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Synthesis, characterization and biological activity of complexes of lanthanum(III) with 2-(1′-phenyl- 2′-carboxyl-3′-aza-*n*-butyl)-1,10-phenanthroline and 2-(1′-*p*-phenol-2′-carboxyl-3′-aza-*n*-butyl)-1,10-phenanthroline

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Abstract—Two novel ligands 2-(1′-phenyl-2′-carboxyl-3′-aza-*n*-butyl)-1,10-phenanthroline (L1) and 2-(1′-*p*-phenol-2′-carboxyl-3′-aza-butyl)-1,10-phenanthroline (L2), and their La(III) complexes of La(III)L1, La(III)$(L1)_2$, La(III)L2, and La(III)$(L2)_2$, were synthesized and characterized by ^{1}H NMR, elemental analysis, IR, thermal analysis and conductance measurement. All complexes have been assayed for anticancer activity in vitro against HL-60 (human leukocytoma) cells, PC-3MIE8 (human prostate carcinoma) cells, BGC-823 (human stomach carcinoma) cells, MDA-MB-435 (human galactophore carcinoma) cells, Bel-7402 (human liver carcinoma) cells, and HeLa (human cervix carcinoma) cells. Results showed that the two complexes La(III)L1 and La(III)$(L1)_2$ exhibited good cytotoxic activity against different cell lines in general; and La(III)$(L1)_2$ is more effective than cisplatin against all six cell lines. DNA-binding studies indicated that, besides the intercalation, the complexes bind to DNA by the other interaction(s).

1. Introduction

cis-Diamminedichloroplatinum(II) (cisplatin) has been widely utilized as antitumor drug in the world. It is highly effective in treating testicular and ovarian cancers,[1,2] and neck and head tumors.[3,4] The great success in the clinical treatment of human malignancies greatly stimulated research in the area of inorganic antitumor agents. But the severe toxic side effects of cisplatin including nephrotoxicity, neurotoxicity, and emetogensis limit the dose that can be given to patients. Another platinum-based antitumor drug, diamine[1,1-cyclobutanedicarboxylato]-*O*,*O*′-platinum(II) (carboplatin), received worldwide approval and achieved routine clinical use. It is less toxic than cisplatin and can be given at a much higher dose. Unfortunately, it is still only active in the same range of tumors as cisplatin and is still administered iv.[5] In order to find more effective and less toxic antitumor drugs, thousands of analogues of cisplatin are synthesized. However, direct structural analogues have not shown a greatly altered spectrum of clinical efficacy in comparison with the parent drug.[6,7] The explanation for these findings is that all *cis*-[PtX_2(amine)$_2$] compounds are similar in terms of their mechanisms of action. Thus, their biological consequences are also expected to be similar.[7,8]

One approach to solve this problem is to identify new classes of complexes having structural features that differ from those of the existing cisplatin analogues. Since 1,10-phenanthroline possesses a rigid planar aromatic ring, it has been used as a DNA intercalator.[9] Some metal complexes containing 1,10-phenanthroline are also known to bind to DNA by an intercalative mode.[10–12] At the same time, several metal complexes with 1,10-phenanthroline and natural products incorporating this heterocyclic nucleus possess interesting anticancer properties.[13–15] Recently, our researches focus on exploring the lanthanum complexes with the antitumor activities. We have found that lanthanum

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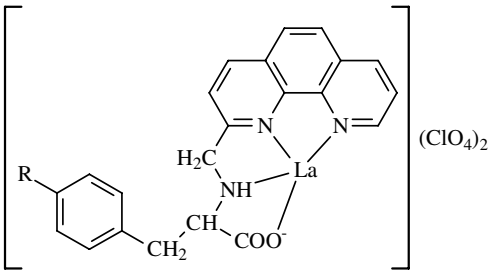
complexes with 1,10-phenanthroline-2,9-bis-α-amino acid conjugates[16] and two novel lanthanum(III) complexes containing 2-methylene-1,10-phenanthroline units bridged by aliphatic diamines[17] show the promising antitumor activity in vitro against a series of cell lines. In the present paper, two novel ligands are synthesized for the first time, which contain three nitrogen atoms from phenanthroline ring and aliphatic amino moiety, and one oxygen atom from carboxylic group. The structures of the two ligands are distinct from those reported previously containing six coordination atoms.[16,17] Four novel lanthanum(III) complexes containing the two ligands were prepared and their anticancer activity was evaluated. These lanthanum complexes possibly represent a new class of potential anticancer agent.

2. Results and discussion

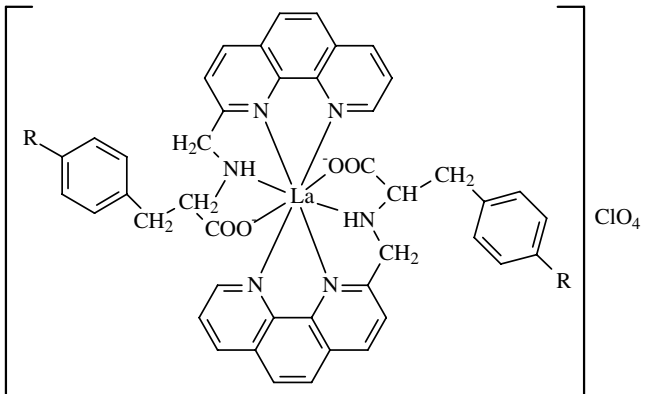
2.1. Preparation for ligands and the La(III) complexes

The synthesis of two ligands 2-(1′-phenyl-2′-carboxyl-3′-aza-*n*-butyl)-1,10-phenanthroline (L1) and 2-(1′-phenol-2′-carboxyl-3′aza-*n*-butyl)-1,10-phenanthroline (L2) was reported in the present study. The two ligands are of special interest because they contain two 'soft' nitrogen atoms from phenanthroline ring, one 'hard' nitrogen atom from aliphatic amino moiety and one 'hard' oxygen atom from carboxylic group. The preparation routes of the novel ligands are outlined in Figure 1. The structures were characterized by elemental analysis, ^{1}H NMR, and IR.

Lanthanum complexes of L1 and L2 were synthesized and characterized by elemental analysis, IR, TG analysis, and conductance measurements (Fig. 2). Their elemental analyses were in accord with their proposed formula. IR spectra provided a lot of valuable information on coordination reaction. The absorption bands 1590–1600, 1330–1350, and 858–852 cm^{-1} of phen ring of ligands were shifted to 1597–1598, 1369–1400, and 854–860 cm^{-1} of complexes after coordination, respectively, suggesting that the phen ring coordinated with



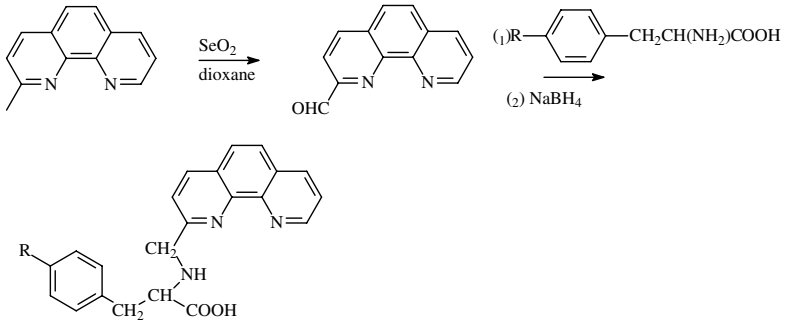
R = H(L1); OH(L2)



R = H(L1); OH(L2)

Figure 2. Proposed structure for the La(III) complexes.

La(III) ion through N.[18] By comparison with the free ligands L1 and L2, the appearance of ν(La–N) at 209–220 cm^{-1} (phen ring) in the complexes La(L1), La(L1)$_2$, La(L2), and La(L2)$_2$ further confirms coordination of phen ring. A very strong broad band of ligand in the 3440–3445 cm^{-1} region, which was assigned to ν(N–H) by comparison to the literature,[19,20] was shifted about 20 cm^{-1} to lower frequencies (3420–3421 cm^{-1}) indicating that the secondary amines in the side chain also coordinate with La(III) ion. The appearance of vibration at 473–493 cm^{-1} is characteristic of ν(La–NH), further supporting this conclusion.



L1: R = H; L2: R=OH

Figure 1. Synthesis of the ligands.

The ClO_4^- group vibrations at 1090–1094 and 625–627 cm^{-1} which is typical for a noncoordinated perchlorate ion[16] are present for the complexes La(L1), $La(L1)_2$, (LaL2), and $(LaL2)_2$. The molar conductivity in methanol for all four complexes La(L1), $La(L1)_2$, La(L2), and $La(L2)_2$ is given in part of Section 4. The results indicate that the complexes La(L1) and La(L2) (169.3–192.5 S cm^2 mol^{-1}) are in the range expected for 1:2 electrolytes, and the complexes $La(L1)_2$ and $La(L2)_2$ (89.1–97.9 S cm^2 mol^{-1}) are in the range expected for 1:1 electrolytes, which also indicated that the perchlorate anions are outside the coordination sphere, agreeing with IR result.

Elemental analysis and thermal analysis (TG) were used to determine the number of coordination water and crystal water molecules. The TG experiment was carried out in flowing N_2 atmosphere at a heating rate of 10 °C per minute. About 5–6 mg of the complexes was used for thermal analysis. Neutral alumina was used as reference material. Temperatures corresponding to water weight loss indicate that the complex La(L1) possesses two crystal water molecules and four coordination water molecules; the complex $La(L1)_2$ possesses four crystal water molecules; the complex La(L2) possesses three crystal water molecules and two coordination water molecules; the complex $La(L2)_2$ possesses two crystal water molecules and one coordination water molecule. This conclusion is consistent with elemental analysis. The four La(III) complexes began to decompose at 325 °C, a result suggesting that the La(III) complexes are very stable possibly because of strong chelation by the two new ligands containing three coordination nitrogen donors. Support for this conclusion comes from the measurements of the stability constants of La(III)(L1), La(III)(L2), La(III)$(L1)_2$, and La(III)$(L2)_2$ by means of pH potential titration showing that $\log\beta$ of La(III)(L1) is 8.36, $\log\beta$ of La(III)(L2) is 11.92, $\log\beta$ of La(III)$(L1)_2$ is 16.75, and $\log\beta$ of La(III)$(L2)_2$ is19.90 in aqueous solution at 25 °C.[21]

2.2. Cytotoxic studies

Free La(III) ion, ligands L1 and L2, and four lanthanum complexes have been tested against six cell lines. They were exposed to cell for 72 h and their growth inhibition assessed using the sulforhodamine B protein-staining assay.[22] The corresponding 50% inhibitory dose (IC_{50}) values are shown in Table 1. Cisplatin is also included for comparison.[16,23–26] Free La(III) ion, and ligands L1 and L2 showed high IC_{50} values (>100 μM) against all test cell lines, indicating that they have no biological activities. Two complexes, La(L1) and $La(L1)_2$, showed IC_{50} values less than 25 μM and another two complexes, La(L2) and $La(L2)_2$, showed IC_{50} values less than 50 μM, suggesting that they have cytotoxic activities. These results further supported the above conclusion that the four complexes were stable in aqueous solution. In contrast to the free La(III) and ligands, the four complexes exhibited cytotoxic activity against tested cell lines, especially more effective against HL-60, BGC-823, Bel-7402, HELA cell lines, MDA-MB-435 and similarly effective against PC-3MIE8 than cisplatin for $La(L1)_2$, respectively. Complex La(L1) displays IC_{50} (4.47 μM) against HL-60 cell line; IC_{50} (7.66 μM) against Bel-7402 cell line, and the complex $La(L1)_2$ displays IC_{50} (3.01μM) against HL-60 cell line; IC_{50} (4.36 μM) against BGC-823 cell line; IC_{50} (3.03 μM) against Bel 7402 cell line; IC_{50} (4.40 μM) against HELA cell line, IC_{50} (13.25 μM) against MDA-MB-435 cell line indicating they have higher cytotoxic activity than cisplatin (6.0 μM for HL-60; 6.8μM for BGC-823; 7.7 μM for Bel-7402; 4.6 μM for HELA; 30.0 μM for MDA-MB-435) (see Table 1). It is also observed that complexes La(L1) and $La(L1)_2$ have higher cytotoxic activity than complexes La(L2) and $La(L2)_2$. For increase of solubility of complexes La(L1) and $La(L1)_2$, the hydroxyl group was been introduced on their corresponding phenyl ring to produce complexes La(L2) and $La(L2)_2$. As expected, the solubility of La(III) complexes was increased, but their cytotoxic activities were pronouncedly decreased. It appears that the solubility and cytotoxic activity of complexes are restricted with each other. Consistent with the finding, reported lanthanum(III) complexes with 1,10-phenanthroline-2,9-bis-α-amino acid conjugates show an increasing activity against a series of cell lines such as the human leukemia (HL-60), the human coloadenocarcinoma (HCT8), the human stomach carcinoma (BGC-823), the human liver carcinoma (Bel-7402), and the human nasopharyngeal carcinoma (KB) as the lipophilicity of nonpolar aliphatic side chains (R groups) increases.[16,21] The same observation was also obtained with palladium complexes containing phenanthroline derivative as a ligand.[19] This finding is of special interest for a design of new anticancer drug.

Table 1. Seventy-two-hour IC_{50} values (μM) obtained for all La(III) complexes and cisplatin against different cell lines

Compound	Cell lines					
	HL60	BGC-823	Bel-7402	PC-3MIE8	HeLa	MDA-MB-435
$La(ClO_4)_3$	>100	>100	>100	>100	>100	>100
L1	>100	>100	>100	>100	>100	>100
L2	>100	>100	>100	>100	>100	>100
LaL1	4.47	20.02	7.66	8.54	9.26	24.57
$La(L1)_2$	3.01	4.36	3.03	5.59	4.40	13.25
LaL2	32.76	38.67	15.40	18.03	26.93	46.17
$La(L2)_2$	28.65	16.86	13.45	11.35	20.47	44.02
Cisplatin	6.0	6.8	7.7	4.66	4.63	30

2.3. Electronic absorption spectroscopy

Since the previous studies have shown that the strong interaction of the lanthanum complexes with DNA might be responsible for their cytotoxic activity,[16,27] the mode of the new synthesized La complexes binding to DNA likewise was carried out to understand the mechanism of their cytotoxic activity. Since the two La(III) complexes La(L1) and La(L2) contain one planar phenanthroline-ring moiety and other two La(III) complexes $La(L1)_2$ and $La(L2)_2$ contain two planar phenanthroline-ring moieties, it is first determined whether there is an intercalative interaction between the complexes and DNA. The binding of the intercalative drugs to DNA has also been characterized classically through absorption titrations, following the hypochromism and red shift associated with binding of the complex to the helix.[28] Figure 3 displays a well-behaved titration of $La(L1)_2$ with calf thymus DNA. The spectra show clearly that the addition of DNA results in evident hypochromism and red shift of two peaks at ~230 and ~273 nm, a result implying that the mode of complex binding to DNA involves a strong stacking interaction between an aromatic chromophore and the base pairs of DNA. The red shift and hypochromism are furthermore commonly found to correlate with the strength of the intercalative interaction.[28] A comparison of red shift found with DNA binding can be seen in Table 2.

Determinations of intrinsic binding constant, K_b, based upon these absorption titrations may be made with the following equation:[29]

$$[DNA]/(\varepsilon_A - \varepsilon_F) = [DNA]/(\varepsilon_B - \varepsilon_F) + 1/K_b(\varepsilon_B - \varepsilon_F),$$

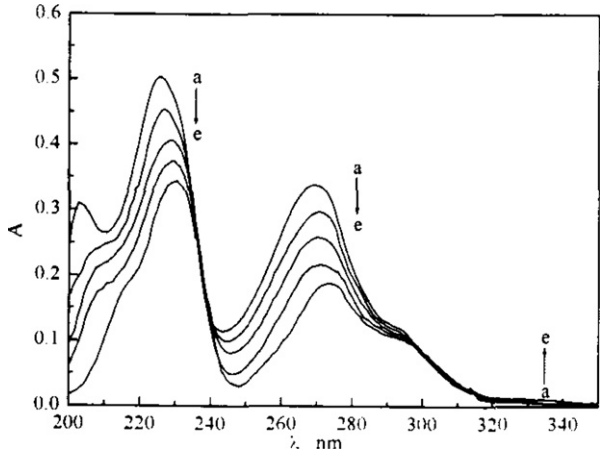


Figure 3. Absorption spectra of $La(L1)_2$ (3.3 μM) in the absence and presence of increasing amounts of CT DNA. [DNA]/[$La(L1)_2$] = 0 (a), 20/1 (b), 40/1 (c), 60/1 (d), and 80/1 (e).

Table 2. Absorbance spectroscopic properties on binding to DNA

Complex	Free (nm)	Bound (nm)	Δλ (nm)	K_b (M^{-1}) ×10^5
La(L1)	268	271	3	5.6
La(L2)	269	272	3	4.4
$La(L1)_2$	268	270	2	10.9
$La(L2)_2$	269	272	3	7.3

where ε_A, ε_F, and ε_B correspond to A_{obsd}/[complex], the extinction coefficient for the free complex, and the extinction coefficient for the complex 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 intercept. This half-reciprocal absorption titration method, which has been used successfully to determine the intrinsic binding constants K_b of molecules as hydrophobic as benzo[*a*]pyrene derivatives,[30] was found to provide a useful route to obtain intrinsic binding constants for the broad range of complexes of differing solubility. Values for K_b, given in Table 2, were obtained. The higher binding affinity is seen for the complex La(L1) and $La(L1)_2$, consistent with its higher cytotoxic activities against the six cancer cell lines as compared to La(L2) and $La(L2)_2$.

2.4. CD spectroscopy

CD spectral variations of calf thymus DNA by the respective addition of the complexes $La(L1)_2$ and $La(L2)_2$ were recorded on a Jasco J-715 spectropolarimeter. Figure 4 shows the CD spectra of calf thymus DNA which was treated with different concentrations of the complexes $La(L1)_2$ and $La(L2)_2$. In Figure 4, DNA solutions incubated with the complexes $La(L1)_2$ and $La(L2)_2$ show CD with opposite signs at near 260 nm, indicating that the DNA remains right-handed.[31] With increasing the concentration of the complexes $La(L1)_2$ and $La(L2)_2$, the peak at 250 nm becomes shallower while the peak at 280 nm being lower, namely, both positive and negative peaks counteract with each other. This result suggests that these two La(III) complexes exhibit a distinct DNA-binding mode from recently reported La(III) complexes, which induced DNA to show a more positive feature instead of positive and negative peaks counteracting with each other.[17] Furthermore, the small red shift of the CD spectra at 250 nm suggests that there exists interaction between aromatic rings of the two complexes and base pairs of DNA. Similar phenomenon was observed with other La(III) complexes,[16,17,26] consistent with the UV measurements (Fig. 3).

2.5. Fluorometric studies

To obtain insight into the binding mode of the La(III) complexes to calf thymus DNA, the fluorescence Scatchard plot was carried out with $La(L1)_2$ and $La(L2)_2$, respectively.[16,32] Saturation curves for fluorescence intensity of a series of DNA–metal complexes at increasing concentrations of the La(III) complex (*R*, the molar ratio of the complex to DNA, is 0–0.339) were obtained by the addition of concentrations of EB (1.67–16.7 μM). As shown in Figure 5, both $La(L1)_2$ and $La(L2)_2$ do not show a typical noncompetitive or competitive inhibition of EB binding in which both the slope that is K_{obs} (the observed association constant) and the intercept of the abscissa that is *n* (number of binding sites per nucleotide) decrease with increasing concentrations of the La(III) complex (Table 3). In contrast, some platinum[10] or palladium[32] metal complexes show a typical competitive or noncompetitive inhibition of EB binding to calf

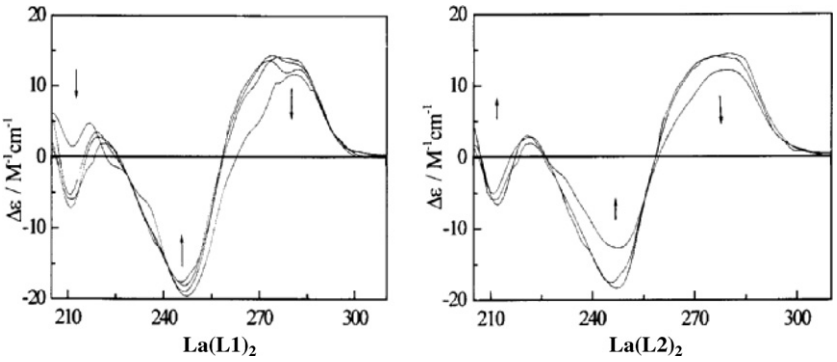


Figure 4. CD spectral variations of calf thymus DNA in the absence and presence of increasing amounts of the respective complexes $La(L1)_2$ and $La(L2)_2$. Conditions: [DNA] = 88.36 μM, $[La(L1)_2]$ = 0, 10, 20, 50 μM; $[La(L2)_2]$ = 0, 10, 20 μM.

Table 3. Binding parameters for the effect of the complexes $La(L1)_2$ on the fluorescence of EB in the presence of calf thymus DNA

Complex	R[a]	K[b] ($\times 10^6$)	n[c]
$La(L1)_2$	0.000	2.028	0.198
	0.113	1.568	0.179
	0.226	1.140	0.167
	0.339	0.893	0.155

[a] R is the formal ratio of the compound concentration to nucleotide concentration.
[b] K is the association constant in M^{-1}.
[c] n is the number of binding sites per nucleotide.

thymus DNA. Therefore, the present results suggest that the newly synthesized La(III) complexes show different DNA-binding mode from well-known platinum including cisplatin and palladium.[10,32] They may represent a new class of metal antitumor drugs.

3. Conclusion

Four lathanum(III) complexes were synthesized and tested in vitro against HL-60, PC-3MIE8, BGC-823, MDA-MB-435, Bel-7402, and HELA cell lines. The results showed that the La(L1) and $La(L1)_2$ complexes exhibit good cytotoxic activity against different cell lines in general. Moreover, the complexes $La(L1)_2$ exhibited better activity than cisplatin against HL-60, BGC-823, Bel-7402, HELA, and MDA-MB-435 cell lines. DNA-binding studies indicate that the complexes possibly interact with calf thymus DNA by a distinct mode from known platinum and palladium complexes. These results may lead to the development of a new class of antitumor agents.

4. Experimental

4.1. Materials and methods

2-Methyl-1,10-phenanthroline,[33] 1,10-phenanthroline-2-carboxaldehyde[34] were prepared by previously published methods. Commercially pure chemicals such as ethidium bromide (E; Fluka, Buchs, Switzerland), calf thymus DNA, and Tris (Sigma Chemical Co., St Louis, MO) were purchased and used as received. Solvents were purified by standard techniques and were freshly distilled prior to use. Other chemicals used were of analytical reagent of high purity grade.

Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer. IR spectra were obtained as KBr disks on a Nicolet 170 SX FT-IR spectrometer.

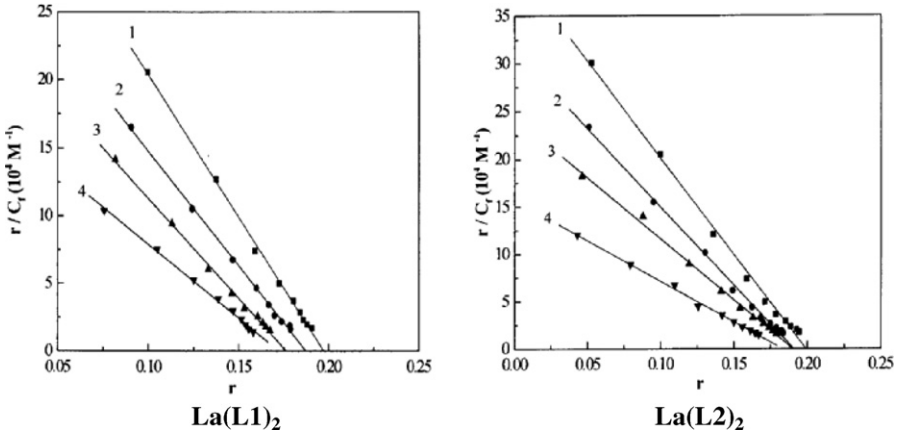


Figure 5. Fluorescence Scatchard plots for the binding of EB (1.67–16.7 μM) to ctDNA in the absence (line 1) and the presence (lines 2–4) of increasing concentrations of the complex $La(L1)_2$ and La(L2), R = 0.000, 0.113, 0.226, and 0.339 for lines 1–4, respectively; [ctDNA] = 44.2 μM.

^{1}H NMR spectra were recorded with a Varian UNITY-plus 400 MHz spectrometer. D_2O was used as a solvent with trace amounts of the reference DDS (3-trimethylsilyl-1-propanesulfonic acid, sodium salt). Mass spectra were obtained with a VG Trio-1 GC mass spectrometer. The quoted *m*/*z* values for metal-containing ions (where the metal has >1 isotope) are the most intense peak of a cluster with an isotope patter in good agreement with the calculated pattern. Thermal analyses were preceded with NETZSCH TG 209 thermal analyzer. Conductance measurements were carried out with a DDS-12A digital conductivity meter. A dip-type conductivity cell containing platinum electrodes was used.

4.2. Preparations of the ligands

The ligand L1 was prepared as follows. α-Phenylpropyl amino acid (5 mmol) and sodium hydroxide (5 mmol) were dissolved in 20 ml water. 1,10-Phenanthroline-2-carboxaldehyde (5 mmol) was stepwise added with stirring to this solution over 2 h. After stirred for 10 h at room temperature, the resulting reaction solution was filtered to remove unreacted solid. One gram of $NaBH_4$ was added to filtrate in small portions over 1 h at 0 °C, then the temperature of system was increased slowly to room temperature, again stirred for 12 h. Concentrated hydrochloric acid was added dropwise to above solution until pH 7.0, then was filtered and washed three times with 95% alcohol. Obtained solid was recrystallized by alcohol–water mixed solvent. The recrystallized product was dissolved in ethanol and protonated by adjusting the solution to 2–3 with concentrated hydrochloric acid. Resultant solution was evaporated to dryness, and final product L1·2HCl·H_2O was kept in desiccator for complex synthesis. Yield, 40–50%. ^{1}H NMR (D_2O): 8.85 (s, 1H, phen), 8.21 (d, 1H, phen), 8.12 (d, 1H, phen), 7.63 (m, 1H, phen), 7.58 (s, 2H, phen), 7.49 (d, 1H, phen), 4.80 (s, 2H, CH_2-phen), 7.21 (s, 4H, benzene ring), 7.14 (s, 1H, benzene ring), 3.50 (t, 1H, OOCCH), 2.99 (d, 2H, CH_2-benzene ring). Anal. Calcd for $C_{22}H_{19}N_3O_2$·2HCl·H_2O: C, 59.05; H, 5.18; N, 9.40. Found: C, 59.04; H, 5.12; N, 9.45. IR 3440, 1510, 1556, 1590, 1330, 858, 1630, 1382.

Ligand L2 was prepared with the same procedure as used for L1 except that pH of the alcoholic solution was adjusted to 8.0 with concentrated hydrochloric acid to obtain a large amount of primrose yellow solid. Final product L2·2HCl·2.5H_2O was also kept in a desiccator for complex synthesis. Yield, 50–60%. ^{1}H NMR (D_2O): 9.08 (m, 2H, phen), 8.95 (d, 1H, phen), 8.47 (d, 1H, phen), 8.09 (m, 3H, phen), 7.75 (d, 1H, phen), 4.78 (s, 2H, CH_2-phen), 6.66 (d, 2H, benzene ring), 5.94 (d, 2H, benzene ring), 4.06 (m, H, OOCCH), 3.09 (d, 2H, CH_2-benzene ring). Anal. Calcd for $C_{22}H_{19}N_3O_3$·2HCl·2.5H_2O: C, 53.88; H, 5.31; N, 8.57. Found: C, 54.11; H, 5.38; N, 8.59. IR (KBr) 3445, 1509, 1559, 1600, 1350, 852, 1640, 1380, 723 cm^{-1}.

4.3. Preparations of the lanthanum complexes

The complex La(L1) was prepared as follows. L1·2HCl·H_2O (0.5 mmol) was dissolved in 100 ml H_2O, and then sodium hydroxide was added to the solution until pH 7. Resulting solution was evaporated to dryness under vacuum and then was dissolved in 50 ml anhydrous methanol. Resultant solution was added dropwise with stirring to 50 ml anhydrous methanol containing 0.5 mmol $La(ClO_4)_3$·6H_2O (0.274 g). *Caution!* $La(ClO_4)_3$·6H_2O is potentially explosive and should be handled with care. After stirred for 2 h, the solution was filtered. The filtrate was condensed to 5 ml under reduced pressure, to which anhydrous ethyl ether was added dropwise with stirring. Formed primrose yellow solid was collected by filtration and washed with anhydrous ethanol three times. Product was recrystallized with methanol/ethyl ether. Yield, 70%. For LaL1 Anal. Calcd for $[La(C_{22}H_{18}N_3O_2)(H_2O)_4](ClO_4)_2$·2$H_2O$: C, 32.96; H, 3.77; N, 5.24; La, 17.34. Found: C, 32.85; H, 3.74; N, 5.20; La, 17.21. IR (KBr) 3420, 220, 489, 417, 453, 1597, 1400, 860, 1620, 1359, 261, 1091, 625; Λ_m(CH_3OH) 169.3 S cm^2 mol^{-1}. TG analysis; lost 4.56% (calcd 4.49%, 2H_2O) in first step at 74.1 °C (the temperature of peak) and lost 9.06% (calcd 8.97%, 4H_2O) in second step at 275.8 °C (the temperature of peak).

The complex $La(L1)_2$ was prepared as follows: 10 ml anhydrous methanol containing 0.5 mmol $La(ClO_4)_3$·6H_2O (0.274 g) was added dropwise with stirring to 50 ml anhydrous methanol containing 1.0 mmol L1. *Caution!* $La(ClO_4)_3$·6H_2O is potentially explosive and should be handled with care. After stirred 2 h, the solution was filtered. The filtrate was concentrated to 5 ml under vacuum. Addition of anhydrous ethyl ether to the above solution resulted in the formation of a large amount of yellow solid, which was washed with anhydrous ethanol for three times. The product was recrystallized with methanol/ethyl ether. Yield, 75%. Anal. Calcd for $[La(C_{22}H_{18}N_3O_2)_2](ClO_4)$·4$H_2O$: C, 32.96; H, 3.77; N, 5.24; La, 17.34. Found: C, 32.85; H, 3.74; N,5.20; La, 17.21. IR (KBr) 3440, 210, 475, 453, 1590, 1400, 856, 1620, 1378, 242, 1090, 636 cm^{-1}; Λ_m(CH_3OH) 89.1 S cm^2 mol^{-1}; TG analysis: lost 7.19% (calcd 7.04%, 4H_2O) at 74.7 °C (the temperature of peak).

The complexes La(L2) and $La(L2)_2$ were prepared under similar conditions as described for the complexes La(L1) and $La(L1)_2$ except that L1 was replaced by L2. For complex La(L2) yield 71%. Anal. Calcd for $[La(C_{22}H_{18}N_3O_3)\ (H_2O)_2]$·$(ClO_4)_2$·3$H_2O$: C: 33.08; H: 3.41; N: 5.26; La: 17.41. Found C, 33.25; H, 3.46; N, 5.20; La, 17.45. IR (KBr) 3450, 209, 493, 419, 449, 1598, 1369, 854, 1620, 1350, 270, 1092, 627 cm^{-1}. Λ_m(CH_3OH) 192.5 S cm^2 mol^{-1}. TG analysis: lost 6.83% (calcd 6.76%, 3H_2O) in first step at 75.1 °C (the temperature of peak); lost 4.68% (calcd 4.50%, 2H_2O) in second step at 231.8 °C (the temperature of peak). For complex $La(L2)_2$ yield 68%. Anal. calcd (%) for $[La(C_{22}H_{18}N_3O_3)_2(H_2O)]\ (ClO_4)$·2$H_2O$: C, 50.95; H, 4.08; N, 8.10; La, 13.39. Found C, 50.83; H, 4.05; N, 8.19; La, 13.53. IR (KBr) 3420, 212, 473, 417, 443, 1598, 1389, 856, 1620, 1350, 270, 1094, 627 cm^{-1}. Λ_m(CH_3OH) 97.9 S cm^2 mol^{-1}. TG analysis: lost 3.59% (calcd 3.47%, 2H_2O) in first step in 73.5 °C (the temperature of peak); lost 1.83% (calcd 1.74%, H_2O) in second step in 237.1 °C (the temperature of peak).

4.4. Cytotoxic studies

Four complexes and cisplatin were assayed for cytotoxicity in vitro against HL-60 (human leukocytoma) cells, PC-3MIE8 (human prostate carcinoma) cells, BGC-823 (human stomach carcinoma) cells, MDA-MB-435 (human galactophore carcinoma) cells, Bel-7402 (human liver carcinoma) cells, HELA (human cervix carcinoma) cells. The six cell lines were provided by the State Key Laboratory of Natural and Biomimetic Drugs, Health Sciences, Peking University. The procedure for cytotoxic studies was similar to that reported earlier.[35] Briefly, in order to calculate the concentration of each drug that produces a 50% inhibition of cell growth (IC_{50}), 100 ml of cell suspension (4×10^5 cell cm^{-3}) was exposed to various concentrations of complexes dissolved in sterile water. After incubation periods of 72 h for all cell lines, the cell concentrations were determined both in control and in drug-treated cultures. All experiments were done in quadruplicate.

4.5. Electronic absorption spectroscopy

All electronic absorption spectra were obtained on a DU-8B UV–visible spectrophotometer, using matched 10 mm path length quartz cells. The blank solution was the corresponding Tris buffer solution. Under room temperature (25 ± 1 °C), 10–40 μl of CT DNA stock solution (19.8 mM) was added, respectively, to 3.0 ml of 3.3 μM $La(L1)_2$ complex in buffer and of the blank solution every time, mixed, and the change of absorption spectra recorded. Each sample solution was scanned in the range 200–500 nm.

4.6. CD spectroscopy

All CD spectra were recorded on a Jasco J-715 spectropolarimeter using matched 10 mm path length quartz cell. The concentration of calf thymus DNA was 80 μM. The respective complex stock solution (1.00×10^{-3} M) 20 μl was added to the 3 ml DNA solution every time. The sample solution was mixed and was allowed to warm up to room temperature (25 ± 1 °C) prior to measurement. Each sample solution was scanned in the range 200–300 nm. A CD spectrum was generated which represented the mean of three scans from which the buffer background had been electronically subtracted.

4.7. Spectrofluorometric studies

The interaction of the complex $La(L1)_2$ with calf thymus DNA in vitro was studied by fluorescence spectroscopic methods as described previously.[10] Fluorescence measurements were carried out on a Shimadzu RF-540 spectrofluorophotometer. For all fluorescence measurements, the entrance and exit slits were maintained at 5.0 and 10 nm, respectively. The sample was excited at 540 nm and its emission observed at 590 nm. The buffer used in the binding studies was 50 mM Tris–HCl, pH 7.0, containing 10 mM NaCl. EB was dissolved in the buffer and concentration determined assuming a molar extinction coefficient of 5600 M cm^{-1} at 480 nm.[27] The sample was incubated for 8 h at room temperature (25 ± 0.1 °C) before spectral measurements. Under the condition, the fluorescence intensity of the respective complexes, calf thymus DNA, and EB was very small and could be ignored.

The fluorescence Scatchard plot was carried out by titrating EB(2.0–20 μM) to a DNA solution (39.7 μM) in the absence and presence of the complex $La(L1)_2$ with different concentrations, respectively. The amount of EB bound (C_b) was then calculated using the equation as previously described.[27]

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