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Synthesis and crystal structure of new dicopper(II) complexes having asymmetric *N*,*N*′-bis(substituted)oxamides with DNA/protein binding ability: *In vitro* anticancer activity and molecular docking studies

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ABSTRACT

Two new dicopper(II) complexes bridged by asymmetric *N*,*N*′-bis(substituted)oxamide ligands: *N*-(5-chloro-2-hydroxyphenyl)-*N*′-[2-(dimethylamino)ethyl]oxamide (H_3chdoxd) and *N*-hydroxypropyl-*N*′-(2-carboxylatophenyl)oxamide (H_3oxbpa), and end-capped with 2,2′-bipyridine (bpy), namely $[Cu_2(ClO_4)(chdoxd)(CH_3OH)(bpy)]\cdot H_2O$ (**1**) and $[Cu_2(pic)(oxbpa)(CH_3OH)(bpy)]\cdot 0.5CH_3OH$ (**2**) (pic denotes picrate anion), have been synthesized and characterized by elemental analysis, molar conductivity measurement, IR and electronic spectral studies, and single-crystal X-ray diffraction. The X-ray diffraction analysis revealed that both the copper(II) ions bridged by the *cis*-oxamido ligands in dicopper(II) complexes **1** and **2** are all in square-pyramidal environments with the corresponding Cu···Cu separations of 5.194(3) and 5.1714(8) Å, respectively. In the crystals of the two complexes, there are abundant hydrogen bonds and π–π stacking interactions contributing to the supramolecular structure. The reactivities toward herring sperm DNA (HS-DNA) and bovine serum albumin (BSA) of the two complexes are studied both theoretically and experimentally, indicating that both the two complexes can interact with the DNA in the mode of intercalation, and effectively bind to BSA *via* the favored binding sites Trp134 for the complex **1** and Trp213 for the complex **2**. Interestingly, the *in vitro* anticancer activities of the two complexes against the selected tumor cell lines are consistent with their DNA/BSA-binding affinities following the order of **1** > **2**. The effects of coordinated counterions in the two complexes on DNA/BSA-binding ability and *in vitro* anticancer activity are preliminarily discussed.

1. Introduction

As is well-known, DNA and protein are the primary molecular targets in the action of drugs, and many drugs exert their drug effects through binding to DNA or protein, which is the critical step for the study of effective drugs. Therefore, understanding the features that determine the binding of metal complexes to DNA/protein is the basis of designing and discovering new and more efficient metal-based antitumor drugs [1–5]. Metal-based drugs play a relevant role in antitumor chemo-therapy. Cisplatin, as one of the leading metal-based drugs targeting DNA, is widely used in treatment of cancers especially testicular and ovarian cancers. However, a major factor that impedes the clinical success of cisplatin is the significant side effects originated from its binding mode to DNA due to the formation of covalent cross-links. Thus, much attention has been paid to the design and synthesis of new metal-based drugs bearing metal ions other than platinum with more-efficacious, target-specific, less-toxic and non-covalently DNA-binding [6,7]. It has been well established that metal complexes can interact with DNA non-covalently through electrostatic interaction, groove binding and intercalation [8]. Among these non-covalent binding modes, the intercalation attracts considerable attention owing to its strong binding ability and various applications in cancer therapy and molecular biology [9,10]. The intercalating ability not only correlates with the planarity and donor atom types of the ligands but also related to the coordination geometry of the metal centers. Furthermore, as attention has focused on developing metal-based therapeutics, there is interest in the analysis of drug-protein interactions that influence the absorption, distribution, metabolism, and excretion properties of drugs. Among the serum albumins, bovine serum albumin (BSA) is one of the major components in plasma protein. With the

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advantages of low cost and ready availability, especially the similarity with human serum albumin in respect of approximately 76% sequence homology, BSA has been widely used as the model protein concerning the interaction between compounds and serum albumins in biophysical and biochemical studies. Compared with the number of organic molecules [11,12], relatively few metal complexes have been investigated the protein BSA-binding activity. Therefore, the reactivity of metal complexes toward DNA and protein BSA are useful in the design and synthesis of metal-based anticancer therapeutics.

So far, in the context of design and synthesis of metal complexes that are capable of binding DNA and protein by non-covalent modes, most attention has centered upon the selection of metal ions and the design of ligands. Because metal complexes can accelerate drug action and increase effectiveness of organic ligands, and medicinal properties of metal complexes depend on the nature of metal ions and ligands. Thus, a combination of suitable metal as well as design of ligand is considered an important prerequisite for the construction of a highly efficient DNA and BSA targeted drug. Along this line, lots of copper(II) complexes with different ligands have been widely studied [13–27], because copper, as a biologically relevant element, shows great effects on the endogenous oxidative DNA damage associated with aging and cancer and relates to many enzymatic activities which have been identified. Comparing the number of studies dealing with monocopper complexes [14–22], relatively few studies on dicopper(II) complexes have been reported to date. However, the enhancement of DNA-binding activity for dinuclear complexes prompts us to design and synthesize new dicopper(II) complexes in order to gain some insight into their DNA/protein binding property and antitumor activity of these kinds of complexes [23–27].

The present work stems from our continuous interest in defining and evaluating DNA and protein binding of dicopper(II) complexes bridged by *N*,*N*′-bis(substituted)oxamides, and also from our efforts to explore the structure–activity relationship of cytotoxicity. Quite recently, we reported the synthesis, structure, DNA/protein-binding property and *in vitro* antitumor activity of two dicopper(II) with symmetric *N*,*N*′-bis(substituted)oxamides [27], and the results suggest that counterions may play an important role in changing the DNA/protein-binding abilities of dicopper(II) complexes. Considering the fact that complexes bridged by asymmetric *N*,*N*′-bis(substituted)oxamides have shown interesting magnetic properties and good anticancer activities [28–30], as well as copper(II) complexes containing perchlorate or picrate (pic) anions exhibited good cytotoxic activities [22–27,30], as a continuation of our ongoing program, we employed *N*-(5-chloro-2-hydroxyphenyl)-*N*′-[2-(dimethylamino)ethyl]oxamide (H_3chdoxd) and *N*-hydroxypropyl-*N*′-(2-carboxylatophenyl)-oxamide (H_3oxbpa) as asymmetric *N*,*N*′-bis(substituted)oxamide bridging ligands, 2,2′-bipyridine (bpy) as terminal ligand, and perchlorate and pic anions as counterions to synthesize and structurally characterize two new complexes with formulae of $[Cu_2(ClO_4)(chdoxd)(CH_3OH)(bpy)]$-$H_2O$ (**1**) and $[Cu_2(pic)(oxbpa)$-$(CH_3OH)(bpy)]\cdot 0.5CH_3OH$ (**2**). The comparative study of the interactions of these complexes with DNA and protein BSA, as well as the antitumor activities was explored both theoretically and experimentally to gain some new insight into the structure–activity relationship of the dicopper(II) complexes with asymmetric *N*,*N*′-bis(substituted)oxamide as bridging ligands. The main results confirmed that coordinated counterions in dicopper(II) complexes bridged by asymmetric *N*,*N*′-bis(substituted)oxamides can influence the DNA/BSA binding events and the *in vitro* anticancer activities, thus suggesting that the DNA/BSA binding properties and the anticancer activities may possibly be tuned through varying counterions in these dicopper(II) systems, which is useful for the design and synthesis of new metal-based drugs.

2. Experimental

2.1. Materials and chemicals

The asymmetric *N*,*N*′-bis(substituted)oxamide bridging ligands, *N*-(5-chloro-2-hydroxylphenyl)-*N*′-[2-(dimethylamino)ethyl]oxamide (H_3chdoxd) and *N*-hydroxypropyl-*N*′-(2-carboxylatophenyl)-oxamide (H_3oxbpa) were synthesized according to the literature method [31]. Ethidium bromide (EB), herring sperm DNA (HS-DNA), and bovine serum albumin (BSA) were purchased from Sigma Corp. and used as received. SRB, foetal bovine serum and all other cell-culture reagents were obtained from Solarbio Science and Technology Co., Ltd. Beijing, China. All other chemical reagents were of AR grade and obtained commercially.

2.2. Physical measurements

The C, H and N microanalyses were performed on a Perkin–Elmer 240 elemental analyzer. Infrared spectra were recorded on a Nicolet-470 spectrophotometer in the spectral range 4000–400 cm^{-1} as KBr pellets. Molar conductance was measured with a Shanghai DDS-11A conductometer. 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. Magnetic susceptibility measurements at room temperature were carried out by Gouy's method using $Hg[Co(SCN)_4]$ as the calibrant. Viscosity experiments were carried out using an Ubbelohde viscometer maintained at a constant temperature of 298.0(±0.1) K in a thermostatic water bath.

Caution! Although we have not encountered any problems, perchlorate and picrate compounds are potentially explosive and should be handled with care.

2.3. Synthesis of the dicopper(II) complexes

2.3.1. Synthesis of $[Cu_2(ClO_4)(chdoxd)(CH_3OH)(bpy)]\cdot H_2O$ (**1**)

An aqueous solution (5 mL) of copper(II) perchlorate hexahydrate (0.0371 g, 0.10 mmol) was added dropwise into a methanol solution (5 mL) containing H_3chdoxd (0.0143 g, 0.05 mmol) and piperidine (0.0128 g, 0.15 mmol). After stirring quickly for 30 min, a methanol solution (5 mL) of bpy (0.0078 g, 0.05 mmol) was added slowly into the reaction system. The resulting solution was stirred at 333 K for 3 h. The obtained brown solution was filtered and dark brown prism crystals of complex **1** suitable for X-ray analysis were obtained by slow evaporation the filtrate at room temperature. Yield: 0.0258 g (72%). Anal. calcd for $Cu_2C_{23}H_{27}N_5O_9Cl_2$: C, 38.61; H, 3.80; N, 9.79%. found: C, 38.31; H, 3.55; N, 9.63%. IR (KBr pellet, cm^{-1}): 1634 [ν(C=O)]; 1443 [ν(C=N)]; 1276 [ν(Ar—O)]; 1121, 1101, 1075 [$\nu(ClO_4)$]. UV–visible: λ_{max}(nm) [ε_{max}(L mol^{-1} cm^{-1})] (in methanol): 203(39,500), 242(20,500), 299(18,000), 310(16,000), and 597(105). Molar conductance (in water over 24 h): 52 S cm^2 mol^{-1}. μ_{eff} (298 K): 2.38 μ_B.

2.3.2. Synthesis of $[Cu_2(pic)(oxbpa)(CH_3OH)(bpy)]\cdot 0.5CH_3OH$ (**2**)

The complex as red–brown crystals suitable for X-ray single-crystal analysis was obtained by the same procedure as above except using H_3oxbpa (0.0133 g, 0.05 mmol) instead of H_3chdoxd, and copper(II) picrate tetrahydrate (0.0592 g, 0.10 mmol) instead of copper(II) perchlorate hexahydrate. Yield: 0.0308 g (75%). Anal. calcd for $Cu_2C_{29.5}H_{26}N_7O_{13.5}$: C 43.07, H 3.31, N 11.92%; found: C 42.85, H 3.16, N 11.75%. IR (KBr pellet, cm^{-1}): 1641 [ν_{as}(COO) + ν(C=O)]; 1470 [ν(C=N)]; 1573, 1538 [$\nu_{as}(NO_2)$]; 1379, 1312 [$\nu_s(NO_2)$]. UV–visible: λ_{max}(nm) [ε_{max}(L mol^{-1} cm^{-1})] (in methanol): 204(44,000), 242(32,000),