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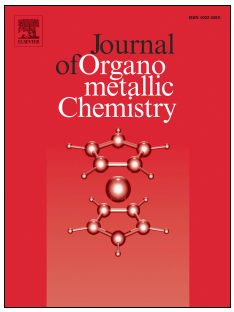

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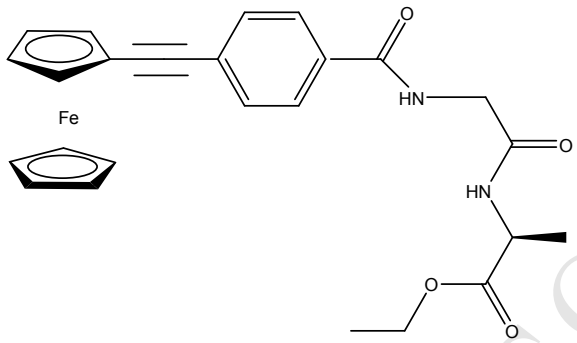

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The synthesis, structural characterization and biological evaluation of novel *N*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide methyl and ethyl esters as anticancer agents.

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Abstract

A series of *N*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide methyl and ethyl esters **4-18** were prepared by coupling *para*-(ferrocenyl) ethynyl benzoic acid **3** to the amino acids GABA(OMe), GABA(OEt) and the dipeptide esters GlyGly(OMe), GlyGly(OEt), Gly-L-Ala(OMe), Gly-L-Ala(OEt), Gly-D-Ala(OMe), Gly-D-Ala(OEt), Gly-L-Leu(OEt), Gly-L-Phe(OEt), SarGly(OMe), SarGly(OEt), Sar-L-Ala(OEt), L-ProGly(OEt) and L-Pro-L-Ala(OEt) using the standard *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBt) protocol. All the compounds were fully characterized using a combination of I.R., UV-Vis, ^{1}H NMR, ^{13}C NMR, DEPT-135, ^{1}H-^{13}C COSY (HMQC) spectroscopy and electrospray ionization mass spectrometry (ESI-MS). Selected compounds **5**, **7**, **9, 11, 16, 17** and **18** showed micromolar activity in the H1299 NSCLC cell line, with IC_{50} values in the range of 3.8 to 8.3 μM.

Keywords : Ferrocene; Bioorganometallic chemistry; Dipeptides; Cytotoxicity; Lung cancer.

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1. Introduction

Organometallic compounds are versatile species due to the range of both structure and bonding modes that are accessible. Ferrocene has shown great promise in the area of medicinal organometallic chemistry due to its aromatic character, redox properties and low toxicity [1-5]. In particular the reversible redox properties of ferrocene have been strogly associated with its biological activity [6]. Ferricenium salts that are known to inhibit tumor growth have been shown to produce hydroxyl ($HO^{\bullet}$) radicals under physiological conditions, leading to oxidatively damaged DNA [7]. The catalytic generation of intracellular reactive oxygen species (ROS) such as the $HO^{\bullet}$ radical offers an attractive and alternative method to target and kill cancer cells [8].

The medicinal application of ferrocene derivatives is currently an active area of research, with countless reports showing their activity *in vitro* and *in vivo* against several diseases including fungal and bacterial infections, human immunodeficiency virus (HIV) and cancer [9-10]. In addition the preparation of artificial ferrocenyl nucleosides have been reported [11-12]. Perhaps the most popular and well-researched application of ferrocene and its derivatives is in the area of cancer research [13]. Over the past decade Jaouen and co-workers have comprehensively investigated the *in vitro* anti-cancer activity of ferrocifen, a ferrocenyl analogue of tamoxifen, and various related derivatives. Their most promising drug candidates contain a [3]-ferrocenophane motif and have a potent *in vitro* anti-proliferative effect in breast and prostate cancer cell lines [14-16]. We have reported the anti-proliferative effects of ferrocenyl benzoyl and ferrocenyl naphthoyl bioconjugates in the H1299 non-small cell lung cancer (NSCLC) cell line [17-23]. These *N*-(ferrocenyl)benzoyl and naphthoyl 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) an amino acid or peptide derivative that can interact with other biomolecules *via* secondary interactions such as hydrogen bonding. In an effort to improve the cytotoxicity of these derivatives, we are currently modifying the conjugated linker moiety and conducting variations of the peptide chain. The compounds prepared in this study have an ethynyl group linked to the ferrocene and the benzoyl spacer group. Herein, we report the synthesis and structural characterization of novel *N*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide ethyl esters. The synthesis of the novel ferrocenyl bioconjugates involved

Sonogashira coupling of ethynyl ferrocene to 4-bromobenzoic acid generating *para*-(ferrocenyl) ethynyl benzoic acid [24]. A series of amino acid and dipeptide esters were coupled to the *para*-(ferrocenyl) ethynyl benzoic acid furnishing the novel ferrocenyl bioconjugates which were characterized by a combination of I.R., UV-Vis, ^{1}H NMR, ^{13}C NMR, DEPT-135, ^{1}H-^{13}C COSY spectroscopy and electrospray ionization mass spectrometry. In addition, we present the *in vitro* anti-cancer activity of compounds **4-18** against the NSCLC human lung carcinoma cell line H1299.

2. Results and Discussion

2.1. Synthesis

*2.1.1. Synthesis of N-{para-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide methyl and ethyl esters **4-18.***

para-(Ferrocenyl) ethynyl benzoic acid **3** was prepared by coupling ethynyl ferrocene to 4-bromobenzoic acid via the Sonogashira reaction [24]. In the ^{1}H NMR spectrum the benzoyl ring protons appeared as two apparent doublets at δ 7.9 and δ 7.58 respectively with a coupling constant of 9.2 Hz. The carboxylic acid proton was observed as a singlet at δ 12.83. The ferrocenyl *ortho* and *meta* protons on the (η^5-$C_5\underline{H}_4$) ring were present at δ 4.61 and δ 4.38, respectively, and an intense signal appeared at δ 4.29 for the (η^5-$C_5\underline{H}_5$) ring. The free *N*-terminal amino acids of GABA(OMe) and GABA(OEt) and the dipeptide esters GlyGly(OMe), GlyGly(OEt), Gly-L-Ala(OMe), Gly-L-Ala(OEt), Gly-D-Ala(OMe), Gly-D-Ala(OEt), Gly-L-Leu(OEt), Gly-L-Phe(OEt), SarGly(OMe), SarGly(OEt), Sar-L-Ala(OEt), L-ProGly(OEt) and L-Pro-L-Ala(OEt) were coupled using EDC and HOBt in the presence of excess triethylamine in dichloromethane (Scheme 1**).** EDC was used in preference to the less expensive coupling reagent *N*,*N*'-dicyclohexylcarbodiimide (DCC) as its reaction by-products are easier to remove compared to those of DCC, namely dicyclohexylurea (DCU). Purification by column chromatography furnished the pure products in yields of 12-38 % and all compounds gave spectroscopic data in accordance with the proposed structures. The relatively low yields of the products obtained under the coupling procedures has been discussed previously [25]. The *N*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide methyl and ethyl esters **4-18** were characterized by a combination of IR, UV-Vis, ^{1}H NMR, ^{13}C NMR, DEPT-135 and ^{1}H-^{13}C COSY (HMQC) spectroscopy. 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 **4-18** were unambiguously assigned by a combination of DEPT-135 and ^{1}H-^{13}C COSY. The ^{1}H and ^{13}C NMR spectra for compounds **4-18** showed peaks in the ferrocene region characteristic of a monosubstituted ferrocene moiety [26-28]. The protons on the ferrocene rings in the *N*-{*para*-(ferrocenyl) ethynyl benzoyl derivatives, are expected to occur as three signals between δ 4.61 and δ 3.85. However, these signals usually overlap with the hydrogen signals of either the methylene groups or the methine groups that may be present in the amino acid and dipeptide esters. However in the Gly L-Ala ethyl ester derivative **7** no overlapping is observed, hence, the *ortho* and *meta* protons on the cyclopentadiene ring attached to the ethynyl spacer (η^5-$C_5\underline{H}_4$-C≡C-) appear as two apparent triplets at δ 4.61 and δ 4.38 respectively . The protons on the unsubstituted cyclopentadiene ring appear as a sharp singlet at δ 4.26 as all the hydrogens are magnetically equivalent. Typical chemical shifts are observed for the *N*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide esters **4-18** including the appearance of amide protons and four aromatic hydrogens on the benzoyl spacer moiety between δ 8.91 and δ 6.65. In the ^{1}H NMR spectra of the Sar Gly **14, 15**, Sar-L-Ala **16,** L-Pro Gly **17** and L-Pro-L-Ala **18** dipeptide derivatives, the amide protons and the aromatic hydrogens on the benzoyl spacer overlap, resulting in multiplets being observed between δ 7.69 and δ 7.35. For the Gly Gly **6, 7**, Gly-L-Ala **8, 9** and Gly-L-Leu **12** derivatives the splitting pattern of the hydrogens on the benzoyl linker follows the second order splitting pattern and do not overlap with the amide proton signals. The methyl protons (-$OCH_2\underline{CH}_3$) of the ethyl ester derivatives appear as a triplet between δ 1.28 and δ 1.17. The methyl protons (-$O\underline{CH}_3$) of the methyl esters appear as a sharp singlet between δ 3.70 and δ 3.63.

In the ^{13}C NMR spectra of *N*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide esters **4-18**, the typical peaks observed include the appearance of carbonyl signals between δ 174.4 to δ 165.2. In the amino acid derivatives **4** and **5** only two carbonyl signals are observed whilst for the dipeptide derivatives **6-18** three carbonyl signals are observed. These carbonyl signals are absent in the DEPT-135 spectra. In

the aromatic region of the spectrum four unique carbons signals are observed between δ 133.8 to δ 125.7. The two quaternary carbon atoms present on the benzoyl spacer moiety can be easily identified by their absence in the DEPT-135 spectrum and these signals appear more downfield than the other aromatic carbons in the range δ 133.8 to δ 130.0. The carbons on the -C≡C- linker appear between δ 91.7 to δ 90.6 for (η^5-C_5H_4-C≡<u>C</u>-) and between δ 85.7 to δ 84.1 for (η^5-C_5H_4-<u>C</u>≡C-). These two quaternary carbon atoms are absent in the DEPT-135 spectrum.

The *ipso* carbon on the cyclopentadiene ring (η^5-<u>C</u>$_5H_4$-C≡C-) attached to the -C≡C- unit appears between δ 67.6 to δ 64.6 and is also absent in the DEPT-135 spectra. The remaining four carbons on the monosubstituted cyclopentadiene rings appear between δ 72.0 to δ 68.0. The *ortho* carbon atoms on the substituted (η^5-<u>C</u>$_5H_4$-C≡C-) ring attached to the *ipso* carbons, appear more downfield than the *meta* carbons (η^5-<u>C</u>$_5H_4$-C≡C-) on the substituted cyclopentadiene ring and the unsubstituted ring carbon signal. This indicates that these carbons have become deshielded by the ethynyl moiety attached to the cyclopentadiene ring. The five carbon atoms on the unsubstituted ring give rise to one unique carbon signal because the carbon atoms are equivalent and appear in the arrow range δ 70.5 to δ 68.8. The methylene carbon of the ethyl esters (-O<u>CH$_2$</u>CH_3) appear between δ 62.7 and δ 60.7 whilst the methyl carbon appears between δ 14.8 and δ 13.7. In the DEPT-135 spectra the methylene carbons appear as a negative resonance. Complete spectroscopic data for all the compounds is presented in the experimental section.

2.3. Mass Spectrometry

Soft ionization techniques such as electrospray ionization (ESI) and matrix assisted laser desorption ionization mass spectrometry permit the analysis of thermolabile and non-volatile analytes [29-30]. The *N*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide methyl and ethyl esters **4-18** were not amenable to electron ionization (EI) or chemical ionization (CI) studies, therefore ESI was employed in the analysis of compounds **4-18.** Electrospray ionization (ESI) mass spectrometry confirmed the correct relative molecular mass for all the compounds. and examination of the mass spectra revealed the presence of intense radical-cation species. The formation of the radical-cation molecular ion species was further confirmed by the detection of the sodium adducts $[M+23]^+$ and potassium adducts $[M+39]^+$ for each of the compounds

analyzed. Similar observations were made in the analysis of the related ferrocenyl ethynyl naphthoyl amino acid and peptide derivatives [25]. Sequence specific fragment ions were not observed or were of low intensity in the ESI mass spectra of compounds **4-18** and therefore tandem mass spectrometry was employed to confirm the integrity of the structures.

In the MS/MS spectrum of N-{*para*-(ferrocenyl) ethynyl benzoyl} glycine glycine ethyl ester **7** the sequence specific fragment ions are present at *m/z* 285, *m/z* 313, *m/z* 341 and *m/z* 369 (Fig. 1). The product ions at *m/z* 285 and *m/z* 313 correspond to the *N*-{*para*-(ferrocenyl) ethynyl benzyl } and the *N*-{*para*-(ferrocenyl) ethynyl benzoyl} subunits respectively. However, the expected a_1 and b_1 product ions at *m/z* 342 and *m/z* 370 were not observed, instead a_1-1 and b_1-1 product ions were observed at *m/z* 341 and *m/z* 369 respectively. 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 [31]. The fragment ion at *m/z* 444 corresponds to loss of C_2H_4 from the molecular ion via a McLafferty rearrangement and is characteristic of these ethyl esters.

2.4. In vitro anti-cancer activity of **4-18.**

The *N*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide methyl and ethyl esters **4-18** have been prepared as part of an ongoing SAR study. In a preliminary screen the *in vitro* anti-proliferative effect of compounds **4-18** was studied at a concentration of 10 μM in the H1299 non-small cell lung cancer (NSCLC) cell line. The results of this biological study are reported in Table 1 and are expressed as percentage cell growth inhibition relative to the untreated controls. The percentage cell growth inhibition was lowest for compound **8** at 22 % and highest for compound **9** at 95 % cell growth inhibition. From the preliminary screen at 10 μM a general trend can be observed, namely, the methyl ester derivatives exhibited lower percentage growth inhibition values between 22 % to 55 % compared to the ethyl ester derivatives which exhibited percentage growth inhibition values between 47 % to 95 %. Generally, the lower the percentage growth value, the lower the anti-proliferative activity. Thus, the methyl ester derivatives were not investigated further. A general

trend was also observed for the ethyl ester derivatives, that is, the Gly L-Leu and Gly L-Phe ethyl esters displayed percentage growth inhibition values ≤ 53 %. For instance, the *N*-{*para*-(ferrocenyl) ethynyl benzoyl} glycine L-leucine ethyl ester **12** displayed a percentage growth inhibition values of 53 % whilst the *N*-{*para*-(ferrocenyl) ethynyl benzoyl} glycine L-phenylalanine ethyl ester **13** displayed a percentage growth inhibition value of 28 %. Thus, the Gly L-Leu and Gly L-Phe ethyl ester derivatives were also not investigated further. As a result, it can be concluded that when chiral α-amino acids with bulky side chains are employed as the second amino acid in the dipeptide moiety, a loss of anti-proliferative activity is observed. Compounds **5, 7, 9, 11, 16, 17** and **18** showed percentage growth inhibition values ≥ 60 %. Therefore, IC_{50} values were then determined for these compounds by the acid phosphatase assay as previously described [32]. 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*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide ethyl esters **5, 7, 9, 11, 16, 17** and **18** 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 IC_{50} values for compounds **5, 7, 9, 11, 16, 17** and **18** had IC_{50} values in the range of 3.8 to 8.3 μM and are displayed in Table 2**.** The *in vitro* cytotoxicity of the platinum(II)-based anti-cancer drugs cisplatin **19** and carboplatin **20** were also evaluated against the H1299 cell line, and were found to have an IC_{50} values of 1.5 ± 0.1 μM and 10.0 ± 1.6 μM respectively (Table 2). Thus, compounds **5, 7, 9, 11, 16, 17** and **18** are less cytotoxic *in vitro* than the clinically employed anti-cancer drug cisplatin but more toxic than the clinically employed anti-cancer drug carboplatin.

It can be seen from Table 2 that the *N*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide ethyl esters **5, 7, 9, 11, 16, 17** and **18** all exert a cytotoxic effect on the human lung carcinoma cell line H1299. All seven derivatives have IC_{50} values that are lower than 8.3 μM. The most active compound was *N*-{*para*-(ferrocenyl) ethynyl

benzoyl} glycine L-alanine ethyl ester **9** which had an IC_{50} value of 3.8 μM. Insertion of the ethynyl group in compound **9** had a slightly positive effect on the anti-proliferative effect compared to *N-{para-*(ferrocenyl)-benzoyl} glycine L-alanine ethyl ester (IC_{50} = 6.8 μM) prepared previously by this research group lacking the ethynyl group [19].

A potential mechanism by which these novel organometallic anticancer compounds may induce DNA damage is by the catalytic generation of ROS. This is possible *via* a Fenton-type reaction, in which $HO^{\bullet}$ radicals are generated from the superoxide dismutation product, hydrogen peroxide (H_2O_2). It was shown that the generation of 8-oxoGua by a compound prepared in a previous SAR study, namely, *N*-(6-ferrocenyl-2-naphthoyl)-glycine-glycine ethyl ester, that the oxidation was occurring by Fenton chemistry and that *N*-(6-ferrocenyl-2-naphthoyl)-glycine-glycine ethyl ester is generating oxidative damage via a ROS-mediated mechanism. Therefore guanine oxidation studies confirmed that *N*-(6-ferrocenyl-2-naphthoyl)-glycine-glycine ethyl ester was capable of causing oxidative damage to guanine, and it does so by the generation of $HO^{\bullet}$ radicals from H_2O_2 [23].

3. Conclusions

In conclusion, the novel *N*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide methyl and ethyl esters **4-18** were synthesized and fully characterized by a range of NMR spectroscopic techniques and mass spectrometry. Compounds **4-18** were tested *in vitro* against the NSCLC human lung carcinoma cell line H1299. Compounds **5, 7, 9, 11, 16, 17** and **18** showed micromolar activity in the H1299 NSCLC cell line, with IC_{50} values in the range of 3.8 to 8.3 μM. Insertion of the ethynyl group in compound **9** had a slightly positive effect on the anti-proliferative effect compared to *N-{para-*(ferrocenyl)-benzoyl} glycine L-alanine ethyl ester (IC_{50} = 6.8 μM). prepared previously by this research group.

4. Experimental

4.1. General Procedures

All chemicals were purchased from Sigma-Aldrich, Lennox Chemicals, Fluorochem Limited or Tokyo Chemical Industry UK Limited and used as received. Commercial grade reagents were used without further purification. When necessary, all solvents

were purified and dried prior to use. Riedel-Haën silica gel was used for thin layer and column chromatography. Melting points were determined using a Stuart melting point (SMP3) apparatus and are uncorrected. Optical rotation measurements were made on a Perkin Elmer 343 Polarimeter and are quoted in units of 10^{-1} deg cm^2 g^{-1}. Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR with ATR. UV-Vis spectra were recorded on a Hewlett Packard 8452 A diode array UV-Vis spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in deuterated solvents on a Bruker Avance 400 NMR. The ^{1}H and ^{13}C NMR chemical shifts are reported in ppm (parts per million). Tetramethylsilane (TMS) or the residual solvent peaks have been used as an internal reference. All coupling constants (*J*) are in Hertz. Electrospray ionisation mass spectra were performed on a Micromass LCT mass spectrometer. Tandem mass spectra were obtained on a Micromass Quattro *micro*™ LC-MS/MS triple quadrupole mass spectrometer.

4.2. General procedure for the synthesis of the starting materials.

4.2.1. (2-Formyl-1-chlorovinyl) ferrocene ***1***

Acetylferrocene (22.8 g, 99.96 mmol) was dissolved in *N*, *N*-dimethylformamide (25 ml) at 0 °C under nitrogen. Phosphorus oxychloride (25 ml) was added to dimethylformamide (25 ml) at 0 °C under nitrogen and stirred for 25 min resulting in a viscous red complex. The viscous complex was added to the acetylferrocene mixture over 2 hr and further stirred for 3 hr at 0 °C. Diethyl ether (80 ml), sodium acetate (116 g, mmol) and deionised water (20 ml) were added to reaction mixture. The reaction mixture was stirred at room temperature for 12 hr and then conc. sodium bicarbonate solution was added. The resulting solution was extracted with ethyl acetate. The solvent was evaporated to yield the crude product which was purified by column chromatography (eluent 9:1 hexane: diethyl ether) yielding the title compound as deep purple crystals. (17 g, 62%), mp. 76 - 77 °C (lit. [33] 76 - 77 °C);
^{1}H NMR (400 MHz) δ (DMSO- d_6): 10.03 (1H, d, *J* = 7.2 Hz, -C<u>H</u>O), 6.32 {1H, d, *J* = 7.2 Hz, η^5-C_5H_4-C=C<u>H</u>(Cl)-}. 4.68 {2H, t, *J* = 2.0 Hz, *ortho* on η^5-C_5<u>H_4</u>-C=CH(Cl)-}, 4.49 {2H, t, *J* = 2.0 Hz, *meta* on η^5-C_5<u>H_4</u>-C=CH(Cl)-}, 4.17 (5H, s, η^5-C_5<u>H_5</u>);

^{13}C NMR (100 MHz) δ (DMSO- d_6): 190.8 (C=O), 155.4 (η^5-C_5H_4-C=<u>C</u>H(Cl)-), 120.4 (η^5-C_5H_4-<u>C</u>=CH(Cl)-), 80.1 (C_{ortho} η^5-<u>C</u>$_5H_4$-C=CH(Cl)-), 72.3 (η^5-<u>C</u>$_5H_5$), 70.1 (C_{meta} η^5-<u>C</u>$_5H_4$-C=CH(Cl)-), 68.9 (C_{ipso} η^5-<u>C</u>$_5H_4$-C=CH(Cl)-).

4.2.2. Ethynyl ferrocene ***2***

Potassium *tert*-butoxide (10.24 g, 91.25 mmol) was added to dry tetrahydrofuran (100 ml) at 0 °C under nitrogen and stirred for 25 min. (2-Formyl-1-chlorovinyl) ferrocene (5 g, 18.25 mmol) was added slowly over 10 min and the reaction mixture was stirred at 0°C for 30 min and refluxed at 80 °C for 4 hr. The reaction mixture was poured into ice then neutralised with conc. hydrochloric acid. The resulting solution was extracted with hexane and the solvent was evaporated to yield the crude product which was purified by column chromatography (eluent 9:1 hexane: diethyl ether) yielding the title compound as a red solid. (1.87 g, 49%) m.p 52 -53 °C, (lit. [31] 52 - 53 °C);

^{1}H NMR (400 MHz) δ (DMSO- d_6): 4.68 (2H, t, J = 2.0 Hz, *ortho* on η^5-C_5<u>H</u>$_4$-C≡CH), 4.25 - 4.15 {7H, m, (*meta* on η^5-C_5<u>H</u>$_4$-C≡CH), (η^5-C_5<u>H</u>$_5$)}, 2.74 (1H, s, η^5-C_5H_4-C≡C<u>H</u>);

^{13}C NMR (100 MHz) δ (DMSO- d_6): 82.5 (η^5-C_5H_4-C≡<u>C</u>H), 73.5 (η^5-C_5H_4-<u>C</u>≡CH), 71.8 (C_{ortho} η^5-<u>C</u>$_5H_4$-C≡CH), 70.1 (η^5-<u>C</u>$_5H_5$), 68.7 (C_{meta} η^5-<u>C</u>$_5H_4$-C≡CH), 63.5 (C_{ipso} η^5-<u>C</u>$_5H_4$-C≡CH).

4.2.3. para-(Ferrocenyl) ethynyl benzoic acid **3**

Ethynyl ferrocene (2.00 g, 9.52 mmol) and 4-bromobenzoic acid (1.91 g, 9.52 mmol) were mixed together and dissolved in 50 ml of a 1:1 mixture of dry triethylamine and tetrahydrofuran under nitrogen for 10 min. Triphenylphosphine (0.20 g, 0.76 mmol), *bis*(triphenylphosphine)palladium(II) dichloride (0.28 g, 0.38 mmol) and copper(I) iodide (0.07 g, 0.38 mmol) were mixed together and added to the reaction mixture. The reaction mixture was stirred for 10 min and refluxed at 80 ^{o}C for 12 hr. The reaction mixture was vacuum filtered. The solvent was removed *in vacuo* to yield the crude product. The crude product was purified by column chromatography (eluent 1:1 hexane: ethyl acetate) yielding the title compound as a red solid (2.53 g, 81%), mp 147 - 149 ^{o}C;

^{1}H NMR (400 MHz) δ (DMSO- d_6): 12.83 (1H, s, -COOH), 7.90 (2H, d, J = 9.2 Hz, ArH), 7.58 (2H, d, J = 9.2 Hz, ArH), 4.61 (2H, t, J = 2.0 Hz, *ortho* on η^5-C_5H_4-C≡C-), 4.38 (2H, t, J = 2.0 Hz, *meta* on η^5-C_5H_4-C≡C-), 4.29 (5H, s, η^5-C_5H_5);

^{13}C NMR (100 MHz) δ (DMSO- d_6): 171.0 (C=O), 138.2 (C_q), 136.1(C_q), 133.0, 131.0, 96.4 (η^5-C_5H_4-C≡C-), 87.0 (η^5-C_5H_4-C≡C-), 71.4 (C_{ortho} η^5-C_5H_4-C≡C-), 70.1 (η^5-C_5H_5), 69.7 (C_{meta} η^5-C_5H_4-C≡C-), 64.0 (C_{ipso} η^5-C_5H_4-C≡C-).

General procedure for the preparation of *N*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acids and dipeptide esters

*4.3.1. N-{para-(ferrocenyl) ethynyl benzoyl} γ-aminobutyric acid methyl ester **4***

γ-Amino butyric acid methyl ester hydrochloride (0.35 g, 3.03 mmol) was dissolved in dichloromethane (100 ml) at 0 ^{o}C. *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (0.58 g, 3.03 mmol), 1-hydroxybenzotriazole (0.35 g, 3.03 mmol), glycine glycine methyl ester hydrochloride (0.44 g, 3.03 mmol) and triethylamine (6 ml) were added and the reaction mixture was allowed to stir at 0 ^{o}C for 45 min. The reaction mixture was then allowed to stir at room temperature for 48 h, and washed with water and brine. The organic layer was dried over $MgSO_4$.. The solvent was removed *in vacuo* to the crude product was purified by column chromatography (eluent 1:1 hexane: ethyl acetate) and recrystallisation from hexane: ethyl acetate yielded the desired product as a red solid (0.41 g, 32%), m.p 88 - 90 ^{o}C;

Mass spectrum: $[M+Na]^+$ found: 452.0937

$C_{24}H_{23}NO_3FeNa$ requires: 452.0925

I.R. υ_{max} (KBr): 3343 (NH), 2207 (-C≡C-), 1731 ($C=O_{ester}$), 1606 ($C=O_{amide}$) cm^{-1};

UV-Vis λ_{max} EtOH: 385, 485 nm;

^{1}H NMR (400 MHz) δ ($CDCl_3$): 7.76 (2H, d, J = 8.4 Hz, ArH), 7.55 (2H, d, J = 8.4 Hz, ArH), 6.65 (1H, t, J = 4.2 Hz, -CONH-), 4.53 (2H, t, J = 2.0 Hz, *ortho* on η^5-C_5H_4-C≡C-), 4.29 - 4.27 {7H, m, (*meta* on η^5-C_5H_4-C≡C-), (η^5-C_5H_5)}, 3.70 (3H, s, -OCH_3), 3.54 {2H, q, J = 6.4 Hz, (-NH$CH_2$$CH_2$$CH_2$-)}, 2.49 {2H, t, J = 6.8 Hz, (-NH$CH_2$$CH_2$$CH_2$-)}, 2.00 {2H, quin, J = 6.4 Hz, (-NH$CH_2$$CH_2$$CH_2$-)};

^{13}C NMR (100 MHz) δ ($CDCl_3$): 174.4 (C=O), 166.9 (C=O), 133.0 (C_q), 131.4 (C_q), 127.3, 126.9, 91.0 (η^5-C_5H_4-C≡C-), 85.1 (η^5-C_5H_4-C≡C-), 71.6 (C_{ortho} η^5-C_5H_4-C≡C-), 70.0 (η^5-C_5H_5), 69.1 (C_{meta} η^5-C_5H_4-C≡C-), 64.8 (C_{ipso} η^5-C_5H_4-C≡C-), 51.9 (-OCH_3),

39.8 (-NH$\underline{CH_2}CH_2CH_2$-, -ve DEPT), 31.8 (-$NHCH_2CH_2\underline{CH_2}$-, -ve DEPT), 24.3 (-$NHCH_2\underline{CH_2}CH_2$-, -ve DEPT).

4.3.2. N-{para-(ferrocenyl) ethynyl benzoyl} γ-aminobutyric acid ethyl ester ***5***

γ-Amino butyric acid ethyl ester hydrochloride (0.40 g, 3.03 mmol) was used as a starting material. The crude product was purified by column chromatography (eluent 1:1 hexane: ethyl acetate) and recrystallisation from hexane: ethyl acetate yielded the desired product as a red solid (0.51 g, 38%), m.p 64 - 66 ^oC;

Mass spectrum: $[M+Na]^+$ found: 466.1101

$C_{25}H_{25}NO_3FeNa$ requires: 466.1082

I.R. υ_{max} (KBr): 3283 (NH), 2205 (-C≡C-), 1726 ($C=O_{ester}$), 1605 ($C=O_{amide}$) cm^{-1};

UV-Vis λ_{max} EtOH: 385, 490 nm;

^{1}H NMR (400 MHz) δ ($CDCl_3$): 7.73 - 7.67 (4H, m, Ar$\underline{H}$), 6.65 (1H, t, *J* = 4.4 Hz, -CON$\underline{H}$-), 4.55 (2H, t, *J* = 3.2 Hz, *ortho* on η^5-$C_5\underline{H_4}$-C≡C-), 4.28 (2H, t, *J* = 3.2 Hz, *meta* on η^5-$C_5\underline{H_4}$-C≡C-), 4.16 (5H, s, η^5-$C_5\underline{H_5}$), 4.07 (2H, q, *J* = 7.2 Hz, -O$\underline{CH_2}CH_3$), 3.45{2H, q, *J* = 6.4 Hz, -NH$\underline{CH_2}CH_2CH_2$-), 2.38 (2H, t, *J* = 6.8 Hz, -$NHCH_2CH_2\underline{CH_2}$-), 1.91 (2H, quin, *J* = 6.4 Hz, -$NHCH_2\underline{CH_2}CH_2$-), 1.19 (3H, t, *J*= 7.2 Hz, -$OCH_2\underline{CH_3}$);

^{13}C NMR (100 MHz) δ ($CDCl_3$): 173.0 (C=O), 167.0 (C=O), 132.7 (C_q), 131.8 (C_q), 127.2, 126.9, 90.8 (η^5-C_5H_4-C≡$\underline{C}$-), 85.4 (η^5-$\underline{C_5}H_4$-$\underline{C}$≡C-), 70.5 (C_{ortho} η^5-$\underline{C_5}H_4$-C≡C-), 68.8 (η^5-$\underline{C_5}H_5$), 68.2 (C_{meta} η^5-$\underline{C_5}H_4$-C≡C-), 67.1 (C_{ipso} η^5-$\underline{C_5}H_4$-C≡C-), 60.7 (-O$\underline{CH_2}CH_3$, -ve DEPT), 39.8 (-NH$\underline{CH_2}CH_2CH_2$-, -ve DEPT), 32.1 (-$NHCH_2CH_2\underline{CH_2}$-, -ve DEPT), 24.3 (-$NHCH_2\underline{CH_2}CH_2$-, -ve DEPT), 14.8 (-$OCH_2\underline{CH_3}$).

4.3.3. N-{para-(ferrocenyl) ethynyl benzoyl} glycine-glycine methyl ester ***6***

N-para-(ferrocenyl) ethynyl benzoic acid (1.00 g, 3.03 mmol) was used as a starting material. The crude product was purified by column chromatography (eluent 1:1 hexane: ethyl acetate) yielding the title compound as an orange solid (0.35 g, 25%), m.p 169 - 171 ^oC;

Mass spectrum: $[M+Na]^+$ found: 481.0814

$C_{24}H_{22}N_2O_4FeNa$ requires: 481.0827

I.R. υ_{max} (KBr): 3261 (NH), 2204 (-C≡C-), 1736 ($C=O_{ester}$), 1658 ($C=O_{amide}$), 1603 ($C=O_{amide}$) cm^{-1};

UV-Vis λ_{max} EtOH: 385, 490 nm;

^{1}H NMR (400 MHz) δ (DMSO- *d_6*): 8.91 (1H, t, *J* = 6.0 Hz, -CONH-), 8.40 (1H, t, *J* = 6.0 Hz, -CONH-), 7.90 (2H, d, *J* = 8.0 Hz, ArH), 7.58 (2H, d, *J* = 8.0 Hz, ArH), 4.60 (2H, s, *ortho* on η^5-C_5H_4-C≡C-), 4.37 (2H, s, *meta* on η^5-C_5H_4-C≡C-), 4.29 (5H, s, η^5-C_5H_5), 3.93 (2H, d, *J* = 5.6 Hz, -NHCH_2CO-), 3.86 (2H, d, *J* = 5.6 Hz, -NHCH_2CO-), 3.63 (3H, s, - OCH_3);

^{13}C NMR (100 MHz) δ (DMSO- *d_6*): 170.2 (C=O), 168.9 (C=O), 167.8 (C=O), 131.9 (C_q), 130.0 (C_q), 127.7, 126.0, 91.2 (η^5-C_5H_4-C≡C-), 84.1 (η^5-C_5H_4-C≡C-), 71.8 (C_{ortho} η^5-C_5H_4-C≡C-), 69.8 (η^5-C_5H_5), 68.2 (C_{meta} η^5-C_5H_4-C≡C-), 64.9 (C_{ipso} η^5-C_5H_4-C≡C-), 51.7 (-OCH_3), 48.7, (-NHCH_2CO-, -ve DEPT), 40.5 (-NHCH_2CO-, -ve DEPT).

4.3.4. N-{para-(ferrocenyl) ethynyl benzoyl} glycine glycine ethyl ester **7**

Glycine glycine ethyl ester hydrochloride (0.48 g, 3.03 mmol) was used as a starting material. The crude product was purified by column chromatography (eluent 1:1 hexane: ethyl acetate) and recrystallisation from hexane: ethyl acetate yielded the desired product as a red solid (0.32 g, 22%), m.p 178 - 180 ^{o}C;

Mass spectrum: $[M+Na]^+$ found: 495.0972

$C_{25}H_{24}N_2O_4FeNa$ requires: 495.0983

I.R. υ_{max} (KBr): 3261(NH), 2204 (-C≡C-), 1736 ($C=O_{ester}$), 1658 ($C=O_{amide}$), 1603 ($C=O_{amide}$) cm^{-1};

UV-Vis λ_{max} EtOH: 385, 490 nm;

^{1}H NMR (400 MHz) δ (DMSO- *d_6*): 8.89 (1H, t, *J* = 6.4 Hz, -CONH-), 8.36 (1H, t, *J* = 6.4 Hz, -CONH-), 7.90 (2H, d, *J* = 9.2 Hz, ArH), 7.58 (2H, d, *J* = 9.2 Hz, ArH), 4.61 (2H, t, *J* = 2.0 Hz, *ortho* on η^5-C_5H_4-C≡C-), 4.38 (2H, t, *J* = 2.0 Hz, *meta* on η^5-C_5H_4-C≡C-), 4.29 (5H, s, η^5-C_5H_5), 4.10 (2H, q, *J* = 7.2 Hz, -OCH_2CH_3), 3.91 (2H, d, *J* = 6.0 Hz, -NHCH_2CO-), 3.84 (2H, d, *J* = 6.0 Hz, -NHCH_2CO-), 1.19 (3H, t, *J* = 7.2 Hz, - OCH_2CH_3);

^{13}C NMR (100 MHz) δ (DMSO- *d_6*): 170.0 (C=O), 169.3 (C=O), 168.2 (C=O), 132.2 (C_q), 130.7 (C_q), 127.7, 126.0, 90.9 (η^5-C_5H_4-C≡C-), 84.3 (η^5-C_5H_4-C≡C-), 72.0 (C_{ortho} η^5-C_5H_4-C≡C-), 70.5 (η^5-C_5H_5), 69.1 (C_{meta} η^5-C_5H_4-C≡C-), 67.6 (C_{ipso} η^5-C_5H_4-C≡C-), 62.0 (-OCH_2CH_3, -ve DEPT), 49.6 (-NHCH_2CO-, -ve DEPT), 42.3, (-NHCH_2CO-, -ve DEPT), 13.9 (-OCH_2CH_3).

4.3.5. *N*-{*para-(ferrocenyl) ethynyl benzoyl}* glycine L-alanine methyl ester ***8***

Glycine L-alanine methyl ester hydrochloride (0.48 g, 3.03 mmol) was used as a starting material. The crude product was purified by column chromatography (eluent 1:1 hexane: ethyl acetate) and recrystallisation from hexane: ethyl acetate yielded the desired product as a red solid (0.25 g, 17%), m.p 112 - 114 ^{o}C;

$[\alpha]_D^{20}$ = -25 ^{o} (*c* 0.1, EtOH);

Mass spectrum: $[M+Na]^+$ found: 495.0965

$C_{25}H_{24}N_2O_4FeNa$ requires: 495.0983

I.R. υ_{max} (KBr): 3336 (NH), 2205 (-C≡C-), 1734 ($C=O_{ester}$), 1660 ($C=O_{amide}$), 1604 ($C=O_{amide}$) cm^{-1};

UV-Vis λ_{max} EtOH: 391, 488 nm;

^{1}H NMR (400 MHz) δ ($CDCl_3$): 7.73 (2H, d, *J* = 8.4 Hz, Ar<u>H</u>), 7.48 (1H, t, *J* = 6.8 Hz, -CON<u>H</u>-), 7.45 (2H, d, *J* = 8.4 Hz, Ar<u>H</u>), 7.33 (1H, d, *J* = 7.2 Hz, -CON<u>H</u>-), 4.55 (1H, quin, *J* = 7.2 Hz, -<u>CH</u>CH_3), 4.44 (2H, t, *J* = 2 Hz, *ortho* on η^5-C_5<u>H_4</u>-C≡C-), 4.21 - 4.13 {9H, m, (*meta* on η^5-C_5<u>H_4</u>-C≡C-), (η^5-C_5<u>H_5</u>), (-NH<u>CH_2</u>CO-)}, 3.66 (3H, s, -O<u>CH_3</u>), 1.36 (3H, d, *J* = 7.2 Hz, -CH<u>CH_3</u>);

^{13}C NMR (100 MHz) δ ($CDCl_3$): 173.2 (C=O), 168.6 (C=O), 167.2 (C=O), 132.0 (C_q), 131.4 (C_q), 127.8, 127.2, 91.7 (η^5-C_5H_4-C≡<u>C</u>-), 85.1 (η^5-C_5H_4-<u>C</u>≡C-), 70.7 (C_{ortho} η^5-<u>C_5</u>H_4-C≡C-), 69.2 (η^5-<u>C_5</u>H_5), 68.2 (C_{meta} η^5-<u>C_5</u>H_4-C≡C-), 66.5 (C_{ipso} η^5-<u>C_5</u>H_4-C≡C-), 52.4 (-OCH_3), 48.3 (-<u>CH</u>CH_3), 43.5 (-NH<u>CH_2</u>CO-, -ve DEPT), 18.1 (-CH<u>CH_3</u>).

4.3.6. N-{para-(ferrocenyl) ethynyl benzoyl} glycine L-alanine ethyl ester ***9***

Glycine L-alanine ethyl ester hydrochloride (0.52 g, 3.03 mmol) was used as a starting material. The crude product was purified by column chromatography (eluent 1:1 hexane: ethyl acetate) and recrystallisation from hexane: ethyl acetate yielded the desired product as a red solid (0.30 g, 20%), m.p 115 - 117 ^{o}C;

$[\alpha]_D^{20}$ = -23 ^{o} (*c* 0.1, EtOH);

Mass spectrum: $[M+Na]^+$ found: 509.1127

$C_{26}H_{26}N_2O_4FeNa$ requires: 509.1140

I.R. υ_{max} (KBr): 3275 (NH), 2204 (-C≡C-), 1739 ($C=O_{ester}$), 1652 ($C=O_{amide}$), 1604 ($C=O_{amide}$) cm^{-1};

UV-Vis λ_{max} EtOH: 389, 489 nm;

^{1}H NMR (400 MHz) δ ($CDCl_3$): 7.81 (2H, d, *J* = 8.0 Hz, ArH), 7.54 - 7.51 {3H, m, (ArH), (-CONH-)} 7.33 (1H, t, *J* = 7.2 Hz, -CONH-), 4.54 (1H, quin, *J* = 6.8 Hz, -CHCH$_3$), 4.53 (2H, t, *J* = 1.6 Hz, *ortho* on η^5-C_5H_4-C≡C-), 4.28 (2H, t, *J* = 1.6 Hz, *meta* on η^5-C_5H_4-C≡C-), 4.26 (5H, s, η^5-C_5H_5), 4.23 - 4.10 {4H, m, (-OCH$_2$CH$_3$), (-NHCH$_2$CO-)}, 1.44 (3H, d, *J* = 7.2 Hz, -CHCH$_3$), 1.28 (3H, t, *J* = 7.2 Hz, -OCH$_2$CH$_3$);

^{13}C NMR (100 MHz) δ ($CDCl_3$): 172.8 (C=O), 168.9 (C=O), 165.2 (C=O), 132.0 (C_q), 130.4 (C_q), 127.5, 126.9, 91.0 (η^5-C_5H_4-C≡C-), 84.9 (η^5-C_5H_4-C≡C-), 71.6 (C_{ortho} η^5-C_5H_4-C≡C-), 70.1 (η^5-C_5H_5), 69.2 (C_{meta} η^5-C_5H_4-C≡C-), 66.0 (C_{ipso} η^5-C_5H_4-C≡C-), 61.6 (-OCH$_2$CH$_3$, -ve DEPT), 48.4 (-CHCH$_3$), 42.0 (-NHCH$_2$CO-, -ve DEPT), 18.7 (-CHCH$_3$), 14.2 (-OCH$_2$CH$_3$).

4.3.7. N-{para-(ferrocenyl) ethynyl benzoyl} glycine D-alanine methyl ester ***10***

Glycine D-alanine methyl ester hydrochloride (0.48 g, 3.03 mmol) was used as a starting material. The crude product was purified by column chromatography (eluent 1:1 hexane: ethyl acetate) and recrystallisation from hexane: ethyl acetate yielded the desired product as a red solid (0.21 g, 15%), m.p 129 - 131 ^{o}C;

$[\alpha]_D^{20}$ = +25 ^{o} (*c* 0.1, EtOH);

Mass spectrum: $[M+Na]^+$ found: 495.0978

$C_{25}H_{24}N_2O_4FeNa$ requires: 495.0983

I.R. υ_{max} (KBr): 3354 (NH), 2207 (-C≡C-), 1735 ($C=O_{ester}$), 1621 ($C=O_{amide}$), 1604 ($C=O_{amide}$) cm^{-1};

UV-Vis λ_{max} EtOH: 387, 492 nm;

^{1}H NMR (400 MHz) δ ($CDCl_3$): 7.73 (2H, d, *J* = 8.4 Hz, ArH), 7.45 (2H, d, *J* = 8.4 Hz, ArH), 7.25 (1H, t, *J* = 5.6 Hz, -CONH-), 7.07 (1H, d, *J* = 6.8 Hz, -CONH-), 4.55 - 4.50 (1H, m, -CHCH$_3$), 4.45 (2H, t, *J* = 1.6 Hz, *ortho* on η^5-C_5H_4-C≡C-), 4.21 - 4.14 {9H, m, (*meta* on η^5-C_5H_4-C≡C-), (η^5-C_5H_5), (-NHCH$_2$CO-)}, 3.68 (3H, s, -OCH$_3$), 1.38 (3H, d, *J* = 7.2 Hz, -CHCH$_3$);

^{13}C NMR (100 MHz) δ ($CDCl_3$): 173.2 (C=O), 168.6 (C=O), 167.2 (C=O), 132.0 (C_q), 131.4 (C_q), 127.8, 127.2, 90.7 (η^5-C_5H_4-C≡C-), 84.2 (η^5-C_5H_4-C≡C-), 70.9 (C_{ortho} η^5-C_5H_4-C≡C-), 69.2 (η^5-C_5H_5), 68.0 (C_{meta} η^5-C_5H_4-C≡C-), 65.9 (C_{ipso} η^5-C_5H_4-C≡C-), 52.0 (-OCH_3), 47.9 (-$CH$$CH_3$), 42.9 (-NH$CH_2$CO-, -ve DEPT), 18.9 (-CHCH_3).

4.3.8. N-{para-(ferrocenyl) ethynyl benzoyl} glycine D-alanine ethyl ester ***11***

Glycine D-alanine ethyl ester hydrochloride (0.52 g, 3.03 mmol) was used as a starting material. The crude product was purified by column chromatography (eluent 1:1 hexane: ethyl acetate) and recrystallisation from hexane: ethyl acetate yielded the desired product as a red solid (0.27 g, 18%), m.p 69 - 71 ^{o}C;

$[\alpha]_D^{20}$ = +23 ^{o} (*c* 0.1, EtOH);

Mass spectrum: $[M+Na]^+$ found: 509.1154

$C_{26}H_{26}N_2O_4FeNa$ requires: 509.1140

I.R. υ_{max} (KBr): 3307 (NH), 2205 (-C≡C-), 1734 ($C=O_{ester}$), 1651 ($C=O_{amide}$), 1604 ($C=O_{amide}$) cm^{-1};

UV-Vis λ_{max} EtOH: 387, 494 nm;

^{1}H NMR (400 MHz) δ ($CDCl_3$): 7.72 (2H, d, *J* = 8.0 Hz, ArH), 7.45 (2H, d, *J* = 8.0 Hz, ArH), 7.30 (1H, t, *J* = 7.2 Hz, -CONH-), 7.05 (1H, d, *J* = 6.8 Hz, -CONH-), 4.53 (1H, quin, *J* = 6.8 Hz, -$CH$$CH_3$), 4.42 (2H, t, *J* = 1.6 Hz, *ortho* on η^5-C_5H_4-C≡C-), 4.20 - 4.18 {7H, m, (*meta* on η^5-C_5H_4-C≡C-), (η^5-C_5H_5)}, 4.15 - 4.10 {4H, m, (-O$CH_2$$CH_3$), (-NH$CH_2$CO-)}, 1.37 (3H, d, *J* = 7.2 Hz, -CHCH_3), 1.20 (3H, t, *J* = 7.2 Hz, -OCH_2CH_3);

^{13}C NMR (100 MHz) δ ($CDCl_3$): 172.7 (C=O), 168.2 (C=O), 167.9 (C=O), 132.1 (C_q), 130.4 (C_q), 127.0, 126.5, 91.0 (η^5-C_5H_4-C≡C-), 84.7 (η^5-C_5H_4-C≡C-), 71.6 (C_{ortho} η^5-C_5H_4-C≡C-), 70.5 (η^5-C_5H_5), 69.1 (C_{meta} η^5-C_5H_4-C≡C-), 66.7 (C_{ipso} η^5-C_5H_4-C≡C-), 61.6 (-O$CH_2$$CH_3$, -ve DEPT), 48.4 (-$CH$$CH_3$), 42.0 (-ve DEPT), 18.2 (-CHCH_3), 14.1 (-OCH_2CH_3).

4.3.9. N-{para-(ferrocenyl) ethynyl benzoyl} glycine L-leucine ethyl ester ***12***

Glycine L-leucine ethyl ester hydrochloride (0.65 g, 3.03 mmol) was used as a starting material. The crude product was purified by column chromatography (eluent 1:1

hexane: ethyl acetate) and recrystallisation from hexane: ethyl acetate yielded the desired product as a red solid (0.19 g, 12%), m.p 132 - 134 ^{o}C;

$[\alpha]_D^{20}$ = -18 ^{o} (*c* 0.1, EtOH);

Mass spectrum: $[M+Na]^+$ found: 551.1617

$C_{29}H_{32}N_2O_4FeNa$ requires: 551.1609

I.R. υ_{max} (KBr): 3275 (NH), 2206 (-C≡C-), 1727 ($C=O_{ester}$), 1640 ($C=O_{amide}$), 1605 ($C=O_{amide}$) cm^{-1};

UV-Vis λ_{max} EtOH: 396, 489 nm;

^{1}H NMR (400 MHz) δ ($CDCl_3$): 7.72 (2H, d, *J* = 8.4 Hz, Ar<u>H</u>), 7.45 {2H, d, *J* = 8.4 Hz, Ar<u>H</u>), 7.30 (1H, t, *J* = 5.2 Hz, -CON<u>H</u>-) 7.00 (1H, d, *J* = 8.0 Hz, -CON<u>H</u>-), 4.57 - 4.51 {1H, m, -<u>CH</u>$CH_2CH(CH_3)_2$)}, 4.45 (2H, t, *J* = 2.0 Hz, *ortho* on η^5-C_5<u>H</u>$_4$-C≡C-), 4.20 - 4.15 { 9H, m, (*meta* on η^5-C_5<u>H</u>$_4$-C≡C-), (-NH<u>CH_2</u>CO-), (η^5-C_5<u>H</u>$_5$)}, 4.13 (2H, q, *J* = 7.2 Hz, -O<u>CH_2</u>CH_3), 1.57 - 1.45 {3H, m, -CH<u>CH_2CH</u>$(CH_3)_2$)}, 1.18 (3H, t, *J* = 7.2 Hz, -OCH_2<u>CH_3</u>), 0.88 - 0.86 (6H, m, -$CHCH_2CH$(<u>CH_3</u>$)_2$);

^{13}C NMR (100 MHz) δ ($CDCl_3$): 171.8 (C=O), 168.9 (C=O), 167.1(C=O), 132.0 (C_q), 131.7 (C_q), 127.3, 126.8, 90.7 (η^5-C_5H_4-C≡<u>C</u>-), 84.1 (η^5-C_5H_4-<u>C</u>≡C-), 71.7 (C_{ortho} η^5-<u>C</u>$_5$H$_4$-C≡C-), 70.5 (η^5-<u>C</u>$_5$H$_5$), 68.2 (C_{meta} η^5-<u>C</u>$_5$H$_4$-C≡C-), 66.1 (C_{ipso} η^5-<u>C</u>$_5$H$_4$-C≡C-), 61.0 (-O<u>CH_2</u>CH_3, -ve DEPT), 50.1 (-<u>CH</u>$CH_2CH(CH_3)_2$), 42.9 (-NH<u>CH_2</u>CO-, -ve DEPT), 41.3 (-CH<u>CH_2</u>$CH(CH_3)_2$, -ve DEPT), 25.8 (-$CHCH_2$<u>CH</u>$(CH_3)_2$), 22.0 (-$CHCH_2CH$(<u>CH_3</u>$)_2$), 21.9 (-$CHCH_2CH$(<u>CH_3</u>$)_2$), 14.0 (-OCH_2<u>CH_3</u>).

4.3.10. N-{para-(ferrocenyl) ethynyl benzoyl} glycine L-phenylalanine ethyl ester ***13***

Glycine L-phenylalanine ethyl ester hydrochloride (0.76 g, 3.03 mmol) was used as a starting material. The crude product was purified by column chromatography (eluent 1:1 hexane: ethyl acetate) and recrystallisation from hexane: ethyl acetate yielded the desired product as a red solid (0.20 g, 12%), m.p 118 - 120 ^{o}C;

$[\alpha]_D^{20}$ = -17 ^{o} (*c* 0.1, EtOH);

Mass spectrum: $[M+Na]^+$ found: 585.1442

$C_{32}H_{30}N_2O_4FeNa$ requires: 585.1453

I.R. υ_{max} (KBr): 3359 (NH), 2205 (-C≡C-), 1743 ($C=O_{ester}$), 1631 ($C=O_{amide}$), 1605 ($C=O_{amide}$) cm^{-1};

UV-Vis λ_{max} EtOH: 390, 492 nm;

^{1}H NMR (400 MHz) δ ($CDCl_3$): 7.81 (2H, d, *J* = 8.0 Hz, Ar<u>H</u>), 7.54 (2H, d, *J* = 8.0 Hz, Ar<u>H</u>), 7.25 - 7.19 {6H, m, (-CON<u>H</u>-), (-CHCH$_2$<u>Ph</u>)}, 6.87 (2H, d, *J* = 8.0 Hz, -CON<u>H</u>-), 4.86 - 4.84 (1H, m, -C<u>H</u>CH$_2$Ph), 4.54 (2H, t, *J* = 1.6 Hz, *ortho* on η^5-C$_5$<u>H</u>$_4$-C≡C-), 4.30 - 4.29 {7H, m, (*meta* on η^5-C$_5$<u>H</u>$_4$-C≡C-), (η^5-C$_5$<u>H</u>$_5$)}, 4.18 (2H, q, *J* = 7.2 Hz, -O<u>CH$_2$</u>CH$_3$), 4.23 (2H, d, *J* = 4.8 Hz, -NH<u>CH$_2$</u>CO-), 3.20 - 3.08 (2H, m, -CH<u>CH$_2$</u>Ph), 1.26 (3H, t, *J* = 7.2 Hz, -OCH$_2$<u>CH$_3$</u>);

^{13}C NMR (100 MHz) δ ($CDCl_3$):171.2 (C=O), 168.1(C=O), 167.0 (C=O), 133.7 (C_q), 132.0 (C_q), 130.1, 129.3, 128.6, 127.8, 127.2, 126.1, 91.7 (η^5-C$_5$H$_4$-C≡<u>C</u>-), 84.9 (η^5-C$_5$H$_4$-<u>C</u>≡C-), 71.1 (C_{ortho} η^5-<u>C</u>$_5$H$_4$-C≡C-), 70.4 (η^5-<u>C</u>$_5$H$_5$), 69.2 (C_{meta} η^5-<u>C</u>$_5$H$_4$-C≡C-), 67.5 (C_{ipso} η^5-<u>C</u>$_5$H$_4$-C≡C-), 62.7 (-O<u>CH$_2$</u>CH$_3$, -ve DEPT), 53.0 (-<u>CH</u>CH$_2$Ph), 42.6 (-NH<u>CH$_2$</u>CO-, -ve DEPT), 38.0 (-CH<u>CH$_2$</u>Ph, -ve DEPT), 13.7 (-OCH$_2$<u>CH$_3$</u>).

4.3.11. N-{para-(ferrocenyl) ethynyl benzoyl} sarcosine glycine methyl ester ***14***

Sarcosine glycine methyl ester hydrochloride (0.49 g, 3.03 mmol) was used as a starting material. The crude product was purified by column chromatography (eluent 1:1 hexane: ethyl acetate) and recrystallisation from hexane: ethyl acetate yielded the desired product as a red solid (0.23 g, 16%), m.p 124 - 126 ^{o}C;

Mass spectrum: $[M+Na]^+$ found: 495.1297

$C_{25}H_{24}N_2O_4FeNa$ requires: 495.0983

I.R. υ_{max} (KBr): 3286 (NH), 2206 (-C≡C-), 1746 (C=O$_{ester}$), 1674 (C=O$_{amide}$), 1620 (C=O$_{amide}$) cm^{-1};

UV-Vis λ_{max} EtOH: 397, 486 nm;

^{1}H NMR (400 MHz) δ ($CDCl_3$): 7.45 - 7.35 {5H, m, (-CON<u>H</u>-), (Ar<u>H</u>)}, 4.44 (2H, s, *ortho* on η^5-C$_5$<u>H</u>$_4$-C≡C-), 4.30 - 3.85 {11H, m, (*meta* on η^5-C$_5$<u>H</u>$_4$-C≡C-), (η^5-C$_5$<u>H</u>$_5$), (-N(CH$_3$)<u>CH$_2$</u>CO-), (-NH<u>CH$_2$</u>CO-)}, 3.70 (3H, s, -OC<u>H$_3$</u>), 3.04 (3H, s, -N(<u>CH$_3$</u>)CH$_2$CO-);

^{13}C NMR (100 MHz) δ ($CDCl_3$): 172.1 (C=O), 168.9 (C=O), 167.2 (C=O), 131.7 (C_q), 130.0 (C_q), 126.9, 125.7, 91.2 (η^5-C$_5$H$_4$-C≡<u>C</u>-), 84.1 (η^5-C$_5$H$_4$-<u>C</u>≡C-), 71.8 (C_{ortho} η^5-<u>C</u>$_5$H$_4$-C≡C-), 69.9 (η^5-<u>C</u>$_5$H$_5$), 68.2 (C_{meta} η^5-<u>C</u>$_5$H$_4$-C≡C-), 65.9 (C_{ipso} η^5-<u>C</u>$_5$H$_4$-C≡C-), 51.7 (-OCH$_3$), 46.2 (-N(CH$_3$)<u>CH$_2$</u>CO-, -ve DEPT), 41.2 (-NH<u>CH$_2$</u>CO-, -ve DEPT), 38.1 (-N(<u>CH$_3$</u>)CH$_2$CO-).

4.3.12. N-{para-(ferrocenyl) ethynyl benzoyl} sarcosine glycine ethyl ester ***15***

Sarcosine glycine ethyl ester hydrochloride (0.53 g, 3.03 mmol) was used as a starting material. The crude product was purified by column chromatography (eluent 1:1 hexane: ethyl acetate) and recrystallisation from hexane: ethyl acetate yielded the desired product as a red solid (0.23 g, 16%), m.p 79 - 81 ^{o}C;

Mass spectrum: $[M+Na]^+$ found: 509.1152

$C_{26}H_{26}N_2O_4FeNa$ requires: 509.1140

I.R. υ_{max} (KBr): 3284 (NH), 2205 (-C≡C-), 1744 ($C=O_{ester}$), 1676 ($C=O_{amide}$), 1620 ($C=O_{amide}$) cm^{-1};

UV-Vis λ_{max} EtOH: 389, 495 nm;

^{1}H NMR (400 MHz) δ ($CDCl_3$): 7.65 - 7.35 {5H, m, (-CONH-), (ArH)}, 4.43 (2H, s, *ortho* on η^5-C_5H_4-C≡C-), 4.30 - 3.85 {13H, m, (*meta* on η^5-C_5H_4-C≡C-), (η^5-C_5H_5), (-$N(CH_3)CH_2CO$-), (-$NHCH_2CO$-) (-OCH_2CH_3)}, 3.15 (3H, s, -$N(CH_3)CH_2CO$-), 1.17 (3H, t, *J* = 7.2 Hz, -OCH_2CH_3);

^{13}C NMR (100 MHz) δ ($CDCl_3$): 173.1 (C=O), 169.3 (C=O), 168.2 (C=O), 132.1 (C_q), 130.7 (C_q), 127.1, 125.9, 90.9 (η^5-C_5H_4-C≡C-), 85.7 (η^5-C_5H_4-C≡C-), 72.0 (C_{ortho} η^5-C_5H_4-C≡C-), 70.1 (η^5-C_5H_5), 69.1 (C_{meta} η^5-C_5H_4-C≡C-), 67.6 (C_{ipso} η^5-C_5H_4-C≡C-), 62.0 (-OCH_2CH_3, -ve DEPT), 49.6 (-$N(CH_3)CH_2CO$-, -ve DEPT), 42.3 (-$NHCH_2CO$-, -ve DEPT), 38.5 (-$N(CH_3)CH_2CO$-), 14.3 (-OCH_2CH_3).

4.3.13. N-{para-(ferrocenyl) ethynyl benzoyl} sarcosine L-alanine ethyl ester ***16***

Sarcosine L-alanine ethyl ester hydrochloride (0.57 g, 3.03 mmol) was used as a starting material. The crude product was purified by column chromatography (eluent 1:1 hexane: ethyl acetate) and recrystallisation from hexane: ethyl acetate yielded the desired product as a red solid (0.21 g, 14%), m.p 99 - 101 ^{o}C;

$[\alpha]_D^{20}$ = -22 ^{o} (*c* 0.1, EtOH);

Mass spectrum: $[M+Na]^+$ found: 523.1292

$C_{27}H_{28}N_2O_4FeNa$ requires: 523.1296

I.R. υ_{max} (KBr): 3270 (NH), 2205 (-C≡C-), 1745 ($C=O_{ester}$), 1675 ($C=O_{amide}$), 1614 ($C=O_{amide}$) cm^{-1} ;

UV-Vis λ_{max} EtOH: 389, 486 nm;

^{1}H NMR (400 MHz) δ ($CDCl_3$): 7.60 - 7.35 {5H, m, (-CONH-), (ArH)}, 4.51- 4.40

{3H, m, (*ortho* on η^5-$C_5\underline{H}_4$-C≡C-), (-C<u>H</u>CH_3)}, 4.19 - 3.90 {11H, m, (*meta* on η^5-$C_5\underline{H}_4$-C≡C-), (η^5-$C_5\underline{H}_5$), (-N(CH_3)<u>CH_2</u>CO-), (-O<u>CH_2</u>CH_3)}, 3.01 (3H, s, -N(<u>CH_3</u>)CH_2CO-), 1.36 (2H, d, *J* = 7.2 Hz, -CH<u>CH_3</u>), 1.21 (3H, t, *J* = 7.2 Hz, -OCH_2<u>CH_3</u>);

^{13}C NMR (100 MHz) δ ($CDCl_3$): 172.7 (C=O), 169.0 (C=O), 168.2 (C=O), 133.8 (C_q), 132.0 (C_q), 128.3, 127.4, 90.6 (η^5-C_5H_4-C≡<u>C</u>-), 85.0 (η^5-C_5H_4-<u>C</u>≡C-), 71.5 (C_{ortho} η^5-<u>C</u>$_5H_4$-C≡C-), 70.0 (η^5-<u>C</u>$_5H_5$), 69.1 (C_{meta} η^5-<u>C</u>$_5H_4$-C≡C-), 64.6 (C_{ipso} η^5-<u>C</u>$_5H_4$-C≡C-), 61.6 (-O<u>CH_2</u>CH_3, -ve DEPT), 48.2 (-C<u>H</u>CH_3), 40.1(-N(CH_3)<u>CH_2</u>CO-, -ve DEPT), 38.3 (-N(<u>CH_3</u>)CH_2CO-), 18.3 (-CH<u>CH_3</u>), 14.2 (-OCH_2<u>CH_3</u>).

4.3.14. N-{para-(ferrocenyl) ethynyl benzoyl} L-proline glycine ethyl ester ***17***

L-Proline glycine ethyl ester hydrochloride (0.61 g, 3.03 mmol) was used as a starting material. The crude product was purified by column chromatography (eluent 1:1 hexane: ethyl acetate) and recrystallisation from hexane: ethyl acetate yielded the desired product as a red solid (0.27 g, 17%), m.p 58 - 60 ^oC; $[\alpha]_D^{20}$ = 17 ^{o};

Mass spectrum: $[M+Na]^+$ found: 535.1282

$C_{28}H_{28}N_2O_4FeNa$ requires: 535.1296

I.R. υ_{max} (KBr): 3313 (NH), 2205 (-C≡C-), 1735 ($C=O_{ester}$), 1652 ($C=O_{amide}$), 1604 ($C=O_{amide}$) cm^{-1};

UV-Vis λ_{max} EtOH: 395, 499 nm;

^{1}H NMR (400 MHz) δ ($CDCl_3$): 7.50 - 7.36 {5H, m, (-CON<u>H</u>-), (Ar<u>H</u>)}, 4.74 (1 H, *t*, *J* = 5.2 Hz, -N($CH_2CH_2CH_2$)C<u>H</u>CO-), 4.45 (2H, s, *ortho* on η^5-$C_5\underline{H}_4$-C≡C-), 4.19 - 4.10 {9H, m, (*meta* on η^5-$C_5\underline{H}_4$-C≡C-), (η^5-$C_5\underline{H}_5$), (-O<u>CH_2</u>CH_3)}, 3.95 (2H, d, *J* = 4.0 Hz, -NH<u>CH_2</u>CO-), 3.51 - 3.40 (2H, m, -N(<u>CH_2</u>CH_2CH_2)CHCO-), 2.40 - 1.70 (4H, m, -N(CH_2<u>CH_2</u><u>CH_2</u>)CHCO-), 1.20 (3H, t, *J* = 7.2 Hz, -OCH_2<u>CH_3</u>);

^{13}C NMR (100 MHz) δ ($CDCl_3$): 171.5 (C=O), 170.6 (C=O), 169.7 (C=O), 133.8 (C_q), 131.2 (C_q), 127.4, 126.0, 90.7 (η^5-C_5H_4-C≡<u>C</u>-), 84.9 (η^5-C_5H_4-<u>C</u>≡C-), 71.9 (C_{ortho} η^5-<u>C</u>$_5H_4$-C≡C-), 70.4 (η^5-<u>C</u>$_5H_5$), 68.1 (C_{meta} η^5-<u>C</u>$_5H_4$-C≡C-), 65.9 (C_{ipso} η^5-<u>C</u>$_5H_4$-C≡C-), 62.4 (-O<u>CH_2</u>CH_3, -ve DEPT), 59.8 (-N($CH_2CH_2CH_2$)<u>CH</u>CO-), 50.4 (-N(<u>CH_2</u>CH_2CH_2)CHCO, -ve DEPT), 41.5 (-NH<u>CH_2</u>CO-, -ve DEPT), 27.5 (-N(CH_2CH_2<u>CH_2</u>)CHCO-, -ve DEPT), 25.4 (-N(CH_2<u>CH_2</u>CH_2)CHCO-, -ve DEPT), 13.7 (-OCH_2<u>CH_3</u>).

4.3.15. N-{para-(ferrocenyl) ethynyl benzoyl} L-proline L-alanine ethyl ester ***18***

L-Proline L-alanine ethyl ester hydrochloride (0.65 g, 3.03 mmol) was used as a starting material. The crude product was purified by column chromatography (eluent 1:1 hexane: ethyl acetate) and recrystallisation from hexane: ethyl acetate yielded the desired product as a red solid (0.21 g, 13%), m.p 52 - 54 ^{o}C;

$[\alpha]_D^{20}$ = -69 ^{o} (*c* 0.1, EtOH);

Mass spectrum: $[M+Na]^+$ found: 549.1467

$C_{29}H_{30}N_2O_4FeNa$ requires: 549.1453

I.R. υ_{max} (KBr): 3285 (NH), 2202 (-C≡C-), 1736 ($C=O_{ester}$), 1672 ($C=O_{amide}$), 1608 ($C=O_{amide}$) cm^{-1};

UV-Vis λ_{max} EtOH: 392, 493 nm;

^{1}H NMR (400 MHz) δ ($CDCl_3$): 7.69 - 7.48 {5H, m, (ArH), (-CONH-)}, 4.68 (1H, t, *J* = 6.4 Hz, -N($CH_2CH_2CH_2$)CHCO-), 4.57 (2H, s, *ortho* on η^5-C_5H_4-C≡C-), 4.46 (1H, quin, *J* = 6.8 Hz, -$CHCH_3$), 4.28 (2H, s, *meta* on η^5-C_5H_4-C≡C-), 4.16 - 4.12 {7H, m, (η^5-C_5H_5), (-OCH_2CH_3)}, 3.60 - 3.40 (2H, m, (-N($CH_2CH_2CH_2$)CHCO-), 2.40 - 1.56 (4H, m, (-N($CH_2CH_2CH_2$)CHCO-)}, 1.36 (3H, d, *J* = 6.4 Hz, -$CHCH_3$), 1.21 (3H, t, *J* = 6.8 Hz, -OCH_2CH_3);

^{13}C NMR (100 MHz) δ ($CDCl_3$): 173.8 (C=O), 171.9 (C=O), 169.1 (C=O), 133.0 (C_q), 131.2 (C_q), 128.9, 127.9, 90.7 (η^5-C_5H_4-C≡C-), 84.4 (η^5-C_5H_4-C≡C-), 72.0 (C_{ortho} η^5-C_5H_4-C≡C-), 70.2 (η^5-C_5H_5), 68.7 (C_{meta} η^5-C_5H_4-C≡C-), 67.3 (C_{ipso} η^5-C_5H_4-C≡C-), 62.4 (OCH_2CH_3, -ve DEPT), 60.1 (-N($CH_2CH_2CH_2$)CHCO-), 50.6 (-N($CH_2CH_2CH_2$)CHCO-, -ve DEPT), 48.4 (-$CHCH_3$), 27.8 (-N($CH_2CH_2CH_2$)CHCO-, -ve DEPT), 25.6 (-N($CH_2CH_2CH_2$)CHCO-, -ve DEPT), 18.9 (-$CHCH_3$), 13.9 (-OCH_2CH_3).

4.4. General procedure for in vitro cytotoxicity assays

4.4.1. Biological Assays. Cell Line.

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

4.4.2. In Vitro Proliferation Assays.

Cells in the exponential phase of growth were harvested by trypsinisation and a cell suspension of 1 x 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 hours in a 37 ^{o}C, 5% CO_2 incubator, to allow cell attachment to the wells. A 10 mM stock solution of a test sample was prepared in dimethyl sulfoxide; dilute solutions of the test sample were prepared at 2*X* final concentration by spiking the cell culture medium with a calculated amount of the stock solution. 100 µL aliquots of each dilute solution was added to each well of the plate, the plate was gently agitated, and then incubated at 37 ^{o}C, 5% CO_2 for 5-6 days, until cell confluency reached 80-90%. Assessment of cell survival in the presence of the compounds **4-18** was determined by the acid phosphatase assay [32]. The acid phosphatase assay is highly sensitive and is easier to perform than the neutral red assay as it involves fewer steps and fewer reagents. It is also more convenient than the MTT assay because of the inherent problem of removal of the medium from the insoluble crystals. The reproducibility between replicate wells is excellent in the acid phosphatase assay and in many cases it has been shown to be better than the neutral red assay and the MTT assay.

The percentage cell growth in the presence of each compound was determined relative to the control cells. The concentration of compounds causing a 50% growth inhibition (IC_{50} of the compound) was determined using Calcusyn (Biosoft, UK).

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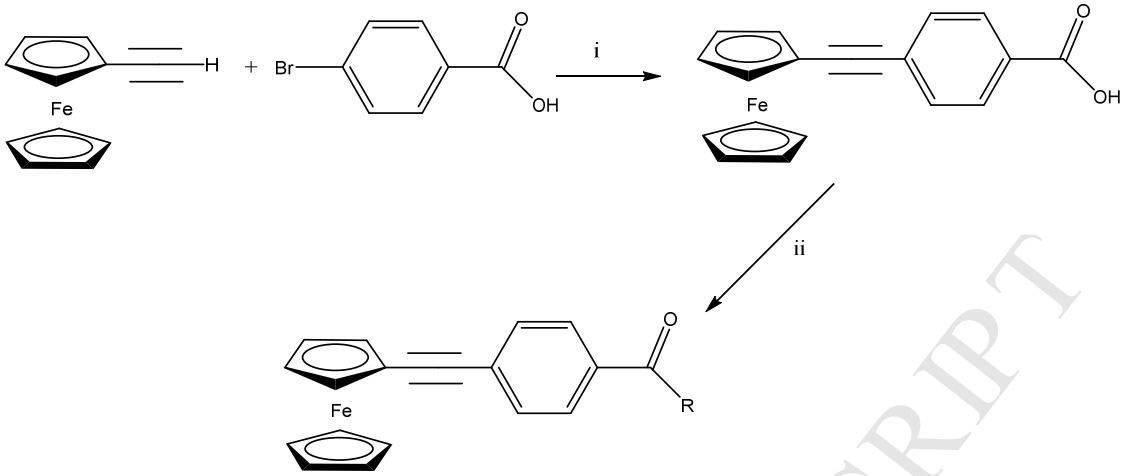


Scheme 1. Synthesis of *N*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide ethyl esters**,** (i) TEA, PPh_3, Bis(triphenylphosphine)palladium(II) dichloride, THF, Cu(I), (ii) EDC, HOBt, triethylamine, amino acid and dipeptide esters, R= GABA(OMe) **4**, GABA(OEt) **5**, GlyGly(OMe) **6**, GlyGly(OEt) **7**, Gly-L-Ala(OMe) **8,** Gly-L-Ala(OEt) **9**, Gly-D-Ala(OMe) **10**, Gly-D-Ala(OEt) **11**, Gly-L-Leu(OEt) **12**, Gly-L-Phe(OEt) **13**, SarGly(OMe) **14**, SarGly(OEt) **15**, Sar-L-Ala(OEt) **16,** L-ProGly(OEt) **17** and L-Pro-L-Ala(OEt) **18**.

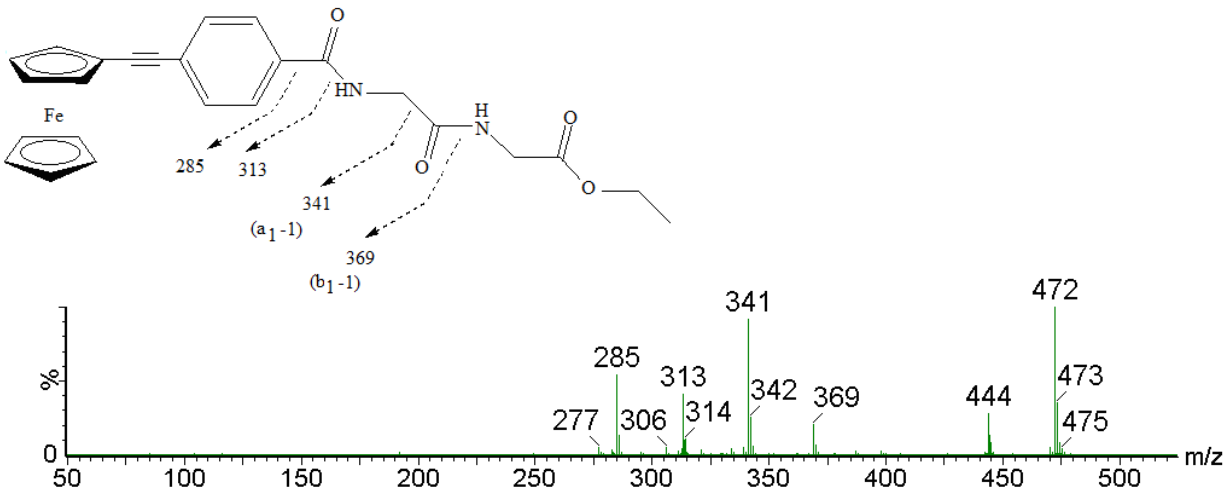


Figure 1. MS/MS spectrum of *N*-{*para*-(ferrocenyl) ethynyl benzoyl) glycine glycine ethyl ester **7.**

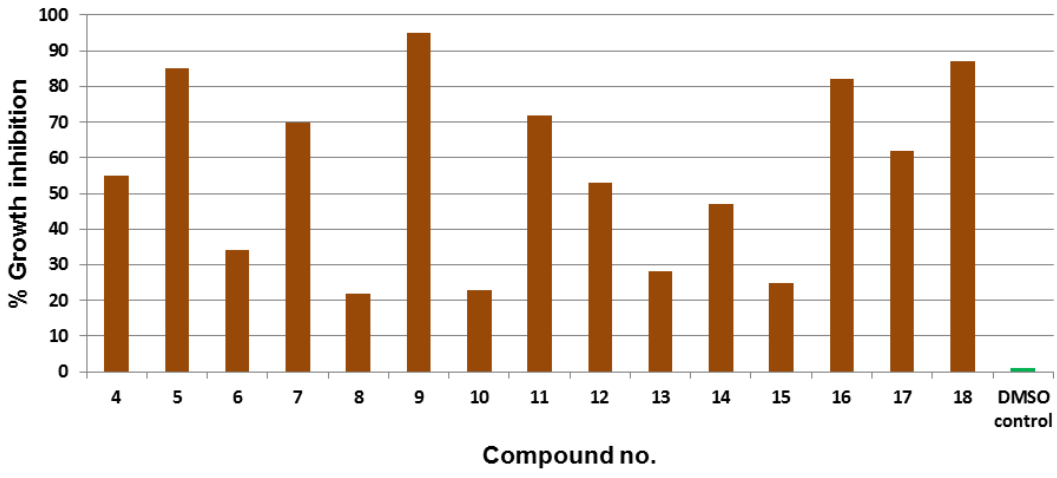


Figure 2. Percentage growth inhibition at 10 µM on H1299 lung cancer cells for *N*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide esters **4-18.**

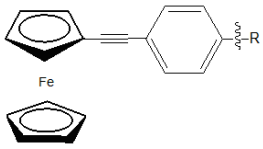
Ferrocenyl bioconjugate	R	Compound no.	% growth inhibition at 10 µM
	GABA(OMe)	**4**	55 ± 3.35
	GABA(OEt)	**5**	85 ± 1.07
	Gly Gly(OMe)	**6**	34 ± 2.49
	Gly Gly(OEt)	**7**	70 ± 2.84
	Gly L-Ala(OMe)	**8**	22 ± 5.47
	Gly L-Ala(OEt)	**9**	95 ± 1.62
	Gly D-Ala(OMe)	**10**	23 ± 4.89
	Gly D-Ala(OEt)	**11**	72 ± 2.01
	Gly L-Leu(OEt)	**12**	53 ± 3.17
	Gly L-Phe(OEt)	**13**	28 ± 1.74
	Sar Gly(OEt)	**14**	47 ± 4.03
	Sar Gly(OMe)	**15**	25 ± 4.10
	Sar L-Ala(OEt)	**16**	82 ± 4.75
	L-Pro Gly(OEt)	**17**	62 ± 5.06
	L-Pro L-Ala(OEt)	**18**	87 ± 1.12

Table 1. Percentage growth inhibition at 10 µM on H1299 lung cancer cells for *N*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide esters **4-18.**

Compound Name	**No.**	**IC_{50}**
Cisplatin	**19**	1.5 ± 0.10
Carboplatin	**20**	10 ± 1.60
N-{*para*-(ferrocenyl) ethynyl benzoyl} GABA(OEt)	**5**	4.9 ± 4.12
N-{*para*-(ferrocenyl) ethynyl benzoyl} Gly Gly(OEt)	**7**	6.9 ± 2.14
N-{*para*-(ferrocenyl) ethynyl benzoyl} Gly L-Ala(OEt)	**9**	3.8 ± 1.92
N-{*para*-(ferrocenyl) ethynyl benzoyl} Gly D-Ala(OEt)	**11**	6.1 ± 3.41
N-{*para*-(ferrocenyl) ethynyl benzoyl} Sar L-Ala(OEt)	**16**	7.1 ± 2.46
N-{*para*-(ferrocenyl) ethynyl benzoyl} L-Pro Gly(OEt)	**17**	8.3 ± 3.10
N-{*para*-(ferrocenyl) ethynyl benzoyl} L-Pro L-Ala(OEt)	**18**	5.7 ± 2.91

Table 2. IC_{50} values for compounds **5, 7, 9, 11, 16, 17** and **18**, cisplatin **19** and carboplatin **20** against human lung carcinoma cell line H1299.

- *N*-{*para*-(ferrocenyl) ethynyl benzoyl} amino acid and dipeptide methyl and ethyl esters were prepared
- The novel structures were characterized by a range of spectroscopic techniques.

- They exhibited cytotoxicity on the human lung carcinoma cell line H1299.

- Compound **9** was the most active and displayed an IC_{50} value of 3.8 µM.