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DNA- and BSA-binding studies and anticancer activity against human breast cancer cells (MCF-7) of the zinc(II) complex coordinated by 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine

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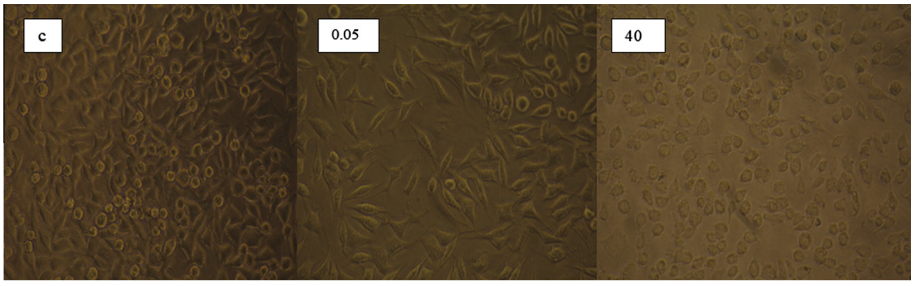
HIGHLIGHTS

- Binding studies of $[Zn(dppt)_2Cl_2]$ (dppt is 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine), with DNA and BSA.
- The intercalative interaction of the complex with DNA.
- A quite strong ability of the complex to quench the fluorescence of BSA.
- The thermodynamic parameters between BSA and the complex were calculated.
- The cytotoxicity of the complex against the human breast adenocarcinoma (MCF-7) cell lines.

GRAPHICAL ABSTRACT



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ABSTRACT

Binding studies of a mononuclear zinc(II) complex, $[Zn(dppt)_2Cl_2]$ (dppt is 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine), with DNA and bovine serum albumin (BSA) have been investigated under physiological conditions. The binding properties of the complex with fish sperm DNA (FS-DNA) have been investigated by UV–Vis absorption, thermal denaturation, competitive DNA-binding studies with ethidium bromide (EB) by fluorescence, and gel electrophoresis techniques. The competitive study with (EB) shows that the complex can displace EB from the DNA-EB system and compete for the DNA-binding sites with EB, which is usually characteristic of the intercalative interaction of compounds with DNA. The value of the fluorescence quenching constant (K_{sv}) was obtained as $3.1 \times 10^4\ M^{-1}$, indicating that this complex shows a high quenching efficiency and a significant degree of binding to DNA.

Moreover, the intercalative binding mode has also been verified by the results of UV–Vis absorption, thermal denaturation and gel electrophoresis. The value of K_b at room temperature was calculated to be $1.97 \times 10^5\ M^{-1}$, indicating that the complex possesses strong tendency to bind with DNA. This value is very greater than to the values obtained for other zinc(II) complexes. The interaction of the complex with BSA has been studied by UV–Vis absorption, fluorescence and circular dichroism (CD) spectroscopic techniques. The results indicate that the complex has a quite strong ability to quench the fluorescence of BSA and the binding reaction is mainly a static quenching process. The quenching constants (K_{SV}), the binding constants (K_b), the number of binding sites at different temperatures, the binding distance between BSA and the complex (r), and the thermodynamic parameters (ΔH^o, ΔS^o and ΔG^o) between BSA and the complex were calculated. The complex exhibits good binding propensity to BSA showing

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relatively high binding constant values. The positive ΔH^o and ΔS^o values indicate that the hydrophobic interaction is main force in the binding of the complex to BSA. Moreover, to evaluate the anticancer properties, the cytotoxicity of the complex has been tested against the human breast adenocarcinoma (MCF-7) cell lines using the MTT assay. The results indicate that the parent complex displays cytotoxicity against human breast cancer cell lines (MCF-7) with an IC_{50} value of 10.44 μM. It is remarkable that the complex can introduce as a potential anticancer drug.

Introduction

In the recent years, the interaction between transition metal complexes with DNA and proteins have been intensively studied [1–5]. In modern medicinal chemistry, transition metal complexes for their binding properties has become suitable candidates as anticancer and antitumor drugs, the most important examples are cisplatin [6], carboplatin, and oxaliplatin [7]. When one compound can be used as drug: firstly, it can bind to carrier proteins in the blood, its solubility increases in the blood plasma and resulting in deliver to its target cells. The most important carrier protein in the blood is serum albumin [8]. Serum albumin has multiple binding sites and is able to interact with drug molecules and form a stable protein–drug complex which could affect the absorption, distribution, activity and toxicity of drugs [9]. Secondly, it arrests the proliferation of the cancer cells. The mechanism of action of metal complexes in cancer cells is that they bind with the DNA of cells and inhibit the division of cancer cells [10,11]. Among three modes of non-covalently bond between metal complexes and DNA, the intercalative binding is stronger than other two binding modes because the surface of intercalative molecule insert between the aromatic and heterocyclic base pairs of DNA [12]. Like intercalators, groove binders also have been used extensively as antitumor, anticancer and antibacterial agents [13].

The effect factors on the medicinal properties of metal complexes are geometry, size and hydrophobicity of the ligands, and also the nature of metal ions [14]. An investigation of new metal complexes by using various metals and ligands is necessary to understand role and importance of the effect factors on the DNA binding. The binding properties of zinc complexes with DNA and BSA have not extensively studied though in the body, Zn^{2+} is the second most abundant transition metal after Fe^{3+} [15], and it plays very important roles in several biological processes [16]. Also, zinc complexes are used for treatment of Alzheimer disease [17], antidiabetic [18], anti-inflammatory [19], antibacterial [20], and anticancer [21,22].

The 1,2,4-triazine derivatives are well-known in the natural materials and they have significant biological and medicinal properties. Some of the 1,2,4-triazine derivatives exhibit antiviral inhibitory, anticancer and anti-HIV activity [23]. One of the important classes of the 1,2,4-triazine derivatives is the 3,5,6-trisubstituted-1,2,4-triazines and 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine (dppt) (Scheme S1 in Supplementary material) is a member of the 3,5,6-trisubstituted-1,2,4-triazines. There is not any report about the interactions of dppt complexes with DNA or BSA and the main aim of this research is to study the bioactivity and anticancer properties of a dppt complex for the first time.

This article reports the DNA- and BSA-binding properties of a mononuclear Zn(II) complex with dppt, $[Zn(dppt)_2Cl_2]$, (Fig. 1). The biological and binding properties of the complex with DNA have been investigated by UV spectroscopy and thermal denaturation experiments. Competitive binding studies with ethidium bromide have been performed by fluorescence spectroscopy in order to investigate ability intercalation of the Zn(II) complex to DNA. In addition, gel electrophoresis assays have been used to investigate the interaction of the complex with DNA. The binding studies of the complex with BSA were studied *in vitro* using UV–Vis absorption, fluorescence (at three different temperatures) and circular dichroism (CD) spectroscopic techniques. Moreover, the *in vitro* cytotoxicity activity of the complex on the human breast (MCF-7) cell lines was evaluated using the MTT assay.

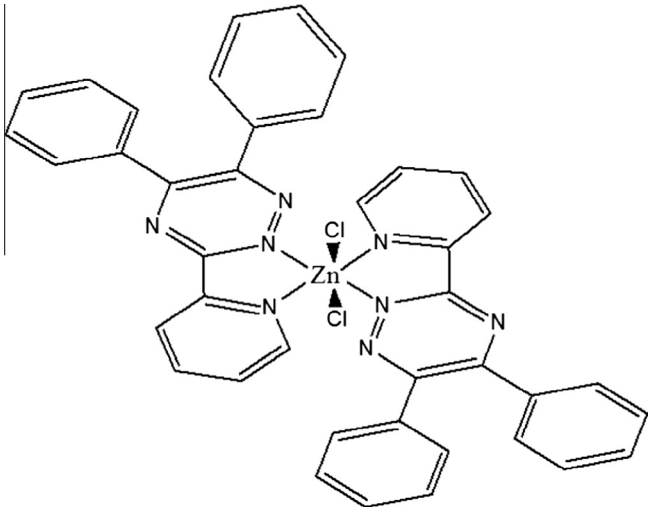

Fig. 1. Structure of $[Zn(dppt)_2Cl_2]$.

Experimental

Materials

All chemicals and solvents were purchased from commercial sources and used as received without further purification. Ethidium bromide and dppt were purchased from Alfa Aesar. Tris–HCl buffer ([tris(hydroxymethyl)-aminomethane]) and BSA were purchased from Merck. FS-DNA was purchased from Acros and its purity was checked by monitoring the ratio of UV absorbance at 260 and 280 nm. The solution gave a ratio 1.89 at A_{260}/A_{280}, indicating that the DNA was sufficiently free from protein contamination [24]. Agarose was purchased from Fermentas. $[Zn(dppt)_2Cl_2]$ was prepared according to the published procedure [25]. Double distilled water was used for preparing all solutions for DNA- and BSA-binding studies.

Apparatus

Electronic absorption spectra were recorded on a Carry 500 UV–Vis spectrophotometer (Varian, USA) using quartz cells with a path length of 1 cm. Fluorescence emission intensity measurements were carried out using a Varian Cary Eclipse spectrophotometer (Varian, USA). CD spectra were recorded on an Aviv Circular Dichroism Spectrometer, model 215 (USA), using a cylindrical cuvette with 0.1 cm path length.