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Cytotoxic activity of new neodymium (III) complexes of bis-coumarins

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Abstract

Complexes of neodymium (III) with bis-coumarins: 3,3'-benzylidene-bis(4-hydroxy-2H-1-benzopyran-2-one); bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-2-yl-methane; bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-4-yl-methane; bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-(1H-pyrazol-3-yl)-methane were synthesized by reaction of neodymium (III) salt and the ligands, in amounts equal to metal:ligand molar ratio of 1:2. The complexes were prepared by adding an aqueous solution of 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 neodymium (III) complexes with bis-coumarins were characterized by different physicochemical methods—elemental analysis, IR-, ¹H- and ¹³C-NMR-spectroscopies and mass-spectral data. The spectral data of neodymium (III) complexes were interpreted on the basis of comparison with the spectra of the free ligands. This analysis showed that in the Nd (III) complexes the ligands coordinated to the metal ion through both deprotonated hydroxyl groups. On the basis of the $\nu(\text{C}=\text{O})$ red shift observed, participation of the carbonyl groups in the coordination to the metal ion was also suggested. Cytotoxic screening by MTT assay was carried out. The complexes were tested on HL-60, HL-60/Dox and SKW-3 cell lines. The overall results from the preliminary screening program revealed that all of the new Nd (III) complexes reach 50% inhibition of the malignant cells proliferation and thus could be considered as biologically active. On the basis of the IC₅₀ values obtained compounds Nd(L₁)(OH).H₂O and Nd(L₃)(OH).2H₂O were found to exert superior activity in comparison to the remaining complexes.

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Keywords: Bis-coumarins; Neodymium (III) complexes; IR- and NMR-spectra; Cytotoxic activity

1. Introduction

Coumarin is used widely as a therapeutic agent and is administered clinically in the treatment of certain lymphedemas and malignancies. 7-hydroxy- and 4-hydroxycoumarins are naturally occurring substances with a variety of biological activities, e.g. antitumoral action.

The antitumor activities of coumarin and its known metabolite 7-hydroxy-coumarin were tested in several human tumor cell lines by Steffen et al. [1]. Both compounds inhibited cell proliferation of a gastric carcinoma cell line, a colon-carcinoma cell line (Caco-2), a hepatoma-derived cell line (Hep-G2) and a lymphoblastic cell line (CCRF cem). Egan et al. [2] have synthesized, characterized and deter-

mined cytostatic and cytotoxic nature of 8-nitro-7-hydroxycoumarin using both human (including K-562 and HL-60) and animal cell lines grown in vitro. Coumarin and its 4-hydroxy and 7-hydroxy derivatives, as well as *o*-, *m*- and *p*-coumaric acid were tested against P-815 and P-388 tumor cells in vitro. All compounds were more or less cytotoxic against tumor cells [3]. The effect of warfarin on tumor cell growth was studied [4]. Warfarin inhibits metastasis of Mtn3 rat mammary carcinoma without affecting primary tumor growth. Seven known coumarins, showing significant cytotoxic activities on P388 cell lines, were isolated from the roots of *Angelica gigas* (Umbelliferae) [5]. Akman et al. [6] had investigated synergistic cytotoxicity between menadione and the related anticoagulant dicumarol, inhibited growth of murine leukemia L1210 in liquid suspension culture. The cytotoxicity of 22 natural and semisynthetic simple coumarins was evaluated in GLC4, a human small cell lung

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carcinoma cell line, and in COLO 320, a human colorectal cancer cell line, using the microculture tetrazolium (MTT) assay [7].

A number of 4-hydroxycoumarin derivatives have been studied as to their HIV integrase inhibitory potency [8]. The main purpose was to simplify the large structure of the compounds while maintaining their potency. It was found that the minimum active pharmacophore consists of a coumarin dimer containing an aryl substituent on the central linker, methylene. The addition of 4- and 7-hydroxy substituents in the coumarin rings improved the potency of the compounds. Among the systems studied, the 3,3'-benzylidene-bis(4-hydroxycoumarin) has been tested as a HIV integrase inhibitor and has shown significant activity [8]. The complexation ability of the 3,3'-benzylidene-bis(4-hydroxycoumarin) with lanthanides was not reported so far. It is expected that the complexes with this ligand and with similar ligands will retain or improve its biological activity as in the case of other lanthanide complexes with hydroxycoumarin derivatives.

The complexes of rare earth ions have aroused much interest. Lanthanides are a subject of increasing interest in bioinorganic and coordination chemistry [9,10].

Nowadays, a lot of studies report complexes of coumarin derivatives with rare earth metals, which possess biological activity. Thus, lanthanide complexes of 3-sulfo-4-hydroxycoumarin [11] and bis-(4-hydroxy-3-coumarinyl)-acetic acid [12] have been synthesised and characterised. The complexes have revealed good anticoagulant action.

Lanthanum chloride manifests an antitumor activity. Furthermore, literature data show that the coumarins have also these properties. These previous data from literature are in accordance with our investigations. They give our reason to suppose that complexes of coumarins with lanthanides could present interesting metalorganic compounds with antitumor activity. As a result from our earlier work the cytotoxic profile of some complexes of mendiixon, warfarin, coumachlor and niffcoumar with lanthanides against P3HR1, K-562 and THP-1 cell lines was proved [13–18]. The complexes of cerium, lanthanum and neodymium with these coumarin ligands induced approximately 30% reduction of the survival of P3HR1 Burkitt lymphoma cells at concentrations 100 and 400 μ M. The cerium and lanthanum complexes of mendiixon and niffcoumar induce similar low cytotoxic effect on AML derived THP-1 myeloleukemia cells. With the relatively resistant CML derived erythroleukemic K-562 cell line we obtained very interesting in vitro results. It is noteworthy that the lanthanum and neodymium complexes with niffcoumar exert more pronounced cytotoxic effects in comparison to cerium complex. They have a strong cell proliferation inhibiting effects (only about 30% of the cells survived). This means that the resistant tumor cells may be inhibited well 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 of resistant tumors. The corresponding lanthanide salts are found to be of very low or missing activity. So far we can conclude that the structure of metal-ligand determines the antitumor spectrum of the newly formed complexes. Those in vitro effects are not so clearly expressed as it is in the case of *cis*-DDP (II). Nevertheless their study is interesting in connection with other cell lines and tumors in order to find out the differences in their spectrum of activity.

Unfortunately, little is known about the complexing ability of neodymium (III) with coumarins. A survey of the literature reveals that no work has been done on the reactions of neodymium (III) with 3,3'-benzylidene-bis(4-hydroxy-2H-1-benzopyran-2-one) and its derivatives. It was, therefore, considered worthwhile to study the complexation and in the first place the objective of this study was to determine whether the new complexes were active as cytotoxic agents.

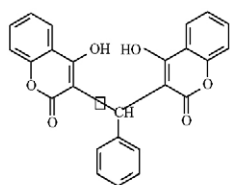
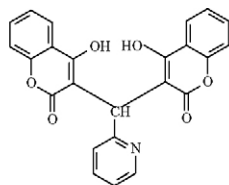
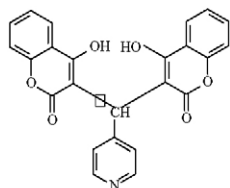
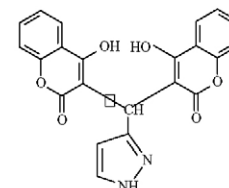
In the present study, we perform investigation of the coordination ability of 3,3'-benzylidene-bis(4-hydroxy-2H-1-benzopyran-2-one); bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-2-yl-methane; bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-4-yl-methane; bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-(1H-pyrazol-3-yl)-methane in complexation reaction with neodymium (III). The obtained Nd (III) complexes with these coumarin ligands was characterized by elemental analysis, physicochemical methods, mass-, NMR- and IR-spectroscopy. The complicated vibrational spectra of neodymium (III) complexes were interpreted on the basis of comparison with the vibrational spectra of the free ligands. The most sensitive to coordination modes of the ligands have been assigned and discussed.

We observed that Nd (III) possess a cytotoxic activity and literature data show that the coumarins have also these properties. That is why our synthesis of complexes of Nd (III) is taken into consideration with cytotoxic screening and further pharmacological study.

2. Chemistry

The compounds used for preparing the solutions were Merck products, p.a. grade: $\text{Nd}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$. 3,3'-benzylidene-bis(4-hydroxy-2H-1-benzopyran-2-one), bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-2-yl-methane, bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-4-yl-methane and bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-(1H-pyrazol-3-yl)-methane were used for the preparation of metal complexes as ligands (Scheme 1). These ligands were synthesized by condensation of 4-hydroxycoumarin and aromatic or heterocyclic aldehyde in ethanol medium at reflux and stirring until crystals appeared to us.

The complexes of neodymium (III) with 3,3'-benzylidene-bis(4-hydroxy-2H-1-benzopyran-2-one) ($\text{H}_2\text{L1}$); bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-2-yl-methane ($\text{H}_2\text{L2}$); bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-4-yl-methane ($\text{H}_2\text{L3}$); bis(4-hydroxy-2-oxo-2H-chromen-3-

H₂L1 = 3,3'-benzylidene-bis(4-hydroxy-2H-1-benzopyran-2-one)H₂L2 = bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-2-yl-methaneH₂L3 = bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-4-yl-methaneH₂L4 = bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-(1H-pyrazol-3-yl)-methane

Scheme 1. Structures of the ligands

yl)-(1H-pyrazol-3-yl)-methane (H₂L4) were synthesized by reaction of neodymium (III) salt and the ligand, in amounts equal to metal:ligand molar ratio of 1:2. The complexes were prepared by adding an aqueous solution of 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 mixtures were 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, washed several times with water and dried in a desiccator to constant weight. The complexes were insoluble in water, slightly soluble in methanol and ethanol and soluble in DMSO.

3. Pharmacology

3.1. Human tumor cell lines and culture conditions

The antineoplastic activity of the tested compounds was assessed on the lymphoid SKW-3 cell line (DSMZ No. ACC 53, cell type: human T-cell leukemia, origin: established from the peripheral blood of a 61-year-old man with T-cell chronic lymphocytic leukemia in 1977, doubling time of ca. 30–40 h, cytogenetics: human near diploid karyotype with 4% polyploidy and on the myeloid HL-60 (DSMZ No. ACC 3, cell type: human acute myeloid leukemia, origin: established from the peripheral blood of a 35-year-old woman with acute myeloid leukemia in 1976, doubling time of ca. 25 h, cytogenetics: human flat-moded hypotetraploid karyotype with hypodiploid sideline and 1.5% polyploidy); as well as on the resistant variant HL-60/Dox, which is characterized by the expression of the multi-drug resistance-associated protein MRP-1, which conditions pleiotropic drug resistance

in this cell line. Cell lines were obtained from the Department of Human and Animal Cell Cultures at the German Collection of Microorganisms and Cell Cultures (DSMZ). They were grown as suspension-type cultures under standard conditions—RPMI 1640 medium (Sigma), supplemented with 10% heat inactivated fetal bovine serum (Sigma) and 2 mM L-glutamine (Sigma), in controlled environment—‘Heraeus’ incubator with humidified atmosphere and 5% carbon dioxide, at 37 °C in cell culture flasks. In order to maintain the cells in log-phase, cell suspension was discarded 2–3 times per week and the remaining culture was supplemented with fresh medium aliquots. The RPMI medium for HL-60/Dox contained 0.2 μM doxorubicin.

3.2. Cytotoxicity determination

The cytotoxic activity of the investigated neodymium complexes was assessed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye-reduction assay as described by Mosmann [19], with some modifications [20]. The method is based on the ability of the vital cells to metabolize the yellow tetrazolium dye MTT to violet, water-insoluble formazan. After the dissolution of the latter, using acidification and organic solvent addition its concentration, which is proportional to dye number of viable cells is determined spectrophotometrically. Briefly logarithmically growing cells were seeded into 96-well microplates (100 μl/well at a density of 1×10^5 cells/ml) and exposed to various concentrations of the investigated compounds for or 72 h. After the incubation with the test-compound, MTT-solution (10 mg/ml in PBS) was added (10 μl/well). Plates were further incubated for 4 h at 37 °C and the formazan crystals formed were dissolved by adding 100 μl/well of 5% formic acid in 2-propanol. Absorption was measured by an ELISA reader (Uniscan–Titertek) at 540 nm, reference filter 690 nm. For each concentration at least eight wells were used. 100 μl RPMI 1640 medium with 10 μl MTT stock and 100 μl 5% formic acid in 2-propanol was used as blank solution.

4. Results and discussion

4.1. Chemistry

The complexes were characterized by elemental analysis. The metal ion was determined after mineralisation. The water content in the complexes was determined by Karl Fisher analysis. The formation of the complexes was confirmed by IR-spectroscopy, ¹H, ¹³C-NMR-spectroscopy and mass-spectral data.

Table 1 shows the data of the elemental analysis of the complexes serving as a basis for the determination of their empirical formulae. The elemental analysis data of the Nd (III) complexes obtained are in agreement with the presented formulas.

Table 1
Elemental analysis data for Nd (III) complexes with bis-coumarins

Complex	Experimental/calculated			%H ₂ O	%Nd
	%C	%H	%N		
Nd(L ₁)(OH).H ₂ O	51.27	3.24	–	3.5	24.18
	50.93	2.89	–	3.06	24.45
Nd(L ₂)(OH).2H ₂ O	47.6	3.42	2.64	6.38	23.45
	47.37	2.96	2.3	5.92	23.68
Nd(L ₃)(OH).2H ₂ O	47.44	3.28	2.55	6.29	23.36
	47.37	2.96	2.3	5.92	23.68
Nd(L ₄)(OH).2H ₂ O	44.24	3.05	5.11	6.3	23.97
	44.22	2.85	4.69	6.03	24.12

L₁ = C₂₅H₁₄O₆²⁻; L₂ = C₂₄H₁₃NO₆²⁻; L₃ = C₂₄H₁₃NO₆²⁻; L₄ = C₂₂H₁₂N₂O₆²⁻.

The suggested formulas were further confirmed by mass-spectral fragmentation analysis. As it is seen from Table 2, the first peaks in the Nd (III) complexes spectra (although with low intensity) correspond to the mass-weight of the complex formation and the next ones to that of the ligands. The results thus obtained are in agreement with metal:ligand ratio 1:1 in the investigated complexes. The data of mass-spectral fragmentation of the ligands and of the complexes are presented in Table 2.

4.2. IR spectra of the complexes

The mode of bonding of the ligands to Nd (III) was elucidated by recording the IR spectra of the complexes as compared with this of the free ligands.

IR-spectra of the compounds were recorded on solid state in Nujol in the range 3800–400 cm⁻¹. The data of the IR

spectra of 3,3'-benzylidene-bis(4-hydroxy-2H-1-benzopyran-2-one) (H₂L1); bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-2-yl-methane (H₂L2); bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-4-yl-methane (H₂L3); bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-(1H-pyrazol-3-yl)-methane (H₂L4) and of the neodymium complexes with these ligands are presented in Table 3.

4.2.1. IR-spectrum of the complex of 3,3'-benzylidene-bis(4-hydroxy-2H-1-benzopyran-2-one) (H₂L1)

The bands appear in the IR spectrum of 3,3'-benzylidene-bis(4-hydroxy-2H-1-benzopyran-2-one) (H₂L1) at 3074, 3032; 1660, 1617; 1605, 1568; 1496, 1182, 1160, 1092, 1074 cm⁻¹. The bands at 1660 and 1617 cm⁻¹ can be attributed to the stretching vibrations of the carbonyl groups of the lacton rings. Bands at 1605 and 1568 cm⁻¹ can be related to the stretching vibrations of the conjugated olefinic system. The vibrations at 1496 cm⁻¹ correspond to the aromatic systems.

A broad band, characteristic of ν_{OH} of coordinated water was observed in the range 3300–3400 cm⁻¹ in the spectrum of the complex. The weak bands observed at 3074 and 3032 cm⁻¹ in the spectrum of the free ligand is missing in the spectrum of the complex. A comparison of the infrared spectra of the ligand and of the complex reveals the disappearance of absorption bands observed in the free ligand at 3074, 3032 cm⁻¹ and 1345, 1336 cm⁻¹ associated with the stretching and deformation OH of the phenolic groups, indicating the loss of phenolic protons on complexation, thus forming

Table 2
Mass-spectral data of bis-coumarins and their Nd (III) complexes

Ligand	m/z	(%)	Complex	m/z	(%)
H ₂ L1=C ₂₅ H ₁₆ O ₆	412	8	Nd(L ₁)(OH).H ₂ O	589	2
	249	100		410	1
	221	17		307	68
	162	20		176	100
	120	37			
H ₂ L2=C ₂₄ H ₁₅ NO ₆	413	7	Nd(L ₂)(OH).2H ₂ O	607	2
	395	2		573	4
	252	7		552	1
	162	30		411	1
	120	28		307	45
	92	38		176	100
H ₂ L3=C ₂₄ H ₁₅ NO ₆	413	0	Nd(L ₃)(OH).2H ₂ O	604	4
	252	18		573	5
	250	50		552	6
	162	62		410	4
	120	74		307	44
	92	86		176	100
H ₂ L4=C ₂₂ H ₁₄ N ₂ O ₆	402	0	Nd(L ₄)(OH).2H ₂ O	600	1
	241	16		579	3
	240	100		562	1
	162	72		410	1
	120	74		307	48
	92	98		176	100

Table 3
Selected experimental IR frequencies of the ligands and their Nd (III) complexes (cm⁻¹)

Compound	$\nu(\text{OH}/\text{H}_2\text{O})$	$\nu(\text{C}=\text{O})$	$\nu(\text{C}=\text{C})$	$\nu(\text{Py})$	$\nu(\text{Ar})$	$\delta(\text{COH})$	$\nu(\text{C}-\text{O})$
$\text{H}_2\text{L1}=\text{C}_{25}\text{H}_{16}\text{O}_6$	3074m	1660s	1605s	–	1496m	1345m 1336m	1182m
	3032m	1617s	1568s				1160m 1092s 1074m 1192w 1150w 1109m 1094w 1181m
$\text{Nd}(\text{L}_1)(\text{OH})\cdot\text{H}_2\text{O}$	3391br	1625sh 1599s	1505s	–	1450m	–	1164m 1111s 1039m 1212w 1150w 1109m 1078w 1181m
							1155m 1107s 1037m 1186w 1150w 1109m 1075w 1187m
$\text{H}_2\text{L2}=\text{C}_{24}\text{H}_{15}\text{NO}_6$	3122m	1696s	1608s	1620 1559	1489m	1350m 1332m	1164m 1111s 1039m 1212w 1150w 1109m 1078w 1181m
	3060m	1635s	1539s				1155m 1107s 1037m 1186w 1150w 1109m 1075w 1187m
$\text{Nd}(\text{L}_2)(\text{OH})\cdot 2\text{H}_2\text{O}$	3400br	1652sh 1598s	1522s	1558 1506 1418 1620	1436m	–	1150w 1109m 1078w 1181m
							1155m 1107s 1037m 1186w 1150w 1109m 1075w 1187m
$\text{H}_2\text{L3}=\text{C}_{24}\text{H}_{15}\text{NO}_6$	3180m	1699s	1610s	1558 1520 1405 1622	1498m	1340m 1315m	1155m 1107s 1037m 1186w 1150w 1109m 1075w 1187m
	3120m	1635s	1538s				1150m 1110s 1044m 1195w 1145w 1108m 1054w
$\text{Nd}(\text{L}_3)(\text{OH})\cdot 2\text{H}_2\text{O}$	3382br	1653sh 1600s	1520s	1558 1516 1418 1620	1436 m	–	1150w 1109m 1075w 1187m
							1150m 1110s 1044m 1195w 1145w 1108m 1054w
$\text{H}_2\text{L4}=\text{C}_{22}\text{H}_{14}\text{N}_2\text{O}_6$	3139m	1669s	1610s	1559 1507 1417 1622	1496m	1360m 1300m	1150m 1110s 1044m 1195w 1145w 1108m 1054w
	3070m	1635s	1539s				1150m 1110s 1044m 1195w 1145w 1108m 1054w
$\text{Nd}(\text{L}_4)(\text{OH})\cdot 2\text{H}_2\text{O}$	3375br	1653sh 1599s	1520s	1559 1505 1420	1460m	–	1150m 1110s 1044m 1195w 1145w 1108m 1054w
							1150m 1110s 1044m 1195w 1145w 1108m 1054w

^a br-broad, s-strong, m-medium, sh-shoulder, w-weak.

metal–oxygen bonds which appear as bands in the far IR region.

The $\nu_{\text{C}=\text{O}}$ bands at 1660 and 1617 cm⁻¹ exhibits a shift of 30–40 cm⁻¹ to lower wavenumber values on complexation which may be taken as evidence for the participation of the C=O groups in coordination.

The C–C and C–O stretch and the C–O–C band are all shifted in the complex. Similar frequency shifts are observed for the other complexes and are attributed to complexation of the positive ion with the carbonyl oxygen [21].

4.2.2. IR-spectrum of the complex of bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-2-yl-methane ($\text{H}_2\text{L2}$)

The bands appear in the IR spectrum of bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-2-yl-methane ($\text{H}_2\text{L2}$) at 3122, 3060; 1696, 1635; 1608, 1539; 1489, 1181, 1164, 1111, 1039 cm⁻¹. The bands at 1696 and 1635 cm⁻¹ can be attributed to the stretching vibrations of the carbonyl groups of the lacton rings. Bands at 1608 and 1539 cm⁻¹ can be related to the stretching vibrations of the conjugated olefinic system. The vibrations at 1489 cm⁻¹ correspond to the aromatic systems. Bands at

1620, 1559, 1505, 1410 cm⁻¹ can be attributed to the stretching vibrations of pyridine and they remain almost the same in the complex.

A broad band, characteristic of ν_{OH} of coordinated water was observed in the range 3300–3400 cm⁻¹ in the spectrum of the complex. The weak bands observed at 3122 and 3060 cm⁻¹ in the spectrum of the free ligand is missing in the spectrum of the complex. A comparison of the infrared spectra of the ligand and of the complex reveals the disappearance of absorption bands observed in the free ligand at 3122, 3060 cm⁻¹ and 1350, 1332 cm⁻¹ associated with the stretching and deformation OH of the phenolic groups, indicating the loss of phenolic protons on complexation, thus forming metal–oxygen bonds which appear as bands in the far IR region.

The $\nu_{\text{C}=\text{O}}$ bands at 1696 and 1635 cm⁻¹ exhibits a shift of 30–40 cm⁻¹ to lower wavenumber values on complexation which may be taken as evidence for the participation of the C=O groups in coordination.

The C–C and C–O stretch and the C–O–C band are all shifted in the complex. Similar frequency shifts are observed for the other complexes and are attributed to complexation of the positive ion with the carbonyl oxygen [21].

4.2.3. IR-spectrum of the complex of bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-4-yl-methane (H_2L3)

The bands appear in the IR spectrum of bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-4-yl-methane (H_2L3) at 3180, 3120; 1699, 1635; 1610, 1538; 1498, 1181, 1155, 1107, 1037 cm^{-1} . The bands at 1699 and 1635 cm^{-1} can be attributed to the stretching vibrations of the carbonyl groups of the lacton rings. Bands at 1610 and 1538 cm^{-1} can be related to the stretching vibrations of the conjugated olefinic system. The vibrations at 1498 cm^{-1} correspond to the aromatic systems. Bands at 1620, 1558, 1520, 1405 cm^{-1} can be attributed to the stretching vibrations of pyridine and they remain almost the same in the complex.

A broad band, characteristic of ν_{OH} of coordinated water was observed in the range 3300–3400 cm^{-1} in the spectrum of the complex. The weak bands observed at 3180 and 3120 cm^{-1} in the spectrum of the free ligand is missing in the spectrum of the complex. A comparison of the infrared spectra of the ligand and of the complex reveals the disappearance of absorption bands observed in the free ligand at 3180, 3120 cm^{-1} and 1340, 1315 cm^{-1} associated with the stretching and deformation OH of the phenolic groups, indicating the loss of phenolic protons on complexation, thus forming metal–oxygen bonds which appear as bands in the far IR region.

The $\nu_{\text{C=O}}$ bands at 1699 and 1635 cm^{-1} exhibits a shift of 30–40 cm^{-1} to lower wavenumber values on complexation which may be taken as evidence for the participation of the C=O groups in coordination.

The C–C and C–O stretch and the C–O–C band are all shifted in the complex. Similar frequency shifts are observed for the other complexes and are attributed to complexation of the positive ion with the carbonyl oxygen [21].

4.2.4. IR-spectrum of the complex of bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-(1H-pyrazol-3-yl)-methane (H_2L4)

The bands appear in the IR spectrum of bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-(1H-pyrazol-3-yl)-methane (H_2L4) at 3139, 3070; 1669, 1635; 1610, 1539; 1496, 1187, 1150, 1110, 1044 cm^{-1} . The bands at 1669 and 1635 cm^{-1} can be attributed to the stretching vibrations of the carbonyl groups of the lacton rings. Bands at 1610 and 1539 cm^{-1} can be related to the stretching vibrations of the conjugated olefinic system. The vibrations at 1496 cm^{-1} correspond to the aromatic systems. Bands at 1620–1417 cm^{-1} can be attributed to the stretching vibrations of pyrazol and they remain almost the same in the complex.

A broad band, characteristic of ν_{OH} of coordinated water was observed in the range 3300–3400 cm^{-1} in the spectrum of the complex. The weak bands observed at 3139 and 3070 cm^{-1} in the spectrum of the free ligand is missing in the spectrum of the complex. A comparison of the infrared spectra of the ligand and of the complex reveals the disappearance of absorption bands observed in the free ligand at 3139,

3070 cm^{-1} and 1360, 1300 cm^{-1} associated with the stretching and deformation OH of the phenolic groups, indicating the loss of phenolic protons on complexation, thus forming metal–oxygen bonds which appear as bands in the far IR region.

The $\nu_{\text{C=O}}$ bands at 1669 and 1635 cm^{-1} exhibits a shift of 20–30 cm^{-1} to lower wavenumber values on complexation which may be taken as evidence for the participation of the C=O groups in coordination.

The C–C and C–O stretch and the C–O–C band are all shifted in the complex. Similar frequency shifts are observed for the other complexes and are attributed to complexation of the positive ion with the carbonyl oxygen [21].

IR-spectra of the compounds were recorded on solid state in Nujol in the range 700–220 cm^{-1} . The spectrum of the complex shows new bands, in comparison with this of the free ligand, which have been assigned to the rocking, wagging and metal–oxygen stretching vibrations.

4.3. ^1H - and ^{13}C -NMR spectra of the ligands and their Nd (III) complexes

Metal ion coordination with ligand by means of oxygen atoms of C=O groups and of the deprotonated hydroxyl groups was shown owing to data of ^1H - 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 ^1H -NMR spectra in DMSO- d_6 are presented in Table 4. As it is seen from Table 4, chemical shifts to higher ppm were observed in the complexes and they were attributed to coordination of ligands to Nd (III).

^{13}C -NMR spectra of the ligands and of the complexes were recorded at 62.9 MHz in DMSO- d_6 . The results of

Table 4

^1H NMR spectral shifts, δ (ppm) of the ligands and their Nd (III) complexes (250 MHz, DMSO- d_6)

Compound	δ (ppm)		
	$\text{H}_5\text{--H}_8^a$	H_9^a	$\text{H}_2\text{--H}_6^a$
$\text{H}_2\text{L1}=\text{C}_{25}\text{H}_{16}\text{O}_6$	7.11–7.39	6.37	7.56–7.92
$\text{Nd}(\text{L}_1)(\text{OH})\cdot\text{H}_2\text{O}$	7.47–7.60	6.64	7.88–8.16
$\text{H}_2\text{L2}=\text{C}_{24}\text{H}_{15}\text{NO}_6$	7.24–7.58	6.54	7.80–8.64
$\text{Nd}(\text{L}_2)(\text{OH})\cdot 2\text{H}_2\text{O}$	7.20–7.75	6.3	8.35–8.81
$\text{H}_2\text{L3}=\text{C}_{24}\text{H}_{15}\text{NO}_6$	7.22–7.58	6.46	7.80–8.68
$\text{Nd}(\text{L}_3)(\text{OH})\cdot 2\text{H}_2\text{O}$	7.20–7.80	6.72	8.40–8.80
$\text{H}_2\text{L4}=\text{C}_{22}\text{H}_{14}\text{N}_2\text{O}_6$	7.23–7.55	6.36	7.83–8.15
$\text{Nd}(\text{L}_4)(\text{OH})\cdot 2\text{H}_2\text{O}$	7.18–7.80	5.89	8.02–8.48

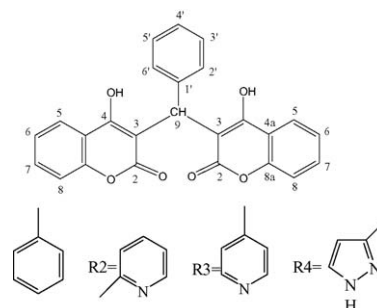


Table 5

¹³C NMR spectral shifts, δ (ppm) of the ligands and their Nd(III) complexes (62.9 MHz, DMSO-d₆)

Atom	δ (ppm)							
	H ₂ L1=C ₂₅ H ₁₆ O ₆	Nd(L ₁)(OH).H ₂ O	H ₂ L2=C ₂₄ H ₁₅ NO ₆	Nd(L ₂)(OH).2H ₂ O	H ₂ L3=C ₂₄ H ₁₅ NO ₆	Nd(L ₃)(OH).2H ₂ O	H ₂ L4=C ₂₂ H ₁₄ N ₂ O ₆	Nd(L ₄)(OH).2H ₂ O
C-2	165.3	167.7	168.6	164.8	168.2	164.7	167.9	169.1
C-4	164.9	164.6	164.0	161.9	164.9	157.0	163.9	157.6
C-8a	152.2	152.5	157.6	157.28	164.2	155.8	152.7	157.1
C-1'	139.9	142.3	152.9	152.58	152.8	148.3	150.5	152.6
C-7	131.9	130.9	146.5	148.3	141.0	146.2	134.3	142.1
C-3'	128.1	127.6	141.9	135.8	131.7	134.5	131.6	132.8
C-5'	128.1	127.6	141.9	135.8	131.7	134.5	–	–
C-4'	126.7	126.6	131.9	130.3	–	–	–	–
C-6'	125.6	124.8	125.9	124.2	125.3	129.8	–	–
C-2'	125.6	124.8	–	–	125.3	129.8	106.1	129.9
C-5	123.9	124.1	124.4	122.4	124.3	126.2	124.3	122.1
C-6	123.8	122.9	123.4	120.4	123.3	121.9	123.3	120.1
C-4a	117.9	119.9	119.3	117.6	119.5	117.1	119.5	117.6
C-8	115.9	115.4	115.9	115.5	115.9	116.8	115.8	117.4
C-3	104.1	103.4	100.5	103.5	101.5	102.7	101.4	103.8
C-9	35.9	36.5	36.7	38.5	37.9	34.4	30.1	38.5

¹³C-NMR spectra of the compounds in δ (ppm) are presented in Table 5.

The ligand 3,3'-benzylidene-bis(4-hydroxy-2H-1-benzopyran-2-one) (H₂L1) showed seven signals in the ¹³C-NMR spectra resonating at δ 131.91, 128.08, 126.70, 125.58, 123.92, 123.76 and 115.95 ppm for 13 methine carbons (Table 5). In agreement with literature data, the peaks at δ 131.91, 123.92, 123.76 and 115.95 ppm were related to C-7, C-5, C-6 and C-8 (the atom numbering is in agreement with the scheme in Table 4 carbons, respectively of the coumarin moieties. The signals at δ 128.08, 126.70 and 125.58 ppm were assigned to C-3' (and C-5'), C-4' and C-2' (and C-6') carbons of the phenyl ring. The chemical shifts at δ 165.36, 164.87, 152.23, 139.94, 117.96 and 104.13 ppm are due to the C-2, C-4, C-8a, C-1', C-4a and C-3 quaternary carbons, respectively. Due to electron transfer from the hydroxyl and carbonyl oxygen atoms to Nd (III), a difference in chemical shifts was observed for the neighboring C-4, C-3 and C-2

carbon atoms of the complex and they confirmed the expected coordination of the ligand through both deprotonated hydroxyl and carbonyl oxygen atoms. The other carbon atoms were only slightly affected from the coordination of the metal. Similar chemical shifts were observed for the other ligands and their complexes (Table 5). On the basis of the results thus obtained, it was suggested that the ligands act as tetradentate ones in the Nd (III) complex formation.

4.4. Pharmacology

The in vitro screening data for the investigated compounds are presented in Tables 6–9 and Figs. 1–12. The investigations carried out enabled the construction of dose–response curves with consequent calculation of the IC₅₀ values for all compounds under investigation. The data for the cytotoxic efficacy of the tested compounds on HL-60 cells indicate that Nd(L₁)(OH).H₂O proved to be the most

Table 6

Spectrophotometric data from MTT assay concerning the cytotoxic activity of the investigated Nd complexes on HL-60 cells after 72 h incubation

Compound	MTT-formazan absorption at 580 nm					
	Untreated control	31.25 (μ M)	62.5 (μ M)	125 (μ M)	250 (μ M)	500 (μ M)
Nd(L ₁)(OH).H ₂ O	0.6047 \pm 0.0351	0.489 \pm 0.0576	0.335 \pm 0.0137	0.2658 \pm 0.0337	0.233 \pm 0.0103	0.1548 \pm 0.0076
Nd(L ₂)(OH).2H ₂ O	0.6047 \pm 0.0351	0.592 \pm 0.0176	0.511 \pm 0.0303	0.3228 \pm 0.0189	0.252 \pm 0.0187	0.2133 \pm 0.0047
Nd(L ₃)(OH).2H ₂ O	0.6047 \pm 0.0351	0.5893 \pm 0.0646	0.5995 \pm 0.0058	0.4863 \pm 0.0504	0.2143 \pm 0.0172	0.1778 \pm 0.6387
Nd(L ₄)(OH).2H ₂ O	0.6047 \pm 0.0351	0.5913 \pm 0.0187	0.5325 \pm 0.0239	0.468 \pm 0.0422	0.3028 \pm 0.0170	0.22 \pm 0.0117

Table 7

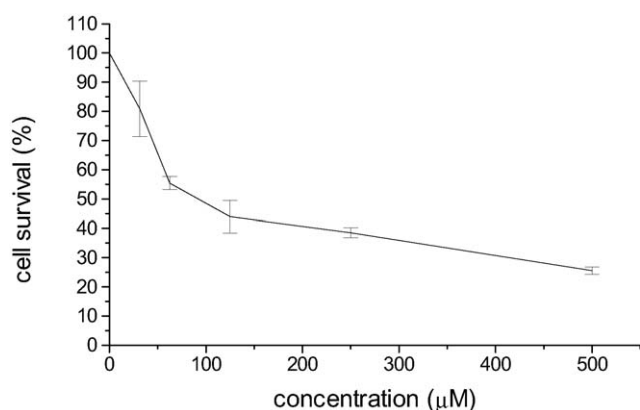
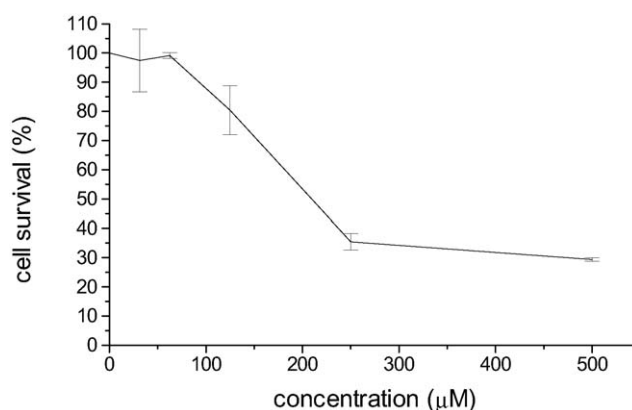
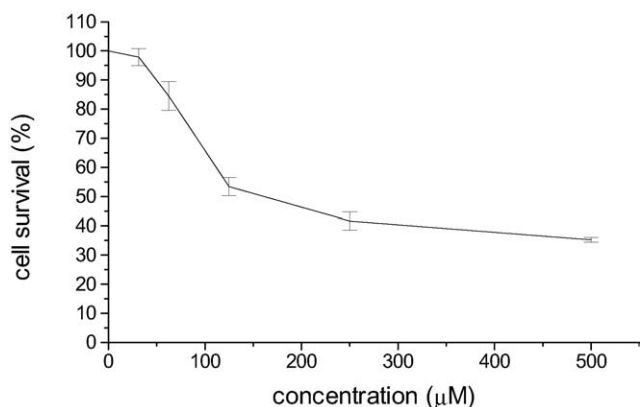
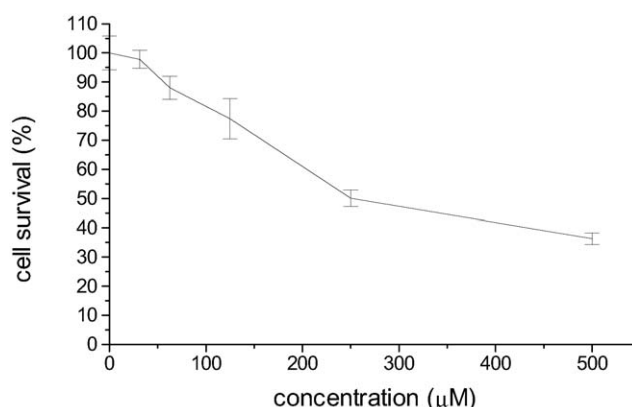
Spectrophotometric data from MTT assay concerning the cytotoxic activity of the investigated Nd complexes on HL-60/Dox cells after 72 h incubation

Compound	MTT-formazan absorption at 580 nm					
	Untreated control	31.25 (μ M)	62.5 (μ M)	125 (μ M)	250 (μ M)	500 (μ M)
Nd(L ₁)(OH).H ₂ O	1.1204 \pm 0.0650	0.8293 \pm 0.0163	0.4068 \pm 0.0415	0.2233 \pm 0.0125	0.1755 \pm 0.0185	0.1888 \pm 0.0199
Nd(L ₂)(OH).2H ₂ O	1.1204 \pm 0.0650	1.244 \pm 0.0360	1.3575 \pm 0.0699	0.8335 \pm 0.1546	0.333 \pm 0.0614	0.2198 \pm 0.0269
Nd(L ₃)(OH).2H ₂ O	1.1204 \pm 0.0650	1.2153 \pm 0.0277	1.2888 \pm 0.0716	1.229 \pm 0.2140	0.6348 \pm 0.0311	0.1823 \pm 0.0222
Nd(L ₄)(OH).2H ₂ O	1.1204 \pm 0.0650	1.1178 \pm 0.0641	0.9735 \pm 0.1152	0.8013 \pm 0.1005	0.8318 \pm 0.0349	0.3663 \pm 0.0627

Table 8

Spectrophotometric data from MTT assay concerning the cytotoxic activity of the investigated Nd complexes on SKW-3 cells after 72 h incubation

Compound	MTT-formazan absorption at 580 nm					
	Untreated control	31.25 (μM)	62.5 (μM)	125 (μM)	250 (μM)	500 (μM)
Nd(L ₁)(OH).H ₂ O	0.2772 \pm 0.0114	0.2973 \pm 0.0220	0.1785 \pm 0.0214	0.1455 \pm 0.0068	0.1073 \pm 0.0119	0.082 \pm 0.0095
Nd(L ₂)(OH).2H ₂ O	0.2772 \pm 0.0114	0.3173 \pm 0.008	0.2995 \pm 0.0244	0.137 \pm 0.0244	0.1215 \pm 0.0019	0.1103 \pm 0.0078
Nd(L ₃)(OH).2H ₂ O	0.2772 \pm 0.0114	0.3045 \pm 0.0121	0.2625 \pm 0.0443	0.1398 \pm 0.0121	0.1158 \pm 0.0102	0.089 \pm 0.0116
Nd(L ₄)(OH).2H ₂ O	0.2772 \pm 0.0114	0.267 \pm 0.0102	0.244 \pm 0.0161	0.1905 \pm 0.0094	0.0958 \pm 0.0213	0.0995 \pm 0.0099

Fig. 1. Cytotoxic activity of Nd(L₁)(OH).H₂O on HL-60 cells after 72 h incubation as assessed by MTT assay. Each data point represents the arithmetic mean of at least six independent experiments.Fig. 3. Cytotoxic activity of Nd(L₃)(OH).2H₂O on HL-60 cells after 72 h incubation as assessed by MTT assay. Each data point represents the arithmetic mean of at least six independent experiments.Fig. 2. Cytotoxic activity of Nd(L₂)(OH).2H₂O on HL-60 cells after 72 h incubation as assessed by MTT assay. Each data point represents the arithmetic mean of at least six independent experiments.Fig. 4. Cytotoxic activity of Nd(L₄)(OH).2H₂O on HL-60 cells after 72 h incubation as assessed by MTT assay. Each data point represents the arithmetic mean of at least six independent experiments.

active compound with IC₅₀ value 90.1 μM , whereas the other compounds could be ranged according to the decrease in potency as follows: Nd(L₂)(OH).2H₂O (IC₅₀ = 161.64 μM) > Nd(L₃)(OH).2H₂O (IC₅₀ = 209.4 μM) > Nd(L₄)(OH).2H₂O (IC₅₀ = 255.24 μM). The most active compounds

Table 9

IC₅₀ values of the investigated Nd complexes on HL-60, HL-60/Dox and SKW-3 cells after 72 h incubation

Compound	IC ₅₀ value (μM)		
	HL-60	HL-60/Dox	SKW-3
Nd(L ₁)(OH).H ₂ O	90.01	51.4	131.59
Nd(L ₂)(OH).2H ₂ O	161.64	161.85	123.1
Nd(L ₃)(OH).2H ₂ O	209.4	290.61	130.8
Nd(L ₄)(OH).2H ₂ O	255.4	396.7	192.4

Nd(L₁)(OH).H₂O and Nd(L₂)(OH).2H₂O also caused the most prominent maximal efficacy at the higher concentration investigated (500 μM)—about 25% of the cells were viable. The other complexes investigated caused less pronounced proliferation inhibition with approximately 35% vital cells at the highest concentration. The results obtained for the effects on the resistant HL-60/Dox cells revealed that Nd(L₁)(OH).H₂O and Nd(L₂)(OH).2H₂O were the most active compounds with IC₅₀ values 51.4 and 161.85 μM , respectively. These data suggest that there is no cross resistance to both complexes in HL-60/Dox and even collateral sensitivity to Nd(L₁)(OH).H₂O was discovered, since its IC₅₀ value in the resistant sub-line is almost twice smaller than that in the sensitive line HL-60. Interestingly we found that HL-60/Dox was quite less sensitive to the other compounds under inves-

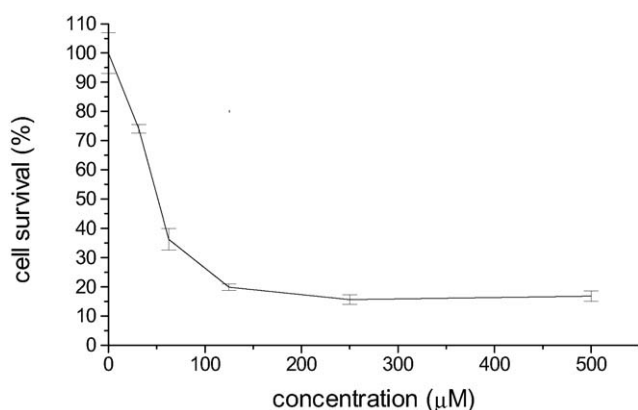


Fig. 5. Cytotoxic activity of $\text{Nd}(\text{L}_1)(\text{OH})\cdot\text{H}_2\text{O}$ on HL-60/Dox cells after 72 h incubation as assessed by MTT assay. Each data point represents the arithmetic mean of at least six independent experiments.

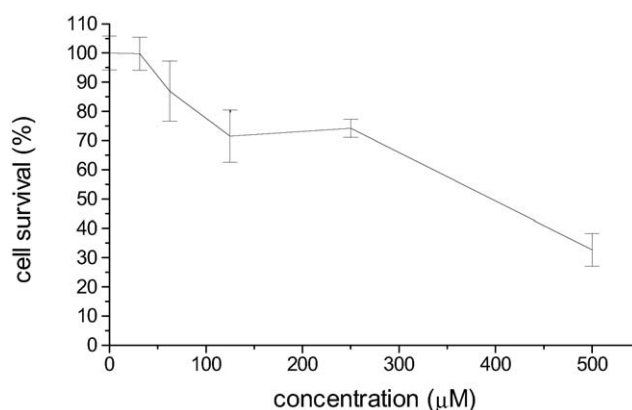


Fig. 8. Cytotoxic activity of $\text{Nd}(\text{L}_4)(\text{OH})\cdot 2\text{H}_2\text{O}$ on HL-60/Dox cells after 72 h incubation as assessed by MTT assay. Each data point represents the arithmetic mean of at least six independent experiments.

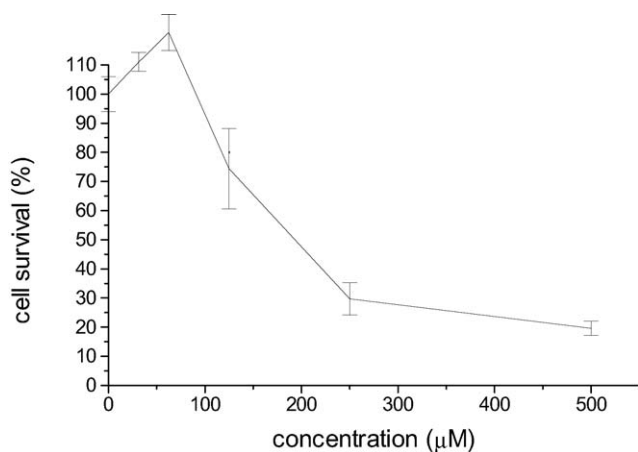


Fig. 6. Cytotoxic activity of $\text{Nd}(\text{L}_2)(\text{OH})\cdot 2\text{H}_2\text{O}$ on HL-60/Dox cells after 72 h incubation as assessed by MTT assay. Each data point represents the arithmetic mean of at least six independent experiments.

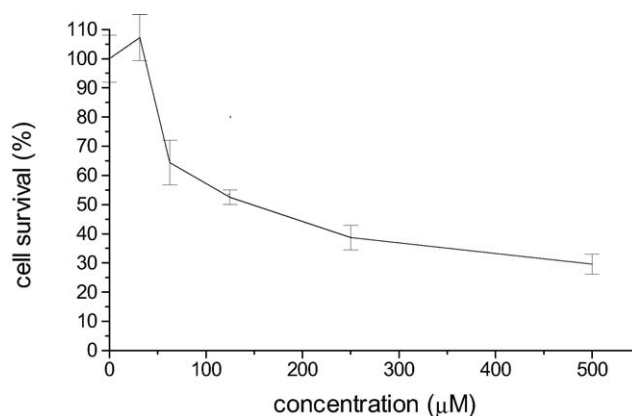


Fig. 9. Cytotoxic activity of $\text{Nd}(\text{L}_1)(\text{OH})\cdot\text{H}_2\text{O}$ on SKW-3 cells after 72 h incubation as assessed by MTT assay. Each data point represents the arithmetic mean of at least six independent experiments.

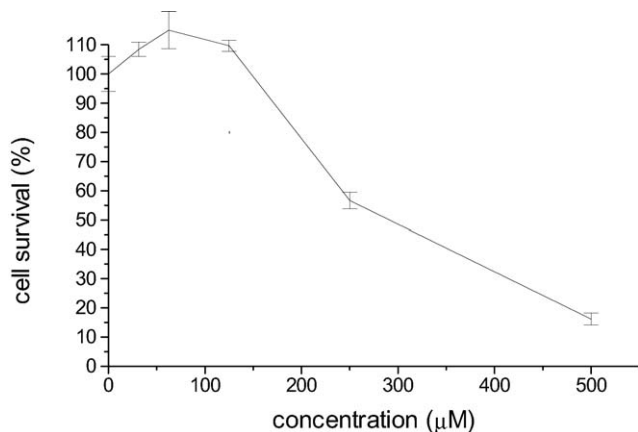


Fig. 7. Cytotoxic activity of $\text{Nd}(\text{L}_3)(\text{OH})\cdot 2\text{H}_2\text{O}$ on HL-60/Dox cells after 72 h incubation as assessed by MTT assay. Each data point represents the arithmetic mean of at least six independent experiments.

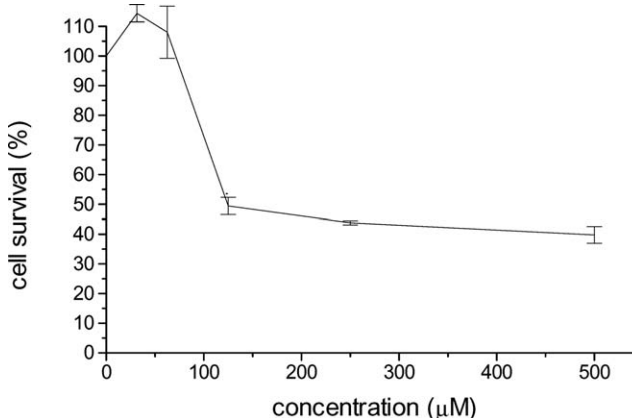


Fig. 10. Cytotoxic activity of $\text{Nd}(\text{L}_2)(\text{OH})\cdot 2\text{H}_2\text{O}$ on SKW-3 cells after 72 h incubation as assessed by MTT assay. Each data point represents the arithmetic mean of at least six independent experiments.

tigation $\text{Nd}(\text{L}_3)(\text{OH})\cdot 2\text{H}_2\text{O}$ and $\text{Nd}(\text{L}_4)(\text{OH})\cdot 2\text{H}_2\text{O}$ with indices of resistance 1.4 and 1.55, respectively. The maximal efficacy of $\text{Nd}(\text{L}_2)(\text{OH})\cdot 2\text{H}_2\text{O}$, $\text{Nd}(\text{L}_1)(\text{OH})\cdot\text{H}_2\text{O}$ and $\text{Nd}(\text{L}_3)(\text{OH})\cdot 2\text{H}_2\text{O}$ did not differ significantly with approximately 16, 17 and 20% vital cells at 500 μM , respectively,

whereas $\text{Nd}(\text{L}_4)(\text{OH})\cdot 2\text{H}_2\text{O}$ caused less pronounced cell survival inhibition with almost 33% viable cells at the highest concentration applied. The antineoplastic efficacy of the individual tested compounds on the lymphoid cell line SKW-3 did not differ to the extent, found for HL-60 and HL-60/Dox.

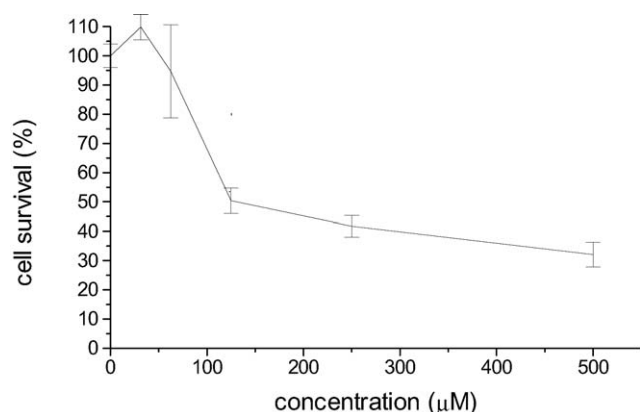


Fig. 11. Cytotoxic activity of $\text{Nd}(\text{L}_3)(\text{OH}) \cdot 2\text{H}_2\text{O}$ on SKW-3 cells after 72 h incubation as assessed by MTT assay. Each data point represents the arithmetic mean of at least six independent experiments.

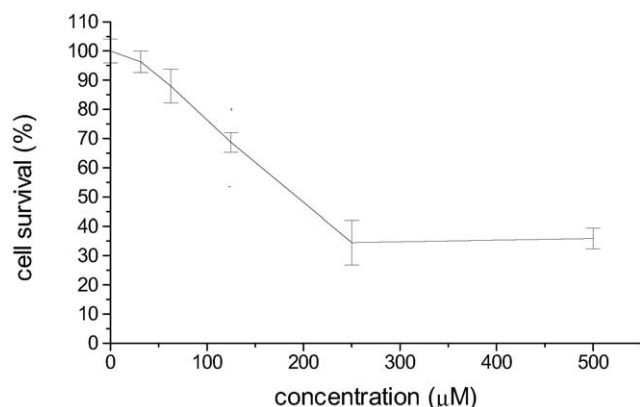


Fig. 12. Cytotoxic activity of $\text{Nd}(\text{L}_4)(\text{OH}) \cdot 2\text{H}_2\text{O}$ on cells after 72 h incubation as assessed by MTT assay. Each data point represents the arithmetic mean of at least six independent experiments.

On the basis of the IC_{50} values obtained $\text{Nd}(\text{L}_2)(\text{OH}) \cdot 2\text{H}_2\text{O}$ was found to be the most active compound (IC_{50} 123.1 μM), whereas the IC_{50} values for $\text{Nd}(\text{L}_1)(\text{OH}) \cdot \text{H}_2\text{O}$ and $\text{Nd}(\text{L}_3)(\text{OH}) \cdot 2\text{H}_2\text{O}$ were approximately 131 μM and for $\text{Nd}(\text{L}_4)(\text{OH}) \cdot 2\text{H}_2\text{O}$ IC_{50} value of 192.4 μM was calculated. As the concentration–response curves indicate, however, there were no large differences in the maximal efficacy of $\text{Nd}(\text{L}_3)(\text{OH}) \cdot 2\text{H}_2\text{O}$, $\text{Nd}(\text{L}_4)(\text{OH}) \cdot 2\text{H}_2\text{O}$ and $\text{Nd}(\text{L}_2)(\text{OH}) \cdot 2\text{H}_2\text{O}$ with 32.1, 35.9 and 39.8% vital cells, respectively, whereas $\text{Nd}(\text{L}_1)(\text{OH}) \cdot \text{H}_2\text{O}$ caused more than 70% inhibition of malignant cell proliferation at the highest concentration investigated.

Cytotoxicity determination by MTT assay shows that the inorganic salt neodymium nitrate did not show any significant activity [13–18] but the complexes with Nd (III) were found to be cytotoxic.

5. Conclusions

The coordination ability of the ligands has been proved in complexation reaction with neodymium (III) ion. ^1H -, ^{13}C NMR- and IR-spectral analysis of the ligands and their Nd

(III) complexes confirmed the suggested coordination of the ligands through both the hydroxyl and carbonyl oxygen atoms.

The overall results from the preliminary screening program revealed that all of the novel Nd complexes reach 50% inhibition of the malignant cells proliferation and thus could be considered as biologically active. On the basis of the IC_{50} values obtained compounds $\text{Nd}(\text{L}_1)(\text{OH}) \cdot \text{H}_2\text{O}$ and $\text{Nd}(\text{L}_3)(\text{OH}) \cdot 2\text{H}_2\text{O}$ were found to exert superior activity in comparison to the remaining complexes. These findings as well as the practical lack of cross-resistance to these agents in HL-60/Dox give us reason to conclude that $\text{Nd}(\text{L}_1)(\text{OH}) \cdot \text{H}_2\text{O}$ and $\text{Nd}(\text{L}_3)(\text{OH}) \cdot 2\text{H}_2\text{O}$ should undergo further thorough pharmacological and toxicological investigations.

According to our expectations the complexes of neodymium (III) possess a cytotoxic activity and their in vitro effects are clearly expressed. These results confirmed our previous observations on the cytotoxicity of neodymium (III) complexes.

6. Experimental protocols

6.1. Chemistry

The carbon, hydrogen and nitrogen contents of the compounds were determined by elemental analysis. The water content was determined by Metrohm Herizall E55 Karl Fisher titrator. IR spectra (Nujol) were recorded on a IR-spectrometer FTIR-8101M Shimadzu (3800 – 400 cm^{-1}) and on a IR-spectrometer Perkin–Elmer GX Auto image system (700 – 200 cm^{-1}). ^1H -NMR spectra were recorded at room temperature on Bruker WP 250 (250 MHz) spectrometer in $\text{DMSO}-d_6$. Chemical shifts are given in ppm. ^{13}C -NMR spectra were recorded at ambient temperature on Bruker 250 WM (62.9 MHz) spectrometer in $\text{DMSO}-d_6$. Chemical shifts are given in ppm, downfield from TMS. Mass spectra were recorded on a Jeol JMS D 300 double focusing mass spectrometer coupled to a JMA 2000 data system. The compounds were introduced by direct inlet probe, heated from 50 to 400 $^{\circ}\text{C}$ at a rate of 100 $^{\circ}\text{C}/\text{min}$. The ionization current was 300 mA, the accelerating voltage 3 kV and the chamber temperature 150 $^{\circ}\text{C}$.

6.2. General method of synthesis

The complexes of neodymium (III) with 3,3'-benzylidene-bis(4-hydroxy-2H-1-benzopyran-2-one) (H_2L_1); bis-(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-2-yl-methane (H_2L_2); bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridin-4-yl-methane (H_2L_3); bis(4-hydroxy-2-oxo-2H-chromen-3-yl)-(1H-pyrazol-3-yl)-methane (H_2L_4) were synthesized by reaction of neodymium (III) salt and the ligand, in amounts equal to metal:ligand molar ratio of 1:2. The complexes were prepared by adding an aqueous solution of 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 mixtures were 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, washed several times with water and dried in a desiccator to constant weight.

6.3. Pharmacology

All of the procedures concerning cell culture maintenance, solution preparation and treatment were carried out in a laminar flow cabinet 'Heraeus'. Stock solutions of the tested compounds were freshly prepared in analytical grade DMSO at a concentration of 50 mM and were thereafter diluted with RPMI-1640 medium to yield the desired final concentrations. For the cell viability assessment MTT-formazan absorption was measured using Uniskan–Titertek ELISA reader at 580 nm.

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