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The synthesis, structural characterization and *in vitro* anticancer activity of novel 1-alkyl-1′-*N*-*meta*-(ferrocenyl) benzoyl dipeptide esters and novel 1-alkyl-1′-*N*-*ortho*-(ferrocenyl) benzoyl dipeptide esters

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ABSTRACT

1-Alkyl-1′-*N*-*meta*-(ferrocenyl) benzoyl dipeptide esters **7**–**18** and 1-alkyl-1′-*N*-*ortho*-(ferrocenyl) benzoyl dipeptide esters **19**–**30** were prepared by coupling the alkyl ferrocenyl benzoic acids **1**–**6** to the dipeptide ethyl esters Gly-Gly(OEt), Gly-L-Ala(OEt), Gly-L-Leu(OEt) and Gly-L-Phe(OEt) using the standard *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBt) protocol. The alkyl groups employed in the synthesis were methyl, ethyl and propyl. The compounds were characterized using a combination of ^{1}H NMR, ^{13}C NMR, DEPT-135 and ^{1}H-^{13}C COSY (HMQC) spectroscopy and electrospray ionization mass spectrometry (ESI-MS). Selected compounds showed micromolar activity in the H1299 NSCLC cell line, with IC_{50} values in the range of 2.6–20.1 μM.

Introduction

Interest in metal complexes with biological applications is currently an active area of research [1–3]. Some of the most promising metal anticancer agents are emerging from the field of bioorganometallic chemistry. A recent review article has categorized the diverse range of metal anticancer compounds according to their mode of action [4]. Also a perspective article on organometallic anticancer compounds has been published [5]. Ferrocene is a particularly attractive candidate for incorporation into biologically active compounds due to its aromaticity, stability, redox properties and low toxicity [6,7]. The reversible redox properties of ferrocene have been strongly associated with its biological activity [8]. One key feature of ferrocene is the ease by which it undergoes oxidation to form the ferricenium cation ($Fc \rightarrow Fc^{+}$). This occurs in a reversible manner and is accommodated readily by the loss/gain of an electron from a non-bonding high energy molecular orbital. Ferricenium salts that are known to inhibit tumor growth, have been shown to produce hydroxyl ($HO^{\bullet}$) radicals under physiological conditions, leading to oxidatively damaged DNA [9]. The catalytic generation of intracellular reactive oxygen species (ROS) such as the $HO^{\bullet}$ radical offers an attractive and alternative method to target and kill cancer cells [10]. To date, the most promising ferrocene-based drug candidate consist of a [3]-ferrocenophane motif and have a potent *in vitro* anti-proliferative effect in breast and prostate cancer cells lines [11–13]. This research group have prepared *N*-ferrocenoyl and *N*-ferrocenyl amino acid and dipeptide derivatives and investigated their potential applications [14–18]. It was shown that *N*-(ferrocenyl)benzoyl and *N*-(ferrocenyl)naphthoyl amino acid and dipeptide derivatives were potential anticancer agents [19–26]. In the case of our ferrocenyl-peptide bioconjugates, the presence of the conjugating aromatic linker between the ferrocene and peptide units lowers the redox potential to within the range of biologically accessible potentials, allowing for the interconversion between the ferrocene and ferricenium species [24,25,27]. The

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anticancer activity of the ferrocenyl bioconjugates is possibly due at least in part to their low redox potentials, which are within the range of biologically accessible potentials. Therefore in order to explore this hypothesis further, alkyl groups were introduced on the previously unsubstituted cyclopentadiene ring to further modify the redox potentials.

Recently, we reported a study of 1-alkyl-1′-*N*-*para*-(ferrocenyl) benzoyl dipeptide esters which found that alkylation of the previously unsubstituted cyclopentadiene ring resulted in an increase in cytotoxicity [28]. We herein report the synthesis, structural characterization and biological evaluation of novel 1-alkyl-1′-*N*-*meta*-(ferrocenyl)benzoyl dipeptide esters and 1-alkyl-1′-*N*-*ortho*-(ferrocenyl)benzoyl dipeptide esters. The bioconjugates consist of four key moieties, namely (i) an electroactive core (ii) a conjugate linker (iii) an alkyl group and (iv) a dipeptide ester. These novel derivatives differ from the previously reported *N*-*meta and N*-*ortho*-(ferrocenyl)benzoyl dipeptide esters by having an alkyl group on the previously unsubstituted cyclopentadiene ring [19,20]. The 1-alkyl-1′-*N*-*meta* and *N*-*ortho*-(ferrocenyl)benzoyl dipeptide esters were prepared by coupling the methyl, ethyl and propyl ferrocenyl benzoic acids **1**–**6** to the dipeptide ethyl esters using the conventional *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC), 1-hydroxybenzotriazole (HOBt) protocol. The dipeptide esters employed in the synthesis were Gly-Gly(OEt), Gly-L-Ala(OEt), Gly-L-Leu(OEt) and Gly-L-Phe(OEt). All the compounds gave spectroscopic data in accordance with the proposed structures. In addition, the *in vitro* anti-cancer activity of the bioconjugates against the human lung carcinoma cell line H1299 was determined.

Results and discussion

Synthesis

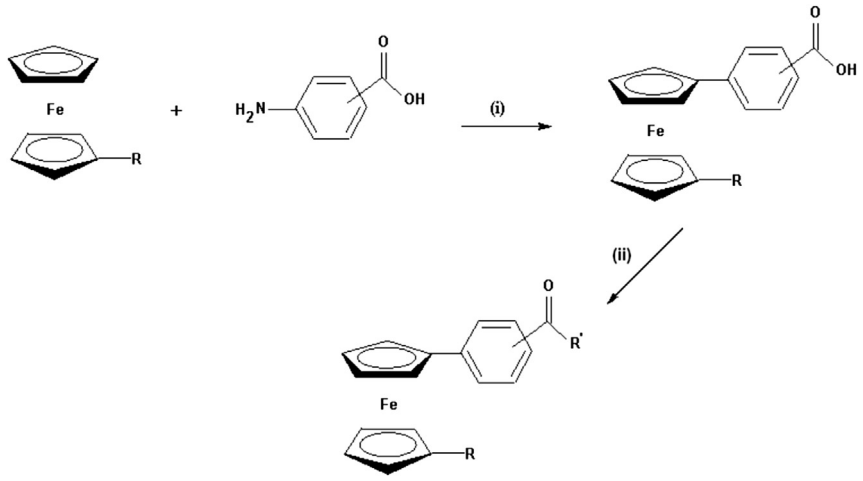
*Synthesis of 1-alkyl-1′-N-meta-(ferrocenyl)benzoyl dipeptide esters **7**–**18** and 1-alkyl-1′-N-ortho-(ferrocenyl)benzoyl dipeptide esters **19**–**30***

1-Alkyl-1′-*N*-*meta*-(ferrocenyl) benzoic acids **1**–**3** were prepared by coupling either methyl, ethyl or propyl ferrocene to 3-amino benzoic acid via diazonium coupling whereas the 1-alkyl-1′-*N*-*ortho*-(ferrocenyl) benzoic acids **4**–**6** were prepared by coupling either methyl, ethyl or propyl ferrocene to 2-amino benzoic acid via diazonium coupling. The free *N*-terminal dipeptide ethyl esters Gly-Gly(OEt), Gly-L-Ala(OEt), Gly-L-Leu(OEt) and Gly-L-Phe(OEt), were coupled to the alkyl ferrocenyl benzoic acids **1**–**6** using EDC and HOBt in the presence of excess triethylamine in dichloromethane (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). Purification by column chromatography furnished the pure products. The 1-alkyl-1′-*N*-*meta*-(ferrocenyl)benzoyl dipeptide esters **7**–**18** were obtained in yields of 14–25% and the 1-alkyl-1′-*N*-*ortho*-(ferrocenyl)benzoyl dipeptide esters **19**–**30** in yields of 12–23%. The chemical structures of compouds **7**–**18** are presented in Table 1 and compounds **19**–**30** in Table 2.

The relatively low yields are partly due to the coupling procedure. The first step in amide bond formation in the coupling protocol is formation of the O-acylisourea ester intermediate. This intermediate is highly reactive, therefore side-reactions can pose a serious problem and can result in extensive racemization resulting in low yields. The addition of HOBt stabilizes the O-acylisourea ester intermediate thus supressing side reactions, however the addition does not result in 100% suppression. As the reaction proceeds, upon addition of the dipeptide ethyl esters the HOBt is displaced resulting in the formation of compounds **7**–**30**. Due to the complexity of the reaction which is associated with the competing reactions, low yields for compounds **7**–**30** were obtained. All of the compounds gave spectroscopic data in accordance with the proposed structures.

^{1}H and ^{13}C spectroscopic analysis

All the proton and carbon chemical shifts for compounds **7**–**30** were unambiguously assigned by a combination of DEPT-135 and ^{1}H-^{13}C COSY (HMQC). The ^{1}H and ^{13}C NMR spectra for compounds **7**–**30** showed peaks in the ferrocene region characteristic of a disubstituted ferrocene moiety [29–31]. For the cyclopentadiene ring attached to the benzoyl spacer moiety ($\eta^5C_5\underline{H}_4$-benzoyl), the *ortho* protons appear as either singlets or triplets between δ 4.85 and δ 4.68, whilst the *meta* protons appear as either singlets or triplets between δ 4.38 and δ 4.2. The protons on the alkylated cyclopentadiene ring (η^5-$C_5\underline{H}_4$-alkyl) overlap with the signals of



Scheme 1. Synthesis of 1-alkyl-1′-*N*-*meta*-(ferrocenyl) benzoyl dipeptide esters **7**–**18** and 1-alkyl-1′-*N*-*ortho*-(ferrocenyl) benzoyl dipeptide esters **19**–**30**. (i) $NaNO_2$, HCl (ii) EDC, HOBt, triethylamine, dipeptide ethyl esters; *meta* series R = CH_3; R′ = Gly-Gly(OEt) **7**, Gly-L-Ala(OEt) **8**, Gly-L-Leu(OEt) **9**, Gly-L-Phe(OEt) **10,** R = CH_2CH_3; R′ = Gly-Gly(OEt) **11**, Gly-L-Ala(OEt) **12**, Gly-L-Leu(OEt) **13**, Gly-L-Phe(OEt) **14**, R = $CH_2CH_2CH_3$; R′ = Gly–Gly(OEt) **15**, Gly-L-Ala(OEt) **16**, Gly-L-Leu(OEt) **17**, Gly-L-Phe(OEt) **18,** *ortho* series R = CH_3; R′ = Gly-Gly(OEt) **19**, R′ = Gly-L-Ala(OEt) **20**, Gly-L-Leu(OEt) **21**, Gly-L-Phe(OEt) **22,** R = CH_2CH_3; R′ = Gly-Gly(OEt) **23**, R′ = Gly-L-Ala(OEt) **24**, Gly-L-Leu(OEt) **25**, Gly-L-Phe(OEt) **26**, R = $CH_2CH_2CH_3$; R′ = Gly-Gly(OEt) **27**, Gly-L-Ala(OEt) **28**, Gly-L-Leu(OEt) **29**, Gly-L-Phe(OEt) **30**.