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Synthesis and biological studies of 4′, 7, 8-trihydroxy-isoflavone metal complexes

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ABSTRACT

A new series of complexes of a ligand 4′, 7, 8-trihydroxy-isoflavone with transition metal (zinc, copper, manganese, nickel, cobalt) and selenium have been synthesized and characterized with the aid of elemental analysis, IR, electron ionization mass spectrum (EI-MS) and ^{1}H NMR spectrometric techniques. The compounds were evaluated for their in vitro antibacterial activities and antitumor properties. The metal complexes were found to be more active than the free ligand. Investigation on the interaction between the complexes and calf-thymus DNA (CT DNA) showed that the absorbance of CT DNA increased and the maximum peak (λ_{max} = 260 nm) red-shifted, while the intensity of fluorescence spectra of Epstein-Bart DNA (EB-DNA) gradually weakened, which indicated that all of these metal complexes tightly combined with CT DNA.

1. Introduction

The clinical success of cisplatin has triggered considerable interest in the search for novel and improved metal-based drugs [1,2]. Several approaches have been applied over the last three decades with common goals, such as the design of new metal compounds that remain active against resistant cell lines, compounds with a wider spectrum of antitumor activity, and compounds with a lower toxicity than cisplatin [3]. Since then numerous non-platinum metal complexes were studied, including ruthenium, iron, and cobalt complexes with different ligands [4–9].

A large number of the literatures related to synthetic metal complexes of flavonoids with strong anti-tumor activity have been reported in recent years [10–12].

Isoflavones (like genistein), a kind of flavonoid, are mainly obtained from soybeans and other plant extracts and possess low toxicity and good potency [13,14]. At the same time, genistein structure possesses many excellent properties, such as having high degree of super-delocalized and complete π-bond conjugated system, containing strong coordination oxygen atoms with the appropriate spatial configuration, which make it as a perfect metal ion chelating ligand. References [15,16] have showed the characterization of the composition of many similar complexes, but no biological activities have been described. Vivek and coworkers [17] have reported the synthesis of four 3-formylchromone Schiff base derivatives and copper complexes, the anticancer activity study results showed that the IC_{50} of these complexes was far less than the ligand itself. Xiang Chen et al. [18] have reported that transition metal complexes with the 4′-methoxy-5, 7-dihydroxy isoflavone possessed greater antitumor activities and selectivities than the parent isoflavone, even more effective than the positive control cisplatin against the selected cell lines.

All the above researches showed that these complexes have a broad prospect to be chosen as anti-tumor drugs. It is known that the metal complexes can bind to DNA via covalent and/or non-covalent interactions [19,20]. The strand scission of the DNA by quercetin and others flavonoids in presence of copper (II) or iron (III) ions was also described by Hadi and coworkers previously [21,22].

The aim of this paper was to obtain selenium (Se), zinc (Zn), copper (Cu), manganese (Mn), nickel (Ni) and cobalt (Co) complexes (**b**, **c**, **d**, **e**, **f**, **g**) with a ligand of 7, 8, 4′-trihydroxy-isoflavone (**a**) as well as evaluation of their anti-bacterial potency and antitumor activities. The six complexes have been tested for their ability to bind to CT DNA.

2. Experimental

2.1. Materials and methods

Pyrogallic acid and p-hydroxyphenylacetic acid were purchased from Merck, zinc acetate, cupric chloride, manganese acetate, nickel chloride, cobalt chloride and selenium oxychloride ($SeOCl_2$) were purchased from Aladdin-reagent Co (Shanghai, China) and used without further purification. CT DNA and EB were purchased from Sigma. All materials and solvents employed in the experiments were of analytical grade. DNA stock solution was prepared by dilution of CT DNA to buffer which contained 150 mmol NaCl and 15 mmol trisodium

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citrate at pH 7.0. Then this solution was exhaustively stirred at 4 °C for 3 days, and kept at 4 °C for no longer than a week. The ratio of UV absorbance at 260 and 280 nm (A260/A280) given by the stock solution of CT DNA was 1.89, indicating that the DNA was sufficiently free of protein contamination. The DNA concentration was determined by the UV absorbance at 260 nm using $\varepsilon = 6{,}600\ M^{-1}\ cm^{-1}$. The slit's width was 10 nm, and fluorescence pool thickness was 1 cm.

Melting points were recorded on a XP4 Electrothermal melting point apparatus without being corrected. Infrared spectra were measured on a 670 FT-IR spectrophotometer equipped with an MCT detector and a diffuse reflectance accessory [23].using the KBr pellet technique (4000–400 cm^{-1}). EI-MS were obtained from an Esquire HCT instrument in the positive/negative mode. Compounds were dissolved in DMSO: methanol (1:1). ^{1}H NMR spectra were recorded on a Bruker Avance-600 using dimethyl sulfoxide-d_6 (DMSO-d_6) as solvent. Elemental analyses were performed on a PE-2400-II Elemental Analyzer. Molar conductivity (Λ) was measured on DDS-11A model digital conductivity meter based on the measurements designed by Geary [24]. A long-term UV–VIS study was carried out to verify the stability of new complexes in DMSO/water solution. The result of antitumor screening was measured at 570 nm on a microplate spectrophotometer (Bio-Tek Instruments, ELx808zu, USA).

2.2. Synthesis of ligand 7,8,4′-trihydroxy-isoflavone, **a**

The reaction of pyrogallic acid (2.25 g, 0.02 mol) and p-hydroxyphenylacetic acid (2.84 g, 0.02 mol) was carried out in boron trifluoride etherate ($BF_3 \cdot Et_2O$ solvent and catalyst) (20 mL) with nitrogen protection. The reaction mixture was stirred at 80 °C for 3 h. Then TLC-point board was used to monitor the reaction progress till the resulting deoxy benzoin intermediate point ($R_f \approx 0.17$, agent for the ethyl acetate: petroleum ether = 1:2) was not changed.

Sequentially the N, N-dimethylformamide (DMF) (30 mL) was added dropwise to the mixture of deoxybenzoin which had been cooled to room temperature. The obtained solution was then cooled to 10 °C. In another flask, DMF (54 mL) was cooled to 10 °C and methylsulfonyl chloride ($MeSO_2Cl$) (14 mL) was added in small portions. The mixture was then allowed to stand at 55 °C for 30 min and added to the above reaction mixture slowly. During the addition, the temperature of the reaction mixture was maintained below 27 °C. The mixture was then stirred at room temperature for 4 h. The following work-up was sequentially carried out: pouring the reaction mixture into methanolic HCl (0.1 mol) followed by heating at 70 °C for 20 min (the reaction progress was monitored by TLC and the R_f of ligand **a** showed 0.07, agent for the ethyl acetate: petroleum ether = 1:2), and extracting the product by acetoacetate after removing the methanol and most of DMF. The solvent was removed under vacuum using a rotary evaporator and product was purified by recrystallization.

a: $C_{15}H_{10}O_5$, Yield 2.4 g, 46.3%. m.p. 280–282 °C. Anal. Calcd. for **a**: $C_{15}H_{10}O_5$ (%): C, 66.67; H, 3.70; Found (%): C, 66.59; H, 3.69; IR (KBr): 3470 (υ_{O-H}), 1621 ($\upsilon_{C=O}$), 1582–1441 ($\upsilon_{C=C}$), 1280 (υ_{C-O-H}), 1175 (υ_{C-O-C}) cm^{-1}. ^{1}H NMR (600 MHz, DMSO-d_6, d = doublet, s = singlet): 6.81 (2H, d, 3′,5′-H), 6.95 (1H, d, 6-H), 7.38 (2H, d, 2′,6′-H), 7.47 (1H, d, 5-H), 8.34 (1H, s, 2-H), 9.42 (1H, s, 8-OH), 9.51 (1H, s, 7-OH), 10.29 (1H, s, 4′-OH) ppm.

2.3. Synthesis of complexes

A pyridine solution (5 mL) of selenium oxychloride (about 3.3 mmol) was added to the pyridine solution of 7, 8, 4′-trihydroxy-isoflavone (ligand **a**, 3.3 mmol in 20 mL dry pyridine). The mixture was stirred at 50 °C for 6 h under nitrogen protection till ligand **a** point disappeared completely and only one point observed through the TLC monitoring. Gradually, the light brown solid precipitated when being cooled to room temperature. The solid products were filtered and washed with methanol, followed by being washed with distilled water to remove inorganic selenium. The selenium compound **b** was then dried in vacuum freeze-drying.

The ethanol solution of ligand **a** (0.270 g, 0.001 mol in 30 mL ethanol) was adjusted to pH 8–9 by addition of triethylamine. The mixture was stirred at 40 °C for 2 h under nitrogen protection, followed by introducing hydrated metal (II) salt (acetate or chloride) (0.001 mol) slowly via syringe which was dissolved in ethanol (10 mL). The reaction mixture was stirred at 60 °C for 24 h, and then stood at room temperature for 2 days. Powered solids were yielded by filtration, rinsed with ethanol and dried under vacuum.

b. SeO(**a**-2H), Yield 0.51 g, 44.44%. m.p.>300 °C. Anal. Calcd. for **b**: $C_{15}H_8O_6Se$ (%): C, 49.59; H, 2.20; Se, 21.75. Found (%): C, 49.56; H, 2.11; Se, 21.92. IR (KBr): 3118 (υ_{O-H}), 1606 ($\upsilon_{C=O}$), 1538–1328 ($\upsilon_{C=C}$), 1206 (υ_{C-O-H}), 1172 (υ_{C-O-C}), 696 (υ_{Se-O}) cm^{-1}. ^{1}H-NMR (600 MHz, DMSO-d_6): 6.73 (2H, d, 3′,5′-H), 7.00 (1H, d, 6-H), 7.21 (2H, d, 2′,6′-H), 7.38 (1H, d, 5-H), 8.30 (1H, s, 2-H), 10.15 (1H, s, 4′-OH) ppm. $\Lambda = 4.6\ S \cdot cm^2 \cdot mol^{-1}$.

c. Zn(**a**-H)$_2$, Yield 0.16 g, 53.2%. m.p.>300 °C. Anal. Calcd. for **c**: $C_{30}H_{18}O_{10}Zn$ (%): C, 59.66; H, 2.98; Zn, 10.84. Found (%): C, 59.46; H, 2.76; Zn, 10.94. IR (KBr) υ: 3347 (υ_{O-H}), 1608 ($\upsilon_{C=O}$), 1558–1387 ($\upsilon_{C=C}$), 1206 (υ_{C-O-H}), 1174 (υ_{C-O-C}) cm^{-1}. ^{1}H-NMR (600 MHz, DMSO-d_6): 5.69 (4H, d, 3′, 5′, L3′, L5′-H), 6.76 (2H, d, 6, L6-H), 7.02 (4H, d, 2′, 6′, L2′, L6′-H), 7.35 (2H, s, 5, L5-H), 8.39 (2H, s, 2, L2-H), 9.83 (2H, s, 7, L7′-OH),10.25 (2H, s, 4, L4′-OH) ppm. $\Lambda = 5.2\ S \cdot cm^2 \cdot mol^{-1}$.

d. Cu(**a**-H)$_2$, Yield 0.19 g, 63.3%. m.p.>300 °C. Anal. calcd. for **d**: $C_{30}H_{18}O_{10}Cu$ (%): C, 59.82; H, 2.99; Cu, 10.56. Found (%): C, 60.16; H, 2.92; Cu, 10.56. IR (KBr) υ: 3371 (υ_{O-H}), 1608 ($\upsilon_{C=O}$), 1567–1387 ($\upsilon_{C=C}$), 1236 (υ_{C-O-H}), 1174 (υ_{C-O-C}) cm^{-1}. ^{1}H-NMR (600 MHz, MSO-d_6): 6.77 (4H, d, 3′, 5′, L3′, L5′-H), 7.34 (2H, d, 6, L6-H), 7.99 (4H, d, 2′, 6′, L2′, L6′-H), 8.09 (2H, s, 5, L5-H), 8.27 (2H, s, 2, L2-H), 9.43 (2H, s, 7, L7′-OH),10.36 (2H, s, 4, L4′-OH) ppm. $\Lambda = 2.8\ S \cdot cm^2 \cdot mol^{-1}$.

e. Mn(**a**-H)$_2$, Yield 0.16 g, 54.1%. m.p.>300 °C. Anal. calcd. for **e**: $C_{30}H_{18}O_{10}Mn$ (%): C, 60.71; H, 3.03; Mn, 9.26. Found (%): C, 61.02; H, 2.96; Mn, 9.15. IR (KBr) υ : 3399 (υ_{O-H}), 1608 ($\upsilon_{C=O}$), 1558–1386 ($\upsilon_{C=C}$), 1222 (υ_{C-O-H}), 1174 (υ_{C-O-C}) cm^{-1}. ^{1}H-NMR (600 MHz, DMSO-d_6): 5.31 (4H, d, 3′, 5′, L3′,L5′-H), 5.68 (2H, d, 6, L6-H), 6.97 (4H, d, 2′, 6′, L2′, L6′-H), 7.47 (2H, s, 5, L5-H), 8.34 (2H, s, 2, L2-H), 9.54 (2H, s, 7, L7′-OH),10.32 (2H, s, 4, L4′-OH) ppm. $\Lambda = 4.3\ S \cdot cm^2 \cdot mol^{-1}$.

f. Ni(**a**-H)$_2$, Yield 0.16 g, 53.8%. m.p.>300 °C. Anal. calcd. for **f**: $C_{30}H_{18}O_{10}Ni$ (%): C, 60.33; H, 3.02; Ni, 9.84. Found (%): C, 60.66; H, 2.88; Ni, 9.79. IR (KBr) υ: 3347 (υ_{O-H}), 1608 ($\upsilon_{C=O}$), 1558–1387 ($\upsilon_{C=C}$), 1206 (υ_{C-O-H}), 1173 (υ_{C-O-C}) cm^{-1}. ^{1}H-NMR (600 MHz, DMSO-d_6): 5.31 (4H, s, 3′, 5′, L3′, L5′-H), 5.88 (2H, s, 6, L6-H), 6.49 (4H, d, 2′, 6′, L2′, L6′-H), 7.47 (2H, d, 5, L5-H), 8.34 (2H, s, 2, L2-H), 9.49 (2H, s, 7, L7′-OH),10.11 (2H, s, 4, L4′-OH) ppm.$\Lambda = 9.0\ S \cdot cm^2 \cdot mol^{-1}$.

g. Co(**a**-H)$_2$, Yield 0.15 g, 53.8%. m.p.>300 °C. Anal. calcd. for **g**: $C_{30}H_{18}O_{10}Co$ (%): C, 60.31 H, 3.02; Co, 9.87. Found (%): C, 60.38; H, 3.01; Co, 10.03. IR (KBr) υ: 3346 (υ_{O-H}), 1608 ($\upsilon_{C=O}$), 1558–1380 ($\upsilon_{C=C}$), 1206 (υ_{C-O-H}), 1173 (υ_{C-O-C}) cm^{-1}. ^{1}H-NMR (600 MHz, DMSO-d_6): 5.81 (4H, d, 3′, 5′, L3′, L5′-H), 6.38 (2H, s, 6, L6-H), 6.95 (4H, d, 2′, 6′, L2′, L6′-H), 7.46 (2H, s, 5, L5-H), 8.44 (2H, s, 2, L2-H), 9.23 (2H, s, 7, L7′-OH),10.43 (2H, s, 4, L4′-OH) ppm.$\Lambda = 8.9\ S \cdot cm^2 \cdot mol^{-1}$.

2.4. Biological studies

2.4.1. Antibacterial activity

The synthesized compounds were tested for their antibacterial activities by means of standard double dilution method using agar media [25]. The assayed collection included the following microorganisms: ATCC25923, *Staphylococcus aureus* 701230, *S.*

aureus 701219, *Staphylococcus epidermidis* 620206, *S. epidermidis* ss, *Enterococcus faecalis* 616247, *E. faecalis* 609230, *Enterococcus faecium* 609205, ATCC25922, *Escherichia coli* 629119, *E. coli* 629247, *Pseudomonas aeruginosa* 630266, *P. aeruginosa* 701218, *Klebsiella pneumonia* 626511, *K. pneumonia* 626238, *Baumanii* 302234-1, *Baumanii* 302234-2.

In the twofold serial dilution method, ligand **a** and complexes **b**–**g** were dissolved by DMSO with Tween-80, which ensured the compounds dissolved completely without precipitation. The maximum of concentration was 2000 μM, and was double diluted sequentially. The concentration of original bacteria liquids were determined to be about 10^7–10^8 CFU·mL^{-1} by nephelometry. Positive control: levofloxacin; negative control: DMSO and Tween-80. Each assay in this experiment was repeated three times.

2.4.2. Antitumor screening studies

By use of MTT [3-(4,5-dimethylthiazole-2-yl)-2,5, diphenyltetrazolium bromide] assay, the antitumor activities of the ligand and its metal complexes were assessed against five cancer cell lines: SW620 (human colon carcinoma cell line), Hela (human cervix cancer cell line), HepG2 (human hepatoma carcinoma cell line), MDA-MB-435 (human breast carcinoma cell line) and A549 (human lung cancer cell line).

The MTT assay was performed by Mosmann [26] as follows:

Tumor cells (180 μL, 4000 cells/well) were plated in 96-wells flat bottom microtiter plates. All compounds were dissolved immediately in dimethyl sulfoxide (DMSO), and diluted with the growth medium. The final concentrations ranged from 0.1 to 600 μM. In addition, the final DMSO concentration in the culture media was lower than 1% showing no obvious cytostatic effect in preliminary tests. Culture medium only containing different concentrations of DMSO was added to the cells in the control wells. Culture medium with the corresponding concentrations of compounds, but void of cells was used as blank. The result was measured at 570 nm on a microplate spectrophotometer.

Assays were performed in triplicate in three independent experiments. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated using the software "Dose–Effect Analysis with Microcomputers". Data were analyzed using Student un-paired *t*-test.

2.4.3. DNA-binding studies

The interaction of ligand **a** and complexes **b**–**g** with CT DNA had been studied with UV spectroscopy in order to investigate the possible binding modes. In UV titration experiments, the spectra of CT DNA with the presence of compounds **a**–**g** have been recorded for a constant CT DNA concentration and diverse concentrations (0, 20, 40, 60, 80, 100 μM) of compounds **a**–**g**.

The competitive properties of each compound with EB have been investigated by UV and fluorescence spectroscopy in order to examine the possibility to displace EB from its CT DNA–EB complex. The CT DNA–EB complex was prepared by adding 20 μM EB and 26 μM CT DNA in buffer (150 mmol NaCl and 15 mmol trisodium citrate at pH 7.0). The interaction of ligand **a** and complexes **b**–**g** with the DNA–EB complex was studied by adding a certain amount of solution of compounds **a**–**g** step by step into the solution of the DNA–EB. The influence of the addition of each compound to the DNA–EB complex solution had been obtained by recording the variation of UV or fluorescence emission spectra.

3. Results and discussion

3.1. Synthesis

Pyrogallol and p-hydroxyphenyl acetic acid were used as the raw materials to obtain ligand **a** (7, 8, 4′-trihydroxy-isoflavone) by multi-step reactions: Friedel–Crafts reaction and cyclization reaction. The corresponding complexes were then synthesized in the presence of $BF_3 \cdot Et_2O$, DMF and $MeSO_2Cl$ (Fig. 1). The synthesis of complexes was achieved via titrating a solution of acetate or chloride metal (II) salt to an ethanol solution of ligand **a** (pH = 7–8) with high purities and acceptable yields. Their molecular structures were studied with the aid of IR, EI-MS, ^{1}H NMR, elemental analysis and melting point determination.

3.2. Spectroscopic data

Compared with the IR spectra of ligand **a**, there were evidences for the coordination between the transition metal ions and ligand molecules. Firstly, the absorption bands of complexes **b**–**g** exhibited strongly downward shifts for the O – H and C – O – H groups, and other groups showed extremely slightly shifts. Moreover, the deprotonation and coordination were also confirmed by the bands that ranged from 400 to 800 cm^{-1}, attributed to M – O linkages [18,27–32].

The EI-MS of the complexes have been investigated by positive/negative mass spectrometric measurements. The major structurally informative molecular ions and fragment ions in the negative-ion mode of compounds **a**–**g** were presented in Table 1. As expected, the data of molecular ions peak of complexes **b**–**g** indicated that each complex contained one metal ion bound with two molecule ligands except complex **b** contained one metal ion with one molecule ligand. The appearance of the characteristic cluster of isotopic peaks of the corresponding metal ion isotopes showed that metal ions have been successfully coordinated to the ligand **a**. The intact negative ion of ligand molecule as a dominant fragment ion appeared at m/z 268.0. Complexes **b** and **g** showed ions obtained by loss of a hydroxy, which was observed at m/z 346.8 and 576.3. It is worth to note that the spectrums of complexes **c**–**g** fragment ion which sequentially lose a ligand were found at m/z 332.3, 331.3, 325.8, 325.9, 326.6.

The ^{1}H NMR spectra of the ligand and complexes have been tested. The resonances of the six complexes are similar, but different from that of the free ligand. The ligand **a** showed resonances of hydrogen atoms in 7, 8, 4′-hydroxyl groups at 9.42, 9.51 and 10.29 ppm. For the corresponding complexes **b**–**g**, the resonances of the 4′, L4′-hydroxyl group protons were found at 10.15, 10.25, 10.36, 10.32, 10.11 and 10.43 ppm respectively, and the signals of the benzopyran and aromatic protons displayed extremely slightly shifts either in comparison with ligand **a**. However, the signals arising from the 8, L8-hydroxyl groups protons disappear completely,

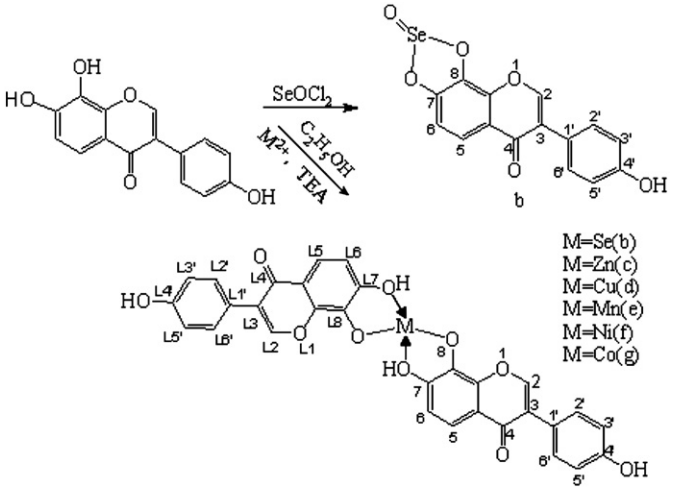

Fig. 1. Scheme of synthesis for compounds **b**–**g** by complexation with Se(II), Zi(II), Cu (II), Mg(II), Ni(II) and Co(II).

Table 1
Main EI-MS data of compounds (**a**–**g**) (m/z).

Compd.	EI-MS	Calcd.	Fragment ions (MS_2)
a	268.8 (M − 1)	270.0	
b	364.7, 363.6 (M + 1)	363.2	346.8, 301.9, 269.9
c	601.1, 602.0, 603.0, 604.0, 605.0 (M − 1)	603.8	567.9, 332.4
d	600.1, 601.0, 602.0 (M − 1)	602.0	568.6, 331.3, 270.2
e	592.1, 593.2 (M − 1)	593.4	325.8, 267.8
f	595.1, 596.1, 597.1 (M − 1)	597.1	560.3, 325.9, 268. 8
g	596.0, 597.0 (M − 1)	597.4	576.3, 326.6, 270.1

pointing out that the binding oxygen atoms of metal ions belong to the 8-hydroxyl groups.

Fig. 2 shows the results of the long-term UV–visible (UV–VIS) study of the ligand and complexes. The UV spectrum of ligand featured a maximum at 262 nm. Compared with ligand **a**, the absorption wavelengths of new complexes (**b**–**g**) exhibited red shift. In addition, it is significant to note that the compounds hardly varied for up to 1 month, meaning that new complexes were stable in DMSO/water solution.

The data of elemental analysis, IR, EI-MS and ^{1}H NMR spectrum confirmed that ligand **a** and metal ions bond at 7, 8-phenolic oxygen group. The elemental analysis and mass spectrum agreed well with the formation of 1:2 (metal/ligand) stoichiometry, except compound **b** with the formation of 1:1 (metal/ligand) stoichiometry.

3.3. Antibacterial activity of the ligand and its complexes

Ligand **a** and complexes **b**–**g** were evaluated for their in vitro antibacterial activity against representative Gram-positive and Gram-negative strains using standard techniques [25]. Minimum inhibitory concentration (MIC) is defined as the concentration of the compound required to give complete inhibition of bacterial growth and MICs of the synthesized compounds along with the standard drugs are compared in Table 2.

From Table 2, the following conclusions were obtained: (1) complexes **b** and **e** exhibited good antimicrobial activities against *S. aureus*, *E. faecalis* and especially against *S. epidermidis* and had great potential in the exploration of new chemotherapy agents. The other complexes also had such antibacterial activity, but a little weaker than complexes **b** and **e**. (2) The antibacterial activities of the complexes were better than those of both the ligand **a** and the starting metal salts. These provided reasons to believe that antibacterial activities of the complexes synthesized do not correlate with the toxicity of the metal (II) ions against the bacterial tested.

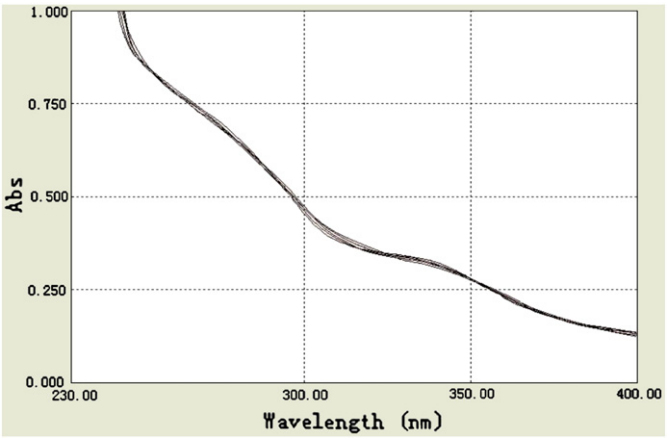

Fig. 2. Long-term UV–VIS spectra (λ_{max}) of complex b in DMSO/water solution during 30 days.

Table 2
The MIC of ligand **a** and its complexes on different microorganisms.[a]

Strains	MIC (μM)								
	LVFX	DMSO	**a**	**b**	**c**	**d**	**e**	**f**	**g**
1	+	−	1000	125	1000	−	250	−	−
2	+	−	1000	125	1000	−	250	−	−
3	125	500	−	+	500	62.5	125	250	1000
4	125	500	500	+	500	125	125	250	1000
5	125	−	500	+	−	−	250	−	−
6	500	−	−	500	−	−	1000	−	−
7	500	−	1000	1000	−	−	1000	−	−
8	62.5	−	1000	125	500	250	250	−	−
9	+	−	1000	125	500	250	250	−	−
10	+	−	1000	−	−	−	1000	−	−
11	+	−	1000	125	500	−	250	1000	1000
12	+	−	−	125	−	−	500	−	−
13	125	−	−	−	−	−	1000	−	−
14	125	−	1000	−	−	−	1000	−	−
15	125	−	1000	−	−	−	250	−	−
16	125	−	500	62.5	1000	−	250	−	−
17	62.5	−	−	−	−	−	1000	−	−

[a] LVFX is levofloxacin; **1** is *Staphylococcus aureus* 701230; **2** is *Staphylococcus aureus* 701209; **3** is *Staphylococcus epidermidis* 620206; **4** *Staphylococcus epidermidis* 619083; **5** is *Enterococcus faecalis* 616247; **6** *Enterococcus faecalis* 609230; **7** is *Escherichia coli* 629119; **8** is *Pseudomonas aeruginosa* 630266; **9** is *Pseudomonas aeruginosa* 701218; **10** is *Escherichia coli* 629247; **11** is *Enterococcus faecium* 609205; **12** is *Klebsiella pneumonia* 626511; **13** is *Klebsiella pneumonia* 626238; **14** is *Baumanii* B5; **15** is *Baumanii* B8; **16** is ATCC25923; **17** is ATCC25922. All strains were from the First Affiliated Hospital of Wenzhou Medical College 2001/01-2009/06 clinical isolates; "−" means the MIC of compounds is more than 2000 μM; "+" means the MIC of compounds is less than 62.5 μM.

3.4. Antitumor activities

The ligand **a** and corresponding complexes (**b**–**g**) were tested for antitumor activities using the MTT assay [26] at concentrations ranging from 0.1 to 600 μM against five different cancer cell lines: human breast carcinoma cell line (MDA-MB-435), human uterine cervix cancer cell line (Hela), human liver cancer cell line (HepG 2), human colon carcinoma cell line (SW620) and human lung carcinoma cell line (A 549). The results, expressed as the concentration of the compound required to kill the tumor cell by 50% (IC_{50}), are listed in Fig. 3. Cisplatin was used as reference substance.

The screening data revealed that all selected compounds possessed antitumor activities. It should be noted that MDA-MB-435 and A549 cells showed higher sensitivities to the anti-proliferative effect of these complexes, while SW620 and HepG 2 cell lines were found to be more resistant. In A549 and MDA-MB-435 cell lines, it was shown that complexes **b**, **d** and **e** induced a more than two times stronger response than ligand **a** and the positive control cisplatin. Moreover, complexes **b** and **e** exhibited slightly more effectiveness, indicating that the described complexes possessed improved antitumor activity. It is interesting to note that, the IC_{50} values of complexes **b** and **e** are lower than the positive control cisplatin displayed in Hela cells. In HepG 2 cells, the IC_{50} values of all complexes exhibited at least three times more effectiveness than that of the ligand **a**. Complex **c** and **g** exhibited the smallest IC_{50} values against the SW620 and MDA-MB-435 cell lines, respectively. All of these results demonstrated that these newly synthesized complexes have elevated antiproliferative activities in comparison with ligand **a** and corresponding metal ions, and some were even more effective than the positive control cisplatin.

The experiments showed that selenium and other metal ions can elevate the antiproliferative activities of ligand **a** and one of the reasons is probably that the combination between the complexes and

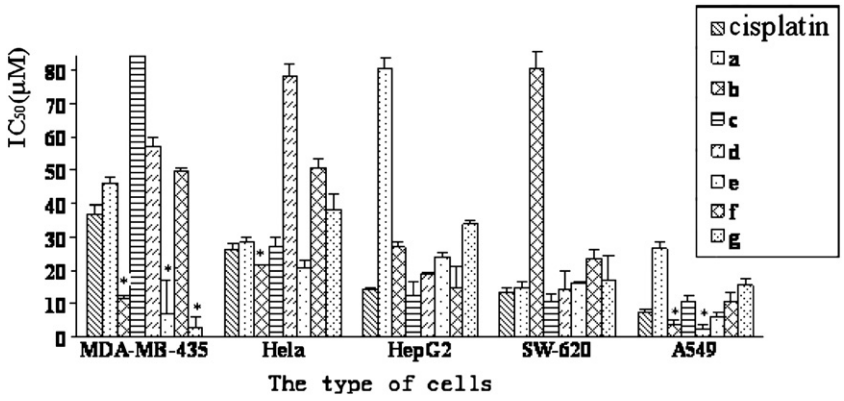

Fig. 3. Inhibition (IC_{50}) of complexes **a–g** against various human cancer cell lines.(*: $p<0.05$, complexes compared with the ligand **a** and cisplatin).

cancer DNA may inhibit DNA replication and transcription and induce cell apoptosis [33–35].

3.5. Interaction with DNA

3.5.1. DNA-binding study with UV spectroscopy

In the case of covalent binding, the labile ligand of the complexes is replaced by a nitrogen base of DNA such as guanine N7, while the non-covalent DNA interactions include intercalative, electrostatic and groove (surface) binding of metal complexes outside of DNA helix, along major or minor groove [36].

An effective method to examine the binding mode of DNA with metal complexes is the titration method accompanied with UV absorption spectroscopy. In general, the binding of an intercalative molecule to DNA is accompanied by hypochromism and/or significant red-shift in the absorption spectra due to strong stacking interaction between the aromatic chromophore of the ligand and DNA base pairs [37]. Spectroscopic titration of a solution of the compounds with CT DNA should be performed in order to obtain the possibility of binding of each compound to CT DNA [38].

The UV spectra of the interaction of CT DNA with ligand **a** and complexes **b–g** have been studied for a constant CT DNA concentration in different compound concentrations. UV spectra of CT DNA in the presence of ligand **a** and complexes **b–g** derived for diverse concentrations are shown in Fig. 4. The differences observed in the absorption spectra of CT DNA with the presence of these compounds, i.e. the increase of the intensity at $\lambda_{max}=260$ nm and the red-shift of the λ_{max} indicate that the interaction of these complexes results in a direct formation of new complex with double-helical CT DNA. The absorption intensity at 260 nm increased due to the fact that the purine and pyrimidine DNA-bases are exposed because of the binding of the compounds to DNA. These characteristics can be attributed to an interaction binding mode [39] which causes the variations of the conformation of DNA [40]. The presence of ligand **a** does not provoke any significant variation of the intensity or the position of the emission band at 260 nm of the CT DNA system, indicating ligand **a** cannot bind to CT DNA. Additionally, the red-shift observed for complexes **b–g** provided evidences of the stabilization of the CT DNA. The results derived from the UV titration experiments suggested that all complexes can bind to CT DNA.

3.5.2. Competitive studies with ethidium bromide

A competitive EB binding study has been undertaken with UV–VIS and fluorescence experiments in order to examine the ability of these compounds to displace EB from its EB–DNA complex [41,42]. Intense fluorescence can be observed in the EB–DNA complex due to the intercalation of the planar phenanthridinium ring between adjacent base pairs on the double helix [43]. The variations observed in the

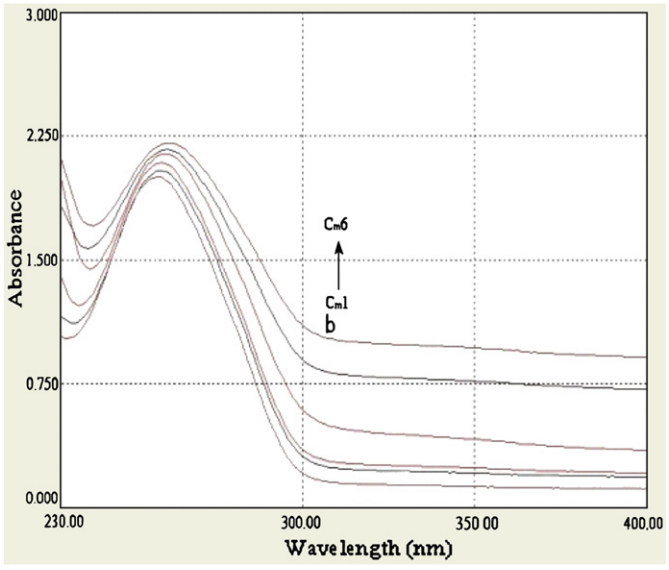

Fig. 4. UV spectra of CT DNA interacted with increasing amounts of complex **b**. The arrow shows the intensity changes on increasing the complex concentration ([complex]=0–100 μM). λmax=260 nm.

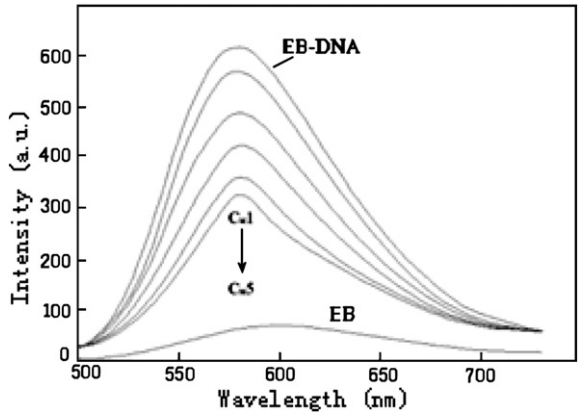

Fig. 5. Fluorescence quenching curves of EB bound to DNA by complex **b**. The arrow shows the intensity changes on increasing the complex concentration. λmax=568 nm.

spectra of EB–DNA are often used for the interaction study between DNA and other compounds, such as metal complexes [44].

The absorption of the mixture solution of EB, CT DNA and complexes **b**–**g** is shown in Fig. 5. The presence of ligand **a** did not provoke any significant variations of the intensity or the position of the emission band at 568 nm of the DNA–EB system, indicating that ligand **a** could not displace EB from the DNA–EB complex. On the other hand, the intensity of the emission band at 568 nm of the DNA–EB system decreased upon the presence of complexes **b**–**g** at diverse concentrations, indicating that there existed competition between the complexes and EB in binding to DNA. The observed quenching of DNA–EB fluorescence for complexes **b**–**g** suggested that they displaced EB from the DNA–EB complexes and these complexes and CT DNA were probably interactive [20,45].

4. Conclusions

The synthesis of ligand 7, 8, 4′-trihydroxy-isoflavone (**a**) and six new complexes (**b**–**g**) have been achieved. All compounds were characterized by IR, ^{1}H NMR, mass spectroscopy and elemental analysis.

The bacteriostatic activities of 7, 8, 4′-trihydroxy-isoflavone and its metal complexes were studied. The results showed that the complexes possessed selective inhibition on some kind of bacteria and broad-spectrum antibacterial activity, which indicated that the bacteriostatic activities of these complexes were positively related to the concentrations, and the metal complexes commonly possessed higher activities than 7, 8, 4′- trihydroxy-isoflavone.

All of the investigated compounds were tested for in vitro antitumor activities against five human cancer cell lines: MDA-MB-435, Hela, HepG2, SW620 and A549. The results showed that these complexes displayed effective anti-tumor activities on those cancer cell lines, especially SW620 and A549. Complexes **b** and **e** possessed greater activity and selectivity than the other described complexes in the antitumor action against the selected cancer cell lines.

The interactions of 7, 8, 4′-trihydroxy-isoflavone and its complexes with CT DNA were studied by fluoroscopy and ultraviolet spectroscopy methods. The results showed that in the presence of these compounds the absorption of CT DNA increased and the maximum peak ($\lambda_{max} = 260$ nm) red-shifted while the intensity of fluorescence spectra of EB–DNA gradually weakened, which consequently indicated that all of these complexes had tighter combined with CT DNA.

Comparing with other metal-natural compound complexes [17,18], these compounds exhibited dose-dependent growth inhibitory effects in some cancer cell lines as well. Moreover, a synergistic enhancement was shown in the antiproliferative activities upon metal conjugation compared to their corresponding parent ligand as well as to the positive control. All of these results would be important to evidence the novelty of these compounds with respect to other findings.

Abbreviations

CT DNA	calf-thymus deoxyribonucleic acid
EB DNA	Epstein–Bart deoxyribonucleic acid
UV-VIS	Ultraviolet-visible spectroscopy
EI-MS	electron ionization mass spectrum
CFU	colony-forming units
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MIC	minimal inhibitory concentration

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.jinorgbio.2011.08.019.

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