Research Article

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Spectral properties and biological activity of La(III) and Nd(III) Monensinates

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Abstract: The present research is focused on evaluation of complexation ability of Monensic acid (MonH) towards La^{3+} and Nd^{3+} ions.

Changes in the SRCD spectrum of Monensinate anion were monitored upon addition of lanthanide(III) ions. The antibiotic undergoes formation of one neutral $[\text{Ln}(\text{Mon})_3(\text{H}_2\text{O})_3]$ and two positively charged complex species of composition $[\text{Ln}(\text{Mon})_2(\text{H}_2\text{O})_2]^+$ and $[\text{Ln}(\text{Mon})(\text{H}_2\text{O})]^{2+}$, respectively ($\text{Ln} = \text{La}^{3+}, \text{Nd}^{3+}$). Neutral complexes were isolated as fine powders and were characterized by IR, FAB-MS and ESI-MS. It is assumed that Monensin acts in bidentate coordination mode *via* monodentate carboxylate moiety and hydroxyl group, both located at the opposite ends of antibiotic molecule.

Activity of Monensic acid and $[\text{Ln}(\text{Mon})_3(\text{H}_2\text{O})_3]$ to decrease visible bacteria growth of *B. subtilis*, *S. Lutea* and *B. mycoides* was evaluated by agar hole diffusion method. Results showed that complexation of lanthanide(III) ions to Monensin enhances the activity of non-coordinated ligand.

Antitumor efficacy of compounds was assayed on human triple negative breast cancer and transplantable sarcoma in rat. The cytotoxicity was accessed by MTT test, NR uptake, CV assay and double AO/PI staining.

Experimental data revealed that Monensic acid and $[\text{Ln}(\text{Mon})_3(\text{H}_2\text{O})_3]$ possess concentration- and time-dependent activity, and express promising cytotoxic properties against human and rat permanent cancer cell lines.

Keywords: polyether ionophore; mononuclear metal(III) complex; Gram-positive microorganisms; human triple negative breast cancer; virus-induced transplantable rat sarcoma.

1 Introduction

Polyether ionophores (PI) as Monensin, Salinomycin, Maduramycin, Narasin, Lasalocid, etc., represent a large group of natural biologically active compounds, produced by *Streptomyces* spp. They show an outstanding potency for the control of coccidiosis caused by protozoan *Eimeria* parasites - a disease, which has been for a long time the major cause of poor performance and lost productivity in poultry and other farm animals. Since discovery of Monensin in 1967, PI are applied as therapeutics in poultry, game birds, sheep, and cattle. An overview of their properties revealed that more animals have been medicated with ionophores for control of disease than any other medicinal agents in the history of veterinary medicine [1]. Apart from antiparasitic efficacy, PI show a broad-spectrum activity as antibacterial, antifungal, herbicidal, antiviral, anti-inflammatory [2-5]. In the last decade it was also found that PI and their derivatives might be promising chemotherapeutic agents for the treatment of cancer [6-10].

Chemically speaking, PI are a unique class of polyketides which reversibly binds metal ions. The structure of Monensin (MonH, **Scheme 1a**) and related compounds consists of a carboxylic acid, functionalized by multiple tetrahydrofuran and tetrahydropyran rings. In acidic form PI exist as a pseudo-cycle, stabilized by “head-to tail” hydrogen bonds. Crystallographic data revealed, that oxygen atoms of the polyether skeleton are internally

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orientated forming a hydrophilic cavity able to host water molecule or monovalent metal ions, respectively (**Scheme 1b**). On the other hand, alkyl substituents of the polyether chain are orientated externally, ensuring the overall lipophilic character of the ligands and their ability to cross cell membranes. The reversible binding and liberation of monovalent metal ions into the intracellular space induce metal homeostasis disturbance, which leads to activation of various energy-dependent processes and ultimate parasite / bacteria death.

PI possess broad similarities in the fundamental mode of action and biology despite of their unique structure and characteristic physicochemical properties. For example, Monensin readily complexes alkali ions with an affinity decreasing in the order of $\text{Na}^+ > \text{K}^+ > \text{Rb}^+ > \text{Li}^+ > \text{Cs}^+$, while Salinomycin is known as a potassium ionophore.

Binding monovalent metal ions, PI transfer them across cell membranes as neutral compounds. The structure of monovalent metal complexes of Monensin, $[\text{MonM}]$ ($\text{M} = \text{Na}^+, \text{K}^+, \text{Li}^+, \text{Cs}^+, \text{Ag}^+$), was proved by X-ray crystallographic analysis on monocrystals. Metal ions are placed into the hydrophilic cavity of the ligand and are coordinated by internal oxygen atoms of the polyether chain. The carboxylic group is deprotonated and participates in H-bond formation with hydroxyl moieties located at the opposite end of the ligand. The only example where the carboxyl group is not deprotonated, is the complex $[\text{MonHNa}^+]\text{Br}$ with bromide ion serving as a counter-ion to form the corresponding neutral species [11-19].

Since 2008, broad research on potential ability of polyether ionophores to bind metal ions of higher oxidation state has been performed at Sofia University [20-27]. It was found that Monensin forms various complexes depending on the ligand form and origin of the metal ions. The reaction of sodium Monensin (MonNa) with divalent metal ions undergoes the formation of heteronuclear complexes of composition $[\text{M}(\text{MonNa})_2\text{Cl}_2]$ ($\text{M} = \text{Mn}^{2+}, \text{Co}^{2+}, \text{Cu}^{2+}$). Complexation of Monensic acid monohydrate leads to the isolation of mononuclear compounds such as $[\text{M}(\text{Mon})_2(\text{H}_2\text{O})_2]$ ($\text{M} = \text{Mn}^{2+}, \text{Co}^{2+}, \text{Mg}^{2+}, \text{Ca}^{2+}, \text{Ni}^{2+}, \text{Zn}^{2+}, \text{Cd}^{2+}$) and $[\text{M}(\text{Mon})(\text{H}_2\text{O})]$ ($\text{M} = \text{Hg}^{2+}$).

Biological assays revealed that the inclusion of divalent biometal ions into the structure of Monensin potentiates its antibacterial properties against Gram(+) - microorganisms. The antitumor activity of the ligand and $[\text{M}(\text{Mon})_2(\text{H}_2\text{O})_2]$ complexes was evaluated against cell lines established from some of the most common and invasive human cancers, drug sensitive and drug resistant squamous cell carcinoma, retrovirus-induced transplantable sarcoma in rat and chicken hepatoma.

Scheme 1: Structure of Monensin A (a) and folding of PI skeleton due to the formation of H-bonds (b).

Monensic acid was found to be less active than metal complexes. The isostructural metal(II) compounds exert enhanced antitumor activity as compared to the non-coordinated ligand and their efficacy seems to depend both on the nature of the metal ions and on the cell lines tested [28-30].

Diversity of Monensinate complex species accompanied by pronounced *in vitro* efficacy provoked us to continue our studies in the field of chemistry and biology of PI. Recently we evaluated the ability of Monensin to bind trivalent metal ions. The present paper reports new data on the coordination of Monensic acid with ions of La^{3+} and Nd^{3+} , spectral characterization of corresponding metal(III) complexes and their antimicrobial / antitumor properties.

2 Experimental

2.1 Materials

Sodium Monensin was cordially supplied by Biovet Ltd. (Peshtera, Bulgaria). Metal salts ($\text{La}(\text{CF}_3\text{SO}_3)_3 \cdot \text{H}_2\text{O}$, $\text{Nd}(\text{CF}_3\text{SO}_3)_3$) were purchased from Merck and Et_4NOH (40% in water) from Fluka. All reagents were of analytical grade, and distilled water ($18.2 \text{ M}\Omega \cdot \text{cm}$) was used in the experiments.

Dulbecco's modified Eagle's medium (D-MEM) and fetal bovine serum were obtained from Gibco-Invitrogen (UK). Dimethyl sulfoxide (DMSO), crystal violet, propidium iodide (PI), acridine orange (AO) and trypsin were purchased from AppliChem (Darmstadt, Germany). Thiazolyl blue tetrazolium bromide (MTT) and 3-amino-7-dimethylamino-2-methylphenazine hydrochloride (Neutral red) were obtained from Sigma-Aldrich Chemie GmbH (Germany). The antibiotics (Penicillin and

Streptomycin) for cell cultures were from Lonza (Belgium). All sterile plastic ware was purchased from Orange Scientific (Belgium).

2.2 Methods

Elemental analysis data (C, H) were obtained with a Vario EL-III Elemental Analyzer, and complexometric titration was used to determine the metal content. Samples of the complexes were decomposed using conc. HNO_3 and the obtained soluble metal nitrates were treated with an excess of Na_2EDTA (standard solution). Unreacted Complexon III was titrated with standard solution of $\text{Zn}(\text{NO}_3)_2$. All titrations were performed in an acetate buffer (1 M, pH 5.5) using xylenol orange as an indicator.

IR spectra of compounds were recorded on Nicolet 6700 FT-IR spectrophotometer, Thermo Scientific ($4000\text{--}400\text{ cm}^{-1}$), in KBr pellets. FAB-MS and ESI-MS spectra were obtained using Fisons VG Autospec and Bruker-microTOF-Q II spectrometers, respectively.

SRCD spectra were recorded at the AU-CD beam line SRCD facility, part of the ASTRID2 storage ring at the Institute for Storage Ring Facilities (ISA), University of Aarhus, Denmark. Compounds were dissolved in methanol, and all spectra were recorded in cuvettes at a 0.014 mm optical pathlength, in 1 nm steps with a dwell time of 2 s per step, with a wavelength range of 170–300 nm and with a resolution of 0.5 nm. A solution of corresponding metal(III) salt was added to MeOH solutions containing an equivalent amount of Monensic acid ($\text{MonH}\times\text{H}_2\text{O}$) and Et_4NOH (1:1 molar ratio) to obtain a series of mixtures at a metal-to-ligand molar ratio of 1–9 to 3–1 (at total 20 mM concentration of the ligand).

2.3 Synthesis of La^{3+} and Nd^{3+} complexes of Monensin

Monensic acid monohydrate ($\text{MonH}\times\text{H}_2\text{O}$) was prepared as previously described [23].

Lanthanide(III) salt (0.1 mmol) was added to the acetonitrile solution containing $\text{MonH}\times\text{H}_2\text{O}$ (0.3 mmol, 206.7 mg) and Et_4NOH (0.3 mmol, 110.4 μL). The reaction mixture was stirred for 1 h at r.t.; the precipitates that formed were filtered off, were washed with MeCN and dried over P_2O_5 . The suggested composition of complexes isolated is $[\text{Ln}(\text{Mon})_3(\text{H}_2\text{O})_3]$ ($\text{Ln} = \text{La}$ (**1**, 115 mg, 52% yield), Nd (**2**, 164 mg, 74% yield)). The complexes were soluble in MeOH.

Isolated species of La^{3+} - and Nd^{3+} -containing Monensin derivatives were analyzed by FAB-MS and ESI-MS spectrometries. Due to the limitations of the FAB-MS technique (we can observe ions of m/z up to 2000), the obtained spectra consisted of limited number of signals, mainly assigned to the formation of $[\text{Ln}(\text{C}_{36}\text{H}_{60}\text{O}_{11})]^+$ and $[\text{Ln}(\text{C}_{36}\text{H}_{61}\text{O}_{11})_2]^+$ ions. ESI-MS allowed the observation of more complex spectra, where the presence of various ions was detected due to the association / dissociation processes as well as to the subtraction of water molecules and anion ligands, or abstraction of metal ions (Na^+ / Ln^{3+}). Details on ESI-MS data are presented in Section 3.1 and in Supplementary material.

2.4 Antibacterial activity

Antimicrobial properties of Monensic acid, metal(III) complexes, as well as corresponding metal(III) salts, were studied against Gram(+)-bacteria using a double layer agar hole diffusion method [31]. Microorganisms *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* spp. and *Micrococcus luteus* (ATCC 10054, *Sarcina lutea*) were obtained from the National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC, Bulgaria).

2.5 Cytotoxicity assays

Permanent cell lines established from human triple negative breast cancer (MDA-MB-231) and transplantable rat sarcoma induced by the Rous sarcoma virus strain Schmidt-Rupin (LSR-SF-SR) were used as model systems in cytotoxicity assays. The cells were grown as monolayer cultures in D-MEM medium, supplemented with 5–10% fetal bovine serum and antibiotics (100 U/mL penicillin and 100 $\mu\text{g/mL}$ streptomycin) at 37°C in humidified CO_2 incubator (Thermo scientific, Hepa class 100).

Compounds tested (Monensic acid and complexes **1–2**) were prepared as stock solutions in DMSO (1 mg/mL) due to their low solubility in water. During the experiments all substances were applied at final concentrations of 1, 5, 10 and 20 $\mu\text{g/mL}$ (working solutions) after the appropriate dilution with D-MEM medium [28–29].

The effect of compounds on the viability and proliferation of the treated cells was evaluated using MTT (thiazolyl blue tetrazolium bromide) test after 24, 48 and 72 h of incubation [32]. In some cases, neutral red (NR) uptake cytotoxicity assay [33] and crystal violet (CV) staining [34] were also performed.

Table 1: Elemental analysis data of lanthanide(III) Monensinates 1-2.

Complex	Composition	MW	H, %	C, %	M, %
[La(Mon) ₃ (H ₂ O) ₃], 1	C ₁₀₈ H ₁₈₉ LaO ₃₆	2202.6	8.65 ^a 7.76 ^b	58.89 ^a 55.07 ^b	6.31 ^a 6.10 ^b
[Nd(Mon) ₃ (H ₂ O) ₃], 2	C ₁₀₈ H ₁₈₉ NdO ₃₆	2207.9	8.63 ^a 8.24 ^b	58.75 ^a 57.52 ^b	6.53 ^a 6.36 ^b

^acalculated; ^bfound

The optical density was measured at 540 nm (MTT, NR) and at 620 nm (CV), respectively, using an automatic microplate reader (TECAN, Sunrise™, Austria). Relative cell viability, expressed as a percentage of the untreated control (100% viability), was calculated for each concentration. “Concentration – response” curves were prepared, and the effective cytotoxic concentration of the compounds as compared to the control - CC₅₀ and CC₉₀ (μM), causing 50% or 90% reduction of cell viability, respectively, was estimated from these curves. All data points represent an average of three independent assays.

The ability of compounds to induce cytopathological changes was assessed using double staining with acridine orange (AO) and propidium iodide (PI) according to the standard procedures using fluorescence microscope (Leika DM 500B, Wetzlar, Germany). The degree of apoptosis was evaluated by the formation of bright green nucleus with chromatin condensation (dense green areas) and/or of orange nucleus (showing condensation of chromatin) indicating early or late apoptotic cell death, respectively.

2.6 Statistical analysis

The data are presented as mean ± standard error of the mean. Statistical differences between control and treated groups were assessed using one-way analysis of variance (ANOVA) followed by Dunnett post-hoc test.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Spectral properties of Monensinate complexes

The coordination of Monensin towards ions of La³⁺ and Nd³⁺ was evaluated at different reaction conditions.

Complexation took place in acetonitrile solutions after the deprotonation of Monensic acid using Et₄NOH. It was observed that at a metal-to-ligand molar ratio of 1:3 a solid phase forms, but it gradually dissolves as the increased concentration of the metal(III) ion was added. The composition of the solid phase was suggested to belong to the neutral species of general formula of [Ln(Mon)₃(H₂O)₃] (Ln = La³⁺ (**1**), Nd³⁺ (**2**)) based on our previous studies of ligand reactivity towards divalent metal ions [20-27] and Gd³⁺ [35]. Elemental analysis data (**Table 1**) align with the proposed composition of metal(III) complexes.

Properties of Monensic acid and complexes **1-2** in solid state were studied by IR spectroscopy (**Figure 1**). Bands at 3530 cm⁻¹ and 3340 cm⁻¹, observed in the spectrum of MonH×H₂O, were attributed to the stretching vibrations (ν_{OH}) of water molecule and hydroxyl groups, respectively, both participating in the formation of H-bonds. These two signals appear as a broad band within the 3550-3400 cm⁻¹ range in the IR spectra of complexes **1-2** due to their coordination with the metal(III) center. A characteristic single band within the Monensic acid spectrum, was observed at 1700cm⁻¹, and originated from the presence of the carboxylic group. In the spectra of metal(III) derivatives it was replaced by two bands at 1560 cm⁻¹ and 1420 cm⁻¹ due to the stretching asymmetric and symmetric vibrations of deprotonated carboxylic moiety, respectively. A monodentate coordination mode of carboxylate anion to Ln(III) center was proposed by the difference between positions of its asymmetric and symmetric stretching vibrations (Δν ~ 140 cm⁻¹) [36]. The assignment of IR data revealed that ligand complexes metal(III) ions through monodentate deprotonated COOH-moiety and a hydroxyl group; in addition, the presence of water molecules into the structure of metal(III) Monensin derivatives was also assumed [35].

ESI-MS spectra of [La(Mon)₃(H₂O)₃] and [Nd(Mon)₃(H₂O)₃] in the gas phase are presented in **Figures 2-3**. The molecular fragmentation of both complexes is shown in **Scheme 2**, the assignment of the most peaks observed is described in detail in **Table S1**. The

Figure 1: IR spectra of Monensic acid and complexes 1-2.

$[\text{Ln}(\text{Mon})_3\text{Na}]^+$ fragment of the complexes was confirmed to exist, which is in accordance with the composition suggested for the neutral lanthanide(III) derivatives of the ligand. The high affinity of Monensin to bind Na^+ , originating from the glassware and the matrix, leads to the signals at 693.4 and 1385.8 m/z , attributed to the formation of $[\text{C}_{36}\text{H}_{62}\text{O}_{11}\text{Na}]^+$ and $[(\text{C}_{36}\text{H}_{62}\text{O}_{11})(\text{C}_{36}\text{H}_{61}\text{O}_{11}\text{Na})\text{Na}]^+$, respectively.

Isolation of $[\text{La}(\text{Mon})_3(\text{H}_2\text{O})_3]$, **1**, and $[\text{Nd}(\text{Mon})_3(\text{H}_2\text{O})_3]$, **2**, in solid state is reasonable due to the formation of neutral species, which preferably precipitate in acetonitrile solutions. On the other hand, it was a matter of interest to monitor the spectral changes of chiral Monensin in solution upon addition of the increasing amount of lanthanide ions to verify if any other species exist at different reaction conditions. The ability of Monensin to bind metal(III) ions in methanolic solutions was monitored using Synchrotron Radiation Circular Dichroism (SRCD) spectroscopy.

Based on our SRCD experimental results it can be concluded that Monensinate anion forms at least three complex species depending on the metal-to-ligand molar ratio. Neutral complexes **1** and **2** exist in the presence of high excess of the ligand. Further addition of metal(III) ions leads to the formation of two positively charged complex ions of composition $[\text{Ln}(\text{Mon})_2(\text{H}_2\text{O})_2]^+$ and $[\text{Ln}(\text{Mon})(\text{H}_2\text{O})]^+$, respectively [35]. These species were

Figure 2: ESI-MS spectrum of complex 1.

Figure 3: ESI-MS spectrum of complex 2.

Scheme 2: Molecular fragmentation of 1 (above) and 2 (bottom).

Figure 4: SRCD spectra of observed complex species at certain metal-to-ligand molar ratio.

observed only in solution, due to their positive charge and high solubility in common solvents. Presently, we were not able to isolate (and characterize) them in solid state. SRCD spectra of Monensinate anion and its complexes in methanolic solutions are placed in **Figure 4**.

3.2 Biological activity of Monensic acid and complexes 1-2

Antibacterial activity of Monensic acid and neutral complexes **1-2** against the test-strains of Gram-positive bacteria was evaluated in terms of their minimum inhibitory concentration (MIC). Results showed that Monensic acid is effective against *B. subtilis* and *S. Lutea* when applied at 23.9 μM , and possesses stronger toxicity against *B. mycoides* at MIC = 11.8 μM . Incorporation of La^{3+} or Nd^{3+} ions into the structure of Monensin improves

the bactericidal activity of the antibiotic, showing *c.a.* four- and twofold enhanced effect towards *B. subtilis* and *S. lutea*, respectively (**1**: MIC = 7 μM , **2**: MIC = 14 μM). It was observed that the Nd(III) complex is more active than the isostructural La(III) Monensinate against *B. mycoides* (**1**: MIC = 7 μM , **2**: MIC = 3.5 μM), but more experiments are required to explain such a difference in behavior of structurally similar metal(III) complex species. On the other hand, metal(III) salts are ineffective towards microorganisms even when applied at high concentration levels (MIC > 1.67 mM).

The bacteria strain of *B. mycoides* is generally more sensitive to the action of Monensic acid and its metal complexes than *B. subtilis* and *S. lutea*. The comparison between antimicrobial efficacy of the ligand and corresponding coordination compounds with di- and three-valent metal ions revealed the following hierarchy order of increasing activity depending on the metal center [21, 23-24]:

B. subtilis:

S. lutea:

B. mycoides:

Current results show that the complexation of Monensin is an efficient strategy to enhance its antibacterial activity towards Gram(+)-microorganisms. The specific mechanism(s) by which metal ions tested affect bacteria life-cycle has not been completely studied. It is likely that Monensin serves as a carrier to transfer them through cell membranes and thus to induce metal homeostasis alterations, which leads to their growth inhibition.

The cytotoxicity of Monensic acid and $[\text{Ln}(\text{Mon})_3(\text{H}_2\text{O})_3]$ was also evaluated in cultured human (MDA-MB-231) and rat (LSR-SF-SR) tumor cells. These permanent cell lines were used as model systems in our study as follows:

i) The cell line MDA-MB-231 was established from a human triple negative breast cancer (TNBC). As a result, TNBC remains a challenging subtype of breast cancer to treat since the cells do not express estrogen / progesterone receptors and human epidermal receptor 2 (HER2), and there is no targeted therapy specifically approved against this malignancy. The treatment of TNBC currently relies on chemotherapy, albeit often with limited efficacy and poor survival outcomes [37-38].

Figure 5: Effect of Monensin (Δ), DMSO (O) and complexes 1-2 (■, ◆) on viability and proliferation of human breast cancer MDA-MB-231 cells (control ▲), MTT test.

ii) The transplantable sarcoma in rats from which the LSR-SF-SR cell line was originated, was induced by the Rous sarcoma virus strain Schmidt-Ruppin (SR-RSV). The virus and the cells that were transformed by it express (viral) *v-src* oncogene. *Src* and *Src*-family protein kinases are proto-oncogenes that are important for cell morphology, motility, proliferation, and survival. The dysregulation of their functioning is involved in the pathogenesis of a wide variety of animal and human cancers [39-40].

The anticancer activity of compounds on the viability and proliferation of tumor cells was assessed

by several methods that varied in cell targets and in the mechanisms of action: i) MTT test (evaluates the ability of mitochondrial enzyme succinate dehydrogenase to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide into formazan); ii) NR uptake (neutral red is a weak cationic dye that readily penetrates cell membranes and distributes to the acidic compartments in the cell, therefore acting as a marker for the integrity of lysosomes); iii) CV assay (crystal violet dye binds to proteins and DNA); iv) double AO/PI staining (AO and PI are DNA intercalating agents, PI cannot cross the membrane of viable cells).

The cells were treated for 24h, 48h and 72h, respectively. The determination of cytotoxic activity of the substances after such short treatment intervals is important because in clinical practice, cancer cells should be rapidly destroyed or physically removed from the body to reduce the risk of selecting drug resistant tumor cells, their metastatic potential, etc.

Monensic acid and complexes 1-2 were studied in the concentration range from 1 to 20 μg/mL [28-29] and from the corresponding “concentration - response” curves their cytotoxic concentration was estimated. Examples using MTT test are presented in **Figures 5-6** and **Tables S2-S3**.

It should be pointed out that the “concentration - response” curves possess a similar profile for all tested compounds if the experimental data are presented in mg/mL units. The molecular weight of Monensic acid is in fact about 3 times lower than that of compounds 1-2. More precise information was retrieved that analyzed the effective molar cytotoxic concentration values (CC_{50} and CC_{90} , respectively; **Tables 2-3**).

A limited set of reliable data relating to CC_{50} and CC_{90} values was obtained applying the compounds in the concentration range studied. Nonetheless, the present results outline the general tendency that metal(III) ions enhance the anticancer activity of the non-coordinated ligand. To fulfill the gaps, one has to increase the number of working solutions, but we designed this experiment according to the available information on cytotoxicity of Monensin and its divalent metal complexes against human and animal tumor cell lines of various origin [28-29].

The cytopathological changes caused by Monensic acid and complexes 1-2 applied at 5 mg/mL, are presented in **Figure 7**. The following changes were observed in LSR-SF-SR cells upon the treatment: a) Monolayer from untreated cells, similar in size and morphology. The presence of a large number of cells with mitotic activity; b) Reduced by about 30% of the control cell composition. The cells are heterogeneous in shape, with increased dimensions and swelling of the cytoplasm. Nuclei show

Figure 6: Effect of DMSO (O) and complexes 1-2 (■, ◆) on viability and proliferation of rat sarcoma LSF-SR-SR cells (control ▲), MTT test.

mild to moderately pronounced nuclear atypism, a dense grain chromatin. Approximately 20% of the cells detect 1-3 optically dense vacuoles of approximately the size of the cell nucleus. These cells have integrity of the cell membrane and show no signs of cell death. Small cell clusters filled with fragments of cytoplasmic organelles and destroyed nuclei with PI-permeabilized membrane, non-vital, no signs of apoptosis were observed; c) About 15% of the cells are present relative to the control,

Table 2: Cytotoxic effect of Monensic acid and complexes 1-2 on MDA-MB-231 cell line.

Compound	24 h	48 h	72 h		
	MTT	MTT	CV	NR	MTT
CC₅₀ [μM]					
MonH×H ₂ O	> 30 ^a	> 30 ^a	6.2	2.4	< 0.75 ^b
Complex 1	> 9 ^a	1.5	1.9	1.9	< 0.23 ^b
Complex 2	> 9 ^a	1.4	1.3	2.5	< 0.23 ^b
CC₉₀ [μM]					
MonH×H ₂ O	> 30 ^c	> 30 ^c	> 30 ^c	2.1	1
[La(Mon) ₃ (H ₂ O) ₃]	> 9 ^c	4.4	> 9 ^c	> 9 ^c	3.3
[Nd(Mon) ₃ (H ₂ O) ₃]	> 9 ^c	4.2	> 9 ^c	> 9 ^c	3.1

Corresponding CC value was not defined since the cell viability was: a) higher than 50%, b) lower than 50%, c) higher than 10%.

Table 3: Cytotoxic effect of Monensic acid and complexes 1-2 on LSR-SF-SR cell line.

Compound	24 h	48 h	72 h	
	MTT	MTT	MTT	CV
CC₅₀ [μM]				
MonH×H ₂ O	> 30 ^a	< 0.75 ^b	< 0.75 ^b	1.00
[La(Mon) ₃ (H ₂ O) ₃]	> 9 ^a	0.45	< 0.23 ^b	0.39
[Nd(Mon) ₃ (H ₂ O) ₃]	7.7	0.98	< 0.23 ^b	0.38
CC₉₀ [μM]				
MonH×H ₂ O	> 30 ^c	< 0.75 ^d	< 0.75 ^d	4.0
[La(Mon) ₃ (H ₂ O) ₃]	> 9 ^c	5.6	1.80	> 9 ^c
[Nd(Mon) ₃ (H ₂ O) ₃]	> 9 ^c	6.4	< 0.23 ^d	> 9 ^c

Corresponding CC value was not defined since the cell viability was: a) higher than 50%, b) lower than 50%, c) higher than 10%, d) lower than 10%.

proportionally increased cell and nuclei sizes. Some cells lack cellular organelles and nuclear fragments. There are shaped apoptotic bodies; d) Cellular composition is reduced to 50%. The cells are heterogeneous in size and shape. There is pronounced nuclear atypism, with nuclear membrane integrity and high cell membrane permeability for PI. Single giant cells with cytoplasmic swelling and signs of necrosis as well as small cell groups with apoptotic bodies are observed.

The data obtained in our study revealed that Monensic acid and its La³⁺ / Nd³⁺ complexes express promising cytotoxic properties against permanent cell lines established from human triple negative breast

Figure 7: Rat sarcoma cell line (LSR-SF-SR) after double staining with AO/PI: a) untreated cells, b) $\text{MonH}\times\text{H}_2\text{O}$, c) complex 1; d) complex 2 (cultivation for 48 h with compounds tested at $5\text{ }\mu\text{g/mL}$, magnification $\times 20$).

cancer and retrovirus-induced rat sarcoma (expressing *v-src* gene). These results are not surprising and could be explained, at least partially, by the ability of the ligand to induce significant alterations in the treated cells such as changes in the pH level and ATP content; cell cycle arrest (in G1/G1-M phase); early mitochondrial damage and energy deficit; apoptosis (associated with changes in Bax, caspase-3, caspase-8 and mitochondrial transmembrane potential) and/or necrosis [41-44]. Additional experiments are underway to characterize the cellular and molecular mechanism(s) of action of the tested compounds.

4 Conclusion

The complexation ability of Monensin towards La^{3+} and Nd^{3+} ions was studied in different phases by using techniques seen in IR and SRCD spectroscopies, FAB-MS / ESI-MS spectrometries and elemental analysis. Experimental data

revealed that the ligand forms at least one neutral and two positively charged complex species of composition $[\text{Ln}(\text{Mon})_3(\text{H}_2\text{O})_3]$, $[\text{Ln}(\text{Mon})_2(\text{H}_2\text{O})_2]^+$ and $[\text{Ln}(\text{Mon})(\text{H}_2\text{O})]^{2+}$ ($\text{Ln} = \text{La}^{3+}, \text{Nd}^{3+}$). Antibacterial and antitumor ability of neutral complex species $[\text{Ln}(\text{Mon})_3(\text{H}_2\text{O})_3]$ was evaluated against selected Gram-positive bacteria strains and tumor cell lines originating from human triple negative breast cancer and virus-induced transplantable rat sarcoma. The cytotoxicity of compounds tested is concentration- and time-dependent, with metal(III) complexes being generally more effective than non-coordinated Monensic acid.

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