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Synthesis, characterization, cytotoxicity, apoptotic inducing activity, cellular uptake, interaction of DNA binding and antioxidant activity studies of ruthenium(II) complexes

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ABSTRACT

Two new ligands APIP, HAPIP and their relative ruthenium(II) complexes $[Ru(bpy)_2(APIP)](ClO_4)_2$ **1** and $[Ru(bpy)_2(HAPIP)](ClO_4)_2$ **2** have been synthesized and characterized. The DNA binding constants for complexes **1** and **2** have been determined to be 6.08 (±0.29) × 10^4 M^{-1} and 1.78 (±0.30) × 10^5 M^{-1}. The results suggest that these complexes intercalate between the base pairs of DNA. The cytotoxicity of complexes has been evaluated by MTT assay. The apoptosis assay was carried out with AO/EB staining methods. The cellular uptake was observed under fluorescence microscopy. The studies on the mechanism of photocleavage demonstrate that superoxide anion radical ($O_2^{\cdot -}$) and singlet oxygen ($^1O_2$) may play an important role. The antioxidant activity against hydroxyl radical (·OH) was also studied.

1. Introduction

In recent years, many researchers have focused on the studies of interaction of small molecules with DNA [1–7]. DNA is generally the primary intracellular target of anticancer drugs, so the interaction between small molecules and DNA can cause DNA damage in cancer cells, blocking the division of cancer cells, and resulting in cell death [8,9]. The studies on the interaction of transition metal complexes with DNA continue to attract the attention of researcher due to their importance in design and development of synthetic restriction enzymes, chemotherapeutic drugs and DNA foot printing agents, DNA cleavage agents and DNA "molecular light switch" [10–14]. Generally, small molecule can interact with DNA through the three non-covalent modes: groove binding, intercalation, and external static electronic effects. The studies on the DNA-binding behaviors of ruthenium complexes have attracted great attention, and some of them exhibit interesting properties. However, little is known about their in vitro behaviors. Only a few studies have focused on their intracellular accumulation and antiproliferative properties [15–19]. These studies found some complexes to possess excellent antitumor activity and might be as potential candidate for drugs. In fact, the activity of many anticancer, antimalarial and antibacterical agents finds its origin in intercalative interactions with DNA [20]. In this study, we report the synthesis, characterization, DNA-binding, cytotoxicity, apoptosis, cellular uptake and antioxidant activity of two new ruthenium(II) polypyridyl complexes $[Ru(bpy)_2(APIP)](ClO_4)_2$ **1** (bpy = 2,2′-bipyridine, APIP = 2-(2-aminophenyl)imidazo[4,5-f][1,10]phenanthroline) and $[Ru(bpy)_2(HAPIP)](ClO_4)_2$ **2** (HAPIP = 2-(2-hydroxyl-5-aminophenyl)-imidazo[4,5-f][1,10]phenanthroline, Scheme 1). The DNA-binding behaviors were studied by spectroscopic titration, viscosity measurements, thermal denaturation and photocleavage. The spectroscopic titration and viscosity changes of calf thymus DNA (CT DNA) show these complexes interact with DNA through intercalative mode. The cytotoxicity of complexes has been evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide) assay. The apoptosis of BEL-7402 cells induced by Ru(II) complexes was investigated. The retardation assay of pGL 3 plasmid DNA by

Abbreviations: APIP, 2-(2-aminophenyl)imidazo[4,5-*f*][1,10]phenanthroline; HAPIP, 2-(2-hydroxyl-5-aminophenyl)imidazo[4,5-f][1,10]phenanthroline; CT DNA, calf thymus DNA; bpy, 2,2′-bipyridine; AO, acridine orange; EB, ethidium bromide; DMSO, dimethylsulfoxide; RPMI, Roswell Park Memorial Institute; MLCT, metal to ligand charge transfer; Tris, tris(hydroxymethyl)aminomethane; ES-MS, electrospray mass spectroscopy; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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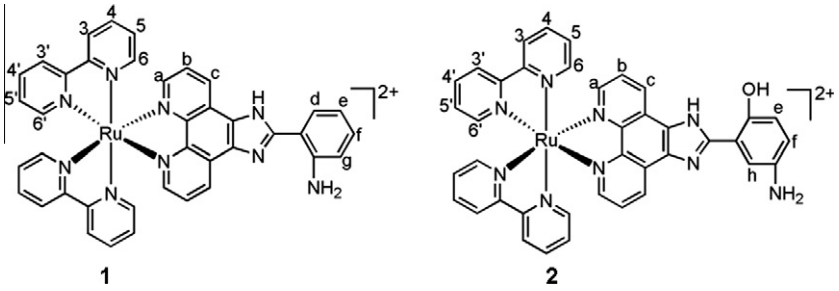

Scheme 1. The structure of complexes.

complexes **1** and **2** was also explored. The cellular uptake shows that complexes can easily enter into the cytoplasm and accumulate in the nuclei. The antioxidant activity of ligands and complexes was performed by hydroxyl radical scavenging method. The studies on the mechanism of photocleavage reveal that singlet oxygen ($^1O_2$) and superoxide anion radical ($O_2^{\cdot-}$) may play an important role.

2. Experimental

2.1. Materials and methods

Calf thymus DNA (CT DNA) was obtained from the Sino-American Biotechnology Company. pBR 322 DNA was obtained from Shanghai Sangon Biological Engineering & Services Co., Ltd. Dimethyl sulfoxide (DMSO) and RPMI 1640 were purchased from Sigma. Cell lines of BEL-7402 (hepatocellular), HepG-2 (hepatocellular) and MCF-7 (breast cancer) were purchased from American Type Culture Collection, agarose and ethidium bromide were obtained from Aldrich. $RuCl_3{\cdot}xH_2O$ was purchased from Kunming Institution of Precious Metals. 1,10-Phenanthroline was obtained from Guangzhou Chemical Reagent Factory. Doubly distilled water was used to prepare buffers (5 mM tris(hydroxymethylaminomethane)–HCl, 50 mM NaCl, pH 7.2). A solution of calf thymus DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of ca. 1.8–1.9:1, indicating that the DNA was sufficiently free of protein [21]. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [22].

Microanalysis (C, H, and N) was carried out with a Perkin-Elmer 240Q elemental analyzer. Fast atom bombardment (FAB) mass spectra were recorded on a VG ZAB-HS spectrometer in a 3-nitrobenzyl alcohol matrix. Electrospray mass spectra (ES-MS) were recorded on a LCQ system (Finnigan MAT, USA) using methanol as mobile phase. The spray voltage, tube lens offset, capillary voltage and capillary temperature were set at 4.50 kV, 30.00 V, 23.00 V and 200 °C, respectively, and the quoted *m/z* values are for the major peaks in the isotope distribution. ^{1}H NMR spectra were recorded on a Varian-500 spectrometer. All chemical shifts were given relative to tetramethylsilane (TMS). UV–Vis spectra were recorded on a Shimadzu UV-3101PC spectrophotometer at room temperature. Luminescence spectra were recorded on a Shimadzu RF-2000 spectrophotometer at room temperature.

2.2. Synthesis of ligands and complexes

2.2.1. 2-(2-Aminophenyl)imidazo[4,5-f][1,10]phenanthroline (APIP)

A mixture of 2-(2-nitrophenyl)imidazo[4,5-f][1,10]phenanthroline (0.171 g, 0.5 mmol) (*NPIP*) [23] was completely dissolved in ethanol (50 cm^3) with stirring for 1 h. Then the Pd/C (0.20 g, 10% Pd) and $NH_2NH_2{\cdot}H_2O$ (8 cm^3) were added in the above solution and refluxed for 6 h. The hot solution was filtered and evaporated to remove the solvent under reduced pressure. The red compound obtained was washed with cold ethanol and dried at 50 °C in vaccuo. Yield: 73%. *Anal.* Calc. for $C_{19}H_{13}N_5$: C, 73.30; H, 4.21; N, 22.49. Found: C, 73.16; H, 4.13; N, 22.38%. FAB–MS: *m/z* = 312 $[M+1]^+$. ^{1}H NMR (500 MHz, DMSO-d_6): 9.05 (d, 2H, H_c, *J* = 8.0 Hz), 8.98 (d, 2H, H_a, *J* = 8.2 Hz), 8.02–8.05 (m, 2H, H_b), 7.83 (d, 1H, H_g, *J* = 8.0 Hz), 7.18–7.35 (m, 1H, H_e), 6.92 (d, 1H, H_f, *J* = 8.0 Hz), 6.74 (t, 1H, H_d, *J* = 7.6 Hz), 3.36 (s, 2H, H_{NH2}).

2.2.2. 2-(2-Hydroxyl-5-aminophenyl)imidazo[4,5-f][1,10]phenanthroline (HAPIP)

This compound was prepared with the similar method described for APIP, with 2-(2-hydroxyl-5-nitrophenyl)imidazo[4,5-f][1,10]phenanthroline (0.179 g, 0.5 mmol) [24] in place of APIP. Yield: 72%. *Anal.* Calc. for $C_{19}H_{13}N_5O$: C, 69.72; H, 4.00; N, 21.39. Found: C, 69.60; H, 4.13; N, 21.52%. FAB–MS: *m/z* = 328 $[M+1]^+$. ^{1}H NMR (500 MHz, DMSO-d_6): 9.06 (d, 2H, H_c, *J* = 8.2 Hz), 8.98 (d, 2H, H_a, *J* = 7.5 Hz), 7.87 (dd, 2H, H_b, *J* = 8.0 Hz), 7.43 (d, 1H, H_d, *J* = 8.0 Hz), 6.84 (d, 1H, H_f, *J* = 8.6 Hz), 6.73–6.75 (m, 1H, H_e), 4.78 (s, 1H, H_{OH}), 3.39 (s, 2H, H_{NH2}).

2.2.3. Synthesis of $[Ru(bpy)_2(APIP)](ClO_4)_2$ (**1**)

$[Ru(bpy)_2(NPIP)](ClO_4)_2$ (0.485 g, 0.5 mmol) [23] was completely dissolved in acetonitrile. Then the ethanol of 45 cm^3, Pd/C (0.20 g, 10% Pd) and $NH_2NH_2{\cdot}H_2O$ (8 cm^3) were added in the above solution and refluxed under argon for 8 h to give a clear red solution. Upon cooling, a red precipitate was obtained by dropwise addition of saturated aqueous $NaClO_4$ solution. The crude product was purified by column chromatography on a neutral alumina with a mixture of CH_3CN-toluene (3:1, v/v) as eluant. The mainly red band was collected. The solvent was removed under reduced pressure and a red powder was obtained. Yield: 71%. *Anal.* Calc. for $C_{39}H_{29}N_9Cl_2O_8Ru$: C, 50.71; H, 3.16; N, 13.65. Found: C, 50.48; H, 3.28; N, 13.59%. ESI-MS [CH_3CN, *m/z*]: 723.4 ($[M-2ClO_4-H]^+$), 362.3 ($[M-2ClO_4]^{2+}$). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.91 (dd, 4H, $H_{3,3'}$, *J* = 8.4, *J* = 8.5 Hz), 8.80 (d, 2H, H_c, *J* = 8.2 Hz), 8.21–8.27 (m, 4H, $H_{4,4'}$), 8.14 (t, 2H, *J* = 7.5 Hz), 7.85 (d, 4H, $H_{6,6'}$, *J* = 6.0 Hz), 7.58–7.66 (m, 2H, H_b), 7.36 (t, 4H, $H_{5,5'}$, *J* = 7.6 Hz), 7.20 (t, 1H, H_d, *J* = 7.5 Hz), 6.98–7.03 (m, 1H, H_f), 6.92 (t, 1H, H_e, *J* = 7.8 Hz), 6.72 (t, 1H, H_g, *J* = 7.8 Hz), 3.37 (s, 2H, H_{NH2}).

2.2.4. Synthesis of $[Ru(bpy)_2(HAPIP)](ClO_4)_2$ (**2**)

A mixture of *cis*-$[Ru(bpy)_2Cl_2]{\cdot}2H_2O$ [25] (0.260 g, 0.5 mmol) and HAPIP (0.164 g, 0.5 mmol) in ethanol (30 cm^3) was refluxed under argon for 8 h to give a clear red solution. Upon cooling, a red precipitate was obtained by dropwise addition of saturated aqueous $NaClO_4$ solution. The crude product was purified by column chromatography on a neutral alumina with a mixture of CH_3CN-toluene (3:1, v/v) as eluant. The mainly red band was collected. The solvent was removed under reduced pressure and a red powder was obtained. Yield: 70%. *Anal.* Calc. for $C_{39}H_{29}N_9Cl_2O_9Ru$: C, 49.85; H, 3.11; N, 13.42. Found: C, 50.28; H, 3.54; N, 13.48%. ESI-MS [CH_3CN, *m/z*]: 739.7 ($[M-2ClO_4-H]^+$),