http://dx.doi.org/10.5935/0103-5053.20150360

J. Braz. Chem. Soc., Vol. 27, No. 6, 1032-1039, 2016.
Printed in Brazil - ©2016 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Antiprotozoal Activity of the Cyclopalladated Complexes Against *Leishmania amazonensis* and *Trypanosoma cruzi*

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The present study describes the antiprotozoal activities of four cyclopalladated compounds, $[Pd(dmba)(\mu\text{-}Cl)]_2$, [Pd(dmba)(NCO)(isn)], $[Pd(dmba)(N_3)(isn)]$ and $[Pd(dmba)(\mu\text{-}NCO)]_2$, (dmba: *N,N'*-dimethylbenzylamine and isn: isonicotinamide), against the diseases leishmaniasis (*Leishmania amazonensis* and *Leishmania infantum*), Chagas disease (*Trypanosoma cruzi*) and human African trypanosomiasis (*Trypanosoma brucei*). $[Pd(dmba)(\mu\text{-}NCO)]_2$ exhibited good leishmanicidal and trypanocidal activities against *L. amazonensis* and *T. cruzi* intracellular amastigote forms, with a 50% inhibitory concentration (IC_{50}) value of less than 9 μM and selectivity indexes of 14.47 and 28.42, respectively. Stability essays were conducted in phosphate buffer saline (PBS) pH 7.0 and showed that $[Pd(dmba)(\mu\text{-}NCO)]_2$ is the most stable molecule. These findings indicate that this compound presented higher selectivity for these parasites than the other tested compounds. The data presented here suggest that this compound should be considered in the development of new and more potent drugs for the treatment of leishmaniasis and Chagas disease.

Keywords: cyclopalladated, leishmaniasis, Chagas disease, *Leishmania amazonensis*, *Trypanosoma cruzi*, trypanosomiasis

Introduction

Leishmaniasis, Chagas disease (American trypanosomiasis, AT) and sleeping sickness (human African trypanosomiasis, HAT) are parasitic diseases caused by flagellated protozoa related to the family Trypanosomatidae. According to the World Health Organization (WHO), 1.3 million new cases of leishmaniasis and 20,000 to 30,000 deaths occur annually in all continents,[1] 7 to 8 million people are infected worldwide with Chagas disease[2] and the estimated number of actual cases of HAT is currently 30,000.[3]

More than 20 species of *Leishmania*[4] are responsible for different clinical manifestations, including cutaneous (CL), mucocutaneous (MCL) and visceral forms (VL).[5] In the Old World, *Leishmania major*, *Leishmania tropica*, *Leishmania aethiopica* and some zymodemes from *Leishmania infantum* are the causative agents of CL. In the New World, CL is mainly caused by *Leishmania amazonensis*, *Leishmania guyanensis*, *Leishmania panamensis* and *Leishmania braziliensis*.[6] MCL can be caused by *L. braziliensis*, *L. panamensis*, *L. guyanensis* and *L. amazonensis* in the New World and by *L. major* and *L. infantum* in the Old World.[7] Pentavalent antimonial drugs are the most frequently prescribed treatments for leishmaniasis.[8] The main adverse effects that occur in systemic administration of these antimonials are arthralgias, myalgias, anorexia, and nausea, and other serious side effects include pancreatitis, liver-enzyme abnormalities, cardiac malfunctions and severe renal toxic effects.[9] Other drugs, such as amphotericin B, pentamidine and miltefosine, are second choice drugs but they also produce side effects that can endanger the patient's life.

Trypanosoma cruzi is the causative agent of Chagas disease, which is present mainly in Latin America but also in North America.[10-12] Nifurtimox and benznidazole are the only drugs available to treat patients in the acute phase of the disease.[13,14] However, these drugs are not

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effective against the chronic phase of the disease, and they present multiple side effects, from dermatitis to bone marrow depression.[15] HAT is caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*.[16] Treatment of the hemolymphatic stage of HAT relies on suramin and pentamidine. In the meningoencephalitis stage, melarsoprol, a highly toxic arsenic-based drug that is effective against both *T. b. gambiense* and *T. b. rhodesiense* is used. Eflornithine is useful only against *T. b. gambiense*. An eflornithine/nifurtimox combined therapy has been used, but this also causes several side effects.[17] In summary, the conventional drugs that have been employed to treat these diseases are antiquated, have high toxicity and are ineffective due to drug resistance. Therefore, is critical to develop new drugs for the treatment of these trypanosomiases.

The use of transition metal-based drugs is increasing significantly in the therapy of cancer and tropical diseases; in fact, many organometallic compounds have been designed to specifically bind to a well-defined target site in different biomolecules.[18-20] The emergence of drug resistance in tropical diseases has led to alternatives to chloroquine and its analogues, such as metallocene derivatives for the treatment of malaria.[19,20] Several scaffolds of metal complexes containing palladium, platinum, gold, iridium, rhodium, osmium and iron have shown leishmanicidal[18-21] and trypanocidal[22,23] activity. For palladium compounds, it was recently verified that a cyclopalladated complex showed a high selectivity index with trypanocidal activity in the treatment of Chagas disease.[24] Literature has reported a cyclopalladated compound with leishmanicidal activity[25] against *L. amazonensis* promastigote and amastigote forms; and the activity of some palladium(II) cyclometalated complexes[26] against *T. cruzi* and *Leishmania*, which indicate on preliminary data that the compounds inhibited the growth of intracellular amastigote forms.

Cyclopalladated complexes have high thermodynamic and kinetic stability compared to others palladium(II) compounds due to the formation of a stable chelate ring.[27] Based on the potential activity of the cyclopalladated complexes reported in the literature,[28-34] in this study, we describe the evaluation of the leishmanicidal and trypanocidal activity of $[Pd(dmba)(\mu\text{-}Cl)]_2$ (**1**), [Pd(dmba)(NCO)(isn)] (**2**), $[Pd(dmba)(N_3)(isn)]$ (**3**) and $[Pd(dmba)(\mu\text{-}NCO)]_2$ (**4**) (Figure 1), dmba: *N,N'*-dimethylbenzylamine and isn: isonicotinamide. All complexes have been previously described[35-38] but their protozoal activities have not been reported yet.

Herein we tested the above mentioned cyclopalladated complexes against *L. amazonensis*, *L. infantum*, *T. cruzi* and *T. brucei*.

Experimental

Infrared spectra (IR) were recorded on a Spectrum 2000, PerkinElmer, in the spectral range 4000-370 cm^{-1}. Samples were prepared in KBr pellets. ^{1}H and $^{13}C\{^{1}H\}$ nuclear magnetic resonance (NMR) spectra were referred to the high field $SiMe_4$ signal, on a INOVA 500 spectrometer; the magnetic field applied was 11.7 T and the resonance of ^{1}H and $^{13}C\{^{1}H\}$ nucleus were at 500 and 125 MHz, respectively. Full spectroscopic data are presented in the Supplementary Information section.

The elemental analysis was performed with an Elemental Analyzer 2400 CHN, PerkinElmer, at Central Analítica, Instituto de Química, USP, São Paulo, Brazil.

Synthesis of cyclopalladated complexes

All synthesis was carried out at room temperature. All reagents were obtained from commercial suppliers and used without further purification.

Compound (**1**), $[Pd(dmba)(\mu\text{-}Cl)]_2$ was prepared as previously described in the literature,[36,37] with some modifications. In summary, 22.6 mmol of LiCl (Carlo Erba) was added to a solution of 11.3 mmol of $PdCl_2$ (Degussa S. A.) in methanol. The mixture was stirred at 60 °C followed by the addition of 11.3 mmol of dmba (*N,N'*-dimethylbenzylamine, Sigma-Aldrich) and 14.4 mmol of triethylamine (Carlo Erba). After 6 h, the

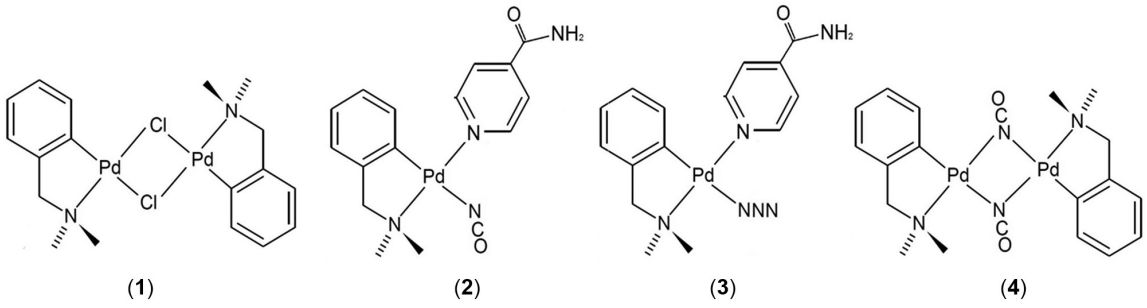

Figure 1. Structures of the cyclopalladated complexes (**1**)-(**4**).

yellow solid formed was filtered off, washed thoroughly with methanol and diethyl ether, and dried *in vacuo*. The yield was 90%; mp 185 °C (dec.); anal. calcd. for $C_{18}H_{24}N_2Cl_2Pd_2$: C 39.1, H 4.3, N 5.1; found: C 39.4, H 4.2, N 5.0; MW = 552.14.

Compound $[Pd(dmba)(\mu\text{-}NCO)]_2$ (**4**) and starting species, $[Pd(dmba)(\mu\text{-}N_3)]_2.H_2O$, for the synthesis of (**2**) and (**3**), were prepared based on Almeida *et al*.[35] by replacement of chlorido ligands in (**1**) by the corresponding pseudohalides. The reactions were carried out in acetone and the obtained yellow solids were filtered off, washed with water and pentane, and dried *in vacuo*. The yield was 79% for compound (**4**) and 87% for the precursor, mp 177 °C (dec.), mp 187 °C (dec.), respectively.

$[Pd(dmba)(\mu\text{-}NCO)]_2$: anal. calcd. for $C_{20}H_{24}N_4O_2Pd_2$: C 42.5, H 4.3, N 9.9; found: C 42.3, H 4.3, N 9.4; MW = 552.14.

$[Pd(dmba)(\mu\text{-}N_3)]_2.H_2O$: anal. calcd. for $C_{18}H_{24}N_8Pd_2.H_2O$: C 37.1, H 4.5, N 19.2; found: C 37.0, H 4.2, N 18.5; MW = 565.28.

Compounds of the type [Pd(dmba)X(isn)]; X = NCO (**2**), N_3 (**3**); isn: isonicotinamide, were prepared according to the literature,[35,38] with some modifications.

In summary, compounds (**2**) and (**3**) were obtained in acetone by reacting the suitable $[Pd(dmba)(\mu\text{-}X)]_2$ precursor with isn (Sigma-Aldrich). The obtained white solids were washed thoroughly with acetone and pentane. The yield was 90% for compound (**2**) and 94% for (**3**), mp 206 °C (dec.), mp 196 °C (dec.), respectively.

[Pd(dmba)(NCO)(isn)] anal. calcd. for $C_{16}H_{18}N_4O_2Pd$: C 47.5, H 4.5, N 13.8; found: C 47.3, H 4.3, N 13.0; MW = 404.76.

[Pd(dmba)(N_3)(isn)] anal. calcd. for $C_{15}H_{18}N_6OPd$: C 44.5, H 4.5, N 20.8; found: C 44.0, H 4.3, N 20.5; MW = 404.76.

Stability assay

The samples **1**- **4** were dissolved in acetonitrile (minimum amount to solubilize) and the solutions then prepared in phosphate buffer saline (PBS) pH 7.0 to give concentrations of 317, 312, 220 and 143 μmol L^{-1}. The solutions were prepared and the samples were left with occasional stirring at room temperature being analyzed aliquots of these solutions in the days immediately after the preparation (time 0) and after 12, 24, 48 and 72 hours. The experiments were performed in HPLC Shimazu LC-20AT CNM-20A UV detector SPD-20A using ODS (C-18) column, particle size 5 μm, 4.6 × 250 mm, mobile phase: methanol:water (70:30, v/v/v), flow 0.8 mL min^{-1} and λ = 254 nm. For more details see Supplementary Information section.

Biological assays

Compounds

Cyclopalladated complexes **1**, **2**, **3**, **4** and controls (pentamidine, amphotericin B and benznidazole (Sigma-Aldrich)) were dissolved in DMSO, dimethyl sulfoxide, (the highest concentration was 1.4%, which was not hazardous to the parasites, as previously determined), added to the parasite suspension to final concentrations between 0.5 and 100.0 μmol L^{-1}.

Parasites: *L. amazonensis*, *L. infantum*, *T. cruzi* and *T. brucei*

Promastigotes of *L. amazonensis* MPRO/BR/1972/M1841-LV-79 strain and epimastigote forms of *T. cruzi* Y strain[39] were maintained at 28 ± 2 ºC in liver-infusion tryptose medium (LIT)[40] supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco). Promastigotes of *L. infantum* (MHOM/BR/72) were freshly isolated from golden hamsters (*Mesocricetus auratus*) and maintained at 28 °C in M199 medium (Cultilab) without phenol red supplemented with 10% FBS (Gibco), penicillin (Sigma-Aldrich) and streptomycin (Sigma-Aldrich). Promastigote and epimastigote forms cultures were carried out until to obtain in the exponential growth phase (1.10^7 parasites mL^{-1}).

Procyclic forms of *T. brucei* 427 strains[41] were cultured at 28 ± 2 °C in SDM-79 medium[42] containing 10% FBS, penicillin (Sigma) and streptomycin (Sigma). Procyclic forms cultures were carried out until to obtain the exponential growth phase (1.10^6 parasites mL^{-1}).

Cytotoxicity using murine macrophages

To determine the cytotoxicity in murine macrophages we used a method previously described.[43] Adult male Swiss albino mice (20 to 35 g) were used in the experiments. They were housed in single-sex cages under a 12 h light/12 h dark cycle in a controlled-temperature room (22 ± 2 °C). The mice had free access to food and water. Groups of three animals were used in each test group. The experiments were performed in concordance to protocol approved by the Institutional Ethics Committee-CEUA (Comissão de Ética no Uso de Animais), protocol CEUA/FCF/CAr No. 20/2013. The mice were stimulated with thioglycolate to collect peritoneal macrophages. Murine peritoneal macrophages were seeded in 96-well flat-bottom plates (TPP) at a density of 1×10^5 cells *per* well in RPMI 1640 medium[44] supplemented with 10% heat-inactivated FBS, 25 mM HEPES, and 2 mM L glutamine and incubated for 4 h at 37 ± 2 °C in a 5% CO_2-air mixture. The medium was removed, and then new medium was added to the cells, which were treated with different

concentrations of compounds and controls. Cells without drugs were used as a negative control. After that, plates were incubated for 24 h at 37 ± 2 °C in a 5% CO_2-air mixture. Subsequently, the MTT (3-4(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) colorimetric assay was carried out as described further on. Absorbance was read in a 96-well plate reader (Robonik) at 595 nm. The drug concentration corresponding to 50% of cell growth inhibition was expressed as the inhibitory concentration (CC_{50}, μM).[45]

MTT colorimetric assay

L. amazonensis and *L. infantum* promastigote, *T. brucei* procyclic and *T. cruzi* epimastigote forms were treated with the cyclopalladated complexes and their respective controls to calculate the half maximal inhibitory concentration (IC_{50}) by MTT colorimetric assay.[46,47] The MTT assay is based on the determination of the ability of living cells to reduce 3-4(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT). To avoid interaction of compounds with the MTT, the cells were gently washed with PBS before the addition of MTT solution on the cells. The compounds were tested in different concentrations and incubated at 28 ± 2 °C for 72 h for *L. amazonensis*, *L. infantum* and *T. cruzi* and 24 h for *T. brucei*.[23,43] The assays were carried out in triplicates. Absorbance was read in a 96-well plate reader (Robonik) at 490 nm for *Leishmania* species and 595 nm for *T. cruzi* and *T. brucei*. The drug concentration corresponding to 50% parasite growth inhibition was expressed as the IC_{50} in μM.

The safety index (SI) was calculated and SI around 10 means that a compound can be better evaluate for further studies. Data obtained were processed with the software Origin 7.0.[48]

Leishmanicidal and trypanocidal activities against intracellular amastigote forms

To determine the leishmanicidal and trypanocidal activities against intracellular amastigote forms we used a method previously described.[43] Murine peritoneal macrophages were plated at 3 × 10^5 cells *per* well on coverslips (13 mm diameter) previously arranged in a 24 well plate in RPMI 1640 medium[44] supplemented with 10% inactivated FBS and allowed to adhere for 4 h at 37 ± 2 °C in 5% CO_2. Adherent macrophages were infected with *Leishmania* promastigotes in the stationary growth phase or *T. cruzi* trypomastigote using a ratio of 5/10:1 parasites *per* cell (*L. amazonensis* or *L. infantum*/*T. cruzi*: macrophages) at 37 °C in 5% CO_2 for 4 h for *L. amazonensis*, 18 h for *L. infantum* and 24 h for *T. cruzi*. After that time, the non-internalized parasites were removed by washing and infected cultures were incubated in RPMI 1640 medium for 24 h at 37 ± 2 °C in 5% CO_2 to allow parasite multiplication. Then, infected cells were treated with different concentrations of compounds, amphotericin B (Sigma-Aldrich), pentamidine (Sigma-Aldrich) or benznidazole (Sigma-Aldrich) for 24 h. The cells were then fixed in a methanol solution and stained with Giemsa. The number of amastigotes/100 macrophage cells and the percent of infected cells were determined. The concentration that caused a 50% decrease of growth inhibition compared to the control was determined by regression analysis and expressed as IC_{50} in μM.[45]

The experiments were performed in accordance with the protocol approved by the Institutional Ethics Committee-CEUA (Comissão de Ética no Uso de Animais), protocol CEUA/FCF/CAr No. 20/2013.

Differentiation of *T. cruzi* epimastigote to trypomastigote forms

In vitro differentiation of *T. cruzi* was made as previously described in literature.[49,50] The epimastigote forms were collected from the LIT culture on the 7th day when the plateau phase of the growth curve was reached and then, re-suspended in artificial triatomine urine (TAU) (190 mmol L^{-1} NaCI, 8 mmol L^{-1} phosphate buffer, pH 6.0, 17 mmol L^{-1} KCl, 2 mmol L^{-1} $CaCI_2$, 2 mmol L^{-1} $MgCI_2$), and incubated for 2 h at room temperature. The parasites were diluted to a final concentration of 5 × 10^6 parasites mL^{-1} in TAU supplemented with 2.5% (v/v) sodium bicarbonate 1.4%, 500 units penicillin mL^{-1}, 10 mmol L^{-1} proline (TAUP medium) and incubated at 27 °C for 10 days.

Results and Discussion

Compounds **1**-**4** (Figure 1) were obtained as stable solids (yields ranging from 79% to 94%), and their structures are fully supported by infrared spectroscopy (IR) and elemental analysis.

The cyclopalladated complexes were tested against *L. amazonensis*, *L. infantum* (promastigote and amastigote), *T. cruzi* (epimastigote and amastigote) and *T. brucei* (procyclic forms) using the MTT colorimetric assay[44-46] and an *in vitro* intracellular amastigote assay[43,49,50] to evaluate the antiprotozoal activity. Amphotericin B and pentamidine were included in the assays as positive controls for *Leishmania* species, benznidazole for *T. cruzi* and pentamidine for *T. brucei*. The MTT assays were carried out after 72 h (*L. amazonensis*, *L. infantum* and *T. cruzi*) and 24 h (*T. brucei*) of incubation. In general, all cyclopalladated complexes showed variable leishmanicidal and trypanocidal activity.

The antiprotozoal effect of the cyclopalladated complexes on intracellular amastigotes was evaluated in murine peritoneal macrophages obtained from adult male Swiss albino mice infected with *Leishmania* species promastigote forms and *T. cruzi* trypomastigote forms. We observed a decrease in the amount of intracellular amastigote forms after treatment with the cyclopalladated complexes. A cytotoxicity assay was also carried out to evaluate the toxicity of the compounds in the peritoneal macrophages, showing lower toxic activity on mammalian cells. All data are presented in Table 1.

The ligands dmba and isn by themselves did not exhibit antiprotozoal activities (Table 1), suggesting that the observed biological activity was due to the cyclopalladated complexes. In the present study, we observed that compound **2** showed *in vitro* activity against *T. cruzi* amastigotes (IC_{50} = 4.45 ± 0.45 μM), while compound **4** showed activity against both *L. amazonensis* (IC_{50} = 9.29 ± 2.08 μM) and *T. cruzi* amastigotes (IC_{50} = 4.73 ± 1.19 μM). It is interesting to note that for *L. amazonensis* amastigote, compound **4** showed almost 3 times higher leishmanicidal activity compared to compounds **2** (IC_{50} = 22.45 ± 1.17 μM) and **3** (IC_{50} = 28.82 ± 2.15 μM) (Table 1), suggesting that the isn group complexed to Pd decreases the anti-*Leishmania* activity but does not influence the anti-*T. cruzi* activity.

The replacement of chlorido by the cyanato ligand (**1** → **4**) did not result in any increase in leishmanicidal activity against the promastigote and amastigote forms; however compound **1** was 2 times more active than compound **4** against the *T. cruzi* epimastigote. On the other hand, different efficacy was observed in the *T. cruzi* amastigote form, where compound **4** was approximately 3 times more active than compound **1**. The presence of

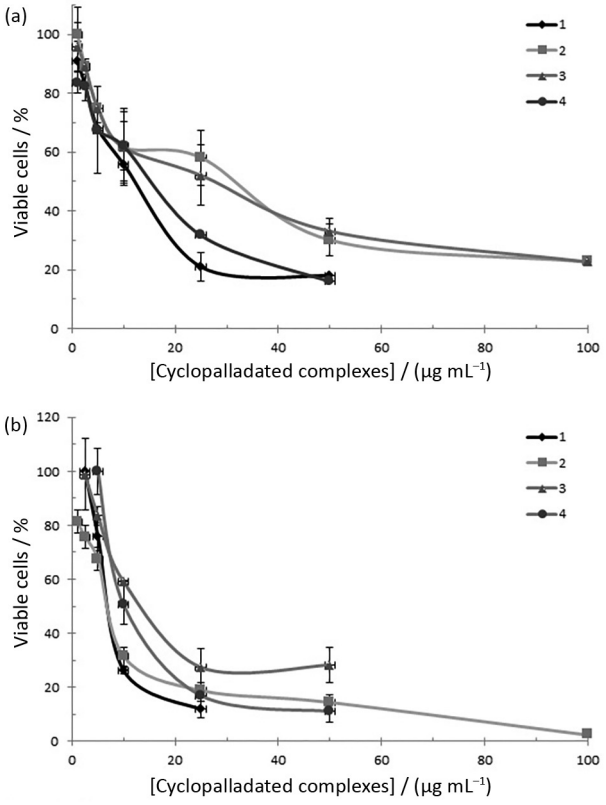

Figure 2. Percentage of viable (a) *L. amazonensis* promastigote; (b) *T. cruzi* epimastigotes forms treated with the cyclopalladated complexes.

Table 1. Antiparasitic activities (IC_{50}, half-maximal inhibitory concentration, μM), mammalian cell toxicity (CC_{50}, half-maximal cytotoxicity concentration, μM) and safety index (SI = CC_{50}/IC_{50}) of the cyclopalladated complexes. The SI values ≥ 10 in bold indicate promising compounds, i.e., those with low mammalian toxicity and high toxicity to parasites. Each value is the mean of three experiments performed in triplicate ± standard error

	Compound	IC_{50} (SI) / μM							CC_{50} / μM
		L. infantum		*L. amazonensis*		*T. cruzi Y*		*T. brucei 427*	Macrophage
		Promastigote	Amastigote	Promastigote	Amastigote	Epimastigote	Amastigote	Procyclic	
1	$[Pd(dmba)(\mu\text{-}Cl)]_2$	24.72 ± 1.40 (2.39)	42.00 ± 1.00 (1.41)	13.53 ± 1.76 (4.37)	9.57 ± 2.60 (6.18)	7.06 ± 1.76 (8.38)	11.82 ± 1.17 (5.01)	7.14 ± 1.54 (8.29)	59.18 ± 5.29
2	[Pd(dmba)(NCO)(isn)]	17.54 ± 0.30 (5.60)	82.30 ± 2.30 (1.19)	32.12 ± 3.09 (3.06)	22.45 ± 1.17 (4.37)	7.72 ± 0.62 (**12.72**)	4.45 ± 0.45 (**22.07**)	41.56 ± 1.68 (2.36)	98.22 ± 11.70
3	$[Pd(dmba)(N_3)(isn)]$	12.35 ± 0.30 (9.56)	59.50 ± 0.80 (1.98)	25.79 ± 2.20 (4.58)	28.82 ± 2.15 (4.10)	14.82 ± 1.09 (7.97)	3.40 ± 0.26 (**34.73**)	46.39 ± 1.17 (2.55)	118.09 ± 14.67
4	$[Pd(dmba)(\mu\text{-}NCO)]_2$	16.57 ± 2.10 (8.11)	53.20 ± 0.10 (2.53)	13.67 ± 1.54 (9.83)	9.29 ± 2.08 (**14.47**)	11.06 ± 1.76 (**12.16**)	4.73 ± 1.19 (**28.42**)	16.38 ± 0.70 (8.21)	134.44 ± 24.53
5	Dmba	–	–	NA	NA	NA	NA	NA	NA
6	Isn	–	–	NA	NA	NA	NA	NA	NA
7	Pentamidine	67.71 ± 8.10 (0.53)	19.77 ± 0.50 (1.81)	7.62 ± 0.10 (4.68)	5.07 ± 1.14 (7.04)	–	–	6.44 ± 0.01 (5.54)	35.69 ± 6.84
8	Benznidazole	–	–	–	–	4.07 ± 0.33 (242.86)	5.28 ± 1.38 (187.20)	–	988.43 ± 38.10
9	Amphotericin B	0.92 ± 0.01 (25.11)	2.98 ± 0.40 (7.75)	3.22 ± 0.03 (7.17)	4.92 ± 0.14 (4.70)	–	–	–	23.10 ± 2.52

NA: not active in the maximal inhibitory concentration tested (100 μmol L^{-1}).

NCO instead of Cl in compound **4** increased its chemical stability after 72 hours and decrease in its content which suggests a profile of Pd release/time without structure change (Figure 3). The advantage of compound **4** is its effectiveness on amastigotes forms that's present in human host while epimastigotes is present in the bugs.

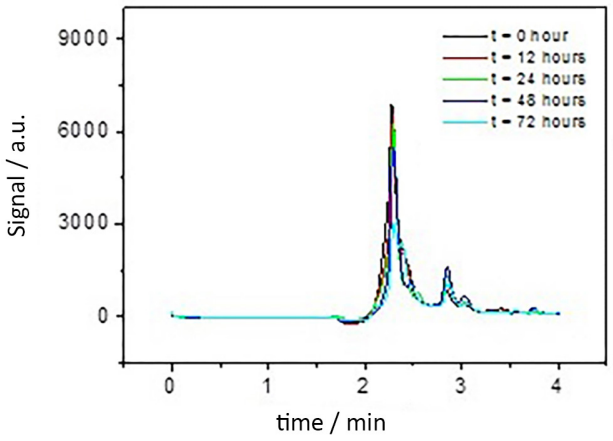

Figure 3. Stability study of compound **4**. Chromatogram profile of compound $[Pd(dmba)(\mu\text{-}NCO)]_2$ (**4**) at 0, 12, 24, 48 and 72 hours in PBS buffer, pH 7, room temperature, C-18 column, particle size 5 μm, 4.6 × 250 mm, mobile phase: methanol:water (70:30, v/v/v), flow 0.8 mL min^{-1}, λ = 254 nm.

All cyclopalladated complexes analyzed showed low mammalian toxicity, mainly for compounds **2**, **3** and **4** (CC_{50} = 98.22, 118.09 and 134.44 μM, respectively) compared to pentamidine and amphotericin B (CC_{50} = 35.69 and 23.10 μM, respectively). The decrease in cytotoxicity of these compounds varies according to their ligands in the following order: Cl < NCO (for dimeric compounds), NCO < N_3 (for mononuclear complexes).

Among the tested complexes, compound **4** showed good activity against the selected pathogens as well as low toxicity, with SI values of approximately 10 and 30, except for the *L. infantum* amastigote. In particular, for *L. amazonensis* promastigote and amastigote forms, compound **4** presented SI values of 9.83 and 14.47, respectively, which are safer than those of amphotericin B (7.17 and 4.70) and pentamidine (4.68 and 7.04).

For *T. brucei* procyclic forms, we did not observe any compound with higher trypanocidal activity than pentamidine. However, the compounds **1** and **4** were more selective (SI = 8.29 and 8.21, respectively) than pentamidine (SI = 5.54).

Cyclopalladated **2**, **3** and **4** evaluated against intracellular amastigote forms of *T. cruzi* showed the best SI values compared to those obtained for the intracellular amastigote forms of the *Leishmania* species (Table 1). This data showed that compounds **2**, **3** and **4** were more selective for *T. cruzi* when compared to other parasites. Further studies on **3** and **4** are required in order to gain a better understanding about their mechanism of action.

NMR analysis of complexes **2** and **3** were recorded in DMSO which indicated that isn is free of its dimeric precursors, $[Pd(dmba)(\mu\text{-}N_3)]_2$ and $[Pd(dmba)(\mu\text{-}NCO)]_2$; besides we do not observe species containing DMSO coordinated to Pd^{II}. According to the literature,[51] cyclopalladated complexes containing pyridine generate its corresponding binuclear precursors in solution at room temperature. Herein, the mononuclear derivatives of cyclopalladated with isn and pseudohalides do not maintain their structure completely intact in solution acting possibly as a prodrug since we observed anti-*T. cruzi* activity, therefore, the activity of compounds **2** and **3** might be the result of fractionation of these species present in solution and their precursors. Probably the observed dissociation in solution would explain the biological activity of the compound and the formation of pro-active species. This could contribute to the bioactivity displayed by compound **4**. This hypothesis can be supported by cisplatin mode of action since it undergoes successive hydrolysis reactions resulting in active species that react more rapidly with the target.[52] Further pharmacokinects studies are needed to prove the pro-action model of the cyclopalladed compound.

Some authors[24] observed that cyclopalladated complex against *T. cruzi* reduced the parasitemia and mortality *in vivo* with very low and nontoxic doses, as well as inhibited amastigote intracellular proliferation, similar to observed for us *in vitro* in *T. cruzi* and *L. amazonensis* intracellular amastigote.

The mechanism of actions proposed by Matsuo *et al.*[24] suggests that cyclopalladated complex interacts with the mitochondria of *T. cruzi*, causing a collapse in the cell extrusion of protons, followed by deoxyribonucleic acid (DNA) fragmentation and exposure of phosphatidylserine on the surface, a process resembling to apoptosis in mammalian cells. Already the same authors mentioned that the cyclopalladated complexes are "much more stable and less toxic than other derivatives of palladium(II)[53] and were able to inhibit cathepsin B (CpB)".[54] Other authors[55-57] reported that cathepsin B and L are involved in the growth of *Leishmania* and their virulence *in vitro* and *in vivo*. Other authors[25] observed that a palladacycle compound showed inhibition of the cysteine protease activity expressed in *L. amazonensis* amastigotes, being significant the inhibition of CpB activity. It was also reported that cyclometalled palladium(II) complexes[26] inhibited cathepsin B in other *Leishmania* species. However, the compounds did not affect the CpB activity of macrophages.[25] Probably, for this latter reason, our compounds were relatively innocuous on

peritoneal macrophages which is very interesting for new drugs evaluation.

Other mechanism of action by palladium complexes[58] described that "the production of oxidative stress as a result of their bioreduction and extensive redox cycling", as well as the interaction with DNA.[21,56]

Studies on the mechanism of action of cyclopalladated complexes **1**-**4** against both *L. amazonensis* and *T. cruzi* are underway, and so are the tests with other *T. cruzi* strains, due to genetic variability, to determine if our compounds may be following the similar mechanisms of action reported in the literature.

Conclusions

The results presented in this work suggest that the cyclopalladated compounds **1**-**4** (in special **4** showed to be the most active compound against *L. amazonensis* and *T. cruzi* intracellular amastigote forms whit low cytotoxicity) should be further considered as potential new hit in the search for new drugs for the chemotherapy of Chagas disease and leishmaniasis. In addition, the cyclopalladated **2** and **3** showed a promising anti-*T. cruzi* activity. Our biological results demonstrated that all cyclopalladated complexes presented low cytotoxicity towards mammalian cells.

Supplementary Information

Supplementary data (IR spectra, ^{1}H NMR spectra, ^{13}C spectra and chromatogram profile) are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

The authors thank FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), process number 201308248-1, for financial support and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), for research fellowship to AMAV, TGP, MLDC and ARR. We are also grateful for Regina M. B. Cicarelli for donate the *T. brucei* 427 strain to our lab.

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Submitted: August 24, 2015
Published online: December 21, 2015

FAPESP has sponsored the publication of this article.