Anti-tumor activity of Titanocene Y in xenografted Caki-1 tumors in mice

Iduna Fichtner[a], Clara Pampillón[b], Nigel J. Sweeney[b], Katja Strohfeldt[b] and Matthias Tacke[b]

The benzyl-substituted unbridged titanocene bis-[(*p*-methoxybenzyl)cyclopentadienyl] titanium(IV) dichloride (Titanocene Y) was tested *in vitro* against human renal cancer cells (Caki-1), in which it showed an IC_{50} value of 36×10^{-6} mol/l. Titanocene Y was then given *in vivo* in doses of 10, 20, 30, 40 and 50 mg/kg on 5 consecutive days to Caki-1-bearing mice, and it showed concentration-dependent and statistically significant tumor growth reduction with respect to a solvent-treated control cohort. The maximum tolerable dose of Titanocene Y was determined to be 40 mg/kg and it showed significantly better tumor volume growth reduction than cisplatin given at a dose of 2 mg/kg. This superior activity of Titanocene Y with respect to cisplatin will hopefully lead to clinical tests against metastatic renal cell cancer in the near future. *Anti-Cancer Drugs* 17:333–336 © 2006 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2006, **17**:333–336

Keywords: anti-cancer drug, Caki-1, cisplatin, renal cell cancer, titanocene

[a]Max Delbrück Center for Molecular Medicine, Berlin, Germany and [b]The UCD School of Chemistry and Chemical Biology, Conway Institute of Biomolecular and Biomedical Research, Centre for Synthesis and Chemical Biology, University College Dublin, Dublin, Ireland.

Correspondence to M. Tacke, The UCD School of Chemistry and Chemical Biology, Conway Institute of Biomolecular and Biomedical Research, Centre for Synthesis and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland.
e-mail: matthias.tacke@ucd.ie

Sponsorship: Science Foundation Ireland (SFI) funded this work through grant (04/BRG/C0682). Additional funding was provided from the Higher Education Authority (HEA) and the Centre for Synthesis and Chemical Biology (CSCB) through the HEA PRTLI cycle 3 as well as COST D20 (WG 0001).

Received 9 November 2005 **Accepted** 22 November 2005

Introduction

Despite the resounding success of cisplatin and closely related platinum anti-tumor agents, the movement of other transition metal anti-cancer drugs towards the clinic has been exceptionally slow [1–3]. Metallocene dichlorides (Cp_2MCl_2) with M = Ti, V, Nb and Mo show remarkable anti-tumor activity [4,5]. Unfortunately, the efficacy of Cp_2TiCl_2 in phase II clinical trials in patients with metastatic renal cell carcinoma [6] or metastatic breast cancer [7] was too low to be pursued. Very recently, more synthetic effort has been employed to increase the cytotoxicity of titanocene dichloride derivatives [8–12]. A novel method starting from titanium dichloride and fulvenes [13–16] allows direct access to highly substituted *ansa*-titanocenes [17–20], i.e. titanocenes containing a carbon–carbon bridge. By using this method we have synthesized [1,2-di(cyclopentadienyl)-1,2-di-(4-*N*,*N*-dimethylaminophenyl)ethanediyl] titanium dichloride (Titanocene **X**), which has an IC_{50} value of 2.7×10^{-4} mol/l when tested for cytotoxic effects on the LLC-PK cell line [21]. It was followed by reports about heteroaryl [22] and methoxyphenyl [23,24] substituted *ansa*-titanocenes, which show similar IC_{50} values. Our most cytotoxic *ansa*-titanocene [1,2-di(cyclopentadienyl)-1,2-bis(*m*-dimethoxyphenyl)ethanediyl] titanium dichloride (Titanocene **Z**) shows an IC_{50} value of 2.1×10^{-4} mol/l when tested on the LLC-PK cell line [23].

The cytotoxic effect was further increased by synthesizing the analogs of unbridged titanocenes by establishing a completely new synthetic route, which has been published recently [25]. Bis-[(*p*-methoxybenzyl)cyclopentadienyl] titanium(IV) dichloride (Titanocene **Y**), which has an IC_{50} value of 2.1×10^{-5} mol/l when tested on the LLC-PK cell line, was synthesized from fulvene and super hydride ($LiBEt_3H$) followed by transmetallation with titanium tetrachloride. The structures of the titanocenes are shown in Fig. 1.

The anti-proliferative activity of Titanocene **X**, **Y** and **Z** was studied in 36 human tumor cell lines [26], and in four freshly explanted human tumors using Titanocene **X** [27]. These *in vitro* and *ex vivo* experiments showed that prostate, cervical and renal cell cancers are prime targets for this novel class of titanocenes.

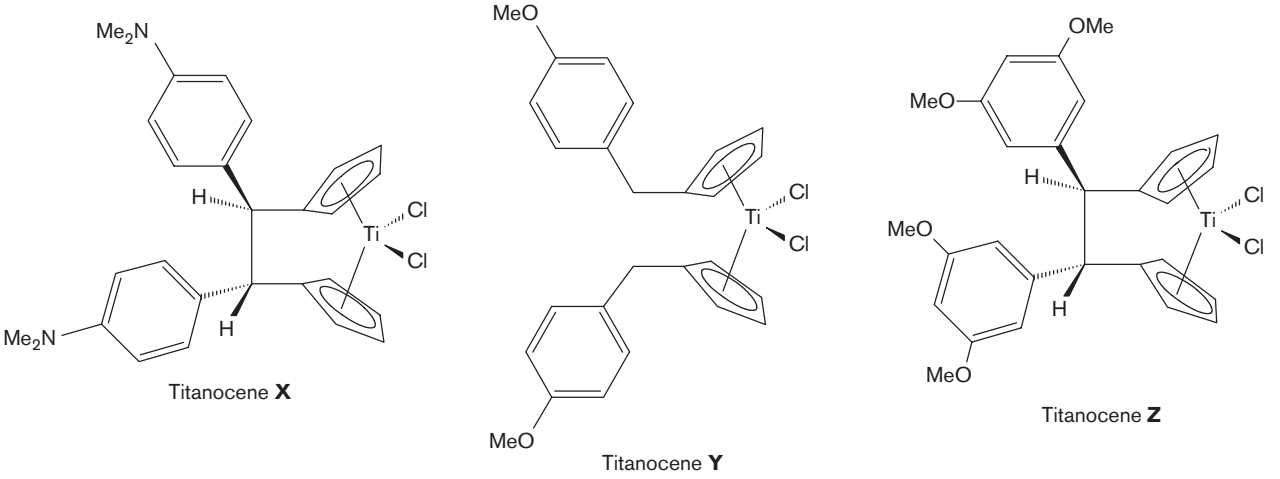
This paper investigates the anti-proliferative effect of Titanocene **Y** against Caki-1 cells *in vitro* and in a Caki-1 xenograft model *in vivo*.

Materials and methods

Caki-1 cell tests

Caki-1 cells (ATCC HTB-46) are derived from a human clear cell carcinoma of the kidney. The cells were seeded in 96-well plates (5000 cells/well), and cultivated with McCoy's 5a medium and 10% FBS (Invitrogen, Karlsruhe,

Fig. 1



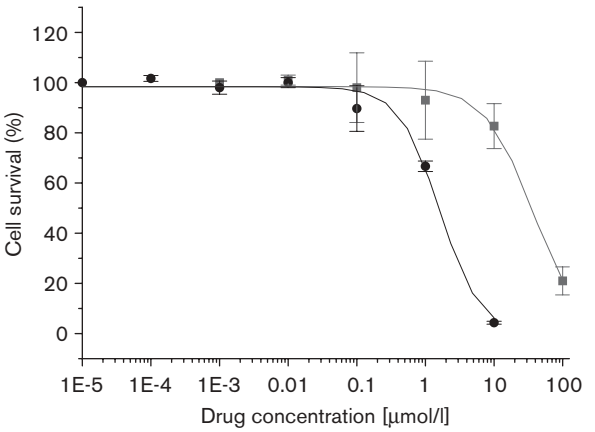
Molecular structure of Titanocenes **X**, **Y** and **Z**.

Germany). At 24 h after seeding, Titanocene **Y** dissolved in 0.1% DMSO or cisplatin dissolved in medium was added in a concentration range of 10^{-4} to 10^{-9} mol/l. The plates were cultivated for 4 days at 37°C and 5% CO_2. Then, a routine MTT assay [28] was performed and IC_{50} values were determined. Each in-vitro assay was performed 3 times identically.

Caki-1 xenografts

For *in vivo* testing, 10^7 Caki-1 cells were injected s.c. to female NMRI:*nu*/*nu* mice (eight mice per group). Treatment was initiated when tumors had grown to a palpable size (5–6 mm diameter). Titanocene **Y** was dissolved in DMSO (final concentration 10%) and diluted with 0.5% Tween-80 in saline. It was injected i.p. in doses of 10, 20, 30, 40 or 50 mg/kg/day once daily for 5 consecutive days. One group of mice was treated with the solvent (negative control) and another group with cisplatin (Medac, Hamburg, Germany) at a dose of 2 mg/kg/day, i.e. at a dose that was determined to be of significant efficacy in other xenograft experiments (unpublished). Tumor size was measured with a caliper-like instrument. Tumor volumes, relative tumor volumes (relative to the first treatment day) and treated/control (T/C) values were calculated. Body weight and mortality of the mice were determined continuously during the experiments for an estimation of tolerability. Additionally, in one experiment blood from the retro-orbital venous plexus was taken at the end of the treatment cycle. Blood parameters were determined with a Coulter counter. Serum creatinine and blood urea nitrogen (BUN) were determined using automated methodology routinely used in the clinic.

Fig. 2



Cytotoxicity curves from three independent MTT assays showing the effect of Titanocene **Y** (squares) and cisplatin (circles) on the viability of Caki-1 cells.

Results and discussion

In-vitro cytotoxicity

The in-vitro cytotoxicity of Titanocene **Y** and cisplatin was determined in three independent experiments using the Caki-1 cell line. Mean IC_{50} values were determined to be 36 ± 4 µmol/l for Titanocene **Y** and 1.6 ± 0.2 µmol/l for cisplatin (Fig. 2).

Renal cancer has already been determined prior to this experiment to be one promising target for the presented titanocene. In an extensive in-vitro test of the antiproliferative activity of different titanocenes in 36 human cancer cell lines, the most promising results could be

Table 1 Overview on results obtained in two independent Caki-1 xenograft experiments

Experiment	Group	Substance	Dose (mg/kg/injection)	Toxic deaths/total	Body weight change (%)	Optimum T/C (%)	White blood cell count (10^6/ml)	Platelets (10^6/ml)	Creatinine (mmol/l)	BUN (mmol/l)
1	A	solvent	0	NT	NT	NT	NT			
	B	Titanocene **Y**	10	0/8	−3	61	NT	NT	NT	NT
	C	Titanocene **Y**	20	0/8	−5	49[a]	NT	NT	NT	NT
	D	Titanocene **Y**	30	0/8	−5	40[a]	NT	NT	NT	NT
2	A	solvent	0	6.9±2.7	1019±44	ND	6.46±0.72			
	B	Titanocene **Y**	40	0/8	−16	42[a]	8.6±1.6	1142±141	ND	4.96±0.58
	C	Titanocene **Y**	50	5/8	−15	39[a]	9.5±3.9	1116±165	ND	5.34±0.96
	D	cisplatin	2	0/8	−2	68	6.8±2.1	1100±90	ND	5.3±0.78

Female nude mice received s.c. tumor cell injections. At a palpable tumor size they were treated with the compounds for 5 consecutive days. Tumor size was measured as a therapeutic marker, and body weight change, white blood cell counts, platelets, creatinine and BUN as toxicity parameters. NT=not tested. ND=not detectable.
[a]Significant compared with solvent.

obtained for renal cancer cell lines with a range of the IC_{50} values from 47 to 120 µmol/l [26]. The best results were obtained for the renal cancer cell line RXF 1781L (IC_{50} 47 µmol/l), whereas the IC_{50} value for cisplatin was 192 µmol/l. The Caki-1 cell line was not tested in the mentioned study, but the even lower IC_{50} value underlines the very promising cytotoxic effect, especially in renal cancer cells.

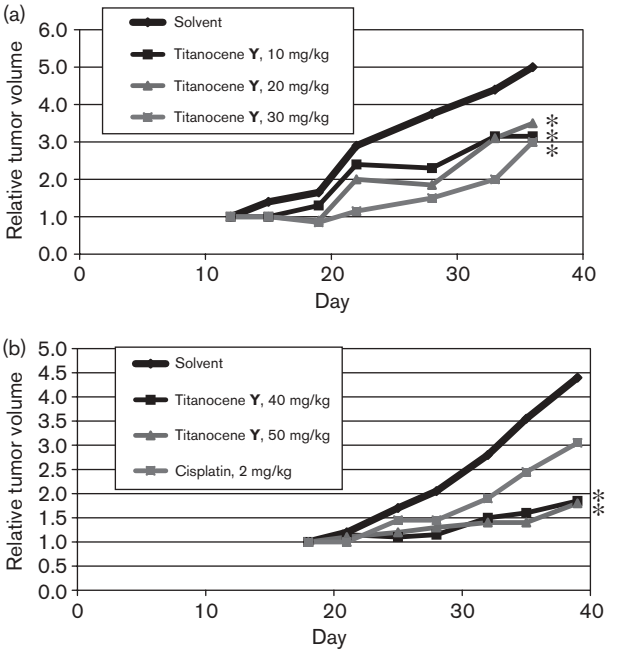
In-vivo efficacy

Results of in-vivo experiments using Caki-1 xenografts can be seen in Table 1. In the first experiment, four groups of eight mice each were treated i.p. with solvent or Titanocene **Y** at doses of 10, 20 or 30 mg/kg for 5 consecutive days. As shown in Fig. 3(A), Titanocene **Y** induced a significant and dose-dependent inhibition of tumor growth with T/C values of 61, 49 and 40%, respectively. As no severe side-effects were observed in this experiment we increased the doses of Titanocene **Y** in a second experiment to 40 and 50 mg/kg, and added a group treated with cisplatin at a dose of 2 mg/kg/day as a positive control. T/C values of 42 and 39% were obtained in this second experiment with Titanocene **Y** (Fig. 3B). In the highest dose used (50 mg/kg/day) five out of eight mice died of toxicity, suggesting that the maximum tolerated dose is about 40 mg/kg/day. Severe body weight loss of 15 or 16% accompanied by diarrhea was observed in both mice cohorts treated with the two highest doses. Dead mice showed signs of inflammation in the gastrointestinal tract at autopsy.

No severe side-effects were noticed at non-lethal doses. The body weight loss was moderate; no influence on hematological (white blood cells, platelets) and renal toxicity-associated (creatinine, BUN) parameters was noticed.

The cisplatin dose used has shown significant anti-tumor efficacy in other xenografts (personal observations). However, it cannot be excluded that the dose could be increased in the Caki-1 model, leading to higher toxicity and/or efficacy. Cisplatin revealed only a non-significant delay of tumor growth at the dose used; it had no significant influence on body weight or hematological and renal parameters. All in-vivo parameters are summarized in Table 1.

Fig. 3



Tumor growth curves of experiments 1 (a) and 2 (b) with Caki-1 xenografts in nude mice.

Conclusion and outlook

The in-vitro experiments using Titanocene **Y** on the Caki-1 cell line resulted in a very promising cytotoxic effect with an IC_{50} of 36 µmol/l, which represents a value in the significantly lower range of IC_{50} values determined up to now for titanocenes on a broad panel of human cancer cell lines.

The in-vivo experiments show that Titanocene **Y** induces a significant and dose-dependent inhibition of tumor growth with no severe side-effects for doses up to 30 mg/kg. In a second experiment with higher doses, a further inhibition of tumor growth was observed and a maximum tolerated dose of about 40 mg/kg/day was determined. No severe side-effects were noticed at non-lethal doses. These results have been compared with cisplatin, which was given at a dose of 2 mg/kg/day, but only a non-significant delay of tumor growth was observed.

This very promising Caki-1 mouse model should be a catalyst to promote Titanocene **Y** into a clinical phase I study against renal cell cancer in the near future.

Acknowledgments

The authors gratefully acknowledge the excellent technical support of M. Lemm, B. Büttner and S. Gromova.

References

1 Gelasco A, Lippard SJ. Anticancer activity of cisplatin and related complexes. *Top Biol Inorg Chem* 1999; **1**:1–43.
2 Jamieson ER, Lippard SJ. Structure, recognition, and processing of cisplatin–DNA adducts. *Chem Rev* 1999; **99**:2467–2498.
3 Farrell N, Qu Y, Roberts JD. Chemistry and biology of multifunctional DNA binding agents. *Top Biol Inorg Chem* 1999; **1**:99–115.
4 Köpf-Maier P, Köpf H. Non-platinum group metal antitumor agents. History, current status, and perspectives. *Chem Rev* 1987; **87**:1137–1152.
5 Köpf-Maier P, Köpf H. Transition and main-group metal cyclopentadienyl complexes: preclinical studies on a series of antitumor agents of different structural type. *Struct Bonding* 1988; **70**:103–194.
6 Lummen G, Sperling H, Luboldt H, Otto T, Rubben H. Phase II trial of titanocene dichloride in advanced renal-cell carcinoma. *Cancer Chemother Pharmacol* 1998; **42**:415–417.
7 Kröger N, Kleeberg UR, Mross KB, Edler L, Saß G, Hossfeld DK. Phase II clinical trial of titanocene dichloride in patients with metastatic breast cancer. *Onkologie* 2000; **23**:60–62.
8 Mokdsi G, Harding MM. Antitumor metallocenes: effect of DMSO on the stability of Cp_2TiX_2 and implications for anticancer activity. *Metal-Based Drugs* 1998; **5**:207–215.
9 Allen OR, Croll L, Gott AL, Knox RJ, McGowan PC. Functionalized cyclopentadienyl titanium organometallic compounds as new antitumor drugs. *Organometallics* 2004; **23**:288–292.
10 Boyles JR, Baird MC, Campling BG, Jain N. Enhanced anti-cancer activities of some derivatives of titanocene dichloride. *J Inorg Biochem* 2001; **84**:159–162.
11 Causey PW, Baird MC. Synthesis, characterization, and assessment of cytotoxic properties of a series of titanocene dichloride derivatives. *Organometallics* 2004; **23**:4486–4494.
12 Meyer R, Brink S, van Rensburg CEJ, Joone GK, Görls H, Lotz S. Synthesis, characterization and antitumor properties of titanocene derivatives with thiophene containing ligands. *J Organomet Chem* 2005; **690**: 117–125.
13 Teuber R, Linti G, Tacke M. The X-ray structure of $Fe(fulvene)_2$: the missing link in the direct synthesis of *ansa*- and Cp^i-metallocenes ($Cp^i = C_5H_4CHMe_2$). *J Organomet Chem* 1997; **545–546**:105–110.
14 Hartl F, Cuffe L, Dunne JP, Fox S, Mahabiersing T, Tacke M. Reduction of substituted fulvenes studied by spectro-electrochemistry and *ab initio* theory. *J Mol Struct* 2001; **559**:331–339.
15 Tacke M, Dunne JP, Fox S, Linti G, Teuber R. The synthesis, X-ray, and DFT structure of the free *ansa*-cyclopentadiene ligand $C_5H_5CMe_2CMe_2C_5H_5$. *J Mol Struct* 2001; **570**:197–202.
16 Fox S, Dunne JP, Dronskowski D, Schmitz D, Tacke M. Synthesis and structural characterisation of a novel chiral *ansa*-cobaltocenium hexafluorophospate. *Eur J Inorg Chem* 2002; 3039–3046.
17 Eisch JJ, Xian S, Owuor FA. Novel synthesis of *ansa*-metallocenes via the reductive dimerization of fulvenes with group 4 metal divalent halides. *Organometallics* 1998; **17**:5219–5221.
18 Eisch JJ, Owuor FA, Xian S. Novel synthesis of unbridged, sterically substituted zirconocene dichlorides from fulvenes and dialkylzirconium dichlorides via zirconium(IV) hydride transfer. *Organometallics* 1999; **18**:1583–1585.
19 Kane KM, Shapiro PJ, Vij A, Cubbon R, Rheingold AL. Reductive coupling of fulvenes with calcium for C2-symmetric *ansa*-metallocenes: syntheses and molecular structures of *trans*-$Ph_2C_2H_2(\eta^5\text{-}C_5H_4)_2Ca(THF)_2$ and *trans*-$Ph_2C_2H_2(\eta^5\text{-}C_5H_4)_2ZrCl_2$. *Organometallics* 1997; **16**:4567–4571.
20 Fox S, Dunne JP, Tacke M, Gallagher JF. Novel derivatives of *ansa*-titanocenes procured from 6-phenylfulvene: a combined experimental and theoretical study. *Inorganica Chim Acta* 2004; **357**:225–234.
21 Tacke M, Allen LT, Cuffe LP, Gallagher WM, Lou Y, Mendoza O, *et al.* Novel titanocene anti-cancer drugs derived from fulvenes and titanium dichloride. *J Organomet Chem* 2004; **689**:2242–2249.
22 Rehmann FJK, Cuffe LP, Mendoza O, Rai DK, Sweeney N, Strohfeldt K, *et al.* Heteroaryl substituted *ansa*-titanocene anti-cancer drugs derived from fulvenes and titanium dichloride. *Appl Organomet Chem* 2005; **19**:293–300.
23 Tacke M, Cuffe LP, Gallagher WM, Lou Y, Mendoza O, Müller-Bunz H, *et al.* Methoxy-phenyl substituted *ansa*-titanocenes as potential anti-cancer drugs derived from fulvenes and titanium dichloride. *J Inorg Biochem* 2004; **98**:1987–1994.
24 Rehmann FJK, Rous AJ, Mendoza O, Pampillon C, Strohfeldt K, Sweeney N, *et al.* Novel substituted *ansa*-titanocene anti-cancer drugs. *Polyhedron* 2005; **24**:1250–1255.
25 Sweeney N, Mendoza O, Müller-Bunz H, Pampillón C, Rehmann F-JK, Strohfeldt K, et al. Novel benzyl substituted titanocene anti-cancer drugs. *J Organomet Chem* 2005; **690**:4537–4544.
26 Kelter G, Sweeney N, Strohfeldt K, Fiebig H-H, Tacke M. *In vitro* anti-tumor activity of bridged and unbridged benzyl-substituted titanocenes. *Anticancer Drugs* 2005; **16**:1091–1098.
27 Oberschmidt O, Hanauske AR, Rehmann F-JK, Strohfeldt K, Sweeney N, Tacke M. Preclinical activity of [1,2-di(cyclopentadienyl)-1,2-di(*p-N,N*-dimethylaminophenyl)-ethanediyl] titanium dichloride against tumor colony forming units. *Anticancer Drugs* 2005; **16**:1071–1073.
28 Stein U, Walther W, Lemm M, Naundorf H, Fichtner I. Development and characterisation of novel human multidrug resistant mammary carcinoma lines *in vitro* and *in vivo*. *Int J Cancer* 1997; **72**: 885–891.