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ORIGINAL ARTICLE / ÖZGÜN MAKALE

IN VITRO CYTOTOXIC ACTIVITIES OF PLATINUM(II) COMPLEXES CONTAINING 1*H*-BENZO[*d*]IMIDAZOLE AND 1*H*-1,3-DIAZOLE DERIVATIVES

1H-BENZO[d]İMİDAZOL VE 1H-1,3-DİAZOL TÜREVLERİ İÇEREN PLATİN(II) KOMPLEKSLERİNİN İN VİTRO SİTOTOKSİK AKTİVİTELERİ

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ABSTRACT

Objective: *This study aimed to synthesize and evaluate the cytotoxic activities of four platinum(II) complexes with 2-substituted or nonsubstituted 1H-benzo[d]imidazole and 1H-1,3-diazole derivatives as carrier ligands (L1-L4), which may have potent cytotoxic activity and low side effects.*
Material and Method: *K1-K4 complexes were synthesized by heating and mixing K_2PtCl_4 and the appropriate L1-L4. The chemical structures of K1-K4 were elucidated by Infrared and ^{1}H Nuclear Magnetic Resonance spectroscopic methods. In vitro, cytotoxic effects of K1-K4 complexes against prostate (DU-145), endometrial adenocarcinoma (Ishikawa), and breast cancer (MCF-7) cell lines were tested by the MTT method.*
Result and Discussion: *According to the IC_{50} values of the tested cell lines, K1 and K2 derivatives bearing unsubstituted 1H-benzo[d]imidazole (L1) and 1H-1,3-diazole (L2) were found to be the most effective compounds among these synthesized complexes.*
Keywords: *1H-1,3-diazole, 1H-benzo[d]imidazole, cisplatin, cytotoxic activity, platinum complexes*

ÖZ

Amaç: *Bu çalışmada, güçlü sitotoksik aktiviteye ve düşük yan etkilere sahip olabilecek, taşıyıcı ligand olarak 2-sübstitüe veya nonsübstitüe 1H-benzo[d]imidazol ve 1H-1,3-diazol türevleri içeren dört platin(II) kompleksinin sentezlenmesi ve sitotoksik aktivitelerinin değerlendirilmesi amaçlanmıştır.*
Gereç ve Yöntem: *K1-K4 kompleksleri, K_2PtCl_4 ve uygun taşıyıcı ligandların ısıtılıp karıştırılmasıyla sentezlenmiştir. K1-K4'ün kimyasal yapıları Infrared ve ^{1}H Nükleer Manyetik Rezonans spektroskopik yöntemleri ile aydınlatılmıştır. İn vitro olarak, K1-K4 komplekslerinin prostat (DU-145), endometrial adenokarsinom (Ishikawa) ve meme kanseri (MCF-7) hücre hatlarına karşı sitotoksik etkileri MTT yöntemi ile test edilmiştir.*
Sonuç ve Tartışma: *Test edilen hücre hatlarının IC_{50} değerlerine göre, sübstitüe olmayan 1H-benzo[d]imidazol veya 1H-1,3-diazol taşıyan K1 ve K2 türevlerinin sentezlenen bu kompleksler arasında en etkili bileşikler olduğu bulunmuştur.*
Anahtar Kelimeler: *1H-1,3-diazol, 1H-benzo[d]imidazol, platin kompleksleri, sisplatin, sitotoksik aktivite*

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INTRODUCTION

Cancer is the leading cause of death worldwide, accounting for around one out of every six deaths and affecting nearly every family. In 2022, there were an estimated 20 million new cases of cancer and 9.7 million cancer-related deaths globally. The cancer burden is expected to rise by approximately 77% by 2050, putting additional strain on healthcare systems, people, and communities [1]. Nowadays, various approaches, including radiotherapy, chemotherapy, surgery, immunotherapy, hormone therapy, and gene therapy, can be used alone or in combination for cancer treatment [2].

Cisplatin, the first platinum complex to be used clinically in the treatment of cancer patients, is the most widely prescribed chemotherapeutic drug for the treatment of testicular, ovarian, bladder, non-small lung cancer, head and neck, esophagus, advanced cervical cancer, lymphomas, metastatic osteosarcoma, and melanoma [3,4].

Despite this clinical success, cisplatin induces several toxic side effects such as nephrotoxicity, neurotoxicity, ototoxicity, nausea, and vomiting [5]. This resistance may be intrinsic or developed during prolonged treatment [6,7]. To overcome these issues, new platinum complexes have been designed and studied for their antitumor properties [8,9].

Even though thousands of complexes have been developed and evaluated, only three platinum drugs, cisplatin, carboplatin, and oxaliplatin, have been approved for clinical use worldwide. In addition, Nedaplatin, lobaplatin, and heptaplatin have only received regional approval [10,11]. These complexes, shown in Figure 1, work through a mechanism of action similar to cisplatin, involving DNA binding and transcription inhibition. They are activated intracellularly by aquation of the leaving groups and then interact with DNA to form DNA adducts by coordination of the cis-[Pt(R-NH_2)$_2$] fragment to the N-7 atom of G residue [12].

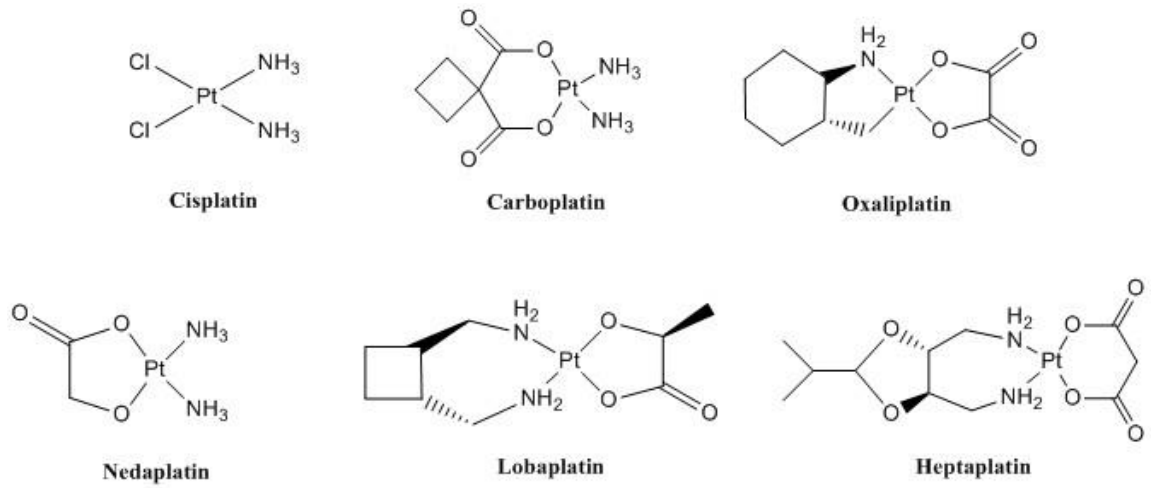

Figure 1. Structural formulas of Pt(II) anticancer metallodrugs: cisplatin, carboplatin, oxaliplatin, nedaplatin, lobaplatin, and heptaplatin

The most significant type of cisplatin-DNA binding is the intrastrand 1,2-d(GpG) cross-link. This type accounts for about 60-65% of the platinum bound to DNA. The resulting Pt-DNA adducts are responsible for distorting and bending the structure of the DNA, which in turn hinders transcription. The inhibitory effects on transcription ultimately lead to the death of the cells [3,13].

Carboplatin, a second-generation platinum (II) complex that carries a slower hydrolyzing 1,1-cyclobutane dicarboxylate ligand instead of the chlorine ligand separated in cisplatin, provided the advantage of fewer side effects in patients despite the use of higher doses than cisplatin, but it was not possible to prevent the development of cross-resistance to carboplatin [14,15]. Oxaliplatin, a third-generation platinum complex, is synthesized by replacing the ammonia and chlorine ligands in the cisplatin structure with 1,2-diaminocyclohexane and oxalate, respectively. There is no development of cross-resistance to oxaliplatin, and nephrotoxic effects are less common in patients using cisplatin and

carboplatin [16].

In our previous studies, we investigated the cytotoxic effect of K1-K4 complexes in cervix cancer (HeLa), laryngeal cancer (HEp-2), and human breast cancer (MCF-7) cell lines, and their plasmid DNA interactions are also investigated using agarose gel electrophoresis [17–19]. Furthermore, the antibacterial and antifungal activity of K1 complex has been evaluated by the macrodilution method [19].

In this study, as an extension of our investigation on the probable anticancer activity of K1-K4 complexes with 2-substituted or nonsubstituted 1*H*-benzo[d]imidazole and 1*H*-1,3-diazole as carrier ligands, were evaluated for their in vitro cytotoxic activities against prostate cancer (DU-145), endometrial adenocarcinoma (Ishikawa) and breast cancer (MCF-7) cell lines using the MTT method, which is called 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide, a tetrazolium salt.

MATERIAL AND METHOD

Chemistry

1*H*-benzo[d]imidazole (L1) was synthesized by the Philips method starting from 1,2-phenylenediamine and formic acid [20]. 1*H*-1,3-diazole (L2), 2-phenyl-1*H*-benzo[d]imidazole (L3), and 2-phenyl-1*H*-1,3-diazole (L4) carrier ligands, solvents, and all starting chemicals were purchased from Sigma-Aldrich. The reactions were verified with thin-layer chromatography using silica gel plates, which were visualized under 254 nm UV light. ^{1}H NMR spectra were recorded in DMSO-d_6 on a Bruker 400 MHz FT-NMR spectrometer using tetramethylsilane as the internal standard. All chemical shifts are reported in ppm (δ). FTIR-ATR spectra were recorded on a Perkin Elmer Spectrum 400 FTIR/FTNIR spectrometer equipped with a Universal ATR Sampling Accessory and were reported in cm^{-1} units. Melting points were determined with an Electrothermal 9200 Melting Point Apparatus and are uncorrected.

Characterization of Carrier Ligands

Detailed structural analyses of carrier ligands 1*H*-benzo[d]imidazole (L1), 1*H*-1,3-diazole (L2), 2-phenyl-1*H*-benzo[d]imidazole (L3) and 2-phenyl-1*H*-1,3-diazole (L4) were carried out previously reported [17–19].

General Procedure for the Synthesis of K1-K4

To a stirred solution of L1-L4 (1.10 mmol) in ethanol-water (7:3 ml) was added dropwise an aqueous solution of K_2PtCl_4 (0.60 mmol) over 30 min at room temperature (Figure 2). The reaction mixture was heated 40-60°C for 2-6 days. The pH was adjusted to 7 and kept constant with the addition of 0.1 M $NaHCO_3$. The resulting crude precipitate formed was filtered off and washed with small portions of water, ethanol, and diethyl ether and dried in vacuo.

cis-Dichloro-bis(1*H*-benzo[d]imidazole)platinum(II) (K1). Yield:85%; Mp: >400 °C; FTIR-ATR υ (cm^{-1}): 3287-3100 (N-H, =C-H); ^{1}H-NMR (DMSO-d_6) δ: 13.44 (s, 2H, 2 x N-H), 8.84 (s, 2H, 2x Ar-H) 7.80 (d, J= 7.2 Hz, 2H, Ar-H), 7.50 (d, J= 7.2 Hz, 2H, Ar-H), 7.25-7.18 (m, 4H, 2x ArH).

cis-Dichloro-di(1*H*-1,3-diazole)platinum(II) (K2). Yield:64%; Mp: >400 °C; FTIR-ATR υ (cm-1): 3243-2888 (N-H, =C-H); ^{1}H-NMR (DMSO-d6) δ: 13.51 (s, 2H, 2x N-H), 8.30 (s, 2H, 2x Ar-H) 7.40 (s, 2H, 2x Ar-H), 7.09 (s, 2H, 2x Ar-H).

cis-Dichloro-bis(2-phenyl-1*H*-benzo[d]imidazole)platinum(II).1.5 H_2O (K3). Yield:70%; Mp: >400 °C; FTIR-ATR υ (cm-1): 3355-3055 (N-H, =C-H, O-H), 1620-1541; ^{1}H-NMR (DMSO-d6) δ: 13.34 (s, 2H, 2x N-H), 8.91-8.89 (m, 1H, ArH), 8.28-8.22 (m, 3H, 2x Ar-H), 7.91-6.79 (m, 14 H, ArH).

cis-Dichloro-bis(2-phenyl-1*H*-1,3-diazole)platinum(II) (K4). Yield:65%; Mp: >400 °C; FTIR-ATR υ (cm^{-1}): 3222-2890 **(**N-H, =C-H); ^{1}H-NMR (DMSO-d_6) δ: 13.13 (broad s, 2H, 2x NH), 8.67-7.99 (m, 4H, 2x ArH), 7.65-7.22 (m, 10H, 2x ArH).

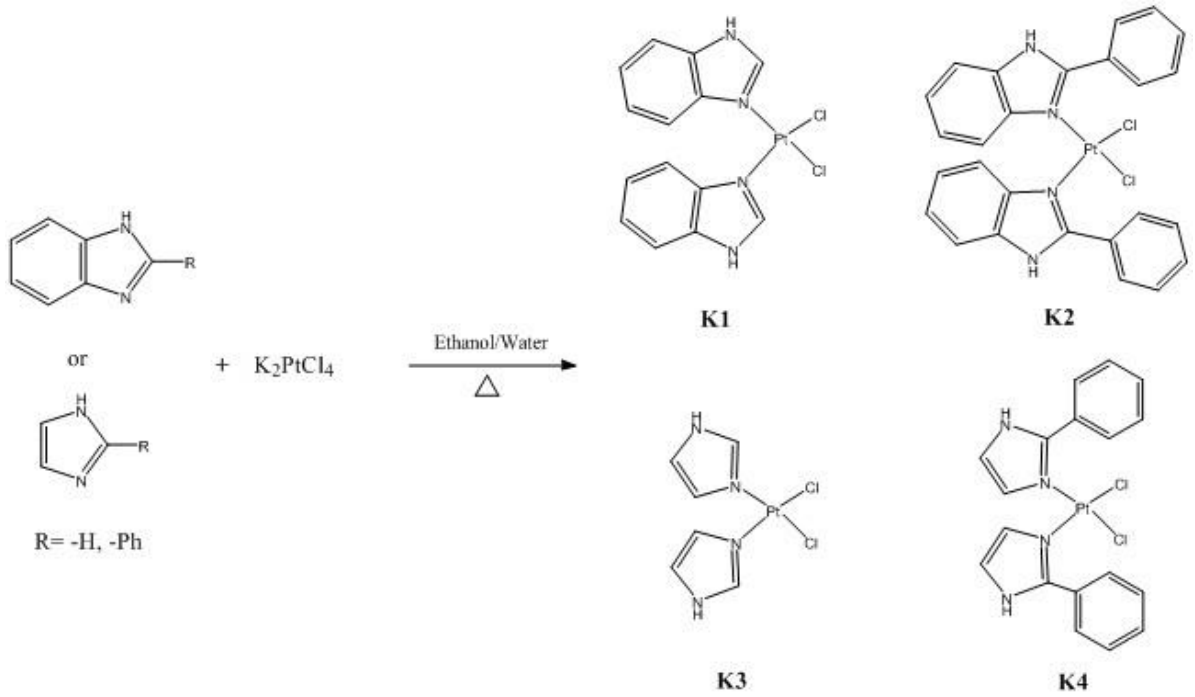

Figure 2. Synthesis of K1-K4

Biological Activity

In our study, commercially purchased MCF-7 breast cancer (The American Type Culture Collection (HTB-81, Manassas, VA, USA)), DU-145 prostate cancer (T.C. Ministry of Agriculture and Forestry Alum Institute, Cell Registration No: 00092502ATCC) and Ishikawa endometrial adenocarcinoma cell lines (Merck, ECACC-99040201, Darmstadt, Germany) were used for cytotoxicity tests. Cells were seeded in 25 cm^2 flasks using either Dulbecco's modified Eagle's medium (HyClone Laboratories, Inc., Logan, UT) or RPMI 1640 Medium (Sartorius,01-106-1A) supplemented with 10% fetal bovine serum (FBS, Gibco, 10082147), %2,5 L-glutamine (Thermo, 25030081), 1% penicillin-streptomycin (Thermo,15140130) and %1 ml amphotericin (Thermo, 15290018) mixture and then grown for 3 days at 37°C in 5% CO_2 in a humidified incubator.

The cytotoxic effect of K1-K4 and cisplatin as a positive control were tested by MTT method. MTT test was performed by ISO 10993-5 standards [21].

Cell culture was performed in 96-well plates with n=3 for each concentration and 7500 cells per well. After incubation in a humidified atmosphere of 5% CO_2 and 37°C for 24 hours, the medium was replaced with medium containing K1-K4 and cisplatin as positive control at concentrations of 2.5, 5, 10, 20, 20, 40, 80, and 160 µM and 1,56, 3.125, 6.25, 12.5, 25, 50, 100 respectively. Cells were incubated with the medicated medium for 72 hours. At the end of this time, the medium on the cells was removed, and 200 µl of medium and 50 µl of medium containing (Sigma-Aldrich, St. Louis, MO, USA)) MTT (5 mg/ml) were added to each well. Cells were incubated at 37°C for 4 hours. The medium was then removed from the cells and 200 µl of DMSO and 50 µl of glycine buffer with a pH of 10.5 was added to each well. To determine the viability of the MCF-7, DU-145, and Ishikawa cells tested, the plates containing the cells were immediately exposed to spectrophotometric measurement at a wavelength of 570 nm in an ELISA microplate reader.

The IC_{50} values of K1-K4 at concentrations of 2.5, 5, 10, 20, 40, 80, and 160 µM; cisplatin 1.56, 3.125, 6.25, 12.5, 25, 50, 100 µM are given in Table 1 and the viability values against MCF-7, DU-145 and Ishikawa cell lines are given in Table 2. The IC_{50} values were using GraphPad Prism software.

Table 1. IC_{50} (μM) values of K1-K4 and cisplatin

Complex No	MCF-7	DU-145	Ishikawa
K1 $[Pt(L1)_2Cl_2]$	3.12±0.0039	2.06±0.006	1.81±0.0102
K2 $[Pt(L2)_2Cl_2]$	1.93±0.0168	4.27±0.0042	1.90±0.0239
K3 $[Pt(L3)_2Cl_2]$	8.75±0.0158	2.16±0.0067	2.87±0.0279
K4 $[Pt(L4)_2Cl_2]$	6.52±0.0099	4.66±0.014	3.55±0.0230
Cisplatin $[Pt(NH_3)_2Cl_2]$	2.08±0.0041	1.92±0.0042	2.05±0.0068

[a] IC_{50} = 50% cytotoxic concentration against in vitro tested cells. Data are presented as mean ± SD

Table 2. K1-K4 and cisplatin % viability values against MCF-7, DU-145 and Ishikawa cell lines

		% Viability (µM ±SS)						
		160	**80**	**40**	**20**	**10**	**5**	**2.5**
MCF-7 (breast cancer)	**K1**	33.48 ±1.01	39.18 ±0.51	35.04 ±0.37	39.24 ±1.30	40.25 ±0.10	54.37 ±1.57	68.91 ± 0.58
	K2	65.54 ±1.94	65.06 ±0.19	70.48 ±2.07	71.04 ±0.62	66.38 ±1.91	71.34 ±1.50	71.80 ± 0.84
	K3	32.73 ±0.95	32.78 ±2.58	43.78 ±0.19	49.94 ±3.61	57.76 ±1.50	78.61 ±9.63	98.69 ± 3.61
	K4	32.45 ±1.11	38.63 ±0.57	39.41 ±2.01	43.35 ±1.05	59.89 ±2.77	71.74 ±3.13	79.75 ± 3.25
	Cisplatin	20.77 ±0.91	22.28 ±0.12	23.63 ±1.28	26.25 ±0.28	28.04 ±0.15	39.32 ±1.65	42.82±1.43
DU-145 (prostate cancer)	**K1**	8.79± 0.22	7.27 ± 0.18	7.95± 0.15	8.44± 0.16	9.91± 0.51	20.11± 0.32	33.63± 1.21
	K2	50.00± 1.41	62.49± 4.01	62.60± 0.54	64.14± 2.66	64.18± 2.33	70.65± 5.64	69.87± 0.63
	K3	10.69± 0.30	9.19± 0.11	9.24± 0.10	9.66± 0.28	8.78± 0.03	10.46± 0.71	20.35± 1.23
	K4	10.68± 0.16	10.81± 0.82	10.27± 0.17	10.98± 0.18	15.53± 2.19	63.56± 1.70	71.03± 0.76
	Cisplatin	7.64 ± 0.32	7.77 ± 0.05	7.94 ± 0.28	8.90 ± 0.11	10.19± 0.22	11.41± 0.40	13.73± 0.36
Ishikawa (endometrial adenocarcinoma cancer)	**K1**	5.95± 0.20	6.25 ± 0.06	7.10 ± 0.36	9.15 ± 0.44	7.45 ± 0.29	16.13± 0.53	17.87± 0.67
	K2	36.92± 0.17	41.04± 1.27	46.33± 0.16	49.09± 0.63	49.11± 0.72	49.65± 1.65	49.39± 1.31
	K3	5.74± 0.03	5.76 ± 0.18	6.02 ± 0.02	6.24 ± 0.18	10.61± 0.98	43.89± 4.61	44.67± 0.90
	K4	5.88± 0.11	6.07 ± 0.06	10.99± 0.19	13.44± 0.57	32.60± 0.44	44.34± 0.91	48.39± 3.44
	Cisplatin	5.36 ± 0.02	5.07 ± 0.07	5.59 ± 0.11	6.86 ± 0.10	13.12± 0.35	22.90± 0.46	31.55± 0.58

RESULT AND DISCUSSION

Platinum complexes represent one of the most successful families of clinically used anticancer drugs. Although cisplatin has a wide spectrum of anticancer activity, it does have significant side toxicity, and its clinical use can also be limited by the existence or development of resistance. Several thousand platinum-based compounds have been synthesized to overcome reduced toxicity and drug resistance [22,23].

In this study, to overcome the disadvantages mentioned above, the ammonia ligands in the

cisplatin structure were replaced with L1-L4 derivatives as carrier ligands, which are known to the body and have ligand properties.

The carrier ligand 1*H*-benzo[d]imidazole (L1) was synthesized using the Phillips method and its melting point was confirmed to be consistent with the literature [20].

The synthesized complexes, which were reported previously by us, were re-synthesized in this study to obtain higher yields using different temperatures and reaction times [17–19]. Our complexes synthesized in 85-64% yield were obtained in higher yields than previous studies.

The melting points of all complexes were >400 °C. The IR spectra of the K1-K4 were shown some characteristic changes when compared to those of the L1-L4 carrier ligands. In the IR spectrum of L1-L4 were observed broad bands in the region of 3400-2500 cm^{-1} due to the 2-substituted/nonsubstituted-1*H*-benzo[d]imidazole and 1*H*-1,3-diazole N-H stretching bands. K1-K4 compounds exhibited N-H stretching bands centered at 3287 and 2880 cm^{-1} sharper than that of L1-L4 carrier ligands, due to the breaking of tautomerism, indicating that the N-H group was not involved in the coordination.

The ^{1}H-NMR spectra of synthesized complexes were consistent with their corresponding protons, both in the chemical shifts and in the number of hydrogens. The spectra of the complexes were compared to those of the free ligands, and significant differences were observed. All complexes showed a large downfield shift in the imidazole N-H signal compared to their ligands, which is due to an increase in the N-H acid character after platinum binding. The N-H chemical shifts of compounds K1-K4 varied between 13.51-13.13 ppm.

The cytotoxic activities of prepared platinum (II) complexes K1-K4 and cisplatin used as reference compound were evaluated in vitro by an MTT assay. Different cancer cell lines, including MCF-7 (breast cancer cell line), DU-145 (prostate carcinoma cell line) and Ishikawa (human endometrial cancer cell line) were used in vitro cytotoxicity test. The corresponding %cell viability and IC_{50} values were shown in Table 1 and 2.

Generally, K1-K4 complexes exhibited considerable cytotoxic activities against MCF-7, DU-145, and Ishikawa cell lines. The IC_{50} values in these tested cell lines were within the range of 1.93-8.75 μM, 2.06-4.66 μM, and 1.81-3.55 μM, respectively. It is noted that complex K1 and K2 are the most effective compounds among the synthesized complexes. The cytotoxic activities of K1 and K2 complexes with the carrier ligand of 1*H*-benzo[d]imidazole and 1*H*-1,3-diazole against Ishikawa and DU-145 cell lines were tested comparable to cisplatin (IC_{50}= 1.81 and 2.06 μM, 1.90 and 4.27, 2.05 and 1.92 μM, respectively). K2 complex with 1*H*-1,3-diazole carrier ligand was tested as more effective complex than cisplatin against MCF-7 cell line (IC_{50}= 1.93 μM vs. IC_{50}=2.08 μM).

In this study, platinum compounds were synthesized in which a selection of biologically active carrier ligands were attached to Pt coordination moieties. Although these complexes exhibited better activity when compared to cisplatin in the cell lines tested, it would be early to recognize them as drug-candidate molecules since advanced anticancer activity tests have not been performed thus far.

According to our previous research, we thought that the steric hindrance of the substituted group would modify the interaction mode between platinum compounds and DNA, influencing the antitumor properties of the platin-DNA adducts and resulting in the absence of cross-resistance with cisplatin [24–26]. The increased steric hindrance of platinum complexes is expected to reduce the interaction between platinum complexes and sulfur-containing molecules while also prolonging the blood cycle, resulting in an enhanced therapeutic impact for tumors [27,28]. However, considering that the steric hindrance in the tested K3 and K4 complexes may disrupt or even prevent the interaction of the compound with DNA, we thought it would be appropriate to include the K1 and K2 complexes, which have nonsubstituted ligands, in the study. Therefore, MTT test results show that the bearing of a phenyl ring at the second position of the benzimidazole or imidazole ring increases the sterically hindered effect of platinum complexes and can reduce the moderately cytotoxic effect against tested cell lines.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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