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Synthesis, structural characterization, *in vitro* anti-proliferative effect and cell cycle analysis of *N*-(ferrocenyl)benzoyl dipeptide esters

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ABSTRACT

N-ortho, meta and *para*-(ferrocenyl)benzoyl dipeptide esters **2–10** were prepared by coupling ferrocenyl benzoic acids **1** *(ortho, meta* and *para)* to the dipeptide ethyl esters GlyAbu(OEt) **2–4**, GlyNva(OEt) **5–7** and GlyNle(OEt) **8–10** in the presence of *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride and 1-hydroxybenzotriazole. The compounds were fully characterized by a range of NMR spectroscopic techniques, mass spectrometry and cyclic voltammetry. The cytotoxicity of **3, 6** and **9** versus H1299 lung cancer cells were 10.5 μM, 19.1 μM and 18.9 μM, respectively, whereas *N*-{*meta*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **11** and *N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **12** gave IC_{50} values of 4.0 and 6.6 μM, respectively. Therefore, an increase in alkyl chain length of the second amino acid also increases the IC_{50} values. Cell cycle analysis of *N*-{*ortho*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **13** suggests a block in the G2/M phase of the cell cycle.

1. Introduction

Organometallic compounds have been successfully incorporated in a wide variety of materials that have diverse applications. Ferrocene is one such compound that is recognized as a promising candidate for use in novel materials due to its ease of use and electrochemical and spectroscopic properties. As a result of this ferrocene research has received a dramatic increase in attention over the past decade. The ultimate goal of this research is the development of novel sensor compounds, peptide mimetic models and unnatural drugs [1–13]. Ferrocene can be conjugated to drugs such as antibiotics, anti-malarials and anti-cancer drugs, such as tamoxifen [14]. It has also been shown that *N*-(ferrocenylmethyl)fluorobenzene-carboxamide derivatives display anti-cancer activity against ER (+) MDA-MB-435-S-F breast cancer cells [15].

We have reported the synthesis and structural characterization of various ferrocene derivatives incorporating natural amino acids and dipeptides [16–22]. The compounds are composed of three key moieties, namely, (i) an electroactive core, (ii) a conjugated aromatic linker and (iii) an amino acid or peptide derivative that can interact with other molecules *via* hydrogen bonding. *N*-{*ortho*-(ferrocenyl)benzoyl}-glycine ethyl ester was initially tested for its *in vitro* anti-proliferative activity towards lung cancer cells (H1299 and H1299 carboplatin resistant variant). This compound was found to be cytotoxic and had an IC_{50} value of 48 μM [23], whereas the starting material, *ortho*-ferrocenyl ethyl benzoate, was completely inactive against this cell line. Therefore, other derivatives were evaluated for their anti-cancer activity against lung cancer cell lines. Initial results showed that the cytotoxicity of the *meta* dipeptide, *N*-{*meta*-(ferrocenyl)-benzoyl}-L-alanine-glycine ethyl ester is *ca.* 2 times higher than the *ortho*-glycine derivative, the IC_{50} value being 26 μM (RSD 20%) [24]; whilst the corresponding *ortho* analog, *N*-{*ortho*-(ferrocenyl)-benzoyl}-L-alanine-glycine ethyl ester has an IC_{50} value of 21 μM (RSD 20%). The dipeptide derivative *N*-{*ortho*-(ferrocenyl)-benzoyl}-glycine-glycine ethyl ester was also shown to have an IC_{50} value of approximately 20 μM, however *N*-{*ortho*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **13** is more active than *N*-{*ortho*-(ferrocenyl)-benzoyl}-L-alanine-glycine ethyl ester with an IC_{50} value of 5.3 μM (RSD 8%) [23]. From this it may be assumed that the glycine residue of the dipeptide that is attached to the benzoyl group is important for activity. The larger amino acid alanine as the second residue also increased activity. To assess the effects of lipophilicity, the alanine residue can be replaced with residues that differ by a methylene (CH_2) unit. By incorporating the amino acid 2-amino butyric acid (Abu) the methyl group of alanine (CH_3) is transformed to an ethyl group (C_2H_5). This process can be extended by using norvaline (Nva) and norleucine (Nle) in the synthesis to introduce propyl (C_3H_7) and butyl (C_4H_9) groups respectively. Here, we now report the synthesis, structural characterization and

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in vitro anti-proliferative activity of novel *N*-(ferrocenyl)benzoyl dipeptide esters.

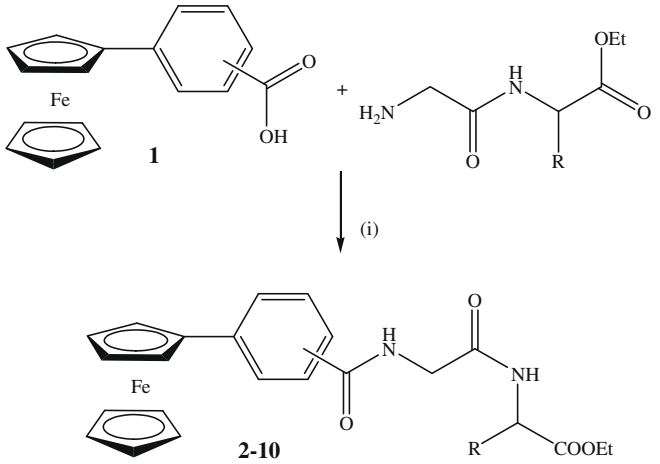
2. Results and discussion

2.1. Synthesis

Coupling reactions were used in the preparation of the dipeptide esters and also to facilitate the introduction of the ferrocenyl benzoyl group onto the dipeptide ester. Ferrocenyl benzoic acids were prepared as previously reported [16,19,24] and were treated with 1-hydroxybenzotriazole (HOBt), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), and triethylamine (TEA) in dichloromethane at 0 °C in the presence of the peptide esters (Scheme 1). EDC was used in preference to the less expensive coupling reagent *N*,*N*′-dicyclohexylcarbodiimide (DCC) as its reaction by-products are easier to remove compared to those of DCC, namely dicyclohexylurea (DCU). In the series of compounds **2–10**, glycine was the first amino acid in the sequence with L-2-aminobutyric acid, L-norvaline and L-norleucine the second amino acids in the sequence. Compounds **2–10** were purified by column chromatography with 1:1 hexane:ethyl acetate as mobile phase in yields of 23–73% and all gave spectroscopic and analytical data in accordance with the proposed structures.

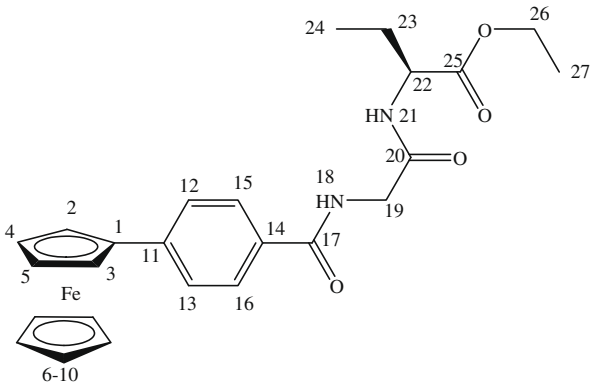
2.2. ^1H and ^{13}C NMR analysis

All proton and carbon chemical shifts were explicitly assigned for **2–10** using various NMR spectroscopic techniques. The proton and carbon peaks are consistent with mono-substituted ferrocenyl compounds [23,24]. The *ortho* peaks of the (η^5-C_5H_4) ring appear between δ 4.92 and δ 4.66 while the *meta* peaks are present between δ 4.51 and δ 4.19. The unsubstituted (η^5-C_5H_5) ring appears in the region δ 4.09–3.87. The aromatic splitting pattern seen in the ^{1}H NMR spectra of **2–10** varied depending on whether *ortho*, *meta* or *para* ferrocenyl benzoic acid was used. The *ortho* derivatives show a doublet, multiplet, doublet splitting pattern. The *meta* derivatives give rise to a singlet, multiplet, triplet splitting pattern where the multiplet integrates for two protons. The *para* derivatives give the archetypal *para* disubstituted aromatic splitting pattern with two doublets that both integrate for two protons. For example in the case of *N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-2-aminobutyric acid ethyl ester **4** the aromatic protons are present as two doublets at δ 7.81 and δ 7.64, respectively, both with coupling constants of 8.4 Hz. In the ^{13}C spectrum of **2–10** the ferrocenyl peaks occur in the region δ 84.3–66.3. The *ipso* carbon of the (η^5-C_5H_4) ring appears in the narrow range of δ 84.3–83.2. This signal is absent from the DEPT 135 spectra as are the other quaternary carbons. A complete assignment of ^{1}H and ^{13}C chemical shifts for compound **4** is given in Table 1.



Scheme 1. Synthesis of *N*-(ferrocenyl)benzoyl dipeptide ethyl esters **2–10** , (i) EDC, HOBt, triethylamine, dipeptide ethyl ester (**2–4** R = C_2H_5, **5–7** R = C_3H_7, **8–10** R = C_4H_9). **2**, **5** and **8** *N*-*ortho*-ferrocenyl benzoic acid used as starting material. **3**, **6** and **9**, *N*-*meta*-ferrocenyl benzoic acid used as starting material. **4**, **7** and **10**, *N*-*para*-ferrocenyl benzoic acid used as starting material.

Table 1
^{1}H and ^{13}C spectroscopic data for **4**.



Site	^{1}H NMR	^{13}C NMR	HMQC
1		83.2	
2 and 3	4.89		66.4
4 and 5	4.41		69.4
6–10	4.04–3.87		69.5
11		142.7	
12 and 13	7.64		125.3
14		131.1	
15 and 16	7.81		127.4
17		166.2	
18	8.66		
19	4.04–3.87		42.2
20		169.2	
21	8.27		
22	4.22–4.17		53.3
23	1.78–1.61		24.4
24	0.90		10.2
25		171.9	
26	4.17–4.07		60.4
27	1.19		14.1

2.3. Mass spectrometry

Electrospray ionization (ESI) mass spectrometry was employed in the analysis of compounds **2–10** and confirmed the correct relative molecular mass for all the compounds. Examination of the mass spectra revealed the presence of both radical-cations, $[M]^{+\cdot}$ as well as $[M+H]^+$ species. Adducts due to sodium were also present 22 Da higher than the protonated molecular ion species. Sequence specific fragment ions were not observed or were of low intensity in the mass spectra of the *meta* and *para*-*N*-(ferrocenyl)benzoyl dipeptide esters. However, an important diagnostic fragment ion at *m*/*z* $[M-65]^+$ was observed in the mass spectra of the *N*-{*ortho*-(ferrocenyl)-benzoyl}dipeptide esters **2, 5** and **8**. This corresponds to the loss of the unsubstituted (η^5-C_5H_5) ring. The formation of this fragment ion is possibly due to steric hindrance between the *ortho* substituted benzoyl substituents and the unsubstituted (η^5-C_5H_5) ring.

2.4. Cyclic voltammetry of N-(ferrocenyl)benzoyl dipeptide esters

All *N*-(ferrocenyl)benzoyl dipeptide compounds (**2–10**) exhibit a one electron reversible redox process in the cyclic voltammograms