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Synthesis and Cytotoxicity Studies of Fluorinated Derivatives of Vanadocene Y

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From the reaction of 6-(2-fluoro-4-methoxyphenyl)fulvene (**1a**), 6-(3-fluoro-4-methoxyphenyl)fulvene (**1b**) and 6-[4-(trifluoromethoxy)phenyl]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[(2-fluoro-4-methoxybenzyl)cyclopentadienyl]vanadium(IV) dichloride (**3a**), bis[(3-fluoro-4-methoxybenzyl)cyclopentadienyl]vanadium(IV) dichloride (**3b**), and bis[(4-trifluoromethoxybenzyl)cyclopentadienyl]vanadium(IV) dichloride (**3c**). The three vanadocenes **3a–c** 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 and Caki-1 cell lines in order to determine their IC_{50} values. Vanadocenes **3a–c** were found to have IC_{50} values of 6.0 (+/–4), 35 (+/–7) and 13 (+/–3) μM on the LLC-PK cell line and IC_{50} values of 78 (+/–11), 18 (+/–16) and 2.2 (+/–0.5) μM on the Caki-1 cell line respectively.

Introduction

Renal Cell Carcinoma (RCC) is the third most common urological cancer and is a particularly aggressive disease which has a poor prognosis and resists conventional chemotherapy.[1] A 5 year survival rate for patients with advanced cases is estimated for 61% of those patients, and the average overall survival rate for patients diagnosed with the metastatic disease is less than a year.[2] In order to find suitable treatments for such diseases, researchers have explored the field of metal-based drugs, where Cisplatin is one of the most successful and widely known compounds. Cisplatin is a very effective anticancer drug and is widely used in the treatment of many different types of cancers, particularly in testicular and ovarian cancer.[3–5] However, problems are encountered when treating RCC with Cisplatin due to its nephrotoxicity and its lack of activity against tumors with natural or acquired resistance.[6,7] As a result further examination into this area is needed.

Beyond the field of platinum antitumor 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.[8] Much more robust in this aspect of hydrolysis is titanocene dichloride (Cp_2TiCl_2), which shows medium antiproliferative activity in vitro but promising results in vivo.[9,10] Titanocene dichloride reached clinical trials, but the efficacy of Cp_2TiCl_2 in Phase II clinical trials in patients with metastatic renal cell carcinoma[11] or metastatic breast cancer[12] was too low to be pursued. 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.[13] Hydridolithiation of 6-anisylfulvene 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. 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,[15] recently and similar to the titanocene complexes mentioned above, vanadocene and vanadocene dichloride complexes have proven to be effective antitumor agents.[16–20] Indeed vanadocene dichloride (Cp_2VCl_2) underwent the same extensive preclinical testing against both animal and human cell lines alongside Cp_2TiCl_2.[21–25] In these studies Cp_2VCl_2 was found to be more active than Cp_2TiCl_2 in vitro. A recent study has examined the action

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mechanism of vanadocene dichloride as an antitumor drug through the binding of V^{IV} to transferrin.[26] More recently, and following on from the work carried out on Titanocene **Y**, highly cytotoxic benzyl-substituted vanadocene dichloride derivatives have been synthesised using the traditional hydridolithiation route, similar to the titanocene derivatives. The most active compound in the series was found to be bis[(*p*-methoxybenzyl)cyclopentadienyl] vanadium(IV) dichloride (Vanadocene **Y**).[27] This gave an IC_{50} of 3.0 μM on the same LLC-PK cell line as Titanocene **Y**. This result warrants further work on these vanadocene dichloride derivatives.

One of the main problems encountered when characterising vanadocene compounds is the paramagnetic nature of the vanadium centre, which hinders the use of classical NMR tools. Along with X-ray crystallography and other methods, electron-spin resonance (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 ^{51}V (I = 7/2; 99.8%) nucleus.

Within this paper we present the synthesis and preliminary cytotoxicity studies of a series of three fluorinated derivatives of Vanadocene **Y** (Figure 1). The compounds were tested on the LLC-PK cell line as well as the human renal cell line Caki-1.

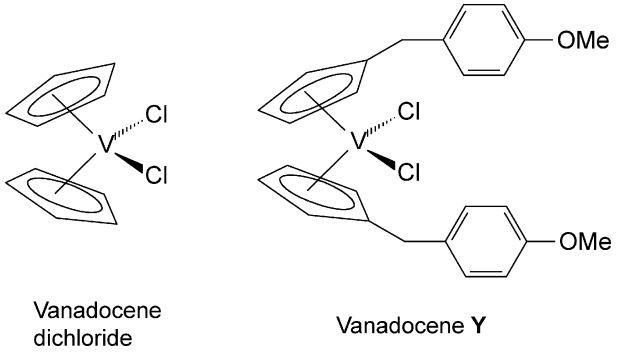


Figure 1. Structures of vanadocene dichloride and Vanadocene **Y**.

Results and Discussion

Synthesis

The lithium intermediates used in the synthesis of vanadocene derivatives **3a**–**c** (Figure 2) were synthesised by the hydridolithiation reaction of aryl-fulvenes with Super Hydride ($LiBEt_3H$) as seen in Scheme 1. 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 arylfulvenes. The lithiated cyclopentadienide intermediate were isolated with good yields of 78% to 86%. Two equivalents of the lithiated cyclopentadienide intermediate were transmetallated with one equivalent of vanadium tetrachloride to form the desired vanadocene dichlorides in yields of 36% to 56% and lithium chloride as a by-product.

The work up of vanadocene **3c** was slightly different compared with the other two compounds due to the difference in solubility caused by the trifluoromethoxy moiety. The derivatives **3a**, **3b**, and **3c** were isolated as air stable green crystalline solids. They are soluble in organic chlori-

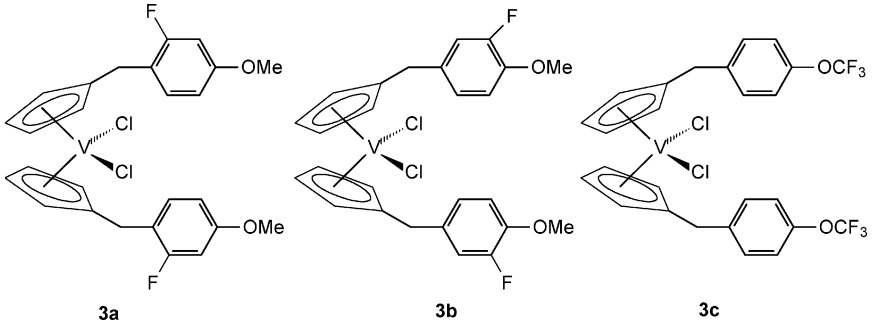
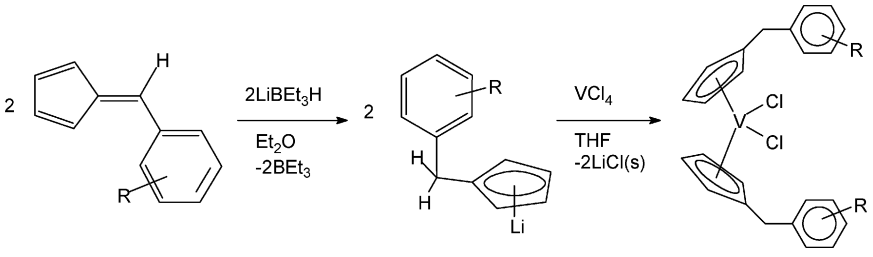


Figure 2. Structures of vanadocenes **3a**–**c**.



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

nated solvents, however all three compounds displayed tendencies to decompose slightly in $CHCl_3$ solutions, when exposed to moist air for 24 h.

Structural Discussion

Suitable crystal structures of compounds **3a**, **3b** and **3c** have been isolated. All three vanadocenes crystallised in the monoclinic space group *C*2/*c* (#15). Both **3a** (Figure 3) and **3b** (Figure 4) contained four molecules in their unit cell with the respective molecule placed on a twofold axis, while **3c** was found to contain eight molecules in the unit cell (Figure 5) with the molecule placed on the general position. The absence of solvent molecules present in all three unit cells is a particular advantage when it comes to biological testing of these compounds. As expected, the bond lengths and angles for these three vanadocene dichloride structures are very similar to those reported for Vanadocene **Y**. Selected bond lengths and angles are displayed in Table 1, while the crystal data and refinement details for all three compounds are found in Table 2.

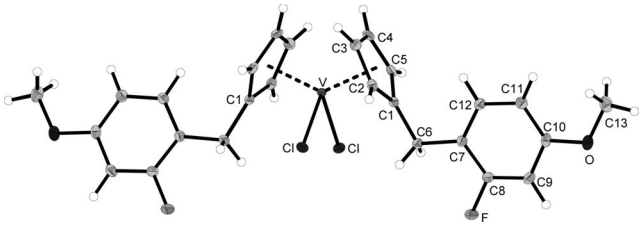


Figure 3. X-ray diffraction structure of **3a**; thermal ellipsoids are drawn on the 50% probability level.

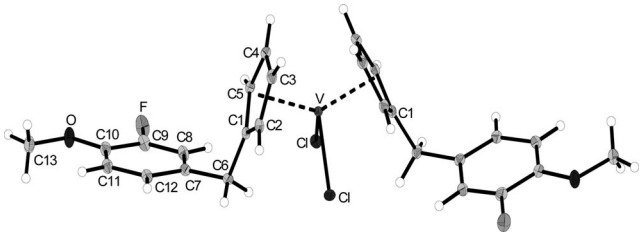


Figure 4. X-ray diffraction structure of **3b**; thermal ellipsoids are drawn on the 50% probability level.

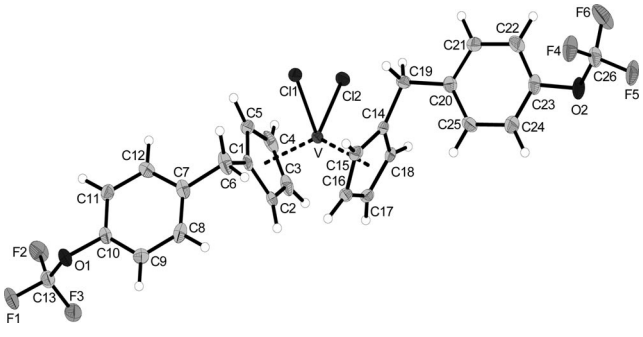


Figure 5. X-ray diffraction structure of **3c**; thermal ellipsoids are drawn on the 50% probability level.

Table 1. Selected bond lengths and angles from the crystal structures of **3a**–**c**.

	3a	**3b**	**3c**
Bond lengths [Å]			
V–C(1)	2.3416(12)	2.3267(12)	2.302(3)
V–C(2)	2.3544(12)	2.3226(12)	2.301(3)
V–C(3)	2.3030(13)	2.3085(13)	2.293(3)
V–C(4)	2.2961(13)	2.3169(13)	2.330(3)
V–C(5)	2.3052(12)	2.3243(12)	2.299(3)
V–Cent(1)	1.985(1)	1.982(1)	1.973(3)
V–Cent(2)	1.985(1)	1.982(1)	1.977(3)
C(1)–C(5)	1.4124(18)	1.4239(17)	1.415(4)
C(1)–C(2)	1.4000(18)	1.4249(18)	1.400(4)
C(1)–C(6)	1.4951(17)	1.5063(17)	1.490(4)
C(2)–C(3)	1.426(2)	1.4071(19)	1.403(4)
C(3)–C(4)	1.4016(19)	1.4268(19)	1.385(4)
C(4)–C(5)	1.4227(17)	1.4036(18)	1.398(4)
C(6)–C(7)	1.5174(18)	1.5135(18)	1.514(4)
V–Cl(1)	2.4000(4)	2.4136(4)	2.4133(8)
V–Cl(2)	2.4000(4)	2.4136(4)	2.3738(8)
Bond angles [°]			
Cent(1)–V–Cent(2)	132.64(1)	133.54(1)	132.89(2)
Cent(1)–V–Cl(1)	105.57(1)	106.87(1)	107.36(2)
Cent(1)–V–Cl(2)	108.14(1)	106.64(1)	106.78(3)
Cent(2)–V–Cl(1)	108.14(1)	106.64(1)	106.28(3)
Cent(2)–V–Cl(2)	105.57(1)	106.87(1)	107.06(3)
Cl(1)–V–Cl(2)	87.602(18)	86.102(18)	86.87(3)

The bond length between the vanadium centre and the centroid of the cyclopentadienyl rings is similar in the **3a**, **3b** and **3c** structures with lengths varying from 1.97–1.99 Å. These lengths are comparable to those found in the literature for Cp–V bonds. These bond lengths are also shorter than the corresponding Ti–Cp(centroid) bond, which has a bond length of 2.06 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 2.37–2.41 Å for all three vanadocene dichloride structures. 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 between 132.6° to 133.5° for the vanadocene compounds when compared to the corresponding bond angle of 130.7° 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 87.6° for **3a**, 86.1° for **3b**, and 86.9° for **3c**, while Titanocene **Y** displayed a Cl–Ti–Cl bond angle of 95.9°. The carbon carbon bond lengths of the cyclopentadienyl rings in **3a** range from 1.43–1.40 Å, **3b** range from 1.43–1.40 Å, with **3c** having lengths in the range of 1.42–1.39 Å. In summary, it means that all three structures display a distorted tetrahedral conformation.

The difference in the V–Cl bond lengths in **3c** is a result of intermolecular π–π and van-der-Waals interactions which are depicted in Figure 6. All other Cl–F distances exceed 3.5 Å. There seems to be a competition between the attractive π–π interaction between the two benzene rings

Table 2. Crystal data and structure refinement for **3a**–**c**.

	3a	**3b**	**3c**
Empirical formula	$C_{26}H_{24}Cl_2F_2O_2V$	$C_{26}H_{24}Cl_2F_2O_2V$	$C_{26}H_{20}Cl_2F_6O_2V$
Formula weight	528.29	528.29	600.26
Crystal system	monoclinic	monoclinic	monoclinic
Space group	*C*2/*c* (#15)	*C*2/*c* (#15)	*C*2/*c* (#15)
Unit cell dimensions	*a* = 18.1247(13) Å	*a* = 23.9387(13) Å	*a* = 38.500(5) Å
	b = 6.6333(5) Å	*b* = 6.8432(4) Å	*b* = 6.5698(8) Å
	c = 18.6186(3) Å	*c* = 13.3830(7) Å	*c* = 22.400(3) Å
	β = 91.305(1)°	*β* = 94.233(1)°	*β* = 124.212(2)°
Volume	2237.9(3) Å^3	2188.8(2) Å^3	4685.4(10) Å^3
Z	4	4	8
Density (calculated)	1.568 Mg/m^3	1.603 Mg/m^3	1.702 Mg/m^3
Absorption coefficient	0.722 mm^{-1}	0.738 mm^{-1}	0.724 mm^{-1}
F(000)	1084	1084	2424
Crystal size	0.60 × 0.20 × 0.01 mm^3	0.60 × 0.40 × 0.05 mm^3	0.80 × 0.25 × 0.02 mm^3
Theta range for data collection	2.19 to 31.88°	1.70 to 32.12°	1.82 to 24.16°
Index ranges	$-26 \le h \le 25$,	$-34 \le h \le 34$,	$-44 \le h \le 44$,
	$-9 \le k \le 9$,	$-10 \le k \le 10$,	$-7 \le k \le 7$,
	$-27 \le l \le 26$	$-19 \le l \le 219$	$-25 \le l \le 225$
Reflections collected	13052	12767	16537
Independent reflections	3654 [*R*(int) = 0.0260]	3568 [*R*(int) = 0.0265]	3752 [*R*(int) = 0.0451]
Completeness to θ_{max}	95.1%	92.8%	99.8%
Max. and min. transmission	0.9928 and 0.8206	0.9640 and 0.8071	0.9857 and 0.7467
Data/restraints/parameters	3654/0/198	3568/12/198	3752/0/334
Goodness-of-fit on F^2	1.069	1.036	1.055
Final *R* indices	R_1 = 0.0337,	R_1 = 0.0342,	R_1 = 0.0377,
[$I > 2\sigma(I)$]	wR_2 = 0.0802	wR_2 = 0.0849	wR_2 = 0.0899
R indices (all data)	R_1 = 0.0407, wR_2 = 0.0832	R_1 = 0.0390, wR_2 = 0.0873	R_1 = 0.0473, wR_2 = 0.0948
Largest diff. peak and hole	0.574 and –0.256 e Å^{-3}	0.596 and –0.228 e Å^{-3}	0.439 and –0.254 e Å^{-3}

and the repulsive van-der-Waals interaction between F(5) and Cl(2). Thus Cl(2) gets pushed closer to the vanadium atom.

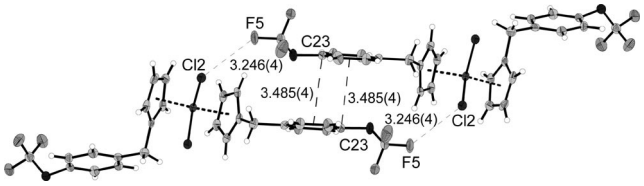


Figure 6. Pair of molecules of **3c** showing the competing van-der-Waals and π–π interactions; distances in Å.

Cytotoxicity Studies

Displaying the lowest cytotoxic effect of the series on the LLC-PK cell line, vanadocene **3b** yielded an IC_{50} value of 35 μM. Vanadocene **3c**, improves on this value by almost a factor of 3 with a value of 13 μM, while compound **3a** gave an IC_{50} value of 6.0 μM. It should be noted that compounds **3a** and **3b** suffered from poor solubility, particularly in the case of **3a**, which is reflected in the larger error margin for that compound as usually obtained (Figure 7).

None of these IC_{50} values surpass 3.0 μM, which was set by Vanadocene **Y** for this cell line. For comparison Cisplatin gave an IC_{50} value of 3.3 μM, the titanium equivalent Titanocene **Y** gave an IC_{50} value of 21 μM and its tin equivalent yielded an IC_{50} value of 15 μM on the same LLC-PK cell line.[28]

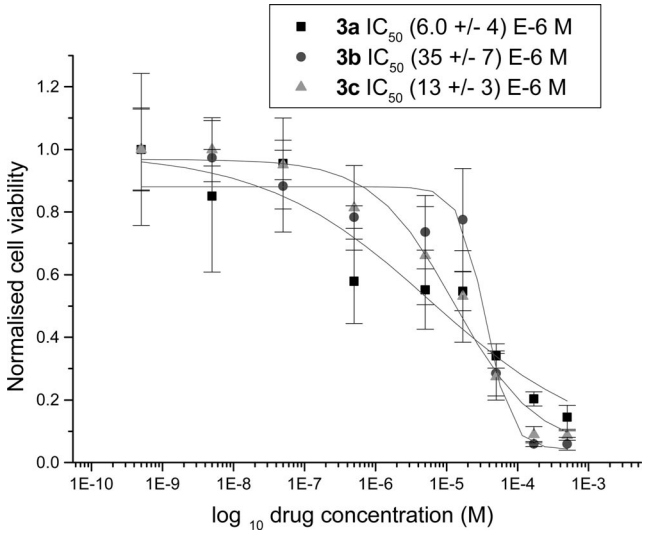


Figure 7. Cytotoxicity curves from typical MTT assays showing the effect of compounds **3a**–**c** on the viability of LLC-PK cells.

Vanadocenes **3a**–**c** were also tested against the human renal cell line, Caki-1. Compound **3a** showed the poorest IC_{50} value of 78 μM, again showing poor solubility, while **3b** showed an improvement in cytotoxicity against this cell line with an IC_{50} value of 18 μM. Likewise vanadocene **3c** displayed a very promising IC_{50} value of 2.2 μM, which is an improvement upon its LLC-PK value by almost a factor of 6, and proved to be the best compound of the this series. Compound **3c** displayed good solubility and satisfactory

margins of error. This improved cytotoxicity value could be due in part to the increased lipophilicity induced in this compound through the incorporation of the three fluorine atoms in the trifluoromethoxy moiety.

This result emphasises the importance of the *p*-methoxyphenyl moiety that is present in both **3a** and **3b** (which also contain additional fluorine in the 2- and 3-position, respectively) and Titanocene **Y**. Compound **3c** also contains a similar structure with a trifluoromethoxy moiety in the *para* position. This result also serves the purpose of 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 Figure 8, cell viability is reduced on a more gradual scale relative to the drug concentration for vanadocene **3c**, with the higher concentrations resulting in almost total cell death for all of the compounds tested.

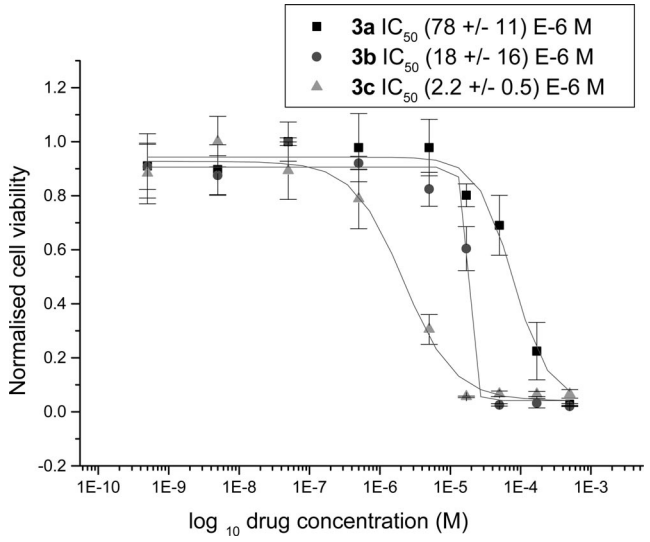


Figure 8. Cytotoxicity curves from typical MTT assays showing the effect of compounds **3a**–**c** on the viability of Caki-1 cells.

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 obtain highly cytotoxic benzyl-substituted vanadocene dichloride complexes. Complexes **3a**–**c** yielded antitumor IC_{50} values of 6.0, 35 and 13 μM, respectively on the LLC-PK cell line. On the Caki-1 cell line, vanadocenes **3a** and **3b** gave IC_{50} values of 78 and 18 μM, vanadocene **3c** gave a superior IC_{50} value of 2.2 μM. Further work is currently underway in order to improve these values by performing formulation experiments to improve solubility of these vanadocene compounds. The results of vanadocene **3c** are important enough to warrant further work in this area leading to in vivo testing against renal cell cancer in the nearby future.

Experimental Section

General Conditions: Manipulations of air- and moisture-sensitive compounds were performed under 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 with a Perkin–Elmer Paragon 1000 FT-IR Spectrometer employing KBr discs. UV/Vis spectra were recorded with a Unicam UV4 Spectrometer. Electron spray mass spectrometry (MS) was performed with 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 with a MiniScope MS200 apparatus in microwave X band (ca. 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 HO2). Spectra obtained were simulated using ESR simulation software Multiplot 2.26 and resulted in the predicted 8-line spectra. X-ray diffraction data for compounds **3a**–**c** were collected at 100 K using Mo-K_α radiation and a Bruker SMART APEX CCD area detector diffractometer. A full sphere of reciprocal space was scanned by ϕ-omega scans. Pseudo-empirical absorption correction based on redundant reflections was performed by the program SADABS.[29] The structures were solved by direct methods using SHELXS-97[30] and refined by full-matrix least-squares on F^2 for all data using SHELXL-97. In **3a** and **3b** all hydrogen atoms were located in the difference fourier map and allowed to refine freely. In **3b** the C–H bond lengths were restrained to their default values using DFIX. 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. Suitable crystals of **3a** and **3b** were formed from the slow evaporation of a saturated dichloromethane solution, while crystals of **3c** were grown in a saturated trichloromethane solution with slow infusion of pentane. Further details about the data collection are listed in Table 2, as well as reliability factors.

CCDC-725054 (for **3a**), -725055 (for **3b**), and -725056 (for **3c**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Syntheses: The syntheses of 6-(2-fluoro-4-methoxyphenyl)fulvene (**1a**), 6-(3-fluoro-4-methoxyphenyl)fulvene (**1b**) and 6-[4-(trifluoromethoxy)phenyl]fulvene (**1c**) were carried out accordingly to already published procedures.[31]

Synthesis of Bis[(2-fluoro-4-methoxybenzyl)cyclopentadienyl]vanadium(IV) Dichloride, [(η^5-C_5H_4–CH_2–C_6H_3F–OCH_3)]$_2VCl_2$ (3a): Super Hydride ($LiBEt_3H$) (15.0 mL, 15.0 mmol, 1 M solution) in THF was concentrated by removal of the solvent by heating 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. The red oil **1a** (2.10 g, 10.4 mmol) was added to a Schlenk flask and was dissolved in 90 mL of dry diethyl ether to give a red solution. The red fulvene solution was transferred to the Super Hydride solution via cannula. The solution was stirred 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 through 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. 1.83 g (8.70 mmol, 83.7% 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. Vanadium tetrachloride (0.46 mL, 4.35 mmol) 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 through 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 (1.03 g, 1.95 mmol, 44.8% yield) **3a**. $C_{26}H_{24}Cl_2F_2O_2V$ (528.32): calcd. C 59.11, H 4.58, Cl 13.42, F 7.19; found C 58.92, H 4.54, Cl 13.60, F 7.10. ESR (CH_2Cl_2 solution, r.t.): 8-line hyperfine coupling, g_{iso} = 2.027, A_{iso} = 7.46 mT. MS (*m*/*z*, QMS-MS/MS): *m*/*z* = 492 $[M – Cl]^+$. IR (KBr): $\tilde{\nu}$ = 3108 (s), 3086 (s), 2956 (w), 2914 (w), 2833 (w), 1623 (s), 1583 (m), 1506 (s), 1474 (w), 1437 (s), 1284 (s), 1261 (s), 1157 (s), 1103 (s), 1030 (s), 932 (w), 869 (s), 851 (m), 791 (m) cm^{-1}. UV/Vis (CH_2Cl_2, nm): λ (ε) = 202 (390), 231 (19895), 280 (17632), 387 (13421) nm.

Synthesis of Bis[(3-fluoro-4-methoxybenzyl)cyclopentadienyl]vanadium(IV) Dichloride, [(η^5-C_5H_4–CH_2–C_6H_3F–OCH_3)]$_2$$VCl_2$ (3b): Super Hydride ($LiBEt_3H$) (15.0 mL, 15.0 mmol, 1 M solution) 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. The red oil **1b** (2.30 g,11.4 mmol) was added to a Schlenk flask and was dissolved in 90 mL of 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 through 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. 2.06 g (9.8 mmol, 85.8% 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. Vanadium tetrachloride (0.52 mL, 4.90 mmol) 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 through 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 (1.45 g, 2.74 mmol, 56.0% yield) **3b**. $C_{26}H_{24}Cl_2F_2O_2V$ (528.32): calcd. C 59.11, H 4.58, Cl 13.42, F 7.19; found C 59.01, H 4.58, Cl 13.51, F 7.12. ESR (CH_2Cl_2 solution, r.t.): 8-line hyperfine coupling, g_{iso} = 2.027, A_{iso} = 7.46 mT. MS (QMS-MS/MS): *m*/*z* = 492 $[M – Cl]^+$. IR (KBr): $\tilde{\nu}$ = 3135 (w), 3109 (m), 2939 (w), 2841 (w), 1621 (w), 1584 (w), 1520 (s), 1479 (m), 1433 (m), 1394 (w), 1319 (m), 1281 (s), 1223 (s), 1122 (s), 1025 (s), 956 (m), 869 (m), 855 (s), 819 (m), 752 (m) cm^{-1}. UV/Vis (CH_2Cl_2, nm): λ (ε) = 202 (336), 233 (20673), 281 (16991), 386 (13100) nm.

Synthesis of Bis{[4-(trifluoromethoxy)benzyl]cyclopentadienyl}vanadium(IV) Dichloride, [(η^5-C_5H_4–CH_2–C_6H_4–OCF_3)]$_2$$VCl$ (3c): Super Hydride ($LiBEt_3H$) (15.0 mL,15.0 mmol, 1 M solution) 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. The red oil **1c** (2.36 g, 9.9 mmol) was added to a Schlenk flask and was dissolved in 90 mL of 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 through 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. 1.91 g (7.76 mmol, 78.3% 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. 0.41 mL (3.88 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 the solvent was removed under reduced pressure. The resulting green/brown solid was washed with 60 mL of dry pentane and redissolved in chloroform. The solution was then filtered through a frit to removed any remaining LiCl. The solvent was removed under reduced pressure and the resulting green solid was again washed with 60 mL of dry pentane and dried under reduced pressure to yield a green crystalline solid (0.82 g, 1.37 mmol, 35.7% yield) **3c**. $C_{26}H_{20}Cl_2F_6O_2V$ (600.28): calcd. C 52.02, H 3.36, Cl 11.81, F 18.99; found C 51.52, H 3.33, Cl 11.75, F 18.86. ESR (CH_2Cl_2 solution, r.t.): 8-line hyperfine coupling, g_{iso} = 2.027, A_{iso} = 7.46 mT. MS (QMS-MS/MS): *m*/*z* = 564 $[M – Cl]^+$. IR (KBr): $\tilde{\nu}$ = 3109 (m), 3088 (m), 2951 (w), 2830 (w), 1509 (s), 1433 (m), 1270 (s, br), 1228 (s, br), 1152 (s), 1105 (m), 1021 (w), 876 (w), 838 (w), 813 (w) cm^{-1}. UV/Vis (CH_2Cl_2, nm): λ (ε) = 207 (657), 230 (12478), 289 (7806), 385 (3090) nm.

Cytotoxicity Studies: Preliminary in-vitro cell tests were performed on the cell line LLC-PK (long-lasting cells–pig kidney), and the human renal cell line Caki-1 in order to compare the cytotoxicity of the compounds presented in this paper. These cell lines were chosen based on their regular and long-lasting growth behaviour, which is similar to the one shown in kidney carcinoma cells. They were 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 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 (dimethyl sulfoxide) 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][32] 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.

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