Synthesis, Physico-Chemical and Toxicological Characterization of New Complexes of Lanthanides (III) with Ampiroxicam and Lornoxicam

SULTANA NITA[1]*, ADRIAN A. ANDRIES[2], LUMINITA PATRON[3], FLAVIAN RADULESCU[2], RADU ALBULESCU[1], ADRIAN BETERINGHE[3], MIHAELA VINTILA[1], NICOLETA RUSU[1]

[1] National Institute of Chemical, Pharmaceutical Research and Development, 112 Vitan Av., 031299, Bucharest, Romania

[2] University of Medicine and Pharmacy "Carol Davila", Faculty of Pharmacy, 37 Dionisie Lupu Str., 070183, Bucharest, Romania

[3] Institute of Physical Chemistry "Ilie Murgulescu", 202 Splaiul Independentei , 060021, Bucharest, Romania

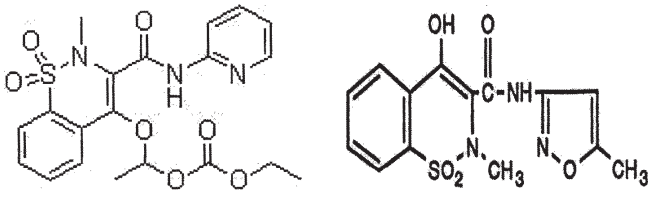
The metallic complexes with pharmacological activity are considered, nowadays, an important research field, in the bioinorganic chemistry. They owe this interest because of the synergy between the ligands effects and those of the metals, which leads to the increase of the therapeutic activity for the new compound. This paper describes the synthesis, physico - chemical and toxicological characterization of new Ln(III) with oxicam ligands namely ampiroxicam and lornoxicam, potential active substances of new antitumoral drugs. The structures and formula proposed for these coordination compounds were established on the basis of Quantitative Structure - Activity Relationships / Computer Assisted Drug Design (QSAR/CADD) studies and correlation with the elemental chemical analysis and other physical – chemical studies (UV-Vis, ICP-MS, IR spectrometry). Cytotoxicity of lanthanide complexes was estimated comparatively to oxicam ligands.

Keywords: Bioactive substances; lanthanides; metallic complexes; oxicams; antitumoral agents

A new concept in design and development of antitumoral / antiinflammatory drugs focuses on the preparation of metallic compounds of the organic structure with therapeutic activity [1-9]. These complex combinations possess the action of both the organic ligand and the metallic ion, so that the gastrointestinal side effects due to the organic compounds' acidity will be eliminated.

The chemical non-steroidal structures with therapeutic properties (AINS) are among the most frequently used drugs as ligands for metallic ions. Mainly they have analgesic, antiinflammatory, antipyretic and antitumoral effects [2-6]. Some recent studies have provided that, when the metallic complexes are administrated together with antiinflammatory / antitumoral drugs, their efficiency is highly increased. In the same time, it has been noticed that some lanthanide (III) complexes, containing as ligands structures with antitumoral / antiinflammatory activity may provide a higher activity than the simple ligands [13]. The non-steroidal antiinflammatory medicines in the oxicams class proved themselves to be strong ligands for a series of lanthanides.

In the present paper are presented studies regarding for the synthesis, analytical and toxicological characterization of new lanthanide (III) coordination compounds with oxicams type drugs, namely ampiroxicam and lornoxicam, which can be used as new antitumoral drugs.



Ampiroxicam **Lornoxicam**

Fig.1. Chemical formula for Ampiroxicam and Lornoxicam

Experimental part

Reagents

All reagents and solvents were of analytical grade: lanthanide (II) chloride (lanthanum, praseodymium and neodymium), potassium bromide (Merck), DMSO(Merk) ampiroxicam and lornoxicam (LKT Laborathories), ethanol 95% was supplied by INCDCF-ICCF (Bucharest, Romania), DMEM-F12 medium and fetal bovine (Sigma), MTS-Cel titer assay (Promega), tissue culture plates (Greiner).

Synthesis of complexes

To the 1, 2 and respectively 3 mmols of ampiroxicam respectively lornoxicam disolved in ethanol by heating to reflux about 1.5 h was added an ethanolic solution of 1 mmol Ln(II) cloride, as lanthan, praseodimium and neodimium. The resulting solutions was refluxed for aprox.1 h.

The formed precipitates were filtered, washed with ethanol and dried in the air.

Physico-chemical measurements

The elemental analysis of C, H, N, S was performed with a Perkin Elmer CHNS/O Analyzer, 2400 Series II and the lanthanide content by ICP-MS using an Elan DRCe Mass Spectrometer.

The electronic spectra were recorded using a Perkin Elmer Lambda 650 UV-Vis Spectrometer, in diffuse reflectance in the range 200 - 900 nm.

The IR spectra were recorded using Perkin Elmer FT-IR Spectrum 100 Spectrometer, in the range 4000 - 350 cm^{-1} (KBr disk method).

Cytotoxicity determinations

Cytotoxicity was estimated in vitro on BalbC / 3T3 cell cultures, using the MTS assay as end point.

* *email: sultananita@yahoo.com; Tel.: 0721811231*

Briefly, subcultures of BalbC / 3T3 cells (passages 4 - 6) were seeded in 96 well plates (Greiner), at a density of 10.000 / well, in 200 μL of DMEM-F12 medium supplemented with 10% fetal bovine serum. After 24 h of cultivation (70% confluence), medium was replaced and cells were exposed to the test compounds, at concentrations 0.01 to 1μM in fresh medium.

Test compounds were solubilized in DMSO, under sterile conditions, and further diluted with serum free medium to create the working stoc solutions (10X concentrated, 10 μM). Two negative controls were prepared, one with 0.1% DMSO in culture medium (corresponding to the maximal content of DMSO in samples), and a second one, at 0.01%. Positive control was 0.1% SDS. After 6 hrs of exposure, culture medium was again replaced and MTS assay was performed according to the specifications of the supplier.

Optical densities were measured on an Anthos-Zenith (LKB) microplate reader at 490 nm.

The toxicity values (CT 50) were estimated based on the double reciprocal plots (cell death^{-1} vs. concentration $^{-1}$).

Results and discussions

Synthesis of complexes

We have studied the systems: Ln(III) – oxicams for the combination ratios Me-L 1:1; 1:2 and respectively 1:3., where Ln(III) = lanthanum, praseodymium and neodymium, and oxicams = ampiroxicam and lornoxicam. From these systems, we were able to isolate and characterize the mononuclear complexes as type: $[Ln(oxicam)_2 (H_2O)_2]$.

Figure 2 presents the structural conformation of the complexes.

The proposed formulas for these compounds were established on the basis of elemental chemical analysis correlated with physical – chemical studies (UV-Vis, IR and ICP-MS spectrometry). Analytical results for the elemental analysis of the ligands and their complexes of lanthanides are presented comparatively in table 1. As can be seen there is a good correlation between the theoretical (*calcd.*) and the experimental values (*found*).

The results of analytical data confirms the combination ratio M:L = 1:2.

UV – Vis Spectra

The UV – Vis electronic spectra in the 250 - 850 nm range of the Nd-ampiroxicam complex, presented in figure 3, show the π - π^*, n - π^* ligand transitions and the bands can be assigned to $4f^n$ transitions of Ln(III) ions, as can be seen in table 2.

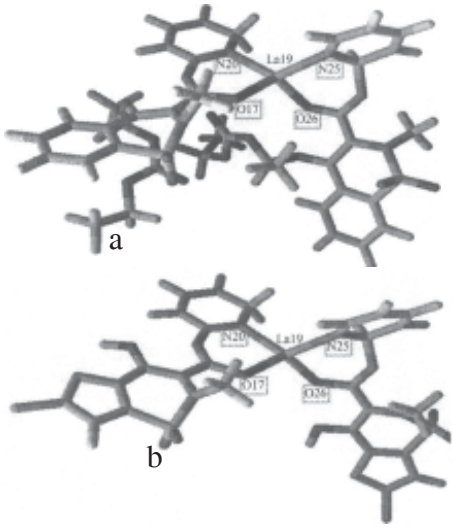


Fig.2. Structural conformation of the complex compounds La(III)-ampiroxicam (a) and La(III)-lornoxicam (b)

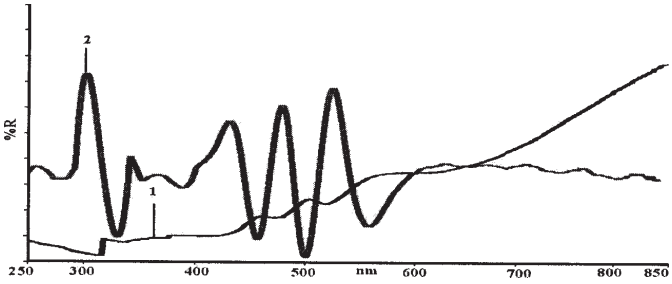


Fig.3. The UV-Vis spectra for Nd-ampiroxicam complex: (1) standard spectra; (2) 2nd-derivative spectra

IR Spectra

It is well know that oxicams can act as a monodentate ligands through the O enolic, as bidentate ligands through the O amide and N pyridyl (pyr) / thiazolyl (thiaz) and as tridentate ligands through the O enolic, O amide and Npyr / thiaz.

In order to obtain some information about the coordination mode of ligands, the 4000-350 cm^{-1} IR spectra of complexes the have been recorded. These spectra were compared with the IR spectra of ligands.

Table 3 presents comparatively the IR bands of ligands and of their lanthanide complexes.

The IR spectra show the coordination mode of ligands. The bands due to $\nu_{C=O(amide)}$ and $\nu_{C=N(pyr/thiaz)}$ shifts to a lower wave number in the complex compounds.

The two bands due to the SO_2 group (ν_{as} and ν_s) shift to higher frequencies in the spectra of complexes. It is

Compound	Chemical formula	% found / calcd.						
		C	H	N	S	La	PrCor respo	Nd
Ampiroxicam (Ampirox)	$C_{20}H_{21}N_3O_7S$	53.69	9.39	7.16	4.73	-		-
Lornoxicam (Lornox)	$C_{13}H_{10}ClN_3O_4S_2$	53.69	9.39	7.16	4.73	-	-	-
$[La(Ampirox)_2(H_2O)_2]$	$LaC_{40}H_{44}N_6O_{16}S_2$	44.95 / 44.28	7.86 / 7.52	5.99 / 6.03	4.12 / 3.92	13.00 / 12.65	-	-
$[La(Lornox)_2(H_2O)_2]$	$LaC_{26}H_{22}Cl_2N_6O_{10}S_4$	34.04 / 33.97	9.16 / 8.93	13.96 / 13.67	2.40 / 2.38	15.15 / 15.02	-	-
$[Pr(Ampirox)_2(H_2O)_2]$	$PrC_{40}H_{44}N_6O_{16}S_2$	45.67 / 4528	7.99 / 8.02	6.08 / 6.13	4.11 / 3.96	-	13.17 / 12.92	-
$[Pr(Lornox)_2(H_2O)_2]$	$PrC_{26}H_{22}Cl_2N_6O_{10}S_4$	33.96 / 39.89	9.14 / 8.97	13.99 / 13.95	2.40 / 2.38	-	15.38 / 14.95	-
$[Nd(Ampirox)_2(H_2O)_2]$	$NdC_{40}H_{44}N_6O_{16}S_2$	44.72 / 44.32	7.82 / 7.75	5.96 / 5.98	4.10 / 3.95	-	-	13.44 / 13.37
$[Nd(Lornox)_2(H_2O)_2]$	$NdC_{26}H_{22}Cl_2N_6O_{10}S_4$	33.84 / 33.75	9.11 / 9.09	13.88 / 13.80	2.38 / 3.35	-	-	15.64 / 15.57

Table 1
ELEMENTAL ANALYSIS RESULTS OF LIGANDS AND THEIR COMPLEXES OF LANTHANIDES

Compound	Absorption bands (λ)	Assignment
Ampiroxicam	210; 260; 293; 380	$\pi - \pi^*$; $\pi - \pi^*$ of ligand
$[La(Ampirox)_2(H_2O)_2]$	205; 255; 294; 370	$\pi - \pi^*$; $n - \pi^*$ of ligand
	439; 478; 526	CT (L→M)
	573; 608; 703	characteristic bands of La^{3+}
$[Pr(Ampirox)_2(H_2O)_2]$	205; 294; 370	$\pi - \pi^*$; $n - \pi^*$ of ligand
	439; 480; 548	CT (L→M)
	592; 703; 1432; 1515	$^3H_4 \rightarrow {}^1D_2$; $^3H_4 \rightarrow {}^3F_4$; $^3H_4 \rightarrow {}^3F_3$ — characteristic bands of Pr^{3+}
$[Nd(Ampirox)_2(H_2O)_2]$	247; 257; 282	$\pi - \pi^*$; $n - \pi^*$ of ligand
	518; 529	$^4I_{9/2} \rightarrow {}^4G_{9/2}$; $^4G_{7/2}$
	540; 567	$^4I_{9/2} \rightarrow {}^2G_{7/2}$
	681	$^4I_{9/2} \rightarrow {}^4F_{9/2}$
	756; 745	$^4I_{9/2} \rightarrow {}^4F_{7/2}$
	788	$^4I_{9/2} \rightarrow {}^4F_{5/2}$
Lornoxicam	211; 280; 323; 440	$\pi - \pi^*$; $n - \pi^*$ of ligand
$[La(Lornox)_2(H_2O)_2]$	205; 293; 331; 361	$\pi - \pi^*$; $n - \pi^*$ of ligand
	436; 481	CT (L⇆M)
	604; 703; 1420; 1532	characteristic bands of La^{3+}
$[Pr(Lornox)_2(H_2O)_2]$	207; 281; 324; 362; 485	of ligand; CT (L⇆M)
	594; 708	characteristic bands of Pr^{3+}
$[Nd(Lornox)_2(H_2O)_2]$	205; 293; 331; 361	$\pi - \pi^*$; $n - \pi^*$ of ligand
	525	$^4I_{9/2} \rightarrow {}^4G_{7/2}$
	573; 608	$^4I_{9/2} \rightarrow {}^2G_{7/2}$
	660	$^4I_{9/2} \rightarrow {}^4F_{9/2}$
	740	$^4I_{9/2} \rightarrow {}^4F_{7/2}$
	780	$^4I_{9/2} \rightarrow {}^4F_{5/2}$

Table 2
UV-Vis SPECTRA OF LIGANDS AND THEIR NEODYMIUM COMPLEXE

Compound	ν_{OH}, (H_2O)	ν_{NH} (amide)	$\nu_{C=O}$ (amide)	$\nu_{C=N}$ (Npir / tiaz)	$\nu_{as\ SO2}$	$\nu_{s\ SO2}$	ν_{M-N}	ν_{M-O}
Ampiroxicam	-	3349	1671	1592	1398	1078	-	-
$[La(Ampirox)_2(H_2O)_2]$	3622	3090	1649	1578	1373	1065	-	-
$[Pr(Ampirox)_2(H_2O)_2]$	3623	3090	1615	1577	1360	1301	488	398
$[Nd(Ampirox)_2(H_2O)_2]$	3621	3438	1647	1557	1410	1115	517	459
Lornoxicam	-	3067	1647	1596	1392	1065	-	-
$[La(Lornox)_2(H_2O)_2]$	3600	2992	1639	1590	1365	1278	492	385
$[Pr(Lornox)_2(H_2O)_2]$	3620	2995	1620	1565	1353	1295	478	373
$[Nd(Lornox)_2(H_2O)_2]$	3588	3223	1634	1586	1400	1100	505	418

Table 3
THE MAIN IR ABSORBTION BANDS FOR COORDINATION COMPOUNDS (cm^{-1})

Compound	CT 50 (mg/mL)
Lanthanum (La)	9.90
Praseodymium (Pr)	4.36
Neodymium (Nd)	5.43
Ampiroxicam	8.43
$[La(Ampirox)_2(H_2O)_2]$	104.56
$[Pr(Ampirox)_2(H_2O)_2]$	16.18
$[Nd(Ampirox)_2(H_2O)_2]$	17.32
Lornoxicam	13.70
$[La(Lornox)_2(H_2O)_2]$	15.86
$[Pr(Lornox)_2(H_2O)_2]$	171.90
$[Nd(Lornox)_2(H_2O)_2]$	14.52

Table 4
VALUES CT 50 OF LIGANDS AND THEIR LANTHANUM, PRASEODYMIUM AND NEODYMIUM COMPLEXES

CT 50 = The concentration of compound for which a cell viability is 50 % La and Pr

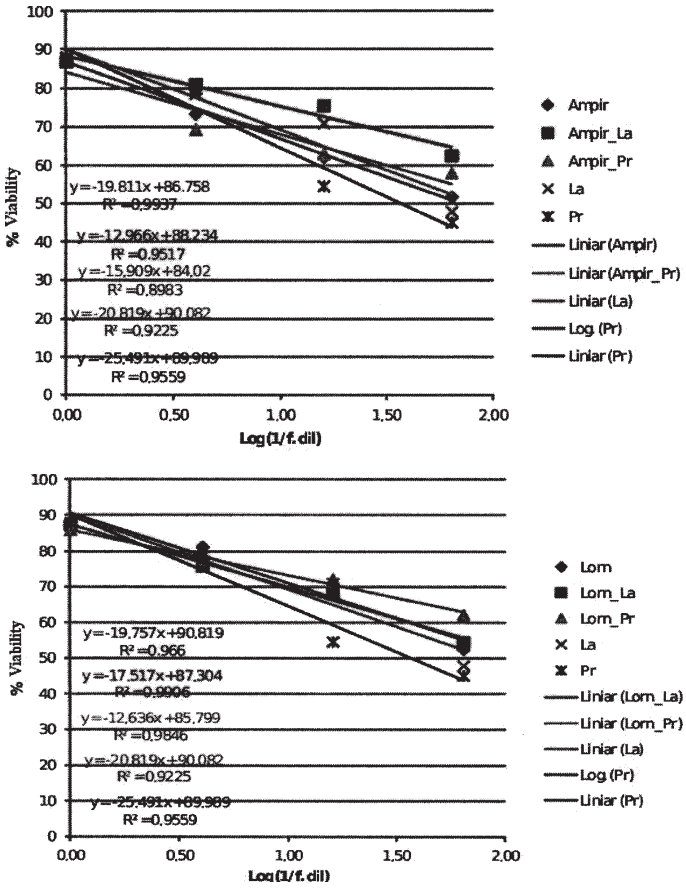


Fig. 4. Cytotoxicity of oxicams and their lanthanum and praseodymium complexes

possible, because of the electronic density charges on the sulphur atom and the ring after complex formation. The appearance of a sharp medium band in the range 3500 - 3600 cm^{-1} indicates the existence of water molecule bound to the metal ion.

The spectra of complexes show the bands due to the M–N and M–O links Ampiroxicam and lornoxicam act as monoanionic chelating ligands, through the amide oxygen and the (thiazolyl) nitrogen.

Cytotoxicity evaluation

Both 0.1% and 0.01% DMSO solutions had no cytotoxic effects on the cultures. The toxicity values (TC 50) estimated for ampiroxicam, lornoxicam, and their lanthanum, praseodymium and neodymium complexes are presented in table 4 and figure 4:

As shown in the graphical representations, complexing with lanthanum and praseodymium induces a moderate modification in the toxicological properties of the compounds. In both cases, the CT50 values of the complexes maintain in the same order of magnitude as this of the free compounds, however, both complexes express a lower cytotoxicity than the free compounds, with a higher modification in the case of lornoxicam. The favorable modification of cytotoxicities by complexing permits advancing the hypothesis that the derivatives are suitable for further investigation of antitumoral / antiinflammatory effects and mechanisms of action.

Conclusions

A some new pharmaceutical coordination compounds of La, Pr, Nd with ampiroxicam and lornoxicam for the antitumoral / antiinflammatory therapy were prepared. The new compounds were characterized from analytical and toxicological point of view and are suitable for further investigation of antitumoral / antiinflammatory effect and mecanisms of action.

Acknowledgements: This research was supported by UEFISCDI– PNCDI – II – 126 / 2012.

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Manuscript received: 3.12.2014