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Searching for Vanadium-Based Prospective Agents against *Trypanosoma cruzi*: Oxidovanadium(IV) Compounds with Phenanthroline Derivatives as Ligands

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Abstract. Searching for new promising metal-based drugs for the treatment of parasitic diseases against *Trypanosoma cruzi*, three related oxidovanadium(IV) complexes, $[V^{IV}O(SO_4)(H_2O)_2(NN)]$, with the phenanthroline derivatives (NN) [1,2,5]thiadiazolo[3,4-*f*][1,10]phenanthroline (tdzp), 1,10-phenanthroline-5,6-dione (phendione), and 5,6-epoxy-5,6-dihydro-1,10-phenanthroline (epoxyphen) are synthesized, characterized, and evaluated in vitro as anti-*T. cruzi* agents. The compounds are characterized in the solid state and in solution by elemental analysis, electrospray ionization mass spectrometry (ESI-MS), conductimetric measurements, and infrared (FTIR), UV/Vis, and electronic paramagnetic resonance (EPR) spectroscopy. EPR spectroscopy suggests that the ligands act as bidentate, binding through both nitro-

gen donor atoms in an axial-equatorial mode. DFT calculations corroborate the structural assignments. The stability of the complexes in solution is evaluated by EPR and ^{51}V- NMR spectroscopy and all complexes show reasonable stability. The anti-*T. cruzi* activity of the complexes was tested by measuring the growth inhibitory effect on the epimastigote life cycle form of the parasite (Dm28c strain). All complexes show IC_{50} values in the micromolar range against *T. cruzi* and display activities of the same order of that of Nifurtimox, but lower than that of the previously reported analogue $[V^{IV}O(SO_4)(H_2O)_2(dppz)]$ (dppz = dipyrido[3,2-a:2′,3′-c]phenazine). Furthermore, DNA was evaluated as a potential target by using atomic force microscopy (AFM), showing that the complexes display ability to interact with this biomolecule.

Introduction

Inorganic medicinal chemistry achievements have demonstrated that the development of bioactive metal-based com-

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pounds is a promising approach in the search for new drugs for the treatment of different pathologies. In particular, pioneering research by *Sánchez-Delgado*, *Biot*, and *Brocard*, followed by investigations by other research groups, led to the identification of some interesting potential metallopharmaceuticals against highly prevalent parasitic illnesses, such as Chagas disease (American Trypanosomiasis) and malaria.[1–12] Although the physiological roles of vanadium in biological systems and its pharmacological activities have led to a considerable amount of research, the main efforts developing the medicinal chemistry of vanadium have focused on improving biodistribution and tolerability of the vanadium insulin-enhancing core or on developing potential anti-tumor compounds.[13–21] Research on vanadium compounds directed towards the potential treatment of parasitic diseases has only arisen in recent years.[21–23]

Chagas disease, caused by *Trypanosoma cruzi* (*T. cruzi*), is recognized by the World Health Organization as one of the world′s thirteen most neglected illnesses. It is endemic of poverty-stricken areas of Latin America, where it affects around 10 million people and causes more deaths in this region than any other parasitic disease. Despite the efficacy of large-scale programs focused on vector (hematophagous triatomine bug) control and quality of transfused blood, the disease is far from being eradicated. In fact, migration flow at the beginning of

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the 2000s caused the spread of the disease in non-endemic areas such as the United States, Australia and some European countries. Currently, only two decades-old non-specific drugs are available for the treatment of Chagas disease, Nifurtimox and Benznidazole. However, they are poorly tolerated and show controversial efficacy. Hence, the development of more efficacious and less toxic drugs, that could also circumvent emerging drug resistance, is urgently needed.[24–27]

Our group has been devoted to the search for prospective metal-based drugs against *T. cruzi* through metal complexation of anti-parasitic organic compounds in an attempt to modulate their activity.[7,8] More recently we have been exploring another strategy based on the generation of metal complexes with ligands bearing DNA intercalating capacity, thus placing this biomolecule as the target in the parasite.[22,23] Compounds able to irreversibly modify nucleic acids have received considerable attention due to their prospective applications in drug design, mainly as anti-tumor agents. The observation that highly-proliferative cells, such as *Trypanosoma* parasites and tumor cells, show metabolic similarities lead in many cases to a correlation between anti-trypanosomal and anti-tumor activities. For instance, some compounds that efficiently interact with DNA in an intercalative mode have been shown to exert anti-trypanosomal activity.[2,8,22,28] Our research following this strategy led to the development of a series of heteroleptic oxidovanadium(IV) complexes, including as ligands a tridentate N,O,O semicarbazone or hidrazone together with a bidentate polypyridyl DNA intercalator (dppz = dipyrido[3,2-a:2′,3′-c]phenazine, bipy = 2,2′-bipyridine, or phen = 1,10-phenanthroline). Most of these complexes displayed IC_{50} values in the micromolar range against *T. cruzi* and showed ability to interact with DNA, hence suggesting that this biomolecule may be a parasite target.[29–32]

Having in mind previous efforts of *Uckum* et al. to develop effective anticancer agents based on oxidovanadium(IV) complexes with phenanthroline derivatives,[33,34] we prepared and characterized in the solid state and in solution $[V^{IV}O(SO_4)(H_2O)_2(dppz)]$.[35] Interestingly, this compound showed an IC_{50} value against *T. cruzi* (Dm28c strain epimastigotes) in the micromolar range and of the same order than that of the reference drug Nifurtimox, the VO-dppz complex being slightly more active. Data from gel electrophoresis and atomic force microscopy studies indicated that the complex interacted with DNA, suggesting that its mechanism of action may involve DNA as a target.

To explore the effect of the nature of the intercalative polypyridyl chelator (N_{py},N_{py} donor) on the anti-trypanosomal activity, we currently designed analogous $[V^{IV}O(SO_4)(H_2O)_2(NN)]$ complexes with the phenanthroline derivatives (NN) [1,2,5]thiadiazolo[3,4-*f*][1,10]phenanthroline (tdzp), 1,10-phenanthroline-5,6-dione (phendione), and 5,6-epoxy-5,6-dihydro-1,10-phenanthroline (epoxyphen) (Figure 1). The compounds were characterized in the solid state and in solution by elemental analysis, electrospray ionization mass spectrometry (ESI-MS), conductometric measurements, and infrared (FTIR), UV/Vis, and electronic paramagnetic resonance (EPR) spectroscopy. The stability of the complexes in

solution was investigated by EPR and ^{51}V nuclear magnetic resonance spectroscopy. Their anti-*T. cruzi* activity was tested by measuring the growth inhibitory effect on the epimastigote life cycle form of the parasite (Dm28c strain). Furthermore, DNA was preliminarily evaluated as a potential target by using atomic force microscopy (AFM).
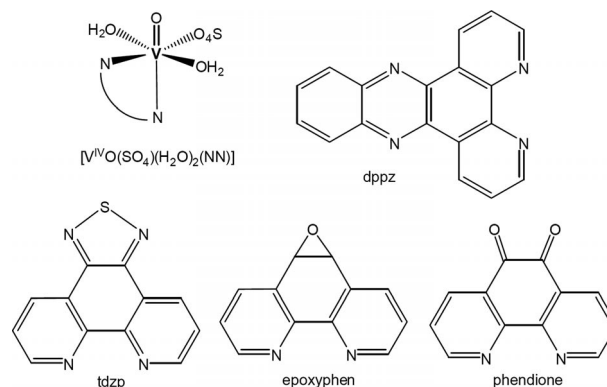

Figure 1. Scheme showing the polypyridyl chelating ligands NN: dppz, tdzp, epoxyphen, and phendione and the proposed structure for the $V^{IV}O$-NN complexes, $[V^{IV}O(SO_4)(H_2O)_2(NN)]$.

Results and Discussion

Three new oxidovanadium(IV) complexes having as ligands the phenanthroline derivatives [1,2,5]thiadiazolo[3,4-*f*][1,10]phenanthroline (tdzp), 1,10-phenanthroline-5,6-dione (phendione), and 5,6-epoxy-5,6-dihydro-1,10-phenanthroline (epoxyphen) (Figure 1) were synthesized with reasonable yields. Analytical data and conductimetric, ESI-MS, FTIR, UV/Vis, and EPR spectroscopic results agree with their formulation as: $[V^{IV}O(SO_4)(H_2O)_2(NN)]$, where NN represents the phenanthroline-derived ligand. Their proposed structural formulae are presented in Figure 1. All of them are non-conducting compounds in DMF, in agreement with the non-charged formula proposed.

The ESI-MS spectra of the complexes dissolved in DMF and diluted with MeOH were measured. For the VO-tdzp complex we propose the following assignments: 239.0 (LH⁺, 239.04, 35%), 335.9 ([VOL(OCH₃)]⁺, 335.99, 75%) and 736.9 ([(VO)₂L₂(OCH₃)₂H]⁺(μ-O)₂CH₃OH, 737.0, 60%).[35] Probably due to its very low solubility, for the VO-phendione complex only a ligand peak [LH(CH₃OH)]⁺ at 242.9 (theoretical: 243.08) (50%) and the oxidation products [VᵛO₂L(HCO₂H)]⁺ at 338.8 (theoretical: 338.98) (13%), and [VᵛO₂L(DMSO)]⁺ at 370.6 (theoretical: 370.99) (35%) could be assigned. For the VO-epoxyphen complex we propose the following assignments: 196.9 (LH⁺; 197.07, 15%), 214.9 (LH⁺H₂O; 215.08, 10%), 293.8 ([VOL(OCH₃)]⁺; 294.02, 25%) and 311.09 ([VOL(OCH₃)(H₂O)]⁺; 312.03, 25%).

FTIR spectroscopic results confirmed the presence of the phenanthroline-derived ligands in the coordination sphere of vanadium. Several bands corresponding to stretching and deformation vibrations of the heterocyclic ligands were observed in the 1700–1300 cm⁻¹ region. In general, most of these bands

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are slightly displaced to higher frequencies upon coordination, this being a typical spectroscopic behavior for phenanthroline complexes.[35–39] In addition, the typical four strong bands pattern for the unidentate coordinated sulfato moiety (C_{3v}) was observed in the 1200–1000 cm^{-1} region, superimposed to skeletal vibrations of the NN ligands.[36,37] A very broad band in the 3422–3405 cm^{-1} region is assigned to hydrogen bonded OH groups of coordinated water molecules. The ν(V=O) band could be identified in the 986–977 cm^{-1} region.[40]

The d-d absorption spectra of oxidovanadium(IV) complexes can be interpreted considering the simple M.O. model proposed by *Ballhausen* and *Gray* for the [VO(H$_2$O)$_5$]$^{2+}$ complex; three (or four) transitions are expected.[40,41] For the present complexes two or three distinct bands may be identified, however most of them are overlapped or superimposed over a strong band tailoring from the UV. The lowest energy band is the one that is best defined in all complexes and it is solvent dependent. Overall, the visible absorption spectra confirm that in solution the complexes are in the +4 oxidation state.

The EPR spectra of frozen solutions of the compounds exhibited a hyperfine pattern typical of V^{IV}O complexes, consistent with the presence of monomeric V^{IV}O bound species with d$^1_{xy}$ ground-state configuration. Figure 2 shows the variation with time of the EPR spectra measured for VO-epoxyphen in DMF, and Table 1 the spin Hamiltonian parameters obtained by either simulation or by an iterative procedure (in the case of VO-epoxyphen). In the anisotropic X-band EPR spectra all complexes present either one or two species. To evaluate the
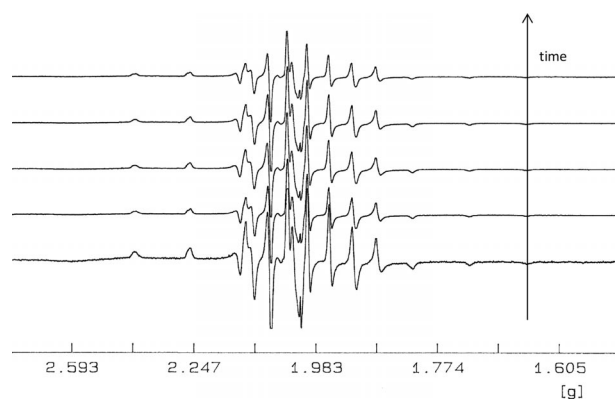
stability of the complexes, solutions were prepared and kept at room temperature in contact with air and their EPR spectra were measured with constant acquisition parameters. The complexes show reasonable stability in the organic solvents, and this was corroborated by ^{51}V NMR spectroscopy (see below). Due to the low solubility of the complexes, in some cases there was an increase in the intensity of the EPR spectra during the first hours (see Supporting Information). Particularly, in the case of VO-tdzp in DMSO, besides this intensity increase, the spectra also showed the presence of a broad band, due to aggregation of the molecules, superimposed on the spectrum of the monomeric species. The VO-tdzp complex is much more resistant to oxidation in DMSO than in DMF, since after 24 h no V^{IV}O species were detected in DMF, while in DMSO after 4 days most of the complex is still present in the +4 oxidation state. For the VO-epoxyphen compound the stability is similar in both solvents and after 24 h only a slight decrease in the spectra intensity is observed. The VO-phendione complex is also stable in DMSO for at least 24 h, while in DMF no paramagnetic species are detected by EPR after this period of time.

The VO-phendione complex shows two species both in DMF and DMSO; however in DMF the amount of the second species (which has higher A_z value) is very low and its spin-Hamiltonian parameters were not determined. VO-tdzp shows one species in DMF and two in DMSO (roughly 1:1) and VO-epoxyphen two in DMF (with very close spin-Hamiltonian parameters) and one in DMSO.

The usual approach, once a particular binding mode is assumed, considers that the values of A_z can be estimated (A_z^{est}) using the "additivity relationship" proposed by *Wüthrich*[42] and *Chasteen*,[43] with estimated accuracy of $\pm 3 \times 10^{-4}$ cm^{-1}. It is often assumed that A_z(O$_{DMF}$) = 43.7 $\times 10^{-4}$ cm^{-1}, A_z(O$_{DMSO}$) $\approx$ 43 $\times 10^{-4}$ cm^{-1} and A_z(O$_{H_2O}$) = 45.7 $\times 10^{-4}$ cm^{-1}. The value of A_z(N$_{phenanthroline}$ = N$_{phen}$ = N$_{py}$) is expected to depend on the angle of the phen rings with the V=O bond,[44] which for pyridine was found to vary between 40.4 (0 or 180°) and 44.0 $\times 10^{-4}$ cm^{-1} (90°). Thus, this contribution will differ if the phen derivatives bind as bidentate in an equatorial-equatorial or equatorial-axial mode.

For the studied vanadium complexes the A_z^{est} values in DMF are 175.4 $\times 10^{-4}$ cm^{-1} for the equatorial-equatorial, and 171.5 $\times 10^{-4}$ cm^{-1} for the equatorial-axial binding mode, which suggests that in DMF the preferred configuration has the ligand bound in an equatorial-axial coordination mode (see

Figure 2. Time changes of the first derivative X-band EPR spectra (ca. 100 K) of the VO-epoxyphen compound in DMF (ca. 3 mM). Spectra were measured at t = 0, 2, 4, 6, and 24 h.

Table 1. Spin Hamiltonian parameters of the studied complexes obtained from the experimental EPR spectra.

Complex	Solvent	g_x, g_y	g_z	A_x, $A_y \times 10^4$ /cm^{-1}	$A_z \times 10^4$ /cm^{-1}	$A_z^{est} \times 10^4$ /cm^{-1}	Binding mode
[VO(SO$_4$)(H$_2$O)$_2$(tdzp)]	DMF	1.976	1.938	60.8	167.7	171.5	(N$_{py}$,3 ×DMF)$_{eq}$(N$_{py}$)$_{ax}$
	DMSO	1.972	1.934	62.7	170.9	169.4	(N$_{py}$,3 ×DMSO)$_{eq}$(N$_{py}$)$_{ax}$
		1.971	1.928	68.5	178.4		(xH$_2$O,(4–x)DMSO)$_{eq}$
[VO(SO$_4$)(H$_2$O)$_2$(phendione)]	DMF	1.980	1.941	61.3	169.7	171.5	(N$_{py}$,3 ×DMF)$_{eq}$(N$_{py}$)$_{ax}$
	DMSO	1.979	1.941	63.6	171.6	169.4	(N$_{py}$,3 ×DMSO)$_{eq}$(N$_{py}$)$_{ax}$
		1.977	1.935	64.8	177.0		(xH$_2$O,(4–x)DMSO)$_{eq}$
[VO(SO$_4$)(H$_2$O)$_2$(epoxyphen)] [a]	DMF	1.967	1.933	59.4	168.7	175.4	(2 ×N$_{py}$,2 ×DMF)$_{eq}$
		1.968	1.931	59.6	167.0	171.5	(N$_{py}$,3 ×DMF)$_{eq}$(N$_{py}$)$_{ax}$
	DMSO	1.966	1.932	61.6	170.8	169.4	(N$_{py}$,3 ×DMSO)$_{eq}$(N$_{py}$)$_{ax}$

a) Parameters obtained by an iterative procedure (see text).

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Table 1). In DMSO one of the species detected in the EPR spectra has an A_z value of ca. 171×10^{-4} cm^{-1}. The A_z^{est} values obtained by the additivity rule for the two isomers in DMSO are: 169.4×10^{-4} cm^{-1} (equatorial-axial) and 174.0×10^{-4} cm^{-1} (equatorial-equatorial) and thus the species with $A_z^{exp} \approx 171 \times 10^{-4}$ cm^{-1} probably corresponds to the complex with the ligand bound in the same configuration as in DMF: equatorial-axial.

Recently, the binding modes and EPR parameters of the V^{IV}O systems involving the ligands phenanthroline and bipyridine (among others) in aqueous solutions were reexamined by a combination of spectroscopic (EPR and UV/Vis), pH potentiometric and computational (DFT calculations) methods.[45] The formation of mono-chelated complexes with equatorial-equatorial and equatorial-axial coordination and bis-chelated species with *cis*-octahedral arrangement, with a water molecule or a hydroxido ion in the fourth equatorial position was demonstrated. The calculated values for the hyperfine coupling constants for the phenanthroline complexes based on the DFT optimized structures were: $A_z(2 \times N_{py}, 2 \times O_{water})_{equat}(O_{water})_{axial} = 173.5 \times 10^{-4}$ cm^{-1} and $A_z(N_{py}, 3 \times O_{water})_{equat}(N_{py})_{axial} = 171.5 \times 10^{-4}$ cm^{-1}. These corroborate the assignments made using the "additivity rule", since the individual contributions to A_z from water, DMSO and DMF are not expected to differ that much (similarly to the contributions to the "additivity rule", see text above).

These assignments do not fully agree with those previously made for the related compound [V^{IV}O(SO$_4$)(H$_2$O)$_2$(dppz)], which was characterized in DMSO,[35] and the results obtained for this system are now re-examined, this being presented in the Supporting Information. For the presented systems the second species found in DMSO has an A_z^{exp} value of ca. 178×10^{-4} cm^{-1}. This value is higher than that found in the VO-dppz system,[35] however we also assign it to the solvolysis species [V^{IV}O(H$_2$O)$_x$(DMSO)$_{4-x}$], since depending on the number of H$_2$O or DMSO molecules the A_z^{est} values vary between 172 and 182×10^{-4} cm^{-1}. Interestingly, this species is present in ca. 50 % in the EPR spectra measured for VO-tdzp in the first 24 h, but its intensity decreases considerably in the spectra measured after this period. Figure 3 depicts this.

In DMF solution the VO-epoxyphen complex presents two species with very similar spin-Hamiltonian parameters. One of them is assigned to the equatorial-axial isomer and the other to the equatorial-equatorial isomer. The experimental values are slightly lower than expected but this may be due to electronic effects from the ligand. Moreover, the two species coexist and the one with binding through (N_{py}, $3 \times O_{DMF})_{equat}(N_{py})_{axial}$ is slightly more predominant than the other.

Having detected partial oxidation of the complexes in solution by EPR, ^{51}V NMR spectroscopy was used to detect probable oxidation products after ageing aerated solutions. The ^{51}V NMR spectrum of VO-phendione dissolved in DMSO after 24 h shows a small peak at $\delta^V = -527$ ppm. The formation of a V^V-species is consistent with the EPR data, which indicated a small decrease with time in the amount of the V^{IV} complexes. In DMF the EPR experiments indicated that all V^{IV}O species
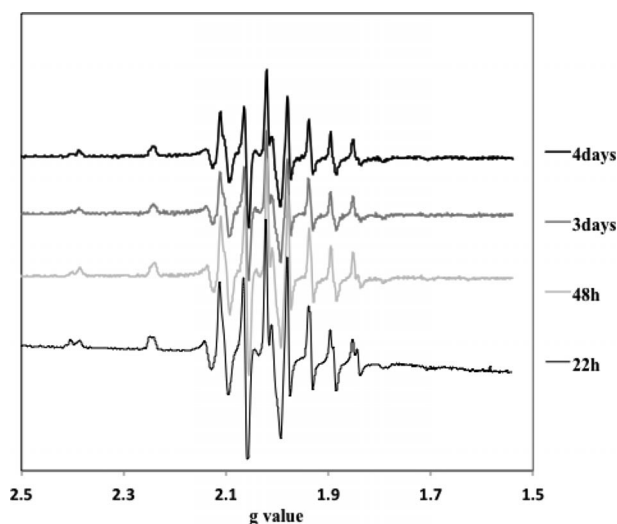

Figure 3. Changes with time of the first derivative X-band EPR spectra (ca. 77 K) of VO-tdzp in DMSO (ca. 1 mM).

were oxidized after 24 h, however, no diamagnetic species were detected in the ^{51}V NMR spectra. This must be due to the low solubility and therefore low concentration of the complex in this solvent, since by ESI-MS we were able to detect oxidation products containing the ligand (see above). For VO-tdzp, in DMSO, after a week a small peak was present at $\delta^V = -527$ ppm and in DMF peaks were observed at $\delta^V = -576.8$ and -546.0 ppm.

For VO-epoxyphen after a week the ^{51}V NMR spectra show peaks at $\delta^V = -527$ ppm (in DMSO) and at -527 and -575 ppm (in DMF). We propose the assignment of the higher field resonances to inorganic vanadate oligomers (V$_1$ and V$_2$) and the one at $\delta^V = -527$ ppm to a decomposition product. Other authors have found the same resonance at ca. $\delta^V = -531$ ppm in DMSO and assigned it to decomposition products.[46] Moreover, theoretical DFT studies (included in the Supporting Information) indicated that V^V complexes containing bound phenanthroline and DMF should appear at δ^V values between -410 and -455 ppm.

In summary, the spectroscopic data indicate that the ligands bind the metal ion as bidentate chelates through N donors in two different coordination arrangements, the axial-equatorial binding arrangement being preferred and that all complexes are reasonably stable in the organic solvents at least for 24 h.

DFT Studies

With the aim to obtain additional information about the structure of the complexes under study in solution, DFT calculations were carried out for the V-phendione systems, namely including two DMF ligands instead of two H$_2$O. The calculations demonstrated that for [V^{IV}O(SO$_4$)(DMF)$_2$(phendione)] four isomeric structures are possible (**a–d**, Figure 4). In the most stable one (**a**), the N-donor atoms of the phendione ligand occupy one axial and one equatorial positions and the sulfate ligand occupies the equatorial *trans* position relative to the nitrogen atom. This finding is in agreement with the experimen-

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tal conclusion about the preferable formation of the complexes with the equatorial-axial coordination of the phenanthroline ligand. Isomers **b** and **c** have similar thermodynamic stabilities and they both are less stable by 4.9–5.0 kcal·mol^{-1} than isomer **a**. The isomer **d** is the least stable one (by 11.3 kcal·mol^{-1} relative to **a**).
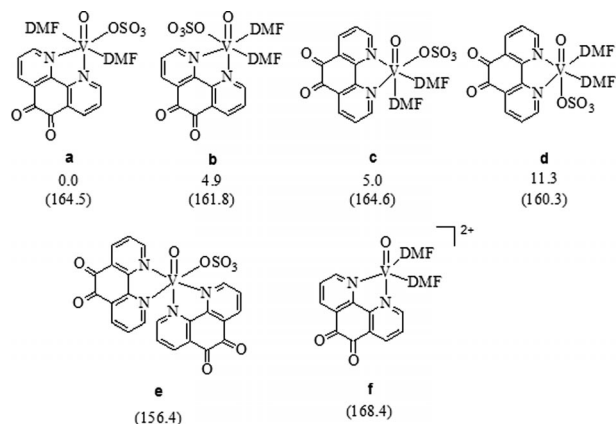

Figure 4. DFT-calculated structures of VO-phendione complexes. Relative energies in terms of Gibbs free energies in DMSO solution (in kcal·mol^{-1}), and DFT-calculated $|A_z|$ values ($\times 10^4$ cm^{-1}, in parentheses), are indicated.

The structure of complex **e**, bearing two phendione ligands, was also calculated. The Gibbs free energy of the reaction: {isomer **a** + phendione → isomer **e** + 2DMF} is 7.3 kcal·mol^{-1}, demonstrating that the formation of the *bis*-phendione species is even less favorable than that of the isomers **b** and **c**.

The calculated ^{51}V hyperfine coupling constants $|A_z|$ for the complexes are also presented in Figure 4. The $|A_z|$ value of the most stable isomer **a** (164.5×10^{-4} cm^{-1}) differs by 3% from the experimental value in DMF (169.7×10^{-4} cm^{-1}). The hyperfine coupling constant of isomer **c** ($|A_z| = 164.5 \times 10^{-4}$ cm^{-1}) is very similar to that of **a**, whereas the $|A_z|$ of **b** is clearly lower (161.8×10^{-4} cm^{-1}). The bis-chelated species shows the lowest $|A_z|$ values. The pentacoordinated complex **f**, with a square pyramidal structure and without any SO$_4^{2-}$ group in the coordination sphere, is the one which has the calculated $|A_z|$ value closer to the experimental one.

These computational data indicate that among the two species detected in the EPR spectrum of VO-phendione solutions, the one exhibiting the higher coupling constant may correspond to the pentacoordinated species **f**. The predominant species with the lower $|A_z|$ value may be assigned to the most stable isomer **a** of the [V^{IV}O(SO$_4$)(DMF)$_2$(phendione)] complexes. Therefore the conclusions formulated above on the basis of the experimental data, previous theoretical calculations[45] and the presented calculations correlate well.

Biological Studies

In vitro anti-Trypanosoma cruzi Activity

The complexes were evaluated in vitro for their activity against *T. cruzi* by analyzing the effect on the growth of epima-

stigotes of Dm28c strain cultures. Cell growth percentages in respect to control at different doses are shown in Figure 5 for [V^{IV}O(SO$_4$)(H$_2$O)$_2$(tdzp)] and tdzp. The inhibition of 50% culture growth (IC$_{50}$) was determined for all the tested compounds. The results were compared to those of the free phenanthroline-derived ligands, to the reference tripanosomicidal drug Nifurtimox (6 µM on DM28c strain *T. cruzi* epimastigotes),[30] and to those previously reported for [V^{IV}O(SO$_4$)(H$_2$O)$_2$(dppz)] and dppz[35] (Table 2). The precursor V^{IV}OSO$_4$·5H$_2$O was not toxic against *T. cruzi* (IC$_{50} >$ 100 µM). The newly synthesized oxidovanadium(IV) complexes showed IC$_{50}$ values of the same order of Nifurtimox. Although active, the NN ligands showed quite different anti-*T. cruzi* activity, which may be ascribed to different solubilities and cellular permeability, and hence different bioavailabilities, and/or different ability to interact with DNA, the proposed biological target. The coordination of these ligands to V^{IV}O forming [V^{IV}O(SO$_4$)(H$_2$O)$_2$(NN)] lead to slight changes in activity for the phendione and epoxyphen complexes but [V^{IV}O(SO$_4$)(H$_2$O)$_2$(tdzp)] showed a significantly better antitrypanosomal activity than free tdzp. The results show that although the complexes are analogous, the nature of the NN ligand is determinant for the biological activity (Table 2).
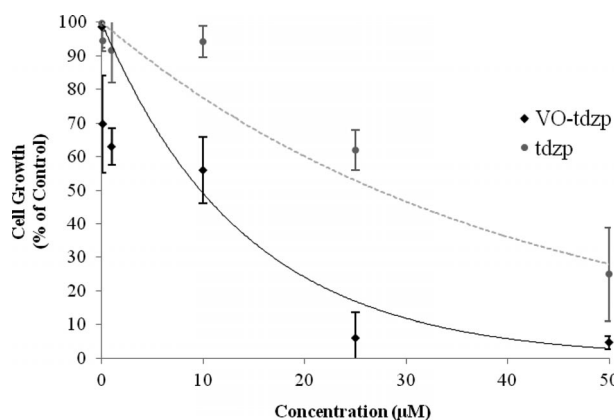

Figure 5. Dose – response curve for [V^{IV}O(SO$_4$)(H$_2$O)$_2$(tdzp)] and tdzp. The inhibition of exponential growth of an initial concentration of 1×10^6 Dm28c *T. cruzi* epimastigotes per mL in the presence of the indicated concentration of the complex or the free tdzp ligand was analyzed as indicated in the Experimental Section. Each point represents the average of three experiments $\pm$ SE.

Table 2. In vitro biological activity on *T. cruzi* (Dm28c strain) of the [V^{IV}O(SO$_4$)(H$_2$O)$_2$(NN)] complexes.

Compound	IC$_{50} \pm$ SD /µM
[V^{IV}O(SO$_4$)(H$_2$O)$_2$(dppz)]	ca. 3 [35]
dppz	> 3 [35]
[V^{IV}O(SO$_4$)(H$_2$O)$_2$(tdzp)]	15.6 $\pm$ 4.88
tdzp	38.7 $\pm$ 16.6
[V^{IV}O(SO$_4$)(H$_2$O)$_2$(phendione)]	7.24 $\pm$ 5.27
phendione	2.95 $\pm$ 2.11
[V^{IV}O(SO$_4$)(H$_2$O)$_2$(epoxyphen)]	21.91 $\pm$ 9.94
epoxyphen	16.27 $\pm$ 10.74

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Atomic Force Microscopy (AFM) Studies

AFM has proved to be a useful tool for imaging DNA and also DNA interactions with metal complexes.[47,48] The presented series of [$V^{IV}O(SO_4)(H_2O)_2(NN)$] compounds was developed aiming to target DNA. Our previous studies by agarose gel electrophoresis and atomic force microscopy methods confirmed DNA as a potential parasite target for the analogous [$V^{IV}O(SO_4)(H_2O)_2(dppz)$].[35] The interaction of the new [$V^{IV}O(SO_4)(H_2O)_2(NN)$] complexes reported herein with DNA was preliminarily studied by AFM using pBR322 plasmid as model molecule. AFM images are depicted in Figure 6. The three complexes modified the tertiary structure of the plasmid. This is visualized as changes in the DNA shape, such as kinks, crosslinking and supercoiling. These observations thus indicate that the new [$V^{IV}O(SO_4)(H_2O)_2(NN)$] compounds also interact with DNA.

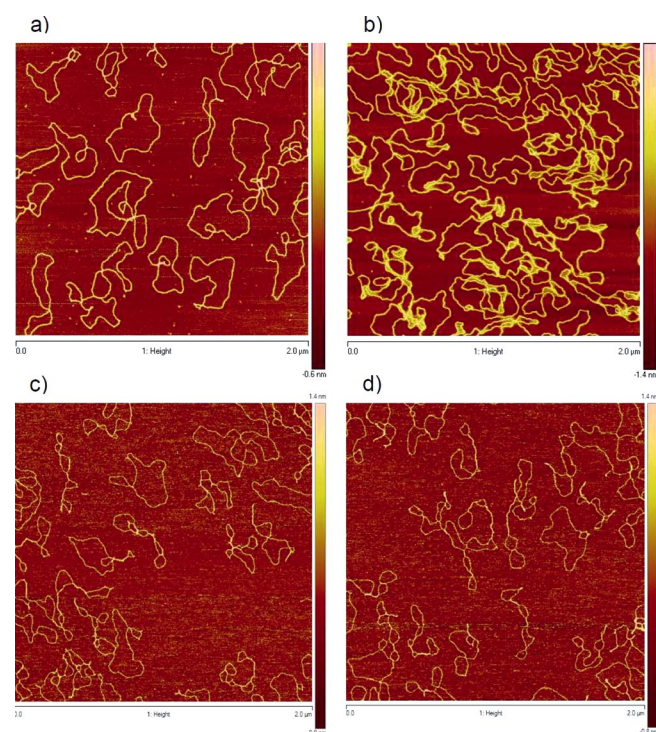**Figure 6.** AFM images showing the modifications suffered by pBR322 DNA (a) due to the interaction with the [$V^{IV}O(SO_4)(H_2O)_2(NN)$] compounds: (b) [$V^{IV}O(SO_4)(H_2O)_2(tdzp)$], (c) [$V^{IV}O(SO_4)(H_2O)_2(epoxyphen)$], (d) [$V^{IV}O(SO_4)(H_2O)_2(phendione)$], for molar ratio compound: DNA base pairs 1:5 and 24 h incubation at 37 °C.

Conclusions

Three new [$V^{IV}O(SO_4)(H_2O)_2(NN)$] complexes, where NN are the potential DNA intercalating phenanthroline-derived ligands tdzp, phendione, or epoxyphen, were prepared and characterized in the solid state and in solution. Globally both EPR data and DFT calculations (this for the VO-phendione system) agree in indicating that the binding mode of the nitrogen atoms of the phenanthroline derivatives is equatorial-axial.

These complexes show IC_{50} values in the micromolar range against *T. cruzi* (Dm28c strain epimastigotes), displaying anti-

trypanosomal activities of the same order of that of Nifurtimox, but lower than that of the previously reported analogue [$V^{IV}O(SO_4)(H_2O)_2(dppz)$]. Although the four complexes show analogous structures the nature of the NN ligand seems to be determinant for the anti-trypanosomal activity.

Improvement in activity due to coordination to the oxidovanadium(IV) core was significant only for the VO-tdzp complex. Nevertheless, in all cases it is possible that coordination to vanadium may also act as a carrier system improving cell uptake due to changes in solubility and/or lipophilicity of the phenanthroline derivatives upon coordination. Further work must be carried out in order to confirm this possibility and to prepare more potent and stable related oxidovanadium(IV) compounds. The complexes were shown to interact with DNA, suggesting that this biomolecule may be the parasite target.

Experimental Section

All common laboratory chemicals were purchased from commercial sources and used without further purification. [1,2,5]Thiadiazolo[3,4-*f*][1,10]phenanthroline (tdzp) was prepared and characterized as previously.[49,50]

Synthesis of the [$V^{IV}O(SO_4)(H_2O)_2(NN)$] Compounds: [$V^{IV}O(SO_4)(H_2O)_2(NN)$] complexes were synthesized by a modification of the procedure previously reported for [$V^{IV}O(SO_4)(H_2O)_2(dppz)$])]·2H$_2$O:[35] NN ligand (0.16 mmol, 38 mg tdzp, or 34 mg phendione or 31 mg epoxyphen) was dissolved in absolute alcohol (12 mL) previously purged with nitrogen for 10 min. $V^{IV}OSO_4$·5H$_2$O (40 mg, 0.16 mmol) was dissolved in the minimum amount of H$_2$O (approximately 2 mL) and the solution was purged with nitrogen for 10 min. This aqueous solution was slowly added to the NN solution. The mixture was stirred at room temperature for 7 days in a nitrogen atmosphere. The solid formed was filtered off from the mixture and was washed with two 3 mL portions of EtOH.

[$V^{IV}O(SO_4)(H_2O)_2(tdzp)$]: Yield: 20 mg, 29%. $C_{12}H_{10}N_4O_7S_2V$: calcd. C 32.96; H 2.30; N 12.81; S 14.66%; found: 33.05; 2.31; 12.77; 14.61%. **ESI-MS** (MeOH) *m/z*: 239.0 (35%), 335.9 (75%), 736.9 (60%). Λ_M(DMSO): 5.0 µS·cm^{-2}. **IR** (KBr): $\tilde{v}$ = 3422 s,br. v(OH) H$_2$O; 986 s v(V=O) cm^{-1}. λ_{max} /nm (ε /M^{-1} cm^{-1}): DMF, 765 (52), 446 sh (406); DMSO, ca. 729 (100), 448 sh (348).

[$V^{IV}O(SO_4)(H_2O)_2(phendione)$]: Yield: 22 mg, 34%. $C_{12}H_{10}N_2O_9SV$: calcd. C 35.22; H 2.46; N 6.85; S 7.84%; found: 35.07; 2.44; 6.83; 7.80%. **ESI-MS** (MeOH) *m/z*: 242.9 (50%), 338.8 (13%), 370.6 (35%), 350.7 (60%), 382.7 (100%). Λ_M(DMSO): 4.0 µS·cm^{-2}. **IR** (KBr): $\tilde{v}$ = 3420 s,br. v(OH) H$_2$O; 978 v(V=O) cm^{-1}. λ_{max} /nm (ε /M^{-1} cm^{-1}): DMSO, ca. 765 (22), 543 sh (26), 433 sh (165).

[$V^{IV}O(SO_4)(H_2O)_2(epoxyphen)$]: Yield: 20 mg, 32%. $C_{12}H_{12}N_2O_8SV$: calcd. C 36.47; H 3.06; N 7.09; S 8.11%; found: C 36.64; H 3.07; N 7.05; S 8.07%. **ESI-MS** (MeOH) *m/z*: 196.9 (15%), 214.9 (10%), 293.8 (25%), 311.09 (25%), 324.8 (100%). Λ_M(DMSO): 6.0 µS·cm^{-2}. **IR** (KBr): $\tilde{v}$ = 3405 s,br. v(OH) H$_2$O; 977 v(V=O) cm^{-1}. λ_{max} /nm (ε /M^{-1} cm^{-1}): DMF, 824 (187), 580 sh (396); DMSO, 779 (33), 562 sh (35).

Physical Properties Measurements: C, H, N and S analyses were carried out with a Carlo Erba Model EA1108 elemental analyzer. Conductimetric measurements were done at 25 °C in 10^{-3} M dimethyl-

formamide (DMF) solutions using a Conductivity Meter 4310 Jenway.[51] A 500-MS Varian Ion Trap Mass Spectrometer was used to measure electrospray ionization mass spectra (ESI-MS) of methanol solutions of the complexes in the positive mode (after dissolution of the complexes in a very small amount of DMF). A combination of several scans was made for each sample. The FTIR absorption spectra (4000–400 cm^{-1}) of the complexes and the free ligands were measured as KBr pellets with a Bomen FTIR model M102 instrument. The UV/Vis absorption spectra were measured with a Perkin-Elmer Lambda 35 spectrophotometer. ^{51}V-NMR spectra of ca. 3 mM solutions of the complexes in DMF or DMSO (p.a. grade) (5–10 % D_2O was added) were recorded with a Bruker Avance III 400 MHz instrument after different time periods standing in aerobic conditions at room temperature. ^{51}V NMR chemical shifts were referenced relative to neat $VOCl_3$ as external standard. EPR spectra were recorded at 77 K (or 100 K) with a Bruker ESP 300E X-band spectrometer coupled to a Bruker ER041 X-band frequency meter (9.45 GHz). Complexes were dissolved at room temperature in DMF or DMSO (p.a. grade) (1–3 mM), previously degassed by passing nitrogen for 10 min. Solutions were immediately frozen in liquid nitrogen prior to recording the EPR spectrum. The spin Hamiltonian parameters were obtained by simulation of the spectra with the computer program of *Rockenbauer* and *Korecz*[52] or by an iterative procedure using equations proposed by *Chasteen*[43] and corrected by *Casella* et al.[53]

Computational Details: The full geometry optimization of the molecular structures was carried out at the DFT level of theory using B3P86[54] functional with the help of the Gaussian-03[55] program package. No symmetry operations were applied for any of the structures calculated. The geometry optimization was carried out using a relativistic Stuttgart pseudopotential which describes 10 core electrons and the appropriate contracted basis set (8s7p6d1f)/[6s5p3d1f][56] for the vanadium atom and the 6-31G(d) basis set for other atoms. The Hessian matrix was calculated analytically for all optimized structures to prove the location of correct minima (no imaginary frequencies) and to estimate the thermodynamic parameters, the latter being calculated at 25 °C.

The total energies corrected for solvent effects (E_s) were estimated at the single-point calculations on the basis of gas-phase geometries at the CPCM-B3P86//gas-B3P86/6-31G(d) level of theory using the polarizable continuum model[57] in the CPCM version[58] with DMSO as solvent. The UAKS model was applied for the molecular cavity. The entropic term in solutions (S_s) was calculated according to the procedure described by *Wertz*[59] and *Cooper* and *Ziegler*[60] (see Supporting Information for details). The enthalpies and Gibbs free energies in solution (H_s and G_s) were estimated using the following equations

$$H_s = E_s - E_g + H_g$$

$$G_s = H_s - TS_s$$

where E_s, E_g and H_g are the total energies in solution and in gas phase and gas-phase enthalpy.

The ^{51}V hyperfine coupling constants in the V^{IV} complex were estimated at the single point calculations using the BHandHLYP functional and 6-311+G* basis set for all atoms on the basis of the equilibrium geometry obtained at the B3P86/6-31G(d)(V-ECP) level of theory. The anisotropic ^{51}V hyperfine coupling constants A_x, A_y, and A_z were estimated as the sum of the isotropic Fermi contact term and corresponding dipolar hyperfine interaction term.[61]

Biological Studies

In vitro anti-Trypanosoma cruzi Activity: *T. cruzi* epimastigotes of the Dm28c strain were maintained in exponential growth at 28 °C in liver infusion tryptose (LIT) medium complemented with 10 % (v/v) fetal calf serum (FCS). The effect on cell growth was analyzed incubating an initial concentration of 1×10^6 cells·mL^{-1} with various concentrations of the compounds for 5 days. Compounds were added as stock DMSO solutions immediately after the preparation of these solutions. The percentage of cell growth was followed by measuring the absorbance, A, of the culture at 595 nm (A_{595}) and calculated as follows: % = $(Ap–A_0p)/(Ac–A_0c) \times 100$, where $Ap = A_{595}$ of the culture containing the drug at day 5; $A_0p = A_{595}$ of the culture containing the drug at day 0; $Ac = A_{595}$ of the culture in the absence of any drug (control) at day 5; $A_0c = A_{595}$ in the absence of the drug at day 0. The results are presented as averages ± SD (standard deviation). The final DMSO concentration in the culture media never exceeded 0.4 % (v/v) and had no effect by itself on the proliferation of the parasites.[29,30] Nifurtimox (Nfx) was used as the reference trypanosomicidal drug. Dose-response curves were recorded and the IC_{50} values (50 % inhibitory concentration) were determined.

Atomic Force Microscopy (AFM) Studies: To optimize the observation of the conformational changes in the tertiary structure of pBR322 plasmid DNA, it was heated at 60 °C for 30 min to obtain a majority of open circular form. 15 ng of pBR322 DNA were incubated in an appropriate volume with the required compound concentration corresponding to the molar ratio base pairs (bp):compound 5:1. Each $V^{IV}O$ complex was dissolved in a minimal amount of DMSO, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (HEPES) pH 7.4 was added up to the required concentration. The different solutions as well as Milli-Q® water were filtered with 0.2 μm FP030/3 filters (Schleicher & Schuell GmbH, Germany). Incubations were carried out at 37 °C for 24 h.

Samples were prepared by placing a drop of DNA solution or DNA-compound solution onto mica (TED PELLA, INC. California, USA). After adsorption for 5 min at room temperature, the samples were rinsed for 10 s in a jet of deionised water (18 MΩ cm^{-1} from a Milli-Q® water purification system) directed onto the surface. The samples were blow dried with compressed argon and then imaged by AFM.

The samples were imaged by a Nanoscope III Multimode AFM (Digital Instrumentals Inc., Santa Barbara, CA) operating in tapping mode in air at a scan rate of 1–3 Hz. The AFM probe was 125 mm-long monocrystalline silicon cantilever with integrated conical shaped Si tips (Nanosensors GmbH Germany) with an average resonance frequency f_o = 330 KHz and spring constant K = 50 N·m^{-1}. The cantilever was rectangular and the tip radius given by the supplier was 10 nm, a cone angle of 35° and high aspect ratio. The images were obtained at room temperature (T = 23 ± 2 °C) and the relative humidity was usually lower than 40 %.[29,30]

Supporting Information (see footnote on the first page of this article): Experimental and simulated EPR spectra, results of the DFT studies.

ARTICLE

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Special Issue

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<div align="right">J. Costa Pessoa, D. Gambino et al.</div>

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H. Gallardo, G. Conte, M. L. Kuznetsov, A. Neves,
V. Moreno, J. Costa Pessoa,* D. Gambino* **1–10**

Searching for Vanadium-Based Prospective Agents against *Trypanosoma cruzi*: Oxidovanadium(IV) Compounds with Phenanthroline Derivatives as Ligands