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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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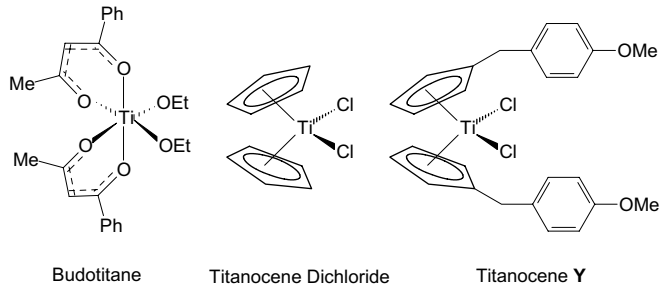


Fig. 1. Structures of Budotitane, Titanocene Dichloride and Titanocene Y.

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.

Table 1
Crystallographic refinement data for titanocenes **3a–c**

Identification code	**3a**	**3b**	**3c**
Empirical formula	$C_{26}H_{26}Cl_2O_2Ti$	$C_{26}H_{26}O_2Cl_2Ti$	$C_{28}H_{30}O_4Cl_2Ti$
Formula weight	489.27	489.27	549.32
Temperature (K)	100(2)	100(2)	100(2)
Wavelength (Å)	0.71073	0.71073	0.71073
Crystal system	Monoclinic	Triclinic	Monoclinic
Space group	*C*2/*c* (#15)	$P\bar{1}$ (#2)	*C*2 (#5)
Unit cell dimensions			
a (Å)	24.011(3)	6.6699(10)	22.168(5)
α (°)	90	111.484(3)	90
b (Å)	6.8180(8)	13.4311(19)	6.4758(15)
β (°)	113.259(2)	100.355(4)	101.074(4)
c (Å)	14.6413(18)	13.927(2)	8.975(2)
γ (°)	90	95.618(4)	90
Volume (Å^3)	2202.1(5)	1123.7(3)	1264.5(5)
Z	4	2	2
D_{calc} (Mg/m^3)	1.476	1.446	1.443
Absorption coefficient (mm^{-1})	0.653	0.640	0.583
F(000)	1016	508	572
Crystal size (mm^3)	0.30 × 0.20 × 0.05	0.50 × 0.05 × 0.05	0.80 × 0.15 × 0.01
Theta range for data collection (°)	1.85–29.00	1.62–23.00	1.87–28.25
Index ranges	$-32 \leqslant h \leqslant 32$, $-9 \leqslant k \leqslant 9$, $-19 \leqslant l \leqslant 19$	$-7 \leqslant h \leqslant 7$, $-14 \leqslant k \leqslant 14$, $-15 \leqslant l \leqslant 15$	$-29 \leqslant h \leqslant 29$, $-8 \leqslant k \leqslant 6$, $-11 \leqslant l \leqslant 11$
Reflections collected	22 773	7395	6065
Independent reflections [R_{int}]	2930 [0.0333]	3111 [0.0407]	2299 [0.0367]
Completeness to theta = 29.00° (%)	99.9	99.4	98.3
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents	Semi-empirical from equivalents
Maximum and minimum transmission	0.9681 and 0.6465	0.9687 and 0.8840	0.9942 and 0.6685
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	2930/0/193	3111/0/282	2299/1/161
Goodness-of-fit on F^2	1.075	1.118	1.059
	$R_1 = 0.0336$, $wR_2 = 0.0849$	$R_1 = 0.0539$, $wR_2 = 0.1167$	$R_1 = 0.0412$, $wR_2 = 0.0960$
R indices (all data)	$R_1 = 0.0365$, $wR_2 = 0.0866$	$R_1 = 0.0682$, $wR_2 = 0.1234$	$R_1 = 0.0480$, $wR_2 = 0.0997$
Largest difference in peak and hole (e Å^{-3})	0.493 and −0.226	0.691 and −0.318	0.699 and −0.417

^{1}H NMR (δ ppm $CDCl_3$, 300 MHz): 3.85 [s, 3H, C_6H_4–OCH_3], 6.43 [m, 2H, C_5H_4], 6.63 [s, 2H, C_5H_4], 6.87 [s, 1H, C_5H_4–CH], 6.89 [d, 1H, $J = 8.4$ Hz, C_6H_4], 6.99 [t, 1H, $J = 7.5$ Hz, C_6H_4], 7.33 [t, 1H, $J = 7.2$ Hz, C_6H_4], 7.58 [d, 1H, $J = 7.5$ Hz, C_6H_4].

^{13}C NMR (δ ppm $CDCl_3$, 100 MHz, proton decoupled): 55.8 [C_6H_4–OCH_3], 110.9, 120.9, 120.9, 127.2, 130.8, 130.9, 132.7, 134.0, 135.1, 145.2, 148.3, 158.8.

IR absorptions (CH_2Cl_2, cm^{-1}): 3054, 1593, 1487, 1248, 760, 702.

UV–Vis (CH_2Cl_2, nm): 248 (ε 1700), 269 (ε 2600), 286 (ε 2500), 307 (ε 2400), 320 (ε 2400), λ_{max} 542 (weak).

Anal. Calc. for $C_{13}OH_{12}$: C, 84.8; H, 6.6. Found: C, 84.8; H; 6.5%.

2.2.2. Synthesis of bis-[(2-methoxy-benzyl) cyclopentadienyl]titanium(IV) dichloride, [(η^5-C_5H_4–CH_2–C_6H_4–OCH_3)]$_2$$TiCl_2$ (**3a**)

16.1 ml (16.1 mmol) of 1 M solution of Super Hydride ($LiBEt_3H$) in THF was concentrated by removal of the solvent by heating it to 60 °C under reduced pressure of 10^{-2} mbar for 40 min and then to 90 °C for 20 min in a Schlenk flask. The concentrated Super Hydride was dissolved in 30 ml of dry diethyl ether to give a cloudy white suspension. 2.29 g (12.4 mmol) of the red oil 6(2-methoxyphenyl) fulvene was added to a Schlenk flask and was dissolved in 30 ml dry diethyl ether to give a red solution. The red fulvene solution was transferred to the Super Hydride solution *via cannula*. The solution was left to stir for 6 h to give a white precipitate of the lithium cyclopentadienide intermediate and the solution had changed colour from orange/red to faint yellow. The precipitate was filtered on to a frit and was washed with 15 ml of diethyl ether. The white precipitate was dried briefly under reduced pressure and was transferred to a Schlenk flask under nitrogen. 1.39 g (7.22 mmol, 58.1% yield) of the lithiated cyclopentadienide intermediate was obtained. 3.60 ml (3.60 mmol) of titanium tetrachloride was dissolved in 60 ml of dry THF to give a yellow solution in a Schlenk flask. The lithium cyclopentadienide intermediate was dissolved in 30 ml of dry THF to give a colourless solution. The solution of titanium tetrachloride solution was added to the lithium cyclopentadienide intermediate solution *via cannula* to give a

dark red solution. The dark red titanium solution was refluxed for 16 h at 85 °C. The solution was then cooled and the solvent was removed under reduced pressure. The remaining brown residue was extracted with trichloromethane (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 1.35 g of a brown/black solid (2.76 mmol, 76.4% yield).

^{1}H NMR (δ ppm $CDCl_3$, 400 MHz): 3.82 [s, 6H, C_6H_4–OCH_3], 4.01 [s, 4H, C_5H_4–CH_2], 6.31 [s, 4H, C_5H_4], 6.38 [s, 4H, C_5H_4], 6.85 [d, 2H, $J = 5.7$ Hz, C_6H_4–OCH_3], 6.89 [t, 2H, $J = 5.4$ Hz, C_6H_4–OCH_3], 7.14 [d, 2H, $J = 5.4$ Hz, C_6H_4–OCH_3], 7.21 [t, 2H, $J = 5.7$ Hz, C_6H_4–OCH_3].
^{13}C NMR (δ ppm $CDCl_3$, 125 MHz, proton decoupled): 31.7 [C_6H_4–OCH_3], 55.3 [C_5H_4–CH_2], 110.4, 116.5, 120.6, 122.6, 127.9, 128.4, 130.5, 136.5, 157.3.
IR absorptions (KBr, cm^{-1}): 3076, 2360, 1585, 1489, 1437, 1250, 1105, 1026, 825, 752, 681.
UV–Vis (CH_2Cl_2, nm): 217 (ε 9500), 229 (ε 7100), 263 (ε 6800), λ_{max} 525 (weak).
Micro Anal. Calc. for $TiC_{26}O_2H_{26}Cl_2$: C, 63.8; H, 5.3; Cl, 14.4. Found: C, 63.5; H, 5.2; Cl, 13.6%.

2.2.3. Synthesis of 6(3-methoxy-phenyl) fulvene, C_5H_4–CH–C_6H_4–OCH_3 (**1b**)

3.01 g (22.3 mmol) of 3-methoxy-benzaldehyde was dissolved in 80 ml of methanol to give a colourless solution. 3.90 ml (47.2 mmol) of fresh cracked cyclopentadiene was added to the reaction solution, which remained colourless. 3.0 ml (36.0 mmol) of pyrrolidine was then added to the solution. The solution slowly changed colour from colourless to yellow and finally reached a red/orange colour. The reaction was left to stir for 28 h. 2.20 ml of acetic acid was added to quench the reaction. 100 ml of water was added to the reaction mixture and the organic product was extracted by 4 × 30 ml ether. The ether solution was dried with 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 3.92 g (95.5% yield, 21.3 mmol) of a red oil.

^{1}H NMR (δ ppm $CDCl_3$, 300 MHz): 3.83 [s, 3H, C_6H_4–OCH_3], 6.41 [m, 2H, C_5H_5], 6.75 [d, 2H, $J = 2.1$ Hz, C_5H_5], 6.79 [s, 1H, C_5H_5–CH], 6.89 [d, 1H, $J = 1.5$ Hz, C_6H_4–OCH_3], 7.11 [s, 1H, C_6H_4–OCH_3], 7.18 [s, 1H, C_6H_4–OCH_3], 7.23 [t, 1H, $J = 3.3$ Hz, C_6H_4–OCH_3].
^{13}C NMR (δ ppm $CDCl_3$, 100 MHz, proton decoupled): 55.9 [C_6H_4–OCH_3], 110.8, 120.8, 120.9, 126.2, 127.1, 130.8, 130.8, 132.3, 132.7, 134.0, 135.0, 145.2.
IR absorptions (CH_2Cl_2, cm^{-1}): 3050, 2838, 1625, 1597, 1465, 1207, 1158, 1049, 767, 697.
UV–Vis (CH_2Cl_2, nm): 270 (ε 2600), 287 (ε 2700), 300 (ε 2200), 318 (ε 2100), λ_{max} 336 (ε 2200).
Micro Anal. Calc. for $C_{13}OH_{12}$: C, 84.7; H, 6.6. Found: C, 84.3; H, 6.5%.

2.2.4. Bis-[(3-methoxy-benzyl) cyclopentadienyl]titanium(IV) dichloride, $[(\eta^5$-C_5H_4-CH_2-C_6H_4–$OCH_3)]_2TiCl_2$ (**3b**)

16 ml (16.0 mmol) of 1 M solution of Super Hydride ($LiBEt_3H$) in THF was concentrated by removal of the solvent by heating it to 60 °C under reduced pressure of 10^{-2} mbar for 40 min and then to 90 °C for 20 min in a Schlenk flask. The concentrated Super Hydride was dissolved in 30 ml of dry diethyl ether to give a cloudy white suspension. 2.03 g (11.0 mmol) of the red oil 6(2-methoxyphenyl) fulvene was added to a Schlenk flask and was dissolved in 30 ml dry diethyl ether to give a red solution. The red fulvene solution was transferred to the Super Hydride solution *via cannula*. The solution was left to stir for 6 h in which time a white precipitate of the lithium cyclopentadienide intermediate formed and the solution had changed its colour from orange/red to yellow. The precipitate was filtered on to a frit and was washed with diethyl ether. The white precipitate was dried briefly under reduced pressure and was transferred to a Schlenk flask under nitrogen. 0.75 g (3.92 mmol, 35.5% yield) of the lithiated cyclopentadienide intermediate was obtained. 2.0 ml (2.0 mmol) of titanium tetrachloride was dissolved in 60 ml of dry THF to give a yellow solution in a Schlenk flask. The lithium cyclopentadienide intermediate was dissolved in 30 ml of dry THF to give a colourless solution. The titanium tetrachloride solution was added to the lithium cyclopentadienide intermediate solution *via cannula* to give a dark red solution. The dark red titanium 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 trichloromethane (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 0.85 g of a brown/black solid (1.74 mmol, 88.6% yield).

^{1}H NMR (δ ppm $CDCl_3$, 300 MHz): 3.78 [s, 6H, C_6H_4–OCH_3], 4.07 [s, 4H, C_5H_4–CH_2], 6.33 [s, 8H, C_5H_4], 6.76 [d, 2H, $J = 4.5$ Hz, C_5H_4], 6.77 [s, 2H, C_5H_4–CH], 6.80 [d, 2H, $J = 5.7$ Hz, C_6H_4], 7.21 [t, 2H, $J = 5.7$ Hz, C_6H_4].
^{13}C NMR (δ ppm $CDCl_3$, 100 MHz, proton decoupled): 36.0 [C_6H_4–OCH_3], 54.2 [C_5H_4–CH_2], 110.8, 113.9, 114.8, 120.3, 121.6, 128.6, 135.8, 140.0, 158.8.
IR absorptions (KBr, cm^{-1}): 3113, 2926, 2360, 1608, 1581, 1487, 1439, 1284, 1252, 1144, 1051, 825, 773.
UV–Vis (CH_2Cl_2, nm): λ_{max} 263 (ε 29 000).
Micro Anal. Calc. for $TiC_{26}O_2H_{26}Cl_2$: C, 63.8; H, 5.4; Cl, 14.5. Found C, 62.2; H, 5.5; Cl, 14.5%.

*2.2.5. Synthesis of 6(3,4-dimethoxy-phenyl) fulvene, [C_5H_4–CH–C_6H_3–(OCH_3)$_2$] (**1c**)*

3.62 g (21.5 mmol) of 3,4-dimethoxy-benzaldehyde was dissolved in 100 ml of methanol to give a colourless solution. 4.0 ml (48.4 mmol) of fresh cracked cyclopentadiene was added to the reaction solution, which remained colourless. 3.0 ml (36.0 mmol) pyrrolidine was added to the solution. The solution slowly changed colour from colourless to yellow and finally reached a red/orange colour. The reaction was left to stir for 16 h. 3.0 ml of acetic acid was added to quench the reaction. 100 ml of water was added to the reaction mixture and the organic product was extracted by 3 × 30 ml ether. The ether solution was dried with 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 4.53 g (98.1% yield, 21.13 mmol) of an orange solid.

^{1}H NMR (δ ppm $CDCl_3$, 300 MHz): 3.93 [s, 6H, C_6H_3–(OCH_3)$_2$], 6.41 [m, 2H, C_5H_4], 6.70 [m, 2H, C_5H_4], 6.90 [d, 1H, $J = 6.3$ Hz, C_5H_4–CH], 7.16 [d, 1H, $J = 6.9$ Hz, C_6H_3–(OCH_3)$_2$], 7.18[s, 1H, C_6H_3–(OCH_3)$_2$], 7.20 [d, $J = 6.0$ Hz, C_6H_3–(OCH_3)$_2$].
^{13}C NMR (δ ppm $CDCl_3$, 100 MHz, proton decoupled): 56.2 [C_6H_3–OCH_3–OCH_3], 56.2 [C_6H_3-OCH_3–OCH_3], 111.4, 113.4, 120.0, 125.0 [C_5H_4–CH], 127.7, 130.0, 130.1, 135.3, 138.7, 143.7, 149.3, 150.6.
IR absorptions (KBr, cm^{-1}): 2929, 2837, 2360, 1618, 1593, 1543, 1462, 1329, 1261, 1022, 891, 812, 769, 631, 584.
UV–Vis (CH_2Cl_2, nm): λ 222 (ε 4460), λ 256 (ε 4683), λ 270 (ε 4348), λ 312 (ε 4794), λ 325 (ε 5329), λ 339 (ε 5017), λ 367 (ε 5001), λ_{max} 386 (ε 3233).
Micro Anal. Calc. for $C_{14}O_2H_{14}$: C, 78.5; H, 6.6. Found: C, 77.5; H, 6.6%.

*2.2.6. Synthesis of bis-[(3,4-bismethoxy-benzyl) cyclopentadienyl]titanium(IV) dichloride, [(η^5-C_5H_4–CH_2–C_6H_3–(OCH_3)$_2$)]$_2TiCl_2$ (**3c**)*

15.8 ml (15.8 mmol) of 1 M solution of Super Hydride ($LiBEt_3H$) in THF was concentrated by removal of the solvent by heating it to 60 °C under reduced pressure of 10^{-2} mbar for 40 min and then to 90 °C for 20 min. The concentrated $LiBEt_3H$ was dissolved in 25 ml of dry diethyl ether to give a colourless solution in a Schlenk flask. 2.55 g (11.9 mmol) of the orange solid 6(3,4-dimethoxy-phenyl) fulvene was added to a Schlenk flask and was dissolved in 125 ml dry diethyl ether to give a red solution. The red solution was transferred to the $LiBEt_3H$ solution *via cannula*. The solution was left to stir for 4 h to give a white precipitate of the lithium cyclopentadienide intermediate and the solution changed colour from orange/red to faint yellow. The precipitate was filtered on to a frit and was washed with 20 ml of diethyl ether. The white precipitate was dried briefly under reduced pressure and was transferred to a Schlenk flask under argon. 2.71 g (11.4 mmol, 95.5% yield) of the lithiated cyclopentadienide intermediate was obtained. 5.6 ml (5.6 mmol) of titanium tetrachloride was dissolved in 50 ml of dry THF to give a yellow solution in a Schlenk flask. The lithium cyclopentadienide intermediate was dissolved in 50 ml of the dried THF to give a colourless solution. The titanium tetrachloride solution was added to the lithium cyclopentadienide intermediate solution *via cannula* to give a dark red solution. The dark red titanium 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 trichloromethane (50 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 2.71 g of an orange/brown solid (4.6 mmol, 80.7% yield).

^{1}H NMR (δ ppm $CDCl_3$, 400 MHz): 3.85 [s, 12H, C_6H_3–(OCH_3)$_2$], 4.05 [s, 4H, C_5H_4–CH_2], 6.32 [s, 8H, C_5H_4], 6.72 [s, 2H, C_6H_3–(OCH_3)$_2$], 6.77 [d, 2H, $J = 11.1$ Hz, C_6H_3–(OCH_3)$_2$], 6.80 [d, 2H, $J = 8.1$ Hz, C_6H_3-(OCH_3)$_2$].
^{13}C NMR (δ ppm $CDCl_3$, 125 MHz, proton decoupled): 36.7 [C_6H_3-(OCH_3)$_2$], 56.1 [C_5H_4–CH_2], 111.5, 112.8, 116.0, 121.2, 122.7, 132.1, 137.8, 147.9, 149.2.
IR absorptions (KBr, cm^{-1}): 3107, 2956, 2933, 2835, 1589, 1514, 1464, 1414, 1333, 1263, 1234, 1140, 1024, 816, 752, 698.
UV–Vis (CH_2Cl_2, nm): 215 (ε 18000), 234 (ε 13000), 264 (ε 14000), 278 (ε 12000), 398 (ε 1000), λ_{max} 523 (ε 180).
Micro Anal. Calc. for $TiC_{28}O_4H_{30}Cl_2$: C, 61.2; H, 5.5; Cl, 12.9. Found: C, 60.0; H, 5.5; Cl, 12.3%.

*2.2.7. Synthesis of bis-[(3,4,5-trismethoxy-benzyl) cyclopentadienyl]titanium(IV) dichloride, [(η^5-C_5H_4–CH_2–C_6H_2–(OCH_3)$_3$)]$_2TiCl_2$ (**3d**)*

16.6 ml (16.6 mmol) of a 1 M solution of Super Hydride ($LiBEt_3H$) in THF was concentrated by removal of the solvent by heating it to 60 °C under reduced pressure of 10^{-2} mbar for 40 min and then to 90 °C for 20 min. The concentrated $LiBEt_3H$ solution was allowed to cool and 25 ml of dry diethyl ether was added to give a cloudy white suspension in the Schlenk flask. 2.70 g (11.0 mmol) of the red oil 6(3,4,5-trimethoxy-phenyl) fulvene was added to another Schlenk flask and was dissolved in 60 ml dry diethyl ether to give a red solution. The red solution was transferred to the $LiBEt_3H$ solution *via cannula*. The solution was left to stir for 6 h to give a white precipitate of the lithium cyclopentadienide intermediate and the solution had changed colour from orange/red to faint yellow. The lithium cyclopentadienide intermediate solution had its solvent decanted off whilst remaining in an inert environment. The white precipitate was washed with 2 × 20 ml dry pentane, which was then also removed by decanting. The

intermediate was dried briefly under reduced pressure. 5.6 ml (5.6 mmol) of titanium tetrachloride was dissolved in 50 ml of dry THF to give a yellow solution in a Schlenk flask. The lithium cyclopentadienide intermediate was dissolved in 80 ml of the dried THF to give a colourless solution. The solution of titanium tetrachloride solution was added to the lithium cyclopentadienide intermediate solution *via cannula* to give a black solution. The black 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 trichloromethane (80 ml) and filtered through celite to remove the remaining LiCl. The black filtrate was filtered twice more by gravity filtration. The solvent was removed under reduced pressure to yield 2.90 g of a black solid (4.76 mmol, 85.0% yield).

^{1}H NMR (δ ppm $CDCl_3$, 300 MHz): 3.82 [s, 6H, $(OCH_3)_2$–C_6H_2–OCH_3], 3.83 [s, 12H, OCH_3–C_6H_2–$(OCH_3)_2$], 4.06 [s, 4H, C_5H_4–CH_2], 6.35 [s, 4H, C_5H_4], 6.46 [s, 4H, C_6H_2–$(OCH_3)_3$].
^{13}C NMR (δ ppm $CDCl_3$, 100 MHz, proton decoupled): 37.5 [C_5H_4–CH_2], 56.4 [C_6H_2–$(OCH_3)_2$–(OCH_3)], 61.1 [C_6H_2–$(OCH_3)_2$–OCH_3], 106.4, 115.5, 123.1, 135.2, 136.9, 137.2, 153.5.
IR absorptions (cm^{-1} KBr): 2960, 1591, 1506, 1460, 1419, 1331, 1261, 1124, 802.
Micro Anal. Calc. for $TiC_{30}O_6H_{34}Cl_2$: C, 59.1; H, 5.6; Cl, 11.6. Found: C, 58.3; H, 5.7; Cl, 11.5% (see Figs. 2 and 3).

2.3. Cytotoxicity studies

Preliminary *in vitro* cell tests were performed on the cell line LLC-PK (long-lasting cells-pig kidney) in order to compare the cytotoxicity of the compounds presented in this paper. This cell line was chosen based on their regular and long-lasting growth behaviour, which is similar to the one shown in kidney carcinoma cells. It was 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 96-well plates containing 200 µl 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 in the minimal amount of DMSO (dimethylsulfoxide) possible and diluted with medium to obtain stock solutions of 5×10^{-4} M in concentration and less than 0.7% of DMSO. 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 (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) in medium. The solution consisted of 30 mg 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. A Wallac Victor (Multilabel HTS Counter) Plate Reader was used to measure absorbance at 540 nm. Cell viability was expressed as a percentage of the absorbance recorded for control wells. The values used for the dose–response curves represent the values obtained from four consistent MTT-based assays for each compound tested. Titanocenes **3a-d** were found to have IC_{50} values of 97, 159, 88 and 253 µM, respectively (see Figs. 4 and 5).

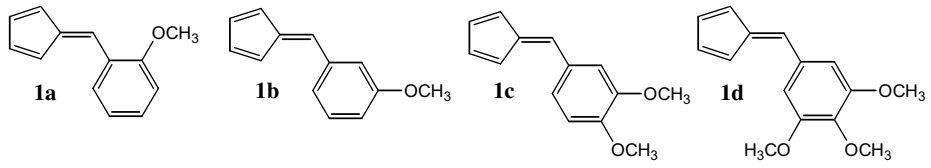


Fig. 2. Structures of fulvenes **1a**–**1d**.

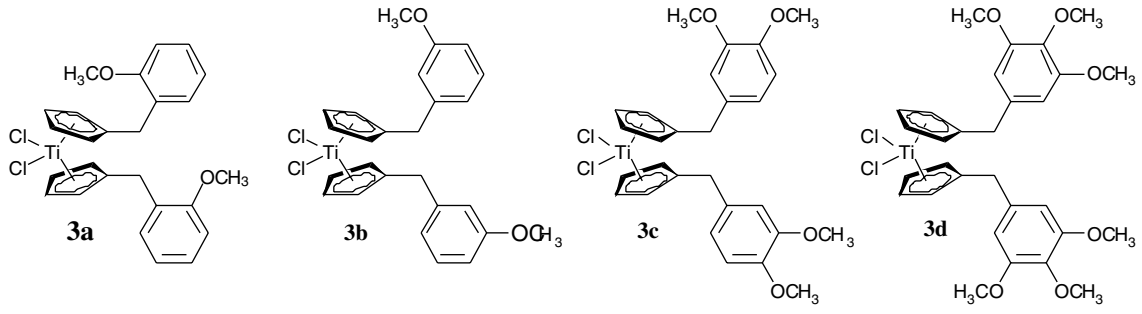


Fig. 3. Structures of titanocenes **3a**–**3d**.

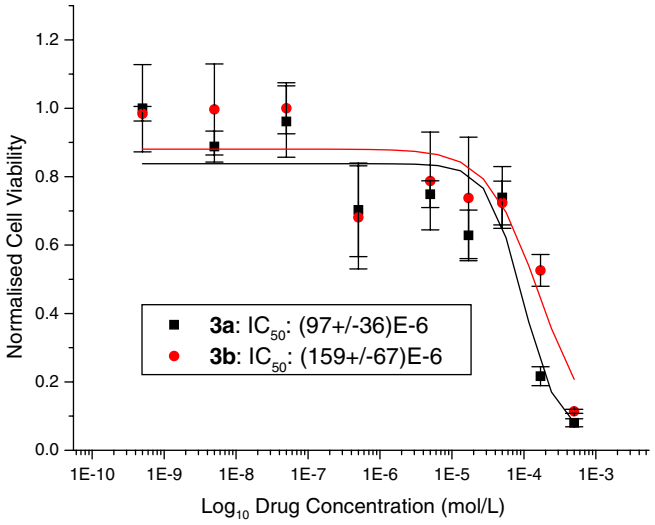


Fig. 4. Cytotoxicity curves from typical MTT assays showing the effect of compounds **3a** and **3b** on the viability of LLC-PK cells.

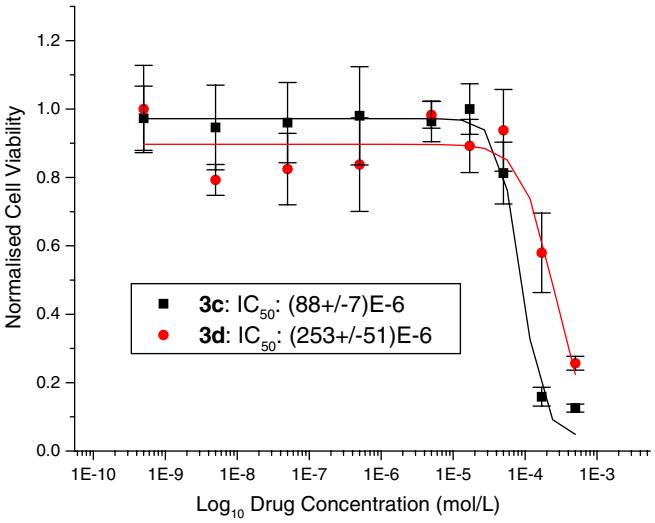


Fig. 5. Cytotoxicity curves from typical MTT assays showing the effect of compounds **3c** and **3d** on the viability of LLC-PK cells.

3. Results and discussion

3.1. Synthesis

The synthesis of the four fulvenes were all done by well established analogous synthetic processes of Stone and Little [37] by the condensation of freshly cracked cyclopentadiene in the presence of a base in yields of 87–98%. Pyrrolidine was used as the base to catalyse this reaction.

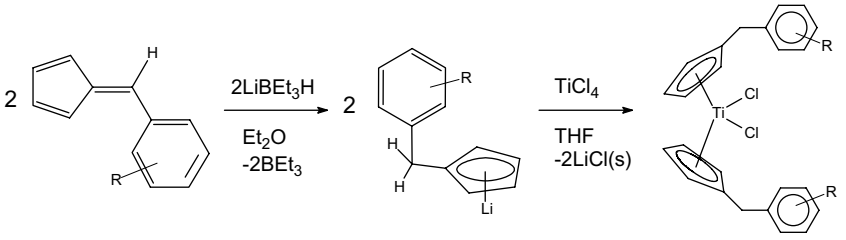
The four titanocenes were synthesised by the nucleophilic addition of hydride to the exocyclic double bond of the fulvenes to form the required isolable lithium cyclopentadienide intermediate in yields of 35–95%. These exocyclic double bonds in the fulvenes have increased polarity, due to the inductive effects of their respective phenyl groups. This increased polarity allows for selective nucleophilic attack at this double bond and not at the diene component of the fulvenes.

Two equivalents of this lithium cyclopentadienide intermediate can be transmetallated to 1 equiv. of $TiCl_4$ resulting in the formation of 1 equiv. of the required benzyl substituted titanocene in yields of 75–88% and 2 equiv. of the by-product of lithium chloride following a 16 hour reflux. The synthesis is shown in Scheme 1.

In the case of 6(3,4,5-trimethoxy-phenyl) fulvene **1d** it could not be isolated following hydridolithiation as the lithiated cyclopentadienide intermediate **2d** formed an extremely compact solid upon precipitation which could not be removed. In this case the $TiCl_4$ solution was added directly to the intermediate once dissolved in THF.

3.2. Structural discussion

The benzyl substituted titanocenes are a particular class of titanocenes that are very promising in crystallisation experiments due to the lack of stereoisomers. 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. The length of the bond between the titanium centre and the carbon atoms of the cyclopentadienyl rings bound to the metal are very similar for **3a** and **3b**. They vary from 2.344 Å to 2.413 Å for **3a** and from 2.342 Å to 2.418 Å for **3b**. In **3c** there is a slight elongation to of these bonds to be seen to the metal ion with the bonds varying from 2.348 Å to 2.487 Å leading to a titanium centroid distance of 2.082 Å. The carbon carbon bond lengths of the cyclopentadienyl rings are more comparable to all three with **3a** having lengths of 1.40–1.42 Å, **3b** having lengths of 1.39–1.41 Å and **3c** having lengths of 1.40–1.42 Å. The titanium to chlorine bond lengths are very



Scheme 1. Synthesis of benzyl substituted titanocenes from fulvenes using the hydridolithiation reaction.

similar varying from 2.355 Å to 2.373 Å. The Cl–Ti–Cl′ bond angle was measured at 93.85° for **3a**, 94.13° for **3b** and 96.03° for **3c** (see Figs. 6–8).

The packing in **3a** features a pronounced z shape of the molecule. This z shape leads to a pseudo chain consisting of two molecules of this titanocene per link of the chain. In **3b** there is no internal symmetry to be seen within the molecule yet it was still possible to crystallise this compound with no solvent present in the packing. The titanium ion is featured on a C_2 axis in **3c**. For all three compounds there are no π–π interactions to be seen between the substituted phenyl groups but some soft Van der Waals

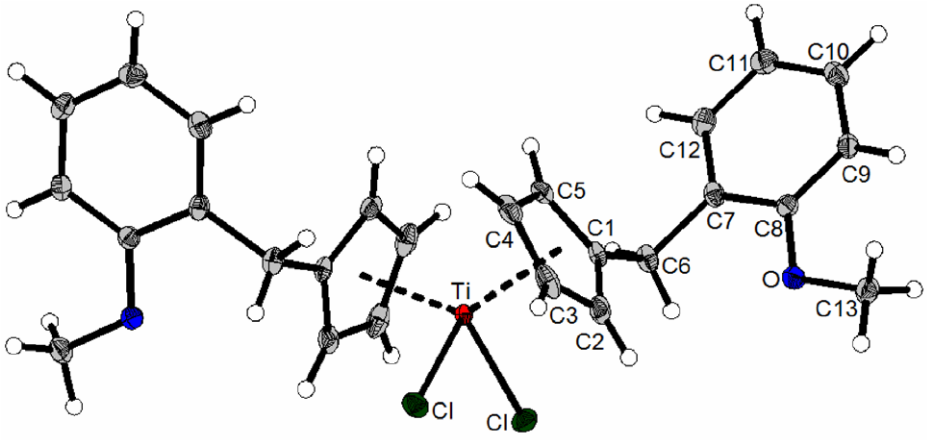


Fig. 6. X-ray diffraction structure of bis-[(2-methoxy-benzyl)cyclopentadienyl]titanium(IV) dichloride **3a** (thermal ellipsoids are drawn on the 50% probability level).

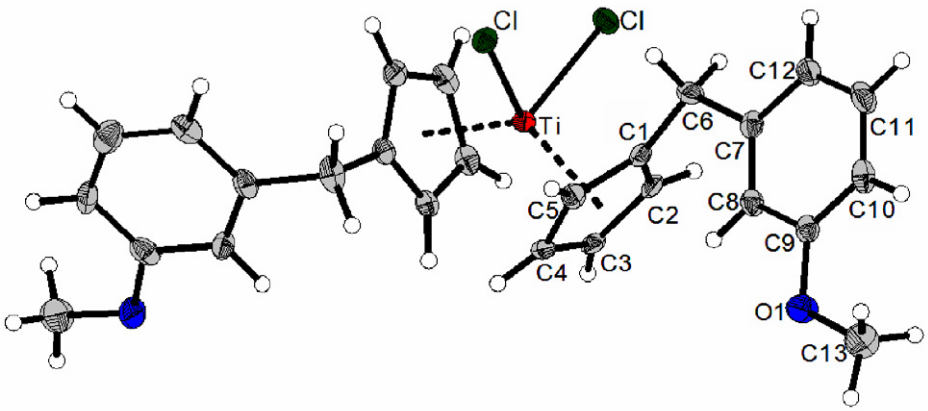


Fig. 7. X-ray diffraction structure of bis-[(3-methoxy-benzyl)cyclopentadienyl]titanium(IV) dichloride **3b** (thermal ellipsoids are drawn on the 50% probability level).

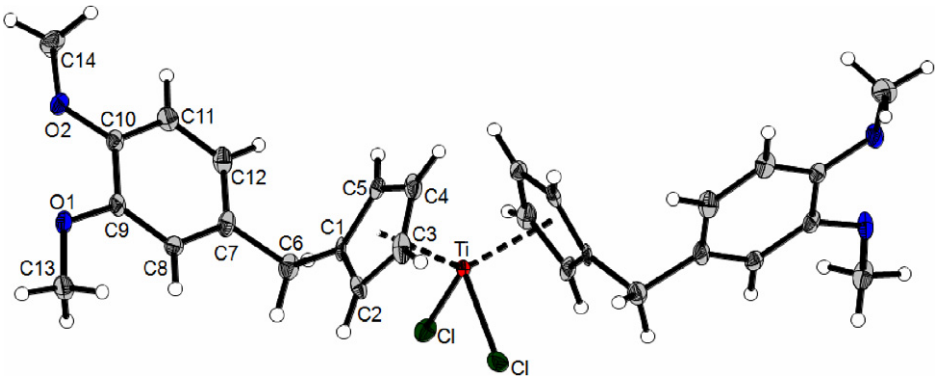


Fig. 8. X-ray diffraction structure of bis-[(3,4-dimethoxy-benzyl)cyclopentadienyl]titanium(IV) dichloride **3c** (thermal ellipsoids are drawn on the 50% probability level).

Table 2
Selected bond lengths and angles from crystallographic structures of titanocenes **3a–c**

Identification code	3a	3b	3c
Bond lengths (Å)			
Ti–C(1)	2.4133(13)	2.418(5)	2.487(3)
Ti–C(2)	2.3921(15)	2.391(5)	2.441(3)
Ti–C(3)	2.3848(15)	2.350(5)	2.376(3)
Ti–C(4)	2.3440(15)	2.342(5)	2.348(3)
Ti–C(5)	2.3858(15)	2.391(5)	2.361(3)
C(1)–C(2)	1.417(2)	1.410(7)	1.409(4
C(2)–C(3)	1.402(2)	1.391(7)	1.421(5)
C(3)–C(4)	1.409(3)	1.400(7)	1.400(5)
C(4)–C(5)	1.423(2)	1.402(7)	1.421(5)
C(5)–C(1)	1.408(2)	1.412(7)	1.412(5)
C(1)–C(6)	1.508(2)	1.488(7)	1.507(4)
C(6)–C(7)	1.517(2)	1.516(7)	1.525(5)
Ti–Cl(1)	2.3726(5)	2.3635(15)	2.3552(9)
Ti–Cl(2)	2.3726(5)	2.3652(15)	2.3551(9)
Ti–Cent	2.059(1)	2.057(5)	2.082(4)
Bond angles (°)			
Cent(1)–Ti–Cl(1)	105.67(1)	105.50(5)	105.87(2)
Cent(1)–Ti–Cl(2)	107.54(1)	106.65(5)	106.04(2)
Cent(1)–Ti–Cent(2)	130.54(1)	130.85(5)	131.48(1)
Cl(1)–Ti–Cl(2)	93.85(1)	94.13(5)	96.03(5)

interactions are present. The absence of solvent molecules present in the three crystal unit cells is a particular advantage when it comes to biological testing of these compounds (see Table 2).

Despite the efforts to crystallise titanocene **3d** no crystal structures were obtained. This could possibly be explained by the solubility problems encountered with this isolated titanocene. In order to acquire a structure density functional theory (DFT) calculations were carried out for this titanocene at the B3LYP level using the 6-31G** basis set.

Selected bond lengths of the optimised structure of this titanocene are listed in Table 3 and the atom numbering scheme is seen in Scheme 2. The calculated structure of titanocene **3d** is presented in Fig. 9.

There are slight variations in the bond length between the metal centre and the cyclopentadienyl carbons with them varying from 2.3373 Å to 2.4431 Å. These slight bond length differences are also seen between the carbon carbon bonds of the cyclopentadienylrings with bond lengths varying from 1.4131 Å to 1.4408 Å. All other bonds within the calculated titanocene are of comparable lengths to those whose X-ray structures were refined. The Cl–Ti–Cl angle was calculated to be 93.32° which is highly comparable to titanocenes **3a**, **3b** and **3c** Cl–Ti–Cl bond angles.

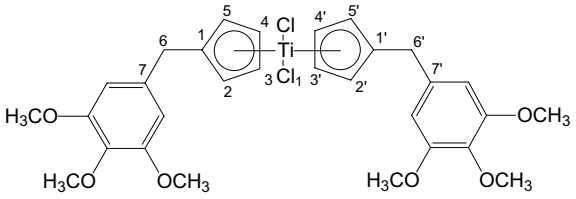
In general, it is possible to calculate the molecular structures of titanocene dichloride derivatives within the error margin of the X-ray crystal structure method, if the DFT method, B3LYP, is used in combination with a large enough basis set like 6-31G**.

3.3. Cytotoxicity studies

The values used for the dose–response curves of Figs. 1 and 2 represent the values obtained from four consistent

Table 3
Selected bond lengths and angles from calculated structure of titanocene **3d**

Identification code	3d
Bond lengths (Å)	
Ti–C(1)	2.4341
Ti–C(2)	2.3753
Ti–C(3)	2.3373
Ti–C(4)	2.4288
Ti–C(5)	2.4431
C(1)–C(2)	1.4395
C(2)–C(3)	1.4131
C(3)–C(4)	1.4279
C(4)–C(5)	1.4341
C(5)–C(1)	1.4199
C(1)–C(6)	1.5143
C(6)–C(7)	1.5211
Ti–Cl(1)	2.3765
Ti–Cl(2)	2.3812
Ti–C(1)*	2.4329
Ti–C(2)*	2.3834
Ti–C(3)*	2.3980
Ti–C(4)*	2.3757
Ti–C(5)*	2.4089
C(1)*–C(2)*	1.4408
C(2)*–C(3)*	1.4211
C(3)*–C(4)*	1.4219
C(4)*–C(5)*	1.4321
C(5)*–C(1)*	1.4201
C(1)*–C(6)*	1.5068
C(6)*–C(7)*	1.5313
Bond angle (°)	
Cl(1)–Ti–Cl(2)	93.3180



Scheme 2. Numbering scheme of **3d** for the structural DFT discussion.

MTT-based assays for each compound tested. As seen in Figs. 1 and 2, titanocenes **3a–d** showed IC_{50} values of 97, 159, 88 and 253 μM, respectively. When compared to unsubstituted titanocene dichloride (IC_{50} value of 2000 μM), each of the newly synthesised titanocenes show greater than an 8-fold decrease and up to 22-fold decrease in magnitude in terms of the IC_{50} value. However, titanocenes **3a–d** do not reach the cytotoxicity with respect to the class leader Titanocene **Y** with an IC_{50} value of 21 μM nor to cisplatin with an IC_{50} value of 3.3 μM. This is likely to be due to the loss of solubility in each of the four titanocenes case showing the importance of the *para* methoxy phenyl moiety in Titanocene **Y** itself. Also the change in shape and size of the new titanocenes might also play a considerable part in the cause of the decrease in cytotoxicity shown in the MTT based cell tests. In comparison to the

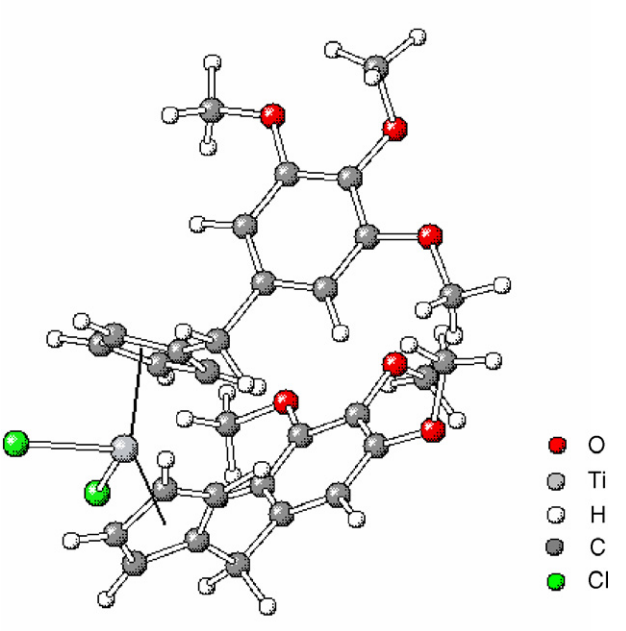


Fig. 9. DFT calculated structure of bis-[(3,4,5-trimethoxy-benzyl)cyclopentadienyl]titanium(IV) dichloride **3d**.

ansa-titanocene equivalent of titanocene **3d**, which has an IC_{50} of 930 μM [12], there is greater than a 3.5-fold decrease in magnitude of the IC_{50}, which is probably due to the less restricted nature of the benzyl substituted titanocene due to the lack of an annular bridge present as in the case of the equivalent *ansa*-titanocene.

4. Conclusions and outlook

The hydridolithiation of 6-aryl substituted fulvenes has been found to be a very effective and reproducible way to cytotoxic benzyl substituted titanocenes of high purity. Following these investigative studies into the synthesis and cytotoxicity of these methoxy phenyl substituted titanocenes we have been able to establish that Titanocene **Y** has the optimum positioning and number of methoxy groups on the phenyl ring of this particular titanocene class. Although all four of these titanocenes show an increased cytotoxicity in comparison to unsubstituted titanocene dichloride they do not compare as favourably against Titanocene **Y** exhibiting an IC_{50} value of 21 μM.

Acknowledgement

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Appendix A. Supplementary material

CCDC 661581, 661583 and 661582 contain the supplementary crystallographic data for **3a**, **3b** and **3c**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2007.11.037.

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