A novel alkenyl-substituted *ansa*-zirconocene complex with dual application as olefin polymerization catalyst and anticancer drug

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ABSTRACT

The alkenyl substituted fulvene compound, $(C_5H_4)=CMe(CH_2CH_2CH=CMe_2)$ (**1**), reacts with one equivalent of LiMe to give the lithium derivative $Li\{C_5H_4(CMe_2CH_2CH_2CH=CMe_2)\}$ (**2**). The reaction of **2** with $Me_2Si(C_5Me_4H)Cl$ gave the *ansa*-ligand precursor $Me_2Si(C_5Me_4H)(C_5H_4(CMe_2CH_2CH_2CH=CMe_2))$ (**3**), which after the subsequent reaction with 2 equivalents of $LiBu^n$ yielded the dilithium salt $Li_2\{Me_2Si(C_5Me_4)(C_5H_3(CMe_2CH_2CH_2CH=CMe_2))\}$ (**4**). The alkenyl-substituted zirconocene complex $[Zr\{Me_2Si(\eta^5-C_5Me_4)(\eta^5-C_5H_3(CMe_2CH_2CH_2CH=CMe_2))\}Cl_2]$ (**5**) was synthesized by the equimolar reaction of **4** and $ZrCl_4$. **5** was characterized by spectroscopic methods and by single crystal X-ray diffraction studies. The zirconocene compound **5** has been tested as a catalyst in the polymerization of ethylene at different temperatures and Al:Zr ratios, and also in the co-polymerization of ethylene and 1-octene, observing modest co-monomer incorporations. In addition, the cytotoxic activity of **5** was tested against tumour cell lines 8505C anaplastic thyroid cancer, A253 head and neck tumour, A549 lung carcinoma, A2780 ovarian cancer and DLD-1 colon carcinoma. Complex **5** showed the best cytotoxic activity on A2780 ovarian cancer (IC_{50} value of 36.8 ± 5.9 μM). This represents the highest reported cytotoxic activity of a zirconocene complex on A2780 ovarian cancer. In addition, the cytotoxic activities of **5**, have been compared with those obtained using cisplatin.

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1. Introduction

Group 4 metallocene dichloride complexes have been extensively studied due to their excellent behaviour as catalysts for the polymerization of α-olefins [1–3]. It is well established that the structural make-up of the catalyst directly influences the physical properties of the polymer [1–3]. In addition, titanocene and zirconocene complexes have been used as catalysts in other processes in organic synthesis such as carbometallation, hydrometallation, carbonylation, acylation, isomerization, hydrogenation, hydroamination, dehydrosilylation and hydrosilylation [4–8].

However, they are not the only applications of these complexes. In pioneering work by Köpf and Köpf-Maier, the potential of group 4 metallocene dihalides as potential anticancer agents was established [9–11]. Recently this field has experienced a renaissance with emphasis being placed on the design and synthesis of new group 4 metallocene complexes with different substituents in or-

der to increase cytotoxic activity as well as improving their overall application as anticancer drugs [12–18]. Titanocene derivatives have been at the forefront of this development and are being considered as alternatives to the actual platinum-based anticancer chemotherapy [12,14,15].

However, in spite of their analogy, zirconocene complexes have not been widely studied for these purposes. Twenty years ago, Köpf and coworkers tested the activity of zirconocene dichloride on Ehrlich's ascites tumour (EAT) *in vitro* [19] and *in vivo* [20], and all experiments showed no anticancer activity. Therefore, these results did not encourage further research on zirconocene complexes. Since that moment, only two different studies on zirconocene anticancer chemistry have been reported [21,22]. These studies by McGowan et al. [21] and Tacke and coworkers [22] have described the cytotoxic activity of different functionalized zirconocene complexes, observing an irregular behaviour in the anticancer tests. From these reports, only the complexes $[Zr\{\eta^5-C_5H_4(CH_2)_2N(CH_2)_5\}_2Cl_2·2HCl]$ and $[Zr\{\eta^5-C_5H_4(CH_2C_6H_4OCH_3)\}_2Cl_2]$ (zirconocene Y) have shown promising activity that needs to be improved in order to apply them in anticancer chemotherapy.

We have recently reported an increase of cytotoxicity for titano-cene and *ansa*-titanocene complexes that have pendant alkenyl substituents on the cyclopentadienyl rings [16–18], and therefore we are interested in observing the behaviour of zirconium complexes containing similar ligands.

Thus, we herein report the synthesis, structural characterization, catalytic behaviour in the polymerization of ethylene and co-polymerization of ethylene and 1-octene and the cytotoxic activity on different human cancer cell lines of a novel alkenyl substituted *ansa*-zirconocene complex and which has proven to be the most active zirconocene complex on human A2780 ovarian cancer cells, reported to date.

2. Results and discussion

2.1. Synthesis and characterization of ansa-zirconocene complex (5)

Methyl lithium reacts with the fulvene $(C_5H_4)=CMe(CH_2-CH_2CH=CMe_2)$ (**1**) via nucleophilic addition at the exocyclic double bond to give the lithium cyclopentadienyl compound $Li\{C_5H_4-(CMe_2CH_2CH_2CH=CMe_2)\}$ (**2**) (Scheme 1).

Complex **2** was characterized by ^1H, ^{13}C NMR spectroscopy and elemental analysis (see Section 4.3). Two multiplets were observed, at 5.55 and 5.53 ppm, in the ^1H NMR spectrum of **2** which were assigned to the cyclopentadienyl ring protons. A singlet due to the two methyl groups substituting the carbon atom adjacent to the cyclopentadienyl ring was observed at 1.20 ppm. The alkenyl fragment exhibited, in the ^1H NMR spectrum, five sets of signals, two corresponding to the CH_2 methylene protons (two multiplets at 1.46 and 1.82 ppm), one corresponding to the alkenylic proton of the C-γ (triplet at 5.06 ppm) and two singlets corresponding to the protons of the terminal methyl groups at 1.50 and 1.60 ppm). The $^{13}C\{^1H\}$ NMR spectrum of **2** showed the expected signals (carbon atom bonded to the cyclopentadienyl ring, one signal at 46.4 ppm; for the two methyl groups attached to this carbon atom one signal at 34.5 ppm; six signals at 16.7, 24.0, 25.0, 30.3, 128.4 and 129.0 ppm for the alkenyl fragment; and for the cyclopentadienyl, three signals at 100.0, 100.8, and 126.5 ppm).

The preparation of the asymmetrically substituted *ansa*-ligand precursor, $Me_2Si(C_5HMe_4)(C_5H_4(CMe_2CH_2CH_2CH=CMe_2))$ (**3**), was achieved by the reaction of **2** with $Me_2Si(C_5HMe_4)Cl$, following pre-viously reported synthetic protocols [23–27] (Scheme 1). **3** was isolated as a mixture of the double bond position isomers, with one isomer being predominant as confirmed by ^1H NMR spectroscopy. The dilithium derivative, $Li_2\{Me_2Si(C_5Me_4)(C_5H_3(C-Me_2CH_2CH_2CH=CMe_2))\}$ (**4**), was obtained, in the traditional manner by the reaction of **3** with *n*-butyllithium (Scheme 1). Complex **4** was isolated as a highly moisture and oxygen sensitive white solid and characterized by elemental analysis (see Section 4.5).

The reaction of the dilithium derivative **4** with $ZrCl_4$, gave the corresponding *ansa*-metallocene dichloride complex $[Zr\{Me_2-Si(\eta^5-C_5Me_4)(\eta^5-C_5H_3(CMe_2CH_2CH_2CH=CMe_2))\}Cl_2]$ (**5**) (Scheme 1), which was isolated as a yellow crystalline solid and characterized spectroscopically. The ^1H NMR spectrum of **5** shows three multiplets for the protons of the alkenyl substituted C_5 ring (between 5.5 and 6.9 ppm), four singlets for the methyl groups of the tetramethyl substituted C_5 ring (between 1.9 and 2.1 ppm), and two singlets corresponding to the methyl groups of the $SiMe_2$ bridging unit (0.81 and 0.85 ppm).

The signals for the alkenyl fragment were observed with similar chemical shifts and identical spectral patterns to those observed in the lithium derivative **2**. However, the methyl groups substituting the carbon atom bonded to the cyclopentadienyl ring are diastereotopic in **5** due to the planar chirality present in the molecule and thus two singlets were observed (1.40 and 1.48 ppm) in the ^1H NMR spectrum. The $^{13}C\{^1H\}$ NMR spectrum of **5**, show the expected signals (see Section 4.6).

Complex **5** was also characterized by FAB-MS. The mass spectrum showed the molecular ion peak, and fragments indicative of the loss of some of the chloride ligands or the alkenyl fragment of the cyclopentadienyl ligand were also observed (see Section 4.6).

2.2. Structural studies

The molecular structure of **5** was established by single crystal X-ray diffraction studies (Fig. 1). The molecular structure and atomic numbering scheme are shown in Fig. 1. Selected bond lengths and angles for **5** are given in Table 1.

Complex **5** crystallizes in the triclinic $P\bar{1}$ space group with two molecules located in the unit cell. The structure of **5** shows the typical bent metallocene conformation observed in other zirconocene
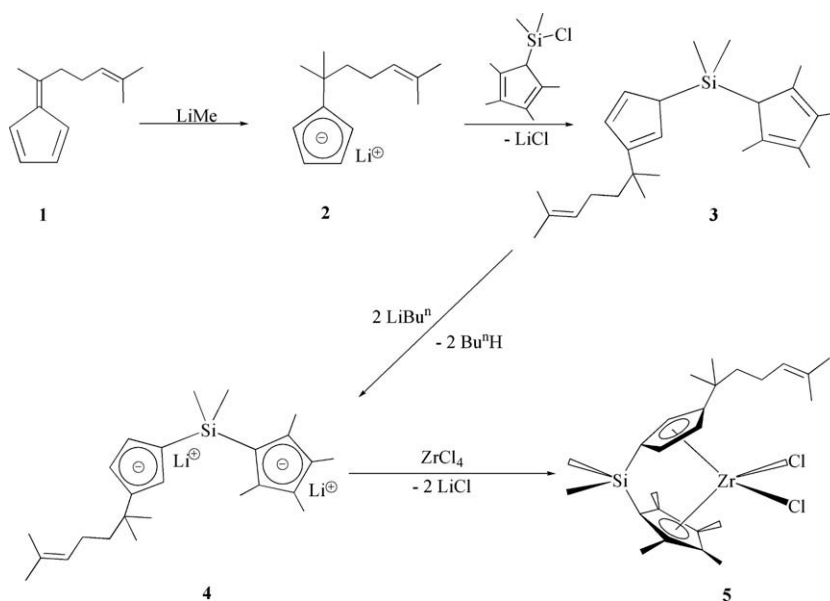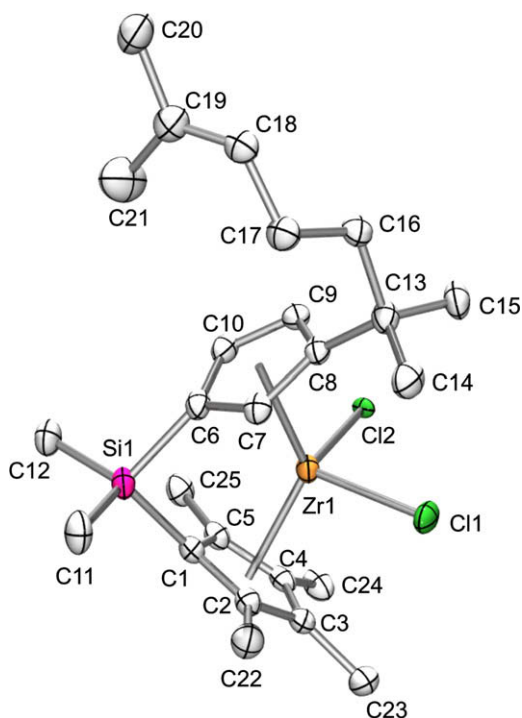

Scheme 1.

Fig. 1. Molecular structure and atom-labelling scheme for [Zr{Me$_2$Si(η^5-C$_5$Me$_4$)(η^5-C$_5$H$_3$(CMe$_2$CH$_2$CH$_2$CH=CMe$_2$))}Cl$_2$] (**5**), with thermal ellipsoids at 50% probability.

Table 1
Selected bond lengths (Å) and angles (°) for **5**.

	5
Zr–Cent(1)	2.223
Zr–Cent(2)	2.235
Zr–C(1)	2.462(4)
Zr–C(2)	2.480(4)
Zr–C(3)	2.611(4)
Zr–C(4)	2.607(4)
Zr–C(5)	2.496(4)
Zr–C(6)	2.471(4)
Zr–C(7)	2.509(4)
Zr–C(8)	2.645(4)
Zr–C(9)	2.588(4)
Zr–C(10)	2.473(4)
Zr–Cl(1)	2.438(1)
Zr–Cl(2)	2.492(1)
C(16)–C(17)	1.533(6)
C(17)–C(18)	1.501(6)
C(18)–C(19)	1.318(7)
C(19)–C(20)	1.506(7)
C(19)–C(21)	1.497(7)
Cent(1)–Zr–Cent(2)	127.1
Si(1)–C(1)–Cent(1)	163.5
Si(1)–C(6)–Cent(2)	163.6
C(1)–Si(1)–C(11)	113.8(2)
C(1)–Si(1)–C(6)	94.0(2)
C(1)–Si(1)–C(12)	116.0(2)
C(6)–Si(1)–C(11)	112.5(2)
C(6)–Si(1)–C(12)	110.2(2)
C(11)–Si(1)–C(12)	109.5(2)
Cl(1)–Zr–Cent(1)	105.4
Cl(1)–Zr–Cent(2)	109.2
Cl(2)–Zr–Cent(1)	107.9
Cl(2)–Zr–Cent(2)	106.0
Cl(1)–Zr–Cl(2)	97.85(4)
C(17)–C(18)–C(19)	127.0(4)
C(18)–C(19)–C(20)	120.4(5)
C(18)–C(19)–C(21)	124.9(5)

Cent(1) and Cent(2) are the centroids of C(1)–C(5) and C(6)–C(10), respectively.

dichloride complexes. The *ansa* ligand chelates the zirconium atom, and both cyclopentadienyl rings are bound to the metal in an η^5 manner.

For complex **5**, the distance from the metal to the alkenyl substituted carbon atom of the cyclopentadienyl ring, Zr(1)–C(8), is 2.645(4) Å. This bond length is significantly longer than those observed between the zirconium atom and the other β-carbon atom of the monosubstituted cyclopentadienyl ring (Zr(1)–C(9) 2.588(4) Å). However, this Zr(1)–C(8) distance is in the range observed for the β-carbon atoms of the tetramethylcyclopentadienyl moiety (Zr(1)–C(3) 2.611(4) Å and Zr(1)–C(4) 2.607(4) Å) and similar to those found for other *ansa*-zirconocene complexes [3,26].

The Cent(1)–Zr(1)–Cent(2) angle of 127.1° for **5**, is similar to those reported for other *ansa*-zirconocene complexes [3,24–28]. The Cl(1)–Zr(1)–Cl(2) angle for **5** (97.85(4)°) is similar to those recorded for related tetramethyl substituted *ansa*-zirconocene complexes [3,24–28]. The distances, Zr–Cl ca. 2.45 Å for **5** are also in the expected range [3,24–28].

The alkenyl substituent is situated nearly above one of the chlorine atoms (Cl(1)) (dihedral angle θ Cl(1)–Zr(1)–C(8)–C(13) = 16.5°) and should therefore be influential in the coordination behaviour of the olefin monomer at this site during polymerization. The alkenyl moiety bond distances are C(16)–C(17) 1.533(6) Å; C(17)–C(18) 1.501(6) Å; C(18)–C(19) 1.318(7) Å; C(19)–C(20) 1.506(7) Å and C(19)–C(21) 1.497(7) Å. The bond length C(18)–C(19) is typical for C–C double bonds [23–25,27] and the angles C(17)–C(18)–C(19) of 127.0(4)°; C(18)–C(19)–C(20) of 120.4(5)° and C(18)–C(19)–C(21) of 124.9(5)° confirm the sp^2 hybridization of C(18) and C(19).

2.3. Ethylene polymerization

The polymerization of ethylene, using zirconocene complex **5**, has been carried out. The polymerization experiments were conducted with MAO using different Al:Zr ratios of 500:1; 1000:1 and 3000:1 at 0, 40 and 70 °C and at olefin pressure of 2 bar during 15 min. The catalytic activities and polymer molecular weight and distribution values are given in Table 2.

These results show that on raising the Al:Zr ratio, from 500 to 3000, the polymerization activity increases substantially at all the studied temperatures, however, the polymer molecular weight decreases notably.

With regards to the temperature, the highest polymerization activity was observed at 40 °C, while the lowest activity was observed at 0 °C. The final polymer molecular weight is higher at lower temperatures, indicating a preference of the chain growth step with respect to chain termination. This is confirmed by the fact that the polymer molecular weights in the polymerization tests

Table 2
Ethylene polymerization results and ethylene–1-octene copolymerization results for **5**.[a,b]

Catalyst	Activity[b]	M_w (g mol^{-1})	M_w/M_n	Al:Ti	T (°C)
5	1038	488 200	4.33	500	0
5	1133	473 200	4.42	1000	0
5	1259	421 100	4.22	3000	0
5	9652	85 200	4.43	500	40
5	10 310	60 900	5.07	1000	40
5	11 078	58 600	4.87	3000	40
5	8286	55 900	3.89	500	70
5	9079	50 700	3.57	1000	70
5	10 992	21 500	3.17	3000	70
5[c,d]	9943	56 800	4.97	3000	40

[a] At 2 bar monomer pressure, 200 mL toluene, t_{Pol} = 15 min.
[b] In kg Pol (mol Zr.h.bar)$^{-1}$.
[c] Starting from 10 mmol of 1-octene in the polymerization mixture.
[d] Final 1-octene incorporation 0.57%.

at 0 °C were of about 450 000 g mol^{-1} while at 40 °C and 70 °C, they were about 60 000 and 50 000 g mol^{-1}, respectively. The polydispersity values, in all cases, of about 4.0 were recorded.

In addition to the ethylene polymerization tests, and using the optimum conditions in terms of activity (40 °C and 3000:1 Al:Zr ratio), a copolymerization run with 1-octene and ethylene was performed. The final polymer shows a modest incorporation of 1-octene of 0.57%, indicating the low copolymerization rate of this complex under these conditions.

2.4. Cytotoxic studies

The cytotoxic activity of the alkenyl-substituted zirconocene complex [Zr{Me$_2$Si(η^5-C$_5$Me$_4$)(η^5-C$_5$H$_3$(CMe$_2$CH$_2$CH$_2$CH=CMe$_2$))}-Cl$_2$] (**5**) has been studied. In titanocene complexes, we have previously observed that the inclusion of an alkenyl group resulted in an increase in the cytotoxic activity [16–18].

The cytotoxic activity of the alkenyl-substituted zirconocene complex **5** was tested against tumour cell lines 8505C anaplastic thyroid cancer, A253 head and neck tumour, A549 lung carcinoma, A2780 ovarian cancer and DLD-1 colon carcinoma. **5** is active against all the studied human cancer cells, presenting moderate activity against 8505C, A253 and A549 (IC$_{50}$ between 136.4 ± 5.4 and 166.7 ± 3.2 μM) good activity on DLD-1 (IC$_{50}$ 107.5 ± 3.7 μM) and a very high activity for a zirconium derivative on A2780 cells (IC$_{50}$ 36.8 ± 5.9 μM) (Table 3). Fig. 2 shows survival of 8505C, A253, A549, A2780 and DLD-1 cells grown for 96 h in the presence of increasing concentrations of complex **5**.

In fact, the cytotoxic activity of complex **5** against ovarian cancer cells A2780 is the highest of all the cytotoxic activities of zirconocene complexes reported in the literature [21].

It is noteworthy, that the cytotoxic behaviour of the zirconium complex **5** is of a similar magnitude to those reported for its tita-

nium analogues, which present IC$_{50}$ values on other human cancer cells between 24 ± 3 and 149 ± 3 μM [16–18].

Experiments carried out by Sadler and coworker using Zr(IV) ions, confirmed the possibility of formation of Transferrin-Zr(IV) adducts [29]. Thus, it seems plausible that zirconium, as in the case of titanium, may reach the cells assisted by the major iron transport protein, "transferrin" [30–33] binding to DNA which is the biological target of these complexes [34–36].

On direct comparison with cisplatin, the cytotoxic activity of complex **5** is significantly lower, however, the tolerance of relatively high zirconium amounts in biological systems, in comparison with the high number of side-effects associated to very low concentrations of platinum, make these results very promising for further *in vivo* experiments.

3. Conclusions

The alkenyl-substituted zirconocene complex [Zr{Me$_2$Si(η^5-C$_5$Me$_4$)(η^5-C$_5$H$_3$(CMe$_2$CH$_2$CH$_2$CH=CMe$_2$))}Cl$_2$] (**5**) has been synthesized and characterized. Complex **5** has been used as catalyst in ethylene polymerization and ethylene–1-octene copolymerization at different temperatures and Al:Zr ratios. The best catalytic activities were found at 40 °C and high Al:Zr ratios of 3000:1.

In addition, the cytotoxic activity of **5** was tested against human tumour cell lines. Complex **5** showed the best cytotoxic activity on A2780 ovarian cancer (IC$_{50}$ value of 36.8 ± 5.9 μM) and represents the highest reported cytotoxic activity of a zirconocene complex on this human cancer cell line. The cytotoxic behaviour of the zirconium complex **5** is of a similar magnitude to those reported for titanium analogues, indicating a similar anticancer mechanism.

Future work, already in progress, will now focus on the improvement of the cytotoxic nature of **5** by the manipulation of the alkenyl group and the introduction of other different functional groups to improve the water solubility and cytotoxic activity of the complexes. In this way, we intend to study the influence of ligand modification on the cytotoxicity of the final complex.

4. Experimental

4.1. General manipulations

All reactions were performed using standard Schlenk tube techniques in an atmosphere of dry nitrogen. Solvents were distilled from the appropriate drying agents and degassed before use. Cyclopentadiene dimer, pyrrolidine, C$_5$H$_2$Me$_4$, Me$_2$Si(C$_5$Me$_4$H)Cl, ZrCl$_4$, LiBu^n (1.6 M in hexane), LiMe (1.6 M in Et$_2$O), MAO (10%wt in toluene), and CH$_3$COCH$_2$CH$_2$CH=CMe$_2$ were purchased from Aldrich. All the commercial reagents were used directly. IR spectra were recorded on a Thermo Nicolet Avatar 330 FT-IR spectrophotometer. ^{1}H, ^{13}C, and ^{29}Si NMR spectra were recorded on a Varian Mercury FT-400 spectrometer. Microanalyses were carried out with a Perkin–Elmer 2400 or LECO CHNS-932 microanalyzer. Mass spectroscopic analyses were preformed on a MASPEC II system [II32/A302] (m/z 50–1000) instrument. Polymer molecular weights and distribution were determined by GPC (Waters 150C Plus or Alliance GPC-2000) in 1,2,4-trichlorobenzene at 150 °C, using standard polystyrene calibration. 1-Octene incorporation was calculated from ^{13}C{^{1}H} NMR spectra of polymer samples dissolved in 1,2,4-trichlorobenzene and C$_6$D$_6$ (1:1).

4.2. Synthesis of (C$_5$H$_4$)=CMe(CH$_2$CH$_2$CH=CMe$_2$) (1)

Complex **1** was prepared using the methodology described by Little and co-workers [37], starting from CH$_3$COCH$_2$CH$_2$CH=CMe$_2$ (4.59 g, 36.30 mmol), freshly cracked cyclopentadiene (6.00 g,

Table 3
IC$_{50}$ (μM) for the 96 h of action of the complex **5** and cisplatin on 8505C anaplastic thyroid cancer, A253 head and neck tumour, A549 lung carcinoma, A2780 ovarian cancer and DLD-1 colon carcinoma determined by sulforhodamine-B microculture colorimetric assay.

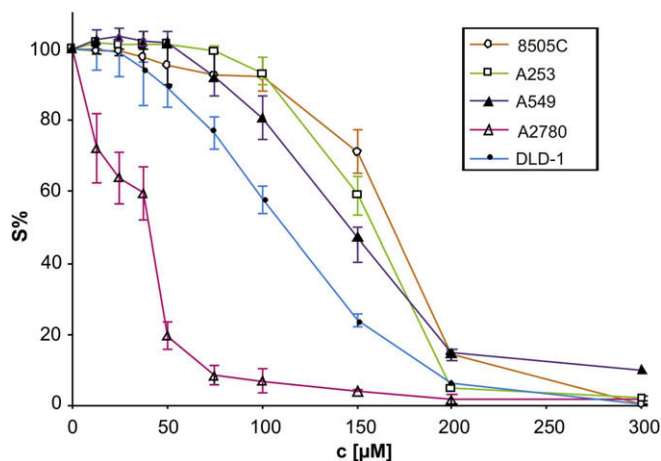| Compound	IC$_{50}$ ± SD				
	8505C	A253	A549	A2780	DLD-1
5	166.7 ± 3.2	154.5 ± 2.2	136.4 ± 5.4	36.8 ± 5.9	107.5 ± 3.7
Cisplatin	5	0.8	1.5	0.5	5

Fig. 2. Representative graphs showing survival of 8505C, A253, A549, A2780 and DLD-1 cells grown for 96 h in the presence of increasing concentrations of complex **5**.

90.75 mmol) and pyrrolidine (3.87 g, 54.45 mmol). Yield: 5.82 g, 92%. ^{1}H NMR (400 MHz, CDCl$_3$, 25 °C): δ = 1.62 and 1.70 (s, each 3H, CH=CMe_2), 2.22 (s, 3 H, C$_5$H$_4$=CMe), 2.25 and 2.55 (2 m, each 2 H, CH_2CH_2), 5.15 (t, ^3J = 5.6 Hz, 1 H, CH=CMe_2), 6.49 and 6.51 (2 m, each 2 H, C$_5$H$_4$) ppm. ^{13}C{^{1}H} NMR (100 MHz, CDCl$_3$, 25 °C): δ = 15.3 (C$_5$H$_4$=CMe), 17.7 and 21.0 (CH=CMe_2) 25.6 and 27.9 (CH_2CH_2), 120.4, 120.7, 130.5 and 130.7 (C$_4$H$_4$), 123.2 and 142.2 (C=C exocyclic), 132.4 and 154.0 (CH=CMe_2) ppm. Anal. Calc. for C$_{13}$H$_{18}$ (174.3): C, 89.59; H, 10.41. Found: C, 89.33; H, 10.27%.

4.3. Synthesis of Li{C$_5$H$_4$(CMe$_2$CH$_2$CH$_2$CH=CMe$_2$)} (2)

LiMe (1.6 M in Et$_2$O) (10.8 mL, 17.23 mmol) was added dropwise to a solution of (C$_5$H$_4$)=CMe(CH$_2$CH$_2$CH=CMe$_2$) (1) (3.00 g, 17.23 mmol) in Et$_2$O (75 mL) at −78 °C. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. Solvent was removed in vacuum to give a white solid which was washed with hexane (2 × 50 mL) and dried under vacuum to yield the title complex. Yield: 2.70 g, 80%. ^{1}H NMR (400 MHz, d$_8$-THF, 25 °C): δ = 1.20 (s, 6 H, CpCMe_2), 1.46 and 1.81 (2 m, each 2 H, CH_2CH_2), 1.50 and 1.60 (s, each 3 H, CH=CMe_2), 5.06 (t, ^3J = 7.2 Hz, 1 H, CH=CMe_2), 5.53 and 5.55 (2 m, each 2 H, C$_5$H$_4$) ppm. ^{13}C{^{1}H} NMR (100 MHz, d$_8$-THF, 25 °C): δ = 16.7 and 30.3 (CH_2CH_2), 24.0 and 25.0 (C=CMe_2), 34.5 (CpCMe_2), 46.4 (CpC), 100.0, 100.8 and 126.5 (C$_5$H$_4$), 128.4, 129.0 (CH=CMe$_2$) ppm. Anal. Calc. for C$_{14}$H$_{21}$Li (196.3): C, 85.68; H, 10.79. Found: C, 85.29; H, 10.58%.

4.4. Synthesis of Me$_2$Si(C$_5$Me$_4$H)(C$_5$H$_4$(CMe$_2$CH$_2$CH$_2$CH=CMe$_2$)) (3)

Me$_2$Si(C$_5$HMe$_4$)Cl (2.19 g, 10.19 mmol) in THF (50 mL) was added to a solution of Li{C$_5$H$_4$(CMe$_2$CH$_2$CH$_2$CH=CMe$_2$)} (2) (2.00 g, 10.19 mmol) in THF (50 mL) at −78 °C. The reaction mixture was allowed to warm to room temperature and stirred for 6 h. Solvent was removed in vacuo and hexane (150 mL) was added to the resulting dark orange oil. The mixture was filtered and solvent removed from the filtrate under reduced pressure to yield the title compound as a dark yellow oil. Yield 3.42 g, 92%. ^{1}H NMR (400 MHz, CDCl$_3$, 25 °C; for the predominant isomer): δ = 0.11 (s, 6 H, SiMe_2), 1.17 (s, 6 H, CpCMe_2), 1.49 and 1.82 (2 m, each 2 H, CH_2CH_2), 1.54 and 1.66 (s, each 3 H, CH=CMe_2), 1.79 and 1.90 (2 s, each 6 H, C$_5$Me$_4$), 2.72 and 2.93 (2 m, each 1 H, 2 × HC$_5$), 5.08 (m, 1 H, CH=CMe_2, 5.97, 6.43 and 6.58 (3 m, each 1 H, C$_5$H$_3$) ppm. Anal. Calc. for C$_{25}$H$_{40}$Si (368.7): C, 81.45; H, 10.94. Found: C, 81.12; H, 10.88%.

4.5. Synthesis of Li$_2${Me$_2$Si(C$_5$Me$_4$)(C$_5$H$_3$(CMe$_2$CH$_2$CH$_2$CH=CMe$_2$))} (4)

LiBu^n (1.6 M in hexane) (10.2 mL, 16.28 mmol) was added dropwise to a solution of 3 (3.00 g, 8.14 mmol) in Et$_2$O (100 mL) at −78 °C. The mixture was allowed to warm to 25 °C and stirred for 15 h. Solvent was removed in vacuum to give a white solid which was washed with hexane (2 × 50 mL) and dried under vacuum to yield the title complex (2.32 g, 75%). Anal. Calc. for C$_{25}$H$_{38}$Li$_2$Si (380.5): C, 78.91; H, 10.07. Found: C, 78.55; H, 9.98%.

4.6. Synthesis of [Zr{Me$_2$Si(η^5-C$_5$Me$_4$)(η^5-C$_5$H$_3$(CMe$_2$CH$_2$CH$_2$CH=CMe$_2$))}Cl$_2$] (5)

Li$_2${Me$_2$Si(C$_5$Me$_4$)(C$_5$H$_3$(CMe$_2$CH$_2$CH$_2$CH=CMe$_2$))} (4) (2.00 g, 5.26 mmol) in THF (50 mL) was added dropwise during 15 min to a solution of ZrCl$_4$ (1.22 g, 5.26 mmol) in THF (50 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. Solvent was removed in vacuum and a toluene:hexane mixture (9:1) (125 mL) added to the resulting solid. The mixture was filtered and the filtrate concentrated (20 mL) and cooled to −30 °C to yield crystals of the title complex. Yield

1.53 g, 56%. IR (ZnSe): ν = 3102 (ν_{CH}), 1628 ($\nu_{CH=CMe_2}$) cm^{-1}. ^{1}H NMR (400 MHz, CDCl$_3$, 25 °C): δ = 0.81 and 0.85 (2 s, each 3 H, SiMe_2), 1.40 and 1.49 (2 s, each 3 H, CpCMe_2), 1.43 and 1.83 (2 m, each 2 H, CH_2CH_2), 1.52 and 1.64 (s, each 3 H, CH=CMe_2), 1.91, 1.99, 2.01 and 2.04 (4 s, each 3 H, C$_5$Me$_4$), 4.99 (t, ^3J = 5.6 Hz, 1 H, CH=CMe_2), 5.57, 5.67 and 6.85 (3 m, each 1 H, C$_5$H$_3$) ppm. ^{13}C{^{1}H} NMR (100 MHz, CDCl$_3$, 25 °C): δ = −0.3 and 0.5 (SiMe_2), 12.3, 12.6, 15.3 and 17.8 (C$_5$Me$_4$), 23.4 and 24.6 (C=CMe_2), 26.0 and 27.5 (CH_2CH_2), 36.7 and 36.8 (CMe_2), 47.2 (CpC), 97.8 and 104.4 (C^1-Cp), 111.6, 124.2, 135.3 and 150.7 (C$_5$H$_3$), 113.8, 126.9, 127.6 and 136.7 (C$_5$Me$_4$), 124.9, 131.4 (CH=CMe$_2$) ppm. ^{29}Si{^{1}H} NMR (79.5 MHz, CDCl$_3$, 25 °C): δ = −21.7 (SiMe_2) ppm. EI MS: m/z (%) = 528 (14) [M$^+$], 489 (6) [M$^+$–H–Cl], 443 (32) [M$^+$–CH$_2$CH$_2$CH=CMe$_2$], 407 (56) [M$^+$–CH$_2$CH$_2$CH=CMe$_2$–Cl]. Anal. Calc. for C$_{25}$H$_{38}$Cl$_2$SiZr (528.8): C, 56.78; H, 7.24. Found: C, 56.44; H, 7.19%.

4.7. Data collection and structural refinement of 5

The data of 5 were collected with a CCD Oxford Xcalibur S (λ(Mo Kα) = 0.71073 Å) using ω and φ scans mode. Semi-empirical from equivalents absorption corrections were carried out with SCALE3 AB-SPACK [38]. All the structures were solved by direct methods [39]. Structure refinement was carried out with SHELXL-97 [40]. All non-hydrogen atoms were refined anisotropically, and hydrogen atoms were calculated with the riding model and refined isotropically. Crystallographic details are listed in Table 4.

4.8. Polymerization studies

4.8.1. Ethylene polymerization

The zirconocene catalyst 5 (6 μmol), the stochiometric amount of MAO (10% in toluene) and toluene (200 mL) were mixed together for 15 min in a 1 l glass autoclave. The N$_2$ pressure inside the autoclave was reduced by applying vacuum. Ethylene pressure of 2 bar was then applied and maintained to the autoclave and stirring of the mixture commenced (1000 rpm). After exactly 15 min, stirring was halted and the ethylene pressure released. Excess MAO was then destroyed by adding cautiously a mixture of meth-

Table 4
Crystal data and structure refinement for 5.

Formula	C$_{25}$H$_{38}$Cl$_2$SiZr
Fw	528.76
T (K)	130(2)
Crystal system	Triclinic
Space group	P$\bar{1}$
a (Å)	9.7118(2)
b (Å)	9.8804(2)
c (Å)	14.9915(3)
α (°)	84.030(2)
β (°)	82.985(2)
γ (°)	66.629(2)
V (Å^3)	1308.18(5)
Z	2
Dc (Mg m^{-3})	1.342
μ (mm^{-1})	0.680
F(0 0 0)	552
Crystal dimensions (mm)	0.3 × 0.2 × 0.2
θ range (deg)	2.57 to 26.37
hkl Ranges	−12 ⩽ h ⩽ 12, −12 ⩽ k ⩽ 12, −18 ⩽ l ⩽ 18
Data/parameters	5347/272
Goodness-of-fit (GOF) on F^2	1.045
Final R indices [I > 2σ(I)]	R_1 = 0.0479, wR_2 = 0.1315
R indices (all data)	R_1 = 0.0627, wR_2 = 0.1401
largest different peak and hole (e Å^{-3})	1.913 and −0.693

$R_1 = \sum ||F_o| - |F_c|| / \sum |F_o|$; $wR_2 = [\sum [w(F_o^2 - F_c^2)^2] / \sum [w(F_o^2)^2]]^{0.5}$.

anol/HCl (90:10). The polymer was isolated by filtration and washed with ethanol and dried under vacuum at 90 °C for 16 h.

4.8.2. Ethylene–1-octene copolymerization

The zirconocene catalyst **5** (6 μmol), the stochiometric amount of MAO (10% in toluene), 10 mmol of 1-octene and toluene (200 mL) were mixed together for 15 min in a 1 l glass autoclave. The N_2 pressure inside the autoclave was reduced by applying vacuum. Ethylene pressure of 2 bar was then applied and maintained to the autoclave and stirring of the mixture commenced (1000 rpm). After exactly 15 min, stirring was halted and the ethylene pressure released. Excess MAO was then destroyed by adding cautiously a mixture of methanol/HCl (90:10). The polymer was isolated by filtration and washed with ethanol and dried under vacuum at 90 °C for 16 h.

4.9. In vitro studies

4.9.1. Preparation of drug solutions

A solution of the investigated zirconium complex was made in dimethyl sulfoxide (DMSO, Sigma Aldrich) at a concentration of 20 mM, filtered through Millipore filter, 0.22 μm, before use, and diluted by nutrient medium to various working concentrations. Nutrient medium was RPMI-1640 (PAA Laboratories) supplemented with 10% fetal bovine serum (Biochrom AG) and penicillin/streptomycin (PAA Laboratories).

4.9.2. Cell lines and culture conditions

The cell lines 8505C, A253, A549, A2780 and DLD-1 were included in this study. All these cell lines were kindly provided by Dr. Thomas Mueller, Department of Hematology/Oncology, Martin Luther University of Halle-Wittenberg, Halle (Saale), Germany. Cultures were maintained as monolayer in RPMI-1640 (PAA Laboratories, Pasching, Germany) supplemented with 10% heat inactivated fetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin/streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5% (v/v) CO_2.

4.9.3. Cytotoxicity assay

The cytotoxic activities of the zirconium complex **5**, were evaluated using the sulforhodamine-B (SRB, Sigma Aldrich) microculture colorimetric assay [41]. In short, exponentially growing cells were seeded into 96-well plates on day zero at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with serial dilutions of the studied compounds for 96 h. Final concentrations achieved in treated wells were 10, 20, 30, 50, 75, 100, 150, 200 and 300 μmol/l. Each concentration was tested in three triplicates on each cell line. The final concentration of DMSO solvent never exceeded 0.5%, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After 96 h treatment, the supernatant medium from the 96-well plates was thrown away and the cells were fixed with 10% TCA. For a thorough fixation, plates were then allowed to stand at 4 °C. After fixation the cells were washed in a strip washer. The washing was carried out four times with water using alternate dispensing and aspiration procedures. The plates were then dyed with 100 μl of 0.4% SRB for about 45 min. After dyeing, the plates were again washed to remove the dye with 1% acetic acid and allowed to air dry overnight. About 100 μl of 10 mM Tris base solutions was added to each well of the plate and absorbance was measured at 570 nm using a 96 well plate reader (Tecan Spectra, Crailsheim, Germany). The IC_{50} values, defined as the concentrations of the compound at which 50% cell inhibition was observed, were estimated from the dose–response curves.

Appendix A. Supplementary material

CCDC 727712 contains the supplementary crystallographic data for the structural analysis of **5**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2009.05.013.

References

[1] H.G. Alt, A. Köppl, Chem. Rev. 100 (2000) 1205–1222.
[2] G.W. Coates, Chem. Rev. 100 (2000) 1223–1252.
[3] S. Prashar, A. Antiñolo, A. Otero, Coord. Chem. Rev. 250 (2006) 133–154.
[4] E. Negishi, T. Takahashi, Aldrichim. Acta 18 (1985) 31–47.
[5] C.L. Buchwald, R.B. Nielsen, Chem. Rev. 88 (1988) 1047–1058.
[6] R.L. Halterman, Chem. Rev. 92 (1992) 965–994.
[7] A.H. Hoveyda, J.P. Morken, in: A. Togni, R.L. Halterman (Eds.), Metallocenes, Wiley-VCH, Weinheim, 1998.
[8] P. Wipf, C. Kendall, Chem. Eur. J. 8 (2002) 1778–1784.
[9] P. Köpf-Maier, H. Köpf, Angew. Chem., Int. Ed. Engl. 18 (1979) 477–478.
[10] P. Köpf-Maier, H. Köpf, Chem. Rev. 87 (1987) 1137–1152.
[11] P. Köpf-Maier, Eur. J. Clin. Pharm. 47 (1994) 1–16.
[12] P.M. Abeysinghe, M.M. Harding, Dalton Trans. (2007) 3474–3482.
[13] E.Y. Tshuva, D. Peri, Coord. Chem. Rev., in press, doi:10.1016/j.ccr.2008.11.015, and references therein.
[14] K. Strohfeldt, M. Tacke, Chem. Soc. Rev. 37 (2008) 1174–1187. and references therein.
[15] C.G. Hartinger, P.J. Dyson, Chem. Soc. Rev. 38 (2009) 391–401. and references therein.
[16] S. Gómez-Ruiz, G.N. Kaluđerović, D. Polo-Cerón, S. Prashar, M. Fajardo, Ž. Žižak, Z.D. Juranić, T.J. Sabo, Inorg. Chem. Commun. 10 (2007) 748–752.
[17] S. Gómez-Ruiz, G.N. Kaluđerović, S. Prashar, D. Polo-Cerón, M. Fajardo, Ž. Žižak, T.J. Sabo, Z.D. Juranić, J. Inorg. Biochem. 102 (2008) 1558–1570.
[18] S. Gómez-Ruiz, G.N. Kaluđerović, Ž. Žižak, I. Besu, Z.D. Juranić, S. Prashar, M. Fajardo, J. Organomet. Chem. 694 (2009) 1981–1987.
[19] P. Köpf-Maier, W. Wagner, H. Köpf, Cancer Chemother. Pharmacol. 5 (1981) 237–241.
[20] P. Köpf-Maier, B. Hesse, H. Köpf, J. Cancer, Res. Clin. Oncol. 96 (1980) 43–51.
[21] O.R. Allen, R.J. Knox, P.C. McGowan, Dalton Trans. (2008) 5293–5295.
[22] D. Wallis, J. Claffey, B. Gleeson, M. Hogan, H. Müller-Bunz, M. Tacke, J. Organomet. Chem. 694 (2009) 828–833.
[23] S. Gómez-Ruiz, D. Polo-Cerón, S. Prashar, M. Fajardo, A. Antiñolo, A. Otero, Eur. J. Inorg. Chem. (2007) 4445–4455.
[24] A. Antiñolo, M. Fajardo, S. Gómez-Ruiz, I. López-Solera, A. Otero, S. Prashar, A.M. Rodríguez, J. Organomet. Chem. 683 (2003) 11–22.
[25] A. Antiñolo, M. Fajardo, S. Gómez-Ruiz, I. López-Solera, A. Otero, S. Prashar, Organometallics 23 (2004) 4062–4069.
[26] S. Gómez-Ruiz, S. Prashar, L.F. Sánchez-Barba, D. Polo-Cerón, M. Fajardo, A. Antiñolo, A. Otero, M.A. Maestro, C.J. Pastor, J. Mol. Catal. A: Chem. 264 (2007) 260–269.
[27] S. Gómez-Ruiz, S. Prashar, M. Fajardo, A. Antiñolo, A. Otero, M.A. Maestro, V. Volkis, M.S. Eisen, C.J. Pastor, Polyhedron 24 (2005) 1298–1313.
[28] S. Gómez-Ruiz, D. Polo-Cerón, S. Prashar, M. Fajardo, V.L. Cruz, J. Ramos, E. Hey-Hawkins, J. Organomet. Chem. 693 (2008) 601–610.
[29] W. Zhong, J.A. Parkinson, M. Guo, P.J. Sadler, J. Biol. Inorg. Chem. 7 (2002) 589–599.
[30] H. Sun, H. Li, R.A. Weir, P.J. Sadler, Angew. Chem., Int. Ed. 37 (1998) 1577–1579.
[31] M. Guo, P.J. Sadler, J. Chem. Soc., Dalton Trans. (2000) 7–9.
[32] M. Guo, H. Sun, S. Bihari, J.A. Parkinson, R.O. Gould, S. Parsons, P.J. Sadler, Inorg. Chem. 39 (2000) 206–215.
[33] M. Guo, H. Sun, H.J. McArdle, L. Gambling, P.J. Sadler, Biochemistry 39 (2000) 10023–10033.
[34] P. Köpf-Maier, D. Krahl, Chem. Biol. Interact. 44 (1983) 317–328.

[35] P. Köpf-Maier, D. Krahl, Naturwissenschaften 68 (1981) 273–274.
[36] P. Köpf-Maier, J. Struct. Biol. 105 (1990) 35–45.
[37] K.J. Stone, R.D. Little, J. Org. Chem. 49 (1984) 1849–1853.
[38] SCALE3 ABSPACK: Empirical Absorption Correction, CrysAlis – Software package, Oxford Diffraction Ltd., 2006.
[39] G.M. Sheldrick, SHELXS-97, Program for Crystal Structure Solution, Göttingen, 1997.
[40] G.M. Sheldrick, SHELXL-97, Program for the Refinement of Crystal Structures, Göttingen, 1997.
[41] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, J. Natl. Cancer Inst. 82 (1990) 1107–1112.