Side-Chain Effects on the 1-(Bis-aryl-methylidene)-[3]ferrocenophane Skeleton: Antiproliferative Activity against TNBC Cancer Cells and Comparison with the Acyclic Ferrocifen Series

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1

Abstract:

As part of our ongoing study of the toxicity of compounds derived from 1,1-di(4-hydroxyphenyl)-2-ferrocenyl-but-1-ene, we recently showed that closely analogous [3]ferrocenophane complexes had an *in vitro* toxicity level substantially higher than that of their ferrocene counterparts, particularly in the case of mono- and diphenol complexes. In the current study we examined whether the presence of a dimethylamino chain, analogous to the chain in hydroxytamoxifen, was capable of producing in the ferrocenophane series the same antiestrogenic effect observed for OH-Tam and Fc-OH-Tam. To this end we synthesized and characterized new complexes bearing various side-chains, $[O(CH_2)_3NMe_2, O(CH_2)_3piperidine, O(CH_2)_3pyrrolidine, NHCO(CH_2)_2NMe_2]$, and studied a number of biochemical properties in those complexes possessing appropriate solubility. The results revealed that the new complexes of [3]ferrocenophane have very strong antiproliferative effects; one of the compounds bearing an $NHCO(CH_2)_2NMe_2$ chain has an IC_{50} value of 0.05 ± 0.02 μM on MDA-MB-231 cells. All these complexes retain affinity for the estradiol receptor. At the low (nanomolar range) concentrations at which the estrogenic/antiestrogenic effect is expressed in these molecules, the presence of an amino side-chain does not induce in the [3]ferrocenophane series the antiestrogenic effect observed with OH-Tam and Fc-OH-Tam. However, this effect is found for the complex with a slightly longer chain $(O(CH_2)_4NMe_2)$.

1- Introduction

Breast cancers remain the principal cause of cancer deaths in Europe causing almost 131,000 deaths in 2012.[1] About two-thirds of primary tumours, those classed as hormone-dependent (ER+) express the estradiol receptor and are stimulated by estrogens. Adjuvant treatment for this type of cancer most commonly includes prescription of antiestrogens (or SERMs, selective estrogen receptor modulators), molecules that can inhibit the growth of these tumours. Hydroxytamoxifen (OH-Tam **1**; Figure 1), the reference antiestrogen, is administered in the form of tamoxifen citrate, the non-hydroxylated pro-drug. The mechanism of action of OH-Tam has been widely studied and extensively described.[2] Thanks to its phenol group, OH-Tam binds at the HBD (hormone binding site) of the estrogen receptor, but the presence of the long amino chain entails a significant modification at this level, namely the displacement of helix 12. This modified receptor binds as a dimer to the estrogen response element (ERE) of the DNA of ER+ cells, but does not permit recruitment of the co-factors necessary to activate DNA transcription. Thus low concentrations (10 nM) of OH-Tam produce a diminution in the proliferation of hormone-dependent breast cancer cells (MCF-7). However, since its effect is entirely linked to an interaction with the estrogen receptor, OH-Tam has no effect, at submicromolar concentration, on the growth of hormone-independent breast cancer cells, such as the triple negative breast cancer (TNBC) MDA-MB-231, which do not express the estrogen receptor. The two chief advantages of OH-Tam are thus its affinity for the estradiol receptor, and the presence of an amino chain that modifies the structure of the receptor such that cellular proliferation is significantly lowered. However, in the related acyclic ferrocifen series the presence of the "ferrocenyl-ene-phenol" unit triggers on cancer cells an additional cytotoxic effect that can be modulated by the basic amine chain.[3] We now describe this behavior in the ferrocenophane series.
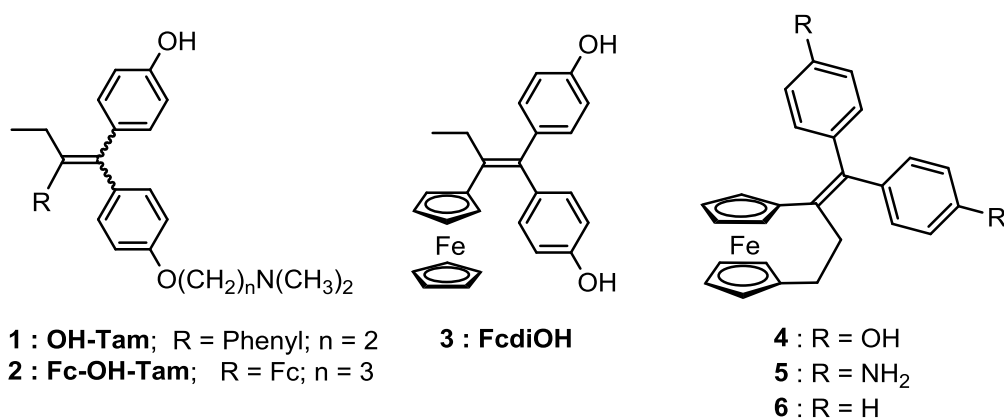
Figure 1 : OH-Tamoxifen and several ferrocenyl and [3]ferrocenophane derivatives

We have shown that Fc-OH-Tam, **2**, in which the phenyl group in hydroxytamoxifen, **1**, has been replaced by a ferrocenyl substituent, has an antiproliferative effect *in vitro* on both hormone-dependent (MCF-7) and hormone-independent (MDA-MB-231) breast cancer cells.[3-7] In hormone-dependent cells this effect is essentially mediated by interaction with the estradiol receptor, and reversed by addition of estradiol. In contrast, the antiproliferative effect observed on hormone-independent cells such as MDA-MB-231 cells is a cytotoxic one.[8] This cytotoxic effect is also observed with the diphenol compound Fc-diOH, **3**.[9] We have found that the most effective compounds (IC$_{50}$ values less than 1 μM) possess, like **2** and **3**, a ferrocene-ene-paraphenol entity.[10, 11] The ferrocene appears to act as a stimulus for a mild intramolecular oxidation involving the phenol group, leading to the facile production of quinone methides whose formation, structure and electrophilic behaviour we have established.[12-15]

A Structure-Activity Relationship (SAR) study extended to include a number of ferrocene complexes has allowed us to identify another molecule, the cyclic diphenol **4** derived from [3]ferrocenophane, whose toxicity on MDA-MB-231 cells is noticeably greater than that of Fc-diOH (IC$_{50}$ values of 0.09 μM for **4** versus 0.6 μM for **3**).[16] In addition, the toxicity of the two diphenols **3** and **4** has been tested on the panel of 60 cell lines of the

National Cancer Institute (Bethesda, MD, USA). The results show that the [3]ferrocenophane **4** is more toxic overall than Fc-diOH, **3** (mean IC_{50} values are 0.18 and 0.52 µM, respectively). The activity profiles of these compounds also show that **3** and **4** probably do not share the same mechanism of action either with each other or with the 171 reference molecules in the database, and in particular that they do not act, like cisplatin, via an alkylation of DNA.[17]

The diphenol **4** possesses, like **3**, a moderate level of affinity for the estradiol receptor. As a result, both show a proliferative effect at low concentrations (10^{-8} M) on hormone-dependent MCF-7 cells, that partially offsets the cytotoxic effect caused by the oxidation of the ferrocene. To better understand the effect of the side chain on the antiproliferative activity of ferrocene and [3]ferrocenophane derivatives, several new compounds containing an amino chain have been synthesized and studied for their cytotoxic and their estrogenic/antiestrogenic effects. The first such molecule was compound **7**; subsequently, we prepared the analogous complexes of pyrrolidine, **10**, of piperidine, **11**, and also molecule **14** which contains an -$O(CH_2)_4N(CH_3)_2$ unit. Interestingly, it has recently been reported that the presence of an amide function on the basic chain of certain SERMs modified their properties by preventing their recognition by the estrogen receptor.[18] We therefore prepared complex **12**, bearing the chain containing an $NHCO(CH_2)_2NMe_2$ amide function. Since we also found that replacement of the OH groups in **4** by NH_2 or H gave cytotoxic products (**5** and **6**), we also synthesized complexes **8**, **9** and **13** that possessed alkoxyamino chains, but no hydroxyl units. Finally, to allow direct comparison between the ferrocene and ferrocenophane series, the ferrocene complexes **15** and **16** were synthesized. In total, ten new complexes, listed in Figure 2, were synthesized and studied.
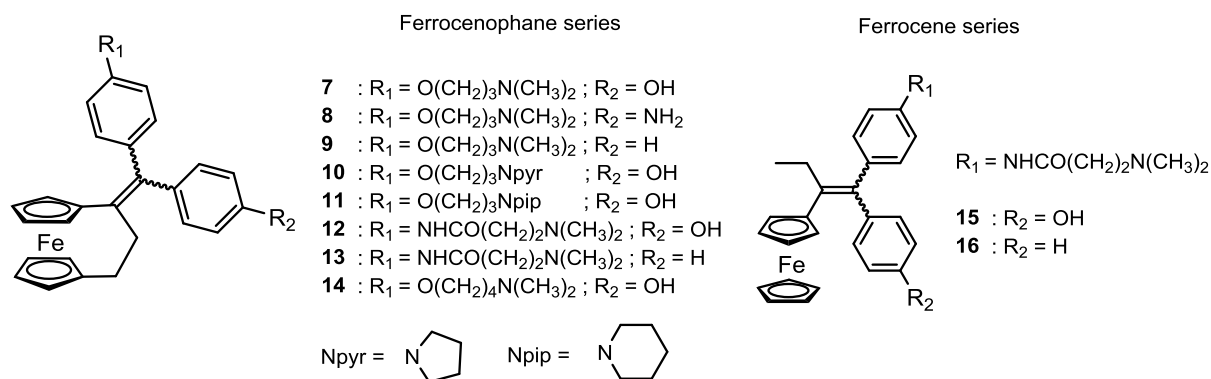
Figure 2 : Newly synthesized complexes

2- Results and Discussion:

2-1 Synthesis of the ferrocenophane and ferrocenyl complexes

2-1-1 Synthesis of the complexes with a dimethylaminopropyl chain 7 – 9

Compounds **7, 8** and **9** were synthesized in a one-step reaction starting from **4**, **17** and **18** by alkylation of the hydroxyl group (Scheme 1). Sodium hydride was used to generate the corresponding phenolate from each of these compounds which, when heated with 3-dimethylamino-1-propyl hydrochloride in DMF at 80°C for 12 hours, furnished **7, 8** and **9** in 27%, 66% and 83% yields, respectively. All the products were obtained as mixtures of (*E+Z*) isomers in various proportions (see Experimental Section).
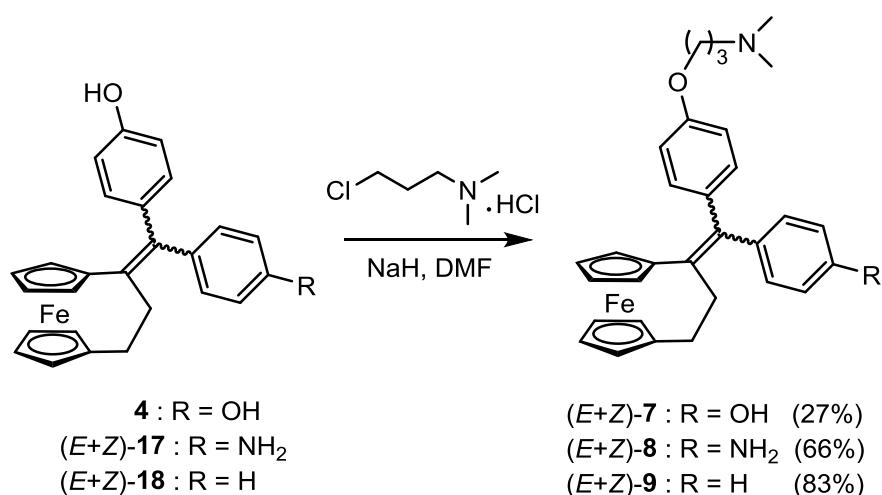
Scheme 1: Synthesis of the [3]ferrocenophane derivatives with an alkyl dimethylamino chain

2-1-2 Synthesis of the [3]ferrocenophane derivatives with pyrrolidine or piperidine side chain

10 and 11

Compounds **10** and **11** were prepared from the bromo compound **20**, obtained in 61% yield from the McMurry coupling of [3]ferrocenophan-1-one and bromo-ketone **19** (Scheme 2). Subsequent replacement of the bromo substituent by amine groups was accomplished by heating **20** with pyrrolidine or piperidine to give **10** or **11** in moderate yields of 27% and 37%, respectively
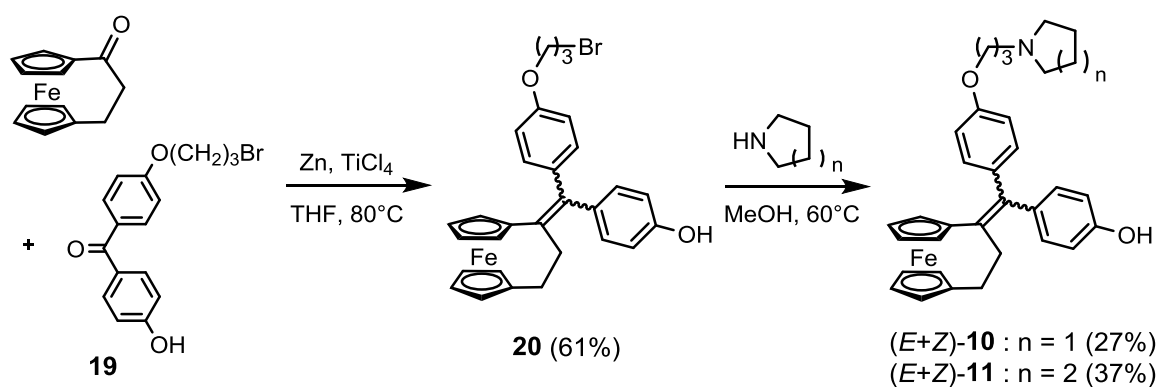
Scheme 2. Synthesis of the [3]ferrocenophane derivatives with a pyrrolidine or piperidine, side-chain.

7

Treatment of **20** with dimethylamine provides an alternative route to **7**, but in this case the overall yield is quite low (13%). Similarly, reaction of the bromo precursor, **21**, (obtained by alkylation of **4** with dibromobutane) with dimethylamine yielded **14** (26%), which contains an -$O(CH_2)_4N(CH_3)_2$ chain (Scheme 3).
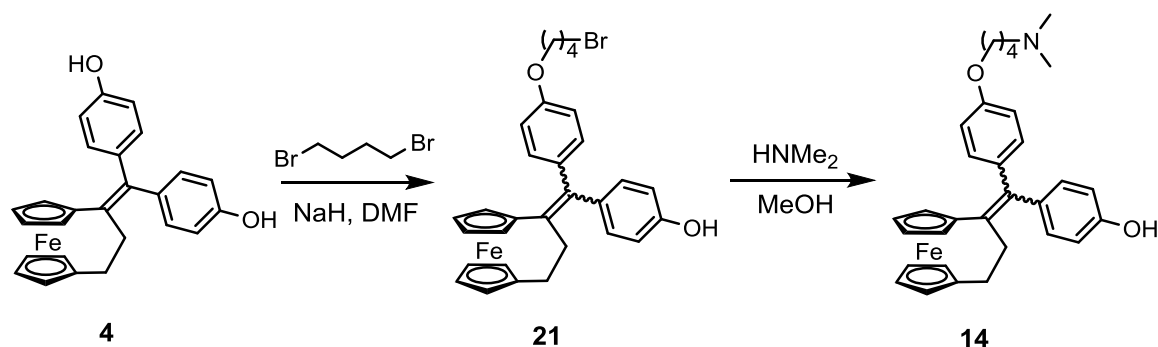
Scheme 3: Synthesis of the [3]ferrocenophane, **14**, containing an -$O(CH_2)_4N(CH_3)_2$ chain.

2-1-3 Synthesis of the amidyl [3]ferrocenophane or ferrocene derivatives 12, 13, 15, and 16

Complexes **12**, **13**, **15**, and **16**, were prepared in two-step reactions starting from **17**[19], **22**[20], **23**[17] and **24**[21] (Scheme 4). The amino function was first transformed into a chloroamido chain by reaction of 3-chloropropionylchloride in the presence of pyridine as a base; in these cases, the acyl chloride reacts exclusively on the amino function. The [3]ferrocenophane chloro compounds **25** and **26** were obtained in high yields (88%, 98%), while the yields of the ferrocenyl chloro compounds **27** and **28** were only moderate (44% and 39%, respectively). Amination of the chloro compounds was carried out by heating a solution of each of the compounds in methanol containing dimethylamine at 60 °C. All compounds were obtained in high yields, 89-97%, as a mixture of *E* and *Z* isomers.
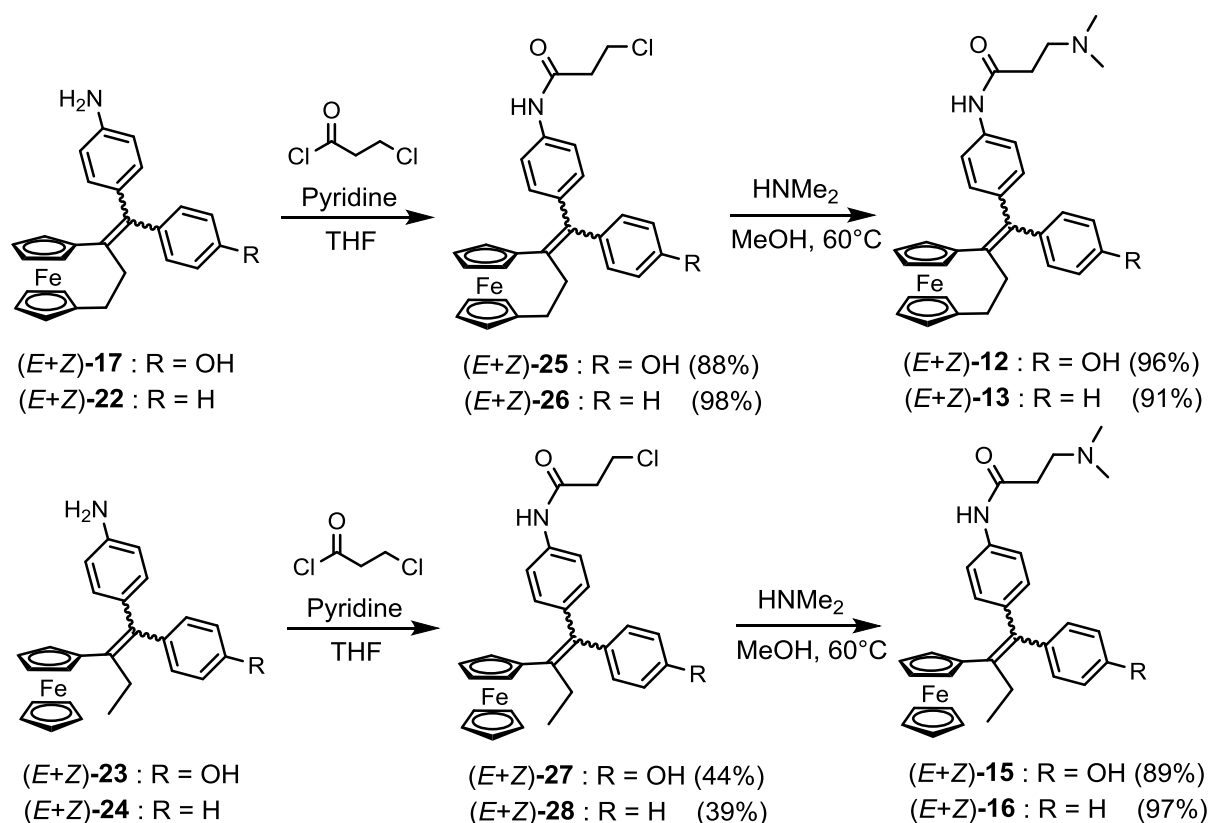
Scheme 4. Synthesis of the amidyl [3]ferrocenophane and ferrocene derivatives **12**, **13**, **15** and **16**

2-2 X-ray crystal structure of 26

To establish the identity of a typical example of one the new compounds unequivocally, the X-ray crystal structure of *Z*-1-[(4-(3-chloropropionyl)aminophenyl-phenyl)-methylidene]-[3]ferrocenophane, **26**, was determined. Crystallization from aceto-nitrile of the *E+Z* mixture gave a suitable sample of the *Z* isomer whose structure is shown in Figure 3. The geometry of the [3]ferrocenophane moiety in **26** closely resembles those reported previously.[19, 22] The system crystallizes with two independent molecules in the unit cell. As expected, the three-carbon bridge between the two cyclopentadienyl rings is too short for them to maintain their coplanarity, and the dihedral angles between the ring planes in the independent molecules are 9.5° and 10°. The Fe-C_{Cp} distances range from 2.028(11) to 2.084(14), and the bond lengths in the three-carbon bridge are C1-C11 = 1.509(13), C11-C12

= 1.507(11), C12-C13 = 1.559(14), and C13-C6 = 1.509(14). The aryl rings attached to C14 are oriented almost orthogonally to each other; the dihedral angle between these ring planes is 80°. Full crystallographic data are provided as Supplementary Information.
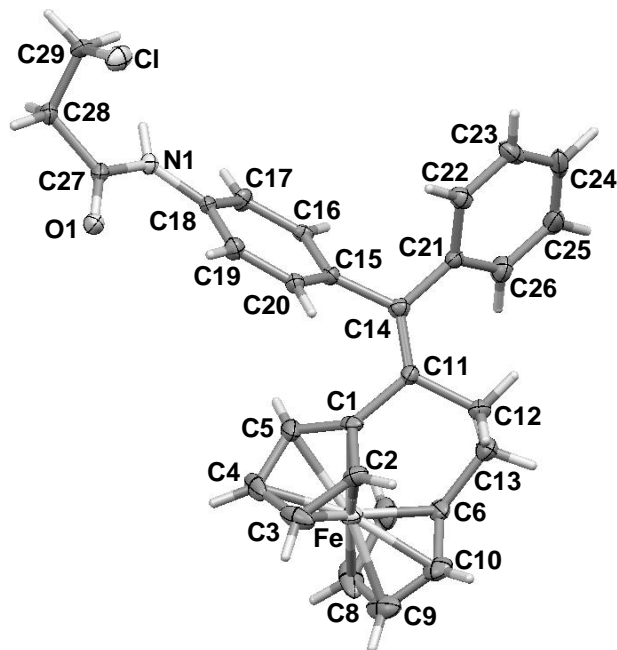
Figure 3. Molecular structure of *Z*-1-[(4-(3-chloropropionyl)aminophenyl-phenyl)-methylidene]-[3]ferrocenophane, **26**.

2.3. Biological Results

The biological effects of the newly synthesized complexes were studied and compared to those of two reference compounds, **2** and **4**. Four types of experiments were carried out: (1) study of the antiproliferative effect on MDA-MB-231 cells, hormone-independent cells which allow the cytotoxicity of the molecules to be tested; (2) determination of Relative Binding Affinity (RBA) values for these compounds, to establish whether or not they possess an affinity for the estradiol receptor; (3) study of the proliferative/antiproliferative effect of these complexes on hormone-dependent MCF-7 cells at low concentrations (in the nanomolar range), to ascertain whether the addition of a long side chain reveals antiestrogenic effects, (4) measurement of the lipophilicity of the complexes.

2.3.1 Study of the antiproliferative effect of the complexes on hormone-independent MDA-MB-231 cells

The IC$_{50}$ values of the new compounds **7**-**16** for the MDA-MB-231 cancer cells are collected in Table 1.

Table 1 IC$_{50}$ values of the ferrocenophane (Series A) and ferrocenyl (Series B) complexes on the hormone-independent breast cancer cell line (MDA-MB-231), Relative Binding Affinity (RBA) on the alpha form of the estrogen receptor, and lipophilicity (LogPo/w)
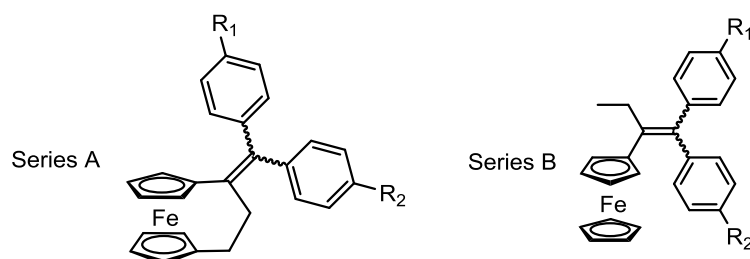
Compound	R$_1$	R$_2$	*Z, E* %[a]	IC$_{50}$ (μM) on MDA-MB-231	RBA(%) on ERα	LogPo/w
Series A						
4	OH	OH	-	0.09 ± 0.01[c]	7.2[c]	4.6
7	O(CH$_2$)$_3$NMe$_2$	OH	72/28	0.18 ± 0.04	17.2	2.4 ; 2.7[e]
8	O(CH$_2$)$_3$NMe$_2$	NH$_2$	52/48	0.17 ± 0.02	14.4	4.4 ; 4.7[e]
9	O(CH$_2$)$_3$NMe$_2$	H	94/6[b]	0.37 ± 0.11	4.8	3.5
10	O(CH$_2$)$_3$Npyr	OH	79/21	0.19 ± 0.05	-	-
11	O(CH$_2$)$_3$Npip	OH	90/10	1.10 ± 0.11	-	-
12	NHCO(CH$_2$)$_2$NMe$_2$	OH	59/41	0.05 ± 0.02	10.3	3.3
13	NHCO(CH$_2$)$_2$NMe$_2$	H	83/17	0.39 ± 0.03	4.4	4.4
14	O(CH$_2$)$_4$NMe$_2$	OH	60/40	0.12 ± 0.06	-	3.0, 3.1[e]
29	NHCO(CH$_2$)$_6$NH$_2$	H	94/6[b,f]	0.84 ± 0.28[f]	-	-
30	NHCO(CH$_2$)$_6$NHOH	H	89/11[b,f]	0.94 ± 0.08[f]	-	-
Series B						
3	OH	OH	-	0.64 ± 0.06	9.6[d]	5.0[g]
Fc-OHTam **2**	O(CH$_2$)$_3$NMe$_2$	OH	50/50[h]	0.50	11.5[d]	4.3 ; 4.5[e]
15	NHCO(CH$_2$)$_2$NMe$_2$	OH	53/47	0.67 ± 0.00	23	4.5 ; 4.6[e]
16	NHCO(CH$_2$)$_2$NMe$_2$	H	67/33	1.13 ± 0.03	4	4.6

a) all the compounds are mixtures of (*E*+*Z*) isomers; b) *Z* configuration attributed to the major isomer (see experimental section); c) values from ref. 16; d) values from ref. 3; e) values for each isomer; f) values from ref. 23; g) values from ref. 9. h) value from ref 4.

We note that all the new compounds are characterized by antiproliferative activity that ranges from good to excellent. Replacement of the OH group by NH_2, as in compounds **7** and **8**, does not change the cytotoxic activity of these compounds. The IC_{50} values of compounds **7**, **8**, and **10** are between 0.17 and 0.19 μM, that is two times less efficacious than the diphenol **4** (0.09 μM), but they are still among the best compounds that we have obtained. The lipophilicity of these four compounds does not appear to play an important role. Once again, it demonstrates that systems lacking an OH group, as in **9**, **13** and **16**, are less active than their analogues that do possess it. Substitution of the amino chain $-O(CH_2)_3NMe_2$ by the amido chain $-NHCO(CH_2)_2NMe_2$ produces different effects. Compounds **9** and **13** have almost the same IC_{50} value, but the amido compound **12** is three times more active than **7** (0.05 μM versus 0.18 μM). At present therefore, molecule **12** is one of the three most efficacious of all the ferrocenyl derivatives that we have prepared.[17] For comparison, we have also included the IC_{50} values for molecules **29** and **30** that were synthesized previously;[23] these latter compounds are less effective than compounds **12** et **13**. The lengthening of the amido chain may be the cause of their diminished activity.

Globally, molecules in the ferrocenophane series are more active than their ferrocene counterparts; to be precise, compounds **4**, **7**, **12** and **13** are, respectively, 7, 3, 13 and 3 times better than compounds **3**, **2**, **15** and **16**. We recently shown that enzymatic oxidation of **4,** the most studied complex in the ferrocenophane series, leads to the formation of a transient short-lived species attributed to a quinone methide radical.[24] On the contrary, the enzymatic oxidation of the ferrocenyl complexes **2** or **3** leads to the formation of quinone methides.[25] Reactivity of a radical is expected to be higher than that of the quinone methide and could explain the higher cytotoxicity of the ferrocenophane complexes. Substitution of the amino chain of **4** by an amido chain gives access to **12,** the most cytotoxic complex of the series. The

precise role of this amido chain has not yet been elucidated and will be the subject of further study.

2.3.2 Relative binding affinity values for the alpha form of the estrogen receptor (ER) and LogPo/w of the compounds

The relative binding affinity values (RBA) of the compounds were measured for the alpha form of the estrogen receptor and are reported in Table 1. All the complexes showed a significant affinity for the estrogen receptor, even those lacking an OH substituent. There is no clear difference between the ferrocenophane and the ferrocenyl complexes. The RBA values found for the disubstituted complexes possessing an amino side chain plus another substituent (OH or NH_2) are high (between 10-23%), the highest value being obtained for **15**, the ferrocenyl derivatives with the $NHCO(CH_2)_2NMe_2$ side-chain. Finally, values of around 4% were found for the three mono substituted complexes bearing only an amino chain.

The lipophilicity values obtained for the different complexes are shown in Table 1. These values are very close for the three ferrocene complexes (4.3 − 4.6) while varying significantly for the ferrocenophane complexes (2.4 − 4.7). In addition, the logPo/w values of the ferrocene complexes are always higher than those of their ferrocenophane counterparts. Finally, complexes **9** and **12** have lipophilicity values very close to those of estradiol (3.5) and OH-Tam (3.2 and 3.4).[3]

2.3.3 Estrogenic/antiestrogenic effect of the complexes *in vitro* on MCF-7 cells

The effect of the new complexes on the growth of MCF-7 hormone dependent cells was then studied and the results are shown in Figure 4. The characteristic proliferative effect of estradiol is observed, and it is clearly evident that the behavior of the complexes of the ferrocenophane family differs from that of the ferrocenyls. In the case of the ferrocene

complexes **15** and **16** an antiproliferative effect is observed analogous to that observed earlier for Fc-OH-Tam, which is attributed to an antiestrogenic effect. This effect is not found in the ferrocenophane series. Instead a slight estrogenic effect is observed in the complexes of this series for **7**, **8**, **12** and **13**, but not for **9** which shows no effect. This result appears to show that the constraint imposed by the presence of a carbon chain linking the two ferrocene rings prevents the expression of the antiestrogenic effect produced by the presence of the long dimethylamino chain in OH-Tam or Fc-OH-Tam. It is interesting to note that in the ferrocene series the antiestrogenic effect of the two complexes **15** and **16**, both of which bear the NHCO(CH$_2$)$_2$NMe$_2$ chain, is identical to that of Fc-OH-Tam **2** and does not depend on the presence of an OH substituent. In addition, it should be mentioned that the analogue of OH-Tam bearing a ferrocenic chain OCH$_2$COFc instead of an amino chain expresses an estrogenic effect at 10 nM.[26]
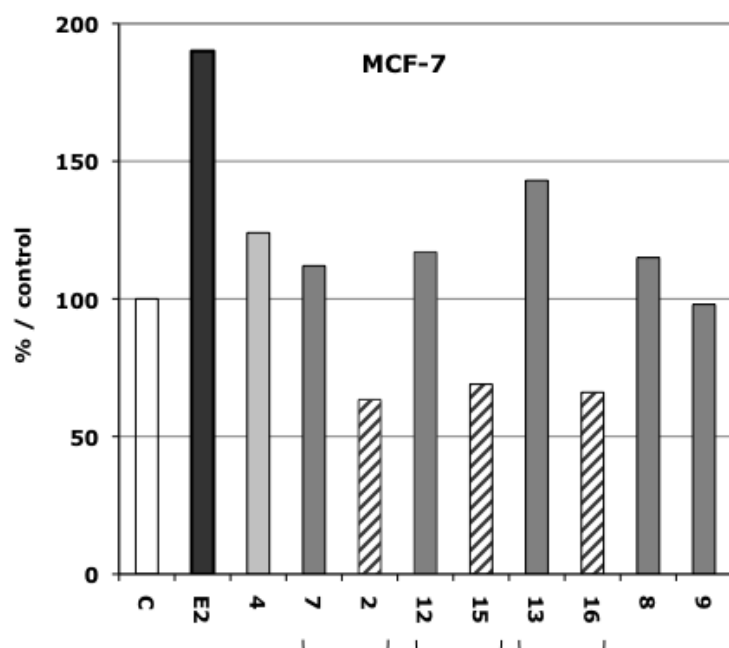
Figure 4. Study of the estrogenic / antiestrogenic effect of the complexes on MCF-7 cells after 5 days of culture in the presence of 1 nM of E$_2$, **7**, **8**, **12** and 10 nM of **2**, **4**, **9**, **13**, **15**, **16,** in a medium without phenol red. The grey bars represent the ferrocenophane complexes and the hatched bars the ferrocenyl complexes. Representative data of one experiment performed twice or three times with similar results.
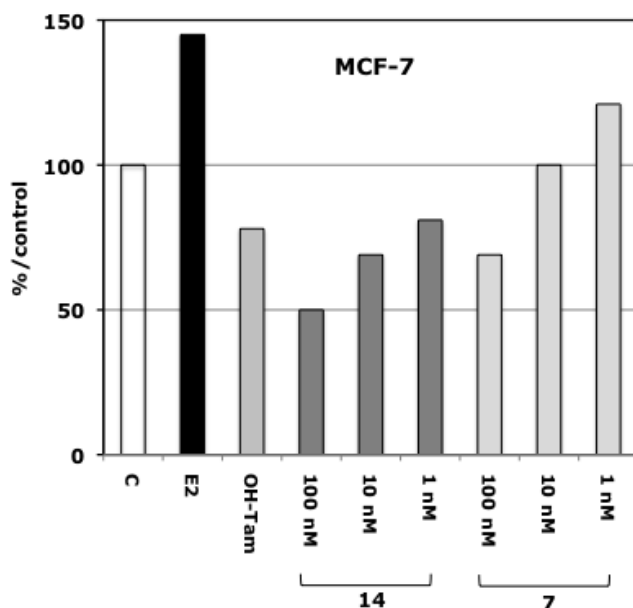
Figure 5. Study of the estrogenic / antiestrogenic effect of **7** and **14** on MCF-7 cells after 4 days of culture in the presence of 1, 10, 100 nM in a medium without phenol red. Representative data of one experiment performed twice with similar results.

We then decided to take a closer look at the estrogenic/antiestrogenic effects of compounds **7** and **14** which differ only in the length of their aminated chain. Figure 5 shows the effect of these two complexes on MCF-7 at 3 concentrations (1, 10, 100 nM). At 1 nM compound **7** provokes an estrogenic effect; this effect diminishes at 10 nM and inverts at 100 nM, without doubt because of the appearance of the cytotoxic effect. By way of contrast, for compound **14** the antiestrogenic effect has already been evident from 1 nM. This result can perhaps be explained by the lengthening of the amino chain, from three to four methylenes, which allows the molecule to bind better with the antiestrogenic conformation of the receptor site, as verified by the modelling study below.

Molecular modeling

15

Molecular docking experiments using the crystal structure of the ligand binding domain (LBD) of human ERα (hERα) as described by Shiau and al.[27] were performed using the program Spartan 14.[28] Only the amino acids that constitute the wall of the cavity have been retained. The organic molecule was removed from the cavity and replaced successively with the organometallic bio-ligands. Only Z isomers were calculated since it had previously been shown that the Z form of ferrocifen **2** shows better recognition for the receptor.[3] Energy minimisation was then carried out using the Merck molecular force field (MMFF) with the heavy atoms (non-hydrogen) of the amino acids of the cavity wall immobilised, then the geometry was optimized using quantum mechanical semi-empirical PM3 methods. This allowed the ideal positions of the bio-ligands to be determined. Quantum mechanical semi-empirical PM3 methods were then used to determine the affinity of the bio-ligands for the cavity. This requires calculation of the energies of bio-ligand cavity group, of the cavity itself, and of the ligand, the latter two in the conformations they had in the molecular assemblies to give the $\Delta rH°$ enthalpy variations of the reactions: bio-ligand + cavity → molecular assembly (Table 2).

For the two compounds, binding to the ER is thermodynamically favoured, as evidenced by the negative enthalpy of formation for the ligand-receptor complex. The most negative value for **14** shows better affinity for the cavity than **7**. Moreover, the calculations reveal a higher possibility for a hydrogen bond between the proton on the carboxylic moiety of the aspartic acid Asp 351 and the nitrogen atom of the bio-ligand for **14** than for **7**. The distances O-H…N- are 1.91 and 2.81 Å respectively and the optimised distance is shown in Figure 6. The bio-ligands, the aspartic acid Asp 351 and two other important amino acids for phenol binding are labelled, and are shown as ball and spoke representations. The other amino acids of the cavity wall are shown merely as sticks.

These results could be explained by the fact that the cyclic character of the ferrocenophane engenders additional steric hindrance, as compared to the ferrocifen, that hampers the formation of the hydrogen bonds with the dimethylaminopropyl chain. The longer dimethylaminobutyl chain in **14** can compensate for this hindrance. This could be the reason for the loss of estrogenicity in **14**, that appears for **7** at low concentrations (10^{-8}, 10^{-9} M).

Table 2 Enthalpy variation values for the Z bio-ligands docked into ERα, and H-N distances

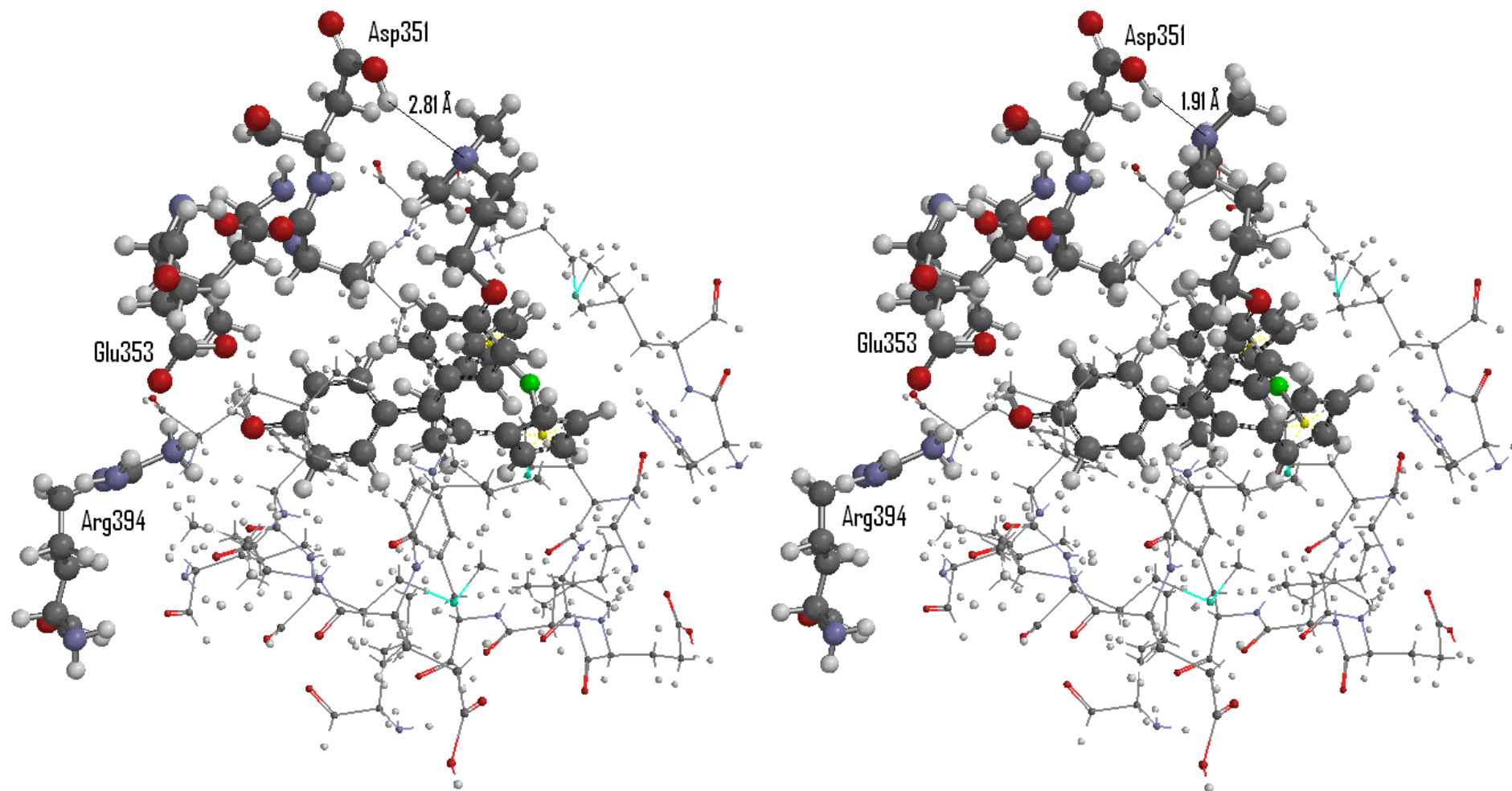| Compounds	$\Delta rH°/kJ\ mol^{-1}$	O-H…N distance (Å)
7	-97	2.81
14	-106	1.91

Figure 6. Docking of Z isomers of **7** (left) and **14** (right) in the antagonist binding site of the α form of the human estrogen receptor (h-ERα). For clarity, the bioligands and three important amino acids are shown as ball and spoke while other amino acids appear as stick representations.

Conclusions

The synthesis of novel complexes in the ferrocene and ferrocenophane series with amino side-chains has produced molecules with very clear cytotoxic effects on MDA-MB-231 hormone-independent breast cancer cells. The mechanistic particularity of **4**, the most studied complex in the ferrocenophane series, has recently been disclosed and is associated with an active radical intermediate.[24]

All the complexes in the series retain an affinity for the estrogen receptor, but only those of the acyclic ferrocenyl series express an antiestrogenic effect analogous to that of OH-Tam on hormone-dependent MCF-7 cells. This effect is not found in their analogs in the ferrocenophane series except for **14**. Actually it is possible to turn the estrogenic effect into an antiestrogenic one by lengthening the amino chain (as in the case of **7** and **14**). However, it is noteworthy that compound **9** is neutral in this respect. At this time, where the issue of SERMs that act as endocrine disrupters is the focus of serious examination, these results illustrate the subtlety of the effects, and emphasise the care that one must bring to their analysis.

Experimental Section

General Remarks. THF was obtained by distillation from sodium/benzophenone. Thin layer chromatography was performed on silica gel 60GF254. The preparative HPLC separations were performed on a Shimadzu apparatus with a Nucleodur C18 column (length 25 cm, diameter 3.2 cm, and particle size 10 mm) using acetonitrile or acetonitrile/water as an eluent. Column chromatography purifications were performed by using silica gel with appropriate eluent. The analytical HPLC controls were performed on a Shimadzu apparatus with a Nucleodur C18 column (length 15 cm, diameter 0.45 cm, and particle size 5 µm) using acetonitrile as an eluent. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz Bruker spectrometer. Mass spectrometry was performed with a Nermag R 10–10C spectrometer.

HRMS measurements were performed on a Thermo Fischer LTQ-Orbitrap XL apparatus equipped with an electrospray source by the Institut Parisien de Chimie Moléculaire (UMR 7201), Université Pierre et Marie Curie, Paris. Elemental analyses were performed by the microanalysis service of ICSN (Gif sur Yvette, France). Cytotoxicity measurements on MCF-7 breast cancer cells, and some of MDA-MB-231 breast cancer cells in vitro, were performed by Institut Parisien de Chimie Moléculaire (UMR 8232), Université Pierre et Marie Curie, Paris and by ImaGIF Ciblothèque Cellulaire (Institut de Chimie des Substances Naturelles). All compounds were isolated as a mixture of *Z:E* isomers. Identification of *Z* and *E* configurations was possible only for the compounds with a large excess of one isomer (**9, 29, 30**).

General Procedure 1: Alkylation of phenol groups

In each case, the appropriate compound was dissolved in DMF and sodium hydride was added. The mixture was stirred for 10 min, 3-dimethylamino-1-propyl chloride hydrochloride was added and the mixture heated at 90°C overnight. Additional 3-dimethylamino-1-propyl chloride hydrochloride was then added, and heating was continued for 4 hours. The mixture was cooled and concentrated under reduced pressure, the residue was dissolved in dichloromethane and was washed twice with a diluted aqueous solution of sodium hydroxide, followed with water. After drying over magnesium sulphate, the solution was concentrated under reduced pressure and the residue was chromatographed on silica gel with a 4/1 solution of chloroform/triethylamine as eluent. The residue was recrystallized from appropriate solution to yield the product.

1-[(4-(3-dimethylaminopropoxy)phenyl-4-hydroxyphenyl)methylidene]-[3]ferroceno-phane, 7. Compound **4** (500 mg, 1.18 mmol), prepared as indicated in ref. 16, sodium hydride (94 mg, 2.36 mmol), 3-dimethylamino-1-propyl chloride hydrochloride (150 mg, 0.95 mmol), 40 mL of DMF were treated as described above. After purification by column

chromatography with acetone/NEt$_3$ 10/1 eluent system and then recrystallization from acetone, **7** (162 mg, 27%) was obtained as a bright yellow product as a mixture of *E* and *Z* isomers (72/28). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.69-1.91 (m, 2H, CH$_2$), 2.10 and 2.15 (s, 6H, NMe$_2$), 2.22-2.41 (m, 4H, CH$_2$N+CH$_2$ cycle), 2.57-2.69 (m, 2H, CH$_2$ cycle), 3.86 and 3.99 (t, *J* = 6.4 Hz, 2H, CH$_2$O), 3.93 (t, *J* = 1.8 Hz, 2H, C$_5$H$_4$), 3.96 (t, *J* = 1.8 Hz, 2H, C$_5$H$_4$), 3.98 (t, *J* = 1.8 Hz, 2H, C$_5$H$_4$), 4.27 (t, *J* = 1.8 Hz, 2H, C$_5$H$_4$), 6.45 and 6.61 (d, *J* = 8.7 Hz, 2H, C$_6$H$_4$), 6.73 and 6.90 (d, *J* = 8.7 Hz, 2H, C$_6$H$_4$), 6.74 and 6.83 (d, *J* = 8.7 Hz, 2H, C$_6$H$_4$), 6.97 and 7.08 (d, *J* = 8.7 Hz, 2H, C$_6$H$_4$), 9.23 and 9.43 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ 26.8 (CH$_2$), 27.9 (CH$_2$), 40.4 (CH$_2$), 45.1 (2CH$_3$, NMe$_2$), 55.6 (CH$_2$), 65.4 (CH$_2$O), 68.0 (2CH, C$_5$H$_4$), 68.4 (2CH, C$_5$H$_4$), 69.6 (2CH, C$_5$H$_4$), 70.0 (2CH, C$_5$H$_4$), 83.6 (C$_{ip}$), 86.6 (C$_{ip}$), 113.0 (2CH, C$_6$H$_4$), 114.9 (2CH, C$_6$H$_4$), 130.0 (2CH, C$_6$H$_4$), 131.2 (2CH, C$_6$H$_4$), 133.9 (C), 135.6 (C), 139.8 (C), 145.4 (C), 156.0 (C), 156.6 (C). MS (EI, 70 eV) m/z: 508 [M]$^{+\cdot}$, 86 [NMe$_2$CH$_2$CH$_2$]$^+$, 58 [NMe$_2$CH$_2$]$^+$. HRMS (ESI, C$_{31}$H$_{34}$FeNO$_2$: [M+H]$^+$) calcd: 508.1939, found: 508.1943.

1-[(4-(3-Dimethylaminopropoxy)phenyl-4-aminophenyl)methylidene]-
[3]ferrocenophane, 8. Analogously, **17** (100 mg, 0.24 mmol) prepared as indicated in ref. 19, sodium hydride (23 mg, 0.9 mmol), 3-dimethylamino-1-propyl chloride hydrochloride (42 mg, 0.3 mmol) were treated as described above. After purification by column chromatography with acetone / NEt$_3$ (10/1) eluent system and then recrystallization from diethylether + pentane solvent system, **8** (79 mg, 66%) was obtained as a bright yellow product as a mixture of *E* and *Z* isomers (52/48). ^{1}H NMR (300 MHz, acetone-d_6): δ 1.84-1.92 (m, 2H, CH$_2$), 2.14 and 2.19 (s, 6H, CH$_3$), 2.30-2.56 (m, 4H, CH$_2$), 2.65-2.69 and 2.72-2.78 (m, 2H, CH$_2$), 3.90-4.07 (m, 8H, CH$_2$O+C$_5$H$_4$), 4.24 (s, 2H, C$_5$H$_4$), 4.49-4.66 (s broad, 2H, NH$_2$), major isomer [6.36, 6.72, 7.14] (d, *J* = 8.7 Hz, 6H, C$_6$H$_4$), minor isomer [6.61 and 6.66] (d, *J* = 8.7 Hz, 4H,

C_6H_4), 6.90-6.95 (m, minor isomer (4H) + major isomer (2H), C_6H_4). ^{13}C NMR (75.4 MHz, acetone-d_6): δ 28.1 (CH_2), 29.0 (CH_2), 41.4 and 41.5 (CH_2), 45.5 ($2CH_3$), 56.7 (CH_2), 66.3 and 66.5 (CH_2), 68.6 (2CH, C_5H_4), 68.9 (2CH, C_5H_4), 70.7 (2CH, C_5H_4), 70.8 (2CH, C_5H_4), 85.0 (C_{ip}), 87.5 (C_{ip}), 113.6 and 113.8 (2CH, C_6H_4), 114.5 (2CH, C_6H_4), 130.7 and 131.0 (2CH, C_6H_4), 132.0 and 132.3 (2CH, C_6H_4), 132.7 (2C), 137.9 (C), 141.6(C), 147.2(C), 158.8 (C). MS (CI, NH_3) m/z : 507 [M+H]$^+$. MS (EI, 70 eV) m/z : 506 [M]$^{+\cdot}$, 86 [$NMe_2CH_2CH_2$]$^+$, 58 [NMe_2CH_2]$^+$. HRMS (ESI, $C_{31}H_{35}FeN_2O$: [M+H]$^+$) calcd: 507.20937, found: 507.20929. Anal. Calcd for $C_{31}H_{34}FeN_2O$.($H_2O)_{0.25}$: C 72.86, H 6.80, N 5.48; found: C 72.61, H 6.88, N 5.33.

1-[(4-(3-Dimethylaminopropoxy)phenyl-phenyl)methylidene]-[3]ferrocenophane, 9.
Analogously, **18** (500 mg, 1.23 mmol) prepared as indicated in ref. 20, sodium hydride (233 mg, 9.85 mmol), 3-dimethylamino-1-propyl chloride hydrochloride (240 mg, 1.48 mmol), 35 mL of DMF were treated as described above. After purification by column chromatography with acetone / NEt_3 (10/1) eluent system and then recrystallization from diethyl ether, **9** (500 mg, 83%) was obtained as a bright yellow product as a mixture of *E* and *Z* isomers (94/6). The major isomer was identified as the *Z* form. ^{1}H NMR (CDCl$_3$, 300 MHz): δ 1.81 (m, 2H, CH_2), 2.15 (s, 6H, NMe_2), 2.25-2.29 (m, 4H, CH_2N+CH_2 cycle), 2.33 (m, 2H, CH_2 cycle), 3.83 (t, J = 6.4 Hz, 2H, CH_2O), 3.89-3.91 (m, 4H, C_5H_4), 3.95 (t, J = 1.8 Hz, 2H, C_5H_4), 4.14 (t, J = 1.8 Hz, 2H, C_5H_4), 6.53 and 6.83 (d, J = 8.8 Hz, 2H,C_6H_4), 6.86 (d, J = 8.8 Hz, 2H,C_6H_4), 7.17-7.26 (m, 5H, C_6H_5). ^{13}C NMR (CDCl$_3$, 75.4 MHz): δ 27.4 and 27.5 (CH_2), 28.7 (CH_2), 40.9 (CH_2), 45.3 ($2CH_3$ NMe_2), 56.4 (CH_2), 65.9 and 66.1 (CH_2O), 68.2 (2CH, C_5H_4), 68.5 and 68.7 (2CH, C_5H_4), 70.2 (2CH, C_5H_4), 70.3 (2CH, C_5H_4), 83.7 (C_{ip}), 86.7 and 86.8 (C_{ip}), 113.2 and 114.0 (2CH, C_6H_4), 125.9 and 126.6 (CH, C_6H_5), 127.2 and 128.1 (2CH$_{arom}$), 129.3 and 130.4 (2CH$_{arom}$), 130.6 and 131.6 (2CH$_{arom}$), 133.6 and 134.3 (C), 135.5

and 135.9 (C), 140.5 and 140.6 (C), 143.4 and 143.8 (C), 157.1 and 157.7 (C). MS (EI, 70 eV) m/z: 491 [M]$^+$, 405 [M-NMe$_2$CH$_2$CH$_2$]$^+$, 86 [NMe$_2$CH$_2$CH$_2$]$^+$, 58 [NMe$_2$CH$_2$]$^+$. HRMS (ESI, C$_{31}$H$_{34}$FeNO: [M+H]$^+$) calcd: 492.1990, found: 492.1998. This compound has been prepared previously by using another synthetic procedure and tested for its antiplasmodial activity.[29]

General procedure 2: Preparation of compounds 7 (second method), 10, 11. Titanium chloride (4.66 g, 2.7 mL, 24.6 mmol) was added dropwise to a suspension of zinc powder (2.41 g, 36.8 mmol) in 100 mL of dry THF at 10-20°C and the mixture was heated at reflux for 2 h. A second solution was prepared by dissolving [3]ferrocenophan-1-one (1.47 g, 6.14 mmol) and bromo compound **19** (2.06 g, 6.14 mmol) in 25 mL of dry THF. This latter solution was added dropwise to the first solution and then the reflux was continued overnight. After cooling to room temperature, the mixture was stirred with water and dichloromethane. The mixture was acidified with diluted hydrochloric acid until the dark color disappeared and was decanted. The aqueous layer was extracted with dichloromethane and the combination of organic layers was dried on magnesium sulphate. After concentration under reduced pressure, the crude product was chromatographed on silica gel column with dichloromethane as an eluent to recover the second of the most colored bands (MS (CI, NH$_3$) m/z: 543 [M+H]$^+$) that contained the compound **20** contaminated by inseparable dehydrohalogenated compound. This crude compound was used without further purification in the second step. It was transferred into a pressure tube and a solution of amine in methanol (60 mmol of amine) was added. The pressure tube was heated at 60°C for 24 hours, then cooled to room temperature. The work-up was done depending on the amine.

1-[(4-(3-Dimethylaminopropoxy)phenyl-4-hydroxyphenyl)methylidene]-[3]ferroceno-phane, 7 (second method). Dimethylamine in methanol (2 M, 30 mL, 60 mmol) was commercially available. The mixture was concentrated under reduced pressure and the residue

was chromatographed on a silica gel column with acetone, then acetone/triethylamine 10/1 as the eluents. After concentration under reduced pressure, the residue was recrystallized from acetone to obtain yellow crystals of **7** (yield 21%).

1-[(4-(3-(N-pyrrolidinyl)propoxy)phenyl-4-hydroxyphenyl)methylidene]-[3]ferroceno-phane, 10. The solution of pyrrolidine was made up from 4.36 g of pyrrolidine in 15 mL of methanol. The compound **10** precipitated and was just filtered off to recover 0.88 g (yield : 27%) as a mixture of *Z* and *E* isomers (79/21). ^{1}H NMR (DMSO-d_6, 300 MHz): δ. 1.79-2.01 (m, 4H, CH_2 pyrrolidine), 2.02-2.21 (m, 2H, CH_2), 2.22-2.36 (m, 2H, CH_2 cycle), 2.55-2.70 (m, 2H, CH_2 cycle), 2.99-3.30 (m, 6H, CH_2N + CH_2 pyrrolidine), 3.93 (s, 2H, C_5H_4), 3.97 (s, 2H, C_5H_4), 3.99 (s, 2H, C_5H_4), 4.03-4.14 (m, 2H, CH_2O), 4.27 (s, 2H, C_5H_4), 6.46 and 6.64 (d, $J = 8.3$ Hz, 2H, C_6H_4), 6.73 and 6.76 (d, $J = 8.3$ Hz, 2H, C_6H_4), 6.85 and 6.92 (d, $J = 8.3$ Hz, 2H, C_6H_4), 6.96 and 7.10 (d, $J = 8.3$ Hz, 2H, C_6H_4), 9.26 and 9.47 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ 22.7 (CH_2 + 2CH_2 pyrrolidine), 25.5 (CH_2, cycle), 27.9 (CH_2, cycle), 51.3 (CH_2N), 52.9 (2CH_2, pyrrolidine), 64.8 (CH_2O), 68.0 (2CH, C_5H_4), 68.4 (2CH, C_5H_4), 69.6 (2CH, C_5H_4), 70.0 (2CH, C_5H_4), 83.6 (C, C_5H_4), 86.4 (C, C_5H_4), 113.1 and 114.0 (2CH, C_6H_4), 114.1 and 114.9 (2CH, C_6H_4), 130.0 (2CH, C_6H_4), 131.1 (2CH, C_6H_4), 132.3 (C), 133.8 (C), 136.0 (C), 139.7 (C), 155.4 (C), 156.9 (C). MS (CI, NH_3) m/z: 534 [M+H]$^+$, 453 [M-$(CH_2)_3N(CH_2)_4$+H]$^+$. HRMS (ESI, $C_{33}H_{36}FeNO_2$: [M+H]$^+$) calcd: 534.2095, found: 534.2111.

1-[(4-(3-(N-piperidinyl)propoxy)phenyl-4-hydroxyphenyl)methylidene]-

[3]ferrocenophane, 11. The solution of piperidine was made up with 5.22 g of piperidine in 15 mL of methanol. The compound **11** precipitated and was simply filtered off to recover 1.23 g (yield : 37%) as a mixture of *Z* and *E* isomers (90/10). ^{1}H NMR (DMSO-d_6, 300 MHz): δ. 1.13-1.25 (m, 2H, CH_2 piperidine), 1.25-1.42 (m, 4H, CH_2 piperidine), 2.06-2.20 (m, 2H,

CH$_2$), 2.39-2.49 (m, 2H, CH$_2$ cycle), 2.59-2.72 (m, 2H, CH$_2$ cycle), 3.06-3.45 (m, 6H, CH$_2$N + CH$_2$ piperidine), 3.76 (s, 2H, C$_5$H$_4$), 3.79 (s, 2H, C$_5$H$_4$), 3.81 (s, 2H, C$_5$H$_4$), 3.80-3.86 (m, 2H, CH$_2$O), 4.09 (s, 2H, C$_5$H$_4$), 6.27 and 6.43 (d, J = 8.6 Hz, 2H, C$_6$H$_4$), 6.56 and 6.66 (d, J = 8.6 Hz, 2H, C$_6$H$_4$), 6.72 and 6.76 (d, J = 8.6 Hz, 2H, C$_6$H$_4$), 6.80 and 6.90 (d, J = 8.6 Hz, 2H, C$_6$H$_4$). ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ δ 23.0 (CH$_2$, piperidine), 24.1 (CH$_2$), 25.6 (2CH$_2$, piperidine), 26.3 (CH$_2$, cycle), 27.9 (CH$_2$, cycle), 54.1 (2CH$_2$, piperidine), 55.1 (CH$_2$N), 65.8 (CH$_2$O), 68.0 (2CH, C$_5$H$_4$), 68.3 (2CH, C$_5$H$_4$), 69.6 (2CH, C$_5$H$_4$), 70.0 (2CH, C$_5$H$_4$), 83.6 (C, C$_5$H$_4$), 86.5 (C, C$_5$H$_4$), 114.0 (2CH, C$_6$H$_4$), 114.1 (2CH, C$_6$H$_4$), 130.0 (2CH, C$_6$H$_4$), 131.1 (2CH, C$_6$H$_4$), 132.3 (C), 133.9 (C), 135.6 (C), 139.8 (C), 155.4 (C), 157.3 (C). MS (CI, NH$_3$) m/z : 548 [M+H]$^+$, 423 [M-(CH$_2$)$_3$N(CH$_2$)$_5$+H]$^+$. HRMS (ESI, C$_{34}$H$_{38}$FeNO$_2$: [M+H]$^+$) calcd: 548.2252, found: 548.2261.

General Procedure 3: Acylation of aniline groups

In a Schlenk flask, the appropriate ferrocenyl amino compound was dissolved in 30 mL of anhydrous THF. Appropriate acetyl chloride derivatives and pyridine were added and the reaction mixture was left to stir at room temperature for 3 hours. The mixture was poured into water and extracted with dichloromethane. The organic layer was washed with water, dried over magnesium sulphate, filtered, and concentrated under reduced pressure. The crude mixture was filtered over silica gel using petroleum ether and CH$_2$Cl$_2$, concentrated under reduced pressure, and purified by HPLC (acetonitrile/H$_2$O). Then, recrystallization from appropriate solvent systems formed desired products as crystals consisting of a mixture of Z and E isomers.

1-[(4-(3-chloropropionyl)aminophenyl)-(4-hydroxyphenyl)methylidene]-[3]ferroceno-phane, 25. 1-[(4-Aminophenyl-4-hydroxyphenyl)methylidene]-[3]ferrocenophane, **17** (0.3 g,

0.71 mmol) prepared as indicated in ref. 19, 3-chloropropionyl chloride (0.11 g, 0.9 mmol) and pyridine (0.068 g, 0.9 mmol) were treated as described above. After purification by HPLC (acetonitrile/water 80/20), recrystallization from acetonitrile/water furnished **25** as yellow crystals (0.32 g, 88% yield) consisting of a mixture of Z and E isomers (58/42). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.32-2.38 (m, 2H, CH_2 cycle), 2.60-2.66 (m, 2H, CH_2 cycle), 2.75 and 2.82 (t, J = 6.2 Hz, 2H, CH_2), 3.83 and 3.89 (t, J = 6.2 Hz, 2H, CH_2), 3.94 (s, 2H, C_5H_4), 3.95-4.05 (m, 4H, C_5H_4), 4.28 (s, 2H, C_5H_4), 6.46, 6.75, 7.12, 7.59 (d, J = 8.5 Hz, 8H, C_6H_4) (minor isomer), 6.75, 6.87, 6.99, 7.30 (d, J = 8.5 Hz, 8H, C_6H_4) (major isomer). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 28.1 (CH_2), 39.3 (CH_2), 40.6 (CH_2), 41.0 (CH_2), 68.2 (2CH, C_5H_4), 68.6 (2CH, C_5H_4), 69.8 (2CH, C_5H_4), 70.2 (2CH, C_5H_4), 83.6 (C_{ip}), 86.7 (C_{ip}), 114.3 and 115.1 (2CH, C_6H_4), 118.0 and 118.9 (2CH, C_6H_4), 129.5 and 130.2 (2CH, C_6H_4), 130.6 and 131.3 (2CH, C_6H_4), 133.7 (C), 137.5 (C), 138.5 (C), 139.8 (C), 141.7 (C), 155.5 and 156.1 (C), 167.7 and 167.9 (CO). MS (EI, 70 eV) m/z : 511 [M]$^{+\cdot}$, 475, 432, 396, 368, 342, 121 [CpFe]$^+$. HRMS (ESI, $C_{29}H_{26}ClFeNO_2$: [M]$^{+\cdot}$) calcd: 511.09960, found: 511.09912. Anal. Calcd for $C_{29}H_{26}ClFeNO_2 \cdot (H_2O)_{0.5}$: C 66.87, H 5.22, N 2.68; found: C 66.45, H 5.18, N 2.65.

1-[(4-(3-chloropropionyl)aminophenyl-phenyl)-methylidene]-[3]ferrocenophane, 26. Analogously, 1-[(4-aminophenyl-phenyl)methylidene]-[3]ferrocenophane, **22** (0.41 g, 1.0 mmol) prepared as indicated in ref. 20, 3-chloropropionyl chloride (0.14 g, 1.1 mmol) and pyridine (0.09 g, 1.1 mmol) were treated as described above. After purification by HPLC (acetonitrile/water 90/10), recrystallization from acetonitrile yielded **26** as dark orange crystals (0.48 g, 98% yield) consisting of a mixture of Z and E isomers respectively (85/15). ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.31-2.39 (m, 2H, CH_2), 2.55-2.65 (m, 2H, CH_2), 2.72 and 2.81 (t, J = 6.2 Hz, 2H, CH_2), 3.82 and 3.88 (t, J = 6.2 Hz, 2H, CH_2), 3.98 (s, 4H, C_5H_4), 4.03 (s, 2H, C_5H_4), 4.22 (s, 2H, C_5H_4), 6.99-7.51 (m, 9H, C_6H_5 + C_6H_4). ^{13}C NMR (75.4 MHz,

CDCl$_3$): δ 28.7 and 29.7 (CH$_2$), 37.4 (CH$_2$), 39.9 (CH$_2$), 40.8 (CH$_2$), 68.9 (2CH, C$_5$H$_4$), 69.5 (2CH, C$_5$H$_4$), 70.4 (4CH, C$_5$H$_4$), 83.6 (C$_{ip}$), 86.9 (C$_{ip}$), 118.8 and 119.7 (2CH, C$_6$H$_4$), 126.1 and 126.7 (CH, C$_6$H$_5$), 127.3 and 128.2 (2CH$_{arom}$), 129.3 and 130.0 (2CH$_{arom}$), 130.5 and 131.2 (2CH$_{arom}$), 134.9 (C), 135.3 (C), 139.6 (C), 140.1 (C), 143.0 and 143.4 (C), 167.6 (CO). MS (EI, 70 eV) m/z: 495 [M]$^{+\cdot}$. Anal. Calcd for C$_{29}$H$_{26}$ClFeNO: C, 70.24; H, 5.28; N, 2.82. Found: C, 69.85; H, 5.41; N, 2.83.

2-Ferrocenyl-1-(4-(3-chloropropionyl)aminophenyl)-1-(4-hydroxyphenyl)-but-1-ene, 27.
Analogously, 2-ferrocenyl-1-(4-aminophenyl)-1-(4-hydroxyphenyl)-but-1-ene, **23** (1 g, 2.36 mmol) prepared as indicated in ref 17, 3-chloropropionyl chloride (0.3 g, 2.4 mmol) and pyridine (0.19 g, 2.4 mmol) were treated as described above. Purified first by flash column chromatography by using diethyl ether / petroleum ether solvent system as an eluent and then HPLC (acetonitrile/water: 80/20), **27** was obtained as dark orange crystals (0.53 g, 44% yield) consisting of a mixture of *Z* and *E* isomers (50/50). ^{1}H NMR (300 MHz, acetone-*d$_6$*): δ 1.01 and 1.02 (t, *J* = 7.5 Hz, 3H, CH$_3$), 2.58-2.67 (m, 2H, CH$_2$), 2.83-2.90 (m, 2H, CH$_2$), 3.87-3.93 (m, 4H, CH$_2$ and C$_5$H$_4$), 4.26 and 4.27 (t, *J* = 1.9 Hz, 2H, C$_5$H$_4$), 4.12 (s, 5H, C$_5$H$_5$), 6.71 and 6.82 (d, *J* = 8.7 Hz, 2H, C$_6$H$_4$), 6.88 and 7.07 (d, *J* = 8.7 Hz, 2H, C$_6$H$_4$), 6.98 and 7.18 (d, *J* = 8.7 Hz, 2H, C$_6$H$_4$), 7.54 and 7.65 (d, *J* = 8.7 Hz, 2H, C$_6$H$_4$), 8.27 and 8.30 (s, 1H, OH), 9.27 and 9.30 (s, 1H, NH). ^{13}C NMR (75.4 MHz, acetone-*d$_6$*): δ 15.8 (CH$_3$), 28.4 and 28.5 (CH$_2$), 40.5 (CH$_2$), 41.1 (CH$_2$), 68.7 (2CH, C$_5$H$_4$), 69.9 (5CH C$_5$H$_5$ + 2CH C$_5$H$_4$), 87.6 (C$_{ip}$), 115.8 and 115.9 (2CH, C$_6$H$_4$), 119.7 and 119.9 (2CH, C$_6$H$_4$), 130.4 and 130.9 (2CH, C$_6$H$_4$), 131.2 and 131.7 (2CH, C$_6$H$_4$), 136.7 and 137.0 (C), 137.5 and 137.7 (C), 138.2 and 138.3 (C), 141.2 (C), 146.0 (C), 156.7 (C), 168.4 (CO). MS (EI, 70 eV) m/z: 513 [M]$^{+\cdot}$, 477 [M-HCl]$^+$, 448 [M-Cp]$^+$. HRMS (ESI, [C$_{29}$H$_{28}$ClFeNNaO$_2$]$^+$: [M+Na]$^+$) calcd: 536.10516, found: 536.10506. C$_{29}$H$_{28}$ClFeNO$_2$.H$_2$O: C, 65.49; H, 5.68; N, 2.63. found: C, 65.45; H, 5.49; N, 2.58.

2-Ferrocenyl-1-(4-(3-chloropropionyl)aminophenyl)-1-phenyl-but-1-ene, **28.**

Analogously, 2-Ferrocenyl-1-(4-aminophenyl)-1-phenyl-but-1-ene, **24** (0.46 g, 1.13 mmol) prepared as indicated in ref. 21, 3-chloropropionyl chloride (0.16 g, 1.2 mmol) and pyridine (0.098 g, 1.2 mmol) were treated as described above. After purification by HPLC (acetonitrile/water 90/10), recrystallization from dichloromethane/hexane yielded **28** as orange crystals (0.22 g, 39% yield) consisting of a mixture of Z and E isomers. ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.89-1.00 (m, 3H, CH$_3$), 2.43-2.55 (m, 2H, CH$_2$), 2.61-2.75 (m, 2H, CH$_2$), 3.73-3.89 (m, 4H, CH$_2$ + C$_5$H$_4$), 3.98-4.06 (m, 7H, C$_5$H$_4$ + C$_5$H$_5$), 6.90-7.51 (m, 9H, C$_6$H$_5$ + C$_6$H$_4$). ^{13}C NMR (75.4 MHz, CDCl$_3$): δ 15.6 (CH$_3$), 28.0 and 28.1 (CH$_2$), 40.0 (CH$_2$), 40.6 (CH$_2$), 68.5 (2CH, C$_5$H$_4$), 69.5 (7CH, C$_5$H$_5$+C$_5$H$_4$), 87.0 (C$_{ip}$), 119.9 and 120.1 (2CH, C$_6$H$_4$), 126.3 (CH, C$_6$H$_5$), 128.4 (2CH$_{arom}$), 129.5 and 130.0 (2CH$_{arom}$), 130.1 and 130.7 (2CH$_{arom}$), 135.7 (C), 137.4 (C), 137.8 (C), 141.2 (C), 144.4 and 144.7 (C), 167.7 (CO). MS (CI, CH$_4$) m/z : 498 [M+H]$^+$. HRMS (ESI, C$_{29}$H$_{28}$ClFeNO: [M]$^{+\cdot}$) calcd: 497.1210, found: 497.1234. Anal. Calcd for C$_{29}$H$_{28}$ClFeNO.(H$_2$O)$_{0.25}$: C 69.33, H 5.71, N 2.78; found: C 69.48, H 5.63, N 2.71.

General Procedure 4: Dimethylamination

In a pressure tube, the appropriate 3-chloropropionylamino compound was dissolved in 2.0 M dimethylamine solution in methanol. The reaction mixture was heated to 60° C with stirring for 1 day. The mixture was concentrated under reduced pressure, a saturated solution of NaHCO$_3$ and dichloromethane were added and, after decantation, the organic layer was washed with water, dried on magnesium sulfate and concentrated under reduced pressure. The crude mixture was purified by flash column chromatography (Acetone/Et$_3$N: 10 / 1); some were recrystallized from appropriate solvent system to yield a mixture of Z and E isomers.

1-[(4-(3-dimethylaminopropionyl)aminophenyl)-(4-hydroxyphenyl)methylidene]-

[3]ferrocenophane, 12. Starting from compound **25** (0.15 g, 0.29 mmol), 2.0 M

dimethylamine solution in methanol (1.47 mL, 2.9 mmol), recrystallization from

dichloromethane/hexane system yielded **12** as a bright yellow compound (0.146 g, 96%)

consisting of a mixture of Z and E isomers (59/41). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.17

and 2.21 (s, 6H, CH$_3$), 2.29 (m, 2H, CH$_2$), 2.39 and 2.46 (t, J = 7.1 Hz, 2H, CH$_2$), 2.53-2.68

(m, 4H, CH$_2$), 3.94 (s, 2H, C$_5$H$_4$), 3.98 (m, 4H, C$_5$H$_4$), 4.28 (s, 2H, C$_5$H$_4$), 6.45, 6.74, 7.10,

7.57 (d, J = 8.4 Hz, 8H, C$_6$H$_4$) (minor isomer), 6.74, 6.85, 6.98, 7.27 (d, J = 8.4 Hz, 8H, C$_6$H$_4$)

(major isomer), 9.25 and 9.46 (s, NH), 9.93 and 10.09 (s, OH). ^{13}C NMR (75.4 MHz, DMSO-

d_6): δ 28.0 (CH$_2$), 34.4 (CH$_2$), 40.6 (CH$_2$), 44.8 (2CH$_3$), 55.1 (CH$_2$), 68.1 (2CH, C$_5$H$_4$), 68.5

(2CH, C$_5$H$_4$), 69.7 (2CH, C$_5$H$_4$), 70.1 (2CH, C$_5$H$_4$), 83.6 (C$_{ip}$), 86.5 (C$_{ip}$), 114.2 and 115.0

(2CH, C$_6$H$_4$), 117.9 and 118.8 (2CH, C$_6$H$_4$), 129.3 and 130.1 (2CH, C$_6$H$_4$), 130.4 and 131.2

(2CH, C$_6$H$_4$), 132.7 (C), 133.8 (C), 137.0 (C), 138.3 (C), 138.7 (2C), 169.9 (CO). MS (EI, 70

eV) m/z : 520 [M]$^+$, 475, 421, 396, 342, 209,121. HRMS (ESI, C$_{31}$H$_{33}$FeN$_2$O$_2$: [M+H]$^+$)

calcd: 521.18860, found: 521.18694. Anal. Calcd for C$_{31}$H$_{32}$FeN$_2$O$_2$.(H$_2$O)$_{0.25}$: C 70.32, H

6.28, N 5.29; found: C 70.15, H 6.58, N 5.08.

1-[(4-(3-dimethylaminopropionyl)aminophenyl-phenyl)-methylidene]-[3]ferroceno-

phane, 13. Analogously, **26** (0.46 g, 0.92 mmol) and 2.0 M dimethylamine solution in

methanol (4.61 mL, 9.2 mmol) were treated as described above. Recrystallization from

diethyl ether/pentane solvent system gave **13** as a bright yellow compound (0.42 g, 91%)

consisting of a mixture of Z and E isomers (83/17). ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.34 and

2.40 (s, 6H, CH$_3$), 2.39 (m, 2H, CH$_2$), 2.46 and 2.54 (t, J = 6.2 Hz, 2H, CH$_2$), 2.62 (t, J = 6.2

Hz, 2H, CH$_2$), 2.70 and 2.74 (m, 2H, CH$_2$), 4.01 (t, J = 1.7 Hz, 2H, C$_5$H$_4$), 4.03 (t, J = 1.7 Hz,

2H, C$_5$H$_4$), 4.07 (t, J = 1.7 Hz, 2H, C$_5$H$_4$), 4.25 (t, J = 1.7 Hz, 2H, C$_5$H$_4$), 7.01 (major

isomer)(d, J = 8.4 Hz, 2H, C$_6$H$_4$), 7.08-7.54 (m, 7H, CH$_{arom}$), 10.78 and 10.97 (s, 1H, NH).

^{13}C NMR (75.4 MHz, CDCl$_3$): δ 28.8 (CH$_2$), 33.5 (CH$_2$), 41.1 (CH$_2$), 44.5 (2CH$_3$), 55.2 (CH$_2$), 68.4 (2CH, C$_5$H$_4$), 68.9 (2CH, C$_5$H$_4$), 70.4 (4CH, C$_5$H$_4$), 83.7 (C$_{ip}$), 86.8 (C$_{ip}$), 118.8 and 119.7 (2CH, C$_6$H$_4$), 126.1 and 126.7 (CH, C$_6$H$_5$), 127.4 and 128.2 (2CH$_{arom}$), 129.4 and 130.0 (2CH$_{arom}$), 130.7 and 131.2 (2CH$_{arom}$), 134.4 and 134.0 (C), 136.7 (C), 138.6 and 139.1 (C), 140.7 (C), 143.7 (C), 170.6 and 170.7 (CO). MS (CI, NH$_3$) m/z : 505 [M+H]$^+$. HRMS (ESI, C$_{31}$H$_{32}$FeN$_2$NaO: [M+Na]$^+$) calcd: 527.17578, found: 527.17403.

2-Ferrocenyl-1-[4-(3-dimethylaminopropionyl)aminophenyl]-(4-hydroxyphenyl)-but-1-ene, 15. Analogously, **27** (0.12 g, 0.24 mmol) and 2.0 M dimethylamine solution in methanol (1.22 mL, 2.4 mmol), were treated as described above, purified by flash column chromatography (acetone/Et$_3$N: 10/1) to give **15** as an orange compound (0.11 g, 89%) consisting of a mixture of Z and E isomers (53/47). ^1H NMR (300 MHz, acetone-d_6): 1.02 (t, J = 7.5 Hz, 3H, CH$_3$), 2.28 and 2.30 (s, 6H, CH$_3$), 2.43-2.48 (m, 2H, CH$_2$), 2.58-2.68 (m, 4H, CH$_2$), 3.91-3.93 (m, 2H, C$_5$H$_4$), 4.06-4.08 (m, 2H, C$_5$H$_4$), 4.12 (s, 5H, C$_5$H$_5$), 6.71 and 6.82 (d, J = 8.7 Hz, 2H, C$_6$H$_4$), 6.88 and 7.06 (d, J = 8.7 Hz, 2H, C$_6$H$_4$), 6.96 and 7.15 (d, J = 8.7 Hz, 2H, C$_6$H$_4$), 7.48 and 7.58 (d, J = 8.7 Hz, 2H, C$_6$H$_4$). ^{13}C NMR (75.4 MHz, acetone-d_6): 15.9 (CH$_3$), 28.4 (CH$_2$), 35.2 (CH$_2$), 45.0 (2CH$_3$), 56.0 (CH$_2$), 68.7 (2CH, C$_5$H$_4$), 69.9 (5CH, C$_5$H$_5$), 70.0 (2CH, C$_5$H$_4$), 87.7 (C$_{ip}$), 115.8 and 115.9 (2CH, C$_6$H$_4$), 119.7 and 119.8 (2CH, C$_6$H$_4$), 130.4 and 130.9 (2CH, C$_6$H$_4$), 131.2 and 131.7 (2CH, C$_6$H$_4$), 137.0 (C), 137.3 (C), 137.5 (C), 138.5 (C), 140.8 (C), 156.7 (C), 170.8 (CO). MS (CI, NH$_3$) m/z : 523 [M+H]$^+$. HRMS (ESI, C$_{31}$H$_{35}$FeN$_2$O$_2$: [M+H]$^+$) calcd: 523.20429, found: 523.20405.

2-Ferrocenyl-1-[4-(3-dimethylaminopropionyl)aminophenyl]-1-phenyl-but-1-ene, 16. Analogously, 2-ferrocenyl-1-(4-(3-chloropropionyl)aminophenyl)-1-phenyl-but-1-ene, **28** (0.1 g, 0.19 mmol) and 2.0 M dimethylamine solution in methanol (0.97 mL, 1.9 mmol) were treated as described above. Recrystallization from dichloromethane/hexane gave **16** as an orange colored compound (0.095 g, 97%) consisting of a mixture of Z and E isomers (67/33).

^{1}H NMR (300 MHz, CDCl$_3$): 1.04 (t, J = 5.4 Hz, 3H, CH$_3$), 2.35 and 2.37 (s, 6H, CH$_3$), 2.50 (t, J = 5.4 Hz, 2H, CH$_2$), 2.58-2.68 (m, 2H, CH$_2$), 2.73-2.83(m, 2H, CH$_2$), 3.88 and 3.95 (t, J = 1.8 Hz, 2H, C$_5$H$_4$), 4.08 (t, J = 1.8 Hz, 2H, C$_5$H$_4$), 4.11 (s, 5H, C$_5$H$_5$), 7.01 and 7.06 (d, J = 8.6 Hz, 2H, C$_6$H$_4$), 7.31 and 7.15 (m, 5H, C$_6$H$_5$), 7.38 and 7.48 (d, J = 8.6 Hz, 2H, C$_6$H$_4$). ^{13}C NMR (75.4 MHz, CDCl$_3$): 15.6 (CH$_3$), 28.1 (CH$_2$), 33.5 (CH$_2$), 44.5 (2CH$_3$), 55.2 (CH$_2$), 68.3 (2CH, C$_5$H$_4$), 69.3 (5CH, C$_5$H$_5$), 69.5 (2CH, C$_5$H$_4$), 86.8 (C$_{ip}$), 119.7 and 119.9 (2CH, C$_6$H$_4$), 126.2 (CH, C$_6$H$_5$), 128.2 and 128.3 (2CH$_{arom}$), 129.5 (2CH$_{arom}$), 130.0 and 130.5 (2CH$_{arom}$), 136.9 (C), 137.4 (C), 137.6 (C), 140.3 (C), 144.7 (C), 170.6 (CO). MS (CI, CH$_4$) m/z : 507 [M+H]$^+$. HRMS (ESI, C$_{31}$H$_{34}$FeN$_2$NaO: [M+Na]$^+$) calcd: 529.191272, found: 529.19006.

1-[(4-(4-dimethylaminobutoxy)phenyl-4-hydroxyphenyl)methylidene]-[3]ferroceno-phane, 14. Compound **4** (4.22 g, 10 mmoles), was dissolved in dry DMF (50 mL) then sodium hydride (60% in oil, 0.060 g, 15 mmoles) was slowly added. The mixture was heated at 80°C, then after 10 min, 1,4-dibromobutane (6.5 g, 30 mmoles) was added. The heating was continued for 4 hours, then the mixture was cooled, water was added dropwise, and the mixture was poured into water. After extraction by diethyl ether twice, the organic layer was concentrated under reduced pressure. The residue was chromatographed on silica gel with a 1/1 dichloromethane/petroleum ether solution as the eluent. The second yellow fraction, containing **21**, was collected, but despite being contaminated with inseparable dehydrohalogenation compounds, was used in the next step without further purification. The residue was transferred into a pressure tube and General Procedure 4 was followed, giving, after crystallization from acetone, compound **14** (1.35 g, 26% overall yield) as a mixture of Z and E isomers (60/40). ^{1}H NMR (DMSO-d_6) : δ 1.44-1.84 (m, 4H, CH$_2$-CH$_2$), 2.16 and 2.19 (s, 6H, NMe$_2$), 2.23-2.39 (m, 4H, CH$_2$N+CH$_2$ cycle), 2.60-2.74 (m, 2H, CH$_2$ cycle), 3.88 and 4.00 (t, J = 5.9 Hz, 2H, CH$_2$O), 3.97 (s, 2H, C$_5$H$_4$), 4.01 (s, 2H, C$_5$H$_4$), 4.02 (s, 2H, C$_5$H$_4$), 4.31 (s, 2H, C$_5$H$_4$), 6.49 and 6.65 (d, J = 8.2 Hz, 2H, C$_6$H$_4$), 6.77 and 6.88 (d, J = 8.2 Hz, 2H,

C_6H_4), 6.78 and 6.94 (d, J = 8.2 Hz, 2H, C_6H_4), 7.01 and 7.11 (d, J = 8.2 Hz, 2H, C_6H_4), 9.27 and 9.47 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) : δ 24.3 and 24.4 (CH_2), 27.4 and 27.5 (CH_2), 28.9 (CH_2), 41.1 and 41.2 (CH_2), 45.9 and 46.0 (2CH_3 NMe_2), 59.5 (CH_2), 66.0 and 68.1 (CH_2O), 69.0 (2CH, C_5H_4), 69.4 (2CH, C_5H_4), 70.6 (2CH, C_5H_4), 71.0 (2CH, C_5H_4), 84.6 (C_{ip}), 87.5 and 87.6 (C_{ip}), 114.1 and 115.0 (2CH, C_6H_4), 115.1 and 115.9 (2CH, C_6H_4), 131.0 (2CH, C_6H_4), 132.2 (2CH, C_6H_4), 133.3 and 133.4 (C), 134.9 and 135.0 (C), 136.5 and 136.6 (C), 140.8 (C), 156.4 and 157.0 (C), 157.6 and 158.3 (C). IR (KBr, ν cm^{-1}): 3434 (OH), 3086, 3034, 2947, 2868, 2781 (CH, CH, CH_3). MS (EI, 70 eV) m/z : 521 [M]$^{+\cdot}$, 436, 422, 343, 100 [$Me_2NCH_2CH_2CH_2CH_2$]$^+$, 58 [Me_2NCH_2]$^+$. HRMS (ESI, $C_{32}H_{36}FeNO_2$: [M+H]$^+$) calcd: 522.2095, found: 522.2119.

Compounds 29 and 30 were prepared as described in ref 23

X-ray crystal structure determination for 26

Crystal data were collected at 200K on a Kappa-CCD Enraf-Nonius diffractometer with graphite monochromated MoK$_\alpha$ radiation (λ = 0.71073 Å). Orientation matrix and lattice parameters were obtained by least-squares refinement of the diffraction data of 105 reflections within the range 3° < θ < 19°. The structure was solved by direct methods and refined with full-matrix least-squares technique on F^2 using the CRYSTALS programs.[30] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were set in calculated positions and isotropically refined. The values of the discrepancy indices R1 (R$_{w2}$) were 0.079 (0.262). The final Flack[31] parameter is 0.45(4). The values of number of variable parameters are 596, and those of the goodness-of-fit are 0.984. $C_{29}H_{26}ClFeNO$; M = 495.50; orthorhombic, Pc21b ; a = 9.6052(19) Å, b = 21.638(6) Å, c = 22.845(6) Å, α = β = γ = 90°. V = 4747.9(20)Å^3; Z = 8; The data were collected in the *h k l* range:-12 to 12, -27 to 28, -29

to 25. Total reflections collected: 43371; independent reflections: 10775; Data was collected up to a $2\Theta_{max}$ value of $60°$. Number of variables: 596; R(I > 2σ(I))= 0.079, wR2(all)= 0.262, S = 0.984).; highest residual electron density 1.56e Å^{-3}).

Biological methods

Materials. Stock solutions (1 x 10^{-3} M) of the ferrocenyl complexes to be tested were prepared in DMSO and were kept at 4°C in the dark; under these conditions they are stable for at least two months. Serial dilutions in DMSO were prepared just prior to use. A stock solution (1 x 10^{-3} M) of 17β- E_2 was prepared in ethanol. (Dulbecco's modified eagle medium (DMEM) was purchased from Gibco BRL, fetal calf serum from Dutscher, Brumath, France, glutamine, E_2 and protamine sulfate were from Sigma.) MCF-7 and MDA-MB-231 cells were from the Human Tumor Cell Bank. Sheep uteri weighing approximately 7g were obtained from the slaughterhouse at Mantes-la-Jolie, France. They were immediately frozen and kept in liquid nitrogen prior to use.

Determination of the Relative Binding Affinity (RBA) of the compounds for ERα

RBA values were measured on ERα from lamb uterine cytosol. 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 previously (ref 3) was used as a source of ERα. Aliquots (200 ml) of ERα in glass tubes were incubated for 3 h at 0°C with [6,7-^{3}H]- E_2 (2 x 10^{-9}M, specific activity 1.62 TBq/mmol, NEN Life Science, Boston MA) in the presence of nine concentrations of the ferrocenyl complexes to be tested (between 6×10^{-7} M and 6×10^{-9} M for the complexes with RBA values higher than 5% and between 6×10^{-6} M and 6×10^{-8} M for the compounds with RBA values lower than 5%) or of 17β- E_2 (between 8×10^{-8} M and 7.5×10^{-10} M). At the end of the incubation period, the fractions of [^{3}H]- E_2 bound to the estrogen receptors (Y values) were precipitated by addition of a 200 ml of a cold solution of protamine sulfate (1 mg/mL in

water). After a 10 min period of incubation at 4°C, the precipitates were recovered by filtration on 25 mm circle glass microfibre filters GF/C filters using a Millipore 12 well filtration ramp. The filters were rinsed twice with cold phosphate buffer and then transferred in 20 mL plastic vials. After addition of 5 mL of scintillation liquid (BCS Amersham) the radioactivity of each fraction was counted in a Packard tri-carb 2100TR liquid scintillation analyzer. The concentration of unlabeled steroid required to displace 50% of the bound $[^3H]$-E_2 was calculated for 17β- E_2 and for each complex by plotting the logit values of Y (logit Y = ln(Y/100-Y) versus the mass of the competing complex. The RBA (relative binding affinity) was calculated as follows: RBA of a compound = concentration of E_2 required to displace 50% of $[^3H]$-E_2 x 100 / concentration of the compound required to displace 50% of $[^3H]$-E_2. The RBA value of E_2 is by definition equal to 100%.

Measurement of octanol/water partition coefficient (logPo/w) of the compounds. The logPo/w values of the compounds were determined by reverse-phase HPLC on a C-8 column (Nucleosil C8, from Macherey Nagel, France) according to the method previously described in reference 4.

4.3.4 Culture Conditions. Cells were maintained in monolayer culture in DMEM with phenol red/Glutamax I, supplemented with 9% of decomplemented fetal calf serum and 0.9% kanamycine, at 37°C in a 5% CO_2 air humidified incubator. For proliferation assays, cells were plated in 24-well sterile plates at a density of $1.1.10^4$ cells for MDA-MB-231 and of 3.10^4 cells for MCF-7 in 1 mL of DMEM without phenol red, supplemented with 9% of fetal calf serum desteroided on dextran charcoal, 0.9% Glutamax I and 0.9% kanamycine, and were incubated for 24 h. The following day (D0), 1 mL of the same medium containing the compounds to be tested diluted in DMSO, was added to the plates (final volumes of DMSO: 0.1% ; 4 wells for each conditions). After three days (D3), the incubation medium was removed and 2 mL of 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 and stained for 1h in methanol with methylene blue (2.5 mg/mL), and then washed thoroughly with water. Two milliliters of HCl (0.1 M) was then added, and the plate was incubated for 1h at 37°C. Then the absorbance of each well was measured at 655 nm with a Biorad spectrophotometer (microplate reader). The results are expressed as the percentage of proteins versus the control. Experiments were performed at least in duplicate. Some cytotoxicity measurements on MDA-MB-231 breast cancer cells were performed by ImaGIF Ciblothèque Cellulaire (Institut de Chimie des Substances Naturelles).
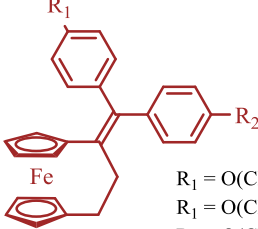
Supporting Information Available

CCDC 913875 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Table of contents

R$_1$ = O(CH$_2$)$_3$N(CH$_3$)$_2$; R$_2$ = OH R$_1$ = O(CH$_2$)$_3$N(CH$_3$)$_2$; R$_2$ = NH$_2$ R$_1$ = O(CH$_2$)$_3$N(CH$_3$)$_2$; R$_2$ = H R$_1$ = NHCO(CH$_2$)$_2$N(CH$_3$)$_2$; R$_2$ = OH R$_1$ = NHCO(CH$_2$)$_2$N(CH$_3$)$_2$; R$_2$ = H R$_1$ = O(CH$_2$)$_4$N(CH$_3$)$_2$; R$_2$ = OH	New [3]ferrocenophane derivatives bearing various side chains have been synthesized and their biochemical properties studied. These complexes have very strong antiproliferative effects against MDA-MB-231 cells (IC$_{50}$ = 0.05 to 0.39 µM)

Captions

Scheme 1: Synthesis of the [3]ferrocenophane derivatives with an alkyl dimethylamino chain

Scheme 2. Synthesis of the [3]ferrocenophane derivatives with a pyrrolidine or piperidine, side-chain

Scheme 3: Synthesis of the [3]ferrocenophane **14** , containing an -O(CH$_2$)$_4$N(CH$_3$)$_2$ chain

Scheme 4. Synthesis of the amidyl [3]ferrocenophane and ferrocene derivatives **12**, **13**, **15** and **16**

Figure 1 : OH-Tamoxifen and several ferrocenyl and [3]ferrocenophane derivatives

Figure 2 : Newly synthesized complexes

Figure 3. Molecular structure of *Z*-1-[(4-(3-chloropropionyl)aminophenyl-phenyl)-methylidene]-[3]ferrocenophane, **26**.

Figure 4. Study of the estrogenic/antiestrogenic effect of the complexes on MCF-7 cells after 5 days of culture in the presence of 1 nM of E$_2$, **7**, **8** and **12** and of 10 nM of **2**, **4**, **9**,

13, **15, 16** in a medium without phenol red. The grey bars represent the ferrocenophane complexes and the hatched bars the ferrocenyl complexes. Representative data of one experiment performed twice or three times with similar results.

Figure 5. Study of the estrogenic/antiestrogenic effect of **7** and **14** on MCF-7 cells after 4 days of culture at 10^{-7}, 10^{-8} and 10^{-9} M

Figure 6. Docking of Z isomers of **7** (left) and **14** (right) in the antagonist binding site of the α form of the human estrogen receptor (h-ERα). For clarity, the bioligands and three important amino acids are shown as ball and spoke while other amino acids appear as stick representations..

Table 1 IC_{50} values of the ferrocenophane (A series) and ferrocenyl (B series) complexes on hormone-independent breast cancer cell line (MDA-MB-231), Relative Binding Affinity (RBA) on the alpha form of the estrogen receptor, and lipophilicity (LogPo/w)

Table 2 Enthalpy variation values for the bio-ligands docked into ERα, and H-N distances