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Synthesis, molecular structure, biological properties and molecular docking studies on Mn^{II}, Co^{II} and Zn^{II} complexes containing bipyridine–azide ligands

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ABSTRACT

Metal complexes of the type $Mn(bpy)_2(N_3)_2$ (**1**), $Co(bpy)_2(N_3)_2 \cdot 3H_2O$ (**2**) and $Zn_2(bpy)_2(N_3)_4$ (**3**) (Where bpy = 2,2-bipyridine) have been synthesized and characterized by elemental analysis and spectral (FT-IR, UV–vis) studies. The structure of complexes (**1**–**3**) have been determined by single crystal X-ray diffraction studies and the configuration of ligand-coordinated metal(II) ion was well described as distorted octahedral coordination geometry for Mn(II), Co(II) and distorted square pyramidal geometry for Zn(II) complexes. DNA binding interaction of these complexes (**1**–**3**) were investigated by UV–vis absorption, fluorescence circular dichroism spectral and molecular docking studies. The intrinsic binding constants K_b of complexes **1**, **2** and **3** with CT-DNA obtained from UV–vis absorption studies were 8.37×10^4, 2.23×10^5 and 5.52×10^4 M^{-1} respectively. The results indicated that the three complexes are able to bind to DNA with different binding affinity, in the order **2** > **1** > **3**. Complexes (**1**–**3**) exhibit a good binding propensity to bovine serum albumin (BSA) proteins having relatively high binding constant values. Gel electrophoresis assay demonstrated the ability of the complexes **1**–**3** promote the cleavage ability of the pBR322 plasmid DNA in the presence of the reducing agent 3-mercaptopropionic acid (MPA) but with different cleavage mechanisms: the complex **3** cleaves DNA via hydrolytic pathway (T4 DNA ligase assay), while the DNA cleavage by complexes **1** and **2** follows oxidative pathway. The chemical nuclease activity follows the order: **2** > **1** > **3**. The effects of various activators were also investigated and the nuclease activity efficacy followed the order MPA > GSH > H_2O_2 > Asc. The cytotoxicity studies of complexes **1**–**3** were tested *in vitro* on breast cancer cell line (MCF-7) and they found to be active.

1. Introduction

The design and development of small- or medium-sized potential therapeutic agents to target nucleic acid cleavage can lead synthesis of novel therapeutic agents for cancer, viral diseases and can act as tool for molecular biology [1–3]. Small molecules interact with double-stranded DNA in a number of ways [4–7] and in most cases it is main non-covalent binding modes such as, intercalation, major/minor groove binding and electrostatic binding interaction. The rationale of our strategy is to design polypyridyl-metal-azide complexes which prove to possess pronounced biological and better pharmacological activity. Bipyridyl containing azide complexes are well known, but there is no information on the solid state structure versus biological activity. Amongst the metal ions chosen, we have opted for biocompatible endogenous metal ions such as Mn, Co and Zn which play essential role in biological living system [8–10]. In particular, the complexes based on essential metals are less toxic than those with non-essential ones. The most important operation formed by manganese in nature is the photolytic oxidation of water to dioxygen within the oxygen evolving complex (OEC) of photosystem (II) (PSII), found in the photosynthetic apparatus of green plants and certain cyanobacteria [11,12]. It is also a cofactor or required metal ion for many enzymes, such as superoxide dismutase (SOD), glutamine synthetase and arginase [13]. It is known that cobalt(III) azide complexes undergo photoreduction [14,15] and are used for reduction of nitric oxide and nitrous acid [16]. Structurally characterized cobalt complexes have been studied as hydrolytic agents for DNA cleavage [17,18] and others showing antitumor, anti-proliferative [19], antimicrobial

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[20], antifungal [21,22], antiviral [23] and antioxidant activity [24]. Zinc complexes with diverse biological activity viz. antibacterial [25], anti-inflammatory [26], for the treatment of Alzheimer disease [27] and anti-proliferative, antitumor activity [28] have been structurally characterized. The azide-pseudohalide ion has been shown to link with two or more metal ions in various symmetric or asymmetric [29] modes: μ–1,1 (end-on), μ–1,3 (end-to-end), μ–1,1,3, and others. The coordination mode of the azido ligand depends on the nature and oxidation state of the central metal ion, as well as the nature of the other coordinated ligands.

In order to have better insight on the factors of DNA binding and cleavage mechanism, Manganese(II), cobalt(II) and zinc(II) complexes of 2,2′-bipyridine and azide ligands were synthesized and characterized. The single crystal structural analyses reveal that metal adopt distorted octahedral MN_6 coordination environments for complexes **1** and **2**; distorted square pyramidal MN_5 coordination environment for complex **3**. All the complexes (**1**–**3**) possess extended aromatic π–π systems due to the coordination of azide ligands with metal atoms. In the present study, the DNA-binding behaviors of the complexes (**1**–**3**) were explored by absorption, emission and circular dichroisim spectroscopies. Computer-aided molecular docking studies were performed to visualize the binding mode of the drug candidate at the molecular level. Their abilities to induce cleavage of pBR322 DNA and *in vitro* cytotoxicity on breast cancer cell line (MCF-7) were also investigated.

2. Experimental

2.1. Materials

2,2′-bipyridyl, manganese(II) acetate tetrahydrate, cobalt(II) acetate tetrahydrate, zinc(II) acetate dihydrate (Sigma–Aldrich), sodium azide (Alfa Aesar) were used as received. The solvents used were of reagent grade. Tris, agarose and ethidium bromide were purchased from Sigma. Calf thymus DNA (CT DNA) and Supercoiled plasmid pBR322 DNA (Genei) were utilized as received. Doubly distilled water was used to prepare buffers.

2.2. Physical measurements

Elemental analysis was carried out using a Carlorerba-1106 microanalyzer. The infrared spectra were recorded by Perkin–Elmer FT-IR spectrophotometer with a KBr disc. The electronic spectra were recorded using Shimadzu UV-3101PC spectrophotometer. The fluorescence study was carried out by Elico SL 174 spectrofluorometer. The CD spectra were recorded on Jasco J-810 spectropolarimeter.

2.3. Synthesis of complexes

2.3.1. $[Mn(bpy)_2(N_3)_2]$ (**1**)

2,2′-bipyridyl (0.32 g, 2 mmol) in methanol (10 mL) was mixed with sodium hydroxide (2 mmol) and the resulting solution was stirred for 15 min at room temperature. Further $(CH_3CO_2)_2Mn\cdot 4H_2O$ (0.25 g, 1 mmol) in methanol (10 mL) was added in to the solution. To the reaction mixture sodium azide (0.13 g, 2 mmol) was added, followed by stirring for 3 h. Finally resultant brown colored precipitate was recrystallized in DMF. Five days later round-shaped brown crystals suitable for X-ray diffraction study were obtained. Yield: 70%. Anal. Calc. for $C_{20}H_{16}N_{10}Mn$: C, 53.22; H, 3.57; N, 31.03. Found: C, 53.48; H, 3.40; N, 30.51%. FT-IR, (ν, cm^{-1}) (KBr Disc): 3370br, 2076s, 1316s (br, broad; s, sharp). UV–vis in DMF [λ_{max}/nm (ε_{max}/mol^{-1} cm^{-1})]: 280 (3870), 390 (40) 539 (1). Conductivity (Λ_M/Ω^{-1} cm^2 mol^{-1}) in DMF: 11. **Caution**! Azide complexes are potentially explosive. Only small amounts of material should be prepared, and these should be handled with care.

2.3.2. $[Co(bpy)_2(N_3)_2]\cdot 3H_2O$ (**2**)

The complex **2** was obtained by the procedure described above for the complex **1** using $(CH_3CO_2)_2Mn\cdot 4H_2O$ instead of $(CH_3CO_2)_2Co\cdot 4H_2O$ (0.25 g, 1 mmol). The resulting dark green colored precipitate formed was recrystallized in DMF. Nine days later needle-shaped green crystals were obtained. Yield: 62%. Anal. Calc. (%) for $C_{20}H_{22}N_{10}O_3Co$: C, 47.16; H, 4.35; N, 27.50. Found (%): C, 46.79; H, 4.50; N, 27.27. FT-IR, (ν, cm^{-1}) (KBr Disc): 3433 br, 2055s, 1315s. UV–vis in DMF [λ_{max}/nm (ε_{max}/mol^{-1} cm^{-1})]: 268 (2260), 305 (4390), 347 (1420), 557 (20), 638(25). Conductivity (Λ_M/Ω^{-1} cm^2 mol^{-1}) in DMF: 16.

2.3.3. $[Zn_2(bpy)_2(N_3)_4]$ (**3**)

The complex **3** was also obtained from the same procedure as described above by using $(CH_3.CO_2)_2Zn.2H_2O$ (0.22 g, 1 mmol) instead of $(CH_3CO_2)_2Mn\cdot 4H_2O$. The white colored precipitate formed was recrystallized in DMF. Six days later, round-shaped colorless crystals were obtained. Yield: 70% Anal. Calc. (%) for $C_{20}H_{16}N_{16}Zn_2$: C, 39.30; H, 2.64; N, 36.67. Found (%): C, 39.42; H, 2.78; N, 36.44. FT-IR, (ν, cm^{-1}) (KBr Disc): 3417br, 2084s, 2050s, 1342s. UV–vis in DMF [λ_{max}/nm (ε_{max}/mol^{-1} cm^{-1})]: 280 (3600), 394(20). Conductivity (Λ_M/Ω^{-1} cm^2 mol^{-1}) in DMF: 14.

2.4. Crystal structure determination and refinement

The X-ray diffraction measurements were made on a Bruker APEX II CCD area detector diffractometer (Mo-Kα radiation, graphite monochromator). Semi-empirical absorption corrections were carried out using the program SADABS [30]. The structures were solved by direct methods using the program SHELXS-97. The refinement and further calculations were carried out using SHELXL-97. The C-bound H-atoms were included in calculated positions and treated as riding atoms using SHELXL-97 default parameters. The non-H atoms were refined anisotropically, using weighted full-matrix least-squares on F^2. Software packages APEX2 (data collection), SAINT (cell refinement and data reduction) and SHELXTL (molecular graphics and publication material) were also used [31]. A summary of the crystal data, experimental details and refinement results have been given in Table 1 and selected bond lengths and bond angles are listed in Table 2.

2.5. DNA-binding studies

The DNA stock solution was prepared by dilution of CT DNA to buffer (containing 5 mM Tris–HCl/NaCl at pH 7.2) followed by exhaustive stirring at 4 °C for 4 days, and kept at 4 °C for no longer than a week. The stock solution of CT DNA gave a ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of 1.89, indicating that the DNA was sufficiently free of protein contamination [32]. The DNA concentration was determined by the UV absorbance at 260 nm after 1:20 dilution using $\varepsilon = 6600$ M^{-1} cm^{-1}. Using the electronic absorption spectral method, the relative binding of the complexes (**1**–**3**) to CT DNA was studied. Absorption titration experiments were made using different concentration of DNA, while keeping the complex concentration as constant. The intrinsic binding constants, K_b, of the compounds with CT DNA have been determined using the absorption spectral technique for the complexes.

The competitive binding studies of each complexes (**1**–**3**) with EB (EB = 3,8-diamino-5-ethyl-6-phenyl-phenanthridinium bromide) have been investigated with fluorescence spectroscopy in order to examine, whether the compound is able to displace EB