New titanocene derivatives with high antiproliferative activity against breast cancer cells

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Abstract

The synthesis and characterization of some new titanocene-complexes, having an ethenyl-phenoxide or a benzyl group as substituents of the cyclopentadienyl rings, are reported. The synthesized compounds have been evaluated for their cytotoxic potential against two human breast cancer cell lines, that is: MCF7 and SkBr3. Most of these compounds have shown significant cytotoxic effects, compared to cisplatin, in MTT-based cell tests.

Keywords:
Metal-based anticancer drugs
Titanocene compounds
Breast cancer cells

The remarkable antitumor activity shown by cisplatin and other platinum complexes has meant that new metal-based anticancer drugs have become a noteworthy subject of research. Among all synthesized compounds, a great deal of research has been focused on titanocene-based complexes, whose cytotoxic activity against solid tumors is well known.

Budotitane, [cis-diethoxybis(1-phenyl-1,3-butanedionato)-titanium(IV)], proved to be very promising in the preclinical evaluation phase, but did not pass phase I clinical trials, due to its rate of hydrolysis.7

Titanocene dichloride (Cp2TiCl2), (Fig. 1a), is much more resistent in this respect, shows moderate antiproliferative activity in vitro, but promising results in vivo,8,9 reaching phase II clinical trials. Unfortunately, its efficiency in patients with metastatic renal cell carcinoma or metastatic breast cancer was too low to be pursued.10,11 As proposed by Sadler and co-workers, titanocene dichloride is able to interact with DNA, binding to phosphate groups instead of binding to nucleotides and nitrogen bases, as does cisplatin.12 Moreover, the titanium(IV) ion, by binding to specific iron(III), forms a strongly-bound complex with transferrin, a protein of human plasma, and thus it is supplied to tumor cells as this complex.13

A lot of analogues of titanocene containing aromatic groups linked to the Cp have been synthesized.13 One of the most interesting of this series, is bis-[[(p-methoxybenzyl)cyclopentadienyl]-titanium-dichloride (titanocene Y), shown in Figure 1a. Its antiproliferative activity was studied in 36 human tumor cell lines14 and in explanted human tumors.15–17 In vitro and ex vivo experiments show that prostate, cervix and kidney tumors are a major target for this novel class of titanocene. Furthermore, titanocene Y has been tested on MCF-7 breast cancer cells, revealing a promising medium–high cytotoxic activity with IC50 values of 76 μM.16

The oxalate complex obtained from titanocene Y by a simple anion exchange reaction was reported to have 13-fold increased activity in relation to titanocene Y during in vitro studies against the LLC-PK pig kidney cells.14

Recently, some of us have reported the synthesis and cytotoxic activity of some titanocene complexes. We have also verified, by substituting chlorine atoms, the influence of other leaving ligands on the activity of the complexes.18 Some of the synthesized compounds showed a good cytotoxicity, in particular, the complexes bis-[methoxy-ethenyl-cyclopentadienyl]-titanium-dichloride (T2) and [methoxy-ethenyl-cyclopentadienyl]-titanium trichloride (T1) (Fig. 1a) gave IC50 values very similar to cisplatin on MCF-7, comparable to the ones reported for titanocene Y.19,20

Complex T1 has been the first example of half-titanocene complex with interesting cytotoxic activity. Therefore, lately, we have synthesized a series of novel half-titanocene complexes and tested them for their regulatory effects on MCF-7 and SkBr3 breast cancer cells.19 Some of these compounds elicited relevant repressive effects on both cell lines if compared to cisplatin. These data provide
evidence that the presence of coordinating groups on cyclopentadienyl ring (substituted aryl or even the phenyl itself) are important for the biological effectiveness of these compounds.\textsuperscript{19}

In order to understand if different ether groups can influence the intramolecular coordination and consequently the stability of the titanocene complexes and whether the ether group is essential for their cytotoxic activity, we synthesized several new-titanocene complexes (Fig. 1b), having as ligands the 2-cyclopentadienyl-ethoxy-benzene [Cp-CH\textsubscript{2}-CH\textsubscript{2}-O-Ph] or the cyclopentadienyl-benzyl [Cp-CH\textsubscript{2}-Ph], and we evaluated their potential antiproliferative effects on MCF7 and SkBr3 breast cancer cells.

In particular, the aim of this paper is to report the synthesis and the characterization by nuclear magnetic resonance (NMR), mass spectroscopy and elemental analysis of bis-[2(cyclopentadienyl-ethoxy-benzene)]-titanium-dichloride (1) and of the homologous complexes obtained by the substitution of chlorides with glycinate (2) and oxalate (3) and of bis-cyclopentadienyl-benzyl-titanium-dichloride (4). In addition we have also synthesized the
(1,2-diphenyl-1,2-dicyclopentadienyl-ethane)-titanium-dichloride (5) in order to have a complex very similar to 4, but with a chelating ligand, which would prevent the intramolecular coordination of phenyl groups to metal, and consequently produce a less stable complex than 4 itself. In Figure 1b all the synthesized compounds are reported.

Complex 1 was synthesized to test the effect of phenoxy group, instead of the methoxy one present in T2, on the effectiveness of this titanocene, considering that this ligand may stabilize the titanium cation either with oxygen atom or with phenyl ring which can be easily verified.

Complexes 2 and 3 were synthesized so as to have different leaving groups on titanocene, compared to chloride, and this could improve the activity of the complexes, by means of favorable pharmacokinetic properties.

Complex 4 was synthesized to test if the ether group, which is present on the titanocene Y, on T1 and T2, as well as on complex 1, is necessary to have a significant higher cytotoxic activity or if a more labile ligand, having greater ability of π-donation, can produce a molecule with likewise interesting cytotoxic properties.

All the synthesized complexes were purified following common procedures and isolated in good yield. Elemental analysis (C, H, N) were in agreement with the proposed formulae. 1H COSY experiments also allowed the assignment of all the proton resonances of the 1H NMR spectrum, whereas DEPT experiments were useful for the attribution of 13C NMR signals. The synthesized titanocenes were also characterized by mass spectrometry. The set of these data (see experimental part in references) allowed us to have an unambiguous structural determination, as reported in Supplementary Figure S1.

The synthesis of (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-dichloride [C5H4(2-O-C6H4)2TiCl2 (1)] was carried out in good yields by reaction of the lithium salt of the ligand Li[C5H4-CH2C6H4OPh] with TiCl4.

The synthesis of (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-bis-glycine [C5H4(2-O-C6H4)2Ti(gly)2 (2)] was carried out by the reaction of 1 with two equivalents of glycine in methanol containing 1% of water (Scheme 1).

Instead, the synthesis of (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-oxalate [C5H4(2-O-C6H4)2Ti(C2O4) (3)] was performed by reaction of 1 with silver-oxalate in dry THF (see Scheme 1).

Complex 4, bis-cyclopentadienyl-benzyl-titanium-dichloride [C5H4(2-C6H4OPh)2TiCl2], was prepared according to Scheme 2. The synthesis of the phenylfulvene was carried out as outlined in the literature22 and its lithium salt was obtained by reaction with Super Hydride (LiBEt3H) in dry diethyl ether. Then it was isolated and subsequently reacted with half-equivalent of TiCl4 to THF in dry THF.

Lastly, complex 5, [1,2-di(cyclopentadienyl)-1,2-bis-1,2-di(phenylethane-di-y)-titanium-dichloride], was prepared according to Scheme 3, following a literature method.23

Hydrolytic stability of the titanocene complexes has been determined in aqueous solution, 90% DMSO by 1H NMR spectroscopy (Supplementary data Figs. S1 and S2), in order to possibly correlate the chemical stability of these complexes with their observed cytotoxic activity. Since we can expect that rapid hydrolysis of the leaving group (Cl) and cyclopentadienyl ligands could give way to biologically inactive species, active species could be generated if the Cp rings remain metal bound.

The hydrolysis of aromatic rings was evaluated by the integration of two signals of protons of cyclopentadienyl bound to metal, compared to newly formed multiplet of substituted cyclopentadiene (Table 1).

In order to investigate the effects on cancer cell growth of the compounds synthesized, MCF7 and SKBr3 breast cancer cells were used as model systems.24,25 Cells were treated for 5 days with each compound and were also exposed to cisplatin, in order to compare the anticancer effects of the chemicals used to this well-known chemotherapeutic. In dose-response and time course experiments, most of the tested compounds showed the capability to inhibit, in a dose-dependent manner, the proliferation of both cell lines, just after a 24 h treatment (Supplementary Figs. S3 and S4). Among all tested titanocene derivatives, complexes 1, 2 and 4 elicited strong repressive effects on the proliferation of both cell lines (Table 2 and supplementary data Figs. S3 and S4). In particular, complex 4 showed quite similar patterns of response across the cell lines.

### Table 1

<table>
<thead>
<tr>
<th>Complex</th>
<th>% Cp ring hydrolysis</th>
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<tbody>
<tr>
<td></td>
<td>5 min</td>
</tr>
<tr>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>4</td>
<td>&lt;1</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
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### Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 [µM]</th>
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<tbody>
<tr>
<td></td>
<td>MCF7</td>
</tr>
<tr>
<td>1</td>
<td>10 (±2)</td>
</tr>
<tr>
<td>2</td>
<td>9 (±1)</td>
</tr>
<tr>
<td>3</td>
<td>&gt;100</td>
</tr>
<tr>
<td>4</td>
<td>10 (±3)</td>
</tr>
<tr>
<td>5</td>
<td>27 (±6)</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>29 (±8)</td>
</tr>
</tbody>
</table>

Hydrolysis results of complexes in DMSO/D2O solution at rt followed by 1H NMR.
two cancer cell lines (with IC_{50} values of 10 μM in both cell lines after 24 h treatment) (Table 2), whereas complex 2 exhibited stronger antiproliferative effects in SKBr3 cells with respect to those elicited in MCF7 cells (IC_{50} values of 9 μM in MCF7 and 6 μM in SKBr3 cells) after a one-day-treatment (Table 2). Remarkably, a 24-h treatment with complex 2 (10 μM) was able to fully prevent SKBr3 cell growth (Supplementary data Fig. S4). Finally, it should be pointed out that complexes 1, 2 and 4 inhibited the proliferation of MCF7 cells more strongly compared to cisplatin (Supplementary Fig. S3).

The low activity of complex 5 can be justified considering that the bridge between the two benzoic carbons prevents the coordination of the phenyl ring on the metal centre, making it less stable, and therefore less active.

In conclusion, in this work, we synthesized and characterized three new titanocene-dichloride complexes having a ethynyl-phenoxide or a benzyl group as substituents of the cyclopentadienyl rings. Both of these groups were able to stabilize the cationic species that is supposed to be the pharmacologically active. Moreover, we also evaluated the influence of different leaving groups (glucinate and oxalate) instead of chloride on the effectiveness of bis-[2-cyclopentadienyl-ethoxy-benzene]titanium–complexes.

The synthesized compounds have been evaluated for their cytotoxic potential against two human breast cancer cell lines. Most of these compounds showed significant anti-proliferative effects compared to cisplatin when tested against both MCF7 and SKBr3 cells in MTT assays. In particular, 1, 2 and 4 represent the organometallic complexes showing the highest inhibitory activity on the growth of tested cancer cell lines. Notably, most of the compounds used have demonstrated a stronger anti-proliferative effects on both MCF7 and SKBr3 breast cancer cells than the titanocene derivatives recently synthesized and evaluated on the same model systems. Therefore, as indicated by our results, the presence of a coordinating group, such as phenyl or ether one, is necessary to stabilize the active species and generate a cytotoxic complex. This conclusion is supported by the lower activity of complex 5 (three times less cytotoxic than the unbridged complex 4), in which the bridge between the Cp ligands prevents the coordination of phenyl group on the metal centre.

Supplementary data
Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.11.058.

References and notes

Synthesis of bis-[{benzyl}-cyclopentadieny]-titanium-dichloride [C5TiCl2 (1,2-(C6H5)2C6H4CH2)]; TiCl2 (0.31 g, 0.93 mmol) was added to 20 mL of dry THF. The solution turned immediately from colourless to pale yellow. The mixture was refluxed overnight and then cooled. The solvent was removed under reduced pressure. The remaining residue was extracted with dichloromethane (30 mL) and filtered twice through celite to remove the LiCl. The filtrate was washed twice with hexane (20 mL) and then dried under reduced pressure to give a dark red solid (yield 55%). 1H NMR (300 MHz, THF-d8) δ 4.06 [s, 4H, C6H5-CH2-C6H4], 6.33–6.44 [m, 8H, C6H5-CH2-C6H4], 7.22–7.24 [m, 10H, C6H5-CH2-C6H4], 1.13 [s, 10H, C6H5-CH2-C6H4]. 13C NMR (75 MHz, THF-d8): δ 36.7 [C5H5-CH2-C6H4], 110.9, 111.5, 122.6, 128.0, 128.6, 140.6 [C6H5-CH2-C6H4], Mass (EL, 70 eV, m/z): 246 [L2TiCl3-Li]+, 357 [L2-Ti]+. Anal. Calcd for C24H22Cl2Ti (%): C, 66.41; H, 5.18. Found (%): C, 66.21; H, 5.21.

The solution turned from yellow to red during addition. After this addition, the mixture was allowed to warm up slowly to room temperature. The colour of the solution became finally black. After 20 h, stirring at r.t., a solution of phenyl-fulvene (0.48 mmol, 1 g) in 10 mL of dry toluene was added to the TiCl2 THF solution at r.t. under nitrogen. Then it was stirred under reflux for another 16 hours. 10 mL of the solvent were removed and washed twice with hexane (20 mL) and then dried under reduced pressure to give a dark red solid (yield 49%).

1H NMR (300 MHz, THF-d8) δ 4.06 [s, 4H, C6H5-CH2-C6H4], 6.33–6.44 [m, 8H, C6H5-CH2-C6H4], 7.22–7.24 [m, 10H, C6H5-CH2-C6H4], 1.13 [s, 10H, C6H5-CH2-C6H4]. 13C NMR (75 MHz, THF-d8): δ 36.7 [C5H5-CH2-C6H4], 110.9, 111.5, 122.6, 128.0, 128.6, 140.6 [C6H5-CH2-C6H4], Mass (EL, 70 eV, m/z): 246 [L2TiCl3-Li]+, 357 [L2-Ti]+. Anal. Calcd for C24H22Cl2Ti (%): C, 67.16; H, 5.17. Found (%): C, 67.24; H, 5.13.

Inhibition of cell proliferation: The effects of each compound on cell viability were determined with the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay,26–29 which is based on the conversion of MTT to MTT-formazan by mitochondrial enzyme. Cells were seeded in quadruplicate in 96-well plates in regular growth medium and grown until 70–80% confluence. Cells were washed once they had attached and then treated with increasing concentrations (2–10 μM) of each compound for indicated time (for one day up to 5 days). Relative cell viability was determined by MTT assay according to the manufacturer’s protocol (Sigma-Aldrich, Milan, Italy). Mean absorbance for each drug dose was expressed as a percentage of the control untreated well absorbance and plotted vs drug concentration. IC50 values represent the drug concentrations that reduced the mean absorbance at 570 nm to 50% of those in the untreated control wells.