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Benzyl-substituted titanocene dichloride anticancer drugs: From lead to hit

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ABSTRACT

Through the reaction of Super Hydride ($LiBEt_3H$) with 6-phenyl-substituted fulvenes followed by transmetallation to $TiCl_4$ ten novel benzyl-substituted titanocene dichloride derivatives were synthesised. 6(4-morpholinomethyl-phenyl) fulvene (**6g**) and (*bis*-[(4-methoxymethyl-benzyl)cyclopentadienyl]titanium(IV) dichloride) (**8a**) were characterised by single crystal X-ray diffraction. All of the titanocenes had their cytotoxicity investigated through preliminary *in vitro* testing on the LLC-PK (pig kidney epithelial) cell line and CAKI-1 human kidney cell human carcinoma cell line in an MTT based assay in order to determine their IC50 values. The titanocenes synthesised were found to have IC50 values ranging from 2.3 (±0.3) μM (comparable to cisplatin) to others which show no anti-proliferative activity on this cell line in standard DMSO formulations on LLC-PK cell line. Eight of the titanocenes were found to be completely water-soluble and had IC50 values of 6.5 (±0.7) μM to no activity when using medium only for formulation. On the CAKI-1 cell line, IC50 values of 7.8 (±1.4) μM to no activity were found using DMSO formulation, while IC50 values of 0.55 (±0.32) μM to no activity were measured using just medium as the formulation reagent. Some of the titanocenes show significant cytotoxicity improvement when compared directly to the lead compound Titanocene **Y** (bis-[(*p*-methoxybenzyl) cyclopentadienyl] titanium(IV) dichloride) and are more cytotoxic than cisplatin. Bis-[(4-diethylaminomethyl-benzyl)cyclopentadienyl]titanium(IV) dichloride (**8d**) at this preliminary stage seems to be the most promising of the ten compounds prepared and exhibits nanomolar activity against CAKI-1.

1. Introduction

In 1967, Barnett Rosenberg and co-workers inadvertently whilst applying electromagnetic radiation to bacterial and mammalian cells to investigate whether electric or magnetic dipole fields might be involved in cell division discovered the anticancer activity of *cis*-diaminedichloroplatinum(II) (cisplatin) [1]. This lead to the first use of a transition metal complex as a chemotherapeutic reagent, when it was approved for use in the clinic in 1978 by the FDA for use on metastatic testicular cancer and metastatic ovarian cancer. In 1993 it was approved by the FDA for use on transitional bladder cancer. Despite the success of cisplatin and its second generation analogue carboplatin (ovarian cancer) in the clinic, platinum drugs have some inherent difficulties associated with their unpredictable and severe nephrotoxicity, lack of oral bioavailability, intrinsic resistance and ototoxicity. This has led to the synthesis and biological evaluation of many thousands of cisplatin analogues and investigation of other inorganic complexes from the periodic table. Transition metal complexes of gold, iron, ruthenium, tin, vanadium, molybdenum and titanium have been shown to have some promising anti-tumour activity *in vitro* and *in vivo* testing but only titanium and ruthenium have been introduced into clinical trials.

Budotitane ([*cis*-diethoxybis(1-phenylbutane-1,3-dionato)titanium (IV)]) reached Phase I clinical trials [2] following a promising early preclinical evaluation but did not progress any further despite the development of a Cremophor EL® based formulation for it. Titanocene dichloride is the only metallocene dichloride so far which has reached clinical trials. Cp_2TiCl_2 shows medium anti-proliferative activity *in vitro* and promising results *in vivo* [3,4] but its efficacy in Phase II clinical trials in patients with metastatic renal cell carcinoma [5] or metastatic breast cancer [6] was too low to be pursued.

McGowan synthesised alkyl ammonium ring-substituted cationic titanocene dichloride derivatives, which are water-soluble and show significant activity against ovarian cancer [7]. This led to a renewal in research interest in titanocene dichlorides as potential anticancer compounds.

The promising anticancer compound bis-[(*p*-methoxybenzyl) cyclopentadienyl] titanium(IV) dichloride (Titanocene **Y**) was synthesised from the hydridolithiation of 6-anisyl fulvene with Super Hydride (lithium triethylborohydride) to give an isolable lithium

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cyclopentadienide, which can then be transmetallated with titanium tetrachloride to isolate it [8]. Titanocene **Y** has an IC50 value of 21 μM when tested on the long life epithelial pig kidney cell line LLC-PK. Titanocene **Y** has had its anti-proliferative activity studied in 36 human tumour cell lines and also against explanted human tumours [9]. These *in vitro* and *ex vivo* experiments showed that renal cell cancer is the prime target for this compound, but it also has activity against ovary, prostate, cervix, lung, colon, and breast cancer. Titanocenes have also been shown to give a positive immune response by up-regulating the number of natural killer (NK) cells in mice [9]. Animal studies reported the successful treatment of mice bearing xenografted Caki-1, MCF-7 [9] and A431 [10] tumours with Titanocene **Y**, where reduction of tumour size was seen.

Recently an oxalate derivative of Titanocene **Y** (Oxali-Titanocene **Y**) has been reported as having an IC50 of 1.6 μM on the LLC-PK cell line [11]. This was also shown to have good anti-angiogenic properties in HUVEC anti-angiogenesis tests and in a mouse model was shown to be cytostatic on xenografted Caki-1 [12] (Fig. 1).

Following the success of Titanocene **Y** in *in vivo* and *in vitro* testing; which showed very promising cytotoxic, anti-angiogenic properties and probable different cytotoxic mechanism than cisplatin; it was determined necessary to make further derivatives of Titanocene **Y** and to do some preliminary *in vitro* biological testing. The synthesis of several titanocene dichloride derivatives is presented in this paper.

2. Experimental

2.1. General conditions

Sodium metal, dimethylamine solution, morpholine, titanocene dichloride, Super Hydride and $TiCl_4$ were obtained commercially from Aldrich Chemical Co. Diethylamine was obtained from Fluka Chemical Co. Diethyl ether, pentane and THF were dried by a Grubbs system PureSolv 400-3-MD 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 a Varian 400 or 500 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. UV–Vis spectra were recorded on a Unicam UV4 Spectrometer, while CHN analysis was done with an Exeter Analytical CE-440 Elemental Analyser, while Cl was determined in mercurimetric titrations. X-ray diffraction data for compounds **6g** and **8a** was 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 [13]. The structure was solved by direct methods using SHELXS-97 [14] and refined by full matrix least-squares on F^2 for all data using SHELXL-97 [14]. All hydrogen atoms were located in the difference Fourier map and allowed to refine freely with isotropic thermal displacement parameters. Anisotropic thermal displacement parameters were refined for all non-hydrogen atoms.

Suitable crystals of **6g** were grown from a pentane solution which was evaporated at room temperature. Crystals of **8a** were grown from a saturated trichloromethane solution with slow infusion of pentane.

2.2. Synthesis

1-Bromo-4-(methoxymethyl) benzene **2a** [15], 1-bromo-4-(ethoxymethyl) benzene **2b** [16], 4-(methoxymethyl) benzaldehyde **5a** [15], 4-bromomethyl benzaldehyde **2** [17], 4-diethylam inomethyl benzaldehyde **5d** [18] and 4-morpholinomethyl ben zaldehyde **5g** [18] were synthesised according to literature methods. Aldehyde **5b** was synthesised in the same manner as aldehyde **5a** and the crude was used without further purification. 4-Dimethylaminomethyl benzaldehyde **5c** [19] and 4-pyrrolidin-1-ylmethyl benzaldehyde **5f** [19] were synthesised in the same procedure as **5d** and **5g** and were found to have the same analytical data as according to previously synthesised literature analytical data. 4-Di-*iso*-propylaminomethyl benzaldehyde **5e**, 4-(4-methyl-piperazin-1-ylmethyl) benzaldehyde **5h**, 4-[4-(2-methoxy-phenyl)-piperazin-1-ylmethyl] benzaldehyde and 4-[4-(4-methoxy-phenyl)-piperazin-1-ylmethyl] benzaldehyde were synthesised in the same manner as aldehydes **5d** and **5g**. The crude yield of aldehydes **5e** and **5h–j** were used in the synthesis of fulvenes **6e** and **6h–j** without further purification.

2.2.1. 4-Ethoxymethyl benzaldehyde, CHO–C_6H_4–CH_2–OCH_2CH_3 (**5b**)

Yellow oil: ^{1}H NMR (δ ppm $CDCl_3$, 400 MHz): 9.98 [1H, s, *CHO*–C_6H_4], 7.85 [2H, d, *J* 7.6, C_6H_4], 7.50 [2H, d, *J* 7.8 C_6H_4], 4.58 [2H, s, C_6H_4–CH_2], 3.68 [2H, q, *J* 7.0, OCH_2CH_3], 1.26 [3H, m, OCH_2CH_3].

2.2.2. 4-Di-iso-propylmethyl benzaldehyde, CHO–C_6H_4–CH_2–N($CH(CH_3)_2$) (**5e**)

Colourless oil: ^{1}H NMR (δ ppm $CDCl_3$, 400 MHz): 9.95 [1H, s, *CHO*–C_6H_4], 7.78 [2H, d, *J* 7.8, C_6H_4], 7.54 [2H, d, *J* 7.8, C_6H_4], 3.69 [2H, s, C_6H_4–CH_2], 3.35 [2H, dq, *J* 12.9, 6.4, N(*CH*$(CH_3)_2$)], 1.37 [12H, d, *J* 6.5, N($CH(CH_3)_2$)].

2.2.3. 4-(4-Methyl-piperazin-1-ylmethyl) benzaldehyde (**5f**)

Yellow oil: ^{1}H NMR (δ ppm $CDCl_3$, 400 MHz): 9.95 [1H, s, *CHO*–C_6H_4], 7.85 [2H, d, *J* 7.7, C_6H_4–CH_2], 7.59 [2H, d, *J* 7.8, C_6H_4–CH_2], 3.66 [2H, s, C_6H_4–CH_2], 2.55 [8H, s (b), N(CH_2CH_2)$_2$–N–CH_3, overlapping signals], 1.81 [3H, s, N(CH_2CH_2)$_2$–N–CH_3].

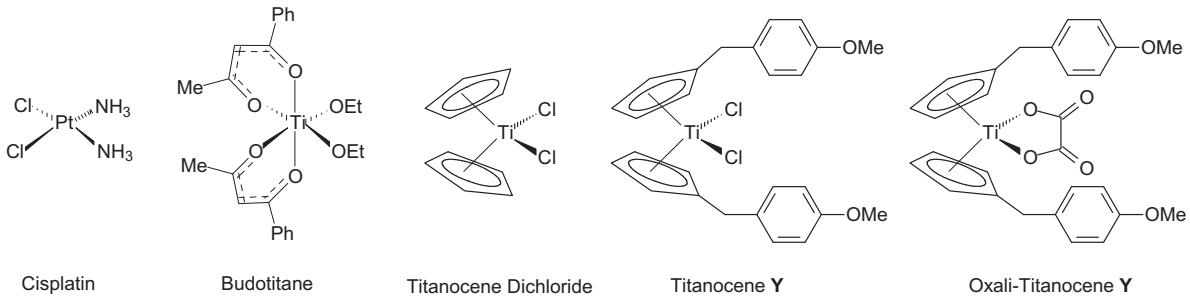

Fig. 1. Structures of Cisplatin, Budotitane, Titanocene Dichloride, Titanocene **Y** and Oxali-Titanocene **Y**.