# Synthesis, characterization and cytotoxic activity of vanadocene dithiocarbamate complexes

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**Highlights**

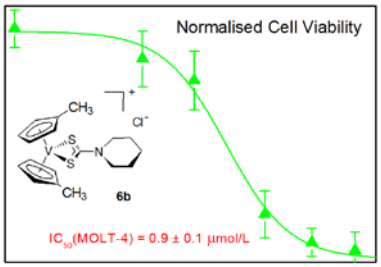
Water-soluble vanadocene dithiocarbamate complexes were prepared.

Spectroscopic and X-ray crystallographic data are presented.

Cytotoxicity toward leukemia cells MOLT-4 was documented.

**Graphical Abstract**

Series of vanadocene compounds bearing dithiocarbamate ligands was synthesized and characterized. The cytotoxicity study was performed on human leukemia cells MOLT-4 revealed strong cytotoxicity for species bearing cyclic dithiocarbamates.




**Keywords:** Metallocene; Vanadium; *S*,*S*-chelating ligands; EPR spectroscopy; Cytotoxicity

**Abstract**

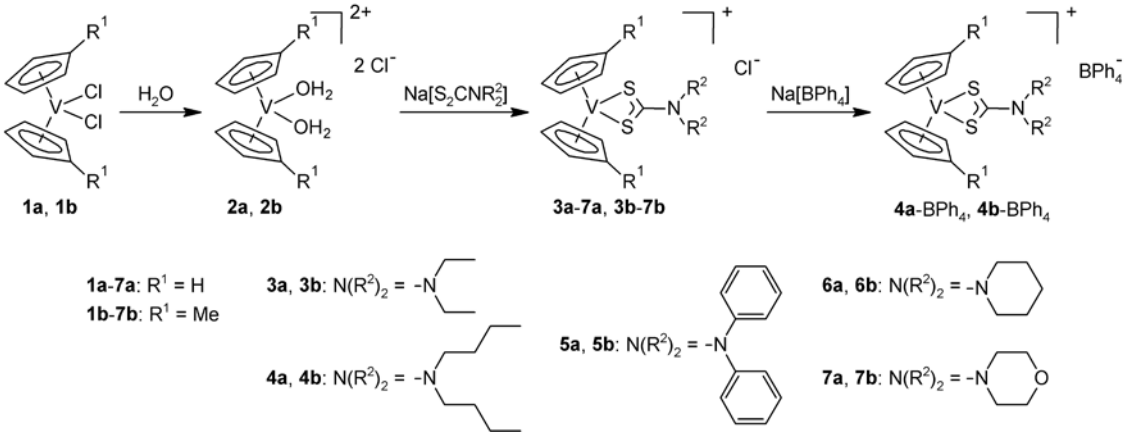
This study describes the synthesis of water-soluble vanadocene and 1,1'-dimethylvanadocene compounds bearing dithiocarbamate ligands. All prepared compounds were characterized by analytical and spectroscopic methods. The structure of $[(\eta^5\text{-}C_5H_4Me)_2V\{S_2CN(CH_2)_4O\}]Cl$ was determined by single-crystal X-ray diffraction analysis. The cytotoxicity of the synthesized compounds was investigated on human leukemia cells MOLT-4. The derivatives bearing cyclic dithiocarbamates show considerably stronger activity than reported for cisplatin.

## 1. Introduction

The vanadocene dichloride, $Cp_2VCl_2$ (Cp = $\eta^5$-$C_5H_5$, **1**) is under comprehensive investigation since its cytostatic activity was discovered by Köpf and Köpf-Maier in 1979 [1]. The early studies have demonstrated *in vivo* activity toward Ehrlich ascites tumor [1] and murine mammary adenocarcinoma [2]. They also brought its basic pharmacological [3-6] and toxicological data [7]. Following studies have given a detailed description of the interactions with biologically relevant ligands and an early insight into mechanism of action [8-17]. In last two decades, several modified vanadocene derivatives have been described. They show improved properties relevant to biological applications [15]. Increased cytotoxicity was observed for several vanadocene species with functional groups in the side chains of substituted cyclopentadienyl rings [18-21]. Water-solubility of vanadocene dichloride and its 1,1'-dimethyl substituted analogue was enhanced through encapsulation into cyclodextrin cavity [22]. Another approach for the design of highly active vanadocene species involves exchange of chloride ligands. Indeed, various metallocene species bearing *N*,*N*-chelating ligands exhibit increased *in vitro* cytotoxicity toward various leukemia cell lines [23-25].

Metal dithiocarbamates have attracted considerable attention for their antibacterial [26, 27] and antifungal properties [28]. They found application in agriculture as pesticides [29]. Insulin mimetic properties were reported for oxovanadium dithiocarbamates [30]. Cationic vanadocene diethyldithiocarbamate complex $[Cp_2V(S_2CNEt_2)]^+$ exhibits a strong spermicidal activity [31-33] without marked mucosal irritation, toxicity or systematic vanadium absorption [33, 34].

The aim of this work is to synthesize new vanadocene dithiocarbamate compounds with increased water-solubility and to determine their *in vitro* cytotoxicity on human leukemia cells MOLT-4 in the attempt to find a highly active complex suitable for a future detailed biological investigation.




**Scheme 1.** Preparation of vanadocene complexes bearing *S*,*S*-coordinated dithiocarbamate ligands.

## 2. Results and discussion

### *2.1 Preparation and characterization*

Our attempt to synthesize new water-soluble dithiocarbamate compounds led us to investigate reaction between vanadocene dichloride (**1a**) and dithiocarbamates in aqueous media by electron paramagnetic resonance (EPR). This method is very suitable for investigation of vanadium(IV) compounds due to strong hyperfine coupling by $^{51}V$ ($I = {}^{7}/_{2}$, 99.8%), which is very sensitive to changes in the inner coordination sphere of vanadium [35, 36].

Dissolution of **1a** in water led to immediate formation of aqua complex $[Cp_2V(OH_2)_2]^{2+}$ (**2a**, Scheme 1), which results in large increase of hyperfine coupling of the EPR signal (*c.f.* Spectrum A and B in Figure 1). The observed isotropic g-factor ($g_{iso}$) and isotropic hyperfine coupling constant ($|A_{iso}|$) of **2a** well correlates with the values reported previously [8]. Addition of one equivalent of sodium diethyldithiocarbamate led to color change from deep bluish green to olive green and partial precipitation of reaction product, which was separated by filtration. EPR spectrum of the solid product dissolved in dichloromethane confirms appearance of desired complex $[Cp_2V(S_2CNEt_2)]Cl$ (**3a**, Figure 1). The species has considerably lower $|A_{iso}|$ than aqua complex **2a**, which well correlates with literature data reported for tetrafluoroborate analogue $[Cp_2V(S_2CNEt_2)][BF_4]$ (**3a**-$BF_4$) [37]. We note that here reported **3a** is soluble in water. Therefore, majority of the product stays in the filtrate as revealed by EPR spectroscopy. Indeed, spectrum of the filtrate was virtually the same as aforementioned spectrum of **3a** in dichloromethane, which clearly proves full conversion in the aqueous solution.

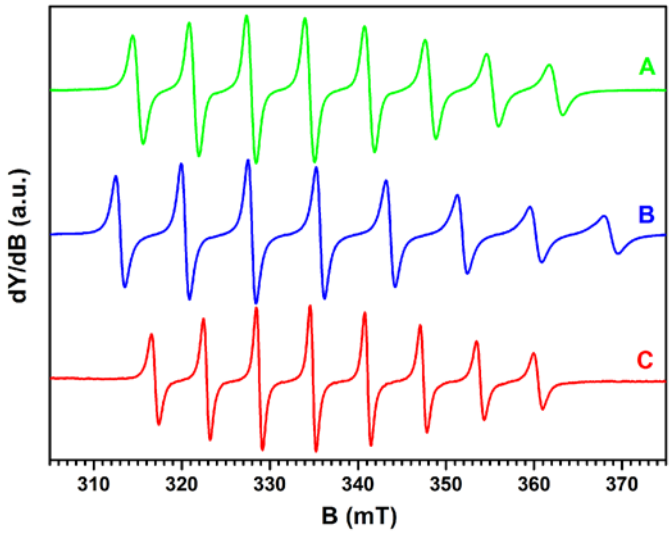



**Fig 1.** EPR spectra: A) $Cp_2VCl_2$ (**1a**, $CH_2Cl_2$ solution); B) $[Cp_2V(OH_2)_2]Cl_2$ (**2a**, aqueous solution); C) $[Cp_2V(S_2CNEt_2)]Cl$ (**3a**, $CH_2Cl_2$ solution). The spectra were measured at $\nu$ = 9.45 GHz.

We note that synthetic protocol for **3a** in scale of hundreds of milligrams was simplified. The final product was not filtered but the volatiles were vacuum evaporated from suspension and then purified by recrystallization. This modified procedure is also suitable for **5a** and 1,1'-dimethylvanadocene analogues **3b** and **5b**. In case of cyclic dithiocarbamates (complexes **6a**, **6b**, **7a** and **7b**), 20–50% excess of given ligand is necessary to reach sufficient yields.

Very low conversions were observed only for butyl derivatives **4a** and **4b**, which led us to detailed study of the reaction between **2a** and $Na[S_2CNBu_2]$. EPR spectra of the reaction mixtures with various molar ratios of the starting compounds (1:1 to 1:10) revealed presence of **2a** in mixture with **4a**. Even the large excess of the ligand did not give sufficient conversion (see Figure 2). Such observation led us to precipitate the complex cation with large anion, which shifts the equilibrium reaction toward products. Purity of the final complexes **4a**-$BPh_4$ and **4b**-$BPh_4$ was checked by EPR spectroscopy (Spectrum C in Figure 2). Although this modification of led to considerable decrease of water-solubility, the product stay suitable for our biological assays.

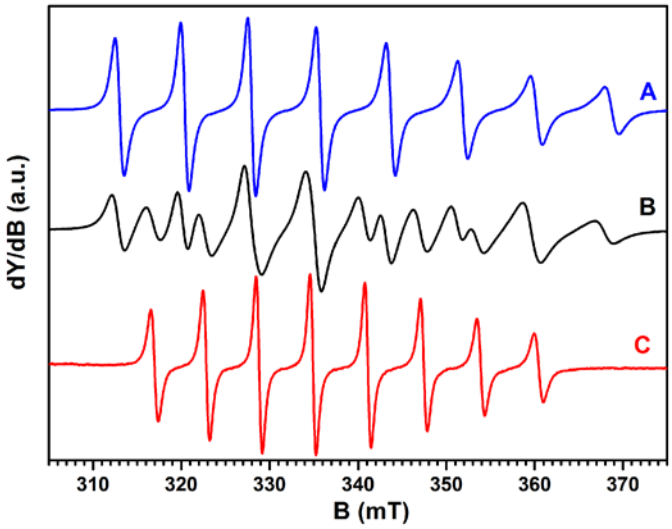



**Fig 2.** EPR spectra: A) $[Cp_2V(OH_2)_2]Cl_2$ (**2a**, aqueous solution); B) Reaction mixture of $[Cp_2V(OH_2)_2]Cl_2$ after addition of 10 eq. of $Na(S_2CNBu_2)$ (aqueous solution); C) $[Cp_2V(S_2CNBu_2)][BPh_4]$ (**4a**, methanolic solution). The spectra were measured at $\nu$ = 9.45 GHz.

The isolated vanadocene complexes (**3a**, **4a**-$BPh_4$, **5a**–**7a**) and their 1,1'-dimethylvanadocene congeners (**3b**, **4b**-$BPh_4$, **5b**–**7b**) were characterized by elemental analysis, electrospray ionization mass spectrometry (ESI-MS) and EPR spectroscopy. Crystal structure of **7b** was determined by single crystal X-ray diffraction analysis (XRD).

Base peaks in ESI-MS spectra were attributed to cationic complexes $[(C_5H_4R^1)_2V\{S_2CN(R^2)_2\}]^+$, which reveals successful assembly of desired compounds. The EPR spectra of isolated dithiocarbamate complexes were measured in dichloromethane or methanolic solutions. The obtained $g_{iso}$ and $|A_{iso}|$ values are summarized in Table 1. The complexes of dialkyl and cyclic dithiocarbamate give $g_{iso}$ and $|A_{iso}|$ in very narrow ranges 1.988–1.989 and 57.7–58.0 × $10^{-4}$ $cm^{-1}$, respectively. Lower values of $|A_{iso}|$ were observed for complexes of aromatic dithiocarbamate (**5a** and **5b**), which is attributed to more efficient delocalization of unpaired electron over *S*,*S*-chelating ligand. The modification of the cyclopentadienyl ring has only a negligible effect on the EPR parameters since the unpaired electron occupies the orbital antibonding to the bonds V–S [35]. We note that all here described complexes show considerably weaker hyperfine coupling than starting dichlorides **1a** and **1b**. It is not only due to higher covalency of the bonds V–S (compared to V–Cl in $Cp_2VCl_2$) but also due to chelate effect, which enables more efficient delocalization of the unpaired electron over the ligands [35].

**Table 1.** Isotropic *g*-factors and hyperfine coupling constants ($10^{-4}$ $cm^{-1}$) of the vanadocene complexes.

| | $g_{iso}$ | $\|A_{iso}\|$ | | $g_{iso}$ | $\|A_{iso}\|$ |
|---|---|---|---|---|---|
| **1a** | 1.991 | 69.5 | **1b** | 1.990 | 69.5 |
| **2a** | 1.979 | 74.0 | **2b** | 1.979 | 74.0 |
| **3a** | 1.989 | 57.9 | **3b** | 1.989 | 57.9 |
| **4a**-$BPh_4$ | 1.989 | 57.8 | **4b**-$BPh_4$ | 1.988 | 57.7 |
| **5a** | 1.990 | 56.7 | **5b** | 1.990 | 56.6 |
| **6a** | 1.989 | 57.8 | **6b** | 1.989 | 57.8 |
| **7a** | 1.989 | 58.0 | **7b** | 1.988 | 57.9 |

Molecular structure of $[(C_5H_4Me)_2V\{S_2CN(CH_2)_4O)\}]^+$ (**7b**), elucidated from XRD analysis, is shown in Figure 3. Important structural parameters, describing coordination sphere of vanadium, are listed in Table 2 together with literature data of **3a**-$BF_4$. We note that unit cell of here described crystal includes two crystallographically independent but essentially the same molecules denoted **7b**-A and **7b**-B. The cationic complex species have a typical bend metallocene structure, in which two $\eta^5$-bonded Cp rings and two sulfur donor atoms of the $\kappa^2$-bonded dithiocarbamate ligand occupy the pseudotetrahedral coordination sites around vanadium atom in the formal oxidation state IV. In both molecules **7b**-A and **7b**-B, the substituted Cp rings near the eclipsed conformation with methyl groups at the same side of the complex.

Distances between the centroid of the cyclopentadienyl rings and vanadium atom [Cg–V = 1.9507(17)–1.9618(18) Å] and Cg–V–Cg angles [133.66(7), 134.55(7)°] are in the range common for the previously reported vanadocene(IV) complexes (Cg–V: 1.95–1.97 Å; Cg–V–Cg: 131–135°) [19, 38-43]. Dithiocarbamate ligand is symmetrically coordinated to vanadium atom with morpholine ring in chair conformation. Bond lengths V–S [2.4726(10)– 2.4904(10) Å] are in line with literature data reported for related compound **3a**-$BF_4$ [31].

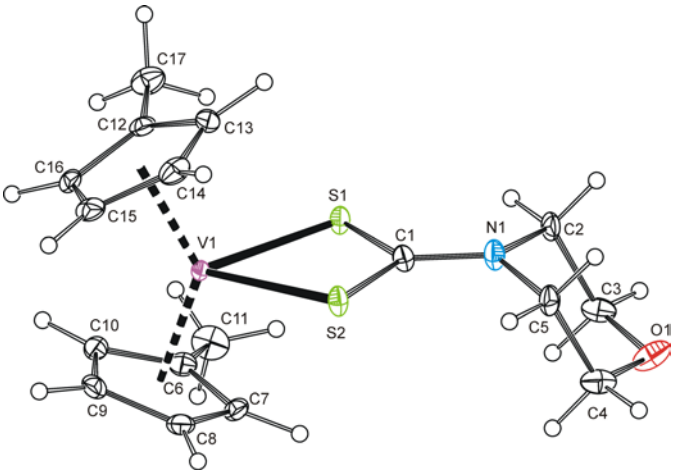



**Fig. 3.** ORTEP drawing of the dicationic complex $[(C_5H_4Me)_2V\{S_2CN(CH_2)_4O)\}]^+$ present in the crystal structure of **7b** (ellipsoids: 30% probability). Numbering of all non-hydrogen atoms is shown.

**Table 2.** Selected bond distanced (Å) and bond angles (°) of the vanadocene dithiocarbamate compounds.

| | **3a**-$BF_4$ [b] | **11b**-A | **11b**-B |
|---|---|---|---|
| V–$Cg_1$ [a] | 1.965 | 1.9548(17) | 1.9507(17) |
| V–$Cg_2$ [a] | 1.965 | 1.9579(16) | 1.9618(18) |
| V–$S_1$ | 2.4566(12) | 2.4821(10) | 2.4726(10) |
| V–$S_2$ | 2.4797(11) | 2.4904(10) | 2.4756(10) |
| $Cg_1$–V–$Cg_2$ [a] | 134.6 | 133.66(7) | 134.55(7) |
| $S_1$–V–$S_2$ | 70.79(4) | 70.39(3) | 70.78(3) |

[a] Cg denotes center of gravity of the five-membered ring.

[b] Data reported elsewhere [31].

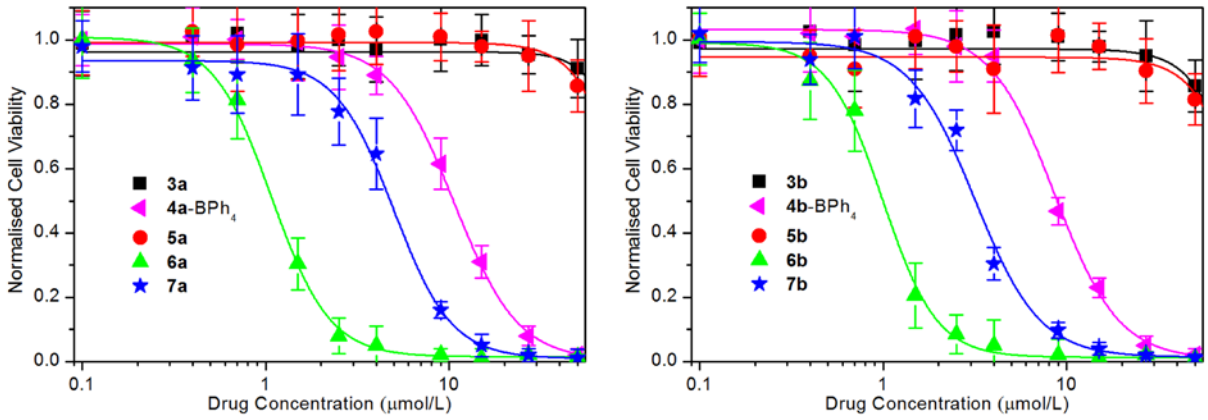



**Fig.4.** Cytotoxicity curves from WST-1 assays showing the effect of vanadocene compounds on the viability of MOLT-4 cells.

*2.2 Cytotoxicity Studies*

The cytotoxic effect of the synthesized dithiocarbamate complexes was established on human T-lymphocytic leukemia cells MOLT-4 using standard WST-1 viability assays, 24 h after the incubation with evaluated compound. Obtained cytotoxicity curves are given in Figure 4 and estimated half-maximal inhibitory concentrations ($IC_{50}$) are summarized in Table 3.

The viability assays show that cytotoxic activity of vanadocene complexes strongly depend on the coordinated *S,S*-chelating ligand while the effect of cyclopentadienyl ring substitution is here negligible. Very strong cytotoxic effect was observed for complexes of dithiocarbamates with piperidine cycle **6a** ($IC_{50}$ = 1.0 ± 0.1 mmol/L) and **6b** ($IC_{50}$ = 1.0 ± 0.1 mmol/L), whose are about 15 times more active than cisplatin (15.8 ± 1.9 µmol/L) [44]. Strong activity was also observed for derivatives bearing morpholine cycle **7a** ($IC_{50}$ = 5.1 ± 0.4 mmol/L) and **7b** ($IC_{50}$ = 3.2 ± 0.3 mmol/L) and dibutyldithiocarbamate complexes **4a**-$BPh_4$ ($IC_{50}$ = 10.4 ± 0.5

mmol/L) and **4b**-$BPh_4$ ($IC_{50}$ = 8.9 ± 0.7 mmol/L). Remaining complexes exhibit $IC_{50}$ values higher than 50 µmol/L implying negligible cytotoxic activity.

**Table 3.** Cytotoxicity toward MOLT-4 cells expressed as the $IC_{50}$ values (µmol/L).

| | $IC_{50}$ | | $IC_{50}$ |
|---|---|---|---|
| **3a** | >50 | **3b** | >50 |
| **4a**-$BPh_4$ | 10.4 ± 0.5 | **4b**-$BPh_4$ | 8.9 ± 0.7 |
| **5a** | >50 | **5b** | >50 |
| **6a** | 1.0 ± 0.1 | **6b** | 0.9 ± 0.1 |
| **7a** | 5.1 ± 0.4 | **7b** | 3.2 ± 0.3 |

## 3. Conclusions

This study brought synthetic protocol for assembly of water-soluble vanadocene complexes bearing *S,S*-chelating ligands. The use of chloride counterion enhances solubility in aqueous media, which is necessary for biological studies. We note that previously reported cationic dithiocarbamate complexes compensated with larger anions, namely $BF_4$, $BPh_4$ or OTf are sparingly soluble or insoluble in water. Our cytotoxic study has shown that activity of the vanadocene compounds relates with the substitution pattern of the dithiocarbamate ligand. The promising activity was observed for derivatives of cyclic dithiocarmatates. Their $IC_{50}$ values are comparable with derivatives bearing modified 1,10-phenantholine ligands [23] and about one order of magnitude lower than reported for cisplatin [44].

## 4. Experimental Section

### *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 [45]. Literature methods were used for syntheses of vanadocene complexes $Cp_2VCl_2$ (**1a**) [46] and ($\eta^5$-$C_5H_4Me)_2VCl_2$ (**1b**) [47]. Starting $Na(S_2CNEt_2)\cdot 3H_2O$ and $Na[BPh_4]$ were obtained from Sigma-Aldrich and used without further purification. The other sodium dithiocarbamates were prepared as described elsewhere [48]. The EPR spectra were recorded on Miniscope MS 300 spectrometer at X-band at ambient temperature. Mass spectrometry was performed on an LCMS 2010 quadrupole mass spectrometer (Shimadzu, Japan). The sample was injected into the mass spectrometer with infusion mode at a constant flow rate of 10 L/min, and electrospray ionization mass spectrometry (ESI-MS) was used for identification of analyzed samples.

### *4.2 Synthesis of [$Cp_2V(S_2CNEt_2)$]Cl (**3a**)*

$Cp_2VCl_2$ (**1a**; 0.200 g, 0.794 mmol) was dissolved in degassed water (20 mL) and treated with solution of $Na(S_2CNEt_2)\cdot 3H_2O$ (0.179 g, 0.794 mmol) in degassed water (15 mL). The reaction mixture was stirred for 15

min and then volatiles were vacuum evaporated. The crude product was extracted with $CH_2Cl_2$ (10 mL) and the filtrate was precipitated with ether (20 mL). The product was decanted, washed ether (2 × 10 mL) and vacuum dried. Yield: 0.192 g (66 %, 0.526 mmol). Olive-green powder. Calc. for $C_{15}H_{20}S_2NVCl$ (MW 364.85): C, 49.40; H, 5.53; N, 3.84; S, 17.58. Anal. Found: C, 49.15; H, 5.48; N, 3.80; S, 17.35. Positive ion MS(MeOH): *m*/*z* = 329 $[M]^+$. EPR($CH_2Cl_2$): $|A_{iso}|$ = 62.4 G, $g_{iso}$ = 1.989.

*4.3 Synthesis of [($\eta^5$-$C_5H_4Me)_2V(S_2CNEt_2)$]Cl (**3b**)*

The reaction was carried out as described for **3a** but with ($\eta^5$-$C_5H_4Me)_2VCl_2$ (**1b**; 0.200 g, 0.714 mmol) and $Na(S_2CNEt_2)\cdot 3H_2O$ (0.161 g, 0.714 mmol). Yield: 0.190 g (68 %, 0.484 mmol). Olive-green powder. Calc. for $C_{17}H_{24}S_2NVCl$ (MW 392.91): C, 51.97; H, 6.16; N, 3.56; S, 16.32. Anal. Found: C, 51.75; H, 6.10; N, 3.25; S, 16.27. Positive ion MS(MeOH): *m*/*z* = 357 $[M]^+$. EPR($CH_2Cl_2$): $|A_{iso}|$ = 62.4 G, $g_{iso}$ = 1.989.

*4.4 Synthesis of [$Cp_2V(S_2CNBu_2)$][$BPh_4$] (**4a**-$BPh_4$)*

$Cp_2VCl_2$ (**1a**; 0.200 g, 0.794 mmol) was dissolved in degassed water (20 mL) and treated with solution of $Na(S_2CNBu_2)$ (0.198 g, 0.873 mmol) in degassed water (5 mL). The reaction mixture was stirred for 15 min. The clear green solution was treated with saturated solution of $Na(BPh_4)$ in methanol (25mL). Appeared precipitate was filtered off, washed with cold water (5 mL) and then with ether (2 × 10 mL) and vacuum dried. Yield: 0.267 g (48 %, 0.379 mmol). Light olive-green powder. Calc. for $C_{43}H_{48}S_2NVB$ (MW 704.73): C, 73.29; H, 6.87; N, 1.99; S, 9.10. Anal. Found: C, 73.15; H, 6.80; N, 1.98; S, 9.25. Positive ion MS(MeOH): *m*/*z* = 386 $[M]^+$. EPR(MeOH): $|A_{iso}|$ = 62.3 G, $g_{iso}$ = 1.989.

*4.5 Synthesis of [($\eta^5$-$C_5H_4Me)_2V(S_2CNBu_2)$][$BPh_4$] (**4b**-$BPh_4$)*

The reaction was carried out as described for **4a**-$BPh_4$ but with ($\eta^5$-$C_5H_4Me)_2VCl_2$ (**1b**; 0.200 g, 0.714 mmol) and $Na(S_2CNBu_2)$ (0.179 g, 0.786 mmol). Yield: 0.230 g (44 %, 0.314 mmol). Olive-green powder. Calc. for $C_{45}H_{52}S_2NVB$ (MW 732.79): C, 73.76; H, 7.15; N, 1.91; S, 8.75. Anal. Found: C, 73.53; H, 7.02; N, 2.01; S, 8.58. Positive ion MS(MeOH): *m*/*z* = 413 $[M]^+$. EPR(MeOH): $|A_{iso}|$ = 62.2 G, $g_{iso}$ = 1.988.

*4.6 Synthesis of [$Cp_2V(S_2CNPh_2)$]Cl (**5a**)*

The reaction was carried out as described for **3a** but with $Cp_2VCl_2$ (**1a**; 0.200 g, 0.794 mmol) and $Na(S_2CNPh_2)$ (0.212 g, 0.794 mmol). Yield: 0.205 g (56 %, 0.444 mmol). Olive-green powder. Calc. for $C_{23}H_{20}S_2NVCl$ (MW 460.94): C, 59.93; H, 4.37; N, 3.04; S, 13.91. Anal. Found: C, 59.68; H, 4.21; N, 3.15; S, 13.76. Positive ion MS(MeOH): *m*/*z* = 425 $[M]^+$. EPR($CH_2Cl_2$): $|A_{iso}|$ = 61.0 G, $g_{iso}$ = 1.990.

*4.7 Synthesis of [($\eta^5$-$C_5H_4Me)_2V(S_2CNPh_2)$]Cl (**5b**)*

The reaction was carried out as described for **3a** but with ($\eta^5$-$C_5H_4Me)_2VCl_2$ (**1b**; 0.200 g, 0.714 mmol) and $Na(S_2CNPh_2)$ (0.191 g, 0.714 mmol). Yield: 0.185 g (53 %, 0.378 mmol). Olive-green powder. Calc. for $C_{25}H_{24}S_2NVCl$ (MW 488.99): C, 61.41; H, 4.95; N, 2.86; S, 13.12. Anal. Found: C, 61.35; H, 4.69; N, 2.98; S, 13.26. Positive ion MS(MeOH): *m*/*z* = 453 $[M]^+$. EPR($CH_2Cl_2$): $|A_{iso}|$ = 60.9 G, $g_{iso}$ = 1.990.

*4.8 Synthesis of [$Cp_2V\{S_2CN(CH_2)_5\}$]Cl (**6a**)*

The reaction was carried out as described for **3a** but with $Cp_2VCl_2$ (**1a**; 0.200 g, 0.794 mmol) and Na{$S_2CN(CH_2)_5$)} (0.175 g, 0.952 mmol). Yield: 0.169 g (56 %, 0.448 mmol). Olive-green powder. Calc. for $C_{16}H_{20}S_2NVCl$ (MW 376.86): C, 50.99; H, 5.35; N, 3.72; S, 17.03. Anal. Found: C, 50.75; H, 5.41; N, 3.68; S, 17.11. Positive ion MS(MeOH): *m*/*z* = 341 $[M]^+$. EPR($CH_2Cl_2$): $|A_{iso}|$ = 62.3 G, $g_{iso}$ = 1.989.

*4.9 Synthesis of [($\eta^5$-$C_5H_4Me)_2V\{S_2CN(CH_2)_5\}$]Cl (**6b**)*

The reaction was carried out as described for **3a** but with ($\eta^5$-$C_5H_4Me)_2VCl_2$ (**1b**; 0.200 g, 0.714 mmol) and Na{$S_2CN(CH_2)_5$)} (0.157 g, 0.857 mmol). Yield: 0.201 g (70 %, 0.496 mmol). Olive-green powder. Calc. for $C_{18}H_{24}S_2NVCl$ (MW 404.92): C, 53.39; H, 3.45; N, 2.86; S, 15.84. Anal. Found: C, 53.25; H, 3.52; N, 2.95; S, 15.91. Positive ion MS(MeOH): *m*/*z* = 369 $[M]^+$. EPR($CH_2Cl_2$): $|A_{iso}|$ = 62.3 G, $g_{iso}$ = 1.989.

*4.10 Synthesis of [$Cp_2V\{S_2CN(CH_2)_4O\}$]Cl (**7a**)*

The reaction was carried out as described for **3a** but with $Cp_2VCl_2$ (**1a**; 0.200 g, 0.794 mmol) and Na{$S_2CN(CH_2)_4O$)} (0.221 g, 1.190 mmol). Yield: 0.222 g (74 %, 0.586 mmol). Olive-green powder. Calc. for $C_{15}H_{18}S_2NOVCl$ (MW 378.84): C, 47.56; H, 4.79; N, 3.70; S, 16.93. Anal. Found: C, 47.45; H, 4.74; N, 3.78; S, 16.83. Positive ion MS(MeOH): *m*/*z* = 343 $[M]^+$. EPR($CH_2Cl_2$): $|A_{iso}|$ = 62.5 G, $g_{iso}$ = 1.989.

*4.11 Synthesis of [($\eta^5$-$C_5H_4Me)_2\{S_2CN(CH_2)_4O\}$]Cl (**7b**)*

The reaction was carried out as described for **3a** but with ($\eta^5$-$C_5H_4Me)_2VCl_2$ (**1b**; 0.200 g, 0.714 mmol) and Na{$S_2CN(CH_2)_4O$)} (0.199 g, 1.071 mmol). Yield: 0.205 g (71 %, 0.506 mmol). Olive-green powder. Calc. for $C_{18}H_{24}S_2NVCl$ (MW 404.92): C, 53.39; H, 3.45; N, 2.86; S, 15.84. Anal. Found: C, 53.25; H, 3.52; N, 2.95; S, 15.91. Positive ion MS(MeOH): *m*/*z* = 369.079 $[M]^+$. EPR($CH_2Cl_2$): $|A_{iso}|$ = 62.4 G, $g_{iso}$ = 1.988. Single crystals suitable for X-ray diffraction analysis were prepared by careful overlayering of dichloromethane solution with hexane.

*4.12 Cytotoxic Studies*

Studies were performed on the human T-lymphocytic leukemia cells MOLT-4 obtained from the European Collection of Authenticated Cell Cultures (UK). The cell was cultured in Iscove's modified Dulbecco's medium supplemented with 20% fetal calf serum and 2 mmol/L L-glutamine, 50 μg/mL peniciline and 50 μg/mL streptomycine (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 were used for this study. Cytotoxicity of vanadium compounds was evaluated by the WST-1 cell viability test (Roche, Germany) according to the manufacturer's instructions. The assay is 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 produces a colored soluble formazan salt [49]. The absorbance at 440 nm was measured using a multiplate reader (Tecan Infinite 200). Given compound was dissolved in cultivation medium to the desired concentrations. The cells were seeded in a 96-well plate, incubated in 1–50 μmol/L solutions of given 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 non-treated cells; half inhibiting

concentration ($IC_{50}$), defined as the concentration of the drug reducing cell viability by 50%, was obtained from the dose-response sigmoid using Origin Pro (version 8, Microcal Software, Inc., Northampton, MA, USA).

### *4.13 Crystallography*

Crystallographic data, summarized in Table 4, were collected on Bruker D8 VENTURE Kappa Duo PHOTON100 by IμS micro-focus sealed tube MoKα (λ = 0.77015 Å) at a temperature of 150(2) K. The structures were solved by direct methods (SHELXL 2014/7) [50] and refined by full matrix least squares based on $F^2$ (SHELXL97) [51]. The hydrogen atoms on carbon were fixed into idealized positions (riding model) and assigned temperature factors either $H_{iso}(H) = 1.2\ U_{eq}$(pivot atom) or $H_{iso}(H) = 1.5$ Ueq (pivot atom) for methyl moiety. CCDC 1856610 contains 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.

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**Table 4.** Crystallographic data of **7b**.

| | **7b** |
|---|---|
| Cryst system | orthorhombic |
| Space group | P$na2_1$ (No. 33) |
| $a$ (Å) | 12.2582(5) |
| $b$ (Å) | 22.2883(8) |
| $c$ (Å) | 13.0608(5) |
| $\alpha$ (deg) | 90 |
| $\beta$ (deg) | 90 |
| $\gamma$ (deg) | 90 |
| $Z$ | 8 |
| V (Å$^3$) | 3568.4(2) |
| $\mu$ (mm$^{-1}$) | 0.942 |
| $D_{calc}$ (g cm$^{-3}$) | 1.515 |
| Cryst color | dark red |
| Cryst shape | block |
| Cryst size (mm) | 0.164×0.455×0.520 |
| θ range (deg) | 2.46-27.52 |
| Index ranges | $-15 \le h \le 15$ |
| | $-26 \le k \le 28$ |
| | $-16 \le l \le 16$ |
| Reflections collected | 42143 |
| Independent reflections | 8161 ($R_{int}$ = 0.0304) |
| Reflections observed [$I > 2\ \sigma(I)$] | 7738 |
| Parameters | 420 |
| $S$ (all data)[a,b] | 1.030 |
| Final R indicates [$I > 2\ \sigma(I)$] | $R1$ = 0.0330 |
| | $wR2$ = 0.0760 |
| Final R indicates (all data)[a,b] | $R1$ = 0.0306 |
| | $wR2$ = 0.0774 |
| Lergest difference in peak and hole (e Å$^{-3}$) | 0.820, –0.478 |

[a] $R1 = \sum \left| |F_o| - |F_c| \right| / \sum |F_o|$.

[b] $wR2 = \sqrt{\sum \left[ w\left(F_o^2 - F_c^2\right)^2 \right] / \sum \left[ w\left(F_o^2\right)^2 \right]}$.

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