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Bimetallic complexes constructed from asymmetrical *N*,*N*′-bis(substituted)-oxamide: Cytotoxicities, and reactivities towards DNA and protein

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ABSTRACT

A new asymmetrical *N*,*N*′-bis(substituted)oxamide ligand, *N*-(5-chloro-2-hydroxyphenyl)-*N*′-[3-(dimethylamino)propyl]oxamide ($C_{13}H_{18}N_3O_3Cl\cdot H_2O$, **$H_3$L**) and its two binuclear complexes $[Cu_2L(H_2O)(bpy)](ClO_4)\cdot CH_3OH$ (**1**) and $[Ni_2L(bpy)_2](ClO_4)$ (**2**) [bpy = 2,2′-bipyridine] have been synthesized and characterized by X-ray single-crystal diffraction. In the crystal structure, **H_3L** adopting a *transoid* conformation occurs as a neutral molecule linked with a water molecule by an intermolecular hydrogen bond. In the two complexes, the *cis*-L^{3-} ligand bridges two metal ions with the corresponding separations of 5.2032(15) Å (**1**) and 5.2466(7) Å (**2**), respectively. *In vitro* cytotoxic activities, and the reactivities of the three compounds towards DNA and protein are investigated.

1. Introduction

Investigations on transition-metal complexes towards DNA and protein are of current interest in connection with information about drug design and tools of molecular biology [1–9]. Modes of DNA non-covalent interaction with metal complexes include electrostatic effect, groove binding, and intercalation. Many important applications of these metal complexes require that they can bind to DNA in an intercalative mode [10]. Both the planarity of ligand and the coordination geometry of the metal ion play important roles in deciding the intercalating ability of complexes to DNA [11,12]. By varying the metal center, it is possible to modify the mode as well as the extent of interaction of the complex with nucleic acids and facilitate individual applications [13]. On the other hand, serum albumins play an important and efficient role in drug delivery due to their remarkable binding properties. With the advantages of low cost and ready availability, bovine serum albumin (BSA) is a widely used serum albumin. Many studies dealing with the interactions between organic molecules and BSA have been carried out because the structures of BSA are similar to human serum albumin (HSA) in 76% [14]. Compared to the number of organic molecules [15–18], relatively few metal complexes [19,20] especially binuclear complexes [21] have been investigated the reactivities towards BSA. With the facts that copper and nickel elements are essential elements to the life process, and binuclear copper and nickel complexes have been found to inhibit DNA replication and tumor cell proliferation [22–26], it is necessary to design and synthesize new bicopper and binickel complexes to evaluate their cytotoxicities, and reactivities towards DNA and protein.

A promising strategy to design and synthesize binuclear complexes is the use of bridging ligand to react with metal salts and terminal ligands. *N*,*N*′-bis(substituted)oxamides are well-known to be good candidates as the binucleating bridging ligand in forming binuclear complexes. Comparatively, due to the synthesis difficulty, the studies on the binuclear complexes involving the bridging asymmetrical *N*,*N*′-bis(substituted)oxamides are very limited [27,28]. However, the fact that those complexes bridged by asymmetrical *N*,*N*′-bis(substituted)oxamides have shown predominant properties [29–33] stimulate us to design and synthesize new binuclear complexes with asymmetrical *N*,*N*′-bis(substituted)oxamides to gain some insight into the influence of metal ions in this kind of complexes on structure, cytotoxic activities and reactivities towards DNA and protein.

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Recently, we reported several transition-metal complexes bridged by asymmetrical *N*,*N*′-bis(substituted)oxamides and found they have significant DNA-binding and cytotoxic activities [34,35]. Enlightened by the above-mentioned facts, and as a continuation of our on going program, in this paper, a new asymmetrical *N*,*N*′-bis(substituted) oxamide ligand, *N*-(5-chloro-2-hydroxyphenyl)-*N*′-[3-(dimethylamino)propyl]oxalamide (**H_3L**), and its two binuclear transition metal complexes, $[Cu_2L(H_2O)(bpy)](ClO_4)\cdot CH_3OH$ (**1**) and $[Ni_2L(bpy)_2](ClO_4)$ (**2**), were synthesized and structurally characterized by X-ray single-crystal diffraction. *In vitro* cytotoxic activities of the three compounds were tested by Sulforhodamine B (SRB) assays against human hepatocellular carcinoma cell SMMC-7721 and human lung adenocarcinoma cell A549. The interactions of the three compounds with herring sperm DNA (*HS*-DNA) are investigated by using UV absorption and fluorescence spectra and viscometry. Furthermore, the protein binding ability has been monitored by using UV absorption and tryptophan fluorescence quenching experiment in the presence of the compounds using bovine serum albumin (BSA) as model protein. To the best of our knowledge, the binding of the asymmetrical *N*,*N*′-bis(substituted)oxamide bridged binuclear complexes to proteins has seldom been investigated.

2. Experimental

2.1. Materials and physical measurements

DNA and BSA were purchased from Sigma and were used as supplied. Other chemicals were of reagent grade. Carbon, hydrogen and nitrogen elemental analyses were performed with a Perkin–Elmer elemental analyzer Model 240. Molar conductance was measured with a Shanghai DDS-11A conductometer. The infrared spectra of the samples were recorded as KBr pellets on a Nicolet model Impact 470 FTIR spectrophotometer in the spectral range 4000–400 cm^{-1}. The mass spectra (ES-MS) were measured with a Waters Q-TOF GLOBLE mass spectrometer. The UV–visible spectrum was recorded in a 1-cm-path length quartz cell on a Cary 300 spectrophotometer. Fluorescence was tested on an Fp-750w fluorometer. Viscosity measurement was carried out using an Ubbelohde viscometer immersed in a thermostatic water bath maintained at 289(±0.1) K.

2.2. Synthesis

2.2.1. Synthesis of the ligand ($C_{13}H_{18}N_3O_3Cl\cdot H_2O$, **$H_3$L**)

The synthesis of *N*-(5-chloro-2-hydroxyphenyl)-*N*′-[3-(dimethylamino)propyl]oxalamide (**H_3L**), consists of two steps. The first step was the preparation of oxamido-2-amino-4-chlorophenol-ethyloxamate (H_3oxch) according to the reported method [27]. For that, a 40 mmol (5.49 g) portion of ethyl oxalyl chloride in 10 mL of tetrahydrofuran (THF) was added dropwise into a 40 mL THF solution of 40 mmol (5.92 g) of 2-amino-4-chlorophenol (97%). The clear solution was concentrated under vacuum and the H_3oxch was precipitated as a white powder then washed with ethanol and dried under vacuum. The second step was the synthesis of *N*-(5-chloro-2-hydroxyphenyl)-*N*′-[3-(dimethylamino)propyl]oxalamide (**H_3L**). A 5 mmol (1.22 g) amount of H_3oxch in 20 mL absolute ethanol was added dropwise into 20 mL absolute ethanol solution containing 6 mmol (0.76 mL) 3-(dimethylamino)propyl at 273 K. The resulting solution was stirred for 2 h, and **H_3L** was precipitated as a white powder and then recrystallized in ethanol and dried under vacuum. Well-shaped colorless single crystals of the ligand were obtained by slow evaporation of the ethanol solution of the recrystallized product. Yield: 72%. Analysis; Calc for $C_{13}H_{18}N_3O_3Cl\cdot H_2O$: C, 49.14; H, 6.34; N, 13.22. Found: C, 49.10; H, 6.32; N, 13.19. IR (KBr, cm^{-1}): 3356 [ν(NH)]; 1686 [ν(C═O)] (oxamidate group). UV–visible (in DMF), λ_{max}(nm) [ε_{max} (L mol^{-1} cm^{-1})]: 301(22 550). ES-MS, *m*/*z*: 300.2 ($[M + H]^+$).

2.2.2. Synthesis of $[Cu_2L(H_2O)(bpy)](ClO_4)\cdot CH_3OH$ (**1**)

To a stirred methanol solution (5 mL) containing $Cu(ClO_4)_2\cdot 6H_2O$ (0.0742 g, 0.2 mmol) was added dropwise a methanol solution (10 mL) of **H_3L** (0.0299 g, 0.1 mmol) and piperidine (0.0256 g, 0.3 mmol) at room temperature. After stirring for 30 min, a methanol solution (5 mL) of bpy (0.0156 g, 0.1 mmol) was added dropwise. The mixture was stirred quickly at 333 K for 6 h, the resulting brown solution was filtered and brown cube crystals of the complex suitable for X-ray analysis were obtained by slow evaporation at room temperature. Yield: 82%. Analysis; Calc for $Cu_2C_{24}H_{29}N_5O_9Cl_2$: C, 39.51; H, 4.01; N, 9.60. Found: C, 39.53; H, 3.98; N, 9.67. IR (KBr, cm^{-1}): 1632 [ν(C═O)]; 1442 [ν(C═N)]; 1104, 622 [$\nu(ClO_4)$]. Molar conductance, Λ_M (DMF solution): 79 Ω^{-1} cm^2 mol^{-1}. UV–visible (in DMF), λ_{max}(nm) [ε_{max}(L mol^{-1} cm^{-1})]: 612(800), 311(40 400), 299(44 300), 243(75 700). ES-MS, *m*/*z*: 598.0 ($[M-ClO_4-CH_3OH]^+$).

2.2.3. Synthesis of $[Ni_2L(bpy)_2](ClO_4)$ (**2**)

Deep reddish purple crystals suitable for X-ray single-crystal analysis were obtained by the same method of preparing complex **1** except using $Ni(ClO_4)_2\cdot 6H_2O$ instead of $Cu(ClO_4)_2\cdot 6H_2O$. Yield: 58%. Analysis; Calc for $Ni_2C_{33}H_{31}N_7O_7Cl_2$: C, 47.99; H, 3.78; N, 11.87. Found: C, 47.92; H, 3.75; N, 11.81. IR (KBr, cm^{-1}): 1633 [ν(C═O)]; 1472, 1443 [ν(C═N)]; 1094, 623 [$\nu(ClO_4)$]. Molar conductance, Λ_M (DMF solution): 87 Ω^{-1} cm^2 mol^{-1}. UV–visible (in DMF), λ_{max}(nm) [ε_{max} (L mol^{-1} cm^{-1})]: 605(912), 420(623), 306(30 300), 295(29 500), 241(30 500). ES-MS, *m*/*z*: 724.1 ($[M - ClO_4]^+$).

2.3. X-ray crystallography

The X-ray diffraction experiments for three compounds were made on a Bruker APEX area-detector diffractometer with graphite monochromatic Mo Kα radiation (λ = 0.71073 Å). The crystal structures were solved by the directed method followed by Fourier syntheses. Structure refinements were performed by full matrix least-squares procedures using SHELXL-97 on F^2 [36].

2.4. In vitro cytotoxic activity evaluation by SRB assays

In vitro cytotoxic activities of the three compounds and *cis*-platin were evaluated against two cancer cell lines including SMMC-7721 and A549 by using the Sulforhodamine B (SRB) assay. All cells were cultured in RPMI 1640 supplemented with 10% (v/v) fetal bovine serum, 1% (w/v) penicillin (104 U/mL) and 10 mg/mL streptomycin. Cell lines are maintained at 310 K in a 5% (v/v) CO_2 atmosphere with 95% (v/v) humidity. Cultures were passaged weekly using trypsin–EDTA to detach the cells from their culture flasks. The three compounds and *cis*-platin were dissolved in DMSO and diluted to the required concentration with culture medium when used. The content of DMSO in the final concentrations did not exceed 0.1%. At this concentration, DMSO was found to be non-toxic to the cells tested. Rapidly growing cells were harvested, counted, and incubated at the appropriate concentration in 96-well micro plates for 24 h. The three compounds and *cis*-platin dissolved in culture medium were then applied to the culture wells to achieve final concentrations ranging from 10^{-4} to 10^2 μg/mL. Control wells were prepared by addition of culture medium without cells. The plates were incubated at 310 K in a 5% CO_2 atmosphere for 48 h. Upon completion of the incubation, the cells were fixed with ice-cold 10% trichloroacetic acid (100 mL) for 1 h at 277 K, washed five times in distilled water and allowed to dry in the air and stained with 0.4% SRB in 1% acetic acid (100 mL) for 15 min. The cells were washed four times in 1% acetic acid and air-dried.