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Selected ferrocenyl chalcones as DNA/BSA-interacting agents and inhibitors of DNA topoisomerase I and II activity

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ABSTRACT

A series of four ferrocenyl chalcone derivatives (**1**-**4**) and one ferrocenyl non-chalcone derivative (**5**) were investigated with regards to their interactions with calf thymus DNA and bovine serum albumin (BSA). Study on the DNA/BSA interactive properties of complexes is one of the active subjects of bioorganic chemistry. It could provide fundamental data for developing potential therapeutic applications and understanding mechanism of action for drug. The interactions and binding characteristics of complexes with ctDNA and BSA were investigated using UV-Vis, CD and fluorescence spectroscopy. Moreover, topoisomerase inhibition assays were used to evaluate the ability of compounds to inhibit the activity of topoisomerases I/II. No interaction was detected between ctDNA and ferrocene derivatives. However, human topoisomerase I (*h*TOPI) was inhibited in a concentration-depend manner in the presence of compounds **1, 3, 4**, although no inhibition was observed with compounds **2** and **5**. In the case of human topoisomerase IIα, inhibition was observed and results indicate that these compounds can be classified as topoisomerase II suppressors, not poisons. The interaction between BSA and the four selected ligands (**1**-**4**) were studied by fluorescence quenching spectra. In addition, this method was used for the calculation of characteristic binding parameters. These results and results obtained by synchronous fluorescence spectra and 3D fluorescence spectra of the ferrocenyl chalcones with BSA determined that compounds create complexes with BSA and induce conformation changes.

1. Introduction

As the archetypal organometallic compounds, ferrocenes (Fc) are of interest for fundamental reasons, such as bonding and structure, as well as their numerous and diverse potential applications. They have many potential medicinal applications as therapeutic agents in the new field of bioorganometalics, including anti-cancer uses. It has been found that the combination of a ferrocenyl moiety with heterocyclic structures may increase their biological activity. Application of ferrocene derivatives in cancer therapeutics is an active research area, and many reports have demonstrated that some ferrocenyl compounds are highly cytotoxic *in vitro* against several cancer cell lines, including breast, prostate, lung, colon and leukaemia [1–3].

Chalcone is a very suitable heterocyclic molecule which bears a structural similarity to stilbene, a synthon found in several selective oestrogen receptor modulators like tamoxifen. Chalcones continue to attract considerable scientific attention due to their association with a variety of biological activities [4–11]. Most chalcones exhibited cytotoxic activity against a variety of tumour cell lines in the low micromolar range. A greater effort had been made to understand how chalcones exert their cytotoxic activity. One such study investigated the interaction of selected chalcone derivatives with DNA and bovine serum albumin. The measurement indicated that compounds behave as effective DNA-interactive agents. Furthermore, electrophoretic separation proved that ligands inhibited topoisomerase I [12,13].

A ferrocenyl chalcone derivative (Fc-chalcone) is formed when one of two chalcone rings is replaced with a ferrocenyl group [14]. Compounds with replaced A rings have different physicochemical properties than the same chalcone derivatives with replaced B

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rings in terms of stability, hydrosolubility or free radical generation [15].

DNA and BSA molecules are prone to be damaged under various conditions, especially by interaction with some molecules and this damage may lead to various pathological changes in living organisms. There are growing interest to explore the binding molecules with these molecules for rational design and construction of new and more efficient drugs targeted to DNA and BSA [16–19].

In previously published study, Vašková et al. [15] examined the effects of our four ferrocene derivatives (**1-4**) and one non-chalcone ferrocene derivative on selected tumour cell lines (Jurkat, HeLa, MCF-7, A549 and MDA cell lines). Compounds **1-4** are ferrocene analogues of the respective benzylidene compounds, which have been reported to show great anti-proliferative activity. Among these derivatives, compound **1** was the most effective; significantly inhibiting Jurkat cell proliferation even at a concentration of 10^{-7} M ($IC_{50} = 2.97 \times 10^{-6}$ M); compound **2** also possessed anti-proliferative effects on Jurkat and HeLa cells ($IC_{50} = 7.23$ and 7.4×10^{-6} M, respectively). To evaluate the effect of the studied compounds on HeLa colony forming ability, a colony formation assay was performed. Obtained results suggest that ferrocene derivatives are highly effective in suppressing the colony-forming ability of human cancer cells.

In this paper, we discuss the biochemical properties of five ferrocene derivatives, four chalcones (**1**–**4**) and one non-chalcone (**5**). The goal of our study is to shed more light on the DNA/BSA binding of these compounds in cell-free media and to explore the effects of these agents on the activity of human topoisomerases I and II. As DNA-binding compounds can influence cell proliferation and therefore gene expression, the interactions described above could be important in providing a better understanding of the mechanism of action of these compounds.

2. Materials and methods

The studied derivatives were dissolved in DMSO to a concentration of 50×10^{-3} M. Chemical structures and characteristics of the derivatives are shown in Table 1. Calf thymus sodium salt (D1501), bovine serum albumin (A7030, essentially fatty acid free) and other reagents were purchased from Sigma Aldrich chemicals (Sigma Aldrich, USA). *p*BR322 plasmid DNA, kinetoplast DNA, topoisomerase I and topoisomerase IIα were purchased from Inspiralis (Ltd., UK).

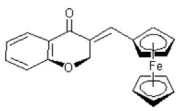
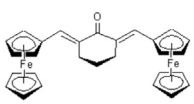
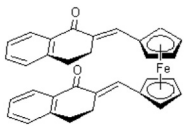
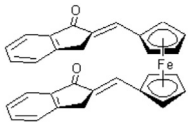
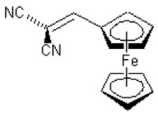
2.1. Interaction of the compounds with calf thymus DNA

A stock solution of calf thymus DNA (ctDNA) was prepared by dissolving 2.5×10^{-3} g/mL in Tris-EDTA buffer (TE buffer, 10×10^{-3} M Tris-HCl pH 7.4 and 1×10^{-3} M EDTA) at 4 °C overnight. The final concentration of ctDNA solution was determined from UV absorption at 260 nm using a molar absorption coefficient $\varepsilon_{260} = 6600\ M^{-1}cm^{-1}$. The purity of ctDNA was determined by the ratio of absorption at 260/280 nm and was found to be 1.82, which indicates that the sample is pure and devoid of protein contamination.

UV-Vis spectra were measured on a Varian Cary 100 UV-Vis spectrophotometer in a 1 cm path-length quartz cuvette, in 10×10^{-3} M Tris-HCl buffer (pH 7.4, 23 °C). Fixed concentrations of samples **1-5** (0.05×10^{-4} M) with gradually increasing concentrations of ctDNA ($c = 4.29 \times 10^{-4}$ M) (ranging from 0 to 4.28×10^{-7} M) were used in the binding experiments. Each absorption spectrum was scanned at a wavelength range of 220–500 nm.

Circular dichroism (CD) measurements were performed by using a Jasco J-810 (Tokyo, Japan) spectropolarimeter in 1 cm path-length quartz cuvette in 10×10^{-3} M Tris-HCl buffer (pH 7.4, 23 °C). The CD spectra were studied at a fixed concentration of ctDNA ($c = 6.34 \times 10^{-5}$ M) and incubated with tested ferrocenyl chalcones **1-5** (9.83×10^{-5} M) at a wavelength range of 220–350 nm under nitrogen atmosphere. Results are presented as a mean of three scans from which the buffer background was electronically subtracted.

Table 1
Chemical structures and characteristics of the investigated compounds.

Sign	Structure	Name	Molecular weight ($g.mol^{-1}$)
1		(*E*)-3-(ferrocenemethylene)-4-chromanone	344.19
2		(2*E*,6*E*)-2,6-bis(ferrocenylemethylidene)-cyclohexanone	490.19
3		1,1′-bis[(1-oxotetralin-2-ylidene)methyl]ferrocene	498.39
4		1,1′-bis[(1-oxoindane-2-ylidene)methyl]ferrocene	470.34
5		2-(ferrocenylmethylidene)malonedinitrile	262.09