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The synthesis, structural characterization and *in vitro* anti-cancer activity of novel *N*-(3-ferrocenyl-2-naphthoyl) dipeptide ethyl esters and novel *N*-(6-ferrocenyl-2-naphthoyl) dipeptide ethyl esters

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ABSTRACT

N-(3-ferrocenyl-2-naphthoyl) dipeptide esters (**5–7**) and *N*-(6-ferrocenyl-2-naphthoyl) dipeptide esters (**8–10**) were prepared by coupling either 3-ferrocenylnaphthalene-2-carboxylic acid **2** or 6-ferrocenylnaphthalene-2-carboxylic acid **4** to the dipeptide ethyl esters GlyAla(OEt) (**5, 8**), AlaGly(OEt) (**6, 9**), and AlaAla(OEt) (**7, 10**) 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). *In vitro*, the cytotoxic effects of compounds **5–10** show improvements over the corresponding *N*-(ferrocenyl)benzoyl derivatives, with IC_{50} values against the H1299 lung cancer cells ranging from 1.2 μM to 8.0 μM. *N*-(6-ferrocenyl-2-naphthoyl)-glycine-L-alanine ethyl ester **8** was found to be the most active derivative of the naphthoyl series so far, displaying an IC_{50} value of 1.3 ± 0.1 μM. This value is slightly lower than that found for the clinically employed anti-cancer drug cisplatin (IC_{50} = 1.5 ± 0.1 μM against H1299).

1. Introduction

Lung cancer is the leading cause of cancer death worldwide (1.4 million deaths per year), with the two main categories being small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [1]. It is estimated for 2007 that 31% of male and 26% of female cancer related deaths in America can be ascribed to lung cancer [2]. Fortunately, many chemotherapeutic agents are effective against both types of lung cancer, with the most active and most commonly used drugs being the platinum(II)-based anti-cancer agents, cisplatin and carboplatin [3]. However, problems with toxicity, and harsh side effects during administration, together with acquired drug resistance problems has prompted the search for alternative anti-cancer drugs with better pharmacological profiles whilst retaining therapeutic efficacy. Some of the most promising novel non-platinum anti-cancer agents are emerging from the field of bioorganometallic chemistry.

Bioorganometallic chemistry is a field devoted to the synthesis and study of organometallic species of biological and medical interest [4]. Notably, the field of medicinal chemistry has benefitted considerably from the incorporation of organometallic moieties into potential drug molecules, with ferrocene receiving particular interest due to its aromatic character, redox properties, stability and low toxicity [5]. A comprehensive review on the bioorganometallic chemistry of ferrocene has been published [6]. Examples of biologically active ferrocenyl derivatives encompass anti-bacterial [7–9], anti-fungal [10,11], anti-malarial [12–15] and anti-cancer therapeutic agents including ferrocifen [16–25].

Our work is focused on the synthesis of ferrocene derivatives incorporating various natural amino acids and peptide derivatives [20,22,26–35]. In particular, several *N*-(ferrocenyl)benzoyl dipeptide ethyl esters have been found to possess good anti-cancer activity *in vitro* against the human lung carcinoma cell line H1299, the most active of which is the *N*-{*ortho*-(ferrocenyl)benzoyl}-glycine-L-alanine ethyl ester (IC_{50} = 5.3 ± 0.4 μM) [20,22]. These *N*-(ferrocenyl)benzoyl dipeptide esters consist of three components, namely: (i) an electroactive core, (ii) a conjugated linker that lowers the oxidation potential of the ferrocene moiety and (iii) a peptide derivative that can interact with other biomolecules *via* secondary interactions such as hydrogen bonding. In order to improve the cytotoxicity of these derivatives, we are currently modifying the conjugated linker and conducting variations of the peptide chain. Herein, we report the synthesis and structural

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characterization of novel *N*-(3-ferrocenyl-2-naphthoyl) dipeptide ethyl esters (**5–7**) and novel *N*-(6-ferrocenyl-2-naphthoyl) dipeptide ethyl esters (**8–10**). In addition, we present the *in vitro* anticancer activity of compounds **5–10** against the human lung carcinoma cell line H1299.

2. Results and discussion

2.1. Synthesis

2.1.1. Synthesis of *N*-(3-ferrocenyl-2-naphthoyl) dipeptide ethyl esters (**5–7**)

The preparation of 3-ferrocenylnaphthalene-2-carboxylic acid **2** employed conventional diazonium salt chemistry. Treatment of the methyl-3-aminonaphthalene-2-carboxylate with sodium nitrite in the presence of hydrochloric acid yielded the diazonium salt which was then reacted with ferrocene *in situ* to furnish the methyl-3-ferrocenylnaphthalene-2-carboxylate **1** as an orange solid. Prior to coupling with the C-protected dipeptide ethyl esters, the methyl ester group was cleanly cleaved by treatment with 10% sodium hydroxide to yield 3-ferrocenylnaphthalene-2-carboxylic acid **2**. The ^{1}H NMR spectrum showed signals for the aromatic ring protons at δ 8.33 (s), δ 8.00 (d), δ 7.99 (s), δ 7.95 (d), δ 7.57 (t) and δ 7.52 (t), integrating for one proton each, characteristic of a 2,3-disubstituted naphthalene ring system. The carboxylic acid proton was present at δ 12.8. The ferrocenyl *ortho* and *meta* protons on the (η^5-C_5H_4) ring were observed at δ 4.70 and δ 4.34, respectively, and an intense signal was present at δ 4.10 for the (η^5-C_5H_5) ring. 3-Ferrocenylnaphthalene-2-carboxylic acid **2** was coupled to the free *N*-terminal dipeptide ethyl esters of GlyAla(OEt) (**5**), AlaGly(OEt) (**6**), and AlaAla(OEt) (**7**) under basic conditions using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt) in dichloromethane (Scheme 1). The *N*-(3-ferrocenyl-2-naphthoyl) dipeptide ethyl esters (**5–7**) were obtained as orange crystals following purification by silica gel column chromatography. The yields obtained ranged from 28% to 55% and all compounds gave spectroscopic data in accordance with the proposed structures. The *N*-(3-ferrocenyl-2-naphthoyl) dipeptide ethyl esters (**5–7**) were characterized by a combination of ^{1}H NMR, ^{13}C NMR, DEPT-135 and ^{1}H-^{13}C COSY (HMQC) spectroscopy and cyclic voltammetry (CV). In addition, electrospray ionization mass spectrometry (ESI) in conjunction with tandem mass spectrometry (MS/MS) was employed in the analysis.

2.1.2. Synthesis of *N*-(6-ferrocenyl-2-naphthoyl) dipeptide ethyl esters (**8–10**)

6-Ferrocenylnaphthalene-2-carboxylic acid (**4**) was prepared in an analogous manner to compound **2** using methyl-6-aminonaphthalene-2-carboxylate (**3**) as starting material. Thus, prior to coupling with the C-protected dipeptide ethyl esters, methyl-6-ferrocenylnaphthalene-2-carboxylate (**3**) was treated with 10% sodium hydroxide, which cleanly cleaved the methyl ester to furnish 6-ferrocenylnaphthalene-2-carboxylic acid (**4**). The ^{1}H NMR spectrum showed signals for the aromatic ring protons at δ 8.57 (1H, s), δ 8.07 (1H, s), δ 8.03 (1H, d), δ 7.94 (2H, s) and δ 7.83 (1H, dd), characteristic of a 2,6-disubstituted naphthalene ring system. The carboxylic acid proton was present at δ 12.8. The ferrocenyl *ortho* and *meta* protons on the (η^5-C_5H_4) ring were observed at δ 4.97 and δ 4.45, respectively, and an intense signal was present at δ 4.04 for the (η^5-C_5H_5) ring. The free *N*-terminal dipeptide ethyl esters of GlyAla(OEt) (**8**), AlaGly(OEt) (**9**), and AlaAla(OEt) (**10**) were coupled to 6-ferrocenylnaphthalene-2-carboxylic acid (**4**) using EDC and HOBt in the presence of excess triethylamine in dichloromethane (Scheme 2). Purification by column chromatography furnished the pure products in yields of 29–79% and all compounds gave spectroscopic data in accordance with the proposed structures. The *N*-(6-ferrocenyl-2-naphthoyl) dipeptide ethyl esters **8–10** were characterized by a combination of ^{1}H NMR, ^{13}C NMR, DEPT-135 and ^{1}H-^{13}C COSY (HMQC) spectroscopy and cyclic voltammetry (CV). Electrospray ionization mass spectrometry (ESI) in conjunction with tandem mass spectrometry (MS/MS) was also employed in the analysis.

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

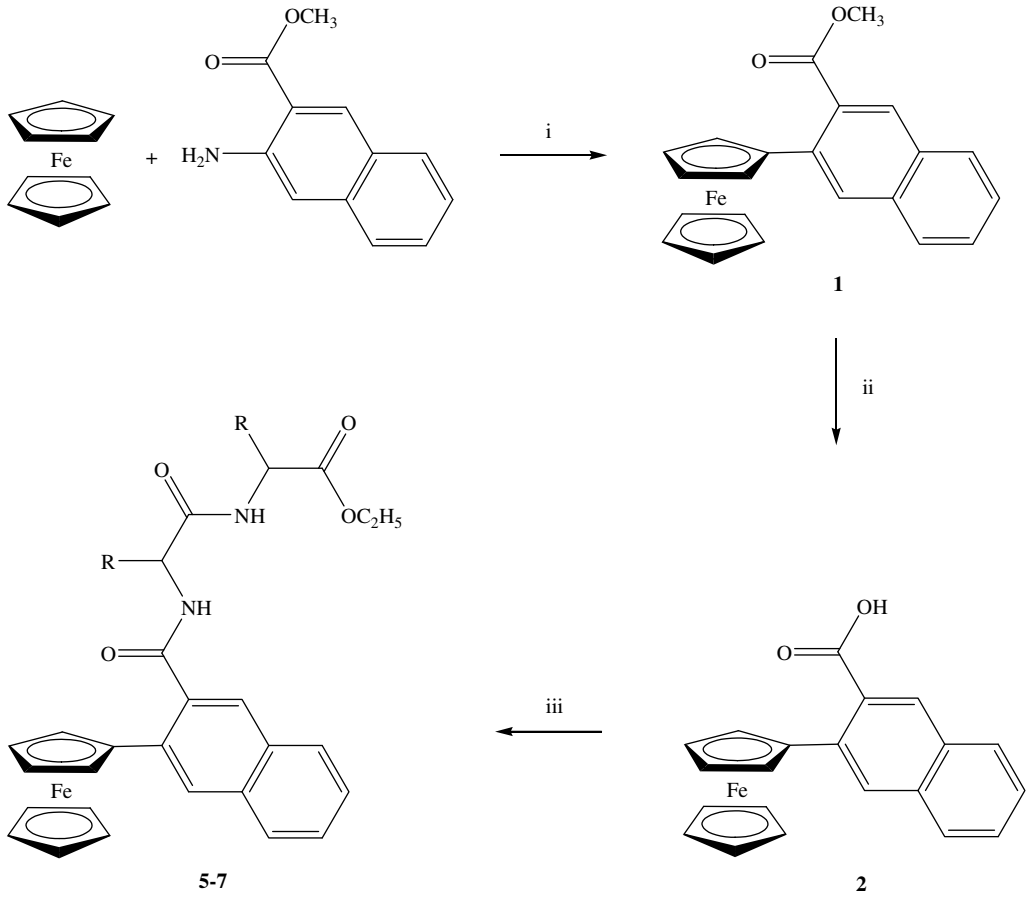
All the proton and carbon chemical shifts for compounds **5–10** were unambiguously assigned by a combination of DEPT-135 and ^{1}H-^{13}C COSY (HMQC). The ^{1}H and ^{13}C NMR spectra for compounds **5–10** showed peaks in the ferrocene region characteristic of a monosubstituted ferrocene moiety. The protons in the *ortho* position of the (η^5-C_5H_4) ring appear in the region δ 4.74–4.96, the *meta* protons occur in the range δ 4.33–4.45, while the unsubstituted (η^5-C_5H_5) ring appears between δ 4.01 and δ 4.11.

For example in the ^{1}H NMR spectrum of *N*-(6-ferrocenyl-2-naphthoyl)-L-alanine-glycine ethyl ester **9** (obtained in DMSO-d_6) the unsubstituted (η^5-C_5H_5) ring appears as a singlet at δ 4.05 whereas the *meta* and *ortho* protons on the (η^5-C_5H_4) ring are present at δ 4.45 and δ 4.96, respectively. Two amide protons appear as a doublet at δ 8.67 and a triplet at δ 8.38, with each signal integrating for one proton. These can be assigned as the L-alanine amide proton and the glycine amide proton, respectively. The signals in the aromatic region confirm the presence of six protons, which appear between δ 7.82 and δ 8.48. The quintet at δ 4.60, which integrates for one proton, represents the α-proton of the L-alanine residue, whilst a quartet at δ 4.12 integrating for two protons corresponds to the methylene protons of the ethyl ester. Two doublets of doublets integrating for one proton each, appear at δ 3.89 and δ 3.82 and can be assigned to the diastereotopic methylene protons of the glycine residue. The methyl group of the L-alanine moiety and the methyl group of the ethyl ester of the dipeptide appear as a doublet at δ 1.41 and a triplet at δ 1.19, respectively.

The ^{13}C NMR spectra of compounds **5–10** show signals in the region δ 66.6 to δ 84.7 indicative of a monosubstituted ferrocene unit. The *ipso* carbon of the (η^5-C_5H_4) ring appears in the narrow range of δ 84.0 to δ 84.7. This signal is absent from the DEPT-135 spectra. The carbon atoms of the aromatic ring are non-equivalent and therefore ten signals are visible in the region of δ 126.0–135.1 for compounds **5–7** and ten signals are observed between δ 122.7 and δ 138.8 for compounds **8–10**. The quaternary carbons of the aromatic ring and the methylene carbon atoms of derivatives **5–10** were identified by DEPT-135. A complete assignment of the ^{1}H and ^{13}C spectra of *N*-(6-ferrocenyl-2-naphthoyl)-L-alanine-glycine ethyl ester **9** is presented in Table 1.

2.3. Mass spectrometry

Soft ionization techniques such as electrospray ionization (ESI) mass spectrometry permit the analysis of thermolabile and non-volatile analytes [36]. Electrospray ionization (ESI) mass spectrometry was employed in the analysis of compounds **5–10** 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. Similar observations were made in the analysis of the ferrocenyl benzoyl amino acid and dipeptide ester derivatives [29–35]. An important diagnostic fragment ion at *m/z* $[M-65]^+$ was only observed in the mass spectra of the *N*-(3-ferrocenyl-2-naphthoyl) derivatives **5–7**. This corresponds to the loss of the unsubstituted (η^5-C_5H_5) ring. The formation of this fragment ion, which was also observed for the *N*-*ortho*-(ferrocenyl)benzoyl dipeptide derivatives, is possibly due

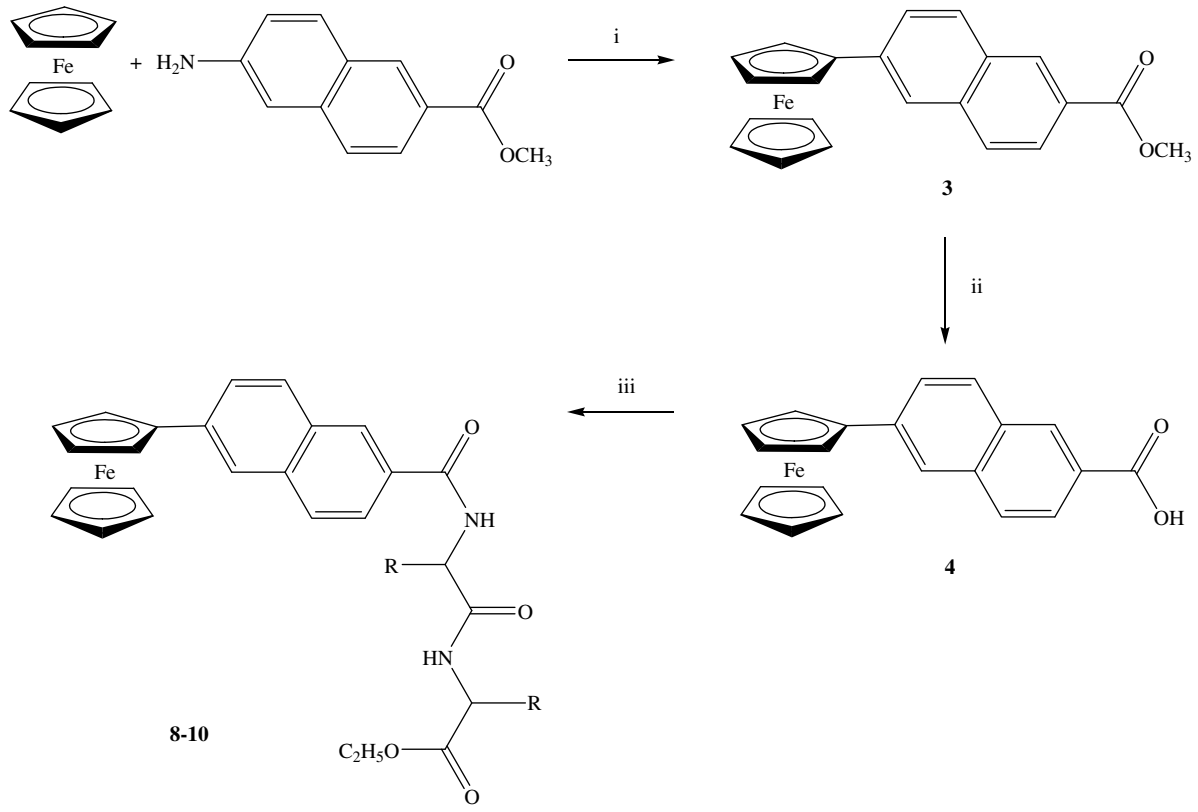


Scheme 1. Synthesis of *N*-(3-ferrocenyl-2-naphthoyl) dipeptide ethyl esters (**5–7**), (i) $NaNO_2$, HCl, 5 °C, (ii) NaOH/MeOH, (iii) EDC, HOBt, triethylamine, dipeptide ethyl ester (GlyAla **5**, AlaGly **6**, AlaAla **7**).

to steric hindrance between the *ortho* substituted naphthoyl substituents and the unsubstituted (η^5-C_5H_5) ring. However, for the *N*-(6-ferrocenyl-2-naphthoyl) derivatives **8–10** sequence specific fragment ions were not observed or were of low intensity in the mass spectra. Tandem mass spectrometry was employed in the analysis of *N*-(3-ferrocenyl-2-naphthoyl)-glycine-L-alanine ethyl ester (**5**) and *N*-(6-ferrocenyl-2-naphthoyl)-glycine-L-alanine ethyl ester (**8**) (Fig. 1). The fragmentation pattern is totally different for the two compounds. The major product ion in the MS/MS spectrum of *N*-(3-ferrocenyl-2-naphthoyl)-glycine-L-alanine ethyl ester **5** is at *m/z* 447 corresponding to the loss of the unsubstituted (η^5-C_5H_5) ring. The product ion at *m/z* 373 is due to $[M-65-H_2O\text{-}Fe]^+$. Sequence specific ions were observed in the MS/MS spectrum of compound **8** confirming that the glycine residue was linked to the naphthoyl spacer group. Important product ions were present at *m/z* 311, *m/z* 339, *m/z* 367 and *m/z* 395 (Fig. 2). The product ions at *m/z* 311 and *m/z* 339 correspond to the ferrocenylnaphthyl and ferrocenylnaphthoyl subunits, respectively (Fig. 2). However, the expected a_1 and b_1 product ions at *m/z* 368 and *m/z* 396 were not observed, instead a_1-1 and b_1-1 product ions were observed at *m/z* 367 and *m/z* 395, respectively. Obviously a hydrogen atom has also been lost during the fragmentation process. This is unusual as these a_1 and b_1 fragment ions are usually produced without loss of a hydrogen atom [37]. The formation of a_1-1 and b_1-1 ions in the mass spectra of *N*-{*para*-(ferrocenyl)benzoyl}-glycine-L-alanine ethyl ester was investigated by tandem mass spectrometry and deuterium labelling studies. The results showed that b_1-1 product ions arise from the loss of a hydrogen atom attached to the nitrogen and not to the α-carbon of the glycine residue [38].

2.4. Electrochemistry

The CV curves for compounds **5–10** exhibit quasi-reversible behaviour similar to the Fc/Fc^+ redox couple. The $E^{\circ\prime}$ (oxidation potential) values for the *N*-(3-ferrocenyl-2-naphthoyl) derivatives **5–7** were in the range of 5–10 mV (versus Fc/Fc^+), whilst the *N*-(6-ferrocenyl-2-naphthoyl) derivatives **8–10** showed values in the 42–56 mV range (versus Fc/Fc^+). The values for compounds **8–10** are comparable with those reported for the *N*-(ferrocenyl)benzoyl dipeptide derivatives (46–59 mV versus Fc/Fc^+), which are significantly lower than ferrocenoyl dipeptide ester derivatives [20,22]. For example, Fc-Ala-Ala-OMe, was reported as $E^{\circ\prime} = 230$ mV (versus Fc/Fc^+) [39]. This difference is explicable in terms of substituent effects, the amide carboxyl group is strongly electron withdrawing and as a result ferrocenoyl dipeptide ester derivatives are more difficult to oxidise. Inserting an aromatic group between the ferrocene moiety and the peptide chain provides extended conjugation to the π-electrons of the Cp rings making these derivatives easier to oxidize with respect to the ferrocenoyl derivatives. The substantial decrease in oxidation potential for derivatives **5–7** is due to steric effects around the 2,3-disubstituted naphthyl ring. The *ortho*-relationship between the two substituents imposes a steric restriction on the bulky ferrocene group, forcing it to adopt a position out-of-plane with regard to the naphthyl ring. Consequently, efficient overlap between the naphthyl p-orbitals and the p-orbitals of the Cp rings is prevented, thus diminishing the extent of π-orbital conjugation between these two moieties. Indeed, since the $E^{\circ\prime}$ values obtained for compounds **5–7** are so close to that of the Fc/Fc^+ redox couple,



Scheme 2. Synthesis of *N*-(6-ferrocenyl-2-naphthoyl) dipeptide ethyl esters (**8–10**), (i) $NaNO_2$, HCl, 5 °C, (ii) NaOH/MeOH, (iii) EDC, HOBt, triethylamine, dipeptide ethyl ester (GlyAla **8**, AlaGly **9**, AlaAla **10**).

it can be surmised that only minimal conjugation is occurring. This is further supported by the UV–Vis spectra of **5–7**, in which the absorbance due to the π–π^* transition of the aromatic spacer group is too weak to be observed. In contrast, for compounds **8–10** an intense absorbance due to this π–π^* transition is observed at 375 nm.

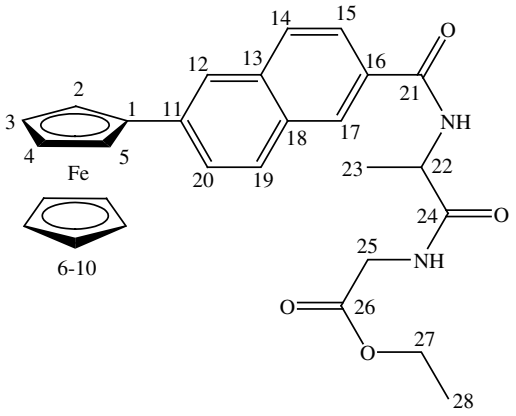
2.5. In vitro anti-cancer activity of *5–10*

The *in vitro* cytotoxicity of the *N*-(ferrocenyl)naphthoyl derivatives **5–10** against the human lung carcinoma cell line H1299 (highly invasive/super invasive) was evaluated by the acid phosphatase assay as previously described [40]. 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 *p*-nitrophenyl phosphate substrate converting it to *p*-nitrophenol which in the presence of strong alkali can be quantified colorimetrically. The cells were treated with the *N*-(ferrocenyl)naphthoyl derivatives **5–10** at a range of concentrations (from 1 μM to 100 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 compounds **5–10** are depicted in Fig. 3 and Table 2 displays the IC_{50} values for derivatives **5–10** and several other compounds.

It can be seen from Fig. 3 that the *N*-(ferrocenyl)naphthoyl derivatives **5–10** all exert a cytotoxic effect on the human lung carcinoma cell line H1299. Indeed, all six derivatives have an IC_{50} value that is lower than 10 μM. The *in vitro* cytotoxicity of the platinum(II)-based anti-cancer drug carboplatin was also evaluated against the H1299 cell line, and was found to have an IC_{50} value of 10.0 ± 1.6 μM (Table 2). Thus, compounds **5–10** are more cytotoxic *in vitro* than the clinically employed anti-cancer drug carboplatin. In addition, compounds **5–10** display improved bioactivity in comparison to the corresponding *N*-(ferrocenyl)benzoyl dipeptide ethyl esters. We have previously reported that *N*-{*meta*-(ferrocenyl)benzoyl}-L-alanine-glycine ethyl ester has an IC_{50} value of 26 μM (RSD 20%), whilst the corresponding *ortho* analog has an IC_{50} value of 21 μM (RSD 20%) [22]. Preliminary results show that cytotoxicity of **6** and **9** are *ca.* 3 times higher than the benzoyl analogues, the IC_{50} values being 6.9 ± 1.5 μM and 7.8 ± 0.2 μM, respectively. Replacing the terminal glycine residue with L-alanine to give the L-alanine-L-alanine ethyl ester derivatives **7** and **10**, does not alter the cytotoxicity to any great extent for the *N*-(3-ferrocenyl-2-naphthoyl) derivatives, the IC_{50} value for **7** being 7.8 ± 0.2 μM. Interestingly, for the *N*-(6-ferrocenyl-2-naphthoyl) derivatives, this simple variation in the peptide chain doubles the cytotoxic effect, with compound **10** showing an IC_{50} value of 3.7 ± 0.6 μM.

The most active benzoyl analogue we have reported to date is the *N*-{*ortho*-(ferrocenyl)benzoyl}-glycine-L-alanine ethyl ester, the IC_{50} being 5.3 ± 0.4 μM [22]. As for all of the *N*-(ferrocenyl)naphthoyl derivatives, the glycine-L-alanine ethyl esters **5** and **8**, display improved *in vitro* anti-cancer activity in comparison to the benzoyl analogues against the H1299 cancer cells. Compound **5** is also around two times more active than the other *N*-(3-ferrocenyl-2-naphthoyl) derivatives **6** and **7** with the IC_{50} value for **5** being 3.7 ± 0.7 μM. The same trend is observed for **8**, which displays a cytotoxicity *ca.* 3 times higher than **10** and *ca.* 6 times higher than **9**. *N*-(6-ferrocenyl-2-naphthoyl)-glycine-L-alanine ethyl ester **8** has an IC_{50} value of 1.3 ± 0.1 μM and is subsequently the most active anti-cancer derivative that we have synthesized

Table 1
^{1}H and ^{13}C spectroscopic data for compound **9**.



Site	^{1}H NMR	^{13}C NMR	HMQC
1		84.1	
2, 5	4.96		66.6
3, 4	4.45		69.4
6–10	4.05		69.5
11		138.8	
12	8.06		122.7
13		134.5[a#]	
14	7.94		127.2
15	7.94		127.7
16		130.4[b#]	
17	8.48		128.7
18		130.6[c#]	
19	7.94		124.8
20	7.82		125.9
21		166.1[a*]	
22	4.60		48.8
23	1.41		17.9
24		169.7[b*]	
25	3.89, 3.82		40.7
26		173.0[c*]	
27	4.12		60.3
28	1.19		14.0

[a–c] Signals may be reversed.

thus far. Interestingly, the *in vitro* cytotoxicity of cisplatin was also evaluated against the H1299 cell line, for which an IC_{50} value of 1.5 ± 0.1 μM was obtained (Table 2). Thus compound **8** is as effective *in vitro* against human H1299 lung carcinoma cells as the clinical drug of choice, cisplatin.

From these results it can be seen that although the order of the amino acids in the dipeptide chain is crucial for activity, the order of importance varies according to the substitution around the conjugated linker. Thus for the *N*-(3-ferrocenyl-2-naphthoyl) derivatives **5–7** a general trend in cytotoxicitiy of GlyAla < AlaGly ~ AlaAla is observed, whereas for the *N*-(6-ferrocenyl-2-naphthoyl) derivatives **8–10** the order observed is GlyAla < AlaAla < AlaGly. We have previously postulated a mechanism of action originating from the low redox potential of these derivatives and their ability to catalyze the generation of reactive oxygenated species (ROS), under physiological conditions, that can oxidatively modify cellular components (*e.g.* DNA) *etc.* via a Fenton-type reaction [20]. However, no clear correlation can be drawn between the $E^{\circ\prime}$ values and the IC_{50} values of compounds **5–10**, since derivatives **5–7** possess considerably lower $E^{\circ\prime}$ values than **8–10** but are not more active *in vitro*. This *per se* does not eliminate the possibility that the redox potential of these derivatives plays a role in cytotoxicity, as the $E^{\circ\prime}$ values are still relatively low when compared to ferrocenoyl dipeptide esters. There is also convincing evidence that ferrocifen exerts its cytotoxic effect via redox processes other than the Fenton-type reaction [41]. However, it does suggest that some other feature of these derivatives may also play an important role, *e.g.* hydrogen bonding ability, lipophilicity, ability to interact with DNA in other ways. It is plausible that these polyaromatic derivatives could intercalate with DNA, as observed for many polyaromatic drugs including the anthracycline class of chemotherapeutics. The hydrogen bond donor and acceptor atoms present in the peptide side chain, could then interact with the nucleotide bases positioned in the centre of the helix. Although the bulky ferrocene substituent (10.5 Å) is too large to fit into the major groove of DNA (depth = 8.5 Å), the low oxidation potential of these derivatives supports the possibility that the ferrocene moiety in its oxidized Fe^{3+} state, could interact with the negatively charged phosphate backbone positioned on the outside of the helix. Thus, it is possible that this series of naphthoyl derivatives could possess two distinct modes of action: the ability to cause oxidative damage to DNA through ROS production and the ability to intercalate with DNA, both of which would result in the disruption of cancer cell replication. Further studies regarding both modes of action must be undertaken, before any definitive conclusions may be drawn.

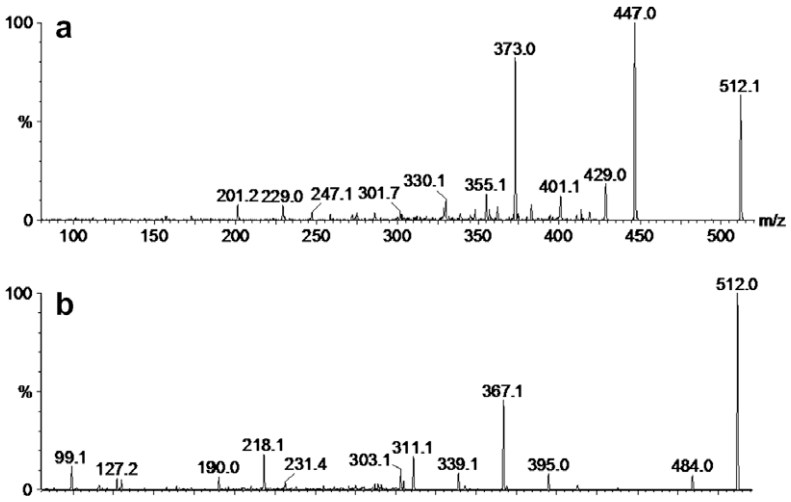


Fig. 1. MS/MS spectrum of (a) *N*-(3-ferrocenyl-2-naphthoyl)-glycine-L-alanine ethyl ester (**5**) and (b) *N*-(6-ferrocenyl-2-naphthoyl)-glycine-L-alanine ethyl ester (**8**).

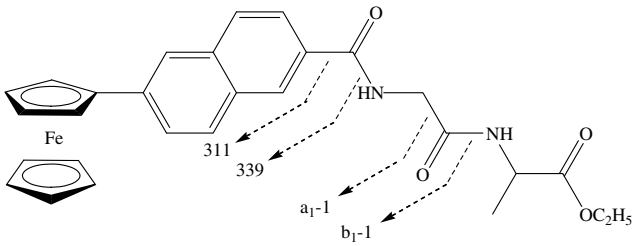


Fig. 2. Product ions observed in the MS/MS spectrum of compound **8**.

Table 2
IC_{50} values for compounds **5–10** and selected other compounds against human lung carcinoma cell line H1299.

Compound	IC_{50} value (μM)
Cisplatin	1.5 ± 0.1
Carboplatin	10.0 ± 1.6
N-{*ortho*-(ferrocenyl)benzoyl}-GlyAla(OEt)	5.3 ± 0.4
N-{*ortho*-(ferrocenyl)benzoyl}-AlaGly(OEt)	21 ± 3.0
N-(3-ferrocenyl-2-naphthoyl)-GlyAla(OEt) (**5**)	3.7 ± 0.7
N-(3-ferrocenyl-2-naphthoyl)-AlaGly(OEt) (**6**)	6.9 ± 1.5
N-(3-ferrocenyl-2-naphthoyl)-AlaAla(OEt) (**7**)	7.8 ± 0.2
N-(6-ferrocenyl-2-naphthoyl)-GlyAla(OEt) (**8**)	1.3 ± 0.1
N-(6-ferrocenyl-2-naphthoyl)-AlaGly(OEt) (**9**)	7.8 ± 0.2
N-(6-ferrocenyl-2-naphthoyl)-AlaAla(OEt) (**10**)	3.7 ± 0.6

3. Conclusions

In conclusion, the novel *N*-(3-ferrocenyl-2-naphthoyl) dipeptide ethyl esters (**5–7**) and the novel *N*-(6-ferrocenyl-2-naphthoyl) dipeptide ethyl esters (**8–10**) were synthesized and fully characterized by a range of NMR spectroscopic techniques, mass spectrometry and cyclic voltammetry. Compounds **5–10** were tested *in vitro* against the human lung carcinoma cell line H1299 and all derivatives showed IC_{50} values below 10 μM. In particular, *N*-(6-ferrocenyl-2-naphthoyl)-glycine-L-alanine ethyl ester (**8**) had an IC_{50} value of 1.3 ± 0.1 μM and is as active *in vitro* as the clinical employed anti-cancer drug cisplatin.

4. Experimental

4.1. General procedures

All chemicals were purchased from Sigma/Aldrich and used as received. Commercial grade reagents were used without further purification, however, solvents were purified prior to use. Melting points were determined using a Griffin melting point apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet 405 FT-IR and UV–Vis spectra on a Hewlett–Packard 8452A diode array UV–Vis spectrophotometer. NMR spectra were obtained on a Bruker AC 400 NMR spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. The ^{1}H and ^{13}C NMR chemical shifts (ppm) are relative to TMS and all coupling constants (*J*) are in Hertz. Electrospray ionization mass spectra were performed on a Micromass LCT mass spectrometer or a Brüker Daltonics Esquire-LC ion trap mass spectrometer. Tandem mass spectra were obtained on a Micromass Quattro *micro*™ LC-MS/MS triple quadrupole mass spectrometer.

Cyclic voltammograms were recorded in acetonitrile (Sigma–Aldrich), with 0.1 M tetrabutylammonium perchlorate (TBAP) as a supporting electrolyte, using a CH Instruments 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/Ag^+ reference electrode was used. The $E^{\circ\prime}$ values obtained for the test samples were referenced to the Fc/Fc^+ couple.

4.2. General procedure for the synthesis of the starting materials

4.2.1. Methyl-3-ferrocenylnaphthalene-2-carboxylate (*1*)

Concentrated hydrochloric acid (4 ml) was added with intermittent cooling to a solution of methyl-3-aminonaphthalene-2-carboxylate (2.62 g, 11 mmol) in 15 ml of water. A solution of sodium nitrite (0.9 g, 13 mmol) in 15 ml of water was then added slowly to this mixture with stirring, keeping the temperature below 5 °C to furnish a pale brown/yellow solution. The resulting diazo salt was added to a solution of ferrocene (2.42 g, 13 mmol) in diethyl ether (90 ml) and allowed to react for 18 h. The reaction was then washed with water, the ether layer was dried over $MgSO_4$ and the solvent was removed *in vacuo* to yield the crude product. The crude product was purified using column chromatography {eluant 3:2 petroleum ether (40–60 °C): diethyl ether} to obtain an orange solid **1** (1.23 g, 30%), m.p. 119–120 °C. Anal. Calc. for

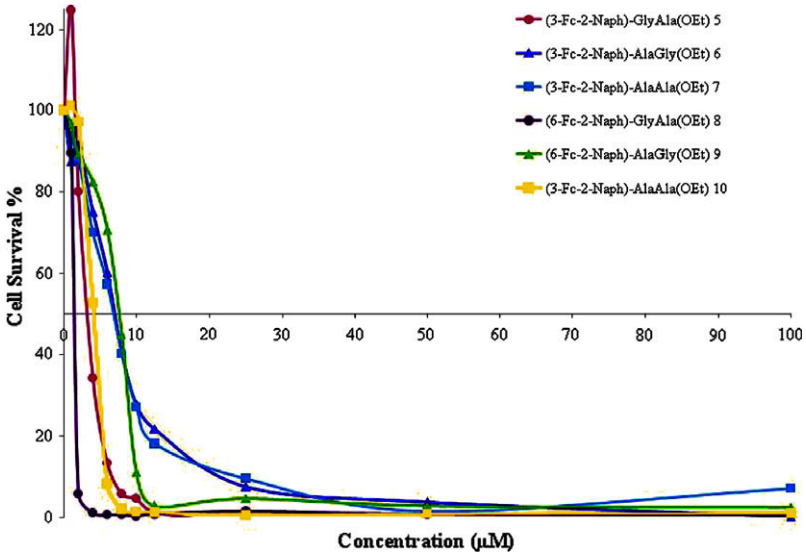


Fig. 3. Cytotoxicity of derivatives **5–10**.

$C_{22}H_{18}O_2Fe$ requires: C, 71.37; H, 4.90. Found: C, 71.35; H, 4.94%. Mass spectrum: found: $[M]^{+\cdot}$ 370.4; $C_{22}H_{18}O_2Fe$ requires: 370.2. I.R. υ_{max} (KBr): 1720, 1494, 1201 cm^{-1}; UV–Vis λ_{max} EtOH: 440 (ε 574) nm; ^1H NMR (400 MHz) δ (DMSO-d_6): 8.40 (1H, s, ArH), 8.07 (1H, s, ArH), 8.05 (1H, d, J = 8 Hz, ArH), 7.97 (1H, d, J = 8 Hz, ArH), 7.61 (1H, t, J = 8 Hz, ArH), 7.54 (1H, t, J = 8 Hz, ArH), 4.59 {2H, t, J = 1.6 Hz, *ortho* on (η^5-C_5H_4)}, 4.35 {2H, t, J = 1.6 Hz, *meta* on (η^5-C_5H_4)}, 4.10 (5H, s, η^5-C_5H_5), 3.77 (3H, s, –OCH_3); ^{13}C NMR (100 MHz) δ (DMSO-d_6): 169.4, 134.2, 133.5, 130.4, 130.3, 129.2, 128.1, 128.0, 127.9, 127.5, 126.4, 85.0, 69.6, 68.9, 68.3, 52.2.

4.2.2. 3-Ferrocenylnaphthalene-2-carboxylic acid (**2**)

Sodium hydroxide (0.1 g, 2.5 mmol) was added to methyl-3-ferrocenylnaphthalene-2-carboxylate (0.92 g, 2.5 mmol) in a 1:1 mixture of water/methanol and was refluxed for 12 h. Concentrated HCl was added until pH 2 was reached. The solution was allowed to cool and filtered to obtain an orange/brown solid **2** (0.81 g, 92%), m.p. (decomp.) at 145 °C; mass spectrum: found: $[M]^{+\cdot}$ 356.4; $C_{21}H_{16}O_2Fe$ requires: 356.2. I.R. υ_{max} (KBr): 3430, 1698 cm^{-1}; UV–Vis λ_{max} EtOH: 445 (ε 568) nm; ^1H NMR (400 MHz) δ (DMSO-d_6): 12.8 (1H, br.s, –COOH), 8.33 (1H, s, ArH), 8.00 (1H, d, J = 8 Hz, ArH), 7.99 (1H, s, ArH), 7.95 (1H, d, J = 8 Hz, ArH), 7.57 (1H, t, J = 8 Hz, ArH), 7.52 (1H, t, J = 8 Hz, ArH), 4.70 {2H, t, J = 1.6 Hz, *ortho* on (η^5-C_5H_4)}, 4.34 {2H, t, J = 1.6 Hz, *meta* on (η^5-C_5H_4)}, 4.10 (5H, s, η^5-C_5H_5); ^{13}C NMR (100 MHz) δ (DMSO-d_6): 170.8, 133.9, 133.1, 130.5, 128.8, 127.9, 127.4, 127.0, 126.1, 85.2, 69.6, 69.1, 68.2.

4.2.3. Methyl-6-ferrocenylnaphthalene-2-carboxylate (**3**)

Concentrated hydrochloric acid (4 ml) was added with intermittent cooling to a solution of methyl-6-aminonaphthalene-2-carboxylate (2.7 g, 11.5 mmol) in 15 ml of water. A solution of sodium nitrite (1.0 g, 14.5 mmol) in 15 ml of water was then added slowly to this mixture with stirring, keeping the temperature below 5 °C furnishing a pale brown/yellow solution. The resulting diazo salt was added to a solution of ferrocene (2.8 g, 14.5 mmol) in diethyl ether (90 ml) and allowed to react for 18 h. The reaction mixture was then washed with water, the ether layer was dried over $MgSO_4$, and the solvent removed *in vacuo* to yield the crude product. The crude product was purified using column chromatography {eluant 3:2 petroleum ether (40–60 °C): diethyl ether} to obtain a red solid **3** (0.88 g, 21%), m.p. 158–159 °C. Anal. Calc. for $C_{22}H_{18}O_2Fe$ requires: C, 71.37; H, 4.90. Found: C, 71.56; H, 5.24%. Mass spectrum: found: $[M]^{+\cdot}$ 370.4; $C_{22}H_{18}O_2Fe$ requires: 370.2. I.R. υ_{max} (KBr): 1708, 1494, 1450, 1219 cm^{-1}; UV–Vis λ_{max} EtOH: 380 (ε 3211), 455 (ε 1523) nm; ^1H NMR (400 MHz) δ (DMSO-d_6): 8.58 (1H, s, ArH), 8.09 (1H, s, ArH), 8.07 (1H, d, J = 8.8 Hz, ArH), 7.95 (2H, m, ArH), 7.85 (1H, dd, J = 1.6 Hz, J = 8.8 Hz, ArH), 4.99 {2H, t, J = 1.6 Hz, *ortho* on (η^5-C_5H_4)}, 4.47 {2H, t, J = 1.6 Hz, *meta* on (η^5-C_5H_4)}, 4.05 (5H, s, η^5-C_5H_5), 3.92 (3H, s, –OCH_3); ^{13}C NMR (100 MHz) δ (DMSO-d_6): 166.4, 140.0, 135.4, 130.6, 130.4, 129.2, 127.8, 126.1, 125.8, 125.1, 122.7, 83.7, 69.6, 69.5, 66.8, 52.2.

4.2.4. 6-Ferrocenylnaphthalene-2-carboxylic acid (**4**)

Sodium hydroxide (0.08 g, 2.0 mmol) was added to methyl-6-ferrocenylnaphthalene-2-carboxylate (0.70 g, 1.9 mmol) in a 1:1 mixture of water/methanol and was refluxed for 12 h. Concentrated HCl was added until pH 2 was reached. The solution was allowed to cool and filtered to obtain an orange solid **4** (0.63 g, 93%), m.p. (decomp.) at 205 °C; mass spectrum: found: $[M]^{+\cdot}$ 356.4; $C_{21}H_{16}O_2Fe$ requires: 356.2. I.R. υ_{max} (KBr): 3435, 1682 cm^{-1}; UV–Vis λ_{max} EtOH: 375 (ε 2635), 450 (ε 1296) nm; ^1H NMR (400 MHz) δ (DMSO-d_6): 12.8 (1H, br.s, –COOH), 8.57 (1H, s, ArH), 8.07 (1H, s, ArH), 8.03 (1H, d, J = 8.4 Hz, ArH), 7.94 (2H, s, ArH), 7.83 (1H, dd, J = 1.6 Hz, J = 8.4 Hz, ArH), 4.97 {2H, t, J = 1.6 Hz, *ortho* on (η^5-C_5H_4)}, 4.45 {2H, t, J = 1.6 Hz, *meta* on (η^5-C_5H_4)}, 4.04 (5H, s, η^5-C_5H_5); ^{13}C NMR (100 MHz) δ (DMSO-d_6): 167.5, 139.7, 135.3, 130.6, 130.4, 129.1, 127.6, 127.0, 125.9, 125.5, 122.7, 83.8, 69.6, 69.5, 66.7.

4.3. General procedure for the synthesis of N-(3-ferrocenyl-2-naphthoyl) dipeptide ethyl esters (**5–7**)

4.3.1. N-(3-ferrocenyl-2-naphthoyl)-glycine-L-alanine ethyl ester (**5**)

Glycine-L-alanine ethyl ester hydrochloride (0.32 g, 1.5 mmol) was added to a solution of 3-ferrocenylnaphthalene-2-carboxylic acid (0.53 g, 1.5 mmol), 1-hydroxybenzotriazole (0.2 g, 1.5 mmol), triethylamine (0.5 ml) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.3 g, 1.6 mmol) in 50 ml of dichloromethane at 0 °C. After 30 min the solution was raised to room temperature and the reaction was allowed to proceed for 48 h. The reaction mixture was then washed with water. The dichloromethane layer was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The product was purified by column chromatography (eluant 1:1 hexane:ethyl acetate – 100% ethyl acetate) to give the title compound as an orange solid (0.42 g, 55%), m.p. 70–71 °C; $E^{\circ\prime} = 5$ mV (versus Fc/Fc^+); $[\alpha]_D^{20} = -35^\circ$ (c 0.01, EtOH); mass spectrum: found: $[M+H]^+$ 513.1487; $C_{28}H_{29}N_2O_4Fe$ requires: 513.1477. I.R. υ_{max} (KBr): 3393, 3291, 1734, 1646, 1544, 1494, 1205 cm^{-1}; UV–Vis λ_{max} EtOH: 450 (ε 525) nm; ^1H NMR (400 MHz) δ (DMSO-d_6): 8.72 (1H, t, J = 5.6 Hz, –CONH–), 8.38 (1H, d, J = 6.8 Hz, –CONH–), 8.32 (1H, s, ArH), 7.99 (1H, d, J = 8 Hz, ArH), 7.90 (1H, d, J = 8 Hz, ArH), 7.83 (1H, s, ArH), 7.49–7.57 (2H, m, ArH), 4.81 {2H, s, *ortho* on (η^5-C_5H_4)}, 4.31–4.36 {3H, m, –NHCHCO–, *meta* on (η^5-C_5H_4)}, 4.11 {7H, m, –OCH_2CH$_3$, (η^5-C_5H_5)}, 3.96 (1H, dd, J = 6 Hz, J = 16.4 Hz, –NHCH_2CO–), 3.87 (1H, dd, J = 6 Hz, J = 16.4 Hz, –NHCH_2CO–), 1.32 (3H, d, J = 7.2 Hz, –CHCH_3), 1.21 (3H, t, J = 7.2 Hz, –OCH$_2$CH_3); ^{13}C NMR (100 MHz) δ (DMSO-d_6): 169.8, 169.4, 168.7, 135.0, 134.3, 132.9, 130.5, 128.1, 127.6, 127.4, 127.0, 126.7, 126.0, 84.3, 69.5, 69.0, 68.3, 60.5 (−ve DEPT), 47.7, 41.8 (−ve DEPT), 17.1, 14.1.

4.3.2. N-(3-ferrocenyl-2-naphthoyl)-L-alanine-glycine ethyl ester (**6**)

For compound **6** L-alanine-glycine ethyl ester hydrochloride (0.32 g, 1.5 mmol) was used as a starting material. The product was purified by column chromatography (eluant 1:1 hexane:ethyl acetate – 100% ethyl acetate) to give the title compound as an orange solid (0.30 g, 59%), m.p. 63–64 °C; $E^{\circ\prime} = 10$ mV (versus Fc/Fc^+); $[\alpha]_D^{20} = -75^\circ$ (c 0.01, EtOH); mass spectrum: found: $[M+H]^+$ 513.1453; $C_{28}H_{29}N_2O_4Fe$ requires: 513.1477. I.R. υ_{max} (KBr): 3397, 3291, 1748, 1646, 1544, 1494, 1197 cm^{-1}; UV–Vis λ_{max} EtOH: 445 (ε 483) nm; ^1H NMR (400 MHz) δ (DMSO-d_6): 8.64 (1H, d, J = 7.6 Hz, –CONH–), 8.38 (1H, s, ArH), 8.35 (1H, t, J = 5.6 Hz, –CONH–), 8.06 (1H, d, J = 8 Hz, ArH), 7.97 (1H, d, J = 8 Hz, ArH), 7.92 (1H, s, ArH), 7.49–7.58 (2H, m, ArH), 4.79 {2H, t, J = 1.6 Hz, *ortho* on (η^5-C_5H_4)}, 4.56 (1H, qt, J = 7.2 Hz, –NHCHCO–), 4.38 {1H, m, *meta* on (η^5-C_5H_4)}, 4.36 {1H, m, *meta* on (η^5-C_5H_4)}, 4.01–4.14 {7H, m, –OCH_2CH$_3$, (η^5-C_5H_5)}, 3.99 (1H, dd, J = 6 Hz, J = 17.6 Hz, –NHCH_2CO–), 3.89 (1H, dd, J = 6 Hz, J = 17.6 Hz, –NHCH_2CO–), 1.37 {3H, d, J = 7.2 Hz, –CH(CH_3)}, 1.27 (3H, t, J = 7.2 Hz, –OCH$_2$CH_3); ^{13}C NMR (100 MHz) δ (DMSO-d_6): 169.7, 169.5, 169.2, 135.1, 134.2, 132.9, 130.5, 128.2, 127.6, 127.4, 126.9, 126.7, 126.0, 84.7, 69.5, 68.6, 68.2, 60.4 (−ve DEPT), 48.4, 40.7 (−ve DEPT), 17.7, 14.0.

4.3.3. N-(3-ferrocenyl-2-naphthoyl)-L-alanine-L-alanine ethyl ester (**7**)

For compound **7** L-alanine-L-alanine ethyl ester hydrochloride (0.29 g, 1.3 mmol) was used as a starting material. The product was purified by column chromatography (eluant 1:1 hexane:ethyl acetate – 100% ethyl acetate) to give the title compound as an orange solid (0.18 g, 28%), m.p. 59–60 °C; $E^{\circ\prime} = 9$ mV (versus Fc/Fc^+); $[\alpha]_D^{20} = -122^\circ$ (c 0.02, EtOH); mass spectrum: found: $[M+H]^+$

527.1649; $C_{29}H_{31}N_2O_4Fe$ requires: 527.1633. I.R. υ_{max} (KBr): 3448, 3292, 1733, 1638, 1550, 1495, 1202 cm^{-1}; UV–Vis λ_{max} EtOH: 445 (ε 498) nm; ^1H NMR (400 MHz) δ (DMSO-d_6): 8.54 (1H, d, J = 7.2 Hz, –CONH–), 8.34 (1H, d, J = 7.2 Hz, –CONH–), 8.32 (1H, s, ArH), 7.99 (1H, d, J = 8 Hz, ArH), 7.91 (1H, d, J = 8 Hz, ArH), 7.81 (1H, s, ArH), 7.49–7.57 (2H, m, ArH), 4.74 {2H, m, *ortho* on (η^5-C_5H_4)}, 4.49 (1H, qt, J = 7.2 Hz, –NHCHCO–), 4.28–4.33 {3H, m, *meta* on (η^5-C_5H_4), –NHCHCO–}, 4.09 {7H, m, –OCH_2CH$_3$, (η^5-C_5H_5)}, 1.32 {6H, m, –CH(CH_3)}, 1.19 (3H, t, J = 7.2 Hz, –OCH$_2$CH_3); ^{13}C NMR (100 MHz) δ (DMSO-d_6): 172.5, 172.2, 169.1, 135.1, 134.2, 132.9, 130.5, 128.2, 127.6, 127.4, 126.9, 126.7, 126.0, 84.6, 69.5, 68.6, 68.2, 68.1, 60.4 (−ve DEPT), 48.3, 47.7, 17.8, 16.9, 14.0.

4.4. General procedure for the synthesis of N-(6-ferrocenyl-2-naphthoyl) dipeptide ethyl esters (**8–10**)

4.4.1. N-(6-ferrocenyl-2-naphthoyl)-glycine-L-alanine ethyl ester (**8**)

Glycine-L-alanine ethyl ester hydrochloride (0.15 g, 0.7 mmol) was added to a solution of 6-ferrocenylnaphthalene-2-carboxylic acid (0.26 g, 0.7 mmol), 1-hydroxybenzotriazole (0.14 g, 1.0 mmol), triethylamine (0.5 ml) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.2 g, 1.0 mmol) in 50 ml of dichloromethane at 0 °C. After 30 min the solution was raised to room temperature and the reaction was allowed to proceed for 48 h. The reaction mixture was then washed with water. The dichloromethane layer was dried over $MgSO_4$ and the solvent removed *in vacuo*. The product was purified by column chromatography (eluant 1:1 hexane:ethyl acetate – 100% ethyl acetate) to give the title compound as an orange solid (0.28 g, 79%), m.p. 130 °C; $E^{\circ\prime} = 56$ mV (versus Fc/Fc^+); $[\alpha]_D^{20} = -78^\circ$ (c 0.01, EtOH). Anal. Calc. for $C_{28}H_{28}N_2O_4Fe$ requires: C, 65.64; H, 5.51; N, 5.47. Found: C, 65.36; H, 5.58; N, 5.40%. Mass spectrum: found: $[M+H]^+$ 513.1486; $C_{28}H_{29}N_2O_4Fe$ requires: 513.1477. I.R. υ_{max} (KBr): 3419, 3284, 1748, 1646, 1546, 1494 cm^{-1}; UV–Vis λ_{max} EtOH: 375 (ε 3451), 450 (ε 1482) nm; ^1H NMR (400 MHz) δ (DMSO-d_6): 8.84 (1H, t, J = 6 Hz, –CONH–), 8.44 (1H, s, ArH), 8.41 (1H, d, J = 7.2 Hz, –CONH–), 8.06 (1H, s, ArH), 7.96 (1H, d, J = 8.8 Hz, ArH), 7.94 (2H, m, ArH), 7.82 (1H, dd, J = 1.6 Hz, J = 8.8 Hz, ArH), 4.95 {2H, t, J = 1.6 Hz, *ortho* on (η^5-C_5H_4)}, 4.44 {2H, t, J = 1.6 Hz, *meta* on (η^5-C_5H_4)}, 4.30 {1H, qt, J = 7.2 Hz, –CH(CH$_3$)–}, 4.09 (2H, m, –OCH_2CH$_3$), 4.02 {6H, m, (η^5-C_5H_5), –NHCH_2CO–}, 3.93 (1H, dd, J = 6 Hz, J = 16.4 Hz, –NHCH_2CO–), 1.31 {3H, d, J = 7.2 Hz, –CH(CH_3)}, 1.19 (3H, t, J = 7.2 Hz, –OCH$_2$CH_3); ^{13}C NMR (100 MHz) δ (DMSO-d_6): 172.5, 168.9, 166.5, 138.8, 134.5, 130.6, 130.4, 128.7, 127.5, 127.3, 125.9, 124.5, 122.7, 84.0, 69.5, 69.4, 66.6, 60.5 (−ve DEPT), 47.7, 42.2 (−ve DEPT), 17.0, 14.0.

4.4.2. N-(6-ferrocenyl-2-naphthoyl)-L-alanine-glycine ethyl ester (**9**)

For the compound **9** L-alanine-glycine ethyl ester hydrochloride (0.24 g, 1.1 mmol) was used as a starting material. The product was purified by column chromatography (eluant 1:1 hexane:ethyl acetate – 100% ethyl acetate) to give the title compound as an orange solid (0.24 g, 67%), m.p. 138–139 °C; $E^{\circ\prime} = 42$ mV (versus Fc/Fc^+); $[\alpha]_D^{20} = -44^\circ$ (c 0.01, EtOH); mass spectrum: found: $[M+H]^+$ 513.1486; $C_{28}H_{29}N_2O_4Fe$ requires: 513.1477. I.R. υ_{max} (KBr): 3415, 3284, 1751, 1637, 1546, 1494, 1262 cm^{-1}; UV–Vis λ_{max} EtOH: 375 (ε 2990), 450 (ε 1266) nm; ^1H NMR (400 MHz) δ (DMSO-d_6): 8.67 (1H, d, J = 7.2 Hz, –CONH–), 8.48 (1H, s, ArH), 8.38 (1H, t, J = 6 Hz, –CONH–), 8.06 (1H, s, ArH), 7.94 (3H, m, ArH), 7.82 (1H, dd, J = 1.6 Hz, J = 8.4 Hz, ArH), 4.96 {2H, t, J = 1.6 Hz, *ortho* on (η^5-C_5H_4)}, 4.60 {1H, qt, J = 7.2 Hz, –CH(CH$_3$)}, 4.45 {2H, t, J = 1.6 Hz, *meta* on (η^5-C_5H_4)}, 4.12 (2H, q, J = 7.2 Hz, –OCH_2CH$_3$), 4.05 (5H, s, η^5-C_5H_5), 3.89 (1H, dd, J = 6 Hz, J = 17.6 Hz, –NHCH_2CO–), 3.82 (1H, dd, J = 6 Hz, J = 17.6 Hz, –NHCH_2CO–), 1.41 {3H, d, J = 7.2 Hz, –CH(CH_3)}, 1.19 (3H, t, J = 7.2 Hz, –OCH$_2$CH_3); ^{13}C NMR (100 MHz) δ (DMSO-d_6): 173.0, 169.7, 166.1, 138.8, 134.5, 130.6, 130.4, 128.7, 127.7, 127.2, 125.9, 124.8, 122.7, 84.1, 69.5, 69.4, 66.6, 60.3 (−ve DEPT), 48.8, 40.7 (−ve DEPT), 17.9, 14.0.

4.4.3. N-(6-ferrocenyl-2-naphthoyl)-L-alanine-L-alanine ethyl ester (**10**)

For the compound **10** L-alanine-L-alanine ethyl ester hydrochloride (0.20 g, 0.9 mmol) was used as a starting material. The product was purified by column chromatography (eluant 1:1 hexane:ethyl acetate – 100% ethyl acetate) to give the title compound as an orange solid (0.12 g, 29%), m.p. 80–81 °C; $E^{\circ\prime} = 42$ mV (versus Fc/Fc^+); $[\alpha]_D^{20} = +58^\circ$ (c 0.01, EtOH); mass spectrum: found: $[M+H]^+$ 527.1616; $C_{29}H_{31}N_2O_4Fe$ requires: 527.1633. I.R. υ_{max} (KBr): 3448, 3288, 1735, 1636, 1546, 1495, 1204 cm^{-1};UV–Vis λ_{max} EtOH: 375 (ε 3233), 450 (ε 1349) nm; ^1H NMR (400 MHz) δ (DMSO-d_6): 8.59 (1H, d, J = 7.2 Hz, –CONH–), 8.46 (1H, s, ArH), 8.40 (1H, d, J = 6.8 Hz, –CONH–), 8.05 (1H, s, ArH), 7.94 (3H, m, ArH), 7.82 (1H, dd, J = 1.6 Hz, J = 8.8 Hz, ArH), 4.96 {2H, t, J = 1.6 Hz, *ortho* on (η^5-C_5H_4)}, 4.59 {1H, qt, J = 7.2 Hz, –CH(CH$_3$)}, 4.45 {2H, t, J = 1.6 Hz, *meta* on (η^5-C_5H_4)}, 4.27 {1H, qt, J = 7.2 Hz, –CH(CH$_3$)}, 4.07 {7H, m, –OCH_2CH$_3$, (η^5-C_5H_5)}, 1.40 {3H, d, J = 7.2 Hz, –CH(CH_3)}, 1.32 {3H, d, J = 7.2 Hz, –CH(CH_3)}, 1.18 (3H, t, J = 7.2 Hz, –OCH$_2$CH_3); ^{13}C NMR (100 MHz) δ (DMSO-d_6): 172.5, 169.4, 166.0, 138.7, 134.5, 130.6, 130.5, 128.7, 127.6, 127.2, 125.9, 124.7, 122.8, 84.1, 69.5, 69.4, 66.6, 60.4 (−ve DEPT), 48.5, 47.7, 17.9, 16.8, 14.0.

4.5. General procedure for in vitro cytotoxicity assays

H1299 lung cancer cells (highly invasive/superinvasive) were harvested by trypsinisation and a cell suspension of 1×10^4 cells/ml was prepared in RPMI medium supplemented with 10% foetal calf serum. 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 stock solution of a test sample was prepared in dimethyl sulfoxide; dilute solutions of the test sample were prepared by spiking the cell culture medium with a calculated amount of the stock solution. Hundred microliters 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 6–7 days, until cell confluency reached 80–90%. Assessment of cell survival in the presence of the ferrocenyl derivatives **5–10** was determined by the acid phosphatase assay. The concentration of drug that kills 50% of the cells (the IC_{50} value) was determined by plotting % survival of cells (relative to the control cells) against concentration of the ferrocenyl derivative.

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