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The synthesis and cytotoxic evaluation of a series of benzodioxole substituted titanocenes

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Using 6-benzo[1,3]dioxolefulvene (1a), a series of benzodioxole substituted titanocenes was synthesized. The benzyl-substituted titanocene bis[(benzo[1,3]dioxole)-5-methylcyclopentadienyl] titanium (IV) dichloride (2a) was synthesized from the reaction of Super Hydride with 1a. An X-ray determined crystal structure was obtained for 2a. The *ansa*-titanocene {1,2-di(cyclopentadienyl)-1,2-di-(benzo[1,3]dioxole)-ethanediyl} titanium(IV) dichloride (2b) was synthesized by reductive dimerisation of 1a with titanium dichloride. The diarylmethyl substituted titanocene bis(di-(benzo[1,3]dioxole)-5-methylcyclopentadienyl) titanium(IV) dichloride (2c) was synthesized by reacting 1a with the *para*-lithiated benzodioxole followed by transmetallation with titanium tetrachloride. When titanocenes 2a–c were tested against pig kidney (LLC-PK) cells inhibitory concentrations (IC_{50}) of 2.8×10^{-4}, 1.6×10^{-4} and 7.6×10^{-5} M, respectively, were observed. These values represent improved cytotoxicity against LLC-PK, when compared with unsubstituted titanocene dichloride, but are not as impressive as values obtained for titanocenes previously synthesized using the above methods. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: anti-cancer drugs; *cis*-platin; titanocene; fulvene; Super Hydride; piperonal; benzodioxole; LLC-PK

INTRODUCTION

Despite the resounding success of *cis*-platin and closely related platinum antitumour 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. Subsequently, 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] allowed direct access to highly substituted *ansa*-titanocenes,[17–21] 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} M when tested for cytotoxic effects on the LLC-PK cell line.[22] Using this method also lead to the most cytotoxic *ansa*-titanocene [1,2-di(cyclopentadienyl)-1,2-bis(m-dimethoxyphenyl)ethanediyl] titanium dichloride (Titanocene **Z**) showing an IC_{50} value of 2.1×10^{-4} M when tested on the LLC-PK cell line.[23]

The cytotoxic effect was further increased by synthesising the analogous unbridged titanocenes.[24] Bis-[(*p*-methoxybenzyl)cyclopentadienyl] titanium(IV) dichloride (Titanocene **Y**) which has an IC_{50} value of 2.1×10^{-5} M when tested on the LLC-PK cell line, was synthesized from 6-methoxyphenylfulvene and Super Hydride ($LiBEt_3H$) followed by transmetallation with titanium tetrachloride. Increased levels of cytotoxicity on the LLC-PK cell lines

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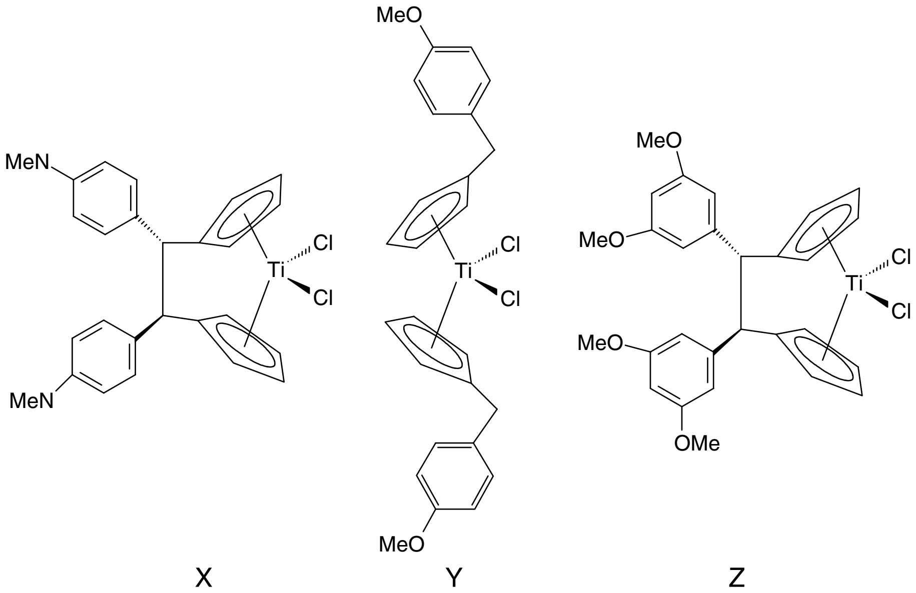

Figure 1. Titanocenes which were tested for cytotoxic activity on a range of human carcinoma cell lines.

(IC_{50} values into the medium to low 10^{-5} M range) are also observed for a new class of titanocenes: diaryl methyl substituted titanocenes most notably bis-[di-(*p*-*N*,*N*-dimethylaminophenyl)-methylcyclopentadienyl] titanium (IV) dichloride with an IC_{50} of 3.1×10^{-5} M.[25] The anti-proliferative activity of titanocenes **X, Y** and **Z** was studied in 36 human tumour cell lines[26] and in four freshly explanted human tumours using Titanocene **X**.[27] The structures of Titanocenes **X, Y** and **Z** are shown in Fig. 1.

These *in vitro* and *ex vivo* experiments showed that prostate, cervix and renal cell cancer are prime targets for this novel class of titanocenes. Preclinical trials using xenografted Caki-1 tumors and xenografted Ehrlich's ascites tumor in mice treated with **X** and **Y** have been recently reported and show an inhibition of tumour growth and tolerable side-effects.[28,29]

In this paper, we present the synthesis and preliminary cytotoxic evaluation of a series of titanocenes: an open benzyl dioxole-substituted titanocene, an *ansa*-titanocene with benzodioxole groups which are part of a chelate system and a highly substituted titanocene which contains two benzodioxole groups attached to each cyclopentadienyl ring. It was of interest to synthesize this class of titanocenes to see if inclusion of benzodioxole rings on the titanocene moiety would increase cytotoxicity, as it is similar to the methoxy functionality seen in Titanocene **Y**. The benzodioxole group is widely used in synthetic medicinal chemistry as a component of enzyme inhibitors; it is present in piperine, a known enzyme inhibitor.[30] It was also hoped that, by varying the degree of substitution, a direct relationship to increased cytotoxicity would be observed. Modification of the substitution pattern on cyclopentadienyl rings may help overcome problems associated with the stability of titanocene compounds under biological conditions, such as solubility and rate of hydrolysis.[31]

EXPERIMENTAL

Titanium tetrachloride (pure for the synthesis of **2a** and as a 1.0 M solution in toluene for the syntheses of **2b** and **2c**), Super Hydride ($LiBEt_3H$, 1.0 M solution so THF), *n*-BuLi (2.0 M solution in pentane), *t*-BuLi (1.7 M solution in pentane) and 4-bromo-1,3-benzodioxole were obtained commercially from Aldrich Chemical Co. Piperonal was received as a gift (see acknowledgements) and was purified by sublimation prior to use. Cyclopentadiene was obtained from freshly cracked dicyclopentadiene. THF and diethyl ether were dried over sodium and benzophenone. 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 a Varian 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. UV–vis spectra were recorded on a Unicam UV2 spectrometer. The gas chromatography–mass spectrometry (GCMS) spectra for fulvenes, **1a**, was measured on a Finnigan Trace GCMS 2000 Series (70 eV), using an ethyl acetate solution. Electron spray mass spectrometry was performed on a quadrupole tandem mass spectrometer (Quattro Micro, Micromass/Water's Corp., USA), using a 50% dichloromethane and 50% methanol solution. Single crystals of **2b** suitable for X-ray diffraction experiments were grown by the diffusion of pentane into saturated solutions

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of each compound in dichloromethane at room temperature. X–ray diffraction data for the two compounds was collected on a Bruker Smart Apex diffractometer. A semi-empirical absorption correction on the raw data was performed using the program SADABS.[32] The crystal structures were then solved by direct methods (SHELXS–NT 97)[33] and refined by full-matrix least squares methods against F^2. Further details about the data collection are listed in Table 1, including the reliability factors. Further details are available free of charge from the Cambridge structural database under the CCDC number 616631. *In vitro* cell tests were performed on LLC-PK cells obtained from the ATCC (American Tissue Cell Culture Collection) and maintained in Dulbecco's modified Eagle medium containing 10% (v/v) FCS (foetal calf serum), 1% (v/v) penicillin streptomycin and 1% (v/v) L-glutamine. Cells were seeded in microtitre wells at a density of 5000 cells/200 µl of medium and were incubated at 37 °C for 24 h to allow for exponential growth. Then the compounds used for the testing were dissolved DMSO and diluted with medium solution to obtain stock solutions of 5×10^{-4} M in concentration with respect to the titanocene, while the DMSO concentration did not exceed 0.7%. A concentration of 0.7% of DMSO does not have a growth effect on the LLC-PK cells. The cells were then treated with varying concentrations of the compounds and incubated for 48 h at 37 °C. Then, the solutions were removed from the wells and the cells were washed with PBS (phosphate buffer solution) and fresh medium was added to the wells. Following a recovery period of 24 h incubation at 37 °C, individual wells were treated with a 200 µl of a solution of MTT in medium, which contained 30mg of MTT in 30 ml of medium. The cells were incubated for 3 h at 37 °C. The medium was then removed and the purple formazan crystals were dissolved in 200 µl DMSO per well. Absorbance was then measured at 540 nm by a Wallac Victor (Multilabel HTS Counter) Plate Reader. Cell viability was expressed as a percentage of the absorbance recorded for control wells. The mean values used for the dose response curves represent the values obtained from four consistent MTT-based assays.

Table 1. Collection and refinement data for **2b**

Empirical formula	$C_{26}H_{22}O_4Cl_2Ti$
Formula weight	517.24
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	Pbcn
Unit cell dimensions	$a = 12.7257(9)$ Å $\quad a = 90°$
	$b = 6.8050(5)$ Å $\quad b = 90°$
	$c = 24.7328(18)$ Å $\quad g = 90°$
Volume	2141.8(3) $Å^3$
Z	4
Density (calculated)	1.604 mg m^{-3}
Absorption coefficient	0.683 mm^{-1}
$F(000)$	1064
Crystal size	$0.40 \times 0.30 \times 0.15$ mm^3
Theta range for data collection	1.65–29.00°.
Index ranges	$-17 \leq h \leq 17, -9 \leq k \leq 9,$ $-32 \leq l \leq 33$
Reflections collected	19 911
Independent reflections	2839 [R(int) = 0.0358]
Completeness to theta = 29.00°	99.8%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9044 and 0.7480
Refinement method	Full-matrix least-squares on F^2
Data/restraints/ parameters	2839/0/194
Goodness-of-fit on F^2	1.151
Final R indices [$I > 2$ sigma(I)]	$R^1 = 0.0439$, $wR^2 = 0.0993$
R indices (all data)	$R^1 = 0.0495$, $wR^2 = 0.1024$
Largest difference peak and hole	0.511 and −0.262 e $Å^{-3}$

6-benzo[1,3]dioxolefulvene (1a)

To a solution of piperonal (4.00 g, 26.6 mmol) in THF (20 ml) was added cyclopentadiene (4.0 ml, 60.5 mmol) and pyrolidine (3.4 ml, 40.7 mmol), under an argon atmosphere. The reaction was left stirring for 8 days, during which time the progress of the reaction was monitored by TLC analysis. The solution turned red in colour during this time. The reaction was then quenched with acetic acid (2.5 ml, 43.7 mmol). The organic layer was washed three times with water and the aqueous washings were then extracted with CH_2Cl_2. The combined organic layers were then washed with brine and then dried over magnesium sulfate. The solvent was removed by rotary evaporation to yield a crude red oil. The crude product was purified by passing through a silica column using a gradient of pentane/dichloromethane as the eleunt and the solvent was removed by rotary evaporation to give a yield of 4.12 g of an orange solid (21 mmol, 79%). Melting Point: 45–47 °C.

^{1}H NMR (δ ppm $CDCl_3$, 400 MHz): 7.16 [C_6H_3(O-CH_2-O), J 1.5Hz, 1H, d]; 7.11 [$C_5H_4 = CH$, 1H, s]; 7.08 [C_6H_3(O-CH_2-O), J_{AB} 1.5 Hz, $J_{A'B'}$ 1.5 Hz, 1H, dd]; 6.84 [C_6H_3(O-CH_2-O), J 8.1 Hz, 1H, d]; 6.60 [C_5H_4, 2H, m]; 6.48 [C_5H_4, J 5.1 Hz, 1H, d]; 6.30 [C_5H_4, J_{AB} 1.8 Hz, J_{BC} 1.8 Hz, J_{AC} 3.6 Hz, $J_{A'B'}$ 1.5 Hz, $J_{B'C'}$ 1.5 Hz, $J_{A'C'}$ 3.3 Hz, $J_{AA'}$ 4.8 Hz, 1H, tt]; 6.02 [O-CH_2-O, 2H, s].

^{13}C NMR (δ ppm $CDCl_3$, 100 MHz): 148.8, 148.2, 143.7, 138.2, 135.2, 131.1, 130.09, 127.43, 126.3, 119.73, 110.05, 108.64 [$C_5H_4 = CH$-C_6H_3(O-CH_2-O)] 101.5 ppm [O-CH_2-O].

IR absorptions (KBr cm^{-1}): 2956 (b), 2901 (b), 1618 (s), 1594 (s), 1503 (s), 1448 (s), 1260 (s), 1037 (s), 819 (s), 762 (s).

UV–vis (CH_2Cl_2): λ 252 nm (ε 16 760 M^{-1} cm^{-1}), λ 308 nm (ε 14 680 M^{-1} cm^{-1}), λ_{max} 358 nm (ε 33 880 M^{-1} cm^{-1}). GCMS: *m/z* 198.0 [M] (83.5% relative abundance). Anal. calcd for $C_{13}H_{10}O_2$: theory, C, 78.77; H, 5.08; found, C; 78.68, H; 5.12.

Bis[(benzo[1,3]dioxole)-5-methylcyclopentadienyl] titanium(IV) dichloride, $[(\eta^5\text{-}C_5H_4)\text{-}CH_2\text{-}C_6H_4(OCH_2O)]_2TiCl_2$ (2a)

$LiBEt_3H$ (11.0 ml of a 1.0 M solution of in THF) was concentrated by removal of the solvent by heating it to 90 °C under reduced pressure of 10^{-2} mbar for 2 h. The concentrated reagent was dissolved in diethyl ether (30 ml) and **1a** (1.87 g, 9.4 mmol) in diethyl ether (30 ml) was added to give a red solution. The solution was left to stir for 2 h to give a white precipitate of the lithium cyclopentadienide intermediate, during which time the solution changed colour from orange/red to yellow. Pentane was added to the solution and more precipitate fell out of solution. The precipitate was filtered onto a frit, was washed with diethyl ether and pentane and was dried briefly *in vacuo* and was then transferred to a Schlenk flask under argon. 1.25 g (6.07 mmol, 62% yield) of the intermediate was obtained and was dissolved in THF (30 ml) and added to a solution of $TiCl_4$ (0.33 ml, 3.04 mmol) in THF (60 ml) at 0 °C to give a dark red solution, which was refluxed (16 h). The solution was then cooled and the solvent was removed under reduced pressure. The remaining residue was extracted with CH_2Cl_2 (30 ml) and filtered through celite to remove the remaining LiCl. The brown filtrate was filtered twice more by gravity filtration. The solvent was removed under reduced pressure to yield 0.93 g of an orange solid (1.8 mmol, 59% yield).

^1H NMR (δ ppm $CDCl_3$, 400 MHz): 6.74 [C_6H_3(O-CH_2-O), *J* 0.4 Hz, 2H, d]; 6.68 [C_6H_3(OCH_2-O), J_{AB} 2.0 Hz, $J_{A'B'}$ 2.0 Hz, $J_{AA'}$ 4.4 Hz, 2H, dd]; 6.66 [C_6H_3(O-CH_2-O), *J* 1.6 Hz, 2H, d]; 6.33 [C_5H_4, 8H, m]; 5.93 [O-CH_2-O, 4H, s]; 4.01 [C_5H_4-CH, 2H, s].

^{13}C NMR (δ ppm $CDCl_3$, 100 MHz): 145.2, 136.4 132.2, 121.4, 120.9, 114.7, 108.4, 107.3 [C_6H_3(O-CH_2-O) and C_5H_4]; 99.9 [O-CH_2-O]; 35.6 [C_5H_4-CH]. IR absorptions (KBr cm^{-1}): 3093 (b), 2890 (b), 1505 (s), 1488 (s), 1441 (m), 1267 (b), 1040 (m), 930 (m), 820 (m). MS (ES-): *m/z* 550.9 $[M + Cl]^-$ (100% relative abundance).

UV–vis (CH_2Cl_2): λ 260 nm (ε 18 980 M^{-1} cm^{-1}), λ 286 nm (ε 16 300 M^{-1} cm^{-1}), λ_{max} 402 nm (ε 1860 M^{-1} cm^{-1}). Anal. calcd for $C_{26}H_{22}O_4Cl_2Ti$: theory, C, 60.35; H, 4.29; found, C, 60.03; H, 4.68.

{1,2-Di(cyclopentadienyl)-1,2-di-(benzo[1,3]dioxole)-ethanediyl} titanium(IV) dichloride, $[1,2\text{-}\{(OCH_2O)C_6H_4\}_2C_2H_2\{\eta^5\text{-}C_5H_4\}_2]TiCl_2$ (2b)

To a solution of $TiCl_4$ (5.25 ml of a 1.0 M solution) dissolved in a 90% toluene–10% THF mixture (70 ml) was added *n*-BuLi (5.5 ml of a 2.0 M solution) dropwise at—78 °C. The solution turned brown then black, was allowed warm up to room temperature and stirred overnight. **1a** (2.08g, 10.49 mmol) was dissolved in a 90% toluene–10%THF mixture (30 ml) and added to the $TiCl_2$ solution at 0 °C. The solution was refluxed (16 h) and then allowed to cool. The solvent was removed *in vacuo* and the remaining residue was extracted with CH_2Cl_2 (30 ml) and filtered through celite to remove the remaining LiCl. The brown filtrate was filtered twice more by gravity filtration. The solvent was removed under reduced pressure to yield 2.82 g of a brown solid (5.4 mmol, 51% yield).

^1H NMR (δ ppm $CDCl_3$, 400 MHz): 7.23, 7.16 [C_6H_3 (O-CH_2-O), 2H, s, (*cis* and *trans*)]; 6.98, 6.85, 6.70–6.60 [C_6H_3(O-CH_2-O), 4H, m, (*cis* and *trans*)]; 6.25 [C_5H_4, J_{AB} 2.4 Hz, J_{AC} 2.7 Hz, $J_{A'B'}$ 2.7 Hz, $J_{A'C'}$ 2.7 Hz, 6H, tt]; 6.12 [C_5H_4, J_{AB} 2.4 Hz, 2H, d]; 5.93–5.91 [O-CH_2-O, 4H, m]; 5.35 [C_5H_4-CH, 1H, s, *cis*]; 4.66 [C_5H_4-CH, 1H, s, *trans*].

^{13}C NMR (δ ppm $CDCl_3$, 100 MHz): 146.8, 145.3, 137.2, 132.9, 131.2, 129.9, 128.0, 127.2, 126.9, 121.0, 120.1, 116.3, 116.0, 108.6, 108.2, 107.3, 107.1 [C_6H_3(O-CH_2-O) and C_5H_4 *cis* and *trans*]; 100.2, 100.1 [O-CH_2-O *cis* and *trans*]; 53.9 [C_5H_4-CH *cis*]; 51.1 [C_5H_4-CH *trans*].

IR absorptions (KBr cm^{-1}): 2951 (s), 2918 (b), 1501 (s), 1484 (s), 1440 (m), 1235 (w), 1037 (s), 927 (m), 809 (m). UV–vis (CH_2Cl_2): λ 258 nm (ε 9560 M^{-1} cm^{-1}), λ 286 nm (ε 6820 M^{-1} cm^{-1}), λ 354 nm (ε 1420 M^{-1} cm^{-1}), λ_{max} 406 nm (ε 740 M^{-1} cm^{-1}). MS (ES-): *m/z* 551.0 $[M + Cl]^-$ (25% relative abundance). Anal. Calcd for $C_{26}H_{20}O_4Cl_2Ti$: theory, C, 60.59; H, 4.17; found, C; 3.91, H; 4.11.

Bis(di-(benzo[1,3]dioxole)-5-methylcyclopentadienyl) titanium(IV) dichloride, $[(\eta^5\text{-}C_5H_4)\text{-}CH\text{-}(C_6H_4(OCH_2O))_2]_2TiCl_2$ (2c)

4-Bromo-1,3-benzodioxole (0.6 ml, 5 mmol) previously purified by distillation under reduced pressure (7×10^{-2} mbar, b.p. 40–42), was dissolved in THF (15 ml). To it was added *t*-BuLi (3.5 ml of a 1.7 M solution) at −78 °C. The solution was then allowed to warm to 0 °C and it was stirred at this temperature for 20 min. Then **1a** (1.00 g, 5 mmol) in THF (20 ml) was added to the solution at −78 °C. The resultant dark red solution was allowed warm to r.t. and was then stirred for 40 min at r.t. $TiCl_4$ (2.5ml of a 1.0 M solution) was added to the solution at 0 °C to give a black solution, which was then refluxed (16 h). The solution was cooled. The solvent was removed *in vacuo* and the remaining residue was extracted with CH_2Cl_2 (30 ml) and filtered through celite to remove the remaining LiCl. The black filtrate was filtered three times more by gravity filtration and the solvent was removed *in vacuo*. The remaining residue was extracted with pentane (15 ml) and diethyl ether (15 ml); 0.78 g of a black solid was obtained which was dried at the pump (1.1 mmol, 46% yield).

^1H NMR (δ ppm $CDCl_3$, 400 MHz): 7.26 [C_6H_3(O-CH_2-O), 4H, s]; 6.98–6.54 [(C_6H_3(O-CH_2-O), 8H, m]; 6.31 [C_5H_4, J_{AB} 2.4 Hz, $J_{AA'}$ 2.4 Hz, $J_{A'B'}$ 2.4 Hz, 4H, dd]; 6.22 [C_5H_4, J_{AB} 2.8 Hz, J_{AC} 2.4 Hz, 4H, t]; 5.99–5.91 [C_6H_3(O-CH_2-O), 8H, m]; 5.09 [C_5H_4-CH-(C_6H_3(O-CH_2-O))$_2$, 2H, s].

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^{13}C NMR (δ ppm $CDCl_3$, 100 MHz): 147.7, 146.3, 137.1 122.2, 120.4, 119.7, 109.3, 108.7, 108.1 107.5, 101.1, 100.9, 100.6 [C_6H_3(O-CH_2-O) and C_5H_4]; 101.9 [O-CH-O]; 51.5 [C_5H_4-CH].

IR absorptions (KBr cm^{-1}): 2885 (b), 1501 (s), 1482 (s), 1438 (m), 1235 (b), 1034 (s), 927 (m). UV–vis (CH_2Cl_2): λ 290 nm (ε 13 940 M^{-1} cm^{-1}), λ_{max} 393 nm (ε 4260 M^{-1} cm^{-1}). MS (ES-): m/z 793.0 $[M + Cl]^-$ (22% relative abundance). Anal. calcd for $C_{34}H_{28}O_8Cl_2Ti$: theory, C, 59.76; H, 4.13; found, C, 59.06; H, 4.55.

RESULTS AND DISCUSSION

Synthesis

The fulvene **1a** (Fig. 2), used in the synthesis of the three titanocenes, was synthesized using the general method of condensation of cyclopentadienes and aldehydes in the presence of a base catalyst, i.e. pyrrolidine.[34] According to this literature method methanol is usually the most appropriate solvent to use, as the reactions proceed to completion more rapidly. However, the yields obtained for **1a** using methanol as the solvent were extremely poor, perhaps due to the conversion of the dioxole group into a diol in the acidic conditions. The optimum conditions were achieved using THF as the solvent and allowing the reaction mixture to stir over a period of 8 days at room temperature.

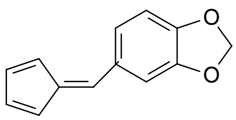
1a

Figure 2. Structure of fulvene **1a**.

For the synthesis of the three titanocenes, the following three established synthetic routes were used: for **2b**, a benzyl substituted lithium cyclopentadienide intermediate was formed by hydride addition to **1a** using the reagent Super Hydride. Two molar equivalents of this intermediate then underwent transmetallation with $TiCl_4$ to yield the benzyl titanocene. For **2a**, $TiCl_2$ was formed *in situ* by reacting *n*-BuLi with $TiCl_4$ in THF/Toluene solution. Addition of two molar equivalents of **1a** resulted in a reductive dimerisation and the formation of the *ansa* (bridged) link between the cyclopentadienyl rings of the titanocene. For **2c** the *para*-lithiated benzodioxole (formed by lithium-halide exchange using *t*-BuLi) added to **1a** to give the diaryl methyl lithium cyclopentadienyl intermediate, which then underwent transmetallation with $TiCl_4$ to yield the appropriate titanocene. The syntheses and structures of **2a, 2b** and **2c** are shown in Fig. 3 and Schemes 1–3, respectively.

Structural discussion

A suitable single crystal of titanocene **2b** (Fig. 4) for X-Ray diffraction experiments was obtained by the slow diffusion of pentane into a saturated solution of the compound. The collection and refinement data for **2b** is shown in Table 1.

Selected bond lengths and angles are shown in Table 2. The lengths of the bonds between the metal centre and the carbon atoms of the cyclopentadienyl rings bound to the metal centre are within the expected ranges for titanocenes. The Ti–carbon bonds vary between 233.2 and 242.2 pm and the carbon–carbon bonds of the cyclopentadienyl rings vary between 140.3 and 141.7 pm. The bond angles listed in Table 2

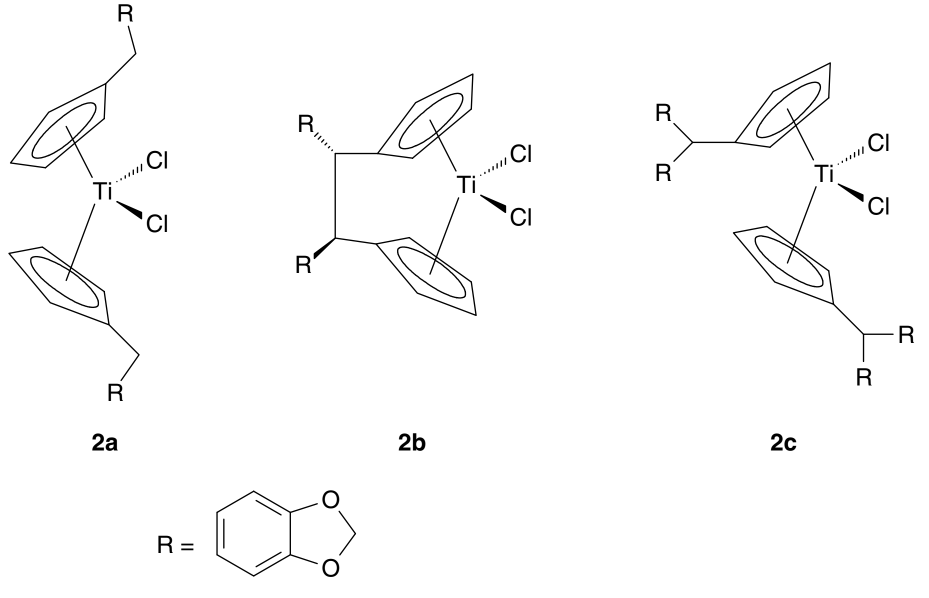

Figure 3. The structures of titanocenes **2a–c**.

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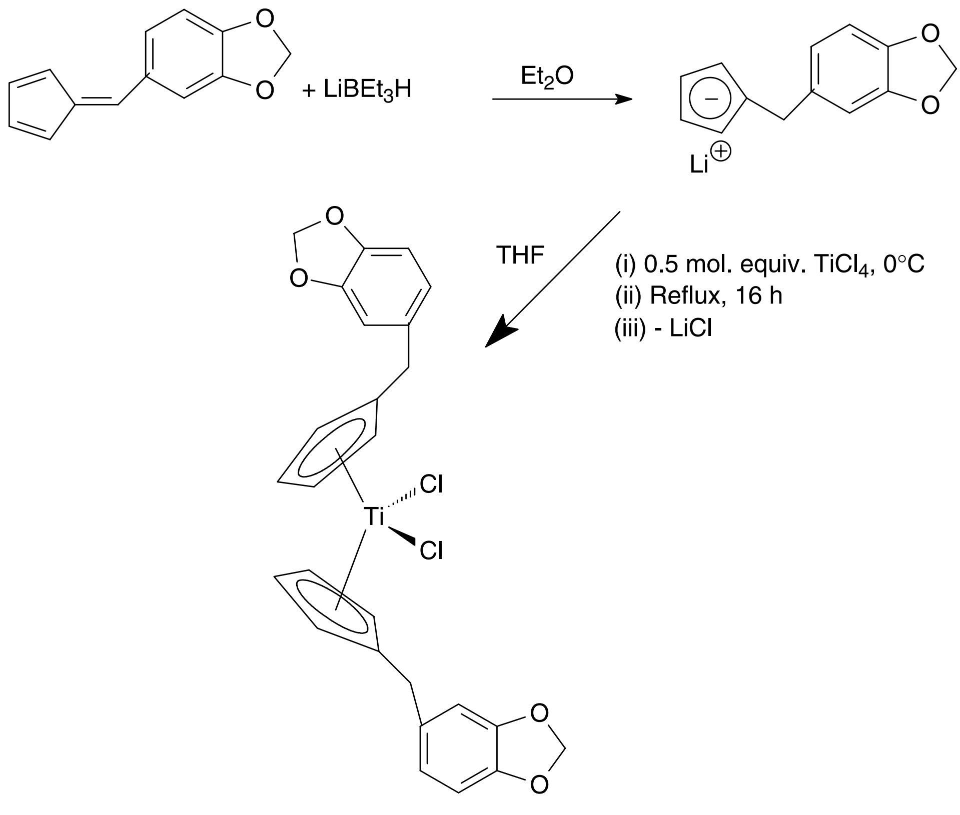

2a

Scheme 1. Synthesis of titanocene **2a**.

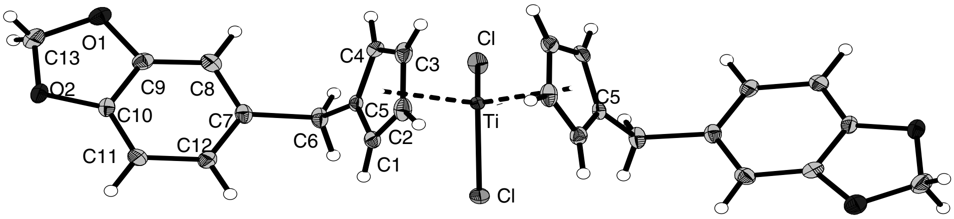

Figure 4. The molecular structure of **2b**, thermal ellipsoids are drawn on the 50% probability level.

also are also within the expected ranges and are indicative of the bent-metallocene geometry adopted by titanocenes.

The molecule occupies a twofold axis. Thus the corresponding bond lengths for the Cp and Cp′ rings as well as the Ti–Cl and Ti–Cl′ bonds are identical. The substituent on C9 breaks the bisecting *pseudo* mirror planes through the TiCl$_2$ moiety. The benzodioxole groups are not co-planar to their corresponding attached cyclopentadienyl ring, as indicated with a dihedral angle of −89.5° along C(5)–C(6)–C(7)–C(12). The packing of the molecules in the unit cell is such that that the benzodioxole rings are orientated away from each other in each molecule. No solvent molecules are present in the crystal structure. In terms of potential biological applications this is advantageous as the compounds are known to be of high purity and that any cytotoxic effects are not due to the presence of chlorinated solvents.

Cytotoxicity studies

The *in vitro* cytotoxicity of compounds **2a**–**c** was determined by MTT-based assays[35] involving a 48 h drug exposure period, followed by 24 h of recovery time. Compounds were tested for their activity on pig kidney (LLC-PK) cells and the results are shown in Fig. 5. In comparison to unsubstituted titanocene dichloride (with the previously determined IC$_{50}$ value of 2.0×10^{-3} M), the three compounds showed increased cytotoxicity. **2a**–**c** had IC$_{50}$ values of 1.6×10^{-4}, 1.5×10^{-4} and 9.1×10^{-5} M, respectively. When compared with titanocene dichloride, for **2a**, the IC$_{50}$ value

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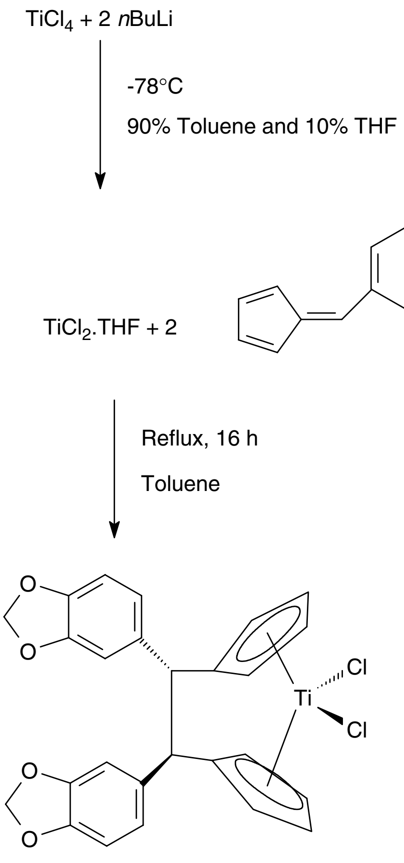
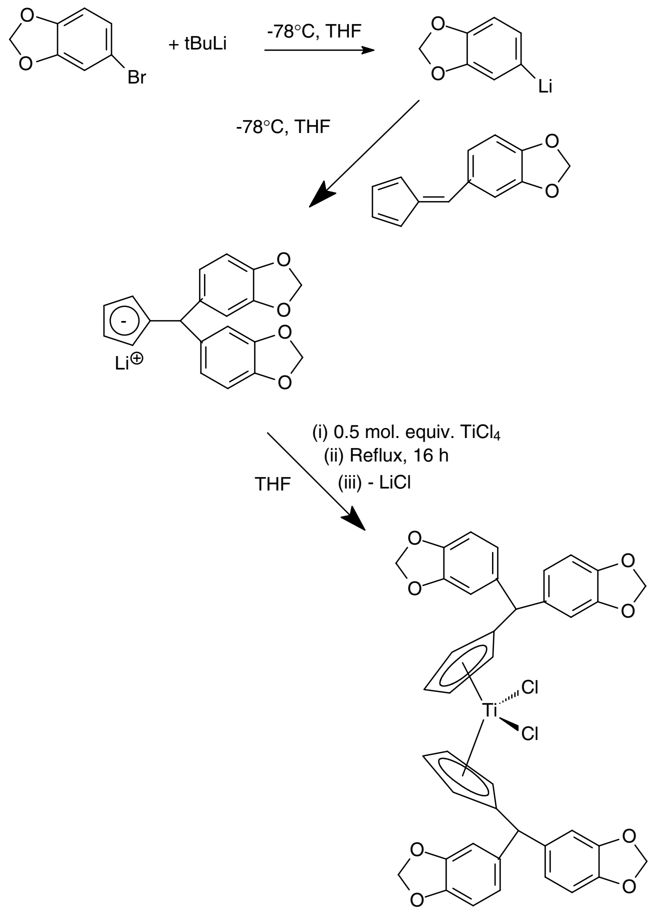


2b

Scheme 2. Synthesis of titanocene **2b**.



2c

Scheme 3. Synthesis of titanocene **2c**.

shows an almost seven-fold decrease in magnitude, for **2b** the decrease in magnitude is approximately 13 times and, most notably, for **2c** the IC_{50} represents a decrease in magnitude of approximately 26 times. As can be seen in the dose–response curve for and **2a,** the cell viability is almost eliminated at a drug concentration of 5×10^{-4} M, but even at concentrations of 10^{-4} M and lower the compound shows little activity. It appears that **2a** had a well-defined switch off point at which cytotoxicity is not observed. For **2b** the dose–response curve differs, in that at 10^{-4} M cell viability is reduced by more than 50%, showing that at concentrations into the higher 10^{-5} M region appreciable reduction in cell viability occurs. Previously a structural activity relationship was suggested for the benzyl substituted titanocenes in relation to their *ansa* analogues, in that their IC_{50} values were lower than their corresponding *ansa* analogues. For **2a** and **2b**, this is not so. For **2c**, the dose–response curve shows an improvement; cell viability is almost eliminated at 10^{-4} M and in the 10^{-5} M region there is an increased cytotoxic response in comparison to **2a** and **2b**.

When compared with Titanocene **Y** and *cis*-platin (with the previously determined IC_{50} values of 2.1×10^{-5} and 3.3×10^{-6} M, respectively), the IC_{50} values for the three compounds are not impressive. In comparison to Titanocene **Y,** the IC_{50} value for **2a** is approximately 13 times more, for **2b** it is an approximately eight-fold increase and for **2c** it is an approximately six-fold increase. Out of the three compounds, the most impressive, **2c** is approximately 20 times less effective when compared with *cis*-platin.

CONCLUSIONS

Using three established synthetic methods, **2a–c** were synthesized starting from fulvene **1a** and subsequently tested for cytotoxicity on the LLC-PK cell line. The IC_{50} values obtained do not show a similar level of cytotoxicity when compared with Titanocene **Y** and are unlikely candidates for studies done *in vivo*. Furthermore, it shows that that the presence of the benzodioxole group does not promote a significantly high level of cytotoxicity on the titanocene moiety in comparison to the methoxy-phenyl group. The presence of several benzodioxole groups on **2c** leads only to a small increase in cytotoxicity, which gives further evidence for the lack of activity due to the benzodioxole groups. Therefore,

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Table 2. Selected bond lengths and angles for **2b**

	Bond length (pm), Angles (deg) **2b**
Ti–C(1)	238.22(2)
Ti–C(2)	233.17(2)
Ti–C(3)	236.34(2)
Ti–C(4)	240.35(2)
Ti–C(5)	242.16(2)
Ti–C(1′)	238.23(2)
Ti–C(2′)	233.17(2)
Ti–C(3′)	236.34(2)
Ti–C(4′)	240.35(2)
Ti–C(5′)	242.16(2)
Ti-Cent	205.8(1)
C(1)–C(2)	140.4(3)
C(2)–C(3)	140.6(3)
C(3)–C(4)	140.3(3)
C(4)–C(5)	141.7(2)
C(5)–C(1)	140.9(3)
Ti–Cl(1)	238.22(2)
Ti–Cl(1′)	238.23(2)
C(5)–C(6)	149.2(3)
Cl-Ti-Cl′	77.2
Cent-Ti-Cent′	131.7
Cent-Ti-Cl	106.1
Cent-Ti-Cl′	106.4

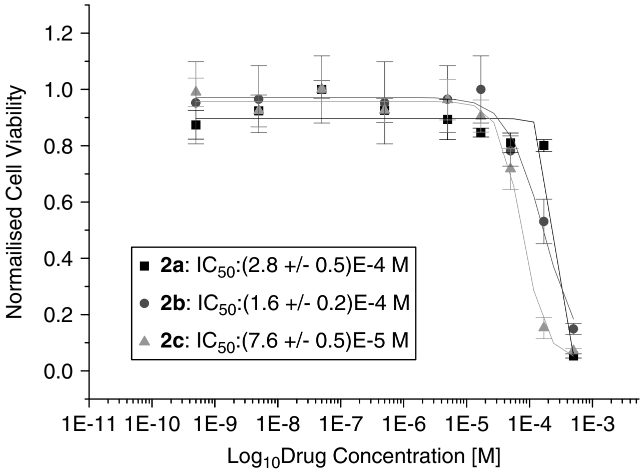

Figure 5. Cytotoxicity curves from typical MTT assays showing the effect of compounds **2a, 2b** and **2c** on the viability of pig kidney (LLC-PK) cells.

the change from the methoxy group as seen in Titanocene **Y** to an ethereal group as seen in the benzodioxole system does not favourably alter the IC_{50} value. This shows that a rational approach to modifying Titanocene **Y** did not result in increased cytotoxicity for **2a–c**.

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