Synthesis, characterisation and biological evaluation of *N*-(ferrocenyl)naphthoyl amino acid esters as anticancer agents†

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A series of *N*-(ferrocenyl)naphthoyl amino acid esters **5–18** has been prepared by coupling ferrocenyl naphthoic acids **3–4** to α-amino acids and linear amino acids in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt). The compounds were fully characterised by a range of NMR spectroscopic techniques, UV-Vis spectroscopy, mass spectrometry and cyclic voltammetry. X-ray crystallographic studies of the intermediate compounds **1–2** were also performed. Biological evaluation of the intermediates **1–2** and *N*-(ferrocenyl)naphthoyl amino acid esters **5–18** was performed in the H1299 non-small cell lung cancer (NSCLC) cell line and the Sk-Mel-28 metastatic melanoma cell line. The intermediates **1–2** failed to produce an effect in either cell line. Compounds **5–18** exhibited a strong anti-proliferative effect in the H1299 cell line, whilst the Sk-Mel-28 cells were slightly more resistant to these compounds. *N*-(6-ferrocenyl-2-naphthoyl)-γ-aminobutyric acid ethyl ester **17** shows a particularly high activity in both the H1299 cell line ($IC_{50} = 0.62 \pm 0.07$ μM) and the Sk-Mel-28 cell line ($IC_{50} = 1.41 \pm 0.04$ μM).

Introduction

Organometallic compounds are versatile species due to the range of both structure and bonding modes that are accessible. As a consequence, they have been incorporated in a wide variety of materials that have diverse applications. One such application is the development of new medicinal agents that exhibit novel modes of action.[1] Ferrocene, the archetypal metallocene, has shown great promise in the area of medicinal organometallic chemistry.[2] Unique properties such as aromaticity, stability, low toxicity and redox activity recommend ferrocene for incorporation in drug molecules.[3] In particular, the reversible redox properties of ferrocene have been strongly associated with its biological activity.[4] Ferrocenyl compounds have shown potential as both antibacterial[5] and antifungal agents[6] however the main focus of research has been centred on antimalarial[7] and anticancer drugs.[8–12] A notable example is that of ferrocifen, an organometallic analogue of the selective estrogen receptor modulator (SERM) tamoxifen, and its derivatives.[13]

Amino acids and peptides play diverse roles in biological systems. Over the past number of years, we have prepared *N*-ferrocenoyl and *N*-ferrocenyl amino acid and peptide derivatives and explored their potential applications.[14–23] Preliminary *in vitro* toxicity testing revealed the *N*-(ferrocenyl)benzoyl peptide derivatives to be potential anticancer agents.[24] This discovery has provided the basis for more in-depth studies which have used rational drug design to explore the structure–activity relationship (SAR).[25,26]

The *N*-(ferrocenyl)benzoyl peptide derivatives are composed of three key moieties, namely, (i) an electroactive core, (ii) a conjugated aromatic linker, (iii) an amino acid or peptide derivative that can interact with other molecules *via* hydrogen bonds. The conjugated linker offers extended conjugation to the π-electrons of the ferrocene rings, lowering the redox potential and making these derivatives easier to oxidise to the ferricenium species. Ferricenium salts known to inhibit tumour growth were shown to produce hydroxyl radicals (HO•) under physiological conditions, leading to oxidative damage to DNA.[27] It is plausible that the anticancer activity of these compounds is due, at least in part, to their low redox potentials, which are within the range of biologically accessible potentials. The peptide chain is also considered to be essential for activity.[26]

We have shown in a previous study that replacing the conjugated linker of *N*-(ferrocenyl)benzoyl dipeptide esters with a naphthoyl linker leads to on average a three-fold improvement in anti-proliferative effect in the H1299 non-small cell lung cancer (NSCLC) cell line.[29] These *N*-(ferrocenyl)naphthoyl dipeptide esters all show a stronger anti-proliferative effect in this cell line than carboplatin. The most promising of these compounds is *N*-(6-ferrocenyl-2-naphthoyl)-glycine-L-alanine ethyl ester ($IC_{50} = 1.3 \pm 0.1$ μM), which displays an *in vitro* anticancer activity comparable with cisplatin ($IC_{50} = 1.5 \pm 0.1$ μM). We have now extended our SAR study to include *N*-(ferrocenyl)naphthoyl amino acid esters, containing both α-amino acids (glycine, L-alanine, L-leucine and L-phenylalanine) and 'linear' amino acids (β-alanine, γ-aminobutyric acid and δ-amino-n-valeric acid).

To date, the biological evaluation of *N*-(ferrocenyl)benzoyl and *N*-(ferrocenyl)naphthoyl derivatives has focussed on determining

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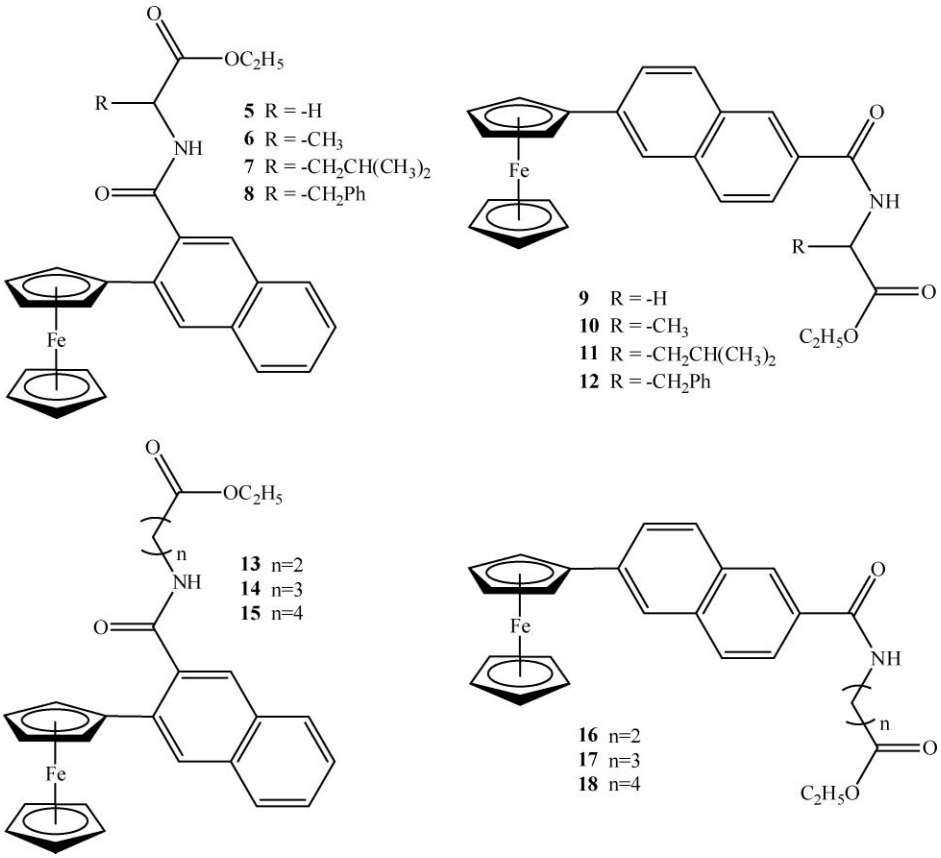


Chart 1

their anti-proliferative effect in the H1299 NSCLC cell line. This is due to the fact that lung cancer is the leading cause of cancer mortality both worldwide and in Europe, with approximately 75–85% of all lung cancer cases being NSCLC.[29] We have now expanded our *in vitro* toxicity testing to include the Sk-Mel-28 malignant melanoma cell line. European national cancer registries have shown a rising incidence of melanoma during the past two decades.[30] Most localised melanoma can be effectively treated early by wide localised excision, however, patients with advanced disease such as those with distant metastasis have a poor prognosis, with a 1 year survival rate of less than 5%. The poor prognosis is due at least in part, to the fact that metastatic melanoma is notoriously resistant to cytotoxic chemotherapy.[31] More efficacious novel chemotherapeutic drugs are urgently required to improve the prognosis for malignant melanoma patients. Thus, we report the synthesis, characterisation and biological evaluation of the *N*-(ferrocenyl)naphthoyl α-amino acid esters **5–12** and *N*-(ferrocenyl)naphthoyl linear amino acid esters **13–18** in lung cancer (non-small cell) and melanoma cell lines (Chart 1).

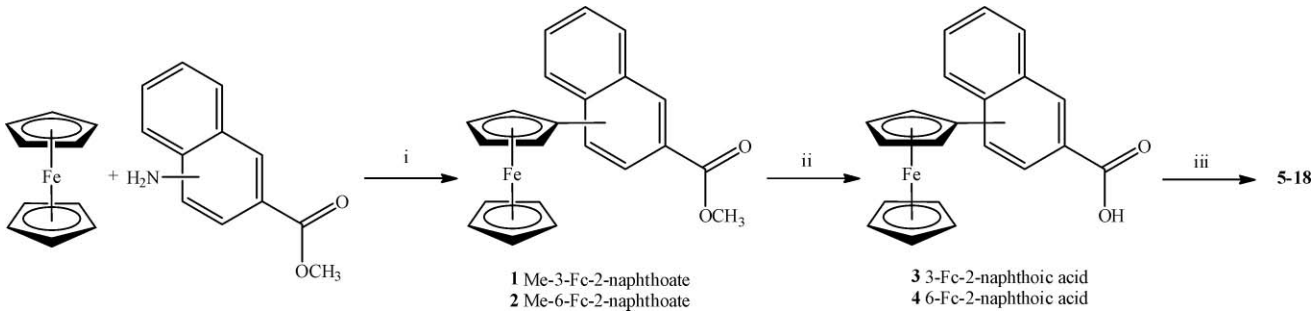
Results and discussion

Synthesis

The synthetic sequence towards the *N*-(ferrocenyl)naphthoyl amino acid esters **5–18** is outlined in Scheme 1. Starting from the appropriate methyl aminonaphthalene-2-carboxylate, the intermediate compounds **1–4** were prepared as previously reported.[28] The ferrocenylnaphthalene units were appended to the free *N*-terminal amino acid ethyl esters of glycine, L-alanine, L-leucine, L-phenylalanine, β-alanine, γ-aminobutyric acid and δ-amino-n-valeric acid under solution-phase peptide coupling conditions to furnish compounds **5–18**. Thus, a solution of ferrocenylnaphthalene carboxylic acid in dichloromethane at 0 °C was treated with *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBt) and triethylamine (Et_3N) prior to the addition of the required amino acid ethyl ester (Scheme 1). Compounds **5–18** were purified by column chromatography with a gradient mixture of hexane and ethyl acetate as mobile phase in yields of 32–83% and all gave spectroscopic and analytical data in accordance with the proposed structures.

Characterisation

Full characterisation of compounds **5–18** was achieved by standard spectroscopic techniques: ^{1}H NMR, ^{13}C NMR, IR, UV, and MS. All proton and carbon chemical shifts were explicitly assigned for **5–18** using various NMR spectroscopic techniques including DEPT 135 and ^{1}H–^{13}C COSY (HMQC). In the ^{1}H and ^{13}C NMR spectra obtained for **5–18**, there was a notable difference in the chemical shift of the ferrocenyl peaks when the substitution pattern around the central naphthoyl linker was altered. The greatest difference is observed for the *ortho* carbons of the substituted (η^5-C_5H_4) ring, which are shifted downfield from approximately δ 66.6 for the *N*-(6-ferrocenyl-2-naphthoyl) derivatives **9–12** and **16–18**, to approximately δ 68.9 for the



Scheme 1 Synthesis of *N*-(ferrocenyl)naphthoyl amino acid esters **5–18**: (i) $NaNO_2$, HCl, 5 °C; (ii) NaOH/MeOH, HCl; (iii) EDC, HOBt, Et_3N, amino acid ethyl ester.

N-(3-ferrocenyl-2-naphthoyl) derivatives **5–8** and **13–15**. In contrast, the *ortho* (η^5-C_5H_4) protons of **5–8** and **13–15** (δ 4.71–4.83) are more shielded than the *ortho* (η^5-C_5H_4) protons of **9–12** and **16–18** (δ 4.94–4.96). The ^{1}H NMR spectra of **6–8**, showed signal multiplicities for either some or all of the characteristic peaks that are observed for a mono-substituted ferrocene moiety (Fig. 1). The ^{1}H NMR spectra of **6** and **7** indicate that the *meta* (η^5-C_5H_4) protons are magnetically inequivalent and in the case of **8**, all four protons of the (η^5-C_5H_4) ring are magnetically inequivalent. The ^{13}C NMR spectra of **6–8** also reflect these observations: in the case of **6** and **7** there are three distinct peaks between δ 68.5 and δ 68.1. For **8**, individual resonances are observed at δ 68.9, δ 68.8, δ 68.3 and δ 68.1.

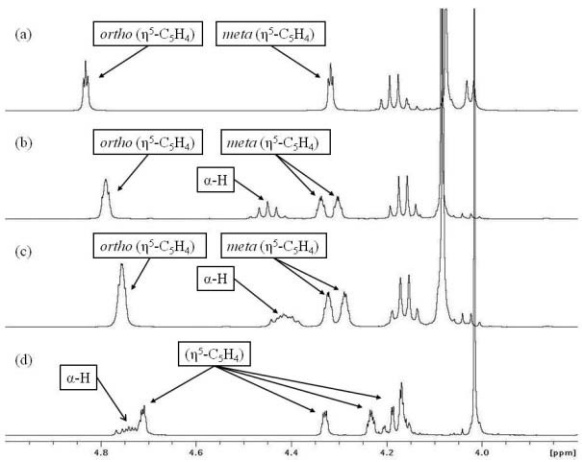


Fig. 1 Ferrocenyl (3.8–5.0 ppm) region of the ^{1}H NMR (25 °C, 400 MHz, DMSO-d_6) spectra of (a) **5**; (b) **6**; (c) **7**; (d) **8**.

The UV-visible spectra of **5–18** are all characterised by an absorbance centred at 450 nm due to metal to ligand charge transfer (MLCT) transitions. For compounds **9–12** and **16–18**, there is also an intense absorbance at 375 nm, which is assigned to a π to π^* transition of the aromatic spacer group. However, in the case of **5–8** and **13–15**, the π to π^* transition of the naphthoyl group is too weak to be observed (Fig. 2). This indicates that for the *N*-(3-ferrocenyl-2-naphthoyl) derivatives, only minimal π conjugation is occurring between the ferrocene and naphthalene units. The UV-visible spectra of **5–18** remain the same after several weeks in solution (ethanol and acetonitrile), thus indicating that the *N*-(ferrocenyl)naphthoyl amino acid esters are stable over long periods of time.

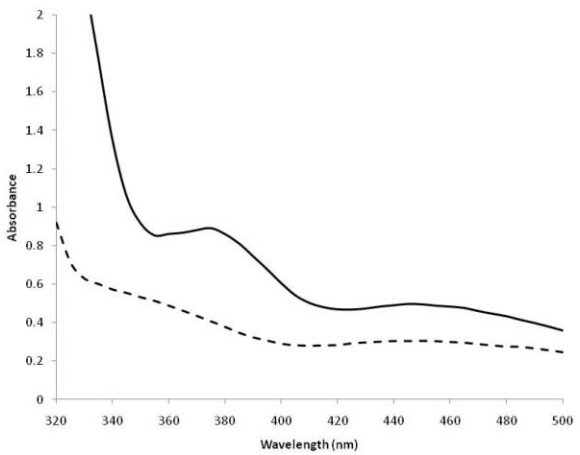


Fig. 2 UV-visible spectra of **15** (dashed line) and **18** (solid line) in acetonitrile (0.2 mM).

X-ray crystallographic studies

The intermediates **1** and **2** have been characterised by single-crystal X-ray crystallographic studies. Crystallographic data and structural refinement parameters are given in Table 1. Some pertinent bond lengths and bond angles are listed in Table 2. Fig. 3 shows the molecular structures and asymmetric unit of **1**, which crystallises in the monoclinic space group $P2_1/c$ with one molecule in the asymmetric unit. The principal dimensions are carboxylate ester C═O 1.199(3) Å, C–O 1.335(2) Å, O–CH_3 1.449(3) Å and O═C–O 123.6(2)°. The cyclopentadienyl rings of the ferrocene unit (Cp1 and Cp2) are almost eclipsed with C1n···Cg1···Cg2···C2n torsion angles (n = 1–5) in the –0.7(42) to 1.3(3)° range. Cg1 and Cg2 are the centroids of the (η^5-C_5H_4) and (η^5-C_5H_5) rings, respectively. Cg1 and Cg2 are equidistant from the iron core, with Fe···Cg1/Cg2 distances of 1.643(3)/1.646(2), respectively, while the Cg1···Fe···Cg2 angle is almost linear at 178.3(3)°. Compound **2** crystallises in the monoclinic space group $P2_1/n$ with one molecule in the asymmetric unit: the molecular structure is depicted in Fig. 4. The principal dimensions are carboxylate ester C═O 1.198(4) Å, C–O 1.343(4) Å, O–CH_3 1.438(4) Å and O═C–O 122.5(4)°. In the case of **2**, the cyclopentadienyl rings of the ferrocene unit (Cp1 and Cp2) are slightly staggered with C1n···Cg1···Cg2···C2n torsion angles (n = 1–5) in the –12.2(4) to –14.9(2)° range. Centroids Cg1 and Cg2 are equidistant from the iron core, with

Table 1 Crystallographic data for intermediates **1** and **2**

	1	**2**
Empirical Formula	$C_{22}H_{18}O_2Fe$	$C_{22}H_{18}O_2Fe$
M/g mol^{-1}	370.21	370.21
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/n$
a/Å	13.9797(15)	14.7851(17)
b/Å	8.7249(9)	6.1301(7)
c/Å	13.9843(15)	18.161(2)
β (°)	103.111(2)	92.273(3)
V/Å^3	1661.2(3)	1644.8(3)
Z	4	4
T/K	273	298
D_c/g cm^{-3}	1.480	1.495
μ/mm^{-1}	0.920	0.929
F_{000}	768	768
Crystal dimensions/mm	0.25 × 0.18 × 0.13	0.27 × 0.19 × 0.13
Max. and min. transmission	0.8898 – 0.8027	0.8888 – 0.7876
Reflections collected/unique	17252/2927	9115/2883
Final R indices [$I > 2\sigma I$]	$R_1 = 0.0285$ w$R_2 = 0.0796$	$R_1 = 0.0469$ w$R_2 = 0.1146$
R indices [all data]	$R_1 = 0.0314$ w$R_2 = 0.0819$	$R_1 = 0.0627$ w$R_2 = 0.1220$
Goodness of fit	1.018	1.02

Table 2 Representative bond lengths (Å) and angles (°)

	1		**2**
Bond Distances/Å		Bond Distances/Å	
Fe2 ··· Cg1[a]	1.643(2)	Fe ··· Cg1	1.650(3)
Fe2 ··· Cg2	1.646(3)	Fe ··· Cg2	1.657(4)
Cg1 ··· Fe ··· Cg2	178.31(3)	Cg1 ··· Fe ··· Cg2	178.1(3)
C14–O25	1.199(3)	C21–O1	1.198(4)
C14–O24	1.335(2)	C21–O2	1.343(4)
O24–C2	1.449(3)	O2–C22	1.438(4)
Bond Angles/°		Bond Angles/°	
Fe2–C21–C15	125.9(1)	Fe–C9–C11	126.4(6)
C17–C19–C14	115.8(2)	C15–C16–C21	122.5(3)
C15–C19–C14	123.8(2)	C17–C16–C21	118.2(3)
O25–C14–O24	123.6(2)	O1–C21–O2	122.5(4)
O25–C14–C19	125.0(2)	O2–C21–C16	112.4(3)
C14–O24–C2	116.2(2)	C21–O2–C22	116.5(3)
O24–C14–C19	111.3(2)	O1–C21–C16	125.1(4)
C20–C21–C15–C19	149.5(2)	Fe–C9–C11–C20	86.7(3)
C28–C21–C15–C19	156.5(2)	C8–C9–C11–C20	–2.5(3)
Fe2–C21–C15–C19	63.3(2)	C10–C9–C11–C12	–4.3(4)
O24–C14–C19–C15	–129.8(2)	C15–C16–C21–O2	3.7(3)
C14–C19–C15–C21	11.6(3)	C17–C16–C21–O1	2.9(5)

[a] Cg1 and Cg2 are the centroids of the (η^5-C_5H_4) and (η^5-C_5H_5) rings, respectively.

Fe ··· Cg1/Cg2 distances of 1.650(3)/1.657(4), respectively, while the Cg1 ··· Fe ··· Cg2 angle is almost linear at 178.1(3)°.

In **1** the $C_{10}H_6$ ring and the four-atom O═C–O–CH_3 plane are oriented at 54.45(12)° and the $C_{10}H_6$/(η^5-C_5H_4) rings at 28.27(15)°; whereas the angle between the (η^5-C_5H_4) ring and the O═C–O–CH_3 plane is 68.87(4)°. This data demonstrates the existence of a large distortion in the molecule, which is evident in Fig. 3. Steric hindrance has forced the atoms of this molecule to adopt this strained conformation in the solid state, resulting in a loss of co-planarity of the conjugating groups. This is supported by the torsion angles for the O25–C14–C19–C15 bonds and C20–C21–C15–C19 bonds which were calculated to be 54.2(3) and 149.5(2)°, respectively. In contrast, the $C_{10}H_6$ ring and the four-atom O═C–O–CH_3 plane of **2** are oriented at 4.45(15)° and the $C_{10}H_6$/(η^5-C_5H_4) rings at 3.13(16)°; whereas the angle between the

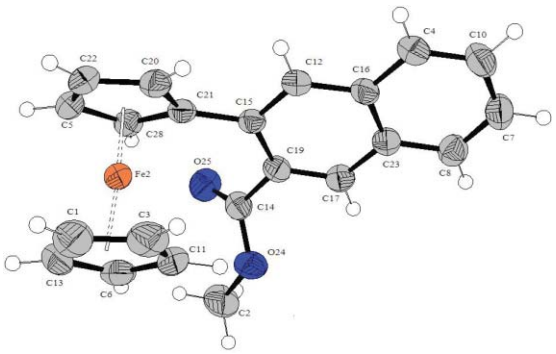


Fig. 3 Molecular drawing of **1** using ORTEP: displacement ellipsoids are drawn at 50% probability level.

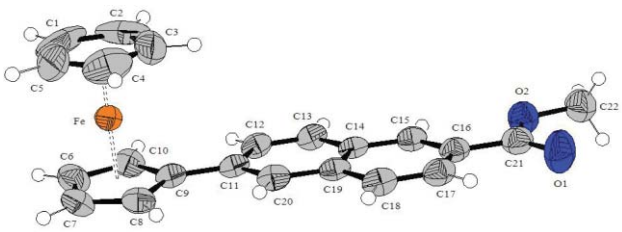


Fig. 4 Molecular drawing of **2** using ORTEP: displacement ellipsoids are drawn at the 50% probability level.

(η^5-C_5H_4) ring and the O═C–O–CH_3 plane is 1.97(5)°. Calculated torsion angles in **2** between the O1–C21–C16–C17 bonds and C10–C9–C11–C12 bonds are minimal at 2.9(5)° and –4.3(5)°, respectively. Thus, the conjugating groups in this molecule lie almost completely within the same plane in the solid state (Fig. 4). A similar observation has been reported for the *para*-substituted ferrocenyl methyl benzoate.[17]

Electrochemical studies

The redox properties of the *N*-(ferrocenyl)naphthoyl amino acid esters **5–18** were investigated by cyclic voltammetry (CV) in

Table 3 Cyclic voltammetry data for compounds **5–18** in acetonitrile (1 mM compound, 0.1 M TBAP *vs.* Ag|AgCl. Scan rate = 100 mV s^{-1})

Compound	E_{pa}/V	E_{pc}/V	$E^{\circ\prime}$ *vs.* Fc/Fc$^+$/mV[a]	I_a/I_c [b]
5	0.502	0.429	13	1.03
6	0.492	0.429	8	1.06
7	0.502	0.435	16	1.09
8	0.495	0.430	10	0.99
9	0.540	0.449	42	1.23
10	0.528	0.461	42	1.06
11	0.527	0.462	42	1.04
12	0.534	0.463	46	1.04
13	0.482	0.417	24	1.02
14	0.489	0.420	29	1.01
15	0.486	0.419	27	1.03
16	0.521	0.445	63	1.03
17	0.518	0.448	58	1.01
18	0.518	0.449	58	1.03

[a] $E^{\circ\prime}$ refers to the formal potential and is calculated by taking the mean of the peak potentials for the anodic (a) and the cathodic wave (c). [b] I_a and I_c refer to the maximum peak currents of the anodic and the cathodic waves, respectively.

Table 4 Effect on the growth of melanoma (Sk-Mel-28) and NSCLC (H1299) cells

Compound	10 μM on Sk-Mel-28[a]	10 μM on H1299	1 μM on H1299
1	103.4 ± 0.6	72.9 ± 8.7	102.1 ± 13.3
2	89.8 ± 10.4	82.6 ± 3.2	117.5 ± 12.2
5	97.1 ± 8.1	43.7 ± 8.1	112.9 ± 14.6
6	99.5 ± 3.2	27.8 ± 3.4	98.8 ± 1.5
7	103.1 ± 3.7	22.4 ± 6.4	103.4 ± 4.5
8	82.9 ± 16.7	3.5 ± 0.6	85.8 ± 11.8
9	31.4 ± 10.1	6.0 ± 3.0	101.1 ± 8.9
10	89.6 ± 21.4	44.4 ± 6.9	91.4 ± 14.4
11	95.7 ± 9.5	57.3 ± 2.3	101.4 ± 2.2
12	95.6 ± 7.9	40.9 ± 4.2	102.3 ± 5.3
13	103.5 ± 2.9	63.8 ± 5.6	112.6 ± 16.5
14	92.3 ± 2.7	39.0 ± 5.4	105.0 ± 24.6
15	98.9 ± 5.5	57.0 ± 9.7	110.3 ± 9.2
16	69.4 ± 24.5	11.6 ± 0.5	109.6 ± 9.5
17	9.6 ± 1.6	3.0 ± 0.6	45.7 ± 3.6
18	33.1 ± 10.5	3.0 ± 0.9	62.1 ± 6.8

[a] The results are expressed relative to the growth of control cells (cells without added compounds, set at 100%) and represent the average of three independent experiments.

acetonitrile solution. Compounds **5–18** were all found to exhibit a one electron, reversible redox process similar to ferrocene, under the same conditions. The key features of their redox behaviour are summarised in Table 3. The peak current ratios (I_a/I_c) are in the 0.99–1.23 range, deviating from the ideal value of 1.0; ferrocene itself exhibits a current ratio of 1.05, under the same conditions. The $E^{\circ\prime}$ (oxidation potential) values for **5–8** and **13–15** were in the range of 8–29 mV, whilst **9–12** and **16–18** showed values in the 42–63 mV range *versus* the ferrocene/ferricenium redox couple (Fc/Fc$^+$). The observed $E^{\circ\prime}$ values are in line with those reported previously for the *N*-(ferrocenyl)naphthoyl dipeptide esters.[28] The naphthoyl linker of the *N*-(6-ferrocenyl-2-naphthoyl) amino acid and 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. In the case of the *N*-(3-ferrocenyl-2-naphthoyl) amino acid and dipeptide esters, only minimal conjugation can occur between the ferrocene and naphthalene units, due to steric restrictions imposed by the *ortho* relationship of the two substituents on the naphthyl ring. These conclusions are supported by observations made in both the UV-visible study of these derivatives and the X-ray crystallographic study of the intermediates **1** and **2**.

Biological evaluation

The *N*-(ferrocenyl)naphthoyl amino acid esters **5–18** have been prepared as part of an ongoing SAR study. Since the precise biological target of the *N*-(ferrocenyl)naphthoyl dipeptide esters has not yet been identified, the best approach is to synthesise a library of compounds in order to identify the molecular features which are favourable and those which are detrimental to the biological activity. Thus, the *in vitro* anti-proliferative effect of **5–18** was studied at a concentration of 10 μM in the Sk-Mel-28 metastatic melanoma cell line and the H1299 non-small cell lung cancer (NSCLC) cell line. The results of this biological study are reported in Table 4 and are expressed as % cell growth relative to the untreated controls; the values determined for the intermediates **1** and **2** have also been included for comparison. On the whole, the H1299 cell line showed a much greater sensitivity to these compounds than the Sk-Mel-28 cell line. Consequently, the *in vitro* anti-proliferative effect of **5–18** was also studied at a concentration of 1 μM in the H1299 cell line.

As expected, the intermediates **1** and **2** showed almost no anti-proliferative effect in either cell line (Table 4). This has also been reported to be the case for the *N*-(ferrocenyl)benzoyl peptide esters.[25] Thus, these results demonstrate conclusively that all three moieties are required for *in vitro* activity. In the Sk-Mel-28 cell line, compounds **5–8** and **10–15** did not have any noticeable effect on cell proliferation. Only the *N*-(6-ferrocenyl-2-naphthoyl) derivatives **9** and **16–18** showed an inhibitory effect on Sk-Mel-28 cell growth: the β-alanine derivative **16** had a weak anti-proliferative effect (~30% inhibition), the glycine derivative **9** and the δ-amino-n-valeric acid derivative **18** had a significant anti-proliferative effect (~70%), whilst the γ-aminobutyric acid derivative **17** displayed a strong anti-proliferative effect (90%).

Compounds **5–18** were shown to exert a significant, if not strong anti-proliferative effect in the H1299 cell line, when tested at the higher concentration of 10 μM. The effect of compounds **5–12** on H1299 cell proliferation follows a distinct pattern that correlates with the structure of these derivatives. For **5–8**, the anti-proliferative effect increases as the side chain of the α-amino acid side chain is enlarged, adding steric bulk. Thus, the L-phenylalanine derivative **8** showed the greatest anti-proliferative effect (~96%). In contrast, the anti-proliferative effect of compounds **9–12** declines as the side chain of the α-amino acid is extended. *N*-(6-ferrocenyl-2-naphthoyl)-glycine ethyl ester **9** displayed a stronger anti-proliferative effect (~94%) than **10–12**. Compounds **13–15** showed a weaker anti-proliferative effect in the H1299 cell line than **5–8**, however, these compounds still achieved an appreciable level of cell growth inhibition (30–60%). Of these compounds, the γ-aminobutyric acid derivative **14** showed the strongest anti-proliferative effect (~60%). On the other hand, compounds **16–18** showed a stronger anti-proliferative effect in

the H1299 cell line than **10–12**; cell growth was inhibited by at least 89%. Compounds **17** and **18** showed a similarly strong anti-proliferative effect (~97%) however, **17** was the only compound that inhibited H1299 cell growth by more than 50% at the lower concentration of 1 μM.

The IC_{50} values for *N*-(6-ferrocenyl-2-naphthoyl)-γ-aminobutyric acid ethyl ester **17** were determined in both cell lines, since this compound has been identified as the most active member of this group of compounds. In the H1299 cell line, compound **17** shows an IC_{50} value of 0.62 ± 0.07 μM; it is two times more potent than both cisplatin (1.5 ± 0.1 μM) and *N*-(6-ferrocenyl-2-naphthoyl)-glycine-L-alanine ethyl ester (1.3 ± 0.1 μM), the most active derivative reported previously.[28] In the Sk-Mel-28 cell line, compound **17** displays an IC_{50} value of 1.41 ± 0.04 μM. This is an extremely encouraging finding as metastatic melanoma is notoriously resistant to chemotherapeutic agents.

These results demonstrate that although the *N*-(3-ferrocenyl-2-naphthoyl) amino acid esters show good anticancer activity in the H1299 cell line, their effect in the Sk-Mel-28 cell line is weaker. For these derivatives, the anti-proliferative effect is greatest when chiral α-amino acids with bulky side chains are employed. This improvement in biological activity for **7** and **8** is not yet fully understood however, it is possible that the conformation adopted by **7** and **8** as a result of steric hindrance, alters the molecular properties in a way that is beneficial to the activity. In comparison, the *N*-(6-ferrocenyl-2-naphthoyl) amino acid esters show a greater anticancer activity in both the H1299 cell line and Sk-Mel-28 cell line. However, substitution at the α-carbon of the amino acid is not beneficial to the biological activity of the *N*-(6-ferrocenyl-2-naphthoyl) amino acid esters. The achiral amino acid derivatives **9** and **16–18**, which are devoid of steric bulk, show a greater anti-proliferative effect. These four derivatives form a homologous series, since they differ only by the number of methylene groups ($n = 1$–4) between the amide and ester functionalities. The weakest anti-proliferative effect is observed when $n = 2$, in the case of the β-alanine derivative **16**, whilst the greatest anti-proliferative effect is observed when $n = 3$, in the case of the γ-aminobutyric acid derivative **17**. In comparison to *N*-(6-ferrocenyl-2-naphthoyl)-glycine-L-alanine ethyl ester, the increased potency of **17** may be a consequence of an increase in lipophilicity or the conformational flexibility afforded by the γ-aminobutyric acid moiety.

With regard to the mode of action of these novel derivatives, we have previously postulated that the low redox potential of these compounds should facilitate the catalytic generation of reactive oxygenated species (ROS), under physiological conditions, leading to oxidative damage to DNA. This is possible *via* a Fenton-type reaction, in which hydroxyl radicals are generated from the superoxide dismutation product, hydrogen peroxide. Preliminary studies performed with *N*-(6-ferrocenyl-2-naphthoyl)-glycine-L-alanine ethyl ester indicate the formation of oxidative lesions typical of Fenton-reaction mediated oxidative damage to DNA. For these studies, DNA was incubated with *N*-(6-ferrocenyl-2-naphthoyl)-glycine-L-alanine ethyl ester and hydrogen peroxide, and 8-oxo-7,8-dihydroguanine, a key biomarker for oxidatively damaged DNA, was generated in a similar way to classical Fenton-mediated oxidative damage to DNA. These findings are extremely encouraging and a more comprehensive study into the mode of action of these derivatives is currently in progress.

Conclusion

A series of *N*-(ferrocenyl)naphthoyl amino acid esters **5–18** has been prepared and characterised. These novel compounds have been subjected to *in vitro* biological evaluation to assess their potential as anticancer agents for the treatment of lung cancer and malignant melanoma. *N*-(6-ferrocenyl-2-naphthoyl)-γ-aminobutyric acid ethyl ester **17** has been identified as having a potent effect on cell proliferation in both the H1299 NSCLC cell line (IC_{50} = 0.62 ± 0.07 μM) and the Sk-Mel-28 melanoma cell line (IC_{50} = 1.41 ± 0.04 μM). This represents a substantial improvement in the anticancer activity with respect to the *N*-(ferrocenyl)naphthoyl dipeptide esters and in addition, demonstrates that the biological activity of these novel compounds is not restricted to only one form of cancer. Further investigations with regard to both the structure–activity relationship and mode of action of these promising anticancer agents are underway.

Experimental

General remarks

All chemicals were purchased from Sigma-Aldrich, 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 either a Griffin melting point apparatus or 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 either a Perkin Elmer GX FT-IR or a Perkin Elmer 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). The residual solvent peaks have been used as an internal reference. All coupling constants (*J*) are quoted in Hz. The abbreviations for the peak multiplicities are as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), qt (quintet), m (multiplet) and br (broad). Electrospray ionisation mass spectra were performed on a Micromass LCT mass spectrometer or a Brüker Daltonics Esquire-LC ion trap mass spectrometer. Elemental analysis was carried out by the microanalytical laboratory at University College Dublin.

General procedure for the synthesis of the intermediates 1–4

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/red solid (1.23 g, 30%), mp 119–120 °C; [Found: C, 71.35; H, 4.9%; $M^{+\bullet}$, 370.4. $C_{22}H_{18}O_2Fe$ requires: C, 71.4; H, 4.9%; $M^{+\bullet}$, 370.4]; λ_{max}(EtOH)/nm 440 (ε/dm^3 mol^{-1} cm^{-1} 574); ν_{max}(KBr)/cm^{-1} 1720, 1494, 1201; δ_H(400 MHz; DMSO-d_6) 8.40 (1H, s, ArH), 8.07 (1H, s, ArH), 8.05 (1H, d, *J* 8.0, ArH), 7.97 (1H, d, *J* 8.0, ArH), 7.61 (1H, t, *J* 8.0, ArH), 7.54 (1H, t, *J* 8.0, ArH), 4.59 {2H, t, *J* 1.6, *ortho* on (η^5-C_5H_4)}, 4.35 {2H, t, *J* 1.6, *meta* on (η^5-C_5H_4)}, 4.10 (5H, s, η^5-C_5H_5), 3.77 (3H, s, –OCH_3); δ_C(100 MHz; DMSO-d_6) 169.4 (*C*=O), 134.2 (C_q), 133.5 (C_q), 130.4 (C_q), 130.3 (C_q), 129.2, 128.1, 128.0, 127.9, 127.5, 126.4, 85.0 (C_{ipso} η^5-C_5H_4), 69.6 (η^5-*C*$_5H_5$), 68.9 (C_{ortho} η^5-C_5H_4), 68.3 (C_{meta} η^5-C_5H_4), 52.2 (–O*C*H$_3$).

Methyl 6-ferrocenylnaphthalene-2-carboxylate (2). 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 (0.88 g, 21%), mp 158–159 °C; [Found: C, 71.6; H, 5.2%; $M^{+\bullet}$, 370.4. $C_{22}H_{18}O_2Fe$ requires: C, 71.4; H, 4.9%; $M^{+\bullet}$, 370.2]; λ_{max}(EtOH)/nm 380 (ε/dm^3 mol^{-1} cm^{-1} 3 211), 455 (1 523); ν_{max}(KBr)/cm^{-1} 1708, 1494, 1450, 1219; δ_H(400 MHz; DMSO-d_6) 8.58 (1H, s, ArH), 8.09 (1H, s, ArH), 8.07 (1H, d, *J* 8.8, ArH), 7.93–7.98 (2H, m, ArH), 7.85 (1H, dd, *J* 1.6 and 8.8, ArH), 4.99 {2H, t, *J* 1.6, *ortho* on (η^5-C_5H_4)}, 4.47 {2H, t, *J* 1.6, *meta* on (η^5-C_5H_4)}, 4.05 (5H, s, η^5-C_5H_5), 3.92 (3H, s, –OCH_3); δ_C(100 MHz; DMSO-d_6) 166.4 (*C*=O), 140.0 (C_q), 135.4 (C_q), 130.6 (C_q), 130.4, 129.2, 127.8, 126.1, 125.8 (C_q), 125.1, 122.7, 83.7 (C_{ipso} η^5-C_5H_4), 69.6 (C_{meta} η^5-C_5H_4), 69.5 (η^5-*C*$_5H_5$), 66.8 (C_{ortho} η^5-C_5H_4), 52.2 (–O*C*H$_3$).

3-Ferrocenylnaphthalene-2-carboxylic acid (3). 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 (0.81 g, 92%), mp (decomp.) at 145 °C; λ_{max}(EtOH)/nm 445 (ε/dm^3 mol^{-1} cm^{-1} 568); ν_{max}(KBr)/cm^{-1} 3430, 1698; δ_H(400 MHz; DMSO-d_6) 12.8 (1H, br.s, –COO*H*), 8.33 (1H, s, ArH), 8.00 (1H, d, *J* 8.0, ArH), 7.99 (1H, s, ArH), 7.95 (1H, d, *J* 8.0, ArH), 7.57 (1H, t, *J* 8.0, ArH), 7.52 (1H, t, *J* 8.0, ArH), 4.70 {2H, t, *J* 1.6, *ortho* on (η^5-C_5H_4)}, 4.34 {2H, t, *J* 1.6, *meta* on (η^5-C_5H_4)}, 4.10 (5H, s, η^5-C_5H_5); δ_C(100 MHz; DMSO-d_6) 170.8 (*C*=O), 134.3 (C_q), 133.9 (C_q), 133.1 (C_q), 130.5 (C_q), 128.8, 127.9, 127.4, 127.0, 126.1, 85.2 (C_{ipso} η^5-C_5H_4), 69.6 (η^5-*C*$_5H_5$), 69.1 (C_{meta} η^5-C_5H_4), 68.2 (C_{ortho} η^5-C_5H_4); *m*/*z* (ESI) 356.4 ($M^{+\bullet}$. $C_{21}H_{16}O_2Fe$ requires 356.2).

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 (0.63 g, 93%), mp (decomp.) at 205 °C; λ_{max}(EtOH)/nm 375 (ε/dm^3 mol^{-1} cm^{-1} 2 635), 450 (1 296); ν_{max}(KBr)/cm^{-1} 3435, 1682; δ_H(400 MHz; DMSO-d_6) 12.8 (1H, br.s, –COO*H*), 8.57 (1H, s, ArH), 8.07 (1H, s, ArH), 8.03 (1H, d, *J* 8.4, ArH), 7.94 (2H, s, ArH), 7.83 (1H, dd, *J* 1.6 and 8.4, ArH), 4.97 {2H, t, *J* 1.6, *ortho* on (η^5-C_5H_4)}, 4.45 {2H, t, *J* 1.6, *meta* on (η^5-C_5H_4)}, 4.04 (5H, s, η^5-C_5H_5); δ_C(100 MHz; DMSO-d_6) 167.5 (*C*=O), 139.7 (C_q), 135.3 (C_q), 130.6 (C_q), 130.4, 129.1, 127.6, 127.0 (C_q), 125.9, 125.5, 122.7, 83.8 (C_{ipso} η^5-C_5H_4), 69.6 (C_{meta} η^5-C_5H_4), 69.5 (η^5-*C*$_5H_5$), 66.7 (C_{ortho} η^5-C_5H_4); *m*/*z* (ESI) 356.4 ($M^{+\bullet}$. $C_{21}H_{16}O_2Fe$ requires 356.2).

General procedure for the synthesis of *N*-(ferrocenyl)naphthoyl amino acid ethyl esters 5–18

***N*-(3-ferrocenyl-2-naphthoyl)-glycine ethyl ester (5).** Glycine ethyl ester hydrochloride (0.17 g, 1.2 mmol) was added to a solution of 3-ferrocenylnaphthalene-2-carboxylic acid (0.43 g, 1.2 mmol), 1-hydroxybenzotriazole (0.22 g, 1.6 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 **5** (0.44 g, 83%), mp 105–106 °C; $E^{\circ\prime}$/mV, 13 *vs.* Fc/Fc^+; [Found: C, 67.7; H, 5.5; N, 3.25%; $M^{+\bullet}$, 441.4. $C_{25}H_{23}NO_3Fe$ requires: C, 68.0; H, 5.25; N, 3.2%; $M^{+\bullet}$, 441.3]; λ_{max}(EtOH)/nm 450 (ε/dm^3 mol^{-1} cm^{-1} 466); ν_{max}(KBr)/cm^{-1} 3296, 1736, 1647, 1544, 1191; δ_H(400 MHz; DMSO-d_6) 8.98 (1H, t, *J* 6.0, –CON*H*–), 8.35 (1H, s, ArH), 8.01 (1H, d, *J* 8.0, ArH), 7.93 (1H, d, *J* 8.0, ArH), 7.82 (1H, s, ArH), 7.56 (1H, t, *J* 8.0, ArH), 7.52 (1H, t, *J* 8.0, ArH), 4.83 {2H, t, *J* 1.6, *ortho* on (η^5-C_5H_4)}, 4.32 {2H, t, *J* 1.6, *meta* on (η^5-C_5H_4)}, 4.18 (2H, q, *J* 7.2, –OCH_2CH$_3$), 4.07 (5H, s, η^5-C_5H_5), 4.03 (2H, d, *J* 6.0, –NHCH_2–), 1.27 (3H, t, *J* 7.2, –OCH$_2$CH_3); δ_C(100 MHz; DMSO-d_6) 170.1 (*C*=O), 169.8 (*C*=O), 134.6 (C_q), 134.3 (C_q), 133.0 (C_q), 130.4 (C_q), 128.1, 127.7, 127.4, 127.1, 126.8, 126.1, 84.1 (C_{ipso} η^5-C_5H_4), 69.6 (η^5-*C*$_5H_5$), 68.9 (C_{ortho} η^5-C_5H_4), 68.3(C_{meta} η^5-C_5H_4), 60.6 (–O*C*H$_2$–, –ve DEPT), 41.1 (–NH*C*H$_2$–, –ve DEPT), 14.1 (–OCH$_2$*C*H$_3$).

***N*-(3-ferrocenyl-2-naphthoyl)-L-alanine ethyl ester (6).** The synthesis followed that of **5** using the following reagents: 3-ferrocenylnaphthalene-2-carboxylic acid (0.36 g, 1.0 mmol), 1-hydroxybenzotriazole (0.14 g, 1.0 mmol), triethylamine (0.5 mL), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.22 g, 1.0 mmol) and L-alanine ethyl ester hydrochloride (0.19 g, 1.2 mmol). 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, 95%), mp 49 °C; $[\alpha]_D^{20}$ +13 (*c* 0.01 in EtOH); $E^{\circ\prime}$/mV 8 *vs.* Fc/Fc$^+$; [Found: C, 68.8; H, 5.8; N, 3.3%; M$^{+\bullet}$, 455.5. $C_{26}H_{25}NO_3Fe$ requires: C, 68.6; H, 5.5; N, 3.1%; M$^{+\bullet}$, 455.3]; λ_{max}(EtOH)/nm 450 (ε/dm^3 mol^{-1} cm^{-1} 512); ν_{max}(KBr)/cm^{-1} 3288, 1734, 1647, 1544, 1494, 1200; δ_H(400 MHz; DMSO-d_6) 8.94 (1H, d, *J* 7.2, –CON*H*–), 8.34 (1H, s, ArH), 8.00 (1H, d, *J* 8.0, ArH), 7.93 (1H, d, *J* 8.0, ArH), 7.80 (1H, s, ArH), 7.50–7.58 (2H, m, ArH), 4.78–4.80 {2H, m, *ortho* on (η^5-C_5H_4)}, 4.45 (1H, qt, *J* 7.2, –NHC*H*–), 4.33–4.35 {1H, m, *meta* on (η^5-C_5H_4)}, 4.29–4.31 {1H, m, *meta* on (η^5-C_5H_4)}, 4.16 (2H, q, *J* 7.2, –OCH_2CH$_3$), 4.09 (5H, s, η^5-C_5H_5), 1.38 (3H, d, *J* 7.2, –CH_3), 1.27 (3H, t, *J* 7.2, –OCH$_2$CH_3); δ_C(100 MHz; DMSO-d_6) 172.5 (*C*═O), 169.3 (*C*═O), 134.7 (C_q), 134.3 (C_q), 132.9 (C_q), 130.4 (C_q), 128.2, 127.7, 127.4, 127.1, 126.8, 126.1, 84.3 (C_{ipso} η^5-C_5H_4), 69.5 (η^5-*C*$_5$H$_5$), 68.5 (C_{ortho} η^5-C_5H_4), 68.3 (C_{meta} η^5-C_5H_4), 68.2 (C_{meta} η^5-C_5H_4), 60.6 (–O*C*H$_2$–, –ve DEPT), 48.0 (α-*C*), 16.7 (–CH_3), 14.1 (–OCH$_2$*C*H$_3$).

***N*-(3-ferrocenyl-2-naphthoyl)-L-leucine ethyl ester (7).** The synthesis followed that of **5** using the following reagents: 3-ferrocenylnaphthalene-2-carboxylic acid (0.36 g, 1.0 mmol), 1-hydroxybenzotriazole (0.22 g, 1.7 mmol), triethylamine (0.5 mL), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.29 g, 1.5 mmol) and L-leucine ethyl ester hydrochloride (0.23 g, 1.2 mmol). 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 (0.16 g, 32%), mp 75–76 °C; $[\alpha]_D^{20}$ +6 (*c* 0.01 in EtOH); $E^{\circ\prime}$/mV 16 *vs.* Fc/Fc$^+$; λ_{max}(EtOH)/nm 445 (ε/dm^3 mol^{-1} cm^{-1} 627); ν_{max}(KBr)/cm^{-1} 3322, 1735, 1649, 1544, 1494, 1209; δ_H(400 MHz; DMSO-d_6) 8.87 (1H, d, *J* 7.6, –CON*H*–), 8.36 (1H, s, ArH), 8.01 (1H, d, *J* 8.0, ArH), 7.94 (1H, d, *J* 8.0, ArH), 7.74 (1H, s, ArH), 7.50–7.58 (2H, m, ArH), 4.75–4.76 {2H, m, *ortho* on (η^5-C_5H_4)}, 4.39–4.44 (1H, m, –NHC*H*–), 4.32–4.33 {1H, m, *meta* on (η^5-C_5H_4)}, 4.28–4.29 {1H, m, *meta* on (η^5-C_5H_4)}, 4.16 (2H, q, *J* 7.2, –OCH_2CH$_3$), 4.08 (5H, s, η^5-C_5H_5), 1.65–1.76 (2H, m, –CH_2CH–), 1.50–1.58 (1H, m, –CH$_2$C*H*–), 1.26 (3H, t, *J* 7.2, –OCH$_2$CH_3), 0.91–0.94 {6H, m, –CH(CH_3)$_2$}; δ_C(100 MHz; DMSO-d_6) 172.5 (*C*═O), 169.7 (*C*═O), 134.9 (C_q), 134.2 (C_q), 132.9 (C_q), 130.4 (C_q), 128.2, 127.7, 127.4, 127.1, 126.5, 126.1, 84.3 (C_{ipso} η^5-C_5H_4), 69.5 (η^5-*C*$_5$H$_5$), 68.4 (C_{ortho} η^5-C_5H_4), 68.2 (C_{meta} η^5-C_5H_4), 68.1 (C_{meta} η^5-C_5H_4), 60.5 (–O*C*H$_2$–, –ve DEPT), 50.8 (α-*C*), 39.4 (–*C*H$_2$–, –ve DEPT), 24.3 (–*C*H–), 22.9 (–CH_3), 21.2 (–CH_3), 14.1(–OCH$_2$*C*H$_3$); *m*/*z* (ESI) 498.1714 ([M+H]$^+$. $C_{29}H_{32}NO_3Fe$ requires 498.1732).

***N*-(3-ferrocenyl-2-naphthoyl)-L-phenylalanine ethyl ester (8).** The synthesis followed that of **5** using the following reagents: 3-ferrocenylnaphthalene-2-carboxylic acid (0.36 g, 1.0 mmol), 1-hydroxybenzotriazole (0.22 g, 1.7 mmol), triethylamine (0.5 mL), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.29 g, 1.5 mmol) and L-phenylalanine ethyl ester hydrochloride (0.24 g, 1.0 mmol). 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 (0.21 g, 40%), mp 48–49 °C; $[\alpha]_D^{20}$ –24 (*c* 0.01 in EtOH); $E^{\circ\prime}$/mV 10 *vs.* Fc/Fc$^+$; (Found: C, 72.15; H, 5.7; N, 2.6. $C_{32}H_{29}NO_3Fe$ requires: C, 72.3; H, 5.5; N, 2.6%); λ_{max}(EtOH)/nm 450 (ε/dm^3 mol^{-1} cm^{-1} 582); ν_{max}(KBr)/cm^{-1} 3342, 1734, 1645, 1544, 1494, 1193; δ_H(400 MHz; DMSO-d_6) 9.01 (1H, d, *J* 8.0, –CON*H*–), 8.29 (1H, s, ArH), 7.98 (1H, d, *J* 8.0, ArH), 7.82 (1H, d, *J* 8.0, ArH), 7.49–7.57 (2H, m, ArH), 7.47 (1H, s, ArH), 7.34–7.43 (5H, m, –CH$_2$*Ph*), 4.71–4.75 {2H, m, –NHC*H*–, (η^5-C_5H_4)}, 4.32–4.34 {1H, m, (η^5-C_5H_4)}, 4.22–4.24 {1H, m, (η^5-C_5H_4)}, 4.15–4.21 {3H, m, –OCH_2CH$_3$, (η^5-C_5H_4)}, 4.01 (5H, s, η^5-C_5H_5), 3.22 (1H, dd, *J* 7.6 and 13.6, –CH_2Ph), 3.01 (1H, dd, *J* 7.6 and 13.6, –CH_2Ph), 1.25 (3H, t, *J* 7.2, –OCH$_2$CH_3); δ_C(100 MHz; DMSO-d_6) 171.5 (*C*═O), 169.4 (*C*═O), 137.5 (C_q), 134.7 (C_q), 134.3 (C_q), 132.9 (C_q), 130.3 (C_q), 129.3, 128.3, 128.1, 127.5, 127.4, 127.1, 126.6, 126.5, 126.1, 83.9 (C_{ipso} η^5-C_5H_4), 69.5 (η^5-*C*$_5$H$_5$), 68.9 (η^5-C_5H_4), 68.8 (η^5-C_5H_4), 68.3 (η^5-C_5H_4), 68.1 (η^5-C_5H_4), 60.7 (–O*C*H$_2$–, –ve DEPT), 53.7 (α-*C*), 36.4 (–*C*H$_2$Ph, –ve DEPT), 14.1 (–OCH$_2$*C*H$_3$); *m*/*z* (ESI) 532.1572 ([M+H]$^+$. $C_{32}H_{30}NO_3Fe$ requires 532.1575).

***N*-(6-ferrocenyl-2-naphthoyl)-glycine ethyl ester (9).** The synthesis followed that of **5** using the following reagents: 6-ferrocenylnaphthalene-2-carboxylic acid (0.37 g, 1.0 mmol), 1-hydroxybenzotriazole (0.2 g, 1.5 mmol), triethylamine (0.5 mL), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.3 g, 1.6 mmol) and glycine ethyl ester hydrochloride (0.1 g, 0.7 mmol). 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 (0.19 g, 62%), mp 114–115 °C; $E^{\circ\prime}$/mV 42 *vs.* Fc/Fc$^+$; [Found: C, 67.8; H, 5.4; N, 3.15; M$^{+\bullet}$, 441.4. $C_{25}H_{23}NO_3Fe$ requires: C, 68.0; H, 5.25; N, 3.2%; M$^{+\bullet}$, 441.3]; λ_{max}(EtOH)/nm 375 (ε/dm^3 mol^{-1} cm^{-1} 2 683), 450 (1 149); ν_{max}(KBr)/cm^{-1} 3306, 1745, 1638, 1545, 1494, 1215; δ_H(400 MHz; DMSO-d_6) 9.08 (1H, t, *J* 6.0, –CON*H*–), 8.44 (1H, s, ArH), 8.07 (1H, s, ArH), 7.97 (1H, d, *J* 8.8, ArH), 7.94 (1H, d, *J* 8.4, ArH), 7.92 (1H, dd, *J* 1.6 and 8.4, ArH), 7.83 (1H, dd, *J* 1.6 and 8.8, ArH), 4.96 {2H, t, *J* 1.6, *ortho* on (η^5-C_5H_4)}, 4.45 {2H, t, *J* 1.6, *meta* on (η^5-C_5H_4)}, 4.15 (2H, q, *J* 7.2, –OCH_2CH$_3$), 4.04–4.07 (7H, m, –NHCH_2–, η^5-C_5H_5), 1.22 (3H, t, *J* 7.2, –OCH$_2$CH_3); δ_C(100 MHz; DMSO-d_6): 169.9 (*C*═O), 166.7 (*C*═O), 138.9 (C_q), 134.6 (C_q), 130.6 (C_q), 130.1 (C_q), 128.8, 127.6, 127.5, 125.9, 124.3, 122.7, 84.0 (C_{ipso} η^5-C_5H_4), 69.5 (η^5-*C*$_5$H$_5$), 69.4 (C_{meta} η^5-C_5H_4), 66.6 (C_{ortho} η^5-C_5H_4), 60.5 (–O*C*H$_2$–, –ve DEPT), 41.4 (–NH*C*H$_2$–, –ve DEPT), 14.1 (–OCH$_2$*C*H$_3$).

***N*-(6-ferrocenyl-2-naphthoyl)-L-alanine ethyl ester (10).** The synthesis followed that of **5** using the following reagents: 6-ferrocenylnaphthalene-2-carboxylic acid (0.37 g, 1.0 mmol), 1-hydroxybenzotriazole (0.2 g, 1.5 mmol), triethylamine (0.5 mL), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.29 g, 1.5 mmol) and L-alanine ethyl ester hydrochloride (0.14 g, 0.9 mmol). 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 (0.34 g, 83.3%), mp 57–58 °C; $[\alpha]_D^{20}$ +32 (*c* 0.01 in EtOH); $E^{\circ\prime}$/mV 42 *vs.* Fc/Fc$^+$; (Found: C, 68.1; H, 5.8; N, 2.95. $C_{26}H_{25}NO_3Fe$ requires: C, 68.6; H, 5.5; N, 3.1%); λ_{max}(EtOH)/nm 375 (ε/dm^3 mol^{-1} cm^{-1} 3 636), 450 (1 614); ν_{max}(KBr)/cm^{-1} 3442, 1734, 1638, 1542, 1494, 1204; δ_H(400 MHz; DMSO-d_6) 8.96 (1H, d, *J* 7.2, –CON*H*–), 8.51 (1H, s, ArH), 8.12 (1H, s, ArH), 8.02 (1H, d, *J* 8.8, ArH), 7.99–8.00 (2H, m, ArH), 7.88 (1H, dd, *J* 1.6 and 8.8, ArH), 5.01 {2H, t, *J* 2.0, *ortho* on (η^5-C_5H_4)}, 4.57 (1H, qt, *J* 7.2, –NHC*H*–), 4.50 {2H, t, *J* 2.0, *meta* on (η^5-C_5H_4)}, 4.19 (2H, q, *J* 7.2, –OCH_2CH$_3$), 4.10 (5H, s, η^5-C_5H_5), 1.51 (3H, d, *J* 7.2, –CH_3), 1.27 (3H, t, *J* 7.2, –OCH$_2$CH_3); δ_C(100 MHz; DMSO-d_6) 172.7 (*C*═O), 166.4 (*C*═O), 138.9 (C_q), 134.6 (C_q), 130.6 (C_q), 130.1 (C_q), 128.7, 127.6, 127.4, 126.0, 124.6, 122.7, 84.0 (C_{ipso} η^5-C_5H_4), 69.5 (η^5-*C*$_5$H$_5$), 69.4

(C_{meta} η⁵-C_5H_4), 66.6 (C_{ortho} η⁵-C_5H_4), 60.4 (–OCH_2–, –ve DEPT), 48.5 (α-*C*), 16.7 (–CH_3), 14.1 (–OCH_2CH_3); *m*/*z* (ESI) 456.1265 ([M+H]⁺. $C_{26}H_{26}NO_3Fe$ requires 456.1262).

***N*-(6-ferrocenyl-2-naphthoyl)-L-leucine ethyl ester (11).** The synthesis followed that of **5** using the following reagents: 6-ferrocenylnaphthalene-2-carboxylic acid (0.30 g, 0.9 mmol), 1-hydroxybenzotriazole (0.14 g, 1.0 mmol), triethylamine (0.5 mL), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.21 g, 1.1 mmol) and L-leucine ethyl ester hydrochloride (0.2 g, 1.0 mmol). 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 (0.14 g, 33.3%), mp 109 °C; $[\alpha]_D^{20}$ +12 (*c* 0.01 in EtOH); *E*°′/mV 42 *vs.* Fc/Fc⁺; (Found: C, 70.35; H, 6.7; N, 2.6. $C_{29}H_{31}NO_3Fe$ requires: C, 70.0; H, 6.3; N, 2.8%); λ_{max}(EtOH)/nm 375 (ε/dm³ mol⁻¹ cm⁻¹ 3 256), 450 (1 395); ν_{max}(KBr)/cm⁻¹ 3437, 1733, 1638, 1544, 1493, 1200; δ_H(400 MHz; DMSO-d_6) 8.82 (1H, d, *J* 7.6, –CON*H*–), 8.45 (1H, s, ArH), 8.06 (1H, s, ArH), 7.97 (1H, d, *J* 8.4, ArH), 7.93 (2H, s, ArH), 7.83 (1H, dd, *J* 1.6 and 8.4, ArH), 4.96 {2H, t, *J* 1.6, *ortho* on (η⁵-C_5H_4)}, 4.52–4.57 (1H, m, –NHC*H*–), 4.45 {2H, t, *J* 1.6, *meta* on (η⁵-C_5H_4)}, 4.09–4.17 (2H, m, –O$CH_2$$CH_3$), 4.05 (5H, s, η⁵-$C_5H_5$), 1.73–1.88 (2H, m, –$CH_2$CH–), 1.58–1.65 (1H, m, –CH_2C*H*–), 1.21 (3H, t, *J* 7.2, –OCH_2CH_3), 0.96 {3H, d, *J* 6.4, –CH(CH_3)$_2$}, 0.91 {3H, d, *J* 6.4, –CH(CH_3)$_2$}; δ_C(100 MHz; DMSO-d_6) 172.7 (*C*═O), 166.7 (*C*═O), 138.9 (C_q), 134.6 (C_q), 130.6 (C_q), 130.2 (C_q), 128.7, 127.6, 127.3, 125.9, 124.6, 122.7, 84.0 (C_{ipso} η⁵-C_5H_4), 69.5 (η⁵-C_5H_5), 69.4 (C_{meta} η⁵-C_5H_4), 66.6 (C_{ortho} η⁵-C_5H_4), 60.4 (–OCH_2–, –ve DEPT), 51.1 (α-*C*), 39.3 (–CH_2–, –ve DEPT), 24.5 (–CH–), 22.9 (–CH_3), 21.2 (–CH_3), 14.1 (–OCH_2CH_3); *m*/*z* (ESI) 498.1712 ([M+H]⁺. $C_{29}H_{32}NO_3Fe$ requires 498.1732).

***N*-(6-ferrocenyl-2-naphthoyl)-L-phenylalanine ethyl ester (12).** The synthesis followed that of **5** using the following reagents: 6-ferrocenylnaphthalene-2-carboxylic acid (0.30 g, 0.9 mmol), 1-hydroxybenzotriazole (0.14 g, 1.0 mmol), triethylamine (0.5 mL), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.21 g, 1.1 mmol) and L-phenylalanine ethyl ester hydrochloride (0.26 g, 1.1 mmol). 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 (0.38 g, 77.8%), mp 151–152 °C; $[\alpha]_D^{20}$ +52 (*c* 0.01 in EtOH); *E*°′/mV 46 *vs.* Fc/Fc⁺; (Found: C, 72.3; H, 5.55; N, 2.6. $C_{32}H_{29}NO_3Fe$ requires: C, 72.3; H, 5.5; N, 2.6%); λ_{max}(EtOH)/nm 375 (ε/dm³ mol⁻¹ cm⁻¹ 3 696), 450 (1 582); ν_{max}(KBr)/cm⁻¹ 3435, 1741, 1623, 1544, 1494, 1208; δ_H(400 MHz; DMSO-d_6) 8.95 (1H, d, *J* 7.6, –CON*H*–), 8.38 (1H, s, ArH), 8.05 (1H, s, ArH), 7.95 (1H, d, *J* 8.4, ArH), 7.92 (1H, d, *J* 8.8, ArH), 7.86 (1H, dd, *J* 1.6 and 8.4, ArH), 7.82 (1H, dd, *J* 1.6 and 8.4, ArH), 7.21–7.35 (5H, m, –CH_2*Ph*), 4.96 {2H, t, *J* 1.6, *ortho* on (η⁵–C_5H_4)}, 4.68–4.74 (1H, m, –NHC*H*–), 4.45 {2H, t, *J* 1.6, *meta* on (η⁵-C_5H_4)}, 4.11 (2H, q, *J* 7.2, –O$CH_2$$CH_3$), 4.05 (5H, s, η⁵-$C_5H_5$), 3.16–3.19 (2H, m, –$CH_2$Ph), 1.14 (3H, t, *J* 7.2, –OCH_2CH_3); δ_C(100 MHz; DMSO-d_6) 171.7 (*C*═O), 166.5 (*C*═O), 138.9 (C_q), 137.7 (C_q), 134.6 (C_q), 130.6 (C_q), 130.1 (C_q), 129.1, 128.7, 128.2, 127.6, 127.4, 126.5, 125.9, 124.5, 122.7, 83.9 (C_{ipso} η⁵-C_5H_4), 69.5 (η⁵-C_5H_5), 69.4 (C_{meta} η⁵-C_5H_4), 66.6 (C_{ortho} η⁵-C_5H_4), 60.5 (–OCH_2–, –ve DEPT), 54.5 (α-*C*), 36.4 (–CH_2Ph, –ve DEPT), 14.0 (–OCH_2CH_3); *m*/*z* (ESI) 532.1552 ([M+H]⁺. $C_{32}H_{30}NO_3Fe$ requires 532.1575).

***N*-(3-ferrocenyl-2-naphthoyl)-β-alanine ethyl ester (13).** The synthesis followed that of **5** using the following reagents: 3-ferrocenylnaphthalene-2-carboxylic acid (0.37 g, 1 mmol), 1-hydroxybenzotriazole (0.16 g, 1.2 mmol), triethylamine (0.5 mL), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.23 g, 1.2 mmol) and β-alanine ethyl ester hydrochloride (0.15 g, 1 mmol). 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, 40%), mp 117 °C; *E*°′/mV 24 *vs.* Fc/Fc⁺; (Found: C, 68.1; H, 5.7; N, 3.05. $C_{26}H_{25}NO_3Fe$ requires: C, 68.6; H, 5.5; N, 3.1%); λ_{max}(CH_3CN)/nm 435 (ε/dm³ mol⁻¹ cm⁻¹ 493); ν_{max}/cm⁻¹ 3234, 3064, 1721, 1629, 1557, 1192; δ_H(400 MHz; DMSO-d_6) 8.50 (1H, t, *J* 5.2, –CON*H*–), 8.32 (1H, s, ArH), 8.00 (1H, d, *J* 8.0, ArH), 7.89 (1H, d, *J* 8.0, ArH), 7.73 (1H, s, ArH), 7.49–7.56 (2H, m, ArH), 4.72 {2H, s, *ortho* on (η⁵-C_5H_4)}, 4.33 {2H, s, *meta* on (η⁵-C_5H_4)}, 4.09–4.13 (7H, m, –O$CH_2$$CH_3$, η⁵-$C_5H_5$), 3.44–3.51 (2H, m, –$CH_2$$CH_2$–), 2.57 (2H, t, *J* 6.8, –CH_2C*H*$_2$–), 1.23 (3H, t, *J* 7.2, –OCH_2CH_3); δ_C(100 MHz; DMSO-d_6) 171.2 (*C*═O), 169.7 (*C*═O), 135.5 (C_q), 134.0 (C_q), 132.8 (C_q), 130.5 (C_q), 128.1, 127.6, 127.4, 126.9, 126.3, 126.0, 84.5 (C_{ipso} η⁵-C_5H_4), 69.5 (η⁵-C_5H_5), 68.8 (C_{ortho} η⁵-C_5H_4), 68.3 (C_{meta} η⁵-C_5H_4), 60.0 (–OCH_2–, –ve DEPT), 35.3 (–$CH_2$$CH_2$–, –ve DEPT), 33.5 (–$CH_2$$CH_2$–, –ve DEPT), 14.1 (–$OCH_2CH_3$); *m*/*z* (ESI) 456.1268 ([M+H]⁺. $C_{26}H_{26}NO_3Fe$ requires 456.1262).

***N*-(3-ferrocenyl-2-naphthoyl)-γ-aminobutyric acid ethyl ester (14).** The synthesis followed that of **5** using the following reagents: 3-ferrocenylnaphthalene-2-carboxylic acid (0.37 g, 1 mmol), 1-hydroxybenzotriazole (0.18 g, 1.3 mmol), triethylamine (0.5 mL), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.25 g, 1.3 mmol) and γ-aminobutyric acid ethyl ester hydrochloride (0.17 g, 1 mmol). 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 (0.17 g, 36%), mp 104–105 °C; *E*°′/mV 29 *vs.* Fc/Fc⁺; (Found: C, 68.8; H, 5.8; N, 2.9. $C_{27}H_{27}NO_3Fe$ requires: C, 69.1; H, 5.8; N, 3.0%); λ_{max}(CH_3CN)/nm 435 (ε/dm³ mol⁻¹ cm⁻¹ 294); ν_{max}/cm⁻¹ 3307, 3086, 1730, 1628, 1535, 1166; δ_H(400 MHz; DMSO-d_6) 8.40 (1H, t, *J* 5.6, –CON*H*–), 8.33 (1H, s, ArH), 7.99 (1H, d, *J* 8.0, ArH), 7.92 (1H, d, *J* 7.6, ArH), 7.76 (1H, s, ArH), 7.48–7.56 (2H, m, ArH), 4.73 {2H, t, *J* 2.0, *ortho* on (η⁵-C_5H_4)}, 4.32 {2H, t, *J* 2.0, *meta* on (η⁵-C_5H_4)}, 4.06–4.11 (7H, m, –O$CH_2$$CH_3$, η⁵-$C_5H_5$), 3.23 (2H, q, *J* 5.6, –$CH_2$$CH_2$$CH_2$–), 2.35 (2H, t, *J* 7.2, –$CH_2$$CH_2$C*H*$_2$–), 1.76 (2H, qt, *J* 7.2, –CH_2C*H*$_2$$CH_2$–), 1.20 (3H, t, *J* 7.2, –OCH_2CH_3); δ_C(100 MHz; DMSO-d_6) 172.6 (*C*═O), 169.7 (*C*═O), 135.8 (C_q), 133.9 (C_q), 132.8 (C_q), 130.6 (C_q), 128.0, 127.6, 127.3, 126.9, 126.1, 126.0, 84.5 (C_{ipso} η⁵-C_5H_4), 69.5 (η⁵-C_5H_5), 68.8 (C_{ortho} η⁵-C_5H_4), 68.2 (C_{meta} η⁵-C_5H_4), 59.7 (–OCH_2–, –ve DEPT), 38.3 (–$CH_2$$CH_2$$CH_2$–, –ve DEPT), 31.0 (–$CH_2$$CH_2CH_2$–, –ve DEPT), 24.2 (–$CH_2CH_2$$CH_2$–, –ve DEPT), 14.1 (–$OCH_2CH_3$); *m*/*z* (ESI) 470.1411 ([M+H]⁺. $C_{27}H_{28}NO_3Fe$ requires 470.1419).

***N*-(3-ferrocenyl-2-naphthoyl)-δ-amino-n-valeric acid ethyl ester (15).** The synthesis followed that of **5** using the following reagents: 3-ferrocenylnaphthalene-2-carboxylic acid (0.37 g, 1 mmol), 1-hydroxybenzotriazole (0.16 g, 1.2 mmol), triethylamine (0.5 mL), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.23 g, 1.2 mmol) and δ-amino-n-valeric acid ethyl

ester hydrochloride (0.18 g, 1 mmol). The product was purified by column chromatography (eluant 1 : 1 hexane: ethyl acetate – 100% ethyl acetate) to give the title compound as an orange oil (0.17 g, 35%), mp 79 °C; $E^{\circ\prime}$/mV 27 *vs.* Fc/Fc$^+$; (Found: C, 69.3; H, 6.0; N, 2.9. $C_{28}H_{29}NO_3Fe$ requires: C, 69.6; H, 6.05; N, 2.9%); λ_{max}(CH_3CN)/nm 450 (ε/dm^3 mol^{-1} cm^{-1} 508); ν_{max}/cm^{-1} 3282, 3093, 1726, 1628, 1638, 1538; δ_H(400 MHz; DMSO-d_6) 8.38 (1H, t, *J* 5.6, –CON*H*–), 8.32 (1H, s, ArH), 7.99 (1H, d, *J* 8.0, ArH), 7.91 (1H, d, *J* 8.0, ArH), 7.74 (1H, s, ArH), 7.48–7.56 (2H, m, ArH), 4.74 {2H, t, *J* 1.6, *ortho* on (η^5-C_5H_4)}, 4.33 {2H, t, *J* 1.6, *meta* on (η^5-C_5H_4)}, 4.05–4.12 (7H, m, η^5-C_5H_5, –OCH_2CH$_3$), 3.22 (2H, q, *J* 5.6, –CH_2CH$_2$CH$_2$CH$_2$–), 2.33 (2H, t, *J* 7.2, –CH$_2$CH$_2$CH$_2$CH_2–), 1.48–1.62 (4H, m, –CH$_2$CH_2CH_2CH$_2$–), 1.20 (3H, t, *J* 7.2, –OCH$_2$CH_3); δ_C(100 MHz; DMSO-d_6) 172.8 (*C*═O), 169.6 (*C*═O), 135.9 (C_q), 133.9 (C_q), 132.8 (C_q), 130.6 (C_q), 127.9, 127.6, 127.3, 126.8, 126.1, 125.9, 84.5 (C_{ipso} η^5-C_5H_4), 69.5 (η^5-*C*$_5$H$_5$), 68.8 (C_{ortho} η^5-C_5H_4), 68.2 (C_{meta} η^5-C_5H_4), 59.7 (–O*C*H$_2$–, –ve DEPT), 38.6 (–*C*H$_2$CH$_2$CH$_2$CH$_2$–, –ve DEPT), 33.2 (–CH$_2$CH$_2$CH$_2$*C*H$_2$–, –ve DEPT), 28.2 (–CH$_2$*C*H$_2$CH$_2$CH$_2$–, –ve DEPT), 22.0 (–CH$_2$CH$_2$*C*H$_2$CH$_2$–, –ve DEPT), 14.1 (–OCH$_2$*C*H$_3$); *m*/*z* (ESI) 484.1566 ([M+H]$^+$. $C_{28}H_{30}NO_3Fe$ requires 484.1575).

***N*-(6-ferrocenyl-2-naphthoyl)-β-alanine ethyl ester (16).** The synthesis followed that of **5** using the following reagents: 6-ferrocenylnaphthalene-2-carboxylic acid (0.37 g, 1 mmol), 1-hydroxybenzotriazole (0.16 g, 1.2 mmol), triethylamine (0.5 mL), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.23 g, 1.2 mmol) and β-alanine ethyl ester hydrochloride (0.15 g, 1 mmol). 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 (0.29 g, 64%), mp 123–124 °C; $E^{\circ\prime}$/mV 63 *vs.* Fc/Fc$^+$; (Found: C, 67.8; H, 5.6; N, 3.0. $C_{26}H_{25}NO_3Fe$ requires: C, 68.6; H, 5.5; N, 3.1%); λ_{max}(CH_3CN)/nm 360 (ε/dm^3 mol^{-1} cm^{-1} 2 818), 435 (1 168); ν_{max}/cm^{-1} 3301, 3095, 1733, 1637, 1625, 1538; δ_H(400 MHz; DMSO-d_6) 8.70 (1H, t, *J* 5.6, –CON*H*–), 8.38 (1H, s, ArH), 8.05 (1H, s, ArH), 7.88–7.96 (3H, m, ArH), 7.81 (1H, dd, *J* 1.6 and 8.4, ArH), 4.95 {2H, t, *J* 2.0, *ortho* on (η^5-C_5H_4)}, 4.44 {2H, t, *J* 2.0, *meta* on (η^5-C_5H_4)}, 4.08 (2H, q, *J* 7.2, –OCH_2CH$_3$), 4.04 (5H, s, η^5-C_5H_5), 3.56 (2H, q, *J* 6.8, –CH_2CH$_2$–), 2.63 (2H, t, *J* 6.8, –CH$_2$CH_2–), 1.19 (3H, t, *J* 7.2, –OCH$_2$CH_3); δ_C(100 MHz; DMSO-d_6) 171.3 (*C*═O), 166.4 (*C*═O), 138.7 (C_q), 134.5 (C_q), 130.8 (C_q), 130.7 (C_q), 128.7, 127.3, 127.2, 125.9, 124.4, 122.7, 84.0 (C_{ipso} η^5-C_5H_4), 69.4 (η^5-*C*$_5$H$_5$, C_{meta} η^5-C_5H_4) 66.6 (C_{ortho} η^5-C_5H_4), 59.9 (–O*C*H$_2$–, –ve DEPT), 35.6 (–*C*H$_2$CH$_2$–, –ve DEPT), 33.8 (–CH$_2$*C*H$_2$–, –ve DEPT), 14.1 (–OCH$_2$*C*H$_3$); *m*/*z* (ESI) 456.1240 ([M+H]$^+$. $C_{26}H_{26}NO_3Fe$ requires 456.1262).

***N*-(6-ferrocenyl-2-naphthoyl)-γ-aminobutyric acid ethyl ester (17).** The synthesis followed that of **5** using the following reagents: 6-ferrocenylnaphthalene-2-carboxylic acid (0.37 g, 1 mmol), 1-hydroxybenzotriazole (0.18 g, 1.3 mmol), triethylamine (0.5 mL), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.25 g, 1.3 mmol) and γ-aminobutyric acid ethyl ester hydrochloride (0.17 g, 1 mmol). 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 (0.38 g, 81%), mp 117 °C; $E^{\circ\prime}$/mV 58 *vs.* Fc/Fc$^+$; (Found: C, 68.9; H, 5.8; N, 3.0. $C_{27}H_{27}NO_3Fe$ requires: C, 69.1; H, 5.8; N, 3.0%); λ_{max}(CH_3CN)/nm 335 (ε/dm^3 mol^{-1} cm^{-1} 2 532), 435 (1 038); ν_{max}/cm^{-1} 3397, 3101, 1729, 1706, 1654, 1626, 1527, 1179; δ_H(400 MHz; DMSO-d_6) 8.63 (1H, t, *J* 5.6, –CON*H*–), 8.39 (1H, s, ArH), 8.04 (1H, s, ArH), 7.89–7.96 (3H, m, ArH), 7.81 (1H, dd, *J* 1.6 and 8.4, ArH), 4.94 {2H, t, *J* 2.0, *ortho* on (η^5-C_5H_4)}, 4.43 {2H, t, *J* 2.0, *meta* on (η^5-C_5H_4)}, 4.03–4.08 (7H, m, –OCH_2CH$_3$, η^5-C_5H_5), 3.34 (2H, q, *J* 5.6, –CH_2CH$_2$CH$_2$–), 2.39 (2H, t, *J* 7.2, –CH$_2$CH$_2$CH_2–), 1.83 (2H, qt, *J* 7.2, –CH$_2$CH_2CH$_2$–), 1.18 (3H, t, *J* 7.2, –OCH$_2$CH_3); δ_C(100 MHz; DMSO-d_6) 172.7 (*C*═O), 166.3 (*C*vO), 138.7 (C_q), 134.4 (C_q), 131.0 (C_q), 130.7 (C_q), 128.7, 127.3, 127.2, 125.9, 124.5, 122.7, 84.1 (C_{ipso} η^5-C_5H_4), 69.4 (η^5-*C*$_5$H$_5$, C_{meta} η^5-C_5H_4), 66.6 (C_{ortho} η^5-C_5H_4), 59.8 (–O*C*H$_2$–, –ve DEPT), 38.6 (–*C*H$_2$CH$_2$CH$_2$–, –ve DEPT), 31.1 (–CH$_2$CH$_2$*C*H$_2$–, –ve DEPT), 24.5 (–CH$_2$*C*H$_2$CH$_2$–, –ve DEPT), 14.1 (–OCH$_2$*C*H$_3$); *m*/*z* (ESI) 470.1417 ([M+H]$^+$. $C_{27}H_{28}NO_3Fe$ requires 470.1419).

***N*-(6-ferrocenyl-2-naphthoyl)-δ-amino-n-valeric acid ethyl ester (18).** The synthesis followed that of **5** using the following reagents: 6-ferrocenylnaphthalene-2-carboxylic acid (0.37 g, 1 mmol), 1-hydroxybenzotriazole (0.16 g, 1.2 mmol), triethylamine (0.5 mL), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.23 g, 1.2 mmol) and δ-amino-n-valeric acid ethyl ester hydrochloride (0.18 g, 1 mmol). 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 (0.22 g, 46%), mp 65–66 °C; $E^{\circ\prime}$/mV 58 *vs.* Fc/Fc$^+$; (Found: C, 69.7; H, 6.1; N, 2.9. $C_{28}H_{29}NO_3Fe$ requires: C, 69.6; H, 6.05; N, 2.9%); λ_{max}(CH_3CN)/nm 370 (ε/dm^3 mol^{-1} cm^{-1} 3 204), 450 (1 304); ν_{max}/cm^{-1} 3321, 3095, 1727, 1634, 1602, 1536, 1157; δ_H(400 MHz; DMSO-d_6) 8.62 (1H, t, *J* 5.6, –CON*H*–), 8.38 (1H, s, –ArH), 8.05 (1H, s, –ArH), 7.86–7.96 (3H, m, –ArH), 7.81 (1H, dd, *J* 2.0 and 8.0, –ArH), 4.96 {2H, t, *J* 1.6, *ortho* on (η^5-C_5H_4)}, 4.45 {2H, t, *J* 1.6, *meta* on (η^5-C_5H_4)}, 4.02–4.08 (7H, m, –OCH_2CH$_3$, η^5-C_5H_5), 3.31 (2H, m, –CH_2CH$_2$CH$_2$CH$_2$–), 2.35 (2H, t, *J* 7.2, –CH$_2$CH$_2$CH$_2$CH_2–), 1.56–1.62 (4H, m, –CH$_2$CH_2CH_2CH$_2$–), 1.17 (3H, t, *J* 7.2, –OCH$_2$CH_3); δ_C(100 MHz; DMSO-d_6) 172.8 (*C*═O), 166.2 (*C*═O), 138.6 (C_q), 134.4 (C_q), 131.1 (C_q), 130.7 (C_q), 128.7, 127.3, 127.1, 125.9, 124.5, 122.7, 84.1 (C_{ipso} η^5-C_5H_4), 69.4 (η^5-*C*$_5$H$_5$, C_{meta} η^5-C_5H_4), 66.6 (C_{ortho} η^5-C_5H_4), 59.7 (–O*C*H$_2$–, –ve DEPT), 38.8 (–*C*H$_2$CH$_2$CH$_2$CH$_2$–, –ve DEPT), 33.2 (–CH$_2$CH$_2$CH$_2$*C*H$_2$–, –ve DEPT), 28.6 (–CH$_2$*C*H$_2$CH$_2$CH$_2$–, –ve DEPT), 22.0 (–CH$_2$CH$_2$*C*H$_2$CH$_2$–, –ve DEPT), 14.1 (–OCH$_2$*C*H$_3$); *m*/*z* (ESI) 484.1553 ([M+H]$^+$. $C_{28}H_{30}NO_3Fe$ requires 484.1575).

X-ray crystallographic studies

Single crystals suitable for X-ray analysis for the starting materials **1** and **2** were grown from a hexane–diethyl ether mixture at room temperature. Good-quality single crystals were secured to a thin glass fibre using immersion oil (NVH) and analysis was made with a Bruker SMART APEX CCD area detector and a normal focus Mo-target X-ray tube (λ = 0.71073 Å). The room temperature data were processed using SAINT,[32] and absorption corrections applied using SADABS.[33] The structure solution was carried out by direct methods, and refinements were performed by full-matrix least-squares on F^2 using the SHELXTL-PLUS suite of programs.[34,35] The non-hydrogen atoms were refined anisotropically, and the hydrogen atoms were fixed using HFIX and refined isotropically.

Electrochemical studies

Cyclic voltammograms were recorded in anhydrous 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|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. Sample solutions (1 mM) containing TBAP (0.1 M) were prepared in acetonitrile. Typically, the sample solution (5 mL) was pipetted into an electrochemical cell, the electrodes were inserted into the receptor solution and the cyclic voltammograms was recorded by scanning voltage in a predefined range (*e.g.*, 0.0–0.8 V) at a scan rate of 100 mV s^{-1}. The concentration range of the ferrocene compounds was 1.0 mM in acetonitrile. A CV scan of a sample of ferrocene (1 mM) with TBAP (0.1 M) in acetonitrile was also recorded before each experiment to obtain $E^{\circ\prime}$ (Fc/Fc^{+}), and the $E^{\circ\prime}$ values obtained for the test samples were referenced relative to the ferrocene/ferricenium redox couple.

Cell culture

Cell lines. Sk-Mel-28 was obtained from the Department of Developmental Therapeutics, National Cancer Institute (NCI) and H1299 from the American Tissue Culture Centre (ATCC). Cell lines were grown in RPMI-1640 supplemented with 10% fetal calf serum (FCS) at 37 °C in a 5% CO_2 humidified chamber.

***In vitro* proliferation assay.** 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 μM stock solution of a test sample was prepared in dimethyl sulfoxide; dilute solutions of the test sample were prepared at 2x 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 growth in the presence of the ferrocenyl derivatives **1–2** and **5–18** was determined by the acid phosphatase assay.[36] The percentage cell growth in the presence of each compound was determined relative to the control cells. The concentration of **17** causing a 50% growth inhibition (IC_{50} of the compound) was determined using Calcusyn (Biosoft, UK).

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