HAL open science

A new series of ferrocifen derivatives, bearing two aminoalkyl chains, with strong antiproliferative effects on breast cancer cells

Pascal Pigeon, Siden Top, Anne Vessières, Michel Huché, Meral Goermen, Mehdi El Arbi, Marie-Aude Plamont, Michael J. Mcglinchey, Gérard Jaouen

To cite this version:

Pascal Pigeon, Siden Top, Anne Vessières, Michel Huché, Meral Goermen, et al.. A new series of ferrocifen derivatives, bearing two aminoalkyl chains, with strong antiproliferative effects on breast cancer cells. New Journal of Chemistry, 2011, 35 (10), pp.2212-2218. 10.1039/c1nj20192a . hal-01230387

HAL Id: hal-01230387

https://hal.science/hal-01230387v1

Submitted on 2 Sep 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License

A new series of ferrocifen derivatives, bearing two aminoalkyl chains, with strong antiproliferative effects on breast cancer cells†

Pascal Pigeon,[a] Siden Top,*[a] Anne Vessières,[a] Michel Huché,[a] Meral Görmen,[a] Mehdi El Arbi,[a] Marie-Aude Plamont,[a] Michael J. McGlinchey[b] and Gérard Jaouen*[a]

We have prepared several organometallic systems whose structures are closely analogous to that of tamoxifen, the drug used in the treatment of hormone-dependent breast cancers, but which now possess two basic aminoalkyl chains: $O(CH_2)_3NMe_2$. Despite the absence of a phenolic functionality, these ferrocenyl compounds **3**, **4** and their organic analogue **5** recognize the estrogen receptor but in addition exhibit strong antiproliferative effects on hormone-dependent breast cancer cells (MCF-7), and also on hormone-independent ones (MDA-MB-231) with, in this case, an IC_{50} value of about 0.4 μM. The ferrocenyl moiety does not create a major effect here compared to a purely organic aromatic group. On the other hand, the presence within the molecule of two vicinal basic entities, potentially allowing complexation to metal ions such as Zn^{2+}, could perhaps be the key to the antiproliferative effectiveness of this series which operates *via* a different mechanism to that of hydroxytamoxifen **1** and hydroxyferrocifen **2**. The behaviour of these new species is discussed. They possess the distinctive feature of combining a strong antiproliferative effect with intense antibacterial and antifungal activity.

Introduction

The unusual reactivity of bioorganometallic species (complexes of biological interest incorporating a direct metal–carbon bond) is fuelling the emergence of a new organometallic-based approach to medicinal chemistry.[1–5] We have recently illustrated the potential of such a topic by changing the tamoxifen series for ferrocifens.[6–9] OH–Tam **1**, *via* its pro-drug tamoxifen, is the medication most widely used to cure hormone-dependent breast cancers (Chart 1).[10–14] The main mechanism of action of this molecule is an antiestrogenic effect which occurs at low concentration (in the sub-micromolar range) *via* a specific interaction with the alpha form of the estrogen receptor (ERα). However non-genomic interactions, *i.e.* an effect not mediated by ER, have also been described,[15] but in this case the IC_{50} value found for OH–Tam on an ER-negative cell line such as MDA-MB-231 is high (29 μM) and not useful for therapeutic purposes.[16] We have prepared a series of ferrocenyl analogues of OH–Tam by substituting the phenyl ring of OH–Tam, and also varying the length of the amino side chain (n = 2, 3–5, 8), and found that the ferrocenyl derivative **2**, with n = 3, is highly cytotoxic to both hormone-dependent and hormone-independent breast cancer cells (IC_{50} values in the range of 0.5 μM), while ferrocene itself has an IC_{50} of 160 μM.[17–19] It has been hypothesized that the novel mechanism of action of this Fc–OH–Tam **2** complex could involve the generation of quinone methide owing to a conjugated redox ferrocenyl antenna.[20–22] Very recently we have fully characterized these quinone species and shown that they could be key metabolites explaining the different behavior between the organic and organometallic series.[23] In cancer therapy the discovery of a new type of mechanism is generally of interest.[24]

Access to quinone methides requires the presence of a phenolic group in the original structure.[20] However, it is also possible to generate these antiproliferative species when the phenol is replaced by an aniline since quinone imines are accessible *via* the same redox process triggered by the ferrocenyl antenna.[25,26] This poses the question as to the unequivocal character of such a mechanism. What happens if one blocks the generation of quinone methides by protecting the phenolic functionality of Fc–OH–Tam, by incorporating a second $–O(CH_2)_3N(CH_3)_2$ aminoalkyl chain identical to the one already present? This is the question we wished to examine.

The first product tested in this new series was, for chronological reasons, the organometallic complex **3**.[7] However, when we found that the ferrocenophanes are often more active

[a] *Ecole Nationale Supérieure de Chimie de Paris (Chimie ParisTech), Laboratoire Charles Friedel UMR CNRS 7223, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05, France. E-mail: siden-top@chimie-paristech.fr, gerard-jaouen@chimie-paristech.fr; Tel: +33 1 43 26 95 55, +33 1 44 27 66 99*

[b] *School of Chemistry and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland*

† Dedicated to Prof. Didier Astruc on the occasion of his 65th birthday.

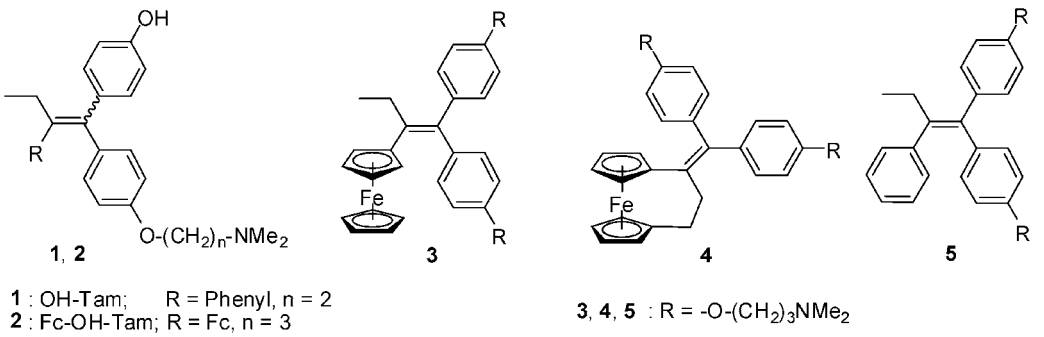


Chart 1

than their open chain counterparts,[27,28] we included the cyclic compound **4**. Finally, since the ferrocene in these structures can only operate conjugatively with difficulty to activate the redox activation process seen in Fc–OH–Tam, **2**, we chose to add the purely organic molecule **5**, where the aryl group is lipophilic but less bulky than the ferrocenyl entity. It is the totality of our results in this domain, leading to unexpected biological effects, that we present herein.

Results and discussion

Synthesis of the compounds

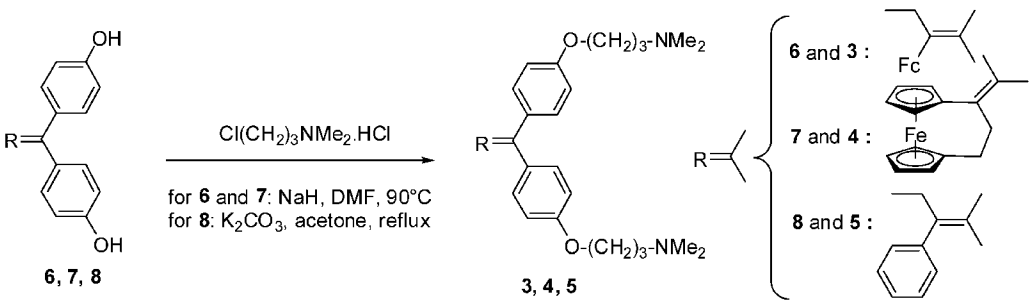
The compounds **3**, **4** and **5** were obtained by dialkylation of the corresponding phenols **6**, **7** and **8**, respectively, with dimethylaminopropyl chloride hydrochloride (Scheme 1). The phenols themselves were prepared by the McMurry coupling procedure that we have used profitably in previous syntheses.[7,9,29]

The dialkylation of the diphenols **6** and **7** was carried out in DMF using sodium hydride as the base to form the diphenolate which, when heated at 90 °C with dimethylaminopropyl chloride hydrochloride, furnished **3** (20%), starting from **6**, and **4** (53%) from **7**. Dialkylation of the diphenol **8** was carried out in acetone at reflux using K_2CO_3 as the base; the isolated yield of **5** was 64%.

Biochemical studies

The study of the effect of the three bis-dimethylaminoalkyl compounds **3**, **4**, **5** on the growth of hormone-independent (MDA-MB-231) and -dependent (MCF-7) breast cancer cells was performed together with the determination of their relative binding affinity (RBA) for ERα and of their lipophilicity. Results are reported in Table 1 and Fig. 1.

Study of the antiproliferative effect of 3, 4 and 5 on MDA-MB-231 cells. The compounds show a strong antiproliferative effect on MDA-MB-231 which can be attributed to their cytotoxicity. Quite surprisingly these IC_{50} values are in the same range, around 0.40 μM, for the three compounds. This is absolutely not the case for their corresponding dimethylamino/phenol molecules, respectively, **1**, **2** (Chart 1) and **9** (Chart 2) which have IC_{50} on MDA-MB-231 of 29, 0.5 and 0.015 μM.[16,18,31] For the organic compound **5**, replacement of the OH of **1** by a dimethylamino chain increases dramatically the cytotoxicity of the compound (ratio = 85), it has almost no effect on the



Scheme 1 Synthesis of the bis-dimethylaminoalkyl compounds **3**, **4** and **5**.

Table 1 Biochemical data of the compounds: IC_{50} values on hormone-independent breast cancer cells MDA-MB-231, relative binding affinity values (RBA) for ERα, enthalpy variation (ΔE) of the association of the molecules in the ERα binding site and lipophilicity ($\log P_{o/w}$)

Compound	IC_{50} (μM) MDA-MB-231[a]	RBA (%) on ERα[b]	ΔE (kcal mol^{-1}) on ERα	$\log P_{o/w}$[d]
1	29	38.5[c]	−151.2	3.2 (*Z*), 3.4 (*E*)
3	0.45 ± 0	2.8 ± 0.1	−89.5	3.56
4	0.40 ± 0.02	2.05 ± 0.08	−92.5	—
5	0.34 ± 0.05	54 ± 12	−136.3	3.86

[a] After 5 days of culture. [b] Mean of two experiments performed on purified ERα, except for **1** (lamb uterus cytosol); the RBA value of estradiol, the compound of reference, is by definition equal to 100%. [c] RBA, value from ref. 9. [d] Measured as described in ref. 30.

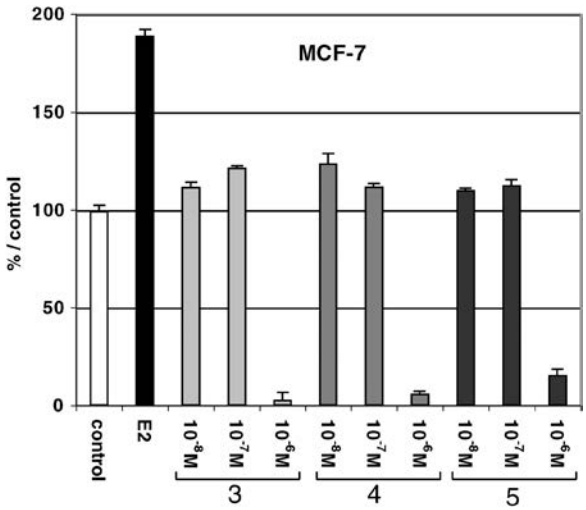


Fig. 1 Effect of various concentrations of the compounds on the growth of hormone dependent breast cancer cells MCF-7 after 72 hours in a medium without phenol red. Representative data of one experiment performed 3 times with similar results (six measurements ± limits of confidence).

3/Fc–OH–Tam couple (ratio = 1.1), and it noticeably decreases in the ferrocenophane series (ratio = 0.04).

Determination of the RBA values of the compounds for the alpha form of the estrogen receptor (ERα) and study of their effect on the growth of MCF-7 cells. All three molecules are recognized by the alpha form of the ER. This is a rather surprising result for these molecules lacking an OH group that is supposed to be one of the essential functional groups allowing the proper anchoring of a molecule in the ER binding site (Table 1). The high RBA value found for **5** was quite unpredictable and the decrease between **5** and **3** and **4** can be attributed to the bulkiness of the ferrocenyl unit. However, the molecular modeling studies (*vide infra*) provide an explanation for this result.

On MCF-7 cells, the three compounds show a slight, but reproducible, proliferative estrogenic effect at low concentrations (1×10^{-8} and 1×10^{-7} M; Fig. 1) after 72 h of incubation. In accordance to what is observed on MDA-MB-231 cells, they become highly cytotoxic at higher concentrations (between 1×10^{-7} M and 1×10^{-6} M). We have previously noted this consecutive dual effect for other series of related complexes albeit possessing diphenolic functions.[28]

Molecular modeling

Having seen that the three molecules **3**, **4**, **5** are recognized by ERα, we decided to use molecular modeling to establish the details of the molecule–receptor interaction. The structure used was that of the ligand binding domain (LBD) of ERα occupied by OH–Tam.[32] Only the amino acids that make up the wall of the cavity were retained. OH–Tam was then removed and replaced successively with **3**, **4** or **5**. An energy minimisation was then carried out, with the heavy atoms of the receptor immobilized, in order to establish the optimal position for the molecule within the active site. The Merck Molecular Force Field (MMFF) was employed for this purpose. Owing to the large number of atoms, in excess of the software limit of 600 for quantum mechanical computations, the calculations were carried out using molecular mechanics rather than quantum mechanics. Energy variation values ΔE corresponding to the binding of the molecules within the active site of the receptor are shown in Table 1. It is interesting to note that these energy values are all very negative, indicating that binding of these molecules in the active site is possible. The value obtained for the organic compound **5** is very close to that of OH–Tam, although less for compounds **3** and **4**, and is easily explained by the steric crowding of the ferrocenyl group. We note also that there is good correlation between these values and the RBA values.

A visualization of the docking of **5** and of OH–Tam in the binding site of the estrogen receptor, in the antiestrogen conformation that corresponds to the interaction with OH–Tam, is shown in Fig. 2. This shows clearly that **5** and OH–Tam dock in a similar way inside the active site. The first anchor point of the ligand is an interaction between the nitrogen of the dimethylamino chain and the aspartic acid Asp 351. This is a strong hydrogen bond between the acid function and the terminal nitrogen of the chain, which, thanks

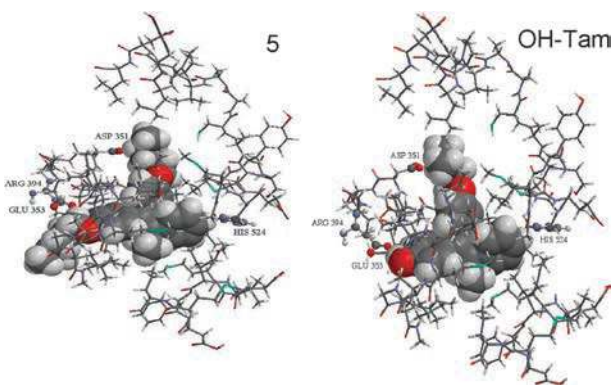


Fig. 2 Docking of **5** (left) and OH–Tam (right) in the antagonist binding site of the α form of the human estrogen receptor (h-ERα).

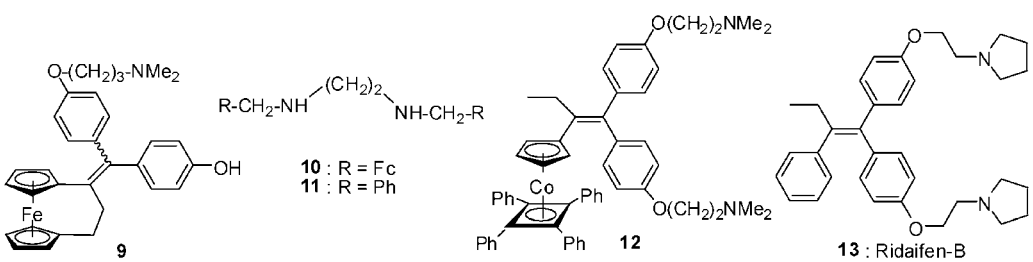
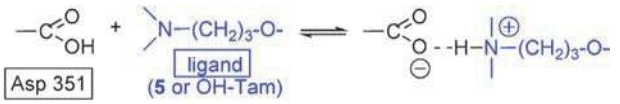
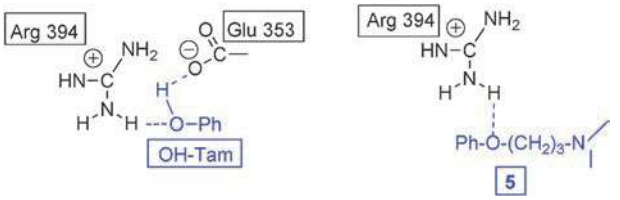


Chart 2

to the difference in their pKa, exist in the form of zwitterions, as shown in the following equation:



In the case of OH–Tam the second anchor point is composed of two hydrogen bonds with Arg 394 and Glu 353 which, owing to their proximity, are also found as zwitterions. The first is a bond between the hydrogen atom of the phenol group and Glu 353 (in carboxylate form) and the second is a bond between the oxygen of the phenol group and Arg 394 (in the form of argininium) *cf.* below. Note that in Shiau's structure, a molecule of water is also present at this level.[32]



In the case of **3**, **4**, or **5**, the phenol function no longer exists, which eliminates the first hydrogen bond, but the second can continue to exist, between the oxygen of the ether function of the amino chain and the argininium. Owing to a displacement of the molecule within the active site, this is however less strong than with OH–Tam. Nevertheless, the whole structure is stabilized by the positioning of the aliphatic chain $-(CH_2)_3-$ in a hydrophobic channel situated in this area. Overall, the replacement of the phenol function by the chain costs around 15 kcal mol^{-1} to the ΔE of the bond, but does not rule it out. These calculations provide a molecular basis for explaining both the RBA values of these compounds and their estrogenic effect as observed on hormone-dependent MCF-7 cells.

Discussion

We prepared a number of compounds selected from the ferrocifen and tamoxifen series, bearing two aminoalkyl chains of the type $O-(CH_2)_3N(CH_3)_2$, and found that they showed a strong antiproliferative effect, probably linked to their cytoxicity, on both hormone-dependent and hormone-independent breast cancer cells. In this series the ferrocenyl unit does not seem to play a specific redox activating role but rather behaves as a compact aromatic unit similar to a lipophilic aryl group. Even though a ferrocene is a compact, lipophilic aromatic metallocene (see the $\log P_{o/w}$ values of the compounds in Table 1), it is bulkier than a simple organic aromatic. This concept of bioisosterism has recently come to the fore to explain in part the behaviour of ferrocenyl groups in biology.[33] In addition, we found recently that **3** and **5** show the same significant bactericidal and fungicidal effects (on *P. aeruginosa*, *S. aureus* and *C. albicans*).[34] Therefore, the driving force of the toxicity of these molecules on cancer cells, bacteria and fungi seems to be connected exclusively to the presence of two aminoalkyl chains. This result differs from that reported for the ferrocenyl-diamine **10**. In that case the ferrocenyl complex is active against the bacterium *Mycobacterium tuberculosis* while its organic equivalent, **11**, is 8 times less effective.[35] The toxicity of these compounds seems closer to that found for polyamines derived from putrescine and spermine than to that of our compounds.[36] We note also that cobaltifen **12** (Chart 2) has been described and an IC_{50} value of 2.5 μM was found on this same cell line, MDA-MB-231.[37] This value, more than six times higher than that of the compounds described here, can be explained by the presence of a very bulky organometallic group which dilutes the relative influence of the aminoalkyl chains.

Finally, Japanese researchers have synthesized and studied ridaifen-B **13** (Chart 2).[38,39] This compound shows a potent antiproliferative effect on both MDA-MB-231 and MCF-7 cells (IC_{50} value of 1.3 μM after 48 h). In addition a COMPARE analysis performed on different cell lines revealed that the mechanism of action of this molecule differs from that of tamoxifen. They also showed that the toxicity of other symmetrical diamines was very similar.[38] This leads us to consider the origin of the significant toxicity demonstrated by this family of molecules.

Since molecules **3**, **4**, **5** and **12** can be excellent complexing agents, we used molecular modelling to estimate their affinities with Zn^{2+}, starting from the idea that this metal is strongly present in the cells and that its displacement, thanks to the presence of basic pincer groups, could lead to their malfunction. A Zn^{2+} cation was placed between the two amino chains of **3**, **4**, and **5**, and an energy minimisation was then carried out by the use of the Merck Molecular Force Field (MMFF) followed by semi-empirical quantum mechanical calculations (PM3). The $\Delta_r H^\circ$ enthalpy of formation of the resulting complex was obtained, and then in each case, the complex was dissociated without geometric modification into two entities: a Zn^{2+} cation and a molecule of **3**, **4** or **5**. The variations in $\Delta_r H^\circ$ enthalpy of the complexation were calculated from the equation: $\Delta_r H^\circ = \Delta_r H^\circ(\text{complex}) - \Delta_r H^\circ(\text{molecule}) - \Delta_r H^\circ(Zn^{2+})$. A $\Delta_r H^\circ$ value around -275 kcal mol^{-1} was obtained for all these compounds. This result confirms that these molecules are likely to be good complexing agents for divalent cations such as Zn^{2+} as well as Ca^{2+}. This hypothesis seems to be verified by the formation of a complex between **3** and $ZnCl_2$. In fact, addition of one equivalent of $ZnCl_2$ dissolved in THF to a THF solution of **3** led immediately to the formation of an orange oil at the bottom of the flask. Addition of water to this orange oil produced a yellow solution proving that the complex is partially soluble in water. The ^{1}H NMR spectrum of the complex in DMSO-d_6 shows a slight deshielding of the protons of the $CH_2CH_2NMe_2$ group (1.83–1.98, 2.46 and 2.48, 2.23 and 2.24 ppm, respectively) compared to that of **3** (1.78–1.94, 2.36 and 2.38, 2.16 and 2.17 ppm, respectively); the remaining protons are unchanged. Other complexing agents for dicationic metals such as hydroxamic acid, present at the end of the SAHA chain, have also been identified.[40–42]

Conclusion

We have shown that in the series of the OH–ferrocifens, where the formation of quinone methides can give rise to a strong cytotoxic effect on cancer cells, there may be another possibility

of action if the phenol function is blocked by an aminoalkyl chain that prevents the generation of quinone methides. This possibility is not specific to organometallic species since it is conserved when the metallocene is replaced by an organic arene. This means of access to new cytotoxic compounds will require new targets to be identified. This will be the subject of further study, although several possible leads have been suggested here, such as the complexing role of two aminoalkyl chains on metallic acid cations like Zn^{2+} or Ca^{2+}. We note that these entities combine an antitumoral effect with an antibiotic effect.[34]

Experimental

General remarks

All reactions took place under argon using standard Schlenk techniques. Anhydrous THF was obtained by distillation from sodium/benzophenone. Thin layer chromatography was performed on silica gel 60 GF254. Infrared spectra were obtained on an IRFT BOMEM Michelson-100 spectrometer equipped with a DTGS detector as a KBr plate. ^{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. Elemental analyses were performed by the microanalysis service of CNRS at Gif sur Yvette. HRMS measurements were performed by a Thermo Fischer LTQ-Orbitrap XL apparatus with an electrospray source.

1,1-Bis[4-(3-dimethylaminopropoxy)phenyl]-2-ferrocenyl-but-1-ene, 3

Compound **6** (0.424 g, 1 mmol) was dissolved in 10 mL of DMF and sodium hydride (0.32 g, 8 mmol) was added. The mixture was stirred for 10 min, then 3-dimethylamino-1-propyl chloride hydrochloride (0.375 g, 2.5 mmol) was added. The mixture was heated at 90 °C overnight. The mixture was cooled and 5 mL of ethanol were slowly added in order to destroy the remaining sodium hydride. Then, the mixture was concentrated under reduced pressure. The residue was dissolved in dichloromethane, washed twice with a diluted aqueous solution of sodium hydroxide followed with water. After drying on magnesium sulfate, the solution was concentrated under reduced pressure and the residue was chromatographed on silicagel with a 4/1 solution of chloroform/triethylamine as eluent to yield product **3** as an oil in 20% yield. An alternative route of synthesis of **3** was published recently.[34] ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.94 (t, J = 7.4 Hz, 3H, CH_3), 1.79–1.95 (m, 4H, CH_2), 2.18 (s, 6H, NMe_2), 2.19 (s, 6H, NMe_2), 2.37 (t, J = 7.2 Hz, 2H, CH_2N), 2.40 (t, J = 7.2 Hz, 2H, CH_2N), 2.50 (q, J = 7.4 Hz, 2H, CH_2), 3.83 (t, J = 1.9 Hz, 2H, C_5H_4), 3.89 (t, J = 6.6 Hz, 2H, CH_2O), 3.91 (t, J = 6.6 Hz, 2H, CH_2O), 3.98 (t, J = 1.9 Hz, 2H, C_5H_4), 4.02 (s, 5H, Cp), 6.66 (d, J = 8.7 Hz, 2H, C_6H_4), 6.77 (d, J = 8.7 Hz, 2H, C_6H_4), 6.86 (d, J = 8.7 Hz, 2H, C_6H_4), 7.02 (d, J = 8.7 Hz, 2H, C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.5 (CH_3), 27.5 (CH_2), 27.6 (CH_2), 27.9 (CH_2), 45.5 ($2NMe_2$), 56.5 ($2CH_2N$), 66.1 ($2CH_2O$), 67.9 (2CH C_5H_4), 69.1 (5CH Cp), 69.3 (2CH C_5H_4), 87.2 (C C_5H_4), 114.1 (2CH C_6H_4), 114.2 (2CH C_6H_4), 130.4 (2CH C_6H_4), 130.9 (2CH C_6H_4), 136.5 (C), 137.2 (C), 137.3 (C), 137.4 (C), 157.3 (2 C). IR (KBr, ν/cm^{-1}): 2948, 2868, 2816, 2764 (CH_2, CH_3). MS (EI, 70 eV) m/z: 594 $[M]^{+\bullet}$, 121 $[CpFe]^{+}$, 86 $[CH_2CH_2CH_2NMe_2]^{+}$, 58 $[CH_2NMe_2]^{+}$. HRMS (ESI, $C_{36}H_{47}FeN_2O_2$: $[M + H]^{+}$) calcd: 595.29815, found: 595.29681. The monoalkylated Fc–OH–TAM **2** was also isolated in 36% yield. Anal. Calcd for $C_{36}H_{46}FeN_2O_2(H_2O)$: C, 70.54; H, 7.80; N, 4.67%. Found: C, 70.58; H, 7.90; N, 4.57%.

1-[Bis(4-(3-dimethylaminopropoxy)phenyl)methylidene]-[3]ferrocenophane, 4

Compound **7** (0.422 g, 1 mmol) was dissolved in 10 mL of DMF and sodium hydride (0.32 g, 8 mmol) was added. The mixture was stirred for 10 min, then 3-dimethylamino-1-propyl chloride hydrochloride (0.375 g, 2.5 mmol) was added. The mixture was heated at 90 °C overnight, then more 3-dimethylamino-1-propyl chloride hydrochloride (0.375 g, 2.5 mmol) was added. The heating was continued for 4 h. The mixture was cooled and 5 mL of ethanol were slowly added in order to destroy the remaining sodium hydride. Then, 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 sulfate, the solution was concentrated under reduced pressure and the residue was chromatographed on silicagel with a 4/1 solution of chloroform/triethylamine as eluent. The residue was recrystallized from ethanol–water solution to yield product **4** in 53% yield. Mp: 119 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.75–1.87 (m, 2H, CH_2), 1.87–2.13 (m, 2H, CH_2), 2.17 (s, 6H, NMe_2), 2.21 (s, 6H, NMe_2), 2.24–2.31 (m, 2H, CH_2 cycle), 2.35 (t, J = 7.2 Hz, 2H, CH_2N), 2.42 (t, J = 7.2 Hz, 2H, CH_2N), 2.54–2.68 (m, 2H, CH_2 cycle), 3.83 (t, J = 6.3 Hz, 2H, CH_2O), 3.89 (s, 4H, C_5H_4), 3.94 (s, 2H, C_5H_4), 3.96 (t, J = 6.3 Hz, 2H, CH_2O), 4.13 (s, 2H, C_5H_4), 6.53 (d, J = 8.3 Hz, 2H, C_6H_4), 6.80 (d, J = 8.3 Hz, 2H, C_6H_4), 6.86 (d, J = 8.3 Hz, 2H, C_6H_4), 7.05 (d, J = 8.3 Hz, 2H, C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.5 (CH_2), 27.6 (CH_2), 28.7 (CH_2 cycle), 41.1 (CH_2 cycle), 45.4 (NMe_2), 45.5 (NMe_2), 56.4 ($2CH_2N$), 65.9 (CH_2O), 66.1 (CH_2O), 68.2 (2CH C_5H_4), 68.6 (2CH C_5H_4), 70.1 (2CH C_5H_4), 70.2 (2CH C_5H_4), 84.0 (C C_5H_4), 86.8 (C C_5H_4), 113.1 (2CH C_6H_4), 114.0 (2CH C_6H_4), 130.4 (2CH C_6H_4), 131.7 (2CH C_6H_4), 133.1 (C), 135.8 (C), 136.2 (C), 140.2 (C), 157.1 (C), 157.7 (C). IR (KBr, ν/cm^{-1}): 2943, 2856, 2814, 2763 (CH_2, CH_3). MS (EI, 70 eV) m/z: 592 $[M]^{+\bullet}$, 86 $[CH_2CH_2CH_2NMe_2]^{+}$, 58 $[CH_2NMe_2]^{+}$. HRMS (ESI, $C_{36}H_{45}FeN_2O_2$: $[M + H]^{+}$) calcd: 593.28250, found: 593.28145. Anal. Calcd for $C_{36}H_{44}FeN_2O_2(H_2O)_{0.25}$: C, 72.42; H, 7.51; N, 4.69%. Found: C, 72.47; H, 7.54; N, 4.56%.

1,1-Bis[4-(3-dimethylaminopropoxy)phenyl]-2-phenyl-but-1-ene, 5

Compound **8** (1.58 g, 5 mmol) and potassium carbonate (2.764 g, 20 mmol) were stirred in 100 mL of acetone, then 3-dimethylamino-1-propyl chloride hydrochloride (1.739 g, 11 mmol) was added. The mixture was refluxed overnight, cooled and concentrated under reduced pressure. The residue was dissolved in dichloromethane then was washed twice with a diluted aqueous solution of sodium hydroxide followed with water.

After drying over magnesium sulfate, the solution was concentrated under reduced pressure and the residue was recrystallized from ethanol–water solution to yield product **5** in 64% yield. Mp: 91 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84 (t, J = 7.4 Hz, 3H, CH_3), 1.71–1.84 (m, 2H, CH_2), 1.84–1.98 (m, 2H, CH_2), 2.13 (s, 6H, NMe_2), 2.18 (s, 6H, NMe_2), 2.30 (t, J = 7.3 Hz, 2H, CH_2N), 2.34–2.47 (m, 4H, CH_2 + CH_2N), 3.78 (t, J = 6.4 Hz, 2H, CH_2O), 3.94 (t, J = 6.4 Hz, 2H, CH_2O), 6.45 (d, J = 8.8 Hz, 2H, C_6H_4), 6.67 (d, J = 8.8 Hz, 2H, C_6H_4), 6.79 (d, J = 8.7 Hz, 2H, C_6H_4), 6.93–7.13 (m, 7H, C_6H_5 + C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.6 (CH_3), 27.5 (CH_2), 27.6 (CH_2), 29.0 (CH_2), 45.2 (NMe_2), 45.5 (NMe_2), 56.4 (CH_2N), 56.5 (CH_2N), 65.9 (CH_2O), 66.1 (CH_2O), 113.3 (2CH C_6H_4), 114.0 (2CH C_6H_4), 125.9 (CH C_6H_5), 127.8 ($2CH_{arom}$), 129.7 ($2CH_{arom}$), 130.6 ($2CH_{arom}$), 131.9 ($2CH_{arom}$), 135.7 (C), 136.2 (C), 137.9 (C), 140.9 (C), 142.7 (C), 156.9 (C), 157.7 (C). IR (KBr, ν/cm^{-1}): 2953, 2871, 2813, 2762 (CH_2, CH_3). HRMS (ESI, $C_{32}H_{43}N_2O_2$: $[M + H]^+$) calcd: 487.33191, found: 487.33075. Anal. Calcd for $C_{32}H_{42}N_2O_2(H_2O)_{0.25}$: C, 78.25; H, 8.72; N, 5.70%. Found: C, 78.26; H, 8.53; N, 5.46%.

Complexation of 1,1-bis[4-(3-dimethylaminopropoxy)phenyl]-2-ferrocenyl-but-1-ene, 3, with $ZnCl_2$

Compound **3** (0.92 g, 1.55 mmol) was dissolved in 4 mL of THF. A solution of $ZnCl_2$ (0.21 g, 1.55 mmol) in THF (4 mL) was slowly added. An orange oil was immediately formed at the bottom of the flask. The solvent was removed to leave the Zn complex as an orange oil that was dried under vacuum.

^{1}H NMR (300 MHz, DMSO-d_6) of compound **3** without addition of $ZnCl_2$: δ 1.02 (t, J = 7.2 Hz, 3H, CH_3), 1.78–1.94 (m, 4H, CH_2), 2.16 (s, 6H, NMe_2), 2.17 (s, 6H, NMe_2), 2.36 (t, J = 7.0 Hz, 2H, CH_2N), 2.38 (t, J = 7.0 Hz, 2H, CH_2N), 2.49 (q, J = 7.2 Hz, 2H, CH_2, partially hidden by DMSO), 3.85 (s, 2H, C_5H_4), 3.96 (t, J = 6.2 Hz, 2H, CH_2O), 4.00 (t, J = 6.2 Hz, 2H, CH_2O), 4.12 (s, 2H, C_5H_4), 4.15 (s, 5H, Cp), 6.83 (d, J = 8.4 Hz, 2H, C_6H_4), 6.91 (d, J = 8.4 Hz, 2H, C_6H_4), 6.93 (d, J = 8.4 Hz, 2H, C_6H_4), 7.12 (d, J = 8.4 Hz, 2H, C_6H_4).

^{1}H NMR (300 MHz, DMSO-d_6) of compound **3** with addition of $ZnCl_2$: δ 1.02 (t, J = 7.2 Hz, 3H, CH_3), 1.83–1.98 (m, 4H, CH_2), 2.23 (s, 6H, NMe_2), 2.24 (s, 6H, NMe_2), 2.46 (t, J = 7.0 Hz, 2H, CH_2N), 2.48 (t, J = 7.0 Hz, 2H, CH_2N), 2.49 (q, J = 7.2 Hz, 2H, CH_2, partially hidden by DMSO), 3.85 (s, 2H, C_5H_4), 3.96 (t, J = 6.2 Hz, 2H, CH_2O), 4.00 (t, J = 6.2 Hz, 2H, CH_2O), 4.12 (s, 2H, C_5H_4), 4.15 (s, 5H, Cp), 6.83 (d, J = 8.4 Hz, 2H, C_6H_4), 6.91 (d, J = 8.4 Hz, 2H, C_6H_4), 6.94 (d, J = 8.4 Hz, 2H, C_6H_4), 7.12 (d, J = 8.4 Hz, 2H, C_6H_4).

Biochemical experiments

Materials

Stock solutions (1×10^{-3} M) and serial dilutions of the compounds to be tested were prepared in DMSO just prior to use. Dulbecco's Modified Eagle Medium (DMEM), fetal calf serum, glutamine and kanamycin were obtained from Invitrogen. MCF-7 and MDA-MB-231 cells were from the Human Tumor Cell Bank. Glutamine, 17β-estradiol and protamine sulfate were from Sigma.

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

RBA values were measured on ERα purchased from Pan Vera (Madison, WI, USA). A volume of 10 μL of the solution containing 3500 pmol mL^{-1} were added to 16 mL of buffer (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 μL) of this solution 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^{-1}, NEN Life Science, Boston MA) in the presence of nine concentrations of the compounds 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%.

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% kanamycin, at 37 °C in a 5% CO_2 air humidified incubator. For proliferation assays, cells were plated in 24-well sterile plates with 1.5×10^4 cells for MDA-MB-231 and with 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% kanamycin, 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 condition). After three days (D3), the incubation medium was removed and 2 mL of fresh medium containing the compounds was added. At different days (D3, D4, D5 and D6), the protein content of each well was quantified by methylene blue staining as follows. Cell monolayers were fixed and stained for 1 h in methanol with methylene blue (2 mg mL^{-1}), and then washed thoroughly with water. Two millilitres of HCl (0.1 M) were then added, and the plate was incubated for 1 h at 37 °C. Then the absorbance of each well was measured at 655 nm with a Biorad microplate reader. The results are expressed as the percentage of proteins *versus* the control. Experiments were performed at least in duplicate.

Molecular modeling

Molecular modeling studies were carried out using the programs Spartan, Trident and Odyssey.[43]

Acknowledgements

We thank Barbara McGlinchey for helpful discussions, and the Agence Nationale de la Recherche (ANR 2010BLAN706 1 blanc, « Mecaferol ») for financial support. M. G. acknowledges a fellowship from the Ministère des Affaires Etrangères (France).

References

1 D. Dive and C. Biot, *ChemMedChem*, 2008, **3**, 383–391.
2 R. H. Fish and G. Jaouen, *Organometallics*, 2003, **22**, 2166–2177.
3 C. G. Hartinger and P. J. Dyson, *Chem. Soc. Rev.*, 2009, **38**, 391–401.
4 G. Jaouen, *Bioorganometallics*, Wiley-VCH, Weinheim (Germany), 2006.
5 G. Jaouen, S. Top, A. Vessières and R. Alberto, *J. Organomet. Chem.*, 2000, **600**, 23–36.
6 G. Jaouen, S. Top, A. Vessières, G. Leclercq and M. J. McGlinchey, *Curr. Med. Chem.*, 2004, **11**, 2505–2517.
7 G. Jaouen, S. Top, A. Vessières, G. Leclercq, J. Quivy, L. Jin and A. Croisy, *C. R. Acad. Sci., Ser. IIc: Chim.*, 2000, **3**, 89–93.
8 S. Top, J. Tang, A. Vessières, D. Carrez, C. Provot and G. Jaouen, *Chem. Commun.*, 1996, 955–956.
9 S. Top, A. Vessières, G. Leclercq, J. Quivy, J. Tang, J. Vaissermann, M. Huché and G. Jaouen, *Chem.–Eur. J.*, 2003, **9**, 5223–5236.
10 E. Badia, J. Oliva, P. Balaguer and V. Cavailles, *Curr. Med. Chem.*, 2007, **11**, 950–964.
11 H. Gronemeyer, J. A. Gustafsson and V. Laudet, *Nat. Rev. Drug Discovery*, 2004, **3**, 950–964.
12 V. C. Jordan, *J. Med. Chem.*, 2003, **46**, 883–908.
13 A. L. LaFrate, J. R. Gunther, K. E. Carlson and J. A. Katzenellenbogen, *Bioorg. Med. Chem.*, 2008, **16**, 10075–10084.
14 M. J. Meegan and D. G. Lloyd, *Curr. Med. Chem.*, 2003, **10**, 181–210.
15 F. Zhang, P. W. Fan, X. Liu, L. Shen, R. B. van Breeman and J. L. Bolton, *Chem. Res. Toxicol.*, 2000, **13**, 53–62.
16 D. Yao, F. Zhang, L. Yu, Y. Yang, R. B. van Breemen and J. L. Bolton, *Chem. Res. Toxicol.*, 2001, **14**, 1643–1653.
17 E. A. Hillard, A. Vessières, L. Thouin, G. Jaouen and C. Amatore, *Angew. Chem., Int. Ed.*, 2006, **45**, 285–290.
18 A. Nguyen, A. Vessières, E. A. Hillard, S. Top, P. Pigeon and G. Jaouen, *Chimia*, 2007, **61**, 761–725.
19 A. Vessières, S. Top, W. Beck, E. A. Hillard and G. Jaouen, *Dalton Trans.*, 2006, 529–541.
20 E. A. Hillard, P. Pigeon, A. Vessières, C. Amatore and G. Jaouen, *Dalton Trans.*, 2007, 5073–5081.
21 E. A. Hillard, A. Vessières, F. Le Bideau, D. Plazuk, D. Spera, M. Huché and G. Jaouen, *ChemMedChem*, 2006, **1**, 551–559.
22 A. Vessières, S. Top, P. Pigeon, E. A. Hillard, L. Boubeker, D. Spera and G. Jaouen, *J. Med. Chem.*, 2005, **48**, 3937–3940.
23 D. Hamels, P. M. Dansette, E. A. Hillard, S. Top, A. Vessières, P. Herson, G. Jaouen and D. Mansuy, *Angew. Chem., Int. Ed.*, 2009, **48**, 9124–9126.
24 A. Vessières, C. Corbet, J. M. Heldt, N. Lories, N. Jouy, I. Laios, G. Leclercq, G. Jaouen and R. A. Toillon, *J. Inorg. Biochem.*, 2010, **104**, 503–511.
25 P. Pigeon, S. Top, O. Zekri, E. A. Hillard, A. Vessières, M. A. Plamont, O. Buriez, E. Labbe, M. Huche, S. Boutamine, C. Amatore and G. Jaouen, *J. Organomet. Chem.*, 2009, **694**, 895–901.
26 O. Zekri, E. A. Hillard, S. Top, A. Vessières, P. Pigeon, M. A. Plamont, M. Huche, S. Boutamine, M. J. McGlinchey, H. Muller-Bunz and G. Jaouen, *Dalton Trans.*, 2009, 4318–4326.
27 M. Görmen, D. Plazuk, P. Pigeon, E. A. Hillard, M. A. Plamont, S. Top, A. Vessières and G. Jaouen, *Tetrahedron Lett.*, 2010, **51**, 118–120.
28 D. Plazuk, A. Vessières, E. A. Hillard, O. Buriez, E. Labbe, P. Pigeon, M. A. Plamont, C. Amatore, J. Zakrzewski and G. Jaouen, *J. Med. Chem.*, 2009, **52**, 4964–4967.
29 S. Top, B. Dauer, J. Vaissermann and G. Jaouen, *J. Organomet. Chem.*, 1997, **541**, 355–361.
30 A. Nguyen, S. Top, P. Pigeon, A. Vessières, E. A. Hillard, M. A. Plamont, M. Huché, C. Rigamonti and G. Jaouen, *Chem.–Eur. J.*, 2009, **15**, 684–696.
31 P. Pigeon, unpublished results.
32 A. K. Shiau, D. Barstad, P. M. Loria, L. Cheng, P. J. Kushner, D. A. Agard and G. L. Greene, *Cell*, 1998, **95**, 927–937.
33 N. Metzler-Nolte and M. Salmain, in *Ferrocenes: ligands, materials and biomolecules*, ed. P. Stepnicka, John Wiley & Sons, Chichester, UK, 2008, pp. 499–639.
34 M. El Arbi, P. Pigeon, S. Top, A. Rhouma, S. Aifa, A. Rebai, A. Vessières, M.-A. Plamont and G. Jaouen, *J. Organomet. Chem.*, 2011, **696**, 1038–1048.
35 D. A. Ralambomanana, D. Razafimahefa-Ramilison, A. C. Rakotohova, J. Maugein and L. Pelinski, *Bioorg. Med. Chem.*, 2008, **16**, 9546–9553.
36 R. A. Casero and P. M. Woster, *J. Med. Chem.*, 2009, **52**, 4551–4573.
37 K. Nikitin, Y. Ortin, H. Muller-Bunz, M. A. Plamont, G. Jaouen, A. Vessières and M. J. McGlinchey, *J. Organomet. Chem.*, 2010, **695**, 595–608.
38 I. Shiina, Y. Sano, K. Nakata, T. Kikuchi, A. Sasaki, M. Ikekita, Y. Nagahara, Y. Hasome, T. Yamori and K. Yamazaki, *Biochem. Pharmacol.*, 2008, **75**, 1014–1026.
39 Y. Nagahara, I. Shiina, K. Nakata, A. Sasaki, T. Miyamoto and M. Ikekita, *Cancer Sci.*, 2008, **99**, 608–614.
40 R. W. Johnstone, *Nat. Rev. Drug Discovery*, 2002, **1**, 287–299.
41 P. A. Marks and R. Breslow, *Nat. Biotechnol.*, 2007, **25**, 84–90.
42 H. Weinmann and E. Ottow, *Annual Reports in Medicinal Chemistry*, 2004, vol. 39, pp. 185–196.
43 Mac Spartan, Spartan, Trident and Odyssey, Wavefunction Co., Irvine CA 92612, USA, http://www.wavefun.com/.