

# New alicyclic thiosemicarbazone chelated zinc(II) antitumor complexes: Interactions with DNA/protein, nuclease activity and inhibition of topoisomerase-I



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## ABSTRACT

Two new zinc(II) complexes,  $Zn(chtsc-N-Me)_2$  and  $Zn(chtsc-N-Ph)_2$  where chtsc = cyclohexanone thiosemicarbazone; chtsc-N-Ph = cyclohexanone N(4)-phenyl thiosemicarbazone, were isolated and characterized by X-ray crystallography. The interaction of these complexes with DNA and protein were studied using calf thymus DNA (CT-DNA) and bovine serum albumin (BSA) as the respective models, and marked activity was observed. The complexes exhibited efficient DNA cleavage activity via the oxidative pathway involving singlet oxygen as the reactive oxygen species. The topoisomerase inhibition assay showed that, even at low concentrations, both  $Zn(chtsc-N-Me)_2$  and  $Zn(chtsc-N-Ph)_2$  are capable of impairing enzymatic occupation of human topoisomerase-I, a significant feature of anticancer drugs. The results of in-vitro anti-proliferation tests carried out against five different human tumor cells lines gave  $GI_{50}$  values lower than 5  $\mu g/mL$ , which indicates that these complexes are potentially advantageous as anticancer agents.

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## 1. Introduction

Cancer is a major health hazard for humankind, causing more than 8 million deaths annually, and it essentially remains an incurable disease [1–3]. Consequently, the development of anticancer drugs has emerged as an indispensable area in medicinal research [4,5]. Researchers have currently shown massive interest in molecules which are proficient in binding/cleaving DNA and protein, and inhibiting topoisomerase activity [6–8]. Cis-platin and its analogues are considered the best available medicines in cancer chemotherapy but they are associated with drug selectivity and resistivity, in addition to high toxicity [9–11]. New metal-based anticancer drugs that are less toxic, more efficient, and target specific are being developed by chemists as a result of shortcomings associated with the platinum based drugs [12–14]. Researchers are giving due focus to the chemistry of thiosemicarbazone metal complexes, since a diverse variety of compounds from this class offer a wide-ranging profile of pharmacological activity [15–17]. Thiosemicarbazone has marked medicinal effects itself

but its complexes have demonstrated even higher potency [18,19]. In the human body, the most abundant heavy metal ion after iron(III) is zinc(II) [20]. Zinc(II) plays a vital role in many cellular processes, and zinc(II) deficiency can effect sexual reproduction and development [21,22]. Furthermore, it holds great significance in the brain, where it performs precise functions, as a neuromodulator [23,24]. Considering the pharmacological value of thiosemicarbazone and the importance of zinc(II) in biological processes, in this paper we report on a study (synthesis, structure, DNA and protein binding, nuclease and topoisomerase-I inhibition activity and cytotoxicity) of two zinc(II) complexes containing alicyclic thiosemicarbazones as the chelating ligand.

## 2. Experimental

### 2.1. Reagents and measurements

The materials were purchased from following sources: 4-methyl-3-thiosemicarbazide, 4-phenyl-3-thiosemicarbazide, cyclohexanone, tris(hydroxymethyl)aminomethane (Tris), calf thymus DNA (CT-DNA), bovine serum albumin (BSA), human DNA topoisomerase-I, phosphate buffer tablets and ethidium bromide (EB) from Sigma–Aldrich (Malaysia); supercoiled pBR322 DNA from

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Fermentas Fisher Scientific (Malaysia); agarose from Vivantis (Malaysia); sodium azide ( $\text{NaN}_3$ ), sodium chloride ( $\text{NaCl}$ ), potassium iodide ( $\text{KI}$ ) from Bendosen Laboratory Chemicals (Malaysia); zinc(II) acetate dihydrate, methanol, hydrochloric acid ( $\text{HCl}$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), dimethylformamide ( $\text{DMF}$ ) and dimethyl sulfoxide ( $\text{DMSO}$ ) from Friedemann Schmidt-Thermoline (Malaysia). All supplies were analytical/HPLC grade and used without further purification. The thiosemicarbazone ligands were prepared by the methods reported earlier [25,26]. Unless otherwise stated, all experiments were carried using solutions of complex in Tris–HCl buffer (5 mM Tris–HCl, 50 mM NaCl, pH 7.2) containing 10% DMF. Concentration and purity of CT-DNA were assessed according to literature methods [27,28]. The ultrapure water with 18.2 M $\Omega$  cm specific resistance was produced by a Cascade LS Ultrapure water system (Pall Corp., USA).

Elemental analysis was carried out on a Perkin Elmer CHNS/O 2400 Series II CHN Analyzer (PerkinElmer, Malaysia). Molar conductance were measured on a Eutech CyberScan CON 510 digital conductivity meter. Infrared spectra were recorded on a Thermo Scientific Nicolet iS10 spectrophotometer (Thermo Scientific, Malaysia). NMR spectra were acquired on a Bruker Avance III spectrometer (Bruker (M) Sdn. Bhd., Malaysia). Fluorescence spectra were measured on a Horiba FluoroMax-4 spectrophotometer (ALV Technologies (M) Sdn Bhd). Viscosity measurements were carried out using a LAUDA iVisc system (Fischer-Intermass (M) Sdn Bhd). Gel imaging was performed with a Syngene gel documentation system (V-BioScience (M) Sdn Bhd).

## 2.2. Synthesis

### 2.2.1. Preparation of $\text{Zn}(\text{chtsc-N-Me})_2$

To a solution of thiosemicarbazone (0.76 mmol) in methanol (15 mL) was added solid zinc(II) acetate dihydrate (0.38 mmol) and the mixture stirred for 5 min. The resulting clear solution was sealed in a small beaker and kept in dark at room temperature for 2 d to give colorless crystals of  $\text{Zn}(\text{chtsc-N-Me})_2$ . Elemental Anal. Calc. for  $\text{C}_{16}\text{H}_{28}\text{N}_6\text{S}_2\text{Zn}$ : C, 44.28; N, 19.37; H, 6.50. Found: C, 44.19; N, 19.09; H, 6.38%. IR: 3348,  $\nu(\text{NH})$ ; 3030, 2921, 2892,  $\nu(\text{CH})$ ; 1572,  $\nu(\text{CN})$ ; 830,  $\nu(\text{CS})$ .  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm, numbering as in Fig. 1): 5.15 (1H, br s,  $\text{N}^3\text{H}$ ), 3.15 (3H, d,  $\text{CH}_3$ ), 2.75 (2H, t,  $\text{C}^1\text{-}^5\text{H}_2$ ), 2.42 (2H, t,  $\text{C}^1\text{-}^5\text{H}_2$ ), 1.59–1.68 (6H, m,

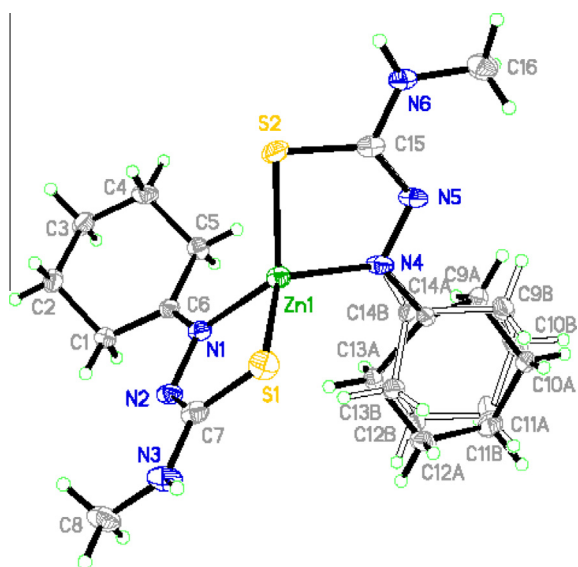


Fig. 1. The structure of  $\text{Zn}(\text{chtsc-N-Me})_2$  along with the atom numbering scheme.

$\text{C}^{2,3}$ ,  $^4\text{H}_2$ ). Molar conductivity ( $10^{-3}$  M, 10% DMF/water): 23  $\text{S cm}^2 \text{mol}^{-1}$ .

### 2.2.2. Preparation of $\text{Zn}(\text{chtsc-N-Ph})_2$

Colorless crystals of  $\text{Zn}(\text{chtsc-N-Ph})_2$  were obtained using the same method. Elemental Anal. Calc. for  $\text{C}_{26}\text{H}_{32}\text{N}_6\text{S}_2\text{Zn}$ : C, 55.95; N, 15.06; H, 5.78. Found: C, 55.91; N, 15.09; H, 5.73%. IR: 3418,  $\nu(\text{NH})$ ; 2934, 2866,  $\nu(\text{CH})$ ; 1595,  $\nu(\text{CN})$ ; 847,  $\nu(\text{CS})$ .  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm, numbering as in Fig. 2): 7.25–7.59 (5H, m, Ph), 6.65 (1H, br s,  $\text{N}^1\text{H}$ ), 2.78 (2H, t,  $\text{C}^1\text{-}^5\text{H}_2$ ), 2.47 (2H, t,  $\text{C}^1\text{-}^5\text{H}_2$ ), 1.61–1.69 (6H, m,  $\text{C}^{2,3}$ ,  $^4\text{H}_2$ ). Molar conductivity ( $10^{-3}$  M, 10% DMF/water): 27  $\text{S cm}^2 \text{mol}^{-1}$ .

## 2.3. X-ray crystallography

The data were collected on a Bruker SMART APEX II CCD diffractometer [29] equipped with a graphite monochromatized  $\text{Mo K}\alpha$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ) at 100(1) K. Multi-scan absorption corrections were applied using the SADABS program [29]. The structure was solved by the direct method using the SHELXS-2008 program [30]. Refinements on  $F^2$  were performed using SHELXL-2014 by the full-matrix least-squares method with anisotropic thermal parameters for all non-hydrogen atoms. Selected crystal data and data collection parameters are presented in Table 1.

## 2.4. DNA binding and cleavage experiments

To a sample containing 4  $\mu\text{M}$  EB and 10  $\mu\text{M}$  CT-DNA in Tris–HCl buffer (pH 7.2), increasing amounts of zinc(II) complex were added. After each addition, the sample was mixed and allowed to equilibrate for 15 min, and then the fluorescence emission spectra were recorded. Emission spectra were recorded between 540 and 700 nm with the excitation wavelength set at 525 nm. Viscosity experiments were carried out on a micro-Ubbelodde viscometer, immersed in a thermostatic water bath maintained at a constant temperature of 30 ( $\pm 0.1$ )  $^\circ\text{C}$ . Data were presented as  $(\eta/\eta_0)^{1/3}$  versus  $[\text{complex}]/[\text{DNA}]$ , where  $\eta_0$  and  $\eta$  represent the viscosity of the CT-DNA solution in the absence and presence of the complexes, respectively. Viscosity values were calculated according to the relation  $\eta = (t - t_0)/t_0$ , where  $t$  is the flow time of samples containing CT-DNA and  $t_0$  that of the buffer alone.

The cleavage of plasmid DNA was monitored using agarose gel electrophoresis. Supercoiled pBR322 DNA (0.5  $\mu\text{g}/\mu\text{l}$ ) was treated with complex (5  $\mu\text{M}$ ) in the presence of  $\text{H}_2\text{O}_2$  (30  $\mu\text{M}$ ). For the inhibition reaction, the scavenging reagent (500  $\mu\text{M}$ ) was added prior to the addition of the complex and  $\text{H}_2\text{O}_2$ . The samples were incubated for 15 min at 37  $^\circ\text{C}$ , followed by addition of loading buffer and electrophoresed for 1 h at 80 V on a 1% agarose gel. The gel was stained with EB, rinsed with water and photographed under UV light.

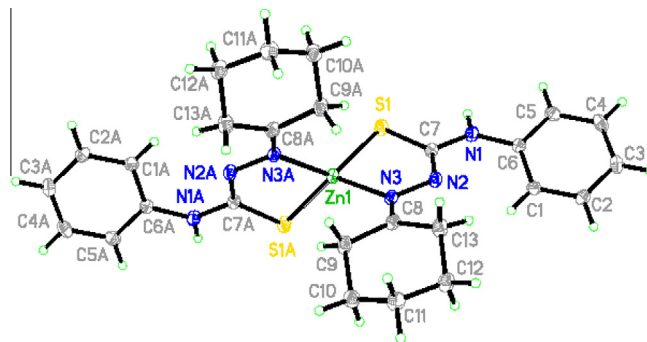


Fig. 2. The structure of  $\text{Zn}(\text{chtsc-N-Ph})_2$  along with the atom numbering scheme.

**Table 1**  
Crystallographic data for Zn(chtsc-N-Me)<sub>2</sub> and Zn(chtsc-N-Ph)<sub>2</sub>.

	Zn(chtsc-N-Me) <sub>2</sub>	Zn(chtsc-N-Ph) <sub>2</sub>
Formula	C <sub>16</sub> H <sub>28</sub> N <sub>6</sub> S <sub>2</sub> Zn	C <sub>26</sub> H <sub>32</sub> N <sub>6</sub> S <sub>2</sub> Zn
Fw	433.93	558.06
T (K)	100 (1)	100 (1)
Crystal system	monoclinic	monoclinic
Space group	P2 <sub>1</sub> /c	C2/c
a (Å)	16.386(2)	24.551(3)
b (Å)	9.2226(12)	7.1948(7)
c (Å)	13.6407(18)	16.412(2)
β (°)	104.087(2)	117.424(4)
V (nm <sup>3</sup> )	1999.4(4)	2573.2(5)
Z	4	4
Dc (Mg m <sup>-3</sup> )	1.442	1.441
F(000)	912	1168
Crystal size (mm)	0.03 × 0.19 × 0.54	0.03 × 0.05 × 0.38
θ range (°)	2.6–32.6	1.9–29.9
hkl ranges	–23 < h < 24 –10 < k < 13 –20 < l < 20	–31 < h < 34 –10 < k < 10 –23 < l < 22
Data/parameters	7173, 291	3716, 163
Goodness-of-fit on $\chi^2$	1.03	1.05
Final R indices	R <sub>1</sub> = 0.0373 [I > 2σ(I)] wR <sub>2</sub> = 0.0949	R <sub>1</sub> = 0.0275 wR <sub>2</sub> = 0.0664
Highest peak/ deepest hole	Δρ <sub>max</sub> = 0.51 e Å <sup>-3</sup> / Δρ <sub>min</sub> = –0.41 e Å <sup>-3</sup>	Δρ <sub>max</sub> = 0.38 e Å <sup>-3</sup> / Δρ <sub>min</sub> = –0.26 e Å <sup>-3</sup>

### 2.5. BSA binding experiments

To the solutions of BSA (2 μM) in a phosphate buffer at pH 7.2 were added increments of the quencher, and the emission signals at 344 nm (excitation wavelength at 295 nm) were recorded after each addition of the quencher. Plots of  $F/F_0$  versus [complex] were constructed, and the quenching constants were determined by linear fitting of the data according to the equation  $F_0/F = 1 + K_{BSA}[\text{complex}]$ , where  $F_0$  is the fluorescence intensity of BSA in the absence of the complex,  $F$  is the fluorescence intensity of BSA in the presence of the complex,  $K_{BSA}$  is the Stern–Volmer quenching constant, and [complex] is the complex concentration.

### 2.6. Topoisomerase inhibition assay

Increasing amounts of the complex (2 μL) were treated with a mixture of 0.1 μg/μL pBR322 DNA and 1 unit of Topoisomerase-I in 8 μL buffer (35 mM Tris–HCl, 72 mM KCl, 5 mM MgCl<sub>2</sub>, 5 mM DTT, 2 mM spermidine, 0.1 mg/mL BSA, pH 8.0). Each sample was aged at 37 °C for 30 min and the reaction was terminated by the addition of loading buffer. The samples were electrophoresed through 1% agarose at 35 V for 8 h.

### 2.7. General in-vitro anti-proliferation assay procedure

The following cell lines were subjected to in-vitro antitumor screening: Caki-2, MCF-7, CaSki, NCI-H322M and Co-115. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and antibiotics, in 96-well culture plates, at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> in a CO<sub>2</sub> incubator. The proliferation of the cells upon treatment with the complex was determined following procedures described previously [31]. In brief, the cultures were exposed to serial dilutions of the complex in DMSO over 48 h and stained with SRB for 20 min. The values of GI<sub>50</sub>, the drug concentration resulting in a 50% reduction in the net protein increase, were calculated from  $[(Ti - Tz)/(C - Tz)] \times 100 = 50$ , where Tz, C and Ti corresponds to time zero, control growth, and test growth in the presence of the drug at various concentration levels.

## 3. Results and discussion

### 3.1. General aspects

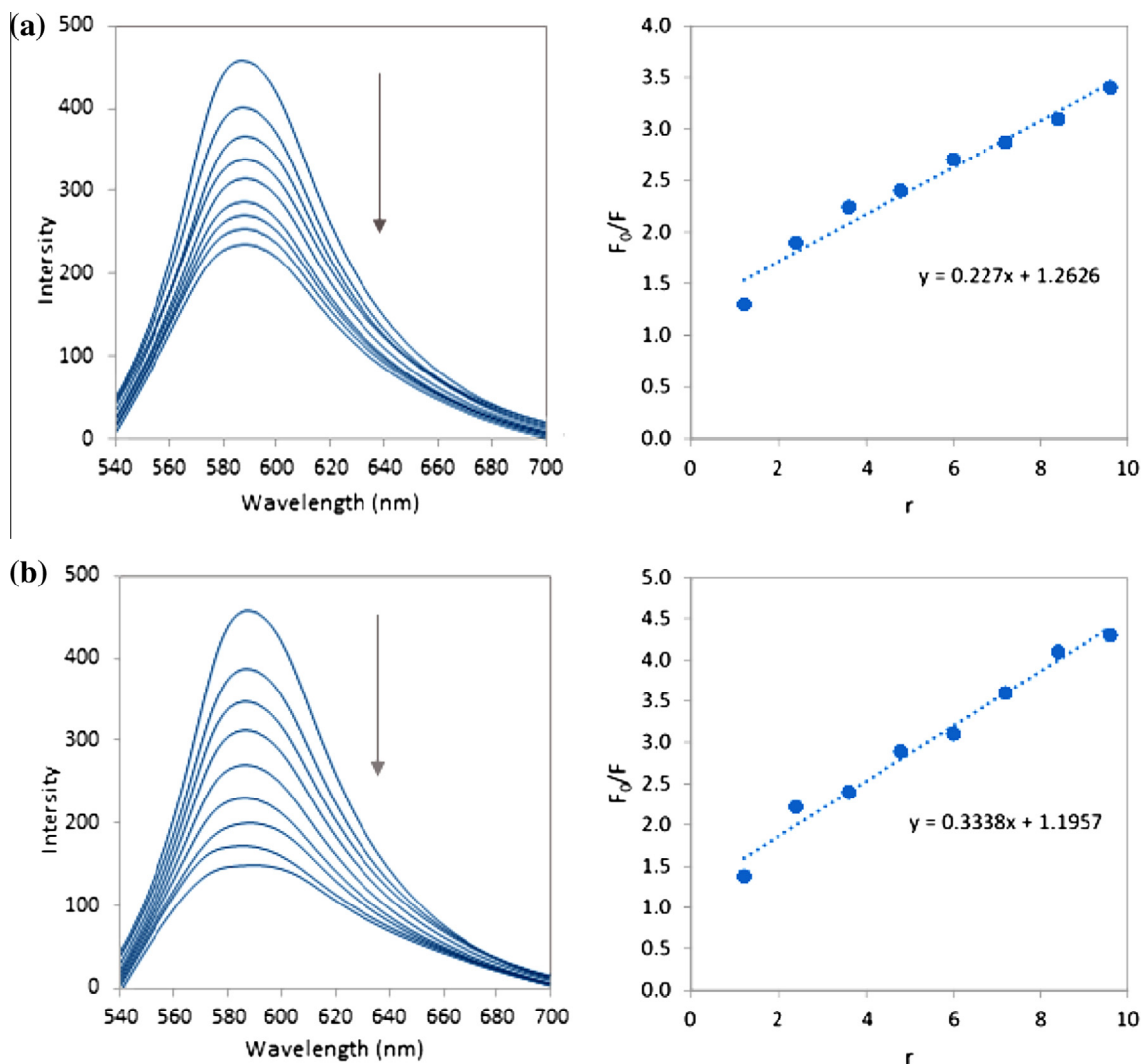
The reaction of the thiosemicarbazones (CH<sub>2</sub>)<sub>5</sub>C=NN(H)C(=S)NHR (R = Me, Ph) with zinc(II) acetate in methanolic solutions proceeds readily under room temperature to form stable mononuclear complexes Zn[(CH<sub>2</sub>)<sub>5</sub>C=NN=C(S)NHR]<sub>2</sub> (R = Me, Ph). The complexes were characterized by CHN, FTIR and <sup>1</sup>H NMR and their structure was confirmed by single crystal X-ray diffraction. Both complexes are sparingly soluble in water but dissolve readily in polar solvents such as methanol, ethanol, DMF and DMSO; their crystals and solution are stable under normal laboratory conditions.

### 3.2. Structure

The molecular structure of Zn(chtsc-N-Me)<sub>2</sub> and Zn(chtsc-N-Ph)<sub>2</sub>, as well as their atom numbering scheme is given in Figs. 1 and 2, respectively. Both complexes presented a tetrahedral geometry that was slightly distorted around the zinc(II) atom with the basal plane occupied by two sulfur and two nitrogen atoms of the two thiosemicarbazone ligands. The distortion in tetrahedral geometry is evident from the following bond angles and bond distances: Zn(chtsc-N-Me)<sub>2</sub>: N1–Zn1–N4 123.69(6)°, N1–Zn1–S2 121.12(4)°, N4–Zn1–S2 86.70(5)°, N1–Zn1–S1 87.30(4)°, N4–Zn1–S1 116.43(5)°, S2–Zn1–S1 125.79(2)°, Zn1–N1 2.0406(16) Å, Zn1–N4 2.0445(16) Å, Zn1–S2 2.2793(5) Å and Zn1–S1 2.2803(6) Å; Zn(chtsc-N-Ph)<sub>2</sub>: N3–Zn1–N3 114.10(7)°, N3–Zn1–S1 125.18(4)°, N3–Zn1–S1 87.52(3)°, N3–Zn1–S1 87.53(3)°, N3–Zn1–S1 125.18(4)°, S1–Zn1–S1 121.33(2)°, Zn1–N3 2.0450(12) Å and Zn1–S1 2.2766(4) Å.

### 3.3. DNA binding

The binding of the complexes to calf thymus DNA was studied using the fluorescence spectral technique using the emission intensity of EB bound to CT-DNA. Free EB is non-emissive but, when bound to DNA through intercalation, it can emit intense fluorescence [32,33]. However, the intense fluorescence of the EB–DNA complex can be quenched by the addition of a new molecule such as complexes [34]. The amount of emission quenched can be used to determine the extent of binding between the added new molecule and DNA [35]. As shown in the left column of Fig. 3 (a) and (b), the emission intensities of EB–DNA complex at 584 nm show a remarkable declining trend with the increasing concentration of the zinc(II) complexes, indicating clearly the binding of the complexes to DNA. In order to determine the quenching efficiency, the linear Stern–Volmer equation is employed [36],  $F_0/F = 1 + K_{sq}r$ , where  $F_0$  and  $F$  represent the fluorescence intensities in the absence and presence of the complex, respectively, and  $r$  corresponds to the concentration ratio of the complex to DNA.  $K_{sq}$  is a linear Stern–Volmer quenching constant and is obtained from the linear regression of  $F_0/F$  with  $r$  [right column of Fig. 3(a) and (b)]. The  $K_{sq}$  values obtained are 0.227 for Zn(chtsc-N-Me)<sub>2</sub> and 0.334 for Zn(chtsc-N-Ph)<sub>2</sub>, following the order Zn(chtsc-N-Ph)<sub>2</sub> > Zn(chtsc-N-Me)<sub>2</sub>. The apparent binding constant ( $K_{app}$ ) is calculated using the equation  $K_{EB}[\text{EB}] = K_{app}[\text{complex}]$ , where [EB] = 4.0 μM,  $K_{EB} = 1.0 \times 10^7 \text{ M}^{-1}$  and [complex] is the value at 50% reduction of the fluorescence intensity of EB. The  $K_{app}$  value for Zn(chtsc-N-Me)<sub>2</sub> is  $5.44 \times 10^5 \text{ M}^{-1}$  while that for Zn(chtsc-N-Ph)<sub>2</sub> is  $8.19 \times 10^4 \text{ M}^{-1}$ . Comparatively, the better DNA affinity of Zn(chtsc-N-Ph)<sub>2</sub> is due to the presence of the planar phenyl groups which facilitate hydrophobic interactions with DNA [37].

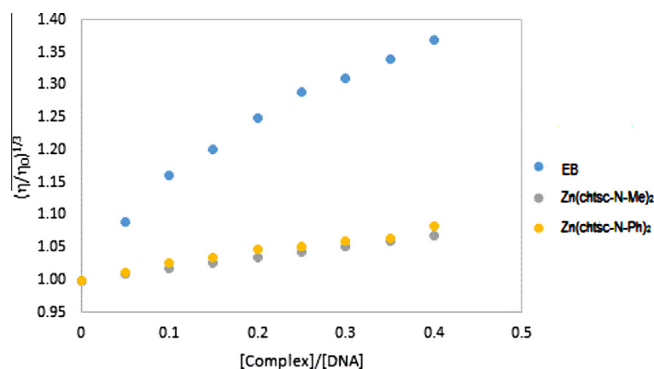


**Fig. 3.** Effect of the addition of Zn(ghtsc-N-Me)<sub>2</sub> (a) and Zn(ghtsc-N-Ph)<sub>2</sub> (b) on the emission intensity of EB (4  $\mu$ M) bound to CT-DNA (10  $\mu$ M) and their respective ( $F_0/F$ ) vs. [complex] plots.

To understand the interaction mode between zinc(II) complexes and CT-DNA, viscosity measurements of DNA were made. In classical intercalation, the DNA helix elongates as the base pairs are separated to lodge the bound ligand leading to a significant increase in DNA viscosity, while in groove binding or the electrostatic mode, the length of the DNA chain is unaffected and, therefore, there is no apparent change in DNA viscosity [38]. Because of these clear indications, viscosity measurement is viewed as the least ambiguous and the most critical technique for studying the binding mode between metal complexes and DNA [39,40]. The effects of the zinc (II) complexes on the viscosity of CT-DNA are shown in Fig. 4. Upon increasing the amount of the complexes, the relative viscosity of CT-DNA increases slightly, which rules out the intercalative binding mode. It can be confirmed that groove binding is the preferred mode of interaction of these zinc(II) complexes because these complexes are uncharged and therefore electrostatically innocent. The DNA viscosity increase, although minor, follows the order of Zn(ghtsc-N-Ph)<sub>2</sub> > Zn(ghtsc-N-Me)<sub>2</sub> which is consistent with the binding affinity results.

### 3.4. DNA cleavage

Gel electrophoresis experiments were performed using pBR322 DNA to ascertain the ability of Zn(ghtsc-N-Me)<sub>2</sub> and Zn



**Fig. 4.** Effect of increasing amount of the EB, Zn(ghtsc-N-Me)<sub>2</sub> and Zn(ghtsc-N-Ph)<sub>2</sub> on the relative viscosities of CT-DNA at 30 °C, [DNA] = 200  $\mu$ M.

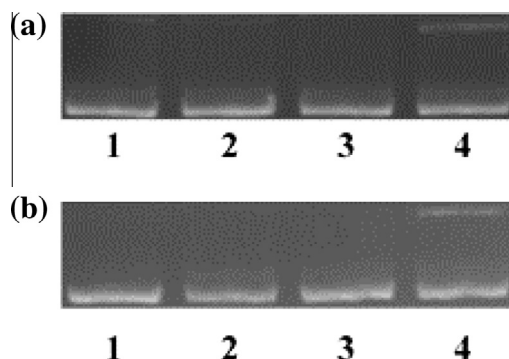
(ghtsc-N-Ph)<sub>2</sub> to serve as artificial nucleases. The naturally occurring supercoiled form (Form I), when nicked, gives rise to an open circular relaxed form (Form II) and upon further cleavage, results in the linear form (Form III). When subjected to gel electrophoresis,

relatively fast migration is observed for Form I. Form II migrates slowly and Form III migrates between Forms I and II [41]. The electrophoretogram of DNA after being treated with  $\text{Zn}(\text{chtsc-N-Me})_2$  and  $\text{Zn}(\text{chtsc-N-Ph})_2$  is shown in Fig. 5. As expected, control samples, free DNA and DNA treated with either hydrogen peroxide or complex fail to show evidence of DNA cleavage (lanes 1–3). However, when the complexes and hydrogen peroxide are together significant nuclease activity is observed at concentrations as low as 5  $\mu\text{M}$ , (evident from the formation of Form II, lane 4) respectively.

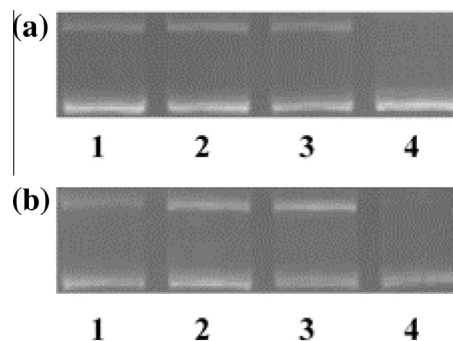
Upon the addition of scavengers of reactive oxygen species (hydroxyl radical scavenger, DMSO [42]; superoxide scavenger, KI [43]; singlet oxygen scavenger,  $\text{NaN}_3$  [44]) to the reaction mixture (Fig. 6(a) and (b)), it was found that DNA cleavage by  $\text{Zn}(\text{chtsc-N-Me})_2$  and  $\text{Zn}(\text{chtsc-N-Ph})_2$  was still active in the presence of DMSO (lane 2) and KI (lane 3) suggesting that hydroxyl and superoxide radicals are absent in the cleavage reaction. However, the cleavage reactions were found to be inhibited by singlet oxygen scavengers  $\text{NaN}_3$  (lane 4) and this reveals that  $\text{Zn}(\text{chtsc-N-Me})_2$  and  $\text{Zn}(\text{chtsc-N-Ph})_2$  can promote DNA cleavage by generating singlet oxygen.

### 3.5. Protein binding

Serum albumin is the protein which helps to solubilize drugs in plasma and controls their transport to the cell [6,45]. Hence, it is of importance to study the protein interaction of every drug candidate. The interaction magnitude between drug and protein influences biological actions such as delivery rate and efficacy of drug. The interaction between serum albumin and the zinc(II) complexes was investigated by tryptophan emission-quenching experiments using BSA as the protein model. BSA is emissive in a buffer medium due to the presence of tryptophan residues. The emission intensity depends on the extent of exposure of the tryptophan residues to the surrounding polar environment and to the quenching groups such as tyrosinate, protonated imidazole, protonated carbonyl, and anions through molecular interaction [46]. The emission intensity of BSA has been found to decay with a gradual increase in the concentration of the complexes (Fig. 7(a) and (b)). The reason is related perturbations in the secondary structure of BSA leading to the exposure of the tryptophan residues to the immediate polar environment [47]. From the molecular structure viewpoint, the bulky cyclohexane groups at the terminal of the complexes might be the protein anchoring tool through hydrophobic interaction, and this is supported by the fact that  $\text{Zn}(\text{chtsc-N-Ph})_2$ , which has an additional hydrophobic moiety, phenyl, scored a higher BSA affinity than  $\text{Zn}(\text{chtsc-N-Me})_2$  bearing the hydrophile, methyl (Fig. 7(c), Table 2). Being avid protein



**Fig. 5.** Gel electrophoretograms showing the cleavage of pBR322 DNA (0.5  $\mu\text{g}/\mu\text{l}$ ) by  $\text{Zn}(\text{chtsc-N-Me})_2$  (a) and  $\text{Zn}(\text{chtsc-N-Ph})_2$  (b) after incubation at 37  $^\circ\text{C}$  for 15 min. Lane 1, DNA control; lane 2, DNA +  $\text{H}_2\text{O}_2$  (30  $\mu\text{M}$ ); lane 3, DNA + complex (5  $\mu\text{M}$ ); lane 4, DNA +  $\text{H}_2\text{O}_2$  (30  $\mu\text{M}$ ) + complex (5  $\mu\text{M}$ ).



**Fig. 6.** Gel electrophoretograms showing the cleavage of pBR322 DNA (0.5  $\mu\text{g}/\mu\text{l}$ ) by  $\text{Zn}(\text{chtsc-N-Me})_2$  (a) and  $\text{Zn}(\text{chtsc-N-Ph})_2$  (b) with different scavenging agents (incubation conditions and concentrations of complex and  $\text{H}_2\text{O}_2$  were identical to those in Fig. 6). Lane 1, DNA + complex +  $\text{H}_2\text{O}_2$ ; Lanes 2–4, DNA + complex +  $\text{H}_2\text{O}_2$  + 500  $\mu\text{M}$  DMSO; KI;  $\text{NaN}_3$ .

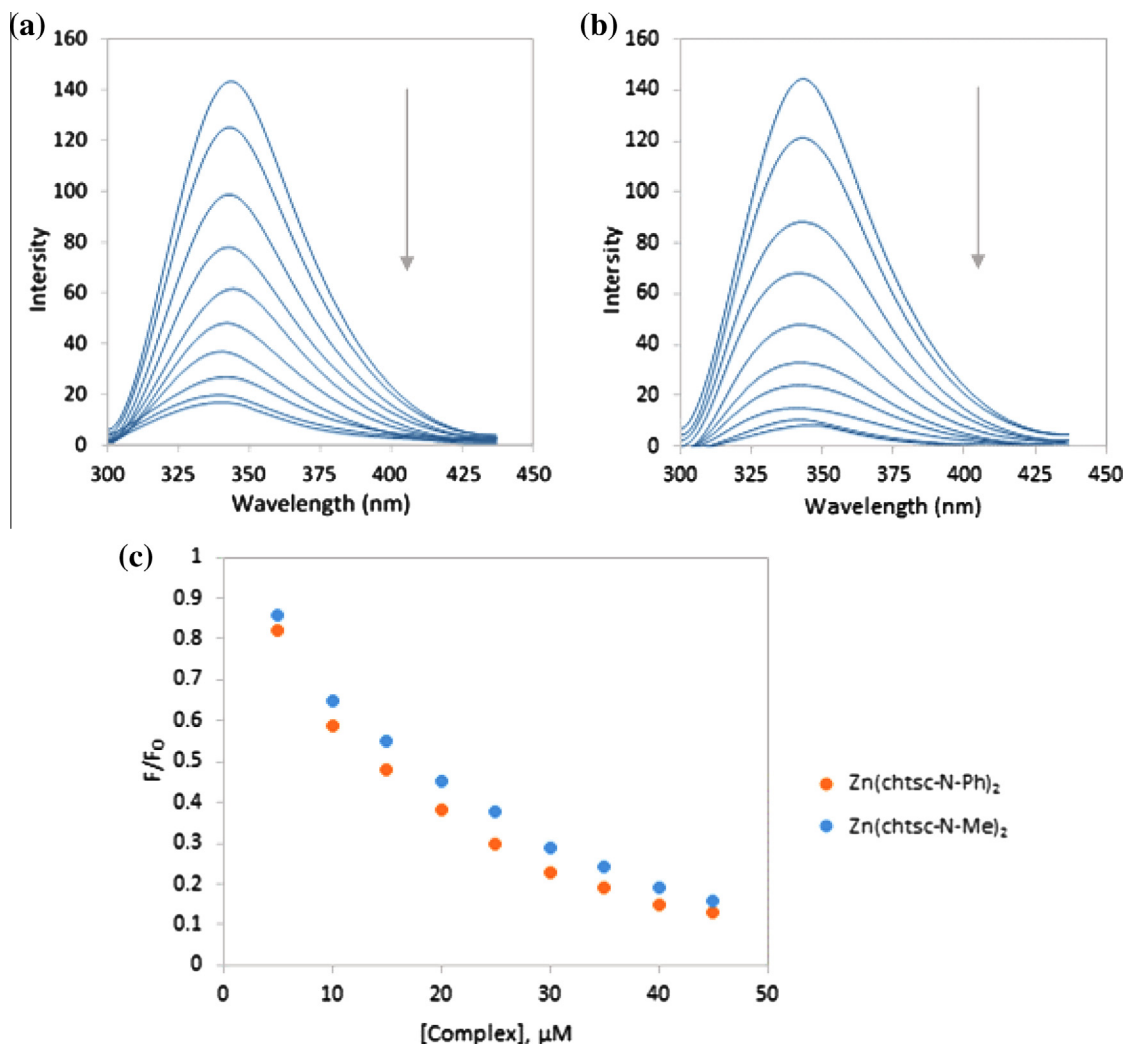
binders, these complexes offer several benefits, such as enhanced lifespan in physiological conditions and precise drug delivery.

### 3.6. Topoisomerase inhibition

Topoisomerases are responsible to solve topological predicaments and optimize functions of DNA [48,49]. Elevated levels of topoisomerase are associated with most cancer cells (esophagus, prostate, ovary, colon and kidney) because of their indispensable role in the rapid and unrestricted proliferation of cells [50]. Treatment of cancer cells with topoisomerase inhibitors leads to programmed cell death (apoptosis) as a result of DNA fragmentation, which interferes with eukaryotic topoisomerase action [51]. In order to assess the ability of  $\text{Zn}(\text{chtsc-N-Me})_2$  and  $\text{Zn}(\text{chtsc-N-Ph})_2$  to act as topoisomerase-I inhibitors, a DNA cleavage assay using pBR322 DNA and human topoisomerase-I was performed. If the compounds affect topoisomerase-I in unwinding the supercoiled DNA to nicked DNA, it can be detected directly from this assay. As shown in Fig. 8(a) and (b), when the complexes were absent (lane 2), topoisomerase-I nicked pBR322 DNA completely. However, the amounts of nicked form diminished gradually (lanes 3–6) with increasing amounts of  $\text{Zn}(\text{chtsc-N-Me})_2$  and  $\text{Zn}(\text{chtsc-N-Ph})_2$  (5–12.5  $\mu\text{M}$ ), and finally at 15.0  $\mu\text{M}$ , topoisomerase-I lost its function totally (evident from disappearance of the nicked band, lane 7). It can be deduced from here that the topoisomerase-I inhibition of  $\text{Zn}(\text{chtsc-N-Me})_2$  and  $\text{Zn}(\text{chtsc-N-Ph})_2$  is concentration-dependent with a potency considerably greater than some of the classical topoisomerase-I inhibitors used as antitumor drugs in clinical practice [52–54].

### 3.7. Antitumor activity

The in vitro antitumor activity of  $\text{Zn}(\text{chtsc-N-Me})_2$  and  $\text{Zn}(\text{chtsc-N-Ph})_2$  was screened on a panel of human tumor cell lines of distinct tissue origin viz., Caki-2 (Kidney), MCF-7 (Breast), CaSki (Cervix), NCI-H322M (Lung) and Co-115 (Colon). The cellular proliferation was assessed by Sulforhodamine-B (SRB) assay; the results in terms of  $\text{GI}_{50}$  values are given in Table 3. Complexes  $\text{Zn}(\text{chtsc-N-Me})_2$  and  $\text{Zn}(\text{chtsc-N-Ph})_2$  were, in general, highly active against all of the five cell lines, with  $\text{GI}_{50}$  values in the range of 0.77–4.7  $\mu\text{g}/\text{mL}$ . Comparatively,  $\text{Zn}(\text{chtsc-N-Me})_2$  showed better activity on Co-115 and NCI-H322M whereas  $\text{Zn}(\text{chtsc-N-Ph})_2$  showed better activity on MCF-7, CaSki and Caki-2. The reason for their selectivity is unclear and, thus, warrants further in-depth investigation particularly in terms of the structure–activity relationship.



**Fig. 7.** Effect of addition of Zn(chtsc-N-Me)<sub>2</sub> (a) and Zn(chtsc-N-Ph)<sub>2</sub> (b) on the emission intensity of the BSA (50 μM) at different concentrations; plots of relative integrated emission intensity ( $F/F_0$ ) vs. [complex] for Zn(chtsc-N-Me)<sub>2</sub> and Zn(chtsc-N-Ph)<sub>2</sub> (c).

**Table 2**

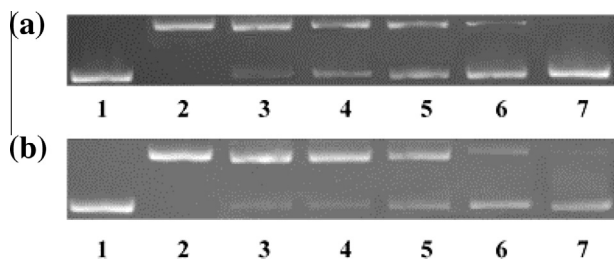
Stern–volmer quenching constants for the interaction of Zn(chtsc-N-Me)<sub>2</sub> and Zn(chtsc-N-Ph)<sub>2</sub> with BSA.

Complex	$K_{BSA}$
1	$3.40 \times 10^5$
2	$3.95 \times 10^5$

**Table 3**

The GI<sub>50</sub> values for Zn(chtsc-N-Me)<sub>2</sub> and Zn(chtsc-N-Ph)<sub>2</sub> against Caki-2 (kidney), MCF-7 (Breast), CaSki (Cervix), NCI-H322M (Lung) and Co-115 (Colon) cell lines.

Complex	GI <sub>50</sub> value (μg/mL)				
	Caki-2	MCF-7	CaSki	NCI-H322M	Co-115
Zn(chtsc) <sub>2</sub>	4.4	4.7	4.5	0.77	1.3
Zn(chtsc-N-Ph) <sub>2</sub>	2.7	3.2	1.6	3.9	4.3



**Fig. 8.** Gel electrophoretograms showing the effect of different concentrations of Zn(chtsc-N-Me)<sub>2</sub> (a) and Zn(chtsc-N-Ph)<sub>2</sub> (b) on the activity of topoisomerase-I. Lane 1, DNA control; lane 2, topoisomerase-I + DNA; lane 3, 5 μM of complex + DNA + topoisomerase-I; lane 4: 7.5 μM of complex + DNA + topoisomerase-I; lane 5: 10 μM of complex + DNA + topoisomerase-I; lane 6: 12.5 μM of complex + DNA + topoisomerase-I; lane 7: 15 μM of complex + DNA + topoisomerase-I.

#### 4. Conclusion

Two zinc(II) complexes containing cyclohexanone N(4)-methyl thiosemicarbazone and cyclohexanone N(4)-phenyl thiosemicarbazone were synthesized and their structures confirmed by X-ray diffraction studies. The new complexes were tested for their binding on CT-DNA and BSA. The complexes, which bound to DNA by the groove binding mode, had a strong binding affinity towards BSA. They cleaved pBR322 DNA via the oxidative pathway and exhibited high inhibition activity against topoisomerase-I, even at low concentrations. The in-vitro antiproliferative activity of the complexes examined on a panel of human tumor cell lines of different histotypes showed promising antitumor effects.

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## Appendix A. Supplementary data

CCDC with numbers 1405552 for Zn(chtsc-N-Me)<sub>2</sub> and CCDC 1405554 for Zn(chtsc-N-Ph)<sub>2</sub>. These 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) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

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