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Original article

Synthesis, characterization, interaction with DNA and cytotoxicity in vitro of the complexes [M(dmphen)(CO_3)]·H_2O [M = Pt(II), Pd(II)]

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ABSTRACT

The complexes [Pt(dmphen)CO_3]·H_2O (**1**), [Pd(dmphen)CO_3]·H_2O (**2**) (dmphen is 2,9-dimethyl-1,10-phenanthroline) have been synthesized and characterized. The binding of the complexes with FS-DNA was investigated by UV spectrum and fluorescence spectrum, showing that the complexes have the ability of interaction with DNA of intercalative mode. The intrinsic binding constant K of the complexes with FS-DNA is 1.8×10^5 M^{-1} (**1**) and 1.6×10^4 M^{-1} (**2**), respectively. Gel electrophoresis assay demonstrated the ability of the complexes to cleave the pBR 322 plasmid DNA. Evaluation of cytotoxic activity of the complexes against four different cancer cell lines proved that the complexes exhibited cytotoxic specificity and significant cancer cell inhibitory rate.

1. Introduction

Cisplatin, *cis*-diamminedichloroplatinum(II) (*cis*-DDP), is an anticancer drug widely used to treat a variety of tumors, especially those of the testes, ovaries, head [1], and neck [2]. It is accepted to mediate its cytotoxic action by forming adducts with deoxyribonucleic acid (DNA), which are believed to be capable of disrupting replication and transcription [3–5]. Though cisplatin remains the most effective [6] utilized antitumor drug in the world, the severe toxic side effects including nephrotoxicity, neurotoxicity, and emetogensise limit the dose that can be given to patients [7]. Therefore, several research groups have investigated with therapeutic activity of cisplatin and its analogues against cancer studying the relationship between the drug structure and biological activity, in order to optimize the active principle both in efficiency and safety [8].

The metal complexes containing multidentate aromatic ligands, with square planar N_4 or N_2O_2 coordination are among the most studied compounds regarding their DNA-binding affinity and their cytostatic properties [9–11]. Also, the bidentate nitrogen ligands 1,10-Phenanthroline (Phen) and its derivatives have been the subject of research interests in recent years [12–17] because of the potential application as DNA cleaving agents and nonradioactive nucleic acid probes.

It is reported dmphen used as ligand, when binding to platinum(II) in a square-planar coordinative environment, is severely distorted because of the steric hindrance of the methyl substituents of dmphen that are in proximity to the other groups coordinated to the metal. Which distortion can be easily described by the dihedral angle that the plane of the chelating moiety forms with the coordination plane. And the biological activity of dmphen was present by describing the fluorescence via interacting with the DNA pretreated with ethidium bromide (EB) [18]. The result represents that the compound can interact with DNA efficiently [19].

In the paper, we synthesized the complexes, [Pt(dmphen)-CO_3]·H_2O (**1**) and [Pd(dmphen)CO_3]·H_2O (**2**), because of both palladium(II) and platinum(II) adopting dsp^2 orbital hybridization, with similar coordination modes and chemical properties [20,21]. The complexes were characterized by elemental analyses, infrared spectrum (IR), nuclear magnetic resonance (NMR) spectroscopy. Studying the interaction of drugs with DNA is one of the most important aspects in biological investigations aimed at discovering and developing new types of antiproliferative agents [22–24]. Binding to DNA is usually accompanied by marked absorbance changes in the UV–vis frequency range and, sometimes, fluorescence emission too, due to excitation of charge transfer transitions [25]. DNA-binding studies of two complexes were researched by ultraviolet spectrum (UV), fluorescence spectroscopy and gel electrophoresis, so as to explore the mode and effect. The cleavage

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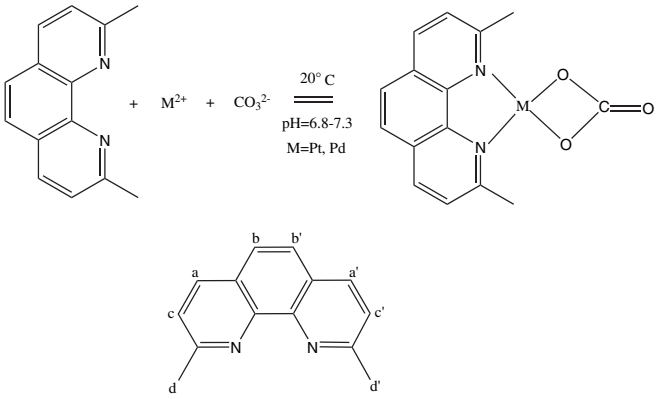

Scheme 1. Synthetic route of the complexes and the numbering scheme for ^{1}H NMR spectroscopy.

behavior toward pBR 322 DNA and cytotoxicity in vitro was also investigated.

2. Chemistry

2.1. Materials

All chemicals and reagents purchased were of reagent grade and used without further purification unless otherwise noted. Fish sperm DNA (FS-DNA) and pBR 322 plasmid DNA were purchased from Sinopharm Chemical Reagent Co., Ltd. The HeLa cells (HeLa human cervix epitheloid carcinoma cells), the Hep-G2 cells (human hepatocellular carcinoma cells), the KB cells (human oral epithelial carcinoma cells) and the AGZY-83a cells (human lung carcinoma cells) were obtained from American Type Culture Collection.

2.2. Preparation of the complexes

2.2.1. Synthesis of complex *1*

Complex **1** was synthesized according to the procedure as follows. 3.0 mmol of dmphen was dissolved in ethanol/DMSO (1:1, 2.0 ml) and diluted with water to 10.0 ml. Then, 3.0 mmol of $K_2[PtCl_4]$ dissolved in 10.0 ml of water was added dropwise. The obtain mixture was stirred for 14 h at room temperature, adjusted pH to 7.1 with 0.4 ml Na_2CO_3 (0.1 mol/l). After five days, red crystals were obtained at room temperature by slow evaporation of mixed solvent. The resulting crystals were filtered, washed with ethanol and dried in vacuo. Synthetic route and the numbering scheme for ^{1}H NMR spectroscopy are given in Scheme 1. Synthesis IR (neat) (cm^{-1}, s, strong; m, medium; w, weak): υ(O–H) 3446(s); υ(=C–H) 3049(s); υ(C–H) 2920(s); υ(C=O) 1625(s); υ(C=C) 1506(s), 1429(m); δ(C–H) 1377(m); υ(C–N) 1355 (m). ^{1}H NMR (DMSO-d_6. s, singlet; m, multiplet): δ 2.809 (s, 6H, 3Hd, 3Hd′), 7.68 (m, 2H, Hc, Hc′), 7.98 (s, 2H, Hb, Hb′), 8.41 (m, 2H, Ha, Ha′) ppm. Anal. Calc. (%) for $C_{15}H_{14}N_2O_4Pt$: C, 37.43; H, 2.93; N, 5.82; O, 13.29. Found (%): C, 37.36; H, 2.91; N, 5.79; O, 13.31.

Table 1
Crystal data and structure refinement for complex **1**.

Empirical formula	$C_{15}H_{14}N_2O_4Pt$
Formula weight	481.36
Temperature/*K*	293(2)
Wavelength/Å	0.71073
Crystal system	Monoclinic
Space group	C_2/c
Unit cell dimensions	
a/Å	14.525(3)
b/Å	27.458(6)
c/Å	13.662(3)
β/°	95.91(3)
Volume/Å^3	5420.1(19)
Z	8
Crystal size	0.14 × 0.12 × 0.10
θ Range for data collection/°	1.48–25.52
Index ranges	$-17 \le h \le 12$, $-32 \le k \le 33$, $-16 \le l \le 15$
Reflections collected	16707
Independent reflections (R_{int})	5024
Data/restraints/parameters	5024/0/404
S	1.1
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0461$, $wR_2 = 0.0951$
R indices (all data)	$R_1 = 0.0676$, $wR_2 = 0.1050$
Largest diffraction peak and hole/e Å^{-3}	1.951 and −1.76

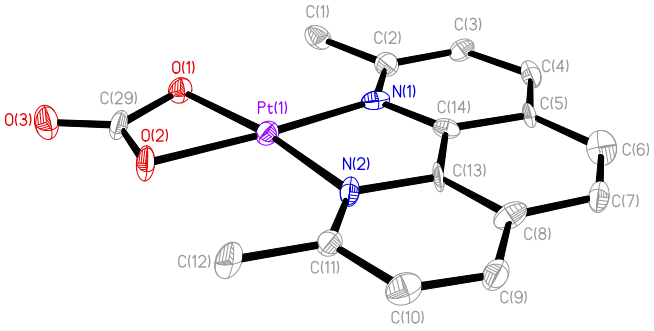

Fig. 1. ORTEP view of complex **1**.

2.2.2. Synthesis of complex ***2***

This complex was synthesized in an identical manner as that described for **1** with $K_2[PdCl_4]$ (3.0 mmol, 10.0 ml of water) in place of $K_2[PtCl_4]$. The product was obtained as a yellow powder. IR(neat) (cm^{-1}, s, strong; m, medium; w, weak): υ(O–H) 3444(s); υ(=C–H) 3057(m); υ(C–H) 2924(s); υ(C=O) 1621(m); υ(C=C) 1508(s), 1426(s); δ(C–H) 1379(m); υ(C–N) 1356(m). ^{1}H NMR (DMSO-d_6. s, singlet; m, multiplet): δ 2.788 (s, 6H, 3Hd, 3Hd′), 7.922 (m, 2H, Hc, Hc′), 8.241 (s, 2H, Hb, Hb′), 8.817 (m, 2H, Ha, Ha′) ppm. Anal. Calc. (%) for $C_{15}H_{14}N_2O_4Pd$: C, 45.88; H, 3.59; N, 7.13; O, 16.30. Found (%): C, 45.80; H, 3.57; N, 7.10; O, 16.35.

2.3. X-ray structure determination for the complex *1*

The crystal structure of complex **1** was determined by singe-crystal X-ray diffraction. A suitable single crystal of approximate dimension 0.14 × 0.12 × 0.10 mm was mounted in a glass fibre capillary. Data were collected on a Brucker Smart 1000 CCD X-ray single-crystal diffractometer with graphite-monochromated MoK_α radiation ($k = 0.71073$ Å) at 293(2) K in the range of $-17 \le h \le 12$, with the ω-scan technique. The structure was solved by direct methods and refined by means of the full-matrix least squares procedures with SHELXTL 97 systems [26,27]. Structure solution and refinement based on 5024 independent reflections with

Table 2
Selected bond lengths/Å and angles/°.

Pt(1)–O(2)	1.996(7)	O(2)–Pt(1)–N(1)	170.2(3)
Pt(1)–N(1)	2.013(9)	O(2)–Pt(1)–O(1)	64.4(3)
Pt(1)–O(1)	2.028(7)	N(1)–Pt(1)–O(1)	106.3(3)
Pt(1)–N(2)	2.047(8)	O(2)–Pt(1)–N(2)	107.2(3)
		N(1)–Pt(1)–N(2)	82.0(3)
		O(1)–Pt(1)–N(2)	171.5(3)