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Syntheses, Structures, Characterization, and Bioactivities of New Cd(II) Complex of 2,3-Pyrazinedicarboxylate with 1,10-Phenanthroline[1]

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Abstract—A novel complex $[Cd(Phen)_2(Pzdc)] \cdot 7.5(H_2O)$ (**I**), where Phen = 1,10-phenanthroline, Pzdc = 2,3-pyrazinedicarboxylic acid, has been synthesized under room temperature conditions and characterized by IR spectroscopy, elemental analysis, and X-ray single-crystal structure analysis (CIF file CCDC no. 1023407). Its mode of interaction with fish sperm DNA (FS-DNA) have been determined by fluorescence. Gel electrophoresis assay demonstrates the ability of complex **I** to cleave the HL-60 DNA. Furthermore, apoptosis assay shows treatment with complex results in morphological changes of cancer cell lines. The results induce apoptosis in JEKO cells.

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INTRODUCTION

Cancer continue to pose a very considerable health problems threat to significant parts of the population. Chemotherapeutics has become an important method in the treatment of cancer cells. Recently, there is a great interest on the binding of transition metal complexes with DNA, owing to their possible applications as new cancer therapeuticagents and their photochemical properties that make them potential probes of DNA structure and conformation [1–3]. Some authors produced evidence to the apoptosis promoting nature of Cd including its ability to induce DNA fragmentation and chromatin condensation in kidney, blood, and liver cells [4–6].

There has been considerable interest in the design and synthesis of complexes with carboxylate ligands because carboxylates can give rise to different coordination modes with metal ions and increase recognition of the metal's role in biological systems [7, 8]. The 2,3-pyrazinedicarboxylate acid (Pzdc) with a number of potential donor atoms commonly bound to transition metals by two carboxylic groups together with two pyrazine nitrogens. 1,10-Phenanthroline (Phen) can coordinate with transition metals and disturb functions of biological systems. Phen has long been used as a component of hemoglobin models with strong coordination ability and biological activity [9–11]. A novel coordination compound has been synthesized under solvent evaporation. In this paper, complex **I** was characterized by IR, elemental analysis, crystal structure determination, fluorescence, gel electrophoresis and apoptotic study.

EXPERIMENTAL

Materials and measurements. All chemicals were of reagent grade quality obtained from commercial sources and used without further purification unless otherwise noted. Elemental analyses (C, H, and N) were performed on a model Finnigan EA 1112 apparatus. Infrared spectroscopy using KBr pellets were performed on a Nicolet FT-IR 470 spectrophotometer. Fluorescence measurements were performed on a PerkinElmer LS55 fluorescence spectrofluorometer. Gel electrophoresis were performed on a JS-380A gel electrophoresis spectrometer.

Synthesis of complex I. Experiments were all reagent concentration 10 mmol/L, $Cd(NO_3)_2 \cdot 7.5H_2O$ aqueous solution (10 mL) and Pzdc solution (10 mL) mixing. With potassium hydroxide solution (0.1 mol/L) pH value of mixed solution will be adjusted to 4.78. After about 3 h of stirring, 10 mL of ethanol solution Phen was added. After about another 3 h of stirring, the solution was filtrated, and the filtrate was evaporated slowly in the air. Colourless crystals were obtained after 30 days.

For $C_{30}H_{34}N_6O_{11.5}Cd$			
anal. calcd, %:	C, 46.68;	H, 3.72;	N, 7.26.
Found, %:	C, 46.70;	H, 3.64;	N, 7.30.

IR spectrum (ν, cm^{-1}): 3077 s, 1671 s, 3498 s, 1496 s, 1516 m, 1310 m, 1629 w, 1356 w.

[1] The article is published in the original.

Table 1. Crystal data and refinement parameters for complex **I**

Parameter	Value
Formula weight	775.04
Crystal system	Monoclinic
Space group	$C2/c$
Unit cell dimensions:	
a, Å	24.436(2)
b, Å	15.8797(15)
c, Å	19.9150(18)
β, deg	124.6570(10)
V, Å^3	6356.6(10)
Z	4
ρ_{calcd}, mg/m^3	1.620
μ, mm^{-1}	0.760
F(000)	3168
θ	1.63–25.06
Limiting indices	$-27 \le h \le 29$, $-18 \le k \le 14$, $-23 \le l \le 23$
Reflections collected/unique	18225/5628
R_{int}	0.0726
Reflections with $I > 2\sigma(I)$	5628
Completeness, %	99.6
Goodness of fit on F^2	1.041
Number of parameters refined	469
Final R indices ($I > 2\sigma(I)$)	$R_1 = 0.0594$, $wR_2 = 0.1276$
R indices (all data)	$R_1 = 0.0946$, $wR_2 = 0.1432$
Residual electronic density (max/min), e Å^{-3}	0.888/–0.844

Table 2. Selected bond lengths and angles for complex **I**

Bond	d, Å	Bond	d, Å
Cd(1)–O(1)	2.267(4)	Cd(1)–N(4)	2.365(5)
Cd(1)–N(3)	2.312(5)	Cd(1)–N(5)	2.371(5)
Cd(1)–N(2)	2.345(5)	Cd(1)–N(1)	2.360(5)
Angle	ω, deg	Angle	ω, deg
O(1)Cd(1)N(3)	91.25(16)	O(1)Cd(1)N(5)	70.69(16)
O(1)Cd(1)N(2)	86.48(16)	N(3)Cd(1)N(5)	149.83(17)
N(3)Cd(1)N(2)	98.38(18)	N(2)Cd(1)N(5)	104.19(17)
O(1)Cd(1)N(1)	147.98(15)	N(1)Cd(1)N(5)	92.20(17)
N(3)Cd(1)N(1)	114.09(17)	O(1)Cd(1)N(4)	113.84(16)
N(2)Cd(1)N(1)	71.31(17)	N(3)Cd(1)N(4)	72.28(17)
N(1)Cd(1)N(4)	93.21(17)	N(2)Cd(1)N(4)	157.28(17)
N(4)Cd(1)N(5)	92.63(16)		

X-ray structure determination. Single-crystal data of complex **I** were collected at 273(2) K on a Rigaku XtaLAB mini X-ray single crystal diffractometer with MoK_α radiation ($\lambda = 0.71073$ Å) in the range of $1.63° < \theta < 25.06°$. The structure was solved by direct methods using SHELXS-97 [12, 13] and refined with SHELXL-97 [14]. All non-hydrogen atoms were determined with successive difference Fourier syntheses and refined by full-matrix least-squares on F^2 [15]. All hydrogens were located at the theoretical positions. Further details of the crystal data and refinement are shown in Table 1. Selected bond distances and bond angles are given in Table 2.

Supplementary material for structure **I** has been deposited with the Cambridge Crystallographic Data Centre (no. 1023407; deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

Fluorescence spectroscopic studies. The buffer solution was 50 mM Tris-HCl, pH 7.4, mixed with 10 mM NaCl. The FS-DNA was pretreated with EtBr for 2 h and then the complex and buffer were added into the DNA–EtBr system. The sample was incubated 2 h at room temperature before spectral measurements. For all fluorescence measurements, the entrance and exit slits were maintained at 10 nm. Fluo-

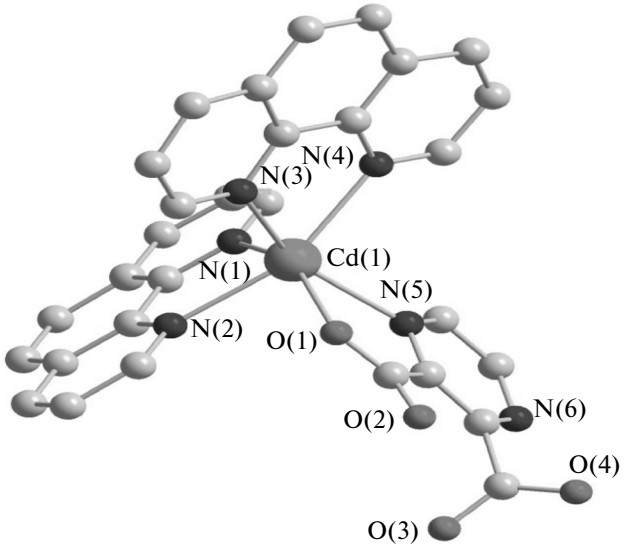

Fig. 1. The molecular structure of complex **I** (H atoms were omitted for clarity).

rescence measurement was done using 526 nm as the excitation wavelength and the emission range was set between 540 and 750 nm.

Cleavage of HL-60 DNA. In this experiment, HL-60 DNA (extracted by ourselves) was treated with complex **I** (dissolved in DMF) in Tris buffer (50 mM Tris–acetate, 18 μM NaCl buffer, pH 7.2) and the contents were incubated for 1 h at room temperature. The samples were electrophoresed for 1.5 h at 120 V on 0.85% agarose gel in Tris-acetate buffer. After electrophoresis, the gel was stained with 1 mg mL^{-1} EtBr and photographed under UV light [16].

Apoptosis assays by flow cytometry. The ability of complex **I** induce apoptosis is evaluated in JEKO cells line using Annexin V conjugated with FITC and propidium iodide (PI) counterstaining by flow cytometry. The JEKO cells in a suitable condition were seeded into a 6-well culture plates at 1×10^6 cells per well in a 3 mL culture medium and 12 h later the medium including the Cd(II) complex was given. After 12 h (24 h) incubation, cells were gathered, wash cells twice with cold phosphate-buffered saline and then resuspended cells in 1× Binging Buffer at a concentration of 1×10^6 cells/mL. Transfer 100 μL of the solution (1×10^5 cells) to a 5 mL culture tube. Futher, add 5 μL of FITC Annexin V and 5 μL PI. Gently vortex the cells were incubated in the dark at 25°C for 15 min. Add 400 μL of 1× Binding Buffer to each tube. Analyze by flow cytometry Accuri C6, USA, within 1 h.

RESULTS AND DISCUSSION

The crystal structure of complex **I** was determined by X-ray crystallography (Fig. 1). The Cd^{2+} ion in **I** is coordinated by a bidentate pzdc and two bidentate Phen. Distorted octahedral coordination geometry is comprised of N and carboxylate O from a doubly deprotonated bidentate Pzdc ligand (Cd(1)–N(5) 2.371(5), Cd(1)–N(1) 2.360(5) Å) and four N atoms from two Phen ligands (Cd(1)–N(1) 2.360(5), Cd(1)–N(2) 2.345(5), Cd(1)–N(3) 2.312(5), Cd(1)–N(4) 2.365(5) Å). The whole structure presents a three-dimensional network arrangement (Fig. 2).

Electronic absorption spectroscopy is an effective method to examine the binding mode of DNA with metal complexes [17]. The absorption spectra of complex **I** in the absence and presence of FS-DNA are given in Fig. 3. As the concentration is increased, changes in emission may arise from the restriction of interannular twisting between Phen and Pzdc. It implies that complex **I** interacts strongly with FS-DNA. According to the classical Stern–Volmer equation: $I_0/I = 1 + K_{sq}r$ [18], where I_0 and I represent the fluorescence intensities in the absence and presence of the complex, respectively, and r is the concentration ratio of complex to DNA. K_{sq} is a linear Stern–Volmer quenching constant dependent on the ratio of the bound concentration of EtBr to the concentration of DNA. The K_{sq} value is obtained as the slope of I_0/I versus r linear plot. The fluorescence quenching curves of DNA-bound EtBr by complex **I** are given in Fig. 4. The K_{sq} values for the complex are $K_{sq1} = 0.8653$. Such values of quenching constant suggest that the interaction of the complex with DNA is moderate intercalation.

In past research, pBR322 plasmid DNA or pUC19 DNA were used but do not originate from cell [19, 20]. However, we analyzed DNA strand breaks in HL-60 DNA treated the complex. As shown in Fig. 5, DNA

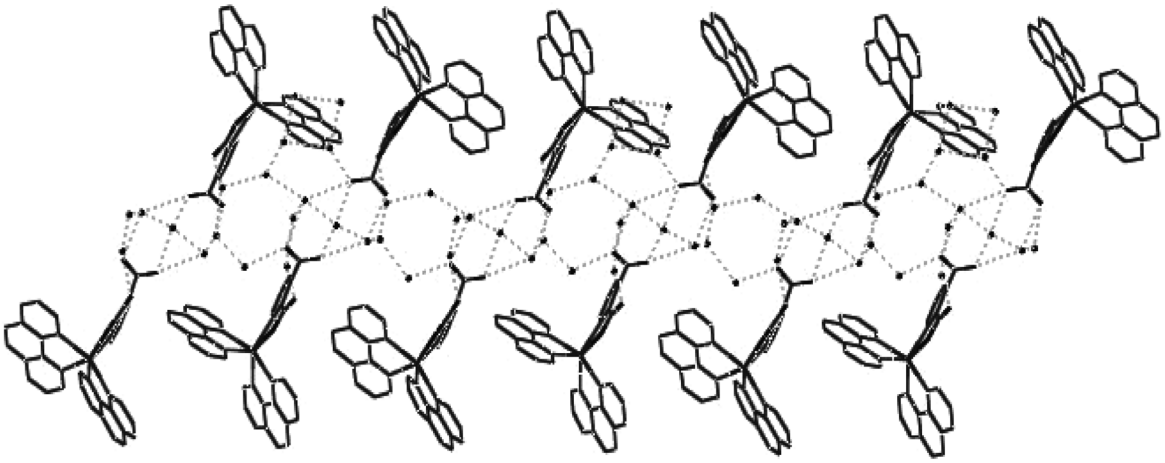

Fig. 2. Network structure of complex **I** based on hydrogen bonds (H atoms were omitted for clarity).

fragmentations with a characteristic laddering pattern were observed for the complex. When HL-60 DNA is conducted by electrophoresis, complex **I** was found to promote the cleavage of HL-60 DNA. The different DNA-cleavage efficiency of complex may be due to the different binding affinity of the complex to DNA. Electrophoretic mobility of the bands is found to decrease slightly with increasing concentration of the complex, which believed to be due to the intercalation of complex with DNA, thus increasing its molecular mass.

Apoptosis is the programmed cell death that controlls the development and homeostasis of multicellular organisms by elimination of aged, damaged, or mutated cells, which has been shown to be the key cellular event responsible for the anticancer activity of the metal anticancer drugs [21]. The interaction of JEKO cells with complex **I** was further discussed using an Annexin V-FITC/PI assay (Fig. 6). Complex **I** improved the apoptotic rate of the JEKO cells as compared with that of the untreated cells (control). When treated with complex **I** after 6, 12, and 18 h, the effects on JEKO cells shown for complex **I** with apoptotic populations of Annexin V-FITC/PI-cells) were 36.7 (6 h), 54.6 (12 h), and 66.4% (18 h). It can be concluded that the complex produce JEKO cells death through an apoptotic pathway.

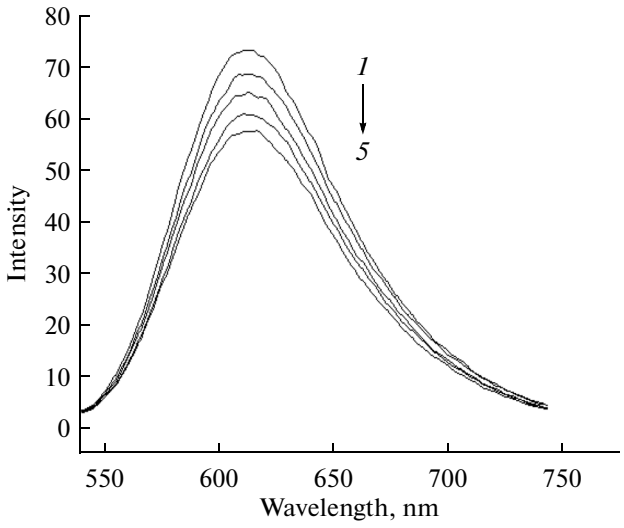

Fig. 3. Fluorescence spectra of the binding of EtBr to DNA in the absence (*1*) and presence of increasing amounts of the complexes: c_M = 2.5 (*2*), 3.75 (*3*), 6.25 (*4*), 10 μM (*5*). [EB] = 1.0 μM, [DNA] = 5.0 μM, λ_{ex} = 526 nm.

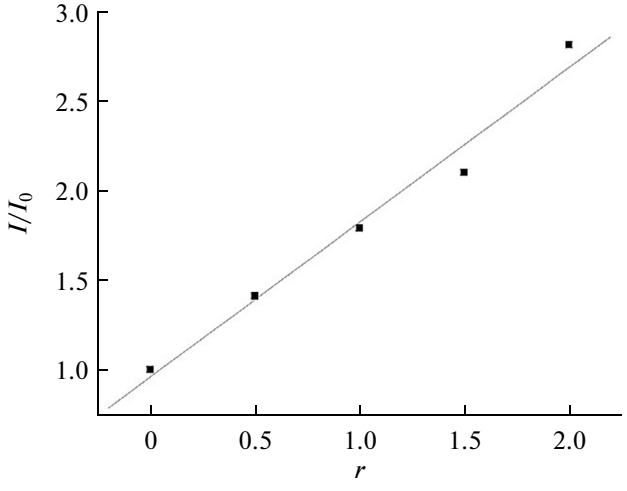

Fig. 4. Stern–Volmer quenching plots of complex **I** with the value of slope 0.8653.

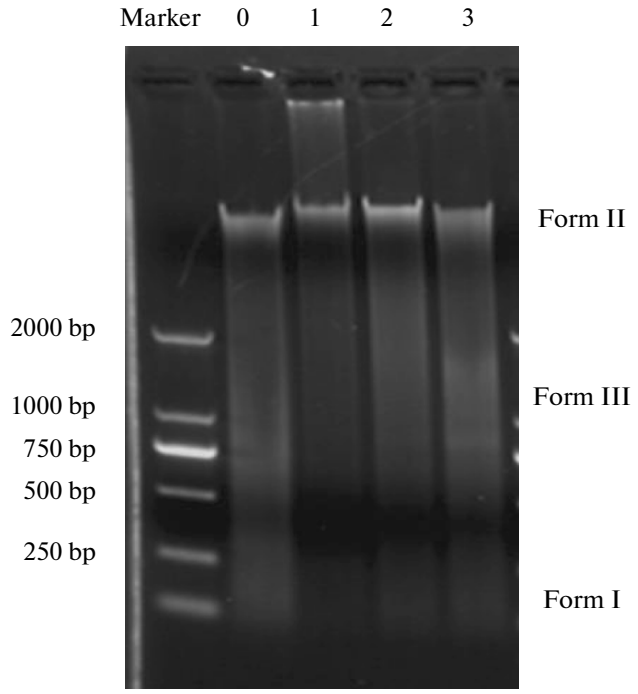

Fig. 5. DNA strand break in HL-60 DNA treated with complex **I** (line 0 is HL-60 DNA alone; lines 1–3 are HL-60 DNA with different concentration of complex **I**).

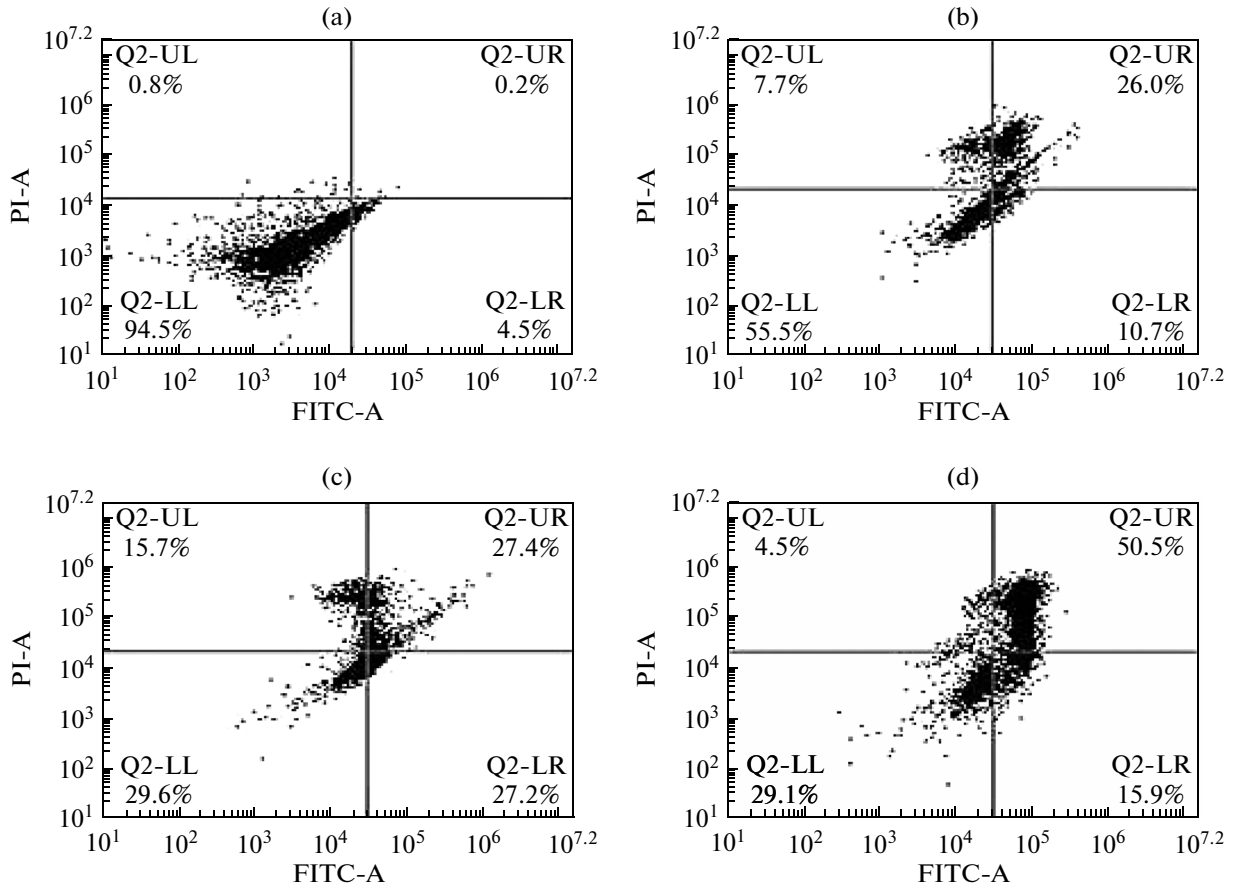

Fig. 6. The apoptotic rate of the JEKO cells cultured with complex **I**: 6 h (b), 12 h (c), 18 h (d) and untreated cells (a). $c_I = 1000$ μg/m. Dot plots show percentages representing the population of cells that are non-apoptotic (c), early apoptotic (d) or late apoptotic/necrotic (b).

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