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Synthesis, structural characterization, modal membrane interaction and anti-tumor cell line studies of nitrophenyl ferrocenes

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ABSTRACT

A series of nitrophenyl ferrocens (**A1** – **A5**) were synthesized and fully characterized in solid state (using CHN analysis, FTIR and single crystal XRD) as well as in solution phase (^{1}H & ^{13}C NMR and UV–visible spectroscopy). Micelle interface interactions of these compounds were explored and found to have ability across a micelle membrane interface. Interestingly, these compounds exhibited π–electronic push pull systems and oxidation of ferrocene to ferrocenium on crossing the negative interface of the micelle membrane. Selective compounds were screened for antitumor activity against parental and drug resistant human ovarian tumor models i.e. A2780 and A2780^{cisR}, A2780^{ZD0473R}. Screened compounds were found to overcome resistance factor compared to cisplatin.

1. Introduction

Ferrocenyl derivatives with low oxidation potentials are attracting attention of many researchers due to their ability to catalyze the production of reactive oxygenated species (ROS), under physiological conditions that generates cytotoxic effects [1,2]. Many ferrocene derivatives have shown cytotoxicity against lung tumors, breast cancer, the anti-proliferative effect in vitro, DNA detection and anti-malarial activity [3,4]. One of the most interesting property of ferrocene is its lipophilic nature that can also be induced to its derivatives, which attracted many bio-organometallic chemists [5].

In the struggle for searching effective anticancer drugs, tremendous attention has been paid to ferrocene and its derivatives in view of their potential applications in medical field [6,7]. It has been established that ferrocenes have appreciable anti-neoplastic activity [8,9]. Many commercial drugs are going to substitute by their ferrocene analogs. Studies show that the insertion of ferrocene in existing drugs enhances their activities many fold. Insertion of ferrocene in peptide structures increased their anticancer activity [10–13], ferrocenyl-cisplatin also been reported [14], chloroquine is going to replaced by ferrochloroquine [15] (ferrocene analog) and a one of the best examples is the discovery of ferrociefen [5], a ferrocene analogue of tamoxifen that is in clinical trials for breast cancer treatment, and give very impressive results [16].

Ovarian carcinoma is the sixth most common malignancy in women and is the leading cause of death from gynecologic malignancy in the western world [17]. It is believed that the vast majority (85–90%) of ovarian cancers are epithelial in origin [18]. Although the exact causes of ovarian cancer remain unknown, a number of genetic and environmental risk factors have been identified. Among the genetic factor, Mutations in the BRCA1 and BRCA2 tumor suppressor genes can cause ovarian cancer. These genes normally help to prevent cancer by producing proteins that carry out functions such as: recognition or repair of certain forms of DNA damage and thus the damaged DNA cause ovarian cancer [19,20]. Cisplatin is vastly employed to treat ovarian cancer, but the A2780

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ovarian cancer cells have gotten the resistance against cisplatin. The mechanisms of resistance to cisplatin are believed to be: (I) reduced drug accumulation in cells, (II) enhanced repair of DNA damage [21–23]. Keeping these considerations in mind, we focus to design such molecules those interact differently with DNA in reference to cisplatin which infect slow down DNA repair. Shah et al. (2010) reported the electrostatic DNA binding (different from cisplatin that is covalent binder) of 4-nitrophenyl ferrocene (**A1**) [24]. So in connection we synthesized a series of similar compounds.

Considering its hydrophobic nature the ferrocene has potential to overcome some of the transport issues and thus reduce toxicity; we checked the interactions with interfaces using micelles—and found confirmation that the concept worked. However, to really solidify this concept—we tested them in cell culture, specifically A2780 and A2780^{cisR}, A2780^{ZD0473R} human ovarian cell lines.

2. Results and discussion

2.1. Synthesis and characterization

2.1.1. Synthesis

A1–**A5** were prepared by reacting ferrocene with diazonium salt of mono and di-substituted anilines as shown in Scheme 1. The reactions were carried out in the ether-water (1:1) mixture using CTAB as a phase transfer catalyst in analogy with previous preparations of many different ferrocene derivatives [25]. The presence of the phase transfer catalyst increase the yields from about 15% to >70% for **A1**–**A5** in agreement with literature reports on other substituted ferrocene derivatives [26,27]. Compounds **A1**–**A5** were characterized by using multinuclear ^{1}H and ^{13}C NMR and FT-IR spectroscopy. The formation of **A1**–**A5** was confirmed by spectral data (^{1}H & ^{13}C NMR and IR) which revealed the characteristic peaks of ferrocene in their usual region [28]. The CHN elemental analysis data showed close agreement with theoretical value as detailed in the experimental section.

2.1.2. Solution characterization

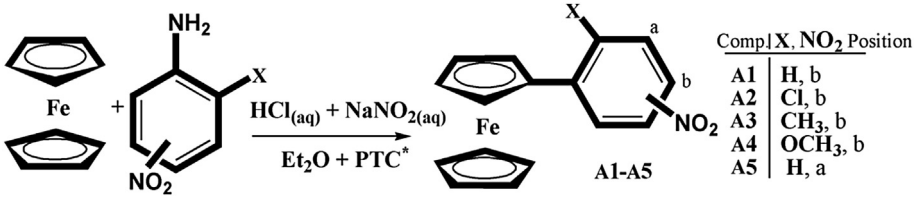
The **A1**-**A5** were soluble in both aprotic solvents such as hexane, heptane, THF, DMSO, DMF, acetonitrile, acetone, dichloromethane and protic solvents such as methanol and ethanol. The solubility of the **A1**-**A5** in isooctane is less than 0.6 mM and observed even lower than that for other solvents. An attempt was made to dissolve **A1**–**A5** in water with very low concentrations with extensive sonication and found to be insoluble.

The UV–Visible spectrum of ferrocene (Fc) showed two absorption bands which is attributed to three spin-allowed ligand field transitions i.e.: $^{1}A_{1g} \rightarrow a^{1}E_{1g}$, $^{1}A_{1g} \rightarrow {}^{1}E_{2g}$, and $^{1}A_{1g} \rightarrow b^{1}E_{1g}$. The first two transitions are unresolved and give rise a band at 442 nm in the absorption spectrum of Fc in THF at room-temperature. The third transition is responsible for the absorption band at 325 nm. Both bands are weak owing to the Laporte-forbidden d–d character of ligand field transitions [29–31]. Higher-energy features in the electronic absorption spectrum of Fc arise from intramolecular charge transfer transitions. In isopentane, the intense band at 200 nm has been assigned as ligand-to-metal charge transfer (LMCT) in character [32]. Shoulders observed at 265 nm and 240 nm are attributed to LMCT and metal-to-ligand charge transfer (MLCT) transitions respectively [31]. The UV–Visible spectrum changed dramatically upon substitution of the cyclopentadienyl ring with conjugated and acceptor groups, as was observed for nitro-phenyl groups. Molar absorptivity values showed that all these compounds exhibit a metal-to-ligand-charge-transfer (MLCT) band about 500 nm and a band below 300 nm which is mainly due to ligand. In these complexes, this MLCT signal is at higher wavelengths compared to Fc and overlapped the d − d transitions of ferrocene moiety.

This wavelength (λ_{max}) is solvent dependent whereas the intensity of the signal changed by mixing of MLCT band. For example, studies for **A1** revealed that the MLCT band in isooctane is at 480 nm with lower signal intensity. This has been observed for other ferrocene derivatives [31]. The nonpolar solvents supported a high energy, low wavelength (λ_{max}) absorption whereas, polar solvent found to have low energy, high wavelength (λ_{max}) as shown in Fig. 1 and Table 1. As explained in the energy level diagram (Fig. 1) electron withdrawing substituent reduce the gap between HOMO and LUMO for the MLCT band which increase the value of λ_{max} drastically in the **A1**–**A5**. The energy gap also affected by the change of solvent, the polar solvent (like acetonitrile, EtOH, and THF) had the ability to donate electrons in the LUMO of the compound and lowered the energy of orbital's and hence band gap between these energy levels decreased as a result the λ_{max} shifted towards higher value. Conversely the electron loving polar solvents (like EtOH have ability of forming H-bonding with π-electronic cloud) did not affect the LUMO energy level, but enhance the energy of HOMO and hence decreased the gap between these two energy levels yielding higher value of λ_{max} with lower energy in comparison to the non-polar solvent. Since the MLCT is at a lower λ_{max} suggesting less conjugation between the ferrocene and phenyl ring in these solvents.

These studies demonstrate that this class of compounds not only had diversity with regard to conjugation between Cp ring and phenyl group, but also with regard to conformation as well. Examples of this type of conformational rotation have been observed in the solid state for **A1** [33,34].

A1–**A5** were examined using ^{1}H NMR spectroscopy. Substituted ferrocene moiety gave rise three signals in the range 4–5 ppm. The intense signal, with an integration of five protons, was assigned to the un-substituted Cp ring, and the two other signals were assigned to the protons of the substituted Cp ring. In ^{13}C NMR, Substituted ferrocene substituted compounds gave rise four signals in the range 60–85 ppm. The highest intensity band was assigned to the un-substituted Cp ring, band with less intensity was assigned to the tertiary carbon on which substitution took place and the two other signals were allocated to the other carbons on the substituted Cp ring. However, all the aromatic protons and carbons fall in their normal expected regions in their respective spectra.



Scheme 1. The reaction scheme for the synthesis nitrophenylferrocenes A1–A5. (PTC = Phase transfer Catalyst i.e., Cetyltrimethylammonium bromide).