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Synthesis and structure of new tetracopper(II) complexes with *N*-benzoate-*N*′-[3-(diethylamino)propyl]oxamide as a bridging ligand: The influence of hydrophobicity on enhanced DNA/BSA-binding and anticancer activity

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ABSTRACT

Two new tetracopper(II) complexes bridged by *N*-benzoate-*N*′-[3-(diethylamino)propyl]oxamide (H_3bdpox), and ended with 4,4′-dimethyl-2,2′-bipyridine (Me_2bpy) or 2,2′-bipyridine (bpy), namely $[Cu_4(bdpox)_2(Me_2bpy)_2](pic)_2$ (**1**) and $[Cu_4(bdpox)_2(bpy)_2](pic)_2\cdot 2H_2O$ (**2**) (where pic denotes the picrate anion) have been synthesized and characterized by X-ray single-crystal diffraction and other methods. In both complexes, four copper(II) ions are bridged alternately by the *cis*-oxamido and the carboxylato groups of two $bdpox^{3-}$ ligands to form a centrosymmetric cyclic tetranuclear cation, in which, the copper(II) ions at the *endo*- and *exo*-sites of *cis*-$bdpox^{3-}$ ligand have square-planar and square-pyramidal coordination geometries, respectively. The reactivity towards DNA/BSA suggests that these complexes can interact with *HS*-DNA through the intercalation mode and the binding affinity varies as **1** > **2** depending on the hydrophobicity, and effectively quench the fluorescence of protein BSA *via* a static mechanism. *In vitro* anticancer activities showed that the two complexes are active against the selected tumor cell lines, and the anticancer activities are consistent with their DNA-binding affinity.

1. Introduction

It is well-known that DNA and protein are not only important biological macromolecules but also commonly considered as the main molecular targets in the action of drugs, and many metal complexes exert pharmacological effects through binding to DNA or protein, which is the basis of designing and discovering new and more efficient metal-based antitumor drugs [1,2]. Thus, the design and synthesis of transition metal complexes which can noncovalently interact with DNA or protein have been particularly active in recent years.

In the context of synthesizing metal complexes with more-efficacious, target-specific, less-toxic and non-covalent DNA/protein binding, much effort has been devoted to the selection of metal ions and the design of the ligands. Copper, as a biologically active metal element for human, shows many correlations with endogenous oxidative deoxyribonucleic acid damage associated with aging and cancer. More importantly, several families of individual copper complexes have been studied as potential antitumor agents, which have been proven to be more specificity and less toxic side effects due to their new mechanisms compared with platinum drugs [3–5]. Thereby, copper-based complexes are considered good alternatives to platinum drugs as potential antitumor agents [6]. Compared to mono- and di-copper(II) complexes [7,8], relatively few studies on tetracopper(II) complexes have been reported [9,10]. However, the enhancement of DNA-binding or cleavage activity for tetranuclear(II) complexes [9–11], together with the fact that relatively few reactivities towards protein of tetranuclear complexes has been investigated, stimulates us to design and synthesize new tetracopper(II) complexes to evaluate the influence of synergetic hydrophobicity of bridging and terminal ligands in these complexes on structure and biological properties. In view of the effective bridging function of asymmetric *N*,*N*′-bis(substituted)oxamides, in this paper, we choose *N*-benzoate-*N*′-[3-(diethylamino)propyl]oxamide (H_3bdpox) as bridging ligand, and 4,4′-dimethyl-2,2′-bipyridine (Me_2bpy) and 2,2′-bipyridine (bpy) as terminal ligands to synthesize and structurally characterize two new tetracopper(II) complexes, $[Cu_4(bdpox)_2(Me_2bpy)_2](pic)_2$ (**1**) and $[Cu_4(bdpox)_2(bpy)_2](pic)_2\cdot 2H_2O$ (**2**). The interactions of these complexes with herring sperm DNA (*HS*-DNA) and bovine serum albumin (BSA), as well as their *in vitro* anticancer activities are studied.

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2. Experimental

2.1. Materials and Chemicals

The ligand *N*-benzoate-*N*′-[3-(diethylamino)propyl]oxamide (H_3bdpox), and Cu(pic)$_2$·6H$_2$O were synthesized according to the literature methods [12,13]. Ethidium bromide (EB), *HS*-DNA and protein BSA were purchased from Sigma Corp. and used as received. All other chemical reagents were of A.R. grade and obtained commercially.

2.2. Physical Measurements

The C, H, and N microanalyses were obtained on a Perkin-Elmer 240 elemental analyzer. Molar conductance was measured with a Shanghai DDS-11A conductometer. The infrared spectrum was recorded with samples as KBr pellets in a Nicolet model Impact 470 FTIR spectrophotometer in the spectral range 4000–400 cm^{-1}. Viscosity experiments were carried out using an Ubbelodhe viscometer maintained at a constant temperature of 298 K in a thermostatic water bath. Electronic spectra were recorded on a Cary 300 spectrophotometer. The fluorescence spectra were measured with an Fp-750w spectrofluorometer equipped with quartz cuvettes of 1-cm-path length. A CHI 832 electrochemical analyzer (Shanghai CHI Instrument, Shanghai, China) in connection with a glassy carbon working electrode (GCE), a saturated calomel reference electrode (SCE), and a platinum wire counter electrode was used for the measurements. The GCE surface was freshly polished to a mirror prior to each experiment with 0.05 μm α-Al_2O_3 paste and then cleaned in water for 5 min.

2.3. Synthesis of [Cu$_4$(bdpox)$_2$(Me$_2$bpy)$_2$](pic)$_2$ (**1**)

To a stirred methanol solution (6 mL) of Cu(pic)$_2$·6H$_2$O (62.70 mg, 0.1 mmol) was added dropwise a methanol solution (8 mL) containing H_3bdpox (16.00 mg, 0.05 mmol) and piperidine (12.80 mg, 0.15 mmol) at room temperature. After stirring for 30 min, a methanol solution (6 mL) of Me$_2$bpy (9.20 mg, 0.05 mmol) was added dropwise. The mixture was stirred quickly at 333 K for 6 h, and then the resulting green solution was filtered and allowed to evaporate at room temperature. Green cube crystals of the complex suitable for X-ray analysis were obtained after seven days. Yield: 35.17 mg (82%). Found (%): C, 47.58; H, 3.93; N, 13.08. Calcd. (%) for $Cu_4C_{68}H_{68}N_{16}O_{22}$: C, 47.61; H, 4.00; N, 13.06. IR (KBr ν/cm^{-1}): 1634, [ν_{as}(COO) + ν(C═O)]; 1443, ν(C═N); 1558, ν_{as}(NO_2); 1363, 1334, ν_s(NO_2). UV–visible: λ_{max} (nm) [ε_{max} (L·mol^{-1}·cm^{-1})]: 235(8119), 298(5367) 355(2069), 622(560). Λ_M (DMF, 298 K): 138 S·cm^2·mol^{-1}.

2.4. Synthesis of [Cu$_4$(bdpox)$_2$(bpy)$_2$](pic)$_2$·2H$_2$O (**2**)

Dark green cube crystals suitable for X-ray single-crystal analysis were obtained by the same method of preparing the complex **1** except using bpy instead of Me$_2$bpy. Yield: 26.70 mg (63%). Found (%): C, 45.29; H, 3.83; N, 13.21. Calcd. (%) for $Cu_4C_{64}H_{64}N_{16}O_{24}$: C, 45.34; H, 3.80; N, 13.22. IR (KBr ν/cm^{-1}): 1635, [ν_{as}(COO) + ν(C═O)]; 1446, ν(C═N); 1559, ν_{as}(NO_2); 1363, 1332, ν_s(NO_2). UV–visible: λ_{max} (nm) [ε_{max} (L·mol^{-1}·cm^{-1})]: 238(6411), 300(4304), 353(2253), 618(550). Λ_M (DMF, 298 K): 145 S·cm^2·mol^{-1}.

2.5. X-ray Crystallography

The X-ray diffraction experiments for the two complexes were made on a Bruker APEX area-detector diffractometer with graphite monochromatic Cu-Kα (λ = 1.54178 Å) for the complex **1** at 296 K and Mo-Kα radiation (λ = 0.71073 Å) for the complex **2** at 130 K. The crystal structures were solved by the direct method followed by Fourier syntheses. Structure refinements were performed by full matrix least-squares procedures using SHELXL-97 on F^2 [14]. In the complex **1**, the oxygen atoms of N8-nitryl group of pic anion is disordered with refined occupancies of 0.58(5) (O10A, O11A) and 0.42(5) (O10B, O11B). For both complexes, the H atoms on carbon atoms were placed at calculated positions with C—H distances of 0.96 (methyl), 0.97 (methylene) and 0.93 Å (aromatic), and refined in riding mode with U_{iso}(H) = 1.2U_{eq}(carrier atoms) or 1.5U_{eq}(methyl C). While the water H atoms in the complex **2** were found in a difference Fourier map and then treated as riding with U_{iso}(H) = 1.5U_{eq}(O). Crystal data and refinement data of the two complexes are summarized in Table 1, and selected bond distances and angles are given in Table 2.

2.6. DNA-interacting Experiments

All experiments involving *HS*-DNA were performed in *Tris*-HCl buffer solution (pH = 7.14). Solutions of *HS*-DNA in *Tris*-HCl buffer gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280}, of *ca.* 1.9, indicating that the DNA was sufficiently free of protein [15]. The concentration of the prepared DNA was determined by UV absorbance at 260 nm. The molar absorption coefficient, ε_{260}, was taken as 6600 M^{-1}·cm^{-1} [16]. Stock solution of *HS*-DNA was stored at 277 K and used after no more than four days. Concentrated stock solution of the two complexes were prepared by dissolving the two complexes in dimethyl sulfoxide (DMSO) and diluted suitably with *Tris*-HCl buffer to obtain the required concentrations for all the experiments. In viscosity measurement, *HS*-DNA samples approximately 200 base pairs in length were prepared by sonication in order to minimize complexities arising from DNA flexibility [17]. The flow times were measured with a digital stopwatch, and each sample was measured three times, and an average flow time was calculated. Relative viscosities for *HS*-DNA in the presence and absence of the two complexes were calculated from the relation $\eta = (t - t_0) / t_0$, where t is the observed flow time of DNA-containing solution and t_0 is that of *Tris*-HCl buffer alone. The data was presented as $(\eta/\eta_0)^{1/3}$ *vs.* binding ratio [18], where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone. Absorption spectral titration was performed by keeping the concentration of the complexes constant while varying the *HS*-DNA concentration. Equal solution of *HS*-DNA was added to the prepared solution and reference solution to eliminate the absorbance of DNA itself. In the EB fluorescence displacement experiment, 5 μL of the EB *Tris*-HCl solution (1 mM) was added to 1 mL of DNA solution (at saturated binding levels) [19], stored in the dark for 2 h. Then the solution of the complexes was titrated into the DNA/EB mixture and then diluted in *Tris*-HCl buffer to 5 mL, producing the

Table 1
Crystal data and details of the structure determination for complexes **1** and **2**.

Complex	**1**	**2**
Formula	$C_{68}H_{68}Cu_4N_{16}O_{22}$	$C_{64}H_{64}Cu_4N_{16}O_{24}$
Formula weight	1715.54	1695.48
Crystal system	Monoclinic	Triclinic
Space group	$P2_1/c$	P-1
a (Å)	11.9688(8)	10.528(2)
b (Å)	28.160(2)	10.949(2)
c (Å)	11.2064(8)	15.510(3)
α (°)	90	76.481(6)
β (°)	107.224(3)	72.712(6)
γ (°)	90	85.449(6)
V (Å^3)	3607.7(4)	1659.7(6)
D (calc) [g cm^{-3}]	1.579	1.696
Z	2	1
μ (mm^{-1})	2.079	1.361
F(000)	1760	868
Crystal size [mm]	0.13 × 0.38 × 0.52	0.05 × 0.11 × 0.27
Temperature (K)	296	130
Radiation [Å]	Cu Kα 1.54178	Mo Kα 0.71073
θ range	3.14–70.31	2.96–27.78
Tot., uniq. data, R(int)	35,753, 6794, 0.0617	27,153, 7587, 0.1665
Observed data [$I > 2\sigma(I)$]	5615	4051
R, ωR_2, S	0.0463, 0.1329, 1.028	0.0779, 0.2167, 0.987
Max., av. shift/error	0.001, 0.000	0.000, 0.000