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Original article

Synthesis, characterization, antioxidant activity and DNA-binding studies of two rare earth(III) complexes with naringenin-2-hydroxy benzoyl hydrazone ligand

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Abstract

Two novel rare earth complexes, Y(III) complex (**1**) and Eu(III) complex (**2**), with naringenin-2-hydroxy benzoyl hydrazone ligand were synthesized and characterized. The interaction of the two metal complexes and the free ligand with calf thymus DNA (CT DNA) was investigated by electronic absorption spectroscopy, fluorescence spectroscopy and viscosity measurement. All the experimental evidences indicate that these three compounds can strongly bind to CT DNA via an intercalation mechanism. The intrinsic binding constants of the Y(III) complex (**1**), Eu(III) complex (**2**) and the free ligand with CT DNA were 2.1×10^4, 8.5×10^4 and 1.6×10^4 M^{-1}, respectively. Furthermore, the antioxidant activity of the metal complexes was determined by hydroxyl radical scavenging method in vitro.

Keywords: Naringenin-2-hydroxy benzoyl hydrazone; Rare earth(III) complex; DNA binding; Antioxidant activity

1. Introduction

It is well known that deoxyribonucleic acid (DNA) is an important genetic substance in organisms. Errors in gene expression can often cause disease and play a secondary role in the outcome and severity of human disease. Medicinal agents can affect gene expression by facilitating, mimicking or inhibiting any one of the properties that exists in typical transcriptional systems [1]. Some researches also show that many chemicals exert their antitumor effects through binding to DNA thereby changing the replication of DNA and inhibiting the growth of the tumor cell, which is the basis of designing new and more efficient antitumor drugs [2–5].

Binding studies of small molecules to DNA are very important in the development of DNA molecular probes and new therapeutic reagents [6–8]. Over the past decades, the DNA-binding metal complexes have been extensively studied as DNA structural probes, DNA-dependent electron transfer probes, DNA footprinting and sequence-specific cleaving agents and potential anticancer drugs [9–12]. The interaction of metal complexes with DNA is an area of intense interest to both inorganic chemists and biochemists. Many transition metal complexes, especially Ru(II) and Cu(II) complexes of polypyridine or 1,10-phenanthroline ligand, have been extensively studied [13–18]. While, rare earth metal complex has not been given much attention, with the research of rare earth compounds, the study for synthesis of rare earth complexes is appealing [19–22].

Naringenin (4′,5,7–trihydroxyflavanone), a predominant flavanone, is widely spread in nature and easily extracted from a lot of different plants. Its protective effect against lipid peroxidation of membranes, involved in several physiological and pathological disorders, as aging, inflammation, atherosclerosis, ischemia, toxicity of oxygen and chemical substances has been largely studied [23]. Our previous work showed that the rare earth complexes of naringenin ramifications can bind to DNA in intercalative mode and they also have certain antioxidative and cytotoxic activities [24,25].

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In order to give a deep research to naringenin ramifications and their metal complexes, in this paper, we synthesized and characterized a ligand, naringenin-2-hydroxy benzoyl hydrazone (H_5L, Fig. 1), and its Y(III), Eu(III) complex. DNA-binding model of these three compounds was investigated by electronic absorption spectroscopy, fluorescence spectroscopy and viscosity measurement. Experimental evidence indicates that these compounds can strongly bind to CT DNA through an intercalative mode. Furthermore, the antioxidant activity of the metal complexes was determined by hydroxyl radical scavenging method in vitro. Results suggest that the complexes possess potent inhibitory activity. Information obtained from this study provides useful insights into the mechanism of interactions of rear earth complex with DNA, and it will also be helpful to develop some new antioxidants.

2. Results and discussion

2.1. Characterization of complexes

The two metal complexes are air stable for extended at least six months and soluble in methanol, ethanol, dimethylsulphoxide (DMSO) and *N*,*N*-dimethylformamide (DMF); slightly soluble in chloroform and acetone; insoluble in water.

The structures of the complexes were characterized by elemental analyses, molar conductivities and IR spectra. The elemental analyses show that the formulas of the complexes conform to $M(H_3L)(H_4L)$ [where M = Y(III) and Eu(III); H_5L is the ligand naringenin-2-hydroxy benzoyl hydrazone]. The molar conductivities in methanol indicate that the Y(III) complex (**1**) and Eu(III) complex (**2**) (65.2 and 30.2 S cm^2 mol^{-1}) are in the range expected for non-electrolytes [26].

IR spectra provide a lot of valuable information on coordination reaction. All the spectra are characterized by vibrational bands mainly due to the NH, OH, C═O and C═N groups. The ν (O—H, N—H) for the ligand appears at 3212 cm^{-1}, and this peak for the complexes shifts to 3384 cm^{-1} or so. The ν (C═O) vibration of the free ligand is at 1638 cm^{-1}; for the complexes **1** and **2**, the peak shifts to 1605 and 1610 cm^{-1}, respectively. $\Delta\nu_{(ligand-complex)}$ is 33 and 28 cm^{-1}, respectively. In the complexes, the band at 579 cm^{-1} or so is assigned to ν (M—O). It demonstrates that the oxygen of carbonyl has formed a coordinative bond with metal ion [27]. The band at 1607 cm^{-1}, for the free ligand, is assigned to the ν (C═N) stretch. It shifts to 1560 cm^{-1} for its complexes; $\Delta\nu_{(ligand-complex)}$ is equal to 47 cm^{-1}. Weak bands at 416 cm^{-1} or so are assigned to ν (M—N) in the complexes. It confirms that the nitrogen of the imino-group bonds to metal ion [28]. For complexes **1** and **2**, the δ_{MOH} vibrations are at 1195 and 1190 cm^{-1}, respectively. It shows that −OH group has formed a coordinative bond with metal ion, so gives a δ_{MOH} vibration [29].

On the basis of elemental analyses, molar conductivities and IR spectra, the likely structure of the complexes is shown in Fig. 2. The complexes have also been unambiguously characterized through ^{1}H NMR spectral and mass spectral analysis. For the complexes, due to the influence of extranuclear electrons of metals, the signals of ^{1}H NMR spectra become broad and weak. Different chemical shifts are also observed in the complexes and these changes are attributed to coordination of the ligand to metal ions. The electrospray ionization (ESI) mass spectrum of complex **1** is shown in Fig. 3. Fig. 4 demonstrates the proposed fragments of complex **1**. The mass spectrum of complex **1** shows peaks at *m*/*z* of 805.3 and 672.5 which can be assigned to fragments $\mathbf{F_1}$ and $\mathbf{F_2}$, respectively.

2.2. Electronic absorption spectroscopy

Electronic absorption spectroscopy is universally employed to determine the binding characteristics of metal complex with DNA [30]. Intercalative mode of binding usually results in hypochromism and red shift due to the strong stacking interaction between an aromatic chromophore and the base pairs of

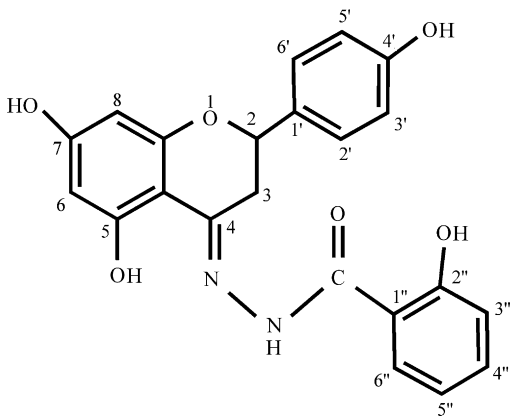

Fig. 1. Chemical structure of the ligand (H_5L).

Fig. 2. Proposed structure of the complexes.

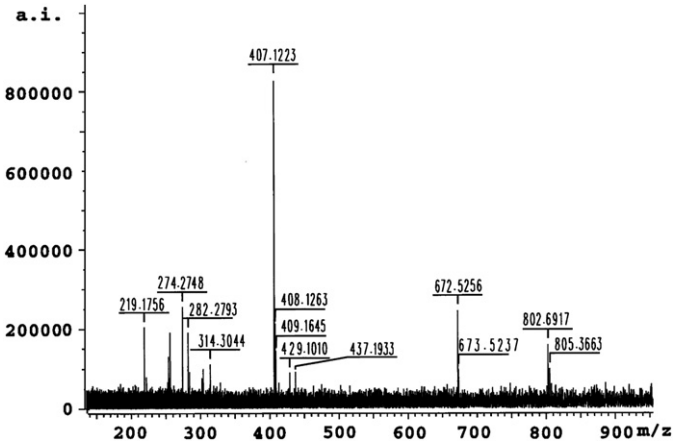

Fig. 3. Electrospray ionization (ESI) mass spectrum of the Y(III) complex (**1**).

DNA. The extent of red shift and hypochromism are commonly found to correlate with the intercalative binding strength. But, metal complexes which bind non-intercalatively or electrostatically with DNA may result in hyperchromism or hypochromism [31–33].

The absorption spectra of complex **1** in the absence and presence of CT DNA are shown in Fig. 5(a). As seen from the figure, for complex **1**, with increasing concentration of DNA hypochromism reaches 43.9% at 224 nm and 29.2% at 365 nm in the ratio of [DNA]/[complex **1**] = 3. Likewise, with increasing DNA concentrations, the absorption bands at 225 and 352 nm for the ligand appear with hypochromism of 43% and 22.4%; the absorption bands at 224 and 354 nm for complex **2** also appear with different hypochromism of 45.7% and 34.5%. The hypochromisms observed for the bands of these three compounds are accompanied by a small red shift by less than 4 nm. The above phenomena imply that these compounds interact with CT DNA by intercalating the compounds into the base pairs of double helical DNA [34–36].

To compare quantitatively the affinity of these three compounds binding to DNA, the intrinsic binding constant (K_b) has been estimated. From absorption data, K_b was determined using the following equation (1) [37] through a plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA]

$$[\mathrm{DNA}]/(\varepsilon_a - \varepsilon_f) = [\mathrm{DNA}]/(\varepsilon_a - \varepsilon_f) + 1/[K_b(\varepsilon_b - \varepsilon_f)], \quad (1)$$

where [DNA] is the concentration of DNA, ε_a, ε_f and ε_b are, respectively, the apparent extinction coefficient (A_{obsd}/[compound]), the extinction coefficient for free compound and the extinction coefficient for compound in the fully bound form. In plots of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA], K_b is given by the ratio of the slope to the intercept.

The K_b value obtained from Fig. 5(b) for complex **1** is $2.1 \times 10^4\ M^{-1}$. Likewise, K_b of the ligand and complex **2** has been estimated to be 1.6×10^4 and $8.5 \times 10^4\ M^{-1}$, respectively. Comparing the intrinsic binding constant (K_b) of these three compounds with those of DNA-intercalative complexes ([Cu(L-Phe)(TATP)(H_2O)]$^+$, $4.7 \times 10^3\ M^{-1}$ [30]; [Ru(bpy)$_2$(DMHBT)]Cl_2 and [Ru(phen)$_2$(DMHBT)]Cl_2, $(2.87 \pm 0.2) \times 10^4$ and $(1.01 \pm 0.2) \times 10^5\ M^{-1}$, respectively [33]; [(phen)Cu(μ-bipp)Cu(phen)]$^{4+}$, $1.6 \times 10^4\ M^{-1}$ [34]; [Ru(phen)$_2$OBIP]$^{2+}$ and [Ru(phen)$_2$PBIP]$^{2+}$, 3.73×10^4 and $5.91 \times 10^4\ M^{-1}$, respectively [37]), we deduce that these compounds bind to DNA by intercalation. These values also show that complex **2** binds to DNA more avidly than complex **1**, and the free ligand has weakest binding affinity.

2.3. Fluorescence spectroscopy

The free ligand, complexes **1** and **2** can emit luminescence in Tris buffer with a maximum wavelength of about 450 nm. Fig. 6 shows the emission spectrum of complex **2** in the absence and presence of varying amounts of DNA. As seen from the figure, the intensity of emission at 450 nm increases appreciably in the presence of DNA. For the ligand, complexes **1** and **2**, the emission intensities of these compounds grow to around 1.11, 1.17, 1.33 times larger than those in the absence of DNA at a [DNA]/[compound] ratio of 1.75, respectively.

The enhancements of emission intensity imply that these compounds can insert between DNA base pair. As a result, these compounds are protected from solvent water molecules by the hydrophobic environment inside the DNA helix; the accessibility of solvent water molecules to these compounds is reduced. The binding of these three compounds to DNA leading to a marked increase in emission intensity also agrees with those observed for other intercalators [24,37]. Compared to the intensity enhancement of these compounds in the presence of DNA, complex **2** can bind to DNA more strongly than complex **1** and the ligand.

For further research the interaction of these three compounds with DNA, the steady-state emission quenching experiment was given. Competitive binding to DNA of the free ligand and its complexes with ethidium bromide (EB) could provide rich information regarding DNA-binding nature and relative DNA-binding affinity [38]. Due to strong intercalation between the adjacent DNA base pairs, EB emits intense fluorescence in the presence of DNA. It was previously reported that the enhanced fluorescence could be quenched, at least partially, by addition of a second intercalative molecule. The quenching extent of fluorescence for EB bound to DNA is used to determine the relative DNA-binding affinity of the second molecule [30].

The emission band at 578 nm of the DNA–EB system decreased in intensity with an increase in the concentration of the compounds, which indicated that the compounds could displace EB from the DNA–EB system. Fig. 7(a) displays a well-behaved emission spectrum of EB bound to DNA in the absence and presence of complex **2**. According to the classical Stern–Volmer equation (2) [39]:

$$F_0/F = K_q[Q] + 1, \quad (2)$$

where F_0 and F represent the emission intensities in the absence and presence of quencher, respectively. [Q] is the quencher concentration. K_q is a linear Stern–Volmer quenching constant. The K_q value is obtained as the slope of F_0/F versus [Q] linear plot. As shown in Fig. 7(b), the K_q for complex **2** is $6.38 \times 10^4\ M^{-1}$. Likewise, the K_q values for the ligand and

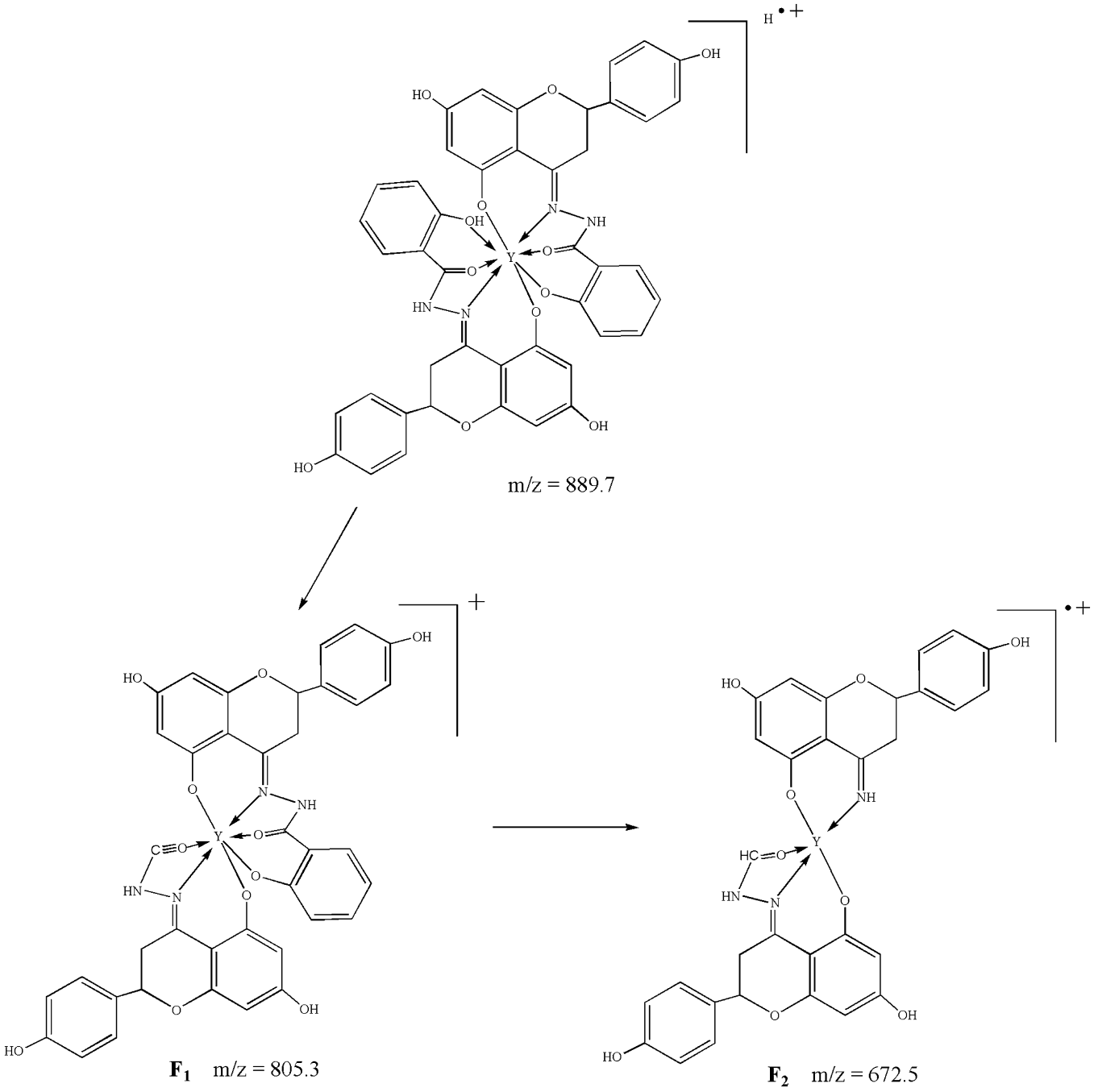

Fig. 4. Proposed degradation of the ligand in Y(III) complex (**1**).

complex **1** are 0.93×10^4 and $1.74 \times 10^4\ M^{-1}$, respectively. Such values of quenching constant suggest that the interaction of these compounds with DNA is of intercalation [24], and the interaction of the complex **2** with DNA is stronger than that of the complex **1** and free ligand.

2.4. Viscosity studies

Hydrodynamic measurements that are sensitive to the length change (i.e., viscosity and sedimentation) are regarded as the least ambiguous and the most critical tests of a binding model in solution in the absence of crystallographic structural data [40,41]. As a means for further clarifying the binding of these compounds with DNA, viscosity studies were carried out. Data are presented as $(\eta/\eta_0)^{1/3}$ versus $1/R$, where $R = [\text{DNA}]/[\text{compound}]$; η and η_0 are the relative viscosities of DNA in the presence and absence of compound, respectively. The relative viscosity values were calculated from the flow time of DNA-containing solution (t) and the flow time of buffer alone (t_0), using the following expression (3) [42]:

$$\eta = (t - t_0)/t_0, \tag{3}$$

Fig. 8 shows the relative viscosity of DNA (50 μM) in the presence of varying amounts of the ligand, complexes **1** and **2**.

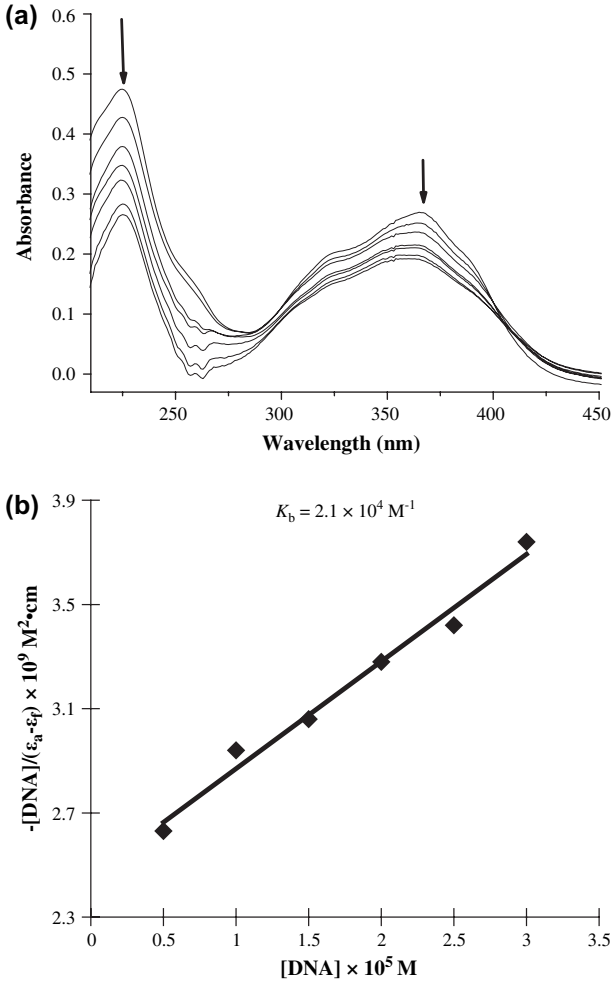

Fig. 5. (a) Absorption spectra of Y(III) complex (**1**) (10 μM) in the absence (top spectrum) and presence of increasing amounts of calf thymus DNA (5, 10, 15, 20, 25 and 30 μM; subsequent spectra). Arrow shows the absorbance changes upon increasing DNA concentration. (b) Plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] for the titration of complex **1** with CT DNA; ◆, experimental data points; full lines, linear fitting of the data. Intrinsic binding constant $K_b = 2.1 \times 10^4\ M^{-1}$.

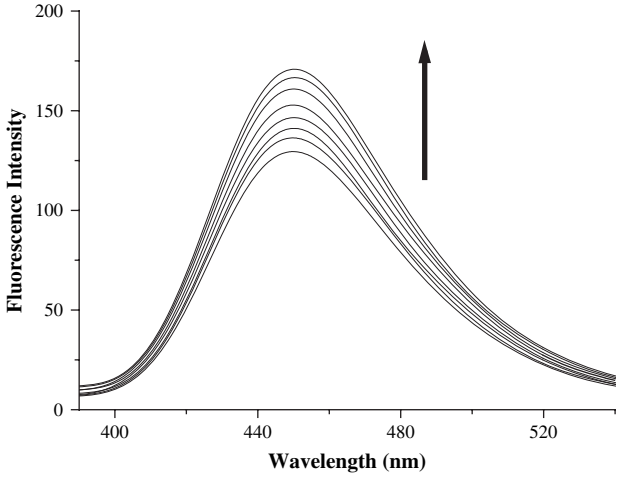

Fig. 6. Emission enhancement spectra of Eu(III) complex (**2**) (10 μM) in the absence (bottom spectrum) and presence of increasing amounts of calf thymus DNA (2.5, 5.0, 7.5, 10.0, 12.5, 15.0 and 17.5 μM; subsequent spectra). Arrow shows the emission intensity changes upon increasing DNA concentration.

It shows that these compounds can intercalate between adjacent DNA base pairs, causing an extension in the helix, and thus increase the relative viscosity of DNA [32,33]. However, the large increase in the relative viscosity values for complex **2** reveals that complex **2** is the best intercalator of the three. The results obtained from viscosity studies validate those obtained from the spectroscopic studies.

2.5. Hydroxyl radical scavenging activity

Generation of reactive oxygen species (ROS) is a normal process in the life of aerobic organisms. It has been estimated that free radical-induced DNA damage in humans is at biologically relevant levels, with approximately 10^4 DNA bases being oxidatively modified per cell per day. Oxidative damage to DNA has been suggested to contribute to aging and various diseases including cancer and chronic inflammation [43]. Since among all reactive oxygen species, the hydroxyl radical ($OH^•$) is by far the most potent and therefore the most dangerous oxygen metabolite, elimination of this radical is one of the major aims of antioxidant administration [44].

The hydroxyl radicals ($OH^•$) in aqueous media were generated through the Fenton system. The hydroxyl radical bleached the safranin, so decreased the absorbance of the reaction mixture indicating a decrease in hydroxyl radical scavenging ability. The scavenging ratio for $OH^•$ was calculated from the following expression (4):

$$\text{Scavenging ratio}(\%) = [(A_i - A_0)/(A_c - A_0)] \times 100, \qquad (4)$$

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.

Fig. 9(a) depicts the inhibitory effect of the complexes on $OH^•$. The inhibitory effect of the complexes is marked and suppression ratio increases with increasing concentration in the range of tested concentration. The order of the suppression ratio for $OH^•$ is complex **1** > complex **2** at different concentrations. In comparison with Cu(II), Ni(II) and Zn(II) complexes of the free ligand that were studied in previous paper [25], the hydroxyl radical scavenging activity of complexes **1** and **2** is slightly weaker than these three transition metal complexes and the ligand at tested concentrations. Moreover, mannitol is a well-known natural antioxidant, so we also studied the scavenging activity of mannitol against hydroxyl radical using the same model. As shown in Fig. 9(b), the 50% inhibitory concentration (IC_{50}) value of mannitol is 9.6 mM. Although the IC_{50} value of complexes **1** and **2** cannot be read in Fig. 9(a), when arrived at similar suppression ratio, concentration of the two complexes is far less than that of mannitol.

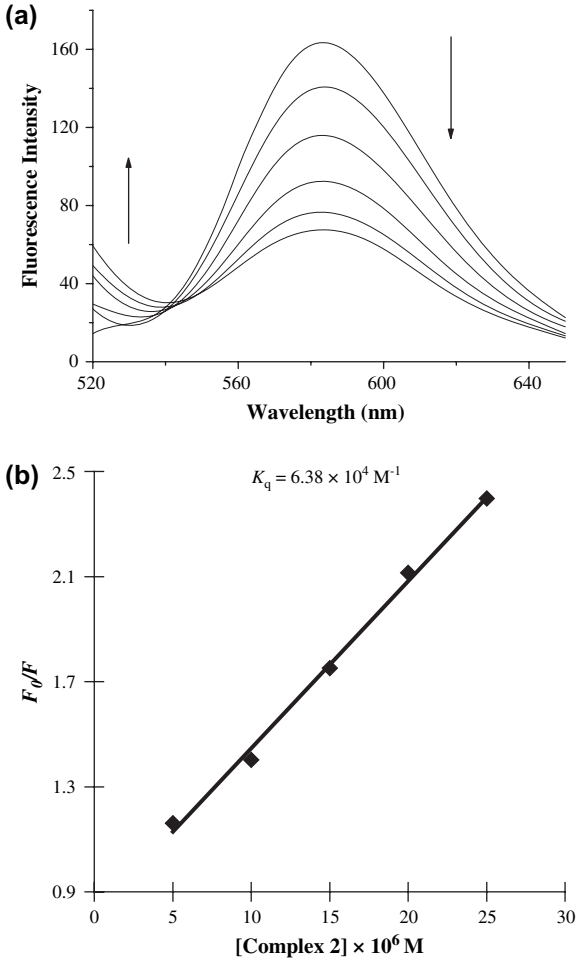

Fig. 7. (a) Emission spectra of DNA–EB system (10 μM DNA and 0.33 μM EB), $\lambda_{ex} = 500$ nm, $\lambda_{em} = 520 - 650$ nm, in the presence of 0, 5, 10, 15, 20 and 25 μM Eu(III) complex (**2**). Arrow shows the emission intensity changes upon increasing Eu(III) complex (**2**) concentration. (b) Stern–Volmer plot of the fluorescence titration data of Eu(III) complex (**2**). Quenching constant $K_q = 6.38 \times 10^4\ M^{-1}$.

3. Conclusion

Two novel rare earth complexes, Y(III) and Eu(III) complex, were synthesized and characterized. DNA-binding studies indicate that the two complexes and their free ligand (naringenin-2-hydroxy benzoyl hydrazone) can interact with calf thymus DNA by intercalation mechanism. The intrinsic binding constant shows that complex **2** ($8.5 \times 10^4\ M^{-1}$) and complex **1** ($2.1 \times 10^4\ M^{-1}$) binds to DNA more avidly than the free ligand ($1.6 \times 10^4\ M^{-1}$). Probably, chelating effect (metal ion to free ligand) can enhance the planar functionality of metal complex, so the complexes can insert and stack between the base pairs of double helical DNA more easily than the free ligand [37].

The intrinsic binding constant of the two complexes is similar to that of some Ru(II) and Cu(II) complexes of polypyridine or 1,10-phenanthroline ligand. It suggests that the two rare earth complexes have similar ability to interact with double-stranded DNA as those transition metal complexes. Furthermore, the two rare earth complexes have active scavenging effect on OH•.

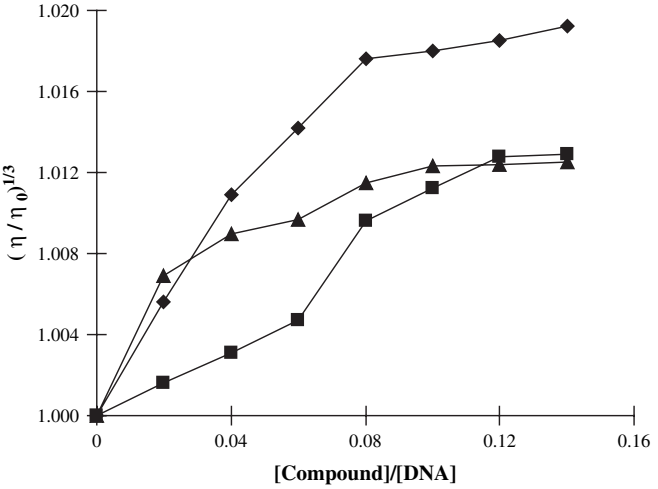

Fig. 8. Effect of increasing amounts of Y(III) complex (**1**) (▲), Eu(III) complex (**2**) (◆) and ligand (■) on the relative viscosity of calf thymus DNA at 30.0 ± 0.1 °C, [DNA] = 50 μM.

4. Experimental

4.1. Materials and methods

Calf thymus DNA (CT DNA) and ethidium bromide (EB) were purchased from Sigma Chemical Co. All other chemicals were of analytical reagent grade and used without further purification unless otherwise noted.

All the experiments involving interaction of the metal complexes and the free ligand with DNA were carried out in doubly distilled water buffer containing 5 mM Tris [Tris(hydroxymethyl)-aminomethane] 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 ($6600\ M^{-1}\ cm^{-1}$) at 260 nm. The free ligand and its metal complexes were dissolved in a mixture solvent of 1% ethanol and 99% Tris–HCl buffer (5 mM Tris–HCl, 50 mM NaCl, pH 7.1) at a concentration of 1.0×10^{-5} M.

Elemental analyses (C, H and N) were performed on an Elemental Vario EL analyzer. The metal contents of the complexes were determined by titration with EDTA. Infrared spectra ($4000 - 400\ cm^{-1}$) were determined with KBr disks on a Thermo Nicolet FT-IR spectrometer. UV–vis spectra were recorded on a Varian Cary 100 Conc spectrophotometer using 10 mm path length quartz cells. Fluorescence spectra were recorded on a Hitachi RF-4500 fluorescence spectrophotometer. ^{1}H NMR spectra were recorded in DMSO-d_6 on a Varian VR 300 MHz spectrometer. Chemical shifts were shown as a δ-value (ppm) with tetramethyl silane (TMS) as an internal standard. Electrospray ionization (ESI) mass spectrometry was recorded on APEX II FT-ICR MS using

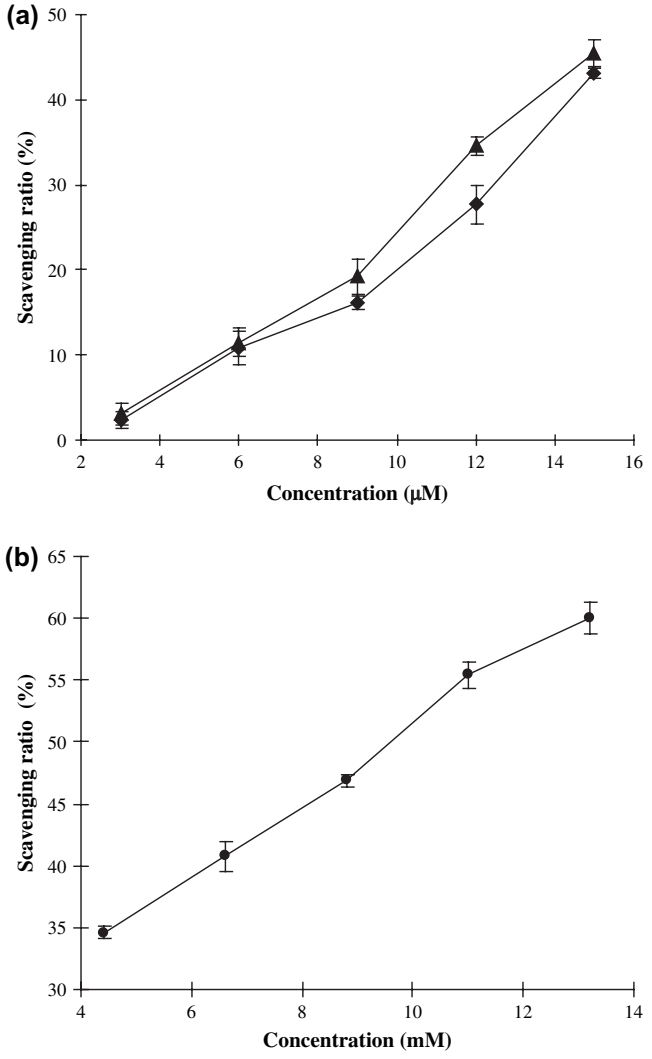

Fig. 9. (a) Scavenging effect of metal complexes on hydroxyl radicals. (▲) Y(III) complex (**1**); (◆) Eu(III) complex (**2**). (b) Scavenging effect of mannitol on hydroxyl radicals. (●) Mannitol. Experiments were performed in triplicate. Values are expressed as mean ± standard deviation ($n = 3$).

methanol as mobile phase. Molar conductance values were determined in methanol on a DDS-11C conductivity meter (Shanghai Leici Factory, China).

4.2. Preparations of the free ligand and its metal complexes

The free ligand, naringenin-2-hydroxy benzoyl hydrazone (H_5L, Fig. 1), was prepared according to the previous literature methods [25]. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.1 (2H, doublet (d), $J = 10.5$ Hz, 3-H), 5.1 (1H, d, $J = 10.5$ Hz, 2-H), 5.9 (1H, singlet (s), 6-H), 6.0 (1H, s, 8-H), 6.8 (2H, d, $J = 8.4$ Hz, 3′,5′-H), 6.9 (2H, triplet (t), $J = 7$ Hz, 3″,5″-H), 7.3 (2H, d, $J = 8.4$ Hz, 2′,6′-H), 7.4 (1H, t, $J = 7$ Hz, 4″-H), 7.9 (1H, d, $J = 7$ Hz, 6″-H), 9.6, 10.0, 11.3, 11.7 (4H, s, O–H, D_2O exchangeable), 12.9 (1H, s, N–H, D_2O exchangeable). IR (KBr): 3212, 1638, 1607 cm^{-1}.

Y(III) complex (**1**) was prepared as follows. The ligand (0.20 g, 0.5 mmol) and $Y(NO_3)_3 \cdot 6H_2O$ (0.096 g, 0.25 mmol) were dissolved in acetone (20 ml). Under stirring, triethylamine (0.076 g, 0.75 mmol) was then dropped to the mixture with caution. Immediately, there was a yellow precipitate in solution. After stirring for 5 h at room temperature, the precipitate, Y(III) complex (**1**), was separated by suction filtration, purified by washing several times with acetone, and dried in vacuum. Eu(III) complex (**2**) was synthesized in a similar manner as that used for complex **1**, reacting a mixture of the ligand (0.20 g, 0.5 mmol) and $Eu(NO_3)_3 \cdot 6H_2O$ (0.112 g, 0.25 mmol).

Complex **1**: yield, 71%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.9 (2H, broad (b), 3-H), 5.0 (1H, b, 2-H), 5.2 (1H, b, 6-H), 5.4 (1H, b, 8-H), 6.6 (2H, b, 3′,5′-H), 6.7 (2H, b, 3″,5″-H), 7.2 (2H, b, 2′,6′-H), 7.3 (1H, b, 4″-H), 7.8 (1H, b, 6″-H), 8.5, 9.5 (2H, b, O-H). Anal. Calcd for $C_{44}H_{33}N_4O_{12}Y$: C, 58.80; H, 3.71; N, 6.24; Y, 9.89. Found: C, 58.97; H, 3.75; N, 6.01; Y, 9.98. IR (KBr): 3384, 1605, 1560, 1195, 579, 413 cm^{-1}. Λ_m (CH_3OH): 65.2 S cm^2 mol^{-1}. Complex **2**: yield, 69%. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.1 (2H, b, 3-H), 5.1 (1H, b, 2-H), 5.8 (1H, b, 6-H), 5.9 (1H, b, 8-H), 6.6 (2H, b, 3′,5′-H), 6.8 (2H, b, 3″,5″-H), 7.3 (2H, b, 2′,6′-H), 7.4 (1H, b, 4″-H), 7.9 (1H, b, 6″-H), 9.4, 9.9 (2H, b, O-H). Anal. Calcd for $C_{44}H_{33}N_4O_{12}Eu$: C, 54.94; H, 3.47; N, 5.83; Eu, 15.80. Found: C, 54.69; H, 3.44; N, 5.61; Eu, 16.05. IR (KBr): 3254, 1610, 1560, 1190, 582, 416 cm^{-1}. Λ_m (CH_3OH): 30.2 S cm^2 mol^{-1}.

4.3. Electronic absorption spectroscopy

Absorption titration experiment was performed by maintaining the free ligand or its metal complex concentration constant (10 μM) and gradually increasing the concentration of nucleic acid. The reference solution was the corresponding Tris–HCl buffer solution (5 mM Tris–HCl, 50 mM NaCl, pH 7.1). While measuring the absorption spectra, equal amount of DNA was added to both compound solution and the reference solution to eliminate the absorbance of DNA itself. Each sample solution was scanned in the range 210–450 nm.

4.4. Fluorescence spectroscopy

For all fluorescence measurements, the entrance and exit slits were both maintained at 2.5 nm. The sample was excited at 322 nm and its emission observed at 450 nm. Fixed amounts of the compound (10 μM) were titrated with increasing amounts of DNA, over a range of DNA concentrations from 2.5 to 17.5 μM. All experiments were conducted at room temperature (25 ± 1 °C) in a buffer containing 5 mM Tris–HCl (pH 7.1) and 50 mM NaCl concentrations. In steady-state emission quenching experiment, a 2 mL solution of 10 μM DNA and 0.33 μM EB was titrated by tested compounds ($\lambda_{ex} = 500$ nm, $\lambda_{em} = 520 - 650$ nm).

4.5. Viscosity measurements

Viscosity experiment was carried out on an Ubbdlodhe's viscometer, immersed in a thermostated water bath maintained

at 30.0 ± 0.1 °C. DNA concentration was kept constant (50 μM) and gradually increased the concentration of tested compounds. Flow time was measured with a stopwatch. Each sample was measured three times and an average flow time was calculated.

4.6. Hydroxyl radical scavenging assay

The hydroxyl radicals ($OH^{\bullet}$) in aqueous media were generated through the Fenton system. Solution of the complexes was prepared with DMF. According to Ref. [45], 5 ml assay mixture contained following reagents: safranin (11.4 μM), EDTA–Fe(II) (40 μM), H_2O_2 (1.76 mM) and the tested compound (3, 6, 9, 12 and 15 μM) in a phosphate buffer (67 mM, pH 7.4). The assay mixtures were incubated at 37 °C for 50 min in a water bath. After which, the absorbance was measured at 520 nm against a blank. All the tests were run in triplicate and expressed as the mean ± standard deviation (SD).

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