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Short Communication

Synthesis, Crystal Structure, Spectral And Electrochemical Characterization, DNA Binding and Antioxidant studies of 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea

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In this manuscript we have synthesized systematically 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea (2F) and successfully determined the structure by single crystal X-rays diffraction analysis. The compound was spectrally characterized using multinuclear ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and fourier transform infra-red (FT-IR) spectroscopic techniques and electrochemical characterization was carried out using cyclic voltammetry. The compound (2F) was then screened for potential deoxyribonucleic acid (DNA) binding and antioxidant activity. The single crystal XRD title compound 2F showed that ferrocenyl moiety had eclipsed conformation while the phenyl ring substituted on ferrocenyl is not in plane with cp-ring. The compound (2F) had shown a reversible process with one electron transfer, two peaks were observed for oxidation and reduction during scan. From voltametric measurements, the shifts in peak potential and peak current were used to review the mode of interaction which is found to be noncovalent electrostatic, drug-DNA binding constant and diffusion coefficients of the compound (2F) and 2F-DNA adduct. The binding constant (M^{-1}) was found to be 2.92 x 10^{3} with binding energy 19.76 kJ mol^{-1}. The diffusion coefficient of free compound (2F) was calculated 1.75 x 10^{-7} whereas diffusion coefficient of 2F-DNA was found to less i.e. 8.96 x 10^{-8} which was obvious as free molecules are easy to diffuse because of its low molecular weight whereas 2F interacted with DNA become heavier and the quantity of remained free molecules became less as a result decrease in current was observed. The compound (2F) showed significant activity with IC_{50} value 41.69 µg mL^{-1} using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay.

Keywords: Crystal structure, Redox nature, Voltammetric titration, DNA interaction, Free radical scavenging.

1. INTRODUCTION

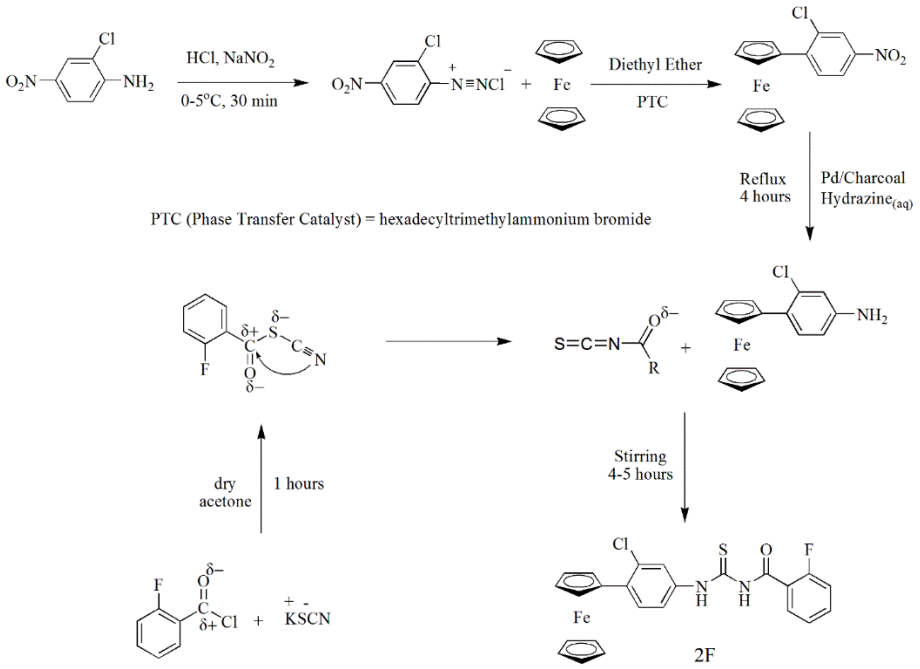
By changing the oxygen atom of urea by sulphur atom produces thiourea which significantly differ in properties of urea and thiourea which may be attributed to difference in electronegativity between sulfur and oxygen [1]. Thioureas and its derivatives were found to have a broad spectrum of biological activities such as antiviral, HDL-elevating, anti-HIV, analgesic properties, antibacterial, and anticancer activities etc [2]. It has been found that many thiourea derivatives posses significant inhibitory activity against receptor protein tyrosine kinases (PTKs), tyrosine kinases (RTKs), and NADH oxidase, which play decisive roles in many aspects of tumor curing agents [3]. The thioureas derivatives like; aroylthioureas, N-nitrosoureas, diarylsulphonylureas, and benzoylureas exhibited activities against different leukemias and solid tumors [4]. One of the major concerns is the use of high dose while using thiourea derivatives because of their very low lipophilicity/hydrophilicity which limits their efficacy and impart lots of fetal side effects [5]. In order to overcome these side effects the dose should be decreased by incorporation of lipophilic moiety in the thiourea structure. The incorporation of ferrocene in the structure of thiourea will minimize the side effects because of being less toxic, kinetically stable, electronically neutral, high lipophilicity and well defined redox behavior [6]. By incorporation of ferrocenyl moiety in the structure of tamoxifen an increased in the anticancer activity has been observed [7]. The substitution of the 2-florobenzoyl group with hydrogen atom of one-sided nitrogen of thiourea and attachment of ferrocenyl moiety to other nitrogen of thiourea will extend the resonance of electron away from the ferrocene. Fluoro-substituted thioureas form effective secondary bonding (i.e. hydrogen bonding) within the molecule and among the molecules, N–H proton-donor to thiocarbonylic =S and carbonylic =O atoms which makes them excellent substrates for studying the hydrogen bonding [8]. The secondary bonding like intermolecular non-bonding interactions are responsible for binding with macromolecules (proteins and nucleic acids etc) which play an important role in determining biological activities of compounds; Those molecules having greater interactions due to secondary bonding are expected to have strong force of binding resulting higher activity [9]. Herein we report synthesis, crystal structure, spectral and electrochemical characterization, DNA binding and free radical scavenging potency of 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea.

2. EXPERIMENT

2.1 Synthesis of 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea (2f)

The compound 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea (2F) was synthesized by immediately reacting prepared positively charged diazonium salt of 2-choloro, 4-nitroaniline with ferrocene in presence of phase transfer catalyst to form 2-chloro, 4-nitrophenyl ferrocene. The nitro moiety was then reduced to amine by the use of hydrazine in presence of Pd/charcoal catalyst resulting in the formation of 4-ferrocenyl, 3-chloroaniline. The 4-ferrocenyl, 3-chloroaniline was then treated with freshly prepared isothiocyanates of 2-florobenzoyl chloride under

N_2 atmosphere in dry acetone to prepare 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea (2F) as shown in scheme 1 [10, 11].



Scheme 1. Schematic representation for the synthesis 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea (2F)

The synthesized compound 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea (2F) was spectrally characterized by FT-IR, ^{1}H and ^{13}C NMR spectroscopy. **Yield** 87%, **FTIR** (**υ cm^{-1}**): Fe-cp (482cm^{-1}), NH (3649-3417 cm^{-1}), C=O (1668 cm^{-1}), C=S (1139-1280 cm^{-1}), C=C Ar (1455-1588 cm^{-1}), sp^2 CH (3057 cm^{-1}). **^{1}H NMR** (300MHz, DMSO): δ 12.64 (s, 1H, CSN*H*), 9.73 (s, 1H, CON*H*),7.66-7.34 (m, 7H,C_6H_3-C_6H_4), 4.77 (t, *J*=1.5Hz, 2H, C_5H_4), 4.38 (t, *J*=1.5Hz, 2H, C_5H_4), 4.17 (s, 5H, C_5H_5) ppm.,**^{13}C NMR** (75MHz, DMSO): δ 177.66, 163.18, 162.23-158.90 (d, *J*=249 Hz, 1C, C_6H_4), 136.11, 135.88, 135.98, 132.20, 131.31, 125.56, 125.22, 121.83, 118.88, 117.02, 116.70, 83.47, 69.73, 68.72, 67.94 ppm. The stepwise appearance and disappearance of absorption bands in FT-IR spectroscopy suggested the formation of desired compounds. The results of FT-IR spectroscopic data is equally supported by multinuclear NMR spectroscopic data. Furthermore the crystal structure of 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea (2F) have justifies all the spectral data. The compound (2F) was synthesized successfully in high yield with high purity [12].

2.2 Single crystal X-rays diffraction analysis

After carful crystallization of synthesized compound 2F in toulene, a single but suitable dark orange crystals of 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea (2F) crystal was screened for the single crystal X-rays diffraction analysis. The instrument used was a Bruker Kappa APEXII CCD diffractometer having Mo-Kα radiation (λ = 0.71073 Å) as a graphite-monochromated source. The ω scans were used to obtain data and after the collection of data application of multi-scan absorption correction was made.

2.3 Cyclic voltammetric measurements

The electrochemical characterization and DNA titration with 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea (2F) were carried out in a three electrode configuration cell enclosed in single compartment with at different scan rate in milli volt per second (mV s^{-1}); 50, 100, 150, 200, and 250 mV s^{-1} without and with CT-DNA having concentrations 30, 60 and 90 µM to understand redox behavior of compound (2F) and also binding potency of compound (2F) with CT-DNA that includes mode of interaction, binding constant and binding energy. The diffusion coefficient of 2F molecules and 2F-DNA adduct is calculated to confirm adduct formation.

2.4 Antioxidant activity

The Free radical scavenging screening of 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea (2F) was determined by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method.

3. RESULTS AND DISCUSSION

3.1 Single crystal X-rays diffraction analysis

The single crystal X-rays diffraction analysis of 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea (2F) obeyed monoclinic system and have space group P21/n. The unit cell dimensions (bond lengths & bond angles) were calculated from these single crystals and it was found that all the cell dimensions are in the acceptable range as compared to theoretical values. The Bond precision used for C-C = 0.0045 A. The other crystal parameters calculated were empirical formula $C_{24}H_{18}FeClFN_2OS$, unit cell dimensions (a≠b≠c in Å) a = 7.148 (10), b = 22.813 (3), c = 13.209 (2), (α=γ≠β) α = 90.0°, β = 104.103 (2)°, γ = 90.0°, Mr = 492.77, Density = 1.567 g/cm^3 , Volume = 2089.1(5) $Å^3$, Z = 4, F(000) = 1008.0, Index ranges $(h, k, l)_{min}$ = (-8, -26, -14), $(h, k, l)_{max}$ = (8, 26, 15), Crystal size = 0.18 x 0.21 x 0.410 mm^3, Total reflections = 3419, Mu = 0.978 mm^{-1}, θ_{max} = 24.440°, R = 0.0358 and wR2 = 0.1121. The ORTEP structure of 2F is shown in figure 1.

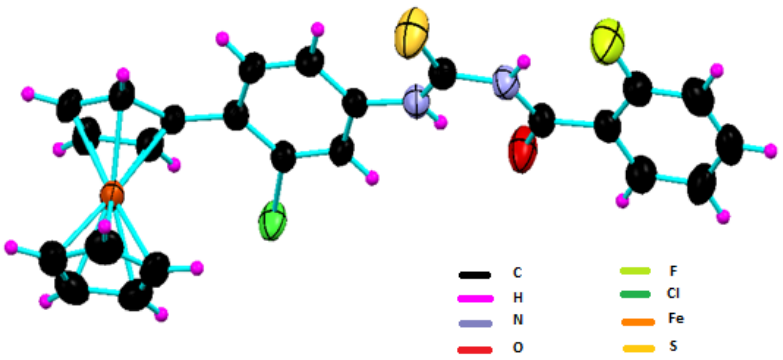


Figure 1. Molecular structure of 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea (2F)

3.2 Cyclic voltammetric measurements

The voltammogram of compound (2F) showed steady redox peaks in the potential range of 0.2-0.8 V in either scan i.e. in forward scan anodic peak and in reverse scan cathodic peak appeared [13]. As the voltammogram was showing each peak in either scan so the next step was to look for the reversibility. The criteria for reversibility is; 1) $\Delta E_p = E_{pc} - E_{pa}$ value should be 0.058, 2) the ratio of anodic and cathodic peak current (I_{pa}/I_{pc}) should be equivalent to one and 3) the scan rate did not produce any change in anodic/cathodic peak position and the voltammograms of 2F obeyed the reversibility criteria of electrochemical reaction. This cyclic voltammogram of compound (2F) showed one electron transfer and the reaction is totally reversible electrochemical reaction as it fulfills all three above requirements [14]. By mixing 30, 60 and 90 µM CT-DNA into 2 mM 2F solution (Figure 2) the change in peak potential and decrease in current i_{pa} was being seen. The decrease in peak current i_{pa} is due to diffusion of drug into double helix DNA which resulted supramolecular complex formation, due to which transfer of electrons was being reduced as a result number of free molecules was being decreased. The change in value of formal potential explained the nature of binding between 2F molecules and DNA. Generally intercalation of small molecules into double helical deoxyribonucleic acid caused positive change in the peak potential, whereas negative change revealed binding of the positively charged drug molecule with the negatively charged phosphate (PO_4^{3-}) of moiety present on DNA backbone called the electrostatic interaction [11]. The negative change in peak potential was observed for 2F by the addition of different concentration of CT-DNA, revealed the electrostatic nature of interaction [15]. The binding constant can be by using following equation; {1/[DNA] = K(1-A)/1-(i/i_o) – K}, where i_0 and i are the peak currents in absence and presence of CT-DNA, K is the binding constant, and A is the proportionality constant. If we plot a graph between 1/[DNA] and 1/(1−i/i0), binding constant (K) can be calculated which was; 2.92 x 10^3 M^{-1}. The changed binding free energy (-ΔG= RT ln K at 25 °C) of compound 2F was calculated to be 19.76 kJ mol^{-1} exhibited the spontaneity of 2F-DNA interaction [16].

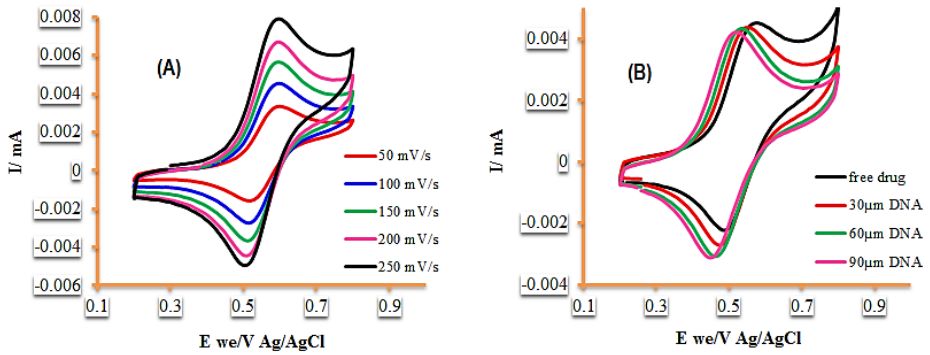


Figure 2. (A) Cyclic voltammograms of 2 mM compound **(2F)** at different scan rates. (B) Cyclic voltammograms of 2 mM compound **(2F)** in the absence and presence of 30 µM, 60 µM and 90 µM DNA showing a decrease in I from Io and a -ve shift in peak potential showing electrostatic interactions.

The diffusion coefficient of free compounds and compound-DNA adduct was calculated from Randles-Sevcik equation; (I_{pa} = 2.69 x 10^5 $n^{3/2}$ A C_o* $Do^{1/2}$ $\upsilon^{1/2}$) where I_{pa} is referred to anodic peak current in ampere, v referred as scan rate in V s^{-1}, $C_o^*$is concentration in mol cm^{-3}, A is cross sectional area of electrode in cm^2, n is number of electrons involved in the reaction, D_o is diffusion coefficient in cm^2 s^{-1}. The diffusion coefficient of free compound (2F) was calculated 1.75 x 10^{-7} whereas diffusion coefficient of 2F-DNA was found to less i.e. 8.96 x 10^{-8}. The decreased diffusion coefficient value for 2F-DNA adduct can be justified as free molecules are easy to diffuse and is of low molecular weight so exhibit more peak current whereas when compound 2F was interacted with DNA, the quantity of free molecules became less as a result decrease in current was observed and which is obvious [17].

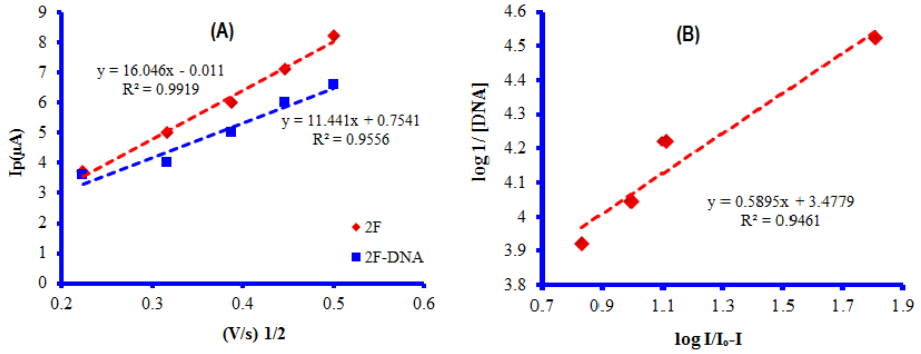


Figure 3. (A) Plots of I vs. v½, for the determination of diffusion coefficients of compound **2F** (0 µM DNA) and **2F-DNA**. (B) Plot of log (1/[DNA]) vs log (I/I_o-I) for determination of binding constant

3.3 Antioxidant activity

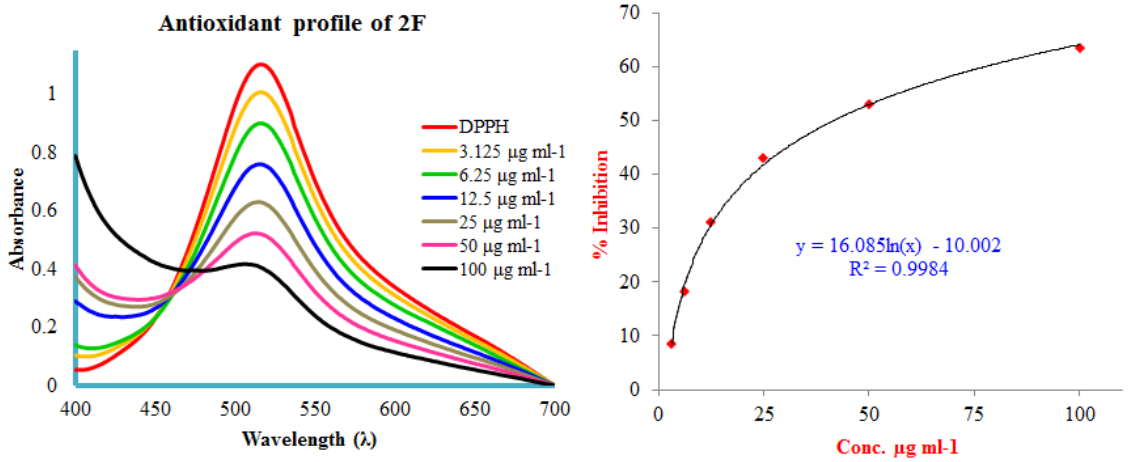


Figure 4. Electronic absorption spectra 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea (2F) (3.125-100 µg mL^{-1}) showing free radical scavenging pattern

The free radical scavenging activity of compound showed a steep increase in percent inhibition by increasing the concentration [18]. By looking at the Figure 4, It had been found that by increasing

the concentration of ferrocene based thioureas the % inhibition is increased. The free radical scavenging caused by 3.125 µg ml-1 is least and is highest for 100 µg ml-1. The 50% inhibitory concentrations (IC_{50}) of the compound (2F) was found to be 41.69 µg ml-1 that is very low which made us to suspect that if furthure needful experiments are performed we may be able to see the utility of these kind of molecules in clinical uses.

4. CONCLUSION

In conclusion 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea (2F) was successfully synthesized and structure was by determined by single crystal X-rays diffraction analysis, multinuclear ^{1}H and ^{13}C NMR and FT-IR spectroscopic techniques. The electrochemical characterization had shown a reversible process with one electron transfer. The compound (2F) had shown significant binding with DNA via electrostatic interactions and impressive free radical scavenging ability.

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