Journal of Organometallic Chemistry 694 (2009) 1981–1987

Contents lists available at ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

Anticancer drugs based on alkenyl and boryl substituted titanocene complexes

Santiago Gómez-Ruiz [a,*], Goran N. Kaluđerović [b,c,*], Željko Žižak [d], Irina Besu [d], Zorica D. Juranić [d], Sanjiv Prashar [a], Mariano Fajardo [a]

[a] Departamento de Química Inorgánica y Analítica, E.S.C.E.T., Universidad Rey Juan Carlos, 28933 Móstoles, Madrid, Spain
[b] Institut für Chemie, Martin-Luther-Universität Halle-Wittenberg, Kurt-Mothes-Straße 2, D-06120 Halle, Germany
[c] Department of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Studentski trg 14, 11000 Belgrade, Serbia
[d] Institute of Oncology and Radiology of Serbia, 11000 Belgrade, Serbia

ARTICLE INFO

Article history:
Received 10 December 2008
Received in revised form 23 January 2009
Accepted 26 January 2009
Available online 7 February 2009

Keywords:
Anticancer drugs
Titanocene complexes
Cytotoxic activity
Hydroboration

ABSTRACT

The alkenyl-substituted titanocene complex [Ti(η^5-C_5H_5)(η^5-C_5H_4{$CMe_2(CH_2CH_2CH{=}CH_2)$})Cl_2] (**1**) has been synthesized and characterized using traditional methods. The reaction of **1** with 9-BBN gave the boryl substituted complex [Ti(η^5-C_5H_5)(η^5-C_5H_4{$CMe_2(CH_2CH_2CH_2CH_2BC_8H_{14})$})$Cl_2$] (**2**). The cytotoxic activity of **1** and **2** was tested against tumour cell lines human adenocarcinoma HeLa, human myelogenous leukemia K562, human malignant melanoma Fem-x, human breast carcinoma MDA-MB-361 and normal immunocompetent cells peripheral blood mononuclear cells PBMC and compared with those of the reference complexes [Ti(η^5-C_5H_5)$_2Cl_2$] (**R1**), [Ti(η^5-C_5H_4Me)$_2Cl_2$] (**R2**) and [Ti(η^5-C_5H_5)(η^5-C_5H_4Si-Me_3)Cl_2] (**R3**). Complex **1** showed higher cytotoxic activities on HeLa, Fem-x and K562 (IC_{50} values from 96.6 ± 3.4 to 149.2 ± 2.9 μM) than the reference complexes **R1**, **R2** and **R3** which presented IC_{50} values from 173.3 ± 6.0 to >200 μM. On the other hand, boryl substituted complex **2**, present slightly lower cytotoxic activities than **1** on HeLa, Fem-x and K562 (IC_{50} values from 155.6 ± 5.5 to 167.9 ± 4.2 μM). However, **2** was the most active of the studied complexes against MDA-MB-361 (IC_{50} value of 161.1 ± 0.1 μM). Structural studies based on DFT calculations of **1** and **2** have also been carried out in order to gain a possible insight into the relationship between metal complex structure and cytotoxicity.

1. Introduction

The fight against cancer is the main and primary target concerning medicinal chemistry. During the last years, investigations in platinum-based anticancer drugs [1–3], have been shifted to non-platinum metal-based agents [4–10]. Thus, an intensive study of metal complexes of Ti, Ga, Ge, Pd, Au, Co, Ru and Sn is helping to improve the problems and side effects associated with the use of platinum compounds [4–10]. Since the discovery of the potential anticancer properties of metallocene dihalides by Köpf-Maier and Köpf [11–20] and the phase I clinical trials carried out for titanocene dichloride in 1993 [21–25] development of similar compounds has been one of the main targets in this field [26,27] due to the absence of any effect on proliferative activity on bone marrow, which is the most usual dose-limiting side-effect of organic drugs. This was, therefore, a very promising result that enhanced the potential of titanocene dichlorides as additives in combination therapy, unfortunately, clinical trials in patients did not have a successful outcome [28,29].

Many biological experiments have demonstrated that titanium, derived from administered titanocene dichloride, accumulates in the nucleic acid rich regions of tumour cells [30–32] and exhibits pronounced inhibition of nucleic acid synthesis [14]. These studies suggested that DNA is the biological target of titanocene compounds. Further work carried out by Sadler and coworkers [33–36], indicated that titanium may reach the cells assisted by the major iron transport protein, "transferrin".

In this context, the current efforts in the titanocene medicinal chemistry are focused on the design of new compounds with different substituents which may increase their cytotoxicity in comparison with that of titanocene dichloride [37–41]. Different research groups have developed alternative synthetic routes starting from fulvenes [42–45] to obtain *ansa*-titanocene complexes with a carbon–carbon bridge [46–49] as well as a variety of other substituted complexes [50–64], all of which were tested as anticancer agents.

Most of the analyzed titanocene complexes present polar substituents or electron withdrawing groups in their structure, which seemed to be the main reason for their high activity in antitumoral tests. In spite of the observations of Köpf and coworkers, which discarded alkyl, alkenyl or aryl substituted *ansa*-titanocene complexes

* Corresponding authors. Address: Department of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Studentski trg 14, 11000 Belgrade, Serbia (G.N. Kaluđerović). Fax: +34 914888143 (S. Gómez-Ruiz); +381 11636061 (G.N. Kaluđerović).
E-mail addresses: santiago.gomez@urjc.es (S. Gómez-Ruiz), goran.kaluderovic@chemie.uni-halle.de, goran@chem.bg.ac.yu (G.N. Kaluđerović).

0022-328X/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jorganchem.2009.01.054

due to their lack of cytotoxicity [65], we have recently reported an increase of the cytotoxicity for titanocene and *ansa*-titanocene complexes that have pendant alkenyl substituents on the cyclopentadienyl rings [66,67]. Following our research on the synthesis, characterization and cytotoxic properties of metal-based anticancer drugs [68–73], we present the synthesis, characterization and the study of the cytotoxicity of different alkenyl and boryl substituted titanocene complexes (Fig. 1) in order to evaluate the influence of these groups on the cytotoxicity of the corresponding complexes. While the cytotoxicity of related alkenyl-substituted complexes has been already determined by us, this work reports the first *in vitro* anticancer tests of boryl substituted titanocene complexes.

2. Experimental

2.1. 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. $[TiCl_4(THF)_2]$, LiMe (1.6 M in Et_2O) and $CH_3COCH_2CH_2CH{=}CH_2$ were purchased from Aldrich. All the commercial reagents were used directly. $Na(C_5H_5)$ and $Na(C_5H_4Me)$ were prepared according to literature procedures [74]. $[Ti(\eta^5\text{-}C_5H_5)_2Cl_2]$ (**R1**) and $[Ti(\eta^5\text{-}C_5H_4Me)_2Cl_2]$ (**R2**) were synthesized by the reaction of two equivalents of $Na(C_5H_5)$ or $Na(C_5H_4Me)$ with $[TiCl_4(THF)_2]$, respectively. $[Ti(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4SiMe_3)Cl_2]$ (**R3**) was prepared by the reaction of $[Ti(\eta^5\text{-}C_5H_5)Cl_3]$ and $Li(C_5H_4SiMe_3)$. 6-(Methyl)-6-(3-butenyl)fulvene was prepared using the methodology described by Little and coworkers [75] and $Li(C_5H_4SiMe_3)$ was prepared as previously reported [76]. $Li\{C_5H_4(CMe_2(CH_2CH_2CH{=}CH_2))\}$ was synthesised by the reaction of one equivalent of 6-(methyl),6-(4-buten-1-yl)fulvene and one equivalent of LiMe [77]. $[Ti(\eta^5\text{-}C_5H_5)Cl_3]$ was prepared according to literature procedures [78]. IR spectra were recorded on a Thermo Nicolet Avatar 330 FT-IR spectrophotometer. ^1H, $^{13}C\{^1H\}$ and $^{11}B\{^1H\}$ NMR spectra were recorded on a Varian Mercury FT-400 spectrometer or on a Bruker AVANCE-400 and referenced to the residual deuterated solvent. Microanalyses were carried out with a Perkin–Elmer 2400 or LECO CHNS-932 microanalyzer. Mass spectroscopic analyses were preformed on a Hewlett–Packard 5988A (*m/z* 50–1000) instrument.

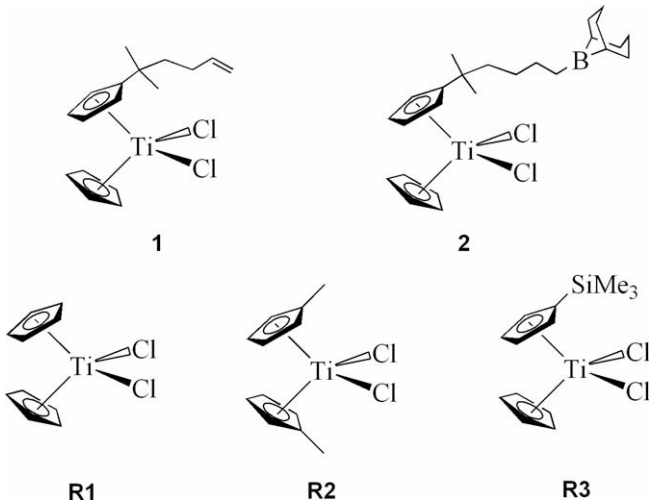


Fig. 1. Titanocene complexes used in the cytotoxicity analysis.

2.2. Preparation of $[Ti(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4\{CMe_2(CH_2CH_2CH{=}CH_2)\})Cl_2]$ (**1**)

$Li\{C_5H_4(CMe_2(CH_2CH_2CH{=}CH_2))\}$ [77] (1.00 g, 5.94 mmol) in THF (50 mL) was added dropwise during 15 min to a solution of $[Ti(\eta^5\text{-}C_5H_5)Cl_3]$ (1.30 g, 5.94 mmol) in THF (150 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. Solvent was removed in vacuo and hexane (400 mL) added to the resulting solid. The mixture was filtered and the filtrate concentrated (30 mL) and cooled to −30 °C to yield crystals of the title complex. Yield 1.70 g, 81%. IR (ZnSe): *ν* 3100 (ν_{CH}), 1640 ($\nu_{CH=CH2}$) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): *δ* 1.36 (s, 6 H, C*Me*$_2$), 1.54, 1.75 (m, C*H*$_2$C*H*$_2$), 4.90 (*cis*), 4.94 (*trans*) (dd, $^3J_{cis}$ 10.8 Hz, $^3J_{trans}$ 17.0 Hz, 1 H, CH_2—CH=C*H*$_2$), 5.70 (m, 1 H, CH_2—C*H*=CH_2), 6.50, 6.61 (m, 2 H, C_5H_4), 6.57 (s, 5 H, C_5H_5) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$, 25 °C): *δ* 26.7, 28.8 (CH_2CH_2), 37.2 (C*Me*$_2$), 46.0 (Cp*C*), 114.4 (CH_2—CH=*C*H$_2$), 118.4, 119.2, 148.4 (C_5H_4), 119.5 (C_5H_5), 138.6 (CH_2—*C*H=CH_2) ppm. EI MS: *m/z* (%) 345 (2) [M^+], 309 (52) [M^+−Cl], 279.0 (39) [$M^+-C_5H_5$], 258 (48) [$M^+-CH_2CH_2CH{=}CH_2-2\times Me$], 218 (100) [$M^+-2\times Cl-CH_2CH_2CH{=}CH_2$))]. Anal. Calc. for $C_{17}H_{22}Cl_2Ti$: C, 59.16; H, 6.43. Found C, 58.97; H, 6.29%.

2.3. Preparation of $[Ti(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4\{CMe_2(CH_2CH_2CH_2CH_2BC_8H_{14})\})Cl_2]$ (**2**)

1 (0.13 g, 0.39 mmol) was dissolved in THF (50 mL), and 9-borabicyclo[3.3.1]nonane (0.5 M in THF) (0.78 mL, 0.39 mmol) was added at room temperature dropwise during 5 min. The solution was then stirred for 15 h. Solvent was removed under reduced pressure to give the title compound as a crystalline solid (0.15 g, 88%). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): *δ* 0.96, 1.02, 1.19, 1.44 (m, 2H, C*H*$_2$C*H*$_2$C*H*$_2$C*H*$_2$-B), 1.35, 1.83 (2 m, 2 H, γ-H of 9-BBN), 1.63, 1.85 (4H) (2 m, 4 H, β and δ-H of 9-BBN), 1.80 (m, 2 H, α-H of 9-BBN), 1.25 (s, 6 H, C*Me*$_2$), 6.42, 6.55 (2 m, each 2 H, C_5H_4), 6.49 (s, 5 H, C_5H_5) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$, 25 °C): *δ* 24.7, 25.5, 27.6, 28.9 (*C*H$_2$*C*H$_2$CH$_2$CH$_2$-B), 23.2, 27.0, 27.1, 33.0 (9-BBN), 36.8 (C*Me*$_2$), 47.4 (Cp*C*), 118.5, 119.4, 148.3 (C_5H_4), 119.8 (C_5H_5) ppm. $^{11}B\{^1H\}$ NMR (85.6 MHz, $CDCl_3$, 25 °C): *δ* 86 ppm. EI MS: *m/z* (%) 439 (20) [$M^+-2\times Me$], 309 (50) [$M^+-Cl-BC_8H_{14}$], 274 (90) [$M^+-2\times Me-BC_8H_{14}$]. Anal. Calc. for $C_{25}H_{37}BCl_2Ti$: C, 64.28; H, 7.98. Found: C, 63.97; H 7.91%.

2.4. Computational details

All DFT calculations were performed by employing the GAUSSIAN 03 program package [79] using the B3LYP functional [80–83]. The 6-31G** basis set was used for all atoms [84–87]. The appropriateness of the chosen functional and basis set for titanium complexes has been stated elsewhere [57,59,88]. All systems have been optimized without symmetry restrictions. The resulting geometries were characterized as equilibrium structures by the analysis of the force constants of normal vibrations (see Supplementary material).

2.5. In vitro studies

2.5.1. Preparation of drug solutions

Stock solutions of the studied titanium complexes were made in dimethyl sulfoxide (DMSO) at a concentration of 20 mM, filtered through Millipore filter, 0.22 μm, before use, and diluted by nutrient medium to various working concentrations. DMSO was used due to solubility problems. Nutrient medium was RPMI 1640 medium, without phenol red, supplemented with L-glutamine (3 mM), streptomycin (100 μg/mL), and penicillin (100 IU/mL), 10% fetal bovine serum (FBS) and 25 mM Hepes, and was adjusted to pH

7.2 by bicarbonate solution. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was dissolved (5 mg/mL) in phosphate buffer saline pH 7.2, and filtered through Millipore filter, 0.22 μm, before use. All reagents were purchased from Sigma Chemicals.

2.5.2. Cell culture

Human cervix adenocarcinoma HeLa, malignant melanoma Fem-x and human breast carcinoma MDA-MB-361 cells were cultured as monolayers in the nutrient medium, while human myelogenous leukemia K562 cells were maintained as suspension culture. The cells were grown at 37 °C in 5% CO_2 and humidified air atmosphere. For the growth of MDA-MB-361 cells and all subsequent experiments, the complete medium was enriched with 1.11 g/L glucose. Peripheral blood mononuclear cells (PBMC) were separated from whole heparinized blood from healthy volunteers by Lymphoprep (Nycomed, Oslo, Norway) gradient centrifugation. Interface cells, washed three times with Haemaccel (aqueous solution supplemented with 145 mM Na^+, 5.1 mM K^+, 6.2 mM Ca^{2+}, 145 mM Cl^- and 35 g/L gelatine polymers, pH 7.4) were counted and resuspended in nutrient medium.

2.5.3. Cell sensitivity analysis

HeLa, Fem-x (2000 cells per well) and MDA-MB-361 cells (10000 cells per well), were seeded into 96-well microtitre plates and 20 h later, after the cell adherence, five different concentrations of the studied compounds were added to the wells. Final concentrations were in the range from 12.5 to 200 μM. The studied compounds were added to a suspension of leukemia K562 cells (3000 cells per well) 2 h after cell seeding, in the same final concentrations applied to HeLa and Fem-x cells. All experiments were carried out in triplicate. PBMC were seeded (150000 cells per well) in nutrient medium enriched with (5 μg/mL) phytohaemaglutinin (PHA – Welcome Diagnostics, England) in 96-well microtitre plates and 2 h later, the studied compounds were added to the wells, in triplicate, to five final concentrations. Only nutrient medium was added to the cells in the control wells. Nutrient medium with corresponding concentrations of compounds, but void of cells was used as blank.

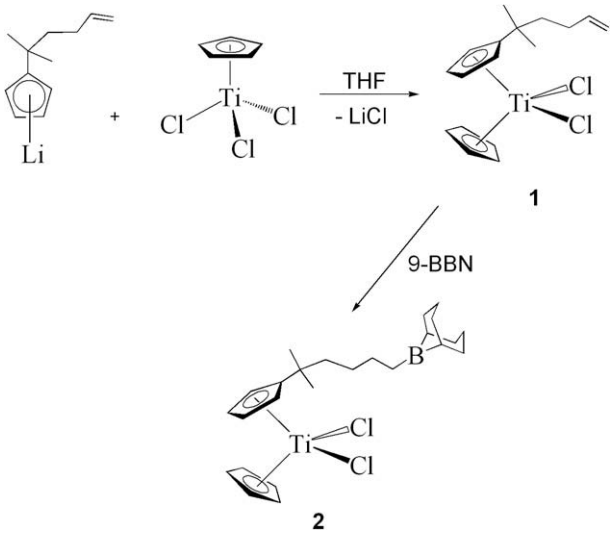
2.5.4. Determination of target cell survival

Cell survival was determined by MTT test according to the method of Mosmann [89] and modified by Ohno and Abe [90], 72 h after drug addition. Immediately afterwards, 20 μL of MTT solution (5 mg/mL in phosphate buffered saline) was added to each well. Samples were incubated for a further 4 h at 37 °C in a humidified atmosphere with 5% CO_2. Then, 100 μL of 10% SDS was added to the wells. Absorbance was measured at 570 nm the next day. To achieve cell survival percentages, absorbance at 570 nm of a sample with cells grown in the presence of various concentrations of agent was divided with absorbance of control sample (the absorbance of cells grown only in nutrient medium), having subtracted from absorbance of a corresponding sample with target cells the absorbance of the blank.

3. Results and discussion

*3.1. Synthesis and characterization of the titanocene complexes **1** and **2***

$[Ti(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4\{CMe_2(CH_2CH_2CH{=}CH_2)\})Cl_2]$ (**1**) was synthesized via the reaction of $Li(C_5H_4\{CMe_2CH_2CH_2CH{=}CH_2\})$ [77] and $[Ti(\eta^5\text{-}C_5H_5)Cl_3]$ in THF (Scheme 1). **1** was isolated as a crystalline solid of high purity. In the ^1H NMR spectrum of **1**, one singlet for the unsubstituted cyclopentadienyl ring protons at 6.57 ppm, two multiplets at 6.50 and 6.61 ppm for the substituted cyclopentadienyl ring protons, and a singlet at 1.36 ppm corresponding to the two methyl groups of the substituent were observed. The alkenyl fragment exhibited four sets of signals, two corresponding to the CH_2 alkylic protons (two multiplets at 1.54 and 1.75 ppm), one for the proton of the C-γ (a multiplet at 5.70 ppm) and two corresponding to the terminal olefinic protons (multiplets at 4.90 and 4.94 ppm). The $^{13}C\{^1H\}$ NMR spectrum of **1** showed one signal at 119.5 ppm assigned to the unsubstituted C_5 ring and three signals at 118.4, 119.2 and 148.4 for the substituted C_5 ring. The carbon atom bonded to the cyclopentadienyl ring, was observed in the spectrum as one signal at 46.0 ppm, and the carbon atoms of the two methyl groups were observed as one signal at 37.2 ppm. In addition, four signals, at 26.7, 28.8, 114.4 and 138.6 ppm, were assigned to the alkenyl moiety. The alkenyl substituent of the cyclopentadienyl ligand of the titanocene dichloride complex **1**, was suitable for hydroboration reactions with the highly selective 9-borabicyclo[3.3.1]nonane (9-BBN). Thus, the reaction of **1** with 9-BBN gave the resulting product from the anti-Markonikoff addition $[Ti(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4\{CMe_2(CH_2CH_2CH_2CH_2BC_8H_{14})\})Cl_2]$ (**2**) (Scheme 1). Complex **2** was isolated as a deep red crystalline solid. Completion of the hydroboration reaction was demonstrated by ^1H NMR spectroscopy. The recorded spectra showed the absence of the olefinic proton signals of the alkenyl moiety and the appearance of four multiplets corresponding to the $-CH_2CH_2CH_2CH_2B-$ fragment in the region between 0.7 and 1.6 ppm. The ^1H NMR spectrum of **2** showed, for the boryl moiety, four multiplets, between ca. 1.2 and 1.9 ppm, corresponding to the four different proton environments. For the protons of the cyclopentadienyl rings, signals with similar patterns than those described for **1** were observed. **1** and **2** were also characterized by mass spectrometry and elemental analysis (see Sections 2.2 and 2.3). In addition, deuterated DMSO solutions of complexes **1** and **2** were prepared under an air atmosphere, in order to observe the stability in solution of these complexes. NMR spectrum of complex **2** showed signals of decomposition after several hours, while spectrum of complex **1** presented total stability in these conditions.



Scheme 1.

3.2. Structural studies

It is well known that the knowledge of the geometry and structural parameters determined by single crystal X-ray diffraction

studies of the complexes, is a tool that may help in the interpretation of the structure–cytotoxicity relationship. However, our efforts to crystallize the titanocene complexes were unsuccessful. In order to circumvent this problem, density functional theory (DFT) calculations were carried out for **1** and **2** at the B3LYP level [80–83] using the 6-31G** basis set [84–87]. Selected bond lengths and angles of the optimized structure of the titanocene compounds are listed in Table 1. The calculated structures of **1** and **2** are presented in Figs. 2 and 3, respectively, and show the distorted tetrahedral geometry of titanium, with the coordination of the cyclopentadienyl rings in an η^5-manner.

The bond lengths between titanium and the cyclopentadienyl carbons of the titanocene complexes **1** and **2** vary from 233.6 to 258.2 pm, observing the longest Ti–C lengths for the substituted carbon atom of the cyclopentadienyl ring (C(1)). The calculated distances Ti–Cent in **1** and **2** are between 210 and 214 pm. The Cent–Ti–Cent angles of about 130° and the Cl(1)–Ti(1)–Cl(2) angles of ca. 97° are similar, and comparable with those recorded for the X-ray crystal structures of related titanocene complexes [91–98].

The C(17)–C(18) distance for the double bonds of the alkenyl chain of **1** shows a similar value (133.4 pm) and is in very good agreement with others reported in X-ray crystal structures of metallocene complexes with alkenyl groups [77,98] and with those of other calculated structures of titanocene derivatives with alkenyl substituents [67]. The bond length C(17)–C(18) is, however, about 22 pm longer in **2** than in **1**, as it corresponds to a C–C single bond (155.4 pm). In addition, the bond length B(1)–C(17) in **2** is close to 158 pm.

Both molecules present similar structural parameters, however, complex **2** is bulkier than **1**. The distance between the titanium centre and the most remote carbon atom in the molecule in **1** is

Table 1
Selected bond lengths (pm) and angles (°) for **1** and **2**.

	1	2
Ti(1)–Cent(1)	212.7	212.7
Ti(1)–Cent(2)	210.0	210.0
av Ti(1)–C(C(1)–C(5))[a]	244.4	244.4
av Ti(1)–C(C(6)–C(10))[a]	242.1	242.1
Ti(1)–C(1)	258.2	258.2
Ti(1)–C(2)	247.2	247.3
Ti(1)–C(3)	235.8	235.9
Ti(1)–C(4)	233.7	233.6
Ti(1)–C(5)	247.2	247.0
Ti(1)–C(6)	243.2	243.3
Ti(1)–C(7)	241.7	241.6
Ti(1)–C(8)	241.0	241.0
Ti(1)–C(9)	239.8	239.7
Ti(1)–C(10)	244.9	244.8
Ti(1)–Cl(1)	234.9	234.8
Ti(1)–Cl(2)	235.0	235.2
C(11)–C(14)	156.4	156.5
C(14)–C(15)	153.2	153.4
C(15)–C(16)	150.9	154.1
C(16)–C(17)	133.4	155.4
C(17)–B(1)		157.7
Cent(1)–Ti–Cent(2)	129.8	132.0
Cl(1)–Ti(1)–Cent(1)	105.3	105.4
Cl(1)–Ti(1)–Cent(2)	106.0	106.1
Cl(2)–Ti(1)–Cent(1)	106.2	106.1
Cl(2)–Ti(1)–Cent(2)	105.0	104.9
Cl(1)–Ti(1)–Cl(2)	97.2	97.2
C(1)–C(11)–C(14)	107.7	107.7
C(11)–C(14)–C(15)	116.6	117.0
C(14)–C(15)–C(16)	115.4	114.0
C(15)–C(16)–C(17)	127.8	114.7
C(16)–C(17)–B(1)		109.7

Cent(1) and Cent(2) are the centroids of C(1)–C(5) and C(6)–C(10), respectively.
[a] Refers to the average bond distance between Ti(1) and the carbon atoms of the C_5 ring of the corresponding cyclopentadienyl moiety.

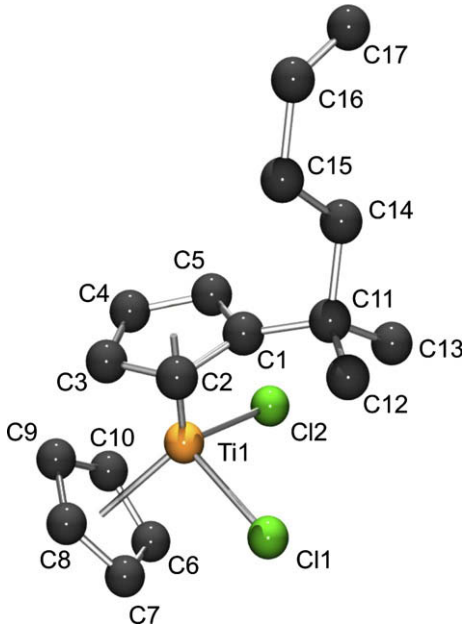


Fig. 2. DFT-calculated structure of **1** (hydrogen atoms are omitted for clarity).

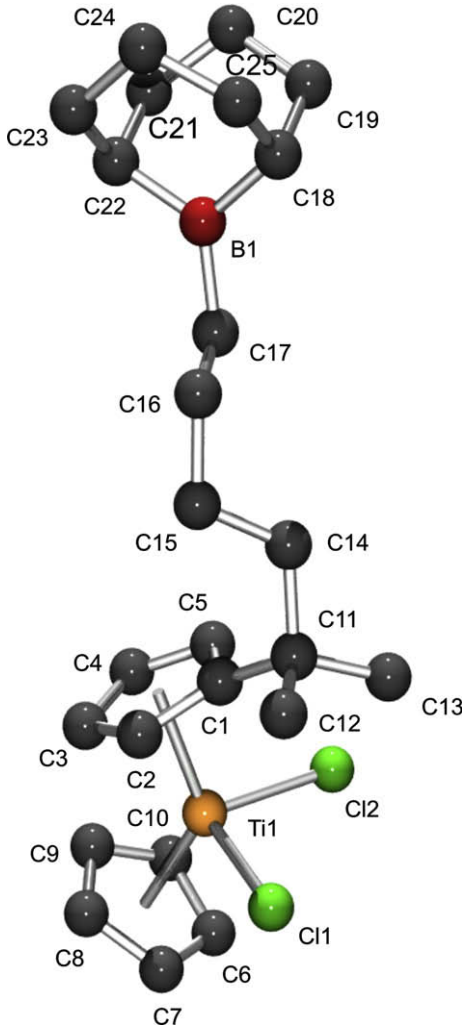


Fig. 3. DFT-calculated structure of **2** (hydrogen atoms are omitted for clarity).

Table 2
IC_{50} (μM) for the 72 h of action of the studied compounds and cisplatin [71] on HeLa, K562, Fem-x, MDA-MB-361, PBMC and PBMC stimulated with PHA determined by MTT test.

Compound	IC_{50} ± SD (μM)					
	HeLa	K562	Fem-x	MDA-MB-361	PBMC−PHA	PBMC+PHA
1	149.2 ± 2.9	96.6 ± 3.4	133.6 ± 9.4	>200	149.8 ± 3.1	142.5 ± 0.9
2	166.3 ± 7.4	155.6 ± 5.5	167.9 ± 4.2	161.1 ± 0.1	>200	>200
R1	>200	>200	177.7 ± 4.9	>200	>200	199.8 ± 9.9
R2	>200	173.3 ± 6.0	198.6 ± 4.3	>200	>200	180.9 ± 4.3
R3	>200	>200	>200	>200	>200	>200
Cisplatin	4.4 ± 0.3	5.7 ± 0.3	4.7 ± 0.3	13.0 ± 1.7	33.6	26 ± 6

ca. 763 pm, while in **2** this distance is much longer, about 1159 pm. It has been previously observed that titanocene complexes that cross the cellular membrane may remain with the cyclopentadienyl ligands intact [35,36]. Therefore, the size and bulkiness of the boryl group may affect the permeation process and the cytotoxicity of the substituted complexes.

3.3. Cytotoxic studies

Titanocene complexes from this study [Ti(η^5-C_5H_5)(η^5-C_5H_4{$CMe_2(CH_2CH_2CH{=}CH_2)$})Cl_2] (**1**) and [Ti(η^5-C_5H_5)(η^5-C_5H_4{$CMe_2(CH_2CH_2CH_2CH_2BC_8H_{14})$})$Cl_2$] (**2**) and the reference complexes [Ti(η^5-C_5H_5)$_2Cl_2$] (**R1**), [Ti(η^5-C_5H_4Me)$_2Cl_2$] (**R2**) and [Ti(η^5-C_5H_5)(η^5-$C_5H_4SiMe_3$)Cl_2] (**R3**) have been used in order to understand the possible relationship between the different substituents on the cyclopentadienyl ring and the cytotoxic activity. In particular, this study was designed to observe the possible influence of a boryl group on the final antiproliferative activity of the synthesized compounds, due to the lack of anticancer studies of metal complexes containing boryl groups. In previous studies, we have observed that the inclusion of an alkenyl group resulted in an increase of the cytotoxic activity of the titanocene complex [66,67]. However, boryl groups, as Lewis acids, should interact with greater ease than titanium(IV) units with the Lewis base-sites of DNA, and this may increase the cytotoxicity of the complexes due to the cooperative effect of titanium and boron. Nevertheless, according to the results, the effect of the alkenyl group in the antiproliferative activity is higher. This can probably be attributed to the facility of the alkenyl-substituted complexes to cross the membrane and reach the cell nucleus. The alkenyl-substituted complex **1**, presents good activity against K562 (IC_{50} 96.6 ± 3.4 μM) and moderate activity on HeLa (IC_{50} 149.2 ± 2.9 μM) and Fem-x (IC_{50} 133.6 ± 9.4 μM), while complex **2** presents only moderate activity on K562, HeLa and Fem-x (Table 2). On the other hand, **2** is the only titanocene complex active against MDA-MB-361 (IC_{50} 161.1 ± 0.1 μM) and this may be due to the possible formation of a B···N adduct of the complex with the DNA base-sites, favouring the further interaction of the titanium unit with the double-helix. Interestingly, **2** is not active against normal inmunocompetent cells, showing an unexpected selectivity on cancer cells (Table 2).

Titanocene complexes **1** and **2** are in all cases more active than the reference complexes **R1**, **R2** and **R3** (Fig. 4). The presence of alkyl groups on the cyclopentadienyl rings (**R2**) leads to a slight increase in the cytotoxicity in some studied cells compared to **R1**, however, the $SiMe_3$ group decreases the cytotoxicity (Table 2). This detriment of the cytotoxicity of the complex with the $SiMe_3$ group (**R3**), contrasts with the enhancement of the antiproliferative activity reported by McGowan and coworkers using trimethylsilyl substituted titanocene complexes [58].

As in anticancer chemotherapy based on titanocene complexes, the search of the increase of the antiproliferative activity compared with [Ti(η^5-C_5H_5)$_2Cl_2$] (**R1**) is very important, titanocene

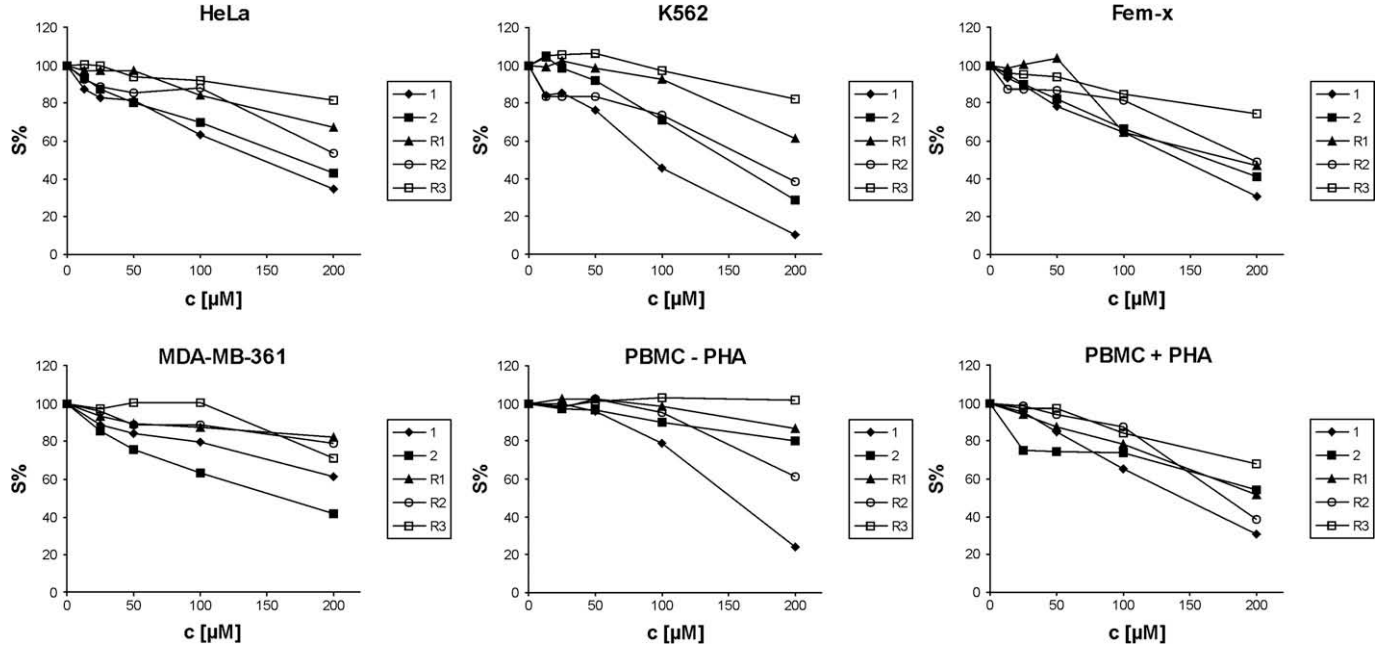


Fig. 4. Representative graphs showing survival of HeLa, K562, Fem-x, MDA-MB-361, PBMC and PBMC + PHA (PBMC stimulated with PHA) cells grown for 72 h in the presence of increasing concentrations of the studied titanium complexes **1**, **2**, **R1**, **R2** and **R3**.

complexes bearing boryl and alkenyl groups are good candidates for further investigations.

4. Conclusions

We have synthesized a new titanocene complex, [Ti(η^5-C_5H_5)(η^5-C_5H_4{$CMe_2(CH_2CH_2CH{=}CH_2)$})Cl_2] (**1**), bearing a long chain alkenyl group, which on reaction with 9-BBN gives the boryl substituted complex [Ti(η^5-C_5H_5)(η^5-C_5H_4(CMe_2{$CH_2CH_2CH_2$-$CH_2BC_8H_{14}$})Cl_2] (**2**). **1** and **2**, as well as the reference complexes [Ti(η^5-C_5H_5)$_2Cl_2$] (**R1**), [Ti(η^5-C_5H_4Me)$_2Cl_2$] (**R2**) and [Ti(η^5-C_5H_5)(η^5-$C_5H_4SiMe_3$)Cl_2] (**R3**), were tested as antitumoral agents in different tumour cell lines, in order to understand the possible relationship between the different groups attached to the cyclopentadienyl ring and the cytotoxic activity. The studied titanocene anti-tumour agents showed a dose-dependent antiproliferative effect towards all cell lines and on human PBMC and stimulated PBMC. The presence of alkyl groups on the cyclopentadienyl rings slightly increases the cytotoxicity of the complexes in some studied cells with respect to **R1**, while the $SiMe_3$ group decreases the cytotoxicity. A more pronounced increase is observed when an alkenyl fragment is included as a substituent of the cyclopentadienyl moiety. The boryl group on the cyclopentadienyl ring examined in this study had a negative influence on the activity on HeLa, Fem-x and K562 and a positive effect on MDA-MB-361. **1** and **2** present comparable if not somewhat lower activities than those described for some previously analyzed titanocene complexes which have polar substituents such as alkoxo-, or amino-groups on the cyclopentadienyl ring and higher activities than all the reference complexes (**R1**, **R2** and **R3**).

Future work, already in progress, will now focus on the improvement of the cytotoxic nature of **1** by the manipulation of the alkenyl groups and introduction of other different functional groups to improve the water solubility and cytotoxic activity of the complexes. In addition, further studies on hydroboration reactions starting from similar alkenyl-substituted complexes and different boryl groups will be carried out, in order to observe the influence of the different groups on the cytotoxicity of the final complex.

Acknowledgements

We gratefully acknowledge financial support from the Ministerio de Educación y Ciencia, Spain (Grant No. CTQ2005-07918-C02-02/BQU) and Ministry of Science and Environmental Protection of the Republic of Serbia (Grant Nos. 142010 and 145006).

Appendix A. Supplementary data

DFT data of the calculated structures are included in the supplementary material. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2009.01.054.

References

[1] B. Lippert, Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug, Wiley Interscience, 1999.
[2] A.S. Abu-Surrah, M. Kettunen, Curr. Med. Chem. 13 (2006) 1337–1357.
[3] C.S. Allardyce, P.J. Dyson, Platinum Metals Rev. 45 (2001) 62–69.
[4] I. Ott, R. Gust, Arch. Pharm. 340 (2007) 117–126.
[5] M.A. Jakupec, M. Galanski, V.B. Arion, C.G. Hartinger, B.K. Keppler, Dalton Trans. (2008) 183–194.
[6] P. Yang, M. Guo, Coord. Chem. Rev. 185–186 (1999) 189–211.
[7] S.K. Hadjikakou, N. Hadjiliadis, Coord. Chem. Rev. 253 (2009) 235–249.
[8] K. Strohfeldt, M. Tacke, Chem. Soc. Rev. 37 (2008) 1174–1187.
[9] P.M. Abeysinghe, M.M. Harding, Dalton Trans. (2007) 3474–3482.
[10] R. Gust, D. Posselt, K. Sommer, J. Med. Chem. 47 (2004) 5837–5846.
[11] P. Köpf-Maier, H. Köpf, Angew. Chem., Int. Ed. Engl. 18 (1979) 477–478.
[12] P. Köpf-Maier, H. Köpf, Z. Naturforsch., B: Anorg. Chem. Org. Chem. 34 (1979) 805–807.
[13] P. Köpf-Maier, M. Leitner, R. Voigtländer, H. Köpf, Z. Naturforsch., Teil C 34 (1979) 1174–1176.
[14] P. Köpf-Maier, B. Hesse, R. Voigtländer, H. Köpf, J. Cancer, Res. Clin. Oncol. 97 (1980) 31–39.
[15] P. Köpf-Maier, B. Hesse, H. Köpf, J. Cancer Res. Clin. Oncol. 96 (1980) 43–51.
[16] P. Köpf-Maier, M. Leitner, H. Köpf, J. Inorg. Nucl. Chem. 42 (1980) 1789–1791.
[17] P. Köpf-Maier, H. Köpf, Naturwissenschaften 67 (1980) 415–416.
[18] P. Köpf-Maier, W. Wagner, H. Köpf, Naturwissenschaften 68 (1981) 272–273.
[19] P. Köpf-Maier, S. Grabowski, H. Köpf, Eur. J. Med. Chem. 19 (1984) 347–352.
[20] P. Köpf-Maier, S. Grabowski, J. Liegener, H. Köpf, Inorg. Chim. Acta 108 (1985) 99–103.
[21] W.E. Berdel, H.J. Schmoll, M.E. Scheulen, A. Korfel, M.F. Knoche, A. Harstrick, F. Bach, J. Baumgart, G. Sass, Onkologie 16 (1993) R172.
[22] W.E. Berdel, H.J. Schmoll, M.E. Scheulen, A. Korfel, M.F. Knoche, A. Harstrci, F. Bach, J. Baumgart, G. Sass, J. Cancer Res. Clin. Oncol. 120 (1994) 172.
[23] A. Korfel, M.E. Scheulen, H.J. Schmoll, O. Gründel, A. Harstrick, M. Knoche, L.M. Fels, M. Skorzec, F. Bach, J. Baumgart, G. Sass, S. Seeber, E. Thiel, W.E. Berdel, Clin. Cancer Res. 4 (1998) 2701–2708.
[24] C.V. Christodoulou, D.R. Ferry, D.W. Fyfe, A. Young, J. Doran, T.M.T. Sheehan, A. Eliopoulos, K. Hale, J. Baumgart, G. Sab, D.J. Kerr, J. Clin. Oncol. 16 (1998) 2761–2769.
[25] T. Schilling, K.B. Keppler, M.E. Heim, G. Niebch, H. Dietzfelbinger, J. Rastetter, A.-R. Hanauske, Invest. New Drugs 13 (1996) 327–332.
[26] M.M. Harding, G. Mokdsi, Curr. Med. Chem. 7 (2000) 1289–1303.
[27] P.M. Abeysinghe, M.M. Harding, Dalton Trans. 20 (2007) 3474–3482.
[28] G. Lummen, H. Sperling, H. Luboldt, T. Otto, H. Rubben, Cancer Chemother. Pharmacol. 42 (1998) 415–417.
[29] N. Kröger, U.R. Kleeberg, K.B. Mross, L. Edler, G. Sass, D.K. Hossfeld, Onkology 23 (2000) 60–62.
[30] P. Köpf-Maier, D. Krahl, Chem.–Biol. Interact. 44 (1983) 317–328.
[31] P. Köpf-Maier, D. Krahl, Naturwissenschaften 68 (1981) 273–274.
[32] P. Köpf-Maier, J. Struct. Biol. 105 (1990) 35–45.
[33] H. Sun, H. Li, R.A. Weir, P.J. Sadler, Angew. Chem. Int. Ed. 37 (1998) 1577–1579.
[34] M. Guo, P.J. Sadler, J. Chem. Soc., Dalton Trans. (2000) 7–9.
[35] M. Guo, H. Sun, S. Bihari, J.A. Parkinson, R.O. Gould, S. Parsons, P.J. Sadler, Inorg. Chem. 39 (2000) 206–215.
[36] M. Guo, H. Sun, H.J. McArdle, L. Gambling, P.J. Sadler, Biochemistry 39 (2000) 10023–10033.
[37] G. Mokdsi, M.M. Harding, Metal-Based Drug 5 (1998) 207–215.
[38] O.R. Allen, L. Croll, A.L. Gott, R.J. Knox, P.C. McGowan, Organometallics 23 (2004) 288–292.
[39] J.R. Boyles, M.C. Baird, B.G. Campling, N. Jain, J. Inorg. Biochem. 84 (2001) 159–162.
[40] P.W. Causey, M.C. Baird, Organometallics 23 (2004) 4486–4494.
[41] R. Meyer, S. Brink, C.E.J. van Rensburg, G.K. Joone, H. Görls, S. Lotz, J. Organomet. Chem. 690 (2005) 117–125.
[42] J.J. Eisch, S. Xian, F.A. Owuor, Organometallics 17 (1998) 5219–5221.
[43] J.J. Eisch, F.A. Owuor, S. Xian, Organometallics 18 (1999) 1583–1585.
[44] K.M. Kane, P.J. Shapiro, A. Vij, R. Cubbon, A.L. Rheingold, Organometallics 16 (1997) 4567–4571.
[45] S. Fox, J.P. Dunne, M. Tacke, J.F. Gallagher, Inorg. Chim. Acta 357 (2004) 225–234.
[46] R. Teuber, G. Linti, M. Tacke, J. Organomet. Chem. 545–546 (1997) 105–110.
[47] F. Hartl, L. Cuffe, J.P. Dunne, S. Fox, T. Mahabiersing, M. Tacke, J. Mol. Struct. Theochem. 559 (2001) 331–339.
[48] M. Tacke, J.P. Dunne, S. Fox, G. Linti, R. Teuber, J. Mol. Struct. 570 (2001) 197–202.
[49] S. Fox, J.P. Dunne, D. Dronskowski, D. Schmitz, M. Tacke, Eur. J. Inorg. Chem. (2002) 3039–3046.
[50] M. Tacke, L.T. Allen, L.P. Cuffe, W.M. Gallagher, Y. Lou, O. Mendoza, H. Müller-Bunz, F.J.K. Rehmann, N. Sweeney, J. Organomet. Chem. 689 (2004) 2242–2249.
[51] F.J.K. Rehmann, L.P. Cuffe, O. Mendoza, D.K. Rai, N. Sweeney, K. Strohfeldt, W.M. Gallagher, M. Tacke, Appl. Organomet. Chem. 19 (2005) 293–300.
[52] M. Tacke, L.P. Cuffe, W.M. Gallagher, Y. Lou, O. Mendoza, H. Müller-Bunz, F.J.K. Rehmann, N. Sweeney, J. Inorg. Biochem. 98 (2004) 1987–1994.
[53] F.J.K. Rehmann, A.J. Rous, O. Mendoza, C. Pampillon, K. Strohfeldt, N. Sweeney, W.M. Gallagher, M. Tacke, Polyhedron 24 (2005) 1250–1255.
[54] N. Sweeney, O. Mendoza, H. Müller-Bunz, C. Pampillón, F.-J.K. Rehmann, K. Strohfeldt, M. Tacke, J. Organomet. Chem. 690 (2005) 4537–4544.
[55] G. Kelter, N. Sweeney, K. Strohfeldt, H.-H. Fiebig, M. Tacke, Anti-Cancer Drug 16 (2005) 1091–1098.
[56] N. Sweeny, W.M. Gallagher, H. Müller-Bunz, C. Pampillón, K. Strohfeldt, M. Tacke, J. Inorg. Biochem. 100 (2006) 1479–1486.
[57] M. Hogan, J. Claffey, C. Pampillón, R.W.G. Watson, M. Tacke, Organometallics 26 (2007) 2501–2506.
[58] O.R. Allen, A.L. Gott, J.A. Hartley, J.M. Hartley, R.J. Knox, P.C. McGowan, Dalton Trans. (2007) 5082–5090.
[59] C. Pampillón, N.J. Sweeney, K. Strohfeld, M. Tacke, J. Organomet. Chem. 692 (2007) 2153–2159.

[60] C. Pampillón, J. Claffey, M. Hogan, K. Strohfeldt, M. Tacke, Transition Met. Chem. 32 (2007) 434–441.
[61] T. Hickey, J. Claffey, E. Fitzpatrick, M. Hogan, C. Pampillón, M. Tacke, Invest. New Drugs 25 (2007) 425–433.
[62] C. Pampillón, J. Claffey, M. Hogan, M. Tacke, Z. Anorg. Allgem. Chem. 633 (2007) 1695–1700.
[63] P.W. Causey, M.C. Baird, S.P.C. Cole, Organometallics 23 (2004) 4486–4494.
[64] G.D. Potter, M.C. Baird, M. Chan, S.P.C. Cole, Inorg. Chem. Commun. 9 (2006) 1114–1116.
[65] P. Köpf-Maier, H. Köpf, Chem. Rev. 20 (1987) 1137–1152.
[66] 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.
[67] 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.
[68] S. Gómez-Ruiz, G.N. Kaluđerović, S. Prashar, E. Hey-Hawkins, A. Erić, Ž. Žižak, Z.D. Juranić, J. Inorg. Biochem. 102 (2008) 2087–2096.
[69] G.N. Kaluđerović, Dj. Miljković, M. Momčilović, V.M. Đinović, M.M. Stojković, T.J. Sabo, V. Trajković, Int. J. Cancer 116 (2005) 479–486.
[70] S. Mijatović, D. Maksimović-Ivanić, J. Radović, D. Miljković, G.N. Kaluđerović, T.J. Sabo, V. Trajković, Cell. Mol. Life Sci. 62 (2005) 1275–1282.
[71] G.N. Kaluđerović, V.M. Đinović, Z.D. Juranić, T.P. Stanojković, T.J. Sabo, J. Inorg. Biochem. 99 (2005) 488–498.
[72] M.E. Kelly, A. Dietrich, S. Gómez-Ruiz, B. Kalinowski, G.N. Kaluđerović, T. Müller, R. Paschke, J. Schmidt, D. Steinborn, C. Wagner, H. Schmidt, Organometallics 27 (2008) 4917–4927.
[73] S. Grurić-Šipka, M.A.M. Alshtewi, D. Jeremić, G.N. Kaluđerović, S. Gómez-Ruiz, Ž. Žižak, Z.D. Juranić, T.J. Sabo, J. Serb. Chem. Soc. 73 (2008) 619–630.
[74] T.K. Panda, M.T. Gamer, P.W. Roesky, Organometallics 22 (2003) 877–890.
[75] K.J. Stone, R.D. Little, J. Org. Chem. 49 (1984) 1849–1853.
[76] W.J. Evans, T.J. Boyle, J.W. Ziller, Organometallics 11 (1992) 3903–3907.
[77] S. Gómez-Ruiz, D. Polo-Cerón, S. Prashar, M. Fajardo, A. Antiñolo, A. Otero, Eur. J. Inorg. Chem. (2007) 4445–4455.
[78] A.M. Cardoso, R.J.H. Clark, S. Moorhouse, J. Chem. Soc., Dalton Trans. (1980) 1156–1160.
[79] GAUSSIAN 03, Revision C.02, Gaussian, M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery, Jr.T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J. B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople, Inc., Wallingford CT, 2004.
[80] A.D. Becke, J. Chem. Phys. 98 (1993) 5648–5652.
[81] C. Lee, W. Yang, R.G. Parr, Phys. Rev. B 37 (1988) 785–789.
[82] S.H. Vosko, L. Wilk, M. Nusair, Can. J. Phys. 58 (1980) 1200–1211.
[83] P.J. Stephens, F.J. Devlin, C.F. Chabalowski, M.J. Frisch, J. Phys. Chem. 98 (1994) 11623–11627.
[84] W.J. Hehre, R. Ditchfield, J.A. Pople, J. Chem. Phys. 56 (1972) 2257–2261.
[85] J.D. Dill, J.A. Pople, J. Chem. Phys. 62 (1975) 2921–2923.
[86] M.M. Francl, W.J. Petro, W.J. Hehre, J.S. Binkley, M.S. Gordon, D.J. DeFrees, J.A. Pople, J. Chem. Phys. 77 (1982) 3654–3665.
[87] V. Rassolov, J.A. Pople, M.A. Ratner, T.L. Windus, J. Chem. Phys. 109 (1998) 1223–1229.
[88] C. Pampillón, J. Claffey, M. Hogan, M. Tacke, BioMetals 21 (2008) 197–204.
[89] T. Mosmann, J. Immunol. Meth. 65 (1983) 55–63.
[90] M. Ohno, T. Abe, J. Immunol. Meth. 145 (1991) 199–203.
[91] P. Beagley, P. Davies, H. Adams, C. White, Can. J. Chem. (2001) 731–741.
[92] S.T. Chacon, E.B. Coughlin, L.M. Henling, J.E. Bercaw, J. Organomet. Chem. 497 (1995) 171–180.
[93] H. Lang, S. Blau, B. Nuber, L. Zsolnai, Organometallics 14 (1995) 3216–3223.
[94] S.-J. Kim, Y.-J. Lee, E. Kang, S.H. Kim, J. Ko, B. Lee, M. Cheong, I.-H. Suh, S.O. Kang, Organometallics 22 (2003) 3958–3966.
[95] B. Douziech, R. Choukroun, C. Lorber, B. Donnadieu, J. Organomet. Chem. 649 (2002) 15–20.
[96] G. Tian, B. Wang, X. Dai, S. Xu, X. Zhou, J. Sun, J. Organomet. Chem. 634 (2001) 145–152.
[97] S. Gómez-Ruiz, A. Garcés, S. Prashar, M. Fajardo, A. Antiñolo, A. Otero, Inorg. Chim. Acta 362 (2009) 1042–1046.
[98] A. Antiñolo, M. Fajardo, S. Gómez-Ruiz, I. López-Solera, A. Otero, S. Prashar, A.M. Rodríguez, J. Organomet. Chem. 683 (2003) 11–22.