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DNA Interactions and Cytotoxicity of the Aryls-Vertexed Zinc(II) Thiosemicarbazone Complex

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A new mononuclear zinc complex of *N*-phenyl-substituted thiophene thiosemicarbazone ligand [Zn(Httsc-*N*-Ph)$_2$] was synthesized and characterized, and investigated as nuclease mimic. As compared to carboplatin, a much greater activity against HeLa cells is exhibited by [Zn(Httsc-*N*-Ph)$_2$] which binds to DNA through an intercalation mechanism.

Several different biological characteristics are demonstrated by thiosemicarbazones and these characteristics are largely dependent on the parent ketone or aldehyde and greater activity is seen if they are heterocyclic aromatic systems.[1,2] Clinicians make use of numerous thiosemicarbazones including triapine or marboran even though different concerns have been raised related to their mechanism of action.[3,4] Still, it has been established that mode of action of heterocyclic thiosemicarbazones involves inhibition of an essential enzyme i.e. ribonucleotide reductase which is required for synthesis of DNA precursors.[5] Besides showing biologically activity, these Schiff bases prove to be potent coordinating agents and their interaction with metals results in formation of stable complexes.[6] Studies indicate that effectiveness and action of organic therapeutic agents can be accelerated if they contain metal ions in the form of complexes.[7–9] Researchers have been recently attracted to the exploration of interactions between nucleic acids and transition-metal complexes as findings of these investigations can be helpful in developing innovative reagents for medicine and biotechnology.[10] Owing to its bioavailability, hard Lewis acid characteristics, low toxicity and redox inertness, zinc ion is known as one of the most suitable metal ion for research studies based on DNA.[11,12] For that reason, in this communication a zinc(II) complex whose coordination sphere is occupied by the *N*-phenyl-substituted thiophene thiosemicarbazone ligands has been synthesized and studied for DNA binding, nuclease activity and cytotoxicity. The molecular structure of [Zn(Httsc-*N*-Ph)$_2$] along with atom numbering scheme is given in Figure 1. [Zn(Httsc-*N*-Ph)$_2$] presents a tetrahedral geometry, which is distorted around the zinc atom, with the basal plane occupied by the S(1), S(1A), N(1), and N(1A) atoms of two ligands. The distortion from tetrahedral geometry is evident from the bond angles S(1)–Zn(1)–N(1) 86.04(3)°, S(1)–Zn(1)–S(1A) 144.33(2)°, S(1)–Zn(1)–N(1A) 115.79(3)°, S(1A)–Zn1–N1 115.79(3)°, N(1)–Zn(1)–N(1A) 106.64(4)(10)°, S(1A)–Zn(1)–N(1A) 86.04(3)° and bond lengths Zn(1)–S(1) 2.2878(3) Å, Zn(1)–N(1) 2.0395(10) Å, Zn(1)–S(1A) 2.2878(3) Å and Zn(1)–N(1A) 2.0395(10) Å.

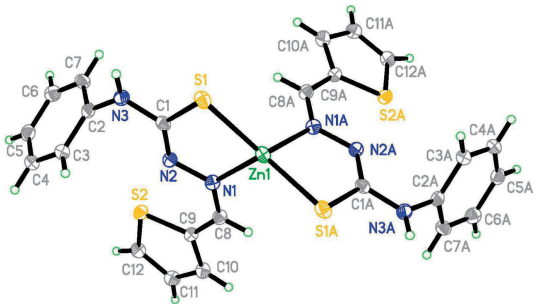

Figure 1. An ORTEP view of [Zn(Httsc-*N*-Ph)$_2$] with 50% ellipsoidal probability, showing the atom numbering scheme.

The DNA binding properties of [Zn(Httsc-*N*-Ph)$_2$] were studied by UV, fluorescence spectroscopy and viscosity measurements. The absorption spectra of [Zn(Httsc-*N*-Ph)$_2$] in the absence and presence of CT-DNA at various concentrations are given in Figure 2a. In the UV region, [Zn(Httsc-*N*-Ph)$_2$] exhibits an intense absorption band around 333 nm which is attributed to the intraligand transition of the coordinated thiosemicarbazonato ligands. When concentration of CT-DNA was increased, red-shift of 5 nm together with hypochromism was noticed. This implies that there occurs a binding interaction between the CT-DNA and [Zn(Httsc-*N*-Ph)$_2$]. The intrinsic binding constant (K_b) for [Zn(Httsc-*N*-Ph)$_2$] is $5.72 \times 10^4\,M^{-1}$ (determined by regression analysis).[13] This value of binding constant shows that [Zn(Httsc-*N*-Ph)$_2$] binds with DNA moderately. To investigate the interaction mode between [Zn(Httsc-*N*-Ph)$_2$] and CT-DNA, ethidium bromide (EB) fluorescence displacement experiments were carried out. As shown in Figure 2b, a prominent reduction trend was noticed in the fluorescence intensity of EB bound to the DNA at 584 nm with increasing concentration of the [Zn(Httsc-*N*-Ph)$_2$]. The reduction in fluorescence occurs because some EB molecules were discharged from the EB–DNA system following an exchange with the complex. This observation is usually attributed to intercalation. Viscosity measurements were carried out to further verify the interaction of the metal complexes with DNA. Alterations in the relative viscosity can be used as an indicator for differentiating electrostatic and intercalative DNA binding. It has been established that viscosity of the DNA solution increases considerably when a ligand intercalates into the DNA. This is because the distance between the base pairs increases at the site of intercalation which in turn results in an increase in

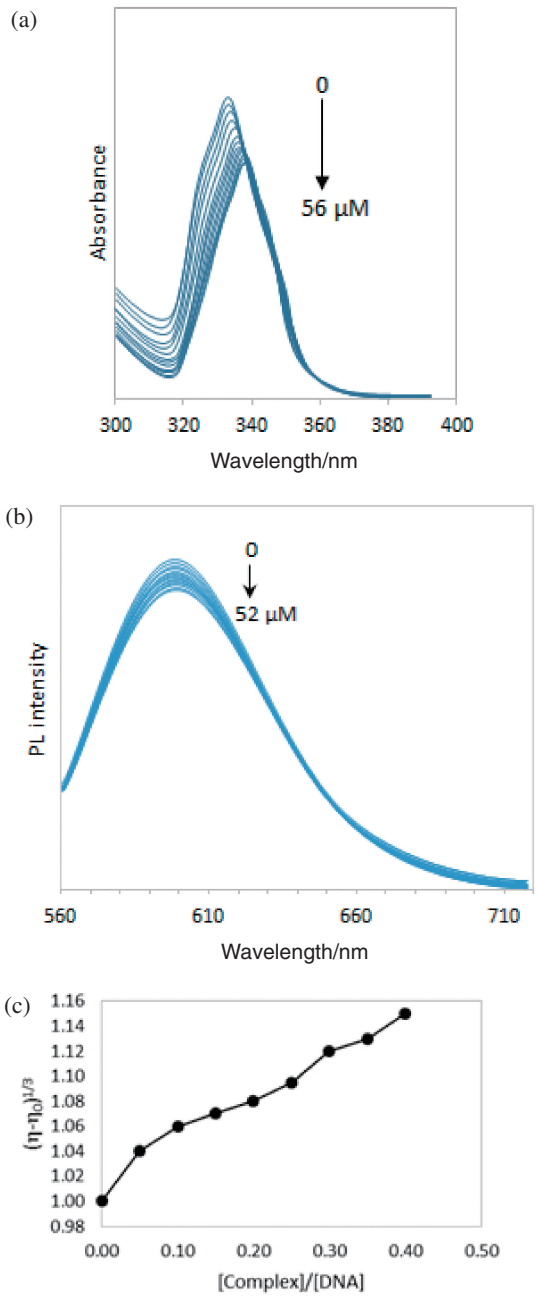

Figure 2. (a) Absorption spectra of [Zn(Httsc-*N*-Ph)$_2$] (10 µM) in the absence and presence of increasing amounts of CT DNA (0–56 µM). (b) The emission spectra of the DNA–EB system in the absence and presence of increasing amounts of [Zn(Httsc-*N*-Ph)$_2$]; [DNA] = 26 µM, [[Zn-(Httsc-*N*-Ph)$_2$]] = 0–52 µM, [EB] = 20 µM. (c) Effect of increasing amounts of [Zn(Httsc-*N*-Ph)$_2$] (0–80 µM) on the relative viscosity of CT-DNA at 30 ± 0.1 °C.

the length of DNA molecule. On the other hand, when a ligand binds in the grooves of DNA, little or no alteration is noticed in the viscosity of the DNA solution.[14] There is a steady increase in the relative viscosity of CT-DNA when concentration of [Zn(Httsc-*N*-Ph)$_2$] is increased as shown in Figure 2c. This shows that intercalative binding occurs between the [Zn(Httsc-*N*-Ph)$_2$] and CT-DNA.

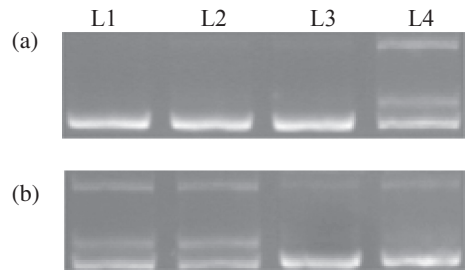

Figure 3. Cleavage of pBR322 supercoiled DNA (0.5 µg µL^{-1}) after incubation at 37 °C for 10 min with [Zn-(Httsc-*N*-Ph)$_2$] (50 µM) and H_2O_2 (0.1 mM). (a) Lane 1, DNA; lane 2, DNA + H_2O_2; lane 3, DNA + [Zn(Httsc-*N*-Ph)$_2$]; lane 4, DNA + [Zn(Httsc-*N*-Ph)$_2$] + H_2O_2. (b) Lane 1, DNA + [Zn(Httsc-*N*-Ph)$_2$] + H_2O_2 + NaN_3 (0.2 mM); lane 2, DNA + [Zn(Httsc-*N*-Ph)$_2$] + H_2O_2 + SOD (4 units); lane 3, DNA + [Zn(Httsc-*N*-Ph)$_2$] + H_2O_2 + KI (0.2 mM); lane 4, DNA + [Zn(Httsc-*N*-Ph)$_2$] + H_2O_2 + DMSO (0.2 mM).

The DNA-cleaving abilities of [Zn(Httsc-*N*-Ph)$_2$] were investigated using pBR322 plasmid DNA in the absence and in the presence of hydrogen peroxide (Figure 3a). As evident from lane 1–3, DNA cleavage did not occur in the case of free DNA, DNA incubated with [Zn(Httsc-*N*-Ph)$_2$] and DNA incubated with H_2O_2. On the other hand, when DNA was incubated with [Zn(Httsc-*N*-Ph)$_2$] and H_2O_2, nuclease activity was detected as shown by lane 4. To gain information about the reactive species involved in the oxidative DNA cleavage reaction, the cleavage experiment was performed using different compounds such as hydrogen peroxide scavenger (KI), superoxide scavenger (SOD), singlet oxygen quencher (NaN_3) and hydroxyl radical scavenger (DMSO) (Figure 3b). NaN_3 and SOD caused no significant inhibition as shown in lane 1 and lane 2 respectively. On the other hand, nuclease activity of [Zn(Httsc-*N*-Ph)$_2$] was found to be considerably inhibited by KI and DMSO as shown in lane 3 and lane 4 respectively. It can therefore be stated that hydroxyl radical and hydrogen peroxide is involved in the DNA cleavage.

Moreover, [Zn(Httsc-*N*-Ph)$_2$] exhibited a very high cytotoxic activity against HeLa cells with the anti-proliferation rate of 81.8% at 1.5×10^{-6} M (2 d). The IC_{50} of [Zn(Httsc-*N*-Ph)$_2$] was found to be 3.2×10^{-7} M and this value is ten times greater than that of carboplatin, a clinical platinum anticancer agent. To inspect whether the cancer cells experience DNA damage during the treatment, alkaline single cell gel electrophoresis (or comet assay) was performed. The cells were treated with IC_{50} concentration of [Zn(Httsc-*N*-Ph)$_2$] for 2 d and harvested for comet assay. The results are displayed in Figure 4.

It was found that the Hela cells treated with [Zn(Httsc-*N*-Ph)$_2$] suffered DNA damage whereas the untreated cells (control) do not. The DNA damage was indicated by the detection of prominent comet-like tails of EB-stained DNA. Findings of this assay validated that the [Zn(Httsc-*N*-Ph)$_2$] complex was able to induce DNA breakage in cells, which is a hallmark of apoptosis. On the whole, the above-mentioned findings show that [Zn(Httsc-*N*-Ph)$_2$] can play a promising role in cancer treatment. Research studies exploring mechanisms involved in its antitumor activity are in progress.

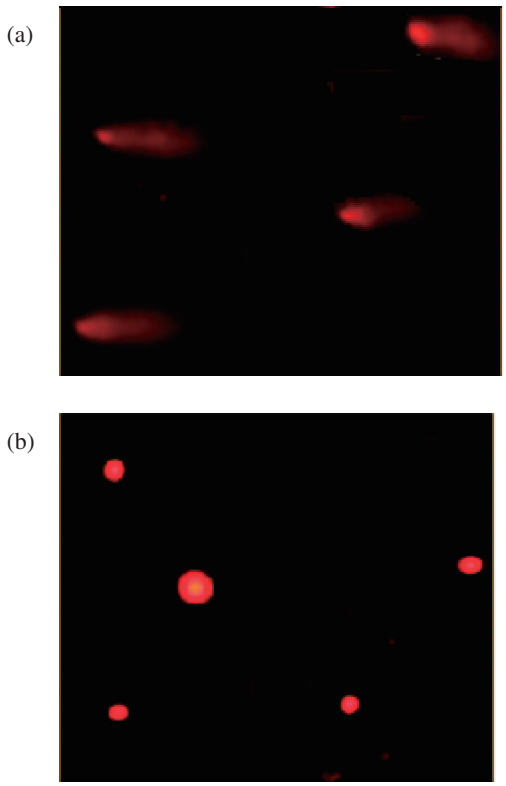

Figure 4. Comet assay for [Zn(Httsc-*N*-Ph)$_2$] treated Hela cells (a) in comparison to control (b).

Experimental

The heterocyclic thiosemicarbazone (Httsc-*N*-Ph) was synthesized according to the method reported in the literature.[15] Its zinc(II) complex [Zn(Httsc-*N*-Ph)$_2$] was obtained by the reaction of Httsc-*N*-Ph with Zn(COO)$_2$(H$_2$O)$_2$ (molar ratio 2:1) in methanol solution for 30 min at 60 °C. Found: C, 49.14%; H, 3.63%; N, 14.15%. Anal Calcd. for $C_{24}H_{20}N_6S_4Zn$ (586.07): C, 49.18%; H, 3.44%; N, 14.34%. IR spectrum (cm^{-1}, KBr pellet): ν(N–H), 3410s (NHPh); ν(C=N) 1546s; ν(C–S), 851m. ^{1}H NMR data (δ, ppm; CDCl$_3$), 6.99 (s, N*H*Ph), 7.92 (s, 1H, *H*C=N), 7.06–7.59 (m, 8H, Ar–H). ESI-MS: 587.37 $[M + H]^+$. Crystallographic data have been deposited with Cambridge Crystallographic Data Centre: Deposition number CCDC-790222 for [Zn(Httsc-*N*-Ph)$_2$]. Copies of the data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; Fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

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