Supplementary data

Structural and Biological Investigation of Ferrocene-Substituted 3-Methylidene-1,3-dihydro-2H-indol-2-ones

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Experimental Section

General

Starting materials and commercial grade solvents were purchased from Sigma-Aldrich or Alfa Aesar and used without further purification. Reactions were carried out under argon. NMR spectra were measured on a Jeol EX270 spectrometer at 270 MHz (^{1}H) and 75 MHz (^{13}C) in $CDCl_3$. Elemental analyses were performed on a CE Instruments apparatus. Mass spectra were recorded by the EPSRC unit (Swansea). Dulbecco's modified eagle medium (DMEM) was purchased from Gibco BRL, fetal calf serum from Dutscher, Brumath, France, glutamine, estradiol and protamine sulfate were from Sigma. MDA-MB-231 cells were from the HumanTumor Cell Bank. Unless otherwise stated all other cell culture reagents were from Invitrogen. Vero cells were from the American Tissue Culture Collection (ATCC-CCL-81). The B16 cells were kindly donated by Professor I. Hart (St Thomas' Hospital)

Compounds (*Z*)- and (*E*)-3.

Oxindole (133 mg, 1.00 mmol), ferrocene carboxaldehyde (214 mg, 1.00 mmol) and piperidine (catalytic, few drops) were combined in ethanol (5 mL) and heated to reflux for 3 h. After cooling, the reaction mixture was concentrated *in vacuo*. The resulting crude material was purified by preparative thin layer chromatography, using a 10:1 CH_2Cl_2: acetone mixture. The respective bands were cut out and the product extracted successively with CH_2Cl_2, EtOH and ethyl acetate (20 mL each) and the silica removed by filtration. After combing the respective extracts and solvent removal, the isomers (*Z*)-**3** (76 mg, 23%, R_f=0.8) and (*E*)-**3** (145 mg, 44%, R_f=0.6) were obtained as dark red solids. Dark red crystals of each isomer, suitable for X-

ray diffraction, were obtained by diffusion of hexane in CH_2Cl_2 solutions. NMR (*Z*)-**3** ($CDCl_3$) δ_H 7.94 (1H, brs, NH), 7.46 (1H, d, ^3J = 4.0 Hz, aryl), 7.38 (1H, s, vinyl CH), 7.18 (1H, m, aryl), 7.08 (1H, m, aryl), 6.82 (1H, d, ^3J = 4.0 Hz, aryl), 5.31 (2H, ^3J = 1.8 Hz, ferrocene), 4.57 (2H, ^3J = 1.8 Hz, ferrocene), 4.17 (5H, s, C_5H_5); δ_c 167.9, 138.9, 138.3, 127.4, 125.7, 121.5, 121.3, 118.2, 109.3, 76.7, 72.4, 69.8 (overlapping carbons). (Found: C, 68.8; H, 4.9; N, 4.1. $C_{19}H_{15}NOFe$ requires C, 69.3; H, 4.6; N, 4.3. NMR (*E*)-**3** ($CDCl_3$) δ_H 7.94 (1H, d, ^3J = 4.0 Hz, aryl), 7.68 (1H, brs, NH), 7.65 (1H, s, vinyl CH), 7.17 (1H, m, aryl), 6.96 (1H, t, ^3J = 4.0 Hz, aryl), 6.85 (1H, d, ^3J = 5.4 Hz, aryl), 4.76 (2H, ^3J = 1.8 Hz, ferrocene), 4.57 (2H, ^3J = 1.8 Hz, ferrocene), 4.21 (5H, s, C_5H_5); δ_c 168.6, 138.9, 137.0, 127.0, 121.4, 121.3, 120.3, 108.3, 76.0, 70.0, 68.5 (overlapping carbons). (Found: C, 68.7; H, 4.7; N, 3.9. $C_{19}H_{15}NOFe.0.05$ CH_2Cl_2 requires C, 68.6; H, 4.6; N, 4.2. HRMS ((*E*)/(*Z*) mixture) Found 327.0544. $C_{19}H_{15}NO^{54}Fe$ requires 327.0544.

Biochemical experiments

MDA-MB-231 hormone independent breast cancer cell line testing. Materials. Stock solutions (1 x 10^{-3} M) of the ferrocenyl complexes to be tested were prepared in DMSO and were kept at 4°C in the dark. Serial dilutions were prepared just prior to use.

Cell Culture. Vero cells were grown in D-MEM containing Glutamax (25mM), penicillin/streptomycin (5mM) and 10% (v/v) FBS and were split 1:20 twice per week using a standard protocol.[1] Similarly, B16 cells[1] were grown in RPMI 1640 containing Glutamax (25mM), penicillin / streptomycin (5mM) and 10% (v/v) FBS and were also split 1:20 twice per week. B16 and Vero cells were also maintained in an atmosphere of 5% (v/v) CO_2 at 37°C. IC_{50} values were obtained using published methodologies.[1,2] Briefly, 5x10^3 cells/well were used to seed 96 well cell culture treated plates (Sigma). Compounds were dissolved at 5 mg/ml in sterile DMSO and further diluted with the appropriate complete cell culture medium. After 72 hrs cell viability was assessed using MTT (Sigma) also following published protocols.[1]

For the commercial IC_{50} determinations at MDS Pharma (http://www.mdsps.com): (Assay reference 171820; Protein Tyrosine Kinase,

KDR (VEGFR-2)), human recombinant insect Sf21cells were employed (1% DMSO vehicle) against the substrate [^{32}P]Poly(Glu:Tyr).

X-Ray Crystallography: (*E*)- **3** and (*Z*)- **3**: Suitable crystals were selected and a dataset for (*Z*)- **3** was measured on a Bruker APEXII CCD diffractometer and for (*E*)- **3** on a Bruker KappaCCD diffractometer both at the windows of a Bruker FR591 rotating anode ($\lambda_{Mo\text{-}K\alpha}$ = 0.71073 Å) at 120 K. The data collections were driven by COLLECT[3] and processed by DENZO.[4] Absorption corrections were applied using SADABS.[5] The structures were solved in SIR2004[6] and were refined by a full-matrix least-squares procedure on F^2 in SHELXL-97.[7] All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were added at calculated positions and refined by use of a riding model with isotropic displacement parameters based on the equivalent isotropic displacement parameter (U_{eq}) of the parent atom. Figures were produced using ORTEP3 for Windows[8] and Mercury CSD 2.0[9] while structural analysis was carried out in PLATON.[10] In the case of (*Z*)- **3** the diffraction data were rather weak, especially at higher angle which has led to a slightly high value of R_{int}. This was the best crystal and the best quality dataset that could be obtained. The CIFs for the crystal structures of (*E*)- **3** and (*Z*)- **3** have been deposited with the CCDC and have been given the deposition numbers 702095 and 702096 respectively.

Table S1. X-Ray Crystallography Experimental Data

	(*E*)- **3**	(*Z*)- **3**
Empirical Formula	$C_{19}H_{15}FeNO$	$C_{19}H_{15}FeNO$
Formula Weight	329.17	329.17
Temperature (K)	120 (2)	120 (2)
Crystal Size (mm)	0.42 x 0.3 x 0.1	0.06 x 0.03 x 0.02
Crystal System	Triclinic	Monoclinic
Space Group	$P\ \bar{1}$	$P2_1/n$
a ; *b* ; *c* (Å)	5.9306 (1) ; 13.5489 (4) ; 19.0452 (5)	15.1939 (14) ; 6.1825 (6) ; 15.4621 (14)
α ; β ; γ(°)	73.846 (1) ; 85.280 (2) ; 86.068 (1)	90 ; 93.168 (5) ; 90

V (Å^3)	1463.27 (6)	1450.2 (2)
Z ; Z'	4 ; 2	4 ; 1
Density (Calculated) (Mg m^{-3})	1.494	1.508
Absorption Coefficient (MoKα, mm^{-1})	1.031	1.040
Max. and Min. Transmission	0.9040 and 0.6714	0.9402 and 0.9795
F(000)	680	680
θ Range for Data Collection (°)	3.02 – 27.48	3.55 – 25.01
Index Ranges	$-7 \le h \le 7$, $-17 \le k \le 17$, $-24 \le l \le 24$	$-18 \le h \le 18$, $-7 \le k \le 7$, $-18 \le l \le 18$
Reflections Collected	30617	10819
Independent Reflections	6708 [R_{int} = 0.0522]	2563 [R_{int} = 0.1211]
Measured Reflections with $I \ge 2\sigma(I)$	5216	1752
Completeness to θ_{max}	99.8	99.6
Data / Restraints / Parameters	6708 / 0 / 397	2563 / 0 / 199
Goodness-of-Fit on F^2	1.044	1.078
Final *R* Indices (Observed Data)	*R1* = 0.0380, *wR2* = 0.0910	*R1* = 0.0996, *wR2* = 0.1877
Final *R* Indices (All Data)	*R1* = 0.0575, *wR2* = 0.0997	*R1* = 0.1522, *wR2* = 0.2191
Largest Diff. Peak ; Hole (e Å^{-3})	0.441 ; -0.579	1.502 ; -0.609

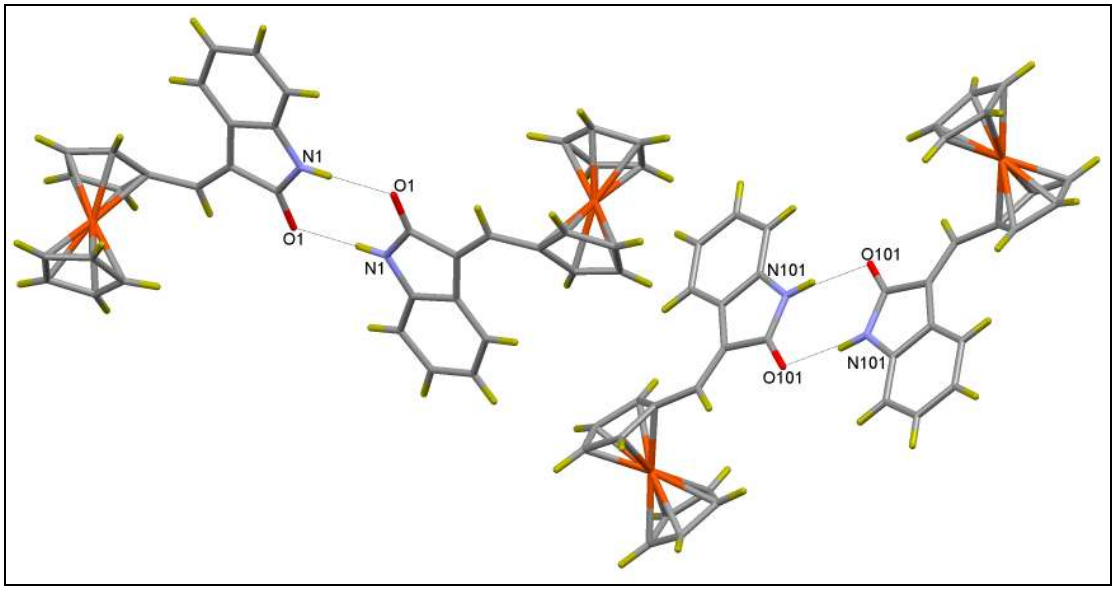

Figure S1. Intermolecular H-bonding in (*E*)-3.

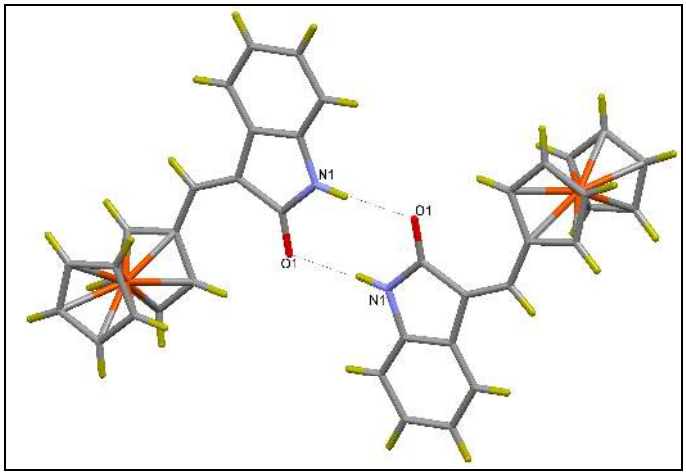

Figure S2. Intermolecular H-bonding in (*Z*)-3.

Table S2. Selected bond lengths (Å), angles (°) and torsion angles (°) for (*E*)-3 and (*Z*)-3

	(*E*)-3	**(*Z*)-3**
C(1)–C(11)	1.449 (3)	1.445 (12)
C(11)–C(12)	1.349 (3)	1.353 (11)
N(1)–C(13)	1.362 (3)	1.378 (11)
N(1)–C(14)	1.400 (3)	1.404 (11)
O(1)–C(13)	1.234 (3)	1.222 (10)
C(101)–C(111)	1.452 (3)	

C(111)–C(112)	1.350 (3)	
N(101)–C(113)	1.354 (3)	
N(101)–C(114)	1.404 (3)	
O(101)–C(113)	1.236 (3)	
C(1)–C(11)–C(12)	130.1 (2)	132.2 (8)
C(13)–N(1)–C(14)	111.2 (2)	112.5 (7)
O(1)–C(13)–N(1)	125.7 (2)	123.9 (8)
C(1)–C(11)–C(12)–C(15)	5.7 (4)	1.0 (10)
C(101)–C(111)–C(112)	131.0 (2)	
C(113)–N(101)–C(114)	111.1 (2)	
O(101)–C(113)–N(101)	125.9 (2)	
C(101)–C(111)–C(112)–C(115)	5.3 (5)	

Table S3. Hydrogen bonds [Å and °]. (*Z*)-3 (a); (*E*)-3 (b,c)

D–H···*A*	*d*(*D*–H)	*d*(H···*A*)	*d*(*D*···*A*)	∠(*D*H*A*)
(a) N1–H1···O1^{i}	0.88	1.99	2.797 (9)	151.0
(b) N1–H1···O1^{ii}	0.88	1.92	2.795 (2)	169.5
(c) N101–H101···O101^{iii}	0.88	1.94	2.815 (2)	170.7

Symmetry transformations used to generate equivalent atoms:

(i) –x+1,–y–1,–z+1 (ii) –x+1,–y,–z (iii) –x+2,–y+1,–z+1

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