

Fluorescence and Biological Evaluation of the La(III) and Eu(III) Complexes with 7-methoxychromone-3-carbaldehyde Benzoyl Hydrazone Schiff Base

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Abstract A novel Schiff base ligand (L=7-methoxychromone-3-carbaldehyde benzoyl hydrazone) and its La(III) and Eu(III) complexes have been successfully prepared. The crystal structure of $[\text{LaL}_2(\text{NO}_3)_3]\cdot\text{H}_2\text{O}$ was characterized by X-ray crystallography. It crystallizes in monoclinic, space group C2/c with crystallographic data: $a=27.7173$ (17) Å, $b=10.0002(6)$ Å, $c=14.7884(9)$ Å, $\beta=102.6870$ (10)° and $Z=4$. In the structure, the La(III) ion satisfies 12 coordination and three nitrate coordinate as bidentate ligand. The biological experiments show that the ligand and its two complexes can strongly bind to DNA through intercalation mode, and the three compounds also exhibit good antioxidant activities against OH^\bullet and $\text{O}_2^{\bullet-}$. Moreover, it is found that the Eu(III) complex exhibits characteristic fluorescence of europium ion in different organic solvent.

Keywords Fluorescence · Biological activities · Rare earth complexes · Schiff base

Introduction

Cisplatin ($\text{cis-}[\text{PtCl}_2(\text{NH}_3)_2]$) is the first and the most widely used metal-based drug in cancer chemotherapy [1]. Then Carboplatin ($[\text{Pt}(\text{cbdc})(\text{NH}_3)_2]$ where cbdc=1,1-cyclobutane dicarboxylate), a second generation Pt-drug appeared soon [2]. Despite their wide clinical use, their severe side effects have stimulated the search for other metal-based anticancer drugs [3]. It is well known that the

ability of Pt-drug to inhibit tumor growth is related with its efficient binding to DNA. In this respect, studying interaction between small metal complexes and DNA may lead to a new kind of active anticancer drugs [4].

More recently, the complexes of rare earth have been attracting attention [5, 6]. They possess interesting photo-physical and photochemical properties which can potentially be harnessed in the development of new photodynamic therapies, and offer exciting opportunities to elucidate structural features within DNA itself [7]. Rare earth complexes with tetracycline, phenanthroline [8], adriamycin and pyridine [9–12] have been synthesized as a probe to study nucleic acids. What we consider is to synthesize the rare earth complexes which can not only emit fluorescence but possess ability to strongly interact with DNA. These complexes may be the excellent candidates for biological application. However, due to the weak absorption capabilities of rare earth ions (e.g. Eu^{3+}), organic ligands are often used to increase ion emission through energy transfer processes [13, 14]. Hence which type of ligand we choose is an intractable problem.

In our previous work, lanthanide complexes with Schiff base derived from 6-hydroxy-3-carbaldehyde chromone were reported and all the complexes can strongly interact with DNA using intercalation mode [15]. We think these DNA-binding properties may be due to the aromatic planar structure of chromone chromophore. As further research, 7-methoxychromone-3-carbaldehyde benzoyl hydrazone Schiff base derived from another new chromone (6-methoxy-3-carbaldehyde chromone) and its La(III), Eu(III) complexes were synthesized. The experiment details suggest that both the two complexes and the ligand can also obviously interact with DNA by intercalation. Fortunately, we find that the Eu(III) complex can emit its characteristic fluorescence in organic solvent. Hence this complex may be

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a candidate of anticancer drugs and nucleic acid probe. Furthermore, the complexes and the ligand have the ability to scavenge OH^\bullet and $\text{O}_2^{\bullet-}$, which also is an important reason of inducing cancer [16].

Experimental section

Instrumentation

Elemental analyses were conducted on a Vario EL analyzer. Infrared spectra ($4,000\text{--}400\text{ cm}^{-1}$) were determined with KBr disks on a Thermo Mattson FTIR spectrometer. The ultraviolet spectra were recorded on a Lambda 35 UV/Vis spectrometer. ^1H NMR spectra were measured on a Bruker Avance Drx 200-MHz spectrometer. The fluorescence spectra were recorded on a RF-5301PC spectrofluorophotometer produced by SHIMADZU. The antioxidant activities were tested on a 721E spectrophotometer.

Materials and methods

Acetic anhydride, 2-hydroxy-4-methoxy-acetophenone, $\text{BF}_3\cdot\text{Et}_2\text{O}$, POCl_3 , transition metal nitrates, Safranin, Mannitol, EDTA, $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ were produced in China. CT-DNA, ethidium bromide (EB), Nitroblue tetrazolium (NBT), methionine (MET), Vitamin B_2 (Vit B_2) were purchased from Sigma Chemicals Co. (USA). All materials and solvents employed in this study were of analytical grade. EDTA-Fe(II) and $\text{Na}_2\text{HPO}_4\text{--KH}_2\text{PO}_4$ buffers were prepared with twice distilled water. All the experiments involving interaction of the complexes with CT-DNA were carried out in doubly distilled water buffer containing 5 mM Tris [Tris (hydroxymethyl)-aminometh-ane] and 50 mM NaCl, and adjusted to pH 7.1 with hydrochloric acid. A solution of CT-DNA in the buffer gave a ratio of UV absorbance of about 1.8–1.9:1 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient ($6,600\text{ M}^{-1}\text{ cm}^{-1}$) at 260 nm.

Viscosity measurements

Viscosity experiments were conducted on an Ubbdlohde viscometer, immersed in a thermostatic water-bath maintained to $25.0\text{ }^\circ\text{C}$. Each compound was introduced into a

DNA solution ($10\text{ }\mu\text{M}$) present in the viscometer. Data was presented as $(\eta/\eta_0)^{1/3}$ versus the ratio of the concentration of the compound and DNA, where η is the viscosity of DNA in the presence of compound and η_0 is the viscosity of DNA alone [17].

Hydroxyl radical scavenging assay

The hydroxyl radicals (OH^\bullet) in aqueous media were generated through the Fenton system. The solution of the tested compound was prepared with DMF. The 5 ml assay mixture contained following reagents: safranin ($10\text{ }\mu\text{M}$), EDTA-Fe(II) ($80\text{ }\mu\text{M}$), H_2O_2 (0.6%), the tested compound ($4\text{--}20\text{ }\mu\text{M}$) and a phosphate buffer (pH 7.4). The assay mixtures were incubated at $40\text{ }^\circ\text{C}$ for 50 min in a water-bath. After which, the absorbance was measured at 520 nm. All the tests were run in triplicate and expressed as the mean \pm standard deviation (SD). The suppression ratio for OH^\bullet was calculated from the following expression:

$$\text{Scavenging effect(\%)} = \frac{A_i - A_0}{A_c - A_0} \times 100$$

(Where A_i = the absorbance in the presence of the tested compound; A_0 = the absorbance in the absence of the tested compound; A_c = the absorbance in the absence of the tested compound, EDTA-Fe(II) and H_2O_2 .)

Superoxide radical scavenging assay

The superoxide radicals ($\text{O}_2^{\bullet-}$) were generated in vitro by non-enzymatic system and determined spectrophotometrically by nitro blue tetrazolium (NBT) photoreduction method with a little modification in the method adopted elsewhere. The amount of $\text{O}_2^{\bullet-}$ and suppression ratio for $\text{O}_2^{\bullet-}$ can be calculated by measuring the absorbance at 560 nm. Solution of MET, Vit B_2 and NBT were prepared at avoiding light. The tested compounds were dissolved in DMF. The assay mixture, in a total volume of 5 ml, contained MET (10 mM), NBT ($46\text{ }\mu\text{M}$), Vit B_2 ($3.3\text{ }\mu\text{M}$), the tested compound ($1\text{--}8\text{ }\mu\text{M}$) and a phosphate buffer (pH 7.8). After illuminating with a fluorescent lamp at $30\text{ }^\circ\text{C}$ for 10 min, the absorbance of the samples (A_i) was measured at 560 nm. The sample without the tested compound was used as control and its absorbance was A_0 . All experimental results were expressed as the mean \pm standard deviation

Fig. 1 synthesis of the Schiff base ligand

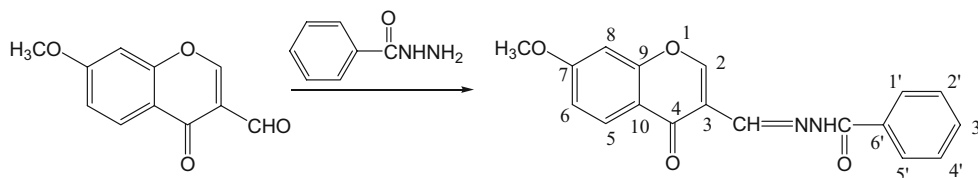


Table 1 Crystal data and experimental data

Empirical formula	C ₃₆ H ₃₂ LaN ₇ O ₁₉
Formula weight	1,005.60
Temperature	298(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, C2/c
<i>a</i> (Å)	27.7173(17)
<i>b</i> (Å)	10.0002(6)
<i>c</i> (Å)	14.7884(9)
α (°)	90
β (°)	102.6870(10)
γ (°)	90
Volume (Å ³)	3,998.9(4)
<i>Z</i>	4
<i>D</i> _{calc} (Mg/m ³)	1.670
<i>F</i> (000)	2,024
μ (mm ⁻¹)	1.159
θ range (°)	1.51–26.00
Reflections collected/unique	11,168/3,909 [<i>R</i> (int)=0.0279]
Goodness-of-fit on <i>F</i> ²	1.041
<i>R</i> indices [<i>I</i> >2 σ (<i>I</i>)]	<i>R</i> ₁ =0.0257, <i>wR</i> ₂ =0.0603
<i>R</i> indices (all data)	<i>R</i> ₁ =0.0302, <i>wR</i> ₂ =0.0619

(SD) of triplicate determinations. The suppression ratio for O₂^{•-} was calculated from the following expression:

$$\text{Scavenging effect(\%)} = \frac{A_0 - A_i}{A_0} \times 100$$

X-ray crystallography

Intensity data were collected on a Bruker Smart Apex II diffractometer using a MoK α radiation ($\lambda=0.71073$ Å). The structure was solved by direct methods with SHELXL-97 [18] program and expanded with difference Fourier technique. The non-hydrogen atoms were refined anisotropically by the full-matrix least-squares method based on *F*² with all observed reflections. The hydrogen atoms were generated geometrically with isotropic thermal parameters.

Table 2 Selected bond lengths

Selected bond	bond lengths (Å)	Selected bond	bond lengths (Å)	Selected bond	bond lengths (Å)
La(1)–O(3)	2.4925(16)	La(1)–O(3a)	2.4925(16)	La(1)–O(4)	2.6386(16)
La(1)–O(4a)	2.6386(16)	La(1)–O(5)	2.6678(18)	La(1)–O(5a)	2.6678(18)
La(1)–O(6)	2.7124(18)	La(1)–O(6a)	2.7124(18)	La(1)–O(8)	2.7167(18)
La(1)–O(8a)	2.7167(18)	La(1)–N(1)	2.873(2)	La(1)–N(1a)	2.873(2)
N(1)–C(11)	1.284(3)	N(1)–N(2)	1.389(3)	N(2)–C(12)	1.339(3)
N(3)–O(7)	1.226(3)	N(3)–O(6)	1.255(3)	N(3)–O(5)	1.267(3)
N(4)–O(9)	1.234(4)	N(4)–O(8)	1.258(2)	N(4)–O(8a)	1.258(2)
O(3)–C(7)	1.240(3)	O(4)–C(12)	1.241(3)	C(6)–C(7)	1.449(3)
C(6)–C(11)	1.455(3)	C(7)–C(8)	1.443(3)		

Preparation of Schiff base ligand and the La(III) and Eu(III) complexes

Scheme of the synthesis of the ligand is shown in Fig. 1. 7-Methoxychromone-3-carbaldehyde was prepared according to the literature [19]. The Schiff base ligand was in accordance with the following method: Benzoyl hydrazine (1.36 g, 10 mmol) in 20 ml ethanol was added dropwise to the 7-methoxychromone-3-carbaldehyde (2.04 g, 10 mmol) of 10 ml trichloromethane solution and a large amount of light yellow precipitate appeared. After continuing stirring for 6 h at room temperature, the precipitate was collected and recrystallized from ethanol to give the pure ligand (L). Yield, 88%. Mp: 176–178 °C. ¹H NMR (DMSO-*d*₆, ppm): δ 11.91 (1H, s, –NH), 8.75 (1H, s, CH=N), 8.60 (1H, s, 2-H), 8.02(1H, d, *J*=8.9 HZ, H-5), 7.90–7.93(2H, d, ph-H (1:5)), 7.48–7.59 (3H, m ph-H(2:3:4)), 7.21 (1H, d, *J*=2.4HZ, 8-H), 7.09–7.14 (1H, dd, *J*=2.4 and *J*=8.9 HZ, H-6), 3.91 (3H, s, –CH₃). IR ν_{max} (cm⁻¹): ν (C=O): 1671, ν (NH–C=O) 1639, ν (C=N): 1603.

La(NO₃)₃·6H₂O (0.476 g, 1.1 mmol) in 5 ml ethanol was added to the 30 ml ethanol solution containing the Schiff base ligand (0.322 g, 1 mmol) and stirred for 1 day at room temperature. The yellow product was collected and washed with ethanol three times, and then dried at vacuo. The Eu(III) complex was prepared using the same method. Lanthanum complex yield, 51%; C₃₆N₇O₁₇H₂₈La requires (%) C, 44.64; N, 10.10; H, 2.89. Found: C, 44.41; N, 10.05, H, 2.72. IR ν_{max} (cm⁻¹): ν (C=O): 1641, ν (NH–C=O): 1623, ν (C=N): 1575, ν (NO₃⁻): 1491, 1284, 1022, 827, 733. ΔM (S cm² mol⁻¹) 10⁻³ M methanol solution 25 °C=40.6. Europium complex yield, 48%; C₃₆N₇O₁₇H₂₈Eu requires (%) C, 44.05; N, 9.97; H, 2.85. Found: C, 43.91; N, 9.86; H, 2.76. IR ν_{max} (cm⁻¹): ν (C=O): 1643, ν (NH–C=O): 1627, ν (C=N): 1571. ν (NO₃⁻): 1491, 1289, 1026, 820, 730. ΔM (S cm² mol⁻¹) 10⁻³ M methanol solution 25 °C=54.2.

Table 3 Selected bond angles

Selected bond	Bond angles (°)
O(3)–La(1)–O(3a)	178.17(8)
O(3a)–La(1)–O(4)	67.76(5)
O(3)–La(1)–O(5a)	66.71(6)
O(4)–La(1)–O(5a)	67.90(6)
O(4a)–La(1)–O(5a)	125.87(5)
O(3)–La(1)–O(6)	69.55(6)
O(4)–La(1)–O(6a)	65.58(6)
O(3a)–La(1)–O(6)	109.52(6)
O(4a)–La(1)–O(6)	65.58(6)
O(6)–La(1)–O(6a)	122.73(8)
O(4)–La(1)–O(8)	125.76(5)
O(5)–La(1)–O(8a)	63.81(6)
O(3a)–La(1)–O(8a)	67.67(5)
O(4a)–La(1)–O(8a)	125.76(5)
O(6a)–La(1)–O(8a)	63.73(6)
O(3)–La(1)–N(1)	63.35(6)
O(4)–La(1)–N(1a)	64.69(5)
O(6)–La(1)–N(1)	120.76(6)
O(8)–La(1)–N(1a)	166.41(5)
O(4a)–La(1)–N(1a)	56.61(5)
O(5a)–La(1)–N(1)	69.68(6)
O(8a)–La(1)–N(1a)	120.06(6)
C(11)–N(1)–N(2)	114.20(2)
C(12)–N(2)–N(1)	117.40(2)
O(6)–N(3)–O(5)	117.20(2)
O(9)–N(4)–O(8a)	121.33(15)
O(3)–La(1)–O(4)	113.43(6)
O(3a)–La(1)–O(4a)	113.43(6)
O(3)–La(1)–O(5)	112.94(6)
O(3a)–La(1)–O(5a)	112.94(6)
O(4a)–La(1)–O(5)	67.90(6)
O(3)–La(1)–O(6a)	109.52(6)
O(5)–La(1)–O(6)	47.17(5)
O(3a)–La(1)–O(6a)	69.55(6)
O(5a)–La(1)–O(6a)	47.17(5)
O(3)–La(1)–O(8)	67.67(5)
O(4)–La(1)–O(8a)	120.89(5)
O(6)–La(1)–O(8)	63.73(6)
O(3a)–La(1)–O(8)	110.52(6)
O(5a)–La(1)–O(8a)	96.83(6)
O(6a)–La(1)–O(8)	64.04(6)
O(3)–La(1)–N(1a)	118.34(5)
O(5)–La(1)–N(1)	129.37(6)
O(6)–La(1)–N(1a)	105.82(6)
O(3a)–La(1)–N(1a)	63.35(6)
O(4a)–La(1)–N(1)	64.69(5)
O(6a)–La(1)–N(1a)	120.76(6)
O(8a)–La(1)–N(1)	166.41(5)
C(11)–N(1)–La(1)	130.23(16)
O(7)–N(3)–O(6)	121.90(2)
O(9)–N(4)–O(8)	121.33(15)
O(3)–La(1)–O(4a)	67.76(5)
O(4)–La(1)–O(4a)	106.60(7)
O(4)–La(1)–O(5)	125.87(5)
O(3a)–La(1)–O(5)	66.71(6)
O(5)–La(1)–O(5a)	159.79(8)

Table 3 (continued)

Selected bond	Bond angles (°)
O(4)–La(1)–O(6)	170.48(5)
O(5)–La(1)–O(6a)	120.77(6)
O(4a)–La(1)–O(6a)	170.48(5)
O(5a)–La(1)–O(6)	120.77(6)
O(3)–La(1)–O(8a)	110.52(6)
O(5)–La(1)–O(8)	96.83(6)
O(6)–La(1)–O(8a)	64.04(6)
O(4a)–La(1)–O(8)	120.86(5)
O(5a)–La(1)–O(8)	63.81(6)
O(8)–La(1)–O(8a)	46.59(8)
O(4)–La(1)–N(1)	56.61(5)
O(5)–La(1)–N(1a)	69.68(6)
O(8)–La(1)–N(1)	120.06(6)
O(3a)–La(1)–N(1)	118.34(5)
O(5a)–La(1)–N(1a)	129.37(6)
O(6a)–La(1)–N(1)	105.82(6)
N(1)–La(1)–N(1)	70.21(8)
N(2)–N(1)–La(1)	114.88(13)
O(7)–N(3)–O(5)	120.80(2)
O(8)–N(4)–O(8a)	117.30(3)

Results and discussion

IR spectra

The IR spectrum of ligand shows bands at 1671, 1639 and 1603 cm^{-1} , which may assign to $\nu(\text{C}=\text{O})$, $\nu(\text{NH}-\text{C}=\text{O})$ and $\nu(\text{C}=\text{N})$ respectively. In the IR spectra of the complexes, these bands shift to 1641, 1623 and 1575 cm^{-1} for La(III) complex, 1643, 1627 and 1571 cm^{-1} for Eu(III) complex. These results indicate the three groups coordinate to the rare earth ions. The absorption bands appear at 1491 and 1284 cm^{-1} for La(III) complex, 1491 and 1289 cm^{-1} for Eu(III) complex assign to nitrate. It indicates that coordinated nitrate groups in the complex are bidentate and there is no free nitrate [20], which is demonstrated by the crystal structures illuminated as follows.

Description of the crystal structure

The single crystal of La(III) complex was grown from ethanol with slow evaporation at room temperature. The crystal is yellow and soluble in DMSO, DMF, methanol, ethanol, slightly soluble in acetone, insoluble in benzene and water. IR spectra indicate the nitrate is C_{2v} symmetric with five absorption peaks, molar conductivity (40.6 $\text{S cm}^2 \text{mol}^{-1}$) show La(III) complex is nonelectrolyte in methanol. All these results are confirmed by crystal structure. The crystal data and refinement results of the La(III) complex is summarized in Table 1. Selected bond lengths and angles

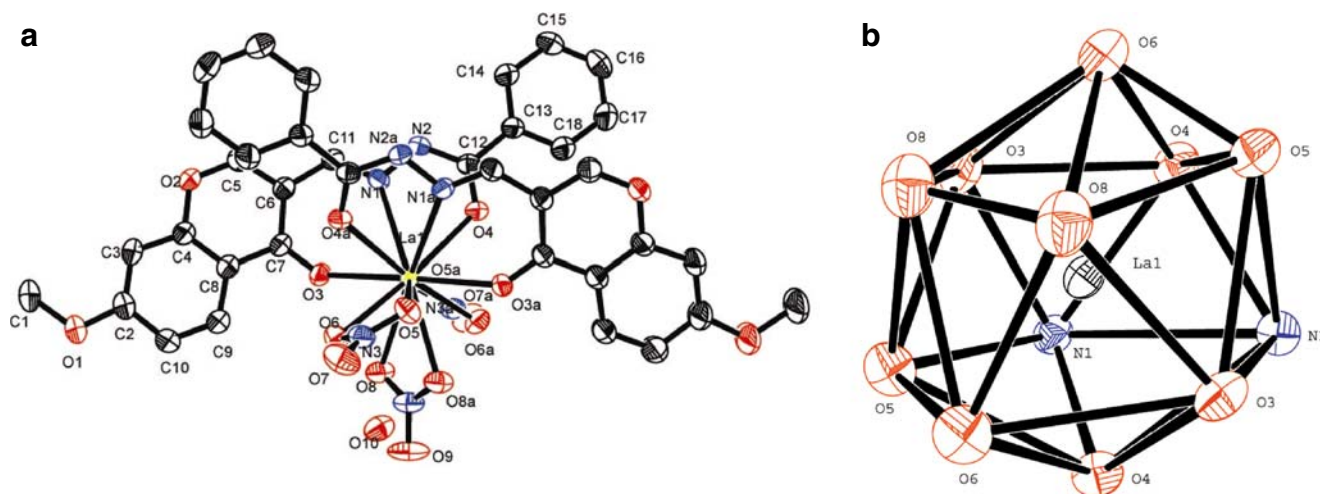


Fig. 2 crystal structure (a) and coordination polyhedron (b) of La(III) complex

are given in Tables 2 and 3. Fig. 2a shows the crystal structure. As seen from the Fig. 2a, La(III) ion is 12 coordination with two ligand and three nitrate. The chromone ring and the benzene ring are nearly at one plane. The distance between La(III) ion and six oxygen atoms of nitrate appear to be the same (2.6922 ± 0.025 Å), but the bond length of La–O(3) and La–O(4) is respectively 2.4925(16) Å and 2.6386(16) Å, which are shorter than the six oxygen of nitrate. It is notable that the distance between La(III) ion and N atoms of imino group is 2.873(2) Å, which is unusually longer than other ten La–O bond length [21]. Three nitrate have two coordination type, the bond angles of O(5a)–La–O(6a) and O(5)–La–O(6) are all $47.17^\circ(5)$, but the bond angle of O(8)–La–O(8a) is 46.59°

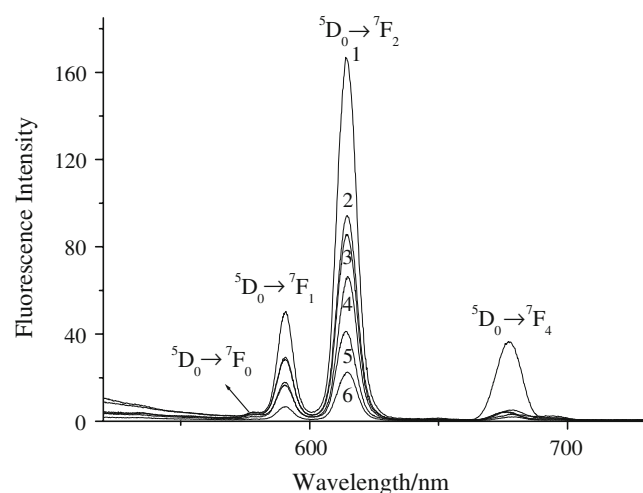


Fig. 3 Emission spectra of the europium complex in different solutions at room temperature concentration: 10^{-4} mol L⁻¹. 1 in CHCl₃, 2 in ethyl acetate, 3 in ethanol, 4 in acetone, 5 in methanol, 6 in MeCN

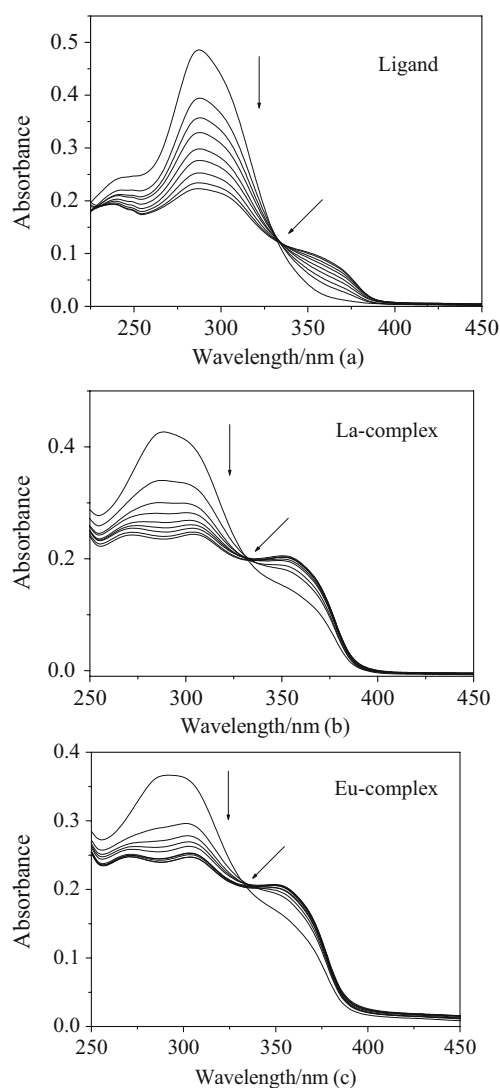


Fig. 4 Electronic spectra of the ligand (a), La(III) (b) and Eu(III) complex (c) (concentration=10 μM) in the presence of increasing amounts of CT-DNA, [DNA]=0–40 μM. The arrow indicates the absorbance changes upon increasing DNA concentration

(8), the bond length of La–O(5)=La–O(5a)=2.6678(18) Å, La–O(6)=La–O(6a)=2.7124(18) Å, La–O(8)=La–O(8a)=2.7167(18) Å. The coordination polyhedron is distorted bicapped pentagonal anti prism as shown in Fig. 2b.

Fluorescence studies

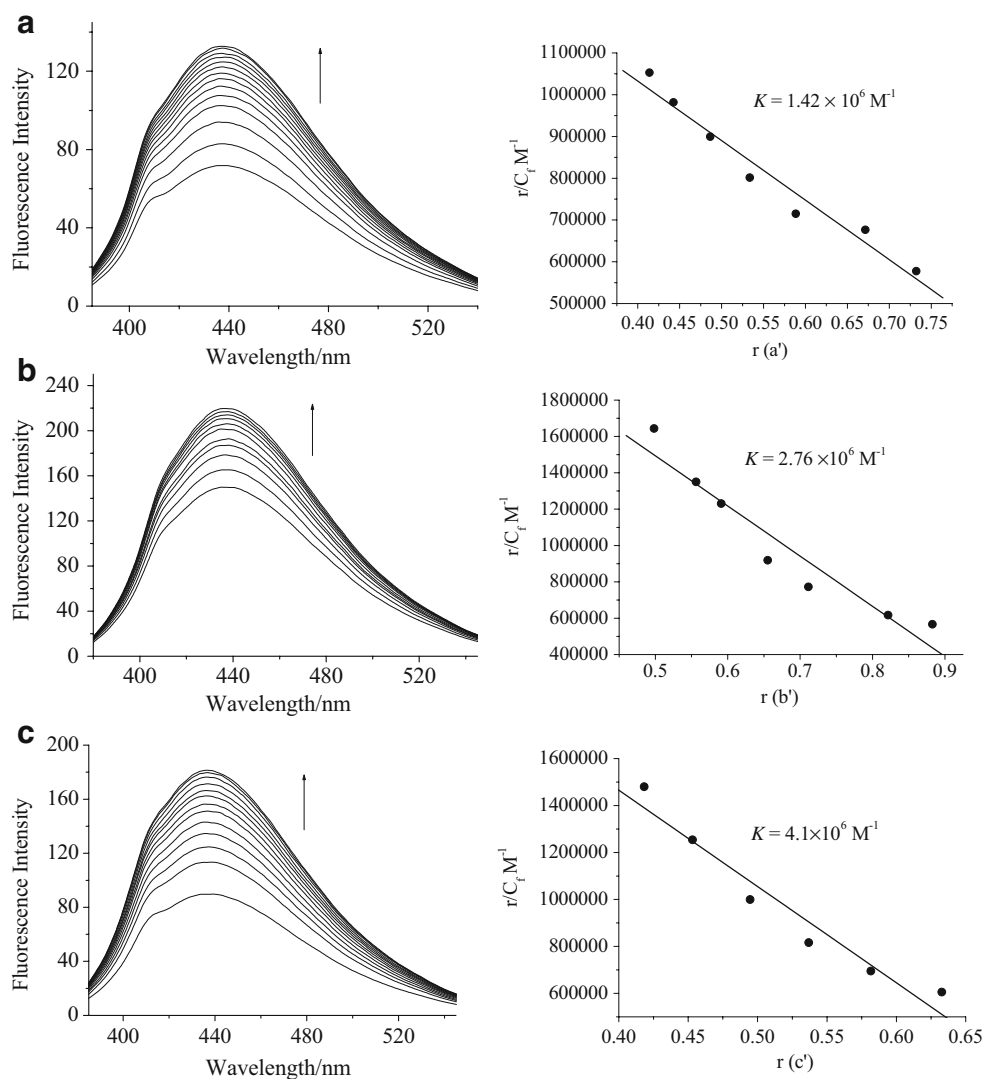
The fluorescence characteristics of the Eu³⁺ complex in organic solvent (concentration: 1×10^{-4} mol L⁻¹, slit: 5 nm) were observed at room temperature. It can be seen from Fig. 3 that the complex has the strongest luminescence in CHCl₃ solution, and then in ethyl acetate, ethanol, acetone, methanol and MeCN. This is due to the coordinating effects of solvents, which is solvate effect [22]. Together with the raising coordination abilities of organic solvent for the lanthanide ions, the oscillatory motions of the entering molecules consume more energy which the ligand triplet level transfer to the emitting level of the lanthanide ion. Thus, the energy transfer could not be carried out perfectly

[23]. The complex is excited at 337 nm in all organic solvent, and emission bands at 577, 590, 614, 677 nm are assigned to ⁵D₀→⁷F₀, ⁵D₀→⁷F₁, ⁵D₀→⁷F₂ and ⁵D₀→⁷F₄. The emission at 614 nm from ⁵D₀→⁷F₂ electronic dipole transition is the strongest, suggesting low symmetry around the Eu(III) ion, in agreement with the crystal structure analysis.

Absorption titration

Various techniques for precise determination of binding constants have been developed and provide information for understanding the nature of the complexes between small molecules and biomacromolecules. Electronic absorption spectroscopy is one of the most useful techniques in DNA-binding studies [24]. The spectrophotometric titration of the ligand, La(III) and Eu(III) complex are shown in Fig. 4a, b and c, which represent its UV–Vis spectra in absence or presence of CT-DNA. The addition of CT-DNA to the

Fig. 5 The emission enhancement spectra of ligand (a), La(III) (b) and Eu(III) complex (c) (concentration=2 μM), in the presence of 0.5–7.0 μM CT-DNA for ligand, 0.5–5.0 μM for La(III) complex and 0.5–6.5 μM for Eu(III) complex. The arrow indicates the emission intensity changes upon increasing DNA concentration. Insert binding constants of ligand (a'), La(III) (b') and Eu(III) complex (c'), 1.42×10^6 M⁻¹ for ligand, 2.76×10^6 M⁻¹ for La(III) complex and 4.1×10^6 M⁻¹ for Eu(III) complex



solutions of the compound at the DNA/compounds molar ratios varying from 0 to 4 induces large hypochromic responses about 54.1% at 287 nm for ligand, 44.9% at 289 nm for La(III) complex and 34.4% at 292 nm for Eu(III) complex. These spectral variation of the complex may be considered as the characteristic features of aromatic chromophore ($\pi \rightarrow \pi^*$) interactions with the nucleic acid bases [25]. In addition, it must be noticed there is an isobathic point observed at 332 nm in ligand, La(III) and Eu(III) complex, respectively. The large hypochromism and isobathic point indicate these three compounds can strongly interact with DNA.

Spectrofluorimetric titration

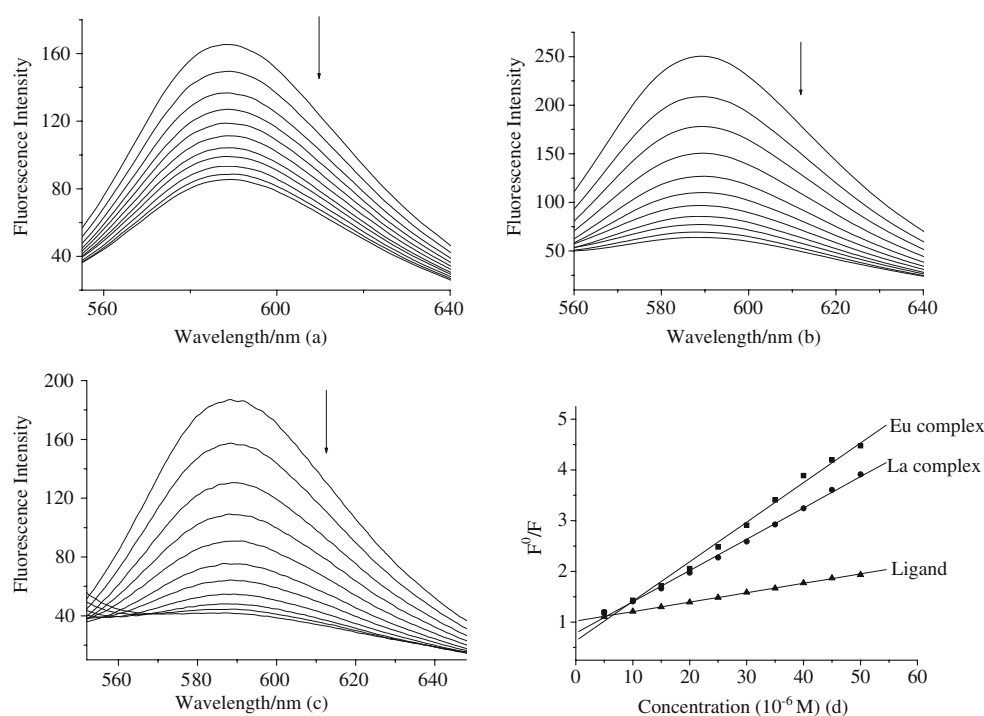
The ligand, La(III) and Eu(III) complex can emit luminescence in Tris buffer at ambient temperature, with a maximum appearing at 437 nm ($\lambda_{\text{ex}}=357$ nm), 435 nm ($\lambda_{\text{ex}}=358$ nm) and 440 nm ($\lambda_{\text{ex}}=360$ nm), respectively. The results of emission titration for three compounds with DNA are illustrated in Fig. 5a, b and c. Upon addition of DNA, the emission intensity of ligand, La(III) and Eu(III) complex grow about 1.9, 1.47 and 2.0 times larger than that in the absence of DNA and saturates at a [DNA]/[compound] ratio of 3.5, 2.5 and 3.25. This implies that the three compounds can interact with DNA and be protected by DNA efficiently, since the hydrophobic environment inside the DNA helix reduces the accessibility of solvent water molecules to the compounds and the compounds' mobility is restricted at the binding site, leading to a

decrease of the vibrational modes of relaxation [26]. Thus, the fluorescence of the three compounds greatly increases. In order to further compare quantitatively the affinity of the compounds bound to CT-DNA, based on the spectrofluorimetric titrations, binding data were cast into the form of a Scatchard plot of r/C_f vs. r , where r is the binding ratio $C_b/[DNA]_t$ and C_f is the free compound concentration. The concentration of the bound compound (C_b) was calculated using Eq [27]:

$$C_b = C_t [(F - F^0)/(F^{\text{max}} - F^0)]$$

Where C_t is the total compounds concentration, F is the observed fluorescence emission intensity at given DNA concentration, F^0 is the intensity in the absence of DNA and F^{max} is the intensity of the totally bound compounds. From the plot of r/C_f versus r (Fig. 5a', b' and c'), the intrinsic binding constants K of the ligand, La(III) and Eu(III) complex were calculated to be $1.42 \times 10^6 \text{ M}^{-1}$, $2.76 \times 10^6 \text{ M}^{-1}$, $4.1 \times 10^6 \text{ M}^{-1}$. The values of the two complexes are somewhat larger than some known DNA intercalators, such as $K=4.8 \times 10^4 \text{ M}^{-1}$ for $[\text{Ru}(\text{bpy})_2(\text{phi})\text{Cl}_2]$ ($\text{phi}=9,10\text{-phenanthrenequinonediimine}$) [28], $2.1 \times 10^4 \text{ M}^{-1}$ for $[\text{Ru}(\text{bpy})_2(\text{ddt})]^{2+}$ ($\text{ddt}=3\text{-}(\text{pyrazin-yl})\text{-5,6-diphenyl-as-triazine}$) [29], $6.3 \times 10^4 \text{ M}^{-1}$ for $[\text{Ru}(\text{bpy})_2(\text{dpt})]^{2+}$ ($\text{dpt}=3\text{-}(\text{pyrazin-yl})\text{-as-triazino}[5,6\text{-f}]\text{phenanthrene}$) [29], and also larger than that observed for $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$ ($>10^6$) [30]. The large K value is the direct evidence that the three compounds have good ability to bind to DNA.

Fig. 6 The emission spectra of DNA-EB system (5 μM DNA and 0.4 μM EB), $\lambda_{\text{ex}}=525$ nm, $\lambda_{\text{em}}=540\text{--}650$ nm, in the presence of 2.5–50 μM ligand (a), La(III) (b) and Eu(III) complex (c). The arrow indicates the emission intensity changes upon increasing compounds concentration. (d) is plots of F_0/F versus $[Q]$. The K_q values for the ligand, La(III) and Eu(III) complex are 1.86×10^4 , 6.17×10^4 and $7.80 \times 10^4 \text{ M}^{-1}$, respectively



EB replacement experiment

The results obtained from absorption titration and spectrofluorimetric titration experiments are enough evidence that the Schiff base ligand, La(III) and Eu(III) complex can effectively bind to DNA. In order to confirm the bind mode, EB replacement was employed. Since EtBr intercalates DNA through interactions with the minor groove, the displacement of EtBr (quantified by fluorescence) by the titration of a compound is suggestive of an intercalative binding [31]. Figure 6a, b and c show the emission spectra of DNA–EB system with increasing amounts of the ligand, La(III) and Eu(III) complex. It is clear that the fluorescence intensity of DNA–EB system greatly decreases upon the addition of the ligand, La(III) and Eu(III) complex. The results imply that the three compounds bind to CT-DNA via the intercalative binding mechanism. According to linear Stern–Volmer Eq. [32]

$$F_0/F = K_q[Q] + 1$$

Where F_0 is the emission intensity in the absence of quencher, F is the emission intensity in the presence of quencher, K_q is the quenching constant, and $[Q]$ is the quencher concentration. The shape of Stern–Volmer plots can be used to characterize the quenching as being predominantly dynamic or static. Plots of F_0/F versus $[Q]$ appear to be linear and K_q depends on temperature. The K_q values for the ligand, La(III) and Eu(III) complex are 1.86×10^4 , 6.17×10^4 and $7.80 \times 10^4 \text{ M}^{-1}$, respectively, as shown in Fig. 6d. This order is well consistent with the results of spectrofluorimetric titration.

Viscosity measurement

Viscosity experiment is the most important method to distinguish between intercalation and groove binding. Since intercalators cause the unwinding and lengthening of DNA, increasing ratios of [intercalator]/[DNA] increase the viscosity

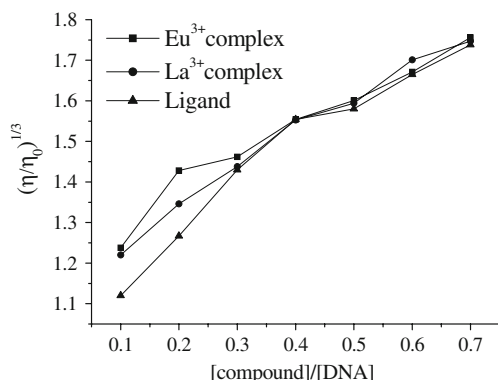


Fig. 7 Effect of increasing amounts of the ligand, La(III) and Eu(III) complex on the relative viscosity of calf thymus DNA at 25.0 °C

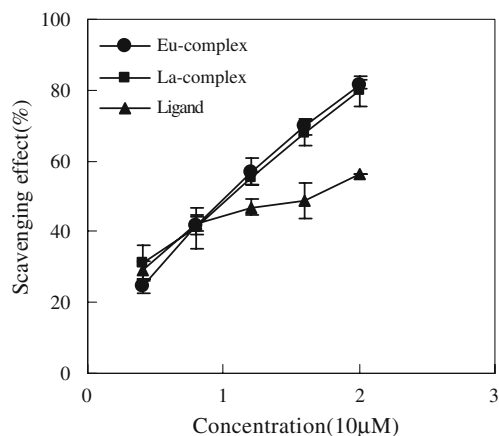


Fig. 8 Scavenging effect of tested compounds on hydroxyl radical. Values are means \pm SD ($n=3$)

of a DNA containing solution [33]. The effects of the ligand, La(III) and Eu(III) complexes on the viscosity of DNA are shown in Fig. 7. It is found that the viscosity of DNA increases steadily with the increase of the concentration of the compounds, which is similar to that of a classical intercalator EB [25]. The results demonstrate that the three compounds bind to DNA through the same way, i.e., the classical intercalation mode, which also parallels the pronounced emission quenching in the EB replacement experiment.

Antioxidant activities

Furthermore, the excellent DNA-binding properties of the Schiff base ligand, La(III) and Eu(III) complex encourage our desire to research their scavenging ability against hydroxyl radical (OH^\bullet) and superoxide radical ($\text{O}_2^{\bullet-}$), which are in close relationship with cancer.

The scavenging ability of the ligand, La(III) and Eu(III) complexes against hydroxyl radical is shown in Fig. 8. It can be seen that the inhibitory effect of three compounds on hydroxyl radical is related to concentration, and at a

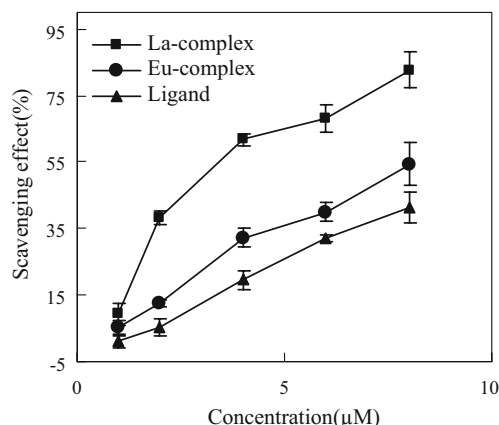


Fig. 9 Scavenging effect of tested compounds on superoxide radicals. Values are means \pm SD ($n=3$)

concentration from 4 to 20 μM , the percentage scavenging effect valued from about 9.11% to 56.33% for ligand, 31.01% to 79.75% for La(III) complex, and 24.68% to 81.65% for Eu(III) complex. It is obvious that scavenging activities of the two complexes are stronger than that of ligand.

Figure 9 shows the scavenging ability of the ligand, La(III) and Eu(III) complexes against superoxide radical. The inhibitory effect of three compounds on superoxide radical is also related to concentration, and at a concentration from 1 to 8 μM , the percentage scavenging effect valued from about 1.32% to 41.68% for ligand, 9.26% to 82.72% for La(III) complex, and 5.06% to 54.43% for Eu(III) complex. Compared to the results of hydroxyl radical experiment, two complexes also exhibit more scavenging ability than free ligand throughout the tested concentration, but the La(III) complex is the strongest, not Eu(III) complex. The results show that the three compounds' ability against superoxide radical is significantly higher than that of previous reported natural products [34].

Conclusion

A new chromone Schiff base, 7-methoxychromone-3-carbaldehyde benzoyl hydrazone, and its La(III) and Eu(III) complexes were reported. The structure of La(III) complex was determined by X-ray crystallography. It is notable that the ligand in La(III) complex has good planar property. It is this planar structure that has result in higher DNA-binding constant of complexes and intercalation mode. What is more, the complexes have excellent ability of antioxidant and the Eu(III) complex can emit its characteristic fluorescence in different organic solvent. These results suggest that the 7-methoxychromone-3-carbaldehyde benzoyl hydrazone is not only a good ligand to form biological rare earth complexes but also a good organic chromophore to absorb and transfer energy to Eu(III) ions. It is clear that the Eu(III) complex can be a good candidate for biological use.

Electronic supplementary materials

Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre, CCDC 670916. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk).

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