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Novel zirconocene anticancer drugs?

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This paper is dedicated to Prof. Dr. Gérard Jaouen – the pioneer in the area of Bioorganometallic Chemistry – on the occasion of his 65th birthday.

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LLC-PK

ABSTRACT

From the reaction of Super Hydride ($LiBEt_3H$) with 6-(4-methoxyphenyl) fulvene (**1a),** 6-(2-fluoro-4-methoxyphenyl) fulvene **(1b)**, and 6-(4-*N*,*N*-dimethylaminophenyl) fulvene (**1c)** lithiated cyclopentadienide intermediates (**2a–c)** were synthesised. These intermediates were then transmetallated to zirconium with $ZrCl_4$ to give benzyl-substituted zirconocenes bis-[(4-methoxybenzyl)cyclopentadienyl] zirconium(IV) dichloride (**3a)**, bis-[(2-fluoro-4-methoxybenzyl)cyclopentadienyl] zirconium(IV) dichloride (**3b)** and bis-[(4-*N*,*N*-dimethylaminobenzyl)cyclopentadienyl] zirconium(IV) dichloride (**3c)**. All three zirconocenes were characterised by single crystal X-ray diffraction and preliminary *in vitro* cell tests were performed with the zirconocene derivatives on the LLC-PK cell line in order to determine their cytotoxicity. Zirconocenes **3b** and **3c** did not show cytotoxicity up to a concentration of 170 μM, while **3a** exhibited an IC50 value of 57 μM against LLC-PK.

1. Introduction

Bioorganometallic compounds like Ferrocifen [1] are now being explored as potential treatment against hormone dependant and hormone independent breast cancer [2] following the pioneering work in this field carried out by Gérard Jaouen.

Another promising bioorganometallic anticancer drug candidate is titanocene dichloride (Cp_2TiCl_2), which showed medium anti-proliferative activity *in vitro* but promising results *in vivo* [3,4]. Titanocene dichloride reached clinical trials, but it's efficacy 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 allow direct access to antiproliferative titanocenes via reductive dimerisation with titanium dichloride, carbolithiation or hydridolithiation of the fulvene followed by transmetallation with titanium tetrachloride in the latter two cases [7]. Hydridolithiation of 6-anisyl fulvene and subsequent reaction with $TiCl_4$ led to bis-[(*p*-methoxybenzyl)cyclopentadienyl] titanium(IV) dichloride (Titanocene **Y**) [8], which has an IC50 value of 21 μM when tested on the LLC-PK cell line. This particular cell line was chosen as it 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. Also, Titanocene **Y** is strongly anti-angiogenic, exhibiting an IC50 value of 4.9 μM in the HUVEC assay [9], which underlines that Titanocene **Y** is a possible drug candidate against advanced renal cell cancer.

In addition, the anti-proliferative activity of Titanocene **Y** and other titanocenes has been studied in 36 human tumor cell lines [10] and against explanted human tumors [11,12]. 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 [13]. Furthermore, it was shown, that titanocene derivatives give a positive immune response by up-regulating the number of natural killer (NK) cells in mice [14]. Recently, animal studies reported the successful treatment of mice bearing xenografted Caki-1 and MCF-7 tumors with Titanocene **Y** [15,16] and the cytotoxicity could be improved by an anion exchange leading to Oxali-Titanocene **Y** [17]. The structures of Ferrocifen and Titanocene **Y** are shown in Fig. 1.

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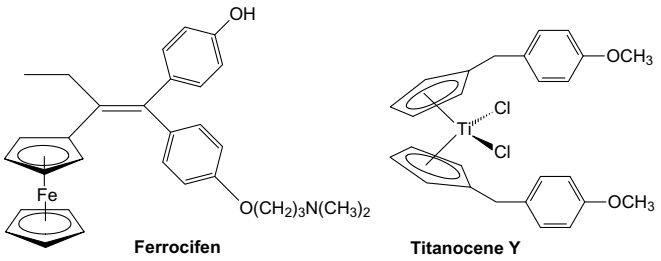


Fig. 1. Structures of Ferrocifen and Titanocene **Y**.

On the other hand, exchange of the titanium to the heavier metal zirconium leads to zirconocene dichloride, which was tested on Ehrlich's ascites tumor (EAT) *in vitro* [18] and *in vivo* [19]. All experiments showed no anticancer activity of the unsubstituted zirconocene, which did not encourage further research into this area. Within this paper, we present the synthesis and preliminary cytotoxicity studies of a series of three substituted zirconocene derivatives, which include the analogous derivative Zirconocene **Y**.

2. Experimental

2.1. General conditions

Zirconium tetrachloride, 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(ethylene–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, 400 or 500 MHz spectrometer. Chemical shifts are reported in ppm and are referenced to TMS. IR spectra were recorded on a Perkin Elmer Paragon 1000 FT-IR Spectrometer employing a KBr disk 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. X-ray diffraction data for the compounds 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 [20]. The structures were solved by direct methods using and refined by full matrix least-squares on F^2 for all data using SHELXL-97 [21]. In **3a** and **3b** all hydrogen atoms were located in the difference fourier map and allowed to refine freely. In **3c** the hydrogen atoms were visible but not freely refined. Instead they were added at calculated positions and refined using a riding model. 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 numbers 690596, 690597 and 690598 for **3a**, **3b** and **3c**, respectively. Suitable crystals of **3a–c** were grown in saturated dichloromethane solution with slow infusion of pentane.

2.2. Synthesis

6-(4-Methoxyphenyl) fulvene (**1a**) was synthesised according to the previously published procedure [22].

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

Identification code	**3a**	**3b**	**3c**
Empirical formula	$C_{26}H_{26}O_2Cl_2Zr$	$C_{26}H_{24}O_2F_2Cl_2Zr$	$C_{28}H_{32}N_2Cl_2Zr$
Formula weight	532.59	568.57	558.68
Temperature (K)	100(2)	100(2)	100(2)
Wavelength (Å)	0.71073	0.71073	0.71073
Crystal system	Orthorhombic	Monoclinic	Orthorhombic
Space group	*Pbca* (#61)	*C2/c* (#15)	$P2_12_12_1$ (#19)
Unit cell dimensions			
a (Å)	6.5846(5)	17.9885(17)	7.3449(6)
b (Å)	25.6492(19)	6.7101(6)	11.7907(10)
c (Å)	27.102(2)	19.3009(18)	28.904(2)
α (°)	90	90	90
β (°)	90	92.508(2)	90
γ (°)	90	90	90
Volume (Å^3)	4577.3(6)	2327.5(4)	2503.1(4)
Z	8	4	4
D_{calc} (mg/m^3)	1.546	1.623	1.482
Absorption coefficient (mm^{-1})	0.735	0.740	0.672
F(000)	2176	1152	1152
Crystal size (mm^2)	0.40 × 0.20 × 0.02	0.50 × 0.20 × 0.05	0.80 × 0.55 × 0.03
θ Range for mm data collection (°)	1.76–26.36	2.11–31.59	1.87–32.02
Index ranges	$-8 \leqslant h \leqslant 6$, $-31 \leqslant k \leqslant 31$, $-33 \leqslant l \leqslant 32$	$-25 \leqslant h \leqslant 25$, $-9 \leqslant k \leqslant 9$, $-28 \leqslant l \leqslant 27$	$-10 \leqslant h \leqslant 10$, $-17 \leqslant k \leqslant 13$, $-35 \leqslant l \leqslant 41$
Reflections collected	26197	13076	21387
Independent reflections	4657 [R_{int} = 0.0376]	3648 [R_{int} = 0.0237]	8074 [R_{int} = 0.0210]
Completeness to θ_{max} (%)	99.7	93.8	94.8
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents	Semi-empirical from equivalents
Maximum and minimum transmission	0.9854 and 0.8211	0.9639 and 0.8045	0.9801 and 0.7205
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	4657/0/384	3648/0/198	8074/0/302
Goodness-of-fit on F^2	1.083	1.050	1.053
Final *R* indices [$I > 2\sigma(I)$]	R_1 = 0.0316, wR_2 = 0.0686	R_1 = 0.0271, wR_2 = 0.0673	R_1 = 0.0296, wR_2 = 0.0702
R indices (all data)	R_1 = 0.0404, wR_2 = 0.0718	R_1 = 0.0297, wR_2 = 0.0686	R_1 = 0.0311, wR_2 = 0.0710
Largest difference peak and hole (e Å^{-3})	0.458 and −0.307	0.762 and −0.305	0.806 and −0.327

2.2.1. Bis-[(4-methoxybenzyl)cyclopentadienyl] zirconium(IV) dichloride, $[(\eta^5\text{-}C_5H_4\text{–}CH_2\text{–}C_6H_4\text{–}OCH_3)]_2ZrCl_2$ (**3a**)

16.0 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. 2.25 g (12.2 mmol) of the orange solid 6-(4-methoxyphenyl) fulvene was added to a Schlenk flask and was dissolved in 30 ml dry diethyl ether to give an orange solution. The fulvene solution was transferred to the Super Hydride solution *via cannula*. The solution was left to stir for 5 h to give a white precipitate of the lithium cyclopentadienide intermediate and the solution had changed colour from orange/red to white. 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. 1.69 g (8.73 mmol, 71.5% yield) of the lithiated cyclopentadienide intermediate was

obtained. 1.02 g (4.36 mmol) of zirconium tetrachloride was dissolved in 60 ml of dry THF to give a colourless 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 zirconium tetrachloride solution was added to the lithium cyclopentadienide intermediate solution *via cannula* to give a yellow solution. The resulting yellow solution was heated under reflux for 20 h. The solution was then cooled and the solvent was removed under reduced pressure. The remaining orange residue was extracted with 100 ml of chloroform to give a green solution and filtered through celite to remove the remaining LiCl. The green filtrate was filtered twice more by gravity filtration. The solvent was removed under reduced pressure to yield 1.22 g of a cream/green solid (2.76 mmol, 52.9% yield).

^{1}H NMR (δ ppm $CDCl_3$, 300 MHz): 3.78 [s, 6H, C_6H_4–OCH_3], 4.94 [s, 4H, C_5H_4–CH_2], 6.17–6.21 [m, 4H, *J* 2.7 C_5H_4], 6.83 [d, 2H, J 8.2 Hz, C_6H_4–OCH_3], 7.15 [d, 2H, J 8.2 Hz, C_6H_4–OCH_3].

^{13}C NMR (δ ppm $CDCl_3$, 100 MHz, proton decoupled): 35.2 [C_6H_4–OCH_3], 55.2 [C_5H_4–CH_2], 112.8, 113.9, 116.7, 129.8, 158.2.

IR absorptions (KBr, cm^{-1}): 3099, 2967, 1690, 1511, 1462, 1300, 1246, 1176, 1029, 818.

UV–Vis (CH_2Cl_2, nm): 210 (ε 22631), 235 (ε 26573), 281 (ε 41176), 426 (ε 1443), λ_{max} 557 (ε 531).

Micro Anal. Calc. for $ZrC_{26}O_2H_{26}Cl_2$: C, 58.9; H, 4.9; Cl, 13.3. Found: C, 59.8; H, 5.2; Cl, 12.1%.

6-(2-Fluoro-4-methoxyphenyl) fulvene (**1b**) has been synthesised according to the established procedure [23].

2.2.2. Bis-[(2-fluoro-4-methoxybenzyl)cyclopentadienyl] zirconium(IV) dichloride [(η^5-C_5H_4–CH_2–C_6H_3F–OCH_3)]$_2ZrCl_2$ (**3b**)

16.0 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. 2.25 g (12.0 mmol) of the orange solid 6-(2-fluoro-4-methoxyphenyl) fulvene was added to a Schlenk flask and was dissolved in 60 ml dry diethyl ether to give an orange solution. The fulvene solution was transferred to the Super Hydride solution *via cannula*. The solution was left to stir for 16 h in which time a white precipitate of the lithium cyclopentadienide intermediate formed and the solution had changed its colour from orange to clear. 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. 1.48 g (7.0 mmol, 71.2% yield) of the lithiated cyclopentadienide intermediate was obtained. 0.82 g (3.5 mmol) of zirconium tetrachloride was dissolved in 30 ml of dry THF to give a clear solution in a Schlenk flask. The lithium cyclopentadienide intermediate was dissolved in 30 ml of dry THF to give a colourless solution. The zirconium tetrachloride solution was added to the lithium cyclopentadienide intermediate solution *via cannula* to give a pale yellow solution. After 24 h of reflux the solution became dark yellow, was then cooled and the solvent was removed under reduced pressure. The remaining pale yellow residue was extracted with 60 ml of trichloromethane and filtered through celite to remove the remaining LiCl. The yellow filtrate was filtered twice more by gravity filtration. The solvent was removed under reduced pressure to yield 1.26 g of a pale yellow solid (22.2 mmol, 63.4% yield).

^{1}H NMR (δ ppm $CDCl_3$, 500 MHz): 3.78 [s, 6H, C_6H_4–OCH_3], 3.94 [s, 4H, C_5H_4–CH_2], 6.22–6.24 [m, 8H, *J* 2.3 Hz C_5H_4], 6.58 [m, 2H, C_6H_4], 7.09 [t, 1H, C_6H_4].

^{13}C NMR (δ ppm $CDCl_3$, 125 MHz, proton decoupled): 55.5 [C_6H_4–OCH_3], 29.1 [C_5H_4–CH_2], 101.8, 109.8, 112.8, 116.8, 131.3, 132.9, 159.7, 160.4, 162.2.

IR absorptions (CH_2Cl_2, cm^{-1}): 3099, 2901, 2805, 2282, 1616, 1524, 1436, 1361, 1233, 1066, 1045, 820, 816.

UV–Vis (CH_2Cl_2, nm): 234 (ε 40641), 280 (ε 32840), 428 (ε 1114), λ_{max} 555 (ε 159).

Micro Anal. Calc. for $ZrC_{26}O_2H_{24}F_2Cl_2$: C, 54.9; H, 4.3; Cl, 13.4. Found C, 54.8; H, 4.2; Cl, 12.5%.

6-(4-*N*,*N*-dimethylaminophenyl) fulvene (**1c**) was synthesised according to the already published procedure [24].

2.2.3. Bis-[4-dimethylaminobenzyl)cyclopentadienyl] zirconium(IV) dichloride [(η^5-C_5H_4–CH_2–C_6H_4–$(N(CH_3)_2)$]$_2ZrCl_2$ (**3c**)

8.4 ml (15.0 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. The concentrated $LiBEt_3H$ was dissolved in 30 ml of dry diethyl ether to give a colourless solution in a Schlenk flask. 1.50 g (7.6 mmol) of the orange solid 6-(4-dimethylaminophenyl) fulvene was added to a 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 5 h to give a yellow precipitate of the lithium cyclopentadienide intermediate and the solution changed colour from orange/red to a clear solution with a yellow precipitate. The precipitate was filtered on to a frit and was washed with 20 ml of diethyl ether. The yellow precipitate was dried briefly under reduced pressure and was transferred to a Schlenk flask under nitrogen. 0.89 g (4.3 mmol, 56.6% yield) of the lithiated cyclopentadienide intermediate was obtained. 0.51 g (2.2 mmol) of zirconium tetrachloride was dissolved in 30 ml of dry THF to give a yellow solution in a Schlenk flask. The lithium cyclopentadienide intermediate was dissolved in 30 ml of the dried THF to give a pale yellow solution. The zirconium tetrachloride solution was added to the lithium cyclopentadienide intermediate solution *via cannula* to give a yellow solution. The yellow zirconium 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 100 ml of trichloromethane and filtered through celite to remove the remaining LiCl. The yellow filtrate was filtered twice more by gravity filtration. The solvent was removed under reduced pressure to yield 0.40 g of a pale yellow/green solid (0.71 mmol, 32.5% yield).

^{1}H NMR (δ ppm $CDCl_3$, 500 MHz): 2.93 [s, 6H, C_6H_4–OCH_3], 3.90 [s, 4H, C_5H_4–CH_2], 6.17–6.20 [m, 8H, *J* 2.45 C_5H_4], 6.79 [d, 2H, *J* 7.3 Hz, C_6H_4], 7.09 [d, 2H, *J* 8.3 Hz, C_6H_4].

^{13}C NMR (δ ppm $CDCl_3$, 100 MHz, proton decoupled): 41.4 [C_6H_4–(OCH_3)], 35.2 [C_5H_4–CH_2], 109.0, 112.7, 113.0, 116.6, 129.7, 129.6, 131.3, 134.4.

IR absorptions (KBr, cm^{-1}): 31071 2959, 2913, 2835, 2359, 2342, 1613, 1506, 1426, 1284, 1259, 1102, 1028, 820.

UV–Vis (CH_2Cl_2, nm): 202 (ε 19974), 210 (ε 21354), 231 (ε 18972), 268 (ε 41439), 424 (ε 1692), λ_{max} 554 (ε 512).

Micro Anal. Calc. for $ZrC_{28}N_2H_{32}Cl_2$: C, 60.4; H, 5.8; Cl, 12.7; N, 5.0. Found: C, 59.3; H, 5.7; Cl 13.2; N, 4.8%.

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 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 1.9×10^{-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 40 mg of MTT in 40 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 [25] for each compound tested. While zirconocenes **3b** and **3c** showed no cytotoxic effects at the concentrations given, zirconocene **3a** was found to be quite cytotoxic and exhibited an IC50 value of 57 μM.

3. Results and discussion

3.1. Synthesis

Fulvenes **1a–c** were synthesised according to published procedures [22–24] and their structures are shown in Fig. 2.

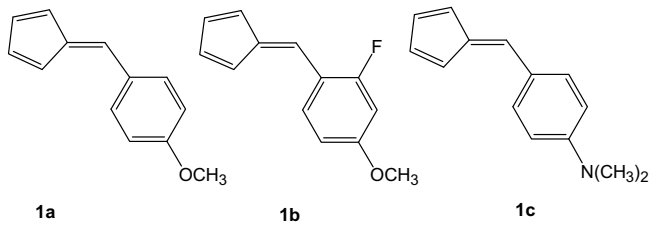


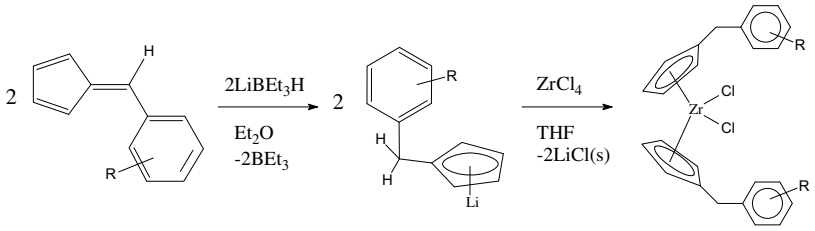
Fig. 2. Structures of fulvenes **1a–c.**

The three zirconocenes were synthesised using the successfully applied hydridolithiation route for the synthesis of titanocenes. This route uses $LiBEt_3H$ to transfer a hydride to a fulvene through nucleophilic addition to the exocyclic double bond of the fulvenes 1a–c to form lithium cyclopentadienyl intermediates. Two molar equivalents of the lithiated intermediate underwent a transmetallation reaction in each case when reacted with one molar equivalent of $ZrCl_4$ in THF under reflux, to give the appropriate non-bridged substituted zirconocenes (Scheme 1). The zirconocenes **3a–c** were synthesised using the unmodified route for the synthesis of the analogous titanocenes. The NMR shifts for these compounds are similar to that of their titanocene analogues [8] apart from the cyclopentadienyl signals, which in the zirconocenes split into a multiplet as opposed to the titanocenes, where they show as one signal. This multiplet appears as a doublet of triplets in all three compounds between 6.17 and 6.24 ppm. The zirconocenes proved to be paler in colour than their titanocene analogues, ranging from cream to pale green in colour and crystallised from saturated dichloromethane solutions (see Fig. 3).

3.2. Structural discussion

For the purpose of X-ray diffraction, suitable single crystals of **3a–c** were grown by vapour diffusion of pentane into a saturated solution of each compound in dichloromethane. The collection and refinement data for each crystal is shown in Table 1. The determined structures for all three zirconocenes did not contain solvent molecules, which is advantageous for cell testing. Selected bond lengths and angles are shown in Table 2.

The zirconocene complexes **3a–c** have distorted tetrahedral shapes with respect to their two halogenido ligands and two cyclopentadienide groups. As their isostructural titanocene analogues these derivatives are stable compounds despite they have only 16 valence electrons. The zirconium-cyclopentadienide centroid distances were measured as 2.201 and 2.202 Å for zirconocene **3a**, 2.197 Å for zirconocene **3b**, and 2.195 and 2.206 Å for zirconocene **3c**. The length of bonds between the zirconium and the carbon atoms of the cyclopentadienyl rings bound to the metal ion are similar for all zirconocenes (Figs. 4–6); they vary only slightly between 2.534 and 2.467 Å for **3a**, between 2.544 and 2.447 Å for **3b** and between 2.533 and 2.466 Å for **3c**. The zirconium-to-chlo-



Scheme 1. Synthesis of benzyl-substituted zirconocenes via the hydridolithiation of fulvenes.

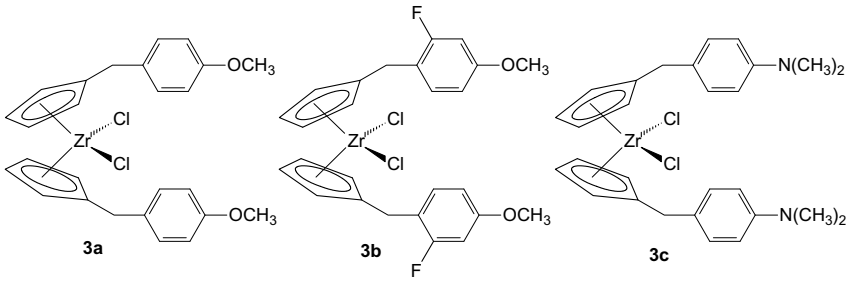


Fig. 3. Structures of zirconocenes **3a–c.**

Table 2
Selected bond lengths and angles of zirconocenes **3a–c**

	3a	3b	3c
Bond length (Å)			
Zr-Cent1	2.201(1)	2.197(2)	2.195(1)
Zr–Cent2	2.202(1)	2.197(2)	2.206(1)
Zr–Cl(1)	2.459(6)	2.449(4)	2.449(5)
Zr–Cl(2)	2.451(6)	2.449(4)	2.455(5)
Bond angle (°)			
Cl(2)–Zr–Cl(1)	98.16(2)	95.305(18)	96.599(17)
Cent(1)–Zr–Cent(2)	129.21(1)	129.59(1)	128.88(1)

rine bond lengths are very similar for all three compounds varying from 2.459 Å to 2.449 Å. All these bond lengths are significantly larger than that of their titanocene analogues [8] due to the larger size of the zirconium atom compared to the titanium atom. The centroid-to-zirconium-to-centroid angle was measured to be 129.59° for **3a**, 129.59° for **3b** and 128.88° for **3c**. These angles are approximately 20° larger than that of a normal tetrahedral angle (109.5°) due to the relatively bulky cyclopentadienide groups on the zirconium atom. The Cl–Zr–Cl′ bond angle on the other side was measured at 98.16° for **3a**, 95.31° for **3b** and 96.60° for **3c**; this compression of between 10° and 15° can again be attributed to the size of cyclopentadienide groups relative to the two chlorine ligands. Interestingly all the angles around the Zr atom are quite similar to the ones around Ti in the corresponding titanium compounds [8].

The packing for zirconocenes **3a** and **3b** is similar and features a z-shaped pattern. The zirconium ion is featured on a C_2 axis in **3b**, however this is not the case for **3a** or **3c**. For zirconocene **3c**, the overall molecular shape is more rod-shaped than z-shaped due to the alignment of the phenyl groups with the cyclopentadienyl rings, although it is not an ideal rod shape because of this alignment. In zirconocene **3c** the aniline groups are not planar, the sum angles for these are 357.56° and 358.60°. In light of this, it is interesting to note that the corresponding titanocene could only be crystallized as its hydrochloride salt. There are no π–π interactions to be seen between the substituted phenyl groups in any of the compounds but some soft Van der Waals interactions are present, this is also the case for the analogous titanocenes.

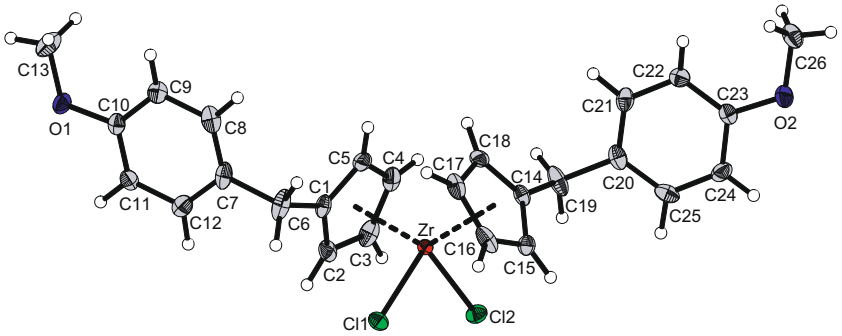


Fig. 4. X-ray diffraction structure of bis-[(*p*-methoxybenzyl)cyclopentadienyl] zirconium(IV) dichloride **3a**; (thermal ellipsoids are drawn at the 50% probability level).

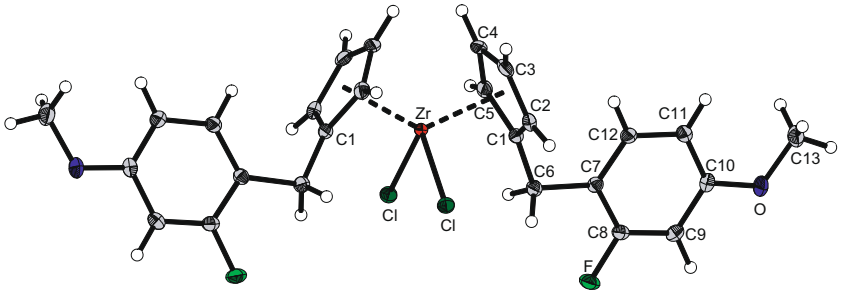


Fig. 5. X-ray diffraction structure of bis-[(2-fluoro-4-methoxybenzyl)cyclopentadienyl] zirconium(IV) dichloride **3b**; (thermal ellipsoids are drawn at the 50% probability level).

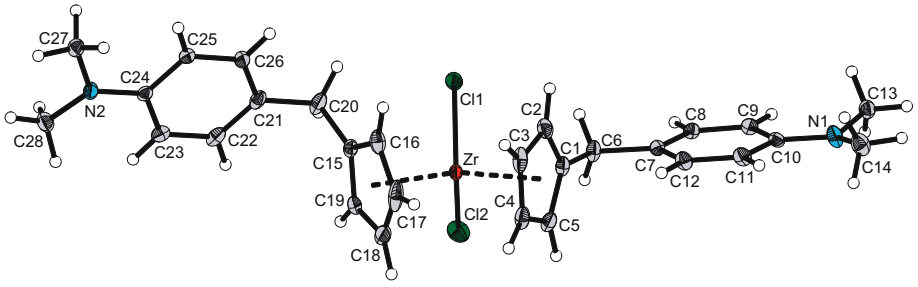


Fig. 6. X-ray diffraction structure of bis-[(4-*N*,*N*-dimethylaminobenzyl)cyclopentadienyl] zirconium(IV) dichloride **3c**; (thermal ellipsoids are drawn at the 50% probability level).

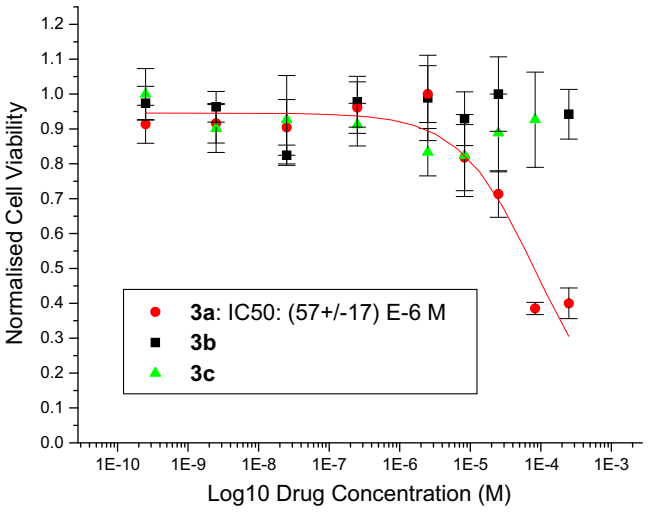


Fig. 7. Cytotoxicity curves from typical MTT assays showing the effect of compounds **3a–c** on the viability of LLC-PK cells.

3.3. Cytotoxicity studies

The solubility of **3a–c** in DMSO is limited. For the cell testing three times the amount of DMSO compared to their isostructural titanocene analogues was required for each compound. In order to keep the DMSO concentration below 0.7%, which protects the cells from a cytotoxic effect through DMSO, three times the amount of medium was added. As a result each solution of the zirconocene was three times less concentrated than that of its titanocene analogue reaching a maximum concentration of 170 μM. The dose response curves shown in Fig. 7 represent the experimental values obtained from two consistent MTT assays for each compound tested. As seen in Fig. 7 compounds **3b** and **3c** did not show a cytotoxic effect at the concentrations given, but **3a** showed good cytotoxic activity and even an IC50 value of 57 μM could be obtained for **3a** despite its limited solubility. The *p*-methoxybenzyl-substituted zirconocene dichloride **3a** (Zirconocene **Y**) does not reach the cytotoxicity shown by its titanocene analogue, Titanocene **Y**, which has an IC50 value of 21 μM, but loses activity by only a factor of three.

4. Conclusions and outlook

The synthesis of titanocenes through the hydridolithiation reaction of aryl-substituted fulvenes with Superhydride can be transferred to zirconocenes without change. Two of the three zirconocenes **3a–b** synthesised did not show cytotoxic activity in the LLC-PK assay, as expected from the literature. But Zirconocene **Y** showed a surprisingly high activity against this kidney cancer cell line leading to an IC50 value of 57 μM. Future experiments on a wider panel of cell lines are planned; these results will show, whether Zirconocene **Y** has antitumor activity or will become an anticancer drug candidate.

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