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Magnetic property, DFT calculation, and biological activity of bis[(μ^2-chloro)chloro(1,10-phenanthroline)copper(II)] complex

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ABSTRACT

The dinuclear complex bis[(μ^2-chloro)chloro(1,10-phenanthroline)copper(II)] (**1**) was synthesized, and characterized by X-ray, FTIR and thermal analysis. The fitting of magnetic susceptibility and magnetization curve of (**1**) indicates the occurrence of weak antiferromagnetic exchange interaction between copper(II) ions. The electronic structure has been also determined by density functional theory (DFT) method. Complex (**1**) displayed potent anticancer activity against B16 (Melanoma), MDA-MB-32 (Breast Adenocarcinoma), A549 (Lung Adenocarcinoma), HT-29 (Colon Adenocarcinoma) and SF (Astrocytoma) cell lines with an average IC_{50} value of 0.726 μg/ml compared to 4.88 μg/ml for cisplatin. Complex (**1**) has a better therapeutic index and toxicological profile than cisplatin, and has demonstrated a potential chemotherapeutic property.

1. Introduction

Metal-based inorganic complexes have gained popularity in the pharmaceutical industry in the past three decades as new chemotherapeutic drugs [1]. The discovery of *cis*-diammine-dichloroplatinum(II), known as cisplatin, was a breakthrough in cancer therapy in the 20th century. It is highly effective in treating a variety of cancers, especially testicular cancer, with a remission rate exceeding 90% [2]. However, cisplatin suffers from severity of side effects such as nephrotoxicity and neurotoxicity [3]. This drawback has stimulated an extensive search for safer alternative metal based antitumor inorganic complexes, with broader spectrum and improved clinical efficacy [4].

Copper complexes are important non-platinum anti-tumor agents for several reasons [5,6]. Copper is a trace element found in all living organisms. It plays a pivotal role as a cofactor for several enzymes and proteins which are involved in energy metabolism, respiration and DNA synthesis [7]. Researchers assumed that the essential element copper is less toxic than the non-essential metal platinum [8]. Several copper complexes with various ligands and donor atoms were synthesized. Among these, copper phenanthroline complexes were found to be several times more cytotoxic than cisplatin against some cervical cancer cell lines [9–11]. Such complexes were reported to inhibit the growth of PC3 human prostate cancer cells and HL-60 human myeloid leukemia cancer cells [12]. Reports on cytotoxicity of copper complexes have shown that the mechanism of action is based on intercalation and cleavage of DNA [13,14] or apoptosis induction [15,16].

On the other hand, organic–inorganic hybrid copper halides have received extensive attention in recent years owing to their electrical, magnetic and optical properties and due to their importance in areas of molecular adsorption and catalysis [17–21]. The copper halides complexes with N-containing organic ligands exhibited one dimensional (1D) chain [22,23] and two dimensional (2D) layer structures [24,25]. The basic copper halide skeletons exhibit several geometrical motifs: cyclic Cu_2X_2 dimeric [21,25], trinuclear Cu_3X_3 [21], cubanetetrameric [26], stair step oligomeric [25], zigzag polymeric [26], and hexagonal $[Cu_3Cl_3]$ 1D polymeric [27] structures.

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The present study aims to elucidate the synthesis and characterization of a dinuclear complex (1) bis[(μ^2-Chloro)chloro (1,10-phenanthroline)copper(II)], [CuCl(phen)μ-Cl]$_2$, determines its magnetic and thermal properties [28–30], study its electronic structure by DFT calculation [31–33] and evaluate its *in vitro* cytotoxic activity against several human cancer cell lines [6,34].

2. Materials and methods

2.1. Physical measurements

Infrared spectra were recorded on a Jasco FTIR 410 instrument by using pressed KBr plates in the range 500–4000 cm^{-1}. Thermogravimetric – differential scanning calorimetry (TG-DSC) curves were recorded on a Setaram Labsys thermal analyzer, at a heating rate of 3°C/min, in the 25–800 °C temperature range, and under nitrogen flow. The magnetic susceptibility of crystalline samples was measured on an Oxford Maglab 2000 magnetic measurement system. Diamagnetic corrections were applied using Pascal's constants. X-ray Analysis was done with a Bruker X8 APEX single-crystal diffractometer using Graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) and Bruker APEX II software suite.

2.2. Synthesis of bis[(μ^2-Chloro)chloro(1,10-phenanthroline)copper(II)] (**1**) [CuCl(phen)μ-Cl]$_2$

Blue powder of complex (1) was obtained by adopting the following experiment: Acetonitrile (30 mL) was added onto $CuCl_2{\cdot}2H_2O$ (1.03 gm, 6.04 mmol) and 1,10-phenanthroline (1.09 gm, 6.06 mmol). The reaction mixture was stirred for 5 h at room temperature. The blue powder precipitate was then collected by filtration and washed with acetonitrile giving 1.68 g of compound (1) (% yield = 88.4%). Crystals of [CuCl(phen)μ-Cl]$_2$ (**1**) has been obtained by the reaction of copper(II) chloride hydrate and phenanthroline ligand as described previously [35,36,21,37]. The crystal were analyzed by single crystal X-ray analysis confirming the formula of complex (**1**). The structural data was summarized on a cif mercury file.

2.3. DFT Computational details

The electronic structure has been determined by the density functional theory (DFT) method. DFT method has shown to be sufficient for geometry optimization and calculation of spectral properties of large molecules [31–33]. The geometry of complex (**1**) has been optimized using B3LYP density functional model [38]. In these calculations, we used the 3-21G* basis set for C and H atoms and the 6-31G* basis for N atoms. For Cu and Cl atoms, the LanL2DZ valence and effective core potential functions were used [39]. All DFT calculations were performed using Gaussian 98 R-A.9 package [40]. X-ray structures were used as input geometries when available.

2.4. Biological activity

2.4.1. Cell culture

B16 (Melanoma), MDA-MB-32 (Breast Adenocarcinoma), A549 (Lung Adenocarcinoma), HT-29 (Colon Adenocarcinoma) and SF (Astrocytoma) cell lines were grown in Dulbecco's modified Eagle's medium (DMEM, Sigma), supplemented with 10% fetal bovine serum and 0.5% penicillin–streptomycin (10,000 units penicillin and 10 mg streptomycin/mL, Sigma). The plates were maintained at 37 °C in a humidified incubator containing 5% CO_2.

Freshly isolated human bone marrow stem cells were grown in Roswell Park Memorial Institute medium (RPMI, Sigma) supplemented with 10% fetal bovine serum, 0.5% penicillin–streptomycin (10,000 units penicillin and 10 mg streptomycin/mL, Sigma) and maintained in an incubator containing 5% CO_2 at 37 °C.

2.4.2. Cell proliferation assay

The cells were harvested by trypsin to obtain a single-cell suspension, counted using Trypan-blue and diluted in the respective media. A volume of 100 μL of 10^5 cells/mL was plated in a 96-well plate and incubated for 24 and 48 h. Cells were then treated with various concentrations (0.5, 1.0, 2.5 and 5.0 μg/ml) of complex (**1**) in DMSO. Cisplatin (Ebewe Pharma, Austria) was used as a positive control throughout the experiments.

Cell proliferation was measured after 24 and 48 h of incubation. Cell viability was measured by water-soluble tetrazolium salt-1 (WST-1) a cell proliferation reagent (Roche Applied Science Penzberg, Germany). This salt is cleaved by mitochondrial dehydrogenases in metabolically active cells, forming formazan. Formazan formation was quantified spectrophotometrically at 440 nm using a microplate reader (Thermo Scientific – Multiskan FC). Experiments were done in triplicates for all cell lines.

2.4.3. Determination of median lethal dose (LD_{50})

The LD_{50} of complex (**1**) and cisplatin was determined using the Dixon's up and down method (Dixon, 1965). Adult male Wistar rats weighing 200–250 g were housed at a constant temperature (22 ± 2 °C) and light (12:12 h light:dark) controlled room. A solution of complex (**1**) was prepared in DMSO to reach the test dose concentration. A single dose of the test chemical was administered intra-peritoneally to 6 rats. The survival or death of animal at the used concentration determine the next dose to be either increased or decreased. Survival was depicted by "o" and death as "x" and the score was made accordingly. The animals were observed after 48 h for survival. Dixon's formula was then applied in order to determine the LD_{50}. The formula used the logarithmic (log) value of the doses administered in mg/kg body weight and is depicted by 'Log (LD50) = $X_f + kd$', where X_f is the last dose administered, k is the tabular value and d is a constant equal to the interval between doses.

3. Results and discussion

3.1. Structure of complex (**1**)

The structure of complex (**1**) was determined by single X-ray diffraction. The basic unit structure is presented in Fig. 1. The solid-state structure of complex (**1**) consists of centrosymmetric

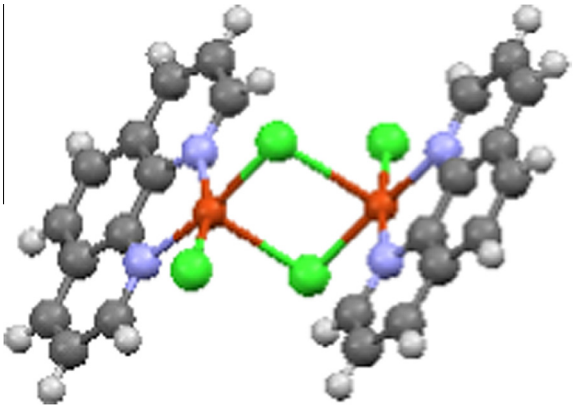

Fig. 1. Ortep figure of the crystal structure of complex (**1**) [CuCl(phen)μ-Cl]$_2$.