Synthesis, antibacterial activity and DNA interactions of lanthanide(III) complexes of N(4)-substituted thiosemicarbazones

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Abstract

This paper reports the synthesis and detailed characterization of six novel lanthanide complexes of La(III), Eu(III) and Nd(III) with N(4)-substituted thiosemicarbazones derived from the 2-carboxybenzaldehyde. The IR, ^{1}H-NMR and ^{13}C-NMR spectroscopic studies confirmed the coordination of the thiocarbonyl (C=S), azomethine (C=N) and carboxylate (COO$^-$) groups to the metal centers, and the carboxylate was coordinated in a bidentate manner. The elemental and thermal analyses suggest that lanthanide complexes were formed in 1:2 molar ratios (metal:ligand). The molar conductivity values confirmed the non-electrolytic nature of the complexes. The interaction of these complexes with calf thymus DNA (CT-DNA) was investigated by UV absorption and viscosity measurements. It was found that the Eu(III) and Nd(III) complexes could roll along the DNA strands through groove interactions. Furthermore, lanthanide complexes could promote the oxidative cleavage of plasmid pBR322 in a high-oxidative stress environment. Finally, the Schiff base ligands (L) and their complexes were evaluated for their antibacterial activities against gram-positive and gram-negative bacteria using a microdilution procedure. The results indicate that the lanthanide complexes exhibit more potent antibacterial activity than the free ligands.

Keywords: Lanthanide complexes; DNA interaction; groove interaction; antibacterial activity; thiosemicarbazones

Introduction

In recent years, metal coordination complexes have been found to have various physicochemical properties which have been exploited in several fields such as catalysis, the synthesis of novel materials and medicinal chemistry [1-4]. Within the medicinal chemistry, many investigations have been conducted to find new antimicrobial agents; in these studies, several metal complexes have been identified as compounds of interest; metal complexes whose biological activities occur via different mechanisms [4]. Previous studies have been

carried out with the aim of discovering alternative treatments for several diseases and fighting antimicrobial resistance, which is a major issue within the field of infectious diseases. This phenomenon is currently increasing and it is now considered a global public health problem [5].

Thiosemicarbazones are Schiff bases, and they have shown potential biological activity against a large range of microorganisms that cause infectious disease, especially bacteria [6]. These types of molecules have also exhibited potent activity against some viruses and multiple types of neoplastic cells [7-9]. The use of Schiff bases for the synthesis of new coordination compounds for biological applications has been widely studied because of their electronic properties, allowing the presence of different coordination sites (electron donor groups, N and S) to which metal ions can bind. Metals bound to these groups can lead to a synergistic effect that improves the biological activity (**Fig. 1**) [10, 11].

Lanthanide complexes have also gained substantial interest for therapeutic applications. The unusual electronic configuration of the lanthanides gives them several features such as fluorescence and redox behavior that can be of high importance for some medicinal treatments including contrast, antimicrobial and chemotherapeutic agents [1, 2, 12].

Additionally, the studies on the interactions between metal complexes and DNA strands have offered some ideas regarding the possible mechanisms of action; numerous compounds that are cytotoxic to tumoral cells can bind to DNA strands. Thereby, the evaluation of the interactions that these types of molecules may have with the DNA provides an initial understanding of their biological activity [13-17].
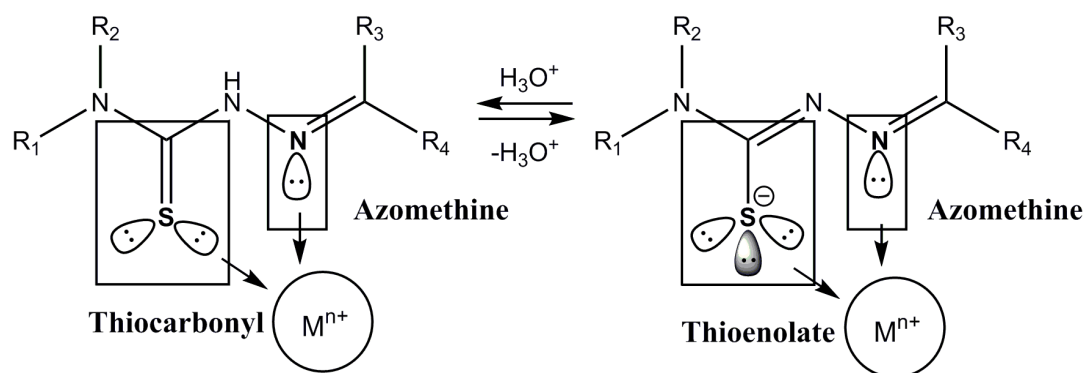
Figure 1. General structure of the thiosemicarbazones and coordination sites with the metal center (M^{n+}).

Thus, considering the potential therapeutic uses of thiosemicarbazones and lanthanide complexes and in order to contribute to the discovery of new molecules able to fight antimicrobial resistance, the aim of this research is to describe the synthesis and characterization of La(III), Nd(III) and Eu(III) complexes of $N(4)$-(4-R-phenyl) thiosemicarbazones derived from the 2-carboxybenzaldehyde. Additionally, to evaluate the interactions between DNA and the complexes by different techniques and to discuss the antibacterial assays.

Material and methods

All reagents for the synthesis were purchased from Sigma Aldrich, Alfa Aesar and Merck and were used without further purification. All solvents used were of analytical grade. The infrared spectra were obtained between 4000 cm^{-1} and 600 cm^{-1} on a Shimadzu Affinity 1 (FT-IR) device with an ATR accessory. For the inorganic region (between 600 cm^{-1} and 250 cm^{-1}) a NICOLET 6700 (FT-IR) Thermo Scientific spectrophotometer was used. IR spectra were acquired using from KBr pellets. Elemental analyses were carried out using a Thermo Scientific EA 112 series CHN Analyzer; and the lanthanide percentage was determined by complexometric titration with EDTA. Melting points were determined using an automatic OptiMelt MPA100 device. Conductivity measurements of the lanthanide complexes were performed in an Orion^{TM} 131S. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in DMSO-d_6 at 25 °C using a Bruker Ultrashield Avance II 400 spectrophotometer operating at 400 MHz, and chemical shifts (δ) are expressed in units of ppm with respect to (tetramethylsilane) TMS. The overall assignment of the NMR signals for the new compounds was supported by analyses of their 2D spectra (HMBC and HSQC). Thermal analyses were performed in a TGA 550 TA Instruments device under a nitrogen atmosphere. Mass spectra were taken with a Shimadzu-GCMS-QP2010 device with electronic impact ionization at 40 eV.

Synthesis of ligands

The syntheses of 2-carboxybenzaldehyde $N(4)$-phenylthiosemicarbazone (L1) and 2-carboxybenzaldehyde $N(4)$-(4-chlorophenyl) thiosemicarbazone (L2) were carried out as shown in **Fig. 2.** The syntheses were conducted following the standard methodologies that were previously reported [6, 18-22].

Synthesis of 2-carboxybenzaldehyde $N(4)$-phenylthiosemicarbazone (L1)

An ethanolic solution (3 mL) of aniline (0.55 mol·L^{-1}, 1.64 mmol) and potassium hydroxide (0.55 mol·L^{-1}, 1.64 mmol) was slowly added to 2 mL of
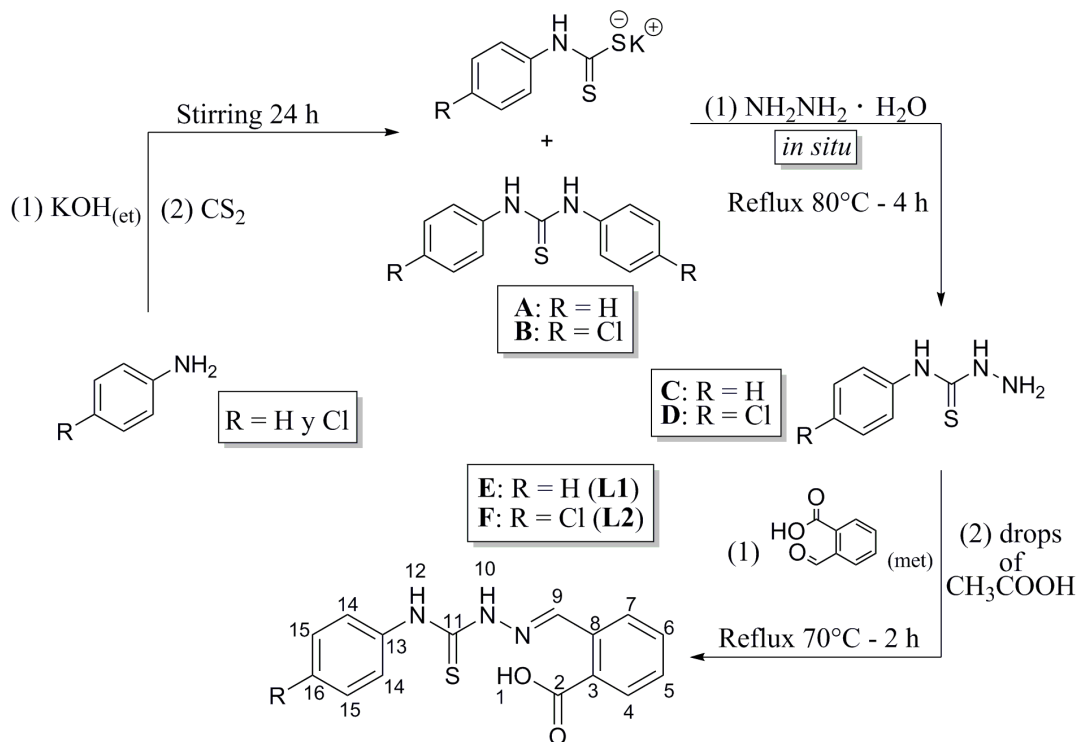
Figure 2. Synthetic route to L1 and L2.

carbon disulfide (33.1 mmol, excess) in an ice bath (4 °C), and the formation of two phases was observed immediately, and the mixture was stirred constantly for 24 hours at room temperature. Once the reaction was complete and a white solid in a yellow solution had been obtained, 80 μL of hydrazine monohydrate (1.65 mmol) was added, and the mixture was refluxed at 80 °C for 4 hours. Then, the solution was concentrated to 3 mL and cooled to 4 °C in an ice bath. Once cool, 5 mL of a cold, 1:1 mixture of hexane and dichloromethane was added to precipitate the *N*(4)-phenylthiosemicarbazide, and the solid was isolated by filtration and washed with 8 mL of cold hexane.

A methanolic solution (2.5 mL) of 2-carboxybenzaldehyde (0.48 mol·L^{-1}, 1.20 mmol) and three drops of glacial acetic acid were added to a methanolic solution (2.5 mL) of *N*(4)-phenylthiosemicarbazide (0.48 mol·L^{-1}, 1.20 mmol), and the system was refluxed at 70 °C for 2 hours. Once the reaction was complete, a white solid was observed (L1); the excess solvent was evaporated under reduced pressure, and the product was washed with 8 mL of cold hexane 3 times. White powder (yield: 341.3 mg, 95 %). m.p.: 203 - 204 °C.

$C_{15}H_{13}N_3O_2S$, Elemental Analysis; C, 59.92 (calc. 60.19); H, 4.32 (4.38); N, 13.97 (14.04); S, 9.57 (10.71) %. IR (ATR cm^{-1}) ν: 3331 m, 3181 m, 3014 m, 2831 w, 2577 w, 2456 w, 2360 w, 2341 w, 1666 s, 1601 w, 1589 w, 1545 m, 1515 m, 1481 m, 1389 m, 1240 s, 1202 s, 1068 s, 943 m, 820 m, 793 m, 745 s, 694 s. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 7.20 (t, J = 7.4 Hz, 1H, H16), 7.37 (t, J = 7.9 Hz, 2H, H15), 7.51 (td, J = 7.5 Hz, 1.2 Hz, 1H, H5), 7.59 (d, J = 8.2 Hz, 2H, H14), 7.61 (td, overlapped with H14, 1H, H6), 7.86 (d, J = 7.7 Hz, 1H, H7), 8.38 (d, J = 7.9 Hz, 1H, H4), 8.90 (s, 1H, H9), 10.08 (s, 1H, H12), 11.98 (s, 1H, H10), 13.32 (s, 1H, H1). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 125.3 (C16), 125.7 (C14), 127.5 (C4), 128.1 (C15), 129.6 (C5), 130.0 (C7), 131.0 (C3), 131.6 (C6), 134.0 (C8), 139.1 (C13), 142.1 (C9), 168.3 (C2), 176.2 (C11). MS (EI, 40 eV): m/z 299 [M+].

Synthesis of 2-carboxybenzaldehyde *N*(4)-(4-chlorophenyl) thiosemicarbazone (L2)

An ethanolic solution (3 mL) of aniline (0.67 mol·L^{-1}, 2.00 mmol) and potassium hydroxide (0.67 mol·L^{-1}, 2.00 mmol) was slowly added to 2 mL of carbon disulfide (33.1 mmol, excess) in an ice bath (4 °C), the formation of two phases was immediately observed, and the mixture was stirred constantly for 24 hours at room temperature. Once the reaction was completed and a white solid in a yellow solution had been obtained and 97 μL (2.00 mmol) of hydrazine monohydrate had been added, the mixture was refluxed at 80 °C for 4 hours. Then, the solution was concentrated to 3 mL and cooled to 4 °C in an ice bath, 5 mL of a cold, 1:1 mixture of hexane and dichloromethane was added to precipitate the *N*(4)-(4-chlorophenyl)thiosemicarbazide. The solid was isolated by filtration and washed with 8 mL of cold hexane.

A methanolic solution (2.5 mL) of 2-carboxybenzaldehyde (0.556 mol·L^{-1}, 1.39 mmol) and four drops of glacial acetic acid were added to a methanolic solution (2.5 mL) of *N*(4)-(4-chlorophenyl)thiosemicarbazide (0.556 mol·L^{-1}, 1.39 mmol), and the system was refluxed at 70 °C for 2 hours. Once the reaction was complete, a white solid was observed (L2), the solvent excess was evaporated under reduced pressure and the product was washed with 8 mL of cold hexane 3 times. Pale yellow powder (yield: 634.2 mg, 95 %). m.p.: 215-216 °C. $C_{15}H_{12}N_3O_2SCl$, Elemental Analysis; C, 54.12 (calc. 53.98); H, 3.60 (3.62); N, 12.42 (12.59); S, 9.20 (9.60)%. IR (ATR cm^{-1}) ν: 3332 w, 3176 m, 3015 m, 2588 w, 2448 w, 1670 s, 1604 w, 1588 w, 1548 s, 1512 m, 1486 m, 1383 m, 1266 m, 1226 s, 1194 s, 1069 s, 1009 m, 950 w, 821 s, 790 m, 757 m, 670 m, 632 m. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 7.42 (d, J = 8.7 Hz, 2H, H14), 7.51 (td, J = 7.5 Hz, 0.9 Hz, 1H, H5), 7.60 (t, J = 7.4 Hz, 1H, H6), 7.63 (d, J = 8.7 Hz, 2H, H15), 7.87 (dd, J = 8.1 Hz, 1.2 Hz, 1H,

H7), 8.38 (d, $J = 7.7$ Hz, 1H, H4), 8.92 (s, 1H, H9), 10.13 (s, 1H, H12), 12.06 (s, 1H, H10), 13.33 (s, 1H, H1). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 127.3 (C15), 127.5 (C4), 127.9 (C14), 129.3 (C16), 129.6 (C5), 130.0 (C7), 131.0 (C3), 131.6 (C6), 134.0 (C8), 138.0 (C13), 142.4 (C9), 168.2 (C2), 176.2 (C11). MS (EI, 40 eV): m/z 333 [M+].

Synthesis of the lanthanide complexes

The syntheses of the lanthanide complexes were carried out with carboxylic acids in aqueous solutions according to methodologies reported in previous investigations (**Fig. 3.**) [1, 2, 19]. The numeration of the protons in the complexes is the same as the one employed for the ligands.

Synthesis of [La(L1)$_2$(Cl)]·2H$_2$O (1)

Distilled water (14 mL) was added to 100 mg (0.330 mmol) of 2-carboxybenzaldehyde N(4)-phenylthiosemicarbazone, and the pH was adjusted to between 7 and 7.5 until all the solid was dissolved. Then, an aqueous solution (5 mL) of LaCl$_3$·7H$_2$O (0.033 mol·L^{-1}, 0.165 mmol) was added dropwise to the ligand solution. A white solid immediately appeared, and the mixture was stirred for 4 hours at 50 °C. Finally, the white solid was isolated by filtration and washed with 10 mL of distilled water and 10 mL of cold hexane. The compound was then placed in a desiccator for 2 days. White powder (yield: 242.6 mg, 90 %). m.p.: 208 - 209 °C. C$_{30}$H$_{28}$N$_6$LaO$_6$S$_2$Cl, Elemental Analysis; C, 44.06 (calc. 44.65); H, 3.69 (3.50); N, 11.54 (10.41); S, 7.71 (7.94); La, 17.27 (17.21) %. IR (KBr pellet cm^{-1}) ν: 3327 w, 3195 w, 1594 w, 1572 w, 1548 s, 1532 s, 1498 m, 1473 w, 1447 m, 1394 s, 1273 m, 1195 m,
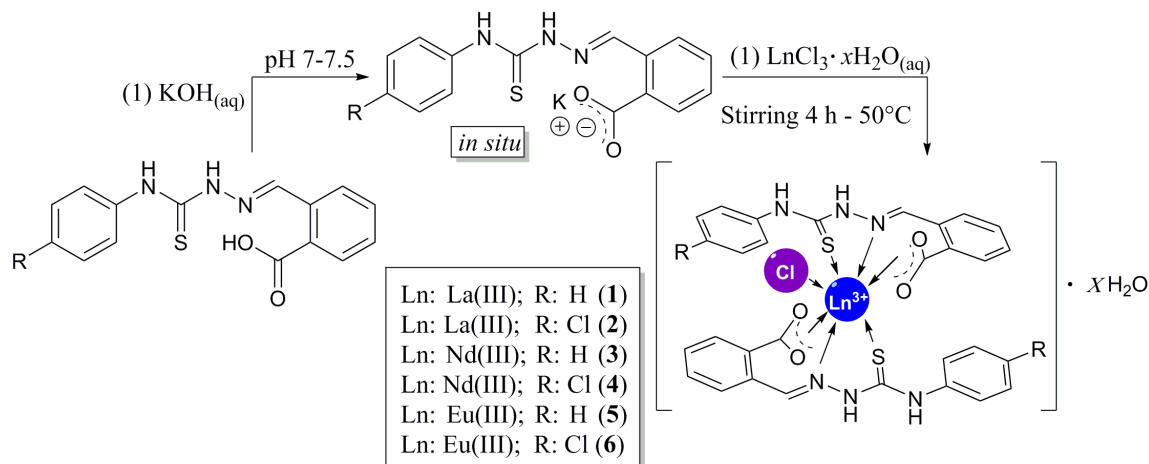
Figure 3. Synthesis of the lanthanide complexes.

1195 m, 1068 w, 938 w, 755 m, 744 m, 691 m, 463 w. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 7.18 (t, $J = 7.3$ Hz, 2H, H16), 7.35 (t, $J = 7.7$ Hz, 4H, H15), 7.40 - 7.42 (m, 4H, H5 and H6), 7.59 (d, $J = 7.5$ Hz, 4H, H14), 7.93 (d, $J = 7.7$ Hz, 2H, H7), 8.17 (d, $J = 8.4$ Hz, 2H, H4), 9.19 (s, 2H, H9), 10.03 (s, 2H, H12), 11.71 (s, 2H, H10). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 125.2, 126.6, 126.8, 128.1, 128.6, 128.8, 129.2, 129.6, 130.2, 139.2, 145.1, 176.0. Λ (DMSO, 28 °C) ($\Omega^{-1} \cdot cm^2 \cdot mol^{-1}$): 17.6.

Synthesis of [La(L2)$_2$(Cl)]·4H$_2$O (2)

Distilled water (14 mL) was added to 200 mg (0.600 mmol) of 2-carboxybenzaldehyde N(4)-(4-chlorophenyl)thiosemicarbazone, and the pH was adjusted to between 7 and 7.5 until all the solid dissolved. Then, an aqueous solution (5 mL) of LaCl$_3$·7H$_2$O (0.060 mol·L^{-1}, 0.300 mmol) was added dropwise to the ligand solution. A white solid immediately appeared, and the mixture was stirred for 4 hours at 50 °C. Finally, the white solid was isolated by filtration and washed with 10 mL of distilled water and 10 mL of cold hexane. The compound was then placed in a desiccator for 2 days. White powder (yield: 459.0 mg, 84 %). m.p.: 205 - 206 °C. C$_{30}$H$_{30}$N$_6$LaO$_8$S$_2$Cl$_3$, Elemental Analysis; C, 39.97 (calc. 39.51); H, 3.80 (3.32); N, 9.83 (9.22); S, 6.75 (7.03); La, 15.19 (15.23) %. IR (KBr pellet cm^{-1}) v: 3317 m, 1584 w, 1571 w, 1517 s, 1451 m, 1412 m, 1395 s, 1287 m, 1263 m, 1187 m, 1069 m, 1010 m, 938 m, 848 w, 829 m, 808 w, 768 m, 755 m, 680 m, 451 w. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 7.35-7.39 (m, 8H, H5, H6 and H14), 7.52 (d, $J = 8.6$ Hz, 4H, H15), 7.72-7.74 (m, 2H, H7), 8.09- 8.11 (m, 2H, H4), 8.95 (s, 2H, H9), 10.00 (s, 2H, H12), 11.55 (s, 2H, H10). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 128.3, 128.5, 129.6, 130.4, 130.5, 131.2, 132.9, 133.6, 134.5, 138.5, 145.7, 167.4, 176.7. Λ (DMSO, 28 °C) ($\Omega^{-1} \cdot cm^2 \cdot mol^{-1}$): 17.0.

Synthesis of [Nd(L1)$_2$(Cl)] (3)

The synthesis of 3 was conducted in a manner identical to the synthesis of 1. Pale violet powder (yield: 207.5 mg, 80 %). m.p.: 207 - 208 °C. C$_{30}$H$_{22}$N$_6$NdO$_4$S$_2$Cl, Elemental Analysis; C, 48.70 (calc. 46.41); H, 3.67 (3.12); N, 11.26 (10.82); S, 7.41 (8.26); Nd, 18.88 (18.58) %. IR (KBr pellet cm^{-1}) v: 3299 w, 1595 w, 1561 w, 1545 m, 1496 m, 1471 w, 1445 m, 1392 s, 1269 w, 1187 m, 1070 w, 940 w, 754 m, 744 s, 690 m, 453 w. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 7.2 (t, $J = 7.6$ Hz, 2H, H16), 7.37 (t, $J = 7.5$ Hz, 4H, H15), 7.62 (d, $J = 7.4$ Hz, 4H, H14), 7.80 (br, 2H, H5), 7.94 (br, 2H, H6), 8.62 (d, $J = 6.4$ Hz, 2H, H7), 9.59 (br, 2H, H4), 10.16 (s, 2H, H12), 10.29 (br, 2H, H9), 11.65 (s, 2H, H10). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 124.9, 125.4, 127.8, 128.0, 129.8, 130.1, 131.0, 27 145.3. Λ (DMSO, 28 °C) ($\Omega^{-1} \cdot cm^2 \cdot mol^{-1}$): 14.4.

Synthesis of [Nd(L2)$_2$(Cl)] (4)

The synthesis of 4 was carried out in a manner identical to the synthesis of 2. Pale violet powder (yield: 436.1 mg, 86 %). m.p.: 203 - 204 °C. $C_{30}H_{22}N_6NdO_4S_2Cl_3$, Elemental Analysis; C, 42.57 (calc. 42.63); H, 3.03 (2.62); N, 10.28 (9.94); S, 7.32 (7.59); Nd, 17.19 (17.06) %. IR (KBr pellet cm^{-1}) ν: 3316 m, 1583 m, 1555 w, 1520 s, 1479 m, 1449 m, 1415 s, 1395 s, 1341 w, 1263 m, 1188 m, 1088 m, 1070 m, 848 m, 828 m, 808 m, 768 m, 756 m, 684 w, 460 m. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 7.12 - 8.21 (br, H chlorophenyl moiety), 8.23 - 8.68 (br, 4H, H5), 9.17 - 9.74 (br, 4H, H6), 10.01 - 10.46 (br, 6H, H9, H12 and H 2-carboxybenzil moiety), 11.71 (s, 2H, H10). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 127.0, 127.6, 128.1, 129.7, 130.0, 131.0. Λ (DMSO, 28 °C) ($\Omega^{-1}\cdot$cm$^2\cdot$mol^{-1}): 12.2.

Synthesis of [Eu(L1)$_2$(Cl)] (5)

The synthesis of 5 was performed in a manner identical to the synthesis of 1. Pale yellow powder (yield: 238.4 mg, 91 %). m.p.: 206 - 207 °C. $C_{30}H_{22}N_6EuO_4S_2Cl$, Elemental Analysis; C, 46.78 (calc. 45.95); H, 3.59 (3.09); N, 11.11 (10.72); S, 7.27 (8.18); Eu, 18.59 (19.00) %. IR (KBr pellet cm^{-1}) ν: 3314 w, 1595 m, 1575 w, 1524 s, 1496 m, 1475 m, 1445 m, 1393 s, 1327 w, 1268 m, 1187 m, 1069 w, 941 w, 757 m, 745 s, 691 m, 640 w, 407 m. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 6.20 (s, 2H, H5), 6.80 (s, 2H, H6), 7.07 (s, 2H, H7), 7.16 (t, J = 7.2 Hz, 2H, H16), 7.32 (t, J = 7.5 Hz, 4H, H15), 7.45 (br, 2H, H9), 7.54 (d, J = 7.5 Hz, 4H, H14), 7.64 (d, J = 5.9 Hz, 2H, H4), 9.86 (s, 2H, H12), 11.72 (s, 2H, H10). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 125.1, 125.6, 128.0, 128.3, 129.2, 132.9, 139.1, 144.2, 175.9. Λ (DMSO, 28 °C) ($\Omega^{-1}\cdot$cm$^2\cdot$mol^{-1}): 13.9.

Synthesis of [Eu(L2)$_2$(Cl)]·H2O (6)

The synthesis of 6 was carried out in a manner identical to the synthesis of 2. Pale yellow powder (yield: 417.5 mg, 80 %). m.p.: 213 - 214 °C. $C_{30}H_{24}N_6EuO_5S_2Cl_3$, Elemental Analysis; C, 41.57 (calc. 41.37); H, 3.07 (2.78); N, 9.93 (9.65); S, 7.16 (7.36); Eu, 18.24 (17.85) %. IR (KBr pellet cm^{-1}) ν: 3320 m, 1584 w, 1563 w, 1522 s, 1477 m, 1449 w, 1395 s, 1324 w, 1287 m, 1262 m, 1186 s, 1089 m, 1070 m, 1011 m, 940 m, 849 w, 828 m, 808 w, 768 m, 756 m, 455 m. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 6.18 (br, 2H), 6.82 - 7.56 (m, 18H), 9.92 (s, 2H, H12), 11.74 (s, 2H, H10). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 127.2, 127.9, 136.0. Λ (DMSO, 28 °C) ($\Omega^{-1}\cdot$cm$^2\cdot$mol^{-1}): 11.4.

DNA interaction assays

The DNA interaction studies with the obtained compounds were accomplished by electronic absorption experiments, oxidative cleavage monitored by agarose gel electrophoresis and viscosity measurements. Highly polymerized and lyophilized calf thymus DNA (CT-DNA) from Sigma Aldrich was employed, and the pBR322 vector purchased from Promega was also used. All the DNA solutions had an A_{260}/A_{280} ratio between 1.8 and 1.9, indicating that the DNA was free of RNA and proteins. DNA resuspensions were carried out using a solution of Tris (10 mmol·L^{-1}) and EDTA (1 mmol·L^{-1}) in deionized water with the pH adjusted to approximately 7.5. The DNA solutions were stored at –5 °C.

Electronic absorption monitoring assays (UV-Vis)

The electronic absorption spectra were recorded in an UV-visible Jasco V-630BIO spectrophotometer equipped with a PAC-743 accessory for temperature control. CT-DNA titrations were carried out to a constant concentration of compound at 25 °C. After each addition of titrant, the electronic absorption spectra of the evaluated compound was taken between 230 nm and 500 nm. A CT-DNA solution (4022 μmol·L^{-1}) in nucleotides was prepared using $\varepsilon_{260} = 6600$ L·cm^{-1}·mol^{-1}· as the molar extinction coefficient and measuring the absorbance at 260 nm; the stock solution was stored at –5 °C.

Oxidative cleavage assays monitored by agarose gel electrophoresis

The oxidative cleavage catalyzed by the lanthanide complexes was studied by incubating different solutions (24 μL each) that contained constant concentrations of pBR322 and H_2O_2 with variable concentrations of the complexes in DMSO. The samples were incubated under physiological conditions (37 °C and pH 7.0) in an Eppendorf Thermomixer® C for 90 minutes. Once incubated, the samples were loaded onto a 1 % agarose gel with TAE 1X buffer, and HyperLadder™ 1 kb from Bioline used as the molecular weight marker. An Owl™ EasyCast™ B1 Mini Gel electrophoresis system was used for the electrophoresis runs, and GelGreen™ was used as the dye to visualize the gel in an UltraBright Led 470 nm MaestroGen transilluminator.

Viscosity measurements

The relative viscosities of the five different DNA solutions were determined. The solutions contained a constant concentration of CT-DNA and increasing amounts of the test compounds. A stock solution of CT-DNA in nucleotides

(402 μmol·L^{-1}) and a Semi-Micro Cannon-Ubbelohde viscometer 75 were used for determining the viscosity; the temperature of the solutions was controlled by using a thermal bath at 22 °C. The efflux times were recorded by processing the videos photograms in Camtasia Studio® 8 software.

Antibacterial assays

The antibacterial activities of all the compounds were evaluated by determining their minimal inhibitory concentrations (MICs); and standard microdilution procedures for antimicrobial susceptibility were followed [23, 24]. All compounds were tested against five different ATCC bacterial strains: ATCC 25923 (*Staphylococcus Aureus*), ATCC 25922 (*Escherichia Coli*), ATCC 14028 (*Salmonella Typhimurium*), ATCC 2146 and ATCC 1705 (*Klebsiella Pneumoniae*); the evaluated concentration range was from 2000 μg·mL to 4 μg·mL. The negative control consisted of Muller-Hinton broth (MHB) in the absence of bacteria, the positive control consisted of MHB with only bacteria, and no commercial bactericide was employed as reference. An additional study was carried out to evaluate the possible effect of the solvent on the biological screening, and it was observed that DMSO did not show any activity against any of the tested strains.

Results and discussion

Synthesis and characterization of ligands

Thiosemicarbazones were obtained through the nucleophilic addition of the corresponding *para*-substituted anilines to the carbon disulphide; in this step, the thiocarbamate potassium salt was synthesized *in situ*. The white solids that precipitated at this stage were identified as 1,3-diphenylthiourea (see **Fig. 2a**) (MS (EI, 40 eV): [M+] m/z 228) and 1,3-bis (4-chlorophenyl)thiourea (see **Fig. 2b**) (MS (EI, 40 eV): [M+] m/z 297), which were also useful for the synthesis of thiosemicarbazides (see **Fig. 2c** and **Fig. 2d**) by the substitution with monohydrated hydrazine. The identification of N(4)-phenylthiosemicarbazide (m.p.: 140 - 141 °C) and N(4)-(4- chlorophenyl) thiosemicarbazide (m.p.: 165 - 167 °C) was conducted by comparing their melting points and IR absorption bands with those reported in previous investigations [18, 20]. After the Schiff base condensation with 2-carboxybenzaldehyde, novel ligands L1 and L2 were obtained as solid powders. They were soluble in DMSO, acetone, DMF and ethyl acetate, and they were slightly soluble in water, ethanol and methanol.

Table 1. Assignments of the characteristic IR absorption bands for the ligands and lanthanide complexes (cm^{-1}).

Compound	ν(C = O) (COOH)	ν(C = N)	ν_{asym}(COO⁻)	ν_{sym}(COO⁻)	$\Delta\nu$(COO⁻)*	ν(C = S)	ν(Ln - O)
L1	1666	1601	-	-	-	793	-
L2	1670	1604	-	-	-	790	-
1	-	1572	1548	1394	154	755	463
2	-	1571	1517	1395	122	768	451
3	-	1561	1545	1392	153	754	453
4	-	1555	1520	1395	125	768	460
5	-	1575	1524	1393	131	757	407
6	-	1563	1522	1395	127	768	455

Synthesis and characterization of lanthanide complexes

All lanthanide complexes (1-6) were isolated as solid powders. They were soluble in DMSO, acetone and DMF, and they were slightly soluble in water, ethanol, methanol and ethyl acetate. The molar conductivities of the complexes in DMSO at 28 °C were found to be between 10 Ω^{-1}·cm²·mol^{-1} and 18 Ω^{-1}·cm²·mol^{-1}, which confirmed the absence of chloride ions (Cl⁻), the non-electrolytic nature of the complexes and the possible coordination of the chloride atom to the metal center [18, 25]. Furthermore, the elemental analyses of the complexes showed a 1:2 (metal to ligand) stoichiometry.

Moreover, the infrared spectroscopic characterization provided important information about the coordination sites in the lanthanide complexes. The shifts to lower energy occurred in the vibrational bands corresponding to the thiocarbonyl (C=S) and azomethine (C=N) groups, which confirmed the participation of these as coordination sites. In addition, the absence of the bands typical of a carboxyl (COOH) group also indicated the coordination to the metal ion. The differences between the symmetric and asymmetric vibrational modes of the carboxylate group in the complexes were between
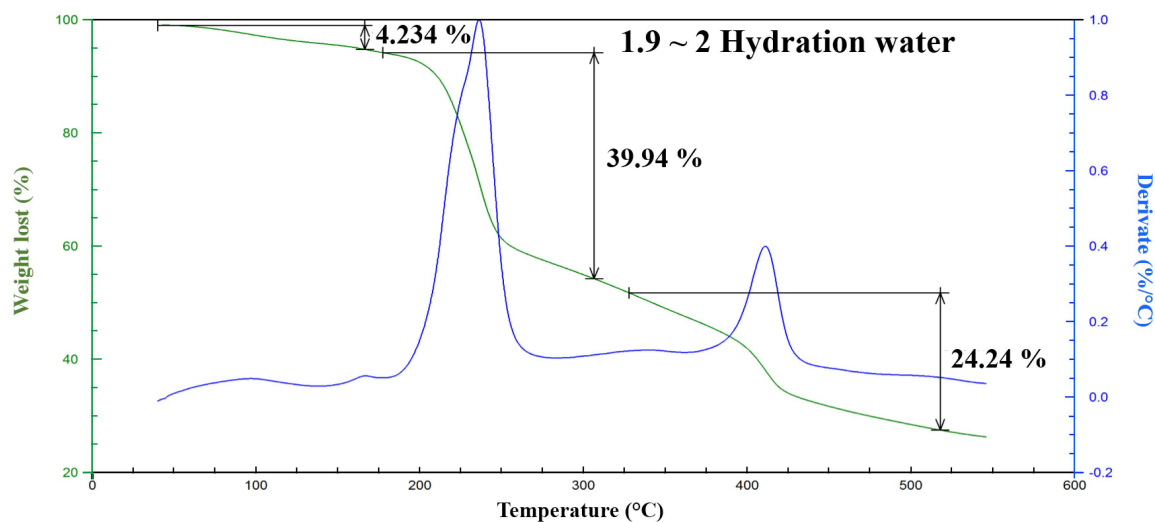
Figure 4. TGA of 1.

120 cm^{-1} and 160 cm^{-1}; this could indicate that the functional group is binding in a bidentate manner. **Table 1** shows the most characteristic frequency assignments for each compound [1, 17, 18, 26].

All proposed molecular formulas for the complexes are consistent with the percentages found in the elemental analyses, and the presented water molecules are placed in the external coordination sphere as waters of hydration according to the thermal analyses. **Fig. 4** and **Fig. 5** show the thermal behaviors of complexes 1 and 6 as representative complexes.

The presence of water molecules in the external coordination sphere was confirmed only for complexes 1, 2 and 6 . All the compounds were highly stable up to 200 °C at which point they thermally decomposed. The first step in this process seems to be the loss of one of the ligands at approximately 230 °C, which coincides with the mass loss observed at that stage. At approximately 300 °C, another thermal degradation event is observed, and in this last stage, the loss of the second ligand is likely and leads to the formation of a carbonate derivate [1, 2].

The complexes (except those of La(III)) are paramagnetic. ^{1}H-NMR and ^{13}C-NMR analyses were performed for La(III), Nd(III) and Eu(III) complexes, and the results confirmed that thiocarbonyl, azomethine and carboxylate participate in coordination to the lanthanide. The absence of the H1 signal in the spectra is an evidence that the proton had been replaced by the metal ion.
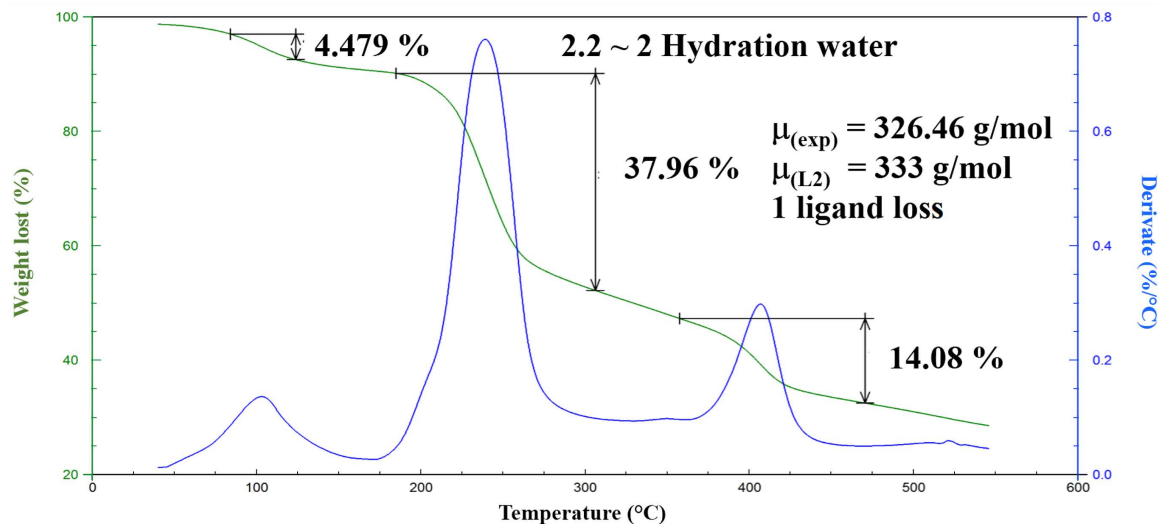
Figure 5. TGA of 6 showing the loss of one ligand.

The signals of H9, H10 and H12 are shifted due to the presence of the metal; for complexes 1 and 2, the signal of H9 is shifted downfield due to the inductive effects after the coordination of the azomethine group (C=N). The signals of H10 and H12 are shifted upfield due to the decrease in the inductive effects form the thiocarbonyl group (C=S) once it participates in metal coordination. Similarly, the signals of H10 and H12 for complexes 5 and 6 are shifted upfield relative to the corresponding signals of the ligands L1 and L2; however, the H9 signal is also shifted upfield, indicating that the Eu(III) complexes may be in a different spatial arrangement with the H9 protons possibly facing the metal center (electron source); although, the participation of the azomethine (C=N) in the coordination was confirmed by the IR spectra. Additional shifts in the proton signals assigned to aromatic systems were found for all complexes due to the different chemical environments of the complexes compared to those in the ligands. The broadening of the NMR signals of complexes 4 and 6 may be a consequence of the paramagnetic character of the lanthanide ions, Nd (III) and Eu (III), which have very short relaxation times due to the small difference between the ground state and the excited state energies. In addition, for the complexes with L2, a slight decrease in solubility was observed; thus, the signal can be a mixture of absorption and dispersion. **Fig. 6** shows the changes in the chemical shifts for complexes 1-6 with respect to their precursors.
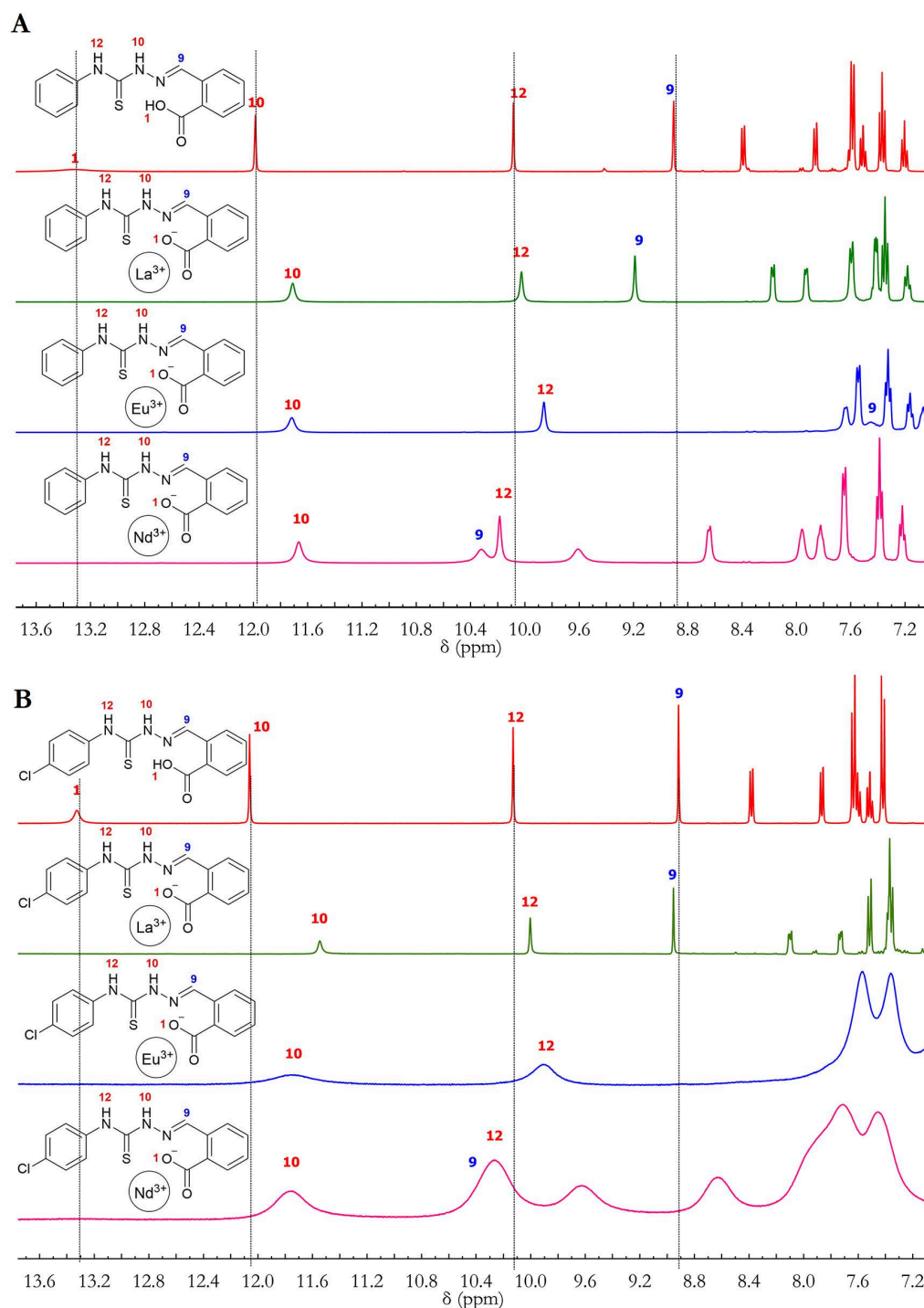
Figure 6. A) ^{1}H-NMR spectra superposition of L1 (red), **1** (green), **3** (purple) and **5** (blue); **B)** ^{1}H-NMR spectra superposition of L2 (red), **2** (green), **4** (purple) and **6** (blue).

The signal assignments were supported by the 2D experiments (HSQC and HMBC). The ^{13}C-NMR spectra also showed shifts in signals of C2, C11 and C9, which supports the participation of the carboxylate (COO-), thiocarbonyl (C=S) and azomethine (C=N) groups in coordination.

DNA interaction assays

The mode of interaction between the DNA strands and the new molecules with biological applications provides an initial indication of its possible biological activity and mechanism of action. On one hand, the binding ability of the studied molecule is a clear signal of its competence for biological applications [17], because the presence of this type of interaction leads to a cytotoxic effect that causes cell apoptosis [14]; however, the absence of this ability does not mean that the evaluated molecule must be discarded as it could exhibit its activity through a different mechanism such as enzymes inhibition or blocking the membrane receptor [27]. Thus, the assays for determining the mode of interaction between the DNA and ligands L1 and L2 and complexes 1-6 were carried out to determine their binding modes and catalytic abilities in the oxidative cleavage of the strands.

Electronic absorption monitoring assays (UV-Vis)

Figure 7 shows the effect of the addition of CT-DNA between 5 μmol·L^{-1} to 40 μmol·L^{-1} in nucleotides to a solution of the compounds at 20 μmol·L^{-1}. The solutions of the complexes contained approximately 10 % DMSO to improve the solubilities of the compounds in the resuspension buffer.

Monitoring the electronic absorption spectra of the studied compound when adding DNA was one of the techniques used to observe the interactions between these two species. The presence of hypochromism and bathochromism is an indicator of nitrogen base intercalation; this is due to the intercalation of the compound between the nitrogen base pairs which hide its chromophores; thus, changing the molecular orbitals, causing minor exposure to radiation and reducing the molar extinction coefficient [15]. Otherwise, a hyperchromic effect is a sign of a change in the tertiary structure of the biopolymer promoted by the compound and could be evidence of DNA scission; this effect is caused by the major exposure of the nitrogen base pairs when unrolling or splitting [28-30].

No net effect was observed for the bands at approximately 315 nm ($\pi \rightarrow \pi^*$) in all the compounds, which suggests that the aromatic groups are not intercalating between the base pairs. However, for complexes 1, 3 and 6, a slight redshift around the 240 nm (n$\rightarrow \pi^*$) is observed. This shift could indicate binding to the DNA groove [17, 31].

This redshift can be explained by considering that electron donor groups are responsible for the n$\rightarrow \pi^*$ transitions, and these groups are involved in metal coordination and are also the one nearest to the metal, which more closely interacts with the phosphate chain of the biopolymer grooves [28].
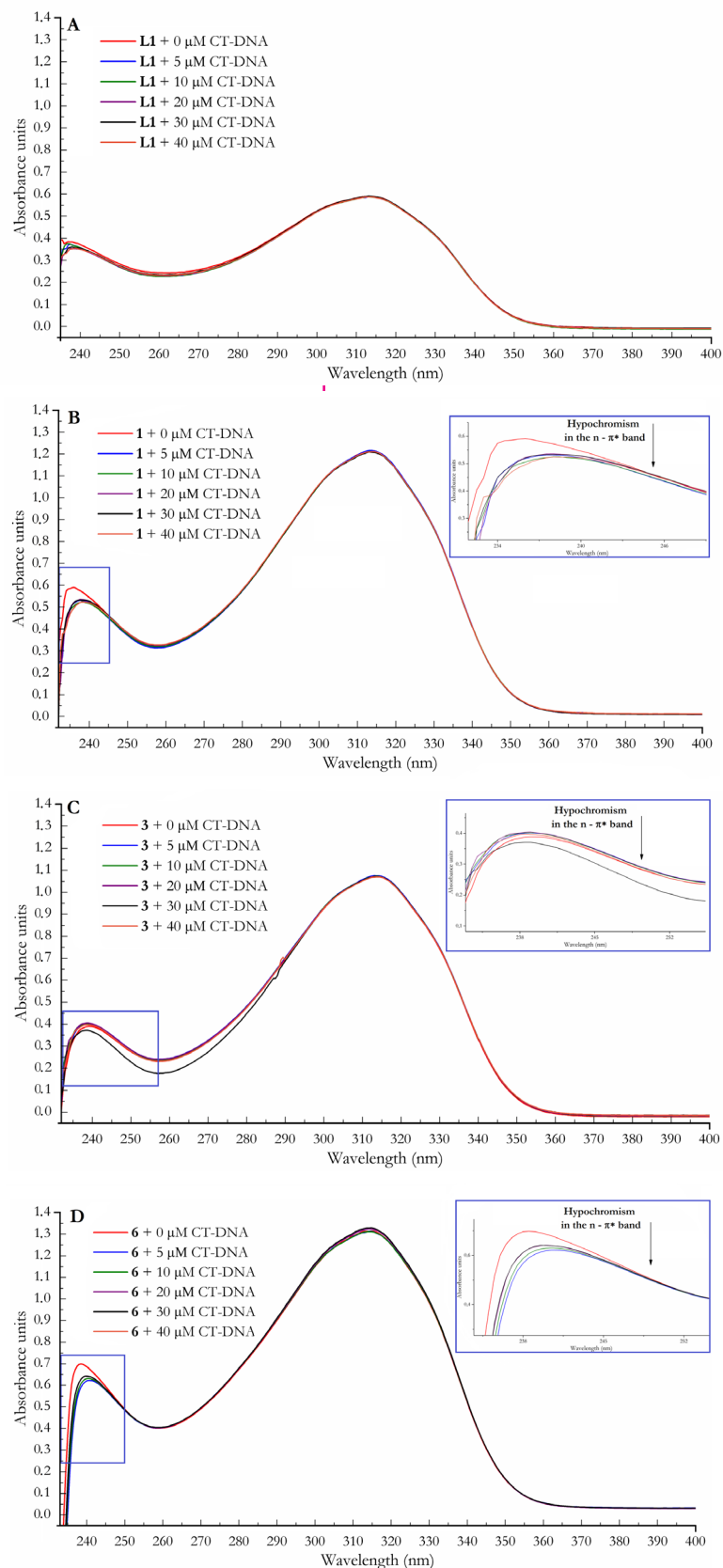
Figure 7. Electronic absorption spectra for (A) L1; (B) L2; (C) 1; (D) 2; (E) 3; (F) 4; (G) 5 and (H) 6 (red) at a constant concentration of 20 μmol·L^{-1} titrated with CT-DNA to concentrations of 5 μmol·L^{-1} (blue), 10 μmol·L^{-1} (green), 20 μmol·L^{-1} (violet), 30 μmol·L^{-1} (black) and 40 μmol·L^{-1} (orange) in nucleotides.

Viscosity measurements

The viscosity of a DNA solution at a certain temperature can change if its structure is altered [32]. To observe the compounds' effect on DNA viscosity, five CT-DNA solutions at 80 μmol·L^{-1} in nucleotides (with different compound quantities) were evaluated. Here, as in the previous analysis, the solutions contained some amount of DMSO below 10 % to solubilize the compounds in the resuspension buffer.

Figure 8 shows the changes in the viscosities of the CT-DNA solutions in the presence of the evaluated compounds. The viscosities were calculated by finding the ratio between the kinetic viscosities of the mixtures and that of free DNA using the viscometer constant and efflux times.

The general trend for compounds L1, L2, and complexes 1, 2, 5 and 6 is that they reduce the relative viscosity of CT-DNA; however, the Nd(III) complexes did not seem to follow any certain pattern. The reduction in the CT-DNA viscosity can mainly be attributed to two three-dimensional alterations in the DNA structure. The first one is the reduction in the size of some linear sections of the DNA caused by a possible rolling; this results in a lower probability of intermolecular crossing, thus, reducing the viscosity. The second cause is DNA cleavage in which the solution with free nucleotides possess a lower viscosity than that with the entire biopolymer [30]. For the case of the evaluated compounds, it is possible that the DNA interactions are occurring through groove binding, which induces partial rolling and no DNA cleavage. This was confirmed because if there was DNA scission occurring, a strong hyperchromism would be observed in all the electronic absorption spectra. However, the changes in the DNA viscosity caused by all the compounds are small in comparison with other types of molecules evaluated in other investigations [17, 29-33]. The strongest interactions were observed for Eu(III) complexes 5 and 6, suggesting that reducing the ionic radio affects the strength of the interactions. This change is because the Pearson acidity increased, enhancing the affinity for the phosphate ions in the DNA backbone.

Oxidative cleavage assays monitored by agarose gel electrophoresis

The catalytic effects of L1, L2, and complexes 1, 2, 5 and 6 in the cleavage of DNA strands, which is initiated by reactive oxygen species (ROS), were tested by agarose gel electrophoresis. **Fig. 9** shows the preference of one of the three pBR322 vector conformations when incubating in several solutions containing plasmid, hydrogen peroxide (ROS generator) and different amounts of the test compounds.
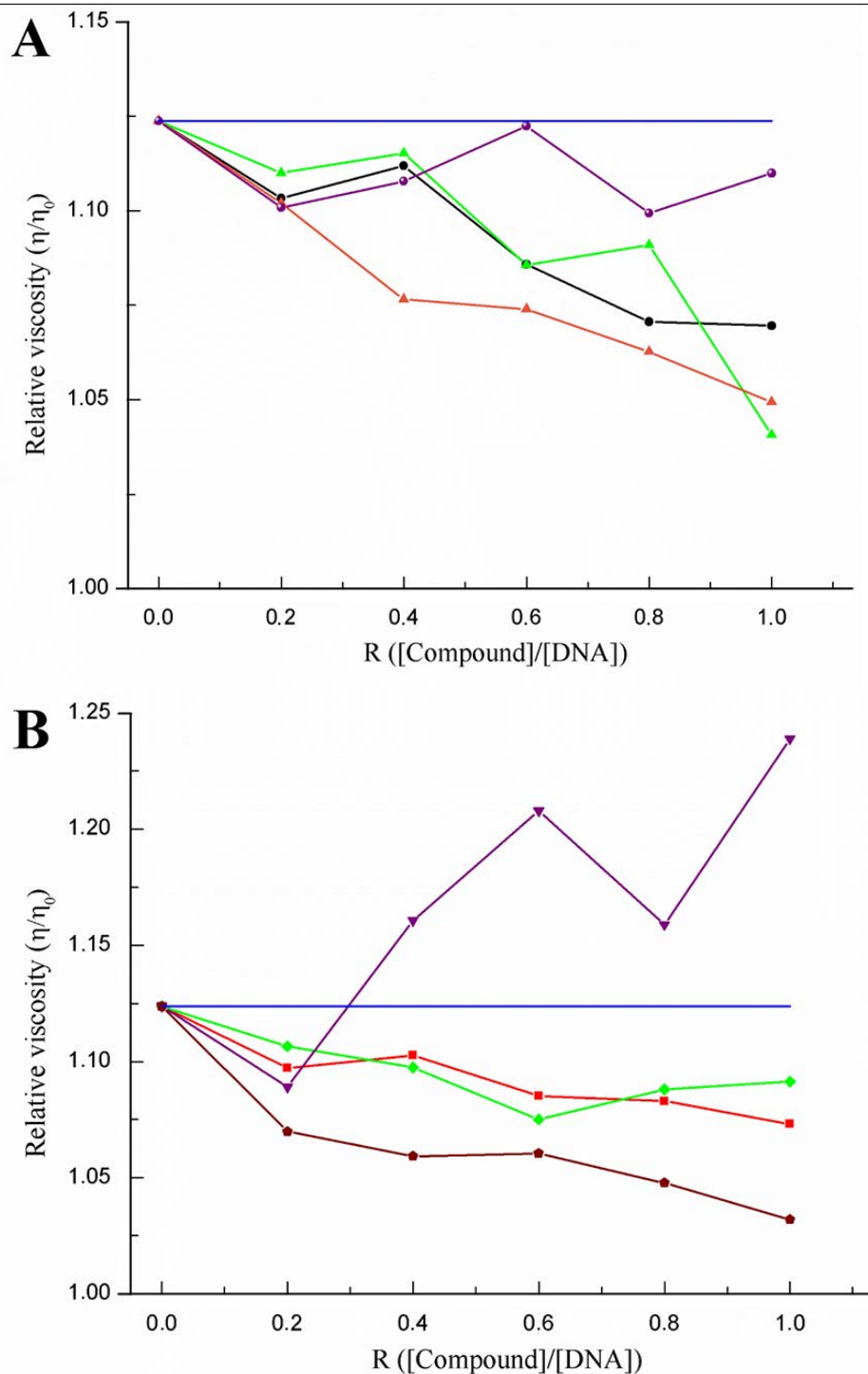
Figure 8. Effect of increasing amounts of compound (R = [compound]/[DNA]) between 0.2 and 1) on the relative viscosities of the CT-DNA solutions at 80 μmol·L^{-1} (blue line). (A) L1 (black), 1 (green), 3 (violet) and 5 (orange); (B) L2 (red), 2 (green), 4 (violet) and 6 (brown).

Plasmids are circular DNA that can adopt several forms or conformations. Form I corresponds to the rolled or bent plasmid, which possesses great mobility in agarose gel; form II is the relaxed circular conformation, which has lower mobility than form I, because the circular form gets stuck more

frequently in the agarose polymer; form III corresponds to the linear conformation that is generated from the cleavage of form II, and this form is much more mobile than form II [15, 34].

For compounds L1, L2, and complexes 3 and 4 (see **Fig. 9a**, **Fig. 9b**, **Fig. 9c** and **Fig. 9d**) no differences in their oxidative cleavage activities compared to that of the peroxide control were observed (Lane 3); however, a slight decrease in intensity in the line that corresponds to form I is seen, which suggests a possible decrease in the amount of this form of the plasmid.

Furthermore, a concentration dependence in the oxidative cleavage by Eu(III) complexes 5 and 6 was observed, and the band corresponding to form II increased in intensity as the amount of complex increases (see , **Fig. 9e** and **Fig. 9f**, lanes 5 to 8), which means that the relaxation of the plasmid to form II is favored. This could be explained by comparing the redox properties of
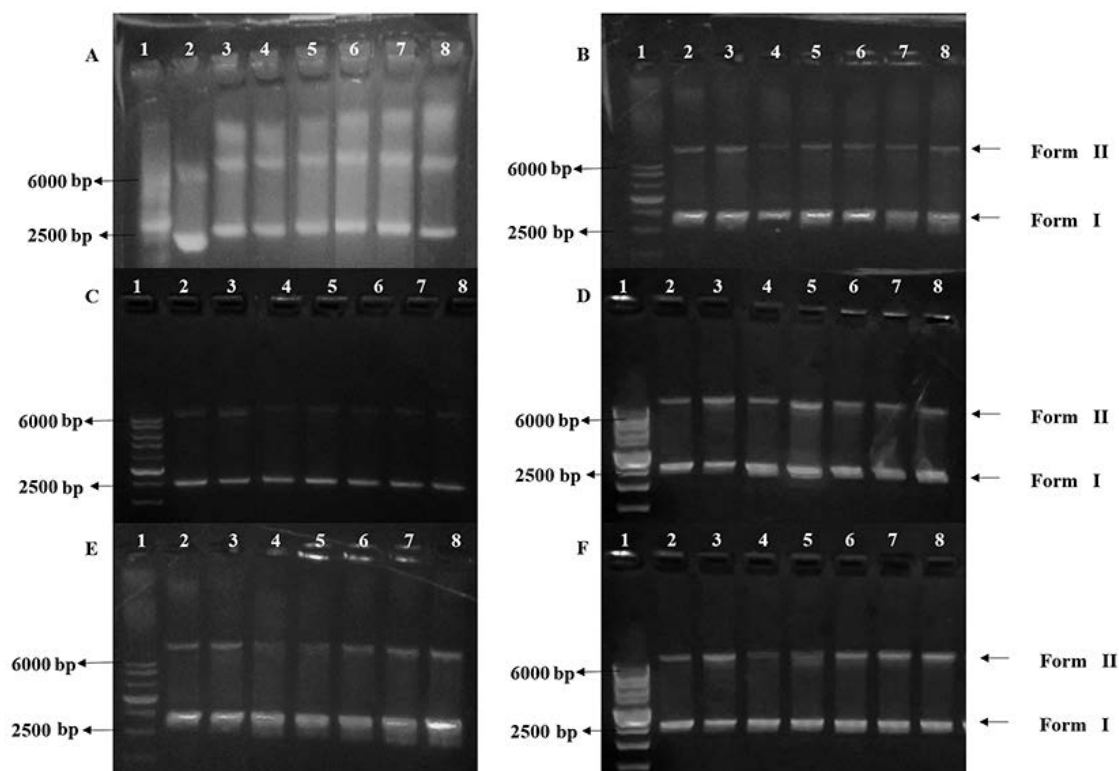
Figure 9. Agarose gel electrophoresis pattern showing the oxidative cleavage of the plasmid pBR322 (5 ng·mL^{-1}) with H$_2$O$_2$ (500 μmol·L^{-1}) and various concentrations of the test compounds. Lane 1: HyperLadder 1 kb (10 μmL); lane 2: only DNA; lane 3: DNA + H$_2$O$_2$; lane 4: DNA + compound (60 μmol·L^{-1}); lane 5: DNA + H$_2$O$_2$ + compound (20 μmol·L^{-1}); lane 6: DNA + H$_2$O$_2$ + compound (50 μmol·L^{-1}); lane 7: DNA + H$_2$O$_2$ + compound (70 μmol·L^{-1}); lane 8: DNA + H$_2$O$_2$ + compound (100 μmol·L^{-1}). (A) L1; (B) L2; (C) 3; (D) 4; (E) 5 and (F)6.

the three metal ions (La(III), Eu(III) and Nd(III)). Eu(III) possesses more stable redox states (it can go from 3+ to 2+ and vice versa) compared to La(III) and Nd(III), which can only stay in the 3+ oxidation state. This behavior leads to a strong interaction between the reactive oxygen species and the DNA strands [34].

Otherwise, a reduction in the intensity in the line corresponding to form II for complexes 3, 5 and 6 (see **Fig. 9c**, **Fig. 9e** and **Fig. 9f**, lane 4) with respect to the DNA control in lane 2 is observed. This indicates that the complexes convert the plasmid from form II to form I as a consequence of induced rolling.

To elucidate the role of reactive oxygen species (ROS) in the cleavage of DNA pBR322 induced by the complexes, a preliminary study was conducted that suggested that H_2O_2 and the hydroxyl radical scavenger (DMSO) have little effect on the scission of DNA. When the experiment was carried out in the presence of KI, the content of form I was markedly reduced. This suggests that the species derived from hydrogen peroxide are those that actually take part in the DNA cleavage, in the experiments carried out using the Eu(III) complexes, the active participation of the complexes in the conversion of form II to I was observed, which confirmed the results obtained in the previous assays.

The interaction modes observed in all the assays could be possible pathways for cytotoxic activity against a range of microorganisms. DNA binding could lead to interruption of the protein transcription mechanisms, which could be very useful against tumoral cells as well [28, 29].

Antibacterial assays

Table 2 presents the MICs determined by the microdilution assays for the selected compounds against *Staphylococcus Aureus* (ATCC 25923).

The antibacterial activities of the compounds were lower for gram-negative bacteria (ATCC 25922 (*Escherichia coli*), ATCC 14028 (*Salmonella Typhimurium*), ATCC 2146 and ATCC 1705 (*Klebsiella pneumoniae*) over the evaluated concentration range; the obtained MICs were 2000 μg·mL^{-1} or higher. However, for the gram-positive strain ATCC 25923 (*Staphylococcus aureus*), complex 6 was found to have a MIC of 63 μg·mL^{-1} and major bactericidal behavior was observed in general. This result suggests that the cytotoxicity activity against bacteria may be dependent on the type of cell wall. Additionally, based on the MICs obtained for the ligands and the complexes, the biological activity was enhanced by the presence of the metal center.

Table 2. Minimal inhibitory concentrations (MICs) found for the obtained compounds (mg·mL⁻¹).

Compound	*Staphylococcus aureus* ATCC 25923	*Escherichia coli* ATCC 25922	*Salmonella Typhimurium* ATCC 14028	*Klebsiella pneumoniae* ATCC 2146	*Klebsiella pneumoniae* ATCC 1705
L1	125	>2000	2000	>2000	>2000
L2	125	>2000	2000	>2000	>2000
1	125	>2000	2000	>2000	>2000
2	125	>2000	2000	>2000	>2000
3	250	>2000	2000	>2000	>2000
4	125	>2000	2000	>2000	>2000
5	125	>2000	2000	>2000	>2000
6	63	>2000	2000	>2000	>2000

According to the experimental results obtained in this study, the antimicrobial activity of the compounds may be influenced by the partial release of small and hard Eu(III) ions that can more efficiently affect the biosynthesis of peptidoglycan in the cell wall of the gram-positive bacteria, causing structural modifications and subsequent cell death. In addition to the conductivity, solubility and dipole moment, which are affected by the presence of metal ions, another possible mechanism that can explain the antimicrobial activity is chelation; and the coordination of thiosemicarbazones to the Lanthanide ions improves the lipophilicity of the complexes, thus favoring their penetration into the lipid membrane of the cell wall. In general, the complexes can inhibit a series of enzymes that play important roles in different metabolic processes that affect the development and growth of microorganisms. This is a similar result to those found in the DNA interaction assays, where the Eu(III) complexes were expected to have substantial biological activities based on the strength of its interaction with the strands.

Conclusions

To evaluate the potential biological activities that novel molecules could present in the fight against antimicrobial resistance and the discovery of new disease treatments, six new lanthanide complexes of La(III), Eu(III) and Nd(III) with *N*(4)-substituted thiosemicarbazones derived from the 2-carboxibenzaldehyde were

synthesized to evaluate their interaction modes with DNA strands. The syntheses of 2-carboxybenzaldehyde N(4)-phenylthiosemicarbazone and 2-carboxybenzaldehyde N(4)-(4-chlorophenyl) thiosemicarbazone were carried out by preparing the corresponding thiosemicarbazides and condensing the subsequent Schiff base with 2-carboxybenzaldehyde. This was an efficient route for obtaining high-pure ligands with yields over 90 %. The lanthanide complexes were also obtained in high yields (over 80 %) and high purity. Additionally, they showed 1:2 (metal to ligand) stoichiometry in which metal ions are coordinated to the thiocarbonyl (C=S), azomethine (C=N) and carboxylate (COO-) groups (with the carboxylate serving as a bidentate ligand) according to IR, ^{1}H-NMR and ^{13}C-NMR spectral analyses. The low molar conductivities corroborated the non-electrolytic nature of the complexes and the possible coordination of one chlorine atom.

According to the results obtained from the viscosity measurements, electronic spectra and oxidative cleavage reactions, complexes 3, 5 and 6 can interact with the DNA strands through the phosphate backbone and promote bending or rolling of the plasmids. Furthermore, the Eu(III) complexes catalyzed the oxidative cleavage of the DNA under high-oxidative-stress conditions. The oxidative cleavage by the complexes was concentration-dependent; the amount of complex increased the amount of cleavage to form II. This behavior was attributed to the redox properties of the Eu(III) ion. The antibacterial activity assays showed that the complexes possess bactericidal properties against the gram-positive strain ATCC 25923 (*Staphylococcus Aureus*) with MICs up to 63 μg·mL^{-1} for complex 6. Additionally, a cell-wall dependence was found in the biological activity of the lanthanide complexes; they may be more active against gram-positive bacteria. A synergistic effect of the metal center was also observed as higher inhibitions were obtained with the Eu(III) complexes compared to those observed with the ligands.

Acknowledgements

Conflict of interest

The authors declare that there are no conflicts of interest.

References

[1] Aragón-Muriel A, Camprubí-Robles M, González-Rey E, Salinas-Castillo A, Rodríguez-Diéguez A, Gómez-Ruiz S, Polo-Cerón D. Dual investigation of lanthanide complexes with cinnamate and phenylacetate ligands: Study of the cytotoxic properties and the catalytic oxidation of styrene, *Polyhedron*, 80: 117-128, 2014.

doi: 10.1016/j.poly.2014.02.040

[2] Aragón-Muriel A, Polo-Cerón D. Synthesis, characterization, thermal behavior and antifungal activity of La(III) complexes with cinnamates and 4-methoxyphenylacetate, *Journal of Rare Earths*, 31: 1106-1113, 2013.

doi: 10.1016/S1002-0721(12)60412-8

[3] Ishida S, Lee J, Thiele DJ, Herskowitz I. Uptake of the anticancer drug cisplatin mediated by the copper transporter Ctr1 in yeast and mammals, *Proceedings of the National Academy of Sciences of the United States of America*, 99: 14298-14302, 2002.

doi: 10.1073/pnas.162491399

[4] Guo Z, Sadler PJ. Metals in Medicine, *Angewandte Chemie International Edition*, 38: 1512-1531, 1999.

doi: 10.1002/(SICI)1521-3773(19990601)38:11<1512::AID-ANIE1512>3.0.CO;2-Y

[5] Diaz Granados CA, McGowan JEJ. Antimicrobial Drug Resistance, Humana Press, New Jersey, USA 2009.

[6] Sriram D, Yogeeswari P, Dhakla P, Senthilkumar P, Banerjee D, Manjashetty TH. 5-Nitrofuran-2-yl derivatives: Synthesis and inhibitory activities against growing and dormant mycobacterium species, *Bioorganic and Medicinal Chemistry Letters*, 19: 1152-1154, 2009.

doi: 10.1016/j.bmcl.2008.12.088

[7] Stefani C, Jansson P J, Gutierrez E, Bernhardt PV, Richardson DR, Kalinowski DS. Alkyl substituted 2'-Benzoylpyridine thiosemicarbazone chelators with potent and selective anti-neoplastic activity: novel ligands that limit methemoglobin formation, *Journal of medicinal chemistry*, 56: 357-370, 2013.

doi: 10.1021/jm301691s

[8] Brodowska K, Correia I, Garribba E, Marques F, Klewicka E, Lodyga-Chruscinska E, Pessoa JC, Dzeikala A, Chruscinski L. Coordination ability and biological activity of a naringenin thiosemicarbazone, *Journal of Inorganic Biochemistry*, 165: 36-48, 2016.

doi: 10.1016/j.jinorgbio.2016.09.014

[9] Pelosi G. Thiosemicarbazone Metal Complexes: From Structure to Activity, *The Open Crystallography Journal*, 3: 16-28, 2010.

doi: 10.2174/1874846501003010016

[10] Tahghighi A. Importance of metal complexes for development of potential leishmanicidal agents, *Journal of Organometallic Chemistry*, 770: 51-60, 2014.

doi: 10.1016/j.jorganchem.2014.08.007

[11] Kalinowski DS, Yu Y, Sharpe PC, Islam M, Liao Y, Lovejoy DB, Kumar N. Design, Synthesis, and Characterization of Novel Iron Chelators: Structure-Activity Relationships of the 2-Benzoylpyridine Thiosemicarbazone Series and Their 3-Nitrobenzoyl Analogues as Potent Antitumor Agents, *Journal of medicinal chemistry*, 50: 3716-3729, 2007.

doi: 10.1021/jm070445z

[12] Fricker SP, Fricker S. The therapeutic application of lanthanides, *Royal Chemical Society*, 35: 524-533, 2006.

doi: 10.1039/b509608c

[13] Hernández-Gil J, Ferrer S, Cabedo N, López-Gresa MP, Castiñeiras A, Lloret F. Two copper complexes from two novel naphthalene-sulfonyl-triazole ligands: Different nuclearity and different DNA binding and cleavage capabilities, *Journal of Inorganic Biochemistry*, 125: 50-63, 2013.

doi: 10.1016/j.jinorgbio.2013.04.007

[14] Saswati, Chakraborty A, Dash SP, Panda AK, Acharyya R, Biswas A, Mukhopadhyay S, Bhutia SK, Crochet A, Patil YP. Synthesis, X-ray structure and *in vitro* cytotoxicity studies of Cu(I/II) complexes of thiosemicarbazone: special emphasis on their interactions with DNA, *Dalton Transactions*, 44: 6140-6157, 2015.

doi: 10.1039/c4dt03764b

[15] Muniyandi V, Pravin N, Raman N. Impact of metallonucleases on DNA interactions: Structural validation and *in-vitro* antibiogram assay, *Inorganic Chemistry* Communications, 46: 60-64, 2014.

doi: 10.1016/j.inoche.2014.05.003

[16] Barone G, Terenzi A, Lauria A, Almerico AM, Leal JM, Busto N, García B. DNA-binding of nickel(II), copper(II) and zinc(II) complexes: Structure-affinity relationships, *Coordination Chemistry Reviews*, 257: 2848-2862, 2013.

doi: 10.1016/j.ccr.2013.02.023

[17] Yang Z, Wang Y, Wang Y. Study on synthesis, structure, and DNA-binding of lanthanide complexes with 2-carboxylbenzaldehyde thiosemicarbazone, *Bioorganic & Medicinal Chemistry* Letters, 17: 2096-2101, 2007.

doi: 10.1016/j.bmcl.2006.10.049

[18] Chandra S, Vandana. Synthesis, spectroscopic, anticancer and antibacterial studies of Ni(II) and Cu(II) complexes with 2-carboxybenzaldehyde thiosemicarbazone*, Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, 129: 333-338, 2014.

doi: 10.1016/j.saa.2014.02.141

[19] Wang B, Yang ZY, Li T. Synthesis, characterization, and DNA-binding properties of the Ln(III) complexes with 6-hydroxy chromone-3-carbaldehyde-(2'-hydroxy) benzoyl hydrazine, *Bioorganic & medicinal chemistry*, 14:6012-6021, 2006.

doi: 10.1016/j.bmc.2006.05.015

[20] Nevagi R, et al. Design, Synthesis and Biological Evaluation of Novel Thiosemicarbazide Analogues as Potent Anticonvulsant Agents, *Bioorganic Chemistry*, 54: 68-72, 2014.

doi: 10.1016/j.bioorg.2014.04.002

[21] Scovill JP. A Facile Synthesis of Thiosemicarbazides and Thiosemicarbazones by the Transamination of 4-methyl-4-phenyl-3-thiosemicarbazide, *Phosphorus, Sulfur, and Silicon and the Related Elements*, 60: 15-19, 1991.

doi: 10.1080/10426509108233920

[22] Serra S, Moineaux L, Vancraeynest C, Masereel B, Wouters J, Pochet L, Frédérick R. Thiosemicarbazide, a fragment with promising indolamine-2, 3-dioxygenase (IDO) inhibition properties, European *Journal of Medicinal Chemistry*, 82: 96-105, 2014.

doi: 10.1016/j.ejmech.2014.05.044

[23] Andrews JM. Determination of minimum inhibitory concentrations, *Journal of Antimicrobial Chemotherapy*, 49:1049, 2002.

doi: 10.1093/jac/dkf083

[24] Clinical and Laboratory Standards Institute. In Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically; approved standard-ninth edition, CLSI, Pennsylvania USA 2012.

doi: 10.1016/j.bmc.2006.05.015

[25] Płowaś I, Świergiel J, Jadżyn J. Electrical conductivity in dimethyl sulfoxide + potassium iodide solutions at different concentrations and temperatures, *Journal of Chemical & Engineering Data*, 59: 2360-2366, 2014

doi: 10.1021/je4010678

[26] Deacon GB, Philibs RJ. Relationships Between The Carbon-Oxygen Stretching Frecuencies of Carboxilato Complexes and The Type of Carboxylate Coordination, *Coordination Chemistry Reviews*, 33: 227-250, 1980.

doi: 10.1016/S0010-8545(00)80455-5

[27] Chen ZF, Gu YQ, Song XY, Liu YC, Peng Y, Liang H. Synthesis, crystal structure, cytotoxicity and DNA interaction of 5,7-dichloro-8-quinolinolato-lanthanides, European *Journal of Medicinal Chemistry*, 59: 194-202, 2013.

doi: 10.1016/j.ejmech.2012.10.037

[28] Biver T. Use of UV-Vis Spectrometry to Gain Information on the Mode of Binding of Small Molecules to DNAs and RNAs, *Applied Spectroscopy Reviews*, 47: 272-325, 2012.

doi: 10.1080/05704928.2011.641044

[29] Suh D, Chaires JB. Criteria for the mode of binding of DNA binding agents, *Bioorganic and Medicinal Chemistry*, 3: 723-728, 1995.

doi: 10.1016/0968-0896(95)00053-J

[30] Raman N, Selvan A, Manisankar P. Spectral, magnetic, biocidal screening, DNA binding and photocleavage studies of mononuclear Cu(II) and Zn(II) metal complexes of tricoordinate heterocyclic Schiff base ligands of pyrazolone and semicarbazide/thiosemicarbazide based derivatives*, Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, 76: 161-173, 2010.

doi: 10.1016/j.saa.2010.03.007

[31] Liu YC, Chen ZF, Song XY, Peng Y, Qin QP, Liang H. Synthesis, crystal structure, cytotoxicity and DNA interaction of 5,7-dibromo-8-quinolinolato-lanthanides, European *Journal of Medicinal Chemistry*, 59: 168-175, 2013.

doi: 10.1016/j.ejmech.2012.11.001

[32] García MA, Pascual-Teresa B. Técnicas empleadas para el estudio de la interacción entre agentes antitumorales y el DNA, *Oncología*, 27: 69-79, 2004.

doi: 10.4321/S0378-48352004000200004

[33] Shahbazy M, Pakravan P, Kompany-Zareh M. Multivariate spectrochemical analysis of interactions of three common Isatin derivatives to calf thymus DNA in vitro, *Journal of Biomolecular Structure and Dynamics*, 35: 2539-2556, 2017.

doi: 10.1080/07391102.2016.1225604

[34] Gökçe C, Gup R. Copper(II) complexes of acylhydrazones: Synthesis, characterization and DNA interaction, *Applied Organometallic Chemistry*, 27: 263-268, 2013.

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Síntesis, actividad antibacterial e interacciones con DNA de complejos lantánidos (III) de Tiosemicarbazonas *N*(4)-sustituídas

Resumen. Este articulo reporta la síntesis y caracterización detallada de seis nuevos complejos lantánidos de La(III), Eu(III) y Nd(III) con tiosemicarbazonas *N*(4)-sustituidas derivadas del 2-carboxibenzaldehído. Los estudios espectroscópicos de IR, ^{1}H-NMR y ^{13}C-NMR confirmaron la coordinación de los grupos tiocarbonilo (C=S), azometina (C=N) y carboxilato (COO^{-}) a los centros metálicos, y el carboxilato se coordinó de forma bidentada. Los análisis elemental y térmico sugieren que los complejos lantánidos se formaron en proporciones molares 1:2 (metal:ligando). Los valores de conductividad molar confirmaron la naturaleza no eléctrica de los complejos. Por medio de medidas de absorción UV y de viscosidad se investigó la interacción entre estos complejos con DNA de timo de ternera (CT-DNA). Se encontró que los complejos Eu(III) y Nd(III) podrían correr a lo largo de cadenas de DNA a través de interacciones en el surco. Además, los complejos lantánidos podrían promover el clivaje oxidativo del plásmido pBR322 en un ambiente de alto estrés oxidativo. Finalmente, se evaluaron las actividades antibacteriales de las bases de Schiff como ligandos (L) y sus complejos contra bacterias gram-positivas y gram-negativas usando un procedimiento de microdilución. Los resultados indican que los complejos lantánidos exhiben una actividad antibacterial más potente que los ligandos libres.

Palabras clave: complejos de lantánidos; interacción del DNA; interacción del surco; actividad antibacterial; tiosemicarbazonas.

Síntese, atividade antibacteriana e interações com DNA de complexos lantanídeos (III) de tiosemicarbazonas *N*(4)-substituídas

Resumo. Este artigo reporta a síntese e caracterização detalhada de seis novos complexos lantanídeos de La(III), Eu(III) e Nd(III) com tiosemicarbazonas *N*(4)-substituídas derivadas do 2-carboxibenzaldeído. Os estudos espectroscópicos de IR, ^{1}H-RMN e ^{13}C-RMN confirmaram a coordenação dos grupos tiocarbonilos (C=S), azometina (C=N) e carboxilato (COO$^-$) com os centros metálicos, e o carboxilato se coordenou de forma bidentada. As análises elementares e térmicas sugerem que os complexos lantanídeos se formaram em proporções molares 1:2 (metal:ligante). Os valores de condutividades molar confirmaram a natureza não-elétrica dos complexos. Se avaliou a interação entre estes complexos com DNA de vitela (CT-DNA) por meio de medidas de absorção UV e de viscosidade. Se observou que os complexos Eu(III) e Nd(III) poderiam deslocar-se ao longo da cadeia de DNA através de interações no sulco. Adicionalmente, os complexos lantanídeos poderiam promover a clivagem oxidativa do plasmídeo pBR322 em um ambiente de alto estresse oxidativo. Finalmente, se avaliaram as atividades antibacterianas das bases de Schiff como ligantes (L) e seus complexos contra bactérias gram-positivas e gram-negativas, usando o método de microdiluição. Os resultados indicam que os complexos lantanídeos exibem uma atividade antibacteriana mais potente que os ligantes livres.

Palavras-chave: complexos de lantanídeos; interação do DNA; interação do sulco; atividade antibacteriana; tiosemicarbazonas.

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