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

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Abstract

Copper complex [Cu(t_{Bu}Phimp)(Cl)] (**1**) derived from tridentate ligand t_{Bu}PhimpH having N_2O donors was synthesized and molecular structure was determined. Phenoxyl radical complex was generated in solution at room temperature using Ce(IV) salt. Nuclease activity and anticancer activity of **1** was investigated. Roles of *tert*-butyl group and singlet oxygen in 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, 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 high affinity for the nucleobases and copper complexes possess biologically accessible redox properties.[3,4] Investigation of literature[5,6] revealed that oxidative DNA cleavage reaction by copper complexes are mediated by reactive oxygen species produced via oxidation of Cu^{II} to Cu^{III} by oxidizing agent and reduction of Cu^{II} to Cu^{I} by reducing agent. Alternatively, a 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, addition of such 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 few reports on nuclease activity via self-activation are available in the literature.[8–10] Recently we have reported mononuclear copper(II) complex which afforded cleavage of pBR322 plasmid DNA via self–activation.[11]

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ASSOCIATED CONTENT

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

As a part of our ongoing research on 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 radical in copper complexes were *not* stable at room tempera ture 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 –SR group at the *ortho* position to the phenolato function and/or bulky alkyl group in the phenyl ring bearing phenolato donor.[14,15]

In this endeavour 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 addition of any oxidizing or reducing agent.[12] Herein we report the synthesis and characterization of copper complex derived from *tert*-butyl group substituted PhimpH (t_{Bu}PhimpH, (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 role of radical stabilizing group in nuclease activity via self-activation as well as anticancer activity was described.

Reaction of the deprotonated t_{Bu}PhimpH with $CuCl_2{\cdot}2H_2O$ afforded [Cu(t_{Bu}Phimp)(Cl)] (**1**). Details of the synthetic procedures and spectroscopic characterization were described in the supporting information. Molecular structure of [Cu(t_{Bu}Phimp)(Cl)]·CH_3OH (**1**·CH_3OH) afforded distorted square planar geometry (Figure 1) around the metal centre having meridional spanning of ligand. The distances around the metal centre were consistent with the reported values.[12] Details of the structural features and magnetic properties were described in the supporting information.

The cyclic voltammogram of complex **1** exhibited a cathodic peak near −0.820 V due to Cu^{II}/Cu^{I} couple. 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. generation of phenoxyl radical (**1**$^{\bullet+}$) complex ($E^{1/2}$, +1.014 V and ΔE_p, 0.158 V).[16] Repeated scans as well as different scan rates clearly expressed the generation of phenoxyl radical complex and regeneration of **1**. This was also supported by UV-vis spectral data (vide infra) (Inset Figure 3).

Generation of phenoxyl radical complex was performed by oxidation of **1** with $(NH_4)_2[Ce^{IV}(NO_3)_6]$ (CAN) in acetonitrile solution at room temperature. The green color of complex **1** was converted into beige colored solution by CAN during ligand centered oxidation and formation of phenoxyl radical complex [**1**]$^{\bullet+}$ was supported by the characteristic peaks in UV–vis spectra (Figure 3a). Upon addition of CAN, the charge transfer band of the copper complex **1** at 422 nm (8075 $M^{-1}cm^{-1}$) decreased and 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 formation of phenoxyl radical complex. The intensity of the characteristic bands near 416 nm and 565 nm were dependent on the concentration of CAN. Two equivalents of CAN were required for the complete conversion of **1** into radical complex [**1**]$^{\bullet+}$ and there was no change in the UV-vis spectra upon further addition of CAN (Figure 3a). We also examined the decomposition of the radical complex and regeneration of **1** (Figure 3b). The decomposition of radical complex [**1**]$^{\bullet+}$ was observed within ~40 min (Inset of Figure 3b).

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 disappearance of both the bands (SC and NC DNA) was observed. Increase in concentration of **1** (~50 μM) afforded disappearance of DNA bands. Hence, **1** exhibited excellent DNA cleavage activity in absence of any external agent. Variation of incubation time for DNA cleavage activity (Figure 4b, lanes 10–15) clearly expressed the activity 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 hydrolytic pathway because nuclease activity happened in absence of any external agent and was not inhibited by radical scavengers. If nuclease activity was inhibited by the presence of radical scavengers one could speculate the possible role of reactive oxygen species (ROS) in nuclease activity[5,6,8–12] hence we investigated nuclease activity in presence of radical scavengers (Figure 4b, lanes 1–9).

In search of mechanism, the inhibition of nuclease activity was studied in presence of DMSO, ethanol, urea, L-histidine, NaN_3, D_2O and catalase.[5,6] Addition of singlet oxygen scavengers NaN_3 and L—histidine (Figure 4b, lanes 6,7) exhibited complete inhibition of nuclease. These results suggested that $^1O_2$ or any other singlet oxygen–like entity may participate in the DNA strand scission.[5–9] Moreover, enhancement of nuclease activity in presence of D_2O also supports the above observation (Figure 4b, lane 8).[7b,9] Comparison of nuclease activity in presence and in absence of oxygen clearly indicated the role of oxygen (Figure S6). On the basis of above observations we speculate that **1** generated singlet oxygen and/or singlet oxygen like reactive oxygen species (ROS) which were responsible for nuclease activity. Hence we found that **1** is a novel example of a copper complex by which nuclease 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) images are shown in Figure 5 and details are reported in the supporting information.

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

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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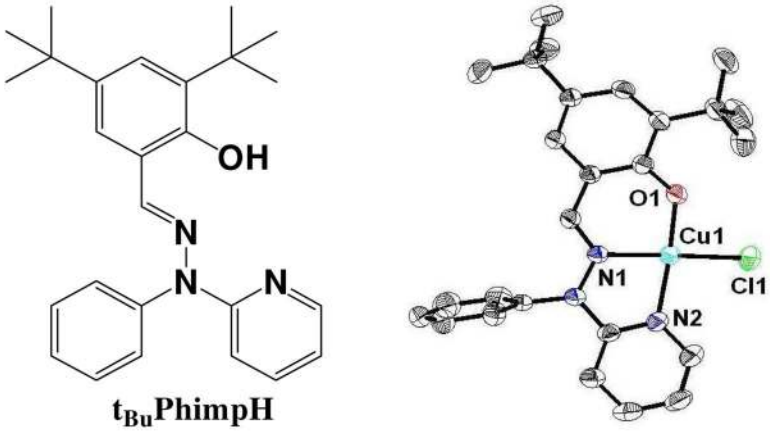

Figure 1.

Schematic representation of t_{Bu}PhimpH and ORTEP drawing (50% probability level) of [Cu(t_{Bu}Phimp)(Cl)]·CH_3OH (**1**·CH_3OH) with atoms numbering scheme. Solvent molecule and hydrogen atoms are omitted for clarity. Selected bond length (Å) and bond angles (°) are: Cu(1)—O(1) 1.8660(9), Cu(1)—N(1) 1.9546(9), Cu(1)—N(2) 1.9812(10), Cu(1)—Cl(1) 2.2479(3); O(1)—Cu(1)—N(1) 91.21(4), O(1)—Cu(1)—N(2) 162.46(4), O(1)—Cu(1)—Cl(1) 92.24(3), N(1)—Cu(1)—N(2) 81.01(4), N(1)—Cu(1)—Cl(1) 172.99(3), N(2)—Cu(1)—Cl(1) 97.27(3).

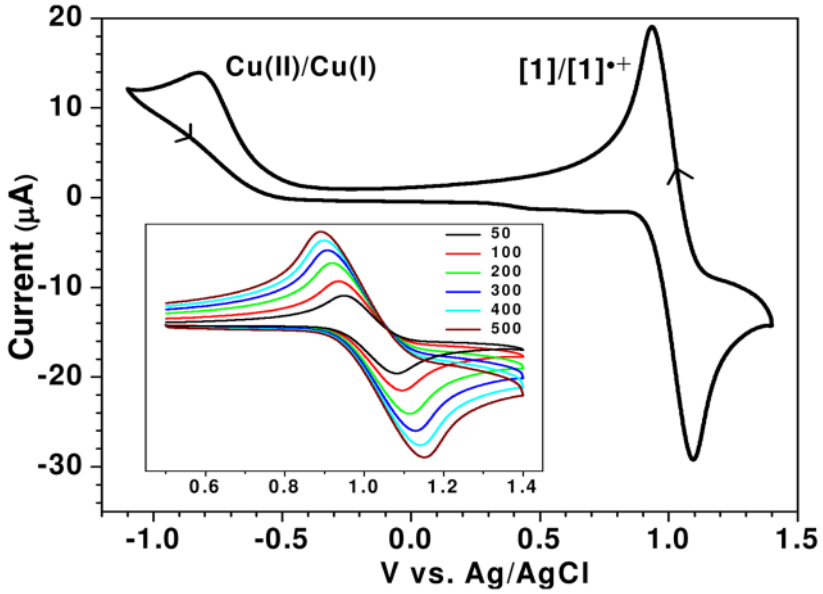

Figure 2.
Cyclic voltammogram of a 10^{-3} M solution of **1** in CH_2Cl_2 in presence of 0.1 M TBAP as a supporting electrolyte and Ag/AgCl as reference electrode; scan rate 0.1 V/s. Inset: Cyclic voltammograms at 50, 100, 200, 300, 400 and 500 mV/s scan rates.

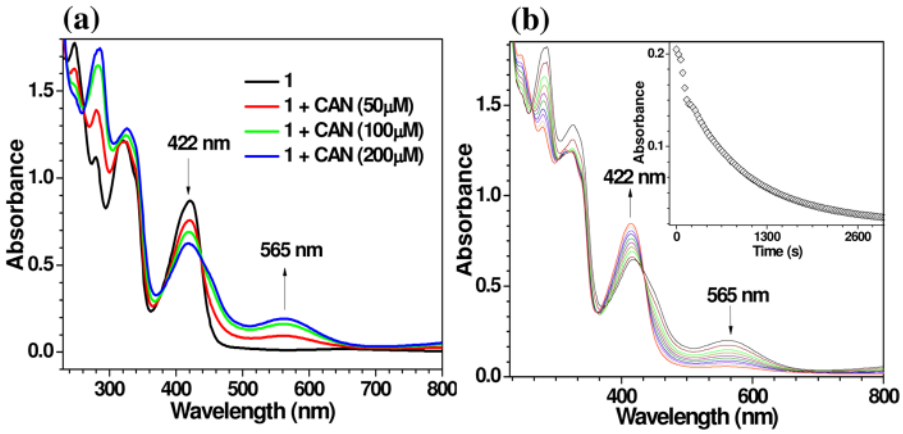

Figure 3.

(a) UV-vis spectra of generation of phenoxyl radical of **1** (100 μM) in the presence of CAN (50–200 μM) in acetonitrile. (b) UV-vis spectra of decomposition of $[\mathbf{1}]^{\bullet+}$ within ~40 min. Inset: Time course of decomposition of $[\mathbf{1}]^{\bullet+}$ ([**1**] = 100 μM, [CAN] = 200 μM) in acetonitrile at room temperature (λ_{max} = 565 nm).

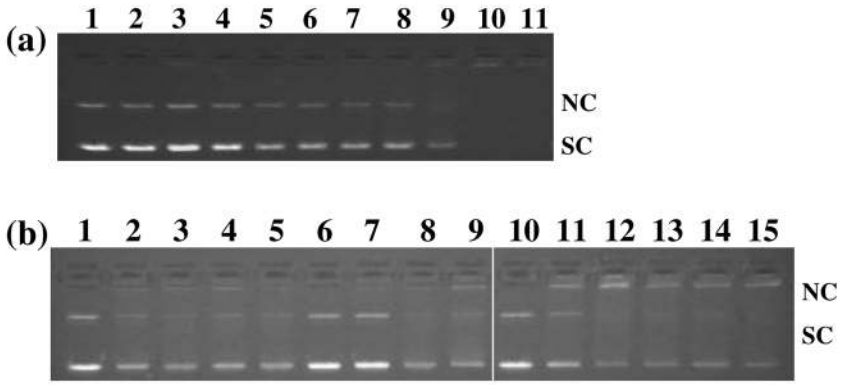

Figure 4.

Gel electrophoresis separations showing the cleavage of supercoiled pBR322 DNA (40 ng) by **1** in 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_{Bu}PhimpH (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, catalase respectively; lane 10, DNA; lanes 11–15, DNA + **1** (50 μM) + 10, 30, 60, 90 and 120 min incubation respectively.

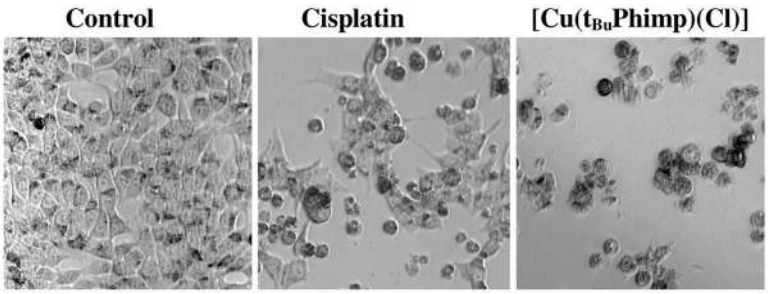

Figure 5.
Differential interference contrast (DIC) images of the MCF-7 cells that were treated with 10 μM of **1** and cisplatin for 16 hrs. Control cells were treated with equivalent volume of vehicle (DMSO).