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Original article

New lanthanide complexes of 4-methyl-7-hydroxycoumarin and their pharmacological activity

Irena Kostova[a]*, Ilia Manolov[b], Irina Nicolova[c], Spiro Konstantinov[c], Margarita Karaivanova[c]

[a]Department of Chemistry, Faculty of Pharmacy, Medical University, 2 Dunav St., Sofia 1000, Bulgaria
[b]Department of Industrial Pharmacy, Faculty of Pharmacy, Medical University, 2 Dunav St., Sofia 1000, Bulgaria
[c]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Medical University, 2 Dunav St., Sofia 1000, Bulgaria

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Abstract – Complexes of cerium(III), lanthanum(III) and neodymium(III) with 4-methyl-7-hydroxycoumarin (Mendiaxon, Hymecromone) were synthesized by the mixing of equimolar amounts of the respective metal nitrates and 4-methyl-7-hydroxycoumarin sodium salt in water. The complexes were characterized and identified by elemental analysis, conductivities, IR, ^{1}H and ^{13}C NMR spectroscopies and mass spectral data. DTA and TGA have been applied to study the compositions of the compounds. The newly synthesized compounds were assayed for acute intraperitoneal and per oral toxicity, influence on blood clotting time and the most active complex was investigated for spasmolytic activity. The complexes of cerium(III) and neodymium(III) showed marginal cytotoxic activity against transformed leukemic cell lines (P3HR1 and THP-1) as compared to the inorganic salts. © 2001 Éditions scientifiques et médicales Elsevier SAS

lanthanide complexes of Mendiaxon / anticoagulants / spasmolytic activity / cytotoxic activity

1. Introduction

Coumarin derivatives are of interest because of their physiological, photodynamic, anticoagulant, spasmolytic and bacteriostatic activity. They are also extensively used as analytical reagents. 7-Hydroxycoumarin is known for its antibiotic and antifungal activities. 8-Substituted-4-methyl-7-hydroxycoumarin [1–3] and 6-substituted-4-methyl-7-hydroxycoumarin [4] have been investigated for complexing ability. These derivatives of coumarin have been found to exhibit anticoagulant and plant-growth regulating property. Racemic sodium Warfarin is widely used in the prevention of thromboembolic disease. Kerr et al. characterized three novel classes of Warfarin analogs and compared them with the Warfarin enantiomers. All three classes of compounds inhibit vitamin K epoxide reductase, the enzyme inhibited by racemic Warfarin [5]. Ammar et al. studied the interaction of oral anticoagulants Warfarin and Dicumarol with methyl xanthines [6]. A series of 7-amino-4-chloro-3-(3-isothioureido-propoxy)isocoumarin derivatives with various substituents at the 7- and 3-positions have been synthesized as inhibitors of several blood coagulation enzymes by Kam et al. [7]. Smirnova et al. reported about new coumarin derivatives useful as injectable anticoagulants [8]. The anticoagulant activity of coumarin derivatives was studied also by Baskaran et al. [9] and Twigg et al. [10].

Yamada et al. evaluated the spasmolytic activity of several coumarin compounds, analogous to aurapten, against Ba(II), acetylcholine and histamine and investigated their structure–activity relationship [11]. The spasmolytic activity of geranyloxycoumarin-related compounds have been described [12]. Chen et al. studied total coumarins in the fruit of Cnidium monnieri. In guinea pigs, oral administration of the total coumarins had a protective effect against bronchospasm induced by histamine [13]. Aminov et al. investigated some coumarins isolated from the plant *Haplophyllum* and it was shown that all compounds exhibited spasmolytic and hypotensive activities [14].

* Correspondence and reprints: Tel.: +359-2-988 3142 ext. 269

Table I. Results of the elemental analyses [a].

Substance	M.p. (°C)	λ (μS cm^{-1})	Elemental analysis (%) (calculated/found)			
			C	H	Met	H_2O
$Ce(HL)_2(OH)\cdot 5H_2O$	162	2.11	40.20 (39.71)	4.19 (3.77)	23.45 (24.80)	15.07 (15.00)
$La(HL)_3\cdot 6H_2O$	250	2.12	46.63 (46.98)	4.27 (3.75)	18.00 (19.50)	13.99 (14.40)
$Nd(HL)_3\cdot 6H_2O$	250	2.12	46.33 (46.34)	4.25 (3.94)	18.53 (20.00)	13.89 (14.00)

[a] $HL = C_{10}H_7O_3$; m.p. >300°C; $\lambda = 39.6$ μS cm^{-1}.

The complexes of rare earth ions have aroused much interest. Lanthanide ion is a subject of increasing interest in bioinorganic and coordination chemistry [15].

Lanthanium chloride [16] manifests an antitumor activity. Furthermore, literature data show that the coumarins also have these properties. As a result from our earlier work the cytotoxic profile of some complexes of Warfarin, Coumachlor and Niffcoumar with lanthanides against P3HR1, K-562 and THP-1 cell lines was proved [17, 18]. 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 [19]. The effect of Warfarin on tumor cell growth was studied [20]. Warfarin inhibits metastasis of Mtln3 rat mammary carcinoma without affecting primary tumor growth. 3,7-Diamino-4-hydroxycoumarin is useful as an antitubercular agent [21]. Akman at al. had investigated synergistic cytotoxicity between menadione and the related anticoagulant Dicumarol, and showed inhibited growth of murine leukemia L1210 in liquid suspension culture [22].

Coumarin derivatives are known to have good complexing ability. Although solution chemistry of transition metal complexes of 7-hydroxy-4-methylcoumarin, substituted at positions 3, 6 or 8 is reported, no work has been done on the synthesis and structural aspects of solid complexes of 4-methyl-7-hydroxycoumarin sodium salt with lanthanides. These data served as a basis of the present study of the possibility of synthesis, isolation and identification of 4-methyl-7-hydroxycoumarin sodium salt complexes with lanthanides in view of the application of these substances as anticoagulants, spasmolytycal agents and cytotoxic agents.

2. Chemistry

The compounds used for preparing the solutions were Merck products, p.a. grade: $Ce(NO_3)_3\cdot 6H_2O$, $La(NO_3)_3\cdot 6H_2O$ and $Nd(NO_3)_3\cdot 6H_2O$. 4-Methyl-7-hydroxy-2*H*-1-benzopyran-2-one sodium salt (Mendiaxon Sodium) was used for the preparation of metal complexes as ligand. It was prepared by the following procedure: 1.76 g (10 mmol) 4-methyl-7-hydroxycoumarin was added to 0.038 g (9.5 mmol) sodium hydroxide in 30 mL water. The mixture was stirred vigorously at room temperature for 1 h until it gets clear. The solution was filtered and the filtrate was evaporated to dryness. The viscous residue was recrystallized from ethylacetate. TLC (hexane–acetone, 2:1). Yield 1.7 g (86%), m.p. above 300°C.

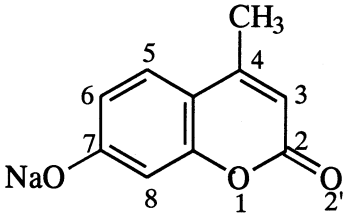
The complexes were synthesized by mixing water solutions of cerium(III), lanthanum(III) and neodymium(III) salts and the ligand, in amounts equal to a metal–ligand molar ratio of 1:3. The reaction mixture was stirred with an electromagnetic stirrer at 25°C for 1 h. At the moment of mixing of the solutions, precipitates were obtained. The precipitates were filtered, washed several times with water and dried in a desiccator to constant weight.

The complexes were insoluble in water, methanol and ethanol and highly soluble in DMSO. Their physicochemical characteristics such as melting points and molar conductances are presented in *table I* .

Table II. Results of the IR spectra.

Substance	νOH	νC=O	νC=C	
Lig	3655	1726	1576	1070–976
Ce	3487	1668	1607	1020–989
La	3480	1669	1607	1022–990
Nd	3493	1671	1607	1022–990

Table III. ^{1}H NMR (100 MHz, DMSO-d_6).



C_n–H	Multiplicity of the signal	Lig	δ (ppm) of the complexes		
			Ce	La	Nd
C_8–H	S, 1H	5.9	6.8	6.7	6.8
C_6–H	D, 1H, $J = 7$ Hz	6.2	6.9	6.8	6.9
C_5–H	D, 1H, $J = 7$ Hz	7.2	7.7	7.6	7.8
C_3–H	S, 1H	5.5	6.1	6.0	6.2
CH_3	S, 3H	2.1	2.3	2.3	2.4

3. Pharmacology

Acute intraperitoneal toxicity (i.p. LD_{50}) of the studied compounds was assessed by dissolving in saline (0.9% NaCl) with one drop of Tween 80, administered to mice via i.p. route. LD_{50} was evaluated for four different doses, each on six animals and calculated by the method of Litchfield–Wilcoxon, using a personal computer.

Acute per oral toxicity (p.o. LD_{50}) of the studied compounds was assessed by dissolving in saline (0.9% NaCl) with one drop of Tween 80, administered to mice via p.o. route. LD_{50} was evaluated for four different doses, each on six animals and calculated by the method of Litchfield–Wilcoxon, using a personal computer.

Index of absorption was calculated by using data from the i.p. and p.o toxicity.

The influence of investigated compounds on blood clotting time was determined by the method of Moravitz [23]. The investigation was performed on 40 male white mice, weighting 20±2 g. The compounds were administered three days in doses of 1/10 of p.o. LD_{50}. On the fourth day (24 h after the last administration) the clotting time was assessed after small incision of sublingual vein and measuring the clotting time of the second drop blood on clean glass.

Spasmolytic effect of the most active complex (Ce^{3+}) was evaluated on isolated guinea pig ileum according to the method of Magnus. Guinea pigs weighing 500–600 g were used. Animals were sacrificed by a

Table IV. Acute i.p. toxicity (LD_{50}) of investigated compounds and Hymecromone.

Compound	LD_{50} (mg kg^{-1})	Range of values (mg kg^{-1})
Hymecromone	189.1	177.8–201.1
$Ce(HL)_2(OH)\cdot 5H_2O$	1485 *	1183.3–1863.7
$La(HL)_3\cdot 6H_2O$	729 *	675.5–787
$Nd(HL)_3\cdot 6H_2O$	>1500 *	

* $P < 0.05$, statistically significant compared to Hymecromone.

Table V. Acute p.o. toxicity (LD_{50}) of investigated compounds and Hymecromone.

Compound	LD_{50} (mg kg^{-1})	Range of values (mg kg^{-1})
Hymecromone	1908.07	1484.75–2452.08
$Ce(HL)_2(OH)\cdot 5H_2O$	>3000 *	
$La(HL)_3\cdot 6H_2O$	>3000 *	
$Nd(HL)_3\cdot 6H_2O$	>3000 *	

* $P < 0.05$, statistically significant compared to Hymecromone.

Table VI. The index of absorption of investigated compounds.

Compound	Index of absorption (%)
Hymecromone	9
$Ce(HL)_2(OH)\cdot 5H_2O$	49
$La(HL)_3\cdot 6H_2O$	24
$Nd(HL)_3\cdot 6H_2O$	50

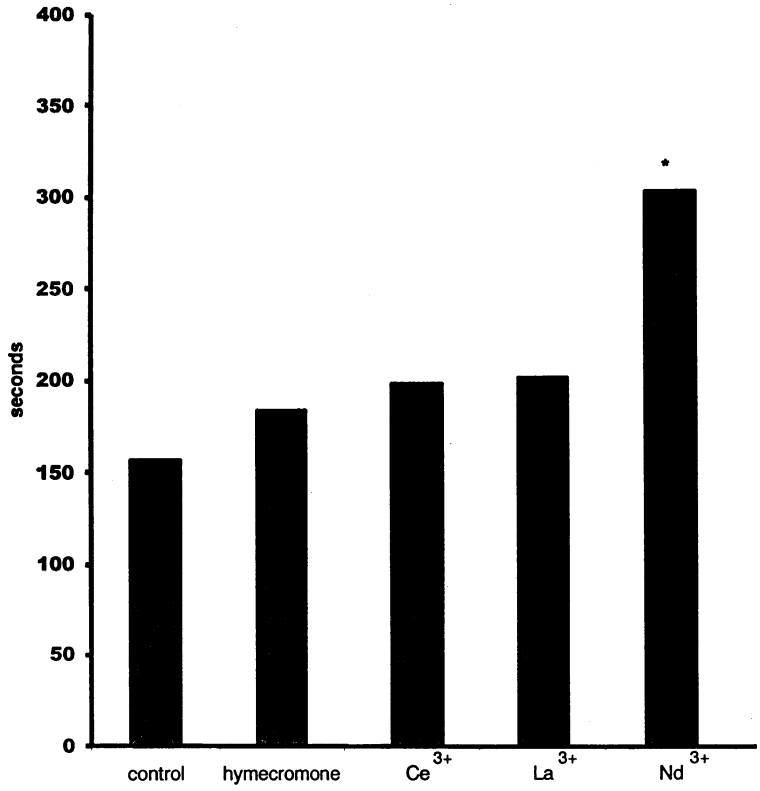


Figure 1. The influence of investigated compounds on clotting time $*P<0.05$, statistically significant compared to control.

Table VII. Influence of investigated compounds on acetylcholine-induced contraction.

Compound	Concentration (Mmol L^{-1})	Relaxation (%)	IC_{50}	pD_2
Hymecromone	1×10^{-6}	0		
	1×10^{-5}	13.4 ± 5		
	3×10^{-5}	22.7 ± 3.6		
	1×10^{-4}	28.9 ± 9.8		
$Ce(HL)_2(OH)\cdot5H_2O$	1×10^{-6}	5.6 ± 1.7	2.64×10^{-5}	4.58
	1×10^{-5}	29.7 ± 2.24		
	3×10^{-5}	38.9 ± 6.26		
	1×10^{-4}	61.1 ± 4.15		
	3×10^{-4}	100		

blow in the head. Then the isolated organ was immersed in aerated Tyrode solution in an organ bath of 30 mL volume aerated with a mixture of 95% O_2 and 5% CO_2 and kept at 37°C. The strips were suspended between two stainless hooks with one end connected to the tissue holder and the other to a force–displacement transducer. The rings were allowed to equilibrate for 60 min at a resting tension of 1*g* and then contracted to reach a plateau with acetylcholine 1×10^{-7} or serotonine 1×10^{-6} and estimated. After 30 min resting time the compounds were added 5 min prior to the contractile agent. Cumulative concentration–effect curves of tested compounds were plotted and mean effective concentrations IC_{50} and pD_2 were calculated using the regression analysis method form the concentration–response curves for the effects of the different concentration against the logarithm of the concentrations using computer programs.

Table VIII. Influence of investigated compounds on serotonine-induced contraction.

Compound	Concentration (Mmol L^{-1})	Relaxation (%)	IC_{50}	pD_2
Hymecromone	1×10^{-6}	0	5.14×10^{-3}	2.29
	1×10^{-5}	16.5 ± 3.8		
	1×10^{-4}	19.9 ± 3.6		
	3×10^{-4}	35.5 ± 5.1		
$Ce(HL)_2(OH)\cdot5H_2O$	1×10^{-6}	0	5.65×10^{-4}	3.25
	1×10^{-5}	18.3 ± 3.5		
	1×10^{-4}	29 ± 5.8		
	3×10^{-4}	47.5 ± 6.46		

Statistical analysis of all data were accomplished by Student's *t*-test and a '*P*' value of less than 0.05 was used as a criterion for statistical significance.

Colorimetric MTT (Tetrazolium) assay is based on the cellular reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] by the mitochondrial dehydrogenases of viable cells to a blue formazan product. The production of formazan can be measured spectrophotometrically following solubilization. We use the method described by Mossmann [24] with some modifications: Burkitt lymphoma P3HR1 and acute myeloid leukemia (AML) derived THP-1 cells were seeded in 96-well plates (100 μL $well^{-1}$ at a density of 1×10^5 cells mL^{-1}) and exposed to various concentrations of the tested compounds. After incubation for 48 h, the 10 mg mL^{-1} MTT solution in PBS was added to each well and was further incubated for 4 h at 37°C. The formazan crystals formed were dissolved by adding 100 μL $well^{-1}$ of 5% formic acid in 2-propanol. After a few minutes at room temperature to ensure that all crystals were dissolved, absorption was measured by an ELISA reader using a test wavelength of 580 nm. For each concentration at least eight wells were used. Cell growth inhibition was calculated according to (OD of drug treatment/OD of untreated control)× 100. Data processing were executed with Microsoft Excel, Microsoft Word and Sigma Plot Windows.

4. Results and discussion

The complexes were characterized by elemental analysis. The metal ions were determined after mineralization and thermogravimetrically. The presence of sodium ion was checked up by means of flame photometry. The water content in the complexes was determined by Karl Fisher analysis and thermogravi-

Table IX. Spectrophotometrical data from MTT assay concerning the cytotoxic activity of complexes of Mendiaxon on P3HR1 cells in comparison with the inorganic salts.

Compound	MTT-formazan absorption at 580 nm			
	Untreated control	25 (μM)	100 (μM)	400 (μM)
$Ce(NO_3)_3\cdot6H_2O$	0.3936	0.4835	0.4216	0.3649
(±)	0.0672	0.0419	0.0511	0.0823
$La(NO_3)_3\cdot6H_2O$	0.3936	0.4429	0.4258	0.4142
(±)	0.0672	0.0579	0.0801	0.0775
$Nd(NO_3)_3\cdot6H_2O$	0.3936	0.4874	0.4143	0.3613
(±)	0.0672	0.0885	0.0245	0.0955
$Ce(HL)_2(OH)\cdot5H_2O$	0.8167	0.7670	0.7508	0.5180
(±)	0.0565	0.0443	0.0397	0.0170
$La(HL)_3\cdot6H_2O$	0.8167	0.6280	0.7164	0.5256
(±)	0.0566	0.0842	0.0877	0.0244
$Nd(HL)_3\cdot6H_2O$	0.8167	1.0138	0.6034	0.4592
(±)	0.0565	0.0565	0.0469	0.0488

Table X. Spectrophotometrical data from MTT assay concerning the cytotoxic activity of complexes of Mendiaxon on THP-1 cells in comparison with the inorganic salts.

Compound	MTT-formazan absorption at 580 nm			
	Untreated control	25 (μM)	100 (μM)	400 (μM)
$Ce(NO_3)_3\cdot6H_2O$	0.374	0.4416	0.4312	0.4230
(±)	0.080	0.010	0.030	0.020
$La(NO_3)_3\cdot6H_2O$	0.3745	0.4078	0.4217	0.4026
(±)	0.080	0.010	0.030	0.040
$Ce(HL)_2(OH)\cdot5H_2O$	0.3745	0.3230	0.2962	0.2612
(±)	0.080	0.008	0.020	0.020
$La(HL)_3\cdot6H_2O$	0.3745	0.3657	0.3335	0.3058
(±)	0.080	0.010	0.008	0.020

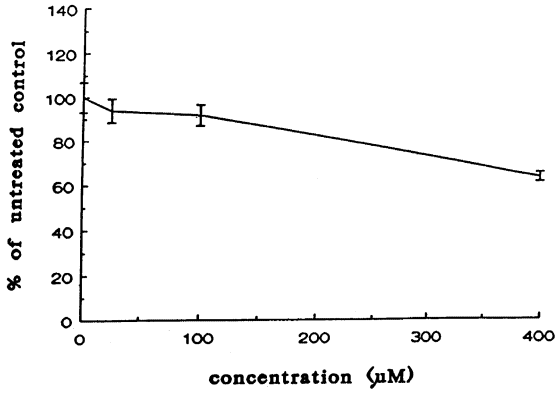


Figure 2. Cytotoxic effect of $Ce(L)_2 \cdot 4H_2O$ on P3HR1 cells (MTT-assay).

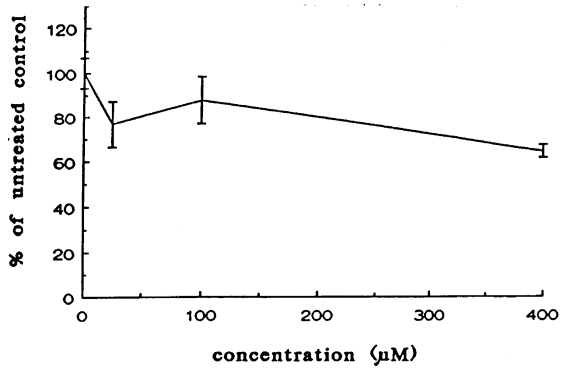


Figure 3. Cytotoxic effect of $La(L)_3 \cdot 2H_2O$ on P3HR1 cells (MTT-assay).

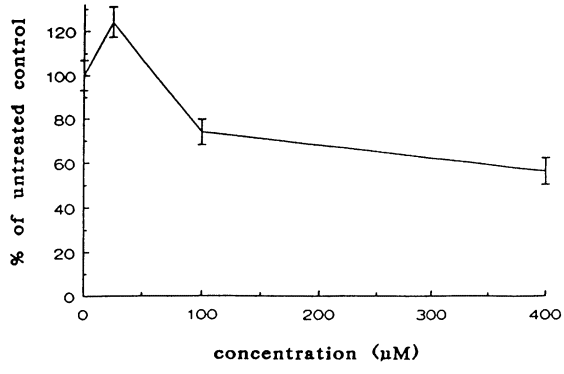


Figure 4. Cytotoxic effect of $Nd(L)_3 \cdot 2H_2O$ on P3HR1 cells (MTT-assay).

metrically. The formation of the complexes was confirmed by IR, ^1H and ^{13}C NMR spectroscopies and mass spectral data. Purities of the compounds were evaluated by thin layer chromatography and the results showed that the complexes were pure.

Table I shows the data of the elemental analysis of the compound obtained serving as a basis for the determination of their empirical formulae and the results of the Karl Fisher analysis, thermal analysis and of the flame photometry analysis. Besides analytical data, in *table I* the molar conductivities and melting points of the compounds are also reported. The molar conductance values of all complexes were in the range of 1.5–3.0 $\mu S\ cm^{-1}$ indicating that the complexes are non-electrolytes.

The compositions of the complexes were confirmed by DTA and TGA. At the beginning of the DTA-curves of the complexes there is a clearly manifested endothermic effect ($\sim 120°C$), which is due to the hygroscopic moisture released. A steady weight loss is

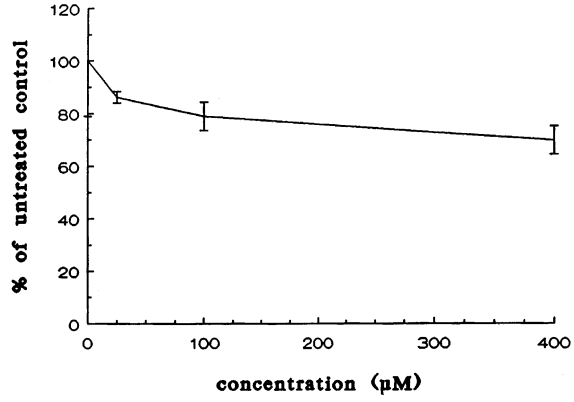


Figure 5. Cytotoxic effect of $Ce(L)_2 \cdot 4H_2O$ on THP-1 cells (MTT-assay).

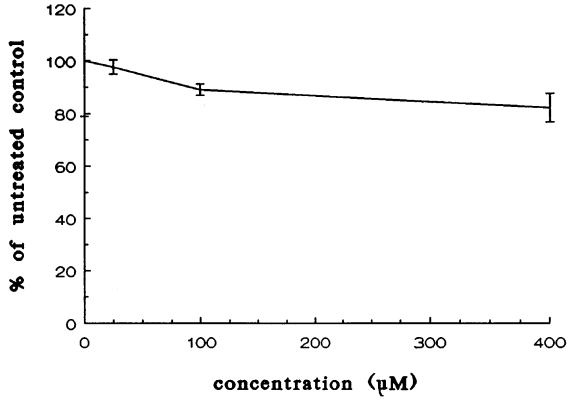


Figure 6. Cytotoxic effect of $La(L)_3 \cdot 2H_2O$ on THP-1 cells (MTT-assay).

recorded on heating up to ~230°C corresponding to the elimination, respectively, of five, six and six molecules of water per molecule of cerium(III), lanthanum(III) and neodymium(III) complexes, respectively. The amount of this weight loss, also determined by Karl Fisher analysis, is correlated with the intensity of these endothermic effects and with the respective decreases in the mass. On heating the complexes the decomposition step in all the cases corresponds to the loss of molecules of the ligands, which is in agreement with the compositions in *table I*. Exothermal effect (500–550°C) dominates in the thermograms of all the complexes, resulting from the decomposition of the organic matter. A further weight loss recorded up to 900°C indicates the formation of thermally stable oxides.

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

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

In the IR spectrum of the ligand the bands appear at 3655, 1726, 1603, 1576, 1290, 1140, 1070–976, 831, 613 and 484 cm^{-1}. A band at 1726 cm^{-1} can be attributed to the stretching vibrations of the carbonyl group; two bands at 1603 and 1576 cm^{-1} can be related to the stretching vibrations of the conjugated olefinic system. In all the complexes the $\nu_{(C=C)}$ band at 1607 cm^{-1} remains.

A broad band, characteristic of ν_{OH} of coordinated water was observed in the range of 3500–3400 cm^{-1} in the spectra of all the complexes. The weak band observed at 3655 cm^{-1} in the spectra of the free ligand shifted to lower wavenumber (3490 cm^{-1}) in the complexes. This assignment is corroborated by the occurrence of the corresponding rocking mode in the range of 840–830 cm^{-1}.

The band at 1159 cm^{-1} assigned as ν_{C-OH} is observed at more or less the same position in the complexes and this may be due to the existence of one non-ionized OH group almost in all cases, but in the ligand this band is at 1140 cm^{-1}.

The $\nu_{C=O}$ band at 1726 cm^{-1} exhibits a shift of 50–60 cm^{-1} to lower wavenumber values on complexation which may be taken as evidence for the participation of the C=O group in coordination.

The most notable change observed upon complex formation is a shift of the C=O stretch to lower frequency. The C–C and C–O stretch and the C–O–C band are all shifted to higher frequency (1277, 1159 and 1076 cm^{-1}) in the complexes. Similar frequency shifts are observed for the other complexes and are attributed to complexation of the positive ion with the carbonyl oxygen [25].

The data of the IR analysis are presented in *table II* and they are in agreement with the compositions in *table I*.

Metal ion coordination with ligand by means of oxygen atom of C=O group was shown by ^{1}H and ^{13}C NMR data.

^{1}H NMR spectra of the compounds recorded at 100 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 III*.

^{13}C NMR spectra of Mendiaxon Sodium and of the cerium complex of Mendiaxon were recorded at 62.9 MHz in DMSO-d_6. The ^{13}C NMR spectra of Mendiaxon Sodium showed signals at (δ ppm): 175.99, 162.02, 157.46, 153.56, 125.03, 118.66, 104.13, 103.03, 101.44, 18.08. The ^{13}C NMR spectra of cerium complex of Mendiaxon showed signals at (δ ppm): 161.16, 160.29, 155.24, 153.57, 126.63, 112.87, 110.29, 109.13, 102.18, 18.11.

The mass spectra analysis confirmed the ratio of metal–ligand in the investigated complexes. Mendiaxon Sodium MS; m/z (% of base peak): 198 (80), 176 (100), 154 (60), 136 (60), 120 (17), 107 (22). Cerium complex of Mendiaxon MS: 352.8 (17), 306.8 (5), 288.8 (5), 176.8 (100), 154 (35), 136 (35), 120 (14), 106.9 (17).

5. Conclusions

Analysis of the obtained data on acute intraperitoneal toxicity (LD_{50}) showed that the compounds are statistically significantly less toxic than Hymecromone (*table IV*).

Analysis of the obtained data on acute per oral toxicity (LD_{50}) showed that the all complexes had an acute p.o. toxicity statistically significantly lower than the standard substance Hymecromone (*table V*).

The index of absorption is shown in *table VI*. The complexes of cerium(III) and neodymium(III) showed the best absorption index of the investigated compounds.

Analysis of the obtained data from blood clotting time showed that the complex of neodymium(III) has the greatest effect on clotting time, which is statistically significant, compared to control (*figure 1*).

In vitro experiments on isolated guinea pig ileum showed that spasmolytic effect on acetylcholine induced contraction was greater in complex of cerium(III), compared to Hymecromone (*table VII*). In experiments with serotonine there were no significant difference between investigated compound and the reference — Hymecromone (*table VIII*).

The performed screening for antitumor activity in vitro (*tables IX and X* and *figures 2–6*) showed that all tested compounds did not have any strong cytotoxic potential. The complexes of cerium(III) and lanthanum(III) with Mendiaxon induced ca. 25% reduction of the survival P3HR1 cells at concentration 400 μM. The complex of neodymium (III) with Mendiaxon reduced the viability of the cells with 30%. Furthermore, the cerium(III) and lanthanum(III) complexes of Mendiaxon were tested on AML derived THP-1 cells. Both compounds induced similar low cytotoxic effect only at the highest applied concentration. So, only the complexes of cerium(III) and neodymium(III) were found to be marginally effective cytotoxic agents that need further investigations.

6. Experimental protocols

6.1. Chemistry

The carbon and hydrogen content of the compounds were determined by elemental analysis.

The water content was determined by Metrohn Herizall E55 Karl Fisher Titrator and thermogravimetrically.

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^{-1} until 900°C. The inert substance was Al_2O_3.

Melting points were determined by using a Boetius melting point apparatus and are uncorrected.

Conductometric measurements were carried out at 25°C on 10^{-3} M solutions in DMSO by using a Metrohn 660 AG-9101 Herisau conductometer with platinum electrode and a cell having cell constant of 0.79 cm^{-1}.

IR spectra (Nujol) were recorded on a IR spectrometer, Shimadzu FTIR-8101M.

^{1}H NMR spectra were recorded at room temperature on a Brucker WP 100 (100 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 a Brucker 250 WM (62.9 MHz) spectrometer in 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°C at a rate of 100°C min^{-1}. The ionization current was 300 mA, the accelerating voltage 3 kV and the chamber temperature 150°C.

General method of synthesis: the complexes were synthesized by mixing water solutions of cerium(III), lanthanum(III) and neodymium(III) salts and the ligand in amounts equal to a metal–ligand molar ratio of 1:3. The reaction mixture was stirred with an electromagnetic stirrer at 25°C for 1 h. At the moment of mixing of the solutions colored precipitates were obtained. The precipitates were filtered, washed several times with water and dried in a desiccator to constant weight.

6.2. Biological evaluation

Experiments on i.p. and p.o. toxicity and anticoagulation activity were conducted on 84 white male mice, weighting 20±2 g, while those concerning spasmolytic activity were performed on 20 guinea pigs weighing 500–600 g.

For cell culture Laminar Flow cabinet Haereus HV 2436 and CO_2 gassed incubator Haereus BB16 were used. For MTT-assay measurement ELISA-reader Labsystems Uniskan I was used.

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