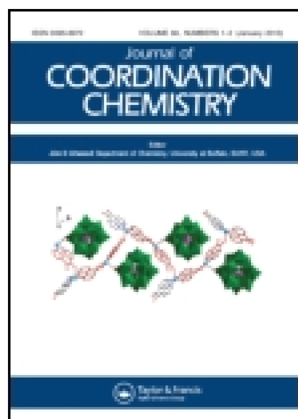
This article was downloaded by: [Renmin University of China]
On: 13 October 2013, At: 10:31
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gcoo20

Crystal structure, DNA interaction, and antiproliferative activity of the cobalt(II) complex of demethylcantharate and imidazole

Lin Qiu-Yue [a b] , Wang Yun-Yun [a b] , Feng Yun-Long [a b] , Yan Dong-Mei [c] , Wang Yan-Jun [b] & Zhang Fan [b]

[a] Zhejiang Key Laboratory for Reactive Chemistry on Solid Surfaces, Institute of Physical Chemistry, Zhejiang Normal University , Jinhua , Zhejiang 321004 , China

[b] College of Chemical and Life Science, Zhejiang Normal University , Jinhua , Zhejiang 321004 , China

[c] Institute of Materia Medica, Zhejiang Academy of Medical Sciences , Hangzhou , Zhejiang 310013 , China

Published online: 26 Feb 2011.

To cite this article: Lin Qiu-Yue , Wang Yun-Yun , Feng Yun-Long , Yan Dong-Mei , Wang Yan-Jun & Zhang Fan (2011) Crystal structure, DNA interaction, and antiproliferative activity of the cobalt(II) complex of demethylcantharate and imidazole, Journal of Coordination Chemistry, 64:5, 920-930, DOI: 10.1080/00958972.2011.557150

To link to this article: http://dx.doi.org/10.1080/00958972.2011.557150

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

Downloaded by [Renmin University of China] at 10:31 13 October 2013

Journal of Coordination Chemistry
Vol. 64, No. 5, 10 March 2011, 920–930

Taylor & Francis
Taylor & Francis Group

Downloaded by [Renmin University of China] at 10:31 13 October 2013

Crystal structure, DNA interaction, and antiproliferative activity of the cobalt(II) complex of demethylcantharate and imidazole

LIN QIU-YUE*†‡, WANG YUN-YUN†‡, FENG YUN-LONG†‡, YAN DONG-MEI§, WANG YAN-JUN‡ and ZHANG FAN‡

†Zhejiang Key Laboratory for Reactive Chemistry on Solid Surfaces, Institute of Physical Chemistry, Zhejiang Normal University, Jinhua, Zhejiang 321004, China
‡College of Chemical and Life Science, Zhejiang Normal University, Jinhua, Zhejiang 321004, China
§Institute of Materia Medica, Zhejiang Academy of Medical Sciences, Hangzhou, Zhejiang 310013, China

(*Received 23 August 2010; in final form 31 December 2010*)

A new cobalt(II) complex, $[Co(C_3H_4N_2)(C_8H_8O_5)(H_2O)_2]\cdot 2H_2O$, of demethylcantharate (7-oxabicyclo[2,2,1]heptane-2,3-dicarboxylate, $C_8H_8O_5$) with imidazole has been synthesized from cobalt chloride, demethylcantharidin (NCTD) and imidazole. The complex was characterized by elemental analysis, IR, and X-ray single crystal diffraction. The complex crystallized in the monoclinic crystal system and $P2_1/m$ space group with $a = 0.634790(10)$ nm, $b = 0.963030(10)$ nm, $c = 1.221770(10)$ nm, $\alpha = 90°$, $\beta = 95.9700(10)°$, $\gamma = 90°$, $V = 0.742844(15)\ \mathrm{nm}^3$, $M_r = 383.22$, $D_c = 1.713\ \mathrm{g\,cm^{-3}}$, $Z = 2$, $F(000) = 398$, $\mu = 1.206\ \mathrm{mm^{-1}}$, the final $R = 0.0291$, and $wR = 0.0837$ $[I > 2\sigma(I)]$. The interaction of the complex with deoxyribonucleic acid (DNA) was studied by electronic absorption spectra, fluorescence spectra, and viscosity measurements, which indicate that the complex binds to calf thymus DNA through a partially intercalative mode. The binding constant K_b for the complex was $2.62 \times 10^4\ \mathrm{L\,mol^{-1}}$. The antiproliferation activity test showed that the complex has high antiproliferative ability against human hepatoma cells SMMC7721 (with IC_{50} being $42.8 \pm 0.9\ \mu\mathrm{mol\,L^{-1}}$) and human lung cancer cells A549 (with IC_{50} being $65.1 \pm 3.2\ \mu\mathrm{mol\,L^{-1}}$). The inhibition rates of the complex are much higher than those of NCTD.

Keywords: Demethylcantharidin; Cobalt(II) complex; Crystal structure; DNA binding; Antiproliferative activity

1. Introduction

Demethylcantharidin (NCTD = 7-oxabicyclo[2,2,1]heptane-2,3-dicarboxylic acid anhydride) and disodium demethylcantharate (Na_2(DCA) = 7-oxabicyclo[2,2,1]heptane-2,3-dicarboxylate) are derivatives of cantharidin. *In vitro* experiments indicate they have a great inhibitive effect on common cancer cells while removing the side effects of cantharidin on the urinary system [1–3]. Meanwhile, complexes of demethylcantharate

*Corresponding author. Email: sky51@zjnu.cn

Journal of Coordination Chemistry
ISSN 0095-8972 print/ISSN 1029-0389 online © 2011 Taylor & Francis
DOI: 10.1080/00958972.2011.557150

Downloaded by [Renmin University of China] at 10:31 13 October 2013

(DCA^{2-}) have been reported [4–6] and have prominent antiproliferative ability. Imidazole, a planar *N*-heterocycle, is favorable to insert into the deoxyribonucleic acid (DNA) base pair. It is reputed as biocatalyst and biological ligand [7]. DNA bears heritage information and instructs the biological synthesis of proteins and enzymes in living cells. The mode of action between anti-cancer drugs with DNA on the molecular level is essential to find the mechanism of anti-cancer drugs. Several complexes of derivatives of imidazole with demethylcantharate have been synthesized and the binding to DNA has been studied [8, 9]. The results showed that binding ability and antiproliferative activity of the complexes of imidazole derivatives is better with DNA. Cobalt is an essential microelement in the human body, playing an important physiological role in the metabolism of iron, synthesis of hemoglobin, and maturation of hematid [10]. Thus, research on the synthesis and physiological activity of cobalt(II) complexes are significant. Recently, Satyanarayan *et al.* [11] reported DNA-binding and photocleavage studies of several polypyridine complexes of cobalt(III) [12]. Cobalt complexes of demethylcantharate and imidazole have been reported [13, 14]. However, no biological activities have been reported about cobalt complexes of demethylcantharate. A cobalt(II) complex of demethylcantharate with imidazole has been synthesized, single crystals obtained, interaction of the complex with DNA was studied by electronic absorption spectra, fluorescence spectra, and viscosity measurements and antiproliferative test was carried out.

2. Experimental

2.1. *Materials and apparatus*

All chemicals were obtained commercially and used without purification. NCTD ($C_8H_8O_4$) was purchased from Nanjing Zelang Medical Technological Co., imidazole ($C_3H_4N_2$) and cobalt chloride ($CoCl_2{\cdot}6H_2O$) from Shanghai Chemical Reagent Co. and calf thymus DNA (ct-DNA) was obtained from Huamei Co. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma corporation. Human hepatoma cells SMMC7721 and human lung cancer cells A549 were obtained from Shanghai Cell Bank of the Chinese Academy of Sciences. Solvents were all of analytic grade. Doubly distilled water was used in the experiments.

Elemental analyses of C, H, and N were carried out in a Vario EL III elemental analyzer. Infrared spectra were recorded as KBr pellets by using a NEXUS-670 FT-IR spectrometer. Diffraction intensities for the complex were collected at 23°C on a Bruker SMART APEX II CCD diffractometer. Fluorescence emission spectra were recorded with a Perkin-Elmer LS-55 spectrofluorometer. Viscosity experiments were carried on an Ubbelodhe viscometer. Sheldon CO_2 culture box and DG3022A ELISA instruments were used to perform antiproliferative activity.

2.2. *Synthesis of the complex*

A mixture of NCTD (0.5 mmol), $CoCl_2{\cdot}6H_2O$ (0.5 mmol), imidazole (1.5 mmol), and distilled water (15 mL) was sealed in a Teflon-lined stainless vessel (25 mL), heated at 160°C for 3 days, and then slowly cooled to room temperature. The solution was

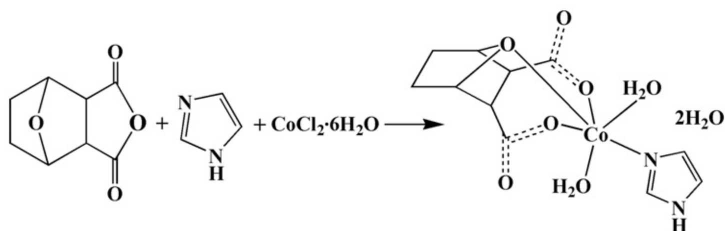


Figure 1. Synthesis of the complex.

Downloaded by [Renmin University of China] at 10:31 13 October 2013

filtered and after 2 weeks, block red crystals were obtained. In solution, NCTD is hydrolyzed and the resulting dianion is a tridentate ligand, which, along with one imidazole and two water molecules forms the octahedrally coordinated Co(II) complex (figure 1). Anal. Calcd for $C_{11}H_{20}N_2O_9Co$ (%): C, 34.48; H, 5.26; N, 7.31. Found: C, 34.13; H, 5.29; N, 7.05. IR (cm^{-1}): 3356 s $\nu(H_2O)$; 1600 s $\nu_{as}(COO^-)$; 1415 s $\nu_s(COO^-)$; 1268 m, 1066 m, 991 w ν(C–O–C). It is predicted that the molecular formula is $[Co(C_3H_4N_2)(C_8H_8O_5)(H_2O)_2]\cdot 2H_2O$ ($C_8H_8O_5$, demethylcantharate ion = DCA^{2-}).

2.3. *DNA binding experiments*

2.3.1. Electronic absorption spectra. The ct-DNA was prepared with 0.1 mol L^{-1} NaCl. The concentration of ct-DNA was 200 µg mL^{-1} ($c(DNA) = 3.72 \times 10^{-4}$ mol L^{-1}) and was kept at 4°C. The ct-DNA solutions gave a ratio of UV absorbance at 260 nm and 280 nm, $A_{260}/A_{280} = 1.8$–2.0, indicating that the DNA was sufficiently free of protein. The DNA solution was used in 4 days. Tris-HCl buffer (5 mmol L^{-1} Tris/50 mmol L^{-1} NaCl, pH = 7.4) was made by conventional method. The complex was dissolved in water.

Different volumes of DNA solution (0–80 µmol L^{-1}) were added to the mixture of complex solution (0.13 mmol L^{-1}) and Tris-HCl buffer. At room temperature, absorption spectra experiments were carried out at 200–400 nm and Tris-HCl buffer solution containing different concentrations of DNA was used as reference.

2.3.2. Fluorescence spectra. Fluorescence experiments were carried out by adding different concentrations (0–6.00 mL) of ct-DNA solution to the complex solution containing 2.00×10^{-4} mol L^{-1} Tris-HCl buffer (pH = 7.4). After incubation for 5 h at 4°C, the fluorescence emission spectra were recorded at an excitation wavelength of 260 nm from 260 to 530 nm using 10 nm slit widths. Fluorescence quenching was carried out by adding complex solution (0–150 µmol L^{-1}) to samples containing 0.25 µmol L^{-1} ethidium bromide (EB) and 74.4 µmol L^{-1} DNA. The solution was diluted by Tris-HCl buffer solution and fluorescence read at an excitation wavelength of 252 nm and emission wavelength between 520 and 700 nm.

2.3.3. Viscosity. Viscosity experiments were carried out on an Ubbelodhe viscometer, immersed in a thermostated water-bath maintained at 25°C. The compounds were added into a DNA solution ($c_{DNA} = 3.72 \times 10^{-4}$ mol L^{-1}) by microsyringe.

Average values of three replicated measurements were used to evaluate the viscosity of the samples. Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* the ratio of the concentration of the complex to DNA ($c_{(\text{complex})}/c_{(\text{DNA})} = 0\text{–}2.20$), where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solution(t) corrected for the flow time of buffer alone (t_0) [15], $\eta = (t - t_0)/t_0$.

2.4. *Crystal structure determination*

A single crystal of the complex with dimensions of $0.254 \times 0.166 \times 0.073\,\text{mm}^3$ was placed on the X-ray diffractometer. Intensity data were collected with graphite monochromated Mo-Kα radiation ($\lambda = 0.071073$ nm) using the ω scan technique in the range $2.70^\circ \leq \theta \leq 27.50^\circ$ at 23(2)°C. A total of 6604 reflections was collected, of which 1805 reflections ($R_{\text{int}} = 0.0218$) were independent and 1675 were observed with $I > 2\sigma(I)$. The structure was solved by direct methods, and the position of non-hydrogen atoms determined by successive Fourier syntheses. Hydrogens of water were located by Fourier methods and the remaining hydrogens were positioned geometrically. The position and anisotropic parameters of all non-hydrogen atoms were refined on F^2 by the full-matrix least-squares method using the SHELXL-97 program package [16]. The complex is monoclinic crystal system and $P2_1/m$ space group with $a = 0.634790(10)$ nm, $b = 0.963030(10)$ nm, $c = 1.221770(10)$ nm, $\alpha = 90^\circ$, $\beta = 95.9700(10)^\circ$, $\gamma = 90^\circ$, $V = 0.742844(15)\,\text{nm}^3$, $D_c = 1.713\,\text{g cm}^{-3}$, $Z = 2$, $F(0\,0\,0) = 398$, $\mu = 1.206\,\text{mm}^{-1}$. The final full-matrix least-squares refinement gave $R = 0.0291$ and $wR = 0.0837$ ($w = 1/[\sigma^2(F_o^2) + (0.0523P)^2 + 0.3702P]$, where $P = (F_o^2 + 2F_c^2)/3$), $(\Delta\rho)_{\max} = 0.714$ and $(\Delta\rho)_{\min} = -0.329\,\text{e}\,\text{Å}^{-3}$.

2.5. *Antiproliferative activity evaluation*

The antiproliferative activities of complex and NCTD were evaluated [17] by using human hepatoma cells SMMC7721 and human lung cancer cells A549. The antiproliferative activity was measured by the MTT assay. The compounds were dissolved in DMSO as $100\,\text{mmol L}^{-1}$ stock solutions. Growth cells in the exponential phase were assayed by adding 100 µL stock solution directly to culture wells. After the cells were seeded for 24 h, the complex and NCTD were added. Then the cells were incubated for 72 h, followed by adding 100 µL MTT into each well. Later, the liquid in each well was discarded and 150 µL acidified isopropanol was added. The mixture was placed in a dark area for 30 min. The inhibition rate and IC_{50} were calculated.

3. Results and discussion

3.1. *Structure description of complex*

Figure 2 shows the molecular structure of the complex. The main bond lengths and angles are listed in table 1. The coordination environment of Co(II) is shown in figure 2. Each Co(II) is six-coordinate by two water molecules, one bridge oxygen, two carboxylate oxygens in two different carboxylate groups from demethylcantharate and one nitrogen from imidazole.

Downloaded by [Renmin University of China] at 10:31 13 October 2013

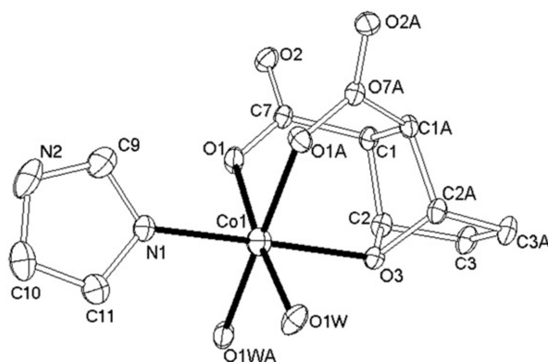


Figure 2. Molecular structure of the complex showing 30% atomic displacement ellipsoids.

Table 1. Select bond lengths (Å) and angles (°) of the complex.

Bond	Distance	Bond	Distance	Bond	Distance
Co1–O1W	2.0649(13)	Co1–N1	2.081(2)	Co1–O1	2.0986(13)
Co1–O3	2.1583(16)				
Angle	(°)	Angle	(°)	Angle	(°)
O1W#1–Co1–O1W	91.15(9)	O1W–Co1–N1	92.32(6)	O1W–Co1–O1#1	92.93(6)
O1W–Co1–O1	174.39(5)	N1–Co1–O1	91.36(6)	O1#1–Co1–O1	82.76(8)
O1W–Co1–O3	86.81(5)	N1–Co1–O3	178.76(7)	O1–Co1–O3	89.57(5)

Symmetry transformations used to generate equivalent atoms: #1, x, $-y+1/2$, z.

The Co(II) lies on a special position in $P2_1/m$, giving the Co(II) complex mirror symmetry. O1, O1WA, O1W, and O1A lie on the equatorial plane with the torsion angle 0.000(49)°. The nitrogen N1 and the bridge oxygen atom O3 are in axial positions. The bond angle of N1–Co1–O3 is 178.76(7)°. Owing to the binding of the bridge oxygen with Co, two six-membered rings (Co1–O1A–C7A–C1A–C2A–O3) and (Co1–O1–C7–C1–C2–O3) are created. In addition, a seven-membered ring (Co1–O1A–C7A–C1A–C1–C7–O1) is formed because of coordination of carboxylate oxygens O1 and O1A, which makes the compound more stable.

Crystal packing (figure 3) shows that there are abundant hydrogen bonds in the complex. Hydrogen bonds between molecules (O1W–H1WA–O1#1, O1W–H1WB–O2#2, O2W–H2WB–O2#3, O2W–H2WA–O2W#4, N2–H2–O2W#5, and N2–H2–O2W#6 (table 2)) indicate that hydrogen bonds form between crystal water molecules and between oxygen of crystal water and hydrogen of imino group in imidazole. The two hydrogens of each coordinated water molecule with coordinated carboxyl oxygen and uncoordinated carboxyl oxygen in DCA^{2-} of two adjacent molecules form intermolecular hydrogen bonds. All the hydrogen bonds form a 3-D network.

3.2. *DNA binding studies*

3.2.1. Electronic absorption spectra. For studying the interaction of DNA with complex, we used titration to test the effect of DNA in UV absorption spectra.

Downloaded by [Renmin University of China] at 10:31 13 October 2013

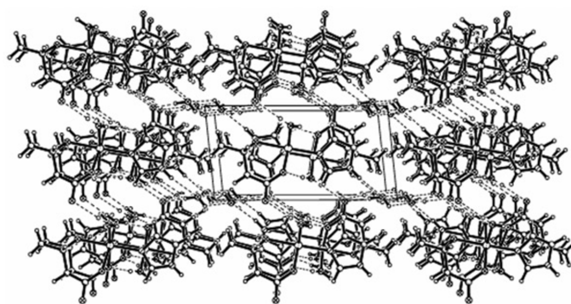


Figure 3. Crystal packing of complex.

Table 2. Hydrogen bonds in the complex (nm and (°)).

D–H···A	d(D–H)	d(H···A)	d(D···A)	∠(DHA)
O1W–H1WA–O1#1	0.852(15)	1.886(16)	2.7331(18)	173(2)
O1W–H1WB–O2#2	0.841(15)	1.973(15)	2.8045(18)	170(2)
O2W–H2WB–O2#3	0.900(17)	1.91(2)	2.798(2)	166(3)
O2W–H2WA–O2W#4	0.832(17)	2.34(3)	2.926(5)	128(3)
N2–H2–O2W#5	0.86	2.51	3.256(3)	145.0
N2–H2–O2W#6	0.86	2.51	3.256(3)	145.0

Symmetry transformations used to generate equivalent atoms: #1, $-x+1$, $y+1/2$, $-z+1$; #2, $x-1$, $-y+1/2$, z; #3, $-x+1$,$-y$,$-z+1$; #4, $-x$,$-y$,$-z$; #5, $x+1$, y, z; and #6, $x+1$, $-y+1/2$, z.

Downloaded by [Renmin University of China] at 10:31 13 October 2013

Absorption spectra of the complex with increasing concentration of ct-DNA are shown in figure 4. On increasing concentration of ct-DNA, absorption bands of the complex at 207 nm were affected, resulting in hyperchromicity.

The intrinsic binding constant is determined according to [18]: $[DNA]/(\varepsilon_A - \varepsilon_F) = [DNA]/(\varepsilon_B - \varepsilon_F) + 1/[K_b(\varepsilon_B - \varepsilon_F)]$. In plots of $[DNA]/(\varepsilon_A - \varepsilon_F)$ *versus* [DNA], K_b is given by the ratio of the slope to intercept. The binding constants K_b for the complex is 2.62×10^4 ($L\,mol^{-1}$), similar to K_b for cobalt complexes of nitrogen heterocycles [19]. The binding intensity of the classical intercalator EB with DNA was 10 times higher than the complex [20]. So, the binding mode between the complex and DNA was not classical intercalation.

3.2.2. Fluorescence spectral study. If the complex emits fluorescence under some conditions and its fluorescence intensity increases after adding DNA, it gives information about the interaction with DNA. The more fluorescence increases, the stronger the interaction between the complex and DNA [21]. Fluorescence spectra of complex in the absence and presence of DNA are shown in figure 5. The complex emits fluorescence at 380 nm and the arrows show the intensity changes upon increasing concentration of DNA. The hyperchromic effect is due to interaction of base pairs of DNA with micromolecules imbedded in DNA. The planar imidazole could intercalate the base pairs of DNA, which decrease collisions between water and the complex and leads to enhancement of fluorescence intensity [22].

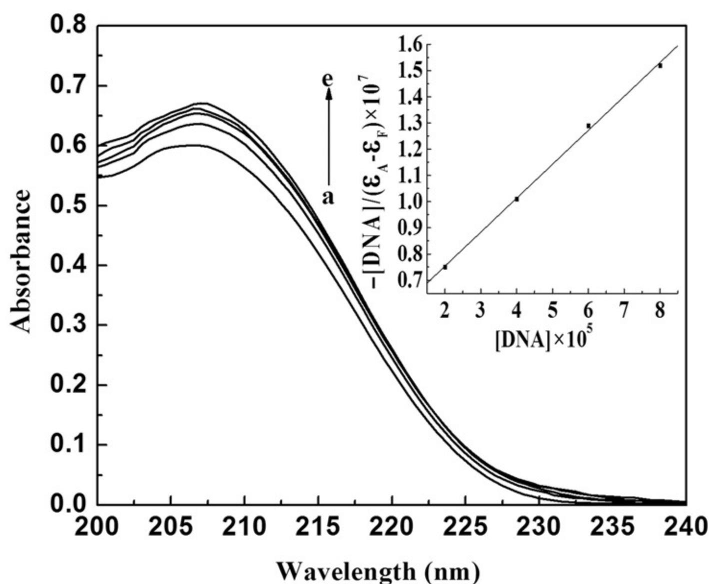


Figure 4. Absorption spectra of the complex in the absence and presence of increasing amounts DNA, $c_{\text{complex}} = 1.30 \times 10^{-4}\,\text{mol L}^{-1}$, $c_{\text{DNA}} \times 10^5\,\text{mol L}^{-1}$: (a–e) = 0, 2.0, 4.0, 6.0, 8.0, respectively.

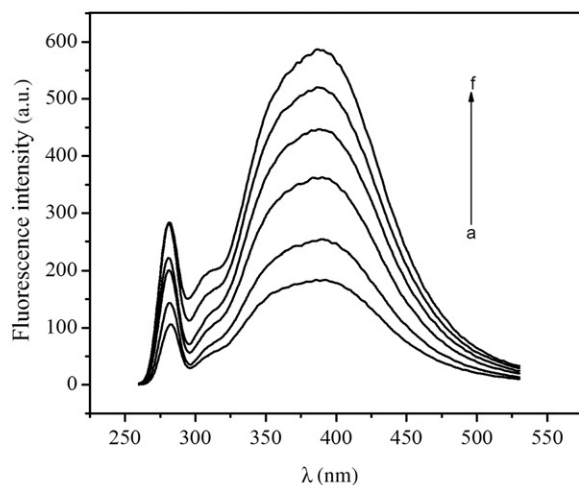


Figure 5. Fluorescence spectra of the complex in the absence and presence of increasing amounts DNA, $\lambda_{ex} = 260\,\text{nm}$, $\lambda_{em} = 260\text{–}530\,\text{nm}$. $c_{\text{complex}} = 2.0010^{-4}\,\text{mol L}^{-1}$, $c_{\text{DNA}} \times 10^5\,\text{mol L}^{-1}$: (a–f) = 0, 1.86, 3.72, 7.44, 14.9, 22.3, respectively.

Quenching of fluorescence of EB-DNA by complex was studied. Reduction in emission intensity of the complex according to increasing concentration of complexes is shown in figure 6. With increasing concentration of the complex, the emission intensity at 589 nm of EB-DNA decreased, indicating that the complex could release some EB

Downloaded by [Renmin University of China] at 10:31 13 October 2013

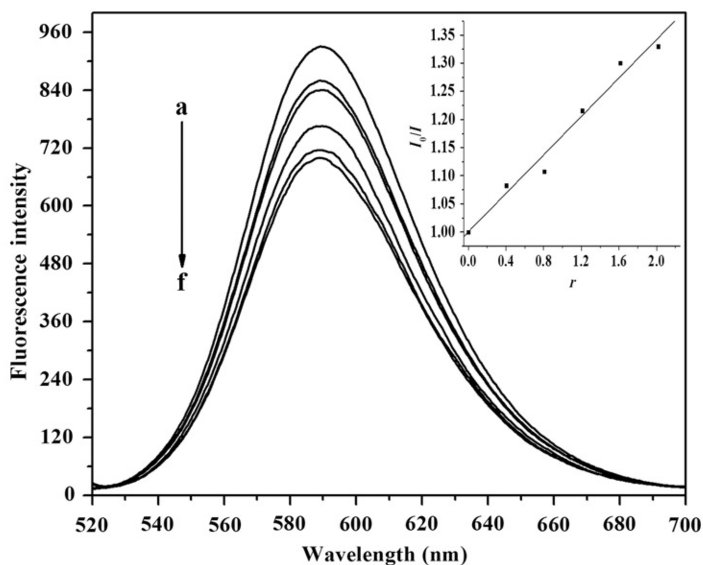


Figure 6. Emission spectra of EB-DNA system in the absence and presence of the complex; inset: fluorescence quenching curve of DNA-bound EB by the complex. $\lambda_{ex} = 252\,\text{nm}$, $\lambda_{em} = 520\text{–}700\,\text{nm}$. $c_{EB} = 2.5 \times 10^{-7}\,\text{mol L}^{-1}$, (*a–f*): $r = c_{complex}/c_{DNA} = 0$, 0.40, 0.81, 1.21, 1.61, 2.01, respectively.

Downloaded by [Renmin University of China] at 10:31 13 October 2013

from EB-DNA because of the planar imidazole ring, but NCTD could not quench the emission intensity of EB-DNA [23].

3.2.3. Viscosity study. To further clarify interactions between the complex and DNA, viscosity measurements were carried out on ct-DNA by varying the concentration of the added complex. In the absence of crystallographic structure data, hydrodynamic measurements, which are sensitive to DNA length increase (e.g., viscosity, sedimentation), are regarded as the least ambiguous and the most critical tests of binding in solution. A classical intercalative mode causes a significant increase in the viscosity of DNA solution due to increase in separation of base pairs at intercalation sites and hence an increase in overall DNA length [24]. By contrast, complexes that bind DNA by partial intercalation, under the same conditions, typically cause decreased viscosity in DNA solution. The values of $(\eta/\eta_0)^{1/3}$ were plotted against $c_{(complex)}/c_{(DNA)}$ (figure 7). NCTD has almost no effect on the viscosity of DNA while the complex decreases the relative viscosity of DNA. The reason may be that NCTD is non-planar, which causes NCTD to interact with DNA by groove binding or electrostatic binding [25]. The decreased relative viscosity of DNA with complex may be explained by imidazole in the axial position intercalating base pairs of DNA while demethylcantharate hinders the intercalation. Therefore, the complex may bind to DNA by partial intercalation. These observations suggest that Co(II) and planar imidazole in the complex play important roles in binding with DNA.

3.3. *Antiproliferative activity evaluation*

Inhibition rates of NCTD and the complex on human hepatoma cells (SMMC7721) and human lung cancer cells (A549) are reported in figures 8 and 9. *In vitro* viability was

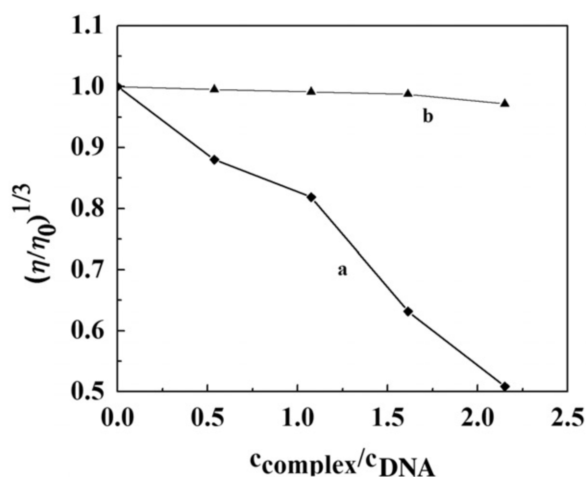


Figure 7. Effect of increasing amounts of NCTD and complex on the relative viscosity of DNA at 25°C. $c_{(DNA)} = 3.72 \times 10^{-4}$ mol L^{-1}, $r = c_{(complex)}/c_{(DNA)}$. $r = 0$, 0.54, 1.08, 1.61, and 2.20, respectively: (a) complex and (b) NCTD.

Downloaded by [Renmin University of China] at 10:31 13 October 2013

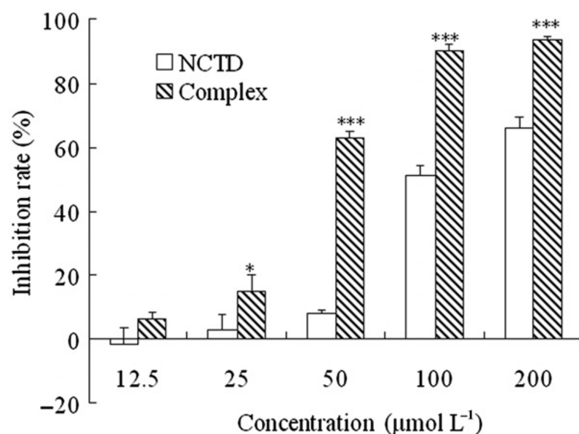


Figure 8. Inhibition rates of NCTD and complex on SMMC7721 cell growth. Data represent mean ± SD and all assays were performed in triplicate for three independent experiments. $*p < 0.05$ and $***p < 0.001$ *vs.* NCTD in the same concentration, *t-test*.

exhibited in a dose-dependent manner. For SMMC7721 cell lines, the complex (0–200 μmol L^{-1}) significantly decreased the tumor cell viability in a dose-dependent manner. At lower doses (50 μmol L^{-1}), inhibition rates of complex (63 ± 2%) is much higher than that of NCTD (8 ± 1%) against SMMC7721 cell lines. At a higher dose (100 μmol L^{-1}), inhibition rate of complex is higher than that of NCTD against A549 cell lines. Therefore, we conclude that the complex has better antiproliferative activity than NCTD.

Downloaded by [Renmin University of China] at 10:31 13 October 2013

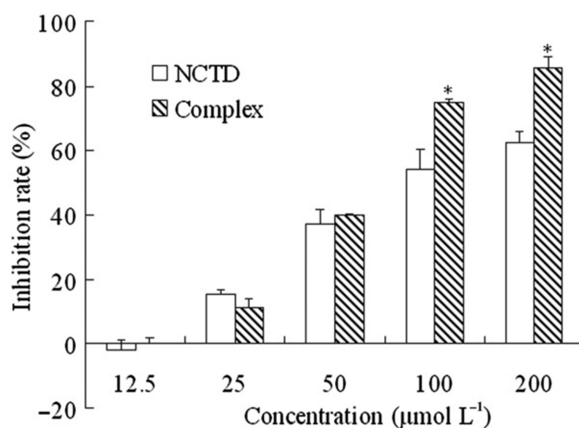


Figure 9. Inhibition rates of NCTD and complex on A549 cell growth. Data represent mean ± SD and all assays were performed in triplicate for three independent experiments. *$p < 0.05$ *vs.* NCTD in the same concentration, *t-test*.

Table 3. IC_{50} ($\mu mol\ L^{-1}$) values (72 h) of test materials on SMMC7721 and A549 cells. (Data represent mean ± SD. All assays were performed in triplicate for three independent experiments.)

IC_{50} ($\mu mol\ L^{-1}$)	SMMC7721	A549
NCTD	$115 \pm 9\ \mu mol\ L^{-1}$	$89 \pm 13\ \mu mol\ L^{-1}$
Complex	$43 \pm 1\ \mu mol\ L^{-1}$	$65 \pm 3\ \mu mol\ L^{-1}$

The concentrations of the compounds for 50% inhibition (IC_{50}) on SMMC7721 and A549 cell lines were determined (table 3). The antiproliferation activity indicates that complex has a strong antiproliferative ability against SMMC7721 ($IC_{50} = 43 \pm 1\ \mu mol\ L^{-1}$) and A549 ($IC_{50} = 65 \pm 3\ \mu mol\ L^{-1}$) cell lines. The inhibition rates of the complex are much higher than those of NCTD; Co(II) and planar imidazole in the complex play important roles in inhibition rates.

4. Conclusion

A new cobalt(II) complex $[Co(C_3H_4N_2)(C_8H_8O_5)(H_2O)_2]\cdot 2(H_2O)$ of demethylcantharate with imidazole has been synthesized and characterized by single crystal X-ray diffraction. Addition of increasing amounts of DNA results in hyperchromicity. Intrinsic binding constant (K_b) of the complex ($10^4\ L\ mol^{-1}$) is smaller than the constants of cobalt(II) with bpy and phen ($10^5\ L\ mol^{-1}$) [11]. The spectral characteristics suggest a stacking interaction between the complex and the base pairs of DNA. Fluorescence spectra and viscosity measurements indicate that the complex can bind to ct-DNA through a partially intercalative mode. The antiproliferation activities indicate that the complex has more antiproliferative ability against human hepatoma cells

SMMC7721 and human lung cancer cells A549 than NCTD. The complex shows much higher DNA-binding affinities than NCTD. These observations suggested that the Co(II) ion and imidazole in the complex play important roles in the antiproliferative effect against cancer cells and in the binding extent with DNA. The results help to understand the relation between binding ability of the complex with DNA and the antiproliferative activities. The new complex has potential to be further developed as effective therapeutic reagents of liver cancer or lung cancer.

Supplementary material

Crystallographic data for the structure reported in this article have been deposited with the Cambridge Crystallographic Data Center CCDC 705663. Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (deposit@ccdc.cam.ac.uk).

Acknowledgments

Financial support from Natural Science Foundation of Zhejiang Province (grant no. Y407301) is gratefully acknowledged.

References

[1] S.N. Yi, J. Wass, P. Vincent, H. Iland. *Leuk. Res.*, **15**, 883 (1991).
[2] W.N. Zeng, Y. Lu. *Chin. J. Org. Chem.*, **26**, 579 (2006).
[3] T.A. Hill, S.G. Stewart, B. Sauer, J. Gilbert, S.P. Ackland, J.A. Sakoff, A. McCluskey. *Bioorg. Med. Chem. Lett.*, **17**, 3392 (2007).
[4] F.L. Yin, J. Shen, J.J. Zou, R.C. Li. *Acta Chim. Sin.*, **61**, 556 (2003).
[5] Y.P. Ho, K.K.W. To, S.C.F.A. Yeung, X.N. Wang, G. Lin, X.W. Han. *J. Med. Chem.*, **44**, 2065 (2001).
[6] Y.Y. Wang, R.D. Hu, Q.Y. Lin, Y.L. Zhao, N. Wang. *Asian J. Chem.*, **22**, 5993 (2010).
[7] F.K. Yang, Z.T. Zhang. *Chem. Bioeng.*, **23**, 6 (2006).
[8] S.K. Li, Q.Y. Lin, T.X. Lv, Y.J. Wang, D. Chen. *Chin. J. Struct. Chem.*, **29**, 1632 (2010).
[9] N. Wang, Q.Y. Lin, J. Feng, Y.L. Zhao, Y.J. Wang, S.K. Li. *Inorg. Chim. Acta*, **363**, 3399 (2010).
[10] X.L. Hu, P.F. Shi, Q. Jiang. *Chin. J. Inorg. Chem.*, **25**, 373 (2009).
[11] K.L. Reddy, K.A. Kumar, S. Vidhisha, P.N. Babu, S. Satyanarayan. *J. Coord. Chem.*, **62**, 3997 (2009).
[12] S.A. Patil, V.H. Naik, A.D. Kulkarni, U. Kamble, G.B. Bagihall, P.S. Badami. *J. Coord. Chem.*, **63**, 688 (2010).
[13] Y.J. Wang, R.D. Hu, Q.Y. Lin, J.P. Cheng. *Acta Cryst., Sect. E*, **65**, m854 (2009).
[14] F.F. Jian, H.L. Xiao, P.P. Sun. *Chin. J. Inorg. Chem.*, **20**, 1339 (2004).
[15] P. Nagababu, S. Satyanarayana. *Polyhedron*, **26**, 1686 (2007).
[16] G.M. Sheldrick. *SHELXL-97. Program for the Refinement of Crystal Structure*, University of Göttingen, Germany (1997).
[17] D.M. Yan, L.L. Tu, X.Y. Peng, W.J. Li, Z.R. Shen. *Chin. JMAP*, **24**, 352 (2007).
[18] A. Wolfe, G.H. Shimer, T. Meehan. *Biochemistry*, **26**, 6392 (1987).
[19] X.W. Huang, X.J. Chen, J.L. Shen, G.J. Su. *J. Coord. Chem.*, **63**, 1570 (2010).
[20] S. Satyanarayana, J.C. Dabrowiak, J.B. Chaires. *Biochemistry*, **31**, 9319 (1992).
[21] W.H. Chen, J.Y. Pang, Y. Qin, Q. Peng, Z. Cai, Z.H. Jiang. *Bioorg. Med. Chem. Lett.*, **15**, 2689 (2005).
[22] H. Yang, W.T. Chen, D.F. Qiu, X.Y. Bao, W.R. Xing, S.S. Liu. *Acta Chim. Sin.*, **65**, 2959 (2007).
[23] W.Z. Zhu, Q.Y. Lin, M. Lu, R.D. Hu, X.L. Zheng, J.P. Cheng, Y.Y. Wang. *J. Fluoresc.*, **19**, 857 (2009).
[24] B.D. Wang, Z.Y. Yang, C. Patrick, D.Q. Wang. *J. Inorg. Biochem.*, **101**, 1492 (2007).
[25] Q.L. Zhang, J.H. Liu, X.Z. Ren, P.X. Zhang, C.H. Li, F. Wang, H. Xu, J.Z. Liu, L.N. Ji. *Acta Chim. Sin.*, **64**, 968 (2006).

Downloaded by [Renmin University of China] at 10:31 13 October 2013