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Journal of Organometallic Chemistry 692 (2007) 1327–1331
Journal of Organometallic Chemistry
www.elsevier.com/locate/jorganchem

Synthesis, structural characterisation and biological activity of novel *N*-(ferrocenylmethyl)benzene-carboxamide derivatives

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Received 31 August 2006; received in revised form 7 November 2006; accepted 7 November 2006
Available online 16 November 2006

Abstract

A series of *N*-(ferrocenylmethyl)benzene-carboxamide derivatives (**4a**–**f**) have been synthesised by coupling ferrocenylmethyl amine **3** with benzoic acid and various substituted fluorobenzoic acids using the standard 1,3-dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt) protocol. All compounds were fully characterised using a combination of ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, DEPT-135, ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY (HMQC) spectroscopy and electrospray ionisation mass spectrometry (ESI-MS). The compounds **4a**, **4d**, **4e** and **4f** exhibited cytotoxic effects on the MDA-MB-435-S-F breast cancer cell line. Single crystal X-ray crystallographic data for **4d** is also presented.

Keywords: Ferrocene; Bioorganometallic chemistry; Breast cancer; MDA-MB-435-S-F; X-ray crystal structure

1. Introduction

We are interested in the synthesis of ferrocene derivatives with a view to finding new cytotoxic agents to aid in the fight against one of the most prolific diseases facing medical science, cancer [1,2]. In the past two decades the use of organometallic compounds in the treatment of cancer has been an active field of research. As early as 1984 the antineoplastic properties of a variety of ferrocenium salts was reported [3,4]. Several reviews on the bioorganometallic chemistry and structural features of ferrocene have recently been published [5–7]. More recently, the pharmacological properties of the tamoxifen derivative, ferrocifen, have been reported [8–10]. The incorporation of C–F bonds is a recognised strategy often employed in the development of pharmaceuticals [11]. The success of fluorine substitution is evident from the large number of fluorinated drugs approved by the FDA and currently being used as anticancer, antidepressant, antiviral and anaesthetic agents.

The synthesis, characterisation and cytotoxicity of a series of *N*-(ferrocenylmethyl)fluorobenzene-carboxamide derivatives which combine the ferrocene moiety with a substituent containing one or more C–F bonds is reported in this communication. The parent compound ferrocenylmethylamine **3** was synthesised from ferrocenecarboxaldehyde **1** *via* the ferrocene oxime **2** intermediate (Scheme 1) [12].

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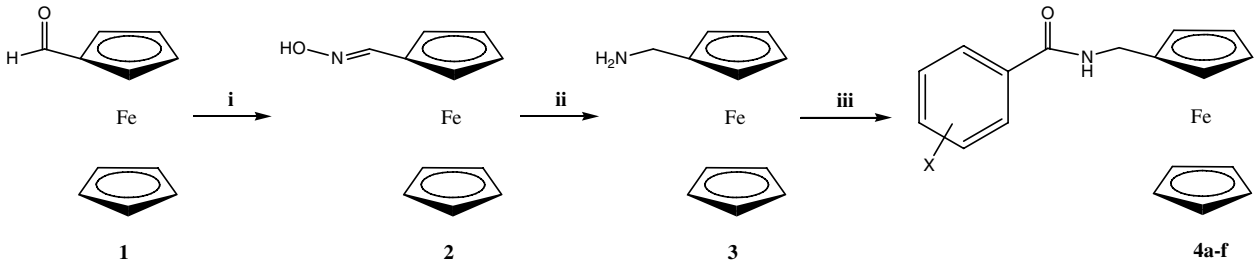
doi:10.1016/j.jorganchem.2006.11.012



Scheme 1. Synthesis of *N*-(ferrocenylmethyl)benzene-carboxamide derivatives (i) $NH_2OH \cdot HCl$, Pyridine, EtOH (ii) $LiAlH_4$, THF (iii) DCC, HOBt, Benzoic acid derivative, CH_2Cl_2, 48 h. **4a** X = H, **4b** X = 2-F, **4c** X = 3-F, **4d** X = 4-F, **4e** X = 2-F and 6-F, **4f** X = 2-F, 3-F, 4-F, 5-F and 6-F.

The condensation of ferrocenylmethylamine with benzoic acid and fluorobenzoic acids in the presence of DCC and HOBt yielded the corresponding *N*-(ferrocenylmethyl)benzene-carboxamides **4a**–**4f** as orange/red coloured crystals. All compounds gave analytical and spectroscopic data in accordance with the proposed structures. All of the proton and carbon chemical shifts for compounds **4a**–**4f** were unambiguously assigned by a combination of DEPT-135 and ^{1}H–^{13}C COSY (HMQC). The ^{1}H NMR and the ^{13}C NMR spectra for the compounds showed peaks in the ferrocene region characteristic of a monosubstituted ferrocene moiety [13–20]. The protons in the *ortho* position appear in the region δ 4.24–4.25, whereas those in the *meta* position occur in the range δ 4.09–4.12. The five protons of the unsubstituted Cp ring (η^5-C_5H_5) appear as a strong singlet in the range δ 4.18–4.20. The protons of the methylene unit adjacent to the ferrocene moiety appear as a doublet (J = 6.4 to 7.2 Hz) in the range δ 4.18–4.20 often overlapping with the singlet of the (η^5-C_5H_5) ring. The aromatic protons of the benzene ring appear in the range δ 7.45–7.87 for the **4a** derivative. For the mono- and difluorobenzene derivatives, the protons of the fluorobenzene rings exhibit ^{1}H–^{19}F coupling interactions and appear as complex multiplets in the range δ 7.28–7.94. The amide protons appear between δ 8.55 and 8.84 as triplets in DMSO-d_6. The ^{13}C NMR spectra of compounds **4a**–**4f** show signals typical of a monosubstituted ferrocene moiety in the range δ 67.2–86.7. The C_{ipso} of the (η^5-C_5H_4) ring appears between δ 85.9 and 86.7 which are in good agreement with previous results [21]. This signal is absent in the DEPT 135 spectra. The ^{13}C–^{19}F coupling can be seen in the ^{13}C NMR and DEPT-135 spectra of the fluorinated compounds. The ESI mass spectra of compounds **4a**–**4f** revealed the formation of the cation adducts due to sodium in addition to radical cation species, protonated molecular ion species and potassium cation adducts. In each case an intense fragment ion was observed at *m*/*z* 199 and is due to the $[FcCH_2]^+$ cation. Fragment ions corresponding to $[M\text{-}65]^+$ were also noted in the spectra of the **4a** and **4e** derivatives. This corresponds to loss of the unsubstituted (η^5-C_5H_5) ring.

A number of crystal structures of ferrocene derivatives have been reported in the Cambridge Structural Database (CSD); however, structures incorporating a ferrocenylmethyl-carboxamide moiety are relatively rare. We have previously reported single crystal X-ray structures of a number of ferrocene benzoyl amino acid derivatives [17,18,20] and a ferrocene benzoyl dipeptide [2]. Herein, we report the single crystal structure of an *N*-(ferrocenylmethyl)benzene-carboxamide derivative **4d**. Molecular drawings using ORTEP are shown in Fig. 1 and the crystallographic details given in Section 2.

Red/orange block shaped crystals of compound **4d**, of a quality suitable for crystallographic determination, were grown from dichloromethane. The compound **4d** crystallises in the orthorhombic space group $P2_12_12_1$ (No. 19), with one molecule per asymmetric unit. The principle interaction is an intermolecular amide–amide hydrogen bonding interaction which is orientated along the *a*-axis (Fig. 2). The carbon atoms of the two Cp rings of the fer-

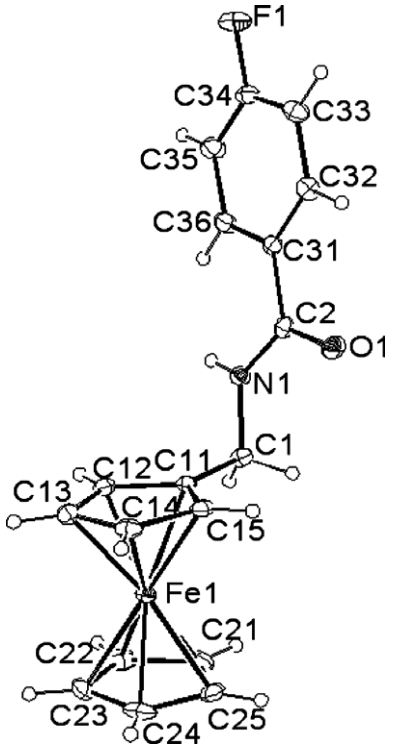


Fig. 1. A view of **4d** using PLATON [22], with atomic displacement ellipsoids depicted at the 30% probability level.

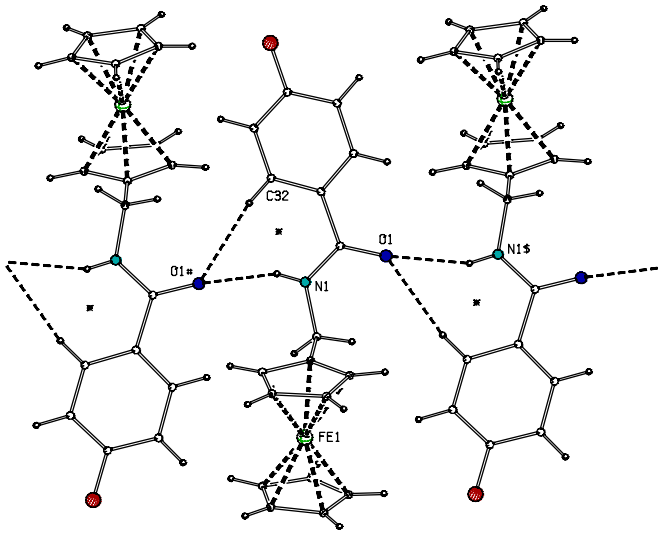


Fig. 2. The primary N–H. . .O=C amide hydrogen bonding interactions in **4d** showing the one-dimensional chain of rings (marked as *, with graph set $R_2^1 7$) along [1 0 0] (symmetry operations: # = 1/2 + *x*, 3/2 − *y*, 2 − *z* and $ = −1/2 + *x*, 3/2 − *y*, 2 − *z*).

rocene moiety are essentially eclipsed with the five C1n–Cg1–Cg2–C2n torsion angles ranging from 1.95° (for the C13/C23 pair) to 2.29° (for the C12/C22 pair). The Fe1–C bond lengths for the substituted Cp ring are in the range 2.041(3)–2.053(2) and those for the unsubstituted Cp ring are quite similar, being [2.034(2)–2.055(2)]. The C–C bonds of the substituted Cp ring are in the range 1.415(4)–1.434(3) while those for the unsubstituted Cp ring are slightly shorter in the range 1.404(4)–1.415(4). The C1–C11–Fe1 angle is 127.51(14)°. The ferrocenyl moiety lies approximately orthogonal to the amide moiety, while the angle between the amide plane and the phenyl ring is 9.43°. The key molecular geometry of the molecule is described by the N1–C1-C11–C12 and the N1–C2–C31–C32 torsion angles (74.61(3)° and 169.54(2)°, respectively). The angle between the two Cp rings is calculated to be 2.96°. The C–C bonds of the fluorinated phenyl ring range from 1.369(4) to 1.394(3) Å. The C=O (C2–O1) distance is 1.240(3) while the C2–N1 distance is 1.332(3), demonstrating significant double bond character.

The *in vitro* activity of four of the compounds (**4a**, **4d**, **4e** and **4f**) against the hormone independent MDA-MB-435-S-F breast cancer cell line was evaluated using the acid phosphatase assay as previously described [22]. 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. These four compounds were selected as a preliminary study of the cytotoxic properties of this series of compounds. The compounds were chosen as a representative sample each containing a different number of fluorine substituents. The cells were treated with the *N*-(ferrocenylmethyl)benzene-carboxamide compounds at two concentrations (1 μM and 10 μM) for the preliminary study. A previous study with a ferrocene pyrazole had shown significant cytotoxicity at concentrations as low as 10 μM in a MCF-7 breast cancer cell line [23]. The MDA-MB-435-S-F cells were incubated for 3 days with the compounds to be evaluated. Cell survival was established through determination of the acid phosphatase activity of surviving cells and growth inhibition calculated relative to controls (untreated cells).

It can be seen from Fig. 1 that all compounds tested exerted inhibitory effects on the growth of MDA-MB-435-S-F cells. Overall, compound **4d** was the most cytotoxic, with 41 ± 3% inhibition achieved at 10 μM. The remaining three compounds demonstrated relatively good inhibition at the same concentration ranging from 27 ± 5% to 37 ± 4%.

Compound **4d** was also tested over a wider range of concentrations (1–40 μM). At a concentration of 40 μM 67% growth inhibition was recorded. The IC_{50} was found to be in the range of 11–14 μM. The results demonstrate that cytotoxicity increased significantly as the concentration of compound **4d** increased, resulting in a dose-dependant relationship (see Fig. 3).

This ease of synthesis and general stability of these new compounds coupled with their encouraging cytotoxic activity against the hormone independent breast cancer cell line MDA-MB-435-S-F clearly promotes further research in this area. We are currently in the process of synthesising other derivatives with the aim of improving cytotoxicity and identifying derivatives with lower IC_{50} values.

2. Experimental

2.1. General procedure for the synthesis of compounds *4a–f*

2.1.1. Synthesis of *N-(Ferrocenylmethyl)-4-fluorobenzene-carboxamide 4d*

Ferrocenylmethyl amine (0.44 g, 2.5 mmol) was added to a stirred solution of 4-fluorobenzoic acid (0.33 g, 2.5 mmol), DCC (0.55 g, 2.5 mmol) and HOBt (0.35 g, 2.5 mmol) in CH_2Cl_2 (40 ml) at 0 °C. After 30 min the temperature was allowed to rise to room temperature and stirring continued for 48 h. The precipitated *N*,*N*′-dicyclohexylurea was removed by filtration and the solvent removed *in vacuo*. The mixture was purified on a silica-gel column using a mobile phase of hexane-ethyl acetate (2:1). Recrystallisation form ethyl acetate-petroleum ether furnished **4d** as orange/red crystals (0.45 g, 54% yield). m.p. 150–152 °C (uncorrected). UV–Vis λ_{max} (435.0 nm, $\varepsilon = 720$); I.R. (KBr, cm^{-1}): $\nu_{C=O}$ 1644; ^1H NMR (400 MHz) δ (DMSO-d_6): 4.09 (2H, t, $J = 1.6$ Hz, η^5-C_5H_4 *meta*), 4.18–4.20 (7H, m, η^5-C_5H_5 and CH_2Fc), 4.25 (2H, t, $J = 1.6$ Hz, η^5-C_5H_4 *ortho*), 7.28–7.35 (2H, m, ArH), 7.92–7.95 (2H, m, ArH), 8.78 (1H, t, $J = 5.8$ Hz, NH). ^{13}C NMR (100 MHz) δ (DMSO-d_6): 38.5 (−ve DEPT), 67.7, 68.6, 68.7, 86.4, 115.5–115.7, 130.1–130.2, 131.2–131.3, 162.9, 165.3. ^{19}F NMR

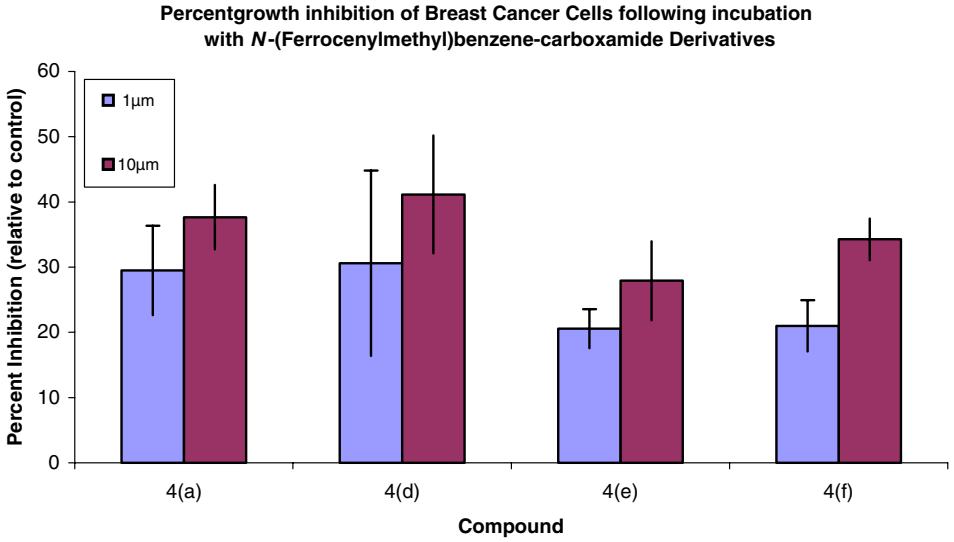


Fig. 3. Bar chart illustrating % growth inhibition of the *N*-(ferrocenylmethyl)benzene-carboxamide compounds at concentrations of 1 μM and 10 μM, $n = 3$.

(376 MHz) δ (DMSO-d_6): −34.5 (broad m). Anal. Calc. for $C_{18}H_{16}NOFFe$ requires: C, 64.12; H, 4.78; N, 4.15. Found: C, 64.01; H, 4.85; N, 4.06%. Mass spectrum: found: $[M + Na]^+$ 360.1. $C_{18}H_{16}NOFFeNa$ requires: 360.17.

Crystallographic data **4d**: chemical formula $C_{18}H_{16}NOFFe$, red/orange block, molecular weight 337.17 g mol^{-1}, orthorhombic, space group $P2_12_12_1$, unit cell dimensions $a = 10.0181(2)$ Å, $b = 11.6928(2)$ Å, $c = 12.2586(2)$ Å, $V = 1435.97(4)$ Å^3, $Z = 4$, density = 1.56 g cm^{-3}, $\mu = 1.061$ mm^{-1}, extinction coefficient = 0.0051(8), BASF component = 0.1429, 10539 reflections collected in the range 2.7–27.5°, 3255 independent reflections, $2983 > 2\sigma(I)$, 205 parameters, R factor = 0.0306, $wR_2 = 0.0653$.

Crystal data were collected using *KappaCCD Server Software* [24]. The structures were solved by direct methods using SHELXS-97 [25] and refined using SHELXL-97 [26]. All H atoms attached to C atoms were treated as riding atoms using the SHELXL97 defaults for 150 K in the refinement and the N–H H atom was allowed to refine freely with isotropic temperature factors to a bond length of 0.79(3) Å. Anisotropic temperature factors were used for all non-hydrogen atoms. Molecules of **4d** are not chiral, however, it crystallises in the chiral space group $P2_12_12_1$. The Flack parameter was determined to be 0.141(17) in the final structure factor calculations and indicating a small but significant fractional contribution from the inverted component of a '*racemic twin*'. Subsequent refinement using the TWIN/BASF SHELXL97 commands of (−1000−1000−1) and 0.143 yielded a final molecular structure. Molecular graphics were produced using PLATON [27].

2.2. General procedure for in vitro cytotoxicity assays

MDA-MB-435-S-F breast cancer cells were grown in RPMI medium supplemented with 10% foetal calf serum and 10 mM sodium pyruvate and seeded at a density of 5×10^4 cells/ml in 96-well plates. After 24 hours incubation in a 37°C, 5% CO_2 incubator 100 μL of medium containing dilute solutions of the ferrocenyl compounds at concentrations of 1 μM and 10 μM from stock solutions in ethanol were added. Controls were supplemented with medium containing ethanol (0.7%). Treatments were set up in six well replicates. Cells were then incubated at 37°C, 5% CO_2 for 3 days. Cell survival was evaluated using the acid phosphatase assay. This assay was shown to be linear with respect to cell number up to 5×10^4 cells/ml (5000 cells/well). The cytotoxic conjugated linoleic acid (CLA) mixture of isomers (NuChek-Prep, Elysian MN, USA) (0–40 μM) [28–30] was used as a reference.

Acknowledgements

P.T.M.K. and P.N.K. thank the National Institute of Cellular Biotechnology, DCU, for a studentship under the Programme for Research in Third Level Institutions (PRTLI, round 3, 2001–2006). A.J.L. thanks the University of Toronto for funds in aid of research.

Appendix A. Supplementary data

CCDC 619909 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2006.11.012.

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