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Novel organotin antibacterial and anticancer drugs

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ABSTRACT

From the reaction of 6-(*p*-methoxyphenyl) fulvene (**1a**), 6-(*p*-*N*,*N*-dimethylaminophenyl) fulvene (**1b**) and 6-(3,4-dimethoxyphenyl) fulvene (**1c**) with $LiBEt_3H$, lithiated cyclopentadienide intermediates (**2a–c**) were synthesised. These intermediates were then transmetallated to tin with $SnCl_4$ to yield tetra-substituted bis(cyclopentadienyl)tin dichloride complexes (**3a–c**). Further reaction with tin tetrachloride yielded the benzyl-substituted derivatives bis-[(*p*-methoxybenzyl)cyclopentadienyl] tin(IV) dichloride (**4a**), bis-[(*p*-*N*,*N*-dimethylaminobenzyl)cyclopentadienyl] tin(IV) dichloride (**4b**), and bis-[(3,4-dimethoxyphenyl)cyclopentadienyl] tin(IV) dichloride (**4c**). Preliminary antibacterial tests were carried out using the Kirby–Bauer disk-diffusion method, in which **4a–c** showed little to no activity against the Gram-negative bacterium *Escherichia coli*, but medium activity against Gram-positive bacteria (MRSA, MSSA). In addition, the organotin complexes had their cytotoxicity investigated through preliminary *in vitro* testing on the LLC-PK (pig kidney epithelial) cell line in order to determine their IC50 values. Compound **4c** showed no cytotoxic activity, while **4a** and **4b** were found to have IC50 values of 15 and 205 μM, respectively.

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*-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 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.

While the use of tin species are known to have an impact on the environment [9], the biological activity and possible antibacterial/antitumor applications of numerous organotin(IV) complexes have been described in the literature [10–16]. A significant amount of the work carried out on these Sn(IV) compounds was in relation to their antibacterial activity against a wide range of both Gram-negative and Gram-positive bacteria. While the results vary considerably depending on the different substitution patterns at the tin centre, a trend in activity emerges inclining towards the Gram-positive bacteria, where a greater antibacterial effect is observed compared to the Gram-negative bacteria [17–18].

With this in mind, and following on from the work carried out on titanocene **Y**, we decided to substitute the titanium metal centre with tin in order to compare the difference in cytotoxicity between titanocene dichloride and bis(cyclopentadienyl)tin dichloride derivatives, along with conducting preliminary antibacterial tests. The synthesis of these organotin compounds requires a slightly different synthetic route than the titanocenes, where by a

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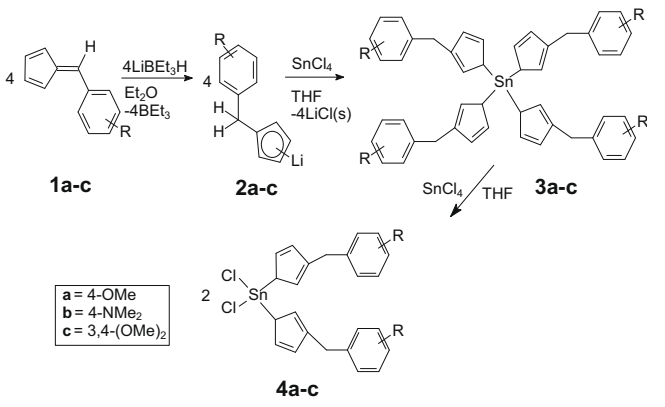
tetrakis cyclopentadienyl tin(IV) intermediate is produced, which undergoes additional transmetallation to isolate the desired organotin dichloride [19]. The reaction scheme can be viewed in Scheme 1.

Within this paper we present the synthesis, preliminary cytotoxicity and antibacterial studies of a series of three organotin 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. Tin tetrachloride ($SnCl_4$ 1.0 M solution in heptane) 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. ^1H and ^{13}C NMR spectra were measured on a Varian 500 MHz spectrometer with C_6D_6 as solvent and TMS as internal standard. ^{119}Sn NMR spectra were measured on a Varian 500 MHz spectrometer with C_6D_6 as solvent and t-But_2SnCl_2 as internal standard. IR spectra were recorded on a Perkin Elmer Paragon 1000 FT-IR Spectrometer employing NaCl 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 negative ionisation) mode for compounds **4a** and **4b**. Compound **4c** was obtained in ES+ (electron spray positive) mode. X-ray diffraction data for compound **5** was 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 SHELXS-97 [21] and refined by full matrix least-squares on F^2 for all data using SHELXL-97 [21]. In **5**, 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. The chloroform molecule could not be located in the unit cell. Platon SQUEEZE [22] was used to compensate for the spread electron density. The solvent is included in the overall formula, $F(000)$, absorption coefficient and density. Further details about the data collection are listed in Table 2, as well as reliability factors. Suitable crystals of **5** were formed from the slow evaporation of a saturated trichloromethane solution.



Scheme 1. General reaction scheme for the synthesis of benzyl-substituted organotin dichloride derivatives.

2.2. Synthesis

6-(*p*-Methoxyphenyl) fulvene **1a**, 6-(*p*-*N*,*N*-dimethylaminophenyl) fulvene **1b** and 6-(3,4-dimethoxyphenyl) fulvene **1c** were synthesised according to already published procedures [23–24].

2.2.1. Synthesis of bis-[(p-methoxybenzyl)cyclopentadienyl] tin(IV) dichloride, $[\eta^1$-C_5H_4–CH_2–C_6H_4–O–$CH_3]_2SnCl_2$ (**4a**)

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 forty minutes 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. 2.06 g (10.7 mmol, 82.2% yield) of the lithiated cyclopentadienide intermediate **2a** was obtained. 2.68 ml (2.68 mmol) of a 1 M solution of tin tetrachloride in heptane was dissolved in 30 ml of dry toluene in a Schlenk flask. The lithium cyclopentadienide intermediate was dissolved in 60 ml of dry toluene to give a colourless solution. The solution of tin tetrachloride was added to the lithium cyclopentadienide intermediate solution slowly at 0 °C *via cannula* to give a yellow solution. The tin solution was allowed to stir for 16 h at room temperature followed by the removal of the solvent under reduced pressure which yielded a yellow solid, tetrakis-[η^1-*(p-methoxybenzyl)cyclopentadienyl] tin(IV)* intermediate **3a**. The yellow solid was dissolved in 30 ml of dry toluene and 2.68 ml (2.68 mmol) of a 1 M solution of tin tetrachloride in heptane was added slowly at 0 °C. The solution was stirred at room temperature for 2 h after which it was allowed to settle, followed by filtration in an inert atmosphere of nitrogen using a frit. The resulting clear yellow solution was reduced under vacuum to yield the title compound as a light yellow, waxy and air sensitive solid (0.960 g, 1.71 mmol, 31.9% yield) **4a**.

^1H NMR (δ ppm C_6D_6, 500 MHz): 3.28 [s, 6H, C_6H_4–OCH_3], 3.61 [s, 4H, C_5H_4–CH_2], 5.15 [s, 4H, C_5H_4–CH_2], 6.05 [s, 4H, C_5H_4–CH_2], 6.75 [d, 4H, J 7.82 Hz, C_6H_4–OCH_3], 7.04 [d, 4H, *J* 7.82 Hz, C_6H_4–OCH_3].

^{13}C NMR (δ ppm C_6D_6, 125 MHz, proton decoupled): 35.5 [C_5H_4–CH_2], 54.4 [C_6H_4–OCH_3], 97.1, 114.0, 123.3, 129.8, 132.3, 145.8, 158.4.

^{119}Sn NMR (δ ppm C_6D_6, 186 MHz, proton decoupled): −36.5.

MS (m/z, QMS-MS/MS): 559 $[M-H]^-$.

IR absorptions (NaCl, cm^{-1}): 2996, 2905, 2833, 1610, 1575, 1511, 1463, 1431, 1300, 1247, 1176, 1036, 919, 803.

UV–Vis (toluene, nm): λ 216 (ε 56000), λ 239 (ε 53000), λ 266 (ε 49000), λ 274 (ε 39000), λ 299 (ε 13000), λ 321 (ε 7800), λ 337 (ε 2000), λ 425 (ε 5900), λ_{max} 555 (ε 600).

2.2.2. Synthesis of bis-[(p-N,N-dimethylaminobenzyl)cyclopentadienyl] tin(IV) dichloride, $[\eta^1$-C_5H_4–CH_2–C_6H_4–N–$(CH_3)_2]_2SnCl_2$ (**4b**)

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 forty minutes 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. 2.38 g (12.1 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 8 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 20 ml of diethyl ether. The white precipitate was dried briefly under reduced pressure and was transferred to a Schlenk flask under nitrogen. 1.94 g (9.45 mmol, 78.2% yield) of the lithiated cyclopentadienide intermediate **2b** was obtained. 2.36 ml (2.36 mmol) of a 1 M solution of tin tetrachloride in heptane was dissolved in 30 ml of dry toluene in a Schlenk flask. The lithium cyclopentadienide intermediate was dissolved in 60 ml of dry toluene to give a colourless solution. The solution of tin tetrachloride was added to the lithium cyclopentadienide intermediate solution slowly at 0 °C *via cannula* to give a yellow solution with a white precipitate. The tin solution was allowed to stir for 16 h at room temperature followed by the removal of the solvent under reduced pressure which yielded a dark yellow solid, tetrakis-[η^1-*(p-dimethylaminobenzyl)cyclopentadienyl] tin(IV)* intermediate **3b**. The dark yellow solid was dissolved in 30 ml of dry toluene and 2.36 ml (2.36 mmol) of a 1 M solution of tin tetrachloride in heptane was added slowly at 0 °C. The solution was stirred at room temperature for 2 h after which it was allowed to settle, followed by filtration in an inert atmosphere of nitrogen using a frit. The resulting clear yellow solution was reduced under vacuum to yield the title compound as a yellow, waxy and air sensitive solid (1.21 g 2.06 mmol, 43.7% yield) **4b**.

^{1}H NMR (δ ppm C_6D_6, 500 MHz): 2.23 [s, 12H, C_6H_4–N(CH_3)$_2$], 3.36 [s, 2H, C_5H_4–CH_2], 3.43 [s, 2H, C_5H_4–CH_2], 4.93 [s, 2H, C_5H_4–CH_2], 4.98 [s, 2H, C_5H_4–CH_2], 5.72 [s, 2H, C_5H_4–CH_2], 5.86 [s, 2H, C_5H_4–CH_2], 6.32 [d, 4H, J 8.31 Hz, C_6H_4–N(CH_3)$_2$], 6.82 [d, 4H, *J* 8.31 Hz, C_6H_4–N(CH_3)$_2$].

^{13}C NMR (δ ppm C_6D_6, 125 MHz, proton decoupled): 35.4 [C_5H_4–CH_2], 40.2 [C_6H_4–N-(CH_3)$_2$], 97.0, 100.3, 113.0, 121.6, 123.4, 129.5, 146.3, 148.1, 149.3.

^{119}Sn NMR (δ ppm C_6D_6, 186 MHz, proton decoupled): −26.8

MS (*m*/*z*, QMS-MS/MS): 585 $[M-H]^-$.

IR absorptions (near, NaCl windows, cm^{-1}): 3007, 2888, 2798, 1614, 1567, 1518, 1478, 1444, 1347, 1224, 1162, 1131, 947, 805.

UV–Vis (toluene, nm): λ 218 (ε 42 000), λ 227 (ε 36 000), λ 236 (ε 43 000), λ 246 (ε 41 000), λ 263 (ε 48 000), λ 299 (ε 20 000), λ 322 (ε 8400), λ 426 (ε 4500), λ 555 (weak).

*2.2.3. Synthesis of bis-[(3,4-dimethoxybenzyl)cyclopentadienyl] tin(IV) dichloride, [η^1-C_5H_4–CH_2–C_6H_3–$(OCH_3)_2$]$_2$$SnCl_2$ (**4c**)*

16.0 ml (16.0 mmol) of 1 molar solution of Super Hydride (Li-BEt_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 **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 8 h to give a white precipitate of the lithium cyclopentadienide intermediate and the solution had changed colour from orange/red to faint yellow 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. 2.16 g (9.72 mmol, 86.8% yield) of the lithiated cyclopentadienide intermediate **2c** was obtained. 2.43 ml (2.43 mmol) of a 1 molar solution of tin tetrachloride in heptane was dissolved in 30 ml of dry toluene in a Schlenk flask. The lithium cyclopentadienide intermediate was dissolved in 60 ml of dry toluene to give a colourless solution. The solution of tin tetrachloride was added to the lithium cyclopentadienide intermediate solution slowly at 0 °C *via cannula* to give a yellow solution. The tin solution was allowed to stir for 16 h at room temperature followed by the removal of the solvent under reduced pressure which yielded a dark brown solid, tetrakis-[η^1-(*p-N,N*-dimethylaminobenzyl)cyclopentadienyl] *tin(IV)* intermediate **3c**. The dark yellow solid was dissolved in 30 ml of dry toluene and 2.43 ml (2.43 mmol) of a 1 M solution of tin tetrachloride in heptane was added slowly at 0 °C. The solution was stirred at room temperature for 2 h after which it was allowed to settle, followed by filtration in an inert atmosphere of nitrogen using a frit. The resulting clear brown solution was reduced under vacuum to yield the title compound as a brown, waxy and air sensitive solid (0.740 g 1.20 mmol, 24.6% yield) **4c**.

^{1}H NMR (δ ppm C_6D_6, 500 MHz): 3.14 [s, 6H, C_6H_3-(OCH_3)$_2$], 3.18 [s, 6H, C_6H_3–(OCH_3)$_2$], 3.46 [s, 4H, C_5H_4–CH_2], 4.77 [s, 4H, C_5H_4–CH_2], 5.95 [s, 4H, C_5H_4–CH_2], 6.37 [d, 2H, J 8.31 Hz, C_6H_3–(OCH_3)$_2$], 6.48 [s, 2H, C_6H_3–(OCH_3)$_2$], 6.52 [d, 2H, J 8.31 Hz, C_6H_3–(OCH_3)$_2$].

^{13}C NMR (δ ppm C_6D_6, 125 MHz, proton decoupled): 35.1 [C_5H_4–CH_2], 54.2 [C_6H_3–(OCH_3)$_2$], 99.2, 111.7, 112.1, 120.0, 120.9, 131.7, 146.3, 149.2.

^{119}Sn NMR (δ ppm C_6D_6, 186 MHz, proton decoupled): −48.9.

MS (*m*/*z*, QMS-MS/MS): 621 $[M+H]^+$.

IR absorptions (neat, NaCl windows, cm^{-1}): 2998, 2934, 2833, 1609, 1590, 1510, 1463, 1417, 1235, 1139, 1028, 804, 733.

UV–Vis (toluene, nm): λ 213 (ε 63 000), λ 230 (ε 53 000), λ 240 (ε 55 000), λ 248 (ε 56 000), λ 266 (ε 58 000), λ 300 (ε 8600), λ 321 (ε 5600), λ 426 (ε 4300), λ_{max} 555 (ε 400) (see Fig. 1).

2.3. Antibacterial Studies

Two different strains of the Gram-positive bacteria *Staphylococcus aureus* (SA) were used; a clinical isolate of Methicillin-resistant SA (MRSA identified as isolate CC), and the commercially available Methicillin-sensitive SA (MSSA ATCC 6538). For comparison, a Gram-negative bacterium was also tested (*Escherichia coli,* lab strain BL21).

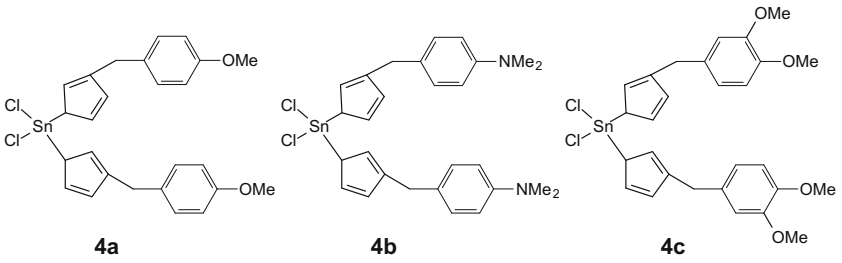


Fig. 1. Structures of η^1 organotin dichlorides **4a–c**.

Table 1
Zone of clearance for compounds **4a–c** in mm

Bacteria	DMSO	**4a** (mm)	**4b** (mm)	**4c** (mm)
Gram-positive				
MRSA	N/A	5	10	14
MSSA	N/A	8	8	14
Gram–negative				
E. coli	N/A	5	6	5

To assess the biological activity of compounds **4a–c**, the Kirby–Bauer disk-diffusion method was applied [25]. All bacteria were individually cultured from a single colony in sterile LB medium [26] overnight at 37 °C (orbital shaker incubator). All the work carried out was performed under sterile conditions.

For each strain, 50 μl of culture were spread evenly on agar-LB medium. Four 5 mm diameter paper discs were placed evenly separated on each plate. 50 mg of each sample (**4a** 0.089 M, **4b** 0.085 M and **4c** 0.081 M) were dissolved in 1 ml of DMSO to prepare the drug formulation. 5 μl of **4a–c** solution (250 μg) were placed on the paper discs and 5 μl of DMSO was placed on the fourth circle of filter paper as a control. The plates were covered and placed in an incubator at 30 °C for 24 h. The plates were then removed and the zone of clearance (defined as the diameter of inhibited bacterial growth around the filter paper) for each sample was measured in mm (see Table 1).

2.4. 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) [27] 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.

Table 2
Crystal data and structure refinement for **5**

Identification code	**5**
Empirical formula	$C_{47}H_{43}N_4O_2Cl_9Sn_2$
Molecular formula	$(C_{23}H_{21}N_2OCl_3Sn)_2xCHCl_3$
Formula weight	1252.28
Temperature (K)	100(2)
Wavelength (Å)	0.71073
Crystal system	monoclinic
Space group	$P2_1/n$ (#14)
Unit cell dimensions	
a (Å)	14.7176(11)
b (Å)	8.2667(6)
c (Å)	20.4595(16)
α (°)	90
β (°)	96.649(2)
γ (°)	90
V (Å^3)	2472.5(3)
Z	2
D_{calc} (Mg/m^3)	1.682
Absorption coefficient (mm^{-1})	1.540
$F(000)$	1244
Crystal size (mm^3)	$0.40 \times 0.05 \times 0.03$
θ range for data collection (°)	1.62–26.43
Index ranges	$-18 \leq h \leq 18$, $-10 \leq k \leq 10$, $-25 \leq l \leq 25$
Reflections collected	21282
Independent reflections	5081 [R(int) = 0.0426]
Completeness to θ = 26.43°	99.8%
Absorption correction	semi-empirical from equivalents
Maximum and minimum transmission	0.9553 and 0.7701
Refinement method	full-matrix least-squares on F^2
Data/restraints/parameters	5081/0/272
Goodness-of-fit on F^2	1.041
Final R indices [I > 2sigma(I)]	R_1 = 0.0347, wR_2 = 0.0777
R indices (all data)	R_1 = 0.0434, wR_2 = 0.0805
Largest difference in peak and hole (e Å^{-3})	0.907 and −0.536

3. Discussion

3.1. Synthesis

The lithium intermediates used in the synthesis of derivatives **4a–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 good yields of 79–87%.

Four equivalents of the lithiated cyclopentadienide intermediate were then transmetallated with one equivalent of tin tetrachloride to form a tetrakis cyclopentadienyl tin(IV) intermediate and lithium chloride as a by-product. This tetrakis cyclopentadienyl tin(IV) intermediate was isolated but not characterised due to extreme air sensitivity. This was then further reacted with 1 equiv. of tin tetrachloride to yield the organotin dichlorides **4a**, **4b**, and **4c** in yields of 25–44%. Elemental analysis was not possible due to the level of air and moisture sensitivity of the compounds.

Derivatives **4a**, **4b**, and **4c** are soluble in organic non-chlorinated solvents, but decompose rapidly in chlorinated solvents such as dichloromethane. The synthesis of organotin dichlorides is dependent upon the solvent. The expected dichloride species were formed only when toluene or benzene was used as the solvent.

A notable feature of the organotin species **4a**, **4b**, and **4c** is the η^1-bonded Cp ligand in these molecules. This results in an interesting ^{1}H NMR spectrum, which appears to suggest a η^5-bonding due to the symmetry of two singlet peaks for the cyclopentadienyl hydrogens, approximately 1 ppm apart. This can be rationalised by fast sigma tropic shift in solution.

3.2. Structural discussion

Despite the effort to crystallise the organotin derivatives **4a-c**, no crystal structures were obtained. 2,2-Bipyridine was success-

fully used as a bidentate nitrogen base in an attempt to crystallise a six co-ordinated tin complex for the organotin dichloride species. This produced single crystals of the bipyridine adduct of η^1-(*p*-methoxybenzyl)cyclopentadienyl tin(IV) trichloride (**5**), where one of the Cp ligands is replaced with a Cl atom, which can be viewed in Fig. 2. This shows that the tin(IV) cyclopentadienyl chlorides undergo more than one exchange reaction: The above mentioned sigma tropic shift and a ligand exchange in a Schlenk-type equilibrium. The structure is monoclinic and contains $CHCl_3$ solvent molecules in the crystal (see Table 3).

The bond lengths for Sn–Cl(1) 2.420 Å, Sn–Cl(2) 2.442 Å, and Sn–Cl(3) 2.414 Å of the six co-ordinated Sn species are slightly longer when compared with other tin(IV) complexes [28], due to the increased electron density surrounding the central Sn atom. Also, the bond length of the Sn–C(3) 2.222 Å is comparable with other organotin complexes such as Me_2SnCl_2 where the Sn–C bond

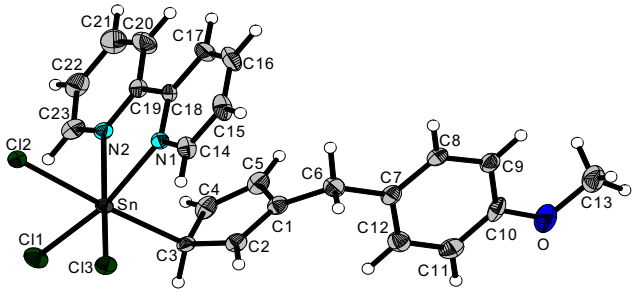


Fig. 2. X-ray diffraction structure of **5**; thermal ellipsoids are drawn on the 50% probability level.

Table 3
Selected bond lengths and angles from the crystal structure of **5**

Identification code	**5**
Bond	*Bond length* (Å)
Sn–C(3)	2.222
Sn–N(1)	2.231
Sn–N(2)	2.278
Sn–Cl(3)	2.414
Sn–Cl(1)	2.420
Sn–Cl(2)	2.442
C(1)–C(2)	1.349
C(1)–C(5)	1.453
C(1)–C(6)	1.494
C(2)–C(3)	1.482
C(3)–C(4)	1.460
C(4)–C(5)	1.346
C(6)–C(7)	1.521
N(1)–C(14)	1.347
N(1)–C(18)	1.355
C(19)–N(2)	1.337
C(19)–C(18)	1.482
C(23)–N(2)	1.342
Bond angles	
C(3)–Sn–N(1)	91.73
C(3)–Sn–N(2)	95.38
N(1)–Sn–N(2)	72.51
C(3)–Sn–Cl(3)	93.98
N(1)–Sn–Cl(3)	93.59
N(2)–Sn–Cl(3)	163.44
C(3)–Sn–Cl(1)	92.82
N(1)–Sn–Cl(1)	165.49
N(2)–Sn–Cl(1)	93.34
Cl(3)–Sn–Cl(1)	99.83
C(3)–Sn–Cl(2)	176.83
N(1)–Sn–Cl(2)	85.48
N(2)–Sn–Cl(2)	82.34
Cl(3)–Sn–Cl(2)	87.72
Cl(1)–Sn–Cl(2)	89.53

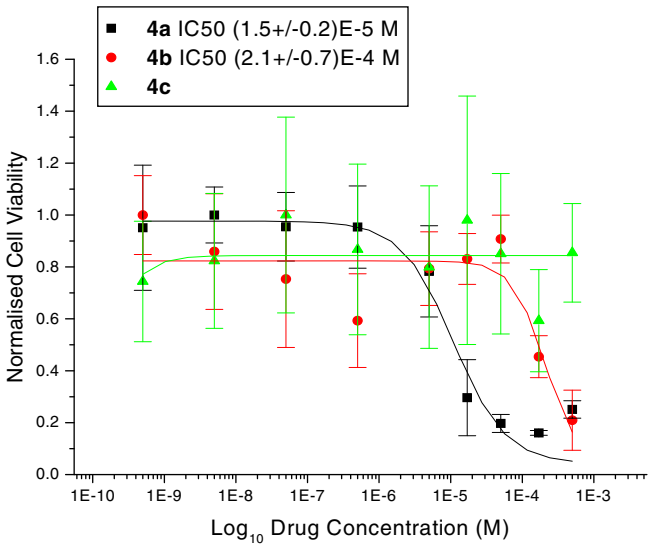


Fig. 3. Cytotoxicity curves from typical MTT assays showing the effect of compounds **4a–c** on the viability of LLC-PK cells.

lengths are in the region of 2.210 Å [29]. This structure also demonstrates the η^1-bonded conformation for the Sn–C bond, which is believed to be present in complexes **4a–c**. The two double bonds present in the cp ring are evident due to the shortening of the carbon-carbon bond lengths, C(1)–C(2) 1.349 Å and C(4)–C(5) 1.346 Å. The longer single CC bonds within the Cp ring were; C(2)–C(3) 1.482 Å, C(3)–C(4) 1.460 Å, and C(1)–C(5) 1.453 Å. The structure displays a distorted octahedral conformation, with the N(1)–Sn–N(2) angle of 72.51° being compressed due to the co-ordinated bipyridine ligand.

3.3. Biological evaluation

Almost no antibacterial effect was observed for complexes **4a–c** against the Gram-negative bacteria *E. coli* using the Kirby–Bauer disk diffusion method. Against the Gram-positive bacteria MRSA and MSSA however, a low to medium effect was observed with the compounds **4b** and **4c** having the greatest effect over 24 h. The solvent used to prepare the stock solutions (DMSO) played no role in growth inhibition. These results are in keeping with other organotin(IV) results found in the literature.

3.4. Cytotoxicity studies

As seen in Fig. 3, compounds **4a** and **4b** showed IC50 values 15 and 210 μM respectively. Complex **4c** displayed no antitumor activity. All three compounds suffered from very poor solubility along with decomposition due to certain air sensitivity of the compounds, which resulted in relatively large error margins for compounds **4b** and **4c**. Compound **4a** showed surprisingly good results of 15 μM with reduced error margins when compared with the titanium equivalent, Titanocene **Y**, which yielded an IC50 value of 21 μM. This result emphasises the importance of the *p*-methoxy phenyl moiety that is present in both **4a** and Titanocene **Y**. However the IC50 value for **4a** did not reach the level set by cisplatin with an IC50 value of 3.3 μM.

4. Conclusion and outlook

The hydridolithiation of 6-aryl substituted fulvenes followed by transmetallation has been found to be a very effective and reproducible way to medium to highly cytotoxic benzyl-substituted

organotin complexes. Although preliminary anti-bacterial results were lower than expected, **4a** yielded a promising anti-tumour IC50 value of 15 μM. While the possibility of **4a** becoming a viable anti-cancer drug are low due to high air sensitivity of **4a** and general toxicity of organotin compounds, these results are encouraging enough to warrant further work in this area.

Supplementary data

CCDC 699232 contains the supplementary crystallographic data for **5**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

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