Analytical and Toxicological Characterization of New Co(II) Coordination Compounds with Antiinflammatory Oxicams Drugs

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A new concept in the process of design and development of antiinflammatory drugs focuses on the preparation of metallic compounds of drugs with therapeutic activity. These metallic coordination compounds 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. This paper describes the synthesis and characterization of new cobalt (II) whith oxicams ligands, piroxicam and meloxicam. 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 correlated with the elemental chemical analysis and other physico – chemical studies (UV-VIS, IR, ICP-MS and magnetic and conductometric measurements). Cytotoxicity of cobalt compounds was estimated, comparatively of oxicams ligands.

Keywords: bioactive substances; metallic coordination complexes; oxicams; anti-inflammatory agents

The high rate of inflammatory diseases throughout the world (especially rheumatoid and osteoarthritis), is one of the most challenging issues that scientists have to deal with. Therefore, it is essential to develop new antiinflammatory drugs, with a high rate of efficiency and less side effects [1-6].

A new concept in design and development of antiinflammatory drugs focuses on metallic compounds of the organic structure with therapeutic activity. 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 [7-8]. New complex combinations of cobalt (II) with ligands, such as oxicams, piroxicam and meloxicam, were investigated.

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 [9].

The chemical non-steroidal structures with therapeutic properties (AINS) are among the most frequently used drugs. Mainly they have analgesic, antiinflammatory and antipyretic effects [12]. Some recent studies have provided that, when the metallic complexes are administrated together with antiinflammatory drugs, their efficiency is highly increased. In the same time, it has been noticed that some cobalt (II) complexes, containing as ligands structures with antiarthritic activity may provide a higher antiinflammatory 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 transitional metals.

In the present paper are presented the studies performed for the synthesis, analytical and toxicological characterization of new cobalt (II) coordination compounds with oxicams type drugs, namely piroxicam and meloxicam, which can be used as new antiinflamatory drugs.

Experimental part

Reagents

All reagents and solvents were of analytical grade: cobalt (II) chloride and potassium bromide (Merck), Piroxicam and Meloxicam (Boehringer-Ingelheim), DMSO (Fluka) while ethanol 95% was supplied by INCDCF_ICCF (Bucharest, Romania).

Synthesis of complexes

To the 1, 2 and respectively 3 mmols of oxicams dissolved in 50 mL ethanol by heating, add a solution of 1mmol cobalt(II) chloride dissolved in 10 mL water and heated to reflux 1 h.

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

Phisico-chemical measurements

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

The electronic spectra were recorded usingPerKin Elmer Lamda 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 Spectometer in the range 4000-350 cm^{-1} (KBr disk method).

The measurements of magnetic susceptibily were determinate at room temperature using the Faraday method.

Cytotoxicity evaluation

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

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DMEM-F12 medium and fetal bovine serum were from Sigma (USA). MTS – Cell titer assay was from PROMEGA, tissue culture 96 wells culture plates from Greiner.

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 microM).

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 microL 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 0.01 to 1 microM concentrations in fresh medium. 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 h of exposure, culture medium was again replaced and MTS assay was performed according to the specifications of the supplier.

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

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

Results and discussions

Synthesis of complexes

We have studied the systems: Co (II) – Piroxicam; Co(II) – Meloxicam for the combination ratio M-L 1:1; 1:2 and respectively 1:3. From these systems, we were able to isolate and characterize the following type of mononuclear complexes: $[Co(pirox)_2(H_2O)_2]$, $[Co(melox)_2(H_2O)_2]$. Figure 1 presents the chemical structure of oxicams ligands (piroxicam, meloxicam). Figure 2 presents the chemical structure of the Co - Piroxicam complex, while figure 3 presents the structural conformation of the complexes.

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

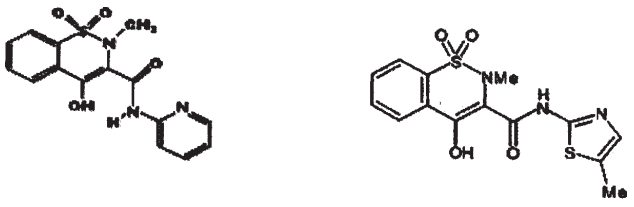


Fig.1. Chemical formula for Piroxicam and Meloxicam

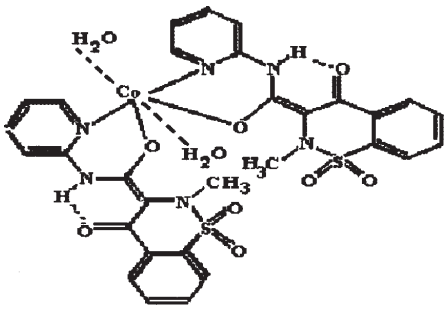
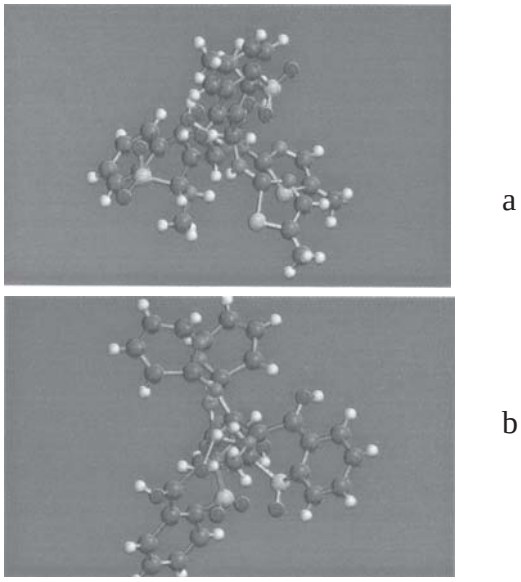


Fig.2. The chemical structure of Co– piroxicam complex



a

b

Fig.3. Structural conformation of the complex compounds Co(II)-meloxicam (a) and Co(II)-piroxicam (b)

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

UV – VIS Spectra

In order to obtain some information about the stereochemistry of Co(II) ion, the electronic spectra in the 250 – 850 nm range have been recorded, as well as their second derivatives .

Table 1
ANALYTICAL DATA OF LIGANDS AND THEIR COBALT COMPLEXES

Compound	Chemical formula	% found / calcd					Magnetic moment (MB)
		C	H	N	S	Co	
Piroxicam (Pirox)	$C_{15}H_{13}N_3O_4S$	**54.21** / **54.38**	**3.90** / **3.92**	**13.61** / **12.68**	**9.52** / **9.66**	-	-
Meloxicam (Melox)	$C_{14}H_{13}N_3O_4S_2$	**50.38** / **50.69**	**3.57** / **3.69**	**12.56** / **12.65**	**19.17** / **19.31**	-	-
$[Co(pirox)_2(H_2O)_2]$	$[Co(C_{15}H_{13}N_3O_4S)_2(H_2O)_2]$	**47.57** / **47.68**	**3.65** / **3.70**	**11.07** / **11.12**	8.52 / 8.47	**7.77** / **7.80**	4.65
$[Co(melox)_2(H_2O)_2]$	$[Co(C_{15}H_{13}N_3O_4S_2)_2(H_2O)_2]$	**42.30** / **42.22**	**3.72** / **3.50**	**10.33** / **10.50**	**15.26** / **16.08**	**7.36** / **7.40**	4.78

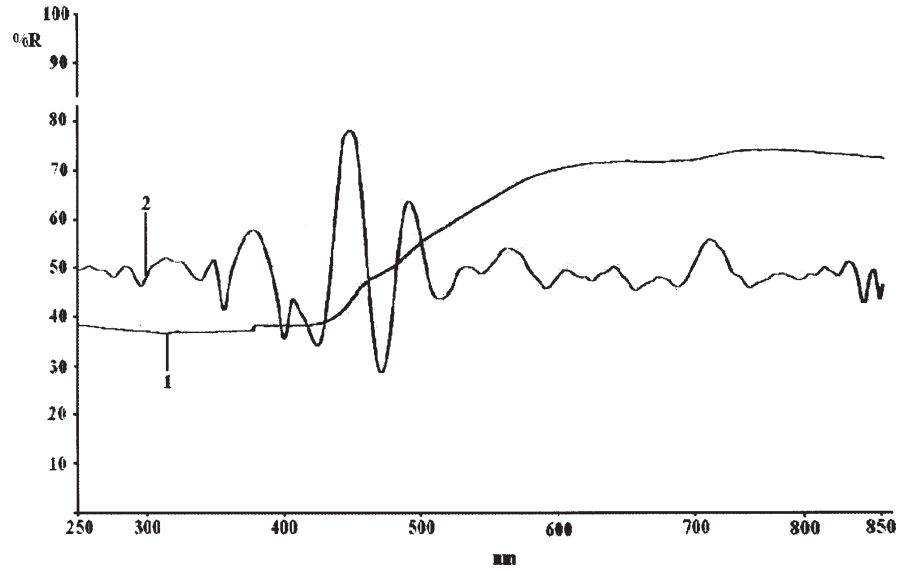


Fig.4. The UV-VIS spectra for Co-meloxicam complex: (1)standard spectra; (2) 2nd-derivative spectra

Table 2
UV-VIS SPECTRA OF LIGANDS AND THEIR COBALT COMPLEXES

Compounds	Absorption bands (λ)	Assignment
Piroxicam	207 241 326	$\pi - \pi^*$, $n - \pi^*$
$[Co(pirox)_2(H_2O)_2]$	424 470	CT (M→L)
	513 563 590	ν_3 ($a^4T_1g \rightarrow b^4T_2g$)
	623 632 687 739	ν_2 ($^4T_1g \rightarrow {}^4A_2g$)
Meloxicam	207 277 353	$\pi - \pi^*$, $n - \pi^*$
$[Co(melox)_2(H_2O)_2]$	400 471	CT (M→L)
	515 561 605	ν_3 ($a^4T_1g \rightarrow b^4T_2g$)
	639 634 701 731	ν_2 ($^4T_1g \rightarrow {}^4A_2g$)

The UV – VIS electronic spectra of the Co-meloxicam complex, presented in figure 4, show the $\pi - \pi^*$, $n - \pi^*$ transitions of ligands, the bands can be assigned to a ligand : metal change transfer, and d – d transitions, in an octahedral configuration of Co(II) ions.

The d-d transition ν_2 and ν_3 are split most likely due to a Jahn Teller or to the binding differencee between Co(II)-O and Co(II)-N.

As can be seen in table 2., the Co-piroxicam complex presents similar spectra.

The spectral data and the value of magnetic moment found for this complex compounds suggest that the Co(II) ion is octahedrelly coordinated, the position 5 and 6 being occupied by water molecules.

IR Spectra

It is well know that piroxicam/meloxicam 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 IR spectra of complexes the 4000-350 cm^{-1} have been recorded .These spectra were compared with the IR spectra of ligands..

Table 3 presents comparatively the bands of ligands and their cobalt complexes, while figure 6 presents the FTIR spectra of piroxicam and the cobalt complex.

The IR spectra show the mode of coordination 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) shifts to higher frequencies in the spectra of complexes. It is 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 3550-3685 cm^{-1} indicate the existence of water molecule bound to the metal ion.

Compounds	ν_{OH}, (H_2O)	ν_{OH}, (enol)	ν_{NH} (amida)	$\nu_{C=O}$ (amida)	$\nu_{C=N}$ (Npir / tiaz)	$\nu_{as\ SO2}$	$\nu_{s\ SO2}$	ν_{M-N}	ν_{M-O}
Piroxicam	-	3337	3330	1628	1576	1349	1038	-	-
$[Co(pirox)_2(H_2O)_2]$	3552	3335	3180	1604	1513	1391	1049	488 445	388 396
Meloxicam	-	3288	3286	1617	1575	1344	1043	-	-
$[Co(melox)_2(H_2O)_2]$	3588	3286	3147	1590	1524	1397	1064	468 472	404 397

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

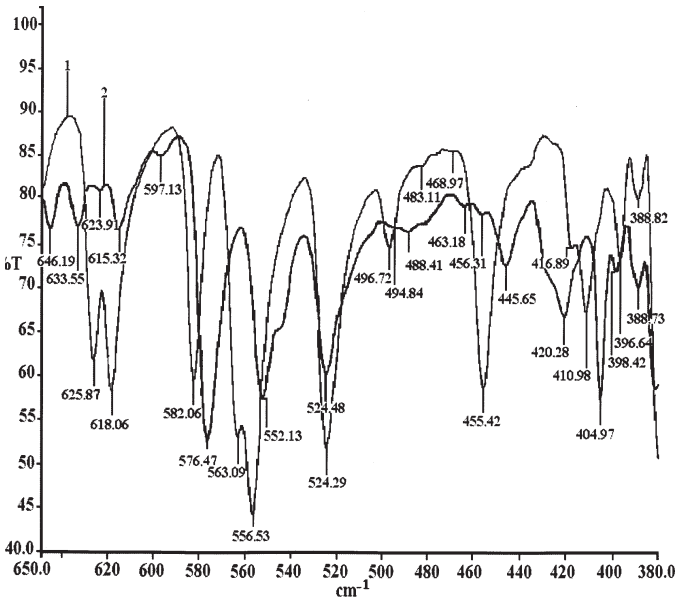
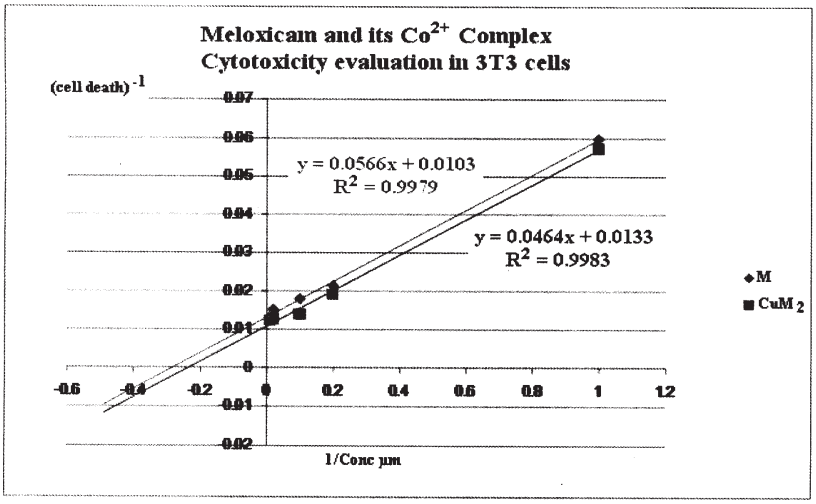


Fig.5. The IR spectra of piroxicam and their cobalt complex compound in 650 – 380 cm^{-1} range 1 = the ligand spectrum; 2 = the complex spectrum

Table 4
VALUES CT 50 OF LIGANDS AND THEIR COBALT COMPLEXES

Compound	CT 50 (microM)
Piroxicam	2,826
$[Co(pirox)_2(H_2O)_2]$	8, 931
Meloxicam	3,489
$[Co(melox)_2(H_2O)_2]$	5,495



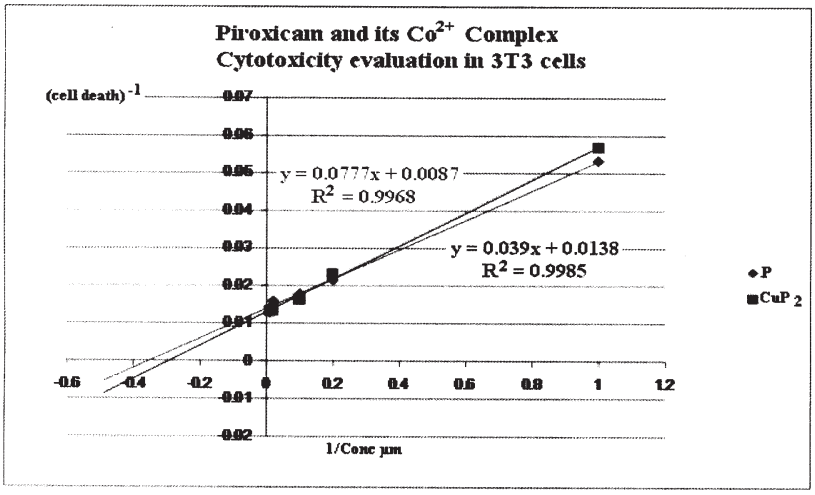


Fig. 6 Cytotoxicity of oxicams and their cobalt complexes

The spectra of complexes show the bands due of the M–N and M–O links. Piroxicam and meloxicam acts as monoanionic chelating ligands, through the amide oxygen and the pyridyl (thiazolyl) nitrogen.

Similar spectrum were obtained for Co - meloxicam compound.

Magnetic Moments

The magnetic moments values calculated from the magnetic susceptibilities, experimentally determined, for the complexes of cobalt is 4.65 MB for piroxicam and 4,78 for meloxicam.These values is in the range 4.3-5.7 MB which corresponds to an octahedral geometry for the ion Co(II)[10].

Cytotoxicity evaluation

Both 0,1% and 0,01% DMSO had no cytotoxic effects on the cultures. The toxicity values estimated for Piroxicam, Meloxicam and their cobalt complexes are presented in table 4 and figure 6.

As shown in the graphical representations (rendered in fig. 6), complexing with cobalt 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 those of the free compounds, however, both complexes express a lower cytotoxicity than the free compounds, with a higher modification in the case of piroxicam. The favourable modification of cytotoxicities by complexing permits advancing the hypothesis that the derivatives are suitable for further investigation of therapeutic effects and mechanisms of actions.

Conclusions

A new methods were developed from obtaining of some pharmaceutical coordination compounds for the antiinflammatory therapy: coordination compounds of cobalt(II) with anti-inflammatory drugs as ligands (oxicams). The new compounds were characterized from analytical and toxicological point of view.

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