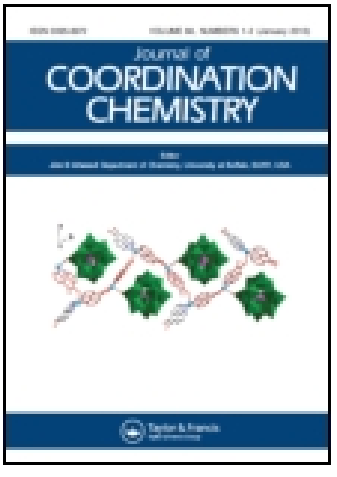

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New supramolecular ferrocene incorporated N,N′-disubstituted thioureas: synthesis, characterization, DNA binding, and antioxidant studies

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New supramolecular ferrocene incorporated N,N′-disubstituted thioureas: synthesis, characterization, DNA binding, and antioxidant studies

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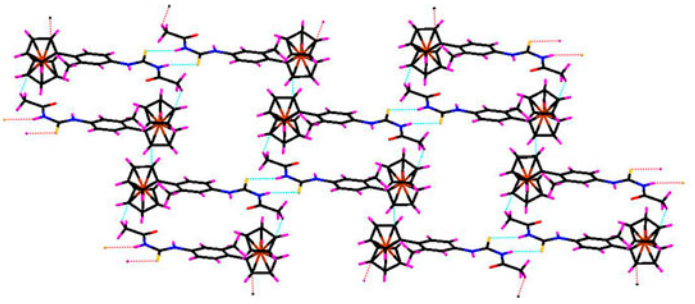

This research article describes the synthesis, structural characterization, electrochemistry, nature and extent of binding with the DNA and free radical scavenging activity of new ferrocene based N, N′-disubstituted thioureas.

Ferrocene incorporated N,N′-disubstituted thioureas (**S1–S6**) were synthesized by allowing 4-ferrocenyl-3-methylaniline to react with freshly prepared aliphatic isothiocyanates and were characterized by using different analytical techniques. Based on single-crystal X-ray analysis compound **S1** shows supramolecular structure mediated by secondary bonding interactions, intermolecular hydrogen bonding NH–O, NH–S, and secondary non-covalent interactions (π–H). Voltammetric measurements were used to study their redox behavior and DNA binding of the compounds. Different binding parameters like binding constants, binding energies, and diffusion coefficients (D_o) were calculated for further insight on the affinity with DNA. All the compounds show electrostatic mode of interactions. The D_o of molecule-DNA adducts were found to be lower than that of free molecule. Compounds **S1**–**S6** having 50% inhibition values less than 37.89 $\mu g\,mL^{-1}$ indicate that they may have value as antioxidants to be used as therapeutic agents.

Keywords: Ferrocene; Crystal structure; Electrochemistry; DNA binding; Antioxidant activity

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1. Introduction

Ferrocene is of interest because of its neutral behavior, stability, and biologically non-toxic effect [1]. Ferrocene and its derivatives have applications as antitumor [2, 3], antimalarial [4], antifungal [5], cytotoxic effects [6, 7], and DNA cleavage activity [8]. An important application of ferrocenyl derivatives is against cancer. Tamoxifen and hydroxytamoxifen are routinely used for the treatment of breast cancer but long-term exposure of clinically available drugs lead to side effects such as development of resistance to the drug, increase in risk of blood clotting in the lungs, and non-effectiveness in hormone-independent tumors [9]. The ferrocifens, which are analogous compounds of tamoxifen with a ferrocenyl moiety, have greater activity against both hormone-dependent and hormone-independent breast cancer cells [10]. The significant increase in activity by incorporation of the ferrocene in basic structure of drug is due to increase in lipophilicity and the ferrocene moiety itself. Some ferrocenyl derivatives like polyphenolic compounds (1,1-bis(4-hydroxyphenyl)-2-ferrocenyl-but-1-ene) have greater anticancer activity than 4-hydroxytamoxifen in hormone-dependent and hormone-independent breast cancer [11, 12]. The anticancer activities of ferrocenyl derivatives generally depend upon the oxidation state of iron (Fe) with Fe^{2+} ferrocenyl derivatives more active than Fe^{3+} derivatives by changing the conformation of receptor protein [13–15]. According to Osella *et al.* [16], anticancer activity arises due to generation of oxygen active radicals by reduction of ferrocenium ion. It means that the good redox properties of ferrocenyl moiety will ultimately exhibit enhanced activity. The anticancer activity of compounds is due to the interaction with DNA by which the physical and chemical nature of DNA alters. The interaction of drug with DNA can be studied by using different analytical techniques, among which electrochemical methods are used because of reliability, high sensitivity and selectivity, and low cost [17, 18]. The interaction of the drug with DNA may be covalent or non-covalent (electrostatic, groove binding, and intercalation) that can be probed by substantial change in peak potential and change in peak current after addition of different concentration of DNA.

Antioxidants have ability to trap free radicals. Highly reactive free radicals and oxygen species (such as hydro peroxides, peroxides, and lipids peroxyl) are found in biological systems from different sources. These free radicals are responsible for oxidation of nucleic acids, proteins, lipids, or DNA and can initiate degenerative disease and thus antioxidant inhibit the oxidative mechanisms that lead to these kinds of diseases [19]. Some ferrocene-based thioureas, selenoureas, guanidines, amides, and bimetallics from our research group have been screened as potential DNA binders and have shown good antitumor and antioxidant activities [20–25]. Herein, we report the synthesis, structural characterization, nature, and extent of binding with the DNA and free radical scavenging activity of new ferrocene-based N,N′-disubstituted thioureas (**S1–S6**).

2. Experimental

2.1. *Materials and methods*

Ferrocene, 3-methyl-4-nitroaniline, potassium thiocyanate, sodium nitrite, acetyl chloride, isobutyryl, phenylacetyl, dichloroacetyl, propionyl, pivaloyl chloride, tetrabutylammonium perchlorate (TBAP), and hydrochloric acid were purchased from Sigma Aldrich and used as

such. Ethanol, methanol, acetone, diethyl ether, and petroleum ether were purified before use according to the standard reported protocols [26, 27].

2.2. *Synthesis of N-acetyl-N'3-(4-ferrocenyl-3-methylbenzyl) thiourea (S1)*

To the solution of acetyl chloride (0.302 mL, 4.25 mM) in dried acetone (100 mL), potassium thiocyanate (0.414 g, 4.25 mM) was added to prepare the acetyl isothiocyanate under N_2. 4-Ferrocenyl-3-methyl aniline (1 g, 4.25 mM) was added to the resulting reaction mixture and kept stirring for 4 h. The reaction mixture was then poured into the ice-cooled water and stirred well. The solid product was filtered off and washed with deionized water. 1-Acetyl-3 (4-ferrocenyl-3-methylbenzyl) thiourea was dried in air. Yield: 74%. δ_H (300 MHz, [D_6] C_3D_6O) 12.65 (s, 1H, CSN*H*), 10.31 (s, 1H, CON*H*), 7.77 (d, *J* 8.4 Hz, 1H, C_6H_3), 7.65 (d, *J* 8.4 Hz, 1H, C_6H_3), 7.42 (s, 1H, C_6H_3), 4.56 (s, 2H, C_5H_4), 4.34 (s, 2H, C_5H_4), 4.15 (s, 5H, C_5H_5), 2.35 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), ppm. δ_C (75 MHz, [D_6]DMSO) 179.13, 172.47, 136.00, 135.65,132.17, 130.53, 125.00, 120.58, 86.55, 69.34, 69.22, 68.02, 28.21, 20.61 ppm. υ_{max}/cm^{-1} NH (3200–3400 cm^{-1}), sp^2 C–H (3080 cm^{-1}), sp^3 C–H (2905 cm^{-1}), C=O (1650–1700 cm^{-1}), C=C Ar (1513–1598 cm^{-1}), C=S (1130–1250 cm^{-1}), Fe–Cp (485 cm^{-1}). Elemental analysis Calcd (%) for $C_{20}H_{20}FeN_2OS$: C, 61.23; H, 5.14; N, 7.14; S, 8.17. Found (%): C, 61.59; H, 5.41; N, 7.43; S, 8.32.

2.3. *Synthesis of N-isobutyryl-N'-(4-ferrocenyl-3-methylbenzyl) thiourea (S2)*

Compound **S2** was prepared using the same method as for **S1** except using isobutyryl chloride (0.445 mL, 4.25 mM) in place of acetyl chloride. Yield: 79%. δ_H (300 MHz, [D_6] DMSO) 12.54 (s, 1H, CSN*H*), 11.45 (s, 1H, CON*H*), 7.68 (d, *J* 8.4 Hz, 1H, C_6H_3), 7.54 (d, *J* 8.4 Hz, 1H, C_6H_3), 7.37 (s, 1H, C_6H_3), 4.57 (s, 2H, C_5H_4), 4.35 (s, 2H, C_5H_4), 4.13 (s, 5H, C_5H_5), 2.75 (m, 1H, C_3H_7), 2.35 (s, 3H, CH_3), 1.15 (d, *J* 6.6 Hz, 6H, C_3H_7) ppm. δ_C (75 MHz, [D_6]DMSO) 179.13, 171.23, 136.03, 135.90, 135.64, 130.50, 124.99, 120.52, 86.57, 69.53, 69.34, 68.02, 35.41, 20.59, 18.43 ppm. υ_{max}/cm^{-1} NH (3262 cm^{-1}), sp^2 C–H (3080 cm^{-1}), sp^3 C–H (2905 cm^{-1}), C=O (1693 cm^{-1}), C=C Ar (1513–1598 cm^{-1}), C=S (1154–1227 cm^{-1}), Fe–Cp (485 cm^{-1}). Elemental analysis Calcd (%) for $C_{22}H_{24}FeN_2OS$: C, 61.23; H, 5.14; N, 7.14; S, 8.17. Found (%): C, 61.46; H, 5.25; N, 7.56; S, 8.37.

2.4. *Synthesis of N-phenylacetyl- N'-(4-ferrocenyl-3-methylbenzyl) thiourea (S3)*

Compound **S3** was prepared using the same method as for **S1** except using phenylacetyl chloride (0.562 mL, 4.25 mM) in place of acetyl chloride. Yield: 67%. δ_H (300 MHz, [D_6] DMSO) 12.40 (s, 1H, CSN*H*), 11.69 (s, 1H, CON*H*), 7.32–7.25 (m, 9H), 4.51 (d, 2H, C_5H_4), 4.30 (s, 2H, C_5H_4), 4.18 (s, 5H, C_5H_5), 4.09 (s, 2H, CH_2), 2.31 (s, 3H, CH3), ppm. δ_C (75 MHz, [D_6]DMSO) 178.25, 169.13, 135.59, 135.21, 133.24, 130.08, 130.45, 129.24, 125.5, 124.18, 120.65, 116.45, 84.53, 69.79, 69.64, 68.03, 52.88, 21.02 ppm. υ_{max}/cm^{-1} NH (3252 cm^{-1}), sp^2 C–H (3064 cm^{-1}), sp^3 C–H (2869 cm^{-1}), C=O (1688 cm^{-1}), C=C Ar (1540–1570 cm^{-1}), C=S (1133–1257 cm^{-1}), Fe–Cp (485 cm^{-1}). Elemental analysis Calcd (%) for $C_{26}H_{24}FeN_2OS$: C, 66.67; H, 5.16; N, 5.98; S, 6.85. Found (%): C, 66.86; H, 5.35; N, 6.06; S, 6.96.

2.5. *Synthesis of N-dichloroacetyl-N'-(4-ferrocenyl-3-methylbenzyl) thiourea (S4)*

Compound **S4** was prepared using the same method as for **S1** except using dichloroacetyl chloride (0.408 mL, 4.25 mM) in place of acetyl chloride. Yield: 69%. δ_H (300 MHz, [D_6] DMSO) 12.71 (s, 1H, CSN*H*), 10.97 (s, 1H, CON*H*), 7.60–7.30 (m, 2H, C_6H_3), 7.27 (s, 1H, C_6H_3), 5.92 (s, 1H, CH), 4.55 (d, *J* 12 2H, C_5H_4), 4.35 (d, *J* 9 Hz,2H, C_5H_4), 4.14 (s, 5H, C_5H_5), 2.34 (s, 3H, CH3) ppm. δ_C (75 MHz, [D_6]DMSO) 178.52, 174.35, 134.63, 132.13, 132.15, 126.15, 121.165 120.05, 86.32, 70.05, 69.88, 69.52, 66.98, 20.71 ppm. υ_{max}/cm^{-1} NH (3302 cm^{-1}), sp^2 C–H (3124 cm^{-1}), sp^3 C–H (2952 cm^{-1}), C=O (1692 cm^{-1}), C=C Ar (1524–1591 cm^{-1}), C=S (1112–1265 cm^{-1}), Fe–Cp (484 cm^{-1}). Elemental analysis Calcd (%) for $C_{20}H_{18}Cl_2FeN_2OS$: C, 61.23; H, 5.14; N, 7.14; S, 8.17. Found (%): C, 61.09; H, 5.33; N, 7.07; S, 8.01.

2.6. *Synthesis of N-propionyl-N'-(4-ferrocenyl-3-methylbenzyl) thiourea (S5)*

Compound **S5** was prepared using the same method as for **S1** except using propionyl chloride (0.371 mL, 4.25 mM) in place of acetyl chloride. Yield: 79%. δ_H (300 MHz, [D_6]DMSO) 12.62 (s, 1H, CSN*H*), 11.59 (s, 1H, CON*H*), 7.73 (d, *J* 8.7 Hz, 1H, C_6H_3), 7.66 (d, *J* 8.7 Hz, 1H, C_6H_3), 7.38 (s, 1H, C_6H_3), 4.59 (s, 2H, C_5H_4), 4.36 (s, 2H, C_5H_4), 4.15 (s, 5H, C_5H_5),2.91 (t, *J* 8.1 Hz, 2H, C_2H_5), 2.37 (s, 3H, CH_3), 1.09 (q, *J* 8.1 Hz, 3H, C_2H_5) ppm. δ_C (75 MHz, [D_6]DMSO) 172.63, 169.04, 137.13, 135.41, 135.16, 132.33, 126.19, 118.32, 85.35, 69.84, 69.52, 67.91, 40.94, 20.57, 10.13 ppm. υ_{max}/cm^{-1} NH (3242 cm^{-1}), sp^2 C–H (3056 cm^{-1}), sp^3 C–H (2912 cm^{-1}), C=O (1674 cm^{-1}), C=C Ar (1531–1565 cm^{-1}), C=S (1122–1256 cm^{-1}), Fe–Cp (482 cm^{-1}). Elemental analysis Calcd (%) for $C_{21}H_{22}FeN_2OS$: C, 62.08; H, 5.46; N, 6.89; S, 7.89. Found (%): C, 62.19; H, 5.23; N, 6.97; S, 7.95.

2.7. *Synthesis of N-pivaloyl-N'-(4-ferrocenyl-3-methylbenzyl) thiourea (S6)*

Compound **S6** was prepared using the same method as for **S1** except using pivaloyl chloride (0.522 mL, 4.25 mM) in place of acetyl chloride. Yield: 73%. δ_H (300 MHz, [D_6]DMSO) 12.65 (s, 1H, CSN*H*), 10.73 (s, 1H, CON*H*), 7.68–7.29 (m, 3H, C_6H_3), 4.56 (s, 2H, C_5H_4), 4.32 (s, 2H, C_5H_4), 4.16 (s, 5H, C_5H_5), 2.35 (s, 3H, CH_3), 1.15 (d, *J* 6.6 Hz, 9H, C_3H_9) ppm. δ_C (75 MHz, [D_6]DMSO) 176.42, 169.19, 135.73, 135.07, 134.91, 129.85, 125.24, 122.02, 84.24, 69.71, 69.52, 67.72, 52.87, 29.01, 20.63 ppm. υ_{max}/cm^{-1} NH (3315 cm^{-1}), sp^2 C–H (3102 cm^{-1}), sp^3 C–H (2921 cm^{-1}), C=O (1669 cm^{-1}), C=C Ar (1516–1591 cm^{-1}), C=S (1131–1262 cm^{-1}), Fe–Cp (483 cm^{-1}). Elemental analysis Calcd (%) for $C_{23}H_{26}FeN_2OS$: C, 63.60; H, 6.03; N, 6.45; S, 7.38. Found (%): C, 63.49; H, 6.13; N, 6.37; S, 7.45.

2.8. *Cyclic voltammetry and DNA binding studies*

The interaction of the drug with DNA was studied by Eco Chemie Auto lab PGSTAT 12 potentiostat/galvanostat (Utrecht, The Netherlands). The investigation of binding ability of drug with DNA was made by using the three electrode system having platinum disk as working electrode, Ag/AgCl as reference electrode and platinum wire as counter electrode. The surface of the working electrode was cleaned and polished each time accompanied by

rinsing with doubly distilled water. The voltammograms were recorded in absence and presence of CT-DNA (2, 4, and 6 μM) at different scan rates (25–300 mV s^{-1}) to probe the electrochemical nature and DNA binding affinities of **S1–S6** in 90% aqueous DMSO.

2.9. *Antioxidant activity*

The antioxidant activities of **S1–S6** were determined by using DPPH. The sample solutions were prepared by keeping constant concentration of DPPH (165 μM) and increasing concentration of compounds (3.1, 6.2, 12.5, 25, 50, 100 μg mL^{-1}) in ethanol and buffered at pH 6 (0.1 M NaH_2PO_4 + 0.1 M NaOH). All these mixtures were kept in the dark for 30 min and absorbance was measured at 517 nm in dim light using a Shimadzu 1800 UV–vis spectrophotometer. From the mean value of three readings, % inhibition was calculated by using following equation [28].

$$\text{Scavenging activity}(\%) = \frac{A_0 - A_s}{A_0} \times 100 \quad (1)$$

3. Results and discussion

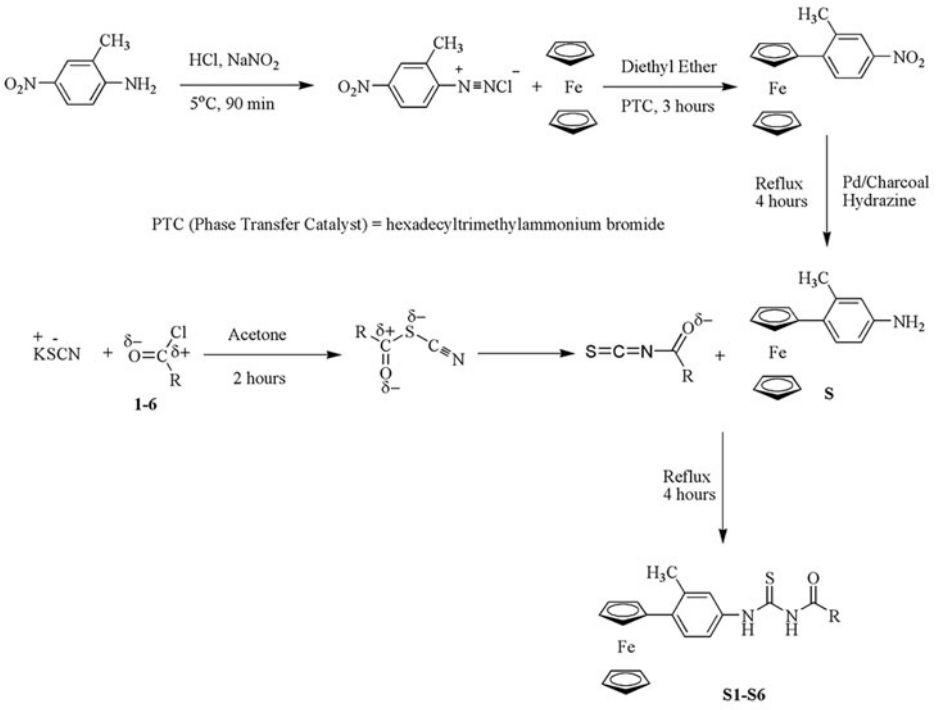
3.1. *Synthesis and spectroscopic analysis*

Synthetic pathway for the synthesis of six new ferrocene-incorporated N,N′-disubstituted thioureas (**S1–S6**) is sketched in scheme 1.

The compounds have been synthesized by reacting 4-ferrocenyl-3-methyl aniline with freshly prepared isothiocyanates under N_2 in dry acetone. The compounds were structurally characterized by using different analytical techniques. In FT-IR spectra N–H gave a broad band at 3400–3200 cm^{-1}. The broadening of the band is due to intermolecular and intramolecular hydrogen bonding and secondary bonding interaction which can be confirmed in crystal structure of **S1**. The diagnostic band at 1700–1650 cm^{-1} corresponds to the carbonyl while thiocarbonyl appeared at 1250–1130 cm^{-1}. In ^{1}H NMR spectra ferrocenyl protons appeared at 4–5 ppm, however, N*H* protons appeared above 11.45 ppm. In the ^{13}C NMR spectra, the (C=O) and (C=S) peaks appeared between 169 and 180 ppm, whereas ferrocenyl carbons appear at 60–90 ppm. The aromatic and aliphatic protons and carbons appear in the usual regions in ^{1}H and ^{13}C NMR [29, 30].

3.2. *Single-crystal X-ray studies of S1*

Careful crystallization of **S1** in acetone yielded orange crystals suitable for single-crystal X-ray diffraction analysis. Data pertaining to the data collection and structure refinement shows that **S1** was crystallized in the triclinic crystal system with *P-1* space group. Multi-scan absorption correction method was used. Crystal parameters were calculated at 296 K using Mo K_α ($\lambda = 0.71073$) radiations with empirical formula $C_{20}H_{20}FeN_2OS$, unit cell dimensions $a = 7.4316(10)$ Å, $b = 10.0064(16)$ Å, $c = 12.3340(18)$ Å, $\alpha = 91.180(6)°$, $\beta = 103.806(5)°$, $\gamma = 101.334(6)°$, Mr = 392.30, volume = 871.1(2) $Å^3$, $Z = 2$, density = 1.496 g/cm^3, F (0 0 0) = 408.0, crystal size = 0.12 × 0.15 × 0.25 mm^3, index ranges (h, k, l)$_{max}$ = (8, 12, 14), (h, k, l)$_{min}$ = (−8, −12, −14), and total reflections = 3235, $\mu = 0.996$ mm^{-1}, $\theta_{max} = 25.50°$, $R = 0.0502$ and $wR_2 = 0.1204$.



Compound	S1	S2	S3	S4	S5	S6
R	$-CH_3$	$-CH(CH_3)_2$	$-CH_2(C_6H_5)$	$-CHCl_2$	$-C_2H_5$	$-C(CH_3)_3$

Scheme 1. Synthetic scheme for ferrocene-incorporated N,N′-disubstituted thioureas (**S1–S6**).

Table 1 provides a selection of important inter atomic distances, bond angles, and torsion angles. Figure 1 represents the molecular structures with numbering scheme of **S1**. The intramolecular hydrogen bonding and intermolecular interactions present in title compound are summarized in table 2 and drawn in figure 2.

The rings A (C6–C10), B (C11–C16), and C (H1A, N1, C18, N2, C19, and O) in **S1** are planar with RMS deviation of 0.0035, 0.0054, and 0.0267 Å, respectively. All these three planes are on the same surface due to the extended resonance over the 17 atoms in the molecule. The bond distance between A and B rings [C10–C11 = 1.485(7)], less than the normal C–C single bond distance 1.54 Å) supports resonance over these rings in **S1**. Intermolecular hydrogen bonding (NH–O type) is responsible for the planarity of six atoms forming ring C in **S1** (figure 2). The interplanar angles between these planes are A/B = 39.88°, A/C = 28.36°, and B/C = 12.93°. This type of intramolecular hydrogen bonding is well known for such aroyl or acoyl substituted organic thioureas as we found in the ferrocene analogs [31, 32].

Two independent molecules exist in an asymmetric unit which are connected alternately by intermolecular NH–S hydrogen bonding and secondary non-covalent interactions (π–H), to mediate a supramolecular structure as shown in figure 3. These intermolecular non-bonding secondary interactions have great importance for biological activities. Literature studies

Table 1. Selective interatomic distances, bond angles, and torsion angles in **S1**.

Bond lengths (Å)		Bond angles (°)	
Fe1–C8	2.033(7)	C4–Fe1–C5	40.59(21)
C8–C9	1.411(6)	C5–Fe1–C9	127.30(19)
C10–C11	1.485(7)	C9–C10–C11	123.54(34)
C16–C17	1.503(6)	C10–C11–C12	119.24(34)
C15–C16	1.383(7)	C13–C14–N1	125.43(34)
C14–N1	1.416(7)	C14–N1–C18	131.16(34)
N1–C18	1.335(6)	N1–C18–N2	114.08(33)
C18–N2	1.397(7)	N1–C18–S1	127.61(31)
C18–S1	1.636(7)	O1–C19–N2	122.88(40)
N2–C19	1.378(7)	S1–C18–N2	118.30(29)
C19–O1	1.209(7)	Torsion angles (°)	
N1–H1A	0.860(4)	C9–C10–C11–C12	−38.34(55)
Fe1–C5	2.034(8)	C12–C11–C10–C6	142.20(42)
C2–C3	1.376(8)	C6–C10–C11–C16	−41.38(62)
C17–C16	1.503(6)	C15–C14–N1–C18	−172.26(38)
N2–H2A	0.861(4)	C14–N1–C18–S1	4.27(63)
C5–H5	0.930(6)	C14–N1–C18–N2	−176.62(36)
C13–H13	0.930(4)	C18–N2–C19–O1	−7.24(66)
C20–H20A	0.960(5)	C18–N2–C19–C20	172.59(37)

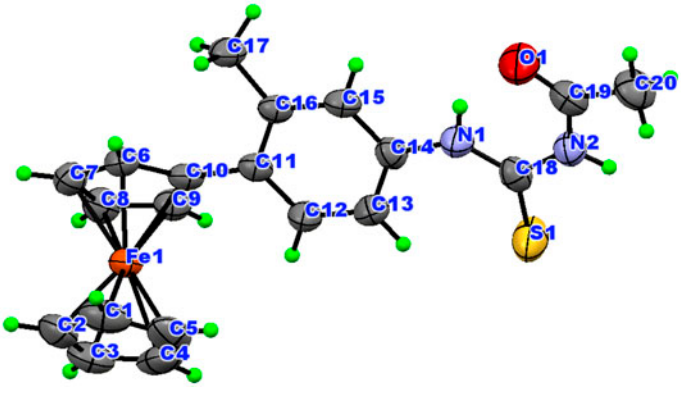


Figure 1. Molecular structure of **S1**.

Table 2. The intramolecular and intermolecular hydrogen bonds interactions in **S1**.

H-bonding	X	H	Y	d(X–H) (Å)	(H–Y) (Å)	d(X–Y) (Å)	<(XHY) (°)
Intramolecular	N1	H1a	O1	0.860(4)	1.888(8)	2.619(12)	141.96(23)
Intermolecular	N2	H2a	S1	0.861(4)	2.511(3)	3.354(3)	166.86(5)

demonstrate that compounds with stronger non-bonding interactions have more ability to bind with macro-biological molecules likes proteins and DNA [33–35]. So, we were expecting good affiliation of **S1** with DNA.

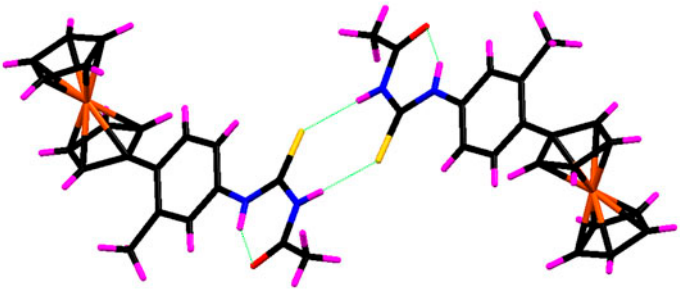


Figure 2. Inter- and intramolecular hydrogen bonding in **S1**.

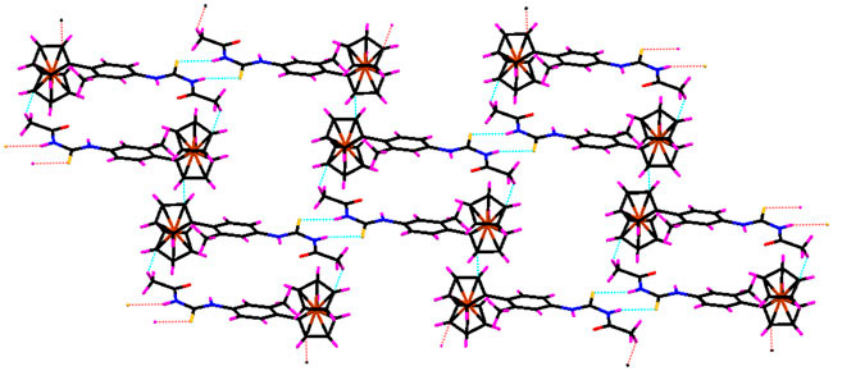


Figure 3. Supramolecular structures of **S1** mediated by secondary bonding interactions.

3.3. *Voltammetric measurements and DNA binding studies*

The interaction of the drug with DNA and investigation of binding ability of drug with DNA were made by voltammetric measurements. The voltammograms were recorded in absence and presence of DNA at different scan rates to probe the electrochemical nature and DNA binding affinities of **S1–S6** in 90% aqueous DMSO. The electrochemical and DNA binding of **S1** is discussed briefly (figures 4 and 5) here, whereas the electrochemical and DNA binding data of other compounds are tabulated in table 3. The cyclic voltammogram of 1 mM **S1** {N-acetyl-N′-(4-ferrocenyl-3-methylbenzyl) thioureas} revealed a couple of well-defined redox peaks from 0 to 0.8 V. Compound **S1** has an oxidation maximum at 0.582 V and reduction maximum at 0.343 V in a quasi-reversible electrochemical process. The quasi reversibility of the process was evident by non-consistency of potential at different scan rates (25, 50, 75, 100, 200, and 300 mV s^{-1}), the ratio of anodic and cathodic current was not unity and the difference between the oxidation and reduction maxima ($E_p = E_{pa} - E_{pc}$) was 0.225 V, which confirms the quasi reversible nature of the system. The number of electrons transferred is one by oxidation of Fe^{2+} of the ferrocenyl moiety to Fe^{3+} in the forward scan and in the reverse scan reduces to Fe^{2+}. On addition of 2 µM of calf thymus DNA (CT-DNA) to **S1**, there is a decrease in peak current from 4.45×10^{-3} to 3.89×10^{-3} mA with a negative shift in oxidation peak potential of 20 and 10 mV for the

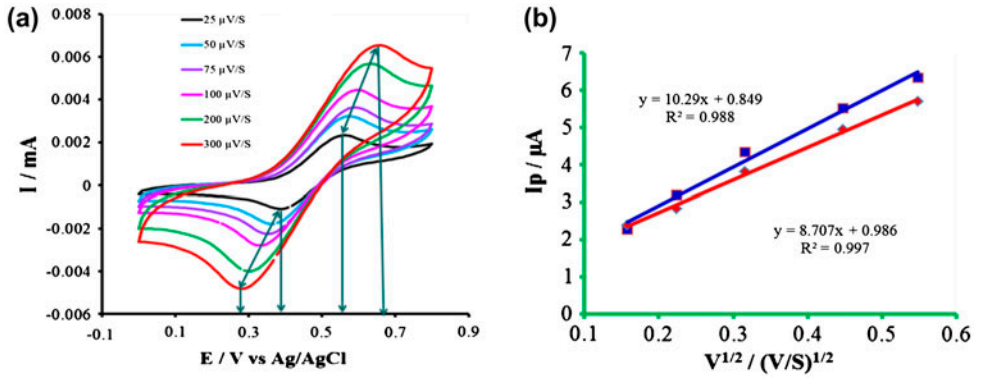


Figure 4. (a) Representative cyclic voltammograms of 1 mM **S1** recorded at different potential sweep rates (25–300 mV s^{-1}) on glassy carbon electrode at 298 K in 10% aqueous DMSO buffer at pH 6.0; supporting electrolyte 0.1 M TBAP (b) I_p *vs.* $v^{1/2}$ plots of 1 mM **S1** in the absence of DNA (–) and presence of 0.6 µM DNA (–) at scan rates ranging from 25 to 300 mV s^{-1} under the experimental condition.

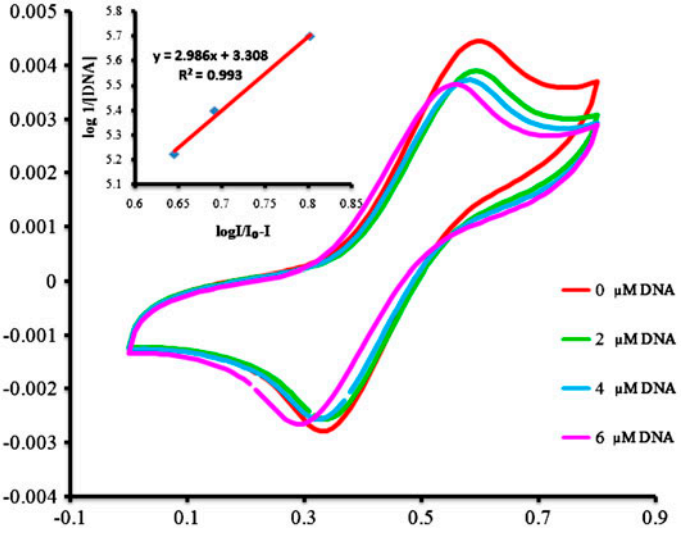


Figure 5. Representative cyclic voltammograms of 1 mM **S1** recorded at 100 mV s^{-1} potential sweep rate on platinum disk electrode at 298 K in the absence and presence of increasing concentration of CT-DNA (2, 4 and 6 µM) in 10% aqueous DMSO buffer at pH 6.0, supporting electrolyte 0.1 M TBAP.

reduction peak. This negative shift in peak potential indicates that the product of oxidized specie of **S1** have more binding ability with DNA than to reduced species. The ferrocenyl derivatives involve Fe^{2+} and Fe^{3+}; electron transfer may generate ROS *in vivo* that cause damage to DNA [36]. The decrease in the current by addition of DNA is due to decrease in the number of free molecules due to formation of S1-DNA complex which is equally supported by the idea that heavy molecules diffuse slowly. The cathodic shift in peak is due to electrostatic interaction of the drug with anionic phosphate backbone of double helical DNA [21]. The diffusion coefficients calculated by using equations (2) and (3) are 2.66×10^{-7} cm^2 s^{-1} for DNA-free drug and 1.47×10^{-7} cm^2 s^{-1} for S1-DNA adduct.

Table 3. The change in oxidation and reduction maxima, formal potential, binding constant, and Gibbs free energies ($-\Delta G$) for **S1**–**S6**, ferrocene, and 4-nitrophenyl ferrocene.

Compounds	$\Delta E_p = E_{pa} - E_{pc}$	$(E_{pc} + E_{pa})/2$	Binding constant (M^{-1})	Binding energy (kJ M^{-1})	D_o [$cm^2\ s^{-1}$] free drug	D_o [$cm^2\ s^{-1}$] drug-DNA
S1	0.22	0.46	2.03×10^3	18.87	2.66×10^{-7}	1.47×10^{-7}
S2	0.22	0.35	1.77×10^2	12.83	4.38×10^{-7}	2.88×10^{-7}
S3	0.24	0.50	1.21×10^2	11.88	_[A]	_[A]
S4	0.20	0.26	2.21×10^4	24.79	_[A]	_[A]
S5	0.22	0.41	3.01×10^4	25.55	2.84×10^{-7}	1.53×10^{-7}
S6	0.21	0.45	1.21×10^4	23.29	4.16×10^{-7}	3.95×10^{-7}
Ferrocene [38]	_[A]	_[A]	3.45×10^2	14.47	1.34×10^{-9}	9.54×10^{-10}
4-Nitrophenyl ferrocene [39]	0.18	0.52	3.85×10^3	20.45	1.03×10^{-5}	7.51×10^{-5}

[A]Not found/calculated.

$$I_{pa} = 2.69 \times 10^5 n^{3/2} A C_o * D^{1/2} \upsilon^{1/2} \tag{2}$$

$$E_{pa} - E_{pa/2} = 47.7/(\alpha_a n) \tag{3}$$

The low diffusion coefficient of S1-DNA adduct compared to **S1** confirmed the interaction of the drug molecule with DNA. Further addition of 4 and 6 μM DNA changed the peak current from 4.45×10^{-3} to 3.73×10^{-3} mA and 3.63×10^{-3} mA, respectively. The negative shift in peak potential attributed to the electrostatic mode of interaction can be explained as Fe^{2+}/Fe^{3+} of the ferrocenyl moiety interacted with negatively charged phosphate backbone of the double helix structure of DNA. The binding constant is calculated by plot of log ($I/I_o - I$) *versus* log (1/[DNA]) [37]. I_o is the current which is observed at E_{pa} for DNA-free drug while I is the current at E_{pa} for drug-DNA adduct. The electrochemical parameters, binding constants, and binding energies are listed in table 3. From the data listed in table 3, the values of binding constants obtained for compounds are fairly impressive. The binding constant of simple ferrocene and 4-nitrophenyl ferrocene is less than **S4**, **S5**, and **S6** which reflects that the linker part of ferrocene also plays a very important role towards the DNA binding affinities. Compounds having binding constants of 10^4 may potentially be used as non-covalent DNA binders [38, 39].

3.4. *Antioxidant activity*

Free radical scavenging activities of **S1**–**S6** along with ascorbic acid were examined by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay in ethanol. A deep violet solution of DPPH gives a strong absorption band at 517 nm which is attributed to its odd electron. As this electron is paired up by a free radical scavenger, the absorbance decreases in accordance with the number of electrons taken up. The decrease in absorbance at 517 nm (figure 6) is used to evaluate the antioxidant potency of compounds [40]. Ascorbic acid was used as standard and was found to have IC_{50} of 10.12 μg mL^{-1}. The free radical scavenging profiles of **S1**–**S6** were fairly impressive. The trend in antioxidant activity obtained by using the DPPH method showed **S1** had the highest DPPH free radical scavenging activity ($IC_{50} = 15.66$ μg mL^{-1}) followed by **S5** ($IC_{50} = 18.22$ μg mL^{-1}) whereas **S6** showed the lowest activity

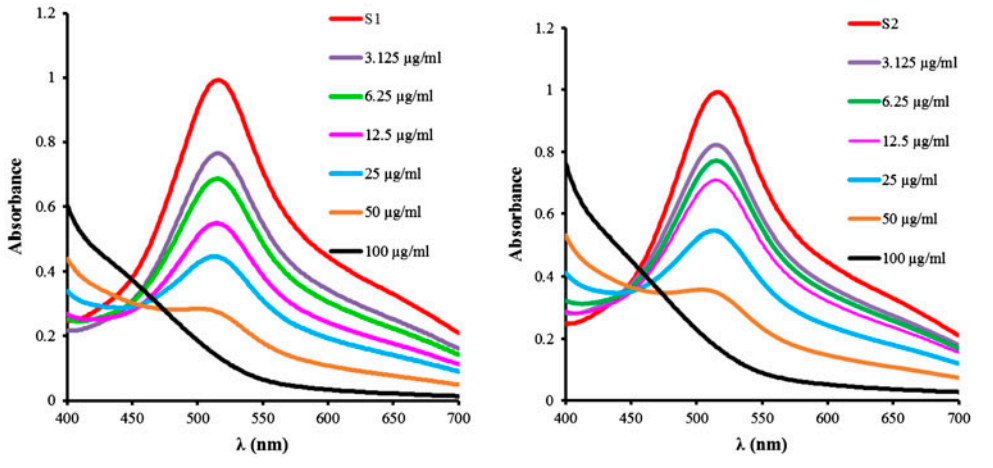


Figure 6. Representative UV–vis absorption spectra of 165 µM DPPH in the absence and presence of increasing concentrations of **S1** and **S2** (3.125–100 µg mL^{-1}).

(IC_{50} = 37.89 µg mL^{-1}). **S2**, **S3**, and **S4** have IC_{50} values 28.70, 23.08, and 24.15 µg mL^{-1} which revealed that substituents decrease antioxidant activity, but having IC_{50} less than 37.89 µg mL^{-1} proved that these compounds have value as antioxidant.

4. Conclusion

Six new ferrocene incorporated N,N′-disubstituted thioureas (**S1–S6**) were synthesized and characterized. The compounds were screened for their potency as DNA binders and antioxidants. The behavior of the series of compounds may suggest electrostatic mode of interactions with DNA as revealed by voltammetric measurements [41]. **S1** has free radical scavenging activity among all screened compounds as revealed by DPPH assay.

Supplementary material

Crystallographic data for the structure reported in this article have been deposited to the Cambridge Crystallographic Data Center as supplementary publication number CCDC-901281. Copies of the data will be available free of charge at deposit@ccdc.cam.ac.uk.

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