Chapter

Supramolecular Chemistry and DNA Interaction Studies of Ferrocenyl Ureas and Thioureas

Samia Kausar, Ataf Ali Altaf, Muhammad Hamayun, Amin Badshah and Abdul Razzaq

Abstract

In this chapter, we have discussed the characteristics and bioapplicabilities of different ferrocene derivatives, for example, amides, amines, sulfonamide, and polymers, focusing mainly on urea and thiourea derivatives due to their autonomous and widespread spectroscopic action and bioactivities. Supramolecular chemistry of ferrocenyl ureas and thioureas is described owing to exploring their mode of interactions within and among the molecules and the role of these supramolecular structures in enhancing the DNA intercalation. DNA interaction studies of these ferrocenyl-based ureas and thioureas are explored with approaches like electrochemical study, viscosity measurements, molecular docking, electronic spectroscopy, dynamic light scattering (DLS), and radical scavenging activity. Attachment of ferrocene moiety to ureas an thiouereas closer to DNA is very promising strategy which most possibly boosts the probability of DNA damage and cell apoptosis which is responsible for enormous biological activities.

Keywords: thioureas, ferrocene, medicinal chemistry, supramolecular chemistry, ureas, DNA interaction

1. Introduction

Soon after discovery of ferrocene in 1951 [1], the main focus of scientists was to determine its accurate structure. Its correct elucidation was carried out individually by Fischer and Hafner and Wilkinson et al. [2, 3]. Woodward et al. termed this iron-containing compound as ferrocene due to resemblance of its reactivity with benzene [4]. The structural determination of ferrocene proved a revolutionary discovery and a progressive revelation in the historical backdrop of chemistry which directed new dimensions in organometallic chemistry. Today, terms like metallocenes and sandwich compounds are used for ferrocene and ferrocenyl derivatives due to significantly more extensive scope of these compounds that assimilate other metals also [5].

Due to impressive stability of ferrocene in aerobic medium and water, its promising electrochemical properties and openness of an extensive assortment of subordinates have made ferrocenyl derivative compounds widespread molecules for biological applicabilities [6–8]. The promising applications of ferrocene in the field of medicine proved a vigorous research area nowadays [9]. Many reports have revealed that some ferrocenyl derivatives are extremely active in vivo and in vitro,

against various diseases, for example, bacterial and fungal contagions [10, 11], malaria [12], cancer [13], and human immunodeficiency virus (HIV) [14].

2. Ferrocene derivatives

Extensive applications of ferrocene and its derivative compounds in material science, homogeneous catalysis [15], nonlinear optics [16], and molecular sensors are observed [17]. Furthermore, an unexpected biological activity is often witnessed upon incorporating a fragment of ferrocene into a molecule of an organic compound [18]. Many ferrocene derivative compounds exhibit stimulating cytotoxic, antimalarial, antitumor, antioxidant, antifungal, and DNA-cleaving activity [19–21].

The anticancer [22] perspective of ferrocenyl derivative compounds was first premeditated around the 1970s. Brynes and collaborators explored the counter tumor action of ferrocenyl compounds containing amide or amine moieties against leukemia P-388 of the lymphocytic system [23]. They administrated these derivative compounds to mice intraperitoneally using either water or surfactant with water as Tween-80: water. The anti-tumor action of these compounds was considerable enough to show that the incorporation of the ferrocenyl moiety into an appropriate bearer could provide a drug with elevated antitumorous activity (**Figure 1**) [23].

Extensive study is carried out about ferrocene and its derivative compounds as efficient chemotherapeutic agents [24]. Stability, electroactivity, and extraordinary spectroscopic actions of ferrocene-incorporated organometallics are reasoned for being auspicious contenders for various biological applicabilities [25–27]. With reversible redox characteristics and elevated cell penetrability owing to its extensive lipophilicity, ferrocenyl moiety is responsible for pronounced characteristics of ferrocenyl derivative

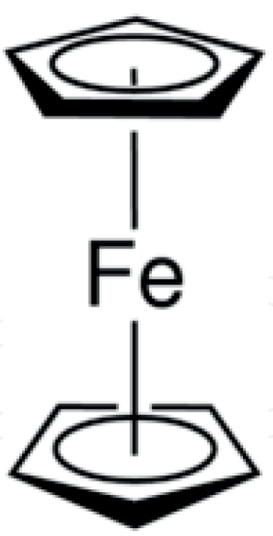


Figure 1.
Structure of ferrocene.

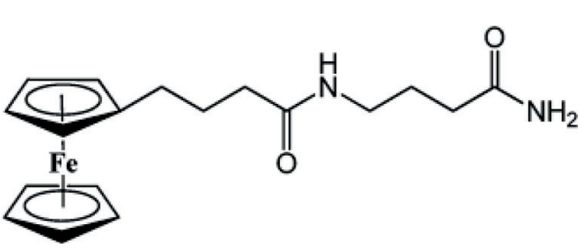


Figure 2.
Ferrocenyl derivative verified against lymphocytic leukemia P-388.

compounds [28]. For example, enhanced anticancer activity was observed when ferrocene was incorporated into tamoxifen which is a potent anticancer drug i.e. Ferrocenyl derivative verified against lymphocytic leukemia P-388 (**Figure 2**) [29].

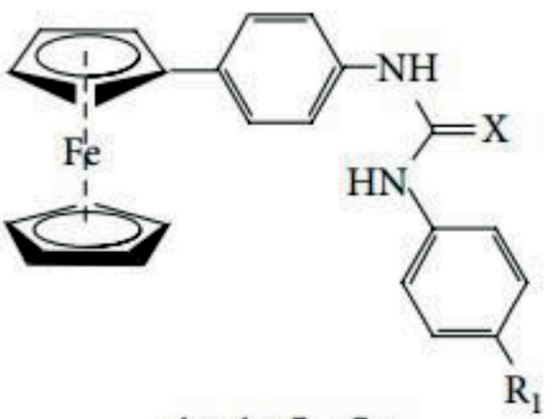
3. Ferrocenyl ureas and thiourea

Among the various ferrocenyl derivative compounds are i.e. amides, amines, polyacids, polymers, ureas, thioureas, and sulfonamide derivatives [30]. A lot of work is happening on the applications of ferrocenyl urea and thiourea compounds due to their pronounced interactions, supramolecular chemistry, electrochemical characteristics, and DNA interactions. Following are some examples of ferrocenyl urea and thiourea derivative compounds checked for their various activities depicted in **Figure 3** [30].

3.1 Ferrocenyl ureas

Urea ($R_1R_2NC{=}ONR_3R_4$) is a striking building block in consequence of its widespread bioactivities and extensive bioavailability from natural products [31]. Among the urea derivative compounds, urea derivatives having aromaticity in them such as N-phenyl-N-(2-chloroethyl)urea and heterocyclic urea derivatives illustrate potential anticancer activities because of their efficient inhibitory effect against the receptor tyrosine kinases (RTKs) [32].

Urea is ascertained to be an appealing building obstruct for receptors of anion as it contributes two comparatively robust H-bonding positions [33]. The two N—H groups in urea are able to make a bond with the only acceptor atom to form a ring structure comprising six-membered chelate or bind with two nearby oxygen atoms in an oxy-anion to give a ring structure consisting of eight-membered chelate as



4a–4e, 5a–5e

	X	R_1		X	R_1
4a	O	–H	**5a**	S	–H
4b	O	–Cl	**5b**	S	–Cl
4c	O	–Br	**5c**	S	–Br
4d	O	$–CH_3$	**5d**	S	$–CH_3$
4e	O	$–OCH_3$	**5e**	S	$–OCH_3$

Figure 3.
Some representative ferrocenyl ureas and thioureas.

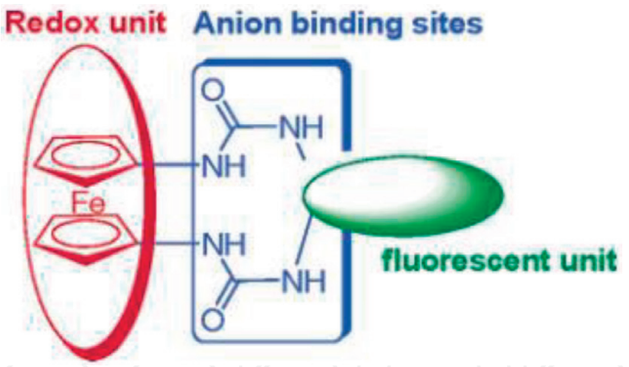


Figure 4.
Ferrocenyl urea [34].

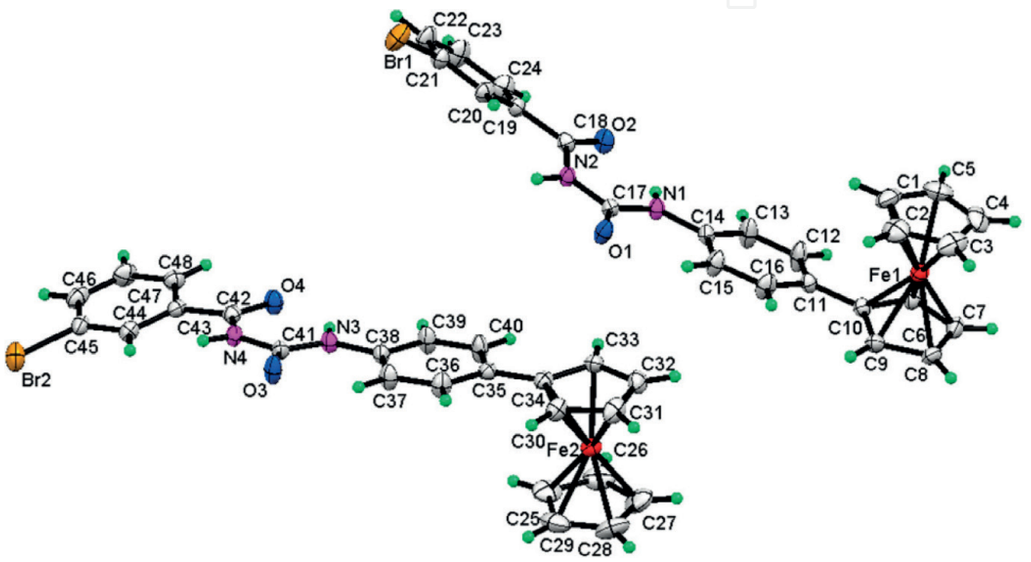


Figure 5.
Molecular structure of 1-(3-bromobenzoyl)-3-(4-ferrocenylphenyl)urea.

depicted in (**Figure 4**). These N-H groups are modified to supplement target anion and minimal intramolecular H-bonding to observe strong and selective binding characteristics [34] (**Figure 4**).

Over the past few years, assortment of urea-based hosts comprising one or more than one urea subunits is premeditated and tested for anion recognition and for being capable of sensing [35]. New perceptions into characteristics of interactions between urea and anionic moiety providing structural measures for considered designing of novel anion-selective receptors which contain two or additional urea binding groups have also been discovered in recent times [36]. On the other hand, there are few instances of ferrocenyl urea derivatives as redox active anionophores [37]. The molecular structure of a representative ferrocenyl urea derivative is presented in **Figure 5** [38].

3.2 Ferrocenyl thioureas

Replacement of an oxygen atom in urea moiety by a sulfur atom results in thiourea formation, the characteristics of which are significantly diverged than those of urea due to the variation in electronegative character among sulfur and oxygen atoms [39]. Thiourea-based compounds and complexes have also been explored for several biological activities because of the thio-carbonyl group, which influences biochemical activity by lipophilic or hydrophilic character and electronic properties of derivative compounds [40].

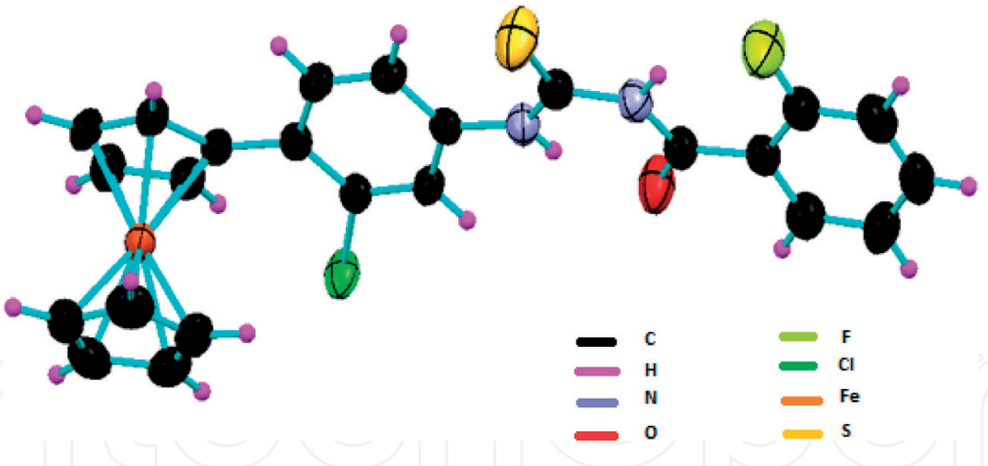


Figure 6.
Molecular structure of 1-(2-florobenzoyl)-3-(2-chloro,4-ferrocenylphenyl)thiourea.

The lipophilicity/hydrophilic characters and the electronic properties of thiourea derivative compounds are greatly inclined due to the presence of thio-carbonyl moiety which in turn affects the labile nature of leaving substituents, hence regulating the biochemical activity. Various ferrocenyl thiourea derivative compounds have been discovered, which are significantly important for various features of antitumorous agents due to their inhibitory rejoinder against receptor tyrosine kinases (RTKs), protein tyrosine kinases (PTKs), and NADH oxidases [41, 42]. Various thiourea derivatives exhibited bioactivities against different infectious diseases, leukemias, and solid tumors, for example, aroylthioureas, diarylsulphonylureas, N-nitrosoureas, and benzoylureas [43]. The molecular structure of a representative ferrocenyl urea derivative compound is depicted in **Figure 6** [40].

4. Supramolecular chemistry of ferrocenyl ureas and thioureas

4.1 Supramolecular chemistry

Supramolecular chemistry [44] focuses on the design and synthesis of "Supramolecular Entities" [45], i.e., compound elements held together by noncovalent connections including hydrogen bonding, bonds with halogens, forces of coordination, or π-π connections (**Figure 7**) [46]. Research in supramolecular chemistry and crystal engineering is principally centered around host-guest arrangements, binding of anion and cation, coordination polymers, developments of self-assembly networks, biological simulators, gels, fibers, liquid polymers, crystals, and other various types of materials [47].

Supramolecular chemistry objects to the considerations of interactions between molecules and packing patterns in molecular crystals and, consequently, usage of information spawned for potential novel material designing gathered with targeted structures and proficient characteristics [48–50]. From this objective, one can consider assembling molecular crystals having a multitude of noncovalent interactions among which a prominent position is occupied by H-bonds owing to their noticeable directionalities and reasonably high strength [51]. Hydrogen bonds are characteristically much weaker in comparison to covalent bonds though and hence have minimum predictability, which often destabilizes the crystal designing process utilizing these interactions [52].

While considering the molecular synthesis, synthetic schemes are confidently planned by researchers for molecules comprising very complex framework. In synthesizing crystalline organic solids, the term engineering can be invoked infrequently in its true sense. Taking from supramolecular structure to design a crystal

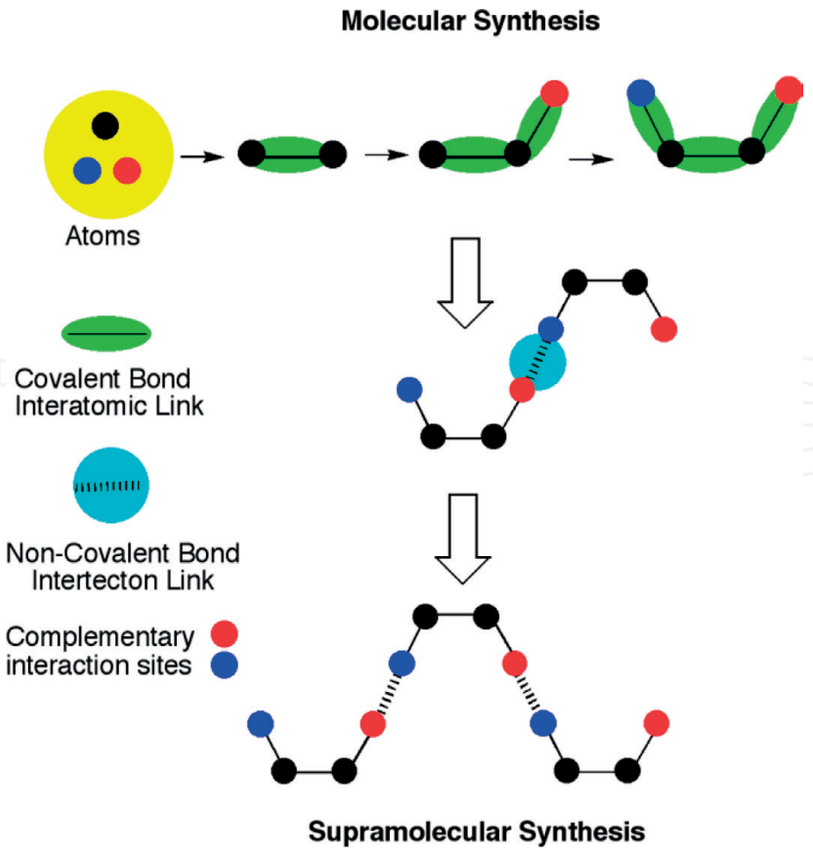


Figure 7.
Noncovalent interactions in supramolecular synthesis.

structure, the whole process considers experiential observations and a posteriori structure of crystal and analytical measurement [53].

4.2 Supramolecules of ureas and thioureas

Urea group is one such powerful building obstruct that formulates obstinate chains that bind through hydrogen bonding in various surroundings, from solutions [54] to gels and fibrous materials [55], also with crystals [47]. An approach that has been comprehensively discovered since the initial research in crystallography of disubstituted ureas in the late 1960s is the use of symmetrical or asymmetrical N,N′-di-substituted ureas that can deliver widespread multiple building congers for designing of organic solids of crystalline nature. N,N′-di-substituted ureas have the ability to act as H-bond donors by using their two N—H protons, and play the role of acceptors through utilizing the presence of the lone electron pairs of CO group [36]. Robust one-dimensional hydrogen bonded chains having self-association reinforced promising complementarity between both groups (**Figure 8**) [56], which have been reconnoitered for developing the crystalline networks repeatedly.

Nanostructured materials on the basis of cylindrical or columnar constructions have been innovated more recently. Thioureas, despite the fact that they can also practice comparatively robust H-bonded motifs [57], have not been as much searched out for the coherent assemblage of solids of crystalline nature as compared to ureas [58, 59].

4.2.1 Example illustrating the supramolecular interactions of ferrocenyl thiourea

Dr. Bhajan Lal Bhatia and coworkers synthesized ferrocenyl-based thiourea compounds, i.e., 1-benzoyl-3-(4-ferrocenylphenyl)thiourea (**B16**) and

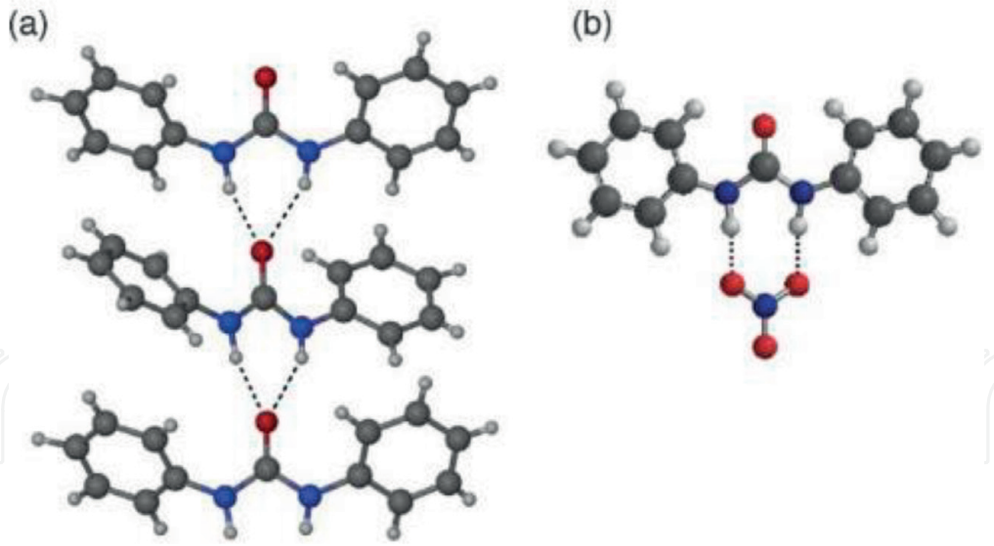


Figure 8.
The contrast of N,N'-disubstituted ureas functioning as: (a) building blocks for the assemblage of H-bonded chains, as depicted by the crystal structure of di-phenylurea [59] or (b) anion binding groups, as represented by calculated di-phenylurea:nitrate complex, optimized with DFT at B3LYP/6-31G level [56].*

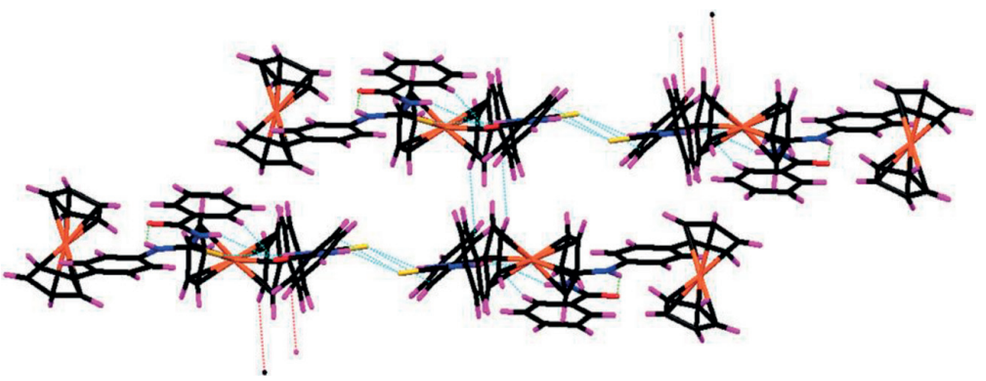


Figure 9.
Supramolecular structures of B16 intervened by secondary bonding.

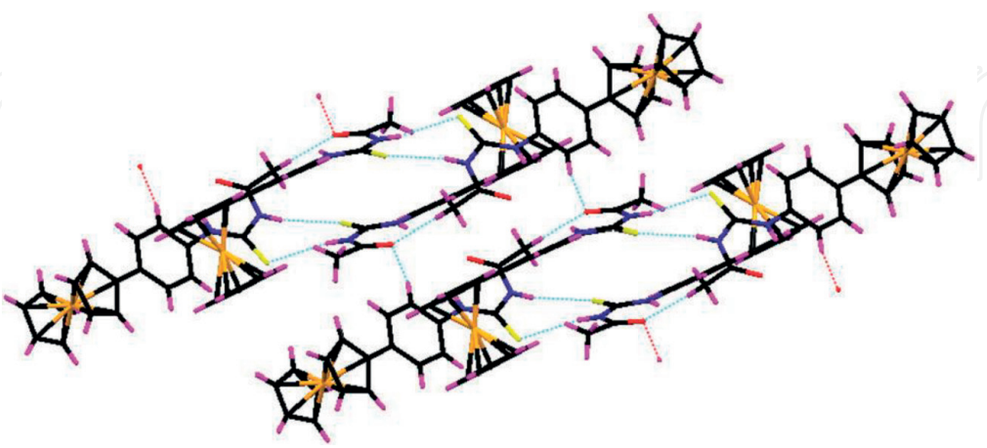


Figure 10.
Supramolecular structures of B3 intervened by secondary bonding.

1-acetyl-3-(4-ferrocenylphenyl)thiourea (**B3**) [60]. Finding displayed two self-regulating molecules which are present in an asymmetrical component in structures of B16 and B3 linked interchangeably to each other through intermolecular NH···O and NH···S types of hydrogen bonding and secondary interactions

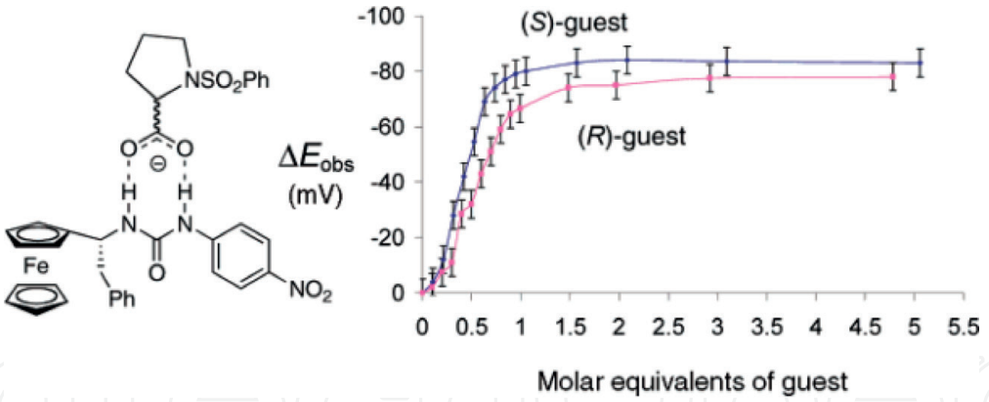


Figure 11.
Novel redox active ferrocenyl urea receptors for the purpose of binding and sensing the chiral carboxylate anions and their molar equivalents of (S)-(blue)(R)-(pink) [66].

non-covalent in nature (π···H), which intervene supramolecular structures for B16 and B3 as shown in **Figures 9** and **10** [60].

Determination of some important biological activities usually depends upon these types of secondary nonbonding interactions between the molecules. Greater ability of compounds to associate with macromolecules such as DNA and proteins is observed due to having an ability to make stronger nonbonding interactions [61]. Among the compounds studied, B16 was reported to be associated with more secondary interactions that were apparent from H-bonding data and formation of a supramolecular structure. Therefore, it can be predictable that B16 can have better strapping interaction with DNA that will transform into more elevated biological activities [60].

4.3 Chiral recognition of ferrocenyl derivatives

Now a days, main focus in supramolecular chemistry is chiral recognitions [62] hence designing of enantioselective sensors growing quickly. The encouragement directed us to search for appropriate ways of ascertaining one enantiomer of a specific chiral target board regarding its mirror image [63–65] (**Figure 11**).

The productions of chiral urea's series attached to the ferrocene group which is redox active are discovered [62]. These are able to bind through hydrogen bonding interactions with chiral carboxylates in organic solvents confirmed by spectroscopic and cyclic voltammetric measurements. Cyclic voltammetric measurements have shown that these guests can be sensed via electrochemical approaches in solution. For example, enantioselective nature is prominent enough in the association of protected amino acid, i.e., N-benzenesulfonylproline, through a ferrocenyl-benzyl host that permits the contrary enantiomers to be distinguished in an electrochemical way as depicted in (**Figure 11**) [66].

5. DNA interaction of ferrocenyl ureas and thioureas

DNA is relatively the key target within cells for research work having small molecular entities important biologically, i.e., steroids, cancer-causing agents and other various modules of drugs [67]. The exploration of interactions of drug with DNA is far more imperative for consideration of characteristic molecular drug action mechanisms to its peculiar target and for fabrication of definite DNA-targeted drugs [68]. Usually, three binding approaches, intercalative mode of binding, groove binding, and electrostatic type of interactions, are involved in the noncovalent interactions

of small molecules with DNA [69]. The intercalative binding mode is resilient than the other two modes of binding due to the surface of intercalating molecule, which is sandwiched among aromatic and heterocyclic DNA base pairs [70–72].

5.1 Activity of ferrocenyl moiety

The introduction of a ferrocenyl group to molecules like urea and thiourea that bind with DNA is an auspicious strategy to take the ferrocene moiety in close proximity of DNA which most possibly boosts the probability of DNA damage and cell apoptosis. The above-discussed anticancer character of ferrocenyl derivatives is found to be reliant on mainly oxidation state of Fe in ferrocene moiety as approximate results clearly exhibited that the Fe(II) ferrocenyl derivative compound is found to have more activity than Fe(III)-containing compounds [73].

One of the Fe(II) compounds, ferrocifen, performs its action by altering the conformation of protein having receptor site as indicated by the results of the study carried out [74]. Binding of ferrocifen to estrogen receptor (ERβ) is considered to result in its dimerization, which is followed by attachment of the dimerized species to a particular targeted area of DNA. Reactive oxygen species (ROS), such as hydroxyl radicals (•OH) produced as a result of electron transfer reactions, result in in-vivo formation of ferrocenium ion or formation of ferrocifen-ERβ complex. ROS produced can be responsible to damage DNA [75, 76] and may also control the anticancer activity by forming the radical metabolites that carry the biological impairment in cancerous regions [77].

5.2 DNA binding studies through cyclic voltammetry

To demonstrate the approach of interaction and the DNA binding constraints, different techniques are employed. Cyclic voltammetry is proved to be one of the most sophisticated and sensitive techniques to carryout DNA binding studies. Investigation of mode of interaction between DNA and derivative compounds is determined through shift in peak potential. Indication of intercalation of derivative compounds into double helix structured DNA comes from slightly positive shift of the peak potential. The ratio of binding of oxidized and reduced molecules is calculated using the following equation Eq. (1) [78, 79]:

$$Eb^{\circ} - Ef^{\circ} = 0.05916\, log\left(\frac{K_{red}}{K_{oxd}}\right) \quad (1)$$

where Eb° and Ef° are proper potentials of bound and free drug candidates, correspondingly. Positive shift is indicative for intercalation of derivative compounds with DNA. The formation of a supramolecular complex due to drug diffusion into DNA results in dropping of current in electrochemical analysis. Drop off in current is observed depending upon the number of transferred electrons, which is decreased upon formation of a supramolecular complex. Binding constant is calculated using the following equation Eq. (2) [80]:

$$\frac{1}{[DNA]} = \frac{K[(1 - A)}{1 - i/i_o} - K \quad (2)$$

where K is the binding constant, i and i_o represent peak currents in the presence and absence of DNA, and A is the proportionality constant. The plot of 1/[DNA] versus $1 - i/i_o$ produces binding constants.

Following are the examples of cyclic voltammetric results in **Figures 12** and **13** for the characteristic ferrocenyl thioureas discussed in Section 3.2 and their molecular structures in **Figures 9** and **10**.

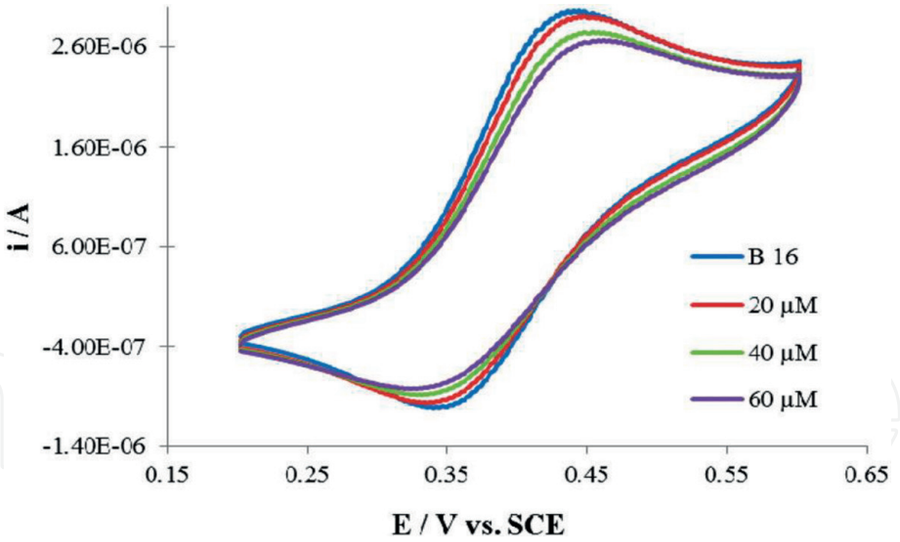


Figure 12.
Cyclic voltammograms of 1 mM B16 at a 0.05 V s^{-1} potential sweep rate on a glassy-carbon electrode at 298 K with and without incorporation of 1 mL of CT-DNA by its increasing concentrations (i.e. 10, 20, and 60 µM) in a 20% aq. DMSO buffer at pH 6.0; supporting electrolyte 0.1 M TBAP [60].

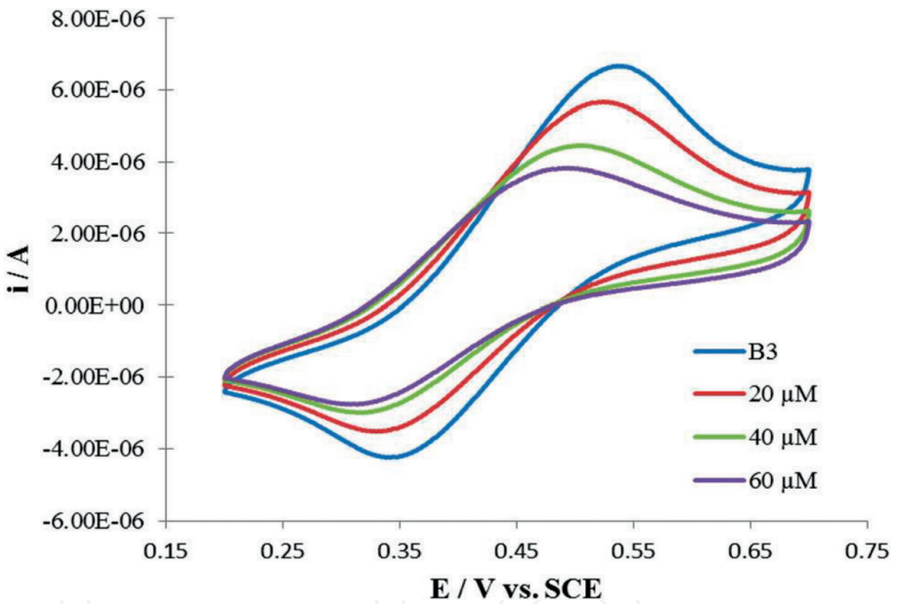


Figure 13.
Cyclic voltammograms of 1 mM B3 at a 0.05 V s^{-1} potential sweep rate on a glassy-carbon electrode at 298 K with and without incorporation 1 mL of CT-DNA by its increasing concentrations (i.e. 10, 20, and 60 µM) in a 20% aq. DMSO buffer at pH 6.0; supporting electrolyte 0.1 M TBAP [60].

Cyclic voltammetric analysis of 1 mM of B16 and B3 was carried out incorporating and without incorporating calf-thymus DNA (CT-DNA). After the addition of CT-DNA, an obvious positive shift in formal potential was observed for B16, which indicated the intercalative mode of interaction, whereas for B3, a formal potential shift toward negative side was observed, which is credited to electrostatic interactions among compound and DNA [60].

5.3 DNA binding studies through viscometry

Viscosity measurement is another beneficial technique to demonstrate intercalation of derivative compounds with DNA. It is sensitive to change in DNA length as base pair active pockets are broadened to provide lodging to binding molecule that ultimately results in lengthening of DNA helix. This technique is considered

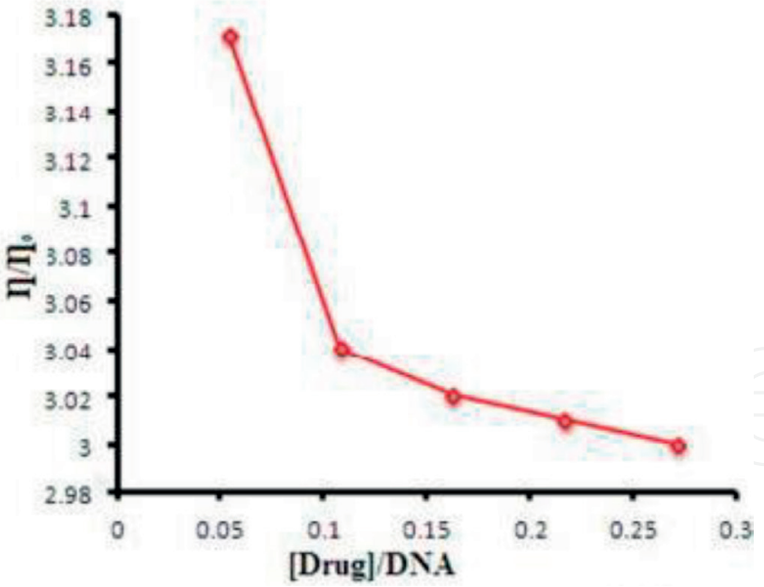


Figure 14.
Relative viscosity versus [Drug]/[DNA] representative plot for mode of interaction determination.

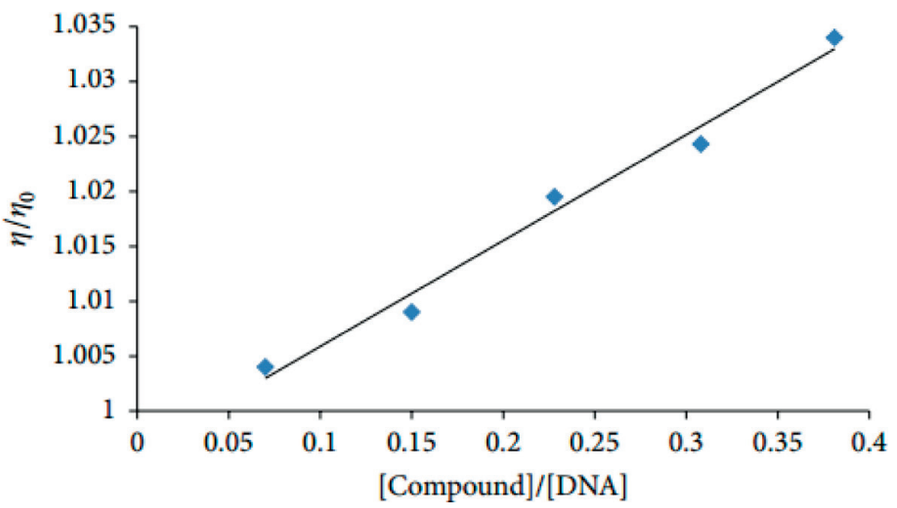


Figure 15.
Effect of increasing concentration of compound 1,1′-(4,4′-di-ferrocenyl)di-phenylthiourea on relative viscosity of DNA at 25°C. [DNA] = 30 μM and [compound] = 5–25 μM.

as a least abstruse and utmost precarious test for binding mode determination in solution phase under suitable conditions, i.e., constant temperature at 25.0 ± 0.1°C in a thermostatic bath [81].

Following is the representative plot (**Figure 14**) [82] which exhibits the relative viscosity (η/ηo) against [compound/[DNA] concentrations to examine the mode of interaction. Derivative compounds have shown that upon increasing binding ratio, relative viscosity decreases which is a representative for electrostatic interactions [82].

Figure 15 [81] is another example of a viscosity measurement result of a ferrocenyl urea derivative compound that shows the consequence of increment in concentration of derivative compound on viscosity [81].

5.4 Molecular docking

Molecular docking approach is one of the most frequently employed approaches in structure-based drug designing, owing to its capability to predict conformation

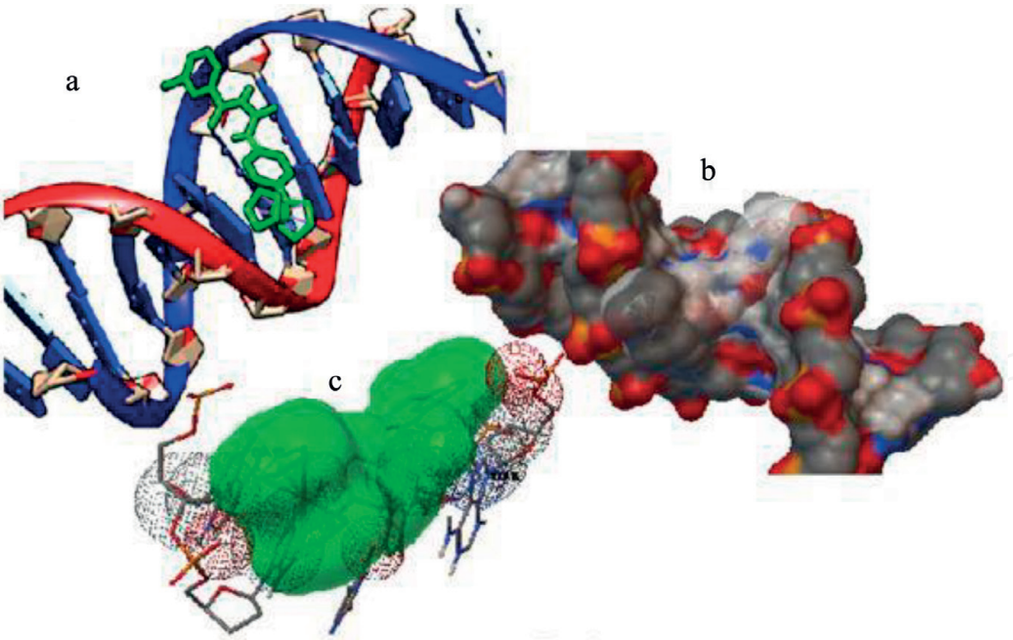


Figure 16.
(a) Docked conformation of representative P3Cl compound with 1-BNA; P3Cl is shown in green color while 1-BNA exhibited a ribbon structure, (b) surface outlook of docked-P3Cl with 1-BNA (color code: grey-carbon, red-oxygen, and blue-nitrogen) and (c) 3D-model representing interactions of P3Cl and DNA [82].

responsible for binding of small molecular entities, i.e., ligands to a suitable target active binding site [83].

The example illustrated in **Figure 16(a)** exhibits the representative docked conformation of compound 1-(3-chlorobenzoyl)-3-(4-ferrocenylphenyl)urea (**P3Cl**) with DNA attributed to have the lowest binding energy, recommended by AutoDock [84], whereas **Figure 16b** depicts the surface view of docked conformation of the represented compound and it is clearly indicated from **Figure 16b** that ferrocenyl group of docked P3Cl is in close interaction with O-atom which is linked to sugar phosphate DNA backbone. This, in another way, suggested that electrostatic interaction force exist among Fe and O-atoms of sugar and phosphate backbone [82]. **Figure 16c** is the close view of DNA atoms that clearly interact with the active surface of the P3Cl compound, and O-atom of the sugar-phosphate backbone can be seen, which prevails among deoxyadenosine (DA)-18 and DA-17, showing electrostatic interactions with ferrocenyl group [85]. The presence of hydrogen bond is also observed in the structure among one of the O atoms of P3Cl and H-atom attached to N-atom of DA5 [86].

5.5 Dynamic light scattering (DLS)

Dynamic light scattering (DLS) approach is employed to govern size distribution of small particles in suspension or polymers in solution. This method is used in medicine to detect molecular changes in the cornea in biology to measure the rate of diffusion on proteins, and in material science to study the orientational fluctuation in the liquid crystals [87].

DLS is, in principle, capable of distinguishing whether a protein is a monomer or dimer; it is much less accurate for distinguishing small oligomers than is classical light scattering or sedimentation velocity. The advantage of using dynamic scattering is the possibility to analyze samples containing broad distributions of species of widely differing molecular masses (e.g. a native protein and various sizes of aggregates), and to detect very small amounts of the higher mass species (<0.01% in many cases) [88].

For example, expansion of DNA helix occurs upon intercalation of a small molecule into DNA helix, as cavities are created to lodge small molecules between the bases which minimize the hydrodynamic radius (Rh) as shown in **Figure 17** [61].

5.6 UV-vis spectroscopy

UV-vis spectroscopy is used frequently for studying ferrocene and ferrocenyl derivative compounds owing to their fairly high stability under visible irradiation, and hence, they are widely used in luminescent systems. They are classical quenchers of excited states. Both energy and electron transfer may be involved, depending on the nature of the excited species [89]. The color of the ferrocene greatly changes upon oxidation, hence permitting spectroscopic measurements in the visible range. UV-vis spectroscopy is proved to be an effective approach for calculation of binding strength of DNA with derivative compounds. **Figure 18** depicts a representative UV-vis plot of ferrocenyl urea depicting a noticeable hypochromic and a slender blue peak shift representing adducts of drug-DNA comparative to free drug entities, which approves the electrostatic nature of interactions (**Figure 18a**) [38].

5.7 Free radical scavenging activity

Substantial binding of derivative compounds with DNA can be observed via electrostatic type of interactions and remarkable free-radical scavenging capability [90].

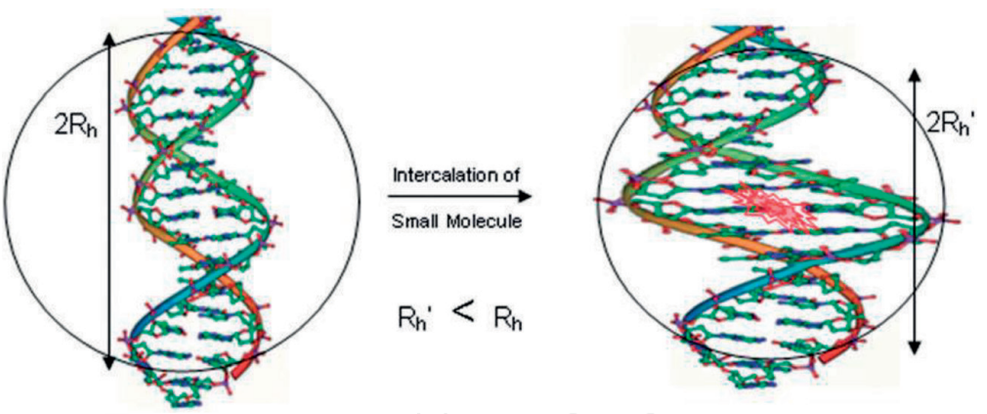


Figure 17.
Decrease in hydrodynamic radius (Rh) upon intercalation of complex in DNA helix.

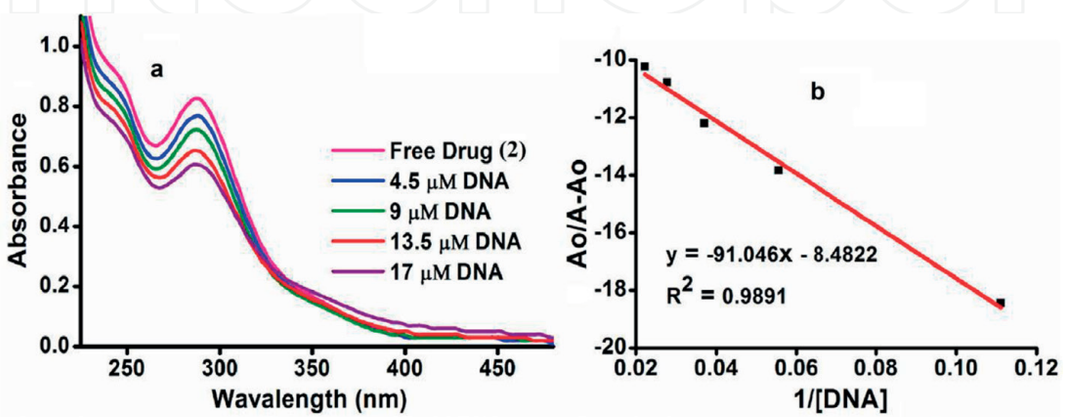


Figure 18.
(a) Representative plots of absorbance versus wavelength of 25 μM 1-(3-bromobenzoyl)-3-(4-ferrocenylphenyl) urea in ethanol with DNA's increasing concentration from (4.5–17 μM) and (b) plot of A°/A A° versus 1/[DNA] for determining binding constant of DNA attached to the compound [38].

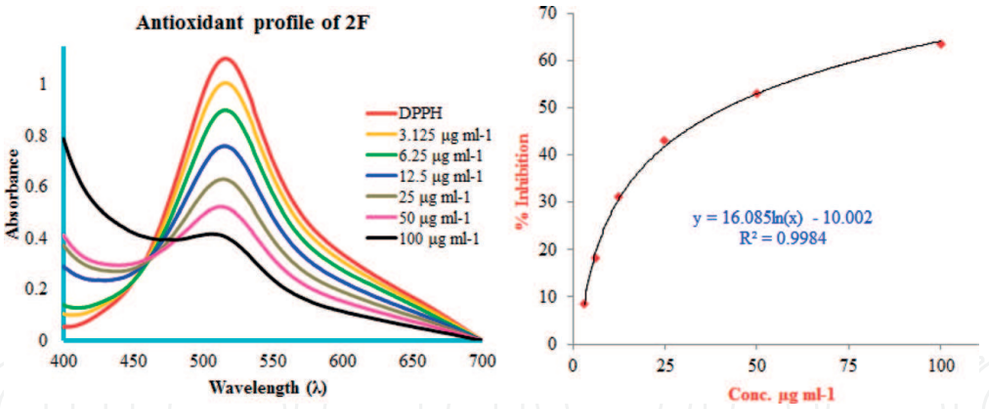


Figure 19.
*Electronic absorption spectra of [1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl)thiourea (***2F***)] (3.125–100 µg mL^{-1}) representing free-radical scavenging outline.*

The free-radical scavenging activity of compound [1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl)thiourea **(2F)**] molecular structure is given in Section 3.2 in **Figure 6**, which exhibits a sheer increment in % inhibition by increment in its concentration. It had been found that with increasing concentration of ferrocenyl thioureas, the % inhibitory activity is increased as depicted in the representative plot in **Figure 19** [40].

Author details

Samia Kausar[1], Ataf Ali Altaf[1*], Muhammad Hamayun[1], Amin Badshah[2] and Abdul Razzaq[3]

1 Department of Chemistry, University of Gujrat, Gujrat, Pakistan

2 Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan

3 Department of Chemistry, Government Postgraduate College, Sahiwal, Pakistan

*Address all correspondence to: atafali.altaf@uog.edu.pk

References

[1] Kealy T, Pauson P. A new type of organo-iron compound. Nature. 1951;**168**(4285):1039

[2] Fischer E, Hafner W. Zeitschrift für Naturforschung. B: Anorganische Chemie, organische Chemie, Biochemie, Biophysik, Biologie. 1962;**17**:774-775

[3] Wilkinson G et al. The structure of iron bis-cyclopentadienyl. Journal of the American Chemical Society. 1952;**74**(8):2125-2126

[4] Woodward R, Rosenblum M, Whiting M. A new aromatic system. Journal of the American Chemical Society. 1952;**74**(13):3458-3459

[5] Rosenblum M. Chemistry of the Iron Group Metallocenes: Ferrocene, Ruthenocene, Osmocene. Vol. 1. Interscience Publishers; 1965

[6] Van Staveren DR, Metzler-Nolte N. Bioorganometallic chemistry of ferrocene. Chemical Reviews. 2004;**104**(12):5931-5986

[7] Allardyce CS et al. Development of organometallic (organo-transition metal) pharmaceuticals. Applied Organometallic Chemistry. 2005;**19**(1):1-10

[8] Hillard EA, Vessieres A, Jaouen G. Ferrocene functionalized endocrine modulators as anticancer agents. In: Medicinal Organometallic Chemistry. Springer; 2010. pp. 81-117

[9] Fouda MF et al. On the medicinal chemistry of ferrocene. Applied Organometallic Chemistry. 2007;**21**(8):613-625

[10] Biot C et al. Synthesis and antifungal activity of a ferrocene–fluconazole analogue. Bioorganic & Medicinal Chemistry Letters. 2000;**10**(8):839-841

[11] Zhang J. Preparation, characterization, crystal structure and bioactivity determination of ferrocenyl–thiazoleacylhydrazones. Applied Organometallic Chemistry. 2008;**22**(1):6-11

[12] Delhaes L et al. Novel ferrocenic artemisinin derivatives: Synthesis, in vitro antimalarial activity and affinity of binding with ferroprotoporphyrin IX. Bioorganic & Medicinal Chemistry. 2000;**8**(12):2739-2745

[13] Ornelas C. Application of ferrocene and its derivatives in cancer research. New Journal of Chemistry. 2011;**35**(10):1973-1985

[14] Kondapi AK, Satyanarayana N, Saikrishna A. A study of the topoisomerase II activity in HIV-1 replication using the ferrocene derivatives as probes. Archives of Biochemistry and Biophysics. 2006;**450**(2):123-132

[15] Andrieux C et al. Determination of the lifetimes of unstable ion radicals by homogeneous redox catalysis of electrochemical reactions. Application to the reduction of aromatic halides. Journal of the American Chemical Society. 1980;**102**(11):3806-3813

[16] Pal SK et al. Schiff base linked ferrocenyl complexes for second-order nonlinear optics. Journal of Organometallic Chemistry. 2000;**604**(2):248-259

[17] Nieto D et al. Pt (II)-activated coupling of aminoethylferrocene with benzonitrile. A facile access route to a new redox-active bis (ferrocenyl-amidine) anion sensor. Chemical Communications. 2011;**47**(37):10398-10400

[18] Chohan ZH et al. Organometallic-based antibacterial and antifungal

compounds: Transition metal complexes of 1, 1′-diacetylferrocene-derived thiocarbohydrazone, carbohydrazone, thiosemicarbazone and semicarbazone. Journal of Enzyme Inhibition and Medicinal Chemistry. 2005;**20**(1):81-89

[19] dos Santos L et al. Synthesis of new 1-phenyl-3-{4-[(2E)-3-phenylprop-2-enoyl] phenyl}-thiourea and urea derivatives with anti-nociceptive activity. Bioorganic & Medicinal Chemistry. 2008;**16**(18):8526-8534

[20] Sharma B et al. A quantitative structure-activity relationship study of novel, potent, orally active, selective VEGFR-2 and PDGFRα tyrosine kinase inhibitors: Derivatives of N-phenyl-N′-{4-(4-quinolyloxy) phenyl} urea as antitumor agents. Journal of Enzyme Inhibition and Medicinal Chemistry. 2008;**23**(2):168-173

[21] Corry AJ et al. Synthesis, characterization and in vitro anti-cancer activity of N-(ferrocenyl) benzoyl tri-and tetrapeptide esters. Inorganica Chimica Acta. 2009;**362**(9):2957-2961

[22] Neuse EW. Macromolecular ferrocene compounds as cancer drug models. Journal of Inorganic and Organometallic Polymers and Materials. 2005;**15**(1):3-31

[23] Fiorina VJ, Dubois RJ, Brynes S. Ferrocenyl polyamines as agents for the chemoimmunotherapy of cancer. Journal of Medicinal Chemistry. 1978;**21**(4):393-395

[24] Sacht C, Datt M. Synthesis and characterisation of mixed-ligand platinum (II)–sulfoxide complexes, [PtCl (DMSO)(L)], for potential use as chemotherapeutic agents (HL= N, N-dialkyl-N′-(3-R-benzoyl) thiourea). Polyhedron. 2000;**19**(11):1347-1354

[25] Osella D et al. On the mechanism of the antitumor activity of ferrocenium derivatives. Inorganica Chimica Acta. 2000;**306**(1):42-48

[26] Abd-Elzaher MM, Hegazy WH, Gaafar AEDM. Synthesis, characterization and biological studies of ferrocenyl complexes containing thiophene moiety. Applied Organometallic Chemistry. 2005;**19**(8):911-916

[27] Lal B et al. Miscellaneous applications of ferrocene-based peptides/amides. Applied Organometallic Chemistry. 2011;**25**(12):843-855

[28] Neidle S. 3 the molecular basis for the action of some DNA-binding drugs. In: Progress in Medicinal Chemistry. Elsevier; 1979. p. 151-221

[29] Pigeon P et al. The replacement of a phenol group by an aniline or acetanilide group enhances the cytotoxicity of 2-ferrocenyl-1, 1-diphenyl-but-l-ene compounds against breast cancer cells. Journal of Organometallic Chemistry. 2009;**694**(6):895-901

[30] Yavuz S, Yıldırım H. Ferrocene derivatives carrying urea, thiourea, and sulfonamide moieties: Synthesis and evaluation of antibacterial and antifungal activities. Journal of Chemistry. 2013;**2013**

[31] Khan KM et al. Unsymmetrically disubstituted urea derivatives: A potent class of antiglycating agents. Bioorganic & Medicinal Chemistry. 2009;**17**(6):2447-2451

[32] Li H-Q et al. Urea derivatives as anticancer agents. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 2009;**9**(4):471-480

[33] Fan E et al. Molecular recognition: Hydrogen-bonding receptors that function in highly competitive solvents. Journal of the American Chemical Society. 1993;**115**(1):369-370

[34] Otón F et al. Ferrocene-based ureas as multisignaling receptors for anions.

The Journal of Organic Chemistry. 2006;**71**(12):4590-4598

[35] Otón F et al. Heteroditopic ferrocene-based ureas as receptors for anions and cations. Dalton Transactions. 2006;**0**(30):3685-3692

[36] Hay BP, Firman TK, Moyer BA. Structural design criteria for anion hosts: Strategies for achieving anion shape recognition through the complementary placement of urea donor groups. Journal of the American Chemical Society. 2005;**127**(6):1810-1819

[37] Pratt MD, Beer PD. Anion recognition and sensing by mono-and bis-urea substituted ferrocene receptors. Polyhedron. 2003;**22**(5):649-653

[38] Asghar F et al. Bioactivity of new ferrocene incorporated N, N′-disubstituted ureas: Synthesis, structural elucidation and DFT study. Inorganica Chimica Acta. 2016;**439**:82-91

[39] Saeed A et al. Synthesis, crystal X-ray diffraction structure, vibrational properties and quantum chemical calculations on 1-(4-(4-Fluorobenzamido) phenyl)-3-(4-fluorobenzoyl) thiourea. Journal of Molecular Structure. 2010;**984**(1):240-245

[40] Lal B et al. SynthesisCrystal structure, spectral and electrochemical characterization, DNA binding and antioxidant studies of 1-(2-florobenzoyl)-3-(2-chloro, 4-ferrocenylphenyl) thiourea. International Journal of Electrochemical Science. 2016;**11**:1632-1639

[41] Lv P-C et al. Synthesis and biological evaluation of pyrazole derivatives containing thiourea skeleton as anticancer agents. Bioorganic & Medicinal Chemistry. 2010;**18**(13):4606-4614

[42] Li H-Q et al. Synthesis and structure–activity relationships of N-benzyl-N-(X-2-hydroxybenzyl)-N′-phenylureas and thioureas as antitumor agents. Bioorganic & Medicinal Chemistry. 2010;**18**(1):305-313

[43] Altaf AA. Synthesis, Characterization and Biological Studies of Ferrocenyl Ureas & Thioureas. Quaid-i-Azam University Islamabad; 2012

[44] Atwood JL, Steed JW. Encyclopedia of Supramolecular Chemistry. Vol. 1. CRC Press; 2004

[45] Dunitz JD. Thoughts on Crystals as Supermolecules. Vol. 2. JM Lehn; 1996

[46] Jouaiti A, Hosseini MW, Kyritsakas N. Molecular tectonics: Design of 1-D coordination networks based on pyrene-bearing pyrazolyl units. European Journal of Inorganic Chemistry. 2003;**2003**(1):57-61

[47] Desiraju GR. The Crystal as a Supramolecular Entity. Vol. 18. John Wiley & Sons; 2008

[48] Lehn J-M. From supramolecular chemistry towards constitutional dynamic chemistry and adaptive chemistry. Chemical Society Reviews. 2007;**36**(2):151-160

[49] Lehn J-M. Towards complex matter: Supramolecular chemistry and self-organization. European Review. 2009;**17**(2):263-280

[50] Stang PJ, Olenyuk B. Self-assembly, symmetry, and molecular architecture: Coordination as the motif in the rational design of supramolecular metallacyclic polygons and polyhedra. Accounts of Chemical Research. 1997;**30**(12):502-518

[51] Ward MD. Design of crystalline molecular networks with charge-assisted hydrogen bonds. Chemical Communications. 2005;**0**(47):5838-5842

[52] Custelcean R. Crystal engineering with urea and thiourea hydrogen-bonding groups. Chemical Communications. 2008;**0**(3):295-307

[53] Crawford PC et al. Synthetic crystallography: Synthon mimicry and tecton elaboration in metallate anion salts. CrystEngComm. 2004;**6**(70):419-428

[54] Lortie F, Boileau S, Bouteiller L. N, N′-disubstituted ureas: Influence of substituents on the formation of supramolecular polymers. Chemistry–A European Journal. 2003;**9**(13):3008-3014

[55] de Loos M et al. Tripodal tris-urea derivatives as gelators for organic solvents. European Journal of Organic Chemistry. 2000;**2000**(22):3675-3678

[56] Custelcean R et al. Anion coordination in metal–organic frameworks functionalized with urea hydrogen-bonding groups. Crystal Growth & Design. 2006;**6**(2):555-563

[57] Pratt RC et al. Exploration, optimization, and application of supramolecular thiourea–amine catalysts for the synthesis of lactide (co) polymers. Macromolecules. 2006;**39**(23):7863-7871

[58] Dove AP et al. Thiourea-based bifunctional organocatalysis: Supramolecular recognition for living polymerization. Journal of the American Chemical Society. 2005;**127**(40):13798-13799

[59] Dannecker W, Kopf J, Rust H. N, N'-Diphenylurea, C13H12N2O. Crystal Structure Communications. 1979;**8**(2):429-432

[60] Lal B et al. Synthesis, characterization and antitumor activity of new ferrocene incorporated N, N′-disubstituted thioureas. Dalton Transactions. 2012;**41**(48):14643-14650

[61] Javed F et al. New supramolecular ferrocenyl amides: Synthesis, characterization, and preliminary DNA-binding studies. Journal of Coordination Chemistry. 2012;**65**(6):969-979

[62] Westwood J et al. Binding and electrochemical recognition of barbiturate and urea derivatives by a regioisomeric series of hydrogen-bonding ferrocene receptors. Organometallics. 2004;**23**(5):946-951

[63] Heo J, Mirkin CA. Pseudo-allosteric recognition of mandelic acid with an enantioselective coordination complex. Angewandte Chemie. 2006;**118**(6):955-958

[64] Abbott AP et al. Electrochemical recognition of chiral species using quaternary ammonium binaphthyl salts. Analytical Chemistry. 2002;**74**(16):4002-4006

[65] Dai Z, Xu X, Canary JW. Rigidified tripodal chiral ligands in the asymmetric recognition of amino compounds. Chirality. 2005;**17**(S1):S227-S233

[66] Willener Y et al. An exploration of ferrocenyl ureas as enantioselective electrochemical sensors for chiral carboxylate anions. The Journal of Organic Chemistry. 2008;**73**(4):1225-1233

[67] Sahoo BK et al. Studies on the interaction of diacetylcurcumin with calf thymus-DNA. Chemical Physics. 2008;**351**(1-3):163-169

[68] Asghar F et al. Facile synthesis of fluoro, methoxy, and methyl substituted ferrocene-based urea complexes as potential therapeutic agents. Bioorganic Chemistry. 2017;**72**:215-227

[69] Tjahjono DH et al. Cationic porphyrins bearing diazolium rings: Synthesis and their interaction with calf thymus DNA. Biochimica et Biophysica Acta (BBA)-General Subjects. 1999;**1472**(1-2):333-343

[70] Strekowski L, Wilson B. Noncovalent interactions with DNA: An overview. Mutation Research/ Fundamental and Molecular Mechanisms of Mutagenesis. 2007;**623**(1):3-13

[71] He X-Q et al. Synthesis, characterization and DNA-binding studies on La (III) and Ce (III) complexes containing ligand of N-phenyl-2-pyridinecarboxamide. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2007;**68**(1):184-190

[72] Verheijen JC et al. Transition metal derivatives of peptide nucleic acid (PNA) oligomers synthesis, characterization, and DNA binding. Bioconjugate Chemistry. 2000;**11**(6):741-743

[73] Cowan DO et al. Organic solid state. VIII. Mixed-valence ferrocene chemistry. Accounts of Chemical Research. 1973;**6**(1):1-7

[74] Kashanian S et al. Interaction of diazinon with DNA and the protective role of selenium in DNA damage. DNA and Cell Biology. 2008;**27**(6):325-332

[75] Sun Y et al. The potential trauma of three kinds of carbamate pesticides to DNA. Journal of Agro-environment Science. 2004;**23**(3):464-466

[76] Top S et al. Studies on organometallic selective estrogen receptor modulators (SERMs) dual activity in the hydroxy-ferrocifen series. Journal of Organometallic Chemistry. 2001;**637**:500-506

[77] Liu J-Z et al. Synthesis and in vitro study of pseudo-peptide thioureas containing α-aminophosphonate moiety as potential antitumor agents. European Journal of Medicinal Chemistry. 2010;**45**(11):5108-5112

[78] Bian C-L et al. Voltammetric studies of the interaction of rutin with DNA and its analytical applications on the MWNTs–COOH/Fe_3O_4 modified electrode. Sensors and Actuators B: Chemical. 2011;**156**(2):615-620

[79] Ibrahim M. Voltammetric studies of the interaction of nogalamycin antitumor drug with DNA. Analytica Chimica Acta. 2001;**443**(1):63-72

[80] Raman N, Pothiraj K, Baskaran T. DNA interaction, antimicrobial, electrochemical and spectroscopic studies of metal (II) complexes with tridentate heterocyclic Schiff base derived from 2′-methylacetoacetanilide. Journal of Molecular Structure. 2011;**1000**(1-3):135-144

[81] Ali S et al. Ferrocene-based bioactive bimetallic thiourea complexes: Synthesis and spectroscopic studies. Bioinorganic Chemistry and Applications. 2015;**2015**

[82] Asghar F et al. Synthesis, structural characterization, DNA binding and antioxidant potency of new ferrocene incorporated acyl ureas. Journal of Organometallic Chemistry. 2015;**797**:131-139

[83] Gul R et al. Ferrocene-based guanidine derivatives: In vitro antimicrobial, DNA binding and docking supported urease inhibition studies. European Journal of Medicinal Chemistry. 2014;**85**:438-449

[84] Namasivayam V, Günther R. PSO@ AUTODOCK: A fast flexible molecular docking program based on swarm intelligence. Chemical Biology & Drug Design. 2007;**70**(6):475-484

[85] Hussain RA et al. Synthesis, chemical characterization, DNA binding and antioxidant studies of ferrocene incorporated selenoure. Journal of Molecular Structure. 2013;**1048**:367-374

[86] Österberg F et al. Automated docking to multiple target structures:

Incorporation of protein mobility and structural water heterogeneity in AutoDock. Proteins: Structure, Function, and Bioinformatics. 2002;**46**(1):34-40

[87] El Hadji Mamour Sakho EA, Oluwafemi OS. Dynamic light scattering (DLS). In: Thermal and Rheological Measurement Techniques for Nanomaterials Characterization. Vol. 3. 2017. p. 37

[88] Ferré-D'Amaré AR, Burley SK. Use of dynamic light scattering to assess crystallizability of macromolecules and macromolecular assemblies. Structure. 1994;**2**(5):357-359

[89] Caballero A et al. Electroactive thiazole derivatives capped with ferrocenyl units showing charge-transfer transition and selective ion-sensing properties: A combined experimental and theoretical study. Inorganic Chemistry. 2007;**46**(3):825-838

[90] Arjmand F, Sayeed F, Muddassir M. Synthesis of new chiral heterocyclic Schiff base modulated Cu (II)/Zn (II) complexes: Their comparative binding studies with CT-DNA, mononucleotides and cleavage activity. Journal of Photochemistry and Photobiology B: Biology. 2011;**103**(2):166-179