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Synthesis and characterization of new ferrocenyl compounds with different alkyl chain lengths and functional groups to target breast cancer cells

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ABSTRACT

A new family of organometallic compounds bearing chains of different lengths and with different functional groups was synthesized and evaluated against triple negative MDA-MB-231 and hormone-dependent MCF-7 breast cancer cells. Biological results comparing suberamides (six-methylene chain length) and succinamides (two methylene chain length) showed that chain shortening does not dramatically impact on their antiproliferative effects. Cytotoxicity of primary amides is not dependent on chain length, as suberamide and succinamide showed almost identical activity against both types of breast cancer cells. However, the possibility that some of the cytotoxic activity of hydroxamides could be related to enzyme inhibition, e.g. histone deacetylase (HDAC) inhibition, is not excluded. This is based on the fact that compounds bearing a longer alkyl chain showed IC_{50} values lower than those with shorter alkyl chains. Succinic and adipic carboxylic acids and a succinimide were also tested and they also showed cytotoxic activity. Interestingly, succinimide was the most active compound against hormone-dependent MCF-7 breast cancer cells, presumably owing to an antagonist effect with the alpha form of the estrogen receptor (ERα). Some new and interesting side chain influences related to anti-proliferative effects can therefore be observed.

1. Introduction

Organometallic compounds show particular physicochemical properties depending on the nature of the metal moieties. Their use in catalysis is very well known and their application in drug synthesis constitutes one of the most common practices of organic chemists. However, at the end of the last century, it was demonstrated that these compounds could themselves be used to treat diseases [1]. Redox properties and 3D structures of organometallic compounds are some of the countless features that have attracted the attention of researchers to design new drug candidates and to explore new mechanisms of action [2]. Recently, we have incorporated the pharmacophore of the known drug (N^1-hydroxy-N^8-phenylsuberamide) **SAHA** into the ferrocifen (**FcTAM3**) molecule as a strategy to explore the mechanism of action of ferrocifen derivatives (Fig. 1) [3]. **SAHA** is a histone deacetylase inhibitor (HDACi) drug used to treat primary cutaneous T-cell lymphoma [4,5] while **FcTAM3** and derived compounds are organometallic tamoxifen-type species which proved to be more active than the corresponding organic analogs against hormone-dependent MCF-7 breast cancer cells. Moreover, they were also found to be strongly cytotoxic against triple negative MDA-MB-231 breast cancer cells, where tamoxifen (**TAM**) and related organic compounds are completely inactive under the same conditions [6].

Biological studies of the resulting organometallic hybrid **FcTAM-SAHA** on triple negative MDA-MB-231 breast cancer cells showed that the combination of both features gave them beneficial effects in terms of antiproliferative activity. Analyzing their cytotoxicities separately, one can see that, in the case of **SAHA**, the presence of the organometallic moiety 2-ferrocenyl-1-phenylbut-1-en-1-yl, as a substituent on the 4 position of its cap, enhanced its activity more than five times (from $IC_{50} = 3.64$ μM to 0.70 μM) while in the case of **FcTAM3**, the presence of the 8-hydroxyamino-8-oxooctanamido group instead of its dimethylaminoalkoxy chain made it over three times more effective (from $IC_{50} = 2.62$ μM to 0.70 μM).

Regarding hormone-dependent MCF-7 breast cancer cells, the positive effect of the structural combination of **SAHA** and **FcTAM3**

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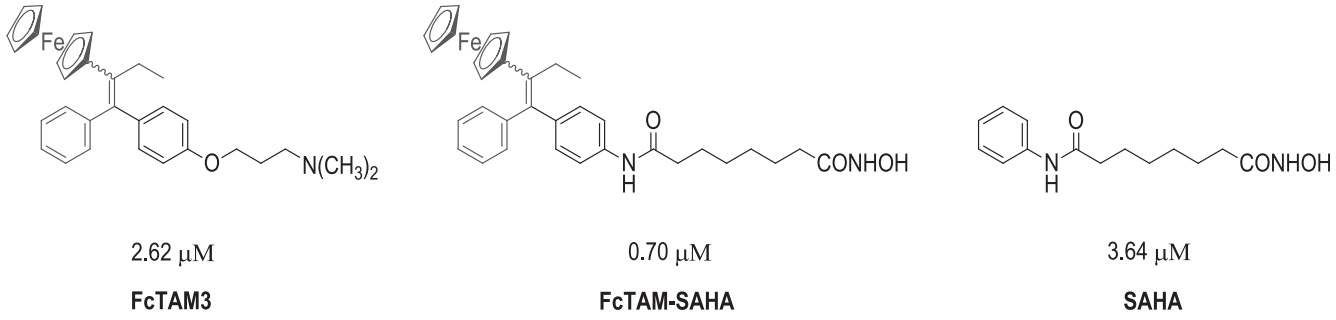


Fig. 1. Structures of **FcTAM3**, **SAHA** and **FcTAM-SAHA** with their corresponding IC_{50} values against triple negative MDA-MB-231 breast cancer cells.

was only observed in the case of the latter. Such modification more than doubled the activity of **FcTAM3** (from IC_{50} = 4.41 μM to 2.01 μM). However, **SAHA** modification did not increase its antiproliferative activity against MCF-7, since on these hormone-dependent breast cancer cells the organometallic derivative **FcTAM-SAHA** remained little more than half as active as **SAHA** (IC_{50} = 2.01 μM for **FcTAM-SAHA** *vs* 1.04 μM for **SAHA** in this study). Interestingly, this behavior on MCF-7 was also reported for two other organometallic **SAHA** analogs, the N^1-ferrocenyl-N^8-hydroxysuberamide (**JAHA**) prepared by Spencer et al. [7] and the tricarbonyl[(8-hydroxyamino-8-oxooctanamido)-η^5-cyclopentadienyl]rhenium(I) prepared by Can et al. [8] (Fig. 2).

On the other hand, both **SAHA** and **FcTAM-SAHA** were shown to chelate metal ions such as Fe^{3+} and they are believed to bind Zn^{2+} as well [9]. This feature plays an important role in biochemistry since these chelating effects could be exploited to inactivate enzymes [10]. For example **FcTAM-SAHA** was shown to be active as HDACi [3]. In this context, it is important to state that some members of the **JAHA** family, which are also ferrocenic, were reported to be effective HDACi against some enzyme isoforms [11].

In an attempt to determine whether the *N*-hydroxamide function on **FcTAM-SAHA** was structurally necessary to produce cytotoxic effects, and thus to estimate the effect of the **SAHA** pharmacophore in the hybrid molecule, the primary amide **FcTAM-PSA** (Fig. 3) was also synthesized and evaluated. Surprisingly, this molecule also showed strong antiproliferative activity against both types of breast cancer cells, with IC_{50} values even slightly lower than those of **FcTAM-SAHA**. Further, its organic analog, the *N*-phenylsuberamide (**PSA**) was very far from achieving the same cytotoxic effects even at higher concentrations (>10 μM).

Interestingly, both **PSA** and **FcTAM-PSA** exhibited neither chelating capacities nor any HDACi activity, seeming to indicate that cytotoxic effects are not strongly dependent on the functionalization on the lateral alkyl chain of both suberamides to produce almost the same response. This also means that the two compounds may share at least one particular mechanistic feature which is responsible for their strong cytotoxic effects. This would correlate with the fact that both **FcTAM-SAHA** and **FcTAM-PSA** were able to generate the expression of the tumor suppressor gene p21 [3].

On the other hand, it is possible to find in the literature that one of the most common strategies in structural–activity relationship studies of biologically active molecules is chain length modification. For example, an idoxifene analog bearing an alkyl chain modified by the presence of eight methylene groups instead of two did not show any improvements in terms of *in vitro* antiestrogenicity or antitumor activity [12]. However, it has been suggested that the chemical nature of the residue located at the end of the long side chain of other related antiestrogens may be an important factor. Another example is the evaluation of 4-phenylbutyric acid analogs, which were believed to act as HDACi. Data suggested that there was no correspondence between their HDACi activity and their cytotoxic effects against a mouse erythroleukemia cell line [13]. The chain length increase, from four carbons to ten, resulted in greater antiproliferative activity but a decline in their HDACi effects, suggesting that they may differ in their mechanism of action. Other studies on derivatives of **SAHA** have explored the chain-length dependence of enzymatic activity [14]. Biological evaluations of compounds combining the bioavailability of short-chain fatty acids such as butyric acid with the binding ability of the hydroxamic function in **SAHA** showed, with modest results, that the most effective species consisted of compounds bearing longer chains with hydroxamic acid groups [15]. Finally, some studies seeking potent multi-acting molecules targeting HDAC, the epidermal growth factor receptor (EGFR) and the human epidermal growth factor receptor 2 (HER2) at the same time for the treatment of cancer, showed that chain length is an important motif for HDAC inhibition, the eight-carbon long-chain analogs being the most active agents [16,17]. Our group has also reported the effects of side-chain modification in structure–activity relationship studies of the ferrocifen family [6,18].

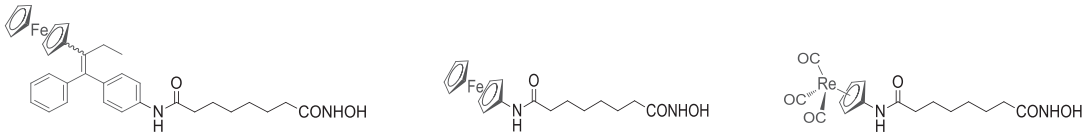
The aim of the present investigation is to explore the impact of this effect in ferrocenyl hydroxy and primary amides derived from adipic and succinic acids on their antiproliferative activity against breast cancer cells. Carboxylic acids and a succinimide are also included in this study in order to evaluate other chemical functionalities.

2. Results and discussion

2.1. Synthesis of adipic compounds

The synthesis of the three adipic derivatives that were tested in biological assays was performed as described in the literature (Scheme 1) [3]. Aniline **1** was previously obtained as a mixture of *Z* and *E* isomers (*Z*/*E* ratio = 85/15) from a McMurry cross coupling between 4-aminobenzophenone and propionylferrocene [3,19]. To obtain carboxylic acid **2**, aniline **1** reacted with adipoyl chloride ($ClCOCH_2CH_2)_2$) in 20 min at room temperature to afford 51% of product. As reported, lower temperatures did not significantly increase the yield, and bisamide **3** is always obtained as byproduct. After aniline **1** was consumed, the mixture was allowed to react with sodium hydroxide (NaOH) to ensure that chlorides transformed into carboxylates and consequently acidification was needed to favor the soluble form in organic solvents for extraction.

To obtain hydroxamide **4**, a solution of hydroxylamine was first prepared at 0 °C from 4 equivalents of hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) and 8 equivalents of potassium hydroxide (KOH). Simultaneously, carboxylic acid **2** reacted with 2 equivalents of ethyl chloroformate ($ClCO_2Et$) and 2.5 equivalents of triethylamine (TEA) to result in a carbonate intermediate. This latter was attacked



Cázares-Marinero[3] [2.01 μM/1.04 μM] *Spencer*[7] [2.44 μM/0.75 μM] *Can*[8] [11.4 μM/3.74 μM]

Fig. 2. Chemical structures of three organometallic **SAHA** derivatives with their corresponding reported IC_{50} values *vs* the reported IC_{50} value for **SAHA** [compound/SAHA] in each study against hormone-dependent MCF-7 breast cancer cells.

by the freshly prepared hydroxylamine to afford **4**. The relatively low yield of the desired product (36%) can be explained by the competitive reaction of MeOH to form the ester from the activated **2** in basic conditions, as was also observed for other series [20]. Finally, to obtain primary amide **5**, the same activation step of carboxylic acid **2** was needed before allowing it to react with an excess of sodium amide ($NaNH_2$). This time, compound **5** was obtained in excellent yield (93%). All the products of this adipic series (**2**–**5**) were characterized by conventional techniques.

2.2. Synthesis of succinic compounds

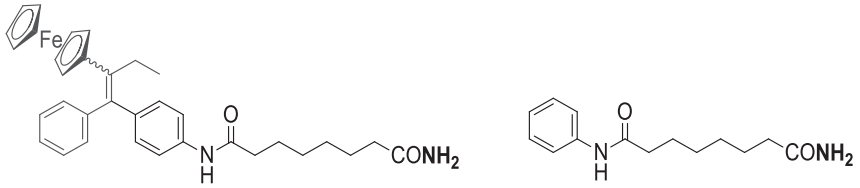
The pathway for the synthesis of the succinic compounds intended for biological evaluations was different from the adipic series since reactivity of aniline **1** with succinyl chloride ($(ClCOCH_2)_2$) preferentially formed the succinimide **6** instead of carboxylic acid **7**. This could be explained by the preference of forming the stable five-membered cycle via the double nucleophilic attack of aniline **1** to succinyl chloride. Thus, given that low temperatures did not significantly improve the yield of carboxylic acid **7**, aniline **1** was allowed to react with succinyl chloride at room temperature. After purification, the nature of the products observed by TLC during the reaction was established as the succinimide **6**, carboxylic acid **7** and bisamide **8** (Scheme 2). Since reaction produced carboxylic acid in low yields, we performed the ring opening of succinimide **6** with NaOH under mild conditions to obtain the desired carboxylic acid **7** in excellent yield (96%). In an effort to explore the possibility of optimizing the production of **7**, bisamide **8** was hydrolyzed with hydrochloric acid (HCl) in an acetone/water mixture to afford quantitatively after 1 h of reflux carboxylic acid **7** and aniline **1**. Having observed the good response of succinimide to nucleophiles such as hydroxide anion (HO^-) we explored the possibility of allowing it to react with NH_2OH to produce hydroxamide **9**. Thus, **6** reacted with an excess of a basic solution of hydroxylamine as previously described to afford **9** in a yield of 19%. This product can also be obtained following the synthetic protocol of **4** but starting from carboxylic acid **7** to afford **9** in a yield of 30%.

Finally, since succinamide **10** could not be obtained by the direct nucleophilic attack of $NaNH_2$ on succinimide **6**, the classic protocol was performed from **7,** activation with ethyl chloroformate followed by reaction with $NaNH_2$ to obtain **10** in 36%. The fact that reaction with $NaNH_2$ did not proceed could be explained by the strong basicity of the amide anion (NH_2^-) over its poor nucleophilicity and because succinimide could be less electrophilic than the activated carboxylate. Instead of obtaining **10** in this attempt, carboxylic acid **7** was the identified product. This could come from the HO^- generation from water in the work-up by the basic $NaNH_2$ to open the succinimide ring.

2.3. Antiproliferative activity of adipamides against breast cancer cells

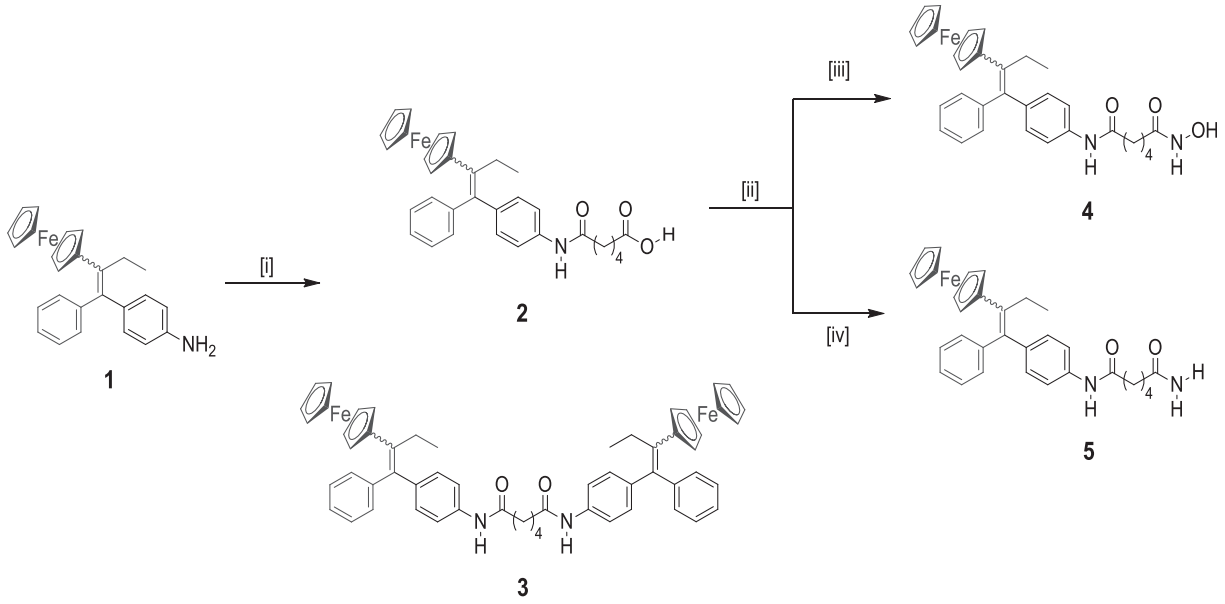
Cytotoxicity studies showed that suberamides **FcTAM-SAHA** and **FcTAM-PSA** which bear an alkyl chain longer than adipamides **4** and **5** remained more active against both breast cancer cell lines, respectively (Table 1 and Chart 1). On triple negative MDA-MB-231 breast cancer cells, for example, it may be seen that this lack of two methylene groups (4.5 Å, approximately) on the lateral alkyl chain of hydroxamide **4** impacted almost fivefold on its antiproliferative activity in comparison with **FcTAM-SAHA**, while on amide **5** it was almost seven times in comparison with **FcTAM-PSA**. On hormone-dependent MCF-7 breast cancer cells, this phenomenon is also true, with almost double the activity.

Interestingly, the attenuated effect (about threefold) observed in the cytotoxicity of suberamides against MCF-7 in comparison with MDA-MB-231 was not present in adipamides. It was shown for suberic compounds **FcTAM-SAHA** and **FcTAM-PSA** that estrogenic effects are developed at concentrations (around 0.01 μM) lower than their IC_{50} values [3]. This hormonal effect could favor cell proliferation and consequently somewhat counteract their cytotoxic effects. However, in the case of adipic compounds this effect

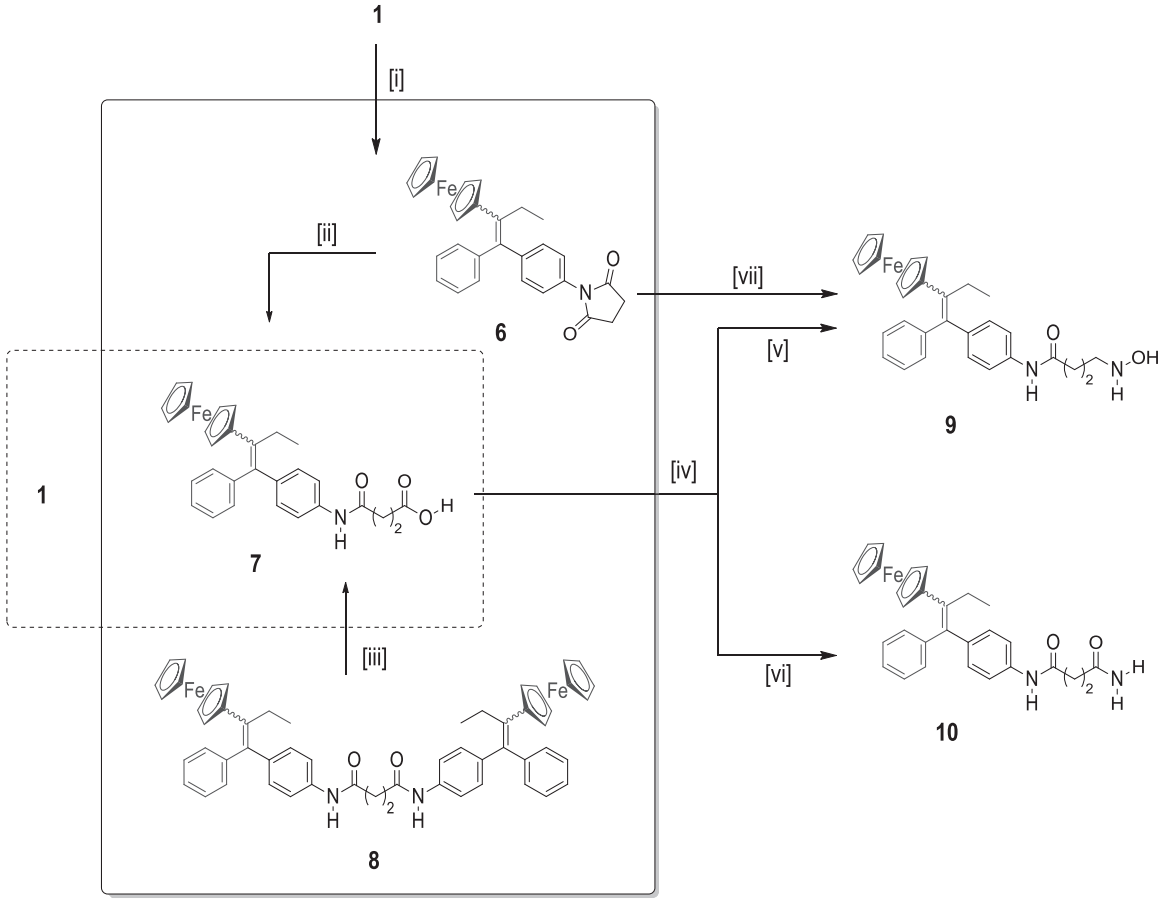


IC_{50} against MDA-MB-231	0.50 μM	>10 μM
IC_{50} against MCF-7	1.81 μM	>10 μM
	FcTAM-PSA	**PSA**

Fig. 3. Chemical structures of primary suberamides **FcTAM-PSA** and **PSA** with IC_{50} values against breast cancer cells.

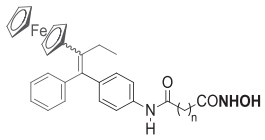


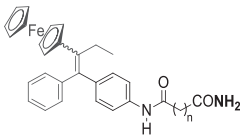
Scheme 1. Synthesis of adipic derivatives. Reagents and conditions: [i] $(ClCOCH_2CH_2)_2$, DCM, rt, 20 min (51%). [ii] $ClCO_2Et$, TEA, THF, 0 °C, 15 min [iii] $NH_2OH{\cdot}HCl$, KOH, MeOH, 0 °C, 20 min then 2.5 h (36%). [iv] $NaNH_2$, rt, 30 min (93%).



Scheme 2. Synthesis of succinic derivatives. Reagents and conditions: [i] $(ClCOCH_2)_2$, THF, rt, 30 min, (48%). [ii] NaOH, MeOH, rt, 15 min (96%). [iii] HCl, $(CH_3)_2CO/H_2O$, reflux, 1 h. [iv] $ClCO_2Et$, TEA, THF, 0 °C, 15 min [v] $NH_2OH{\cdot}HCl$, KOH, MeOH, 0 °C, 20 min then 2 h (30%). [vi] $NaNH_2$, rt, 30 min (36%). [vii] Excess of NH_2OH, 20 min, 19%.

Table 1
IC_{50} (μM) for adipic ($n = 4$) and suberic ($n = 6$) amides against breast cancer cell lines.[a]





n	Compound	MDA-MB-231	MCF-7	Compound	MDA-MB-231	MCF-7
6	**FcTAM-SAHA**	0.70 ± 0.07	2.01 ± 0.07	**FcTAM-PSA**	0.50 ± 0.11	1.81 ± 0.88
4	**4**	3.31 ± 0.46	3.71 ± 0.76	**5**	3.35 ± 0.68	3.14 ± 0.43

[a] Measurements were performed in duplicate after 72 h. Values are reported with SD.

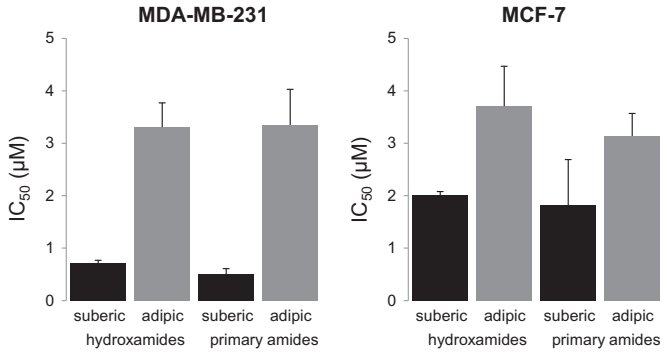


Chart 1. IC_{50} (μM) for suberic and succinic amides against MDA-MB-231 and MCF-7 breast cancer cells.

was not present, with equal cytotoxicity on both hormone-dependent and hormone-independent breast cancer cells. Given the estrogenicity of related compounds and the fact that one of the major differences between MDA-MB-231 and MCF-7 is the presence of ERα, adipic compounds may be less estrogenic but less cytotoxic as well.

2.4. Antiproliferative activity of succinamides against breast cancer cells

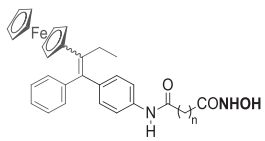
The two ferrocenyl succinamides were shown to be antiproliferative against both breast cancer cell lines (Table 2 and Chart 2). In the case of succinic hydroxamide **9**, the attenuation of cytotoxicity due to the chain shortening was also true as it occurred with adipic hydroxamide **4**. Surprisingly, primary succinamide **10** was as active as primary suberamide **FcTAM-PSA**, even though it lacks four methylene groups in comparison with this latter. Moreover, it was six times more active than adipamide **5** against MDA-MB-231 and twice as active against MCF-7, which lacks two methylene groups in comparison with **5**. This could indicate that primary succinamide **10** may show a particular behavior different from that of adipamides. We can take as an example the study on phenylbutyrate derivatives [13] that suggest that phenylbutyrate was more effective than other acids bearing longer chains. This is also consistent with the activities observed in the present study for succinic derivatives with shorter chain lengths but more cytotoxic effects than adipic ones.

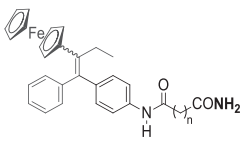
2.5. Antiproliferative activity of succinimide and carboxylic acids

Succinimide **6** and carboxylic acids **2** and **7** were also tested against both breast cancer cell lines (Table 3 and Chart 3). Interestingly, with more modest IC_{50} values than the amides, carboxylic acids were also cytotoxic against cancer cells. Contrary to the behavior of adipic and succinic amides, carboxylic acid **2** bearing a longer chain than **7** seems to be the more cytotoxic of the two. In both carboxylic compounds **2** and **7**, we can see a similar cytotoxicity against both breast cancer cell lines. However, succinimide **6** was, interestingly, more active than either **2** or **7**. Moreover, it was the most active compound of all the three series – suberic, adipic and succinic derivatives – against hormone-dependent MCF-7 breast cancer cells with an IC_{50} value of 1.06 μM. The crystal structure of hERα LBD bound to 4-OHTAM, determined by Shiau et al. [21] has shown that the dimethylamino chain is stabilized in the active site by contacts with Thr-347, Ala-350 and Trp-383. The good activity of **6** against MCF-7 cells could be explained by the potential ability of the succinimide group to bind to these amino acids and by the steric effect of succinimide unit.

In these three examples, it can be seen that shortening the chain length did not dramatically modify the activity of compounds as it was observed in the reported study of multifunctional species towards HDAC, EGFR and HER2 demonstrating that EGFR and HER2 inhibition were largely unaffected by the change in carbon chain length and concluding that side chain on the molecular structure of such compounds is not critical for EGFR/HER2 inhibition activity

Table 2
IC_{50} (μM) for succinic ($n = 2$) and suberic ($n = 6$) amides against breast cancer cell lines.[a]





n	Compound	MDA-MB-231	MCF-7	Compound	MDA-MB-231	MCF-7
6	**FcTAM-SAHA**	0.70 ± 0.07	2.01 ± 0.07	**FcTAM-PSA**	0.50 ± 0.11	1.81 ± 0.88
2	**9**	1.43 ± 0.05	2.78 ± 0.82	**10**	0.54 ± 0.11	1.30 ± 0.18

[a] Measurements were performed in duplicate after 72 h. Values are reported with SD.

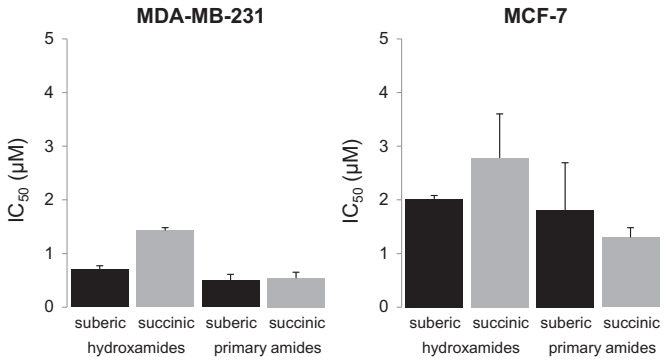
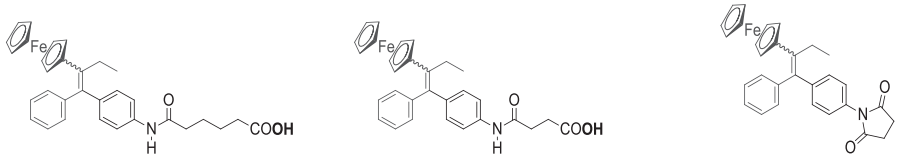


Chart 2. IC_{50} (μM) for succinic and suberic amides against MDA-MB-231 and MCF-7 breast cancer cells.

3. Conclusions

We have synthesized seven new ferrocenyl compounds bearing different chain lengths and functional groups. All of them showed strong antiproliferative activities against triple negative MDA-MB-231 and hormone-dependent MCF-7 breast cancer cell lines with IC_{50} values ranging from 0.5 μM to 4.12 μM. Primary amides **FcTAM-PSA** and **10** were the most cytotoxic compounds of all the three series (suberic, adipic and succinic) against MDA-MB-231 cells, with IC_{50} values of 0.50 μM and 0.54 μM, respectively; while succinimide **6** was the most active compound against hormone-dependent MCF-7 breast cancer cells. This study showed that MCF-7 and MDA-MB-231 are differently sensitive to the effects of compounds. Cytotoxic activities of primary amides are not strongly affected by chain length shortening. It seems that some of the cytotoxic effects of hydroxamides could be related to enzymatic inhibition processes. This could be of interest in our efforts to identify the target proteins for these species.

Table 3
IC_{50} (μM) of acids and succinimide breast cancer cell lines.[a]



2 **7** **6**

MDA-MB-231	MCF-7	MDA-MB-231	MCF-7	MDA-MB-231	MCF-7
1.89 ± 0.01	2.69 ± 0.78	3.48 ± 0.76	4.12 ± 0.09	1.74 ± 0.21	1.06 ± 0.06

[a] Measurements were performed in duplicate after 72 h. Values are reported with SD.

[16]. This is also consistent with our results, showing that chain shortening does not significantly affect the cytotoxicity of products and even the less cytotoxic products in this paper (**7**, for example, showed an IC_{50} = 4.12 μM against MCF-7) are antiproliferative species against breast cancer cells. We have already reported that HDAC are not the principal targets of such compounds. This study might confirm that hypothesis. However, it seems that there is still the possibility that some of the cytotoxic effects of hydroxamides could be related to enzymatic inhibition processes. This opens the possibility of study of the above compounds on diverse biological targets.

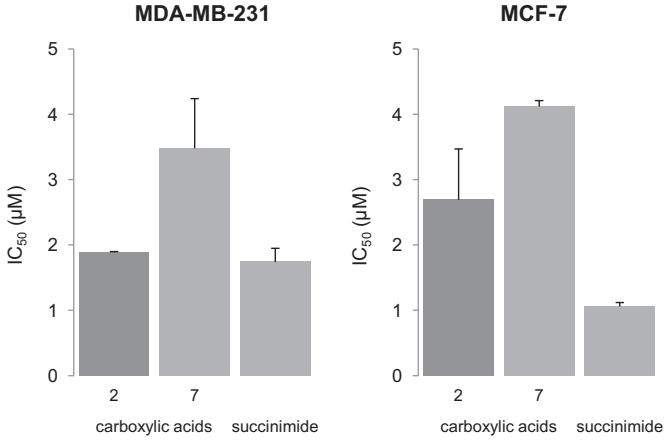


Chart 3. IC_{50} (μM) for succinimide and carboxylic acids against MDA-MB-231 and MCF-7 breast cancer cells.

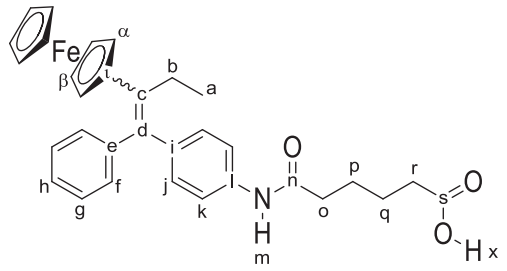
4. Experimental

4.1. General procedures

All reagents and solvents were obtained from commercial suppliers and used without further purification. Tetrahydrofuran (THF) was distilled from Na/benzophenone and dichloromethane (DCM) from P_2O_5, both under argon atmosphere. Thin layer chromatography (TLC) 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. Infrared (IR) spectra were recorded on a Jasco FT/IR-4100 Fourier transform infrared spectrometer by potassium bromide (KBr) pellets technique and all data are expressed in wave numbers (cm^{-1}). Melting points (mp) were obtained with a Kofler device and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a 300 MHz Bruker spectrometer and chemical shifts (δ) are expressed in *ppm*. The mass spectra (MS) were obtained on a DSQII and ITQ 1100 Thermo Scientific spectrometer for both electronic impact (EI) and chemical ionization (CI) methods and API 3000 PE Sciex Applied Biosystems for electrospray ionization (ESI) method. Purity of >99% was confirmed by analytical reverse phase HPLC with a column Kromasil C18, 10 μm, L = 25 cm, D = 4.6 mm using MeOH and mixtures of MeOH/H_2O as mobile phase, flow rate = 1 mL/min, λ = 254 nm. Elemental analyses were performed by the Laboratory of Microanalysis at ICSN of CNRS at Gif sur Yvette, France.

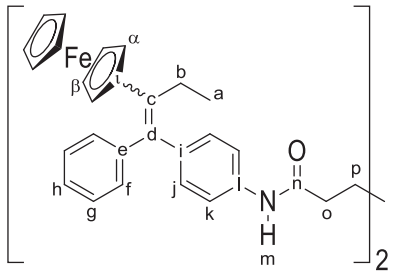
4.2. Synthesis

4.2.1. 6-[4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)anilino]-6-oxohexanoic acid, **2**



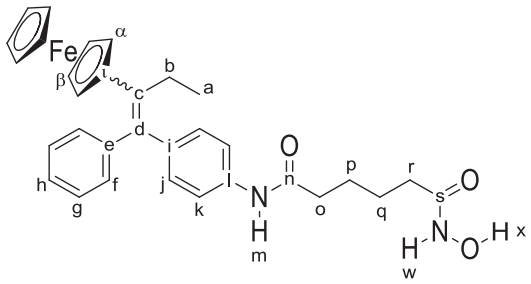
A solution of aniline **1** (5.53 mmol, 2.25 g) in 30 mL of DCM was slowly added at room temperature into a stirred solution of (11.05 mmol, 1.6 mL) adipoyl chloride in 10 mL of DCM. After 20 min of stirring, the mixture was poured into an aqueous solution of sodium hydroxide (NaOH) and then it was slightly acidified with a 10% aqueous solution of hydrochloric acid (HCl). The product was extracted with ethyl acetate (AcOEt), the organic layer was dried over magnesium sulfate ($MgSO_4$), filtered and evaporated. The crude product was separated by column chromatography using a 1:1 mixture of hexane and AcOEt. The first fraction was identified as the byproduct bisamide **3**. The last fraction was the carboxylic acid **2**. Thus, 1.50 g (51%) of **2** were obtained as an orange-red solid in a *Z*/*E* isomer ratio = 78/22. mp: 85–87 °C. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm)**:** *Z* isomer, δ 0.99 (1.00 for *E* isomer, t, J = 7.4 Hz, 3H: a), 1.42–1.67 (m, 4H: q, p), 2.18–2.36 (m, 4H: r, o), 2.48 (q, J = 7.4 Hz, 2H: b), 3.84 (3.78 for *E*, t, J = 1.9 Hz, 2H: α), 4.11 (4.07 for *E*, t, J = 1.9 Hz, 2H: β), 4.12 (s, 5H: Cp), 6.96 (7.04 for *E*, d, J = 8.5 Hz, 2H: j), 7.21 (d, J = 7.5 Hz, 2H: f), 7.25 (t, J = 7.5 Hz, 1H: h), 7.34 (t, J = 7.5 Hz, 2H: g), 7.49 (7.56 for *E*, d, J = 8.5 Hz, 2H: k), 9.86 (9.89 for *E*, s, 1H: m), 12.04 (s, 1H: x). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$, ppm): *Z* isomer, δ 15.4 (a), 24.1 (q), 24.7 (p), 27.1 (b), 33.4 (r), 36.1 (o), 68.1 (β), 68.8 (α), 69.1 (Cp), 85.5 (ι), 119.1 (k), 126.2 (h), 128.4 (129.1 for *E*, g), 128.8 (129.2 for *E*, f), 129.5 (j), 136.6 (c), 137.0 (d), 137.5 (l), 139.1 (i), 144.3 (e), 170.9 (n), 174.4 (s). IR (KBr, υ_{max}/cm^{-1}): 3298 (N–H and O–H stretch), 3093, 3051 (C_{Ar}–H stretch), 2970, 2962 (C_{Alk}–H stretch), 1709 (OC=O stretch), 1662 (NC=O stretch), 1593 (C_{Ar}=C_{Ar} stretch), 1523 (N–H bend), 1404 (C–N stretch), 1242 (C–O stretch). MS (CI, *m/z*): 553 $[MNH_4]^+$, 536 $[MH]^+$, 408 [FPBA-H]$^+$. Anal. Calc. for $C_{32}H_{33}FeNO_3$ (%): C, 71.78; H, 6.21; N, 2.62. Found: C, 71.16; H, 6.78; N, 2.22. HPLC (R_T, min): 2.97 (MeOH), 4.17 (MeOH/H_2O, 90:10).

4.2.2. N^1,N^6-bis[4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)phenyl] adipamide, **3**



Bisanilide **3** was identified as byproduct for **2** synthesis. mp: 96–98 °C. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): major isomer (68%), δ 0.98 (t, J = 7.4 Hz, 6H: a), 1.55–1.70 (m, 4H: p), 2.24–2.38 (m, 4H: o), 2.48 (q, J = 7.4 Hz, 4H: b), 3.88 (3.77 for minor isomer, t, J = 1.9 Hz, 4H: α), 4.10 (4.07 for minor, t, J = 1.9 Hz, 4H: β), 4.12 (s, 10H: Cp), 6.96 (7.04 for minor, d, J = 8.5 Hz, 4H: j), 7.21 (d, J = 7.5 Hz, 4H: f), 7.24 (t, J = 7.5 Hz, 2H: h), 7.34 (t, J = 7.5 Hz, 4H: g), 7.49 (7.56 for minor, d, J = 8.5 Hz, 4H: k), 9.88 (9.91 for minor, s, 1H: m). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$, ppm): major isomer, δ 15.4 (a), 24.9 (p), 27.1 (b), 36.3 (o), 68.1 (β), 68.8 (α), 69.1 (Cp), 85.5 (ι), 119.1 (k), 126.2 (h), 128.4 (129.1 for *E*, g), 128.8 (129.2 for *E*, f), 129.5 (j), 136.6 (c), 137.0 (d), 137.6 (l), 139.1 (i), 144.4 (e), 171.0 (n). IR (KBr, υ_{max}/cm^{-1}): 3297 (N–H stretch), 3093, 3040 (C_{Ar}–H stretch), 2927, 2866 (C_{Alk}–H stretch), 1662 (NC=O stretch), 1593 (C_{Ar}=C_{Ar} stretch), 1520 (N–H bend), 1401 (C–N stretch). MS (CI, *m/z*): 925 $[MH]^+$, 408 [FPBA-H]$^+$. Anal. Calc. for $C_{58}H_{56}Fe_2N_2O_2 \cdot 3H_2O$ (%): C, 71.17; H, 6.38; N, 2.86. Found: C, 70.99; H, 6.43; N, 2.57. HPLC (R_T, min): 8.52 min (MeOH).

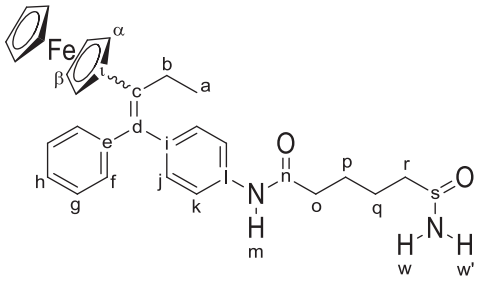
4.2.3. N^1-[4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)phenyl]-N^6-hydroxyadipamide, **4**



A solution of hydroxylamine hydrochloride ($NH_2OH \cdot HCl$, 4.0 mmol, 0.278 g) in 10 mL of methanol (MeOH) was added to a stirred solution of potassium hydroxide (KOH, 8.0 mmol, 0.448 g) in 10 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 **2** (1.0 mmol, 0.535 g) in 15 mL of anhydrous THF, cooled to 0 °C, ethyl chloroformate ($ClCO_2Et$, 2.0 mmol, 0.19 mL) and triethylamine (TEA, 2.5 mmol, 0.35 mL) were added and the mixture was stirred for 15 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 2.5 h. After that, water was added and the mixture was slightly acidified with HCl. 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 AcOEt as eluent. 0.200 g (36%) of **4** was obtained as an orange-red solid in a *Z*/*E* isomer ratio = 71/29. mp: 130–132 °C. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): *Z* isomer, δ 0.99 (1.00 for *E* isomer, t, J = 7.4 Hz, 3H: a), 1.42–1.67 (m, 4H: q, p), 1.91–2.04 (m, 2H: r), 2.18–2.36 (m, 2H: o), 2.48 (q, J = 7.4 Hz, 2H: b), 3.83 (3.77 for *E*, t, J = 1.9 Hz, 2H: α), 4.11 (4.08 for *E*, t, J = 1.9 Hz, 2H: β), 4.12 (s, 5H: Cp), 6.96 (7.04 for *E*, d, J = 8.5 Hz, 2H: j), 7.21 (d, J = 7.5 Hz, 2H: f), 7.25 (t, J = 7.5 Hz, 1H: h), 7.35 (t, J = 7.5 Hz, 2H: g), 7.48 (7.56 for *E*, d, J = 8.5 Hz, 2H: k), 8.69 (s, 1H: w), 9.86 (9.89 for *E*, s, 1H: m), 10.37 (s, 1H: x). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$, ppm): *Z* isomer, δ 15.4 (a), 24.9 (q, p), 27.1 (b), 32.2 (r), 36.2 (o), 68.1 (β), 68.8 (68.7 for *E*, α), 69.1 (Cp), 85.5 (ι), 119.1 (k), 126.2 (h), 128.4 (129.1 for *E*, g), 128.8 (129.2 for *E*, f), 129.5 (j), 136.6 (c), 137.0 (d), 137.5 (l), 139.1 (i), 144.3 (e), 169.0 (s), 171.0 (n). IR (KBr, υ_{max}/cm^{-1}): 3236 (N–H and O–H stretch), 3101 (C_{Ar}–H stretch), 2958, 2931 (C_{Alk}–H stretch), 1643 (NC=O stretch), 1597 (C_{Ar}=C_{Ar} stretch), 1523 (N–H

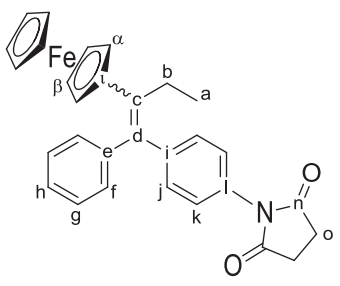
bend), 1401 (C–N stretch). MS (ESI, *m/z*): 549 $[M - H]^-$. Anal. Calc. for $C_{32}H_{34}FeN_2O_3 \cdot ¼H_2O$ (%): C, 69.25; H, 6.27; N, 5.05. Found: C, 69.51; H, 6.55; N, 5.05. HPLC (R_T, min): 3.78 (MeOH), 7.85 ($MeOH/H_2O$, 90/10).

4.2.4. N^1-[4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)phenyl] adipamide, **5**



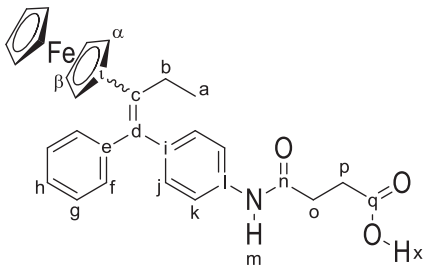
To a solution of **2** (1.0 mmol, 0.535 g) in 10 mL of anhydrous THF, cooled to 0 °C, $ClCO_2Et$ (2.0 mmol, 0.19 mL) and TEA (2.5 mmol, 0.35 mL) were added and the mixture was stirred for 15 min. The solid was filtered off and an excess of sodium amide ($NaNH_2$) was added to the filtrate. After 30 min of stirring at room temperature, 15 mL of water were 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 AcOEt as eluent. 0.500 g (93%) of amide **5** was obtained as an orange-red solid in a *Z/E* isomer ratio: 60/40. mp: 95–96 °C. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): *Z* isomer, δ 0.99 (1.00 for *E* isomer, t, $J = 7.4$ Hz, 3H: a), 1.42–1.67 (m, 4H: q, p), 2.00–2.13 (m, 2H: r), 2.16–2.36 (m, 2H: o), 2.48 (q, $J = 7.4$ Hz, 2H: b), 3.83 (3.77 for *E*, t, $J = 1.9$ Hz, 2H: α), 4.11 (4.08 for *E*, t, $J = 1.9$ Hz, 2H: β), 4.12 (s, 5H: Cp), 6.72 (s, 1H: w'), 6.96 (7.04 for *E*, d, $J = 8.5$ Hz, 2H: j), 7.21 (d, $J = 7.5$ Hz, 2H: f), 7.20–7.30 (m, 4H: f, w, h), 7.34 (t, $J = 7.5$ Hz, 2H: g), 7.48 (7.55 for *E*, d, $J = 8.5$ Hz, 2H: k), 9.86 (9.89 for *E*, s, 1H: m). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$, ppm): *Z* isomer, δ 15.4 (a), 24.9 (q), 25.0 (p), 27.1 (b), 35.0 (r), 36.3 (o), 68.1 (β), 68.8 (α), 69.1 (Cp), 85.5 (ι), 119.1 (k), 126.2 (h), 128.4 (129.1 for *E*, g), 128.8 (129.2 for *E*, f), 129.5 (j), 136.6 (c), 137.0 (d), 137.6 (l), 139.1 (i), 144.4 (e), 171.1 (n), 174.2 (s). IR (KBr, υ_{max}/cm^{-1}): 3433, 3294 (N–H stretch), 3097 (C_{Ar}–H stretch), 2950, 2873 (C_{Alk}–H stretch), 1658 (NC=O stretch), 1600 (C_{Ar}=C_{Ar} stretch), 1519 (N–H bend), 1404 (C–N stretch). MS (CI, *m/z*): 552 $[MNH_4]^+$, 535 $[MH]^+$. Anal. Calc. for $C_{32}H_{34}FeN_2O_2 \cdot ½\ H_2O$ (%): C, 70.72; H, 6.49; N, 5.15. Found: C, 70.71; H, 6.48, N, 5.08. HPLC (R_T, min): 3.77 (MeOH), 7.79 ($MeOH/H_2O$, 90/10).

4.2.5. N-[4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)phenyl] succinimide, **6**



A solution of **1** (5.13 mmol, 2.09 g) in 45 mL of THF was added in 15 min into a stirred solution of succinyl chloride (6.35 mmol, 0.7 mL) in 15 mL of anhydrous THF at room temperature. After 30 min of stirring, the mixture was poured into 50 mL of a saturated aqueous solution of sodium bicarbonate ($NaHCO_3$). The product was extracted with AcOEt, the organic layer was dried over $MgSO_4$, filtered and evaporated. The crude was purified on a silica gel column using a 1:1 mixture of hexane and AcOEt as mobile phase. The first fraction was identified as the bisamide **8**, the second one was the succinimide **6** and the last one was the carboxylic acid **7**. Thus, 1.2 g (48%) of succinimide **6** were obtained as an orange-red solid in a *Z/E* isomer ratio = 72/28. mp : 89–90 °C. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): *Z* isomer, δ 1.01 (1.05 for *E* isomer, t, $J = 7.4$ Hz, 3H: a), 2.41–2.60 (q, $J = 7.4$ Hz, 2H: b), 2.76 (2.78 for *E*, s, 4H: o), 3.85 (3.80 for *E*, t, $J = 1.9$ Hz, 2H: α), 4.13 (4.10 for *E*, t, $J = 1.9$ Hz, 2H: β), 4.16 (4.15 for *E*, s, 5H: Cp), 7.17 (s, 4H: k, j), 7.21–7.33 (t, 1H: h), 7.26 (d, $J = 7.5$ Hz: f), 7.37 (t, $J = 7.5$ Hz, 2H: g). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$, ppm): *Z* isomer, δ 15.4 (a), 27.1 (b), 28.4 (o), 68.2 (β), 68.8 (α), 69.1 (Cp), 85.0 (ι), 126.4 (h), 126.9 (k), 128.5 (129.0 for *E*, g), 128.8 (129.1 for *E*, f), 129.4 (j), 130.6 (l), 136.3 (d), 137.4 (c), 143.9 (i), 144.0 (e), 176.9 (n). IR (KBr, υ_{max}/cm^{-1}): 3086 (C_{Ar}–H stretch), 2966, 2873 (C_{Alk}–H stretch), 1712 (C=O stretch), 1381 (C–N stretch). MS (CI, *m/z*): 507 $[MNH_4]^+$, 490 $[MH]^+$. Anal. Calc. for $C_{30}H_{27}FeNO_2 \cdot ½H_2O$ (%): C, 72.30; H, 5.66; N, 2.81. Found: C, 72.40; H, 5.56; N, 2.82. HPLC (R_T, min): 3.57 (MeOH), 7.13 ($MeOH/H_2O$, 90/10).

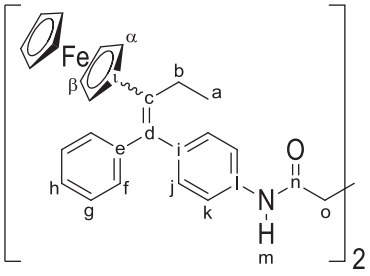
4.2.6. 4-[4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)anilino]-4-oxobutyric acid, **7**



An excess of NaOH was added to a solution of **6** (0.51 mmol, 0.25 g) in MeOH at room temperature. After 15 min of stirring, water was added and the mixture was slightly acidified with HCl. 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 a 1:1 mixture of hexane and AcOEt. 0.25 g (96%) of **8** was obtained as an orange-red solid in a *Z/E* isomer ratio = 70/30. This product can also be obtained, but in low yields, following the same protocol to synthesized succinimide **6**. However, the acid hydrolysis of bisamide **8** is also an alternative way to produce more of **7**. This hydrolysis can be performed directly on the reaction medium or on bisamide itself. Thus, an excess of HCl was added to a stirred solution of **8** in acetone/water. After 1 h of reflux, carboxylic acid **7** and amine **1** were obtained. mp: 210–213 °C. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): *Z* isomer, δ 0.99 (1.00 for *E* isomer, t, $J = 7.4$ Hz, 3H: a), 2.41–2.60 (m, 6H: b, p o), 3.83 (3.78 for *E*, t, $J = 1.9$ Hz, 2H: α), 4.11 (4.08 for *E*, t, $J = 1.9$ Hz, 2H: β), 4.13 (s, 5H: Cp), 6.96 (7.04 for E, d, $J = 8.5$ Hz, 2H: j), 7.21 (d, $J = 7.5$ Hz, 2H: f), 7.24 (t, $J = 7.5$ Hz, 1H: h), 7.34 (t, $J = 7.5$ Hz, 2H: g), 7.48 (7.55 for *E*, d, $J = 8.5$ Hz, 2H: k), 9.94 (9.97 for *E*, s, 1H: m), 12.17 (s, 1H: x). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$, ppm): *Z* isomer, δ 15.4 (a), 27.1 (b), 28.8 (p), 31.0 (o), 68.1 (β), 68.8 (α), 69.1 (Cp), 85.5 (ι), 118.9 (k), 126.2 (h),

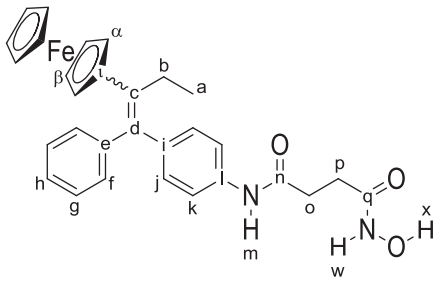
128.4 (129.1 for *E*, g), 128.7 (129.2 for *E*, f), 129.5 (j), 136.6 (c), 137.0 (d), 137.5 (l), 139.1 (i), 144.3 (e), 170.0 (n), 173.8 (q). IR (KBr, υ_{max}/cm^{-1}): 3321 (N–H and O–H stretch), 3097 (C_{Ar}–H stretch), 2966 (C_{Alk}–H stretch), 1712 (OC = O stretch), 1662 (NC═O stretch), 1601 (C_{Ar}═C_{Ar} stretch), 1531 (N–H bend), 1404 (C–N stretch), 1250 (C–O stretch). MS (CI, *m/z*): 525 $[MNH_4]^+$, 508 $[MH]^+$, 408 [FPBA-H]$^+$. Anal. Calc. for $C_{30}H_{29}FeNO_3$ (%): C, 71.01; H, 5.76; N, 2.76. Found: C, 69.91 H, 5.86; N, 2.75. HPLC (R_T, min): 3.46 (MeOH), 5.98 (MeOH/H_2O, 90:10).

4.2.7. N^1,N^4-bis[4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)phenyl] succinamide, **8**



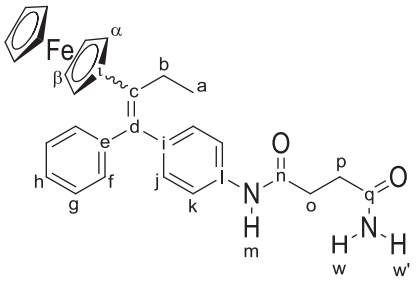
Bisanilide **8** was identified as byproduct for **6** and **7** synthesis. mp: 98–99 °C. ^{1}H-NMR (300 MHz, $(CD_3)_2CO$, ppm): major isomer (78%), δ 1.02 (1.04 for minor isomer, t, J = 7.4 Hz, 6H: a), 2.60 (q, J = 7.4 Hz, 4H: b), 2.72 (2.73 for minor, s, 4H: o), 3.92 (3.87 for minor, t, J = 1.9 Hz, 4H: α), 4.08 (4.06 for minor, t, J = 1.9 Hz, 4H: β), 4.13 (s, 5H: Cp), 6.99 (7.08 for minor, d, J = 8.5 Hz, 2H: j), 7.10-7.29 (m, 3H: f, h), 7.34 (t, J = 7.5 Hz, 2H: g), 7.55 (7.65 for minor, d, J = 8.5 Hz, 2H: k), 9.24 (9.28 for minor, s, 1H: m). ^{13}C-NMR (75 MHz, $(CD_3)_2CO$, ppm): major isomer, δ 15.8 (a), 28.4 (b), 32.4 (o), 68.9 (β), 69.9 (Cp), 70.0 (α), 87.2 (ι), 119.7 (k), 127.0 (h), 129.1 (g), 130.0 (f), 130.8 (j), 138.0 (c), 138.4 (d), 138.7 (l), 140.6 (i), 145.6 (e), 171.1 (n). MS (CI, *m/z*): 897 $[MH]^+$, 408 [FPBA-H]$^+$.

4.2.8. N^1-[4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)phenyl]-N^4-hydroxysuccinamide, **9**



A solution of $NH_2OH{\cdot}HCl$ (10.0 mmol, 0.695 g) in 10 mL of methanol (MeOH) was added to a stirred solution of potassium hydroxide (KOH, 20.0 mmol, 1.120 g) in 15 mL of MeOH at 0 °C. After it was stirred for 20 min, the precipitate was removed and the filtrate was placed in a flask. In another flask, to a solution of **8** (1.18 mmol, 0.600 g) in 15 mL of anhydrous THF, cooled to 0 °C, $ClCO_2Et$ (5.7 mmol, 0.55 mL) and TEA (6.84 mmol, 0.95 mL) were added and the mixture was stirred for 15 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 2 h. After that, water was added and the mixture was slightly acidified with HCl. 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 AcOEt as eluent. 0.185 g (30%) of **9** was obtained as an orange-red solid in a *Z*/*E* isomer ratio = 73/27. This compound can also be obtained by the direct attack of hydroxylamine to the succinimide **6** as follows. A solution of succinimide **6** (1.02 mmol, 0.50 g) reacted with an excess of basic NH_2OH freshly prepared in MeOH as described above at room temperature for 20 min. After that, water was added and the mixture was slightly acidified with HCl. 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 AcOEt as eluent. 0.100 g (19%) of product **9** was obtained. mp: 200–202 °C. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): *Z* isomer, δ 0.99 (1.00 for *E* isomer, t, J = 7.4 Hz, 3H: a), 2.27 (t, J = 7.0 Hz, 2H: p), 2.43–2.60 (m, 4H: b, o), 3.82 (3.77 for *E*, t, J = 1.9 Hz, 2H: α), 4.11 (4.08 for *E*, t, J = 1.9 Hz, 2H: β), 4.12 (s, 5H: Cp), 6.96 (7.04 for *E*, d, J = 8.5 Hz, 2H: j), 7.21 (d, J = 7.5 Hz, 2H: f), 7.24 (t, J = 7.5 Hz, 1H: h), 7.34 (t, J = 7.5 Hz, 2H: g), 7.48 (7.55 for *E*, d, J = 8.5 Hz, 2H: k), 8.72 (s, 1H: w), 9.95 (9.98 for *E*, s, 1H: m), 10.43 (s, 1H: x). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$, ppm): *Z* isomer, δ 15.4 (a), 27.1 (b), 27.4 (p), 31.5 (o), 68.1 (β), 68.8 (α), 69.1 (Cp), 85.5 (ι), 118.9 (k), 126.2 (h), 128.4 (129.1 for *E*, g), 128.7 (129.2 for *E*, f), 129.5 (j), 136.6 (c), 137.0 (d), 137.6 (l), 139.1 (i), 144.3 (e), 168.3 (q), 170.1 (n). IR (KBr, υ_{max}/cm^{-1}): 3255 (N–H and O–H stretch), 3097 (C_{Ar}–H stretch), 2966 (C_{Alk}–H stretch), 1658 (NC═O stretch), 1600 (C_{Ar}═C_{Ar} stretch), 1523 (N–H bend), 1404 (C–N stretch). MS (ESI, *m/z*): 521 $[M-H]^-$. Anal. Calc. for $C_{30}H_{30}FeN_2O_3{\cdot}H_2O$ (%): C, 66.67; H, 5.97; N, 5.18. Found: C, 66.17; H, 5.63; N, 5.22. HPLC (R_T): 3.55 (MeOH), 7.07 (MeOH/H_2O, 90:10).

4.2.9. N^1-[4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)phenyl] succinimide, **10**



To a solution of **8** (0.59 mmol, 0.300 g) in 10 mL of anhydrous THF, cooled to 0 °C, $ClCO_2Et$ (3.0 mmol, 0.29 mL) and TEA (3.4 mmol, 0.47 mL) were added and the mixture was stirred for 15 min. The solid was filtered off and an excess of sodium amide ($NaNH_2$) was added to the filtrate. After 30 min of stirring at room temperature, 15 mL of water were 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 AcOEt as eluent. 0.102 g (36%) of amide **10** was obtained as an orange-red solid in a *Z*/*E* isomer ratio: 65/35. mp: 235–236 °C. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm): *Z* isomer, δ 0.99 (1.00 for *E* isomer, t, J = 7.4 Hz, 3H: a), 2.33–2.42 (m, J = 7.0 Hz, 2H: p), 2.48 (q, J = 7.4 Hz, 2H: b), 2.46–2.60 (m, 2H: o), 3.82 (3.77 for *E*, t, J = 1.9 Hz, 2H: α), 4.11 (4.08 for *E*, t, J = 1.9 Hz, 2H:

β), 4.13 (s, 5H: Cp), 6.77 (s, 1H: w'), 6.96 (7.04 d, $J = 8.5$ Hz, 2H: j), 7.21 (d, $J = 7.5$ Hz, 2H: f), 7.24 (t, $J = 7.5$ Hz, 1H: h), 7.31–7.36 (s, 1H: w), 7.34 (t, $J = 7.5$ Hz, 2H: g), 7.48 (7.55 for *E*, d, $J = 8.5$ Hz, 2H: k), 9.91 (9.94 for *E*, s, 1H: m). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$, ppm): *Z* isomer, δ 15.4 (a), 27.1 (b), 29.9 (p), 31.5 (o), 68.1 (β), 68.8 (α), 69.1 (Cp), 85.5 (ι), 118.9 (k), 126.2 (h), 128.4 (129.1 g), 128.8 (f), 129.5 (j), 136.6 (c), 137.0 (d), 137.6 (l), 139.0 (i), 144.4 (e), 170.5 (n), 173.4 (q). IR (KBr, υ_{max}/cm^{-1}): 3433, 3282 (N–H stretch), 3093 (C_{Ar}–H stretch), 2966, 2927, 2873 (C_{Alk}–H stretch), 1654 (NC=O stretch), 1604 (C_{Ar}=C_{Ar} stretch), 1516 (N–H bend), 1404 (C–N stretch). MS (CI, *m/z*): 524 $[MNH_4]^+$, 507 $[MH]^+$, 408 $[FPBA\text{-}H]^+$. Anal. Calc. for $C_{30}H_{30}FeN_2O_2 \cdot 3H_2O$ (%): C, 64.29; H, 6.47; N, 5.00. Found: C, 64.79; H, 6.07; N, 4.86. HPLC (R_T, min): 3.59 (MeOH), 7.32 (MeOH/H_2O, 90:10).

4.2.9.1. Cytotoxicity assays. 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 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 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 compound concentrations ranged 5 nM to 100 μM in separate duplicate experiments.

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Abbreviations

EGFR epidermal growth factor receptor
FcTAM3 ferrocifen
FcTAM-PSA N^1-[4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)phenyl] suberamide
FcTAM-SAHA N^1-[4-(2-ferrocenyl-1-phenylbut-1-en-1-yl) phenyl]-N^8-hydroxysuberamide
FPBA 4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)aniline
HDAC histone deacetylase
HDACi histone deacetylase inhibitor
HER2 human epidermal growth factor receptor 2
JAHA N^1-ferrocenyl-N^8-hydroxysuberamide
PSA N^1-phenylsuberamide
SAHA N^1-hydroxy-N^8-phenylsuberamide
TAM tamoxifen
TEA triethylamine
THF tetrahydrofuran

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