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New cerium(III) and neodymium(III) complexes as cytotoxic agents

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The cerium(III) and neodymium(III) complexes of 5-aminoorotic acid were synthesized and characterized by means of spectral data (IR, Raman, ^{1}H NMR and ^{13}C NMR) and elemental analysis. Significant differences in the IR spectra of the complexes were observed as compared with the spectrum of the ligand. A comparative analysis of the Raman spectra of the complexes with that of the free 5-aminoorotic acid allowed a straightforward assignment of the vibrations of the ligand groups involved in coordination. ^{1}H NMR and ^{13}C NMR spectra confirmed the formation of the complexes. The ligand and the complexes were tested for the cytotoxic activities on the chronic myeloid leukemia-derived K-562, overexpressing the BCR-ABL fusion protein, and the non-Hodgkin lymphoma-derived DOHH-2, characterized by a rexpression of the antiapoptotic protein bcl-2 cell lines. The results obtained indicate that the tested compounds exerted a considerable cytotoxic activity upon the evaluated cell lines in a concentration-dependent manner, which enabled the construction of dose–response curves and the calculation of the corresponding IC_{50} values. Cytotoxicity towards tumor cells was determined for a broad concentration range. The inorganic salts exerted a very weak cytotoxic effect on these cells that is in contrast to the lanthanide complexes, which exhibited potent cytotoxic activity towards K-562 and DOHH-2 cell lines. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: lanthanide(III) complexes; 5-aminoorotic acid; IR, Raman; NMR; cytotoxic activity

INTRODUCTION

Orotic acid (2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylic acid, vitamin B_{13}, H_3L') occupies a unique position biologically by being the only effective precursor in the biosynthesis of the pyrimidine-based nucleic acids.[1–17] Metal orotates are also widely applied in medicine. Recent interest has focused on the proposed biological carrier function of orotic acid and the corresponding anionic species for metal ions, which is held responsible for the obviously successful application of metal orotates in curing syndromes associated with a deficiency of a variety of metals.[18–22] Platinum, palladium and nickel orotate complexes have been screened as therapeutic agents for cancer.[23]

The multifunctionality of the hydroorotate, $H_2L'^{-}$, and orotate, HL'^{2-}, anions offer interesting possibilities in crystal engineering as a versatile ligand for supramolecular assemblies. Metal ion coordination may occur through the two nitrogen atoms of the pyrimidine ring as well as the two carbonyl oxygen atoms or the carboxylic group, which results in a multi-faceted coordination chemistry. The coordinated orotate anions exhibit a ligand surface with double or triple hydrogen-bonding capabilities, depending on the metal coordination mode, and has thus a potential to adopt several modes of interligand hydrogen bonding to allow the formation of extended, self-assembled structures.

Orotic acid, H_3L' (see Fig. 1), acts as a diacid in aqueous solution, with the acidic function suggested to be located on the carboxylic group ($pK_{a1} = 2.09$) and on the N1 site ($pK_{a2} = 9.45$).[24] The monobasic form, hydroorotate, $H_2L'^{-}$, appears to predominantly form complexes with coordination through the carboxyl oxygen atoms. With the Cu(I)[25] and Zn(II)[26] cations, a bidentate coordination is observed whereas UO_2(II) coordinates monodentately.[27] Compounds with the Zn(II)[28] and Mg(II)[29] cations, where the $H_2L'^{-}$ anion does not

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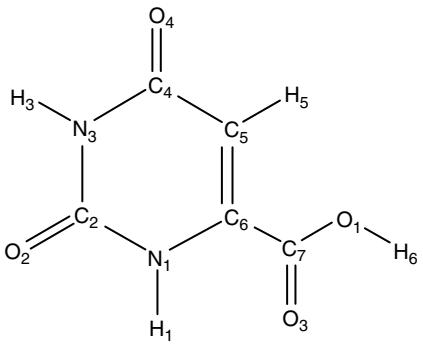

Figure 1. The structure of orotic acid (H_3L', HOA).

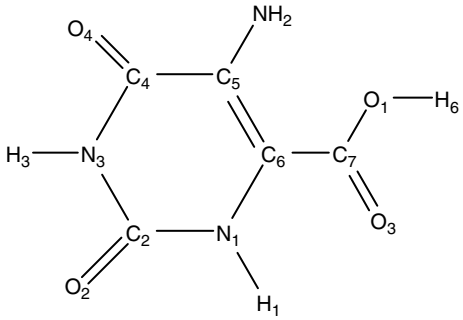

Figure 2. The structure of the ligand 5-aminoorotic acid (H_4L, HAOA).

enter the inner coordination sphere of the cation have also been reported.

With the dibasic form HL'^{2-}, the predominant coordination mode is a bidentate O3–N1 mode as observed for the Cu(II),[30] Ni(II),[31] Pt(II),[32,33] Pd(II),[34] Ir(I) and Rh(I)[35] cations. This coordination mode is also observed with Group 6 metal carbonyls.[36] In some cases, mixed coordination modes are observed where the orotate anion acts as a bridging ligand to form polymeric structures. In $[Mn_2(HL')_2(H_2O)_6]$, for example, polymeric chains result where the HL'^{2-} anion links three manganese atoms via O3–N1, O1 and O4.[37] One rare example of N3 coordination is found in hexaamine bis(5-nitroorotato)tricopper(II), where tribasic L'^{3-} ligands bridge two copper atoms via N1–O1 and N3 coordination.[38] Recently, a complex containing both $H_2L'^{-}$ and HL'^{2-} has been structurally characterized, namely $[Fe_3(\mu_3\text{-}OH)(H_2L')_3(HL')_3]$.[39] The $H_2L'^{-}$ anion bridges two Fe(III) cations via the carboxylate group, whereas HL'^{2-} chelates one Fe(III) by the bidentate O3–N1 mode and is bonded to a second Fe(III) through the adjacent carbonyl oxygen atom. In short, the hydroorotate and orotate anions exhibit several different coordination modes in the compounds so far crystallographically characterized.

Despite the interest in orotate metal complexes, the coordination chemistry of the derivatives of orotic acid has received rather scant attention.[1,10–12,33,40–47] One of these derivatives is 5-aminoorotic acid (5-amino-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylic acid, H_4L), which has relatively unknown coordination chemistry.[33,45,48,49]

We have recently synthesized lanthanide complexes with a number of biologically active ligands, and we reported their significant cytotoxic acitivity in different human cell lines.[50–59] These promising results prompted us to search for new lanthanide complexes with 5-aminoorotic acid (see Fig. 2). Thus, the aim of this work was to synthesize and characterize complexes of cerium(III) and neodymium(III) with 5-aminoorotic acid and to determine the cytotoxic activities of these complexes in the selected tumor cell lines. In the present study the following cell lines were exploited as *in vitro* tumor test systems: the chronic myeloid leukemia-derived K-562, overexpressing the BCR-ABL fusion protein, and the non-Hodgkin lymphoma-derived DOHH-2, characterized by a rexpression of the antiapoptotic protein bcl-2 cell lines.

EXPERIMENTAL

Chemistry

Reagents

The compounds used for preparing the solutions were Merck (Germany) products, *p.a.* grade: $Ce(NO_3)_3.6H_2O$ and $Nd(NO_3)_3.6H_2O$. 5-Aminoorotic acid (Fig. 2) was purchased from the Aldrich Chemical Co, USA. It was used for the preparation of the metal complexes as a ligand.

Synthesis

The complexes were synthesized by reaction of cerium(III) and neodymium(III) salts and the ligand, in amounts equal to metal: ligand molar ratio of 1:3. The synthesis of the complexes was made in different ratios (1:1, 1:2, 1:3), but in all the cases the product was with the composition 1:3. The complexes were prepared by adding an aqueous solution of cerium(III) and neodymium(III) salt to an aqueous solution of the ligand subsequently raising the pH of the mixture gradually to ca. 5.0 by adding dilute solution of sodium hydroxide. 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 (pH of the filtrate was 5.0), washed several times with water and dried in a desicator to constant weight. The complexes were insoluble in water, methanol and ethanol and well 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. IR spectra (Nujol) were recorded on a IR-spectrometer FTIR-8101M Shimadzu (Japan) (range 3800–400 cm^{-1}) and in KBr by using FTIR

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IFS25 Bruker spectrometer (Germany) in the 4000–400 cm^{-1} wavenumbers range.

The Raman spectra of 5-aminoorotic acid and its cerium(III) and neodymium(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 is 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 for each figure caption.

^{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.

Pharmacology

The cytotoxic effects of the tested lanthanide complexes and of the corresponding nitrate salts were assessed on the chronic myeloid leukemia-derived K-562, overexpressing the BCR-ABL fusion protein and the non-Hodgkin lymphoma-derived DOHH-2, characterized by a rexpression of the antiapoptotic protein bcl-2 cell lines. 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/ml and after 24 h incubation at 37 °C they were exposed to various concentrations of the lanthanide complexes for 48 h. After the incubation with the tested 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 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 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%. The stock solutions (20 mM, in water) of the nitrate salts of the lanhanides were freshly prepared and following antibacterial filtration they were accordingly diluted in RPMI-1640 medium.

Data processing was performed using Microsoft Excel 2002 (SP-1) and the plots were generated using Microcal Origin, version 3.5.

RESULTS AND DISCUSSION

The new complexes were characterized by elemental analysis. The metal ions were determined after mineralization. Their metal content in chemical analysis was determined complexonometrically. The water content in the complexes was determined by Karl Fisher analysis. The nature of the complexes was confirmed by IR and Raman spectroscopy, ^{1}H and ^{13}C NMR spectroscopy.

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 are presented in Table 1. The complexes were characterized by means of vibrational (IR, Raman), ^{1}H NMR and ^{13}C NMR) spectral analyses. The spectral data of the ligand have been compared with these of the complexes and the most probable sites for coordination with lanthanide(III) ions have been suggested. The spectral data of the obtained compounds are in good agreement with the data available in the literature.[60–68]

IR spectra of the ligand and its lanthanide complexes

The monoanions of orotic acid and its derivatives show a preference for monodentate oxygen carboxylate

Table 1. Elemental analysis of the lanthanide(III) complexes of 5-aminoorotic acid (HAOA)[a]

Compound formulae	Calculated/found C	(%) H	N	H_2O	Ce/Nd
$Ce(AOA^b)_3 \cdot 3H_2O$	25.57/25.38	2.56/2.78	17.90/17.52	7.67/7.34	19.89/20.10
$Nd(AOA)_3 \cdot 3H_2O$	25.42/25.62	2.54/2.15	17.80/17.37	7.63/7.28	20.34/20.15

[a] HAOA = $C_5N_3O_4H_5$; [b] AOA = $C_5N_3O_4H_4^-$.

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Table 2. Assignments of the more relevant absorption bands of 5-aminoorotic acid and its complexes in the 3800–1300 cm^{-1} IR-region (in Nujol)

ν (cm^{-1})	HAOA	$Ce(AOA)_3 \cdot 3H_2O$	$Nd(AOA)_3 \cdot 3H_2O$
$\nu(OH/H_2O)$	—	3442	3449
$\nu_{asym}(NH_2)$	3457	3442	3449
$\nu_{sym}(NH_2)$	3322	3340	3338
ν(N–H)	3196	3258	3251
		3166	3169
ν(C2=O2)	1689	1718	1719
ν(C4=O4)	1667	1684	1687
$\nu_{asym}(COO^-)$	—	1636	1644
ν(C=C)	1566	1555	1556
	1604	—	—
ν(C–N)	1436	1499	1500
	1457		
$\nu_{sym}(COO^-)$	—	1424	1424
δ(N–H)	1405	1390	1391

coordination.[26] In our new lanthanide(III) complexes with the monoanion of 5-aminoorotic acid as ligand, monodentate coordination via one of the carboxylate oxygen is suggested, which is in accordance with the literature data.[26,60]

Tentative assignments of selected IR bands of the complexes are given in Table 2. The assignments have been given by studying literature reports[45] and comparing the spectra of the ligand and of the metal complexes. As a general remark we must emphasize that some stretching and deformation modes are coupled, so that the proposed assignments should be regarded as approximate descriptions of the vibrations.

In the $\nu(OH)/H_2O$ region the spectra of Ce(III) and Nd(III) complexes show one medium band at 3442 and 3449 cm^{-1}, respectively, attributed to the presence of coordinated water.[61] This band overlaps with the $\nu_{asym}(NH_2)$ band.[60]

In the spectrum of Ce(III) complex the bands due to the $\nu_{asym}(NH_2)$, $\nu_{sym}(NH_2)$, ν(C2=O2) and ν(C4=O4) vibrations appear[45] at 3442, 3340, 1718 and 1684 cm^{-1}, respectively; and in the spectrum of Nd(III) complex the bands due to the $\nu_{asym}(NH_2)$, $\nu_{sym}(NH_2)$, ν(C2=O2) and ν(C4=O4) vibrations appear[45] at 3449, 3338, 1719 and 1687 cm^{-1}, respectively. The absence of large systematic shifts of these bands in the spectra of the complexes implies that there is no interaction between the amino nitrogen or the carbonyl oxygens and the lanthanide(III) ions. It was not possible to differentiate clearly the spectroscopic behavior of the different carbonyl groups of these complexes.[60]

The $\nu_{asym}(COO^-)$ and $\nu_{sym}(COO^-)$ bands are at 1636 and 1424 cm^{-1} for Ce(III) complex and at 1644 and 1424 cm^{-1} for Nd(III) complex, respectively; the possible participation of the monodentate carboxylate group of H_3L^- in hydrogen bonds may be responsible for the observed Δ value (210–220 cm^{-1}).[60]

The assignments of the more relevant absorption bands of 5-aminoorotic acid and its complexes in the 3500–1300 cm^{-1} IR-region are presented in Table 2 and also in Fig. 3(a), and they are in agreement with the literature data for such kind of complexes. The IR spectra of the presented new lanthanide complexes are similar to that of other complexes of this type. Notwithstanding in these studies it was not possible to differentiate clearly the spectroscopic behavior of the different functional groups present in these systems. Therefore, we have attempted to advance in that direction, using Raman spectroscopy.

Raman spectra of the ligand and its lanthanide complexes

The Raman spectra of the free 5-aminoorotic acid and its lanthanide complexes, in the pertinent range, are shown in Fig. 3(b). In the well-defined high-frequency field present in the spectra, dramatic intensity changes are observed, in going from the acid to the complexes (Table 3).

The 3500–2000 cm^{-1} spectral region

In this region the O–H (coordinated H_2O), N–H, and C–H stretches give rise to intense IR bands [Fig. 3(a), Table 2). The assignment of the O–H and N–H stretching bands is rather difficult. These bands appear overlapped in the same spectral region, and the involvement of these groups in hydrogen bonds affects their wavenumbers and produces a relevant band broadening in the IR and Raman spectra.

The 1800–900 cm^{-1} spectral region

The bands that appear in this region are mainly due to in-plane vibrations. Some mixed modes with ring and carboxylic contributions are observed in this region (Table 3); a significant vibrational coupling between the ring and carboxylic moieties is observed. The double bond stretching vibrations ν(C=O) and ν(C=C) are the internal coordinates that dominate in the modes with fundamentals in the 1800–1600 cm^{-1} spectral range.

The infrared spectra of the title complexes show a broad and relatively strong band with a maximum at 1718 cm^{-1} (Ce complex) and 1719 cm^{-1} (Nd complex). The considerable width of this band is due to a superposition of ν(C=O) and ν(C=C) stretching modes and bending modes of water molecules, of very similar frequencies. Fortunately, in the Raman spectrum, bands arising from $\delta(H_2O)$ vibrations are very weak, hence, the ν(C=O) and ν(C=C) stretching vibrations can be clearly observed. The Raman band of the highest frequency, 1680 cm^{-1} (Ce complex) and 1669 cm^{-1} (Nd complex), should be assigned to the ν(C7=O3) stretching vibrations in the carboxylate group, whereas the next two strong bands at 1625 and 1543 cm^{-1} (Ce complex) and at 1620 and 1543 cm^{-1} (Nd complex) are mainly caused by the ν(C4=O4) and ν(C5=C6) stretching vibrations.

The medium intensity infrared band at 1499–1500 cm^{-1} and Raman band at 1488 cm^{-1} (Ce complex) and 1493 cm^{-1} (Nd complex) arise mainly from carbon–nitrogen stretching

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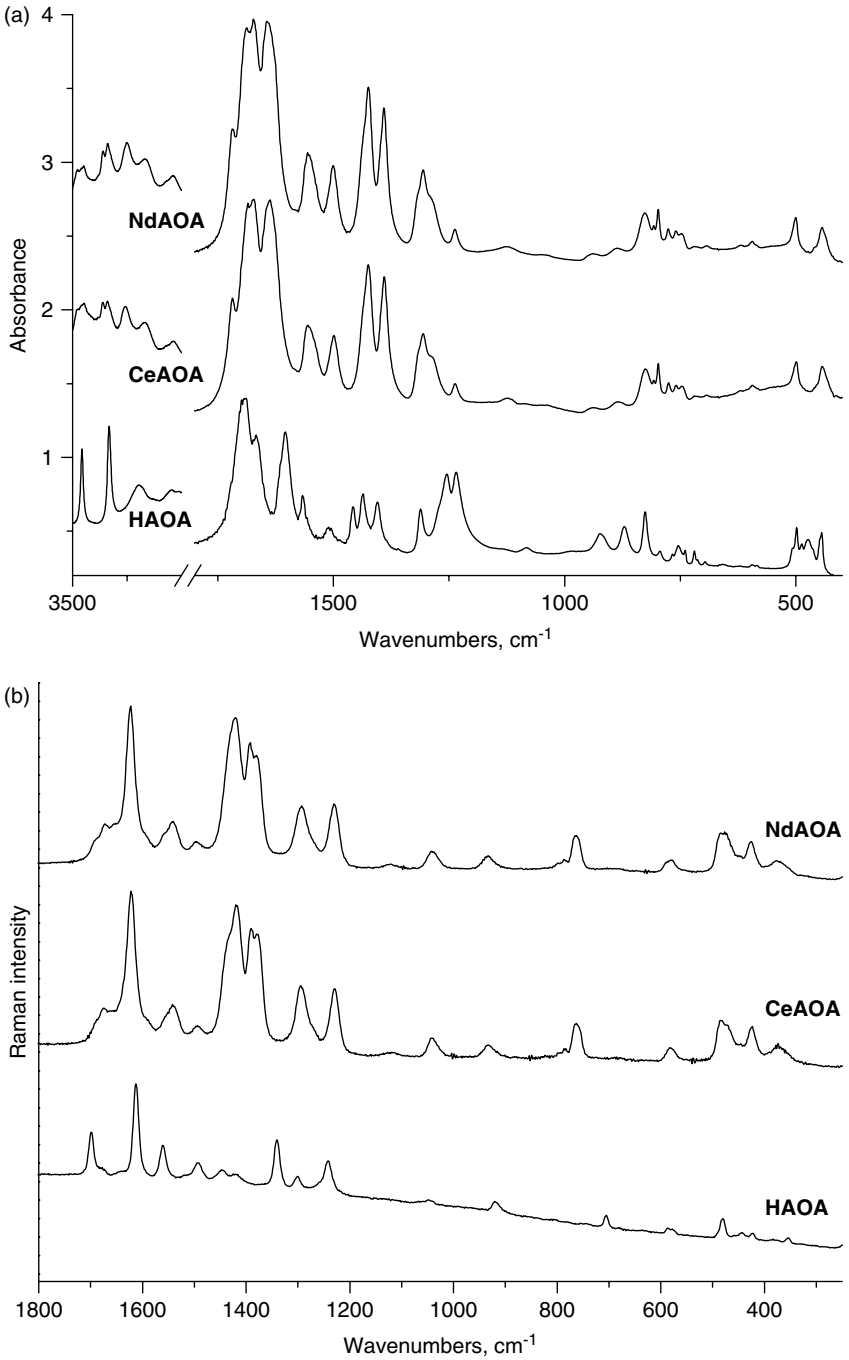

Figure 3. IR spectra of 5-aminoorotic acid and its cerium(III) and neodymium(III) complexes (in KBr) in the range (a) 3500–400 cm^{-1} and (b) 1800–200 cm^{-1}.

vibrations in the uracilate ring. These frequencies are higher than the stretching vibrations of single C–N bonds.[67] The strong bands at 1424 cm^{-1} (IR) can be also assigned to the C–N bonds and this vibration corresponds to the so-called $\nu_{sym}(COO^-)$ vibration in the monodentate metal–carboxylate complexes.[67] The medium band at 1390–1391 cm^{-1} in the IR spectra of the both complexes is assigned to the N3–H3 in-plane bending vibration coupled to the C2═O2 stretching vibrations, which is in agreement with the literature data.[68] The C7–O1 stretching vibrations in the title complexes are assigned at 1306 (IR) and 1296 (Raman) cm^{-1}. These stretching vibrations are coupled with water deformations.[68]

Different stretches of the uracil ring contribute to the bands in the 1600–900 cm^{-1} region. The assignment of all observed IR and Raman bands in this spectral range is difficult, because of the presence of highly coupled modes and combination

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Table 3. Experimental infrared and Raman bands (cm^{-1}) for 5-aminoorotic acid and its Ce(III) and Nd(III) complexes in the 2000–200 cm^{-1} region

Band assignment (cm^{-1})	HAOA		$Ce(AOA)_3 \cdot 3H_2O$		$Nd(AOA)_3 \cdot 3H_2O$	
	IR	R	IR	R	IR	R
δ(HOH), ν(C2═O2), ν(C7═O3)	1689s	1699m	1718m	—	1719m	—
ν(C7═O3), ν(C4═O4)	1667w	1670w	1684s	1680m	1687s	1669m
ν(C4═O4), ν(C5═C6)	1604s	1609vs	—	1625vs	—	1620vs
$\nu_{asym}(COO^-)$	—	—	1636s	—	1644s	—
ν(C5═C6), ν(C4═O4)	1566w	1560m	1555m	1543m	1556m	1543m
ν(N1–C2), ν(N1-C6), ν(N3-C2)	1511w	1492w	1499m	1488w	1500m	1493w
ν(N3–C2), ν(N1-C6), $\nu_{sym}(COO^-)$	1457m	1440w	1424s,br	1416s	1424s,br	1422s
	1436m	1420w				
δ(N3–H3), ν(C2═O2)	1405m	1341m	1390m	1384s	1391m	1389s
ν(C7–O1), ρ(HOH)	1311m	1300w	1306m	1296s	1306m	1296s
ν(Ura ring)	1255m	1247m	1236w	1230s	1238w	1230s
	1235m					
ν(Ura ring)	923w	920w	937w	934w	940w	928w
ρ(HOH), γ(C2═O2)	871w	—	884br,w	—	887br,w	—
δ(O3═C7–O1)	826m	780w	826m	764m	826m	764m
γ(C7═O3)	794w	—	798m	—	798m	—
δ(Ura ring), δ(C2═O2)	—	584w	595w	583w	594w	577w
γ(C7═O3)	499m	483m	499m	480m	500m	484m
δ(C4═O4), δ(Ura ring)	445m	430w	443m	425m	444m	418m
ν(Ln[a]–O1)	—	—	No data	373w	No data	374w

[a] Ln = Ce(III); Nd(III).

bands that may overlap with those due to fundamentals, and they interact with one another, leading to distortions of the observed bands.

The medium bands at 1255 cm^{-1} in the IR spectrum and at 1247 cm^{-1} in the Raman spectrum of the ligand are assigned to the ν(uracil ring), δ(N1–H1) and δ(C═O) of the carboxyl ion and they are shifted in the spectra of the complexes to lower wavenumbers. The strong Raman band at 1230 cm^{-1} in the Raman spectra of the both complexes can be assigned to the stretching vibration of the uracil ring. The weak bands at 1150 cm^{-1} in the IR spectrum and at 1130 cm^{-1} in the Raman spectrum of the ligand are assigned to the ν(N3–C4), ν(N3–C2), ν(N1–C2) and ν(uracil ring) and they are also shifted in the spectra of the complexes to lower wavenumbers [Fig. 3(a, b)]. The same shifts were observed for the bands at 1083 cm^{-1} in the IR spectrum and at 1045 cm^{-1} in the Raman spectrum of the ligand which are assigned to the δ(HOH), ν(C–C) and ν(C–O) [Fig. 3(a, b)].

Below 900 cm^{-1} spectral region

In this spectral region the normal modes appear to be rather delocalized. Nevertheless, the IR bands at 923, 755, 719 and 445 cm^{-1} are mainly due to vibrations of the uracil ring, whereas the IR bands at 826, 794 and 499 cm^{-1} are mostly due to vibrations of the carboxylic group in the spectrum of the ligand.

The Raman bands at about 780 cm^{-1} are assigned to the in-plane δ(O3C7O1) vibration of the carboxylate group in the spectrum of the ligand.[68] The corresponding infrared band is observed at 826 cm^{-1}. The out-of-plane vibration of the carboxylate C7═O3 groups is observed as the medium intensity infrared band at 798 cm^{-1} in the spectrum of the complexes. They are not observed in the Raman spectra. The bands of the C2═O2, C4═O4, C–O and C–C bonds contribute to the bands observed in the range of 790–750 cm^{-1} [Fig. 3(a, b)].

The spectra in the region below 600 cm^{-1} are particularly interesting, since they provide information about the metal–ligand vibrations. The bands centered at about 595 cm^{-1} arise from the uracil ring deformation coupled with δ(C2═O2) vibration.[68] The infrared bands at 443–444 cm^{-1} are caused by the in-plane δ(C4═O4) vibration[68] coupled with ring deformation, as shown in Table 3.

In the Raman spectrum, the new bands at 373–374 cm^{-1} can be assigned to the in phase (symmetric) stretching vibration of Ln–O1 bonds [where Ln is Ce(III) or Nd(III) and O1 is the carboxylate oxygen atom], which is in accordance with the literature data for similar coordination compounds.[68] The Raman spectra are particularly useful in studying the metal–oxygen stretching vibrations, since these vibrations give rise to medium intensity bands in Raman, but are weak in the infrared spectra. The new bands for Ce(III) and Nd(III) complexes in the Raman spectra can be assigned to the ν(Ln–O1) vibrations. The detailed assignment of the remaining bands in the vibrational spectra is shown in Table 3.

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^{1}H NMR and ^{13}C NMR spectra of the ligand and its lanthanide complexes

Metal ion coordination with ligand by means of oxygen atom of carboxyl ion is shown owing to data from ^{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 complexes. The typical chemical shifts of the ^{1}H NMR spectra in DMSO-d_6 are presented in Table 4. The chemical shifts of the protons vary in the lanthanide complexes, because of the shift properties of these metals. The paramagnetism of lanthanide complexes, which might affect the NMR spectra, should be mentioned. It is evidence that in the neodymium(III) complex there is observable negative shift effect on the protons. A strong positive shift effect in the cerium(III) complex was observed.

The ^{1}H NMR spectrum of the ligand in DMSO-d_6 shows the expected three resonances due to the NH protons. The carboxamido and imido protons give singlets at δ_H 11.47 and 9.44 and the amino protons give a broad signal at δ_H 6.00.[60] In the spectra of the complexes in the same solvent the amido and imido protons are observed. Their spectra show a very broad peak at *ca* δ_H 3.4, which is due to the intermolecular exchange of protons between the amino group and water (contained in the solvent). The facts that we did not observe two sharp signals of the separated species or an averaged signal are attributed to the intermediate rate of the exchange.[62,63]

The presence of the N1H and N3H proton resonances in the spectra of the complexes (Table 4) clearly shows that these nitrogen atoms are not engaged in complex formation. So far we can conclude that lanthanide(III) ions appear to bind the 5-aminoorotic acid at the carboxylato group, as reported for most orotato complexes.

We should mention that, as reported earlier in the literature for similar complexes,[60] the ^{1}H NMR spectra of the new Ce(III) and Nd(III) complexes in DMSO-d_6 showed the presence of one carboxamido, one imido and two amino protons. Moreover, the signals of the complexes in this solvent are very similar in the regions of the resonances due to NH and NH_2 protons. The spectral behaviour of Ce(III) and Nd(III) complexes strongly suggests that the aminoorotate is monoanionic (H_3L^-). Recently published literature data are in accordance with our suggestions.[60]

^{13}C NMR spectra of 5-aminoorotic acid and of the Ce(III) complex were recorded at 62.9 MHz in DMSO-d_6. The results of ^{13}C NMR spectra are presented in Table 5. Owing to electron transfer from the carboxyl ion to lanthanide(III), changes of chemical shifts were observed for the neighboring carbon atoms of Ce(III) complex and they confirmed the expected coordination of the ligand through oxygen atom of the the carboxyl ion.[64,65] The literature values[66] of the ^{13}C chemical shifts of orotic acid are included in Table 5 for comparison purposes.

Table 4. ^{1}H-NMR (250 MHz, DMSO-d_6), δ (ppm)

N_n–H	HAOA	$Ce(AOA)_3{\cdot}3H_2O$	$Nd(AOA)_3{\cdot}3H_2O$
N_1–H	11.47	11.25	11.55
N_3–H	9.44	10.20	8.24
C_5NH_2–2H	6.00	6.69	5.88

Table 5. ^{13}C-NMR (62.9 MHz, DMSO-d_6), δ (ppm)

C_n	HOA[a]	HAOA	$Ce(AOA)_3{\cdot}3H_2O$
C_4	164.1	173.9	183.3
COO^-	161.8	170.8	178.9
C_2	150.9	157.1	163.5
C_6	142.6	138.7	149.5
C_5	103.3	118.8	128.0

[a] HOA = orotic acid.

Summarizing the data from the vibrational and NMR spectra, we can conclude that the lanthanide ions are coordinated with 5-aminoorotic acid via the oxygen atoms from the carboxyl group, basing our arguments on the discussion of the vibrational spectroscopic data obtained. We have to take into consideration that the coordination number 6 for these central metal ions indeed is too low, but not impossible for lanthanide(III) ions.[55–59] One plausible mode of coordination might involve also the neutral amino group, taking into account the data of X-ray crystal structures of other metal complexes with 5-aminoorotic acid, reported in the literature.[60]

Pharmacology

The screening performed revealed that both of the lanthanide complexes evaluated exerted cytotoxic effects against the chronic myeloid leukemia-derived K-562, overexpressing the BCR-ABL fusion protein and the non-Hodgkin lymphoma-derived DOHH-2, characterized by a rexpression of the antiapoptotic protein bcl-2 cell lines in a concentration-dependent manner, which enabled the construction of concentration response curves as depicted in Figs 4–11 and Tables 6–9. The corresponding lanthanide(III) nitrate salts

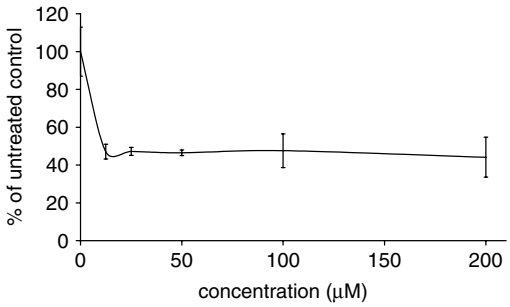

Figure 4. Cytotoxic effects of HAOA on the chronic myeloid leukemia-derived K-562 cell line after 48 h exposure, as assessed by the MTT-dye reduction assay. Each data point represents the mean ± SD ($n \geq 6$).

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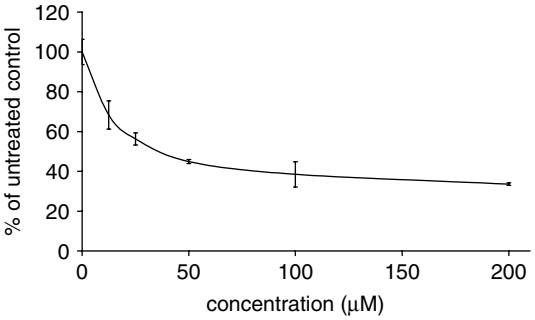

Figure 5. Cytotoxic effects of HAOA on the non-Hodgkin lymphoma-derived DOHH-2 cell line after 48 h exposure, as assessed by the MTT-dye reduction assay. Each data point represents the mean ± SD ($n \geq 6$).

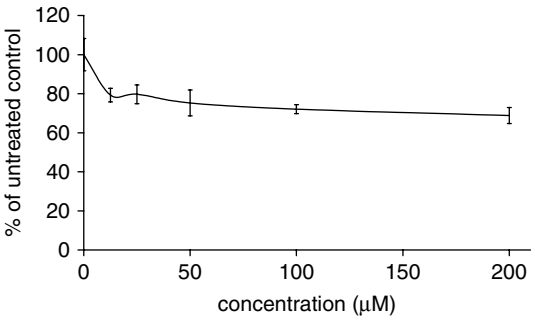

Figure 6. Cytotoxic effects of $Ce(AOA)_3 \cdot 3H_2O$ on the chronic myeloid leukemia-derived K-562 cell line after 48 h exposure, as assessed by the MTT-dye reduction assay. Each data point represents the mean ± SD ($n \geq 6$).

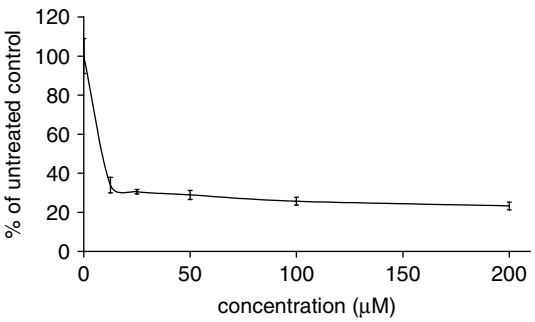

Figure 7. Cytotoxic effects of $Ce(AOA)_3.3H_2O$ on the non-Hodgkin lymphoma-derived DOHH-2 cell line after 48 h exposure, as assessed by the MTT-dye reduction assay. Each data point represents the mean ± SD ($n \geq 6$).

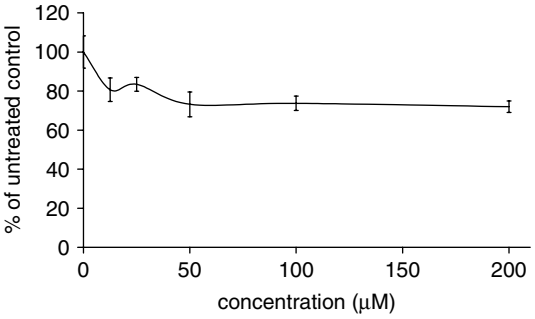

Figure 8. Cytotoxic effects of $Nd(AOA)_3 \cdot 3H_2O$ on the chronic myeloid leukemia-derived K-562 cell line after 48 h exposure, as assessed by the MTT-dye reduction assay. Each data point represents the mean ± SD ($n \geq 6$).

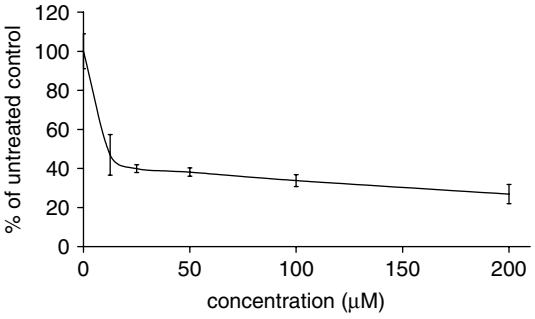

Figure 9. Cytotoxic effects of $Nd(AOA)_3.3H_2O$ on the non-Hodgkin lymphoma-derived DOHH-2 cell line after 48 h exposure, as assessed by the MTT-dye reduction assay. Each data point represents the mean ± SD ($n \geq 6$).

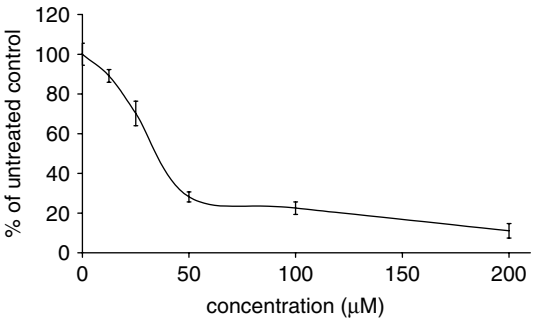

Figure 10. Cytotoxic effects of cisplatin on the chronic mywloid leukemia-derived K-562 cell line after 48 h exposure, as assessed by the MTT-dye reduction assay. Each data point represents the mean ± SD ($n \geq 6$).

were found to be inactive in the investigated concentration range (data not shown).[50–58]

An even more intriguing discrepancy between the cytotoxic efficacy of the lanthanide(III) complexes of 5-aminoorotic acid and cisplatin was encountered in DOHH-2 cells. The new Ce(III) and Nd(III) complexes show the practically equivalent relative potencies (in terms of IC_{50}) with the referent drug cisplatin. This encounter could be generally ascribed to the well-established high level of bcl-2 expression in DOHH-2 cells.[69] This anti-apoptotic protein abolishes

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Table 6. Spectrophotometrical data from MTT assay concerning the cytotoxic activity of 5-aminoorotic acid, lanthanide complexes of 5-aminoorotic acid and cisplatin on K-562 cells

	MTT-formazan absorption at 580 nm					
Compound	Untreated control	12.5 μM	25 μM	50 μM	100 μM	200 μM
HAOA	0.616 ± 0.080	0.290 ± 0.024	0.291 ± 0.013	0.286 ± 0.009	0.293 ± 0.055	0.272 ± 0.065
$Ce(AOA)_3 \cdot 3H_2O$	0.475 ± 0.039	0.376 ± 0.017	0.378 ± 0.023	0.357 ± 0.031	0.342 ± 0.011	0.327 ± 0.019
$Nd(AOA)_3 \cdot 3H_2O$	0.475 ± 0.039	0.383 ± 0.029	0.396 ± 0.017	0.347 ± 0.030	0.350 ± 0.017	0.342 ± 0.014
Cisplatin	0.814 ± 0.045	0.726 ± 0.026	0.572 ± 0.050	0.230 ± 0.021	0.183 ± 0.025	0.090 ± 0.030

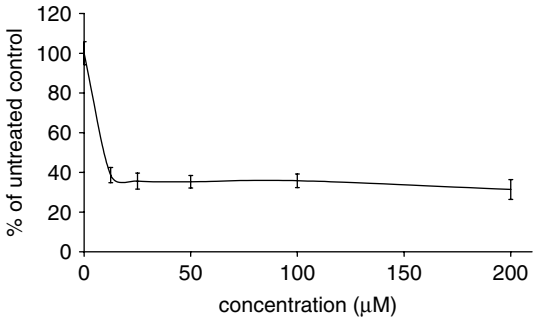

Figure 11. Cytotoxic effects of cisplatin on the non-Hodgkin lymphoma-derived DOHH-2 cell line after 48 h exposure, as assessed by the MTT-dye reduction assay. Each data point represents the mean ± SD ($n \geq 6$).

Table 7. Relative potency of the investigated compounds in the panel of human tumor cell line K-562, following 48 h treatment

Compound	IC_{50} value
HAOA	11.81 μM
$Ce(AOA)_3 \cdot 3H_2O$	>200 μM
$Nd(AOA)_3 \cdot 3H_2O$	>200 μM
Cisplatin	36.68 μM

several of the cell-death signaling pathways, which are involved in the cytotoxic mode of action of cisplatin, and conversely higher levels of bcl-2 are well established to confer resistance to platinum-based drugs.[70–72] Thus the established superior inhibition of DOHH-2 proliferation by the novel compounds implies that this class of tumor-inhibiting metal coordination compounds bypasses the bcl-2 mechanisms of cellular resistance.

CONCLUSION

The complexes of cerium(III) and neodymium(III) with 5-aminoorotic acid have been synthesized by new method for the first time. It has been proved that the lanthanide ions are coordinated with 5-aminoorotic acid via the oxygen atoms from the carboxyl group, basing our arguments on the discussion of the spectroscopic results, which is in accordance with the literature data obtained for similar compounds. One plausible mode of coordination through the neutral amino group can be also suggested, taking into consideration the data of X-ray crystal structures of other metal complexes with 5-aminoorotic acid, previously reported in the literature.

In conclusion, the complexes described above demonstrate once more the versatility of the 5-aminoorotate ligand, which adopts different coordination modes. The different charge and

Table 9. Relative potency of the investigated compounds in the panel of human tumor cell line DOHH-2, following 48 h treatment

Compound	IC_{50} value
HAOA	38.67 μM
$Ce(AOA)_3.3H_2O$	9.46 μM
$Nd(AOA)_3.3H_2O$	11.78 μM
Cisplatin	10.25 μM

Table 8. Spectrophotometrical data from MTT assay concerning the cytotoxic activity of 5-aminoorotic acid, lanthanide complexes of 5-aminoorotic acid and cisplatin on DOHH-2 cells

	MTT-formazan absorption at 580 nm					
Compound	Untreated control	12.5 μM	25 μM	50 μM	100 μM	200 μM
HAOA	0.903 ± 0.057	0.617 ± 0.064	0.508 ± 0.027	0.405 ± 0.009	0.348 ± 0.057	0.303 ± 0.006
$Ce(AOA)_3.3H_2O$	0.251 ± 0.022	0.085 ± 0.010	0.077 ± 0.003	0.073 ± 0.006	0.065 ± 0.005	0.059 ± 0.005
$Nd(AOA)_3.3H_2O$	0.251 ± 0.022	0.118 ± 0.026	0.100 ± 0.005	0.096 ± 0.005	0.085 ± 0.008	0.068 ± 0.012
Cisplatin	1.264 ± 0.073	0.488 ± 0.048	0.450 ± 0.051	0.446 ± 0.040	0.462 ± 0.044	0.397 ± 0.063

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coordination modes of the ligand have a major effect on the supramolecular structures adopted by the complexes. From previous results and this work, it is clear that the nature of 5-aminoorotic acid makes its various anionic forms versatile ligands for use with a variety of metals and for a variety of objectives/advantages, including variable coordination modes, high-nuclearity aggregate formation and/or linking of aggregates into polymeric arrays. Thus, we believe that H_4L has great potential as a generally useful new polyfunctional ligand in metal chemistry and it will prove attractive to a variety of coordination chemists.

As the results obtained clearly indicate, the lanthanide(III) complexes of 5-aminoorotic acid are very active cytotoxic agents against the non-Hodgkin lymphoma-derived DOHH-2, characterized by a rexpression of the antiapoptotic protein bcl-2 cells. The lanthanide(III) complexes caused very good inhibition of DOHH-2 viability, with *ca* 30% viable cells at the highest concentration of 200 μM. These results confirmed our previous observations on the cytotoxicity of cerium(III) and neodymium(III) complexes with other biologically active ligands.

Taken together the results from the cytotoxicity screening give us reason to conclude that the lanthanide(III) complexes with 5-aminoorotic acid, being very active cytotoxic agents, necessitate further more detailed pharmacological evaluation.

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