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Synthesis and cytotoxicity studies of new dimethylamino-functionalised and heteroaryl-substituted titanocene anti-cancer drugs

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Abstract

From the carbolithiation of *N*,*N*-dimethylamino fulvene (**3a**) and different *ortho*-lithiated heterocycles (furan, thiophene and *N*-methylpyrrole), the corresponding lithium cyclopentadienide intermediate (**4a**–**c**) was formed. These three lithiated intermediates underwent a transmetallation reaction with $TiCl_4$ resulting in dimethylamino-functionalised titanocenes **5a**–**c**. When these titanocenes were tested against LLC-PK cells, the IC_{50} values obtained were of 240, and 28 μM for titanocenes **5a** and **5b**, respectively. The most cytotoxic titanocene **5c** with an IC_{50} value of 5.5 μM is found to be almost as cytotoxic as *cis*-platin, which showed an IC_{50} value of 3.3 μM, when tested on the LLC-PK cell line, and titanocene **5c** is approximately 400 times better than titanocene dichloride itself.

Keywords: Anti-cancer drugs; *cis*-Platin; Titanocene; Fulvene; Dimethylamino-functionalised metallocenes; Heteroaryl-substituted metallocenes; RCC; LLC-PK

1. Introduction

Cancer is a class of diseases or disorders characterised by uncontrolled cell division and the ability of these cells to invade other tissues. In developed countries, this is one of the leading causes of death. Transition metal-based anti-cancer drugs have found widespread use, as the well known *cis*-platin, 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. 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 [1–4]. Other metal based drugs, for example metallocene dichlorides (Cp_2MCl_2) with M = Ti, V, Nb and Mo also show remarkable antitumor activity [5,6]. 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 [7] or metastatic breast cancer [8] 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 from that of these drugs. 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 synthesis of substituted titanocene dichloride anti-cancer drugs. By using a novel method starting from titanium dichloride and fulvenes [9–12] highly substituted *ansa*-titanocenes [13–20], 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 270 μM when tested for cytotoxic effects against the LLC-PK cell line [17].

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One of our best titanocenes, Titanocene **Y**, was obtained through a different synthetic pathway, which was recently published [20]: bis-[(*p*-methoxybenzyl)cyclopentadienyl] titanium(IV) dichloride (Titanocene **Y**), which has an IC_{50} value of 21 μM when tested on the LLC-PK 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 [21]. 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 38 μM when tested for cytotoxic effects on the LLC-PK cell line [22].

Surprisingly, a change in the substitution pattern of titanocenes can also lead to proliferative effects, as seen in the case of some glycol methyl ether and glycol amine substituted titanocenes [23].

The anti-proliferative activity of Titanocene **X** and **Y** has been studied in 36 human tumor cell lines [24] and in four freshly explanted human tumors using Titanocene **X** [25]. 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 [26]. 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 Titanocene **X** and **Y** [27,28].

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 metal centre cation or dication before an interaction with DNA occurs [29–31]. Within this paper we present a series of *N*,*N*-dimethylamino-functionalised titanocenes, their synthesis 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 it 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 disk. UV/Vis spectra were recorded on a Unicam UV4 Spectrometer.

2.2. Synthesis

6-*N*,*N*-Dimethylamino fulvene was synthesised according to the already published procedure [32].

2.2.1. Bis-(*N*,*N*-dimethylamino-2-thiophenylmethylcyclopentadienyl) titanium(IV) dichloride, $\{\eta^5\text{-}C_5H_4\text{–}CH[N(CH_3)_2][C_5H_3S]\}_2TiCl_2$ (**5a**)

To a Schlenk flask with 1.34 g (8.25 mmol) 2-bromothiophene, 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 14.0 ml (8.25 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.

In a second Schlenk flask 1.00 g (8.25 mmol) of 6-*N*,*N*-dimethylamino fulvene were dissolved in 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 (4.1 ml, 4.13 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 green oil that was dissolved in dichloromethane and filtered through celite to remove the LiCl. The black filtrate was filtered additionally twice by gravity filtration. The solvent was removed under reduced pressure forming a shiny black solid, which was washed with 20 ml of pentane and then dried *in vacuo* (1.22 g, 2.31 mmol, 56.0% yield).

^{1}H NMR (δ ppm $CDCl_3$, 300 MHz): 7.04–6.79 [m, 6 H, C_4H_3S]; 6.36–6.30 [m, 8H, C_5H_4]; 5.6, 5.4 [s, 2H, C_5H_4–CH–$(C_4H_3S)(NCH_3)_2$].

^{13}C NMR (δ ppm $CDCl_3$, 125 MHz, proton decoupled): 140, 133, 124, 123, 111, 108, 105 [C_5H_4 and C_4H_3S]; 42, 40 [C_5H_4–CH–$(C_4H_3S)_2$].

IR absorptions (cm^{-1} KBr): 2931, 2832, 1606, 1509, 1461, 1425, 1299, 1174, 1108, 1031, 831.

Anal. Calc. for $C_{24}H_{28}N_4S_2Cl_2Ti$: C, 54.65; H, 5.35; S, 12.16; Cl, 13.44. Found: C, 54.63; H, 5.36; N, 12.15; Cl, 13.46%.

UV–Vis (CH_2Cl_2): λ 230 nm (ε 33440), λ 412 nm (ε 3120), λ 499 nm (ε 216), λ_{max} 520 nm (weak).

2.2.2. Bis-(*N*,*N*-dimethylamino-2-furylmethylcyclopentadienyl) titanium(IV) dichloride, $\{\eta^5\text{-}C_5H_4\text{–}CH[N(CH_3)_2][C_5H_3O]\}_2TiCl_2$ (**5b**)

To a Schlenk flask with 0.5 ml (8.25 mmol) furan, 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 4.8 ml (8.25 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.

In a second Schlenk flask 1.00 g (8.25 mmol) of 6-*N*,*N*-dimethylamino fulvene were dissolved in 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 (4.1 ml, 4.1 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 green oil that was dissolved in dichloromethane and filtered through celite to remove the LiCl. The black filtrate was filtered additionally twice by gravity filtration. The solvent was removed under reduced pressure forming a shiny black solid, which was washed with 20 ml of pentane and then dried *in vacuo* (1.14 g, 2.27 mmol, 56.0% yield).

^{1}H NMR (δ ppm $CDCl_3$, 300 MHz): 6.20–6.98 [m, 6H, C_5H_4–CH–(C_4H_3O)($N(CH_3)_2$)]; 6.36–6.30 [m, 8H, C_5H_4]; 4.92, 4.86 [s, 2H, C_5H_4–C*H*–(C_4H_3O)($N(CH_3)_2$)]; 3.72, 3.75 [s, 6H, $N(CH_3)_2$].

^{13}C NMR (δ ppm $CDCl_3$, 125 MHz, proton decoupled): 135, 129, 127, 126, 120, 119, 116, 110 [C_5H_4 and (C_4H_3O)($N(CH_3)_2$)]; 44, 41 [C_5H_4 – *C*H–(C_4H_3O)($N(CH_3)_2$)]; 35, 32 [$N(CH_3)_2$].

IR absorptions (cm^{-1} KBr): 3412, 2964, 2780, 1608, 1460, 1405, 1259, 1179, 1086, 1023, 828, 614.

Anal. Calc. for $C_{24}H_{28}N_2O_2Cl_2Ti$: C, 58.20; H, 5.70; N, 5.66; Cl, 14.32. Found: C, 58.21; H, 5.68; N, 5.70; Cl, 13.96%.

UV–Vis (CH_2Cl_2): λ 230 nm (ε 22770), λ 402 nm (ε 2020), λ 499 nm (ε 210), λ_{max} 523 nm (weak).

2.2.3. Bis-(*N*,*N*-dimethylamino-2(*N*-methylpyrrolyl)methylcyclopentadienyl) titanium(IV) dichloride, {η^5–C_5H_4–CH[$N(CH_3)_2$][C_5H_3–*N*–CH_3]}$_2TiCl_2$ (**5c**)

To a Schlenk flask with 1.23 g (13.77 mmol) *N*-methylpyrrole, 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 8.1 ml (13.77 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.

In a second Schlenk flask 1.67 g (13.77 mmol) of 6-*N*, *N*-dimethylamino fulvene were dissolved in 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 (6.88 ml, 6.88 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 green oil that was dissolved in dichloromethane and filtered through celite to remove the LiCl. The black filtrate was filtered additionally twice by gravity filtration. The solvent was removed under reduced pressure forming a shiny black solid, which was washed with 20 ml of pentane and then dried *in vacuo* (2.01 g, 3.83 mmol, 55.7% yield).

^{1}H NMR (δ ppm $CDCl_3$, 400 MHz): 6.20, 6.43 [m, 8H, C_5H_4]; 6.30, 6.82 [m, 6H, $CH_3NC_4H_3$]; 4.90, 4.87 [s, 2H, C_5H_4–C*H*($C_4H_3NCH_3$)($N(CH_3)_2$)]; 3.72, 3.75 [s, 12H, $N(CH_3)_2$]; 3.49, 3.56 [s, 6H, CH_3NCCHCHCH].

^{13}C NMR (δ ppm $CDCl_3$, 125 MHz, proton decoupled): 135, 129, 127, 126, 120, 119, 116, 110 [C_5H_4 and ($C_4H_3NCH_3$)($N(CH_3)_2$)]; 44 [C_5H_4–*C*H–(C_8H_5N–CH_3)-($N(CH_3)_2$)]; 35 [($C_4H_3NCH_3$)($N(CH_3)_2$)]; 30 [$C_4H_3NCH_3$].

IR absorptions (cm^{-1} KBr): 3412, 2964, 2780, 1608, 1460, 1405, 1259, 1179, 1086, 1023, 828, 614.

Anal. Calc. for $C_{26}H_{34}N_4Cl_2Ti$: C, 59.89; H, 6.57; N, 10.74; Cl, 13.59. Found: C, 59.63; H, 6.50; N, 10.64, Cl, 12.92%.

UV–Vis (CH_2Cl_2): λ 230 nm (ε 22770), λ 402 nm (ε 2020), λ 499 nm (ε 210), λ_{max} 523 nm (weak).

3. Results and discussion

3.1. Synthesis

6-*N*,*N*-Dimethylamino fulvene (**3a**) was synthesised according to the already published procedure [32], and its structure is shown in Scheme 1.

The use of aryl lithium in the synthesis of other metallocenes is well known [31–35], and it has recently been used for the synthesis of achiral titanocene dichlorides [20]. This time, the carbolithiation method led to the synthesis of a new group of titanocenes that contain stereo centres (**5a**–**c**).

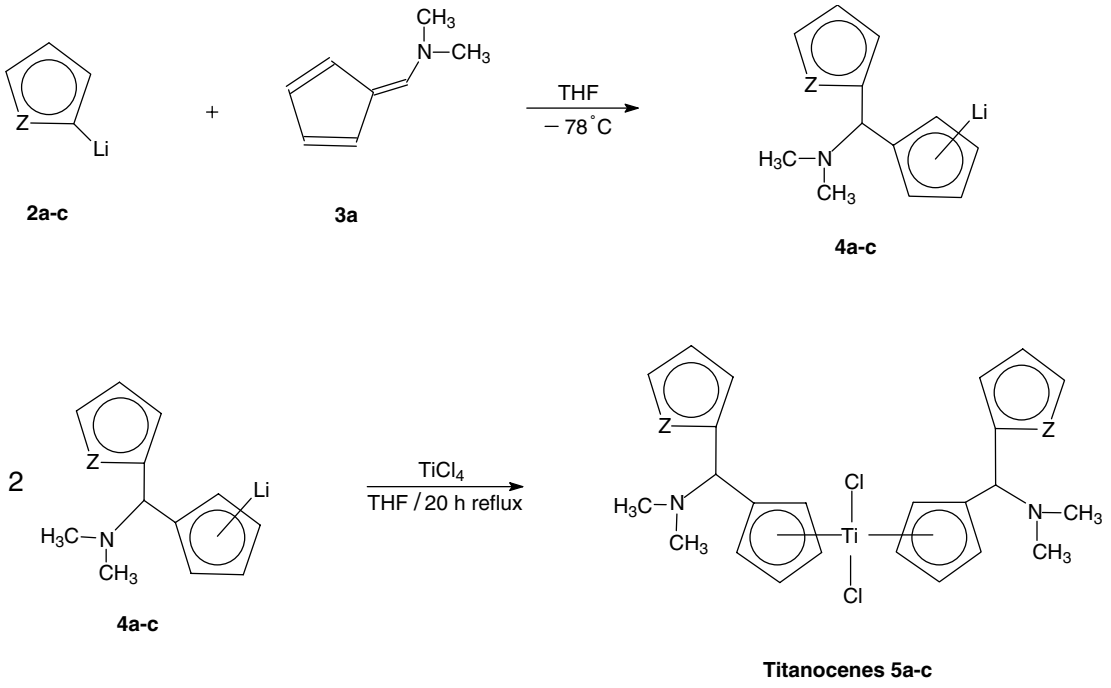
The first step of the reaction consists on the formation of the functionalised lithium intermediates (**2a**–**c**) by reacting the corresponding heterocycles (**1a**–**c**) with *tert*-butyl lithium. Side reactions were avoided by cooling the reaction down to −78 °C during the addition of *tert*-butyl 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 stereogenic carbon.

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 over 20 h in THF, to give titanocenes **5a**–**c**.

The compounds obtained are shiny dark red solids. The synthesis of these compounds is 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. The R,R and S,S isomers are enantiomers and thus give identical NMR spectra, whereas for protons or carbons corresponding to R,S (same as S,R)



Scheme 1. Synthesis of titanocenes **5a**–**c** (Z = S, O, N–CH_3).

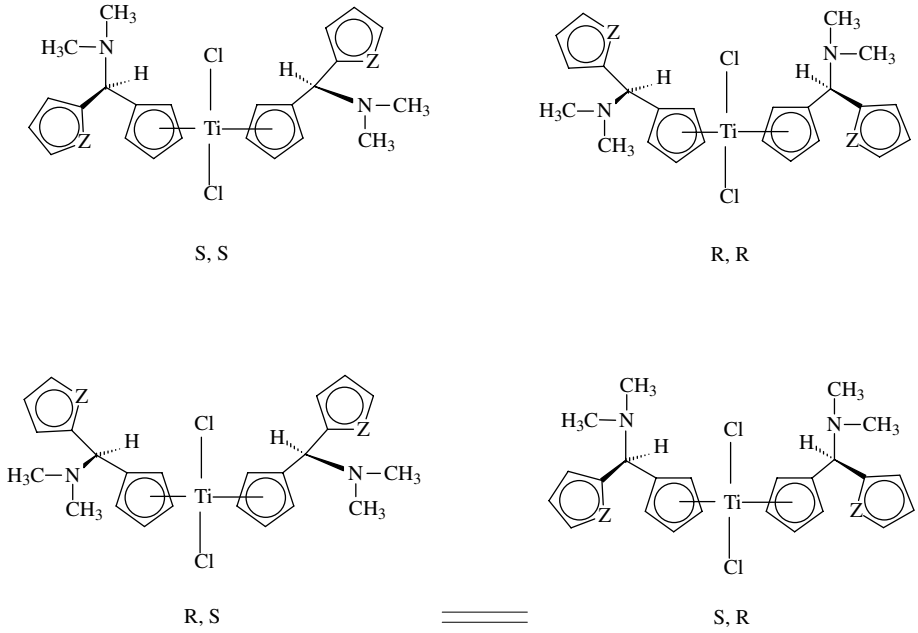


Fig. 1. Expected isomers for titanocenes **5a**–**c** (note that in this case R,S = S,R).

isomer, two signals can be observed, as the environment of the two cyclopentadienyl rings is different. A relation 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.

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 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) 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. 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 of Fig. 2 represent the values obtained from four consistent MTT-based assays for each compound tested.

As seen in Fig. 2, titanocenes **5a** and **5b** showed an IC_{50} value of 240 μM and 28 μM, respectively, whereas **5c** with an IC_{50} value of 5.5 μM against LLC-PK is the most promising candidate in this paper and the most cytotoxic titanocene tested against LLC-PK so far. When compared to unsubstituted titanocene dichloride, titanocene **5c** has a 400-fold decrease in magnitude in terms of the IC_{50} value, and it is very similar to *cis*-platin itself (IC_{50} value = 3.3μM). The increase in cytotoxicity is likely to be due to the extra *N*,*N*-dimethylamino groups, as the mono-substituted *N*-methylpyrrolyl analogue of **5c** shows a higher IC_{50} value of 91 μM [22]. It is believed that, once passed the cell membrane, a mono- or dication is formed by hydrolysis of one or two of the chlorine groups. At this point, the coordination of the extra NMe_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 **5c** is shown in Fig. 3.

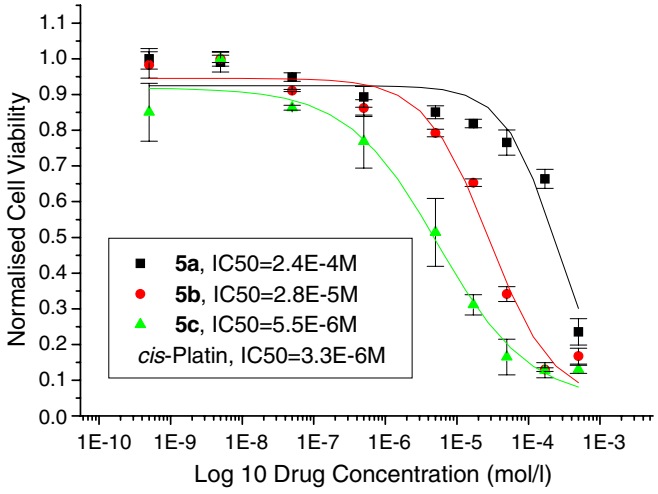


Fig. 2. Cytotoxicity studies of titanocenes **5a**–**c** against LLC-PK cells.

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 titanocene **5c** at the B3LYP level using the 6-31G** basis set [36].

Selected bond lengths of the optimised structure of this titanocene are listed in Table 1 (for atom numbering see Scheme 2). The calculated structure of (S,S)-titanocene **5c** 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 (250.4 and 247.8 pm, respectively). The same applies for the carbon–carbon bonds of the cyclopentadienyl rings with bond lengths between 140.2 and 143.2 pm.

The bond length between the methylic carbon centre and the carbon centre of the Cp group is of 152.2 and 152.0 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 of 148.3 and 148.4 pm, respectively. The steric impediment of the aryl groups and dimethylamino groups attached to

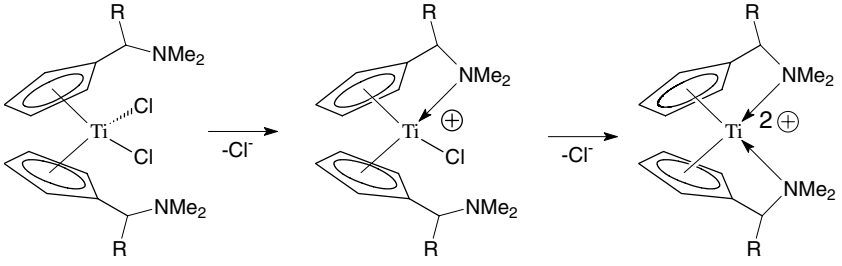


Fig. 3. Intramolecular stabilisation of the mono- or dications of dimethylamino-functionalised titanocenes.

the methylic carbons causes a lengthening of the bond, in order to relieve the resultant steric strain.

The bond length between both methylic carbons is too large to suggest any bridge formation, 559.5 pm.

The Cl–Ti–Cl angle was calculated to be 95.1°. The angle formed between C_1 and $C_{1'}$, the respective methylic carbons (C_6 or $C_{6'}$), 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.

Table 1
Selected bond lengths from the DFT- calculated structure of **5c** and X-ray crystal structure of **6**

	DFT structure (**5c**)	X-ray structure (**6**)
	Bond length (pm)	Bond length (pm)
Ti–C_1	250.4	252.8
Ti–C_2	242.8	243.1
Ti–C_3	240.0	232.6
Ti–C_4	237.4	234.2
Ti–C_5	242.9	243.6
Ti–$C_{1'}$	247.8	249.3
Ti–$C_{2'}$	239.0	239.3
Ti–$C_{3'}$	233.1	232.7
Ti–$C_{4'}$	243.7	239.4
Ti–$C_{5'}$	249.3	244.6
C_1–C_2	143.2	
C_2–C_3	141.5	
C_3–C_4	141.3	
C_4–C_5	142.3	
C_5–C_1	141.4	
$C_{1'}$–$C_{2'}$	141.4	
$C_{2'}$–$C_{3'}$	142.4	
$C_{3'}$–$C_{4'}$	142.2	
$C_{4'}$–$C_{5'}$	140.2	
$C_{5'}$–$C_{1'}$	143.0	
C_1–C_6	152.2	
$C_{1'}$–$C_{6'}$	152.0	
C_6–$C_{6'}$	559.5	
C_6–C_7	152.0	
$C_{6'}$–$C_{7'}$	151.5	
C_6–N_1	148.3	149.8
$C_{6'}$–N_2	148.4	149.6
Ti–Cl_1	234.9	235.7
Ti–Cl_2	236.1	237.2

The DFT calculated structure of **5c** was then compared to the X-ray structure of a titanium(IV) complex found in the literature $(Me_2N–CMe_2–C_5H_4)_2TiCl_2$ (**6**) [31]. In this complex, the length of the bond between the titanium centre and the two Cl atoms appeared to differ in only 1 pm approximately from the one found for **5c**, and of 235.7 and 237.2 pm, respectively. The same applies to the bond length between the N_1 or N_2 and C_6 or $C_{6'}$ respectively, and to the length of the bond between the Cp carbon atoms and the titanium centre.

The Cl–Ti–Cl angle in **6**, is very similar to the one calculated for **5c**, and of 94.9°, and so is the angle formed between the titanium centre and the centre of the Cp rings (with a difference of 0.3°).

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

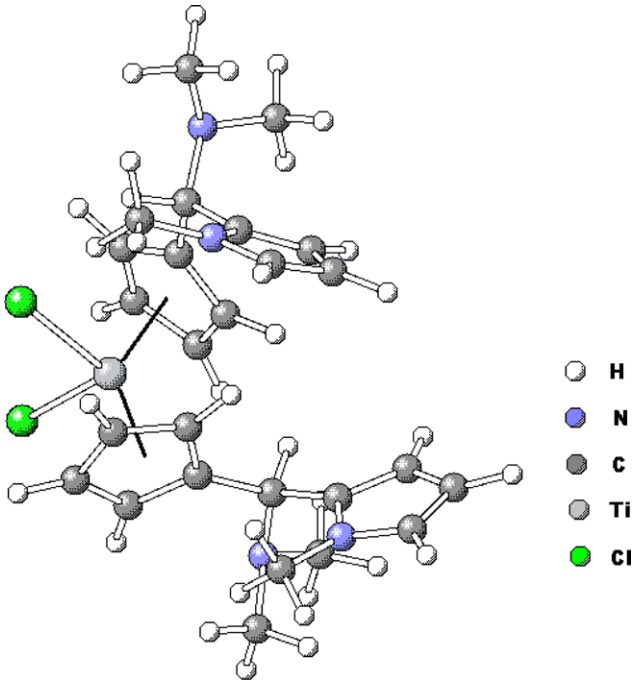
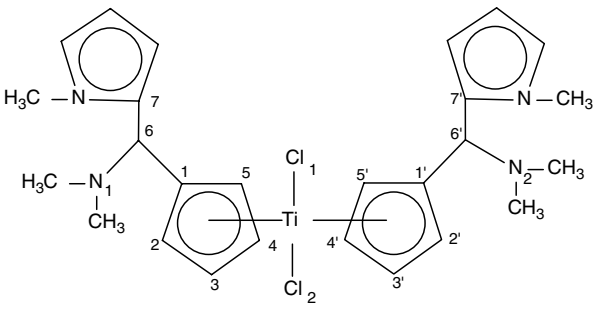
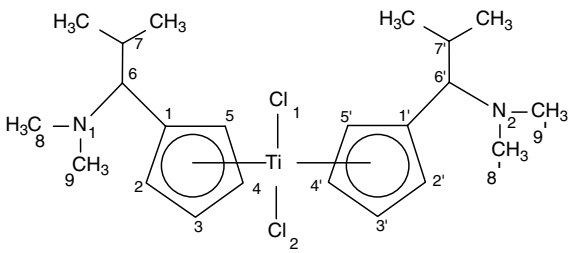


Fig. 4. DFT calculated structure of (S,S)-isomer of **5c**.



5c



6

Scheme 2. Numbering scheme of **5c** and **6** for the structural DFT discussion of **5c**.

4. Conclusions and outlook

The carbolithiation of 6-*N*,*N*-dimethylamino fulvene with lithiated heteroaryl species followed by transmetallation offers a general way into the synthesis of new chiral heteroaryl-substituted and dimethylamino-functionalised metallocenes. The most promising compound **5c** (Titanocene **C**) shows the highest cytotoxicity for a titanocene against LLC-PK indicating its high potential as an anticancer drug. It is intended to employ the carbolithiation of 6-*N*,*N*-dimethylamino fulvene for future synthesis of titanocenes with even improved cytotoxicities enabling the first chemotherapy against renal cell cancer (RCC) in the nearby future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2007.01.045.

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