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Sulfonamide–metal complexes endowed with potent anti-*Trypanosoma cruzi* activity

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Abstract

In this article, we describe that mononuclear complexes composed of (5-chloro-2-hydroxybenzylidene)aminobenzenesulfonamides (**L1–3**) of general formula ($L_2(M)2H_2O$, where M is Co, Cu, Zn, Ni or Mn) reduced epimastigote proliferation and were found cidal for trypomastigotes of *Trypanosoma cruzi* Y strain. Complexes **C5** and **C11** have IC_{50} of 2.7 ± 0.27 and $4.8 \pm 0.47\,\mu M$, respectively, for trypomastigotes, when the positive control Nifurtimox, which is also an approved drug for Chagas disease, showed IC_{50} of $2.7 \pm 0.25\,\mu M$. We tested whether these complexes inhibit the enzyme *T. cruzi* trypanothione reductase or acting as DNA binders. While none of these complexes inhibited trypanothione reductase, we observed some degree of DNA binding, albeit less pronounced than observed for cisplatin in this assay. Unfortunately, most of these complexes were also toxic for mouse splenocytes. Along with the present studies, we discuss a number of interesting structure–activity relationships and chemical features for these metal complexes, including computational calculations.

Keywords

Cobalt, copper, DFT, metal complexes, prodrugs, sulfonamides, *Trypanosoma cruzi*

History

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Introduction

Trypanosoma cruzi, the parasitic protozoan agent of Chagas disease or American trypanosomiasis, affects about 17 million people, and around 100 million are at risk of infection across America[1]. *T. cruzi* infection initially leads to a generally mild acute phase, followed by a long but asymptomatic phase, while during the time it is estimated around 30% infected patients develop the symptomatic chronic phase of Chagas disease. It is characterized by the presence of myocarditis (often called chagasic cardiomyopathy) and in some cases, pathological disorders of the peripheral nervous and gastrointestinal systems[2,3]. Benznidazole is the only chemotherapeutic agent used for the treatment, but it has issues relating to toxicity and limited efficacy during the chronic phase of the disease[4]. As a consequence, it is essential to discover and identify novel therapies that could fight more aggressively as anti-*T. cruzi* agents.

A number of studies have proved the usefulness of metal complexes as an important chemical class of anti-*T. cruzi* agents[5]. The DNA structure was the first drug target recognized for anti-trypanosomal metal complexes[6], but other drug targets of *T. cruzi* were recently identified, such as the cysteine protease cruzain[7], trypanothione reductase[8], NADH-fumarate reductase[9] and also the biochemical path of nitric oxide[10]. In the search for new therapeutic agents, the incorporation of a metallic atom into anti-*T. cruzi* compounds can lead to metal complexes with a strong anti-trypanosomal effect on cell cultures[11,12]. From the structure–activity studies of these metal complexes, it appeared that increase in the hydrophobicity of the resulting complexes is the cause for the potency enhancement of the anti-trypanosomal effects[13–16].

In this paper, we report the evaluation of metal complexes bearing a sulfonamide moiety. We used the sulfonamides, denoted **L1–3** as the ligand to form metal complexation. We decided to use a sulfonamide group on **L1–3** because of the plenty of works highlighting their pharmacological potential as therapeutic agents[17,18]. For instance, sulfonamides are important structural component of the carbonic anhydrase inhibitors, which are important drug targets in widespread pathological conditions[19,20]. Besides, they undergo chemical transformations in physiological medium and therefore behaving as prodrugs[21].

We report here that while the metal-free ligands **L1–3** did not have potent trypanosomicidal activity, we noted the striking ability of the metal complexes in inhibiting epimastigote proliferation and showing toxicity for trypomastigotes of Y strain *T. cruzi*. A detailed computational study and attempt to elucidate the action mechanism were performed for these metal complexes. Emphasizing these studies could potentially offer further mechanism of drug designing in this significant area of research for the next generation of anti-*T. cruzi* metal complexes. For the accomplishment of this task, the sulfonamides and their metal-based (cobalt, copper, nickel or zinc) compounds were

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synthesized as described in Figures 1 and 2[22,23], by generating the respective mononuclear chelated complexes of metal:ligand ratio of 1:2, and having the general formula $[M(\mathbf{L1–3})_2(H_2O)_2]$.

All metallo-sulfonamides were evaluated against Y strain *T. cruzi*. We first measured cell viability of mouse splenocytes treated with metal-free ligand **L1–3** and their complexes, with determination of highest non-toxic concentration. Once the toxicity for mouse splenocytes was measured, we tested these compounds against epimastigotes (axenic culture) and for blood-stream trypomastigotes of Y strain *T. cruzi*, using Nifurtimox as a control drug. The ability of representative of these metallic compounds to inhibit the enzymatic activity of cruzain or trypanothione reductase of *T. cruzi* was also performed in parallel to the parasite cell assays. Table 1 summarizes the enzymatic, trypanosomicidal activity as well as the toxicity to splenocytes.

Nifurtimox (Nfx) displayed an IC_{50} value of 2.75 and 1.8 μM for trypomastigotes and epimastigotes, respectively. These IC_{50} values we found for Nifurtimox were reliable, since in our previous works, we have generally observed IC_{50} values below 5.0 μM[24]. The metal-free ligands **L1–3** were far less potent than Nifurtimox, at least fivefold.

Having ascertained the IC_{50} values for **L1–3** and Nifurtimox, we moved forward and tested the metal complexes. We found very promising trypanosomicidal activities for the metal complexes prepared from the sulfonamide compounds **L1–3**. The complexes **C1**, **C5**, **C7** and **C11** showed pronounced antiproliferative activity for the replicative form of epimastigote as well as toxicity for the infective form trypomastigote of Y strain *T. cruzi*. For example, complexes **C5** and **C11** have IC_{50} of 2.7 and 4.8 μM for trypomastigotes Y strain, when our positive control Nifurtimox showed IC_{50} of 2.7 μM. Although these complexes were less toxic for mouse splenocytes than gentian violet (positive control), significant toxicity for mouse splenocytes was observed for metal complexes when we compared to that observed for Nifurtimox (Table 1).

With the intention of broadening our understanding on the functional activity of metal complexes on *T. cruzi*, we selected and tested some of these compounds against the recombinant cysteine protease of *T. cruzi* cruzain as well as against *T. cruzi* trypanothione reductase. The complexes were used at concentrations of 50 and 100 μM in the enzymatic assays with trypanothione reductase and cruzain, respectively (data not shown). We did not observe any substantial inhibition of catalytic activity for these metal complexes neither for the metal-free ligand **L1** against trypanothione reductase and cruzain. We were surprised with these results, because these enzymes have been largely studied as important *T. cruzi* molecular targets[24,25] and their well-known metallic compounds are reported as inhibitors of these enzymes. Because of the findings in the literature, a possibility is that these compounds may inhibit *T. cruzi*-carbonic anhydrase.

Finally, we attempted to see whether these complexes bind to DNA. Using an UV-Vis-based titration assay[26], we observed that complexes **C3** and **C11** strongly interact with calf-thymus DNA in a time- and dose–response way (data not shown). But we cannot state that there is a clear correlation between DNA binding and trypanosomicidal activity, so we might suggest that the DNA pathway can be a way for further investigation of these trypanosomicidal complexes.

With the intent of broadening our understanding of anti-*T. cruzi* activity of metal complexes, we decided to perform an extensive work of structural characterization using theoretical calculation.To our knowledge, structural data of complexes **1–12**

Table 1. Antiprotozoal activity, toxicity and enzyme inhibition for the sulfonamides and their metal complexes.

Compounds	Metal	Y strain *T. cruzi* (IC_{50} μM) trypomastigotes*	epimastigotes†	Splenocytes (μM)‡	Trypanothione reductase inhibition (%)§
L1	–	20.4	32.9	10	NT
L2	–	39.2	20.7	25	NT
L3	–	65.8	>100	10	0
C1	Co	5.6	7.0	10	10 ± 2
C2	Ni	29.8	10.1	10	5
C3	Cu	10.5	1.1	<1.0	NT
C4	Zn	ND	ND	<1.0	NT
C5	Co	2.7	1.9	<1.0	5
C6	Ni	>100	>100	<1.0	0 ± 1
C7	Cu	5.9	10.8	<1.0	8 ± 0.5
C8	Zn	>100	>100	<1.0	NT
C9	Co	10.5	14.3	<1.0	NT
C10	Ni	45.8	59.8	<1.0	0 ± 1.5
C11	Cu	4.8	3.1	<1.0	0 ± 3
C12	Zn	ND	ND	<1.0	5 ± 3
Nfx	–	2.75	1.8	1.0	–
GV	–	–	–	0.01	–

Nfx, Nifurtimox; GV, gentian violet; ND, not determined, due to the lack of activity in the tested concentrations.

*After 24 h of incubation of cell cultures in the presence of the inhibitor. Values were calculated from seven concentrations using data obtained from at least two independent experiments (SD ± 10%).

†After 11 days of incubation of cell cultures in the presence of the inhibitor. Calculated from seven concentrations using data obtained from at least two independent experiments (SD ± 10%).

‡LC_{50} values were calculated for BALB/c splenocytes after 24 h of incubation of the inhibitor.

§Compounds were added at 50 μM.

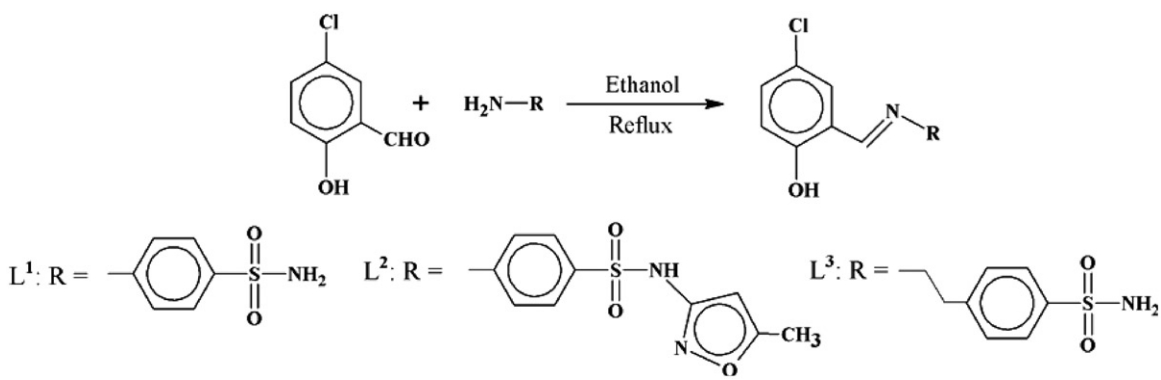

Figure 1. Preparation of ligands (**L1–3**).

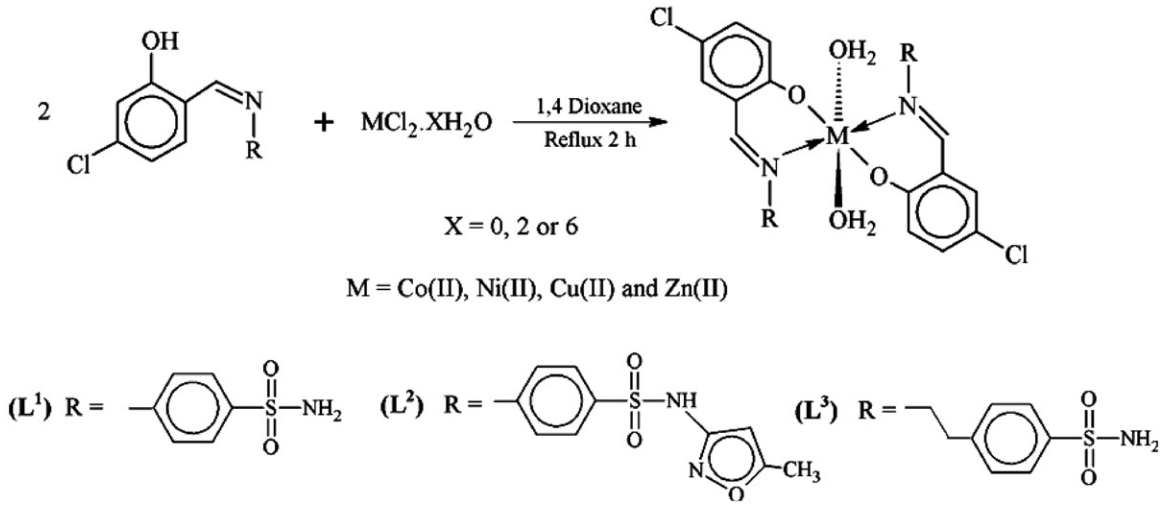

Figure 2. Proposed structure of the metal(II) complexes, **C1–12**.

Table 2. Calculated geometric and electronic parameters for complexes **C1–12**.

Complex	M–O1*	M–O2	M–N1	M–N2	M–W1	M–W2	*q* (M) NBO	*q* (M) APT	Spin (M)
1	1.981	1.999	2.217	2.212	2.240	2.272	1.40	1.44	2.68
2	2.001	2.013	2.145	2.142	2.177	2.199	1.41	1.31	1.67
3	1.950	1.953	2.082	2.073	2.553	2.660	1.31	1.23	0.58
4	2.022	2.037	2.260	2.244	2.244	2.285	1.68	1.51	
5	1.986	1.986	2.186	2.184	2.274	2.272	1.40	1.42	2.67
6	1.999	2.011	2.145	2.143	2.177	2.196	1.38	1.32	1.65
7	1.952	1.952	2.071	2.071	2.601	2.576	1.31	1.25	0.58
8	2.022	2.033	2.263	2.243	2.240	2.285	1.69	1.52	
9	2.010	2.004	2.155	2.163	2.303	2.274	1.41	1.47	2.68
10	2.029	2.022	2.106	2.108	2.223	2.199	1.38	1.33	1.65
11	1.957	1.971	2.058	2.047	3.045	2.484	1.29	1.23	0.57
12	2.058	2.044	2.194	2.177	2.319	2.288	1.68	1.56	

*Bond lengths are given in Angstrom (M = Co for **1**, **5** and **9**; Ni for **2**, **6** and **10**; Cu for **3**, **7** and **11**, and Zn for **4**, **8** and **12**). W1 and W2 are related to water molecules.

are absent in the literature. From our DFT calculations, complexes **1–12** were found to have approximate C_2 symmetry in which the metal centre (Co, Ni, Cu and Zn) is coordinated in a pseudo-octahedral fashion. In particular, the metal centre is located at a centre of inversion and coordinated by two chelating L^n ($n = 1$, 2 or 3) ligands in the equatorial plane and by two water molecules (hereafter W1 and W2) in the axial positions. The ligands coordinated to metal in their nonprotonated form leads to a six-membered chelation ring. In the equatorial plane, O atoms (N atoms) are *trans* to each other. A molecular representation of compounds **1–12** and the corresponding atom-labeling scheme are given in Figures 3 and 4. Some relevant bond distances around the metal centre are presented in Table 2. NBO and APT charges as well as spin densities on the metal centre are also given in Table 2.

Due to an approximate C_2 symmetry, the M–O1 and M–O2 bond distances are very similar to each other in all complexes. They differ by no more than 0.02 Å. A similar finding is observed for M–N1 and M–N2 bond distances. Nevertheless, differences of up to 0.1 Å are found when comparing M–W1 and M–W2 bond distances for a given complex. The M–O and M–N bond distances in complexes **1–12** are depend on the nature of the ligands (L^1, L^2 or L^3). For all complexes, M–O bond distances are found to be slightly shorter than the corresponding M–N bond. We also observed that, for all ligands, the M–O bond length increases in the following order: Cu–O < Co–O < Ni–O < Zn–O. The corresponding sequence for the M–N bond distances is Cu–O < Ni–O < Co–O < Zn–O. It can be observed that for a given metal, on passing from L^1 to L^2, the M–X (X = O or N) bond length is virtually unchanged. The only exception is observed for the Co–N bond, in which there is a decrease of 0.03 Å. In any case, in going from L^2 to L^3 the M–O bonds increase slightly while an opposite effect is observed for the M–N ones.

Main bond angles around the metal centre are listed in Table 3. We also inferred that in passing from L^1 to L^2, O1–M–N1 and N2–M–O2 bond angles do not change significantly. The largest increasing of about 1.7° is predicted for O2–Co–N2 bond angle. However, all chelation bond angles increased with the replacement of L2 by L3. The largest changing was predicted for O2–Zn–N2 angle (3.8°). Our calculations also reveal significant intramolecular hydrogen bonding between the hydrogen atom from the water molecule W1 and the oxygen atom O2 (W1H–O2) and other hydrogen bonding between the hydrogen atom from W2 and the oxygen atom O1 (W2H–O1).

We also calculated NPA and APT charges (Table 2). For a given ligand, APT charges on metal are found to increase monotonically in the order Cu < Ni < Co < Zn. NBO scheme predicts the same trend, but they gave a similar charge distribution for both Ni and Co centres.

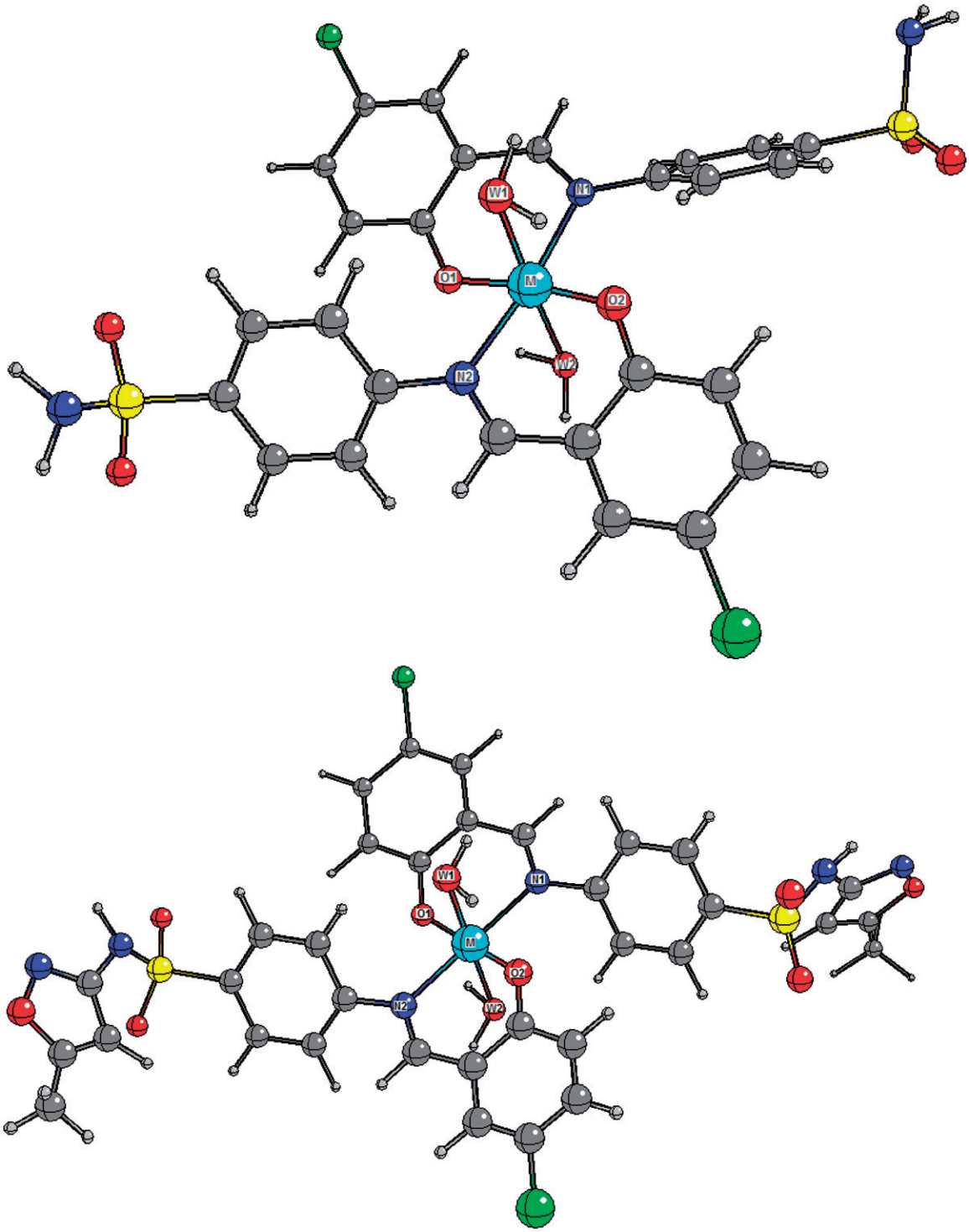

Figure 3. Top: Calculated molecular structure of complexes **C1–4**. Central ''M'' spheres represent metal (Co, Ni, Cu or Zn). W1 and W2 describe oxygen atoms belonging to water molecules. Bottom: Calculated molecular structure of complexes **C5–8**.

Experimental section

Synthesis: Ligands L1–3 and their metal complexes were previously prepared and fully characterized by spectral analysis[22,23].

Pharmacological evaluation

Parasites: Epimastigotes of *T. cruzi* (Y strain) were maintained at 26 °C in LIT medium (Liver Infusion Tryptose) supplemented with 10% fetal calf serum (FCS; Cultilab, Campinas, SP, Brazil), 1% hemin (Sigma Chemical Co., St. Louis, MO), 1% R9 medium, and 50 μg/mL of gentamycin (Novafarma, Anápolis, GO, Brazil). Bloodstream trypomastigotes forms of *T. cruzi* were obtained from supernatants of LLC-MK_2 cells previously infected and maintained in the RPMI-1640 medium (Sigma) supplemented with 10% FBS, 1% hemin, 1% R9 medium and 50 mg/mL gentamycin at 37 °C and 5% CO_2.

Cytotoxicity to mice splenocytes: BALB/c mice splenocytes were placed into 96-well plates at a cell density of 5×10^6 cells/well in the RPMI-1640 medium supplemented with 10% of FCS

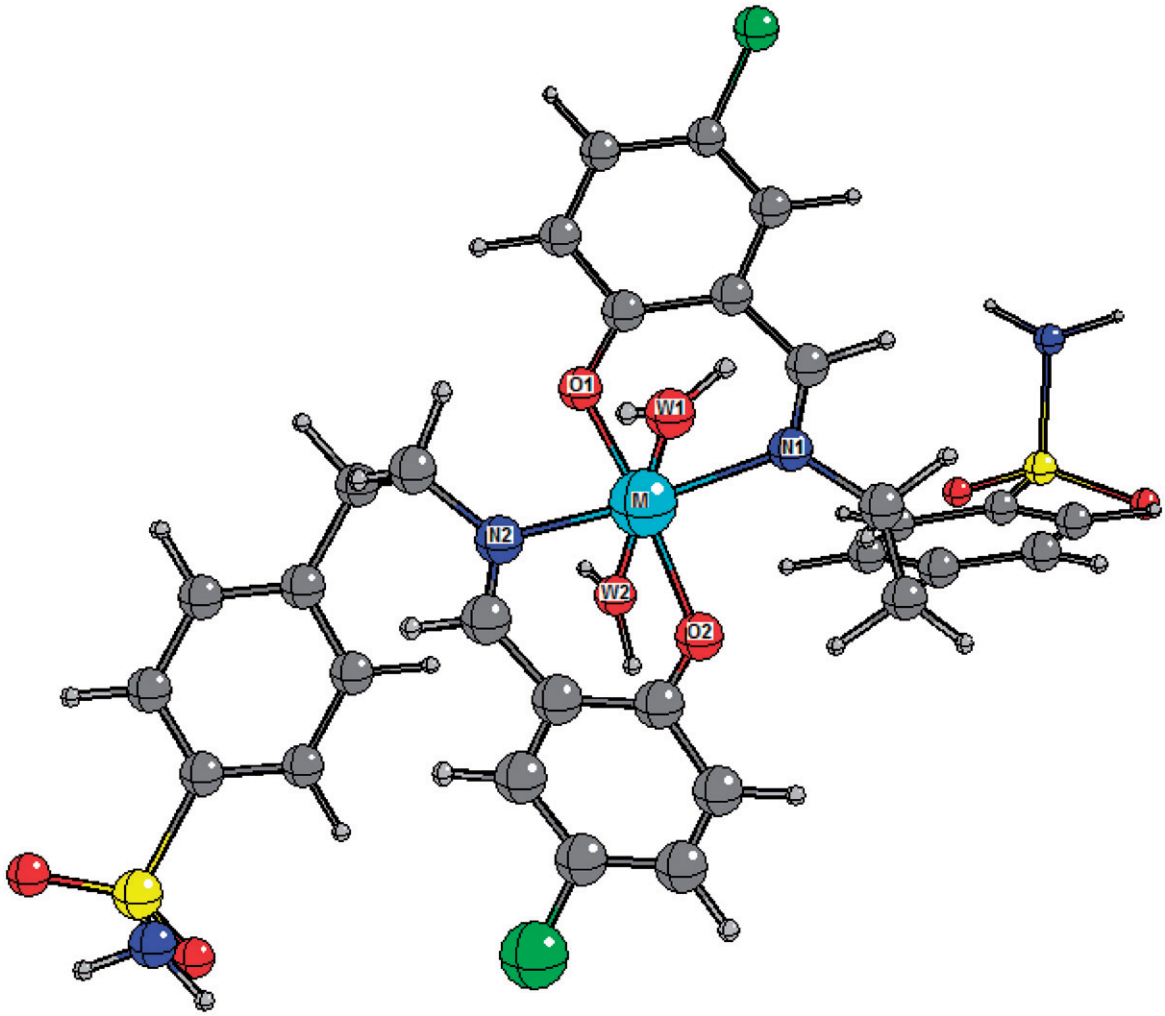

Figure 4. Calculated molecular structure of complexes **C9–12**.

Table 3. Calculated bond angles (°) and intramolecular hydrogen bonds (Å) for complexes **C1–12**.

Complex	O1–M–N1	O2–M–N2	O1–M–N2	O2–M–N1	W1–M–W2	O1–M–O2	N1–M–N2	W1H–O2	W2H–O1
1	86.2	84.5	90.5	98.8	180.0	174.8	176.6	2.033	2.078
2	84.4	86.5	90.2	95.9	179.0	176.6	178.6	1.982	2.014
3	90.2	88.1	85.5	92.2	177.5	177.4	178.9	1.962	1.990
4	85.5	83.3	90.1	101.1	177.7	173.3	173.6	1.981	2.021
5	86.2	86.2	97.7	93.9	179.6	179.8	179.9	2.048	2.035
6	88.4	86.5	90.1	95.0	179.2	176.7	178.5	2.003	2.030
7	88.8	89.0	99.9	91.2	179.5	180.0	179.2	1.987	1.978
8	85.6	83.2	89.9	101.4	177.6	172.9	173.2	1.995	2.041
9	87.1	87.6	93.9	91.5	178.7	177.3	171.6	2.022	1.935
10	88.6	88.7	91.5	91.2	178.2	178.5	177.8	1.986	1.912
11	90.6	89.6	88.6	90.8	169.4	173.6	176.6	2.035	1.933
12	86.6	87.0	93.7	92.8	177.1	177.6	178.5	1.980	1.908

and 50 μg/mL of gentamycin. Each test inhibitor was used in five concentrations (1.23, 3.70, 11.11, 33.33 and 100 μg/mL) in triplicate. To each well, an aliquot of test inhibitor suspended in DMSO was added, in addition to wells only containing either solvent (untreated cells) or gencian violet (drug control). Then, the plate was incubated for 24 h at 37 °C and 5% CO_2. After incubation, 1.0 μCi of ^{3}H-thymidine (Perkin Elmer, Waltham, MA) was added to each well, and the plate was returned to the incubator. The plate was then transferred to a beta-radiation counter (Multilabel Reader, Finland), and the percent of ^{3}H-thymidine was determined. Cell viability was measured as the percent of ^{3}H-thymidine incorporation for treated-cells in comparison to untreated cells.

Antiproliferative activity for epimastigotes: Epimastigotes were counted in a hemocytometric and then dispensed into 96-well plates at a cell density of 10^6 cells/well. Test inhibitors, dissolved in DMSO, were diluted into five different concentrations (1.23, 3.70, 11.11, 33.33 and 100 μg/mL) and added to the respective wells in triplicate. The plate was incubated for 11 days at 26 °C, and aliquots of each well were collected and the number

of viable parasites were counted in a Neubauer chamber, and compared to untreated parasite culture. IC_{50} calculation was carried out using a nonlinear regression on GraphPad Prism version 5.00 for Windows, GraphPad Software (San Diego, CA). This experiment was done in duplicate, and Nifurtimox (Nfx) was used as the reference inhibitor.

Toxicity for trypomastigotes: Trypomastigotes collected from the supernatants of LLC-MK_2 cells were dispensed into 96-well plates at a cell density of 4×10^5 cells/well. Test inhibitors, dissolved in DMSO, were diluted into five different concentrations and added into their respective wells, and the plate was incubated for 24 h at 37 °C and 5% of CO_2. Aliquots of each well were collected and the number of viable parasites, assessed as parasite motility, was counted in a Neubauer chamber. The percentage of inhibition was calculated in relation to untreated cultures. IC_{50} calculation was also carried out using a nonlinear regression on Prism 4.0 GraphPad software. This experiment was repeated once, and Nifurtimox (Nfx) was used as the reference inhibitor.

Inhibition of recombinant trypanothione reductase: The enzymatic activity was measured spectrophotometrically at 25 °C in protein assay buffer [40 mM N-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (Hepes), 1 mM EDTA, pH 7.5] as previously described[8]. Stock solutions of the complexes were dissolved in DMSO. The assay mixtures (1 mL) contained 100 mM NADPH, 100 mM of trypanothione disulfide (TS2) and various concentrations of the inhibitor. NADPH, enzyme and inhibitor were mixed. The reaction was started by adding TS2 and the absorption decrease at 340 nm due to NADPH consumption was followed. Control assays contained the respective amount of DMSO instead of inhibitor.

Computational calculations: Electronic structure calculations were performed using the Gaussian 03 quantum chemistry software[27]. DFT calculations were carried out using de Becke's three-parameters functional and the correlation function of Lee, Yang and Parr (B3LYP)[28,29]. A pseudo-potential LANL2DZ basis set[30] was used to describe the metallic centres, whereas all electron 6-31G(d,p) was used for main group elements. The corresponding valence basis sets for Co, Ni, Cu and Zn were augmented by an f-type polarization function with the coefficients $\xi = 2.780$, 3.130, 3.525 and 3.031, respectively[31,32]. All geometry optimizations were performed without any symmetry constraints. Stationary points were characterized by the calculation of vibrational frequencies. We calculated net atomic charges via two approaches: natural population analysis[33] and from the atomic polar tensor[34]. A MOLDRAW[33] program was used to draw the molecular structures[35].

Conclusion

Overall, we have demonstrated that the complexation of bioactive sulfonamides with transition metals leads to a new set of anti-*T. cruzi* agents with an attractive range of efficacy against the aforementioned parasite. Structure–activity relationships, chemical reactivity as well predicted geometry we determined here in details for complexes **C1–12** might be used for further structural design of complexes as well to explain the difference of pharmacological property between different used metals.

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Declaration of interest

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