ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2017, *62*(3), 227-236

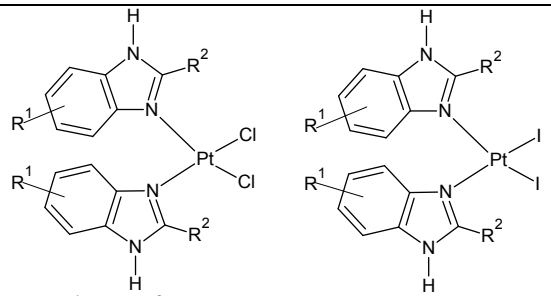
SYNTHESIS, CHARACTERIZATION AND DNA INTERACTION OF NOVEL PLATINUM(II) COMPLEXES CONTAINING SUBSTITUTED BENZIMIDAZOLE LIGANDS

Abdoul NZEYIMANA,[a] Semra UTKU,[a,*] Leyla AÇIK,[b] Ayten ÇELEBİ KESKİN[c]

[a]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mersin University, Mersin 33169, Turkey
[b]Department of Biology, Faculty of Science, Gazi University, Ankara 06500 Turkey
[c] Department of Bioengineering, Faculty of Engineering, Kırıkkale University, Kırıkkale, 71450 Turkey

Received July 27, 2016

Eight novel Pt(II) complexes corresponding to the following general formula $[PtCl_2(L^1\text{-}L^4)_2]$ (**C1-C4)** and $[PtI_2(L^1\text{-}L^4)_2]$ (**C5-C8**) in which 5(6)-chloro/or-methyl-2-H/or-methylbenzimidazole (L^1-L^4) played the key role as carrier ligands were synthesized and characterized by elemental analysis, IR and ^{1}H NMR. Considering leaving group functions, the anionic ligand iodido and chloro were utilized with the purpose of studying the interaction between the synthesized complexes and pBR322 plasmid DNA by using cisplatin as positive control throughout the Agarose Gel Electrophoresis method. Therefore, looking after plasmid DNA interacting outcomes, synthesized complexes modified the tertiary structure of pBR322 plasmid DNA, and the results showed that the complex **C2** ($[PtCl_2(L^2)_2]$) was highly active compound regarding to all synthesized complexes.



INTRODUCTION

Cancer is one of the leading causes of morbidity and mortality in the worldwide, with approximately 14 million new cases and 8.2 million deaths from cancer were reported in 2012. By 2030, it is estimated that there will be 27 million new cancer cases**,** 17 million cancer deaths annually with 75 million persons alive with cancer within five years of diagnosis.[1]

An extensive research has been done to characterize antiproliferative effect of various classes of compounds ranging from naturally occurring molecules and their derivatives, and to discover organic and/or inorganic compounds basically for their application in cancer therapy.[2]

The fortuitous discovery of cisplatin (*cis*-diamminedichloroplatinum(II)) in 1965, and by the 1978, it had been approved by FDA for treatment of the different types of cancer such as testicular, ovarian, head and neck, colon, bladder, gastric, and lung cancer.[3] Cisplatin is one of the most successful anticancer drug, being specifically effective in the treatment of testicular cancer with a cure rate of 90-95%.[4]

However, there are two considerable problems associated with clinical cisplatin usage: intrinsic or acquired resistance and side effects including nephrotoxicity, ototoxicity, nausea and emetogenicity.[5] These have led to the development of cisplatin analogs that would be clinically effective without and/or less toxicity.[6]

* Corresponding author: utkusemra@hotmail.com or utkusemra@mersin.edu.tr

Therapeutically, cisplatin enters passively into the cells by a simple diffusion or actively by protein-mediated transport systems; for instance, human organic cation transporter (hOCT2) and the copper transport protein (Ctr1).[7] Cisplatin undergoes hydrolysis within the cell, and producing a highly reactive charged platinum complexes species. Then after, this reactive complex binds to DNA bases through the N7 atom preferably guanine or adenine forming the monofunctional adduct $[PtCl(DNA)(NH_3)_2]$. The second chloride ligand can be displaced by a water molecule to form the adduct $[Pt(H_2O)(DNA)(NH_3)_2]^{2+}$. These species may re-interact with DNA by forming 1,2-d(GpG) and 1,2-d(ApG) intrastrand crosslink and preventing DNA replication and transcription. Consequently, they activate the apoptotic pathways to cell death.[7-9]

Benzimidazole moiety is structurally related to purine bases and found out in a variety of natural compounds like vitamin B_{12}.[10] It also serves as a good ligand in various transition metal complexes where it has been considerably found retaining efficacy against cisplatin-resistant ovary cell lines.[11-16]

From this context, our research group reported the synthesis, characterization, cytotoxic activity and DNA binding affinity of Pt(II) and Pt(IV) complexes, in which both ammine and chloro ligands in cisplatin were exchanged by substituted benzimidazole or imidazole carrier ligands and iodido, oxalato, malonato, methylmalonato or malate leaving ligands respectively.[17-27] Among them showed remarkable cytotoxic activity toward RD,[18] HeLa,[20-22,24,25,27] HEp-2,[24,28] HRT-18,[29] MDA-MB231,[25,30,31] Caco-2,[30] SK-Hep1[30] MCF-7,[19-22,24,25,30,31] and OVCAR-3[27] cancer cells and DNA binding affinity[22,24-26,31] comparatively to cisplatin.

In this paper, we report on the synthesis and spectral characterization of eight new platinum(II) complexes of the type $[Pt(L^1\text{-}L^4)_2Cl_2]$ **C1-C4** and $[Pt(L^1\text{-}L^4)_2I_2]$ **C5-C8** (**L**^{1}=5(6)-chlorobenzimidazole, **L**^{2}= 5(6)-methylbenzimidazole, **L**^{3}=5(6)-chloro-2-methylbenzimidazole, **L**^{4}= 5(6)-methyl-2-methyl benzimidazole) by their elemental analyses, Infrared (IR) and ^{1}H Nuclear Magnetic Resonance (NMR) technigue. The interactions with pBR322 plasmid DNA and inhibition of the *BamH*I and *Hind*III restriction enzyme activity through the synthesized complexes were also studied. Detailed studies on anticancer activity of synthesized complexes will be reported on a following research.

RESULTS AND DISCUSSION

The carrier ligands L^1-L^4 were prepared according to the Phillips method[32] as shown in Scheme 1, and their melting points were in accordance with the literature.[33-36]

Complexes **C1-C4** or **C5-C8** were prepared by the reaction of the corresponding ligand and K_2PtCl_4 or K_2PtI_4 which were obtained in situ by mixing aqueous solutions of K_2PtCl_4 and KI respectively in ethanol/water solution as shown in Scheme 2. The melting points of all complexes were above 400 °C.

C1-C8 were characterized by elemental analysis, IR and ^{1}H NMR spectra. Elemental analysis suggested a 1:2 (metal:ligand) stoichiometry for **C1**-**C8**, as expected empirical formula. For complexes **C1**, **C3**, **C4** and **C5**, molecules of water were included as justified by analytical results.

The IR spectra of the complexes have characteristically shown changes comparing to free ligands in the measured region between 4000-600 cm^{-1} and the **L**^{1}**-L**^{4} show broad bands in the region between 3500-2300 cm^{-1} due to the intermolecular hydrogen bonded imidazole N—H.

All the complexes exhibited N—H stretching bands centered at 3240 cm^{-1} sharpers than those with their ligands brought from breaking tautomerism at which N—H benzimidazoles did not involve in the coordination.[20,25]

The insolubility character of the complexes makes them to be solved in dimethylsulfoxide (DMSO)-d_6 and immediately recorded by ^{1}H NMR in order to avoid the ligand exchange reaction between the synthesized platinum complexes and DMSO-d_6. The ^{1}H NMR spectral data of the ligands and **C1**-**C8** are presented in the experimental section, and all signals were shifted upon as the result of the electric field effect caused by complexation.

The large downfield shifts N-H signal of carrier ligand benzimidazoles in the spectra of the **C1**-**C8** as result of an increase of the N-H acid character after platinum binding.[37] Chemical shifts of these synthesized complexes to N-H signals vary between 13.64-13.00 ppm.

Agarose gel electrophoresis is extensively simple technique used to visualize interactions between platinum based complexes and plasmid DNA. Nuclear DNA is an important molecular target for platinum anticancer compounds, which bind purine bases at the N7 position. The resulting Pt-DNA damage triggers downstream effects, including inhibition of replication and transcription. If the cell cannot remove the damage, then it dies by one of several pathways. It is widely

documented that platinum-based complexes can untwist and/or bend plasmid DNA, depending on the kind of specific adducts formed on the DNA. Monofunctional or intercalating adducts may untwist dsDNA, whereas bifunctional adducts and intra- and interstrand cross-links, in addition, bend DNA.[4] Normally, untreated pBR322 which is mixture of mainly covalently closely circular form I and a small amount of open circular form II DNA was used as a control. After platinum complexes binding to DNA changes in DNA conformation and DNA damage may relatedly occur with changes to mobility and intensity of DNA bands. [24,31]

When circular pBR322 plasmid DNA is subjected to electrophoresis, fast migration will be relatively observed from the intact covalent closed circular form I and if scission occurs on one strand via platinum complex, the supercoiled form will be relaxed to generate a slower-moving single nicked circular form II.[38]

Fig. 1 shows the results of electrophoretic separation of pBR322 plasmid DNA after incubating with **C1-C8** and cisplatin in a range of concentrations from 160 to 2.5 μM at 37 °C for 24 h where the cisplatin and the untreated pBR322 plasmid DNA were used respectively as positive and negative controls.

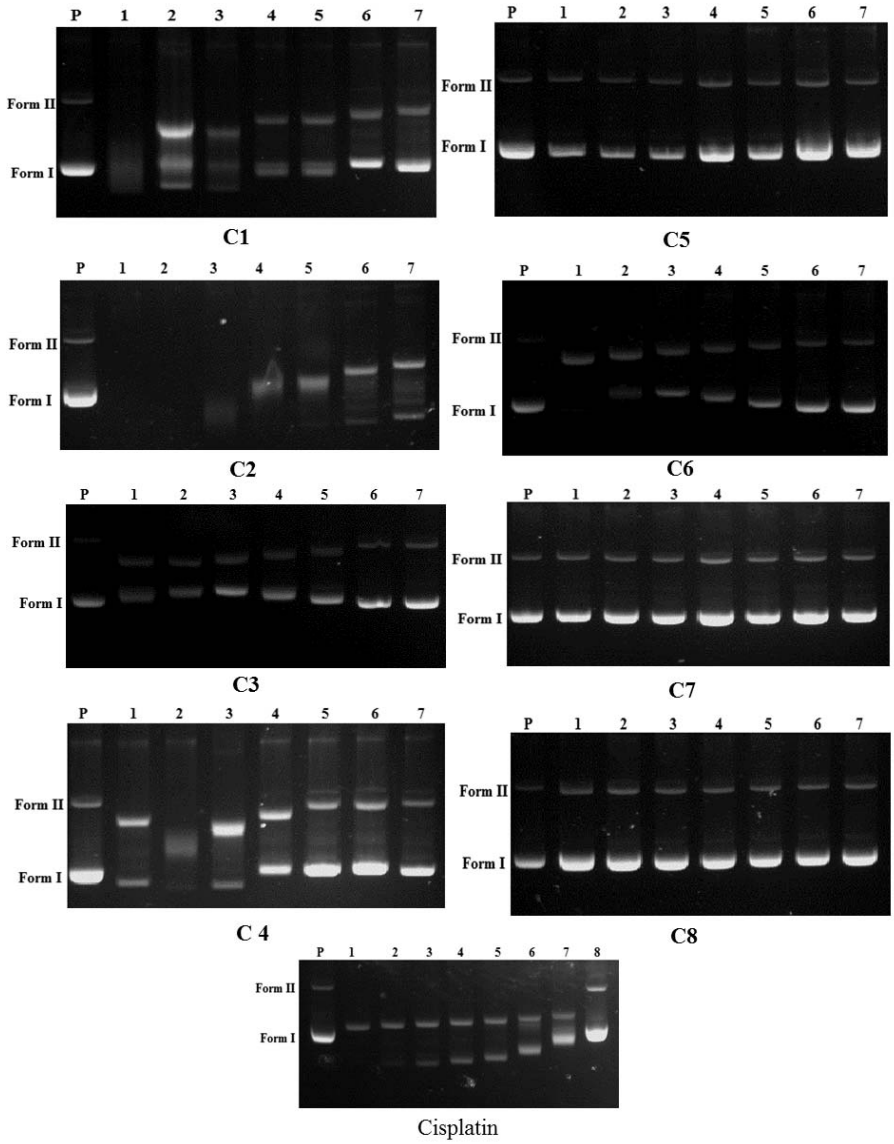


Fig. 1 – Modification of gel electrophoretic mobility of pBR322 plasmid DNA when incubated with various concentrations of complexes **C1-C8** and cisplatin. Concentrations (in μM) are as follows: (P) untreated pBR322 plasmid DNA; for complexes **C1-C8**: (line 1) 160; (line 2) 80; (line 3) 40; (line 4) 20; (line 5) 10; (line 6) 5; (line 7) 2.5. (P) and 8 untreated pBR322 plasmid DNA; for cisplatin: (line 1) 160; (line 2) 80; (line 3) 40; (line 4) 20; (line 5) 10; (line 6) 5; (line 7) 2.5. The top and the bottom bands correspond to form II (open circular) and form I (covalently closed circular) plasmids, respectively. Roman numerals I, and II indicate form I (covalently closed circular) and form II (open circular), plasmids.

When pBR322 plasmid DNA was incubated with cisplatin at decreased concentration from 160 to 2.5 μM, the mobility of both forms I and II bands decreased, and only one band corresponding to form II was determined at 160 and 80 μM concentration. Besides, the intensity of the form I band (compared to untreated DNA, P) decreased with the increasing concentrations of cisplatin. The behavior of the gel electrophoretic mobility of both forms of pBR322 DNA plasmid and cisplatin adducts were comparatively consistent to the previous published reports.[24,39]

While the mobility of form II relatively decreased with the decreasing concentration of **C1,** the intensity was slightly increased, and a faint band of form I and form II was observed at the 160 μM concentration. This can partially be attributed from the unwinding supercoiled DNA to opened circular DNA by demonstrating the binding between DNA and the platinum complexes.[26,40]

At the range concentration between 160-40 μM of **C2** complex, both form I and form II bands disappeared, this implies the degradation of plasmid DNA into undetectable single-stranded pieces. As the concentration of **C2** decreased, intensity of both forms I and II bands increased at different rates (lane 4-7).

When plasmid DNA was interacted with decreasing concentration of **C3** and **C6,** the intensity and the mobility of form I and II bands changed. While the intensity and mobility of form II band decreased, form I band increased comparatively to untreated pBR322 plasmid DNA.

The mobility of both forms I and II bands increased but intensity was slightly increased as the concentration of **C4** ranging from 160 to 2.5 μM concentrations.

The **C5, C7** and **C8** having iodide leaving groups did not alter the electrophoretic mobility at the form I and form II bands of pBr322 plasmid DNA through different concentrations.

The affinity assessment between the **C1**-**C8** and guanine-guanine (GG) and/or adenine-adenine (AA) regions was carried out through the restriction endonuclease analysis of the complex-pBR322 plasmid DNA adducts digested by *BamH*I and *Hind*III enzymes. This analysis was used to provide further information into changes regarding DNA conformation.

Figs. 2 and 3 gave the electropherograms applied to the interaction among the pBR322 plasmid DNA with decreasing concentration of **C1**-**C8** and cisplatin for a period of 4 h at 37 °C followed by *BamH*I and *Hind*III digestion for a further period of 1 h at the same temperature.

*BamH*I or *Hind*III enzymes bind at the recognition sequence 5′-G/GATCC-3′ or 5′-A/AGCTT-3′ and cleaves these sequences at 5′-guanine/5′-adenine sites respectively. As a result, they convert form I and form II bands to linear form III band.[38] When pBR322 DNA plasmid DNA interacted with **C1**-**C8** following by *BamH*I or *Hind*III digestion, the **C1** and **C2** increasingly prevented enzymes digestion at high concentrations by the fact that they binded at A/A and A/G site of pBR322 plasmid DNA. The **C4** was slightly restricted by *BamH*I and *Hind*III enzymes for all tested concentrations. Among the tested Pt(II) complexes **C1**-**C8**, compound **C2** having 5(6)-methylbenzimidazole non leaving carrier ligands and chloro as leaving ligands inhibit *BamH*I and *Hind*III restriction enzyme activity to a greater extent than the others. This result implies that **C3** was specifically binding to GG/AA regions of the plasmid DNA like cisplatin.

It was emerged that platinum–DNA adducts formed from the uptake of platinum based drugs throughout the nucleus cells activate several cellular processes that mediate the cytotoxicity of these platinum drugs. The platinum complexes bind to DNA through covalent attachment with purine bases and finally produce cooperative changes in double helix. This means that if the cells don't physiologically recover a damage, the damage directly causes the death to cells by proceeding one of cellular pathways mediated cytoxicity.[41]

In recent years, of great interest unconventional metal based drugs are the iodide-platinum complexes as anticancer agents. It was believed that the derivative iodido cisplatin analogues become inactive anticancer agents basically the fact that they contain higher stability and lower reactivity of Pt-I bonds compared to those of Pt-Cl bonds in physiological environment. In fact, a few reports can be only found in the literature that show a different reactivity of Pt-I complexes versus some important biological components. Probably these platinum complexes exhibit anticancer activity by different mechanism of action which has been shown by research groups.[42,43] This results illustrates why the **C5**-**C8** containing iodido leaving group did not bind to plasmid DNA, but still looking another way they could exhibit activities by focusing on mechanisms of action.

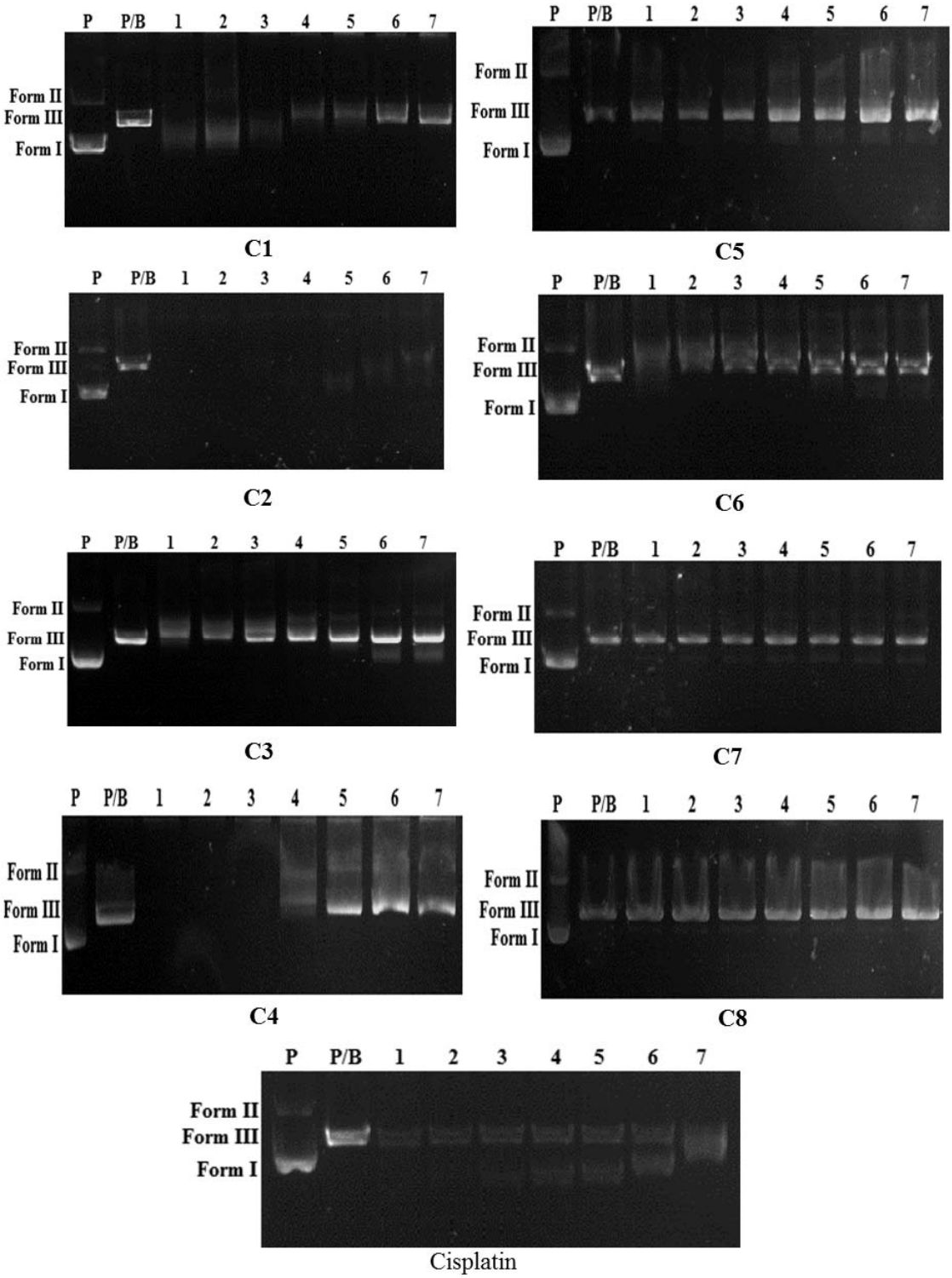


Fig. 2 – Electrophoretograms for the *BamH*I digested mixtures of pBR322 plasmid DNA after their treatment with various concentrations of **C1-C8** and cisplatin. Concentrations (in μM) are as follows: (P) untreated pBR322 plasmid DNA; (P/B) pBR322 DNA linearized by *BamH*I; for C**1-C8** and cisplatin: (line 1) 160; (line 2) 80; (line 3) 40; (line 4) 20; (line 5) 10; (line 6) 5; (line 7) 2.5. The top, the bottom and in the middle bands indicate form I (covalently closed circular) and form II (open circular) and form III (linear) plasmids, respectively.

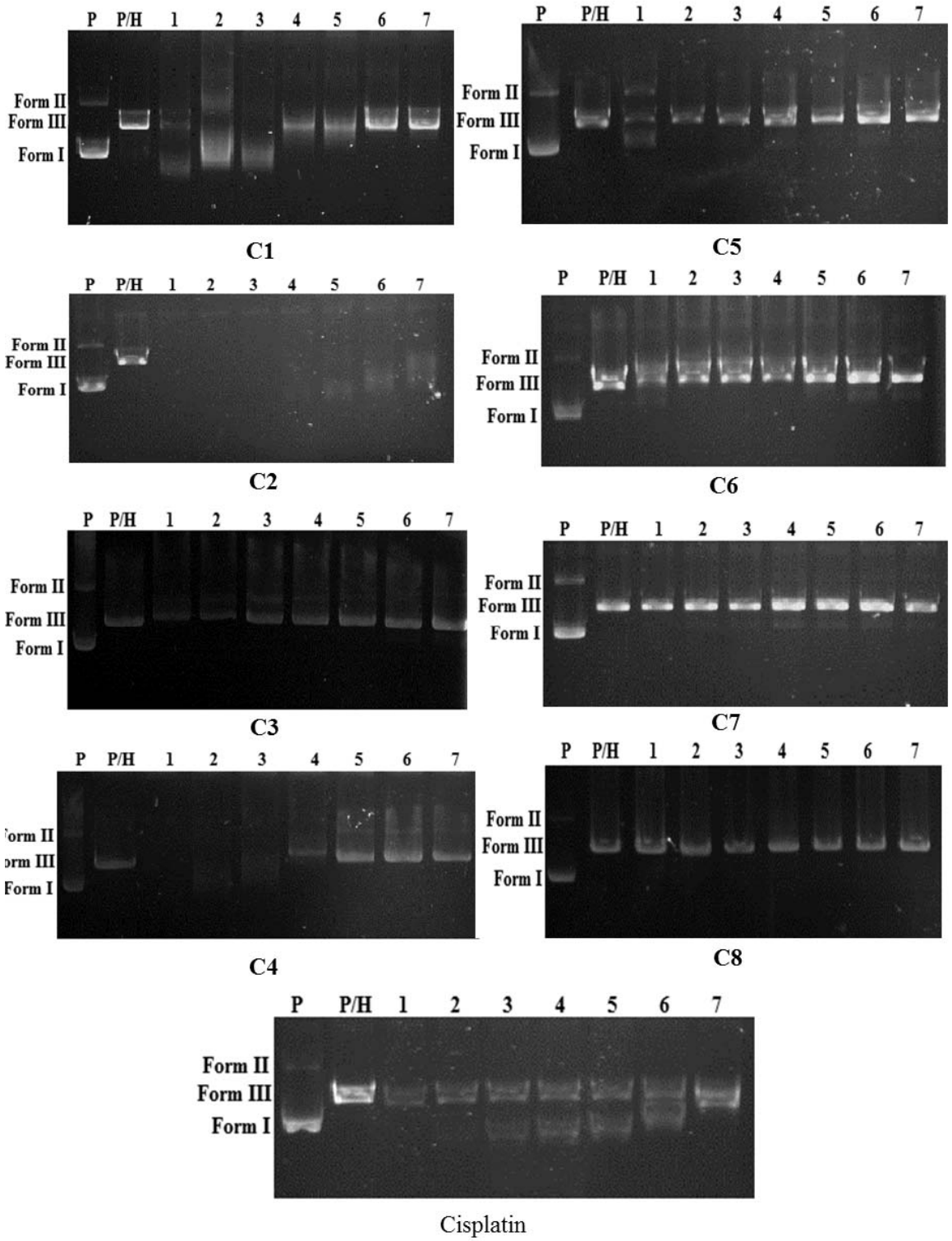


Fig. 3 – Electrophoretograms for the *Hind*III digested mixtures of pBR322 plasmid DNA after their treatment with various concentrations of **C1-C8** and cisplatin. Concentrations (in μM) are as follows: (P) untreated pBR322 plasmid DNA; (P/H) pBR322 DNA linearized by *Hind*III; for C**1-C8** and cisplatin: (line 1) 160; (line 2) 80; (line 3) 40; (line 4) 20; (line 5) 10; (line 6) 5; (line 7) 2.5. The top, the bottom and in the middle bands indicate form I (covalently closed circular) and form II (open circular) and form III (linear) plasmids, respectively.

EXPERIMENTAL

All chemicals and solvents were purchased from Merck, Aldrich or Sigma. Melting point was measured using open capillary on a Electrothermal 9200 melting point apparatus. IR spectra were recorded on Perkin Elmer Spectrum FT-IR/NIR spectrometer equipped with a Universal ATR Sampling Accessory at Mersin University Advanced Technology, Training, Research and Application Center (Mersin, Turkey). Elemental analyses were carried out in the LECO 932 CHNS analyzer and ^{1}H NMR spectra were recorded on a Varian Mercury 400 MHz FT-NMR with the chemical shift (δ) reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS) at Central Laboratuvary of the Faculty of Pharmacy, Ankara University (Ankara, Turkey) and the coupling constants (*J*) quoted in Hz. TMS was used as an internal standard with chemical shifts δ in ppm from downfield to upfield.

Synthesis of carrier ligands

L^1-L^4 used as carrier ligands in the structure of complexes **C1**–**C8** were prepared using formerly described method (Scheme 1).[32]

5(6)-chlorobenzimidazole (**L^1**) m.p. 118-120°C, (122–123°C);[33] yield 41%; IR (ATR) υ cm^{-1}: 3084-2560 (N-H, =CH and -CH), 1626-1288 (C=N and C=C). ^{1}H-NMR (DMSO-d$_6$) δ: 8.34 (s, 1H), 7.70 (d, *J*= 2.0 Hz, 1H), 7.66 (d, *J*=8.4 Hz, 1H), 7.29 (dd, *J*=8.4 Hz, *J*= 2 Hz, 1H).

5(6)-methylbenzimidazole (**L^2**) m.p. 112-113°C, (115–116°C);[34] yield 26%; IR (ATR) υ (cm^{-1}): 3230-2557 (N-H, =CH and -CH), 1618-1245 (C=N and C=C). ^{1}H-NMR (DMSO-d$_6$) δ: 9.53 (s, 1H), 7.75 (d, *J*= 8.8 Hz, 1H), 7.65 (s, 1H), 7.41 (d, *J*=8.4 Hz, 1H), 2.49 (s, 3H, C*H*$_3$).

5(6)-chloro-2-methylbenzimidazole (**L^3**) m.p. 201-202°C, (199–200°C);[35] yield 45%; IR (ATR) υ (cm^{-1}): 3100-2533 (N-H, =CH and -CH), 1618-1278 (C=N and C=C). ^{1}H-NMR (DMSO-d$_6$) δ: 7.51 (d, *J* = 2 Hz, 1H), 7.47 (d, *J* = 8.8 Hz, 1H), 7.14 (dd, *J* = 8.8, *J* = 2 Hz, 1H), 2.49 (s, 3H, C*H*$_3$).

5(6)-methyl-2-methylbenzimidazole (**L^4**) m.p. 198-200°C, (201–202°C);[36] yield 52%; IR (ATR) υ (cm^{-1}): 3146-2563 (N-H, =CH and -CH), 1630-1281 (C=N and C=C). ^{1}H-NMR (DMSO-d$_6$) δ: 7.33 (d, *J* = 8 Hz, 1H), 7.23 (s, 1H), 6.90 (d, *J* = 8 Hz, 1H), 2.43 (s, 3H, C*H*$_3$), 2.37 (s, 3H, C*H*$_3$)

General procedure for the synthesis of C1-C4

To solution of K_2PtCl_4 (1.204 mmol) in distilled water (8 mL) was added to an ethanol—water solution (5:15 mL) of the corresponding L^1-L^4 derivatives respectively. The reaction mixture was heated at 50 °C and stirred for 2 or 3 days in the dark. The pH was adjusted to ~7 and kept constantly with the addition of 0.1 M $NaHCO_3$. Light brown solid formed gradually during this period. The reaction mixture was then cooled to room temperature, and resulting crude precipitates were filtered off, washed with distilled water, ethanol, and diethyl ether and dried in vacuo. The resulting product was stable in dry air and in the dark (Scheme 2).

[dichloro-bis(5(6)-chlorobenzimidazole)platinum(II)] [Pt(L$^{1)}_2$Cl$_2$].H$_2$O (**C1**)

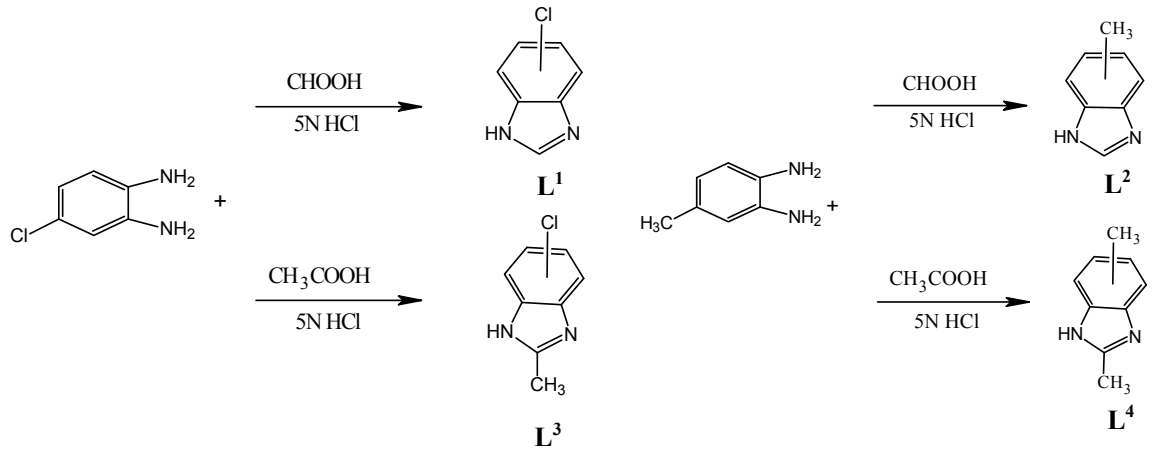
Yield 33%, IR (ATR) υ(cm^{-1}): 3210-2981(N–H, =CH, -CH and –OH), 1622-1296 (C=N and C=C). ^{1}H-NMR (DMSO-d$_6$) δ: 13.64 (s, 2H, 2x N-H, exchangeable with D_2O), 8.91 (d, *J* = 1,6 Hz, 1H), 8.85 (s, 1H), 7.89 (dd, *J* = 1.6 Hz, *J* = 2 Hz, 1H), 7.83-7.77 (m, 1H), 7.61 (dd, *J* = 1.6 Hz, *J*= 1.6 Hz, 1H), 7.56-7.53 (m, 1H), 7.32-7.25 (m, 2H). Anal. calcd. for $C_{14}H_{10}Cl_4N_4Pt.H_2O$: C, 28.54; H, 2.05; N, 9.51. Found: C, 28.79; H, 2.00; N, 9.79.

[dichloro-bis(5(6)-methylbenzimidazole)platinum(II)] [Pt(L^2)$_2$Cl$_2$] (**C2**)

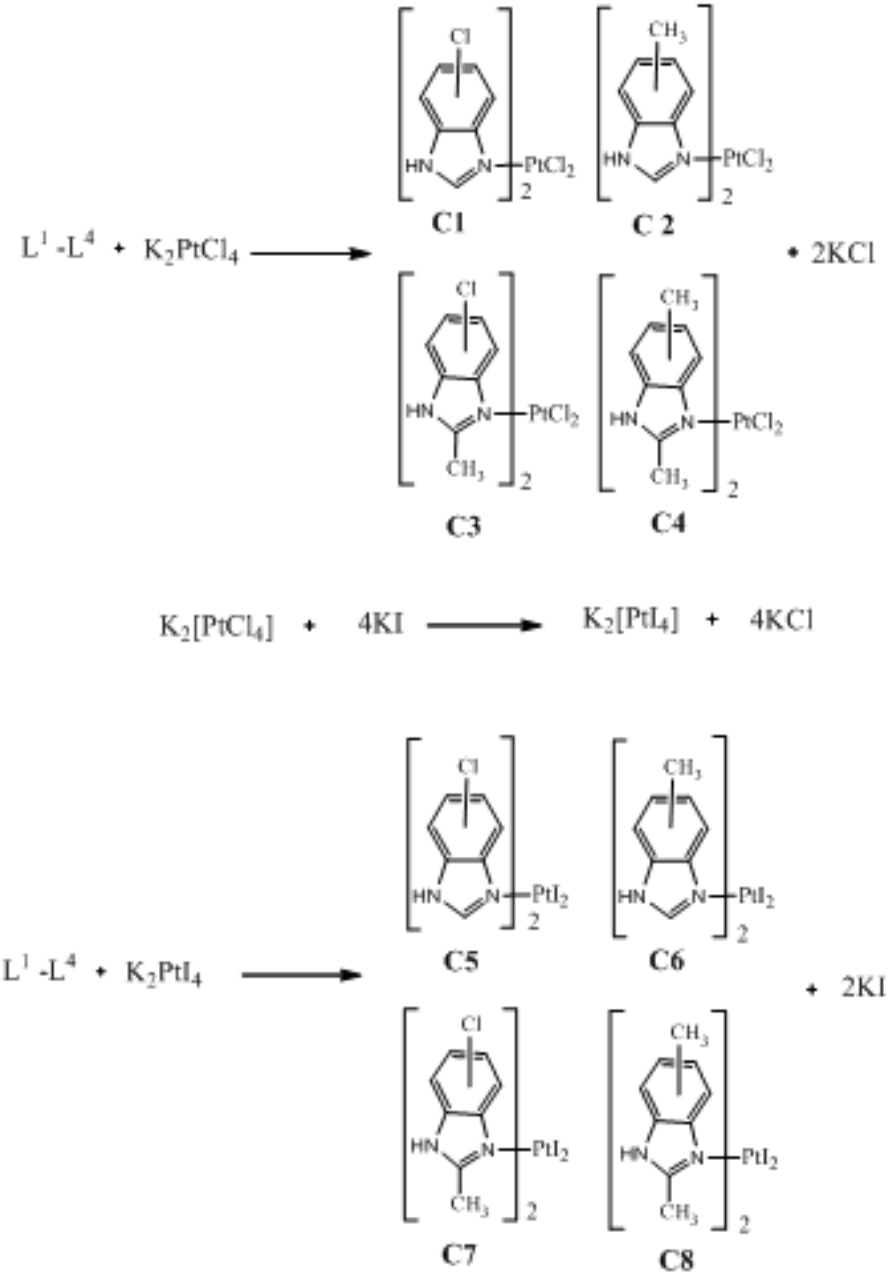
Yield: 29%, IR (ATR) υ (cm^{-1}): 3277-2980 (N–H, =CH and -CH), 1631-1248 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 13.26 (s, 2H, 2x N-H, exchangeable with D_2O), 8.76-8.71 (m, 2H), 7.72-7.57 (m, 2H), 7.36-7.25 (m, 2H), 7.06-6.98 (m, 2H), 2.48-2.30 (m, 6H, 2x C*H*$_3$). Anal. calcd. for $C_{16}H_{16}Cl_2N_4Pt$: C, 36.24; H, 3.04; N, 10.56. Found: C, 35.98; H, 3.03; N, 10.54.

[dichloro-bis(5(6)-chloro-2-methylbenzimidazole)platinum(II)] [Pt(L^3)$_2$Cl$_2$].1.5 H$_2$O (**C3**)

Yield: 25%, IR (ATR) υ (cm^{-1}): 3286-2981 (N–H, =CH, -CH and –OH), 1623-1221 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 13.44 (broad s, 2H, 2x N-H, exchangeable with D_2O), 8.27-7.96 (m, 2H), 7.62- 7.23 (m, 4H), 3.04-2.45 (m, 6H, 2x C*H*$_3$). Anal. Calcd. for $C_{16}H_{14}Cl_4N_4Pt.1.5\ H_2O$: C, 30.69; H, 2.73; N, 8.94. Found: C, 29.82; H, 2.41; N, 8.63.



Scheme 1 – Synthesis of carrier ligands **L^1-L^4**.



Scheme 2 – Synthesis of platinum(II) complexes **C1**-**C8**.

[dichloro-bis(5(6)-methyl-2-methylbenzimidazole)platinum(II)] [Pt(L^4)$_2$Cl$_2$] .H_2O (**C4**)
Yield: 46%, IR (ATR) υ (cm^{-1}): 3266-2982 (N–H, =CH, -CH and –OH), 1626-1226 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 13.04 (s, 2H, 2x N-H, exchangeable with D_2O), 7.97-7.85 (m, 2H), 7.28- 7.00 (m, 4H), 2.81-2.74 (m, 6H, 2x CH_3), 2.48-2.43 (m, 6H, 2x CH_3). Anal. calcd. for $C_{18}H_{20}Cl_2N_4Pt.H_2O$: C, 37.51; H, 3.85; N, 9.72. Found: C, 37.65; H, 3.47; N, 9.86.

General procedure for the synthesis of C5-C8

To a solution of K_2PtCl_4 (1.204 mmol) in distilled water (15 mL) was added KI (4.80 mmol) and stirred at 60 °C for 45 minutes. Two equivalents of **L^1-L^4** (2.408 mmol) corresponding substituebenzimidazole derivatives in ethanol—water mixture (5:15 mL) was added dropwise over 30 minutes at room temperature to the resulting K_2PtI_4. The reaction mixture was heated at 60 °C and stirred for 3 or 4 days in the dark. The resulting crude yellow precipitate was filtered off and purified by repeated washing with small portions of distilled water-ethanol, diethyl ether. The resulting product was stable in dry air and in the dark (Scheme 2).

[diiodo-bis(5(6)-chlorobenzimidazole)platinum(II)] [Pt(L^1)$_2$$I_2$].2$H_2O$ (**C5**)
Yield: 36%, IR (ATR) υ (cm^{-1}): 3244-1613 (N–H, =CH, –CH and –OH), 1621-1291 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 13.61 (broad s, 2H, 2x N-H, exchangeable with D_2O), 9.09-8.59 (m, 2H), 8.12-8.05 (m, 1H), 7.88-7.82 (m, 1H), 7.71-7.40 (m, 4H). Anal. calcd. for $C_{14}H_{10}Cl_2I_2N_4Pt$ .2H_2O: C, 21.28; H, 1.78; N, 7.09. Found: C, 20.84; H, 1.37; N, 6.74.

[diiodo-bis(5(6)-methylbenzimidazole)platinum(II)] *[Pt(L^2)$_2I_2$]* (**C6**)
Yield: 27%, IR (ATR) υ (cm^{-1}): 3293-2918 (N–H, =CH and -CH), 1597-1247 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 13.43 (broad s, 2H, 2x N-H, exchangeable with D$_2$O), 9.15-8.75 (m, 2H), 8.08-7.88 (m, 1H), 7.69-7.03 (m, 5H), 2.48-2.25 (m, 6H, 2x CH_3). Anal. calcd. for $C_{16}H_{16}I_4N_4Pt$: C, 26.94; H, 2.26; N, 7.86. Found: C, 26.58; H, 2.20; N, 7.52.

[diiodo-bis(5(6)-chloro-2-methylbenzimidazole)platinum(II)] *[Pt(L^3)$_2I_2$]* (**C7**)
Yield: 26%, IR (ATR) υ (cm^{-1}): 3197-2981 (N–H, =CH and –CH), 1594-1217 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 13.41 (s, 2H, 2x N-H, exchangeable with D$_2$O), 8.26-8.12 (m, 2H), 7.54-7.21 (m, 4H), 2.91-2.84 (m, 3H, CH_3), 2.68-2.63 (m, 3H, CH_3). Anal. calcd. for $C_{16}H_{14}Cl_2I_2N_4Pt$: C, 24.57; H, 1.80; N, 7.16. Found: C, 24.28; H, 1.93; N, 7.34.

[diiodo-bis(5(6)-methyl-2-methylbenzimidazole)platinum(II)] *[Pt(L^4)$_2Cl_2$]* (**C8**)
Yield: 37%, IR (ATR) υ (cm^{-1}): 3218-2981 (N–H, =CH and –CH), 1631-1223 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 13.00 (s, 2H, 2x N-H, exchangeable with D$_2$O), 8.15-8.02 (m, 2H), 7.28-6.97 (m, 4H), 2.87-2.70 (m, 6H, 2x CH_3), 2.49-2.41(m, 6H, 2x CH_3). Anal. Calcd. For $C_{18}H_{20}I_2N_4Pt$: C, 29.17; H, 2.72; N, 7.56. Found: C, 29.48; H, 3.00; N, 7.01.

Gel electrophoresis study

pBR322 plasmid DNA, *Bam*HI, *Hind*III restriction enzyme, ethidium bromide, agarose, and positive control cisplatin were obtained from Sigma.

Interaction of **C1-C8** and cisplatin with pBR322 plasmid DNA, with and without *Bam*HI or *Hind*III digestion, was studied by agarose gel electrophoresis method described by Stellwagen.[44] Briefly speaking, 1 µL of pBR322 plasmid DNA (concentration of 0.5 µg/mL) in solution was added to varied amounts of **C1-C8** and cisplatin at different concentration ranging from 2.5 to 160 µM in a shaking water bath at 37°C for 24h and electrophoresed in 1% agarose gel. Electrophoresis was carried under 1× TAE (Tris acetic acid EDTA) buffer for approximately 5h at 40V. At the end of the electrophoresis, the gel was stained in the same buffer containing ethidium bromide (0.5 µg/mL). The gel was photographed using a video camera (GelDoc-It Imaging System, UVP). The experiments were repeatedly done for three times.

In these series experiments, a same set of the drug-DNA mixtures as that described previously,[26] were first incubated for 24 h on a shaking water bath and then subjected to enzyme *BamH*I or *Hind*III digestion for 1h at 37°C. To each 8 µL of incubated drug-DNA mixtures were added 1 µL of 10 x digestion buffer and then 0.1 µL of *BamH*I or *Hind*III (1 unit). The digestion reactions were terminated by rapid cooling. The restricted DNA was run in 1% agarose gel electrophoresis for 3 h at 40 V in TAE buffer. The gel was stained with ethidium bromide. The gels were photographed following method described previously.

CONCLUSION

Several attempts of synthesizing new potent anticancer drugs were done by combining Pt(II) chloro and iodido compounds with benzimidazole derivatives ligands **L^1-L^4**. The description of compounds after the synthesis was assumed by using spectroscopic characterization, pBr322 plasmid DNA interaction and then *BamH*I and *Hind*III restriction enzymes. It was profound that the labile ligands (leaving groups) containing chloro (**C1-C4**) are more active than those containing iodido (**C5-C8**). Promising biological activity from synthesized complexes provides useful information for further cytotoxic evaluation including cisplatin resistant cell lines and future platinum-drug design strategies. Detailed studies on anticancer activity of the synthesized compounds will be reported in the next studies.

Acknowledgements: We would like to thank Prof. Dr. Fatma Gümüş for her supervision and guidance. This research was financially supported by Mersin University Scientific Research Funds (Grant nos. 2015-TP2-1175).

REFERENCES

1. J. Ferlay, I. Saerjomartaram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D. M. Parkin, D. Forman and F. Bray, *Int. J. Cancer*, 2015, *136*, E359-E386.
2. S. Kumar, M. K. Ahmad, M. Waseem and A. K. Pandey, *Med. Chem*., 2015, *5*, 115123.
3. E. Wong and C. M. Giandomenico. *Chem. Rev*., 1999, *99*, 2451-2466.
4. M. Kartalou and J. M. Essigmann, *Mutat. Res*., 2001, *478*, 1-21.
5. C. A. Rabik and M. E. Dolan, *Cancer Treat. Rev*., 2007, *33*, 9-23.
6. M. Fanelli, M. Formica, V. Fusi, L. Giorgi, M. Micheloni and P. Paoli, *Coord. Chem. Rev*., 2016, *310*, 41-79.
7. E. Wexselblatt, E. Yavin and D. Gibson. *Inorg. Chim. Acta*, 2012, *393*, 75-83.
8. F. Trudu, F. Amato, P. Vanhara, T. Pivetta, E.M. Pena-Mendez and J. Havel, *J. Appl. Biomed*., 2015, *13*, 79-103.
9. S. Dasari and P. B. Tchounwou, *Eur. J. Pharmacol*. 2014, *740*, 364-378.
10. L. B. Townsend and G. R. Revankar, *Chem. Rev*., 1970, *70*, 389-438.
11. X. F. He, C. M. Vogels and A. Decken and S. A. Westcott, *Polyhedron*, 2004, *23*, 155-160.
12. M. Z. Wisniewski, T. Glowiak, A. Opolski and J. Wietrzyk, *Met. Based Drugs*, 2001, *8*, 189-194.
13. S. Mylonas, A. Valavanidis, K. Dimitropoulos, M. Polissiou, A. S. Tsiftsoglou and I. S. Vizirianakis, *J. Inorg. Biochem*., 1988, *34*, 265-275.
14. J. S. Casas, A. Castiñeiras, E. García-Martínez, Y. Parajó, M. L. Pérez-Parallé, A. Sánchez-González and J. Sordo, *Z. Inorg. Allg. Chem*., 2005, *631*, 2258-2264.
15. D. Qui, Y. Guo, H. Wang, X. Bao, Y. Feng, Q. Huang, J. Zeng and G. Qiu. *Inorg. Chem. Commun*., 2011, *14*, 1520-1524.
16. N.T.A. Ghani and A.M. Mansour, *Inorg. Chim. Acta*, 2011, *373*, 249-258.

17. F. Gümüş, A. B. Demirci, T. Özden, H. Eroğlu and N. Diril, *Die Pharmazie*, 2003, *58*, 303-307.
18. F. Gümüş, İ. Pamuk, T. Özden, S. Yıldız, N. Diril, E. Öksüzoğlu, S. Gür and A. Özkul, *J. Inorg. Biochem*., 2003, *94*, 255-262.
19. F. Gümüş F, Ö. Algül, G. Eren, H. Eroğlu, N. Diril, S. Gür and A. Özkul, *Eur. J. Med. Chem*., 2003, *38*, 473-480.
20. M. Gökçe, S. Utku, S. Gür, A. Özkul and F. Gümüş, *Eur. J. Med. Chem*., 2005, *40*, 135-141.
21. S. Utku, F. Gümüş, S. Gür and A. Özkul, *Turk. J. Chem*., 2007, *31*, 503-514.
22. F. Gümüş, G. Eren, L. Açık, A. Çelebi, F. Öztürk, Ş. Yılmaz, R.I. Sağkan, S. Gür, A. Özkul, A. Elmalı and Y. Elerman, *J. Med. Chem*., 2009, *52*, 1345-1357.
23. S. Utku, M. Topal, A. Döğen and M.S. Serin, *Turk. J. Chem*., 2010, *34*, 427-436.
24. S. Utku, F. Gümüş, S. Tezcan, M.S. Serin and A. Özkul, *J. Enzym Inhib. Med. Chem*., 2010, *25*, 502-508.
25. S. Utku, A.B. Özçelik, F. Gümüş, Ş. Yılmaz, T. Arsoy, L. Açık and A. Çelebi Keskin, J. *Pharm. Pharmacol*., 2014, *66*, 1593-1605.
26. Ç. Boğatarkan, S. Utku and L. Açık, *Rev. Roum. Chim*., 2015, *60*, 59-64.
27. A. B. Özçelik, F. Gümüş, R.I. Sağkan and U. Musabak, *Z. Naturforsch*., 2015, *70*, 243-250.
28. S. Utku, F. Gümüş, T. Karaoğlu and A. Özkul, *J. Fac. Pharm. Ankara*, 2007, *36*, 21-30.
29. S. Utku, T. Karaoğlu and F. Gümüş, *Fabad J. Pharm. Sci*., 2008, *33*, 16-21.
30. G. Eren, F. Gümüş and Ş. Yılmaz, *Fabad J. Pharm. Sci*., 2011, *36*, 69-73.
31. A. B. Özçelik, S. Utku, F. Gümüş, A. Çelebi Keskin, L. Açık, Ş. Yılmaz and A. Özgüngör, *J. Enzym Inhib. Med. Chem*., 2012, *27*, 413-418.
32. M. A. Phillips. *J. Chem. Soc*., 1928, 2393-2399.
33. G. Aridoss and K. K. Laali, *Eur. J. Org. Chem*., 2011, *15*, 2827-2835.
34. L. Wang, J. Sheng, H.Tian and C. Qian, *Synth. Commun*., 2004, *34*, 4265-4272.
35. Z. H. Zhang, J. J. Li, Y. Z. Gao and Y. H. Liu, *J. Heterocyclic Chem*., 2007, *44*, 1509-1512.
36. S. Khaksar, A. Heydari, M. Tajbakhsh and S. M. Vahdat, *J. Fluor. Chem*., 2010, *131*, 1377-1381.
37. B. Lippert, *Prog. Inorg. Chem*., 1989, *37*, 1-94.
38. H. Tayyem, F. Huq, J. Q. Yu, P. Beale and K. Fisher. *Chem. Med. Chem*., 2008, *3*, 145-151.
39. H. Cheng, F. Huq, P. Beale and K. Fisher, *Eur. J. Chem*., 2006, *41*, 896-903.
38. L. Fang, S. Gou, J. Zhao, Y. Sun and L. Cheng, *Eur. J. Med. Chem*., 2013, *69*, 842-847.
39. L. Amable, *Pharmacol Res*., 2016, *106*, 27-36.
40. L. Messori, L. Cubo, C. Gabbiani, A. Alvarez-Valdes, E. Michelucci, G. Pieraccini, C. Rios-Luci, L. G. Leon, J. M. Padron, C. Navarro-Ranninger, A. Casini and A.G. Quiroga, *Inorg Chem*., 2012, *51*, 1717-1726.
41. T. Paro, M.A. Medrano, L. Cubo, S. Munoz-Galván, A. Carnero, C. Navarro-Runninger and A.G. Quiroga, *J. Inorg. Biochem*., 2013, *127*, 182-187.
42. N. Stellwagen, "Nucleic Acid Electrophoresis", Springer, New York, 1998, p. 1-53.