Journal of Organometallic Chemistry 694 (2009) 874–879

Contents lists available at ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

Proliferative and anti-proliferative effects of titanium- and iron-based metallocene anti-cancer drugs

Anne Vessières [a,*], Marie-Aude Plamont [a], Claude Cabestaing [a], James Claffey [b], Sandra Dieckmann [b], Megan Hogan [b], Helge Müller-Bunz [b], Katja Strohfeldt [b], Matthias Tacke [b]

[a] Laboratoire de Chimie et Biochimie des Complexes Moléculaires, École Nationale Supérieure de Chimie de Paris, UMR CNRS 7576, 11, rue Pierre et Marie Curie, 75231 Paris 05, France
[b] Conway Institute of Biomolecular and Biomedical Research, Centre for Synthesis and Chemical Biology, UCD School of Chemistry and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland

ARTICLE INFO

Article history:
Received 24 August 2008
Received in revised form 6 November 2008
Accepted 7 November 2008
Available online 25 December 2008

For publication in the Journal of Organometallic Chemistry ISBOMC'08 special issue dedicated to Prof. Dr. Gérard Jaouen.

Keywords:
Bioorganometallic chemistry
Anti-cancer drugs
Cisplatin
Titanocene
Ferrocene
Breast cancer

ABSTRACT

In previous work we have found that Cp_2TiCl_2 and its corresponding derivative of tamoxifen, Titanocene tamoxifen, show an unexpected proliferative effect on hormone dependent breast cancer cells MCF-7. In order to check if this behavior is a general trend for titanocene derivatives we have tested two other titanocene derivatives, Titanocene Y and Titanocene K, on this cell line. Interestingly, these two titanocene complexes behave in a totally different manner. Titanocene K is highly proliferative on MCF-7 cells even at low concentrations (0.5 μM), thus behave almost similarly to Cp_2TiCl_2. This proliferative effect is also observed in the presence of bovine serum albumin (BSA). In contrast, Titanocene Y alone has almost no effect on MCF-7 at a concentration of 10 μM, but exhibits a significant dose dependent cytotoxic effect of up to 50% when incubated with BSA (20–50 μg/mL). This confirms the crucial role played by the binding to serum proteins in the expression of the *in vivo*, cytotoxicity of the titanocene complexes. From the hydridolithiation reaction of 6-*p*-anisylfulvene with $LiBEt_3H$ followed by transmetallation with iron dichloride [bis-[(*p*-methoxy-benzyl)cyclopentadienyl]iron(II)] (Ferrocene Y) was synthesised. This complex, which was characterised by single crystal X-ray diffraction, contains the robust ferrocenyl unit instead of Ti associated with easily leaving groups such as chlorine and shows only a modest cytotoxicity against MCF-7 or MDA-MB-231 cells.

1. Introduction

Beyond the field of platinum and ruthenium anti-cancer drugs there is significant unexplored space for further metal-based drugs targeting cancer. Titanium-based reagents have significant potential against solid tumors. Budotitane ([*cis*-diethoxybis(1-phenylbutane-1,3-dionato)titanium (IV)]) (Chart 1) looked very promising during its preclinical evaluation, but did not go beyond Phase I clinical trials, although a Cremophor EL® based formulation was found for this rapidly hydrolysing molecule [1]. Much more robust in this aspect of hydrolysis is titanocene dichloride (Cp_2TiCl_2), which shows medium anti-proliferative activity *in vitro* but promising results *in vivo* [2,3]. Titanocene dichloride reached clinical trials, but its efficacy in Phase II clinical trials in patients with metastatic renal cell carcinoma [4] or metastatic breast cancer [5] was too low to be pursued.

The field gained renewed interest with an elegant synthesis of ring-substituted cationic titanocene dichloride derivatives developed by McGowan and co-workers, which produced water-soluble compounds showing significant activity against ovarian cancer [6]. More recently, novel methods starting from fulvenes and other precursors [7,8] allow direct access to anti-proliferative titanocenes via reductive dimerisation with titanium dichloride [9–13], hydridolithiation [14–17] or carbolithiation [18–26] of the fulvene followed by transmetallation with titanium tetrachloride in the latter two cases.

Hydridolithiation of 6-anisyl fulvene and subsequent reaction with $TiCl_4$ led to bis-[(*p*-methoxybenzyl)cyclopentadienyl] titanium(IV) dichloride (Titanocene Y) [17], which has an IC_{50} value of 21 μM when tested on the LLC-PK cell line, which has proven to be a good mimic of a kidney carcinoma cell line and a reliable tool for the optimisation of titanocenes against this type of cancer.

In addition, the anti-proliferative activity of Titanocene Y and other titanocenes has been studied in 36 human tumor cell lines [27] and against explanted human tumors [28,29]. These *in vitro* and *ex vivo* experiments showed that renal cell cancer is the prime target for this novel class of titanocenes, but there is significant activity against ovary, prostate, cervix, lung, colon, and breast cancer as well. These results were underlined by first mechanistic

* Corresponding author. Tel.: +33 1 44 27 67 29; fax: +33 1 43 26 00 61.
E-mail address: a-vessieres@enscp.fr (A. Vessières).

0022-328X/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jorganchem.2008.11.071

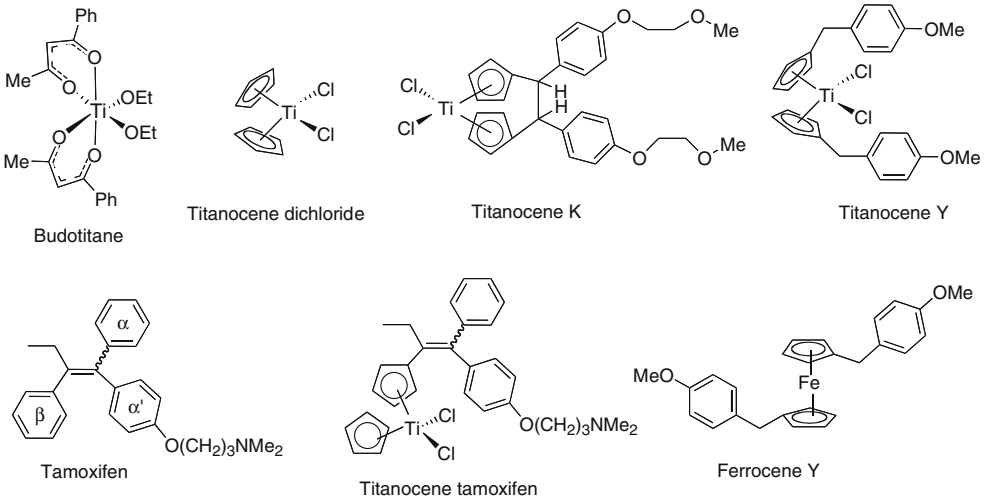


Chart 1. Structure of the molecules of reference and of the molecules under study.

studies concerning the effect of these titanocenes on apoptosis and the apoptotic pathway in prostate cancer cells [30]. Furthermore, it was showed, that titanocene derivatives give a positive immune response by up-regulating the number of natural killer (NK) cells in mice [31]. Recently, animal studies reported the successful treatment of mice bearing xenografted Caki-1 and MCF-7 tumors with Titanocene Y [32,33].

Some time ago Jaouen and co-workers reported the unexpected strong proliferative effect on hormone dependent breast cancer cells (MCF-7) of the titanocene derivative of tamoxifen (Titanocene tamoxifen; Chart 1) [34]. In addition we also found that Cp_2TiCl_2 alone showed a strong proliferative effect even at the very low concentration of 10 nM. The only plausible explanation for this behavior is that these titanocene complexes act as estrogens and this hypothesis was confirmed on MVLN cells, another hormone dependent cell line in which expression of luciferase is proportional to the estrogenicity of the tested compounds. The mechanism underlying this effect could be the generation in the cell of Ti(IV), which could be able to mimic the interaction of estradiol with its specific receptor. More recently, Tacke and co-workers observed a proliferative effect of Titanocene K on LLC-PK cells but only at high concentrations (>10 μM) [14]. Therefore, we thought that it could be of interest to check the behavior of these organometallic titanium complexes Titanocene K and Titanocene Y on hormone dependent breast cancer cell line. It has also been demonstrated that following administration in blood, titanocene derivatives rapidly loose their chloride groups and bind to serum proteins [35,36]. This encouraged us to study a possible stabilisation of the complexes, *in vitro*, via their binding to serum albumin. Finally as some of us have described the cytotoxicity of a wide range of ferrocenyl derivatives [37–41] we decided to prepare and fully characterise Ferrocene Y, the ferrocenyl analog of Titanocene Y, and to study its effect on breast cancer cell lines.

2. Experimental

2.1. General conditions

Anhydrous iron dichloride and Super Hydride ($LiBEt_3H$, 1.0 M solution in THF) were obtained from Aldrich Chemical Company and used without further purification. Pentane, diethyl ether and THF were dried over Na and benzophenone (pentane: + di(ethylene-glycol)ethyl-ether) and they were freshly distilled and collected under an atmosphere of nitrogen prior to use. Manipulations of air and moisture sensitive compounds were done using standard Schlenk techniques, under a nitrogen atmosphere. NMR spectra were measured on either a Varian 300 or a 400 MHz spectrometer. Chemical shifts are reported in ppm and are referenced to TMS. IR spectra were recorded on a Perkin–Elmer Paragon 1000 FT-IR Spectrometer employing a KBr disk or a liquid IR cell. UV–Vis spectra were recorded on a Unicam UV4 Spectrometer, while CHN analysis was done with an Exeter Analytical CE-440 Elemental Analyser. Molecular modeling calculations were carried out at the PM3 level for the optimisation of the titanocene using the program package HyperChem [42] and for the albumin-Titanocene Y conjugate using MOLVIEW [43]. X-ray diffraction data for compound **3** were collected using a Bruker SMART APEX CCD area detector diffractometer. A full sphere of reciprocal space was scanned by phi–omega scans. Pseudo-empirical absorption correction based on redundant reflections was performed by the program SADABS [44]. The structures were solved by direct methods using SHELXS-97 [45] and refined by full-matrix least-squares on F^2 for all data using SHELXL-97. All hydrogen atoms were located in the difference fourier map and allowed to refine freely. Anisotropic thermal displacement parameters were used for all non-hydrogen atoms. Further details about the data collection are listed in Table. 1, as well as reliability factors. Suitable crystals of **3** were grown from a saturated trichloromethane solution by slow evaporation.

2.2. Synthesis

Titanocene Y and Titanocene K ({1,2-di(cyclopentadienyl)-1,2-di[4-(2-methoxyethoxy)phenyl]ethanediyl} titanium dichloride; with *cis–trans* ratio at the carbon–carbon bridge of 44:56) were synthesised as described in [17,14] while 6-*p*-anisylfulvene was synthesised according to the literature method [13].

2.2.1. Synthesis of bis-[(p-methoxybenzyl)cyclopentadienyl]iron(II) (Ferrocene Y)

Sixteen microlitres of Superhydride solution (13 mmol, 14.27 g, 1.6 mL in THF) were heated under vacuum for 45 min at 60 °C and 30 min at 90 °C to remove most of the THF. The concentrated reagent was re-dissolved in 75 mL of diethyl ether. 2.4 g 6-*p*-anisylfulvene (13 mmol) were dissolved in 25 mL diethyl ether and was added to the Superhydride solution *via cannula* during 5 min and left to stir for 4 h. The colour of the solution changed during this time from orange to pale yellow, while the insoluble lithium cyclopentadienide intermediate precipitated from the solution. The

Table 1
Crystallographic refinement data for **3**.

Empirical formula	$C_{26}H_{26}O_2Fe$
Formula weight	426.32
Temperature (K)	293(2)
Crystal system	Monoclinic
Space group	$P2_1/n$ (#14)
Unit cell dimensions	
a (Å)	11.8726(12)
b (Å)	13.9370(14)
c (Å)	12.5593(13)
β (°)	93.509(2)
V (Å^3)	2074.3(4)
Z	4
D_{calc} (Mg/m^3)	1.365
Absorption coefficient (mm^{-1})	0.746
$F(000)$	896
Crystal size (mm^3)	0.50 × 0.20 × 0.15
Theta range for data collection (°)	2.19–26.41
Index ranges	$-14 \leqslant h \leqslant 14$, $-17 \leqslant k \leqslant 17$, $-15 \leqslant l \leqslant 15$
Reflections collected	18110
Independent reflections [R_{int}]	4255 [0.0266]
Goodness-of-fit (GOF) on F^2	1.044
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0342$, $wR_2 = 0.0825$
R indices (all data)	$R_1 = 0.0427$, $wR_2 = 0.0872$
Largest difference peak and hole (e Å^{-3})	0.367 and −0.213

solution was filtered through a Schlenk glass frit under N_2 atmosphere and after washing with small portions of diethyl ether followed by pentane, a white solid was dried *in vacuo* (1.1 g, 5.7 mmol, 44% yield).

The white solid was transferred into a Schlenk flask and dissolved in 25 mL THF and added to a solution of 0.36 g iron dichloride (2.85 mmol, in 50 mL THF) *via cannula* during 5 min. The dark brown solution was refluxed for 16 h. The solution was cooled down and the solvent was removed *in vacuo*. The remaining residue was extracted with dichloromethane and filtered through celite in a Büchner funnel to remove the LiCl. The brown filtrate was filtered again by gravity filtration and the solvent removed *in vacuo* to obtain a yellow solid (1.0 g, 82.1% yield). ^{1}H NMR: (δ in ppm, 300 MHz, $CDCl_3$): 7.08 (d, 2H, J = 8.6 Hz, C_6H_4); 6.80 (d, J = 8.6 Hz, 2H, C_6H_4); 4.01 (d, 4H, J = 1.8 Hz, C_5H_4); 3.80 (s, 3H, C_6H_4–OCH_3); 3.59 (s, 2H, C_5H_4–CH_2). ^{13}C NMR (δ in ppm, 100 MHz, $CDCl_3$, proton decoupled): 158.0 (C_i–OCH_3); 134.1 (C_5H_4–CH_2–C_i); 129.5, 113.9 (*C*H, Ph); 88.7 ($MeOC_6H_4$–CH_2–C_i), 69.5, 68.5 (*C*H, Cp); 55.5 (OCH_3); 35.2 (CH_2). Anal. Calc. for $FeC_{26}O_2H_{26}$: C: 73.3; H, 6.2. Found: C, 73.5; H, 6.4%.

2.3. Cell studies

2.3.1. Materials

Fresh stock solutions (1×10^{-3} M) of the compounds to be tested were prepared for each set of experiments in DMSO. Serial dilutions in DMSO were prepared just prior to use. Dulbecco's modified eagle medium (DMEM) was purchased from Gibco BRL, fetal calf serum from Dutscher, Brumath, France, glutamine, estradiol and protamine sulfate and BSA were from Sigma. MCF-7 and MDA-MB-231 cells were from the Human Tumor Cell Bank.

2.3.2. Culture conditions

Cells were maintained in monolayer in DMEM with phenol red (Gibco BRL) supplemented with 8–9% fetal calf serum (Gibco BRL) and glutamine 2 mM (Sigma) at 37 °C in a 5% CO_2 air humidified incubator. For proliferation assays, cells were plated in 24-well sterile plates at a density of 1.1×10^4 cells for MDA-MB-231 and of 3×10^4 cells for MCF-7 in 1 mL of DMEM medium without phenol red, supplemented with 10% decomplemented and hormone-depleted fetal calf serum and 2 mM glutamine and incubated. The following day (D0), 1 mL of the same medium containing the compounds to be tested was added to the plates (final volumes of DMSO: 0.1%; four wells for each condition). After 3 days (D3) the incubation medium was removed and fresh medium containing the compounds was added. After 5 days (D5) the total protein content of the plate was analysed by methylene blue staining as follows. Cell monolayers were fixed for 1 h in methanol, stained for 1 h with methylene blue (1 mg/mL) in PBS, and then washed thoroughly with water. One millilitre of HCl (0.1 M) was then added and the absorbance of each well was measured at 620 nm with a Bio-Rad spectrophotometer. The results are expressed as the percentage of proteins vs. the control.

2.4. Molecular modeling studies

In order to test the possibility of albumin interacting with Titanocene Y it was decided to perform preliminary docking studies by using the crystal structure of human serum albumin (HSA) co-crystallised with medium-sized fatty acids [46]. The pdb file of this crystal structure was used as a starting point for MOLVIEW [43] and a suitably looking palmitate ligand, which is around 1.6 nm in length, was removed to create a possible free site for Titanocene Y. Titanocene Y was modelled in an elongated confirmation exhibiting a length of 1.8 nm using HyperChem [42] at the PM3 level of theory.

2.5. Determination of the relative binding affinity (RBA) for estrogen receptor alpha (ERα)

RBA values were measured on ERα from lamb uterine cytosol. Sheep uterine cytosol prepared in buffer A (0.05 M Tris–HCL, 0.25 M sucrose, 0.1% β-mercaptoethanol, pH 7.4 at 25 °C) as described previously [40] was used as a source of ERα. Aliquots (200 mL) of ERα were incubated for 3 h at 0 °C with [6,7-^{3}H]-estradiol (2×10^{-9} M, specific activity 1.62 TBq/mmol, NEN Life Science, Boston, MA) in the presence of nine concentrations of the hormones to be tested. At the end of the incubation period, the free and bound fractions of the tracer were separated by protamine sulfate precipitation. The percentage reduction in binding of [^{3}H]-estradiol (Y) was calculated using the logit transformation of Y (logit$Y$: $\ln[Y/1-Y]$ vs. the log of the mass of the competing steroid. The concentration of unlabeled steroid required for displacing of 50% of the bound [^{3}H]-estradiol was calculated for each steroid tested, and the results expressed as RBA. The RBA value of estradiol is by definition equal to 100%.

3. Results and discussion

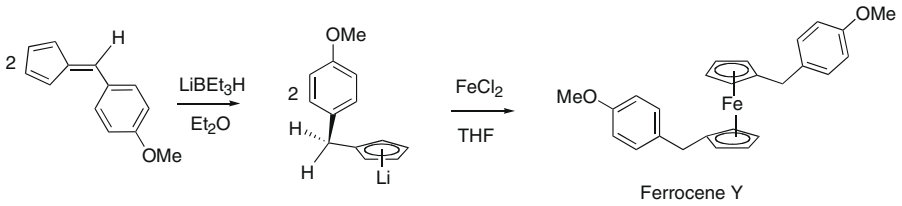
3.1. Chemical studies

3.1.1. Synthesis

3.1.1.1. Synthesis of Ferrocene Y. The hydridolithiation reaction of 6-aryl substituted fulvenes followed by transmetallation of titanium tetrachloride could successfully be transferred to iron(II). Two equivalents of this lithium cyclopentadienide intermediate was transmetallated to 1 equiv. of iron dichloride resulting in the formation of 1 equiv. of the required benzyl-substituted Ferrocene Y in a yield of 82% and 2 equiv. of the by-product of lithium chloride following a 16 h reflux. The synthesis is shown in Scheme 1.

3.1.2. Structural discussion

The molecular structure of Ferrocene Y is determined by X-ray diffraction and shown in Fig. 1.



Scheme 1. Structure and synthesis of Ferrocene Y.

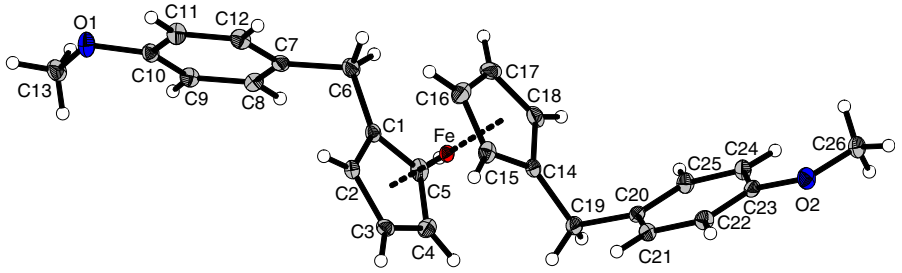


Fig. 1. Molecular structure of Ferrocene Y (thermal ellipsoids are drawn on the 15% probability level).

Ferrocene Y crystallises in the monoclinic space group $P2_1/n$ with one molecule in the asymmetric unit. Details of the refinement are shown in Table 1. The molecules exhibit an overall *Z*-like shape in the solid state and form an efficient packing without solvent molecules. The Fe–centroid distance is 1.648 Å and compares well with distance seen in other ferrocene derivatives, e.g. ferrocene itself with 1.66 Å. Also the centroid–Fe–centroid angle of 178.7° is as close to linear as in other ferrocenes. This forces the molecule into the above-mentioned *Z* shape. All other structural features are very similar indeed to the ones in Titanocene Y.

3.2. Biochemical studies

3.2.1. Determination of relative binding affinity of the compounds for the estrogen receptor (ERα)

The relative binding affinity (RBA) values found for Titanocene Y and Titanocene K are very low, 0.001% and 0.006%, respectively, and even equal to zero for Ferrocene Y. This is not very surprising, as the chemical structure of these molecules does not mimic at all that of estradiol, the natural ligand. An RBA value of zero was also found previously for Cp_2TiCl_2 [34]. It is important to notice that these measurements were performed at 4 °C after an incubation period of 2 h i.e. in experimental conditions totally different to those of cell cultures (37 °C, 5 days).

3.2.2. Effect of Titanocene Y and Titanocene K on the growth of MCF-7 cells

The effect of the two titanium complexes was tested on the growth of hormone-dependent breast cancer cells MCF-7, which are estrogen receptor positive (ER+) cancer cells (Figs. 2 and 3). The proliferative estrogenic effect of estradiol on these cells is known to be mediated by its interaction with this specific receptor.

The results obtained on the growth of MCF-7 cells with various concentration of Titanocene K alone (0.5–5 μM) and with 10 μM of Titanocene K incubated in the presence of various amount of BSA (20–50 μg/mL) are shown in Fig. 2.

As expected, estradiol shows a strong proliferative effect on this cell line, which possesses estrogen receptor and BSA alone has no effect. On this cell line Titanocene K has a significant proliferative effect (55–80% of the effect of estradiol) and the addition of BSA does not induce any significant change. This complex exhibits, at low concentration, an estrogenic effect similar to that previously found for Cp_2TiCl_2 and Titanocene tamoxifen [34], although it is a little bit less potent. As the RBA value of this compound is very low we can also suggest that this estrogenic effect is connected to the generation of *in situ* Ti(IV). Eventually, its behavior on MCF-7 cells differs from that observed previously on LLC-PK [14].

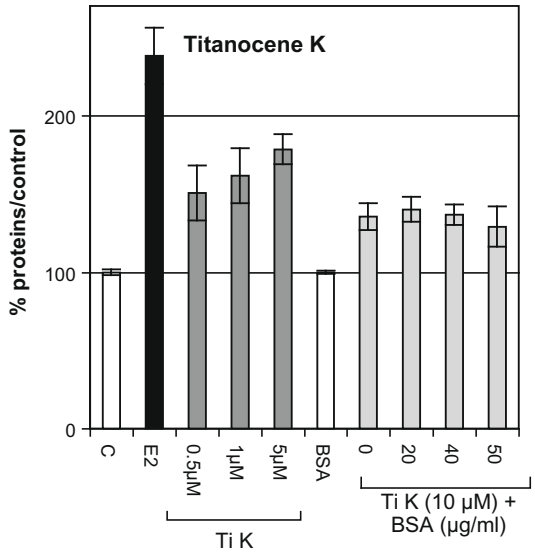


Fig. 2. Effect of 1 nM of estradiol (E_2), of various concentrations of Titanocene K and of 10 μM of Titanocene K incubated alone or in the presence of various concentration of bovine serum albumin (BSA; 20–50 μg/mL) on the growth of MCF-7 cells (hormone-dependent breast cancer cells) after 5 days of culture. Comparison with the control (C) set at 100%. Mean of at least two independent experiments (six measurements for each one) ± mean.

The results obtained on MCF-7 with the second complex, Titanocene Y are shown in Fig. 3. This complex behaves in a totally different way than Titanocene K. It does not exhibit a significant proliferative effect at 10 μM and interestingly incubation in the presence of BSA (20–50 μg/mL) induces a significant anti-proliferative effect. For example a 50% decrease of the growth (i.e. the IC_{50} value) is obtained after incubation of 10 μM of the complex with 50 μg/mL of BSA. An IC_{50} value of 21 μM has been found previously

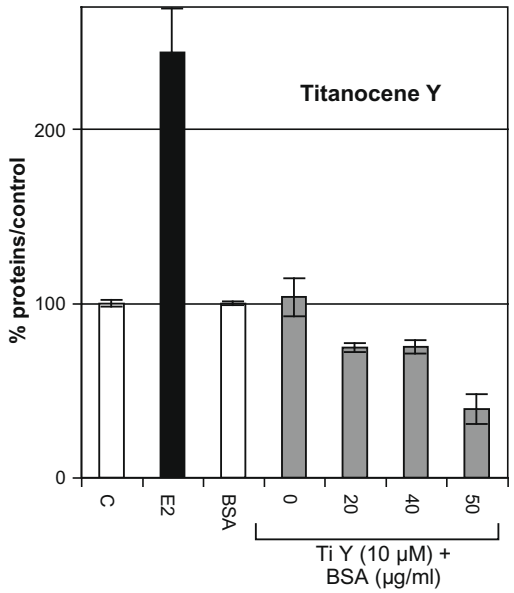


Fig. 3. Effect of 1 nM of estradiol (E_2), of 10 μM of Titanocene Y incubated alone or in the presence of various concentration of bovine serum albumin (BSA; 20–50 μg/mL) on the growth of MCF-7 cells (hormone-dependent breast cancer cells) after 5 days of culture. Comparison with the control (C) set at 100%. Mean of at least two independent experiments (six measurements for each one) ± range.

for this compound alone on LLC-PK cells [17]. Our results indicate that the cytotoxicity of the complex is significantly enhanced by its binding to albumin. Interestingly, Titanocene Y alone has no estrogenic effect suggesting that this molecule is more stable to total hydrolysis than Titanocene K.

3.2.3. Molecular modeling studies of Titanocene Y in the free cavity of human serum albumin (HSA)

Cancer cells are known to be leaky for albumin and therefore one possible strategy for the targeted delivery of anti-cancer drugs are drug–albumin conjugates. The preliminary docking studies of Titanocene Y using a free cavity in HSA resulted in a surprisingly well-fitting conjugate despite the slight difference in length between the palmitate, the ligand originally in the structure and Titanocene Y in its elongated form (1.6 nm vs. 1.8 nm). In addition, the bulky Cp_2TiCl_2 unit of Titanocene Y gave no steric problems. The most striking feature of the interaction is the hydrogen bond formed by O1 of Titanocene Y with Ser 285 of albumin for which a distant of 3.10 Å is observed between the involved oxygen atoms. In addition O1 shows a short interaction with Ala 256 of 3.70 Å as well. O2 of Titanocene Y interacts with Ph–Ala 68 at 2.87 Å and with Arg 255 of the albumin backbone at 3.77 Å. Further van der Waals interactions are seen from the chlorides of Titanocene Y: both of them approach Leu 20 and Ala 149 by just under 3.0 Å and interact with Pro 150 at a distance of just over 3.0 Å. Fig. 4 shows the area of interest, in which Titanocene Y is found to bind to albumin. Overall, it can be summarised that Titanocene Y fits well into the albumin cavity and forms a relatively weak and obviously reversible complex, which is likely to be carried into the cancer cell and released there. This result could also explain the protective role played by BSA in the *in vitro* test on MCF-7 cells.

In future experiments, systematic molecular modeling studies in combination with NMR spectroscopic experiments will reveal the exact binding site of Titanocene Y in HSA and will hopefully give access to the binding energy.

3.2.4. Effect of Ferrocene Y on the growth of MCF-7 and MDA-MB-231 cells

The effect of Ferrocene Y has been tested on the growth of MCF-7 and MDA-MB-231, the two breast cancer cells lines which are routinely used to study the estrogenicity (MCF-7) and the cytotoxicity (MDA-MB-231) of the ferrocenyl derivatives [39]. On MCF-7 cells Ferrocene Y shows no proliferative effect at 1 μM and becomes slightly cytotoxic at 10 μM with an anti-proliferative effect of 18% at this concentration. The absence of estrogenicity of this molecule is expected has it lacks a phenol ring that is considered to be essential for the binding of a molecule to the estrogen receptor. Not surprisingly also it is unable to displace estradiol from its specific receptor (RBA = 0). The IC_{50} value found for this compound on MDA-MB-231 cells is 95 μM. So this value is a little bit lower than the value found for ferrocene alone (IC_{50} = 160 μM) but it is much higher than the value found for Ferrocifen, the ferrocenyl

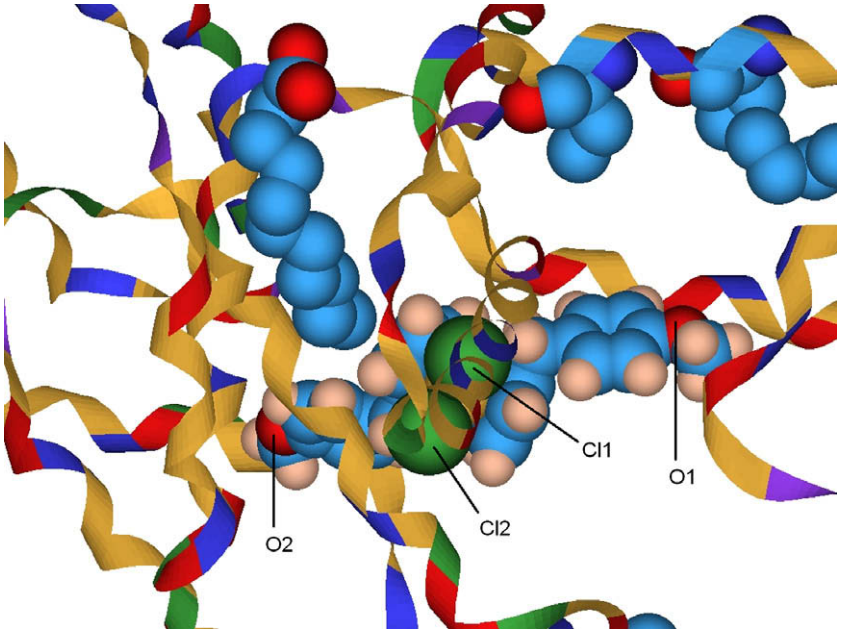


Fig. 4. Plot of the relevant structural part of the albumin–Titanocene Y conjugate obtained by molecular modeling using MOLVIEW.

analog of Tamoxifen, which has an IC_{50} value of 0.5 μM [39]. This result could easily be explain by the fact that we have demonstrated that this cytotoxicity is connected to the oxidative formation of quinoid species [47]. Ferrocene Y possessing methoxy rather than hydroxy groups and a non-conjugated system cannot undergo this transformation [48]. Then, one can predict that it will behave like ferrocene and this is the experimental observation.

4. Conclusion

The *in vitro* study of the cytotoxicity of three metallocenes on the hormone dependent breast cancer cell line MCF-7 shows three different behaviors. Titanocene K shows, at concentrations as low as 0.5 μM, an estrogenic effect similar to that observed with Cp_2TiCl_2 and this effect is not changed by the addition of albumin. By contrast, Titanocene Y alone at 10 μM has almost no effect on the growth of these cells but it becomes cytotoxic in the presence of albumin. Finally, Ferrocene Y is only slightly cytotoxic at the same concentration. These results underline the interest of *in vitro* study of potential drugs in the presence of serum proteins and might lead to an albumin-based formulation of Titanocene Y for further xenograft experiments.

5. Supplementary material

CCDC 669818 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Acknowledgments

We thank A. Cordaville for technical assistance and COST D39 for support. M.T. thanks the Université Paris 6 for a 1-month professorship.

References

[1] T. Schilling, B.K. Keppler, M.E. Heim, G. Niebch, H. Dietzfelbinger, J. Rastetter, A.R. Hanauske, Invest. New Drugs 13 (1996) 327.
[2] F. Caruso, M. Rossi, Met. Ions Biol. Syst. 42 (2004) 353.
[3] E. Melendez, Crit. Rev. Oncol. Hematol. 42 (2002) 309.
[4] G. Lummen, H. Sperling, H. Luboldt, T. Otto, H. Rubben, Cancer Chemother. Pharmacol. 42 (1998) 415.
[5] N. Kröger, U.R. Kleeberg, K. Mross, L. Edler, D.K. Hossfeld, Onkologie 23 (2000) 60.
[6] O.R. Allen, L. Croll, A.L. Gott, R.J. Knox, P.C. McGowan, Organometallics 23 (2004) 288.
[7] P.W. Causey, M.C. Baird, S.P.C. Cole, Organometallics 23 (2004) 4486.
[8] R. Meyer, S. Brink, C.E.J. van Rensburg, G.K. Joone, H. Gorls, S. Lotz, J. Organomet. Chem. 690 (2005) 117.
[9] S. Fox, J.P. Dunne, M. Tacke, J.F. Gallagher, Inorg. Chim. Acta 357 (2004) 225.
[10] F.-J.K. Rehmann, L.P. Cuffe, O. Mendoza, D.K. Rai, N. Sweeney, K. Strohfeldt, W.M. Gallagher, M. Tacke, Appl. Organomet. Chem. 19 (2005) 293.
[11] F.-J.K. Rehmann, A.J. Rous, O. Mendoza, N.J. Sweeney, K. Strohfeldt, W.M. Gallagher, M. Tacke, Polyhedron 24 (2005) 1250.
[12] M. Tacke, L.T. Allen, L. Cuffe, W.M. Gallagher, Y. Lou, O. Mendoza, H. Müller-Bunz, F.J.K. Rehmann, N. Sweeney, J. Organomet. Chem. 689 (2004) 2242.
[13] M. Tacke, L.P. Cuffe, W.M. Gallagher, Y. Lou, O. Mendoza, H. Müller-Bunz, F.-J.K. Rehmann, N. Sweeney, J. Inorg. Biochem. 98 (2004) 1987.
[14] K. Strohfeldt, H. Müller-Bunz, C. Pampillon, N.J. Sweeney, M. Tacke, Eur. J. Inorg. Chem. (2006) 4621.
[15] N. Sweeney, W.M. Gallagher, H. Müller-Bunz, C. Pampillon, K. Strohfeldt, M. Tacke, J. Inorg. Biochem. 100 (2006) 1479.
[16] N.J. Sweeney, J. Claffey, H. Müller-Bunz, C. Pampillon, K. Strohfeldt, M. Tacke, Appl. Organomet. Chem. 21 (2007) 57.
[17] N.J. Sweeney, O. Mendoza, H. Müller-Bunz, C. Pampillon, F.-J.K. Rehmann, K. Strohfeldt, M. Tacke, J. Organomet. Chem. 690 (2005) 4537.
[18] T. Hickey, J. Claffey, E. Fitzpatrick, M. Hogan, C. Pampillon, M. Tacke, Invest. New Drugs 25 (2007) 425.
[19] M. Hogan, J. Claffey, C. Pampillon, R.W.G. Watson, M. Tacke, Organometallics 26 (2007) 2501.
[20] C. Pampillon, J. Claffey, M. Hogan, K. Strohfeldt, M. Tacke, Trans. Met. Chem. 32 (2007) 434.
[21] C. Pampillon, J. Claffey, M. Hogan, M. Tacke, Z. Anorg. Allg. Chem. 633 (2007) 1695.
[22] C. Pampillon, J. Claffey, M. Hogan, M. Tacke, BioMetals 21 (2008) 197.
[23] C. Pampillon, J. Claffey, K. Strohfeldt, M. Tacke, Eur. J. Med. Chem. 43 (2008) 122.
[24] C. Pampillon, O. Mendoza, N.J. Sweeney, K. Strohfeldt, M. Tacke, Polyhedron 25 (2006) 2101.
[25] C. Pampillon, N.J. Sweeney, K. Strohfeldt, M. Tacke, Inorg. Chim. Acta 359 (2006) 3969.
[26] C. Pampillon, N.J. Sweeney, K. Strohfeldt, M. Tacke, J. Organomet. Chem. 692 (2007) 2153.
[27] G. Kelter, N.J. Sweeney, K. Strohfeldt, H.-H. Fiebig, M. Tacke, Anti-Cancer Drugs 16 (2005) 1091.
[28] O. Oberschmidt, A.-R. Hanauske, F.-J.K. Rehmann, K. Strohfeldt, N. Sweeney, M. Tacke, Anti-Cancer Drugs 16 (2005) 1071.
[29] O. Oberschmidt, A.R. Hanauske, C. Pampillon, N.J. Sweeney, K. Strohfeldt, M. Tacke, Anti-Cancer Drugs 18 (2007) 317.
[30] K. O'Connor, C. Gill, M. Tacke, F.-J.K. Rehmann, K. Strohfeldt, N. Sweeney, J.M. Fitzpatrick, R.W.G. Watson, Apoptosis 11 (2006) 1205.
[31] M.C. Valadares, A.L. Ramos, F.-J.K. Rehmann, N.J. Sweeney, K. Strohfeldt, M. Tacke, M.L.S. Queiroz, Eur. J. Pharmacol. 534 (2006) 264.
[32] P. Beckhove, O. Oberschmidt, A.R. Hanauske, C. Pampillon, V. Schirrmacher, N.J. Sweeney, K. Strohfeldt, M. Tacke, Anti-Cancer Drugs 18 (2007) 311.
[33] I. Fichtner, C. Pampillon, N.J. Sweeney, K. Strohfeldt, M. Tacke, Anti-Cancer Drugs 17 (2006) 333.
[34] S. Top, E.B. Kaloun, A. Vessières, I. Laïos, G. Leclercq, G. Jaouen, J. Organomet. Chem. 643–644 (2002) 350.
[35] A.R. Timerbaev, C.G. Hartinger, S. Aleksenko, B.K. Keppler, Chem. Rev. 106 (2006) 2224.
[36] M. Ravera, C. Cassino, E. Monti, M. Gariboldi, D. Osella, J. Inorg. Biochem. 99 (2005) 2264.
[37] G. Jaouen, S. Top, A. Vessières, G. Leclercq, M.J. McGlinchey, Curr. Med. Chem. 11 (2004) 2505.
[38] G. Jaouen, S. Top, A. Vessières, G. Leclercq, J. Quivy, L. Jin, A. Croisy, C.R. Acad. Sci. Paris Sér. IIc (2000) 89.
[39] A. Nguyen, A. Vessieres, E.A. Hillard, S. Top, P. Pigeon, G. Jaouen, Chimia 61 (2007) 761.
[40] S. Top, A. Vessières, G. Leclercq, J. Quivy, J. Tang, J. Vaissermann, M. Huché, G. Jaouen, Chem. Eur. J. 9 (2003) 5223.
[41] A. Vessières, S. Top, P. Pigeon, E.A. Hillard, L. Boubeker, D. Spera, G. Jaouen, J. Med. Chem. 48 (2005) 3937.
[42] HyperChem 7.0, Hypercube, Alberta, Canada, 2002.
[43] J.M. Cense, Phys. Theor. Chem. 71 (1990) 763.
[44] G.M. Sheldrick, University of Göttingen, Germany, 2002.
[45] G.M. Sheldrick, University of Göttingen, Germany, 1997.
[46] A.A. Bhattacharya, T. Grune, S. Curry, J. Mol. Biol. 303 (2000) 721.
[47] E.A. Hillard, A. Vessières, L. Thouin, G. Jaouen, C. Amatore, Angew. Chem., Int. Ed. 45 (2006) 285.
[48] E.A. Hillard, A. Vessières, F. Le Bideau, D. Plazuk, D. Spera, M. Huché, G. Jaouen, ChemMedChem 1 (2006) 551.