Oxidovanadium(IV) and dioxidovanadium(V) complexes of tridentate salicylaldehyde semicarbazones: Searching for prospective antitrypanosomal agents

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phenazine

A B S T R A C T

As a contribution to the identification of the relevant species for biological activity and the understanding of structure–activity relationships of \([\text{V}^{\text{IV}}\text{O}-(\text{L}-2\text{H})(\text{NN})]\) antitrypanosomal complexes (\(\text{NN}\) is a bidentate polypyridyl DNA intercalator; \(L\) is a tridentate salicylaldehyde semicarbazone derivative), new \([\text{V}^{\text{IV}}\text{O}-(\text{L}-2\text{H})(\text{NN})]\) complexes and \([\text{V}^{\text{V}}\text{O}-(\text{L}-2\text{H})(\text{NN})]\) complexes including bipy or dppz (dipyrano[3,2-a: 2′,3′-c]phenazine co-ligands are prepared and characterized in the solid state and in solution. Their activity is evaluated on Trypanosoma cruzi. The lipophilicity, as structural descriptor related to bioactivity, of the whole \([\text{V}^{\text{IV}}\text{O}-(\text{L}-2\text{H})(\text{NN})]\) series is determined. Furthermore, the antiproliferative effect of those new compounds showing activity against \(T.\) cruzi is evaluated on the genetically related parasite \(T.\) brucei with the aim to develop broad spectrum agents. The new \([\text{V}^{\text{IV}}\text{O}-(\text{L}-2\text{H})(\text{dppz})]\) complexes are about ten to fifteen times more toxic to \(T.\) cruzi than the bipy analogues and show quite good in vitro activity on \(T.\) brucei brucei. They are shown to interact with DNA, suggesting that this biomolecule may be the parasite target. The stability of the \(\text{V}^{\text{IV}}\text{O}-\) complexes in solution is accessed by several techniques. Globally the data suggest that the relevant species for biological activity are the \([\text{V}^{\text{IV}}\text{O}-(\text{L}-2\text{H})(\text{NN})]\) compounds, their order of activity being dependent on the \(\text{NN}\) nature, but not much on the substitution on the salicylaldehyde semicarbazone moiety. A parabolic relationship between biological response and lipophilicity (determined as \(R_M = \log \left(\frac{1}{R_f} - 1\right)\)) by a TLC method) is obtained. From this correlation an optimum \(R_M\) value, close to 1.44, was found, which may be used as design guide for future development of antitrypanosomal compounds.

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1. Introduction

The possible physiological roles of vanadium in biological systems and its pharmacological activities have led to a considerable amount of research. Efforts developing the medicinal chemistry of vanadium have mainly focused whether on improving biodistribution and tolerability of the vanadium insulin-enhancing core or on developing potential anti-tumor compounds [1–9]. Despite the fact that parasitic diseases are among the most prevalent illnesses worldwide, work on vanadium compounds for the potential treatment of some of these diseases has only arisen in recent years [10,11].

Inorganic medicinal chemists have demonstrated that the development of bioactive metal-based compounds could be a promising approach in the search for new drugs against some parasitic diseases [10,12–19]. In particular, pioneering research by Sánchez-Delgado, Riot and Brocard led to the identification of some interesting potential metallopharmaceuticals for Chagas disease and malaria [13,20–24]. Among the most neglected diseases are American trypanosomiasis (Chagas disease), human African trypanosomiasis (sleeping sickness) and Leishmaniasis. They are caused by genetically related single-celled protozoa parasites that belong to the family Trypanosomatidae. In particular, American trypanosomiasis and human African trypanosomiasis constitute major health concerns in the poorest tropical and subtropical regions of the world [25–28]. The trypanosomiasis and leishmaniasis are among the ten most prevalent diseases caused by protozoan parasites.

American trypanosomiasis (etiologic agent: Trypanosoma cruzi) is endemic of Latin America where it affects around 10 million people and causes more deaths in this region than any other parasitic disease. Globalization and immigration has also led to the appearance of several...
infection cases in developed countries [26,29]. Sleeping sickness, which is caused by parasites from the Trypanosoma brucei complex (e.g. Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense), represents a major disease burden in sub-Saharan regions of Africa. Most of the available treatments against both diseases are based on decades-old non-specific drugs that give rise to undesirable collateral toxic effects, show limited and variable efficacy depending on the type or stage of the disease and suffer from parasite’s development of resistance. Therefore, the development of more efficacious and less toxic drugs, that could also circumvent emerging drug resistance, is urgently needed [25,27,29–31].

Several attempts to develop anti-parasitic metal-based drugs are currently in progress through distinct approaches. Although our group has been mainly devoted to the search for new metal-based antitypanosomal drugs through metal complexation of anti-parasitic organic compounds in an attempt to modulate their activity [10,18,19], more recently we also began exploring another strategy which consists in binding to DNA metal complexes containing ligands with intercalating capacity, thus placing this biomolecule as the target in the parasite [10].

Molecules able to irreversibly modify nucleic acids have received considerable attention due to their prospective applications in drug design. This strategy is based on the observation that highly-proliferative cells such as Trypanosoma parasites and tumor cells show metabolic similarities that lead in many cases to a correlation between antitypanosomal and antitumor activities. For instance, some compounds that efficiently interact with DNA in an intercalative mode have been shown to exert antileishmanial and/or antitypanosomal activity [10,12,13,19,32]. Our research following this strategy led to the development of [VIVO(O)SO4](H2O)2(dppz)]-2H2O and a series of mixed-ligand oxidovanadium(IV) complexes, [VIVO(L-2H)(NN)], including as ligands: (i) a bidentate polypyridyl DNA intercalator, abbreviated as NN (NN = dppz = dipyrido[3,2-a:2′,3′-c]phenazine, bipy = 2,2′-bipyridine, phen = 1,10-phenanthroline), and (ii) a tridentate salicylaldehyde semicarbazone derivative (L). These complexes displayed IC50 values in the micromolar range against T. cruzi (Dm28c strain, epimastigote form of the parasite life cycle), in both T. cruzi and T. brucei (T. brucei rhodesiense and T. brucei gambiense) parasites, together with the previously developed [VIVO(L-2H)(NN)] series showed a clear correlation with the nature of the NN ligand and not with the substitution on the salicylaldehyde semicarbazone moiety [33,35]. Therefore, in the current work three new VIVO-semicarbazone complexes [VIVO(L-H)] with L = L3–L5, compounds 1–3, were synthesized, characterized and evaluated against T. cruzi together with the previously reported analogous VIVO-compounds of L1 and L2 [36–38].

As a contribution to the understanding of structure-activity relationships in the above mentioned systems, in this work we further address a similar type of mixed-ligand complexes by synthesizing six new [VIVO(L-2H)(NN)] complexes, hereafter named compounds 4–9, with L = L3–L5 and NN = bipy or dppz (Fig. 1). The complexes were characterized in the solid state and in solution by several techniques, and their biological activity was evaluated on T. cruzi. The lipophilicity of the compounds was determined and then correlated with the observed activity in order to perform a preliminar QSAR (quantitative structure–activity relationship) study. Furthermore, the antiproliferative effect of those new compounds showing activity against T. cruzi was evaluated on the genetically related parasite T. brucei with the aim to evaluate the approach of developing new broad spectrum antitypanosomal agents based on the presence of common targets in both parasites.

Previous EPR and 51V NMR studies carried out in solvents having moderate coordinating ability towards metal ions (DMSO or DMF (dimethylformamide)) suggested that the complexes could undergo hydrolysis, oxidation and release of the NN ligand in solution, leading to dioxidovanadium(V) semicarbazone complexes that could be responsible for the bioactivity [33,35]. Nevertheless, the antitypanosomal activity of the previously developed [VIVO(L-2H)(NN)] series showed a clear correlation with the nature of the NN ligand and not with the substitution on the salicylaldehyde semicarbazone moiety [33,35].

2. Materials and methods

2.1. Materials

All common laboratory chemicals were purchased from commercial sources and used without further purification. Semicarbazone ligands were synthesized from an equimolar mixture of the corresponding aldehyde and semicarbazide and characterized by C, H and N elemental analyses, and by FTIR and 1H and 13C NMR spectroscopies [34–39].

| Fig. 1. a) Salicylaldehyde semicarbazone derivatives L selected as ligands (L1–L5); b) new [VIVO(L-H)] complexes 1–3; c) new [VIVO(L-2H)(NN)] complexes, where NN = bipy (4–6) or dppz (7–9). |
and characterized as previously described [36–38]. Previously reported complexes, namely VO\textsubscript{5}O\textsubscript{4}(L-2H)(NN) with NN = bipy, dppz or phen, were also synthesized and characterized as described in the literature [34,35].

2.2. Syntheses of the dioxidovanadium(V) complexes, [V\textsubscript{2}O\textsubscript{2}(L-H)], 1–3

The new [V\textsubscript{2}O\textsubscript{2}(L-H)] complexes, where L = 2-hydroxy-3-methoxybenzaldehyde semicarbazone (L3), 3-ethoxysalicylaldehyde semicarbazone (L5), were prepared by first mixing [V\textsubscript{2}O\textsubscript{2}(acac)\textsubscript{2}] (100 mg, 0.375 mmol, acac = acetylacetonate) with L (0.375 mmol) in ethanol (10 mL) and then keeping for 24 h under reflux. The reaction mixture was then stirred during 5–10 days at room temperature. In each case a yellow solid was isolated by centrifugation and recrystallized from boiling ethanol.

\textbf{[V\textsubscript{2}O\textsubscript{2}(L3-H)]}, 1. Yield: 42 mg, 39%. Anal (%) calc. for C\textsubscript{27}H\textsubscript{18}BrN\textsubscript{7}O\textsubscript{4}V: C, 51.0; H, 2.9; N, 15.4. Found: C, 50.8; H, 2.9; N, 15.5.

\textbf{[V\textsubscript{2}O\textsubscript{2}(L4-H)]}, 2. Yield: 94 mg, 82%. Anal (%) calc. for C\textsubscript{27}H\textsubscript{19}N\textsubscript{7}O\textsubscript{4}V: C, 58.3; H, 3.4; N, 17.6. Found: C, 58.2; H, 3.3; N, 17.6.

\textbf{[V\textsubscript{2}O\textsubscript{2}(L5-H)] \cdot} H\textsubscript{2}O, 3. Yield: 61 mg, 44%. Anal (%) calc. for C\textsubscript{27}H\textsubscript{19}N\textsubscript{7}O\textsubscript{4}V: C, 50.7; H, 2.9; N, 13.2. ESI-MS (MeOH) \(\Lambda\) \(m/z\) \([\text{Found (Calcd)}]\): 282.9 (283.1) (45%) (Hdppz\textsuperscript{+}), 307.9 (308.2) (36%) (Hdppz\textsuperscript{+} • H\textsubscript{2}O).

2.3. Syntheses of the oxidovanadium(IV) complexes, [V\textsubscript{2}O\textsubscript{4}(L-2H)(NN)], NN = bipy, 4–6, or dppz, 7–9

The new [V\textsubscript{2}O\textsubscript{4}(L-2H)(NN)] complexes, where L = L3–L5 and NN = bipy or dppz, were synthesized by the following procedure: 0.375 mmol of L (78 mg L3, 84 mg L4 or 108 mg L5) and 0.375 mmol of NN (0.375 mmol bipy or 110 mmol dppz) were suspended in 15 mL of absolute alcohol previously purged with nitrogen for 10 min. [V\textsubscript{2}O\textsubscript{4}(acac)\textsubscript{2}] (0.375 mmol, 100 mg) was suspended in 6 mL of absolute alcohol, previously purged with nitrogen, and was added to the previous mixture. This was then heated at reflux under nitrogen for 3.5 h. The brown-red solid formed was filtered off from the hot mixture, and then washed three times with 2 mL portions of EtOH:Et\textsubscript{2}O (1:1).

\textbf{[V\textsubscript{2}O\textsubscript{4}(L3-2H)(bipy)] \cdot} 2H\textsubscript{2}O, 4. Yield: 48 mg, 27%. Anal. calc. for C\textsubscript{19}H\textsubscript{12}N\textsubscript{3}O\textsubscript{4}V: C, 48.9; H, 4.5; N, 15.0. Found: C, 48.7; H, 4.0; N, 14.9.

\textbf{[V\textsubscript{2}O\textsubscript{4}(L4-2H)(bipy)] \cdot} H\textsubscript{2}O, 5. Yield: 80 mg, 46%. Anal. calc. for C\textsubscript{20}H\textsubscript{13}N\textsubscript{3}O\textsubscript{4}V: C, 51.9; H, 4.6; N, 15.1. Found: C, 51.7; H, 4.6; N, 14.8.

\textbf{[V\textsubscript{2}O\textsubscript{4}(L5-2H)(dppz)] \cdot} H\textsubscript{2}O, 6. Yield: 97 mg, 49%. Anal. calc. for C\textsubscript{28}H\textsubscript{18}N\textsubscript{3}O\textsubscript{4}V: C, 49.0; H, 3.7; N, 17.2. Found: C, 48.8; H, 3.6; N, 17.1.

2.4. Physicochemical characterization

C, H and N analyses were carried out with a Carlo Erba Model EA1108 elemental analyzer. Thermogravimetric measurements were done with a Shimadzu TGA 50 thermobalance, with a platinum cell, working under flowing nitrogen (50 mL/min) and at a heating rate of 0.5 °C/min (RT–80 °C) and 1.0 °C/min (80–350 °C). Conductometric measurements were done at 25 °C in 10−3 M DMF solutions using a Conductivity Meter 4310 Jenway [40]. Conductivity measurements of DMSO–H\textsubscript{2}O solutions were also done over time in order to access the stability of the complexes in such medium. A 500-MS Varian Ion Trap Mass Spectrometer was used to measure ESI-MS of methanolic 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 ESI-MS spectra (4000–400 cm\textsuperscript{−1}) of the complexes and the free ligands were measured as KBr pellets with a Bomen FTIR model M102 instrument. \(\Lambda\) NMR spectra of the free ligands and of the V\textsubscript{2}O\textsubscript{2}-complexes in DMSO-d\textsubscript{6} were recorded at 30 °C on a Bruker DPX-400 instrument (at 400 MHz). Heteronuclear correlation experiments (2D-HETCOR), HMOC (heteronuclear multiple quantum correlation) and HBMC (heteronuclear multiple bond correlation), were carried out with the same instrument. The UV–vis absorption spectra were measured with a Perkin Elmer Lambda 35 spectrophotometer. \(\Lambda\) V NMR spectra of ca. 3 mM solutions of the complexes in DMF (p.a. grade) (5–10% D\textsubscript{2}O was added) were recorded on a Bruker Avance III 400 MHz instrument. \(\Lambda\) V chemical shifts were referenced relative to neat VOCI\textsubscript{3} as external standard. EPR spectra were recorded either at 77 K or at 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 p.a. grade (3 mM), previously degassed by passing N\textsubscript{2} for 10 min, and the solutions were immediately frozen in liquid nitrogen. The spin Hamiltonian parameters were obtained by simulation of the spectra with the computer program of Rockenbauer and Korecz [41] or by an iterative procedure using equations proposed by Chasteen [42] and corrected by Casella [43].

2.5. Crystallographic study

Suitable crystals for structural single crystal X-ray diffraction studies of [V\textsubscript{2}O\textsubscript{4}(L3-H)]\textsubscript{•}CH\textsubscript{3}CH\textsubscript{2}OH were obtained by recrystallization from boiling ethanol. The measurements were performed on an Oxford Xcalibur Gemini, Eos CCD diffractometer with graphite-monochromated CuK\textsubscript{α} (\(\lambda\) = 1.54178 Å) radiation. X-ray diffraction intensities were collected (\(\omega\) scans with \(\delta\) and \(\kappa\)-offsets), integrated and scaled with the CrysAlisPro suite of programs [44]. The unit cell parameters were obtained by least-squares refinement (based on the angular settings for all collected reflections with intensities larger than seven times the standard deviation of measurement errors) using CrysAlisPro. Data were corrected empirically for absorption employing the multi-scan method implemented in CrysAlisPro. The structure was solved by direct methods with SHELXS-97 [45] and the molecular model refined by full-matrix least-squares procedure on \(\chi^{2}\) with SHELXL-97 [46]. The hydrogen atoms were located stereo-chemically and refined with the riding model. The methyl H-positions in [V\textsubscript{2}O\textsubscript{4}(L3-H)] and ethanol solvent molecules were optimized by treating them as rigid groups which were allowed to rotate during the refinement around the corresponding C–O and C–C bonds. As a result, both CH\textsubscript{3} groups converged to staggered conformations. The position of the ethanol hydroxyl H-atom was refined by rigid rotation of the O–H group around the corresponding O–C bond. The crystal structure data was deposited as CCDC 879491 and contains the supplementary crystallographic data for the structure reported. The data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 361 122.
Trypanosomes were grown to late exponential phase and diluted at DMSO as solvent and then diluted in sterile phosphate buffered saline. Ten mM stock solutions of the test compounds were prepared using complemented with 10% (v/v) fetal calf serum (FCS). The effect on exponential growth at 28 °C in liver infusion tryptose (LIT) medium was followed by measuring the absorbance, A, of the culture at 595 nm in the absence of any drug (control) at day 5; A0c=A 595 in the absence of the parasites [34,35]. Nifurtimox (Nfx) was used as the reference drug today. 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 [34,35].

2.7. Lipophilicity studies

Reversed-phase TLC experiments were done on pre-coated TLC plates SIL RP-18W/UV254 and eluted with MeOH:DMSO:buffer Tris-HCl pH 7.4 (85:5:10, v/v/v). Stock solutions were prepared in pure methanol (Aldrich) prior to use. The plates were developed in a closed chromatographic tank, dried and the spots were located under UV light. The Rf values were averaged from two to three determinations, and converted into RM via the relationship: RM = log [(1 / Rf) – 1] [49–53].

2.8. Atomic force microscopy (AFM) studies

To optimize the observation of the conformational changes in the tertiary structure of p8322 plasmid DNA, it was heated at 60 °C for 30 min to obtain a majority of open circular form. 15 ng of p8322 DNA were incubated in an appropriate volume with the required compound concentration corresponding to the molar ratio base pairs (bp): compound 5:1. Each VVO-complex was dissolved in a minimal amount of DMSO as solvent and then diluted in sterile phosphate buffered saline (PBS pH 7.4) to obtain working solutions at 0.5, 0.25 and 0.031 mM. Trypanosomes were grown to late exponential phase and diluted at a cell density of 5 × 10⁵ cells/mL (mid exponential phase) in fresh culture medium. One ml of the cell suspension was plated into each well of a 24-well culture plate. Compounds were immediately added at final concentrations of 25, 5, 3.5, 2.5, 1.25, 0.62 and 0.12 μM. Controls included DMSO at 0.025% (v/v) and culture medium (growth control). Each condition was tested in triplicate. After 24 h incubation at 37 °C with 5% CO₂, living parasites were counted twice with a Neubauer chamber under the light microscope. IC₅₀ values were obtained from dose response curves fitted to a sigmoidal equation (Boltzmann model) or extrapolated from linear fitting plots.

3. Results and discussion

Nine vanadium complexes of the tridentate salicylaldehyde semicarbazone derivatives L3–L5 (Fig. 1a) were synthesized with reasonable yields. All of them are non conducting compounds in DMSF. Analytical, TGA (thermogravimetric analysis) and FTIR and ¹H NMR spectroscopic results of the three VVO₂-complexes are in agreement with the proposed formula, [VVO₂(L³-H)]·xH₂O, and their molecular formulae are presented in Fig. 1b. Moreover, analytical, TGA and ESI-MS, FTIR and EPR spectroscopic results of the six new VVO₃-complexes are in agreement with the proposed formulation: [VVO₃(L⁻²⁻H⁻¹)(N)]·xH₂O, and their molecular formulae are also depicted in Fig. 1c. ESI-MS experiments allowed the clear detection of the molecular ion for each VVO₃-complex. In the case of complexes 6 and 9, containing the brominated ligand L₅, two peaks were detected for M + H, reflecting the isotopic distribution of ⁷⁹Br and ⁸¹Br.
3.1. Characterization of the complexes in the solid state

3.1.1. Thermal analysis

Thermogravimetric curves for [V\text{O}_2(L-H)]\text{⋅}CH_3CH_2OH, where L = L3 or L4, demonstrated the absence of crystallization solvent molecules. [V\text{O}_2(L5-H)]\text{⋅}CH_3CH_2OH showed a single weight loss of 5.8\% (calcd. 5.6\%) centered near 50 °C, that corresponds to one water molecule. On the other hand, the complexes of the [V\text{O}_2(L2-H)(bipy)] series showed a weight loss around 1667 cm\(^{-1}\), that corresponds to one water molecule. On the other hand, the complexes of the [V\text{O}_2(L2-H)(bipy)] series showed also a single weight loss that corresponds to the number of water molecules assigned in each case (see above).

3.1.2. IR spectroscopic studies

Based on our previous reports on vibrational behavior of metal complexes of salicylaldehyde semicarbazone derivatives [34–39,54] and other reports [55,56], tentative assignments were made. Some selected IR bands and their tentative assignments are presented in Table 2. The absence of the ν(=O) bands, present in the ligands at around 1667–1676 cm\(^{-1}\), indicates the enolization of the amide functionality upon coordination to vanadium. Instead strong bands at ca. 1600–1664 cm\(^{-1}\) are observed which are characteristic of the coordination of the ligand enolate forms [55].

In the case of the [V\text{O}_2(L1-H)]\text{⋅}CH_3CH_2OH complexes, the shift of ν(=O) and ν(C=O) bands and the non-observation of the ν(=OH) (in the 3430–3500 cm\(^{-1}\) region) are in agreement with tridentate coordination through the carboxylic oxygen (O\text{carboxylate}), the azomethyne nitrogen (N\text{azomethine}) and the phenolic oxygen (O\text{phenolate}), and with deprotonation of the semicarbazone ligand at the phenolic hydroxyl group. The [V\text{O}_2(L2-H)(NN)] complexes show a similar spectroscopic behavior but in addition deprotonation of the NH group is confirmed by the non-observation of the NH stretching band in the 3160–3180 cm\(^{-1}\) region upon complexation. In these V\text{O}_2-complexes the semicarbazones act as double deprotonated tridentate ligands. In addition, [V\text{O}_2(L1-H)]\text{⋅}CH_3CH_2OH complexes show two bands assigned to the symmetric and antisymmetric stretching that are characteristic of the VO\text{2}\text{+} moiety. [V\text{O}_5(L2-H)(NN)] complexes show a characteristic intense band around 960 cm\(^{-1}\) assigned to ν(V=O). All the spectral modifications observed upon complexation agree with those previously reported for the L1 and L2 analogous complexes, upon coordination, the de-shielding effect of the metal is apparent in some protons (i.e., protons 1, 8, 10 and 11), causing a magnetic anisotropy of the C=O double bond than in the free ligand. when L is coordinated, the azomethyne moiety is fixed in an opposite spatial distribution to the magnetic anisotropy of the C≡N double bond in the free ligand.

Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>ν(V\text{O}_3)\text{⋅}CH_3CH_2OH</th>
<th>ν(V\text{O}_3)\text{⋅}L3</th>
<th>ν(V\text{O}_3)\text{⋅}L4</th>
<th>ν(V\text{O}_3)\text{⋅}L5</th>
<th>ν(=O)</th>
<th>ν(C=O)</th>
<th>ν(C=O)</th>
<th>ν(C≡N)</th>
<th>ν(=O−H)</th>
<th>ν(N−H)</th>
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<td>–</td>
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<td>1586</td>
<td>3466</td>
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<td>1607</td>
<td>1550</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>[V\text{O}_2(L2-H)(dpz)]\text{⋅}CH_3CH_2OH</td>
<td>959</td>
<td>1607</td>
<td>1550</td>
<td>–</td>
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<tr>
<td>[V\text{O}_5(L2-H)(NN)]\text{⋅}L4</td>
<td>984</td>
<td>1644</td>
<td>1550</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[V\text{O}_5(L2-H)(NN)]\text{⋅}L5</td>
<td>983</td>
<td>1644</td>
<td>1550</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As the biological activity of the complexes was tested in vitro in aerated diluted solutions and with incubating periods of several days, efforts were done to characterize the complexes in solution and, particularly, to understand the stability of the [V\text{O}_5(L2-H)(NN)] complexes towards hydrolysis and/or oxidation of V\text{O}_4. In fact the bio- logically active species may differ significantly from the solid complex that undergoes dissolution.

The V\text{O}_2-complexes were characterized in solution by 1H NMR and HETCOR experiments. Their stability in solution was followed by the same technique. To characterize the ligand substitution ability of the [V\text{O}_5(L2-H)(NN)] complexes in solution, EPR and UV NMR studies were also carried out with fresh and aged DMF (and in some cases DMSO) solutions of the complexes. In addition, the results were compared with those obtained from the stability studies done with both series of mixed-ligand compounds by measuring the conductivity of DMSO-H\text{2}O and DMSO solutions during 5 days.

3.2.1. NMR characterization of the [V\text{O}_5(L2-H)] complexes, 1–3

NMR spectroscopic data show narrow signals, typical of diamagnetic complexes. HETCOR experiments allowed the assignment of all 1H signals for the studied complexes. The 1H NMR chemical shift values along with the chemical shift differences between each complex and the corresponding ligand (expressed as Δδ) are listed in Table 4. The figure depicted in the table shows the numbering scheme of the salicylaldehyde semicarbazone moiety. 1H NMR integrations and signal multiplicities are in agreement with the proposed molecular formula. The three complexes show similar 1H chemical shifts of the salicylaldehyde semicarbazone common fragment of their molecules. As previously discussed for V\text{O}_2-complexes of L1 and L2 derivatives, upon coordination, the de-shielding effect of the metal is apparent in some protons (i.e., protons 1, 8, 10 and 11), causing a down-field shift of the corresponding 1H NMR peaks [36,37]. The up-field shift of proton 3 may be the result of a decreasing azomethyne anisotropic effect in the coordinated form of L. When L is coordinated, the azomethyne moiety is fixed in an opposite spatial distribution to proton 3. Therefore, upon coordination this proton is less affected by the magnetic anisotropy of the C≡N double bond than in the free ligand.
This effect has been also previously observed for V\textsuperscript{IV}\textsubscript{O}\textsubscript{2}-complexes of the related salicylaldehyde semicarbazones L1 and L2\textsuperscript{[36,37]}. As expected, proton 7 is not observed in the spectra of the three complexes.

The V\textsuperscript{IV}\textsubscript{O}-compounds showed similar NMR spectra after 5 days of preparation of the solutions, which indicates their high stability in solution.

### 3.2.2. EPR characterization of the [V\textsuperscript{IV}\textsubscript{O}(L-2H)(NN)] complexes, 4–9

EPR spectroscopy of V\textsuperscript{IV}\textsubscript{O}-complexes is a powerful tool to get structural information on the binding mode of the species present in solution\textsuperscript{[58]}. The additivity relationship, first proposed by Wüthrich\textsuperscript{[59]} and later refined by Chasteen\textsuperscript{[42]}, correlates the hyperfine coupling constant, namely \(A_z\), with the electron-donating ability of the ligands present in the equatorial plane of the V\textsuperscript{IV}\textsubscript{O}-center. The \(A_z\) value can be estimated as \(A_z^{est} = \sum A_{ij} \) (\(i = 1 \) to \(4\)), \(A_{ij}\) being the specific contribution of each donor atom equatorially bound to V\textsuperscript{IV}. Care must be taken when applying this relationship as it may be difficult to distinguish between different donor groups with similar \(A_{ij}\) values\textsuperscript{[42,58,60–62]}. The estimated error in the \(A_{ij}\) values is \(\pm 3 \times 10^{-4}\) cm\(^{-1}\).

The spectra of the V\textsuperscript{IV}\textsubscript{O}-mixed-ligand complexes exhibit a hyperfine pattern typical of V\textsuperscript{IV}\textsubscript{O}-complexes, consistent with the presence of mononumeric V\textsuperscript{IV}\textsubscript{O}-bound species with \(d^1\)\(xy\) ground-state configuration.

**Table 3**

| Bond lengths [Å] and angles around vanadium(V) [°] in [V\textsuperscript{IV}\textsubscript{O}(L3-H)] \(CH_3CH_2OH.\) |
|---------------------------------|-----------------|
| **Bond distances**               |                  |
| \(V-O(1)\)                       | 1.590(2)         |
| \(V-O(2)\)                       | 1.649(2)         |
| \(V-O(3)\)                       | 1.888(2)         |
| \(V-O(4)\)                       | 2.000(2)         |
| \(V-N(1)\)                       | 2.173(2)         |
| **Bond angles**                  |                  |
| \(O(1)-V-O(2)\)                  | 108.4(1)         |
| \(O(1)-V-O(3)\)                  | 104.6(1)         |
| \(O(2)-V-O(3)\)                  | 99.7(1)          |
| \(O(1)-V-O(4)\)                  | 98.0(1)          |
| \(O(2)-V-O(4)\)                  | 90.7(1)          |
| \(O(3)-V-O(4)\)                  | 150.5(1)         |
| \(O(1)-V-N(1)\)                  | 106.2(1)         |
| \(O(2)-V-N(1)\)                  | 143.5(1)         |
| \(O(3)-V-N(1)\)                  | 82.42(9)         |
| \(O(4)-V-N(1)\)                  | 73.15(9)         |

**Fig. 2.** ORTEP drawing depicting a view of [V\textsuperscript{IV}\textsubscript{O}(L3-H)] showing the labeling of the non-H atoms and their displacement ellipsoids at the 50% probability level.

**Fig. 3** shows the EPR spectra (at 77 K) obtained for 3 mM solutions of the bipy-binding complexes in DMF. The spectra of these complexes show the presence of two species, particularly evident in the case of 4 and 5. The spin Hamiltonian parameters of the spectra are included in Table 5. The \(g\) and \(A\) parameters obtained for the dppz-containing complexes and the inner (major) species of the bipy-containing complexes are the same within experimental error indicating the same binding set for all of them. They are also very close to those previously reported for the related phenanthroline compounds\textsuperscript{[35]} and for the bipy-containing complexes of L1 and L2, for which two species were also detected. Thus we propose the same binding set: the semicarbazone acting as a tridentate ligand, binding through Ophenolate, N\textsuperscript{Azomethyne} and O\textsuperscript{Enolate} in the equatorial plane and the NN co-ligands binding with one N in the equatorial position and the other trans to the O\textsuperscript{Enolate} donor. The \(A_z^{est}\) for this binding set is \(158.5 \times 10^{-4}\) cm\(^{-1}\) (considering Ophenolate = \(38.9 \times 10^{-4}\) cm\(^{-1}\), N\textsuperscript{Azomethyne} = \(41.6 \times 10^{-4}\) cm\(^{-1}\), O\textsuperscript{Enolate} = \(37.5 \times 10^{-4}\) cm\(^{-1}\))\textsuperscript{[2,42,58,60,62,63]} and fits the experimental values, within the estimated error.

The minor species, present in the spectra of the bipy-containing complexes shows higher \(A_z\) values (ca. \(166.7 \times 10^{-4}\) cm\(^{-1}\)), and its spin Hamiltonian parameters are similar to those also obtained for the minor species of V\textsuperscript{IV}\textsubscript{O}(L-2H)(bipy) (with \(L = L1\) and \(L2\))\textsuperscript{[34]} suggesting significant solvolysis and substitution of the bipy ligand by DMF. Nevertheless, the main complex species detected in freshly prepared solutions correspond to the formulation [V\textsuperscript{IV}\textsubscript{O}(L-2H)(bipy)]. The V\textsuperscript{IV}\textsubscript{O}-dppz-containing complexes show only the presence of one species, [V\textsuperscript{IV}\textsubscript{O}(L-2H)(dppz)], and no solvolysis was detected for the EPR active species.

### 3.3. Accessing the stability of the complexes in solution

#### 3.3.1. Conductivity measurements of the [V\textsuperscript{IV}\textsubscript{O}(L-2H)(NN)] complexes, 4–9

Conductivity measurements carried out with \(10^{-4}\) M solutions in DMSO and 8% DMSO-H\(_2\)O show that changes due to substitution of ligands due to solvolysis or oxidation processes occur significantly more slowly in 8% DMSO-H\(_2\)O than in neat DMSO solution. After 5 days the conductivity of the [V\textsuperscript{IV}\textsubscript{O}(L-2H)(NN)] solutions showed only a very slight increase in respect to \(t = 0\) value, probably due to generation of hydrolyzed V\textsuperscript{IV} charged species. On the other hand, the solutions in neat DMSO showed a continuous increase of their conductivity which was clearly detectable after 24 h of preparation of the solution.
3.2. Stability of the $V^{IV}$-complexes towards solvolysis and/or oxidation

To evaluate the stability of the $V^{IV}$-complexes towards oxidation, the EPR and visible absorption spectra were measured during one day period.

Strong charge transfer bands appear up to ca. 650 nm in the visible absorption spectra of the solutions of $[V^{IV}O(2H\cdot2H)(NN)]$ in DMF, therefore distinct detectable maxima corresponding to d–d transitions cannot be identified. The visible absorption spectra change with time but faster for the solutions of the bipy-containing complexes than for the corresponding dppz-containing compounds; e.g. with a 3 mM solution of $[V^{IV}O(L\cdot2H)(bipy)]$, the absorption at 780 nm decreased ca. 52% after 3 h, while with $[V^{IV}O(L\cdot2H)(dppz)]$ this decrease was ca. 30%.

Fig. 4 shows the changes in the EPR spectra observed for complex 7. After 3 h the intensity of the spectrum decreases, but ca. 25% of the complex is still in the +4 oxidation state and the spin Hamiltonian parameters are the same. After 6 h the oxidation is almost complete. For the other complexes the behavior was similar.

Having detected solvolysis and oxidation of the complexes in solution by EPR, $^{51}$V NMR spectroscopy was used to detect probable oxidation products after aging aerated DMF solutions. All bipy-containing samples show quite intense peaks at ca. $-533$ ppm after 24 h (Table 5). The dppz samples, besides this resonance, show the presence of other peaks, which can be tentatively assigned to vanadate oligomers [at ca. $-557$ ppm (V$_1$) and $-570$ ppm (V$_2$)]. The main peak is in the expected range for monomeric $V^{IV}$O-complexes involving N,O-ligands [2]. Moreover, chemical shifts of $V^{IV}$O–semicarbazone complexes have been reported and theoretically predicted in the range $-530$ to $-550$ ppm range [64]. Thus the $V^{IV}$ complexes formed with $\delta_V$ values in the range of $-529$ to $-534$ ppm probably correspond to species formulated as $V^{IV}O(2H\cdot2H)(solvent)$.

From the experiments made we can conclude that the $[V^{IV}O(L\cdot2H)(bipy)]$ complexes, where L = L$_1$–L$_5$, are significantly more susceptible to oxidation and solvolysis in DMF solution than the corresponding $[V^{IV}O(L\cdot2H)(dppz)]$ complexes [35], and similar to the structurally related $[V^{IV}O(L\cdot2H)(phen)]$ complexes [34], to the structurally related $[V^{IV}O(L\cdot2H)(phen)]$ complexes of L$_1$ and L$_2$ [34]. The $[V^{IV}O(L\cdot2H)(dppz)]$ complexes are less stable towards oxidation than the corresponding phenanthroline complexes, since e.g. complex $[V^{IV}O(L\cdot2H)(phen)]$ shows no considerable oxidation even after 72 h in DMF [35], and the $V^{IV}$-dppz complexes oxidize within 6 h.

### 3.4. Lipophilicity studies

Among the prime factors controlling transport to and interaction with biological receptors are lipophilic, polar, electronic and steric ones. Hence, many quantitative structure activity relationships contain terms representing more than one of these factors [65]. Herein, due to the chemodiversity of the used ligands and, thus, their effect on physicochemical properties such as lipophilicity, this property was determined for the whole series of chemically related $V^{IV}$O mixed-ligand complexes developed up to now to analyze its effect on the biological activity. Lipophilicity was experimentally determined using reversed-phase TLC experiments where the stationary phase, pre-coated TLC-C$_{18}$, simulates lipids of biological membranes or receptors, and the mobile phase, MeOH:DMF:buffer Tris–HCl pH 7.4 (85:5:10, v/v/v), resembles the aqueous biological milieu. Several attempts were done to select the adequate mobile phase, which allowed it to differentiate complexes according to their lipophilicity, the more adequate one being a combination of polar organic solvents, MeOH and DMF, with a buffer that simulates the physiological pH.

### Table 4

$^1$H NMR chemical shifts ($\delta$, ppm) of $[V^{IV}O(2H\cdot2H)]$ complexes in DMSO-d$_6$ at 30 °C.

<table>
<thead>
<tr>
<th>Complex</th>
<th>H</th>
<th>$\delta$ligand</th>
<th>$\delta$complex</th>
<th>$\Delta\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>8.17</td>
<td>8.59</td>
<td>0.42</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>8.17</td>
<td>8.59</td>
<td>0.42</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>7.34</td>
<td>7.05</td>
<td>-0.29</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>6.75</td>
<td>6.77</td>
<td>-0.02</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>6.92</td>
<td>7.06</td>
<td>0.14</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>10.21</td>
<td>12.48</td>
<td>2.26</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>6.41</td>
<td>7.97</td>
<td>1.56</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>6.41</td>
<td>7.97</td>
<td>1.56</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>9.30</td>
<td>12.48</td>
<td>3.18</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>6.41</td>
<td>7.09</td>
<td>0.68</td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>3.80</td>
<td>3.75</td>
<td>-0.05</td>
</tr>
</tbody>
</table>

$^a$ $\Delta\delta = \delta_{\text{complex}} - \delta_{\text{ligand}}$.
value. The $R_f$ values obtained were converted into $R_M$ values via the relationship: $R_M = \log \left( \frac{1}{R_f} \right) - 1$. Table 6 summarizes $R_M$ for each compound.

When the complete series of evaluated compounds, i.e. complexes, ligands and Nifurtimox (Table 6), were analyzed to find a correlation between $R_M$ and the anti-

$T. cruzi$ activity no statistically significant equations were obtained. However, when the population under study was restricted to the $\text{VIVO(O)}$-complexes a clear quadratic correlation was found ($R_{\text{M}} = 2495 \pm 781 \, \mu M$ + 1208 ± 425 $R_M$, $r^2_{\text{adj}} = 0.6381$, $F = 13.34$, $p = 0.0009$, Fig. 5). Similar nearly parabolic relationship between biological response and lipophilicity has been previously described for a large number of biologically relevant families of compounds [49,53,66]. From this correlation an optimum $R_M$ value, close to 1.44 (Fig. 5), was obtained as a design tool for further development of new compounds that could possess a better biological profile.

### 3.5. Biological results

#### 3.5.1. In vitro anti-

$T. cruzi$ activity

The complexes were evaluated in vitro for their anti-

$T. cruzi$ activities against epimastigotes of Dm28c strain. The results were compared to that of the reference drug Nifurtimox and to those previously reported for related $\text{VIVO(O)}$-[$(\text{NN})$] complexes (Table 6). The free semicarbazones $L_5$–$L_7$ were previously tested by the same procedure (see text). The free semicarbazones $L_1$–$L_4$ showed low anti-

$T. cruzi$ activity with similar $IC_{50}$ values to the previously reported $L_1$ and $L_2$ analogous bipy-containing complexes and higher $IC_{50}$ values than Nifurtimox.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$IC_{50} \pm SD , (\mu M)$</th>
<th>$R_M$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{VIVO(O)}(L1-2H),(\text{bipy})$</td>
<td>19 [34]</td>
<td>1.590</td>
</tr>
<tr>
<td>$\text{VIVO(O)}(L2-2H),(\text{bipy})$</td>
<td>23 [35]</td>
<td>1.352</td>
</tr>
<tr>
<td>$\text{VIVO(O)}(L3-2H),(\text{bipy})$</td>
<td>3.8 [35]</td>
<td>1.340</td>
</tr>
<tr>
<td>$\text{VIVO(O)}(L4-2H),(\text{bipy})$</td>
<td>6.0 [35]</td>
<td>1.221</td>
</tr>
<tr>
<td>$\text{VIVO(O)}(L5-2H),(\text{bipy})$</td>
<td>8.4 [34]</td>
<td>1.199</td>
</tr>
<tr>
<td>$\text{VIVO(O)}(L5-2H),(\text{phen})$</td>
<td>2.0 [35]</td>
<td>1.559</td>
</tr>
<tr>
<td>$\text{VIVO(O)}(L1-2H),(\text{dppz})$</td>
<td>12.7 ± 8.27</td>
<td>1.149</td>
</tr>
<tr>
<td>$\text{VIVO(O)}(L4-2H),(\text{dppz})$</td>
<td>3.8 ± 1.60</td>
<td>1.537</td>
</tr>
<tr>
<td>$\text{VIVO(O)}(L5-2H),(\text{dppz})$</td>
<td>1.6 ± 1.30</td>
<td>1.221</td>
</tr>
<tr>
<td>$\text{VIVO(O)}(L5-2H),(\text{dppz})$</td>
<td>1.352</td>
<td></td>
</tr>
<tr>
<td>$\text{VIVO(O)}(L5-2H),(\text{phen})$</td>
<td>1.509</td>
<td></td>
</tr>
<tr>
<td>$\text{VIVO(O)}(L1-2H),(\text{phen})$</td>
<td>1.221</td>
<td></td>
</tr>
<tr>
<td>$\text{VIVO(O)}(L2-2H),(\text{phen})$</td>
<td>1.559</td>
<td></td>
</tr>
<tr>
<td>Nifurtimox</td>
<td>6.0 [35]</td>
<td>1.221</td>
</tr>
</tbody>
</table>

### Table 5

$^{51}$V NMR chemical shifts, $\delta$, 24 h after dissolution in DMF, and EPR spin Hamiltonian parameters.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\delta$ ($ppm^a$)</th>
<th>$g_x$</th>
<th>$g_y$</th>
<th>$g_z$</th>
<th>$A_{\text{M}}$</th>
<th>$A_{\text{M}}$ $\times 10^4 , cm^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{VIVO(O)}(L3-2H),(\text{dppz})$</td>
<td>5</td>
<td>1.982</td>
<td>1.915</td>
<td>55.2</td>
<td>160.2</td>
<td>166.7b</td>
</tr>
<tr>
<td>$\text{VIVO(O)}(L4-2H),(\text{dppz})$</td>
<td>6</td>
<td>1.979</td>
<td>1.947</td>
<td>56.3</td>
<td>151.2</td>
<td>161.2</td>
</tr>
<tr>
<td>$\text{VIVO(O)}(L5-2H),(\text{dppz})$</td>
<td>7</td>
<td>1.976</td>
<td>1.947</td>
<td>54.8</td>
<td>159.2</td>
<td>159.7</td>
</tr>
</tbody>
</table>

$^a$ See Section 3.3.2.  
$^b$ $A_{\text{M}}$ for the minor (outer) species.  
$^c$ Minor peaks.

### Table 6

In vitro biological activity on $T. cruzi$ (Dm28c strain) and lipophilicity of the oxidovanadium(IV) complexes. Nifurtimox was included for comparison.

![Fig. 4. Changes observed with time in the EPR spectra of a frozen solution (100 K) of complex \([\text{VIVO(O)}(L3-2H)\,(\text{dppz})]\). T. cruzi: 3h, 6h.](image)

![Fig. 5. $IC_{50}$ values (in $\mu M$ units) for the anti-

$T. cruzi$ activity of the compounds vs $R_M$ values as a measure of their lipophilicity.](image)
On the other hand, the $[\text{VIVO(L-2H)(dppz)}]$ complexes showed IC$_{50}$ values lower or of the same order than Nifurtimox and similar to those previously reported for the L1 and L2 analogous dppz compounds [34]. The substitution on the phenol moiety of the semicarbazone ligand seems to have only a low incidence on the antitrypanosomal activity. In addition, the activity of the bipy mixed-ligand complexes is significantly lower than that of the analogous compounds with other NN ligands, like dppz and phen, indicating that the nature of this co-ligand is determinant of the biological activity (Table 6) [33,35].

The biological activity of the compounds thus appears to correlate with the stability of the $[\text{VIVO(L-2H)(NN)}]$ complexes. Globally the phen-containing compounds are the most stable and the most active. These are followed in stability and activity by the dppz-containing ones, and finally the bipy-containing complexes, which are the least stable and with lower activity. Whether this is due to an intrinsic bioactivity of the $[\text{VIVO(L-2H)(NN)}]$ species or to a higher bioavailability is not presently known, however, as shown, the lipophilicity of the compounds appears to be correlated with the biological activity. Inside cells the vanadium speciation (not addressed in this work) may yield species containing or not the original ligands. The NN moieties may be relevant in this respect, and there are several examples where phen and other related compounds, which may act as DNA intercalators, improve the biological activity of the metal-based systems.

### 3.5.2. In vitro anti-*T. brucei* activity

The new dppz-containing $\text{VIVO}$-complexes showed growth inhibitory activity towards *T. brucei brucei*. In fact they induced a dose-dependent antiproliferative effect on parasites treated for 24 h.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ ± SD (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\text{VIVO(L3-2H)(bipy)}]$</td>
<td>$&gt;25$</td>
</tr>
<tr>
<td>$[\text{VIVO(L4-2H)(bipy)}]$</td>
<td>$&gt;25$</td>
</tr>
<tr>
<td>$[\text{VIVO(L5-2H)(bipy)}]$</td>
<td>$&gt;25$</td>
</tr>
<tr>
<td>$[\text{VIVO(L3-2H)(dppz)}]$</td>
<td>$2.8 ± 0.1$</td>
</tr>
<tr>
<td>$[\text{VIVO(L4-2H)(dppz)}]$</td>
<td>$3.0 ± 0.2$</td>
</tr>
<tr>
<td>$[\text{VIVO(L5-2H)(dppz)}]$</td>
<td>$1.5 ± 0.1$</td>
</tr>
</tbody>
</table>

Please cite this article as: M. Fernández et al., Oxidovanadium(IV) and dioxidovanadium(V) complexes of tridentate salicylaldehyde semicarbazones: Searching for prospective antitrypanosomal agents, *J. Inorg. Biochem.* (2013), http://dx.doi.org/10.1016/j.jinorgbio.2013.02.010
(Table 7). They show IC₅₀ values in the low micromolar range. On the contrary, the analogous bipy-containing complexes showed no significant activity against bloodstream T. brucei.

The behavior of these bipy- and dppz-containing complexes against T. brucei is similar to the one observed against T. cruzi. These results support our drug design approach that involves the development of compounds that could show activity towards both genetically related parasites.

3.6. Atomic force microscopy (AFM) results

AFM has proved to be a useful tool for imaging DNA and also DNA interactions with metal complexes [67,68]. As mentioned above, the present series of [V⁴O(O-L-2H)(NN)] complexes was developed aiming to target DNA. To confirm that DNA is a potential parasite target, the interaction of the new bioactive complexes [V⁴O(O-L-2H)(dppz)] with DNA was preliminarily studied by AFM using pBR322 plasmid as model molecule. AFM images are depicted in Fig. 6. The three complexes modified the tertiary structure of the plasmid. This is visualized as changes in the DNA shape, such as crossinglinking and supercoiling. The observed effect is only slightly affected by the nature of the semicarbazone co-ligand. These observations thus indicate that the [V⁴O(O-L-2H)(dppz)] compounds interact with DNA but more studies involving other techniques are needed in order to clearly establish DNA as a target.

4. Conclusions

A series of [V⁴O₂(L-2H)] and of mixed-ligand V⁴O-complexes, [V⁴O(O-L-2H)(NN)], including tridentate salicylaldehyde semicarbazone derivatives as ligands (L) and either bipy or dppz as co-ligands (NN), were prepared and characterized. The new [V⁴O(O-L-2H)(dppz)] complexes showed IC₅₀ values in the low micromolar range against T. cruzi epimastigotes being about ten to fifteen times more toxic to the parasite than the bipy-containing analogues. The former also showed quite good activity on T. brucei (strain 427). The corresponding V⁴O₂-complexes, [V⁴O₂(O-L-2H)] do not show activity towards T. cruzi epimastigotes (Dm28c strain).

Globally the data suggest that the relevant species for biological activity are the [V⁴O(O-L-2H)(NN)] complexes including the bidentate NN co-ligand, the order of activity of the complexes being phen-containing > dppz-containing > bipy-containing compounds. This order correlates with the order of stability of the VIVO-complexes.

Appendix A. Supplementary data

Tables containing crystallographic information for [V⁴O₂(L-2H)]·CH₃CH₂OH, including atomic coordinates and equivalent isotropic displacement parameters (Table 5), intra-molecular bond distances and angles (Table 4), anisotropic full displacement parameters (Table 3), hydrogen atom parameters (Tables S1 and S2) and H-bond distances and angles (Table 7) are available from the authors upon request. A CIF file with details of the crystal structure reported in the paper has been deposited with the Cambridge Crystallographic Data Centre (12 Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223/336-033; Email: deposit@ccdc.cam.ac.uk) and can be obtained free of charge at www.ccdc.cam.ac.uk/contents/retrieving.html, reference number CCDC 879419.

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