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clarez@ula.ve
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Aragón-Muriel, Alberto; Upegui, Yulieth; Muñoz, JulyA.; Robledo, Sara M.; Polo-Cerón, Dorian

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Artículo científico

Synthesis, characterization and biological evaluation of rare earth complexes against tropical diseases Leishmaniasis, Malaria and Trypanosomiasis

Alberto Aragón-Muriel[1], Yulieth Upegui[2], JulyA. Muñoz[2], Sara M. Robledo[2], Dorian Polo-Cerón[1]*

[1)] Departamento de Química, Universidad del Valle, Cali, Colombia

[2)] PECET, Instituto de Investigaciones Médicas, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia. Calle 70 # 52-21, Medellín, Colombia

(*) dorian.polo@correounivalle.edu.co

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Resumen

Complejos lantánidos de Y(III), Ce(III), Nd(III), Sm(III) e Yb(III) con ligandos 4-R-cinamato y 4-R-fenilacetato (R = Cl, CH_3O-) fueron sintetizados y caracterizados empleando análisis elemental, conductancia molar, mediciones espectroscópicas (infrarrojo, UV-Vis) y análisis térmico. La evaluación de la actividad citotóxica en células U-937 mostró que los complejos lantánidos no son tóxicos contra esta línea promonocítica humana. Todos los compuestos sintetizados fueron examinados para su actividad *in vitro* contra tres parásitos protozoarios: *Leishmania panamensis*, *Plasmodium falciparum y Trypanosoma cruzi*. El complejo de Ce(III) con ligando 4-metoxicinamato, a 20 µg/ml, redujo la infección en 22 % y puede considerarse moderadamente activo contra *L. panamensis*. Todos los complejos seleccionados demostraron actividad anti-parasitaria contra *T. cruzi*.

Palabras Clave: citotoxicidad; lantánidos; *L. panamensis*; *P. falciparum*; *T. cruzi*

Abstract

The lanthanide complexes Y(III), Ce(III), Nd(III), Sm(III) and Yb(III) with 4-R-cinnamate and 4-R-phenylacetate (R = Cl, CH_3O-) ligands have been synthesized and characterized using elemental analysis, molar conductance, spectroscopic measurements (IR, UV-Vis spectra) and thermal analysis. The evaluation of cytotoxic activity in U-937 cells has revealed that lanthanide complexes are not toxic against this human promonocytic line. All synthesized compounds have been screened for *in vitro* activity against three parasitic protozoa: *Leishmania panamensis*, *Plasmodium falciparum* and *Trypanosoma cruzi*. The Ce(III) complex with the 4-methoxycinnamate ligand at 20 µg/ml reduces infection by 22 % and may be considered moderately active against *L. panamensis*. All of the selected complexes demonstrate anti-parasitic activity against *T. cruzi*.

Keywords: Cytotoxicity; Lanthanides; *L. panamensis*; *P. falciparum*; *T. cruzi*

Introduction

Tropical diseases such as leishmaniasis, malaria and trypanosomiasis, referred to as 'neglected diseases' by the World Health Organization (WHO), are responsible for the high morbidity and mortality that affect almost a third of the world's population and is considered to be the primary public health problem in the poorest tropical and subtropical regions of developing countries. Due to the high cost of treatment, the resistance to conventional drugs and the toxicity generated by drugs used against these diseases, it is necessary to search for new effective and reliable compounds that can act as anti-parasitic agents. A few metal-based drugs offer great opportunities as potential agents against the major protozoan diseases (malaria, leishmaniasis and trypanosomiasis)[1], metal-containing compounds for the treatment of protozoan diseases has been extensively studied by Sánchez-Delgado *et al.*[2,3], showing that coordination and organometallic complexes can be employed as potential drugs. Positively charged metallic centers in coordination compounds are attracted to negatively charged biomolecules, such as proteins and nucleic acids that act as ligands in the presence of metallic ions. Mechanisms involved in the anti-parasitic activity of coordination compounds have been studied, including the formation of hemozoin as the main target of antimalarial drugs. Recent reports have led to an increasing interest in developing new drugs that involve coordination complexes with lanthanide ions (Ln^{3+}). Bioinorganic chemistry studies on lanthanide complexes have shown that these compounds have anti-tumor and anti-microbial properties that make them potential precursors in the development of new drugs.

Cinnamates are widely used in the production of food, cosmetics and pharmaceuticals[4,5]; their derivatives have been evaluated as antimalarial agents. Given our interest in this medical application and our ongoing development of new metal complexes with biological activity[6-8], we have

synthesized lanthanide complexes Y(III), Ce(III), Nd(III), Sm(III) and Yb(III) with 4-R-cinnamate and 4-R-phenyl-acetate (R = chlorine and methoxy) ligands (Fig. 1) and have evaluated *in vitro* biological activity against the tropical diseases leishmaniasis, malaria and American trypano-somiasis (Chagas disease).

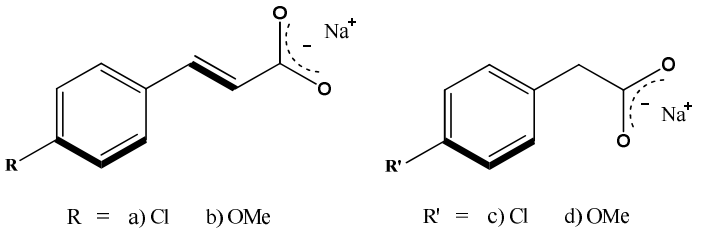


Fig. 1: Structural formula of sodium ligand precursors (NaL): a) sodium 4-chloro-cinnamate, Na(4-Clcinn); b) sodium 4-methoxy-cinnamate, Na(4-OMecinn); c) sodium 4-chloro-phenylacetate, Na(4-Clphac); d) 4-methoxy-phenylacetate, Na(4-OMephac).

Experimental

General

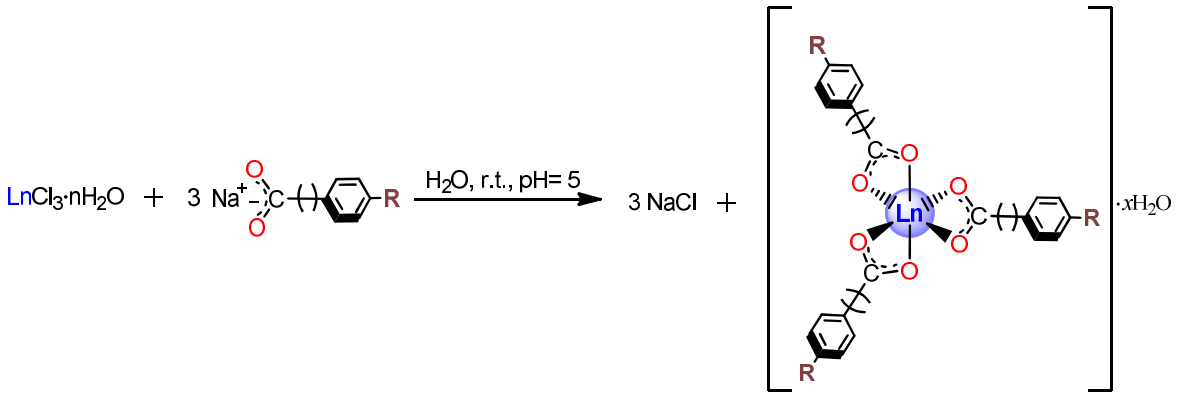
4-chloro-cinnamic acid (4-ClcinnH), 4-methoxy-cinnamic acid (4-OMecinnH), 4-chloro-phenylacetic acid (4-ClphacH), 4-methoxy-phenylacetic acid (4-OMephacH) and $LnCl_3 \cdot xH_2O$ (Ln = Y, Ce, Nd, Sm, Yb) were purchased from Sigma-Aldrich, and other chemicals were obtained comercially and used without further purification. Sodium cinnamates were synthesized by neutralization of its respective acid with a NaOH solution in water. Microanalyses were performed with a Flash EA 1112 Series CHN Analyzer. Metal analyses were performed in duplicate by ethylene-diamine tetraacetic acid (EDTA) titration (direct complex-metric titration)[9] and using xylenol orange as indicator. Molar conductance of 10^{-3} M solutions of the complexes in DMF was recorded on a Thermo Orion 150 conductivity meter by using 0.01 M KCl water solution as calibrant. A Shimadzu UV-1700 spectrophotometer was used to record the electronic spectra. Infrared spectra were recorded with a NICOLET 6700 Spectrometer (4000-400 cm^{-1} with a recording accuracy of 1 cm^{-1}). Thermogravimetric analyses were performed with a NETZSCH TG 209 F1 thermal analyzer. Heating was conducted under static conditions in nitrogen with a range of 20-700 °C at 20 °C·min^{-1} in an Al_2O_3 crucible.

Synthesis of rare earth compounds

The Ln(III) complexes were prepared using similar methods to those described in the literature (Fig. 2)[10].

[Y(4-Clcinn)$_3$] (1). A 10 ml aqueous solution of Na (4-Clcinn) (3 eq: 1012 mg, 4.95 mmol) was slowly added to a 15 ml aqueous solution of $YCl_3 \cdot 6H_2O$ (500 mg, 1.65 mmol); a precipitate formed instantly upon the addition of the sodium salt. The solution was adjusted to pH 5 with drops of a 0.1 M HCl or NaOH solution controlled by a pH-meter and then stirred for 3 h and filtered. The white precipitate was then washed with distilled water and dried in a desiccator for 2 days. Yield: 82 %. $C_{27}H_{18}Cl_3O_6Y$, elemental analysis: C 51.21 (calc. 51.17), H 2.91 (2.86), Y 13.85 (14.03) %. IR (KBr pellet cm^{-1}): 3038 w, 1640 vs, 1592 m, 1525 s, 1492 s, 1417 vs, 1395 s, 1285 w, 1248 m, 1202 w, 1176 w, 1091 s, 1011 m, 982 s, 880 m, 855 w, 825 vs, 759 m, 731 m, 693 w, 664 w, 557 m, 496 s, 458 s. Conductivity (µS·cm^{-1}, 0.001 M, DMF): 11.7. UV–Vis (ethanol): λ_{max} = 270 nm.

[Y(4-OMecinn)$_3$] (2). The synthesis of 2 was performed in an identical manner to the synthesis of 1. White powder, yield: 80%. $C_{30}H_{27}O_9Y$, elemental analysis: C 58.16 (calc. 58.08), H 4.40 (4.39), Y1 4.32 (14.33) %. IR (KBr pellet cm^{-1}): 3006 w, 2953 w, 2837 w, 1635 s, 1605 s, 1574 m, 1511 s, 1459 m, 1426 s, 1405 s, 1306 m, 1246 vs, 1172 s, 1109 w, 1027 m, 988 m, 876 w, 830 s, 782 w, 728 w, 699 w, 560 m, 516 w, 479 w, 456 w. Conductivity (µS·cm^{-1}, 0.001 M, DMF): 7.3. UV–Vis (ethanol): λ_{max}= 280 nm.



(1) Ln = Y(III), R = Cl, ⇿ = ≈ x = 0
(2) Ln = Y(III), R = OMe, ⇿ = ≈ x = 0
(3) Ln = Ce(III), R = Cl, ⇿ = ≈ x = 0
(4) Ln = Ce(III), R = OMe, ⇿ = ≈ x = 1
(5) Ln = Ce(III), R = Cl, ⇿ = ∧ x = 1
(6) Ln = Ce(III), R = OMe, ⇿ = ∧ x = 1
(7) Ln = Nd(III), R = Cl, ⇿ = ≈ x = 2
(8) Ln = Nd(III), R = OMe, ⇿ = ≈ x = 1
(9) Ln = Sm(III), R = Cl, ⇿ = ≈ x = 1
(10) Ln = Sm(III), R = OMe, ⇿ = ≈ x = 1
(11) Ln = Yb(III), R = Cl, ⇿ = ≈ x = 1
(12) Ln = Yb(III), R = OMe, ⇿ = ≈ x = 0

Fig. 2: General reaction scheme for the synthesis of rare earth complexes.

[Ce(4-Clcinn)$_3$] (3). The synthesis of 3 was performed in an identical manner to the synthesis of 1. White powder, yield: 88 %. $C_{27}H_{18}Cl_3O_6Ce$, elemental analysis: C 47.59 (calc. 47.35), H 2.66 (2.65), Ce 20.02 (20.46) %. IR (KBr pellet cm^{-1}): 3034 w, 2926 w, 1639 vs, 1590 m, 1516 s, 1492 s, 1416 vs, 1393 vs, 1284 w, 1247 m, 1175 w, 1091 s, 1012 m, 982 s, 880 m, 854 w, 825 s, 748 m, 693 w, 665 w, 545 m, 495 m, 456 m. Conductivity (μS·cm^{-1}, 0.001 M, DMF): 4.4. UV–Vis (ethanol): λ_{max} = 270 nm.

[Ce(4-OMecinn)$_3$]·H$_2$O (4). The synthesis of 4 was performed in an identical manner to the synthesis of 1. White powder, yield: 90 %. $C_{30}H_{27}O_9Ce(OH_2)$, elemental analysis: C 51.89 (calc. 52.25), H 4.21 (4.24), Ce 20.31 (20.32) %. IR (KBr pellet cm^{-1}): 3420 w, 3003 w, 2934 w, 2836 w, 1638 s, 1606 s, 1573 m, 1512 s, 1426 s, 1401 s, 1304 m, 1245 vs, 1173 s, 1108 w, 1031 m, 983 m, 877 w, 830 vs, 779 m, 720 m, 637 w, 558 s, 518 m, 476 w, 458 w. TGA mass loss 2.80 % (70 - 150 °C, 1 step, calc. 1 × H_2O = 2.61 %), 4.70 % (180 - 270 °C, 1 step, calc. 1 × OCH_3 [$C_{29}H_{25}O_8Ce$ formation] = 4.62 %), 52.0% (310 - 600 °C, 1 step, calc. Ce_2O_3 formation = 52.4 %). Conductivity (μS·cm^{-1}, 0.001 M, DMF): 5.9. UV–Vis (ethanol): λ_{max} = 280 nm.

[Ce(4-Clphac)$_3$]·H$_2$O (5). The synthesis of 5 was performed in an identical manner to the synthesis of 1. Pale yellow powder, yield: 74 %. $C_{24}H_{18}Cl_3O_6Ce(OH_2)$, elemental analysis: C 43.04 (calc. 43.22), H 3.01 (3.02), Ce 21.13 (21.01) %. IR (KBr pellet cm^{-1}): 3440 s, 3044 w, 1631 s, 1544 vs, 1493 m, 1422 s, 1398 s, 1286 m, 1200 w, 1092 m, 1017 w, 935 w, 857 w, 809 m, 743 m, 683 m, 594 w, 507 w, 476 m, 424 w. TGA mass loss 2.40% (75- 50 °C, 1 step, calc. 1 × H_2O = 2.70 %), 64.6 % (300-600 °C, 1 step, calc. $CeOCO_3$ formation = 67.6 %). Conductivity (μS·cm^{-1}, 0.001 M, DMF): 5.5.

[Ce(4-OMephac)$_3$]·H$_2$O (6). The synthesis of 6 was performed in an identical manner to the synthesis of 1. Pale yellow powder, yield: 81 %. $C_{27}H_{27}O_9Ce(OH_2)$, elemental analysis: C 49.96 (calc. 49.61), H 4.32 (4.47), Ce 21.47 (21.44) %. IR (KBr pellet cm^{-1}): 3460 s, 3058 w, 2905 w, 2836 w, 1636 vs, 1574 m, 1543 s, 1517 vs, 1430 s, 1400 s, 1284 m, 1251 vs, 1178 m, 1106 w, 1034 m, 949 w, 859 w, 821 w, 730 m, 697 w, 588 w, 538 w, 481 w, 446 w. TGA mass loss 3.10 % (95-160 °C, 1 step, calc. 1 × H_2O = 2.76 %), 70.5 % (280-600 °C, 1 step, calc. CeO_2 formation = 73.7%). Conductivity (μS·cm^{-1}, 0.001 M, DMF): 11.6.

[Nd(4-Clcinn)$_3$]·2H$_2$O (7). The synthesis of 7 was performed in an identical manner to the synthesis of 1. White powder, yield: 85 %. $C_{27}H_{18}Cl_3O_6Nd(2OH_2)$, elemental analysis: C 45.02 (calc. 44.73), H 2.99 (3.06), Nd 19.97 (19.89) %. IR (KBr pellet cm^{-1}): 3344 m, 3046 w, 1640 vs, 1591 w, 1507 vs, 1420 vs, 1393 s, 1249 m, 1093 s, 1014 w, 984 s, 826 s, 756 m, 542 w, 496 m, 456 m. TGA mass loss 6.00 % (80-150 °C, 1 step, calc. 2 × H_2O = 4.97 %), 48.0 % (340-580 °C, 1 step, calc. $Nd_2O_2CO_3$ formation = 47.5 %). Conductivity (μS·cm^{-1}, 0.001 M, DMF): 3.9. UV–Vis (ethanol): λ_{max} = 268 nm.

[Nd(4-OMecinn)$_3$]·H$_2$O (8). The synthesis of 8 was performed in an identical manner to the synthesis of 1. White powder, yield: 89 %. $C_{30}H_{27}O_9Nd(OH_2)$, elemental analysis: C 52.31 (calc. 51.94), H 4.23 (4.21), Nd 20.15 (20.79) %. IR (KBr pellet cm^{-1}): 3340 w, 3004 w, 2932 w, 2837 w, 1682 m, 1637 s, 1606 s, 1573 m, 1512 vs, 1427 s, 1401 vs, 1304 m, 1245 vs, 1173 s, 1109 w, 1029 m, 984 m, 879 w, 831 s, 779 m, 722 m, 558 s, 520 m, 459 w. Conductivity (μS·cm^{-1}, 0.001 M, DMF): 6.1. UV–Vis (ethanol): λ_{max} = 279 nm.

[Sm(4-Clcinn)$_3$]·H$_2$O (9). The synthesis of 9 was performed in an identical manner to the synthesis of 1. White powder, yield: 82 %. $C_{27}H_{18}Cl_3O_6Sm(OH_2)$, elemental analysis: C 45.66 (calc. 45.47), H 2.90 (2.83), Sm 21.48 (21.08) %. IR (KBr pellet cm^{-1}): 3353 m, 3041 w, 2925 w, 1639 s, 1590 m, 1510 s, 1494 s, 1417 vs, 1394 s, 1285 w, 1248 m, 1203 w, 1091 s, 1013 m, 983 s, 880 m, 854 w, 825 s, 755 m, 689 w, 667 w, 544 m, 496 m, 457 m. Conductivity (μS·cm^{-1}, 0.001 M, DMF): 2.7. UV–Vis (ethanol): λ_{max} = 267 nm.

[Sm(4-OMecinn)$_3$]·H$_2$O (10). The synthesis of 10 was performed in an identical manner to the synthesis of 1. White powder, yield: 96 %. $C_{30}H_{27}O_9Sm(OH_2)$, elemental analysis: C 51.08 (calc. 51.48), H 4.34 (4.18), Sm 21.18 (21.48) %. IR (KBr pellet cm^{-1}): 3346 w, 2962 w, 2931 w, 2838 w, 1683 m, 1637 s, 1605 s, 1575 s, 1511 vs, 1427 s, 1403 vs, 1305 m, 1245 vs, 1172 s, 1110 w, 1027 m, 985 m, 881 w, 832 s, 780 m, 723 m, 557 m, 520 m, 459 w. Conductivity (μS·cm^{-1}, 0.001 M, DMF): 10.5. UV–Vis (ethanol): λ_{max} = 278 nm.

[Yb(4-Clcinn)$_3$]·H$_2$O (11). The synthesis of 11 was performed in an identical manner to the synthesis of 1. White powder, yield: 84 %. $C_{27}H_{18}Cl_3O_6Yb(OH_2)$, elemental analysis: C 43.98 (calc. 44.07), H 2.68 (2.74), Yb 23.24 (23.52) %. IR (KBr pellet cm^{-1}): 3355 m, 3026 w, 1640 s, 1590 m, 1523 s, 1493 s, 1417 vs, 1396 s, 1290 w, 1248 m, 1202 w, 1091 s, 1011 m, 984 s, 880 m, 825 s, 764 m, 748 m, 726 m, 666 w, 557 w, 497 m, 458 m. Conductivity (μS·cm^{-1}, 0.001 M, DMF): 4.9. UV–Vis (ethanol): λ_{max} = 269 nm.

[Yb(4-OMecinn)$_3$] (12). The synthesis of 12 was performed in an identical manner to the synthesis of 1. White powder, yield: 80 %. $C_{30}H_{27}O_9Yb$, elemental analysis: C 51.36 (calc. 51.14), H 3.82 (3.86), Yb 24.72 (24.56) %. IR (KBr pellet cm^{-1}): 2932 w, 2837 w, 1634 s, 1603 s, 1573 m, 1512 vs, 1427 vs, 1405 vs, 1307 m, 1248 vs, 1173 s, 1110 w, 1027 m, 991 m, 875 w, 830 s, 783 w, 730 w, 697 w, 558 m, 517 m, 459 w. Conductivity (μS·cm^{-1}, 0.001 M, DMF): 3.5. UV–Vis (ethanol): λ_{max} = 280 nm.

Cytotoxicity assay

Cytotoxic activity of the compounds was evaluated based on the viability of the human promonocytic cell line U-937 (ATCC CRL-1593.2^{TM}) determined using the MTT enzymatic micro-method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide). U-937 cells were grown in 96-wells plates at 1x10^5 cells/ml in RPMI-1640 enriched with

10 % FBS and incubated at 37 °C with 5 % CO_2 in the presence of lanthanide complexes. After 72 h of incubation, the viability of cells was determined by assaying the reduction of MTT to formazan as described previously by adding 10 µl/well of an MTT solution (5 mg/ml) and incubating the solution for 3 h. The enzymatic reaction was terminated by the addition of 100 µl of a solution of 50% isopropanol with 10% sodium dodecyl-sulfate. Formazan production was measured at 570 nm on a 96-well microplate reader (BioRad Benchmark). Cells cultivated in the absence of lanthanide complexes and maintained under the same conditions were used as a control. All of the compounds were tested in triplicate in two independent experiments. The results are expressed as 50 % lethal concentration (LC_{50}), calculated by the probit probabilistic method[11].

Anti-leishmanial and anti-trypanocidal activity

The *in vitro* leishmanicidal activity of the lanthanide complexes against intracellular amastigotes was evaluated using flow cytometry based on methodology described by Pulido *et al.*[12]. Promastigotes of *L.(V.) panamensis* UA140-epirGFP, which are responsible for the mucocutaneous form of the disease, were grown in a modified biphasic NNN environment with phosphate-buffered saline (PBS) and glucose in the liquid phase. Infection of U-937 cells involved adjusting the number of cells to $1x10^5$ cells/ml and adding 0.1 µg/ml of phorbol myristate acetate (PMA) to induce the transformation of cells to macrophages. The experiment was conducted in 24-well plates where cells were infected with promastigotes in the growth stationary phase. After 4 h of incubation at 34 °C (5 % CO_2), free parasites were removed by two successive washes with PBS. Adapted dilutions of lanthanide complexes were added, and cultures were incubated for 72 h at 37 °C (5 % CO_2). Infected cells cultivated in the absence of lanthanide complexes served as a control. Amphotericin B was used as a reference drug. The anti-parasitic effect was measured with an argon laser in a flow cytometer at 488 nm (excitation) and 525 nm (emission). Each lanthanide complex concentration, as well as each control, was tested in two independent experiments. The results are expressed as the percentages of inhibition[11].

In vitro anti-trypanosomal activity of lanthanide complexes were evaluated on *Trypanosoma cruzi* cells (Tulahuen strain) for intracellular amastigotes that express the *Escherichia coli* beta-galactosidase gene. After U-937 cells were infected by epimastigotes and incubated for 24 h, lanthanide compounds were added at concentrations of 20 µg/ml. The experiments were performed in duplicate two times, and the results are expressed as the percentages of inhibition. The LC_{50} of benznidazole, a compound conventionally used for the treatment of Chagas disease, was used as a control[12-14].

Anti-plasmodial assay

The *P. falciparum* 3D7 strain was grown asynchronously in standard conditions described by Trager and Jensen[15]. Compound activity affecting parasite growth as determined in 24-well suspension cultures using fluorometry and quantifying the DNA parasite with ethidium bromide (BrEt) staining[16]. The intensity of fluorescence is directly proportional to the amount DNA, which is proportional to the amount of viable parasites. The compounds were dissolved in RPMI 1640 at a concentration of 20 µg/ml. The parasites were incubated with the compounds for 72 h at 37 °C in an atmosphere of 5 % O_2, 5 % CO_2, and 90 % N_2. Chloroquine was used as a positive control to measure activity. The stained DNA was quantified with a Varioskan Flash spectrofluorometer at an excitation wavelength of 510 nm and an emission wavelength of 595 nm. For each concentration, the experiment was performed in duplicate two times. The results are expressed as EC_{50} (effective concentration 50), estimated by the probit method[17].

Results and discussion

Chemistry

Lanthanide complexes ***1-12*** have been prepared using methods that involve a metathesis or double displacement reaction, similar to those described in the literature. Complexes ***1-12*** were isolated as solids in powder, soluble in DMSO, DMF, and acetone, and partially soluble in water and hexane. The results obtained by elemental analysis (carbon and hydrogen), by EDTA complexometry and by TG-DTG analysis, agree with all structures proposed.

Elemental analysis

The experimental values of the percentage of lanthanide, carbon and hydrogen obtain showed sufficient correspondence with the calculated values for the suggested general formula of the complexes such as $[Ln(L)_3]\cdot xH_2O$.

Molar conductance

Molar conductance (Λ_M) values of complexes ***1-12*** (3.5–11.7 $S\cdot cm^2.mol^{-1}$) were measured in dimethylformamide (DMF) at room temperature. These conductance values are much lower than the values reported for 1:1 electrolytes in these solvents[18]. The results indicated a non-ionic nature of the compounds, and hence, they are neutral complexes. The overall charge of the trivalent metal ions was balanced by three negatively charged coordinated ligands (bidentate coordination of the carboxylates ligands).

UV-Visible spectra

The UV-Visible spectra of the complexes with cinnamic ligands were recorded by UV-Visible spectroscopy in the wavelength range of 200-800 nm. The band observed at 265 nm for (4-ClcinnH) and at 275 nm for (4-OMecinnH) is due to π-π* transition of conjugated system in the ligand and it was shifted to higher wavelength (red shift) upon complexation. The amounts of these shifts however are small, i.e., 2–5 nm. The occurrence of red shifts for lanthanide chelates indicates the decrease in the corresponding energy

gaps in the electronic energy levels of the ligands as a result of chelation.

Infrared spectroscopy analysis

Infrared spectra of all of the complexes obtained show characteristic absorption bands of the cinnamate and phenylacetate ligands. The absence of the absorption band (COOH) at approximately 1700 cm^{-1} of the ligands in all of the spectra indicates coordination of the ligands to the metal. The experimental data obtained by IR spectroscopy of the Ln (III) complexes, as well as of the respective ligands, are presented in Table 1.

For complexes 4-11, the bands in the 3460-3340 cm^{-1} region are assigned to the stretching of the OH group, $(OH)_w$, with the hydration water molecules. For the precursors and complexes derived from cinnamic acid 1-4 and 7-12, bands assigned to the υ(C=C) vibrations are observed in the 1600 cm^{-1} region, confirming that the π electron system of the olefinic double bond does not participate in the coordination[10,19-21]. Stretching of the Ln-O bond (metal-carboxylate) has been attributed to the ν(Ln-O) bands that have been reported at 780 cm^{-1} for the lanthanum complex[22], and differences have been found among all of the compounds due to the presence of the substituents (-Cl, -OMe) and the $(C=C)_{propenyl}$ bond. A significant change to lower frequencies (bathochromic effect) is observed for the stretching $\upsilon(CO_2)$ compared to the sodium salts. Frequency analysis of the bands corresponding to the COO^- group demonstrates that $\Delta\upsilon COO^- = \upsilon_{as}(COO^-) - \upsilon_s(COO^-)$. The calculated values of $\Delta\upsilon COO^-$ are in the range of 100-122 cm^{-1}, indicating that the ligand is in a chelating environment[23,24]. This finding confirms that the ligands and the lanthanide ions are coordinated through the oxygen atoms of the carboxylate groups in a bidentate fashion[24].

Thermogravimetric analysis

Thermal decomposition results of compounds 4–7 in nitrogen are presented in Table 2 and Figure 3. For all compounds, the TG curves show endothermic decomposition between 70 °C and 160 °C, corresponding to the loss of hydration water molecules in one step. For the neodymium complex (**7**), the loss of two water molecules has been calculated; other compounds studied using TG are monohydrate. In the case of 4, the possible loss of the methoxy substituent on one of the ligands indicates the formation of $C_{29}H_{25}O_8Ce$ at 180-270 °C.

Table 1: Select IR bands of Na ligands and their Ln (III) complexes.

Compound	Main bands (cm^{-1})								
	$\upsilon(OH)_w$	υ(C=C)	$\upsilon_{as}(CO_2)$	$\upsilon_s(CO_2)$	$\Delta\upsilon^*$	$\delta(C\text{-}H)_{ip}$	δ(=CH)	$\delta(C\text{-}H)_{op}$	υ(La-O)
Na(4-Clcinn)	3424	1639	1562	1429	133	1238	970	827	-
Na(4-OMecinn)	-	1642	1547	1399	148	1241	971	829	-
Na(4-Clphac)	3455	-	1567	1390	177	1289	-	815	-
Na(4-OMephac)	3411	-	1560	1404	156	1248	-	816	-
1	-	1640	1525	1417	108	1248	982	825	759
2	-	1635	1511	1405	106	1246	988	830	782
3	-	1639	1516	1416	100	1247	982	825	748
4	3420	1638	1512	1401	111	1245	983	830	779
5	3440	-	1544	1422	122	1286	-	809	743
6	3460	-	1517	1400	117	1251	-	821	730
7	3344	1640	1507	1393	114	1249	984	826	756
8	3340	1637	1512	1401	111	1245	984	831	779
9	3353	1639	1510	1394	116	1248	983	825	755
10	3346	1637	1511	1403	108	1245	985	832	780
11	3355	1640	1523	1417	106	1248	984	825	764
12	-	1634	1512	1405	107	1248	991	830	783

Table 2: Thermal decomposition data on complexes ***4-7*** in nitrogen

Compound	Range of decomposition / °C	Mass loss* / %		Product
		Meas.	Calc.	
$[Ce(4\text{-}OMecinn)_3]\cdot H_2O$ (***4***)	70 – 150	2.80	2.61	$Ce(4\text{-}OMecin)_3$
	180 – 270	4.70	4.62	$C_{29}H_{25}O_8Ce$
	310 – 600	52.0	52.4	Ce_2O_3
$[Ce(4\text{-}Clphac)_3]\cdot H_2O$ (***5***)	75 – 150	2.40	2.70	$Ce(4\text{-}Clfen)_3$
	300 – 600	64.6	67.6	$CeOCO_3$
$[Ce(4\text{-}OMephac)_3]\cdot H_2O$ (***6***)	95 – 160	3.10	2.76	$Ce(4\text{-}OMefen)_3$
	280 – 600	70.5	73.7	CeO_2
$[Nd(4\text{-}Clcinn)_3]\cdot 2H_2O$ (***7***)	80 – 150	6.00	4.97	$Nd(4\text{-}Clcin)_3$
	340 – 580	48.0	47.5	$Nd_2O_2CO_3$

* Measured and calculated values of the percentage of mass loss from the initial sample (initial temperature at 20 °C) to the final product.

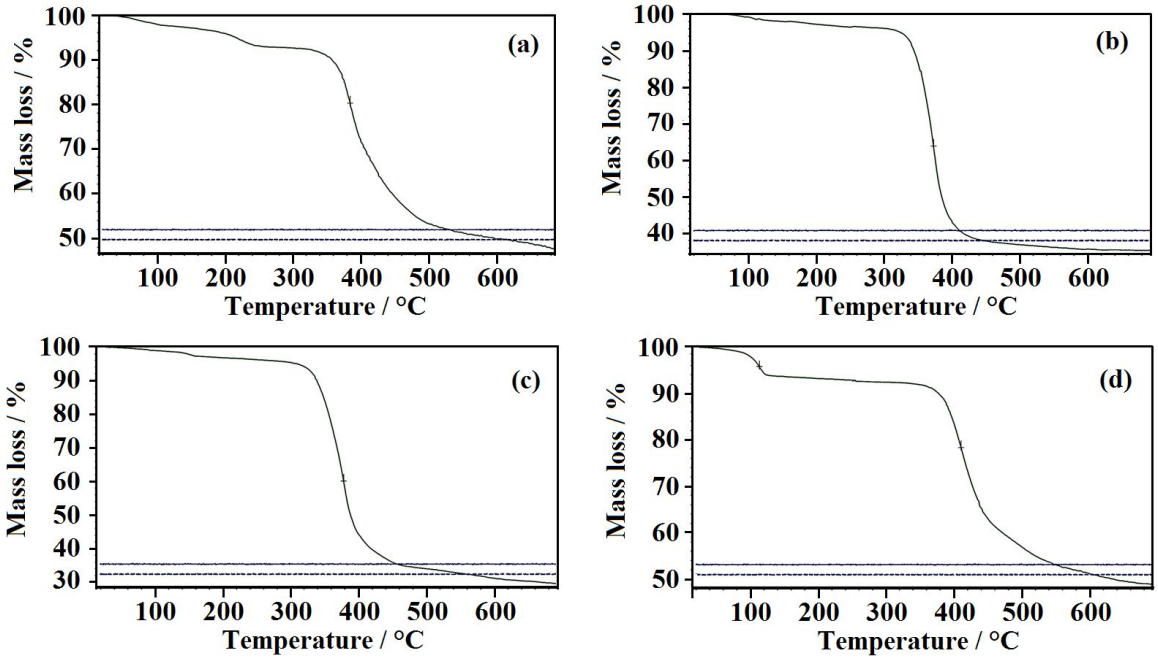


Fig. 3: TG curves of compounds: (a) $[Ce(4\text{-}OMecinn)_3]\cdot H_2O$; (b) $[Ce(4\text{-}Clphac)_3]\cdot H_2O$; (c) $[Ce(4\text{-}OMephac)_3]\cdot H_2O$; (d) $[Nd(4\text{-}Clcinn)_3]\cdot 2H_2O$

Thermal analysis of Ce(III) anhydrous compounds shows three decomposition oxides between 280 °C and 600 °C: Ce_2O_3 to 4, $CeOCO_3$[25] to ***5*** and CeO_2 to 6 (common for Ce(IV) oxides). Between 340-580 °C, complex 7 shows the decomposition of anhydrous cinnamate to neodymium(III) carbonate oxide ($Nd_2O_2CO_3$), a phase which has been reported in other studies of lanthanide complexes[26,27].

Biological evaluation

To evaluate *in vitro* cytotoxicity of lanthanide complexes, experiments were performed using the human promonocytic cellular line (ATCC CRL-1593.2™) and the methyl thiazol tetrazolium (MTT) enzymatic micro-method. Results show that lanthanide complexes are potentially non-toxic, with values of $LC_{50} > 200$ µg/ml. The *in vitro* anti-parasitic activity of lanthanide complexes against *L. panamensis, P. falciparum* and *T. cruzi* are shown in Table 3. *In vitro* anti-plasmodial activity has been evaluated on an asynchronous culture of the *P. falciparum* strain 3D7 (sensitive to chloroquine), according to a previously described procedure[15]. Percentages of inhibition vary between 0 and 17 %. Ce(III) complexes with phenylacetate ligands (***5*** and ***6***) display a higher percentage of inhibition against *P. falciparum*. All of the synthesized compounds have been evaluated *in vitro* against intracellular amastigotes of *Leishmania (viannia) paramensis*, according to a previously described procedure[12]. Results displayed in Table 3 indicate that Ce(III) complexes 3-6 significantly inhibit the growth of *Leishmania* parasites. Compound ***4***, which was able to reduce the infection by 22 %, could have leishmanicidal potential if the effective concentration 50 (EC50), i.e. the concentration of compound that achieves decrease infection in at least 50 %, less than 50 µg/ml, a concentration classified as moderately active compound. Factors that most likely contribute to this anti-parasitic

Table 3: *In vitro* anti-parasitic and cytotoxic activities of lanthanide complexes 1-12.

Compound	% inhibition of growth[a]			
	LC_{50} (µg/ml)	*L. panamensis*	*P. falciparum*	*T. cruzi*
1	> 200	0 ± 0	1.5 ± 0.8	45.3 ± 0.6
2	> 200	4.7 ± 1.0	0 ± 0	46.2 ± 3.8
3	> 200	9.1 ± 2.0	0 ± 0	27.6 ± 7.8
4	> 200	22.0 ± 5.0	0 ± 0	41.5 ± 3.1
5	> 200	9.7 ± 2.7	16.7 ± 8.05	42.9 ± 5.7
6	> 200	16.7 ± 3.5	14.4 ± 3.3	49.5 ± 8.1
7	> 200	5.7 ± 0.2	6.8 ± 2.1	33.4 ± 5.6
8	> 200	6.8 ± 0.6	3.9 ± 1.3	45.7 ± 0.7
9	> 200	0 ± 0	0 ± 0	33.2 ± 2.6
10	> 200	8.5 ± 2.0	0 ± 0	46.6 ± 9.2
11	> 200	12.0 ± 2.5	2.4 ± 0.3	28.8 ± 6.4
12	> 200	6.3 ± 1.3	12.3 ± 2.9	42.8 ± 5.3
Anphotericin B[b]	35.1±3.1	49.8 ± 7.5[b]	NA	NA
Chloroquine[c]	> 200	NA	60.1 ±2.3	NA
Benznidazole[d]	33.4 ± 5.1	NA	NA	50.1 ± 7.4[c]

[a] Growth inhibition of U-937 cells or parasites determined by flow cytometry for intracellular amastigotes of *L. panamensis*, by fluorometry for all forms of *P. falciparum* and by the colorimetric β-galactosidase method for intracellular amastigotes of *T. cruzi*. Data are expressed as the average of at least two independent experiments, each performed in triplicate.

[b] B amphotericin was applied at 0.05 µg/ml, the concentration at which infection is reduced by 50 %.

[c] EC_{50} = 0.1 µg/ml

[d] Benznidazole was applied at 10 µg/ml, the concentration at which infection is reduced by 50 %.

activity are the higher solubility of these complexes in culture media and the possibility that this metal is involved in biological oxidation-reduction reactions (Ce^{3+}/Ce^{4+}), has shown that the cerium cinnamate presents corrosion resistance, and according XPS curves, 3+ and 4+ oxidation states are involved[28]. The activity of these cerium compounds could increase intracellular oxidative stress which could affect parasite redox metabolism[29]. Lanthanides in complex with methoxy groups display higher anti-leishmanial activity than complexes with chorine substituents, however, no significant correlation has been found between modifications of the C=C double bond of an alkenyl substituent (compounds 3 and 4) to an alkyl fragment (compounds 5 and 6) and the leishmanicidal activity of the Ce(III) complexes. Lanthanide complexes 1-12 have been evaluated for *in vitro* trypanocidal activity against *T. cruzi* (tulahuen strain). All complexes show anti-parasitic activity against *T. cruzi*. Compounds 2, 4, 6, 8, 10 and 12 with methoxy groups in the aromatic ring show the highest inhibition percentages (> 40 %), based on their different abilities to interact with vital biomolecules (DNA and specific proteins) as shown in other studies[30], even its mechanism of action could be related to the ability to produce toxic free radical species in the parasite[31]. The results show that although the structures of the complexes are similar, the biological activity depends on the ligands and the metal center.

Conclusions

We have described the synthesis and characterization of twelve lanthanide complexes containing different cinnamate and phenylacetate ligands. These compounds have been evaluated as potential anti-parasitic agents against leishmaniasis, malaria and trypanosomiasis. The toxicity of the lanthanide complexes in U-937 cells has been evaluated and, at the concentrations used, none were found to be toxic. Ce(III) complexes displayed high anti-parasitic activity against *L. panamensis*, *P. falciparum* and *T. cruzi*. These results indicate that lanthanide coordination compounds with organic ligands could be used to develop novel therapeutic tools against tropical diseases.

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