SUPPORTING INFORMATION

Facile synthesis and strong antiproliferative activity of disubstituted diphenylmethylidenyl-[3]ferrocenophanes on breast and prostate cancer cell lines

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General Remarks. The syntheses of all compounds were performed under argon atmosphere, using standard Schlenk techniques. THF was obtained by distillation from sodium/benzophenone. Thin layer chromatography was performed on silica gel 60GF254. The preparative HPLC separations were performed on a Shimadzu apparatus with a Nucleodur C18 column (length of 25 cm, diameter of 3.2 cm, and particle size of 10 mm) using acetonitrile as an eluent. The analytic HPLC controls were performed on a Shimadzu apparatus with a Nucleodur C18 column (length of 15 cm, diameter of 0.45 cm, and particle size of 5 µm) using acetonitrile as an eluent. Infrared spectra were obtained on a JASCO FT/IR-4100 spectrometer as a KBr plate. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz Bruker spectrometer. Mass spectrometry was performed with a Nermag R 10–10C spectrometer. HRMS measurements were performed by the Institut Parisien de Chimie Moléculaire (UMR 7201), Université Pierre et Marie Curie, Paris. Elemental analyses were performed by the microanalysis services of ICSN (Gif sur Yvette, France). Melting points were measured with a Kofler device. Cytotoxicity measurements on PC3 prostate cancer cells in vitro were performed by ImaGIF Ciblothèque Cellulaire (Institut de Chimie des Substances Naturelles).

Synthesis

Compounds **1**, **2**, **3**, and **4** have been previously prepared.[1, 2] [3]ferrocenophane-1-one,[3] 4-hydroxy-4'nitrobenzophenone[4, 11] and 4-4'diacetylamino benzophenone[9] were prepared as previously described.

General synthesis of 5, 6, and 7. Zinc powder was suspended in THF at r.t. and titanium tetrachloride was added slowly via a syringe while stirring. The reaction mixture was refluxed for 2 h, after which a THF solution containing ferrocenophane-1-one and the appropriate benzophenone was added, and mixture was refluxed. The reaction mixture was poured into water, acidified with HCl and extracted with dichloromethane. The organic phase was washed with water, dried over magnesium sulfate, filtered and the solvent was evaporated. The oil was purified by HPLC and recrystallized with the appropriate solvents.

1-diphenylmethylidenyl-[3]ferrocenophane, **5**. Zinc powder (1.96 g, 30 mmol), titanium tetrachloride (2.2 mL, 20 mmol), [3]ferrocenophane-1-one (1.2 g, 5 mmol), benzophenone (0.91 g, 5 mmol), reflux duration was 2 h. Recrystallization from hexane gave **5** as bright yellow crystals (1.81 g, 93%, m.p. = 181 °C). 1H NMR (300 MHz, $CDCl_3$): δ 2.17 (broad s, 2H, CH_2), 2.50 (broad s, 2H, CH_2), 3.96 (s, 2H, C_5H_4), 3.99 (s, 2H, C_5H_4), 4.04 (s, 2H, C_5H_4), 4.22 (s, 2H, C_5H_4), 7.01-7.29 (m, 10H, 2 C_6H_5). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 28.7 (CH_2), 40.5 (CH_2), 69.6 (2CH C_5H_4), 70.1 (2CH C_5H_4), 70.6 (2x2CH C_5H_4), 83.9 (C_{ip}), 87.2 (C_{ip}), 126.1 (CH_{arom}),

126.6 (CH_{arom}), 127.4 ($2CH_{arom}$), 128.2 ($2CH_{arom}$), 129.3 ($2CH_{arom}$), 130.5 ($2CH_{arom}$), 134.8 (C), 140.6 (C), 143.2 (C), 143.6 (C). IR (KBr, ν cm^{-1}): 3077, 2924 (CH2). MS (EI, 70 eV) m/z : 390 [M]+.. Anal. Calcd for C26H22Fe: C 80.01, H 5.68; Found: C 79.61, H 5.59.

1-[(4-aminophenyl-4'-hydroxyphenyl)methylidenyl]-[3]ferrocenophane, 6. Zinc powder (9.152 g, 140 mmol), titanium tetrachloride (8.79 mL, 80 mmol), [3]ferrocenophane-1-one (4.8 g, 20 mmol), 4-hydroxy-4'nitrobenzophenone (4.86 g, 20 mmol). Reflux duration was 1 day. Recrystallization from EtOH gave **6** as bright yellow crystals (1.75 g, 21% yield) consisting of a mixture of *Z* and *E* isomers (58/42). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.26 (m, 2H, CH_2), 2.58 and 2.65 (m, 2H, CH_2), 3.93 (s, 2H, C_5H_4), 3.97 (m, 4H, C_5H_4), 4.24 (s, 2H, C_5H_4), 4.89 and 5.06 (s broad, 2H, NH_2), 6.23 and 6.44 (d, *J*=8.5, 2H, C_6H_4), 6.53 and 6.60 (d, *J*=8.5, 2H, C_6H_4), 6.73 (d, *J*=8.5, 2H, C_6H_4), 6.83 and 6.97 (d, *J*=8.5, 2H, C_6H_4), 9.17 and 9.38 (s, 1H, OH). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 28.0 (CH_2), 40.6 (CH_2), 68.0 (2CH, C_5H_4), 68.3 (2CH, C_5H_4), 69.7 (2CH, C_5H_4), 70.0 (2CH, C_5H_4), 84.2 (C_{ip}, C_5H_4), 86.6 and 86.7 (C_{ip}, C_5H_4), 112.7 and 113.5 (2CH, C_6H_4), 114.0 and 114.8 (2CH, C_6H_4), 129.7 and 130.1 (2CH, C_6H_4), 130.4 (2C), 130.9 and 131.3 (2CH, C_6H_4), 134.5 (C), 140.6 and 140.7 (C), 146.6 and 147.3 (C), 155.3 and 155.9 (C). IR (KBr, ν cm^{-1}): 3390 (OH, NH_2), 3086, 2928, 2845 (CH_2), 1608, 1509, 1434, 1260, 1171, 835, 804. MS (CI, NH_3) *m/z*: 422 $[M+H]^+$. HRMS (ESI, $C_{26}H_{23}FeNNaO$) calcd: 444.10224, found: 444.10213. Anal. Calcd for $C_{26}H_{23}FeNO.(0.6\ H_2O)$: C 72.27, H 5.64, N 3.24; found: C 72.12, H 5.28, N 3.21.

1-[bis(4,4'-N-acetylaminophenyl)methylidenyl]-[3]ferrocenophane, 7. Zinc powder (5.72 g, 87.5 mmol), titanium tetrachloride (6.87 mL, 62.5 mmol), [3]ferrocenophane-1-one (3.0 g, 12.5 mmol), 4-4'-bis-N-acetylaminobenzophenone (2.96 g, 10 mmol). Reflux duration was 3 days. Recrystallization from the acetonitrile/water eluent used in HPLC gave **7** as bright yellow crystals (2.18 g, 44% yield).
m. p. =181 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.29 (s, 2H, CH_2), 2.61 (s, 2H, CH_2), 3.96 (s, 2H, C_5H_4), 3.98 (s, 2H, C_5H_4), 4.02 (s, 2H, C_5H_4), 4.22 (s, 2H, C_5H_4), 6.93 (d, *J*=8.4, 2H, C_6H_4), 7.10 (d, *J*=8.4, 2H, C_6H_4), 7.20 (d, *J*=8.4, 2H, C_6H_4), 7.46 (d, *J*=8.4, 2H, C_6H_4), 7.90 (s, 1H, NH), 8.18 (s, 1H, NH). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 24.5 ($2CH_3$), 28.8 (CH_2), 41.0 (CH_2), 66.8 (2CH, C_5H_4), 69.3 (2CH, C_5H_4), 70.4 (2CH, C_5H_4), 70.5 (2CH, C_5H_4), 83.7 (C_{ip}, C_5H_4), 87.0 (C_{ip}, C_5H_4), 119.0 (2CH, C_6H_4), 119.8 (2CH, C_6H_4), 130.0 (2CH, C_6H_4), 131.2 (2CH, C_6H_4), 134.9 (C), 136.0 (C), 136.8 (C), 139.3 (C), 139.4 (C), 139.7 (C), 168.8 (CO), 169.1 (CO). IR (KBr, ν cm^{-1}): 3421, 3357 (NH), 2951, 2922, 2898 (CH_2), 1671 (CO), 1596. MS (CI, NH_3) m/z : 505 $[M+H]^+$, 522 $[M+NH_4]^+$. HRMS (ESI, $C_{30}H_{28}FeN_2O_2$: $[M]^{+\cdot}$) calcd: 504.1501, found: 504.14947. Anal. Calcd for $C_{30}H_{28}FeN_2O_2.(\ 0.5\ H_2O)$: C 70.18, H 5.69, N 5.46; found: C 69.83, H 5.94, N 5.33.

1-[(4-acetylaminophenyl-4'-hydroxyphenyl)methylidenyl]-[3]ferrocenophane, 8. In a Schlenk flask **6** (0.2g, 0.47 mmol) was dissolved in anhydrous THF. Acetyl chloride (0.037 g, 1.104 g/mL, 0.5 mmol) and pyridine (0.038 g, 0.5 mmol) were added and the reaction mixture was left to stir for 3 h. The mixture was poured into water and extracted with dichloromethane. The organic phase was washed with water, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude mixture was filtered over silica gel using petroleum ether and CH_2Cl_2, concentrated, and purified by HPLC (acetonitrile/H_2O) to yield a

mixture of *Z* and *E* isomers and recrystallized from acetonitrile to yield **8** as orange-yellow crystals (0.14 g, 63 % yield) consisting of a mixture of *Z* and *E* isomers (75/25, *E*/Z respectively). ^{1}H NMR (300 MHz, acetone-d_6): Z isomer: δ 2.09 (s, 3H, CH_3), 2.32-2.38 (m, 2H, CH_2), 2.66-2.74 (m, 2H, CH_2), 3.93 (s, 2H, C_5H_4), 3.95 (s, 2H, C_5H_4), 3.96 (s, 2H, C_5H_4), 4.26 (s, 2H, C_5H_4), 6.55, 6.85, 7.16 and 7.64 (d, *J*=8.7, 8H, C_6H_4), 8.23 (s, 1H, OH), 9.22 (s broad, 1H, NH); *E* isomer: δ 2.01 (s, 3H, CH_3), 2.32-2.38 (m, 2H, CH_2), 2.66-2.71 (m, 2H, CH_2), 3.93 (s, 2H, C_5H_4), 3.95 (s, 2H, C_5H_4), 3.96 (s, 2H, C_5H_4), 4.26 (s, 2H, C_5H_4), 6.84, 6.93, 7.07 and 7.35 (d, *J*=8.7, 8H, C_6H_4), 8.44 (s, 1H, OH), 9.04 (s broad, 1H, NH). ^{13}C NMR (75.4 MHz, acetone-d_6): *Z* isomer: δ 24.2 (CH_3), 29.2 (CH_2), 41.5 (CH_2), 68.9 (2CH, C_5H_4), 69.2 (2CH, C_5H_4), 70.9 (2CH, C_5H_4), 71.0 (2CH, C_5H_4), 84.7 (C_{ip}, C_5H_4), 87.7 (C_{ip}, C_5H_4), 115.0 (2CH, C_6H_4), 119.5 (2CH, C_6H_4), 130.3 (2CH, C_6H_4), 132.5 (2CH, C_6H_4), 134.5 (C), 135.7 (C), 138.4 (C), 139.8 (C), 143.2 (C), 157.2 (C), 172.7 (CO); *E* isomer: δ 24.2 (CH_3), 29.2 (CH_2), 41.5 (CH_2), 69.0 (2CH, C_5H_4), 69.4 (2CH, C_5H_4), 71.0 (2CH, C_5H_4), 71.1 (2CH, C_5H_4), 84.8 (C_{ip}, C_5H_4), 87.8 (C_{ip}, C_5H_4), 115.8 (2CH, C_6H_4), 118.6 (2CH, C_6H_4), 131.2 (2CH, C_6H_4), 131.6 (2CH, C_6H_4), 134.5 (C), 135.7 (C), 138.4 (C), 139.6 (C), 141.3 (C), 157.2 (C), 168.7 (CO). IR (KBr, ν cm^{-1}): 3395 (OH, NH_2), 2924 (CH_2), 1664 (CO), 1607, 1508, 1268, 834. MS (EI, 70 eV) m/z: 463 $[M]^{+\cdot}$, 384, 121. HRMS (ESI, $C_{28}H_{25}FeNNaO_2$) calcd: 486.11282, found: 486.11269.

1-[bis(4,4'-aminophenyl)methylidenyl]-[3]ferrocenophane, 9. 1-[bis(4,4'-acetylaminophenyl) methylidenyl]-[3]ferrocenophane **7** (1.3 g, 2.58 mmol) was dissolved in EtOH and HCl (16.93 mL, 80 eq) was added. The solution was refluxed for 4 h. After cooling, the solution was poured into a saturated $NaHCO_3$ solution portion by portion. Water was added and the solution was extracted with dichloromethane, the solvent was removed under reduced pressure, and the product was purified with HPLC. Recrystallization from the acetonitrile/water eluent used gave 3 as bright yellow crystals (0.84 g, 78 %).
m.p. = 126°C. ^{1}H NMR (300 MHz, acetone-d_6): δ 2.28-2.34 (m, 2H, CH_2), 2.67-2.73 (m, 2H, CH_2), 3.91 (t, *J*=1.9, 2H, C_5H_4), 3.94-3.98 (m, 4H, C_5H_4), 4.22 (t, *J*=1.9, 2H, C_5H_4), 4.44 (s broad, 2H, NH_2), 4.61 (s broad, 2H, NH_2), 6.35 (d, *J* =8.7, 2H, C_6H_4), 6.65 (d, *J* =8.7, 2H, C_6H_4), 6.72 (d, *J* =8.7, 2H, C_6H_4), 6.93 (d, *J* =8.7, 2H, C_6H_4). ^{13}C NMR (75.4 MHz, acetone-d_6): δ 29.2 (CH_2), 41.8 (CH_2), 68.7 (2CH, C_5H_4), 69.0 (2CH, C_5H_4), 70.9 (2CH, C_5H_4), 71.0 (2CH, C_5H_4), 85.7 (C_{ip}, C_5H_4), 100.4 (C_{ip}, C_5H_4), 113.9 (2CH, C_6H_4), 114.7 (2CH, C_6H_4), 130.9 (2CH, C_6H_4), 131.3 (2C, C-NH_2, C_6H_4), 132.3 (2CH, C_6H_4), 139.8 (C), 147.2 (2C), 159.1 (C). IR (KBr, ν cm^{-1}): 3439, 3376 (NH_2), 2926 (CH_2). MS (EI, 70 eV) *m/z*: 420 $[M]^{+\cdot}$, 341, 284, 208. HRMS (ESI, $C_{26}H_{25}FeN_2$: $[M+H]^+$) calcd: 421.1368, found: 421.13532. Anal. Calcd for $C_{26}H_{24}FeN_2$.(0.5 H_2O): C 72.74, H 5.87, N 6.52; found: C 72.37, H 5.88, N 6.23.

Cell culture MDA-MB-231

Cells were maintained in a monolayer culture in DMEM with phenol red/Glutamax I supplemented with 9% fetal bovine serum at 37 °C in a 5% CO_2/air-humidified incubator. For proliferation assays, MDA-MB-231 cells were plated in 1 mL of DMEM without phenol red, supplemented with 9% decomplemented and hormone-depleted fetal bovine serum, 0.9% kanamycin, 0.9% Glutamax I and incubated. The following day (D0), 1 mL of the same medium containing the compounds to be tested was added to the plates. After 3 days (D3) the incubation medium was removed and 2 mL of the fresh medium containing the compounds was added. After 5 days the total protein content of the plate was analyzed as follows: cell monolayers were fixed

for 1 h at room temperature with methylene blue (1 mg mL^{-1} in 50:50 water/MeOH mixture), then washed with water. After addition of HCl (0.1 M, 2 mL), the plate was incubated for 1 h at 37 °C and then the absorbance of each well (three wells for each concentration) was measured at 655 nm with a Biorad microplate reader. The results are expressed as the percentage of proteins vs. the control. Experiments were performed at least in duplicate

Cell culture PC3

The human cell PC-3 (prostate adenocarcinoma) purchased from ATCC was grown in RPMI medium supplemented with 10% fetal calf serum, in the presence of penicilline, streptomycine and fungizone in 75cm² flasks under 5% CO_2. For cytotoxicity/antiproliferative determinations, cells were plated in 96-well tissue culture microplates in 200µl complete medium and treated 24h later with compounds dissolved in DMSO using a Biomek 3000 (Beckman-Coulter). Controls received the same volume of DMSO (1% final volume). After 72h exposure, MTS reagent (Promega) was added and incubated for 3h at 37°C: the absorbance was monitored at 490 nm and results expressed as the inhibition of cell proliferation calculated as the ratio [(1-(OD490 treated/OD490 control))×100] in triplicate experiments. For IC50 experiments performed in duplicate, compounds were added in the range 0.5 nM-10 µM in a fixed volume of DMSO.

X-ray crystallography

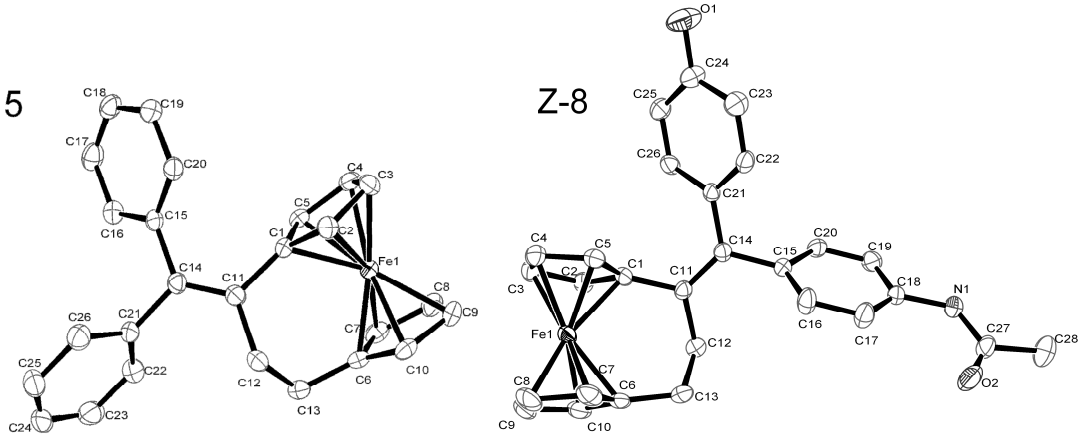

Table 1. Selected bond distances and angles for **5** and **8**.		
	5	**8**
Fe-C_{Cp}	2.032(2)-2.085(2)	2.0363(19)-2.080(2)
C_{Cp}-C_{Cp}	1.413(4)-1.444(4)	1.426(4)-1.447(3)
C6-C13	1.515(4)	1.530(3)
C12-C13	1.552(4)	1.562(3)
C11-C12	1.535(3)	1.527(3)
C1-C11	1.504(3)	1.503(3)
C11-C14	1.354(3)	1.358(3)
D_{Cp}-Fe-D_{Cp}	173.0(1)	173.4(1)
C6-C13-C12	114.3(2)	114.17(17)
C11-C12-C13	115.5(2)	114.91(18)
C1-C11-C12	115.1(2)	115.73(17)
C1-C11-C14	122.2(2)	121.34(18)

C11-C14-C12	122.6(2)	122.91(17)