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Selective Estrogen Receptor Modulators in the Ruthenocene Series.

Synthesis and Biological Behavior

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A series of ruthenocene derivatives, 1-[4-(O(CH₂)_{*n*}N(CH₃)₂)phenyl]-1-(4-hydroxyphenyl)-2-ruthenocenylobut-1-ene, with *n* = 2-5, based on the structure of the breast cancer drug tamoxifen has been prepared. These compounds were obtained, via a McMurry cross-coupling reaction, as a mixture of *Z* and *E* isomers that could not be separated by HPLC. The relative binding affinity values for estrogen receptor α (ER α) for *n* = 2 and 3 were very high (85 and 53%) and surpassed even that of hydroxytamoxifen (38.5%), the active metabolite of tamoxifen. Ruthenocene derivatives act as anti-estrogens as effective (*n* = 2) or slightly more effective (*n* = 3-5) than hydroxytamoxifen on ER α -positive breast cancer cell lines but, unlike ferrocifens, do not show antiproliferative effects on ER α -negative breast cancer cell lines. Electrochemical studies showed that the ruthenocifen radical cations are unstable, which may account for this behavior. Some of these compounds could be useful as radiopharmaceuticals for ER α -positive breast cancer tumors.

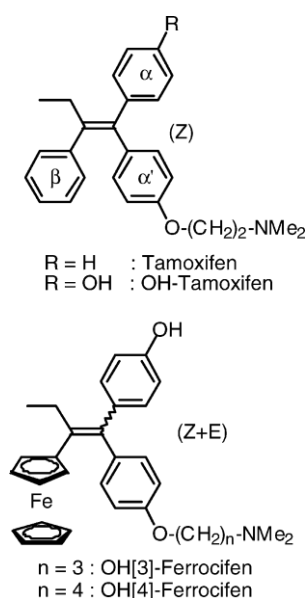
1. Introduction

The development of new selective estrogen receptor modulators (SERMs) is a field of active

study in the fight against breast cancer, a disease that strikes about one in eight¹ women in the Western world. Approximately two-thirds of these cases are classified as hormone-dependent, in which the estrogen receptor is present, (ER-positive). For these patients, an anti-estrogen treatment is prescribed, commonly the drug tamoxifen, the prodrug of the active metabolite hydroxytamoxifen, OH-Tam (Chart 1). However, tamoxifen is not effective against the remaining third of breast cancer cases classified as hormone-independent, where the estrogen receptor is not detected, (ER-negative). For these reasons, the discovery of new molecules that are active toward these tumors would supply a much-needed innovation in an area where the current therapeutic arsenal is deficient.²

The activity of OH-Tam in the breast has been illuminated by recent developments in the complex endocrinology of breast cancer.³ A second estrogen receptor, $ER\beta$, was discovered in 1996.⁴ Tumors which had been classified as ER-negative due to the lack of $ER\alpha$ have been shown to contain $ER\beta$,⁵ which may be important in the proliferation of tamoxifen-resistant tumors, although the role of this receptor is still poorly understood.⁶

Chart 1. Tamoxifen and Ferrocifen Derivatives



The conformational effect of the bioligand on the ER has been elucidated by the publication of the X-ray structure of the ligand binding domain crystallized with both

estrogens and anti-estrogens in the site.⁷ These studies underscore the importance of OH-Tam's hydroxy group for receptor affinity, due to its interaction with Asp351,^{2b} and of the basic chain in changing the secondary structure of the protein, thus modifying transcriptional activity. Two different mechanisms for gene expression have been discovered. The estrogen receptor/bioligand complex can dimerize and interact directly with the DNA at the estrogen response element (ERE), or in the activated protein pathway (AP1), the monomer can interact with two proteins (Jun and Fos) to form a complex that binds to DNA.⁸ The recruitment of coactivators or corepressors that determine proliferative or antiproliferative effects seems to be dependent upon the pathway at the DNA level, the nature of the bioligand, and the organ tissue.

Because there is currently no satisfactory therapy for ER-negative breast tumors, and because these tumors are often heterogeneous, containing both ER-positive and ER-negative cells, it is currently of great interest to develop SERMs that are effective on both cell types. Our strategy has been to couple the vector, tamoxifen, with a potentially toxic organometallic moiety. In this way, the estrogen receptors (α and β) can be targeted by the shape and the binding properties of hydroxytamoxifen, which can simultaneously deliver a cytotoxic metal unit to the cancer cell nucleus.

Using these concepts, we have previously shown that, in the ferrocifen series (Chart 1), complexes such as OH[3]-ferrocifen and OH[4]-ferrocifen are anti-estrogenic in the hormone-dependent breast cancer cell line MCF-7 due to their conformational effects on ER α , even surpassing the activity of OH-Tam. More important, however, is their efficacy on the hormone-independent breast cancer cell line MDA-MB231,⁹ likely due to the advent of a cytotoxic moiety from the oxidation of Fe(II) in ferrocene,¹⁰ possibly via a pathway that involves ER β .¹¹ Although OH-Tam and its metabolites show cytotoxic behavior at high concentrations,¹² the ferrocene compounds show a much greater activity. We are currently studying the role of the ferrocene moiety in modulating the cytotoxicity of these compounds via electrochemical means.

We now turn our attention to the ruthenocene series. Due to ruthenium's position below iron in group 8, the ionization potential of such molecules should be considerably higher when iron is replaced by ruthenium. Thus, if oxidation is in fact the necessary precursor to the cytotoxic phenomena in ER-negative breast cancer cell lines, one would expect enhanced results when ruthenium is substituted for iron. Furthermore, anticancer properties have

already been shown for a number of rutheno-arene complexes, such as Ru(III) imidazole and indazole compounds, which are currently in clinical trials.¹³ Because the activity of the latter compounds is thought to arise from the in situ reduction of Ru(III) to Ru(II),¹⁴ Sadler and co-workers have focused on the anticancer properties of Ru(II) monoarene compounds,¹⁵ and a Ru(II)-cymene complex has been shown to damage DNA.¹⁶ Finally, ruthenium is of special interest due to the availability of two γ -emitting isotopes, ⁹⁷Ru and ¹⁰³Ru, which may be useful in the radioimaging of cancer tumors.¹⁷

In this work we present the synthesis and characterization of a series of hydroxytamoxifen-ruthenocene compounds, or “hydroxyruthenocifens”, 1-[4-(O(CH₂)_nN(CH₃)₂)phenyl]-1-(4-hydroxyphenyl)-2-ruthenocenylobut-1-ene, with $n = 2-5$. We have also included the results from a series of biological tests to evaluate their antiproliferative effects against both hormone-dependent and -independent breast cancer cell lines and their suitability for use in radioimaging, as well as electrochemical and molecular mechanics studies.

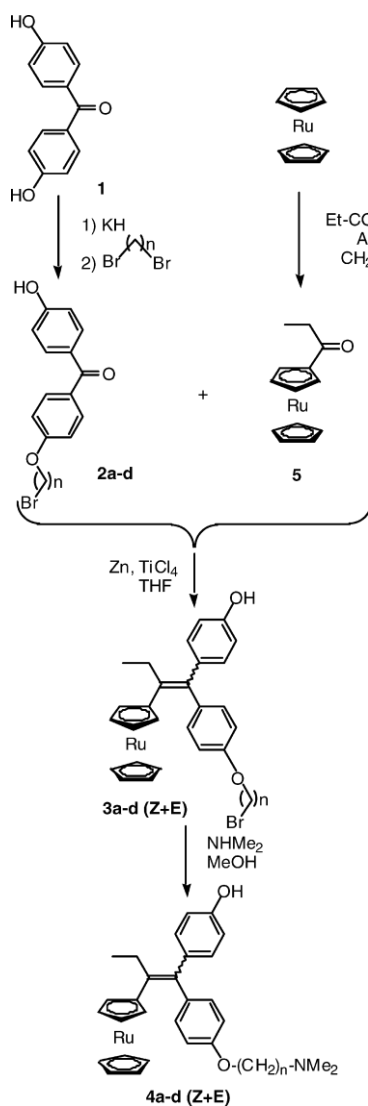
2. Results and Discussion

2.1. Synthesis. The outline of the synthetic procedure is shown in Scheme 1. As the length of the basic side chain proved to be of importance on the antiproliferative effects of the ferrocifen series, we have varied the length of the side chain from $n = 2-5$ carbon atoms in the ruthenocene series. We have found that, for the rhenium¹⁸ and iron¹⁹ series of compounds, a McMurry coupling reaction is most effective for preparing the alkene derivatives.²⁰ In this case, as with the rhenium series, we have chosen to attach the alkyl chain, in the form of a halogenated chain, to the dihydroxybenzophenones (**2a-d**) prior to the coupling reaction with the propionylruthenocene **5**. The coupling reaction was then carried out with a 2:1 ratio of bromoalkylhydroxybenzophenone to propionylruthenocene in order to diminish the yield of the homogeneous propionyl-ruthenocene coupled compound. The halogen function was then converted to a dimethylamino function in the last step.

In the first step, 4,4'-dihydroxybenzophenone **1** was monoalkylated with the appropriate halogenoalkyl chain to prepare ketones **2a-d**. This occurred by the combination of the monopotassium salt of **1**, prepared with KH, with the dibromoalkyl derivative. McMurry coupling of the corresponding ketone **2a-d** with the ruthenocene ketone **5**, prepared by the

action of AlCl_3 and propionyl chloride on ruthenocene, gave the alkenes **3a-d** in good yields (>70%). In the final step, the alkenes were converted to the amines **4a-d** in an autoclave by the action of dimethylamine in methanol at 60 °C.

Scheme 1. Synthesis of Hydroxyruthenocifens **4a-d** ($n = 2-5$)



The McMurry coupling reaction furnished mixtures of *Z* and *E* isomers of the bromo derivatives **3a-d**, which were obtained as oils and converted to the amino compounds **4a-d**. In both the rhenium¹⁸ and ferrocene^{9b} series, these isomers could be separated by chromatography or fractional crystallization, and while the ferrocifens interconverted rapidly in chloroform, this tendency to isomerize was not observed in the case of rhenium. The ruthenocene compounds, however, could not be separated by chromatography, as only one broad peak was

seen in both semipreparative and analytical HPLC. This phenomenon can be explained by the rapid interconversion between *Z* and *E* isomers in the ruthenium series. We have suggested, in a previous study of the ferrocifens,^{9b} that the intermediate species in the isomerization mechanism is the α -carbenium ion formed by protonation of the double bond, and thus, greater stability of this species would enhance isomerization. An evaluation of the pK_{R+} values for a series of organometallic compounds showed that the stability of $[\text{CpRu}(\eta^5\text{-C}_5\text{H}_4\text{CH}_2)]^+$ was 2 orders of magnitude higher than that of the corresponding iron cation.²¹ Presumably, the enhanced stability of this species derives from greater back-bonding from the more diffuse ruthenium orbitals as compared with iron, and DFT calculations on similar compounds have supported this interpretation.²² Prevention of isomerization in order to evaluate the isomers separately may be accomplished through the use of fixed ring systems in the future.²³

Table 1. Relative Binding Affinities (RBAs) for ER α (cytosol) and ER β (purified) and lipophilicity (log $P_{o/w}$) of the Ruthenocene Derivatives of OH-Tamoxifen

compound ^a	ER α	ER β	log $P_{o/w}$
E2	100 ^b	100 ^b	3.5
OH-Tam	38.5 ^c	18.5 ^c	<i>Z</i> = 3.2 ^c <i>E</i> = 3.4
4a	85 \pm 4	19 \pm 0.4	4.5
4b	53 \pm 6	13 \pm 4	4.6
4c	10.6 \pm 0.2	10.9 \pm 0.1	4.7
4d	14.5 \pm 0.3	13 \pm 4	4.8

^a OH-Tam and the ruthenocifens **4a-d** are the mixture of both *Z* and *E* isomers.

Measurements were performed with stock solution in DMSO for 3 h at 0 °C. Mean of two experiments \pm range. ^b Value by definition. ^c Value from ref 9b.

2.2. Biochemical Studies. a. Measurement of the Relative Binding Affinities (RBAs) of the Complexes for the Estrogen Receptors ER α and ER β . As the separation of the *Z* and

E isomers was impossible, all the biochemical studies were carried out on the mixture of the two isomers. The RBA values obtained on the α form of the receptor were remarkably high for the complexes with the side chain with $n = 2$ and 3 (Table 1). These values are higher than the RBA value found for OH-Tam, which is unprecedented in our studies of other organometallic SERMs. The RBA values obtained for the β form were also high, and in this case, they are comparable to that found for OH-Tam.

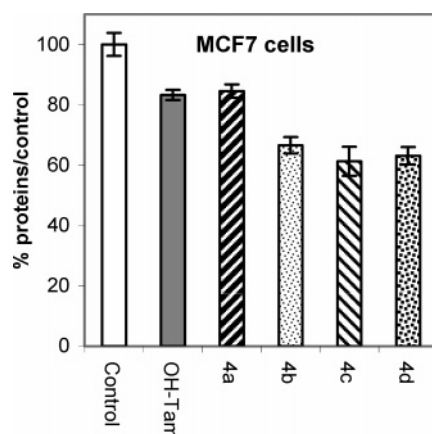


Figure 1. Antiproliferative effect of 1 μ M OH-Tam and **4a-d** on MCF7 cells (breast cancer cell line, ER α -positive) after 5 days of culture. In this experiment E₂ (1 nM) has a proliferative effect (176%; data not shown). Representative data of one experiment performed twice with similar results (eight measurements \pm limit of confidence; $P = 0.1$, $t = 1.895$).

b. Determination of log $P_{o/w}$ Values. The lipophilicity of the complexes was determined by reverse phase HPLC. Again, we were unable to separate the *E* and *Z* isomers in the ruthenocene derivatives; we always observed a broad peak in the HPLC, instead of the two distinct peaks observed for OH-Tam or the Re-Tam and Fc-Tam series. Thus, the log $P_{o/w}$ values correspond to the values of the mixtures, and they are given in Table 1. These values are higher than the value found for estradiol, and they increased slowly with the length of the side chain. These values are very similar to those found in the rhenium series.¹⁸

c. Study of the Effect of Ruthenocifen Complexes on the Proliferation of Hormone-Dependent (MCF7) and -Independent (MDA-MB231) Breast Cancer Cell Lines. On the MCF-7 (ER α -positive) breast cancer cell line the complexes have an antiproliferative

effect, as shown in Figure 1. The effect of **4a** is similar to that observed with OH-Tam, while it is stronger for the three other ruthenium compounds **4b-d**. On the MDA-MB231 (ER α -negative) breast cancer cell line, these complexes had no effect. E₂ and OH-Tam also had no effect (Figure 2). Therefore, the ruthenocene complexes of tamoxifen seem to act in an anti-estrogenic fashion similar to OH-Tam.

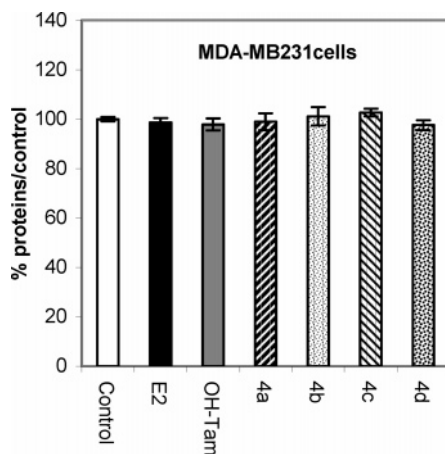


Figure 2. Effect of 1 μ M OH-Tam and **4a-d** and of E₂ (1 nM) on MDA-MB231 cells (breast cancer cell line, ER α -negative) after 6 days of culture. Representative data of one experiment performed twice with similar results (eight measurements \pm limit of confidence; $P = 0.1$, $t = 1.895$).

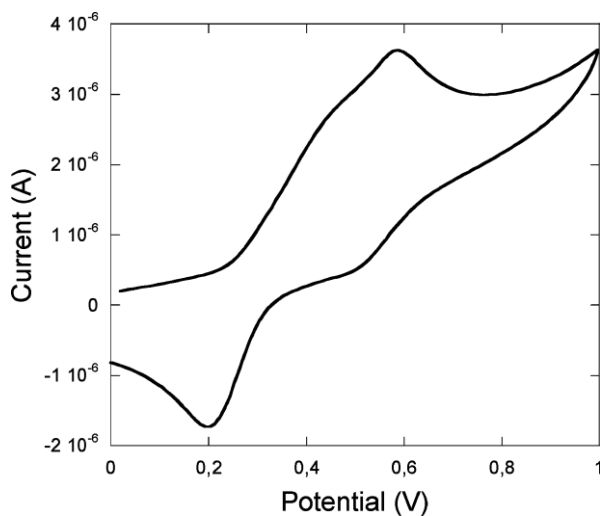


Figure 3. Cyclic voltammogram of **4b** in MeOH. Scan rate = 0.5 V/s.

2.3. Electrochemical Studies. Electrochemical studies were carried out upon ruthenocene and compounds **4a-d** in MeOH at room temperature. The behaviors of compounds **4a-d** were quite similar. A representative example, **4b**, is shown in Figure 3. At low scan rates, these compounds all yielded a poorly formed oxidation wave, consisting of a lower potential shoulder and a sharper, irreversible higher potential wave. The shoulder featured more prominently with increasing n and may arise from slow electron transfer from the amino group; this feature diminished with scan rate. In general, the potential of the main peak, which we ascribe to the Ru(II) to Ru(III) oxidation, increased with scan rate, typical of an irreversible electrochemical process. The oxidation potentials of this peak varied from about 0.55 to 0.68 V, depending on the compound and scan rate. There was an uncoupled reduction wave that ranged in potential from 0.26 to 0.13 V, depending on the compound and scan rate. These data are indicative of a fast chemical reaction following the oxidation of the ruthenocene moiety. No coupled reverse wave was observed up to 20 V/s, and thus, the kinetics could not be studied in this scan rate range. The half-life of the radical cation can be estimated to be less than 1.3 ms.

We believe that the cytotoxicity of the ferrocifen SERMs toward the ER β -positive breast cancer cell line is due to the production of radical species in situ, following the preliminary oxidation of Fe(II) to Fe(III). Genotoxicity and DNA cutting has been shown in the hydroxyferrocifen series and for other ferrocene-containing molecules.^{9a,10b,c} The mechanism of radical production is not currently understood and may be associated with ER β , the Fenton reaction, and/or the generation of reactive organic radicals or quinones via ferrocene-modulated phenol oxidation. The lack of cytotoxicity of the ruthenocene series can be rationalized along these lines. Although the oxidation potentials are reasonably accessible in vivo, the highly reactive cation likely transforms into another species before it has the opportunity to reach the target. On the other hand, the ferrocenium cation is stable in most media. It is worth noting that the ruthenocenium cation itself is generally unstable in all but the most innocent media. A reversible one-electron oxidation to [RuCp₂]⁺ was obtained only in the CH₂Cl₂/[NBu₄][B(C₆H₃(CF₃)₂)₄] solvent/electrolyte system.²⁴

2.4. Molecular Modeling Studies. Molecular mechanics studies were performed on compound **4b**, with an amino chain consisting of three carbon atoms, to determine the stability of the idealized orientation of both the *E* and *Z* isomers in the ligand binding domain of the estrogen receptor. We utilized the crystal structure of the ligand binding domain of

human ER α occupied by OH-Tam, published by Shiau et al.;^{7d} the current resolution of the human ER β structures does not permit these types of studies. Only the amino acids forming the wall of the cavity were retained, a total of 757 atoms. The hydroxytamoxifen was digitally removed and replaced with the *E* and *Z* isomers of compound **4b**. With all the heavy atoms of the cavity immobilized, a position search was carried out to determine the energetically optimal location for the bioligand. Next, the lateral chain of the amino acid Met343, which represented a steric hindrance for the organometallic moiety, was liberated. This was justified by the fact that this part of the cavity has been shown to be flexible.²⁵ An energy-minimization routine was then carried out with all of the heavy atoms immobilized except those of the bioligand and the Met343 side chain, using the Merck Molecular Force Field (MMFF). A conformational study in the cavity was also carried out to determine the best position for the organometallic moiety in relation to the rest of the molecule. This determined the ideal position for the bioligand. Next, the affinity of the bioligand for the cavity was also determined using MMFF molecular mechanics, with calculations for the bioligand-cavity combination and for the cavity and the bioligand done separately, with the latter two retaining the conformations they had in the molecular complex. This gave the ΔE energy for the reaction bioligand + cavity \rightarrow molecular complex. The result for the *Z* isomer of compound **4b** was -52 kcal/mol, while that for the *E* isomer was -10 kcal/mol. The calculation shows that affinity for the cavity is considerably higher for the *Z* isomer than for the *E* isomer. These results are shown in Figure 4.

While the calculated values for all of the organometallic SERMs that we have examined so far show a greater cavity affinity for the *Z* isomer, the disparity between the values for the ruthenium compound is quite dramatic. For example, the *Z*:*E* ratio value for OH[3]-ferrocifen is -27:-21 kcal/mol, and that for OH[3]-rhenium is -42:-36 kcal/mol. Furthermore, the *Z* isomer of tamoxifen has been shown to be the most strongly anti-estrogenic, although, because the molecule isomerizes in solution, the mixture is administered. In the case of the ruthenium compound **4b**, its rapid isomerization coupled with the results of the thermodynamic calculations suggest that the *Z* isomer is the active isomer in the biochemical studies. While RBA and enthalpy values cannot be simplistically correlated, the great stability of the *Z* isomer in the cavity supports the high observed RBA value for this compound. Our modeling studies have also shown that the hydrogen bond between the N atom of the side chain and Asp351 is 1.857 Å for (*Z*)-**4b** and 1.999 Å for (*E*)-**4b**. The

importance of this stabilizing effect has been previously recognized in related antagonist series.^{2b}

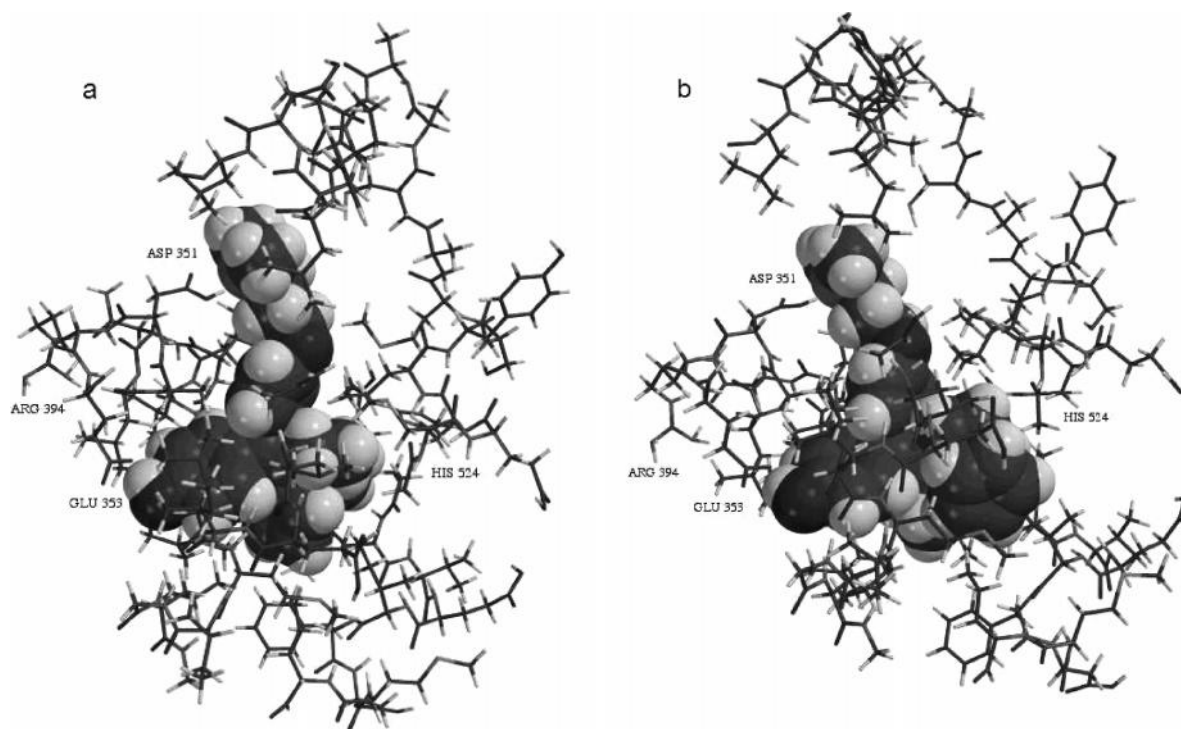


Figure 4. Representations of the (a) *E* and (b) *Z* isomer of **4b** docked in ER α .

3. Experimental Section

3.1. General Comments. Starting materials were synthesized using standard Schlenk techniques, under an argon atmosphere. Anhydrous THF and diethyl ether were distilled from sodium/benzophenone. Thin layer chromatography was performed on silica gel 60 GF254. FT-IR spectra were recorded on a BOMEM Michelson-100 spectrometer as a KBr plate. ¹H and ¹³C NMR spectra were acquired on Bruker 200 and 400 spectrometers by using CDCl₃ as a solvent. Mass spectrometry was performed on a Nermag R 10-10C spectrometer. High-resolution mass spectrometry (HRMS) was performed on a JEOL MS 700 instrument. Melting points were measured with a Kofler device. Cyclic voltamograms were obtained by utilizing an Autolab PGStat potentiostat driven by GPES software,²⁶ a platinum wire counter electrode, a 500 μ M platinum disk working electrode, and an aqueous standard calomel reference electrode. The electrolytic cell was connected to a Schlenk line, and all experiments

were performed under an inert atmosphere of argon gas. Analyte solutions were 1-2 mM in MeOH with 0.1 M Bu₄NBF₄ supporting electrolyte. Molecular modeling studies were carried out utilizing Mac Spartan Pro.²⁷

3.2 Synthesis. a. The formation of ketones **2a-d** has been reported elsewhere.¹⁸

b. The Formation of Ethyl Ruthenocenyl Ketone **5.** Ruthenocene (1.00 g, 4.32 mmol) was dissolved in dry dichloromethane (20 mL) while being stirred. Aluminum chloride (0.464 g, 3.45 mmol) was slowly added and then propionyl chloride (0.25 mL, 2.86 mmol) was added over 15 min with a syringe. The solution was stirred for 3 h and then poured in water. Dichloromethane (40 mL) was added and the solution was decanted. The aqueous layer was extracted with dichloromethane and the combination of organic layers was dried on magnesium sulfate and concentrated under reduced pressure. The residue was chromatographed on silica gel plates with dichloromethane as eluent to obtain the pure ketone **5** with a yield of 66% and a great amount of starting ruthenocene. The ketone **5** was recrystallized from ethanol. mp: 78 °C. IR: 2967 (CH₃), 2932 (CH₂), 1663 (C=O) cm⁻¹; ¹H NMR: δ 1.07 (t, 3H, CH₃, J = 7.4 Hz), 2.55 (q, 2H, CH₂, J = 7.4 Hz), 4.52 (s, 5H, C₅H₅), 4.69 (t, 2H, C₅H₄, J = 1.8 Hz), 5.04 (t, 2H, C₅H₄, J = 1.8 Hz). ¹³C NMR: δ 8.9 (CH₃), 31.9 (CH₂), 70.5 (2CH), 71.7 (5CH), 73.2 (2CH), 83.6 (C), 203.0 (CO). MS (IE, 70 eV) m/e : 288 (M⁺•).

c. The Formation of Alkenes **3a-d.** Titanium tetrachloride (1.2 mL, 11 mmol) was added dropwise to a suspension of zinc powder (1.40 g, 22 mmol) in 20 mL of THF at 0 °C. The mixture obtained was heated at reflux for 2 h. A second solution was prepared by dissolving ketone **5** (0.50 g, 1.74 mmol) and 4-bromoalkoxy-4'-hydroxybenzophenone **2a-d** (3.47 mmol) in 30 mL of THF. This latter solution was added dropwise to the first solution and the resulting mixture was heated for 2 h. After cooling to room temperature, the mixture was stirred with water and dichloromethane. The mixture was acidified with dilute hydrochloric acid and decanted. The aqueous layer was extracted with dichloromethane and the combination of organic layers was dried over magnesium sulfate. After concentration under reduced pressure, the crude product was chromatographed on silica gel plates with dichloromethane as eluent to give a pure, oily mixture of *Z* + *E* **3a-d**.

1-[4-(2-Bromoethoxy)phenyl]-1-(4-hydroxyphenyl)-2-ruthenocenylbut-1-ene, **3a.**
Yield: 76%. IR: 3420 (OH), 2964, 2925, 2861 (CH₃) cm⁻¹. MS (IE, 70 eV) m/e : 576 (M⁺•).

Z + E Isomers. ^1H NMR: δ 0.91 (t, $J = 7.6$ Hz, 3H, CH_3), 2.25 + 2.26 (q, $J = 7.6$ Hz, 2H, CH_2), 3.53 + 3.56 (t, $J = 6.0$ Hz, 2H, CH_2Br), 4.17 (t, $J = 6.0$ Hz, 2H, CH_2O), 4.23 (d, $J = 1.5$ Hz, 2H, C_5H_4), 4.33 (d, $J = 1.5$ Hz, 2H, C_5H_4), 4.45 (s, 5H, Cp), 6.56-7.02 (m, 8H, $2\text{C}_6\text{H}_4$). ^{13}C NMR: δ 15.5 (CH_3), 29.2 (CH_2), 53.7 (CH_2), 67.8 (CH_2), 69.7 (2 CH, C_5H_4), 71.1 (5 CH, Cp), 72.0 (2 CH, C_5H_4), 92.7 (C, C_5H_4), 114.1 + 114.3 (2 CH, C_6H_4), 114.8 + 115.0 (2 CH, C_6H_4), 130.5 (2 CH, C_6H_4), 131.2 (2 CH, C_6H_4), 135.8 (C), 136.8 + 137.1 (C), 137.2 (C), 137.7 + 138.0 (C), 153.9 + 154.0 (C), 156.3 + 156.4 (C).

1-[4-(3-Bromopropoxy)phenyl]-1-(4-hydroxyphenyl)-2-ruthenocenylbut-1-ene, 3b. Yield: 91%. IR: 3423 (OH), 2964, 2924, 2856 (CH_3) cm^{-1} . MS (IE, 70 eV) m/e : 590 (M^+).

Z + E Isomers. ^1H NMR: δ 0.99 (t, $J = 7.6$ Hz, 3H, CH_3), 1.45-2.15 (m, 2H, CH_2), 2.20-2.45 (m, 2H, CH_2), 3.59 + 3.61 (t, $J = 6.4$ Hz, 2H, CH_2Br), 4.05 + 4.07 (t, $J = 6.7$ Hz, 2H, CH_2O), 4.25-4.65 (m, 9H, C_5H_4 + Cp), 6.60-7.15 (m, 8H, $2\text{C}_6\text{H}_4$). ^{13}C NMR: δ 15.4 (CH_3), 30.0 (CH_2), 32.4 (CH_2), 56.6 (CH_2), 65.0 (CH_2), 69.6 (2 CH, C_5H_4), 71.0 (5 CH, Cp), 71.9 (2 CH, C_5H_4), 92.6 (C, C_5H_4), 113.7 + 113.9 (2 CH, C_6H_4), 114.7 + 114.9 (2 CH, C_6H_4), 130.3 + 130.4 (2 CH, C_6H_4), 131.1 (2 CH, C_6H_4), 135.4 (C), 136.8 (C), 137.1 (C), 137.3 (C), 154.1 (C), 156.8 (C).

1-[4-(4-Bromobutoxy)phenyl]-1-(4-hydroxyphenyl)-2-ruthenocenylbut-1-ene, 3c. Yield: 83%. IR: 3414 (OH), 2958, 2927, 2869 (CH_3) cm^{-1} . MS (IE, 70 eV) m/e : 604 (M^+).

Z + E Isomers. ^1H NMR: δ 1.01 (t, $J = 7.5$ Hz, 3H, CH_3), 1.75-2.25 (m, 4H, CH_2CH_2), 2.35 (q, $J = 7.5$ Hz, 2H, CH_2), 3.50 + 3.52 (t, $J = 6.4$ Hz, 2H, CH_2Br), 3.95 + 3.99 (t, $J = 6.7$ Hz, 2H, CH_2O), 4.30-4.38 (m, 2H, C_5H_4), 4.40 (t, $J = 1.6$ Hz, 2H, C_5H_4), 4.52 (s, 5H, Cp), 6.50-7.15 (m, 8H, $2\text{C}_6\text{H}_4$). ^{13}C NMR: δ 15.4 (CH_3), 27.8 (CH_2), 29.2 (CH_2), 29.4 (CH_2), 33.4 (CH_2), 66.6 (CH_2), 69.5 (2 CH, C_5H_4), 70.9 (5 CH, Cp), 71.8 (2 CH, C_5H_4), 92.6 (C, C_5H_4), 113.7 + 113.9 (2 CH, C_6H_4), 114.7 + 114.9 (2 CH, C_6H_4), 130.3 + 130.4 (2 CH, C_6H_4), 131.0 + 131.2 (2 CH, C_6H_4), 135.4 (C), 136.7 + 136.9 (C), 137.1 + 137.2 (C), 137.3 + 137.4 (C), 154.1 + 154.2 (C), 156.8 + 158.9 (C).

1-[4-(5-Bromopentoxy)phenyl]-1-(4-hydroxyphenyl)-2-ruthenocenylbut-1-ene, 3d. Yield: 94%. IR: 3422 (OH), 2954, 2931, 2868 (CH_3) cm^{-1} . MS (IE, 70 eV) m/e : 618 (M^+).

Z + E Isomers. ^1H NMR: δ 0.91 (t, $J = 7.5$ Hz, 3H, CH_3), 1.19-1.96 (m, 6H, $-(\text{CH}_2)_3-$), 2.25 (q, $J = 7.5$ Hz, 2H, CH_2), 3.34 + 3.35 (t, $J = 6.7$ Hz, 2H, CH_2Br), 3.83 + 3.87 (t, $J = 6.9$ Hz, 2H, CH_2O), 4.23 (d, $J = 1.5$ Hz, 2H, C_5H_4), 4.31 (d, $J = 1.5$ Hz, 2H, C_5H_4), 4.43 (s, 5H, Cp),

5.65 (s broad, 1H, OH), 6.51-7.04 (m, 8H, 2C₆H₄). ¹³C NMR: δ 15.5 (CH₃), 24.9 (CH₂), 28.5 (CH₂), 29.2 + 29.3 (CH₂), 32.5 (CH₂), 33.6 (CH₂), 67.5 (CH₂), 69.7 (2 CH, C₅H₄), 71.1 (5 CH, Cp), 72.0 (2 CH, C₅H₄), 92.8 (C, C₅H₄), 113.8 + 114.0 (2 CH, C₆H₄), 114.8 + 115.0 (2 CH, C₆H₄), 130.4 + 130.5 (2 CH, C₆H₄), 131.1 + 131.3 (2 CH, C₆H₄), 135.5 (C), 136.8 + 136.9 (C), 137.1 + 137.2 (C), 137.4 (C), 154.0 + 154.1 (C), 157.2 + 157.3 (C).

d. The Formation of Amines 4a-d. Halides **3a-d** (3.0 mmol) and a solution of dimethylamine in methanol (2 M, 15 mL, 30 mmol) were heated with stirring in an autoclave at 60 °C for 1 day. After cooling, the solution was concentrated under reduced pressure. The residue was chromatographed on silica gel plates (chloroform/triethylamine 80/20 as eluent). After filtration, the solution was concentrated under reduced pressure. The residue was dissolved in dichloromethane and the solution was washed three times with water. The organic layer was dried on magnesium sulfate and then the solution was concentrated under reduced pressure to furnish pure amines **4a-d** as a mixture of *Z* and *E* isomers.

1-[4-(2-Dimethylaminoethoxy)phenyl]-1-(4-hydroxyphenyl)-2-ruthenocenylbut-1-ene, 4a. Yield: 81%. IR: 3430 (OH), 2925, 2856 (CH₃) cm⁻¹. HRMS: C₃₀H₃₄O₂NRu calcd 542.1642, found 542.1630.

Z + *E* isomers: ¹H NMR: δ 0.89 + 0.90 (t, *J* = 7.5 Hz, 3H, CH₃), 2.16-2.35 (m, 8H, N(CH₃)₂ + CH₂), 2.70 + 2.73 (t, *J* = 5.2 Hz, 2H, CH₂N), 3.91 + 3.96 (t, *J* = 5.2 Hz, 2H, CH₂O), 4.22 + 4.26 (t, *J* = 1.6 Hz, 2H, C₅H₄), 4.30 (t, *J* = 1.6 Hz, 2H, C₅H₄), 4.43 (s, 5H, Cp), 6.29-6.97 (m, 8H, 2C₆H₄). ¹³C NMR: δ 14.1 (CH₃), 27.8 + 28.1 (CH₂), 43.8 + 43.9 (N(CH₃)₂), 56.7 (CH₂), 63.0 + 63.2 (CH₂), 68.2 (2 CH, C₅H₄), 69.6 (5 CH, Cp), 70.6 (2 CH, C₅H₄), 91.6 + 91.7 (C, C₅H₄), 112.1 + 112.4 (2 CH, C₆H₄), 113.9 + 114.1 (2 CH, C₆H₄), 129.0 + 129.1 (2 CH, C₆H₄), 129.8 + 129.9 (2 CH, C₆H₄), 133.5 + 133.6 (C), 134.3 + 134.8 (C), 135.8 + 136.0 (C), 136.3 + 136.4 (C), 153.8 + 154.0 (C), 155.4 + 155.5 (C).

1-[4-(3-Dimethylaminopropoxy)phenyl]-1-(4-hydroxyphenyl)-2-ruthenocenylbut-1-ene, 4b. Yield: 71%. IR: 3437 (OH), 2957, 2928, 2871 (CH₃) cm⁻¹. HRMS: C₃₁H₃₆O₂NRu calcd 556.1798, found 556.1800.

Z + *E* isomers: ¹H NMR: δ 1.02 + 1.03 (t, *J* = 7.4 Hz, 3H, CH₃), 1.80-2.15 (m, 2H, CH₂), 2.25-2.75 (m, 10H, CH₂N(CH₃)₂ + CH₂), 3.95 + 3.99 (t, *J* = 6.9 Hz, 2H, CH₂O), 4.30-4.40 (m, 2H, C₅H₄), 4.40-4.48 (m, 2H, C₅H₄), 4.54 (s, 5H, Cp), 6.55-7.20 (m, 8H, 2C₆H₄). ¹³C NMR: δ

15.5 (CH₃), 26.9 (CH₂), 29.2 (CH₂), 45.0 (N(CH₃)₂), 56.3 (CH₂), 65.9 (CH₂), 69.6 (2 CH, C₅H₄), 71.0 (5 CH, Cp), 71.9 (2 CH, C₅H₄), 92.8 (C, C₅H₄), 113.7 + 113.9 (2 CH, C₆H₄), 115.1 + 115.2 (2 CH, C₆H₄), 130.3 + 130.4 (2 CH, C₆H₄), 131.0 + 131.2 (2 CH, C₆H₄), 135.0 (C), 135.7 + 136.0 (C), 137.0 + 137.3 (C), 137.7 (C), 155.1 + 155.2 (C), 157.0 + 157.1 (C).

1-[4-(4-Dimethylaminobutoxy)phenyl]-1-(4-hydroxyphenyl)-2-ruthenocenylbut-1-ene, 4c.

Yield: 65%. IR: 3430 (OH), 2949, 2869 (CH₃) cm⁻¹. HRMS: C₃₂H₃₈O₂NRu calcd 570.1955, found, 570.1942.

Z + E isomers: ¹H NMR: δ 0.99 (t, *J* = 7.0 Hz, 3H, CH₃), 1.60-1.82 (m, 4H, -(CH₂)₂-), 2.20-2.52 (m, 10H, CH₂N(CH₃)₂ + CH₂), 3.88 + 3.92 (t, *J* = 6.5 Hz, 2H, CH₂O), 4.25-4.35 (m, 2H, C₅H₄), 4.35-4.45 (m, 2H, C₅H₄), 4.51 (s, 5H, Cp), 5.35 (s broad, 1H, OH), 6.52-7.13 (m, 8H, 2C₆H₄). ¹³C NMR: δ 15.4 (CH₃), 23.5 (CH₂), 27.1 (CH₂), 29.1 (CH₂), 44.8 (N(CH₃)₂), 59.1 (CH₂), 67.3 (CH₂), 69.5 (2 CH, C₅H₄), 70.9 (5 CH, Cp), 71.9 (2 CH, C₅H₄), 92.7 (C, C₅H₄), 113.7 + 113.9 (2 CH, C₆H₄), 115.0 + 115.1 (2 CH, C₆H₄), 130.3 + 130.4 (2 CH, C₆H₄), 131.0 + 131.1 (2 CH, C₆H₄), 135.1 (C), 135.9 + 136.2 (C), 136.9 + 137.2 (C), 137.6 (C), 154.9 + 155.0 (C), 157.0 (C).

1-[4-(5-Dimethylaminopentoxy)phenyl]-1-(4-hydroxyphenyl)-2-ruthenocenylbut-1-ene, 4d.

Yield: 72%. IR: 3434 (OH), 2939, 2867 (CH₃) cm⁻¹. HRMS: C₃₃H₄₀O₂NRu calcd 584.2112, found 584.2101.

Z + E isomers: ¹H NMR: δ 0.90 + 0.91 (t, *J* = 7.5 Hz, 3H, CH₃), 1.24-1.80 (m, 8H, (CH₂)₄N), 2.11-2.40 (m, 8H, N(CH₃)₂ + CH₂), 3.77 + 3.81 (t, *J* = 7.0 Hz, 2H, CH₂O), 4.18-4.27 (m, 2H, C₅H₄), 4.27-4.35 (m, 2H, C₅H₄), 4.42 (s, 5H, Cp), 6.42-7.07 (m, 8H, 2C₆H₄), 7.34 (s broad, 1H, OH). ¹³C NMR: δ 15.6 (CH₃), 24.1 (CH₂), 26.7 (CH₂), 29.2 (CH₂), 29.3 (CH₂), 45.0 (N(CH₃)₂), 59.5 (CH₂), 67.6 (CH₂), 69.6 (2 CH, C₅H₄), 71.0 (5 CH, Cp), 72.0 (2 CH, C₅H₄), 92.9 (C, C₅H₄), 113.8 + 114.0 (2 CH, C₆H₄), 115.2 + 115.3 (2 CH, C₆H₄), 130.4 + 130.5 (2 CH, C₆H₄), 131.1 + 131.2 (2 CH, C₆H₄), 135.0 + 135.1 (C), 135.8 + 136.1 (C), 137.0 + 137.3 (C), 137.8 (C), 155.3 + 155.4 (C), 157.2 + 157.3 (C).

4.3 Biochemical Experiments. a. Materials. 17β-Estradiol and OH-Tam (*Z + E*) were obtained from Sigma-Aldrich (France). Stock solutions (1 × 10⁻³ M) of the compounds to be tested were prepared in DMSO and were kept at 4 °C in the dark; under these conditions they are stable at least 2 months. Serial dilutions in ethanol were prepared just prior to use.

Dulbecco's modified eagle medium (DMEM) was purchased from Gibco BRL; fetal calf serum was from Dutscher, Brumath, France; glutamine, estradiol, and protamine sulfate were from Sigma. MCF7 and MDA-MB231 cells were from the Human Tumor Cell Bank. Sheep uteri weighing approximately 7 g were obtained from the slaughterhouse at Mantes-la-Jolie, France. They were immediately frozen and kept in liquid nitrogen prior to use.

b. Determination of the Relative Binding Affinity (RBA) of the Compounds for ER α and ER β . RBA values were measured on ER α from lamb uterine cytosol and on ER β purchased from Pan Vera (Madison, WI). Sheep uterine cytosol prepared in buffer A (0.05 M Tris-HCL, 0.25 M sucrose, 0.1% β -mercaptoethanol, pH 7.4 at 25 °C) as described in ref 28 was used as a source of ER α . For ER β , 10 μ L of the solution containing 3500 pmol/mL was added to 16 mL of buffer B (10% glycerol, 50 mM Bis-Tris-Propane pH = 9, 400 mM KCl, 2 mM DTT, 1 mM EDTA, 0.1% BSA) in a silanized flask. Aliquots (200 μ L) of ER α in glass tubes or ER β in polypropylene tubes were incubated for 3 h at 0 °C with [6,7- 3 H]estradiol (2×10^{-9} M, specific activity 1.62 TBq/mmol, NEN Life Science, Boston MA) in the presence of nine concentrations of the hormones to be tested. At the end of the incubation period, the free and bound fractions of the tracer were separated by protamine sulfate precipitation. The percentage reduction in binding of [3 H]estradiol (Y) was calculated using the logit transformation of Y (logit Y : $\ln[y/1 - Y]$) versus the log of the mass of the competing steroid. The concentration of unlabeled steroid required to displace 50% of the bound [3 H]estradiol was calculated for each steroid tested, and the results were expressed as RBA. The RBA value of estradiol is by definition equal to 100%.

Measurement of Octanol/Water Partition Coefficient (log $P_{o/w}$) of the Compounds. The log $P_{o/w}$ values of the compounds were determined by reverse-phase HPLC on a C-8 column (Nucleosil 5.C8, from Macherey Nagel, France) according to the method previously described by Minick²⁹ and Pomper.³⁰ Measurement of the chromatographic capacity factors (kN) for each compounds was done at various concentrations in the range 85%-60% methanol (containing 0.25% octanol) and an aqueous phase consisting of 0.15% *n*-decylamine in 0.02 M MOPS (3-morpholinopropanesulfonic acid) buffer pH 7.4 (prepared in 1-octanol-saturated water). These capacity factors (kN) are extrapolated to 100% of the aqueous component given the value of kN_w. log $P_{o/w}$ (y) is then obtained by the formula: $\log P_{o/w} = 0.13418 + 0.98452 \log kN_w$.

Culture Conditions. Cells were maintained in monolayer in DMEM with phenol red (Gibco BRL) supplemented with 8-9% fetal calf serum (Gibco BRL) and 2 mM glutamine (Sigma) at 37 °C in a 5% CO₂ air humidified incubator. For proliferation assays, cells were plated in 1 mL of DMEM medium with phenol red (for MCF7 cells) and without phenol red (for MDA-MB231 cells), supplemented with 10% decomplexed and hormone-depleted fetal calf serum and 2 mM glutamine, and incubated. The following day (D0) 1 mL of the same medium containing the compounds to be tested was added to the plates (final volumes of alcohol 0.1%; four wells for each conditions, one plate per day). After 3 days (D3), the incubation medium was removed and fresh medium containing the compounds was added. After 5 or 6 days the total protein content of the plate was analyzed by methylene blue staining as follows. Cell monolayers were fixed for 1 h in methanol, stained for 1 h with methylene blue (1 mg/mL) in PBS, and then washed thoroughly with water. One milliliter of HCl (0.1 M) was then added and the absorbance of each well was measured at 620 nm with a BioRad spectrophotometer. The results are expressed as the percentage of proteins versus the control.

4. Conclusions

We have synthesized a series of ruthenocene-substituted tamoxifen derivatives with varying carbonaceous chain lengths in high yield via the McMurry coupling reaction and tested their efficacy toward ER-positive and ER-negative breast cancer cell lines. These molecules act as anti-estrogens toward the ER-positive MCF7 breast cancer cell line and have no cytotoxic effect on the ER-negative MDA-MB231 breast cancer cell line. This result is surprising in that it contrasts with the ferrocene derivatives, which show a cytotoxic effect in the ER-negative breast cancer cell line. Electrochemical studies show that the ruthenium radical cation quickly decomposes after electron transfer, which is not the case for the stable ferrocenium radical. Molecular modeling and RBA studies, however, show that these compounds would be eminently suitable for isotopic radioimaging of tumor cells.

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