Dalton Transactions

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Cite this: *Dalton Trans.*, 2013, **42**, 15489

Efficient new constructs against triple negative breast cancer cells: synthesis and preliminary biological study of ferrocifen–SAHA hybrids and related species

José de Jesús Cázares Marinero,[a,b] Marion Lapierre,[c,d,e,f] Vincent Cavaillès,*[c,d,e,f] Rénette Saint-Fort,[a,b] Anne Vessières,[a,b] Siden Top*[a,b] and Gérard Jaouen[a,b]

Chemotherapeutic agents combining several active groups within a single molecule can modulate multiple cellular pathways and, thus, exhibit higher efficacy than single-target drugs. In this study, six new hybrid compounds combining tamoxifen (**TAM**) or ferrocifen (**FcTAM**) structural motifs with suberoylanilide hydroxamic acid (**SAHA**) were synthesised and evaluated. Antiproliferative activity was first explored in cancer cell lines. Combining **FcTAM** and **SAHA** structural motifs to form the unprecedented **FcTAM–SAHA** hybrid molecule led to an increased cytotoxicity (IC_{50} = 0.7 μM) in triple-negative MDA-MB-231 breast cancer cells when compared to **FcTAM** or **SAHA** alone (IC_{50} = 2.6 μM and 3.6 μM, respectively), while the organic hybrid analogue **TAM–SAHA** was far less cytotoxic (IC_{50} = 8.6 μM). In hormone-dependent MCF-7 breast cancer cells, **FcTAM–SAHA** was more active (IC_{50} = 2.0 μM) than **FcTAM** (IC_{50} = 4.4 μM) and **TAM–SAHA** (IC_{50} > 10 μM), but less toxic than **SAHA** (IC_{50} = 1.0 μM). Surprisingly, **FcTAM–PSA**, an N^1-phenylsuberamide derivative, also possessed strong antiproliferative activity (IC_{50} = 0.5 μM and 1.8 μM in MDA-MB-231 and MCF-7 cells, respectively). Subsequent biochemical studies indicate that estrogen receptor alpha (ERα) and histone deacetylases (HDAC) are not the main targets of the hybrid compounds for their antiproliferative effect. Interestingly, both organometallic compounds were able to induce $p21^{waf1/cip1}$ gene expression in MCF-7 breast cancer cells in accordance with their antiproliferative activity.

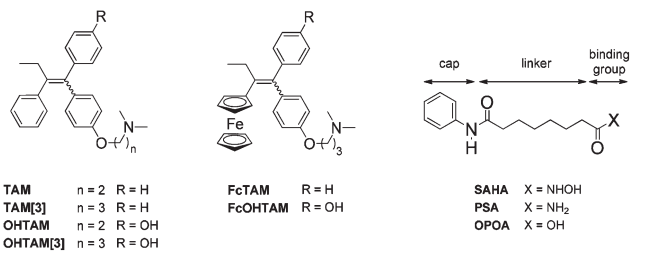
Received 15th July 2013,
Accepted 22nd August 2013

DOI: 10.1039/c3dt51917a

www.rsc.org/dalton

Introduction

Organometallic chemical biology has recently been defined as a new field of research.[1,2] In this context, organometallic compounds, which are complexes containing at least one metal–carbon covalent bond, have also been recognised – in favorable situations – as strong candidates for use as anticancer drugs.[3,4] They offer a wide range of possibilities for the design of new classes of medicinal compounds with novel mechanisms of action compared to conventional drugs.[5,6] Our group previously described the synthesis of the first organometallic derivatives with potent antiproliferative activities, particularly against breast cancer cells.[7–10] Replacement of the β-phenyl group in tamoxifen (**TAM**) – the primary antitumor drug currently used to treat hormone-dependent breast cancer – or in its active metabolite, hydroxytamoxifen (**OHTAM**), with a ferrocenyl group generates the organometallic compounds ferrocifen (**FcTAM**) and hydroxyferrocifen (**FcOHTAM**) (Chart 1).[11] It is worthwhile to note that **FcOHTAM** significantly inhibits proliferation in both hormone-dependent MCF-7 and hormone-



TAM n = 2 R = H
TAM[3] n = 3 R = H
OHTAM n = 2 R = OH
OHTAM[3] n = 3 R = OH

FcTAM R = H
FcOHTAM R = OH

SAHA X = NHOH
PSA X = NH_2
OPOA X = OH

Chart 1 Chemical structure of ferrocifen (**FcTAM**), hydroxyferrocifen, (**FcOHTAM**), tamoxifen (**TAM**), hydroxytamoxifen (**OHTAM**), suberoylanilide hydroxamic acid (**SAHA**), N^1-phenylsuberamide (**PSA**), and 8-oxo-8-(phenylamino)octanoic acid (**OPOA**).

[a]*Chimie ParisTech, Laboratoire Charles Friedel, 11, rue Pierre et Marie Curie, F75231 Paris cedex, France*
[b]*CNRS, UMR 7223, Paris, France. For medicinal chemistry (S.T.). E-mail: siden-top@chimie-paristech.fr; Fax: (+33)143260061; Tel: (+33)144276699*
[c]*IRCM, Institut de Recherche en Cancérologie de Montpellier, Montpellier, F-34298, France*
[d]*INSERM, U896, Montpellier, F-34298, France*
[e]*Université Montpellier1, Montpellier, F-34298, France*
[f]*CRLC Val d'Aurelle Paul Lamarque, Montpellier, F-34298, France. For molecular biology (V.C.). E-mail: vincent.cavailles@inserm.fr; Fax: (+33)467613787; Tel: (+33)467612405*

independent MDA-MB-231 breast cancer cells but has no effect on normal cells.[12] As a triple-negative breast cancer (TNBC) cell line, MDA-MB-231 lacks expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), making it more aggressive than other cancer subtypes since no available molecularly targeted agents are effective.[13,14]

We have postulated that the ferrocenyl group (Fc) acts as a redox antenna for phenol oxidation *via* an intramolecular mechanism producing cytotoxic species under mild oxidizing conditions.[15] This hypothesis is supported by results obtained using other ferrocenyl analogues comprising modifications at different structural levels: the alkyl chain,[16] the organometallic moiety,[17,18] the phenol position,[19] conjugation,[20] and aromatic substituents.[21] Moreover, the proposed hypothesis is consistent with the fact that cancer cells possess different redox properties compared to healthy cells.[22,23]

On the other hand, recent research has reflected a keen interest in the study of compounds that carry out their therapeutic effects by enzyme inhibition mechanisms. Histone deacetylase inhibitors (HDACi) are an important class of epigenetic drugs. Specifically, they inhibit the activity of histone deacetylases (HDACs), which catalyse the deacetylation of histones. Histone deacetylation silences gene expression by inducing DNA to adopt a closed conformation with histones, restricting its access to the transcription factors.[24,25] In addition to their effects on histones, HDACi favor hyperacetylation of non-histone targets, such as transcription factors and other proteins involved in cell cycle progression. They promote DNA repair, cell differentiation, arrest of uncontrolled growth and cancer cell death.[26,27] Based on their chemical structure, HDACi can be classified into six main groups.[28] Among them, hydroxamic acids have been the most widely studied agents, and suberoylanilide hydroxamic acid (**SAHA**, Chart 1) is a model compound. **SAHA** was validated in 2007 as a new therapeutic option for the treatment of cutaneous T cell lymphoma. The mechanism of action involves binding of its hydroxamate group to the zinc cation (Zn^{2+}) located in the HDAC cavity.[29]

HDAC inhibition represents a potentially exploitable mechanism to design new antitumor agents with the goal of extending their therapeutic spectra to other cancer types, such as breast cancer. For instance, tetrahydroisoquinoline-based hydroxamic acid derivatives,[30] *N*-hydroxy-7-(2-naphthylthio)-heptanomide[31] and **SAHA**,[32,33] were observed to be active against breast cancer cells. In an effort to improve **SAHA** HDACi activity, to enhance its cytotoxicity and to better understand the structural requirements for the design of new drugs, research has been directed towards the synthesis of analogous compounds with subtle modifications to its cap, linker and binding group (Chart 1).[34] The phenyl group has been modified by the presence of heterocycles,[35,36] nuclear receptor agonists,[37] phenyl substituents,[38] platinum agents,[39] and most recently with the incorporation of the ferrocene moiety.[40] Similarly, previous studies have also been conducted on the effect of substituents,[41] the chain length, and the presence of stereogenic centers.[42] Finally, the binding group has been replaced by different functional groups, including oximes[43] and sulfur derivatives.[44]

Both *in vitro* and *in vivo* studies have shown that **SAHA** is able to synergize with other drugs to improve antitumor activity.[45–50] One example of an active hybrid compound is triciferol, which combines an HDACi motif with 1α,25-dihydroxyvitamin-D_3 (1,25D).[37] Hence, incorporation of the **SAHA** pharmacophore into selected agents may produce new hybrid bifunctional drugs with improved efficacy. In order to evaluate the impact of the structural combinations of selected pharmacophores with the **FcTAM** molecule and to investigate their biological responses, we felt it would be interesting to prepare an **FcTAM–SAHA** hybrid and its corresponding organic analogue **TAM–SAHA** by replacing the 3-(dimethylamino)propan-1-oxy group of **FcTAM** or **TAM** with the 8-hydroxyamino-8-oxo-octanamido group of **SAHA** (Chart 2). In addition to **FcTAM–SAHA** and **TAM–SAHA**, hybrids derived from two **SAHA**-type molecules, N^1-phenylsuberamide (**PSA**) and 8-oxo-8-(phenylamino)octanoic acid (**OPOA**), bearing primary amide ($CONH_2$) and carboxylic acid (COOH) functions, respectively, were also prepared (Charts 1 and 2).

In total, six new hybrid compounds corresponding to three ferrocenyl (**FcTAM-**) and phenyl (**TAM-**) couples were synthesised. Some biological properties associated with each part of the hybrids were then tested: (i) the antiproliferative effect on cancer cells, which is very well documented for the **FcTAM** family, (ii) the interaction with estrogen receptor ERα, which is associated with the **TAM** and **OHTAM** skeleton and (iii) the ability to inhibit histone deacetylase activity to increase p21 mRNA expression, which is commonly observed using **SAHA** alone, was investigated for all hybrids to determine any possible synergistic effects.

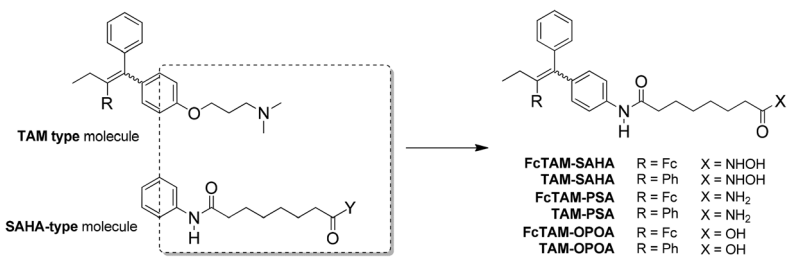


Chart 2 Design of the newly synthesised hybrid molecules.

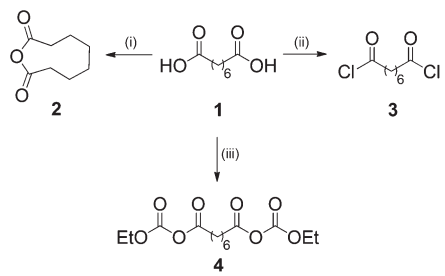
Results and discussion

Synthesis

Suberic anhydride **2** was obtained in good yields from the thermal reaction of suberic acid with acetic anhydride.[51] Suberoyl chloride **3** was obtained using modified published methods,[52,53] in which oxalyl chloride and dimethylformamide (DMF) were used as catalysts for the chlorination reaction. Carbonate **4** was produced in good yield following a modified literature method[54] by the reaction of ethyl chloroformate ($ClCO_2Et$) with suberic acid **1** in the presence of triethylamine (Et_3N) (Scheme 1).

4-(2-Ferrocenyl-1-phenylbut-1-en-1-yl)aniline **5** and 4-(1,2-diphenylbut-1-en-1-yl)aniline **6** were obtained as a mixture of *Z* and *E* isomers (*Z*/*E* ratio = 85/15 for **5** and 95/5 for **6**) by McMurry cross coupling reactions between 4-aminobenzophenone and the corresponding ketone as previously described,[55,56] N^1-[4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)phenyl]-N^8-hydroxysuberamide **FcTAM–SAHA** can be prepared from the reaction of the aniline **5** with one of the activated forms of **1**, such as suberic anhydride **2**, suberoyl chloride **3** or suberoyl ethylcarbonate **4** (Scheme 2). The nucleophilic substitution reaction of **2**, **3** or **4** with **5** resulted in similar yields (50%) of carboxylic acid **FcTAM–OPOA**. Chloride **3** was more suitable for the synthesis of carboxylic acid **FcTAM–OPOA** due to its ease of formation and rapid reaction with aniline **5**. Under the same reaction conditions, the organic carboxylic acid **TAM–OPOA** was obtained from **6** in 51% yield. In both cases, undesirable formation of bisanilides **7** and **8** (20–50%) from the reaction with **2**, **3** and **4** were observed. To obtain the ferrocenyl hybrid compound **FcTAM–SAHA** and the organic hybrid compound **TAM–SAHA**, carboxylic acids **FcTAM–OPOA** and **TAM–OPOA** were first activated by reaction with $ClCO_2Et$ and Et_3N. Addition of freshly prepared hydroxylamine (NH_2OH) to the activated carboxylic acids **FcTAM–OPOA** and **TAM–OPOA** produced **FcTAM–SAHA** and **TAM–SAHA**, respectively, each in 30% yield. In a similar reaction, primary amides **FcTAM–PSA** and **TAM–PSA** were synthesised by addition of sodium amide ($NaNH_2$) in excess to the activated carboxylic acids **FcTAM–OPOA** and **TAM–OPOA**.

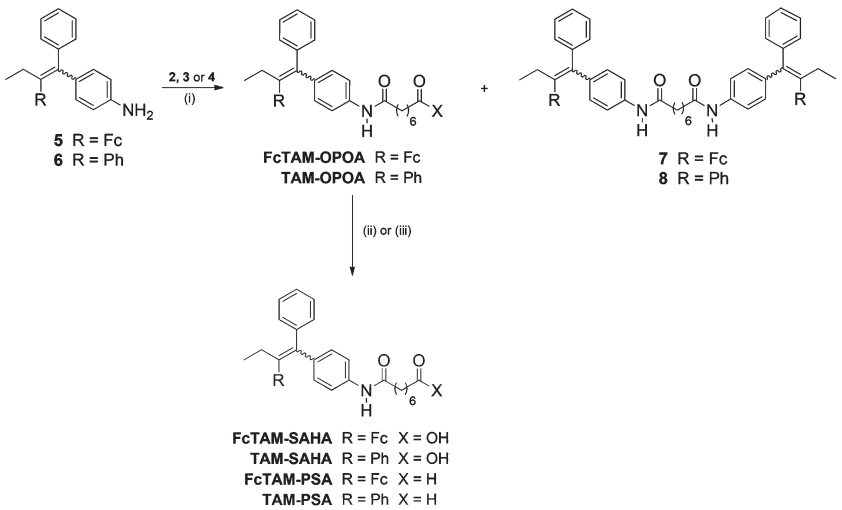
All compounds were obtained as a mixture of *Z* and *E* isomers that could not be separated by flash chromatography. Identification of *E* and *Z* isomers was performed by 2D NMR experiments. Proportions of *E* and *Z* isomers are summarised in Table 1. A large excess of the *Z* isomer, similar to that of **5** and **6**, was observed in all mixtures (Table 1). Stability of the compounds was assessed by ^1H NMR, which indicated that all compounds were stable in DMSO-d_6 at room temperature for at least 10 days. During this period, no isomerization was observed. The organic carboxylic acid **OPOA** and the organic amide **PSA** (Chart 1), corresponding to the simplest **SAHA** analogues, were also synthesised for comparison.



Scheme 1 Synthesis of suberic acid derivatives. Reagents and conditions: (i) acetic anhydride, 160 °C, 2 h, 99%. (ii) $(COCl)_2$, DMF, CH_2Cl_2, 1 h, 92%. (iii) $ClCO_2Et$, Et_3N, THF, 30 min, rt, 95%.

Biological evaluation

1. Antiproliferative effect. The antiproliferative effect of 10 μM of the compounds was first evaluated in three cancer cell lines, including MDA-MB-231 and MCF-7 breast cancer cells, which are the archetypes of hormone-independent and

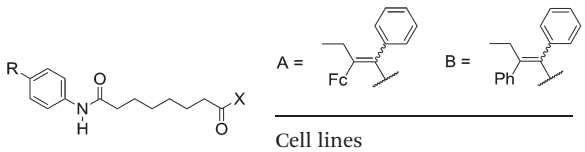


Scheme 2 Synthesis of hybrid compounds. Reagents and conditions: (i) THF, 48–55 °C, 1 h with **2**; rt, 15 min with **3**; rt, 1 h with **4**, 50% and 51% yield for **FcTAM–OPOA** and **TAM–OPOA**, respectively. (ii) THF, $ClCO_2Et$, Et_3N, 10 min, 0 °C and then $NH_2OH{\cdot}HCl$, KOH, MeOH, 15 min, 0 °C to rt, 30% for **FcTAM–SAHA** and **TAM–SAHA**. (iii) THF, $ClCO_2Et$, Et_3N, 10 min, 0 °C then $NaNH_2$, rt, 30 min, 45% and 40% yield for **FcTAM–PSA** and **TAM–PSA**, respectively.

Table 1 *Z*/*E* isomer proportions (%) of organometallic and organic compounds

Compound	*Z*	*E*	Compound	*Z*	*E*
FcTAM–SAHA	94	06	**TAM–SAHA**	89	11
FcTAM–PSA	82	18	**TAM–PSA**	92	08
FcTAM–OPOA	86	14	**TAM–OPOA**	91	09

Table 2 Cell growth inhibition (%) using 10 μM of each compound in three cancer cells lines after 72 h[a] and lipophilicity (log $P_{o/w}$)



			Cell lines			
Compound	R	X	MDA-MB-231	MCF-7	PC-3	log $P_{o/w}$
SAHA	H	NHOH	90 ± 1	87 ± 1	77 ± 1	1.7
FcTAM–SAHA	A	NHOH	83 ± 1	75 ± 2	47 ± 3	6.8
TAM–SAHA	B	NHOH	58 ± 3	43 ± 4	49 ± 10	5.8
PSA	H	NH_2	21 ± 3	41 ± 5	23 ± 9	2.1
FcTAM–PSA	A	NH_2	92 ± 1	76 ± 4	37 ± 3	6.6
TAM–PSA	B	NH_2	52 ± 4	36 ± 4	<5	5.6
OPOA	H	OH	7 ± 3	32 ± 3	26 ± 2	3.7
FcTAM–OPOA	A	OH	57 ± 6	59 ± 1	26 ± 3	7.5
TAM–OPOA	B	OH	41 ± 1	32 ± 7	<5	6.5

[a] Data are the means ± standard deviation (SD) of two independent experiments performed in triplicate.

hormone-dependent breast cancer cells, respectively, and one hormone-independent prostate cancer cell line, PC-3 (Table 2). All experiments were performed using mixtures of both isomers, taking into account that *Z* isomers comprised more than 80%.

At 10 μM, all hybrid compounds showed significant antiproliferative effect on breast cancer cells with a slightly greater effect against MDA-MB-231 (ER−) cells than MCF-7 (ER+) cells. **SAHA** was also very effective on MDA-MB-231 cells, while **PSA** and **OPOA**, differing only by the functional group, were significantly less active against these cell lines. The antiproliferative effects of the ferrocenyl hybrids **FcTAM–SAHA**, **FcTAM–PSA** and **FcTAM–OPOA** were always greater than those of their corresponding organic phenyl hybrids (**TAM–SAHA**, **TAM–PSA** and **TAM–OPOA**). Cytotoxicities of **SAHA** and **PSA** hybrids were quite similar and significantly higher than those of **OPOA** derivatives. In PC-3 cells, all compounds showed lesser efficiency but following a similar trend. Interestingly, findings of the current study are not consistent with our previous study using a series of ferrocenophane compounds, which exhibited similar antiproliferative effects in MDA-MB-231 and PC-3 cells.[57]

IC_{50} values for MDA-MB-231 and MCF-7 breast cancer cells were determined for the most active compounds, and the results are summarised in Table 3. The superior antiproliferative effect of ferrocenyl hybrids (IC_{50} = 0.7 μM for **FcTAM–SAHA** and 0.5 μM for **FcTAM–PSA**) over their corresponding organic

Table 3 IC_{50} values (μM) of selected compounds in MDA-MB-231 and MCF-7 breast cancer cell lines after 72 h[a]

Compound	MDA-MB-231	MCF-7
SAHA	3.6 ± 0.5	1.0 ± 0.2
FcTAM	2.6 ± 0.7	4.4 ± 0.9
FcTAM–SAHA	0.7 ± 0.1	2.0 ± 0.1
TAM–SAHA	8.6 ± 0.8	>10
FcTAM–PSA	0.5 ± 0.1	1.8 ± 0.9
TAM–PSA	25.9 ± 4.9	>10

[a] Data are the means ± standard deviation (SD) of two independent experiments performed in duplicate.

analogues (IC_{50} = 8.6 μM for **TAM–SAHA** and 25.9 μM for **TAM–PSA**) was confirmed. These IC_{50} values of **FcTAM–SAHA** and **FcTAM–PSA** have been mentioned by us for the sake of comparison with other ferrocenyl complexes bearing succinic and adipic chains.[58] Interestingly, these results suggest a synergistic effect between **FcTAM** and **SAHA** or **PSA**. IC_{50} values of **FcTAM–SAHA** and **FcTAM–PSA** were four to seven times lower than those of their parent molecules (IC_{50} = 0.5 μM or 0.7 μM for **FcTAM–PSA** and **FcTAM–SAHA** *vs.* 2.6 μM or 3.6 μM for **FcTAM** and **SAHA**, respectively). Such a synergistic effect was not observed with organic **TAM** hybrids.

Superiority of organometallic hybrid compounds over organic derivatives was also observed in hormone-dependent MCF-7 breast cancer cells. However, antiproliferative effects were still lower compared to those in MDA-MB-231 cells. This may be due to the estrogenic effects expressed by these hybrid compounds on MCF-7 cells at low concentrations which slightly counteract their antiproliferative activity. This point will be further discussed.

Regarding the lipophilicity of the compounds (Table 2, log $P_{o/w}$), the ferrocene derivatives were, as expected, more lipophilic than their corresponding hybrid organic analogues. Both series were much more lipophilic than **SAHA** and its **PSA** and **OPOA** analogues. In terms of functionality, the order of lipophilicities is: carboxylic acid > hydroxamic acid > amide, except for amide **PSA**, which was slightly more lipophilic than **SAHA**.

2. Analysis of the effects on estrogen signaling. Part of the antiproliferative activity of the compounds in MCF-7 cells, the archetype of estrogen receptor positive (ER+) breast cancer cells, may be related to an anti-estrogenic effect. Thus, the expression of the latter was studied *via* three different evaluations: (i) the determination of their relative binding affinity (RBA) for the alpha form of the estrogen receptor (ERα), (ii) the regulation of estrogen signalling, and (iii) the effect of compounds at low concentration on the growth of MCF-7 cells.

2.1 Relative binding affinity (RBA) for ERα. RBA of the six hybrids for ERα was measured and summarised in Table 4. **TAM–SAHA**, **TAM–PSA** and **TAM–OPOA** organic derivatives exhibited high affinity for ERα, ranging from 21.7 to 25%. These values are higher than would be expected for compounds lacking the 4-hydroxy group. The latter is considered essential for receptor–ligand interactions, such as in **OHTAM**. RBA values for **FcTAM–SAHA**, **FcTAM–PSA** and **FcTAM–OPOA**

Table 4 Relative binding affinity (RBA) of compounds for ERα

Compounds	RBA (%)
β-Estradiol (E_2)	100
TAM–SAHA	21.7
TAM–PSA	25.0
TAM–OPOA	22.3
FcTAM–SAHA	9.5
FcTAM–PSA	6.9
FcTAM–OPOA	4.1

ferrocene derivatives were lower than the organic compounds; however, they were reasonably well-recognized by the receptor with RBA values of 9.5%, 6.9% and 4.1%, respectively. The lower affinity of organometallic compounds compared to that of their organic analogues was previously reported for this class of organometallic species.[11] The difference in affinity between the carboxylic acid, hydroxamic acid and amide functions became more pronounced in the ferrocene series, where the affinity followed the order: hydroxamic acid > amide > carboxylic acid.

2.2 Regulation of estrogen signaling. The effects of the two hybrid hydroxamic acids, **FcTAM–SAHA** and **TAM–SAHA**, and the two hybrid amides, **FcTAM–PSA** and **TAM–PSA**, on ERα transcriptional activity were investigated. To do so, we used stably transfected bioluminescent reporter HELN-ERα cells, which are derived from HeLa cells stably expressing full-length ERα and an ERE-driven luciferase plasmid.[59] As shown in Fig. 1, strong anti-estrogenic activity was observed for 10 nM **OHTAM[3]**, while a moderate effect was only observed at higher concentrations of the four hybrid compounds. This result confirms that the strong antiproliferative effects of ferrocene complexes are not related to their anti-estrogenic activity, and ERα is not their principal target for its cytotoxicity.

2.3 Estrogenic and anti-estrogenic effects in MCF-7 cells. The estrogenic and anti-estrogenic effects of compounds were tested at a low concentration (10 nM) on hormone-dependent MCF-7 breast cancer cells. 17β-Estradiol (E_2) and **OHTAM** were used as references for estrogenic and anti-estrogenic activities,

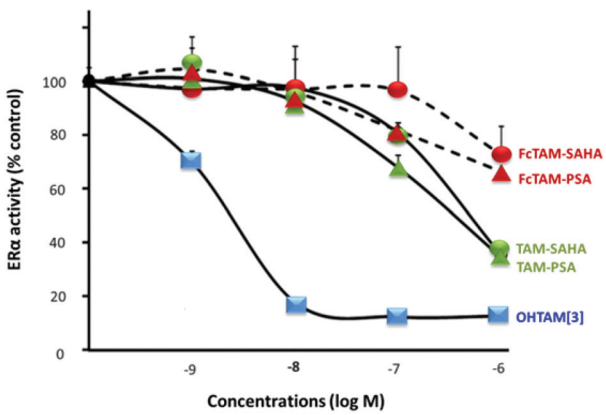


Fig. 1 Effect of hybrid hydroxamic acids (**FcTAM–SAHA** and **TAM–SAHA**) and amides (**FcTAM–PSA** and **TAM–PSA**), as compared to **OHTAM[3]**, on ERα activity in HELN-ERα cells.

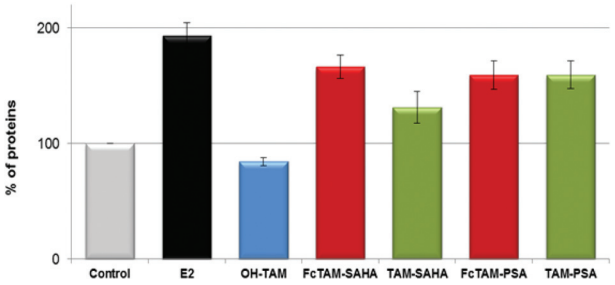


Fig. 2 Effect of 10 nM 17β-estradiol (E_2), **OHTAM** and hybrid compounds on the growth of MCF-7 cells after five days of culture. The mean of three separate experiments is reported.

respectively. A significant estrogenic effect was induced by all hybrid compounds (Fig. 2). Ferrocenyl compounds **FcTAM–SAHA** and **FcTAM–PSA** showed similar effects as an organic compound **TAM–PSA**, while **TAM–SAHA** possessed slightly lower estrogenic activity. Thus, as previously observed, the presence of a TAM-like structure is not systematically associated with an anti-estrogenic effect, and RBA values do not correlate with estrogenicity of molecules. Eventually, this estrogenic effect could explain the increase of IC_{50} values from MDA-MB-231 to MCF-7 (*vide supra*).

3. Effect on histone deacetylase activity. The ability of different compounds to act as histone deacetylase inhibitors (HDACi) was then measured experimentally using a fluorescent assay based on the deacetylation reaction of a substrate bearing an acetylated lysine side chain (BML-AK500 kit from Enzo Life Sciences). 10 μM trichostatin A (**TSA**) was used as a control. As expected, the results show that **SAHA** inhibited HDAC activity (Fig. 3). In contrast, its organic analogue, amide **PSA**, was inactive. The latter proves that hydroxamic function is important for HDAC inhibition. Both hybrid hydroxamic acids, **FcTAM–SAHA** and **TAM–SAHA**, showed significant enzymatic inhibition; however, this activity was lower than that of **SAHA**. Similar to **PSA**, **TAM–PSA** and **FcTAM–PSA** amides, which both lack hydroxamic function, were not active. This

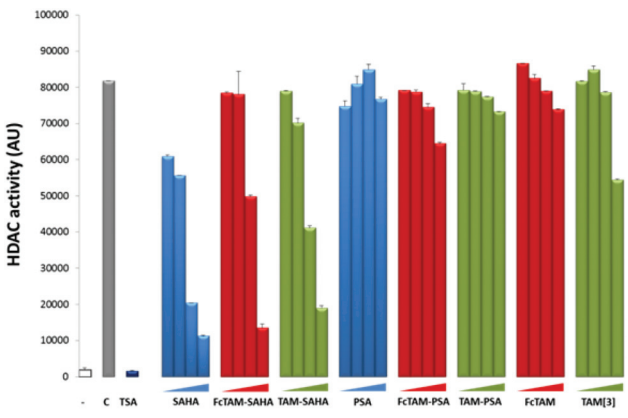


Fig. 3 Effect of hydroxamic acids (**SAHA**, **FcTAM–SAHA**, **TAM–SAHA**), amides (**PSA**, **FcTAM–PSA**, **TAM–PSA**), **TAM[3]** and **FcTAM** at 0.1, 1, 10, and 100 μM on HDAC activity. The background activity is denoted by (–). 10 μM trichostatin A (**TSA**) was used as a control.

was also the case for **TAM[3]** and **FcTAM**, which had no effect on HDAC activity. Therefore, only hydroxamic compounds inhibited HDAC activity. Interestingly, contrary to the remarkable difference in the antiproliferative effects of **FcTAM–SAHA** and **TAM–SAHA**, both organometallic and organic compounds exhibited similar HDAC inhibition activity, indicating that HDAC enzymes are not their principal target.

In this context, it is well documented that the HDACi activity of **SAHA** is related to the interaction of hydroxamate with Zn^{2+} in the enzyme pocket of HDAC.[17] In addition, it is also known that hydroxamic acids are used in the extraction of certain metals, such as iron and zinc.[60–62] Furthermore, hydroxamic acids are considered to be siderophores because of their powerful chelating capacity and transport of Fe^{3+} in microbial metabolism,[63] and they are easily revealed by qualitative colorimetric tests with ferric salts.[64] Therefore, we evaluated the chelating ability of hydroxamic acids **FcTAM–SAHA**, **TAM–SAHA** and **SAHA** by reaction with Fe^{3+}. The colourless THF solutions of **SAHA** and **TAM–SAHA**, as well as the orange solution of **FcTAM–SAHA**, immediately turned dark brown upon the addition of $FeCl_3$. This colour change was not observed with amides and carboxylic acids. As such, only the hydroxamic acids **FcTAM–SAHA** and **TAM–SAHA** could form chelates with Fe^{3+}. This observation is consistent with a recent crystallographic study that demonstrated interaction of **SAHA** with metals, such as Fe^{3+} and Zn^{2+}, to form tris- and bis-hydroxamato complexes, respectively.[65]

4. Effect on the expression of p21. Effects on the expression of an endogenous HDACi target gene, $p21^{waf1/cip1}$ (CDKN1A), in MCF-7 breast cancer cells were investigated for the hydroxamic acids **SAHA**, **TAM–SAHA** and **FcTAM–SAHA**, as well as the amides **PSA**, **TAM–PSA** and **FcTAM–PSA** using previously described methods.[66,67] As shown in Fig. 4, the levels of p21 mRNA increased upon treatment with **SAHA**, but only moderately with **PSA**. The high level of p21 mRNA after **SAHA** treatment may be related to its HDAC inhibition activity.[68] Thus, low levels of p21 mRNA resulting from treatment with **TAM–SAHA** compared to **SAHA** may be a consequence of its low HDACi activity (Fig. 3). Interestingly, **FcTAM–SAHA** led to increased expression of p21 mRNA compared to **TAM–SAHA**, while both had similar HDACi activity. Surprisingly, organometallic amide **FcTAM–PSA** also led to increased p21 mRNA compared to **FcTAM–SAHA**, while organic amide **TAM–PSA** displayed a low effect similar to **PSA**.

Similar to **SAHA**, **FcTAM–SAHA** and **FcTAM–PSA** ferrocene derivatives are also able to induce p21 gene expression. Since amide **FcTAM–PSA** had no HDACi activity, ferrocene derivatives and **SAHA** may not follow the same mechanism to increase p21 gene expression. Consequently, the high activity of this type of organometallic complexes may be attributed to the specific properties of the ferrocenyl antenna. A possible explanation for upregulation of p21 mRNA induced by ferrocene derivatives is that the conjugated metallocene may induce production of reactive oxygen species.[69,70] One of the transcriptional targets of redox status is the tumour suppressor gene p53,[71] which is known to be a major inducer of p21 gene expression.[72] As such, ferrocifen derivatives may be able to differentially impact the p53 signaling pathway. Preliminary results indicate that the ferrocene derivative **FcTAM–PSA** is able to upregulate the expression of other p53 target mRNAs (namely PIG and PUMA[73]) in MCF-7 breast cancer cells.

Conclusion

This study describes a new family of hybrid compounds, which combine select structural motifs of tamoxifen (**TAM**), ferrocifen (**FcTAM**) and **SAHA**. Results indicate that ferrocene derivatives are far more active than organic analogues in triple-negative MDA-MB-231 cells and hormone-dependent MCF-7 breast cancer cell lines, confirming the importance of this organometallic moiety in the anticancer activity of the compounds. The antiproliferative activity of **FcTAM** can be improved by replacing its 3-(dimethylamino)propan-1-oxy group with an 8-hydroxyamino-8-oxooctanamido or an 8-amino-8-oxooctanamido group. **FcTAM–SAHA** and amide **FcTAM–PSA** exhibited better antiproliferative effects than **SAHA** against triple-negative MDA-MB-231 breast cancer cells, but both were observed to be less active than **SAHA** in hormone-dependent MCF-7 breast cancer cells. In contrast to its strong antiproliferative effects, the hybrid amide **FcTAM–PSA** was less active than **SAHA**, **TAM–SAHA** and **FcTAM–SAHA** as an HDAC inhibitor. **FcTAM–SAHA** and **TAM–SAHA** exhibit similar HDACi activity yet display a large difference in their

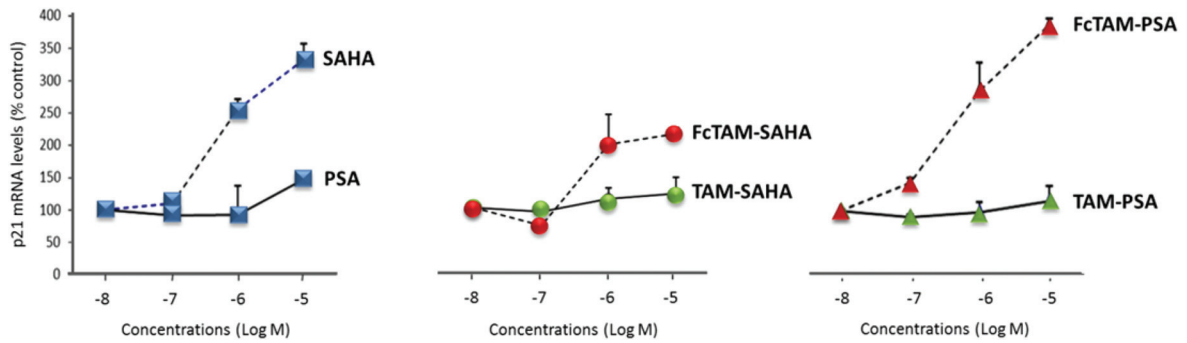


Fig. 4 Effect of hydroxamic acids and amides on the expression of p21 mRNA in MCF-7 cells.

cytotoxicities, while **FcTAM–PSA** shows strong toxic effects against cancer but does not produce HDAC inhibition. This enables us to infer that the antiproliferative activity of such ferrocenyl compounds is likely due to specific properties of the organometallic antenna. Anti-estrogenic effects and HDAC inhibition may not play a primary role in the antiproliferative activity of such complexes. In other words, ERα and HDAC are not the principal targets whereby the ferrocene derivatives exert their cytotoxic effects as described in this work. Interestingly, both organometallic compounds were able to induce p21 gene expression. The redox properties of ferrocene compounds and the production of reactive oxygen species, which we have already explored on other related series,[69] could be the key to their activity *via* a mechanism that may involve the modulation of p53 activity. The fact remains that the hybrid constructs **FcTAM–SAHA** and **FcTAM–PSA**, despite the lack of a phenol function in the molecule as in **FcOHTAM**,[69] are astonishingly active against the subtype of breast cancer cells called triple negative and whose vital prognosis is bleak. The reasons for this efficacy have to be investigated.

Experimental section

Chemical procedures

General considerations. THF was distilled from Na/benzophenone under an argon atmosphere and CH_2Cl_2 was distilled from P_2O_5. All reagents and solvents were obtained from commercial suppliers and used without further purification. Thin layer chromatography was performed on silica gel 60 GF_{254}. Column chromatography was performed on silica gel Merck 60 (40–63 μm). All of the products were characterized by conventional techniques. IR spectra were recorded using a Jasco FT/IR-4100 Fourier transform infrared spectrometer by the KBr technique and all data are expressed in wave numbers (cm^{-1}). Melting points were obtained using a Kofler device and are uncorrected. ^1H and ^{13}C NMR spectra were recorded using a 300 MHz Bruker spectrometer and chemical shifts (δ) are expressed in ppm. The mass spectra were obtained using a DSQII and ITQ 1100 Thermo Scientific spectrometer for both the electronic impact (EI) and chemical ionization (CI) methods and an API 3000 PE Sciex from Applied Biosystems for the electrospray ionization (ESI) method. A purity of >99% was confirmed by elemental analysis and analytical reverse phase HPLC with a column Kromasil C18, 10 μm, L = 25 cm, D = 4.6 mm using MeOH as an eluent, flow rate = 1 mL min^{-1}, λ = 254 nm. Elemental analyses were performed by the Laboratory of Microanalysis at ICSN of CNRS at Gif sur Yvette, France. **FcTAM**, **OPOA** and **SAHA** were prepared according to literature procedures.[11,38]

Procedures and analytical data

Suberoyl chloride (3). A suspension of **1** (28.7 mmol, 5.00 g) in 50 mL of CH_2Cl_2 was stirred at room temperature. Oxalyl chloride (63.2 mmol, 8.05 g) was added dropwise and then 0.5 mL of DMF was added. After 1 h of stirring at room temperature, all the volatile compounds were evaporated. 6.45 g (92%) of desired product **3** were obtained. ^1H-NMR (300 MHz, $CDCl_3$, ppm): δ 1.38 (m, 4H, CH_2), 1.71 (m, 4H, CH_2), 2.89 (t, J = 7.2 Hz, 4H, CH_2). ^{13}C-NMR (75 MHz, $CDCl_3$, ppm): δ 24.7 (CH_2), 27.9 (CH_2), 46.9 (CH_2), 173.7 (CO). IR (CH_2Cl_2, υ_{max}/cm^{-1}): 2943, 2866 (Alkyl C–H stretch), 1797 (C═O stretch). MS (EI, m/z): 211 $[M]^{+\bullet}$, 175 $[M - Cl]^+$, 139 $[M - Cl - HCl]^+$.

Suberoyl ethylcarbonate (4). To a solution of **1** (31.4 mmol, 5.473 g) in 60 mL of THF, $ClCO_2Et$ (69.2 mmol, 6.6 mL) was added followed by Et_3N (75.5 mmol, 10.5 mL). After 1 h of stirring at room temperature, the mixture was filtered, washed with water and extracted with AcOEt. The organic layer was dried with $MgSO_4$, filtered and the solvents were evaporated under vacuum. 9.5 g (95%) of a yellowish transparent oil were obtained. ^1H-NMR (300 MHz, $(CD_3)_2CO$, ppm): δ 1.32 (t, J = 7.1 Hz, 6H, CH_3), 1.43 (m, 4H, CH_2), 1.69 (m, 4H, CH_2), 2.55 (t, J = 7.3 Hz, 4H, CH_2), 4.30 (q, J = 7.1 Hz, 4H, CH_2). ^{13}C-NMR (75 MHz, $(CD_3)_2CO$, ppm): δ 14.2 (CH_3), 24.7 (CH_2), 28.1 (CH_2), 33.4 (CH_2), 66.3 (CH_2), 149.9 (CO), 168.9 (CO).

8-[4-(2-Ferrocenyl-1-phenylbut-1-en-1-yl)phenyl]amino-8-oxo-octanoic acid (FcTAM–OPOA). A solution of **5** (5.2 mmol, 2.1 g) in 50 mL of CH_2Cl_2 was added in 10 min into a stirred solution of **2** (7.7 mmol, 1.2 g) in 20 mL of CH_2Cl_2. After it was stirred at 50–55 °C for 1 h, the mixture was cooled at room temperature, poured into a solution of KOH and acidified with HCl. The product was extracted with AcOEt, the organic phase was dried over $MgSO_4$ and filtered. Solvents were evaporated and the crude product was purified by column chromatography using AcOEt as an eluent. 1.45 g (50%) of a brownish solid of **FcTAM–OPOA** was obtained. With **4**, the reaction proceeded for 1 h as well, but at room temperature, and with **3** the reaction finished in 10 min at room temperature. Z/E ratio: 58/42, mp. 153–154 °C. Z isomer ^1H-NMR (300 MHz, $(CD_3)_2SO$, ppm): δ 0.97 (t, J = 7.0 Hz, 3H, CH_3), 1.26 (m, 4H, $2CH_2$), 1.47 (m, 2H, CH_2), 1.54 (m, 2H, CH_2), 2.16 (m, 2H, CH_2), 2.24 (m, 2H, CH_2), 2.46 (q, J = 7.0 Hz, 2H, CH_2), 3.81 (t, J = 1.9 Hz, 2H, Cp_{subst}), 4.09 (t, J = 1.9 Hz, Cp_{subst}), 4.11 (s, 5H, Cp), 6.94 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 7.10–7.35 (m, 5H, $5CH_{Ar}$), 7.46 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 9.82 (s, 1H, NH), 11.97 (s, 1H, OH). E isomer ^1H-NMR (300 MHz, $(CD_3)_2SO$, ppm): δ 0.98 (t, J = 7.0 Hz, 3H, CH_3), 1.26 (m, 4H, $2CH_2$), 1.47 (m, 2H, CH_2), 1.54 (m, 2H, CH_2), 2.16 (m, 2H, CH_2), 2.24 (m, 2H, CH_2), 2.46 (q, J = 7.0 Hz, 2H, CH_2), 3.75 (t, J = 1.9 Hz, 2H, Cp_{subst}), 4.06 (t, J = 1.9 Hz, 2H, Cp_{subst}), 4.10 (s, 5H, Cp), 7.02 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 7.10–7.35 (m, 5H, $5CH_{Ar}$), 7.53 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 9.85 (s, 1H, NH), 11.97 (s, 1H, OH). ^{13}C-NMR (75 MHz, $(CD_3)_2CO$, ppm): δ 15.9 (CH_3), 25.5 (CH_2), 26.1 (CH_2), 28.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 34.1 (CH_2), 37.7 (CH_2), 68.9 (Cp_{subst}), 69.9 (Cp), 70.0 (Cp_{subst}), 87.2 (Cp_{ipso}), 119.7 (C_{Ar}), 127.0 (C_{Ar}), 129.1 (C_{Ar}), 130.4 (C_{Ar}), 130.8 (C_{Ar}), 138.0 (C═C), 138.4 (C═C), 138.8 (C_{Ar}), 140.5 (C_{Ar}), 145.7 (C_{Ar}), 171.9 (C═O), 174.7 (C═O). IR (KBr, υ_{max}/cm^{-1}): 3320 (N–H and O–H stretch), 3094, 3043 (aromatic C–H stretch), 2931, 2873 (alkyl C–H stretch), 1705 (OC═O stretch), 1647 (NC═O stretch), 1593 (aromatic C═C stretch), 1523 (N–H bend), 1400 (C–N stretch). MS (EI, m/z):

563 $[M]^{+\bullet}$, 498 $[M - Cp]^+$, 480 $[M - H_2O - Cp]^+$. Anal. Calc. for $C_{34}H_{37}FeNO_3 \cdot H_2O$ (%): C, 70.22; H, 6.76; N, 2.41. Found: C, 70.12; H, 6.66; N, 2.15. R_F: 0.81 (Me_2CO). HPLC (R_T), 3.44 min.

8-[4-(1,2-Diphenylbut-1-en-1-yl)phenyl]amino-8-oxooctanoic acid (FcTAM–OPOA). A solution of **6** (4.0 mmol, 1.19 g) and **2** (10.0 mmol, 1.56 g) in 30 mL of distilled THF was stirred at 48–50 °C for 1 h. The mixture was cooled at room temperature, poured into a solution of KOH and acidified with HCl. The product was extracted with AcOEt, and the organic phase was dried over $MgSO_4$ and filtered. Solvents were evaporated and the crude product was purified by column chromatography using AcOEt as an eluent. 0.927 g (51%) of a white solid was obtained as the desired product **8**. With **4**, the reaction proceeded for 1 h at room temperature. With **3**, the reaction finished in 10 min at room temperature. *Z*/*E* ratio: 91/9, mp: 169–170 °C. *Z* isomer ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): δ 0.83 (t, J = 7.2 Hz, 3H, CH_3), 1.23 (m, 4H, $2CH_2$), 1.47 (m, 4H, $2CH_2$), 2.12–2.20 (m, 4H, $2CH_2$), 2.36 (q, J = 7.2 Hz, 2H, CH_2), 6.71 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 7.08–7.29 (m, 10H, $10CH_{Ar}$), 7.34 (t, J = 7.5 Hz, 2H, $2CH_{Ar}$), 9.69 (s, 1H, NH), 11.93 (s, 1H, OH). ^{13}C-NMR (75 MHz, $(CD_3)_2CO$, ppm): δ 13.9 (CH_3), 25.5 (CH_2), 26.1 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 34.2 (CH_2), 37.7 (CH_2), 119.0 (C_{Ar}), 127.1 (C_{Ar}), 127.6 (C_{Ar}), 128.9 (C_{Ar}), 129.2 (C_{Ar}), 130.2 (C_{Ar}), 130.6 (C_{Ar}), 131.8 (C_{Ar}), 138.5 (C_{Ar}), 138.8 (C_{Ar}), 139.6 (C═C), 142.6 (C═C), 143.3 (C_{Ar}), 144.6 (C_{Ar}), 171.8 (C═O), 174.7 (C═O). IR (KBr, υ_{max}/cm^{-1}): 3336 (N–H and O–H stretch), 3060 (aromatic C–H stretch), 2931, 2862 (alkyl C–H stretch), 1705 (OC═O stretch), 1643 (NC═O stretch), 1597 (aromatic C═C stretch), 1531 (N–H bend), 1408 (C–N stretch). MS (CI, *m*/*z*): 473 $[MNH_4]^+$, 456 $[MH]^+$. Anal. Calc. for $C_{30}H_{33}NO_3$ (%): C, 79.09; H, 7.30; N, 3.07. Found: C, 78.60; H, 7.32; N, 3.12. R_F: 0.73 (Me_2CO). HPLC (R_T), 2.90 min.

***N*^{1},*N*^{8}-Bis[4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)phenyl]suberamide (7).** This compound is the byproduct of the reaction to obtain **FcTAM–OPOA**. mp: 208–210 °C. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): δ 0.99 (t, J = 7.4 Hz, 6H, $2CH_3$), 1.27–1.41 (m, 4H, $2CH_2$), 1.50–1.68 (m, 4H, $2CH_2$), 2.20–2.37 (m, 4H, $2CH_2$), 2.40–2.60 (q, J = 7.4, 4H, $2CH_2$), 3.83 (t, J = 1.9 Hz, 4H, Cp_{subst}), 4.10 (t, J = 1.9 Hz, 4H, Cp_{subst}), 4.12 (s, 10H, 2Cp), 6.96 (d, J = 8.4 Hz, 4H, $4CH_{Ar}$), 7.06–7.25 (m, 6H, $6CH_{Ar}$), 7.34 (t, J = 7.4 Hz, 4H, $4CH_{Ar}$) 7.49 (d, J = 8.4 Hz, 4H, $4CH_{Ar}$), 9.84 (s, 1H, NH). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$, ppm): δ 15.4 (CH_3), 25.1 (CH_2), 27.1 (CH_2), 28.5 (CH_2), 36.3 (CH_2), 68.0 (Cp_{subst}), 68.8 (Cp_{subst}), 69.1 (Cp), 85.5 (Cp_{ipso}), 119.0 (C_{Ar}), 126.2 (C_{Ar}), 128.4 (C_{Ar}), 128.8 (C_{Ar}), 129.5 (C_{Ar}), 136.6 (C═C), 137.0 (C═C), 137.6 (C_{Ar}), 139.1 (C_{Ar}), 144.4 (C_{Ar}), 171.1 (C═O). IR (KBr, υ_{max}/cm^{-1}): 3398, 3290 (N–H stretch), 3093, 3040 (aromatic C–H stretch), 2931, 2866 (alkyl C–H stretch), 1662 (NC═O stretch), 1593 (aromatic C═C stretch), 1520 (N–H bend), 1400 (C–N stretch). MS (ESI, *m*/*z*): 953 $[MH]^+$. HRMS for $C_{60}H_{60}Fe_2N_2O_2$ $[M]^+$, calc.: 952.3354; found: 952.3379. R_F: 0.58 (hexane–AcOEt: 50/50). HPLC (R_T), 5.00 min.

***N*^{1},*N*^{8}-Bis[4-(1,2-diphenylbut-1-en-1-yl)phenyl]suberamide (8).** This compound is the byproduct of the reaction to obtain **TAM–OPOA**. mp: 212–214 °C. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): δ 0.85 (t, J = 7.3 Hz, 6H, $2CH_3$), 1.18–1.36 (m, 4H, $2CH_2$), 1.42–1.65 (m, 4H, $2CH_2$), 2.19 (t, J = 7.3 Hz, 4H, $2CH_2$), 2.37 (q, J = 7.3 Hz, 4H, $2CH_2$), 6.72 (d, J = 8.5 Hz, 4H, $4CH_{Ar}$), 7.05–7.30 (m, 20H, $20CH_{Ar}$), 7.38 (t, J = 7.4 Hz, 4H, $4CH_{Ar}$), 9.69 (s, 1H, NH). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$, ppm): δ 13.4 (CH_3), 25.1 (CH_2), 28.5 ($2CH_2$), 36.3 (CH_2), 118.1 (C_{Ar}), 126.3 (C_{Ar}), 126.8 (C_{Ar}), 128.0 (C_{Ar}), 128.3 (C_{Ar}), 129.0 (C_{Ar}), 129.4 (C_{Ar}), 130.5 (C_{Ar}), 138.0 ($2C_{Ar}$), 138.9 (C═C), 141.1 (C═C), 141.7 (C_{Ar}), 142.6 (C_{Ar}), 171.0 (C═O). IR (KBr, υ_{max}/cm^{-1}): 3271 (N–H stretch), 3097, 3047 (aromatic C–H stretch), 2962, 2931, 2862 (alkyl C–H stretch), 1655 (NC═O stretch), 1596 (aromatic C═C stretch), 1527 (N–H bend), 1400 (C–N stretch). MS (CI, *m*/*z*): 754 $[MNH_4]^+$, 737 $[MH]^+$. HRMS for $C_{52}H_{53}N_2O_2$ $[MH]^+$, calc.: 737.4107; found: 737.4110. R_F: 0.48 (hexane–AcOEt: 50/50). HPLC (R_T) 3.28 min (Macherey-Nagel C18, 5 micron, 4.6 × 150 mm).

***N*^{1}-[4-(2-Ferrocenyl-1-phenylbut-1-en-1-yl)phenyl]-*N*^{8}-hydroxysuberamide (FcTAM–SAHA).** A solution of $NH_2OH \cdot HCl$ (4.2 mmol, 0.293 g) in 5 mL of MeOH was added to a stirred solution of KOH (4.2 mmol, 0.236 g) in 5 mL of MeOH at 0 °C. After it was stirred for 15 min, the precipitate was removed and the filtrate was placed in a flask. In another flask, to a solution of **FcTAM–OPOA** (1.4 mmol, 0.80 g) in 15 mL of anhydrous THF, cooled to 0 °C, $ClCO_2Et$ (2.1 mmol, 0.2 mL) and Et_3N (2.5 mmol, 0.35 mL) were added and the mixture was stirred for 10 min and filtered. The filtrate was added to the freshly prepared solution of NH_2OH in MeOH. The resulting mixture was stirred at room temperature for 15 min. After that, water was added, the mixture was slightly acidified with HCl and the product was extracted with AcOEt; the organic phase was dried over $MgSO_4$ and filtered. The solvents were evaporated and the crude product was purified by column chromatography using AcOEt–petroleum ether as an eluent. 0.246 g (30%) of **FcTAM–SAHA** was obtained. *Z*/*E* isomer ratio: 94/6, mp: 142–144 °C. *Z* isomer ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): δ 0.97 (t, J = 7.4 Hz, 3H, CH_3), 1.24 (m, 4H, $2CH_2$), 1.46 (m, 2H, CH_2), 1.53 (m, 2H, CH_2), 1.91 (t, J = 7.4 Hz, 2H, CH_2), 2.24 (t, J = 7.4 Hz, 2H, CH_2), 2.46 (q, J = 7.4 Hz, 2H, CH_2), 3.81 (t, J = 1.9 Hz, 2H, Cp_{subst}), 4.09 (t, J = 1.9 Hz, 2H, Cp_{subst}), 4.10 (s, 5H, Cp), 6.94 (d, J = 8.6 Hz, 2H, $2CH_{Ar}$), 7.18–7.23 (m, 3H, $3CH_{Ar}$), 7.32 (t, J = 7.4 Hz, 2H, $2CH_{Ar}$), 7.46 (d, J = 8.6 Hz, 2H, $2CH_{Ar}$), 8.65 (s, 1H, NH), 9.82 (s, 1H, NH), 10.31 (s, 1H, OH). *E* isomer ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): δ 0.97 (t, J = 7.4 Hz, 3H, CH_3), 1.24 (m, 4H, $2CH_2$), 1.46 (m, 2H, CH_2), 1.53 (m, 2H, CH_2), 1.91 (t, J = 7.4 Hz, 2H, CH_2), 2.24 (t, J = 7.4 Hz, 2H, CH_2), 2.46 (q, J = 7.4 Hz, 2H, CH_2), 3.75 (t, J = 1.9 Hz, 2H, Cp_{subst}), 4.06 (t, J = 1.9 Hz, 2H, Cp_{subst}), 4.10 (s, 5H, Cp), 6.94 (d, J = 8.6 Hz, 2H, $2CH_{Ar}$), 7.18–7.23 (m, 3H, $3CH_{Ar}$), 7.32 (t, J = 7.4 Hz, 2H, $2CH_{Ar}$), 7.46 (d, J = 8.6 Hz, 2H, $2CH_{Ar}$), 8.65 (s, 1H, NH), 9.82 (s, 1H, NH), 10.31 (s, 1H, OH). ^{13}C-NMR (100 MHz, $(CD_3)_2SO$, ppm): δ 15.7 (CH_3), 25.9 (CH_2), 26.0 (CH_2), 28.3 (CH_2), 29.3 (CH_2), 29.5(CH_2), 33.0 (CH_2), 37.5 (CH_2), 68.8 (Cp_{subst}), 69.8 (Cp), 69.9 (Cp_{subst}), 87.1 (Cp_{ipso}), 119.6 (C_{Ar}), 126.8 (C_{Ar}), 129.0 (C_{Ar}), 129.9 (C_{Ar}), 130.7 (C_{Ar}), 137.9 (C═C), 138.3 (C═C), 138.7 (C_{Ar}), 140.4 (C_{Ar}), 145.5 (C_{Ar}), 170.6 (C═O), 171.7 (C═O). IR (KBr, υ_{max}/cm^{-1}): 3266 (N–H and O–H stretch), 3095, 3035 (aromatic C–H stretch), 2927, 2862 (alkyl C–H stretch), 1655

(NC═O stretch), 1593 (aromatic C═C stretch), 1519 (N–H bend), 1400 (C–N stretch). MS (EI, *m*/*z*): 578 $[M]^{+\bullet}$, 562 $[M - O]^{+\bullet}$, 544 $[M - H_2O_2]^{+\bullet}$, 497 $[M - O - Cp]^{+}$, 407 $[FcEtC{=}CPhPhNH_2]^{+\bullet}$. (ESI, MeCN, *m*/*z*): 578 $[M]^{+\bullet}$. Anal. Calc. for $C_{34}H_{38}FeN_2O_3{\cdot}\frac{1}{3}H_2O$ (%): C, 69.86; H, 6.77; N, 4.79. Found: C, 70.02; H, 6.64; N, 4.62. R_F: 0.74 (Me_2CO). HPLC (R_T), 3.70 min.

***N*^{1}-[4-(1,2-Diphenylbut-1-en-1-yl)phenyl]-*N*^{8}-hydroxysuberamide (TAM–SAHA).** A solution of $NH_2OH{\cdot}HCl$ (4.2 mmol, 0.293 g) in 5 mL of MeOH was added to a stirred solution of KOH (4.2 mmol, 0.236 g) in 5 mL of MeOH at 0 °C. After it was stirred for 15 min, the precipitate was removed and the filtrate was placed in a flask. In another flask, to a solution of **8** (1.0 mmol, 0.455 g) in 10 mL of anhydrous THF, cooled to 0 °C, $ClCO_2Et$ (1.4 mmol, 0.13 mL) and Et_3N (1.7 mmol, 0.24 mL) were added and the mixture was stirred for 10 min and filtered. The filtrate was added to the freshly prepared solution of NH_2OH in MeOH. The resulting mixture was stirred at room temperature for 15 min. After that, water was added, the mixture was slightly acidified with HCl and the product was extracted with AcOEt; the organic phase was dried over $MgSO_4$ and filtered. The solvents were evaporated and the crude product was purified by column chromatography using AcOEt–petroleum ether as an eluent. 0.141 g (30%) of **TAM–SAHA** was isolated. *Z*/*E* isomer ratio: 89/11, mp. 135–137 °C. *Z* isomer ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): δ 0.83 (t, J = 7.3 Hz, 3H, CH_3), 1.20 (m, 4H, $2CH_2$), 1.46 (m, 4H, $2CH_2$), 1.89 (t, J = 7.4 Hz, 2H, CH_2), 2.18 (t, J = 7.4 Hz, 2H, CH_2), 2.36 (q, J = 7.3 Hz, 2H, CH_2), 6.71 (d, J = 8.5 Hz, 2H, $2CH_{Ar}$), 7.09–7.29 (m, 10H, $10CH_{Ar}$), 7.36 (t, J = 7.2 Hz, 2H, $2CH_{Ar}$), 8.64 (s, 1H, NH), 9.69 (s, 1H, NH), 10.30 (s, 1H, OH). ^{13}C-NMR (75 MHz, $(CD_3)_2CO$, ppm): δ 13.8 (CH_3), 26.0 (CH_2), 26.1 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 33.1 (CH_2), 37.6 (CH_2), 118.0 (C_{Ar}), 127.0 (C_{Ar}), 127.5 (C_{Ar}), 128.8 (C_{Ar}), 129.1 (C_{Ar}), 130.1 (C_{Ar}), 130.5 (C_{Ar}), 131.7 (C_{Ar}), 138.3 (C_{Ar}), 138.7 (C_{Ar}), 139.5 (C═C), 142.5 (C═C), 143.2 (C_{Ar}), 144.5 (C_{Ar}), 170.9 (C═O), 171.8 (C═O). IR (KBr, υ_{max}/cm^{-1}): 3240 (N–H and O–H stretch), 3051 (aromatic C–H stretch), 2931, 2862 (alkyl C–H stretch), 1655 (NC═O stretch), 1597 (aromatic C═C stretch), 1523 (N–H bend), 1400 (C–N stretch). MS (CI, *m*/*z*): 488 $[MNH_4]^+$, 471 $[MH]^+$. Anal. Calc. for $C_{30}H_{34}N_2O_3{\cdot}\frac{1}{2}H_2O$ (%): C, 75.13; H, 7.36; N, 5.84. Found: C, 75.06; H, 7.35; N, 4.70. R_F: 0.70 (Me_2CO). HPLC (R_T), 3.34 min.

***N*^{1}-[4-(2-Ferrocenyl-1-phenylbut-1-en-1-yl)phenyl]suberamide (FcTAM–PSA).** To a solution of **Fc-TAM–OPOA** (1.4 mmol, 0.800 g) in 15 mL of anhydrous THF, cooled to 0 °C, $ClCO_2Et$ (2.1 mmol, 0.2 mL) and Et_3N (2.5 mmol, 0.35 mL) were added and the mixture was stirred for 10 min. The solid was filtered off and an excess of $NaNH_2$ was added to the filtrate. After 30 min of stirring, 20 mL of water was slowly added. The product was extracted with AcOEt; the organic layer was dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography using mixtures of AcOEt and petroleum ether. 0.350 g (45%) of **FcTAM–PSA** was obtained. *Z*/*E* isomer ratio: 82/18, mp: 98–100 °C. *Z* isomer ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): δ 0.97 (t, J = 7.4 Hz, 3H, CH_3), 1.25 (m, 4H, $2CH_2$), 1.45 (m, 2H, CH_2), 1.54 (m, 2H, CH_2), 2.00 (t, J = 7.4 Hz, 2H, CH_2), 2.25 (t, J = 7.4 Hz, 2H, CH_2), 2.46 (q, J = 7.4 Hz, 2H, CH_2), 3.81 (t, J = 1.9 Hz, 2H, Cp_{subst}), 4.09 (t, J = 1.9 Hz, 2H, Cp_{subst}), 4.11 (s, 5H, Cp), 6.67 (s, 1H, NH), 6.94 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 7.10–7.25 (m, 4H, $3CH_{Ar}$ and NH), 7.32 (t, J = 7.3 Hz, 2H, $2CH_{Ar}$), 7.46 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 9.82 (s, 1H, NH). *E* isomer ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): δ 0.97 (t, J = 7.4 Hz, 3H, CH_3), 1.25 (m, 4H, $2CH_2$), 1.45 (m, 2H, CH_2), 1.54 (m, 2H, CH_2), 2.00 (t, J = 7.4 Hz, 2H, CH_2), 2.25 (t, J = 7.4 Hz, 2H, CH_2), 2.46 (q, J = 7.4 Hz, 2H, CH_2), 3.75 (t, J = 1.9 Hz, 2H, Cp_{subst}), 4.06 (t, J = 1.9 Hz, 2H, Cp_{subst}), 4.11 (s, 5H, Cp), 6.67 (s, 1H, NH), 7.02 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 7.10–7.25 (m, 4H, $3CH_{Ar}$ and NH), 7.32 (t, J = 7.3 Hz, 2H, $2CH_{Ar}$), 7.53 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 9.85 (s, 1H, NH). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$, ppm): δ 15.4 (CH_3), 24.9 (CH_2), 25.0 (CH_2), 27.0 (CH_2), 28.4 (CH_2), 28.5 (CH_2), 35.1 (CH_2), 36.3 (CH_2), 68.0 (Cp_{subst}), 68.7 (Cp_{subst}), 69.0 (Cp), 85.4 (Cp_{ipso}), 118.9 (C_{Ar}), 126.1 (C_{Ar}), 128.3 (C_{Ar}), 128.7 (C_{Ar}), 129.4 (C_{Ar}), 136.5 (C═C), 136.9 (C═C), 137.5 (C_{Ar}), 139.0 (C_{Ar}), 144.3 (C_{Ar}), 171.1 (C═O), 174.2 (C═O). IR (KBr, υ_{max}/cm^{-1}): 3410 (N–H stretch), 3097 (aromatic C–H stretch), 2931, 2862 (alkyl C–H stretch), 1662 (NC═O stretch), 1601 (aromatic C═C stretch), 1523 (N–H bend), 1408 (C–N stretch). MS (EI, *m*/*z*): 562 $[M]^{+\bullet}$, 497 $[M - Cp]^{+}$, 479 $[M - H_2O - Cp]^{+}$, 407 $[FcEtC{=}CPhPhNH_2]^{+\bullet}$, 342 $[FeCpEtC{=}CPhPhNH_2]$. Anal. Calc. for $C_{34}H_{38}FeN_2O_2{\cdot}H_2O$ (%): C, 70.34; H, 6.94; N, 4.83. Found: C, 70.30; H, 7.00, N, 4.58. R_F: 0.70 (Me_2CO). HPLC (R_T) 3.68 min.

***N*^{1}-[4-(1,2-Diphenylbut-1-en-1-yl)phenyl]suberamide (TAM–PSA).** To a solution of **TAM–OPOA** (2.0 mmol, 0.910 g) in 15 mL of anhydrous THF, cooled to 0 °C, $ClCO_2Et$ (3.0 mmol, 0.29 mL) and Et_3N (3.4 mmol, 0.47 mL) were added and the mixture was stirred for 10 min. The solid was filtered off and an excess of $NaNH_2$ was added to the filtrate. After 30 min of stirring, 20 mL of water was slowly added. The product was extracted with AcOEt, and the organic layer was dried over $MgSO_4$, filtered and evaporated. The crude was purified by column chromatography using mixtures of AcOEt and petroleum ether. 0.363 g (40%) of **TAM–PSA** was isolated. *Z*/*E* isomer ratio, 92/8, mp: 178–180 °C. *Z* isomer ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): δ 0.83 (t, J = 7.4 Hz, 3H, CH_3), 1.21 (m, 4H, $2CH_2$), 1.40–1.51 (m, 4H, $2CH_2$), 1.98 (t, J = 7.4 Hz, 2H, CH_2), 2.18 (t, J = 7.5 Hz, 2H, CH_2), 2.36 (q, J = 7.4 Hz, 2H, CH_2), 6.66 (s, 1H, NH), 6.71 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 7.09–7.29 (m, 11H, $10CH_{Ar}$ and NH), 7.36 (t, J = 7.3 Hz, 2H, $2CH_{Ar}$), 9.69 (s, 1H, NH). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$, ppm): δ 13.3 (CH_3), 24.9 (CH_2), 25.0 (CH_2), 28.4 (CH_2), 28.5 (CH_2), 28.6 (CH_2), 35.0 (CH_2), 36.3 (CH_2), 118.0 (C_{Ar}), 126.2 (C_{Ar}), 126.7 (C_{Ar}), 127.9 (C_{Ar}), 128.2 (C_{Ar}), 128.9 (C_{Ar}), 129.3 (C_{Ar}), 130.4 (C_{Ar}), 137.0 (C_{Ar}), 137.2 (C_{Ar}), 138.0 (C═C), 141.0 (C═C), 141.7 (C_{Ar}), 143.0 (C_{Ar}), 171.0 (C═O), 174.2 (C═O). IR (KBr, υ_{max}/cm^{-1}): 3464, 3278, 3182 (N–H stretch), 3101, 3043 (aromatic C–H stretch), 2927, 2858 (alkyl C–H stretch), 1662 (NC═O stretch), 1601 (aromatic C═C stretch), 1523 (N–H bend), 1400 (C–N stretch). MS (CI, *m*/*z*): 472 $[MNH_4]^+$, 455 $[MH]^+$. Anal. Calc. for $C_{30}H_{34}N_2O_2{\cdot}\frac{1}{2}H_2O$ (%): C, 77.72; H, 7.61; N, 6.04.

Found: C, 77.65; H, 7.60; N, 5.78. R_F: 0.68 (Me_2CO). HPLC (R_T), 3.28 min.

N^1-Phenyloctanediamide (PSA). To a solution of **OPOA** (3.0 mmol, 0.747 g, prepared as **FcTAM–OPOA** and **TAM–OPOA**) in 5 mL of anhydrous THF, cooled to 0 °C, $ClCO_2Et$ (4.5 mmol, 0.44 mL) and Et_3N (5.1 mmol, 0.71 mL) were added and the mixture was stirred for 10 min. The solid was filtered off and an excess of $NaNH_2$ was added to the filtrate. After 30 min of stirring, 20 mL of water was slowly added. The product was extracted with AcOEt, and the organic layer was dried over $MgSO_4$, filtered and evaporated. The product was purified by column chromatography using mixtures of AcOEt and petroleum ether as an eluent. 0.372 g (50%) of the product **PSA** was isolated. mp: 160–161 °C. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): δ 1.26 (m, 4H, $2CH_2$), 1.46 (quint, J = 7.0 Hz, 2H, CH_2), 1.56 (quint, J = 7.1 Hz, 2H, CH_2), 2.00 (t, J = 7.4 Hz, 2H, CH_2), 2.27 (t, J = 7.3 Hz, 2H, CH_2), 6.67 (s, 1H, NH), 6.99 (t, J = 7.8 Hz, 1H, CH_{Ar}), 7.21 (s, 1H, NH), 7.26 (t, J = 7.8 Hz, 2H, $2CH_{Ar}$), 7.56 (d, J = 7.8 Hz, 2H, $2CH_{Ar}$), 9.83 (s, 1H, CONH). ^{13}C-NMR (300 MHz, $(CD_3)_2SO$, ppm): δ 25.0 ($2CH_2$), 28.5 ($2CH_2$), 35.0 (CH_2), 36.3 (CH_2), 119.0 (C_{Ar}), 122.8 (C_{Ar}), 128.6 (C_{Ar}), 139.3 (C_{ipso}), 171.2 (C═O), 174.3 (C═O). IR (KBr, υ_{max}/cm^{-1}): 3406, 3309, 3201 (N–H stretch), 3055 (aromatic C–H stretch), 2939, 2856 (alkyl C–H stretch), 1658 (NC═O), 1604 (aromatic C═C stretch), 1527 (N–H bend), 1419 (C–N stretch). MS (EI, m/z): 248 $[M]^{+\bullet}$, 232 $[M - NH_2]^+$, 190 $[M - CONH_2]^+$, 93 $[PhNH_2]^{+\bullet}$. HRMS for $C_{14}H_{21}N_2O_2$ $[MH]^+$, calc.: 249.1603; found: 249.1596. R_F: 0.73 (Me_2CO). HPLC (R_T), 2.68 min.

3-[4-(1,2-Diphenylbut-1-en-1-yl)phenoxy]-*N*,*N*-dimethylpropan-1-amine (TAM[3]). In a Schlenk tube, 4-(1,2-diphenylbut-1-en-1-yl)phenol (1 mmol, 300 mg) was dissolved in anhydrous DMF (8 mL). NaH (60% in oil, 1.1 mmol, 44 mg) was added as a powder into the solution within 10 min. In another Schlenk tube, Et_3N (3 mmol, 303 mg) was added to a suspension of $Cl(CH_2)_3NMe_2$·HCl (3 mmol, 474 mg) in 15 mL of THF. After stirring for 30 min, the solution was filtered and concentrated to 1 mL. After this, 2 mL of DMF was added. This chloroamine solution was added to the first solution and the mixture was heated at 110 °C with an oil bath. After 1.5 h of heating, the mixture was allowed to cool to room temperature, and then 100 mL of AcOEt was added. The solution was washed with 2 × 40 mL of water. After solvent removal, the crude product was first purified by a silica gel flash column. Compounds were first eluted with acetone and then with acetone–Et_3N (10 : 1). 346 mg of a pure compound was isolated as a colourless oil (90%, Z/E or $E:Z$ = 69 : 31). ^{1}H NMR (300 MHz, $(CD_3)_2CO$, ppm): δ 0.89 (minor) and 0.91 (major) (t and t, 3 H, J = 7.4 Hz, CH_2CH_3), 1.80 (minor) and 1.90 (major) (quin and quin, 2 H, $CH_2CH_2CH_2$), 2.12 (minor) and 2.17 (major) (s and s, 6 H, $N(CH_3)_2$), 2.29–2.53 (m, 4 H, CH_2CH_3 + $CH_2CH_2CH_2N$), 3.88 (minor) and 4.05 (t and t, 2 H, J = 6.4 Hz, $OCH_2CH_2CH_2$), 6.55–7.37 (m, 14 H, C_6H_4 + $2C_6H_5$). ^{13}C NMR (75 MHz, $(CD_3)_2CO$, ppm): δ 13.8 (CH_3), 28.3 (CH_2), 29.6 ($CH_2CH_2CH_2$), 45.7 ($N(CH_3)_2$), 56.8 ($CH_2CH_2CH_2N$), 66.5 and 6.7 ($OCH_2CH_2CH_2$), 114.2 (CH, C_{Ar}), 115.0 (CH, C_{Ar}), 126.5 (CH, C_{Ar}), 126.9 (CH, C_{Ar}), 127.4 (CH, C_{Ar}), 128.1 (CH, C_{Ar}), 128.6 (CH, C_{Ar}), 128.7 (CH, C_{Ar}), 129.0 (CH, C_{Ar}), 130.1 (CH, C_{Ar}), 130.5 (CH, C_{Ar}), 131.2 (CH, C_{Ar}), 131.4 (CH, C_{Ar}), 132.5 (CH, C_{Ar}). 136.1, 136.5, 139.6, 142.0, 142.6, 143.2, 144.4, 144.7 and 159.0 (C_q, C_6H_4 + $2C_6H_5$ + C═C). (CI, m/z): 386.24 $[M + H]^+$. Anal. Calc. For $C_{27}H_{31}NO$: C, 84.11; H, 8.10; N, 3.63. Found: C, 83.68; H, 8.12; N, 3.66.

3-[4-(2-Ferrocenyl-1-phenylbut-1-en-1-yl)phenoxy]-*N*,*N*-dimethylpropan-1-amine (FcTAM). In a Schlenk tube, (*E* + *Z*)-4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)phenol (1 mmol, 408 mg) was dissolved in anhydrous DMF (8 mL). NaH (60% in oil, 1.1 mmol, 44 mg) was added as a powder into the solution within 10 min. In another Schlenk tube, Et_3N (3 mmol, 303 mg) was added to a suspension of $Cl(CH_2)_3NMe_2$·HCl (3 mmol, 474 mg) in 15 mL of THF. After stirring for 30 min, the solution was filtered and concentrated to 1 mL. 2 mL of DMF was added. This chloroamine solution was added to the first solution and the mixture was heated at 110 °C with an oil bath. After 1 h 30 min of heating, the mixture was allowed to cool to room temperature, and then 100 mL of ethyl acetate was added. The solution was washed with 2 × 40 mL of water. After removal of solvent, the crude product was first purified by a silica gel flash column. Compounds were first eluted with acetone and then with acetone–Et_3N (10 : 1). 350 mg of a pure compound was isolated as an orange oil (70%, Z/E = 50 : 50). ^{1}H NMR (300 MHz, $(CD_3)_2CO$, ppm): δ 1.01 and 1.03 (t and t, J = 7.4 Hz, 3H, CH_3), 1.89 (m, 2 H, $CH_2CH_2CH_2$), 2.15 and 2.16 (s and s, 6 H, $N(CH_3)_2$), 2.38 (m, 2 H, CH_2CH_3), 2.60 (m, 2 H, $CH_2CH_2CH_2N$), 4.00 (m, 2 H, $OCH_2CH_2CH_2$), 3.85 and 3.91 (t and t, J = 1.9 Hz, 2H, Cp_{subst}), 4.04 and 4.07 (t and t, J = 1.9 Hz, 2H, Cp_{subst}), 4.12 and 4.12 (s and s, 5H, Cp), 6.77–7.34 (m, 9 H, C_6H_4 + C_6H_5). ^{13}C NMR (75 MHz, $(CD_3)_2CO$, ppm): δ 15.8 (CH_3), 28.3 (CH_2), 45.7 ($N(CH_3)_2$), 56.9 ($CH_2CH_2CH_2N$), 66.7 ($OCH_2CH_2CH_2$), 68.8 (Cp_{subst}), 69.9 (Cp + Cp_{subst}), 87.3 (C_{ip}, C_5H_4), 115.0 (CH, C_{Ar}), 115.1 (CH, C_{Ar}), 126.9 (CH, C_{Ar}), 129.0 (CH, C_{Ar}), 129.1 (CH, C_{Ar}), 130.0 (CH, C_{Ar}), 131.1 (CH, C_{Ar}), 131.6 (CH, C_{Ar}), 137.7, 137.9, 138.0, 138.5, 145.9, 146.1, and 158.6 (C_q, C_6H_4 + C_6H_5 + C═C). MS (CI, m/z): 494.20 $[M + H]^+$. Anal. Calc. for $C_{31}H_{35}FeNO$: C, 75.45; H, 7.15; N, 2.84. Found: C, 75.22; H, 7.20; N, 2.62.

Lipophilicity. Measurements of the octanol/water partition coefficient ($\log P_{o/w}$) were made by the HPLC technique according to a method described previously.[74] Measurement of the chromatographic capacity factors (k') for each molecule was done at various concentrations in the range of 95–75% methanol containing 0.25% (v/v) 1-octanol and an aqueous phase consisting of 0.15% (v/v) *n*-decylamine in the buffering agent MOPS (3-morpholinopropane-1-sulfonic acid, prepared in 1-octanol saturated water) adjusted to pH 7.4. These capacity factors (k') are extrapolated to 100% of the aqueous component given the value of k'_w. The $\log P_{o/w}$ is obtained by the formula $\log P_{o/w} = 0.13418 + 0.98452 \log k'$.

Biological methods

Relative binding affinity (RBA). Stock solutions (1 mM) of the compounds were prepared in DMSO and kept at 4 °C in the dark. It has already been proven that the compounds

FcTAM–OPOA, **TAM–OPOA**, **FcTAM–SAHA**, **TAM–SAHA**, **FcTAM–PSA** and **TAM–PSA** are stable under these conditions. Serial dilutions in DMSO were prepared just prior to use. RBA values were measured on ERα from lamb uterine cytosol purchased from Pan Vera (Madison, WI, USA). Aliquots (200 μL) of ERα in glass tubes were incubated for 3 h at 0 °C with [6,7-^{3}H]-estradiol (2 nM, specific activity 1.62 TBq mmol^{-1}, NEN Life Science, Boston, MA) in the presence of nine concentrations of compounds. 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 compound tested and the results are expressed as RBA values. The RBA value of 17β-estradiol is by definition equal to 100%.

IC_{50} determination. The breast adenocarcinoma cell lines MDA-MB-231 and MCF7 were obtained respectively from ATCC and Dr Matthias Kassack (Bonn, Germany). Cells were grown in RPMI medium supplemented with 10% fetal calf serum, in the presence of penicillin, streptomycin and fungizone in a 75 cm^2 flask under 5% CO_2. Cells were plated in 96-well tissue culture plates in 200 μL medium and treated 24 h later with 2 μL stock solution of compounds dissolved in DMSO using a Biomek 3000 (Beckman-Coulter). Controls received the same volume of DMSO (1% final volume). After 72 h of exposure, MTS reagent (Promega) was added and incubated for 3 h at 37 °C: the absorbance was monitored at 490 nm and results are expressed as the inhibition of cell proliferation calculated as the ratio [(1 − (OD490 treated/OD490 control) × 100] in triplicate experiments. For IC_{50} determination [50% inhibition of cell proliferation], cells were incubated for 72 h following the same protocol with the compound concentration range 5 nM to 100 μM in separate duplicate experiments.

Estrogenic/antiestrogenic effect of compounds on MCF-7 cells. Cells were maintained in monolayer culture in DMEM with phenol red/Glutamax I, supplemented with 9% decomplemented fetal calf serum and 0.9% kanamycin, at 37 °C in a 5% CO_2 air humidified incubator. Cells were plated on 24-well sterile plates at a density of 3 × 10^4 cells in 1 mL of DMEM without phenol red, supplemented with 9% 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. On 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 (2.5 mg mL^{-1}), and then washed thoroughly with water. 2 mL of HCl (0.1 M) was then added, and the plate was incubated for 1 h at 37 °C. Then the absorbance of each well was measured at 655 nm using a Biorad spectrophotometer (microplate reader). The results are expressed as the percentage of proteins *versus* the control. Experiments were performed in triplicate.

Luciferase assays. HELN-ERα cells[58] were seeded at a density of 4 × 10^4 cells per well on 96-well white opaque tissue culture plates (Greiner CellStar, D. Dutscher, Brumath, France) and maintained in 5% DCC–FCS. Compounds were added 20 h later and the cells were incubated with the compounds for 16 h. Experiments were performed in quadruplicate. At the end of the incubation, the medium was removed and replaced by 0.3 mM luciferin containing 5% DCC–FCS. The 96-well plate was then introduced into a microplate luminometer (Microbeta, Wallac) and intact living cell luminescence was measured for 2 s.

mRNA quantification. MCF7 human breast cells were grown in steroid-free DMEM/F12 medium supplemented with 3% DCC–FCS, 100 U mL^{-1} penicillin, 100 mg mL^{-1} streptomycin and 100 mg mL^{-1} sodium pyruvate during 5 days. Cells were then stimulated in media containing 3% DCC–FCS with 10 nM of estradiol for 1 day. Total RNA was extracted from the cells using a High Pure RNA Isolation kit (Roche) according to the manufacturer's instructions. Total RNA (1 μg) was subjected to reverse-transcription using Superscript II reverse transcriptase (Invitrogen). Quantitative RT-PCR (qPCR) was then performed on specific primers for the indicated genes, using LightCycler 480 SYBR Green I Master (Roche Diagnostics). qPCR was carried out in a final volume of 10 μL using 0.25 μL of each primer (25 μM), 5 μL of the supplied enzyme mix, 3 μL of H_2O, and 2 μL of the template diluted at 1 : 10. After a 10 min pre-incubation at 95 °C, runs corresponded to 35 cycles of 15 s each at 95 °C, 5 s at 60 °C and 15 s at 72 °C. Melting curves of the PCR products were analyzed using the LightCycler software system to exclude the amplification of unspecific products. Results were normalized to the RS9 housekeeping gene transcripts. Primers are available upon request.

HDACi assay. The HDACi activity was measured using the *Fluor de Lys®* Fluorescent Assay System (Enzo Life Sciences) according to the manufacturer's instructions. Briefly, the *Fluor de Lys®* Substrate, which comprises an acetylated lysine side chain, was incubated with a nuclear extract prepared from HeLa cells and containing HDAC. Different concentrations of compounds were added and the reaction mixture was left for 30 minutes at room temperature. The reaction was enhanced by the addition of the *Fluor de Lys®* Developer. Deacetylation of the substrate sensitizes the substrate which produces a fluorophore. Arbitrary fluorescence units (AFU) of each sample were quantified using a microtiter-plate reading fluorimeter at 460 nm.

Acknowledgements

We thank the Agence Nationale de la Recherche for financial support (ANR 2010 BLAN 7061 blanc "Mecaferrol") and the

Institut National du Cancer (INCa TRANSLA 2011-054). We also thank the National Council of Science and Technology of Mexico (CONACyT) for the PhD scholarship for JJCM.

Notes and references

1 M. Patra and G. Gasser, *ChemBioChem*, 2012, **15**, 1232–1252.
2 S. S. David and E. Meggers, *Curr. Opin. Chem. Biol.*, 2008, **12**, 194–196.
3 G. Jaouen, *Bioorganometallics*, John Wiley & Sons, Germany, 2006.
4 *Medicinal Organometallic Chemistry. Topics in Organometallic Chemistry*, ed. G. Jaouen, N. Metzler-Nolte, Springer, 1st edn, 2010, 32.
5 E. Meggers, *Chem. Commun.*, 2009, 1001–1010.
6 G. Gasser, I. Ott and N. Metzler-Nolte, *J. Med. Chem.*, 2011, **54**, 3–25.
7 S. Top, J. Tang, A. Vessières, D. Carrez, C. Provote and G. Jaouen, *Chem. Commun.*, 1996, 955–956.
8 G. Jaouen, S. Top, A. Vessières, G. Leclercq, J. Quivy, L. Jin and A. Croisy, *C. R. Acad. Sci. Paris*, 2000, **Série IIc**, 89–93.
9 E. A. Hillard, A. Vessières, S. Top, P. Pigeon, K. Kowalski, M. Huché and G. Jaouen, *J. Organomet. Chem.*, 2007, **692**, 1315–1326.
10 G. Jaouen, S. Top, A. Vessières, G. Leclercq and M. J. McGlinchey, *Curr. Med. Chem.*, 2004, **11**, 2505–2517.
11 S. Top, A. Vessières, G. Leclercq, J. Quivy, J. Tang, J. Vaissermann, M. Huché and G. Jaouen, *Chem.–Eur. J.*, 2003, **9**, 5223–5236.
12 A. Nguyen, A. Vessières, E. A. Hillard, S. Top, P. Pigeon and G. Jaouen, *Chimia*, 2007, **61**, 716–724.
13 B. D. Lehmann, J. A. Bauer, X. Chen, M. E. Sanders, A. B. Chakravarthy, Y. Shyr and J. A. Pietenpol, *J. Clin. Invest.*, 2011, **121**, 2750–2767.
14 D. A. Ferraro, N. Gaborit, R. Maron, H. Cohen-Dyashi, Z. Porat, F. Pareja, S. Lavi, M. Lindzen, N. Ben-Chetrit, M. Sela and Y. Yarden, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 1815–1829.
15 E. A. Hillard, A. Vessières, L. Thouin, G. Jaouen and C. Amatore, *Angew. Chem., Int. Ed.*, 2006, **45**, 285–290.
16 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.
17 A. Vessières, S. Top, W. Beck, E. Hillard and G. Jaouen, *Dalton Trans.*, 2006, 529–541.
18 D. Plażuk, A. Vessières, E. A. Hillard, O. Buriez, E. Labbé, P. Pigeon, M. A. Plamont, C. Amatore, J. Zakrewski and G. Jaouen, *J. Med. Chem.*, 2009, **52**, 4964–4967.
19 A. Vessières, S. Top, P. Pigeon, E. A. Hillard, L. Boubeker, D. Spera and G. Jaouen, *J. Med. Chem.*, 2005, **48**, 3937–3940.
20 E. A. Hillard, A. Vessières, F. Le Bideau, D. Plażuk, D. Spera, M. Huché and G. Jaouen, *Chem. Med. Chem.*, 2006, **1**, 551–559.
21 O. Zekri, E. A. Hillard, S. Top, A. Vessières, P. Pigeon, M. A. Plamont, M. Huché, S. Boutamine, M. J. McGlinchey, H. Müller-Bunz and G. Jaouen, *Dalton Trans.*, 2009, 4318–4326.
22 E. A. Hillard, F. C. Caxico de Abreu, D. C. M. Ferreira, G. Jaouen, M. O. F. Goulart and C. Amatore, *Chem. Commun.*, 2008, 2612–2628.
23 H. Hagen, P. Marzenell, E. Jentzsch, F. Wenz, M. R. Veldwijk and A. Mokhir, *J. Med. Chem.*, 2012, **55**, 924–934.
24 S. B. Baylin, *Nat. Clin. Pract. Oncol.*, 2005, **2**, S4–S11.
25 W. J. Pearce, *Transl. Stroke Res.*, 2011, **2**, 243–247.
26 M. Paris, M. Porcelloni, M. Binaschi and D. Fattori, *J. Med. Chem.*, 2008, **51**, 1505–1529.
27 H. De Schutter and S. Nuyts, *Anti-Cancer Agents Med. Chem.*, 2009, **9**, 99–109.
28 J. E. Bolden, M. J. Peart and R. W. Johnstone, *Nat. Rev. Drug Discovery*, 2006, **5**, 769–784.
29 P. A. Marks and R. Breslow, *Nat. Biotechnol.*, 2007, **25**, 84–90.
30 Y. Zhang, H. Fang, J. Feng, Y. Jia, X. Wang and W. Xu, *J. Med. Chem.*, 2011, **54**, 5532–5539.
31 K. C. Park, S. W. Kim, J. H. Park, E. H. Song, J. W. Yang, H. J. Chung, H. J. Jung, J. S. Suh, H. J. Kwon and S. H. Choi, *Cancer Sci.*, 2011, **102**, 343–350.
32 P. N. Münster, T. Troso-Sandoval, N. Rosen, R. Rifkind, P. A. Marks and V. M. Richon, *Cancer Res.*, 2001, **61**, 8492–8497.
33 P. Bali, M. Pranpat, R. Swaby, W. Fiskus, H. Yamaguchi, M. Balasis, K. Rocha, H. G. Wang, V. Richon and K. Bhalla, *Clin Cancer Res.*, 2005, **11**, 6382–6389.
34 T. A. Miller, D. J. Witter and S. Blevedere, *J. Med. Chem.*, 2003, **46**, 5097–5116.
35 S. Belvedere, D. J. Witter, J. Yan, J. P. Secrist, V. Richon and T. A. Miller, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 3969–3971.
36 A. P. Kozikowski, S. Tapadar, D. N. Luchini, K. Hwan Kim and D. D. Billadeau, *J. Med. Chem.*, 2008, **51**, 4370–4373.
37 L. E. Tavera-Mendoza, T. D. Quach, B. Dabbas, J. Hudon, X. Liao, A. Palijan, J. L. Gleason and J. H. White, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 8250–8255.
38 F. Oger, A. Lecorgne, E. Sala, V. Nardese, F. Demay, S. Chevance, D. C. Desravines, N. Aleksandrova, R. Le Guével, S. Lorenzi, A. R. Beccari, P. Barath, D. J. Hart, A. Bondon, D. Carettoni, G. Simonneaux and G. Salbert, *J. Med. Chem.*, 2010, **53**, 1937–1950.
39 D. Griffith, M. P. Morgan and C. J. Marmion, *Chem. Commun.*, 2009, 6735–6737.
40 J. Spencer, J. Amin, M. Wang, G. Packham, S. S. Syed Alwi, G. J. Tizzard, S. J. Coles, R. M. Paranal, J. E. M. Bradner and T. D. Heightman, *ACS Med. Chem. Lett.*, 2011, **2**, 358–362.
41 A. V. Bieliauskas, S. V. Weerasinghe and M. K. Pflum, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 2216–2219.
42 S. Hanessian, L. Auzzas, G. Giannini, M. Marzim, W. Cabri, M. Barbarino, L. Vesci and C. Pisano, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 6261–6265.
43 C. B. Botta, W. Cabri, E. Cini, L. De Cesare, C. Fattorusso, G. Giannini, M. Persico, A. Petrella, F. Rondinelli, M. Rodriguez, A. Russo and M. Taddei, *J. Med. Chem.*, 2011, **54**, 2165–2182.

44 F. Dehmel, T. Ciossek, T. Maier, S. Weinbrenner, B. Schmidt, M. Zoche and T. Beckers, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 4746–4752.
45 L. Hodges-Gallagher, C. D. Valentine, S. El Bader and P. J. Kushner, *Breast Cancer Res. Treat.*, 2007, **105**, 297–309.
46 T. K. Owonikoko, S. S. Ramalingam, B. Kanterewicz, T. E. Balius, C. P. Belani and P. A. Hershberger, *Int. J. Cancer*, 2010, **126**, 743–755.
47 C. S. Dietrich, V. L. Greenberg, C. P. DeSimone, S. C. Modesitt, J. R. van Nagell, R. Craven and S. G. Zimmer, *Gynecol. Oncol.*, 2010, **116**, 126–130.
48 Y. K. Shi, Z. H. Li, X. Q. Han, X. Q. Han, J. H. Yi, Z. H. Wang, J. L. Hou, C. R. Feng, Q. H. Fang, H. H. Wang, P. F. Zhang, F. S. Wang, J. Shen and P. Wang, *Cancer Chemother. Pharmacol.*, 2010, **66**, 1131–1140.
49 P. N. Münster, K. T. Thurn, S. Thomas, P. Raha, M. Lacevic, A. Miller, M. Melisko, R. Ismail-Khan, H. Rugo, M. Moasser and S. E. Minton, *Br. J. Cancer*, 2011, **104**, 1828–1835.
50 S. Thomas, K. T. Thurn, E. Biçaku, D. C. Marchion and P. N. Münster, *Breast Cancer Res.*, 2011, **130**, 437–447.
51 A. Mai, M. Esposito, G. Sbardellat and S. Massat, *Org. Prep. Proced. Int.*, 2001, **33**, 391–394.
52 Y. Okunoi, K. Horita, O. Yonemitsu, K. Shibata and T. A. Amemiya, *J. Chem. Soc., Perkin Trans. 1*, 1984, 1115–1118.
53 L. Kubicova, K. Waisser, J. Kunes, K. Kralova, Z. Odlerova, M. Slosarek, J. Janota and Z. Svodoba, *Molecules*, 2000, **5**, 714–726.
54 T. B. Windholz and J. B. Clements, *J. Org. Chem.*, 1964, **29**, 3021–3023.
55 O. Buriez, E. Labbé, P. Pigeon, G. Jaouen and C. Amatore, *J. Electroanal. Chem.*, 2008, **619**, 169–175.
56 P. Pigeon, S. Top, O. Zekri, E. A. Hillard, A. Vessières, M. A. Plamont, O. Buriez, E. Labbé, M. Huché, S. Boutamine, C. Amatore and G. Jaouen, *J. Organomet. Chem.*, 2009, **694**, 895–901.
57 M. Görmen, P. Pigeon, S. Top, A. Vessières, M. A. Plamont, E. Hillard and G. Jaouen, *MedChemComm*, 2010, **1**, 149–151.
58 J. J. Cázares-Marinero, S. Top and G. Jaouen, *J. Organomet. Chem.*, DOI: 10.1016/j.jorganchem.2013.07.031.
59 A. Escande, A. Pillon, N. Servant, J. P. Cravedi, F. Larrea, P. Muhn, J. C. Nicolas, V. Cavaillès and P. Balaguer, *Biochem. Pharmacol.*, 2006, **71**, 1459–1469.
60 F. Vernon and J. H. Khorassani, *Talanta*, 1978, **25**, 410–412.
61 M. Hojjatie, T. Cecconie and H. Freiser, *Anal. Chim. Acta*, 1987, **199**, 49–57.
62 B. K. Tait, K. E. Mdlalose and I. Taljaard, *Hydrometallurgy*, 1995, **38**, 1–6.
63 M. J. Miller, *Chem. Rev.*, 1989, **89**, 1563–1579.
64 D. Vincent, H. Schwal and M. C. Vincent, *Ann. Pharm. Fr.*, 1959, **17**, 579–584.
65 D. M. Griffith, B. Szöcs, T. Keogh, K. Y. Suponitsky, E. Farkas, P. Buglyó and C. J. Marmion, *J. Inorg. Biochem.*, 2011, **105**, 763–769.
66 Y. Sowa, T. Orita, S. Minimikawa, K. Nakano, T. Mizuno, H. Nomura and T. Sakai, *Biochem. Biophys. Res. Commun.*, 1997, **241**, 142–150.
67 R. Margueron, A. Licznar, G. Lazennec, F. Vignon and V. Cavaillès, *J. Endocrinol.*, 2003, **179**, 41–53.
68 V. M. Richon, T. W. Sandhoff, R. A. Rifkind and P. Marks, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 10014–10019.
69 A. Vessières, C. Corbet, J. M. Heldt, N. Lories, N. Jouy, I. Laïos, G. Leclercq, G. Jaouen and R. A. Toillon, *J. Inorg. Biochem.*, 2010, **104**, 503–511.
70 K. Jomova and M. Valko, *Toxicology*, 2011, **283**, 65–87.
71 D. H. Kim, J. K. Kundu and Y. J. Suth, *Mol. Carcinog.*, 2011, **50**, 222–234.
72 W. S. el-Deiry, T. Tokino, V. E. Velculescu, D. B. Levy, R. Parsons, J. M. Trent, W. E. Mercer, K. W. Kinzler and B. Vogelstein, *Cell*, 1993, **75**, 817–825.
73 A. Mallet and S. Pervaiz, *Antioxid. Redox Signaling*, 2012, **16**, 1285–1294.
74 O. Payen, S. Top, A. Vessières, E. Brulé, A. Lauzier, M. A. Plamont, M. J. McGlinchey, H. Müller-Bunz and G. Jaouen, *J. Organomet. Chem.*, 2011, **696**, 1049–1056.