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Synthesis, Cytotoxicity, and COMPARE Analysis of Ferrocene and [3]Ferrocenophane Tetrasubstituted Olefin Derivatives against Human Cancer Cells

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Herein we report the antiproliferative effects of a series of 28 compounds against the MDA-MB-231 breast cancer cell line, including the synthesis of seven new [3]ferrocenophanyl and four new ferrocenyl compounds. For each *p*-R-phenyl substitution pattern investigated, the [3]ferrocenophanyl derivatives were more cytotoxic than the corresponding ferrocenyl derivative, with the highest activity found for compounds with protic substituents. Theoretical calculations of the HOMO-LUMO gap for the molecules in the Fe³⁺ oxidation state suggest a higher reactivity for the [3]ferrocenophanyl derivatives. A lead compound from each series, a [3]ferrocenophanyl and a ferrocenyl compound, possessing two phenol groups, were screened against the NCI/DTP 60-cell-line panel. The mean activity over all cell lines was better than cisplatin for both compounds, and both compounds showed subpanel selectivity for leukemia, CNS cancer, and renal cancer. Low systemic toxicity and lack of interaction with DNA (when in the reduced form), suggest that the compounds may act as prodrugs.

Introduction

The development of organometallic compounds for cancer therapeutics is one of the fastest growing areas of bioorganometallic chemistry.^[1-4] These molecules can be generally categorized based on their mode of action, for example those where direct interaction of the metal with a biological target, such as DNA or proteins after ligand hydrolysis, is implicated.^[5-9] Modeled after the action of cisplatin, these metal-centered organometallic compounds, notably containing ruthenium^[9, 10] or titanium,^[11] possess judiciously chosen ligands or substituents on the phenyl ring to optimize the pharmacokinetic properties of the molecule. Another class demonstrates the enthusiasm researchers have for grafting metallocenes and metal carbonyls to a variety of biomolecules to modify their biological effects.^[12] These therapeutic bioconjugates include steroidal^[13-15] and nonsteroidal^[16-23] endocrine modulators, natural products,^[24-27] and others.^[28-34] In these cases, the often covalently grafted organometallic unit is usually inert to ligand substitution, but potentiates the activity of the biomolecule via modification of the pharmacokinetic profile or acts as a structural mimic.^[35] A variety of other compounds are between these two classes, possessing labile biomolecule ligands, such as nucleobases.^[33, 36-41] Currently in press, the book “Medicinal Organometallic Chemistry” demonstrates that the growth of structural and mechanistic diversity of organometallic compounds for cancer therapy is in full bloom.^[42]

Among organometallic compounds based on endocrine modulators, the most active and well-studied are the ferrocenyl derivatives of tamoxifen (with or without the side chain present).^[43] We have shown that the reversible ferrocene/ferricenium redox couple can catalyze the intramolecular formation of quinones when the ferrocenyl and phenol groups are linked by a conjugated system.^[44] This mechanism is not limited to phenolic compounds: ferrocenyl moieties conjugated to an aniline or acetanilide group also show high activity against cancer cells, with IC_{50} values of the order of $10^{-7}M$.^[45] The structural requirements for activity seem to be 1) the presence of a ferrocene group, 2) a conjugated linker, 3) aromatic para substitution by a protic function, and 4) an ethyl group residing on the same carbon as the ferrocene group.

As a part of our studies of organometallic compounds with this motif, [3]ferrocenophanes have recently emerged as a class of notable antiproliferative agents. The [3]ferrocenophane

structure consists of a ferrocene, where the two cyclopentadienyl rings are linked by a three atom bridge, in this case carbon atoms. Molecules possessing this motif have been discussed in reviews,^[46, 47] and have been studied in the context of their structures,^[48-57] catalytic properties,^[56, 58-63] and conjugation to biomolecules,^[64, 65] inter alia. For the [3]ferrocenophane compounds based on the tamoxifen skeleton, it is supposed that their biological activity is due to a mechanism of activation similar to that of their ferrocenyl analogues. Among organometallic compounds studied so far, the highest in vitro cancer activity is exhibited by those based on the 1-(diphenylmethylidene)-[3]ferrocenophane skeleton, with para substitution on both phenyl rings^[66-68] (Table 1).

Table 1. Compounds based on the 1-(diphenylmethylidene)-[3]ferrocenophane skeleton that show high cytotoxicity against hormone-independent breast and prostate cancer cells.

R ¹	R ²	IC ₅₀ [μM]	
		MDA-MB-231	PC3
OH	OH	0.09 ± 0.01	0.14 ± 0.01
NH ₂	OH	0.06 ± 0.01	0.03 ± 0.01
NHAc	OH	0.09 ± 0.02	0.02 ± 0.00
NH ₂	NH ₂	0.05 ± 0.01	0.05 ± 0.00

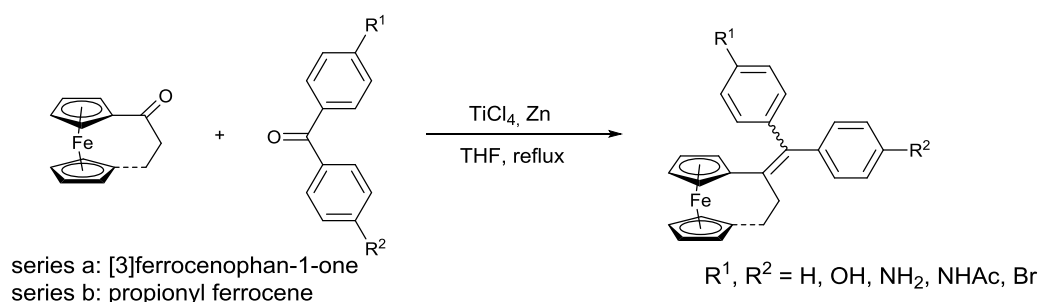
For compounds possessing a phenol, an aniline, and/or an acetanilide, with all other parameters being equal, the presence of a [3]ferrocenophane in lieu of an *n*-propylferrocene yields more toxic molecules. As this phenomenon has been studied with a limited number of compounds, herein we report the results from a 28-molecule structure-activity study, which involved the synthesis and biological evaluation of 11 new compounds. These molecules are all based on the 1-(diphenylmethylidene)-[3]ferrocenophane or the 1-

(diphenylmethylidene)ethylferrocene skeleton and were tested on hormone-independent MDA-MB-231 cells. A lead compound (with two hydroxy substituents) from each series was further evaluated by the National Cancer Institute Developmental Therapeutics Program (NCI/DTP) against their panel of 60 human tumor cell lines, and a COMPARE analysis was performed on these results. Cytotoxicity results have also been interpreted in light of theoretical calculations on the HOMO-LUMO gap of the oxidized molecule (Fc^+) and the interaction with DNA of a reduced molecule.

Results

Synthesis

A simple and key step to obtain the desired tetrasubstituted olefins involves a McMurry cross-coupling reaction between the appropriate benzophenone and [3]ferrocenophan-1-one or propionyl ferrocene to generate the [3]ferrocenophanes (**1a-17a**) and the corresponding ferrocenes (**1b-15b**), respectively (Scheme 1, Table 2). The reaction time depends on the substituents and can vary from 90 min to three days. One caveat of the McMurry reaction is that there is a competition between the formation of the desired cross-coupled product and the two homo-coupled compounds, and this is reflected in the variety of the reaction yields. In most cases, the reactions with [3]ferrocenophan-1-one were more efficient than those using propionyl ferrocene. This can be attributed to the great avidity propionyl ferrocene shows for itself, resulting in a relatively high proportion of homo-coupled diferrocenyl species under McMurry conditions.



Scheme 1. General scheme of the McMurry cross-coupling reaction.

Table 2. Reaction conditions, yields, and isomer ratios of selected compounds.^[a]

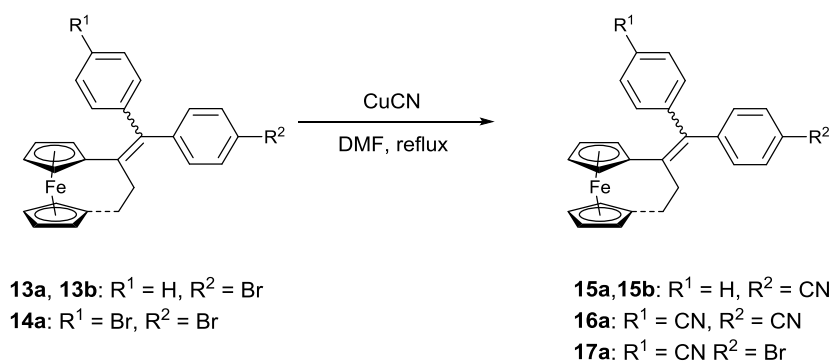
Compd	R ¹	R ²	TiCl ₄ [equiv]	Zn [equiv]	t _{reaction} [h]	Yield [%]	Isomer ratio	Ref.
1a	H	H	4	6	2	93	n.a.	[68]
1b	H	H	3	6	2	25	n.a.	[84]
(<i>E+Z</i>)- 2a	OH	H	4	6	2	74	81:19	[67]
(<i>E+Z</i>)- 2b	OH	H	3	6	2	26	50:50	[22]
(<i>E+Z</i>)- 3a	OAc	H	Acetylation of 2a			66	82:18	
(<i>E+Z</i>)- 3b	OAc	H	Acetylation of 2b			47	54:46	
(<i>E+Z</i>)- 4a	NH ₂	H	5	7	48	54	87:13	[67]
(<i>E+Z</i>)- 4b	NH ₂	H	3	6	4	51	55:45	[45]
(<i>E+Z</i>)- 5a	NHAc	H	Acetylation of 4a			75	80:20	[67]
(<i>E+Z</i>)- 5b	NHAc	H	Acetylation of 4b			91	60:40	[45]
6a	OH	OH	4	6	2	55	n.a.	[66]
6b	OH	OH	4	6	2	53	n.a.	[85]
(<i>E+Z</i>)- 7a	NH ₂	OH	4	7	24	21	58:42	[68]
(<i>E+Z</i>)- 7b	NH ₂	OH	13	24	4	62	50:50	
(<i>E+Z</i>)- 8a	NHAc	OH	Acetylation of 7a			63	75:25	[68]
(<i>E+Z</i>)- 8b	NHAc	OH	Acetylation of 7b			86	50:50	
9a	NH ₂	NH ₂	Deprotection of 11a			78	n.a.	[68]
9b	NH ₂	NH ₂	various	various	various	0	n.a.	
10a	NHAc	NHAc	5	7	72	44	n.a.	[68]
10b	NHAc	NHAc	various	various	various	0	n.a.	
(<i>E+Z</i>)- 11a	OAc	OH	Protection of 6a			46	54:46	
(<i>E+Z</i>)- 11b	OAc	OH	Protection of 6b			65	50:50	
12a	OAc	OAc	Protection of 6a			33	n.a.	
12b	OAc	OAc	Protection of 6b			90	n.a.	[86]
(<i>E+Z</i>)- 13a	Br	H	4	6	3.5	89	62:38	
(<i>E+Z</i>)- 13b	Br	H	3	6	4	13	64:36	[72]
14a	Br	Br	4	6	3.5	81	n.a.	
14b	Br	Br	various	various	various	0	n.a.	
15a	CN	H	Cyanation of 13a			74	61:39	
15b	CN	H	Cyanation of 13b			62	67:33	[72]
16a	CN	CN	Cyanation of 14a			44	n.a.	
17a	CN	Br	Cyanation of 14a			15	44:56	

[a] McMurry reaction, if not otherwise indicated; n.a.=not applicable.

In some cases, the coupling reaction did not yield the desired product. For example, the reaction between propionyl ferrocene and 4,4'-diaminobenzophenone did not lead to any trace of the expected 2-ferrocenyl-1,1-bis(4-aminophenyl)but-1-ene, **9b**. Attempts to similarly obtain the NHAc-substituted compound **10b** were likewise unsuccessful. However, when

propionyl ferrocene was replaced by [3]ferrocenophan-1-one, the amido compound **10a** was obtained in moderate yield, although only after a reaction time of 72 h. On the other hand, the reaction between [3]ferrocenophan-1-one and 4,4'-diaminoacetophenone did not yield any desired product and therefore **9a** was instead obtained by deacetylation of **10a**. The McMurry reaction between propionyl ferrocene and the dibromobenzophenone also failed to yield the desired product **14b**. This is surprising, as the reaction between [3]ferrocenophan-1-one and dibromobenzophenone gave the desired **14a** in high yield. It should be mentioned that the failure of the McMurry reaction was only observed for the disubstituted compounds; the monosubstituted NH₂ (**4**), NHAc (**5**) and Br (**13**) derivatives were obtained in both the ferrocenophane and ferrocene series without difficulty.

We routinely used an excess of TiCl₄ for the McMurry reaction, between three and five equivalents, and a 1:1 proportion of the ferrocenyl ketone and benzophenone. In the case of **4a** and **10a**, five equivalents of TiCl₄ were used, because our experience shows that the nitrogen-containing benzophenones are less reactive than the corresponding hydroxy benzophenones. This is also reflected in the long reaction time necessary to obtain these compounds. In some cases amino-substituted compounds were obtained by an in situ reduction of nitro-substituted benzophenones. We compared the reactions using such reagents for the synthesis of **4a** and **4b**. For **4a**, 4-aminobenzophenone gave a yield of 54% after two days of reaction and 4-nitrobenzophenone gave a yield of 21 %. For **4b**, the trend was reversed, with yields of 26% and 51%, using 4-amino and 4-nitrobenzophenone, respectively. In the case of **7a** and **7b**, 4-nitro-4-hydroxybenzophenone, formed via a Friedel-Crafts reaction, was used directly.^[68] In this case, a large excess of Zn was used to reduce the NO₂ to NH₂.^[69]



Scheme 2. Cyanation of monosubstituted and disubstituted bromo derivatives.

Nitriles **15a**, **15b**, and **16a** were obtained by treating the corresponding bromo derivatives with CuCN (Scheme 2). Compound **16b** was inaccessible, as its precursor **14b** was not obtained via the McMurry reaction as previously discussed. In our hands the cyanation of **14a** was incomplete, giving access to the mixed CN/Br derivative **17a** in 15% yield with a 44:56 isomer ratio (this compound was not tested for its antiproliferative effects).

X-ray crystallography of **7b**

X-ray quality crystals of *E*-**7b** were obtained from a mixture of *E* and *Z* isomers in an EtOH/H₂O solution by slow evaporation. Reflections were recorded using MoK_α radiation at 200 K. The ORTEP diagram, with selected bond distances and angles, is shown in Figure 1 and crystallographic data are available from the Cambridge Crystallographic Data Centre (CCDC-783005).^[70] The crystal structure shows almost no distortion around the ferrocene group, but a significant deviation from planarity around the ethylene center, with an average torsion angle of 13°. The phenyl rings are twisted out of the plane created by C1-C15-C21 by 52.8 and 48.1° which is typical of such compounds.^[67, 71]

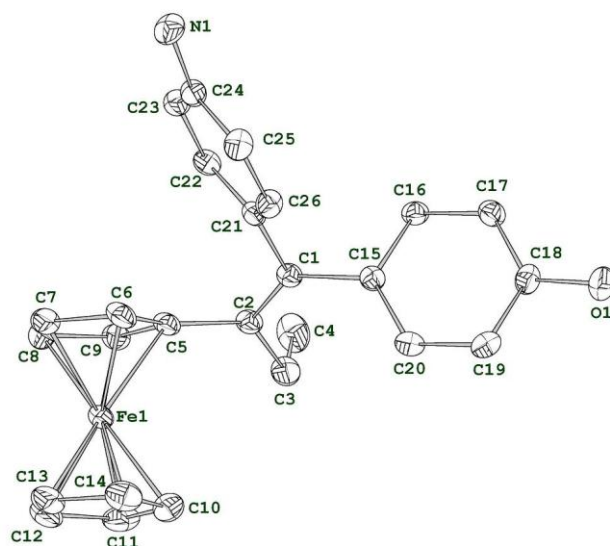


Figure 1. ORTEP diagram of (*E*)-**7b**. Thermal ellipsoids shown at 50% probability level, and hydrogen atoms have been omitted for clarity. Selected bond distances (Å) and bond angles (°): Fe1–C₅=2.061(2), C1–C2=1.372(2), C18–O1=1.376(2), C24–N1=1.420(2), Fe1–

$C_{\text{Centroid}}=179.25$, $C_p-C_p=2.34$; $C3-C2-C1-C21=164.1$, $C5-C2-C1-C15=169.8$, $C3-C2-C1-C15=-13.54$, $C5-C2-C1-C21=-12.58$.

Table 3. Cytotoxicity of compounds **1–16** against hormone-independent MDA-MB-231 breast cancer cells.

Compd	R ¹	R ²	IC ₅₀ [μM]		Cytotoxicity ratio ^[d]
			Ferrocenophane derivatives (a)	Ferrocene derivatives (b)	
1	H	H	0.92 ± 0.11	7.54 ± 0.7	8.2
(<i>E+Z</i>)- 2	OH	H	0.47 ± 0.06	1.54 ± 0.13	3.3
(<i>E+Z</i>)- 3	OAc	H	0.26 ± 0.004	1.97 ± 0.2	7.6
(<i>E+Z</i>)- 4	NH ₂	H	0.21 ± 0.03	0.86 ± 0.04	4.1
(<i>E+Z</i>)- 5	NHAc	H	0.47 ± 0.04	0.65 ± 0.01	1.4
6	OH	OH	0.089 ± 0.006	0.64 ± 0.06	7.2
(<i>E+Z</i>)- 7	NH ₂	OH	0.061 ± 0.005	0.55 ± 0.05	9.0
(<i>E+Z</i>)- 8	NHAc	OH	0.092 ± 0.019	0.56 ± 0.06	6.1
9	NH ₂	NH ₂	0.047 ± 0.01	n.o. ^[b]	-
10	NHAc	NHAc	5.64 ± 1.13	n.o. ^[b]	-
(<i>E+Z</i>)- 11	OAc	OH	0.049 ± 0.003	0.42 ± 0.03	8.6
12	OAc	OAc	0.044 ± 0.001	0.64 ± 0.05	14.5
(<i>E+Z</i>)- 13	Br	H	2.93 ± 0.62	>10 ^[a]	>3.4
14	Br	Br	>10 ^[a]	n.o. ^[b]	-
15	CN	H	0.85 ± 0.07 (<i>E+Z</i>)	10.9 ± 0.2 (<i>Z</i>)	12.8
16	CN	CN	7.98 ± 1.2	n.s. ^[c]	-

[a] Precise IC₅₀ values were not obtained because of low activity in the single-dose test (**13b**: 86% cell viability at 10⁻⁵M, **14a**: 89% cell viability at 10⁻⁵M); [b] n.o.=molecule not obtained; [c] n.s.=molecule not synthesized; [d] IC₅₀(**b**)/IC₅₀(**a**).

Antiproliferative effects on MDA-MB-231 cells

All new compounds, except **17a**, were screened for their activity against the MDA-MB-231 human breast cancer cell line. This line is hormone independent, that is, it does not express the estrogen receptor, and therefore cell culture results are not confounded with estrogenic or antiestrogenic effects. Cells were incubated with at least three concentrations of the test compound for five days, and cell viability was determined by the staining of proteins in living cells. Cytotoxicity results (IC₅₀ values) are given in Table 3. In all cases, the ferrocenophane derivatives are more cytotoxic than their ferrocenyl analogues. In the case of the NHAc-

substituted compound **5**, the difference between the two compounds is small, but significant. Conversely, the diacetoxy compound **12a** is almost 15 times more potent than its ferrocenyl analogue. The difference in cytotoxicity results for the nitriles **15a** and **15b** is even more dramatic than shown in Table 3. With an IC₅₀ value of 60 μM, the *E* isomer of **15b** is much less active than its *Z* isomer.^[72] Therefore, given a 50:50 mixture of isomers for **15b**, one could predict a cytotoxicity ratio of approximately 40.

Antiproliferative effects of **6a** and **6b** on the NCI cell line panel

Compounds **6a** and **6b** were selected for further study on the human tumor cell line panel of the Developmental Therapeutics Program of the National Cancer Institute (Bethesda, MD, USA).^[73] The compounds were tested on approximately 60 cell lines within nine tumor type subpanels. The cells were treated for 48 h at five concentrations ranging from 0.01 to 100 μM. Three endpoints were calculated: GI₅₀ (the concentration where the compound inhibits cell growth by 50%), TGI (the concentration at which the compound inhibits cell growth by 100%), and LC₅₀ (the concentration at which the drug decreases the original cell number by 50%), shown in Figure 2, as well as the MG-MID (full-panel mean-graph midpoint). Compounds **6a** and **6b** have a broad spectrum of activity, with MG-MID values (representing the average of the MG-MIDs for two experiments, using almost identical panels) lower than those for cisplatin (Table 4). The correlation between the full-panel results for the two compounds was 78% for GI₅₀, 58% for TGI, and 58% for LC₅₀ values.

Table 4. Full-panel mean-graph midpoint (MG-MID) GI ₅₀ , TGI, LC ₅₀ values of compounds in the NCI cell panel. ^[a]			
Compd	GI ₅₀ [μM]	TGI [μM]	LC ₅₀ [μM]
6a	0.18 ± 0.03	11 ± 1	52 ± 3
6b	0.52 ± 0.04	4.3 ± 0.4	23 ± 2
Cisplatin ^[b]	10.3	50.7	90.5

[a] Data from two experiments. [b] Data from NCI/DTP screening: October 2009, maximum concentration: 100 μM, after 48 h.

The GI₅₀ results varied over approximately three orders of magnitude for **6a**, and two orders of magnitude for **6b**, suggesting a more varied selectivity profile for **6a**. Comparing the mean GI₅₀ for each histological subpanel gives an indication of tumor selectivity, and a compound is considered selective for a tumor type if the mean GI₅₀ value is below the MG-

MID for all cell lines (here 0.18 μM for **6a** and 0.52 μM for **6b**). Table 5 shows the mean values over each subpanel for two different experiments. Compound **6a** is selective for leukemia, CNS cancer, and renal cancer, and was particularly active against melanoma M14, renal cancer ACHN, and CNS cancer SF-539, with two experiments giving GI_{50} values less than 20 nM. Compound **6b** also showed selectivity for leukemia, CNS cancer, and renal cancer. Similar to **6a**, this compound was particularly active against ACHN and SF-539, but also against UACC-62 melanoma, HL60 (TB), and SR leukemia cell lines with GI_{50} values falling below 0.2 μM for two experiments. Compound **6a** was more active than **6b** for every subpanel of cells. The most dramatic difference in activity was noted for the renal cancer subpanel.

Table 5. Histological subpanel mean-graph midpoint (MG-MID) GI_{50} values of compounds in the NCI cell panel. ^[a]					
Cell panel	6a (exp. 1)	6a (exp. 2)	6b (exp.1)	6b (exp. 2)	6b/6a
Leukemia	0.14	0.11	0.27	0.17	1.8
NSCL Cancer	0.31	0.16	0.84	0.50	2.9
Colon Cancer	0.40	0.34	0.80	0.61	1.9
CNS Cancer	0.074	0.092	0.31	0.29	3.6
Melanoma	0.19	0.15	0.83	0.55	4.1
Ovarian Cancer	0.43	0.30	1.09	0.53	2.2
Renal Cancer	0.072	0.048	0.43	0.20	5.3
Prostate Cancer	0.41	0.048 ^[b]	0.78	0.56	1.6
Breast Cancer	0.58	0.49	1.0	0.84	1.7

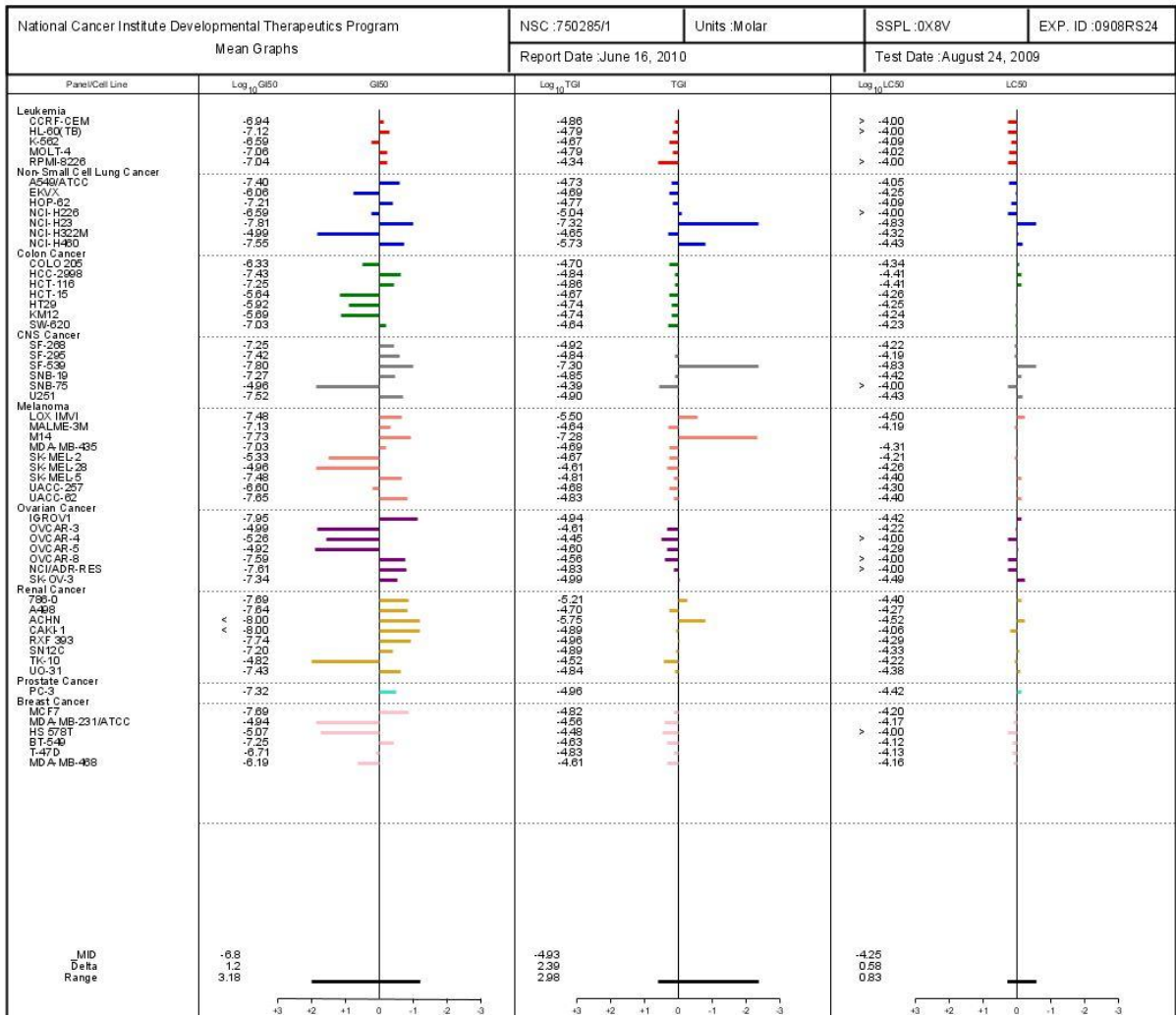
[a] Data shown are from two experiments and are the ratio of averaged values (μM); [b] data only for PC3 (excluded from averaging).

Table 6. COMPARE results using the NCI/DTP Standard Agents database.						
Compd	Endpoint	Molecule with best correlation			Tamoxifen PCC	Cisplatin PCC
		Name	PCC	Mechanism		
6a	GI_{50}	topotecan	0.70	topoisomerase I inhibitor	0.06	0.51
	TGI	3HP	0.62	DNA antimetabolite	0.15	0.50
	LC_{50}	bleomycin	0.72	DNA strand breaks	0.36	0.38
6b	GI_{50}	aphidicolin glycinate	0.81	DNA antimetabolite	0.23	0.59
	TGI	topotecan	0.67	topoisomerase II inhibitor	0.39	0.38
	LC_{50}	tamoxifen	0.56	selective estrogen receptor modulator	0.56	0.40

To gain insight into a possible mechanism of action for **6a** and **6b**, a COMPARE analysis^[74] was carried out against the NCI/DTP Standard Agents database, a collection of

171 known molecules. The three endpoints for each seed molecule were compared with the same endpoint for the database molecules (GI_{50} , TGI, and LC_{50}). The molecule with the best correlation [Pearson correlation coefficient (PCC)] and the values for tamoxifen and cisplatin are given in Table 6. The full-panel correlations with cisplatin and tamoxifen were weak ($R^2 < 0.6$) for all endpoints. The best PCCs for each endpoint belong to molecules which inhibit DNA replication or cause DNA damage.

Each standard agent in the database appears with an associated mechanism: alkylating agent, antimetabolic agent, DNA antimetabolite, RNA/DNA antimetabolite, topoisomerase II inhibitor, or not given/other. Averaging over the top 20 hits in each category over each endpoint, the dominant suggested mechanism for **6a** is RNA and/or DNA antimetabolite (36 %), followed by an DNA alkylation (32 %), not given/other (20 %), topoisomerase II inhibition (9 %), or as a antimetabolic agent (3 %). The dominant suggested mechanism for **6b** was DNA alkylation (36 %), followed by acting as a RNA and/or DNA antimetabolite (22 %), topoisomerase II inhibition (18 %), not given/other (14 %), or as an antimetabolic agent (9 %).



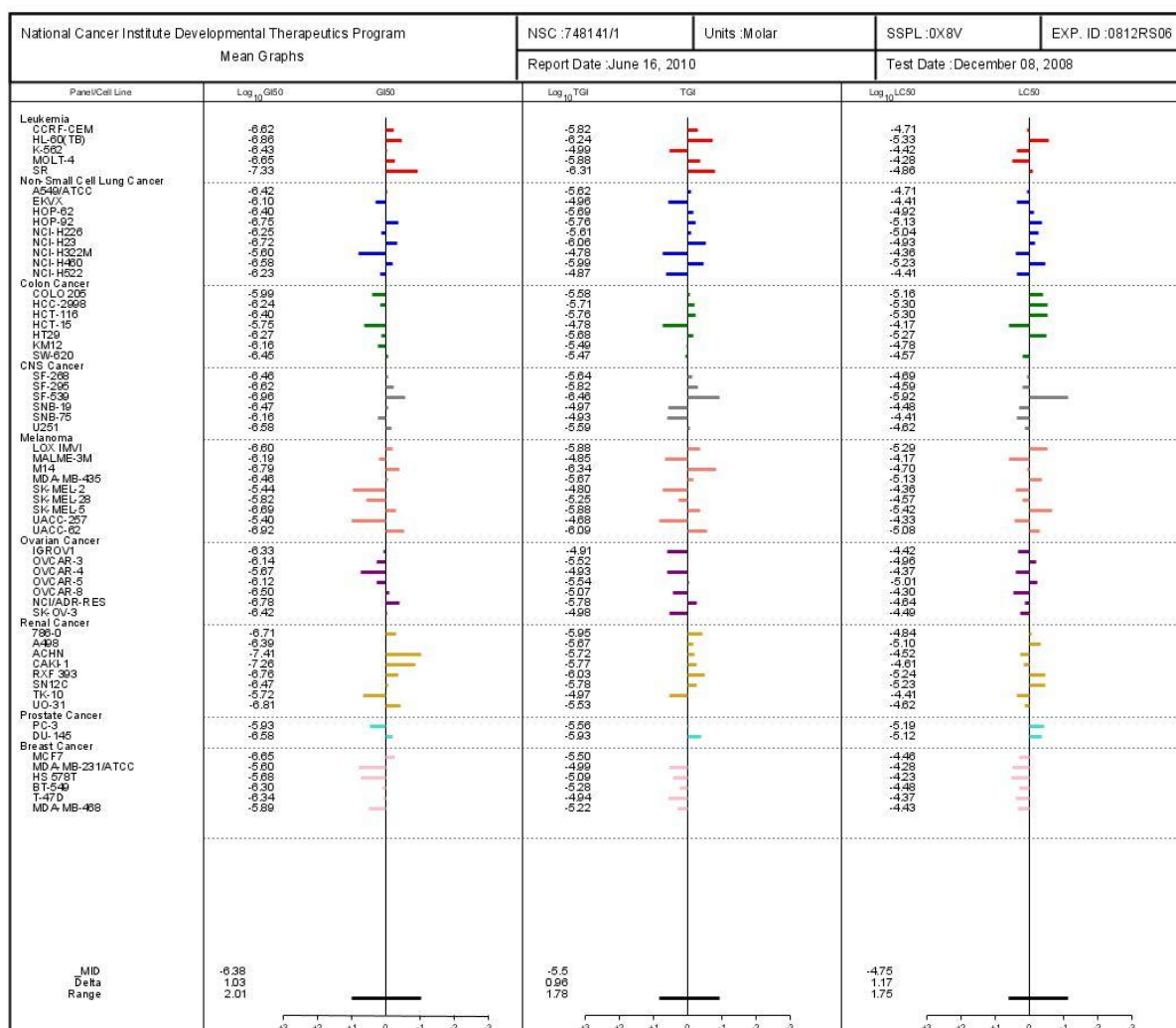


Figure 2. Mean graphs for compounds a) **6a** and b) **6b** from the National Cancer Institute Developmental Therapeutic Program; data from one experiment shown.

Acute toxicity of **6a** and **6b**

Mice were treated by interperitoneal injection of **6a** and **6b** in DMSO by the NCI/DPT program.^[75] One mouse was injected with 400 mg kg⁻¹, the second with 200 mg kg⁻¹, and the third with 100 mg kg⁻¹ of the test compound. The mice were observed for two weeks and sacrificed if more than 20% weight loss or other signs of toxicity were noted. For **6a**, the mice given doses of 400 and 200 mg kg⁻¹ were sacrificed. For **6b**, only the mouse given the highest dose of 400 mg kg⁻¹ was sacrificed. Thus the determined maximum tolerated dose was 100 mg kg⁻¹ for **6a** and 200 mg kg⁻¹ for **6b**.

Interaction of **6b** with nucleotides

Compound **6b** (0.5 mM) was incubated in 10 mM NaH₂PO₄-Na₂HPO₄ buffer (pH 7.4) with the nucleotides 5'-GMP, 5'-AMP, or 5'-CMP to obtain molar ratios of 1:2 complex/nucleotide). Analyses of the solutions were performed on a Hewlett-Packard ^{3D}Capillary Electrophoresis system equipped with a diode array detector at λ 200 nm for 10 h. As shown in Figure 3, there is no significant change in the intensity of the peaks over time, indicating that no adducts between the nucleotides and **6b** were formed. Similar studies with transition metal complexes featuring leaving groups, including cisplatin and Pt^{II} analogues,^[76] Ru anticancer drug candidates and developmental compounds,^[77] exclusively form adducts with nucleobases, normally with highest binding preference to the N7 atoms of guanine and adenine.^[78]

Calculation of HOMO-LUMO gaps for oxidized molecules

Relative differences in the HOMO-LUMO gaps of molecules can be used as a predictor of their reactivity.^[79, 80] Using as a starting point our proposal that quinone methide metabolites of the test molecules are the toxic species, and that quinone methide formation involves an initial oxidation of ferrocene to ferricenium, we calculated the HOMO-LUMO gap of the oxidized (ferricenium) forms of the molecules, Table 7. The HOMO-LUMO gap of the ferrocenophanes is quite small, with an average of 1.6 eV and a range of 1.4-1.9 eV. The calculated HOMO-LUMO gaps for the oxidized ferrocene compounds are more variable, but consistently larger, with an average HOMO-LUMO gap of 6.2 eV and a range of 3.9 to 7.2 eV.

Discussion

The [3]ferrocenophane series is more active than the ferrocene series on the MDA-MB-231 cells and the 60 cell line panel overall. We previously proposed that quinone methides, formed via initial oxidation of the ferricenium moiety, could be the active metabolite in the cell, and higher cytotoxicity of the ferrocenophane molecules is consistent with this mechanism for the following reasons. Firstly, the additional electron density imparted to the ferrocene group by the bridge lowers the oxidation potential of the ferrocene, the first step in the proposed mechanism.^[66] Secondly, due to the rigid conformation of the bridge, the formed quinone methide is expected to be highly strained and reactive.^[67] Thirdly, Hartree-Fock

calculations suggest that the difference in activity between the ferrocenophane and ferrocene series could be due to differences in the HOMO and LUMO energy levels for the oxidized (Fc^+) form of the compound. With a narrower HOMO-LUMO gap, the oxidized ferrocenophane complexes can more easily attain the first excited state and are therefore more reactive. These compounds could evolve to the quinone methide, when possible, or another reaction such as fragmentation giving rise to toxic molecules.

Indeed, the compounds that can be oxidized to a quinone methide, such as the hydroxy compounds **2** or **6**, or quinone imines, such as **4** or **9**, are very active. Although the best activity was provided by the diacetoxy **12a**, this species should be easily transformed into **6a** by hydrolysis in the cell. By contrast, comparison of the diamino compound **9a** with diacetamido **10a** shows a dramatic decrease in activity from 0.047 to 5.64 μM , probably because the hydrolysis of the acetamido compound is more difficult than the hydrolysis of acetoxy compound. The presence of an electron withdrawing group such as Br or CN decreases the antiproliferative activity for the ferrocene series, as previously observed,^[72] and also for the [3]ferrocenophane compounds.

There are also several indications that these compounds act as prodrugs, that is, innocuous compounds which are activated in the cell to form toxic species. For example, NCI/DPT acute toxicity tests on mice for **6a** and **6b** showed mouse death only at high drug concentrations (200 mg kg^{-1} and 400 mg kg^{-1} , respectively).^[75] This suggests a selectivity for cancer cells which is consistent with our previous observation that **6b** is 100x less toxic on normal cells than on cancer cells,^[81] although it may also be due to a lack of bioavailability. Secondly, incubation of **6b** with nucleotides did not show any direct interaction between the two, as measured by capillary zone electrophoresis (CZE). The low toxicity and lack of reaction with DNA, (though alteration of DNA after metabolism cannot be excluded), suggest that these compounds are only activated in cancer cells to form reactive species.

The COMPARE results suggest interference with DNA as the primary mechanism. These results should be regarded with caution, as mechanisms involving DNA dominate the pool of common anticancer agents. Direct attack on DNA is consistent with our proposed mechanism of quinone methide formation, as quinone methides are known to react with DNA via Michael addition.^[82] Indeed, compound **6b** has been shown to damage DNA, but only when incubated in living cells,^[83] where it is presumably metabolized to the quinone methide or other active species. However, these results do not exclude the possibility of other targets. For example,

we have found (unpublished results) that the quinone methide forms of some of these compounds can react with glutathione and other thiols, such as those present in many proteins.

Conclusions

We have described the synthesis and antiproliferative effects for 11 new compounds based on the 1-(diphenylmethylidene)-[3]ferrocenophane or the 1-(diphenylmethylidene)ethylferrocene skeletons. Of the full 28-member library, compounds possessing the [3]ferrocenophane motif are, in every case, more active against the MDA-MB-231 hormone-independent breast cancer cell line than the corresponding ferrocenyl compounds. This is consistent with the smaller HOMO-LUMO gap present in the oxidized form of the [3]ferrocenophane compounds. Furthermore, those compounds which can form quinone or imine methides (with or without preliminary hydrolysis) are the most active of both the [3]ferrocenophane and ferrocene series. Results on the NCI/DPT 60 cell line panel show greater cell line selectivity for **6a** than for **6b**, although the qualitative subpanel selectivity patterns are similar. Possible mechanisms of action, as gleaned from a COMPARE analysis include the compounds acting as DNA alkylating agents or DNA antimetabolites. The low systemic toxicity and inertness with regard to DNA may be due to their putative behavior as oxidation-activated prodrugs.

Experimental Section

General remarks

All reactions were performed under argon using standard Schlenk techniques. Anhydrous THF was obtained by distillation from sodium/benzophenone. Thin layer chromatography was performed on silica gel 60 GF₂₅₄. The preparative HPLC separations were performed on a Shimadzu apparatus with a Nucleodur C₁₈ column (*l*=25 cm, Ø=3.2 cm, particle size=10 µm) using CH₃CN as an eluent. The analytical HPLC controls were performed on a Shimadzu apparatus with a Nucleodur C₁₈ column (length of 15 cm, diameter of 0.45 cm, and particle size of 5 µm) using CH₃CN as an eluent. IR spectra were obtained on an IRFT BOMEM Michelson-100 spectrometer equipped with a DTGS detector as a KBr plate. ¹H and ¹³C NMR spectra were recorded on a 300 MHz Bruker spectrometer. Mass spectrometry was performed with a Nermag R 10-10C spectrometer. Elemental analyses were performed by the

microanalysis service of CNRS at Gif-sur-Yvette (France). HRMS measurements were performed with an LTQ-Orbitrap XL of Thermo Fischer used in positive mode with an electrospray source.

General synthesis of 7b, 13a, and 14a

Zinc powder was suspended in dry THF at room temperature, and TiCl₄ was added slowly via a syringe while stirring. The reaction mixture was held at reflux for 2 h, after which a THF solution containing the appropriate ketones was added, and reflux was continued. The reaction mixture was poured into H₂O, acidified with HCl until the black precipitate disappeared, and extracted with CH₂Cl₂. The organic layer was washed with H₂O, dried over magnesium sulfate, filtered, and concentrated under reduced pressure, and work-up was done as described below.

1-(4-aminophenyl)-1-(4-hydroxyphenyl)-2-ferrocenylbut-1-ene, 7b. Zinc powder (17 g, 260 mmol), TiCl₄ (15.4 mL, 140 mmol), propionyl ferrocene (2.58 g, 10.67 mmol), 4-hydroxy-4'-nitrobenzophenone (2.59 g, 10.67 mmol), and reflux for 4 h. The reaction mixture was separated on a silica gel column with CH₂Cl₂ as the eluent. Recrystallization of the crude product as a mixture of *Z* and *E* isomers (50:50) from CH₃CN yielded **7b** as an orange-red solid (2.8 g, 62%). ¹H NMR (300 MHz, [D₆]DMSO): δ=0.99 and 1.02 (t, *J*=7.4 Hz, 3H, CH₃), 2.50 (q, *J*=7.4 Hz, 2H, CH₂), 3.80 and 3.86 (t, *J*=1.8 Hz, 2H, C₅H₄), 4.07 and 4.08 (t, *J*=1.8 Hz, 2H, C₅H₄), 4.09 and 4.11 (s, 5H, Cp), 4.99 and 5.01 (s broad, 2H, NH₂), 6.42 and 6.54 (d, *J*=8.5 Hz, 2H, C₆H₄), 6.64 and 6.92 (d, *J*=8.5 Hz, 2H, C₆H₄), 6.71 and 6.73 (d, *J*=8.5 Hz, 2H, C₆H₄), 6.98 and 7.01 (d, *J*=8.5 Hz, 2H, C₆H₄), 9.25 and 9.31 (s, 1H, OH); ¹³C NMR (75.4 MHz, [D₆]DMSO): δ=15.4 (CH₃), 27.1 (CH₂), 67.7 (2CH C₅H₄), 68.6 (2CH C₅H₄), 68.9 (5CH Cp), 86.1 (C_{ip}), 115.1 (2CH C₆H₄), 119.2 (2CH C₆H₄), 129.7 (2CH C₆H₄), 129.8 (2CH C₆H₄), 134.9 (C), 136.0 (C), 137.3 (C), 139.4 (C), 149.2 (C), 155.6 (C); IR (KBr, $\tilde{\nu}$ cm⁻¹): 3495, 3469, 3428, 3399, 3319 (OH, NH₂), 3088, 2980, 2959, 2923, 2866 (CH₂, CH₃); MS (CI, NH₃) *m/z*: 424 [M+H]⁺; HRMS (EI, 70 eV, C₂₆H₂₅FeNO: [M]⁺) calcd: 423.1286, found: 423.1299; Anal. calcd for C₂₆H₂₅FeNO · 0.5H₂O: C 72.23, H 6.06, N 3.24, found: C 72.16, H 5.95, N 3.01.

1-[(4-bromophenyl)phenylmethylidene]-[3]ferrocenophane, 13a. Zinc powder (1.96 g, 30 mmol), TiCl₄ (2.2 mL, 20 mmol), [3]ferrocenophane-1-one (1.2 g, 5 mmol), *p*-bromobenzophenone (1.31 g, 5 mmol), and reflux for 3.5 h. Purification by HPLC with

CH₃CN as eluent. Recrystallization from hexane yielded **13a** as bright yellow crystals (2.08 g, 89%) as an unidentified mixture of *Z* and *E* isomers (62:38). ¹H NMR (300 MHz, CDCl₃): δ=2.15 (s, 2H, CH₂), 2.46 (s, 2H, CH₂), 3.86 (s, 2H, C₅H₄), 3.89 (s, 2H, C₅H₄), 3.94 (s, 2H, C₅H₄), 4.12 (s, 2H, C₅H₄), 6.72 (d, *J*=8.4 Hz, 2H, C₆H₄), 6.79 (d, *J*=8.4 Hz, 2H, C₆H₄), 6.88-7.33 (m, 5H, C₆H₅); ¹³C NMR (75.4 MHz, CDCl₃): δ=28.7 (CH₂), 40.6 and 40.8 (CH₂), 69.1 (2CH C₅H₄), 69.7 (2CH C₅H₄), 70.4 (2CH C₅H₄), 70.5 (2CH C₅H₄), 83.3 (C_{ip}), 86.9 (C_{ip}), 120.1 and 120.7 (C-Br), 126.3 and 126.9 (CH C₆H₅), 127.5 and 128.3 (2CH_{arom}), 129.3 and 131.0 (2CH_{arom}), 130.5 (2CH_{arom}), 131.4 and 132.2 (2CH_{arom}), 135.7 (C), 139.5 and 139.6 (C), 142.0 and 142.5 (C), 142.6 and 143.0 (C); IR (KBr, $\bar{\nu}$ cm⁻¹): 3081, 2953, 2920, 2842 (CH₂); MS (EI, 70 eV) *m/z*: 468 [M]⁺ (⁷⁹Br); Anal. calcd for C₂₆H₂₁BrFe: C 66.55, H 4.51, found: C 66.35, H 4.53.

1-[(di-4-bromophenyl)methylidene]-[3]ferrocenophane, 14a. Zinc powder (1.96 g, 30 mmol), TiCl₄ (2.2 mL, 20 mmol), [3]ferrocenophane-1-one (1.2 g, 5 mmol), di-*p*-bromobenzophenone (1.7 g, 5 mmol), and reflux for 3.5 h. Purification by HPLC with CH₃CN as eluent. Recrystallization from CH₂Cl₂ yielded **14a** as yellow crystals (2.21 g, 81%); mp: 142°C; ¹H NMR (300 MHz, CDCl₃): δ=2.33 (m, 2H, CH₂), 2.64 (m, 2H, CH₂), 3.99 (s, 2H, C₅H₄), 4.02 (s, 2H, C₅H₄), 4.08 (s, 2H, C₅H₄), 4.25 (s, 2H, C₅H₄), 6.90 (d, *J*=8.3, 2H, C₆H₄), 7.09 (d, *J*=8.3, 2H, C₆H₄), 7.21 (d, *J*=8.3, 2H, C₆H₄), 7.48 (d, *J*=8.3, 2H, C₆H₄); ¹³C NMR (75.4 MHz, CDCl₃): δ=28.9 (CH₂), 41.0 (CH₂), 69.0 (2CH C₅H₄), 69.6 (2CH C₅H₄), 70.4 (2CH C₅H₄), 70.6 (2CH C₅H₄), 83.0 (C_{ip}), 86.7 (C_{ip}), 120.5 (C-Br), 121.1 (C-Br), 130.7 (2CH C₆H₄), 131.1 (2CH C₆H₄), 131.6 (2CH C₆H₄), 132.3 (2CH C₆H₄), 136.7 (C), 138.6 (C), 141.6 (C), 142.0 (C); IR (KBr, $\bar{\nu}$ cm⁻¹): 2923, 2843 (CH₂); MS (EI, 70 eV) *m/z*: 546 [M]⁺; Anal. calcd for C₂₆H₂₀Br₂Fe: C 56.97, H 3.67, found: C 56.99, H 3.67.

General synthesis of 15a, 16a, 17a

In a Schlenk tube **13a** or **14a** were dissolved in anhydrous DMF. Copper(I) cyanide, dissolved in anhydrous DMF, was added dropwise and the reaction mixture was held at reflux for 12 h. The mixture was poured in 10 mL of a 10% NaCN solution and was extracted with Et₂O (10 mL). The organic phase was washed with 20 mL of a 10% NaCN solution, followed by 20 mL of saturated NaCl solution. The solution was dried over magnesium sulfate, filtered, and concentrated under reduced pressure. After being passed through a small filter (filled to 3 cm with silica gel) by using a 4:1 solution of petroleum ether and CH₂Cl₂, the solvent was

evaporated. The desired compounds were purified with HPLC with CH₃CN as eluent, and recrystallization was performed using the appropriate solvent system as described below.

1-[(4-cyanophenyl)phenylmethylidene]-[3]ferrocenophane, 15a. **13a** (0.25 g, 0.53 mmol), anhydrous DMF (15 mL), copper(I) cyanide (0.286 g, 3.2 mmol), anhydrous DMF (12 mL), recrystallization from CH₂Cl₂-hexane solvent system. Orange crystals, **15a** (0.162 g, 74%) as an unidentified mixture of *Z* and *E* isomers (61:39). ¹H NMR (300 MHz, [D₆]acetone): δ=2.40-2.46 (m, 2H, CH₂), 2.70-2.73 (m, 2H, CH₂), 3.96-3.98 (m, 2H, C₅H₄), 4.01-4.07 (m, 4H, C₅H₄), 4.32-4.34 (m, 2H, C₅H₄), 7.04-7.80 (m, 9H, C₆H₄ and C₆H₅); ¹³C NMR (75.4 MHz, [D₆]acetone): δ=29.3 (CH₂), 41.1 and 41.5 (CH₂), 69.2 (2CH C₅H₄), 69.6 and 69.9 (2CH C₅H₄), 71.0 and 71.1 (2CH C₅H₄), 71.2 (2CH C₅H₄), 83.4 and 83.6 (C_{ip}), 87.3 and 87.7 (C_{ip}), 110.3 (C), 119.5 (C), 127.3 and 128.0 (CH C₆H₅), 128.5-129.4 (2CH_{arom}), 130.1 and 131.1 (2CH_{arom}), 131.2 and 132.0 (2CH_{arom}), 132.1 and 133.0 (2CH_{arom}), 138.2 and 139.0 (C), 140.2 and 140.3 (C), 143.3 and 143.4 (C), 149.1 and 149.4 (C); IR (KBr, $\bar{\nu}$ cm⁻¹): 3080, 2954, 2923, 2852 (CH₂), 2226 (CN); MS (EI, 70 eV) *m/z*: 415 [M]⁺, 336, 121; Anal. calcd for C₂₇H₂₁FeN: C 78.08, H 5.09, N 3.37, found: C 77.69, H 5.02, N 3.29.

1-[(di-4-cyanophenyl)methylidene]-[3]ferrocenophane, 16a and 1-[(4-bromophenyl)-(4-cyanophenyl)methylidene]-[3]ferrocenophane, 17a. **14a** (1.7 g, 3.1 mmol), anhydrous DMF (15 mL), copper(I) cyanide (3.33 g, 37.2 mmol), anhydrous DMF (12 mL). Recrystallization from CH₂Cl₂ gave orange crystals of **16a** (0.6 g, 44%) and orange crystals of **17a** (0.22 g, 15%) as an unidentified mixture of *Z* and *E* isomers (56:44).

16a; mp: 190°C. ¹H NMR (300 MHz, [D₆]acetone): δ=2.45 (m, 2H, CH₂), 2.74 (m, 2H, CH₂), 3.96 (t, *J*=1.8, 2H, C₅H₄), 4.05 (t, *J*=1.8, 2H, C₅H₄), 4.08 (t, *J*=1.8, 2H, C₅H₄), 4.35 (t, *J*=1.8, 2H, C₅H₄), 7.24 (d, *J*=8.5, 2H, C₆H₄), 7.52 (d, *J*=8.5, 4H, C₆H₄), 7.82 (d, *J*=8.5, 2H, C₆H₄); ¹³C NMR (75.4 MHz, [D₆]acetone): δ=29.3 (CH₂), 41.3 (CH₂), 69.3 (2CH C₅H₄), 70.0 (2CH C₅H₄), 71.1 (2CH C₅H₄), 71.3 (2CH C₅H₄), 82.9 (C_{ip}), 87.4 (C_{ip}), 110.1 (2C), 119.3 (2C), 131.3 (2CH C₆H₄), 132.2 (2CH C₆H₄), 132.3 (2CH C₆H₄), 133.3 (2CH C₆H₄), 138.7 (C), 141.1 (C), 148.2 (2C); IR (KBr, $\bar{\nu}$ cm⁻¹): 2948, 2922, 2848 (CH₂), 2222 (CN); MS (EI, 70 eV) *m/z*: 440 [M]⁺, 361, 278, 199, 134, 121; HRMS (ESI, C₂₈H₂₀FeN₂: [M]⁺) calcd: 440.09704, found: 440.09657; Anal. Calcd for C₂₈H₂₀FeN₂ · 0.05 H₂O: C 76.22, H 4.59, N 6.35, found: C 75.95, H 4.74, N 6.17.

17a. ^1H NMR (300 MHz, $[\text{D}_6]$ acetone): δ =2.40-2.45 (m, 2H, CH_2), 2.68-2.76 (m, 2H, CH_2), 3.96 (s, 2H, C_5H_4), 4.03-4.09 (m, 4H, C_5H_4), 4.33 (s, 2H, C_5H_4), 6.98 and 7.23 (d, J =8.5, 2H, C_6H_4), 7.25 and 7.29 (d, J =8.5, 2H, C_6H_4), 7.49 and 7.50 (d, J =8.5, 2H, C_6H_4), 7.59 and 7.80 (d, J =8.5, 2H, C_6H_4); ^{13}C NMR (75.4 MHz, $[\text{D}_6]$ acetone): δ =29.2 (CH_2), 41.5 (CH_2), 69.2 (2CH C_5H_4), 69.8 and 69.9 (2CH C_5H_4), 71.0 (2CH, C_5H_4), 71.2 (2CH C_5H_4), 83.3 (C_{ip}), 87.3 and 87.6 (C_{ip}), 110.5 (C), 119.3 (C), 120.9 (C), 131.2 and 131.5 (2CH C_6H_4), 132.1 (2CH C_6H_4), 132.2 and 132.5 (2CH C_6H_4), 133.1 and 133.2 (2CH C_6H_4), 139.0 (C), 139.3 and 140.0 (C), 142.5 (C), 148.8 (C); IR (KBr, $\bar{\nu}$ cm^{-1}): 3066, 2955, 2917, 2902, 2842 (CH_2), 2232 (CN); HRMS (ESI, $\text{C}_{27}\text{H}_{20}\text{BrFeN}$: $[\text{M}]^+$) calcd: 493.01231, found: 493.01266; Anal. calcd for $\text{C}_{27}\text{H}_{21}\text{FeNBr} \cdot 0.26 \text{H}_2\text{O}$: C 65.00, H 4.15, N 2.81, found: C 64.91, H 4.44, N 2.53.

General synthesis of 11a and 12a

In a Schlenk flask, **6a** (0.84 g, 2 mmol) was dissolved in anhydrous THF and NaH (0.12 g, 5 mmol, 60% suspension in oil) was added. After 10 min stirring, acetyl chloride (0.3 mL, 4.2 mmol) was added and the reaction mixture was stirred for 3 h. The mixture was poured in H_2O , extracted twice with CH_2Cl_2 , and concentrated under reduced pressure. Products were separated by HPLC with a solution 90:10 of $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ as the eluent to yield the mono- and diacetylated products. Then the products were recrystallized from EtOH to yield bright yellow crystals of **11a** (0.43 g, 46%) consisting of an unidentified mixture of *Z* and *E* isomers (54:46) and bright yellow crystals of **12a** (0.33 g, 33 %).

1-[(4-acetyloxyphenyl)-(4-hydroxyphenyl)methylidene]-[3]ferrocenophane, 11a. ^1H NMR (300 MHz, $[\text{D}_6]$ acetone): δ =2.18 and 2.27 (s, 3H, CH_3), 2.30-2.40 (m, 2H, CH_2), 2.62-2.74 (m, 2H, CH_2), 3.94 (t, J =1.7, 2H, C_5H_4), 3.96-4.05 (m, 4H, C_5H_4), 4.27 (t, J =1.7, 2H, C_5H_4), 6.56 and 6.83 (d, J =8.8, 2H, C_6H_4), 6.86 and 7.04 (d, J =8.8, 2H, C_6H_4), 6.87 and 7.09 (d, J =8.8, 2H, C_6H_4), 7.13 and 7.28 (d, J =8.8, 2H, C_6H_4), 8.18 and 8.38 (s, 1H, OH); ^{13}C NMR (75.4 MHz, $[\text{D}_6]$ acetone): 21.0 (CH_3), 29.2 (CH_2), 41.3 and 41.6 (CH_2), 68.9 (2CH C_5H_4), 69.3 and 69.4 (2CH C_5H_4), 70.9 (2CH C_5H_4), 71.1 (2CH C_5H_4), 84.4 and 84.5 (C_{ip}), 87.5 and 87.8 (C_{ip}), 115.1 and 115.9 (2CH C_6H_4), 121.3 and 122.3 (2CH C_6H_4), 130.9 and 131.2 (2CH C_6H_4), 132.1 and 132.4 (2CH C_6H_4), 134.8 and 135.3 (C), 135.5 (C), 140.8 and 140.9 (C), 142.0 and 142.3 (C), 150.0 and 150.6 (C), 156.7 and 157.3 (C), 169.4 and 169.6 (CO); IR (KBr, $\bar{\nu}$ cm^{-1}): 3434 (OH), 3077, 2915, 2853 (CH_3 , CH_2), 1731 (CO); MS (EI, 70 eV) m/z :

464 [M]⁺, 422, 343; HRMS (ESI, C₂₈H₂₄FeO₃: [M]⁺) calcd: 464.1076, found: 464.10694; Anal. Calcd for C₂₈H₂₄FeO₃ · 0.54 H₂O: C 70.94, H 5.33, found: C 70.66, H 5.42.

1-[(di-4-acetyloxyphenyl)methylidene]-[3]ferrocenophane, 12a; mp: 192°C (dec.) ¹H NMR (300 MHz, CDCl₃): δ=2.23 (s, 3H, CH₃), 2.31 (s, 3H, CH₃), 2.36 (m, 2H, CH₂), 2.68 (m, 2H, CH₂), 3.97 (s, 4H, C₅H₄), 4.03 (s, 2H, C₅H₄), 4.22 (s, 2H, C₅H₄), 6.81 (d, *J*=8.7, 2H, C₆H₄), 7.03 (d, *J*=8.7, 2H, C₆H₄), 7.07 (d, *J*=8.4, 2H, C₆H₄), 7.24 (d, *J*=8.4, 2H, C₆H₄); ¹³C NMR (75.4 MHz, CDCl₃): δ=21.3 (2CH₃), 28.9 (CH₂), 41.1 (CH₂), 68.5 (2CH C₅H₄), 69.1 (2CH C₅H₄), 70.3 (2CH C₅H₄), 70.4 (2CH C₅H₄), 83.3 (C_{ip}), 86.7 (C_{ip}), 120.4 (2CH C₆H₄), 121.4 (2CH C₆H₄), 130.5 (2CH C₆H₄), 131.7 (2CH C₆H₄), 136.0 (C), 139.3 (C), 140.5 (C), 140.9 (C), 149.0 (C), 149.6 (C), 169.4 (2CO); IR (KBr, $\bar{\nu}$ cm⁻¹): 3089, 3047, 2942, 2850 (CH₃, CH₂), 1755 (CO); MS (EI, 70 eV) *m/z*: 506 [M]⁺, 464, 343; Anal. calcd for C₃₀H₂₆FeO₄: C 71.15, H 5.17, found: C 70.95, H 5.25.

1-(4-acetoxyphenyl)-1-(4-hydroxyphenyl)-2-ferrocenylbut-1-ene, 11b. The same procedure as for synthesis of **11a** and **12a** was used with **6b** (0.84 g, 2 mmol), NaH (0.12 g, 5 mmol, 60% suspension in oil), and acetyl chloride (0.2 mL, 2.4 mmol) to yield **11b** (0.60 g, 65%) as an orange solid consisting of an unidentified mixture of *Z* and *E* isomers (50:50). ¹H NMR (CDCl₃): δ=0.92 (t, *J*=6.8 Hz, 3H, CH₃), 2.20 and 2.23 (s, 3H, CH₃), 2.31-2.51 (m, 2H, CH₂), 3.92 and 3.95 (s, 2H, C₅H₄), 4.02-4.19 (m, 7H, C₅H₄ + Cp), 4.72 and 4.77 (s, 1H, OH), 6.62 and 6.71 (d, *J*=7.9 Hz, 2H, C₆H₄), 6.86 (d, *J*=8.5 Hz, 2H, C₆H₄), 6.92-7.14 (m, 4H, C₆H₄); ¹³C NMR (CDCl₃): δ=15.4 and 15.5 (CH₃), 21.2 (CH₃), 27.9 and 28.1 (CH₂), 68.9 and 69.0 (2CH, C₅H₄), 69.7 and 69.8 (2CH C₅H₄), 70.0 and 70.2 (5CH Cp), 86.9 (C_{ip}), 115.1 and 115.2 (2CH C₆H₄), 121.1 and 121.2 (2CH C₆H₄), 130.4 and 130.8 (2CH C₆H₄), 131.1 and 131.4 (2CH C₆H₄), 136.6 and 136.9 (C), 136.9 and 137.4 (C), 138.5 and 138.9 (C), 142.0 and 142.3 (C), 148.9 (C), 154.0 and 154.1 (C), 169.5 and 169.6 (CO). R (KBr, $\bar{\nu}$ cm⁻¹): 3367 (OH), 3093, 3035, 2966, 2931, 2870 (CH₂, CH₃), 1728 (CO); MS (CI, CH₄) *m/z*: 467 [M+H]⁺, 495 [M+C₂H₅]⁺, 401; Anal. calcd for C₂₈H₂₆FeO₃: C, 72.11; H, 5.61, found: C, 72.11; H, 5.67.

1-[(4-acetyloxyphenyl)(phenyl)methylidene]-[3]ferrocenophane, 3a. The same procedure as for the synthesis of **11a** and **12a** was used with **2a** (0.251 g, 0.62 mmol), NaH (0.05 g, 1.24 mmol, 60% suspension in oil), and acetyl chloride (0.065 mL, 0.91 mmol). After concentration under reduced pressure, the crude product was directly purified by preparative

HPLC with an CH₃CN/H₂O (95:5) solution as the eluent (because of decomposition on silica gel) to give **3a** (0.183 g, 66% yield) as a light yellow solid consisting of an unidentified mixture of *Z* and *E* isomers (82:18). ¹H NMR (300 MHz, CDCl₃): δ=2.15 (s, 3H, CH₃), 2.16-2.26 (m, 2H, CH₂), 2.47-2.59 (m, 2H, CH₂), 3.95 (s broad, 2H, C₅H₄), 3.97 (s broad, 2H, C₅H₄), 4.03 (s, 2H, C₅H₄), 4.20 (s, 2H, C₅H₄), 6.75 (d, *J*=8.4 Hz, 2H, C₆H₄), 6.99 (d, *J*=8.4 Hz, 2H, C₆H₄), 7.11-7.31 (m, 5H, C₆H₅); ¹³C NMR (75.4 MHz, CDCl₃): δ=21.2 (CH₃), 26.5 and 28.7 (CH₂), 40.7 (CH₂), 69.3 (2CH C₅H₄), 69.9 (2CH C₅H₄), 70.5 (2CH C₅H₄), 70.6 (2CH C₅H₄), 83.5 (C_{ip}), 87.0 (C_{ip}), 120.3 and 121.2 (2CH C₆H₄), 126.2 and 126.8 (CH C₆H₅), 127.4 and 128.2 (2CH_{arom}), 129.3 and 130.3 (2CH_{arom}), 130.5 and 131.5 (2CH_{arom}), 135.3 (C), 139.8 (C), 140.5 (C), 143.3 (C), 148.8 (C), 169.2 (CO); IR (KBr, $\bar{\nu}$ cm⁻¹): 1762 (CO), 1501, 1206; HRMS (ESI, C₂₈H₂₄FeO₂: [M]⁺): calcd: 448.11202, found: 448.11206.

1-(4-acetyloxyphenyl)-1-phenyl-2-ferrocenylbut-1-ene, 3b. The same procedure as for synthesis of **11a** and **12a** was used with **2b** (0.5 g, 1.23 mmol), NaH (0.098 g, 2.46 mmol, 60% suspension in oil), and acetyl chloride (0.13 mL, 1.8 mmol). After concentration under reduced pressure, the crude product was directly purified by preparative HPLC with an CH₃CN/H₂O (95:5) solution as the eluent (because of decomposition on silica gel) to give **3b** (0.26 g, 47% yield) a dark red oil consisting of an unidentified mixture of *Z* and *E* isomers (54:46). ¹H NMR (300 MHz, CDCl₃): δ=0.90 and 0.93 (t, *J*=7.2 Hz, 3H, CH₃), 2.16 and 2.19 (s, 3H, CH₃), 2.37 and 2.38 (q, *J*=7.2 Hz, 2H, CH₂), 3.90 (s, 2H, C₅H₄), 4.08 (s, 7H, C₅H₄+Cp), 6.85 and 6.95 (d, *J*=8.3 Hz, 2H, C₆H₄), 6.98 (d, *J*=8.3 Hz, 2H, C₆H₄), 7.03-7.25 (m, 5H, C₆H₅); ¹³C NMR (75.4 MHz, CDCl₃): δ=15.4 and 15.5 (CH₃), 21.2 (CH₃), 27.8 and 28.1 (CH₂), 68.9 (2CH C₅H₄), 69.7 and 69.9 (5CH Cp), 70.0 (2CH C₅H₄), 87.5 (C_{ip}), 121.2 and 121.3 (2CH C₆H₄), 126.3 (CH C₆H₅), 128.2 and 128.3 (2CH_{arom}), 129.5 and 130.0 (2CH_{arom}), 130.3 and 140.0 (2CH_{arom}), 137.2 (C), 137.9 (C), 141.8 and 142.0 (C), 144.1 and 144.3 (C), 149.0 (C), 169.3 and 169.4 (CO); IR (KBr, $\bar{\nu}$ cm⁻¹): 1757 (CO), 1496, 1193; HRMS (ESI, C₂₈H₂₆FeO₂: [M]⁺): calcd: 450.12767, found: 450.12779.

1-(4-*N*-acetamidophenyl)-1-(4-hydroxyphenyl)-2-ferrocenylbut-1-ene, 8b. In a flask **7b** (0.67 g, 1.59 mmol) was dissolved in 20 mL of anhydrous THF. Acetyl chloride (0.249 g, 0.23 mL, 3.2 mmol) and pyridine (0.251 g, 0.26 mL, 3.2 mmol) were added and the reaction mixture was left to stir for 3 h. The mixture was poured into H₂O and extracted with CH₂Cl₂. The organic phase was washed with H₂O, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The crude mixture was filtered over silica gel using

petroleum ether and CH_2Cl_2 , concentrated, and purified by HPLC ($\text{CH}_3\text{CN}/\text{H}_2\text{O}$ 7:3) and recrystallized from ether/pentane to yield **8b** as orange-yellow crystals (0.628 g, 86% yield) consisting of a mixture of *Z* and *E* isomers (50:50). ^1H NMR (300 MHz, $[\text{D}_6]\text{acetone}$): δ =0.88 and 0.89 (t, J =7.4 Hz, 3H, CH_3), 2.48 and 2.50 (q, J =7.4 Hz, 2H, CH_2), 2.74 (s, 3H, CH_3), 3.78 and 3.79 (t, J =1.9 Hz, 2H, C_5H_4), 3.93 and 3.94 (t, J =1.9 Hz, 2H, C_5H_4), 3.99 (s, 5H, Cp), 6.58 and 6.69 (d, J =8.7 Hz, 2H, C_6H_4), 6.75 and 6.93 (d, J =8.7 Hz, 2H, C_6H_4), 6.83 and 7.02 (d, J =8.7 Hz, 2H, C_6H_4), 7.38 and 7.49 (d, J =8.7 Hz, 2H, C_6H_4), 8.15 and 8.18 (s, 1H, OH), 8.99 and 9.03 (s broad, 1H, NH); ^{13}C NMR (75.4 MHz, $[\text{D}_6]\text{acetone}$): δ =16.5 and 16.6 (CH_3), 24.9 (CH_3), 29.0 and 29.1 (CH_2), 69.4 (2CH C_5H_4), 70.5 (5CH Cp), 70.6 (2CH C_5H_4), 88.3 and 88.4 (C_{ip}), 116.5 and 116.6 (2CH C_6H_4), 120.2 and 120.4 (2CH C_6H_4), 131.0 and 131.5 (2CH C_6H_4), 131.9 and 132.4 (2CH C_6H_4), 134.4 and 137.7 (C), 138.0 and 138.2 (C), 139.0 and 139.1 (C), 139.1 and 139.2 (C), 141.5 and 141.8 (C), 157.4 and 157.5 (C), 169.5 (CO); IR (KBr, $\bar{\nu}$ cm^{-1}): 3397 (OH, NH), 3097, 2967, 2930, 2871 (CH_2 , CH_3), 1664 (CO); MS (CI, NH_3) m/z : 466 $[\text{M}+\text{H}]^+$, 483 $[\text{M}+\text{NH}_4]^+$; HRMS (CI, CH_4 , $\text{C}_{28}\text{H}_{28}\text{FeNO}_2$: $[\text{M}+\text{H}]^+$) calcd: 466.1470, found: 466.1453; Anal. calcd for $\text{C}_{28}\text{H}_{27}\text{FeNO}_2 \cdot \text{H}_2\text{O}$: C 69.57, H 6.05, N 2.90, found: C 69.48, H 5.75, N 2.89.

MDA-MB-231 cytotoxicity tests

Materials

Stock solutions (1×10^{-3} M and 1×10^{-2} M) of the compounds to be tested were prepared in DMSO and were kept at -20°C . Under these conditions they are stable for at least two weeks. Serial dilutions in DMSO were prepared just prior to use. Dulbecco's modified Eagle's medium (DMEM), fetal calf serum, glutamine, and kanamycin were purchased from Invitrogen (France). Breast cancer cells MDA-MB-231 were from the American Type Culture Collection (ATCC-LGC Standards).

Culture conditions

Cells were maintained in monolayer culture in DMEM with phenol red/Glutamax I™ supplemented with 9% of decompemented fetal bovine serum and 0.9% kanamycin, at 37°C in a 5% CO_2 air-humidified incubator. For proliferation assays, cells were seeded in 24-well sterile plates in 1 mL of DMEM without phenol red, supplemented with 9% of decompemented and hormone-depleted on dextran charcoal fetal bovine serum, 0.9%

Glutamax ITM and 0.9% kanamycin. MDA-MB-231 were incubated 24 h at a density of 20000-30000 cells per mL. The following day (D0), 1 mL of the same medium containing the compounds to be tested, which were diluted in DMSO, was added to the plates (final volume of DMSO: 0.1%, three wells for each conditions, one plate per day). After 3 d (D3) the incubation medium was removed and 2 mL of fresh medium containing the compounds were added. At different days (D3, D4, D5 and D6), the total proteins content of each well were quantified by methylene blue staining as follows: cell monolayers were fixed and stained for 1 h in MeOH with methylene blue (2 mg mL⁻¹) and then washed thoroughly with H₂O. HCl (2 mL, 0.1M) was then added, and the plate was incubated for 1 h at 37°C. Then the absorbance of each well was measured at λ 655 nm with a Bio-Rad microplate reader. The results are expressed as the percentage of protein versus the DMSO control. Experiments were performed at least in duplicate.

NCI/DTP cytotoxicity and acute toxicity tests

The protocol for the determination of cytotoxicity on the 60 cell line panel can be found at <http://dtp.nci.nih.gov/branches/btb/ivclsp.html>; the protocol for acute toxicity testing in mice can be found at http://dtp.nci.nih.gov/branches/btb/acute_tox.html. The DTP homepage can be accessed at <http://dtp.cancer.gov/>.

Capillary electrophoresis

All analyses were performed on a Hewlett-Packard ^{3D}Capillary Electrophoresis system equipped with a diode-array detector. Fused-silica capillaries (50 μ m ID, total length 50 cm, effective length 42 cm) were purchased from Polymicro Technologies Inc. (Phoenix, AZ, USA). Before the first use, the capillary was flushed with 1 mM NaOH solution (HPCE grade, Fluka, Buchs, Switzerland) and H₂O (HPLC grade, Roth, Karlsruhe, Germany) for 30 min each. Afterward, the capillary was conditioned for 30 min with the background electrolyte (BGE) (NaH₂PO₄-Na₂HPO₄ buffer, 50 mM, pH 7.4). Before each analysis, the capillary was purged for 1 min with H₂O, for 2 min with 0.1 mM NaOH, and for 2 min with the BGE which was replenished after every third run. The temperature of the capillary as well as of the sample tray was kept constant at 37 °C. Injection was done by applying pressure (10 mbar) for 15 s. Voltage was maintained constant at 15 kV. The compound **6b** (0.5 mM) was incubated in 10 mM NaH₂PO₄-Na₂HPO₄ buffer (pH 7.4) with 5'-GMP, 5'-AMP or 5'-CMP to

obtain molar ratios of 1:2 (complex/nucleotide). The repeated runs were recorded at λ 200 nm for 10 h. The experiment was repeated three times.

Theoretical calculations

Theoretical calculations were performed with Titan software [Wavefunction Inc., 18401 Von Karman Avenue, Suite 370, Irvine, CA 92612 (USA)]. A geometry optimization was performed on each molecule to find the absolute energetic minimum using the semi-empirical PM3 Hamiltonian.

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- [1] G. Jaouen, *Bioorganometallics*, Wiley-VCH, Weinheim (Germany), **2006**.
- [2] G. Jaouen, P. Dyson, in *Comprehensive Organometallic Chemistry III, Vol. 12* (Eds.: R. H. Crabtree, D. M. P. Mingos), Elsevier Ltd., Oxford, **2007**, pp. 445.
- [3] C. G. Hartinger, P. J. Dyson, *Chem. Soc. Rev.* **2009**, 38, 391.
- [4] C. S. Allardyce, A. Dorcier, C. Scolaro, P. J. Dyson, *Appl. Organomet. Chem.* **2005**, 19, 1.
- [5] T. Gianferrara, I. Bratsos, E. Alessio, *Dalton Trans.* **2009**, 7588.
- [6] K. Strohfeldt, M. Tacke, *Chem. Soc. Rev.* **2008**, 37, 1174.
- [7] Y. K. Yan, M. Melchart, A. Habtemariam, P. J. Sadler, *Chem. Commun.* **2005**, 4764.
- [8] S. Schäfer, I. Ott, R. Gust, W. S. Sheldrick, *Eur. J. Inorg. Chem.* **2007**, 3034.

- [9] M. Melchart, P. J. Sadler, in *Bioorganometallics* (Ed.: G. Jaouen), Wiley-VCH, **2005**, pp. 39.
- [10] G. Süß-Fink, *Dalton Trans.* **2010**, 39, 1673.
- [11] U. Olszewski, J. Claffey, M. Hogan, M. Tacke, R. Zeillinger, P. Bednarski, G. Hamilton, *Invest. New Drugs* **2010**, DOI: 10.1007/s10637-010-9395-5.
- [12] N. Metzler-Nolte, *Angew. Chem.* **2001**, 113, 1072; *Angew. Chem. Int. Ed.* **2001**, 40, 1040.
- [13] S. Top, C. Thibaudeau, A. Vessières, E. Brulé, F. L. Bideau, J.-M. Joerger, M.-A. Plamont, S. Samreth, A. Edgar, J. Marrot, P. Herson, G. Jaouen, *Organometallics* **2009**, 28, 1414.
- [14] A. Vessières, S. Top, A. A. Ismail, I. S. Butler, M. Louër, G. Jaouen, *Biochemistry* **1988**, 27, 6659.
- [15] F. Le Bideau, E. B. Kaloun, P. Haquette, U. Kernbach, J. Marrot, E. Stéphan, S. Top, A. Vessières, G. Jaouen, *Chem. Commun.* **2000**, 211.
- [16] A. P. Ferreira, J. L. F. da Silva, M. T. Duarte, M. F. M. da Piedade, M. P. Robalo, S. G. Harjivan, C. Marzano, V. Gandin, M. M. Marques, *Organometallics* **2009**, 28, 5412.
- [17] S. Top, A. Vessières, G. Leclercq, J. Quivy, J. Tang, J. Vaissermann, M. Huché, G. Jaouen, *Chem. Eur. J.* **2003**, 9, 5223.
- [18] O. Payen, S. Top, A. Vessières, E. Brulé, M. Plamont, M. McGlinchey, H. Muller-Bunz, G. Jaouen, *J. Med. Chem.* **2008**, 51, 1791.
- [19] S. Top, J. Tang, A. Vessières, D. Carrez, C. Provot, G. Jaouen, *Chem. Commun.* **1996**, 955.
- [20] S. Top, E. B. Kaloun, A. Vessières, I. Laios, G. Leclercq, G. Jaouen, *J. Organomet. Chem.* **2002**, 643-644, 350.
- [21] A. Vessières, S. Top, W. Beck, E. A. Hillard, G. Jaouen, *Dalton Trans.* **2006**, 529.

- [22] S. Top, A. Vessières, C. Cabestaing, I. Laios, G. Leclercq, C. Provot, G. Jaouen, *J. Organomet. Chem.* **2001**, 637, 500.
- [23] P. Pigeon, S. Top, A. Vessières, M. Huché, E. A. Hillard, E. Salomon, G. Jaouen, *J. Med. Chem.* **2005**, 48, 2814.
- [24] A. Arezki, E. Brulé, G. Jaouen, *Organometallics* **2009**, 28, 1606.
- [25] S. C. B. Gnoatto, A. Dassonville-Klimpt, S. Da Nascimento, P. Galéra, K. Boumediene, G. Gosmann, P. Sonnet, S. Moslemi, *Eur. J. Med. Chem.* **2008**, 43, 1865.
- [26] S. Knauer, B. Biersack, M. Zoldakova, K. Effenberger, W. Milius, R. Schobert, *Anti-Cancer Drugs* **2009**, 20, 676.
- [27] B. Long, S. Liang, D. Xin, Y. Yang, J. Xiang, *Eur. J. Med. Chem.* **2009**, 44, 2572.
- [28] M. Jung, D. E. Kerr, P. D. Senter, *Arch. Pharm.* **1997**, 330, 173.
- [29] M. Schlenk, I. Ott, R. Gust, *J. Med. Chem.* **2008**, 51, 7318.
- [30] M. Patra, G. Gasser, A. Pinto, K. Merz, I. Ott, Julia E. Bandow, N. Metzler-Nolte, *ChemMedChem* **2009**, 4, 1930.
- [31] A. Houlton, R. Roberts, J. Silver, *J. Organomet. Chem.* **1991**, 418, 107.
- [32] C. W. Ong, J. Y. Jeng, S. S. Juang, C. F. Chen, *Bioorg. Med. Chem. Lett.* **1992**, 2, 929.
- [33] Y. Yamamoto, K. Yamashita, M. Nakamura, *Organometallics* **2010**, 29, 1472.
- [34] M. C. Semenčić, K. Heinze, C. Foerster, V. Ropic, *Eur. J. Inorg. Chem.* **2010**, 1089.
- [35] E. Meggers, *Chem. Commun.* **2009**, 1001.
- [36] A. Alama, B. Tasso, F. Novelli, F. Sparatore, *Drug Discovery Today* **2009**, 14, 500.
- [37] H. Struthers, A. Hagenbach, U. Abram, R. Schibli, *Inorg. Chem.* **2009**, 48, 5154.
- [38] L. Wei, J. Babich, W. C. Eckelman, J. Zubieta, *Inorg. Chem.* **2005**, 44, 2198.

- [39] D. Schlawe, A. Majdalani, J. Velcicky, E. Heßler, T. Wieder, A. Prokop, H.-G. Schmalz, *Angew. Chem.* **2004**, *116*, 1763; *Angew. Chem. Int. Ed.* **2004**, *43*, 1731.
- [40] J. C. Franke, M. Plötz, A. Prokop, C. C. Geilen, H.-G. Schmalz, J. Eberle, *Biochem. Pharmacol.* **2010**, *79*, 575.
- [41] P. James, J. Neudörfl, M. Eissmann, P. Jesse, A. Prokop, H.-G. Schmalz, *Org. Lett.* **2006**, *8*, 2763.
- [42] *Medicinal Organometallic Chemistry*, 1st ed., (Eds. G. Jaouen, N. Metzler-Nolte), Springer, Berlin/Heidelberg, **2010**.
- [43] A. Nguyen, A. Vessières, E. A. Hillard, S. Top, P. Pigeon, G. Jaouen, *Chimia* **2007**, *61*, 716.
- [44] E. A. Hillard, A. Vessières, L. Thouin, G. Jaouen, C. Amatore, *Angew. Chem.* **2006**, *118*, 291; *Angew. Chem. Int. Ed.* **2006**, *45*, 285.
- [45] P. Pigeon, S. Top, O. Zekri, E. A. Hillard, A. Vessières, M. A. Plamont, O. Buriez, E. Labbe, M. Huché, S. Boutamine, C. Amatore, G. Jaouen, *J. Organomet. Chem.* **2009**, *694*, 895.
- [46] G. Erker, *Macromolecular Symposia* **2006**, *236*, 1.
- [47] G. Erker, *Polyhedron* **2005**, *24*, 1289.
- [48] A. Csámpai, A. Z. Györfi, G. I. Túrós, P. Sohár, *J. Organomet. Chem.* **2009**, *694*, 3667.
- [49] O. N. Kadkin, H. Han, Y. G. Galyametdinov, *J. Organomet. Chem.* **2007**, *692*, 5571.
- [50] L. Tebben, M. Neumann, G. Kehr, R. Fröhlich, G. Erker, S. Losi, P. Zanello, *Dalton Trans.* **2006**, 1715.
- [51] G. Erker, G. Kehr, R. Fröhlich, *J. Organomet. Chem.* **2004**, *689*, 1402.
- [52] C. Arisandy, A. R. Cowley, S. Barlow, *J. Organomet. Chem.* **2004**, *689*, 775.

- [53] T. J. Brunker, C. Arisandy, A. R. Cowley, L. H. Rees, S. Barlow, D. O'Hare, *J. Organomet. Chem.* **2004**, 689, 252.
- [54] P. Liptau, M. Neumann, G. Erker, G. Kehr, R. Frohlich, S. Grimme, *Organometallics* **2004**, 23, 21.
- [55] O. Kadkin, C. Näther, W. Friedrichsen, *J. Organomet. Chem.* **2002**, 649, 161.
- [56] P. Liptau, S. Knüppel, G. Kehr, O. Kataeva, R. Fröhlich, G. Erker, *J. Organomet. Chem.* **2001**, 637-639, 621.
- [57] S.-J. Jong, J.-M. Fang, *Org. Lett.* **2000**, 2, 1947.
- [58] R. Sebesta, A. Skvorcová, *J. Organomet. Chem.* **2009**, 694, 1898.
- [59] P. Liptau, L. Tebben, G. Kehr, R. Fröhlich, G. Erker, F. Hollmann, B. Rieger, *Eur. J. Org. Chem.* **2005**, 1909.
- [60] N. Faux, D. Razafimahefa, S. Picart-Goetgheluck, J. Brocard, *Tetrahedron: Asymmetry* **2005**, 16, 1189.
- [61] P. Liptau, D. Carmona, L. A. Oro, F. J. Lahoz, G. Kehr, G. Erker, *Eur. J. Inorg. Chem.* **2004**, 4586.
- [62] P. Liptau, L. Tebben, G. Kehr, B. Wibbeling, R. Fröhlich, G. Erker, *Eur. J. Inorg. Chem.* **2003**, 3590.
- [63] P. Liptau, T. Seki, G. Kehr, A. Abele, R. Frohlich, G. Erker, S. Grimme, *Organometallics* **2003**, 22, 2226.
- [64] L. Tebben, K. Bussmann, M. Hegemann, G. Kehr, R. Froehlich, G. Erker, *Organometallics* **2008**, 27, 4269.
- [65] L. Tebben, G. Kehr, R. Fröhlich, G. Erker, *Eur. J. Inorg. Chem.* **2008**, 2654.
- [66] D. Plažuk, A. Vessières, E. A. Hillard, O. Buriez, E. Labbe, P. Pigeon, M. A. Plamont, C. Amatore, J. Zakrzewski, G. Jaouen, *J. Med. Chem.* **2009**, 52, 4964.

- [67] M. Gormen, P. Pigeon, E. A. Hillard, M. A. Plamont, D. Plažuk, S. Top, A. Vessières, G. Jaouen, *Tetrahedron Lett.* **2010**, *51*, 118.
- [68] M. Görmen, P. Pigeon, S. Top, A. Vessières, M.-A. Plamont, E. Hillard, G. Jaouen, *MedChemComm* **2010**, *1*, 149.
- [69] J. E. McMurry, *Chem. Rev.* **1989**, *89*, 1513.
- [70] CCDC-783005 contains the supplementary crystallographic data (excluding structure factors) for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via <http://www.ccdc.cam.ac.uk>.
- [71] Y. L. K. Tan, P. Pigeon, E. A. Hillard, S. Top, M.-A. Plamont, A. Vessières, M. J. McGlinchey, H. Müller-Bunz, G. Jaouen, *Dalton Trans.* **2009**, 10871.
- [72] O. Zekri, E. A. Hillard, S. Top, A. Vessières, P. Pigeon, M. A. Plamont, M. Huché, S. Boutamine, M. J. McGlinchey, H. Muller-Bunz, G. Jaouen, *Dalton Trans.* **2009**, 4318.
- [73] <http://dtp.nci.nih.gov/branches/btb/ivclsp.html> (accessed September 19, 2010).
- [74] K. D. Paull, R. H. Shoemaker, L. Hodes, A. Monks, D. Scudiero, L. Rubenstein, J. Plowman, M. R. Boyd, *J. Natl. Cancer Inst.* **1989**, *81*, 1088.
- [75] http://dtp.nci.nih.gov/branches/btb/acute_tox.html (accessed September 19, 2010).
- [76] C. G. Hartinger, P. Schluga, M. Galanski, C. Baumgartner, A. R. Timerbaev, B. K. Keppler, *Electrophoresis* **2003**, *24*, 2038.
- [77] M. Groessl, C. G. Hartinger, P. J. Dyson, B. K. Keppler, *J. Inorg. Biochem.* **2008**, *102*, 1060.
- [78] A. Dorcier, C. G. Hartinger, R. Scopelliti, R. H. Fish, B. K. Keppler, P. J. Dyson, *J. Inorg. Biochem.* **2008**, *102*, 1066.
- [79] N. T. Anh, *Orbitales Frontières, Manuel Pratique*, 2nd edition ed., CNRS éditions and EDP sciences, Paris, 2007.
- [80] Z. Zhou, R. G. Parr, *J. Am. Chem. Soc.* **1990**, *112*, 5720.

- [81] E. Allard, C. Passirani, E. Garcion, P. Pigeon, A. Vessières, G. Jaouen, J. P. Benoit, *J. Controlled Release* **2008**, *130*, 146.
- [82] P. W. Fan, J. L. Bolton, *Drug Inf. News Drug Metabol. Disp.* **2001**, *29*, 891.
- [83] I. Zanellato, J. M. Heldt, A. Vessières, G. Jaouen, D. Osella, *Inorg. Chim. Acta* **2009**, *362*, 4037.
- [84] E. A. Hillard, P. Pigeon, A. Vessières, C. Amatore, G. Jaouen, *Dalton Trans.* **2007**, 5073.
- [85] A. Vessières, S. Top, P. Pigeon, E. A. Hillard, L. Boubeker, D. Spera, G. Jaouen, *J. Med. Chem.* **2005**, *48*, 3937.
- [86] J. B. Heilmann, E. A. Hillard, M. A. Plamont, P. Pigeon, M. Bolte, G. Jaouen, A. Vessières, *J. Organomet. Chem.* **2008**, *693*, 1716.