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Synthesis, characterization and cytotoxic effect of ring-substituted and *ansa*-bridged vanadocene complexes

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ABSTRACT

The cytotoxic effect of vanadocene dichloride (Cp_2VCl_2, **1**) and its ring-substituted, $(\eta^5\text{-}C_5H_4Me)_2VCl_2$ (**2**), $(\eta^5\text{-}C_5Me_5)_2VCl_2$ (**3**), $(\eta^5\text{-}C_5H_4R)_2VCl_2$ (**4**: R = $MeOCH_2CH_2$–, **5**: R = 2-$MeOC_6H_4CH_2$–, **6**: R = 4-$MeOC_6H_4CH_2$–) and *ansa*-bridged analogs $Me_2C(\eta^5\text{-}C_5H_4)_2VCl_2$ (**7**) and $Me_4C_2(\eta^5\text{-}C_5H_4)_2VCl_2$ (**8**) was investigated. Synthesis of two new methoxy-functionalized compounds (**4** and **5**) is described. They were characterized by spectroscopic methods and X-ray diffraction analysis. The cytotoxicity studies were performed with leukemic cells MOLT-4.

1. Introduction

Chemotherapy is the major strategy in the treatment of human leukemia. This area has experienced intensive advance during recent years. For example, the frequency of complete response achieved with initial treatment of chronic lymphocytic leukemia has increased from less than 5% with single-agent alkylators to more than 50% with multiagent chemoimmunotherapy over the past 20 years [1]. But even now, the prognosis of some leukemia types is not satisfactory. Resistant forms of chronic lymphocytic leukemia still exist. The adults suffering from the acute lymphoblastic leukemia almost universally relapse after the conventional chemotherapeutical protocols [2]. Therefore new chemotherapeutical strategies are desperately needed.

Bent metallocene complexes (Cp_2MX_2; M = Ti, V, Mo; X = halide) are known as the potent antitumor agents with low general toxicity [3,4]. So far the main attention has been focused on titanocene dichloride (Cp_2TiCl_2; TDC). It has given promising results in preclinical [5] and phase I of the clinical trials [6]. However, disappointing results from phase II of the trials [7,8] stimulated enhanced interest in the modified compounds. The modifications of the dihalides Cp_2MX_2 done by exchange of X for X′ = pseudohalide, carboxylate, thiolate, amino acid led to limited improvements because putative active species "Cp_2M" stay unchanged. However, this type of substitution can overcome the problems of the parent compounds with biological incompatibility [9] and low water solubility [10].

Modification of the cyclopentadienyl rings is a more promising approach for solving above mentioned problems. Moreover, it could be a potent tool for drug design enabling a fine tuning of the cytostatic effects or a design of the bifunctional drugs [11,12]. Indeed, recent preclinical tests made with some new titanocene compounds show improved activity toward the cisplatin resistant tumor cell lines depending on the substituents used [13–16]. The Cp ring modifications could be also used for studies elucidating the mechanism of antitumor action. For this purpose, the compounds with luminescent markers connected to Cp rings were designed [17]. In the case of the group V and group VI metallocenes, only few ring-substituted compounds were the subjects of the biological studies [18–20].

Currently ongoing comprehensive study of the antitumor activity mechanism of bent metallocenes [21–28] and our long-standing interest in chemistry of Cp_2VCl_2 (**1**) led us to examine its cytotoxic activity toward T-lymphocytic leukemia cells MOLT-4. This study is focused on the substitution effect. It includes methyl-substituted $(\eta^5\text{-}C_5H_4Me)_2VCl_2$ (**2**), $(\eta^5\text{-}C_5Me_5)_2VCl_2$ (**3**), methoxy-substituted $(\eta^5\text{-}C_5H_4R)_2VCl_2$ (**4**: R = $MeOCH_2CH_2$–,

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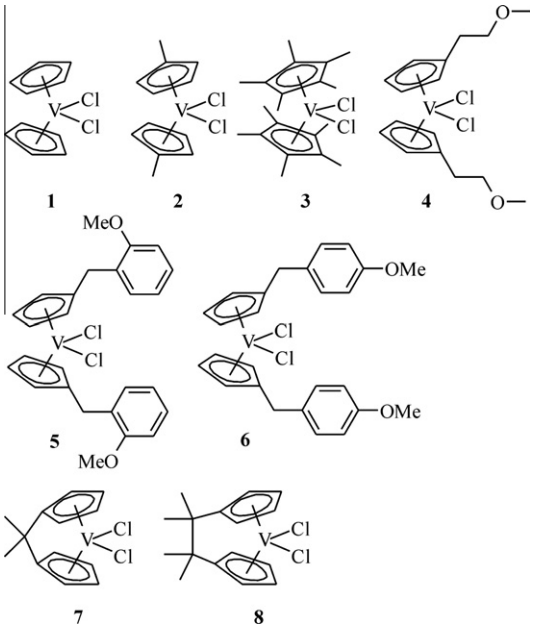
5: R = 2-$MeOC_6H_4CH_2$–, **6**: R = 4-$MeOC_6H_4CH_2$–), *ansa*-bridged vanadocene dichlorides $Me_2C(\eta^5\text{-}C_5H_4)_2VCl_2$ (**7**) and $Me_4C_2(\eta^5\text{-}C_5H_4)_2VCl_2$ (**8**), Scheme 1. While this work was in progress, Tacke et al. reported two largely complementary studies of the antitumor properties of the six methoxybenzyl-substituted vanadocene derivatives [18,19], one of which (**6**) is among those investigated here.

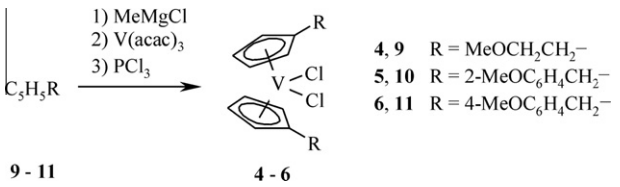
2. Results and discussion

2.1. Preparation and characterization

Literature procedures were used for synthesis of vanadocene(IV) dichlorides **1**–**3** and **7**–**8** [29–33]. Compounds **4**–**6** were prepared according to the procedure previously used for the preparation of *ansa*-vanadocene compounds (Scheme 2) [32,34]. Deprotonation of methoxy-substituted cyclopentadienes (**9**–**11**) with MeMgCl produces appropriate cyclopentadienides that react with $V(acac)_3$ to give appropriate vanadocene(III) monochlorides $(\eta^5\text{-}C_5H_4R)_2VCl$. These intermediates were not isolated. They were treated with PCl_3 to give desired dichloride compounds **4**–**6**. Of course, one may expect appearance of acetylacetonates $(\eta^5\text{-}C_5H_4R)_2V(acac)$ as the intermediates instead of monochlorides. However, the previous study on *ansa*-vanadocenes have shown that the vanadocene(III) acetylacetonates are very reactive toward ligand exchange. If they appear they readily react with $MgCl_2$ to give chloride analogs [34].



Scheme 1. Vanadocene complexes used in the cytotoxicity study.



Scheme 2. Synthesis of ring-substituted vanadocene dichlorides **4**–**6**.

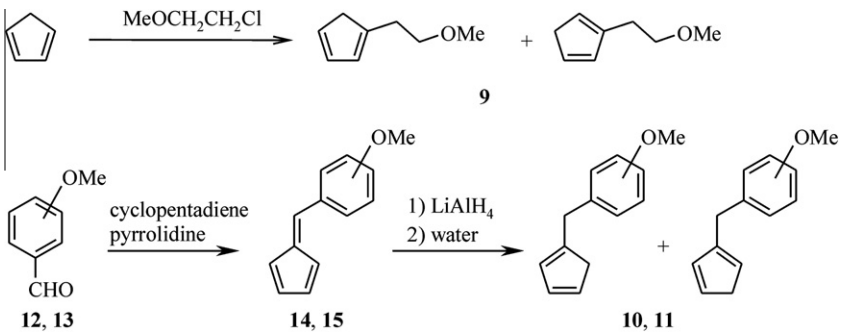
Starting cyclopentadiene **9** was prepared from sodium cyclopentadienide and 2-chloroethyl methyl ether according to procedure published elsewhere [35]. Fulvene protocol was used for synthesis of cyclopentadienes **10** and **11**, see Scheme 3 [36]. Methoxybenzaldehydes **12** and **13** react with cyclopentadiene in presence of pyrrolidine to give fulvenes **14** and **15**, respectively. These were obtained in high yield and characterized by NMR spectroscopy. The addition of $LiAlH_4$ to fulvenes **14** and **15** produces cyclopentadienes **10** and **11**, respectively. The NMR spectroscopic measurements prove that $CDCl_3$ solutions of cyclopentadienes **10** and **11** consist of 1- and 2-isomer in molar ratio 1:1, see Scheme 3. Both these cyclopentadienes show two signals of the cyclopentadiene CH_2 protons (**10**: 3.29 and 3.23 ppm; **11**: 3.00 and 2.88 ppm) in ^1H NMR spectrum. No information about the assignment of the signals among 1- and 2-isomer was obtained.

New methoxy-substituted compounds **4** and **5** were characterized by EPR and IR spectroscopy. The EPR spectra measured in dichloromethane showed expected eight-line hyperfine coupling (HFC), corresponding to the nuclear spin value of ^{51}V ($I = {}^7/_2$), see Fig. 1. Isotropic HFC constant and isotropic *g*-factor (**4**: $|A_{iso}| = 69.4 \times 10^{-4}\ cm^{-1}$, $g_{iso} = 1.990$; **5**: $|A_{iso}| = 69.5 \times 10^{-4}\ cm^{-1}$, $g_{iso} = 1.991$) were found to be in the narrow range close to the values of the unsubstituted analog **1** ($|A_{iso}| = 69.1 \times 10^{-4}\ cm^{-1}$, $g_{iso} = 1.989$). The substitution in the cyclopentadienyl ring has only low effect on these parameters, because the unpaired electron occupies the orbital that is antibonding to the bonds V–Cl [27,30].

Infrared spectra of the compounds **4** and **5** show a pattern that is consistent with proposed structures. The presence of the substituted η^5-bonded cyclopentadienyl rings is evident mainly from medium absorption bands of C–H stretching ($\nu_{C-H} \sim 3105\ cm^{-1}$). The methoxyethyl compound **4** shows the band of C–O stretching at $\sim$1113 cm^{-1}. The spectrum of the compounds **5** show C–C stretching of benzene ring at $\sim$1600 cm^{-1} and C–O stretching at $\sim$1245 cm^{-1}. These vibration bands are characteristic for methoxybenzyl-substituted compounds.

2.2. X-ray structures of compounds *4* and *5*

Structures of the compounds **4** and **5** were determined by single-crystal X-ray diffraction analysis, see Figs. 2 and 3. These



Scheme 3. Synthesis of cyclopentadienes **9**–**11**.

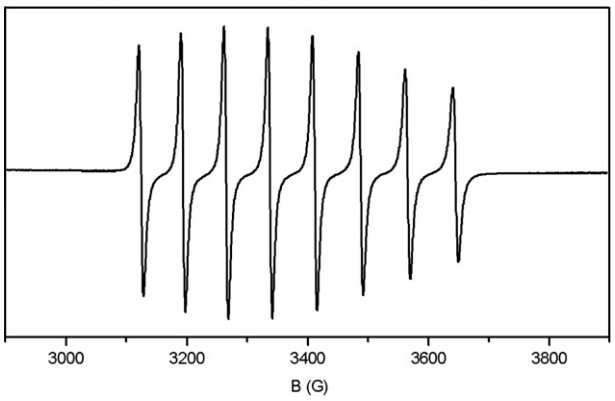


Fig. 1. EPR spectrum of dichloromethane solution of compound **4** (ν = 9.428 GHz).

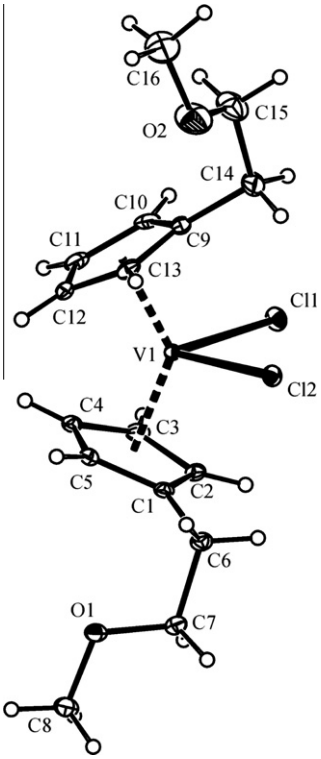


Fig. 2. ORTEP drawing of the compound **4** with atom numbering (ellipsoids: 30% probability). Selected bond lengths (Å) and angles (°): Cg(C1–C5)–V1 1.9812(12), Cg(C9–C13)–V1 1.9811(12), V1–Cl1 2.4018(7), V1–Cl2 2.3868(7), C7–O1 1.417(3), C8–O1 1.420(3), C15–O2 1.364(4), C16–O2 1.442(4), Cg(C1–C5)–V1–Cg(C9–C13) 132.21(5), Cl1–V1–Cl1 86.70(3), C7–O1–C8 111.43(19), C15–O2–C16 111.6(3). Cg – centroid of the cyclopentadienyl ring.

compounds have a distorted tetrahedral coordination around vanadium(IV) with two η^5-bonded substituted cyclopentadienyl rings and two chlorides. The distances V–Cg(Cp) (**4**: 1.9811(12), 1.9812(12) Å; **5**: 1.9743(7) Å), V–Cl (**4**: 2.3868(7), 2.4018(7) Å; **5**: 2.4135(4) Å) and the bond angles Cg(Cp)–V–Cg(Cp) (**4**: 132.21(5)°; **2**: 132.23(3)°) and Cl–V–Cl (**4**: 86.70(3)°; **5**: 86.76(2)°) were found to be in the range usual for vanadocene dichloride [37] and its ring-substituted analogs [18,19,38]. The Cp rings of the compounds **4** and **5** are in the staggered conformation. The compound **5** has a rigorous overall C_2 molecular symmetry with the methoxybenzyl groups placed far apart on the sides of the molecule. The compound **4** shows different conformation with one

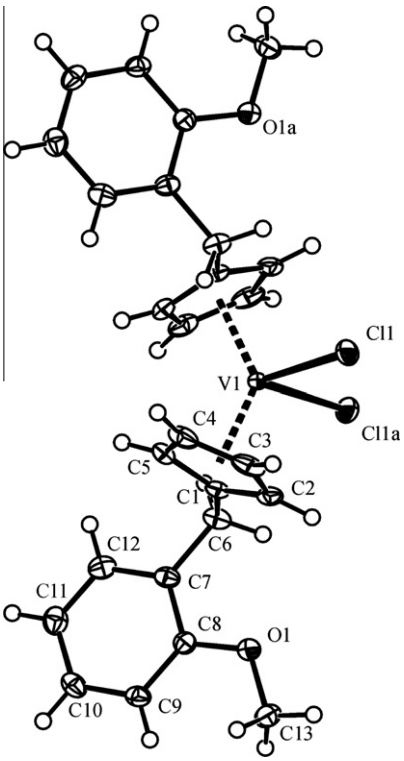


Fig. 3. ORTEP drawing of the compound **5** with atom numbering (ellipsoids: 50% probability). Selected bond lengths (Å) and angles (deg): Cg(C1–C5)–V1 1.9743(7), V1–Cl1 2.4135(4), C8–O1 1.3664(16), C13–O1 1.4302(17), Cg(C1–C5)–V1–Cg(C1–C5) 132.23(3), Cl1–V1–Cl1a 86.76(2), C8–O1–C13 116.71(11). Cg – centroid of the cyclopentadienyl ring.

methoxyethyl group above the Cl–V–Cl moiety and the second group placed on the side of the molecule.

2.3. Cytotoxicity studies

We used standard WST-1 viability assays [39] to examine the cytotoxic activity of vanadocene dichloride (**1**) and its derivatives **2**–**8**. Cytotoxic effect was evaluated on human T-lymphocytic leukemia cells MOLT-4 in exponential grow phase, 24 h after the incubation with the cytostatic drugs.

It was observed that substitution with methyl groups in the cyclopentadienyl rings causes significant decrease of cytotoxicity as was evidenced at compounds **2** and **3** (Fig. 4). Both dimethyl- (**2**) and decamethyl- (**3**) derivatives showed lower cytotoxic effect (**2**: IC_{50} = 96 ± 12 μmol/L; **3**: IC_{50} = 152 ± 10 μmol/L) than the unsubstituted analog **1** (70 ± 7 μmol/L). Effect of methoxyethyl substituent on cytotoxicity is very similar. The IC_{50} value of the compound **4** was found to be 105 ± 10 μmol/L (Fig. 4).

The highest cytotoxic effect was detected at methoxybenzyl derivatives **5** and **6** (Fig. 5). Both ortho- (**5**) and para- (**6**) derivatives have shown increased cytotoxic effect with IC_{50} values 33 ± 11 μmol/L and 11 ± 7 μmol/L, respectively. This observation is in line with the recently published tests on cell line LLC-PK (long-lasting cells-pig kidney) with compound **6** [19] and analogous titanocene derivative [40]. Our experiment clearly shows the importance of the methoxy group position in the benzene ring, since the compound **6** has three times lower IC_{50} value compared to the compound **5**.

The effect of the connection of the cyclopentadienyl rings by interannular bridge was studied on the *ansa*-vanadocenes **7** and **8** (Fig. 6). Both compounds exhibit similar cytotoxic effect (**7**: IC_{50} = 80 ± 10 μmol/L; **8**: IC_{50} = 65 ± 14 μmol/L) to the unbridged vanadocene analog **1**.

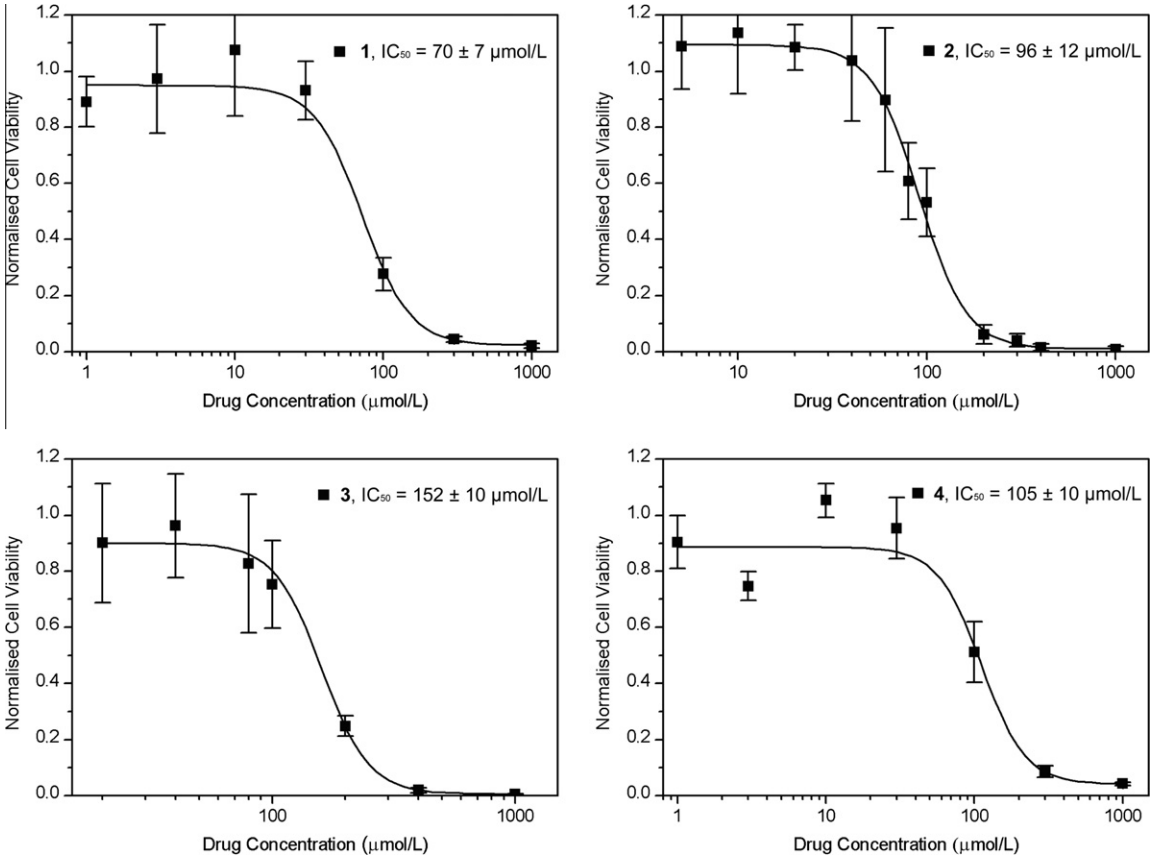


Fig. 4. Cytotoxicity curves showing the effect of the compounds **1**–**4** on the viability of the leukemic cells MOLT-4.

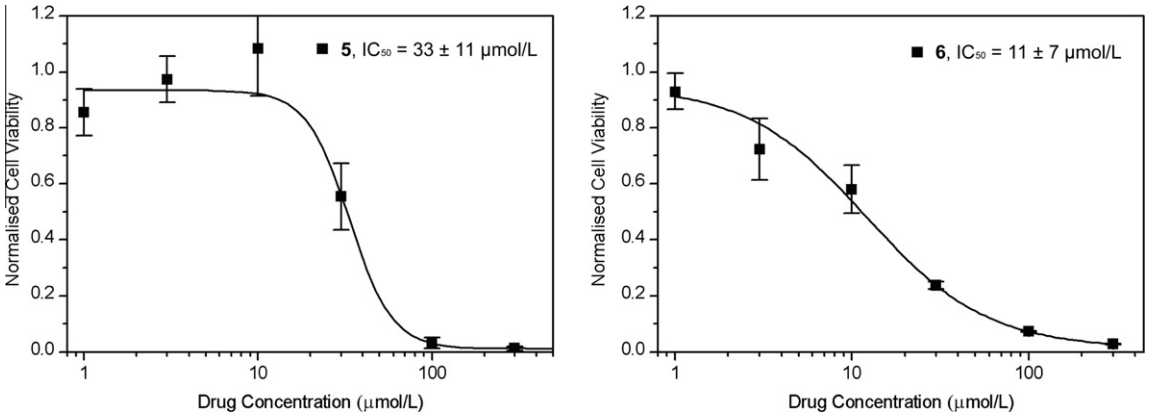


Fig. 5. Cytotoxicity curves showing the effect of the compounds **5**, **6** on the viability of the leukemic cells MOLT-4.

3. Conclusions

Recently published studies have shown that substitution with various methoxybenzyls in the cyclopentadienyl generates vanadocene compounds with increased cytotoxicity and brings promising candidates for anticancer drugs [19,20].

The obvious assumption that the increased cytotoxicity could be promoted with the better cell uptake of more lipophilic compounds led us to investigate a larger set of the modified compounds with various unpolar groups. However, the improved properties were observed only for the methoxybenzyl-substituted compounds **5** and **6**. The other modified vanadocenes under this study have shown similar (**7** and **8**) or lower cytotoxicity (**2**–**4**) than parent compound (**1**). This discrepancy led us to search for the differences in hydrolytic behavior of the most effective vanadocene (**5**) and its unsubstituted vanadocene **1**.

Previous experiments have shown that compound **1** hydrolyzes immediately after dissolution in water to give appropriate aquacomplexes $[Cp_2V(OH_2)_2]^{2+}$ [41,32]. The behavior in the mixtures DMSO/water in the air atmosphere is very similar. Our EPR measurements have shown appearance $[Cp_2V(OH_2)Cl]^{+}$ ($|A_{iso}| = 71.9 \times 10^{-4}\,cm^{-1}$, $g_{iso} = 1.985$) and $[Cp_2V(OH_2)_2]^{2+}$ ($|A_{iso}| = 74.0 \times 10^{-4}\,cm^{-1}$, $g_{iso} = 1.979$). As expected, the degree of hydrolysis depends on the DMSO/water ratio.

Hydrolysis of compound **5** was studied only in the mixtures DMSO/water because benzyl-substituted vanadocenes are insoluble

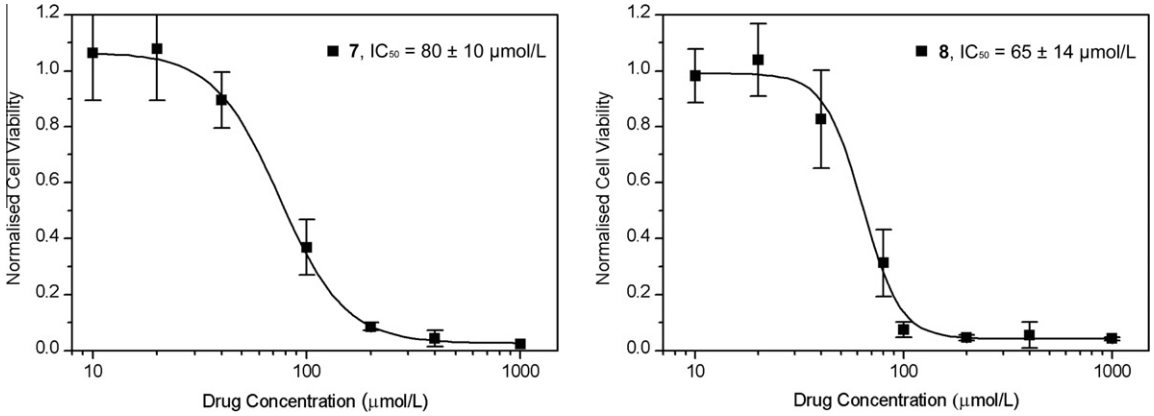


Fig. 6. Cytotoxicity curves showing the effect of the compounds **7** and **8** on the viability of the leukemic cells MOLT-4.

in water. The EPR spectroscopic measurements have proven lower stability of the $(\eta^5\text{-}C_5H_4R)_2V$ moiety in case of the compound **5** in the air atmosphere. The expected hydrolytic products $[(\eta^5\text{-}C_5H_4R)_2V(OH_2)Cl]^+$ ($|A_{iso}| = 72.0 \times 10^{-4}$ cm^{-1}, $g_{iso} = 1.983$) and $[(\eta^5\text{-}C_5H_4R)_2V(OH_2)_2]^{2+}$ ($|A_{iso}| = 74.0 \times 10^{-4}$ cm^{-1}, $g_{iso} = 1.980$) are formed only as minor products. The major products have considerably higher $|A_{iso}|$ constants than the known vanadocene complexes. $[VO(H_2O)_3Cl]^+$ ($|A_{iso}| = 101.0 \times 10^{-4}$ cm^{-1}, $g_{iso} = 1.973$) is formed at high DMSO concentrations. The full hydrolysis was observed at low DMSO concentrations. It gives $[VO(H_2O)_4]^{2+}$ ($|A_{iso}| = 106.7 \times 10^{-4}$ cm^{-1}, $g_{iso} = 1.968$) as the major product.

The experiments described here suggest that benzyl-substituted vanadocenes have different mechanism of the cytotoxic action on the molecular level than unsubstituted vanadocene dichloride. Of course, it is not conclusive, which of the hydrolytic products ($[Cp'_2V(OH_2)_2]^{2+}$, $[VO(H_2O)_4]^{2+}$ or Cp′H) is the "active species" or the precursor of the "active species" that reaches the target in the tumor cell. Nevertheless, this study has proven that the substitution in the cyclopentadienyl can considerably change not only the biological properties of the metallocene complexes but also their hydrolytic behavior.

4. Experimental

4.1. Methods and materials

All operations were performed under nitrogen using conventional Schlenk-line techniques. The solvents were purified and deoxygenated by standard methods [42]. Cp_2VCl_2 (**1**) [29], $(\eta^5\text{-}C_5H_4Me)_2VCl_2$ (**2**) [30], $(\eta^5\text{-}C_5Me_5)_2VCl_2$ (**3**) [31], $Me_2C(\eta^5\text{-}C_5H_4)_2VCl_2$ (**7**) [32], $Me_4C_2(\eta^5\text{-}C_5H_4)_2VCl_2$ (**8**) [33], 6-(4-methoxyphenyl)fulvene [43] and methoxyethylcyclopentadiene (**9**) [35] were prepared according literature procedures. IR spectra were recorded in the 4000–400 cm^{-1} region on a Nicolet Magna 550 FT-IR spectrometer using KBr pellets or Nujol mull between KBr windows. ^{1}H NMR spectra were recorded on Bruker 360 and 500 MHz spectrometers at room temperature. The EPR spectra were recorded on Miniscope MS 200 and Miniscope MS 300 spectrometers at X-band at ambient temperature.

4.2. Cytotoxicity studies

The studies were performed on the human T-lymphocytic leukemia cells MOLT-4 obtained from the American Type Culture Collection (USA). The cells were cultured in Iscove's modified Dulbecco's medium supplemented with a 20% fetal calf serum and 0.05% L-glutamine (all Sigma–Aldrich, USA) in a humidified incubator at 37 °C and a controlled 5% CO_2 atmosphere. The cell lines in the maximal range of up to 20 passages have been used for this study.

Cytotoxicity of the compounds **1**–**8** was evaluated by the WST-1 cell viability test (Roche, Germany) according to manufacturer's instructions. The assay is based on the reduction of WST-1 (4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) by viable cells. The reaction produces a colored soluble formazan salt [39]. The absorbance at 440 nm was measured using multiplate reader (Tecan Infinite 200). Compounds **1**–**8** were dissolved in DMSO and diluted by cultivation medium to desired concentrations. The MOLT-4 cells were seeded in 96-wells plate, incubated in 1–1000 μmol/L solutions of the compounds **1**–**8** for 24 h, then washed in pure media and incubated for 180 min in WST-1 solution. The same cells incubated in the cultivation media only were used as the control. High concentrations (>300 μmol/L) of the compounds **5** and **6** form significant clouding and therefore could not be measured. However, the compounds are cytotoxic in the concentrations below these limits.

4.3. Synthesis of 6-(2-methoxyphenyl)fulvene

Pyrrolidine (25 mL, 0.3 mol) was added dropwise to the mixture of freshly monomerized cyclopentadiene (42 mL, 0.5 mol) and 2-methoxybenzaldehyde (27.2 g, 0.2 mol) of in 200 mL of methanol. After the addition was complete, the solution was stirred at room temperature for 60 min and then 18 mL (0.32 mol) of acetic acid was added. The dark red reaction mixture was diluted with diethyl ether and water. The aqueous layer was washed twice with pentane. The combined organic portions were washed three-times with saline and dried over anhydrous $MgSO_4$. The volatiles were evaporated *in vacuo*. The crude product (dark red liquid) was used without further purification. Yield: 35.8 g (97%). ^{1}H NMR ($CDCl_3$): δ 7.73 [d, 1H, C_6H_4], 7.71 [s, 1H, CpC*H*Ph], 7.44 [t, 1H, C_6H_4], 7.11 [t, 1H, C_6H_4], 6.98 [d, 1H, C_6H_4], 6.78 [s, 2H, C_5H_4], 6.65 [d, 1H, C_5H_4], 6.51 [d, 1H, C_5H_4], 3.93 [s, 3H, OCH_3)$_2$].

4.4. Synthesis of (2-methoxybenzyl)cyclopentadiene (***10***)

6-(2-Methoxyphenyl)fulvene (18.4 g, 0.1 mol) of was added dropwise to the mixture of $LiAlH_4$ (3.8 g, 0.1 mol) in ether (250 mL) at 0 °C. After the addition was complete, the mixture was stirred at room temperature for 12 h. The reaction mixture was then slowly poured onto the mixture ice-water. The organic layer was separated. The water layer was then washed with 200 mL of diethyl ether. The combined organic portions were washed four times with saline, dried over anhydrous $MgSO_4$ and

concentrated *in vacuo*. The product was distilled at 40 °C (50 Pa) giving light orange liquid. Yield: 12.7 g (68%). ^{1}H NMR($CDCl_3$): δ 7.52, 7.46 [2 × t, 2H of A and 2H of B, C_6H_4], 7.22 [t, 1H of A and 1H of B, C_6H_4], 7.17, 7.16 [2 × d, 1H of A and 1H of B, C_6H_4], 6.82 [m, 1H of A or B, C_5H_5], 6.75 [m, 1H of A or B, C_5H_5], 6.74 [m, 1H of A or B, C_5H_5], 6.59 [d, 2H of A or B, C_5H_5], 6.50 [s, 1H of A or B, C_5H_5], 6.35 [s, 1H of A or B, C_5H_5], 4.11, 4.10 [2 × s, 2H of A and 2H of B, CpCH_2Ph], 4.09 [s, 3H of A and 3H of B, OCH_3], 3.29, 3.23 [2 × m, 2H of A and 2H of B, C_5H_5].

4.5. Synthesis of (4-methoxybenzyl)cyclopentadiene (**11**)

The steps of synthesis followed the procedure for compound **10**. Reagents: 6-(4-methoxyphenyl)fulvene (18.4 g, 0.1 mol), $LiAlH_4$ (3.8 g, 0.1 mol). The product was distilled at 45 °C (50 Pa) giving light orange liquid. Yield: 16.9 g (91%). ^{1}H NMR($CDCl_3$): δ 7.17, 7.14 [2 × d, 2H of A and 2H of B, C_6H_4], 6.89, 6.87 [2 × d, 2H of A and 2H of B, C_6H_4], 6.47 [d, 1H of A or B, C_5H_5], 6.46 [d, 2H of A or B, C_5H_5], 6.31 [dd, 1H of A or B, C_5H_5], 6.20 [t, 1H of A or B, C_5H_5], 6.04 [t, 1H of A or B, C_5H_5], 3.78 [s, 3H of A and 3H of B, OCH_3], 3.74, 3.70 [2 × s, 2H of A and 2H of B, CpCH_2Ph], 3.00, 2.88 [2 × d, 2H of A and 2H of B, C_5H_5].

4.6. Synthesis of ($MeOCH_2CH_2C_5H_4)_2VCl_2$ (**4**)

MeMgCl (9.6 mL, 3 M solution in THF) was added dropwise to the solution of the compound **9** (3.6 g; 28 mmol) in THF (80 mL). The reaction mixture was stirred overnight. This solution was added *via* cannula to the suspension of $V(acac)_3$ (5 g, 14 mmol) in THF (100 mL) precooled at −78 °C. The reaction mixture charged to dark brown. After stirring for 1 h at the room temperature, the reaction mixture was heated at reflux for 30 min. The solvents were removed *in vacuo* and the solid residuum was extracted with diethyl ether. The brown extract was treated with PCl_3 (3.8 g, 28 mmol) and stirred overnight. The green precipitate of the complex **4** was collected on the glass frit and washed with diethyl ether (2 × 20 mL). The crude product was recrystallized from CH_2Cl_2/hexane. Yield: 0.91 g (17%). *Anal*. Calc. for $C_{16}H_{22}Cl_2O_2V$: C, 52.19; H, 6.02. Found: C, 52.12; H, 6.09%. EPR(CH_2Cl_2): g_{iso} = 1.990, $|A_{iso}|$ = 74.8 G; IR(cm^{-1}, Nujol mull): 3109m, 3097m, 3085m, 1113vs.

4.7. Synthesis of (2-$MeOC_6H_4CH_2C_5H_4)_2VCl_2$ (**5**)

The steps of synthesis followed the procedure for compound **4**. Reagents: **10** (5.4 g, 28 mmol), 9.6 mL of MeMgCl (3 M solution in THF), $V(acac)_3$ (5 g, 14 mmol), PCl_3 (3.8 g, 28 mmol). The crude product was recrystallized from CH_2Cl_2/hexane. Yield: 1.22 g (17%). *Anal*. Calc. for $C_{26}H_{26}Cl_2O_2V$: C 63.43; H 5.32. Found: C, 63.24; H, 5.29%. EPR(CH_2Cl_2): g_{iso} = 1.991, $|A_{iso}|$ = 74.8 G; IR(cm^{-1}, KBr pellet): 3105m, 2963m, 2928m, 2838s, 1629m, 1600s, 1494vs, 1246vs. IR(cm^{-1}, Nujol mull): 3109m, 1598m, 1585m, 1244vs. Single crystal of **5** suitable for X-ray diffraction analysis was prepared by careful layering of the CH_2Cl_2 solution with double volume of hexane.

4.8. Synthesis of (4-$MeOC_6H_4CH_2C_5H_4)_2VCl_2$ (**6**)

The steps of synthesis followed the procedure for compound **4**. Reagents: **11** (5.4 g, 28 mmol), 9.6 mL of MeMgCl (3 M solution in THF), $V(acac)_3$ (5 g, 14 mmol), PCl_3 (3.8 g, 28 mmol). Yield: 1.08 g (15%). *Anal*. Calc. for $C_{26}H_{26}Cl_2O_2V$: C, 63.43; H, 5.32. Found: C, 63.31; H, 5.28%. EPR(CH_2Cl_2): g_{iso} = 1.990, $|A_{iso}|$ = 74.8 G. IR(cm^{-1}, KBr pellet): 3101m, 3077s, 2964s, 2936m, 2838m, 1610s, 1583m, 1512vs, 1247vs.

Table 1
Crystallographic data for the compounds **4** and **5**.

Compounds	**4**	**5**
Formula	$C_{16}H_{22}Cl_2O_2V$	$C_{26}H_{26}Cl_2O_2V$
Crystal system	monoclinic	monoclinic
Space group	$P2_1/c$	$C2/c$
a (Å)	20.5657(5)	23.7980(6)
b (Å)	6.9761(5)	6.82004(10)
c (Å)	11.3962(13)	14.6928(4)
β (°)	99.506(4)	113.7262(12)
Z	4	4
μ (mm^{-1})	0.947	0.721
D_x (g cm^{-3})	1.517	1.498
Crystal size (mm)	0.26 × 0.20 × 0.14	0.40 × 0.27 × 0.25
Crystal color	green	green
Crystal shape	block	prism
θ range (°)	2.0–27.5	1.9–27.5
h, k, l range	−24/26, −9/8, −14/14	−30/30, −8/8, −18/18
Number of reflections measured	13 459	14 682
Number of unique reflections; R_{int}[a]	3600, 0.044	2502, 0.026
Number of observed reflections [$I > 2\sigma(I)$]	2923	2279
Number of parameters	190	142
S[b] all data	1.033	1.025
R[c], wR[c]	0.0399, 0.0885	0.0268, 0.0631
$\Delta\rho$, maximum, minimum (e Å^{-3})	1.697, −0.525	0.294, −0.354

[a] $R_{int} = \Sigma|F_o^2 - F_{o,mean}^2|/\Sigma F_o^2$.
[b] $S = [\Sigma(w(F_o^2 - F_c^2)^2)/(N_{diffrs} - N_{params})]^{1/2}$ for all data.
[c] $R(F) = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ for observed data, $wR(F^2) = [\Sigma(w(F_o^2 - F_c^2)^2)/(\Sigma w(F_o^2)^2)]^{1/2}$ for all data.

4.9. Crystallography

The X-ray data for crystals of **4** and **5** were obtained at 150 K using Oxford Cryostream low-temperature device on a Nonius KappaCCD diffractometer with Mo Kα radiation (λ = 0.71073 Å), a graphite monochromator, and the φ and χ scan mode. Data reductions were performed with DENZO-SMN [44]. In the case of **4**, the absorption was corrected by integration methods [45]. Structures were solved by direct methods (SIR92) [46] and refined by full matrix least-square based on F^2 (SHELXL97) [47]. Crystallographic data are summarized in Table 1.

Hydrogen atoms were mostly localized on a difference Fourier map, however to ensure uniformity of treatment of crystal, all hydrogen were recalculated into idealized positions (riding model) and assigned temperature factors $H_{iso}(H) = 1.2\ U_{eq}$(pivot atom) or of 1.5 U_{eq} for the methyl moiety with C–H = 0.96, 0.97, and 0.93 Å for methyl, methylene and hydrogen atoms in Cp ring, respectively.

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Appendix A. Supplementary material

CCDC 747083 and 747084 contain the supplementary crystallographic data for compounds **5** and **4**, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via 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.ica.2011.03.034.

References

[1] N. Lamanna, Curr. Hematol. Malig. Rep. 5 (2010) 45.
[2] S.H. Kaufmann, D.P. Steensma, Leukemia 19 (2005) 2195.

[3] P. Köpf-Maier, H. Köpf, Struct. Bonding (1988) 103.
[4] I. Kostova, Anticancer Agents Med. Chem. 9 (2009) 827.
[5] P. Köpf-Maier, H. Köpf, Chem. Rev. 87 (1987) 1137.
[6] K. Mross, P. Robben-Bathe, L. Edler, J. Baumgart, W.E. Berdel, H. Fiebig, C. Unger, Onkologie 23 (2000) 576.
[7] N. Kröger, U.R. Kleeberg, K. Mross, L. Edler, G. Sass, D.K. Hossfeld, Onkologie 23 (2000) 60.
[8] G. Lümmen, H. Sperling, H. Luboldt, T. Otto, H. Rübben, Cancer Chemother. Pharmacol. 42 (1998) 415.
[9] J. Vinklárek, H. Paláčková, J. Honzíček, J. Holubová, M. Holčapek, I. Císařová, Inorg. Chem. 45 (2006) 2156.
[10] J.B. Waern, P. Turner, M.M. Harding, Organometallics 25 (2006) 3417.
[11] O.R. Allen, L. Croll, A.L. Gott, R.J. Knox, P.C. McGowan, Organometallics 23 (2004) 288.
[12] O.R. Allen, A.L. Gott, J.A. Hartley, J.M. Hartley, R.J. Knox, P.C. McGowan, Dalton Trans. (2007) 5082.
[13] P.W. Causey, M.C. Baird, S.P.C. Cole, Organometallics 23 (2004) 4486.
[14] K. Strohfeldt, M. Tacke, Chem. Soc. Rev. 37 (2008) 1174.
[15] L.M. Gao, J. Matta, A.L. Rheingold, E. Meléndez, J. Organomet. Chem. 694 (2009) 4134.
[16] S. Gómez-Ruiz, G.N. Kaluđerović, S. Prashar, D. Polo-Cerón, M. Fajardo, J. Inorg. Biochem. 102 (2008) 1558.
[17] A. Gansäuer, D. Franke, T. Lauterbach, M. Nieger, J. Am. Chem. Soc. 127 (2005) 11622.
[18] B. Gleeson, J. Claffey, A. Deally, M. Hogan, L.M.M. Méndez, H. Müller-Bunz, S. Patil, D. Wallis, M. Tacke, Eur. J. Inorg. Chem. (2009) 2804.
[19] B. Gleeson, J. Claffey, M. Hogan, H. Müller-Bunz, D. Wallis, M. Tacke, J. Organomet. Chem. 694 (2009) 1369.
[20] B. Gleeson, J. Claffey, A. Deally, M. Hogan, L.M.M. Méndez, H. Müller-Bunz, S. Patil, M. Tacke, Inorg. Chim. Acta 363 (2010) 1831.
[21] J. Aubrecht, R.K. Narla, P. Ghosh, J. Stanek, F.M. Uckun, Toxicol. Appl. Pharmacol. 154 (1999) 228.
[22] J.L. Vera, F.R. Román, E. Meléndez, Anal. Bioanal. Chem. 379 (2004) 399.
[23] J.L. Vera, F.R. Román, E. Meléndez, Bioorg. Med. Chem. 14 (2006) 8683.
[24] A. Erxleben, L. Yovkova, Inorg. Chim. Acta 359 (2006) 2350.
[25] K.S. Campbell, A.J. Foster, C.T. Dillon, M.M. Harding, J. Inorg. Biochem. 100 (2006) 1194.
[26] J.B. Waern, M.M. Harding, J. Organomet. Chem. 689 (2004) 4655.
[27] J. Honzíček, J. Vinklárek, P. Nachtigall, Chem. Phys. 305 (2004) 291.
[28] J. Vinklárek, J. Honzíček, J. Holubová, Magn. Reson. Chem. 42 (2004) 870.
[29] G. Wilkinson, J.M. Birmingham, J. Am. Chem. Soc. 76 (1954) 4281.
[30] J. Petersen, F. Dahl, J. Am. Chem. Soc. 97 (1975) 6422.
[31] M. Morán, J.R. Masaguer, V. Fernández, J. Organomet. Chem. 291 (1985) 311.
[32] J. Honzíček, H. Paláčková, J. Vinklárek, I. Císařová, J. Organomet. Chem. 691 (2006) 202.
[33] B. Dorer, J. Diebold, O. Weyand, H.H. Brintzinger, J. Organomet. Chem. 427 (1992) 245.
[34] B. Dorer, M.H. Prosenc, U. Rief, H.H. Brintzinger, Collect. Czech. Chem. Commun. 62 (1997) 265.
[35] J. Honzíček, A. Mukhopadhyay, T.S. Silva, M.J. Romão, C.C. Romão, Organometallics 28 (2009) 2871.
[36] K.J. Stone, R.D. Little, J. Org. Chem. 49 (1984) 1849.
[37] J. Honzíček, J. Vinklárek, I. Císařová, M. Erben, Inorg. Chim. Acta 362 (2009) 83.
[38] J.A. Belot, R.D. McCullough, A.L. Rheingold, G.P.A. Yap, Organometallics 15 (1996) 5062.
[39] M. Ishiyama, M. Shiaga, K. Sasamoto, M. Mizoguchi, P. He, Chem. Pharm. Bull. 41 (1993) 1118.
[40] N.J. Sweeney, O. Mendoza, H. Müller-Bunz, C. Pampillón, F.-J.K. Rehmann, K. Strohfeldt, M. Tacke, J. Organomet. Chem. 690 (2005) 4537.
[41] J.H. Toney, T.J. Marks, J. Am. Chem. Soc. 107 (1985) 947.
[42] W.L.F. Armarego, D.D. Perrin, Purification of Laboratory Chemicals, Butterworth-Heinemann, Oxford, 1996.
[43] M. Tacke, L.T. Allen, L. Cuffe, W.M. Gallagher, Y. Lou, O. Mendoza, H. Müller-Bunz, F.-J.K. Rehmann, N. Sweeney, J. Organomet. Chem. 689 (2004) 2242.
[44] Z. Otwinowski, W. Minor, Macromolecular Crystallography, Pt A, vol. 276, Academic Press Inc., San Diego, 1997, p. 307.
[45] P. Coppens, W.C. Hamilton, Acta Crystallogr., Sect. A 26 (1970) 71.
[46] A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, J. Appl. Crystallogr. 26 (1993) 343.
[47] G.M. Sheldrick, SHELXL97, University of Göttingen, Germany, 1997.