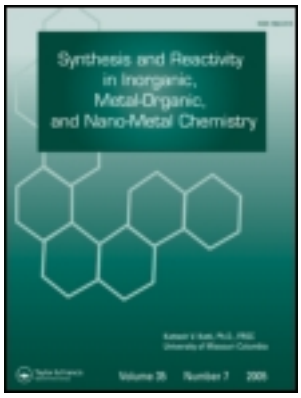


Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsrt20

Zn(II)ferrocenylthiosemicarbazones: DNA Binding and Nuclease Activity

Vikneswaran Rajamuthy [a], Siang Guan Teoh [a], Amin Malik Shah Abdul Majid [b], Chin Sing Yeap [c], Hoong-Kun Fun [c], Chew Hee Ng [d] & Seik Weng Ng [e]

[a] School of Chemical Sciences, Universiti Sains Malaysia, Penang, Malaysia

[b] School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia

[c] X-ray Crystallography Unit, School of Physics, Universiti Sains Malaysia, Penang, Malaysia

[d] Faculty of Science, Universiti Tunku Abdul Rahman, Perak, Malaysia

[e] Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia

Accepted author version posted online: 09 Oct 2012.Published online: 28 Dec 2012.

To cite this article: Vikneswaran Rajamuthy , Siang Guan Teoh , Amin Malik Shah Abdul Majid , Chin Sing Yeap , Hoong-Kun Fun , Chew Hee Ng & Seik Weng Ng (2013) Zn(II)ferrocenylthiosemicarbazones: DNA Binding and Nuclease Activity, Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry, 43:2, 149-156, DOI: 10.1080/15533174.2012.680138

To link to this article: http://dx.doi.org/10.1080/15533174.2012.680138

Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry, 43:149–156, 2013

ISSN: 1553-3174 print / 1553-3182 online
DOI: 10.1080/15533174.2012.680138

Taylor & Francis
Taylor & Francis Group

Zn(II)ferrocenylthiosemicarbazones: DNA Binding and Nuclease Activity

Vikneswaran Rajamuthy,[1] Siang Guan Teoh,[1] Amin Malik Shah Abdul Majid,[2] Chin Sing Yeap,[3] Hoong-Kun Fun,[3] Chew Hee Ng,[4] and Seik Weng Ng[5]

[1]*School of Chemical Sciences, Universiti Sains Malaysia, Penang, Malaysia*
[2]*School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia*
[3]*X-ray Crystallography Unit, School of Physics, Universiti Sains Malaysia, Penang, Malaysia*
[4]*Faculty of Science, Universiti Tunku Abdul Rahman, Perak, Malaysia*
[5]*Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia*

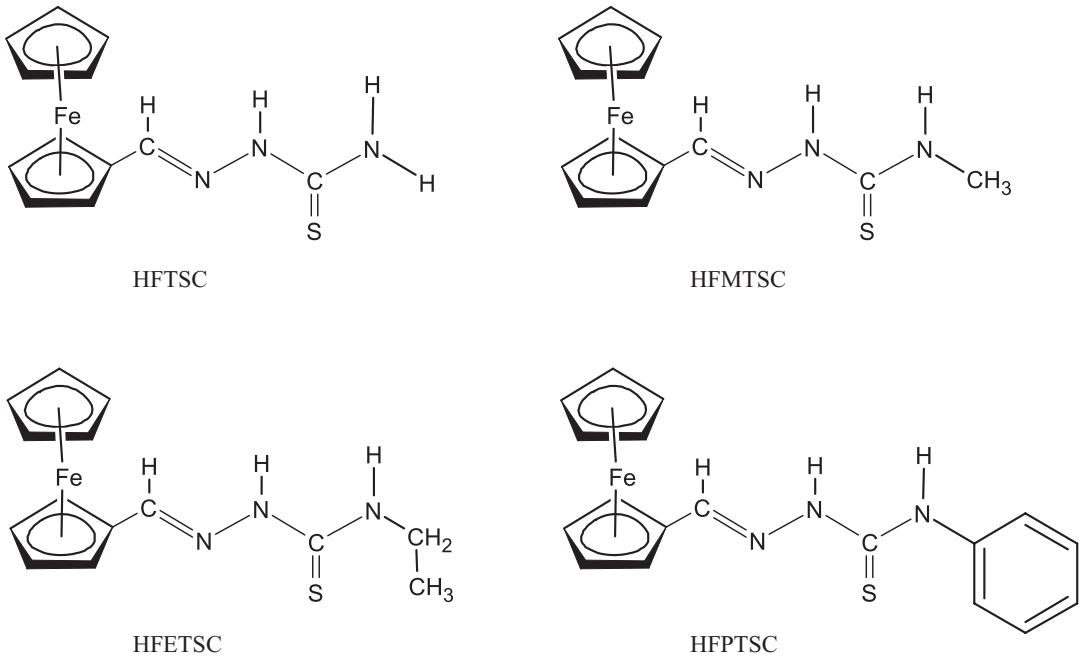
A series of Zn(II) ferrocenylthiosemicarbazones complexes derived from thiosemicarbazide and 4-methyl-, 4-ethyl-, and 4-phenyl-3-thiosemicarbazide were evaluated for their DNA binding propensity and chemical nuclease activity. The equilibrium binding constants, K_b, of the complexes for binding with calf thymus DNA (CT DNA) were in the range of 0.68×10^3 to 2.8×10^4 M^{-1}. The complexes do not intercalate into the nucleobases of CT DNA, as evident from viscosity measurements. They exhibit efficient nuclease activity in the absence of an activating agent and cleave supercoiled DNA into nicked and linear circular forms of DNA at very low concentrations.

Keywords crystal structure, DNA binding, ferrocene, nuclease activity, Zn(II) complex

INTRODUCTION

An important criteria for the development of metallodrugs as chemotherapeutic agents are the ability of the metallodrug to bring about DNA cleavage.[1] In general, anticancer agents that are approved for clinical use are molecules which damage DNA, block DNA synthesis indirectly through the inhibition of nucleic acid precursor biosynthesis, or disrupt the hormonal stimulation of cell growth.[2] The cleavage of DNA can be achieved by targeting its basic constituents such as base and/or sugar by an oxidative pathway or by the hydrolysis of phosphoester linkages.[3] Transition-metal complexes, which are characterized by high stability, structural versatility, and unique spectroscopic and redox properties, are exploited in many of these efforts. They are capable of binding to DNA by a multitude of interactions and cleaving DNA by virtue of their intrinsic chemical, electrochemical, and photochemical reactivities.[4–7] Redox active complexes are known to be useful for the oxidative cleavage of DNA involving nucleobase oxidation and/or the degradation of sugar by the abstraction of deoxyribose hydrogen atom(s) while complexes containing strong Lewis acids are suitable for the hydrolytic cleavage of DNA.[8] The oxidative method of cleaving DNA has the disadvantage of affecting biomolecules indiscriminately,[9] which is undesirable in treating diseases and studying the interactions of DNA with other molecules. For example, both carbon-centered and hydroxyl radicals are known to modify histone proteins, causing either protein-DNA crosslinking[10–12] or the dissociation of protein-DNA assemblies[13] in addition to cleaving DNA. Furthermore, oxidative damage results in DNA strand termini which prevent subsequent enzymatic manipulation.[14] The hydrolytic cleavage of DNA in particular is challenging because the phosphoester linkages have remarkable stabilities and are extraordinarily resistant to hydrolysis under uncatalyzed physiological conditions.[15] Among the various metal ions that have been studied and are undergoing studies with nucleic acids and nucleobases, Zn(II) is regarded as one of the best suited metal ion for the development of artificial metallonucleases. This is because Zn(II) is a strong Lewis acid and exchange ligands very rapidly, it is of low toxicity and it is not redox active, catalyzing only the hydrolytic cleavage of DNA.[16,17] The selection of ligands in preparing Zn(II) complex is very important as it should favor metal center-DNA interaction while maintaining the stability of the complex. Ferrocene is suitable to be coupled with Zn(II) because it has excellent stability in biological media and is composed of Fe(II), the only redox active metal ion found in hydrolytic enzymes.[16] Recent studies show that ferrocene moiety plays a significant role in the nuclease activity of Cu(II) complexes.[18–20] Here in this study, we investigate the DNA binding and nuclease activity of zinc complexes made with ferrocenylthiosemicarbazone ligands (Scheme 1).

Received 2 September 2011; accepted 28 December 2011.
The authors would like to thank Malaysian Ministry of Higher Education for financial support.
Address correspondence to Siang Guan Teoh, School of Chemical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia. E-mail: sgteoh@usm.my



SCH. 1. The ferrocenylthiosemicarbazone ligands.

EXPERIMENTAL

Materials and Measurements

All the reagents and chemicals were obtained from commercial sources (Acros Chemicals, Aldrich) and used as such. Supercoiled (SC) pBR 322 DNA and loading dye were purchased from Fermentas. Tris(hydroxylmethyl)aminomethane-HCl (Tris-HCl) buffer solution was prepared using Barnstead Nanopure ultrapure water (18.2 MΩ.cm). Calf thymus (CT) DNA, agarose (molecular biology grade), and ethidium bromide (EB) were from Sigma (St. Louis, MO, USA). The ferrocenylthiosemicarbazone ligands were prepared according to the literature procedure.[21,22] The elemental analysis was carried out using Perkin-Elmer 2400 series-11 CHN/O analyzer (Waltham, MA, USA). The infrared, electronic, and fluorescent spectral were recorded on Perkin-Elmer 2000, Perkin Elmer-Lambda 35, and Jasco FP-750 spectrophotometers, respectively. Cyclic voltammetric measurements were done at room temperature on a BAS-Epsilon electrochemical system using a three electrode setup (West Lafayette, IN, USA) comprising a platinum disc working, platinum wire auxiliary and a Ag/AgCl reference electrode. Ferrocene ($E_{1/2} = 0.46$ V) was used as a standard in MeCN/0.1 M $TBAPF_6$.

Preparation of Complexes

Complexes **1–4** were prepared by following a general synthetic procedure in which $Zn(CH3COO^{-})_2 \cdot 2H_2O$ (1 mmol) dissolved in methanol was added drop wise at room temperature to a mixture of appropriate ferrocenylthiosemicarbazone (1 mmol) and KOH (2 mmol) in absolute methanol (15 mL). The resulting orange suspension was stirred under reflux for 4 h and filtered. After several days, brown crystals were obtained from the filtrate.

$Zn\{(\eta^5\text{-}C_5H_5)Fe(\eta^5\text{-}C_5H_4)C(H){=}NN{=}C(S)NH_2\}_2$ (**1**)
Yield: 79%. Anal. Calcd. for $C_{24}H_{24}N_6S_2Fe_2Zn$ (%): C, 45.20; H, 3.79; N, 13.18. Found: C, 45.08; H, 3.48; N, 12.79. IR data (KBr pellet, cm^{-1}): 3453, 3344, ν(N–H); 1596, ν(C=N); 834, ν(C−S).

$Zn\{(\eta^5\text{-}C_5H_5)Fe(\eta^5\text{-}C_5H_4)C(H){=}NN{=}C(S)NHCH_3\}_2 \cdot CH_3OH$ (**2**)
Yield: 83%. Anal. Calcd. for $C_{27}H_{32}Fe_2N_6OS_2Zn$ (%): C, 46.48; H, 4.62; N, 12.04. Found: C, 46.10; H, 4.58; N, 12.36. IR data (KBr pellet, cm^{-1}): 3368, ν(N–H); 1601, ν(C=N); 835, ν(C−S).

$Zn\{(\eta^5\text{-}C_5H_5)Fe(\eta^5\text{-}C_5H_4)C(H){=}NN{=}C(S)NHC_2H_5\}_2$ (**3**)
Yield: 81%. Anal. Calcd. for $C_{28}H_{32}N_6S_2Fe_2Zn$ (%): C, 48.47; H, 4.65; N, 12.11. Found: C, 48.12; H, 4.29; N, 11.71. IR data (KBr pellet, cm^{-1}): 3339, ν(N–H); 1601, ν(C=N); 820, ν(C−S).

$Zn\{(\eta^5\text{-}C_5H_5)Fe(\eta^5\text{-}C_5H_4)C(H){=}NN{=}C(S)NHC_6H_5\}_2 \cdot H_2O$ (**4**)
Yield: 77%. Anal .Calcd. for $C_{36}H_{34}Fe_2N_6OS_2Zn$ (%): C, 53.52; H, 4.24; N, 10.40. Found: C, 53.51; H, 4.41; N, 10.01. IR data (KBr pellet, cm^{-1}): 3319, ν(N–H); 1601, ν(C=N); 828, ν(C−S).

X-Ray Crystallographic Procedure

The determination of the cell constant and data collection were carried out at 100.0(1) K using the Oxford Cryosystem Cobra low-temperature attachment (Long Hanborough Oxford, United Kingdom) with Mo-Kα radiation ($\lambda = 0.71073$) on a Bruker SMART APEXII CCD area-detector diffractometer (Billerica, MA, USA) equipped with a graphite

TABLE 1
Crystal data and structure refinement of **3**

Empirical formula	$C_{28}H_{32}Fe_2N_6S_2Zn$
Formula weight	693.79
Temperature	100.0(1) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	$a = 9.3383(1)$ Å
	$\alpha = 90^\circ$
	$b = 21.3792(3)$ Å
	$\beta = 90.158(1)^\circ$
	$c = 15.3796(2)$ Å
	$\gamma = 90^\circ$
Volume	3070.45(7) Å^3
Z	4
Density (calculated)	1.501g/cm^3
Absorption coefficient	1.871mm^{-1}
$F(000)$	1424
Crystal size	0.40 × 0.21 × 0.18 mm
Theta range for data collection	1.32–25.00°
Index ranges	$-8 \le h \le 11$, $-25 \le k \le 23$, $-17 \le l \le 18$
Reflections collected	21524
Independent reflections	9416 [R (int) = 0.032]
Completeness to theta = 25.00°	99.5%
Absorption correction	Multiscan
Max. and min. transmission	0.7342 and 0.5186
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3851/435/746
Goodness-of-fit onF^2	1.06
Final R indices [$I > 2$sigma (I)]	$R1 = 0.0412$, $wR2 = 0.0837$
R indices (all data)	$R1 = 0.0649$, $wR2 = 0.0977$
Largest diff. peak and hole	0.527 and −0.245 e Å^{-3}

monochromator.[23] The data were reduced using SAINT.[23] A semiempirical absorption correction was applied to the data using SADABS.[23] The structure was solved by direct methods and refined against F^2 by full-matrix least-squares using SHELXTL.[24] Non-hydrogen atoms were anisotropically refined and all the hydrogen atoms were positioned geometrically and refined using a riding model with isotropic temperature factors fixed at 1.2 times U_{eq} of the parent atoms (1.5 times for ethyl groups). The crystal study is a pseudomerohedral twin with the twin matrix (-1 0 0 0 -1 0 0 0 1) and the refined ratio of the twin components was 0.371 (1)/0.729 (1). All the carbon and nitrogen atoms were restrained so that their U_{ij} components approximated isotropic behavior. The C27A–C28A bond distance was restrained to 1.50 (1) Å. Table 1 summarizes the crystal data, data collection, and refinement parameters for **3**.

DNA Binding Method

The experiments were carried out in Tris-HCl buffer (5 mM Tris-HCl, pH 7.1) using the complex solution in 10% DMF. The CT DNA in the buffer medium gave a ratio of UV absorbance at 260 and 280 nm of *ca.* 1.9:1 suggesting that the DNA was apparently free from protein.[25] The concentration of the DNA was estimated from its absorption intensity at 260 nm using its known molar absorption coefficient value of 6600 M^{-1} cm.[26] Absorption and emission titration experiments were carried out by varying the concentration of the CT DNA while keeping the complex concentration constant. The intrinsic equilibrium binding constant (Kb) of the complexes to CT DNA were determined from the plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] where [DNA] is the concentration of DNA in base pairs and the apparent absorption coefficients, ε_a, ε_f, ε_b corresponded to A_{obs}/[Zn], the extinction coefficient for the free zinc complex, and the extinction coefficient of the zinc complex in the totally bound form, respectively. The data were fitted to Eq. 1 with a slope that equaled 1/($\varepsilon_b - \varepsilon_f$) and the intercept equaled 1/[$K_b(\varepsilon_b - \varepsilon_f)$] and K_b was obtained from the ratio of the slope to the intercept.[27]

$$[\text{DNA}]/(\varepsilon_a - \varepsilon_f) = [\text{DNA}]/(\varepsilon_b - \varepsilon_f) + 1/[K_b(\varepsilon_b - \varepsilon_f)] \quad [1]$$

Viscosity measurements were made using a Cannon Manning Semi-Micro viscometer (State College, PA, USA). The viscometer was thermostated at 30°C in a constant temperature bath. The concentration of CT DNA was 40 μM in NP and the

TABLE 2
Selected bond distances (Å) and angles (°)

Zn1A—N4A	2.060 (7)
Zn1A—N1A	2.068 (8)
Zn1A—S1A	2.287 (3)
Zn1A—S2A	2.294 (3)
Zn1B—N1B	2.028 (8)
Zn1B—N4B	2.067 (6)
Zn1B—S2B	2.271 (3)
Zn1B—S1B	2.293 (3)
N4A—Zn1A—N1A	108.4 (3)
N4A—Zn1A—S1A	125.2 (2)
N1A—Zn1A—S1A	84.7 (2)
N4A—Zn1A—S2A	85.3 (2)
N1A—Zn1A—S2A	124.5 (2)
S1A—Zn1A—S2A	131.09 (12)
N1B—Zn1B—N4B	107.2 (3)
N1B—Zn1B—S2B	123.0 (2)
N4B—Zn1B—S2B	86.3 (3)
N1B—Zn1B—S1B	85.9 (2)
N4B—Zn1B—S1B	122.2 (2)
S2B—Zn1B—S1B	133.26 (12)

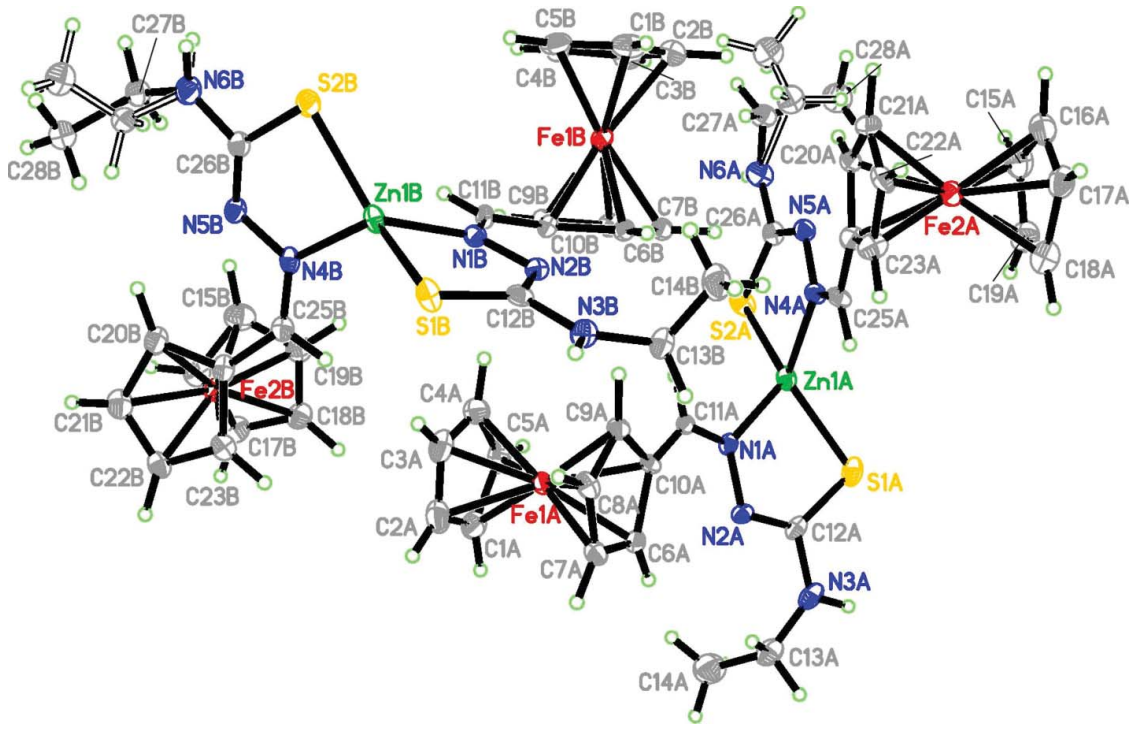


FIG. 1. The asymmetric unit of **3** with 10% probability ellipsoids for non-H atoms. Open bonds show minor disorder component (color figure available online).

flow times were measured manually with a digital stopwatch. The viscosity values were calculated from the observed flowing time of DNA-containing solutions (t) corrected for that of the solvent mixture used (t_0), $\eta = (t - t_0)/t_0$.

DNA Cleavage Experiments

The cleavage of supercoiled pBR322 DNA (0.5 μg/μL) was studied by agarose gel electrophoresis using metal complexes in 10% DMF/5 mM Tris-HCl/50 mM NaCl buffer at pH 7.1. All

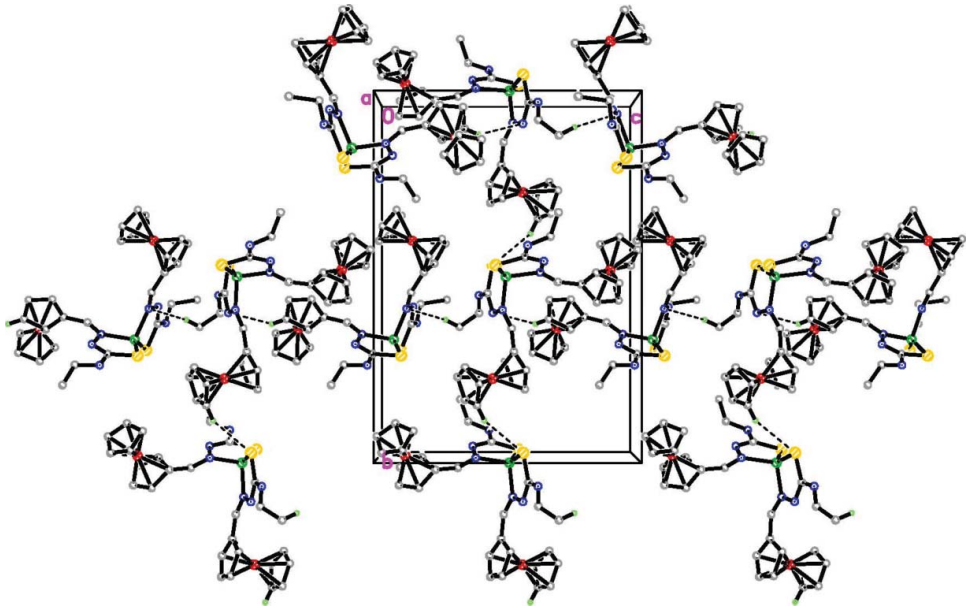


FIG. 2. The crystal packing of **3**, viewed down the *a*-axis showing a 2-D plane parallel to *bc* plane. H atoms not involved in hydrogen-bonding (dashed lines) have been omitted for clarity (color figure available online).

TABLE 3
Hydrogen-bonding geometry (Å, °)

D-H···A	Distance (Å) D-H	Distance (Å) H···A	Distance (Å) D···A	Angle (°) D-H···A
C17—H17A···S2A[a]	1.00	2.86	3.791(15)	155
C7B—H7BA···N5A	1.00	2.61	3.436(12)	140
C28A—H28B···N4B[b]	0.98	2.51	3.48(3)	170

[a] $2 - x, 1/2 + y, 1 - z$.
[b] $x, y, -1 + z$.

the samples were incubated at 37°C under argon atmosphere for 2 h in the dark. After incubation, the sample was added with a loading dye and the solution was loaded on 1% agarose gel with a running time of 1 h at a constant voltage of 50 V. After the electrophoresis step, the resultant DNA bands were stained with ethidium bromide before being photographed under UV light.

RESULTS AND DISCUSSION

The binary Zn(II) ferrocenylthiosemicarbazone complexes (**1–4**) were isolated by the addition of a methanolic solution of hydrated Zn(II) acetate to a mixture of KOH and Schiff base in methanol. The crystal structure descriptions of 1, 2, and 4 have been reported elsewhere and that of **3** is determined in this study.[28–30]

Structure Description of $Zn\{(\eta^5\text{-}C_5H_5)Fe(\eta^5\text{-}C_5H_4)C(H)=NN=C(S)NHC_2H_5\}_2$ (3)

The asymmetric unit of complex **3** consists of two crystallographic independent molecules with similar conformation (Figure 1) and the zinc coordinate environment is comparable to its related structure (Table 2).[30] The Cp (cyclopentadiene) rings of each ferrocene residue are parallel, with dihedral angles of Cp1/Cp2 [C1A—C5A/C6A—C10A] = 2.2(8)°, Cp3/Cp4 [C15A—C19A/C20A—C24A] = 0.6(8)°, Cp5/Cp6 [C1B—C5B/C6B—C10B] = 3.5(7)° and Cp7/Cp8 [C15B—C19B/C20B—C24B] = 2.7(7)°. The Cp rings adopt closely to eclipse conformation [average torsion angles for C—Cg—Cg—C being 15.05, 0.98, –6.64, and 3.52°]. In both of the ligands, the thiosemicarbazone chain and the substituted ethyl group are practically perpendicular with the torsion angles C12A—N3A—C13A—C14A = 81.7(14)°, C26A—N6A—C27A—C28A = 80(2)°, C12B—N3B—C13B—C14B = 96.0(13)° and C26B—N6B—C27B—C28B = 86(2)°. Each of the thiosemicarbazone ligands coordinate almost perpendicularly to the zinc atom with dihedral angles between the mean plane of Zn1A—S1A—C12A—N2A—N1A/Zn1A—S2A—C26A—N5A—N4A and Zn1B—S1B—C12B—N2B—N1B/Zn1B—S2B—C26B—N5B—N4B, equaling 84.8(3) and 86.2(3)°, respectively. The intermolecular C17A—H17A···S2A hydrogen bonds (Table 3) link the molecules into infinite one-dimensional chains along the *b*-axis. The molecules are also linked into infinite one-dimensional chains along the *c*-axis *via* the intermolecular C7B—H7BA···N5A and C28A—H28B···N4B hydrogen bonds (Table 3). All these hydrogen bonds consolidate the crystal structure into supramolecular two-dimensional planes that are parallel to the *bc* plane (Figure 2).

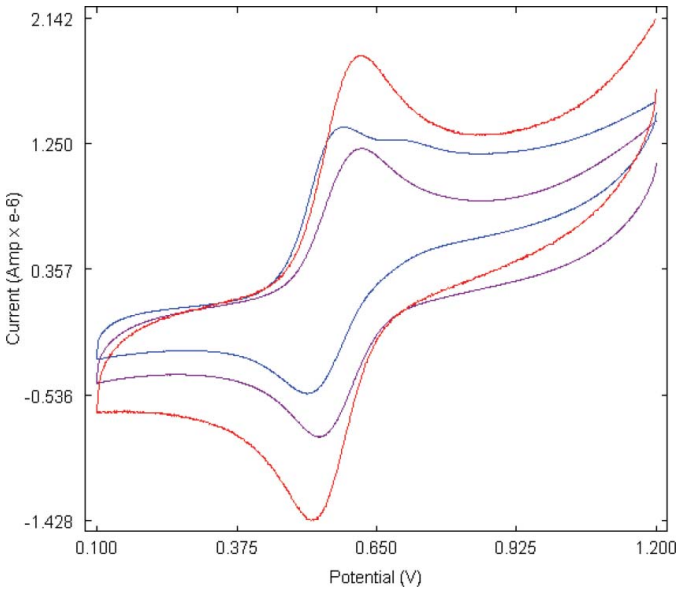


FIG. 3. Cyclic voltammogram of **2** (—), **3** (—), and **4** (—) (color figure available online).

Electrochemistry

The cyclic voltammetric behavior of **2**, **3**, and **4** were carried out in MeCN-0.1 M $TBAPF_6$ (Figure 3). All the complexes showed redox activity (Table 4). The Fe(III)−Fe(II) couple of the ferrocenyl moiety was observed as a reversible response. The

TABLE 4
Electrochemical data (V)

Complex	$E_{1/2}$ (ΔE_p)
2	0.551 (94)
3	0.572 (71)
4	0.580 (81)

TABLE 5
Equilibrium binding constant (K_b)

Complex	K_b/M^{-1}
1	2.3×10^3
2	0.68×10^3
3	3.6×10^3
4	2.8×10^4

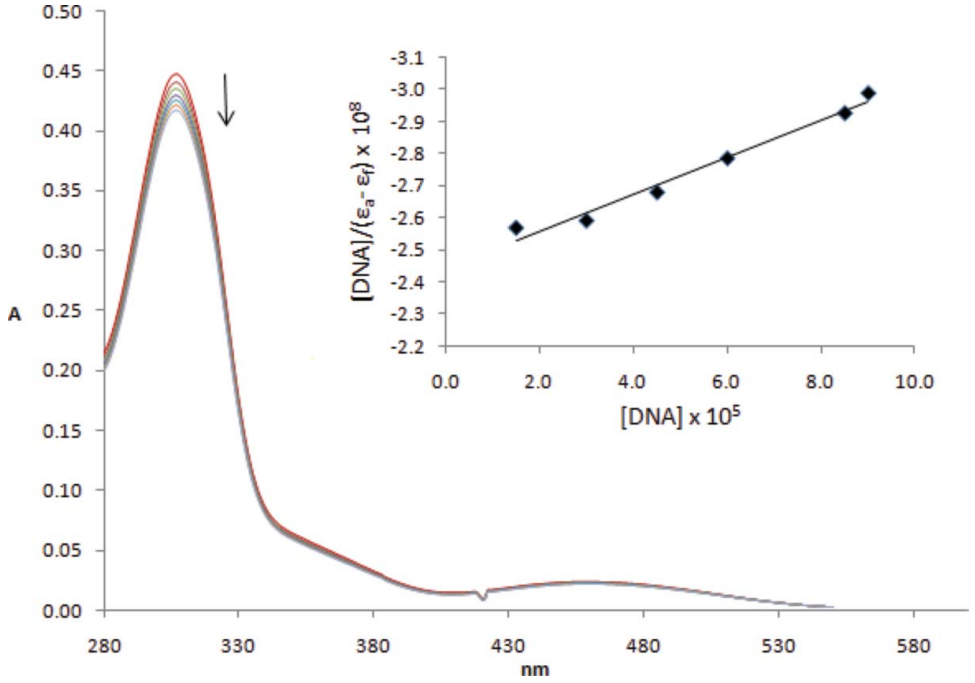


FIG. 4. Electronic spectra of **1** (10 μM) in the presence of increasing amounts of CT DNA. [DNA] = 0–90μM. Arrow shows the absorbance changes upon increasing DNA concentration. Inset: plot [DNA]/(ε_a-ε_f) versus [DNA] (color figure available online).

conjugation of the {C(H)NN(H)C(S)NHR}Zn unit to ferrocenyl moiety caused a *ca.* 100 mV positive shift of the Fe(III)−Fe(II) potential from 0.46 V of the ferrocene ([(η^5-C_5H_5)$_2$Fe]). The shift toward more positive values suggests that the inductive effect of zinc, when bound to the thiosemicarbazone chain, was transferred to the iron atom thus reducing the proclivity of Fe(II) to oxidize.

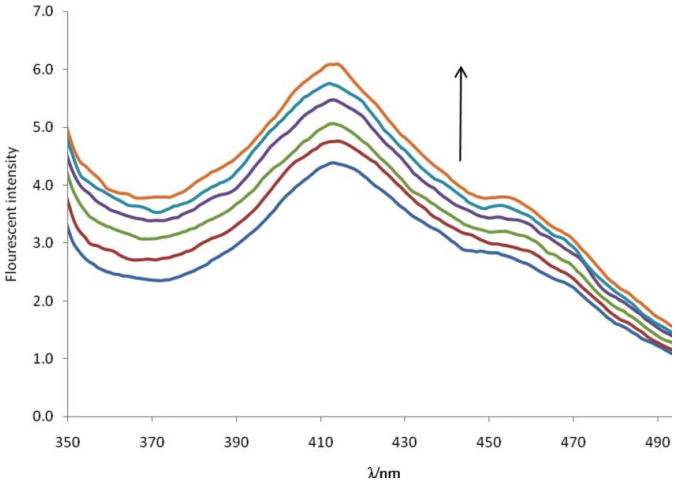


FIG. 5. Magnified view on the fluorescent spectra of **4** (10 μM) in the presence of increasing amounts of CT DNA, [DNA] = 0–60μM. Arrow shows the emission enhancement upon increasing DNA concentration (color figure available online).

DNA Binding Properties

UV-visible absorption spectral measurements were performed to determine the equilibrium binding constant (K_b) of the complexes to CT DNA by monitoring the change in the absorption intensity of the ligand-centered band (Figure 4). The K_b values of the complexes **1–4** are in the range of 0.68×10^3 to 2.8×10^4 M^{-1}, giving an order of **4** > **3** > **1** > **2** (Table 5). The values are comparable with some reported nonintercalators[31,32] and much lower than those observed for typical classical intercalators (EthBr, K_b, 7.0×10^7 M^{-1})[33] and partially intercalating metal complexes ($[Ru(bipy)_2(dppz)]^{2+}$ where dppz is dipyrido-[3,2-*d*:2′,3′-*f*]-phenazine, $K_b > 10^6$ M^{-1}) bound to CT DNA.[34] Fluorescent titration showed emission enhancement

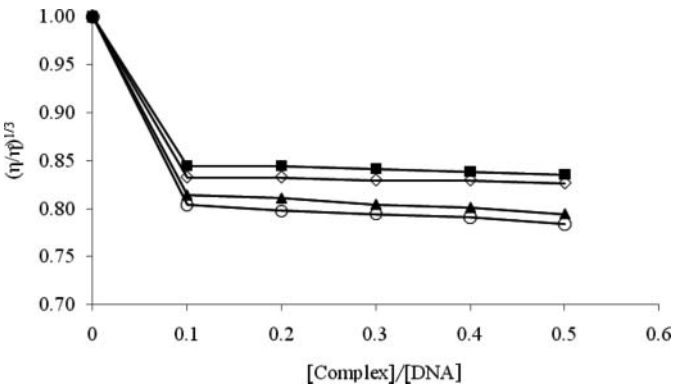


FIG. 6. Effect of complexes [**1**(○), **2** (■), **3**(◇), **4**(▲)] on the viscosity of CT DNA. Relative specific viscosity versus [complex]/[DNA].

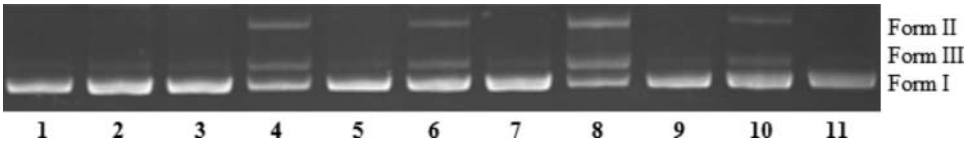


FIG. 7. Cleavage of pBR322 supercoiled plasmid DNA (0.5 μg/μL) by the zinc(II) complexes (100 μM) in 10% DMF/5 mM Tris-HCl/50 mM NaCl buffer at pH 7.1 and 37°C with an incubation time of 2 h under argon atmosphere in dark. Lane 1 = DNA; lane 2 = DNA + $Zn(CH_3COO^-)_2$; lane 3 = DNA + ferrocene; lane 4 = DNA + **1**; lane 5 = DNA + HFTSC; lane 6 = DNA + **2**; lane 7 = DNA + HFMTSC; lane 8 = DNA + **3**; lane 9 = DNA + HFETSC; lane 10 = DNA + **4**; lane 11 = DNA + HFPTSC. Forms I, II, and III are supercoiled and nicked and linear circular forms of DNA, respectively.

(Figure 5), which implies that the interaction between DNA and the complexes occur due to the hydrophobicity of both the molecules.[35]

To understand the nature of the DNA binding of the complexes, viscosity measurements were carried out on CT DNA by varying the concentration of the added complexes. The values of the relative specific viscosity (η/η_0), where η and η_0 are the specific viscosities of DNA in the presence and absence of the complex are plotted against [complex]/[DNA] for **1–4** (Figure 6). The viscosity decreases with an increase in the [complex]/[DNA] ratio indicating that these complexes do not intercalate within the base pairs as expected because of their nonplanar nature. So, surface binding has led to the formation of kinks or bends in the DNA chain.[36]

Chemical Nuclease Activity

To assess the DNA cleavage ability of the complexes, SC pBR322 DNA (0.5 μg/μL) was incubated with **1–4** (100 μM) in 10% DMF/5 mM Tris-HCl/50 mM NaCl buffer at pH 7.1 for 2 h under argon atmosphere without the addition of an activator in the dark. Upon the gel electrophoresis of the reaction mixture, the Zn(II) complexes displayed efficient cleavage activities and cleaved the double-stranded SC DNA (form I) into the nicked circular form (form II) and linear circular form (form III; Figure 7). The precursor species, $Zn(CH_3COO^-)_2$, ligands, and ferrocene alone did not show any cleavage of DNA under similar experimental conditions. At this stage, it was presumed that the hydrolytic cleavage mechanism supported the fact that DNA cleavage occurred under an argon atmosphere for all of the complexes.

CONCLUSION

We have presented here a series of the complexes designed to have redox active ferrocene conjugated to strong Lewis acid Zn(II) through bioactive thiosemicarbazone chelant showing efficient chemical nuclease activity and found to interact in a nonintercalative manner with CT DNA.

SUPPLEMENTARY MATERIALS

CCDC-784760 contains the supplementary crystallographic data for **3**. This data can be obtained free of charge *via* www. ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Center, 12, Union Road, Cambridge CB21EZ, UK; fax: +44-1223-336033; or deposit@ccdc.cam.ac.uk).

REFERENCES

1. Sathyaraj, G.; Weyhermüller, T.; Nair, B.U. Synthesis, characterization and DNA binding studies of new ruthenium(II)bisterpyridine complexes. *Eur. J. Med. Chem.* **2010**, *45*, 284–291.
2. Rajendiran, V.; Karthik, R.; Palaniandavar, M.; Periasamy, V.S.; Akbarsha, M.A.; Srinag, B.S.; Krishnamurthy, H. Mixed-Ligand Copper(II)-phenolate Complexes: Effect of Coligand on Enhanced DNA and Protein Binding, DNA Cleavage, and Anticancer Activity. *Inorg. Chem.* **2007**, *46*, 8208–8221.
3. Chakravarty, A. Photocleavage of DNA by copper(II) complexes. *J. Chem. Sci.* **2006**, *118*, 443–453.
4. Varadarajan, U.; Munusamy, E.; Balachandran Unni, N. Copper(II) terpyridine complexes: effect of substituent on DNA binding and nuclease activity. *Eur. J.Inorg. Chem.* **2007**, 3484–3490.
5. Turro, N.J.; Barton, J.K.; Tomalia, D.A. Molecular recognition and chemistry in restricted reaction spaces. Photophysics and photoinduced electron transfer on the surfaces of micelles, dendrimers, and DNA. *Acc. Chem. Res.* **1991**, *24*, 332–340.
6. Jennette, K.W.; Lippard, S.J.; Vassiliades, G.A.; Bauer, W.R. Metallointercalation reagents. 2-hydroxyethanethiolato(2,2′,2′-terpyridine)-platinum(II) monocation binds strongly to DNA by intercalation. *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 3839–43.
7. Tu, C.; Shao, Y.; Gan, N.; Xu, Q.; Guo, Z. Oxidative DNA strand scission induced by a trinuclear copper(II) complex. *Inorg. Chem.* **2004**, *43*, 4761–4766.
8. Rao, R.; Patra, A.K.; Chetana, P.R. Synthesis, structure, DNA binding and oxidative cleavage activity of ternary (l-leucine/isoleucine) copper(II) complexes of heterocyclic bases. *Polyhedron* **2008**, *27*, 1343–1352.
9. Griffiths, H.R. Chemical modifications of biomolecules by oxidants. *Handbook of Environmental Chemistry* **2005**, 33–62.
10. Gajewski, E.; Fuciarelli, A.F.; Dizdaroglu, M. Structure of hydroxyl radical-induced DNA-protein crosslinks in calf thymus nucleohistone in vitro. *Int. J. Radiat. Biol.* **1988**, *54*, 445–459.
11. Luxford, C.; Dean, R.T.; Davies, M.J. Radicals derived from histone hydroperoxides damage nucleobases in RNA and DNA. *Chem. Res. Toxicol.* **2000**, *13*, 665–672.
12. Schuessler, H.; Jung, E. Protein-DNA crosslinks induced by primary and secondary radicals. *Int. J. Radiat. Biol.* **1989**, *56*, 423–435.
13. Maddox, M.P.; Colton, C.M.; Patterson, M.J.; Mohler, D.L. Dissociation of DNA-histone assemblies resulting from protein side-chain functionalization. *J. Am. Chem. Soc.* **2007**, *129*, 11328–11329.
14. Shell, T.A.; Mohler, D.L. Hydrolytic DNA cleavage by non-lanthanide metal complexes. *Curr. Org. Chem.* **2007**, *11*, 1525–1542.
15. Camargo, M.A.; Neves, A.; Bortoluzzi, A.J.; Szpoganicz, B.; Fischer, F.L.; Terenzi, H.N.; Serra, O.A.; Santos, V.G.; Vaz, B.G.; Eberlin, M.N. Efficient phosphodiester hydrolysis by luminescent

terbium(III) and europium(III) complexes. *Inorg. Chem.* **2010**, *49*, 6013–6025.
16. Mancin, F.; Tecilla, P. Zinc(II) complexes as hydrolytic catalysts of phosphate diester cleavage: from model substrates to nucleic acids. *New J. Chem.* **2007**, *31*, 800–817.
17. Li, J.-H., Wang, J.-T., Zhang, L.-Y., Chen, Z.-N., Mao, Z.-W., Ji, L.-N. Structure, speciation, DNA binding and nuclease activity of two bipyridylzinc complexes bearing trimethylaminomethyl groups. *Inorg. Chim. Acta* **2009**, *362*, 1918–1924.
18. Goswami, T.K.; Roy, M.; Nethaji, M.; Chakravarty, A.R. Photoinduced DNA and protein cleavage activity of ferrocene-appended l-methionine reduced Schiff base copper(II) complexes of phenanthroline bases. *Organometallics* **2009**, *28*, 1992–1994.
19. Maity, B.; Roy, M.; Chakravarty, A.R. Ferrocene-conjugated copper(II) dipyridophenazine complex as a multifunctional model nuclease showing DNA cleavage in red light. *J. Organomet. Chem.* **2008**, *693*, 1395–1399.
20. Maity, B.; Roy, M.; Saha, S.; Chakravarty, A.R. Photoinduced DNA and protein cleavage activity of ferrocene-conjugated ternary copper(II) complexes. *Organometallics* **2009**, *28*, 1495–1505.
21. Casas, J.S.; Castaño, M.V.; Cifuentes, M.C.; GarcIa-Monteagudo, J.C.; Sánchez, A., Sordo, J.; Abram, U. Complexes of dichloro[2-(dimethylaminomethyl)phenyl-C1,N]gold(III), [Au(damp-C1,N)Cl_2], with formylferrocene thiosemicarbazones: synthesis, structure and cytotoxicity. *J. Inorg. Biochem.* **2004**, *98*, 1009–1016.
22. Mariño, M.; Gayoso, E.; Antelo, J.M.; Adrio, L.A.; Fernández, J.J.; Vila, J.M. Synthesis and crystal structure analysis of ferrocenylthiosemicarbazone complexes of palladium(II): unusual σ Pd-C bond cleavage. *Polyhedron* **2006**, *25*, 1449–1456.
23. Bruker, *APEX2, SAINT and SADABS*; Bruker AXS Inc., Madison, WI, **2009**.
24. Sheldrick, G. A short history of SHELX. *Acta Crystallogr. Sect. A* **2008**, *64*, 112–122.
25. Marmur, J. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol.* **1961**, *3*, 208–218.
26. Reichmann, M.E.; Rice, S.A.; Thomas, C.A.; Doty, P. A Further examination of the molecular weight and size of desoxypentose nucleic acid. *J. Am. Chem. Soc.* **1954**, *76*, 3047–3053.
27. Wolfe, A.; Shimer, G.H.; Meehan, T. Polycyclic aromatic hydrocarbons physically intercalate into duplex regions of denatured DNA. *Biochemistry* **1987**, *26*, 6392–6396.
28. Vikneswaran, M.R.; Teoh, S.G.; Razak, I.A.; Fun, H.-K. Bis(ferrocenecarbaldehyde thiosemicarbazonato-κ^2N^1,S)zinc. *Acta Crystallogr. Sect. E* **2009**, *65*, m373–m374.
29. Vikneswaran, M.R.; Teoh, S.G.; Quah, C.K.; Fun, H.K. Bis(ferrocenecarbaldehyde 4-methylthiosemicarbazonato-κ^2N^1,S)zinc(II) methanol solvate. *Acta Crystallogr. Sect. E* **2009**, *65*, 1027.
30. Vikneswaran, M.R.; Teoh, S.G.; Yeap, C.S.; Fun, H.-K. Bis(1-ferrocenylmethylidene-4-phenylthiosemicarbazidato-κ^2N^1,S)zinc(II) monohydrate. *Acta Crystallogr. Sect. E* **2009**, *65*, 1524–1525.
31. Arjmand, F.; Jamsheera, A. DNA binding studies of new valine derived chiral complexes of tin(IV) and zirconium(IV). *Spectrochim. Acta A* **2011**, *78*, 45–51.
32. Li, J.-H., Wang, J.-T., Mao, Z.-W., Ji, L.-N. Synthesis, interaction with DNA and nuclease activity of zinc complexes of 2,2-bipyridine derivatives with tetraalkylammonium groups. *J. Coord. Chem.* **2009**, *62*, 446–455.
33. Waring, M.J. Complex formation between ethidium bromide and nucleic acids. *J. Mol. Biol.* **1965**, *13*, 269–282.
34. Friedman, A.E.; Chambron, J.C.; Sauvage, J.P.; Turro, N.J.; Barton, J.K. A molecular light switch for DNA: Ru(bpy)2(dppz)$^{2+}$. *J. Am. Chem. Soc.* **1990**, *112*, 4960–4962.
35. Tabassum, S.; Khan, R.A.; Arjmand, F.; Sen, S.; Kayal, J.; Juvekar, A.S.; Zingde, S.M. Synthesis and characterization of glycoconjugate tin(IV) complexes: in vitro DNA binding studies, cytotoxicity, and cell death. *J. Organomet. Chem.* **2011**, *696*, 1600–1608.
36. Ramakrishnan, S.; Rajendiran, V.; Palaniandavar, M.; Periasamy, V.S.; Srinag, B.S.; Krishnamurthy, H.; Akbarsha, M.A. Induction of cell death by ternary copper(II) complexes of l-tyrosine and diimines: role of coligands on DNA binding and cleavage and anticancer activity. *Inorg. Chem.* **2009**, *48*, 1309–1322.