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Ferrocenyl flavonoid-induced morphological modifications of endothelial cells and cytotoxicity against B16 murine melanoma cells

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Abstract

With the aim of improving the cytotoxic and vascular disrupting activities of flavonoids, several classes of ferrocenyl-modified flavonoids were prepared and tested on cancer and endothelial cells. Three ten-member series of ferrocenyl flavonoids: chalcones ((*E*)-1-(*R*-2'-hydroxyphenyl)-3-ferrocenylprop-2-en-1-ones), aurones ((*Z*)-*R*-2-(ferrocenylidene) benzofuran-3-ones) and flavones (*R*-2-ferrocenyl-chromen-4-ones) were synthesized by recently reported methods. Three ferrocenyl flavonols (*R*-3-hydroxy-2-ferrocenyl-chromen-4-ones) and four ferrocenyl flavanones (3-ferrocenylmethylidenyl-*R*-2-phenyl-chroman-4-ones) were also obtained. All compounds were evaluated for their cytotoxic effects on a cancer cell line (B16 murine melanoma) and for their morphological effects on endothelial cells (EAhy 926). Some interesting structure-activity relationships were disclosed: of all the compounds, the halogen-substituted aurones showed the best cytotoxic activity, with IC_{50} values ranging between 12 and 18 μM. Ferrocenyl flavonols and ferrocenyl flavanones with substitution in the 3-position (-OH and =C-Fc respectively) were not active against cancer or endothelial cells. Some of the ferrocenyl flavones caused the endothelial cells to adopt a round shape ("rounding up") at submicromolar concentrations, which can be predictive of vascular disrupting activity. The most morphologically active flavones showed only moderate cytotoxicity against cancer cells, indicating that they may primarily act as antivascular agents.

Keywords: Flavones; Aurones; Ferrocene; Cytotoxicity; Antivascular agents; Bioorganometallic chemistry

1. Introduction

Flavonoids are small molecules, widespread in the plant kingdom, consisting of two phenyl rings linked by a 3-carbon atom bridge, and represent the most common polyphenols in the human diet. They are currently enjoying enormous interest as nutraceuticals and drug candidates for diabetes, cognitive function, blood pressure, cancer, obesity, and other diseases [1]. Appearing with the first plants on earth and thus having accompanied the evolution of animal life, flavonoids interact with many biological pathways. In

cancer research, flavonoids have been shown to inhibit various pathways related to drug resistance [2], apoptosis [3], division [4] and metastasis [5]. Currently, there is great interest in flavonoids as angiogenesis inhibitors. Flavonoids are an important class of vascular disrupting agents, an experimental treatment involving rapid destruction of the tumor vasculature and thus preventing oxygen and nutrient flow to the tumor [6]. One flavonoid, ASA404 (vadimezan), is currently undergoing Phase II clinical trials as a vascular disrupting agent [7]. ASA404 acts by disrupting the actin cytoskeleton of tumor vascular endothelial cells, making the vessels more permeable. When used in combination, this molecule has been shown to enhance the efficacy of carboplatin and paclitaxel [8].

Organometallic medicinal chemistry is one of the most mature and active areas in bioorganometallic chemistry [9], and related applications in enzyme inhibition, bioimaging, immunoassay, small molecule delivery, and *in cellulo* catalysis are growing topics [10]. The use of organometallic compounds in anticancer drug discovery continues to receive a great deal of attention [11], as such molecules are often more stable to hydrolysis than coordination compounds. The introduction of organometallics can alter the physicochemical characteristics of a bioactive compound by acting as a structural element [12], controlling hydrolysis kinetics, or modifying its lipophilicity [13]. Organometallic units can also add supplementary reactivity to the biomolecule by imparting redox properties [14] or acting as an alkylating function to proteins [15] or DNA [16]. From another point of view, bioligands can be used to vectorize cytotoxic metal moieties, bringing them into the mitochondria or nucleus, and allowing them to interact with receptors [17]. Ferrocene in particular has gained considerable interest as a substituent in medicinal chemistry due to its stability in aqueous and aerobic media, its lipophilicity, its ease of derivation, and its redox activity [18]. Some ferrocene derivatives have shown promising activity against proteins, cells or organisms associated with diseases including fungal and bacterial infections [19], malaria [20], HIV [21], and cancer [22].

While there are several examples of biologically active flavonoid metal complexes [23], few examples of organometallic flavonoids, apart from ferrocenyl chalcones [24], can be found in the literature [25]. Notably, however, ruthenium arenes coordinated to flavonols recently displayed particularly interesting cytotoxic activity against CH1 ovarian cells, possibly due to binding to DNA accompanied by topoisomerase IIa inhibitory activity [26]. As part of our ongoing project studying ferrocene-modified biomolecules, we have recently obtained access to a variety of ferrocenyl flavonoids [27]. We now present our studies on the antiproliferative effects on B16 murine melanoma and the potential vascular disrupting effects on the EAhy 926 endothelial cell model of a number of ferrocenyl chalcones, aurones, flavones, flavanols and flavanones. Some interesting structure-activity relationships were observed and a clear differential activity between the cytotoxic effects on cancer cells and the morphological effects on endothelial cells was observed for certain compounds, suggesting that these activities are not correlated.

2. Material and methods

2.1. General remarks

All reactions were carried out under argon. THF was distilled over sodium/benzophenone. All other chemical reagents and solvents were used without further purification. Silica gel chromatography was performed with Merck 60 (40-63 µm) silica. ^{1}H and ^{13}C NMR spectra were recorded with a 300 or 400 MHz Bruker Avance spectrometer, and δ are given in ppm and referenced to the residual solvent peaks (^{1}H, δ 7.26 and $^{13}C\{^{1}H\}$, δ 77.1 for $CDCl_3$ and ^{1}H, δ 2.05 and $^{13}C\{^{1}H\}$, δ 29.7 for acetone d_6). Mass spectra were measured on a Thermoscientific ITQ1100 spectrometer using the direct exposure probe method by the mass spectrometry service at ENSCP Chimie ParisTech. High resolution mass spectra were obtained by ESI/ESCI-TOF using a Waters LCT Premier XE or a Thermo Fischer LTQOrbitrap XL at the Institut Parisien de Chimie Moléculaire (IPCM). Melting points were determined using an Electrothermal 9100 apparatus. IR data were collected on a JASCO FT/IR-4100 using a KBr pellet. The purity of all compounds (>98%) was confirmed using a Shimadzu HPLC LC20 series equipped with LC-20AB pump and SPD-20A UV detector prior to biological testing.

2.2. Synthesis

The synthesis and characterization of the ferrocenyl chalcones **1a-j** and ferrocenyl aurones **2a-j** have been previously reported [28].

*2.2.1. General synthesis of ferrocenyl flavones **3a-j***

Ferrocenyl aurone (30 mg) and potassium cyanide (1.5 equiv) were dissolved in ethanol (25 mL) and stirred at reflux for 2 h. The solution was cooled to rt and poured into an aqueous solution of 1 M NaOH (100 mL). The mixture was extracted with EtOAc (3 x 50 mL) and washed with water. The organic phase was dried over $MgSO_4$, filtered, and evaporated. The crude product was purified using a silica gel column, with 60/40 petroleum ether/ethyl acetate as an eluent and was isolated as an orange solid. Characterization of **3a**, **3c** and **3h** has been previously reported [27].

*2.2.1.1. 6-chloro-2-ferrocenyl-chromen-4-one, **3b***. Yield: 72%; mp 202 °C, ν_{max}/cm^{-1} 1639 (C=O), 1608 (C=C), δ_H (300 MHz; $CDCl_3$) 4.17 (s, 5H, C_5H_5), 4.54 (s, 2H, C_5H_4), 4.85 (s, 2H, C_5H_4), 6.45 (s, 1H, vinyl), 7.45 (d, 1H, *J* = 8.5 Hz, C_6H_3), 7.59 (d, 1H, J = 8.5 Hz, C_6H_3), 8.16 (s, 1H, C_6H_3). δ_C (75 MHz; $CDCl_3$) 67.5 (C_5H_4), 70.3 (C_5H_5), 71.6 (C_5H_4), 74.5 (C_5H_4 *ipso*), 105.6 (C=C), 111.6 (C_6H_3), 119.4 (C_6H_3), 125.1 (C_6H_3), 130.9 (C_6H_3), 133.4 (C_6H_3), 154.5 (C_6H_3), 168.7 (C=C), 176.2 (C=O). HRMS (ESI) calcd. for $C_{19}H_{14}ClFeO_2^+$: 365.0032, found: 365.0027.

*2.2.1.2. 6,8-difluoro-2-ferrocenyl-chromen-4-one, **3d***. Yield: 60%; mp 155 °C, ν_{max}/cm^{-1} 1639 (C=O), 1585 (C=C), δ_H (400 MHz; $CDCl_3$) 4.18 (s, 5H, C_5H_5), 4.56 (t, 2H, *J* = 1.9 Hz, C_5H_4), 4.88 (t, 2H, *J* = 1.9 Hz, C_5H_4), 6.44 (s, 1H, vinyl), 7.18 (dd, 1H, *J* = 8.8 Hz, *J* = 2.4 Hz, C_6H_2), 7.63 (d, 1H, *J* = 8.8 Hz, C_6H_2). δ_C (100 MHz; $CDCl_3$) 67.7 (C_5H_4), 70.5 (C_5H_5), 71.9 (C_5H_4), 74.2 (C_5H_4 *ipso*), 105.4 (C=C), 106.0 (dd, ^2J = 23.7 Hz, ^4J = 4.0 Hz, C_6H_2) 108.7 (dd, ^3J = 20.1 Hz, ^3J = 28.5 Hz, C_6H_2), 126.8 (s, C_6H_2), 141.9 (d, ^2J = 9.4 Hz, C_6H_2), 151.3 (dd, ^1J = 256 Hz, ^3J = 11.6 Hz, C_6H_2), 158.3 (dd, ^1J = 249 Hz, ^3J = 9.7 Hz, C_6H_2), 168.7 (C=C), 176.0 (C=O). HRMS (ESI) calcd. for $C_{19}H_{13}F_2FeO_2^+$: 367.0233, found: 367.0244.

*2.2.1.3. 6,8-dichloro-2-ferrocenyl-chromen-4-one, **3e***. Yield: 60%; mp 193 °C, ν_{max}/cm^{-1} 1646 (C=O), 1612 (C=C), δ_H (300 MHz; $CDCl_3$) 4.18 (s, 5H, C_5H_5), 4.58 (t, 2H, *J* = 1.9 Hz, C_5H_4), 4.88 (t, 2H, *J* = 1.9 Hz, C_5H_4), 6.44 (s, 1H, vinyl), 7.71 (d, 1H, *J* = 1.8 Hz, C_6H_2), 8.07 (d, 1H, *J* = 1.8, C_6H_2). δ_C (75 MHz; $CDCl_3$); 67.7 (C_5H_4), 70.4 (C_5H_5), 71.9 (C_5H_4), 74.1 (C_5H_4 *ipso*), 105.3 (C=C), 123.5 (C_6H_2), 123.9 (C_6H_2), 125.9 (C_6H_2), 130.5 (C_6H_2), 133.3 (C_6H_2), 150.4 (C_6H_2), 168.9 (C=C), 175.4 (C=O). HRMS (ESI) calcd. for $C_{19}H_{13}Cl_2FeO_2^+$: 398.9642, found: 398.9642.

*2.2.1.4. 6,8-dibromo-2-ferrocenyl-chromen-4-one, **3f***. Yield: 55%; mp 194 °C, ν_{max}/cm^{-1} 1643 (C=O), 1612 (C=C), δ_H (300 MHz; $CDCl_3$) 4.22 (s, 5H, C_5H_5), 4.59 (t, 2H, *J* = 1.9 Hz, C_5H_4), 4.93 (t, 2H, *J* = 1.9 Hz, C_5H_4), 6.44 (s, 1H, vinyl), 8.01 (d, 1H, *J* = 2.4 Hz, C_6H_2), 8.27 (d, 1H, *J* = 2.4 Hz, C_6H_2). δ_C (75 MHz; $CDCl_3$), 67.8 (C_5H_4), 70.4 (C_5H_5), 71.8 (C_5H_4), 74.1 (C_5H_4 *ipso*), 105.2 (C=C), 112.5 (C_6H_2), 118.1 (C_6H_2), 127.8 (C_6H_2), 136.6 (C_6H_2), 138.8 (C_6H_2), 151.7 (C_6H_2), 169.0 (C=C), 175.3 (C=O). HRMS (ESI) calcd. for $C_{19}H_{13}{}^{79}Br_2FeO_2^+$: 486.8632, found: 486.8629.

*2.2.1.5. 7-methoxy-2-ferrocenyl-chromen-4-one, **3g***. Yield: 70%; mp 174 °C, ν_{max}/cm^{-1} 1631 (C=O), 1604 (C=C), δ_H (300 MHz; $CDCl_3$) 3.93 (s, 3H, OMe) 4.17 (s, 5H, C_5H_5), 4.50 (t, 2H, *J* = 1.8 Hz, C_5H_4), 4.83 (t, 2H, *J* = 1.8 Hz, C_5H_4), 6.40 (s, 1H, vinyl), 6.89 (d, 1H, *J* = 2.3 Hz, C_6H_3), 6.95 (dd, 1H, *J* = 2.3 Hz, *J* = 8.9 Hz, C_6H_3), 8.10 (d, 1H, *J* = 8.9 Hz, C_6H_3). δ_C (75 MHz; $CDCl_3$) 55.6 (OMe), 67.1 (C_5H_4), 70.0 (C_5H_5), 71.0 (C_5H_4), 75.1 (C_5H_4 *ipso*), 100.1 (C_6H_3), 105.4 (C=C), 113.7 (C_6H_3), 117.7 (C_6H_3), 126.7 (C_6H_3), 157.7 (C_6H_3), 163.6 (C_6H_3), 167.4 (C=C), 176.8 (C=O). HRMS (ESI) calcd. for $C_{20}H_{17}FeO_3^+$: 361.0520, found: 361.0527.

*2.2.1.6. 5-methoxy-2-ferrocenyl-chromen-4-one, **3i**.* Yield: 68%; mp 165 °C, ν_{max}/cm^{-1} 1635 (C=O), 1608 (C=C), δ_H (300 MHz) 3.99 (s, 3H, OMe) 4.16 (s, 5H, C_5H_5), 4.49 (t, 2H, *J* = 1.8 Hz, C_5H_4), 4.82 (t, 2H, *J* = 1.8 Hz, C_5H_4), 6.38 (s, 1H, vinyl), 6.80 (d, 1H, *J* = 8.2 Hz, C_6H_3), 7.06 (d, 1H, *J* = 8.4 Hz, C_6H_3), 7.54 (t, 1H, *J* = 8.4 Hz, C_6H_3). δ_C (75 MHz; $CDCl_3$) 55.3 (OMe), 67.0 (C_5H_4), 69.9 (C_5H_5), 70.9 (C_5H_4), 74.8 (C_5H_4 *ipso*), 106.1 (C_6H_3), 107.7 (C_6H_3), 109.7 (C_6H_3), 114.4 (C=C), 133.0 (C_6H_3), 158.1 (C_6H_3), 159.5 (C_6H_3), 165.4 (C=C), 177.4 (C= O). HRMS (ESI) calcd. for $C_{20}H_{17}FeO_3^+$: 361.0527, found: 361.0527.

*2.2.1.7. 5,7-dimethoxy-2-ferrocenyl-chromen-4-one, **3j**.* Yield: 67%; mp 179 °C, ν_{max}/cm^{-1} 1643 (C=O), 1608 (C=C), δ_H (300 MHz; $CDCl_3$) 3.90 (s, 3H, OMe), 3.93 (s, 3H, OMe), 4.14 (s, 5H, C_5H_5), 4.45 (t, 2H, *J* = 1.8 Hz, C_5H_4), 4.77 (t, 2H, *J* = 1.8 Hz, C_5H_4), 6.30 (s, 1H, vinyl), 6.34 (d, 1H, *J* = 2.3 Hz, C_6H_2), 6.48 (d, 1H, *J* = 2.3 Hz, C_6H_2). δ_C (75 MHz; $CDCl_3$) 55.4 (OMe), 56.1 (OMe),66.8 (C_5H_4), 69.8 (C_5H_5), 70.6 (C_5H_4), 74.6 (C_5H_4 *ipso*), 92.4 (C_6H_2), 95.6 (C_6H_2), 106.9 (C_6H_2), 109.0 (C=C), 159.5 (C_6H_2), 160.5 (C_6H_2), 163.4 (C_6H_2), 164.5 (C=C), 176.6 (C=O). HRMS (ESI) calcd. for $C_{21}H_{19}FeO_4^+$: 391.0622, found: 391.0633.

*2.2.2. General synthesis of ferrocenyl flavonols **3a**, **3b** and **3h***

Carbonate buffer (15 mL) was added to a solution of ferrocenyl flavone (40 mg) in CH_2Cl_2/acetone (2:1,10 mL) and stirred vigorously at rt. A 5 mL aqueous solution of oxone (0.6 g) was added in four portions every 10 min, and the pH of the solution was monitored to ensure that it remained alkaline. The consumption of starting material was followed by TLC. After the reaction was complete, the two phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layer was washed with sodium thiosulfate solution, dried with $MgSO_4$, filtered, and evaporated. The crude product was purified using a silica gel column with 80/20 petroleum ether/ethyl acetate as eluent. Characterization of **3a** and **3b** has previously been reported [27].

*2.2.2.1. 7-methoxy-3-hydroxy-2-ferrocenyl-chromen-4-one, **5h**.* Yield: 78%; mp 208 °C, ν_{max}/cm^{-1} 3255 (OH), 1716 (C=O), 1608 (C=C), δ_H (400 MHz; $CDCl_3$) 3.95 (s, 3H, OMe), 4.16 (s, 5H, C_5H_5), 4.50 (t, ^3J = 1.9 Hz, 2H, C_5H_4), 5.15 (t, ^3J = 1.9 Hz, 2H, C_5H_4), 6.70 (s, 1H, OH), 6.91 (d, ^4J = 2.3 Hz, 1H, C_6H_3), 6.95 (dd, ^3J = 8.9 Hz, 1H, C_6H_3), 8.08 (d, ^3J = 8.9 Hz, 1H, C_6H_3). δ_C (100 MHz; $CDCl_3$) 55.9 (OMe), 68.4 (C_5H_4), 70.0 (C_5H_5), 70.7 (C_5H_4), 74.5 (C_5H_4-C *ipso*), 100.0 (C_6H_3), 114.5 (C_6H_3), 115.2 (C_6H_3), 126.7 (C_6H_3), 136.5 (C=C), 149.4 (C=C), 157.3 (C_6H_3), 163.8 (C_6H_3), 171.2 (C=O). HRMS (ESI) calcd. for $C_{20}H_{16}FeO_4^+$: 376.03980, found: 376.03939.

*2.2.3. Synthesis of organic flavones **4a-j***

Organic flavones **4a-j** were synthesized by treating chalcones with I_2 in DMSO according to a literature procedure [29]. The ^{1}H NMR spectra of **4-c** [30], **4e** [31], **4f** [32], **4g**, **4h** [33], **4i** [34] and **4j** [35] were consistent with those found in the literature. Compound **4d** was not found in the literature, and its structure was deduced from its ^{1}H NMR spectrum: δ_H (300 MHz; $CDCl_3$) 6.84 (s, 1H, vinyl), 7.23-7.30 (m, 2H, C_6H_5), 7.54-7.56 (m, 1H, C_6H_5), 7.65-7.69 (m, 1H, C_6H_2), 7.93-7.96 (m, 2H, C_6H_5/C_6H_2).

2.2.4. Synthesis of ferrocenyl flavanones

Flavanone was purchased from Sigma Aldrich. Starting materials 7-methoxy-2-phenylchroman-4-one, 5-methoxy-2-phenylchroman-4-one and 6-chloro-2-phenylchroman-4-one were prepared via treatment of the chalcone with pyridine in MeOH and water according to a reported procedure [36].

*2.2.4.1. 3-ferrocenylmethylidenyl-2-phenylchroman-4-one, **6a**.*

2-phenylchroman-4-one (100 mg, 0.45 mmol) and ferrocene carboxaldehyde (96 mg, 0.45 mmol) were dissolved in 15 mL EtOH in a round bottom flask. HCl gas, generated from concentrated sulfuric acid addition to NaCl, was bubbled into the flavanone solution for 1 h and the solution was stirred at 0° for 72 h. The solution was extracted with CH_2Cl_2 and washed with a saturated solution of NaCl before being purified using a silica gel column using CH_2Cl_2/petroleum ether (3:2) as an eluent. Yield = 18%; mp 163 °C, ν_{max}/cm^{-1} 1461, 1596, 1662, δ_H (300 MHz; $CDCl_3$) 4.16 (s, 5H, C_5H_5), 4.22 (s, 1H, C_5H_4), 4.47 (s, 2H,

C_5H_4), 4.55 (s, 1H, C_5H_4), 6.62 (s, 1H), 6.90 (m, 2H), 7.27-7.37 (m, 4H), 7.46 (m, 2H), 7.94 (m, 2H). δ_C (75 MHz; $CDCl_3$) 68.5.2 (C_5H_5), 69.5 (C_5H_4), 71.8 (C_5H_4), 72.0 (C_5H_4, C *ipso*) 74.0, 118.5, 121.6, 122.4, 127.6, 127.8, 128.5, 128.6, 135.5, 138.2, 140.5, 158.8, 181.3. MS (APEI) *m/z* 420.18 $(M)^+$.

2.2.4.2. 3-ferrocenylmethylidenyl-7-methoxy-2-phenylchroman-4-one, ***6b***. 7-methoxy-2-phenylchroman-4-one (50 mg, 0.20 mmol) and ferrocene carboxaldehyde (43 mg, 0.20 mmol) were dissolved in 10 mL EtOH in a round bottom flask and a spatula of $MgSO_4$ was added. HCl gas was bubbled into the flavanone solution for 1 h and the solution was stirred at rt for 48 h. The solution was extracted with CH_2Cl_2 and washed with a saturated solution of NaCl before being purified using a silica gel column with 50/50 CH_2Cl_2/petroleum ether as an eluent. Yield = 6%; δ_H (300 MHz; acetone d_6): 4.05 (s, 3H, OMe), 4.40 (s, 5H, C_5H_5), 4.50 (d, *J* = 1.6 Hz, 1H, C_5H_4), 4.75 (t, *J* = 1.6 Hz, 2H, C_5H_4), 4.87 (d, *J* = 1.6 Hz, 1H, C_5H_4), 6.72 (d, *J* = 2.4 Hz, 1H), 6.8 (dd, *J* = 2.2 Hz, 8.8 Hz, 1H), 6.91 (s, 1H), 7.58 (d, *J* = 7.3 Hz, 1H), 7.65 (t, *J* = 1.6 Hz, 2H), 7.75 (d, *J* = 7.3 Hz, 2H), 8.01 (d, *J* = 8.8 Hz, 1H), 8.1 (s, 1H). MS (APCI) *m/z* 451.04 $(M + H)^+$.

2.2.4.3. 3-ferrocenylmethylidenyl-6-methoxy-2-phenylchroman-4-one, ***6c***. 6-methoxy-2-phenylchroman-4-one (25 mg, 0.1 mmol) and ferrocene carboxaldehyde (22 mg, 0.1 mmol) were dissolved in 15 mL EtOH in a round bottom flask and a spatula of $MgSO_4$ was added. HCl gas was bubbled into the flavanone solution for 5 min and the solution was stirred at 50 °C for 20 h. The solution was extracted with CH_2Cl_2 and washed with a saturated solution of NaCl before being purified using a silica gel column with 50/50 CH_2Cl_2/ petroleum ether as an eluent. Yield = 4%; δ_H (300 MHz; acetone d_6) 4.06 (s, 3H, OMe), 4.16 (s, 5H, C_5H_5), 4.41 (t, *J* = 1.8 Hz, 1H, C_5H_4), 4.50 (t, *J* = 1.8 Hz, 2H, C_5H_4), 4.67 (t, *J* = 1.8 Hz, 1H, C_5H_4), 6.52 (s, 1H), 6.81 (s, 1H), 7.16 (d, *J* = 2.8 Hz, 2H), 7.31 (t, *J* = 1.2 Hz, 3H), 7.38 (d, *J* = 7.3 Hz, 2H), 7.77 (s, 1H). MS (APCI) *m/z* 451.04 $(M + H)^+$.

2.2.4.4. 3-ferrocenylmethylidenyl-6-chloro-2-phenylchroman-4-one, ***6d***. 6-chloro-2-phenylchroman-4-one (150 mg, 0.58 mmol) and ferrocenecarboxaldehyde (124 mg, 0.58 mmol) were dissolved in 20 mL EtOH in a round bottom flask. HCl gas was bubbled into the flavanone solution for 1 h and the solution was stirred at 40° for 72 h. The solution was extracted with CH_2Cl_2 and washed with a saturated solution of NaCl before being purified using a silica gel column with 10/90 ethyl acetate/petroleum ether as an eluent. Yield = 15%; mp 192 °C, ν_{max}/cm^{-1} 1727, 1662, 1585, 1461; δ_H (300 MHz; $CDCl_3$) 4.13 (t, *J* = 4.0 Hz, 5H, C_5H_5), 4.18 (d, *J* = 1.5 Hz, 1H, C_5H_4), 4.47 (t, *J* = 1.5 Hz, 2H, C_5H_4), 4.53 (dd, *J* = 1.5 Hz, 3.8 Hz, 1H, C_5H_4), 6.60 (s, 1H), 6.87 (d, *J* = 8.8 Hz, 1H), 7.29-7.34 (m, 3H), 7.36 (s, 1H), 7.43-7.46 (m, 2H), 7.88 (d, *J* = 2.6 Hz, 1H), 7.97 (s, 1H). δ_C (75 MHz; $CDCl_3$) 68.4, 69.7, 72.1, 72.3, 74.2, 78.2, 120.2, 123.3, 126.9, 127.6, 128.7, 128.7, 135.3, 137.7, 141.5, 157.2, 180.2. MS (APCI) *m/z* 454.94 $(M + H)^+$.

2.3. X-ray crystallography

Intensity data were collected at 200 K with an Enraf-Nonius Kappa-CCD diffractometer equipped with a CCD two-dimensional detector. Data reduction was performed with DENZO/SCALEPACK. Data were corrected for Lorentz and polarization effects, and a semi-empirical absorption correction based on symmetry equivalent reflections was applied using SADABS. Lattice parameters were obtained from least-squares analysis of 188 reflections. The structures were solved by direct methods and refined by full matrix least squares (5725 reflections/263 parameters), based on F^2, using the Crystals software package. All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were located with geometrical restraints in riding mode.

2.4. Biological assays

2.4.1. Cytotoxicity on cancer cells

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, in an incubator set at 37 °C with a humidified atmosphere containing 5% CO_2. Exponentially growing

cancer cells were plated onto 96-well plates at 5000 cells per well in 200 µL DMEM; 24 h later, the cells were exposed for 48 h to the solvent alone (1% DMSO final concentration for the controls) or to the flavonoid at the indicated concentrations. 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, Fisher Bioblock Scientific, Illkirch, France). Experiments were run in triplicate and repeated 3-6 times. Results are presented as the inhibitory concentrations for 50% (IC_{50}) of cells for a 48 h exposure time.

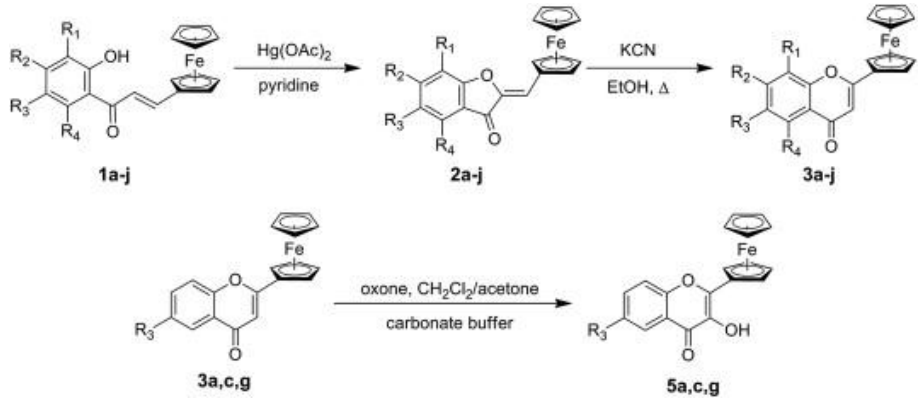
2.4.2. Morphologic effects on EAhy 926 endothelial cells

To assess the effects of flavonoids on the morphology of endothelial cells, we used the EAhy 926 endothelial cell line which is derived from the fusion of human umbilical vein endothelial cells (HUVEC) with the permanent human cell line A549 [37]. The EAhy 926 cell line is considered as one of the best immortalized HUVEC cell lines because these cells express most of the biochemical markers of parental HUVEC EAhy 926 cells [38]. EAhy 926 cells were grown in DMEM containing 2 mM L-glutamine, 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin (37 °C, 5% CO_2). Exponentially growing EAhy 926 cells were plated onto 96-well plates at 5000 cells/100 µL/well. Twenty-four hours after plating, the medium was aspirated, and 100 mL of medium containing the test compound was added to the well containing the cells (in triplicate) in 10-fold dilutions, and incubated for 2 h. After the 2 h incubation period, digital photographs were taken of representative centre areas of each well at a magnification of 100x and 200x. The antivascular agent combretastatin A4 was routinely included in the experiments as internal standard. Results are presented as the efficacious concentration for the change in cell shape (rounding up) for more than 20% of cells after a 2 h exposure time (EC_{20}).

3. Results and discussion

3.1. Synthesis of compounds

Although ferrocenyl chalcones, where ferrocene replaces the phenyl ring in the chalcone skeleton, have been studied since the 1950s [39], only recently have ferrocene-substituted higher flavonoids been obtained. Access to these molecules was accomplished by the synthesis of the ferrocenyl aurone via an oxidative 1,5-cyclization of 2-hydroxychalcone. The latter being obtained via a condensation of ferrocenecarboxaldehyde and 2-hydroxyacetophenone, a variety of substitution patterns can be acquired by selecting appropriate commercially-available 2-hydroxyacetophenones. A series of ten ferrocene-substituted chalcones, **1a-j**, with halogen and methoxy substitution were synthesized (Scheme 1) and subsequently treated with $Hg(OAc)_2$ in pyridine to form the corresponding series of ferrocenyl aurones **2a-j** (cf. Table 1) [28]. Isomerization of the aurones by refluxing the latter in EtOH with KCN gave ferrocenyl flavones, **3a-j**, in yields ranging from 55% to 88% (cf. Table 1) [27]. Flavonols **5a**, **5c** and **5g** were obtained from the corresponding flavones by treatment with oxone and acetone in a biphasic CH_2Cl_2/carbonate buffer solution as previously reported [27].



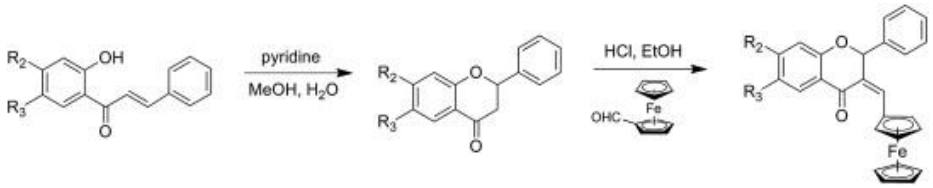
Scheme 1. Synthesis of ferrocenyl aurones, flavones and flavonols. a: R = H; b: R_3 = Cl; c: R_3 = Br; d: R_1 = R_3 = F; e: R_1 = R_3 = Cl; f: R_1 = R_3 = Br; g: R_2 = OMe; h: R_3 = OMe; i: R_4 = OMe; j: R_2 = R_4 = OMe.

Table 1. Cell and refinement parameters for compound **6a**.

Sum formula	$C_{26}H_{20}FeO_2$
Molecular weight	420.29
Crystal system	orthorhombic
Space group	Pcab
a, Å	14.706(1)
b, Å	15.632(2)
c, Å	17.146(3)
V, Å^3	3941.5(8)
Z	8
T, K	200(2)
λ, Å	0.71073
D_{calc}, g/cm^3	1.416
$\mu_{MoK\alpha}$, mm^{-1}	0.785
F(000)	1744
θ range, °	2.95–30.00
Reflections collected	23,720
Refinement reflections/restraints/parameters	5725/0/263
GooF on F^2	0.9286
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0406[a] wR_2 = 0.0924[b]
R indices (all data)	R_1 = 0.0848[a] wR_2 = 0.1116[b]

$R_1 = {}^a\Sigma||F_0| - |F_C||/\Sigma|F_o|$, and $wR_2 = {}^b[\Sigma w(F_0^2 - F_C^2)^2/\Sigma w(F_0^2)^2]^{1/2}$, $w = 1/[\sigma^2(F_o^2) + (aP)^2 + bP]$ and $P = (F_o^2 + 2F_C^2)/3$.

Ferrocenyl flavanones **6a-d** were synthesized by coupling organic flavanones - formed by base-catalyzed cyclization of the corresponding chalcone - with ferrocene carboxaldehyde in an ethanolic solution of HCl. The yields of these reactions were poor, due to decomposition of the desired adduct over the long reaction times necessary. Attempts to synthesize the condensation products using neat piperidine, sulfuric acid or NaOH in EtOH or diethylamine or piperidine [40] in DMF yielded no traces of the desired compounds (Scheme 2).



Scheme 2. Synthesis of ferrocenyl flavanones. **6a** = unsubstituted; **6b**: R_2 = OMe; **6c**: R_3 = OMe; **6d**: R_3 = Cl.

One of the ferrocenyl flavanones, **6a**, gave crystals suitable for X-ray analysis by slow diffusion of pentane into a saturated dichloromethane solution (Table 1). The structure of **6a** (Fig. 1) is essentially comprised of a quasi-planar arrangement of the C_5H_4 ring of the ferrocene and the

chromanone, while the FeCp and phenyl moieties extend below this plane. The ferrocene group is slightly twisted out of the quasi-plane of the chromanone moiety with a torsion angle of 15.5° for C2-C1-C11-C12. All of the atoms of the chromanone share one plane, except for C16 and O1, where torsion angles of between 13.7° (C14-C13-C12-C16) and 176.7° (C20-C14-C15-O1) are observed. The phenyl ring is almost perpendicular to the plane of the molecule, with an angle of 88° between the plane formed by the phenyl ring and that formed by the C_5H_4-C-chromanone.

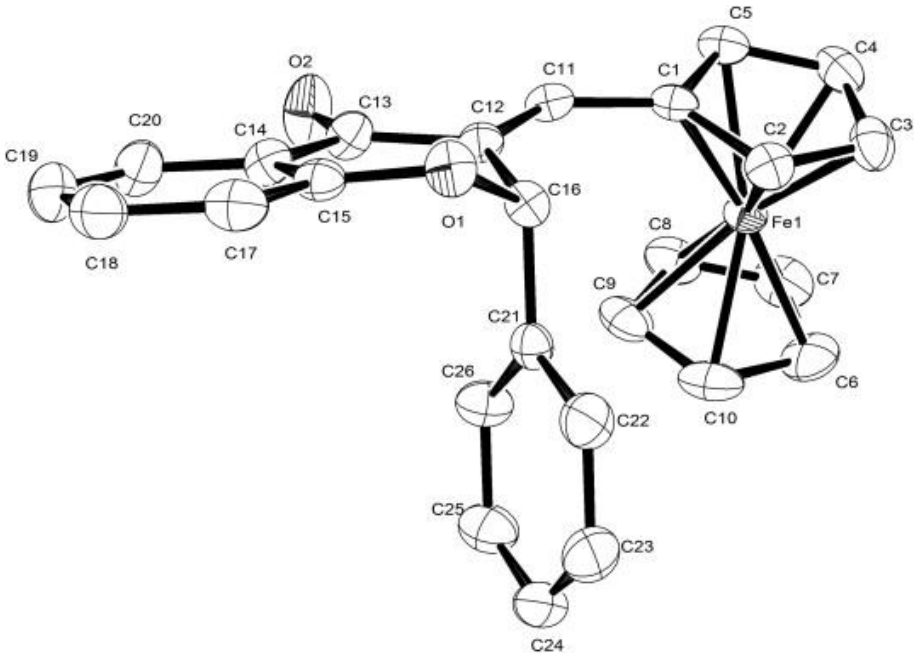
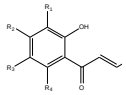
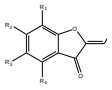
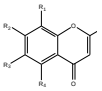


Fig. 1. Ortep diagram of **6a**. Thermal ellipsoids shown at 50% probability, hydrogen atoms omitted for clarity. Selected bond distances (Å): C13–O2 = 1.224(2); Fe1–C_{Cp} = 2.027(2)–2.049(2) (range), 2.036(2) (avg); C1–C11 = 1.439(3); C11–C12 = 1.339(3); and angles (°): $Cp_{centroid}$–Fe–$Cp_{centroid}$ = 178.10°; C1–C11–C12 = 131.05(18)°; C13–C12–C16–O1 = −41.13°.

3.2. Cytotoxicity of ferrocenyl chalcones, aurones and flavones

The antiproliferative effects, expressed as IC_{50} values, of the ferrocenyl chalcones, aurones and flavones against B16 melanoma cells for a 48 h exposure time, are presented in Table 2. For the compounds where R = H or OMe, the geometry of the flavonoid and the number or position of the substituents have only a modest effect on the cytotoxicity. For example, the unsubstituted compounds **1a**, **2a** and **3a** presented similar activity, with an average IC_{50} value of 44 ± 5 μM. The methoxylated compounds **1g-j**, **2g-j** and **3g-j**, all have IC_{50} values ranging within a factor of 2 (between 36 and 63 μM), except for the bismethoxychalcone **1j**, which is not active at 100 μM.

Table 2. Cytotoxicity on B16 murine melanoma cells (IC_{50} in μM) and lowest concentration causing morphological changes on EAhy 926 endothelial cells (EC_{20} in μM) of ferrocenyl compounds.

		**IC_{50} (μM)[a]**	**EC_{20} (μM)[b]**		**IC_{50} (μM)[a]**	**EC_{20} (μM)[b]**		**IC_{50} (μM)[a]**	**EC_{20} (μM)[b]**
Unsubstituted	**1a**	50 ± 1	>100	**2a**	45 ± 4	25	**3a**	39 ± 1	25
R_3 = Cl	**1b**	30 ± 2	>100	**2b**	16 ± 1	>100	**3b**	41 ± 1	1.56
R_3 = Br	**1c**	45 ± 3	>100	**2c**	16 ± 1	>100	**3c**	41 ± 1	0.8
R_1 = R_3 = F	**1d**	49 ± 1	12.5	**2d**	18 ± 1	>100	**3d**	54 ± 2	100
R_1 = R_3 = Cl	**1e**	74 ± 3	50	**2e**	12 ± 1	>100	**3e**	55 ± 4	6.25
R_1 = R_3 = Br	**1f**	84 ± 5	25	**2f**	14 ± 1	50	**3f**	91 ± 8	0.8
R_2 = OMe	**1g**	43 ± 2	50	**2g**	48 ± 1	100	**3g**	36 ± 1	50
R_3 = OMe	**1h**	39 ± 4	50	**2h**	46 ± 2	6.25	**3h**	38 ± 1	12.5
R_4 = OMe	**1i**	42 ± 2	50	**2i**	53 ± 2	6.25	**3i**	63 ± 2	6.25
R_2 = R_4 = OMe	**1j**	>100	50	**2j**	46 ± 1	50	**3j**	49 ± 2	>100
Mean IC_{50}		56 ± 44			31 ± 22			51 ± 40	

[a] IC_{50} is the cytotoxicity for 50% of cells and was assessed using the MTT viability test for a 48 h exposure time.

[b] EC_{20} is the lowest concentration (μM) showing more than 20% rounded cells (negative controls with 1% DMSO ≈15% rounded cells).

The compounds comprising the halogen series have a more diverse profile. The halogenated chalcones and flavones show low activity, with IC_{50} values ranging between 30 μM and 91 μM. However, the aurones **2b-f** all show cell growth inhibition at concentrations below 20 μM. The IC_{50} values are similar, regardless of the type of halogen substitution, ranging between 12 μM and 18 μM.

Indeed, it is the presence of the active halogenated aurones which drive down the mean IC_{50} value for the aurone class. The ferrocenyl chalcones and flavones, on the other hand, have a similar mean and range of activity (55.5 ± 44.5 and 50.6 ± 40.6 μM, respectively). We should mention that B16 cells seem to be particularly resistant to these compounds. Previous studies [28] on MDA-MB-231 breast cancer cells found IC_{50} values in the low micromolar range (1-4 μM) for methoxylated aurones **2g-j**.

3.3. Morphological effects of ferrocenyl chalcones, aurones and flavones

The effect of these compounds on the morphology of endothelial cells (EAhy 926), which could be predictive of antivascular activity, was assessed. Fig. 2 shows the characteristic "rounding up" of endothelial cells after a brief 2-h exposure time to a representative active flavonoid compared to controls. It can be observed that compound **3b** causes a rounding up of endothelial cells for 53% of cells at a concentration of 3 μM, whereas 14% of control cells adopted a round shape.

Remarkably, many of the compounds showed morphological activity after a 2 h exposure time at non-cytotoxic concentrations. The exposure time was deliberately chosen to be short to simulate more closely the acute effects of these flavonoids upon *in vivo* administration. Because most of these compounds

have a short half-life the endothelial cells are therefore exposed to high concentrations for a short time *in vivo*.

The ferrocenyl flavonoids show a wide range in morphological activity, and the geometry of the flavonoid skeleton has an important impact on their potency. The ferrocenyl chalcones have little effect on the endothelial cells, with only two molecules, **1d** and **1f**, showing activity at concentrations below 50 µM. The ferrocenyl aurones are more effective, with three compounds changing the cell morphology at concentrations below 50 µM, and two, **2h** and **2i**, showing activity at 6.25 µM. The flavones show by far the best morphological activity of all of the ferrocenyl flavonoid classes examined. Indeed, most of the compounds are active at concentrations below 25 µM. Most notably, the halogenated compounds **3b**, **3c** and **3f**, are active at low or submicromolar concentrations, which compare favorably to compounds in the combretastatin series, which are considered as the reference compounds in the antivascular series [41].

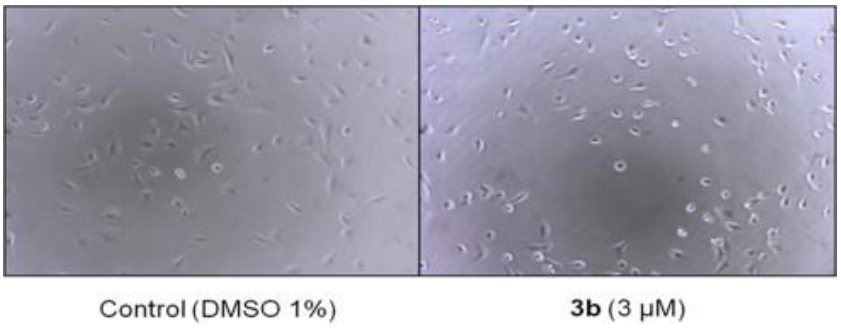


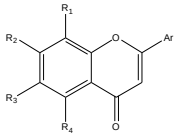
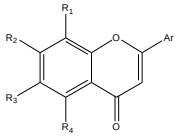
Fig. 2. Representative photographs depicting the morphological effects of compound **3b** on endothelial cells (EAhy 926). Cells in exponential growth were exposed for 2 h to either 1% DMSO (control) or compound **3b** at 3 µM. Original magnification was 360×.

3.4. Comparison of the biological effects of ferrocenyl and organic flavones

As shown above, some of the ferrocenyl flavones can induce changes in the morphology of endothelial cells at low concentrations. To investigate the influence of the ferrocenyl group on morphological effects and cytotoxicity, the corresponding series of organic flavones was synthesized by a 1,6-oxidative cyclization of chalcones with I_2 in DMSO [24]. We observed that the presence of a ferrocene in lieu of a phenyl group generally enhanced the cytotoxicity on cancer cells, as presented in Table 3. This enhanced cytotoxicity against cancer cells has already been observed by us and by others in various ferrocene functionalized biomolecules [12,13,18b,42].

Concerning the morphological effects on endothelial cells, the comparison of ferrocenyl flavones to their organic counterparts did not allow us to draw clear structure-activity relationships. For the compound pairs **3a**/**4a**, **3d**/**4d**, **3g**/**4g** and **3j**/**4j**, the presence of ferrocene inhibited the morphological activity of compound compared to its phenyl analogue. For example, the simple unsubstituted flavone **4a** shows surprisingly good morphological activity on endothelial cells, whereas its ferrocenyl counterpart **3a** was active at a 32-fold higher concentration. On the other hand, for the pairs of compounds **3c**/**4c**, **3e**/**4e**, **3f**/**4f**, **3h**/**4h** and **3i**/**4i** the ferrocenyl derivative clearly showed a stronger morphological effect on the endothelial cells compared to its unsubstituted counterpart. Therefore, in summary, while the addition of ferrocene to the ferrocenyl flavones tends to enhance the compound's cytotoxicity, there does not seem to be a clear effect on their morphological activity.

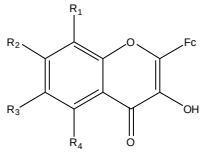
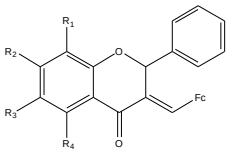
Table 3. Cytotoxicity on B16 murine melanoma cells and lowest concentration causing morphological changes on EAhy 926 endothelial cells of organic flavones **4a**–**j** compared to ferrocenyl flavones **3a**–**j**.

		**IC_{50} (µM)**[a]	**EC_{20} (µM)**[b]			**IC_{50} (µM)**[a]	**EC_{20} (µM)**[b]
Unsubstituted	**3a** (Fc)	39 ± 1	25	$R_1 = R_3$ = Br	**3f** (Fc)	91 ± 8	0.8
	4a (Ph)	49 ± 3	0.78		**4f** (Ph)	>100	6.3
R_3 = Cl	**3b** (Fc)	41 ± 1	1.56	R_2 = OMe	**3g** (Fc)	36 ± 1	50
	4b (Ph)	>100	n.d.		**4g** (Ph)	>100	1.5
R_3 = Br	**3c** (Fc)	41 ± 1	0.8	R_3 = OMe	**3h** (Fc)	38 ± 1	12.5
	4c (Ph)	>100	3.1		**4h** (Ph)	>100	25
$R_1 = R_3$ = F	**3d** (Fc)	54 ± 2	100	R_4 = OMe	**3i** (Fc)	63 ± 2	6.25
	4d (Ph)	>100	50		**4i** (Ph)	89 ± 9	12.5
$R_1 = R_3$ = Cl	**3e** (Fc)	55 ± 4	6.25	$R_2 = R_4$ = OMe	**3j** (Fc)	49 ± 2	>100
	4e (Ph)	>100	25		**4j** (Ph)	98 ± 11	12.5

[a] IC_{50} is the cytotoxicity for 50% of cells and was assessed using the MTT viability test for a 48 h exposure time.

[b] EC_{20} is the lowest concentration (µM) showing more than 20% rounded cells (negative controls with 1% DMSO ≈15% rounded cells).

Table 4. Cytotoxicity on B16 murine melanoma cells (IC_{50} in µM) and lowest concentration causing morphological changes on EAhy 926 endothelial cells (EC_{20} in µM) of ferrocene flavonols **5a**, **5b** and **5h**, and of ferrocenyl flavanones (**6a**–**c**).

			**IC_{50} (µM)**[a]	**EC_{20} (µM)**[b]			**IC_{50} (µM)**[a]	**EC_{20} (µM)**[b]
5a	Unsub.		>100	>100	**6a**	Unsub.	36 ± 2	50
5c	R_3 = Br		>100	>100	**6b**	R_2 = OMe	56 ± 8	>100
5h	R_2 = OMe		>100	>100	**6c**	R_3 = OMe	52 ± 7	100
					6d	R_3 = Cl	44 ± 1	25

[a] Cytotoxicity was assessed using the MTT viability test for a 48 h exposure time.

[b] Lowest rounding up concentration (µM) showing more than 20% rounded cells (negative controls with 1% DMSO = 15% rounded cells).

3.5. Ferrocenyl flavonoids with 3-substitution

Of the many studies undertaken on the biological properties of flavonoids, those compounds with substitution in the 3-position of the ketone, whether OH or a glycoside, have raised the most interest. It is therefore rather surprising that none of the three flavonols tested in this study had any effect on cell viability or cell morphology below 100 μM (Table 4). This is in contradiction with the behavior of natural flavonoids. From a biosynthesis point of view, natural 3-substituted flavonoids, such as flavonols and isoflavones, possessing respectively a hydroxyl and an aromatic ring in this position, are more "evolved" than the others, since their biosynthetic enzymes appeared last [43]. Currently, the majority of natural flavonoids possess substitutions in the 3-position. Moreover, flavonols and flavanonols possess a 1,2-ketol motif, which allows chelation of certain metals, such as iron or zinc [44], known to have an important role in many biological functions.

Likewise, no antitumoral or antivascular effects were observed for the flavanones possessing a ferrocenylmethylidenyl group in the 3-position. This result was also unexpected, as several 3-benzylidenyl flavanones have recently exhibited IC_{50} values in the low to submicromolar range against cancer cells [45]. In this case the ferrocene does not seem to act as a good mimic of a phenyl ring. Moreover, the absence of activity of the 3-substituted ferrocenyl flavonoids (whether OH or =C-Fc) suggest an entirely different mode of action than that of the organic compounds.

4. Conclusions

The cytotoxic and morphological effects of some three dozen ferrocenyl flavonoids have been reported. Some broad structure-activity relationships can be discerned. Overall, the halogenated aurones showed the best cytotoxic activity, although their morphological activity was modest. For the flavones, the presence of a ferrocenyl group led to a clear increase in cytotoxic activity on cancer cells, whereas for the morphological effects on endothelial cells, the presence of the ferrocenyl substituent could not be linked to a definite structure activity relationship. Surprisingly, the presence of substitution in the 3-position of ferrocenyl flavonoids yielded biologically inactive compounds.

Although the mechanism of action of these novel compounds is not presently known, they possibly act in a similar fashion as other flavonoids. For their cytotoxic action, flavonoids can act through a myriad of signaling pathways. For example, flavonoids and chalcones can regulate expression of VEGF, matrix metalloproteinases (MMPs), EGFR and inhibit NFkappaB, PI3-K/Akt, ERK1/2 signaling pathways [46]. Regarding the flavonoids morphological activity on endothelial cells, it appears that interaction with the tubulin is needed and results in a disorganization of the cytoskeleton which ultimately cause the endothelial cell to adopt a round shape. This cell morphology ultimately may translate into an antitumoral antivascular action in vivo [47].

Overall, there was no correlation observed between cytotoxic effects and potential antiangiogenic effects. For example, the most cytotoxic compounds, the halogenated ferrocenyl aurones, show only mild morphological effects, while the most active compounds, the chloro and bromo ferrocenyl flavones, are not very cytotoxic. Thus we expect that these ferrocenyl flavones will act as pure antivascular agents in vivo, that is, destroying tumor vasculature without directly interfering with cancer cell growth or division. Ideally, however, the most promising drug leads will possess both cytotoxic and antivascular properties, which do not seem to be correlated in this class of molecules. Thus future work will focus on optimizing the ferrocenyl flavones possessing both increased cytotoxic and morphologic activities compared to their organic analogues, such as **3c**, **3e**, **3f**, **3h** and **3i**.

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Appendix A. Supplementary data

CCDC 906156 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Appendix B. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jorganchem.2012.12.031.

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Supporting information

Ferrocenyl flavonoid-induced morphological modifications of

endothelial cells and cytotoxicity against B16 murine melanoma cells

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1H NMR characterization of organic flavones 4b-j.

6-chloro-2-phenyl-4*H*-chromen-4-one, 4b. δ_H (300 MHz; $CDCl_3$) 6.83 (s, 1H, vinyl), 7.52-7.55 (m, 4H, C_6H_5), 7.64 (dd, *J* = 2.3 Hz, *J* = 8.8 Hz, 1H, C_6H_5), 7.92 (dd, *J* = 2.3 Hz, *J* = 8.8 Hz, 2H, C_6H_3), 8.20 (d, *J* = 2.3 Hz, 1H, C_6H_3).

6-bromo-2-phenyl-4*H*-chromen-4-one, 4c. δ_H (300 MHz; $CDCl_3$) 6.77 (s, 1H, vinyl), 7.42 (d, *J* = 8.8 Hz, 1H, C_6H_3), 7.46-7.49 (m, 3H, C_6H_5), 7.72 (dd, *J* = 2.2 Hz, *J* = 8.8 Hz, 1H, C_6H_3), 7.83-7.87 (m, 2H, C_6H_5), 8.30 (d, *J* = 2.2 Hz, 1H, C_6H_3).

6,8-difluoro-2-phenyl-4*H*-chromen-4-one, 4d. δ_H (300 MHz; $CDCl_3$) 6.84 (s, 1H, vinyl), 7.23-7.30 (m, 2H, C_6H_5), 7.54-7.56 (m, 1H, C_6H_5), 7.65-7.69 (m, 1H, C_6H_2), 7.93-7.96 (m, 2H, C_6H_5/C_6H_2).

6,8-dichloro-2-phenyl-4*H*-chromen-4-one, 4e. δ_H (300 MHz; $CDCl_3$) 6.87 (s, 1H, vinyl), 7.56 (m, 3H, C_6H_5), 7.75 (d, *J* = 2.6 Hz, 1H, C_6H_2), 7.97-8.00 (m, 2H, C_6H_5), 8.11 (d, *J* = 2.6 Hz, 1H, C_6H_2).

6,8-dibromo-2-phenyl-4*H*-chromen-4-one, 4f. δ_H (300 MHz; $CDCl_3$) 6.87 (s, 1H, vinyl), 7.56 (m, 3H, C_6H_5), 8.00 (m, 2H, C_6H_5), 8.03 (d, *J* = 2.2 Hz, 1H, C_6H_2), 8.30 (d, *J* = 2.2 Hz, 1H, C_6H_2).

7-methoxy-2-phenyl-4*H*-chromen-4-one, 4g. δ_H (300 MHz; $CDCl_3$) 3.90 (s, 3H, OMe), 6.74 (s, 1H, vinyl), 6.96 (m, 2H, C_6H_3), 7.46-7.52 (m, 3H, C_6H_5), 7.87 (m, 3H, C_6H_5), 8.10 (d, *J* = 8.4 Hz, 1H, C_6H_3).

6-methoxy-2-phenyl-4*H*-chromen-4-one, 4h. δ_H (300 MHz; $CDCl_3$) 3.91 (s, 3H, OMe), 6.83 (s, 1H, vinyl), 7.29 (dd, *J* = 2.8 Hz, *J* = 8.8 Hz, 1H, C_6H_3), 7.50 (s, 1H, C_6H_3), 7.54 (m, 3H, C_6H_5), 7.60 (d, *J* = 2.8 Hz, 1H, C_6H_3), 7.91-7.94 (m, 2H, C_6H_5).

5-methoxy-2-phenyl-4*H*-chromen-4-one, 4i. δ_H (300 MHz; $CDCl_3$) 4.00 (s, 3H, OMe) 6.74 (s, 1H, vinyl), 6.82 (d, *J* = 8.2 Hz, 1H, C_6H_3), 7.14 (dd, *J* = 0.9 Hz, *J* = 8.2 Hz, 1H, C_6H_3), 7.51-7.60 (m, 4H, C_6H_5), 7.90 (m, 2H, C_6H_5/ C_6H_3).

5,7-dimethoxy-2-phenyl-4*H*-chromen-4-one, 4j. δ_H (300 MHz; $CDCl_3$) 3.92 (s, 3H, OMe), 3.96 (s, 3H, OMe), 6.39 (d, *J* = 2.2 Hz, 1H, C_6H_2), 6.58 (d, *J* = 2.2 Hz, 1H, C_6H_2), 6.69 (s, 1H, vinyl), 7.50 (m, 3H, C_6H_5), 7.89 (dd, *J* = 2.2 Hz, *J* = 6.0 Hz, 2H, C_6H_5).

Table SI1 Bond distances and angles for 6a.

Bond distances (Å)	Angles (°)	Angles (°)
Fe1 . C1 . 2.0366(19)	C1 . Fe1 . C2 . 41.31(8)	C2 . C3 . Fe1 . 69.27(12)
Fe1 . C2 . 2.032(2)	C1 . Fe1 . C3 . 69.08(8)	C2 . C3 . C4 . 108.26(19)
Fe1 . C3 . 2.043(2)	C2 . Fe1 . C3 . 40.69(8)	Fe1 . C3 . C4 . 69.58(12)
Fe1 . C4 . 2.040(2)	C1 . Fe1 . C4 . 69.15(8)	C3 . C4 . Fe1 . 69.76(12)
Fe1 . C5 . 2.027(2)	C2 . Fe1 . C4 . 68.68(9)	C3 . C4 . C5 . 108.19(18)
Fe1 . C6 . 2.032(2)	C3 . Fe1 . C4 . 40.66(9)	Fe1 . C4 . C5 . 69.28(12)
Fe1 . C7 . 2.033(2)	C1 . Fe1 . C5 . 41.49(8)	C1 . C5 . C4 . 108.81(18)
Fe1 . C8 . 2.049(2)	C2 . Fe1 . C5 . 69.06(8)	C1 . C5 . Fe1 . 69.61(11)
Fe1 . C9 . 2.032(2)	C3 . Fe1 . C5 . 68.40(9)	C4 . C5 . Fe1 . 70.28(12)
Fe1 . C10 . 2.031(2)	C4 . Fe1 . C5 . 40.44(9)	Fe1 . C6 . C7 . 69.61(14)
C1 . C2 . 1.435(3)	C1 . Fe1 . C6 . 157.66(9)	Fe1 . C6 . C10 . 69.64(13)
C1 . C5 . 1.439(3)	C2 . Fe1 . C6 . 120.77(9)	C7 . C6 . C10 . 108.1(2)
C1 . C11 . 1.439(3)	C3 . Fe1 . C6 . 105.75(10)	C6 . C7 . Fe1 . 69.56(13)
C2 . C3 . 1.417(3)	C4 . Fe1 . C6 . 121.90(10)	C6 . C7 . C8 . 108.0(2)
C3 . C4 . 1.418(3)	C5 . Fe1 . C6 . 158.61(10)	Fe1 . C7 . C8 . 70.45(13)
C4 . C5 . 1.406(3)	C1 . Fe1 . C7 . 160.46(10)	C7 . C8 . Fe1 . 69.23(13)
C6 . C7 . 1.418(4)	C2 . Fe1 . C7 . 156.64(10)	C7 . C8 . C9 . 107.7(2)
C6 . C10 . 1.410(4)	C3 . Fe1 . C7 . 120.97(10)	Fe1 . C8 . C9 . 69.05(13)
C7 . C8 . 1.407(4)	C4 . Fe1 . C7 . 106.67(9)	C8 . C9 . Fe1 . 70.34(13)
C8 . C9 . 1.416(3)	C5 . Fe1 . C7 . 123.15(9)	C8 . C9 . C10 . 108.4(2)
C9 . C10 . 1.410(4)	C1 . Fe1 . C8 . 124.77(9)	Fe1 . C9 . C10 . 69.65(13)
C11 . C12 . 1.339(3)	C2 . Fe1 . C8 . 161.18(10)	C6 . C10 . C9 . 107.7(2)
C12 . C13 . 1.476(3)	C3 . Fe1 . C8 . 157.45(9)	C6 . C10 . Fe1 . 69.76(13)
C12 . C16 . 1.495(3)	C4 . Fe1 . C8 . 122.73(9)	C9 . C10 . Fe1 . 69.74(13)
C13 . C14 . 1.474(3)	C5 . Fe1 . C8 . 108.93(9)	C1 . C11 . C12 . 131.05(18)
C13 . O2 . 1.224(2)	C1 . Fe1 . C9 . 108.62(9)	C11 . C12 . C13 . 118.21(17)
C14 . C15 . 1.388(3)	C2 . Fe1 . C9 . 124.01(9)	C11 . C12 . C16 . 124.98(18)
C14 . C20 . 1.397(3)	C3 . Fe1 . C9 . 159.26(10)	C13 . C12 . C16 . 116.71(16)
C15 . C17 . 1.387(3)	C4 . Fe1 . C9 . 159.43(10)	C12 . C13 . C14 . 115.59(17)
C15 . O1 . 1.360(2)	C5 . Fe1 . C9 . 124.40(9)	C12 . C13 . O2 . 123.04(18)
C16 . C21 . 1.519(3)	C1 . Fe1 . C10 . 122.45(9)	C14 . C13 . O2 . 121.38(19)
C16 . O1 . 1.458(2)	C2 . Fe1 . C10 . 106.72(9)	C13 . C14 . C15 . 120.37(18)
C17 . C18 . 1.371(3)	C3 . Fe1 . C10 . 121.99(10)	C13 . C14 . C20 . 120.89(19)
C18 . C19 . 1.380(3)	C4 . Fe1 . C10 . 158.05(10)	C15 . C14 . C20 . 118.71(18)
C19 . C20 . 1.372(3)	C5 . Fe1 . C10 . 159.91(10)	C14 . C15 . C17 . 120.46(19)
C21 . C22 . 1.382(3)	C6 . Fe1 . C7 . 40.83(10)	C14 . C15 . O1 . 122.08(17)
C21 . C26 . 1.382(3)	C6 . Fe1 . C8 . 68.14(10)	C17 . C15 . O1 . 117.40(18)
C22 . C23 . 1.384(3)	C7 . Fe1 . C8 . 40.32(10)	C12 . C16 . C21 . 114.47(15)
C23 . C24 . 1.377(3)	C6 . Fe1 . C9 . 68.13(10)	C12 . C16 . O1 . 111.75(15)
C24 . C25 . 1.367(3)	C7 . Fe1 . C9 . 68.21(10)	C21 . C16 . O1 . 108.29(14)
C25 . C26 . 1.386(3)	C8 . Fe1 . C9 . 40.61(9)	C15 . C17 . C18 . 119.6(2)
	C6 . Fe1 . C10 . 40.60(11)	C17 . C18 . C19 . 120.8(2)
	C7 . Fe1 . C10 . 68.58(10)	C18 . C19 . C20 . 119.7(2)
	C8 . Fe1 . C10 . 68.38(10)	C14 . C20 . C19 . 120.7(2)
	C9 . Fe1 . C10 . 40.60(11)	C16 . C21 . C22 . 119.14(18)
	Fe1 . C1 . C2 . 69.19(11)	C16 . C21 . C26 . 122.38(17)
	Fe1 . C1 . C5 . 68.90(11)	C22 . C21 . C26 . 118.47(19)
	C2 . C1 . C5 . 106.36(17)	C21 . C22 . C23 . 120.5(2)
	Fe1 . C1 . C11 . 121.28(14)	C22 . C23 . C24 . 120.2(2)
	C2 . C1 . C11 . 131.32(17)	C23 . C24 . C25 . 119.9(2)
	C5 . C1 . C11 . 121.96(18)	C24 . C25 . C26 . 119.9(2)
	C1 . C2 . Fe1 . 69.50(11)	C25 . C26 . C21 . 121.0(2)
	C1 . C2 . C3 . 108.38(18)	C16 . O1 . C15 . 115.28(14)
	Fe1 . C2 . C3 . 70.04(12)	