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Novel benzyl substituted titanocene anti-cancer drugs

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Abstract

From the novel reaction of Super Hydride ($LiB(Et)_3H$) with 6-(*p*-*N*,*N*-dimethylanilinyl)fulvene (**1a**) or 6-(*p*-methoxyphenyl)fulvene (**1b**) the corresponding lithium cyclopentadienide intermediates (**2a**, **2b**) were obtained. When reacted with $TiCl_4$, bis-[(*p*-dimethylaminobenzyl)cyclopentadienyl]titanium (IV) dichloride (**3a**) and bis-[(*p*-methoxybenzyl)cyclopentadienyl]titanium (IV) dichloride (**3b**) were obtained. Titanocene **3a** was reacted with an ethereal solution of HCl, by which its dihydrochloride derivative (**3c**) was formed and isolated. Titanocenes **3b** and **3c** were characterised by X-ray crystallography. When the titanocenes **3a**–**c** were tested against pig kidney carcinoma (LLC-PK) cells inhibitory concentrations (IC_{50}) of 1.2×10^{-4} M, 2.1×10^{-5} M and 9.0×10^{-5} M, respectively, were observed. These values represent improved cytotoxicity against LLC-PK, most notably for **3b** (Titanocene Y), which is a hundred times more cytotoxic than titanocene dichloride itself.

Keywords: Anti-cancer drug; *cis*-platin; Titanocene; Fulvene; Super Hydride; LLC-PK

1. Introduction

Despite the resounding success of *cis*-platin and closely related platinum antitumor 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 antitumor 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]. By using this method we have synthesised [1,2-di(cyclopentadienyl)-1,2-di-(4-*N*,*N*-dimethylaminophenyl)-ethanediyl]titanium dichloride (**4**, Titanocene X), which has an IC_{50} value of 2.7×10^{-4} M 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. This paper reports the synthesis of three novel benzyl substituted titanocene dichlorides, which leads to an improved cytotoxicity against LLC-PK cells, when compared to their analogue *ansa*-titanocenes.

2. Experimental

2.1. General conditions

Titanium tetrachloride and Super Hydride [$LiB(Et)_3H$, 1.0 M solution in THF] were obtained commercially from Aldrich Chemical Co. In the synthesis of **3a**, titanium tetrachloride as a 1.0 M solution in toluene, for

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the synthesis of **3b** it was used the pure reagent. THF and diethyl ether were dried over Na and benzophenone. For the synthesis of **3c**, CH_2Cl_2 was dried over calcium hydride. Solvents were freshly distilled and collected under an atmosphere of argon prior to use. Manipulations of air and moisture sensitive compounds were done using standard Schlenk techniques, under an argon atmosphere. NMR spectra were measured on either a Varian 300 or a 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. A single crystal of titanocene **3b** suitable for X-ray diffraction experiments was grown by the diffusion of pentane into a saturated solution of **3b** in dichloromethane at room temperature. A single crystal of **3c** was grown by slow evaporation from a saturated chloroform solution of **3a**, to which was added an ethereal solution of HCl. X-ray diffraction data for the two compounds was collected on a BRUKER Smart Apex diffractometer at 100 K. A semi-empirical absorption correction on the raw data was performed using the program SADABS [25]. The crystal structures were then solved by direct methods (SHELXS-NT97) [26] and refined by full-matrix least squares methods against F^2. Further details about the data collection are listed in Table. 1, as well as reliability factors. Further details are available free of charge from the Cambridge structural database under the CCDC Nos. 264345 and 264344 for **3b** and **3c**, respectively.

3. Synthesis

6-(*p*-*N*,*N*-dimethylanilinyl)fulvene **(1a)** and 6-*p*-(methoxyphenyl)fulvene (**1b**) were synthesised according to the procedures used previously [21,23].

*3.1. Bis-[(p-dimethylaminobenzyl)cyclopentadienyl] titanium (IV) dichloride, [(η^5-C_5H_4–CH_2–C_6H_4–$N(CH_3)_2$)]$_2$$TiCl_2$ (**3a**)*

$LiB(Et)_3H$ (12.4 ml of a 1.0 M solution in THF) was concentrated by removal of the solvent by heating it to 90 °C under a vacuum of 10^{-2} mbar for 2 h. The concentrated reagent was dissolved in diethyl ether (75 ml) and was transferred to a solution of **1a** (2.30 g, 11.7 mmol) in diethyl ether (200 ml). The solution was stirred (12 h), during which time the lithium cyclopentadienide intermediate **2a** precipitated from the solution and the colour of the solution changed from red to orange. After stirring, the precipitate was allowed to settle and was filtered to remove the filtrate. **2a** was then collected on a frit and washed with diethyl ether (75 ml), dried briefly in vacuo and transferred to a Schlenk flask under argon.

The yellow lithium cyclopentadienide intermediate **2a** (1.65 g, 7.9 mmol, 67.5% yield) was dissolved in THF (80 ml), followed by drop wise addition of $TiCl_4$ (4.0 ml of a 1.0 M solution in toluene) at 0 °C. The resultant red solution was refluxed for 16 h during which time it darkened in colour. The solution was then cooled and the solvent was removed under reduced pressure. The remaining residue was extracted with dichloromethane (75 ml) and filtered through celite to remove the LiCl. The dark red filtrate was filtered twice more by gravity filtration. The solvent was removed under reduced pressure to yield a very dark red solid, which was dried in vacuo (1.25 g, 2.0 mmol, 50.1% yield).

^{1}H NMR (δ ppm $CDCl_3$, 300 MHz): 7.10 [$C_6H_4N(CH_3)_2$, *J* 6.6 Hz, 4H, d]; 6.72 [$C_6H_4N(CH_3)_2$, *J* 6.0 Hz, 4H, d]; 6.33–6.31 [C_5H_4, 8H, m]; 3.99 [Cp–CH_2–$C_6H_4N(CH_3)_2$, 4H, s]; 2.95 [$C_6H_4N(CH_3)_2$, 12H, s].

^{13}C NMR (δ ppm $CDCl_3$, 125 MHz), 149.4, 138.4, 130.0, 129.8, 122.2, 116.8, 113.3 [C_5H_4 and C_6H_4]; 41.1 [$C_6H_4N(CH_3)_2$]; 36.2 [Cp–CH_2–$C_6H_4N(CH_3)_2$].

IR absorptions (cm^{-1} KBr): 3113, 3085, 2890, 2803, 1611, 1521, 1488, 1440, 1354, 821, 805, 752, 677.

Anal. Calc. for $C_{28}H_{32}N_2Cl_2Ti$: Theory: C, 65.26; H, 6.26; N, 5.43. Found: C, 65.60; H, 6.38; N, 5.31%.

UV–Vis (CH_2Cl_2): λ 265 nm (ε 39,000), λ 315 nm (ε 11,000), λ_{max} 400 nm (ε 1000), λ_{max} 540 nm (weak).

*3.2. Bis-[(p-methoxybenzyl)cyclopentadienyl]titanium (IV) dichloride, [(η^5-C_5H_4–CH_2–C_6H_4–O–CH_3)]$_2$-$TiCl_2$ (**3b**)*

$LiB(Et)_3H$ (14.0 ml of a 1.0 M solution in THF) was concentrated by removal of the solvent by heating it to 90 °C under vacuum of 10^{-2} mbar for 2 h. The concentrated reagent was dissolved in diethyl ether (80 ml) and to this solution was added **1b** (2.27 g, 12.3 mmol) in diethyl ether (40 ml); the solution was stirred (12 h), during which time lithium cyclopentadienide intermediate **2b** precipitated from the solution and the colour of the solution changed from orange to yellow. **2b** was allowed to settle and was filtered to remove the filtrate. **2b** was then collected on a frit and washed with diethyl ether (75 ml), dried briefly in vacuo and transferred to a Schlenk flask under argon.

The white lithium cyclopentadienide intermediate **2b** (1.01 g, 5.27 mmol, 42.7% yield) was dissolved in THF (40 ml) and it was added to a solution of $TiCl_4$ (0.3 ml, 2.65 mmol) in THF (80 ml) at 0 °C. The resultant dark red solution was refluxed for 16 h. The solution was then cooled and the solvent was removed under reduced pressure. The remaining residue was extracted with chloroform (50 ml) and filtered through celite to remove the LiCl. The brown filtrate was filtered twice more by gravity filtration. The solvent was removed under reduced pressure to yield a red-brown solid, which was dried in vacuo (0.70 g, 1.4 mmol, 54.0% yield).

Table 1
Crystal data and structure refinement for the salt derivative of **3b** and **3c**

Identification code	**3b**	**3c**
Empirical formula	$C_{26}H_{26}O_2Cl_2Ti$	$C_{31}H_{37}N_2Cl_{13}Ti$
Molecular formula	$C_{26}H_{26}O_2Cl_2Ti$	$[C_{28}H_{34}N_2Cl_2Ti]Cl_2 \cdot 3CHCl_3$
Formula weight	489.27	946.38
Temperature (K)	100(2)	100(2)
Wavelength (Å)	0.71073	0.71073
Crystal system	Orthorhombic	Orthorhombic
Space group	*Pbca* (#61)	$Pca2_1$ (#29)
Unit cell dimensions	$A = 6.4295(7)$	$a = 15.5743(13)$
	$B = 25.896(3)$	$b = 20.6939(18)$
	$C = 27.126(3)$	$c = 13.0797(11)$
	$\alpha = \beta = \gamma = 90°$	$\alpha = \beta = \gamma = 90°$
Volume (Å^3)	4516.4(9)	4215.5(6)
Z	8	4
D_{calc} (Mg/m^3)	1.439	1.491
Absorption coefficient (mm^{-1})	0.637	1.053
$F(000)$	2032	1920
Crystal size (mm^3)	$0.50 \times 0.10 \times 0.02$	$0.60 \times 0.25 \times 0.03$
θ Range for data collection	1.50–25.00.	1.64–28.28
Index ranges	$-7 \leqslant h \leqslant 7$, $-30 \leqslant k \leqslant 30$, $-32 \leqslant l \leqslant 32$	$-20 \leqslant h \leqslant 20$, $-27 \leqslant k \leqslant 27$, $-17 \leqslant l \leqslant 16$
Reflections collected	29196	49162
Independent reflections [R_{int}]	3972 [0.0552]	9711 [0.0396]
Completeness to θ_{max} (%)	99.9	97.4
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents
Maximum and minimum transmission	0.9874 and 0.6368	0.9691 and 0.7229
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	3972/0/282	9711/1/429
Goodness-of-fit on F^2	1.141	1.089
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0448$, $wR_2 = 0.1047$	$R_1 = 0.0677$, $wR_2 = 0.1716$
R indices (all data)	$R_1 = 0.0540$, $wR_2 = 0.1096$	$R_1 = 0.0826$, $wR_2 = 0.1856$
Absolute structure parameter	–	0.52(5)[a]
Largest difference in peak and hole (e Å^{-3})	0.713 and −0.348	1.590 and −1.049

[a] Due to that refined as an inversion twin.

^{1}H NMR (δ ppm $CDCl_3$, 300 MHz): 7.13 [C_6H_4-(OCH_3), J 8.4 Hz, 4H, d]; 6.83 [$C_6H_4(OCH_3)$, J 8.4 Hz, 4H, d]; 6.30 [C_5H_4, 8H, s]; 4.02 [Cp–CH_2–C_6H_4-(OCH_3), 4H, s]; 3.78 [$C_6H_5(OCH_3)$, 6H, s].

^{13}C NMR (δ ppm $CDCl_3$, 75 MHz): 157.3, 136.7, 130.5, 129.0, 128.9, 121.2, 115.1 [C_5H_4 and C_6H_4]; 54.3 [$C_6H_4(OCH_3)$]; 35.0 [Cp–CH_2–$C_6H_4(OCH_3)$].

IR absorptions (cm^{-1} KBr): 3099, 2956, 1609, 1577, 1511, 1459, 1438, 1256, 1247, 1020, 820, 801, 764.

Anal. Calc. for $C_{26}H_{26}O_2Cl_2Ti$: Theory: C, 63.83; H, 5.36. Found: C, 64.48; H, 5.87%.

UV–Vis (CH_2Cl_2): λ 260 nm (ε 16,000), λ 310 nm (ε 1000), λ 405 nm (ε 700), λ_{max} 540 nm (weak).

3.3. Dihydrochloride derivative of **3a**, $[(\eta^5\text{-}C_5H_4\text{–}CH_2\text{–}C_6H_4\text{–}N(CH_3)_2)]_2TiCl_2 \cdot 2HCl$, (**3c**)

3a (0.49 g, 0.9 mmol) was dissolved in CH_2Cl_2 (10 ml) and to the solution was added an excess ethereal solution of hydrogen chloride (2.5 ml of a 2-molar solution). A precipitate immediately formed. Diethyl ether (30 ml) was then added. After 15 min stirring, the pale yellow supernatant liquid was decanted and the remaining brown powder was dried in vacuo (0.38 g, 0.6 mmol, 66.6% yield.).

^{1}H NMR (δ ppm D_2O, 300 MHz): 7.34 [C_6H_4N-(CH_3)$_2$, J 8.7 Hz, 4H, d], 7.22 [$C_6H_4N(CH_3)_2$, J 8.4 Hz, 4H, d]; 6.24 [C_5H_4, J_{AB} 2.7 Hz, J_{BC} 2.1 Hz, J_{AC} 4.8 Hz, 4H, t]; 6.21 [C_5H_4, J_{AB} 2.4 Hz, J_{BC} 2.4 Hz, J_{AC} 4.8 Hz, 4H, t]; 3.62 [Cp–CH_2–$C_6H_4N(CH_3)_2$, 4H, s); 3.04 [$C_6H_4N(CH_3)_2$, 12H, s).

^{13}C NMR (δ ppm D_2O, 75 MHz): 141.6, 140.5, 130.9, 120.5, 118.3, 116.4 [C_5H_4 and C_6H_4]; 46.3 [$C_6H_4N(CH_3)_2$]; 34.5 [Cp–CH_2–$C_6H_4N(CH_3)_2$].

IR absorptions (cm^{-1} KBr): 3115, 3081, 3067, 2927, 2918, 2359, 2343, 1631, 1627, 1511, 1475, 1132, 816, 680, 617.

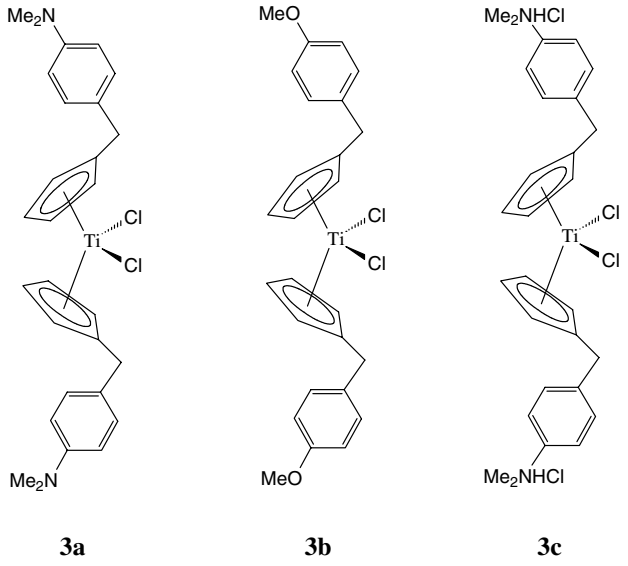
Anal. Calc. for $C_{28}H_{34}N_2Cl_4Ti$: Theory: C, 57.17; H, 5.83; N, 4.76. Found: C, 57.57; H, 5.67; N, 4.31%.

UV–Vis (H_2O): λ 210 nm (ε 44,000), λ 250 nm (ε 32,000), λ 320 nm (ε 11,000), λ_{max} 555 nm (weak).

4. MTT-based cytotoxicity tests

The cytotoxic activities of titanocenes **3a**–**c** were determined using an MTT-based assay. In more detail, cells were seeded into a 96-well plate (5000 cells/well) and allowed to attach for 24 h. Subsequently, the cells were treated with various concentrations of the cytotoxic

agents. In order to prepare drug solutions, drugs were firstly dissolved in DMSO, and medium was added to obtain a stock solution with a concentration 5×10^{-4} M, with a final concentration of DMSO not exceeding 0.7%. From these stock solutions, solutions with lower concentrations were prepared by further dilution with medium. Care was taken that the drug solutions were applied within 1 h on the cells to avoid interference with already hydrolysed compounds. After 48 h, the relevant drug was removed, the cells washed twice with PBS and fresh medium was added for another 24 h for recovery. Viability of cells was determined by treatment with MTT in medium (5 mg/11 ml) for 3 h. The purple formazan crystals formed were dissolved in DMSO and absorbance measured at 540 nm using a VICTOR^2 multilabel plate reader (Wallac). IC_{50} (inhibitory concentration 50%) values were determined from the drug concentrations that induced a 50% reduction in light absorbance.



3a **3b** **3c**

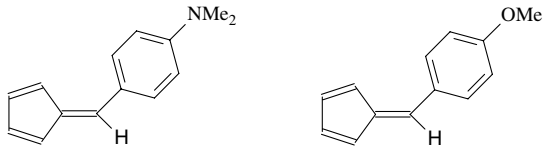
Fig. 2. The structures of titanocenes **3a**, **3b** and **3c**.

5. Results and discussion

5.1. Synthesis

Fulvenes **1a** and **1b** were synthesised by reacting the corresponding benzaldehyde with cyclopentadiene in the presence of pyrrolidine as a base [21,23] and their structures are shown in Fig. 1.

The use of $LiB(Et)_3H$, otherwise known as Super Hydride, in the transfer of a hydride to a fulvene is a novel method to obtain synthetically very interesting, functionalised lithium cyclopentadienide intermediates. This is a new and highly useful synthetic approach to the synthesis of benzyl-substituted metallocenes, as seen with titanocenes **3a** and **3b** (Fig. 2). The nucleophilic addition of a hydride to the exocyclic double bond of the fulvenes **1a** or **1b**, using $LiB(Et)_3H$ as the hydride transfer reagent, resulted in the formation of the appropriate substituted lithium cyclopentadienyl intermediates, **2a** and **2b**. Two molar equivalents of either **2a** or **2b** underwent a transmetallation reaction when reacted with one molar equivalent of $TiCl_4$ in THF under reflux, to give the appropriate non-bridged substituted titanocenes, **3a** or **3b** (Scheme 1). Super Hydride is one of the most powerful nucleophilic reducing agents available, capable of reducing many functional groups [27]. It is also highly selective: The exocyclic double bonds in the fulvenes **1a** and **1b** have increased polarity, due to the inductive effects of their respective aryl groups. This increased polarity allows for selective nucleophilic attack at this double bond and not at the diene component of the fulvenes. Other examples of the nucleophilic addition of hydrides to substituted fulvenes (albeit with alkyl or unsubstituted phenyl group functionality) include the use of lithium aluminium hydride and the use of alkyllithium species as highly reactive-hydride transfer reagents [28,29].



1a **1b**

Fig. 1. The structures of fulvenes **1a** and **1b**.

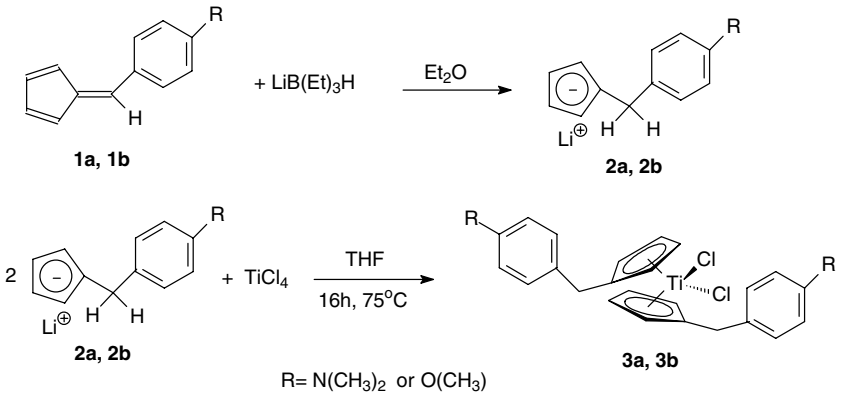
In comparison, the protonated titanocene **3c** (Fig. 2) was synthesised by reacting **3a** (dissolved in dichloromethane) with an ethereal solution of HCl. **3c** then readily precipitates out of this solution. Due to the presence of quaternary ammonium cations on the molecule, it is highly water soluble when compared to the non-ionic **3a** and **3b**.

5.2. Structural discussion

For the purpose of X-ray diffraction, suitable single crystals of **3b** were grown by vapour diffusion of pentane into a saturated solution of **3b** in dichloromethane and for **3c**, suitable single crystals were grown from a saturated chloroform solution of **3a**, to which was added an ethereal solution of HCl. The collection and refinement data for these compounds are listed in Table 2.

For **3c**, in the unit cell can be found three chloroform molecules per titanocene molecule. In comparison, the determined structure for **3b** contains no solvent molecules in the unit cell, which is an advantage for potential biological applications. Selected bond lengths of these structures are listed in Table 2.

The length of bonds between the metal centre and the carbon atoms of the cyclopentadienyl rings bound to the metal ion are similar for both **3b** and **3c** (Figs. 3 and 4).



Scheme 1. Synthesis of titanocenes **3a** and **3b**.

They vary between 233.6 and 242.8 pm for **3b** and between 233.2 and 243.7 pm for **3c**. The same applies for the carbon–carbon bonds of the cyclopentadienyl rings with bonds length between 138.1 and 141.6 pm for **3b** and for **3c** between 139.7 and 143.9 pm. There are different bond lengths and angles for the Cp and Cp′ rings for both structures. These values suggest the titanocenes have no plane of symmetry bisecting the Cl–Ti–Cl′ plane and that the structures exhibit C_2 symmetry only. This is also indicated by comparison of dihedral angles: The dihedral angle created by C(1)–C(6)–C(7)–C(8) is 101.4(3)° for **3b** and is 91.6(6)° for the salt of **3c**, whereas the dihedral angle C(1′)–C(6′)–C(7′)–C(8′) is 88.7(3)° for **3b** and 101.6(5)° for **3c**. These values also show that the benzyl ring substituents are not co-planar with the cyclopentadienyl rings, but approximately perpendicular in their arrangement. The titanium–chlorine bond length are almost identical for both structures with values between 236.3 and 237.3 pm. The Cl–Ti–Cl′ angle was measured for **3b** as 95.94(3)° and for **3c** to be 90.82(4)°.

Table 2
Selected bond lengths from the crystal structure determinations of **3b** and **3c**

	Bond length (pm) **3b**	Bond length (pm) **3c**
Ti–C(1)	238.5(3)	242.2(5)
Ti–C(2)	240.9(3)	239.5(5)
Ti–C(3)	240.3(3)	235.4(5)
Ti–C(4)	234.4(3)	235.5(4)
Ti–C(5)	237.2(3)	239.4(5)
Ti–C(1′)	242.8(3)	243.7(4)
Ti–C(2′)	241.3(3)	242.3(5)
Ti–C(3′)	238.6(3)	233.2(4)
Ti–C(4′)	233.6(3)	234.2(5)
Ti–C(5′)	235.2(3)	239.7(4)
C(1)–C(2)	141.2(4)	140.5(6)
C(2)–C(3)	139.3(4)	139.7(7)
C(3)–C(4)	140.5(4)	143.9(8)
C(4)–C(5)	140.9(4)	140.3(7)
C(5)–C(1)	140.5(4)	141.9(6)
C(1′)–C(2′)	141.7(4)	142.0(6)
C(2′)–C(3′)	140.1(4)	140.7(6)
C(3′)–C(4′)	138.1(4)	142.2(7)
C(4′)–C(5′)	141.6(4)	140.9(7)
C(5′)–C(1′)	139.6(4)	142.8(6)
Ti–Cl(1)	236.8(1)	236.3(1)
Ti–Cl(1′)	236.6(1)	237.3(1)
C(1)–C(6)	150.9(4)	151.3(6)
C(6)–C(7)	151.7(4)	152.1(6)
C(1′)–C(6′)	150.2(4)	150.9(6)
C(6′)–C(7′)	151.9(4)	149.5(6)

The two structures show similar conformations. The benzyl substituents are orientated away from each other, so that steric hindrances are minimised. The data collection for the structures was done at 100 K, so rotational isomers do not exist in the unit cell, leading to an efficient crystal lattice packing system, with adjacent benzyl rings exhibiting π stacking. In solution rotation of the cyclopentadienyl rings is possible, as indicated in the ^{1}H NMR spectra for **3b** (measured at 25 °C), where the cyclopentadienyl proton peaks resonate as a broad singlet for **3b**. The analogous *ansa*-titanocenes, with a carbon-carbon bridge, do not have freedom of rotation and as a result exists as a mixture of *cis* and *trans* isomers. The presence of stereoisomers, which could not be separated, meant that crystallisation was not possible for the majority of the *ansa*-titanocenes previously synthesised. Even when crystallisation was possible, the *trans* isomers with *S*,*S* and *R*,*R* configurations had inefficient packing in their crystal lattices, leading to low quality refinements. [21] The benzyl-substituted titanocenes do not have stereo centres at the C(6) and C(6′) positions, which represents amongst others, an advantage for crystallisation.

5.3. Cytotoxicity studies

The in vitro cytotoxicities of compounds **3a**–**c** were determined by MTT-based assays [30] involving a 48-h drug exposure period, followed by 24 h of recovery time. Compounds were tested for their activity on pig kidney carcinoma (LLC-PK) cells and the results are shown in Fig. 5. Compound **3a**, which contains dimethyl amino groups, has an IC_{50} value of 1.2×10^{-4} M, showing slightly more cytotoxicity than its *ansa* analogue. The *ansa* analogue of **3a**, compound **4**, has an IC_{50} value of

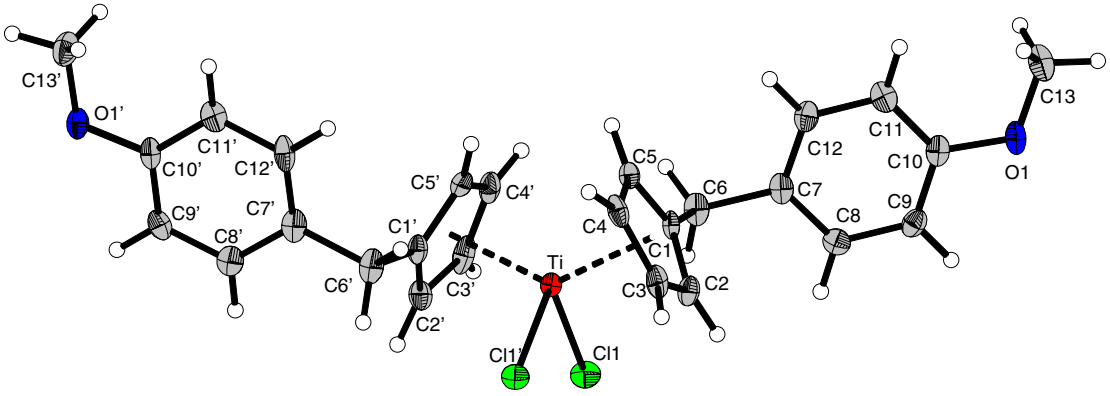


Fig. 3. Molecular structure of **3b**; thermal ellipsoids are drawn on the 50% probability level.

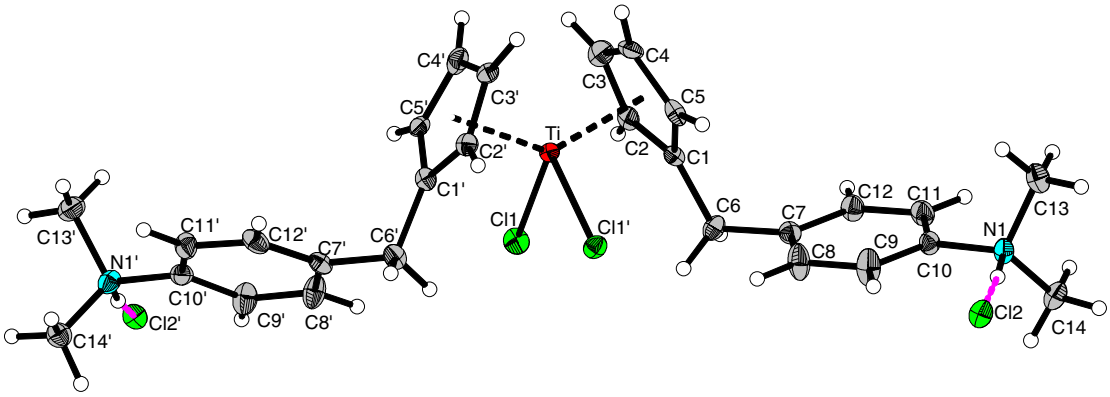


Fig. 4. Molecular structure of **3c**; thermal ellipsoids are drawn on the 50% probability level.

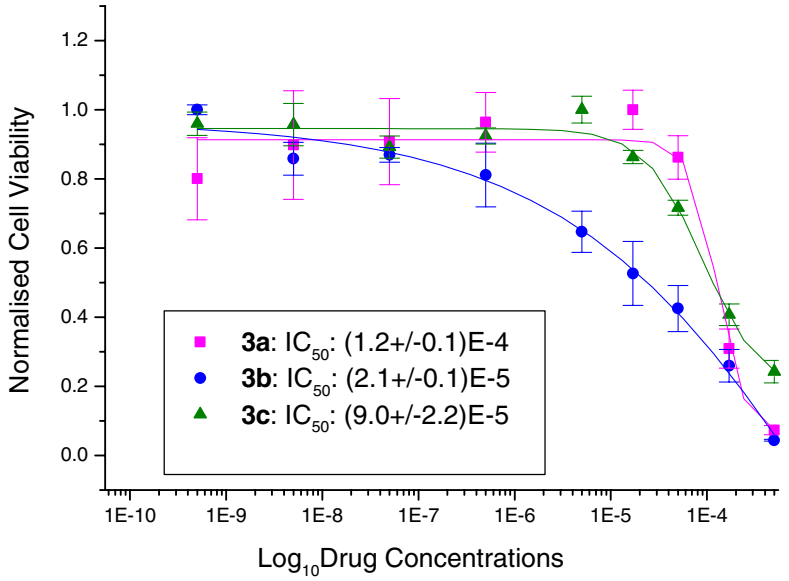


Fig. 5. Cytotoxicity curves from typical MTT assays showing the effect of compounds **3a**, **3b** and **3c** on the viability of pig kidney carcinoma (LLC-PK) cells.

2.7×10^{-4} M as is shown in Fig. 6. **3a** has over a 10-fold decrease in magnitude in terms of IC_{50} values when compared to unsubstituted titanocene dichloride (Fig. 6). Compound **3b**, which contains methoxy groups, shows the most significant IC_{50} value of 2.1×10^{-5} M. This value is approximately a hundred-fold decrease in magnitude, when compared to that of titanocene dichloride. When compared to the value for *cis*-platin, the IC_{50}

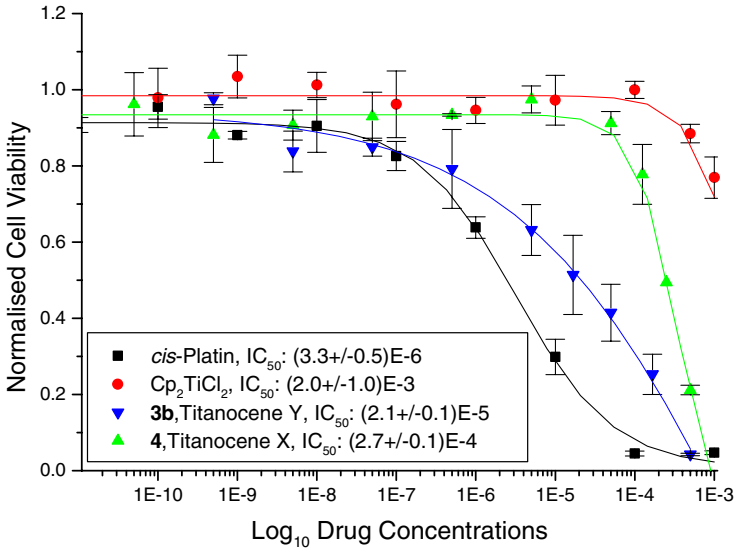


Fig. 6. Cytotoxicity curves from typical MTT assays showing the effect of *cis*-platin, Cp_2TiCl_2, **3b** and **4** on the viability of pig kidney carcinoma (LLC-PK) cells.

value for **3b** shows an increase of approximately 6.4 in the order of magnitude (Fig. 6). The protonated compound **3c**, which has as a result of the positive charges at the nitrogen centres an increased aqueous solubility, has an IC_{50} value of 9.0×10^{-5} M, which shows a slight decrease in magnitude in comparison its non-ionic precursor **3a**. However, the use of phosphate buffer solution throughout the cell testing may result in deprotonation of **3c** in solution. Therefore, the non-ionic precursor may the active species. This may explain the similarities in IC_{50} values for **3a** and **3c**. It must also be noted that the cytotoxic action of **3c** differs: at higher concentrations the compound is not as effective as the other two compounds at inducing cell death (Fig. 5). However, in terms of formulation of titanocene compounds in aqueous solution, the presence of ionic groups is desirable.

As mentioned previously, the benzyl-substituted titanocenes presented in this paper do not have stereocentres and therefore stereoisomers do not exist, unlike their *ansa* analogues. In terms of in vivo and in vitro cell testing this is advantageous. Previously, the presence of unseparated stereoisomers means that the issue of whether the compounds′ cytotoxicities are related to specific isomers was not addressed. This is not of concern in the achiral benzyl-substituted titanocenes **3a**, **3b** and **3c**.

6. Conclusions and outlook

The novel reaction of Super Hydride and phenyl-substituted fulvenes results in the formation of lithium cyclopentadienide intermediates, which is in general an interesting and applicable method towards the synthesis of a wide range of new benzyl-substituted metallocenes. By employing titanium tetrachloride, the titanocenes **3a** and **3b** were synthesised, whereas **3c** was obtained as the ionic and even better water-soluble dihydrochloride derivative of **3a**, which might be of benefit for in vitro and in vivo biological testing and applications.

All three titanocenes have a possible application as anti-cancer drugs and when tested on the LLC-PK cell line, compounds **3a**, **3b** and **3c** show substantial cytotoxicity with IC_{50} values in the range from 1.2×10^{-4} to 2.1×10^{-5} M, which represents a slight improvement when compared to the IC_{50} values of the related *ansa*-titanocenes previously synthesised and tested for cytotoxicity in this group (Fig. 6). Titanocene **3b** shows the best cytotoxicity effect and the IC_{50} values for **3a** and the protonated analogue **3c** are only slightly different as a result of the buffer system used for the cell tests (Fig. 5). Next to the increased cytotoxicity of the new titanocenes the loss of any stereocentre is in terms of a potential biological application a big advantage compared to the analogues *ansa*-titanocenes, which only could be obtained as mixtures of stereoisomers.

Compared to the unsubstituted titanocene dichloride, which reached Phase II clinical trials and failed there, the most effective titanocene **3b** shows an over a 100-fold decrease of the IC_{50} value (Fig. 6). Additionally, the cytotoxicity of **3b** is just slightly lower compared to *cis*-platin, which underlines the high potential of **3b** as a novel anti-cancer drug.

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References

[1] A. Gelasco, S.J. Lippard, Top. Biol. Inorg. Chem. 1 (1999) 1.
[2] E.R. Jamieson, S.J. Lippard, Chem. Rev. 99 (1999) 2467.
[3] N. Farrell, Y. Qu, J.D. Roberts, Top. Biol. Inorg. Chem. 1 (1999) 99.
[4] P. Köpf-Maier, H. Köpf, Chem. Rev. 87 (1987) 1137.
[5] P. Köpf-Maier, H. Köpf, Struct. Bonding 70 (1988) 103.
[6] G. Lummen, H. Sperling, H. Luboldt, T. Otto, H. Rubben, Cancer Chemother. Pharmacol. 42 (1998) 415.
[7] N. Kröger, U.R. Kleeberg, K. Mross, L. Edler, G. Saß, D.K. Hossfeld, Onkologie 23 (2000) 60.
[8] G. Mokdsi, M.M. Harding, Metal-Based Drugs 5 (1998) 207.
[9] O.R. Allen, L. Croll, A.L. Gott, R.J. Knox, P.C. McGowan, Organometallics 23 (2004) 288.
[10] R.J. Boyles, M.C. Baird, B.G. Campling, N. Jain, J. Inorg. Biochem. 84 (2001) 159.
[11] P.W. Causey, M.C. Baird, Organometallics 23 (2004) 4486.
[12] R. Meyer, S. Brink, C.E.J. van Rensburg, G.K. Joone, H. Görls, S. Lotz, J. Organomet. Chem. 690 (2005) 117.
[13] R. Teuber, G. Linti, M. Tacke, J. Organomet. Chem. 545–546 (1997) 105.
[14] F. Hartl, L. Cuffe, J.P. Dunne, S. Fox, T. Mahabiersing, M. Tacke, J. Mol. Struct. 559 (2001) 331.
[15] M. Tacke, J.P. Dunne, S. Fox, G. Linti, R. Teuber, J. Mol. Struct. 570 (2001) 197.
[16] S. Fox, J.P. Dunne, R. Dronskowski, D. Schmitz, M. Tacke, Eur. J. Inorg. Chem. (2002) 3039.
[17] J.J. Eisch, S. Xian, F.A. Owuor, Organometallics 17 (1998) 5219.
[18] J.J. Eisch, F.A. Owuor, S. Xian, Organometallics 18 (1999) 1583.
[19] K.M. Kane, P.J. Shapiro, A. Vij, R. Cubbon, A.L. Rheingold, Organometallics 16 (1997) 4567.
[20] S. Fox, J.P. Dunne, M. Tacke, J.F. Gallagher, Inorg. Chim. Acta 357 (2004) 225.
[21] M. Tacke, L.T. Allen, L.P. Cuffe, W.M. Gallagher, Y. Lou, O. Mendoza, H. Müller-Bunz, F.-J.K. Rehmann, N. Sweeney, J. Organomet. Chem. 689 (2004) 2242.
[22] 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.
[23] 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.
[24] F.-J.K. Rehmann, A.J. Rous, O. Mendoza, N.J. Sweeney, K. Strohfeldt, W.M. Gallagher, M. Tacke, Polyhedron 24 (2005) 1250.
[25] G.M. Sheldrick, SADABS Version 2.03, University of Göttingen, Germany, 2002.
[26] G.M. Sheldrick, SHELXS97 and SHELXL97, University of Göttingen, Germany, 1997.
[27] H.C. Brown, S.C. Kim, S. Krishnamurthy, J. Org. Chem. 45 (1980) 1.
[28] J.C. Leblanc, C. Moise, J. Organomet. Chem. 120 (1976) 65.
[29] G. Erker, R. Nolte, R. Aul, S. Wilker, C. Krüger, R. Noe, J. Am. Chem. Soc. 113 (1991) 7594.
[30] T. Mosmann, J. Immunol. Meth. 65 (1983) 55.