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Synthesis, crystal structure and Hirshfeld surface analysis of copper(II) complexes: DNA- and BSA-binding, molecular modeling, cell imaging and cytotoxicity

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ABSTRACT

In order to explore the structure–activity relationship of complexes, two copper(II) complexes, [Cu(dimethoxybpy)$_2$](PF$_6$)$_2$ (**1**) and [Cu(dimethylbpy)$_2$Cl]PF$_6$ (**2**) (dimethoxybpy is 4,4′-dimethoxy-2,2′-bipyridine and dimethylbpy is 4,4′-dimethyl-2,2′-bipyridine), have been synthesized and characterized by several physicochemical techniques. The single-crystal X-ray structures of **1** and **2** exhibit distorted square-planar and distorted square-pyramidal structure, respectively. The Hirshfeld surface analysis and the associated 2D fingerprint plots of **1** and **2** have been also studied to evaluate intermolecular interactions. The DNA binding properties of **1** and **2** have been investigated by absorption and emission spectra, viscosity, cyclic voltammetry, circular dichroism and competitive DNA–binding studies, which indicate that the complexes interact with DNA through partial intercalation. The results of absorption and emission spectra, synchronous fluorescence and circular dichroism show that the complexes bind with BSA. The results exhibit that the complex **1** has stronger binding ability to DNA and BSA than the complex **2**. Molecular docking technique has been used to evaluate and understand the interaction mode of **1** and **2** with DNA and BSA. The *in vitro* cytotoxicity of the complexes against MCF-7, A-549 and HT-29 cell lines has been assayed by MTT method. The complexes exhibit significant cytotoxicity in cell lines with IC$_{50}$ values ranging from 1.5 to 53 μM. Based on the results of cytotoxicity, it would appear that the complex **2** has better cytotoxicity than the complex **1** under the same experimental conditions, suggesting that the hydrophobicity of methyl groups on the complex enhances the anticancer activity. The results of the microscopic analyses of cancer cells confirm the results of cytotoxicity.

1. Introduction

According to the recent reports, cisplatin is one of the most widely used and world's best-selling metal-based anticancer drugs which binds to DNA and inhibits the division of cancer cells [1,2]. The widespread success of cisplatin in the clinical treatment has placed metal-based drugs in the frontline in the fight against cancer, numerous mononuclear platinum(II) complexes have been synthesized and their anticancer activities carefully evaluated on appropriate biological models [3]. Unfortunately, in contrast to general expectations, most of the platinum(II) complexes investigated so far exhibit biological activities very similar to those of cisplatin thus, with no significant therapeutic advantage. Also, the cancers that can be treated with platinum drugs are very few, and the drugs suffer from side effects and resistance phenomena [1,4]. Therefore, development of new alternative strategies for treatment of cancers is immediately required by finding out new transition metal complexes based on different metals and ligands with an aim of reducing toxicity, enhancing specificity, and thereby enhancing the therapeutic efficacy through noncovalent binding with DNA [2]. However, design concepts for such drugs are still in their primary steps; mainly because an understanding of structure–activity relationships has not yet reached a level that allows providing general rules.

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Much attention has been paid to copper complexes, as the best promising alternatives to platinum drugs as anticancer drugs because of their rich synthetic chemistry, rich variety of coordination complexes from four to six with oxidation states Cu(II) and Cu(I), multiple mechanisms of action that are distinct from those of platinum-based drugs and low systemic toxicity [5]. In contrast to exogenous platinum, copper is found in all living organisms and is a crucial trace element in redox chemistry, growth, and development. However, copper can also be toxic to cells, due to its redox activity and its affinity for binding sites that should be occupied by other metals. Thus, several families of copper complexes have been studied as potential antitumor agents in recent years [5–7]. Copper(II) polypyridyl complexes are an important class of biologically active drugs and very interesting compounds with wide range of biological actions. In recent years many authors have published their findings about these complexes [8,9]. The planner hetrocyclic molecule of 2,2′-bipyridine and its derivatives are a kind of polypyridyl ligands, which plays an important role both chemists and biochemists. Ruiz-Azuara synthesized a class of mixed-chelate, cationic complexes named Casiopeinas having general formula [Cu(4,4′-dimethyl-2,2′-bipyridine)(acetylacetonate)]NO_3 and [Cu(4,4′-dimethyl-2,2′-bipyridine)(glycinato)]NO_3 [10,11]. In other reports, wide range of biological actions of Casiopeinas complexes have been investigated [12,13]. Fei et al. reported synthesis, DNA binding and cytotoxic activity of two novel copper-2,2′-bipyridine complexes [14]. 2,2′-Bipyridine and its derivatives complexes have shown mainly antibacterial activity, good affinity in DNA binding, and anticancer activity against human cancer cells [15–18].

We present in this article the synthesis and characterization of two Cu(II) complexes, [Cu(dimethoxybpy)$_2$]$(PF_6)_2$ (**1**) and [Cu(dimethylbpy)$_2$Cl]PF_6 (**2**). The crystal structures of the complexes have been determined by X-ray crystallography. The biological properties of **1** and **2** have been studied, including: (i) the binding properties of **1** and **2** with DNA by UV–Vis absorption, fluorescence titration, viscosity, cyclic voltammetry (CV) and circular dichroism studies (CD) (ii) competitive binding studies with GelRed (GR) by fluorescence spectroscopy in order to investigate the existence of a potential intercalation of **1** and **2** to DNA, (iii) the binding properties of **1** and **2** with BSA by UV–Vis absorption, fluorescence, synchronous and circular dichroism, (iv) the interaction of **1** and **2** with DNA and BSA by molecular docking, (v) microscopic analyses of the cancer cells treated with **1** and **2** using an inverted microscope without any treatment or staining and (vi) the *in vitro* cytotoxicity activity of **1** and **2** on the human carcinoma cell lines (MCF-7, A-549 and HT-29) by the MTT assay.

2. Experimental

2.1. Materials

All reagents and solvents were purchased commercially and used without further purification unless otherwise noted. Tris (hydroxymethyl)-aminomethane (Tris) and BSA were purchased from Merck. Fish sperm DNA (FS-DNA) and 4,4′-dimethoxy-2,2′-bipyridine were purchased from Acros. Solution of DNA in Tris buffer (5 mM Tris/50 mM NaCl) gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280}, of 1.9, indicating that the DNA was sufficiently free of protein [19]. 4,4′-Dimethyl-2,2′-bipyridine was purchased from Alfa Aesar. GelRed was purchased from Biotium.

2.2. Physical measurements

Elemental analyses were performed using a costech 4010 elemental analyzer. Fourier transform infrared spectra were recorded on a FT-IR Bruker-Tensor 27 spectrometer. Electronic absorption spectra were recorded on a Mecasys Optizen 3220 UV spectrophotometer using quartz cells with a path length of 1 cm. Fluorescence emission intensity measurements were carried out using a Varian Cary Eclipse spectrophotometer. The electrochemical measurements were performed with an Autolab potentiostat/galvanostat. CD spectra were recorded on an Aviv spectropolarimeter, model 215 using a cylindrical cuvette with 0.1 cm path length.

2.3. Syntheses

2.3.1. Synthesis of [Cu(dimethoxybpy)$_2$](PF$_6$)$_2$ complex (**1**)

0.17 g (1 mmol) of $CuCl_2{\cdot}H_2O$ and 0.43 g (2 mmol) of dimethoxybpy were dissolved in 15 mL of ethanol and stirred for 5 h at room temperature. The product was obtained by addition of a saturated aqueous NH_4PF_6 solution. The blue product, [Cu(dimethoxybpy)$_2$]$(PF_6)_2$, was collected by suction filtration, washed with cold water and ether and then air-dried. For further purification, the blue precipitate was recrystallized by slow evaporation of an acetonitrile/dioxane solution of the complex gave shiny blue single crystals suitable for crystallography in a yield of 75%. *Anal.* Calc. for $C_{24}H_{24}CuN_4O_4P_2F_{12}$ (MW = 785.95): C, 36.6; H, 3.05; N, 7.12. Found: C, 36.49; H, 3.25; N, 6.97%. IR (KBr, cm^{-1}): 1615(s) ν(C=N) and 838 (s) ν(P–F); UV–Vis λ_{max}/nm ($\varepsilon_{max} \times 10^3$/mol^{-1} cm^{-1}): 231 (68), 276 (22), 285 (21) and 297 (17).

2.3.2. Synthesis of [Cu(dimethylbpy)$_2$Cl]PF$_6$ complex (**2**)

The complex was prepared using a procedure similar to that for **1** by using dimethylbpy (2 mmol, 0.37 g) instead of dimethoxybpy. The blue product, [Cu(dimethylbpy)$_2$Cl]PF_6, was collected by suction filtration, washed with cold water and ether and then air-dried. For further purification, the blue precipitate was recrystallized by slow diffusion of diethyl ether into an acetonitrile solution of the complex gave shiny blue single crystals suitable for crystallography in a yield of 83%. *Anal.* Calc. for $C_{24}H_{24}ClCuF_6N_4P$ (MW = 612.43): C, 47.02; H, 3.92; N, 9.14. Found: C, 47.26; H, 3.96; N, 9.28%. IR (KBr, cm^{-1}): 3070 (w) ν(C–H_{cycle}), 2924 (w) ν(C–H_{Me}), 1615(s) ν(C=N) and 838(s) ν(P–F); UV–Vis λ_{max}/nm ($\varepsilon_{max} \times 10^3$/mol^{-1} cm^{-1}): 235 (34), 295 (29) and 306 (26).

2.4. X-ray crystallographic procedure

Single crystal X-ray diffraction measurements of **1** were carried out at 295 (2) K on a four-circle KUMA KM4 diffractometer equipped with a two-dimensional CCD area detector. Graphite monochromatised Mo-Kα radiation (λ = 0.71073 Å) and ω-scan technique with step $\Delta\omega = 1^\circ$ were used for data collection and **2** carried out at 294 K on a Bruker APEX-II CCD diffractometer equipped with graphite monochromated Mo Kα radiation (λ = 0.71073 Å. Data collection and reduction of **1** were performed using the *CrysAlis* software package [20] and **2** were performed using the SADABS software [21]. The structures were solved by direct methods using SHELXT program [22] which revealed the positions of almost all non-hydrogen atoms. The remaining atoms were located from subsequent difference Fourier synthesis. The structure was refined using SHELXL-2014/7 program [23] with anisotropic thermal displacement parameters. The hydrogen atoms were refined using the riding model with isotropic thermal displacement parameters 20% greater than aromatic carbon atoms linked directly the H atoms, and 50% greater than for carbon atom of CH_3. Visualization of the structure of **1** was made with Diamond 3.0 program [24]. Molecular plots of **2** were obtained using the ORTEP-3 [25] and SCHAKAL-99 [26] programs.