# Available online at <u>www.pelagiaresearchlibrary.com</u>



**Pelagia Research Library** 

Der Chemica Sinica, 2013, 4(2):170-176



# Metallonuclease activity of copper(II) complexes containing benzimidazolyl terpyridine

**R.** Elayaperumal<sup>1</sup> and **P.** Dharmalingam<sup>\*2</sup>

<sup>1</sup>Department of Chemistry, J. J. College of Engineering and Technology, Tiruchirappalli, Tamil Nadu, India <sup>2</sup>Department of Chemistry, Urumu Dhanalakshmi College, Tiruchirappalli, Tamil Nadu, India

## ABSTRACT

Mononuclear copper(II) complexes  $[Cu(bitpy)_2](ClO_4)_2$  (1) and [Cu(bitpy)(Cl)](Cl) (2) (where bitpy = 4'-(benzimidazolyl)-2,2':6',2''-terpyridine) have been synthesized. Physico-chemical techniques like elemental analysis, ESI-MS, UV–Visible, Infra red and EPR spectroscopic techniques were employed to characterize the synthesized complexes. The complexes are one-electron paramagnetic and exhibit quasi-reversible cyclic voltammetric response at 100 mVs<sup>-1</sup> in DMSO-TBAP for the Cu<sup>II</sup>/Cu<sup>I</sup> couple. Oxidative DNA cleavage of the complexes has also been studied. Both the complexes however successfully promoted an oxidative cleavage of plasmid DNA, producing single strand break.

Keywords: benzimidazolyl terpyridine, copper(II), oxidative cleavage, plasmid DNA.

## INTRODUCTION

Copper is a biologically relevant element and many enzymes that depend on copper for their activity have been identified. Because of possessing biologically accessible redox potential and relatively high nucleobase affinity, the investigation of copper complexes as chemical nucleases is well renowned.[1] Recent reports show that some of the mono-, di- and multinuclear metal complexes of  $Cu^{2+}$ ,  $Fe^{3+}$ ,  $Zn^{2+}$ ,  $Ru^{2+}$ ,  $Co^{3+}$  with pyridyl, benzimidazolyl, ferrocenyl and others exhibit strong chemical nucleases activity under physiological conditions.[2-5] Being a transition metal, Cu gets biologically converted between different redox states namely oxidized Cu (II) and reduced Cu (I). This unique attribute has made copper metal to get manifested as an important catalytic co-factor for a variety of metabolic reactions in biological systems. Numerous Cu(II) complexes showing enhanced antiinflammatory and anti-ulcerogenic activity, as well as reduced gastrointestinal toxicity compared to the uncomplexed drug, have been prepared and structurally characterized.[6] Other studies have concentrated on the potential chemotherapeutic properties of copper-based compounds.[7-8] Copper enzymes are widely distributed within the body; they perform several diverse functions.[9] It includes transport of oxygen and electrons, catalysis in oxidation reduction reactions and the protection of the cell against damaging oxygen radicals. The interaction of transition metals (Mn, Fe, Cu) with dioxygen (in the presence of a reductant) often generates reactive oxygen species that ultimately may cleave DNA.[10] The single- or double-strand oxidative DNA cleavage by redox-active metal complexes like  $[Fe(edta)]_2$  or Cu(1,10-phenanthroline)<sub>2</sub>Cl<sub>2</sub>, is initiated by the production of reactive oxygen species through a Fenton-type mechanism.[11-13] We recently reported the oxidative cleavage of DNA by furyl

terpyidine containing copper complex. In continuation of our work, herein, we report the synthesis and nuclease activity of Cu(II) complexes (1 and 2) containing benzimidazolyl terpyridine ligand.

## MATERIALS AND METHODS

2-acetyl pyridine, copper(II) chloride dihydrate and copper(II) perchlorate hexahydrate, agarose (molecular biology grade) and ethidium bromide were procured from Sigma Aldrich, USA and used as received. Other materials like sodium hydroxide, ammonium acetate and solvents like methanol, acetonitrile were of reagent grade. The ligand, bitpy (benzimidazolyl terpyridine) was prepared using published procedure.[14] Buffers were prepared using deionised and sonicated triple distilled water. Tris (hydroxymethyl) aminomethane–HCl (Tris–HCl) buffer (pH, 7.2) was used for DNA cleavage studies. UV–visible spectra of the complexes were recorded on a Perkin–Elmer Lambda 35 double beam spectrophotometer at 25°C. Electron paramagnetic resonance spectra of the copper(II) complexes were obtained on a Varian E 112 EPR spectrometer. IR spectra were recorded as KBr pellets in the 400 - 4000 cm<sup>-1</sup> region using a Shimadzu FT–IR 8000 spectrophotometer. Cyclic voltammetric studies of the complexes were carried out by using three electrode system in a single compartment comprising of glassy-carbon working electrode and potentials were referenced to standard calomel electrode. Positive ion electrospray ionization mass spectra of the complexes were analysed using UVITEC gel documentation system and fragments were analysed using UVIchem and UVI-band software.

### 2.1 Synthesis of [Cu (bitpy)<sub>2</sub>](ClO<sub>4</sub>)<sub>2</sub> (1)

**Caution**! During handling of the perchlorate salts of metal complexes with organic ligands, care should be taken because of the possibility of explosion.

The ligand benzimidazolyl terpyridine (bitpy) was prepared by slight modification of the reported procedure and the authenticity was confirmed from ESI-MS, m/z = 350 [bitpy+H<sup>+</sup>]. The complex was prepared in high yield. To a hot solution of Cu(ClO<sub>4</sub>)<sub>2</sub>. 6H<sub>2</sub>O (0.5 g, 1.3 mmol) in methanol, bitpy (0.94 g, 2.6 mmol) was added slowly and the reaction mixture was refluxed for 15 min. A green solid that separated out upon slow evaporation of the solvent, was filtered and washed with diethyl ether. Yield: 1.05 g (84 %). Anal. Calc. for C<sub>44</sub>H<sub>30</sub>CuN<sub>10</sub>O<sub>8</sub>Cl<sub>2</sub>: C, 54.98; H, 3.15; N, 7.38; Cu, 6.61. Found: C, 54.92; H, 3.11; N, 7.32; Cu, 6.57. ESI-MS: m/z = 760.2,  $[M-(L)_2]^{2+}$ -H<sup>+</sup>.

## 2.2 Synthesis of [Cu(bitpy)(Cl)]Cl (2)

The complex was prepared in good yield from the reaction of  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  in methanol with benzimidazolyl terpyridine (1.03 g, 3 mmol) and  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  (0.5 g, 3 mmol) were dissolved in methanol individually and the solutions were warmed. To the hot solution of bitpy, copper chloride was added slowly with constant stirring when the colour changed to intense green. The green precipitate of the copper–bitpy complex obtained was filtered and dried. Yield: 1.13 g (78 %). Anal. Calc. for  $\text{C}_{22}\text{H}_{15}\text{Cl}_2\text{CuN}_5$ : C, 54.61; H, 3.12; N, 14.47; Cu, 13.13; found: C, 54.58; H, 3.08; N, 14.39; Cu, 13.09. ESI-MS:  $m/z = 447.07 \text{ [M - L·Cl]}^+$ .

### 2.3 DNA Cleavage Experiment

The cleavage of DNA in the absence and presence of an activating agent  $H_2O_2$  was monitored using agarose gel electrophoresis. Supercoiled pUC18 plasmid DNA in 5 mM Tris-HCl buffer at pH 7.2 was treated with copper(II) complex. The samples were incubated for 1 h at 37°C. The reactions were quenched using loading buffer (0.25 % bromophenol blue, 40 % (w/v) sucrose and 0.5 M EDTA (Ethylene Diamine Tetra Acetic Acid) and then loaded on 0.8 % agarose gel containing 0.5 mg/mL ethidium bromide. Another set of experiment was also performed using DMSO (Dimethyl sulphoxide) and histidine in order to find out the type of molecule involved in the cleavage mechanism. The gels were run at 50 V for 3 h in Tris-boric acid-ethylenediamine tetraacetic acid (TBE) buffer and the bands were photographed by a UVITEC gel documentation system.

### **RESULTS AND DISCUSSION**

### 3.1 Synthesis and spectral characterization

The complex  $[Cu(bitpy)_2](ClO_4)_2$ , where bitpy is the tridentate ligand 4'-(benzimidazolyl)-2,2':6',2"-terpyridine have been isolated from methanolic solution containing hexahydrated copper(II) perchlorate as the starting material. Whereas the complex [Cu(bitpy)Cl]Cl have been prepared by reacting copper(II) chloride dehydrate with 4'-(benzimidazolyl)-2,2':6',2"-terpyridine in a 1:1 ratio in methanol. Both the complexes were obtained in good yield and characterized by using elemental analysis, UV-Vis, ESI-MS and EPR spectral techniques. The analytical data obtained for the new complexes agree well with the proposed molecular formula. The ESI mass spectra of  $[Cu(bitpy)_2](CIO4)_2$  and [Cu(bitpy)CI]CI, which are shown in figure 3 displayed the molecular ion isotopic peak at m/z 760.2 and 447.07 respectively. These peaks are reliable with the proposed molecular formula of the corresponding copper(II) complexes. The structures of both the complexes are shown in figure 1.



#### 3.2 Electronic spectral analysis

The electronic spectra of both the complexes in acetonitrile showed two bands in the region of 287-357 nm and a broad band in the 594-698 nm region. The electronic spectra of 1 and 2 showed two types of transitions, the first one appeared at range 287-357 nm which can be assigned to  $\pi$ - $\pi$ \* and n- $\pi$ \* transitions due to transitions involving molecular orbitals located on the benzimidazolyl terpyridine ligand. For an unsubstituted terpyridine complex, these bands are in the region of 228-324 nm. These bands have also been shifted in the spectra of the new complexes indicating the involvement of the lowering of the LUMO (Lowest Unoccupied Molecular Orbital) ( $\pi^*$ ) energy in the bitpy ligand owing to a more extended conjugation relative to the terpy ligand. Both the complexes are paramagnetic, indicating the presence of copper in the +2 oxidation state. Three d-d transitions are possible for copper(II) complexes. They are  $d_{xz}, d_{yz} = d_{x}^{2} - d_{x}^{2} - y^{2}$  and  $d_{xy} - d_{x}^{2} - y^{2}$ . However, only a single broad band is observed for both the copper(II) complexes. This indicates the total sum of all the above transitions. The broadness associated with the d-d bands is generally taken as an indication of the geometrical distortion of the complex from perfect planar symmetry. Spectrum of 1 shows a band at 287.4 nm and a shoulder at 312.5 nm, which can be attributed to intra ligand transitions of the bitpy ligand. Broad metal to ligand charge transfer (MLCT) transition has been observed at 344 nm. Complex 2 shows the intra ligand transitions at 291.6 nm and a shoulder at 342 nm. Broad MLCT band has been observed at 356.2 nm for complex 2. Complexes 1 and 2 show their ligand field transitions as broad bands at 688 nm and 697.5 nm respectively.

## P. Dharmalingam *et al*

## 3.3 IR and EPR spectral analysis

IR spectra provide the valuable information about the nature of the binding mode and functional group attached to the metal ion. The IR spectra spectra show bands at 3381 and 3406 cm<sup>-1</sup> which was assigned as N-H stretching frequency for complex 1 and 2. Medium intensity bands at 3061 and 3045 cm<sup>-1</sup> for 1 and 2 were attributed to C-H stretching vibration. Presence of perchlorate ion and chloride ion in the IR spectra of complex 1 and 2 were confirmed by the appearance of a band at 1087 and 511 cm<sup>-1</sup> respectively. In both the complex, other medium intensity bands appeared at 1614-1616 and 1469-1473 cm<sup>-1</sup> and have been assigned to C=C and C=N stretching vibrations.



Complex 2

Figure.2. EPR Spectra of Cu(II) Complexes

The epr spectra of complex 1 and 2 (figure 2) show axial signal at 300 K from a static copper (II) centre with  $dx^2-y^2$ as the ground state. And also the spectra show one intense band in the high field region, which are isotropic due to tumbling motion of the molecules. The g value for complex 1 and 2 are 2.11 and 2.08 respectively.

## **3.4 Electrochemical studies**

The redox behavior of copper complexes is studied with the help of cyclic voltammetry. Cyclic voltammograms of the copper complexes were recorded in DMSO (Dimethyl sulphoxide) solution at 300 K using tetrabutyl ammonium perchlorate (TBAP) as supporting electrolyte. The cyclic voltammogram of complex 1 in DMSO solution shows a quasi reversible peak at -0.16 V and for complex 2 at -0.8 V with large  $\Delta$ Ep values of 320 and 121 mV respectively at a scan rate of 100 mVs<sup>-1</sup>. The redox process is assigned to Cu<sup>II</sup>/Cu<sup>I</sup> couple.[15-16]

## 3.5 DNA Cleavage Studies of Cu(II) Complexes

The characterization of DNA recognition by transition metal complex has been aided by the DNA cleavage chemistry that is associated with redox-active or photo-activated metal complexes.[17] DNA cleavage is controlled by relaxation of supercoiled circular form of pUC18 DNA into nicked circular form and linear form. When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be observed for the supercoiled form (Form I). If one of the strands is cleaved, the supercoils will relax to produce a slower-moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) will be generated that migrates in between Form II and Form III. Many copper complexes have been shown to cleave DNA in the presence of  $H_2O_2$  due to their ability to behave like a Fenton catalyst.[18] DNA cleavage was monitored by gel electrophoresis on plasmid pUC18 DNA and so the metallonuclease activity of the newly synthesized copper complexes have been ascertained.



Figure. 4. Schematic representation of unselective cleavage on a DNA plasmid

Control experiments suggest that untreated DNA and DNA incubated with either complexes 1 and 2 or peroxide alone did not show any significant DNA cleavage (lanes 1–4 in figure 5). However, in the presence of hydrogen peroxide, copper complex was found to exhibit nuclease activity. As shown in figure 6, with the increase of complex concentration, the intensity of supercoiled DNA band was found to decrease and the intensity of nicked form DNA was found to increase apparently. When the complex concentration was 6  $\mu$ M in the presence of hydrogen peroxide, no cleavage was observed (lanes 2-3 in figure 6). But on increasing the complex concentration to 48  $\mu$ M, supercoiled DNA was completely cleaved into nicked form (lanes 6 and 9 in figure 6). It is believed that when the present redox active copper complexes were interacted with DNA in the presence of hydrogen peroxide as an oxidant hydroxyl radicals might be produced.[19-22] These hydroxyl radicals are responsible for cleavage of DNA. In order to establish the reactive species responsible for the cleavage of DNA, we carried out the experiment in the presence of histidine and DMSO. When the standard hydroxyl radical scavenger DMSO was added to the reaction

## P. Dharmalingam et al

mixture of the complex and DNA, the DNA cleavage activity of 1 and 2 decreases significantly (lanes 6 and 9 in figure 5). Interestingly, on addition of histidine to the reaction mixture, the DNA cleavage activity was not inhibited greatly (lanes 7 and 10 in figure 5). This conclusively shows the involvement of the hydroxyl radical in the observed nuclease activity of complex 1 and 2 in the presence of peroxide.



Figure. 5. Cleavage of pUC18 DNA by 48  $\mu$ M complex (1 and 2) in the presence of peroxide (100  $\mu$ M). DNA was incubated with complex for 60 min in Tris buffer (pH 7.2) at 37°C. Lane 1, DNA control; lane 2, DNA + peroxide (100  $\mu$ M); lane 3, DNA + 1 (48  $\mu$ M) alone; lane 4, DNA + 2 (48  $\mu$ M) alone; lane 5, DNA + 1 (48  $\mu$ M) + peroxide (100  $\mu$ M); lane 6, DNA + 1 (48  $\mu$ M) + peroxide (100  $\mu$ M) + 10 mM DMSO ; lane 7, DNA + 1 (48  $\mu$ M) + peroxide (100  $\mu$ M) + 10 mM Histidine; lane 8, DNA + 2 (48  $\mu$ M) + peroxide (100  $\mu$ M); lane 9, DNA + 2 (48  $\mu$ M) + peroxide (100  $\mu$ M); lane 9, DNA + 2 (48  $\mu$ M) + peroxide (100  $\mu$ M) + 10 mM DMSO ; lane 10, DNA + 2 (48  $\mu$ M) + peroxide (100  $\mu$ M) + 10 mM Histidine.



Figure. 6. Concentration dependent DNA cleavage by complex 1 & 2 (6-48  $\mu$ M) in Tris buffer (pH 7.2) at 37°C in the presence of H<sub>2</sub>O<sub>2</sub>. Lane 1, DNA + peroxide (100  $\mu$ M); lane 2, DNA + 1 (6  $\mu$ M) + peroxide (100  $\mu$ M); lane 3, DNA + 2 (6  $\mu$ M) + peroxide (100  $\mu$ M); lane 4, DNA + 1 (12  $\mu$ M) + peroxide (100  $\mu$ M); lane 5, DNA + 1 (24  $\mu$ M) + peroxide (100  $\mu$ M); lane 6, DNA + 1 (48  $\mu$ M) + peroxide (100  $\mu$ M); lane 7, DNA + 2 (12  $\mu$ M) + peroxide (100  $\mu$ M); lane 8, DNA + 2 (24  $\mu$ M) + peroxide (100  $\mu$ M); lane 9, DNA + 2 (48  $\mu$ M) + peroxide (100  $\mu$ M); lane 7, DNA + 2 (12  $\mu$ M) + peroxide (100  $\mu$ M); lane 8, DNA + 2 (24  $\mu$ M) + peroxide (100  $\mu$ M); lane 9, DNA + 2 (48  $\mu$ M) + peroxide (100  $\mu$ M); lane 9, DNA + 2 (12  $\mu$ M) + peroxide (100  $\mu$ M); lane 8, DNA + 2 (24  $\mu$ M) + peroxide (100  $\mu$ M); lane 9, DNA + 2 (48  $\mu$ M) + peroxide (100  $\mu$ M); lane 9, DNA + 2 (12  $\mu$ M) + peroxide (100  $\mu$ M); lane 9, DNA

### CONCLUSION

We have prepared two copper(II) complexes 1 and 2 having 4'-(benzimidazolyl)-2,2':6',2"-terpyridine ligand with a metal to heterocyclic base ratio as 1:2 and 1:1 respectively. Both the complexes were characterized by various physico-chemical techniques and a six coordinated distorted octahedral environment has been proposed for the complex 1 and four-coordinate geometry with considerable tetrahedral distortion in the square plane has been tentatively proposed for complex 2. DNA cleavage was brought about by the copper complexes in the presence of hydrogen peroxide. The involvement of hydroxyl radical in the oxidative cleavage reactions is evidenced from the inhibition reactions in presence of DMSO.

#### Acknowledgement

The authors thank the Head, Department of Chemistry, UDC, Trichy for providing Laboratory facilities.

#### REFERENCES

[1] (a) Pogozelski WK, Tullius TD, *Chem. Rev.*, **1998**, 98, 1089. (b) Burrows CJ, Muller JG, *Chem. Rev.*, 1998, 98, 1109.

[2] Neves A, Lanznaster M, Bortoluzzi AJ, Peralta RA, Casellato A, Castellano EE, Herrald P, Riley MJ, Schenk G, *J. Am. Chem. Soc.*, **2007**, 129, 7486.

[3] Metcalfe C, Thomas JA, Chem. Soc. Rev., 2003, 32, 215.

[4] Clarke MJ, Zhu F, Frasca DR, Chem. Rev., 1999, 99, 2511.

[5] Zeglis BM, Pierre VC, Barton Chem. Commun., 2007, 4565.

[6] Weder JE, Dillon CT, Hambley TW, Chem. Rev., 2002, 232, 95.

[7] (a) Gonzalez-Vilchez F, Vilaplana R, <sub>29</sub>Cu chemotherapeutic copper compounds, Metallotherapeutic Drugs and Metal—Based Diagnostic Agents, Gielen M, Tiekink ERT, Eds., John Wiley & Sons, New York, USA, chapter 12, **2005**, pp 219.

[8] Tisato F, Marzano C, Porchia M, Pellei M, Santini C, Med. Res. Rev., 2010, 30, 708.

[9] Harris ED, Copper in human and animal health, Butterworths, London, 1983, p.44.

[10] Tullius TD, Greenbaum JA, Curr. Opin. Chem. Biol., 2005, 9, 127.

[11] Hall KB, Fox RO, Hall, K.B. and Fox, R.O. Methods Enzymol., 1999, 18, 78.

[12] Kobayashi H, Oikawa S, Hirakawa K, Kawanishi S, Mutat Res., 2004, 558, 111.

[13] Li MX, Li NQ, Chem. J. Chin. Univ., 2001, 22, 1230.

[14] Cave GWV, Raston CL, J. Chem. Soc., Perkin Trans. I, 2001, 3258.

[15] Jeyasubramanian K, Sampath SA, Thambidurai S, Murugesan R, Ramalingam SK, *Tran. Met. Chem.*, **1996**, 20, 76.

[16] Mishra L, Pandey AK, Polyhedron, 1992, 11, 423.

[17] Sitlani AS, Long EC, Pyle AM, Barton JK, J. Am. Chem. Soc., 1992, 114, 2303.

[18] Sreedhara A, Cowan JA, J. Chem. Soc. Chem Commun., 1998, 1737.

[19] Thyagarajan S, Murthy, NN, Narducci-Sarjeant, AA, Karlin KD, Rokita, SE, J. Am. Chem. Soc., 2006, 128, 7003.

[20] Pyle AM, Barton JK, Prog. Inorg. Chem., 1990, 38, 413.

[21] Morrow JR, Iranzo O, Curr. Opin. Chem. Biol., 2004, 8, 192.

[22] Perrin DM, Mazumder A, Sigman DS, In Progress in Nucleic Acid Chemistry and Molecular Biology, Cohnk

W, Moldave ed.; Academic Press: New York Orlando, 1996, pp.123.