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Spectroscopic evidence of La(III) complex of coumarin-3-carboxylic acid with cytotoxic activity

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The complex of La(III) with coumarin-3-carboxylic acid (HCCA) has been synthesized and characterized with different physicochemical data, elemental analysis, DTA and TGA data, IR, Raman, ^{1}H NMR and ^{13}C NMR spectra. B3LYP method with 6-31G(d) and 6-31++G(d,p) basis sets was successfully applied to study the molecular, electronic and vibrational structures as well as the conformational behavior of the neutral and deprotonated ligand (CCA). FTIR and FT Raman spectra of the compounds were recorded in regions 4000 cm^{-1} to 400 cm^{-1} and 4000 cm^{-1} to 100 cm^{-1} respectively. Significant differences in the IR and Raman spectra of the complex were observed as compared to the spectra of the ligand. A detailed assignment of the observed fundamental bands of HCCA and La(III)-HCCA has been analyzed in terms of peak positions and relative intensities from the recorded FTIR and FTR spectra. The metal-ligand binding mode has been extensively studied on the basis of obtained spectral data. Detailed vibrational analysis of HCCA and La(III) complex gave evidence for bidentate coordination of CCA to La(III) - the metal ion binds through the oxygen atoms of the carbonylic and the deprotonated carboxylic groups of CCA in the complex formation. The new La(III) complex has revealed a strong cell proliferation inhibiting effect on K-562 cells and it may be used for treatment of resistant tumor cells.

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Keywords: La(III) complex, Coumarin-3-carboxylic acid, FTIR, FT Raman spectra, Cytotoxic activity

1. Introduction

Coumarins, both naturally occurring as well as synthetic derivatives, have found widespread applications in biochemistry and medicine. Consequently, a wealth of experimental and theoretical data on spectral characteristics, photophysics and photochemistry of coumarin derivatives is available [1–5]. A coumarin ligand belongs to the class of compounds with remarkable biological significance. The antitumor effects of coumarin and its major metabolite, 7-hydroxycoumarin, were tested in several human tumor cell lines by Weber et al. [6].

A number of coumarins have been investigated for complexing ability. It was found that in some cases the metal complexes obtained revealed higher biological activity than their ligands.

Lanthanum chloride [7] possesses an antitumor activity. Furthermore, literature data show that the coumarins have also these properties. As a result from our earlier work the cytotoxic profile of some new complexes of coumarin derivatives with lanthanides against different tumor cell lines was proved [8-14]. We reported their significant cytotoxic acitivity in different human cells lines. These promising results prompted us to search for new lanthanide complexes with coumarin-3-carboxylic acid (see Fig. 1). Thus, the aim of this work was to synthesize and characterize lanthanum (III) complex of coumarin-3-carboxylic acid and to determine the cytotoxic activity of the obtained complex on the selected tumor cell lines. In the present study the chronic myeloid leukemia derived K-562, overexpressing the BCR-ABL fusion protein cell line was exploited as in vitro tumor test system.

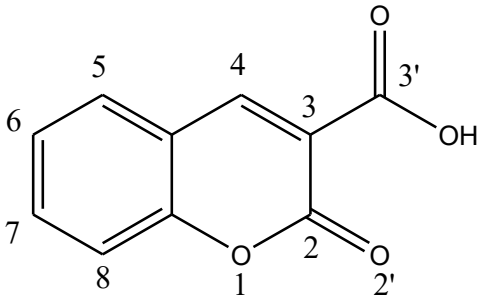

Fig. 1. Atom numbering used in discussion of ^{1}H NMR and ^{13}C NMR spectra.

In our previous published work, the metal-ligand binding mode of coumarin-3-carboxylic acid (HCCA) was studied on the basis of theoretical data [15]. B3LYP method with 6-31G(d) and 6-31++G(d,p) basis sets was successfully applied to study the molecular, electronic and vibrational structures as well as the conformational behavior of the neutral and deprotonated ligand (CCA). Molecular electrostatic potential (MEP) calculations of CCA in solvent environment suggested bidentate binding

mode through the carboxylic and the carbonylic oxygens [15]. Energy calculations of La(III)-CCA model structures performed both with large and small quasi-relativistic effective core potentials predicted the same bidentate binding mode of CCA [15]. The calculated atomic charges and the bonding orbital polarizations pointed to strongly ionic metal-ligand bonding in La(III)-CCA complex and insignificant donor acceptor interaction [15].

In this paper we report spectroscopic and biological results about a new La(III) complex of HCCA. The complex was identified and characterized with physicochemical data, elemental analysis, DTA and TGA data, IR, Raman, ^{1}H NMR and ^{13}C NMR spectra. Detailed vibrational analysis of HCCA and La(III)-CCA systems based on the experimental spectra confirmed the theoretically suggested metal-ligand binding mode. The new La(III) complex has revealed a strong cell proliferation inhibiting effect on K-562 cells and it may be used for treatment of resistant tumor cells.

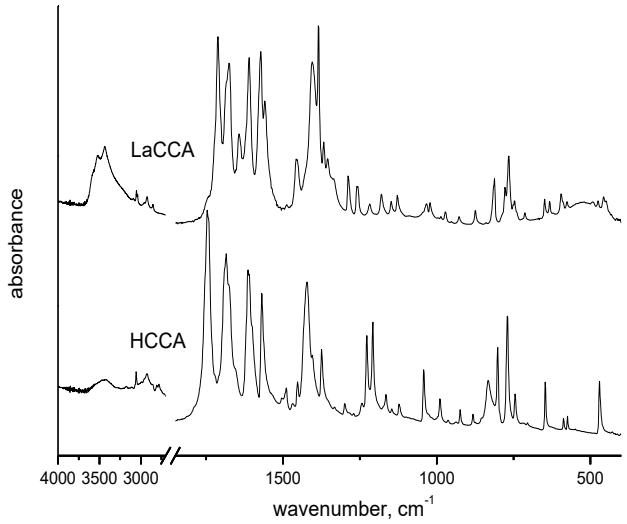

Fig. 2. Experimental IR spectra of HCCA and its lanthanum(III) complex.

2. Experimental

2.1. Chemistry

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

The complex was synthesized by reaction of lanthanum (III) salt and the ligand, in amounts equal to metal: ligand molar ratio of 1: 2. The synthesis of the complex was made in different ratio (1:1, 1:2, 1:3) but in all the cases the product was with the composition 1:2. The complex was prepared by adding the ethanol solution of Ln(III) salt to ethanol solution of the ligand. The reaction mixture was stirred with an electromagnetic stirrer at 25 °C for one hour. At the moment of mixing of the solutions, a precipitate was obtained. The precipitate was filtered, washed several times with ethanol and dried in a desicator to constant weight. The complex was insoluble in water, methanol and ethanol and well soluble in DMSO.

The carbon, hydrogen and nitrogen contents of the compound were determined by elemental analysis. The water content was determined by Metrohn Herizall 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.

^{1}H NMR spectra were recorded at room temperature on Bruker 250 WM (250 MHz) spectrometer in DMSO-d_6. Chemical shifts are given in ppm, downfield from TMS.

^{13}C NMR spectra were recorded at ambient temperature on Bruker 250 WM (62.9 MHz) spectrometer in DMSO- d_6. Chemical shifts are given in ppm, downfield from TMS.

The FT-IR spectra were recorded in KBr (4000-400 cm^{-1}) using IFS25 Bruker spectrometer. The resolution was 1 cm^{-1}.

The Raman spectra of the ligand and its new La(III) complex were recorded with a Dilor microspectrometer (Horiba-Jobin-Yvon, model LabRam) equipped with a 1800 grooves/mm holographic grating. The 514.5 nm line of an argon ion laser (Spectra Physics, model 2016) was used for the probes excitation. The spectra were collected in a backscattering geometry with a Raman microscope equipped with an Olympus LMPlanFL 50x objective and with a resolution of 2 cm^{-1}. The detection of Raman signal was carried out with a Peltier-cooled CCD camera. The laser output power was 100 mW.

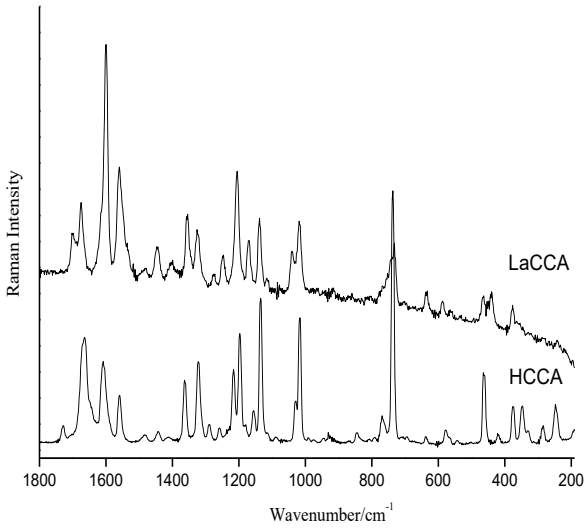

Fig. 3. Experimental Raman spectra of HCCA and its lanthanum(III) complex.

2.2. Pharmacology

The cytotoxic effects of the tested lanthanum complex and of the corresponding nitrate salt were assessed in a panel of human leukemic cell lines, consisting of the acute myeloid leukemia-derived HL-60, the chronic lymphoid leukemia-derived SKW-3, the pre-B cell leukemia-derived REH cells and the chronic myeloid leukemia derived K-562, overexpressing the BCR-ABL fusion protein 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 2mM L-glutamine (Sigma), in an '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 2 or 3 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 lanthanum complex for 72h. After the incubation with the test compounds MTT solution (10mg/ml in PBS) was added (10 µl/well). The plates were further incubated for 4h at 37 °C and the formazan crystals formed were dissolved through addition of 100µl/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 100µl RPMI 1640 medium (Sigma), 10µl MTT stock and 100 µl 5% formic acid in 2-propanol. The survival fractions were calculated as percentage of the untreated control using the formula:

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

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 lanthanum complex (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%. The stock solution (20 mM, in water) of lanthanum nitrate was freshly prepared and following antibacterial filtration they were accordingly diluted in RPMI-1640 medium.

Data processing was performed using Microsoft Excel and the plots were generated using Microcal Origin, version 3.5.

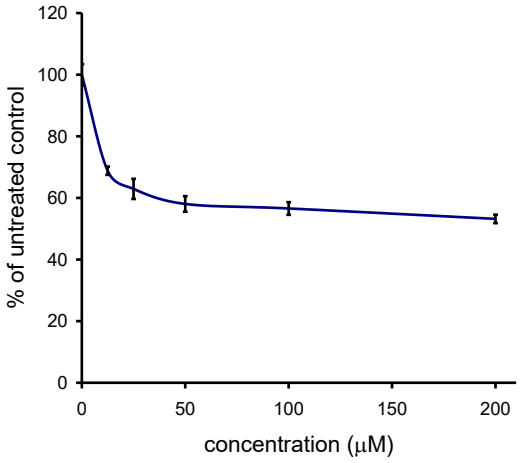

Fig. 4. Cytotoxic effect of $La(CCA)_2(NO_3)(H_2O)_2$ on the chronic myeloid leukemia-derived K-562 cell line after 48 h exposure, as assessed by the MTT-dye reduction assay. Each data point represents the mean ± sd (n≥6).

3. Results and discussion

3.1. Analytical and physicochemical data

The new complex was characterized by elemental analysis. The metal ion was determined after mineralization and was confirmed by TGA. The water content in the complex was determined by Karl Fisher analysis and by TGA. The nature of the complex was confirmed by ^{1}H NMR, ^{13}C NMR, IR and Raman spectroscopy.

The data of the elemental analysis of the compound obtained serving as a basis for the determination of its empirical formula are presented below.

Elemental analysis (calculated/found):

$La(CCA)_2(NO_3).2H_2O$: % C = 39,02, % H = 2.28, % N = 2.28 % C = 38,99, % H = 2.48, % N = 2.19

where CCA = $C_{10}H_5O^-_4$

The composition of the complex was confirmed by DTA and TGA. At the beginning of the DTA-curve of the complex there is a clearly manifested endothermic effect (~120 °C), which is due to the hygroscopic moisture released. A steady weight loss is recorded on heating up to ~210 °C corresponding to the elimination of two molecules of water per molecule of lanthanum (III) complex. The amount of the weight loss, determined also by Karl Fisher analysis, is correlated with the intensity of the endothermic effect and with the respective decrease in the mass. It is obvious that the water eliminated at this temperature is coordinated one. A similar feature has often been observed in lanthanide complexes of coumarins. It was confirmed by comparison with data of related compounds known in the literature [16, 17]. Unfortunately the product does not give crystals suitable for X-ray

molecular structure determination, therefore it is not possible to establish with certainly whether the water is coordinated. However it seems reasonable to assume that the coordination number is six at least in the complex and thus at least one water molecule is coordinated. On heating the complex the decomposition step corresponds to the loss of molecules of the ligand, which is in agreement with the empirical formula. Exothermal effects (360 °C; 500 °C) dominate in the thermogram of the complex, resulting from the decomposition of the organic matter. A further weight loss recorded up to 650 °C indicates the formation of thermally stable oxide.

3.2. ^{1}H NMR and ^{13}C NMR spectra

Metal ion coordination with ligand by means of oxygen atoms of carboxyl and carbonyl groups was shown owing to data of ^{1}H NMR and ^{13}C NMR spectra.

Proton spectra of the compounds recorded at 250 MHz in DMSO - d_6, confirmed the formation of the complex. The typical chemical shifts of the ^{1}H NMR spectra in DMSO - d_6 are presented below. It is evidence that in the lanthanum (III) complex there is observable weak negative shift effect on the protons. These data confirm formation of the complex and the coordination of metal ion with the oxygen atoms of the lactone carbonyl and of the carboxyl ion.

^{13}C NMR spectra of coumarin-3-carboxylic acid and of the lanthanum complex were recorded at 62.9 MHz in DMSO - d_6. The results of ^{13}C NMR spectra are presented below. Due to electron transfer from the carboxyl ion and lactone carbonyl oxygen to lanthanum (III), changes of chemical shifts were observed for the neighboring C-2, C-3 and C-3' carbon atoms of the complex and they confirmed the expected coordination of the ligand through oxygen atoms of the lactone carbonyl and of the carboxyl ion [16]. The other carbon atoms were only slightly affected from the coordination of the metal.

$C_{10}H_6O_4$:
^{1}H-NMR (DMSO-d_6): 8.68 (C_4 – H), 7.83 (C_5 – H), 7.67 (C_7 – H), 7.38 (C_8 – H), 7.32 (C_6 – H), 13.2 ($C_{3'}$ – H); ^{13}C-NMR (DMSO-d_6): 164 (C-3'), 157 (C-2), 155 (C-8'), 148 (CH-7), 134 (CH-4), 130 (CH-5), 125 (CH-6), 118 (C-3), 117 (C-4'), 116 (CH-8).
$La(CCA)_2(NO_3).2H_2O$:
^{1}H-NMR (DMSO-d_6): 8.53 (C_4 – H), 7.70 (C_5 – H), 7.57 (C_7 – H), 7.24 (C_8 – H), 7.19 (C_6 – H); ^{13}C-NMR (DMSO-d_6): 169 (C-3'), 160 (C-2), 154 (C-8'), 147 (CH-7), 134 (CH-4), 130 (CH-5), 125 (CH-6), 123 (C-3), 119 (C-4'), 116 (CH-8).

3.3. Vibrational analysis

Depending on the orientation of the two donor groups, C=O and COO^-, different binding of the anion of coumarin-3-carboxylic acid (CCA^-) is possible. The binding mode of CCA^- to the metal cation depends on the type of the metal and the character of the metal-ligand interaction [18-21]. However, in most of the known lanthanide complexes, the metal-ligand interaction is mainly electrostatic by nature and therefore more suitable and informative characteristic of the reactive sites of the ligand is the molecular electrostatic potential (MEP). The calculated MEPs for CCA were presented by us earlier [15]. According to these results, there are two regions with comparatively large negative MEP values; they are concentrated between the oxygen atoms of the carboxylic group and between the carboxylic and carbonyl oxygen atoms of the ligand. The MEP calculations predicted that in solvent environment, where the reaction between CCA^- and La(III) occurs, the second region will be preferred for electrophilic attack and an electrophile will prefer to bind CCA^- through the COO^- and C=O groups but not through the carboxylate group and the metal-ligand bonding in La-CCA is classified as predominantly ionic.

The analysis of the geometrical parameters obtained for CCA^-, HCCA and La-CCA species revealed that the bond length changes in the coumarin ring produced from the H-CCA and La-CCA interactions followed the same direction as compared to the free anion [15]. As it was expected, the most affected coumarin bond lengths were those that take part in the formation of six-membered H-bonded or chelate ring with H^+ or La(III) respectively.

To help further the binding mode elucidation in the new La(III) complex of HCCA, detailed vibrational analysis was performed on the basis of comparison of experimental vibrational spectra of HCCA and its La(III) complex with theoretically predicted by us earlier as well as with literature data about related compounds. Since in HCCA the vibrational modes related to the C=O and COOH groups are affected from the intramolecular H-bonding, the available solid state IR spectrum of HCCA is not a suitable base for direct tracking of the ligand vibrational changes in the complex as well as for understanding of the binding mode in the lanthanum complex. Therefore, the vibrational structure of non H-bonded conformer, calculated by us earlier [15], was also considered in the discussion.

The experimental FT-IR and FT-Raman spectra of HCCA and its La(III) complex are given in Figs. 2 and 3, respectively. Selected experimental IR and Raman frequencies of the ligand and its complex are presented in Table 1.

Table 1. Experimental vibrational frequencies of HCCA and its La(III) complex.

HCCA	La-HCCA	Assignments

———————		———————		
IR	Raman	IR	Raman	
3176w	-	-	-	$\nu(OH)_{coum}$
3057w	3066w	3054w	-	$\nu(CH)$
2956w	-	2956w	-	$\nu(CH)$
2926w	-	2924w	-	$\nu(CH)$
1746vs	1729m	1712vs	1690sh	$\nu(C=O)_{carboxylic}$
1685s	1676m, 1663m	1684sh, 1675s	1674m	$\nu(C=O)_{carbonylic}$
1613s	1608vs	1610vs	1601vs	$\nu(CC)$
1569s	1559m	1572vs, 1559s	1558s, 1535m	$\nu(CC)$
-	-	1492m	1486br	$\delta(HOH)$
1489w	1483w	1487vw	1446m	$\nu(CC) + \delta(CCH)_{ip}$
1453w	1442w	1456m, 1405s	1402w	$\nu(CC) + \delta(CCH)_{ip}$
1422s	1413vw	-	-	$\delta(COH)_{ip}$
1374m	1363s	1385vs	1327m	$\nu(CC) + \delta(CCH)_{ip}$
1228s	1216s	1218w	1206s	$\nu(C\text{-}O)_{lactone}$
1208s	1197vs	1258m	1275w	$\nu(C\text{-}O)_{carboxylic}$
-	-	1260m	-	$\nu(NO)^{as}$
-	-	1053w	1044m	$\delta(ONO)$
989m	-	976w	966w	$\delta(CCH)_{op}$
802s	-	-	-	$\delta(COH)_{op}$
-	-	786w	777w	$\delta(ONO)$
-	-	766s, 751w	-	$\delta(OCO)_{ip(cabox)} + \nu(La\text{-}O)_{carboxylic}$
-	-	725vw	-	$\delta(ONO)$
-	-	457w, 449w	465w	$\nu(La\text{-}O)_{carbonylic}$
-	-	No data	198vw	$\nu(La\text{-}O_{NO3})$

The broad band at 3176 cm^{-1} in the IR spectrum of the ligand was assigned to the ν(OH) vibrational mode. It should be mentioned that due to the intramolecular O…H-O hydrogen bond in HCCA, the ν(OH) band is shifted to lower frequency (with 273 cm^{-1}) in comparison with that of non-bonded conformer [15]. This band was not detected in the La(III) spectrum, indicating that the deprotonated ligand form participates in the complex. The bands in the 3060-2920 cm^{-1} region were assigned to ν(CH) modes of HCCA. In the IR spectrum of the La(III) complex they remain unchanged.

The strong IR bands at 1746 cm^{-1} 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 retained in La(III) complex spectrum and the $\nu(C=O)_{carboxylic}$ band was shifted to lower frequency (1712 cm^{-1}), while the $\nu(C=O)_{carbonylic}$ band showed insignificant position change (1684sh, 1675s cm^{-1}). The same shift effects were observed in the Raman spectrum of the complex. The calculated ν(C=O) frequencies of HCCA give good coincidence with the experimental values [15]. In our previous paper [15] the effect of intramolecular H-bond on C=O and COOH frequencies modes of HCCA were estimated: the carboxylic ν(C=O) and ν(C-O) were positive shifted with ~ 40 and 30 cm^{-1}, respectively, whereas the carbonyl ν(C=O) was negative shifted with 90 cm^{-1} [15]. An inspection of the calculated and experimental $\nu(C=O)_{carboxylic}$ mode of CCA and of La(III) complex revealed that in the complex $\nu^{as}(COO)$ mode was shifted to higher frequencies, indicating monodentate binding of COO^- group to La(III). Since the $\nu(C=O)_{carboxylic}$ band was shifted to higher frequency it is expected that the La–O-C=O interaction is strong. The bidentate and bridging binding of COO^- group to La(III) is also possible [22, 23]. According to the spectroscopic criteria for bidentate COO^- binding in metal-ligand complexes, $\nu^{as}(COO^-)$ should appear at lower frequency than that of the ligand anion form (in the region 1500-1520 cm^{-1}) [24-26]. Such a lowering was not observed in our La(III) complex spectrum and therefore bidentate binding of the carboxylate group of CCA in the complex was not suggested.

In agreement with literature data, the bands observed in the 1650-1330 cm^{-1} frequency range are due to the ν(CC) stretching vibrations of HCCA coumarin ring [27]. The bands that are typical for the coumarin vibrations were not shifted significantly in the La(III) complex spectrum, which indicated that the La(III) cation did not produce substantial polarization on the coumarin ring. The strong IR (at 1613 and 1569 cm^{-1}) and Raman (at 1608 and 1559 cm^{-1}) bands are attributed to the ν(C=C) stretching vibrations of HCCA coumarin fragment. Their positions and intensities are almost retained and the second band is split in the complex. The bands at 1489, 1453 and 1374 cm^{-1} (IR) and at 1483, 1442 and 1363 cm^{-1} (Raman), which also are assigned to the ν(CC) modes of HCCA, show negative shifts (20-50 cm^{-1}) in the La(III) complex and at the same time the intensity of these bands increases. The induced polarization by La(III) – CCA interaction produces electron density distribution in the conjugated coumarin ring and as a result the ν(CC) frequencies change their positions and intensity.

The strong IR bands at 1422 cm^{-1} and at 802 cm^{-1} were assigned to the in-plane $\delta(COH)_{ip}$ and out-of-plane $\delta(COH)_{op}$ deformation modes of HCCA, respectively. Our calculations revealed that due to the intramolecular H-bonds, both the in-plane and the out-of-plane δ(COH) modes were shifted to higher frequencies. The bands due to δ(COH) modes were not observed in the spectrum of the complex.

The strong IR band at 1208 cm^{-1} and the very strong Raman band at 1197 cm^{-1} were assigned to the $\nu(C\text{-}O)_{carboxylic}$ mode for HCCA. The frequency changes of $\nu(C\text{-}O)_{carboxylic}$ mode is in relation with the strength of the $H^{+}\text{-}O\text{-}C=O$ interaction in HCCA or of $La–^{-}O\text{-}C=O$ interaction in La(III)-CCA complex. In the CCA form (free COO^- group) the $\nu(C\text{-}O)_{carboxylic}$ mode corresponds to the $\nu^{s}(COO)$ mode and it was calculated at 1296 cm^{-1} [15]. In the La(III) complex the $\nu(C\text{-}O)_{carboxylic}$ band was shifted to lower frequencies, with 38 cm^{-1}, (in comparison with theoretically predicted) in agreement with La(III) – COO^- interaction. The $\nu(C\text{-}O)_{carboxylic}$ mode in the La(III)

complex appeared at higher frequency (1258 cm^{-1}) in comparison with that in HCCA (1208 cm^{-1}), indicating weaker electrostatic La(III) – COO^- interaction than the covalent one in the free (due to the H- bonding in HCCA). The spectra comparison showed that the intramolecular H-bonding in HCCA and the La(III) – $O_{carbonylic}$ (CCA) binding produced similar $\nu(C=O)_{carbonylic}$ frequency changes and the bands due to the $\nu(C=O)_{carbonylic}$ mode of HCCA and La(III)-CCA complex should appear at similar frequencies (as it was shown above).

The strong bands at 1228 cm^{-1} (IR spectrum of HCCA) and at 1216 cm^{-1} (Raman spectrum of HCCA) and the medium one at 989 cm^{-1}, in the IR spectrum of HCCA, were assigned to the lactone ν(C-O) modes, respectively. In the complex, these modes were shifted to lower frequency. In agreement with La(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 in a direction mentioned above.

The CH in-plane bending modes, $\delta(CCH)_{ip}$ of HCCA were observed in the region 1170 -1000 cm^{-1} with weak IR intensity and medium Raman activity (Figs. 2 and 3). The CH out-of-plane bending modes, $\delta(CCH)_{op}$ appear in the 990-750 cm^{-1} region with medium IR intensity. The bands typical for the coumarin vibrations are shifted insignificantly in the La(III) complex.

The following bands, observed in the IR spectrum of the complex are assigned to the vibrational modes of the NO_3 group: 1260 cm^{-1} - $\nu(NO)_{bonded}$; 1053 - δ(ONO); 786 - δ(ONO) and 725 cm^{-1} - δ(ONO). Some of them also appear in the Raman spectrum of the complex: 1044 - δ(ONO); 777 - δ(ONO). The observed NO_3 vibrational modes are in good agreement with the calculated ones [15] and with the literature data [16].

Because of the predominant electrostatic character of the La–O bonding the bands corresponding to the ν(La-O) modes have low intensities, they are coupled with other modes and hence, their assignment is unreliable. The doublet bands observed in the IR spectrum of the complex at 766, 751 cm^{-1} and 457, 449 cm^{-1} were assigned to $\nu(La-O)_{carboxylic}$ and $\nu(La-O)_{carbonylic}$ modes, respectively. The theoretical calculations predict that the bands due to $\nu(La-O)_{water}$ and $\nu(La-O)_{NO3}$ modes should appear at about 200 cm^{-1} [15]. At the same position (198 cm^{-1}) a new band in the Raman spectrum of the complex was observed.

3.4. Pharmacology

The screening performed revealed that the new La(III) complex of HCCA exerted cytotoxic effect against the chronic myeloid leukemia K-562 cells, overexpressing the BCR-ABL fusion protein cell line in a concentration dependent matter, which enabled the construction of concentration response curve as depicted on Fig. 4 and Table 2. Even at lower concentrations of 12.5 and 25 μM the La(III) complex reduced the viable cells by ca. 30% whereas at the highest concentration of 200μM an approximately 40-50% decrease of the cell survival was encountered (Table 2 and Fig. 4). The screening performed revealed that the new La(III) complex with CCA showed only marginal cytotoxic effects against the acute myeloid leukemia-derived HL-60, the chronic lymphoid leukemia-derived SKW-3, the pre-B cell leukemia-derived REH cells. The corresponding La(III) nitrate was found to be inactive in the investigated concentration range [9-13]. Taken together the results from the cytotoxicity screening give us reason to conclude that the lanthanum (III) complex with coumarin-3-carboxylic acid, being active cytotoxic agent against the chronic myeloid leukemia derived K-562, overexpressing the BCR-ABL fusion protein cell line necessitates further more detailed pharmacological evaluation.

Table 2. Spectrophotometrical data from MTT assay concerning the cytotoxic activity of La(III) complex of HCCA on SKW-3 cells, REH cells, HL-60 cells and K-562 cells.

MTT- formazan absorption at 580 nm						
Cell lines	Untreated control	12.5 μM	25 μM	50 μM	100 μM	200 μM
SKW-3	1.1505 ± 0.4882	-	-	1.1320 ± 0.3681	0.9825 ± 0.0541	0.7870 ± 0.0892
REH	0.8726 ± 0.1059	-	-	0.7187 ± 0.1420	0.7705 ± 0.0721	0.6982 ± 0.0502
HL-60	1.4535 ± 0.1641	-	-	1.4585 ± 0.0770	1.4700 ± 0.1875	1.3060 ± 0.0763
K-562	1.6390 ± 0.0560	1.1280 ± 0.0220	1.0310 ± 0.0530	0.9510 ± 0.0410	0.9270 ± 0.0340	0.8720 ± 0.0230

4. Conclusions

A new La(III) complex with coumarin-3-carboxylic acid (HCCA) was synthesized and characterized with different physicochemical data, elemental analysis, DTA and TGA data, IR, Raman, ^1H NMR and ^{13}C NMR spectra. The metal-ligand binding mode in the new La(III) complex of coumarin-3-carboxylic acid was elucidated. The metal-ligand bonding in La(CCA) appeared to be strongly ionic with very small donor-acceptor character. The vibrational analysis performed for the species studied, helped to explain the vibrational behavior of the ligand vibrational modes, sensitive to H-bonding and to interaction with La(III). The vibrational study gave evidence for bidentate coordination of CCA^- to La(III) ion through the carbonylic oxygen and the carboxylic oxygen and hence it was in agreement with the other theory prediction.

With the relatively resistant K-562 cell line we obtained once again very interesting in vitro results which are in accordance with our previously published data concerning the activity of lanthanide complexes with other coumarin derivatives [9-13]. It is noteworthy that the Ln(III) complexes have a strong cell proliferation inhibiting effects on K-562 cells. This means that the

resistant tumor cells may be very good inhibited with lanthanide complexes. This means also that the spectrum of cytotoxicity of these complexes is different from cis-DDP (II) and from Pt (II) complexes. These results are of some interest as a possibility to influence the resistant tumors. The corresponding lanthanide salts and ligand are found to be of very low or missing activity. So far we can conclude that the structure metal-ligand determines the antitumor spectrum of the new complex.

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