Polyhedron 161 (2019) 298–308

Contents lists available at ScienceDirect

Polyhedron

journal homepage: www.elsevier.com/locate/poly

Studies on the synthesis, characterization, cytotoxic activities and plasmid DNA binding of platinum(II) complexes having 2-subsituted benzimidazole ligands

Mahmut Gozelle [a], Aysun Kılıç Süloğlu [b], Güldeniz Selmanoğlu [b], Nagehan Ramazanoğlu [c], Leyla Açık [c], Fatma Gümüş [a,*]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, 06560 Etiler-Ankara, Turkey
[b] Department of Biology, Faculty of Science, Hacettepe University, 06800 Beytepe-Ankara, Turkey
[c] Department of Biology, Faculty of Science, Gazi University, 06560 Ankara, Turkey

ARTICLE INFO

Article history:
Received 23 November 2018
Accepted 10 January 2019
Available online 24 January 2019

Keywords:
Benzimidazoles
Platinum complexes
Cytotoxic activity
pBR322
Restriction enzymes

ABSTRACT

The aim of this study was to synthesize and evaluate the cytotoxic activities and plasmid DNA interaction of some new platinum(II) complexes, which may have potent cytotoxic activity and low side effects, with benzimidazole derivatives containing some amino acid residues as substituents in their 2 position. Seven 2-subtituted benzimidazole derivatives (**1**–**7**) (2-(H, methyl isopropyl, isobutyl, sec-butyl 1H-imidazol-4-ylmethyl or 4-hydroxybenzyl)benzimidazole, respectively), seven platinum(II) complexes with two chlorido leaving groups (**1a**–**7a**) and six platinum(II) complexes with an oxalato leaving group (**2b**–**7b**) bearing the benzimidazole carrier ligand were synthesized. The chemical structures of the compounds were characterized by their elemental analysis, IR, ^{1}H NMR, and HRMS spectra. The compounds were evaluated for their cytotoxic activities against human HeLa cervical cancer cell lines. The interaction of all the ligands and their complexes with plasmid DNA and their restriction endonuclease reactions with *Bam*HI and *Hind*III enzymes were investigated by agarose gel electrophoresis. Compounds **1a**, **3a**, **3b**, **6**, **7a** and **7b**, having 2-(H, isopropyl, 1H-imidazol-4-ylmethyl or 4-hydroxybenzyl)benzimidazole carrier ligands, have moderate in vitro cytotoxic activity, close to that of carboplatin.

1. Introduction

Platinum complexes as antitumor agents have received significant attention due to the serendipitous discovery of the antiproliferative activity of cisplatin (*cis*-diamminedichloroplatin(II)) by Rosenberg [1,2]. Cisplatin, carboplatin and oxaliplatin, which are DNA damaging agents, have become the most noteworthy drugs for cancer treatments. Cisplatin is used to treat lung cancer, mesothelioma, brain tumours and neuroblastoma [1,3–5]. Most notably, long-term survival rates of testicular cancer patients using cisplatin improved from less than 10% to greater than 90% [3,6]. Despite the great success in treating most cancers, there are several side effects reported, such as nephrotoxicity, neurotoxicity, ototoxicity and myelosuppression [7]. Therefore, its clinical utility is restricted by both toxicological and especially tumour resistance considerations [8]. The need for cisplatin analogues, which are less toxic and have a broader spectrum of activity, has led to the synthesis of a large number of platinum complexes over the past four decades. The second and third generation platinum complexes are obtained by the replacement of the leaving groups with carboxylate groups which are more stable and chelating agents [4,9]. Replacement of the NH_3 groups with cyclic amines, especially those involving large rings, generally reduces the toxicity of the platinum compounds [9]. Several platinum complexes with N-heterocyclic ligands, such as thiazole, benzoxazole, benzimidazole, imidazole and benzothiazole, have been reported [10–14].

Studies have been carried out on the development of new platinum complexes having anticancer activity and less toxicity. Platinum(II) complexes carrying some amino acids, peptides or glucosamines have been synthesized and some of them have anticancer activity. The activities of the aforementioned complexes are associated with the carrying ligands as they are natural substances and used by cells in the organism [15]. Cancer cells have high requirements for amino acids for their existence [16,17] and amino acids could be transported into the cell by special transport systems [18]. Therefore, platinum(II) complexes having amino

* Corresponding author.
E-mail address: fgumus@gazi.edu.tr (F. Gümüş).

https://doi.org/10.1016/j.poly.2019.01.028
0277-5387/

acids as carrier ligands were synthesized and tested for their biological activities [19–27].

The benzimidazole nucleus is structurally similar to purine bases and it is in a variety of naturally occurring compounds, such as vitamin B12 and its derivatives. Compounds having benzimidazole moieties are used in the area of pharmaceuticals. Various benzimidazole derivatives have been found to possess anticancer, antiviral, antihypertension and some other properties [28–30]. In the analytical area, benzimidazole moieties have been investigated as reagents for the separation, concentration and selective determination of many first-row transition metal cations. It is known that coordination with metals can modify the toxicological and pharmacological properties of benzimidazole moieties [4,9].

In previous papers, we reported the synthesis, characterization and in vitro cytotoxic activities of complexes of the structure *cis*-[Pt(L)$_1$ or $_2$ C$_2$], where L is a mono- or bidentate 2-substituted benzimidazole [12,31–34]. It was found that some of these new benzimidazole Pt(II) complexes have in vitro cytotoxic activities equal to cisplatin.

The aim of this study is the synthesis and evaluation of the cytotoxic effect of new platinum(II) complexes having 2-substituted benzimidazoles which have some amino acid residues as substituents. For this purpose, seven benzimidazole ligands, seven platinum(II) complexes having two chlorido leaving groups and six platinum(II) complexes having an oxalato leaving group were synthesized and characterized by their elemental analysis, mass, IR and ^{1}H NMR spectra. Furthermore, we report in vitro testing of their cytotoxic activities on HeLa cervical cancer cell lines and the plasmid DNA interactions of the new platinum(II) complexes.

2. Results and discussion

2.1. Chemistry

The first step in the synthetic sequence to obtain the platinum (II) complexes was the synthesis of the 2-substituted benzimidazole derivatives as carrier ligands (**1**–**7**). The melting points of the carrier ligands, except **6**, were in accordance with the literature. Ligands **1**, **2**, **6** and **7** were achieved by the Philips method [35], **3** and **4** were synthesized with an appropriate aldehyde and $Co(OH)_2$ [36], and **5** was synthesized with an appropriate aldehyde and $NaHSO_3$ [37], as shown in Scheme 1. Ligands **1**–**5** and **7** were structurally characterized by using their melting points along with ^{1}H NMR, IR and HRMS spectra. The new ligand **6** was structurally characterized by using its melting point, elemental analysis, ^{1}H NMR, IR and HRMS spectra.

The platinum(II) complexes **1a**–**7a**, with two chlorido leaving groups, were synthesized as shown in Scheme 2 and characterized using melting point, elemental analysis, ^{1}H NMR, IR and HRMS spectroscopy methods. Complexes **1a** and **2a** were synthesized as previously reported [38,39]. Platinum(II) complexes **2b**–**7b**, with oxalato leaving groups, were synthesized as shown in Scheme 3 and characterized by using melting point, elemental analysis, ^{1}H NMR, IR and HRMS spectroscopy methods. All characterization data suggested a 1:2 (metal:ligand) stoichiometry for the Pt(II) complexes except **6a** and **6b**. All characterization data for complexes **6a** and **6b** suggested a 1:1 (metal:ligand) stoichiometry.

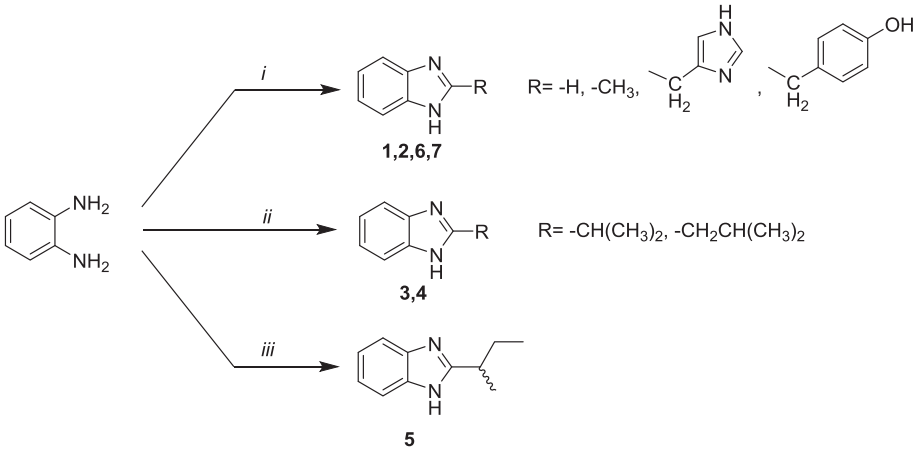
The IR spectra of the compounds were measured in the range 4000–40 cm^{-1} and featured characteristic changes when compared to those of the free ligands. The Pt–Cl vibrations are considered to be specific for platinum(II) complexes with two chlorido leaving groups. The synthesis method used is expected to yield complexes with a cis geometry, in compliance with the kinetic trans effect [40,41]. In the far-IR region, the spectra of complexes **1a**–**7a** differ from the spectra of the carrier ligands by the presence of a new broad band at ~320 cm^{-1}, which is assigned to the ν(Pt–Cl) mode. The C=O, C–O and Pt–O vibrations are considered to be specific for platinum(II) complexes with an oxalato leaving group, having two strong absorption peaks [42]. In the IR spectra of **2b**–**7b**, different from those of carrier ligands' spectra, new broad bands appeared that are assigned to ν(C=O) at ~1700–1600 cm^{-1}, ν(C–O) at ~1300–1200 cm^{-1} and ν(Pt–O) at ~570–550 cm^{-1}.

The ^{1}H NMR spectra of the compounds were measured in dimethyl sulfoxide-d_6 (DMSO-d_6) due to the insolubility of complexes in other NMR solvents. In order to avoid ligand exchange between the platinum(II) complexes and DMSO-d_6, all measurements of the solutions were recorded immediately.

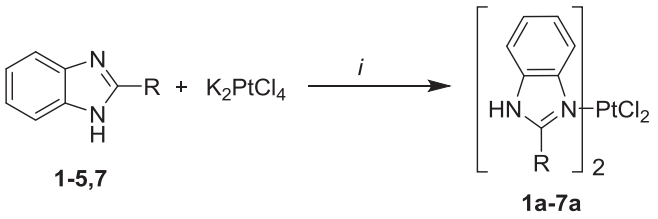
The HRMS spectra displayed peaks having m/z values and isotopic distributions which were congruent with those calculated for the cationic/anionic ligands and the platinum complexes. The mass errors of all the compounds were less than 5 ppm.

2.2. Cytotoxicity assay

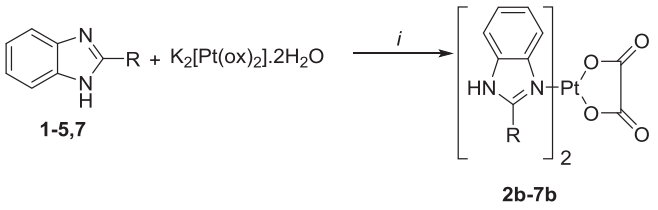
To analyse the potential in vitro cytotoxic activity of the ligands **1**–**7**, the dichloro-platinum(II) complexes **1a**-**7a** and the oxalato-platinum(II) complexes **2b**–**7b**, we evaluated their cytotoxic activity on HeLa cervical cancer cell lines using cisplatin, carboplatin and oxaliplatin as reference drugs. An MTT (3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay was used to estimate the inhibition percentage values of the deter-



Scheme 1. Synthesis of the 2-substituted benzimidazole ligands. (*i*) RCOOH, 4–5 N HCl, Δ; (*ii*) RCHO, $Co(OH)_2$, EtOH, rt; (*iii*) RCHO, $NaHSO_3$, DMA, 100 °C.



Scheme 2. Synthesis of the *cis*-[dichloro-bis(ligand)platin(II)] complexes with 2-substituted benzimidazole ligands. (*i*) EtOH (or iPrOH)/water, 40–50 °C.



Scheme 3. Synthesis of the oxalato-bis(ligand)platin(II)] complexes with 2-substituted benzimidazole ligands. (*i*) EtOH (or iPrOH)/water, 50–60 °C.

mined compound concentrations (10, 20, 40, 80 and 160 μM) after 48 and 72 h incubation. The percentage of surviving cells was defined as the treatment group/control group where the control group was assumed as 100% survival. The percentage of inhibition was calculated via the surviving cell percentage. The percentage of inhibition of cell viability in HeLa cell lines treated with different concentrations of the compounds and positive controls are presented in Figs. 1 and 2.

Our results showed that cytotoxic activity of the carrier ligand **6** at 80 μM concentration after 48 h incubation showed moderate activity compared with carboplatin. The carrier ligand **6** was found to be more cytotoxic than its platinum complexes **6a** and **6b** which have bidentate carrier ligands. In addition, the carrier ligands **4** and **5** at 80 and 160 μM concentrations, after 72 h incubation, showed moderate activity.

The results of the preliminary cytotoxic activities indicated that, overall, the cytotoxic activities of the complexes, except **5a**, after 72 h incubation were higher than 48 h incubation. **5a** showed no inhibition on HeLa cell lines after 72 h incubation.

Complexes **3b**, **5b** and **7b** showed more cytotoxic activity than the complexes with chloride leaving groups after 72 h incubation. This might be because the oxalato ligand leaves the platinum complexes slower than the chloride ligand [43]. In this study, the complexes with chloride leaving groups were partially found to be more cytotoxic than the complexes with the oxalato group.

Having low cytotoxic activity after 48 h incubation, compounds **3a**, **3b** and **5** showed more inhibition after 72 h incubation. The percentage inhibition values of those compounds at 160 μM concentration after 72 h incubation were 51.26 ± 7.69, 46.62 ± 7.43 and 43.34 ± 14.92, respectively.

2.3. Interaction with plasmid DNA

As platinum-DNA adducts damage cancer cell division rapidly after they bind to DNA, platinum complexes have a cytotoxic effect [44]. In order to reveal whether conformational changes in the DNA helix are induced by the synthesized ligands **1**–**7**, dichloro-platinum(II) complexes **1a**–**7a** and oxalato-platinum(II) complexes **2b**–**7b**, and whether there is an association between the binding affinity and the cytotoxicity of the ligands and complexes with plasmid DNA, we researched the ability of the ligands and

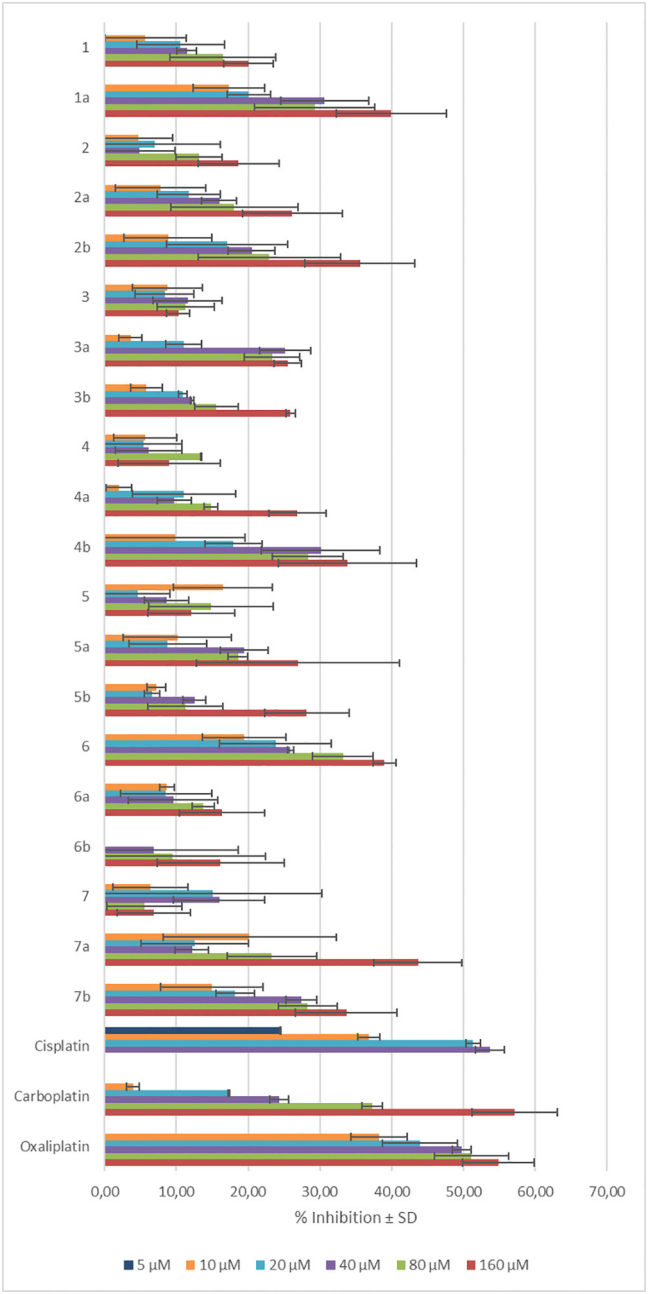


Fig. 1. Percentage inhibition of cell viability in HeLa cell lines treated with different concentrations of the compounds and positive controls for 48 h.

complexes to remove and reverse closed circular plasmid DNA supercoiling using electrophoretic mobility of DNA on agarose gels. The patterns of the electrophoretic mobility of Form I (covalently closed circular DNA) and Form II (open circular DNA) after incubation in concentrations from 10 to 320 μM at 37 °C for 24 h, where untreated plasmid DNA was used as a negative control and cisplatin, carboplatin and oxaliplatin were used as positive control drugs, are shown in Fig. 3.

Nuclear DNA is a crucial molecular target for platinum complexes; platinum complexes bind purine bases (adenine and guanine) at the N7 position [45–47]. The resulting Pt-DNA damage initiates the formation of various responses, such as replication and transcription inhibition, cell cycle arrest, DNA repair and apoptosis. When the damage is not repaired by the DNA repair mechanism, the cell dies by one of the several pathways [48].

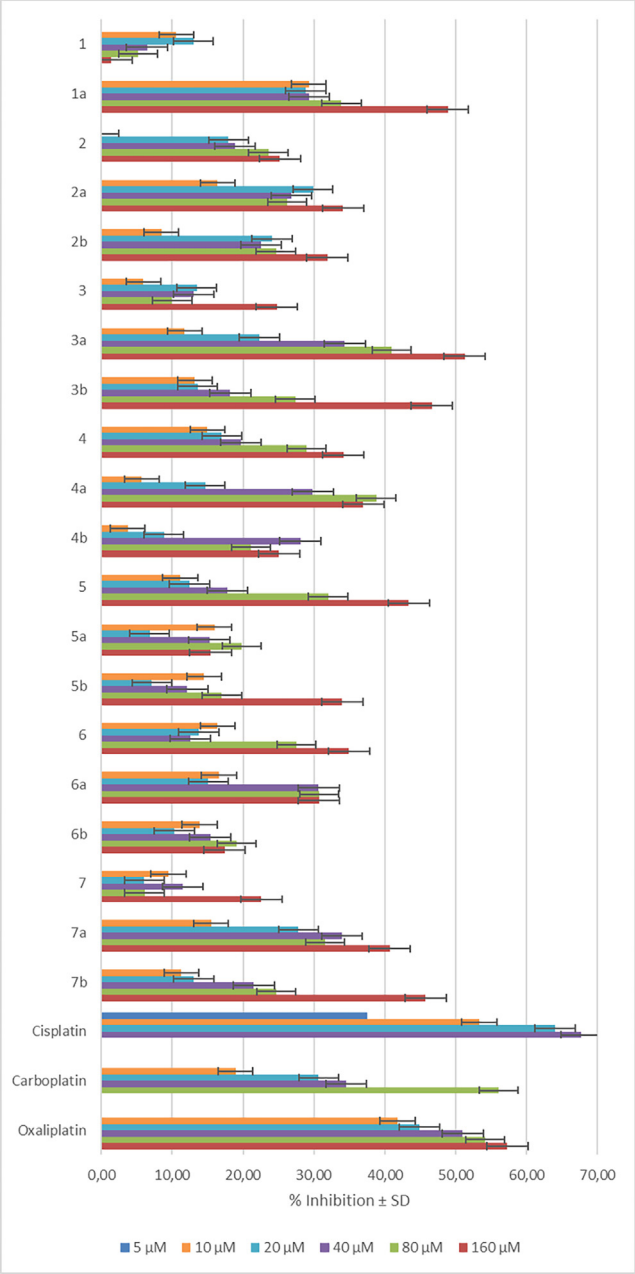


Fig. 2. Percentage inhibition of cell viability in HeLa cell lines treated with different concentrations of the compounds and positive controls for 72 h.

All platinum complexes which are used in clinic have a *cis* configuration and they mostly bond intrastrand. Furthermore, interstrand, monofunctional and intercalating DNA adducts are formed as well [47]. Pt-DNA adducts cause structural changes in DNA, such as untwisting or bending [45,49,50], prevent transcription [51], which then leads to cell death. Although cross-linked intrastrand and interstrand bifunctional adducts bend DNA, dsDNA (double-stranded DNA) may be untwisted by monofunctional or intercalating adducts.

Due to the interaction between increasing concentrations of the complexes, cisplatin, carboplatin, and oxaliplatin with plasmid DNA, the DNA experiences a decrease in the Form I DNA mobility. Since this reduces the number of supercoils, a decrease in the migration rate of the Form I DNA is the result of untwisting of the DNA. Particularly, **1a**, **2a**, **3a**, **5a**, **6a**, cisplatin and oxaliplatin resulted in an important decrease in the rate of migration of Form I DNA, which is shown Fig. 3. Furthermore, complex **4a** and carboplatin had a medium effect on the rate of Form I DNA's migration. On the contrary, all the synthesized ligands (**1**–**7**), complexes **7a** and all the platinum complexes having the oxalato group as a leaving ligand (**2b**–**7b**) did not untwist plasmid DNA meaningfully. The bifunctional binding of the synthesized compounds shortened and condensed the DNA helix similar to cisplatin [52] and these synthesized compounds expedited the mobility of Form II DNA.

It was observed that complexes **1a**, **2a**, **3a**, **5a** and **6a** meaningfully altered the electrophoretic mobility and a coalescence of the two forms of plasmid DNA was detected that was similar to that of oxaliplatin and greater than that of carboplatin. The required concentration to achieve overall unwinding of DNA for complexes **1a**, **2a**, **3a**, **6a**, cisplatin and oxaliplatin were 80, 80, 80, 160, 20 and 80 µM, respectively. A strong unwinding of the supercoiled DNA is indicated by the presence of a coalescence point [53]. This coalescence point suggests which compounds have a stronger interaction than both oxaliplatin and carboplatin with plasmid DNA. Structural factors (electronic and steric, etc.) caused by the isopropyl or methyl moieties may further stabilize the Pt-plasmid DNA adduct.

The interaction with cisplatin leads to an alteration in the plasmid DNA's mobility and this is thought to occur because of adducts which are intrastrand bifunctional Pt(GG) and monofunctional Pt (G) [53,54]. The bifunctional DNA-cisplatin adduct unwinds DNA more efficiently than monoadducts [53]. Monofunctional binding to DNA may be the reason for the small alterations in the mobilities of Form II and particularly of Form I DNA treated with the other synthesized complexes. Moreover, the complexes with chloro leaving groups were found to be more active than the complexes with oxalato leaving groups.

2.4. BamHI and HindIII digestion of drug-plasmid DNA

We carried out restriction endonuclease analysis of the compounds to evaluate whether the synthesized compounds show an affinity to adenine-adenine (AA) and/or guanine-guanine (GG) regions. Linear Form III DNA is formed after cutting Form I and Form II DNA by *Bam*HI and *Hind*III restriction enzymes, which bind at the recognition sequence 5′-G|GATCC-3′ and 5′-A|AGCTT-3′, and cut these sequences just next to the 5′-G and 5′-A sites, respectively [55].

The major cisplatin-DNA adduct is formed by a 1,2-intrastrand crosslink between contiguous guanine and/or adenine [56]. It is generally believed that the 1,2-intrastrand DNA adduct accounts for the cytotoxic effect of cisplatin [57]. X-ray crystallography and NMR spectroscopy results of the cisplatin-DNA adduct show that 65% 1,2-(GG), 25% 1,2(AG) and 5–10% 1,3-(GNG) intrastrand crosslinks and 1–3% interstrand crosslink are formed [47,49,58].

It was observed that *Bam*HI and *Hind*III (except for carrier ligands **1**–**7** and the oxalato-platinum(II) complexes **3b**, **4b** and **6b**) digestions were increasingly prevented (Figs. 4 and 5) with increasing concentrations of the tested compounds. The reason for this might be due to a conformational change in the DNA caused by covalent binding of the compounds with plasmid DNA.

It was determined that compounds **1a**, **2a**, **3a**, and **6a** have potently inhibited both *Bam*HI and *Hind*III restriction enzyme activity. Among the platinum(II) complexes tested, compounds **1a**, **2a**, **3a** and **6a** inhibit the *Bam*HI restriction enzyme activity to a greater extent than the *Hind*III restriction enzyme activity; thus, demonstrating their affinity for the GG regions of plasmid DNA. On the other hand, it is interesting to note that the inhibition of *Bam*HI and *Hind*III restriction enzyme activities by compounds **7a** and **7b** was greater than by the other compounds.

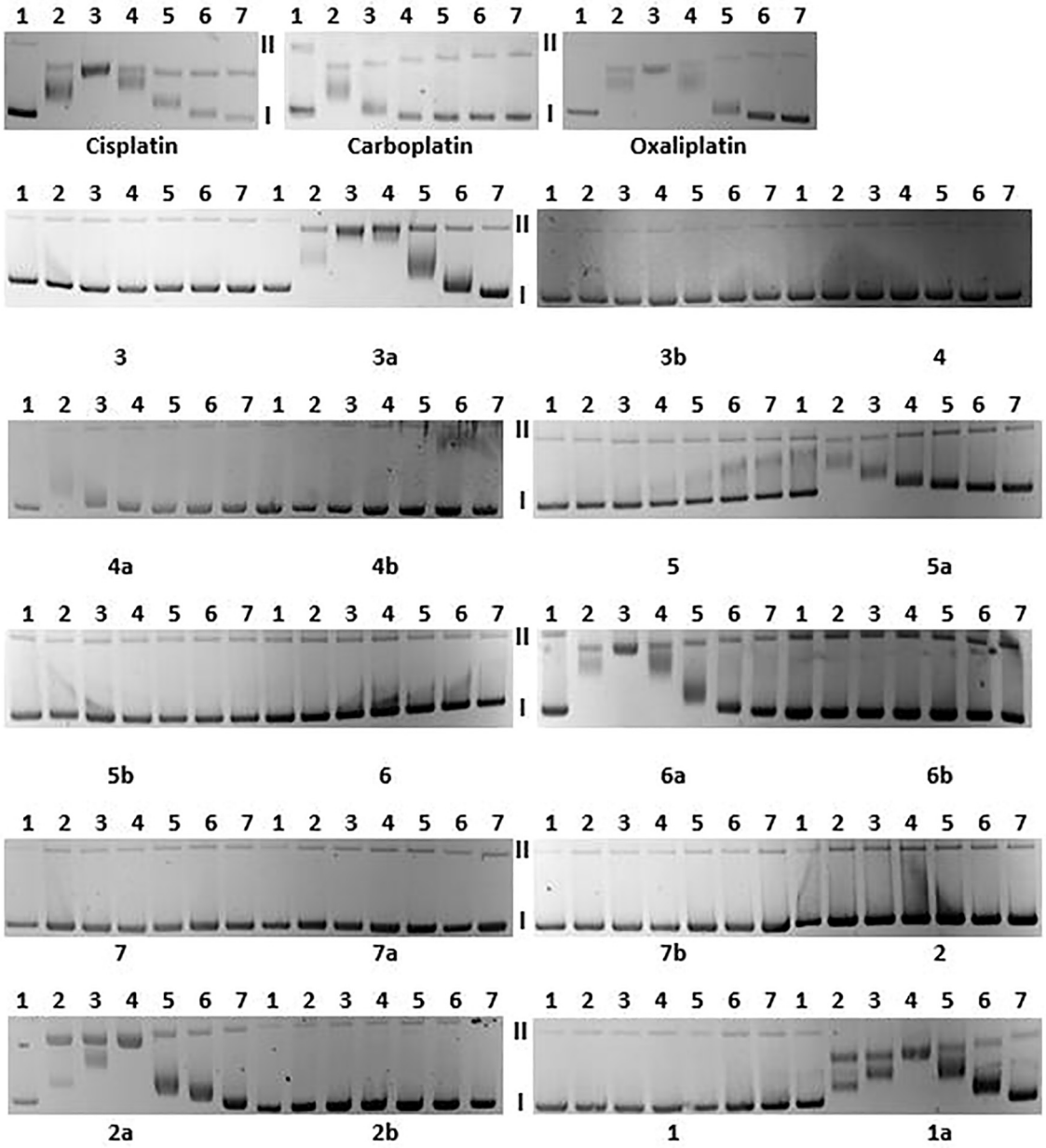


Fig. 3. Modification of the gel electrophoretic mobility of plasmid DNA when incubated with various concentrations of the carrier ligands (**1**–**7**), platinum(II) complexes (**1a**–**7a** and **2b**–**7b**), cisplatin, carboplatin and oxaliplatin. Concentrations (in μM) are as follows: (line 1) untreated plasmid DNA. For cisplatin; (line 2) 40 μM, (line 3) 20 μM, (line 4) 10 μM, (line) 5 μM, (line 2) 2.5 μM, (line 2) 1.25 μM. For oxaliplatin; (line 2) 160 μM, (line 3) 80 μM, (line 4) 40 μM, (line 5) 20 μM, (line 6) 10 μM, (line 7) 5 μM. For carboplatin and the compounds **1**–**7**, **1a**–**7a** and **2b**–**7b**; (line 2) 20 μM, (line 3) 160 μM, (line 4) 80 μM, (line 5) 40 μM, (line 6) 20 μM, (line 7) 10 μM.

The findings of the plasmid DNA interaction and restriction studies recommend that the DNA binding mode and the sequence selectivity may be modulated by changing the chemical structure of benzimidazole ligands.

3. Conclusion

In the present study, the in vitro cytotoxic activity and interactions with DNA of several new platinum(II) complexes were evaluated. The preliminary data obtained on the cytotoxic activity, plasmid DNA interaction and restriction in this study led us to conclude that the DNA binding mode and the sequence selectivity may be modulated by changing the chemical structure of the benzimidazole ligands.

It was determined that complexes **1a**, **2a**, **3a** and **6a** have a stronger interaction than both carboplatin and oxaliplatin with plasmid DNA. The compounds **1a**, **3a**, **3b**, **6**, **7a** and **7b**, having glycine, valine, histidine and tyrosine residues on the carrier ligands, have been found to be noteworthy for further studies due to moderate in vitro cytotoxic activity, close to that of carboplatin.

4. Experimental

Formic acid (Merck, NJ, USA), acetic acid (Merck, NJ, USA), m-anisaldehyde (Sigma-Aldrich, Hong Kong, China), isovaleraldehyde (Sigma-Aldrich, Germany, Hamburg), isobutyraldehyde (Sigma-Aldrich, MO, USA), 4-imidazoleacetic acid hydrochloride (Sigma-Aldrich, Bangalore, India), methyl 4-hydrophenylacetate (Sigma-Aldrich, MO, USA), 1,2-phenylendiamine (Merck, NJ, USA),

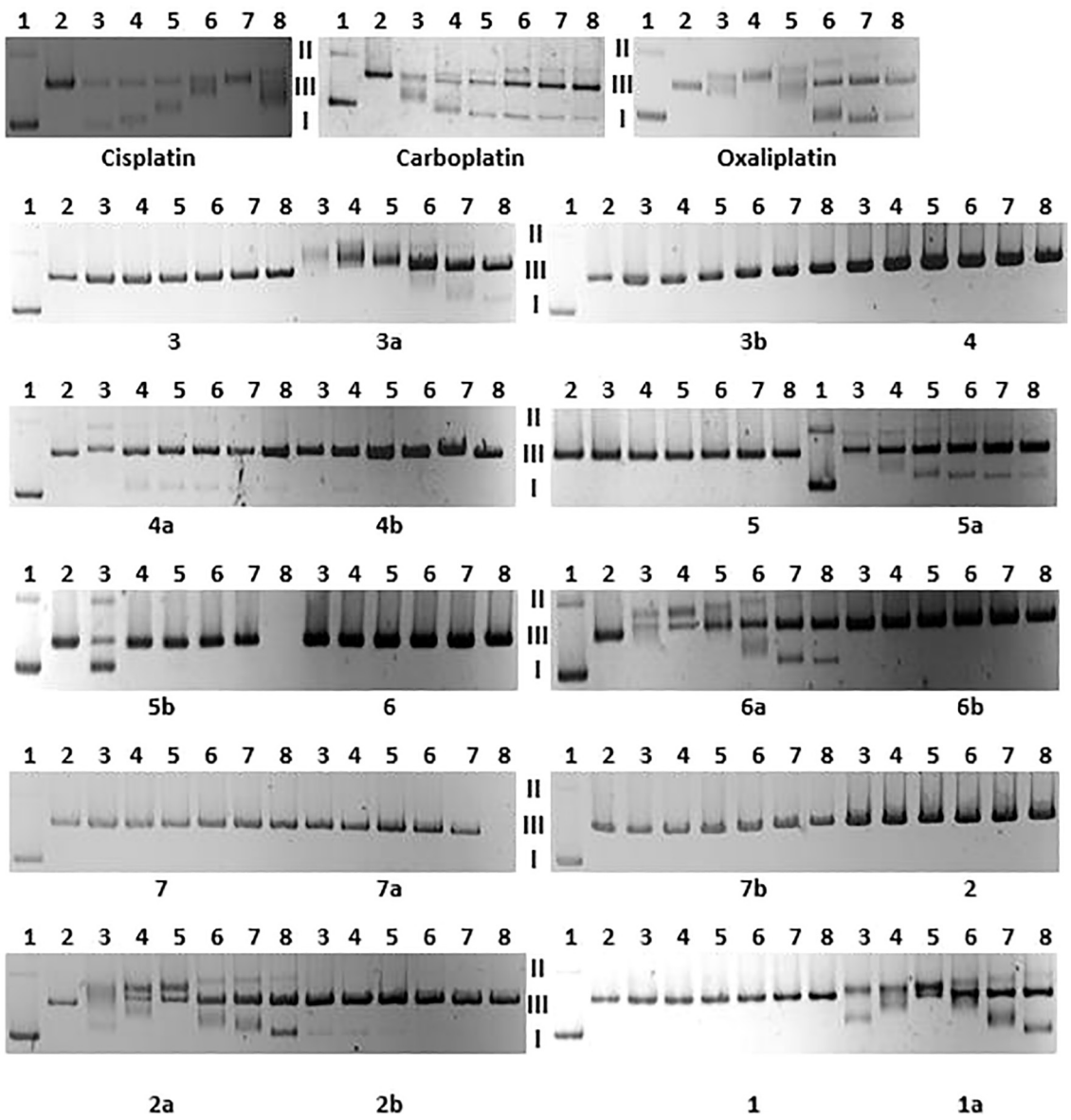


Fig. 4. Electrophoretograms for *Bam*HI digested mixtures of plasmid DNA after their treatment with various concentrations of the carrier ligands (**1**–**7**), platinum(II) complexes (**1a**–**7a** and **2b**–**7b**), cisplatin, carboplatin and oxaliplatin. Concentrations (in μM) are as follows: (line 1) untreated plasmid DNA, (line 2) plasmid DNA linearized by *Bam*HI. For cisplatin: (line 3) 40 μM, (line 4) 20 μM, (line 5) 10 μM, (line 6) 5 μM, (line 7) 2.5 μM, (line 8) 1.25 μM. For oxaliplatin: (line 3) 160 μM, (line 4) 80 μM, (line 5) 40 μM, (line 6) 20 μM, (line 7) 10 μM, (line 8) 5 μM. For carboplatin and **1**–**7**, **1a**–**7a** and **2b**–**7b**: (line 3) 320 μM, (line 4) 160 μM, (line 5) 80 μM, (line 6) 40 μM, (line 7) 20 μM, (line 8) 10 μM.

sodium bisulfite (Sigma-Aldrich, Tokyo, Japan), potassium oxalate monohydrate (Sigma-Aldrich, Hamburg, Germany) and cobalt(II) hydroxide (Sigma-Aldrich, Hong Kong, China) were obtained and used without further purification. All solvents were obtained commercially (Merck and Sigma-Aldrich) and were used without further purification. The reactions were monitored by thin layer chromatography (TLC), which was performed on pre-coated aluminium plates (Silicagel F_{254}, Merck). Plates were visualized by UV light, Dragendorff's reagent and iodine vapor. ^{1}H NMR spectra were recorded in DMSO-d_6 on a Varian Mercury 400 MHz High-Performance Digital FT-NMR spectrometer (Agilent Technologies Inc, Santa Clara, CA, USA) using tetramethylsilane as the internal standard. All chemical shifts were reported in ppm (δ). Infrared absorption (IR) spectra were recorded on a Perkin Elmer Spectrum 400 FTIR/FTNIR spectrometer equipped with a Universal ATR Sampling Accessory (Perkin Elmer Inc., Waltham, Ma, USA) and a Bruker IFS 66/S, FRA 106/S, HYPERION 1000, RAMANSCOPE II spectrometer (Bruker Corporation, Billerica, MA, USA), and were reported in cm^{-1} units. Carbon, nitrogen and hydrogen analyses were performed on a LECO CHNS-932 elemental analyser (Leco Corporation, Saint Joseph, MI, USA) and were within ±0.4% of the theoretical values. High resolution mass spectra data (HRMS) were collected in-house using a Waters LCT Premier XE mass spectrometer (high sensitivity orthogonal acceleration time-of-flight instrument) operating in either the ESI (+) or ESI (−) mode, also coupled to an AQUITY Ultra Performance Liquid Chromatography system (Waters Corporation, Milford, MA, USA). Flash chromatography was performed with a Combiflash®Rf automated flash chromatography system with RediSep columns (Teledyne-Isco, Lincoln, NE, USA) using hexane–EtOAc solvent gradients. The purity of the final compounds was determined to be >97% by UPLC with a UV detector. Melting points were determined with an SMP-II Digital Melting Point Apparatus (Schorpp Geraetetechnik, Überlingen, Germany) and are uncorrected.

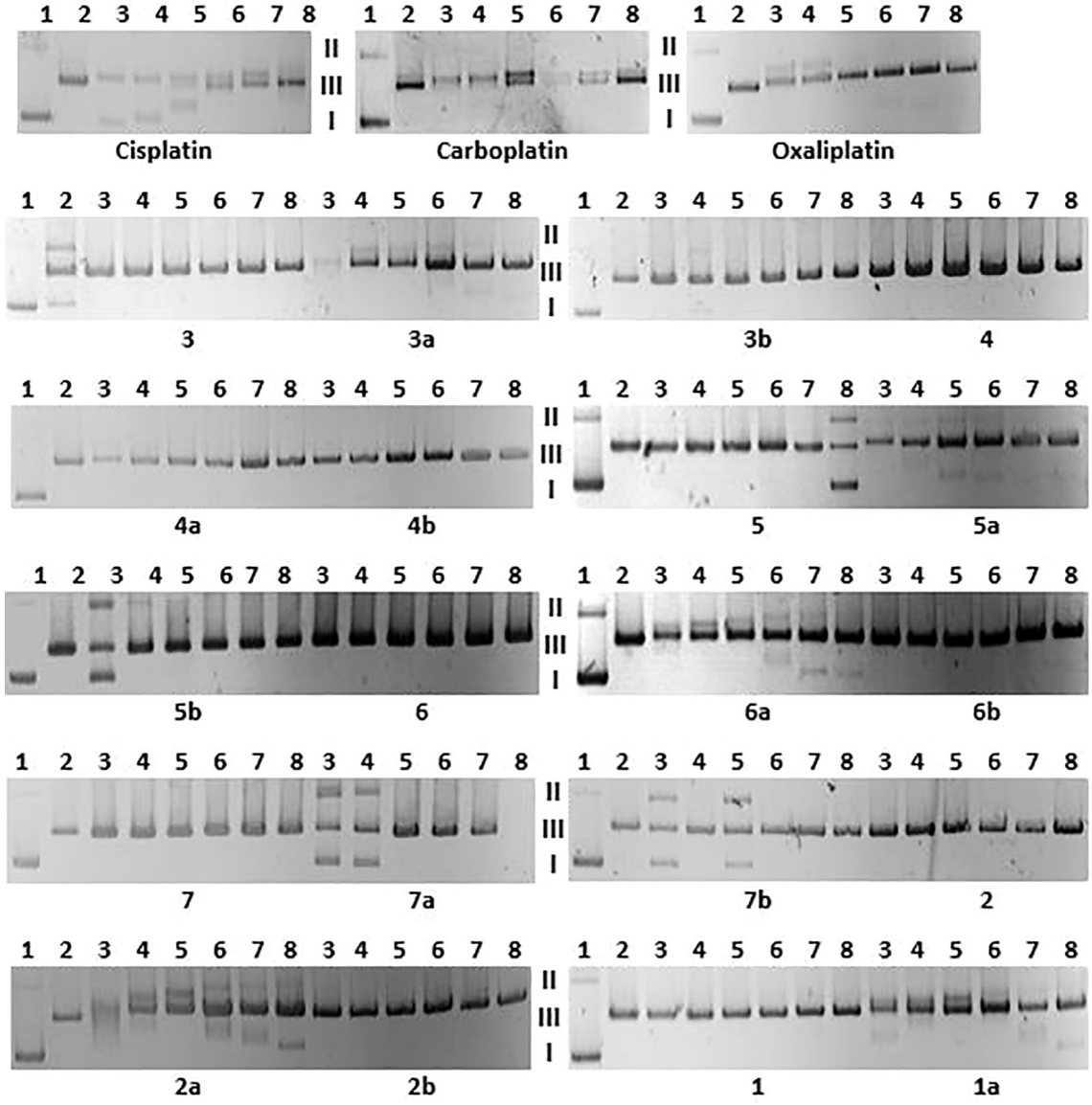


Fig. 5. Electrophoretograms for *Hind*III digested mixtures of plasmid DNA after their treatment with various concentrations of the carrier ligands (**1**–**7**), platinum(II) complexes (**1a**–**7a** and **2b**–**7b**), cisplatin, carboplatin and oxaliplatin. Concentrations (in μM) are as follows: (line 1) untreated plasmid DNA, (line 2) plasmid DNA linearized by *Hind*III. For cisplatin: (line 3) 40 μM, (line 4) 20 μM, (line 5) 10 μM, (line 6) 5 μM, (line 7) 2.5 μM, (line 8) 1.25 μM. For oxaliplatin: (line 3) 160 μM, (line 4) 80 μM, (line 5) 40 μM, (line 6) 20 μM, (line 7) 10 μM, (line 8) 5 μM. For carboplatin and **1**–**7**, **1a**–**7a**, and **2b**–**7b**: (line 3) 320 μM, (line 4) 160 μM, (line 5) 80 μM, (line 6) 40 μM, (line 7) 20 μM, (line 8) 10 μM.

4.1. Chemical experiments

4.1.1. Synthesis of 2-substituted benzimidazoles

General procedure A (Philips Method) [35]: A solution of 1,2-phenylenediamine (1.0 mmol) and the appropriate carboxylic acid (1.1–1.5 mmol) in 4–5 N HCl (1 ml) was heated under reflux until condensation was finished, as judged by TLC and LC–MS. The reaction mixture was cooled down and then quenched by aqueous saturated $NaHCO_3$ or K_2CO_3. The crude mixture was washed with iced water until a neutral pH was obtained. The crude mixture was recrystallized with an appropriate solvent.

General procedure B [36]: To a solution of 1,2-phenylenediamine (1.0 mmol) and the appropriate aldehyde (1.2 mmol) in ethanol (2–3 ml), $Co(OH)_2$ (0.1 mmol) was added and the mixture was stirred at rt until condensation was finished, as judged by TLC and LC–MS. The reaction mixture was concentrated by evaporation under vacuum to give the crude product. Flash column chromatography was used for purification (*n*-hexane:ethyl acetate, gradient flow from 100-0% to 30–70%).

General procedure C [37]: To a solution of 1,2-phenylenediamine (1.0 mmol) and $NaHSO_3$ (1.0 mmol) in N,N-dimethylacetamide (DMA) (1 ml), a solution of the appropriate aldehyde (1.0–1.1 mmol) in DMA (1 ml) was added and the mixture was stirred at 100 °C until condensation was finished, as judged by TLC and LC–MS. The reaction mixture was quenched with iced water to give the crude product, which was recrystallized with an appropriate solvent.

Benzimidazole (L^1) (**1**). General procedure A was followed using 1,2-phenylenediamine, formic acid and a reaction time of 3 h. The crude product was recrystallized with water.

Yield: 76.7%; mp: 172.6–173.0 °C (170 °C [39]). ^{1}H NMR (DMSO-d_6) δ ppm: 12.48 (br s, 1H, N–H, exchangeable with D_2O), 8.23 (s, 1H, H^2), 7.60 (dd, J = 5.6 and 3.2 Hz, 2H, Ar-H), 7.22–7.18 (m, 2H, Ar-H). IR (ATR) ν (cm^{-1}): 3113 (N–H), 3062 (=C—H). HRMS (m/z), [M+H] *calcd* for $C_7H_7N_2$: 119.0609, *found*: 119.0609.

2-Methylbenzimidazole (L^2) (**2**). General procedure A was followed using 1,2-phenylenediamine, acetic acid and a reaction time of 3 h. The crude product was recrystallized with water.

Yield: 65.6%; mp: 178.0–178.2 °C (176–178 °C [59]). ^{1}H NMR (DMSO-d_6) δ ppm: 12.15 (br s, 1H, N–H, exchangeable with D_2O), 7.44 (s, 2H, Ar-H), 7.11–7.07 (m, 2H, Ar-H), 2.50 (s, 3H, –CH_3). IR (ATR) ν (cm^{-1}): 3100 (N–H), 3062 (=C—H), 2994 (–C–H). HRMS (*m*/*z*), [M+H] *calcd* for $C_8H_9N_2$: 133.0766, *found*: 133.0760.

2-Isopropylbenzimidazole (L^3) (**3**). General procedure B was followed using 1,2-phenylenediamine, isobutyraldehyde and a reaction time of 7 h. The crude product was purified by flash column chromatography.

Yield: 68.0%; mp: 234.0–235.5 °C (237–239 °C [36], 232–234 °C [60]). ^{1}H NMR (DMSO-d_6) δ ppm: 12.10 (br s, 1H, N–H, exchangeable with D_2O), 7.51–7.41 (m, 2H, Ar-H), 7.11–7.10 (m, 2H, Ar-H), 3.13 (heptet, J = 7.0 Hz, 1H, –$CH(CH_3)_2$), 1.34 (d, J = 6.8 Hz, 6H, –$CH(CH_3)_2$). IR (ATR) ν (cm^{-1}): 3158 (N–H), 3048 (=C—H), 2969 (–C–H). HRMS (*m*/*z*), [M+H] *calcd* for $C_{10}H_{13}N_2$: 161.1079, *found*: 161.1073.

2-Isobutylbenzimidazole (L^4) (**4**). General procedure B was followed, using 1,2-phenylenediamine, isovaleraldehyde and a reaction time of 5 h. The crude product was purified by flash column chromatography.

Yield: 68.0%; mp: 190.2–191.0 °C (196–198 °C [61]). ^{1}H NMR (DMSO-d_6) δ ppm: 12.13 (br s, 1H, N–H, exchangeable with D_2O), 7.52–7.39 (m, 2H, Ar-H), 7.10 (d, J = 7.0 Hz, 2H, Ar-H), 2.65 (d, J = 7.2 Hz, 2H, –CH_2CH-), 2.16 (heptet, J = 6.7 Hz, 1H, –$CH_2CH(CH_3)_2$), 0,93 (d, J = 6.4 Hz, 6H, –$CH(CH_3)_2$). IR (ATR) ν (cm^{-1}): 3107 (N–H), 3054 (=C—H), 2952 (–C–H). HRMS (*m*/*z*), [M+H] *calcd* for $C_{11}H_{15}N_2$: 175.1235, *found*: 175.1229.

R,S-2-(*sec*-butyl)benzimidazole (L^5) (**5**). General procedure C was followed, using 1,2-phenylenediamine, R,S-2-metylbutanal and a reaction time of 2 h. The crude product was recrystallized with ethanol–water.

Yield: 70.0%; mp: 225.8–226.0 °C (226–227 [62], 228–230 °C [63]). ^{1}H NMR (DMSO-d_6) δ ppm: 12.11 (s, 1H, N–H, exchangeable with D_2O), 7.46 (s, 2H, Ar-H), 7.12–7.08 (dd, J = 5.8 and 2.9 Hz, 2H, Ar-H), 2.91 (sextet, J = 7.0 Hz, 1H, –$CH(CH_3)CH_2CH_3$), 1.86–1.61 (m, 2H, –$CH(CH_3)CH_2CH_3$), 1.31 (d, J = 6.8 Hz, 3H, –$CH(CH_3)CH_2CH_3$), 0.83 (t, J = 7.6 Hz, 3H, –$CH(CH_3)CH_2CH_3$). IR (ATR) ν (cm^{-1}): 3115 (N–H), 3046 (=C—H), 2959 (–C–H). HRMS (*m*/*z*), [M+H] *calcd* for $C_{11}H_{15}N_2$: 175.1235, *found*: 175.1227.

2-((1H-Imidazol-4-yl)methyl)benzimidazole (L^6) (**6**). General procedure A was followed, using 1,2-phenylenediamine, (4-imidazolyl)acetic acid HCl and a reaction time of 24 h. The purified compound **6** was obtained.

Yield: 60.0%; mp: 236.2 °C (decomp), 251.0 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 12.01 (br s, 2H, 2× N–H, exchangeable with D_2O), 7.57 (d, J = 1.2 Hz, 1H, Ar-H), 7.47–7.44 (m, 2H, Ar-H), 7.11–7.09 (dd, J = 6.0 and 3.2 Hz, 2H, Ar-H), 6.92 (s, 1H, Ar-H), 4.08 (s, 2H, Ar-CH_2-Ar). IR (ATR) ν (cm^{-1}): 3141, 3115 (N–H), 3061 (=C—H), 2997 (–C–H). HRMS (*m*/*z*), [M+H] *calcd* for $C_{11}H_{10}N_4$: 199.0984, *found*: 199.0981; *Anal. Calc.* for $C_{11}H_{10}N_4$: C, 66.65; H, 5.08; N, 28.26. Found: C, 66.26; H, 5.29; N, 27.91%.

2-((4-Hydroxyphenyl)methyl)benzimidazole (L^7) (**7**). General procedure A was followed, using 1,2-phenylenediamine, methyl 2-(4-hydroxyphenyl)acetate and a reaction time of 27 h. The purified compound **7** was obtained.

Yield: 88.0%; mp: 240–242 °C (decomp), 260–263 °C (264–266 °C [64]). ^{1}H NMR (DMSO-d_6) δ ppm: 9.33 (br s, 1H, O–H, exchangeable with D_2O), 7.50 (dd, J = 6.0 and 3.2 Hz, 2H, Ar-H), 7.16 (dd, J = 6.0 and 3.2 Hz, 2H, Ar-H), 7.14 (dd, J = 8.4 and 3.2 Hz, 2H, Ar-H), 6.72 (dd, J = 8.4 and 3.2 Hz, 2H, Ar-H), 4.09 (s, 2H, Ar-CH_2-Ar). IR (ATR) ν (cm^{-1}): 3258 (O–H), 3123 (N–H), 3053 (=C—H), 2973 (–C–H), 1251 (C–O). HRMS (*m*/*z*), [M+H] *calcd* for $C_{14}H_{13}N_2O$: 225.1028, *found*: 225.1028.

4.1.2. Synthesis of the platinum(II) complexes

Synthesis of potassium bis(oxalato)platinate(II) dihydrate ($K_2[Pt(ox)_2]\cdot 2H_2O$): This compound was obtained similar to a previously published approach [65]. Yield 78.6%. IR (ATR) ν (cm^{-1}): 3352 and 3474 (OH), 1696 and 1670 (C=O), 1232 (C–O), 567 (Pt–O). *Anal. Calc.* for $C_4O_8K_2Pt\cdot 2H_2O$: C, 9.90; H, 0.83. Found: C, 9.51; H, 0.95%.

*General procedure for the preparation of complexes **1a**–**7a**:* To a solution the appropriate 2-substituted benzimidazole (1.8 mmol for monodentate ligands; 0.9 mmol for bidentate ligands) in ethanol (10 ml), a solution of K_2PtCl_4 (1 mmol) in water (10 ml) was added dropwise, and the mixture was stirred at 50–60 °C until complexation was finished, as judged by TLC and LC-MS. The precipitate was filtered and the crude product was washed with cold water (4–5 °C), cold ethanol (4–5 °C), cold acetone (4–5 °C) and then diethyl ether (4–5 °C). The purified complex was dried in vacuo.

Cis-[dichloro-bis(benzimidazole)platinum(II)] [$Pt(L^1)_2Cl_2$] (**1a**). [38,39] The general procedure was followed, using benzimidazole (L^1), K_2PtCl_4 and a reaction time of 24 h.

Yield: 80.8%; mp: >360 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 13.45 (br s, 2H, 2× N–H, exchangeable with D_2O), 8.83 (s, 2H, 2× H^2), 7.78 (d, J = 8.0 Hz, 2H, Ar-H), 7.48 (d, J = 7.2 Hz, 2H, Ar-H), 7.24–7.17 (m, 4H, Ar-H). IR (ATR) ν (cm^{-1}): 3270 (N–H), 3099 (=C—H), 321 and 313 (Pt–Cl). HRMS (*m*/*z*), [M+H] *calcd* for $C_{14}H_{13}N_4Cl_2Pt$: 503.0165, *found*: 503.0167; *Anal. Calc.* for $C_{14}H_{13}N_4Cl_2Pt$: C, 33.48; H, 2.41; N, 11.15. Found: C, 33.27; H, 2.59; N, 11.51%.

Cis-[dichloro-bis(2-methylbenzimidazole)platinum(II)] [$Pt(L^2)_2Cl_2]\cdot 1.5H_2O$ (**2a**). [49] The general procedure was followed, using 2-methylbenzimidazole (L^2), K_2PtCl_4 and a reaction time of 7 days.

Yield: 77.3%; mp: 330 °C (decomp). ^{1}H NMR (DMSO-d_6) δ ppm: 13.20 (br s, 2H, 2× N–H, exchangeable with D_2O), 8.08–8.05 (m, 2H, Ar-H), 7.41 (d, J = 8.4 Hz, 2H, Ar H), 7.33 (m, 2H, Ar-H), 7.26–7.21 (m, 2H, Ar-H), 2.85 (s, 3H, –CH_3), 2.83 (s, 3H, –CH_3). IR (ATR) ν (cm^{-1}): 3518 (H_2O), 3187 (N–H), 3054 (=C—H), 2925 (C–H), 326 and 319 (Pt–Cl). HRMS (*m*/*z*), [M−H] *calcd* for $C_{16}H_{15}N_4Cl_2Pt$: 529.0323, *found*: 529.0305; *Anal. Calc.* for $C_{16}H_{15}N_4Cl_2Pt\cdot 1.5H_2O$: C, 34.48; H, 3.44; N, 10.05. Found: C, 34.73; H, 3.52; N, 10.09%.

Cis-[dichloro-bis(2-isopropylbenzimidazole)platinum(II)] [$Pt(L^3)_2Cl_2]\cdot 1.5H_2O$ (**3a**). The general procedure was followed, using 2-isopropylbenzimidazole (L^3), K_2PtCl_4 and a reaction time of 7 days.

Yield: 19.0%; mp: 275 °C (decomp). ^{1}H NMR (DMSO-d_6) δ ppm: 13.17 (br s, 2H, 2× N–H, exchangeable with D_2O), 8.32 (d, J = 7.2 Hz, 1H, Ar-H), 8.14–8.12 (m, 1H, Ar-H), 7.56–7.20 (m, 6H, Ar-H), 4.21–4.14 (m, 2H, 2× –$CH(CH_3)_2$), 1.69–1.17 (m, 12H, 2× –$CH(CH_3)_2$). IR (ATR) ν (cm^{-1}): 3500 (H_2O), 3211 (N–H), 3049 (=C—H), 2969, 2879 (C–H), 325 and 317 (Pt–Cl). HRMS (*m*/*z*), [M−H] *calcd* for $C_{20}H_{23}N_4Cl_2Pt$: 585.0949, *found*: 585.0942; *Anal. Calc.* for $C_{20}H_{24}N_4Cl_2Pt\cdot 1.5H_2O$: C, 39.16; H, 4.44; N, 9.13. Found: C, 39.09; H, 4.60; N, 8.99%.

Cis-[dichloro-bis(2-isobutylbenzimidazole)platinum(II)] [$Pt(L^4)_2Cl_2]\cdot CH_3CH_2OH$ (**4a**). The general procedure was followed, using 2-isobutylbenzimidazole (L^4), K_2PtCl_4 and a reaction time of 2 days.

Yield: 16.0%; mp: 275 °C (decomp). ^{1}H NMR (DMSO-d_6) δ ppm: 13.11 and 12.91 (2× br s, 2H, 2× N–H, exchangeable with D_2O), 8.36 and 8.20 (2× d, J = 8.4 Hz, 2H, 2× Ar-H), 7.62–7.23 (m, 6H,

Ar-H), 5.4 (br s, 1H, CH_3CH_2OH, exchangeable with D_2O), 3.38 (q, J = 6.8 Hz, 2H, CH_3CH_2OH), 3.23 (d, J = 7.6 Hz, 4H, 2× –CH_2CH-), 2.74–2.70 (m, 2H, 2× –$CH_2CH(CH_3)_2$), 1.11–0.35 (m, 15H, 2× –CH_2-$CH(CH_3)_2$), CH_3CH_2OH). IR (ATR) ν (cm^{-1}): 3188 (N–H), 3041 (=C—H), 2958 (C–H), 320 (Pt–Cl). HRMS (m/z) [M−H] *calcd* for $C_{22}H_{27}N_4Cl_2Pt$: 613.1262, *found*: 613.1249; *Anal. Calc.* for $C_{22}H_{28}N_4Cl_2Pt{\cdot}CH_3CH_2OH$: C, 42.73; H, 4.99; N, 8.67. Found: C, 42.82; H, 5.16; N, 8.82%.

Cis-[dichloro-bis(R,S-2-(*sec*-butyl)benzimidazole)platinum(II)] $[Pt(L^5)_2Cl_2]{\cdot}1.5H_2O$ (**5a**). The general procedure was followed, using R,S-2-(*sec*-butyl)benzimidazole (L^5), K_2PtCl_4 and a reaction time of 6 days.

Yield: 39.3%; mp: 246 °C (decomp). ^{1}H NMR (DMSO-d_6) δ ppm: 13.11 and 12.82 (2× br s, 2H, 2× N–H, exchangeable with D_2O), 8.37 and 8.01 (2× d, J = 8.0 Hz, 2H, 2× Ar-H), 7.55–7.24 (m, 6H, Ar-H), 3.80–3.37 (m, 2H, 2× Ar-CH$(CH_3)CH_2CH_3$), 2.09–1.63 (m, 4H, 2× –$CH(CH_3)CH_2CH_3$), 1.44–1.28 (m, 6H, 2× $CH(CH_3)CH_2CH_3$), 1.09–0.81 (m, 6H, –$CH(CH_3)CH_2CH_3$). IR (ATR) ν (cm^{-1}): 3186 (N–H), 3059 (=C—H), 2966 (C–H), 323 (Pt–Cl). HRMS (m/z) [M−H] *calcd* for $C_{22}H_{27}N_4Cl_2Pt$: 613.1262, *found*: 613.1260; *Anal. Calc.* for $C_{22}H_{28}N_4Cl_2Pt{\cdot}1.5H_2O$: C, 41.19; H, 4.87; N, 8.73. Found: C, 41.01; H, 4.76; N, 8.39%.

Cis-[dichloro-(2-((1*H*-imidazol-4-yl)methyl)benzimidazole)-platinum(II)] $[Pt(L^6)Cl_2]{\cdot}2H_2O$ (**6a**). The general procedure was followed, using 2-((1*H*-imidazol-4-yl)methyl)benzimidazole (L^6), K_2PtCl_4 and a reaction time of 6 days.

Yield: 85.0%; mp: >360 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 13.73 (br s, 1H, N–H, exchangeable with D_2O), 13.01 (br s, 1H, N–H, exchangeable with D_2O), 8.27–8.16 (m, 2H, Ar-H), 7.57–7.27 (m, 4H, Ar-H), 4.29 (s, 2H, Ar-CH_2-Ar). IR (ATR) ν (cm^{-1}): 3508 (H_2O), 3219, 3133 (N–H), 3042 (=C—H), 2976 (C–H), 321 and 317 (Pt–Cl). HRMS (m/z), [M−H] *calcd* for $C_{11}H_9N_4Cl_2Pt$: 462.9854, *found*: 462.9836; *Anal. Calc.* for $C_{11}H_{10}N_4Cl_2Pt{\cdot}2H_2O$: C, 26.41; H, 2.82; N, 11.20. Found: C, 26.68; H, 2.73; N, 11.06%.

Cis-[dichloro-bis(2-((4-hydroxyphenyl)methyl)benzimidazole)-platinum(II)] $[Pt(L^7)_2Cl_2]{\cdot}3.25H_2O$ (**7a**). The general procedure was followed, using 2-((4-hydroxyphenyl)methyl)benzimidazole (L^7), K_2PtCl_4 and a reaction time of 2 days.

Yield: 84.0%; mp: >360 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 12.16 (br s, 2H, 2× N–H, exchangeable with D_2O), 9.26 (br s, 2H, 2× O–H, exchangeable with D_2O), 7.50–7.38 (m, 8H, Ar-H), 7.11 (d, J = 8.0 Hz, 4H, Ar-H), 6.69 (d, J = 8.0 Hz, 4H, Ar-H), 4.03 (s, 4H, 2× Ar-CH_2-Ar). IR (ATR) ν (cm^{-1}): 3528 (O–H and H_2O), 3193 (N–H), 3053 (=C—H), 2958 (C–H), 1224 (C–O), 325 and 318 (Pt–Cl). HRMS (m/z), [M−H] *calcd* for $C_{28}H_{23}N_4O_2Cl_2Pt$: 713.0848, *found*: 713.0868; *Anal. Calc.* for $C_{28}H_{24}N_4O_2Cl_2Pt{\cdot}3.25H_2O$: C, 43.50; H, 3.98; N, 7.25. Found: C, 43.82; H, 3.67; N, 6.92%.

General procedure for the preparation of complexes ***2b*–*7b***: To a solution the appropriate 2-substituted benzimidazole (1.8 mmol for monodentate ligands; 0.9 mmol for bidentate ligands) in ethanol/isopropanol (10 ml) at 50–60 °C, a solution of $K_2[Pt(ox)_2]{\cdot}2H_2O$ (1 mmol) in water (10 ml) at 50–60 °C was added dropwise, and the mixture was stirred at 60–70 °C until complexation was finished, as judged by TLC and LC–MS. The precipitate was filtered and the crude product was washed with hot water (70 °C), cold water (4–5 °C), hot ethanol/isopropanol (70 °C), cold ethanol/isopropanol (4–5 °C) and then diethyl ether (4–5 °C). The purified complex was dried in vacuo.

Oxalato-bis(2-methylbenzimidazole)platinum(II)] $[Pt(L^2)_2(ox)]{\cdot}0.35H_2O$ (**2b**). The general procedure was followed, using 2-methylbenzimidazole (L^2), $K_2[Pt(ox)_2]{\cdot}2H_2O$ and a reaction time of 9 days.

Yield: 56.4%; mp: 267–268 °C (decomp). ^{1}H NMR (DMSO-d_6) δ ppm: 13.40 (br s, 2H, 2× N–H, exchangeable with D_2O), 7.79–7.77 (m, 2H, Ar-H), 7.50–7.48 (m, 2H, Ar-H), 7.29–7.24 (m, 4H, Ar-H), 2.72 (s, 6H, 2× –CH_3). IR (ATR) ν (cm^{-1}): 3507 (H_2O), 3186 (N–H), 3060 (=C—H), 2929 (C–H), 1698, 1647 (C=O), 1225 (C–O), 563 (Pt–O). HRMS (m/z), [M−H] *calcd* for $C_{18}H_{17}N_4O_4Pt$: 548.0898, *found*: 548.0914; *Anal. Calc.* for $C_{18}H_{16}N_4O_4Pt{\cdot}0.35H_2O$: C, 39.04; H, 3.04; N, 10.12. Found: C, 39.49; H, 3.54; N, 10.52%.

Oxalato-bis(2-isopropylbenzimidazole)platinum(II)] $[Pt(L^3)_2(ox)]{\cdot}0.5CH_3CH_2OH$ (**3b**). The general procedure was followed, using 2-isopropylbenzimidazole (L^3), $K_2[Pt(ox)_2]{\cdot}2H_2O$ and a reaction time of 7 days.

Yield: 71.8%; mp: 296–297 °C (decomp). ^{1}H NMR (DMSO-d_6) δ ppm: 13.39 (br s, 2H, 2× N–H, exchangeable with D_2O), 7.98 (s, 2H, Ar-H), 7.53–7.51 (m, 2H, Ar-H), 7.34–7.32 (m, 4H, Ar-H), 4.34 (br s, 0.5H, 0.5× CH_3CH_2OH, exchangeable with D_2O), 3.97–3.92 and 3.79–3.76 (two separate m, 1.5H ve 0.5H, 2× –CH-$(CH_3)_2$), 3.44 (q, J = 7.2 Hz, 1H, 0.5× CH_3CH_2OH), 1.45–1.03 (m, 13.5H, 2× –$CH(CH_3)_2$, 0.5× CH_3CH_2OH). IR (ATR) ν (cm^{-1}): 3521 (O–H), 3164 (N–H), 3059 (=C—H), 2970 (C–H), 1695, 1624 (C=O), 1260 (C–O), 560 (Pt–O). HRMS (m/z), [M−H] *calcd* for $C_{23}H_{25}N_4O_4Pt$: 604.1524, *found*: 604.1495; *Anal. Calc.* for $C_{23}H_{24}N_4O_4Pt{\cdot}0.5CH_3CH_2OH$: C, 43.62; H, 4.23; N, 9.04. Found: C, 44.31; H, 4.97; N, 9.77%.

Oxalato-bis(2-isobutylbenzimidazole)platinum(II)] $[Pt(L^4)_2(ox)]{\cdot}0.2\ L^4$ (**4b**). The general procedure was followed, using 2-isobutylbenzimidazole (L^4), $K_2[Pt(ox)_2]{\cdot}2H_2O$ and a reaction time of 9 days.

Yield: 64.0%; mp: 299 °C (decomp). ^{1}H NMR (DMSO-d_6) δ ppm: 13.37 (br s, 2H, 2× N–H, exchangeable with D_2O), 8.14 (d, J = 6.8 Hz, 2H, Ar-H), 7.55 (d, J = 7.6 Hz, 2H, Ar-H), 7.43–7.36 (m, 4H, Ar-H), 7.11–7.09 (m, 0.4H, 0.2× L^4 Ar-H), 2.73–2.62 (m, 4H, 2× –CH_2CH), 2.41–2.38 (m, 2H, 2× –$CH_2CH(CH_3)_2$), 0.93 (d, J = 6.4 Hz, 1.2H, 0.2× L^4 –$CH(CH_3)_2$), 0.92–0.50 (m, 12H, 2× CH–$(CH_3)_2$). IR (ATR) ν (cm^{-1}): 3198 (N–H), 3097 (=C—H), 2960 (C–H), 1710, 1657 (C=O), 1221 (C–O), 564 (Pt–O). HRMS (m/z), [M−H] *calcd* for $C_{24}H_{27}N_4O_4Pt$: 630.1680, *found*: 630.1690; *Anal. Calc.* for $C_{24}H_{28}N_4O_4Pt{\cdot}0.2\ L^4$: C, 47.22; H, 4.66; N, 9.25. Found: C, 47.37; H, 4.97; N, 9.29%.

Oxalato-bis(R,S-2-(*sec*-butyl)benzimidazole)platinum(II)] $[Pt(L^5)_2(ox)]{\cdot}0.5H_2O$ (**5b**). The general procedure was followed, using R,S-2-(*sec*-butyl)benzimidazole (L^5), $K_2[Pt(ox)_2].2H_2O$ and a reaction time of 7 days.

Yield: 77.8%; mp: 308 °C (decomp). ^{1}H NMR (DMSO-d_6) δ ppm: 13.32 (br s, 2H, 2× N–H, exchangeable with D_2O), 8.10 (br s, 2H, Ar-H), 7.52 (m, 2H, Ar-H), 7.37–7.36 (m, 4H, Ar-H), 3.52 (m, 2H, 2× –CH$(CH_3)CH_2CH_3$), 2.00–1.10 (m, 10H, 2× –$CH(CH_3)CH_2CH_3$, 2× $CH(CH_3)CH_2CH_3$), 1.0–0.7 (m, 6H, 2× –$CH(CH_3)CH_2CH_3$). IR (ATR) ν (cm^{-1}): 3570 (O–H), 3184 (N–H), 3041 (=C—H), 2970 (C–H), 1694, 1650 (C=O), 1252 (C–O), 561 (Pt–O). HRMS (m/z), [M−H] *calcd* for $C_{24}H_{27}N_4O_4Pt$: 630.1680, *found*: 630.1674; *Anal. Calc.* for $C_{24}H_{28}N_4O_4Pt{\cdot}0.5H_2O$: C, 45.00; H, 4.56; N, 8.75. Found: C, 44.93; H, 4.64; N, 9.06%.

Oxalato-bis(2-((1*H*-imidazol-4-yl)methyl)benzimidazole)platinum(II)] $[Pt(L^6)(ox)]0.0.3CH_3CH_2OH$ (**6b**). The general procedure was followed, using 2-((1*H*-imidazol-4-yl)methyl)benzimidazole (L^6), $K_2[Pt(ox)_2]{\cdot}2H_2O$ and a reaction time of 8 days.

Yield: 82.0%; mp: 290–291 °C. ^{1}H NMR (DMSO-d_6) δ ppm: 13.70 (br s, 1H, N–H, exchangeable with D_2O), 13.20 (s, 1H, N–H, exchangeable with D_2O), 8.20 (d, J = 1.2 Hz, 1H, Ar-H), 7.97–7.94 (m, 1H, Ar-H), 7.62–7.60 (m, 1H, Ar-H), 7.45–7.36 (m, 3H, Ar-H), 4.39 (s, 2H, Ar-CH_2-Ar), 3.42 (q, J = 6.8 Hz, 0.6H, 0.3× CH_3CH_2OH),

1.08 (t, J = 6.8 Hz, 0.9H, 0.3× C<u>H</u>$_3$CH$_2$OH). IR (ATR) ν (cm^{-1}): 3212 (N–H), 3042 (=C—H), 2888 (C–H), 1687, 1647 (C=O), 1232 (C–O), 561 (Pt–O). HRMS (m/z): [M−H] *calcd* for $C_{13}H_9N_4O_4Pt$: 480.0272, *found*: 480.0266; *Anal. Calc.* for $C_{13}H_{10}N_4O_4Pt{\cdot}0.3CH_3CH_2OH$: C, 32.54; H, 2.30; N, 11.41. Found: C, 32.04; H, 2.79; N, 11.85%.

Oxalato-bis(2-((4-hydroxyphenyl)methyl)benzimidazole)platinum(II) [Pt(L^7)$_2$(ox)] (**7b**). The general procedure was followed, using 2-((4-hydroxyphenyl)methyl)benzimidazole (L^7), K_2[Pt(ox)$_2$]·2H$_2$O and a reaction time of 7 days.

Yield: 35.0%; mp: 255–256 °C (decomp). ^{1}H NMR (DMSO-d_6) δ ppm: 9.45 (br s, 2H, 2× O–<u>H</u>, exchangeable with D$_2$O), 8.02–7.78 (m, 2H, Ar-<u>H</u>), 7.42–7.28 (m, 2H, Ar-<u>H</u>), 7.18–6.98 (m, 4H, Ar-<u>H</u>), 6.85–6.69 (m, 2H, Ar-<u>H</u>), 6.61–6.55 (m, 2H, Ar-<u>H</u>), 6.49 (d, J = 8.4 Hz, 2H, Ar <u>H</u>), 6.32, 6.23, 6.09 and 5.63 (four separate d, J = 8.0; 8.8; 8.0; 8.4 Hz, 2H, Ar <u>H</u>), 4.72–4.25 (m, 4H, 2× Ar-C<u>H</u>$_2$-Ar). IR (ATR) ν (cm^{-1}): 3559 (O–H), 3180 (N–H), 3027 (=C—H), 2961 (C–H), 1652, 1611 (C=O), 1228 (C–O), 552 (Pt–O). HRMS (m/z), [M−H] *calcd* for $C_{30}H_{25}N_4O_6Pt$: 733.1410, *found*: 733.1424; *Anal. Calc.* for $C_{30}H_{24}N_4O_6Pt$: C, 49.25; H, 3.31; N, 7.66. Found: C, 49.84; H, 3.95; N, 7.97%.

4.2. Biological assays

4.2.1. MTT cytotoxicity assay

The HeLa cell line was obtained from HUKUK, Foot and Mouth Disease Institute (Ankara, Turkey). HeLa cells were cultured in complete Dulbecco's modified Eagle medium (DMEM) (HyClone Laboratories, Inc. Logan, UT) containing 10% Fetal Bovine Serum (FBS) (Gibco, Invitrogen, USA), 1% Penicillin and 1% Streptomycin (Gibco, Invitrogen, USA) at 37 °C and 5% CO_2 in a humidified incubator. Cells were seeded into 96-well microculture plates and incubated for 24 h. Stock solutions of the tested substances were prepared with DMF, which did not exceed 0.1% and different concentrations of the compounds were prepared. The different concentrations of the newly synthesized drugs were then added. After 48 and 72 h incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma, St. Louis, MO, USA) was added. After incubation for 4 h, an ammonia:DMSO (5:100) solution was added and the optical density at 550 nm was recorded with a microplate reader (Bio Tek Instruments Inc., Winooski, VT). The experiments were repeated in triplicate and the surviving cell percentage was defined as the treatment group/-control group where the control group was assumed as 100% survival. The inhibition percentage was calculated via the surviving cell percentage.

4.2.2. Interaction with plasmid DNA

Cisplatin, carboplatin and oxaliplatin were used as reference compounds, pBR322 plasmid DNA, ethidium bromide, agarose and the enzymes *BamH*I and *Hind*III were purchased from Sigma.

The interaction of the ligands **1**–**7**, the platinum(II) complexes **1a**–**7a** and **2b**–**7b**, and the reference compounds with plasmid DNA were studied by agarose gel electrophoresis. Stock solutions of the tested substances in DMF were prepared and used within 1 h. The final amount of DMF never exceeded 0.1%. Briefly, 40 μL aliquots of increasing concentrations of the compounds, ranging from 0 to 320 μM, were added to 1 μL of plasmid DNA (conc: 0.5 μg/mL) in a buffer solution containing TAE (0.05 M Tris–HCl, 0.05 M glacial acetic acid, 1 mM EDTA, pH = 8.0). The samples were incubated at 37 °C for 24 h in the dark. 10 μL aliquots of the drug-DNA mixtures were mixed with the loading buffer (0.1% bromophenol blue, 0.1% xylene cyanol) and loaded onto the 1% agarose gel with or without ethidium bromide. Electrophoresis was carried under TAE buffer (0.05 M Tris base, 0.05 M glacial acetic acid, 1 mM EDTA, pH = 8.0) for 2.5 h at 70 V. At the end of the electrophoresis, the gel without ethidium bromide was stained in the same buffer containing ethidium bromide (0.5 μg/mL). The gels were then viewed with a transilluminator and the image was captured by a video camera (GelDoc-It Imaging System, UVP) as a JPEG file. The experiments were repeated three times.

4.2.3. Compound-DNA mixture digestion with BamHI and HindIII restriction enzymes.

Stock solutions of the tested substances in DMF were prepared and used within 1 h. The final amount of DMF never exceeded 0.1%. The drug-DNA mixtures were first incubated for 24 h and then subjected to the enzyme *BamH*I or *Hind*III digestion for 1 h at 37 °C. To each 8 μL of the incubated drug-DNA mixtures were added 1 μL of 10× digestion buffer and then 0.1 μL of *BamH*I or *Hind*III (1 unit). The mixtures were left shaking in a water bath for 1 h at 37 °C. The digestion was terminated by rapid cooling. The restricted DNA was run in 1% agarose gel electrophoresis for 1.5 h at 70 V in TAE buffer. The gel was stained with ethidium bromide and the gels were then viewed with a transilluminator and the image captured by a video camera as a JPEG file.

Acknowledgement

We would like to thank the Research Foundation of Gazi University, Turkey (02/2015-01) for financial support of this work.

Declaration of interest

The authors have declared no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.poly.2019.01.028.

References

[1] B. Rosenberg, L. Van Camp, T. Krigas, Inhibition of cell division in escherichia coli by electrolysis products from a platinum electrode, Nature 205 (1965) 698.
[2] B. Rosenberg, L. VanCamp, J.E. Trosko, V.H. Mansour, Platinum compounds: a new class of potent antitumour agents, Nature 222 (1969) 385.
[3] E. Wong, C.M. Giandomenico, Current status of platinum-based antitumor drugs, Chem. Rev. 99 (1999) 2451.
[4] Cisplatin: chemistry and biochemistry of a leading anticancer drug, Verlag Helv. Chim. Acta, Zürich, 1999.
[5] L. Kelland, The resurgence of platinum-based cancer chemotherapy, Nat. Rev. Cancer 7 (2007) 573.
[6] T.C. Johnstone, J.J. Wilson, S.J. Lippard, Monofunctional and higher-valent platinum anticancer agents, Inorg. Chem. 52 (2013) 12234.
[7] J.M. Pérez, I. López-Solera, E.I. Montero, M.F. Braña, C. Alonso, S.P. Robinson, C. Navarro-Ranninger, Combined effect of platination and intercalation upon DNA binding of novel cytotoxic Pt-Bis(naphthalimide) complexes, J. Med. Chem. 42 (1999) 5482.
[8] E.R. Jamieson, S.J. Lippard, Structure, recognition and processing of cisplatin–DNA adducts, Chem. Rev. 99 (1999) 2467.
[9] N.J. Wheate, S. Walker, G.E. Craig, R. Oun, The status of platinum anticancer drugs in the clinic and in clinical trials, Dalton Trans. 39 (2010) 8113.
[10] M.J. Bloemink, H. Engelking, S. Karentzopoulos, B. Krebs, J. Reedijk, Synthesis, crystal structure, antitumor activity and DNA-binding properties of the new active platinum compound (Bis(N-methylimidazol-2-yl)carbinol) dichloroplatinum(II), lacking a NH moiety, and of the inactive analog dichloro(N1, N1′-dimethyl-2,2′-biimidazole)platinum(II), Inorg. Chem. 35 (1996) 619.
[11] E.S. Domina, V.N. Varopayev, G.G. Skvorsov, S.M. Minakova, B.A. Çernov, Sıntez ı nrotivopuholevaya aktıvnost komleksnıh soyedneny Pt(II), Pt(IV), Pd(IV) 1-vinil-azıkamı, Zh. Neorg. Khim 17 (1983) 700.
[12] F. Gümüş, Ö. Algül, DNA binding studies with cis-dichlorobis (5(6)-non/chlorosubstituted-2-hydroxymethyl-benzimidazole) platinum(II) complexes, J. Inorg. Biochem. 68 (1997) 71.
[13] M.M. Muir, O. Cox, L.A. Rivera, M.E. Cadiz, E. Medina, Synthesis and characterization of new platinum(II) complexes containing thiazole and imidazole donors III. dichlorobis(styrylbenzazole)platinum(II) complexes, Inorg. Chim. Acta 191 (1992) 131.

[14] S. Mylonas, A. Valavanidis, K. Dimitropoulos, M. Polissiou, A.S. Tsiftsoglou, I.S. Vizirianakis, Synthesis, molecular structure determination, and antitumor activity of platinum(II) and palladium(II) complexes of 2-substituted benzimidazole, J. Inorg. Biochem. 34 (1988) 265.
[15] A. Iakovidis, N. Hadjiliadis, Complex compounds of platinum (II) and (IV) with amino acids, peptides and their derivatives, Coord. Chem. Rev. 135 (1994) 17.
[16] D.F.H. Wallach, Some biochemical anomalies that can contribute to the malignant behavior of cancer cells, J. Mol. Med. 1 (1976) 97.
[17] D.R. Williams, Metals, ligands, and cancer, Chem. Rev. 72 (1972) 203.
[18] L.J. Hanka, Proceedings of the workshop on amino Acid imbalance in the treatment of cancer. Introduction: possibilities for biochemically rational chemotherapy for some malignancies with depleting enzymes and antimetabolites of specific amino acids, Cancer Treat. Rep. 63 (1979) 1009.
[19] E. Bersanetti, A. Pasini, G. Pezzoni, G. Pratesi, G. Savi, R. Supino, F. Zunino, Antitumor complexes of platinum with carrier molecules. 2 [1]. Mixed complexes of amino acids and tert-butylamine, Inorg. Chim. Acta 93 (1984) 167.
[20] A. Colombo, R. di Gioia, A. Pasini, T. Dasdia, F. Zunino, Antitumor complexes of platinum with carrier molecules. 3. Cytotoxicity of some platinum amino acid complexes against cisplatin-sensitive and resistant L1210 leukemia cells, Inorg. Chim. Acta 125 (1986) L1.
[21] L. Kumar, N.R. Kandasamy, T.S. Srivastava, A.J. Amonkar, M.K. Adwankar, M.P. Chitnis, Synthesis and spectroscopic studies of potential anticancer [platinum (II)(2,2′-bipyridine)(amino acid)]n+ (n = 1 or 2) complexes, J. Inorg. Biochem. 23 (1985) 1.
[22] R. Mital, T.S. Srivastava, H.K. Parekh, M.P. Chitnis, Synthesis, characterization, DNA binding and cytotoxic studies of some mixed-ligand palladium(II) and platinum(II) complexes of α-diimine and amino acids, J. Inorg. Biochem. 41 (1991) 93.
[23] V.X. Jin, J.D. Ranford, Complexes of platinum(II) or palladium(II) with 1,10-phenanthroline and amino acids, Inorg. Chim. Acta 304 (2000) 38.
[24] J.M. Vujić, G.N. Kaluđerović, M. Milovanović, B.B. Zmejkovski, V. Volarević, D. Živić, P. Đurđević, N. Arsenijević, S.R. Trifunović, Stereospecific ligands and their complexes. Part VII. Synthesis, characterization and in vitro antitumoral activity of platinum(II) complexes with O,O′-dialkyl esters of (S, S)-ethylenediamine-N,N′-di-2-(4-methyl)pentanoic acid, Eur. J. Med. Chem. 46 (2011) 4559.
[25] S.R. Chaudhuri, G.N. Kaluđerović, M. Bette, J. Schmidt, H. Schmidt, R. Paschke, D. Steinborn, Synthesis, characterization and cytotoxicity studies of platinum (II) complexes with amino acid ligands in various coordination modes, Inorg. Chim. Acta 394 (2013) 472.
[26] M.A. Carvalho, S.M. Shishido, B.C. Souza, R.E.F. de Paiva, A.F. Gomes, F.C. Gozzo, A.L.B. Formiga, P.P. Corbi, A new platinum complex with tryptophan: Synthesis, structural characterization, DFT studies and biological assays in vitro over human tumorigenic cells, Spectrochim. Acta Part A 122 (2014) 209.
[27] A. Alsalme, S. Laeeq, S. Dwivedi, M.S. Khan, K. Al Farhan, J. Musarrat, R.A. Khan, Synthesis, characterization of α-amino acid Schiff base derived Ru/Pt complexes: Induces cytotoxicity in HepG2 cell via protein binding and ROS generation, Spectrochim. Acta Part A 163 (2016) 1.
[28] W.A. Denny, G.W. Rewcastle, B.C. Baguley, Potential antitumor agents. 59. Structure-activity relationships for 2-phenylbenzimidazole-4-carboxamides, a new class of minimal DNA-intercalating agents which may not act via topoisomerase II, J. Med. Chem. 33 (1990) 814.
[29] G.L. Gravatt, B.C. Baguley, W.R. Wilson, W.A. Denny, DNA-directed alkylating agents. 6. Synthesis and antitumor activity of DNA minor groove-targeted aniline mustard analogs of pibenzimol (Hoechst 33258), J. Med. Chem. 37 (1994) 4338.
[30] M. Gaba, C. Mohan, Development of drugs based on imidazole and benzimidazole bioactive heterocycles: recent advances and future directions, Med. Chem. Res. 25 (2016) 173.
[31] F. Gümüş, A.B. Demirci, T. Özden, H. Eroğlu, N. Diril, Synthesis, characterization and mutagenicity of new *cis*-[Pt(2-substitutedbenzimidazole)$_2$Cl$_2$ complexes, Die Pharmazie 58 (2003) 303.
[32] F. Gümüş, İ. Pamuk, T. Özden, S. Yıldız, N. Diril, E. Öksüzoğlu, S. Gür, A. Özkul, Synthesis, characterization and in vitro cytotoxic, mutagenic and antimicrobial activity of platinum(II) complexes with substituted benzimidazole ligands, J. Inorg. Biochem. 94 (2003) 255.
[33] F. Gümüş, G. Eren, L. Açık, A. Çelebi, F. Öztürk, Ş. Yılmaz, R.I. Sağkan, S. Gür, A. Özkul, A. Elmalı, Y. Elerman, Synthesis, cytotoxicity, and DNA interactions of new cisplatin analogues containing substituted benzimidazole ligands, J. Med. Chem. 52 (2009) 1345.
[34] G. Eren, S. Yilmaz, F. Gumus, In vitro cytotoxic activities of a platinum(II) complex with 1-methyl-2-(3'-hydroxypropyl)benzimidazole and 2-(3'-hydroxypropyl)benzimidazolium hexa- and tetrachloroplatinate salts, Lett. Drug Des. Discov. 15 (2018) 65.
[35] M.A. Phillips, The formation of 2-substituted benziminazoles, J. Chem. Soc. (Resumed) (1928) 2393.
[36] M. Adharvana Chari, D. Shobha, T. Sasaki, Room temperature synthesis of benzimidazole derivatives using reusable cobalt hydroxide (II) and cobalt oxide (II) as efficient solid catalysts, Tetrahedron Lett. 52 (2011) 5575.
[37] T. Yamashita, S. Yamada, Y. Yamazaki, H. Tanaka, New procedure for the synthesis of 2-alkylbenzimidazoles, Synth. Commun. 39 (2009) 2982.
[38] F. Gümüş, Ö. Algül, G. Eren, H. Eroğlu, N. Diril, S. Gür, A. Özkul, Synthesis, cytotoxic activity on MCF-7 cell line and mutagenic activity of platinum(II) complexes with 2-substituted benzimidazole ligands, Eur. J. Med. Chem. 38 (2003) 473.
[39] S. Utku, F. Gumus, S. Tezcan, M.S. Serin, A. Ozkul, Synthesis, characterization, cytotoxicity and DNA binding of some new platinum(II) and platinum(IV) complexes with benzimidazole ligands, J. Enzyme Inhib. Med. Chem. 25 (2010) 502.
[40] Z. Chval, M. Sip, J.V. Burda, The trans effect in square-planar platinum(II) complexes—A density functional study, J. Comput. Chem. 29 (2008) 2370.
[41] B.J. Coe, S.J. Glenwright, Trans-effects in octahedral transition metal complexes, Coord. Chem. Rev. 203 (2000) 5.
[42] P. Štarha, J. Marek, Z. Trávníček, Cisplatin and oxaliplatin derivatives involving 7-azaindole: structural characterisations, Polyhedron 33 (2012) 404.
[43] M.F.A. Lucas, M. Pavelka, M.E. Alberto, N. Russo, Neutral and acidic hydrolysis reactions of the third generation anticancer drug oxaliplatin, J. Phys. Chem. B 113 (2009) 831.
[44] M.D. Hall, M. Okabe, D.-W. Shen, X.-J. Liang, M.M. Gottesman, The role of cellular accumulation in determining sensitivity to platinum-based chemotherapy, Annu. Rev. Pharmacol. Toxicol. 48 (2008) 495.
[45] R.C. Todd, S.J. Lippard, Structure of duplex DNA containing the cisplatin 1,2-{Pt(NH3)2}2+-d(GpG) cross-link at 1.77 Å resolution, J. Inorg. Biochem. 104 (2010) 902.
[46] A. Casini, J. Reedijk, Interactions of anticancer Pt compounds with proteins: an overlooked topic in medicinal inorganic chemistry?, Chem Sci. 3 (2012) 3135.
[47] Y. Jung, S.J. Lippard, Direct cellular responses to platinum-induced DNA damage, Chem. Rev. 107 (2007) 1387.
[48] D. Wang, S.J. Lippard, Cellular processing of platinum anticancer drugs, Nat. Rev. Drug Discov. 4 (2005) 307.
[49] P.M. Takahara, A.C. Rosenzweig, C.A. Frederick, S.J. Lippard, Crystal structure of double-stranded DNA containing the major adduct of the anticancer drug cisplatin, Nature 377 (1995) 649.
[50] P.M. Takahara, C.A. Frederick, S.J. Lippard, Crystal structure of the anticancer drug cisplatin bound to duplex DNA, J. Am. Chem. Soc. 118 (1996) 12309.
[51] W.H. Ang, M. Myint, S.J. Lippard, Transcription inhibition by platinum–DNA cross-links in live mammalian cells, J. Am. Chem. Soc. 132 (2010) 7429.
[52] G.L. Cohen, W.R. Bauer, J.K. Barton, S.J. Lippard, Binding of cis- and trans-dichlorodiammineplatinum(II) to DNA: Evidence for unwinding and shortening of the double helix, Science 203 (1979) 1014.
[53] M.V. Keck, S.J. Lippard, Unwinding of supercoiled DNA by platinum-ethidium and related complexes, J. Am. Chem. Soc. 114 (1992) 3386.
[54] A.M.J. Fichtinger-Schepman, J.L. Van der Veer, J.H.J. Den Hartog, P.H.M. Lohman, J. Reedijk, Adducts of the antitumor drug cis-diamminedichloroplatinum(II) with DNA: formation, identification, and quantitation, Biochemistry 24 (1985) 707.
[55] R.J. Roberts, G.A. Wilson, F.E. Young, Recognition sequence of specific endonuclease BamHI from Bacillus amyloliquefaciens H, Nature 265 (1977) 82.
[56] V. Brabec, J. Kasparkova, Modifications of DNA by platinum complexes: Relation to resistance of tumors to platinum antitumor drugs, Drug Resist. Updat. 8 (2005) 131.
[57] M.A. Fuertes, J. Castilla, C. Alonso, J.M. Pérez, Cisplatin biochemical mechanism of action: from cytotoxicity to induction of cell death through interconnections between apoptotic and necrotic pathways, Curr. Med. Chem. 10 (2003) 257.
[58] D. Yang, S.S.G.E. van Boom, J. Reedijk, J.H. van Boom, A.H.J. Wang, Structure and isomerization of an intrastrand cisplatin-cross-linked octamer DNA duplex by NMR analysis, Biochemistry 34 (1995) 12912.
[59] H. Thakuria, G. Das, An expeditious one-pot solvent-free synthesis of benzimidazole derivatives, ARKIVOC 15 (2008) 321.
[60] Y. Kim, M.R. Kumar, N. Park, Y. Heo, S. Lee, Copper-catalyzed, one-pot, three-component synthesis of benzimidazoles by condensation and C-N bond formation, J. Org. Chem. 76 (2011) 9577.
[61] N.A. Weires, J. Boster, J. Magolan, Combined Pd/C and Montmorillonite catalysis for one-pot synthesis of benzimidazoles, Eur. J. Org. Chem. 2012 (2012) 6508.
[62] S.R. Landor, P.D. Landor, Z.T. Fomum, G.W.B. Mpango, Allenes. Part 38. Imidazolines, benzimidazoles and hexahydrobenzimidazoles from 1,2-diamines and allenic or acetylenic nitriles, J. Chem. Soc., Perkin Trans. 1 (1972–1999) (1979) 2289.
[63] N. Vinot, Synthesis of α-substituted 2-cyanomethylbenzimidazoles. Their use for the preparation of benzimidazoles with other functions, Bull. Soc. Chim. Fr. 12 (1966) 3989.
[64] P. Boggu, E. Venkateswararao, M. Manickam, D. Kwak, Y. Kim, S.-H. Jung, Exploration of 2-benzylbenzimidazole scaffold as novel inhibitor of NF-κB, Bioorg. Med. Chem. 24 (2016) 1872.
[65] P. Štarha, Z. Trávníček, I. Popa, Platinum(II) oxalato complexes with adenine-based carrier ligands showing significant in vitro antitumor activity, J. Inorg. Biochem. 104 (2010) 639.