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Original article

Synthesis and cytotoxicity studies of new dimethylamino-functionalised and aryl-substituted titanocene anti-cancer agents

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Abstract

From the carbolithiation of 6-*N*,*N*-dimethylamino fulvene (**3a**) and different lithiated aryl species [*p*-*N*,*N*-dimethylanilinyl lithium, *p*-anisyl lithium and 4-lithio-benzo[1.3]dioxole (**2a–c**)], the corresponding lithium cyclopentadienide intermediates **4a–c** were formed. These three lithiated intermediates underwent a transmetallation reaction with $TiCl_4$ resulting in dimethylamino-functionalised and aryl-substituted titanocenes **5a–c**. When these titanocenes were tested against LLC-PK cells, the IC_{50} values obtained were of 54, 45 and 26 μM for titanocenes **5a**, **b** and **c**, respectively. The most cytotoxic titanocene in this paper, **5c** is approximately 10 times less cytotoxic than cis-platin, which showed an IC_{50} value of 3.3 μM, when tested on the LLC-PK cell line, but approximately 100 times better than titanocene dichloride.

Keywords: Anti-cancer agents; Cis-platin; Titanocene; Fulvene; Dimethylamino-functionalised metallocenes; LLC-PK

1. Introduction

In developed countries, cancer is one of the leading causes of death. However, there is no chemotherapy available for the treatment of many varieties of this disease.

Metal based drugs have been used in medicine for many centuries, but very often only in an empirical fashion. Nowadays there is enormous scope for the design of novel therapeutic compounds, for example, the well known cis-platin is a transition metal based drug which forms highly reactive, charged, platinum complexes that bind to nucleophilic groups such as GC-rich sites in DNA, inducing DNA cross-links that result in apoptosis and cell growth inhibition [1]. Due to the severe adverse effects of cis-platin, research moved to a second-generation of platinum compounds like carboplatin, nedaplatin, satraplatin and other closely related platinum antitumor agents, some of which are still used for the treatment of certain types of tumors [2–5]. Other metal based drugs, for example metallocene dichlorides (Cp_2MCl_2) with M = Ti, V, Nb and Mo also show remarkable antitumor activity [6,7]. Titanocene dichloride is a potent anti-cancer agent. 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. In contrast to cis-platin and other metal-containing drugs, the mechanism and biological action of Cp_2TiCl_2 seems to be different. Nevertheless, little synthetic effort has been employed to overcome the mentioned efficacy problems. This is the reason why recent research of our group has focussed on the syntheses of substituted titanocene dichloride anti-cancer drugs. By using a novel method starting from titanium dichloride and fulvenes [10–13] highly substituted *ansa*-titanocenes [14–21], containing a carbon–carbon bridge, have been synthesised, such as [1,2-bis(cyclopentadienyl)-1,2-bis-(4-*N*,*N*-dimethylaminophenyl)-ethanediyl] titanium dichloride (titanocene **X**), which has shown an IC_{50} value of 2.7×10^{-4} M when tested for cytotoxic effects against LLC-PK cells (a type of pig kidney epithelial cell line) [18].

One of our best titanocenes, titanocene **Y**, was obtained through a different synthetic pathway, which was recently published [21]. Bis-[(*p*-methoxybenzyl)cyclopentadienyl]

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titanium (IV) dichloride (titanocene **Y**), which has an IC_{50} value of 2.1×10^{-5} M when tested on the LLC-PK (pig kidney epithelial) cell line, was synthesised from fulvene and super hydride ($LiBEt_3H$) followed by transmetallation with titanium tetrachloride.

A third method leads to diarylmethyl and diheteroaryl substituted titanocenes, which can be obtained using a carbolithiation reaction of the respective 6-arylfulvenes with the corresponding aryl lithium species followed by a transmetallation with titanium tetrachloride [22]. An example of a titanocene synthesised using this new method, bis-[di-(*p*-*N*,*N*-dimethylaminophenyl)methylcyclopentadienyl] titanium (IV) dichloride, shows an IC_{50} value of 3.8×10^{-5} M when tested for cytotoxic effects on the LLC-PK cell line [23].

Surprisingly, a change in the substitution pattern in *ansa*-titanocenes can also lead to proliferative effects, as seen in the case of some glycol methyl ether and glycol amine substituted titanocenes [24].

The anti-proliferative activity of titanocenes **X** and **Y** has been studied in 36 human tumor cell lines [25] and in four freshly explanted human tumors using titanocene **X** [26]. These *in vitro* and *ex vivo* experiments showed that prostate, cervix and renal cell cancer are prime targets for these novel classes of titanocenes. These results were underlined by first mechanistic studies concerning the effect of these titanocenes on apoptosis and the apoptotic pathway in prostate cancer cells [27]. Furthermore, first animal studies have been published recently reporting the successful treatment of xenografted Caki-1 tumors and xenografted Ehrlich's ascites tumor in mice with titanocenes **X** and **Y** [28,29].

The main idea behind the research presented in this paper was to improve the cytotoxicity of titanocene **Y** and its analogues by adding extra dimethylamino groups close to the titanium centre, helping to solve solubility problems, and stabilising the metallocene cation or dication before an interaction with DNA occurs [30–32]. Within this paper we present a series of *N*,*N*-dimethylamino-functionalised and aryl-substituted titanocenes, their syntheses and preliminary cytotoxicity studies.

2. Experimental

2.1. General conditions

Titanium tetrachloride (1.0 M solution in toluene) and *tert*-butyl lithium (1.7 M solution in cyclohexane) were obtained commercially from Aldrich Chemical Co. THF was dried over Na and benzophenone and was freshly distilled and collected under an atmosphere of argon prior to use. Manipulations of air and moisture sensitive compounds were done using standard Schlenk techniques, under an argon atmosphere. NMR spectra were measured on either a Varian 300 or a 500 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 disc. UV–vis spectra were recorded on a Unicam UV4 Spectrometer, while CHN analysis was done with an Exeter Analytical CE-440 Elemental Analyser.

2.2. Synthesis

6-*N*,*N*-Dimethylamino fulvene was synthesised according to the already published procedure [33].

2.2.1. Bis-(N,N-dimethylamino-p-N,N-dimethylanilylmethyl-cyclopentadienyl) titanium (IV) dichloride, $\{\eta^5\text{-}C_5H_4\text{–}CH[N(CH_3)_2][C_6H_4\text{–}N(CH_3)_2]\}_2TiCl_2$ (**5a**)

To a Schlenk flask with 1.01 g (5.07 mmol) 4-bromo-*N*,*N*-dimethylaniline (**1a**), 20 ml of THF were added until a transparent solution was formed, while stirring. The solution was cooled down to −78 °C for 15 min and 3.30 ml (5.57 mmol) of *tert*-butyl lithium were added. The solution was warmed up to 0 °C for 20 min, resulting in the formation of the yellow lithium intermediate (**2a**).

In a second Schlenk flask 0.61 g (5.07 mmol) of 6-*N*,*N*-dimethylamino fulvene (**3a**) were dissolved in 25 ml of THF and the resultant red solution was added *via cannula* at −78 °C to the Schlenk flask containing the lithiated intermediate (**4a**). The reaction mixture was allowed to warm up to room temperature and left stirring for 40 min. Afterwards 2.5 ml (2.53 mmol) titanium tetrachloride were added at room temperature and the mixture was refluxed for 20 h. The solvent was removed under vacuum, resulting in a dark brown precipitate. This precipitate was dissolved in dichloromethane and filtered through celite to remove the LiCl, followed by two gravity filtrations. The solvent was removed under reduced pressure forming a shiny black solid (**5a**), which was washed with 150 ml of pentane and then dried *in vacuo* (1.14 g, 1.93 mmol, 76.1% yield).

^{1}H NMR (δ ppm $CDCl_3$, 300 MHz): 7.56–6.25 [m, 8H, C_5H_4 and $C_6H_4N(CH_3)_2$]; 3.83, 3.40 [s, 2H, $C_5H_4\text{–}CH(C_6H_4N(CH_3)_2)(N(CH_3)_2)$]; 3.03, 2.95 [s, 12H, $C_6H_4N(CH_3)_2$]; 2.72, 2.39 [s, 6H, $N(CH_3)_2$]. IR absorptions (cm^{-1} KBr): 3396, 2960, 2780, 2434, 1610, 1588, 1467, 1355, 1256, 1190, 1023, 941, 806, 625. Anal. Calc. for $C_{32}H_{34}N_4Cl_2Ti$: theory: C, 64.76; H, 5.77; N, 9.44; Cl, 11.94; found: C, 64.39; H, 5.50; N, 9.60; Cl, 12.01. UV–vis (CH_2Cl_2): λ 238 nm (ε 24,770), λ 382 nm (ε 2020), λ 509 nm (ε 210), λ_{max} 521 nm (weak).

2.2.2. Bis-(p-anisyl-N,N-dimethylaminomethylcyclopentadienyl) titanium (IV) dichloride, $\{\eta^5\text{-}C_5H_4\text{–}CH[N(CH_3)_2][C_6H_4\text{–}O\text{–}CH_3]\}_2TiCl_2$ (**5b**)

To a Schlenk flask with (1.1 ml, 4.12 mmol) of 4-bromoanisole (**1b**), 20 ml of THF were added until a transparent solution was formed, while stirring at room temperature. The solution was cooled down to −78 °C for 15 min and 2.7 ml (4.54 mmol) of *tert*-butyl lithium were added. The solution was allowed to warm up to 0 °C for 20 min, resulting in the formation of the yellow lithium intermediate (**2b**).

In a second Schlenk flask 0.5 g (4.12 mmol) of 6-*N*,*N*-dimethylamino fulvene (**3a**) was dissolved in 25 ml of THF and the resultant red solution was added *via cannula* at −78 °C to the Schlenk flask containing the lithiated

intermediate. The reaction mixture was then allowed to warm up to room temperature and left stirring for 40 min. Titanium tetrachloride (2.0 ml, 2.06 mmol) was added afterwards *in situ* at room temperature and the mixture was refluxed for 20 h. Subsequently, the solvent was removed under vacuum, resulting in the formation of a dark brown to black precipitate. This precipitate was dissolved in dichloromethane and filtered through celite to remove the LiCl, followed by two gravity filtrations. The solvent was removed under reduced pressure forming a shiny black solid (**5b**), which was washed with pentane and then dried *in vacuo* (0.70 g, 1.23 mmol, 59.7% yield).

^{1}H NMR (δ ppm $CDCl_3$, 300 MHz): 7.56–6.37 [m, 8H, C_5H_4 and $C_6H_4OCH_3$]; 4.25, 4.23, 4.20 [s, 2H, C_5H_4–$CH(C_6H_4OCH_3)(N(CH_3)_2)$]; 2.99, 2.95, 2.93 [s, 6H, $C_6H_4OCH_3$]; 2.72 [s_b, 6H, $N(CH_3)_2$]. IR absorptions (cm^{-1} KBr): 3429, 2956, 2925, 2852, 1621, 1440, 1465, 1386, 1248, 1119, 1108, 802, 623. Anal. Calc. for $C_{30}H_{30}N_2O_2TiCl_2$: theory: C, 62.62; H, 6.47; N, 4.86; Cl, 12.32; found: C, 62.27; H, 6.47; N, 5.0; Cl, 12.28. UV–vis (CH_2Cl_2): λ 243 nm (ε 18,932), λ 393 nm (ε 1070) λ 404 nm (ε 1120), λ 500 nm (ε 210), λ_{max} 527 nm (weak).

2.2.3. Bis-[(4-benzo[1,3]dioxole)(N,N-dimethylamino)-methylcyclopentadienyl] titanium (IV) dichloride, {η^5-C_5H_4–$CH[N(CH_3)_2][C_6H_3$–O–CH_2–$O]$}$_2TiCl_2$ (5c)

To a Schlenk flask with 0.60 ml (4.98 mmol) 4-bromo-1,3-benzodioxole (**1c**), 20 ml of THF were added until a transparent solution was formed, while stirring. The solution was cooled down to −78 °C for 15 min and 3.22 ml (5.47 mmol) of *tert*-butyl lithium were added. The solution was warmed up to 0 °C for 20 min, resulting in the formation of the yellow lithium intermediate (**2c**).

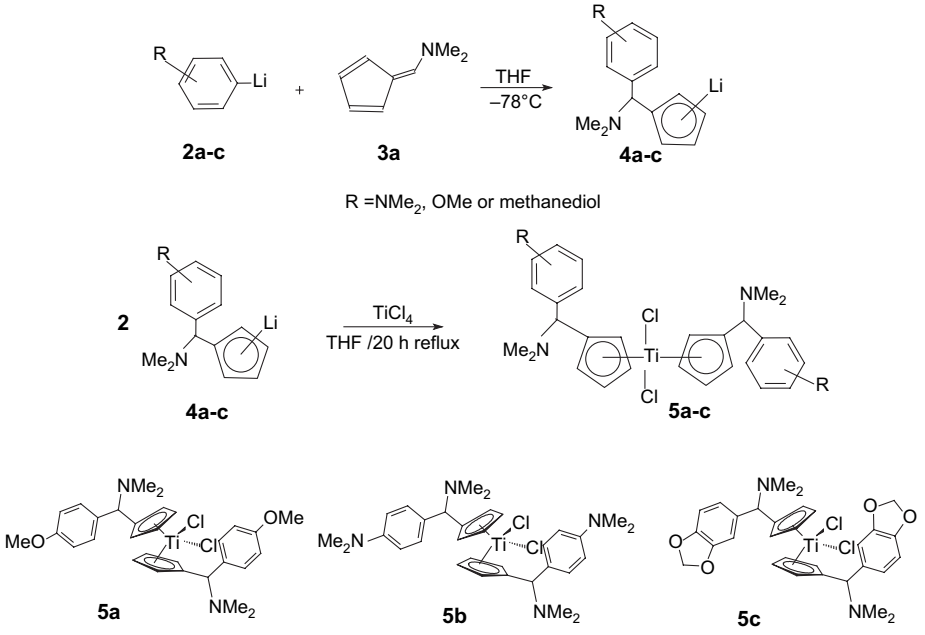
In a second Schlenk flask 0.60 g (4.98 mmol) of 6-*N*,*N*-dimethylamino fulvene (**3a**) were dissolved in 25 ml of THF and the resultant yellow solution was added *via cannula* at −78 °C to the Schlenk flask containing the lithiated intermediate (**4c**). The reaction mixture was allowed to warm up to room temperature and left stirring for 60 min. Afterwards 2.50 ml (2.49 mmol) titanium tetrachloride were added at room temperature and the mixture was refluxed for 20 h. The solvent was removed under vacuum, resulting in a dark brown precipitate. This precipitate was dissolved in dichloromethane and filtered through celite to remove the LiCl, followed by four gravity filtrations. The solvent was removed under reduced pressure forming a shiny black solid (**5c**), which was washed with 50 ml of pentane and then dried *in vacuo* (1.21 g, 2.00 mmol, 80.3% yield).

^{1}H NMR (δ ppm $CDCl_3$, 400 MHz): 7.20–6.25 (m, 14H, C_5H_4 and C_6H_3); 6.05–5.90 (m, 4H, OCH_2O); 4.90–4.95 (m, 2H, $C_5H_4CHC_6H_3$); 2.85–2.65 [m, 12H, $N(CH_3)_2$]. ^{13}C NMR (δ ppm $CDCl_3$, 125 MHz): 147, 146, 131, 122, 120, 116, 115, 105, 103 [C_5H_4 and C_6H_3]; 91, 91, 90 [OCH_2O]; 45, 44, 42 [C_5H_4–CH–$C_6H_3OCH_2O$]; 42, 40, 41 [$N(CH_3)_2$]. IR absorptions (cm^{-1} KBr): 3066 (s), 2994 (m), 2642 (d), 2445 (s), 1619 (s), 1500 (s), 1488 (d), 1446 (s), 1220 (s), 1101 (s), 1036 (s), 811 (s), 723 (s), 623 (s). Anal. Calc. for $C_{30}H_{32}Cl_2N_2O_4Ti$: theory: C, 59.72; H, 5.35; N, 4.64; Cl, 11.75; found: C, 59.67; H, 5.33; N, 4.64; Cl, 11.70. UV–vis (CH_2Cl_2): λ 220 nm (ε 16,596), λ 285 nm (ε 13,759), λ 342 nm (ε 8511), λ_{max} 527 nm (ε 922). MS (ES−): 638.83 ($M + Cl^-$).

3. Results and discussion

3.1. Synthesis

6-*N*,*N*-Dimethylamino fulvene (**3a**) was synthesised according to the already published procedure [33], and its structure is shown in Scheme 1.



Scheme 1. Syntheses and structures of titanocenes **5a–c**.

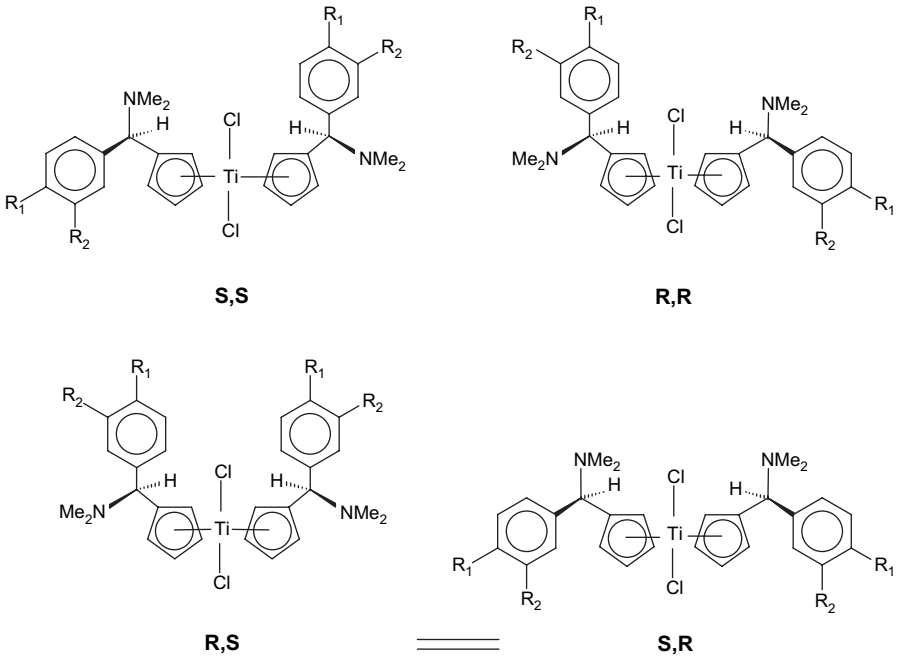


Fig. 1. Expected isomers for titanocenes **5a–c** (note that *S*,*R* = *R*,*S*).

The use of aryl lithium in the synthesis of other metallocenes is well known [31–36], and it has recently been used for the synthesis of achiral titanocene dichlorides [21]. This time, the carbolithiation method led to the synthesis of a new group of titanocenes that contain stereo centres (**5a–c**).

The first step of the reaction consists of the formation of the functionalised lithium intermediates (**2a–c**) by reacting the corresponding benzyl bromides (**1a–c**) with *tert*-butyl lithium. Side reactions were avoided by cooling the reaction down to −78 °C during the addition of *tert*-butyl lithium, and subsequent warming up to 0 °C.

This step was followed by a nucleophilic addition of the lithiated intermediate to the double bond of 6-*N*,*N*-dimethylamino fulvene at −78 °C. Then, the reaction mixture was allowed to warm up to 0 °C, resulting in the formation of the appropriately substituted lithium cyclopentadienyl intermediates **4a–c**. This reaction occurs with no stereo selectivity, and the intermediates **4a–c** already contain a stereo centre.

After stirring the reaction mixture for 40 min, two molar equivalents of **4a**, **4b** or **4c**, underwent a transmetallation reaction when reacted with $TiCl_4$ under reflux for 20 h in THF, to give titanocenes **5a–c**.

The compounds obtained are shiny dark red solids. The syntheses of these compounds and their structures are shown in Scheme 1.

All three titanocenes shown in this paper have different isomers as seen in Fig. 1. As a result of this, three different signals should be seen for every proton and carbon in the ^{1}H and ^{13}C NMR spectra. In the case of protons or carbons contained in the *S*,*S* and *R*,*R* isomers, the NMR shifts will overlap, whereas for protons or carbons corresponding to *R*,*S* (same as *S*,*R*) isomer, two signals can be observed, as the environment of the two cyclopentadienyl rings is different. A ratio of 2:1:1 for *S*,*S* and *R*,*R*, and the two signals for the *S*,*R* (or *R*,*S*) isomers can be observed in the integration pattern.

The absorption bands in the UV–vis spectra show a red shift with respect to the parent compound titanocene dichloride due to the extra aromatic substituents on the cyclopentadienyl rings and the additional dimethylamino groups.

3.2. Cytotoxicity studies

Preliminary *in vitro* cell tests were performed on LLC-PK cells in order to compare the cytotoxicity of the compounds presented in this paper. This cell line was chosen based on their long-lasting growth behaviour, similar to the one shown in carcinoma cells. It was obtained from the ATCC (American

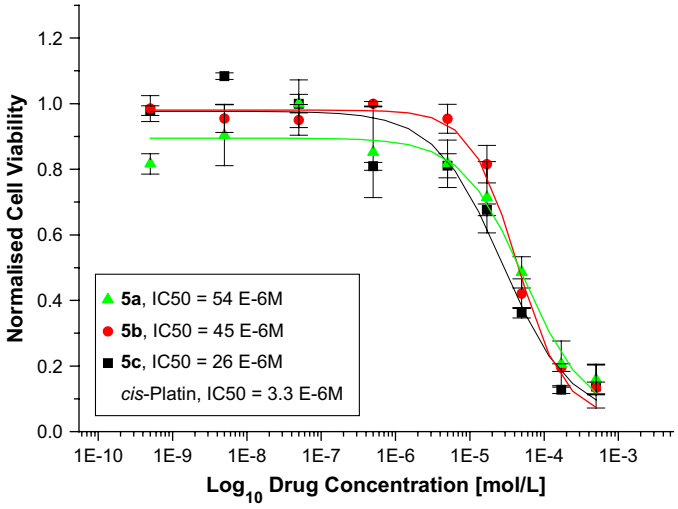


Fig. 2. Cytotoxicity studies of titanocenes **5a–c** against LLC-PK cells.

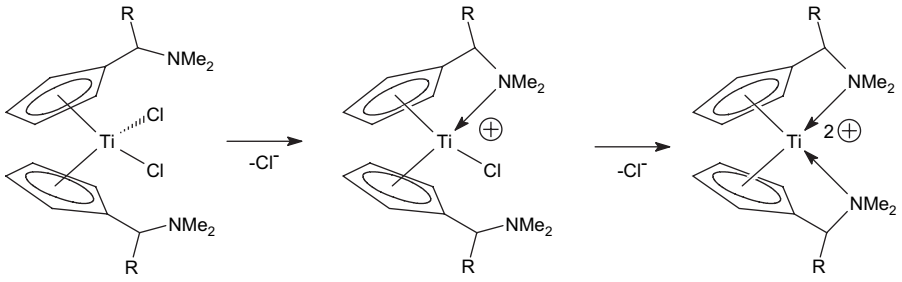


Fig. 3. Intramolecular stabilisation of the monocations or dications of dimethylamino-functionalised titanocenes.

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) 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 200 μl of a solution of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) in the 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. Absorbance was then measured at 540 nm by a Wallac Victor (Multilabel HTS Counter) Plate Reader. 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.

As seen in Fig. 2, titanocenes **5a–c** showed an IC_{50} value of 54 μM, 45 μM and 26 μM, respectively, against LLC-PK. When compared to unsubstituted titanocene dichloride, these titanocenes have approximately a 100-fold decrease in magnitude in terms of the IC_{50} value, and a 10-fold increase in magnitude compared to cis-platin itself (IC_{50} value = 3.3 μM). **5a** and **5b** show a very similar IC_{50} value to their mono-substituted analogues bis-[di-(*p*-*N*,*N*-dimethylaminophenyl)-methylcyclopentadienyl] titanium (IV) dichloride and titanocene **Y**, respectively, whose IC_{50} values are 38 μM and 21 μM [21]. The mono-substituted analogue of **5c** (titanocene **6**) shows a higher IC_{50} value of 280 μM [37]. It is believed that, once passed the cell membrane, a monocation or dication is formed by hydrolysis of one or two of the chlorine groups. At this point, the coordination of the extra $N(CH_3)_2$ donor groups to the titanium centre [31] could stabilise these cationic intermediates and finally increase the number of titanocene–DNA interactions leading to cell death at a lower concentration. The possible intramolecular stabilisation of the monocation of titanocene **5a** is shown in Fig. 3.

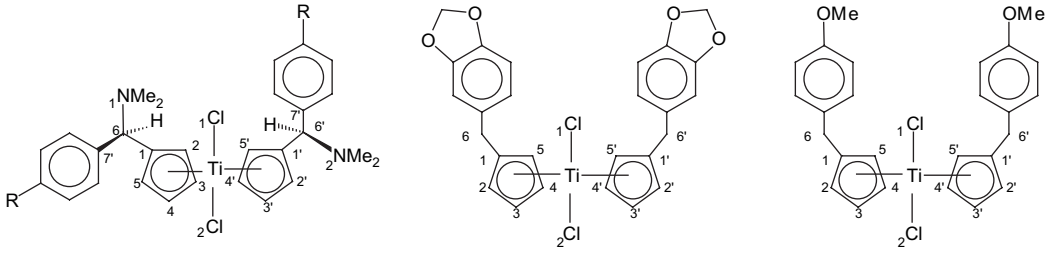
3.3. Structural DFT discussion

Despite our efforts to crystallise these three titanocenes, no crystal structures were obtained. This might be explained by the existence of different isomers in the racemic mixture. In order to overcome this problem, density functional theory (DFT) calculations were carried out for titanocenes **5a** and **5b** at the B3LYP level using the 6-31G** basis set [38].

Selected bond lengths of the optimized structures of these titanocenes are listed in Table 1 (for atom numbering see

Table 1
Selected bond lengths (pm) from the DFT calculated structures **5a** and **5b** and X-ray crystal structures **6** and **7**

	DFT structure [bond length (pm)]		X-ray structure [bond length (pm)]	
	5a	**5b**	**6**	**7**
Ti–C_1	251.7	253.1	238.2	238.5
Ti–C_2	239.4	239.3	233.2	240.9
Ti–C_3	232.0	231.6	236.3	240.3
Ti–C_4	242.6	242.2	240.3	234.4
Ti–C_5	250.0	250.6	242.2	237.2
Ti–$C_{1'}$	249.9	250.3	238.2	242.8
Ti–$C_{2'}$	243.4	241.4	233.2	241.3
Ti–$C_{3'}$	240.9	237.5	236.3	238.6
Ti–$C_{4'}$	236.7	241.2	240.3	233.6
Ti–$C_{5'}$	241.1	242.9	242.2	235.2
C_1–C_2	141.4	141.4	140.4	141.2
C_2–C_3	143.0	142.8	140.6	139.3
C_3–C_4	142.0	142.0	140.3	140.5
C_4–C_5	140.6	140.6	141.7	140.9
C_5–C_1	143.0	142.8	140.9	140.5
$C_{1'}$–$C_{2'}$	143.1	141.4		
$C_{2'}$–$C_{3'}$	141.3	142.6		
$C_{3'}$–$C_{4'}$	141.1	140.9		
$C_{4'}$–$C_{5'}$	142.5	141.5		
$C_{5'}$–$C_{1'}$	141.4	143.1		
C_1–C_6	151.8	151.8	149.2	150.9
$C_{1'}$–$C_{6'}$	153.4	152.0		
C_6–$C_{6'}$	570.3	568.9		
C_6–C_7	152.9	153.0		
$C_{6'}$–$C_{7'}$	153.4	153.5		
C_6–N_1	148.1	148.0		
$C_{6'}$–N_2	148.3	148.2		
Ti–Cl_1	236.0	235.7	238.2	236.8
Ti–Cl_2	235.5	235.4	238.2	236.6



Scheme 2. Numbering scheme of **5a**, **5b**, **6** and **7** for structural discussion of **5a** and **5b**.

Scheme 2). The calculated structure of titanocenes **5a** and **5b** is presented in Fig. 4.

The length of the bond between the metal centre and the cyclopentadienyl carbons is slightly different for the different Cp rings, and between 242.2 and 253.1 pm. The same applies for the carbon–carbon bonds of the cyclopentadienyl rings with bond lengths between 140.6 and 143.0 pm.

The bond length between C_1 and C_6 and $C_{1'}$ and $C_{6'}$ is very similar for both structures, between 151.8 and 153.4 pm, respectively. As well, the length of the bond between the methylic carbon and the nitrogen of the dimethylamino group is almost identical in both cases, and ranges from 148.0 to 148.3 pm. The steric impediment of the aryl groups and dimethylamino groups attached to the methylic carbons causes a lengthening of the bond, in order to relieve the resultant steric strain.

The Cl–Ti–Cl angle was calculated to be 95.0° and 95.1°, respectively. The angle formed between C_1 and $C_{1'}$, the respective methylic carbons, and C_7 or $C_{7'}$, respectively, was of 114.2° in both cases, and almost identical to the one formed between each nitrogen of the dimethylamino group, C_6 or $C_{6'}$, and C_1 and $C_{1'}$, respectively.

The DFT calculated structures of **5a** and **5b** were then compared to the X-ray structure of two different titanium (IV) complexes previously synthesised by our group, {1,2-dicyclopentadienyl-1,2-di(benzo[1,3]dioxole)-ethanediyl} titanium (IV) chloride (**6**) and titanocene **Y** (**7**) [22]. In these complexes, the length of the bond between the titanium centre and the two Cl atoms appeared to be very similar to that of **5a** and **5b**, from 238.2 to 236.8 pm. The same applies to the bond lengths between the Cp carbon atoms and the titanium centre (from 210.5 to 211.7 pm).

The Cl–Ti–Cl angle in all structures, appeared to be very similar, 96° approximately. The same applies to the angle formed between the titanium centre and the centre of the Cp rings, 131.7° for **5a** and **5b**, 130.5° for **6**, and 132.6° for **7**.

Selected bond lengths from the X-ray molecular structures of **6** and **7** are listed in Table 1. For atom numbering see Scheme 2.

4. Conclusions and outlook

The carbolithiation of 6-*N*,*N*-dimethylamino fulvene with lithiated aryl species followed by transmetallation offers a general way for the syntheses of new chiral aryl-substituted and dimethylamino-functionalised metallocenes. The titanocene compounds presented in this paper show high cytotoxicity against LLC-PK indicating their high potential as anticancer agents. It is intended to employ the carbolithiation of

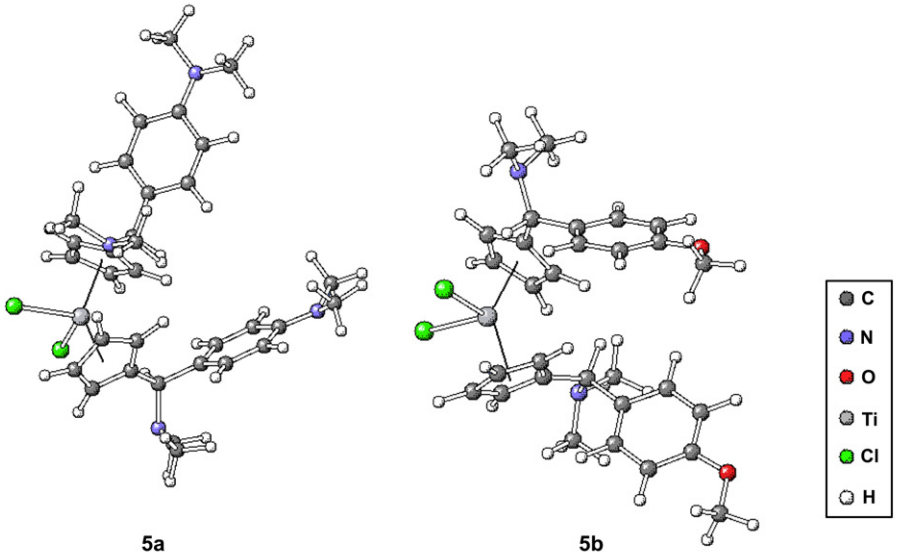


Fig. 4. DFT calculated structure of **5a** and **5b**.

6-*N*,*N*-dimethylamino fulvene for future synthesis of titanocenes with even improved cytotoxicities enabling chemotherapy against renal cell cancer (RCC) in the nearby future.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2007.02.011.

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