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Keywords

Anticancer agents; Ferrocifen; Organometallics; MDA-MB-231; quinone methides.

Abstract

As previously reported, the ferrocenyl derivative $HO(CH_2)_3C(Fc)=C(C_6H_4OH)_2$ (**2**) shows an excellent cytotoxic effect against MDA-MB-231 (TNBC) cancer cell lines. Building on an analysis of this molecular framework, a series of novel hydroxypropyl-ferrociphenol derivatives with modified terminal hydroxyl groups were synthesized, and their antiproliferative activities against MDA-MB-231 cell lines were evaluated. Biological results showed that compound **8**, whose terminal hydroxyl was protected by acetylation, exhibited the greatest cytotoxic effect among this series of hydroxypropyl derivatives. Furthermore, the impact of acetyl as a protecting group on the cytotoxicity of hydroxypropyl-ferrociphenol compounds by incorporating it to alkyl or phenyl hydroxyl positions of the core structure has been studied. Several of compounds presented in this study revealed lipophilicity more suitable for formulation in lipid nanocapsules (LNCs) for subsequent in vivo studies. They also inhibit the cancer cell growth of MDA-MB-231 at a submicromolar IC_{50} value, providing an interesting potential for further development as innovative anticancer agents.

Introduction

Since it was first proposed as a topic in 1985 [1], bioorganometallic chemistry has become increasingly popular owing to the unveiling of several therapeutically active organometallic compounds currently undergoing clinical trials [2-4]. In this context, compounds possessing metal-carbon covalent bonds have been widely studied as drug candidates or imaging agents for the treatment of various diseases [5-11]. It provides a broad space for the Medicinal Chemist to develop novel molecules that are different from conventional drugs, in terms of chemical structure and mechanisms of biological action [12-17]. We have previously developed a series of compounds, entitled ferrociphenols, resulting from the incorporation of a ferrocenyl group into the tamoxifen backbone, and have shown the [ferrocenyl-ene-phenol] motif to be sensitive to an oxidative environment [18]. Many of these organometallic compounds exhibit a marked antiproliferative effect against MDA-MB-231 triple negative breast cancer cells (TNBC); an archetypical example is compound **1** with an IC_{50} value around 0.6 μM (Figure 1). Moreover, compounds in this series inhibit the proliferation of breast cancer cells but have no cytotoxic effect on normal cells (IC_{50} > 100-200 μM) [19, 20], which makes them more attractive in terms of selective toxicity as compared to platinum complexes. We have also demonstrated that the ferrociphenols exert their cytotoxic activity by formation of quinone methides (such as **1-QM**) [21-23] that are easily attacked by nucleophiles present in the cancer cell [24], at least partly affecting the cellular redox balance [25]. It should be emphasized that the ferrocenyl group plays a significant role in the oxidation of ferrociphenols to give corresponding the quinone methides [21, 24].

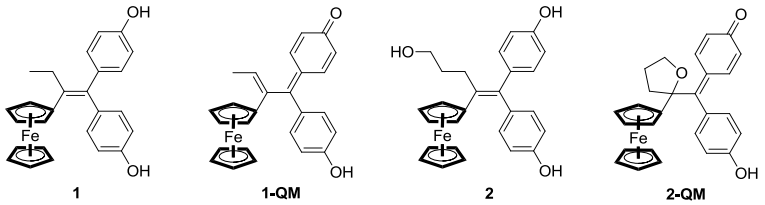


Figure 1. Structures of ferrocenyl compounds **1**, **2** and their corresponding quinone methides.

More recently, based on an investigation of the metabolites of ferrocenyl derivatives, we have prepared the hydroxypropyl derivative of ferrociphenol, $HO(CH_2)_3C(Fc)=C(C_6H_4OH)_2$ (**2**), which displays excellent antiproliferative activity against a range of primary and metastic neoplasms including MDA-MB-231 TNBC cancer cells (IC_{50} value approximately 0.11 μM) [26]. Formation of the new tetrahydrofuran-substituted quinone methide (**2-QM**) via internal cyclization of the hydroxy-alkyl chain may explain its greater cytotoxic activity. The stronger and broad-spectrum antitumor effect of hydroxypropyl-ferrociphenol **2** prompted us towards further chemical exploration of this framework to elucidate the active motif. An increase in the lipophilicity of compound **2** by modification of its hydroxyl group may increase the quantity of this molecule encapsulated in lipid nanocapsules (LNCs), thus increasing the quantity of drug available for administration [27]. As part of this examination, we first chose to examine the influence of its alkyl chain, for example, by replacing the terminal hydroxyl substituent with its classical bioisosteres including chloro- and thiol- to test their influence on cytotoxic activity (Figure 2). Hydroxamate-based analogues [28] or oxime ether fragments [29-31] are widely applied in medicinal chemistry as ubiquitous pharmacophores that display a broad range of biological activity. By combining our hydroxypropyl-ferrociphenol skeleton with some N-substituted hydroxylamine derivatives, we were also able to probe how such substituents influence the lipophilicity and cytotoxicity of these types of molecules. Protecting groups are often used in drug development to improve the stability and bioavailability of molecules; these so-called prodrugs take action only after *in situ* hydrolysis by appropriate

enzymes *in vivo*. Inspired by this concept, two different acyl protecting groups were incorporated at the hydroxyl position with the aim of monitoring the formation of their quinone methides, and also their cytotoxic behavior.

We here present the syntheses and antiproliferative evaluation of a series of novel hydroxypropyl-ferrociphenol derivatives with modified hydroxyl substituents. The effect of several pharmacophores, such as chloro, thiol and hydroxamate, on the lipophilicity and cytotoxicity of ferrocenyl-containing molecules is explored. The selective introduction of one or more acyl groups at different positions within the hydroxypropyl-ferrociphenol skeleton, and the comprehensive structure-activity-relationship of the resulting compounds is also reported.

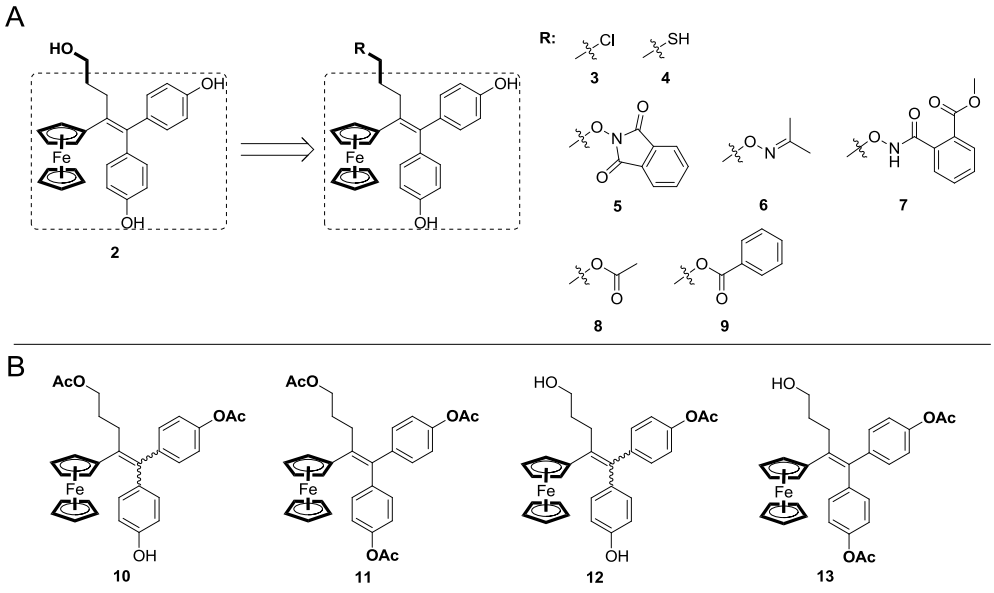
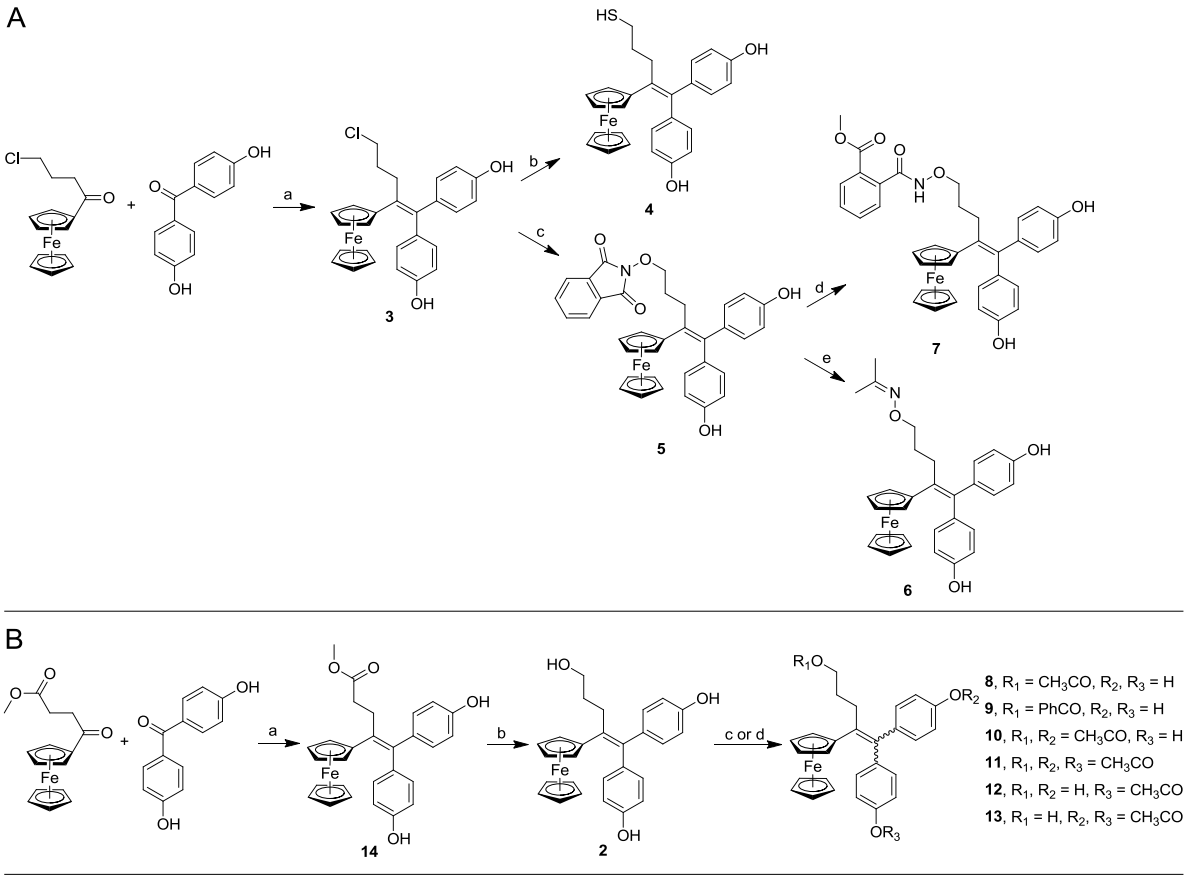


Figure 2. New hydroxypropyl-ferrociphenol compounds. **A**. Structural modification of the terminal hydroxyl on the alkyl chain of compound **2**; **B**. A range of hydroxypropyl-ferrociphenol derivatives possessing multiple acetyl groups.

Results and Discussion

Synthesis



Scheme 1. A. Syntheses of hydroxypropyl-ferrociphenol derivatives with modified terminal hydroxyl groups: a) Zn, $TiCl_4$, reflux; b) thiourea, KI, CH_3CN, reflux; then NaOH, $EtOH/H_2O$, reflux; c) 2-hydroxyisoindoline-1,3-dione, KI, K_2CO_3, CH_3CN, reflux; d) CH_3OH, rt; e) hydrazine hydrate, CH_3OH/acetone, rt. **B**. Selective introduction of one or more acyl groups to different positions in the hydroxypropyl-ferrociphenol skeleton: a) Zn, $TiCl_4$, reflux; b) $LiAlH_4$, THF, reflux; c) Ac_2O, pyridine, rt; d) Ac_2O, TMSOTf, CH_2Cl_2.

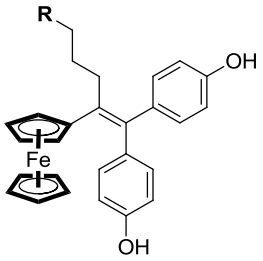
An efficient synthesis for the desired alkenes was established based on McMurry cross-coupling by the method previously developed in our group. As depicted in **Scheme 1**, 4,4'-dihydroxybenzophenone was coupled with 4-chloro-1-ferrocenylbutan-1-one or methyl 4-oxo-4-ferrocenylbutanoate using $TiCl_4$/Zn in dry THF, to give compounds **3** [32] or **14**, respectively, as brown solids. Compound **4** bearing a terminal thiol unit was prepared *via* a two-step procedure involving an isothiuronium intermediate. After initial nucleophilic substitution of chloride in **3** by thiourea in the presence of KI, followed by removal of solvent,

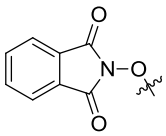
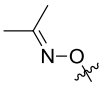
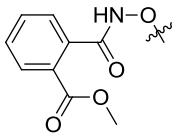
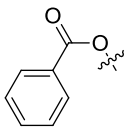
the crude isothiuronium obtained was then hydrolyzed in NaOH solution ($EtOH/H_2O$) to give compound **4** in an overall yield of 89% for the two steps. Similarly, the isoindoline-1,3-dione analog **5** was synthesized by nucleophilic displacement of Cl in **3**. Compounds **6** and **7,** respectively, were obtained by treatment of **5** with a solution of hydrazine hydrate in acetone/methanol, or by the alcoholysis in methanol.

Hydroxypropyl-ferrociphenol, **2**, was obtained from the ester **14** by reduction using $LiAlH_4$ in THF solution. However, since compound **2** possesses one aliphatic and two phenolic hydroxyl groups, it was necessary to find suitable reagents to control acetylation at only the desired positions. Attempted acetylation at a single phenolic site, as in **12**, by treatment of compound **2** with AcCl or Ac_2O in the presence of TEA gave instead the triacetylated molecule **11** as the major product. There was no selectivity of the esterification between aliphatic and phenolic hydroxyl groups when using the Ac_2O/TEA system. Gratifyingly, when the weaker base pyridine was allowed to react with one equivalent of **2** and two equivalents of Ac_2O, the mono- and di-acetylated compounds **12** (42%) and **13** (42%) were selectively obtained, and could be separated by chromatography. In general, the phenols are predominantly acylated in the presence of aliphatic alcohols due to the greater nucleophilicity of phenols under basic conditions. Fortunately, reversal of chemoselectivity has been reported in a number of Lewis acid catalyzed esterifications [33, 34]. Thus, for compounds **8** and **10** whose phenolic –OH groups were apparently exposed, the Lewis acid trimethylsilyl hydroxyl group using two equivalents of Ac_2O [34] to give compounds **8**, **10** and **11** that were easily purified by chromatography. When the same method was used for benzoylation, the single product was compound **9** (82% yield), whereby reaction occurred only at the aliphatic site.

Biological evaluation of new hydroxypropyl-ferrociphenol derivatives

Table 1. IC_{50} values (μM) of new hydroxypropyl-ferrociphenol compounds with modified terminal hudroxyl groups against breast MDA-MB-231 cancer cells, and also their lipophilicity (log $P_{o/w}$).



Compd.	R	MDA-MB-231[a]	log $P_{o/w}$
2[b]	OH	0.11 ± 0.02	4.2
3	Cl	1.94 ± 0.43	5.7
4	SH	> 20	9.5
5		13.31 ± 1.35	5.6
6		1.97 ± 0.63	5.4
7		12.71 ± 1.3	5.0
8		0.28 ± 0.07	5.0
9		0.43 ± 0.05	6.2

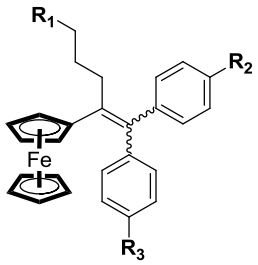
[a] IC_{50}: Data are means ± standard deviation (SD) from two independent experiments running in quadruplicate. [b] IC_{50} and log $P_{o/w}$ value from Ref [24].

The antiproliferative effect of the new hydroxypropyl ferrociphenol derivatives were evaluated on the triple negative breast cancer cell line MDA-MB-231, and the IC_{50} and log$P_{o/w}$ values of these compounds are summarized in Table 1. All of the hydroxypropyl ferrociphenol derivatives tested inhibit the growth of MDA-MB-231 cancer cells from the micromolar to the submicromolar level, except for compound **4** which possesses a terminal thiol group. This compound does not show a significant cytotoxic effect on MDA-MB-231 cells even at the concentration of 20 μM moreover, its log$P_{o/w}$ value was 9.5 indicating the high hydrophobic property of this molecule. Mass spectrometric experiments have shown that the molecule present in the incubation medium for cancer cells during IC_{50} test and the stock solution for lipophilicity measurement was the dimer of compound **4**, formed by the oxidative coupling of thiols. This observation not only provides a reasonable explanation of its high log$P_{o/w}$ value, but also the increased size of the S-S coupled derivative of thiol **4** may inhibit its ability to bind peptides or to proteins, even it can form a quinone methide, thus leading to a non-cytotoxic effect. The behavior of the alkyl thiol moiety on the ferrociphenol skeleton is similar to what we have previously reported for thiophenol analogues of compound **1** [35].

Compounds **5** and **7,** which each bear a bulky aromatic group at the terminal hydroxyl position, displayed weak cytotoxic activity (IC_{50} values around 13 μM). In the case of compounds **3** and **6**, whereby the terminal hydroxyl has been replaced by a chlorine or propan-2-one oxime substituent, they both displayed only moderate cytotoxic activity on MDA-MB-231 cells (IC_{50} values around 2 μM). In contrast, the antiproliferative activity of the benzoylated compound **9** was superior to those of compound **5** and **7**, even though a bulky aromatic group is sited at the terminal hydroxyl position, and its lipophilicity is higher. This result is perhaps attributable to the behavior of the ester, which may be prone to hydrolysis by

enzymes in the living cells, thereby easily regenerating the parent hydroxypropyl-ferrociphenol, **2**. It also emphasizes the crucial role of the terminal hydroxyl substituent in ferrociphenol motif in terms of antiproliferative effect. Among this series of hydroxypropyl-ferrociphenol derivatives with a modified terminal hydroxyl group, compound **8** showed the best antiproliferative effect on MDA-MB-231 cells (IC_{50}: 0.28 μM), apparently because of the ready hydrolysis of the acetyl group. It is now evident that the bulky aromatic groups at terminal hydroxyl position of the alkyl chain are disfavored, except when they can be deprotected easily in living cells. However, the acetyl group has proven to be a valuable protecting group for aliphatic alcohols with respect to the cytotoxicity of hydroxypropyl-ferrociphenol derivatives since it can be removed in vitro conditions.

Table 2. IC_{50} values (μM) of new acetyl hydroxypropyl-ferrociphenol compounds against MDA-MB-231 cancer cells, and also their lipophilicity (log $P_{o/w}$).



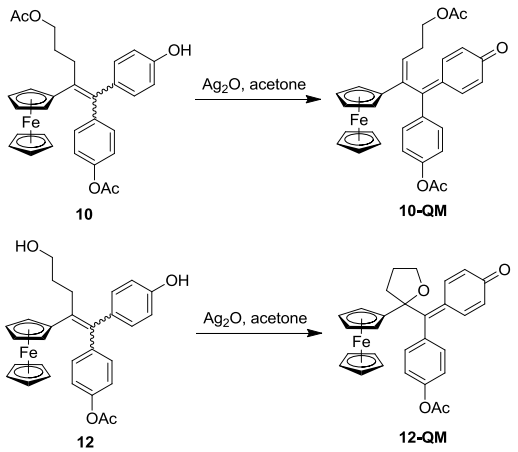
Compd.	**R_1**	**R_2**	**R_3**	MDA-MB-231[a]	log $P_{o/w}$
2[b]	OH	OH	OH	0.11 ± 0.02	4.2
12	OH	AcO	OH	0.26 ± 0.05	4.6 / 5.1
13	OH	AcO	AcO	0.38 ± 0.1	5.3
8	AcO	OH	OH	0.28 ± 0.07	5.0
10	AcO	AcO	OH	0.29 ± 0.1	5.5 / 5.7
11	AcO	AcO	AcO	0.38 ± 0.05	6.1

[a] IC_{50}: Data are means ± standard deviation (SD) from two independent experiments running in quadruplicate. [b] IC_{50} and log $P_{o/w}$ value from Ref [24].

Since the acetyl group has proven to be a useful tool as a protecting group for aliphatic alcohols, it was therefore interesting to explore its controlling influence on the whole structure of hydroxypropyl-ferrociphenols. Several novel compounds resulting from acetylation of one or more phenol or alcohol substituents were successfully prepared by use of a pyridine/Ac_2O or TMSOTf/Ac_2O system, and their biological activities were also tested on MDA-MB-231 cancer cells. All these acetate derivatives displayed excellent inhibitory activity against MDA-MB-231 cells, but are slightly weaker than that of their parent compound **2** (Table 2), even when the acetyl group could be easily hydrolyzed within cancer cells. Evidently, there is a small diminution of the cytotoxicity of hydroxypropyl-ferrociphenol compounds upon introducing acetyl as protecting group either for aliphatic alcohols or phenols. For the compounds **8**, **10** and **11**, whose aliphatic hydroxyl groups were all protected by acetyl, they displayed antiproliferative activity of the same magnitude (from 0.28 μM to 0.38 μM). This observation is consistent with the behavior of acetoxy derivatives of compound **1** that we reported previously [35, 36], as they can form the same chemotype of quinone methide as **1-QM** by possible oxidation inside cells. Conversely, the cytotoxicity of compounds **2**, **12** and **13** which bear a terminal hydroxyl moiety group decreased with the introduction of one or two acetyl groups onto the phenol(s). In these cases, it is noteworthy that the acetyl group has a more significant effect on the cytotoxicity of hydroxypropyl-ferrociphenols. Nevertheless, even with their slightly decreased cytotoxicity, the better lipophilicity of these acetyl hydroxypropyl-ferrociphenol compounds may enhance their ability to be formulated in lipid nanocapsules (LNCs) for subsequent in vivo studies.

The cytotoxic activity of compounds **11** and **13**, in which both phenols were protected by acetyl groups, was also slightly inferior to that of other ferrocenyl compounds bearing at least

one phenolic hydroxyl substituent. This may be rationalized by the fact that neither of these two compounds can be directly oxidized to quinine methides. Presumably, they need a short window of time to be hydrolyzed so as to release the free phenols inside the cell. Indeed, we were unable to observe formation of the corresponding quinone methide by chemical oxidation of compounds **11** and **13** using silver oxide. In contrast, oxidation of compounds **10** and **12** by silver oxide furnished their corresponding quinone methides **10-QM** and **12-QM**, respectively (Scheme 2).



Scheme 2. Formation of the quinone methides **10-QM** and **12-QM**.

Conclusion

Starting from the hydroxypropyl-ferrociphenol, **2**, a range of new derivatives with modified terminal hydroxyl substituents displaying significant antiproliferative activities have been successfully synthesized, and their structure-activity-relationship was clearly explicated. The presence of a terminal aliphatic hydroxyl group, or protection by a small and easily hydrolyzed group made the molecule better able to exert its cytotoxic activity. Furthermore, the effect of acetyl as a protecting group for both alkyl and phenolic hydroxyls was explored. The better lipophilic effect resulting from the incorporation of acetyl groups to the

hydroxypropyl-ferrociphenol skeleton has the potential to increase the quantity of the drug candidate inside the lipid nanocapsules (LNCs) for in vivo studies [27]. Moreover, since ferrocenyl groups have been recently recognised as important hydrophobic features of biologically active compounds, the improvement of this effect described herein is of interest [37]. Several of the compounds described in this study showed excellent cytotoxicity against MDA-MB-231 (TNBC) cells (IC_{50} values range from 0.26 μM to 0.38 μM), providing a great potential for further development as innovative anticancer agents.

Experimental section

General Synthetic Methods. Unless otherwise stated, the ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 300-MHz spectrometer. The data are reported in parts per million relative to the referenced solvent. Mass spectra were obtained on a DSQII and ITQ 1100 Thermo Scientific spectrometer for both electron ionization (EI) and chemical ionization (CI) methods on API 3000 PE Sciex Applied Biosystems using the electrospray ionization (ESI) method. Flash column chromatography was performed on silica gel Merck 60 (40-63 μm). Anhydrous solvents were obtained by standard procedures. The purity of products was more than 95% characterized by elemental analysis and analytical reverse phase HPLC (Shimadzu instrument) with Nucleodur C18 column (4.6 × 150 mm) using MeOH as eluent (except compound **11**, using CH_3CN as eluent), flow rate = 1 mL/min, λ = 254 nm. Elemental analyses were performed by the Laboratory of Microanalysis at ICSN of CNRS at Gif sur Yvette, France.

Methyl 5,5-bis(4-hydroxyphenyl)-4-ferrocenylpent-4-enoate (14). $TiCl_4$ (3.8 ml, 33 mmol) was added dropwise to a suspension of zinc powder (4.5 g, 66 mmol) in 40 ml of THF at 0

°C. The dark grey mixture obtained was heated at refluxing for 2 hrs. A solution of THF (20 ml) containing 4,4'-dihydroxybenzophenone (4.91 g, 22 mmol) and 4-oxo-4-ferrocenylbutanoate (3.13 g, 10 mmol) was added dropwise to the first solution and the resulting mixture was heated for 2 hrs. After cooling to room temperature, the mixture was acidified by addition of diluted HCl solution. The aqueous layer was extracted with EtOAc for three times. The combined organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluting with hexane:EtOAc (2:1) to give methyl 5,5-bis(4-hydroxyphenyl)-4-ferrocenylpent-4-enoate as a red solid 2.8 g, yield: 58%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (d, J = 8.5 Hz, 2H, C_6H_4), 6.88 (d, J = 8.5 Hz, 2H, C_6H_4), 6.79 (d, J = 8.5 Hz, 2H, C_6H_4), 6.66 (d, J = 8.6 Hz, 2H, C_6H_4), 4.77 (s, 1H, OH), 4.67 (s, 1H, OH), 4.12 (s, 5H, C_5H_5), 4.11 – 4.06 (m, 2H, C_5H_4), 3.90 (m, 2H, C_5H_4), 3.63 (s, 3H, OCH_3), 2.98 – 2.86 (t, J = 7.5 Hz, 2H, CH_2), 2.46 – 2.35 (t, J = 7.5 Hz, 2H, CH_2).

4,4'-(5-Hydroxy-2-ferrocenylpent-1-ene-1,1-diyl)diphenol (2). $LiAlH_4$ (0.16 g, 4.2 mmol) was added slowly to the solution of methyl 5,5-bis(4-hydroxyphenyl)-4-ferrocenylpent-4-enoate (0.4 g, 0.83 mmol) in 10 ml of THF. The mixture obtained was heated at reflux for 12 hrs. The reaction was then quenched by water. The aqueous layer was extracted with EtOAc for three times. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluting with hexane:EtOAc (2:1) to give 4,4'-(5-hydroxy-2-ferrocenylpent-1-ene-1,1-diyl)diphenol as a brown solid 0.29 g, yield: 77%. ^{1}H NMR (300 MHz, Acetone-d_6) δ 8.23 (s, 1H, OH), 8.19 (s, 1H, OH), 7.05 (d, J = 8.5 Hz, 2H, C_6H_4), 6.86 (d, J = 8.6 Hz, 2H, C_6H_4), 6.80 (d, J = 8.6 Hz, 2H, C_6H_4), 6.70 (d, J = 8.6 Hz, 2H, C_6H_4), 4.12 (s, 5H, C_5H_5), 4.07 – 4.01 (m, 2H, C_5H_4), 4.02 – 3.93 (m, 2H, C_5H_4), 3.51 – 3.36 (m, 3H, CH_2 and OH), 2.73 – 2.64 (m, 2H, CH_2), 1.66 (m, 2H, CH_2).

Acetylation of 2. Method **A**. Ac_2O (0.1 ml, 1.8 mmol) was added to a solution of **2** (0.41 g, 0.9 mmol) in pyridine (2 mL) at 0 °C. The mixture was stirred overnight at room temperature. The mixture was diluted with EtOAc and washed with water and brine, dried, and evaporated. The residue was purified by column chromatography on silica gel eluting with hexane:EtOAc (2:1) to give compoud **12** (yield: 32%) and **13** (yield: 42%) respectively (The ratio is approached 3:4) as brown solids. **4-(5-hydroxy-1-(4-hydroxyphenyl)-2-ferrocenylpent-1-en-1-yl)phenyl acetate (12).** ^{1}H NMR (300 MHz, Acetone-*d*$_6$) δ 8.30 and 8.28 (s, 1H, OH), 7.27 (d, *J* = 8.7 Hz, 2H, C_6H_4), 7.14 – 6.85 (m, 4H, C_6H_4), 6.73 (d, *J* = 8.6 Hz, 2H, C_6H_4), 4.14 and 4.13 (s, 5H, C_5H_5), 4.10 – 4.06 (m, 2H, C_5H_4), 4.02 – 3.99 (m, 1H, C_5H_4), 3.99 – 3.95 (m, 1H, C_5H_4), 3.49 – 3.41 (m, 3H, CH_2 and OH), 2.76 - 2.66 (m, 2H, CH_2), 2.25 and 2.23 (s, 3H, OCH_3), 1.73 - 1.65 (m, 2H, CH_2) [*isomer present in 7:9*]; ^{13}C NMR (75 MHz, Acetone-*d*$_6$) δ 169.60 (C=O), 156.91 (C, C_6H_4), 150.37 (C, C_6H_4), 143.66 and 143.34 (C, C_6H_4), 138.21 (C, C_6H_4), 136.90 and 136.78 (C=C), 136.55 and 136.46 (C=C), 131.74 (CH, C_6H_4), 131.51 (CH, C_6H_4), 131.34 (CH, C_6H_4), 130.99 (CH, C_6H_4), 122.43 (2 CH, C_6H_4), 116.03 (2 CH, C_6H_4), 88.06 and 87.88 (C, C_5H_4), 70.15 (2 CH, C_5H_4), 70.00 (5 CH, C_5H_5), 68.87 (2 CH, C_5H_4), 60.50 (OCH_2), 34.87 (CH_2), 32.14 (CH_2), 21.03 (2 CH_3); MS-CI *m/z*: 497 $(M+H)^+$; HPLC tR = 1.76 min, 98.4%; Anal. Calc. for $C_{29}H_{28}FeO_4$•1/2H_2O: C 68.92, H 5.78, found: C 68.85, H 5.77. **(5-hydroxy-2-ferrocenylpent-1-ene-1,1-diyl)bis(4,1-phenylene) diacetate (13).** ^{1}H NMR (300 MHz, Acetone-*d*$_6$) δ 7.30 (d, *J* = 8.6 Hz, 2H, C_6H_4), 7.13 (d, *J* = 2.0 Hz, 2H, C_6H_4), 7.10 (d, *J* = 2.1 Hz, 2H, C_6H_4), 7.01 (d, *J* = 8.7 Hz, 2H, C_6H_4), 4.16 (s, 5H, C_5H_5), 4.12 – 4.08 (m, 2H, C_5H_4), 4.01 – 3.97 (m, 2H, C_5H_4), 3.57 – 3.39 (m, 3H, CH_2 and OH), 2.78 - 2.66 (m, 2H, CH_2), 2.25 (s, 3H, OCH_3), 2.23 (s, 3H, OCH_3), 1.77 - 1.63 (m, 2H, CH_2); ^{13}C NMR (75 MHz, Acetone-*d*$_6$) δ 169.60 (2 C=O), 150.37 (2 C, C_6H_4), 143.03 (C, C_6H_4), 142.70 (C, C_6H_4), 137.92 (C=C), 137.24 (C=C), 131.54 and 131.10 (4 CH, C_6H_4), 122.53 and 122.45 (4 CH, C_6H_4), 87.43 (C, C_5H_4), 70.23 (2 CH, C_5H_4), 70.04

(5 CH, C_5H_5), 69.12 (2 CH, C_5H_4), 62.46 (OCH_2), 34.81 (CH_2), 32.10 (CH_2), 21.09 (2 CH_3); MS-CI *m/z*: 556 $(M+NH_4)^+$; HPLC tR = 1.79 min, 97.6%; Anal. Calc. for $C_{31}H_{30}FeO_5$•1/2H_2O: C 68.02, H 5.71, found: C 68.25, H 5.82.

Method **B**. Ac_2O (0.12 ml, 1.3 mmol) was added to a solution of **8** (0.3 g, 0.66 mmol) dissolved in CH_2Cl_2 (10 mL) at 0 °C. TMSOTf (6 µL, 0.04 mmol) was added and the mixture was stirred overnight at room temperature. The mixture was washed with water and brine, dried, and evaporated. The residue was purified by column chromatography on silica gel eluting with hexane:EtOAc (2:1) to give compoud **8** (yield: 32%), **10** (yield: 30%) and **11** (yield: 28%) respectively (The ratio is approached 1:1:1) as brown solids. **5,5-bis(4-hydroxyphenyl)-4-ferrocenylpent-4-en-1-yl acetate (8).** ^{1}H NMR (300 MHz, Acetone-*d*$_6$) δ 8.32 (s, 1H, OH), 8.27 (s, 1H, OH), 7.05 (d, *J* = 8.6 Hz, 2H, C_6H_4), 6.87 (d, *J* = 8.6 Hz, 2H, C_6H_4), 6.81 (d, *J* = 8.6 Hz, 2H, C_6H_4), 6.70 (d, *J* = 8.6 Hz, 2H, C_6H_4), 4.13 (s, 5H, C_5H_5), 4.07 (m, 2H, C_5H_4), 3.95 (m, 4H, C_5H_4 and CH_2), 2.77 – 2.67 (m, 2H, CH_2), 1.91 (s, 3H, CH_3), 1.84 - 1.21 (m, 2H, CH_2); ^{13}C NMR (75 MHz, Acetone-*d*$_6$) δ 170.90 (C=O), 156.73 (2 C, C_6H_4), 139.75 (C, C_6H_4), 137.29 (C, C_6H_4), 136.93 (C=C), 134.30 (C=C), 131.75 (2 CH, C_6H_4), 131.28 (2 CH, C_6H_4), 115.90 (2 CH, C_6H_4), 115.80 (2 CH, C_6H_4), 88.28 (C, C_5H_4), 69.88 (5 CH, C_5H_5), 68.71 (4 CH, C_5H_4), 64.52 (CH_2), 31.80 (CH_2), 30.61 (CH_2), 20.84 (CH_3); MS-EI *m/z*: 496 $[M]^{+\bullet}$; HPLC tR = 1.82 min, 99.4%; Anal. Calc. for $C_{29}H_{28}FeO_4$•1/2H_2O: C 68.92, H 5.78, found: C 68.84, H 5.69. **4-(5-acetoxy-1-(4-hydroxyphenyl)-2-ferrocenylpent-1-en-1-yl)phenyl acetate (10).** ^{1}H NMR (300 MHz, Acetone-*d*$_6$) δ 8.48 and 8.44 (s, 1H, OH), 7.32 – 7.03 (m, 4H, C_6H_4), 7.02 – 6.86 (m, 2H, C_6H_4), 6.78 (m, 2H, C_6H_4), 4.14 (s, 5H, C_5H_5), 4.09 (m, 2H, C_5H_4), 3.99 – 3.88 (m, 4H, CH_2 and C_5H_4), 2.81 – 2.61 (m, 2H, CH_2), 2.25 and 2.23 (s, 3H, CH_3), 1.92 and 1.91 (s, 3H, CH_3), 1.74 - 1.85 (m, 2H, CH_2) [*isomer present in 1:1*]; ^{13}C NMR (75 MHz, Acetone-*d*$_6$) δ 171.03 and 170.98 (C=O), 169.65

and 169.59 (C=O), 157.65 and 156.97 (C, C_6H_4), 150.24 (C, C_6H_4), 143.45 and 143.13 (C, C_6H_4), 138.82 (C, C_6H_4), 136.64 and 136.25 (C=C), 135.84 and 135.49 (C=C), 131.70 (CH, C_6H_4), 131.49 (CH, C_6H_4), 131.32 (CH, C_6H_4), 131.00 (CH, C_6H_4), 122.41 and 122.26 (2 CH, C_6H_4), 116.09 and 116.02 (2 CH, C_6H_4), 87.73 and 87.53 (C, C_5H_4), 69.97 (5 CH, C_5H_5), 68.98 and 68.95 (4 CH, C_5H_4), 64.53 and 64.45 (CH_2), 31.94 and 31.75 (CH_2), 30.62 and 30.53 (CH_2), 21.04 (CH_3), 20.87 (CH_3); MS-EI *m/z*: 538 $[M]^{+\bullet}$; HPLC tR = 1.89 min, 98.2%; Anal. Calc. for $C_{31}H_{30}FeO_5 \bullet 1/2H_2O$: C 68.02, H 5.71, found: C 68.13, H 5.81. **(5-acetoxy-2-ferrocenylpent-1-ene-1,1-diyl)bis(4,1-phenylene) diacetate (11).** ^{1}H NMR (300 MHz, Acetone-*d*$_6$) δ 7.30 (d, *J* = 8.4 Hz, 2H, C_6H_4), 7.13 (d, *J* = 2.2 Hz, 2H, C_6H_4), 7.10 (d, *J* = 2.4 Hz, 2H, C_6H_4), 7.01 (d, *J* = 8.6 Hz, 2H, C_6H_4), 4.16 (s, 5H, C_5H_5), 4.14 – 4.09 (m, 2H, C_5H_4), 4.00 – 3.89 (m, 4H, CH_2 and C_5H_4), 2.77 - 2.68 (m, 2H, CH_2), 2.25 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 1.91 (s, 3H, CH_3), 1.81 (m, 2H, CH_2); ^{13}C NMR (75 MHz, Acetone-*d*$_6$) δ 169.53 (2 C=O), 163.41 (C=O), 150.45 (2 C, C_6H_4), 146.37 (C, C_6H_4), 142.47 (C, C_6H_4), 137.82 (C=C), 136.95 (C=C), 131.47 (2 CH, C_6H_4), 131.08 (2 CH, C_6H_4), 122.59 (2 CH, C_6H_4), 122.48 (2 CH, C_6H_4), 87.04 (C, C_5H_4), 70.04 (5 CH, C_5H_5), 69.17 (4 CH, C_5H_4), 64.39 (CH_2), 31.89 (CH_2), 30.36 (CH_2), 21.00 (2 CH_3), 20.82 (CH_3); MS-EI *m/z*: 580 $[M]^{+\bullet}$; HPLC tR = 1.96 min, 95.4%; Anal. Calc. for $C_{33}H_{32}FeO_6 \bullet 2/5H_2O$: C 67.45, H 5.63, found: C 67.42, H 5.61.

5,5-Bis(4-hydroxyphenyl)-4-ferrocenylpent-4-en-1-yl benzoate (9). 9 was prepared as the same procedure of **8**, giving a brown solid, yield 82%. ^{1}H NMR (300 MHz, Acetone-*d*$_6$) δ 8.47 (s, 1H, OH), 8.43 (s, 1H, OH), 7.93 (d, *J* = 7.1 Hz, 2H, C_6H_5), 7.64 (t, *J* = 7.3 Hz, 1H, C_6H_5), 7.54 (t, *J* = 7.4 Hz, 2H, C_6H_5), 7.09 (d, *J* = 8.5 Hz, 2H, C_6H_4), 6.85 (dd, *J* = 16.8, 8.5 Hz, 4H, C_6H_4), 6.71 (d, *J* = 8.5 Hz, 2H, C_6H_4), 4.23 (t, *J* = 6.1 Hz, 2H, CH_2), 4.10 (s, 5H, C_5H_5), 4.09 – 4.04 (m, 2H, C_5H_4), 4.00 - 4.96 (m, 2H, C_5H_4), 2.90 – 2.80 (m, 2H, CH_2), 2.01 - 1.90 (m, 2H, CH_2); ^{13}C NMR (75 MHz, Acetone-*d*$_6$) δ 169.08 (C=O), 156.85 (2 C, C_6H_4),

143.61 (C, C_6H_5), 139.84 (C, C_6H_4), 137.25 (C, C_6H_4), 136.94 (C=C), 134.25 (C=C), 133.77 (CH, C_6H_5), 131.73 (2 CH, C_6H_4), 131.28 (2 CH, C_6H_4), 130.19 (2 CH, C_6H_5), 129.41 (2 CH, C_6H_5), 115.98 (2 CH, C_6H_4), 115.81 (2 CH, C_6H_4), 88.22 (C, C_5H_4), 69.88 (5 CH, C_5H_5), 68.70 (4 CH, C_5H_4), 65.48 (CH_2), 32.26 (CH_2), 30.50 (CH_2); MS-EI *m/z*: 558 $[M]^{+\bullet}$; HPLC tR = 1.99 min, 100%; Anal. Calc. for $C_{34}H_{30}FeO_4 \bullet 3/4H_2O$: C 71.40, H 5.55, found: C 71.73, H 6.04.

4,4'-(5-Chloro-2-ferrocenylpent-1-ene-1,1-diyl)diphenol (3). 3 was prepared as the same procedure of **14**, giving a brown solid, yield 95%. ^{1}H NMR (300 MHz, Acetone-*d*$_6$) δ 8.24 (s, 1H, OH), 8.23 (s, 1H, OH), 7.03 (d, *J* = 8.6 Hz, 2H, C_6H_4), 6.83 (d, *J* = 8.6 Hz, 2H, C_6H_4), 6.78 (d, *J* = 8.6 Hz, 2H, C_6H_4), 6.66 (d, *J* = 8.6 Hz, 2H, C_6H_4), 4.10 (s, 5H, C_5H_5), 4.06 – 4.01 (m, 2H, C_5H_4), 3.95 – 3.90 (m, 2H, C_5H_4), 3.47 (t, *J* = 6.6 Hz, 2H, CH_2), 2.82 – 2.70 (m, 2H, CH_2), 1.82 (m, 2H, CH_2); ^{13}C NMR (75 MHz, Acetone-*d*$_6$) δ 157.5 (2 C, C_6H_4), 140.7 (C, C_6H_4), 138.0 (C, C_6H_4), 137.6 (C=C), 134.7 (C=C), 132.5 (2 CH, C_6H_4), 132.0 (2 CH, C_6H_4), 116.7 (2 CH, C_6H_4), 116.5 (2 CH, C_6H_4), 89.1 (C, C_5H_4), 70.7 (2 CH, C_5H_2), 70.6 (5 CH, C_5H_5), 69.5 (2 CH, C_5H_4), 46.7 (CH_2), 35.1 (CH_2), 33.8 (CH_2); MS-CI *m/z*: 473 $(M+H)^+$; HR-MS ESI calc. for $C_{27}H_{25}ClFeO_2$: 472.0892, found: 472.0887.

4,4'-(5-Mercapto-2-ferrocenylpent-1-ene-1,1-diyl)diphenol (4). To a stirred solution of **3** (0.34 g, 0.72 mmol) in MeCN (8 mL) were successively added thiourea (274 mg, 3.6 mmol) and KI (120 mg, 0.72 mmol) at room temperature. The reaction was then refluxed overnight, after which the mixture was evaporated under reduced pressure. The residue was dissolved in a 1/2 (v/v) mixture of EtOH (3 mL) and H_2O (6 mL) to which, powered NaOH (0.7 g, 18 mmol) was added portionwised at room temperature. The reaction was then refluxed for one day. The resulting solution was carefully acidified (at 0 ºC) to pH ~ 2 by the addition of concentrated (37%) HCl and then extracted with EtOAc for three times. The combined

organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluting with PE:EtOAc (2:1) to give 4,4'-(5-mercapto-2-ferrocenylpent-1-ene-1,1-diyl)diphenol as a brown solid 0.3 g, yield: 89%. ^{1}H NMR (300 MHz, Acetone-d_6) δ 8.33 (s, 1H, OH), 8.28 (s, 1H, OH), 7.06 (d, J = 8.5 Hz, 2H, C_6H_4), 6.87 (d, J = 8.5 Hz, 2H, C_6H_4), 6.82 (d, J = 8.5 Hz, 2H, C_6H_4), 6.70 (d, J = 8.5 Hz, 2H, C_6H_4), 4.13 (s, 5H, C_5H_5), 4.07 (m, 2H, C_5H_4), 3.99 – 3.94 (m, 2H, C_5H_4), 2.75 (t, J = 8.0 Hz, 2H, CH_2), 2.61 (t, J = 7.1 Hz, 2H, CH_2), 1.90 – 1.80 (m, 2H, CH_2); ^{13}C NMR (75 MHz, Acetone-d_6) δ 156.73 (2 C, C_6H_4), 139.74 (C, C_6H_4), 137.33 (C, C_6H_4), 136.96 (C=C), 134.41 (C=C), 131.82 (2 CH, C_6H_4), 131.33 (2 CH, C_6H_4), 115.98 (2 CH, C_6H_4), 115.79 (2 CH, C_6H_4), 88.49 (C, C_5H_4), 70.07 (2 CH, C_5H_2), 69.91 (5 CH, C_5H_5), 68.74 (2 CH, C_5H_4), 39.39 (CH_2), 34.46 (CH_2), 30.92 (CH_2); MS-EI *m/z*: 470 $[M]^{+\bullet}$; HPLC tR = 1.96 min, 100%; Anal. Calc. for $C_{27}H_{26}FeO_2S$•3/4H_2O: C 67.01, H 5.73, found: C 67.10, H 5.71.

2-((5,5-Bis(4-hydroxyphenyl)-4-ferrocenylpent-4-en-1-yl)oxy)isoindoline-1,3-dione (5). To a stirred solution of 2-hydroxyisoindoline-1,3-dione (0.133 g, 0.8 mmol) in MeCN (6 mL) K_2CO_3 (115 mg, 0.8 mmol) was added at room temperature. The reaction was then refluxed for 1 hr, after which **3** (230 mg, 0.4 mmol) and KI (70 mg, 0.4 mmol) was added successively. The reaction was then refluxed for overnight. The mixture was diluted with EtOAc, washed with water and brine, dried, and evaporated. The residue was purified by column chromatography on silica gel eluting with PE:EtOAc (2:1) to give 2-((5,5-bis(4-hydroxyphenyl)-4-ferrocenylpent-4-en-1-yl)oxy)isoindoline-1,3-dione as a brown solid 0.16 g, yield: 67%. ^{1}H NMR (300 MHz, Acetone-d_6) δ 8.27 (s, 1H, OH), 8.24 (s, 1H, OH), 7.87 (t, J = 2.6 Hz, 4H, C_6H_4), 7.06 (d, J = 8.6 Hz, 2H, C_6H_4), 6.87 (d, J = 8.6 Hz, 2H, C_6H_4), 6.77 (d, J = 8.6 Hz, 2H, C_6H_4), 6.70 (d, J = 8.6 Hz, 2H, C_6H_4), 4.13 (s, 5H, C_5H_5), 4.13 (m, 2H, CH_2), 4.09 – 4.04 (m, 2H, C_5H_4), 4.03 – 3.96 (m, 2H, C_5H_4), 2.90 (m, 2H, CH_2), 1.95 – 1.81 (m, 2H,

CH_2); ^{13}C NMR (75 MHz, Acetone-*d*$_6$) δ 164.14 (2 C=O), 156.74 (2 C, C_6H_4), 139.70 (C, C_6H_4), 137.31 (C, C_6H_4), 136.88 (C=C), 135.44 (2 CH, C_6H_4), 134.46 (C=C), 131.82 (2 CH, C_6H_4), 131.22 (2 CH, C_6H_4), 130.09 (2 C, C_6H_4), 123.93 (2 CH, C_6H_4), 115.96 (2 CH, C_6H_4), 115.75 (2 CH, C_6H_4), 88.58 (C, C_5H_4), 78.96 (CH_2), 70.08 (2 CH, C_5H_4), 69.91 (5 CH, C_5H_5), 68.73 (2 CH, C_5H_4), 31.80 (CH_2), 30.63 (CH_2); MS-EI *m/z*: 599 $[M]^{+\bullet}$; HPLC tR = 1.87 min, 99.69%; Anal. Calc. for $C_{35}H_{29}FeNO_5$•9/10H_2O: C 68.28, H 5.04, N 2.28, found: C 68.61, H 5.54, N 2.12.

Propan-2-one O-(5,5-bis(4-hydroxyphenyl)-4-ferrocenylpent-4-en-1-yl) oxime (6). N_2H_4-H_2O (0.01 ml, 0.2 mmol) was added to a solution of **5** (0.06 g, 0.1 mmol) dissolved in a mixture of CH_3OH (3 mL) and acetone (3 mL) at room temperature. The mixture was stirred for 1 hr and concentrated. The residue was purified by column chromatography on silica gel eluting with PE:EtOAc (2:1) to give propan-2-one O-(5,5-bis(4-hydroxyphenyl)-4-ferrocenylpent-4-en-1-yl) oxime as a brown solid, the yield: 80%. ^1H NMR (300 MHz, Acetone-*d*$_6$) δ 8.24 (s, 1H, OH), 8.21 (s, 1H, OH), 7.05 (d, *J* = 8.5 Hz, 2H, C_6H_4), 6.87 (d, *J* = 8.6 Hz, 2H, C_6H_4), 6.80 (d, *J* = 8.6 Hz, 2H, C_6H_4), 6.70 (d, *J* = 8.6 Hz, 2H, C_6H_4), 4.11 (s, 5H, C_5H_5), 4.08 – 4.03 (m, 2H, C_5H_4), 3.97 – 3.93 (m, 2H, C_5H_4), 3.88 (t, *J* = 6.1 Hz, 2H, CH_2), 2.76 – 2.65 (m, 2H, CH_2), 1.80 (m, 8H, CH_2 and 2 CH_3); ^{13}C NMR (75 MHz, Acetone-*d*$_6$) δ 156.72 (C=N), 156.62 (C, C_6H_4), 153.84 (C, C_6H_4), 139.35 (C, C_6H_4), 137.44 (C, C_6H_4), 137.09 (C=C), 134.94 (C=C), 131.75 (2 CH, C_6H_4), 131.25 (2 CH, C_6H_4), 115.86 (2 CH, C_6H_4), 115.78 (2 CH, C_6H_4), 88.43 (C, C_5H_4), 73.44 (CH_2), 70.00 (2 CH, C_5H_4), 69.85 (5 CH, C_5H_5), 68.62 (2 CH, C_5H_4), 32.13 (CH_2), 31.26 (CH_2), 21.74 (CH_3), 15.37 (CH_3); MS-CI *m/z*: 510 $(M+H)^+$; HPLC tR = 1.88 min, 100%; Anal. Calc. for $C_{30}H_{31}FeNO_3$•1/2H_2O: C 69.50, H 6.22, N 2.70, found: C 69.81, H 6.45, N 2.75.

Methyl 2-(((5,5-bis(4-hydroxyphenyl)-4-ferrocenylpent-4-en-1-yl)oxy)carbamoyl) benzoate (7). 5 (95 mg, 0.16 mmol) was dissolved in CH_3OH (4 mL) and the mixture was stirred for 4 days and then concentrated. The residue was purified by column chromatography on silica gel eluting with PE:EtOAc (2:1) to give methyl 2-(((5,5-bis(4-hydroxyphenyl)-4-ferrocenylpent-4-en-1-yl)oxy)carbamoyl)benzoate as a brown solid, yield: 68%. ^{1}H NMR (300 MHz, Acetone-*d*$_6$) δ 10.22 (s, 1H, NH), 8.28 (s, 1H, OH), 8.23 (s, 1H, OH), 7.86 (d, *J* = 6.0 Hz, 1H, C_6H_4), 7.61 (dd, *J* = 10.9, 7.8 Hz, 2H, C_6H_4), 7.49 (d, *J* = 8.6 Hz, 1H, C_6H_4), 7.08 (d, *J* = 7.6 Hz, 2H, C_6H_4), 6.87 (d, *J* = 7.9 Hz, 2H, C_6H_4), 6.80 (d, *J* = 8.5 Hz, 2H, C_6H_4), 6.70 (d, *J* = 8.5 Hz, 2H, C_6H_4), 4.15 (s, 5H, C_5H_5), 4.06 (m, 2H, C_5H_4), 4.02 (m, 2H, C_5H_4), 3.96 (m, 2H, CH_2), 3.82 (s, 3H), 2.82 (m, 2H, CH_2), 1.91 – 1.78 (m, 2H, CH_2); ^{13}C NMR (75 MHz, Acetone-*d*$_6$) δ 166.80 (C=O), 157.16 (C=O), 156.74 (2 C, C_6H_4), 139.41 (C, C_6H_4), 137.39 (C, C_6H_4), 137.03 (C=C), 136.10 (C, C_6H_4), 135.88 (C, C_6H_4), 134.84 (C=C), 132.69 (2 CH, C_6H_4), 131.80 (2 CH, C_6H_4), 131.30 (2 CH, C_6H_4), 130.52 (CH, C_6H_4), 129.21 (CH, C_6H_4), 115.94 (2 CH, C_6H_4), 115.77 (2 CH, C_6H_4), 88.49 (C, C_5H_4), 75.26 (CH_2), 70.10 (2 CH, C_5H_4), 69.91 (5 CH, C_5H_5), 68.70 (2 CH, C_5H_4), 52.61 (OCH_3), 30.98 (CH_2), 30.62 (CH_2); MS-EI *m/z*: 631 $[M]^{+\bullet}$; HPLC tR = 1.66 min, 99.74%; Anal. Calc. for $C_{36}H_{33}FeNO_6 \bullet H_2O$: C 66.57, H 5.43, N 2.16, found: C 66.43, H 5.57, N 2.11.

Chemical Oxidation of 4-(5-hydroxy-1-(4-hydroxyphenyl)-2-ferrocenylpent-1-en-1-yl)-phenyl acetate (12). Freshly made Ag_2O (0.22 g, 0.9 mmol) was added to a solution of **12** (0.09 g, 0.26 mmol) in 10 ml of acetone. The dark grey mixture obtained changed to be dark red suspension about 5 minutes later. The reaction was monitored by TLC until complete conversion of the starting material (40 minutes). Filtration was followed by removal of the solvent under reduced pressure to give **12-QM** as a dark red solid 80 mg, yield 90%. ^{1}H NMR (300 MHz, Acetone-*d*$_6$) δ 8.80 (dd, *J* = 10.4, 2.7 Hz, 1H, CH=C), 7.32 (d, *J* = 9.0 Hz, 1H,

C_6H_4), 7.24 (d, *J* = 8.3 Hz, 1H, C_6H_4), 7.11 (d, *J* = 8.2 Hz, 1H, C_6H_4), 6.75 (d, *J* = 8.7 Hz, 1H, C_6H_4), 6.62 (dd, *J* = 10.2, 2.7 Hz, 1H, CH=C), 6.30 (dd, *J* = 10.4, 2.2 Hz, 1H, CH=C), 6.04 (dd, *J* = 10.2, 2.2 Hz, 1H, CH=C), 4.33 (s, 1H, C_5H_4), 4.28 (s, 1H, C_5H_4), 4.22 (s, 5H, C_5H_5), 4.20 (m, 1H, C_5H_4), 4.18 – 4.11 (m, 1H, OCH_2), 4.06 (s, 1H, C_5H_4), 3.98 (m, 1H, OCH_2), 2.40 – 2.30 (m, 1H, CH_2), 2.28 (s, 3H, CH_3), 2.26 (m, 1H, CH_2), 2.23 – 2.08 (m, 2H, CH_2); ^{13}C NMR (75 MHz, Acetone-*d*$_6$) δ 186.43 (C=O), 169.53 (C=O), 164.61 (C, C_6H_4), 151.57 (C, C_6H_4), 140.67 (2 C, CH=C), 138.11 (2 C, CH=C), 137.12 (C=C), 130.79 (C=C), 130.71 (CH, C_6H_4), 128.65 and 128.56 (2 CH, C_6H_4), 122.01 and 121.83 (CH, C_6H_4), 88.83 (C, C_5H_4), 85.35 (C), 69.91 (5 CH, C_5H_5), 69.27 (2 CH, C_5H_4), 68.10 (OCH_2), 67.82 (2 CH, C_5H_4), 39.24 (CH_2), 25.69 (CH_2), 21.02 (CH_3); MS-EI *m/z*: 494 $[M]^{+\bullet}$.

Chemical Oxidation of 4-(5-acetoxy-1-(4-hydroxyphenyl)-2-ferrocenylpent-1-en-1-yl)-phenyl acetate (10). Freshly made Ag_2O (0.11 g, 0.3 mmol) was added to a solution of **10** (0.04 g, 0.07 mmol) in 3 ml of acetone. The dark grey mixture obtained changed to be dark red suspension about 5 minutes later. The reaction was monitored by TLC until complete conversion of the starting material (20 minutes). Filtration was followed by removal of the solvent under reduced pressure to give **10-QM** as a dark red solid 80 mg, yield 90%. ^1H NMR (300 MHz, Acetone-*d*$_6$) δ 7.69 – 7.59 (m, 3H, CH=C and C_6H_4), 7.52 (dd, *J* = 10.2, 2.7 Hz, 1H, CH=C), 7.35 – 7.27 (m, 2H, C_6H_4), 6.45 – 6.36 (m, 3H, CH=C), 4.30 (s, 1H, CH_2), 4.17 (m, 3H, C_5H_4 and CH_2), 4.11 (m, 2H, C_5H_4), 3.99 (s, 5H, C_5H_5), 2.46 – 2.35 (m, 2H, CH_2), 2.28(s, 3H, CH_3), 2.01 (s, 3H, CH_3); ^{13}C NMR (75 MHz, Acetone-*d*$_6$) δ 186.83 (C=O), 170.97 (C=O), 169.46 (C=O), 156.02 (C, C_6H_4), 153.16 (C, C_6H_4), 140.07(C, C_6H_4), 139.65 (CH, CH=C), 138.13 (CH, CH=C), 135.90 (C=C), 132.88 (2 CH, C_6H_4), 130.81 (C=C), 129.93 (CH, CH=C), 129.47 (CH, CH=C), 127.35 (CH, CH=C), 122.87 (2 CH, C_6H_4), 87.39 (C,

C_5H_4), 70.22 (5 CH, C_5H_5), 69.11 (2 CH, C_5H_4), 68.08 (CH, C_5H_4), 67.58 (CH, C_5H_4), 63.81 (OCH_2), 30.37 (CH_2), 21.02 (CH_3), 20.92 (CH_3); MS-EI *m/z*: 536 $[M]^{+\bullet}$.

Biological evaluations

Lipophilicity. Measurements of the octanol/water partition coefficient (log Po/w) were made by the HPLC technique according to a method described previously [38, 39]. Measurement of the chromatographic capacity factors (k) for each molecule was done at various concentrations in the range of 95–75% methanol containing 0.25% (v/v) 1-octanol and an aqueous phase consisting of 0.15% (v/v) n-decylamine in the buffering agent MOPS (3-morpholinopropane-1-sulfonic acid, prepared in 1-octanol saturated water) adjusted to pH 7.4. These capacity factors (k′) are extrapolated to 100% of the aqueous component given the value of k′w. The log Po/w is obtained by the formula log Po/w = 0.13418 + 0.98452 log k′.

Culture cells. Stock solutions (10 mM) of the compounds to be tested were prepared in DMSO and were kept at -20°C in the dark. Serial dilutions in Dulbecco's modified eagle medium (DMEM) without phenol red/Glutamax I were prepared just prior to use. DMEM without phenol red, Glutamax I and fetal bovine serum were purchased from Life Technologies; MDA-MB-231 cells were obtained from ATCC (Manassas, VA, USA). 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, 1% kanamycin, 1% 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. At different days (D4, D5), the protein content of each well was quantified by methylene blue staining as

follows: cell monolayers were fixed for 1 h at room temperature with methylene blue (1mg 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 (4 wells for each concentration) was measured at 655 nm with a Biorad spectrophotometer. The results are expressed as the percentage of proteins versus the control. Two independent experiments, run in quadruplicate, were performed.

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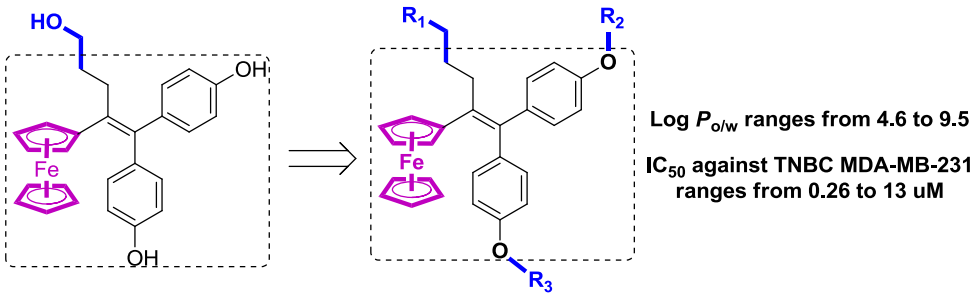
Graphical Abstract



Highlights

- 11 New hydroxypropyl ferrociphenol compounds synthesized and characterized.
- The lipophilicity of ferrocenyl compounds was investigated.
- Evaluation of cytotoxic activity of ferrocenyl compounds on TNBC MDA-MB-231 carried out.