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

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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

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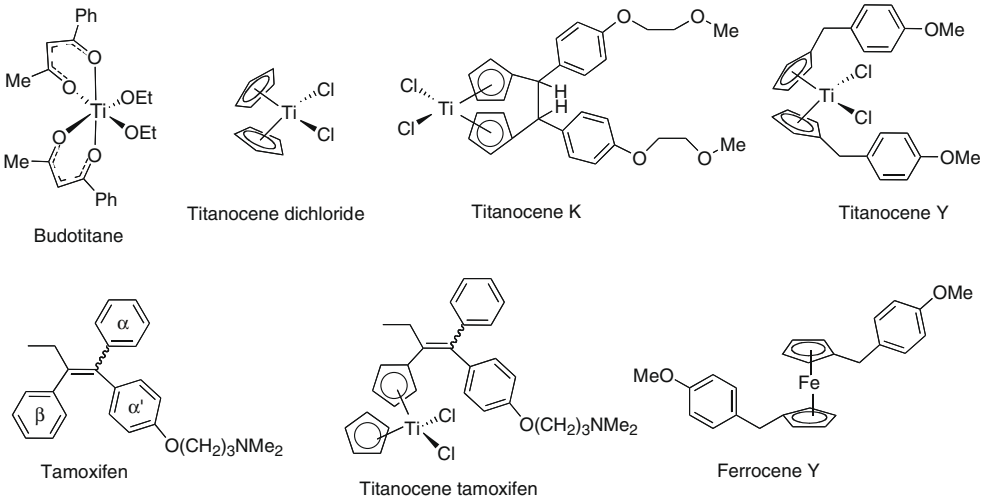

Chart 1. Structure of the molecules of reference and of the molecules under study.

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