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One pot synthesis of Cu(II) 2,2′-bipyridyl complexes of 5-hydroxy-hydurilic acid and alloxanic acid: Synthesis, crystal structure, chemical nuclease activity and cytotoxicity

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ABSTRACT

A barbiturate derivative [1,5-dihydro-5-[5-pyrimidine-2,4(1H,3H)-dionyl]-2H-chromeno[2,3-d] pyrimidine-2,4 (3H)-dione] (LH_4) was allowed to react with 2,2′-bipyridyl-dinitrato-Copper(II)-dihydrate which provides two complexes, characterized as $[Cu(bpy)(L1)]\cdot 3H_2O$ (**1**) and $[Cu(bpy)(L2)]\cdot H_2O$ (**2**), where bpy = 2,2′-bipyridine, L1 = 5-hydroxy-hydurilic acid and L2 = alloxanic acid. In a separate reaction of LH_4 with $Cu(NO_3)_2\cdot H_2O$ another type of complex $[Cu(LH_3)_2\cdot(H_2O)_2]\cdot 4H_2O$ (**3**) is formed. The complexes were characterized by single crystal X-ray crystallography, physicochemical and electrochemical studies. The interaction of complexes **1** and **3** with DNA was monitored using absorption and emission titrations as well as circular dichroism spectroscopy. The complexes were found to cleave supercoiled plasmid DNA to nicked circular and linear DNA. Complexes **1** and **3** were also tested against T-cell lymphoma (Dalton lymphoma DL) and showed significant cytotoxic activity with IC_{50} values of ~9.0 nM and 0.6 nM.

1. Introduction

Chemical nucleases show potential applications in the fields of biotechnology and therapeutic reagents [1–5]. They are efficient tools for the cleavage of DNA. A large number of transition metal complexes have been explored with good DNA cleavage activities through either hydrolytic or oxidative pathways [6–13]. The transition metal complexes are known to bind DNA via both covalent and non-covalent interactions. In covalent binding, the labile ligand of the complexes is replaced by a DNA nucleobase, e.g. coordination via guanine N7. On the other hand, the non-covalent interactions with DNA include intercalative, electrostatic and groove (surface) binding of cationic metal complexes along the major or minor grooves of DNA helices. The transition metal complexes can also induce cleavage of DNA under physiological conditions. This property is of interest, especially in the areas of genomic research, footprinting and development of therapeutic agents [14]. Hydrolytic cleavage of DNA involves scission of phosphodiester bonds to generate fragments which can subsequently be re-ligated. The compounds which enable hydrolytic cleavage mimic restriction enzymes. The oxidative DNA cleavage involves either oxidation of the deoxyribose moiety by abstraction of sugar hydrogen atoms or oxidation of nucleobases, followed by cleavage of the nucleosidic bond and subsequent strand breakage. Among purine nucleobases, guanine is most susceptible to oxidation. However, most of the cleavage reagents that exhibited outstanding DNA cleavage activity require the addition of either external oxidant (dihydrogen peroxide, molecular oxygen) or external reductant (ascorbic acid, 3-mercaptopropionic acid). However, in few cases, the photo-induction of DNA cleavage was also reported. Thus, *in vivo* applications of these reagents are limited [15–18]. Therefore, the development of chemical nucleases that work without any external stimuli is a challenge for chemists, and only few examples are known to us [19–22].

In this context, it was noted that barbiturates besides their biological significance, can also be exploited as building blocks in the construction of supramolecular structures owing to their both H-bond donor and acceptor capabilities. Therefore, such molecules lie at the forefront of modern chemical research [23–30].

Thus, in view of excellent precedence of barbiturate chemistry, it was considered worthwhile to synthesize a barbiturate derivative [1,5-dihydro-5-[5-pyrimidine-2,4(1H,3H)-dionyl]-2H-chromeno[2,3-d] pyrimidine-2,4(3H)-dione)] LH_4 which was initially complexed with $Zn(bpy)(NO_3)_2\,2H_2O$. The X-ray diffraction study of the resulting complex provided a supramolecular structure of type $[Zn(bpy)_2\cdot 2H_2O]\cdot(LH_3)_2\cdot 7H_2O$ [31]. Enthused by this interesting observation, a reaction of barbiturate ligand (LH_4) was carried out with a Cu(II) ion coordinatively protected with 2,2′-bipyridine and bearing two substitutable NO_3^- groups. Ligand LH_4 on reaction with 2,2′-bipyridyl-dinitrato-copper(II)-dihydrate, yielded two new metal complexes in a

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one-pot-reaction. Complex **1** possesses 5-hydroxy-[5,5′]-bipyrimidinyl-2,4,6,2′,4′,6′-hexaone (5-hydroxy-hydurilic acid) whereas the other complex, **2** contains 4-hydroxy-2,5-dioxo-imidazolidine-4-carboxylic acid (alloxanic acid) as co-ligand. Since none of the complexes bear the framework of LH_4, the original ligand must have been transformed during the reaction. In order to explore the effect of 2,2′-bipyridyl groups on the transformation of LH_4, a reaction between LH_4 and copper nitrate salt was also carried out. This reaction provided complex **3** of type $[Cu(LH_3)_2(H_2O)_2]$, which retained the original ligand framework.

In view of the aforementioned reports and owing to the biological significance of Cu(II) ions [32], the nuclease property of the novel complexes was studied. In addition, the present article embodies the spectroscopic and single crystal characterization of the newly synthesized complexes. The DNA binding and cleavage properties of copper(II) complexes **1** and **3** have been studied (complex **2** is insoluble in common organic solvents and was not investigated further). The cytotoxic effects of complexes **1** and **3** against Dalton's lymphoma cell lines are also reported.

2. Experimental section

2.1. Materials

Barbituric acid, 2,2′-bipyridine and salicylaldehyde were purchased from Sigma Aldrich Chem. Co and copper(II) nitrate dihydrate was purchased from S.D. Fine Chemicals, India and used as received. Solvents were purchased from E. Merk and were freshly distilled prior to their use. The barbiturate ligand (LH_4) was synthesized using slight modification of the reported procedure [33]. Calf thymus (CT) DNA and supercoiled (SC) plasmid DNA pBR322 (as a solution in Tris buffer and cesium chloride purified), with a length of 4361 base pairs were purchased from Bangalore Genei, India. pUC19 plasmid DNA with a length of 2686 base pairs was purchased from Fermentas. Restriction enzymes were purchased from New England Biolabs and DNA oligonucleotide primers were purchased from Sigma Aldrich Chem. Co.

2.2. Physical measurements

IR (KBr disc, 400–4000 cm^{-1}) spectra were recorded on a Varian FTIR 3100 spectrometer; elemental analysis was done on Carbo-Erba 1108 elemental analyzer, UV-visible (UV-vis) spectra were recorded on a Shimadzu UV-1601 spectrometer while TGA plots were taken on a DU-PONT9900thermalanalyzingsystem(heatingrate10 °C/min)upto400 °C. Cyclic voltammetric measurements were performed on a CHI 620c Electrochemical Analyzer using glassy carbon as working electrode, a platinum wire auxiliary electrode, and Ag/Ag^+ reference electrode in a standard three-electrode configuration. Tetrabutylammonium perchlorate (TBAP) was used as the supporting electrolyte, and the concentration of solutions of the complexes in DMSO was maintained as 10^{-3} M. ESR spectra were recorded at 273 K and 77 K on a Varian E-line Century Series ESR spectrometer equipped with a dual cavity and operating at X-band of 100 kHz modulation frequency. Tetracyanoethylene was used as the field marker ($g = 2.00277$). The CD measurements of DNA with and without complexes were carried out with a Jasco J500 spectropolarimeter calibrated with ammonium (+)-10-camphorsulfonate.

2.3. Equipments used for DNA cleavage studies

PCR amplification was performed on an Eppendorf Mastercycler ep gradient S. Polyacrylamide gel electrophoresis was carried out with 20 × 30 cm self-cast denaturing polyacrylamide gels (5–20% acrylamide, 7 M urea, 1× TBE (89 mM Tris, 89 mM boric acid, and 2 mM EDTA, pH 8.3) on CBS Scientific DNA sequencing systems using PowerPac HV power supply from Biorad. Gels were dried on a Whatman 3MM filter paper using a gel dryer model 583 from Biorad at 80 °C for 30 min. Phosphorimaging was performed with a Storm 820 Phosphorimager from GE Healthcare.

2.4. Synthesis of complex 1

A methanolic solution (10 mL) of $Cu(bpy)(NO_3)_2 \cdot 2H_2O$ (0.361 g, 1 mmol) was added drop wise to a solution of LH_4 (0.374 g, 1 mmol) in 5 mL dimethyl formamide (DMF). The reaction mixture was then refluxed for 2 h. The resulting solution was kept at room temperature for slow evaporation. After 5–6 days, the dark green coloured crystals were obtained. These crystals were washed with methanol and dried in air. Yield: 54%, M.P. 220 °C, elemental analysis calculated for $C_{18}H_{11}CuN_6O_{10}$ (%): C, 40.44; H, 2.05; and N, 15.73. Found (%): C, 41.24; H, 2.17; and N, 16.21. UV–vis absorptions: λ_{max} (DMSO, 10^{-4} M), nm ($\varepsilon/10^4$ M^{-1} cm^{-1}) 266 (4.0), 312 (2.922) and 620 (0.023). IR (KBr): ν_{max}, cm^{-1} 3436 (OH, H_2O), 3201 (NH), 3086 (CH, Ph), 1695 (CO), and 1603 (2,2′-bpy).

2.5. Synthesis of complex 2

After isolation of complex **1**, the filtrate thus obtained provided a bluish brown solid product after two weeks. It was then redissolved in MeOH and left for slow evaporation at room temperature. After 24 h, block shaped blue colour crystals were formed which were found insoluble in all common organic solvents. The crystals were then washed with diethyl ether and dried in air. Yield: 25%, M.P. >250 °C, elemental analysis calculated for $C_{14}H_{10}CuN_4O_6$ (%): C, 42.74; H, 2.54; and N, 14.24. Found (%): C, 42.85; H, 3.08; and N, 14.84. IR (KBr): ν_{max}, cm^{-1} 3303 (NH), 1731 and 1656 (CO), 3037 (CH, 2,2′-bpy), 2929 (CH), 1266, 1024 (C–O–C), and 3378 (OH, water).

2.6. Synthesis of complex 3

A solution of $Cu(NO_3)_2 \cdot 2H_2O$ (0.241 g, 1 mmol) in MeOH (10 mL) was added drop wise to a solution of LH_4 (0.374 g, 1 mmol) in DMF (5 mL). The reaction mixture after stirring for 5–6 h at room temperature was left for slow evaporation. Fluorescent block shaped green colour crystals were grown in solution after 4–5 days. The crystals were washed with MeOH followed by diethyl ether and then dried in air. Yield: 72%, M.P. >250 °C, elemental analysis calculated for $C_{30}H_{38}CuN_8O_{22}$ (%): C, 38.87; H, 4.10; and N, 12.09. Found (%): C, 39.20; H, 4.76; and N, 12.98. UV–vis absorptions: λ_{max} (DMSO, 10^{-4} M), nm ($\varepsilon/10^4$ M^{-1} cm^{-1}) 329 (4.059), 379 (0.088) and 408 (0.056). IR (KBr): ν_{max}, cm^{-1} 3225 (NH), 1705 and 1658 (CO), 3020 (CH, Ph), 2937 (CH), 1266, 1039 (C–O–C), and 3409 (OH, water).

2.7. X-ray structural studies

Single crystal X-ray diffraction data for the complexes were collected in the temperature range of 100(2) K to 293(2) K on an Enraf Nonius MACH 3 diffractometer using graphite monochromatized Mo Kα radiation ($\lambda = 0.71073$) from block shaped crystals in the ω–2θ scan mode for complexes **1**, **2** and **3**. Intensities of these reflections were measured periodically to monitor crystal decay. The crystal structures were solved by direct methods and refined by full matrix least squares (SHELX-97) [34]. Due to high degree of hydration, thermal motion and disorder, hydrogen atoms of water of crystallization could not be located. Drawings were carried out using MERCURY [35] and special computations were carried out with PLATON [36]. The crystal refinement data are collected in Table 1 while selected bond distances and bond angles are reported in Table 2.

2.8. Interaction of complexes 1 and 3 with DNA

2.8.1. Absorption titration

The binding of complexes **1** and **3** with DNA was measured in a Na-phosphate buffer solution (pH 7.2). The absorption ratio at 260 nm and 280 nm of calf thymus DNA (CT DNA) solutions was found as 1.9:1, demonstrating that DNA is sufficiently free of protein. The concentration of DNA was then determined by UV-visible absorbance