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Methyl-substituted enhance the antitumor activity in Square-Planar Metal Complex, analysis of ΔE, ΔG, CV, UV-vis and Luminescence

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Abstract

Two novel Ala-based copper(II) compounds of formulas [(1,10-Phen)Cu(Ala)·(H_2O)]·Cl·H_2O (1) and [(2,9-DMP)Cu(Ala)·(H_2O)]·NO_3·H_2O (2) have been synthesized and determined by X-ray diffraction. The two complexes stack in Square-Planar structure and exhibit excellent anticancer activities. Especially, the complex 2 with methyl-substituted-phenanthroline presents higher anticancer properties than the complex 1 with Phen. Computational ΔE ($\Delta E_{1,10\text{-Phen}} > \Delta E_1$ and $\Delta E_{2,9\text{-DMP}} > \Delta E_2$) has showed the two substituted methyl groups can activate the conjugated system of π_{14}[14] in the aromatic phenanthroline ring to inhibit the growth of cancer cells. From ΔG calculation, it illustrates that the complex 2 is easier to decompose into free Cu^{II} and ligands than 1. UV-vis and luminescent spectra also reveal the 2,9-DMP in the complex 2 is partially dissociated. The partially dissociated 2,9-DMP might be responsible for the superiority of anticancer activities. In addition, the $E_{2pCu(II)/Cu(I)}$ is more positive than $E_{1pCu(II)/Cu(I)}$, which might be a reason that Cu^{II}-SOD is more effective to transfer O_2^{-}• into O_2 to inhibit the growth of cancer cells.

Introduction

As functional metal in living organisms, copper presents in many proteins and enzymes, especially plays an important role in the electron transfer of many cellular processes. [1, 2] From early 1960s, the copper complex of thiosemicarbazone (TSC) was reported as potential antitumor drug. [3] Then many studies showed that the modification in ligands and structures could produce better Cu-complexes with higher

cytotoxicity to cancer cells and lower toxicity to normal cells. [4-11] Furthermore, Cu-complexes with Phen and Bipy were also tested as anticancer compounds, and some with general formula $[Cu(N–N)(O–O)]NO_3$ and $[Cu(N–N)(O–N)]NO_3$ have been patented and registered under the name of Casiopeínas which have shown promising antineoplastic activity against cancer cells in vitro and in vivo, respectively. [12, 13] However, their molecular structures have not been determined by X-ray single crystal diffraction, [14-17] therefore, it doesn't have precise bond lengths and angles which are significant for the research of anticancer mechanism. And the assistant ligand is quite different from our ligand (Ala) which possesses harmonization with living system and effect. Moreover, though the mechanisms in anticancer are described as binding, cleavage, and oxidative modification to DNA, [18-28] whereas it is still needed to be clarified. The ligands in Cu-complexes affect the anticancer activities greatly; especially the planar ligands which can intercalate the grooves of DNA therefore disturb the growth of cancer cells. [29-33] However, the more efficient phenanthroline ligands for anticancer are needed to be chosen, which will be benefit for the design and synthesis of Cu-complexes as anticancer drugs. Fortunately, copper is an active center that combines with varieties ligands, [34, 35] which provide a splendid field for the synthesis and choices of the Cu-complex as anticancer drugs. In our previous works, we have found that Phen is much better than Bipy as ligands for Cu-complexes as anticancer drugs. [36] Hence, we start to research the difference between 1,10-phenanthroline-based and substituted-phenanthroline-based Cu-complexes as anticancer drugs. Fortunately we have synthesized two complexes

{[(1,10-Phen)Cu(Ala)(H_2O)]·Cl·H_2O and [(2,9-DMP)Cu(Ala)(H_2O)]·NO_3·H_2O} and tested their anticancer activity. As expected, the two complexes showed excellent anticancer activities against A-549, Bel-7402, HCT-8 cancer cell lines, even better than 5-Fu which has been applied to clinical, and their compositions[1,10-Phen, 2,9-DMP, Alanine (Ala) and $Cu(NO_3)_2$]. More importantly, the complex 2 with dimethyl-substituted phenanthroline ligand exhibited much higher cytotoxicity than the complex 1. To reveal the mechanisms that caused the difference of cytotoxicity between the two complexes and their free phenanthroline ligands, we have determined the molecular structure by single crystal X-ray diffraction, calculated the energy gap (ΔE) between π-π^* of Phen and 2,9-DMP in free and coordinated state, computed the Gibbs free energies (ΔG) of the complexes which decompose into Cu^{II} and free ligands, represented UV-vis and luminescent spectra in solution. At last, we have revealed the relationships between the anticancer activities and their molecular structures, ΔE, ΔG, UV-vis and luminescent properties.

Experimental

Materials and Methods

All reagents were directly obtained from commercial supplies and analytical grade. All manipulations were carried out in the laboratory atmosphere. IR spectra were obtained from the Bruker EQUINOX55 spectrometer with KBr disks. UV-vis spectra were determined using SHIMADZU UV-2550 spectrometer in aqueous solution. Luminescence was detested by EDINBURGH FLS-920 Instrument in aqueous solution. Redox potential $E_{pCu(II)/Cu(I)}$ were measured using CHI-832 electrochemical

workstation in 0.1M KCl was used as a supporting electrolyte; 1.25×10^{-3}M aqueous solutions of these complexes. The potential was scanned from −0.65 to 0.5 V with a scan rate of 50mV/s, glassy carbon, Pt foil, and Ag wire as working, auxiliary, and reference electrodes, respectively.

Synthesis of [(1,10-Phen)Cu(Ala)·(H_2O)]·Cl·H_2O (1). the complex were synthesized by the reaction of copper(II) chlorate/Ala/Phen (1:1:1 molar ratio) in 30 ml ethyl alcohol (84%), twelve hours later, the final solution was filtrated and transferred into a tube, then diffused by ether. Last blue needles crystals were obtained in 2 weeks later. Elemental analysis (%), calc (exp): C 43.70 (43.66), H 3.88 (3.80), N 13.60 (13.46), Cu 15.54 (15.32). IR (selected data, KBr, ν/cm^{-1}): 3442 m, 3291 w, 3126 w, 2971 w, 2930 w, 1648 s, 1519 s, 1455 s, 1430 s, 1392 vs, 1115 m, 858 m, 723 m, 559 w.

Synthesis of [(2,9-DMP)Cu(Ala)·(H_2O)]·NO_3·H_2O (2). Complex 2 was synthesized in the same procedure by the reaction of copper(II) nitrate/Ala/2,9-DMP (1:1:1 molar ratio). Two weeks later, the green block crystals were obtained. Elemental analysis (%), calc (exp): C 44.75 (44.76), H 4.39 (4.30), N 12.29 (12.26), Cu 14.04 (14.02). IR (selected data, KBr, ν/cm^{-1}): 3405m, 3269 w, 2976 w, 2931 w, 1604 s, 1501 s, 1453 s, 1409 s, 1384 vs, 1115 m, 864 m, 729 m, 549 w.

Computational ΔG and △E

The calculations for the relative changes of Gibbs free energies (ΔG) were carried out with Gaussian 03 program. The hybrid density functional B3LYP and 6-31+G(d) basis set were used to fully optimized the structures of complexes 1 and 2 to obtain

their Gibbs free energies. Solvent effect was considered through PCM model for all the calculations. And the π* and π energies of phenanthroline at free and coordinated situation are calculated under B3LYP/6-31+G* method using Gaussian 03 program.

X-ray crystallography

Single crystal X-ray analyses were performed on a Bruker APEXII area detector device with Mo-Kα radiation (l = 0.71073 Å) by the Φ–ω scan method. Bruker SMART CCD diffract meter equipped with a Mo anode and graphite monochromator (λ=0.70713 Å). The crystals with approximate dimensions 0.06×0.18×0.26 mm^3 were mounted on a glass fiber at 293(2) K. The initial unit cell was determined using a least squares analysis of a set of random reflections obtained from three short (20 data frame) series of 0.3°-wide ω-scans which were well distributed in space. The intensities were collected using ω-scans with a crystal-to-detector distance of 5cm to yield the complete sphere of data to a resolution of 0.75 Å. The final unit cell was calculated using a least squares refinement of reflections culled from the entire data set. Empirical absorption corrections were made from ψ-scan data using the program SHELXTL 97 at the data reduction stage along with the correction for Lorentz and polarization effects. The structure was solved using direct methods and refined against $|F2|$ using the routines included in the APEX-2 software suite. Hydrogen positions were not readily discernible from electron density maps. All non-hydrogen atoms were refined anisotropic ally.

MTT assay test

Tests of the cytotoxicity of complex 1, 2 and 5-Fu against three cancer cell lines, i.e.

A-549, Bel-7402 and HCT-8 were carried out by MTT assay. The cells in logarithmic growth were cultured in a RPMI 1640 medium with 10% fetal bovine serum. The cell suspension (ca. 5000 cells mL^{-1}) was redistributed into 96-well micro plates equivalently, 100 μL per well. After preincubation (24 h at 37°C, 5% CO_2), 10 μL test compound solution (dissolved in DMSO, and then diluted by saline to 0.005 μg mL^{-1}, 0.05 μg mL^{-1}, 0.5 μg mL^{-1}, 5 μg mL^{-1}, respectively) was added to each well and then each well was finally made up to 200 μL by RPMI 1640 medium. Incubation of cells with the test compound lasted for 72 h at 37°C, in a humidified atmosphere of 5% CO_2. Then the supernatant was removed, and 100 μL MTT was added to each well. After 4h incubation at 37°C, the supernatant was removed again and the formazan precipitates formed were dissolved with 200 μL of DMSO by sonic oscillation. The optical density of each well was measured at 544 nm wavelength using a microplate reader. The inhibition ratio (%) was calculated as eqn (1):

Inhibition ratio (%) = [(ODc–ODt) /(ODc-OD0)]×100% (1),

Where ODc presents the absorbance of the contrast sample; ODt represents the absorbance of the test compound; OD0 represents the absorbance in the blank solution. All the anticancer experiments have been completed by the Institute of Materia Medica, Chinese Academy of Medical Science, and all the dates can be checked from there.

Results and discussion

Anticancer activities in vitro

These complexes and ligands were evaluated for anti-proliferative activity against A-549, Bel-7402 and HCT-8 cancer cells in vitro. As the shown in Table 1, the metal

salt and ligands exhibit low activities against cancer cells, nonetheless the two new complexes have shown excellent anticancer properties, particularly the complex 2 with methyl substituent. The copper(II) nitrate and Ala showed low inhibition against cancer cells, because the moderate copper(II) and Ala are benefited for most living organisms, even cancer cell, and enter to cells easily. As an organic molecule, the Phen is hard to pass the tympanic membrane to enter cancer cells. However, the Phen coordinate with the copper(II) and Ala which might lead to the complexes easier to pass the bio-membrane for anti-proliferation. From the data of inhibition ratios and $IC_{50,}$ the complex 2 have excellent anticancer properties than complex 1 which should be related with the two substituted methyl groups.

Table 1 The anticancer activities of complex 1, 2, 5-Fu, $Cu(NO_3)_2$ and ligand against human cancer cells

Complex	A-549 (lung cancer)		Bel-7402 (liver cancer)		HCT-8 (colonic cancer)	
	Inhibition ratios(%)	IC_{50} (μg/ml)	Inhibition ratios(%)	IC_{50} (μg/ml)	Inhibition ratios(%)	IC_{50} (μg/ml)
1						
5 μg/ml	76.13	0.60	72.09	0.72	78.60	0.75
0.5 μg/ml	16.77		0.89		45.53	
0. 05 μg/ml	0.81		-5.22		-5.12	
0. 005 μg/ml	8.63		-4.58		-9.88	
2						
5 μg/ml	94.08	0.10	96.32	0.09	97.73	0.08
0.5 μg/ml	90.16		91.11		91.26	
0. 05 μg/ml	10.28		56.97		45.39	
0. 005 μg/ml	9.48		-7.21		-3.19	
5-Fu						
5 μg/ml	69.04	0.61	74.23	0.47	77.57	0.62
0.5 μg/ml	50.29		64.23		42.71	
0. 05 μg/ml	13.29		6.15		3.24	
0. 005 μg/ml	14.33		-6.70		0.32	
$Cu(NO_3)$						
5 μg/ml	3.67		-0.54		11.12	

Alanine			
5 μg/ml	5.82	15.61	22.35
Phen [38]			
5 μg/ml	2.94		

*1: The anticancer experiments have been completed by the instituted of Material Medical, Chinese Academy of Medical Science, and all the date can be checked from there.

Molecular structure of complex 1 and 2 related with anticancer activities

To reveal the mechanism of anticancer activities in the two complexes, especially the superiority of complex 2, we analyzed and determined the molecular structures by X-ray single crystal diffraction, and the crystal data are summarized in Tables 2 and 3. As shown in Fig. 1A, the complex 1 {[(1,10-Phen)Cu(Ala)·(H_2O)]·Cl·H_2O} stack in tetragonal pyramid coordination geometry with two five-membered rings. One coordinates a phenanthroline with two N atoms, the other ring is coordinated by the second ligand (Ala) with one N and one O. Cl is counter ions, and the other H_2O is lattice molecule. The coordinated model of mono-copper complex [(2,9-DMP)Cu(Ala)·H_2O]·NO_3·H_2O (2) is similar to the complex 1 , whereas, the bond length and angle has been changed because of the substitution of the two methyl in coordinated phenanthroline of complex 2 (Table 3). It deserves to be mentioned that complex 2 still stack in the tetragonal pyramid geometry structure. Compared to the two molecular structures, they have the similar coordinated structure and spatial configuration (Fig. 1) except two substituted methyl groups in the complex 2. Hence, we hold the opinion that methyl can enhance antitumor activity.

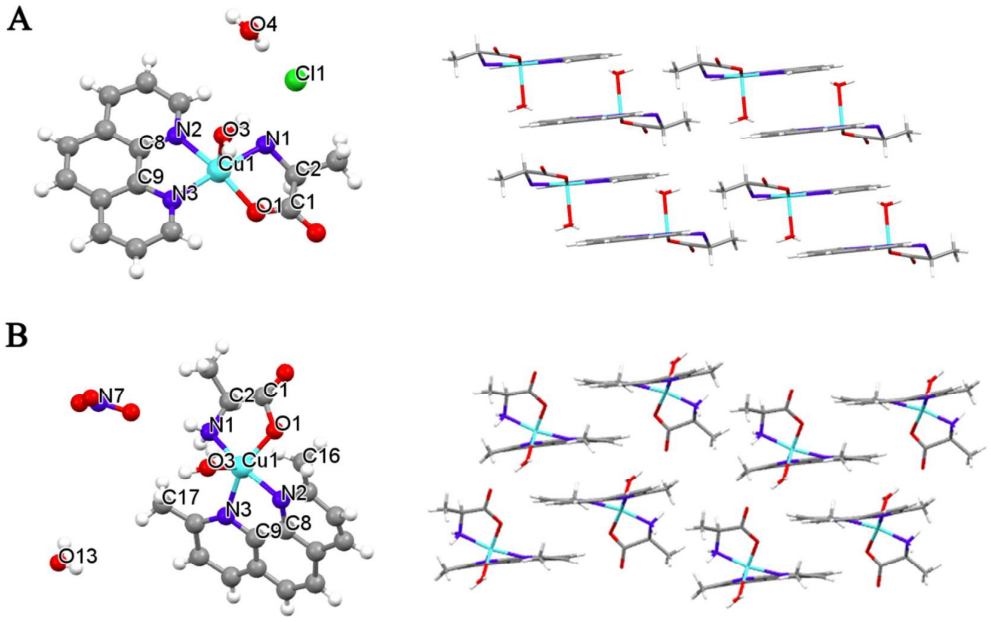

Fig. 1 Molecular structure of complex 1, 2 and their stacking diagram have been synthesized and structurally characterized by X-ray diffraction methods.

Table 2 Crystallographic data for complex 1 and 2

Complex	**1**	**2**
Formula	$C_{15}H_{16}ClCuN_3O_4$	$C_{17}H_{20}CuN_4O_7$
F_w	401.30	455.91
Crystal system	Triclinic	Triclinic
Space group	P-1	P-1
a (Å)	7.2802(7)	7.3950(8)
b (Å)	11.7250(10)	10.7830(11)
c (Å)	11.8101(12)	12.3600(14)
α (°)	119.471(2)	102.2790(10)
β (°)	101.9220(10)	90.4280(10)
γ (°)	91.0860(10)	106.080(2)
V (Å 3)	850.09(14)	923.05(17)
Z	2	2
Dcalc (g cm^{-3})	1.568	1.640
T (K)	298(2)	298(2)
collected reflns	4381	3131
unique reflns	2635	3131
R	0.0358	0.1210
R_w	0.0948	0.2882

S	1.136	1.056

Table 3 Selected bond distances (Å) and angles (◦) for complex 1 and 2

	1	**2**
Bond length		
Cu1-O1	1.953(2)	1.953(9)
Cu1-O3	2.307(3)	2.174(9)
Cu1-N1	2.000(3)	1.982(9)
Cu1-N2	2.024(3)	1.998(9)
Cu1-N3	2.003(3)	2.068(10)
Bond Angle		
O1-Cu1-N1	84.13(11)	83.7(4)
O1-Cu1-N2	167.62(11)	90.4(4)
O1-Cu1-N3	94.17(10)	149.3(4)
O1-Cu1-O3	98.08(10)	107.4(4)
N1-Cu1-N2	98.59(11)	168.0(4)
N1-Cu1-N3	175.05(13)	99.1(4)
N1-Cu1-O3	94.34(12)	93.0(4)
N2-Cu1-N3	82.10(11)	80.8(4)
N2-Cu1-O3	93.77(10)	98.7(4)
N3-Cu1-O3	90.50(10)	103.0(4)

Computational ΔE and ΔG related with anticancer activities

To further reveal how the two substituted methyl groups cause the differences in anticancer, the changes of the energy gaps between π*–π as free and coordinated states are calculated by Gaussian 03 program. As shown in Fig. 2, the data ($\Delta E_{1,10\text{-Phen}} > \Delta E_{2,9\text{-DMP}}$) illustrates that the ΔE of free 2,9-DMP is decreased by the existence of the two methyl groups located at the 2nd and 9th position. In other words, the two substituted methyl groups can activate the conjugated system of π_{14}[14] in the aromatic phenanthroline ring. Furthermore, $\Delta E_{1,10\text{-Phen}} > \Delta E_1$ and $\Delta E_{2,9\text{-DMP}} > \Delta E_2$ show that the conjugated system of phenanthroline is activated by the Cu^{II}-coordination. More importantly, the sensitized value of complex 2 [$\Delta(\Delta E)_2=(\Delta E)_{2,9\text{-DMP}}-(\Delta E)_2=0.36$eV] is higher than complex1 [$\Delta(\Delta E)_1=(\Delta E)_{1,10\text{-phen}}-(\Delta E)_1=0.10$eV] by CuII-coordination

which indicate that the complex 2 are more sensitized than the 1. In other words, the instable complex 2 conduce to cause the partial dissociation, and the more active complex 2 exhibited higher inhibition than complex 1, thus, we conclude that the methyl substituent can enhance the antitumor activity.

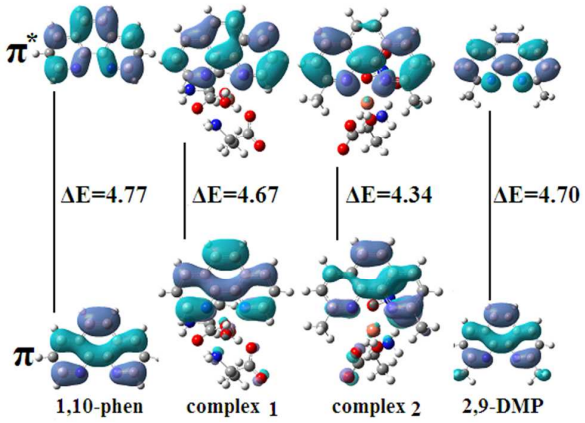

Fig. 2 Energy gaps between π-π* of the coordinated and free phenanthroline.

In our previous paper, we have found that the higher ΔG is negative to the anticancer activity for the copper and molybdenum complexes with similar planar ligand [36-38]. Hence, the relative changes of the Gibbs free energies (ΔG) for the complexes decomposed into Cu^{II} and free ligands are calculated by Gaussian 03 program. As shown in Scheme 1, ΔG_1 (6.9 Kcal/mol) > ΔG_2 (0 Kcal/mol), the computation indicates that 2 is easier to dissociate into Cu^{II} and free ligands which agrees with the conclusion of $\Delta(\Delta E)_n$. Through the comparison and analysis of the two complexes, the existence of the two methyl substituent in complex 2 (ΔG=0 Kcal/mol) lead to the reaction in a balanced state and this reaction provides the active molecule to inhibit the cancer cell, nevertheless 1(ΔG=6.9 Kcal/mol) cannot. Therefore we conclude that the methyl substituent can enhance the antitumor activity.

$$[(1,10-\mathrm{Phen})\mathrm{Cu(Ala)(H_2O)}]\cdot\mathrm{Cl}\cdot\mathrm{H_2O} \rightarrow 1,10-\mathrm{Phen} + \mathrm{Cu^{II}} + \mathrm{Ala} + \mathrm{Cl^-} + 2\mathrm{H_2O} \quad \Delta G = 6.9\ \mathrm{Kcal/mol}$$

$$[(2,9-\mathrm{DMP})\mathrm{Cu(Ala)(H_2O)}]\cdot \mathrm{NO_3}\cdot \mathrm{H_2O} \rightarrow 2,9-\mathrm{DMP} + \mathrm{Cu^{II}} + \mathrm{Ala} + \mathrm{NO_3^-} + 2\mathrm{H_2O} \quad \Delta G = 0.0\ \mathrm{Kcal/mol}$$

Scheme 1 The Gibbs free energies (ΔG) of complex 1 and 2 have been computed.

UV-vis and luminescent spectra related with anticancer activities

To further study the difference in anticancer activity among the compounds and their ligands, UV-vis spectroscopy is employed for studying the complexes and free ligands in aqueous solution (Fig. 3A). Phen shows a peak at 323 nm, and 2,9-DMP exhibits at 327 nm. The main peaks of complexes 1 and 2 present at 611 and 675nm respectively, which result from the transfer of the electrons between d-d orbital of Cu^{II}. Significantly, a new different signal appear at 445 nm which can be assigned to the partially dissociation of the coordinated 2,9-DMP with Cu^{II}. It also supports the conclusion that the coordinated 2,9-DMP is easier to depart from Cu^{II} than coordinated 1,10-phen.

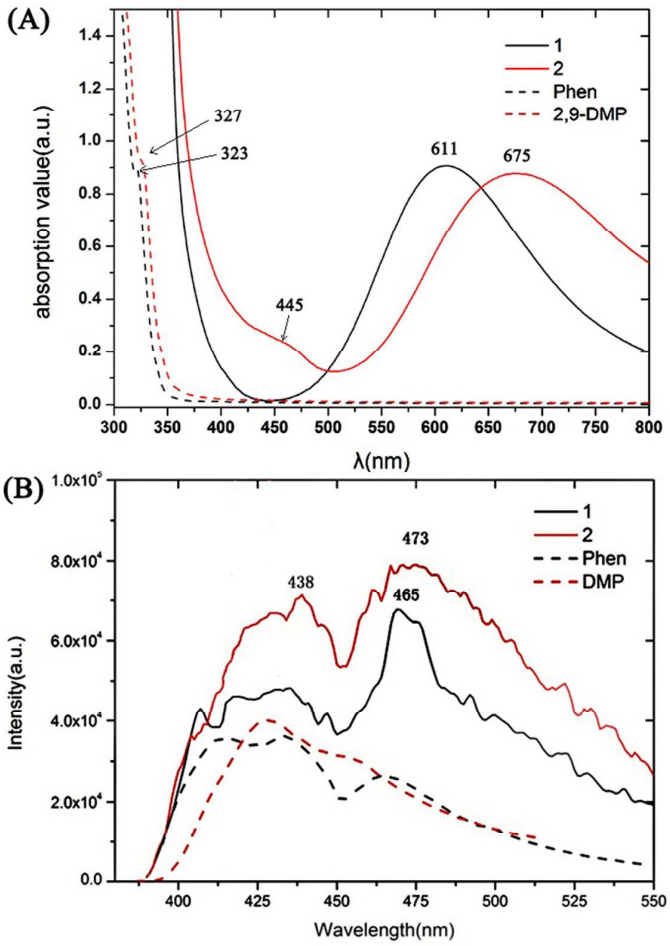

Fig. 3 UV-vis spectra and Luminescent of complexes **1** (black solid line) and **2** (red solid line), free phen (black dash-dot line), and 2,9-dmp (red dash-dot line) in aqueous solution at room temperature.

The luminescent spectra of the two complexes and their ligands are shown in Fig. 4B. The two complexes exhibit similar emission bands from 400 nm to 550 nm and the main peaks present at 465 and 473 nm respectively. However, a new peak shown at 438 nm in the complex 2 which could correlate with the new peak at 445 nm in UV-vis spectra (Fig 3). Similarly, it also can be assigned to the partially dissociated 2,9-DMP which depart from Cu^{II}.

$E_{pCu(II)/Cu(I)}$ of complex 1 an 2 with anti-cancer activities

Copper, as an essential element in living organisms, plays vital roles in electron transfer of many cellular processes by the Cu^{II}/Cu^{I} redox process[1, 2]. Therefore, the redox potential is an important factor to reflect the redox ability of the Cu^{II}-complex. The CV experiment reveals that the two complexes have the property of redox-active and quasi-reversible (Fig. 5). The $\Delta_1E_{2paCu(II)/Cu(I)}$ (-0.475eV) is more negative than $\Delta_1E_{1paCu(II)/Cu(I)}$ (-0.342eV), which can be assigned to the donated electrons of methyl in 2. As general, the biological potential in cancer cells is higher than the normal cells which can attract the complex 2 to access cancer cells efficiently. In addition, Cu^{II} can transfer O_2^{-}• into O_2 (Cu^{II}-SOD + O_2^{-}• = Cu^{I}-SOD + O_2) for anti-proliferation, and the higher $\Delta E_{2pCu(II)/Cu(I)}$ tend to oxidize O_2^{-}• into O_2 more efficiently and reduce the growth of cancer cells. Hence, we deem the CV result can be used as an index to predict the anticancer activities of CuII-complexes with similar dominant ligands.

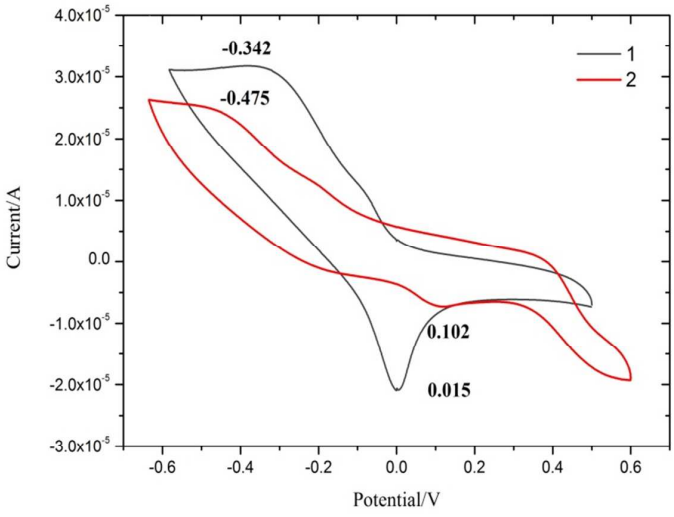

Fig. 5 the cyclic voltammetry (CV) curves of the two complexes (0.1M KCl was used as a supporting electrolyte; 1.25×10^{-3}M aqueous solutions of these complexes. The potential was scanned from −0.65 to 0.5 V with a scan rate of 50mV/s.)

For the anticancer mechanism of phenanthroline molecule, it is explained that the planar aromatic ring can insert into the groove of DNA and disturb DNA Replication to inhibit the growth of cancer cells. However, how the methyl-substituted-phenanthroline possesses the higher inhibition toward cancer cells? From geometric hindrance, the methyl-substituted-phenanthroline should be more difficult to insert into the grooves of DNA than 1,10-phen, so there should be other mechanisms for the methyl-substituted-phenanthroline. We suggested that the partially dissociated 2,9-DMP is more flexible than the coordinated phenanthroline ligands by the weak and soft linkages with copper. Thus the partially dissociated 2,9-DMP is easier to intercalate into the grooves of DNA to disturb the replication of DNA.

Furthermore, the methyl radicals might sustain the phenanthroline to insert into the grooves of DNA by super-conjugated forces between the π-conjugated system of aromatic ring and the H atoms from methyl, which might be similar to the methyl radical of thymine in DNA. In living cells, DNA is a macro and long double-strains, the way to maintain the structure may originate the super-conjugated forces between methyl and the π-conjugated system from the thymine-methyl-substituent, such as purines and pyrimidine. Whereas, RNA is difficult to couple into long double-strain, since RNA is without methyl groups.

Conclusions

We have synthesized complexes [(1,10-Phen)Cu(Ala)(H_2O)]·Cl·H_2O (1) and [(2,9-DMP)Cu(Ala)(H_2O)]·NO_3·H_2O (2), tested their anticancer activities in vitro,

determined their crystal structures by X-ray diffraction, measured their UV-vis, luminescent spectra and CV, and calculated their ΔE and ΔG. The two complexes stack in Square-Planar structure and exhibit excellent anticancer activities. Especially 2 with methyl-substituted-phenanthroline presents much better anticancer properties than the complex 1 with 1,10-phen. Computational ΔE ($\Delta E_{1,10\text{-Phen}} > \Delta E_1$ and $\Delta E_{2,9\text{-DMP}} > \Delta E_2$) illustrate that both phenanthroline are activated by Cu^{II}-coordination, especially the complex 2 with two substituted methyl groups can activate the conjugated system of π_{14}[14] in the aromatic phenanthroline ring for antiproliferative. ΔG_1 (6.9 Kcal/mol) > ΔG_2 (0 Kcal/mol) indicate the complex 2 is easier to decompose into free Cu^{II} and ligands than 1, the partially dissociation of the coordinated 2,9-dmp with Cu^{II} has availed for antiproliferation and against cancer cells. UV-vis and luminescent spectra in solution also reveals the methyl-substituted-phenanthroline cause partially dissociated in complex 2. Also, the $E_{2pCu(II)/Cu(I)}$ is more positive than $E_{1pCu(II)/Cu(I)}$, which might be more effective to transfer O_2^- into O_2 to inhibit the growth of cancers. In summarize, the substitution of the methyl in phenanthroline has increased the activation of the coordinated phenanthroline, changed the ΔE, ΔG, absorption value, luminescence intensity, value of CV and enhanced the anticancer activities of the phenanthroline-based Cu^{II}-complex. The contribution in anticancer properties by substituted methyl at phenanthroline offers a functional radical which might be helpful for the innovation of other anticancer drugs.

Abbreviations

2, 9-DMP	2, 9-Dimethyl-1, 10-phenanthroline
1, 10-Phen	1, 10-phenanthroline
Ala	Alanine

CV	cyclic voltammetry
SOD	superoxide dismutase
TSC	Thiosemicarbazone
A-549	human lung carcinoma
Bel-7402	human hepatocellular carcinoma cells
HCT-8	human ileocecal adenocarcinoma
5-Fu	Fluorouracil
MTT	3-(4,5-dimethylthiazoyl-2-yl)-2,5-diphenyltetrazolium bromide
FBS	fetal bovine serum

Conflict of interest

The authors have no conflict of interest to declare.

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Notes and references

CCDC: 996601 for complex 1 and 996599 for complex 2. Copies of the data may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 (0)1223 762911; e-mail: kamila@ccdc.cam.ac.uk or www.ccdc.cam.ac.uk

References

1. M. Valko, H. Morris and M. T. Cronin, Curr. Med. Chem., 2005, **12**, 1161.
2. E. I. Solomon, R. K. Szilagyi, G. S. DeBeer and L. Basumallick, Chem. Rev., 2004, **104**, 419.
3. S. Padhye and G. B. Kauffman, Coord. Chem. Rev., 198, **563**, 127.

4. J. Easmon, G. Purstinger, G. Heinisch, T. Roth, H. H. Fiebig, W. Holzer, W. Jager, M. Jenny and J. Hofmann, J. Med. Chem., 2001, **44**, 2164.

5. M. R. Taylor, E. J. Gabe, J. P. Glusker, J. A. Minkin and A. L. Patterson, J. Am. Chem. Soc., 1966, **88**, 1845.

6. J. A. Crim and H.G. Petering, Cancer. Res., 1967, **27**, 1278.

7. W. E. Antholine, J. M. Knight and D. H. Petering, Inorg. Chem., 1977, **16**, 569.

8. D. L. Klayman, J. P. Scovill, J. F. Bartosevich and J. Bruce, J. Med. Chem., 1983, **26**, 35.

9. D. A. Boothman, D. K. Trask and A. B. Pardee, Cancer. Res., 1989, **49**, 605.

10. B. Frydman, L. J. Marton and J.S. Sun, Cancer. Res., 1997, **57**, 620.

11. D. X. West and A. E. Liberta, Coord. Chem. Rev., 1993, **123**, 49.

12. US. Pat 005107, 1992. US Pat., 35 458, 1997. US. Pat. l 326 5576, 1996.

13. Mark. Tittle. Casiopeína. Reg. 407543. SECOFI, 1992, 2002.

14. M. E. Bravo-Gómez, J. C. García-Ramos, I. racia-Mora and L. Ruiz-Azuara, J. Inorg. Biochem., 2009, **103**, 299.

15. L. Ruiz-Azuara and M. E. Bravo-Gómez, Curr. Med. Chem., 2010, **17**, 3606.

16. R. Alemón-Medina, M. E. Bravo-Gómez, M. I. Gracia-Mora and L. Ruiz-Azuara, Toxicol. In. Vitro., 2011, **25**, 868.

17. M. Devereux, D. O'Shea, M. O'Connor, H.Grehan, G. Connor, M. McCann, G. Rosair, F. Lyng, A. Kellett, M. Walsh, D. Egan and B. Thati, Polyhedron., 2007, **26**, 4073.

18. D. S. Sigman, Biochem., 1990, **29**, 9097.

19. D. S. Sigman, D. R. Graham, V. D. Aurora and A.M. Stern, J. Biol. Chem., 1979, **254**, 12269.

20. S. C. Zhang, Y. G. Zhu, C. Tu, H. Y. Wei, Z. Yang, L. P. Lin, J. Ding, J.F. Zhang and Z. J. Guo, J. Inorg. Biochem., 2004, **98**, 2099.

21. B. C. Bales, T. Kodama, Y. N. Weledji, M. Pitie, B. Meunier and M. M Greenberg, Nucleic. Acids. Res., 2005, **33**, 5371.

22. T. Wang and Z. J. Guo, Curr. Med. Chem., 2006, **13**, 525.

23. A. K. Patra, M. Nethaji and A. R. Chakravarty, J. Inorg. Biochem., 2007, **101**, 233.

24. R. Alemón-Medina, M. Bre-Valle, J. Mu-Sánchez, I. Gracia-Mora and L. Ruiz-Azuara, Cancer. Chemoth. Pharm., 2007, **60**, 219.

25. Q. Jiang, N. Xiao, P. F. Shi, Y. G. Zhu and Z. J. Guo, Coordin. Chem. Rev., 2007, **251**, 1951.

26. R. Alemón-Medina, J. L. Muz-Sánchez, L. Ruiz-Azuara and I. Gracia-Mora, Toxicol. In. Vitro., 2008, **22**, 710.

27. A.Rivero-Muller, A. D. Vizcaya-Ruiz, N. Plant, L. Ruiz and M. Dobrota, Chem. Biol. Interact., 2007,**165**, 189.

28. R. Kachadourian, H. M. Brechbuhl, L. Ruiz-Azuara, I. Gracia-Mora and B. J. Day, Toxicology., 2010, **268**,176.

29. J. Reedijk, Proc. Natl. Acad. Sci. USA., 2003, **100**, 3611.

30. T. W. Hambley, Dalton. Trans., 2007, **43**, 4929.

31. H. J. Yu, S. M. Huang, L. Y. Li, H. N. Jia, H. Chao, Z.W. Mao, J. Z. Liu and N. Ji, J. Inorg. Biochem., 2009, **103**, 881.

32. V. G. Vaidyanathan and B. U. Nair, J. Inorg. Biochem., 2003, **95**, 334.

33. Y. J. Sun, S. N. Collins, L. E. Joyce and C. Turro, Inorg, Chem., 2010, **49**, 4257.

34. X. M. Chen, M. Chen and M. L. Tong, Acc. Chem. Res., 2007, **40**, 162.

35. J. P. Zhang, Y. B. Zhang, J.B. Lin and X. M. Chen, Chem. Rev., 2012, **112**, 1001.

36. J. Feng, X. M. Lu, G. Wang, S. Z. Du and Y. F. Cheng, Dalton Trans., 2012, **41**, 8697.

37. Y. F. Cheng, X. M. Lu and G. Wang, Dalton Trans. 2014, **43**, 5357.

38. B. Zhang, X. M. Lu, G. Wang, W. C. Zhang, S. F. Xia and Y.U. Chen, J. Inorg. Biochem., 2014, **140**, 213.