaliplatin.[54] It has also been postulated that the hydrophobic diaminocyclohexan moiety in oxaliplatin may direct drug reactivity toward cellular proteins with sulfydryl groups within hydrophobic pockets, which may be poorly reactive with cisplatin. Considering the data reported in the literature and the preliminary results obtained in this study, it may be concluded that the mechanism of antiproliferative activity of compounds **1** and **2** against MCF-7 and HeLa cell lines, respectively, is probably different from that of cisplatin. These differences reflect the distinct nature of the carrier ligands and might be promising agents, and further studies on compounds **1** and **2** are warranted.

To determine whether the hydrophobicity of the Pt(II) complexes influences their cytotoxicities and accumulations on the cell lines tested and their plasmid DNA interactions, theoretical calculations of hydrophobicity of the carrier ligands $\mathbf{L_1}-\mathbf{L_3}$, $\mathbf{L_5}$, $\mathbf{L_6}$ as log *P* were performed using the software ACD/LogP. It has been reported in a systematic study of the hydrophobicity of *cis*-diam(m)ineplatinum(II) complexes that when the leaving groups are unchanged, the hydrophobicity of a platinum complex is linearly related to that of the am(m)ine ligand.[56] It was also shown that the calculated log P_{oct} correlates well with observed cellular uptake for the complexes. The log *P* values of the ligands $\mathbf{L_1}-\mathbf{L_3}$, $\mathbf{L_5}$, $\mathbf{L_6}$ are 2.02 ± 0.25, 1.56 ± 0.28, 0.87 ± 0.23, 1.86 ± 0.59, 1.13 ± 0.57, respectively. If compounds **1**−**3**, **5**, and **6** in order of decreasing cytotoxicity against MCF-7 and HeLa cell lines (**1**, **2**, **6** > **3**, **5** and **2** > **6** > **1**, **3**, **5**, respectively) are compared with the order of the log *P* values of the corresponding ligands ($\mathbf{L_1} > \mathbf{L_5} > \mathbf{L_2} > \mathbf{L_6} > \mathbf{L_3}$), although it is not clear, there seems to be a positive correlation (except for compound **5**) between log *P* and antiproliferative activity of the compounds against MCF-7 cell line. However, in the case of the cytotoxicity against HeLa cell line, it is more difficult to establish a correlation. In addition, it is very possible to conclude that there seems to be an inverse correlation (except for compound **3**) between the log *P* ($\mathbf{L_1}$ >

$\mathbf{L_5} > \mathbf{L_2} > \mathbf{L_6} > \mathbf{L_3}$) and the plasmid DNA binding affinity (**6** > **3** > **2** > **5**, **1**) of the compounds.

In summary, while an inverse correlation between the cellular accumulation and the log *P* values of the compounds **3**, **5**, and **6** was observed, there seems to be a positive correlation between platinum uptake and the antiproliferative activities of compounds **1**, **5**, and **6** for both cell lines used.

Conclusion

The preliminary data obtained in this study lead us to conclude that (i) compounds **1**, **2**, and **6**, which were found to be in vitro antiproliferative active higher or equal to carboplatin against the human MCF-7 and HeLa cell lines, may be regarded as having potential antitumor activity. (ii) The results of the plasmid DNA interaction and the restriction studies suggest that changing the chemical structure of the benzimidazole ligands may modulate DNA binding mode and the sequence selectivity. Although it is not possible to make a clear conclusion about the necessity of the free N1−H moiety on the cytotoxic activities of the compounds tested against the cell lines used, the presence of a hydrogen-bond donor, such as a hydroxyl group or N1−H moiety on the benzimidazole carrier ligands of the Pt(II) complexes, seems to have a significant effect on the DNA unwinding. (iii) The antiproliferative activity mechanism of compounds **1** and **2** against MCF-7 and HeLa cells, respectively, is probably different from that of cisplatin. These differences reflect the distinct nature of the carrier ligands and might make them promising agents, and further studies on compounds **1** and **2** are warranted. (iv) In general, there is no clear correlation between the plasmid DNA binding affinity and the stability and the antiproliferative activity of the compounds tested. (v) In addition, it also has to be examined whether antiproliferative activity and the mode of action of the compounds including cell cycle modification and apoptosis induction are cell-type dependent.

Experimental Section

Synthesis. All chemicals and solvents used were reagent grade (Merck or Aldrich or Sigma) and were used without further purification. Analytical thin-layer chromatography (TLC) was performed on 60 F_{254} precoated silica gel plates (Merck). Plates were visualized by UV light, Dragendorff reagent, or iodine vapor.

Instruments. Melting points were determined with an Electrothermal 9200 melting point apparatus and are uncorrected. Elemental analyses for C, H, N were performed by TÜBİTAK Laboratory (Ankara, Turkey) on a CHNS analyzer, model Leco-932, and were within ±0.4% of theoretical values. IR spectra were recorded on KBr pellets and Nujol mulls on a Mattson 1000 FTIR spectrometer in the range of 4000−200 cm^{-1}. For the region 400−200 cm^{-1}, the samples were prepared as Nujol mulls on CsI windows. ^{1}H NMR spectra were recorded in DMSO-d_6 (Merck) on a Bruker 400 MHz spectrometer. FAB^+ mass spectra were recorded on a ZapSpec spectrometer using 3-nitrobenzyl alcohol (NBA) as the matrix solvent. The ESI-LC/MS analyses for the identification of the benzimidazole−Pt(II) complexes synthesized and the determination of the reactivity of the compounds against model nucleophile I^- were performed with an Agilent 1100 HPLC system (Waldbronn, Germany). ICP-MS analyses for the determination of platinum levels of samples for the reactivity of the compounds against I^- and cellular uptake studies were performed by using a standard Agilent 7500a benchtop ICP/MS fitted with a cross-flow nebulizer and a quartz spray chamber (experimental details of the spectral analyses are presented in the Supporting Information). The purity of the synthesized compounds was established as >95% by elemental analysis and ^{1}H NMR.

Synthesis of the Ligands. Ligands $\mathbf{L_1}$−$\mathbf{L_6}$, used as carrier ligands in the structures of the Pt(II) complexes, were prepared according to the published procedures as shown in the Scheme 1. Melting points and ^{1}H NMR data of the ligands are presented in the Supporting Information.

Synthesis of the Platinum(II) Complexes. ESI-LC/MS, IR, ^{1}H NMR, and FAB-MS data of compounds **1**−**6** are presented in the Supporting Information.

$[Pt(L_1)_2Cl_2]\cdot 3H_2O$ (1). To a stirred solution of $\mathbf{L_1}$ (0.180 g, 1.08 mmol) in 0.5 N HCl (15 mL) was added a solution of K_2PtCl_4 (0.250 g, 0.60 mmol) in 0.5 N HCl (5 mL) dropwise over 30 min at room temperature. The reaction mixture protected from light was heated at 60 °C for 5 days. After that time the mixture was kept in the refrigerator at 4 °C for 12 h. The resulting crude precipitate was filtered off and purified by repeated washing with small portions of water, ethanol, and diethyl ether and dried in vacuo to yield 0.150 g (41.66%) of **1**. Anal. ($C_{16}H_{14}Cl_4N_4Pt\cdot 3H_2O$) C, H, N.

$[Pt(L_2)_2Cl_2]\cdot 2DMF$ (2). To a stirred solution of $\mathbf{L_2}$ (0.125 g, 1.69 mmol) in an ethanol−water mixture (5:10 mL) was added an aqueous solution of K_2PtCl_4 (0.162 g, 0.39 mmol in 5 mL of water) dropwise over 30 min at room temperature. The pH was adjusted to ∼7 and kept constant with the addition of 0.1 M KOH. The reaction mixture protected from light was stirred at room temperature for 7 days. After that time the mixture was kept in the refrigerator at 4 °C for 12 h. The resulting crude precipitate was filtered off and purified by repeated washing with small portions of water, ethanol, and diethyl ether and dried in vacuo and recrystallized from DMF by slow evaporation. Yield: 0.082 g (32.86%) of **2**. Anal. ($C_{20}H_{20}Cl_2N_4O_4Pt\cdot 2DMF$) C, H, N.

$[Pt(L_3)_2Cl_2]\cdot H_2O$ (3). A similar procedure was carried out as described for compound **2** using $\mathbf{L_3}$ (0.250 g, 1.50 mmol) and K_2PtCl_4 (0.366 g, 0.88 mmol) at 60 °C for 5 days. Yield: 21.19%, 0.110 g (21.19%). Anal. ($C_{18}H_{20}Cl_2N_4O_2Pt\cdot H_2O$) C, H, N.

$[Pt(L_4)_2Cl_2]$ (4). $\mathbf{L_4}$ (0.122 g, 0.68 mmol) and K_2PtCl_4 (0.160 g, 0.39 mmol) was dissolved in 7 mL of DMF. The reaction mixture protected from light was heated at 50 °C for 7 days. Precipitated KCl was removed by filtration. A solution of 5% aqueous KCl (3 mL) was then added to the filtered solution, and the mixture was stirred for 2 h. The resulting crude precipitate was filtered off and purified by repeated washing with small portions of water, ethanol, and diethyl ether and dried in vacuo to yield 0.050 g (20.00%) of **4**. Anal. ($C_{18}H_{18}Cl_4N_4Pt$) C, H, N.

$[Pt(L_5)_2Cl_2]\cdot 6H_2O$ (5). A similar procedure was carried out as described for compound **2** using $\mathbf{L_5}$ (0.200 g, 0.98 mmol) and K_2PtCl_4 (0.240 g, 0.58 mmol) at room temperature for 10 days. Yield: 0.184 g (24.02%). Anal. ($C_{22}H_{24}Cl_2N_4O_4Pt\cdot 6H_2O$) C, H, N.

$[Pt(L_6)_2Cl_2]\cdot H_2O$ (6). A similar procudure was carried out as described for compound **2** using $\mathbf{L_6}$ (0.070 g, 0.40 mmol) and K_2PtCl_4 (0.094 g, 0.23 mmol) at 60 °C for 3 days. Yield: 0.083 g (32.59%). Anal. ($C_{20}H_{26}Cl_2N_4O_3Pt\cdot H_2O$) C, H, N.

Determination of the Reactivity of the Compounds against Model Nucleophile I^-, Preliminary Cytotoxicity Test, Cellular Uptake Studies, Interaction with pBR322 Plasmid DNA, *Bam*H1 and *Hind*III Restriction Enzyme Digestion and Cell Cycle Analysis, and SubG1 Detection by Flow Cytometry. Experimental details are presented in the Supporting Information.

Acknowledgment. We are grateful to Prof. Dr. İbrahim Burgu (Faculty of Veterinary Medicine, Ankara University), Prof. Dr. Ali Şengül (Department of Immunology, Gülhane Military Medical Academia, Ankara) and Dr. Recep Ergül (Foot and Mouth Disease Institute, Ankara, Turkey) for their valuable support. For helpful assistance we thank Research Assistant Humay Akın (Faculty of Veterinary Medicine, Ankara University). We thank the Research Foundation of Gazi University (EF-02/2002-06, EF-02/2004-28 and 02/2007-24) for financial support.

Supporting Information Available: Experimental details of the spectral analyses, melting points, and ^{1}H NMR data of ligands $\mathbf{L_1}$−$\mathbf{L_6}$, elemental analysis, IR, ^{1}H NMR, FAB-MS, and ESI-LC/MS data of compounds **1**−**6**, crystallographic data for compound

2, experimental details of the determination of the reactivity of the compounds against model nucleophile I^-, preliminary cytotoxicity test, cellular uptake, interaction with pBR322 plasmid DNA, BamH1 and HindIII restriction enzyme digestion, cell cycle analysis, and SubG1 detection by flow cytometry studies. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Chaney, S. G.; Sancar, A. DNA Repair: Enzymatic Mechanisms and Relevance to Drug Response. *J. Natl. Cancer Inst.* **1996**, *88*, 1346–1360.

(2) Xin Zhang, C.; Lippard, S. J. New Metal Complexes as Potential Therapeutics. *Curr. Opin. Chem. Biol.* **2003**, *7*, 481–489.

(3) Jamieson, E. R.; Lippard, S. J. Structure, Recognition, and Processing of Cisplatin−DNA Adducts. *Chem. Rev.* **1999**, *99*, 2467–2498.

(4) Wang, D.; Lippard, S. J. Cellular Processing of Platinum Anticancer Drugs. *Nat. Rev. Drug Discovery* **2005**, *4*, 307–320.

(5) Brabec, V.; Kasparkova, J. Molecular Aspects of Resistance to Antitumor Platinum Drugs. *Drug Resist. Updates* **2002**, *5*, 147–161.

(6) Ohndorf, M. A.; He, R. Q.; Pabo, C. O.; Lippard, S. J. Basis for Recognition of Cisplatin-Modified DNA by High-Mobility-Group Proteins. *Nature* **1999**, *399*, 708–712.

(7) He, Q.; Liang, C. H.; Lippard, S. J. Steroid Hormones Induce HMG1 Overexpression and Sensitize Breast Cancer Cells to Cisplatin and Carboplatin. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5768–5772.

(8) Zamble, D. B.; Mikata, Y.; Eng, C. H.; Sandman, K. E.; Lippard, S. J. Testis-Specific HMG-Domain Protein Alters the Responses of Cells to Cisplatin. *J. Inorg. Biochem.* **2002**, *91*, 451–462.

(9) Brown, S. J.; Kellet, P. J.; Lippard, S. J. Ix1, a Yeast Protein That Binds to Platinated DNA and Confers Sensitivity to Cisplatin. *Science* **1993**, *261*, 603–605.

(10) Tallen, G.; Mock, C.; Gangopadhyay, S. B.; Kangarloo, B.; Krebs, B.; Wolff, J. E. A. Overcoming Cisplatin Resistance: Design of Novel Hydrophobic Platinum Compounds. *Anticancer Res.* **2000**, *20*, 445–450.

(11) Reedijk, J. New Clues for Platinum Antitumor Chemistry: Kinetically Controlled Metal Binding to DNA. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3611–3616.

(12) Reardon, J. T.; Vaisman, A.; Chaney, S.; Sancar, A. Efficient Nucleotide Exicion Repair of Cisplatin, Oxaliplatin, and Bis-aceto-ammine-dichlorohexylamine-platinum(IV) (JM216) Platinum Intrastrand DNA Diadducts. *Cancer Res.* **1999**, *59*, 3968–3971.

(13) Reedijk, J. Why Does Cisplatin Reach Guanine-N7 with Competing S-Donor Ligands Available in the Cell? *Chem. Rev.* **1999**, *99*, 2499–2510.

(14) Kostova, I. Platinum Complexes as Anticancer Agents. *Recent Pat. Anti-Cancer Drug Discovery* **2006**, *1*, 1–22.

(15) Gümüş, F.; Aİzgü, F.; Algül, Ö. Synthesis and Structural Characterization of Some 5(6)-Substituted-2-Hydroxymethylbenzimidazole Derivatives and Their Platinum(II) Complexes and Determination of Their in Vitro Antitumor Activities by "Rec-Assay Test". *FABAD Farm. Bil. Der.* **1996**, *21*, 7–15.

(16) Gümüş, F.; Pamuk, İ.; Özden, T.; Yıldız, S.; Diril, N.; Öksüzoğlu, E.; Gür, S.; Özkul, A. Synthesis, Characterization and in Vitro Cytotoxic, Mutagenic and Antimicrobial Activity of Platinum(II) Complexes with the Substituted Benzimidazole Ligands. *J. Inorg. Biochem.* **2003**, *94* (3), 255–262.

(17) Gümüş, F.; Algül, Ö.; Eren, G.; Eroğlu, H.; Diril, N.; Gür, S.; Özkul, A. Synthesis, Cytotoxic Activity on MCF-7 Cell Line and Mutagenic Activity of Platinum(II) Complexes with 2-Substituted Benzimidazole Ligands. *Eur. J. Med. Chem.* **2003**, *38*, 473–480.

(18) Gümüş, F.; Özçelik, A. B.; Özden, T.; Eroğlu, H.; Diril, N. Synthesis, Characterization and Mutagenicity of New *cis*-[Pt(2-substituted-benzimidazole)$_2$Cl$_2$] Complexes. *Pharmazie* **2003**, *58* (5), 303–307.

(19) Gökçe, M.; Utku, S.; Gür, S.; Özkul, A.; Gümüş, F. Synthesis, in Vitro Cytotoxic and Antiviral Activity of *cis*-[Pt(*R*(−) and *S*(+)-2-α-Hydroxybenzyl-benzimidazole)$_2$Cl$_2$] Complexes. *Eur. J. Med. Chem.* **2005**, *40*, 135–141.

(20) Algül, Ö.; Özçelik, B.; Abbasoğlu, U.; Gümüş, F. Synthesis, Characterization and Genotoxicity of Platinum(II) Complexes with Substituted Benzimidazole Ligands. *Turk. J. Chem.* **2005**, *29*, 607–615.

(21) Gümüş, F.; Algül, Ö. DNA Binding Studies with *cis*-Dichlorobis5(6)-non/chlorosubstituted-2-hydroxymethylbenzimidazole Platinum(II) Complexes. *J. Inorg. Biochem.* **1997**, *68* (1), 71–74.

(22) Knobloch, W. Synthese von Substituierten Benzimidazolen mit Potentieller Antitumorwirkung. *Chem. Ber.* **1958**, *91*, 2557–2561.

(23) Jones, J. B.; Taylor, K. E. Hydroxymethylbenzimidazole Carboxylic Acid Models of the Asp-His-Ser Charge Relay System of Serine Proteases. *Can. J. Chem.* **1977**, *55* (10), 1653–1657.

(24) Bachman, G. B.; Heisey, L. V. Monomers and Polimers: The Preperation of Vinyl Derivatives of Five-Atom Heterocyclic Rings. *J. Am. Chem. Soc.* **1949**, *71*, 1985–1988.

(25) Bednyagina, N. P.; Postovskii, I. Ya. Hydrolytic Cleavage of Some Sulfones of Heterocyclic Series. Sythesis and Properties of *p*-Nitrophenylsulfonyl-*N*-methylbenzimidazolylmethane and *p*-Nitrophenylsulfonylbenzothiazolylmethane. *Zh. Obshch. Khim.* **1960**, *30*, 3193–3196 (*Chem Abstr*. **1961**, *55*, 19908i).

(26) Porai-Koshits, B. A.; Kvitko, I. YA.; Shutkova, E. A. Synthesis of Aminoesters of Benzimidazole Derivatives. *Zh. Prikl. Khim.* **1964**, *37* (6), 1386–1388.

(27) Tertov, B. A.; Koblik, A. V.; Avdyunina, N. I. Organosodium Compounds of N-Substituted Benzimidazoles. *Chem. Heterocycl. Compd.* **1971**, *7* (9), 1163–1167.

(28) Tunalı, N. K.; Özkar, S. Anorganik Tepkime Mekanizmaları. In *Anorganik Kimya*, 4th ed.; Gazi Publishing: Ankara, Turkey, 1999; pp 373−402.

(29) Mylonas, S.; Valavanidis, A.; Polyssiou, V. V. Platinum(II) and Palladium(II) Complexes with Amino Acid Derivatives. Sythesis, Interpretion of IR and ^{1}H-NMR Spectra and Conformational Implications. *Inorg. Chim. Acta* **1981**, *55*, 125–128.

(30) Kammermeier, T.; Wıegrebe, W. ^{1}H-NMR and IR Spectroscopic Data of 1,3-Diphenylpropane-1,3-diamines and Their Pt(II) Complexes: Stereochemical Assigments and Binding Mode of the Non-Amine Ligands. *Arch. Pharm.* **1994**, *327*, 697–707.

(31) Lippert, B. Platinum Nucleobase Chemistry. *Prog. Inorg. Chem.* **1989**, *37*, 1–97.

(32) Cini, R.; Fanizzi, F. P.; Intini, F. P.; Maresca, L.; Natile, G. Platinum Amides from Platinum Nitriles: X-ray Crystal Structures of the Unbridged Dinuclear Compounds Bis[bis(1-imino-1-hydroxy-2,2-dimethylpropane)(1-amino-1-oxo-2,2-dimethylpropane)dichloroplatinum(II)]. *J. Am. Chem. Soc.* **1993**, *113*, 5123–5131.

(33) Schreiber, A.; Hillgeris, E. C.; Lippert, B. On Metal Modified Nucleobase Pairs and Triples. *Z. Naturforsch.* **1993**, *48b*, 1603–1612.

(34) Wheate, N. J.; Collins, J. G. Multi-Nuclear Platinum Drugs: A New Paradigm in Chemotherapy. *Curr. Med. Chem.: Anti-Cancer Agents* **2005**, *5*, 267–279.

(35) Knipp, M.; Karotki, A. V.; Chesnov, S.; Natile, G.; Sadler, P. J.; Brabec, V.; Vasak, M. Reaction of Zn_7Metallothionein with *cis*- and *trans*-[Pt(N-donor)$_2$Cl$_2$] Anticancer Complexes: *trans*-Pt^{II} Complexes Retain Their N-Donor Ligands. *J. Med. Chem.* **2007**, *50*, 4075–4086.

(36) Gust, R.; Schnurr, B.; Krauser, R.; Bernhardt, G.; Koch, M.; Schmid, B.; Hummel, E.; Schonenberger, H. Stability and Cellular Studies of [*rac*-1,2-Bis(4-fluorophenyl)-ethylenediamine][cyclobutane-1,1-dicarboxylato]platinum(II), a Novel, Highly Active Carboplatin Derivative. *J. Cancer Res. Clin. Oncol.* **1998**, *124*, 585–597.

(37) Gust, R.; Krauser, R.; Schmid, B.; Schonenberger, H. Breast Cancer Inhibiting Diastereomeric Diacetato[1,2-bis(4-fluorophenyl)ethylenediamine]platinum(II) Derivatives: Synthesis and Studies on the Relationship between Reactivity and Antitumor Activity. *Inorg. Chim. Acta* **1996**, *250*, 203–218.

(38) Gust, R.; Krauser, R.; Schmid, B.; Schonenberger, H. Synthesis and Antitumor Activity of [1,2-Bis(4-fluorophenyl)ethylenediamine][dicarboxylato]platinum(II) Complexes. *Arch. Pharm. (Weinheim, Ger.)* **1998**, *331*, 27–35.

(39) Gust, R.; Heinrich, H.; Krauser, R.; Schonenberger, H. [*meso*- and *rac*-1,2-Bis(4-fluorophenyl)ethylenediamine]chloro[sulfinyl-bis(methane)-*S*]platinum(II) Chloride New Water Soluble Platinum Complexes with High Anti-Breast Cancer Activities. *Inorg. Chim. Acta* **1999**, *285*, 184–189.

(40) Bernhardt, G.; Koch, M.; Spruss, T.; Gust, R.; Krauser, R.; Schlemmer, R.; Hollstein, M.; Lux, F.; Schonenberger, H. [*meso*-1,2-Bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]sulfato-platinum(II). Pharmacokinetic Studies. *Arch. Pharm. (Weinheim, Ger.)* **1999**, *332*, 195–200.

(41) Gust, R.; Keilitz, R.; Krauser, R.; Schmidt, K.; Schnurr, B. Structure Activity Studies on Leaving Group Derivatives of [*meso*-1,2-Bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]-platinum(II). *Arch. Pharm. (Weinheim, Ger.)* **1999**, *332*, 261–270.

(42) Schertl, S.; Hartmann, R. W.; Batzl-Hartmann, C.; Bernhardt, G.; Spruss, T.; Beckenlehner, K.; Koch, M.; Krauser, R.; Schlemmer, R.; Gust, R.; Schonenberger, H. [1,2-Bis(2,6-difluoro-3-hydroxyphenyl) ethylenediamine]platinum(II) Complexes, Compounds for the Endocrine Therapy of Breast Cancer. Mode of Action II: Contribution of Drug Inactivation, Cellular Drug Uptake and Sterical Factors in the Drug−Target Interaction to the Antitumor Activity. *Arch. Pharm. (Weinheim, Ger.)* **2004**, *337*, 349–359.

(43) Cohen, G. L.; Bauer, W. R.; Barton, J. K.; Lippard, S. J. Binding of *cis*- and *trans*-Dichlorodiammineplatinum(II) to DNA Evidence for Unwinding and Shortening of the Double Helix. *Science* **1979**, *203*, 1014–1016.

(44) Keck, M. V.; Lippard, S. J. Unwinding of Supercoiled DNA by Platinum−Ethidium and Related Complexes. *J. Am. Chem. Soc.* **1992**, *114*, 3386–3390.
(45) Neplechova, K.; Kasparkova, J.; Vrana, O.; Novakova, O.; Habtemariam, A.; Watchman, B.; Sadler, P. J.; Brabec, V. DNA Interactions of New Antitumor Aminophosphine Platinum(II) Complexes. *Mol. Pharmacol.* **1999**, *56*, 20–30.
(46) Fuertes, M. A.; Alonso, C.; Pérez, J. M. Biochemical Modulation of Cisplatin Mechanisms of Action: Enhancement of Antitumor Activity and Circumvention of Drug Resistance. *Chem. Rev.* **2003**, *103*, 645–662.
(47) Khazanov, E.; Barenholz, Y.; Gibson, D.; Najajreh, Y. Novel Apoptosis-Including trans-Platinum Piperidine Derivatives: Synthesis and Biological Characterization. *J. Med. Chem.* **2002**, *45* (24), 5196–5204.
(48) Roberts, R. S.; Wilson, G. A.; Young, F. E. Recognition Sequence of Specific Endonuclease *Bam*HI.1 Form *Bacillus amyloliquefaciens*. *Nature* **1977**, *265*, 82–84.
(49) Gonzales, V. M.; Fuertes, M. A.; Alonso, C.; Perez, J. M. Is Cisplatin-Induced Cell Death Always Produced by Apoptosis? *Mol. Pharmacol.* **2001**, *59*, 657–663.
(50) Mueller, S.; Schittenhelm, M.; Honecker, F.; Malenke, E.; Lauber, K.; Wesselborg, S.; Hartmann, J. T.; Bokemeyer, C.; Mayer, F. Cell-Cycle Progression and Responce of Germ Cell Tumors to Cisplatin in Vitro. *Int. J. Oncol.* **2006**, *29*, 471–479.
(51) Ishibashi, T.; Lippard, S. J. Telomere Loss in Cells Treated with Cisplatin. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4219–4223.
(52) Tardito, S.; Bussolati, O.; Gaccioli, F.; Gatti, R.; Guizzardi, S.; Uggeri, J.; Marchio, L.; Lanfranchi, M.; Franchi-Gazzola, R. Non-Apoptotic Programmed Cell Death Induced by a Copper(II) Complex in Human Fibrosarcoma Cells. *Histochem. Biol.* **2006**, *126*, 473–482.
(53) Perez, R. P. Cellular and Molecular Determinants of Cisplatin Resistance. *Eur. J. Cancer* **1998**, *34* (10), 1535–1542.
(54) Raymond, E.; Faivre, S.; Chaney, S.; Woynarowski, J.; Cvitkovic, E. Cellular and Molecular Pharmacology of Oxaliplatin. *Mol. Cancer Ther.* **2002**, *1*, 227–235.
(55) Spingler, B.; Whittington, D. A.; Lippard, S. J. 2.4 Å Crystal Structure of an Oxaliplatin 1,2-d(GpG) Intrastrand Cross-Link in a DNA Dodecamer Duplex. *Inorg. Chem.* **2001**, *40*, 5596–5602.
(56) Souchard, J. P.; Ha, T. T. B.; Cros, S.; Johnson, N. P. Hydrophobicity Parameters for Platinum Complexes. *J. Med. Chem.* **1991**, *34*, 863–864.

JM8000983