

Supporting information

Synthesis of Cytotoxic Ferrocenyl Flavones via a Ferricenium-Mediated 1,6-Oxidative Cyclization

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General remarks

All aldolic condensation reactions were carried out under argon, using standard Schlenk techniques. Cyclization reactions were done in air. THF was distilled over sodium-benzophenone. All other chemical reagents and solvents were used without further purification. Preparative HPLC was performed using a Shimadzu LC-8A machine. Silica gel chromatography was done with Merck 60 (40-63 μm) silica. ^1H and ^{13}C NMR spectra were measured with a 300 or 400 MHz Bruker Advance spectrometer, and δ are given in ppm. LR-MS mass spectra were measured on a ThermoScientific ITQ1100 spectrometer using the direct exposure probe method by the mass spectrometry service at the Ecole Nationale Supérieure de Chimie de Paris.

General preparation of ferrocenyl chalcones

Ferrocenyl chalcones were prepared following the literature procedure.¹ Ferrocene carboxaldehyde (1 equiv, (50-200 mg)) and the appropriate 2-hydroxyacetophenone (1 equiv) were dissolved in absolute EtOH in a round bottom flask. After stirring the mixture 10 to 15 min at r.t., NaOH (3 equiv) was added, and the solution was stirred overnight. The mixture was poured into H_2O and HCl 12 M, extracted with CH_2Cl_2 , and washed with H_2O . The organic phase was dried over MgSO_4 , filtered, and the solvent removed by evaporation. The product was purified by silica gel chromatography, using a mixture of petroleum ether/ CH_2Cl_2 4:1 as an eluent, and again using HPLC in MeCN/ H_2O (90:10). After HPLC purification, the acetonitrile was removed under reduced pressure and the aqueous phase extracted with CH_2Cl_2 . The NMR spectra of all purified compounds showed residual CH_2Cl_2 , even after being dried under vacuum for several hours.

(E)-1-(2'-hydroxyphenyl)-3-ferrocenylprop-2-en-1-one 1a. Violet solid. Found: C 64.43, H 4.89. Calc. for $\text{C}_{19}\text{H}_{16}\text{O}_2\text{Fe}\cdot 0.35 \text{CH}_2\text{Cl}_2$: C 64.22, H 4.65. δ_{H} (300 MHz; CDCl_3 ; Me_4Si) 7.90 (d, $J=15.1$ Hz, 1H, H_{vinyl}), 7.86 (d, $J=7.7$ Hz, 1H, H_{ar}), 7.48 (t, $J=7.8$ Hz, 1H, H_{ar}), 7.25 (d, $J=15.1$ Hz, 1H, H_{vinyl}), 7.02 (d, $J=7.8$ Hz, 1H, H_{ar}), 6.93 (t, $J=7.7$ Hz, 1H, H_{ar}), 4.64 (s, 2H, C_5H_4), 4.55 (s, 2H, C_5H_4), 4.20 (s, 5H, C_5H_5). δ_{C} (75 MHz; CDCl_3 ; Me_4Si) 192.7 (C_{ketone}), 162.6 (C_{ar}), 147.9 (C_{vinyl}), 135.9 (C_{ar}), 129.3 (C_{ar}), 120.0 (C_{vinyl}), 118.7 (C_{ar}), 118.6 (C_{ar}), 116.7 (C_{ar}), 78.9 (C_5H_4 , C_{quad}), 71.8 (C_5H_4), 69.9 (C_5H_5), 69.3 (C_5H_4). MS (APCI) m/z 332.07 $[\text{M}+\text{H}]^+$. This compound has been previously reported.¹

(E)-1-(5'-chloro-2'-hydroxyphenyl)-3-ferrocenylprop-2-en-1-one 1b. Violet solid. Found: C 61.75, H 4.02. Calc. for $\text{C}_{19}\text{H}_{15}\text{O}_2\text{FeCl}\cdot 0.06 \text{CH}_2\text{Cl}_2$: C 61.59, H 4.1. δ_{H} (300 MHz; CDCl_3 ; Me_4Si) 7.94 (d, $J=15$ Hz, 1H, H_{vinyl}), 7.80 (d, $J=2.4$ Hz, 1H, H_{ar}), 7.42 (dd, $^3J=9$ Hz, $^4J=2.4$ Hz, 1H, H_{ar}), 7.14 (d, $J=15$ Hz, 1H, H_{vinyl}), 6.96 (d, $J=9$ Hz, 1H, H_{ar}), 4.66 (s, 2H, C_5H_4), 4.51 (s, 2H, C_5H_4), 4.22 (s, 5H, C_5H_5). δ_{C} (75 MHz; CDCl_3 ; Me_4Si) 191.7 (C_{ketone}), 162.1 (C_{ar}), 149.4 (C_{vinyl}), 135.6 (C_{ar}), 128.5 (C_{ar}), 123.3 (C_{vinyl}), 120.7 (C_{ar}), 120.2 (C_{ar}), 115.9 (C_{ar}), 78.6 (C_5H_4 , C_{q}), 72.2 (C_5H_4), 70.0 (C_5H_5), 69.5 (C_5H_4). MS (APCI) m/z 366.03 $[\text{M}+\text{H}]^+$.

(E)-1-(5'-bromo-2'-hydroxyphenyl)-3-ferrocenylprop-2-en-1-one 1c. Violet solid. Found: C 55.04, H 3.73. Calc. for $C_{19}H_{15}O_2FeBr \cdot 0.05 CH_2Cl_2$: C 55.09, H 3.66. δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.94 (d, $J=14.4$ Hz, 1H, H_{vinyl}), 7.92 (m, 1H, H_{ar}), 7.55 (d, $J=8.1$ Hz, 1H, H_{ar}), 7.13 (d, $J=14.5$ Hz, 1H, H_{vinyl}), 6.92 (d, $J=8.1$ Hz, 1H, H_{ar}), 4.66 (s, 2H, C_5H_4), 4.58 (s, 2H, C_5H_4), 4.22 (s, 5H, C_5H_5). δ_C (75 MHz; $CDCl_3$; Me_4Si) 191.6 (C_{ketone}), 162.5 (C_{ar}), 149.4 (C_{vinyl}), 138.4 (C_{ar}), 131.6 (C_{ar}), 121.3 (C_{vinyl}), 120.6 (C_{ar}), 115.9 (C_{ar}), 110.3 (C_{ar}), 78.6 (C_5H_4 , C_q), 72.3 (C_5H_4), 70.1 (C_5H_5), 69.5 (C_5H_4). MS (APCI) m/z 410.05 $[M+H]^+$.

(E)-1-(3',5'-dichloro-2'-hydroxyphenyl)-3-ferrocenylprop-2-en-1-one 1d. Violet solid. Found: C 55.72, H 3.49. Calc. for $C_{19}H_{14}O_2FeCl_2 \cdot 0.125 CH_2Cl_2$: C 55.80, H 3.49. δ_H (300 MHz; $CDCl_3$; Me_4Si) 8.00 (d, $J=15$ Hz, 1H, H_{vinyl}), 7.74 (s, 1H, H_{ar}), 7.57 (s, 1H, H_{ar}), 7.01 (d, $J=15$ Hz, 1H, H_{vinyl}), 4.67 (s, 2H, C_5H_4), 4.62 (s, 2H, C_5H_4), 4.23 (s, 5H, C_5H_5). δ_C (75 MHz; $CDCl_3$; Me_4Si) 190.2 (C_{ketone}), 157.0 (C_{ar}), 149.8 (C_{vinyl}), 134.1 (C_{ar}), 126.1 (C_{ar}), 122.9 (C_{ar}), 122.0 (C_{ar}), 120.2 (C_{ar}), 114.2 (C_{vinyl}), 77.3 (C_5H_4 , C_q), 71.6 (C_5H_4), 69.1 (C_5H_5), 68.7 (C_5H_4). MS (APCI) m/z 400.08 $[M+H]^+$.

(E)-1-(3',5'-dibromo-2'-hydroxyphenyl)-3-ferrocenylprop-2-en-1-one 1e. Violet solid. Found: C 45.24, H 2.70. Calc. for $C_{19}H_{14}O_2FeBr_2 \cdot 0.25 CH_2Cl_2$: C 45.23, H 2.86. δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.99 (d, $J=14.7$ Hz, 1H, H_{vinyl}), 7.91 (d, $^4J=1.8$ Hz, 1H, H_{ar}), 7.86 (d, $^4J=1.8$ Hz, 1H, H_{ar}), 7.10 (d, $J=14.7$ Hz, 1H, H_{vinyl}), 4.67 (s, 2H, C_5H_4), 4.62 (s, 2H, C_5H_4), 4.23 (s, 5H, C_5H_5). δ_C (75 MHz; $CDCl_3$; Me_4Si) 191.0 (C_{ketone}), 159.3 (C_{ar}), 150.8 (C_{vinyl}), 140.7 (C_{ar}), 130.8 (C_{ar}), 121.7 (C_{ar}), 115.1 (C_{vinyl}), 113.3 (C_{ar}), 110.1 (C_{ar}), 78.37 (C_5H_4 , C_q), 72.6 (C_5H_4), 70.1 (C_5H_5), 69.7 (C_5H_4). MS (APCI) m/z 488.01 $[M+H]^+$.

((E)-1-(3',5'-difluoro-2'-hydroxyphenyl)-3-ferrocenylprop-2-en-1-one 1f. Violet solid. Found: C 60.25, H 4.04. Calc. for $C_{19}H_{14}O_2FeF_2 \cdot 0.2 CH_2Cl_2$: C 59.88, H 3.77. δ_H (400 MHz; $CDCl_3$; Me_4Si) 7.98 (d, $J=15$ Hz, 1H, H_{vinyl}), 7.34 (s, 1H, H_{ar}), 7.06 (m, 1H, H_{vinyl} , H_{ar}), 4.66 (s, 2H, C_5H_4), 4.61 (s, 2H, C_5H_4), 4.22 (s, 5H, C_5H_5). δ_C (100 MHz; $CDCl_3$; Me_4Si) 191.6 (C_{ketone}), 153.3 (dd, $^1J=238.6$ Hz, $^3J=9$ Hz, C_{ar}), ~151 (C_{ar} overlap) 150.5 (C_{vinyl}), 148.8 (d, $^2J=11.9$ Hz, C_{ar}) 120.9 (C_{ar}), 115.7 (C_{vinyl}), 110.5 (dd, $^2J=26$ Hz, $^2J=21$ Hz, C_{ar}), 109.6 (d, $^2J=23$ Hz, C_{ar}), 78.4 (C_5H_4 , C_q), 72.6 (C_5H_4), 70.2 (C_5H_5), 69.7 (C_5H_4). MS (APCI) m/z 368.07 $[M+H]^+$.

General preparation of ferrocenyl flavones

Ferrocene chalcone (10 mg) was dissolved in THF (15 mL) in a 50 mL two-necked round bottom flask. NaH (3 equiv) was added and the solution went from deep violet to a light red. After stirring for 5 min at room temperature, AgOTf (2.5 equiv) was added, and the mixture was stirred for another 5 min, before being poured into a H_2O (100 mL) and HCl 12 M (15 mL). The mixture was extracted with CH_2Cl_2 (3x50 mL), and washed with water. The organic phase was dried over $MgSO_4$, filtered and evaporated. The product was purified using a silica gel column, using a mixture of petroleum ether/dichloromethane 1/1, and again by HPLC using CH_3CN/H_2O , and the 1H NMR spectra showed the presence of residual H_2O . Yields were calculated after purification on the silica gel column.

2-ferrocenyl-chromen-4-one 2a. Yield: 8.0 mg, 80%. Violet solid. Found: C 67.60, H 4.66. Calc. for $C_{19}H_{14}O_2Fe \cdot 0.5 H_2O$: C 67.28, H 4.46. δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.80 (d, $J=7.5$ Hz, 1H, H_{ar}), 7.64 (t, $J=7.2$ Hz, 1H, H_{ar}), 7.29 (d, $J=7.2$ Hz, 1H, H_{ar}), 7.19 (t, $J=7.5$ Hz, 1H, H_{ar}), 6.89 (s, 1H, H_{vinyl}), 4.92 (s, 2H, C_5H_4), 4.60 (s, 2H, C_5H_4), 4.22 (s, 5H, C_5H_5). δ_C (75 MHz; $CDCl_3$; Me_4Si) 182.9 (C_{ketone}), 165.4

(C_{ar}), 146.0 (C_{vinyl}), 136.1 (C_{ar}), 124.5 (C_{ar}), 123.1 (C_{vinyl}), 122.6 (C_{ar}), 116.4 (C_{ar}), 112.9 (C_{ar}), 75.1 (C₅H₄, C_q), 71.8 (C₅H₄), 71.5 (C₅H₄), 70.0 (C₅H₅). MS (APCI) *m/z* 331.07 [M+H]⁺.

6-chloro-2-ferrocenyl-chromen-4-one 2b. Yield: 7.8 mg, 78%. Violet solid. Found: C 61.65, H 3.67. Calc. for C₁₉H₁₃O₂FeCl·0.25 H₂O: C 61.66, H 3.69. δ_H(300 MHz; CDCl₃; Me₄Si) 7.77 (d, *J*=2.1 Hz, 1H, H_{ar}), 7.57 (dd, ⁴*J*=2.1 Hz, ³*J*=8.7 Hz, 1H, H_{ar}), 7.24 (m, 1H, H_{ar}), 6.92 (s, 1H, H_{vinyl}), 4.91 (s, 2H, C₅H₄), 4.63 (s, 2H, C₅H₄), 4.24 (s, 5H, C₅H₅). δ_C(75 MHz; CDCl₃; Me₄Si) 181.4 (C_{ketone}), 163.5 (C_{ar}), 146.1 (C_{vinyl}), 135.8 (C_{ar}), 128.8 (C_{ar}), 124.0 (C_{vinyl}), 123.8 (C_{ar}), 117.9 (C_{ar}), 114.2 (C_{ar}), 74.7 (C₅H₄, C_q), 72.2 (C₅H₄), 71.6 (C₅H₄), 70.0 (C₅H₅). MS (APCI) *m/z* 364.02 [M+H]⁺.

6-bromo-2-ferrocenyl-chromen-4-one 2c. Yield: 6.4 mg, 64%. Violet solid. Found: C 55.77, H 3.65. Calc. for C₁₉H₁₃O₂FeBr·0.165 H₂O: C 55.39, H 3.26. δ_H(300 MHz; CDCl₃; Me₄Si) 7.93 (d, *J*=2.1 Hz, 1H, H_{ar}), 7.72 (dd, ⁴*J*=2.1 Hz, ³*J*=8.7 Hz, 1H, H_{ar}), 7.20 (d, *J*=8.7 Hz, 1H, H_{ar}), 6.94 (s, 1H, H_{vinyl}), 4.86 (m, 2H, C₅H₄), 4.60 (m, 2H, C₅H₄), 4.19 (s, 5H, C₅H₅). δ_C(75 MHz; CDCl₃; Me₄Si) 181.2 (C_{ketone}), 163.9 (C_{ar}), 145.9 (C_{vinyl}), 138.5 (C_{ar}), 127.1 (C_{ar}), 124.4 (C_{vinyl}), 118.0 (C_{ar}), 115.9 (C_{ar}), 114.7 (C_{ar}), 74.7 (C₅H₄, C_q), 72.2 (C₅H₄), 71.7 (C₅H₄), 70.1 (C₅H₅). MS (APCI) *m/z* 408.08 [M+H]⁺.

6,8-dichloro-2-ferrocenyl-chromen-4-one 2d. Yield: 6.2 mg, 62%. Blue solid. Found: C 56.05, H 3.31. Calc. for C₁₉H₁₂O₂FeCl₂·0.5 H₂O: C 55.93, H 3.21. δ_H(300 MHz; CDCl₃; Me₄Si) 7.69 (d, *J*=2.1 Hz, 1H, H_{ar}), 7.64 (d, *J*=2.1 Hz, 1H, H_{ar}), 7.06 (s, 1H, H_{vinyl}), 4.93 (s, 2H, C₅H₄), 4.66 (s, 2H, C₅H₄), 4.22 (s, 5H, C₅H₅). δ_C(75 MHz; CDCl₃; Me₄Si) 179.3 (C_{ketone}), 158.2 (C_{ar}), 144.8 (C_{vinyl}), 134.0 (C_{ar}), 127.9 (C_{ar}), 124.1 (C_{ar}), 121.4 (C_{vinyl}), 118.9 (C_{ar}), 118.4 (C_{ar}), 73.3 (C₅H₄, C_q), 71.7 (C₅H₄), 71.0 (C₅H₄), 69.2 (C₅H₅). MS (APCI) *m/z* 398.00 [M+H]⁺.

6,8-dibromo-2-ferrocenyl-chromen-4-one 2e. Yield: 8.0 mg, 80%. Blue solid. Found: C 46.47, H 2.60. Calc. for C₁₉H₁₂O₂FeBr₂·0.25 H₂O: C 46.34, H 2.56. δ_H(300 MHz; CDCl₃; Me₄Si) 7.92 (d, *J*=1.5 Hz, 1H, H_{ar}), 7.86 (d, *J*=1.5 Hz, 1H, H_{ar}), 7.03 (s, 1H, H_{vinyl}), 4.93 (s, 2H, C₅H₄), 4.67 (s, 2H, C₅H₄), 4.22 (s, 5H, C₅H₅). δ_C(75 MHz; CDCl₃; Me₄Si) 179.3 (C_{ketone}), 159.9 (C_{ar}), 144.4 (C_{vinyl}), 139.2 (C_{ar}), 129.8 (C_{ar}), 125.0 (C_{vinyl}), 124.5 (C_{ar}), 119.0 (C_{ar}), 115.0 (C_{ar}), 73.3 (C₅H₄, C_q), 71.7 (C₅H₄), 71.0 (C₅H₄), 69.2 (C₅H₅). MS (APCI) *m/z* 485.99 [M+H]⁺.

6,8-difluoro-2-ferrocenyl-chromen-4-one 2f. Yield: 8.6 mg, 86%. Violet-blue solid. Found: C 61.04, H 3.22. Calc. for C₁₉H₁₂O₂FeF₂·0.5 H₂O: C 60.83, H 3.49. δ_H(400 MHz; CDCl₃; Me₄Si) 7.31-7.29 (m, 1H, H_{ar}), 7.22-7.15 (m, 1H, H_{ar}), 7.02 (s, 1H, H_{vinyl}), 4.91 (s, 2H, C₅H₄), 4.65 (s, 2H, C₅H₄), 4.22 (s, 5H, C₅H₅). δ_C(100 MHz; CDCl₃; Me₄Si) 180.7 (C_{ketone}), 157.9 (dd, ¹*J*=246.5 Hz, ³*J*=7.3 Hz, C_{ar}), 148.8 (d, ²*J*=11.3 Hz, C_{ar}), 148.1 (dd, ¹*J*=254.7 Hz, ³*J*=11.2 Hz, C_{ar}), 145.9 (C_{vinyl}), 125.6 (d, ³*J*=7.7 Hz, C_{ar}), 119.8 (C_{vinyl}), 110.9 (dd, ²*J*=19.7 Hz, ²*J*=24.3 Hz, C_{ar}), 105.5 (dd, ²*J*=23.8 Hz, ⁴*J*=4.1 Hz, C_{ar}), 74.3 (C₅H₄, C_q), 72.6 (C₅H₄), 71.9 (C₅H₄), 70.2 (C₅H₅). MS (APCI) *m/z* 366.04 [M+H]⁺.

Theoretical calculations

A coarse drawing of the structure led to a geometry that was first improved using Hartree-Fock ab-initio calculations at a STO3G level; the resulting structure was then optimized using a 321G basis (a more sophisticated, but time and memory consuming, 631G basis only led to minor improvements over 321G). No symmetry constraint was imposed during optimization, allowing the full (3N - 6) degrees of freedom. The energy minimum was obtained by minimizing the gradient norm using the default (BFGS) algorithm; for the neutral **1a** and derived anionic species we used the RHF method and the ROHF for the treatment of the radical species.

This suite of programs in GAMESS² was run on RS/6000 System Regatta Power4 machines.

Biological evaluation.

Murine B16 melanoma cells were grown in Dulbecco's modified essential medium (DMEM) containing 2 mM L-glutamine, 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin (37°C, 5% CO₂). Stock solutions of the compounds were prepared in DMSO and further diluted in DMEM at the indicated concentrations with a final DMSO concentration of not exceeding 1%. Exponentially growing cells were plated onto 96-well plates at a density of 5000 cells per well in 200 µL DMEM, and 24 h later the compounds were added for another 48 h. Control cells were exposed to 1% DMSO. Viability was assessed using the MTT (1-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium) test and absorbance was read at 562 nm in a microplate reader (BioKinetics Reader, EL340).³ Results are presented as the inhibitory concentrations for 50% of cells (IC₅₀) for a 48 h exposure time (mean ± S.D. of three determinations).

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