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Novel titanocene anti-cancer drugs derived from fulvenes and titanium dichloride

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Abstract

Starting from 6-($p-N,N$-dimethylanilinyl)fulvene (**1a**) or 6-(pentamethylphenyl)fulvene (**1b**) [1,2-di(cyclopentadienyl)-1,2-di($p-N,N$-dimethylaminophenyl)ethanediyl] titanium dichloride (**2a**) and [1,2-di(cyclopentadienyl)-1,2-bis(pentamethylphenyl)ethanediyl] titanium dichloride **(2b)** and their corresponding dithiocyanato complexes (**3a, 3b**) were synthesized. Titanocene **2b** did not show a cytotoxic effect, but when **2a** was tested against pig kidney carcinoma cells (LLC-PK) or human ovarian carcinoma cells (A2780/cp70) inhibitory concentrations (IC_{50}) of 2.7×10^{-4} and 1.9×10^{-4} M, respectively, were observed.

Keywords: Anti-cancer drug; *cis*-Platinum; Titanocene; Fulvene; LLC-PK; A2780/cp70

1. Introduction

Despite the resounding success of *cis*-platinum and closely related platinum anti-tumor agents, the movement of other transition-metal anti-cancer drugs towards the clinic has been exceptionally slow [1–3]. Metallocene dichlorides (Cp_2MCl_2) with M = Ti, V, Nb and Mo show remarkable anti-tumor activity [4,5]. However, only titanocene dichloride has reached Phase I clinical trials so far, with a maximum tolerable dose of 315 mg/m^2 per week. The dose limiting effects of titanocene dichloride include nephrotoxicity and elevation of creatine and bilirubin levels [6,7]. Unfortunately, the efficacy of Cp_2TiCl_2 in Phase II clinical trials in patients with metastatic renal-cell carcinoma [8] or metastatic breast cancer [9] was too low to be pursued. Nevertheless, little synthetic effort has been employed to increase the cytotoxicity of any titanocene dichloride derivative [10–12], despite the existence of a novel method starting from titanium dichloride and fulvenes [13–16], which allows direct access to highly substituted *ansa*-titanocenes [17,18]. This paper reports the synthesis of novel [(1,2-diaryl-1,2-dicyclopentadienyl)-ethanediyl] titanium dichlorides [19,20], which combine the reactivity of the titanium dichloride moiety with the ability of hydrogen bonding towards DNA of the ammine ligand of *cis*-platinum, if the aryl group is substituted accordingly.

2. Experimental

Titanium tetrachloride and *n*-butyl lithium (2 mol solution in hexane) were obtained commercially from Aldrich Chemical Co. Potassium thiocyanate (KNCS) was obtained commercially from Aldrich Chemical Co; it was well ground before the reaction and dried under vacuum. Acetone was dried over magnesium sulfate. THF and toluene were dried over and distilled from Na/benzophenone prior to use. *p*-(N,N-dimethyl-

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amino)benzaldehyde and pentamethylbenzaldehyde were obtained commercially from Aldrich Chemical Co. Cyclopentadiene was collected under an atmosphere of nitrogen from freshly cracked dicyclopentadiene and pyrrolidine was distilled under argon prior to use. Manipulation of air and moisture sensitive compounds was carried out using standard Schlenk techniques under an argon atmosphere. NMR spectra were measured on a Varian 300 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. UV/Vis spectra were recorded on an UNICAM UV/VIS Spectrometer in either methanol (fulvenes) or acetonitrile (titanocenes). The GC mass spectra were measured on a FINNIGAN TRACE GC MS 2000Series (70 eV) and a 1×10^{-5} M solution in ethyl acetate was used. A Gallenkamp Melting Point Apparatus was used for melting point measurement.

A single crystal of fulvene **1b** suitable for X-ray diffraction experiments was grown by slow evaporation of petroleum spirit (40–60) from an open sample bottle. For the *ansa*-titanocene **3b**, slow evaporation of chloroform-d from an NMR tube was used. X-ray diffraction data for **1b** and **3b** were collected on a BRUKER Smart Apex diffractometer at room temperature for **1b** and 100 K for **3b**, respectively. A semi-empirical absorption correction on the raw data was performed using the program SADABS [21]. The crystal structure was then solved by direct methods (SHELXS-NT 97 [22] and refined by full-matrix least squares methods against F^2. 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 Nos. 232680 (**1b**) and 232681 (**3b**). In the crystal structure of **3b** additionally roughly one-third of a molecule of chloroform was found per asymmetric unit. All non-hydrogen atoms except the solvent carbon were refined anisotropically. For the refinement of the partially occupied $CHCl_3$ positions all chlorine–chlorine distances and carbon–chlorine bond lengths were restrained to be equal. The hydrogen atoms were geometrically idealised and refined using a riding model. Their isotropic temperature factors were fixed to 150% (methyl hydrogens) or 120% (all other hydrogens) of the equivalent temperature factor of the atom the hydrogen is attached to. A careful examination of the electron density map of **1b** showed that the methyl groups are disordered.

Table 1
Crystal data and structure refinement for **1b** and **3b**

Identification code	**1b**	**3b**
Empirical formula	$C_{17}H_{20}$	$C_{36}H_{40}N_2S_2\ Ti \times 0.35\ CHCl_3$
Formula weight	224.33	654.68
Temperature	293(2) K	100(2) K
Wavelength	0.71073 Å	0.71073 Å
Crystal system	Monoclinic	Triclinic
Space group	$P2_1/n$ (#14)	$P\bar{1}$ (#2)
Unit cell dimensions	$a = 11.374(2)$ Å	$a = 9.4666(15)$ Å
	$b = 5.7535(11)$ Å	$b = 10.9852(18)$ Å
	$c = 20.851(4)$ Å	$c = 17.271(3)$ Å
	$\alpha = 90°$	$\alpha = 92.463(3)°$
	$\beta = 93.027(3)°$	$\beta = 93.033(4)°$
	$\gamma = 90°$	$\gamma = 104.951(3)°$
Volume	1362.5(4) Å^3	1729.9(5) Å^3
Z	4	2
Density (calculated)	1.094 Mg/m^3	1.257 Mg/m^3
Absorption coefficient	0.061 mm^{-1}	0.472 mm^{-1}
$F(000)$	488	689
Crystal size	$0.40 \times 0.30 \times 0.05$ mm^3	$0.20 \times 0.10 \times 0.05$ mm^3
θ range for data collection	1.96–24.00°	2.20–22.50°
Index ranges	$-13 \leqslant h \leqslant 12$, $-6 \leqslant k \leqslant 6$, $-23 \leqslant l \leqslant 16$	$-10 \leqslant h \leqslant 10$, $-11 \leqslant k \leqslant 11$, $-18 \leqslant l \leqslant 18$
Reflections collected	5809	9459
Independent reflections	2138 [R(int) = 0.0277]	4388 [R(int) = 0.1379]
Completeness to max. θ	99.4%	96.9%
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9970 and 0.5061	0.9768 and 0.8526
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/parameters	2138/0/154	4388/18/412
Goodness-of-fit on F^2	1.034	0.791
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0483$, $wR_2 = 0.1325$	$R_1 = 0.1357$, $wR_2 = 0.2180$
R indices (all data)	$R_1 = 0.0828$, $wR_2 = 0.1515$	$R_1 = 0.2279$, $wR_2 = 0.2563$
Largest diff. peak and hole	0.140 and −0.136 e Å^{-3}	0.454 and −0.401 e Å^{-3}

With a view to elucidate the structures, spectroscopic data, bonding properties and energies of formation, the application of theoretical methods is advantageous. For this purpose, the GAUSSIAN 98 Revision A11 [23] running under Red Hat Linux was used. DFT calculations were performed at the B3LYP level using the 6-31G** basis set for the species of interest.

2.1. 6-(p-N,N-Dimethylanilinyl)fulvene (**1a**)

The syntheses of fulvenes **1a** and **1b** were carried out under argon as outlined in [23]. Pyrrolidine (2.5 ml, 30.0 mmol) was added to a solution of *p*-(*N*,*N*-dimethylamino)benzaldehyde (3.0 g, 30.0 mmol) and cyclopentadiene (4.1 ml, 50.0 mmol) in 30 ml of methanol. After addition, the colour of the solution immediately turned from colourless to red-orange. Large amounts of an orange solid precipitated out of the solution. When TLC analysis showed only one product band after 15 min, acetic acid (1.8 ml, 32.0 mmol) was added. The reaction mixture was diluted with 20 ml of a mixture of diethyl ether and water (1:1). The resultant organic layer was separated and the aqueous layer was washed with diethyl ether (3×20 ml). The combined organic extracts were washed with a saturated aqueous NaCl solution. The organic solution was dried over magnesium sulfate. When the solvent was removed under reduced pressure, 3.6 g of an orange product were obtained (90% yield). m.p. 102 °C.

^{1}H NMR (δ ppm $CDCl_3$): 6.79, 6.63, 6.43, 6.31 (C_5H_4, 4H m); 7.58, 7.55, 6.71, 6.68 (C_6H_4, 4H m); 3.03 ($N(CH_3)_2$, 6H s); 7.13 (Ph–C*H*–Cp, 1H s).

^{13}C NMR (δ ppm $CDCl_3$): 140.9, 133.7, 128.1, 127.5, 119.5 (C_5H_4); 151.2, 132.8, 124.8, 112.0 (C_6H_4); 40.1 (CH_3); 139.8 (Ph–*C*H–Cp).

IR absorption (cm^{-1} KBr): 3072 (w); 2918 (w); 2816 (w); 1605 (C=C m); 1450 (m); 1373 (m); 816 (s).

GCMS: 197.2 (M 100%); 182.2 ($M^+ - CH_3^+$ 25%); 153.1 ($M^+ - N(CH_3)_2^+$ 92%).

UV/Vis (methanol): $\lambda_{max} = 399$ nm.

Anal. Calc. for $C_{14}H_{15}N$: C, 85.24; H, 7.66; N, 7.10. Found: C, 84.40; H, 7.69; N, 6.86%.

2.2. 6-(Pentamethylphenyl)fulvene (**1b**)

Pyrrolidine (1.3 ml, 15.0 mmol) was added to a solution of pentamethylbenzaldehyde (1.8 g, 10.0 mmol) and cyclopentadiene (2.1 ml, 26.0 mmol) in 30 ml of methanol. After this addition the solution turned from colourless to clear orange-yellow. When TLC analysis showed only one product band after 20 h, acetic acid (0.9 ml, 16.0 mmol) was added. The reaction mixture was diluted with 20 ml of a mixture of diethyl ether and water (1:1). The resultant organic layer was separated and the aqueous layer was washed with diethyl ether (3×20 ml). The combined organic extracts were washed with a saturated aqueous NaCl solution. The organic solution was dried over magnesium sulphate. The crude product was redissolved in petroleum spirit (40–60) and purified by column chromatography over silica gel 60 (0.063–0.200) and petroleum spirit (40–60) as the eluent, yielding 2.0 g (89% yield). m.p. 100 °C.

^{1}H NMR (δ ppm $CDCl_3$): 6.49, 6.36, 6.02 (C_5H_4, 4H m); 2.27 (*o*, *m*-CH_3, 12H s); 2.16 (*p*-CH_3, 3H s); 7.30 (Ph–C*H*–Cp, 1H s).

^{13}C NMR (δ ppm $CDCl_3$): 139.6, 133.7, 131.4, 125.1, 121.9 (C_5H_4); 18.5, 16.4 (*o*, *m*-CH_3); 16.8 (*p*-CH_3).

IR absorption (cm^{-1} KBr): 3064 (w); 2922 (w); 2860 (w); 1636 (C=C m); 1472 (w); 1367 (m); 1066 (m); 898 (m); 768 (s); 616 (s).

GCMS: 224.2 (M 60%); 209.2 ($M^+ - CH_3^+$ 100%); 194.2 ($M^+ - 2CH_3^+$ 90%); 179.2 ($M^+ - 3CH_3^+$ 70%).

UV/Vis (methanol): $\lambda_{max} = 302$ nm.

Anal. Calc. for $C_{17}H_{20}$: C, 91.01; H, 8.99. Found: C, 90.57; H, 9.06%.

2.3. [1,2-Di(cyclopentadienyl)-1,2-di(p-N,N-dimethylaminophenyl)ethanediyl] titanium dichloride [1,2-(4-Me_2N-C_6H_4)$_2$ C_2H_2{η^5-C_5H_4}]$TiCl_2$ (**2a**)

$TiCl_4$ (0.70 ml, 6.3 mmol) was added to 40 ml of dry toluene containing 5% dry THF. The solution turned immediately from colourless to pale yellow. The solution was stirred and cooled down to −78 °C, followed by drop wise addition of *n*-butyl lithium (6.4 ml, 12.7 mmol). The solution turned from yellow to brown during addition. After this addition, the mixture was allowed to warm up slowly to room temperature. The colour of the solution became finally black. After 20 h stirring at r.t., a solution of **1a** (2.5 g, 12.6 mmol) in 35 ml of dry toluene was added to the $TiCl_2 \cdot 2THF$ solution at r.t. under argon. Then it was stirred under reflux for another 20 h. The solvent was removed under vacuum leaving a black residue. The residue was washed with chloroform and the solution was filtered through celite under reduced pressure. The colour of the filtrate reddened slightly. It was filtered using gravity filtration for at least four times until no further black precipitate appeared on the filter paper and the filtrate turned to dark red. Chloroform was removed to leave dark-red solid **2a** with 1.5 g (3.0 mmol, 47% yield). The ratio of *trans* and *cis* isomers is 60% and 40%, respectively.

^{1}H NMR (δ ppm $CDCl_3$): 7.15–6.68 (C_6H_4, 8H m and C_5H_4, 4H m); 6.30–6.12 (C_5H_4, 4H m); 5.40 (*trans*-PhC*H*Cp, 2H s); 4.76 (*cis*-PhC*H*Cp, 2H s); 2.91 (*cis*-$N(CH_3)_2$, 12H s); 2.89 (*trans*-$N(CH_3)_2$, 12H s).

^{13}C NMR (δ ppm $CDCl_3$): 139.6, 138.4, 133.8, 130.3, 129.7, 128.5, 128.2, 126.3, 117.0, 116.9, 114.0, 113.2, 109.9 ($C_6H_4$and C_5H_4); 53.0 (*cis*-Ph*C*HCp, 2H); 50.7 (*trans*-Ph*C*HCp); 41.1 ($N(CH_3)_2$).

IR absorption (cm^{-1} KBr): 3059 (w); 2956 (w); 2916 (w); 2853 (w); 1520 (s); 1480 (m); 1351 (m); 1261 (m); 1130 (m); 1019 (m); 946 (m); 802 (s); 694 (m); 596 (m).

UV/Vis (MeCN): $\lambda_{max} = 265$ nm.

Anal. Calc. for $C_{28}H_{30}N_2Cl_2Ti$:C, 65.53; H, 5.89; N, 5.46. Found: C, 64.94; H, 6.31; N, 4.94%.

2.4. [1,2-Di(cyclopentadienyl)-1,2-bis(pentamethylphenyl)ethanediyl] titanium dichloride [1,2-(Me_5C_6)$_2$ C_2H_2{η^5-C_5H_4}]$TiCl_2$ (**2b**)

$TiCl_4$ (0.5 ml, 4.5 mmol) was added to 40 ml of dry toluene containing 5% dry THF. The solution turned immediately from colourless to pale yellow. The solution was stirred and cooled down to −78 °C, and then was treated dropwise with *n*-butyllithium (4.5 ml, 8.9 mmol). The solution turned from yellow to brown during the addition. After this addition, the mixture was allowed to warm up slowly to r.t. The colour of the solution finally became black. After 20 h stirring, a solution of **1b** (2.0 g, 8.9 mmol) in dry toluene was added to the solution of $TiCl_2 \cdot 2THF$ at r.t. under argon. Then it was stirred under reflux for another 20 h. After performing the extraction procedure for **2a**, the product was washed with hexane yielding 0.3 g (10%) of a copper-red product **2b**. The ratio of *trans* and *cis* isomers is 93% and 7%, respectively.

^{1}H NMR (δ ppm $CDCl_3$): 6.98–6.56 (C_5H_4, 8H m); 6.32 (*trans*-PhC*H*Cp, 2H s); 4.23 (*cis*-PhC*H*Cp, 2H s); 2.23 (*p*-CH_3, 6H s); 2.15 (*o*,*m*-CH_3, 24H).

^{13}C NMR (δ ppm $CDCl_3$): 138.7, 134.7, 134.0, 131.2, 126.4, 118.2, 113.6, 111.3 (C_5H_4); 48.5 (*trans*-Ph*C*HCp); 31.8 (*cis*-Ph*C*HCp); 17.5 (CH_3).

IR absorption (cm^{-1} KBr): 3126 (w); 2990 (w); 2920 (s); 2872 (w); 1632 (m); 1570 (m); 1452 (s); 1380 (m); 1260 (w); 1062 (m); 926 (w); 812 (s).

UV/Vis (MeCN): $\lambda_{max} = 293$ nm.

Anal. Calc. for $C_{34}H_{40}Cl_2Ti$: C, 71.96; H, 7.11; Cl, 12.50. Found: C, 71.44; H, 7.68; Cl, 12.05%.

2.5. [1,2-Di(cyclopentadienyl)-1,2-di(p-N,N-dimethylaminophenyl)ethanediyl]titanium dithio-cyanate [1,2-(4-Me_2N-C_6H_4)$_2$$C_2$ H_2(η^5-C_5H_4)$_2$]$Ti(NCS)_2$ (**3a**)

KNCS (0.1 g, 1.3 mmol) was added to a solution of *ansa*-titanocene dichloride **2a** (0.3 g, 0.5 mmol) in 30 ml acetone. The mixture was refluxed for 3 h, filtered while still hot and the solvent was removed under reduced pressure. This gave 0.3 g (62% yield) of a dark-brown solid. The ratio of *trans* and *cis* isomers is 60% and 40%, respectively.

^{1}H NMR (δ ppm $CDCl_3$): 7.13–6.69 (C_6H_4, 8H m and C_5H_4, 3H m); 6.83, 6.71, 6.66, 6.53, 6.34, 6.26, 6.07 (C_5H_4, 5H m); 5.50 (*trans*-PhC*H*Cp, 2H s); 4.85 (*cis*-PhC*H*Cp, 2H s); 2.91 (*trans*-N(CH_3)$_2$, 12H s); 2.89 (*cis*-N(CH_3)$_2$, 12H s).

^{13}C NMR (δ ppm $CDCl_3$): 149.7, 149.0, 141.4, 129.5, 129.1, 128.3, 126.3, 126.0, 124.4, 116.9, 114.3, 112.4, 110.9 (C_6H_4 and C_5H_4); 122.1 (N*C*S); 53.4 (*cis*-Ph*C*HCp, 2H); 50.8 (*trans*-Ph*C*HCp); 40.5 (N(CH_3)$_2$).

IR absorption (cm^{-1} KBr): 3075 (w); 2952 (w); 2920 (w); 2886 (w); 2852 (w); 2795 (w); 2048 ($\nu_{s(CN)}$); 2006 ($\nu_{as(CN)}$); 1611 (m); 1519 (s); 1479 (w); 1351 (m); 1204 (w); 1163 (w); 1059 (w); 946 (m); 820 (s).

UV/Vis (MeCN): $\lambda_{max} = 285$ nm.

Anal. Calc. for $C_{30}H_{30}N_4S_2Ti$: C, 64.51; H, 5.41; N, 10.03; S, 11.48. Found: C, 63.89; H, 5.94; N, 10.52; S, 10.07%.

2.6. [1,2-Di(cyclopentadienyl)-1,2-bis(pentamethylphenyl)ethanediyl]titanium dithiocyanate [1,2-(Me_5C_6)$_2$ C_2H_2(η^5-C_5H_4)$_2$]$Ti(NCS)_2$ (**3b**)

KNCS (0.2 g, 2.6 mmol) was added to a solution of *ansa*-titanocene dichloride **2b** (0.31 g, 0.5 mmol) in 30 ml acetone. The mixture was refluxed for 3 h, filtered while still hot and the solvent was removed under reduced pressure. This gave 0.26 g (79% yield) of a red-brown solid. The ratio of *trans* and *cis* isomers is 93% and 7%, respectively.

^{1}H NMR (δ ppm $CDCl_3$): 6.89–6.56 (C_5H_4, 8H m); 6.45 (*trans*-PhC*H*Cp, 2H s); 4.12 (*cis*-PhC*H*Cp, 2H s); 2.17 (*p*-CH_3,6H s); 2.15 (*o*,*m*-CH_3, 24H d).

^{13}C NMR (δ ppm $CDCl_3$): 141.7,139.6, 134.5, 127.1, 117.8, 114.2, 111.2, 108.6 (C_5H_4); 122.7 (N*C*S); 49.2 (*trans*-Ph*C*HCp); 36.9 (*cis*-Ph*C*HCp); 17.5 (CH_3).

IR absorption (cm^{-1} KBr): 3096 (w); 2921 (m); 2870 (w); 2048 ($\nu_{as(CN)}$ vs); 2001 ($\nu_{s(CN)}$ vs); 1618 (m); 1452 (m); 1379 (w); 1260 (w); 1063 (m); 819 (m); 747 (w).

UV/Vis (MeCN): $\lambda_{max} = 253$ nm.

Anal. Calc. for $C_{36}H_{40}N_2S_2Ti$: C, 70.57; H, 6.58; N, 4.57; S, 10.47. Found: C, 69.77; H, 7.08; N, 9.95; S, 9.81%.

2.7. MTT-based cytotoxicity tests

The pig kidney carcinoma cell line, LLC-PK, was obtained from the American Tissue Culture Collection. The platinum-resistant human ovarian carcinoma cell line, A2780/cp70, was kindly provided by Prof. Robert Brown, Centre for Oncology and Applied Pharmacology, University of Glasgow, Cancer Research UK Beatson Laboratories.

The cytotoxic activities of titanocenes **2a** and **2b** were determined using an MTT-based assay. In more detail, cells were seeded into a 96-well plate (5000 cells/well) and allowed to attach for 24 h. Subsequently, the cells were treated with various concentrations of the cytotoxic agents. After 48 h, the relevant drug was removed, the cells washed with PBS and fresh medium was added for another 24 h for recovery. Viability of cells was determined by treatment with MTT in medium (5 mg/11

ml) for 3 h. The purple formazan crystals formed were dissolved in DMSO and absorbance measured at 540 nm using a VICTOR^2 multilabel plate reader (Wallac). IC_{50} (inhibitory concentration 50%) values were determined from the drug concentrations that induced a 50% reduction in light absorbance.

3. Results and discussion

3.1. Synthesis

Fulvenes **1a** and **1b** (Fig. 1) were synthesized, according to [24], by reacting the corresponding benzaldehyde with cyclopentadiene in the presence of pyrrolidine as a base. The compounds were formed in high yields, of about 90%.

Titanocenes **2a** and **2b** (Fig. 2) were synthesized by reductive dimerisation of fulvenes **1a** and **1b** with titanium dichloride, respectively. $TiCl_2$ was obtained by reduction of $TiCl_4$ with *n*BuLi as described in [17,18]. The determined *cis–trans* ratio at the bridge is 60:40 for **2a**, and 93:7 for **2b**. The high *trans*-ratio in **2b** reflects the steric bulk of the pentaphenyl rings making the *trans* geometry highly favoured. This steric strain might also be the cause for the very small yield of only 10% obtained for **2b**, compared with 47% for **2a**. Substitution of the chloride ligands is easily achieved by reaction of **2a** and **2b** with potassium thiocyanate in acetone under reflux to obtain the corresponding dithiocyanato complexes **3a** and **3b** (Fig. 2), respectively [25].

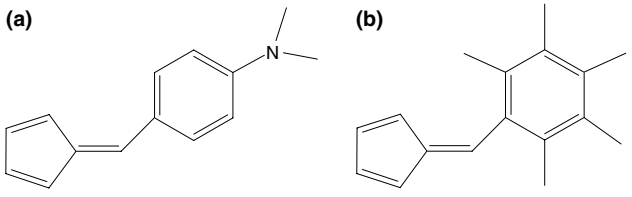


Fig. 1. Structures of fulvenes **1a** and **1b**.

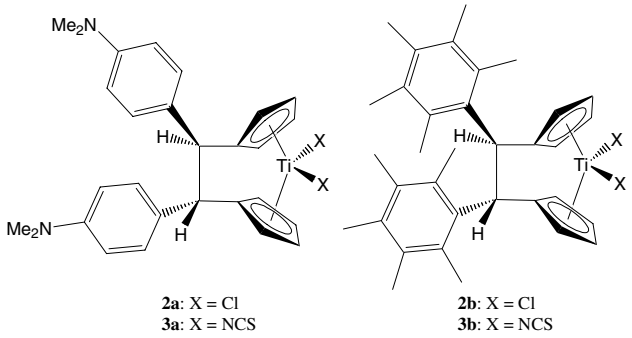


Fig. 2. Structures of *ansa*-titanocene dichlorides **2a/b**, and *ansa*-titanocene dithiocyanates **3a/b**.

3.2. Structural discussion

Density functional theory calculations were carried out for compounds **1b**, **2a**, and **2b** at the B3LYP level using the 6-31G** basis set. In addition, X-ray diffraction measurements were carried out for compounds **1b** and **3b** (see Table 1 for further details about the data collection). For compound **3b** in accordance with the centrosymmetric space group $P\bar{1}$ the *R*,*R*- and *S*,*S*-isomers are found in equal amounts in the crystal.

Selected bond lengths of the optimised structure of fulvene **1b** (Fig. 3) can be found in Table 2. As expected, the carbon–carbon bond lengths in the cyclopentadiene system of fulvene **1b** vary significantly, demonstrating the absence of a significant resonance system within the five-membered ring. This is confirmed by the length of the regular exocyclic double bond C(1)–C(6) found at 135.4 pm and the single bond carrying the phenyl substituent C(6)–C(7) is calculated as 148.1 pm (Table 2). The corresponding values of the crystal structure determination tend to be around 1–3 pm shorter than the calculated values (Table 2, Fig. 3). Especially outstanding is the dihedral angle of 61.7°, which shows that

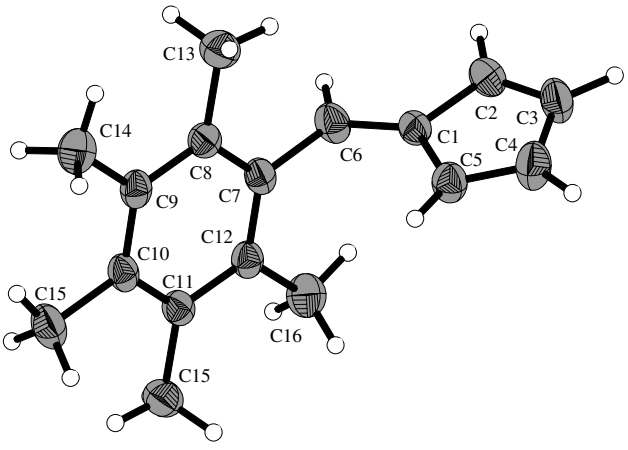


Fig. 3. Molecular structure of **1b**; thermal ellipsoids are drawn on the 50% probability level; from disordered methyl groups only one orientation is shown.

Table 2
Selected bond lengths of the DFT-calculated structure and crystal structure of fulvene **1b**

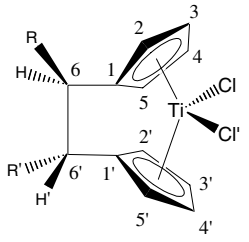
	Bond length (pm) (**1b**) DFT structure	Bond length (pm) (**1b**) crystal structure
C(1)–C(2)	147.1	146.2
C(2)–C(3)	135.5	132.1
C(3)–C(4)	147.2	143.8
C(4)–C(5)	135.6	133.6
C(5)–C(1)	147.1	143.9
C(1)–C(6)	135.4	134.5
C(6)–C(7)	148.1	148.7

the bulky aryl group does not allow co-planarity of the full aromatic ring system. The crystal structure confirms this twist with an even larger angle of 82.2°. The significant difference between calculated and experimental angle may be due to packing effects in the crystal.

Optimised structures were also calculated for titanocenes **2a** and **2b** (Scheme 1) at the B3LYP level using the 6-31G** basis set. Selected bond lengths of these structures are listed in Table 3.

The length of bonds between the metal centre and the carbon atoms of the cyclopentadienyl rings bound to the metal ion are similar for both titanocene complexes. They vary between 236.8 and 244.5 pm for **2a** and 237.0–245.1 pm for **2b** with values slightly different for the different cyclopentadienyl rings. The same applies for the carbon-carbon bonds of the cyclopentadienyl rings with bonds length between 140.3 and 143.0 pm for **2a** and 140.2–142.9 pm for **2b**. The higher steric bulk in titanocene **2b** leads to a elongated bond of the carbon bridge (C(6)–C(6′)) compared to **2a** with values of 157.1 and 156.2 pm, respectively. The titanium–chlorine bond length are almost identical for **2a** and **2b**, with values for 234.7 pm and 235.1 pm for **2a** and identical values for **2b** of 234.8 pm. For **2a** the $TiCl_2$ angle was calculated to be 97.5° and the dihedral angle between the aryl rings to be 62.1°. The corresponding values for **2b** are 97.5° and 83.8°, respectively. For **2b**, the angles formed by the bonds between C(1), C(6) and C(6′) is 106.0°, between C(7), C(6), and C(6′) is 120.6, and between C(7), C(6) and C(1) 114.9°, compared with the values found for the X-ray structure of 110.4°, 125.6°, and 115.0° for **3b** (Fig. 4), the dithiocyanato derivative of **2b**. The corresponding calculated values of **2a** are 108.0°, 113.9°, and 113.5°. The significantly larger angle formed between C(7), C(6), and C(6′) in **2b** compared with **2a** demonstrates the bulky overload given by the pentamethylphenyl substituent. In contrast, the angles formed between the centroids of the cyclopentadienyl rings are almost identical, with values of 128.0° for **2a** and 128.3° in **2b**. The carbon–carbon bonds of the cyclopentadienyl rings found in the crystal structure of complex **3b** tend to be around 2–3 pm shorter than the calculated values of **2b**. And also the titanium–carbon bond distances of 232.4–237.9 pm found in the crystal structure of **3b** tend to be significantly shorter (up to 10 pm) compared to the values calculated for **2b**. This difference is even more accentuated for the carbon–carbon bridge, which was measured by X-ray diffraction with 146.3 pm for **3b** compared to the calculated value of 157.1 pm in **2b**.

Due to the large, unexpected differences between the DFT-calculated structure of **2b** and the crystal structure



Scheme 1. Numbering scheme of atoms for the discussed titanocene complexes **2a/b** and **3b**.

Table 3
Selected bond lengths from the DFT-calculated structures of complexes **2a/b**, and from the crystal structure determination of complex **3b**

	Bond length (pm) (**2a**) DFT structure	Bond length (pm) (**2b**) DFT structure	Bond length (pm) (**3b**) crystal structure
Ti–C(1)	241.2	242.2	235.3
Ti–C(2)	236.8	237.0	235.2
Ti–C(3)	243.7	244.0	237.9
Ti–C(4)	244.5	245.2	237.7
Ti–C(5)	241.4	240.1	233.2
Ti–C(1′)	242.6	242.2	236.6
Ti–C(2′)	237.3	237.0	232.4
Ti–C(3′)	243.1	244.0	237.8
Ti–C(4′)	244.9	245.2	236.2
Ti–C(5′)	240.8	240.1	234.5
C(1)–C(2)	143.0	142.9	144.1
C(2)–C(3)	142.0	142.1	140.0
C(3)–C(4)	140.5	140.2	140.3
C(4)–C(5)	142.4	142.3	139.3
C(5)–C(1)	141.4	142.0	140.8
0C(1′)–C(2′)	142.3	142.9	140.9
C(2′)–C(3′)	142.4	142.1	139.9
C(3′)–C(4′)	140.3	140.2	138.7
C(4′)–C(5′)	142.4	142.3	139.3
C(5′)–C(1′)	141.9	142.0	140.0
Ti–Cl	235.1	234.8	Ti–N: 204.8
Ti–Cl′	234.7	234.8	Ti–N′: 201.8
C(6)–C(6′)	156.2	157.1	146.3
C(1)–C(6)	151.4	151.8	150.6

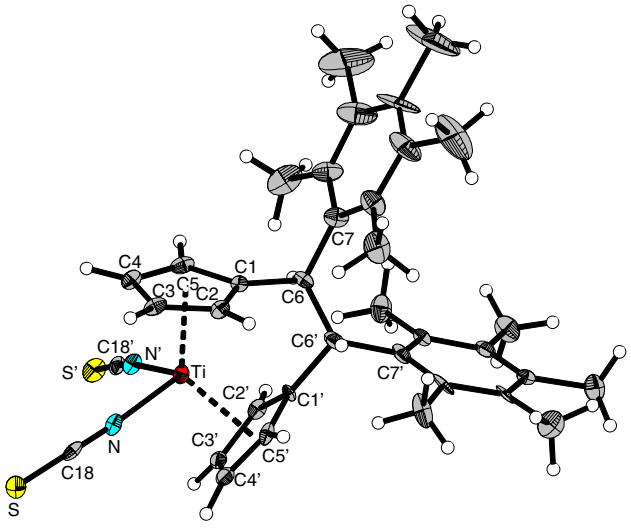


Fig. 4. Molecular structure of **3b** showing the atom numbering scheme; thermal ellipsoids are drawn on the 25% probability level.

found for **3b**, the refinement of **3b** will be discussed in detail. The aromatic substituents form a channel in which solvent molecules are situated. As adjacent $CHCl_3$ locations are very close to each other (the shortest intermolecular Cl–Cl-distance is 155 pm), these locations must be partially occupied. As the solvent positions are so close to each other, a molecule can easily hop from one position to the next. Therefore, at room temperature a solution failed completely due to this easy movement of the solvent. Even at 100 K large thermal parameters indicate a high mobility of the $CHCl_3$ molecules, which causes a general haziness of the electron density map. All components in this crystal structure arrange in layers, which leads to plate-shaped crystals with a low mechanical stability. The growth of crystals is significantly hindered by such an arrangement. In fact, the size of the crystal was at the lower limit of being suitable for an X-ray structure analysis.

The most important consequence of these two difficulties is the very short C(6)–C(6′) distance, compared with the calculated value for the chlorine compound **2b** (Table 3). A close look on their thermal ellipsoids reveals that both are elongated along the C–H bond axis which probably is a hint on a slight disorder. This is supported by the position of these carbon atoms, which are found to be nearly in the plane of their non-hydrogen-neighbours. However, the low quality of the crystal did not allow the refinement of any disorder of this kind.

3.3. Cytotoxicity studies

The in vitro cytotoxicity of compounds **2a** and **2b** were determined by an MTT-based assay [26] involving a 48 h drug exposure period, followed by 24 h of recovery time. Initially, compounds **2a** and **2b** were tested for their activity on pig kidney carcinoma (LLC-PK) cells. Due to the low solubility of **2b** even in DMSO, the saturated solution of **2b** in DMSO was applied in a dilution series with medium, starting with a concentration of 1% of the **2b**-saturated DMSO solution in medium. However, no cytotoxic effect was observed even at the highest concentration of the drug. In contrast, **2a** showed a significant inhibition of cell growth at higher concentrations, with an IC_{50} value of 2.7×10^{-4} M. Under identical conditions, *cis*-platin showed an IC_{50} value of 3.3×10^{-6} M, whereas the activity of Cp_2TiCl_2 was at least one order of magnitude lower (Fig. 5). The cytotoxicity of **2a** is therefore very promising, since it is shows significantly higher activity compared to Cp_2TiCl_2, the latter compound already having reached use in Phase I/II clinical trials.

Additionally, the cytotoxic effect of **2a** was also tested against the *cis*-platin-resistant human ovarian carcinoma cell line, A2780/cp70, with an IC_{50} value of 1.9×10^{-4} M being obtained (Fig. 6). Under equivalent conditions, the IC_{50} values of *cis*-platin and Cp_2TiCl_2 were determined to

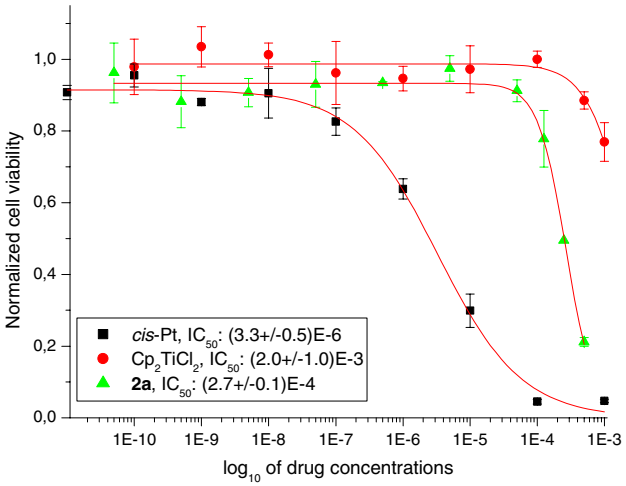


Fig. 5. Cytotoxicity curves from typical MTT assays showing the effect of *cis*-platin, Cp_2TiCl_2, and **2a** on the viability of pig kidney carcinoma (LLC-PK) cells.

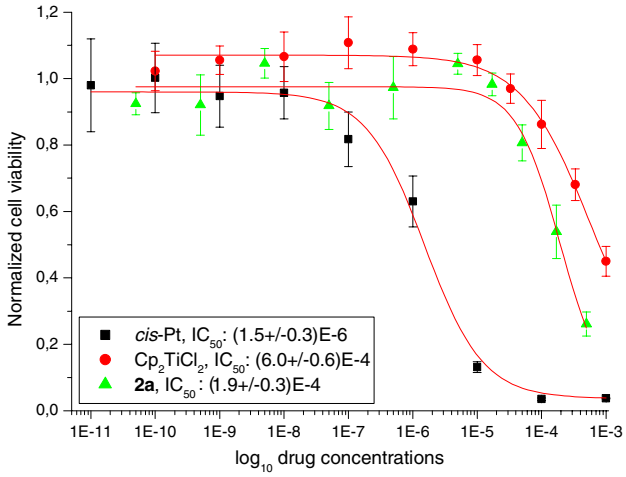


Fig. 6. Cytotoxicity curves from typical MTT assays showing the effect of *cis*-platin, Cp_2TiCl_2, and **2a** on the viability of platinum-resistant human ovarian carcinoma (A2780CP/cp70) cells.

be 1.5×10^{-6} and 6.0×10^{-4} M, respectively, which are similar to values published previously [11,27]. Consequently, for human ovarian carcinoma cells, the observed activity for **2a** is higher than for Cp_2TiCl_2, but still significantly less than for *cis*-platin.

4. Conclusion and outlook

The water-insoluble, non-cytotoxic titanocene **2b** was synthesised using the aryl-substituted fulvene **1b**. In contrast, when the NMe_2 substituted fulvene **1a** was used, compound **2a**, which resembles *cis*-platinum to a certain degree, was produced. In **2a**, the two NMe_2 substituents resemble the ammine ligands of *cis*-platinum and the $TiCl_2$ is equivalent to the $PtCl_2$ group. This

compound **2a** is significantly more cytotoxic against LLC-PK and A2780/cp70 cells than titanocene dichloride, for which Phase I/II clinical trials have been performed. It is intended to perform further in vitro cellular assays with the compound to evaluate its potential for testing in animal models and additionally to search for differently substituted titanocenes also derived from fulvenes.

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