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Bioactivity of new ferrocene incorporated N,N′-disubstituted ureas: Synthesis, structural elucidation and DFT study

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ABSTRACT

We report here the synthesis, structural characterization and biological assessment of three new ferrocene incorporated ureas (**1**–**3**). The synthesis of these complexes was accomplished by the deprotection of ferrocene-based thioureas to the corresponding oxo analogues using $NaOH_{(aq)}$ and mercuric chloride. The new ferrocenyl ureas were characterized by FT-IR, multinuclear (^1H and ^{13}C) NMR, AAS and elemental analysis. Furthermore, the single-crystal X-ray structure of compound **2** was also determined. The DNA binding potency of these ureas was evaluated by UV–Vis spectroscopy and cyclic voltammetry (CV). The three complexes interact electrostatically with DNA and have impressive binding constants ranging from 3.42×10^4 to $8.15 \times 10^4\ M^{-1}$. The diffusion coefficients of the drug–DNA adducts are lower than is that for the free drug indicating the formation of a high molecular weight complex that diffuses slowly towards the electrode. The small binding site size of 0.509 (**1**), 0.528 (**2**) and 0.473 (**3**) base pairs is also indicative of an electrostatic mode of interaction. The DFT calculated HOMO and LUMO energies correlate well with the experimentally determined redox potential values. The synthesized ureas (**1**–**3**) were screened for their antibacterial, antifungal and protein kinase inhibition potency. These compounds play a significant role in arresting microbial growth and are potent protein kinase inhibitors.

1. Introduction

The development of new anticancer and antimicrobial therapeutic agents is one of the fundamental goals in medicinal organometallic chemistry. Among the metallocenes, ferrocene has proved itself to be an excellent choice to design new drugs [1–3], most probably because of its small size, aromaticity, hydrophobicity, low cytotoxicity and redox behavior [4–7]. Moreover, ferrocene derivatives have already found significant uses as anticancer, antimalarial, antiviral and antibiotic agents [4]. DNA binding is a pre-requisite for a compound to be used as an antitumor agent. Some ferrocene derivatives have also been evaluated for their DNA binding affinity and display good DNA binding constants [8–12]. Generally, ferrocene derivatives interact electrostatically with DNA, but partial intercalation also has been reported [11]. Insertion of a ferrocene moiety into an organic compound not only improves the electrochemical and spectroscopic behavior but also augments the possible applications of the compound in which it is integrated by making it more lipophilic [13].

The urea group, $R_1R_2NCONR_3R_4$, is an attractive structural unit owing to its broad scope in bio-activities and is extensively found in natural products [14]. Consequently, urea derivatives have attracted considerable attention as anti-proliferative agents [15], anticancer (renal cancer, colon cancer, lungs cancer, prostate cancer and breast cancer) [16], anticonvulsant [17], antifungal [18] and antibacterial agents [19]. Aromatic urea derivatives, such as N-phenyl-N-(2-chloroethyl) urea and heterocyclic urea derivatives, show good anticancer activity due to their good inhibitory activity against receptor tyrosine kinases (RTKs) [20]. In addition, hydroxyurea has been investigated for the treatment of a wide range of solid tumors as well as acute and chronic leukemia [21]. Recently the N,N′-disubstituted urea functionality has received substantial attention as a moiety that is incorporated into the compounds with numerous biological activities and resultant therapeutic

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applications [22]. Inspired by the important applications of both ferrocene and ureas separately, we have combined them in a new class of molecules, i.e., ferrocene incorporated ureas.

We present here the synthesis, characterization and *in vitro* biological activities of three new ferrocene-based ureas. The drug–DNA binding activities of the three compounds have been determined by cyclic voltammetry (CV) and the proposed mode of interaction has been substantiated by UV–Vis spectroscopy. 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, protein kinase inhibition and antimicrobial activities of the compounds.

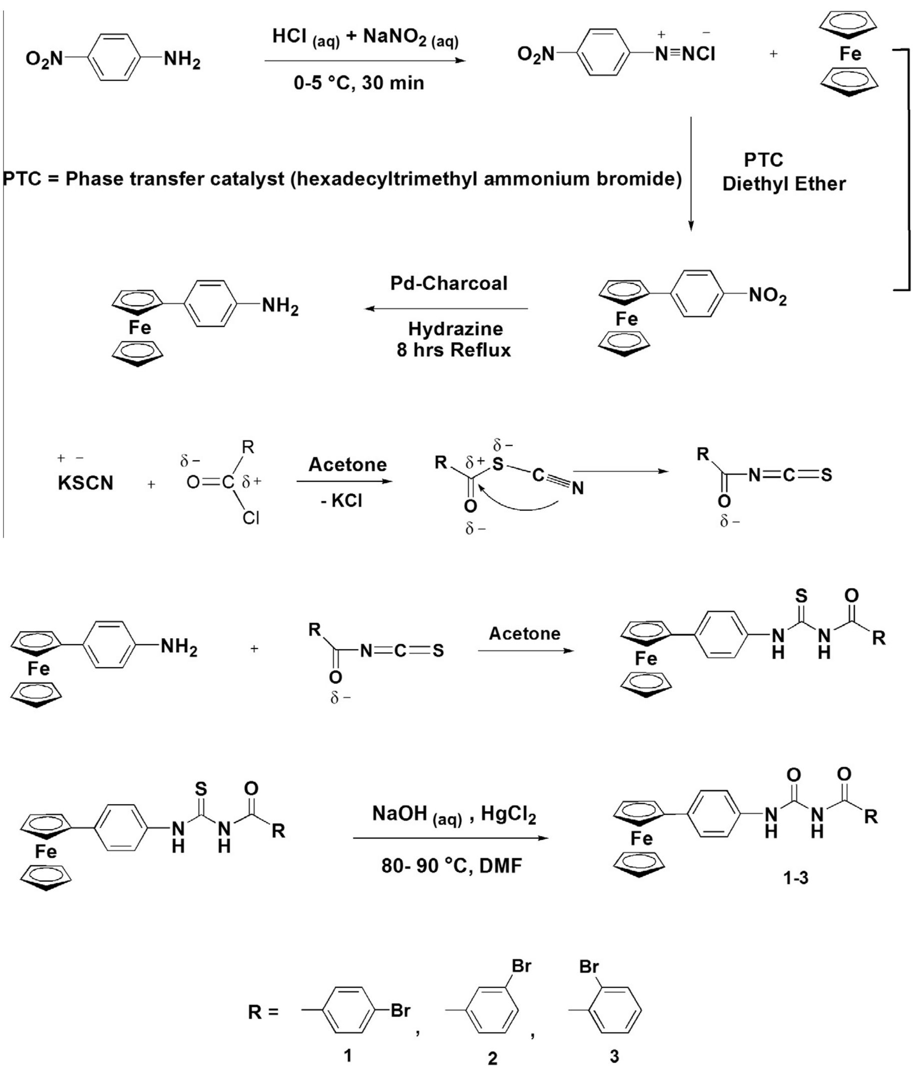
2. Experimental

2.1. Materials and methods

Ferrocene, 4-nitroaniline, HCl, $NaNO_2$, KSCN, $HgCl_2$, NaOH and acid chlorides such as *p*-bromobenzoyl chloride, *m*-bromobenzoyl chloride and *o*-bromobenzoyl chloride were obtained from Sigma Aldrich/Fluka and were used without further purification. All the solvents were dried and purified before use according to established methods. Para ferrocenyl aniline and ferrocene incorporated N,N′-disubstituted thioureas were synthesized by methods reported previously by our group (Part 1 Scheme 1) [11,23]. 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 AV 500 MHz spectrometer in DMSO using TMS (tetramethylsilane) as internal reference. FT-IR data was obtained on a Thermo Scientific NICOLET 6700 FT-IR instrument in the 4000–400 cm^{-1} range. 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 synthesis of ferrocene incorporated N,N′-disubstituted ureas **1–3**

To the solution of ferrocene incorporated N,N-disubstituted thioureas in 20 ml DMF, $HgCl_2$ was introduced in 1:1 molar 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 filtered off and the filtrate was then poured into ice cold water and stirred well in order to remove any water soluble impurities. The solid product was separated by filtration, washed with deionized water and recrystallized from ethanol (Scheme 1).



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