Journal of Organometallic Chemistry 694 (2009) 1369–1374

Contents lists available at ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

Novel benzyl-substituted vanadocene anticancer drugs

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ARTICLE INFO

Article history:
Received 13 October 2008
Received in revised form 3 December 2008
Accepted 11 December 2008
Available online 25 December 2008

Keywords:
Anticancer drug
Vanadium
Hydridolithiation
Fulvene
LLC-PK

ABSTRACT

From the reaction of 6-(*p*-methoxyphenyl) fulvene (**1a**), 6-(3,4-dimethoxyphenyl) fulvene (**1b**) and 6-(3,4,5-trimethoxyphenyl) fulvene (**1c**) with $LiBEt_3H$, lithiated cyclopentadienide intermediates (**2a–c**) were synthesised. These intermediates were then transmetallated to vanadium with VCl_4 to yield the benzyl-substituted vanadocenes bis-[(*p*-methoxybenzyl)cyclopentadienyl] vanadium(IV) dichloride (**3a**), bis-[(3,4-dimethoxybenzyl)cyclopentadienyl] vanadium(IV) dichloride (**3b**), and bis-[(3,4,5-trimethoxybenzyl)cyclopentadienyl] vanadium(IV) dichloride (**3c**). The two vanadocenes **3a** and **3c** were characterised by single crystal X-ray diffraction. All three vanadocenes had their cytotoxicity investigated through MTT based preliminary *in vitro* testing on the LLC-PK (pig kidney epithelial) cell line in order to determine their IC50 values and compare them with the corresponding titanocene dichloride derivatives. Vanadocenes **3b–c** were found to have IC50 values of 9.1 and 8.3 μM, while **3a** showed a superior value of 3.0 μM, respectively.

1. Introduction

Beyond the field of platinum anticancer drugs there is significant unexplored space for further metal-based drugs targeting cancer. Titanium-based reagents have significant potential against solid tumors. Budotitane ([*cis*-diethoxybis(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 allow direct access to anti-proliferative 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.

Though many applications have been explored for vanadocene compounds such as catalyses in polymerisation experiments [9], recently and similar to the titanocene complexes mentioned above, vanadocene and vanadocene dichloride complexes have proven to be effective antitumor agents [10–14]. Indeed vanadocene dichloride (Cp_2VCl_2) underwent the same extensive preclinical testing against both animal and human cell lines alongside Cp_2TiCl_2 [15–19]. In these studies Cp_2VCl_2 was found to be more active than Cp_2TiCl_2 *in vitro*.

The main problem encountered when characterising vanadocene compounds is the paramagnetic nature of the vanadium centre, which hinders the use of classical NMR tools. Along with other methods, ESR spectroscopy was used as a very efficient method for the investigation of such paramagnetic d^1-complexes, which resulted in the expected 8-line spectrum caused by interaction of the unpaired electron with the ^{51}V ($I = 7/2$; 99.8%) nucleus.

With this in mind, and following on from the work carried out on Titanocene **Y**, we decided to substitute the titanium metal centre with vanadium in order to compare the difference in cytotoxicity between the titanocene dichloride compounds and vanadocene dichloride compounds. The vanadocene dichloride compounds undergo the traditional hydridolithiation route, similar to the titanocene derivatives.

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doi:10.1016/j.jorganchem.2008.12.033

Within this paper, we present the synthesis and preliminary cytotoxicity studies of a series of three vanadocene dichloride derivatives, modelled on Titanocene **Y**.

2. Experimental

2.1. General conditions

Manipulations of air and moisture sensitive compounds were performed under an inert atmosphere of nitrogen or argon using standard Schlenk techniques. Vanadium tetrachloride (VCl_4) and Super Hydride ($LiBEt_3H$, 1.0 M solution in THF) were obtained commercially from Sigma–Aldrich. All solvents were dried and distilled according to standard methods. IR spectra were recorded on a Perkin Elmer Paragon 1000 FT-IR Spectrometer employing KBr discs. UV–Vis spectra were recorded on a Unicam UV4 Spectrometer. Electron spray mass spectrometry (MS) was performed on a quadrupole tandem mass spectrometer (Quattro Micro, Micromass/Water's Corp., USA), using solutions made up in 50% dichloromethane and 50% methanol. MS spectra were obtained in the ES+ (electron spray positive ionisation) mode for compounds **3a–c**. ESR spectra were measured on a MiniScope MS200 apparatus in microwave X band (~9.5 GHz). Isotropic spectra were measured in 5 mM solutions of **3a–c** in CH_2Cl_2 at room temperature (20 °C set using Magnettech temperature controller HO_2). Spectra obtained were simulated using ESR simulation software Multiplot 2.26. X-ray diffraction data for compounds **3a** and **3c** were collected using a Bruker SMART APEX CCD area detector diffractometer. A full sphere of reciprocal space was scanned by φ-ω scans. Pseudo-empirical absorption correction based on redundant reflections was performed by the program SADABS [20]. The structures were solved by direct methods using SHELXS-97 [21] and refined by full matrix least-squares on F^2 for all data using SHELXL-97 [21]. In **3a** all hydrogen atoms were located in the difference fourier map and allowed to refine freely. In **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 formed from the slow evaporation of a saturated trichloromethane solution, while crystals of **3c** were grown in a saturated dichloromethane solution with slow infusion of pentane.

Table 1
Crystal data and structure refinement for **3a** and **3c**.

Identification code	**3a**	**3c**
Empirical formula	$C_{26}H_{26}O_2Cl_2V$	$C_{30}H_{34}O_6Cl_2V$
Formula weight	492.31	612.41
Temperature (K)	100(2)	293(2)
Wavelength (Å)	0.71073	0.71073
Crystal system	Orthorhombic	Monoclinic
Space group	*Pbca* (#61)	*Pc* (#7)
Unit cell dimensions		
a (Å)	6.5480(17)	19.515(4)
b (Å)	25.124(6)	6.5893(11)
c (Å)	26.984(7)	11.795(2)
α (°)	90	90
β (°)	90	107.587(3)
γ (°)	90	90
Volume (Å^3)	4439.1(19)	1445.8(4)
Z	8	2
D_{calc} (Mg/m^3)	1.473	1.404
Absorption coefficient (mm^{-1})	0.709	0.569
F(000)	2040	636
Crystal size (mm^3)	0.60 × 0.05 × 0.02	0.90 × 0.25 × 0.20
θ Range for data collection (°)	1.51–24.13	1.09–29.09
Index ranges	$-7 \leqslant h \leqslant 7$, $-28 \leqslant k \leqslant 21$, $-20 \leqslant l \leqslant 30$	$-26 \leqslant h \leqslant 26$, $-8 \leqslant k \leqslant 8$, $-15 \leqslant l \leqslant 15$
Reflections collected	14799	26979
Independent reflections	3507 [R_{int} = 0.0507]	7012 [R_{int} = 0.0199]
Completeness to θ_{max} (%)	99.0	92.8
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents
Maximum and minimum transmission	0.9860 and 0.7918	0.8946 and 0.7466
Refinement method	Full-matrix least-squares on 0^2	Full-matrix least-squares on F^2
Data/restraints/parameters	3507/0/282	7012/2/358
Goodness-of-fit on F^2	1.026	1.047
Final *R* indices [$I > 2\sigma(I)$]	R_1 = 0.0451, wR_2 = 0.1000	R_1 = 0.0280, wR_2 = 0.0705
R indices (all data)	R_1 = 0.0615, wR_2 = 0.1063	R_1 = 0.0285, wR_2 = 0.0709
Absolute structure parameter	N/A	0.000(11)
Largest difference in peak and hole (e Å^{-3})	0.468 and −0.322	0.311 and −0.227

2.2. Synthesis

The syntheses of 6-(*p*-methoxyphenyl) fulvene (**1a**), 6-(3,4-dimethoxyphenyl) fulvene (**1b**) and 6-(3,4,5-trimethoxyphenyl) fulvene (**1c**) were carried out accordingly to already published procedures [8,22].

2.2.1. Synthesis of bis-[(p-methoxybenzyl)cyclopentadienyl] vanadium(IV) dichloride [η^5-C_5H_4–CH_2–C_6H_4–O–CH_3]$_2VCl_2$ (**3a**)

15.0 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 in a Schlenk flask. The concentrated Super Hydride was dissolved in 30 ml of dry diethyl ether to give a cloudy white suspension. 2.40 g (13.0 mmol) of the red solid **1a** was added to a Schlenk flask and was dissolved in 90 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 8 h to give a white precipitate of the lithium cyclopentadienide intermediate and the solution had changed colour from orange/red to colourless with a white precipitate formed. 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 nitrogen. 2.01 g (10.4 mmol, 80.4% yield) of the lithiated cyclopentadienide intermediate **2a** was obtained. The lithium cyclopentadienide intermediate was dissolved in 60 ml of dry THF to give a colourless solution. 0.56 ml (5.25 mmol) of vanadium tetrachloride was added to the lithium cyclopentadienide intermediate solution slowly at −78 °C to give a dark red solution. The dark red vanadium solution was refluxed for 20 h at 88 °C. After refluxing, the solution was allowed to return to room temperature and then cooled to −78 °C where a light green precipitate formed. The precipitate was filtered on to a frit and washed with 20 ml of THF and small quantities of chloroform. The light green solid was then dissolved in chloroform and filtered through a frit to remove any remaining LiCl. The solvent was removed under reduced pressure to yield a light green crystalline solid (1.23 g, 2.51 mmol, 48.0% yield) **3a**.

Micro Anal. Calc. for $VCl_2O_2C_{26}H_{26}$: C, 63.43; H, 5.32; Cl, 14.40. Found: C, 62.80; H, 5.16; Cl, 13.98%.

ESR (CH_2Cl_2 solution, RT): 8-line hyperfine coupling, g_{iso} = 2.012, A_{iso} = 7.47 mT.

MS (*m*/*z*, QMS-MS/MS): 456 [M–Cl]$^+$.

IR absorptions (KBr, cm^{-1}): 3084, 3001, 2959, 2931, 2835, 1608, 1511, 1462, 1439, 1301, 1246, 1178, 1030, 833, 764.

UV–Vis (CH_2Cl_2, nm): λ 234 (ε 22,800), λ 284 (ε 12,400), λ 383 (ε 2,860), λ 655 (ε 460).

*2.2.2. Synthesis of bis-[(3,4-dimethoxybenzyl)cyclopentadienyl] vanadium(IV) dichloride [η^5-C_5H_4–CH_2–C_6H_3–$(OCH_3)_2$]$_2VCl_2$ (**3b**)*

15.0 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 in a Schlenk flask. The concentrated Super Hydride was dissolved in 30 ml of dry diethyl ether to give a cloudy white suspension. 2.40 g (11.2 mmol) of the red solid **1b** was added to a Schlenk flask and was dissolved in 90 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 16 h to give a white precipitate of the lithium cyclopentadienide intermediate and the solution had changed colour from orange/red to colourless with a white precipitate formed. 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 nitrogen. 1.92 g (8.6 mmol, 77.1% yield) of the lithiated cyclopentadienide intermediate **2b** was obtained. The lithium cyclopentadienide intermediate was dissolved in 60 ml of dry THF to give a colourless solution. 0.46 ml (4.3 mmol) of vanadium tetrachloride was added to the lithium cyclopentadienide intermediate solution slowly at −78 °C to give a dark red solution. The dark red vanadium solution was refluxed for 20 h at 88 °C. After refluxing, the solution was allowed to return to room temperature and then cooled to −78 °C where a light green precipitate formed. The precipitate was filtered on to a frit and washed with 20 ml of THF and small quantities of chloroform. The light green solid was then dissolved in chloroform and filtered through a frit to remove any remaining LiCl. The solvent was removed under reduced pressure to yield a light green crystalline solid (0.89 g, 1.6 mmol, 37.5% yield) **3b**.

Micro Anal. Calc. for $VCl_2O_4C_{28}H_{30}$: C, 60.88; H, 5.84; Cl, 12.84. Found: C, 59.91; H, 5.37; Cl, 13.03%.

ESR (CH_2Cl_2 solution, RT): 8-line hyperfine coupling, g_{iso} = 2.019, A_{iso} = 7.47 mT.

MS (*m*/*z*, QMS-MS/MS): 516 $[M-Cl]^+$.

IR absorptions (KBr, cm^{-1}): 3103, 3083, 2953, 2931, 2835, 1588, 1514, 1462, 1444, 1416, 1261, 1142, 1022, 874, 848, 767.

UV–Vis (CH_2Cl_2, nm): λ 236 (ε 26,560), λ 283 (ε 17,820), λ 386 (ε 3,580), λ 655 (ε 620).

*2.2.3. Synthesis of bis-[(3,4,5-trimethoxybenzyl)cyclopentadienyl] vanadium(IV) dichloride [η^5-C_5H_4–CH_2–C_6H_2–$(OCH_3)_3$]$_2VCl_2$ (**3c**)*

15.0 ml (15.0 mmol) of 1 molar 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. 2.00 g (8.2 mmol) of the dark red solid **1c** was added to a Schlenk flask and was dissolved in 90 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 16 h to give a white precipitate of the lithium cyclopentadienide intermediate and the solution had changed colour from orange/red to colourless with a white precipitate formed. 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 nitrogen. 1.58 g (6.3 mmol, 76.3% yield) of the lithiated cyclopentadienide intermediate **2c** was obtained. The lithium cyclopentadienide intermediate was dissolved in 60 ml of dry THF to give a colourless solution. 0.34 ml (3.2 mmol) of vanadium tetrachloride was added to the lithium cyclopentadienide intermediate solution slowly at −78 °C to give a dark red solution. The dark red vanadium solution was refluxed for 20 h at 88 °C. After refluxing, the solution was allowed to return to room temperature and then cooled to −78 °C where a light green precipitate formed. The precipitate was filtered on to a frit and washed with 20 ml of THF and small quantities of chloroform. The light green solid was then dissolved in chloroform and filtered through a frit to remove any remaining LiCl. The solvent was removed under reduced pressure to yield a light green crystalline solid (0.89 g, 1.1 mmol, 35.2% yield) **3c**.

Micro Anal. Calc. for $VCl_2O_6C_{30}H_{34}$: C, 58.83; H, 5.60; Cl, 11.58. Found: C, 57.87; H, 5.65; Cl, 11.42%.

ESR (CH_2Cl_2 solution, RT): 8-line hyperfine coupling, g_{iso} = 2.015, A_{iso} = 7.47 mT.

MS (*m*/*z*, QMS-MS/MS): 576 $[M-Cl]^+$.

IR absorptions (KBr, cm^{-1}): 3099, 2947, 2832, 1637, 1589, 1506, 1457, 1419, 1330, 1238, 1140, 1116, 1054, 1000, 919, 803, 731.

UV–Vis (CH_2Cl_2, nm): λ 236 (ε 24,620), λ 288 (ε 12,380), λ 386 (ε 3,040), λ 655 (ε 520).

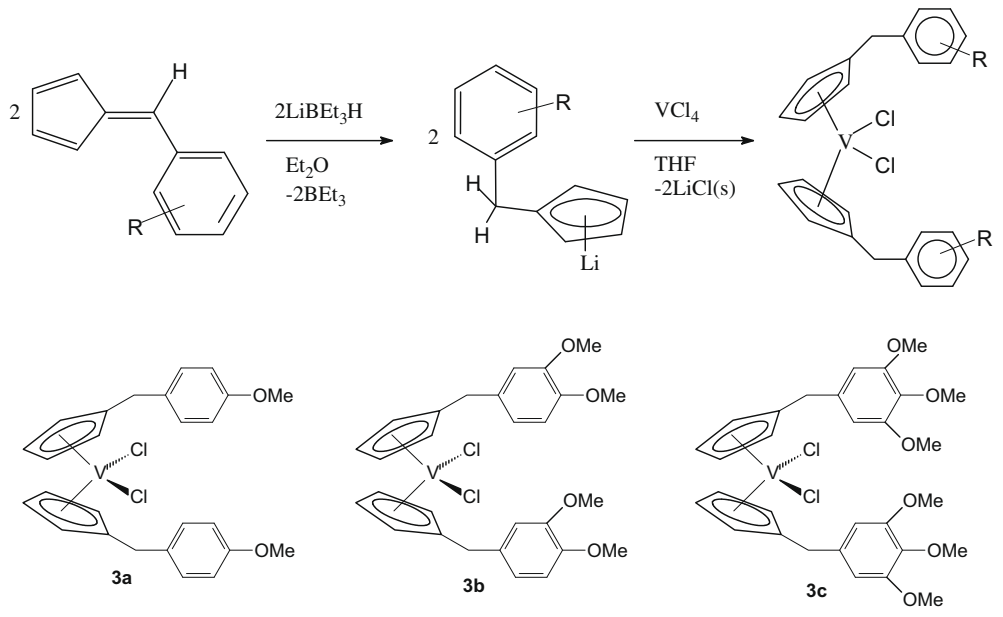
2.3. Cytotoxicity studies

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 (fetal 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 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) [23] 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.

3. Results and discussion

3.1. Synthesis

The lithium intermediates used in the synthesis of vanadocene derivatives **3a–c** were synthesised by the hydridolithiation reaction of aryl-fulvenes with Super Hydride ($LiBEt_3H$). This form of nucleophilic addition to the exocyclic double bond of the fulvene is highly selective due to the increased polarity as a result of the inductive effect of the corresponding phenyl ring. There is no nucleophilic attack seen at the diene element of the aryl-fulvenes. The lithiated cyclopentadienide intermediate was isolated with



Scheme 1. General reaction scheme for the synthesis of benzyl-substituted vanadocene dichloride derivatives **3a–c**.

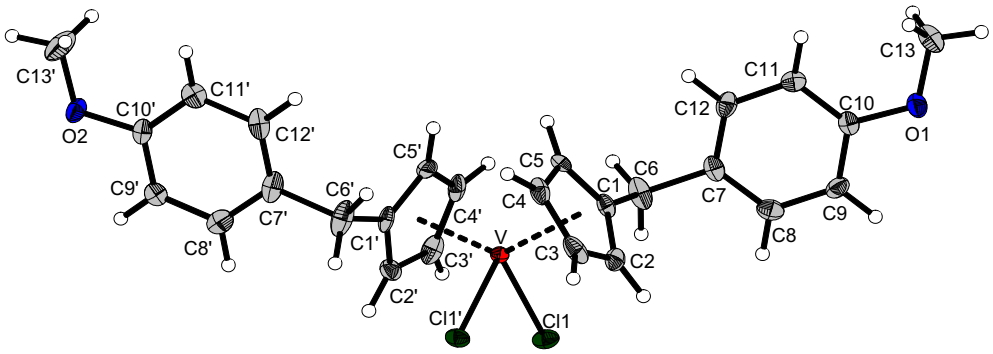


Fig. 1. X-ray diffraction structure of **3a**; thermal ellipsoids are drawn on the 50% probability level.

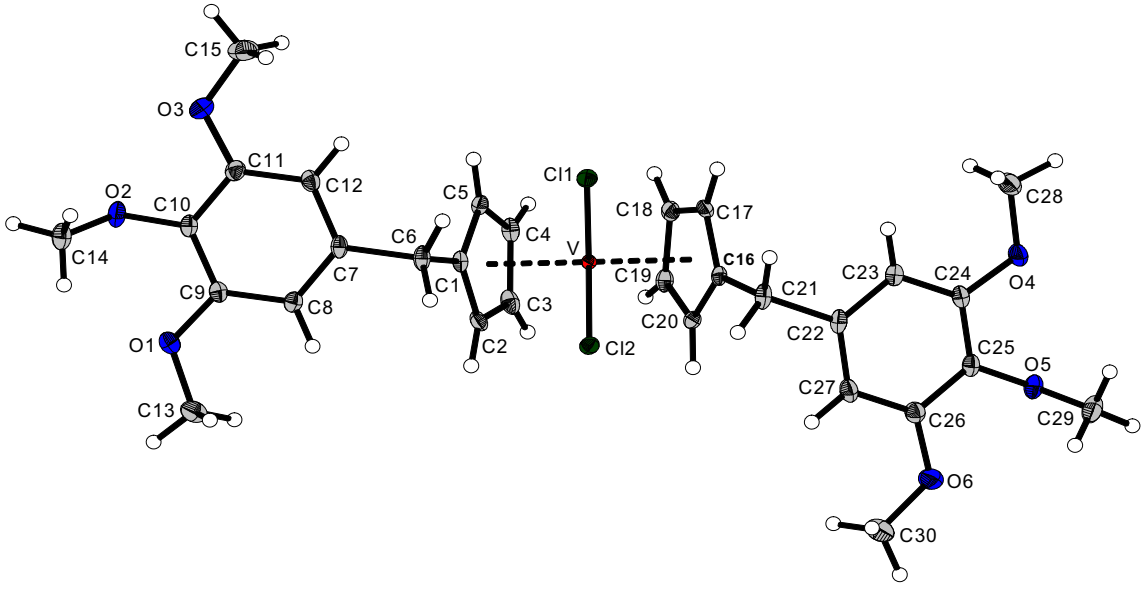


Fig. 2. X-ray diffraction structure of **3c**; thermal ellipsoids are drawn on the 15% probability level.

good yields of 76–80%. Two equivalents of the lithiated cyclopentadienide intermediate were transmetallated with one equivalent of vanadium tetrachloride to form the desired vanadocene dichlorides in yields of 35–48% and lithium chloride as a by-product.

As shown in Scheme 1, derivatives **3a**, **3b**, and **3c** were isolated as air stable light green crystalline solids. They are soluble in organic chlorinated solvents, however all three compounds displayed tendencies to decompose slightly in $CHCl_3$ solutions, when exposed to moist air for 24 h.

3.2. Structural discussion

Despite the effort to crystallise the vanadocene **3b**, no single crystals could be isolated and hence no structures were obtained. This could possibly be explained by the slow decomposition of the compound in solution as stated previously. However, suitable crystal structures of compounds **3a** and **3c** have been isolated. **3a** crystallises in the orthorhombic space group *Pbca* (#61) with eight molecules in the unit cell (see Fig. 1), while the structure for **3c** is found to be monoclinic using space group *Pc* (#7) with two molecules in the unit cell as shown in Fig. 2. The absence of solvent molecules present in both unit cells is a particular advantage when it comes to biological testing of these compounds.

Table 2
Selected bond lengths and angles from the crystal structure of **3a** and **3c**.

	3a	3c
Bond lengths (Å)		
V–C(1)	2.304(3)	2.333(16)
V–C(2)	2.331(3)	2.336(15)
V–C(3)	2.335(3)	2.311(17)
V–C(4)	2.278(3)	2.304(16)
V–C(5)	2.288(3)	2.317(16)
V–Cent(1)	1.974(1)	1.987(3)
V–Cent(2)	1.979(1)	1.992(1)
C(1)–C(5)	1.410(5)	1.419(2)
C(1)–C(2)	1.414(5)	1.410(2)
C(2)–C(3)	1.391(5)	1.394(3)
C(3)–C(4)	1.397(5)	1.418(3)
C(4)–C(5)	1.408(5)	1.398(2)
C(1)–C(6)	1.502(5)	1.496(2)
C(6)–C(7)	1.527(4)	1.516(2)
V–Cl(1)	2.4103(10)	2.3973(5)
V–Cl(2)	2.4185(10)	2.3847(6)
Bond angles (°)		
Cent(1)–V–Cent(2)	132.79(3)	132.80(1)
Cent(1)–V–Cl(1)	106.49(3)	107.19(1)
Cent(1)–V–Cl(2)	106.78(3)	106.41(1)
Cl(1)–V–Cl(2)	88.47(4)	86.41(17)

The bond length between the vanadium centre and the centroid of the cyclopentadienyl rings is similar for both **3a** and **3c** structures with lengths varying from 1.974 Å to 1.992 Å. These lengths are comparable to those found in the literature for Cp–V bonds [9]. These bond lengths are also shorter than the corresponding Ti–Cp(Centroid) bond, which has a bond length of 2.060 Å for Titanocene **Y**. This is due to the unpaired electron at the vanadium d^1 centre, which leads to back bonding at the Cp ligands, resulting in shorter bond lengths. The same characteristic has the effect of slightly lengthening the V–Cl bonds to a range of 2.385–2.419 Å for both vanadocene dichloride structures, whereas the Ti–Cl bond lengths for Titanocene **Y** are in the region of 2.370 Å. This back bonding, along with the bulkiness of the Cp ligands, also has an effect on the overall conformation of the molecule, whereby the Cp(centroid)–V–Cp(centroid) bond angle was widened to around 132.8° for both vanadocene compounds when compared to the corresponding bond angle of 130.70° in Titanocene **Y**. Conversely the Cl–V–Cl bond angles are narrowed in order to accommodate the broadened Cp(centroid)–V–Cp(centroid) bond angles, and were measured at 88.47° for **3a** and 86.41° for **3c**, while titanocene Y displayed a Cl–Ti–Cl bond angle of 95.90°. The carbon carbon bond lengths of the cyclopentadienyl rings in **3a** range from of 1.414 Å to 1.391 Å with **3c** having lengths in the range of 1.419–1.394 Å. In summary it means, that both structures display a distorted tetrahedral conformation; their crystal data and refinement details are found in Table 1, while selected bond lengths and angles are displayed in Table 2.

ESR spectra of all three vanadocenes were taken using 5 mM solutions in CH_2Cl_2 at room temperature and resulted in the predicted 8-line spectra as mentioned above. Fig. 3 displays the ESR spectrum of vanadocene **3a**, which corresponds to ESR studies carried out on similar vanadocene complexes [13,14,24–26].

3.3. Cytotoxicity studies

While displaying the lowest cytotoxic effect of the series, vanadocene **3b** yielded an IC50 value of 9.1 μM, which improves upon its corresponding titanocene with an IC50 value of 88 μM. Similarly **3c**, with a value of 8.3 μM, also proves to be a greater cytotoxic agent when compared to its poorly soluble titanocene counterpart which provided an IC50 value of 253 μM. Compound **3a**, which gave an IC50 value of 3.0 μM, shows a dramatic improvement in cytotoxicity when compared to its titanium equivalent, Titanocene **Y**, which yielded in an IC50 value of 21 μM and also its tin equivalent, which yielded an IC50 value of 15 μM [27]. Along with these comparisons, vanadocene **3a** displayed a value, which is equivalent to that of cisplatin, which gave an IC50 value of 3.3 μM

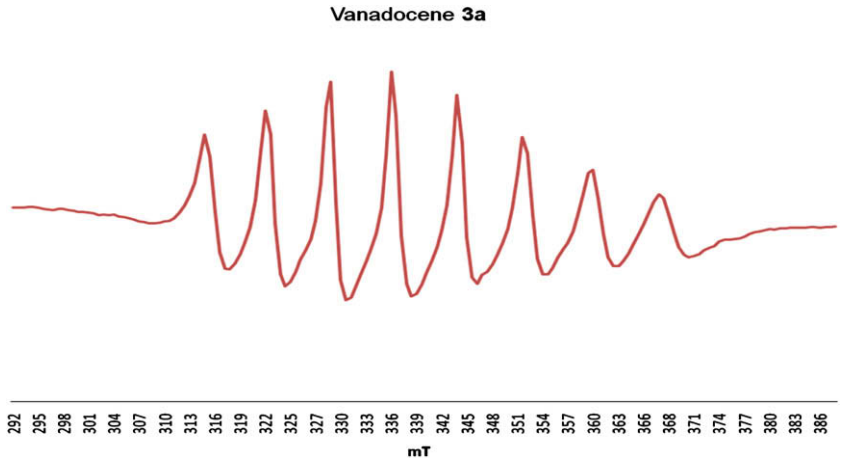


Fig. 3. 8-Line ESR spectrum of **3a**.

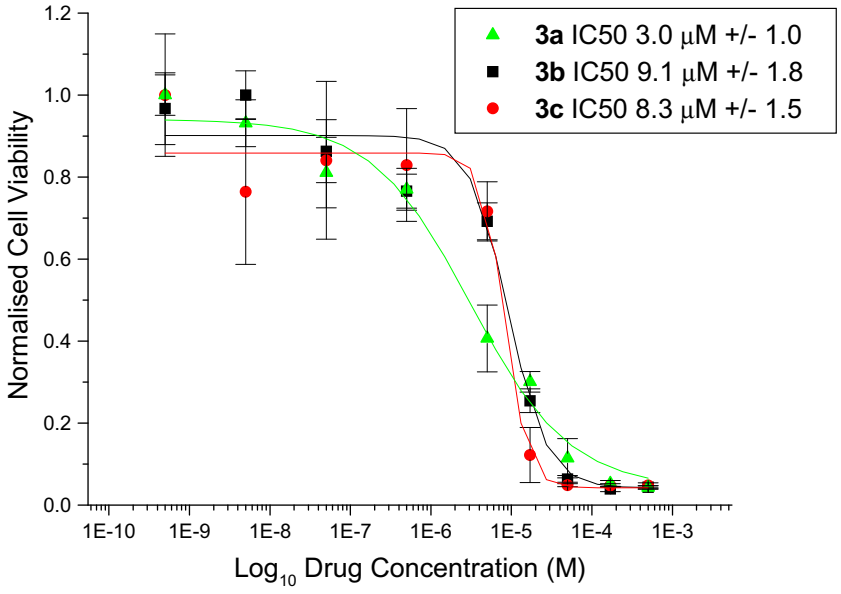


Fig. 4. Cytotoxicity curves from typical MTT assays showing the effect of compounds **3a–c** on the viability of LLC-PK cells.

against the same LLC-PK cell line. This result emphasises the importance of the *p*-methoxy phenyl moiety that is present in both **3a** and Titanocene **Y**, as well as highlighting the difference, in cytotoxic effects, of substituting the metal centre of these metallocenes with different transition metals, in this case vanadium. As seen in Fig. 4, cell viability is reduced on a more gradual scale relative to the drug concentration for compound **3a**, with the higher concentrations resulting in almost total cell death for all three compounds. In general, **3a–c** displayed good solubility and satisfactory margins of error.

4. Conclusions and outlook

The hydridolithiation of 6-aryl substituted fulvenes followed by transmetallation has been found to be a very effective and reproducible way to highly cytotoxic benzyl-substituted titanocene and also vanadocene dichloride complexes. Complexes **3b–c** yielded very promising anti-tumour IC50 values of 9.1 and 8.3 μM, respectively. But these two compounds are outperformed by **3a** (Vanadocene **Y**), which exhibits an IC50 value of 3.0 μM against the LLC-PK cell line. This is particularly encouraging, since Vanadocene **Y** is performing slightly better against this cell line than cisplatin, which shows an IC50 value of 3.3 μM. The results of Vanadocene **Y** are important enough to warrant further work in this area along with future experiments on a wider panel of cell lines hopefully leading to *in vivo* testing against the most promising cancer in the nearby future.

Supplementary material

CCDC 704917 and 704918 contain the supplementary crystallographic data for compounds **3a** and **3c**, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Acknowledgements

The authors thank the Higher Education Authority (HEA), the Centre for Synthesis and Chemical Biology (CSCB), University College Dublin (UCD), COST D39 and the Central European Society for Anticancer Drug Research (CESAR) for funding.

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