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Letters

Modification of the Estrogenic Properties of Diphenols by the Incorporation of Ferrocene. Generation of Antiproliferative Effects in Vitro

Anne Vessières, Siden Top, Pascal Pigeon, Elizabeth Hillard, Leila Boubeker, Daniela Spera, and Gérard Jaouen*

Laboratoire de Chimie et Biochimie des Complexes Moléculaires, UMR CNRS 7576, Ecole Nationale Supérieure de Chimie de Paris, 11, rue Pierre et Marie Curie, 75231 Paris Cedex 05, France

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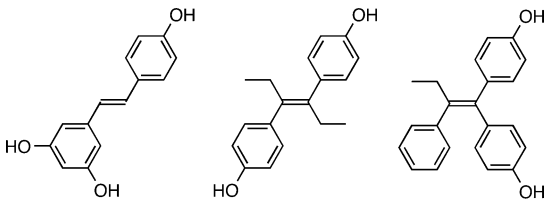
Abstract: We report here the synthesis and the strong and unexpected antiproliferative effect of the organometallic diphenolic compound 1,1-bis(4′-hydroxyphenyl)-2-ferrocenyl-but-1-ene (**4**) on both hormone-dependent (MCF7) and -independent (MDA-MB231) breast cancer cells (IC_{50} = 0.7 and 0.6 μM). Surprisingly, **6** [1,2-bis(4′-hydroxyphenyl)-2-ferrocenyl-but-1-ene], the regioisomer of **4**, shows only a modest effect on these cell lines. This pertinent organometallic modification seems to trigger an intracellular oxidation of the structurally favorable compound **4**, leading to the generation of a potent cytotoxic compound.

Small polyphenolic molecules such as stilbenes, flavonoids, proanthocyanidines, and their derivatives are found throughout the vegetable world (for example, in grapes, green tea, and cocoa) and are recognized for their beneficial effects.[1,2] Although they are most well-known for their antioxidant action[3] against free radicals which have been associated with diseases related to aging (certain cancers, cardiac, ocular, and degenerative problems, etc.),[4−6] these entities also act as specific modulators of certain protein functions.[4,6]

For example, resveratrol **1** (3,4′,5-trihydroxy-*trans*-stilbene), present in red wine, is an endocrine modulator that recognizes estrogen receptors alpha and beta (ERα and ERβ),[7,8,9] as well as a free radical scavenger.[10,11]



1: Resveratrol **2** : DES **3**

Another structurally similar stilbene, diethylstilbestrol (DES), **2** [(*E*)-3,4-(bis)(4′-hydroxyphenyl)-3-hexene], can act as a powerful estrogen via ERα and ERβ.[12] This compound was clinically prescribed from 1938 to 1971 for the prevention of pregnancy complications, but was subsequently prohibited due to its many adverse effects, including carcinogenicity and teratogenicity.[13] In addition, the offspring of women treated by DES showed an increased incidence of reproductive and genital abnormalities.[14] Among synthetic diphenolethylenes, compound **3**, (1,1-bis(4′-hydroxyphenyl)-2-phenylbut-1-ene, likewise presents estrogenic effects,[15,16] a feature that we have confirmed in our biological experiments to be discussed below.

As a part of our program investigating the biological activity of organometallic moieties tethered to selective estrogen receptor modulators, (SERMs),[17−19] we have modified compounds **2** and **3** by incorporating a ferrocenyl substituent. Ferrocene is lipophilic, compact, stable in nonoxidizing media, and has shown some antitumor potential, albeit at rather high concentrations (10^{-4} M), when oxidized to the ferrocenium ion.[20,21] Due to the receptor-mediated properties of **2** and **3**, we hoped that their structures could act as vectors to facilitate the introduction of the potentially cytotoxic ferrocenyl group to cells containing the estrogen receptor. We show here, for the first time, that these known estrogenic properties can be profoundly modified, indeed reversed, by the grafting of an organometallic moiety to the organic diphenol skeleton. Furthermore, the addition of a ferrocenyl group generates surprising in vitro antiproliferative effects on breast cancer cells classified as hormone-independent (ERα negative). These results are

* To whom correspondence should be addressed. Phone: +33-1-43-26-95-55. Fax: +33-1-43-26-00-61. E-mail: gerard-jaouen@enscp.fr.

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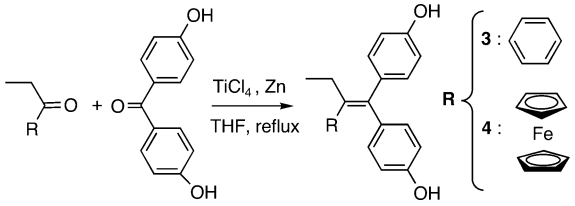
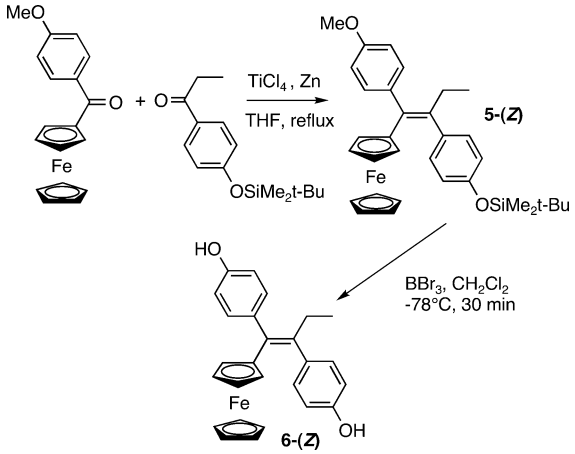
Scheme 1. Synthesis of Diphenols **3** and **4** by McMurry Cross-Coupling



Scheme 2. Synthesis of Diphenol (Z)-**6** via McMurry Cross-Coupling



very significant as treatment of hormone-independent breast tumors suffers from few effective therapeutic solutions.

We found that a McMurry coupling (Zn, $TiCl_4$, THF) between the commercially available propiophenone and 4,4′-dihydroxybenzophenone allowed the direct synthesis of **3** with a yield, after purification and recrystallization, of 73% (Scheme 1). This synthesis is simpler than the five-step procedure described previously for **3**.[15] This strategy was also used to obtain **4** in 53% yield.[19]

Finally, the McMurry coupling of 4-*tert*-butyldimethylsiloxyphenyl ethyl ketone and 4-methoxyphenyl ferrocenyl ketone provided compound **5** in 74% yield, as shown in Scheme 2. The coupling reaction was stereoselective; **5** was obtained mainly as the *Z* isomer (*Z*/*E*: 93/7). The pure (Z) isomer was separated by crystallization and identified by 2D NMR techniques (COSY, NOESY). The two protecting groups, methoxy and 4-*tert*-butyldimethylsiloxy, were eliminated simultaneously by BBr_3, yielding (*Z*)-**6**, in 97% yield.

The relative binding affinity (RBA) values for the organic diphenol **3** and the organometallic diphenols **4** and **6** with respect to ERα (from lamb uterine cytosol and purified) and ERβ (purified) are reported in Table 1. All of the products showed an acceptable recognition for both forms of the receptor. The RBA values found for ERα from uterus and purified were similar for **4** and **6** (values between 9.6 and 5.4) while for **3** this value is significantly higher for purified ERα (6.8 versus 47.5). This is probably due to higher nonspecific interactions of **3** in cytosol. The RBA values were at least twice as high for ERβ than for ERα with **3** showing a RBA value similar to that of estradiol. The lipophilicity was found to rise upon addition of the ferrocene unit, as expected, but the difference between the compounds is limited and thus could not account for the differences in biological activity

Table 1. Relative Binding Affinities (RBAs) for ERα (cytosol and purified), ERβ (purified) and Lipophilicity of the Diphenols Derivatives

	RBA (DMSO, 0 °C, 3 h 30 min)[a,b]			
compound	ERα (uterus)	ERα (purified)	ERβ	log $P_{o/w}$[c]
3	6.8 ± 0.9	47.5 ± 5.8	101 ± 5	4.4
4	9.6 ± 0.9	8.6 ± 1.5	16.3 ± 1.5	5.0
6	5.4 ± 0.9	8.6 ± 1.3	31 ± 1	4.6

[a] RBA value of E_2, the compound of reference is by definition equal to 100%. [b] Mean of at least two experiments. [c] Measured by reversed-phase HPLC.

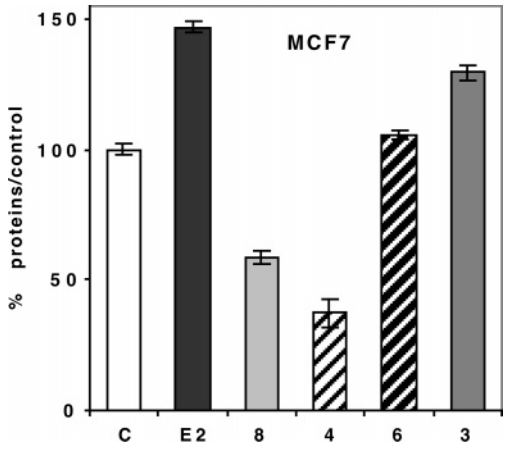


Figure 1. Effect of 1 μM of **8** (OH-tamoxifen), **4**, **6**, **3** and of 10 nM of estradiol (E_2) (C = control) on MCF7 cells (breast cancer cell line, ERα-positive) after 5 days of culture in medium with phenol red. Representative data of one experiment performed twice with similar results (eight measurements ± limits of confidence; $P = 0.1$, $t = 1.895$).

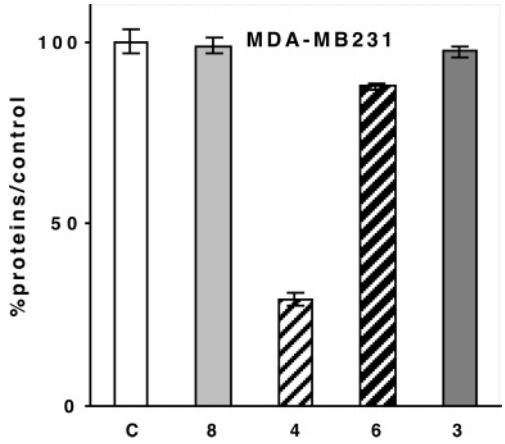


Figure 2. Effect of 1 μM of **8** (OH-Tamoxifen), of the ferrocenyl diphenols **4** and **6**, and of the organic phenol **3** (C = control) on MDA-MB231 cells (breast cancer cell line, ERα-negative) after 5 days of culture in medium without phenol red. Representative data of one experiment performed twice with similar results (eight measurements ± limits of confidence; $P = 0.1$, $t = 1.895$).

The proliferative/antiproliferative effects of **3**, **4**, and **6** at a concentration of 1 μM were studied on hormone dependent (MCF7) and hormone-independent (MDA-MB231) breast cancer cell lines. The results are represented in Figures 1 and 2. For the hormone-dependent MCF7 breast cancer cells, in medium with phenol red, which is best suited for the expression of an antiestrogenic behavior, the two organometallic diphenols **4** and **6** showed rather different behavior. Whereas **6** displayed a slight proliferative effect, as one would predict from

its diphenol ethylene structure, complex **4** showed a remarkably strong antiproliferative effect ($IC_{50} = 0.7$ μM). This antiproliferative effect was even stronger than that observed for 4-hydroxytamoxifen, the antiestrogen of reference. The strong antiproliferative effect of **4** was also observed in the hormone independent MDA-MB231 breast cancer cell line ($IC_{50} = 0.6$ μM), while compound **6** showed a very weak antiproliferative effect. Ferrocene alone has been previously shown to have no effect on these two cell lines.[19] Finally, in a medium without phenol red which is best suited for the expression of an estrogenic behavior, the proliferative effect of **3** and **6** is stronger (respectively 173% and 145% of the control with E_2 at 257%) while **4** still shows an antiproliferative effect although less marked (73% of the control).

Diphenol compounds **1**−**3** are known to be estrogenic, contributing to cell proliferation through interactions with the ER. In our experiments, compound **3** indeed showed a proliferative effect on the hormone dependent cells and had no significant effect on the hormone-independent cells. Conversely, ferrocene compounds, after oxidation to ferrocenium species, have been shown to be cytotoxic via generation of OH radical. These opposing properties have been combined to yield the ferrocenyl diphenols **4** and **6**. Therefore there is competition between the two effects: a proliferative effect from the estrogenicity of the diphenol and an antiproliferative/cytotoxic effect from the ferrocenyl substituent. Compound **4** yielded strong antiproliferative effects in *both* hormone-dependent and -independent breast cancer cells, suggesting that the cytotoxic activity of the ferrocenyl group surpasses that of the estrogenic proliferative effect of the diphenol moiety. Surprisingly, **6** shows only a weak proliferative effect on MCF7 (ER positive) cells and a modest antiproliferative effect on MDA-MB231 (ER negative) cells. It is thus clear that the presence of a ferrocene group is necessary, but not sufficient, for the generation of antiproliferative effects, and that the positioning of the oxidizable ferrocenyl group is important. It should be noted that the increased activity of compound **4** cannot be solely attributed to higher receptor affinity, as the values for **4** and **6** are very similar for ERα, while the RBA is actually higher for **6** than for **4** for ERβ.

There are two notable structural differences between compounds **4** and **6**. First, one of the two phenol groups is necessarily always oriented trans to the ferrocene group in compound **4**, while there is a cis relationship between the ferrocene and phenol in compound **6**. Second, the two phenol groups share the same carbon atom in **4**, while, in compound **6**, one phenol group resides on each of the alkene carbon atoms. As is clear from the disparate biochemical results for these compounds, cytotoxicity does not arise from the ferrocenyl group in isolation, and we must therefore consider ferrocene's influence on the organic portion of the molecule. It should be noted that the oxidation of SERMs such as tamoxifen and raloxifene to quinoids is a recognized pathway to cytotoxicity,[22−24] and it is suggestive that the loss of two hydrogen atoms from compound **4** could theoretically yield quinone methide **7**, Figure 3. It is possible that, in **4**, the initial and easy oxidation of the ferrocenyl group to the ferrocenium radical cation promotes a transformation of the phenol

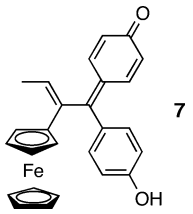


Figure 3. Putative cytotoxic quinone methide compound **7**.

group, and preliminary electrochemical results strongly support this hypothesis.[25] The transformation of a phenol to a quinoid via ferrocene oxidation has been reported in the literature,[26] and current work to isolate oxidation products of compounds **4** and **6** is underway. The cytotoxicity of **7** would be the consequence of its sensitivity to nucleophilic attack as shown in other quinoid examples.[22−24]

The proposed oxidative intracellular activation of compound **4** is further suggested by the observation that ERβ plays a role in controlling redox processes in the cell[27] on the level of quinonases and in the mitochondria.[28] In terms of the cytotoxic effect, the role of ERα can be excluded, as this receptor is not present in the MDA-MB231 cell line. However, ERβ is present in both the MCF7 and MDA-MB231 cells and may mediate the cytotoxic effect[29] although this has not been proved. Should ERβ, in fact, play a role in cell death, compounds such as **4** would be a much needed weapon against currently intractable ER negative breast tumors which are frequently ERβ positive.[30] Whatever is the actual mechanism, it remains that the powerful observed antiproliferative effects of compound **4** supply an impetus for the continued work in the burgeoning area of bioorganometallic chemistry.

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Supporting Information Available: Synthesis and complete characterization of **3**, **4**, and **6**; experimental procedure for biochemical experiments. This material is available free of charge via the Internet at http//:pubs.acs.org

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