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Original article

Synthesis, crystal structure, antioxidant activities and DNA-binding studies of the Ln(III) complexes with 7-methoxychromone-3-carbaldehyde-(4′-hydroxy) benzoyl hydrazone

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ABSTRACT

A novel chromone Schiff base, 7-methoxychromone-3-carbaldehyde-(4′-hydroxy) benzoyl hydrazone (L) and its Ln(III) complexes (Ln = La, Eu) were synthesized and characterized. The crystal structure of the La(III) complex was determined by single-crystal X-ray diffraction: crystallized in the orthorhombic system, space group *Pbcn*, $Z = 8$, $a = 14.595(2)$ Å, $b = 20.055(3)$ Å, $c = 30.078(5)$ Å, $R_1 = 0.0657$. Fluorescence titration spectra, electronic absorption titration spectra, EB displacement and viscosity measurement indicated that both the ligand and the complexes can bind to DNA via the intercalation mode, and that the binding affinity of the La(III) complex is higher than that of the Eu(III) complex and of the ligand (L). The antioxidant activity experiments show that these compounds also exhibit good antioxidant activities against $^{\bullet}OH$ and $O_2^{\bullet-}$. Moreover, the Eu(III) complex exhibits characteristic fluorescence of europium ion in different organic solvents.

1. Introduction

Binding of small molecules with DNA has been studied extensively since DNA is the material of inheritance and controls the structure and function of cells [1]. Small molecules possessing DNA-binding abilities include metal complexes, porphyrins, natural antibiotics, simple aromatic hydrocarbons and some heterocyclic cations [2–7]. The process, denoted as intercalation, can produce deep alterations in the nucleotide secondary structure [8,9] with major consequences for DNA replication and transcription [10]. Actually, the activity of many anticancer, antimalarial and antibacterical agents, and that of aromatic carcinogens finds its origin in intercalative interactions [11]. Therefore, in order to generate new drugs for novel applications, many efforts have been devoted to design and synthesize small molecules that could specifically target polynucleotide sites or sequences through intercalating to DNA. The tested molecules are usually concentrated on condensed aromatic compounds, since the presence of a planar hydrophobic residue is an essential requirement for intercalation. Moreover, lanthanide ions are subjects of increasing interest in bioinorganic and coordination chemistry [12,13]. Lanthanide complexes with tetracycline, phenanthroline [14], adriamycin and pyridine [15–18] have been synthesized as a probe to study nucleic acids.

Of particular interests, chromone derivatives which have aromatic planar structure show wide variety of biological activities [19–21]. Based on the above consideration, we suppose that complexes of chromone with lanthanides could present interesting biological activities. In our previous work, the lanthanide complexes derived from 6-hydroxy-3-carbaldehyde chromone Schiff base have been reported and all the complexes can strongly interact with DNA through intercalation mode [22–24]. It is well known that the different substitute and its different situation can affect the pharmacological activities and DNA-binding abilities of compound. More importantly, a recent epidemiological study provides strong support that the methoxylated chromones have potential cancer preventive properties [25]. In this regard, as a further research, the Schiff base ligand, 7-methoxychromone-3-carbaldehyde-(4′-hydroxy) benzoyl hydrazone (L), and its Ln(III) complexes (Ln = La, Eu) were synthesized and their DNA-binding modes were investigated systematically. In addition, the antioxidant activities of the metal complexes were studied by the hydroxyl radical ($^{\bullet}OH$) and superoxide anion ($O_2^{\bullet-}$) which are highly reactive species implicated in cell membrane disintegration, membrane protein damage, DNA mutation [26,27]. Furthermore, the Eu(III)

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complex can emit intrinsic spectra under excitation both in solid and organic solvents. That may provide more advantages to apply the Eu(III) complex as a candidate of anticancer drugs and nucleic acid probe.

2. Results and discussion

The complexes were prepared by direct reaction of ligand with the appropriate mole ratios of Ln(III) nitrate in ethanol. The yields were good to moderate. The complexes were air stable for extended periods and soluble in methanol, DMF (*N*,*N*-dimethylformamide) and DMSO (dimethylsulfoxide); slightly soluble in ethanol, acetone, acetonitrile, THF (tetrahydrofuran) and water; insoluble in benzene, ethyl acetate. The elemental analyses show that formula of the complexes in powder conform to $[LnL_2(NO_3)_3]$ (Ln = La, Eu), while the single-crystal structure of La(III) complex was $[LaL_2(NO_3)_3]\cdot C_2H_5OH\cdot CH_3OH$ according to the single-crystal X-ray analysis.

2.1. IR spectra

Since the characteristic absorption peaks of the La(III) complex and the Eu(III) complex were very similar, the structure of the Eu(III) complex could be talked about in the same way. Take the La(III) complex for example, in the La(III) complex, $\nu_{(\text{hydrazonic})\ C=O}$, $\nu_{(\text{carbonyl})\ C=O}$, $\nu_{C=N}$ vibrations were at 1667, 1626 and 1613 cm^{-1}, respectively, while for the free ligand, these peaks were at 1643, 1623 and 1589 cm^{-1}, and ν(ligand – complex) was equal to 24, 3 and 24 cm^{-1}. These shifts demonstrated that the above three groups have taken part in coordination to La(III) ion. The absorption bands of the coordinated nitrates were observed at about 1452, 1289, 1022, 783, 733 cm^{-1} suggesting that the nitrate group took part in coordination [28,29]. The above speculations were consistent with the crystal structure of the La(III) complex by X-ray analysis.

2.2. Crystal structure of the La(III) complex

On the basis of the single-crystal X-ray analysis of the complex $[LaL_2(NO_3)_3]\cdot C_2H_5OH\cdot CH_3OH$, each La(III) ion was deca-coordinated by two tridentate ligand, two monodentate nitrate and one bidentate nitrate. The complex crystallized in the orthorhombic lattice with a space group *Pbcn*. Each unit cell contained eight molecules. The crystal structure is shown in Fig. 1. The X-ray diffraction data for the La(III) complex are given in Table 1 and the selected bond lengths and angles are summarized in Table 2.

The La(1)–O(2) and La(1)–O(7) hydrazonic interactions were found to be 2.577(4) and 2.541(3) Å, respectively, the carbonyl distances were found to be 2.501(4) and 2.449(4) Å for La(1)–O(4) and La(1)–O(9). The average Ln–O carbonyl distance (2.475 Å) was significantly shorter than the average hydrazonic Ln–O distance (2.564 Å), which suggested that the carbonyl bond was stronger than the hydrazonic bond. The La–imine distances were found to be 2.841(5) and 2.951(5) Å for La(1)–N(2) and La(1)–N(4), which was unusually longer than the other La–O bond lengths [30]. The nitrate anion had proved to be a very useful ligand for the construction of coordination complex. In the crystal structure of the La(III) complex, three nitrates had two coordination types, monodentate and bidentate; the monodentate nitrate bond lengths of La–O(11) and La–O(14) were 2.648(5) and 2.625(5) Å, respectively, while the bidentate nitrate bond lengths of La–O(17) and La–O(18) were 2.683(5) and 2.591(5) Å, respectively. In $[LaL_2(NO_3)_3]\cdot C_2H_5OH\cdot CH_3OH$ complex, the bond lengths of C(8)–N(2) and C(26)–N(4) (mean 1.286(7) and 1.275(7) Å, respectively) were consistent with double bond while bond lengths of C(7)–N(1) and C(25)–N(3) (mean 1.369(7) and 1.350(7) Å, respectively) were corresponding to a single bond. Take the crystal structure of the $[LaL'_2(CH_3OH)(H_2O)(NO_3)]\cdot[NO_3]\cdot H_2O$ (L'_2 = 6-hydroxychromone-3-carbaldehyde benzoyl hydrazone) [24] for comparison, observe their constructions of coordination in the two crystal structures, both La(III) ions were ten coordinated and both the structures have two tridentate ligands. However, in $[LaL_2(NO_3)_3]\cdot C_2H_5OH\cdot CH_3OH$, all the three nitrates took part in coordination, while in $[LaL'_2(CH_3OH)(H_2O)(NO_3)]\cdot[NO_3]\cdot H_2O$ only one nitrate took part in coordination. The reason may be the difference of substitute and its situation.

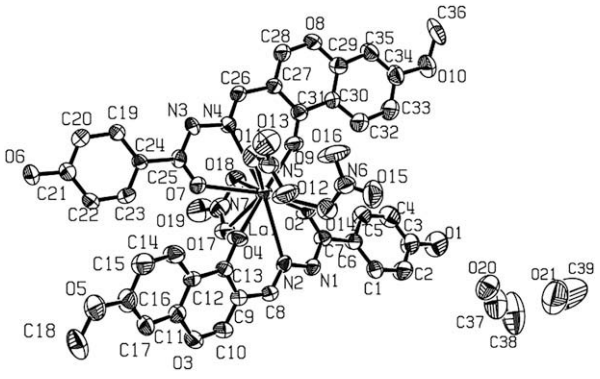


Fig. 1. The structure of the La(III) complex.

Table 1
Crystal data and experimental data.

Empirical formula	$C_{39}H_{35.31}\cdot LaN_7O_{21}$
Formula weight	1076.96
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	*Pbcn*
a (Å)	14.595(2)
b (Å)	20.055(3)
c (Å)	30.078(5)
α (°)	90
β (°)	90
γ (°)	90
Volume ($Å^3$)	8804(2)
Z	8
Calculated density (Mg/m^3)	1.62
Absorption coefficient (mm^{-1})	1.062
F(000)	4347
θ Range (°)	1.35–28.29
Reflections collected/unique	50,933/10,742 [$R_{int} = 0.1304$]
Goodness of-fit on F^2	1.015
R indices [$I > 2\sigma(I)$]	$R_1 = 0.0657$, $wR_2 = 0.1241$

2.3. DNA-binding studies

2.3.1. Fluorescence spectra

The fluorescence titration spectra have been confirmed to be effective for characterizing the binding mode of the metal complexes to DNA [31,32]. The enhancements in the emission intensity of the ligand and its Ln(III) complexes (Ln = La, Eu) with increasing CT-DNA concentrations are shown in Fig. 2. The ligand and its Ln(III) complexes (Ln = La, Eu) could emit luminescence in Tris–HCl buffer with a maximum appearing at 438 nm (λ_{ex} = 360 nm). This phenomenon was related to the extent to which the compounds penetrate into the hydrophobic environment inside the DNA, thereby avoiding the quenching effect of solvent water molecules. The marked increase in emission intensity of these three compounds were also in accordance with those observed in the fluorescence titration spectra studies of other intercalators [33,34]. Consequently, it might be concluded that the ligand and the La(III) and Eu(III) complexes bound DNA via intercalation mode and

Table 2
Selected bond lengths (Å) and angles (°) for $[LaL_2(NO_3)_3]\cdot C_2H_5OH\cdot CH_3OH$ complex.

Selected bond	Bond length or angle	Selected bond	Bond length or angle	Selected bond	Bond length or angle
La(1)–O(2)	2.577(4)	La(1)–O(4)	2.501(4)	La(1)–O(7)	2.541(4)
La(1)–O(9)	2.449(4)	La(1)–O(11)	2.648(5)	La(1)–O(14)	2.625(5)
La(1)–O(17)	2.683(5)	La(1)–O(18)	2.591(5)	La(1)–N(2)	2.841(5)
La(1)–N(4)	2.951(5)	C(7)–O(2)	1.228(6)	C(13)–O(4)	1.239(6)
C(18)–O(5)	1.429(9)	C(25)–O(7)	1.225(6)	C(31)–O(9)	1.245(7)
C(36)–O(10)	1.406(9)	C(7)–N(1)	1.369(7)	C(8)–N(2)	1.286(7)
C(25)–N(3)	1.350(7)	C(26)–N(4)	1.275(7)	N(1)–N(2)	1.376(6)
N(3)–N(4)	1.395(6)	N(5)–O(11)	1.249(7)	N(5)–O(12)	1.223(6)
N(5)–O(13)	1.220(6)	N(6)–O(14)	1.278(7)	N(6)–O(15)	1.232(7)
N(6)–O(16)	1.220(7)	N(7)–O(17)	1.245(7)	N(7)–O(18)	1.287(7)
N(7)–O(19)	1.206(7)	O(9)–La(1)–O(4)	165.53(16)	O(9)–La(1)–O(7)	115.73(13)
O(4)–La(1)–O(7)	66.59(14)	O(9)–La(1)–O(2)	67.22(13)	O(4)–La(1)–O(2)	121.00(13)
O(7)–La(1)–O(2)	140.55(15)	O(9)–La(1)–O(18)	70.95(16)	O(4)–La(1)–O(18)	121.89(15)
O(7)–La(1)–O(18)	72.00(14)	O(2)–La(1)–O(18)	72.64(14)	O(9)–La(1)–O(14)	84.42(16)
O(4)–La(1)–O(14)	88.15(15)	O(7)–La(1)–O(14)	148.98(15)	O(2)–La(1)–O(14)	67.58(15)
O(18)–La(1)–O(14)	138.92(15)	O(9)–La(1)–O(11)	90.76(16)	O(4)–La(1)–O(11)	76.29(15)
O(7)–La(1)–O(11)	71.41(14)	O(2)–La(1)–O(11)	146.25(15)	O(18)–La(1)–O(11)	125.62(15)
O(14)–La(1)–O(11)	85.77(15)	O(9)–La(1)–O(17)	116.11(16)	O(4)–La(1)–O(17)	78.20(14)
O(7)–La(1)–O(17)	66.41(14)	O(2)–La(1)–O(17)	77.13(14)	O(18)–La(1)–O(17)	48.08(14)
O(14)–La(1)–O(17)	127.81(15)	O(11)–La(1)–O(17)	136.62(14)	O(9)–La(1)–N(2)	74.09(11)
O(4)–La(1)–N(2)	83.40(10)	O(7)–La(1)–N(2)	63.85(10)	O(2)–La(1)–N(2)	126.51(9)
O(18)–La(1)–N(2)	72.69(10)	O(14)–La(1)–N(2)	101.16(11)	O(11)–La(1)–N(2)	67.86(8)
O(17)–La(1)–N(2)	168.15(10)	O(9)–La(1)–N(4)	116.16(10)	O(4)–La(1)–N(4)	158.40(10)
O(7)–La(1)–N(4)	115.31(9)	O(2)–La(1)–N(4)	65.91(8)	O(18)–La(1)–N(4)	118.57(10)
O(14)–La(1)–N(4)	71.13(10)	O(11)–La(1)–N(4)	69.63(9)	O(17)–La(1)–N(4)	125.78(8)
N(2)–La(1)–N(4)	49.34(9)				

according to the previous literature [22–24], we can also find that the fluorescence spectra of other Ln(III) complexes reported by Wang [22–24] do not differ significantly with the substitutions on the chromone hydrazone ligand.

2.3.2. Electronic absorption titration

Electronic absorption spectroscopy is one of the most useful techniques for DNA-binding studies of metal complexes [35,36]. The absorption spectra of the ligand and its Ln(III) complexes (Ln = La, Eu) in presence of CT-DNA are given in Fig. 3(a), (b) and (c), respectively. The addition of CT-DNA to the solutions of the compound at the DNA/compounds molar ratios varying from 0 to 3.5 induced large hypochromic responses about 39.3% at 295 nm for ligand, 66.6% at 296 nm for La(III) complex and 60.9% at 296 nm for Eu(III) complex, respectively, and the bathochromism responses about 8, 8, 10 nm. The phenomenon of large hypochromism and appreciable bathochromism which were similar to our previously reported complexes [22–24] were both attributed to the strong stacking interaction between aromatic group and the base pairs of CT-DNA, when the compound intercalate to the CT-DNA [37]. The spectroscopic changes suggested that both the ligand and the complexes interacted with DNA most likely through an intercalation

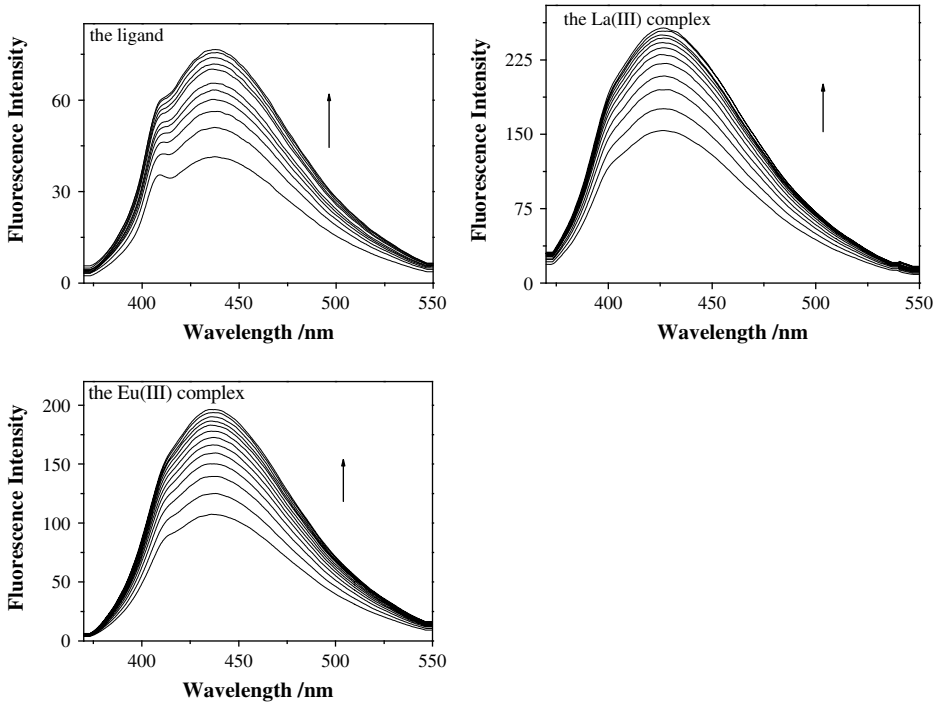


Fig. 2. Emission enhancement spectra of the ligand, the La(III) and Eu(III) complexes in the presence of increasing amounts of CT-DNA.

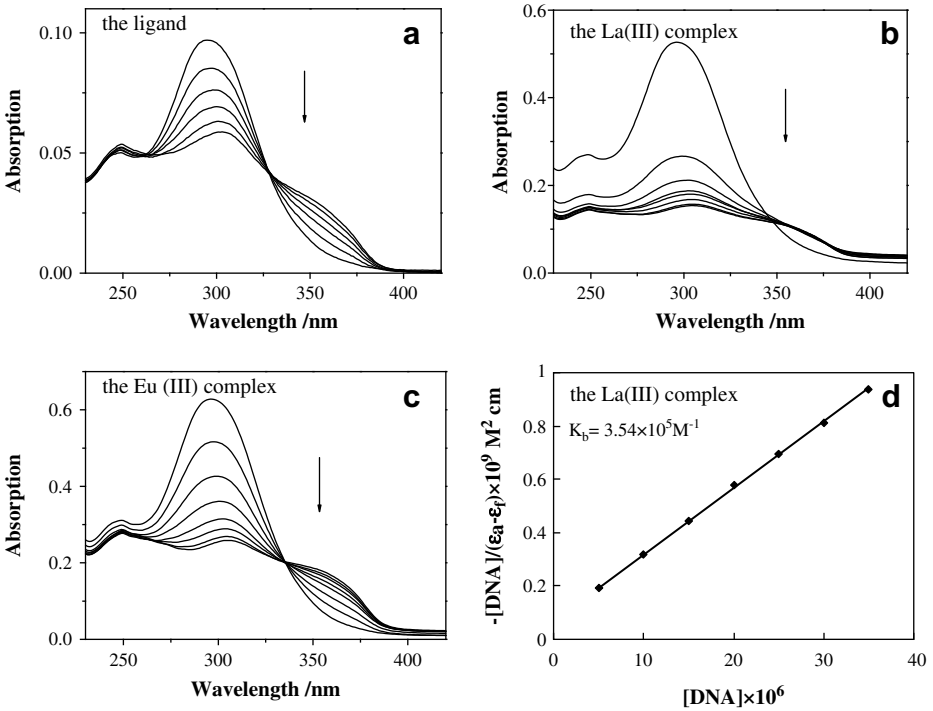


Fig. 3. Electronic absorption spectra of (a) the ligand, (b) the La(III) complex, (c) the Eu(III) complex in the presence of increasing amounts of CT-DNA. (d) Plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] for the titration of the La(III) complex with CT-DNA.

mode. To further illustrate the binding affinity of the compounds quantitatively, the intrinsic DNA-binding constants K_b of the compounds were determined by monitoring the changes in absorbance at 300 nm with increasing concentrations of DNA, according to Eq. (1). As an example of the La(III) complex, Fig. 3(d) showed the mathematic method of evaluating K_b value, which was calculated from the plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA]. Take this method, the intrinsic DNA-binding constants K_b were found to be the La(III) complex, $3.54 \times 10^5\ M^{-1}$; the Eu(III) complex, $5.11 \times 10^4\ M^{-1}$; the ligand, $3.89 \times 10^4\ M^{-1}$, in the order of the La(III) complex > the Eu(III) complex > the ligand.

Furthermore, comparing the K_b values of the compounds in title with those of other known DNA-intercalative complexes, (for instance, $[(bpy)_2Ru(\mu\text{-}bipp)Ru(bpy)_2](ClO_4)_4$, $2.6 \times 10^5\ M^{-1}$ [38]; $[Ru(NH_3)_4dppz]^{2+}$, 1.24×10^5 [39]; $[Ru(phen)_2(PPIP)]^{2+}$, 1.1×10^5; $[Ru(bpy)_2(PPIP)]^{2+}$, 4.3×10^4 [40]; $[Pd_2(\mu\text{-}bzta)_4] \cdot 1.5DMSO$, 1.2×10^4 [41]; $[Sr(Phen)_2(DMF)_2]$ $[Fe(NO)(CN)_5]$, 1.6×10^4 [42]; $[Ru(dmb)_2(NMIP)]^{2+}$, $5.46 \times 10^3\ M^{-1}$; $[Ru(bpy)_2(NMIP)]^{2+}$, $1.15 \times 10^4\ M^{-1}$ [43]), it was obviously that the K_b value of the La(III) complex was much higher than all of the above DNA intercalators. However, the K_b values of the Eu(III) complex and the ligand were only higher than some of them. In spite of that, the results implied that the three compounds had good ability to bind to DNA.

2.3.3. EB displacement experiment

The results obtained from absorption titration and spectrofluorimetric titration experiments indicated that the ligand, the La(III) and Eu(III) complexes could effectively bind to DNA. In order to confirm the binding mode and compare their binding affinities, EB displacement experiment was carried out. EB (ethidium bromide) is a conjugate planar molecule. Its fluorescence intensity is very weak, but it is greatly increased when EB is specifically intercalated into the base pairs of double stranded DNA [1]. In previous studies, the fluorescent light could be quenched by the addition of the complex which can compete with EB to bind with DNA. This is a proof that the complexes intercalate to base pairs of DNA [44–46]. Therefore, the binding of the compounds in title were evaluated using competitive binding studies involving EB. Fig. 4(a–c) showed the emission spectra of DNA–EB system with increasing amounts of the ligand, the La(III) and Eu(III) complex. It was clear that the fluorescence intensity of DNA–EB system greatly decreases upon the addition of the ligand, La(III) and Eu(III) complex and there were more significant decreases (>50%) in the emission spectra of DNA–EB system of the La(III) and Eu(III) complex. These results indicated that EB was partially replaced by the compounds intercalate into the DNA. As an example of the La(III) complex, Fig. 4(d) showed the mathematic method of evaluating K_q (the quenching constant) value, which was calculated from the plot of F_0/F versus [Q]. According to linear Stern–Volmer equation, K_q was given by the ratio of the slope to the intercept. The K_q values for the ligand, the La(III) and Eu(III) complexes were 2.81×10^4, 7.02×10^4 and $5.55 \times 10^4\ M^{-1}$, respectively. These data suggested that the interaction of the La(III) complexes with CT-DNA was strongest and the interaction of the Eu(III) complexes with CT-DNA was stronger then that of the free ligand, which were consistent with the above absorption spectral results. Compare their K_q values with those of other known DNA-intercalative complexes which possess analogical structure, $[LaL'_2(CH_3OH)(H_2O)(NO_3)][NO_3] \cdot H_2O$ $5.28 \times 10^4\ M^{-1}$; $[SmL'_2(NO_3)_2] \cdot [NO_3] \cdot 0.5H_2O \cdot 2CH_3OH$ $1.37 \times 10^4\ M^{-1}$ (L'_2 = 6-hydroxychromone-3-carbaldehyde benzoyl hydrazone) [24], the La(III) and Eu(III) complexes in our paper have stronger affinities with CT-DNA. The reason may be the difference of substitute and its situation.

2.3.4. Viscosity measurement

Optical photophysical probes provide necessary but not sufficient clues to support a binding mode and viscosity experiment is considered as one of the least ambiguous and the most critical tests of the binding modes of complexes to DNA [47]. Therefore, the viscosity experiments were performed to investigate the binding mode of these complexes to DNA. In general, a classical intercalation mode might increase the relative viscosity of DNA, because the

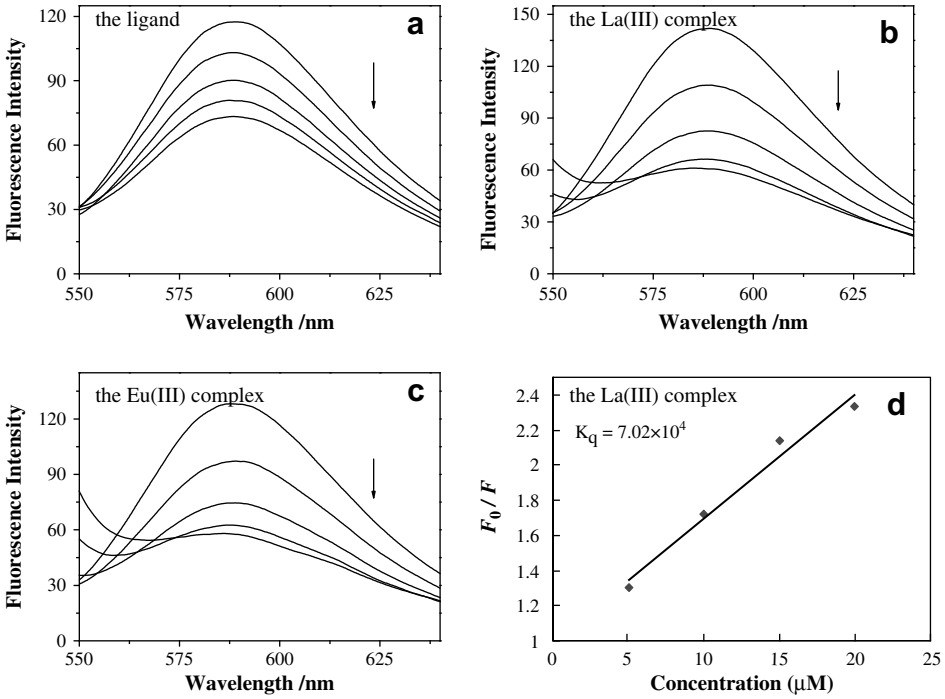


Fig. 4. The emission spectra of DNA–EB system in the presence of (a) the ligand, (b) the La(III) complex, (c) the Eu(III) complex. (d) Plot of F_0/F versus [Q] for the titration of the La(III) complex with DNA–EB.

binding compounds would separate base pairs of double helix DNA, and thus lengthened the DNA helix. On the contrary, a partial and/or non-classical intercalation of compounds would reduce the relative viscosity of DNA, since the binding ligand might bent (or kinked) the DNA helix and reduce its effective length [48]. As shown in Fig. 5, the specific viscosity of the DNA sample increased obviously with the addition of the compounds, which provided a further support that the binding modes of ligand and the complexes were intercalative in the interactions with CT-DNA.

2.4. Antioxidant activity

Since the ligand and its lanthanide complexes exhibit good DNA-binding affinity, it is considered worthwhile to study other potential aspects of these compounds, such as antibacterial, antioxidant and antiradical activity. The antiradical properties of

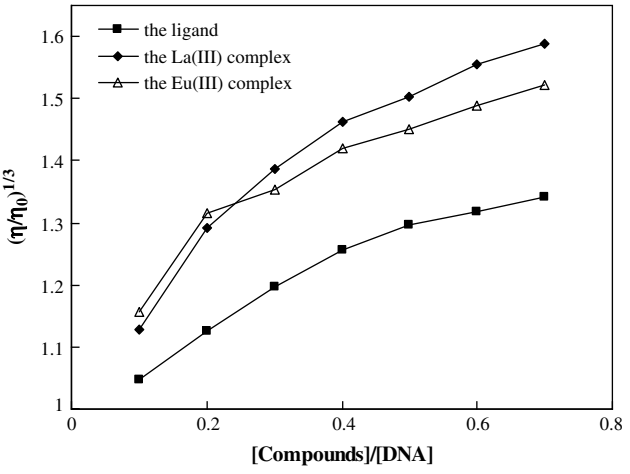


Fig. 5. Effect of increasing amounts of the ligand, the La(III) and Eu(III) complexes on the relative viscosity of CT-DNA at 25.0 °C.

chromones have attracted increased interest and consequently have been extensively investigated, mainly in the in vitro systems [49,50]. It has been reported that their free radical-scavenging ability is excellently directed toward hydroxyl radical (•OH) [51] and superoxide radical ($O_2^{\bullet-}$) [52,53] which are highly reactive products of reactive oxygen species (ROS) implicated with pathogenic processes including carcinogenesis through direct effects on DNA directly and by acting as a tumor promoter [54]. Consequently, in this paper, the ligand and its Ln(III) complexes (Ln = La, Eu) studied for its antioxidant activity by comparing their scavenging effect on hydroxyl radical (•OH) and superoxide radical ($O_2^{\bullet-}$).

As shown in Fig. 6(a) and (b), the inhibitory effect of the tested compounds on •OH and $O_2^{\bullet-}$ was concentration related and the suppression ratio increased with the increasing of sample concentration in the range of 1.0–8.0 μM. The suppression ratio against •OH valued from 4.85 ± 1.63% to 30.30 ± 1.13% for the ligand, 6.17 ± 2.06% to 47.31 ± 1.87% for the La(III) complex and 16.17 ± 0.99% to 50.82 ± 1.56% for the Eu(III) complex. Mannitol is nearly no scavenging effect at the same concentration lift. Mannitol was employed as a standard since it is known to selectively inhibit the •OH radical [55,56]. According to previous literature [57], the experiment was carried out again at higher concentration. The suppression ratio of mannitol was valued from 24.10 ± 0.95% to 71.79 ± 1.78% at a concentration from 5 to 40 μM. The results clearly suggest that the ligand and its the La(III) and Eu(III) complex have stronger scavenging activity against hydroxyl radical than mannitol. The suppression ratio against $O_2^{\bullet-}$ valued from about 1.98 ± 1.54% to 17.39 ± 1.52% for ligand, 10.08 ± 1.92% to 45.38 ± 0.99% for La(III) complex and 5.93 ± 1.09% to 33.20 ± 1.42% for the Eu(III) complex. The results show that the three compounds' ability against superoxide radical is significantly higher than that of nitroxide Tempo which has been recently used in biological system for its capacity to mimic superoxide dismutase [58].

It could be seen clearly that the Eu(III) complex was the most efficient scavenger against •OH, and the suppression ratio took the order of the Eu(III) complex > the La(III) complex > the ligand at the same concentration. However, in the experiment of

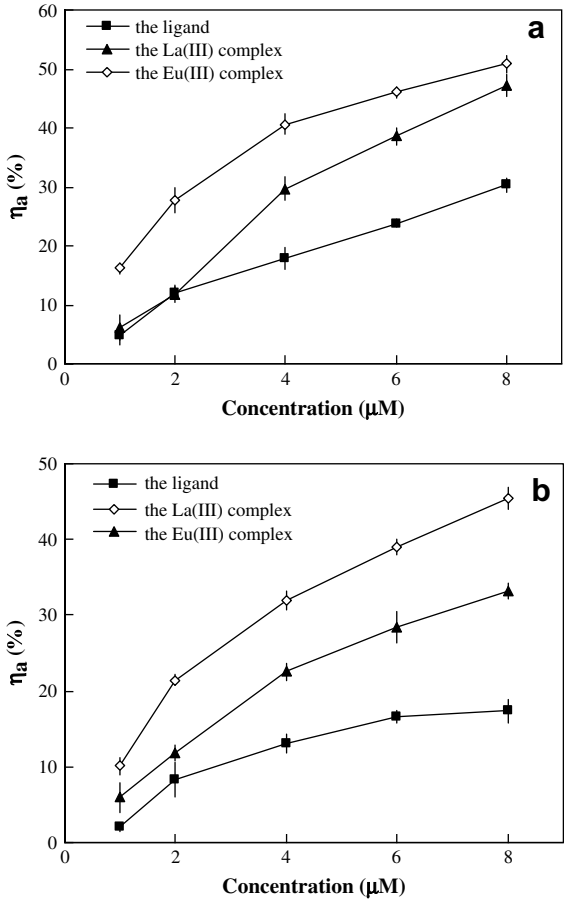


Fig. 6. (a) Scavenging effect of the tested compounds on ·OH by the Fenton system; (b) scavenging effect of the tested compounds on $O_2^{\cdot-}$ by MET/VitB$_2$/NBT system.

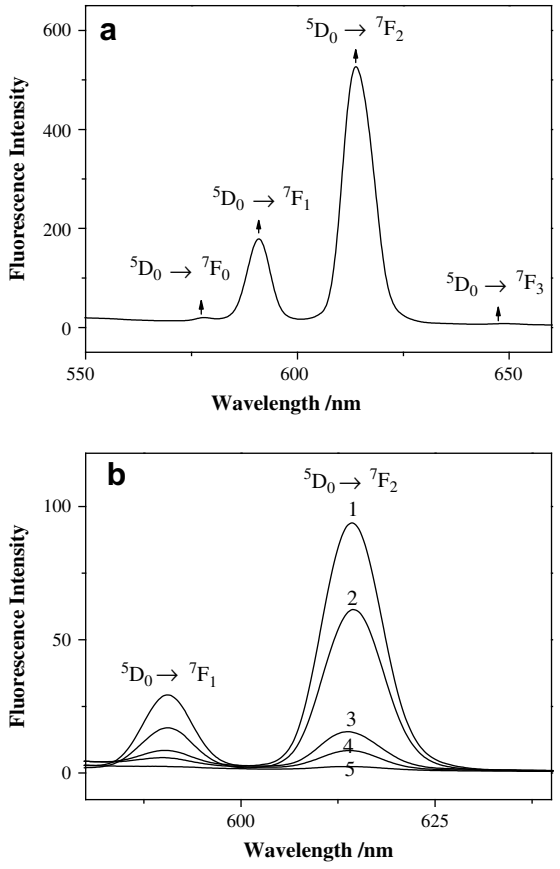


Fig. 7. (a) The fluorescence emission spectrum of the Eu(III) complex in solid state at room temperature; (b) the fluorescence emission spectrum of the Eu(III) complex in different solutions (1, ethanol; 2, acetone; 3, methanol; 4, cyclohexane; 5, THF).

investigating superoxide radical ($O_2^{\cdot-}$) scavenging activity, the La(III) complex showed excellent activity. The order of the suppression ratio for $O_2^{\cdot-}$ was the La(III) complex > the Eu(III) complex > the ligand. The results suggested that the scavenging effect on ·OH and $O_2^{\cdot-}$ could be enhanced by the formation of metal–ligand coordination complexes and the lanthanide ions had differential and selective nature for scavenging $O_2^{\cdot-}$ and ·OH. It was believed that the information obtained from the present work would ultimately be helpful to develop new potential antioxidants and new therapeutic reagents for some diseases.

2.5. Fluorescence studies

As shown by DNA-binding and antioxidant activity studies, the lanthanide complexes in title possess outstanding biological properties. Furthermore, luminescence of several Ln(III) ions has been a useful probe of metal ion binding sites in biomolecules. The Eu(III) ion, in particular, is valuable for use in determination of specific features of binding, since the non-degenerate $^5D_0$ excited state results in relatively simple emission spectra [59]. Excitation of the $^7F_0 \rightarrow {}^5D_0$ transition results in a characteristic and unique fluorescence in the red, with its strongest lines associated with $^5D_0 \rightarrow {}^7F_J$ transitions at 580, 593, 614, 650 and 700 nm, for $J = 0$–4. The hypersensitive $^5D_0 \rightarrow {}^7F_2$ transition at 614 nm is typically monitored because each chemically distinct Eu(III) species gives rise to a unique excitation spectrum, the intensity of which is directly related to its concentration [60].

The emission spectra of the Eu(III) complexes in solid state (the excitation and emission slit widths were 3.0 nm, Fig. 7(a)) and in several organic solvents solutions (concentration: 1.0×10^{-4} mol L^{-1}, the excitation and emission slit widths were 5.0 nm, Fig. 7(b)) were recorded at room temperature.

It could be seen from Fig. 7(a) that the Eu(III) complex showed strong emission when excited with 349 nm in the solid state. This indicated that the ligand in title was a good organic chelator to absorb energy and transfer it to Eu(III) ion. The most intensity ratio value η ($^5D_0 \rightarrow {}^7F_1/{}^5D_0 \rightarrow {}^7F_2$) was 2.9, indicating a low symmetry for the Eu(III) site [61]. The result was in agreement with the crystal structure analysis.

As shown in Fig. 7(b), the emission spectra of the Eu(III) complex in different solvents were similar. They all showed characteristic emission bands of an Eu(III) ion at about 590 and 614 nm, corresponding to $^5D_0 \rightarrow {}^7F_1$ and $^5D_0 \rightarrow {}^7F_2$ transitions, respectively. From Fig. 7(b), it could be found that the Eu(III) complex had the strongest fluorescence in ethanol, and then in acetone, methanol, cyclohexane and THF. This was probably due to the coordinating effects of solvents, namely solvate effect [62]. The energy transfer from the triplet excited state of the ligand to the emitting level of the europium ion could not be carried out perfectly because the oscillatory motions of the entering solvent molecules may consume more energy. It was clear that excellent luminescence properties made the Eu(III) complex to be a good candidate for biological use.

3. Conclusion

It is well documented that 7-methoxychromone-3-carbaldehyde-(4′-hydroxy) benzoyl hydrazone (Schiff base ligand, L) and its Ln(III) complexes (Ln = La, Eu) can interact with calf thymus DNA by intercalation mechanism, and the La(III) complex has a greater affinity. Additionally, the ligand and its Ln(III) complexes (Ln = La,

Eu) also show antioxidant activities. In particular, it is observed the ligand is a good organic chromophore to absorb and transfer energy to Eu(III) ion. The outstanding luminescence properties of the Eu(III) complex provide more advantages to apply it in probing metal-binding properties of some biomolecules. These results suggest that the combination of a chromone and a hydrazone has formed a good ligand. It is not only a good ligand to form biological lanthanide complexes but also a good organic chromophore to absorb and transfer energy to Eu(III) ions.

4. Experimental

4.1. Instrumentation

The metal contents of the complexes were determined by titration with EDTA. Elemental analyses were carried out on an Elemental Vario EL analyzer. The IR spectra were obtained in KBr discs on a Therrno Mattson FTIR spectrometer in the 4000–400 cm^{-1} region. ^1H NMR spectra were recorded on a Bruker Avance Drx 200-MHz spectrometer with TMS as an internal standard. The melting points of the compounds were determined on a Beijing XT4-100× microscopic melting point apparatus. Fluorescence spectra were obtained on a Shimadzu RF-5301 spectrophotometer at room temperature. The UV–vis spectra were recorded on a Perkin–Elmer Lambda-35 UV–vis spectrophotometer. The antioxidant activities were performed in DMF with a 721E spectrophotometer (Shanghai Analytical Instrument factory China).

4.2. Materials

CT-DNA (Calf thymus DNA), ethidium bromide (EB), NBT (nitroblue tetrazolium), MET (methionine), and $VitB_2$ (vitamin B_2) were purchased from Sigma Chemical Co. EDTA, Safranin, $BF_3 \cdot Et_2O$, $POCl_3$, and $Ln(NO_3)_3 \cdot 6H_2O$ (Ln = La, Eu) were produced in China. All the chemicals used were of analytical grade. Tris–HCl buffer, KH_2PO_4–Na_2HPO_4 buffers and EDTA–Fe(II) were prepared with twice distilled water.

All the experiments involving the interaction of the ligand and the complexes with CT-DNA were carried out in doubly distilled water buffer containing 5 mM Tris and 50 mM NaCl and adjusted to pH = 7.1 with hydrochloric acid. Solution of CT-DNA gave ratios of absorbance at 260 and 280 nm of about 1.8–1.9, indicating that the CT-DNA was sufficiently free of protein [63,64]. The CT-DNA concentration per nucleotide was determined spectrophotometrically by employing an extinction coefficient of 6600 $M^{-1}\,cm^{-1}$ at 260 nm.

4.3. Preparation of the ligand and its complexes

4.3.1. Synthesis of the ligand

As shown in Scheme 1, Compound **1** and Compound **2** were prepared according to the literature [65]. Synthesis of the ligand was based on the following method: an ethanol solution (25 ml) which contained 4-hydroxy benzoyl hydrazine (0.76 g, 5 mmol) was added dropwise to compound **2** (1.02 g, 5 mmol) of chloroform solution (10 ml). After stirring at 70–80 °C for 6 h, the light yellow precipitates were collected then recrystallized from 1:1 (V/V) DMF/H_2O. Yield: 80%. ^1H NMR (DMSO-d_6, ppm): δ 11.72 (1H, s, –NH), 10.12 (1H, s, –OH), 8.72 (1H, s, 2-H), 8.56 (1H, s, CH═N), 8.02 (1H, d, J = 8.9 Hz, H-5), 7.81 (2H, d, J = 8.7 Hz, ph-H(2,4)), 7.21 (1H, d, J = 2.3 Hz, 8-H), 7.11 (1H, dd, J = 2.3 and J = 8.9 Hz, H-6), 6.84 (2H, d, J = 8.7 Hz, ph-H(1,5)), 3.90 (3H, s, $-CH_3$). The labelled numbers of H-atoms are shown in Fig. 7. IR (KBr) cm^{-1}: $\nu_{(hydrazonic)\ C=O}$: 1667; $\nu_{(carbonyl)C=O}$: 1626; $\nu_{C=N}$: 1613.

4.3.2. Synthesis of lanthanide complexes

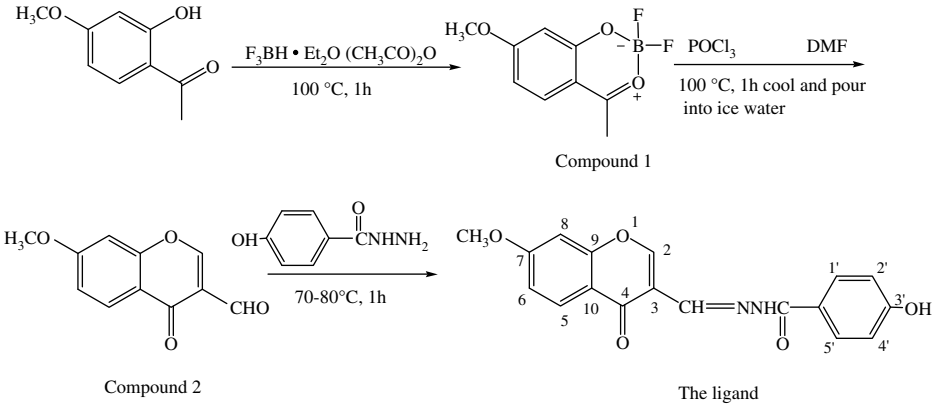
The ligand (1 mmol, 0.34 g) was dissolved in anhydrous ethanol (20 ml) and another ethanol solution (10 ml) containing $La(NO_3)_3 \cdot 6H_2O$ (1 mmol, 0.45 g) was then added dropwise with stirring. Then the mixture solution was refluxed on an oil-bath at 80 °C for 4 h with stirring. After cooling to room temperature, a large amount of light yellow precipitate appeared. It was separated from the solution by suction filtration, purified by washing several times with ethanol, and dried for 48 h in vacuum. The Eu(III) complex was prepared by the same method.

$[LaL_2(NO_3)_3]$. Yield: 50%. IR (KBr) cm^{-1}: $\nu_{(hydrazonic)\ C=O}$: 1643; $\nu_{(carbonyl)C=O}$: 1623; $\nu_{C=N}$: 1589; $\nu_{C=N}$: 1589; ν_{NO_3}: 1452, 1289, 1022, 783, 733. Anal. Calcd for $C_{28}H_{36}N_7O_{21}La$: C, 43.17; H, 2.82; N, 9.79; La, 13.87. Found: C, 43.36; H, 3.029; N, 9.680; La, 13.53.

$[EuL_2(NO_3)_3]$. Yield: 55%. IR (KBr) cm^{-1}: $\nu_{(hydrazonic)\ C=O}$: 1643; $\nu_{(carbonyl)C=O}$: 1623; $\nu_{C=N}$: 1572; ν_{NO_3}: 1452, 1287, 1024, 781, 748. Anal. Calcd for $C_{21}H_{25}N_6O_{15}Eu$: C, 42.62; H, 2.78; N, 9.66; Eu, 14.98. Found: C, 42.71; H, 3.104; N, 9.533; Eu, 14.62.

4.4. X-ray crystallography

A yellow crystal of $[LaL_2(NO_3)_3] \cdot C_2H_5OH \cdot CH_3OH$ obtained from a methanol and ethanol mixture solution at room temperature of dimensions $0.23 \times 0.25 \times 0.29$ mm was centered on a Bruker Smart-1000 CCD diffractometer operating in $\omega/2\theta$ scan mode with graphite monochromated Mo Kα radiation (0.71073 Å). The structure was solved by direct methods. The positions of non-hydrogen atoms were determined from successive Fourier syntheses. The hydrogen atoms were placed in their geometrically calculated positions. The positions and anisotropic thermal parameters of all



Scheme 1. The preparation of the ligand.

non-hydrogen atoms were refined on F^2 by full-matrix least-squares techniques with the SHELX-97 program package. Absorption correction was employed using semi-empirical methods from equivalents.

4.5. DNA-binding study methods

4.5.1. Fluorescence spectroscopy

The ligand and the complexes were dissolved in a mixture solvent of 1% methanol and 99% Tris–HCl buffer (5 mM Tris–HCl; 50 mM NaCl, pH = 7.1). The example solution of fixed amounts compounds (2 μM) were titrated with increasing amounts of DNA, over a range of DNA concentrations from 0.5 to 6.0 μM. An excitation wavelength of 360 nm was used.

4.5.2. Electronic absorption spectroscopy

In order to affirm quantitatively the affinity of the compounds binding to DNA, the intrinsic binding constant K_b of the ligand and its Ln(III) complexes (Ln = La, Eu) were obtained by the electronic absorption spectroscopy method. The ligand and the complexes were dissolved in a mixture solvent of 1% methanol and 99% Tris–HCl buffer (5 mM Tris–HCl; 50 mM NaCl, pH = 7.1) at concentration 10 μM. Absorption titration experiments were performed with fixed concentration of drugs (10 μM) while gradually increasing the concentration of CT-DNA with the range from 5 to 35 μM. The reference solution was the corresponding Tris–HCl buffer solution. While measuring the absorption spectra, equal amount of DNA was added to both the compound solution and the reference solution to eliminate the absorbance of DNA itself. Each sample solution was scanned in the range of 210–450 nm. From absorption data, K_b was determined using the following Eqs. (1) [66] through a plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA]

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

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

4.5.3. EB displacement experiment

Further support for the ligand and its Ln(III) complexes (Ln = La, Eu) binding to DNA via intercalation is given through the emission quenching experiment. A 2 ml solution of 50 μM DNA and 0.4 μM EB was titrated against 10–40 μM of compounds (λ_{ex} = 525 nm, λ_{em} = 540.0–650.0 nm, slit = 10 nm). According to the classical Stern–Volmer equation [67]:

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

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 Eq. (2) 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.

4.5.4. Viscosity measurements

To further confirm the binding modes of the compounds in the interaction with CT-DNA, hydrodynamic method was used. Viscosity experiments were conducted on an Ubbelodhe viscometer, immersed in a thermostated water bath maintained to 25.0 °C. Titrations were performed for the ligand and its Ln(III) complexes (Ln = La, Eu) (0.5–3.0 μM), and each compound was introduced into CT-DNA solution (5.0 μM) present in the viscometer. Data were presented as $(\eta/\eta_0)^{1/3}$ versus the ratio of the concentration of the compound and CT-DNA, where η was the viscosity of CT-DNA in the presence of the compound and η_0 was the viscosity of CT-DNA alone [68,69].

4.6. Scavenger measurements of hydroxyl radical (·OH) and superoxide radical ($O_2^{\cdot-}$)

The hydroxyl radical (·OH) in aqueous media was generated by the Fenton system [34]. The solution of the tested compound was prepared with DMF (*N*,*N*-dimethylformamide). The 5 ml assay mixture contained following reagents: safranin (11.4 μM), EDTA–Fe(II) (40 μM), H_2O_2 (17.6 μM), the tested compound (1.0–8.0 μM) and a phosphate buffer (67 mM, pH = 7.4). The assay mixtures were incubated at 37 °C for 30 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 and (±) standard deviation (S.D.). A_i was the absorbance in the presence of the tested compound; A_0 was the absorbance in the absence of the tested compound; A_c was the absorbance in the absence of the tested compound, EDTA–Fe(II), H_2O_2. The suppression ratio (η_a) was calculated on the basis of $(A_i - A_0)/(A_c - A_0) \times 100\%$.

The superoxide radical ($O_2^{\cdot-}$) was produced by the system of MET/VitB_2/NBT and determined spectrophotometrically by nitro-blue tetrazolium (NBT) photoreduction method with a little modification in the method adopted elsewhere [70,71]. The amount of $O_2^{\cdot-}$ and suppression ratio for $O_2^{\cdot-}$ could be calculated by measuring the absorbance at 560 nm. Solution of VitB_2 and NBT were prepared under the condition of 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 μM), VitB_2 (3.3 μM), the tested compound (1.0–8.0 μM) and a phosphate buffer (67 mM, pH = 7.8). After illuminating with a fluorescent lamp at 30 °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 and (±) standard deviation (S.D.) of triplicate determinations. The suppression ratio for $O_2^{\cdot-}$ was calculated from the following expression. The suppression ratio $\eta_a = (A_0 - A_i)/A_0 \times 100\%$.

5. Supplementary data

Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC (695464). Copy 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 or http://www.ccdc.cam.ac.uk/deposit).

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