Synthesis, Characterization, Cytotoxic Activities, and DNA-Binding Studies of Ternary Copper(II) Complexes with New Coumarin Derivatives

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Received October 9, 2009; accepted May 3, 2010; published online May 13, 2010

In this study, two novel complexes [Cu(MCVH)phen(H_2O)]·ClO_4 (1) and [Cu(MCLH)phen(H_2O)]·ClO_4 (2) (here, $MCVH_2$=(7-hydroxy-4-methyl-8-coumarinyl) valine, $MCLH_2$=(7-hydroxy-4-methyl-8-coumarinyl) leucine) have been synthesized and characterized by elemental analyses, molar conductance, infrared spectra (IR), ^{1}H-NMR and UV–Vis measurements. The interactions of them with calf thymus DNA (ct DNA) have been investigated by absorption spectroscopy, fluorescence spectroscopy, circular dichroism spectroscopy and viscosity measurements. Experimental results reveal an intercalative interaction with DNA for the complexes, furthermore the binding affinity of 2 is higher than that of 1 according to the calculated binding constant values. In addition, they were evaluated for their cytotoxic activities toward human prostate cancer cell (PC3), human liver cell (L02) and human myeloid leukemia cancer cell (HL-60) by acid phosphoatase assay. Both of them showed significant cytotoxic potency.

Key words copper(II) complex; coumarin derivative; DNA-binding; intercalative mode; cytotoxic activity

Currently, cancer is the second cause of death, accounting for about a quarter of all deaths. Nearly one half of all cancers that are diagnosed result in the death of the patient, so that the development of new anticancer therapies is one of the fundamental goals in medicinal chemistry.

Coumarin (1,2-benzopyrone) is structurally the least complex member of a class of compounds known as benzopyrones.[1)] Nowadays, Coumarins have long been recognized to possess anti-inflammatory, antioxidant, antithrombotic, antiallergic, hepatoprotective, antiviral and anticarcinogenic activities.[2—5)] Regarding their high fluorescence ability, they are widely used as fluorescent probes in biology and medicine.[6,7)] More recently, coumarin derivatives have been evaluated in the treatment of human immunodeficiency virus, due to their ability to inhibit human immunodeficiency virus integrase.[8,9)] Interest in metal coumarin complexes has arisen from the search for novel lead compounds along with the desire to improve the pharmacological profile. For example, some interesting lanthanide complexes of coumarin derivatives like bis(4-hydroxy-3-coumarinyl) acetic acid,[10)] the ligand 8,8′-[1,2-ethanediylbis(nitriloethylidyne)]bis[7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one] and coumarin-3-carboxylic acid have been reported.[11—13)] Transition metal complexes with coumarin Schiff bases have also been reported recently.[14,15)] 1,10-Phenanthroline (phen) and substituted derivatives, both in the metal-free state and as ligands coordinated to transition metals, disturb the functioning of a wide variety of biological systems.[16)] Copper complexes of 1,10-phenanthroline and its derivatives are of great interests since they exhibit numerous biological activities such as antitumor, anti-Candida, antimycobacterial and antimicrobial activity *etc.*[17—20)] Moreover, considerable attention has been focused on the use of phenanthroline complexes as intercalating agents of DNA and as artificial nucleases.[21—24)]

Amino acids are the basic structural units of proteins, and some copper complexes of amino acids were reported to exhibit potent antitumor and artificial nuclease activity.[25,26)] In this context, we focused our interests on the development of ternary copper(II) complexes of amino-coumarins with phenanthroline, and investigated their *in vitro* cytotoxicity. We report here the preparation of two novel ternary copper(II) complexes of amino-coumarins with phenanthroline. The selection of 7-hydroxyl-4-methyl coumarine as a second ligand in the ternary copper(II) complexes may enhance the affinity of the complex towards DNA because of the formation of hydrogen bonds between the hydroxyl group of 7-hydroxyl-4-methyl coumarine and DNA double helix and increase the biocompatibility of the complex.[27)]

Experimental

Materials 7-Hydroxy-4-methyl coumarine, calf thymus DNA (ct DNA) and ethidium bromide (EB) were obtained from Sigma Chemical Co. All the experiments involving interaction of the complexes with ct DNA were carried out in doubly distilled water buffer containing 5 mM Tris[Tris(hydroxymethyl)aminomethane] and 50 mM NaCl, and adjusted to pH 7.2 with hydrochloric acid. The concentration of DNA solution was determined from UV absorption at 260 nm using a molar absorption coefficient ε_{260}=6600 $mol^{-1}\,cm^{-1}$. Purity of the DNA was checked by monitoring the ratio of the absorbance at 260 nm to that at 280 nm. The solution gave a ratio of A_{260}/A_{280}>1.80, indicating that DNA was sufficiently free from protein.[28)]

Physical Measurements The UV–Vis absorption measurements were conducted by using a Varian Cary 100 spectrophotometer equipped with quartz cells. All fluorescence emission spectra were measured using a Hitachi F-4500 spectrofluorophotometer equipped with a xenon lamp source and a quartz cell of 1 cm path length. Viscosity experiments were carried out on an Ubbelodhe viscometer. The circular dichroism (CD) spectra were recorded on a Jasco J-810 spectropolarimeter. The elemental analyses were performed in the microanalytical laboratory, Department of Chemistry, National University of Singapore. The ^{1}H-NMR spectra were recorded with a Bruker ACF300 FT-NMR instrument using tetramethylsilane (TMS) as an internal reference in D_2O for the ligand. Electrospray ionization (ESI) mass spectra were recorded on a Finnigan MAT LCQ mass spectrometer using the syringe pump method.

Methods Absorption titration experiments were performed by fixing

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concentrations of **1** and **2** as constant at 10 μM while varying the concentration of ct DNA. Fitting was completed using an Origin 6.0 spreadsheet, where values of the binding constants K_b were calculated.

Fluorescence spectra of the competitive binding experiments were carried out by maintaining the EB and ct DNA concentration at 3 μM and 30 μM, respectively, while increasing the concentrations of the complexes. Fitting was completed using an Origin 6.0 spreadsheet, where values of K_q were calculated.

Viscosity experiments were carried out on an Ubbelodhe viscometer, immersed in a thermostated water-bath maintained at 25.0±0.1 °C. Titrations were performed for the complexes (1—5 μM), and each compound was introduced into DNA solution (50 μM) present in the viscometer. Flow time was measured with a digital stopwatch and each sample was measured three times and an average flow time was calculated.

The CD spectra of DNA were recorded on a Jasco J-810 spectropolarimeter at 25.0±0.1 °C. Calf thymus DNA used were 120 μM in concentration and compounds solutions was added to a ratio of 1 : 1 (DNA/compound). Each sample solution was scanned in the range of 200—350 nm. CD spectrum was generated which represented the average of three scans from which the buffer background had been subtracted.

Acid Phosphoatase Assay The reagent, *p*-nitrophenyl phosphate (*p*-NPP), was obtained from Amresco. The compounds synthesized were dissolved in dimethyl sulphoxide (DMSO) and diluted in culture medium. The final concentration of DMSO in cultures was always not exceeding 0.5% (v/v), which did not cause toxicity. The PC3 and HL-60 cells obtained from ATCC were maintained in Dulbecco's modified Eagle's medium (DMEM) medium with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin. Cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Two different cell lines, human prostate cancer cell (PC3) and human myeloid leukemia cancer cell (HL-60) were plated in 96-well plates, at a density of 5×10^4 cells/ml, and then treated with varied concentration (10, 50, 100, 200, 300 μM) of the compounds. The culture medium was removed from the plates after 24, 48 and 72 h, and each well was washed once with 200 μl phosphate-buffered saline (PBS, pH=7.2). To each well, 100 μl of buffer containing 0.1 M sodium acetate (pH=5.0), 0.1% Triton X-100, and 5 mM *p*-NPP was added. The reaction was stopped with the addition of 10 μl of 1 M NaOH, and color development was assayed at 405 nm using a microplate reader (Victor^3 1420-050). The nonenzymatic hydrolysis of the *p*-NPP substrate was determined for each assay by including wells that did not contain cells as blank wells. Cell survival was expressed as an absorbance (A) percentage defined by ($A_{\text{drug-blank}}/A_{\text{control-blank}}\times100$).

Preparation of the Ligands The preparation of (7-hydroxy-4-methyl-8-coum arinyl)valine ($MCVH_2$) was prepared as follows: a mixture of 7-hydroxy-8-coumarine (8.1 g, 0.05 mol) and L-valine (0.58 g, 0.05 mol) was taken in 100 ml of 80% ethanol and 3 ml formaldehyde was added. The resulting mixture was refluxed on a water bath for 8 h. The white solid $MCAH_2$ was filtered and washed with ethanol. Yield: 89%. mp: 252 °C. $MCVH_2$ (D_2O) δ (ppm): 0.81—0.85 (m, 6H), 1.72—1.83 (m, 1H), 2.36—2.38 (d, 3H), 2.87—2.89 (d, 1H, J=6.4 Hz), 3.67—3.82 (m, 2H, J=8.1 Hz), 5.84—5.86 (d, 1H, J=4.1 Hz), 6.56—6.59 (m, 1H), 7.37—7.41 (m, 1H). IR (KBr) cm^{-1}: ν (NH) 3160, ν (CH_2) 2974, ν (COO^-) 1606, 1384, ν (C=O of lactone ring) 1720, ν (O–C–O of lactone ring) 1081. electrospray ionization (ESI): m/z=304 $[M-H]^+$. *Anal.* Calcd for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59. Found: C, 62.87; H, 6.55; N, 4.56.

The preparation of (7-hydroxy-4-methyl-8-coumarinyl)leucine ($MCLH_2$) is similar to that of $MCVH_2$, buy using L-leucine in place of L-valine. Yield: 82%. mp: 253 °C. $MCLH_2$ (D_2O) δ (ppm): 0.81—0.85 (m, 6H), 1.35—1.40 (m, 2H), 1.75—1.86 (m, 1H), 2.36—2.38 (d, 3H), 2.83—2.85 (d, 1H, J=6.4 Hz), 3.69—3.87 (m, 2H, J=8.1 Hz), 5.84—5.86 (d, 1H, J=4.1 Hz), 6.53—6.59 (m, 1H), 7.31—7.39 (m, 1H). IR (KBr) cm^{-1}: ν (NH) 3147, ν (CH_2) 2959, ν (COO^-) 1607, 1389, ν (C=O of lactone ring) 1722, ν (O–C–O of lactone ring) 1081. ESI: m/z=318 $[M-H]^+$. *Anal.* Calcd for $C_{17}H_{21}NO_5$: C, 63.94; H, 6.63; N, 4.39. Found: C, 63.76; H, 6.72; N, 4.51.

Preparation of the Complexes Complex **1**: To the green solution formed from $Cu(ClO_4)_2\cdot6H_2O$ (0.37 g, 1 mmol) in MeOH (8 ml) and phen (0.18 g, 1 mmol) in MeOH (8 ml) was added a filtered solution of $MCVH_2$ (0.3 g, 1 mmol) in H_2O (20 ml) containing NaOH (0.04 g, 1 mmol). The resulting blue solution was stirred for 1 h and then filtered and left for a week, after which time the dark blue crystals were isolated by filtration. Yield: 91%. ESI: m/z=549 $[M-ClO_4-H_2O+H]^+$. *Anal.* Calcd for $C_{28}H_{28}ClCuN_3O_{10}$: C, 50.53; H, 4.24; N, 6.31. Found: C, 50.75; H, 4.51; N, 6.66. IR (KBr) cm^{-1}: ν (H_2O) 3435, ν (NH) 3065, ν (CH_2) 2960, ν (COO^-) 1631, 1386. ν (Cl–O) 1118, ν (phen) 850 and 724, ν (C=O of lactone ring) 1736, ν (phenolic C–O) 1312, ν (O–C–O of lactone ring) 1089. Complex **2** was prepared using $MCLH_2$ through the same way as that of complex **1**. Yield: 91%. ESI: m/z=563 $[M-ClO_4-H_2O+H]^+$. *Anal.* Calcd for $C_{29}H_{30}ClCuN_3O_{10}$: C, 51.26; H, 4.45; N, 6.18. Found: C, 51.55; H, 4.68; N, 6.39. IR (KBr) cm^{-1}: ν (H_2O) 3433, ν (NH) 3067, ν (CH_2) 2965, ν (COO^-) 1632, 1388. ν (Cl–O) 1118, ν (phen) 850 and 724, ν (C=O of lactone ring) 1736, ν (phenolic C–O) 1310, ν (O–C–O of lactone ring) 1089.

Results and Discussion

Characterization of the Complexes The complexes **1** and **2** are air stable for extended periods and soluble in methanol, ethanol, acetone, *N*,*N*-dimethylformamide (DMF) and DMSO; slightly soluble in water; insoluble in benzene and diethyl ether. Since the crystal structure of the Cu(II) complexes have not been obtained yet, we characterized the complexes and speculated their possible structure by elemental analyses, molar conductivities, IR data and UV–Vis measurements. The likely structure of the Cu(II) complexes are shown in Fig. 1.

Conductivity of the Cu(II) Complexes in Solution: The stability of Cu(II) complexes in DMSO solution have been studied by observing the UV–Vis spectrums and estimating the molar conductivities at different time intervals for any possible change. The tested Cu(II) complexes are prepared in DMSO and for experiments freshly diluted in phosphate buffer system (at pH 7.4). Then, the UV–Vis spectrums and molar conductivities are researched at different time intervals. The investigations reveal that the UV–Vis spectra have remained unaltered for the solutions and its molar conductance values have no obvious change for very freshly prepared and for over the whole experiment (72 h). It indicates that the Cu(II) complexes are quite stable in solution. The molar conductance values in DMF are 75.51 and 81.26 S cm^2 mol^{-1} for 1 and 2, respectively, which suggest that the two complexes are 1 : 1 electrolytes in DMF.[29]

IR Spectra: The IR spectra of the reduce Schiff bases show characteristic bands due to ν (NH), ν (C=O lactonic carbonyl), ν (phenolic C–O) and ν (COO–) around 3000, 1720, 1312 and 1600 cm^{-1}, respectively.[29] In comparison with the spectra of the reduce Schiff bases, the ν (NH) group exhibits shift towards higher wavenumber values on complexation which may be taken as suggestion for the participation of the NH group in coordination. The bands observed around 1630 and 1380 cm^{-1} are assigned to ν $(COO–)_{asym}$ and ν $(COO–)_{sym}$ of carboxylate group, respectively. The magnitude of separation between these two vibrations (>200 cm^{-1}) suggests the coordination of carboxylate group in unidentate fashion.[30] This suggests that the hydroxyl group of –COOH has involved in coordination *via* deprotonation. The high intensity band due to phenolic C–O appears in the region at 1312 cm^{-1} in the reduce Schiff bases appear as a medium to high intensity band in the 1310 cm^{-1} region in the complexes. These observations indicate that the phenolic proton did not

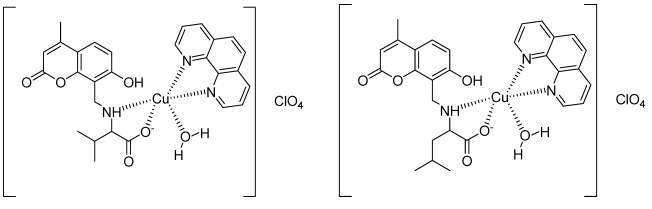

Fig. 1. The Proposed Structure of the Complexes **1** (Left) and **2** (Right)

lose on complexation. The broad bands at 3430 cm^{-1} are ascribed to the vibration of the O–H stretching of the water ligands. The topical band around 1145 cm^{-1} is assigned to ν (Cl–O) of anion. Two topical bands in the region *ca.* 850 and 724 cm^{-1} are assigned to ν (C=N) of phen. In all the complexes, the lactone carbonyl has shifted to higher energy (14 cm^{-1}), due to the strong intermolecular association between the lactone carbonyl and –NH in the solid state. Thus, the IR spectral data provide strong evidence for the complexation of the ternary copper complexes.

UV–Vis Spectra: The study of the electronic spectra in the ultraviolet and visible ranges for the ligand $MCVH_2$ and its Cu(II) complex is carried out in a buffer solution. The electronic spectra of $MCVH_2$ has a strong band at λ_{max}=206 and 361 nm, a medium band at λ_{max}=229 nm. The complex also yields three bands, but the two bands at 229 and 361 nm in the ligand are shifted to 226 and 359 nm or so in its Cu(II) complex. In addition, a new band at λ_{max}=269 nm appeared, these indicate that the Cu(II) complex has been formed.

DNA-Binding Mode and Affinity. Electronic Absorption Spectra Electronic absorption spectroscopy is universally employed to examine the binding mode of DNA with small molecules. The absorption spectra of **1** and **2** in the absence and presence of ct DNA are given in Figs. 2a and b. In the absence of ct DNA, the UV–Vis absorption spectra of the complex **1** has strong π–π* transitions band at λ_{max}=206, 226 and 269 nm and a strong n–π* transitions band at λ_{max}= 359 nm, while the complex **2** has strong π–π* transitions band at λ_{max}=205, 226 and 269 nm and a strong n–π* transitions band at λ_{max}=358 nm. With increasing DNA concentration, the absorption bands of the two complexes show decreases in molar absorptivity (hypochromism) as well as slight bathochromism. These variations are strongly indicative of the intercalation mode of the complexes with ct DNA, involving a strong π-stacking interaction between the complexes and DNA base pairs.[31,32)]

In order to study the binding ability of the compounds with DNA quantitatively, the binding constant K_b was determined using the Eq. 1,[33)]

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

where [DNA] is the concentration of DNA in base pairs, ε_a, ε_f, and ε_b are the apparent extinction coefficient correspond to $A_{obsd}/[M]$, the extinction coefficient for the free compound and the extinction coefficient for the compound in the fully bound form, respectively. In plots of $[DNA]/(\varepsilon_a-\varepsilon_f)$ *versus* [DNA], K_b is given by the ratio of slope to the intercept (Figs. 2a and b, inset). The binding constants K_b for complexes **1** and **2** are found to be 7.3×10^4 M^{-1} and 8.8×10^4 M^{-1}, respectively. The results indicate that the binding strength of complex **2** is stronger than that of complex **1**. The K_b value obtained here is lower than that reported for classical intercalator (for ethidium bromide and [Ru(phen)DPPZ] whose binding constants have been found to be in the order of 10^6—10^7 M).[34,35)]

Fluorescence Spectra In order to further investigate the interaction mode between the ternary complexes and ct DNA, the fluorescence titration experiments were performed. The fluorescence titration experiments, especially the EB fluorescence displacement experiment, have been widely used to characterize the interaction of complexes with DNA by

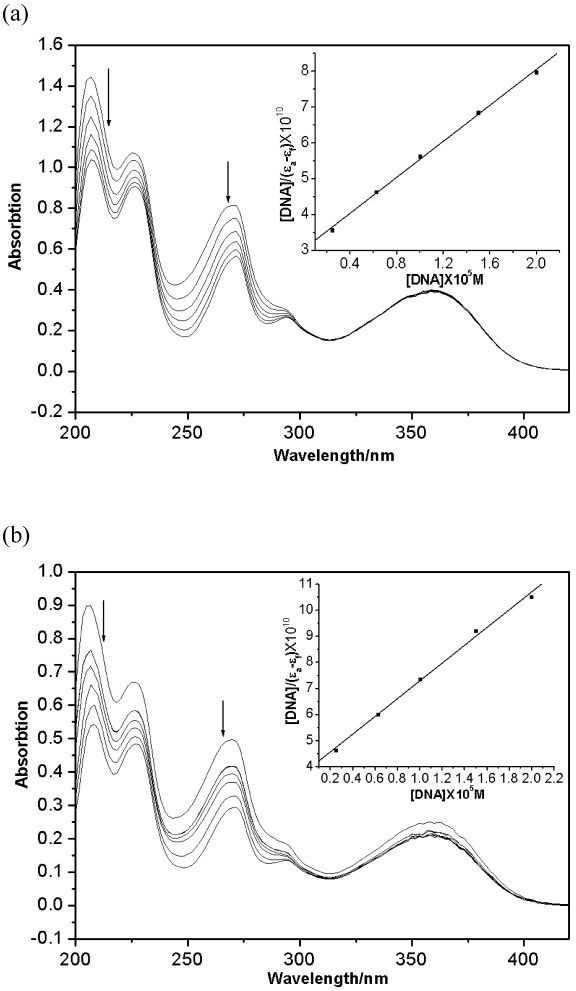

Fig. 2. UV-Vis Absorption Spectra of **1** (a) and **2** (b) (10 μM) in the Presence of Increasing Amounts of ct DNA; [DNA]=0, 10, 20, 30, 40, 50 μM

The arrow indicates the absorbance changes upon increasing DNA concentration. The inset is plot of $[DNA]/(\varepsilon_b-\varepsilon_f)$ *vs.* [DNA] for the titration of DNA to complex.

following the changes in fluorescence intensity of the complexes. The intrinsic fluorescence intensities of DNA and that of EB in Tris–HCl buffer are low, while the fluorescence intensity of EB will be enhanced on addition of DNA as its intercalation into the DNA. Therefore, EB can be used to probe the interaction of complexes with DNA. If the complexes can intercalate into DNA, the binding sites of DNA available for EB will be decreased, and hence the fluorescence intensity of EB will be quenched.[36)]

The emission spectra of DNA–EB system in the presence of increasing amounts of complexes **1** and **2** are shown in Figs. 3a and b, the fluorescence intensity of EB at 584 nm showed a remarkable decreasing trend with the increasing concentration of the complex **1** and **2**, indicating that some EB molecules are released from EB–DNA after an exchange with the complexes **1** and **2** which result in the fluorescence quenching of EB. This may be due either to the metal complex competing with EB for the DNA binding sites thus displacing the EB (whose fluorescence is enhanced upon DNA binding) or it could be a more direct quenching interaction on the DNA itself. We assume it is the former which implies that both of the complexes bind more strongly to DNA than EB at 50 mM NaCl concentration. Such a quenched fluores-

cence behavior of EB bound to DNA caused by the interaction between the copper(II) complexes and DNA is also found in other ternary copper complexes.[37,38]

According to the classical Stern–Volmer Eq. 2[39]:

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

where F_0 and F represent the emission intensity in the absence and presence of quencher, respectively, K_q is a linear Stern–Volmer quenching constant and [Q] is the quencher concentration. The quenching plots illustrate that the quenching of EB bound to DNA by the complexes is in good agreement with the linear Stern–Volmer equation (Figs. 3a and b, inset). In the plots of F_0/F *versus* [Q], K_q is given by the ratio of the slope to the intercept. The K_q values of ternary copper(II) complexes are $2.75\times10^4\,M^{-1}$ and $4.45\times10^4\,M^{-1}$ for complex **1** and **2**, respectively. This result suggests that complex **2** is more able than complex **1** in replacing the strong DNA intercalator EB, being accordance with above absorption titration results.

CD Spectroscopy Circular dichroic spectral techniques may give us useful information on how the conformation of the ct DNA chain is influenced by the bound complex. The CD spectrum of ct DNA consists of a positive band at 274 nm that can be due to base stacking and a negative band at 243 nm that can be due to helicity and it is also characteristic of DNA in a right-handed B form.[40] The changes in CD signals of ct DNA observed on interaction with drugs may often be assigned to the corresponding changes in ct DNA structure.[41] Thus simple groove binding and electrostatic interaction of small molecules show less or no perturbation on the base-stacking and helicity bands, whereas intercalation enhances the intensities of both the bands stabilizing the right-handed B conformation of ct DNA as observed for the classical intercalator methylene blue.[42]

The CD spectrum of ct DNA is monitored in the presence of complexes **1** and **2**, the changes observed in the two cases are shown in Fig. 4. On addition of complexes to ct DNA, the negative-band position are shift to 244 and 246 nm for **1** and **2**, respectively, while the intensity of the positive band in the CD spectrum of DNA is perturbed remarkably with no shift. These observations are supportive of the intercalative mode of binding of the complexes, where in the stacking of the complex molecules between the base pairs of DNA leads to an enhancement in the positive band and the partial unwinding of the helix is reflected in the decreased intensity of the negative band, which attributed to a strong conformational change in DNA helix.[43,44] It is similar to that observed for $[Ru(NH_3)_4(qdppz)]^{2+}$ and $[Co(NH_3)_6]^{3+}$ bound to DNA of short lengths with 160 base pairs.[45,46] The results obtained here validate those obtained from the UV–Vis spectral studies.

Viscosity Studies Hydrodynamic measurements that are sensitive to the length change (*i.e.*, viscosity and sedimentation) are regarded as the least ambiguous and the most critical tests of a binding model in solution in the absence of crystallographic structural data.[8,35] As a means for further clarifying the binding of these compounds with DNA, viscosity studies are carried out. Data are presented as $(\eta/\eta_0)^{1/3}$ *versus* $1/R$, where R=[DNA]/[compound]; η and η_0 are the relative viscosities of DNA in the presence and absence of complexes, respectively. The relative viscosity values were

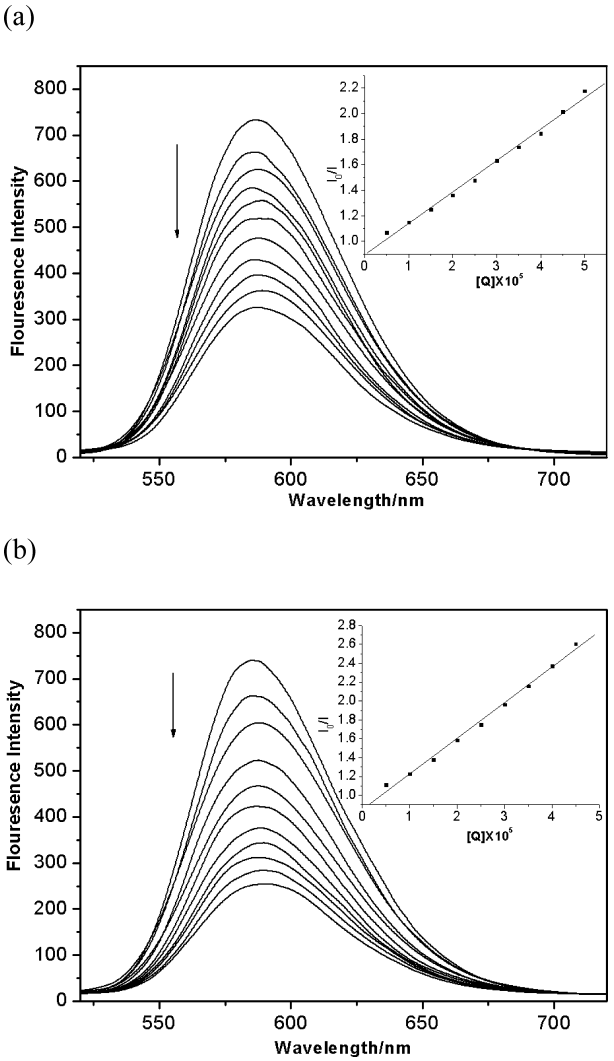

Fig. 3. Fluorescence Emission Spectra of DNA–EB in the Presence of 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 μM of **1** (a) and **2** (b)

(λ_{ex}=500 nm, λ_{em}=520—700 nm) [EB]=3 μM, [DNA]=30 μM. The inset is Stern–Volmer quenching plots of the fluorescence titration.

calculated from the flow time of DNA-containing solution (t) and the flow time of buffer alone (t_0), using the following expression (3)[47]

$$\eta=(t-t_0)/t_0 \quad (3)$$

Intercalating agents are expected to elongate the double helix to accommodate the ligands in between the base leading to an increase in the viscosity of DNA. In contrast, complexes that bind exclusively in the DNA grooves by partial and/or non-classical intercalation, under the same conditions, typically cause less pronounced (positive or negative) or no change in DNA solution viscosity.[48]

The effect of the two complexes on the viscosity of DNA at 25.0±0.1 °C are shown in Fig. 5. It can be observed that the viscosity of the DNA increase steadily with increasing amounts of complex **1** and **2**. Such behavior is in accordance with other intercalators, which increases the relative specific viscosity for the lengthening of the DNA double helix resulting from intercalation. These results indicate that the two complexes can intercalate between adjacent DNA base pairs, causing an extension in the helix, and thus increase the vis-

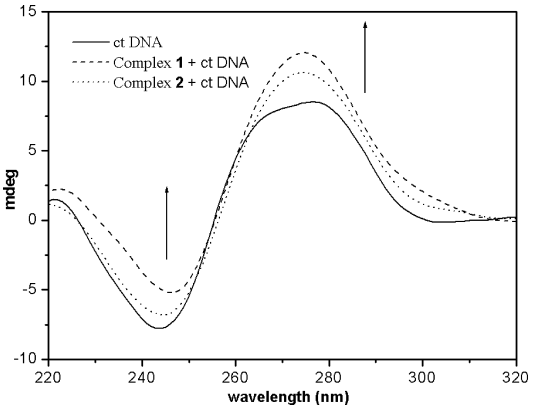

Fig. 4. CD Spectra of ct DNA (120 μM) in the Absence and Presence of **1** (120 μM) and **2** (120 μM)

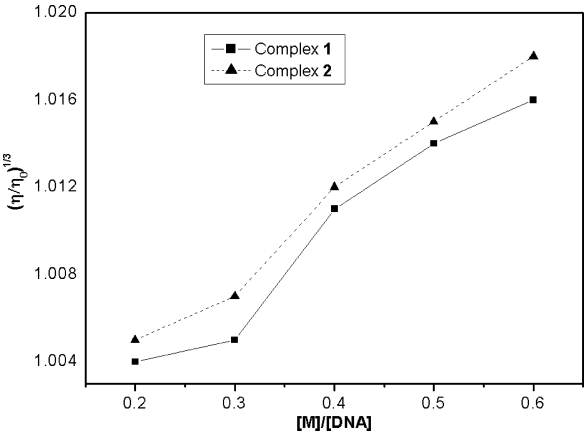

Fig. 5. Effect of Increasing Amounts of the Complexes **1** and **2** on the Relative Viscosity of ct DNA at 25 °C

[DNA]=50 μM, [compound]=1, 2, 3, 4, 5 μM.

cosity of DNA, and complex **2** can intercalate more deeply than **1**. The results obtained from viscosity studies also validate those obtained from the spectroscopic studies. On the basis of all the spectroscopic studies together with the viscosity measurements, it is suggested that the two complexes can bind to ct DNA in intercalative mode.

Cytotoxic Activity Evaluation of the two complexes for cytotoxic activity in incline was performed by acid phosphatase assay using two different human cancer cell lines, human prostate cancer cell (PC3), human liver cell (L02) and human myeloid leukemia cancer cell (HL-60). The assay is based on the hydrolysis of the *p*-nitrophenyl phosphate by intracellular acid phosphatases in viable cells to produce *p*-nitrophenol. For the cell lines examined, absorbance of *p*-nitrophenol at 405 nm is directly proportional to the cell number in the range of 10^3—10^5 cells. The cultured cell lines are divided into multi-well microplate and treated with the synthesized compounds. All test agents are incubated with the three model cell lines for a period of 72 h. Results obtained for phen, $MCVH_2$, $MCLH_2$, the ternary copper complexes and simple metal salt were determined and the IC_{50} values are presented in Table 1. Phen and the two metal complexes displayed a concentration-dependent cytotoxic profile in all cell lines. Since the IC_{50} values for complex **1** and **2** were statistically lower than that for metal-free phen

Table 1. Antiproliferative Effects (IC_{50}) of **1** and **2** on PC3 Cells and HL-60 Cells

Cell line	IC_{50} (μM)					
	$Cu(ClO_4)_2\cdot 6H_2O$	$MCVH_2$	$MCLH_2$	phen	**1**	**2**
PC3	>500	>100	>100	>100	5.4	3.1
HL-60	>500	>100	>100	6.2	3.6	2.4
L02	>500	>100	>100	35	4.8	3.4

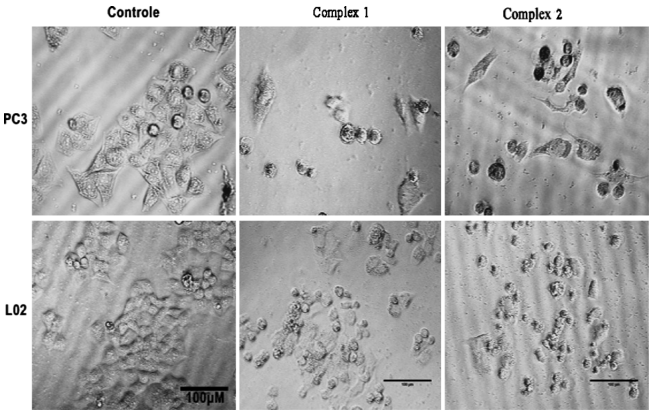

Fig. 6. Phase-Contrast Micrographs of Cells Treated with Complexes **1** and **2**

and two ligands in all of the tested cells, it suggested that coordinated copper(II) ion plays a major role in mediating potency of the complex. The IC_{50} values for phen on all the cells are not statistically different, it displayed a greater effect against HL-60 cell line, while the IC_{50} values for the metal complexes on HL-60 cell are relatively lower than PC3 cell lines, this result suggested that in general, HL-60 may be more sensitive PC3 cells. In order to prove that the cytotoxicity observed is due to the ternary copper complexes, rather than to simple aquated metal ions $[Cu(H_2O)_x]^{y+}$, the ligand-free metal salts $Cu(ClO_4)_2\cdot 6H_2O$, was screened against two model cell lines. The data showed that $Cu(ClO_4)_2\cdot 6H_2O$ displayed significantly less effective than phen, $MCVH_2$, $MCLH_2$ and their metal complexes. The results also suggested that **2** had more significant cytotoxic activities than **1** against the human cancer cell lines and the inhibitory effect *in vitro* is consistent with the result of DNA binding studies above, which may be due to the complexes inducing DNA damage in cancer cells and the nature of the metal ions.[49—51] However, the complexes can also inhibit the normal human liver cell (L02), which needs to be further researched by modifying the ligands or other methods. Some more research are still in doing in our laboratory.

The morphology examination also showed that the proliferation of the cells were significant inhibited and the cells exhibited morphological changes such as cell shrinkage and cell detachment (Fig. 6).

Conclusion

Two novel reduced Schiff base ligand $MCVH_2$, $MCLH_2$ and their ternary copper(II) complexes are prepared and characterized, the DNA-binding properties of the complexes are investigated by absorption, fluorescence, circular dichroic spectra and viscosity measurements. The binding constant

shows that the DNA-binding affinity increases in the order: complex **1**<complex **2**. The two complexes show considerable cytotoxic activity against human prostate cancer cell (PC3), human liver cell (L02) and human myeloid leukemia cancer cell (HL-60) lines, and the IC_{50} values of all the metal complexes are lower than that of phen, $MCVH_2$, $MCLH_2$ and $Cu(ClO_4)_2 \cdot 6H_2O$. Studies are currently underway in our laboratory to investigate more fully the mechanisms by which phen, $MCVH_2$, $MCLH_2$ and their metal complexes control cancer cell viability. It is intended that the results from these studies will allow identification of key molecular targets, and in doing so will assist in elucidating their mechanisms of action, along with facilitating the development of highly effective anti-cancer therapies.

Acknowledgements This work was supported by the Fundamental Research Funds for the Central Universities and we are grateful to Prof. Jagadese J. Vittal of National University of Singapore for physical measurements.

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