

Synthesis, antiproliferative activity and plasmid DNA interaction studies of [Pt(1-substituted-1*H*-1,3-diazole)₂Cl₂] and [Pt(1-substituted-1*H*-1,3-diazole)₂I₂] analogs

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ABSTRACT: Researchers have accelerated subject their research on platinum complexes since the serendipitous discovery of cisplatin's anticancer properties. Their goal is to synthesize more potent platinum complexes by replacing the carrier ammonia ligands in the structure of cisplatin with biologically active heterocyclic compounds. This approach is a key strategy for increasing the efficacy of cancer treatments and better targeting cancer types that develop resistance to current therapies. For this purpose, 1*H*-1,3-diazoles are heterocyclic compounds, well-known to biological systems and have exceptional pharmacological activities, were chosen carrier ligands in our study. In this paper, six novel platinum(II) complexes with the structures of [Pt(L1-3)₂Cl₂] (**1a-c**), and [Pt(L1-3)₂I₂] (**2a-c**), (L1= 1-(4-methoxyphenyl)-1*H*-1,3-diazole, L2= 1-phenyl-1*H*-1,3-diazole and L3= 1-benzyl-1*H*-1,3-diazole) were synthesized for cisplatin analogues. The chemical structures of **1a-c** and **2a-c** were elucidated by Fourier Transform Infrared (FT-IR), Proton Nuclear Magnetic Resonance (¹H-NMR), Electrospray Ionization Mass Spectrometry (ESI-MS), and elemental analysis. The interactions of the complexes with pBR322 plasmid DNA and *Bam*HI and *Hind*III restriction endonuclease enzymes were investigated by agarose gel electrophoresis. The cytotoxic effects of **1a-c** and **2a-c** against human non-small cell lung cancer (A549), human colon adenocarcinoma (CaCo-2), and mouse fibroblast cells (L929) cells were tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Compound **2c** was as effective as cisplatin against the CaCo-2 cell line with 17.07 µM IC₅₀ values.

KEYWORDS: Cytotoxic activity; cisplatin; platinum(II) complexes; 1*H*-1,3-diazole; gel electrophoresis

1. INTRODUCTION

Cancer, a leading health issue, is defined by the uncontrolled proliferation of abnormal cells that invade adjacent tissues and spread to other organs. The global cancer burden is rapidly increasing due to the aging and growth of the population and changes in people's exposure to risk factors [1]. Accordingly, it is estimated that more than 35 million new cancer cases will occur worldwide by 2050. This represents an increase of 77% compared to an estimated 20 million cases in 2022. Despite the progress made in the early detection of cancer and the treatment and care of cancer patients, the need to develop new chemotherapeutics continues today [2].

Since ancient times, metals have been used to treat various infectious and fatal diseases despite their toxic effects. In later years, metal complexes, especially those containing d-series metal ions, have attracted much attention as anticancer drugs due to their extraordinary potential against cancer cells [3–7].

Originally called Peyrone's chloride, cisplatin (cis-diamminedichloroplatinum(II)), one of the most commonly used anticancer drugs today, was first synthesized by Italian chemist Michele Peyrone in 1844 [8]. In 1965, Barnett Rosenberg and colleagues serendipitously discovered that cisplatin or cis-[Pt(NH₃)₂Cl₂] induced cell growth but inhibited cell division [9]. Clinical trials for cisplatin began in 1971, and in 1978, the FDA approved it as the first platinum complex for use as an effective chemotherapeutic drug for the treatment of advanced and metastatic testicular and ovarian cancer [10, 11]. Nowadays, cisplatin, carboplatin, and oxaliplatin are platinum-based metal complexes used worldwide as anticancer agents, while nedaplatin, lobaplatin, and heptaplatin are used locally [12].

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Cisplatin, when taken into the cell, displaces either one or two chlorine atoms and is replaced by water, forming monocationic ($[\text{PtCl}(\text{H}_2\text{O})(\text{NH}_3)_2]^+$) and/or dicationic ($[\text{Pt}(\text{H}_2\text{O})_2(\text{NH}_3)_2]^{2+}$) reactive derivatives. These derivatives preferentially bind to the nitrogen atom 7 of the guanine and adenine bases in the DNA double strand, with approximately 60-65% binding to 1,2-d(GpG), 20-25% to 1,2-d(ApG), ~2% to single bonds, and 2% to 1,3-d(GpXpG) [10].

However, the occurrence of toxic side effects like nephrotoxicity, bone marrow depression, nausea, vomiting, peripheral neuropathy, sudden seizures, and acquired/intrinsic resistance in patients treated with platinum-based drugs have led researchers to develop new platinum complexes [10, 13, 14]. Numerous platinum complexes containing biologically active heteroaromatic N donor ligands, including benzimidazole, imidazole, and thiazole, have been developed to address the deficiencies and increase the efficacy; some of these complexes have been shown to exhibit anticancer activity. It has been reported that the activity results exhibited by platinum complexes with the above-mentioned carrier ligands are because the ligands are natural substances and are used by cells in the organism [15-17].

The core 1*H*-1,3-diazole is essential for various physiological actions and vital biological functions. Vitamin B₁₂, histamine, histidine, various metalloproteins, iron-heme systems, DNA, and hemoglobin contain the core unit of 1*H*-1,3-diazole in their structures [18, 19]. Drugs containing the biologically active heterocyclic 1*H*-1,3-diazole ring, which has various pharmacological activities, are used in current treatments such as antiinflammatory, antihistaminic, antimicrobial, and anticancer activities [20, 21].

Previous studies reported that platinum iodido complexes have long been recognized as less effective than those with chlorido and carboxylate leaving ligands, leading researchers to overlook these complexes initially. At the same time, bioinorganic chemists have ignored platinum iodido complexes because several pioneer studies determined that the iodido ligand was unsuitable for developing novel platinum-based complexes [22-24]. Due to the rapid development and advancement of molecular pharmacology and cell biology over the past 20 years, this situation has drastically changed. Several studies have revealed that various platinum iodido complexes are substances with promising chemical, physical, and cytotoxic properties [25-30].

Particularly several platinum (II) iodido complexes exhibited greater activity than their chlorido counterparts, surpassing even the activity of the traditional platinum-based drugs [28]. Furthermore, many studies have shown significant differences in the mechanisms of action between platinum iodido agents, their chlorido analogs, and clinically used platinum complexes. As a result of these findings, researchers have begun to reassess iodide- carrying platinum complexes as potential anticancer drug candidates [25, 31-35].

In previous papers, we reported the synthesis, characterization, in vitro cytotoxic activities, and plasmid DNA interaction of platinum complexes of the structure $[\text{Pt}(\text{L})_2\text{X}]$ L= 1*H*-1,3-diazole or 2-phenyl-1*H*-1,3-diazole, X= Cl₂, I₂ or oxalate). It was found that some of these 1*H*-1,3-diazole Pt(II) complexes modified the tertiary structures of pBR322 plasmid DNA and in vitro cytotoxic activities equal to cisplatin [36, 37]. In this study, as a continuation of our previous work, six new platinum (II) chlorido or iodido complexes (**1a-c** and **2a-c**) were designed, synthesized, and characterized with spectral and elemental analysis (Figure 1).

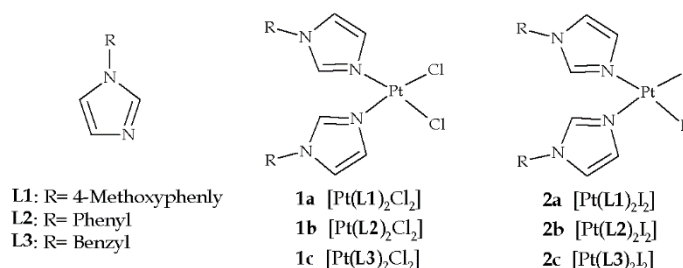


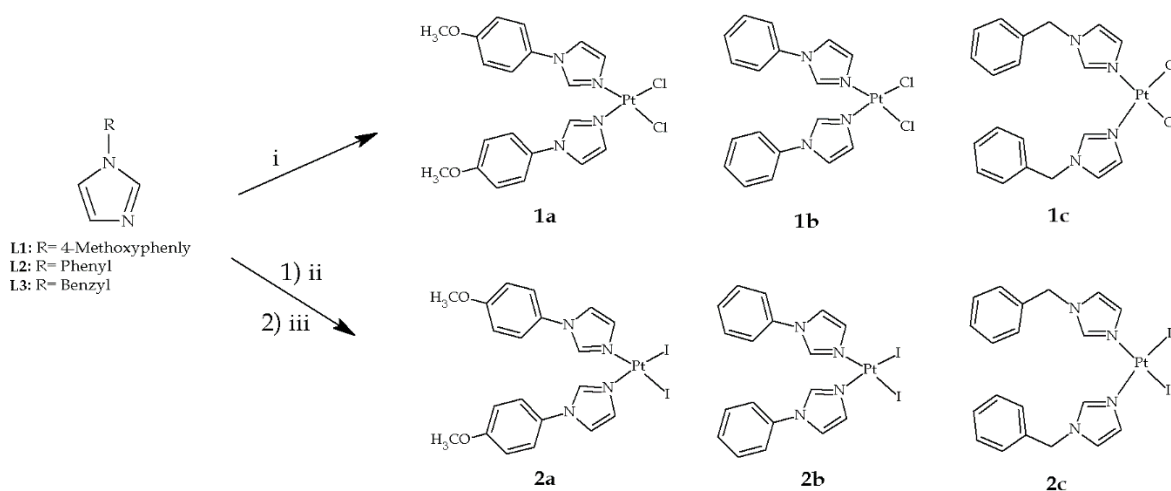
Figure 1. The structure of a cis- $[\text{Pt}(1\text{-substituted-1H-1,3-diazole})_2\text{Cl}_2]$ and cis- $[\text{Pt}(1\text{-substituted-1H-1,3-diazole})_2\text{I}_2]$ complexes.

The interactions of the complexes with DNA were studied using agarose gel electrophoresis, examining their interaction with pBR322 plasmid DNA strands and identifying the binding region with *Bam*HI and *Hind*III restriction endonuclease enzymes. Additionally, their cytotoxic effects against human A549, CaCo-2, and L929, cells were tested using the MTT method [38, 39].

2. RESULTS AND DISCUSSION

2.1. Chemistry

The six new platinum(II) complexes, which chlorido and iodido (**1a-c** and **2a-c**) complexes, synthesized as shown in Figure 2. The three platinum(II) dichlorido complexes (**1a-c**) were obtained by the reaction of the carrier ligands with K_2PtCl_4 in ethanol:water solution [40, 41]. For the synthesis of the other three platinum(II) diiodido complexes (**2a-c**), K_2PtI_4 was first synthesized from K_2PtCl_4 and KI. Then, complexes **2a-c** were synthesized by dropwise addition of appropriate carrier ligands to this solution [36, 40].



(i) K_2PtCl_4 , 0.5 N HCl, 40-50°C; (ii) K_2PtCl_4 , KI, water 60°C; (iii) K_2PtI_4 , ethanol/water, 40°C

Figure 2. Synthesis of Pt(II) complexes.

The suggested empirical formulas and the elemental analysis data for the **1a-c** and **2a-b** complexes agreed on properly. The analytical results confirmed that complexes **1a**, **1c**, and **2b** included crystalline water in their structures. When the FT-IR spectra of the synthesized Pt(II) complexes and carrier ligands were compared, slight shifts in the absorption bands were observed upon complex formation. The FT-IR spectrum reveals moderate intensity bands in the range of 1610-1599 cm^{-1} for free **L1-3**, which can be attributed to the stretching vibrations of the C=N bond. In the spectra of the complexes (**1a**, **1c**, **2a**, **2c**) and (**1b**, **2b**), these bands exhibited shifts of 1-3 cm^{-1} towards higher frequencies and 2-3 cm^{-1} towards lower frequencies, respectively. This observation suggests that the tertiary nitrogen of the 1H-1,3-diazoles was coordinated with the platinum ion. The broad band centered at around 3345-3120 cm^{-1} was attributed to ν O-H of the hydration for compounds **1a**, **1c**, and **2b**. The ν Pt-Cl and ν Pt-I vibrational bands of the **1a-c** and **2a-c** complexes are expected to appear at 330-320 and 195-183 cm^{-1} in the far-IR region. However, these bands could not be observed as the spectrophotometer measured in the 4000-600 cm^{-1} range [42-44]. ^1H -NMR spectra in dimethylsulfoxide- d_6 (DMSO- d_6) had to be recorded as the complexes were insoluble in the other organic solvents. In the ^1H -NMR spectra of **1a-c** and **2a-c**, we observed three proton signals of the 1H-1,3-diazole ring (C2H, C4H, C5H) and protons of the substituted and/or nonsubstituted phenyl ring. Coordination to the platinum center leads to a lower field shift for 1H-1,3-diazole protons compared to free 1-substituted-1H-1,3-diazole ligands. The assignment of the C2H proton signals in the spectrum of the free carrier ligands are seen at δ 8.13, 8.24, and 7.74 ppm. The C2H proton peaks of the platinum(II) dichlorido (**1a-c**) and diiodido (**2a-c**) complexes with the same carrier ligands were observed at δ 8.65, 8.79-8.72, 8.26, 8.87, 8.99 and 8.91, and 8.35 ppm, respectively. The coordination with Pt(II) resulted in the magnetic nonequivalence of the methyl protons in **L1**. Consequently, the singlet methyl signal at δ 3.77 ppm observed in free **L1** was split into two multiplet peaks, appearing at about δ 3.82-3.78 and 3.80-3.78 ppm in the **1a** and **2a** complexes, respectively. As compared to the signals of all protons in the synthesized complexes with

those of the **L1-3** carrier ligand, it was observed that the protons shifted to a lower field as a result of the electric field effect caused by the platinum atom [17, 42, 45]. The ESI-MS spectra of the peaks in the platinum complexes have displayed m/z values and were observed in the mass spectrum.

2.2. Cytotoxic activity evaluation

To investigate the effect of carrier and leaving ligands on viability of cells, each synthesized complex was individually applied to (10-160 μ M) each cancer cell line CaCo-2, A549, and noncancerous cell line L929. MTT analysis shows that the viability of cells decreases concentration-dependent when cells are exposed to derivatives. Cisplatin was used as the reference drug (6.25-100 μ M). The cytotoxicity findings obtained from the MTT test results are shown in the Table 1. On the A549 cell line, it was found that the platinum(II) complex **1a-c**, which had chlorido ligands as leaving groups, was slightly more active than **2a-c**, which had iodido ligands as leaving groups. Compound **2c** was as effective as the reference drug cisplatin against the CaCo-2 cell line with IC_{50} values of 17.07 and 16.74 μ M, respectively. Compounds **1c** and **2c**, which contain a benzyl group at position 1 of the 1*H*-1,3-diazole, demonstrated the highest efficacy against the CaCo-2 cell line, with IC_{50} values of 28.4 and 17.07 μ M, respectively. In the A549 cell line, compounds **1a** and **2a**, featuring a methoxyphenyl group at position 1 of the 1*H*-1,3-diazole, exhibited greater potency compared to complexes with unsubstituted phenyl or benzyl groups. Additionally, compound **2a** showed a selectivity index of 1.26 against the A549 cell line, similar to cisplatin.

Table 1. IC_{50} (μ M) values of the compounds against A549, CaCo-2, and L929.

Compound No	A549		CaCo-2		L929
	IC_{50}^a	SI ^b	IC_{50}^a	SI ^b	IC_{50}^a
1a	38.1 $\pm$ 0.04	0.75	39.22 $\pm$ 0.04	0.72	28.42 $\pm$ 0.02
1b	50.09 $\pm$ 0.04	0.50	39.41 $\pm$ 0.04	0.64	25.14 $\pm$ 0.04
1c	40.34 $\pm$ 0.06	0.58	28.4 $\pm$ 0.03	0.82	23.25 $\pm$ 0.04
2a	39.76 $\pm$ 0.06	1.26	59.73 $\pm$ 0.02	0.84	50.28 $\pm$ 0.05
2b	55.67 $\pm$ 0.09	0.54	34.8 $\pm$ 0.04	0.86	29.96 $\pm$ 0.02
2c	43.08 $\pm$ 0.06	0.79	17.07 $\pm$ 0.02	1.99	33.91 $\pm$ 0.06
Cisplatin	26.39 $\pm$ 0.06	1.26	16.74 $\pm$ 0.04	1.98	33.13 $\pm$ 0.08

^a IC_{50} =50% cytotoxic concentration against in vitro tested cells. Data are presented as mean $\pm$ SD.

^bSI=Selectivity Index – IC_{50} value relative to a healthy cell.

2.3. Determination of the interactions of plasmid DNA with the complexes

When plasmid DNA interacted with increasing concentrations of compounds **1a-c**, **2a-c**, and cisplatin, two distinct DNA bands, indicating Forms I and II, were seen in both untreated and treated plasmid DNA (Figure 3). As the compound concentration increased, the mobility of the Form I and Form II bands also increased, though at different rates. For compounds **1a**, **2a**, and **2c**, the separation between the bands was smallest at a concentration of 3.1 μ M (lane 6), above which the separation increased (lanes 4 and 5). At higher concentrations (100-25 μ M; lanes 1-3), a faint coalesced band was seen, indicating a transition from Form II to Form I. The most active compound, **2b**, exhibited slightly greater DNA damage, showing a linear DNA form at the highest concentration of 100 μ M (lane 1).

2.4. BamHI and HindIII digestion of complexes-plasmid DNA

To investigate changes in DNA conformation, plasmid DNA was incubated with compounds **1a-c**, **2a-c**, and cisplatin, followed by digestion with *Bam*HI and *Hind*III. Figure 4 presents the electrophoretograms illustrating the interaction of plasmid DNA with varying concentrations of the compounds over a 24 h period at 37°C, followed by a 1 h restriction digestion at the same temperature. Upon digestion with *Bam*HI or *Hind*III, only one band corresponding to Form III (linear band) was observed, whereas untreated and undigested plasmid DNA displayed two bands of supercoiled and open circular (Forms I and II).

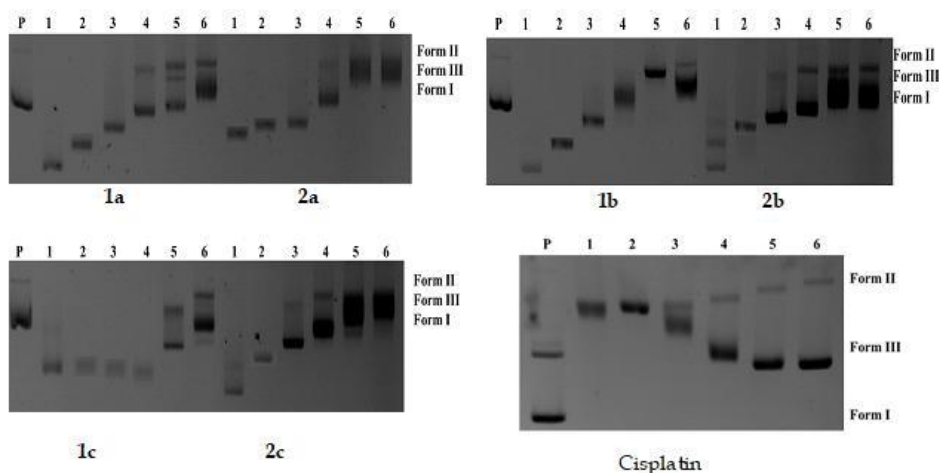


Figure 3. The interaction of plasmid DNA with decreasing concentrations synthesized complexes and cisplatin. Lane P applied to untreated plasmid DNA as a control, lanes 1 to 6 applied to plasmid DNA interacted with decreasing concentrations of the synthesized complexes and cisplatin. Lane 1: 100 μ M; Lane 2: 50 μ M; Lane 3: 25 μ M; Lane 4: 12.5 μ M; Lane 5: 6.25 μ M; Lane 6: 3.1 μ M

For compound **1a**, two bands corresponding to Forms I and II were seen in lanes 2 and 3 at concentrations of 6.25 and 3.1 μ M, respectively. In contrast, three bands were noted in lanes 2 and 3 for compound **2a** at the same concentrations. For compounds **1b**, **1c**, **2b**, and **2c**, both two and three bands corresponding to Forms I, II, and III were observed in lane 3 and lane 1 for the 3.1 μ M concentration of compounds **1b** and **2b**, respectively. Three bands were recorded for compounds **1c** and **2c** (lane 3) at 25 μ M, while two bands representing Forms I and II were observed (lane 2) at 50 μ M. No bands were observed in lanes 1 and 2 for the 100 μ M concentration of the compounds. Digestion with *Bam*HI showed two bands for compound **1a** and Forms I, II, and III for compound **2a**. Similar results were obtained with the other compounds. These results suggest that compounds **1a-c** and **2a-c** have the ability to alter the conformation of DNA, probably by forming GG and AA adducts.

3. CONCLUSION

The synthesized compounds **1a-c**, **2a-c**, and cisplatin were evaluated for their interactions with plasmid DNA and their effects on DNA conformation and integrity. Upon interaction with plasmid DNA, agarose gel electrophoresis revealed distinct DNA bands corresponding to different forms, namely Forms I, II, and III. As the concentration of the synthesized complexes increased, both Forms I and II exhibited altered mobility, indicating conformational changes in the DNA. The affinity of the compounds for the guanine-guanine (GG) and adenine-adenine (AA) parts of DNA has been investigated further with restriction endonuclease studies with *Bam*HI and *Hind*III enzymes. Following a 24 h incubation period, the compound-plasmid DNA mixtures were subjected to enzymatic digestion. The *Bam*HI enzyme, successfully converted supercoiled Form I and open circular Form II into linear DNA (Form III). Similarly, *Hind*III also facilitated the conversion of Forms I and II to Form III. Notably, **2b** exhibited the most pronounced DNA damage at a concentration of 100 μ M, as evidenced by the presence of linear DNA. The presence of GG and AA adducts suggests that these compounds induce conformational changes in the DNA, implicating their potential as effective agents in DNA targeting. Overall, the results indicate that compounds **1a-c** and **2a-c** are capable of causing significant conformational changes in DNA. Furthermore, their ability to induce DNA damage through adduct formation underscores their potential applications in therapeutic settings. The synthesized compounds demonstrated varying levels of cytotoxicity against different cancer cell lines, with notable efficacy observed in the CaCo-2 cell line. Compounds **1c** and **2c**, featuring a benzyl group at position 1 of the imidazole ring, exhibited the highest potency, with IC_{50} values of 28.4 μ M and 17.07 μ M, respectively. Additionally, platinum (II) complexes **1a-c**, which possess chlorido ligands as leaving groups, were found to be slightly more active than their iodido counterpart (**2a-c**) in the A549 cell line. In this cell line, compounds **1a** and **2a**, which contain methoxyphenyl substituents at position 1 of the imidazole ring, showed superior activity compared to those with unsubstituted phenyl and benzyl groups. These results underscore the significance of both the type of substituent and the nature of the leaving group in enhancing the antitumor

activity of these compounds. In conclusion, the obtained biological activity test results suggest that Pt(II) complexes have the potential for further development as effective anticancer agents in clinical applications.

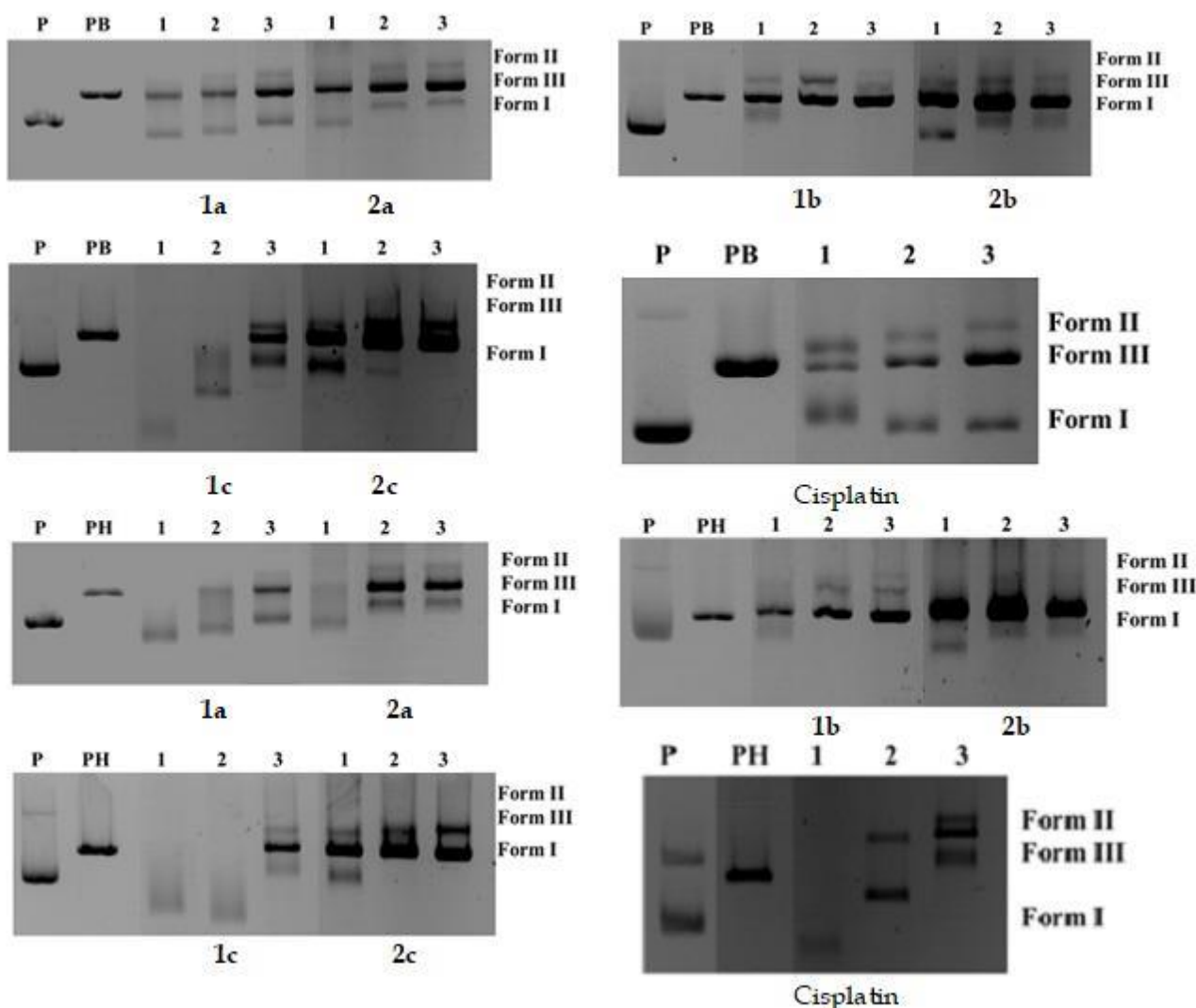


Figure 4. The incubated mixtures of plasmid DNA and varying concentrations of synthesized complexes and cisplatin followed by digestion with *Bam*HI and *Hind*III. Lane P applied to the untreated plasmid DNA and undigested, lane PB or PH applied to untreated but digestion with *Bam*HI and *Hind*III respectively. Lanes 1-3: apply to 12.5, 6.25, 3.1 μ M, plasmid DNA interacted with decreasing concentrations of the compounds followed by enzyme digestion.

4. MATERIALS AND METHODS

4.1. Chemistry

All chemicals and solvents were reagent-grade quality, obtained from such as Merck, Sigma, and Aldrich were utilized for the synthesis process. To determine the melting points, an Electrothermal 9200 capillary melting point apparatus was employed. Infrared spectra were recorded using a Perkin Elmer/MID Spectrum two/UATR spectrometer in the range of 4000-600 cm^{-1} . ^1H -NMR spectra were recorded in $\text{DMSO}-d_6$ on a VARIAN Mercury 400 FT-NMR spectrometer operating at frequencies of 400 MHz. Elemental analyses (C, H, N) were performed on a Leco CHNS 932 instrument and were within $\pm 0.4\%$ of the theoretical values. ESI-LC/MS spectra were obtained using the ESI⁺ technique on a Waters Micro-mass ZQ connected with Waters Alliance HPLC instrument.

4.1.1. Spectral analysis of **L1-3**

1-(4-Methoxyphenyl)-1*H*-1,3-diazole (**L1**)

FT-IR/ATR: (ν , cm^{-1}) 3127-2837 (=C-H, C-H), 1610-1518 (C=N, C=C), 1050 (=C-O-C). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$, ppm): δ 8.13 (s, 1H, 1,3-diazole-H), 7.62 (bs, 1H, 1,3-diazole-H), 7.52 (d, J = 8 Hz, 2H, ArH), 7.07 (s, 1H, 1,3-diazole-H), 7.03 (d, J = 8 Hz, 2H, ArH), 3.77 (s, 3H, -OCH₃).

1-Phenyl-1*H*-1,3-diazole (**L2**)

FT-IR/ATR: (ν , cm^{-1}) 3114-3054 (=C-H), 1599-1504 (C=N, C=C). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$, ppm): δ 8.24 (s, 1H, 1,3-diazole-H) 7.72 (s, 1H, 1,3-diazole-H), 7.62 (d, 2H, J = 8 Hz ArH), 7.52- 7.47 (m, 2H, ArH), 7.34 (t, 1H, J = 8 Hz, ArH), 7.10 (s, 1H, 1,3-diazole-H).

1-Benzyl-1*H*-1,3-diazole (**L3**)

FT-IR/ATR: (ν , cm^{-1}) 3113-2883 (C-H, =C-H), 1602-1505 (C=N, C=C). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$, ppm): δ 7.74 (s, 1H, 1,3-diazole-H), 7.35-7.22 (m, 5H, ArH), 7.16 (s, 1H, 1,3-diazole-H), 6.89 (s, 1H, 1,3-diazole-H), 5.17 (s, 2H, -CH₂-).

4.1.2. Synthesis of dichloroplatinum(II) complexes (**1a-c**)

To a solution of potassium tetrachloroplatinate (0.8 mmol) in 0.5 N HCl (2 mL) was added dropwise a solution of an appropriate carrier ligand (**L1**, **L2**, or **L3**) (1.5 mmol) in 0.5 N HCl (5 mL) within 25 minutes. The reaction mixture was stirred at 40-50 °C in the dark for 5-7 days. After the completion of the reaction was confirmed by TLC, the precipitate was filtered. The crude products were washed with water, ethanol, and diethyl ether, respectively, and dried in a vacuum to yield dichloroplatinum(II) complexes (**1a-c**).

Dichloro-di(1-(4-methoxyphenyl)-1*H*-1,3-diazole)platinum(II) 0.5 H₂O [Pt(**L1**)₂Cl₂] (**1a**)

Yield 62.16% (310 mg); m.p. >400 °C; elemental analysis for C₂₀H₂₀Cl₂N₄O₂Pt. 0.5 H₂O: calculated C, 38.53; H, 3.40; N, 8.99; found: C, 38.39; H, 3.49; N, 9.11. FT-IR/ATR: (ν , cm^{-1}) 3120-2836 (C-H, =C-H, O-H), 1611-1524 (C=N, C=C), 1068 (=C-O-C). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$, ppm): δ 8.65 (s, 2H, 2x 1,3-diazole-H), 7.80 (s, 2H, 2x 1,3-diazole-H), 7.63-7.57 (m, 4H, 2x ArH), 7.19 (s, 2H, 2x 1,3-diazole-H), 7.10-7.06 (m, 4H, 2x ArH), 3.82-3.78 (m, 6H, -OCH₃). HRMS (ESI⁺): m/z calculated 614.4 for C₂₀H₂₀Cl₂N₄O₂Pt; found: 655.7 [M+2H+K]⁺.

Dichloro-di(1-phenyl-1*H*-1,3-diazole)platinum(II) [Pt(**L2**)₂Cl₂] (**1b**)

Yield 53.44% (237 mg); m.p. >400 °C; elemental analysis for C₁₈H₁₆Cl₂N₄Pt: calculated C, 39.00; H, 2.91; N, 10.11; found: C, 39.15; H, 2.99; N, 9.97. FT-IR/ATR: (ν , cm^{-1}) 3127-3059 (=C-H), 1597-1522 (C=N, C=C). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$, ppm): δ 8.79-8.72 (m, 2H, 2x 1,3-diazole-H), 7.99-7.90 (m, 2H, 2x 1,3-diazole-H), 7.77-7.67 (m, 4H, 2x ArH), 7.58-7.52 (m, 4H, 2x ArH), 7.46-7.40 (m, 2H, 2x ArH), 7.26-7.24 (m, 2H, 2x 1,3-diazole-H). HRMS (ESI⁺): m/z calculated 554.3 for C₁₈H₁₆Cl₂N₄Pt; found 595.8 [M+2H+K]⁺.

Dichloro-di(1-benzyl-1*H*-1,3-diazole)platinum(II) [Pt(**L3**)₂Cl₂]. H₂O (**1c**)

Yield 39.06% (182 mg); m.p. >400 °C; elemental analysis for C₂₀H₂₀Cl₂N₄Pt.H₂O: calculated C, 40.01; H, 3.69; N, 9.33; found: C, 39.93; H, 3.58; N, 9.26. FT-IR/ATR: (ν , cm^{-1}) 3122-2881 (C-H, =C-H, OH), 1604-1530 (C=N, C=C). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$, ppm): δ 8.26 (bs, 2H, 2x 1,3-diazole-H), 7.39-7.30 (m, 10H, 2x ArH), 7.27-7.25 (m, 2H, 2x 1,3-diazole-H), 6.82 (s, 2H, 2x 1,3-diazole-H), 5.28-5.22 (m, 4H, 2x -CH₂-). HRMS (ESI⁺): m/z calculated 582.4 for C₂₀H₂₀Cl₂N₄Pt; found 623.9 [M+2H+K]⁺.

4.1.3. Synthesis of diiodoplatinum(II) complexes (**2a-c**)

Potassium tetrachloroplatinate (0.6 mmol), and potassium iodide (2.4 mmol) were dissolved in water (10 mL) and stirred at 60 °C in the dark for 45 minutes. The appropriate carrier ligand (**L1**, **L2**, or **L3**) (1.2 mmol) dissolved in ethanol-water (3:5 mL) was added dropwise at room temperature to the resulting potassium tetraiodoplatinate solution. The reaction mixture was stirred at 40 °C in the dark for 2-4 days. After the completion of the reaction was confirmed by TLC. The yellowish crude products were filtered and washed with water, ethanol, and diethyl ether, respectively, and dried in a vacuum to yield diiodoplatinum(II) complexes (**2a-c**).

Diiiodido-di(1-(4-methoxyphenyl)-1*H*-1,3-diazole)platinum(II) [Pt(L1**)₂I₂] (**2a**)**

Yield 50.38% (241mg); m.p. >400 °C; elemental analysis for C₂₀H₂₀I₂N₄O₂Pt: calculated C, 30.13; H, 2.53; N, 7.03; found: C, 30.09; H, 2.66; N, 7.14. FT-IR/ATR: (ν, cm⁻¹) 3120-2836 (C-H, =C-H), 1613-1522 (C=N, C=C), 1066 (=C-O-C). ¹H-NMR (400 MHz, DMSO-*d*₆, ppm): δ 8.87 (s, 2H, 2x 1,3-diazole-H), 7.97 (s, 2H, 2x 1,3-diazole-H), 7.64-7.58 (m, 4H, 2x ArH), 7.29 (s, 2H, 2x 1,3-diazole-H), 7.11- 7.08 (m, 4H, 2x ArH), 3.80-3.78 (m, 6H, -OCH₃). HRMS (ESI⁺): *m/z* calculated 797.2 for C₂₀H₂₀I₂N₄O₂Pt; found 797.0 [M]⁺.

Diiiodido-di(1-phenyl-1*H*-1,3-diazole)platinum(II).H₂O [Pt(L2**)₂I₂] (**2b**)**

Yield 55.61% (246 mg); m.p. >400 °C; elemental analysis for C₁₈H₁₆I₂N₄Pt.H₂O: calculated C, 28.63; H, 2.40; N, 7.42; found: C, 28.76; H, 2.32; N, 7.53. FT-IR/ATR: (ν, cm⁻¹) 3345-3122 (=C-H, O-H), 1596-1518 (C=N, C=C). ¹H-NMR (400 MHz, DMSO-*d*₆, ppm): δ 8.99 and 8.91 (s, 2H, 2x 1,3-diazole-H) 8.08-7.92 (m, 2H, 2x 1,3-diazole-H), 7.73- 7.66 (m, 4H, 2x ArH), 7.59- 7.52 (m, 4H, 2x ArH), 7.49- 7.41 (m, 2H, 2x ArH), 7.35-7.33 (m, 2H, 2x 1,3-diazole-H). HRMS (ESI⁺): *m/z* calculated 737.2 for C₁₈H₁₆I₂N₄Pt; found 754.9 [M+H₂O]⁺ and 688.9 [M-I+DMSO]⁺.

Diiiodido-di(1-benzyl-1*H*-1,3-diazole)platinum(II) [Pt(L3**)₂I₂] (**2c**)**

Yield 48.35% (222 mg); m.p. >400 °C; elemental analysis for C₂₀H₂₀I₂N₄Pt: calculated C, 31.39; H, 2.63; N, 7.32; found: C, 31.29; H, 2.76; N, 7.44. FT-IR/ATR: (ν, cm⁻¹) 3122-2890 (C-H, =C-H), 1605-1521 (C=N, C=C). ¹H-NMR (400 MHz, DMSO-*d*₆, ppm): δ 8.35 (bs, 2H, 2x 1,3-diazole-H), 7.32-7.30 (m, 10H, 2x ArH), 7.22-7.17 (m, 2H, 2x 1,3-diazole-H), 6.96 (bs, 2H, 2x 1,3-diazole-H), 5.28-5.22 (m, 4H, 2x -CH₂-). HRMS (ESI⁺): *m/z* calculated 765.3 for C₂₀H₂₀I₂N₄Pt; found 844.7 [M+2K+H]⁺ and 716 [M-I+DMSO]⁺.

4.2. Biological activity

4.2.1. MTT assay

Cisplatin was obtained from Sigma with a purity of more than 99.9%. Human non-small cell lung cancer (A549, ATCC CCL185), human colon adenocarcinoma (CaCo-2, An1/ HUKUK, Foot and Mouth Disease Institute, Ankara, Türkiye), and mouse fibroblast cells (L929, An2 Mouse C3, HUKUK, Foot and Mouth Disease Institute, Ankara, Türkiye), cells were tested using the MTT method [38, 39].

The L929, A549, and CaCo-2 cell lines were incubated in Dulbecco's Modified Eagle and Eagle's Minimal Essential, respectively. The mediums were supplemented with broth containing 10% fetal bovine serum, 100 IU/mL penicillin/streptomycin with 4 μM glutamine at 37 °C in a humidified incubator with 5% CO₂. Cells were seeded into 96-well microculture plates and incubated for 24 h. To determine cytotoxicity by the MTT method, stock solutions of **1a-c**, **2a-c** (160, 80, 40, 20, and 10 μM), and cisplatin (100, 50, 25, 12.5, and 6.25 μM) were diluted with the supplemented medium to obtain final concentrations. These solutions were prepared with DMF, which did not exceed 0.1%. Cisplatin was used as a positive control. After incubating the cells with the samples for 24 h, 30 μL of MTT solution was added. After 2 h of incubation at 37 °C, 100 μL of isopropanol was added to the wells, and the absorbance values of the 96-well plate were recorded at 570 nm using an ELISA microplate reader. All experiments were repeated in triplicate, and the results were presented as a mean ± standard deviation. The IC₅₀ values of samples were calculated using a percentage of relative cell viability via GraphPad Prism 5.

4.2.2. Interaction with pBR322 plasmid DNA

pBR322 plasmid DNA, ethidium bromide agarose, *Hind*III, and *Bam*HI enzymes were purchased from Sigma. The interaction of **1a-c**, **2a-c**, and cisplatin with plasmid DNA were examined by agarose gel electrophoresis [46]. The compound-DNA mixtures were loaded onto a 1% agarose gel using a loading buffer containing 0.1% bromophenol blue and 0.1% xylene cyanol. Using TAE buffer (0.05 M Tris base, 0.05 M glacial acetic acid, and 1.0 mM EDTA, pH 8.0), agarose gel electrophoresis was carried out for two hours at a voltage of 70 V. After electrophoresis, the gel was stained with ethidium bromide at a concentration of 0.5 μg/mL and visualized under UV light using a transilluminator (BioDoc Analyzer, Biometra).

4.2.3. *Hind*III and *Bam*HI digestions of the synthesized complexes-plasmid DNA

Platinum complexes-plasmid DNA adducts were subjected to restriction endonuclease analyses using *Hind*III and *Bam*HI enzymes to assess the compounds' affinity for guanine-guanine (GG) and/or adenine-adenine (AA) regions of DNA. The compounds-plasmid DNA mixtures were incubated for 24 h and

subsequently restricted with either *Hind*III or *Bam*HI at 37 °C to assess the binding of the compounds to DNA. The *Bam*HI enzyme targets the sequence 5'-G/GATCC-3' and cleaves this sequence, as plasmid DNA contains a single copy of it. Consequently, *Bam*HI converts the supercoiled Form I and open circular Form II into a linear form of DNA (Form III). Similarly, *Hind*III recognizes and cleaves the sequence 5'-A/AGCTT-3', converting Forms I and II to Form III. The restricted DNA was then analyzed using 1% agarose gel electrophoresis for 1 h at 70 V in TAE buffer. After electrophoresis, the gel was stained with ethidium bromide (0.5 µg/mL) and visualized with a transilluminator (BioDoc Analyzer, Biometra) [46].

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