Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 128 (2014) 686–693

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

DNA binding, cytotoxicity and apoptosis induction activity of a mixed-ligand copper(II) complex with taurine Schiff base and imidazole

Mei Li [a,b], Lin Lin kong [b], Yi Gou [b], Feng Yang [b,*], Hong Liang [a,b,*]

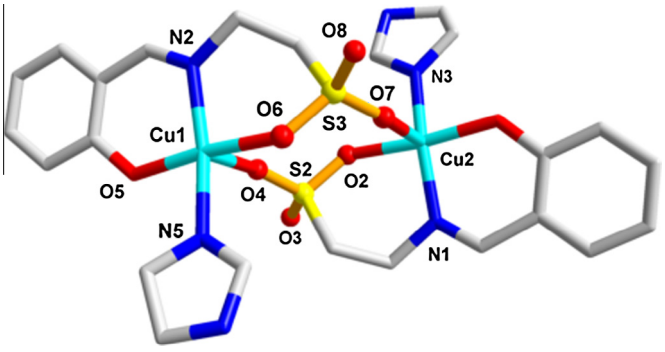
[a] College of Chemistry and Chemical Engineering, Central South University, Changsha, Hunan, China
[b] State Key Laboratory Cultivation Base for the Chemistry and Molecular Engineering of Medicinal Resources, Ministry of Science and Technology of China, Guangxi Normal University, Guilin, Guangxi, China

HIGHLIGHTS

- A novel mixed-ligand copper(II) complex has been synthesized and characterized.
- Two copper ions are bridged by sulfonate and exhibit square pyramidal geometries.
- **1** could bind to CT-DNA *via* intercalation and show efficient cleavage activity.
- **1** could arrest cell cycle in the S phase and induce apoptosis of MGC-803 cells.

GRAPHICAL ABSTRACT

A novel binuclear copper(II) complex (complex **1**) with taurine Schiff base and imidazole has been synthesized and characterized. The interaction between **1** and DNA was investigated by UV–vis, fluorescence, circular dichroism (CD) spectra and agarose gel electrophoresis. In addition, **1** showed an antitumor effect on cell cycle and apoptosis.



ARTICLE INFO

Article history:
Received 6 November 2013
Received in revised form 26 February 2014
Accepted 27 February 2014
Available online 14 March 2014

Keywords:
Taurine Schiff base
Imidazole
Copper(II) complex
CT-DNA
Cytotoxicity
Apoptosis

ABSTRACT

A novel binuclear copper(II) complex (complex **1**) with taurine Schiff base and imidazole has been synthesized and structurally characterized by single crystal X-ray diffraction, elemental analysis, ESI-MS spectrometry, UV–vis and IR spectroscopy. Single-crystal analysis revealed that **1** displays the sulfonate-bridged dinuclear copper(II) centers. Both copper atoms are five-coordinated and exhibit slightly distorted square pyramidal geometries. Each of copper atom is surrounded by three oxygen atoms and one nitrogen atom from different taurine Schiff base ligands, and one nitrogen atom from one imidazole ligand. The interaction between **1** and calf thymus DNA (CT-DNA) was investigated by UV–vis, fluorescence, circular dichroism (CD) spectra and agarose gel electrophoresis. The experimental results indicated that **1** could bind to CT-DNA *via* an intercalative mode and show efficient cleavage activity. In addition, **1** showed an antitumor effect on cell cycle and apoptosis. Flow cytometric analysis revealed that MGC-803 cells were arrested in the S phase after treatment with **1**. Fluorescence microscopic observation indicated that **1** could induce apoptosis of MGC-803 cells.

Abbreviations: CT-DNA, calf thymus DNA; EB, ethidium bromide; FBS, fetal bovine serum; MTT, 3-(4,5-dimathylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide; PBS, phosphate buffered saline; PI, propidium iodide; TBE, tris-boracic-EDTA; JC-1, 5,5,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide.

* Corresponding authors. Address: 15 Yucai Road, Guilin, Guangxi 541004, China. Tel./fax: +86 773 212 0958.
E-mail address: hliang@mailbox.gxnu.edu.cn (H. Liang).

http://dx.doi.org/10.1016/j.saa.2014.02.197
1386-1425/

Introduction

Platinum-based antitumor agents such as cisplatin, carboplatin, and oxaliplatin have achieved great successes. However, theses possess inherent limitations such as resistance, serious toxicity and other side effects [1–7]. These shortcomings make inorganic chemists attempt to replace these drugs with more effective, less toxic, target specific, and preferably noncovalently bound anticancer drugs. Copper complexes have emerged as an attractive alternative to cisplatin as anticancer substances for their essential physiological activity and oxidative nature [8–11]. As is well known, some antitumor drugs exert their activities by binding to, modifying and cleaving DNA [12]. Basically, the three non-covalent modes of the complex-DNA interaction are intercalation, groove binding and external electrostatic effects [13]. During the last few decades, the binding behavior of copper(II) complexes with DNA have been extensively studied [14–18]. Mechanism for copper(II) complexes-mediated cytotoxicity may be caused by their ability of binding to and cleaving DNA which leads to cell cycle arrest and apoptosis or generation of reactive oxygen species (ROS) then in turn to cell death [19,20].

Taurine (Fig. 1), the sulphonic acid analogue of β-alanine, has raised increasing interest to chemists as an ingredient in dietary supplements and functional foods and beverages. A few of taurine Schiff base complexes have been reported to have antiviral, anticancer and antibacterial activities [21–23]. Schiff bases derived from taurine have manifold coordination modes [24]. In a ternary complex, an aromatic-ring stacking interaction is an important characteristic, and it can stabilize the double-helical structure and the interaction between anticancer drugs and DNA [25].

Imidazole group plays an important role in numerous bioactive compounds and the pharmacological interest of the imidazole ring has already been established [26]. The study of mixed ligand complexes is becoming increasingly more important [27]. Herein, we designed and synthesized a new mixed-ligand copper(II) complex (Fig. 1) bridged by two sulfonates ligands. In order to investigate the biological activities of **1**, such as DNA binding, DNA cleavage, cell cycle analysis, cell apoptosis, measurement of mitochondrial transmembrane potential, several indexes were employed. Most importantly, our current results showed the potential mechanisms of action of **1** conducting on cell proliferation and apoptosis of MGC-803 cells.

Experimental

Reagents and instrumentation

All chemicals and reagents were purchased from commercial sources and were all used as received without further purification unless noted specifically. Taurine, imidazole and salicylaldehyde were purchased from Alfa Aesar Chemicals Co. (USA). Hoechst 33258, JC-1, Ethidium bromide (EB), 3-(4,5-dimathylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), Propidium iodide (PI), calf thymus DNA (CT-DNA), AO/EB (Acridine orange/Ethidium bromide), RNase A and plasmid pBR322 DNA were purchased from Sigma Chemicals Co. (USA). The Tris–HCl buffer solution was prepared with triple-distilled water. Fetal bovine serum (FBS) and RPMI 1640 were obtained from Hyclone (USA). A Tris-buffer solution of CT-DNA gave a ratio of 1.8–1.9 of UV absorbance at 260 and 280 nm, indicating that the DNA was sufficiently free of protein [28]. The DNA concentration per nucleotide in base pairs was determined spectrophotometrically by employing a molar absorptivity (6600 M^{-1} cm^{-1}) at 260 nm [29]. CT-DNA stock solution was prepared by diluting DNA to Tris–HCl/NaCl buffer (pH = 7.2, 5 mM Tris–HCl, 50 mM NaCl).

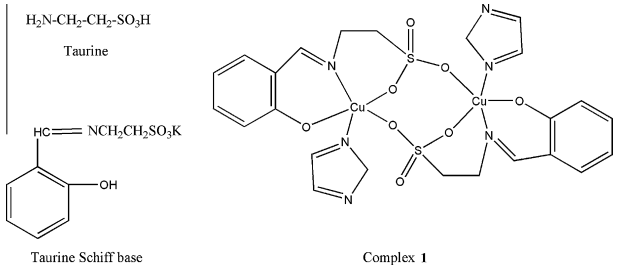


Fig. 1. Chemical structures of taurine, taurine Schiff base and **1**.

Elemental analyses (C, H, N, S) were carried out on a Perkin Elmer Series II CHNS/O 2400 elemental analyzer. ESI-MS (electrospray ionization mass spectrum) spectra were recorded on a Bruker HCT Electrospray Ionization Mass Spectrometer. UV–vis absorption spectra were performed on a Varian Cary100 UV–vis spectrophotometer. Infrared spectra were obtained on a PerkinElmer FT-IR Spectrometer. Fluorescence measurements were obtained by using a Shimadzu RF-5301/PC spectrofluorophotometer. The circular dichroic spectra of CT-DNA were performed on a JASCO J-810 automatic recording spectropolarimeter operating at 25 °C. The fluorescence microscope (Nikon MF30 LED, Japan). Flow cytometry using a BectoneDickinson FACSCalibur.

*Synthesis of **1***

Salicylaldehyde (122.12 mg, 1 mmol) was slowly added to a solution containing KOH (56.10 mg, 1 mmol) and taurine (125.15 mg, 1 mmol) in MeOH (30 mL), and the mixture was stirred for 4 h at 50 °C. Then a solution of $Cu(OAc)_2{\cdot}H_2O$ (199.65 mg, 1 mmol) in H_2O (8 mL) was added, still stirred for 2 h, and then imidazole (68.07 mg, 1 mmol) was added and refluxed for 4 h, resulting in a dark green solution. It was then filtered to discard any insoluble precipitates. The clear filtrate was left to stand at room temperature for slow evaporation, and the dark green crystals of **1** suitable for X-ray analysis were collected after several days. Yield: 419 mg, 58.4%. Selected IR (KBr cm^{-1}): 3151 (Ar—H), 1628 (C=N), 1541, 1471, 1451, 1251 (SO_3^-), 1151 (SO_3^-), 1033, 758; UV–vis in DMSO, λ nm (ε M^{-1} cm^{-1}): 357 (7624), 267 (26,083); Elemental analysis (%): calc. for $C_{24}H_{26}Cu_2N_6O_8S_2$: C, 40.13; H, 3.62; N, 11.70; S, 8.92. Found: C, 40.47; H, 3.58; N, 11.60; S, 8.31. ESI-MS (in DMSO): m/z 716.9, 650.9, 588.0, 640.9, 642.9, 652.9.

X-ray crystallography

Crystal data were collected at 25 °C on a Bruker Smart Apex II CCD diffractometer equipped with graphite monochromated Mo Kα radiation (λ = 0.71073 Å). The structure was solved by direct methods and refined with SHELX-97 programs [30]. The non-hydrogen atoms were located in successive difference Fourier synthesis. The final refinement was carried out by full-matrix least-squares methods with anisotropic thermal parameters for non-hydrogen atoms on F^2 [30]. The hydrogen atoms were added theoretically.

DNA-binding and cleavage experiments

The 2.1 mM CT-DNA stock solution was stored at 4 °C for no more than 4 days before use. **1** was prepared as 2 mM DMSO stock solution and was diluted by Tris–HCl/NaCl buffer for DNA binding studies.

To investigate the binding affinity between CT-DNA and **1**, absorption spectra titrations were carried out in Tris–HCl/NaCl buffer at room temperature by maintaining **1** concentration as