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Copper(II)–fluoroquinolone complexes with anti-*Trypanosoma cruzi* activity and DNA binding ability

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Abstract Copper(II) complexes of fluoroquinolone antibacterial agents levofloxacin (LEV) and sparfloxacin (SPAR), containing or not a nitrogen donor heterocyclic ligand, 2,2′-bipyridine (bipy) or 1,10-phenathroline (phen), were prepared and characterized. The complexes are of the type $[CuCl_2(H_2O)(L)]$, $[CuCl(bipy)(L)]Cl$ and $[CuCl_2(phen)(L)]$, where L = LEV or SPAR. The data suggest that LEV and SPAR act as zwitterionic bidentade ligands coordinated to Cu(II) through the carboxylate and ketone oxygen atoms. The electron paramagnetic resonance spectra of the $[CuCl(bipy)(L)]Cl$ and $[CuCl_2(phen)(L)]$ complexes (L = LEV and SPAR) in aqueous and DMSO solutions indicate mixture of mononuclear and binuclear forms. The Cu(II) complexes, together with the corresponding ligands, were evaluated for their trypanocidal activity in vitro against *Trypanosoma cruzi*, the causative agent of Chagas disease. The assays performed against bloodstream trypomastigotes showed that all complexes were more active than their corresponding ligands. Complexes $[CuCl_2(phen)(LEV)]$ and $[CuCl_2(phen)(SPAR)]$ were revealed, among all studied compounds, to be the most active with IC_{50} = 1.6 and 4.7 μM, respectively, both presenting a superior effect than benznidazole. The interactions of fluoroquinolones and their Cu(II) complexes with calf-thymus DNA were investigated. These compounds showed binding properties towards DNA, with moderated binding constants values, suggesting that this structure may represent a parasite target.

Keywords Fluoroquinolones · Copper(II) complexes · Anti-*T. cruzi* activity · UV–Vis spectroscopy · Interaction with calf-thymus DNA

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Introduction

Fluoroquinolones (FQs) are a group of synthetic antibacterial agents active against various microorganisms (Sharma et al. 2009). Some works regarding the antiparasitary activity of FQs can also be found in the literature, including chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* (Anquetin et al. 1993) *Trypanosoma cruzi* (Castora et al. 1983), *Trypanosoma brucei* (Chollet et al. 2009; Nenortas et al. 2003), *Leishmania* spp. (Romero

et al. 2005; Cortázar et al. 2007) and *Toxoplasma gondii* (Anquetin et al. 1993; Gozalbes et al. 2000).

Trypanosoma cruzi is the etiological agent of Chagas disease, affecting approximately eight million individuals in Latin America (Coura and Dias 2009). Also, Chagas disease is emerging in non-endemic areas due to the globalization of immigration and non-vectorial transmission routes (Soeiro and Castro 2011). However, although this neglected tropical disease still poses an important public health problem, resulting in high morbidity and considerable mortality rates, few investments have been allocated towards developing novel anti-*T. cruzi* agents. In fact, the available therapy for this illness, based on only two nitro derivatives, nifurtimox ([3-methyl-4-(5′-nitrofurfurylideneamine) tetrahydro-4*H*-1,4-tiazine-1, 1-dioxide], Nf) and benznidazole (*N*-benzyl-2-nitroimidazole acetamide, Bz), presents limited efficacy, especially during the later chronic phase, besides displaying undesirable secondary side effects (Coura and Castro 2002). Additionally, both require long periods of treatment (30–60 days), and natural resistance of different parasite strains to both nitro-derivatives has also been reported (Coura and Castro 2002). Thus, all these drawbacks justify the urgent need to identify better drugs to treat chagasic patients.

With that in mind, not long ago some members of out team developed a series of Co(II) and Cu(II) complexes with norfloxacin (NOR) and sparfloxacin (SPAR). At the time, we managed to demonstrated that, while the free ligands were poorly effective against *T. cruzi*, $[CoCl_2(NOR)(H_2O)_2]$ and $[CoCl_2(SPAR)(H_2O)_2]$ were active against intracellular forms of the parasite and that $[CuCl_2(phen)(NOR)]$ and $[CuCl_2(phen)(SPAR)]$ present trypanocidal effect against both bloodstream trypomastigotes and intracellular forms of *T. cruzi* (Batista et al. 2011).

In the present work we prepare a new series of Cu(II) complexes with levofloxacin (LEV) and SPAR (Fig. 1), containing or not 2,2′-bipyridine (bipy) or 1,10-phenanthroline (phen), in order to verify the importance of the presence of a nitrogen-donor for the anti-*T. cruzi* activity. $[CuCl_2(phen)(SPAR)]$ which had been studied by some members of our team (Batista et al. 2011) was included in this study for comparison. The binding properties of the free fluoroquinolones and their Cu(II) complexes to DNA investigated using UV–Vis spectroscopy.

Experimental

Materials

LEV, SPAR, calf-thymus DNA (CT DNA), 2,2′-bipyridine, $CuCl_2{\cdot}2H_2O$ and $CuCl_2$(phen) were purchased from Sigma-Aldrich. All solvents were purchased from Merck.

DNA stock solution was prepared by dilution of CT DNA into universal buffer at pH 7.4 (Perrin and Dempsey 1974), followed by exhaustive stirring at 4 °C for 3 days, and storage at 4 °C for no longer than a week. The stock solution of CT DNA gave a ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of 1.90, indicating that DNA was sufficiently free of protein contamination (Marmur 1961). The DNA concentration was determined by the UV, absorbance at 260 nm after 1:20 dilution using $\varepsilon = 6{,}600\ M^{-1}\ cm^{-1}$ (Reichmann et al. 1954).

The solutions of fluoroquinolones and Cu(II) complexes were prepared in universal buffer at pH 7.4. Universal Buffer consists of citric acid (Sigma), potassium phosphate monobasic (Synth), sodium tetraborate (Ecibra), tris(hydroxymethyl)aminomethane (Sigma) and potassium chloride (Synth). For pH

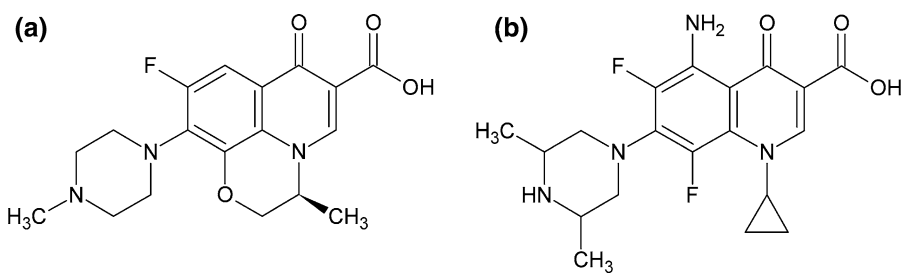

Fig. 1 Structures of **a** levofloxacin and **b** sparfloxacin

regulation the required amount of a 0.4 mol L^{-1} sodium hydroxide solution (Nuclear) was added (Perrin and Dempsey 1974).

Apparatus

Elemental analyses were performed on a CE Instruments, model EA 1110. Molar conductivity measurements were performed in H_2O or DMF solutions, 1×10^{-3} mol L^{-1} concentration, using a Quimis, model *Q405M*, conductivity meter. The IR spectra were acquired on a Mattson Instruments Galaxy 3000 spectrophotometer using KBr pellets. X-band electron paramagnetic resonance (EPR) spectra were obtained with a Bruker ESP300E spectrometer with modulation frequency of 100 kHz and modulation amplitude of 1 mT. Frozen aqueous and DMSO solutions of the complexes (~1 mM) were measured at liquid N_2 temperature (77 K) in Teflon® tubes of 3 mm internal diameter. A Hewlett Packard 8451-A UV–Vis spectrophotometer was used to obtain UV–Vis spectra with a 1.00 cm quartz cell.

Syntheses of [$CuCl_2$(bipy)] precursor

Equimolar amounts of $CuCl_2{\cdot}2H_2O$ and 2,2′-bipyridine (1.0×10^{-3} mol) were dissolved in acetone (20 mL). The reaction mixture was kept under stirring for 24 h. The solid which precipitated was filtered and washed with diethyl ether and dried.

Synthesis of the complexes

The complexes were obtained by dissolving LEV or SPAR (0.31 mmol) in acetone (20 mL) with gentle heating and stirring. After cooling the solution to room temperature $CuCl_2{\cdot}2H_2O$, [$CuCl_2$(bipy)] or [$CuCl_2$(phen)] (0.31 mmol) were added. The mixture was stirred at room temperature for 24 h. The solids which precipitated were filtered and washed with diethyl ether and dried in vacuo.

[$CuCl_2(H_2O)$(LEV)] (**1**): Green solid. Yield: 71 %. Anal.: found, C 42.3, H 4.6, N 8.1. Calc. for $C_{18}H_{22}N_3O_5Cl_2FCu$, C 42.1 %, H 4.3 %, N 8.2 %. Molar conductivity (1×10^{-3} mol L^{-1}, DMF): 40.8 μS cm^{-1}. IR (cm^{-1}): 3469 s ν(OH); 1592 m $\nu(C{=}O)_p$; 1630 s $\nu_{as}(COO^-)$; 1344 m $\nu_s(COO^-)$.

[CuCl(bipy)(LEV)]Cl·$3H_2O$ (**2**): Green solid. Yield: 61 %. Anal.: found, C 47.4, H 4.6, N 9.8. Calc. for $C_{28}H_{34}N_5O_7Cl_2FCu$, C 47.6 %, H 4.8 %, N 9.9 %. Molar conductivity (1×10^{-3} mol L^{-1}, H_2O): 142 μS cm^{-1}. IR (cm^{-1}): 3422 s ν(OH); 1518 s $\nu(C{=}O)_p$; 1617 s $\nu_{as}(COO^-)$; 1343 m $\nu_s(COO^-)$.

[$CuCl_2$(phen)(LEV)]·$4H_2O$ (**3**): Green solid. Yield: 65 %. Anal.: found, C 47.4, H 5.0, N 9.0. Calc. for $C_{30}H_{36}N_5O_8Cl_2FCu$, C 48.2 %, H 4.8 %, N 9.4 %. Molar conductivity (1×10^{-3} mol L^{-1}, DMF): 38 μS cm^{-1}. IR (cm^{-1}): 3442 s ν(OH); 1584 s $\nu(C{=}O)_p$; 1643 s $\nu_{as}(COO^-)$; 1395 m $\nu_s(COO^-)$.

[$CuCl_2(H_2O)$(SPAR)]·H_2O (4): Green solid. Yield: 78 %. Anal.: found, C 40.4, H 4.9, N 9.7. Calc. for $C_{19}H_{28}N_4O_6Cl_2F_2Cu$, C 40.6 %, H 4.7 %, N 10.0 %. Molar conductivity (1×10^{-3} mol L^{-1}, DMF): 29 μS cm^{-1}. IR (cm^{-1}): 3431 s ν(OH); 1573 s $\nu(C{=}O)_p$; 1636 s $\nu_{as}(COO^-)$; 1385 m $\nu_s(COO^-)$.

[CuCl(bipy)(SPAR)]Cl·$2H_2O$ (**5**): Green solid. Yield: 92 %. Anal.: found, C 47.6, H 4.4, N 11.5. Calc. for $C_{29}H_{30}N_6O_3Cl_2F_2Cu$, C 48.4 %, H 4.8 %, N 11.7 %. Molar conductivity (1×10^{-3} mol L^{-1}, H_2O): 148 μS cm^{-1}. IR (cm^{-1}): 3445 s ν(OH); 1568 s $\nu(C{=}O)_p$; 1611 s $\nu_{as}(COO^-)$; 1384 m $\nu_s(COO^-)$.

[$CuCl_2$(phen)(SPAR)]·$3H_2O$ (**6**): Green solid. Yield: 76 %. Anal.: found, C 49.1, H 4.8, N 10.9. Calc. for $C_{31}H_{36}N_6O_6Cl_2F_2Cu$, C 48.9 %, H 5.8 %, N 11.0 %. Molar conductivity (1×10^{-3} mol L^{-1}, DMF): 37 μS cm^{-1}. IR (cm^{-1}): 3422 s ν(OH); 1574 s $\nu(C{=}O)_p$; 1632 m $\nu_{as}(COO^-)$; 1383 m $\nu_s(COO^-)$.

Parasites

Y strain of *T. cruzi* was used throughout the experiments. Bloodstream forms were harvested by heart puncture from *T. cruzi*-infected Swiss mice at the peak of parasitemia (Meirelles et al. 1982).

Trypanocidal analysis

For the in vitro analysis on trypomastigotes, the parasites were incubated at 37 °C in the presence of increasing doses (0–200 μM) of each compound diluted in Dulbecco's modified medium supplemented with 5 % fetal bovine serum and 1 mM L-glutamine (DMES) (Batista et al. 2010). After 24 h, death rates were determined by light microscopy through the direct quantification of live parasites using a Neubauer chamber, and IC_{50} values (drug concentration that reduces 50 % of the number of lived parasites) were then calculated as reported (Daliry et al. 2009).

Mammalian cell cultures and toxicity assays

Primary cultures of embryonic cardiomyocytes (CM) were obtained following the previously described method (Meirelles et al. 1986). After purification, the CM were seeded at a density of 0.1×10^6 cells/well into 24-well culture plates, or 0.05×10^6 cell/well into 96-well microplates, containing gelatin-coated cover slips and sustained in Dulbecco's modified medium supplemented with 10 % horse serum, 5 % fetal bovine serum, 2.5 mM $CaCl_2$, 1 mM L-glutamine and 2 % chicken embryo extract (DMEM). All procedures were carried out in accordance with the guidelines established by the FIOCRUZ Committee of Ethics for the Use of Animals (License 0099/01). All the cultures were maintained at 37 °C in an atmosphere of 5 % CO_2, and the assays were run at least three times in duplicate. In order to rule out toxic effects of the compounds on mammalian host cells, uninfected CMs were incubated for 24 h at 37 °C in presence or absence of the compounds (up to 200 μM) diluted in DMEM, then their morphology evaluated by light microscopy and the cell viability measured by the MTT colorimetric assay (Mosmann 1983). The absorbance was measured at 490 nm wavelength with a spectrophotometer (VERSAmax tunable, Molecular Devices, USA) allowing the determination of LC_{50} values (drug concentration that reduces 50 % of cellular viability) and the respective selective indexes ($SI = LC_{50}/IC_{50}$).

DNA binding studies

The interaction of complexes **1**–**6** with CT DNA has been studied with UV spectroscopy in order to investigate the possible binding modes to CT DNA and to calculate the binding constants (K_b). In UV titration experiments, the spectra of each compound in the presence of CT DNA have been recorded for a constant compound concentration in diverse [compound]/[CT DNA] mixing ratio (r). K_b values were obtained by monitoring absorbance changes in the compounds spectra at the corresponding λ_{max} (287, 288, 272, 288, 291, and 272 nm for complexes **1**, **2**, **3**, **4**, **5** and **6**, respectively), with increasing concentrations of CT DNA. K_b is given by the ratio of slope to the y intercept in plots [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA], according to the Eq. 1 (Pyle et al. 1989):

$$\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)_q} + \frac{1}{[K_b(\varepsilon_b - \varepsilon_f)]} \quad (1)$$

where [DNA] is the concentration of DNA in base pairs, ε_a, ε_f and ε_b correspond to the apparent absorption coefficient A_{obsd}/[compound], the extinction coefficient for the free compound and the extinction coefficient for the compound in the fully bound form, respectively.

Results and discussion

Microanalyses and molar conductivity studies

Microanalyses suggest the formation of [$CuCl_2$(H_2O)(LEV)] (**1**), [CuCl(bipy)(LEV)]Cl (**2**), [$CuCl_2$(phen)(LEV)] (**3**), [$CuCl_2$(H_2O)(SPAR)] (**4**), [CuCl(bipy)(SPAR)]Cl (**5**) and [$CuCl_2$(phen)(SPAR)] (**6**) in which the fluoroquinolones LEV and SPAR coordinate as bidentate ligands. The molar conductivity data reveal that the complexes (**1**), (**3**), (**4**) and (**6**) are non-electrolytes and that complexes (**2**) and (**5**) are 1:1 electrolytes, in accordance with the proposed formulations.

Infrared spectral studies

In the IR spectra of LEV and SPAR the valence vibration of the carboxylic stretch $\nu(C{=}O)_{carb}$ was found at 1,725 and 1,716 cm^{-1}, respectively, and the pyridone stretch $\nu(C{=}O)_p$ at 1,620 and 1,641 cm^{-1}, respectively (Efthimiadou et al. 2006). The characterization of metal–quinolone complexes can be achieved by studying the most typical vibrations that are characteristic of the coordination type of quinolones. In the IR spectra of the complexes **1**–**6** the absorption of the $\nu(C{=}O)_{carb}$ vibration has disappeared. Two very strong characteristic bands are present in the 1,611–1,636 and 1,344–1,395 cm^{-1} range assigned to ν(O–C–O) asymmetric and symmetric stretching vibrations, respectively, whereas $\nu(C{=}O)_p$ is shifted from 1,620 and 1,641 to 1,522–1,592 cm^{-1} range upon coordination.

The difference $\Delta = \nu(O{-}C{-}O)_{asym} - \nu(O{-}C{-}O)_{sym}$ is a useful characteristic for determining the coordination mode of the quinolone ligands. The Δ values for complexes **1**–**6** fall in the 227–286 cm^{-1} range, indicating a monodentate coordination mode of

the carboxylate group of the LEV and SPAR ligands (Deacon and Phillips 1980). The overall changes of the IR spectra suggest that the LEV and SPAR ligands are coordinated to Cu(II) via the pyridone and one carboxylate oxygen in the neutral zwitterionic form.

EPR spectral properties of the Cu(II) complexes

Room temperature EPR powder spectra

Room temperature X-band EPR spectra of the powdered samples were obtained (Fig. 2; parameters in Table 1). The EPR spectra of **1** and **4** are characteristic of axial symmetry, with $g_{\|}$ and $g_{\perp}$ presented in Table 1, and lack the hyperfine splitting as frequently observed in concentrated solid samples of Cu(II) complexes where exchange interactions are present (Hathaway and Billing 1970).

Complex **2** presents a well resolved doublet at the perpendicular region of the spectrum, associated with the triplet state ($S = 1$, $\Delta m_s = \pm 1$) of binuclear complexes (Weil et al. 1993; Singh et al. 2010). At the parallel region a seven-line hyperfine structure also appears. The much weaker half-field resonance from the forbidden transitions $\Delta m_s = \pm 2$ also exhibits the seven line hyperfine structure ($2I + 1$, from the two copper nuclei $I = 3/2 + 3/2$), with an average hyperfine splitting of $A = 90$ G (inset in Fig. 2b), which confirms the presence of a binuclear complex. In this case, there was no contribution from mononuclear complex. On the other hand, the room temperature EPR spectrum of complex **5** is a superposition of binuclear and mononuclear Cu(II) complexes.

The distance between the two Cu(II) ions can be estimated from the zero field splitting parameter D. The average distance r between the two coupled unpaired electrons can be calculated by using the equation $D = 3/2\ g\beta/r^3 = 1.39 \times 10^4\ (g/r^3)$, where D is in Gauss and r is in Angstrom (Eaton et al. 1983). The estimated distances r for the binuclear complexes are 4.1 Å (**2**) and 4.5 Å (**5**).

The room temperature EPR spectra of complexes **3** and **6** also suggest the presence of two components. The mononuclear component of **3** lacks the hyperfine splitting at the parallel region but **6** has a hyperfine splitting constant $A_{\|} \sim 130$ G. Besides these, wide components that can be due to binuclear complexes are present.

EPR spectra of aqueous and DMSO solutions frozen at 77 K

The EPR spectra of aqueous and DMSO solutions of the complexes were obtained at 77 K. Aqueous solutions produced very wide spectra (Fig. 3), because ice crystals segregate the paramagnetic species and cause cluster formation with strong dipole–dipole interactions. In one experiment, glycerol was added as glass-forming agent and the EPR spectrum became similar to that in DMSO, but since glycerol can act as a

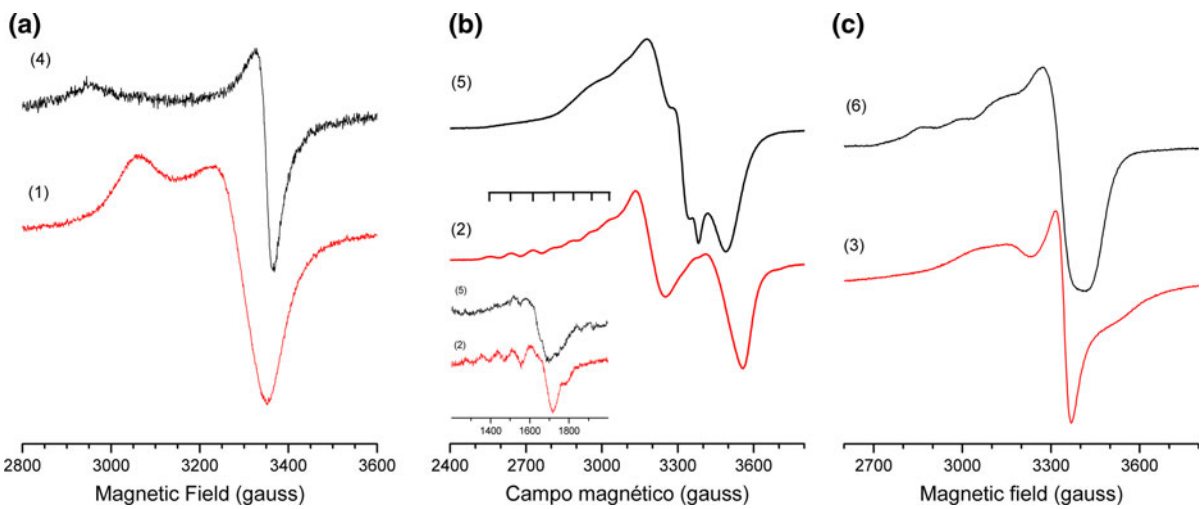

Fig. 2 Solid state powder EPR spectra (X-band), at room temperature, of **a** $[CuCl_2(H_2O)(LEV)]$ (**1**) and $[CuCl_2(H_2O)(SPAR)]$ (**4**), **b** [CuCl(bipy)(LEV)]Cl (**2**) and [CuCl(bipy)(SPAR)]Cl (**5**), and **c** $[CuCl_2(phen)(LEV)]$ (**3**) and $[CuCl_2(phen)(SPAR)]$ (**6**)

Table 1 EPR parameters of the Cu(II) complexes (powder at room temperature)

	$g_{\perp}$	$g_{\parallel}$	$A_{\parallel}$ (G)	g_{binucl}	D	A_{binucl} (G)
[$CuCl_2$(phen)]	2.074	2.292	155	–		
[$CuCl_2$(H_2O)(LEV)] (**1**)	2.133	2.274	0			
[$CuCl_2$(H_2O)(SPAR)] (**4**)	2.082	2.360	0			
[CuCl(bipy)(LEV)]Cl (**2**)	–	–	–	2.08	425	75
[CuCl(bipy)(SPAR)]Cl (**5**)	2.087	2.36	0	2.08	320	–
[$CuCl_2$(phen)(LEV)] (**3**)	2.08	2.28	0	Very wide		
[$CuCl_2$(phen)(SPAR)] (**6**)	2.08	2.28	130			

ligand, it can modify the aqueous environment of the complex. In aqueous solutions, complexes showed little evidence of binuclear complexes, excepting **3**, which exhibited the doublet with a zero-field splitting constant D equal to 475 G (see the arrows in Fig. 3c).

In DMSO solutions the EPR spectral components are much more defined, because there is no aggregation upon freezing (Fig. 4). All the spectra represent mixtures of mononuclear and binuclear complexes. The half-field lines due to the forbidden transitions $\Delta m_s = \pm 2$ were detected in these complexes (see insets in Fig. 4). The first derivative central line of the spectrum at $g \sim 2.08$ is assigned to the $g_{\perp}$ component of mononuclear Cu(II). The $g_{\parallel}$ component is around $g \approx 2.2$, and part of the expected fourfold hyperfine splitting is clearly seen in Fig. 4 for complexes **2** and **5**, but is less clear for **3** and **6**.

The estimated distances *r* for the binuclear complexes in DMSO are 3.9 Å for **2** and **3**, and 4.0 Å, for **5** and **6**. These distances are similar to those obtained for others Cu(II) dinuclear complexes (Batista et al. 2011; Saha et al. 2004; Psomas et al. 2006).

Anti-*T. cruzi* activity

Table 2 shows the effect of all the evaluated compounds and of Bz, the reference drug, against bloodstream trypomastigote forms of *T. cruzi* (Y strain) expressed as IC_{50}, as well as their corresponding selectivity index (SI, ratio between LC_{50} and IC_{50} values).

LEV and SPAR exerted a low trypanocidal effect against bloodstream trypomastigotes, exhibiting IC_{50} values of 211 and 114 μM, respectively. The association of the Cu(II) ion with LEV and SPAR led to an increase of the trypanocidal activity. Complexes [$CuCl_2$(H_2O)(LEV)] (**1**) and [$CuCl_2$(H_2O)(SPAR)] (**4**) are more active than the correspondent free ligands

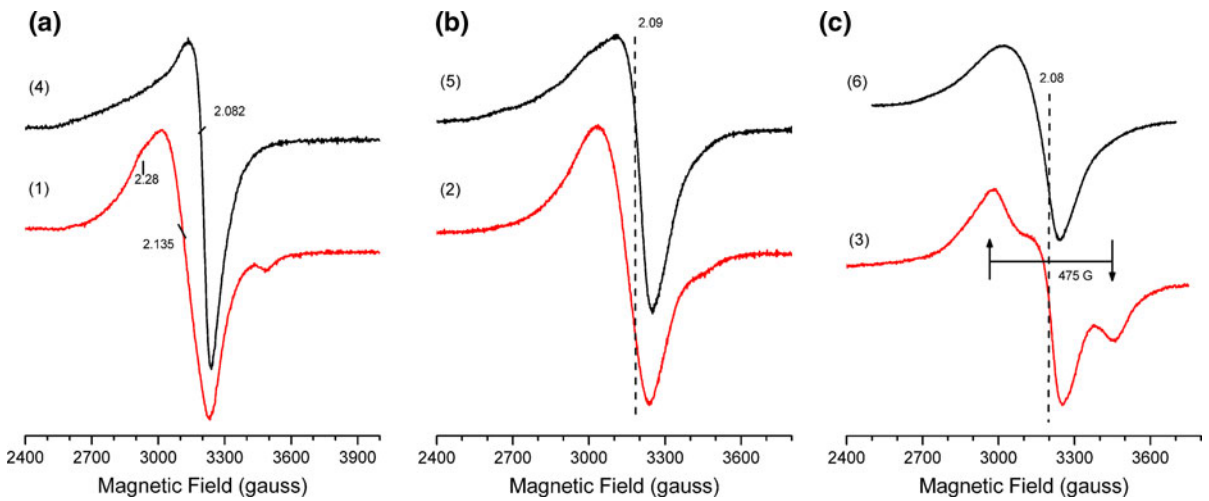

Fig. 3 EPR spectra (X-band) in aqueous solutions at 77 K of **a** [$CuCl_2$(H_2O)(LEV)] (**1**) and [$CuCl_2$(H_2O)(SPAR)] (**4**), **b** [CuCl(bipy)(LEV)]Cl (**2**) and [CuCl(bipy)(SPAR)]Cl (**5**), and **c** [$CuCl_2$(phen)(LEV)] (**3**) and [$CuCl_2$(phen)(SPAR)] (**6**)

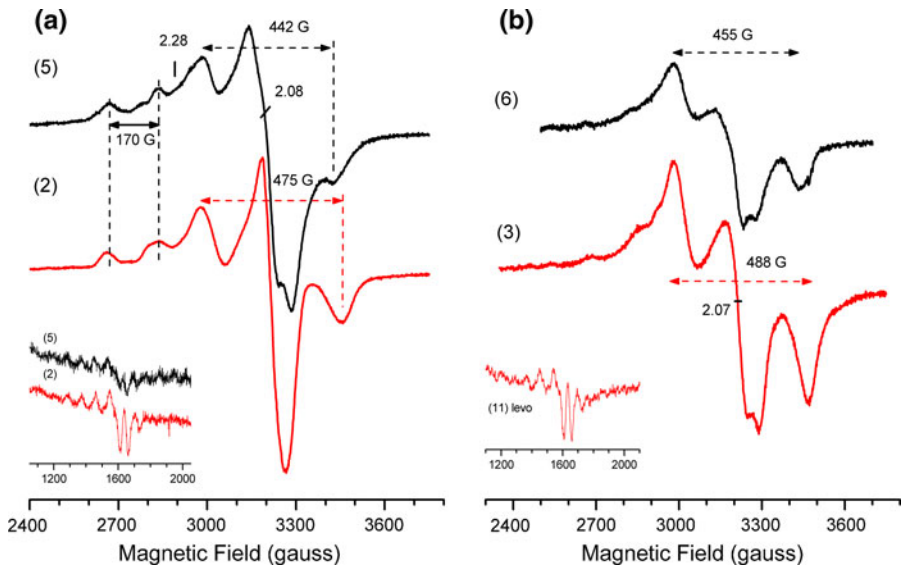

Fig. 4 EPR spectra (X-band) in DMSO solutions at 77 K of **a** [CuCl(bipy)(LEV)]Cl (**2**) and [CuCl(bipy)(SPAR)]Cl (**5**) and **b** $[CuCl_2(phen)(LEV)]$ (**3**) and $[CuCl_2(phen)(SPAR)]$ (**6**)

Table 2 Activity (mean ± SD) and selectivity index (SI) of the compounds and of benznidazole (Bz) upon bloodstream trypomastigotes (BT) forms of *T. cruzi* (Y strain), in vitro (24 h of incubation at 37 °C)

Compound	IC_{50} (μM)	Selectivity index (SI)
Levofloxacin (LEV)	211 ± 38	nd
$[CuCl_2(H_2O)(LEV)]$ (**1**)	60 ± 9	nd
[CuCl(bipy)(LEV)]Cl (**2**)	15 ± 3	4.18
$[CuCl_2(phen)(LEV)]$ (**3**)	1.6 ± 1.4	19
Sparfloxacin (SPAR)	114 ± 20	>1.75
$[CuCl_2(H_2O)(SPAR)]$ (**4**)	45 ± 30	nd
[CuCl(bipy)(SPAR)]Cl (**5**)	18 ± 4	3.4
$[CuCl_2(phen)(SPAR)]$ (**6**)	4.7 ± 0.1	2.86
$CuCl_2$	83 ± 3	6
$[CuCl_2(bipy)]$	134 ± 7	4.5
$[CuCl_2(fen)]$	7 ± 5	<4
Benznidazole	13 ± 2	77

SD standard deviation of multiple experimental measurements, *SI* selective index: ratio between LC_{50}/IC_{50} values, LC_{50} drug concentration that reduces 50 % the viability of mammalian cell, IC_{50} drug concentration that reduces 50 % of the number of the parasites, *nd* not done

and the $CuCl_2{\cdot}2H_2O$ salt, exhibiting IC_{50} values of 59.7 and 45 μM, respectively.

The association of 2,2′-bipyridine (bipy) with Cu(II) and LEV and SPAR improves the anti-*T. cruzi* activity. Complexes $[CuCl_2(bipy)(LEV)]$ (**2**) and $[CuCl_2(bipy)(SPAR)]$ (**5**) exhibit IC_{50} values of 14.9 and 18.2 μM, respectively. However, the precursor $[CuCl_2(bipy)]$ exhibits a IC_{50} value of the same order, $IC_{50} = 13.8$ μM.

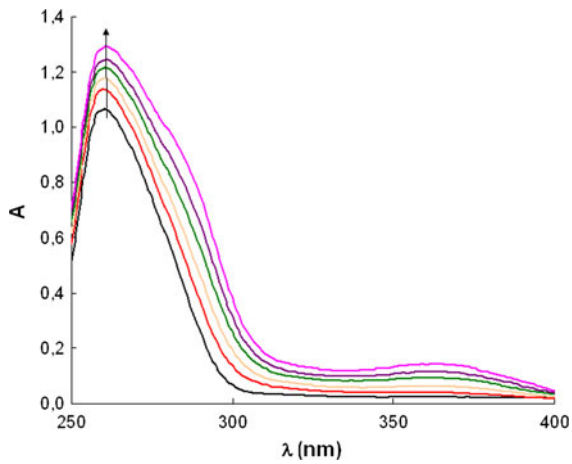

Fig. 5 UV spectra of CT DNA in buffer solution, pH 7.4, in the absence and in the presence of $[CuCl_2(H_2O)(SPAR)]$ (**4**). The *arrow* shows the changes upon increasing amounts of complex

Complexes $[CuCl_2(phen)(LEV)]$ (**3**) and $[CuCl_2(phen)(SPAR)]$ (**6**) were revealed to be the most active among all studied compounds, exhibiting IC_{50} values of 1.6 and 4.7 μM, respectively, while the precursor $[CuCl_2(phen)]$ exhibits a IC_{50} value of 7.2 μM.

[$CuCl_2$(phen)(LEV)] (**3**) is 36 times more active than complex (**1**), 9 times more active than complex (**2**) and 8 times more active than Bz, being the most promising anti-*T. cruzi* agent. Moreover, its SI is the highest one (SI = 19). So, the association of the LEV and SPAR with Cu(II) and 1,10-phenanthroline (phen) in one same complex proved to be a good strategy of activity improvement.

Interaction with DNA

Metal–quinolone complexes can bind to DNA via noncovalent interactions: intercalative, electrostatic and groove binding (Zeglis et al. 2007; Chao et al. 2002). Changes in the UV spectra of the complexes give evidences of the existing interaction and its mode (Hathaway and Billing 1970). In general hyperchromism means the breakage of the secondary structure of DNA and hypochromism shows that binding of complex to DNA can be due to electrostatic effect or intercalation which may stabilize the DNA duplex (Chao et al. 2002).

UV spectra of CT DNA in the presence of complexes **1**–**6** are in Fig. 5. The intensity of the band at λ_{max} = 258 nm increases for all compounds, indicating that the interaction with CT DNA results in the

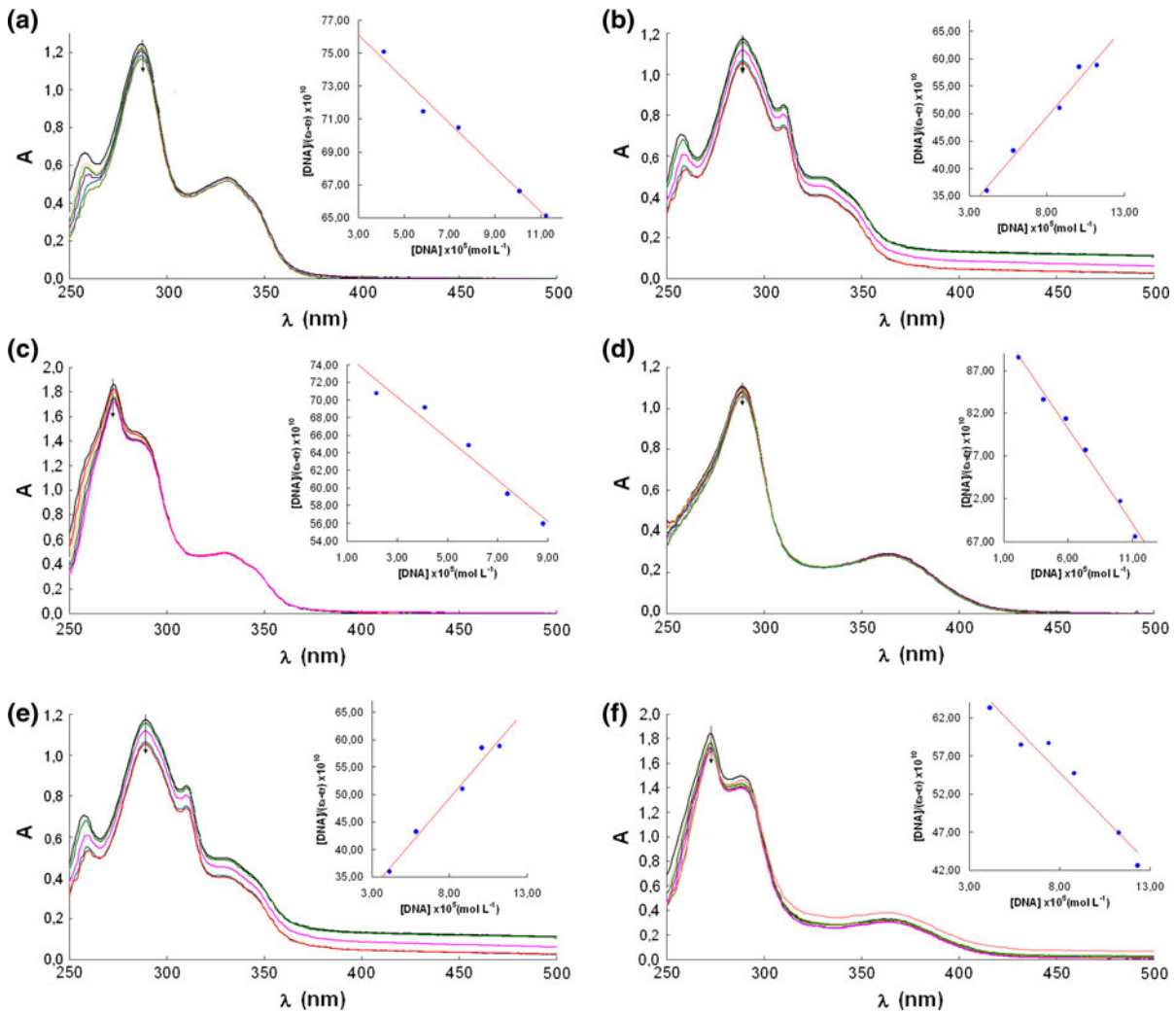

Fig. 6 UV spectra of **a** [$CuCl_2(H_2O)$(LEV)] (**1**), **b** [CuCl(bipy)(LEV)]Cl (**2**), **c** [$CuCl_2$(phen)(LEV)] (**3**), **d** [$CuCl_2(H_2O)$(SPAR)] (**4**), **e** [CuCl(bipy)(SPAR)]Cl (**5**) and **f** [$CuCl_2$(phen)(SPAR)] (**6**) in buffer solution, pH 7.4, in the absence and in the presence of CT DNA at increasing amounts. Inset: plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA]. The *arrows* show the changes upon increasing amounts of CT DNA

direct formation of a new complex with double-helical CT DNA (Efthimiadou et al. 2010; Katsarou et al. 2008; Son et al. 1998).

The changes observed in the UV spectra of complexes **1–6** after addition of increasing amounts of DNA are shown in Fig. 6. The initial spectrum refers to free complex in the absence of CT DNA. In the UV region the intense absorption bands observed in the spectra of the Cu(II) complexes can be attributed to the intra-ligand transitions of LEV and SPAR. Any interaction between each complex and CT DNA could perturb the intra-ligand bands of the complex (Long and Barton 1990).

In the UV-spectra of complexes **1–6** no appreciable changes in the position of the intra-ligand band centred in the 272–291 nm range were observed upon addition of CT DNA. However, the intensity of these bands decrease, resulting in hypochromism (see Fig. 6), and suggesting binding to CT DNA, maybe through intercalation.

The K_b values of Cu(II) complexes are similar ($\sim 10^3$ M^{-1}) and suggest a moderated binding to CT DNA.

Conclusions

The developed Cu(II) complexes (**1–6**) present a significant trypanocidal activity in vitro against *T. cruzi*, the causative agent of Chagas disease. The strategy of joining in one same complex LEV or SPAR with Cu(II) and 1,10-phenanthroline (phen) proved to be a good strategy of activity improvement. It must be pointed out that previous data from our group had already demonstrated the activity of novel complexes of $MnCl_2$, $CoCl_2$ and [$CuCl_2$(phen)] coordinated with NOR and SPAR against *T. cruzi* (Batista et al. 2011) indicating the promising trypanocidal effect of this class of compounds.

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References

Anquetin G, Greiner J, Mahmoudi N, Santillana-Hayat M, Gozalbes R, Farhati K, Tripathi KD, Sharma AK, Valecha N, Biswas S (1993) In vitro activity of fluoroquinolones against chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum*. Indian J Malariol 30:67–73

Batista DGJ, Pacheco MGO, Kumar A, Branowska D, Ismail MA, Hu L, Boykin DW, Soeiro MNC (2010) Biological, ultrastructural effect and subcellular localization of aromatic diamidines in *Trypanosoma cruzi*. Parasitology 137: 251–259

Batista DGJ, Silva PB, Stivanin L, Lachter DR, Silva RS, Felcman J, Louro SRW, Teixeira LR, Soeiro MNC (2011) Co(II), Mn(II) and Cu(II) complexes of fluoroquinolones: synthesis, spectroscopical studies and biological evaluation against *Trypanosoma cruzi*. Polyhedron 30:1718–1725

Castora FJ, Vissering FF, Simpson MV (1983) The effect of bacterial DNA gyrase inhibitors on DNA synthesis in mammalian mitochondria. Biochim Biophys Acta 740: 417–427

Chao H, Mei W, Huang Q, Ji L (2002) DNA binding studies of ruthenium(II) complexes containing asymmetric tridentate ligands. J Inorg Biochem 92:165–170

Chollet C, Baliani A, Wong PE, Barrett MP, Gilbert IH (2009) Targeted delivery of compounds to *Trypanosoma brucei* using the melamine motif. Bioorg Med Chem 17:2512–2523

Cortázar TM, Coombs GH, Walker J (2007) *Leishmania panamensis*: comparative inhibition of nuclear DNA topoisomerase II enzymes from promastigotes and human macrophages reveals anti-parasite selectivity of fluoroquinolones, flavonoids and pentamidine. Exp Parasitol 116: 475–482

Coura JR, Castro SL (2002) A critical review on chagas disease chemotherapy. Mem Inst Oswaldo Cruz 97:3–24

Coura JR, Dias JC (2009) Epidemiology, control and surveillance of Chagas disease: 100 years after its discovery. Mem Inst Oswaldo Cruz 104:31–40

Daliry A, Da Silva PB, Da Silva CF, Batista MM, Castro SL, Tidwell RR, Soeiro MNC (2009) In vitro analyses of the effect of aromatic diamidines upon *Trypanosoma cruzi*. J Antimicrob Chemother 64:747–750

Deacon GB, Phillips RJ (1980) Relationships between the carbon-oxygen stretching frequencies of carboxylato complexes and the type of carboxylate coordination. Coord Chem Rev 33:227–250

Eaton SS, More KM, Sawant BM, Eaton GR (1983) Use of the ESR half-field transition to determine the interspin distance and the orientation of the interspin vector in systems with two unpaired electrons. J Am Chem Soc 105:6560–6567

Efthimiadou EK, Sanakis Y, Raptopoulou CP, Karaliota A, Katsarosa N, Psomas G (2006) Crystal structure, spectroscopic, and biological study of the copper(II) complex with third-generation quinolone antibiotic sparfloxacin. Bioorg Med Chem Lett 16:3864–3867

Efthimiadou EK, Karaliota A, Psomas G (2010) Metal complexes of third-generation quinolone antimicrobial drug sparfloxacin: structure and biological evaluation. J Inorg Biochem 104:455–466

Gozalbes R, Brun-Pascaud M, Garcia-Domenech R, Galvez J, Girard PM, Doucet JP, Derouin F (2000) Anti-toxoplasma activities of 24 quinolones and fluoroquinolones in vitro: prediction of activity by molecular topology and virtual computational techniques. Antimicrob Agents Chemother 44:2771–2776

Hathaway BJ, Billing DE (1970) The electronic properties and stereochemistry of mono-nuclear complexes of the copper(II) ion. Coord Chem Rev 5:143–207

Katsarou ME, Efthimiadou EK, Psomas G, Karaliota A, Vourloumis D (2008) Novel copper(II) complex of N-propyl-norfloxacin and 1,10 phenanthroline with enhanced antileukemic and DNA nuclease activities. J Med Chem 51(3):470–478

Long EC, Barton JK (1990) On demonstrating DNA intercalation. Acc Chem Res 23:271–273

Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from micro-organisms. Mol Biol 3:208–218

Meirelles MN, Araujo-Jorge TC, Souza W (1982) Interaction of *Trypanosoma cruzi* with macrophages in vitro: dissociation of the attachment and internalization phases by low temperature and cytochalasin B. Z Parasitenkd 68:7–14

Meirelles MN, Araujo-Jorge TC, Miranda CF, Souza W, Barbosa HS (1986) Interaction of *Trypanosoma cruzi* with heart muscle cells: ultrastructural and cytochemical analysis of endocytic vacuole formation and effect upon myogenesis in vitro. Eur J Cell Biol 41:198–206

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–56

Nenortas E, Kulikowicz T, Burri C, Shapiro TA (2003) Antitrypanosomal activities of fluoroquinolones with pyrrolidinyl substitutions. Antimicrob Agents Chemother 47: 3015–3027

Perrin DD, Dempsey B (1974) Buffers for pH and metal ion control. Chapman and Hall, New York

Psomas G, Tarushi A, Efthimiadou EK, Sanakis Y, Raptopoulou CP, Katsaros N (2006) Synthesis, structure and biological activity of copper(II) complexes with oxolinic acid. J Inorg Biochem 100:1764–1773

Pyle AM, Rehmann JP, Meshoyrer R, Kumar CV, Turro NJ, Barton JK (1989) Mixed-ligand complexes of ruthenium(II): factors governing binding to DNA. J Am Chem Soc 111:3053–3063

Reichmann ME, Rice SA, Thomas CA, Doty P (1954) A further examination of the molecular weight and size of desoxypentose nucleic acid. J Am Chem Soc 76:3047–3053

Romero IC, Saravia NG, Walker J (2005) Selective action of fluoroquinolones against intracellular amastigotes of *Leishmania (Viannia) panamensis* in vitro. J Parasitol 91: 1474–1479

Saha DK, Sandbhor U, Shirisha K, Padhye S, Deobagkar D, Anson CE, Powell AK (2004) A novel mixed-ligand antimycobacterial dimeric copper complex of ciprofloxacin and phenanthroline. Bioorg Med Chem Lett 14:3027–3032

Sharma PC, Jain A, Jain S (2009) Fluoroquinolone antibacterials: a review on chemistry, microbiology and therapeutic prospects. Acta Pol Pharm 66:587–604

Singh LJ, Devi NS, Devi SP, Devi WB, Singh RKH, Rajeswari B, Kadam RM (2010) Spectroscopic studies on bis(1-amidino-O-alkylurea) copper(II) sulfate complexes where alkyl = methyl, ethyl, n-propyl or n-butyl: EPR evidence for binuclear complexes. Inorg Chem Commun 13: 365–368

Soeiro MNC, Castro SL (2011) Screening of potential anti-*Trypanosoma cruzi* candidates: in vitro and in vivo. Open Med Chem J 5:21–30

Son GS, Yeo JA, Kim MS, Kim SK, Holmem A, Akerman B, Nordin B (1998) Binding mode of norfloxacin to calf-thymus DNA. J Am Chem Soc 120:6451–6457

Weil JA, Bolton JR, Wertz JE (1993) Electron paramagnetic resonance, elementary theory and practical applications. Wiley, New York

Zeglis BM, Pierre VC, Barton JK (2007) Metallo-intercalators and metallo-insertors. Chem Commun 4565–4579