Inorganic Chemistry

Communication

pubs.acs.org/IC

Nuclease Activity via Self-Activation and Anticancer Activity of a Mononuclear Copper(II) Complex: Novel Role of the Tertiary Butyl Group in the Ligand Frame

Kaushik Ghosh,*[,†] Pramod Kumar,[†] Varun Mohan,[†] Udai P. Singh,[†] Sahba Kasiri,[‡] and Subhrangsu S. Mandal[‡]

[†]Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee 247667, India

[‡]Department of Chemistry and Biochemistry, The University of Texas at Arlington, Arlington, Texas 76012, United States

Supporting Information

ABSTRACT: The copper complex [Cu(^{t}BuPhimp)(Cl)] (**1**) derived from tridentate ligand ^{t}BuPhimpH having N_2O donors was synthesized, and its molecular structure was determined. A phenoxyl radical complex was generated in solution at room temperature using Ce(IV). The nuclease and anticancer activities of **1** were investigated. The roles of the *tert*-butyl group and singlet oxygen in the DNA cleavage activity were also discussed.

Interaction of metal complexes with DNA and transition-metal-complex-mediated DNA damage are important areas of chemical research because of their applications in nucleic acid chemistry and cancer research.[1] Moreover, DNA/RNA cleavage is a fundamental reaction in gene regulation and gene therapy and is important for programmed cell death.[2] Among complexes of first-row transition elements, copper complexes received special attention because copper is one of the essential elements in biology and several metalloproteins need copper for their activity.[3] Biologically relevant copper has a high affinity for the nucleobases and copper complexes possess biologically accessible redox properties.[3,4] Investigation of the literature[5,6] revealed that oxidative DNA cleavage reactions by copper complexes are mediated by reactive oxygen species (ROS) produced via the oxidation of Cu^{II} to Cu^{III} by an oxidizing agent and the reduction of Cu^{II} to Cu^{I} by a reducing agent. Alternatively, transient-metal-bound species formulated as $[CuOH]^{2+}$, $[CuOOH]^{+}$, and $[CuO]^{+}$ are also reported to be responsible for DNA strand scission.[6] It has also been reported that the copper complexes, derived from proper ligand(s), could cleave DNA without any external agent in the presence of light.[7]

However, the addition of such an oxidizing or reducing agent or illumination of light is not conducive for in vivo applications of such complexes;[8] hence, it would be of interest to find a self-activated system that would not require any type of activation to generate reactive species for DNA cleavage activity. Only a few reports on the nuclease activity via self-activation are available in the literature.[8−10] Recently, we have reported a mononuclear copper(II) complex that afforded cleavage of pBR322 plasmid DNA via self-activation.[11]

As a part of our ongoing research on the interaction of copper complexes with DNA,[11,12] we were exploring the phenoxyl radical complexes and this radical-mediated cleavage of DNA.[13] In our recent report, we found that generated phenoxyl radicals in copper complexes were *not* stable at room temperature; however, stabilization of phenoxyl radical complexes was found in zinc complexes. This was due to the formation of phenoxo-bridged dinuclear zinc complexes.[12,13] Investigation of the literature revealed that phenoxyl radical complexes were stabilized by a −SR group at the ortho position to the phenolato function and/or bulky alkyl group in the phenyl ring bearing a phenolato donor.[14,15]

In this endeavor, we tried to examine the increased stability of the phenoxyl radical at room temperature and study the nuclease activity. No complex among the family of complexes derived from ligand PhimpH ([Cu(Phimp)(X)] where X = H_2O, $Phimp^-$, CH_3COO^-, SCN^-, and NO_2^-) exhibited DNA cleavage activity without the addition of an oxidizing or reducing agent.[12] Herein we report the synthesis and characterization of a copper complex derived from a *tert*-butyl-group-substituted PhimpH {^{t}BuPhimpH, (*E*)-2,4-di-*tert*-butyl-6-[[phenyl(pyridin-2-yl)hydrazono]methyl]phenol; Figure 1} and its nuclease activity studies. To the best of our knowledge, there is no report where the role of the radical stabilizing group in the nuclease activity via self-activation as well as the anticancer activity was described.

Reaction of the deprotonated ^{t}BuPhimpH with $CuCl_2·2H_2O$ afforded [Cu(^{t}BuPhimp)(Cl)] (**1**). Details of the synthetic procedures and spectroscopic characterization are described in the Supporting Information. The molecular structure of [Cu(^{t}BuPhimp)(Cl)]·CH_3OH (**1**·CH_3OH) afforded a distorted square-planar geometry (Figure 1) around the metal center having meridional spanning of ligand. The distances around the metal center were consistent with the reported values.[12] Details of the structural features and magnetic properties are described in the Supporting Information.

The cyclic voltammogram of complex **1** exhibited a cathodic peak near −0.820 V due to a Cu^{II}/Cu^{I} couple (Figure 2). Phenolato oxygen stabilized higher oxidation states, and this negative potential was consistent with the reported data.[12] Interestingly, we found a quasi-reversible redox couple near +1.0 V, which clearly indicated ligand-centered oxidation, i.e., the generation of a phenoxyl radical ($\mathbf{1}^{\bullet+}$) complex ($E_{1/2}$ =

Received: August 2, 2011
Published: February 28, 2012

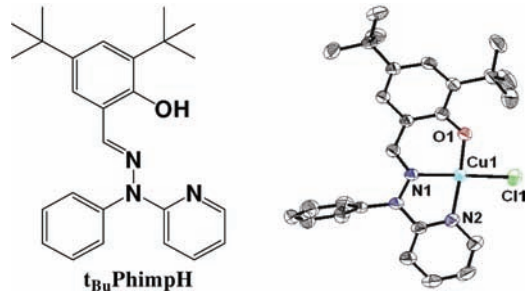

Figure 1. Schematic representation of ^{t}BuPhimpH and ORTEP drawing (50% probability level) of $1{\cdot}CH_3OH$ with an atom numbering scheme. Solvent molecules and hydrogen atoms are omitted for clarity. Selected bond length (Å) and bond angles (deg): Cu1−O1 1.8660(9), Cu1−N1 1.9546(9), Cu1−N2 1.9812(10), Cu1−Cl1 2.2479(3); O1−Cu1−N1 91.21(4), O1−Cu1−N2 162.46(4), O1−Cu1−Cl1 92.24(3), N1−Cu1−N2 81.01(4), N1−Cu1−Cl1 172.99(3), N2−Cu1−Cl1 97.27(3).

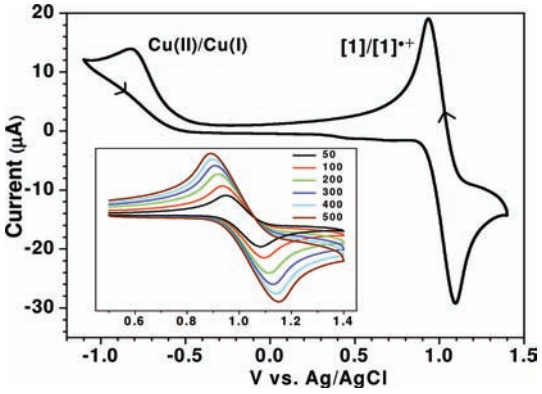

Figure 2. Cyclic voltammogram of a 10^{-3} M solution of **1** in CH_2Cl_2 in the presence of 0.1 M TBAP as the supporting electrolyte and Ag/AgCl as the reference electrode; scan rate 0.1 V s^{-1}. Inset: Cyclic voltammograms at 50, 100, 200, 300, 400, and 500 mV s^{-1} scan rates.

+1.014 V and ΔE_p = 0.158 V).[16] Repeated scans as well as different scan rates clearly expressed the generation of a phenoxyl radical complex and regeneration of **1**. This was also supported by UV−vis spectral data (Figure S5).

The generation of a phenoxyl radical complex was performed by oxidation of **1** with $(NH_4)_2[Ce^{IV}(NO_3)_6]$ (CAN) in an acetonitrile solution at room temperature. The green color of complex **1** was converted into a beige-colored solution by CAN during ligand-centered oxidation, and the formation of phenoxyl radical complex $[\mathbf{1}]^{\bullet+}$ was supported by the characteristic peaks in UV−vis spectra (Figure S5(a)). Upon the addition of CAN, the charge-transfer band of the copper complex **1** at 422 nm (8075 M^{-1} cm^{-1}) decreased and the appearance of a new band at 416 nm (6240 M^{-1} cm^{-1}) and a broad band at 565 nm (1990 M^{-1} cm^{-1}) with an isosbestic point at 437 nm was observed because of the formation of a phenoxyl radical complex. The intensities of the characteristic bands near 416 and 565 nm were dependent on the concentration of CAN. A total of 2 equiv of CAN was required for the complete conversion of **1** into radical complex $[\mathbf{1}]^{\bullet+}$, and there was no change in the UV−vis spectra upon the further addition of CAN (Figure S5(a)). We also examined the decomposition of the radical complex and regeneration of **1** (Figure S5(b)). The decomposition of radical complex $[\mathbf{1}]^{\bullet+}$ was observed within ∼40 min (Figure S5(b), inset).

The nuclease activity of **1** has been studied using supercoiled (SC) pBR322 DNA, and the extent of DNA cleavage was measured by gel electrophoresis. Contrary to our previous results,[12] we found a different type of nuclease activity where the disappearance of both bands (SC and NC DNA) was observed. An increase in the concentration of **1** (∼50 μM) afforded the disappearance of DNA bands. Hence, **1** exhibited excellent DNA cleavage activity in the absence of an external agent. Variation of the incubation time for the DNA cleavage activity (Figure 4b, lanes 10−15) clearly expressed the activity

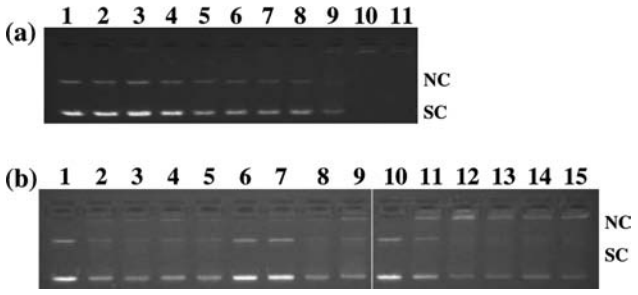

Figure 4. Gel electrophoresis separations showing the cleavage of SC pBR322 DNA (40 ng) by **1** in a buffer containing 10% acetonitrile and incubated at 37 °C for 2 h. (a) Lane 1: DNA control. Lane 2: DNA + 10% acetonitrile. Lane 3: DNA + ^{t}BuPhimpH (100 μM). Lanes 4−11: DNA + **1** = 5, 10, 20, 30, 40, 50, 75, and 100 μM, respectively. (b) Lane 1: DNA control. Lane 2: DNA + **1** (50 μM). Lanes 3−9: DNA + **1** (50 μM) + DMSO, urea, ethanol, NaN_3, L-histidine, D_2O, and catalase, respectively. Lane 10: DNA control. Lanes 11−15: DNA + **1** (50 μM) + 10, 30, 60, 90, and 120 min of incubation, respectively.

within 30 min. These observations indicated extensive DNA degradation and DNA cleavage at multiple positions.[17]

In certain reports,[5b,11] authors explained self-activated DNA cleavage via a hydrolytic pathway because the nuclease activity happened in the absence of any external agent and was not inhibited by radical scavengers. If the nuclease activity was inhibited by the presence of radical scavengers, one could speculate the possible role of ROS in the nuclease activity;[5,6,8−12] hence, we investigated the nuclease activity in the presence of radical scavengers (Figure 4b, lanes 1−9).

In the search for a mechanism, the inhibition of the nuclease activity was studied in presence of dimethyl sulfoxide (DMSO), ethanol, urea, NaN_3, L-histidine, D_2O, and catalase.[5,6] The addition of singlet-oxygen scavengers NaN_3 and L-histidine (Figure 4b, lanes 6 and 7) caused complete inhibition of the nuclease activity. These results suggested that $^1O_2$ or any other singlet-oxygen-like entity may participate in DNA strand scission.[5−9] Moreover, enhancement of the nuclease activity in the presence of D_2O also supports the above observation (Figure 4b, lane 8).[7b,9] A comparison of the nuclease activity in the presence and absence of oxygen clearly indicated the role of oxygen (Figure S7 in the Supporting Information). On the basis of above observations, we speculate that **1** generated singlet oxygen and/or singlet-oxygen-like ROS, which were responsible for the nuclease activity. Hence, we found that **1** is a novel example of a copper complex by which nuclease activity happened via self-activation.

These data prompted us to study the anticancer activity of **1** on MCF-7 cells. Preliminary data afforded an IC_{50} value of 4.76 ± 0.14 μM in cell viability assay, which was found to be better than the IC_{50} value obtained for cisplatin (17.98 ± 1.18) in the same experiment.[18] Differential interference contrast (DIC)

dx.doi.org/10.1021/ic2016676 | Inorg. Chem. 2012, 51, 3343−3345

images are shown in Figure 5, and details are reported in the Supporting Information.

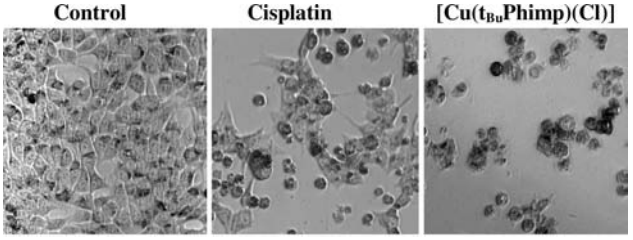

Figure 5. DIC images of the MCF-7 cells that were treated with 10 μM of **1** and cisplatin for 16 h. Control cells were treated with an equivalent volume of the vehicle (DMSO).

In summary, novel complex **1** exhibited quasi-reversible redox couple $[\mathbf{1}]/[\mathbf{1}]^{\bullet+}$ near 1.0 V due to ligand-centered oxidation. The generated phenoxyl radical from **1** was stable for ~40 min in room temperature, which was due to the presence of a *tert*-butyl group in the ligand frame. Retention of the $E_{1/2}$ value at different scan rates in cyclic voltammetry[16] and an isosbestic point during the formation of $[\mathbf{1}]^{\bullet+}$ and regeneration of **1** in UV−vis spectra clearly expressed the formation of a stable radical. We have found that the complexes derived from PhimpH[12] did not show nuclease activity in the absence of an external agent; on the other hand, **1** itself is enough to cleave DNA efficiently. Investigation of the mechanism indicated a possible role of singlet oxygen and/or singlet-oxygen-like species. The IC_{50} value for **1** was found to be 4.76 ± 0.14 μM in cell viability assay. Hence, an electron-donating *tert*-butyl group not only gave stability to the phenoxyl radical complex but also increased the electron density around metal center and imparted a self-activating mechanism through the generation of singlet oxygen and/or singlet-oxygen-like species that were possibly responsible for the DNA cleavage activity, and complex **1** afforded excellent anticancer activity. Details of this work are underway.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Details of the synthesis, characterization, X-ray analysis, and a CIF file. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: ghoshfcy@iitr.ernet.in.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

K.G. is thankful to DST, New Delhi, India, for SERC FAST Track project (SR/FTP/CS-44/2006) support. P.K. and V.M. are thankful to CSIR, MHRD and UGC India, respectively, for financial assistance. U.P.S. is thankful to IIT Roorkee for its single-crystal X-ray facility. S.S.M. is thankful for NIH Grant 1R15 ES019129-01.

■ REFERENCES

(1) (a) Chen, J.; Stubbe, J. *Nat. Rev. Cancer* **2005**, *5*, 102. (b) Mancin, F.; Scrimin, P.; Tecilla, P.; Tonellato, U. *Chem. Commun.* **2005**, 2540 and references cited therein.

(2) Hernandez-Gil, J.; Llusar, S. F.; Maldonado, C. R.; Mareque-Rivas, J. C. *Chem. Commun.* **2011**, *47*, 2955 and references cited therein.

(3) Lippard, S. J.; Berg, J. M. *Principles of Bioinorganic Chemistry*; University Science Books, Panima Publishing Corp.: New Delhi, India, 2005.

(4) Chen, J.; Wang, X.; Shao, Y.; Zhu, J.; Zhu, Y.; Li, Y.; Xu, Q.; Guo, Z. *Inorg. Chem.* **2007**, *46*, 3306.

(5) (a) Tan, J.; Wang, B.; Zhu, L. *J. Biol. Inorg. Chem.* **2009**, *14*, 727. (b) Rajendiran, V.; Karthik, R.; Palaniandavar, M.; Stoeckli-Evans, H.; Periasamy, V. S.; Akbarsha, M. A.; Srinag, B. S.; Krishnamurthy, H. *Inorg. Chem.* **2007**, *46*, 8208. (c) Shao, Y.; Zhang, J.; Tu, C.; Dai, C.; Xu, Q.; Guo, Z. *J. Inorg. Biochem.* **2005**, *99*, 1490. (d) Sheng, X.; Guo, X.; Lu, X.-M.; Lu, G.-Y.; Shao, Y.; Liu, F.; Xu, Q. *Bioconjugate Chem.* **2008**, *19*, 490.

(6) (a) Tu, C.; Shao, Y.; Gan, N.; Xu, Q.; Guo, Z. *Inorg. Chem.* **2004**, *43*, 4761. (b) Pitie, M.; Pratviel, G. *Chem. Rev.* **2010**, *110*, 1018.

(7) (a) Lahiri, D.; Bhowmick, T.; Pathak, B.; Shameema, O.; Patra, A. K.; Ramakumar, S.; Chakravarty, A. R. *Inorg. Chem.* **2009**, *48*, 339. (b) Patra, A. K.; Dhar, S.; Nethaji, M.; Chakravarty, A. R. *Chem. Commun.* **2003**, 1562. (c) Dhar, S.; Senapati, D.; Das, P. K.; Chattopadhyay, P.; Nethaji, M.; Chakravarty, A. R. *J. Am. Chem. Soc.* **2003**, *125*, 12118.

(8) Maheswari, P. U.; van der Ster, M.; Smulders, S.; Barends, S.; van Wezel, G. P.; Massera, C.; Roy, S.; den Dulk, H.; Gamez, P.; Reedijk, J. *Inorg. Chem.* **2008**, *47*, 3719.

(9) Maheswari, P. U.; Roy, S.; den Dulk, H.; Barends, S.; van Wezel, G.; Kozlevčar, B.; Gamez, P.; Reedijk, J. *J. Am. Chem. Soc.* **2006**, *128*, 710.

(10) (a) Tonde, S. S.; Kumbhar, A. S.; Padhye, S. B.; Butcher, R. J. *J. Inorg. Biochem.* **2006**, *100*, 51. (b) Li, K.; Zhou, L.-H.; Zhang, J.; Chen, S.-Y.; Zhang, Z.-W.; Zhang, J.-J.; Lin, H.-H.; Yu, X.-Q. *Eur. J. Med. Chem.* **2009**, *44*, 1768. (c) Sissi, C.; Mancin, F.; Gatos, M.; Palumbo, M.; Tecilla, P.; Tonellato, U. *Inorg. Chem.* **2005**, *44*, 2310. (d) Kellett, A.; O'Connor, M.; McCann, M.; McNamara, M.; Lynch, P.; Rosair, G.; McKee, V.; Creaven, B.; Walsh, M.; McClean, S.; Foltyn, A.; O'Shea, D.; Howe, O.; Devereux, M. *Dalton Trans.* **2011**, *40*, 1024. (e) Lamour, E.; Routier, S.; Bernier, J.-L.; Catteau, J.-P.; Bailly, C.; Vezin, H. *J. Am. Chem. Soc.* **1999**, *121*, 1862.

(11) Ghosh, K.; Kumar, P.; Tyagi, N.; Singh, U. P.; Goel, N. *Inorg. Chem. Commun.* **2011**, *14*, 489.

(12) Ghosh, K.; Kumar, P.; Tyagi, N.; Singh, U. P.; Aggarwal, V.; Baratto, M. C. *Eur. J. Med. Chem.* **2010**, *45*, 3770.

(13) Ghosh, K.; Kumar, P.; Tyagi, N.; Singh, U. P. *Inorg. Chem.* **2010**, *49*, 7614.

(14) (a) Bill, E.; Muller, J.; Weyhermuller, T.; Wieghardt, K. *Inorg. Chem.* **1999**, *38*, 5795. (b) Chaudhuri, P.; Hess, M.; Muller, J.; Hildenbrand, K.; Bill, E.; Weyhermuller, T.; Wieghardt, K. *J. Am. Chem. Soc.* **1999**, *121*, 9599. (c) Benisvy, L.; Blake, A. J.; Collison, D.; Davies, E. S.; Garner, C. D.; McInnes, E. J. L.; McMaster, J.; Whittaker, G.; Wilson, C. *Chem. Commun.* **2001**, 1824.

(15) (a) Mukherjee, A.; Lloret, F.; Mukherjee, R. *Inorg. Chem.* **2008**, *47*, 4471. (b) Orio, M.; Philouze, C.; Jarjayes, O.; Neese, F.; Thomas, F. *Inorg. Chem.* **2010**, *49*, 646. (c) Thomas, F. *Eur. J. Inorg. Chem.* **2007**, 2379 and references cited therein. (d) Orio, M.; Jarjayes, O.; Kanso, H.; Philouze, C.; Neese, F.; Thomas, F. *Angew. Chem., Int. Ed.* **2010**, *49*, 4989.

(16) Shimazaki, Y.; Yajima, T.; Shiraiwa, T.; Yamauchi, O. *Inorg. Chim. Acta* **2009**, *362*, 2467.

(17) Roy, S.; Maheswari, P. U.; Lutz, M.; Spek, A. L.; den Dulk, H.; Barends, S.; van Wezel, G. P.; Hartl, F.; Reedijk, J. *Dalton Trans.* **2009**, 10846.

(18) de Hoog, P.; Boldron, C.; Gamez, P.; Sliedregt-Bol, K.; Roland, I.; Pitie, M.; Kiss, R.; Meunier, B.; Reedijk, J. *J. Med. Chem.* **2007**, *50*, 3148.