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Full Length Article

New zinc(II) N4 tetradentate Schiff base complex: A potential cytotoxic metallodrug and simple precursor for the preparation of ZnO nanoparticles

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ABSTRACT

A novel Schiff base Zn(II) complex using a tetradentate Schiff base ligand was synthesized and characterized. The results of cytotoxicity assay revealed that the prepared complex induced cytotoxicity in a breast cancer cell line. Thus, the binding of the Zn(II) complex to human serum albumin (HSA) was investigated using spectroscopic and molecular docking methods. The fluorescence data showed that the Zn(II) complex quenched protein fluorescence by a static quenching mechanism. The binding constant (K_b), number of binding sites (n), and thermodynamic parameters were calculated and showed that the Zn(II) complex binds to HSA through hydrogen bond and Vander Waals interactions with one binding site. Protein-ligand docking analysis confirmed that the Zn(II) complex binds to residues located in the subdomain IIA of HSA. This Zn(II) complex was used for the preparation of ZnO nanoparticles, and their photocatalytic activities were examined via photocatalytic degradation of ethidium bromide (EBr) in the absence of UV–vis irradiation. The results showed that these nanoparticles are promising materials for photocatalytic degradation.

1. Introduction

Schiff base ligands, which can form stable coordinate bonds with metal ions via imine nitrogen and other groups, have received considerable attention in recent years [1,2]. Studies have demonstrated that transition-metal complexes of Schiff base ligands function as excellent homogeneous- and heterogeneous-phase catalysts [3], precursors for electrocatalytic processes [4,5], and chemical sensors [6]. These compounds exhibit biological activity, with anticancer, antifungal, antibacterial, antiviral, and anti-parasitic properties [7,8]. Furthermore, a broad variety of Schiff base ligands and their complexes can be used in metal bio-site modelling, models of reaction centers of metalloenzymes, nonlinear optical materials, and luminescence reagents. Symmetrical Schiff bases, particularly tetradentate species with four donor heteroatoms, have been extensively studied due to their ability to stabilize many different metals in various oxidation states [9]. Diamino tetradentate Schiff base ligands and their complexes have also been used as biological models in studies of the structure of biomolecules and biological processes [10].

To design effective chemotherapeutic agents and better anticancer drugs, an understanding of the interaction between small molecules and bio-macromolecules, such as DNA and albumin, is very important. Human serum albumin (HSA) is a transporter for different types of endogenous and exogenous compounds, such as drugs, fatty acids, and dyes in the bloodstream. Binding between HSA and small molecules, such as drugs, can significantly affect the absorption, distribution, metabolism, and toxicity of drugs and may change the functions and structures of HSA [11]. Thus, studies of the interaction between HSA and small molecules are not only important to comprehend their transport and metabolism in the body but also to provide insight the toxicity of drugs at the molecular level [12–16].

Recently, the synthesis of nanoparticles with inorganic compounds has attracted attention [17]. Reducing the size of particles to the nanometer scale results in different and interesting properties compared with bulk properties [18]. Due to their large surface area, metal oxide and hydroxide nanoparticles have many benefits as compared with conventional materials and can be used in various applications. For example, ZnO nanoparticles have been used in the treatment of diseases, including cancer, as well as against bac-

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terial infections in agriculture [19]. They also have uses in water treatment and in cosmetics [19].

In the present paper, we report the synthesis and characterization of a novel tetradentate N_4 donor Schiff base ligand and its Zn(II) complex. *In vitro* cytotoxic effects of the complex on a breast cancer MCF-7 cell line were examined using an MTT assay and showed that this complex induced cytotoxicity in the above-mentioned cell line. The interaction of the Zn(II) complex with HSA was investigated by various spectroscopic and molecular modeling methods. The Zn(II) complex was used as a simple precursor for the synthesis of ZnO nanoparticles. The photocatalytic activity of these nanoparticles was examined via degradation of ethidium bromide (EBr) in the absence of ultraviolet irradiation.

2. Experimental

2.1. Materials and methods

Diacetyl monoxime, $ZnCl_2$, 2,2-dimethyl-1,3-propanediamine and Tris-HCl buffer were purchased from Merck (Germany). Human serum albumin and ethidium bromide obtained from Aldrich (England). Other used chemicals and solvents were of high purity and used without any further purification.

^{1}H NMR spectra were measured on a Bruker DRX-500 Avance spectrometer at 500 MHz, using TMS as the internal reference in DMSO-*d6*. Infrared spectra (4000–400 cm^{-1}) were determined with KBr disks on a JASCO-460 plus FT-IR spectrophotometer. Electronic absorption spectra were recorded on a JASCO UV/Vis-7850 double-beam spectrophotometer using quartz cells with a path length of 1.0 cm. Elemental analysis (C, H and N) was performed by a Leco, CHN-932 elemental analyzer. Conductivity measurements of the above Zn(II) complex was carried out on a Systronics Conductivity Bridge 305, using a conductivity cell of cell constant 1.0. SEM images of ZnO powders prepared in the present work were recorded using scanning electron microscope (TESCAN Vega Model). Crystal structures of ZnO nanoparticles were analyzed by Powder X-Ray Diffractometer (Bruker AXS-D8 Advance).

2.2. Synthesis and characterization of Zn compounds

2.2.1. Schiff base ligand

The ligand (L) was prepared by condensation of 2,2-dimethyl-1,3-propanediamine (10 mmol, 1.2 mL) and diacetyl monoxime (20 mmol, 2.02 g) in methanol (30 mL) under reflux for 3 h. The color changes from colorless to clear yellow occurred but no precipitate was obtained. After all of the solvent was evaporated at 35–40 °C, the desired product was collected and washed with methanol and acetone, and dried at 50 °C. The product was recrystallized from methanol. Anal. Calculated for $C_{13}H_{24}N_4O_2$ (268.08): Calc. C, 58.20; H, 8.95; N, 20.89%. Found. C, 57.97; H, 8.65; N, 21.11%. ^{1}H NMR (500 MHz, DMSO-*d6*): 0.67–1.73 (12H, $4CH_3$ oxime), 1.76–2.33 (6H, $2CH_3$ diamine), 2.55–2.59 (4H, $2CH_2$ diamine), 11.01 (2H, 2NOH oxime) (Fig. 1S). FT-IR (KBr, ν/cm^{-1}): 3285 [ν(OH) of oxime], 1617 [ν(C=N) of Schiff-base], 1485 [ν(C=N) of oxime], 1183 [ν(N—O)] (Fig. 3SA).

2.2.2. Zn(II) complex

Methanol solution (10 mL) containing $ZnCl_2$ (10 mmol) was added to methanol solution (30 mL, 10 mmol) of the Schiff base ligand with constant stirring for 4 h. A yellow precipitate separated, which was filtered, washed with methanol and few acetone, and finally dried in desiccator. Yield (2.71 g, 67%) with a melting point of 209–211 °C, Anal. Calculated for $C_{13}H_{23}N_4O_2Cl_2Zn$ (404.3): Calc. C, 38.58; H, 5.68; N, 13.85%. Found. C, 37.98; H, 5.77; N, 13.43%. ^{1}H NMR (500 MHz, DMSO-*d6*): The spectra of the Zn(II) complex show a broad resonance at δ 11.42 ppm due to the H_d (2H). Four singlets due to the H_a (12H) appear at δ 0.65–1.10, two singlets at δ 1.91–1.95 and a multiplet at δ 2.26–2.49 are assigned to H_c (4H) and H_b (6H), respectively (Fig. 2S). The downfield shift of 0.41 ppm related to oxime hydrogen atom as compared to the free Schiff base ligand indicates the coordination of ligand to Zn(II) and is consistent with the IR spectral data. The IR spectra of Zn(II) complex is shown in Fig. 3SB. In the IR spectrum of ligand (Fig. 3SA) two bands in the 1617 cm^{-1} and 1485 cm^{-1} regions are correspond to the ν(C=N) stretching vibrations of Schiff-base and oxime groups, respectively. These bands are shifted to higher wavenumbers (1629 and 1488 cm^{-1}, respectively) after complexation with Zn(II) atom (Fig. 3SB). Also, the infrared spectrum of complex exhibits a broad band at 3314 cm^{-1}, due to oxime ν(OH) stretching vibrations of Shiff base ligand and a band at 1197 cm^{-1} due to the N—O stretching vibration in this complex. The electronic spectra of this complex exhibit three bands (Fig. 4S). The bands at 234 (logε = 3.21) and 273 nm (logε = 2.09) assigned to intraligand $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the azomethine C=N bond [20] and the band at 349 nm (logε = 3.07) assigned to metal to ligand charge transfer transition [21,22].

2.2.3. ZnO nanoparticles

The above Zn(II) complex was used as a new precursor for preparation of the ZnO nanoparticles. These nanoparticles were obtained by subjecting 0.30 g of the as-prepared Zn(II) complex, powder to heat treatment at a relatively low temperature (400 °C) in air. An average temperature increase of 10 °C every minute was selected before the temperature reached 400 °C, and after keeping the thermal treatment at 400 °C for 4 h, the white powder was allowed to cool to room temperature naturally. Furthermore, the same experiment was done at 600 °C for 2 h. The ZnO nanoparticles sample showed a weak IR band at 443 cm^{-1} and shoulders at 539 cm^{-1} (Fig. 3SC). Fig. 1A and B show the SEM images of the ZnO nanoparticles which obtained by calcination Zn(II) complex at 400 °C for 4 h and 600 °C for 2 h. These figures confirm that prepared Zn(II) complex can act as a precursor for preparation ZnO nanoparticles. The particle size is increased with an increase in the calcination temperature. Also, Fig. 5S shows the particle-size histograms of the ZnO nanoparticles; the average particle size is calculated to be ~98 nm. To confirm purity of the products the chemical compositions of ZnO nanoparticles was analyzed using Energy-dispersive X-ray spectroscopy (EDX). The EDX patterns of nanoparticles are shown in Fig. 6S and the related data are listed in Table 1S. The results verify the high purity of the nanoparticles.

Fig. 1C shows the XRD patterns of the as-obtained nanoparticles. All the diffraction peaks can be well indexed to the hexagonal phase ZnO reported in the JCPDS. The results indicate that the samples consist of pure phase and no characteristic peaks were observed for other impurities. The narrow width of the peaks confirms that above sample is of high purity.

2.3. In vitro *cytotoxicity analysis by MTT assay*

Human breast cancer MCF-7 cells were grown on the DMEM medium supplemented with L-glutamine (2 mM), streptomycin and penicillin (5 μg/mL) under 5%: 95% CO_2: air atmosphere. The harvested cells were seeded into a 96-well plate (1×10^4 cell/mL) and were left to adhere overnight. Then, cancer cells were incubated with various concentrations of synthsized Zn (II) complex (0–250 μM) and incubated for 24 h. Four hours to the end of the incubation, 25 μL of the MTT solution (5 mg/mL in PBS) was added to each well containing fresh and cultured media. At the end, the insoluble formazan produced was dissolved in a solution containing 10% SDS and 50% DMF (left for 2 h at 310 K in darkness) and optical density (OD) was read against reagent blank with a multiwell scanning spectrometer (ELISA reader, Model Expert 96, Asys