Antitumor Agents

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Ferrocene-Mediated Proton-Coupled Electron Transfer in a Series of Ferrocifen-Type Breast-Cancer Drug Candidates**

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The archetypical selective estrogen receptor modulator (SERM) tamoxifen, (*Z*)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethanamine, is widely prescribed for patients diagnosed with hormone-dependent breast cancer, that is, cancer in which the estrogen receptor (ER) is present (ER(+)). The antiproliferative action in the breast of the hydroxylated form of tamoxifen (OH-Tam) arises primarily from an antiestrogenic effect caused by competitive binding to the ER, which represses estradiol-mediated DNA transcription.[1] Unfortunately, some breast-cancer cells are resistant to tamoxifen because they either do not express ER (classified as ER(−)) or have developed resistance following prolonged exposure to the drug. To fight both ER(+) and ER(−) breast cancer, we have focused on the creation of dual-function drugs that combine antiestrogenicity and estrogen-independent cytotoxicity in the same molecule.

The discovery of the inorganic complex cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) has revolutionized the treatment of testicular cancer[2] and has led to increased research into organometallic complexes as antitumor agents.[3] Our strategy, therefore, has been to incorporate a potentially cytotoxic moiety, ferrocene, by grafting it onto the tamoxifen skeleton. A series of these molecules, called "hydroxyferrocifens" by analogy, have been prepared (Scheme 1) and their antiproliferative effects have been studied in ER(+) and ER(−) breast-cancer cell lines.[4] In the ER(+) MCF7 cells, the hydroxyferrocifens behave similarly to OH-Tam in that they express an antiestrogenic effect, although they also possess a cytotoxic component.[5] However, the hydroxyferrocifens present a remarkable anti-

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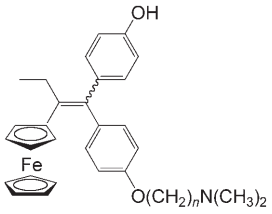


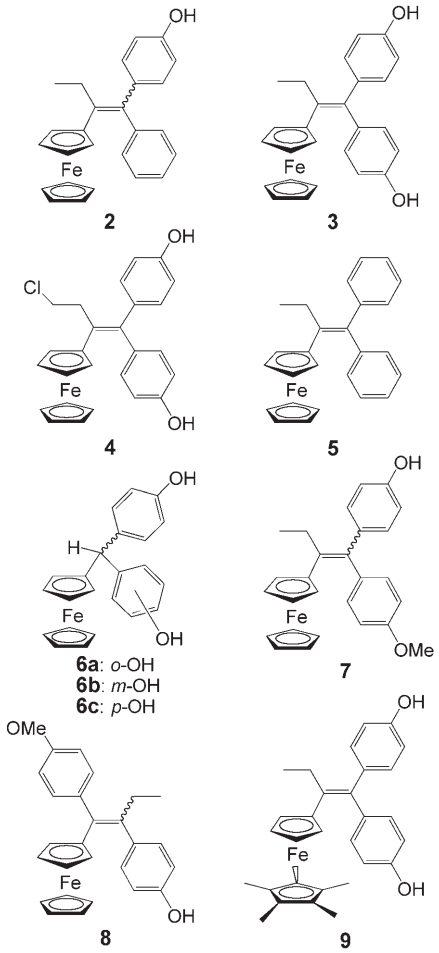
Z + *E*
1a: *n* = 2
1b: *n* = 3
1c: *n* = 5

Scheme 1. Hydroxyferrocifens.

proliferative behavior on the ER(−) MDA-MB231 cell line, with IC_{50} values on the order of 0.5 μM, whereas OH-Tam is completely inactive at this concentration range; this effect can be attributed only to cytotoxicity of the ferrocenyl complexes. Thus, the observed antiproliferative effect of the hydroxyferrocifens can be divided into two components: antiestrogenic in ER(+) cells and cytotoxic in both ER(+) and ER(−) cells; the cytotoxicity likely arises from the oxidation in situ of Fe^{2+} to Fe^{3+} ions.[6]

Although hydroxytamoxifen does not show cytotoxic activity towards MCF7 or MDA-MB231 breast-cancer cell lines at sub-micromolar concentrations, a cytotoxic effect has been observed at higher concentrations (IC_{50} = 34 μM for the ER(−) MDA-MB231 cell line).[7] The fact that hydroxyferrocifens show cytotoxic activity at much lower concentrations than hydroxytamoxifen suggests that the ferrocene group somehow modulates this effect. We have synthesized several compounds based on the hydroxyferrocifen structure (Scheme 2) and assessed their antiproliferative effects in vitro to further understand the role of ferrocene and the structure–activity relationship in the cytotoxicity of these molecules in both hormone-dependent (MCF7) and hormone-independent (MDA-MB231) breast-cancer cell lines. In the MDA-MB231 cell line, an antiproliferative effect was observed for **1**, **2**, **3**, and **4**, whereas the remaining compounds showed little or no effect towards the MDA-MB231 cells. These results are compiled in Table 1.

As cell death has been linked with the oxidation in situ of phenol groups and the ferrocenium cation, we have used electrochemistry to monitor the reactivity of these compounds in a model environment. First, the cyclic voltammograms were obtained in methanol, and then pyridine was added to determine the reactivity of the electrochemically generated cations towards nucleophiles; the standard potentials ($E°$) are shown in Table 2. In pure MeOH, the compounds exhibited voltammograms that are essentially due to the ferrocene/ferrocenium (Fc/Fc^+) redox couple, often followed by the irreversible oxidation of the phenolic moiety.[8] However, two distinct types of electrochemical behavior were observed when pyridine was added. Very little change was observed in the cyclic voltammograms upon the addition of pyridine for the compounds that showed slight or no cytotoxic effects in vitro. However, the addition of pyridine caused two major changes to the voltammograms of the biologically active compounds. Firstly, the Fc/Fc^+ couple became irreversibile at low scan rates, which indicates



Scheme 2. Hydroxyferrocifen-type molecules used for electrochemical and bioactivity screening.

Table 1: Biological and electrochemical results for the ferrocenyl derivatives.

Compound	Antiproliferative effect on MDA-MB231 cells [% of inhibition][a]	Cytotoxic effect?[b]	Electron transfer?[c]
OH-Tam	none[g]	–	n.a.
1a	53[g]	+	Y
1b	77[g]	+	Y
1c	80[g]	+	Y
2	35[h]	+	Y
3	71[i]	+	Y
4[d]	49[j]	+	Y
6a[e]	26[k]	slight	N
6b[e]	18[k]	slight	N
6c[e]	16[k]	slight	N
7[d]	16[j]	slight	Y
8[d]	3[j]	–	N
9[f]	none[j]	–	N

[a] Defined as the effect of 1 μM of the compound after 6 days of culturing relative to the control, set by definition at 100%. [b] The cytotoxic effect is considered positive (+) for an antiproliferative effect (% inhibition) greater than 30%, slight for an effect 10–30%, and negative (−) for an effect less than 10%. [c] Observed by the loss of the ferrocenium reduction wave in the presence of pyridine. [d] Prepared as described in Ref. [17]. [e] Prepared as described in Ref. [18]. [f] Prepared as described in Ref. [19]. [g] Ref. [4c]. [h] Ref. [4b]. [i] Ref. [16]. [j] Ref. [17]. [k] Ref. [20].

Table 2: Standard oxidation potentials in methanol and methanol/pyridine (6:1 v/v).

Compound	Solvent	E°, (Fc/Fc^+) [V vs. SCE][a]	Second-wave peak potential [V vs. SCE][a]
ferrocene	MeOH	0.417(2)	–
	MeOH/py	*0.432(2)*	–
1a	MeOH	0.395(3)	very broad
	MeOH/py	*0.393(3)*	*0.482(3)*
1b	MeOH	0.401(2)	0.92(3)
	MeOH/py	*0.420(3)*	*0.513(3)*
1c	MeOH	0.385(2)	0.93(4)
	MeOH/py	*0.383(3)*	*0.476(2)*
2	MeOH	0.397(2)	0.96(3)
	MeOH/py	*0.423(4)*	*0.510(3)*
3	MeOH	0.373(2)	0.88(3)
	MeOH/py	*0.387(3) (sh)*	*0.480(2)*
4	MeOH	0.413(2)	0.87(4)
	MeOH/py	*0.460(2)*	*0.584(3)*
5	MeOH	0.421(3)	–
	MeOH/py	*0.442(3)*	–
6a	MeOH	0.379(3)	not observed
	MeOH/py	*0.408(3)*	*not observed*
6b	MeOH	0.404(3)	not observed
	MeOH/py	*0.428(3)*	*not observed*
6c	MeOH	0.391(2)	not observed
	MeOH/py	*0.414(2)*	*not observed*
7	MeOH	0.387(2)	0.93(3)
	MeOH/py	*0.407(2) (sh)*	*0.513(3)*
8	MeOH	0.39(2)	not observed
	MeOH/py	*0.461(2)*	*1.03(4)*
9	MeOH	0.153(3)	not observed
	MeOH/py	*0.164(2)*	*0.83(4)*

[a] The uncertainty of last digit given in parentheses.

that the ferrocenium cation was scavenged chemically prior to the reverse sweep. The loss of reversibility was accompanied by an increase of the Fc oxidation wave, which is indicative of secondary electron transfer following a chemical reaction of the primary cation radical. Secondly, any phenol-oxidation waves present in the cyclic voltammogram in MeOH underwent a dramatic cathodic shift. An example of this phenomenon for **3** is shown in Figure 1. The fact that all of the compounds displaying significant cytotoxic effects in vitro also showed reactivity with pyridine in the electrochemical experiments (Table 1) validates the use of this electrochemical model.

The ferrocenium group appears to play the role of an intramolecular electron acceptor in the cytotoxicity in vitro, as evidenced by the transience of the electrochemically produced Fe^{3+} center in the presence of pyridine. The inertness of the non-phenolic compound **5** to pyridine in our electrochemical model system shows that a phenolic group is necessary for electron transfer to take place (Figure 2). Furthermore, the fact that chemical reduction of the Fe^{3+} centers was not observed for the nonconjugated compounds **6a–c** suggests that the electron-transfer process is intramolecular and occurs through a slight coupling in the molecular π system. A further electrochemical experiment to support this interpretation was performed using a mixture of ferrocene and 1,1-di-*p*-hydroxyphenyl-2-phenylbut-1-ene in pyridine/MeOH. The Fc/Fc^+ couple was conventional and reversible, and the phenol-oxidation wave was observed at a potential typical of the hydroxyferrocifens when pyridine is not present. These results confirm the conclusion that the electron transfer from the phenol moiety to the ferrocenyl group is intramolecular and relies on weak electron delocalization within the π system. It is important to note, however, that the π electron delocalization in the initial cation radical is quite weak. This is attested to by the observation that the standard oxidation potentials of the Fc/Fc^+ couple of the hydroxyfer-

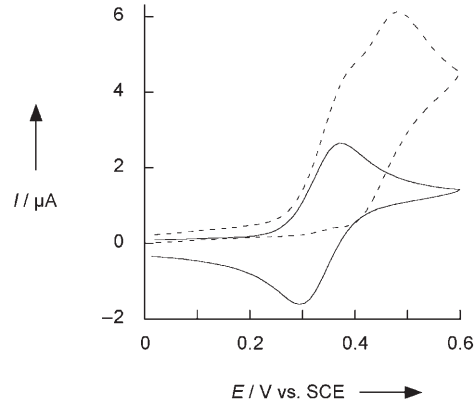


Figure 1. Cyclic voltammograms of **3** (2 mM in 0.1 M Bu_4NBF_4/MeOH) in the absence (solid line) and presence (dashed line) of pyridine in 1:6 volume ratio. Scan rate 0.5 Vs^{-1}. Pt electrode of 0.5 mm diameter. The oxidations of the phenol groups in MeOH occur at 0.88 and 1.17 V versus SCE (not shown).

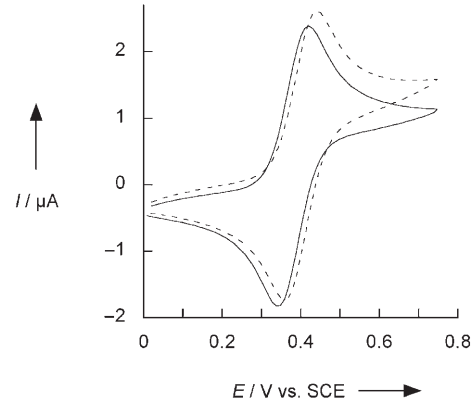


Figure 2. Cyclic voltammograms of **5** in the absence (solid line) and presence (dashed line) of added pyridine. Same conditions as for Figure 1.

rocifens **2** (0.397 V), **3** (0.373 V), and **7** (0.387 V) are not very much lower than that of the non-hydroxylated analogue **5** (0.421 V). The standard oxidation potentials of the non-conjugated compounds **6a** (0.379 V), **6b** (0.404 V), and **6c** (0.391 V) suggest that any stabilization of the radical species in the hydroxyferrocifens is due to inductive effects, rather than resulting from extended electron-delocalization effects, and that the cation radical is primarily ferrocene-centered. However, weak delocalization is sufficient to allow significant interaction between the phenolic and ferrocenium substituents during intramolecular electron transfer. Thus, as soon as an adequate base is present, a relatively fast intramolecular electron transfer may occur, thereby leading to the oxidation of the phenolic moiety becoming easier because of its displacement by the reaction of the phenoxy cation with the pyridine base.[9–11]

Pyridine could conceivably contribute to the irreversible electron-transfer process either by proton abstraction or nucleophilic attack. To rule out a nucleophilic mechanism, the nucleophilic, but nonbasic, electrolyte Me_4NCl was substituted for pyridine and Bu_4NBF_4. In this experiment, the ferrocene moiety displayed a reversible redox event, which suggests that pyridine acts as a base, not as a nucleophile. Further experiments have shown that the pKa value of the added base influences the reaction pathway. For example, dimethylformamide (DMF) and pyrazole are too weak to deprotonate the phenol moiety in the activated molecule, as the cyclic voltammograms are not qualitatively different from those in MeOH, whereas pyridine ($pK_a = 5.14$)[12a] and imidazole ($pK_a = 6.95$)[12b] are sufficiently basic. In this respect, it may be envisioned that DNA bases act as proton scavengers in vitro. Alternatively, the relatively high pK_a values (>7) of α-NH_2 groups present in most peptides suggest that these groups could also act as bases in vitro in proteinic environments.[12c] However, this point was not tested in this work because the low solubility of the ferrocenyl compounds precludes electrochemical experiments in aqueous solution.

Finally, the importance of the position of the ethyl group with respect to the ferrocene moiety was assessed by comparing the electrochemical results for the geometric isomers **7** and **8**. Only the compound in which the ethyl group and the ferrocene group are attached to the same carbon atom (**7**) showed the characteristic irreversible Fc oxidation and shift of the phenol oxidation wave upon addition of pyridine (Figure 3). These results strongly suggest the participation of the ethyl group in the mechanism.

In view of the role of the ferrocene moiety as an intramolecular hole reservoir,[10] the conjugated π system, the basic action of pyridine, and the interesting constraints on the placement of the ethyl group, we propose the mechanism shown in Scheme 3 for the generation of the quinone methide species most likely responsible for the cytotoxicity in the MDA-MB231 cell line. The ferrocene moiety is electrochemically oxidized, and the electron may to a small extent be delocalized over the π system. This imparts a partial positive charge to the hydroxy group, thus acidifying the proton, which may then be easily abstracted by pyridine.[11] The small delocalization is almost not observable in the absence of

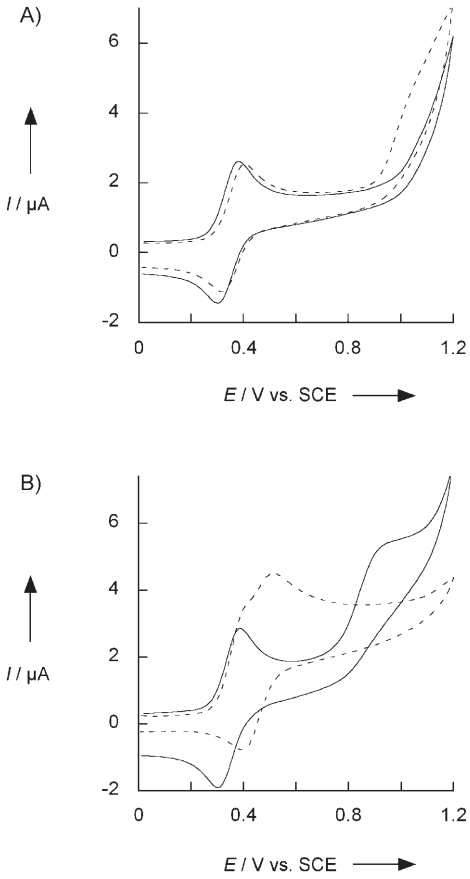
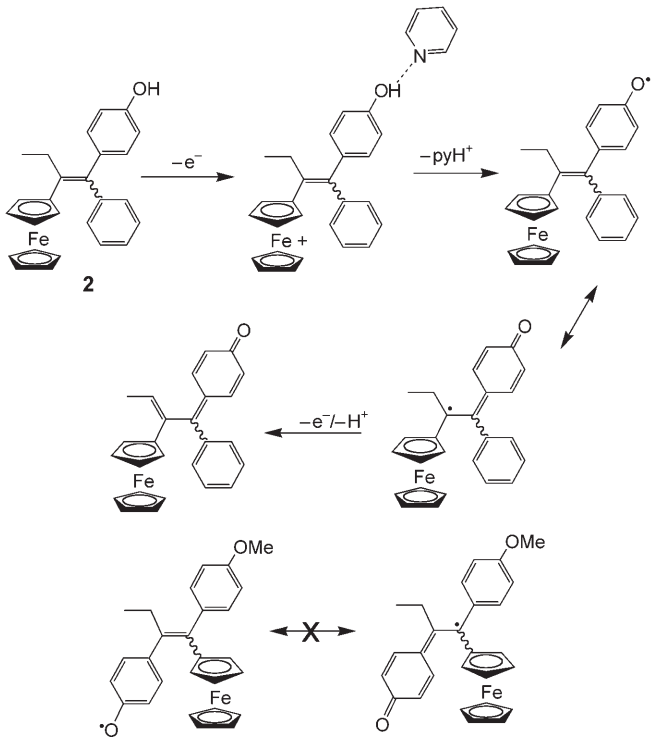


Figure 3. Cyclic voltammograms of A) **8** and B) **7** in the absence (solid line) and presence (dashed line) of added pyridine. Same conditions as for Figure 1.

pyridine, as evidenced by the small change in the oxidation potential of the ferrocene moiety. In the presence of pyridine, the transient existence of an adduct may encourage a strong coupling in the deprotonated transition state. The resulting phenoxy radical species can be described by many mesomeric structures, one of the most stable being the quinoid in which the radical is positioned on the α carbon atom with respect to the ferrocene moiety. This species may then be oxidized, observed as the second oxidation wave at slightly higher potential than the ferrocene oxidation wave. This second oxidation may then be followed by another proton abstraction from the ethyl group, which results in a quinone methide structure. This mechanism is the only possibility that we can envision to account for the difference in reactivity between **7** and **8**. In the latter case, there is no possibility to stabilize the α-carbenium radical through back-bonding into the iron d orbital, and simultaneously abstract an adjacent proton to form a double bond (see bottom of Scheme 3).[13]

The potential cell-damaging pathways for tamoxifen include metabolism to electrophilic *o*-quinones, quinone methides, or carbocations, which may form adducts with DNA, GSH, or proteins.[14] We believe that the biologically active ferrocifen-type compounds undergo similar oxidative metabolism, which is enhanced by the easier oxidation of ferrocene in comparison to phenol. Thus, the ferrocene



Scheme 3. Proposed mechanism for transformation of **2** to a quinone methide species in the presence of pyridine. As indicated in the lower part of the Scheme, compound **8** cannot follow the same mechanism.

moiety may be oxidized far from the biological target and may thus serve as an intramolecular carrier of the hole while the activated drug finds its way to its target. Furthermore, the small degree of coupling between the ferrocenium-centered radical and the oxo radical yields a relatively high energy transition state, which may stabilize the ferrocenium species on its path to the target.[15] In this way the ferrocene acts as a kind of intramolecular oxidation "antenna" and may oxidize the phenol group through a intramolecular pathway, thus producing cytotoxic species in milder oxidizing conditions. Only when an adequate base is present will the electron transfer proceed, presumably in a concerted fashion with deprotonation, to yield the neutral phenoxy radical. DMF, for example, is not basic enough to allow such electron transfer. Therefore, the drug will only be activated to form the quinone methide species in the presence of basic species such as DNA nucleobases or peptides, the harm of which may lead to cell death.

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