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The Effect of Protic Electron Donor Aromatic Substituents on Ferrocenic and [3] Ferrocenophanic Anilines and Anilides: Some Aspects of Structure-Activity Relationship Studies on Organometallic Compounds with Strong Antiproliferative Effects

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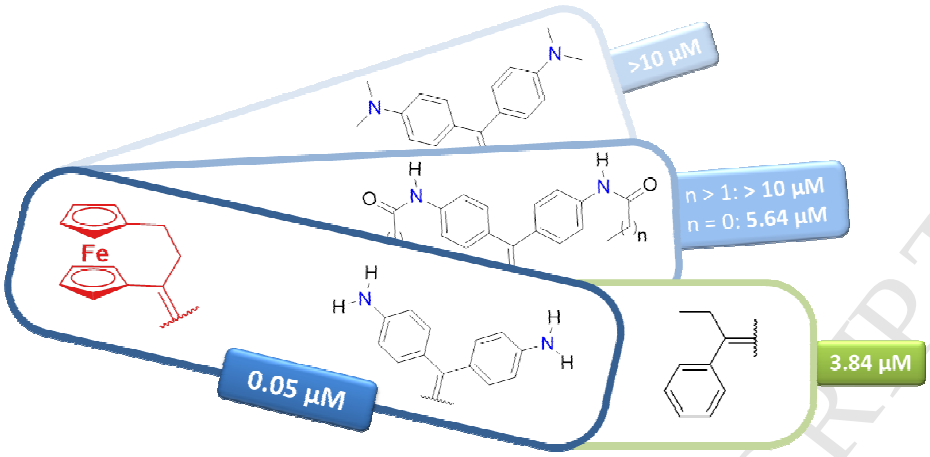

IC_{50} values against triple-negative MDA-MB-231 breast cancer cells

Highlights:

1. Strongly cytotoxic ([3]ferrocenophan-1-ylidenemethylene)dianiline was prepared.
2. Ferrocenophane compounds derived from Michler's ketone were synthesized.
3. *N*-acylation and *N*-methylation decreases activity of dianilines.
4. Intramolecular electron transfer process is observed in nitrogenous derivatives.
5. Aniline oxidation is influenced by the nature of organometallic moiety

The Effect of Protic Electron Donor Aromatic Substituents on Ferrocenic and [3]Ferrocenophanic Anilines and Anilides: Some Aspects of Structure-Activity Relationship Studies on Organometallic Compounds with Strong Antiproliferative Effects

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Dedicated to Prof. W. A. Herrmann on the occasion of his 65th birthday

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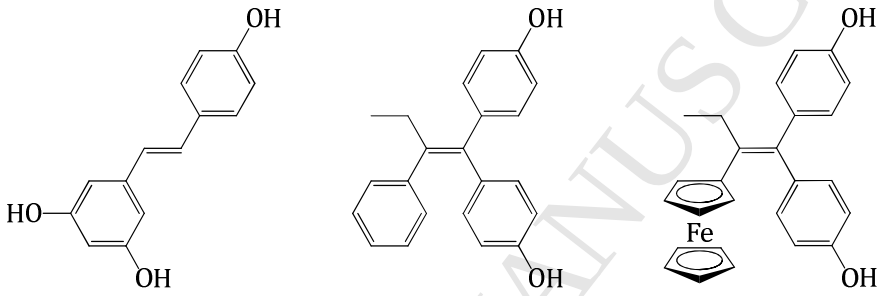
A B S T R A C T

A new family of nitrogenous derivatives is synthesized, characterized and evaluated for the investigation of the impact of some structural motifs such as functionalization and conjugation on the antiproliferative activity of ferrocenic complexes against cancer cells,. These compounds are 4,4'-([3]ferrocenophan-1-ylidenemethylene)dianilides and tetramethylated dianilines derived from Michler's ketone. An alternative McMurry direct heterocoupling method for 4,4'-([3]ferrocenophan-1-ylidenemethylene)dianiline synthesis is described and electrochemical studies are also discussed.

1. Introduction

Bioorganometallic chemistry, a burgeoning discipline involving the study of molecules bearing at least one carbon-metal covalent bond (C-M) and their biological applications, has grown exponentially in the last few years [1, 2]. A pertinent model of an organometallic moiety is ferrocene, which has been shown to be a desirable motif for the design of new products in medicinal chemistry research [3, 4].

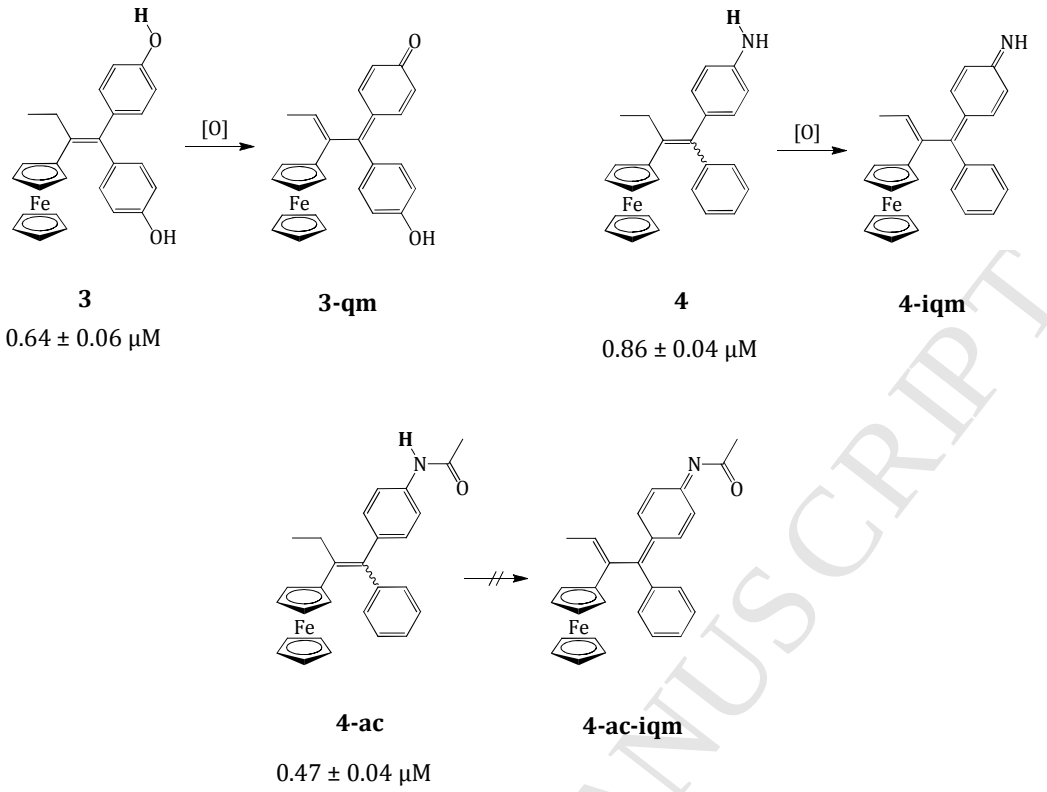
Generally, the antiproliferative properties of metal-based drugs are attributed to their direct interaction with deoxyribonucleic acid (DNA), as proposed from the coordination complex *cisplatin* [5]. Nevertheless, our group has focused on the synthesis of complexes that appear to be related to non-genomic mechanisms, and which could represent an alternative for the treatment of drug-resistant or incurable cancers. We found that organic polyphenols such as **1** (resveratrol) and **2** could be activated by judicious incorporation of organometallic units on their skeletons. For example, the ferrocene derivative **3** (Figure 1) showed strong antiproliferative activity, not only against MCF-7 cells, but also against hormone-independent MDA-MB-231 breast cancer cells (IC_{50} = 0.6 µM), while **2** stimulated hormone-dependent MCF-7 breast cancer cell growth and had no effect on MDA-MB-231 cells [6,7].



1 **2** **3**

Figure 1. Chemical structures of resveratrol **1**, 4,4'-(2-phenylbut-1-ene-1,1-diyl)diphenol **2** and 4,4'-(2-ferrocenylbut-1-ene-1,1-diyl)diphenol **3**.

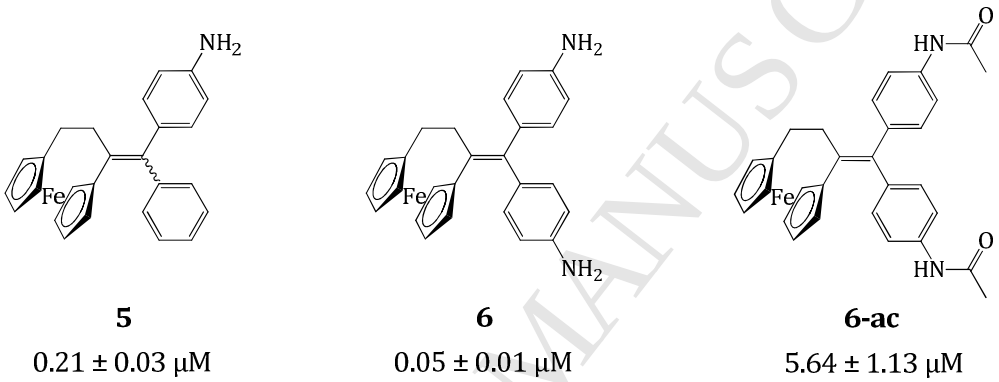
It has been proposed that the antiproliferative activity of ferrocene compounds is related to the generation of reactive oxygen species (ROS) in cancer cells [8, 9, 10, 11], and quinone methides such as **3-qm** (Scheme 1) [12]. Therefore, the conjugation of the ferrocenyl group to the 4-hydroxyphenyl group is essential for their activity [13].



Scheme 1. Formation of 4-[2-ferrocenyl-1-(4-hydroxyphenyl)but-2-en-1-ylidene]cyclohexa-2,5-dienone **3-qm** and 4-(2-ferrocenyl-1-phenylbut-2-en-1-ylidene)cyclohexa-2,5-dienimine **4-iqm** through the oxidation of diphenol **3** and monoaniline **4**, respectively. No oxidation of **4-ac** to **4-ac-iqm** occurs under the same conditions. IC_{50} values of **3**, **4** and **4-ac** against MDA-MB-231.

According to the mechanism shown in scheme 1, other protic and oxidizable functionalities should also exhibit the same behavior. Indeed, during our investigations, it was observed that compounds bearing amino (**4**) and acetamido (**4-ac**) functions also exhibited strong cytotoxic effects against breast cancer cell lines [14,15]. It has been reported that **4**, similarly to **3**, could readily undergo a base-promoted oxidation sequence [16], and the formation of iminoquinone methide (iqm) intermediates under biological conditions has been proposed [17,18]. The high reactivity of these species towards nucleophiles, in particular, towards thiols, polyhydroxylated compounds and cytosine, as well as electron-deficient dienophiles [19], has also been reported. The cytotoxic properties of amino analogues may be strongly related to the electron donor capacity of the heteroatom to activate the π-conjugated system, and therefore, acidic protons in such substituents should be an important element in the molecular design to assure cytotoxic effects. For **4-ac**-like amide derivatives, it was proposed that same oxidative pathway may be followed after enzymatic hydrolysis of the amide functionality to afford **4**-like compounds [20].

On the other hand, we have recently shown that the replacement of the 1-ferrocenylpropylidene (Fcpd) group by [3]ferrocenophan-1-ylidene (Fpd) yielded more effective species against cancer cells (Figure 2) [21,22,23]. For example, the monoaniline **5** (IC_{50} = 0.21 ± 0.03 μM)[24] was four times more active than **4** (IC_{50} = 0.86 ± 0.04 μM) against triple negative MDA-MB-231 breast cancer cells. Moreover, the additional amine substituent produced the dianiline **6** (0.05 ± 0.01 μM), which, in turn, was four times more active than the monoaniline **5**. Nonetheless, it was suspected that these constricted species do not undergo the same redox activation patterns as the Fcpd derivatives, and consequently, exert their antiproliferative effects through different mechanisms of action.



5 0.21 ± 0.03 μM | **6** 0.05 ± 0.01 μM | **6-ac** 5.64 ± 1.13 μM

Figure 2. Chemical structures of monoaniline **5**, dianiline **6** and diacetanilide **6-ac** with their IC_{50} values against hormone-independent MDA-MB-231 breast cancer cells.

Herein, we describe the synthesis and characterization of a new series of three organometallic amides bearing non-functionalized lateral alkyl chains. With these species, we explored the impact of chain length, the role of functionalization and the importance of organometallic moieties on their antiproliferative activities. We also describe the synthesis and characterization of three new tetramethylated dianilines derived from Michler's ketone; with these, we evaluated the importance of the hydrogen atoms on primary dianilines such as **6**. To explore the impact of the Fpd motif on the cytotoxicity, we also synthesized the organic dianiline **7**. We expected these new compounds to show very low antiproliferative effects to support our proposal regarding the formation of iminoquinone methide-type derivatives as active metabolites. An alternative direct method for the synthesis of the known 4,4'-([3]ferrocenophan-1-ylidenemethylene)dianiline **6** is also described. Finally, a mechanism based on electrochemical studies explaining the impediments to iminoquinone methide-type derivative formation from **4-ac**-like *N*-[4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)phenyl]alkanamides is proposed.

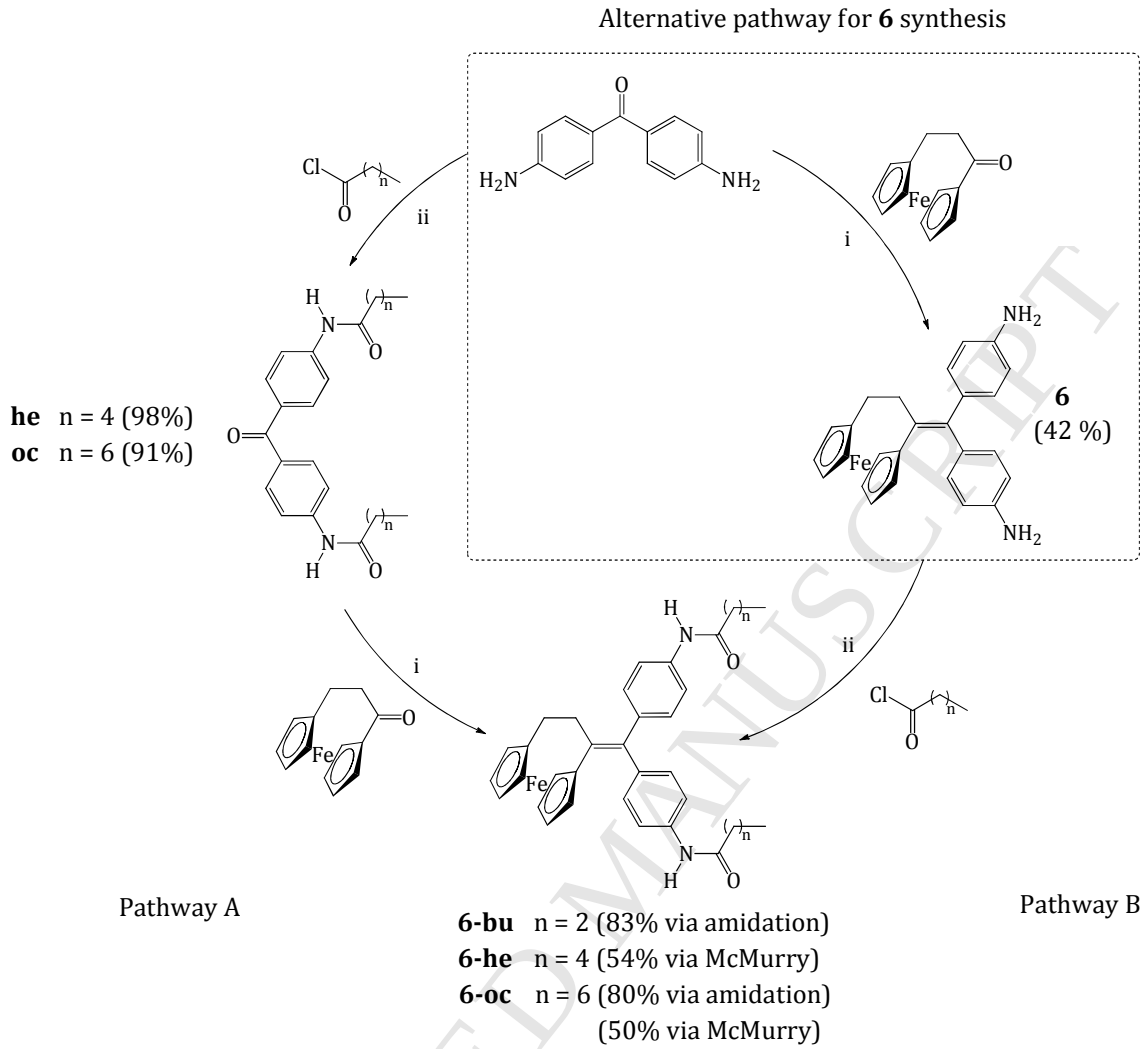
2. Results and discussion

2.1 Synthesis

*2.1.1 Alternative synthesis of **6** and synthesis of derived diamides **6-bu**, **6-he** and **6-oc**.*

The protocol reported for the organometallic dianiline **6** involves two steps [24]. First, a 3-day McMurry cross-coupling reaction took place between [3]ferrocenophan-1-one and *N*,*N*'-[carbonylbis(4,1-phenylene)]diacetamide to afford the intermediate diamide. This was followed by a 4-hour hydrolysis step to give the final product in 34% yield. We now describe its synthesis in only one step by using a McMurry cross-coupling reaction between [3]ferrocenophan-1-one and 4,4'-diaminobenzophenone, which gave a 42% yield in just 15 min (Scheme 2).

On the other hand, *N*,*N*'-[([3]ferrocenophan-1-ylidenemethylene)bis(4,1-phenylene)]dialkanamides derived from dianiline **6** and butyric (**bu**), hexanoic (**he**) and octanoic (**oc**) acids could be prepared through two pathways (Scheme 2). Pathway A involved a McMurry cross-coupling reaction between [3]ferrocenophan-1-one and the corresponding *N*,*N*'-[carbonylbis(4,1-phenylene)]dialkanamide, which was prepared previously through the amidation reaction (ii) of 4,4'-diaminobenzophenone with acyl chlorides, to produce dialkanamides **6-he** and **6-oc** in 54% and 50% yields, respectively. In pathway B, **6** reacted with the corresponding acyl chloride to form dialkanamides **6-he** and **6-oc** in 83% and 80% yields, respectively.

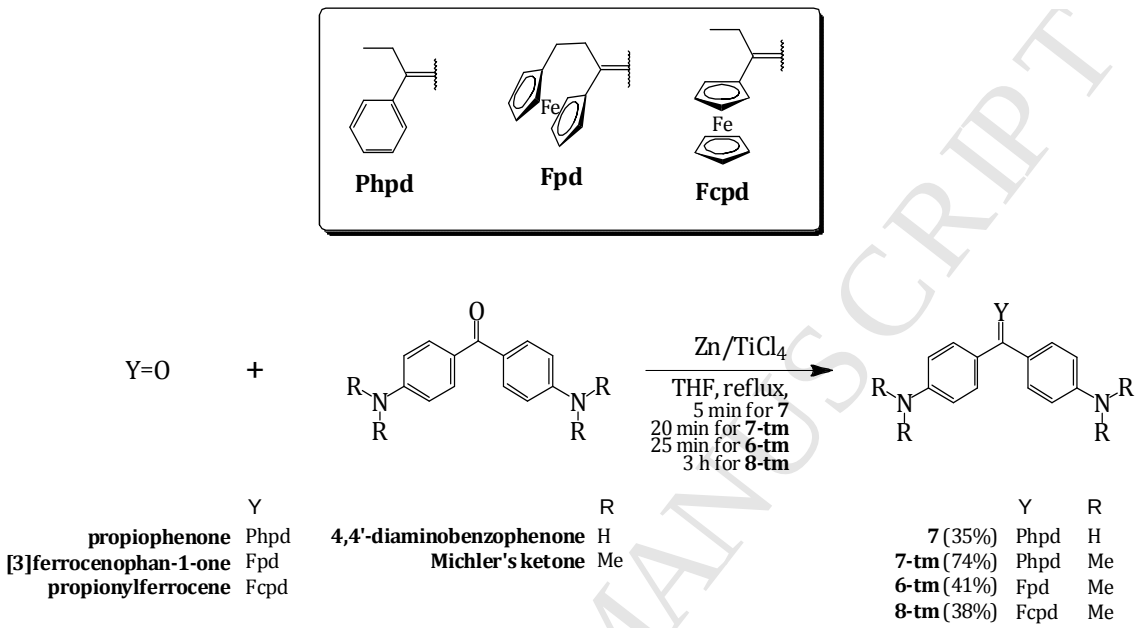


Scheme 2. *Alternative synthetic pathway for dianiline **6** and synthesis of N,N'-[([3]ferrocenophan-1-ylidenemethylene)bis(4,1-phenylene)]dialkanamides **6-bu**, **6-he** and **6-oc**.* Reagents and conditions: (i) THF, TEA, 15 min. (ii) Zn/$TiCl_4$, THF, 90-100°C, 15 min.

*2.1.2. Synthesis of organic dianiline **7** and tetramethylated dianilines **6-tm**, **7-tm** and **8-tm**.*

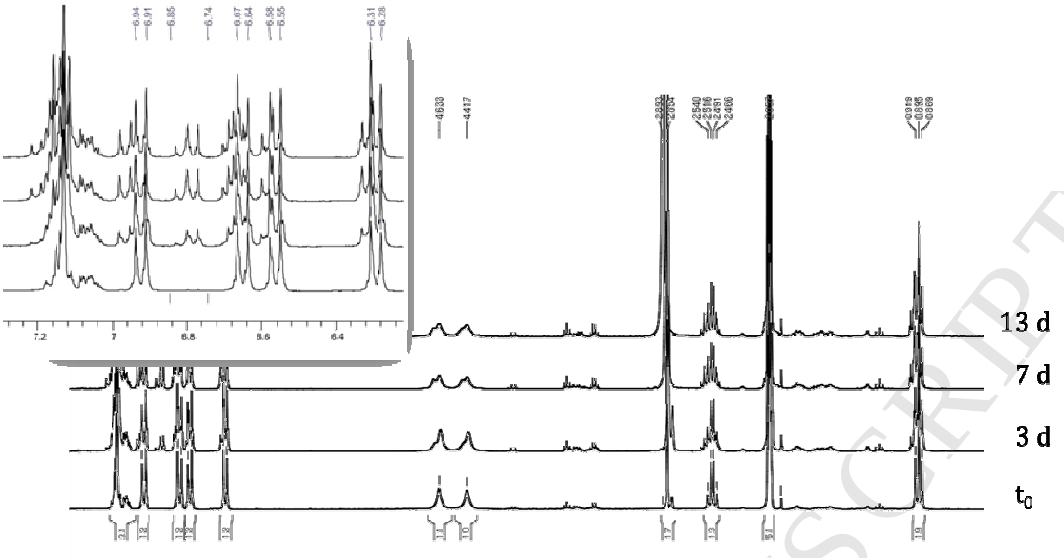
For the evaluation of the effect of the [3]ferrocenophan-1-ylidene (Fpd) motif on the antiproliferative activity, an organic analogue of dianiline **6**, bearing the 1-phenylpropilydene (Phpd) group instead of Fpd, *i.e.* dianiline **7**, was also prepared via the McMurry cross-coupling between 4,4'-diaminobenzophenone and propiophenone in just 5 min after catalyst preparation (Scheme 3). Finally, for evaluation of the effect of the removal of protons from the nitrogen atoms in dianilines **6** and **7**, [bis(4-(dimethylamino)phenyl]methanone (Michler's ketone) was reacted with [3]ferrocenophan-1-one and propiophenone, respectively, under McMurry cross-coupling conditions to produce the corresponding methylated analogues **6-tm** and **7-tm**. Surprisingly, the tetramethylated dianiline **8-tm**

was formed in 3 h, while we were unable to isolate its protic analogue, the 4,4'-(2-ferrocenylbut-1-en-1,1-diyl)dianiline.

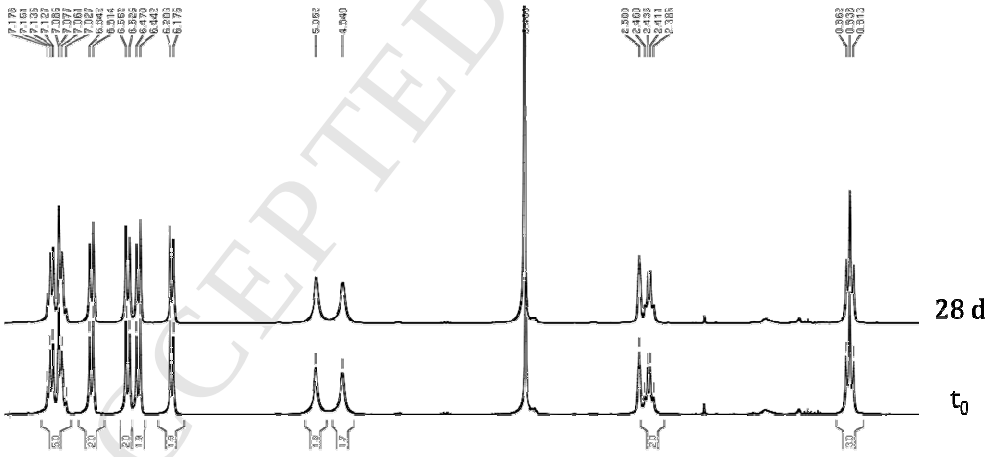


Scheme 3. Synthesis of primary dianiline **7** and tertiary dianilines **6-tm, 7-tm** and **8-tm**.

It is important to mention that dianilines such as **6** and **7** showed particular behaviors in acetone-*d*. For example, ^{1}H-NMR analysis (Spectrum 1) of a sample of **7** stored in acetone-*d* for 13 days showed a deformation of the triplet at 0.90 ppm (CH_3) and the quartet at 2.50 ppm (CH_2). An integral decrease of the singlets at 4.42 ppm (NH_2) and 4.63 ppm (NH_2) and partially overlapped signals at 4.44 ppm and 4.67 ppm were also observed. In the aromatic region, new signals appeared, especially in the ranges 6.74-6.85 ppm and 6.91-7.00 ppm. ^{1}H-NMR analysis in DMSO-*d* did not show any changes, even over a longer period (Spectrum 2). It was suspected that this particular behavior with acetone could be the result of imine formation, highlighting the capability of the dianiline to react with electrophilic species such as ketones.



Spectrum 1. ^{1}H-NMR (300 Hz) analysis of dianiline **7** stored in $(CD_3)_2CO$ for 13 days



Spectrum 2. ^{1}H-NMR (300 Hz) analysis of dianiline **7** stored in $(CD_3)_2SO$ for 28 days

2.2 Biological activity

*2.2.1 Comparison of the antiproliferative activities of diamides **6-bu**, **6-he** and **6-oc** and diamine **6**.*

The cytotoxic effects of diamides **6-bu**, **6-he** and **6-oc** bearing the [3]ferrocenophan-1-ylidene (Fpd) motif on hormone-independent MDA-MB-231 breast cancer cells were determined first. Figure 3 shows the percentage of cell growth inhibition at 1 µM. The values for **6-bu**, **6-he**, and **6-oc** were 27%, 23%, and 18%, respectively. These results prove that the efficiencies of such species vary with the chain length. Thus, the longer the alkyl side-chain of the compound, the lower its cytotoxicity; the order of cytotoxicity was **6-bu** > **6-he** > **6-oc**. At 10 µM, the inhibition value for **6-he** was 51%, while those of **6-bu** and **6-oc** were below 50% which indicates that they were less active than the diacetanilide **6-ac** (IC_{50} = 5.64 µM). It should be noted that these four anilides were far less active than the diamine **6** (IC_{50} = 0.05 µM).

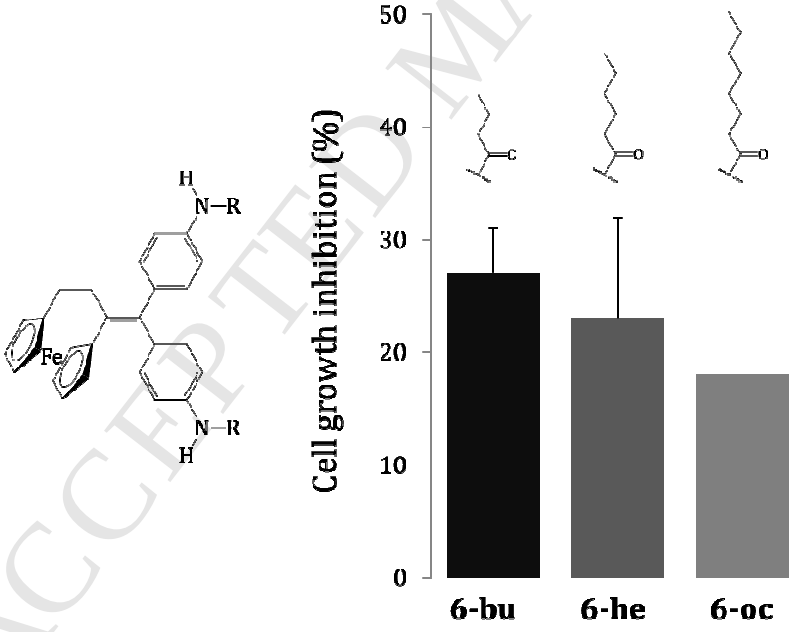


Figure 3. MDA-MB-231 breast cancer cell growth inhibition by dianilides **6-bu**, **6-he** and **6-oc** at 1 µM.

*2.2.1 Comparison of the antiproliferative activity of primary (**6**-**7**) and tertiary (**6-tm**, **7-tm** and **8-tm**) dianilines.*

For the evaluation of the impact of the [3]ferrocenophan-1-ylidene (Fpd) motif in the skeleton of the organometallic compound **6**, the organic analogue **7** (bearing the 1-phenylpropylidene (Phpd) motif instead of Fpd) was also tested against hormone-independent MDA-MB-231 breast cancer cells. The results (Figure 4) show the prominent antiproliferative effect of the organometallic amine **6** (IC_{50} = 0.05 ± 0.01 μM) against these cancer cell lines (76 times more active than aniline **7**, with IC_{50} = 3.84 ± 0.23 μM). However, the results of the Fpd series seem to indicate that aromatic substituent modification with electron-withdrawing groups such as acyl moieties has a greater impact on the antiproliferative activity than Fpd replacement with the Phpd group. Compound **7** was also tested against hormone-dependent MCF-7 breast cancer cells, and showed an IC_{50} value of 5.01 ± 0.21 μM.

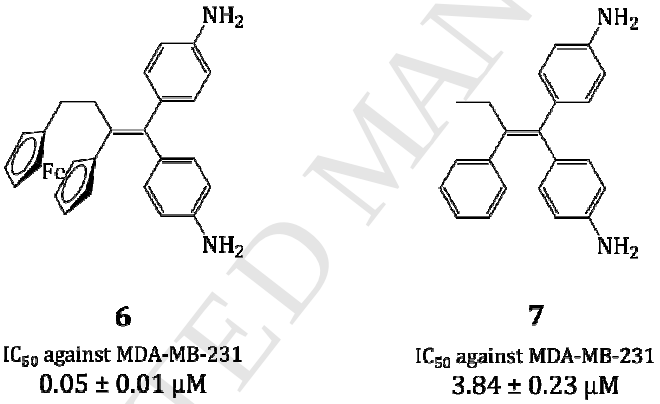


Figure 4. Chemical structures of primary dianilines **6** and **7** with their IC_{50} values against hormone-independent MDA-MB-231 breast cancer cells.

Having seen the differences between the primary amines **6** and **7**, and having recognized the importance of the organometallic unit Fpd and the amine function in the production of strong cytotoxic effects, we now evaluate the impact of the absence of protons on their nitrogenous substituents. The results on MDA-MB-231 (Figure 5) show that the tetramethylated analogue of the organic dianiline **7**, **7-tm**, was unable to inhibit cell growth, even at a concentration of 10 μM. The tetramethylated dianiline **6-tm** inhibited only 20% cell growth at a concentration of 10 μM, while its protic analogue **6** inhibited 50% cell growth at a concentration of just 0.05 μM. The tetramethylated aniline **8-tm**, inhibited 15% of cell growth at a concentration of 10 μM.

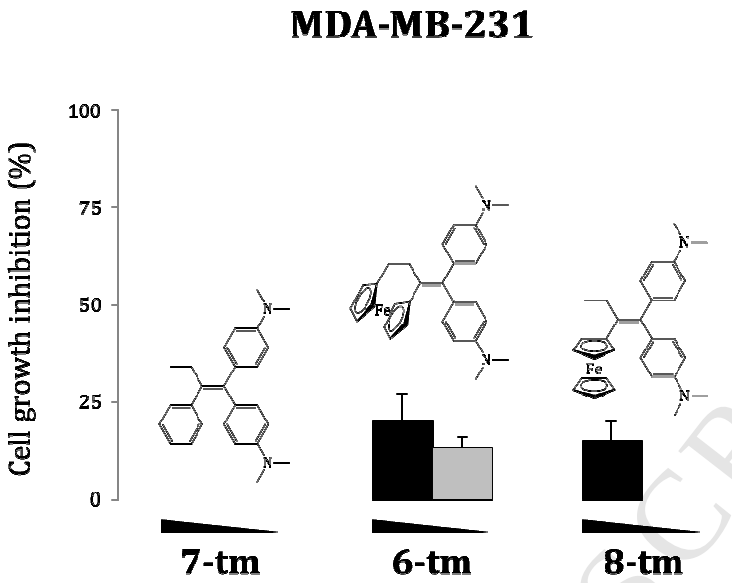


Figure 5. MDA-MB-231 cell growth inhibition (%) by tetramethylated dianilines **6-tm, 7-tm** and **8-tm** at concentrations of 1 µM (gray) and 10 µM (black).

Once again, we highlight the impact of aromatic substituent modification on the cytotoxic responses. Thus, we can assume that these modifications on the Fpd series, particularly on **6**, affect the compound activity negatively in the order *N*-dimethylation >> *N*-diacylation > Phpd replacement. The ferrocenophanic dianiline **6**, combining both structural imperatives, *i.e.*, an Fpd motif and protic amino substituents, was consequently the most cytotoxic compound investigated in this study, and one of the most cytotoxic organometallic compounds reported against breast cancer cells.

2.3 Electrochemistry

The redox properties of the ferrocifen family are key issues for understanding their oxidative metabolism. In this context, these compounds are usually investigated by means of cyclic voltammetry in the absence and presence of a base model having a pKa value close to those of peptides or DNA nitrogen intracellular bases [25,26]. Previous electrochemical studies have established that the monoaniline **4** undergoes a base-promoted oxidation sequence to form iminoquinone methide-type metabolites, which could play a very important role in the cytotoxic properties of the compound. First, we explored the electrochemical behavior of the Fpd compound, the dianiline **6**. The corresponding CVs are displayed in Figure 6. Two oxidation waves were observed at 0.43 V (O1) and 0.90 V (O2). Comparison with the electrochemical behavior reported for **4** [14, 19] prompted us to ascribe wave O1 to the monoelectronic oxidation of the ferrocene group, and wave O2 to the oxidation of both amine groups of the ferricenium cation electrogenerated at O1 (the intensity of wave O2 is ca. twice that of O1).

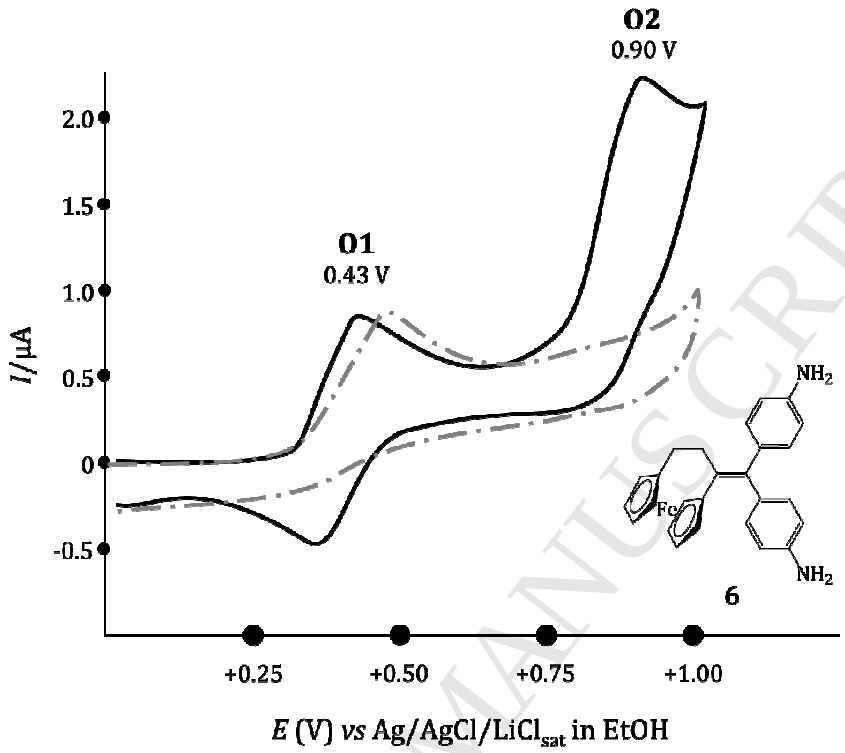


Figure 6. Cyclic voltammogram for **6** with (dashed) and without (bold) 50 equivalents of imidazole. Recorded in a solution of 0.1 M Bu_4NBF_4 in MeOH, with Pt (0.5 mm) working electrode, stainless steel rod counter electrode, and Ag/AgCl/$LiCl_{sat}$ in EtOH reference electrode, at a scan rate of 0.5 Vs^{-1}.

Interestingly, peculiar behavior was observed when the model compound imidazole was added. Ferrocene oxidation became irreversible and shifted slightly to a higher potential, and the prominent second wave assigned to aniline oxidation disappeared. This modification of the cyclic voltammogram upon the addition of imidazole shows the occurrence of a base-triggered oxidation sequence, similar to the one reported for the aniline **4**, featuring a base-promoted intramolecular proton-coupled electron transfer between the amine group and ferricenium moiety, ultimately leading to an aminyl radical, which grafts onto the Pt electrode [19].

In the absence of a base, the difference in *E* of the first oxidation wave [O1] for both compounds **4** and **6** was 0.04 V; the organometallic moiety in **6** was the easiest to oxidize (*i.e.*, at less positive potential values). However, the difference in the oxidation potential values for the second oxidation wave [O2] was 0.13 V; this time, the aniline function in **4** was the most easily oxidized. Cyclic voltammetry was also performed for organic compounds **7** and aniline ($C_6H_5NH_2$), and the potential values are reported in Table 1. Compound **7** showed an irreversible oxidation wave at 0.79 V assigned to the aniline moiety transformation, while aniline itself (recorded in MeCN) oxidized at 1.20 V, which was consistent with a study showing the aniline potential oxidation to be around 1.00 V [27].

Table 1. Oxidation waves [O1] and [O2] for selected compounds.

Compound	O1 (V)	O2 (V)
$C_6H_5NH_2$	-	1.20
6	0.43	0.90
7	-	0.79
4	0.47	0.77

The Fc^+ antenna in compound **4** makes aniline oxidation "easier", *i.e.*, oxidation is observed at less positive potential values (0.77 V *vs.* 1.20 V for aniline itself). This effect was also observed for the ferrocenophanic compound **6**; however, the effect was attenuated (0.90 V *vs.* 1.20 V for aniline itself) when compared with **4**. Interestingly, this oxidation potential value for organic **7** was rather similar to that of **4**. This seems to indicate that the overall electronic effects are prone to affect the energetics of the oxidation of the aniline moiety, and therefore its oxidation potential values were similar for compounds **4** and **7**. Thus, the primary aniline oxidation potential may be influenced by the substituent, decreasing in the following order: 1.20 V for H > 0.90 V for Fpd in **6** >> 0.79 V for Phpd in **7** > 0.77 V for Fcpd in **4**. Rather similar potential values related to the oxidation of the amine moiety in aniline and compound **6** seem to indicate that the dianiline **6** could only be partially conjugated to the ferrocenophanic unit (its oxidation being "blind" towards the electronic influence of the ferricenium species electrogenerated at O1), while in **4** and even **7**, the electronic delocalization is possible in this planar and flexible structures. The voltammograms of the tetramethylated dianilines **6-tm**, **7-tm** and **8-tm** support this observation. Their potential values for the oxidation of the amine group were 0.83 V, 0.70 V and 0.56 V, respectively, thus respecting the established order Fpd > Phpd > Fcpd.

Despite showing intermediate behavior between ferrocenophanic and ferrocenic compounds, the organic dianiline **7** did not exhibit the same strong cytotoxic effects as the dianiline **6** and monoanilines **4** and **5**. This means that, despite the differences in reactivity observed in both the Fpd and Fcpd series, which are both electrochemically active and therefore oxidizable, Fpd and Fcpd compounds can be converted into ferricenium species that promote ROS production, which, in turn, can activate tumor suppressor genes such as p53, leading to apoptosis or senescence in cancer cells, or may cause direct DNA damage.

Neither the anilide **4-ac** nor dianilides **6-bu, 6-he** and **6-oc** displayed the second oxidation wave [O2] under the same conditions. **6-bu** was chosen as the representative example (Figure 7). The reversible wave observed at 0.48 V (a potential value slightly more positive than that of **6**) was assigned to the oxidation of the organometallic moiety displaying the expected energetic effect of electron-withdrawing substituents on the oxidation of ferrocene.

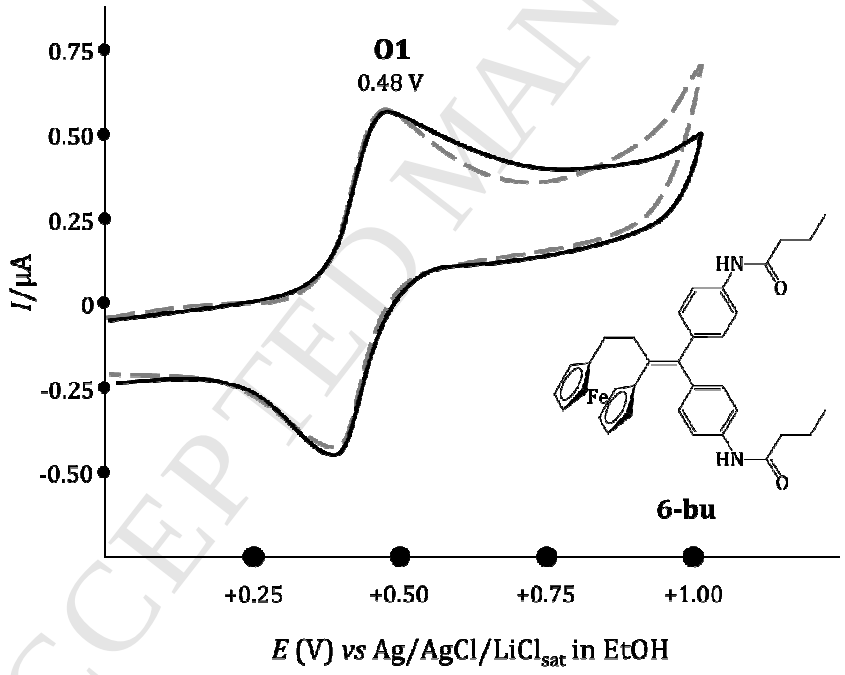
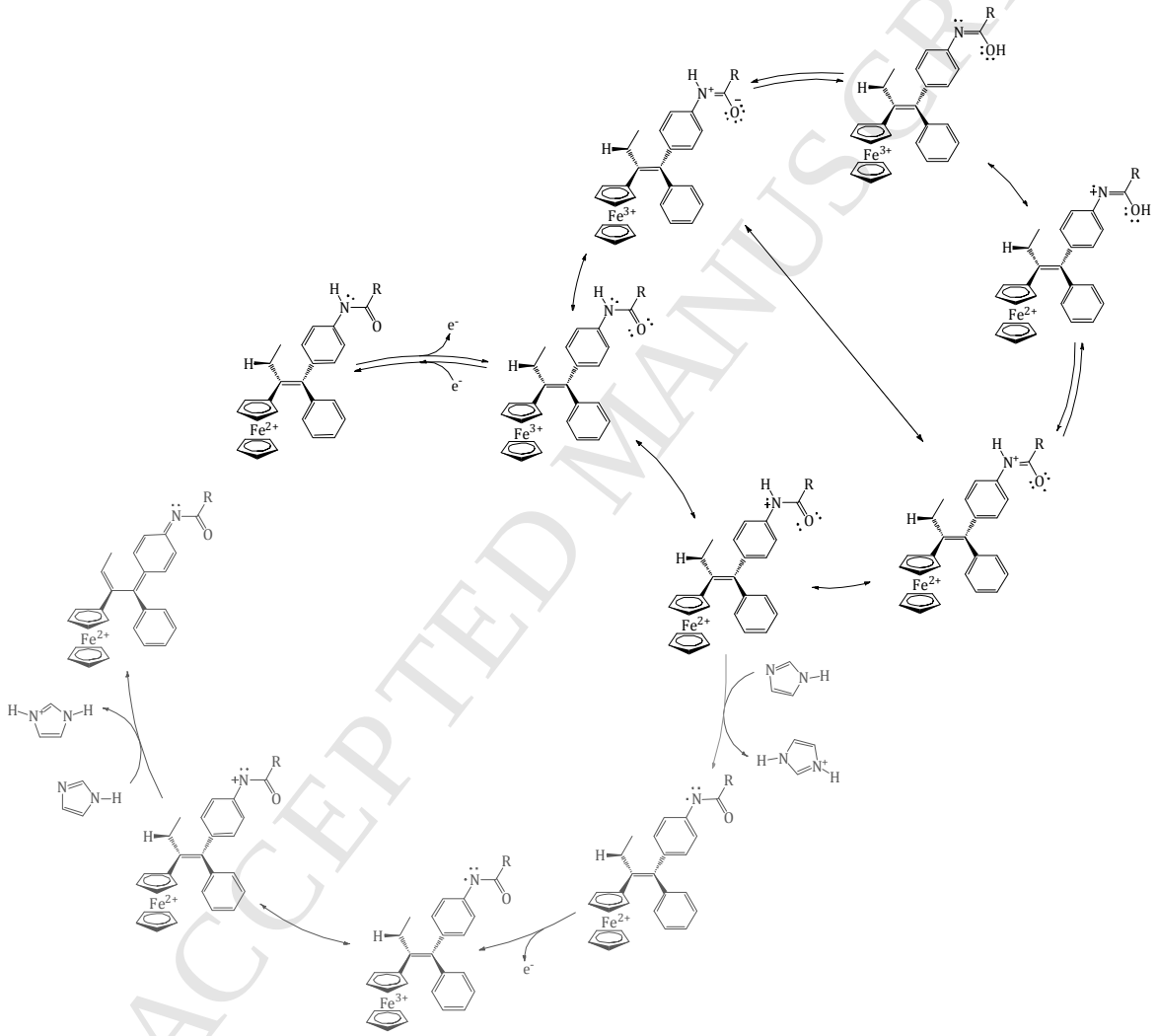


Figure 7. Cyclic voltammogram for **6-bu** with (dashed) and without (bold) 50 equivalents of imidazole. Recorded in a solution 0.1 M of Bu_4NBF_4 in MeOH, with a Pt (0.5 mm) working electrode, stainless steel rod counter electrode, and $Ag/AgCl/LiCl_{sat}$ in EtOH reference electrode, at a scan rate of 0.5 Vs^{-1}.

This could be explained by the fact that, after ferrocene oxidation, the nitrogen atom in **6-bu** does not undergo intramolecular electron transfer with ferricenium (as was proposed for aniline **4** activation) owing to a resonance effect with the adjacent carbonyl group of the amide function, considering that the oxygen atom is more electronegative than nitrogen (the Pauling values for O and N are 3.5 and 3.0, respectively [28]) (Scheme 4). Thus, nitrogen electrons could competitively and formally displace towards the oxygen atom, resulting in an iminolic form. One can also envision that, if the electron transfer occurs between nitrogen and ferricenium, the aminyl radical cation would also experience the resonance effect and result in the formation of iminolic radical species, offering a competitive parallel pathway.



Scheme 4. Proposed mechanism for the electrochemical oxidation of *N*-[4-(2-ferrocenyl-1-phenylbut-1-en-1-yl)phenyl]alkanamides.

3. Conclusions

We have demonstrated that the blockade of protic substituents by acyl or methyl groups in nitrogenous aromatic derivatives dramatically decreases the antiproliferative activity of the compounds. Notably, organic derivatives are far less cytotoxic than organometallic compounds. However, aromatic substituent modification has a greater impact on the antiproliferative activity of organometallic compounds than the replacement of the Fpd motif with the organic Phpd group. The mechanism of activation seems to follow *mutatis mutandis* the intramolecular electron transfer process previously observed for phenol derivatives. Despite their differences in terms of redox activation pathways, ferrocenophanic and ferrocenic compounds are thought to produce ferric metabolites capable of generating ROS in cancer cells. These latter species may be responsible for cytotoxic effects *via* tumor suppressor gene activation, such as p53, or by direct DNA damage.

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 under an argon atmosphere. Thin-layer chromatography (TLC) was performed on silica gel 60 GF254. 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 using the potassium bromide (KBr) pellets technique, and all data are expressed in wavenumbers (cm^{-1}). Melting points (mp) were obtained with a Kofler device and are uncorrected. ^{1}H 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 the electrospray ionization (ESI) method. A purity of >99% was confirmed by analytical reverse phase HPLC with columns Kromasil C18, 10 μm, L = 25 cm, D = 4.6 mm and Macherey-Nagel C18, 5 μm, L = 15 cm, D = 4.6 mm, using different mixtures of MeOH/H_2O and MeCN/H_2O as eluents (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 and characterization of compounds

4.2.1 Alternative pathway for synthesis of 4,4'-([3]ferrocenophan-1-ylidenemethylene)dianiline, **6**[24]

In a Schlenk tube, Zn powder (36 mmol, 2.35 g) was suspended in THF (60 mL) at room temperature and $TiCl_4$ (18 mmol, 2.0 mL) was added slowly with a syringe while stirring. The reaction mixture was heated to 90-100°C. After 1 h at reflux, a solution of [3]ferrocenophan-1-one (3.9 mmol, 0.94 g) and 4,4'-diaminobenzophenone (3.9 mmol, 0.83 g) in THF (30 mL) and DMSO (1mL) was added. DMSO was used to improve the solubility of 4,4'-diaminobenzophenone. The reflux was maintained for 15 min. Then, the mixture was poured into water and extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified on a silica gel column by using petroleum ether/AcOEt (75:25) as eluent. The product was obtained in 42% yield (0.70 g).

4.2.2 Amidation reactions

The corresponding primary amine was dissolved in 30 mL of THF (in the case of 4,4'-diaminobenzophenone, DMSO was used to complete its solubilisation). With stirring, acyl chloride and triethylamine (TEA) were added successively. After 15 min, water was added and the crude product was extracted with AcOEt. The organic phase was washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$ and filtered. The solvents were evaporated under reduced pressure and the product was purified on a silica gel column.

N,N'-[carbonylbis(4,1-phenylene)]dihexanamide, ***he***

Reagents and quantities: 4,4'-diaminobenzophenone (4.72 mmol, 1.00 g), hexanoyl chloride (9.44 mmol, 1.3 mL), TEA (9.44 mmol, 1.3 mL). The mixture was heated at 55°C. Eluent for column: petroleum ether/ethyl acetate (1:1). The product was obtained in 98% yield (1.90 g). **^{1}H-NMR (300 MHz, $(CD_3)_2SO$, ppm):** δ 0.88 (t, J = 7.0 Hz, 6H, $2CH_3$), 1.20-1.40 (m, 8H, $4CH_2$), 1.55-1.70 (quint, J = 7.3 Hz, 4H, $2CH_2$), 2.35 (t, J = 7.4, 4H, $2CH_2CO$), 7.69 (d, J = 8.8 Hz, 4H, $4CH_{Ar}$), 7.76 (d, J = 8.8 Hz, 4H, $4CH_{Ar}$), 10.23 (s, 2H, 2NH).

N,N'-[carbonylbis(4,1-phenylene)]dioctanamide, ***oc***

Reagents and quantities: 4,4'-diaminobenzophenone (4.72 mmol, 1.00 g), octanoyl chloride (9.44 mmol, 1.6 mL), TEA (9.44 mmol, 1.3 mL). The mixture was heated at 55°C. Eluent for column: petroleum ether/ethyl acetate (1:1). The product was obtained in 91% yield (2.00 g). **^{1}H-NMR (300**

MHz, $(CD_3)_2CO$, ppm): δ 0.88 (t, J = 7.0 Hz, 6H, $2CH_3$), 1.20-1.40 (m, 16H, $8CH_2$), 1.69 (quint, J = 7.3 Hz, 4H, $2CH_2$), 2.44 (t, J = 7.4, 4H, $2CH_2CO$), 7.73 (d, J = 8.8 Hz, 4H, $4CH_{Ar}$), 7.87 (d, J = 8.8 Hz, 4H, $4CH_{Ar}$), 9.69 (s, 2H, 2NH).

N,N'-[([3]ferrocenophan-1-ylidenemethylene)bis(4,1-phenylene)]dibutyramide, ***6-bu***

Reagents and quantities: **6** (2.36 mmol, 0.99 g), butyryl chloride (4.72 mmol, 0.5 mL), TEA (4.72 mmol, 0.7 mL). Eluent for column: chloroform/ethyl acetate/acetic acid (2:1:1). The first fraction was the desired product **6-bu**, the second fraction was identified as the monoamide/monoamine, and the third was the remaining unreacted **6**. **6-bu** was obtained in 83% yield (1.10 g). **mp**: 147-148 °C (methanol), **^{1}H-NMR (300 MHz, $(CD_3)_2CO$, ppm):** δ 0.91 (t, J = 7.4 Hz, 3H, CH_3), 0.96 (t, J = 7.4 Hz, 3H, CH_3), 1.64 (sext, J = 7.4 Hz, 2H, CH_2), 1.70 (sext, J = 7.4 Hz, 2H, CH_2), 2.27 (t, J = 7.4 Hz, 2H, CH_2CO), 2.35 (t, J = 7.4 Hz, 2H, CH_2CO), 2.32-2.42 (m, 2H, CH_2Cp'), 2.67-2.76 (m, 2H, CH_2C=C), 3.93 (t, J = 1.8 Hz, 2H, $CpH_{\beta'}$), 3.98 (t, J = 1.8 Hz, 2H, CpH_{β}), 4.01 (t, J = 1.8 Hz, 2H, CpH_{α}), 4.27 (t, J = 1.8 Hz, 2H, $CpH_{\alpha'}$), 6.94 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 7.17 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 7.39 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 7.68 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 8.99 (s, 1H, NH_{cis}), 9.16 (s, 1H, NH_{trans}). **^{13}C-NMR (75 MHz, $(CD_3)_2CO$, ppm):** δ 14.0 (CH_3), 14.5 (CH_3), 19.5 (CH_2), 19.6 (CH_2), 29.2 (CH_2Cp'), 39.6 ($2CH_2CO$), 41.5 (CH_2C=C), 68.9 ($Cp_{\beta'}$), 69.4 (Cp_{β}), 70.9 (Cp_{α}), 71.1 ($Cp_{\alpha'}$), 84.5 (Cp_{ipso}), 87.6 (Cp'_{ipso}), 118.7 ($2C_{Ar}$), 119.6 ($2C_{Ar}$), 130.4 ($2C_{Ar}$), 131.6 ($2C_{Ar}$), 135.1 (C=C), 138.5 (C_{Ar}), 139.2 ($2C_{Ar}$), 139.4 (C_{Ar}), 141.1 (C=C), 171.6 (CO_{cis}), 171.8 (CO_{trans}). **IR (KBr, υ_{max}/cm^{-1}):** 3298 (N-H stretch), 3101 (aromatic C-H stretch), 2958, 2873 (alkyl C-H stretch), 1662 (N=CO stretch), 1597 (aromatic C=C stretch) 1523 (N-H bend), 1400 (C-N stretch). **MS (CI, m/z):** 578 $[MNH_4]^+$, 561 $[MH]^+$. **Anal. Calc.** for $C_{34}H_{36}FeN_2O_2 \bullet CH_3OH$ (%): C, 70.94; H, 6.80; N, 4.73. Found, C, 71.01; H, 6.84; N, 4.40. **HPLC** (R_T), 2.09 min (Macherey-Nagel C18, 5 micron, 4.6 x 150 mm, MeOH, 1mL/min).

N,N'-[([3]ferrocenophan-1-ylidenemethylene)bis(4,1-phenylene)]dioctanamide, ***6-oc***

Pathway B. Reagents and quantities: **6** (0.59 mmol, 0.25 g), THF (15 mL), octanoyl chloride (1.18 mmol, 0.2 mL), TEA (1.43 mmol, 0.2 mL). Eluent: chloroform/ethyl acetate/acetic acid (2:1:1). The product was obtained in 80% yield (0.32 g). The first fraction was the desired product, the second fraction was identified as the monoamide/monoamine, and the third was the remaining starting material. **mp** : 119-120°C, **^{1}H-NMR (300 MHz, $(CD_3)_2CO$, ppm):** δ 0.86 (t, J = 7.0 Hz, 3H, CH_3), 0.88 (t, J = 7.0 Hz, 3H, CH_3), 1.21-1.45 (m, 16H, $8CH_2$), 1.55-1.78 (m, 4H, $2CH_2$), 2.29 (t, J = 7.4 Hz, 2H, CH_2CO), 2.37 (t, J = 7.4 Hz, 2H, CH_2CO), 2.34-2.40 (m, 2H, CH_2Cp'), 2.68-2.76 (m, 2H, CH_2C=C), 3.93 (t, J = 1.8 Hz, 2H, $CpH_{\beta'}$), 3.98 (t, J = 1.8 Hz, 2H, CpH_{β}), 4.02 (t, J = 1.8 Hz, 2H, CpH_{α}), 4.27 (t, J = 1.8 Hz, 2H, $CpH_{\alpha'}$), 6.93 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 7.17 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 7.39 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 7.67 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 8.98 (s, 1H, NH_{cis}), 9.15 (s, 1H, NH_{trans}). **^{13}C-NMR (75 MHz, $(CD_3)_2CO$, ppm):** δ 14.3

($2CH_3$), 23.3 ($2CH_2$), 26.2 (CH_2), 26.3 (CH_2), 29.2 (CH_2Cp'), 29.8 ($2CH_2$), 29.9 ($2CH_2$), 32.4 ($2CH_2$), 37.7 (CH_2CO), 37.8 (CH_2CO), 41.5 (CH_2C=C), 68.9 ($Cp_{\beta'}$), 69.4 (Cp_{β}), 70.9 (Cp_{α}), 71.1 ($Cp_{\alpha'}$), 84.6 (Cp_{ipso}), 87.6 (Cp'_{ipso}), 118.7 ($2C_{Ar}$), 119.6 ($2C_{Ar}$), 130.4 ($2C_{Ar}$), 131.6 ($2C_{Ar}$), 135.1 (C=C), 138.5 (C_{Ar}), 139.1 (C_{Ar}), 139.2 (C_{Ar}), 139.4 (C_{Ar}), 141.1 (C=C), 171.8 (CO_{cis}), 172.0 (CO_{trans}). **IR (KBr, υ_{max}/cm^{-1}):** 3298 (N-H stretch), 3101 (aromatic C-H stretch), 2924, 2854 (alkyl C-H stretch), 1662 (NC=O stretch), 1597 (aromatic C=C stretch), 1523 (N-H bend), 1400 (C-N stretch). **MS (CI, m/z):** 690 $[MNH_4]^+$, 673 $[MH]^+$. **Anal. Calc.** for $C_{42}H_{52}FeN_2O_2$ (%): C, 74.99; H, 7.79; N, 4.16. Found: C, 74.54; H, 7.90; N, 4.04. **HPLC** (R_T), 3.14 min (Macherey-Nagel C18, 5 micron, 4.6 x 150 mm, MeOH, 1 mL/min).

4.2.3 McMurry cross-coupling reactions

In a Schlenk tube under argon, zinc powder (Zn) was suspended in THF at room temperature. With stirring, titanium chloride ($TiCl_4$) was added slowly with a syringe, and the reaction mixture was heated to 90-100°C. After 1 h of reflux, a THF solution of both ketones was added. The reaction was monitored by TLC. The mixture was poured into water and extracted with AcOEt. The organic layer was washed, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified on a silica gel column.

*Pathway A for the synthesis of **6-oc***

Reagents and quantities: Zn (20.8 mmol, 1.36 g), $TiCl_4$ (10.4 mmol, 1.1 mL), THF (30 mL), ketones (in 17 mL of THF): ***oc*** (2.08 mmol, 0.97 g), [3]ferrocenophanon-1-one (2.08 mmol, 0.50 g). Reaction was stopped 15 min after the addition of ketones. After purification, the product was obtained in 50% yield (0.70 g).

*N,N'-[([3]ferrocenophan-1-ylidenemethylene)bis(4,1-phenylene)]dihexanamide, **6-he***

Reagents and quantities: Zn (24 mmol, 1.57 g), $TiCl_4$ (12 mmol, 1.3 mL), THF (30 mL), ketones (in 20 mL of THF): ***he*** (2.4 mmol, 0.98 g) and [3]ferrocenophanon-1-one (2.4 mmol, 0.58 g). Reaction was stopped 15 min after the addition of ketones. Eluent for column: petroleum ether/ethyl acetate (7:3). The product was obtained in 54% yield (0.80 g). **mp**: 133-134°C. **^{1}H-NMR (300 MHz, $(CD_3)_2CO$, ppm):** δ 0.86 (t, J = 7.0 Hz, 3H, CH_3), 0.89 (t, J = 7.0 Hz, 3H, CH_3), 1.25-1.40 (m, 8H, $4CH_2$), 1.57-1.74 (m, 4H, $2CH_2$), 2.29 (t, J = 7.4 Hz, 2H, CH_2CO), 2.37 (t, J = 7.4 Hz, 2H, CH_2CO), 2.30-2.40 (m, 2H, CH_2Cp'), 2.68-2.75 (m, 2H, CH_2C=C), 3.93 (t, J = 1.8 Hz, 2H, $CpH_{\beta'}$), 3.98 (t, J = 1.8 Hz, 2H, CpH_{β}), 4.01 (t, J = 1.8 Hz, 2H, CpH_{α}), 4.27 (t, J = 1.8 Hz, 2H, $CpH_{\alpha'}$), 6.93 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 7.16 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 7.39 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 7.68 (d, J = 8.7 Hz, 2H, $2CH_{Ar}$), 9.05 (s, 1H, NH_{cis}), 9.22 (s, 1H, NH_{trans}). **^{13}C-NMR (75 MHz, $(CD_3)_2CO$, ppm):** δ 14.2 (CH_3), 14.3 (CH_3), 23.1 ($2CH_2$), 25.9 (CH_2), 26.0 (CH_2), 29.2

(CH_2Cp'), 32.2 (2CH_2), 37.6 (CH_2CO), 37.7 (CH_2CO), 41.5 (CH_2C=C), 68.9 ($Cp_{\beta'}$), 69.3 (Cp_{β}), 70.9 (Cp_{α}), 71.0 ($Cp_{\alpha'}$), 84.5 (Cp_{ipso}), 87.6 (Cp'_{ipso}), 118.7 (2C_{Ar}), 119.7 (2C_{Ar}), 130.4 (2C_{Ar}), 131.6 (2C_{Ar}), 135.0 (C=C), 138.5 (C_{Ar}), 139.1 (2C_{Ar}), 139.4 (C_{Ar}), 141.0 (C=C), 171.9 (CO_{cis}), 172.1 (CO_{trans}). **IR (KBr, υ_{max}/cm^{-1}):** 3298 (N-H stretch), 3097, 3043 (aromatic C-H stretch), 2927, 2858 (alkyl C-H stretch), 1662 (NC=O stretch), 1597 (aromatic C=C stretch), 1523 (N-H bend), 1400 (C-N stretch). **MS (CI, m/z):** 634 $[MNH_4]^+$, 617 $[MH]^+$. **Anal. Calc.** for $C_{38}H_{44}FeN_2O_2$ (%): C, 74.02; H, 7.19; N, 4.54. Found: C, 73.92; H, 7.47; N, 4.49. **HPLC** (R_T), 2.47 min. (Macherey-Nagel, C18, 5 micron, 4.6 x 250 mm, MeOH, 1 mL/min).

*4,4'-(2-phenylbut-1-ene-1,1-diyl)dianiline, **7***

Reagents and quantities: Zn (48 mmol, 3.14 g) and $TiCl_4$ (24 mmol, 2.6 mL). THF (80 mL), ketones (in 40 mL of THF): propiophenone (6.78 mmol, 0.91 g) and 4,4'-diaminobenzophenone (5.09 mmol, 1.08 g), 1 mL of DMSO. The reaction was stopped 5 min after addition of ketones. Eluent for column : petroleum ether/ethyl acetate (1:1). The product was obtained in 35% yield (0.57 g). **mp** : 150-151°C, **^{1}H-NMR (300MHz, $(CD_3)_2SO$, ppm):** δ 0.84 (t, J = 7.3 Hz, 3H, CH_3), 2.42 (q, J = 7.3 Hz, 2H, CH_2), 4.84 (s, 2H, NH_{2cis}), 5.05 (s, 2H, NH_{2trans}), 6.19 (d, J = 8.7 Hz, 2H, 2CH_{Ar}), 6.46 (d, J = 8.7 Hz, 2H, 2CH_{Ar}), 6.54 (d, J = 8.5 Hz, 2H, 2CH_{Ar}), 6.83 (d, J = 8.5 Hz, 2H, 2CH_{Ar}), 7.02-7.10 (t, J = 7.5 Hz, 1H, CH_{Ar}), 7.07 (d, J = 7.5 Hz, 2H, 2CH_{Ar}), 7.15 (t, J = 7.5 Hz, 2H, 2CH_{Ar}). **^{13}C-NMR (75 MHz, $(CD_3)_2SO$, ppm):** δ 13.7 (CH_3), 28.6 (CH_2), 112.9 (2C_{Ar}), 113.5 (2C_{Ar}), 125.5 (Ph_p), 127.8 (Ph_m), 129.5 (Ph_o), 129.8 (2C_{Ar}), 131.1 (C_{Ar}), 131.3 (2C_{Ar}), 131.4 (C_{Ar}), 137.4 (Ph*C*=C), 139.3 (C=C), 143.1 (Ph_{ipso}), 146.3(C_{Ar}N), 147.2(C_{Ar}N). **IR (KBr, υ_{max}/cm^{-1}):** 3432, 3352, 3213 (N-H stretch), 3024 (aromatic C-H stretch), 2962, 2927, 2870 (alkyl C-H stretch), 1616 (NH_2 scissoring), 1508 (aromatic C=C stretch), 1280 (C_{Ar}-N stretch). **MS (CI, m/z):** 332 $[MNH_4]^+$, 315 $[MH]^+$. **Anal. Calc.** for $C_{22}H_{22}N_2 \bullet \frac{1}{3}H_2O$(%): C, 82.46; H, 7.13; N, 8.74. Found: C, 82.49; H, 6.96; N, 8.73. **HPLC** (R_T), 2.79 min (Kromasil C18, 10 microns, 4.6 x 250 mm, MeOH, 1 mL/min).

*4,4'-(2-phenylbut-1-ene-1,1-diyl)bis(N,N-dimethylaniline), **7-tm***

Reagents and quantities: Zn (72 mmol, 4.71 g), $TiCl_4$ (36 mmol, 3.9 mL), THF (100 mL). Ketones in (60 mL of THF): propiophenone (7.6 mmol, 1.02 g) and 4,4'-bis(dimethylamino)benzophenone (7.6 mmol, 2.04 g). The reaction was stopped 20 min after addition of ketones. Eluent for column : petroleum ether/ethyl acetate (9:1). The product was obtained in 74.6% yield (2.10 g). **mp**: 123-125°C, **^{1}H-NMR (300 MHz, $CDCl_3$, ppm):** δ 0.94 (t, J = 7.4 Hz, 3H, CH_3), 2.52 (q, J = 7.4 Hz, 2H, CH_2), 2.83 (s, 6H, 2NCH_3), 2.97 (s, 6H, 2NCH_3), 6.39 (d, J = 8.9 Hz, 2H, 2CH_{Ar}), 6.72 (d, J = 8.9 Hz, 2H, 2CH_{Ar}), 6.73 (d, J = 8.9 Hz, 2H, 2CH_{Ar}), 7.12 (d, J = 8.9 Hz, 2H, 2CH_{Ar}), 7.05-7.18 (m, 5H, 5CH_{Ar}). **^{13}C-NMR (75 MHz, $CDCl_3$, ppm):** δ 14.0 (CH_3), 29.3 (CH_2), 40.6 (2NCH_3), 40.8 (2NCH_3), 111.4 (2C_{Ar}), 112.1 (2C_{Ar}), 125.6 (Ph_p), 127.9 (Ph_m), 130.0 (Ph_o), 130.6 (2C_{Ar}), 132.0 (2C_{Ar}), 132.1 (C_{Ar}), 132.9 (C_{Ar}), 138.8 (Ph*C*=C),

139.2 (C=C), 143.6 (Ph_{ipso}), 148.3 ($C_{Ar}N$), 149.2 ($C_{Ar}N$). **IR (KBr, υ_{max}/cm^{-1}):** 3074, 3032 (aromatic C-H stretch), 2962, 2877 (alkyl C-H stretch), 1608, 1515 (aromatic C=C stretch), 1342 (C_{Ar}-N stretch). **MS (EI, m/z):** 370 (100%) $[M]^{+\bullet}$, 355 $[M\text{-}CH_3]^+$. **Anal. Calc.** for $C_{26}H_{30}N_2$(%): C, 84.28; H, 8.16; N, 7.56. Found: C, 83.91; H, 8.16; N, 7.48. **HPLC** (R_T), 3.62 min (Kromasil 10 microns, 4.6 x 250 mm, MeCN, 1 mL/min).

4,4'-([3]ferrocenophan-1-ylidenemethylene)bis(N,N-dimethylaniline), ***6-tm***

Reagents and quantities: Zn (72 mmol, 4.71 g), $TiCl_4$ (36 mmol, 3.9 mL), THF (100 mL), Ketones (in 60 mL of THF): 4,4'-bis(dimethylamino)benzophenone (7.6 mmol, 2.04 g) and [3]ferrocenophan-1-one (7.6 mmol, 1.82 g). The reaction was stopped 25 min after addition of ketones. Eluent for column : petroleum ether/ethyl acetate (85:15). The product was obtained in 41.4% yield (1.50 g). **mp** : 210-212°C. **^{1}H-NMR (300 MHz, $CDCl_3$, ppm):** δ 2.30-2.40 (m, 2H, CH_2Cp'), 2.69-2.74 (m, 2H, CH_2C=C), 2.87 (s, 6H, $2NCH_3$), 2.98 (s, 6H, $2NCH_3$), 3.97 (t, J = 1.8 Hz, 2H, $CpH_{\beta'}$), 4.00 (t, J = 1.8 Hz, 2H, CpH_{α}), 4.03 (t, J = 1.8 Hz, 2H, CpH_{β}), 4.21 (t, J =1.8 Hz, 2H, $CpH_{\alpha'}$), 6.45 (d, J = 8.8 Hz, 2H, $2CH_{Ar}$), 6.71 (d, J = 8.8 Hz, 2H, $2CH_{Ar}$), 6.92 (d, J = 8.8 Hz, 2H, $2CH_{Ar}$), 7.12 (d, J = 8.8 Hz, 2H, $2CH_{Ar}$). **^{13}C-NMR (75 MHz, $CDCl_3$, ppm):** δ 28.8 (CH_2Cp'), 40.5 ($2NCH_3$), 40.7 ($2NCH_3$), 41.5 (CH_2C=C), 68.2 ($Cp_{\beta'}$), 68.6 (Cp_{β}), 70.3 (Cp_{α}), 70.4 ($Cp_{\alpha'}$), 85.0 (Cp_{ipso}), 87.2 (Cp'_{ipso}), 111.1 ($2C_{Ar}$), 112.0 ($2C_{Ar}$), 130.4 ($2C_{Ar}$), 131.0 (C_{Ar}), 131.8 ($2C_{Ar}$), 132.2 (C_{Ar}), 132.6 (C=C), 141.1 (C=C), 148.5 ($C_{Ar}N$), 149.2 ($C_{Ar}N$). **IR (KBr, υ_{max}/cm^{-1}):** 3085, 3039 (aromatic C-H stretch), 2935, 2893 (alkyl C-H stretch), 1604, 1516 (aromatic C=C stretch), 1342 (C_{Ar}-N stretch). **MS (EI, m/z):** 476 (100%) $[M]^{+\bullet}$, 397, 238, 199. **Anal. Calc.** for $C_{30}H_{32}FeN_2$(%): C, 75.63; H, 6.77; N, 5.88. Found: C, 75.45; H, 6.89; N, 5.60. **HPLC** (R_T), 4.24 min (Kromasil 10 microns, 4.6 x 250 mm, MeCN, 1 mL/min).

4,4'-(2-ferrocenylbut-1-ene-1,1-diyl)bis(N,N-dimethylaniline), ***8-tm***

Reagents and quantities: Zn (72 mmol, 4.71 g), $TiCl_4$ (36 mmol, 3.9 mL), THF (100 mL). Ketones (in 60 mL of THF): 4,4'-bis(dimethylamino)benzophenone (7.6 mmol, 2.04 g) and propionylferrocene (7.6 mmol, 1.84 g). The reaction was stopped 3 h after the addition of ketones. Eluent for column: petroleum ether/ethyl acetate (9:1). The product was obtained in 38.5% yield (1.40 g). **mp** : 168-170°C. **^{1}H-NMR (300 MHz, $CDCl_3$, ppm):** δ 1.04 (t, J = 7.5 Hz, 3H, CH_3), 2.62 (q, J = 7.5 Hz, 2H, CH_2), 2.91 (s, 6H, $2NCH_3$), 2.96 (s, 6H, $2NCH_3$), 3.96 (t, J = 1.9 Hz, 2H, CpH_{α}), 4.06 (t, J = 1.9 Hz, 2H, CpH_{β}), 4.11 (s, 5H, $CpH_{unsubst}$), 6.59 (d, J = 8.9 Hz, 2H, $2CH_{Ar}$), 6.70 (d, J = 8.9 Hz, 2H, $2CH_{Ar}$), 6.92 (d, J = 8.9 Hz, 2H, $2CH_{Ar}$), 7.05 (d, J = 8.9 Hz, 2H, $2CH_{Ar}$). **^{13}C-NMR (75 MHz, $CDCl_3$, ppm):** δ 15.8 (CH_3), 28.2 (CH_2), 40.7 ($4NCH_3$), 67.8 (Cp_{β}), 69.2 ($Cp_{unsubst}$), 69.4 (Cp_{α}), 88.2 (C_{ipso}), 112.2 ($2C_{Ar}$), 112.3 ($2C_{Ar}$), 130.4 ($2C_{Ar}$), 130.9 ($2C_{Ar}$), 133.8 (C_{Ar}), 134.0 (C_{Ar}), 135.0 (Fc*C*=C), 138.5 (C=C), 148.8 ($2C_{Ar}N$). **IR (KBr, υ_{max}/cm^{-1}):** 3089, 3031 (aromatic C-H stretch), 2951, 2920, 2862 (alkyl C-H stretch), 1604, 1515 (aromatic C=C

stretch), 1346 (C_{Ar}-N stretch). **MS (EI, m/z):** 478 (100%) $[M]^{+\bullet}$, 449 $[M\text{-}Et]^{+}$, 413 $[M\text{-}Cp]^{+}$. **Anal. Calc.** for $C_{30}H_{34}FeN_2$(%): C, 75.31; H, 7.16; N, 5.86. Found: C, 75.15; H, 7.25; N, 5.86. **HPLC** (R_T), 4.72 min (Kromasil 10 microns, 4.6 x 250 mm, MeCN, 1 mL/min).

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 penicilline, streptomycine and fungizone in a 75 cm^3 flask under 5% CO_2. Cells were plated in 96-well tissue culture plates in 200 µL medium and treated 24 h later with a 2 µL stock solution of compounds dissolved in DMSO with a Biomek 3000 apparatus (Beckman-Coulter). The control samples received the same volume of DMSO (1% final volume). After 72h exposure, MTS reagent (Promega) was added, and the sample was incubated for 3 h at 37°C; the absorbance was monitored at 490 nm and the 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 in the range 5 nM to 100 µM in separate duplicate experiments.

Electrochemistry. Cyclic voltammograms (CVs) were obtained using a three-electrode cell with a 0.5 mm Pt working electrode, stainless steel rod counter electrode and Ag/AgCl/$LiCl_{sat}$ in ethanol reference electrode, with an µ-Autolab 3 potentiostat driven by General Purpose Electrochemical System (GPES) version 4.8, EcoChemie B.V., Utrecht, the Netherlands. Solutions consisted of 5 mL MeOH, analyte (approx. 0.5 mM) and Bu_4NBF_4 (0.1 M) as supporting electrolyte. After the CV was obtained in MeOH, 50 equivalents of imidazole were added to the cell, and new data were obtained.

5. Abbreviations

Fcpd	1-ferrocenylpropylidene
Fpd	[3]ferrocenophan-1-ylidene

6. Acknowledgments

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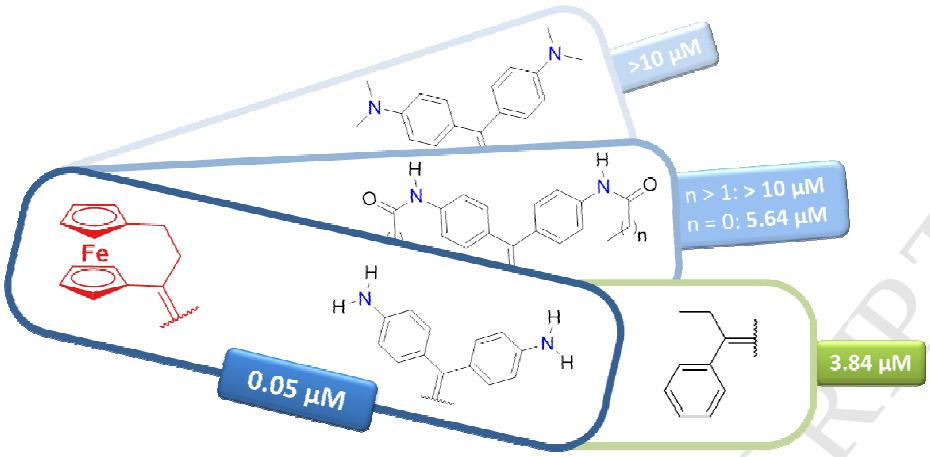
Ferrocenic and [3]Ferrocenophanic Anilines and Anilides have been synthesized and evaluated against triple negative MBA-MB-231 breast cancer cells. Electrochemical studies were discussed.



IC_{50} values against triple-negative MDA-MB-231 breast cancer cells

Highlights:

1. The strongly cytotoxic 4,4'-([3]ferrocenophan-1-ylidenemethylene)dianiline can be obtained in 15 min by McMurry cross coupling.

2. *N*,*N*'-[([3]ferrocenophan-1-ylidenemethylene)bis(4,1-phenylene)]dialkanamides and tetramethylated dianilines derived from Michler's ketone were synthesized.

3. The replacement of hydrogen by acyl or methyl groups in nitrogenous aromatic derivatives dramatically decreases the antiproliferative activity of compounds.

4. The mechanism of activation of nitrogenous derivatives seems to follow the intramolecular electron transfer process observed for phenol derivatives.

5. Primary aniline oxidation potential is influenced by the nature of the *trans* organometallic or organic moiety.