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Heteroaryl substituted *ansa*-titanocene anti-cancer drugs derived from fulvenes and titanium dichloride

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Starting from 2-furylfulvene (1a), 2-thiophenylfulvene (1b), and 1-methyl-2-pyrrolylfulvene (1c), [1,2-di(cyclopentadienyl)-1,2-di-(2-furyl)ethanediyl] titanium dichloride (2a), [1,2-di(cyclopentadienyl)-1,2-di-(2-thiophenyl)ethanediyl] titanium dichloride (2b), and [1,2-di(cyclopentadienyl)-1,2-bis-(1-methyl-2-pyrrolyl)ethanediyl] titanium dichloride (2c) were synthesized. When titanocenes (2a–c) were tested against pig kidney carcinoma cells (LLC-PK), inhibitory concentrations (50%) of 4.5×10^{-4} M, 2.9×10^{-4} M and 2.0×10^{-4} M respectively were observed. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: anti-cancer drug; *cis*-platinum; titanocene; fulvene; LLC-PK; DFT calculations

INTRODUCTION

Despite the resounding success of *cis*-platinum and closely related platinum antitumor agents, the movement of other transition-metal anti-cancer drugs towards clinical use has been exceptionally slow.[1–3] Metallocene dichlorides (Cp_2MCl_2; M = Ti, V, Nb, Mo) show remarkable antitumor 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 creatinine 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 new method starting from titanium dichloride and fulvenes,[13–16] which allows direct access to highly substituted *ansa*-titanocenes.[17–20] Recently, using this method we have synthesized [1,2-di(cyclopentadienyl)-1,2-di-(4-*N*,*N*-dimethylaminophenyl)ethanediyl] titanium dichloride, which has an inhibitory concentration (50%, IC_{50}) value of 2.7×10^{-4} M when tested for cytotoxic effects on the LLC-PK cell line.[21] This paper reports the synthesis of new 1,2-diheteroarylsubstituted ethanediyl-*ansa*-titanium dichlorides, which combine the reactivity of the titanium dichloride moiety with the ability of hydrogen bonding towards DNA of the ammine ligand of *cis*-platinum with the aryl heteroatom.

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Experimental

Titanium tetrachloride (1 mol solution in toluene) and *n*-butyl lithium (2 mol solution in cyclohexane) were obtained commercially from Aldrich Chemical Co. Tetrahydrofuran (THF) and toluene were dried over and distilled from sodium–benzophenone prior to use. 2-Furaldehyde, 2-thiophenecarboxaldehyde and 1-methyl-2-pyrrolecarboxaldehyde 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

parts per million and are referenced to tetramethylsilane. IR spectra were recorded on a Perkin Elmer Paragon 1000 FT-IR spectrometer employing a KBr disk.

The gas chromatography–mass spectrometry (GCMS) spectra for fulvenes **1a–c** were measured on a Finnigan Trace GCMS 2000 Series (70 eV) and 1×10^{-5} M solutions in ethyl acetate were used.

For mass spectrometric analysis of titanocenes **2a–c**, stock solutions of the samples were prepared by dissolving the compounds in 0.5 ml dichloromethane. A ten fold dilution of these solutions was made in acetonitrile and electrospray mass spectrometry was performed on a quadrupole tandem mass spectrometer (Quattro Micro, Micromass/Waters Corp., USA) in negative ion mode.

With a view to elucidating 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 (Gaussian Inc., Carnegie, PA) running under Red Hat Linux was used. Density functional theory (DFT) calculations were performed at the B3LYP level using the 6-31G** basis set for the species of interest.

2-Furylfulvene (1a)

The syntheses of fulvenes **1a–c** were carried out under argon as outlined in Ref. 22. Pyrrolidine (5.0 ml, 60.0 mmol) was added to a solution of 2-furaldehyde (5.0 ml, 5.80 g, 60.4 mmol) and cyclopentadiene (10 ml, 151 mmol) in 80 ml of methanol. After addition, the colour of the solution immediately turned from colourless to red–orange. When thin-layer chromatography (TLC) analysis showed only one product band after 3 h, acetic acid (3.5 ml, 60.0 mmol) was added. The reaction mixture was diluted with 40 ml of a mixture of CH_2Cl_2 and water (1 : 1). The resultant organic layer was separated and the aqueous layer was washed with CH_2Cl_2 (3×30 ml). The combined organic extracts were washed with a saturated aqueous NaCl solution. The organic solution was dried over sodium sulfate, filtered and the solvent removed under reduced pressure. The crude product was redissolved in pentane/CH_2Cl_2 (2 : 1) and purified by column chromatography over silica gel 60 (0.063–0.200), using pentane as the eluent. When the solvent was removed under reduced pressure, 4.6 g of a red product was obtained (32 mmol, 52% yield relative to 2-furaldehyde).

^{1}H NMR (δ ppm, $CDCl_3$): 7.58 (OCH, 1H, d, 1.61 Hz); 6.69 (OCHCHC*H*, 1H, d, 3.37 Hz); 6.49 (OCHC*H*CH, 1H, dd, 3.37 Hz, 1.76 Hz); 6.99, 6.60, 6.45, 6.25 (C_5H_4, 4H, m); 6.81 (Ph–CH–Cp, 1H, s).

^{13}C NMR (δ ppm, $CDCl_3$): 151.9, 144.6, 140.4, 133.4, 129.7, 125.3, 121.4, 120.2, 115.9, 111.4 (C_5H_4, C_4H_3, Ph–CH–Cp).

IR absorptions (cm^{-1}, KBr): 3440 (s); 2962 (w); 2923 (w); 2852 (w); 1621 (C=C, m); 1449 (w); 1383 (m); 1369 (w); 1262 (m); 1152 (w); 1081 (s); 1019 (s); 933 (w); 801 (m); 765 (m); 746 (m); 620 (w), 590 (w).

GCMS: 144.1 (M^+, 32%); 115.1 ($M^+ - OCH$, 100%); 89.1 ($M^+ - OC_3H_3$, 22%).

Anal. Found: C, 84.12; H, 5.37. Calc. for $C_{10}H_8O$: C, 83.31; H, 5.59%.

2-Thiophenylfulvene (1b)

Pyrrolidine (4.5 ml, 53.9 mmol) was added to a solution of 2-thiophenecarboxaldehyde (6.0 g, 53.5 mmol) and cyclopentadiene (6.0 ml, 90.8 mmol) in 80 ml of methanol. After this addition the solution turned from colourless to clear red. When TLC analysis showed only one product band after 3 h, acetic acid (3.5 ml, 53.5 mmol) was added. The reaction mixture was diluted with 40 ml of a mixture of dichloromethane and water (1 : 1). The resultant organic layer was separated and the aqueous layer was washed with dichloromethane (3×40 ml). The combined organic extracts were washed with a saturated aqueous NaCl solution. The organic solution was dried over sodium sulfate and the solvent removed under reduced pressure, 7.9 g of a red product was obtained (49.3 mmol, 92% yield relative to 2-thiophenecarboxaldehyde).

^{1}H NMR (δ ppm, $CDCl_3$): 7.47 (SCH, 1H, d, 5.13 Hz); 7.29 (SCHCHCH, 1H, d, 3.66 Hz); 7.05 (SCHCHCH, 1H, dd, 5.13 Hz, 3.66 Hz); 6.85, 6.62, 6.44, 6.26 (C_5H_4, 4H, m); 7.23 (Ph–CH–Cp, 1H, s).

^{13}C NMR (δ ppm, $CDCl_3$): 142.5, 140.7, 135.2, 133.3, 130.8, 130.6, 129.9, 120.2, 127.8, 127.3 (C_5H_4, C_4H_3, Ph–CH–Cp).

IR absorptions (cm^{-1}, KBr): 3429 (s); 3093 (w); 3071 (w); 2956 (w); 2918 (w); 2851 (w); 1654 (C=C, m); 1605 (m); 1460 (m); 1413 (m); 1383 (m); 1325 (w); 1287 (w); 1235 (m); 1218 (w); 1086 (m); 1039 (w); 990 (w); 905 (w); 897 (m); 856 (w) 801 (m); 760 (s); 702 (s); 617 (s), 584 (w); 552 (w); 491 (w).

GCMS: 160.1 (M^+, 85%); 134.1 ($M^+ - C_2H_2$, 57%); 115.1 ($M^+ - SCH$, 100%); 89.1 ($M^+ - SC_3H_3$, 35%).

Anal. Found: C, 74.38; H, 5.01; S, 19.70. Calc. for $C_{10}H_8S$: C, 74.96; H, 5.03; S, 20.01%.

1-Methyl-2-pyrrolylfulvene (1c)

Pyrrolidine (3.1 ml, 37.3 mmol) was added to a solution of 1-methyl-2-pyrrolecarboxaldehyde (4.1 g, 37.3 mmol) and cyclopentadiene (6.0 ml, 90.8 mmol) in 80 ml of methanol. After this addition the solution turned from colourless to clear red. When TLC analysis showed only one product band after 3 h, acetic acid (2.1 ml, 37.3 mmol) was added. The reaction mixture was diluted with 40 ml of a mixture of dichloromethane and water (1 : 1). The resultant organic layer was separated and the aqueous layer was washed with dichloromethane (3×40 ml). The combined organic extracts were washed with a saturated aqueous NaCl solution. The organic solution was dried over sodium sulfate and the solvent removed under reduced pressure, 4.8 g of a red product was obtained (30.5 mmol, 82% yield relative to 1-methyl-2-pyrrolecarboxaldehyde).

^{1}H NMR (δ ppm, $CDCl_3$): 6.89 (Ph–CH–Cp, 1H, s); 6.79, 6.74, 7.05, 6.68, 6.58, 6.40, 6.25, 6.18 (C_4H_3S, C_5H_4, 7H, m); 3.55 (N–CH_3, s, 3H).

^{13}C NMR (δ ppm, $CDCl_3$): 140.2, 133.9, 130.8, 129.1, 127.1, 126.8, 125.0, 119.4, 115.8, 110.3 (C_5H_4, C_4H_3, Ph–CH–Cp); 34.3 (CH_3).

IR absorptions (cm^{-1}, KBr): 3445 (s); 3132 (w); 3104 (w); 3071 (w); 3049 (w); 2923 (w); 2852 (w); 1648 (C═C, m); 1608 (s); 1523 (w); 1482 (m); 1460 (m); 1402 (s); 1383 (m); 1336 (m); 1314 (m); 1287 (w); 1251 (w); 1182 (w); 1100 (m); 1075 (w); 1062 (w); 993 (w); 905 (m); 886 (w) 792 (w); 760 (s); 743 (w); 730 (m); 724 (s); 653 (w), 642 (w); 626 (s); 595 (w).

GCMS: 157.1 (M^+, 100%); 141.1 ($M^+ - CH_4$, 15%); 115.1 (M^+ − MeNCH, 57%); 89.1 ($M^+ - MeNC_3H_3$, 40%).

Anal. Found: C, 83.45; H, 7.00; N, 8.99. Calc. for $C_{11}H_{11}N$: C, 84.04; H, 7.05; N, 8.91%.

[1,2-Di(cyclopentadienyl)-1,2-di(2-furyl)ethanediyl] titanium dichloride [1,2-(2-C_4H_3O)$_2$ $C_2H_2\{\eta^5$-$C_5H_4\}_2$]$TiCl_2$ (2a)

$TiCl_4$ (5.9 ml, 5.9 mmol) was added to 90 ml of dry toluene containing 10 ml dry THF. The solution turned immediately from colourless to pale yellow. The solution was stirred and cooled to −78 °C, followed by dropwise addition of *n*-butyllithium (5.9 ml, 11.8 mmol). The solution turned from yellow to brown during addition. After this addition, the mixture was allowed to warm slowly to room temperature (r.t.). The colour of the solution became black finally. After 20 h stirring at r.t., a solution of **1a** (1.7 g, 11.8 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 under reduced pressure, the crude product redissolved in 10 ml chloroform and filtered. After removal of the solvent under reduced pressure, a dark-red product **2a** with 1.5 g (3.7 mmol, 63% yield) was obtained. The ratio of trans to cis isomers is 52% to 48%.

^{1}H NMR (δ ppm, $CDCl_3$): 7.42–5.86 (C_4H_3O, 6H, m, and C_5H_4, 8H, m); 5.23 (*cis*-furylCHCp, 2H, s); 5.01 (*trans*-furylCHCp, 2H, s).

^{13}C NMR (δ ppm, $CDCl_3$): 151.8, 150.6, 141.4, 141.2, 134.5, 132.8, 132.6, 128.6, 128.5, 125.4, 116.1, 114.4, 113.9, 109.9, 109.4, 109.3, 107.9, 105.9 (C_4H_3O and C_5H_4); 45.4, 45.0 (PhCHCp).

IR absorptions (cm^{-1}, KBr): 3390 (m); 3110 (m); 2956 (w); 2923 (w); 2863 (w); 1629(w); 1588(w); 1501 (w); 1484 (w); 1424 (w); 1383 (w); 1259 (w); 1144 (m); 1100 (w); 1070 (m); 1043 (w); 1012(s); 924 (w); 883 (w); 815 (s); 733 (s); 590 (m).

MS: 440.9 ($M + Cl^-$).

Anal. Found: C, 57.05; H, 4.23. Calc. for $C_{20}H_{16}Cl_2O_2Ti$: C, 59.00; H, 3.96%.

[1,2-Di(cyclopentadienyl)-1,2-bis(2-thiophenyl)ethanediyl] titanium dichloride [1,2-(2-C_4H_3S)$_2C_2H_2\{\eta^5$-$C_5H_4\}_2$]$TiCl_2$ (2b)

$TiCl_4$ (7.8 ml, 7.8 mmol) was added to 90 ml of dry toluene containing 10 ml dry THF. The solution turned immediately from colourless to pale yellow. The solution was stirred and cooled to −78 °C, followed by dropwise addition of *n*-butyllithium (7.8 ml, 15.6 mmol). The solution turned from yellow to brown during addition. After this addition, the mixture was allowed to warm slowly to r.t. The colour of the solution became black finally. After 20 h stirring at r.t., a solution of **1a** (2.5 g, 15.6 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 under reduced pressure, the crude product redissolved in 10 ml chloroform and filtered. After removal of the solvent under reduced pressure, a dark-red product **2b** with 1.8 g (4.1 mmol, 53% yield) was obtained. The ratio of trans to cis isomers is 58% to 42%.

^{1}H NMR (δ ppm, $CDCl_3$): 7.35–6.14 (C_4H_3S, 6H, m, and C_5H_4, 8H, m); 5.52 (*cis*-thiophenylCHCp, 2H, s); 5.01 (*trans*-thiophenylCHCp, 2H, s).

^{13}C NMR (δ ppm, $CDCl_3$): 143.9, 140.6, 136.7, 135.9, 134.1, 130.1, 128.1, 127.3, 126.9, 126.8, 126.6, 125.7, 125.5, 125.3, 117.0, 116.1, 115.1, 109.8 (C_4H_3S and C_5H_4); 50.9, 49.2 (thiophenylCHCp).

IR absorptions (cm^{-1}, KBr): 3434 (s); 3104 (m); 2950 (w); 2923 (w); 2857 (w); 1638 (m); 1484 (w); 1456 (w); 1423 (w); 1399 (w); 1383 (w); 1234 (w); 1078 (m); 1039 (m); 851 (m); 815 (s); 755 (m); 694 (s); 499 (w).

MS: 472.8 ($M + Cl^-$).

Anal. Found: C, 56.69; H, 4.15; S, 13.53. Calc. for $C_{20}H_{16}Cl_2S_2Ti$: C, 54.69; H, 3.67; S, 14.60%.

[1,2-Di(cyclopentadienyl)-1,2-bis(1-methyl-2-pyrrolyl)ethanediyl] titanium dichloride [1,2-(2-C_4H_3N–CH_3)$_2C_2H_2\{\eta^5$-$C_5H_4\}_2$]$TiCl_2$ (2c)

$TiCl_4$ (8.0 ml, 8.0 mmol) was added to 90 ml of dry toluene containing 10 ml dry THF. The solution turned immediately from colourless to pale yellow. The solution was stirred and cooled to −78 °C, followed by dropwise addition of *n*-butyllithium (8.0 ml, 16.0 mmol). The solution turned from yellow to brown during addition. After this addition, the mixture was allowed to warm slowly to r.t. The colour of the solution became black finally. After 20 h stirring at r.t., a solution of **1a** (2.5 g, 15.9 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 rapidly filtered through Celite under reduced pressure. The colour of the filtrate reddened slightly. It was rapidly 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 under reduced pressure, the crude product redissolved in 10 ml chloroform and filtered. After removal of the solvent under reduced pressure, a dark-red product **2b** with 1.4 g (3.2 mmol, 40% yield) was obtained. The ratio of trans to cis isomers is 70% to 30%.

^{1}H NMR (δ ppm, $CDCl_3$): 7.23–5.86 (C_4H_3N, 6H, m, and C_5H_4, 8H, m); 5.20 (*cis*-pyrrolylCHCp, 2H, s); 4.66 (*trans*-pyrrolylCHCp, 2H, s), 3.39 (*cis*-N–CH_3, 3H, s); 3.05 (*trans*-N–CH_3, 3H, s).

^{13}C NMR (δ ppm, $CDCl_3$): 137.0, 135.5, 134.0, 132.4, 130.0, 129.2, 128.0, 126.4, 122.6, 122.5, 116.7, 116.1, 114.4, 110.2, 110.1, 107.7, 107.5, 107.2 (C_4H_3N and C_5H_4); 46.9 (*trans*-pyrrolylCHCp); 44.2 (*cis*-pyrrolylCHCp); 34.1 (*cis*-N–CH_3); 33.0 (*trans*-N–CH_3).

IR absorptions (cm^{-1}, KBr): 3434 (m); 3098 (w); 2951 (w); 2923 (w); 2857 (w); 1638 (w); 1487 (m); 1465 (w); 1449 (w); 1421 (w); 1399 (w); 1383 (w); 1262 (w); 1235 (w); 1089 (m); 1056 (w); 1040 (w); 818 (s); 749 (s); 711 (s); 663 (w); 606 (w).

MS: 467.1 ($M + Cl^-$).

Anal. Found: C, 58.74; H, 5.34. Calc. for $C_{22}H_{22}Cl_2N_2Ti$: C, 61.00; H, 5.12%.

Methylthiazoletetrazolium-based cytotoxicity tests

The pig kidney carcinoma cell line, LLC-PK, was obtained from the American Tissue Culture Collection.

The cytotoxic activities of titanocenes **2a–c** were determined using an methylthiazoletetrazolium (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. In order to prepare drug solutions, drugs were first dissolved in dimethylsulfoxide (DMSO), followed by dilution with medium to the required maximum concentration, 1.5×10^{-3} M for **2a** and 5×10^{-4} M for **2b** and **2c**, with a final concentration of DMSO not exceeding 0.7%. From these stock solutions, solutions with lower concentrations were prepared by further dilution with medium. Care was taken that the drug solutions were applied within 1 h on the cells to avoid interference with already hydrolysed compounds. After 48 h, the relevant drug was removed, the cells washed twice with phosphate-buffered saline 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} values were determined from the drug concentrations that induced a 50% reduction in light absorbance.

RESULTS AND DISCUSSION

Synthesis

Heteroarylfulvenes **1a–c** (Fig. 1) were synthesised, according to Ref. 22, by reacting the corresponding carbaldehydes with cyclopentadiene in the presence of pyrrolidine as a base.

For the synthesis of *ansa*-titanocene dichlorides by reductive dimerization of fulvenes with titanium dichloride it is essential that the fulvenes chosen are easily reduced to form a stable radical anion intermediate, which subsequently dimerizes.[20] Therefore, arylsubstituted fulvenes have been chosen so far for this kind of synthesis. In contrast, dimerization of dimethylfulvene in order to obtain *ansa*-metallocenes resulted in the formation of unbridged metallocenes as a side product. Analogously to the reductive dimerization of phenylsubstituted fulvenes with titanium dichloride to yield the corresponding *ansa*-titanocene dichlorides, titanocenes **2a–c** (Scheme 1) were synthesized starting with the heteroarylsubstituted fulvenes **1a–c** (Scheme 1). $TiCl_2$ was obtained by reduction of $TiCl_4$ with *n*-butyllithium as described in References 17 and 18 (Scheme 1). The cis : trans ratios determined at the bridge are 52 : 48 for **2a**, 58 : 42 for **2b**, and 70 : 30 for **2c**. The higher trans content in **2c** reflects the steric bulk of the methyl group bound to the nitrogen heteroatom, making the trans geometry more favoured.

Theoretical studies

DFT calculations were carried out for fulvenes **1a–c** and titanocenes **2a–c** at the B3LYP level using the 6-31G** basis set.

Selected bond lengths of the optimized structure of fulvenes **1a–c** (Fig. 2; for atom numbering scheme see Fig. 1) can be found in Table 1. As expected, the carbon–carbon bond lengths in the cyclopentadiene system of these fulvenes 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 bonds C(1)–C(6) calculated with 136.6–137.1 pm and the single bond carrying the aryl substituent C(6)–C(7) is calculated as 143.0–144.0 pm (Table 1). For fulvenes **1a** and **1b** the carbon–carbon bonds within the heterocycle also alter significantly. In contrast, the carbon–carbon bonds within

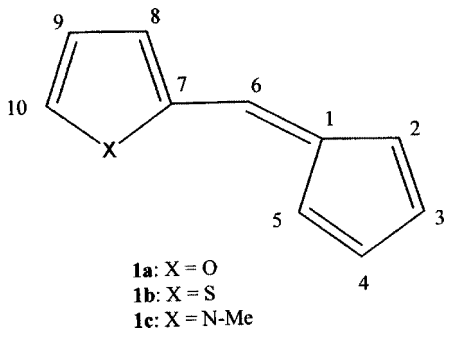
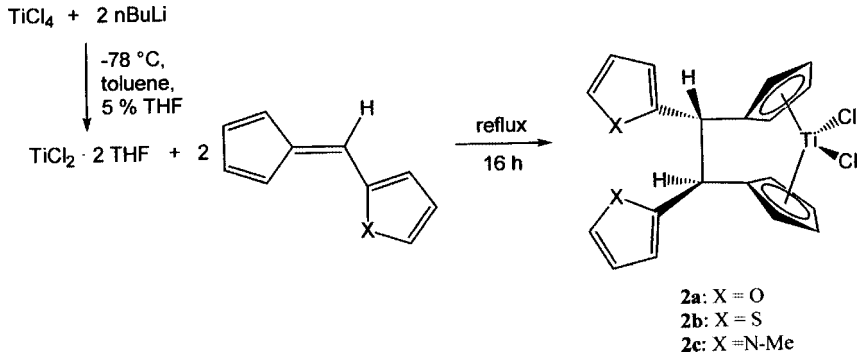


Figure 1. Structures of fulvenes **1a–c**.



Scheme 1. Synthesis of titanocenes **2a**–**c**.

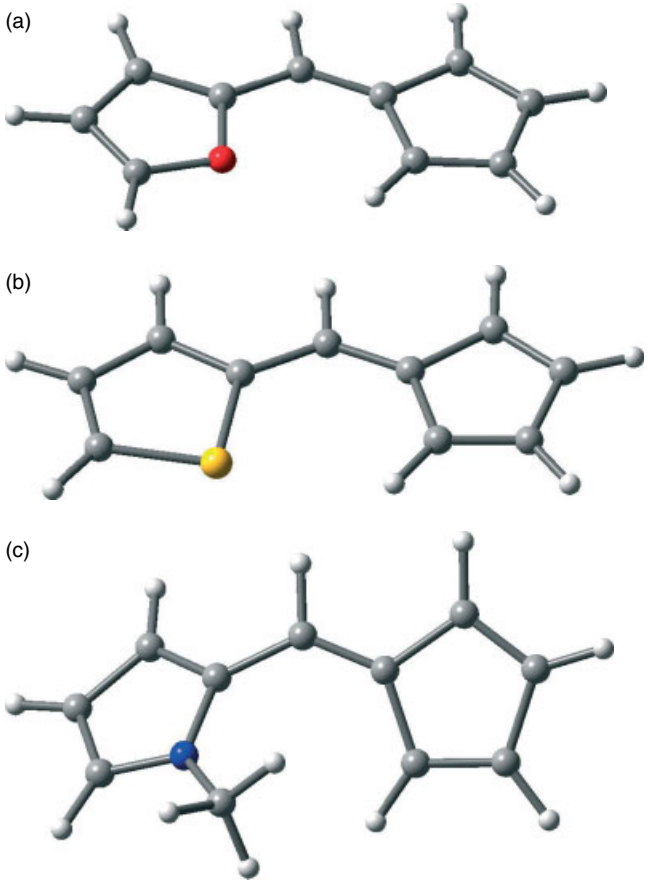


Figure 2. Gaussview plot of the optimized structures of fulvenes **1a** (a), **1b** (b) and **1c** (c).

the heterocycle of fulvene **1c** are rather similar, indicating a significant resonance system in the pyrrole ring. Both fulvenes **1a** and **1b** have a completely planar structure. For fulvene **1c**, a distortion from planarity is seen, with a dihedral angle between the heteroaryl ring and the five-membered dienyl ring of 31.1°. Obviously, the bulky methyl group bound to the nitrogen heteroatom of the pyrrole ring prevents the fulvene from adopting a planar structure.

Table 1. Selected bond lengths of the DFT-calculated structures **1a**–**c**

	Bond length DFT structure (pm)		
	1a	**1b**	**1c**
C(1)–C(2)	147.8	147.9	147.6
C(2)–C(3)	136.5	136.5	136.7
C(3)–C(4)	147.7	147.0	147.0
C(1)–C(6)	136.6	136.7	137.1
C(6)–C(7)	143.0	143.6	144.0
C(7)–C(8)	138.4	138.5	140.6
C(8)–C(9)	143.3	142.8	141.5
C(9)–C(10)	137.0	137.0	139.1
C(10)–X	139.1	179.5	138.1
X–C(7)	140.6	182.4	140.7

Table 2. LUMO energies of fulvenes **1a**–**c**, 6-phenylfulvene and 6,6-dimethylfulvene

	LUMO energy (eV)
1a	−2.43
1b	−2.58
1c	−2.13
6-Phenylfulvene	−2.46
6,6-Dimethylfulvene	−1.75

Additionally, the lowest unoccupied molecular orbital (LUMO) energies of **1a**–**c**, phenylfulvene and dimethylfulvene were calculated (Table 2). The values calculated for fulvenes **1a**–**c** vary between −2.58 eV for fulvene **1b** and −2.13 eV for fulvene **1c**, which are similar to the value calculated for 6-phenylfulvene (−2.46 eV), but much lower than the LUMO energy calculated for 6,6-dimethylfulvene (−1.75 eV). These results help to explain why titanocenes **2a**–**c** are the only products observed from the reductive dimerization of fulvenes **1a**–**c** with titanocene dichloride, with no unbridged titanocenes being observed. This result is analogous to the formation of *ansa*-titanocenes starting from phenylsubstituted

fulvenes. In contrast, dimethylfulvene, with its significantly higher lying LUMO, forms a less stable radical anion intermediate and, therefore, an unbridged titanocene as a side product.[23,24]

Optimized structures were also calculated for titanocenes **2a–c** (Fig. 3; for atom numbering, see Scheme 2) at the B3LYP level using the 6-31G** basis set. Selected bond lengths of these structures are listed in Table 3.

The lengths of bonds between the metal centre and the carbon atoms of the cyclopentadienyl rings bound to the metal ion are similar for the three titanocene complexes. They vary between 238.0 and 245.1 pm for **2a**, between 237.3 and 245.4 pm for **2b** and between 237.2 and 244.9 pm for **2c**, 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

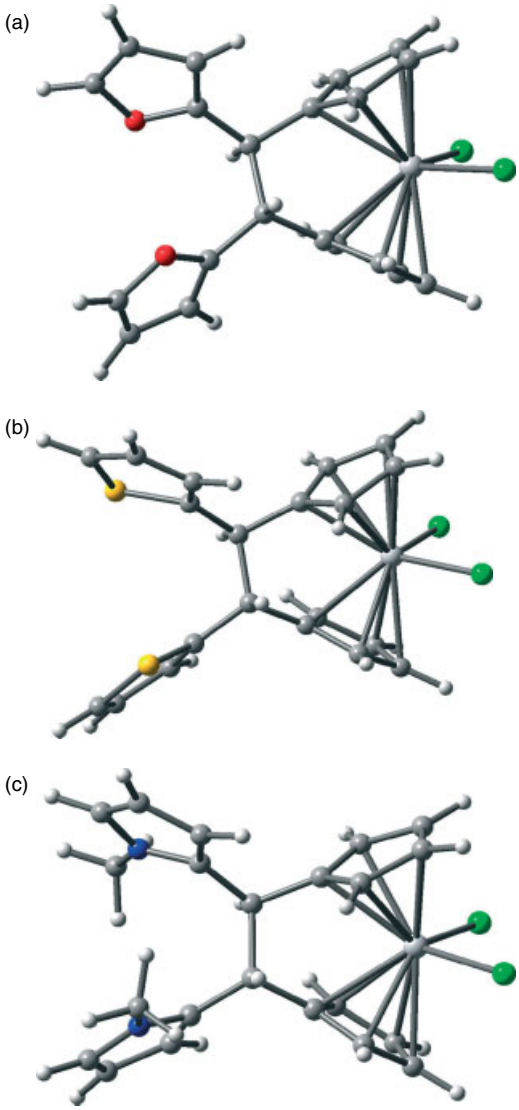
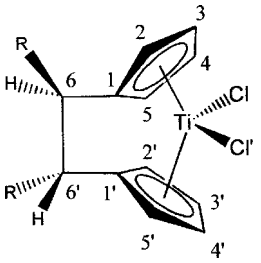


Figure 3. Gaussview plot of the optimized structures of titanocenes **2a** (a), **2b** (b) and **2c** (c).



Scheme 2. Numbering scheme for structural discussion of titanocenes **2a–c**.

Table 3. Selected bond lengths from the DFT-calculated structures of complexes **2a–c**

	Bond length DFT structure (pm)		
	2a	**2b**	**2c**
Ti–C(1)	241.7	241.4	241.7
Ti–C(2)	238.0	237.3	237.2
Ti–C(3)	244.3	244.3	244.0
Ti–C(4)	245.1	245.3	244.9
Ti–C(5)	240.9	241.4	241.0
Ti–C(1′)	241.4	241.7	241.7
Ti–C(2′)	238.0	237.3	237.2
Ti–C(3′)	244.5	244.1	243.9
Ti–C(4′)	244.8	245.4	244.9
Ti–C(5′)	241.0	241.4	240.9
C(1)–C(2)	142.5	142.7	142.6
C(2)–C(3)	142.2	142.1	142.1
C(3)–C(4)	140.4	140.4	140.4
C(4)–C(5)	142.4	142.4	142.4
C(5)–C(1)	141.6	141.5	141.5
C(1′)–C(2′)	142.7	142.6	142.6
C(2′)–C(3′)	142.1	142.2	142.2
C(3′)–C(4′)	140.5	140.4	140.4
C(4′)–C(5′)	142.4	142.4	142.4
C(5′)–C(1′)	141.5	141.6	141.6
Ti–Cl	234.1	234.2	234.6
C(6)–C(6′)	157.4	157.0	157.4
C(1)–C(6)	150.9	151.2	151.2

140.4 and 142.7 pm for **2a**, between 140.4 and 142.7 pm for **2b** and between 140.4 and 142.6 for **2c**. These values suggest the titanocenes have a plane of symmetry bisecting the Cl–Ti–Cl plane and that the calculated structures exhibit C_2 symmetry. **2a, 2b** and **2c** have similar ansa-bridge lengths (C(6)–C′(6)) of 157.4 pm, 157.0 pm and 157.4 pm respectively. These values are in agreement with the corresponding value calculated previously for [(1,2-diphenyl-1,2-dicyclopentadienyl)ethanediyl} titanium dichloride.[20] The steric bulk of the heteroaryl rings attached to the bridging carbon atoms causes a lengthening of the bond, in order to relieve the resultant steric strain. The titanium–chlorine bond

lengths are almost identical for **2a, 2b** and **2c**, with values of 234.1 and 234.2 pm for **2a,** 234.2 pm for **2b,** and 234.6 pm for **2c**. The $TiCl_2$ angle was calculated for **2a** to be 97.8°, for **2b** as 98.0° and for **2c** as 97.9°. The dihedral angle between the aryl rings was calculated as 63.3° for **2a**, 62.4° for **2b**, and 64.4° for **2c**. The dihedral angles between the cyclopentadienyl rings show an inverse order: the lowest value calculated was for **2c** with 45.0°, for **2a** it was 45.8° and the highest value was for **2b** with 46.1°. For **2a**, the angle formed by the bonds between C(1), C(6) and C(6′) is 107.8°, between C(7), C(6), and C(6′) it is 114.2°, and between C(7), C(6) and C(1) it is 112.3°. The corresponding calculated values of **2b** are 107.5°, 113.9°, and 112.7°, and for **2c** they are 107.7°, 114.3° and 112.1°.

Cytotoxicity studies

The *in vitro* cytotoxicity of compounds **2a–c** were determined by MTT-based assays[25] involving a 48 h drug exposure period, followed by 24 h of recovery time. Compounds were tested for their activity on pig kidney carcinoma (LLC-PK) cells. The IC_{50} values found decreased from 4.5×10^{-4} M, found for **2a**, over 2.9×10^{-4} M for **2b**, to 2.0×10^{-4} M for **2c** (Fig. 4) and are similar to the inhibition value found previously for [1,2-di(cyclopentadienyl)-1,2-di(4-*N*,*N*-dimethylaminophenyl)ethanediyl] titanium dichloride, which has an IC_{50} value of 2.7×10^{-4} M.[21] Under identical conditions, *cis*-platinum 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 than **2a–c**.[21]

CONCLUSIONS AND OUTLOOK

Compounds **2a–c** have IC_{50} values in the lower 10^{-4} M region, which are significantly more cytotoxic than unsubstituted titanocene dichloride against LLC-PK, for which Phase I/II clinical trials have been performed. The use of heteroarylsubstituted ligands may overcome the problem of water insolubility. Also, the heteroatoms resemble the ammine ligands of *cis*-platinum and the $TiCl_2$ is equivalents to the $PtCl_2$ group. It is intended to perform further *in vitro* cellular tests with the compounds to evaluate their potential for testing in animal models and additionally to search for differently substituted titanocenes also derived from fulvenes. In addition, experiments to separate the *ansa*-titanocene isomers and to test them individually against LLC-PK cells are under way.

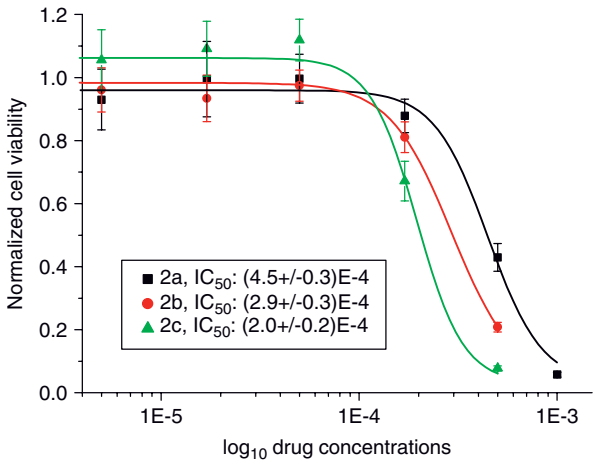


Figure 4. Cytotoxicity curves from typical MTT assays showing the effect of compounds **2a–c** on the viability of pig kidney carcinoma (LLC-PK) cells.

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