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Synthesis, characterization and cytotoxicity evaluation of new cerium(III), lanthanum(III) and neodymium(III) complexes

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Complexes of cerium(III), lanthanum(III) and neodymium(III) with coumarin-3-carboxylic acid (HCCA) were synthesized by mixing of equimolar amounts of the respective metal nitrates and coumarin-3-carboxylic acid in ethanol. The complexes were characterized and identified by elemental analysis, IR and Raman spectroscopy. DTA and TGA were applied to study the compositions of the compounds. The vibrational study showed bidentate coordination of CCA^- to Ln(III) ions through the carbonyl oxygen and the carboxylic oxygen atoms. The newly synthesized compounds were assayed for cytotoxicity against SKW-3, HL-60 and Reh cells. The complexes of cerium(III) and lanthanum(III) showed marginal cytotoxic activity against SKW-3 and Reh cells as compared with the inorganic salts at concentration 200 μM. The complex of neodymium(III) induced approximately 50% reduction of the survival HL-60 and SKW-3 cells at concentration 200 μM.

KEYWORDS: lanthanide(III) complexes; coumarin-3-carboxylate metal complexes; IR, Raman; cytotoxic activity

INTRODUCTION

New compounds are constantly being screened for their potential anticancer properties. The need for new antitumor drugs is underscored by the usefulness of the classical antitumor agents in chemotherapy and the resistance of many tumors to these compounds. Among the categories of new drugs that are receiving a great deal of attention are metal-based drugs.[1–5] Metal centers of the coordination complexes, being positively charged, are favored to bind to negatively charged biomolecules; the constituents of proteins and nucleic acids offer excellent ligands for binding to metal ions. The pharmaceutical use of metal complexes therefore has excellent potential. Broad arrays of medicinal applications of metal complexes have been investigated. Testing of these complexes for their anticancer properties would shed some light on the effects of the ligands and the metals on the anticancer properties of the complexes. However, despite the numerous attempts for creating novel metal complexes as anticancer agents, only a few complexes have been introduced as antineoplastic drugs worldwide.

Coumarins, both naturally occurring as well as synthetic derivatives, have found widespread applications in medicine.[6–12] The coumarin ligand belongs to the class of compounds with remarkable biological significance. Consequently, a wealth of experimental data on antitumor properties of coumarin derivatives is available.[13–23] Coumarins have attracted significant attention as appropriate ligands for synthesis of new coordination compounds. The coumarin derivatives have been the focus of our recent research concerning the design of new cytotoxic agents. Furthermore, cytotoxic effects of complexes of coumarin derivatives with rare earth metals were examined by us on different tumor cell lines.[24–34]

Chemistry of lanthanides in living systems is more than just a matter of metal–ligand bond formation and metal–ligand stability. It needs no discussion that the lanthanides, investigated and widely described in the literature, provide fascinating new possibilities for research in the coming decade. It is generally appreciated that enormous progress has been made in the understanding of the mode of action of most lanthanide antitumor agents. Application of this knowledge

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in drug design is close, and it is generally expected that in the next decade improved antitumor drugs will be developed based on this knowledge. Our recently published data on the new coumarins and their lanthanide complexes indicate that a number of these compounds have demonstrated antiproliferative activity on various cancer cell lines.[24–34]

The complexation of lanthanides with heterocyclic ligands possessing more than one donor atom is of great interest in the chemistry of coordination compounds, since they can act either as neutral molecules or as deprotonated anions. Furthermore, the complexation can lead to mono- or polydentate binding. This has prompted us to investigate the metal binding properties of coumarin derivatives. The aim of this work was to synthesize and characterize complexes of cerium(III), lanthanum(III) and neodymium(III) with coumarin-3-carboxylic acid (see Fig. 1) and to determine the cytotoxic activities of these complexes in the selected tumor cell lines.

EXPERIMENTAL

Chemistry

The compounds used for preparing the solutions were Merck products, p.a. grade: $Ce(NO_3)_3 \cdot 6H_2O$, $La(NO_3)_3 \cdot 6H_2O$ and $Nd(NO_3)_3 \cdot 6H_2O$. Coumarin-3-carboxylic acid (Fig. 1) was used for the preparation of metal complexes as a ligand.

The complexes were synthesized by reaction of cerium(III), lanthanum(III) and neodymium(III) salts and the ligand, in amounts equal to metal : ligand molar ratio of 1 : 2. The synthesis of the complexes was made in a different ratio (1 : 1, 1 : 2, 1 : 3), but in all the cases the product was with the composition 1 : 2. The complexes were prepared by adding ethanol solutions of Ln(III) salts to ethanol solutions of the ligand. The reaction mixture was stirred with an electromagnetic stirrer at 25 °C for 1 h. At the moment of mixing of the solutions, precipitates were obtained. The precipitates were filtered (the pH of the filtrate was 5.0), washed several times with water and ethanol and dried in a dessicator to constant weight. The complexes were insoluble in water, methanol and ethanol and soluble in DMSO.

Analytical methods and instruments

The carbon, hydrogen and nitrogen contents of the compounds were determined by elemental analysis using Vario EL V2.3 CHNS Modus (Elementar Analysen Systeme, Germany). Water content was determined by Metrohm (Switzerland) E55 Karl Fisher Titrator and was confirmed by TGA.

The experiments of DTA and TGA were carried out using a derivatograph produced by the firm MOM (Budapest). Samples with particle size below 0.25 mm were placed in platinum crucibles. The heating rate was 10 °C/min until 900 °C. The inert substance was Al_2O_3.

IR spectra (Nujol) were recorded on a IR-spectrometer FTIR-8101M Shimadzu (Japan) (range 3800–400 cm^{-1}) and in KBr using FTIR IFS25 Bruker spectrometer (Germany) in the 4000–400 cm^{-1} wavenumber range.

The Raman spectra of the ligand and its new Ln(III) complexes were recorded in Würzburg (Germany) with a Dilor Labram spectrometer (Horiba-Jobin-Yvone) using the 514.5 nm excitation line from a Spectra Physics argon ion laser. The Labram integrated system was coupled through an Olympus LMPlanFL 100× objective to the optical microscope. The spectra were collected in the back-scattering geometry with a resolution of 2 cm^{-1}. The detection of Raman signal was carried out with a CCD camera (Photometric model 9000). The laser power varied from 100 to 250 mW as indicated in the figure caption.

Pharmacology

The cytotoxic effects of the tested lanthanide complexes and of the corresponding nitrate salts were assessed in a panel of human leukemic cell lines, consisting of the acute myeloid leukemia-derived HL-60, the T-cell leukemia-derived SKW-3 and the pre-B cell leukemia-derived Reh cells. They were all grown as suspension-type cultures in a controlled environment: RPMI 1640 medium (Sigma), with 10% heat inactivated fetal bovine serum (Sigma) and 2 mM L-glutamine (Sigma), in a 'Heraeus' incubator with humidified atmosphere and 5% carbon dioxide, at 37 °C. In order to maintain the cells in log phase, cell suspension was discarded two or three times per week and the cell culture was re-fed with fresh RPMI-1640 aliquots.

The cell viability was determined using the MTT-dye reduction assay. Briefly, exponentially growing cells were seeded in 96-well microplates (100 µl/well) at a density of 1×10^5 cells per ml and after 24 h incubation at 37 °C they were exposed to various concentrations of the lanthanide complexes for 72 h. After the incubation with the test compounds MTT solution (10 mg/ml in PBS) was added (10 µl/well). The plates were further incubated for 4 h at 37 °C and the formazan crystals formed were dissolved through addition of 100 µl/well 5% solution of formic acid in 2-propanol (Merck). The absorption of the samples was then measured using an ELISA reader (Uniscan Titertec) at wavelength of 580 nm. The blank solution consisted of 100 µl RPMI 1640 medium (Sigma), 10 µl MTT stock and 100 µl 5% formic acid in 2-propanol. The survival fractions were calculated as a percentage of the untreated control using the formula:

$$SF\ \% = A_{test}/A_{control} \times 100$$

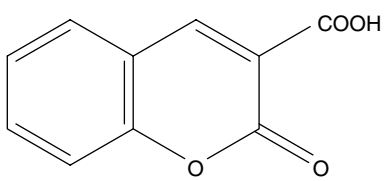

Figure 1. The structural formula of the ligand.

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where A_{test} is the average value for the absorption at a given concentration and $A_{control}$ is the average absorption of the untreated control, respectively.

The stock solutions of the tested lanthanide complexes (at 20 mM) were freshly prepared in DMSO, and thereafter consequently diluted in RPMI-1640 medium, in order to achieve the desired final concentrations. At the final dilutions obtained, the concentration of DMSO never exceeded 1%.

Data processing was performed using Microsoft Excel and the plots were generated using Microcal Origin, version 3.5. The statistical significance of the results was examined using Student's *t*-test with $p \leq 0.05$ taken as significance level.

RESULTS AND DISCUSSION

The new complexes were characterized by elemental analysis. The metal ions in their complexes were determined after mineralization. The metal content in chemical analysis was estimated complexometrically[24–34] and was confirmed by TGA. The water content in the complexes was determined by Karl Fisher analysis and by TGA. The data of the elemental analysis of the compounds obtained serving as a basis for the determination of their empirical formulas and the results of the Karl Fisher analysis and thermal analysis are presented in Table 1. The experimental data were in very good agreement with the calculated ones.

The compositions of the complexes were confirmed by DTA and TGA. The derivatograms of lanthanide complexes are similar. At the beginning of the DTA curves of the complexes there was a clearly manifested endothermic effect (~120 °C), due to the hygroscopic moisture released. A steady weight loss was recorded on heating up to ~210 °C corresponding to the elimination of one molecule of water per molecule of cerium(III), lanthanum(III) and neodymium(III) complexes. The observed temperature indeed was too high, but not impossible for the coordinated H_2O in these compounds. It was reported in the literature that the monohydrated lanthanide complexes of Tb(III) and Dy(III) with coumarin-3-carboxylic acid lose their water at about 210 °C.[35] The second endothermic peak corresponded to the simultaneous loss of remaining one water molecule at ~270 °C (observed only in the derivatogram of lanthanum complex). The amount of this weight loss, determined also by Karl Fisher analysis, correlated with the intensity of these endothermic effects and with the respective decreases in the mass. It is obvious that the water eliminated at this temperature was coordinated one. A similar feature has often been observed in lanthanide complexes of coumarins.[35] Unfortunately the products do not give crystals suitable for X-ray molecular structure determination, therefore it is not possible to establish with certainty whether the water is coordinated. However it seems reasonable to assume that the coordination number is six at least in all the complexes and thus at least one water molecule is coordinated. On heating the complexes the decomposition step in all the cases corresponds to the loss of molecules of the ligands, which is in agreement with the compositions in Table 1. Exothermal effects (360; 500 °C) dominate in the thermograms of all the complexes, resulting from the decomposition of the organic matter. A further weight loss recorded up to 650 °C indicates the formation of thermally stable oxides.

Table 1. Elemental analysis of Ln(III) complexes of coumarin-3-carboxilic acid

Compound Formulae	Calculated/found, %				
	C	H	N	H_2O	Ln
$Ce(CCA)_2(NO_3) \cdot H_2O$	40.13/ 39.83	2.00/ 2.36	2.34/ 2.86	3.01/ 3.42	23.41/ 22.95
$La(CCA)_2(NO_3) \cdot 2H_2O$	39.02/ 38.99	2.28/ 2.48	2.28/ 2.19	5.85/ 5.63	22.60/ 22.34
$Nd(CCA)_2(NO_3) \cdot H_2O$	39.88/ 39.61	1.99/ 2.27	2.32/ 2.22	2.99/ 2.58	23.92/ 23.74

HCCA = $C_{10}H_6O_4$; CCA = $C_{10}H_5O_4^-$.

The mode of bonding of the ligand to Ce(III), La(III) and Nd(III) ions was elucidated by recording the IR and Raman spectra of the complexes as compared with those of the free ligand.

Vibrational analysis

To help further the binding mode elucidation in the new Ce(III), La(III) and Nd(III) complexes of HCCA, detailed vibrational analysis was performed on the basis of comparison of experimental vibrational spectra of HCCA and its Ln(III) complexes with theoretically predicted by us earlier[36] as well as with literature data about related compounds. The vibrational spectra of the complexes showed new bands in comparison with those of the free ligand (HCCA) which have been assigned to the rocking, wagging and metal–oxygen stretching vibrations. The data of the experimental FT-IR and FT-Raman spectra of HCCA and its Ce(III), La(III) and Nd(III) complexes are given in Table 2. The recorded FT-IR and FT-Raman spectra of the ligand and its Ln(III) complexes are presented in Figs 2 and 3, respectively.

Since some vibrational modes exhibit different IR intensity and Raman activity, therefore the combination of IR and Raman experimental spectroscopy afford an opportunity for more precise characterization of the vibrational spectra of the Ln(III) complexes.

As seen from Table 2, the weak and broad band at 3176 cm^{-1} in the IR spectrum of the ligand is assigned to the ν(OH) vibrational mode of the ligand. This band was not detected in the spectra of the complexes, indicating that the deprotonated ligand form participates in the complexes.

The strong IR bands at 1746 and 1685 cm^{-1} and the medium Raman bands at 1729, 1676 and 1663 cm^{-1} were assigned to ν (C═O) modes of the carboxylic and carbonylic group, respectively. The high IR intensity of these bands was retained

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Table 2. Experimental vibrational frequencies of HCCA and its Ln(III) complexes

HCCA		Ce-HCCA		La-HCCA		Nd-HCCA		
IR	Raman	IR	Raman	IR	Raman	IR	Raman	Assignments
3176wb	—	—	—	—	—	—	—	ν (OH)$_{coum}$
1746vs	1729m	1715m	1691sh	1712vs	1690sh	1706s	1693m	ν(C═O)$_{carboxylic}$
		1702vs				1701s		[ν^{as}(COO$^-$) for CCA$^-$]
1685s	1676m,	1683vs		1684sh,	1674m	1673s		ν(C═O)$_{carbonylic}$
	1663m		1676m	1675s			1659m	
1569s	1559m	1570s	1559s	1572vs	1558s	1571s	1561s	ν(CC)
		1556s	1535m	1559s	1535m	1555s	1540m	ν(NO)$_{free}$
1489w	1483w	1486m	1445m	1487vw	1446m	1488m	1448m	ν(CC) + δ(CCH)$_{ip}$
1453w	1442w	1456m	1401w	1456m	1402w	1458m	1407m	ν(CC) + δ(CCH)$_{ip}$
		1398s		1405s		1407s	1380br	
1422s	1413vw	—	—	—	—	—	—	δ(COH)$_{ip}$
1374m	1363s	1355w	1326m	1385vs	1327m	1356w	1326m	ν(CC) + δ(CCH)$_{ip}$
							1321m	
1228s	1216s	—	1205s	1218w	1206s	—	1205s	ν(C−O)$_{lactone}$
1208s	1197vs	1299sh	1276w	1258m	1275w	1287sh	1275w	ν(C−O)$_{carboxylic}$
		1284s	1270w			1280m		[ν^{s}(COO$^-$) for CCA$^-$]
—	—	1260m	—	1260m	—	1262m	—	ν(NO)^{as}
—	—	1053w	1044m	1053w	1044m	1049w	1039m	δ(ONO)
—	—	786w	777w	786w	777w	791vw	772w	δ(ONO)
—	—	753w	—	751w	—	749w		ν(Ln−O)$_{carboxylic}$
—	—	725vw	—	725vw	—	—	—	δ(ONO)
—	—	459w	465w	457w	465w	457w	—	ν(Ln−O)$_{carbonylic}$
		449w		449w		449w		
—	—	No data	198w	No data	198vw	No data	210w	ν(Ln−O$_{NO3}$)

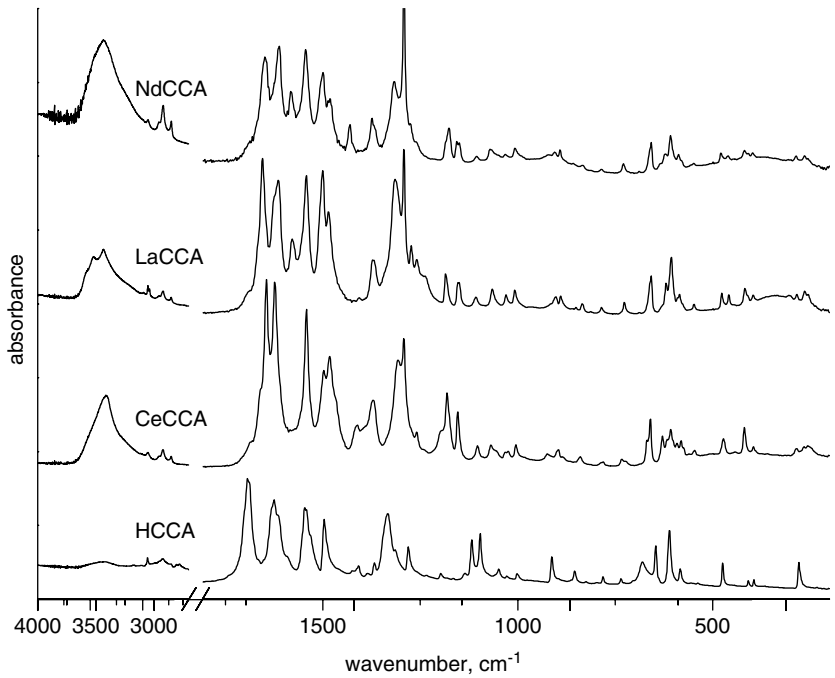

Figure 2. FT-IR spectra of HCCA and its Ce(III), La(III) and Nd(III) complexes in KBr in the 4000–400 cm^{-1} frequency region.

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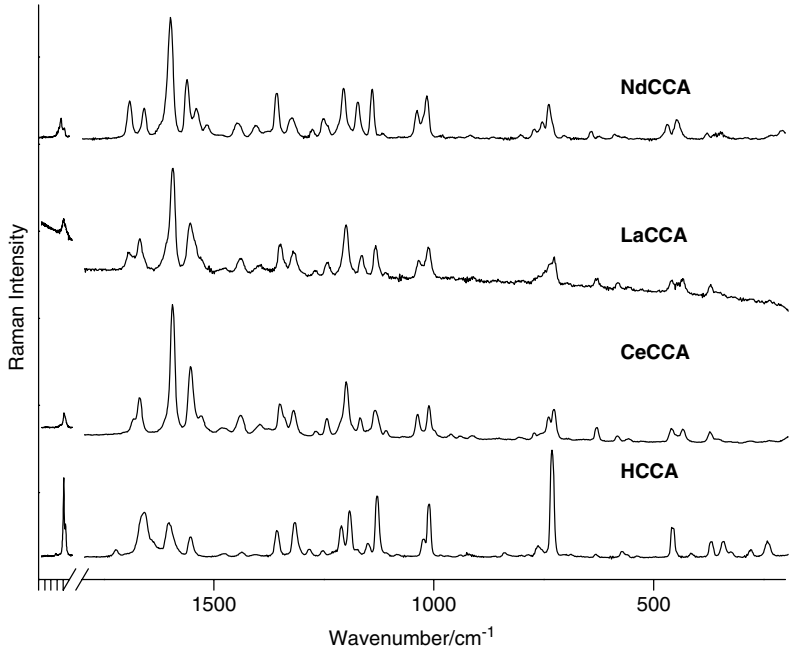

Figure 3. Experimental Raman spectra of HCCA and its Ce(III), La(III) and Nd(III) complexes. Excitation: 514.5 nm, 100 mW.

in the spectra of Ce(III), La(III) and Nd(III) complexes; the $\nu(C{=}O)_{carboxylic}$ and the $\nu(C{=}O)_{carbonylic}$ bands showed position changes and shifts to lower frequency (Table 2). The same shift effects were observed in the Raman spectra of the complexes (Table 2, Fig. 3). The HCCA vibrational spectrum appears not to be a suitable base for direct prediction of its coordination mode to the Ln(III). This finding could be explained by the intramolecular H-bond effect on vibrational modes of HCCA, reported previously.[36] Moreover, the deprotonated ligand form, CCA^- is bonded to Ln(III) in the complexes, but not in the HCCA form. It has already been observed by several authors that the difference $\Delta\nu$ between the asymmetric and symmetric COO^- stretching frequencies is greater in complexes than in simple salts. That is why, for comparison purposes, the vibrational spectrum of isolated CCA^- ligand was calculated and discussed.[36] A detailed theoretical and vibrational investigation based on FTIR and DFT/B3LYP/SVP studies of HCCA and its deprotonated form (CCA^-) was performed and published recently by us to suggest the binding mode of HCCA.[36] In accordance with the theoretical calculations, the appearance of new sharp bands in the lanthanide complexes of coumarin-3-carboxylic acid can be reasonably assigned to the asymmetric and symmetric COO^- stretching vibrations (Table 2, Figs 2 and 3). Since a bidentate binding of the COO^- group was also reported in the literature,[35] we checked whether this binding is also possible for the La(III) complex with COO^-.[36] In line with the model calculations, the bidentate binding of the carboxylic group of CCA^- in the complex could not be suggested.

The bands observed in the 1650–1330 cm^{-1} frequency range are due to the ν(CC) stretching vibrations of HCCA coumarin ring. The bands that are typical for the coumarin vibrations were not shifted significantly in the spectra of Ce(III), La(III) and Nd(III) complexes, which indicated that the Ln(III) cations did not produce substantial polarization on the coumarin ring.

In agreement with $Ln(III)-O_{carbonyl}$ interaction, the induced polarization on CCA^- leads to changes of the C–O lactone bond lengths as well as of their frequencies. The strong bands at 1228 cm^{-1} (IR spectrum of HCCA) and at 1216 cm^{-1} (Raman spectrum of HCCA) were assigned to the lactone ν(C–O) modes, respectively. In the complexes, these modes were shifted to lower frequency.

The following bands, observed in the IR spectra of the complexes are assigned to the vibrational modes of the NO_3 group: 1555–1559 cm^{-1} for $\nu(NO)_{free}$; 1260–1262 cm^{-1} for $\nu(NO)_{bonded}$; 1049–1053 cm^{-1} for δ(ONO); 786–791 cm^{-1} for δ(ONO) and at 725 cm^{-1} for δ(ONO). Some of them also appear in the Raman spectra of the complexes (Table 2, Fig. 3). This suggests that the nitrate group is present inside the coordination sphere. The magnitude of the splitting of the two bands at higher energies suggests that the nitrate group is bidentate and these results are in good agreement with the literature data.[35]

Because of the predominant electrostatic character of the Ln–O bonding the bands corresponding to the ν(Ln–O) modes have low intensities, they are coupled with other modes and hence their assignment is unreliable. The doublet bands observed in the IR spectra of the complexes at 767 and 753 cm^{-1} (Ce complex), 766, 751 cm^{-1} (La complex), 768, 749 cm^{-1} (Nd complex); the bands at about 460 cm^{-1}, and the bands at 449 cm^{-1} for all the Ln(III) complexes were assigned

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to $\nu(Ln-O)_{carboxylic}$ and $\nu(Ln-O)_{carbonylic}$ modes, respectively. Our previous theoretical calculations[36] predict that the bands due to $\nu(Ln-O)_{water}$ and $\nu(Ln-O)_{NO3}$ modes should appear at about 200 cm^{-1}. At the same positions are the observed corresponding vibrational modes in the Raman spectra of Ln(III) complexes.

The vibrational study gave evidence for bidentate coordination of CCA^- to Ce(III), La(III) and Nd(III) ions through the carbonylic oxygen and the carboxylic oxygen and also the nitrate group behaves as a bidentate chelating ligand in the complexes. On the basis of the above detailed vibrational study we could suggest the most probable structural formula of the investigated complexes (see Fig. 4).

Pharmacology

The screening performed revealed that all of the lanthanide complexes evaluated exerted marginal cytotoxic effects against the panel of leukaemic cell lines which enabled the construction of concentration response curves as depicted on Figs 5–7 and Tables 3–5. The corresponding Ln(III) nitrate salts were found to be inactive in the investigated concentration range.[30,31,37]

The results obtained indicate that the neodymium(III) complex of coumarin-3-carboxylic acid is slightly more active cytotoxic agent against the lymphoid SKW-3 cells as compared with the other two compounds, although the difference between their effects failed to reach statistical significance. Even at the lower concentrations of 50 and 100 μM Nd(III)complex reduced the viable cells by ca. 12 and 20% respectively whereas at the highest concentration of 200 μM an approximately 47% decrease of the cell survival was encountered (Fig. 5). The cerium(III) and lantanum(III) complexes caused only marginal inhibition of SKW-3 viability, with ca. 75 and 68% viable cells at the highest concentration of 200 μM.

The data for Reh cells, depicted in Fig. 6, revealed that all complexes exhibit only marginal activity, whereby the cellular viability did not differ significantly among the individual compounds, when applied at given concentrations.

The results obtained for the myeloid HL-60 cells demonstrated significant cytotoxic activity of the Nd(III) complex only at the highest concentration, causing a significant, near 56% inhibition of cell viability (Fig. 7). Both the Ce(III) and the La(III) complexes with coumarine-3-carboxylic acid did not demonstrate any significant cytotoxic effects on this cell line.

Taken together, the results from the cytotoxicity screening give us reason to conclude that the neodymium(III) complex with coumarin-3-carboxylic acid, being the most active cytotoxic agent amongst the lanthanide complexes studied, necessitates further more detailed pharmacological evaluation.

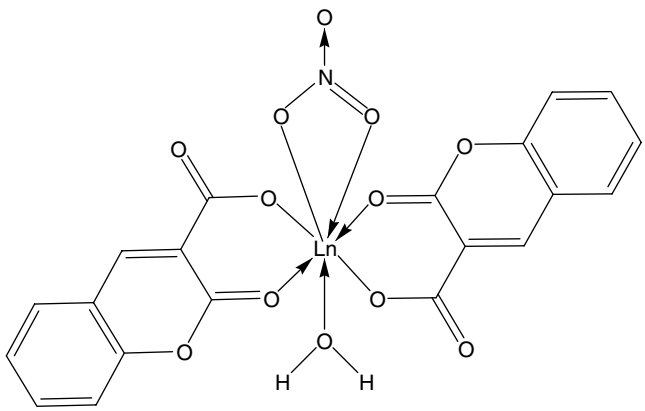

Figure 4. The most probable structural formula of the Ln(III) complexes.

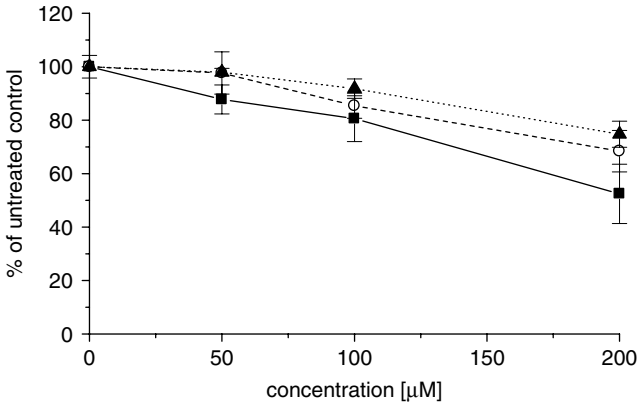

Figure 5. Cytotoxic effects of the lanthanide complexes $Ce(CCA)_2(NO_3) \cdot H_2O$ (▲), $La(CCA)_2(NO_3) \cdot 2H_2O$ (○) and $Nd(CCA)_2(NO_3) \cdot H_2O$ (■) against SKW-3 cells after 72 h incubation. Each data point represents the arithmetic mean ± SD of at least eight independent experiments.

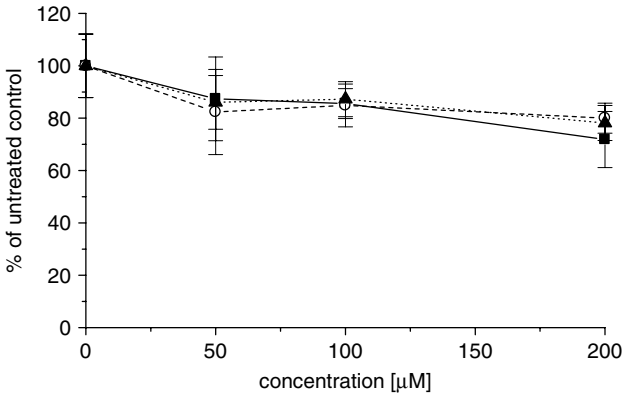

Figure 6. Cytotoxic effects of the investigated lanthanide complexes $Ce(CCA)_2(NO_3) \cdot H_2O$ (▲), $La(CCA)_2(NO_3) \cdot 2H_2O$ (○) and $Nd(CCA)_2(NO_3) \cdot H_2O$ (■) against Reh cells after 72 h incubation. Each data point represents the arithmetic mean ± SD of at least eight independent experiments.

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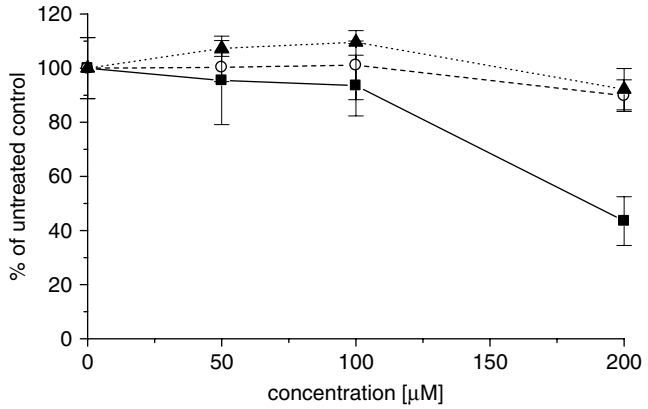

Figure 7. Cytotoxic effects of the investigated lanthanide complexes $Ce(CCA)_2(NO_3) \cdot H_2O$ (▲), $La(CCA)_2(NO_3) \cdot 2H_2O$ (○) and $Nd(CCA)_2(NO_3) \cdot H_2O$ (■) against HL-60 cells after 72 h incubation. Each data point represents the arithmetic mean ± SD of at least eight independent experiments.

Table 3. Spectrophotometrical data from MTT assay concerning the cytotoxic activity of complexes of coumarin-3-carboxylic acid on SKW-3 cells

MTT-formazan absorption at 580 nm				
Compound	Untreated control	50 μM	100 μM	200 μM
$Ce(CCA)_2(NO_3) \cdot H_2O$ (±)	1.1505	1.1270	1.0560	0.8600*
	0.4882	0.0165	0.0419	0.0563
$La(CCA)_2(NO_3) \cdot 2H_2O$ (±)	1.1505	1.1320	0.9825	0.7870*
	0.4882	0.3681	0.0541	0.0892
$Nd(CCA)_2(NO_3) \cdot H_2O$ (±)	1.1505	1.0095	0.9270	0.6035*
	0.4882	0.0620	0.0984	0.1274

Statistical significance: * $p \leq 0.05$ vs the untreated control (*t*-test).

Table 4. Spectrophotometrical data from MTT assay concerning the cytotoxic activity of complexes of coumarin-3-carboxylic acid on Reh cells

MTT-formazan absorption at 580 nm				
Compound	Untreated control	25 μM	100 μM	400 μM
$Ce(CCA)_2(NO_3) \cdot H_2O$ (±)	0.8726	0.7508	0.7615	0.6818*
	0.1059	0.0895	0.0585	0.0585
$La(CCA)_2(NO_3) \cdot 2H_2O$ (±)	0.8726	0.7187	0.7705	0.6982*
	0.1059	0.1420	0.0721	0.0502
$Nd(CCA)_2(NO_3) \cdot H_2O$ (±)	0.8726	0.7625	0.7467	0.6270*
	0.1059	0.1361	0.0936	0.0936

Statistical significance: * $p \leq 0.05$ vs the untreated control (*t*-test).

Table 5. Spectrophotometrical data from MTT assay concerning the cytotoxic activity of complexes of coumarin-3-carboxylic acid on HL-60 cells

MTT-formazan absorption at 580 nm				
Compound	Untreated control	50 μM	100 μM	200 μM
$Ce(CCA)_2(NO_3) \cdot H_2O$ (±)	1.4535	1.5600	1.5920	1.3405
	0.1641	0.0424	0.0070	0.1108
$La(CCA)_2(NO_3) \cdot 2H_2O$ (±)	1.4535	1.4585	1.4700	1.3060
	0.1641	0.0770	0.1875	0.0763
$Nd(CCA)_2(NO_3) \cdot H_2O$ (±)	1.4535	1.3880	1.3605	0.6325*†
	0.1641	0.2375	0.1632	0.1308

Statistical significance: * $p \leq 0.05$ vs the untreated control; † $p \leq 0.05$ vs $Ce(CCA)_2(NO_3) \cdot H_2O$ (*t*-test).

CONCLUSION

The coordination ability and the binding mode of coumarin-3-carboxylic acid to Ln(III) was investigated in the new obtained complexes. On the basis of detailed vibrational study of the spectral behavior of HCCA and its Ce(III), La(III) and Nd(III) complexes it was suggested that coumarin-3-carboxylic acid binds to the Ln(III) ions through both oxygen atoms of the carboxylic and carbonylic groups from the ligands and through the oxygen atoms of NO_3^-, and thus, the central ion Ln(III) is at least six-coordinated. The analysis of the vibrational spectra suggests predominantly ionic character of the Ln–CCA bond with slight ligand–metal charge transfer.

The obtained *in vitro* results regarding the cytotoxicity of lanthanide(III) complexes of coumarin-3-carboxylic acid are in accordance with our previously published data concerning the activity of lanthanide complexes with other coumarin derivatives.[19–29] It is noteworthy that the neodymium(III) complex with coumarin-3-carboxylic acid exerted more pronounced cytotoxic effects in comparison to other lanthanide complexes. This means that there is a selectivity of the inhibition of tumor cells to the respective lanthanide complexes. So far we can conclude that the structure metal–ligand determines the antitumor spectrum of the newly synthesized lanthanide(III) complexes.

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