Dalton Transactions

RSC Publishing

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/c3dt50512j

A new series of heteroleptic oxidovanadium(IV) compounds with phenanthroline-derived co-ligands: selective *Trypanosoma cruzi* growth inhibitors

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Searching for prospective metal-based drugs for the treatment of Chagas disease, a new series of ten mixed-ligand oxidovanadium(IV) complexes, [V^{IV}O(L-2H)(NN)], where L is a tridentate salicylaldehyde semicarbazone derivative (**L1–L5**) and NN is either 5-amine-1,10-phenanthroline (aminophen) or 5,6-epoxy-5,6-dihydro-1,10-phenanthroline (epoxyphen), were synthesized. The compounds were characterized in the solid state and in solution. EPR spectroscopy suggests that the NN ligands act as bidentate through both nitrogen donor atoms in an axial–equatorial mode. The stability of the complexes in solution was investigated by EPR and ^{51}V-nuclear magnetic resonance spectroscopies. The complexes were evaluated *in vitro* for their activities against *Trypanosoma cruzi* (*T. cruzi*), the parasite responsible for the disease, and their selectivity was analyzed using J-774 murine macrophages, as a mammalian model. All the complexes are more active than both the reference drug Nifurtimox and the previously reported [V^{IV}O(L-2H)(NN)] complexes. In general they are more active than the corresponding free NN ligands. Complexation led to highly increased selectivities towards the parasite. In addition, the lipophilicity of the compounds was determined and correlated with the observed activity in order to perform a QSAR (quantitative structure–activity relationship) study. A clear quadratic correlation is found. This study also confirms the influence of the structure of the co-ligand on the anti-*T. cruzi* effect. To get insight into the mechanism of action of the compounds, the changes in biochemical pathways promoted by two of the most active and most selective complexes are studied by analyzing a few of the parasite excreted metabolites by ^{1}H NMR spectroscopy. The combined information suggests that the mitochondrion could be a target for these complexes. Furthermore, DNA was preliminarily evaluated as a potential target by using atomic force microscopy (AFM), which showed that the complexes display an ability to interact with this biomolecule.

Received 25th February 2013,
Accepted 28th May 2013

DOI: 10.1039/c3dt50512j

www.rsc.org/dalton

Introduction

Chagas disease is a systemic parasitic infection caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*) that is recognized by the World Health Organization as one of the world's thirteen most neglected illnesses. It is autochthonous of Latin America where it affects around 10 million people and causes more deaths than any other parasitic disease. It is endemic to this region affecting particularly poverty-stricken areas. Despite the efficacy of large-scale programs focused on vector (hematophagous triatomine bug) control and screening of organ and blood donors, the disease is far from being eradicated. Moreover, Chagas disease is becoming an emerging health problem in non-endemic areas, such as United States, Australia and some European countries, because of growing population movements. Currently, only two decades-old non-specific

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drugs are available for the treatment of Chagas disease: Nifurtimox and Benznidazole. However, they show several collateral toxic effects and controversial efficacy. Hence, the design of more efficacious and less toxic alternative drugs capable of circumventing emerging drug resistance and the assessment of new strategies of treatment are a global health priority.[1–4]

The development of bioactive metal compounds is a promising approach in the search for new drugs. In particular, inorganic medicinal chemistry research has led to the identification of some prospective metal-based drugs against highly prevalent parasitic illnesses, such as Chagas disease (American Trypanosomiasis) and malaria.[5–13] Even though the roles of vanadium in biological systems and the pharmacological activities of vanadium compounds have led to a considerable number of investigations, research on medicinal chemistry of vanadium has mainly focused on improving biodistribution and tolerability of vanadium insulin-enhancing complexes or on developing potential anti-tumor compounds.[14–20] Despite the fact that parasitic diseases are among the most prevalent illnesses worldwide, research on vanadium bioactive compounds directed towards their use for such a purpose has only arisen in recent years.[20–22] Vanadium offers interesting chemical and biochemical properties for the development of antiparasitic drugs. In particular, potential parasite targets related to enzymatic inhibition, interaction with biomolecules and ROS generation have been described.[21,22] In addition, bioactivity and/or bioavailability of organic bioactive ligands could be favorably modified through their coordination to vanadium due to changes in their physicochemical properties upon complexation.[21]

Our group has been searching for prospective metal-based drugs against *T. cruzi* and genetically related parasites mainly through two different strategies: metal complexation of antiparasitic organic compounds in an attempt to modulate their activity and, more recently, generation of metal complexes with ligands bearing DNA intercalating capacity, thus placing this biomolecule as the target in the parasite.[10,11,21,22] Regarding the last approach, compounds able to irreversibly modify DNA structure have been extensively studied as prospective anti-tumor drugs. Moreover, highly-proliferative cells such as protozoan parasites and tumor cells show metabolic similarities leading in many cases to a correlation between antiparasitic and anti-tumor activities. For instance, some compounds that efficiently interact with DNA in an intercalative mode have been shown to exert anti-trypanosomatid activity.[23] Following this strategy our group has developed two series of oxidovanadium(IV) complexes with bidentate polypyridyl DNA intercalators (NN) as ligands. One of the series involves bioactive $[V^{IV}O(SO_4)(H_2O)_2(NN)]$ complexes, where NN = dipyrido-[3,2-*a*:2′,3′-*c*]phenazine (dppz), [1,2,5]thiadiazolo[3,4-*f*][1,10]-phenanthroline (tdzp), 1,10-phenanthroline-5,6-dione (phendione) or 5,6-epoxy-5,6-dihydro-1,10-phenanthroline (epoxyphen).[24,25] The other series comprises heteroleptic $[V^{IV}O(L\text{-}2H)(NN)]$ complexes, including as ligands a tridentate N,O,O semicarbazone or hidrazone (L) together with a bidentate

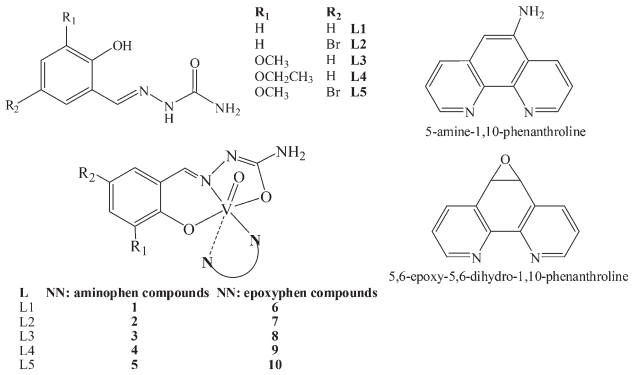

Fig. 1 Selected tridentate salicylaldehyde semicarbazone ligands and phenanthroline-derived co-ligands.

polypyridyl DNA intercalator (dppz, 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 an ability to interact with DNA, hence suggesting that this biomolecule may be a parasite target.[26–29]

To further explore the effect of the nature of the intercalative polypyridyl chelator (N_{py},N_{py} donor) on the anti-trypanosomal activity and also the mechanism of action in the parasite, we currently designed, prepared and characterized in the solid state and in solution a new series of ten $[V^{IV}O(L\text{-}2H)(NN)]$ complexes with the tridentate salicylaldehyde semicarbazone derivatives **L1–L5** and the phenanthroline derivatives (NN) 5-amine-1,10-phenanthroline (aminophen) and 5,6-epoxy-5,6-dihydro-1,10-phenanthroline (epoxyphen) (Fig. 1). The complexes were evaluated *in vitro* for their anti-*T. cruzi* activities against the epimastigote life cycle form of the parasite (Tulahuen 2 strain) and their selectivity of action was analyzed using murine macrophages, J-774, as a mammalian model. In addition, to carry out a QSAR (quantitative structure–activity relationship) study, the lipophilicity of the compounds was determined and then correlated with the observed activity. To get insight into the mechanism of action the changes in biochemical pathways promoted by two of the most active and selective complexes were studied by analyzing the parasite excreted metabolites by ^{1}H NMR spectroscopy. Furthermore, DNA was preliminarily evaluated as a potential target by using atomic force microscopy (AFM).

Experimental

General considerations

All common laboratory chemicals were purchased from commercial sources and used without further purification. The 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 ^{1}H and ^{13}C NMR spectroscopies.[27,30,31]

Physical measurements

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^{-1}) and at a heating rate of 0.5 °C min^{-1} (RT–80 °C) and 1.0 °C min^{-1} (80–350 °C). Conductimetric measurements were done at 25 °C in 10^{-3} M dimethylsulfoxide (DMSO) solutions using a Conductivity Meter 4310 Jenway.[32] A 500-MS Varian Ion Trap Mass Spectrometer was used to measure electrospray ionization mass spectra (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 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. ^{51}V-NMR spectra of *ca.* 3 mM solutions of the complexes in DMF (p.a. grade) (5–10% D_2O was added) were recorded on a Bruker Avance III 400 MHz instrument. ^{51}V chemical shifts were referenced relative to neat $V^{V}OCl_3$ as the external standard. EPR spectra were recorded at 77 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_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.[33]

Syntheses of the oxidovanadium(IV) complexes, [V^{IV}O(L-2H)(NN)], NN = aminophen, 1–5, or epoxyphen, 6–10

The new [V^{IV}O(L-2H)(NN)] complexes, where L = salicylaldehyde semicarbazone (**L1**), 5-bromosalicylaldehyde semicarbazone (**L2**), 2-hydroxy-3-methoxybenzaldehyde semicarbazone (**L3**), 3-ethoxysalicylaldehyde semicarbazone (**L4**) or 5-bromo-2-hydroxy-3-methoxybenzaldehyde semicarbazone (**L5**) and NN = aminophen or epoxyphen, were synthesized by the following procedure: 0.375 mmol of L (67 mg **L1**, 97 mg **L2**, 78 mg **L3**, 84 mg **L4** or 108 mg **L5**) and 0.375 mmol of NN (73 mg aminophen or 74 mg epoxyphen) were suspended in 15 mL of absolute ethanol previously purged with nitrogen for 10 min. [V^{IV}O-$(acac)_2$] (0.375 mmol, 100 mg), where acac = acetylacetonate, was suspended in 6 mL of absolute ethanol, previously purged with nitrogen, and was added to the previous mixture. This was then heated at reflux under nitrogen for 4 h. The reddish brown solids formed were filtered off from the hot mixture, and washed three times with 2 mL portions of EtOH–Et_2O (1 : 1).

[V^{IV}O(L1-2H)(aminophen)], 1. Yield: 112 mg, 68%. Found: C, 54.50; H, 3.65; N, 19.30. Calc. for $C_{20}H_{16}N_6O_3V$: C, 54.68; H, 3.67; N, 19.13. ESI-MS (MeOH) *m/z* [Found (Calcd)]: 441.1 (441.06) (10%) $[M + H]^+$. Λ_M(DMSO): 1.5 μS cm^{-2} mol^{-1}.

[V^{IV}O(L2-2H)(aminophen)], 2. Yield: 132 mg, 68%. Found: C, 46.60; H, 2.86; N, 16.30. Calc. for $C_{20}H_{15}BrN_6O_3V$: C, 46.35; H, 2.92; N, 16.22. ESI-MS (MeOH) *m/z* [Found (Calcd)]: 518.0 (517.99) (50%) $[M + H]^+$. Λ_M(DMSO): 1.9 μS cm^{-2} mol^{-1}.

[V^{IV}O(L3-2H)(aminophen)], 3. Yield: 134 mg, 76%. Found: C, 53.79; H, 3.81; N, 17.96. Calc. for $C_{21}H_{18}N_6O_4V$: C, 53.74; H, 3.87; N, 17.91. ESI-MS (MeOH) *m/z* [Found (Calcd)]: 470.1 (470.09) (100%) $[M + H]^+$. Λ_M(DMSO): 1.4 μS cm^{-2} mol^{-1}.

[V^{IV}O(L4-2H)(aminophen)]·H_2O, 4. Yield: 148 mg, 79%. Found: C, 52.64; H, 4.70; N, 16.88. Calc. for $C_{22}H_{20}N_6O_4V{\cdot}H_2O$: C, 52.70; H, 4.72; N, 16.76. ESI-MS (MeOH) *m/z* [Found (Calcd)]: 484.2 (484.11) (100%) $[M + H]^+$. Λ_M(DMSO): 1.4 μS cm^{-2} mol^{-1}.

[V^{IV}O(L5-2H)(aminophen)], 5. Yield: 120 mg, 58%. Found: C, 45.91; H, 3.10; N, 15.39. Calc. for $C_{21}H_{17}BrN_6O_4V$: C, 46.01; H, 3.13; N, 15.33. ESI-MS (MeOH) *m/z* [Found (Calcd)]: 548.0 (548.00) (30%) $[M + H]^+$. Λ_M(DMSO): 1.7 μS cm^{-2} mol^{-1}.

[V^{IV}O(L1-2H)(epoxyphen)]·H_2O, 6. Yield: 68 mg, 40%. Found: C, 52.50; H, 3.67; N, 15.32. Calc. for $C_{20}H_{15}N_5O_4V{\cdot}H_2O$: C, 52.41; H, 3.74; N, 15.28. ESI-MS (MeOH) *m/z* [Found (Calcd)]: 441.1 (441.06) (100%) $[M + H]^+$. Λ_M(DMSO): 2.8 μS cm^{-2} mol^{-1}.

[V^{IV}O(L2-2H)(epoxyphen)], 7. Yield: 60 mg, 31%. Found: C, 46.36; H, 2.70; N, 13.56. Calc. for $C_{20}H_{14}BrN_5O_4V$: C, 46.27; H, 2.72; N, 13.49. ESI-MS (MeOH) *m/z* [Found (Calcd)]: 519.0 (518.97) (95%) $[M + H]^+$. Λ_M(DMSO): 2.1 μS cm^{-2} mol^{-1}.

[V^{IV}O(L3-2H)(epoxyphen)], 8. Yield: 120 mg, 68%. Found: C, 53.80; H, 3.69; N, 14.98. Calc. for $C_{21}H_{17}N_5O_5V$: C, 53.63; H, 3.64; N, 14.89. ESI-MS (MeOH) *m/z* [Found (Calcd)]: 471.1 (471.07) (100%) $[M + H]^+$. Λ_M(DMSO): 2.3 μS cm^{-2} mol^{-1}.

[V^{IV}O(L4-2H)(epoxyphen)], 9. Yield: 128 mg, 70%. Found: C, 54.59; H, 3.91; N, 14.55. Calc. for $C_{22}H_{19}N_5O_5V$: C, 54.55; H, 3.95; N, 14.46. ESI-MS (MeOH) *m/z* [Found (Calcd)]: 485.1 (485.09) (100%) $[M + H]^+$. Λ_M(DMSO): 1.1 μS cm^{-2} mol^{-1}.

[V^{IV}O(L5-2H)(epoxyphen)], 10. Yield: 128 mg, 62%. Found: C, 46.02; H, 2.89; N, 12.80. Calc. for $C_{21}H_{16}BrN_5O_5V$: C, 45.92; H, 2.94; N, 12.75. ESI-MS (MeOH) *m/z* [Found (Calcd)]: 549.0 (548.99) (25%) $[M + H]^+$. Λ_M(DMSO): 1.7 μS cm^{-2} mol^{-1}.

Biological studies

Anti-*T. cruzi* activity. *Trypanosoma cruzi* epimastigotes (Tulahuen 2 strain) were grown at 28 °C in an axenic milieu (BHI-Tryptose) supplemented with 5% fetal bovine serum (FBS) as previously described.[34,35] Cells from a 10-day-old culture (stationary phase) were inoculated into 50 mL of fresh culture milieu to give an initial concentration of 1×10^6 cells per mL. Cell growth was followed by measuring the absorbance of the culture at 600 nm every day. Before inoculation, the milieu was supplemented with the indicated quantity of the studied complexes from a freshly prepared stock solution in DMSO. The activity of the co-ligands epoxyphen and aminophen was also determined. Nifurtimox (Nfx) was used as the reference trypanosomicidal drug. The final concentration of DMSO in the culture milieu never exceeded 0.4%, and the control was run in the presence of 0.4% DMSO and in the absence of the studied compounds. No effect on epimastigote growth was observed due to the presence of up to 1% DMSO in the culture milieu. The percentage of growth inhibition (PGI) was

calculated as follows: PGI (%) = {1 − [(Ap − A0p)/(Ac − A0c)]} × 100, where Ap = $A_{600\ nm}$ of the culture containing the studied compound at day 5; A0p = $A_{600\ nm}$ of the culture containing the studied compound just after addition of the inocula (day 0); Ac = $A_{600\ nm}$ of the culture in the absence of the studied compound (control) at day 5; A0c = $A_{600\ nm}$ in the absence of the studied compound at day 0. To determine IC_{50} values (50% inhibitory concentrations) parasite growth was followed in the absence (control) and presence of increasing concentrations of the corresponding compound. At day 5, the absorbance of the culture was measured and related to the control. The IC_{50} value was taken as the concentration of the compound under study necessary to reduce the absorbance ratio to 50%.

Cytotoxicity on mammalian cells. J-774 murine macrophage-like cells (ATCC, USA) were maintained by passage in Dulbecco's modified Eagle's milieu (DMEM) containing 4 mM L-glutamine, and supplemented with 10% heat inactivated fetal calf serum and 1% of antibiotics (10 000 U mL^{-1} penicillin and 10 000 μg mL^{-1} streptomycin). J-774 cells were seeded (1×10^5 cells per well) in 96 well microplates with 200 μL of RPMI 1640 milieu supplemented with 20% heat-inactivated fetal calf serum. Cells were allowed to attach for 48 h in a humidified 5% CO_2/95% air atmosphere at 37 °C and, then, exposed to the studied complexes (1.0–50.0 μM) for 48 h. The cytotoxicity of co-ligands epoxyphen and aminophen was also determined. Afterwards, cell viability was assessed by measuring the mitochondrial-dependent reduction of MTT (Sigma) to formazan. For that purpose, MTT was added to cells to a final concentration of 0.4 mg mL^{-1} and cells were incubated at 37 °C for 3 h. After removing the milieu, formazan crystals were dissolved in DMSO (180 μL), and the absorbance at 595 nm was read using a microplate spectrophotometer. Cytotoxicity percentages (% C) were determined as follows: % C = [100 − (OD_d − OD_{dm})/(OD_c − OD_{cm})] × 100, where OD_d is the mean of $OD_{595\ nm}$ of wells with macrophages and different concentrations of the compounds; OD_{dm} is the mean of $OD_{595\ nm}$ of wells with different compound concentrations in the milieu; OD_c is the growth control and OD_{cm} is the mean of $OD_{595\ nm}$ of wells with milieu only. Results are expressed as IC_{50} (compound concentration that reduces 50% control absorbance at 595 nm). Every reported IC_{50} is the average of three different experiments. The selectivity indexes, SI, were expressed as the ratio between IC_{50} in macrophages and IC_{50} in *T. cruzi* (Tulahuen 2 strain).[36]

Accessing the mechanism of action

^{1}H NMR study of the excreted metabolites. For the ^{1}H NMR spectroscopic studies,[37–40] 5 mL of 2-day-treated *T. cruzi* (Y strain), with each studied compound at concentrations corresponding to the IC_{50} values, were centrifuged at 1500*g* for 10 min at 4 °C. The pellet was discarded, and the parasite-free supernatant was stored at −20 °C until use. Before measuring, 0.1 mL of DMF (10 mM) as the internal standard and 0.1 mL of D_2O were added to 0.3 mL of the supernatant. The spectra were registered with water suppression in 5 mm NMR sample tubes. The chemical displacements used to identify the respective metabolites were previously confirmed by adding each analyzed metabolite to the studied supernatant as well as by the study of a control solution with 4 μg mL^{-1} of each metabolite in buffer (phosphate, pH = 7.4). Each run was done at least in triplicate and the Student *t* test was used to analyse the significance of the changes. The chemical shifts (δ, ppm) and multiplicity of the analysed catabolites are: Ala (alanine), 1.316, d; Lac (lactate), 1.466, d; Ace (acetate), 1.904, s; Pyr (pyruvate), 2.357, s; Succ (succinate), 2.392, s; Gly (glycine), 3.547, s.

Lipophilicity studies. Reversed-phase TLC experiments were done on precoated TLC plates SIL RP-18W/UV_{254} and eluted with MeOH–DMF–Tris-HCl buffer, 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 R_f values were averaged from two to three determinations, and converted to R_M *via* the relationship: $R_M = \log_{10}[(1/R_f) - 1]$.[41–43]

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 of 5 : 1. Each $V^{IV}O$-complex was dissolved in a minimal amount of DMSO, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (HEPES, with pH 7.4) was then 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 five minutes 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 a 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, and a cone angle of 35° and high aspect ratio were used. The images were obtained at room temperature (t = 23 ± 2 °C) and the relative humidity was usually lower than 40%.[26,27]

Results and discussion

Syntheses and characterization of the oxidovanadium(IV) complexes

Ten new mixed-ligand oxidovanadium(IV) ($V^{IV}O$) complexes, **1–10**, of the tridentate salicylaldehyde semicarbazone

derivatives **L1–L5** (Fig. 1), with the phenanthroline derivatives 5-amine-1,10-phenanthroline and 5,6-epoxy-5,6-dihydro-1,10-phenanthroline as co-ligands, were synthesized in good to reasonable yields. Analytical, TGA, FTIR, conductimetric, ESI-MS and EPR spectroscopic results for the $V^{IV}O$-complexes are in agreement with the proposed formulation: $[V^{IV}O(L\text{-}2H)(NN)]\cdot xH_2O$. Their structural formulae are depicted in Fig. 1. All of them are 'nonconducting' compounds in DMSO. ESI-MS measurements allowed the detection of the molecular ion for all $V^{IV}O(L\text{-}2H)(NN)$ complexes. In the case of complexes **2**, **5**, **7** and **10**, which contain brominated ligands **L2** or **L5**, two peaks were detected for $[M + H]^+$, in agreement with the ^{79}Br and ^{81}Br isotopic distribution.

Characterization of the complexes in the solid state

Thermal analysis. Thermogravimetric curves for the complexes $[V^{IV}O(L\text{-}2H)(aminophen)]$, where L = **L1–L3** and **L5**, and $[V^{IV}O(L\text{-}2H)(epoxyphen)]$, where L = **L2–L5**, demonstrated the absence of crystallization solvent molecules. On the other hand, the other two complexes of the series showed a single weight loss corresponding to loss of water: (i) $[V^{IV}O(\mathbf{L4}\text{-}2H)(aminophen)]$ **7** showed a weight loss of 3.8% centered near 150 °C that corresponds to one crystallization water molecule (calcd 3.6%), and (ii) $[V^{IV}O(\mathbf{L1}\text{-}2H)(epoxyphen)]$ **2** showed a weight loss of 4.1% centered near 115 °C that also corresponds to one crystallization water molecule (calcd 3.9%).

IR spectroscopic studies. 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 are slightly displaced to higher frequencies upon coordination, as commonly observed for phenanthroline complexes.[25,44] Based on previous reports on metal complexes of salicylaldehyde semicarbazone derivatives[24,27,30,31] and related compounds,[45,46] tentative assignments were made, which are presented in Table 1. The absence of the ν(C═O) bands, present in the semicarbazone compounds at around 1667–1676 cm^{-1}, indicates the enolization of the amide functionality upon coordination to vanadium. Instead, strong bands at *ca.* 1600–1640 cm^{-1} are observed, which are characteristic of the coordination of the ligand in the enolate form.[45] The shift of ν(C═O) and ν(C═N) bands and the non-observation of the ν(OH) and ν(NH) are in agreement with tridentate coordination through the carbonylic oxygen ($O_{C=O}$), the azomethine nitrogen ($N_{azomethine}$) and the phenolic oxygen ($O_{phenolate}$), and with double deprotonation of the semicarbazone ligand at the phenolic hydroxyl and NH groups. The FT-IR spectra of the complexes show characteristic intense bands around 960 cm^{-1} assigned to ν(V═O).

Characterization of the complexes in solution

EPR characterization of the $[V^{IV}O(L\text{-}2H)(NN)]$ complexes. EPR spectroscopy is a very powerful tool to obtain structural information on the solution behaviour of $V^{IV}O$-complexes.[47–50] The spectra of frozen solutions (at 77 K) of the $V^{IV}O$ mixed-ligand complexes exhibit a hyperfine pattern consistent with axial-type spectra of monomeric $V^{IV}O$-bound species with d^1_{xy} ground-state configuration. Fig. 2 shows the X band EPR spectra (at 77 K) measured for 3 mM solutions of the $[V^{IV}O(L\text{-}2H)(NN)]$ compounds in DMF. The spectra are very similar for all complexes and their simulation[33] thus yielded similar spin-Hamiltonian parameters: g_x,g_y = 1.981 (±0.001), g_z = 1.950 (±0.001), A_x,A_y = 54.7 (±0.3) × 10^{-4} cm^{-1} and A_z = 159.4 (±0.3) × 10^{-4} cm^{-1}. These suggest the same binding set for all complexes. They are also very close to those previously reported for other $[V^{IV}O(L\text{-}2H)(NN)]$ related compounds in which (NN) refers to phen,[27] dppz[26,28] or bipy.[26,28]

The values of the hyperfine coupling constant A_z can be estimated (A_z^{est}) using the additivity relationship proposed by Wüthrich[48] and Chasteen,[49] with an estimated accuracy of ±3 × 10^{-4} cm^{-1}. However, for some of the donor groups their contributions to the A_z are not straightforward, particularly the contributions of $N_{semicarbazone}$ (═N_{smc}), O_{CO} and N_{phen}. This was discussed in previous papers,[26–28] and considering the similar A_z values obtained for the aminophen, epoxyphen, phen, dppz

Table 1 Tentative assignment of selected IR bands of the $[V^{IV}O(L\text{-}2H)(NN)]$ complexes **1–10**. Bands for the free semicarbazone ligands are included for comparison.[27,31] Band positions are given in cm^{-1}. Measured ^{51}V NMR chemical shifts, δ^V, 24 h after dissolution in DMF

	ν(VO)	ν(C═O)	ν(C═N)	ν(O–H)	ν(N–H)	δ^V/ppm
L1	—	1695	1593	3493	3155	
[VO(**L1**-2H)(aminophen)], **1**	959	1609	1501	—	—	−532
[VO(**L1**-2H)(epoxyphen)], **2**	957	1613	1518	—	—	−532
L2	—	1698	1596	3470	3170	
[VO(**L2**-2H)(aminophen)], **3**	954	1610	1505	—	—	−530
[VO(**L2**-2H)(epoxyphen)], **4**	959	1613	1506	—	—	−529
L3	—	1676	1586	3466	3160	
[VO(**L3**-2H)(aminophen)], **5**	956	1638	1551	—	—	−529
[VO(**L3**-2H)(epoxyphen)], **6**	956	1614	1553	—	—	−535
L4	—	1667	1595	3433	3160	
[VO(**L4**-2H)(aminophen)], **7**	958	1599	1551	—	—	−535
[VO(**L4**-2H)(epoxyphen)], **8**	954	1611	1552	—	—	−533
L5	—	1672	1572	3477	3191	
[VO(**L5**-2H)(aminophen)], **9**	959	1618	1542	—	—	−534
[VO(**L5**-2H)(epoxyphen)], **10**	960	1617	1542	—	—	−532

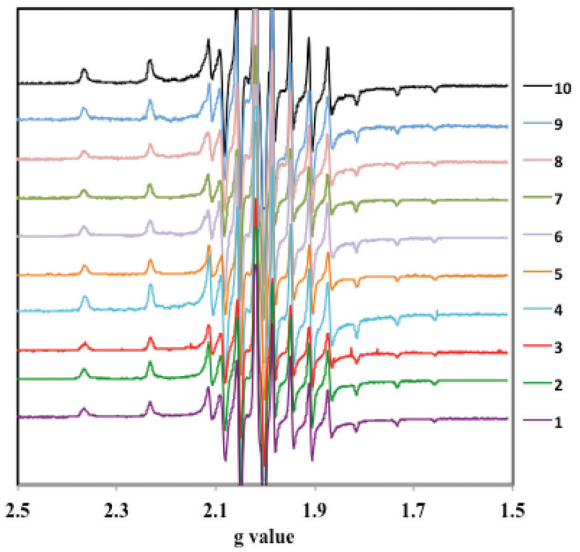

Fig. 2 First derivative X-band EPR spectra of frozen solutions (in DMF, *ca.* 3 mM at 77 K) of [$V^{IV}O$(L-2H)(NN)] complexes, **1–10**. Acquisition parameters: modulation frequency 100 kHz, receiver gain 6.3 × 10^4, modulation amplitude 2.86 and time constant 2.56.

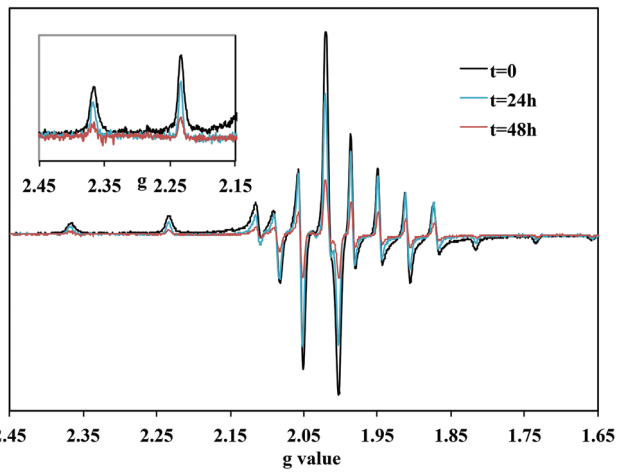

Fig. 3 Changes observed with time in the 1st derivative EPR spectra of a frozen solution (77 K) of complex **7** upon dissolution in DMF (*ca.* 3 mM). Acquisition parameters: modulation frequency 100 kHz, receiver gain 6.3 × 10^4, modulation amplitude 2.86 and time constant 2.56.

and bipy containing complexes of salicylaldehyde semicarbazone ligands **L1–L5**, we propose the same binding set for all of them: the semicarbazone acting as a tridentate ligand, the binding involving O_{phen}, N_{smc} and O_{CO} in the equatorial plane, and the phenanthroline derivatives binding as bidentate ligands through the two N donors, in an equatorial–axial mode. The contribution of N_{phen} to A_z^{est} depends on the angle of the phen rings with the V=O bond.[50] We expect this angle to be close to zero for an axial–equatorial bound phenanthroline and took $A_z(N_{phen})$ = 40.4 × 10^{-4} cm^{-1}. Assuming that CO contributes as O-enolate(−1) (37.6 × 10^{-4} cm^{-1}),[14] and taking the average value for N_{imine} (41.6 × 10^{-4} cm^{-1})[14,51] and $O_{phenolate}$ (O_{Ph}) = 38.9 × 10^{-4} cm^{-1},[49] we obtained the value for A_z^{est} = 158.5 × 10^{-4} cm^{-1}, which fits the experimental values reasonably well.

Stability of the $V^{IV}O$-complexes towards solvolysis and/or oxidation. To evaluate the stability of the $V^{IV}O$-complexes towards oxidation, solutions of complexes **1** and **7** in DMF (3 mM) were studied by EPR over a 2 day period. Fig. 3 shows the spectra obtained for complex **7**. The epoxyphen complex **7** is moderately stable since after 24 h there is a decrease of *ca.* 30% in the intensity of the EPR spectra, but after 48 h *ca.* 65% of the V^{IV} is oxidized to V^V-species. The aminophen complex **1** is much more stable, as after 48 h most of the vanadium is still in the +4 oxidation state.

To corroborate the oxidation and to identify which types of V^V-species were formed, all vanadium solutions were additionally evaluated by ^{51}V NMR spectroscopy, after a one-day period under air. The ^{51}V NMR spectra measured for all complexes showed the presence of one peak at $\delta^V \approx -530$ ppm. Previously studied $V^{IV}O$ semicarbazone complexes of phen, bipy and dppz also showed the presence of an oxidized species in the same δ^V range.[26–28] Moreover, chemical shifts of V^VO_2-semicarbazone complexes have been found and also theoretically predicted in the −515 to −540 ppm range.[52] Thus, the V^V-complexes formed with δ^V values in the range −529 to −535 ppm could be assigned to species formulated as V^VO_2(L-2H).

From the spectroscopic characterization made in solution we can conclude that the [$V^{IV}O$(L-2H)(epoxyphen)] complexes are slightly more susceptible to oxidation in DMF solution than the corresponding [$V^{IV}O$(L-2H)(aminophen)] analogous complexes. In solution both types of complexes form only one V^{IV}-species in DMF with a binding set (O_{Ph}, O_{CO}, N_{smc}, $N_{imine})_{eq}(N_{imine})_{ax}$, no V^{IV}-solvolysis products being detected, at least for 72 h. The stability of the V^{IV}-complexes is comparable to the structurally related [$V^{IV}O$(L-2H)(phen)] complexes,[27] which also oxidize slowly and do not form V^{IV}-solvolysis products. After 24 h under air, the complexes partially oxidize forming V^VO_2(L-2H)(solvent) species, after displacement of the NN heteroligand, similarly to what was found for the systems: [$V^{IV}O$(L-2H)(dppz)],[26,28] [$V^{IV}O$(L-2H)(phen)][27] and [$V^{IV}O$(L-2H)-(bipy)].[26,28]

The relative stability of metal complexes in biological media, along with their lipophilicity, is a crucial factor that may determine their biological activity.[52] In the present study we found that all V^{IV}-complexes **1–5**, containing the aminophen ligand, were more stable and more cytotoxic to parasitic cells and less toxic to mammalian cells than the corresponding complexes **6–10**, containing the epoxyphen ligand. On the other hand, the corresponding V^VO_2(L-2H)(solvent) complexes are not active.

For several V^{IV}-complexes diluted in neutral aqueous solution, such as $V^{IV}O(maltolato)_2$, their oxidation to their V^V counterparts is frequently accompanied by ligand loss and formation of $H_nVO_4^{(3-n)}$.[53] The present set of salicylaldehyde semicarbazones act as tridentate ligands and extrapolation of the solubility and stability data to neutral aqueous media,

namely cell culture media, or to carry out spectroscopic studies in very dilute solutions, is not straightforward. Notwithstanding, in previous studies in aqueous–organic solvents[26,27,54,55] with $V^{IV}O(L)(NN)$ and $V^{V}O_2(L)$ complexes (L = salicylaldehyde semicarbazone derivatives), no evidence for significant formation of $V^{IV}O$- or V^{V}-complexes other than $V^{IV}O(L)(NN)$ and $V^{V}O_2(L)$ was obtained, namely no $H_nVO_4^{(3-n)}$, $V^{V}O_2(L)(NN)$ or $V^{V}O_2(NN)_n$ species were clearly detected. Thus, in the present set of complexes one of the relevant factors determining the biological activity is indeed stability of the $V^{IV}O(L\text{-}2H)(NN)$ towards hydrolysis and oxidation, and a global correlation of biological activity with stability is found.

Biological results

***In vitro* anti-*Trypanosoma cruzi* activity and unselective cytotoxicity.** The $V^{IV}O$-complexes and the NN co-ligands were evaluated *in vitro* for their anti-*T. cruzi* activities against epimastigotes of Tulahuen 2 strain and their selectivities were analyzed using murine macrophages, J-774, as a mammalian model. Results are included in Table 2.

Both free 1,10-phenanthroline derivatives were tested in order to evaluate the effect of vanadium coordination on the biological activity. Phenanthroline and its substituted derivatives have shown to disturb the functioning of a wide variety of biological systems.[56,57] It has been usually assumed that DNA damage, resulting from structural features like planarity, hydrophobicity and rigidity, and/or the sequestering of trace metals *in situ*, the resulting metal complexes being the active species, might be involved in their biological action.[56–58]

The epoxyphen and aminophen showed IC_{50} values of *ca.* 2.3 μM and 5 μM, respectively. Neither the precursor $[V^{IV}O(acac)_2]$ (IC_{50} > 25 μM)[43] nor the salicylaldehyde semicarbazones **L1–L5** are toxic to *T. cruzi* at these low micromolar concentrations.[26]

In molar units all vanadium complexes were more active than both the reference drug Nifurtimox (Nfx) and the previously reported $[V^{IV}O(L\text{-}2H)(NN)]$ complexes (Table 2).[28] Except for **9**, the $[V^{IV}O(L\text{-}2H)(epoxyphen)]$ complexes were 1.9 to 2.6 times more active than epoxyphen itself. Additionally, complexes **6**, **7** and **10** were more selective to *T. cruzi* than epoxyphen with selectivity indexes (SI) 1.5 to 2.0 higher than the value for this ligand, showing that the complexation to V^{IV} leads to more cytotoxic agents for the parasite. The lower selectivity observed, when compared to the aminophen analogues, could be due to the known epoxide pharmacophore that, under nucleophilic biological conditions, could suffer ring-opening promoting unselective cytotoxic events. However, complexes $[V^{IV}O(\mathbf{L1}\text{-}2H)(epoxyphen)]$ and $[V^{IV}O(\mathbf{L2}\text{-}2H)(epoxyphen)]$ were as selective as Nfx.

On the other hand, the $[V^{IV}O(L\text{-}2H)(aminophen)]$ complexes showed quite low IC_{50} values against *T. cruzi* displaying 9.6 to 18.5 times more activity than the aminophen ligand. Furthermore, none of these complexes displayed relevant toxicity against J-774 macrophages at doses down to 50 μM (Table 3) showing in all cases much higher SI values than the corresponding value for aminophen itself. The selectivities for the *T. cruzi* parasite were at least 50 times higher than that for aminophen, and *ca.* 2.3 times better than the Nfx selectivity.

Table 2 *In vitro* biological activity on *T. cruzi* (Tulahuen 2 strain) and on macrophages J-774 of the oxidovanadium(IV) complexes, Nfx and the NN ligands

Compound	IC_{50} (μM) *T. cruzi* (Tulahuen 2)	IC_{50} (μM) J-774 murine macrophages	SI
$[V^{IV}O(\mathbf{L1}\text{-}2H)(aminophen)]$, **1**	0.50 ± 0.02	50 ± 1	100.0
$[V^{IV}O(\mathbf{L2}\text{-}2H)(aminophen)]$, **2**	0.27 ± 0.09	50 ± 2	185.0
$[V^{IV}O(\mathbf{L3}\text{-}2H)(aminophen)]$, **3**	0.52 ± 0.02	50 ± 1	96.0
$[V^{IV}O(\mathbf{L4}\text{-}2H)(aminophen)]$, **4**	0.51 ± 0.02	>50	>98.0
$[V^{IV}O(\mathbf{L5}\text{-}2H)(aminophen)]$, **5**	0.51 ± 0.02	>50	>98.0
$[V^{IV}O(\mathbf{L1}\text{-}2H)(epoxyphen)]$, **6**	1.0 ± 0.1	40 ± 1	40.0
$[V^{IV}O(\mathbf{L2}\text{-}2H)(epoxyphen)]$, **7**	0.9 ± 0.2	41 ± 2	45.5
$[V^{IV}O(\mathbf{L3}\text{-}2H)(epoxyphen)]$, **8**	1.2 ± 0.3	9.0 ± 0.5	7.5
$[V^{IV}O(\mathbf{L4}\text{-}2H)(epoxyphen)]$, **9**	3.9 ± 0.6	54 ± 2	13.8
$[V^{IV}O(\mathbf{L5}\text{-}2H)(epoxyphen)]$, **10**	1.1 ± 0.3	37 ± 2	34.0
Aminophen	5.0 ± 0.7	9.7 ± 0.4	1.9
Epoxyphen	2.3 ± 0.6	50 ± 5	22.0
Nfx	7.7 ± 0.3	316.0 ± 0.5[36]	41.0

Table 3 ^{1}H NMR signal-integrations of the end-products excreted to the milieu in the different treatments, expressed with respect to DMF,[62] by *T. cruzi* epimastigote (Y strain) (for details see the Experimental section)

	Excreted catabolite					
Treatment with	Gly	Succ	Pyr	Ace	Ala	Lac
$[V^{IV}O(\mathbf{L2}\text{-}2H)(epoxyphen)]$	3.12 ± 0.05[a]	11.3 ± 0.1	18.5 ± 0.2	25.4 ± 0.1[b]	24.89 ± 0.04[b]	9.84 ± 0.05
$[V^{IV}O(\mathbf{L2}\text{-}2H)(aminophen)]$	2.95 ± 0.06	11.4 ± 0.7	18.6 ± 0.9	26 ± 1	25 ± 1	10.1 ± 0.5
Untreated	2.94 ± 0.01	11.47 ± 0.02	18.6 ± 0.1	24.67 ± 0.03	24.57 ± 0.01	9.87 ± 0.01

[a] $p = 0.14$ (different with respect to untreated *T. cruzi*); [b] $p = 0.06$ (different with respect to untreated *T. cruzi*). Student *t* test.

Complex **2**, [$V^{IV}O$(**L2**-2H)(aminophen)], was the most active and selective compound of the series. It may also be noted that for the [$V^{IV}O$(**L**-2H)(epoxyphen)] series, complex **7** is the most active, suggesting that the 5-bromosalicyl-motive present in **L2** seems to play a relevant role in the desired biological activities, since in both families the most active derivatives contain **L2** as a ligand.

Accessing the mechanism of action

Changes in *T. cruzi* excreted catabolites when the parasite cells are exposed to a bioactive compound can be indicative of the biochemical pathway modified by the agent.[59–62] In order to study the changes in the biochemical pathways promoted by the most active complexes of each series, [$V^{IV}O$(**L2**-2H)(epoxyphen)] **7** and [$V^{IV}O$(**L2**-2H)(aminophen)] **2**, we studied the modifications in the excreted metabolites by ^{1}H NMR spectroscopy. The spectra of the cell-free milieu of complex-treated parasites were compared with those of the untreated *T. cruzi*-free milieu as the control. We focused mainly on the changes of the excreted salts of the carboxylic acids, lactate (Lac), acetate (Ace), pyruvate (Pyr), and succinate (Succ) and the amino acids, alanine (Ala) and glycine (Gly), among the most relevant modified metabolites. Fig. 4 shows the changes in the excreted end-products without or after treatment with **2** and **7**. Clearly, the treatment with these complexes produced an increment of excreted Gly, Ace and Ala that is significantly higher for [$V^{IV}O$(**L2**-2H)(epoxyphen)], when compared to the untreated control. These increments may be an indication that the integrity of the *T. cruzi* mitochondrion was affected due to two of the catabolites produced in this organelle, Ace and Gly.[63] Additionally, Ala is produced in the cytosol of the parasite cell from Pyr,[64] that is originated in the glycosome, *via* phosphoenolpyruvate. This Pyr could also be used, into the mitochondrion, in the Krebs-like cycle.[63] Consequently, an increment in the amount of Ala may be indicating an increment of cytosolic Pyr concentration as a result of a deficient mitochondrion uptake due to modifications of the organelle integrity. Consequently, the collected information indicated that the mitochondrion could be a target for these complexes.

Lipophilicity studies and QSAR. Lipophilic, polar, electronic and steric effects are among the prime factors controlling transport to and interaction with biological receptors. Hence,

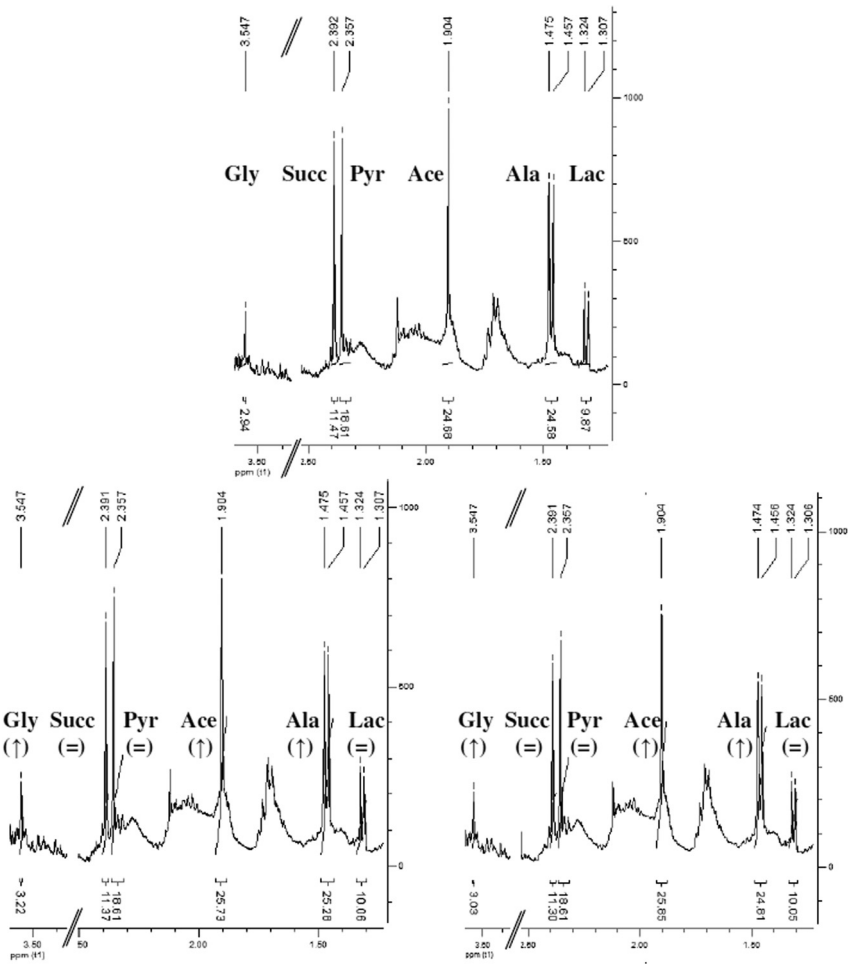

Fig. 4 Region of the ^{1}H NMR spectra used to analyze the excreted catabolites (in these cases the integrations were expressed with respect to Pyr). Above: experiment of *T. cruzi* without treatment. Below: left, experiment of *T. cruzi* treated with [$V^{IV}O$(**L2**-2H)(epoxyphen)] (**7**); right, experiment of *T. cruzi* treated with [$V^{IV}O$(**L2**-2H)(aminophen)] (**2**).

Table 4 R_M values obtained for the $V^{IV}O$-complexes **1–10** (see the text)

Compound	R_M	Compound	R_M
$[V^{IV}O(\mathbf{L1}\text{-}2H)(aminophen)]$, **1**	1.35	$[V^{IV}O(\mathbf{L1}\text{-}2H)(epoxyphen)]$, **6**	0.91
$[V^{IV}O(\mathbf{L2}\text{-}2H)(aminophen)]$, **2**	1.50	$[V^{IV}O(\mathbf{L2}\text{-}2H)(epoxyphen)]$, **7**	0.95
$[V^{IV}O(\mathbf{L3}\text{-}2H)(aminophen)]$, **3**	1.32	$[V^{IV}O(\mathbf{L3}\text{-}2H)(epoxyphen)]$, **8**	1.69
$[V^{IV}O(\mathbf{L4}\text{-}2H)(aminophen)]$, **4**	1.32	$[V^{IV}O(\mathbf{L4}\text{-}2H)(epoxyphen)]$, **9**	1.88
$[V^{IV}O(\mathbf{L5}\text{-}2H)(aminophen)]$, **5**	1.63	$[V^{IV}O(\mathbf{L5}\text{-}2H)(epoxyphen)]$, **10**	0.95
Aminophen	0.91	Epoxyphen	0.63

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 newly developed $V^{IV}O$ mixed-ligand complexes to analyze its effect on the biological activity and to compare the results with those previously reported for analogous compounds. Lipophilicity was experimentally determined using reversed-phase TLC experiments where the stationary phase, precoated TLC-C_{18}, may be considered to simulate lipids of biological membranes or receptors, and the mobile phase, MeOH–DMF–Tris-HCl buffer pH 7.4 (85 : 5 : 10, v/v/v), resembles the aqueous biological milieu. The composition of the mobile phase was adjusted in order to allow differentiating complexes according to their lipophilicity. The most adequate one was a combination of polar organic solvents, MeOH and DMF, together with a buffer that simulates the physiological pH value. Table 4 summarizes the R_M values for each compound.

When the evaluated compounds (complexes, ligands and Nifurtimox[28]) (Table 4) were analyzed to find a quantitative structure–activity relationship (QSAR) 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 new $V^{IV}O$-complexes a clear quadratic correlation was found (eqn (1), Fig. 5):

$$IC_{50,T.\ cruzi} = (14 \pm 3) - (22 \pm 4)\ R_M + (9 \pm 2)\ {R_M}^2 \quad (1)$$
$$r_{adj}{}^2 = 0.7927,\ n = 10,\ F = 18.21,\ p = 0.0017$$

A similar nearly parabolic relationship between biological response and lipophilicity has been previously described for a large number of biologically relevant families of compounds[42,59,66] as well as for the series of $[V^{IV}O(L\text{-}2H)(NN)]$ complexes previously described.[28] From this correlation an optimal R_M value, close to 1.29 (Fig. 5), was obtained as a design tool for further development of new compounds that could possess a better biological profile. This value is close to that obtained for a previously described series of $V^{IV}O$-complexes.[28]

When attempting to obtain QSAR for the complete series of $V^{IV}O$-complexes, $[V^{IV}O(L\text{-}2H)(NN)]$, which includes those previously reported,[24,27,28] as well as those described herein, we need to pay attention to the different structural features of the co-ligands (NN, Fig. 6). These potential scaffolds may display different biological behaviours: (i) the highly electrophilic epoxyphen may react with biological nucleophiles from

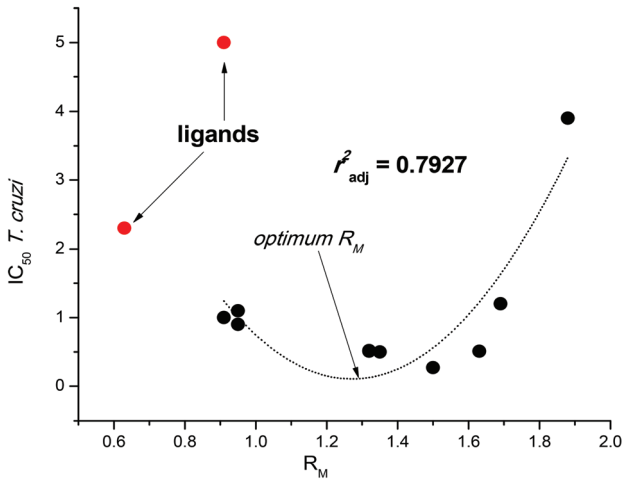

Fig. 5 IC_{50} values (in μM units) for the anti-*T. cruzi* activity of the compounds *vs.* R_M values as a measure of the lipophilicity of the compounds.

proteins and DNA through the epoxide-moiety, while (ii) the nucleophile aminophen may react with biological electrophiles through the amino-moiety. Moreover, (iii) the π-expanded systems dppz, phen and phen derivatives may also intercalate to DNA. In view of these effects, we defined an indicator variable, I_{col}, to quantify them for the analysis of the possible correlations. The use of indicator variables has been shown to be very useful to improve the ability to formulate QSAR relationships for different molecules interacting with complex biological systems. According to the expected different biological behaviours of the co-ligands, we arbitrarily assigned for the most reactive epoxyphen co-ligand an I_{col} of 4, for the nucleophile aminophen a value of 3, for the intercalators dppz and phen a value of 2, and for bipy a value of 1, when each of them are part of the structure of a complex.[41,67]

When the complete series of 25 $[V^{IV}O(L\text{-}2H)(NN)]$ complexes are considered, including lipophilicity and the structural descriptor of the co-ligands, I_{col}, a modest statistical correlation was obtained ($r_{adj}{}^2$ = 0.5842). Three compounds could be identified as outliers: $[V^{IV}O(\mathbf{L4}\text{-}2H)(bipy)]$, $[V^{IV}O(\mathbf{L1}\text{-}2H)(phen)]$ and $[V^{IV}O(\mathbf{L3}\text{-}2H)(epoxyphen)]$, which probably correspond to different mechanisms of action from that of the rest of the complexes. Thus, when the population under study was restricted by not including these three compounds the following satisfactory correlation was found:

$$IC_{50,T.\ cruzi} = (506 \pm 97) - (592 \pm 137)R_M + (205 \pm 51){R_M}^2 - (31 \pm 4)\ I_{col} \quad (2)$$
$$r_{adj}{}^2 = 0.7847,\ n = 22,\ F = 26.52,\ p = 8 \times 10^{-7}$$

Clearly, eqn (2) highlights the relevance of the lipophilic parameter to the desired bioactivity, as it was found previously[28] and as eqn (1) does. Additionally it also reflects the significance of the structure of the co-ligand on the anti-*T. cruzi* effect. According to eqn (2), complexes with DNA-

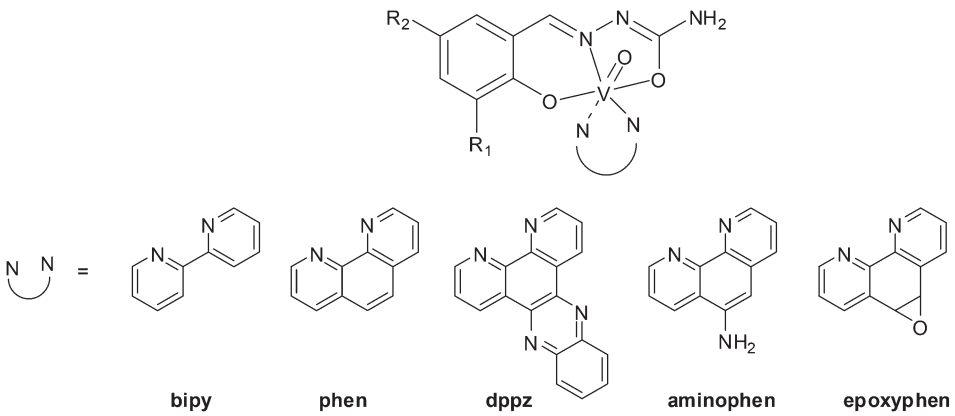

Fig. 6 Structural formulae of the co-ligands NN in the [$V^{IV}O$(L-2H)(NN)] complexes under study.

modifying co-ligands, *i.e.* with the higher I_{col} values, were the most cytotoxic for the parasite, *i.e.* showed the lowest IC_{50} against *T. cruzi*.

Atomic force microscopy (AFM) results. AFM is a useful tool for imaging DNA and also DNA interactions with metal complexes.[68,69] The present series of [$V^{IV}O$(L-2H)(NN)] compounds, where NN is aminophen or epoxyphen, was developed with DNA as a possible target. Our previous studies by AFM methods, supported by other techniques, confirmed DNA as a potential parasite target for the analogous [$V^{IV}O$(L-2H)(NN)] compounds, with NN = phen, dppz and bipy.[26–28] The interaction of the new series of aminophen and epoxyphen complexes with DNA was preliminarily studied here by AFM using pBR322 plasmid as a model molecule. Selected AFM images are depicted in Fig. 7. All 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 compounds also interact with DNA.

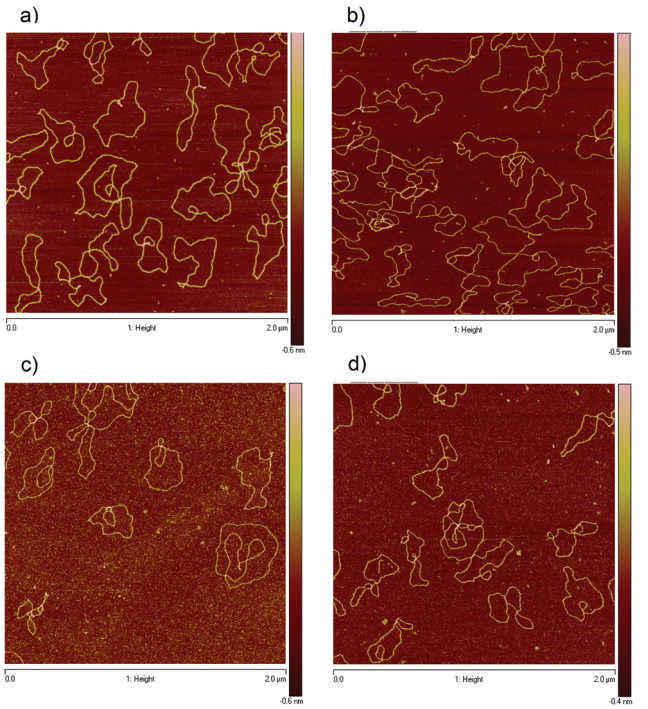

Fig. 7 AFM images showing the modifications suffered by pBR322 DNA (a) due to the interaction with selected [$V^{IV}O$(L-2H)(NN)] compounds: (b) [$V^{IV}O$(**L1**-2H)(epoxyphen)], (c) [$V^{IV}O$(**L1**-2H)(aminophen)], and (d) [$V^{IV}O$(**L3**-2H)(aminophen)]. The used molar ratio compound : DNA base pairs was 1 : 5, and incubation was done for 24 h at 37 °C (for details see the Experimental section).

Conclusions

A new series of mixed-ligand $V^{IV}O$-complexes, [$V^{IV}O$(L-2H)(NN)], including tridentate salicylaldehyde semicarbazone derivatives as ligands (L) and either aminophen or epoxyphen as co-ligands (NN), were prepared and characterized in the solid state and in solution by a combination of different techniques. The V^{IV}-center displays an octahedral environment with the NN ligand coordinated in an equatorial–axial mode and the tridentate semicarbazone ligand occupying the remaining equatorial positions.

These new complexes showed IC_{50} values in the low micromolar or submicromolar range against *T. cruzi* epimastigotes and were more toxic to the parasite than the phen-, bipy- and dppz-containing analogues, the free NN ligands and the anti-trypanosomal drug Nifurtimox. The aminophen complexes were significantly more active than the epoxyphen analogues, the results clearly showing the influence of the nature of the intercalative N_{py},N_{py} polypyridyl chelator on the anti-trypanosomal activity. Additionally, the new series of compounds displayed good to high selectivities towards the parasite, the aminophen compounds being non-toxic on J-774 murine macrophages at doses up to 50 μM.

QSAR studies are not very commonly reported for metal-based bioactive compounds since relatively large series of closely structurally related complexes are needed. The results of the QSAR study of the whole series of [$V^{IV}O$(L-2H)(NN)] complexes developed by our group highlight the relevance of the lipophilicity of the compounds, but also indicates the significance of the structure of the NN co-ligand on the anti-*T. cruzi* activity. A parabolic relationship between biological response

and lipophilicity was obtained and, from this correlation, an optimum R_M value was determined, which may be a design guide for the future development of new compounds bearing a better biological profile.

The information emerging from the NMR follow-up of the amount of catabolites produced by the parasites exposed to selected $V^{IV}O$-compounds suggests that the mitochondrion may be a target for these complexes. In addition, the complexes were shown to interact with DNA, suggesting that this biomolecule may be an alternative/additional parasite target.

Acknowledgements

The authors would like to thank RIIDFCM and RIDIMEDCHAG CYTED networks for supporting collaborative research on the development of bioactive metal-based compounds and on anti-*T. cruzi* agents, respectively, and also acknowledge the financial support from Fundação para a Ciência e Tecnologia (FCT, Portugal), PEst-OE/QUI/UI0100/2011 and CIÊNCIA2007, the Portuguese NMR and Mass Spectrometry Networks (IST-UTL Centers) and the Comisión Sectorial de Investigación Científica (CSIC)-UdelaR (CSIC-No. 610, Uruguay). ANII (Agencia Nacional de Investigación e Innovación, Uruguay) is also thanked for scholarships to M. F., J. V. and E. B. AFM studies were supported by Ministerio de Economía y Competitividad (MINECO, CTQ2011-27929-C02-01) and performed by Rosa Brissos.

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