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Synthesis, characterization, DNA studies of copper(II) complexes of (2*E*)-3-phenylprop-2-enal thiosemicarbazones

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ABSTRACT

*A series of substituted of thiosemicarbazones derived from (2E)-3-phenylprop-2-enal with thiosemicarbazides [NH_2-NH-C(S)-NHR, R= H (**HL1**), Me (**HL2**), Et (**HL3**) and Ph (**HL4**)] and their copper(II) complexes [**1-4**] have been synthesized and characterized by analytical, magnetic susceptibility, molar conductivity, infrared, electronic, ESR and cyclic voltammetric data. In the electronic spectra of the complexes one d-d band in 13333–12658 cm^{-1} region observed and is assigned to $2E_g \rightarrow 2T_{2g}$ transition in an distorted octahedral field with moderate Jahn-Teller effect. In ESR, the trend $g_{\parallel} > g_{\perp} > 2.0023$, suggests that the unpaired electron lies predominantly in the $d_{x^2-y^2}$ orbital and the axial symmetry parameter (except to **4**) values suggests no interaction between the copper centers in solid state, where as in **4**, G is less than 4 suggesting a negligible exchange. In cyclic voltametry, $E_{1/2}$ values of complexes are observed in potential range of 0.462–0.565 V vs Ag/AgCl is corresponding to Cu(II)/Cu(I) reduction couple. The absorption studies revealed that each of these complexes is avid binder to calf thymus-DNA. The apparent binding constants for complexes are in the order of 10^7 M^{-1}. The nucleolytic cleavage activity of ligands and their complexes were carried out on pUC18 plasmid DNA by using gel electrophoresis experiment in the presence and absence of H_2O_2. Ligands showed increased nuclease activity when administered as copper complexes. All these copper (II) complexes behave as efficient chemical nucleases with hydrogen peroxide activation. They revealed that the complexes employ both oxidative and hydrolytic chemistry exhibiting ambivalent function in DNA cleavage. The hydrolytic cleavage of DNA is evident from the control experiments showing cleavage inhibition in the presence of the hydroxyl radical inhibitor, DMSO or the singlet oxygen quencher, azide ion.*

Key words: Copper(II) complexes, (2*E*)-3-phenylprop-2-enal thiosemicarbazones, binding constants, and hydrolytic cleavage activity.

INTRODUCTION

Thiosemicarbazone ligands have been extensively explored Schiff bases in the development of coordination chemistry with biological and radiopharmaceutical applications [1, 2]. This study continues our development of the coordination chemistry of the copper(II) metal with thio-containing ligands in order to provide new materials for the artificial chemical nucleases [3-7]. Artificial metallo nucleases are the metal complexes that require ligands which effectively deliver metal ion to the vicinity of the DNA strand [8-10]. Studies on chemical modification of nucleic acids by transition metal complexes are of paramount important for the designing of chemotherapeutic drugs, regulating gene expression and designing tools for molecular biology [11, 12]. Reports on DNA interactions of copper complexes with ligands derived from modifications of 2, 2'-bipyridine (Bpy) and 1, 10–phenanthroline (Phen), Salen–type Schiff base families are numerous in the literature, those of interactions of transition metal complexes of substituted thiosemicarbazones is limited [3,13-17]. The literature survey reveals that the molecular structure of **HL1** and **HL2** [18, 19] and their Pd(II), Zr(IV), Tin(IV), Si(IV), Fe(II) and Zn(II) complexes of (2*E*)-3-phenylprop-2-enalthiosemicarbazones were reported [20-25], however detailed structural investigation of substituted

thiosemicarbazone ligands or Cu(II) complexes are not reported so far. As a part of our ongoing work [3-7, 26, 27], we report here the synthesis, structural characterization and biological evaluation of copper(II) complexes of a series of (2*E*)-3-phenylprop-2-enalthiosemicarbazones.

MATERIALS AND METHODS

2.1. Materials and methods

Thiosemicarbazide, 4-methyl-3-thiosemicarbazide, 4-ethyl-3-thiosemicarbazide, 4-phenyl-3-thiosemicarbazide and (2*E*)-3-phenylprop-2-enal were of reagent grade purchased from Sigma-Aldrich. All other chemicals were of AR grade and used as supplied. The solvents used in the synthesis of ligands, their metal complexes and in spectral measurements were distilled before use. All other chemicals were of AR grade and were used without further purification. Calf thymus DNA was purchased from Genie Bio labs, Bangalore, India. The plasmid pUC18 DNA was isolated from *E-coli* DH5α strains in Luria Broth (LB) medium supplemented by ampicillin cells from 5 ml culture by Qiagen column following manufacturer's protocol.

2.2. Preparation of the ligands and their complexes

2.2.1. Preparation of ligands (HL1-HL4)

The ligands (**HL1-HL4**) were prepared by the following general procedure described in the literature [28]. To a hot ethanolic solution (30 ml) of (2*E*)-3-phenylprop-2-enal (4.9g , 0.03 mol) in 250 ml round bottom flask, 5% $CH_3COOH–H_2O$ solution of thiosemicarbazide (0.03 mol) is mixed and heated the reaction mixture under reflux on a steam bath for 30-45 minutes. The crystalline product which formed was collected by filtration, washed several times with hot water and dried in *Vacuo*. All the ligands were recrystallized from methanol (**Scheme 1)**.

2.2.2. Preparation of the complexes

The complexes (**1-4**) were prepared by mixing a hot methanolic solution of copper(II) chloride (1 mol) and ligands (**HL1-HL4**) (2 mol) in 1:2 ratio in methanol medium. The reaction mixture was heated under reflux for about 1 hour. Crystalline complexes which separated out were collected by filtration, washed with hot water and small amount of methanol and dried under *vacuo*.

2.3. Physical measurements

The elemental analysis was carried out on a Perkin-Elmer 2400 CHNS elemental analyser. Magnetic susceptibility measurements were carried out on a magnetic susceptibility balance (Sherwood scientific, Cambridge, England). High purity hydrated Copper sulphate was used as a standard. Molar conductance of the complexes (10^{-3} M) in dimethylformide at $(30\pm2)^0$C with CC180 model (ELICO) direct reading conductivity bridge, electronic spectra were recorded in DMSO with a Schimadzu UV-160A spectrophotometer. FT-IR spectra were recorded in the range 4000-270 cm^{-1} in KBr disc on Nicolet protégé 460 IR Spectrometer. ESR spectra were recorded on Varian E–122 X-band spectrophotometer in liquid nitrogen temperature in DMSO. The cyclic voltammetric measurements were performed on a Bio Analytical System (BAS) CV-27 assembly in conjugation with an X-Y recorder. Measurements were made on the degassed (N_2 bubbling for 5 minutes) ligand/complex solution (10^{-3}M) in dimethylformamide and ethanol containing tetra butyl ammonium perchlorate (0.1M) as a supporting electrolyte. The three-electrode system consisted of a glassy carbon (working), platinum wire (auxiliary) and Ag/AgCl (reference). The ^{1}H and ^{13}C{^{1}H} NMR spectra were recorded on a Bruker Spectrospin DPX-300 NMR spectrometer at 300.13 and 75.47 MHz respectively.

2.4. DNA binding experiment

Isolation of DNA

DNA was isolated using the procedure mentioned below. The fresh bacterial culture (1.5 ml) was centrifuged to obtain the pellet. So obtained pellet was dissolved in 0.5 ml of lysis buffer (100 mM tris pH 8.0, 50 mM EDTA, 50 mM lysozyme) and 0.5 ml of saturated phenol was added to it and incubated at 55 ^{0}C for 10 min, and then centrifuged at 10,000 rpm for 10 min. To the supernatant liquid equal volume of chloroform: isoamyl alcohol (24:1) mixture and 1/20th volume of 3 M sodium acetate (pH 4.8) was added.

Again it was centrifuged at 10,000 rpm for 10 min and to the supernatant 3 volumes of chilled absolute alcohol added. The precipitated DNA was separated by centrifugation. The pellet was dried and dissolved in TE buffer (10 mM tris pH 8.0, 1 mM EDTA) and stored in cool place.

Concentration measurement: The concentration of DNA per nucleotide was measured by using its known extinction coefficient at 260 nm (6600 M^{-1} cm^{-1}) [29]. The absorbance at 260 nm (A260) and at 280 nm (A280) for *E-coli* was measured to check its purity. The ratio A260/A280 was found to be 1.8-1.9, indicating that E. coli was satisfactorily free from protein [30]. Buffer [5 mM tris(hydroxymethyl) amino methane, tris, pH 7.2, 50 mM NaCl]

was used for the absorption experiments.

Stock solutions were stored at 4^{o}C and were used after no more than four days. Doubly distilled water was used to prepare buffer solutions. Solutions were prepared with the appropriate copper complexes (20 µM of 1 mM solution in DMF)), CT-DNA (diluted from concentrated DNA solution) diluted with DMF. After equilibrium (ca 5 min) the spectra were recorded against an analogous blank solution containing the same concentration of DNA.

The data were then fitted to equation (1) to obtain the intrinsic binding constant (K_b) [31].

$[DNA]/(\varepsilon_A-\varepsilon_{B)} = [DNA]/(\varepsilon_A-\varepsilon_{F)} + 1/ K_b (\varepsilon_B-\varepsilon_F)$ -(1)

Where $\varepsilon_{A,}$ ε_B and ε_F correspond to apparent, bound and free metal complexes extinction coefficients respectively. A plot of $[DNA]/(\varepsilon_A-\varepsilon_{F)}$ Vs [DNA] gave a slope of $1/(\varepsilon_B-\varepsilon_{F)}$ and a Y-intercept equal to $1/ K_b (\varepsilon_B-\varepsilon_{F)}$; K_b is the ratio to the Y- intercept.

2.5. Assay of nuclease activity

The DMF solution containing metal complexes taken in clean eppendroff tube and 1µg of pUC18 DNA was added to it. The contents were incubated for 30 minutes at 37^{o}C and loaded on 0.8% Agarose gel after mixing 5 µl of loading buffer (0.25% bromophenol blue + 0.25% Xylene cyanol+30% glycerol sterilized distilled water). Electrophoresis was performed at constant voltage (100V) until the bromophenol blue reached to the ¾ of the gel. Further the gel is stained for 10 minutes by immersing it in ethidium bromide solution. The gel was then destained for 10 minutes by keeping it in sterilized distilled water and plasmid band were by viewing the gel under transilluminator and photographed. The efficiency of DNA cleavage was measured by determining the ability of the complex to form open circular (OC) or nicked circular (NC) DNA from its super coiled (SC) form. The reactions were carried out under oxidative and/or hydrolytic condition. Control experiment was done in the presence of hydroxyl scavenger DMSO and oxygen quencher Sodium Azide.

RESULTS AND DISCUSSION

3.1. Characterization of thiosemicarbazones ligands (HL1-HL4)

The ligands (**HL1-HL4**) (**Fig. 1**) are pale yellow coloured air stable solids and their analytical data are given in **Table 1**. The pK_a values of the lignads are calculated by Phillips-Merritt method, are 6.1, 8.2 and 8.1 for HL1, HL2 and HL3 respectively and data indicate that at lower pH the ligands which were in thione form are converted into thiol (II) form and at higher pH the ligands may loose one proton to become mono anionic (III) ligand (**Scheme 2**). The IR spectra of ligands show two medium bands in 3463-3261 cm^{-1} region due to terminal –NH_2/NHR vibrational modes. Strong bands are observed in 1205-1121 cm^{-1} and 1621-1624 cm^{-1} regions are respectively assigned to υ(C=S) and >C=N- stretching vibration, No band is observed near 2575 cm^{-1} suggesting that the ligands remain in thione form in solid state.

The ^{1}H-NMR and ^{13}C{^{1}H}-NMR of the ligands are recorded in d_6-DMSO solvent and the data is: **HL1**: **^{1}H NMR** (δ, ppm) 7.41-7.61 (m, 2H, $H_{4, 9}$), 8.24-8.26 (m, 6H, $H_{2, 3, 5, 6-8}$) 8.75 (bs, 3H, NH and NH_2); **^{13}C{^{1}H}:** C_4 (123.86), $C_{2, 6}$ (127.14), $C_{3, 5}$ (128.90), C_7 (129.37), C_1 (135.53), C_9 (141.14), C_8 (145.68), C_{12} (178.17); **HL2**: **^{1}H NMR** (δ, ppm) 3.23-3.25 (s, 3H, H_{14}), 6.76-6.94 (m, 2H, $H_{7, 8}$), 7.29-7.47 (m, 5H, H_{2-6}), 7.63-7.66 (d, *J* = 12 Hz, 1H, H_9), 9.52 (bs, 2H, $H_{11, 13}$); **^{13}C{^{1}H}:** C_{14} (31.97), C_4 (124.38), $C_{2, 6}$ (128.79), C_7 (128.87), $C_{3, 5}$ (129.04), C_1 (135.53), C_9 (142.54), C_8 (146.27), C_{12} (176.43); **HL3**: **^{1}H NMR (δ, ppm):** 1.31 (t, 3H, H_{15}), 3.70-3.79 (qt, 2H, H_{14}), 6.78-6.94 (m, 2H, $H_{7, 8}$), 7.34-7.47 (m, 5H, H_{2-6}), 7.70-7.73 (d, *J* = 12 Hz, 1H, H_9), 9.92 (bs, 2H, $H_{11,13}$); **^{13}C{^{1}H}:** C_{15} (23.75), C_{14} (34.16), C_4 (124.67), C_6 (127.02), C_5 (128.85), C_7 (130.71), C_1 (137.85), C_9 (143.40), C_8 (152.11), C_{12} (175.64), **HL4: ^{1}HNMR (δ, ppm):** 6.82-6.98 (m, 2H, $H_{7, 8}$), 7.25 (t, 1H, H_{17}), 7.32-7.40 (m, 6H, $H_{3, 5, 15, 16, 18, 19}$), 7.46 (t, 1H, H_4), 7.65-7.68 (d, *J* = 12 Hz, 2H, $H_{2, 6}$), 7.76-7.79 (d, *J* = 12 Hz, 1H, H_9), 9.15 (s, H, H_{11}), 10.23 (s, H, H_{13}); **^{13}C{^{1}H}:** C_{17} (124.05), $C_{15, 19}$ (124.48), C_7 (126.21), $C_{3, 5}$ (127.18), $C_{2, 6}$ (128.83), $C_{16, 18}$ (128.93), C_4 (129.34), C_{14} (135.67), C_1 (137.80), C_9 (141.08), C_8 (144.97), C_{12} (175.25). A typical NMR spectrum of **HL2** is given in **Fig.2**. The mass spectrum of **HL1 (Fig. 3)** shows a molecular ion peak at 206 (*m/z*) corresponding to its molecular weight. The other important peaks at *m/z* 189, 147, 132, 130, 117, 103 are due to loss of -NH_3, C(S)-NH_2, HN-C(S)-NH_2, H_2N-C(S)-NH_2, =HN-HN-C(S)-NH_2, HC=HN-HN-C(S)-NH_2 from the molecular ion peak. Fragmentation pattern of the HL1 is shown in **Scheme 3**. Similarly the other ligands gave the m/z peaks at 220, 234 and 282 are corresponding to their molecular weights. The presence of only cathodic peaks at -1.50, -1.63, -1.17, and -1.39 in cyclic voltammograms of **HL1-HL4** respectively in DMF suggests an irreversible reduction of the azomethine group. Each peak corresponds to a one electron reduction. A possible mechanism (**Scheme 2**) is proposed for the reduction of these ligands.

3.2. Characterization of complexes (1-4)

The analytical data of complexes support the proposed molecular formula $[Cu(\mathbf{HL})_2Cl_2nH_2O]$, (where **HL** = thiosemicarbazone, n=1 for complex 1 and n=0 for 2-4). The ligands and their complexes are stable at room temperature, non-hygroscopic and insoluble in water and partially soluble in methanol and readily soluble in chloroform, pyridine, dimethyl formamide, and dimethyl sulfoxide. The colour, melting point, analytical, magnetic susceptibility and molar conductivity data are summarized in **Table 1**. The molar conductivity data indicate that the complexes are non-electrolytes. The magnetic susceptibility value of the complexes indicates the presence of one unpaired electron. Magnetic moment of the complex **1** is 1.58 BM which is lower than the spin-only value and is due to mixing of orbital angular momentum from excited state via spin-orbit coupling.The electronic spectra of the complexes (**Table 1)** has one charge transfer band and one d-d band in 22222–29412 cm^{-1} and 12658–13333 cm^{-1} region respectively. The charge transfer may occur due to the migration of electrons from the ligand orbitals to metal orbitals. The presence of single d-d band may be attributed to the symmetric nature of the ligand field. The band observed in the visible region is assigned to $^2E_g \rightarrow {}^2T_{2g}$ transition in a distorted octahedral ligand field [32] with moderate Jahn-Teller effect. The IR spectra of ligands show two medium bands in 3463-3261 cm^{-1} region due to terminal–NH_2/NHR vibrational modes. These bands are not affected in complexes suggesting non-participation of terminal -NH_2 group in coordination. Strong band is observed in 1205-1121 cm^{-1} region assigned to υ(C=S) stretching vibration, but in IR spectra of complexes the shift (Δυ=±19-68 cm^{-1}) in υ(C=S) frequency suggests the involvement of thioketo sulphur in coordination [3]. A strong band is observed in 1544- 1590 cm^{-1} region is due to >C=N- in the ligands is shifted to lower frequency in the complexes suggesting the involvement of azomethine nitrogen (>C=N-) in chelation [33]. These data suggest that the thiosemicarbazone act as neutral bidentate ligand. In IR spectrum of complex **1**, an additional band at 3732 cm^{-1} is observed due to presence of water molecule. The non-ligand bands in 409-445; 374-384 and 229-236 cm^{-1} regions are tentatively assigned to υ(M-N), υ(M-S) and υ(M-Cl) modes respectively.

The ESR spectra were recorded in DMSO at liquid nitrogen temperature. A typical ESR spectrum of complex **1** is given in **Fig.4**. The spectra of the complexes exhibit a set of four well- resolved signals at low field and one or two signals at high field, corresponding to $g_{\parallel}$ and $g_{\perp}$ respectively. The $g_{\parallel}$ and $g_{\perp}$ values are computed from the spectrum using the tetracyano ethylene (TCNE) free radical as the 'g' marker. Kivelson and Nieman [34] have reported that $g_{\parallel}$ value is less than 2.3 for covalent character and is greater than 2.3 for ionic character of the metal ligand bond in complexes. As seen in the **Table 2**, in present complexes (except complex 4) $g_{\parallel}$ values suggesting the covalent character for the metal-ligand bond. For complex 4, the $g_{\parallel}$ value is slightly higher than the 2.3 suggesting the small amount ionic character of the metal–ligand bond. The trend $g_{\parallel}>g_{\perp}>2.0023$, suggests that the unpaired electron lies predominantly in the $d_{x^2-y^2}$ orbital [35] characteristic of square planar or octahedral geometry of copper(II) complexes [36]. The g_{av} value for these complexes is greater than 2 indicating the presence of covalent property [37]. The axial symmetry parameter G factor higher than 4 (exception to complex 4) suggesting the absence of interaction between the copper centers in solid state, while in 4, G is less than 4 suggesting a negligible exchange interaction [38]. The ESR parameters $g_{\parallel}$, $g_{\perp}$, $A_{\parallel}$ and $A_{\perp}$ of the complexes and the energies of d-d transitions are used to evaluate the orbital reduction parameters ($K_{\parallel}$, $K_{\perp}$) and the dipolar interaction (p) [38-40].

Hathaway has pointed out that for the pure σ–bonding $K_{\parallel} \approx K_{\perp} \approx 0.77$, for in-plane π-bonding $K_{\perp}<K_{\parallel}$, while for out-of-plane π-bonding $K_{\perp}>K_{\parallel}$. From table, the orbital reduction parameters observed $K_{\perp}<K_{\parallel}$ relationship, indicates the presence of in plane π-bonding. Giordano and Bergman [41] suggest the identification of bonding groups from the values of dipolar term P. The reduction of P values from the free ion value (0.036 cm^{-1}) might be attributed to the strong covalent bonding. The values of P obtained for the present complexes lie between 0.015-0.019 cm^{-1} and are consistent with bonding of copper to nitrogen donor atoms. Analysis of ESR spectrum of 4, indicates that $g_{\parallel}>g_{\perp}>2.0023$ and $A_{\parallel}=172 \times 10^{-4}$ cm^{-1}, This observation suggests an elongation in octahedral geometry [42]. The shape of ESR lines indicates the geometry around the coper(II) ions. It is not trigonal bipyramidal for the present complexes since the low field side of the ESR spectrum is less intense than the high field side. The ESR spectra of present complexes may suggest axial symmetry around copper(II) ion. EPR parameters also reflect the degree of tetrahedral distortion of square planar copper(II) complexes. The empirical factor ($f = g_{\parallel}/A_{\parallel}$ cm) index of deviation from idealized geometry. The values of f factor is in the 110-130 range are typical of square planar complexes. The values in 130-150 range are characteristic of slight to moderate distortion and 180-250 cm^{-1} indicate considerable distortion [43]. Values from 135-242 cm^{-1} for the present complexes suggest a moderate to considerable distortion geometry.

Based on the molar conductance, electronic, infrared and ESR spectral data, it is suggested that all these complexes have six coordinated tetragonal structure **(Fig. 5**).

The redox behavior of these complexes was recorded in DMF in 0.1M tetrabutyl ammonium perchlorate (TBAP) as supporting electrolyte and the data is given in **Table 3**. A typical cyclic voltammetric profile of the complex 4 is

given in **Fig.6**. Repeated scans as well as various scan rates showed that dissociation does not takes place in these complexes. $E_{1/2}$ values of the complexes are observed in potential range of 0.462–0.565 V vs Ag/AgCl [44-46] is corresponding Cu(II) to Cu(I) reduction couple. The non-equivalent current intensity of cathodic and anodic peaks (i_c/i_a= 0.821–0.970 V) indicates quasi reversible behaviour of these complexes [47]. The difference $\Delta Ep = E_{pc} - E_{pa}$ values exceeds the Nernstian values ($\Delta E_p \approx 59mV$) for one electron redox system. This indicates a considerable reorganization of the coordination sphere during electron transfer as has been observed for a number of other copper (II) complexes [47]. The higher ΔG° values indicate that the complexes are more stable in DMF medium. Comparison of the $E_{1/2}$ values of present complexes with other related complexes reveals that the complexes undergo more facile redox change which seems to be a requirement to the DNA cleavage [48, 49].

3.3. Electronic absorption titrations

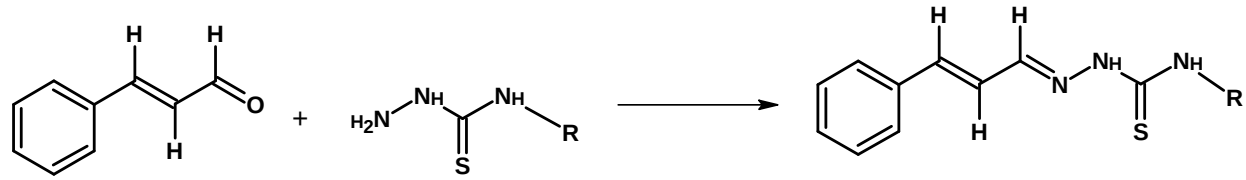
The binding interaction of complexes with CT-DNA was monitored by comparing their absorption spectra with and without CT-DNA. In the UV region of spectra, all the copper complexes exhibit an intense absorption band around 329-370 nm with increasing in the DNA concentration. The intrinsic binding constant (Kb) values for the complexes were determined from the decay of the absorbance in the region 329 to 370 nm with increasing concentration of DNA by using equation (1). The intrinsic binding constant values suggests that interactions of complexes with CT-DNA. Electronic absorption data upon addition of CT-DNA to the complexes is tabulated in **Table 4**. The percentage hyperchromicity for the CTDNA/complex was determined from $(\varepsilon_F-\varepsilon_B)/\varepsilon_F \times 100$, where ε_F is the extinction coefficient of the free complex and ε_B is the extinction coefficient of the bound complex. In the presence of increasing amount of DNA , absorption spectra of 1-4 complexes showed red-shift ($\Delta\lambda_{max}$: 1–5nm) and hyperchromism [hyperchromism: -8.41% for 1, -8.27% for 2, -33.33% for 3 and -20.39 % for 4] indicates that the interaction of the complexes with CT-DNA leading to the formation of new complex with double-helical CT-DNA. The hyperchromic shift observed for complex is due to smaller size metal ions which enhances binding affinity with DNA. From Table 7 the order of binding constants of complexes are 3<1<2<4. Highest binding constant of complex 4 is due to the presence of additional phenyl group that facilitate pi-stacking interaction [50].

3.4. Nuclease activity

Gel electrophoresis experiments using pUC18 plasmid DNA were performed with the ligands and their complexes in the presence and absence of H_2O_2 as an oxidant. A micro-molar concentration for 30 min incubation periods, the ligands exhibits no significant activity in the absence and presence of the oxidant. But due to presence of phenyl rings in thiosemicarbazone moiety, the ligands show little activity. It is evident form **Fig. 7(a)**, the intensity of nicked form slightly enhanced. The nuclease activity is greatly enhanced by the incorporation of copper ion in the respective ligands. The nuclease activity of the complexes was also investigated in the presence of a free radical scavenger viz. dimethyl sulphoxide.

From **Fig 7(b)** it is evident that the complexes cleave DNA more effectively in the presence of an oxidant (even no. lanes, except lane 2), which may be due to the reaction of hydroxyl radical (OH.) with DNA. These hydroxyl free radicals participate in the oxidation of the deoxyribose moiety, followed by hydrolytic cleavage of the sugar phosphate back bone [49].The more pronounced nuclease activity of the complexes in the presence [**Fig 7(b);** lanes 4, 6, 8 and 10] of oxidant indicates the participation of hydroxyl radical in DNA cleavage. Even in the absence of oxidant, the complexes exhibit significant DNA cleavage activity [**Fig 7(b)** lanes 3, 5, 7 and 9]. This may be due to hydrolytic cleavage of DNA catalyzed by copper complexes.

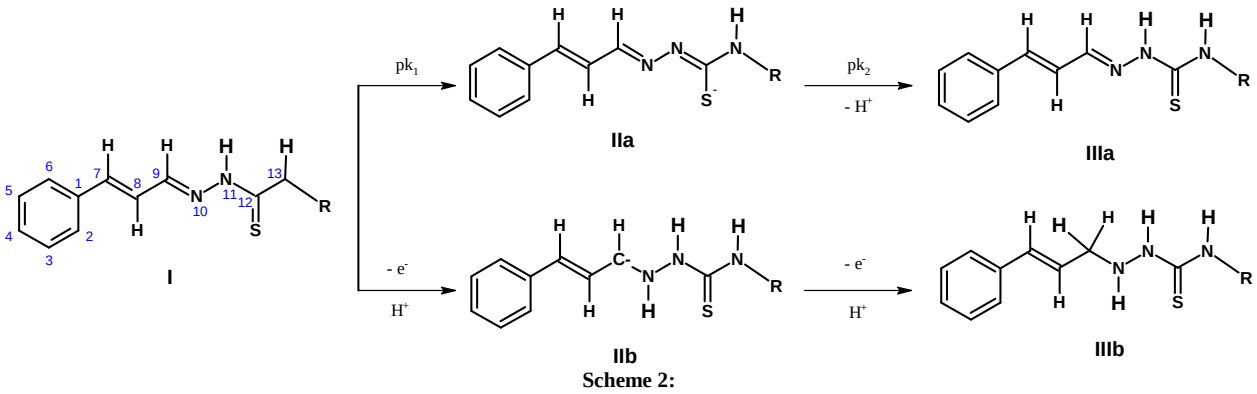
The nuclease activity of the complexes was also investigated in the presence of a free radical scavenger, viz. dimethyl sulfoxide (DMSO). Appreciable nuclease activity of complexes in the presence of DMSO (Hydroxyl free radical scavenger) suggests hydrolytic cleavage of DNA catalysed by copper complexes. The complexes may be arranged in the increasing order of nuclease activity, 3>4>1>2.



(2*E*)-3-phenylprop-2-enal

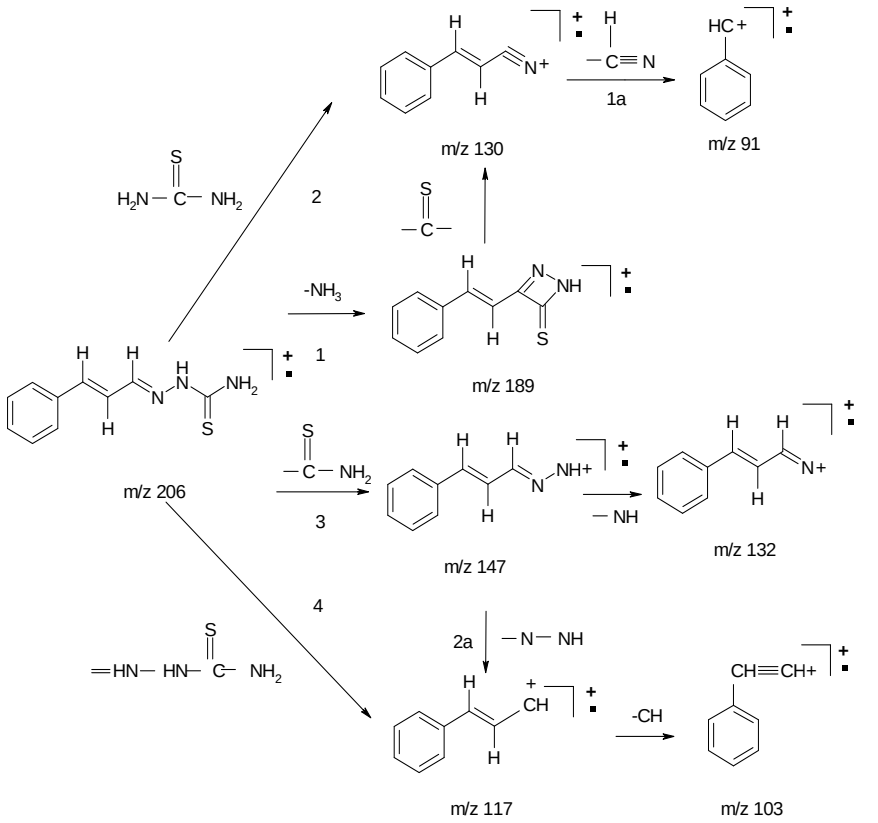
Where R= H (HL1); CH_3 (HL2); C_2H_5 (HL3); C_6H_5 (HL4)

Scheme 1: Synthesis of Ligands (HL1-HL4)



Scheme 2:

Scheme 3 Mass fragmentation of HL1



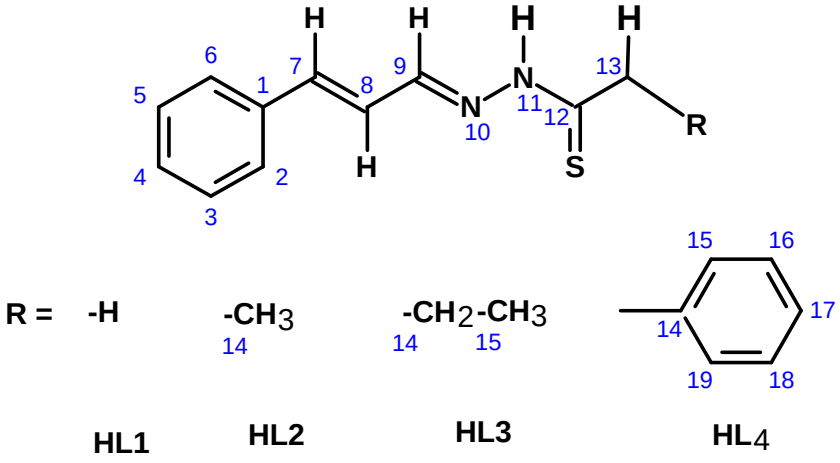


Fig. 1 Structure of the Thiosemicarbazone(s) (HL1-HL4)

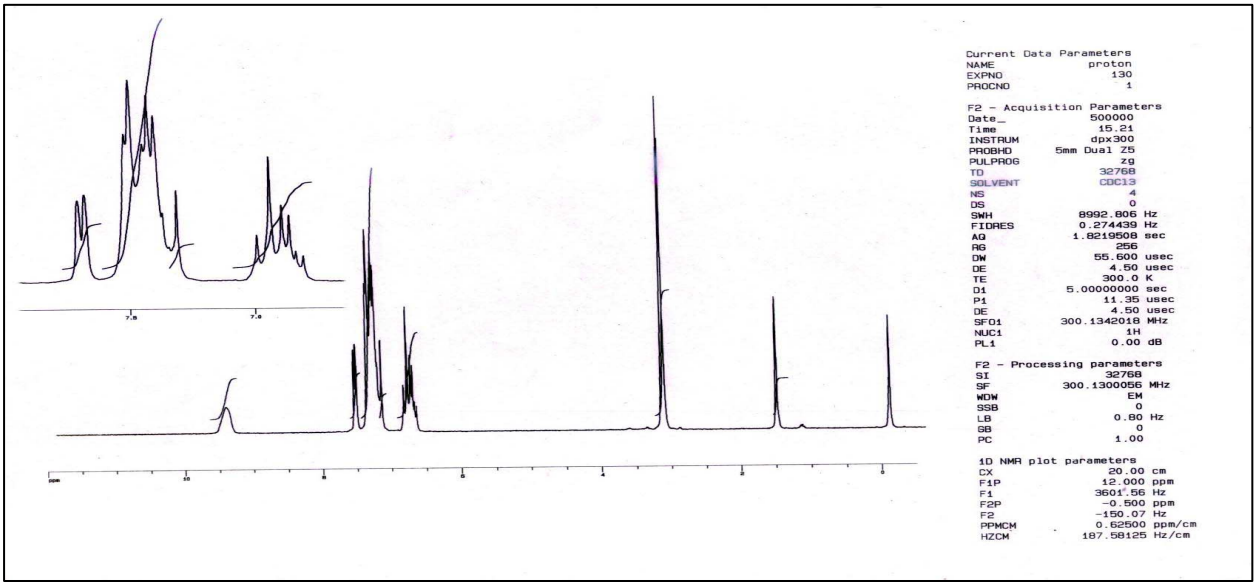


Fig. 2 Proton NMR spectrum of HL2

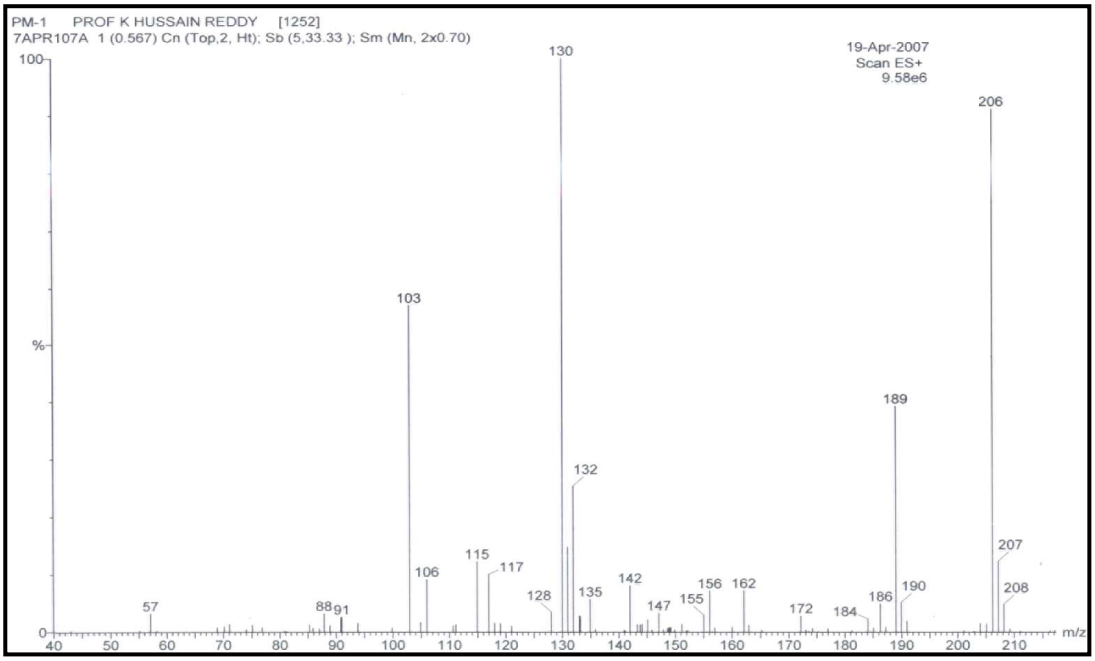


Figure 3 Mass spectrum of HL1

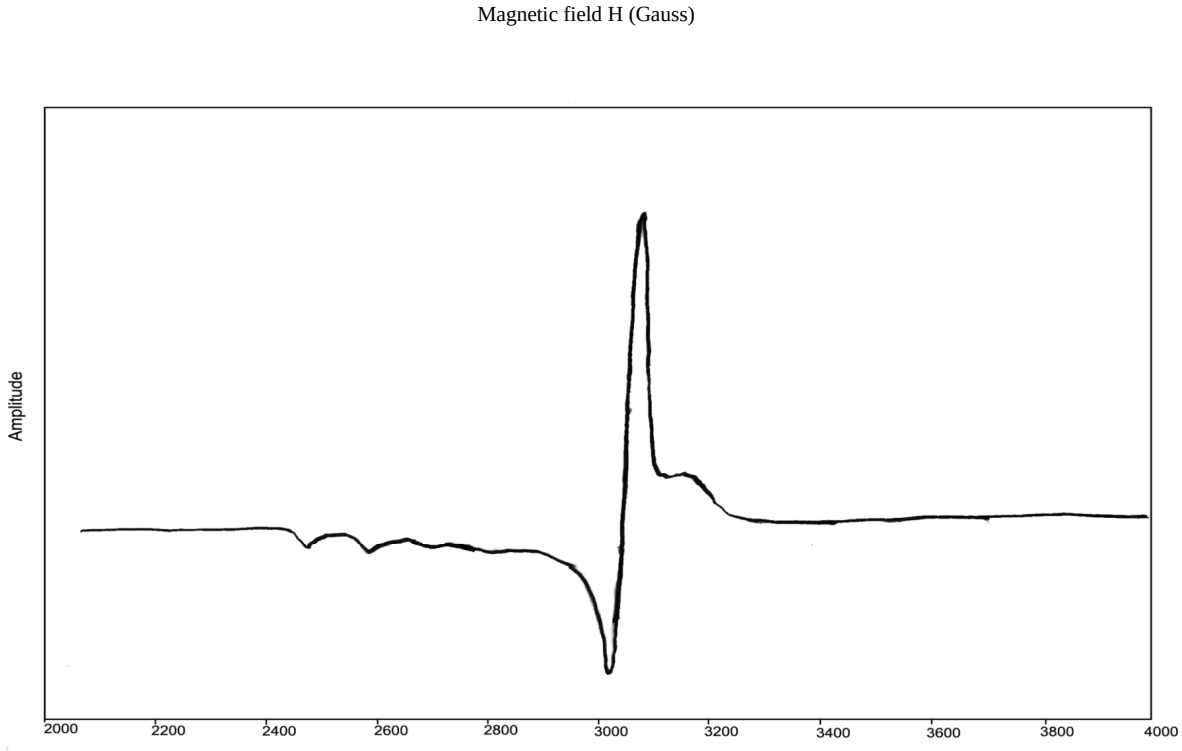


Fig. 4 X- Band ESR spectra of the Complex 1 at liquid nitrogen temperature (LNT) in DMS

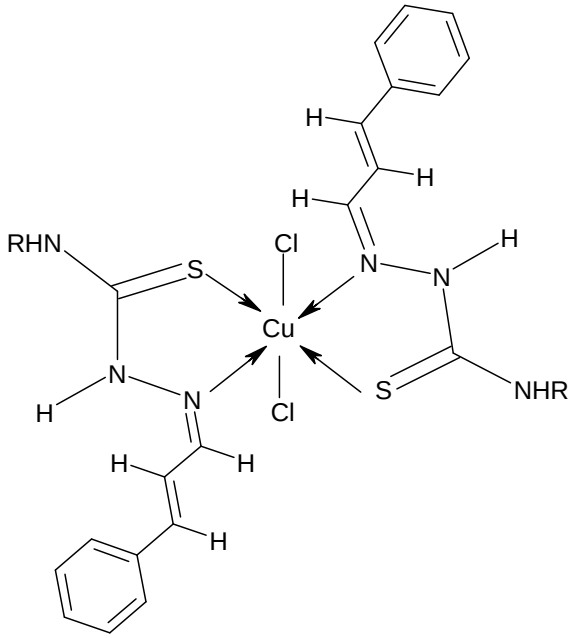


Fig. 5 A Tentative structure of [Cu(L1-4)$_2$Cl$_2$]

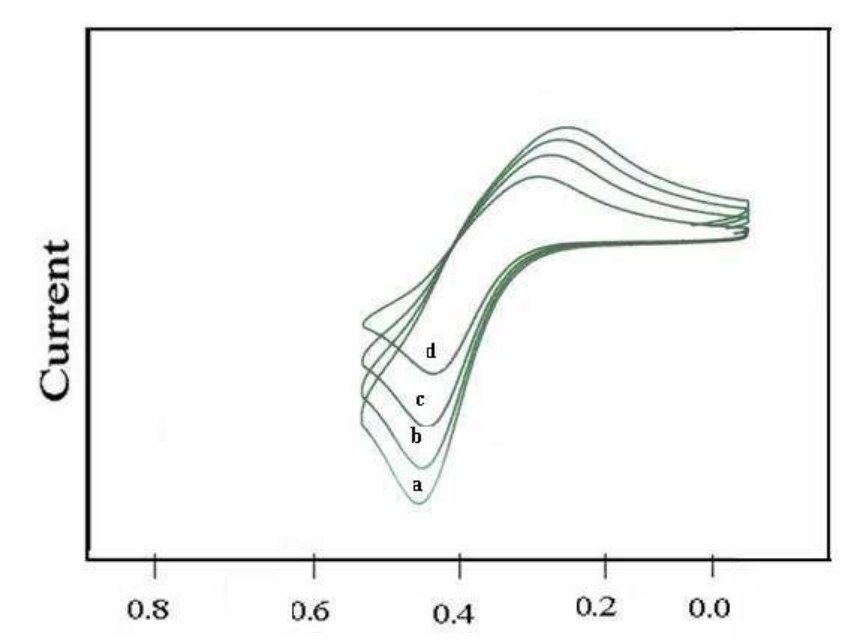


Fig. 6 Cyclic voltammetric profile of complex 4 at scan rates (a) 100 (b)75 (c) 50 and (d) 25 mVs^{-1}

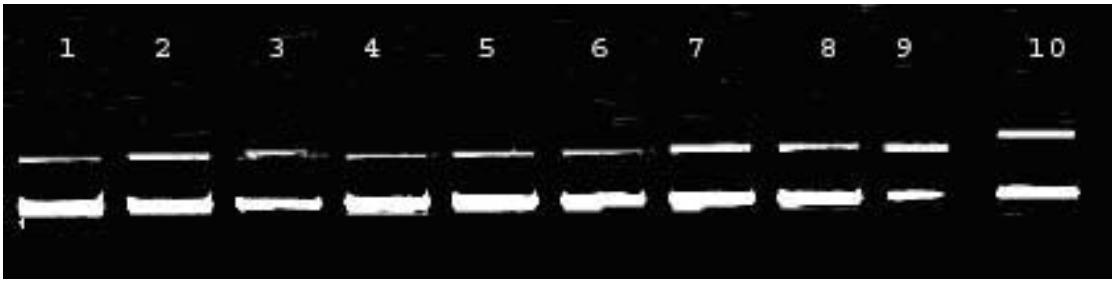


Fig. 7(a) Agarose gel (0.8%) showing results of electrophoresis of 4 µL of pUC18 DNA; 2 µL 0.1M TBE buffer (pH8); 2 µL ligand in DMF (10^{-3}M); 10 µL water, 2 µL H_2O_2 (Total volume 20 µL) were added respectively, incubated at 37^0C (30 min): Lane 1: DNA control, 2. DNA+H_2O_2, 3. HL1 + DNA, 4. HL1 + H_2O_2, 5. HL2 + DNA, 6. HL2 + H_2O_2, 7. HL3 + DNA, 8. HL3+ DNA + H_2O_2, 10. HL4 + DNA 10. HL4 + DNA + H_2O_2

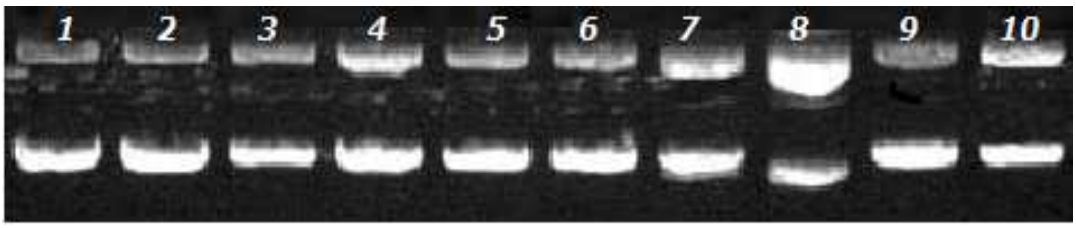


Fig. 7(b) Agarose gel (0.8%) showing results of electrophoresis of 4 µL of pUC18 DNA; 2 µL 0.1M TBE buffer (pH8); 2 µL complex in DMF (10^{-3}M); 10 µL water, 2 µL H_2O_2 (Total volume 20 µL) were added respectively, incubated at 37^0C (30 min): Lane 1: DNA control, Lane 2. DNA+H_2O_2, Lane 3. Complex 1+DNA, Lane 4. Complex 1+DNA + H_2O_2, Lane 5. Complex 2+DNA, Lane 6. Complex 2+DNA+H_2O_2, Lane 7. Complex 3+DNA, Lane 8. Complex 3+DNA+ H_2O_2, Lane 9. Complex 4+DNA, Lane 10 . Complex 4+DNA+H_2O_2.

Table.1. Analytical, melting point, conductance, magnetic susceptibility and electronic spectral data of ligands and their copper complexes

**Measured at room temperature and units $Ohm^{-1}cm^{-2}mol^{-1}$*

Ligand/ Complexes	Yield (%)	M.P (^oC)	Elemental analysis Found (Cal.)				μ_{eff} (BM)	Molar Conductance	Electronic spectral data	
			Carbon	Hydrogen	Nitrogen	Sulphur			Electronic Transition	Assignment
HL1	90	114-115	58.41 (58.50)	5.20 (5.40)	20.49 (20.48)	15.51 (15.62)	-	-	30769	Charge transfer Band
1	72	185-188	41.60 (41.34)	4.23 (4.51)	14.45 (14.46)	11.20 (11.03)	1.58	10	22222 12658	Charge transfer band d–d band
HL2	88	164-166	60.24 (59.97)	5.97 (5.63)	19.16 (19.32)	17.62 (17.99)	-	-	31250	Charge transfer Band
2	65	185-186	45.71 (46.11)	4.61 (4.57)	14.33 (14.66)	11.27 (11.19)	1.91	56	29412 13245	Charge transfer band d–d band
HL3	67	157-158	61.76 (61.47)	6.47 (5.94)	18.00 (18.03)	16.34 (16.40)	-	-	31152	Charge transfer Band
3	48	175-177	48.09 (47.95)	4.93 (5.03)	13.94 (13.98)	10.24 (10.66)	1.96	46	29412 13157	Charge transfer band d–d band
HL4	67	162-164	68.70 (68.29)	5.37 (5.37)	15.01 (14.93)	11.27 (11.39)	-	-	28571	Charge transfer band
4	52	188-190	55.13 (55.12)	4.14 (4.33)	12.22 (12.05)	9.16 (9.19)	Dia	24	28571 13333	Charge transfer band d–d band

Table 2: Spin Hamiltonian and orbital reduction parameters of Cu(II) complexes

Complex	$g_{\parallel}$	$g_{\perp}$	g_{av}	G	$K_{\parallel}$	$K_{\perp}$	$^aA_{\parallel}$ $_{(10^{-4})}$	$^aA_{\perp}$ $_{(10^{-4})}$	$^aA_{av(10^{-4})}$	P	$f = g_{\parallel}/A_{\parallel}$ cm
1	2.272	2.065	2.134	4.30	0.718	0.346	160	51	68	0.0200	142
2	2.237	2.044	2.108	5.673	0.665	0.288	130	17	55	0.0177	172
3	2.239	2.063	2.122	4.352	0.690	0.349	136	17	57	0.0155	164
4	2.327	2.131	2.196	2.530	0.808	0.508	172	-	-	-	135

[a] Units cm^{-1}

Table 3: Cyclic voltommetric data of copper complexes for the couple $Cu^{II/I}$ [a]*

Complex	Redox couple	CV cathodic, E_{pc}	CV anodic, E_{pa}	ΔE_p (mV)	$E_{1/2}$	$-i_c/i_a$	$logK_c$ [a]	$-\Delta G^{ob}$
1	II/I	0.46	0.55	90	0.505	0.821	0.363	2140
2	II/I	0.44	0.60	160	0.520	0.970	0.204	1203
3	II/I	0.485	0.645	160	0.565	0.760	0.204	1203
4	II/I	0.395	0.530	135	0.462	0.868	0.242	1427

[a] Recorded in ethanol and DMF at room temperature with (t-Bu_4NClO_4) as supporting electrolyte, glassy carbon as working electrode, Pt wire as auxiliary electrode and Ag/AgCl as reference electrode, Scan rate 50 mVs^{-1}

** Parenthesis indicates data in DMF.*

[b] $logK_c = 0.434ZF/RT\Delta E_p$ [c] $\Delta G^o = -2.303RTlogK$

Table 4 Absorption parameters of DNA binding studies

Entry	$\lambda_{max/nm}$		$\Delta\lambda$ /nm	H (%)	K_b (M^{-1})x10^7
	Free	Bound			
HL1	-	-	-	-	-
1	365	366.5	1.5	-8.41	4.15
HL2	-	-	-	-	-
2	365	360	5.0	-8.27	9.18
HL3	-	-	-	-	-
3	365	366	1.0	-33.33	1.95
HL4	-	-	-	-	-
4	365	367	2	-20.39	9.71

CONCLUSION

The utility of transition metal complexes of substituted heterocyclic thiosemicarbazones in DNA binding and destruction is very limited. In this study, we have attempted to unravel the DNA interactions and cleavage activity of these complexes. The increasing binding constants of complexes may be due the presence of phenyl ring of ligands that facilitate pi-stacking interaction. Since the complexes are found to cleave DNA even in the presence of free radical scavenger (DMSO) these may be regarded as hydrolytic cleavage agents.

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