SYNTHESIS, CHARACTERIZATION AND pBR322 PLASMID DNA INTERACTION OF PLATINUM(II) COMPLEXES WITH IMIDAZOLE AND 2-PHENYLIMIDAZOLE AS CARRIER LIGANDS

Çağla BOĞATARKAN,[a] Semra UTKU[a,*] and Leyla AÇIK[b]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mersin University, Mersin, Turkey
[b] Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey

A series of platinum(II) complexes involving a physiologically active imidazole (Im) and 2-phenylimidazole (Pim) as carrier ligands of the types $[Pt(Im)_2X_2]$ and $[Pt(Pim)_2X_2]$ (X= -Cl or -I) were synthesized and characterized by their elemental analyses, IR, ^{1}H NMR and ESI-LC/MS. The plasmid DNA interactions of the platinum compounds were also investigated using Agarose Gel Electrophoresis method. According to plasmid DNA interaction studies, synthesized complexes modified the tertiary structure of pBR322 plasmid DNA.

R= -H **C1**
R= -C$_6$H$_5$ **C2**
R= -H **C3**
R= -C$_6$H$_5$ **C4**
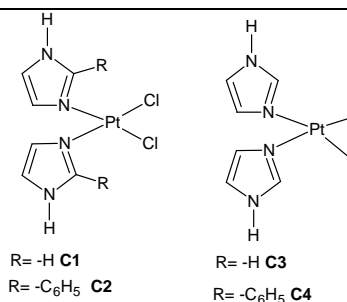
INTRODUCTION

Since the discovery that cisplatin (*cis*-diamminedichloroplatinum(II)) promotes cancer cell death by binding to DNA, thousands of platinum complexes have been synthesized and evaluated in the last 30 years.[1,2] Among these complexes, only carboplatin and oxaliplatin have received worldwide approval so far, nedaplatin, loboplatin and heptaplatin have gained regionally limited approval.[3,4]

Cisplatin and its close analogues have a broad range of activity in malignant disease and are used to treat many types of cancers, including testicular, ovarian, head and neck, colon, bladder, gastric, and lung cancer.[5] Broader applications of platinum-based anticancer drugs, however, exhibit two main disadvantages: intrinsic or acquired resistance and side effects including nephrotoxicity, ototoxicity, nausea and emetogenicity.[2,6] In an effort to overcome these problems, new platinum complexes are being developed that have broader spectra of activity, improved clinical efficacy, and reduced toxicity.[7,8]

It is well established that cisplatin binds to DNA through covalent bonding, known as platinum-DNA adducts, which deforms the DNA structure, preventing DNA replication and transcription, activate the apoptotic pathway, resulting in cell death.[9]

Cisplatin forms bifunctional adducts (interstrand and intrastrand cross-link), monofunctional adducts, as well as protein-cisplatin-DNA cross-links.[10] The formation of cisplatin-DNA cross links distorts the structure of DNA and interferes with normal transcription and/or DNA replication mechanisms. Intrastrand cross-links are probably the most toxic lesions because 85-90% of total cisplatin lesions account for 1,2-intrastrand ApG and GpG cross-links.[11]

The replacement of ammine groups can result in different structural and formational alterations in

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

target DNA, which may affect the character of biological effects of the analogues. It has been shown that increasing cytotoxicity of cisplatin analogues, in which NH_3 groups were replaced by more hydrophobic amine ligands, correlated with growing hydrophobicity of these analogues.[12]

One noteworthy approach in the design of new platinum anticancer drugs is to use physiologically active compounds as ligands. The imidazole ring is a physiologically active ligand, as a histidine moiety, function as ligands toward transition metal ions in a variety of biologically important molecules including iron-heme systems, vitamin B_{12} and its derivatives and several metalloproteins.[13,14] It also serves as a good ligand in various transition metal complexes. Several platinum complexes with imidazole as carrier ligands have been reported[15-21] and some of these complexes have been found to retain considerable efficacy against cisplatin-resistant ovary cell lines.[19-21]

In previous studies, with the consideration that variations in the chemical structure of the ammine groups of cisplatin might have a significant effect on the cytotoxic activity and toxicity of platinum complexes and with the aim of determining the role of the substituents on position 2 of the benzimidazole carrier ligands of Pt(II) and Pt(IV) complexes on cytotoxic properties, we synthesized some Pt(II) and Pt(IV) complexes with 2-substituted benzimidazole ligands.[22-32] It was determined that some of these platinum complexes have *in vitro* cytotoxic activities on RD,[23] HeLa,[26, 28-31] MCF-7,[24,26,28-31] HEp-2,[30] MDA-MB231[31] and SK-Hep1[32] cell lines.

In this paper, we report the synthesis and spectral characterization by their elemental analyses, Infrared (IR), [1]H Nuclear Magnetic Resonance (NMR) and electrospray ionization liquid chromatography/mass spectrometry (ESI-LC/MS) of four platinum(II) complexes of the type $[Pt(Im)_2Cl_2]$ **C1**, $[Pt(Pim)_2Cl_2]$ **C2**, $[Pt(Im)_2I_2]$ **C3** and $[Pt(Pim)_2I_2]$ **C4** where **Im** or **Pim** stands for imidazole or 2-phenylimidazole and a brief overview of their ability to modify the electrophoretic mobility of the form I and II bands of pBR322 plasmid DNA.

RESULTS AND DISCUSSION

The Pt(II) complexes **C1**, **C2** and **C3**, **C4** were synthesized by the reaction of **Im** or **Pim** as carrier ligands with K_2PtCl_4 or K_2PtI_4 respectively in ethanol/water solution as shown in Scheme. The melting points of all complexes were above

400 °C. Among the compounds synthesized, **C1** and **C3**, which were reported previously by other researchers,[15,18,21] were synthesized in this study as reported previously.

The complexes obtained were characterized by elemental analysis, spectral data, such as IR, ^{1}H NMR and ESI-LC/MS spectra. The elemental analyses data for each complex were in good agreement with the empirical formula proposed. IR spectrums of the complexes have shown some characteristic changes when compared to the free ligands.

In the IR spectrum **Im** and **Pim** show broad bands in the region of 3400-2500 cm^{-1} due to the imidazole N–H. All the complexes which have free **Im** and **Pim** N–H were exhibited N–H stretching bands 3124-2616 and 3053-2770 cm^{-1} respectively, sharper than those of the ligands due to breaking of tautomerism, indicating that **Im** and **Pim** N–H was not involved in the coordination.[21,33]

According to the kinetic trans effect,[34] the synthesis method used are expected to yield complexes with *cis* geometry. The υ (Pt–Cl) and υ (Pt–I) bands of the dichloro and diiodo complexes **C1-C4** should show at 320-330 and 195-183 cm^{-1} in the far-IR region of the complex's spectra[28,35] but the ν (Pt–Cl) and ν (Pt–I) stretching bands for synthesized complexes could not be measured on the spectrophotometer used.

The insolubility of the complexes in the other organic solvents made it necessary to record ^{1}H NMR spectra in dimethylsulfoxide (DMSO)-d_6. All ^{1}H NMR measurements were recorded immediately in order to avoid the ligand exchange reaction between the platinum complexes synthesized and DMSO-d_6. The ^{1}H NMR spectral data of the ligands and complexes are presented in the experimental section. Almost all signals were shifted upon complexation as the result of the electric field effect caused by complexation.

The spectra of the **C1-C4** complexes compared to those of the free ligands showed considerable difference. The large downfield shifts in the **Im** and **Pim** N-H signal in the spectra of the complexes with respect to the ligands are a result of an increase in the N-H acid character after platinum binding.[36] N-H chemical shifts of **C1-C4** complexes of vary between 13.51-12.93 ppm.

Both the retention times and the ESI-LC/MS spectra of the peaks in samples are evidence of the purity and the excepted structures of the synthesized compounds **C1-C4**. Because of three isotopes of Pt element, all the ESI-LC/MS spectra of the platinum complexes were found with three protonated ion isotopic peaks.[37]

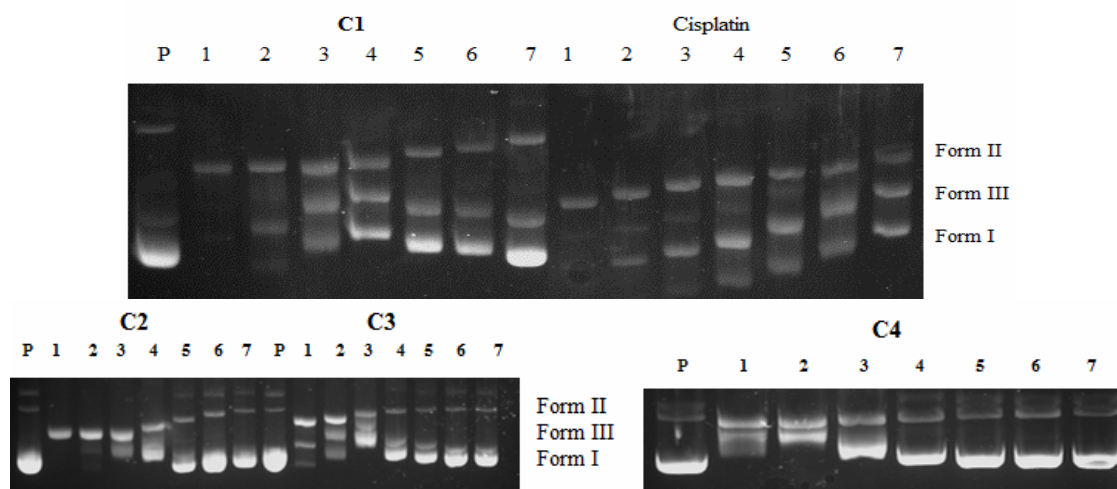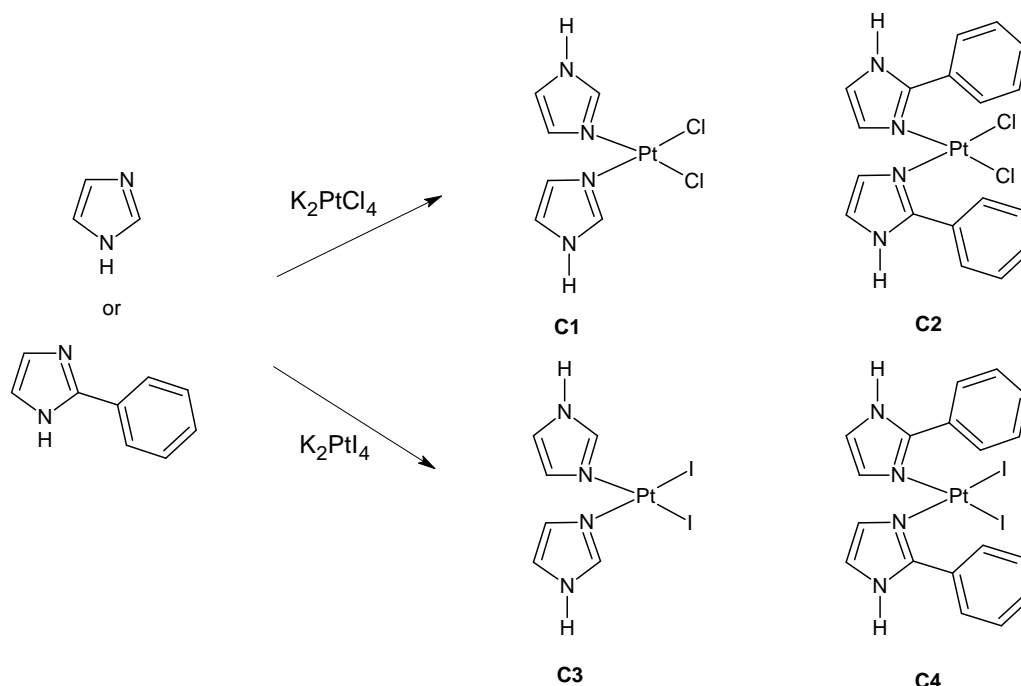Scheme 1– Synthesis of platinum(II) complexes **C1-C4**.

Fig. 1 – Modification of gel electrophoretic mobility of pBR322 plasmid DNA when incubated with various concentrations of complexes **C1-C4** and cisplatin. Concentrations (in μM) are as follows: (P) untreated pBR322 plasmid DNA; for complexes **C1-C4** 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 and the bottom bands correspond to form II (open circular) and form I (covalently closed circular) plasmids, respectively. Roman numerals I, II, and III indicate form I (covalently closed circular), form II (open circular), and form III (linear) plasmids, respectively.

The effect of the platinum(II) complexes **C1-C4** and cisplatin binding on pBR322 plasmid DNA tertiary structure were evaluated by their ability to alter the electrophoretic mobility of supercoiling of closed circular pBR322 plasmid DNA on agarose gels. When circular pBR322 plasmid DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact covalently closed circular form I. If scission occurs on one strand, the supercoiled form will relax to generate a slower-moving single nicked circular form II. If

both strands are cleaved, doubly nicked linear form III that migrates between form I and form II will be generated.[38]

Figure gives the electrophoretograms applying to the interaction of pBR322 plasmid DNA after incubation with **C1-C4** and cisplatin in a range of concentrations from 160 to 2.5 μM at 37 °C for 24 h. For comparison, cisplatin and untreated pBR322 plasmid DNA were used as positive and negative controls, respectively.

When pBR322 plasmid DNA was incubated with cisplatin increased from 2.5 to 160 µM concentration, an increase in mobility for form I and II bands were observed, and only one band corresponding to form II was determined at 160 µM concentration. Besides, the intensity of the form I band (compared with that of the untreated DNA, P) decreased with the increase in concentrations of cisplatin. The behavior of the gel electrophoretic mobility of both forms of pBR322 plasmid DNA–cisplatin adducts are consistent with previous reports.[30,39]

When plasmid DNA was interacted with decreasing concentration of **C1** the intensity and the mobility of form I band changed. The intensity and mobility of form I band decreased with the increase in concentration of **C1**. While the mobility of form II increased with the increase in concentration of **C1.** A faint band of form I and form II was observed at the 160 µM concentration. This can partially be attributed to the unwinding of supercoiled DNA to open circular DNA, demonstrating the binding between DNA and the platinum complexes.[40]

In case of **C2-C3** complexes, for **C2**, only single band form III band was observed at 160 and 80 µM concentrations. The mobility of form II was increased with the increasing concentrations of compound while form I band slightly decreased. Form I, II and III bands were also observed at three high concentrations of **C3** complex-pBR322 plasmid DNA interaction. The mobility of form I decreased, while form II increased.

In case of **C4**, the mobility of form I was sharply decreased but not for form II. However, the intensity of form I decreased, form II and III bands were observed at two concentration of 160 and 80 µM.

The antitumor platinum anticancer compounds bind covalently to DNA with concomitant bending and local unwinding of the double helix. It is generally accepted now that the antineoplastic activity of the drug is based on its interaction with cellular DNA leading to the formation of various types of adducts. If the cell cannot remove the damage, then it dies by one of several pathways.[41,42]

In general one noteworthy approach in the design of platinum anticancer drugs is to use physiologically active compounds as ligands. For this purpose, in this study we synthesized and characterized of **C1-C4** complexes which have physiologically active imidazole (Im) and 2-phenylimidazole (Pim) as the carrier ligands. The nature of interaction with pBR322 plasmid DNA has also been studied. The gel electrophoresis results show that **C1-C4** have been able to cause conformational changes in DNA and DNA damage at higher concentrations. This is believed to be due to covalent interstrand binding of the compound with DNA. Detailed studies on anticancer activity of the synthesized compounds **C1-C4** will be reported in a separate paper.

EXPERIMENTAL

Chemistry

All chemicals and solvents used in the synthesis were purchased from Merck or Aldrich. IR spectra were obtained using a 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 performed with LECO 932 CHNS analyzer and ^{1}H NMR spectra were recorded in DMSO-d[6] (Merck) on a Varian Mercury 400 MHz FT-NMR spectrometer using tetramethylsilane as the internal standard and ESI-LC/MS spectra were taken on a Water Micromass ZQ connected with Waters Alliance HPLC, using ESI (+) method at Central Laboratuvary of the Faculty of Pharmacy, Ankara University (Ankara, Turkey). All chemical shifts were recorded as δ (ppm). Thin-layer chromatography was performed on pre-coated aluminium plates (Merck) Silica Gel 60 F_{254}. Plates were visualized by ultraviolet light, Dragendorff reagent or iodine vapour.

General procedure for the synthesis of *[dichloro-bis(imidazole) platinum(II)]* *[Pt(Im)₂Cl₂]* (**C1**) and *[dichloro-bis(2-phenylimidazole)platinum(II)]* *[Pt(Pim)₂Cl₂]* (**C2**)

To a stirred aqueous solution of K_2PtCl_4 (1.204 mmol) was added a solution of imidazole (**Im**) or 2-phenylimidazole (**Pim**) (2.408 mmol) in ethanol–water mixture (8:12 mL) dropwise over 2 hours at room temperature. The pH was adjusted to ~7 and kept constant with the addition of 0.1 M NaHCO$_3$. The reaction mixture protected from light was heated at 60 °C for 2 days. After that time the mixture was cooled to room temperature. The resulting crude precipitate was filtered off and purified by repeated washing with small portions of water, ethanol, and diethyl ether and dried in vacuo.

[dichloro-bis(imidazole)platinum(II)] *[Pt(Im)₂Cl₂]* (**C1**)
Yield: 54%, IR (ATR) υ (cm⁻¹): 3243, 1672, 1542 ^{1}H NMR (400 MHz, DMSO-d₆): δ 13.51 (s, 2H, 2x N-H, exchangeable with D₂O), 8.30 (s, 2H, 2x ArH), 7.40 (s, 2H, 2x ArH), 7.09 (s, 2H, 2x ArH). For $C_6H_8Cl_2N_4Pt$ calculated: C 17.92, H 2.01, N 13.93; found: C 17.76, H 2.26, N 14.09. MS (ESI +) *m/z*: calculated for [M+Na]+ 425.13; [M+H] 403.15; [M-Cl]+ 366.69; found 425.70, 403.70, 366.30.

[dichloro-bis(2-phenylimidazole)platinum(II)] *[Pt(Pim)₂Cl₂]* (**C2**)
Yield: 57.50%, IR (ATR) υ (cm⁻¹): 3222, 2888, 1607, 1566. ^{1}H NMR (400 MHz, DMSO-d₆): δ 13.13 (s, 2H, 2x N-H, exchangeable with D₂O), 8.67-7.99 (m, 4H, 2x ArH), 7.65-7.22 (m, 10H, 2x ArH). For $C_{18}H_{16}Cl_2N_4Pt$ calculated: C 39.00, H 2.91, N 10.11; found: C 38.77, H 3.25, N, 9.94. MS (ESI +) *m/z*: calculated for [M+2Na]+ 600.31, [M-H]+ 553.32; found 600.30, 553.00.

General procedure for the synthesis *of [diiodo-bis(imidazole)platinum(II)] [Pt(Im)₂I₂]* (**C3**) and *[diiodo-bis(2-phenylimidazole)platinum(II)] [Pt(Pim)₂I₂]* (**C4**)

K_2PtCl_4 (1.204 mmol) and KI (4.80 mmol) were dissolved in water (15 mL) and stirred at 60 °C for 45 minutes. Then a solution of **Im** or **Pim** (2.408 mmol) in ethanol–water mixture (6:10 mL) was added dropwise over 2 hours at room temperature to the resulting K_2PtI_4. The reaction mixture, protected from light, was heated at 50 °C for 2 days. The resulting crude yellowish precipitate was filtered off and purified by repeated washing with small portions of water, ethanol, and diethyl ether and dried in vacuo.

[Diiodo-bis(imidazole)platinum(II)] [Pt(Im)₂I₂] (**C3**)
Yield: 16.64%, IR (ATR) υ (cm⁻¹): 3277, 2888, 1655, 1540.
¹H NMR (400 MHz, DMSO-d₆) δ 12.93 (broad s, 2H, 2x N-H, exchangeable with D_2O), 8.04 (s, 2H, 2x ArH), 7.23 (s, 2H, 2x ArH), 6.86 (s, 2H, 2x ArH). Anal. Calcd. for $C_6H_8I_2N_4Pt$: C 12.32, H 1.38, N 9.58; found: C 13.44, H 1.85; N 8.71. MS (ESI +) *m/z*: calculated for [M+Na]+ 608.03; [M+H] 586.05; found 608.29, 586.91.

[Diiodo-bis(2-phenylimidazole)platinum(II)] [Pt(Pim)₂I₂] (**C4**)
Yield: 29.60%, IR (ATR) υ (cm⁻¹): 3235, 2889, 1610, 1565.
¹H NMR (400 MHz, DMSO-d₆) δ 13.18 (broad s, 2H, 2x N-H, exchangeable with D_2O), 8.67-7.93 (m, 4H, 2x ArH), 7.56-7.32 (m, 10 H, 2x ArH). Anal. Calcd. for $C_{18}H_8I_{16}N_4Pt$: C, 29.32; H, 2.19; N, 7.60; found: C 30.55, H 3.06, N, 7.31. MS (ESI +) *m/z*: [M+Na+H]+ 761.23, [M+] 737.23, [M—I+H]+ 611.34; found 761.50, 737.50, 611.70.

IR and ¹H NMR spectral data of imidazole (Im) and 2-phenylimidazole (Pim) ligands

Imidazole (Im): IR (ATR) υ (cm⁻¹): 3124-2616 (N-H, =C-H), 1670-1542 (C=N, C=C). ¹H NMR (400 MHz, DMSO-d₆): δ, 12.22 (broad s, 1H, N-H), 7.69 (s, 1H, ArH), 7.05 (s, 2H, ArH).

2-Phenylimidazole (Pim): IR (ATR) υ (cm⁻¹): 3053-2770 (N-H, =C-H), 1680-1565 (C=N, C=C). ¹H NMR (400 MHz, DMSO-d₆): δ, 12.52 (broad s, 1H, N-H), 7.95-7.93 (d, 2H, ArH), 7.45-7.42 (m, 2H, ArH), 7.34-7.14 (m, 3H, ArH).

Studies of interaction with pBR322 plasmid DNA

The cisplatin used as reference compound, pBR322 plasmid DNA, ethidium bromide, and agarose were purchased from Sigma. The interaction of platinum(II) complexes **C1-C4**, and cisplatin with pBR322 plasmid DNA was studied by agarose gel electrophoresis.[43] Stock solutions to the tested complexes in dimethylformamide (DMF) were prepared and used within 1 h. The final amount of DMF never exceeded 0.1%. In brief, 40 μL aliquots of increasing concentrations of the complexes, ranging from 2.5 to 160 μM, were added to 1 μL of plasmid DNA (concentration of 0.5 μg/mL) in a buffer solution containing TE (10 mM Tris-HCl, 0.1 mM EDTA, pH=7.4). The samples were incubated at 37 °C for 24 h in the dark, and then 10 μL aliquots of drug-DNA mixtures were mixed with loading buffer (0.1% bromophenol blue, 0.1% xylene cyanol) and loaded into 1% agarose gel with or without ethidium bromide. Electrophoresis was carried out under TAE buffer (0.05 M Tris base, 0.05 M glacial acetic acid, 1mM EDTA, pH=8.0) for 5 h at 40 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 gel was then viewed with a transilluminator and the image was captured with a video camera (GelDoc-It Imaging System, UVP) as a TIFF file. The experiments were repeated three times.

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