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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 a 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.

The remarkable antitumor activity shown by *cisplatin* and other platinum complexes[1–6] 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 titanium-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 (Cp_2TiCl_2), (Fig. 1a), is much more resistant 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.[12]

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 lines[14] 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 IC_{50} 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 (**T_2**) and [methoxy-ethenyl-cyclopentadienyl]-titanium trichloride (**T_1**) (Fig. 1a) gave IC_{50} values very similar to *cisplatin* on MCF-7, comparable to the ones reported for titanocene Y.[19,20]

Complex **T_1** 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

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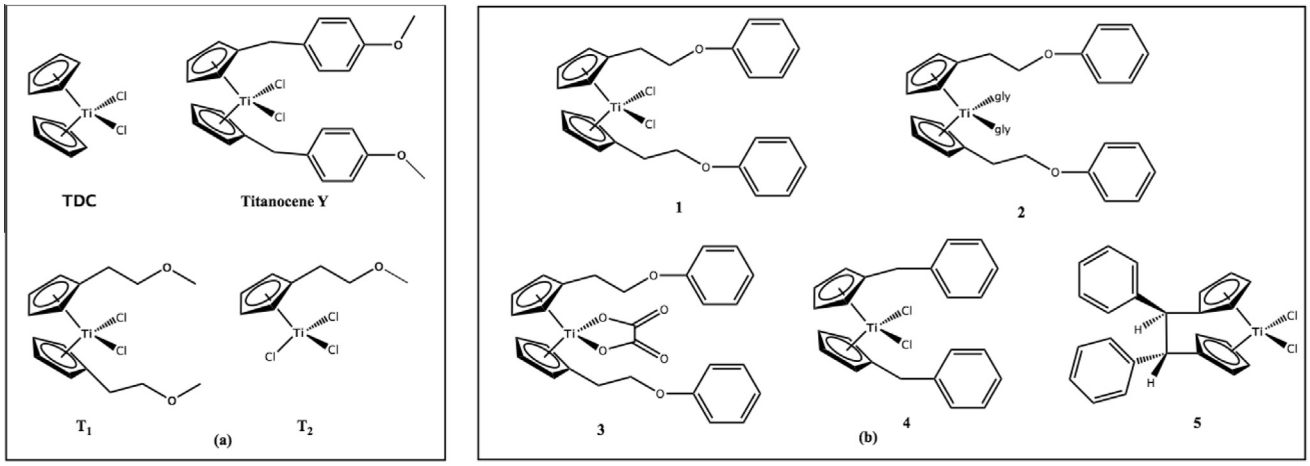
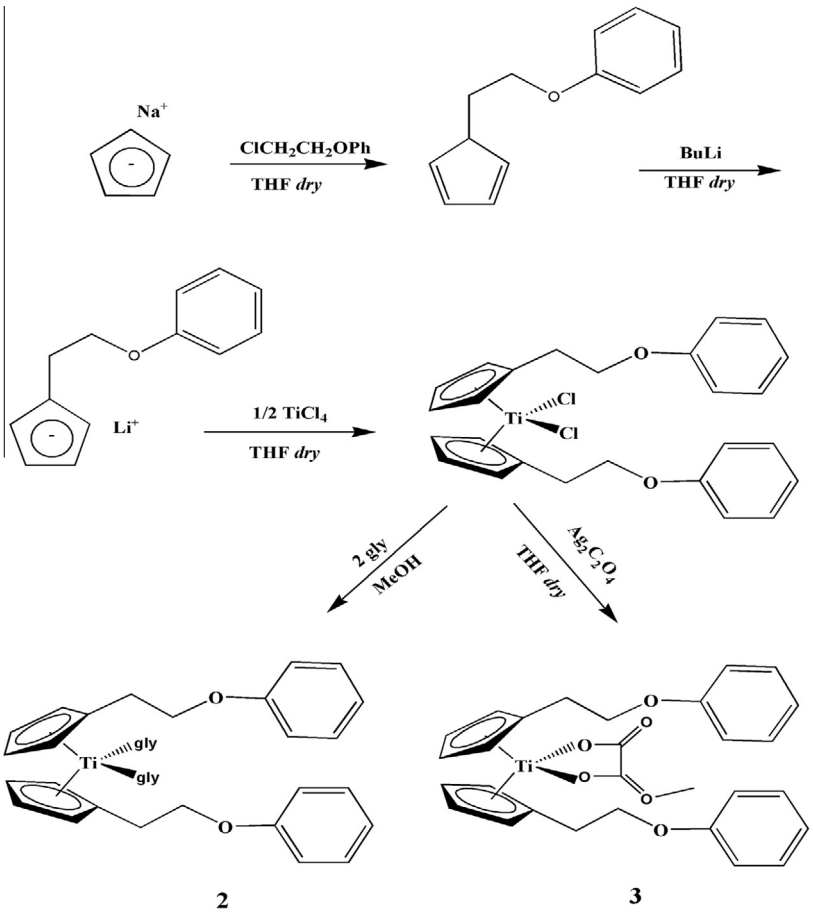


Figure 1. (a) Titanocene dichloride (TDC); bis-[(*p*-methoxybenzyl)cyclopentadienyl]titanium dichloride (titanocene Y); bis-[methoxy-ethenyl-cyclopentadienyl]-titanium-dichloride ($\mathbf{T_2}$) and [methoxy-ethenyl-cyclopentadienyl]-titanium trichloride ($\mathbf{T_1}$); (b) structures of synthesized complexes.

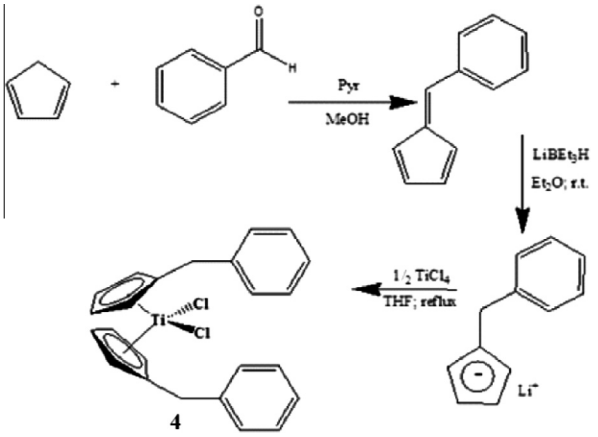
evidence that the presence of coordinating groups on cyclopentadienyl ring (substituted aryl or even the phenyl itself) are important for the biological effectiveness of these compounds.[19]

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_2CH_2-O-Ph] or the cyclopentadienyl-benzyl [Cp-CH_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



Scheme 1. Synthetic route for the preparation of ligands and complexes **1**–**3**.



Scheme 2. Synthetic route for the preparation of complex **4**.

(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 $\mathbf{T_2}$, 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 $\mathbf{T_1}$ and $\mathbf{T_2}$, 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. ^{1}H COSY experiments allowed the assignment of all the proton resonances of the ^{1}H NMR spectrum, whereas DEPT experiments were useful for the attribution of ^{13}C NMR signals. The synthesized titanocenes were also characterized by mass spectrometry. The set of these data (see experimental part in references)[21] allowed us to have an unambiguous structural determination, as reported in Supplementary Figure S1.

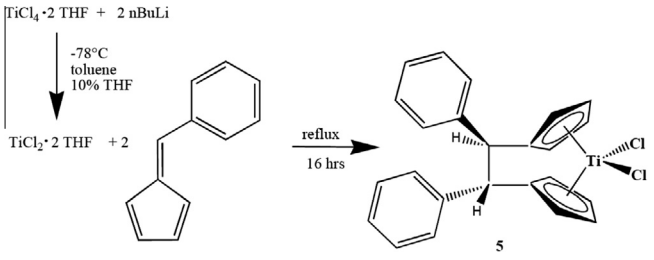
The synthesis of (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-dichloride $[C_5H_4\text{-}CH_2CH_2OPh]_2TiCl_2$ (**1**) was carried out in good yields by reaction of the lithium salt of the ligand $Li[C_5H_4\text{-}CH_2CH_2OPh]$ with $TiCl_4$.

The synthesis of (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-bis-glycine $[C_5H_4\text{-}CH_2CH_2OPh]_2Ti(gly)_2$ (**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 $[C_5H_4CH_2CH_2OPh]_2Ti(C_2O_4)$ (**3**) was performed by reaction of **1** with silver-oxalate in dry THF (see Scheme 1).

Complex **4**, bis-cyclopentadienyl-benzyl-titanium-dichloride $[C_5H_4\text{-}CH_2\text{-}Ph]_2TiCl_2$, was prepared according to Scheme 2. The synthesis of the phenylfulvene was carried out as outlined in the literature[22] and its lithium salt was obtained by reaction with Super Hydride ($LiBEt_3H$) in dry diethyl ether. Then it was isolated and subsequently reacted with half-equivalent of $TiCl_4{\cdot}2THF$ in dry THF.

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



Scheme 3. Synthesis of complex **5**.

Hydrolytic stability of the titanocene complexes has been determined in aqueous solution, 90% DMSO by ^{1}H 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

Table 1
Hydrolysis results of complexes in DMSO/D_2O solution at rt followed by ^{1}H NMR

Complex	% Cp ring hydrolysis			
	5 min	4 h	8 h	24 h
1	<1	<1	<1	<5
2	<1	<1	<1	<5
3	<1	<1	<1	<5
4	<1	<1	13	40
5	25	40	48	60

Table 2
Cytotoxic activity of tested compounds on MCF7 and SkBr3 breast cancer cells after one 24 h treatment, as determined by using the MTT assay. IC_{50} values were calculated by probit analysis ($P < 0.05$, χ^2 test)

Compound	IC_{50} [μM]	
	MCF7	SkBr3
1	10 (±2)	10 (±1)
2	9 (±1)	6 (±2)
3	>100	38 (±5)
4	10 (±3)	10 (±1)
5	27 (±6)	25 (±5)
Cisplatin	29 (±8)	10 (±2)

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 benzilic 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 ethenylphenoxide 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 (glycinate 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.[19] 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.

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21. (a) *Experimental section:* The elemental analyses for C and H were recorded on a ThermoFinnigan Flash EA 1112 series and performed according to standard microanalytical procedures. ^{1}H NMR, homodecoupled ^{1}H NMR, ^{1}H COSY and ^{13}C NMR spectra were recorded at 298 K on a Bruker Avance 300 Spectrometer operating at 300 MHz (^{1}H) and 75 MHz (^{13}C) and referred to internal tetramethylsilane. Molecular weights were determined by ESI mass spectrometry. ESI-MS analysis in positive and negative ion mode, were made using a Finnigan LCQ ion trap instrument, manufactured by Thermo Finnigan (San Jose, CA, USA), equipped with the Excalibur software for processing the data acquired. The sample was dissolved in acetonitrile and injected directly into the electrospray source, using a syringe pump, which maintains constant flow at 5 μl/min. The temperature of the capillary was set at 220 °C. GC analyses were carried out with a GC–MS 7890A/5975C spectrometer (Agilent Technologies) equipped with an OPTIMA 17MS column (diphenylpolysiloxane/dimethylpolysiloxane, 1:1, 30 m, 0.25 mm ID) and a mass-selective detector. All manipulations were carried out under oxygen- and moisture-free atmosphere in an MBraun MB 200 glove-box. All the solvents were thoroughly deoxygenated and dehydrated under argon by refluxing over suitable drying agents, while NMR deuterated solvents (Euriso-Top products) were kept in the dark over molecular sieves. $TiCl_4$, Titanium(IV) chloride tetrahydrofuran complex and all chemicals were obtained from Aldrich chemical Co. and used without further purification. Silver oxalate was prepared by following the reported procedure.[21b]
Synthesis of (2-(cyclopenta-2,4-dienyl)ethoxy)benzene [C_5H_5-$CH_2CH_2OC_6H_5$] (**I**): 5.23 mL of 1-(2-chloroethoxy)benzene (38.45 mmol), previously distilled on CaH_2, were dissolved in 50 mL of dry THF to give a colorless solution. 25 mL of a solution 2 M of sodium cyclopentadienide in THF were added drop-wise at −78 °C. The solution was warmed up to room temperature and left stirred overnight to give a white precipitate and a dark pink solution. Afterwards the mixture was quenched with methanol and cold water. The organic product was extracted by 3x 50 mL ether fraction. The solution was dried over magnesium sulphate and had its solvent removed at reduced pressure to yield a brown oil (yield 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.88 [t, 2H, C_5H_5-($CH_2CH_2OC_6H_5$),], 4.14 [t, 2H, C_5H_5-($CH_2CH_2OC_6H_5$)], 3.01-3.20 [m, 1H, C_5H_5-($CH_2CH_2OC_6H_5$)], 6.17–6.52 [m, 4H, C_5H_5-($CH_2CH_2OC_6H_5$)], 6.92–7.32 [m, 5H, C_5H_5-($CH_2CH_2OC_6H_5$)]. ^{13}C NMR (75 MHz, C_6D_6) δ 30.1 [C_5H_5-(CH_2CH_2OPh)], 67.8 [C_5H_5-(CH_2CH_2OPh)], 44.1 [C_5H_5-(CH_2CH_2OPh)], 114.8, 120.9, 128.1, 129.7, 134.4, 134.9, 159.1 [C_5H_5-(CH_2CH_2O*Ph*)]. Mass (GC–MS): 186.1 [M]$^+$.
Synthesis of (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-dichloride [C_5H_4-$CH_2CH_2OC_6H_5$]$_2$.$TiCl_2$ (**1**): To a solution of neutral ligand (0.45 g, 2.41 mmol) in dry THF (40 mL), a stoichiometic amount of *n*-BuLi (2.5 M solution in hexane, 1.5 mL) was slowly added at 0 °C. The solution was warmed up to room temperature and left stirred overnight, obtaining a yellow lithium intermediate. Afterward the solution was treated at −78 °C with 0.13 mL (1.21 mmol) of $TiCl_4$ and stirred overnight. The solvent was then removed under reduced pressure. The remaining residue was extracted with dichloromethane (30 mL) and filtered through celite to remove the LiCl. The deep red filtrate was washed twice with hexane (20 mL) and then dried under reduced pressure to give a deep red solid (yield 37%). ^{1}H NMR (300 MHz, THF-d_8) δ 3.58 [t, 4H, C_5H_4-($CH_2CH_2OC_6H_5$)], 4.68 [t, 4H, C_5H_4-(CH_2CH_2O C_6H_5)], 6.75–6.85 [m, 8H, C_5H_4-($CH_2CH_2OC_6H_5$),], 6.90–7.10 [m, 10H, C_5H_4-($CH_2CH_2OC_6H_5$)]. ^{13}C NMR (75 MHz, C_6D_6) δ 25.1 [C_5H_4-($CH_2CH_2OC_6H_5$)], 67.5 [C_5H_4-($CH_2CH_2OC_6H_5$)], 114.0, 114.9, 128.9 [C_5H_4-($CH_2CH_2OC_6H_5$)], 115.2, 118.6, 120.2, 157.8, 159.4 [C_5H_4-($CH_2CH_2OC_6H_5$)]. Mass (E.I., 70 eV, *m/z*): 496 [L_2-$TiCl_2$-Li]$^+$. Anal Calcd for $C_{26}H_{26}Cl_2O_2Ti$ (%): C, 63.83; H, 5.36. Found (%): C, 64.02; H 5.32.
Synthesis of (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-bis-glycine [C_5H_4-$CH_2CH_2OC_6H_5$]$_2$Ti(gly)$_2$ (**2**): In a round-bottom flask 50 mg of (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-dichloride (0.16 mmol) was dissolved in 30 mL of methanol (containing 1% of water). To this brown solution 17 mg of glycine (0.23 mmol) was added at room temperature and the mixture was stirred for 4 h. The solvent was removed in vacuum, obtaining a red/orange solid, the yield was quantitative. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.16 [t, 4H, C_5H_4-($CH_2CH_2OC_6H_5$)], 4.11 [t 4H, C_5H_4-($CH_2CH_2OC_6H_5$)], 6.70–7.20 [m, 18H, C_5H_4-($CH_2CH_2OC_6H_5$)]; 3.30 [s, 4H, Ti-OCO-CH_2-NH_2] ^{13}C NMR (75 MHz, THF-d_8) δ 29.7 [C_5H_4-($CH_2CH_2OC_6H_5$)], 68.3 [C_5H_4-($CH_2CH_2OC_6H_5$)], 112.5, 114.3, 120.5, 129.1, 130.0, 159.5, 160.2 [C_5H_4-($CH_2CH_2OC_6H_5$)], 185.6 [Ti-*OCO*-CH_2-NH_2], 47.1 [Ti-OCO-CH_2-NH_2]. Anal. Calcd for $C_{30}H_{34}N_2O_6Ti$ (%): C, 63.61; H 6.05. Found (%): C, 63.45; H 6.09.
Synthesis of (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-oxalate [$C_5H_4CH_2CH_2OC_6H_5$]$_2$Ti(C_2O_4) (**3**): Silver oxalate (0.04 g, 0.12 mmol) and (bis-2-cyclopentadienyl-ethoxy-benzene)-titanium-dichloride (0.04 g, 0.08 mmol) were dissolved in THF (30 mL) in a round-bottom flask, shielded from the light. The solution was left stirring for 24 h at room temperature. The suspension was gravity filtered to give a red–orange colored filtrate. The solvent was removed in vacuum and a red–orange solid was obtained (yield 59.4%). ^{1}H NMR (300 MHz, THF-d_8) δ 3.16 [t, 4H, C_5H_4-($CH_2CH_2OC_6H_5$)], 4.07 [t, 4H, C_5H_4-(CH_2CH_2O C_6H_5)], 6.73–7.16 [m 18H, C_5H_4-($CH_2CH_2OC_6H_5$)]. ^{13}C NMR (75 MHz, C_6D_6) δ 30.8 [C_5H_4-($CH_2CH_2OC_6H_5$)], 68.3 [C_5H_4-($CH_2CH_2OC_6H_5$)], 115.4, 116.2, 119.7, 121.2, 130.0,

130.2, 134.0, 159.1 [C_5H_4-($CH_2CH_2OC_6H_5$)], 160.2 [Ti-C_2O_4]. Anal. Calcd for $C_{28}H_{26}O_6Ti$ (%): C, 66.41; H 5.18. Found (%): C, 66.21; H 5.21.

Synthesis of bis-[(benzyl)-cyclopentadienyl]-titanium-dichloride [C_5H_4-CH_2-C_6H_5]$_2TiCl_2$ (**4**): $TiCl_4$·2 THF (0.31 g, 0.93 mmol) was added to 20 mL of dry THF. The solution turned immediately from colourless to pale yellow. 0.30 g (1.86 mmol) of the lithium cyclopenadienide intermediate were dissolved in 30 mL of dry THF and added *via cannula* to the solution containing the $TiCl_4$.The solution turned from yellow to red during addition. After this addition ,the mixture was refluxed overnight and then cooled. The solvent was removed under reduced pression. The remaining residue was extracted with dichlorometane (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 pression to give a dark red solid (yield 55%). ^{1}H NMR (300 MHz, THF-d_8) δ 4.06 [s, 4H, C_5H_4-CH_2-C_6H_5], 6.33–6.44 [m, 8H, C_5H_4-CH_2-C_6H_5], 7.22–7.24 [m, 10H, C_5H_4-CH_2-C_6H_5]. ^{13}C NMR (75 MHz, THF-d_8): δ 36.7 [C_5H_4-CH_2-C_6H_5], 110.9, 115.1, 122.6, 128.0, 128.6, 140.6 [C_5H_4-CH_2-C_6H_5]. Mass (E.I., 70 eV, *m*/*z*): 426 [L_2-$TiCl_2$-Li]$^+$; 357 [L_2-Ti]$^+$. Anal. Calcd for $C_{24}H_{22}Cl_2Ti$ (%): C, 67.16; H, 5.17. Found (%): C, 67.24; H 5.13.

Synthesis of [1,2-di(cyclopentadienyl)-1,2-bis-1,2-di(phenyl)ethanediyl] titanium-dichloride [1,2-$(C_6H_5)_2C_2H_2\{\eta^5$-$C_5H_4\}_2$]$TiCl_2$ (**5**): $TiCl_4$·2 THF (1.08 g, 3.24 mmol) was added to 20 mL of dry toluene containing 10% dry THF. The solution turned immediately from colourless to pale yellow. The mixture was stirred and cooled down to −78 °C, followed by drop wise addition of *n*-butyllithium (4 mL, 6.48 mmol). The solution turned from yellow to brown 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 (6.48 mmol, 1 g) in 10 mL of dry toluene was added to the $TiCl_2$·2 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 then the remaining mixture was filtered through celite. The solvent was then removed under vacuum and washed with dichlorometane and finally twice with hexane to give a dark brown solid (yield 49%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.54 [s, 1H, *anti*-C_5H_4-$C(H_{anti})$-*H*-C_6H_5], 4.85 [s, 1H, *syn*-C_5H_4-$C(H_{syn})$-*H*-C_6H_5], 6.20–6.37 [m, 8H, C_5H_4-CH_2-C_6H_5], 6.89–7.29 [m, 10H, C_5H_4-CH_2-C_6H_5]. ^{13}C NMR (75 MHz, $CDCl_3$) δ 54.2 [*anti*-C_5H_4-$C(H_{anti})H$-C_6H_5], 51.0 [*syn*-C_5H_4-$C(H_{syn})$-*H*-C_6H_5], 110.0, 113.4, 117.1, 127.5, 128.1, 128.3, 128.6, 133.8, 138.3 [C_5H_4-CH_2-C_6H_5]. Mass (E.I., 70 eV, *m*/*z*): 433.04 [L_2-$TiCl_2$-Li]$^+$. Anal.Calcd for $C_{24}H_{20}Cl_2Ti$ (%): C, 67.48; H, 4.72. Found (%): C, 67.39; H, 4.77.

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25. *Cell culture:* MCF7 breast cancer cells were maintained in DMEM/F-12 supplemented with 10% fetal bovine serum (FBS), 100 mg/mL penicillin/streptomycin and 2 mM L-glutamine (Invitrogen, Gibco, Milan, Italy). SkBr3 breast cancer cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 mg/mL penicillin/streptomycin and 2 mM L-glutamine (Invitrogen, Gibco, Milan, Italy). Cells were switched to medium without serum the day before experiments and thereafter treated in medium supplemented with 1% FBS.
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. IC_{50} values represent the drug concentrations that reduced the mean absorbance at 570 nm to 50% of those in the untreated control wells.
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