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Synthesis and cytotoxicity studies of methoxy benzyl substituted titanocenes

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Abstract

From the reaction of 6(2-methoxy-phenyl)fulvene (**1a**), 6(3-methoxy-phenyl)fulvene (**1b**), 6(3,4-dimethoxy-phenyl)fulvene (**1c**) and 6(3,4,5-trimethoxy-phenyl)fulvene (**1d**) with $LiBEt_3H$, lithiated cyclopentadienide intermediates **2a–d** were synthesised. These intermediates were then transmetallated to titanium with $TiCl_4$ to give benzyl substituted titanocenes bis-[(2-methoxy-benzyl)cyclopentadienyl]titanium(IV) dichloride (**3a**), bis-[(3-methoxy-benzyl)cyclopentadienyl]titanium(IV) dichloride (**3b**), bis-[(3,4-dimethoxy-benzyl)cyclopentadienyl]titanium(IV) dichloride (**3c**) and bis-[(3,4,5-trimethoxy-benzyl)cyclopentadienyl]titanium(IV) dichloride (**3d**). The three titanocenes **3a–c** were characterised by single crystal X-ray diffraction, while the structure of the fourth titanocene **3d** was elucidated through a DFT calculation. All four titanocenes had their cytotoxicity investigated through preliminary *in vitro* testing on the LLC-PK (pig kidney epithelial) cell line in order to determine their IC_{50} values. Titanocenes **3a–d** were found to have IC_{50} values of 97, 159, 88 and 253 μM, respectively. All four titanocene derivatives show significant cytotoxicity improvement when compared to unsubstituted titanocene dichloride.

Keywords: Anti-cancer drugs; Cisplatin; Titanocene; Hydridolithiation; Fulvene; RCC; LLC-PK

1. Introduction

Beyond the field of platinum 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*-diethoxy-bis(1-phenylbutane-1,3-dionato)titanium(IV)]) 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 the efficacy of Cp_2TiCl_2 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 got renewed interest with P. McGowan's elegant synthesis of ring-substituted cationic titanocene dichloride derivatives, which are water-soluble and show significant activity against ovarian cancer [6]. More recently, novel methods starting from fulvenes and other precursors [7,8] allow direct access to antiproliferative 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**) [14], 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. The structures of

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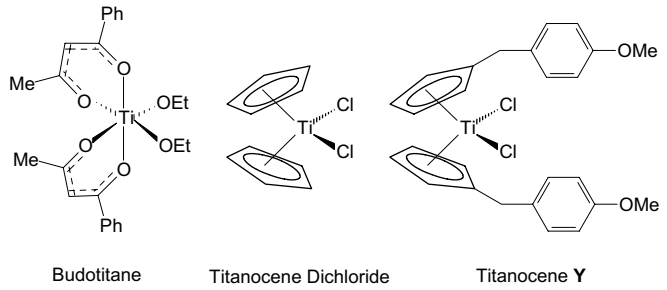

Fig. 1. Structures of Budotitane, Titanocene Dichloride and Titanocene Y.

Budotitane, Titanocene Dichloride and Titanocene **Y** are shown in Fig. 1.

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 studies concerning the effect of these titanocenes on apoptosis and the apoptotic pathway in prostate cancer cells [30]. Furthermore, it was shown, 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].

Within this paper we present the synthesis and preliminary cytotoxicity studies of a series of four derivatives of Titanocene **Y**.

2. Experimental

2.1. General conditions

Titanium tetrachloride (1.0 M solution in toluene), Super Hydride ($LiBEt_3H$, 1.0 M solution in THF) and the benzaldehyde derivatives were obtained from Aldrich Chemical Company and used without further purification. Diethyl ether and THF were dried over Na and benzophenone and they were freshly distilled and collected under an atmosphere of nitrogen prior to use. Pentane was dried over sodium, benzophenone and di(ethlene-glycol)ethyl-ether and it was 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. CHN analysis was done with an Exeter Analytical CE-440 Elemental Analyser, while Cl was determined in mercurimetric titrations. Density functional theory (DFT) calculations were carried out for titanocene **3d** at the B3LYP level using the 6-31G** basis set implemented in the *ab-initio* programme package GAUSSIAN 03 [34]. X-ray diffraction data for the compounds **3a**, **3b** and **3c** 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 [35]. The structures were solved by direct methods using SHELXS-97 [36] and refined by full matrix least-squares on F^2 for all data using SHELXL-97 [36]. In **3a** all hydrogen atoms were located in the difference fourier map and allowed to refine freely. In **3b** and **3c** hydrogen atoms were added at calculated positions and refined using a riding model. Their isotropic temperature factors were fixed to 1.2 times (1.5 times for methyl groups) the equivalent isotropic displacement parameters of its parent atom. 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 **3a** were grown from a saturated dichloromethane solution, crystals of **3b** were grown in saturated dichloromethane solution with slow infusion of pentane and **3c** formed crystals from slow evaporation of a saturated trichloromethane solution.

2.2. Synthesis

Fulvene **1c** was synthesised according to previously used literature method [12].

2.2.1. Synthesis of 6(2-methoxy-phenyl) fulvene, C_5H_4–CH–C_6H_4–OCH_3 (*1a*)

3.02 g (22.5 mmol) of 2-methoxy-benzaldehyde was dissolved in 100 ml of methanol to give a colourless solution. 4.00 ml (48.5 mmol) of freshly cracked cyclopentadiene was added to the reaction solution, which remained colourless. 3.10 ml (37.2 mmol) pyrrolidine was added to the solution. The solution immediately changed colour from colourless to yellow and finally reached a red colour. The reaction was left to stir whilst being monitored by thin layer chromatography (silica/dichloromethane), which showed only one product spot after 3 h. 2.5 ml of acetic acid was added to quench the reaction. One hundred milliliter of water was added to the reaction mixture and the organic product was extracted by 3×30 ml ether fractions. The ether fractions were combined and the solution was dried over magnesium sulphate and had its solvent removed at reduced pressure to yield a red oil. The red oil was purified by column chromatography with dichloromethane used as the eluent. The dichloromethane was removed at reduced pressure to yield 3.61 g (87.0% yield, 19.6 mmol) of a red oil.