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The synthesis, structural characterization and *in vitro* anti-cancer activity of novel 1-alkyl-1′-*N*-*para*-(ferrocenyl) benzoyl dipeptide esters

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ABSTRACT

1-Alkyl-1′-*N*-*para*-(ferrocenyl) benzoyl dipeptide esters **4**–**12** were prepared by coupling the alkyl ferrocenyl benzoic acids **1**–**3** to the dipeptide ethyl esters 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. All the compounds were fully characterized using a combination of ^{1}H NMR, ^{13}C NMR, DEPT-135 and ^{1}H–^{13}C COSY (HMQC) spectroscopy, electrospray ionization mass spectrometry (ESI-MS) and cyclic voltammetry (CV). Selected compounds showed micromolar activity in the H1299 NSCLC cell line, with IC_{50} values in the range of 4.5–6.6 μM.

1. Introduction

Organometallic compounds have been incorporated in a wide variety of materials that have diverse applications. Ferrocene is a particularly attractive candidate for incorporation into biologically active compounds due to its aromaticity, stability, redox properties and low toxicity [1,2]. The medicinal application of ferrocene derivatives is an active area of research, with applications in the area of cancer research being the most popular and well-researched [3,4].

The reversible redox properties of ferrocene have been strongly associated with its biological activity [5]. To date, the most promising ferrocene-based drug candidates have been reported by Jaouen and co-workers [6]. These anticancer compounds contain a [3]-ferrocenophane motif and have a potent *in vitro* antiproliferative effect in breast and prostate cancer cells lines [7,8].

This research group have prepared *N*-ferrocenoyl and *N*-ferrocenyl amino acid and peptide derivatives and investigated their potential applications [9–13]. *In vitro* toxicity testing showed that *N*-(ferrocenyl)benzoyl and *N*-(ferrocenyl)naphthoyl amino acid and peptide derivatives were potential anticancer agents [14–21]. These ferrocenyl-peptide bioconjugates 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 bonds. 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. 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. Ferricenium salts that are known to inhibit tumour growth, have been shown to produce hydroxyl ($HO^{\bullet}$) radicals under physiological conditions, leading to oxidatively damaged DNA [5]. The catalytic generation of intracellular reactive oxygenated species (ROS) such as the $HO^{\bullet}$ radical offers an attractive and alternative method to target and kill cancer cells [22]. The anticancer activity of the ferrocenyl bioconjugates is possibly due at

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least in part to their low redox potentials, which are within the range of biologically accessible potentials. In order to explore this hypothesis further, alkyl groups were introduced on the previously unsubstituted cyclopentadiene ring to further modify the redox potentials.

We now report the synthesis and structural characterization of novel 1-alkyl-1′-*N*-*para*-(ferrocenyl)benzoyl dipeptide esters which consist of four key moieties, namely (i) an electroactive core (ii) a conjugate linker (iii) an alkyl group which further lowers the oxidation potential of the ferrocene moiety and (iv) a dipeptide ester. These novel derivatives differ from the previously reported *N*-*para*-(ferrocenyl)benzoyl dipeptide esters by having an alkyl group on the previously unsubstituted cyclopentadiene ring. The 1-alkyl-1′-*N*-*para*-(ferrocenyl)benzoyl dipeptide esters were prepared by coupling the alkyl ferrocenyl benzoic acids **1**–**3** 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-L-Ala(OEt), Gly-L-Leu(OEt) and Gly-L-Phe(OEt). All the compounds were fully characterized using a combination of ^{1}H NMR, ^{13}C NMR, DEPT-135 and ^{1}H–^{13}C COSY (HMQC) spectroscopy, electrospray ionization mass spectrometry (ESI-MS) and cyclic voltammetry (CV). In addition, we present the *in vitro* anti-cancer activity of selected compounds against the human lung carcinoma cell line H1299.

2. Results and discussion

2.1. Synthesis

2.1.1. Synthesis of 1-alkyl-1′-N-para-(ferrocenyl)benzoyl dipeptide esters 4–12

1-Alkyl-1′-*N*-*para*-(ferrocenyl) benzoic acids **1**–**3** were prepared by coupling either methyl, ethyl or propyl ferrocene to 4-aminobenzoic acid via diazonium coupling. The ^{1}H NMR spectra showed signals for the aromatic ring protons between δ 7.86 and δ 7.62. The carboxylic acid proton was present in the narrow range of δ 12.85–12.8. For the disubstituted ferrocene moiety, 3 splitting patterns are observed. For the cyclopentadiene ring attached to the benzoyl spacer moiety (η^5-$C_5\underline{H}_4$-benzoyl), the protons appear as either singlets or triplets between δ 4.89 and δ 4.31, whilst the protons on the alkylated cyclopentadiene ring (η^5-$C_5\underline{H}_4$-alkyl) appear as doublets of doublets between δ 3.92–3.9.

The free *N*-terminal dipeptide ethyl esters Gly-L-Ala(OEt), Gly-L-Leu(OEt) and Gly-L-Phe(OEt), were coupled to the 1-alkyl-1′-*para*-(ferrocenyl) benzoic acids **1**–**3** 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 in yields of 14–25%. The relatively low yields of the products is 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 and thus, side-reactions can pose a serious problem and can result in extensive racemisation 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 amino acid and dipeptide ethyl esters the HOBt is displaced resulting in the formation of compounds **4**–**12**. As a result of the complexity of the reaction which is associated with the competing reactions, low yields for compounds **4**–**12** were obtained.

All compounds gave spectroscopic data in accordance with the proposed structures. The 1-alkyl-1′-*N*-*para*-(ferrocenyl)benzoyl dipeptide esters **4**–**12** were characterized by a combination of ^{1}H NMR, ^{13}C NMR, DEPT-135 and ^{1}H–^{13}C COSY (HMQC) spectroscopy, cyclic voltammetry (CV) and electrospray ionization mass spectrometry (ESI).

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

All the proton and carbon chemical shifts for compounds **4**–**12** 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 **4**–**12** showed peaks in the ferrocene region characteristic of a disubstituted ferrocene moiety [23–25]. For the disubstituted ferrocene moiety, 3 splitting patterns are observed. For the cyclopentadiene ring attached to the benzoyl spacer moiety (η^5-$C_5\underline{H}_4$-benzoyl), the protons appear as either singlets or triplets between δ 4.88 and δ 4.27, whilst the protons on the alkylated cyclopentadiene ring (η^5-$C_5\underline{H}_4$-alkyl) overlap with the signals of the methylene groups of the glycine moiety, resulting in a multiplet being observed between δ 3.97 to δ 3.79. For the alkyl groups attached to the cyclopentadiene ring, the methyl ($-\underline{CH}_3$) group proton signals are present between δ 1.63 to δ 1.4. The methylene and methyl protons of the ethyl group appear as a quartet between δ 2.1 to δ 2.0 and as a triplet between δ 0.98 to δ 0.76 respectively. For the propyl group the protons on the methylene groups ($-\underline{CH}_2\underline{CH}_2CH_3$) appear as a triplet between δ 2.07 to δ 1.9 and as a multiplet between δ 1.4 to δ 1.27 whilst, the protons on the methyl group ($-CH_2CH_2\underline{CH}_3$) appear as a triplet between δ 0.74 to δ 0.66.

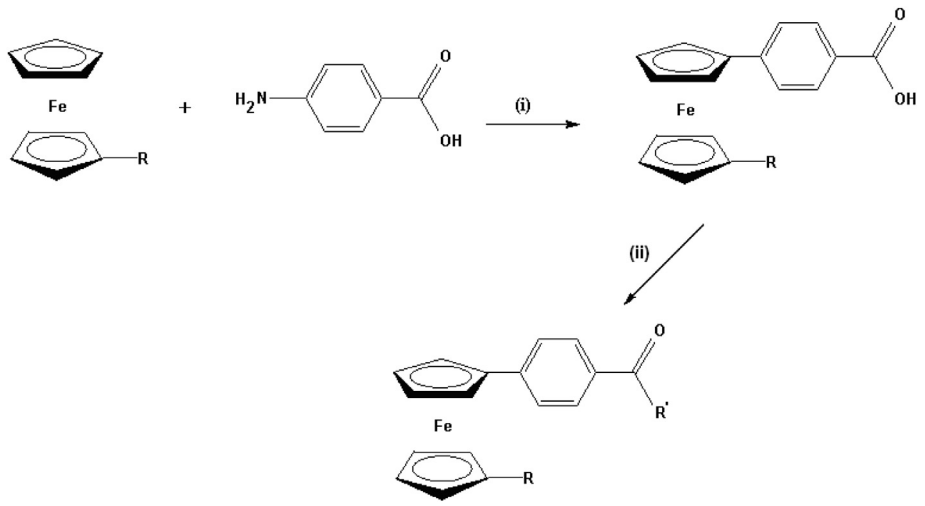
The ^{13}C NMR spectra of compounds **4**–**12** show signals in the region between δ 93.1 to δ 66.0 indicative of a disubstituted ferrocene unit. The *ipso* carbon atom on the (η^5-$\underline{C}_5H_4$-alkyl) ring appears between δ 93.1 to δ 89.8 whilst the *ipso* on the (η^5-$\underline{C}_5H_4$-benzoyl) ring appears in the range δ 84.2 to δ 82.0. These signals are absent in the DEPT-135 spectra. The carbon atoms on the benzoyl linker appear between δ 142.5 and δ 124.0 for compounds **4**–**12**. The quaternary carbon atoms of the aromatic rings and the methylene carbon atoms of derivatives **4**–**12** were identified by DEPT-135. Complete spectroscopic data for all the compounds is presented in the experimental section.

2.3. Mass spectrometry

Since the introduction of soft ionization techniques such as matrix assisted laser desorption ionization (MADLI) and electrospray ionization (ESI), a wide range of thermolabile and non-volatile compounds can be subjected to mass spectrometric analysis [26–28]. Compounds **4**–**12** were not amenable to electron ionization studies, therefore electrospray ionization (ESI) mass spectrometry was employed in the analysis and confirmed the correct relative molecular mass for all the compounds. The ESI mass spectra displayed both radical cation and $[M + H]^+$ species and adducts due to sodium and potassium were present 22 Da and 38 Da higher than the protonated molecular ion species.

2.4. Electrochemistry

The CV curves for compounds **4**–**12** exhibit quasi-reversible behaviour similar to the ferrocene/ferricenium redox couple (Fc/Fc^+). The $E^{\circ\prime}$ (oxidation potential) values for compounds **4** (20 mV versus Fc/Fc^+), **7** (30 mV versus Fc/Fc^+) and **10** (30 mV versus Fc/Fc^+) are lower than that reported for *N*-{*para*(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester (73 mV versus Fc/Fc^+) [29]. It is also considerably lower than the ferrocenoyl dipeptide ester derivatives.



Scheme 1. Synthesis of 1-alkyl-1′-*N*-*para*-(ferrocenyl) benzoyl dipeptide esters (i) $NaNO_2$, HCl (ii) EDC, HOBt, triethylamine, dipeptide ethyl esters, R = CH_3; R′ = Gly-L-Ala(OEt) **4**, Gly-L-Leu(OEt) **5**, Gly-L-Phe(OEt) **6**, R = CH_2CH_3; R′ = Gly-L-Ala(OEt) **7**, Gly-L-Leu(OEt) **8**, Gly-L-Phe(OEt) **9**, R = $CH_2CH_2CH_3$; R′ = Gly-L-Ala(OEt) **10**, Gly-L-Leu(OEt) **11**, Gly-L-Phe(OEt) **12.**

For example, Fc-Ala-Ala-OMe, was reported as $E^{\circ\prime} = 230$ mV (vs Fc/Fc^+) [30]. The benzoyl linker of the 1-methyl-1′-*N*-{para-(ferrocenyl)-benzoyl}dipeptide esters provides extended conjugation to the π-electrons of the Cp rings making initial oxidation of the iron centre easier, relative to *N*-ferrocenoyl dipeptide esters. The methyl substituent on the Cp ring further lowers the oxidation potential.

2.5. *In vitro* anti-cancer activity of **4**–**12**

The *in vitro* anti-proliferative effect of compounds **4**–**12** was studied in the H1299 non-small cell lung cancer (NSCLC) cell line. Proliferation was measured using the acid phosphatase assay as previously described [31]. In a preliminary screen the cells were treated with the 1-alkyl-1′-*N*-*para*-(ferrocenyl) benzoyl dipeptide esters **4**–**12** at a concentration of 10 μM and were incubated for 5–6 days until cell confluency reached 80–90%. Cell survival was established through determination of the acid phosphatase activity of surviving cells and growth inhibition calculated relative to controls (untreated cells). The results for the preliminary screen of compounds **4**–**12** are depicted in Table 1. Of the nine compounds screened the three most active compounds which showed % growth inhibition values ≥70%, were selected for further evaluation. A general trend was also observed in these derivatives, that is, the Gly-L-Ala(OEt) derivatives are more active than the Gly-L-Leu and Gly-L-Phe ethyl esters which display % growth inhibition values ≤ 55%. For instance 1-ethyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **7** displayed a % growth inhibition value of 84 ± 6.3% whereas the 1-ethyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-leucine ethyl ester **8** and the 1-ethyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-phenylalanine ethyl ester **9** displayed % growth inhibition values of 34 ± 10.6% and 19 ± 13.6% respectively. Therefore the 1-methyl, 1-ethyl and 1-propyl-1′-*N*- {*para*-(ferrocenyl)-benzoyl}-glycine-L-leucine ethyl esters and the 1-methyl, 1-ethyl and 1-propyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-phenylalanine ethyl ester derivatives were not investigated further. It can be concluded that when chiral α-amino acids with bulky side chains are used as the second amino acid in the dipeptide moiety a loss of anti-proliferative activity is observed. The IC_{50} values were determined for compounds **4**, **7** and **10** using the acid phosphatase assay. This colorimetric end-point assay is an indirect measure of cytotoxicity which evaluates the enzyme activity of cells after a given treatment period. Acid phosphatase is an enzyme which dephosphorylates the *p*-nitrophenyl phosphate substrate converting it to *p*-nitrophenol which in the presence of strong alkali can be quantified colorimetrically. The acid phosphatase assay is highly sensitive and is easier to perform than the neutral red assay as it involves fewer steps and fewer reagents. It is also more convenient than the MTT assay because of the inherent problem of removal of the medium from the insoluble crystals. The reproducibility between replicate wells is also excellent in the acid phosphatase assay. To determine the IC_{50} values of the three compounds, individual 96-well plates containing H1299 cells were treated with the test compound at concentrations ranging from 0.1 μM to 100 μM. The cells were then incubated for 5–6 days, until cell confluency was reached. Cell survival was determined by performing the acid phosphatase assay. The IC_{50} value for each compound was calculated using Calcusyn software, and standard deviations have been calculated using data obtained from three independent experiments. The values obtained are listed in Table 2.

From the IC_{50} values of the 1-alkyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl esters **4**, **7** and **10** it can be seen

Table 1
% Growth inhibition values for compounds **4**–**12** against human lung carcinoma cell line H1299.

Compound name	% Growth inhibition at 10 μM
1-Methyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **4**	83 ± 1.0
1-Methyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-leucine ethyl ester **5**	28 ± 18.6
1-Methyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-phenylalanine ethyl ester **6**	20 ± 9.9
1-Ethyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **7**	84 ± 6.3
1-Ethyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-leucine ethyl ester **8**	34 ± 10.6
1-Ethyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-phenylalanine ethyl ester **9**	19 ± 13.6
1-Propyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **10**	80 ± 4.5
1-Propyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-leucine ethyl ester **11**	55 ± 10.6
1-Propyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-phenylalanine ethyl ester **12**	34 ± 10.6

Table 2
IC_{50} values for compounds **4**, **7**, **10**, **15,** carboplatin **13** and cisplatin **14** against human lung carcinoma cell line H1299.

Compound	IC_{50} value (μM)
Carboplatin	10.0 ± 1.6
Cisplatin	1.5 ± 0.1
1-Methyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **4**	4.5 ± 0.4
N-{*para*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **15**	6.6 ± 0.7
1-Ethyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **7**	5.6 ± 1.6
1-Propyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **10**	6.6 ± 2.1

that the cytotoxicity of the derivatives decreases with an increase of the size of the alkyl group incorporated (propyl < ethyl < methyl). The *in vitro* cytotoxicity of the platinum(II)-based anti-cancer drug carboplatin **13** was also evaluated against the H1299 cell line, and was found to have an IC_{50} value of 10.0 ± 1.6 μM. Thus compounds **4**, **7** and **10** are more cytotoxic *in vitro* than the clinically employed anti-cancer drug carboplatin. 1-Methyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **4** displayed an IC_{50} value of 4.5 μM (Fig. 1). This is lower than *N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **15** which displayed an IC_{50} value of 6.6 μM [16]. Therefore alkylation of the previously unsubstituted cyclopentadiene ring increases the cytotoxicity of the ferrocenyl bioconjugates. However compounds **4**, **7** and **10** are less active compared to cisplatin **14** which displays an IC_{50} value of 1.5 μM against human H1299 lung carcinoma cells. A potential mechanism by which these novel organometallic anticancer compounds may induce DNA damage is by the catalytic generation of reactive oxygenated species (ROS). This is possible via a Fenton-type reaction, in which HO• radicals are generated from the superoxide dismutation product, hydrogen peroxide (H_2O_2). It was shown that the generation of 8-oxo-7,8-dihydroguanine (8-oxoGua) by a compound prepared in a previous SAR study, *N*-(6-ferrocenyl-2-naphthoyl)-glycine-glycine ethyl ester, that the oxidation was occurring by Fenton chemistry and that *N*-(6-ferrocenyl-2-naphthoyl)-glycine-glycine ethyl ester is generating oxidative damage via a ROS-mediated mechanism. Therefore guanine oxidation studies confirmed that *N*-(6-ferrocenyl-2-naphthoyl)-glycine-glycine ethyl ester was capable of causing oxidative damage to guanine, and it does so by the generation of HO• radicals from H_2O_2 [20].

3. Conclusion

In conclusion, the novel 1-alkyl-1′-*N*-*para*-(ferrocenyl)benzoyl dipeptide esters were synthesized and fully characterized by a range of NMR spectroscopic techniques, mass spectrometry and cyclic voltammetry. Compounds **4, 7** and **10** were tested *in vitro* against the human lung carcinoma cell line H1299. The compounds showed micromolar activity in the H1299 NSCLC cell line, with IC_{50} values of 4.5, 5.6 and 6.6 μM respectively. Alkylation of the previously unsubstituted cyclopentadiene ring increases the cytotoxicity of the ferrocenyl bioconjugates. The cytotoxicity of the derivatives decreases with an increase of the size of the alkyl group on the previously unsubstituted cyclopentadiene ring.

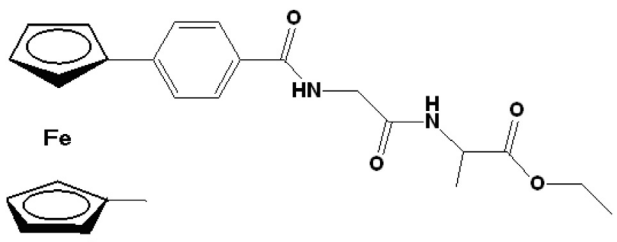


Fig. 1. 1-Methyl-1′-*N*-{*para*-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **4**.

4. Experimental

4.1. General procedures

All chemicals were purchased from Sigma–Aldrich, Lennox Chemicals, Fluorochem Limited or Tokyo Chemical Industry UK Limited; and used as received. Commercial grade reagents were used without further purification. When necessary, all solvents were purified and dried prior to use. Riedel-Haën silica gel was used for thin layer and column chromatography. Melting points were determined using a Stuart melting point (SMP3) apparatus and are uncorrected. Optical rotation measurements were made on a Perkin Elmer 343 Polarimeter and are quoted in units of 10^{-1} deg cm^2 g^{-1}. Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR with ATR. UV–Vis spectra were recorded on a Hewlett Packard 8452 A diode array UV–Vis spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in deuterated solvents on a Bruker Avance 400 NMR. The ^1H and ^{13}C NMR chemical shifts are reported in ppm (parts per million). Tetramethylsilane (TMS) or the residual solvent peaks have been used as an internal reference. All coupling constants (*J*) are in Hertz. Electrospray ionisation mass spectra were performed on a Micromass LCT mass spectrometer.

Cyclic voltammograms were recorded in anhydrous acetonitrile (Sigma–Aldrich), with 0.1 M tetrabutylammonium hexafluorophosphate ($TBAPF_6$) as a supporting electrolyte, using a CH Instruments 600a electrochemical analyzer (Pico-Amp Booster and Faraday Cage). The experiments were carried out at room temperature. A three-electrode cell consisting of a glassy carbon working-electrode, a platinum wire counter-electrode and an Ag|AgCl reference electrode was used. The glassy carbon electrode was polished with 0.3 μm alumina followed by 0.05 μm alumina, between each experiment to remove any surface contaminants. The scan rate was 0.1 V s^{-1}. The concentration range of the ferrocene compounds was 1.0 mM in acetonitrile. The $E^{0\prime}$ values obtained for the test samples were referenced relative to the ferrocene/ferricenium redox couple.

4.2. General procedure for the synthesis of the starting materials

4.2.1. 1-Methyl-1′-para-ferrocenyl benzoic acid **1**

4-Aminobenzoic acid (3.43 g, 25.00 mmol) was dissolved in deionised water (30 ml) at 0 °C followed by the addition of concentrated HCl (7 ml). Sodium nitrite (1.72 g, 25.00 mmol) was dissolved in deionised water (20 ml) at 0 °C and was then added to the 4-aminobenzoic acid solution with stirring at a temperature less than 5 °C. The resulting pale yellow diazonium salt was then added to a solution of methylferrocene (5.00 g, 25.00 mmol) in dry diethyl ether (100 ml) and the reaction mixture was allowed to stir at room temperature for 48 h. The reaction mixture was then washed with water and brine and the organic layer was dried over $MgSO_4$. The solvent was removed *in vacuo* to yield the crude product. The crude product was purified by column chromatography {eluant 1:1 hexane: ethyl acetate} yielding the title compound as a red oil (1.65 g, 21%).

^1H NMR (400 MHz) δ (DMSO-d_6): 12.8 (1H, s, –COOH), 7.79 (2H, d, J = 7.2 Hz, ArH), 7.67 (2H, d, J = 7.2 Hz, ArH), 4.89 (2H, s, *ortho* on

η^5-C_5H_4-benzoyl), 4.41 (2H, s, *meta* on η^5-C_5H_4-benzoyl), 3.9 (4H, dd, η^5-C_5H_4-alkyl), 1.62 (3H, s, $-CH_3$).

^{13}C NMR (100 MHz) δ (DMSO-d_6): 174.3 (C=O), 140.1 (C_q), 132.4 (C_q), 126.5, 123.9, 91.6 (C_{ipso} η^5-C_5H_4-alkyl), 80.7 (C_{ipso} η^5-C_5H_4-benzoyl), 73.2 (C_{meta} η^5-C_5H_4-benzoyl), 69.8(C_{meta} η^5-C_5H_4-alkyl), 68.0 (C_{ortho} η^5-C_5H_4-alkyl), 67.5 (C_{ortho} η^5-C_5H_4-benzoyl), 13.1($-CH_3$).

4.2.2. 1-Ethyl-1′-para-ferrocenyl benzoic acid ***2***

4-Aminobenzoic acid (3.20 g, 23.36 mmol) and ethylferrocene (5.00 g, 23.36 mmol) were used as starting materials. Recrystallisation from chloroform furnished the desired product as an orange solid (1.80 g, 23%).

^{1}H NMR (400 MHz) δ (DMSO-d_6): 12.85 (1H, s, $-COOH$), 7.86 (2H, d, J = 7.6 Hz, ArH), 7.62 (2H, d, J = 7.6 Hz, ArH), 4.82 (2H, t, J = 1.6 Hz, *ortho* on η^5-C_5H_4-benzoyl), 4.31 (2H, t, J = 1.6 Hz, *meta* on η^5-C_5H_4-benzoyl), 3.91 (4H, dd, η^5-C_5H_4-alkyl), 2.04 (2H, q, J = 7.2 Hz, $-CH_2CH_3$), 0.97 (3H, t, J = 7.2 Hz, $-CH_2CH_3$).

^{13}C NMR (100 MHz) δ (DMSO-d_6): 167.3 (C=O), 144.3 (C_q), 129.4 (C_q), 127.5, 125.4, 91.6 (C_{ipso} η^5-C_5H_4-alkyl), 81.3 (C_{ipso} η^5-C_5H_4-benzoyl), 70.2 (C_{meta} η^5-C_5H_4-benzoyl), 68.8 (C_{meta} η^5-C_5H_4-alkyl), 68.6 (C_{ortho} η^5-C_5H_4-alkyl), 67.0 (C_{ortho} η^5-C_5H_4-benzoyl), 20.7 ($-CH_2CH_3$, −ve DEPT), 14.4 ($-CH_2CH_3$).

4.2.3. 1-Propyl-1′-para-ferrocenyl benzoic acid ***3***

4-Aminobenzoic acid (3.01 g, 21.93 mmol) and propyl ferrocene (5.00 g, 21.93 mmol)) were used as starting materials. Recrystallisation from chloroform furnished the desired product as an orange solid (1.61 g, 21%).

^{1}H NMR (400 MHz) δ (DMSO-d_6): 12.85 (1H, s, $-COOH$), 7.86 (2H, d, J = 7.4 Hz, ArH), 7.62 (2H, d, J = 7.4 Hz, ArH), 4.82 (2H, s, *ortho* on η^5-C_5H_4-benzoyl), 4.38 (2H, s, *meta* on η^5-C_5H_4-benzoyl), 3.92 (4H, dd, η^5-C_5H_4-alkyl), 1.94 (2H, t, J = 7.6 Hz, $-CH_2CH_2CH_3$), 1.4–1.27 (2H, m, $-CH_2CH_2CH_3$), 0.75 (3H, t, J = 7.6 Hz, $-CH_2CH_2CH_3$).

^{13}C NMR (100 MHz) δ (DMSO-d_6): 167.3 (C=O), 144.3 (C_q), 129.3 (C_q), 127.5, 125.4, 89.3 (C_{ipso} η^5-C_5H_4-alkyl), 81.7 (C_{ipso} η^5-C_5H_4-benzoyl), 70.1 (C_{meta} η^5-C_5H_4-benzoyl), 68.8 (C_{meta} η^5-C_5H_4-alkyl), 68.5 (C_{ortho} η^5-C_5H_4-alkyl), 67.0 (C_{ortho} η^5-C_5H_4-benzoyl), 32.3 ($-CH_2CH_2CH_3$, −ve DEPT), 22.0 ($-CH_2CH_2CH_3$, −ve DEPT), 14.4 ($-CH_2CH_2CH_3$).

4.3. General procedure for the synthesis of 1-alkyl-1′-N-ferrocenyl benzoyl dipeptide ethyl esters

4.3.1. 1-Methyl-1′-N-{para-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester ***4***

1-Methyl-1′-*N*-*para*-ferrocenyl benzoic acid (0.25 g, 0.78 mmol) was dissolved in dichloromethane (100 ml) at 0 °C. *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.15 g, 0.78 mmol), 1-hydroxybenzotriazole (0.11 g, 0.78 mmol), glycine-L-alanine ethyl ester hydrochloride (0.14 g, 0.78 mmol). and triethylamine (3 ml) were added and the reaction mixture was allowed to stir at 0 °C for 45 min. The reaction mixture was then washed with water and brine. The organic layer was then dried over $MgSO_4$. The solvent was removed *in vacuo* to yield the crude product. The crude product was purified by column chromatography {eluant 1:1 hexane: ethyl acetate} yielding the title compound as a red oil (0.08 g, 22%), $E^{\circ\prime}$ = 20 mV (vs Fc/Fc^+); $[\alpha]_D^{20} = -10^\circ$ (c 0.1, EtOH).

Mass spectrum: $[M + Na]^+$ found: 499.1394.

$C_{25}H_{28}N_2O_4FeNa$ requires: 499.1396.

I.R. υ_{max} (KBr): 3295 (NH), 1743 (C=O_{ester}), 1636 (C=O_{amide}), 1608 (C=O_{amide}) cm^{-1}.

UV–Vis λ_{max} EtOH: 360, 451 nm.

^{1}H NMR (400 MHz) δ (DMSO- d_6): 8.67 (1H, t, J = 6.4 Hz, $-CONH-$), 8.41 (1H, d, J = 7.0 Hz, $-CONH-$), 7.8 (2H, d, J = 8.4 Hz, ArH), 7.61 (2H, d, J = 8.4 Hz, ArH), 4.81 (2H, s, *ortho* on η^5-C_5H_4-benzoyl), 4.36 (2H, s, *meta* on η^5-C_5H_4-benzoyl), 4.31–4.24 (1H, m, $-CHCH_3$), 4.05 (2H, q, J = 6.8 Hz, $-OCH_2CH_3$), 3.96–3.85 {6H, m, (η^5-C_5H_4-alkyl), ($-NHCH_2CO-$)}, 1.6 (3H, s, $-CH_3$), 1.31 (3H, d, J = 6.2 Hz, $-CHCH_3$), 1.18 (3H, t, J = 6.8 Hz, $-OCH_2CH_3$).

^{13}C NMR (100 MHz) δ (DMSO-d_6): 172.5 (C=O), 169.0 (C=O), 166.5 (C=O), 142.5 (C_q), 130.8 (C_q), 127.4, 125.2, 92.3 (C_{ipso} η^5-C_5H_4-alkyl), 82.0 (C_{ipso} η^5-C_5H_4-benzoyl), 71.0 (C_{meta} η^5-C_5H_4-benzoyl), 69.8 (C_{meta} η^5-C_5H_4-alkyl), 68.5 (C_{ortho} η^5-C_5H_4-alkyl), 66.9 (C_{ortho} η^5-C_5H_4-benzoyl), 60.9 ($-OCH_2CH_3$, −ve DEPT), 47.6 ($-CHCH_3$), 42.0 ($-NHCH_2CHO-$, −ve DEPT), 17.0 ($-CHCH_3$), 14.5 ($-OCH_2CH_3$), 14.0 ($-CH_3$).

4.3.2. 1-Methyl-1′-N-{para-(ferrocenyl)-benzoyl}-glycine-L-leucine ethyl ester ***5***

Glycine-L-leucine ethyl ester hydrochloride (0.17 g, 0.78 mmol) was used as a starting material. The crude product was purified by column chromatography {eluant 1:1 hexane: ethyl acetate} and recrystallisation from hexane: ethyl acetate yielded the desired product as an orange solid (0.07 g, 17%), m.p 142–144 °C; $E^{\circ\prime}$ = 25 mV (vs Fc/Fc^+); $[\alpha]_D^{20} = -17^\circ$ (c 0.1, EtOH).

Mass spectrum: $[M + Na]^+$ found: 541.1884.

$C_{28}H_{34}N_2O_4FeNa$ requires: 541.1888.

I.R. υ_{max} (KBr): 3267 (NH), 1742 (C=O_{ester}), 1640 (C=O_{amide}), 1610 (C=O_{amide}) cm^{-1}.

UV–Vis λ_{max} EtOH: 358, 450 nm.

^{1}H NMR (400 MHz) δ (DMSO-d_6): 8.65 (1H, t, J = 6 Hz, $-CONH-$), 8.33 (1H, d, J = 7.6 Hz, $-CONH-$), 7.8 (2H, d, J = 8.0 Hz, ArH), 7.6 (2H, d, J = 8.0 Hz, ArH), 4.82 (2H, s, *ortho* on η^5-C_5H_4-benzoyl), 4.37–4.29 {3H, m, (*meta* on η^5-C_5H_4-benzoyl), ($-CH(CH_2CH(CH_3)_2)$}, 4.08 (2H, q, J = 7.2 Hz, $-OCH_2CH_3$), 3.96–3.85 {6H, m, (η^5-C_5H_4-alkyl), ($-NHCH_2CO-$)}, 1.63–1.4 {6H, m, ($-CH_3$), ($-CH(CH_2CH(CH_3)_2)$}, 1.18 (3H, t, J = 7.2 Hz, $-OCH_2CH_3$), 0.91–0.89 {6H, m, $-CH(CH_2CH(CH_3)_2$};

^{13}C NMR (100 MHz) δ (DMSO-d_6): 172.5 (C=O), 169.2 (C=O), 166.2 (C=O), 142.2 (C_q), 130.8 (C_q), 127.4, 125.2, 90.4 (C_{ipso} η^5-C_5H_4-alkyl), 84.2 (C_{ipso} η^5-C_5H_4-benzoyl), 70.1 (C_{meta} η^5-C_5H_4-benzoyl), 70.0 (C_{meta} η^5-C_5H_4-alkyl), 68.4 (C_{ortho} η^5-C_5H_4-alkyl), 66.9 (C_{ortho} η^5-C_5H_4-benzoyl), 60.4 ($-OCH_2CH_3$, −ve DEPT), 50.3 {$-CH(CH_2CH(CH_3)_2$}, 42.0 ($-NHCH_2CHO-$, −ve DEPT), 39.4 {$-CH(CH_2CH(CH_3)_2)$, −ve DEPT}, 24.2 {$-CH(CH_2CH(CH_3)_2$}, 22.7 ($-CH(CH_2CH(CH_3)_2$}, 21.4 {$-CH(CH_2CH(CH_3)_2$}, 14.0 ($-OCH_2CH_3$), 13.2 ($-CH_3$).

4.3.3. 1-Methyl-1′-N-{para-(ferrocenyl)-benzoyl}-glycine-L-phenylalanine ethyl ester ***6***

Glycine-L-phenylalanine ethyl ester hydrochloride (0.20 g, 0.78 mmol) was used as a starting material. The crude product was purified by column chromatography {eluant 1:1 hexane: ethyl acetate} and recrystallisation from hexane: ethyl acetate yielded the desired product as an orange solid (0.07 g, 16%), m.p 82–83 °C; $[\alpha]_D^{20} = +6^\circ$ (c 0.1, EtOH).

Mass spectrum: $[M + Na]^+$ found: 575.1794.

$C_{31}H_{32}N_2O_4FeNa$ requires: 575.1796.

I.R. υ_{max} (KBr): 3263 (NH), 1735 (C=O_{ester}), 1663 (C=O_{amide}), 1609 (C=O_{amide}) cm^{-1}.

UV–Vis λ_{max} EtOH: 358, 448 nm.

^{1}H NMR (400 MHz) δ (DMSO-d_6): 8.68 (1H, t, J = 6.4 Hz, $-CONH-$), 8.34 (1H, d, J = 7.6 Hz, $-CONH-$), 7.81 (2H, d, J = 8.4 Hz, ArH), 7.60 (2H, d, J = 8.4 Hz, ArH), 7.27–7.21 {5H, m, $-CH(CH_2Ph)$}, 4.8 (2H, s, *ortho* on η^5-C_5H_4-benzoyl), 4.47–4.45 {1H, m, $-CH(CH_2Ph)$}, 4.35 (2H, s, *meta* on η^5-C_5H_4-benzoyl), 4.04 (2H, q, J = 7.2 Hz, $-OCH_2CH_3$), 3.96–3.85 {6H, m, (η^5-C_5H_4-alkyl), ($-NHCH_2CO-$)}, 3.06–2.93 {2H, m, $-CH(CH_2Ph)$}, 1.57 (3H, s, $-CH_3$), 1.12 (3H, t, J = 7.2 Hz, $-OCH_2CH_3$).

^{13}C NMR (100 MHz) δ (DMSO-d_6): 172.4 (C=O), 169.2 (C=O), 166.3 (C=O), 142.0 (C_q) 136.9 (C_q), 130.7 (C_q), 129.1, 128.0, 127.4, 126.0, 125.2, 90.2 (C_{ipso} η^5-$\underline{C}_5H_4$-alkyl), 82.0 (C_{ipso} η^5-$\underline{C}_5H_4$-benzoyl), 71.4 (C_{meta} η^5-$\underline{C}_5H_4$-benzoyl), 71.0 (C_{meta} η^5-$\underline{C}_5H_4$-alkyl), 68.0 (C_{ortho} η^5-$\underline{C}_5H_4$-alkyl), 66.4 (C_{ortho} η^5-$\underline{C}_5H_4$-benzoyl), 61.4 (−O$\underline{C}H_2CH_3$, −ve DEPT), 53.6 {−$\underline{C}$H(CH_2Ph)}, 42.9 (−NH$\underline{C}H_2$CHO−, −ve DEPT), 36.7 {−CH($\underline{C}H_2$Ph), −ve DEPT}, 14.0 (−OCH$_2\underline{C}H_3$), 13.2 (−$\underline{C}H_3$).

4.3.4. 1-Ethyl-1′-N-{para-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **7**

Glycine-L-alanine ethyl ester hydrochloride (0.13 g, 0.75 mmol) was used as a starting material. The crude product was purified by column chromatography {eluant 1:1 hexane: ethyl acetate} yielding the title compound as a red oil (0.09 g, 25%), $E^{\circ\prime} = 30$ mV (vs Fc/Fc$^+$); $[\alpha]_D^{20} = -16^\circ$ (*c* 0.1, EtOH).

Mass spectrum: $[M + Na]^+$ found: 513.1454.

$C_{26}H_{30}N_2O_4FeNa$ requires: 513.1453.

I.R. υ_{max} (KBr): 3290 (NH), 1753 (C=O$_{ester}$), 1626 (C=O$_{amide}$), 1608 (C=O$_{amide}$) cm^{-1}.

UV−Vis λ_{max} EtOH: 359, 449 nm.

^{1}H NMR (400 MHz) δ (DMSO-d_6): 8.68 (1H, t, $J = 6$ Hz, −CONH−), 8.4 (1H, t, $J = 7.2$ Hz, −CONH−), 7.81 (2H, d, $J = 7.8$ Hz, ArH), 7.6 (2H, d, $J = 7.8$ Hz, ArH), 4.82 (2H, t, $J = 1.6$ Hz, *ortho* on η^5-C_5H_4-benzoyl), 4.37 (2H, t, $J = 1.6$ Hz, *meta* on η^5-C_5H_4-benzoyl), 4.28−4.25 (1H, m, −$\underline{C}$HCH$_3$), 4.15 (2H, q, $J = 6.8$ Hz, −O$\underline{C}H_2$CH$_3$), 3.9−3.85 {6H, m, (η^5-C_5H_4-alkyl), (−NH$\underline{C}H_2$CO−)}, 2.1 (2H, q, $J = 7.6$ Hz, −$\underline{C}H_2$CH$_3$), 1.31 (3H, d, $J = 6.4$ Hz, −CH$\underline{C}$H$_3$), 1.18 (3H, t, $J = 6.8$ Hz, −OCH$_2\underline{C}$H$_3$), 0.98 (3H, t, $J = 7.6$ Hz, −CH$_2\underline{C}$H$_3$).

^{13}C NMR (100 MHz) δ (DMSO-d_6): 171.7 (C=O), 168.7 (C=O), 167.2 (C=O), 142.5 (C_q), 130.8 (C_q), 127.4, 125.2, 92.3 (C_{ipso} η^5-$\underline{C}_5H_4$-alkyl), 82.0 (C_{ipso} η^5-$\underline{C}_5H_4$-benzoyl), 71.0 (C_{meta} η^5-$\underline{C}_5H_4$-benzoyl), 69.8 (C_{meta} η^5-$\underline{C}_5H_4$-alkyl), 68.7 (C_{ortho} η^5-$\underline{C}_5H_4$-alkyl), 66.0 (C_{ortho} η^5-$\underline{C}_5H_4$-benzoyl), 61.4 (−O$\underline{C}H_2$CH$_3$, −ve DEPT), 48.0 (−$\underline{C}$HCH$_3$), 43.0 (−NH$\underline{C}H_2$CHO−, −ve DEPT), 22.8 (−$\underline{C}H_2$CH$_3$, −ve DEPT), 18.0 (−CH$\underline{C}$H$_3$), 14.5 (−OCH$_2\underline{C}$H$_3$), 13.9 (−CH$_2\underline{C}$H$_3$).

4.3.5. 1-Ethyl-1′-N-{para-(ferrocenyl)-benzoyl}-glycine-L-leucine ethyl ester **8**

Glycine-L-leucine ethyl ester hydrochloride (0.16 g, 0.75 mmol) was used as a starting material. The crude product was purified by column chromatography {eluant 1:1 hexane: ethyl acetate} and recrystallisation from hexane: ethyl acetate yielded the desired product as an orange solid (0.07 g, 18%), m.p 97−99 °C; $E^{\circ\prime} = 10$ mV (vs Fc/Fc$^+$); $[\alpha]_D^{20} = -12^\circ$ (*c* 0.1, EtOH).

Mass spectrum: $[M + Na]^+$ found: 555.1919.

$C_{29}H_{36}N_2O_4FeNa$ requires: 555.1922.

I.R. υ_{max} (KBr): 3280 (NH), 1740 (C=O$_{ester}$), 1641 (C=O$_{amide}$), 1615 (C=O$_{amide}$) cm^{-1}.

UV−Vis λ_{max} EtOH: 357, 446 nm.

^{1}H NMR (400 MHz) δ (DMSO-d_6): 8.68 (1H, t, $J = 6$ Hz, −CONH−), 8.34 (1H, d, $J = 7.6$ Hz, −CONH−), 7.82 (2H, d, $J = 8.2$ Hz, ArH), 7.61 (2H, d, $J = 8.2$ Hz, ArH), 4.81 (2H, t, $J = 1.6$ Hz, *ortho* on η^5-C_5H_4-benzoyl), 4.36 (2H, t, $J = 1.6$ Hz, *meta* on η^5-C_5H_4-benzoyl), 4.3−4.25 {1H, m, −$\underline{C}$H(CH$_2$CH(CH$_3$)$_2$)}, 4.15 (2H, q, $J = 7.2$ Hz, −O$\underline{C}H_2$CH$_3$), 3.97−3.87 {6H, m, (η^5-C_5H_4-alkyl), (−NH$\underline{C}H_2$CO−)}, 2.0 (2H, q, $J = 7.2$ Hz, −$\underline{C}H_2$CH$_3$), 1.69−1.5 {3H, m, −CH($\underline{C}H_2\underline{C}$H(CH$_3$)$_2$)}, 1.15 (3H, t, $J = 7.2$ Hz, −OCH$_2\underline{C}$H$_3$), 0.9−0.85 {6H, m, −CH(CH$_2$CH($\underline{C}$H$_3$)$_2$)}, 0.76 (3H, t, $J = 7.2$ Hz, −CH$_2\underline{C}$H$_3$).

^{13}C NMR (100 MHz) δ (DMSO-d_6): 173.4 (C=O), 170.0 (C=O), 166.9 (C=O), 140.1 (C_q), 130.8 (C_q), 127.4, 125.2, 92.1 (C_{ipso} η^5-$\underline{C}_5H_4$-alkyl), 83.1 (C_{ipso} η^5-$\underline{C}_5H_4$-benzoyl), 71.0 (C_{meta} η^5-$\underline{C}_5H_4$-benzoyl), 69.9 (C_{meta} η^5-$\underline{C}_5H_4$-alkyl), 68.1 (C_{ortho} η^5-$\underline{C}_5H_4$-alkyl), 67.2 (C_{ortho} η^5-$\underline{C}_5H_4$-benzoyl), 61.4 (−O$\underline{C}H_2$CH$_3$, −ve DEPT), 51.3 {−$\underline{C}$H(CH$_2$CH(CH$_3$)$_2$)}, 42.6 (−NH$\underline{C}H_2$CHO−, −ve DEPT), 39.3 {−CH($\underline{C}H_2$CH(CH$_3$)$_2$), −ve DEPT}, 28.1 {−CH(CH$_2\underline{C}$H(CH$_3$)$_2$)}, 23.4 (−$\underline{C}H_2$CH$_3$, −ve DEPT), 22.9 {−CH(CH$_2$CH($\underline{C}$H$_3$)$_2$)}, 21.0 {−CH(CH$_2$CH($\underline{C}$H$_3$)$_2$)}, 15.1 (−OCH$_2\underline{C}$H$_3$), 13.0 (−CH$_2\underline{C}$H$_3$).

4.3.6. 1-Ethyl-1′-N-{para-(ferrocenyl)-benzoyl}-glycine-L-phenylalanine ethyl ester **9**

Glycine-L-phenylalanine ethyl ester hydrochloride (0.19 g, 0.75 mmol) was used as a starting material. The crude product was purified by column chromatography {eluant 1:1 hexane: ethyl acetate} and recrystallisation from hexane: ethyl acetate yielded the desired product as an orange solid (0.07 g, 17%), m.p 92−94 °C; $E^{\circ\prime} = -30$ mV (vs Fc/Fc$^+$); $[\alpha]_D^{20} = +12^\circ$ (*c* 0.1, EtOH).

Mass spectrum: $[M + Na]^+$ found: 589.1770.

$C_{32}H_{34}N_2O_4FeNa$ requires: 589.1769.

I.R. υ_{max} (KBr): 3303 (NH), 1742 (C=O$_{ester}$), 1629 (C=O$_{amide}$), 1608 (C=O$_{amide}$) cm^{-1}.

UV−Vis λ_{max} EtOH: 356, 450 nm.

^{1}H NMR (400 MHz) δ (DMSO-d_6): 8.6 (1H, t, $J = 6.0$ Hz, −CONH−), 8.37 (1H, d, $J = 7.6$ Hz, −CONH−), 7.85 (2H, d, $J = 7.4$ Hz, ArH), 7.63 (2H, d, $J = 7.4$ Hz, ArH), 7.27−7.21 {5H, m, −CH($\underline{C}H_2$Ph)}, 4.83 (2H, t, $J = 1.6$ Hz, *ortho* on η^5-C_5H_4-benzoyl), 4.48−4.46 {1H, m, −$\underline{C}$H(CH$_2$Ph)}, 4.37 (2H, t, $J = 1.6$ Hz, *meta* on η^5-C_5H_4-benzoyl), 4.00 (2H, q, $J = 6.8$ Hz, −O$\underline{C}H_2$CH$_3$), 3.9−3.83 {6H, m, (η^5-C_5H_4-alkyl), (−NH$\underline{C}H_2$CO−)}, 3.06−2.93 {2H, m, −CH($\underline{C}H_2$Ph)}, 2.08 (2H, q, $J = 7.6$ Hz, −$\underline{C}H_2$CH$_3$), 1.12 (3H, t, $J = 6.8$ Hz, −OCH$_2\underline{C}$H$_3$), 0.98 (3H, t, $J = 7.6$ Hz, −CH$_2\underline{C}$H$_3$).

^{13}C NMR (100 MHz) δ (DMSO-d_6): 170.8 (C=O), 168.2 (C=O), 166.7 (C=O), 137.0 (C_q), 132.7 (C_q), 130.1 (C_q), 128.9, 127.2, 126.9, 124.0, 120.2, 93.1 (C_{ipso} η^5-$\underline{C}_5H_4$-alkyl), 84.0 (C_{ipso} η^5-$\underline{C}_5H_4$-benzoyl), 71.4 (C_{meta} η^5-$\underline{C}_5H_4$-benzoyl), 68.4 (C_{meta} η^5-$\underline{C}_5H_4$-alkyl), 67.5 (C_{ortho} η^5-$\underline{C}_5H_4$-alkyl), 66.4 (C_{ortho} η^5-$\underline{C}_5H_4$-benzoyl), 61.2 (−O$\underline{C}H_2$CH$_3$, −ve DEPT), 51.6 (−$\underline{C}$H(CH_2Ph)), 43.0 (−NH$\underline{C}H_2$CHO−, −ve DEPT), 38.0 {−CH($\underline{C}H_2$Ph), −ve DEPT}, 24.5 (−$\underline{C}H_2$CH$_3$, −ve DEPT), 13.3 (-OCH$_2\underline{C}$H$_3$), 13.0 (−CH$_2\underline{C}$H$_3$).

4.3.7. 1-Propyl-1′-N-{para-(ferrocenyl)-benzoyl}-glycine-L-alanine ethyl ester **10**

Glycine-L-alanine ethyl ester hydrochloride (0.13 g, 0.72 mmol) was used as a starting material. The crude product was purified by column chromatography {eluant 1:1 hexane: ethyl acetate} yielding the title compound as a red oil (0.08 g, 22%), $E^{\circ\prime} = 30$ mV (vs Fc/Fc$^+$); $[\alpha]_D^{20} = -14^\circ$ (*c* 0.1, EtOH).

Mass spectrum: $[M + Na]^+$ found: 527.1632.

$C_{26}H_{30}N_2O_4FeNa$ requires: 527.1609.

I.R. υ_{max} (KBr): 3295 (NH), 1743 (C=O$_{ester}$), 1636 (C=O$_{amide}$), 1608 (C=O$_{amide}$) cm^{-1};

UV−Vis λ_{max} EtOH: 360, 451 nm.

^{1}H NMR (400 MHz) δ (DMSO-d_6): 8.68 (1H, t, $J = 6$ Hz, −CONH−), 8.41 (1H, d, $J = 7.0$ Hz, −CONH−), 7.78 (2H, d, $J = 7.4$ Hz, ArH), 7.64 (2H, d, $J = 7.4$ Hz, ArH), 4.82 (2H, s, *ortho* on η^5-C_5H_4-benzoyl), 4.37 (2H, s, *meta* on η^5-C_5H_4-benzoyl), 4.3−4.25 (1H, m, −$\underline{C}$HCH$_3$), 4.08 (2H, q, $J = 6.8$ Hz, −O$\underline{C}H_2$CH$_3$), 3.95−3.88 {6H, m, (η^5-C_5H_4-alkyl), (−NH$\underline{C}H_2$CO−)}, 2.07 (2H, t, $J = 7.6$ Hz, −$\underline{C}H_2$CH$_2$CH$_3$), 1.4−1.27 {5H, m, (−$\underline{C}H_2\underline{C}H_2CH_3$), (−CH$\underline{C}H_3$)}, 1.18 (3H, t, $J = 6.8$ Hz, −OCH$_2\underline{C}$H$_3$), 0.74 (3H, t, $J = 7.2$ Hz, −CH$_2$CH$_2\underline{C}$H$_3$).

^{13}C NMR (100 MHz) δ (DMSO-d_6): 171.0 (C=O), 169.2 (C=O), 168.6 (C=O), 141.5 (C_q), 132.8 (C_q), 128.4, 124.2, 90.3 (C_{ipso} η^5-$\underline{C}_5H_4$-alkyl), 83.0 (C_{ipso} η^5-$\underline{C}_5H_4$-benzoyl), 71.5 (C_{meta} η^5-$\underline{C}_5H_4$-benzoyl), 68.7 (C_{meta} η^5-$\underline{C}_5H_4$-alkyl), 68.2 (C_{ortho} η^5-$\underline{C}_5H_4$-alkyl), 67.9 (C_{ortho} η^5-$\underline{C}_5H_4$-benzoyl), 62.4 (−O$\underline{C}H_2$CH$_3$, −ve DEPT), 49.6 (−$\underline{C}$HCH$_3$), 43.0 (−NH$\underline{C}H_2$CHO−, −ve DEPT), 32.0 (−$\underline{C}H_2$CH$_2$CH$_3$, −ve DEPT), 22.8 (−CH$_2\underline{C}$H$_2$CH$_3$, −ve DEPT), 17.6 (−CH$\underline{C}$H$_3$), 14.8 (−OCH$_2\underline{C}$H$_3$), 13.9 (−CH$_2$CH$_2\underline{C}$H$_3$).

4.3.8. 1-Propyl-1′-N-{para-(ferrocenyl)-benzoyl}-glycine-leucine ethyl ester **11**

Glycine-L-leucine ethyl ester hydrochloride (0.16 g, 0.72 mmol) was used as a starting material. The crude product was purified by column chromatography {eluant 1:1 hexane: ethyl acetate} and recrystallisation from hexane: ethyl acetate yielded the desired product as an orange solid (0.06 g, 15%), m.p 76–78 °C; $E^{\circ\prime}$ = 20 mV (vs Fc/Fc^{+}); $E^{\circ\prime}$ = 20 mV (vs Fc/Fc^{+}); $[\alpha]_D^{20} = -15^{\circ}$ (*c* 0.1, EtOH).

Mass spectrum: $[M + Na]^{+}$ found: 569.2197.

$C_{30}H_{38}N_2O_4FeNa$ requires: 569.2181.

I.R. υ_{max} (KBr): 3275 (NH), 1749 (C=O_{ester}), 1629 (C=O_{amide}), 1613 (C=O_{amide}) cm^{-1}.

UV–Vis λ_{max} EtOH: 360, 451 nm.

^{1}H NMR (400 MHz) δ (DMSO-d_6): 8.67 (1H, t, J = 6 Hz, –CONH–), 8.33 (1H, d, J = 7.6 Hz, –CONH–), 7.85 (2H, d, J = 7.6 Hz, ArH), 7.66 (2H, d, J = 7.6 Hz, ArH), 4.88 (2H, s, *ortho* on η^5-C_5H_4-benzoyl), 4.37 (2H, s, *meta* on η^5-C_5H_4-benzoyl), 4.33–4.27 {1H, m, –CH($CH_2CH(CH_3)_2$)}, 4.14 (2H, q, J = 7.2 Hz, –OCH_2CH_3), 3.97–3.86 {6H m, (η^5-C_5H_4-alkyl), (–NHCH_2CO–)}, 2.0 (2H, t, J = 7.6 Hz, –$CH_2CH_2CH_3$), 1.69–1.45 {3H, m, –CH($CH_2CH(CH_3)_2$)}, 1.40–1.27 (2H, m, –$CH_2CH_2CH_3$), 1.15 (3H, t, J = 7.2 Hz, –OCH_2CH_3), 0.91–0.83 {6H, m, –CH($CH_2CH(CH_3)_2$)}, 0.72 (3H, t, J = 7.2 Hz, –$CH_2CH_2CH_3$).

^{13}C NMR (100 MHz) δ (DMSO-d_6): 171.4 (C=O), 169.4 (C=O), 168.1 (C=O), 140.1 (C_q), 131.8 (C_q), 129.4, 126.2, 89.8 (C_{ipso} η^5-C_5H_4-alkyl), 84.1 (C_{ipso} η^5-C_5H_4-benzoyl), 71.2 (C_{meta} η^5-C_5H_4-benzoyl), 69.9 (C_{meta} η^5-C_5H_4-alkyl), 68.5 (C_{ortho} η^5-C_5H_4-alkyl), 67.9 (C_{ortho} η^5-C_5H_4-benzoyl), 62.8 (–OCH_2CH_3, –ve DEPT), 52.1 {–CH($CH_2CH(CH_3)_2$)}, 42.5 (–NHCH_2CHO–, –ve DEPT), 40.0 {–CH($CH_2CH(CH_3)_2$), –ve DEPT}, 32.0 (–$CH_2CH_2CH_3$, –ve DEPT), 26.4 {–CH($CH_2CH(CH_3)_2$)}, 23.5 (–$CH_2CH_2CH_3$, –ve DEPT), 23.7 {–CH($CH_2CH(CH_3)_2$)}, 22.4 {–CH($CH_2CH(CH_3)_2$)}, 14.9 (–OCH_2CH_3), 14.0 (–$CH_2CH_2CH_3$).

4.3.9. 1-Propyl-1′-N-{para-(ferrocenyl)-benzoyl}-glycine-L-phenylalanine ethyl ester **12**

Glycine-L-phenyalanine ethyl ester hydrochloride (0.18 g, 0.72 mmol) was used as a starting material. The crude product was purified by column chromatography {eluant 1:1 hexane: ethyl acetate} and recrystallisation from hexane: ethyl acetate yielded the desired product as an orange solid (0.06 g, 14%), m.p 74–75 °C.

$[\alpha]D^{20} = +11^{\circ}$ (*c* 0.1, EtOH).

Mass spectrum: $[M + Na]^{+}$ found: 603.2046.

$C_{33}H_{36}N_2O_4FeNa$ requires: 603.2034.

I.R. υ_{max} (KBr): 3292 (NH), 1737 (C=O_{ester}), 1630 (C=O_{amide}), 1609 (C=O_{amide}) cm^{-1}.

UV–Vis λ_{max} EtOH: 360, 452 nm.

^{1}H NMR (400 MHz) δ (DMSO-d_6): 8.56 (1H, t, J = 6.0 Hz, –CONH–), 8.27 (1H, d, J = 7.6 Hz, –CONH–), 7.71 (2H, d, J = 8.0 Hz, ArH), 7.51 (2H, d, J = 8.0 Hz, ArH), 7.18–7.12 {5H, m, –CH(CH_2Ph)}, 4.74 (2H, s, *ortho* on η^5-C_5H_4-benzoyl), 4.4–4.38 {1H, m, –CH(CH_2Ph)}, 4.27 (2H, s, *meta* on η^5-C_5H_4-benzoyl), 3.96 (2H, q, J = 7.2 Hz, –OCH_2CH_3), 3.84–3.79 {6H, m, (η^5-C_5H_4-alkyl), (–NHCH_2CO–)}, 2.92–2.85 {2H, m, –CH(CH_2Ph)}, 1.9 (2H, t, J = 7.6 Hz, –$CH_2CH_2CH_3$), 1.3–1.29 (2H, m, –$CH_2CH_2CH_3$), 1.04 (3H, t, J = 7.2 Hz, –OCH_2CH_3), 0.66 (3H, t, J = 7.2 Hz, –$CH_2CH_2CH_3$).

^{13}C NMR (100 MHz) δ (DMSO-d_6): 169.8 (C=O), 168.7 (C=O), 166.3 (C=O), 140.6 (C_q), 137.0 (C_q), 134.1 (C_q), 128.8, 128.5, 126.2, 124.2, 124.1, 91.5 (C_{ipso} η^5-C_5H_4-alkyl), 83.1 (C_{ipso} η^5-C_5H_4-benzoyl), 70.0 (C_{meta} η^5-C_5H_4-benzoyl), 68.8 (C_{meta} η^5-C_5H_4-alkyl), 68.0 (C_{ortho} η^5-C_5H_4-alkyl), 66.2 (C_{ortho} η^5-C_5H_4-benzoyl), 60.1 (–OCH_2CH_3, –ve DEPT), 52.8 (–CH(CH_2Ph), 42.8 (–NHCH_2CHO–, –ve DEPT), 36.4 {–CH(CH_2Ph), –ve DEPT}, 31.0 (–$CH_2CH_2CH_3$, –ve DEPT), 20.6 (–$CH_2CH_2CH_3$, –ve DEPT), 14.4 (–OCH_2CH_3), 14.0 (–$CH_2CH_2CH_3$).

4.4. General procedure for in vitro cytotoxicity assays

4.4.1. Biological assays – cell line

H1299 was obtained from the American Tissue Culture Centre (ATCC). The cell line was grown in RPMI-1640 supplemented with 10% foetal calf serum (FCS) at 37 °C in a 5% CO_2 humidified chamber.

4.4.2. In vitro proliferation assays

Cells in the exponential phase of growth were harvested by trypsinisation and a cell suspension of 1×10^4 cells per ml was prepared in fresh culture medium. The cell suspension (100 μL) was added to a flat bottom 96-well plate (Costar, 3599), plates were agitated gently in order to ensure even dispersion of cells over the surface of the wells, and then cells were incubated for an initial 24 h in a 37 °C, 5% CO_2 incubator, to allow cell attachment to the wells. A 10 mM stock solution of a test sample was prepared in dimethyl sulfoxide; dilute solutions of the test sample were prepared at 2× final concentration by spiking the cell culture medium with a calculated amount of the stock solution. 100 μL aliquot of each dilute solution was added to each well of the plate, the plate was gently agitated, and then incubated at 37 °C, 5% CO_2 for 5–6 days, until cell confluency reached 80–90%. Assessment of cell survival in the presence of the compounds **4**–**12** was determined by the acid phosphatase assay [31]. The percentage cell growth in the presence of each compound was determined relative to the control cells. The concentration of compounds causing a 50% growth inhibition (IC_{50} of the compound) was determined using Calcusyn (Biosoft, UK).

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