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Facile synthesis of fluoro, methoxy, and methyl substituted ferrocene-based urea complexes as potential therapeutic agents

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ABSTRACT

In the present work, the synthesis, characterization (FT-IR, multinuclear (^{1}H and ^{13}C) NMR, AAS, Raman, and elemental analysis), DNA binding (cyclic voltammetry, UV–Vis spectroscopy and viscometry), and *in vitro* biological assessment of nine new ferrocene-based ureas are reported. The desulphurization of ferrocenyl thioureas to the corresponding oxo analogues using aqueous sodium hydroxide and mercuric chloride led to the ferrocenyl ureas **(F1–F9)** in high yields. The DNA binding studies performed by cyclic voltammetry and UV–Vis spectroscopy produced results that are in close agreement with one another for the binding constants (K) and an electrostatic mode of interaction was observed. The nature and the extent of interaction with DNA was further investigated by viscometry. The DFT/B3LYP method was used to determine the charge distribution and HOMO/LUMO energies of the optimized structure. The DFT calculated HOMO and LUMO energies correlate well with the experimentally determined redox potential values. The synthesized ferrocenyl derivatives exhibited good scavenging activity against 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). These complexes were also scanned for their *in vitro* cytotoxicity against human carcinoma cell line THP-1 (leukemia cells). The results showed a moderate level of cytotoxicity against the subjected cancer cell line as compared with the standard chemotherapeutic drug (cisplatin).

1. Introduction

During the past two decades, there has been a growing interest in the synthesis of more potent chemotherapeutic drugs containing oxygen and nitrogen donor ligands capable of higher cytotoxic activity with reduced or no side effects relative to cisplatin [1]. Among these the urea derivatives comprising of nitrogen and oxygen donor atoms offer multitude of bonding potentials, play a key role in cancer therapy [2,3]. The ureido moiety exists in numerous biologically essential molecules [4]. The prevalence of the urea functionality in medicinal chemistry is accredited to the metabolic stability of the N—C(O)—N linkage and to the large number of variations possible. The urea analogs such as N-nitrosoureas, benzoylureas, diarylsulphonylureas (DSUs), and heterocyclic urea derivatives signify one of the most useful classes of anticancer agents with an extensive range of activities against several leukemias and solid tumors [5,6]. The conformational rigidity and exclusive hydrogen bonding properties of ureas empowered the design of highly sophisticated functional molecules such as ion receptors, dyes, self-assembled supramolecules, herbicides, and corrosion inhibitors [7–9]. Recently, Realista et al. reported the redox behavior and *in vitro* antiproliferative activity of a family of functionalized ferrocene complexes containing nitrogen donors. Complexes bearing benzimidazole backbone exhibited substantial cytotoxicity against HeLa cells with IC_{50} values comparable to that of the standard chemotherapeutic drug i.e. cisplatin [9].

The cytotoxic impact of various drugs depends upon the proficiency of the drugs to bind with the DNA. Thus, the drugs need to approach the DNA in the nucleus. The interaction of small molecules with DNA is one of the fundamental aspects of biological exploration in drug discovery and pharmaceutical development processes [10–13]. Furthermore, the study of these interactions affords immense help in comprehending the structural features of DNA, the mutation of genes, the origin of some diseases and the mechanism of action of antitumor and antivirus drugs and, therefore, in the design of new and more competent DNA-targeted drugs to treat genetic diseases [14]. A well-built synthetic chemistry of ferrocene tolerates its conjugation with a plethora of functional groups of biological relevance. The ferrocene moiety

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might function as a hydrophobic spacer that is, being an appropriate arene bioisostere, whose existence in a molecule would boost lipophilicity and thus permeability into the cells [15,16]. On the other hand, the chemically stable ferrocene fragment may become redox active in the biological environment and produce reactive species, such as the ferrocenium ion (Fe^{3+}) and free radicals that contribute to pharmacodynamic properties [17].

Motivated from the diverse applications of ferrocene and ureas separately we have combined them in a new class of molecules i.e. ferrocene-based ureas. We report herein, the synthesis, characterization, and *in vitro* biological screening of some new ferrocene incorporated ureas. The drug-DNA binding activities of the nine compounds have been measured by cyclic voltammetry (CV) and the proposed mode of interaction has been validated by UV–Vis spectroscopy and viscometry. A density functional theory (DFT) study was also conducted on these structures to predict theoretically the redox potentials. In addition, we report here the antioxidant and cytotoxic activities of the compounds.

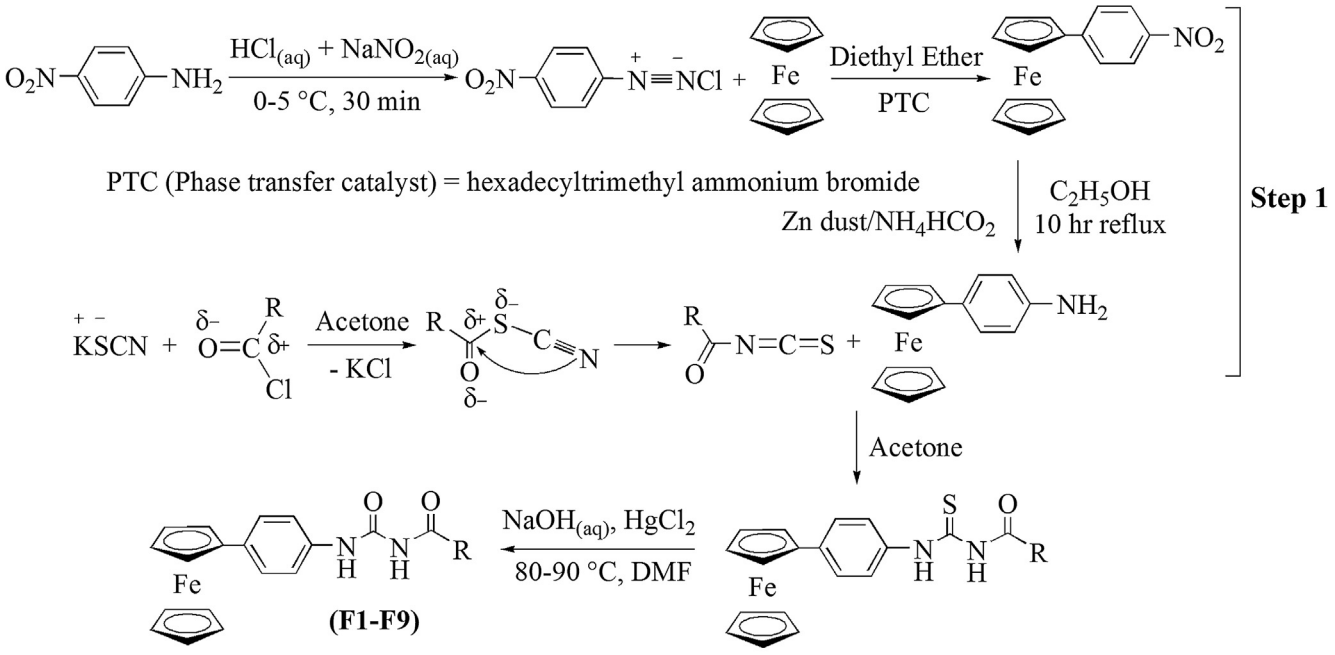
2. Experimental

2.1. Materials and methods

Ferrocene (Fc), *p*-nitroaniline, HCl, $NaNO_2$, KSCN, $HgCl_2$, NaOH and acid chlorides such as *o*-fluorobenzoyl chloride, *m*-fluorobenzoyl chloride, *p*-fluorobenzoyl chloride, *o*-methoxybenzoyl chloride, *m*-methoxybenzoyl chloride, *p*-methoxybenzoyl chloride, *o*-methyl benzoyl chloride, *m*-methylbenzoyl chloride, and *p*-methylbenzoyl chloride were obtained from Sigma Aldrich/Fluka and used without further purification. All the solvents were dried and purified before use according to established methods [18]. *Para*-ferrocenyl aniline and ferrocene-based N,N′-disubstituted thioureas were synthesized by methods reported earlier by our group (Step 1, Scheme 1) [19–20]. Melting points were determined in a capillary tube using an electrothermal melting point apparatus model MP-D Mitamura Riken Kogyo (Japan). NMR measurements were carried out on a Bruker AV500 MHz spectrometer in DMSO using TMS (tetramethylsilane) as an internal reference. FT-IR data was obtained on a Thermo Scientific NICOLET 6700 FT-IR instrument in the 4000–400 cm^{-1} range and Raman spectra (±1 cm^{-1}) were recorded on an InVia Renishaw spectrometer, using argon-ion (514.5 nm) and near-infrared diode (785 nm) lasers. The Renishaw WiRE 2.0 software was used for the Raman data acquisition and spectral manipulation. Elemental analyses were performed using a LECO-932 CHNS analyzer, while the Fe concentrations were determined on Perkin-Elmer Atomic absorption spectrophotometer model 2380.

2.2. General procedure for the synthesis of ferrocene-based N,N′-disubstituted ureas (F1–F9)

To the solution of ferrocene incorporated N,N′-disubstituted thioureas in 20 ml DMF, $HgCl_2$ was introduced in 1:1 M ratio. The reaction mixture was stirred for 30 min, 3 mL of 100 mM $NaOH_{(aq)}$ was then added dropwise with constant magnetic stirring and the suspension was allowed to reflux for about 8 h. The progress of the reaction was monitored by thin layer chromatography (TLC). On completion of the reaction, the black precipitate of HgS were



Code	R	Code	R
F1	2-FC_6H_4	F6	4-$OCH_3C_6H_4$
F2	3-FC_6H_4	F7	2-$CH_3C_6H_4$
F3	4-FC_6H_4	F8	3-$CH_3C_6H_4$
F4	2-$OCH_3C_6H_4$	F9	4-$CH_3C_6H_4$
F5	3-$OCH_3C_6H_4$		

Scheme 1. Synthetic scheme for ferrocene-based N,N′-disubstituted ureas.