Journal of Photochemistry & Photobiology, B: Biology 173 (2017) 650–660

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Synthesis, structures, nuclease activity, cytotoxicity, DFT and molecular docking studies of two nitrato bridged homodinuclear (Cu-Cu, Zn-Zn) complexes containing 2,2′-bipyridine and a chalcone derivative

Ruchi Gaur[a], Diksha Kumari Choubey[b], Mohammad Usman[c], Benzamin D. Ward[d], Jagat Kumar Roy[b], Lallan Mishra[a,*]

[a] Department of Chemistry, Institute of Science, Banaras Hindu University, Varanasi 221 005, U.P., India
[b] Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi 221 005, U.P., India
[c] Department of Chemistry, Aligarh Muslim University, Aligarh 202002, U.P., India
[d] Department of Chemistry, University of Cardiff, Cardiff CF10 3AT, UK

ARTICLE INFO

Keywords:
Dinuclear complex
X-ray crystallography
DFT
DNA cleavage
Cytotoxicity
Molcular docking

ABSTRACT

Nitrato briged dinuclear complexes of type $[Cu_2(L)_2(bpy)_2(NO_3)](NO_3)\cdot 4H_2O$, **1** and $[Zn_2(L)_2(bpy)_2(NO_3)](NO_3)\cdot 4H_2O$, **2** (L = deprotonated form of free ligand LH, [1-(2-hydroxyphenyl)-3-(9-anthracenyl) propenone; bpy = 2,2′bipyridine] are synthesized and characterized using a battery of physicochemical techniques and X-ray crystallography. A distorted square pyramidal geometry is assigned to them with N_2O_3 coordination core around the metal ion. The co-ligand L binds the metal ions through its *O,O′* atoms in anti-syn mode. The metal centers in complexes **1** and **2** are separated via bridging nitrato group at a distance of 6.073 Å and 5.635 Å respectively. Their structures and absorption spectra are supported by the computational studies using density functional theory (DFT) and TD-DFT. Both complexes exhibit nuclease activity and cleave supercoiled (form I) DNA. The complex **1** preferentially binds major groove of DNA and follows an oxidative pathway whereas complex **2** binds with minor groove of DNA via hydrolytic pathway. Both complexes inhibit topoisomerase I relaxation activity with IC_{50} values of 7 and 35 μM. Molecular docking studies support the groove binding and topoisomerase I binding of the complexes. The complex **1** showed a significant cytotoxicity against HeLa cell lines (a cervical cancer cell lines) in vitro with IC_{50} value calculated as 2.9 ± 0.021 μM as compared to 28.2 ± 0. 044 μM for complex **2**. Complex **2** induces the cell apoptosis at a later-stage as compared to complex **1**. The cell apoptosis and topoisomerase inhibition by complexes enable them to be potential candidates as future anticancer drugs.

1. Introduction

The search for potential anticancer drugs without side effects has always been demanding in drug development processes. In this context, cellular/molecular targets have primarily identified. Recently, the biological action of transition metal complexes are found capable of site specific DNA cleavage and DNA topoisomerase inhibition and elicited considerable interest for diagnostic and anti-cancer chemotherapy [1]. The site specific DNA cleavage by metal complexes is of contemporary interest as these complexes follow either oxidative or hydrolytic DNA cleavage pathways depending on the central metal atom and co-ordinated ligands [2]. In particular, redox inactive metals such as zinc [3] and zirconium [4] prefer hydrolytic cleavage mechanism, whereas redox-active metal centers like iron and copper initiate oxidative cleavage mechanism owing to the generation of reactive oxygen species (ROS) [5]. Oxidative damage of DNA in the presence of few ternary Cu (II) complexes is attributed to reactive hydroxyl radicals (•OH) generated through site-specific Fenton reactions [6]. Among transition metals, zinc and copper perform various chemical, biological and medicinal roles and are the second and third most abundant metal ions present in the cellular body after iron [7]. Copper homeostasis and metabolism are crucial to various cancerous cells. Moreover, Cu (II) ion can also interact with DNA through intercalation or surface association via N7 guanine residue of DNA [8] and it can significantly propagate reactive oxygen species (ROS) and subsequently induce DNA damage. On the other hand, Zn (II) ion being biologically important [9] and involved in several metalloenzymes, has recently been discovered to play its significant role in tumor suppression [10]. In this context,

* Corresponding author.
E-mail address: lmishrabhu@yahoo.co.in (L. Mishra).

http://dx.doi.org/10.1016/j.jphotobiol.2017.07.005
Received 11 April 2017; Received in revised form 30 June 2017; Accepted 5 July 2017
Available online 08 July 2017
1011-1344/

dinuclear and multinuclear complexes are found to possess synergistic effect as compared to their corresponding mononuclear analogues in terms of DNA binding, cleavage, cytotoxicity and their cellular uptake [11,12]. Recently, Spingler *et al.* reported that dinuclear Cu(II) and Ni (II) complexes containing 1,3-bis(1,5,9-triazacyclododecyl) propane, change the conformation of right handed B-DNA into left-handed *Z*-DNA, but their analogous mononuclear complexes could not induce such conformational changes [13]. On the other hand, Khalil *et al.* described the presence of an oxolating source in dinuclear curcumin–metal complexes (Zn and Cu). They inhibited the synergistic effect by pre-exhaustion of curcumin reducing potential effect of cytotoxic activity against four cancer cell lines [14]. Thus, the design and synthesis of dinuclear complexes especially of Cu(II) and Zn(II) was found interesting. The tuning to highly efficient anticancer drugs which could regulate metal uptake, trafficking, function and excretion in biological systems and provide better activities in drug resistance cells [15] requires a proper manipulation of both the metal ions and ligand frameworks. A suitable anionic linker or template effects have been successfully exploited as the most effective tools in the direct synthesis of dinuclear complexes. Anions do not function only to balance the charge in metallorganic hybrid species, but they also play a crucial role in creating structural versatility by bridging the components together and provide structures of higher dimensionality and also stabilize their structures. For example, the planar anions such as NO_3^- and CO_3^{2-} originate supramolecular networks together with cyclic structures [16,17]. The nitrato ligand (NO_3^-), which behaves as monodentate or a bidentate chelating agent [18] particularly shows the μ-(*O*,*O*′)-bridging mode in its copper(II) complexes. On the other hand, nitrate represents very common bridging ligand acting through different coordination modes [19] yet structural and biological studies of metal complexes bridged by nitrate are scarce. Thus, based on such precedence, and in view of remarkable DNA binding, cleavage and cytotoxicity [20,21] exhibited by copper bipyridine complexes planar aromatic organics species such as 2,2′ bipyridine was selected as a ligand. The selectivity of a hydroxyl chalcone embedded with a conjugated anthracenyl frameworks as ligand was also considered important owing to its pharmaceutical importance [22] and ability to act as bidentate *O*,*O*' donor which may probably involve in the construction of H-bonded networks in view of our earlier report [23,24]. Thus, in anticipation of some new unprecedented structural frame works, it was thought worthwhile to condense a chalcone LH (protonated form of L) separately with Cu(bpy)$(NO_3)_2{\cdot}H_2O$ and Zn(bpy)$(NO_3)_2{\cdot}H_2O$. As expected, such reactions allowed to generate new rare homodinuclear complexes containing Cu (II)-Cu(II) and Zn(II)-Zn(II) metal centers bridged via nitrato groups in each case. We further investigated these nitrato bridged dinuclear complexes for their applications in DNA cleavage studies. The structural tunability of metal complexes was also considered as a beneficial feature to exploit this framework in cytotoxicity and apoptotic studies.

2. Experimental Section

2.1. Materials and Methods

Dinitrato trihydrate salt of Cu(II) and dinitrato hexahydrate salt of Zinc(II), 2,2′-bipyridine, and 9-anthracenaldehyde were purchased from Sigma Aldrich Chem. Co. and used as received without further purification. The solvents were purchased from E. Merck and were freshly distilled prior to their use. IR (KBr disc, 400–4000 cm^{-1}) spectra were recorded on a Varian FTIR 3100 spectrometer; elemental analysis was carried out using Carbo-Erba 1108 elemental analyzer, UV-visible (UV-vis) were recorded on a Jasco V-630 spectrometer. However, ^{1}H NMR spectra were recorded in DMSO-d_6 using JEOL AL 300 MHz spectrometer and TMS was used as internal reference. ESI-MS measurements were performed by using a Waters Q-TOF Premier mass spectrometer. ESR spectrum of Cu(II) complex was 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).

2.2. X-ray Structural Studies

Single crystal X-ray diffraction data of the ligand and complexes were collected in the temperature range of 293(2) K and 150(2) K using an Oxford diffraction XCALIBUR-S CCD area detector diffractometer and graphite monochromatized Mo Kα radiation (λ = 0.71073) from needle shaped crystals in ω–2θ scan mode for all the complexes. 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) [25]. 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 [26] and special computations were carried out using PLATON [27]. The precursor complexes of type M(2,2′-bipy)$(NO_3)_2{\cdot}H_2O$ (M = Cu(II), Zn(II))were prepared and characterized using reported procedure [28].

2.3. Synthesis of Ligand 1-(2-hydroxyphenyl)-3-(9-anthracenyl)propenone (LH)

The ligand 1-(2-hydroxyphenyl)-3-(9-anthracenyl)propenone (LH) was synthesized by the condensation of 2′-hydroxy acetophenone and 9-anthracenaldehyde in the presence of 50% NaOH solution using a method reported earlier [29]. Yield: 226 mg (70%), M.P. 166 °C, IR (KBr pellet, cm^{-1}): 2924(m) υ(C—H), 1634(s) υ(C═O), 1299(s) υ(O—H); ^{1}H NMR ($CDCl_3$,δ ppm): 12.85(s, 1H, —OH), 6.88–8.93 (m, 13H, Ar), 7.70 (d, 1H, H_α), 6.91 (d, 1H, H_β), ^{13}C NMR ($CDCl_3$, δ ppm): 193.19 (C═O) 163.83 (Ar—CO), 142.66 (C-α), 125.12 (C-β),118.69, 118.99, 125.48, 126.62, 128.78, 128.98, 129.37, 129.67, 129.75, 129.88, 131.28, 136.64 (—Ar), UV-vis (methanol, 10^{-4} M): λ_{max}(nm) ($\varepsilon_{max} \times 10^4\ M^{-1}\ cm^{-1}$) 251 (3.638), 352(0.7377), 425 (1.0915), $\lambda_{emission}$ 511 nm at $\lambda_{excitation}$ 424 nm.

2.4. Synthesis of Complex $[Cu_2(L)_2(bpy)_2(NO_3)](NO_3){\cdot}4H_2O$ **1**

A methanolic solution (20 mL) of Cu(bpy)$(NO_3)_2{\cdot}H_2O$ (0.361 g, 1 mmol) was added drop wise to a solution of LH (0.324 g, 1 mmol) in dichloromethane (5 mL). The reaction mixture was initially stirred for 12 h then refluxed for another 2 h. The resulting solution was then kept in a refrigerator. After 5–6 days, the brown colored needle shaped crystals were obtained. These crystals were washed with diethyl ether and dried in air. Yield: 0.503 g(40%), M.P. > 230 °C, elemental analysis calculated for $C_{66}H_{54}N_6O_{14}Cu_2$ (%): C 61.82; H 4.24; N 6.55, Found: C, 61.45; H, 4.68; N, 6.64. ESI-MS: (*m*/*z*) calcd.: 1146 found: 1147 $[M]^+$. IR (KBr): ν_{max}/cm^{-1} 3436 (OH, H_2O), 2926(m) υ(CH, Ph), 1616 υ(CO, 2,2′-bpy), and 1382, 1243υ(NO_3, $\mu_{1,3}$-bridging nitrate). UV-vis. absorptions: λ_{max} (methanol, 10^{-4} M)/nm ($\varepsilon \times 10^{-4}/M^{-1}\ cm^{-1}$) 295 (3.01), 306 (3.08), 454 (2.93) and 657 (0.0389) $\lambda_{emission}$, 526 nm at $\lambda_{excitation}$ 454 nm.

2.5. Synthesis of Complex $[Zn_2(L)_2(bpy)_2(NO_3)](NO_3){\cdot}4H_2O$ **2**

A solution of Zn(bpy)$(NO_3)_2{\cdot}H_2O$ (0.363 g, 1 mmol) in methanol (20 mL) was added dropwise to a solution of LH (0.324 g, 1 mmol) in DCM (5 mL) with stirring at room temp. The stirring was further continued up to 12 h then the solution was refluxed for 6 h. After cooling the reaction mixture, methanol (10 mL) was added to it and the solution was stored in a refrigerator. After 5–6 days, the light yellow needle shaped crystals were obtained. These crystals were washed with methanol and dried in air. Yield: 0. 435 g (34%), M.P. > 230 °C, elemental analysis calculated for $C_{66}H_{54}N_6O_{14}Zn_2$ (%): C, 61.65; H, 4.23; N 6.54, Found: C, 61.98; H, 4.59; N, 6.78. ESI-MS: (*m*/*z*) calcd.: 1171 found: