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Synthesis and characterization of ruthenium(III) complex containing 2-aminomethyl benzimidazole, and its anticancer activity of *in vitro* and *in vivo* models

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ABSTRACT

A novel ruthenium(III) complex with 2-aminomethyl benzimidazole (AMBI) has been synthesized and characterized by elemental analysis, spectroscopic (FTIR, UV–Vis and XRD), thermal, magnetic and electrochemical techniques. Characterization shown that the ruthenium ion is octahedral coordinated by three H_2O molecules, one chloride ion and a bidentate AMBI. The calf thymus DNA binding activity of complex was studied by absorption spectra and viscosity measurements. The strong interaction between Ru(III) complex and CT-DNA was investigated and the K_b value equals 8.3×10^{-5}. The cytotoxicity effect of water soluble $[RuCl(AMBI)(H_2O)_3]Cl_2$ complex on breast adenocarcinoma (MCF-7) and human colon carcinoma (HCT-116) cell lines (*in vitro*) was investigated by the MTT method. The Ru(III) complex shows good cytotoxic activity and the IC_{50} values are 57.20 and 18.08 μg/mL toward MCF-7 and HCT-116, respectively. The antiproliferative effect of Ru(III) complex on HCT-116 cells was nearly as the cisplatin value; that indicates its efficiency as anticancer agents. Apoptosis was studied by AO/EB staining and the Ru(III) complex shows apoptotic effect against the two cancer cell lines by percent to 23.1 and 33.1%. DNA damage assay by gel electrophoresis was done and the DNA fragmentation that appears in the two treated lanes indicates that there was destruction in the nuclear DNA. The cell cycle arrest by flow cytometry showed that the Ru(III) complex stops the two cancer cell lines proliferation by decrease the S and G0/G1 phases and increase in G2/M indicates that the interactions of our complex with DNA prevents the entry of cells into the DNA synthesis phase as well as into a new cell cycle. Moreover, the Ru(III) complex stops the proliferation of Ehrlich ascites carcinoma (EAC) bearing female mice (*in vivo* study) with low toxicity to the kidney. In addition, the antioxidant enzymes' activity in the treated and untreated groups' blood samples were also investigated. The decrease in topoisomerase I (*Top I*) levels in treated mice groups than the EAC group indicates the effect of our compound as topoisomerase I inhibitor.

1. Introduction

Cancer is a devastating disease that strikes different people all over the world. Breast cancer is the most second common cancer worldwide after lung cancer [1]. Delay screening with late diagnosis of breast cancer leads to metastasis all over the body causing death [2]. Cisplatin is used clinically in the treatment of a variety of cancers; but with long times of treatment, it causes nephrotoxicity, hepatotoxicity and drug resistance [3]. These side effects encourage the scientists to invent new anticancer drugs with less toxicity like ruthenium complexes. The new anticancer drugs based on ruthenium metal take large attention because the coordination and redox properties of ruthenium metal [4]. Chemical properties of ruthenium metal make it a promising anticancer drug to be used as a core with certain ligand complexes [5]. Recent studies have used Ru(III) complexes as an anticancer drug against different human cell lines (*in vitro*) with a great bioactivity of these complexes [6,7]. Unlike cisplatin; the *in vivo* studies of ruthenium complexes proved that they have minimal kidney toxicity that can be reversed after quitting [8]. Minor number of Ru(III) complexes get into pre-clinical trials [9,10] as an anti-breast cancer [11,12], but still there was a lack in *in vivo* studies with the development of safer, water soluble Ru(III) complexes.

Benzimidazoles ligand has the structure similar to purine base of the DNA and the nucleus of vitamin B12 [13]. That similarity allows the biological systems to recognize the benzimidazole ring without any sign of allergy [14]. Also, it shows anti-inflammatory, DNA cleaving properties and anticancer activities against various cancer cell lines alone and

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with metal chelators such as ruthenium(I), iron(II), platinum(II) and zinc(II) [15–17]. Benzimidazole compounds were also reported as topoisomerase I, tyrosine kinase and serine protease inhibitors [18]. Different complexes of transition metals with 2-substituted benzimidazole were proven to be effective as anticancer [19]. Some benzimidazole derivatives show diverse biological activities in the treatment of leukemia and cancer. In addition, the stability of the 2-aminomethyl benzimidazole due to aromatic ring π-π stacking effects made it promising to be the choice ligand for ruthenium(III). The innovation of new anticancer drug design with special properties is currently needed in clinical fields. Therefore, this study aimed to synthesize a safe and effective anticancer drug with ruthenium core by selecting a heterocyclic ligand with known safety and anticancer activity like the benzimidazoles ring structure. The calf thymus DNA binding activity of water soluble $[RuCl(AMBI)(H_2O)_3]Cl_2$ complex was studied by absorption spectra and viscosity measurements. MCF-7 cells (breast adenocarcinoma) are considered a violent drug resistant cancer cells. The chemotherapeutic drug that can affect these cells will consider a potent anticancer. Thus, we examine the cytotoxic effect of our complex on MCF-7 as well as on HCT-116 (human colon carcinoma) cell lines (*in vitro*). Then, we study the apoptotic analysis and cell cycle arrest of our complex on the treated cell lines. Besides its effect on Ehrlich ascites carcinoma (EAC) transplanted in female mice as a mammary adenocarcinoma model (*in vivo* study) with the toxicity profile of this complex on different mice organs in treated and healthy mice. Finally, the topoisomerase I level was determined in all mice groups.

2. Materials and methods

2.1. Chemicals and reagents

Hydrated ruthenium trichloride ($RuCl_3 \cdot 3H_2O$/10452), 2-aminomethyl benzimidazole dihydrochloride (AMBI/165638), tetrabutylammonium hexafluorophosphate (TBHFP/281026), acridine orange (AO/A6014) and ethidium bromide (EB/E7637) were obtained from Sigma-Aldrich and used without further purification. Other chemicals and solvents were obtained from Fluka and used as received. Serum glutamate-pyruvate transaminase (SGPT), serum glutamate-oxaloacetate transaminase (SGOT), albumin, total bilirubin, creatinin, urea and uric acid kits were from Spinreact, Spain. Super oxide dismutase (SOD) kit, malondialdehyde (MDA) kit, reduced glutathione (GSH), catalase (CAT) and total antioxidant capacıty (TAC) kits were from Biodiagnostic, Egypt. Hemoglobin kit was from Randox Laboratory Ltd., UK. White blood cells count reagent was from Egyptian Diagnostic Media Co., Egypt.

2.2. Instrumentation

Microanalytical data (C, H and N) were collected on Automatic Analyzer CHNS Vario ELIII, Germany. Infrared spectra (KBr discs, 4000–400 cm^{-1}) were recorded on Jasco FTIR-4100 spectrophotometer. UV–Visible spectra were recorded by Perkin-Elmer AA800 spectrophotometer Model AAS. Fluorescence microscopy BK6000, China. Magnetic susceptibility measurements were determined at room temperature on a Johnson Matthey magnetic susceptibility balance using $Hg[Co(SCN)_4]$ as calibrant. X-ray diffraction analysis of compound in powder forms were recorded on X-ray diffractometer in the range of diffraction angle $2\theta = 10–80°$. This analysis was carried out using CuKα radiation ($\lambda = 1.540598$ Å). The applied voltage and the tube current are 40 kV and 30 mA, respectively. Scherer's equation [20] calculates the average crystallite size, t, using the Eq.: $t = 0.95\lambda\ \gamma/\cos\ \Theta$, where γ is the width measured in radians of the half-maximum peak intensity, λ is the X-ray wavelength and Θ is the Bragg's angle. Thermal analysis of $[RuCl(AMBI)(H_2O)_3]Cl_2$ complex was carried out using a Shimadzu thermogravimetric analyzer under a nitrogen atmosphere with heating rate of 20 °C/min over a temperature range from room temperature up to 1000 °C. Conductivity measurements of the complex at 25 ± 1 °C were determined in water (10^{-3} M) using conductivity/TDS meter model Lutron YK-22CT. The electrochemical behavior of the complex was studied using an electrochemical analyzer CHI 610A (HCH Instrument) under nitrogen atmosphere and at room temperature. The electrochemical cell that was used in this work contains three electrodes: platinum wire was used as a working electrode, Ag^+/AgCl was used as a reference electrode and a platinum wire was used as a counter electrode.

2.3. Synthesis of $[RuCl(AMBI)(H_2O)_3]Cl_2$ complex

2-aminomethyl benzimidazole (0.440 g, 2 mmol) was dissolved in 20 cm^3 of ethanol. $RuCl_3 \cdot 3H_2O$ (0.261 g, 1 mmol) was dissolved and refluxed in ethanol (20 cm^3) until the initial black color turned into green. The aqueous of the ligand previously prepared was added to this green solution, then the reaction mixture was refluxed for 3 h. As a result of refluxing green microcrystals was formed which were collected by filtration using filtered glass gooch, washed with ethanol (10 cm^3) and dried in a vacuum desiccator over anhydrous $CaCl_2$. The analytical data for that complex are well agreed with its formula. Anal. Calcd. for $C_8H_{15}Cl_3N_3O_3Ru$ (%): C, 23.91; H, 3.73; N, 10.70. Found (%): C, 23.71; H, 3.70; N, 10.55.

$$RuCl_3 \cdot 3H_2O + AMBI \rightarrow [RuCl(AMBI)(H_2O)_3]Cl_2$$

2.4. DNA binding

The binding properties of complex to calf thymus DNA have been studied using electronic absorption spectroscopy. The stock solution of CT-DNA was prepared in (1×10^{-3} M) Tris–HCl/50 mM NaCl buffer (pH 7.2), which a ratio of UV absorbances at 260 and 280 nm (A_{260}/A_{280}) of CT-DNA 1.8–1.9, indicating that the DNA was sufficiently free of protein [21], and the concentration was determined by UV absorbance at 260 nm ($\varepsilon = 6600\ M^{-1}\ cm^{-1}$) [22]. Electronic absorption spectra (200–700 nm) were carried out using 1 cm quartz cuvettes at 25 °C by fixing the concentration of complex (3×10^{-4} M), while gradually increasing the concentration of CT-DNA (0.00–1.15×10^{-4} M). An equal amount of CT-DNA was added to both the complex solutions and the references buffer solution to eliminate the absorbance of CT-DNA itself. The intrinsic binding constant K_b of the complex with CT-DNA was determined using Eq. (1) [23]:

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_a - \varepsilon_f) \quad (1)$$

where [DNA] is the concentration of CT-DNA in base pairs, ε_a is the extinction coefficient observed for the A_{obs}/[complex] at the given DNA concentration, ε_f is the extinction coefficient of the free complex in solution and ε_b is the extinction coefficient of the complex when fully bond to DNA. In plots of [DNA]/($\varepsilon_a - \varepsilon_f$) *vs.* [DNA], K_b is given by the ratio of the slope to the intercept.

2.5. Viscosity measurements

Viscosity measurements were performed at compound concentration within the range of ($0.1–1 \times 10^{-3}$ M) and each compound was added into a DNA solution (1×10^{-3} M) present in the viscometer. The average flow times of three replicates were measured with a digital stopwatch. The data were presented as $(\eta/\eta_o)^{1/3}$ *vs.* [complex]/[DNA] ratio of the concentration of the compound to DNA [24], where η and η_o are the viscosity of the DNA in the presence and absence of complex, respectively [25]. The relative viscosities η were calculated using Eq. (2) [26]:

$$\eta = (t - t_o)/t_o \quad (2)$$

where t is the observed flow time of DNA containing solution and t_o is the flow time of buffer alone.