Synthesis and Cytotoxicity Studies of New Morpholino-Functionalised and *N*-Heteroaryl-Substituted Titanocene Anticancer Drugs

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Abstract: From the carbolithiation of 6-morpholino fulvene (**3**) and different lithiated nitrogen containing heterocycles (2-*N*-methylimidazolyl, 2-*N*-(*N,N*-dimethylamino)methyl-imidazolyl, and 2-*N*-methylindolyl), the corresponding lithium cyclopentadienide intermediate (**4a-c**) was formed. These three lithiated intermediates underwent a transmetallation reaction with $TiCl_4$ resulting in morpholino-functionalised titanocenes **5a-c**. When these titanocenes were tested against LLC-PK cells, the IC_{50} values obtained were of 24, 36, and 41 μM respectively. The most cytotoxic titanocene in this paper (**5a**) with an IC_{50} value of 24 μM is found to be almost ten times less cytotoxic than *cis*-platin, which showed an IC_{50} value of 3.3 μM when tested on the epithelial pig kidney LLC-PK cell line, and approximately 2 times less cytotoxic than its dimethylamino-functionalised analogue. Encouragingly however, the IC_{50} value obtained for titanocene **5a** is approximately 100 times better than titanocene dichloride itself.

Key Words: Anti-cancer drugs, *cis*-platin, titanocene, fulvene, morpholino-functionalised metallocenes, nitrogen containing heteroaryl-substituted metallocenes, RCC, LLC-PK.

1. INTRODUCTION

Titanium-based reagents have significant potential against solid tumors. Budotitane ([*cis*-diethoxybis(1-phenylbutane-1,3-dionato)titanium (IV)]) looked very promising during its preclinical evaluation, but did not go beyond Phase I clinical trials, although a Cremophor EL® based formulation was found for this rapidly hydrolysing molecule [1]. Much more robust in this aspect of hydrolysis is titanocene dichloride (Cp_2TiCl_2), which shows medium anti-proliferative activity *in vitro* but promising results *in vivo* [2,3]. Titanocene dichloride reached clinical trials, but the efficacy of Cp_2TiCl_2 in Phase II clinical trials in patients with metastatic renal cell carcinoma [4] or metastatic breast cancer [5] was too low to be pursued.

More recently, novel methods starting from fulvenes [6-17] and other precursors [18-20] allow direct access to highly substituted titanocenes *via* reductive dimerisation, carbolithiation or hydridolithiation of the fulvene followed by transmetallation in the last two cases.

Titanocene **Y** (bis-[(*p*-methoxybenzyl)cyclopentadienyl] titanium dichloride), was obtained through hydridolithiation of fulvenes with Superhydride ($LiBEt_3H$), which has been published recently [12]: 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. The anti-proliferative activity of Titanocene **Y** has been studied in 36 human tumor cell lines [21] and in explanted human tumors [22]. These *in vitro* and *ex vivo* experiments showed that prostate, cervix and renal cell cancer are prime targets for these novel classes of titanocenes, whereas the IC_{50} values for the breast cancer cell lines were very promising as well. These results were underlined by first mechanistic studies concerning the effect of these titanocenes on apoptosis and the apoptotic pathway in prostate cancer cells [23]. Furthermore first animal studies have been published recently reporting the successful treatment of xenografted Ehrlich's ascites tumor in mice with an ansa-titanocene [24] and xenografted Caki-1 tumors with Titanocene **Y** [25]. The effect of Titanocene **Y** against xenograft Caki-1 tumors in mice was shown to be superior to cisplatin.

So far our most cytotoxic titanocene, Titanocene **C** (bis-(*N*,*N*-dimethylamino-2-(*N*-methylpyrrolyl)methylcyclopentadienyl) titanium (IV) dichloride, was obtained through carbolithiation of fulvenes which has been published recently [26]. It has an IC_{50} value of 5.5 μM when tested on the LLC-PK cell line. This was significant progress, since Cp_2TiCl_2 exhibits an IC_{50} value of only 2000 μM against LLC-PK [8], which explains partly the failed Phase II clinical trials against renal cell carcinoma.

The main idea behind the research presented in this paper was to evaluate the cytotoxic effects of morpholino-functionalised titanocenes with respect to Titanocene **C** and its analogues, as these groups provide a restriction in the possible coordination of the dimethylamino groups with the titanium centre together with an increase in the water-solubility of the molecule. Furthermore, it is believed that suitable modification of the cyclopentadienyl rings may help overcome problems associated with the stability of titanocene compounds under biological conditions, as kinetic and mechanistic studies of the hydrolysis and uptake of titanocene dichloride and its derivatives under physiological conditions give evidence towards the rapid hydrolysis of the chloride ligands and less rapid hydrolysis of the cyclopentadienyl rings at physiologi-

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cal pH [27]. Within this paper we present a new series of *N*-heteroaryl-substituted and morpholino-functionalised titanocenes, their synthesis and preliminary cytotoxicity studies.

2.EXPERIMENTAL SECTION

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 400 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, while CHN analysis was done with an Exeter Analytical CE-440 Elemental Analyser.

2.2. Synthesis

6-morpholino fulvene (**3**) was synthesised according to the already published procedure in 52% yield [28], and its structure is shown in Scheme **1**. The synthesis of *N*(*N,N*-dimethylamino)methylimidazole was based on the typical Mannich reaction, following the conditions found in the literature [29], and was produced in 51% yield.

The use of aryl lithium in the synthesis of other metallocenes is well known [30-34], and it has recently been used for the synthesis of achiral titanocene dichlorides [13, 14]. 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-morpholino 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.

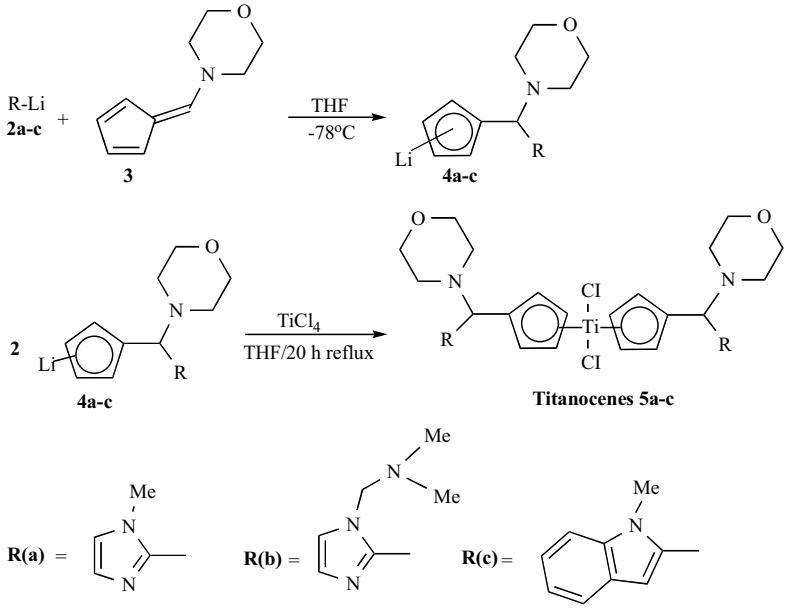
After stirring the reaction mixture for 40 min, two molar equivalents of **4a**, **4b** or **4c**, underwent a transmetallation reaction when reacted with $TiCl_4$ under reflux over 20 h in THF, to give titanocenes **5a-c**.

The compounds obtained are shiny dark brown solids. The synthesis of these compounds is shown in Scheme **1**.

*Bis-[morpholino-2-(*N*-methyl)imidazolylmethylcyclopentadienyl] Titanium (IV) Dichloride, {η^5-C_5H_4-CH[(C_4H_8NO)][$C_3H_2N_2$-CH_3]}$_2TiCl_2$ (5a)*

To a Schlenk flask with 0.49 ml (6.1 mmol) *N*-methylimidazole, 20 ml of THF were added until a transparent solution was formed, while stirring at room temperature. The solution was cooled down to –78ºC and 3.6 ml (6.1 mmol) of *tert*-butyl lithium were added. The solution was allowed to warm up to 0ºC for 20 min, resulting in the formation of the yellow lithium intermediate.

In a second Schlenk flask 1.00 g (6.1 mmol) of 6-morpholinofulvene were dissolved in THF, and the resultant orange solution was added *via cannula* at –78ºC to the Schlenk flask containing the lithiated intermediate. The reaction mixture was then allowed to warm up to 0ºC and left stirring for 40 min. Titanium tetrachloride (3.0 ml, 3.0 mmol) was added afterwards *in situ* at room temperature and



Scheme 1. Synthesis of Titanocenes **5a-c**.

the mixture was refluxed for 20 h. The solvent was subsequently removed under vacuum, resulting in the formation of a dark brown precipitate that was dissolved in dichloromethane and filtered through celite to remove the LiCl. The black filtrate was filtered twice more by gravity filtration. The solvent was removed under reduced pressure forming a dark brown solid, which was washed with pentane and then dried *in vacuo* (0.80 g, 1.3 mmol, 43.9% yield).

^{1}H NMR (δ ppm $CDCl_3$, 300 MHz): 7.02 [s_b, 4H, $CH_3N_2C_3H_2$]; 6.55-6.31 [m, 8H, C_5H_4]; 4.06, 4.04, 4.02 [s, 2H, C_5H_4-C*H*($CH_3N_2C_3H_2$)]; 3.84-3.61 [m, 22H, C_5H_4-CH(N(C*H*$_2$)$_4$O)($CH_3N_2C_3H_2$) and C*H*$_3N_2C_3H_2$].

^{13}C NMR (δ ppm $CDCl_3$, 125 MHz, proton decoupled): 147, 127, 126, 120, 117, 116, 114 [*C*$_5H_4$ and (*C*$_3H_2N_2CH_3$)]; 66, 64 [C_5H_4-CH[N(*C*H$_2$)$_4$O][$C_3H_2N_2CH_3$]]; 50 [C_5H_4-*C*H[N(CH$_2$)$_4$O][$C_3H_2N_2CH_3$]]; 44 [($C_3H_2N_2$*C*H$_3$].

IR absorptions (cm^{-1} KBr): 3405, 3063, 2965, 2849, 1614, 1445, 1387, 1350, 1242, 1112, 1006, 743.

Anal. Calc. for $C_{28}H_{36}O_2N_6Cl_2Ti$: C, 55.37; H, 5.97; N, 13.84; Cl, 11.67. Found: C, 54.72; H, 7.86; N, 13.32; Cl, 11.55.

UV-Vis (CH_2Cl_2): λ 240 nm (ε 11800), λ 280 nm (ε 9000), λ 300 nm (ε 11000), λ 358 nm (ε 10600), λ 386 nm (ε 8600), λ_{max} 400 nm (6200).

*Bis-[(morpholino-2-*N*-(*N,N*-dimethylamino)methylimidazolyl) Methyl Cyclopentadienyl] Titanium (IV) Dichloride, {η^5-C_5H_4-CH[(C_4H_8NO)][$C_3H_2N_2$-CH_2-N(CH_3)$_2$]}$_2$TiCl$_2$ (5b)*

To a Schlenk flask with 0.61 g (4.9 mmol) *N*-(*N,N*-dimethylamino)methylimidazole, 20 ml of THF were added until a transparent solution was formed, while stirring at room temperature. The solution was cooled down to –78ºC and 2.9 ml (4.9 mmol) of *tert*-butyl lithium were added. The solution was allowed to warm up to 0ºC for 20 min, resulting in the formation of the yellow lithium intermediate.

In a second Schlenk flask 0.80 g (4.9 mmol) of 6-morpholinofulvene were dissolved in THF, and the resultant orange solution was added *via cannula* at –78ºC to the Schlenk flask containing the lithiated intermediate. The reaction mixture was then allowed to warm up to 0ºC and left stirring for 40 min. Titanium tetrachloride (2.4 ml, 2.4 mmol) was added afterwards *in situ* at room temperature and the mixture was refluxed for 20 h. The solvent was subsequently removed under vacuum, resulting in the formation of a dark brown liquid that was dissolved in dichloromethane and filtered through celite to remove the LiCl. The black filtrate was filtered twice more by gravity filtration. The solvent was removed under reduced pressure forming a shiny black solid, which was washed with pentane and then dried *in vacuo* (0.63 g, 0.91 mmol, 37.9% yield).

^{1}H NMR (δ ppm $CDCl_3$, 300 MHz): 7.67 – 7.00 [s_b, 4H, $C_3H_2N_2$-CH_2-N(CH_3)$_2$]; 6.96 – 6.32 [m, 8H, C_5H_4]; 4.51 [s_b, 4H, C_5H_4-CH(N(CH$_2$)$_4$O)($C_3H_2N_2$-C*H*$_2$-N(CH_3)$_2$)], 4.10 [s_b, 2H, C_5H_4-C*H*(N(CH$_2$)$_4$O)($C_3H_2N_2$-CH_2-N(CH_3)$_2$)] 3.79 - 3.42 [m, 16H, C_5H_4-CH(N(C*H*$_2$)$_4$O)(C_8H_6N-CH_3]; 3.62 [m, 12H, $C_3H_2N_2$-CH_2-N(C*H*$_3$)$_2$].

^{13}C NMR (δ ppm $CDCl_3$, 125 MHz, proton decoupled): 147, 137, 132, 127, 126, 124, 122,120 [*C*$_5H_4$ and *C*$_3H_2N_2$-CH_2-N(CH_3)$_2$]; 66, 64 [C_5H_4-CH(N(*C*H$_2$)$_4$O)($C_3H_2N_2$-CH_2-N(CH_3)$_2$)]; 62 [$C_3H_2N_2$-*C*H$_2$-N(CH_3)$_2$]; 50 [C_5H_4-CH(N(*C*H$_2$)$_4$O)($C_3H_2N_2$-CH_2-N(CH_3)$_2$)]; 43 [$C_3H_2N_2$-CH_2-N(*C*H$_3$)$_2$].

IR absorptions (cm^{-1} KBr): 3410, 2958, 2763, 1619, 1567, 1467, 1371, 1091, 797.

Anal. Calc. for $C_{32}H_{46}N_8O_2Cl_2Ti$: C, 55.41; H, 6.69; N, 16.16; Cl, 10.22 Found: C, 55.40; H, 6.63; N, 16.55; Cl, 9.98.

UV-Vis (CH_2Cl_2): λ 230 nm (ε 13245), λ 330 nm (ε 14569), λ 360 nm (ε 10331), λ_{max} 510 nm (weak).

*Bis[morpholino-2-(*N*-methylindolyl) Methyl Cyclopentadienyl] Titanium (IV) Dichloride, {η^5-C_5H_4-CH[C_4H_8NO][C_8H_6N-CH_3]}$_2$TiCl$_2$ (5c)*

To a Schlenk flask with 0.64 mL (4.9 mmol) *N*-methylindole, 20 ml of THF were added until a transparent solution was formed, while stirring at room temperature. The solution was cooled down to –78ºC and 2.9 ml (4.9 mmol) of *tert*-butyl lithium were added. The solution was allowed to warm up to 0ºC for 20 min, resulting in the formation of the yellow lithium intermediate.

In a second Schlenk flask 0.80 g (4.9 mmol) of 6-morpholino fulvene were dissolved in THF, and the resultant orange solution was added *via cannula* at –78ºC to the Schlenk flask containing the lithiated intermediate. The reaction mixture was then allowed to warm up to 0ºC and left stirring for 40 min. Titanium tetrachloride (2.45 ml, 2.45 mmol) was added afterwards *in situ* at room temperature and the mixture was refluxed for 20 h. The solvent was subsequently removed under vacuum, resulting in the formation of a dark brown precipitate 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 pentane and then dried *in vacuo* (1.00 g, 1.42 mmol, 57.9% yield).

^{1}H NMR (δ ppm $CDCl_3$, 300 MHz): 7.65-6.32 [m, 20H, C_5H_4 and C_8H_6N-CH_3]; 4.37 [s_b, 2H, C_5H_4-C*H*(N(CH$_2$)$_4$O)(C_8H_6N-CH_3)]; 3.98-3.22 [m, 16H, C_5H_4-CH(N(C*H*$_2$)$_4$O)(C_8H_6N-CH_3]; 3.80[s_b, 6H, C_5H_4-CH(N(CH$_2$)$_4$O)(C_8H_6N-C*H*$_3$].

^{13}C NMR (δ ppm $CDCl_3$, 125 MHz, proton decoupled): 145, 139, 136, 135, 131, 128, 125, 121, 120, 109, 107 [*C*$_5H_4$ and (*C*$_8H_6$N-CH_3)]; 64, 44 [C_5H_4-CH[N(*C*H$_2$)$_4$O][C_8H_6N-CH_3]]; 50 [C_5H_4-*C*H[N(CH$_2$)$_4$O][C_8H_6N-CH_3]]; 30 [(C_8H_6N-*C*H$_3$].

IR absorptions (cm^{-1} KBr): 3366, 2924, 2711, 2448, 1610, 1465, 1345, 1318, 1234, 1152, 1105, 1069, 1047, 909, 872, 734.

Anal. Calc. for $C_{38}H_4O_2N_4Cl_2Ti$: Theory: C, 64.70; H, 6.00; N, 7.94, Cl, 10.05 Found: C, 64.89; H, 5.95; N, 7.90; Cl, 10.08.

UV-Vis (CH_2Cl_2): λ 279 nm (ε 10600), λ 320 nm (ε 11600), λ 354 nm (ε 10000), λ 386 (ε 9200), λ 400 nm (ε 7600), λ_{max} 430 nm (6600).

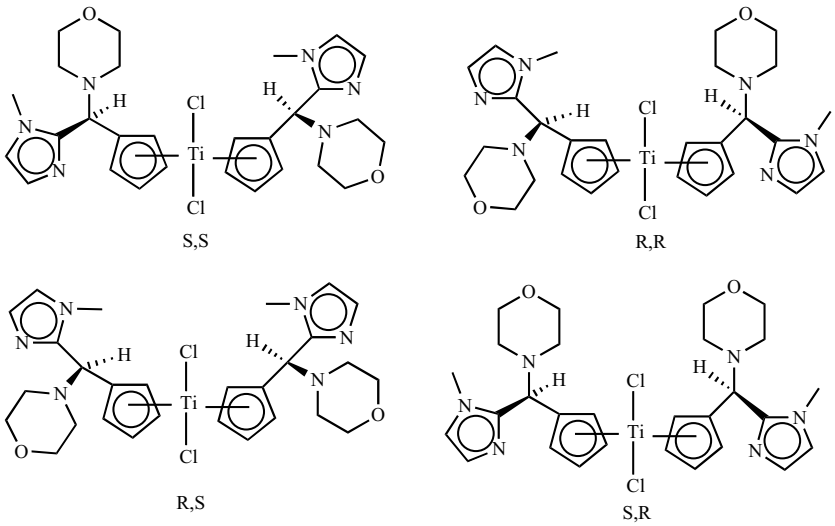


Fig. (1). Expected isomers for titanocene **5a** (note that in this case R,S=S,R).

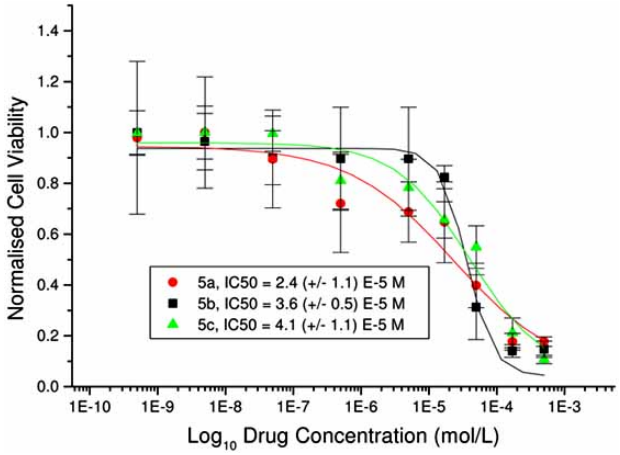


Fig. (2). Cytotoxicity studies of titanocenes **5a-c** against LLC-PK cells.

2.3. MTT-Based Assay

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. The cytotoxic activities of titanocenes **5a-c** were determined using an MTT-based assay [35]. Specifically, cells were seeded in 96-well plates containing 200 µl microtitre wells at a density of 5,000-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 x 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-diphenyl-tetrazolium 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.

3. RESULTS AND DISCUSSION

3.1. Spectroscopic Data

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

Supplementary Information

DFT Calculated Structure and Energy of **5a** using B3LYP/6-31G**

E=-3320.524310 Hartrees

Center Number	Atomic Number	Atomic Type	Coordinates(Angstroms)		
			X	Y	Z
1	22	0	0.103425	-1.025290	0.919543
2	1	0	0.897854	-1.005822	-2.065311
3	6	0	0.027942	-1.157763	-1.450647
4	6	0	-0.916685	-0.149896	-1.075310
5	1	0	0.031146	-3.362458	-1.087749
6	6	0	-1.948699	-0.764600	-0.317784
7	6	0	-1.641247	-2.168585	-0.240739
8	1	0	-2.196713	-2.907226	0.312829
9	6	0	-0.458101	-2.410313	-0.977183
10	17	0	1.137448	-3.119471	1.438339
11	17	0	-1.459990	-1.277890	2.712871
12	6	0	1.996685	0.398176	0.438871
13	6	0	2.355048	-0.363640	1.586180
14	1	0	0.369522	1.936425	0.183651
15	1	0	3.152109	-1.083260	1.639862
16	6	0	1.438141	-0.063564	2.635071
17	1	0	1.408752	-0.532926	3.604857
18	6	0	0.536891	0.938443	2.155914
19	1	0	-0.287654	1.370691	2.693636
20	6	0	0.864585	1.209480	0.803171
21	1	0	-0.873213	0.899729	-1.297145
22	6	0	-3.242693	-0.155966	0.180595
23	1	0	-3.497495	-0.631032	1.135472
24	6	0	2.810059	0.631931	-0.824635
25	1	0	2.121602	0.782196	-1.673598
26	1	0	5.493537	0.331230	0.417997
27	6	0	3.480433	1.975492	-0.638194
28	7	0	3.004732	3.107748	-1.164720
29	6	0	3.849091	4.124775	-0.700231
30	6	0	4.826046	3.592133	0.104718
31	7	0	4.583241	2.219669	0.168547
32	1	0	3.705891	5.151903	-0.984672
33	1	0	5.651063	4.039029	0.630913

(Suppl. Info. Contd….)

Center Number	Atomic Number	Atomic Type	Coordinates(Angstroms)		
			X	Y	Z
34	6	0	-3.173425	1.332597	0.365154
35	7	0	-2.057876	2.074951	0.315835
36	6	0	-2.436712	3.392896	0.623528
37	6	0	-3.794736	3.440182	0.802730
38	7	0	-4.275876	2.139052	0.631617
39	1	0	-1.723124	4.196343	0.664451
40	1	0	-4.457537	4.258853	1.020274
41	6	0	-5.717230	1.769829	0.730306
42	1	0	-6.017438	1.075601	-0.060401
43	1	0	-6.284093	2.697500	0.632255
44	1	0	-5.924930	1.329343	1.711668
45	6	0	5.365246	1.258439	0.978147
46	1	0	4.844205	1.049330	1.917430
47	1	0	6.336297	1.709664	1.194757
48	7	0	3.834354	-0.399545	-1.061009
49	6	0	3.475484	-1.826954	-1.098528
50	6	0	4.732013	-2.594219	-0.590908
51	8	0	5.949429	-1.789925	-0.771748
52	6	0	5.921654	-1.173770	-2.097847
53	6	0	4.779523	-0.110468	-2.163742
54	1	0	3.205726	-2.151925	-2.120916
55	1	0	4.822916	-3.555665	-1.114481
56	1	0	5.781929	-1.938502	-2.875341
57	1	0	4.285451	-0.158100	-3.152160
58	1	0	6.898331	-0.706132	-2.234573
59	1	0	5.182558	0.894024	-2.028754
60	1	0	4.665029	-2.770364	0.484286
61	1	0	2.635852	-2.034746	-0.440308
62	7	0	-4.393439	-0.391109	-0.697954
63	6	0	-5.039506	-1.707352	-0.677692
64	6	0	-6.557009	-1.456580	-0.940796
65	8	0	-6.785801	-0.157647	-1.602177
66	6	0	-5.792176	0.058606	-2.659478
67	6	0	-4.385085	0.248575	-2.031572
68	1	0	-4.922212	-2.150364	0.316322
69	1	0	-7.093001	-1.390239	0.008621

(Suppl. Info. Contd….)

Center Number	Atomic Number	Atomic Type	Coordinates(Angstroms)		
			X	Y	Z
70	1	0	-6.107080	0.959935	-3.189449
71	1	0	-4.213545	1.318226	-1.875661
72	1	0	-3.611039	-0.145978	-2.709562
73	1	0	-5.800794	-0.782720	-3.364864
74	1	0	-4.598141	-2.399250	-1.412215
75	1	0	-6.982105	-2.263890	-1.552050

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 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

As seen in Fig. **2**, titanocenes **5a**, **5b**, and **5c** show an IC_{50} value of 24 μM, 36 μM, and 41 μM, respectively. When compared to unsubstituted titanocene dichloride, titanocene **5a** has a 100-fold decrease in magnitude in terms of the IC_{50} value, and, approximately, a 10-fold increase in magnitude with respect to *cis*-platin itself (IC_{50} value = 3.3 μM). Titanocene **5a** also shows a two-fold decrease in cytotoxicity with respect to its *N*,*N*-dimethylamino-substituted counterpart (an analogue of Titanocene **C**) [36]. The increased polarity of the new titanocene, brought on by the morpholino group, together with an increase in size might explain the decrease in cytotoxicity. This is consistent for titanocene **5b**, as the dimethylamino-functionalised analogue showed a higher IC_{50} value of 5.4 μM [37]. But, there is no general trend: when comparing titanocene **5c** with its dimethylamino-functionalised analogue, with an IC_{50} value of 71 μM [38], titanocene **5c** is almost twice as cytotoxic (having an IC_{50} value of 41 μM).

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 **5a** at the B3LYP level using the 6-31G** basis set [39].

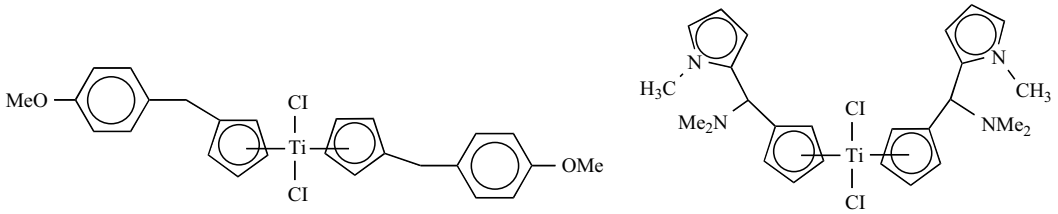
Selected bond lengths of the optimized structure of this titanocene are listed in Table **1** (for atom numbering see Scheme **2**). The calculated structure of (S,S)-titanocene **5a** is presented in Fig. **4**.

The length of the bond between the metal centre and the cyclopentadienyl carbons is similar for the different Cp rings (241.0 and 241.7 pm respectively), whereas the carbon–carbon bonds of the two cyclopentadienyl rings are slightly different, with bond lengths between 141.4 and 144.0 pm.

The bond length between the methylic carbon centre and the carbon centre of the Cp group is of 151.4 and 152.1 pm respectively. As well, the length of the bond between the methylic carbon and the nitrogen of the morpholino group is similar in both cases, 146.7 and 147.3 pm respectively. The steric impediment of the aryl groups and morpholino 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 91.6º. The angle formed between C_1 (or $C_{1'}$), the corresponding methylic carbon C_6 (or $C_{6'}$), and C_7 (or $C_{7'}$), was of 113.5º and 105.7º, respectively. The angles formed between each nitrogen of the morpholino group, C_6 or $C_{6'}$, and C_1 or $C_{1'}$ measured 114.1º and 113.4º, respectively.

The DFT calculated structure of **5a** was then compared to the calculated structure of our most cytotoxic titanocene, Titanocene **C** [26]. In this complex, the length of the bond between the titanium centre and the two Cl atoms (234.9 and 236.1 pm) appeared to differ in only 3 pm approximately from the one found for **5a**. The same applies to the bond length between the N_1 or N_2 and C_6 or $C_{6'}$ respectively, and



Titanocene **Y** Titanocene **C**

Fig. (3). Structure of Titanocenes **Y** and **C**.

Table 1. Selected Bond Lengths from the DFT- Calculated Structure of 5a and Titanocene C

	DFT Structure (5a)	DFT Structure (Titanocene C)
	Bond Length (pm)	Bond Length (pm)
Ti-C_1	241.0	250.4
Ti-C_2	238.7	242.8
Ti-C_3	241.5	240.0
Ti-C_4	237.5	237.4
Ti-C_5	240.5	242.9
Ti-$C_{1'}$	241.7	247.8
Ti-$C_{2'}$	244.0	239.0
Ti-$C_{3'}$	237.7	233.1
Ti-$C_{4'}$	236.1	243.7
Ti-$C_{5'}$	236.4	249.3
C_1-C_2	143.9	143.2
C_2-C_3	141.4	141.5
C_3-C_4	142.4	141.3
C_4-C_5	141.3	142.3
C_5-C_1	142.0	141.4
$C_{1'}$-$C_{2'}$	142.3	141.4
$C_{2'}$-$C_{3'}$	142.5	142.4
$C_{3'}$-$C_{4'}$	143.0	142.2
$C_{4'}$-$C_{5'}$	141.8	140.2
$C_{5'}$-$C_{1'}$	144.0	143.0
C_1-C_6	151.4	152.2
$C_{1'}$-$C_{6'}$	152.1	152.0
C_6-$C_{6'}$	618.6	152.0
C_6-C_7	150.2	151.5
$C_{6'}$-$C_{7'}$	151.3	148.3
C_6-N_1	146.7	148.4
$C_{6'}$-N_2	147.3	234.9
Ti-Cl_1	239.2	236.1
Ti-Cl_2	239.2	237.2

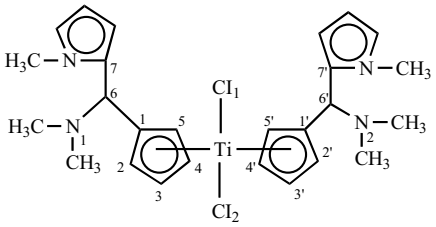
to the length of the bond between the Cp carbon atoms and the titanium centre.

The Cl–Ti–Cl angle in Titanocene **C** (95.1º) is similar to the one calculated for **5a**, and so is the angle formed between the titanium centre and the centre of the Cp rings (within 1º).

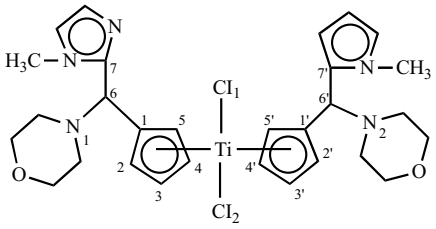
Selected bond lengths from the calculated DFT structure of Titanocene **C** are listed in Table **1**. For atom numbering see Scheme **2**.

4. CONCLUSIONS AND OUTLOOK

The carbolithiation of 6-morpholino fulvene with lithiated *N*-heteroaryl species followed by transmetallation offers a general way into the synthesis of new chiral *N*-heteroaryl-substituted and morpholino-functionalised metallocenes. The cytotoxicity, however, is not maintained consistently with respect to the dimethylamino-functionalised analogues of this new class of titanocene dichlorides. For this reason, fu-



Titanocene **C**



5a

Scheme 2. Numbering scheme of Titanocene **C** and **5a** for the structural DFT discussion of **5a**.

ture work will focus on the carbolithiation of different fulvenes for future synthesis of titanocenes with even improved cytotoxicities enabling the first chemotherapy against metastatic or advanced renal cell cancer (RCC) in the nearby future.

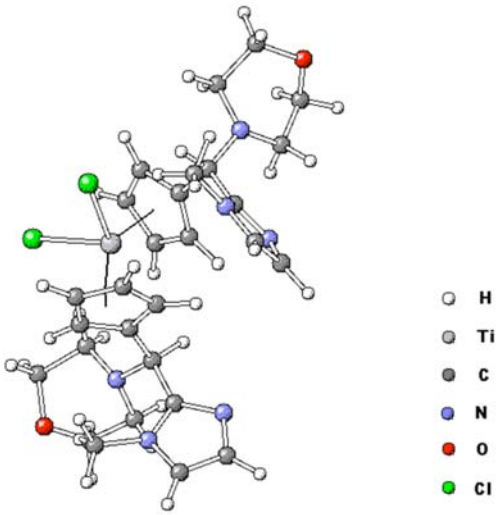


Fig. (4). DFT calculated structure of (S,S)-isomer of **5a**.

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