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Can Substitution of Chlorides Enhance the Cytotoxicity of Vanadocene Dichloride?

Jaromír Vinklárek,[a] Hana Hurychová,[a] Jan Honzíček,*[b] Lucie Šebestová,[c] Zdeňka Padělková,[a] and Martina Řezáčová[d]

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A series of vanadocene complexes $[Cp'_2V(\mathbf{L})][OTf]_2$ (Cp′ = η^5-C_5H_5, η^5-C_5H_4Me; **L** = phen, 5-NO_2-phen, 5-NH_2-phen, 4,7-Ph_2-phen) was prepared and characterized by mass spectrometry and EPR spectroscopy. Structures of two complexes that contain an *N*,*N*′-chelating ligand, $[(\eta^5\text{-}C_5H_4Me)_2V(phen)][OTf]_2{\cdot}0.5Me_2CO$ and $[(\eta^5\text{-}C_5H_5)_2V(5\text{-}NH_2\text{-}phen)][OTf]_2$, and the triflate intermediate $[(\eta^5\text{-}C_5H_5)_2V(OTf)_2]$ were further confirmed by X-ray diffraction analysis. The cytotoxicity study has shown that complexes that contain phenanthroline ligands have considerably higher in vitro activity toward human leukemia cells MOLT-4 and HL-60 than their parent dichlorides ($[Cp'_2VCl_2]$). The high activity of these complexes coheres with their higher stability in various aqueous media. This study has further shown that substitution of chloride ligands in the vanadocene dichloride has a considerably higher effect on cytotoxicity than expected on the basis of established mechanistic rules.

Introduction

Despite our constantly deepening knowledge about pathobiochemical mechanisms of various hematological malignancies, many leukemias remain incurable and have very bad survival prognoses. Although targeted therapy, such as tyrosin kinase inhibitors or specific monoclonal antibodies, was a great revolution for the therapy of some types of leukemias, others rely fully on classical chemotherapy.[1] For example, the core of the treatment regimen of acute myeloid leukemia has remained nearly unchanged for 40 years, and the prognosis remains poor, mainly in elderly patients. Therefore, constant research continues on novel cytostatics that would provide fewer undesirable side effects while maintaining potent antitumor activity.[2]

Vanadocene dichloride (**1**, $[Cp_2VCl_2]$; Cp = η^5-C_5H_5) is known as a potent antitumor agent with low general toxicity.[3] In the last few years, drug design in this area has been focused mainly on compounds that have been substituted on the cyclopentadienyl rings because such an approach can considerably enhance cytotoxicity, as was already evidenced in various derivatives.[4] Recently, we have shown that **1** and its ring-substituted and *ansa*-bridged derivatives are active toward leukemia cells MOLT-4.[5] So far, only minor improvements have been achieved through the substitution of the chlorides.[6] It is probably a result of the low hydrolytic stability of the V–X bonds that results in the formation of the same "active species" as their chloride parent. Although the vanadocene framework is stable in various aqueous media, the hydrolytic chemistry of **1** is relatively complex.[7] The aqua complex $[Cp_2V(OH_2)_2]^{2+}$ appears immediately after dissolution and reacts with carbonates and phosphates to give chelate complexes $[Cp_2V(O,O\text{-}CO_3)]$[8,9] and $[Cp_2V(O,O\text{-}PO_4H)]$,[10] respectively. Furthermore, several structure types were described as the products of the reaction with α-amino acids.[9,11,12] So far, it has been not concluded which of the hydrolytic products is the "active species" that reaches the target in the diseased cell.

Our focus on the phenanthroline derivatives follows the previous study that reports that $[Cp_2V(phen)][OTf]_2$ (**3**) is stable in phosphate-buffered saline (PBS) and that it shows high permeability in the liposomal membranes.[13] The unusual electrochemical properties of the vanadocene complexes that contain 2,2′-bipyridine ligands were described in detail theoretically[14] and experimentally.[15] High stability, water solubility, and permeability in membranes, which were reported for the phenanthroline complexes, could overcome the problems of the metallocene-based drugs that are associated with medical applications and with transport into the diseased cell, thus finally leading to an enhance-

[a] Department of General and Inorganic Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 53210 Pardubice, Czech Republic
[b] Institute of Chemistry and Technology of Macromolecular Materials, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 53210 Pardubice, Czech Republic
Fax: +420-46-603-7068
E-mail: jan.honzicek@upce.cz
Homepage: http://www.upce.cz/en/fcht/uchtml.html
[c] Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 53210 Pardubice, Czech Republic
[d] Department of Medical Biochemistry, Faculty of Medicine in Hradec Králové, Charles University in Prague, Šimkova 870, 50001 Hradec Králové, Czech Republic
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ment of their activity. This study reports the synthesis, characterization, and a detailed stability study of vanadocene–phenanthroline complexes. Their cytotoxicity was evaluated in vitro on human leukemia cells MOLT-4 and HL-60.

Results and Discussion

Preparation and Characterization

Phenanthroline complexes $[Cp_2V(\mathbf{L})][OTf]_2$ (**L** = 5-NO_2-phen (**4**), 5-NH_2-phen (**5**), 4,7-Ph_2-phen (**6**)) and $[(\eta^5\text{-}C_5H_4Me)_2V(\mathbf{L})][OTf]_2$ (**L** = phen (**3a**), 5-NO_2-phen (**4a**), 5-NH_2-phen (**5a**), 4,7-Ph_2-phen (**6a**)) were prepared in a two-step procedure according to the method developed for $[Cp_2V(phen)][OTf]_2$ (**3**)[13] (see Scheme 1). The reaction of dichloride complexes **1** and $[(\eta^5\text{-}C_5H_4Me)_2VCl_2]$ (**1a**) with silver triflate gives reactive intermediates $[Cp_2V(OTf)_2]$ (**2**) and $[(\eta^5\text{-}C_5H_4Me)_2V(OTf)_2]$ (**2a**), respectively. The weakly bonded ligands are exchanged in the second step with the appropriate phenanthroline to give cationic vanadocene complexes **3**–**6** and **3a**–**6a**.

The reaction process was followed by EPR spectroscopy. The addition of silver triflate to the solution of vanadocene dichloride **1** in THF was accompanied with the precipitation of silver chloride and the appearance of a new signal in the EPR spectrum ($|A_{iso}| = 73.7 \times 10^{-4}$ cm^{-1}, $g_{iso} = 1.980$) that was assigned to triflate complex **2**. The disappearance of the signal of the starting compound (**1**: $|A_{iso}| = 69.5 \times 10^{-4}$ cm^{-1}, $g_{iso} = 1.991$), after about one hour, proved that the first reaction step is complete (compare spectra A and B in Figure 1). The reaction mixture was filtered from the precipitated silver chloride. The addition of 1,10-phenanthroline to the filtrate led to the appearance of the signal of **3** ($|A_{iso}| = 62.8 \times 10^{-4}$ cm^{-1}, $g_{iso} = 1.986$) and slow formation of a brown precipitate. The reaction was complete after 18 h when the signal of triflate complex had disappeared (see spectrum C in Figure 1). The precipitate of **3** was isolated and characterized. The obtained analytical and spectroscopic data are in line with those published elsewhere.[13] A similar reaction process was observed for substituted vanadocene dichloride **1a** and for substituted phenanthroline ligands (5-NO_2-phen and 5-NH_2-phen). In the case of reactions with 4,7-Ph_2-phen only the products do not precipitate from solution because compounds **6** and **6a** are much more soluble in THF than the other derivatives.

The triflate intermediates **2** and **2a** were isolated from the reaction mixture and characterized by elemental analysis. We have checked that the species were not changed during isolation. The samples dissolved in acetone display the EPR spectra with the same $|A_{iso}|$ and g_{iso} parameters as the species in the reaction mixture. The $|A_{iso}|$ values of compounds **2** and **2a** are in range of the vanadocene complexes that contain two monodentate *O*-donor ligands (73.5–74.2 $\times 10^{-4}$ cm^{-1}).[16] It suggests that both triflate ligands are coordinated through an oxygen atom rather than by forming some type of chelate or ionic structure. The X-ray analysis of compound **2** proves that this structure remains in the solid state (see Figure 2).

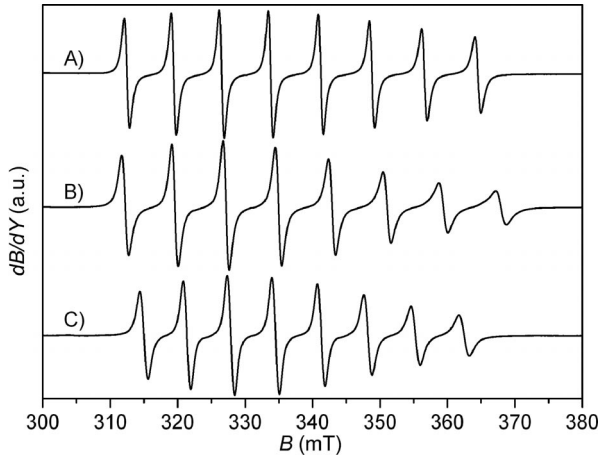


Figure 1. EPR spectra of the solutions of (A) **1**, (B) **2**, and (C) **3** in THF. The spectra were measured at $\nu = 9.45$ GHz.

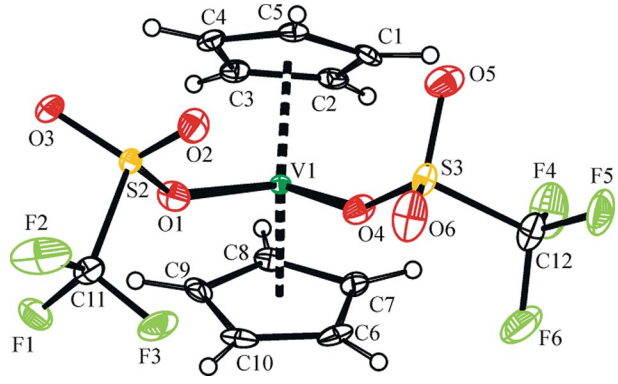
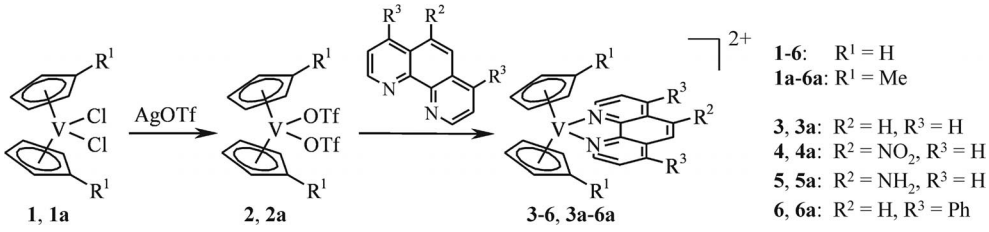


Figure 2. ORTEP drawing of the molecule $[Cp_2V(OTf)_2]$ present in the crystal structure of **2** (ellipsoids: 30% probability). Numbering of all non-hydrogen atoms is shown.



Scheme 1. Preparation of phenanthroline complexes **3**–**6** and **3a**–**6a**.

Phenanthroline complexes **3**–**6** and **3a**–**6a** were characterized by positive-ion ESI mass spectrometry. These compounds give peaks that were assigned to the parent dicationic species $[M]^{2+}$. The EPR spectra of the phenanthroline complexes were measured in acetone and methanol. The obtained $|A_{iso}|$ and g_{iso} values are summarized in Table 1. Although these solvents might coordinate, the negligible differences in the $|A_{iso}|$ and g_{iso} values show that they are inert for this type of species. The complexes of 5-nitro-1,10-phenanthroline (**4** and **4a**) and 4,7-diphenyl-1,10-phenanthroline (**6** and **6a**) display a slightly lower $|A_{iso}|$ parameter than complexes of 1,10-phenanthroline (**3** and **3a**) and 1,10-phenanthroline-5-amine (**5** and **5a**). The effect of the electron-withdrawing substituents in the phenanthroline framework lowers the spin density on the metal. The substitution of the cyclopentadienyl ring with a methyl group has a negligible effect on these parameters because the unpaired electron occupies the orbital that is *anti* bonding to the V–N bonds.[17,18] Structures of the complexes $[(\eta^5\text{-}C_5H_4Me)_2V(phen)][OTf]_2\cdot 0.5Me_2CO$ (**3a**·0.5Me_2CO) and $[Cp_2V(5\text{-}NH_2\text{-}phen)][OTf]_2$ (**5**) were determined by X-ray diffraction analysis.

Table 1. Isotropic *g* factors and isotropic hyperfine coupling constants [10^{-4} cm^{-1}] of the vanadocene complexes.

	g_{iso}	$\|A_{iso}\|$		g_{iso}	$\|A_{iso}\|$
1	1.991	69.5	**1a**	1.990	69.5
2	1.980	73.7	**2a**	1.981	73.5
3	1.986	62.8	**3a**	1.986	62.9
4	1.986	61.9	**4a**	1.986	62.1
5	1.986	62.5	**5a**	1.986	62.7
6	1.987	62.1	**6a**	1.987	62.0

X-ray Structures of Compounds 2, 3a·0.5Me_2CO and 5

Molecular structures of compounds **2**, **3a**·0.5Me_2CO, and **5** are shown in Figures 2, 3, and 4. Important structural parameters are listed in Table 2. These complexes have a typical bent metallocene structure in which two η^5-bonded cyclopentadienyl rings and two other donor atoms occupy the pseudotetrahedral coordination sites around the vanadium(IV) center. The Cg–V distances [1.949(2)–1.963(2) Å] and Cg–V–Cg angles [132.63(9)–134.38(7)°] lay in the range common for the other known vanadocene(IV) complexes (Cg–V: 1.95–1.97 Å; Cg–V–Cg: 131–135°).[19]

Compound **2** has two OTf ligands bonded through oxygen atoms to vanadium. The V–O bonds [2.044(3), 2.057(3) Å] are longer than in the vanadocene complexes of carboxylic acids {$[Cp'_2V(OOCR)_2]$: V–O 2.014(2)–2.042(3) Å}.[12,16] The O–V–O angle was found to be smaller [82.88(12)°] than the O–Ti–O angle in the titanocene counterpart $[Cp_2Ti(OTf)_2]$ [90.74(8)°].[20] The M–O–S angles in these compounds are very similar {**2**: V–O–S 146.7(2), 151.3(2)°; $[Cp_2Ti(OTf)_2]$: 145.7(1), 152.3(1)°}.

Complexes **3a**·0.5Me_2CO and **5** have the chelating phenanthroline ligand bonded to the vanadocene moiety. The V–N bond lengths [2.125(3)–2.152(4) Å] and N–V–N bond angles [76.46(13)–76.66(12)°] were found to be close to the values reported for unsubstituted analogue **3**[13] and the 2,2′-bipyridine complexes $[Cp_2V(bpy)][BPh_4]$ (bpy = 2,2′-bipyridyl)[15] and $[Cp_2V(bpy)][OTf]_2$.[13] The small effect of substitution and the oxidation state of vanadium on these structural parameters is in line with rigid chelate structure in these complexes.

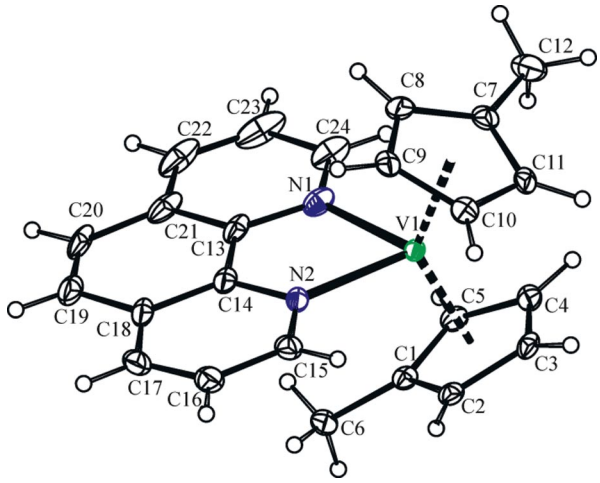


Figure 3. ORTEP drawing of the dicationic complex $[(\eta^5\text{-}C_5H_4Me)_2V(phen)]^{2+}$ present in the crystal structure of **3a**·0.5Me_2CO (ellipsoids: 30% probability). Numbering of all non-hydrogen atoms is shown.

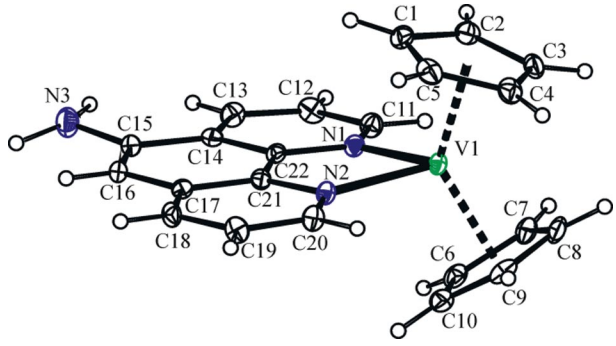


Figure 4. ORTEP drawing of the dicationic complex $[Cp_2V(5\text{-}NH_2\text{-}phen)]^{2+}$ present in the crystal structure of **5** (ellipsoids: 30% probability). Numbering of all non-hydrogen atoms is shown.

Table 2. Structural parameters (bond lengths in Å, angles in °) of vanadocene compounds describing the coordination around the vanadium.

	2[a]	**3**[b]	**3a**[c]	**5**[d]
V–Cg1	1.954(2)	1.96	1.965(2)	1.954(2)
V–Cg2	1.963(2)	1.96	1.957(2)	1.949(2)
V–X1	2.044(3)	2.134(2)	2.136(4)	2.152(4)
V–X2	2.057(3)	2.139(2)	2.131(3)	2.125(3)
Cg1–V–Cg2	132.63(9)	133.63	134.38(7)	133.40(10)
X1–V–X2	82.88(12)	76.66(7)	76.66(12)	76.46(13)

[a] X1 = O1, X2 = O2, Cg1 = C1–C5, Cg2 = C6–C10. [b] X = N, data reported in the literature.[13] [c] Compound **3a**·0.5Me_2CO: X1 = N1, X2 = N2, Cg1 = C1–C5, Cg2 = C7–C11. [d] X1 = N1, X2 = N2; Cg1 = C1–C5, Cg2 = C6–C10.

Cytotoxicity Studies

The cytotoxic effect of phenanthroline complexes **3**–**6** and **3a**–**6a** was evaluated on human T-lymphocytic leukemia cells MOLT-4 and human promyelocytic leukemia cells HL-60 in exponential grow phase, 24 h after the incubation with the cytostatic drugs.

The IC_{50} values were obtained from the standard WST-1 viability assays. The assay is based on reduction of the tetrazolium salt WST-1 to a colored substance by mitochondrial dehydrogenases of viable cells, and thus reflects combined changes in proliferation and viability. The obtained IC_{50} values are listed in Table 3. The effect of the drug concentration on the viability of the leukemic cells relative to untreated control cells is given in Figures 5 and 6.

All phenanthroline complexes under study (**3**–**6** and **3a**–**6a**) showed considerably higher activity than the parent dichloride complexes (**1** and **1a**). High cytotoxicity was observed against both leukemic cell lines. It was further shown that the activity of the vanadocene complexes is markedly improved through the substitution of the phenanthroline framework. For example, complex **5**, which contained an amino-substituted phenanthroline ligand, has an IC_{50} value that is about two times lower for MOLT-4 cells and about three times lower for HL-60 relative to unsubstituted analogue **3**. Substitution of the cyclopentadienyl ring with a methyl group has a rather negative effect. Compounds **1a**, **4a**, **5a**, and **6a** have activity that is similar to or lower than their unsubstituted analogues **1**, **4**, **5**, and **6**, respectively. Only the methyl-substituted derivative **3a** has higher cytotoxicity than its unsubstituted counterpart **3**; but even here the improvement nears the accuracy of the assay.

Table 3. Cytotoxicity of the vanadocene complexes and cisplatin toward MOLT-4 and HL-60 cells expressed as the IC_{50} values [μM].

	MOTL-4	HL-60		MOTL-4	HL-60
1	70 ± 7[a]	60.5 ± 3.3	**1a**	96 ± 12[a]	88.6 ± 5.2
3	7.8 ± 1.4	9.6 ± 1.3	**3a**	5.0 ± 0.7	7.6 ± 0.5
4	15.2 ± 1.8	26.2 ± 1.6	**4a**	17.4± 3.1	28.2 ± 3.3
5	3.1 ± 0.4	2.8 ± 0.4	**5a**	15.5 ± 1.0	20.3 ± 3.0
6	5.6 ± 0.5	7.1 ± 0.7	**6a**	6.0 ± 0.7	16.8 ± 4.1
cisplatin	15.8 ± 1.9	11.3 ± 2.5			

[a] Data reported in literature.[5]

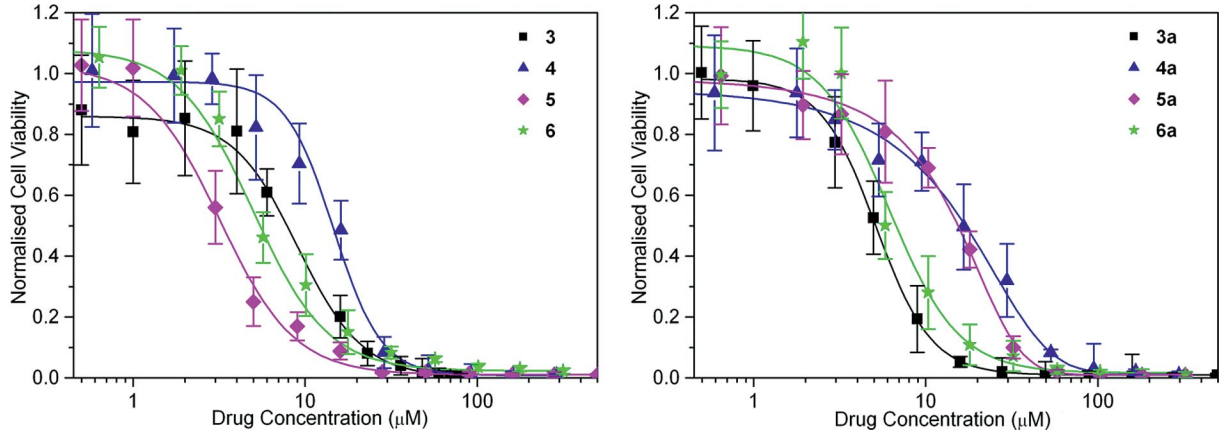


Figure 5. Cytotoxicity curves from WST-1 assays showing the effect of compounds **3**–**6** and **3a**–**6a** on the viability of MOLT-4 cells. The representation of viable cells was determined by cell proliferation reagent WST-1 24 h after the application of the compounds. To calculate cell viability, the value of the signal from the treated culture well was expressed as a part of that of the control well with untreated cells.

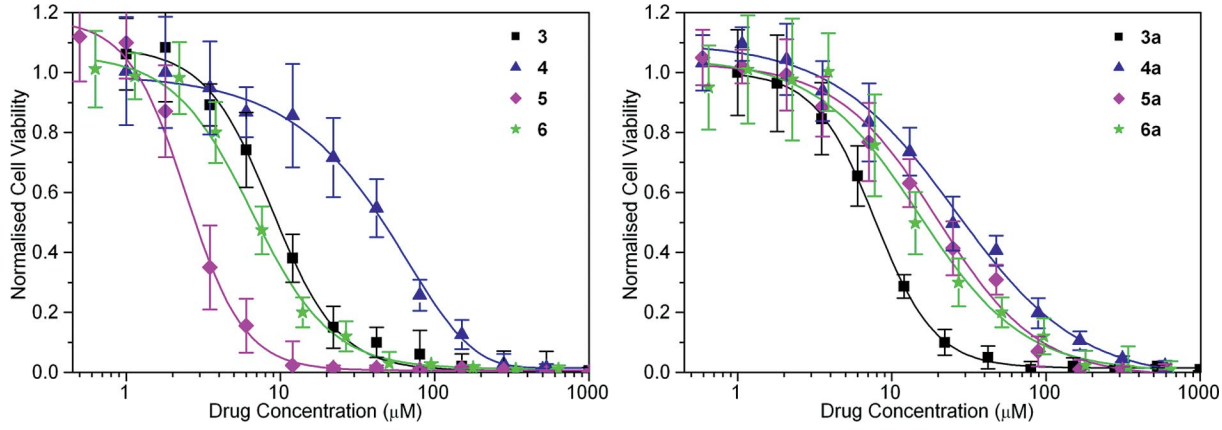


Figure 6. Cytotoxicity curves from WST-1 assays showing the effect of compounds **3**–**6** and **3a**–**6a** on the viability of HL-60 cells. The representation of viable cells was determined by cell proliferation reagent WST-1 24 h after the application of the compounds. To calculate cell viability, the value of the signal from the treated culture well was expressed as a part of that of the control well with untreated cells.

The highest cytotoxic effect was detected with the complex of 1,10-phenanthroline-5-amine (**5**). The IC_{50} values against both MOLT-4 [(3.1 ± 0.4) μM] and HL-60 [(2.8 ± 0.4) μM] are considerably lower than those observed for cisplatin (Table 3). The large improvement is observed mainly for the HL-60 cells because cisplatin exhibits a very slow decrease in the cytotoxicity curve (see Figure S1 in the Supporting Information).

Stability of the Phenanthroline Complexes

The behavior of phenanthroline complexes **3**–**5** and **3a**–**5a** was monitored in water, physiological saline, PBS, Iscove's Modified Dulbecco's Medium (IMDM), and Krebs–Ringer solution for seven days. Complexes **6** and **6a** were rejected from this part of the study owing to their very low solubility in water. Complexes **3**–**5** and **3a**–**5a** were fully dissolved without addition of organic solvents. The dissolution was checked by eye and by solution EPR spectroscopy.

The EPR spectra of compounds **3**–**5** and **3a**–**5a**, obtained immediately after dissolution in these media, show the same $|A_{iso}|$ and g_{iso} values as the solutions in the inert solvents such as acetone. It proves that the dicationic species stay unchanged because all expected hydrolysis products display very different values of these parameters.[8–10] The spectra in water, physiological saline, and PBS stay unchanged for seven days. Hence, the signal does not lower the intensity, and the appearance of another species was also not observed. Slightly lower stability of phenanthroline complexes **3**–**5** and **3a**–**5a** was observed in IMDM and Krebs–Ringer solution. These species were found to be stable in these media for six days. After seven days, a trace of carbonate complex [$Cp_2V(O,O\text{-}CO_3)$] and [$(\eta^5\text{-}C_5H_4Me)_2V(O,O\text{-}CO_3)$] were detected for compounds **3**–**5** and **3a**–**5a**, respectively (for example, see Figure 7).

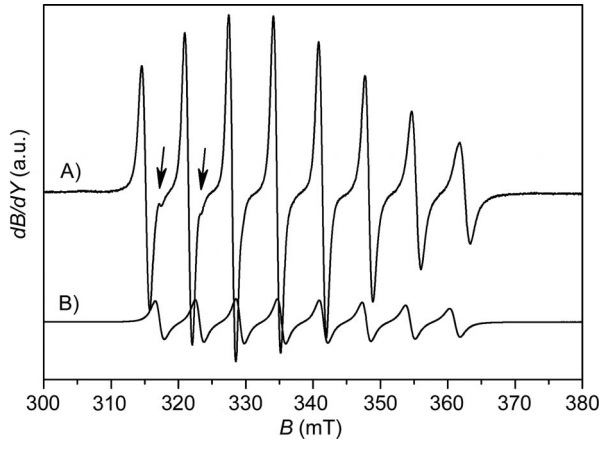


Figure 7. EPR spectra of (A) **3** in IMDM obtained 7 d after dissolution; (B) aqueous solution of [$Cp_2V(O,O\text{-}CO_3)$] prepared according to the literature.[8] The spectra were measured at ν = 9.45 GHz.

The behavior observed here in water, therapeutic, and physiological solutions proves that phenanthroline complexes **3**–**5** and **3a**–**5a** stay unchanged in the application media used in the preclinical in vitro testing. These compounds should be able to enter into the organism without hydrolyzing the phenanthroline or cyclopentadienyl ligand. The inertness toward ionic components of the human plasma at physiological pH contrasts with dichloride complexes **1** and **1a**. This might be the main reason for higher activity of phenanthroline complexes presented here.

Conclusion

Vanadocene complexes that contain phenanthroline ligands are promising cytotoxic agents, mainly owing to their high cytotoxicity, high water solubility, and stability in the application media. Their cytotoxic properties are easily modified through substitution of the phenanthroline framework. Substitution of the cyclopentadienyl ring described here has only a minor effect on the cytotoxicity. Nevertheless, it is possible that the attachment of substituents with functional groups can bring additional advantages, such as was previously successfully done with various metallocene compounds.[21]

The high activity of the phenanthroline compounds proves that stabilization of the vanadocene complexes through the substitution of chlorides can lead to considerable improvement of their cytotoxic properties. This finding contrasts with established mechanistic rules, which suggests that the modification of the putative active Cp_2M fragment is the only way to develop new, highly active metallocene anticancer drugs.

Experimental Section

Methods and Materials: All operations were performed under nitrogen using conventional Schlenk-line techniques. The solvents were purified and deoxygenated by standard methods.[22] Complexes **1**,[23] **1a**,[17] and **3**[13] were prepared according literature procedures. 1,10-Phenanthroline, 5-nitro-1,10-phenanthroline, 1,10-phenanthroline-5-amine, 4,7-diphenyl-1,10-phenanthroline, and Iscove's Modified Dulbecco's Medium were obtained from Sigma–Aldrich and used without further purification. Physiological saline (0.154 mM NaCl), PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2.0 mM KH_2PO_4, pH = 7.4), and Krebs–Ringer solution (115 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM Na_2SO_4, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 10 mM glucose, pH = 7.4) were prepared from analytical-grade chemicals and deionized redistilled water.

Infrared spectra were recorded in the 4000–400 cm^{-1} region with a Nicolet Magna 550 FTIR spectrometer using Nujol mull between KBr windows. The EPR spectra were recorded with Miniscope MS 300 spectrometers at the X band at ambient temperature. Mass spectrometry was performed with a quadruple mass spectrometer (LCMS 2010, Shimadzu, Japan). The sample was injected into the mass spectrometer with infusion mode at a constant flow rate of 10 μL min^{-1}, and electrospray ionization-mass spectrometry (ESI-MS) was used for the identification of analyzed samples.

[$Cp_2V(OTf)_2$] (2): Complex **1** (0.253 g, 1.00 mmol) was dissolved in THF (20 mL), treated with AgOTf (0.515 g, 2.00 mmol), and stirred for 90 min in the dark. The reaction mixture was filtered through Celite to remove the fine precipitate of silver chloride. The

clear green solution was evaporated under vacuum and recrystallized from a mixture of acetone/diethyl ether to give a grass-green powder. Yield: 144 mg (30%, 0.30 mmol). $C_{12}H_{10}F_6O_6S_2V$ (M_r = 479.27): C 30.07, H 2.10; found C 30.01; H 2.14. EPR (acetone): $|A_{iso}|$ = 79.7 G; g_{iso} = 1.980. Single crystals suitable for X-ray diffraction analysis were prepared by careful overlayering of the solution of compound **2** in acetone with diethyl ether.

[(η^5-$C_5H_4Me)_2V(OTf)_2$] (2a): The reaction was carried out as described for **2** but with **1a** (0.253 g, 0.90 mmol) and AgOTf (0.464 g, 1.80 mmol). Yield: 162 mg (35%, 0.31 mmol). $C_{14}H_{14}F_6O_6S_2V$ (M_r = 507.32): calcd. C 33.15, H 2.78; found C 33.32, H 2.89. EPR (acetone): $|A_{iso}|$ = 79.5 G; g_{iso} = 1.981.

[(η^5-$C_5H_4Me)_2V(phen)][OTf]_2$ (3a): Complex **1a** (0.253 g, 0.90 mmol) was dissolved in THF (20 mL), treated with AgOTf (0.464 g, 1.80 mmol), and stirred for 90 min in the dark. The reaction mixture was filtered through Celite to remove the fine precipitate of silver chloride. The clear green solution was treated with 1,10-phenanthroline (0.244 g, 1.35 mmol), which caused an immediate color change from green to brown. The reaction mixture was stirred for 18 h. The solid product was separated by decantation. It was washed with THF and diethyl ether and dried under vacuum to give a brown powder. Yield: 247 mg (40%, 0.36 mmol). $C_{26}H_{24}VS_2N_2F_6O_6$ (M_r = 689.54): calcd. C 45.29, H 3.51, N 4.06; found C 45.42, H 3.47, N 4.10. Positive-ion MS (acetone): *m*/*z* = 194.5 $[M]^{2+}$. EPR (acetone): $|A_{iso}|$ = 67.8 G, g_{iso} = 1.986; EPR (water): $|A_{iso}|$ = 67.8 G, g_{iso} = 1.985; EPR (physiological saline): $|A_{iso}|$ = 67.8 G, g_{iso} = 1.985; EPR (IMDM): $|A_{iso}|$ = 67.9 G, g_{iso} = 1.985; EPR (Krebs–Ringer solution): $|A_{iso}|$ = 67.9 G; g_{iso} = 1.986; EPR (PBS): $|A_{iso}|$ = 67.9 G; g_{iso} = 1.986. Single crystals of **3a**·0.5Me_2CO suitable for X-ray diffraction analysis were prepared by careful overlayering of the solution of compound **3a** in acetone with diethyl ether.

[Cp_2V(5-NO_2-phen)][OTf]$_2$ (4): The reaction was carried out as described for **3a** but with **1** (0.253 g, 1.00 mmol), AgOTf (0.515 g, 2.00 mmol), and 5-NO_2-phen (0.338 g, 1.5 mmol). Yield: 225 mg (32%; 0.32 mmol). $C_{24}H_{17}F_6N_3O_8S_2V$ (M_r = 704.47): calcd. C 40.92, H 2.43, N 5.96; found C 41.04, H 2.39, N 5.92. Positive-ion MS (acetone): *m*/*z* = 203 $[M]^{2+}$. EPR (acetone): $|A_{iso}|$ = 66.8 G, g_{iso} = 1.986; EPR (water): $|A_{iso}|$ = 67.0 G, g_{iso} = 1.985; EPR (physiological saline): $|A_{iso}|$ = 67.0 G, g_{iso} = 1.985; EPR (IMDM): $|A_{iso}|$ = 66.9 G, g_{iso} = 1.986; EPR (Krebs–Ringer solution): $|A_{iso}|$ = 67.0 G; g_{iso} = 1.985; EPR(PBS): $|A_{iso}|$ = 67.0 G; g_{iso} = 1.985.

[(η^5-$C_5H_4Me)_2V$(5-NO_2-phen)][OTf]$_2$ (4a): The reaction was carried out as described for **3a** but with 5-NO_2-phen (0.305 g, 1.35 mmol). Yield: 228 mg (35%, 0.31 mmol). $C_{26}H_{21}F_6N_3O_8S_2V$ (M_r = 732.52): calcd. C 42.63, H 2.89, N 5.74; found C 42.76, H 3.02, N 5.91. Positive-ion MS (acetone): *m*/*z* = 217 $[M]^{2+}$. EPR (acetone): $|A_{iso}|$ = 66.9 G; g_{iso} = 1.986; EPR (water): $|A_{iso}|$ = 67.0 G, g_{iso} = 1.985; EPR (physiological saline): $|A_{iso}|$ = 67.0 G, g_{iso} = 1.985; EPR (IMDM): $|A_{iso}|$ = 67.0 G, g_{iso} = 1.986; EPR (Krebs–Ringer solution): $|A_{iso}|$ = 67.0 G; g_{iso} = 1.986; EPR (PBS): $|A_{iso}|$ = 66.9 G; g_{iso} = 1.985.

[Cp_2V(5-NH_2-phen)][OTf]$_2$ (5): The reaction was carried out as described for **3a** but with **1** (0.253 g, 1.00 mmol), AgOTf (0.515 g, 2.00 mmol), and 5-NH_2-phen (0.293 g, 1.5 mmol). Yield: 202 mg (30%; 0.30 mmol). $C_{24}H_{19}F_6N_3O_6S_2V$ (M_r = 674.49): calcd. C 42.74, H 2.84, N 6.23; found C 42.80, H 2.77, N 6.18. Positive-ion MS (acetone): *m*/*z* = 188 $[M]^{2+}$. EPR (acetone): $|A_{iso}|$ = 67.4 G, g_{iso} = 1.986; EPR (water): $|A_{iso}|$ = 67.4 G, g_{iso} = 1.986; EPR (physiological saline): $|A_{iso}|$ = 67.4 G, g_{iso} = 1.986; EPR (IMDM): $|A_{iso}|$ = 67.5 G, g_{iso} = 1.986; EPR (Krebs–Ringer solution): $|A_{iso}|$ = 67.4 G; g_{iso} = 1.986; EPR (PBS): $|A_{iso}|$ = 67.4 G; g_{iso} = 1.986. Single crystals suitable for X-ray diffraction analysis were prepared by careful overlayering of the solution of compound **5** in acetone with diethyl ether.

[(η^5-$C_5H_4Me)_2V$(5-NH_2-phen)][OTf]$_2$ (5a): The reaction was carried out as described for **3a** but with 5-NH_2-phen (0.264 g, 1.35 mmol). Yield: 216 mg (34%, 0.31 mmol). $C_{26}H_{23}F_6N_3O_6S_2V$ (M_r = 702.54): calcd. C 44.45, H 3.30, N 5.98; found C 44.52, H 3.34, N 6.01. Positive-ion MS (acetone): *m*/*z* = 202 $[M]^{2+}$. EPR (acetone): $|A_{iso}|$ = 67.6 G, g_{iso} = 1.986; EPR (water): $|A_{iso}|$ = 67.6 G, g_{iso} = 1.986; EPR (physiological saline): $|A_{iso}|$ = 67.6 G, g_{iso} = 1.986; EPR (IMDM): A_{iso} = 67.7 G, g_{iso} = 1.986; EPR (Krebs–Ringer solution): $|A_{iso}|$ = 67.5 G; g_{iso} = 1.986; EPR (PBS): $|A_{iso}|$ = 67.6 G; g_{iso} = 1.986.

[Cp_2V(4,7-Ph_2-phen)][OTf]$_2$ (6): The reaction was carried out as described for **3a** but with **1** (0.253 g, 1.00 mmol), AgOTf (0.515 g, 2.00 mmol), and 4,7-Ph_2-phen (0.499 g, 1.50 mmol). The product did not precipitate from THF solution. The solvent was vacuum-evaporated, then the crude product was washed with diethyl ether and dried under vacuum to give a green-brown powder. Yield: 269 mg (33%, 0.33 mmol). $C_{36}H_{26}F_6N_2O_6S_2V$ (M_r = 811.66): calcd. C 53.27, H 3.23, N 3.45; found C 53.35, H 3.09, N 3.61. Positive-ion MS (acetone): *m*/*z* = 256.5 $[M]^{2+}$. EPR (acetone) $|A_{iso}|$ = 66.9 G, g_{iso} = 1.987.

[(η^5-$C_5H_4Me)_2V$(4,7-Ph_2-phen)][OTf]$_2$ (6a): The reaction was carried out as described for **6** but with 4,7-Ph_2-phen (0.450 g, 1.35 mmol). Yield: 245 mg (32%, 0.29 mmol). $C_{38}H_{30}F_6N_2O_6S_2V$ (M_r = 839.72): calcd. C 54.35, H 3.60, N 3.34; found C 54.53, H 3.55, N 3.22. Positive-ion MS (acetone): *m*/*z* = 270.5 $[M]^{2+}$. EPR (acetone): $|A_{iso}|$ = 66.8 G; g_{iso} = 1.987.

Stability Studies: The appropriate compound (50 μmol) was dissolved in the medium (e.g., water, physiological saline, PBS, IMDM, or Krebs–Ringer solution; 5 mL) under an air atmosphere. The composition and concentration of the vanadium(IV) species were monitored by EPR spectroscopy (Miniscope MS 300) immediately after dissolution, and then after each 12 h for 7 d. Samples were measured in 50 μL capillaries (fixed adapter) at room temperature. The concentration was calculated by the integration method.

Cytotoxicity Studies: The studies were performed on the human T-lymphocytic leukemia cells MOLT-4 obtained from the American Type Culture Collection (USA) and human promyelocytic leukemia cells HL-60 obtained from the European Collection of Cell Cultures (Porton Down, Salisbury, Great Britain). The cells were cultured in IMDM supplemented with a 20% fetal calf serum and 0.05% L-glutamine (Sigma–Aldrich) in a humidified incubator at 37 °C and a controlled 5% CO_2 atmosphere. Cell lines in the maximal range of up to 20 passages were used for this study. The cytotoxicity of compounds **1**, **3**–**6**, **1a**, **3a**–**6a**, and cisplatin were evaluated by the WST-1 cell viability test (Roche) according to the manufacturer's instructions. The assay was based on the reduction of WST-1 {4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate} by viable cells. The reaction produced a colored soluble formazan salt.[24] The absorbance at 440 nm was measured with a multiplate reader (Tecan Infinite 200). Compounds **1**, **3**–**5**, **1a**, **3a**–**5a**, and cisplatin were dissolved in cultivation medium to the desired concentrations. Compounds **6** and **6a** were dissolved in DMSO and diluted by cultivation medium to the desired concentrations. The cells were seeded in a 96-well plate, incubated in 1–1000 μM solutions of the evaluated compound 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. Absorbance data were normalized to 100%

cell viability for untreated cells. Half-inhibiting concentration (IC_{50}), which is defined as the concentration of the drug-reducing cell viability by 50%, and standard deviations were calculated from three independent measurements. They were obtained from the dose–response sigmoid using Origin Pro 8.0 (Microcal Software Inc., Northampton, MA, USA).

Crystallography: The X-ray data for crystals of compounds **2**, **3a**·0.5Me_2CO, and **5** were obtained at 150 K with an Oxford Cryostream low-temperature device on a Nonius Kappa CCD diffractometer with Mo-K_α radiation (λ = 0.71073 Å), a graphite monochromator, and the ϕ and χ scan mode. Data reductions were performed with DENZO-SMN.[25] Structures were solved by direct methods (SIR92)[26] and refined by full-matrix least-squares on the basis of F^2 (SHELXL97).[27] Crystallographic data are summarized in Table 4. Hydrogen atoms were mostly localized on a difference Fourier map. However, to ensure uniform treatment of the 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 the Cp ring, respectively.

Table 4. Crystallographic data of the compounds **1**, **3a**·0.5Me_2CO, and **5**.

	2	**3a**·0.5Me_2CO	**5**
Crystal system	monoclinic	triclinic	monoclinic
Space group	$P2_1/c$	$P\bar{1}$	$P2_1/c$
a [Å]	8.2350(3)	8.43701(5)	16.9301(12)
b [Å]	12.5260(7)	8.6150(7)	9.7960(8)
c [Å]	17.4559(9)	22.5601(16)	16.0840(11)
α [°]	90	80.896(7)	90
β [°]	115.327(5)	79.383(5)	107.336(6)
γ [°]	90	73.252(6)	90
Z	4	2	4
μ [mm^{-1}]	0.962	0.546	0.646
$D_{calcd.}$ [$g\,cm^{-3}$]	1.956	1.615	1.759
Crystal color	dark green	brown	red
Crystal shape	plate	rod	block
Size [mm]	0.29; 0.22; 0.07	0.76; 0.38; 0.19	0.35; 0.22; 0.10
θ range [°]	2.08–27.49	2.48–27.50	2.43–27.40
h range	–10/10	–10/10	–21/20
k range	–15/16	–11/11	–10/12
l range	–21/22	–29/29	–19/20
Reflns. measured	16609	28140	15321
Unique reflections	3707	6914	5683
R_{int}[a]	0.0514	0.0779	0.0650
Obsd. reflections	2842	5708	3788
Parameters	244	388	379
S[b] (all data)	1.074	1.101	1.148
R[c], wR[c]	0.0494, 0.1272	0.0554, 0.1333	0.0654, 0.1441
$\Delta\rho$ max./min. [$e\,Å^{-3}$]	1.138/–0.608	0.591/–0.484	0.788/–0.598

[a] $R_{int} = \Sigma|F_o^2 - F_{o(mean)}^2|/\Sigma F_o^2$. [b] $S = \{\Sigma[w(F_o^2 - F_c^2)^2]/(N_{diff.} - N_{params.})\}^{1/2}$ 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.

There is disordered solvent (acetone) in the structure of **3a**·0.5Me_2CO. Attempts were made to model this disorder or split it into two positions, but were unsuccessful. Platon/Squeeze[28] was used to correct the data for the presence of disordered solvent. A potential solvent volume of 240 $Å^3$ was found. Forty-four electrons per unit cell worth of scattering were located in the void. The calculated stoichiometry of solvent was calculated to be one molecule of acetone per unit cell, which results in 32 electrons per unit cell.

CCDC-904714 (for **2**), -904715 (for **3a**·0.5Me_2CO), and -904716 (for **5**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Supporting Information (see footnote on the first page of this article): Cytotoxicity curves of the WST-1 assays on MOLT-4 for cisplatin and on HL-60 for **1**, **1a**, and cisplatin.

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