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Synthesis, Spectral Properties, Antibacterial and Antitumor Activity of Salinomycin Complexes with the Transition Metal Ions Co(II), Ni(II), Cu(II) and Zn(II)

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Abstract: SalNa (sodium salinomycin) reacts with divalent transition metal ions of Co(II), Ni(II), Cu(II) and Zn(II) to produce novel compounds characterized by various spectroscopic methods. The interaction of metal (II) ions with SalNa results in the formation of mononuclear complexes of a general composition of $[M(Sal)_2 \cdot (H_2O)_2]\ nH_2O$ (n = 0 or 2) where the divalent cations replace Na^+ ions from the cavity of initial compound. The new compounds (disalinomycinates) possess an enhanced antibacterial activity against Gram-positive microorganisms as compared to both SalNa and SalH (salinomycinic acid), respectively. The metal (II) complexes manifest strong concentration dependent cytotoxic effect in experiments using human leukemia cell lines. The complexes of Co(II) and Cu(II) proved to exert superior activity as compared to the Ni(II) and Zn(II) analogues and are much more cytotoxic than SalNa and SalH. Further studies should be conducted to determine the therapeutic indexes of the new compounds.

Key words: Salinomycin, divalent metal complexes, antibacterial properties, antitumor activity.

1. Introduction

$C_{42}H_{70}O_{11}$ (Salinomycinic acid, Fig. 1a) is a monocarboxylic polyether ionophorous antibiotic belonging to the group of monensin, maduramicin, semduramicin, narasin, etc.. The structure of these compounds, consisting of a monocarboxylic group and several heterocyclic ether-containing rings, determines their ability to complex with monovalent metal ions [1-4]. Salinomycin, monensin, narasin are referred to as monovalent polyether ionophores due to their high binding affinity to alkali cations [4]. The antibiotics mediate the transport of alkali ions across cell membranes and initiate a cascade of biochemical processes leading to cell death [5-11]. The polyether ionophores, applied mainly in their sodium form, possess a broad spectrum of biological activity as antibacterial, herbicidal, antifungal, anti-inflammatory, etc. [12-22]. Our systematic studies, performed in the last five years, reveal that the antibacterial activity of MonH (monensic acid) and its sodium complex -MonNa (sodium monensin) can be significantly enhanced via complex formation with divalent biometal ions. Higher toxicity towards

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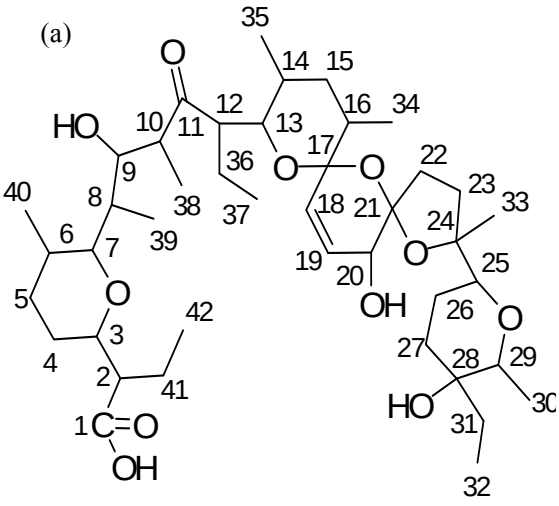
Gram(+)- microorganisms than the initial compounds have been shown for all studied biometal(II) complexes of MonH and MonNa [23-27]. The enhanced antimicrobial effect of new metal (II) compounds necessitates a detailed study on complexation of monovalent polyether ionophores with biometal ions in order to obtain new species with improved biological activity.

The study on chronic oral toxicity of polyether ionophores in animals shows that the relative toxicity of these antibiotics decreases in the order of maduramycin > monensin > narasin > lasalocid > salinomycin [28]. Despite being the least toxic representative among this ionophore group, it has been recently demonstrated that salinomycin causes apoptosis of breast cancer stem cells and is more effective than the traditional anti-cancer drug paclitaxel [29-35]. It has been also suggested that salinomycin may provide a promising approach for chemotherapy of apoptosis resistant cancer cells, colorectal cancer lines or lung cancer [36-40].

To the best of our knowledge no literature data concerning the ability of salinomycin to bind divalent biometal ions has been published so far. The question whether salinomycin reacts with metal cations of higher valence to form new species provokes our interest in order to be able to compare the results with those already observed when monensins (MonH, MonNa) are applied as starting reagents.



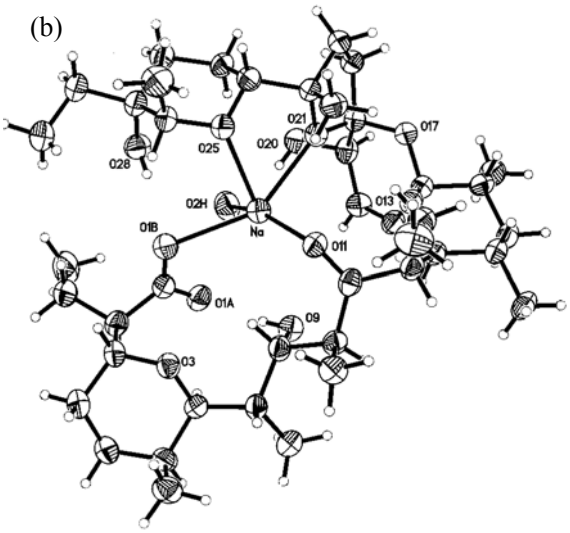


Fig. 1 (a) Structural formula of SalH (salinomycinic acid) with a numbering scheme; (b) crystal structure of SalNa (sodium salinomycin).

In the present paper we report the results on synthesis and spectral properties of new Co(II), Ni(II), Cu(II), and Zn(II) complexes using sodium salinomycin (SalNa, Fig. 1b) as a starting compound. The promising results for biological activity of monensin biometal(II) complexes as well as the strong antitumor effect of salinomycin motivated us to investigate also in some details the biological properties of these compounds and to compare their effect with that possessed by salinomycinic acid and sodium salinomycin.

2. Experiment

2.1 Materials and Measurements

All chemicals used were of reagent grade. The commercially available sodium salinomycin ($C_{42}H_{69}O_{11}Na$) was obtained from BIOVET Ltd, Bulgaria and was used without further purification. Solvents (MeCN, MeOH, DMSO, hexane, THF) and metal salts ($Co(NO_3)_2{\cdot}6H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$, $Cu(CH_3COO)_2{\cdot}H_2O$, $Zn(NO_3)_2{\cdot}6H_2O$) were purchased from Merck, Germany. In all experiments distilled water was used.

Elemental analysis (C, H) was conducted with a VarioEL V5.18.0 Elemental Analyzer. For the AAS determination of metal content the samples were

digested with conc. HNO_3. AAS was performed with Perkin Elmer 1100 B using a stock standard solution (Merck, 1,000 μg/mL); the working reference solutions were prepared after suitable dilution. Infrared spectra (4,000-400 cm^{-1}) were obtained on a Specord 75-IR in a nujol mull. FAB-MS spectra were acquired using a Fisons VG Autospec.

The X-band EPR spectra of Cu(II) complex of salinomycin were obtained on a ERS 220/Q spectrometer within the temperature range 85-410 K, using Mn^{2+}/ZnS as a standard. The experimental data were analyzed with WINEPR SimFonia program (Bruker Analytische Messtechnik GmbH).

^1H (600.13 MHz) and ^{13}C (150.92 MHz) spectra of SalH, SalNa and the Zn(II) complex were recorded on an AVANCE AV600 II+ NMR spectrometer. All spectra were acquired in CD_3CN at 293 K ± 0.1 K. TMS was used as an internal standard for the ^1H and ^{13}C spectra. Unambiguous assignment of the signals was made on the basis of the gradient enhanced versions of COSY, TOCSY, HSQC, HMBC and ROESY experiments (Bruker pulse library programs: cosygpmfqf, dipsi2etgpsi, hsqcedetgpsisp2.2, hmbcgplpndqf, roesyph.2, 2007). The chemical shift values of the overlapped individual protons in the complexes have been determined from the HSQC spectra.

2.2 Synthetic Procedures

2.2.1 Synthesis of SalH (Salinomycinic Acid)

Sodium salinomycin (2 mmol, 1.55 g in 10 mL chloroform) reacts with excess of HCl (10 mL 1 M in H_2O) to afford the formation of SalH (salinomycinic acid). The organic layer was separated and washed three times with water to remove NaCl formed; after concentration of the solution the organic compound was dissolved in acetone and was isolated by precipitation from water. Yield 933 mg, 62%. Anal. Calcd. for $C_{42}H_{70}O_{11}$ (MW 751.0): H, 9.39; C, 67.17. Found: H, 9.44; C, 67.17%. ^1H-NMR (600.13 MHz, δ (assignment), CD_3CN): 6.06 (18CH), 5.92 (19CH), 4.09 (9CH), 3.93 (20CH), 3.90 (3CH), 3.72 (29CH), 3.69 (13CH), 3.64 (25CH), 3.60 (7CH), 2.95 (2CH, 10CH), 2.66 (12CH), 2.24 ($22CH_2$", $23CH_2$"), 1.88 ($22CH_2$', $4CH_2$", $36CH_2$"), 1.85 ($5CH_2$"), 1.80 (6CH), 1.71 (14CH, $23CH_2$'), 1.70 ($26CH_2$"), 1.61 ($27CH_2$"), 1.59 (16CH), 1.58 ($15CH_2$"), 1.54 ($27CH_2$'), 1.50 ($26CH_2$'), 1.47 (8CH), 1.45 ($5CH_2$'), 1.44 ($41CH_2$, $4CH_2$'), 1.42 ($36CH_2$'), 1.36 ($33CH_3$), 1.29 ($31CH_2$), 1.16 ($30CH_3$), 1.12 ($15CH_2$'), 0.94 ($40CH_3$), 0.91 ($42CH_3$), 0.86 ($35CH_3$), 0.85 ($32CH_3$), 0.79 ($37CH_3$), 0.77 ($38CH_3$), 0.73 ($39CH_3$), 0.69 ($34CH_3$). $^{13}C\{^1H\}$-NMR (150.92 MHz, δ (assignment), CD_3CN): 215.53 (11C), 177.86 (1C), 133.65 (19C), 123.43 (18C), 107.72 (21C), 100.14 (17C), 89.04 (24C), 78.60 (13C), 77.83 (29CH), 75.51 (3C), 75.05 (25CH), 72.73 (7C), 71.47 (28C), 69.89 (9C), 68.10 (20C), 56.80 (12C), 49.25 (2C), 49.09 (10C), 41.42 (16C), 39.09 (15C), 37.49 (22C), 37.06 (8C), 33.66 (14C), 31.92 (31C), 31.53 (23C), 30.19 (27C), 29.06 (6C), 26.03 (33C), 26.94 (5C), 23.47 (41C), 22.72 (26C), 20.77 (4C), 18.68 (36C), 17.86 (35C), 15.99 (34C), 15.31 (30C), 14.15 (37C), 13.47 (38C), 12.20 (42C), 11.65 (40C), 7.83 (39C), 6.79 (32C).

The ^1H and ^{13}C NMR signals of sodium salinomycin are unambiguously assigned as follows: ^1H-NMR (600.13 MHz, δ (assignment), CD_3CN): 6.05 (18CH), 5.79 (19CH), 4.06 (9CH), 4.05 (29CH), 4.02 (20CH), 3.78 (3CH), 3.67 (13CH), 3.63 (7CH), 3.51 (25CH), 2.79 (10CH), 2.77 (2CH), 2.75 (12CH), 2.17 ($22CH_2$", $26CH_2$"), 1.94 ($22CH_2$'), 1.90 ($23CH_2$"), 1.88 ($36CH_2$"), 1.86 ($4CH_2$", $23CH_2$'), 1.84 ($5CH_2$"), 1.71 (6CH), 1.69 (14CH), 1.67 ($15CH_2$"), 1.62 (16CH, $33CH_3$), 1.58 ($27CH_2$), 1.43 ($5CH_2$'), 1.40 ($4CH_2$'), 1.42 (8CH), 1.35 ($26CH_2$'), 1.33 ($41CH_2$"), 1.32 ($36CH_2$'), 1.30 ($31CH_2$"), 1.26 ($31CH_2$'), 1.24 ($30CH_3$),1.23 ($41CH_2$'), 1.16 ($15CH_2$'), 0.91 ($40CH_3$), 0.89 ($35CH_3$), 0.87 ($32CH_3$), 0.85 ($42CH_3$), 0.79 ($38CH_3$), 0.76 ($37CH_3$), 0.70 ($34CH_3$, $39CH_3$). $^{13}C\{^1H\}$-NMR (150.92 MHz, δ (assignment), CD_3CN): 220.23 (11C), 185.06 (1C), 130.58 (19C), 123.57 (18C), 107.52 (21C), 100.00 (17C), 89.20 (24C), 77.88

(29CH), 76.99 (3C), 76.87 (13C), 75.11 (25CH), 72.12 (7C), 70.80 (28C), 69.24 (9C), 67.46 (20C), 56.55 (12C), 51.62 (2C), 49.91 (10C), 41.48 (16C), 39.16 (15C), 38.10 (22C), 36.76 (8C), 33.44 (14C), 33.15 (23C), 32.86 (31C), 29.57 (27C), 28.94 (6C), 27.76 (33C), 27.43 (5C), 24.17 (41C), 20.72 (4C), 20.42 (26C), 17.87 (35C), 16.29 (34C), 16.20 (36C), 15.12 (30C), 13.31 (37C), 12.94 (38C, 42C), 11.27 (40C), 7.18 (39C), 6.87 (32C).

2.2.2 Synthesis of Salinomycin Complexes with Co(II) (**1**), Ni(II) (**2**), Cu(II) (**3**), Zn(II) (**4**)

The addition of a solution of corresponding metal(II) salt to a solution of sodium salinomycin at metal-to-ligand molar ratio = 1:1 or 2:1 results in the formation of complex compounds with a general formula of $[M(Sal)_2(H_2O)_2]\cdot nH_2O$ (M = Co, **1**; Ni, **2**; Cu, **3**; Zn, **4**; n = 0 or 2). The reactions occur in MeOH or in solvent mixtures (MeCN:MeOH = 1:10, H_2O: MeCN:MeOH = 1:1:10, H_2O:MeCN = 1:10). The new complexes are sparingly soluble in water and possess particular solubility in MeOH, MeCN, EtOH, $CHCl_3$, DMSO and hexane.

Generally, the metal salt ($Co(NO_3)_2\cdot 6H_2O$, $Ni(NO_3)_2\cdot 6H_2O$, $Cu(CH_3COO)_2\cdot H_2O$, $Zn(NO_3)_2\cdot 6H_2O$) (0.5 mmol dissolved in 2 mL H_2O) was slowly added to a solution of SalNa (0.25 mmol, 193 mg, in 22 mL mixed solvent). The reaction mixture was stirred for 15 min to produce the corresponding complexes **1-4** which were isolated after concentration of the initial solutions at room temperature as pink **(1)**, green **(2)**, blue **(3)** and white **(4)** solids. The solid phases were filtered off, washed with MeCN and dried over P_4O_{10}.

$[Co(Sal)_2(H_2O)_2]\cdot 2H_2O$ **(1):** Yield 163 mg, 80%. Anal. Calcd. for $C_{84}H_{146}O_{26}Co$ (MW = 1631.0): H, 9.02; C, 61.86; Co, 3.61. Found: H, 8.85; C, 61.81; Co, 3.68%.

$[Ni(Sal)_2(H_2O)_2]\cdot 2H_2O$ **(2):** Yield 163 mg, 80%. Anal. Calcd. for $C_{84}H_{146}O_{26}Ni$ (MW = 1631.8): H, 9.02; C, 61.87; Ni, 3.60. Found: H, 8.69; C, 61.91; Ni, 3.21%.

$[Cu(Sal)_2(H_2O)_2]\cdot 2H_2O$ **(3):** Yield 150 mg, 73%. Anal. Calcd. for $C_{84}H_{146}O_{26}Cu$ (MW = 1635.5): H, 9.00; C, 61.68; Cu, 3.89. Found: H, 8.88; C, 61.44; Cu, 3.67%.

$[Zn(Sal)_2(H_2O)_2]$ **(4):** Yield 170 mg, 85%. Anal. Calcd. for $C_{84}H_{142}O_{24}Zn$ (MW = 1601.4): H, 8.94; C, 63.00; Zn, 4.08. Found: H, 8.58; C, 62.65; Zn, 3.77%.

2.3 Bactericidal Activity Assay

The antibacterial activity of compounds studied was evaluated using the double layer agar hole diffusion method described in details in Refs. [23-27]. The microorganisms *Bacillus subtilis* NBIMCC 1709 (ATCC 6633) *Bacillus cereus* NBIMCC 1085 (FDA strain PCI 213, *Bacillus mycoides*) and *Micrococcus luteus* NBIMCC 159 (FDA strain PCI 1001, *Sarcina lutea*) were obtained from the national bank for industrial microorganisms and cell cultures (nbimcc, Bulgaria).

2.4 Cell Lines, Culture Conditions and Cytotoxicity Assessment

The human tumor cell lines BV-173 (chronic myeloid leukemia in pre-B cell blast crisis), K-562 (chronic myeloid leukemia) and SKW-3 (a KE-37 derivative; T-cell leukemia) were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ GmbH, Braunschweig, Germany). The cells were cultured in controlled environment - 90% RPMI-1640 medium supplemented with 10% fetal bovine serum, in culture flasks at 37 ^oC in an incubator BB 16-Function Line Heraeus (Kendro, Hanau, Germany) with humidified atmosphere and 5% CO_2. Cells were kept in *log* phase by supplementation with fresh medium after removal of cell suspension aliquots, two or three times a week.

The cellular viability was assessed using the standard MTT-dye reduction assay as described by Mosmann [41] with minor modifications [42]. Exponentially growing cells were seeded in 96-well flat-bottomed microplates (100 μL/well) at a density of 1×10^5 cells/mL and after 24 h incubation at 37 °C they

were exposed to various concentrations of the tested compounds (solved in DMSO) for 72 h. For each concentration a set of at least 8 wells were used. After the exposure period 10 μL aliquots of MTT solution (10 mg/mL in PBS) were added to each well. Thereafter the microplates were incubated for 4 h at 37 °C and the MTT-formazan crystals formed were dissolved through addition of formic acid (5% in 2-propanol, 100 μL/well). The absorption at 580 nm was measured using a LabeximLMR-1 microplate reader. Cell survival fractions were calculated as a percentage of the untreated control.

3. Results and Discussion

3.1 Chemistry

In the present study we report the results on preparation of new complexes of salinomycin with transition metal ions of Co(II) (**1**), Ni(II) (**2**), Cu(II) (**3**) and Zn(II) (**4**) and their characterization by various spectroscopic methods. The complexes **1-4** isolated in solid state using sodium salinomycin as a starting compound do not contain sodium ions in contrast to sodium monensin [23, 25] SalNa forms complex species of different composition with the divalent cations studied. These findings are in agreement with previously reported data on selectivity of both antiobiotics toward sodium ions. Unlike monensin, known as a sodium ionophore, salinomycin is mentioned as a potassium ionophore and forms weaker complex species with Na^+ ions [4]. The divalent metal ions abstract sodium ions from the cavity of salinomycin causing formation of homometallic mononuclear complexes.

In order to obtain crystals suitable for X-ray single crystal diffraction to refine the solid state structure of the new metal(II) compounds a variety of experimental conditions has been tested. Crystallization of **1-4** from both single solvents (MeOH, MeCN, DMSO, hexane) and multi-solvent systems (H_2O/MeCN; MeOH/MeCN; THF/hexane) leads to the formation of thin plate-like crystals. Change of the crystallization temperature range did not affect the quality of the crystals obtained. Our attempts to grow single crystals from the newly prepared complexes are in line with previous observations that in contrast to other polyether ionophores, salinomycin and its derivatives do not easily yield crystals suitable to perform X-ray structure analysis. Since 1975 only two single crystal structures of sodium salinomycin and salinomycin p-iodophenacyl ester have been refined [43, 44]. The structure characterization of complexes **1-4** was evaluated using IR and FAB-MS spectroscopies; in addition the Cu(II) complex **3** was analyzed by EPR (electronic paramagnetic resonance), and the properties of the diamagnetic Zn(II) complex **4** were evaluated using NMR spectroscopy.

*3.2 IR and FAB-MS Analysis of SalNa, SalH and Complexes **1-4***

The broad band at 3,300 cm^{-1} in the IR spectrum of sodium salinomycin is characteristic for the stretching vibrations of hydroxyl groups engaged in intramolecular hydrogen bonds. The presence of band at 1,710 cm^{-1} is indicative for the stretching vibrations of carbonyl group. Bands observed at 1,550 cm^{-1} and 1,400 cm^{-1} due to the presence of carboxylate anion are assigned to its asymmetric and symmetric stretching vibrations, respectively, and confirm that the carboxylic function of salinomycin is deprotonated. The last two bands cannot be observed in the corresponding IR spectrum of salinomycinic acid.

The IR spectra of new divalent metal complexes of salinomycin **1-4** show similar absorbance suggesting that all compounds isolated are isostructural. The broad band at 3,450 cm^{-1} observed in the IR spectra of complexes **1-4** is attributed to the presence of water molecules in the composition of metal(II) compounds. The shift of the band responsible for the stretching vibrations of carbonyl group towards lower frequencies (1,690 cm^{-1}) compared to the IR spectrum of SalNa is most likely due to its participation in additional weak bonds of intramolecular origin. The bands at 1,550 cm^{-1}

and 1,400 cm^{-1} are assigned to asymmetric ($\nu(CO_2)_{assym}$) and symmetric ($\nu(CO_2)_{sym}$) stretching vibrations of the carboxylate anion and their presence confirms that the carboxylic group in the complexes remains deprotonated. The value of $\Delta\nu$ = 150 cm^{-1} ($\Delta\nu = \nu(CO_2)_{assym} - \nu(CO_2)_{sym}$) indicates that the carboxylate function reacts with the metal(II) center in a monodentate coordination mode [45]. It has been reported that monensin also forms mononuclear complexes with some divalent biometal ions where the carboxylate function is bound in the same manner [24, 26, 27].

The FAB-MS data obtained for complexes **1-4** are summarized in Fig. 2. The presence of species assigned to ions of $[M(C_{42}H_{69}O_{11})]^+$ and of $[M_2(C_{42}H_{67}O_{11}]^+$ (M = Co, Ni, Cu, Zn) is observed in the FAB-MS spectra of all complex compounds. The peak at m/z 773.6 recorded in the FAB-MS spectra of **1-4** is attributed to the presence of $[(C_{42}H_{70}O_{11})Na]^+$. It must be noted that the same peak is also observed in the mass spectrum of salinomycinic acid resulting from its relatively high affinity to bind sodium ions originating from the glass and/or matrix used (nitrobenzylalcohol) [46].

*3.3 Magnetic Properties of Complexes **3** and **4***

The X-band EPR spectrum of the paramagnetic Cu(II) complex **3** recorded in the solid state at room temperature (293 K) is shown in Fig. 3. The spectrum of **3** consists of a signal with an axial symmetry and a resolved hyperfine structure. The EPR parameters are as follows: $g_{\|} = 2.310$, $g_{\perp} = 2.046$, $A_{\|} = 149 \times 10^{-4}$ cm^{-1} and $A_{\perp} \leq 7 \times 10^{-4}$ cm^{-1}. These values are characteristic for mononuclear Cu(II)-containing species possessing

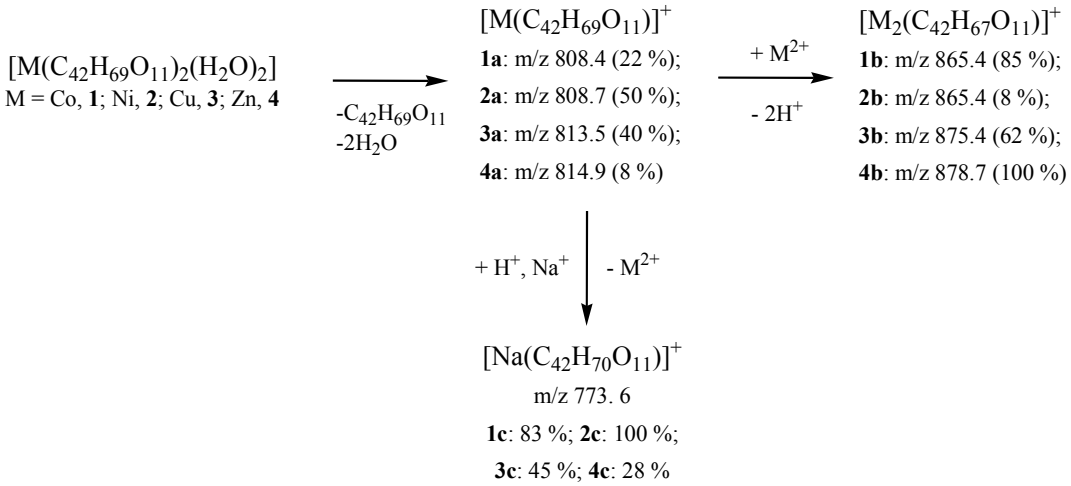


Fig. 2 Main molecular ions of complexes 1-4 observed in the corresponding FAB-MS spectra.

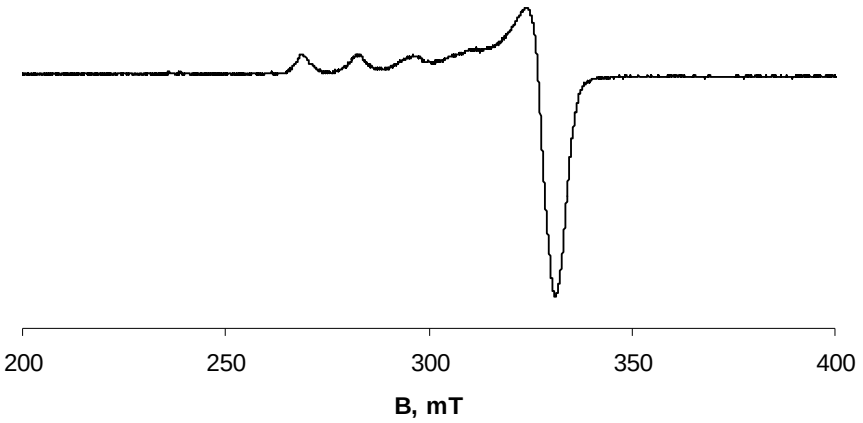


Fig. 3 The EPR spectrum of complex 3 (solid state, 273 K).

a distorted tetragonal symmetry and are in agreement with the structure suggested for divalent metal complexes reported in the present paper. The low solubility of **3** in common solvents did not allow the performing of EPR analysis in solution.

The ^{1}H and ^{13}C resonances of sodium salinomycin, of salinomycinic acid and of the diamagnetic Zn(II) complex **4** were assigned using one- and two-dimensional NMR techniques. The proton and carbon-13 chemical shifts of SalH and SalNa in acetonitrile-d_3 are unambiguously assigned and presented in Section 2 (Experimenta) using the numbering shown in Fig. 1a.

The assignment of the NMR signals for both compounds is in a good agreement with the literature data [8, 47, 48]. Deprotonation and coordination of the carboxylic function is responsible for the main observed difference of the ^{13}C-1 NMR chemical shifts between salinomycinic acid and sodium salinomycin. Various smaller upper- and downfield shifts observed in the spectra of SalNa compared to SalH show that additional conformational changes take place in the structure of the sodium complex in solution.

The spectroscopic data for the zinc(II) complex **4** indicate the presence of at least two exchanging species in the NMR spectra both in $CDCl_3$ and acetonitrile-d_3. NOESY spectra manifest that the main signals should be assigned to monomer species with chemical shift values in close correspondence to the values of salinomycinic acid under the same registration conditions. Most likely complex **4** undergoes dissociation in these solvents, but evaluation of the behaviour of complex **4** in solution necessitates a more detailed NMR investigation, a challenge, which we hope to report in the near future.

*3.4 Structure of Complexes **1-4***

At present we are not able to discuss X-ray single crystal diffraction data for the mononuclear complexes of salinomycin with ions of Co(II), Ni(II), Cu(II) and Zn(II) (**1-4**). However, based on spectral studies and elemental analysis data, as well as on the experience, gained studying the complexes of monensin, we suggest that the metal(II) center in the new divalent metal(II) disalinomycinates is placed in an octahedral environment forming complexes of composition $[M(Sal)_2(H_2O)_2]\cdot nH_2O$ (n = 0 or 2) (Fig. 4). Two salinomycin monoanions react with metal(II) ions in the equatorial plane of the complexes via terminal deprotonated carboxylic group and one of the secondary hydroxyl groups, both situated at the opposite ends of the ligand molecule. Two water molecules occupy the axial positions in the octahedron and their participation in various intramolecular hydrogen bonds supports the pseudo-cyclization of salinomycin similarly to the metal(II) dimonensinates which structures were refined in the solid state [24, 26, 27]. The elemental analysis data revealed that an

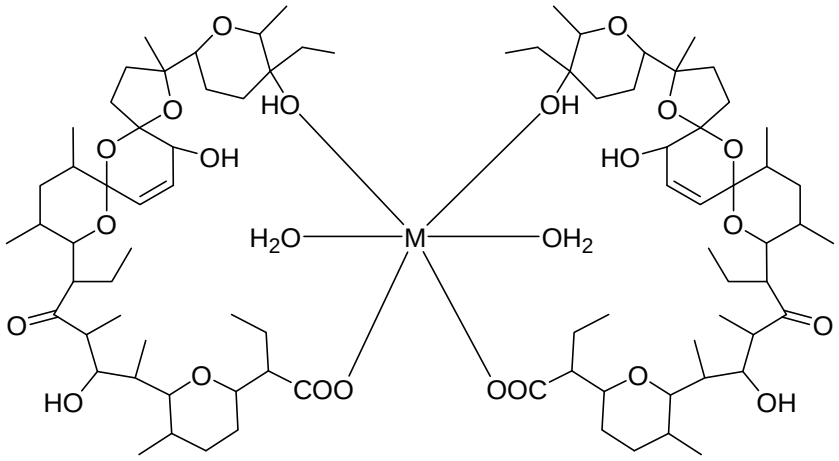


Fig. 4 Proposed structure of biometal(II) complexes of salinomycin.

additional inclusion of two crystallization water molecules is observed in the complexes **1-3,** which do not affect the main coordination mode of salinomycin.

*3.5 Biological Properties of SalH, SalNa and Complexes **1-4***

3.5.1 Antibacterial Activity

The bactericidal activity of acidic and sodium forms of salinomycin, new complexes **1-4** and corresponding metal(II) salts was tested against three Gram(+)-microorganisms. The data on the MIC (minimum inhibitory concentration) of compounds studied are summarized in Table 1. The metal salts have shown to possess no significant activity against the bacteria strains in the concentration range studied from 0.25 μg/mL to 1 mg/mL.

Complexes **1-4** display two- to twenty-fold higher antimicrobial activity against the test microorganisms in comparison to SalH and SalNa, respectively. These data confirm that the antibacterial effect of polyether ionophores can be improved via complexation reactions with divalent biometal ions. Cobalt(II) (**1**) and zinc(II) (**4**) disalinomycinates have shown the strongest bactericidal activity with MIC varying from 1 to 5 μM and from 1 to 10 μM, respectively. It should be mentioned that the bacteria strain of *B. cereus (B. mycoides)* is the most sensitive among the studied microorganisms towards the action of salinomycins and complexes **1-4**. The results presented in Table 1 are in agreement with the suggestion reported previously [23-27] that the bactericidal activity of metal complexes of polyether ionophorous antibiotics depends both on the bacterial strain and on the nature of the coordinated metal (II) ion. At the same time our preliminary studies on an acute toxicity of new salinomycin complexes have demonstrated that some divalent metal disalinomycinates are less toxic than sodium salinomycin (V. Atanasov, R. Zhorova, I.N. Pantcheva, J. Ivanova, M. Mitewa, L. Tancheva, unpublished results).

The findings that metal (II) complexes of polyether ionophores improve the antimicrobial activity and decrease the toxicity of antibiotics by themselves refer to the possible application of these new species as potential drugs in veterinary medicine.

3.5.2 Antitumor Activity of Salinomycins and Complexes **1-4**

Due to the high efficacy of salinomycin killing cancer stem cells and apoptosis resistant cancer cells [29-40], it is of great importance to evaluate the influence of metal (II) ions on the antitumor properties of the antibiotic. The cytotoxicity of SalH, SalNa, the four novel complexes **1-4** and the corresponding metal (II) salts was investigated in a panel of three human tumor cell lines after 72 h continuous exposure. The data were fitted to sigmoidal dose response curves (Fig. 5) and the equieffective inhibitory concentration value (IC_{50}, μM) was derived thereof using the non-linear regression analysis (Table 2).

As can be seen from the concentration response curves (Fig. 5), SalH, SalNa and the corresponding new coordination compounds possess strong concentration dependent cytotoxic effects, leading to total eradication of viable cells at low micromolar

Table 1 MIC (minimum inhibitory concentration) of salinomycins and metal(II) disalinomycinates expressed as [μg/mL] and [μM].

Bacteria / Compound	*B. subtillis*		*B. cereus*		*M. luteus*	
	μg/mL	μM	μg/mL	μM	μg/mL	μM
SalH	32	43	16	21	32	43
SalNa	16	20	8	10	16	20
1	8	5	2	1	8	5
2	16	10	8	5	16	10
3	16	10	4	2	16	10
4	16	10	2	1	16	10

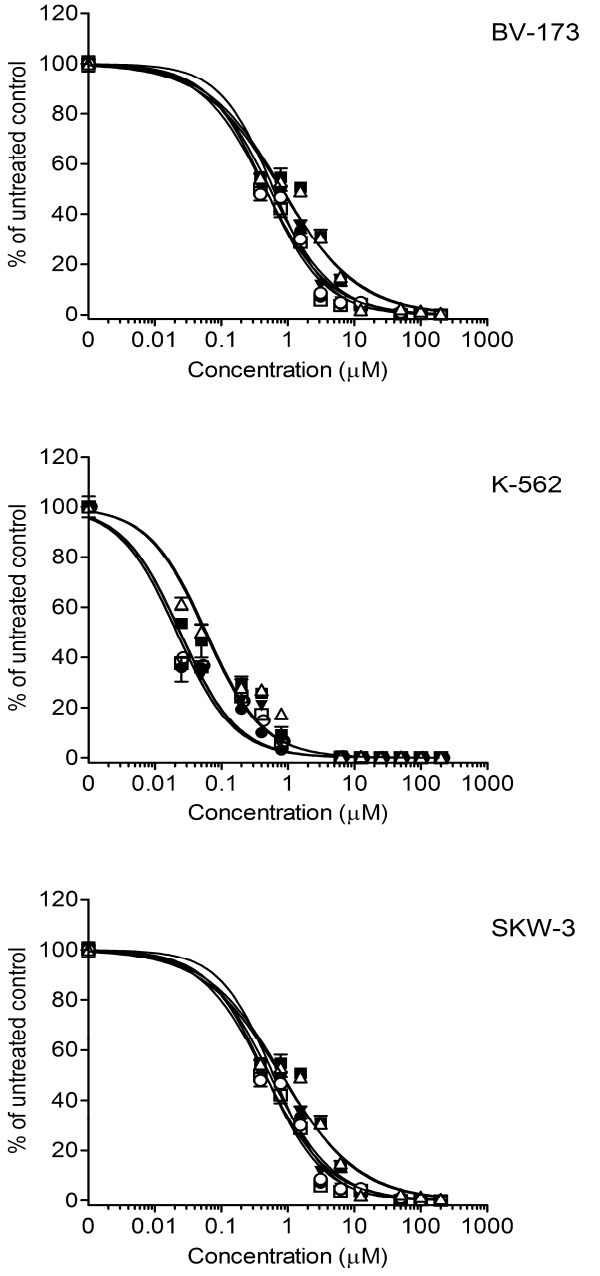


Fig. 5 Cytotoxicity of SalH (open triangles), SalNa (solid squares) and the corresponding metal(II) complexes as follows: 1 (open circles), 2 (solid triangles), 3 (open squares) and 4 (solid circles) against human tumor cell lines (72 h continuous exposure, MTT-dye reduction assay).

concentrations. Based on the juxtaposition of the IC_{50} values obtained, the complexes with Co(II) (**1**) and Cu(II) (**3**) proved to exert superior activity as compared to the Ni(II) (**2**) and Zn(II) (**4**) compounds and display four-fold higher cytotoxicity than the initial compounds. In our hands the metal salts (Co(II), Ni(II) and Zn(II) nitrates, and Cu(II) acetate) proved to be far less active causing 50% decrease in cellular viability at substantially higher concentrations as compared to the corresponding salinomycin coordination compounds. Invariably the myeloid cell lines K-562 and BV-173 showed higher chemosensitivity to SalH, SalNa and the tested complexes with nanomolar IC_{50} values as compared to the lymphoid T-cell leukemia derived cell line SKW-3.

4. Conclusions

Four novel complexes of salinomycin with divalent metal ions of Co(II), Ni(II), Cu(II) and Zn(II) are synthesized and characterized. The new compounds are isostructural and are of a composition $[M(Sal)_2(H_2O)_2]\cdot nH_2O$ (n = 0 or 2). It is shown that if sodium salinomycin is used as a starting compound, the sodium ion is replaced by the divalent cation. Corroborated by the spectral studies performed we suggest that the metal (II) center is placed in an octahedral environment realized by the coordination of two salinomycin monoanions and by two water molecules. Salinomycin reacts with metal (II) ions in a bidentate coordination mode via a deprotonated terminal carboxylic group and one of the secondary hydroxyl groups, both located at the opposite ends of the ligand molecule. The carboxylate and hydroxyl oxygen atoms occupy the equatorial places in the metal (II) octahedron. Two water molecules placed at the axial positions stabilize the pseudo-cyclization of salinomycin through intramolecular hydrogen bonds.

The novel metal (II) complexes possess enhanced antibacterial activity as compared to acidic and sodium forms of salinomycin against Gram-positive microorganisms, especially towards the strain of *B. cereus.* The new coordination compounds proved to be exceptionally active cytotoxic agents against human leukemic cell lines within nanomolar IC_{50} values. All coordination compounds, especially the complexes of Co(II) and Cu(II), are more effective agents compared

Table 2 Cytotoxic activity of SalH, SalNa, complexes 1-4 and the corresponding metal(II) salts against human tumor cell lines expressed as IC_{50} [μM].

Compound	IC_{50} (μM)		
Cell line	BV-173	K-562	SKW-3
SalH	0.052	0.059	0.830
SalNa	0.033	0.056	0.791
Complex **1**	0.012	0.021	0.461
Complex **2**	0.034	0.024	0.544
Complex **3**	0.012	0.022	0.468
Complex **4**	0.061	0.024	0.612
$Co(NO_3)_2 \cdot 6H_2O$	31.27	21.71	14.74
$Ni(NO_3)_2 \cdot 6H_2O$	125.60	19.70	107.00
$Cu(CH_3COO)_2 \cdot H_2O$	67.56	10.83	51.89
$Zn(NO_3)_2 \cdot 6H_2O$	64.71	52.81	73.34

to salinomycinic acid and sodium salinomycin despite the detection of some variability in the responsiveness of the different cell lines. To assess the therapeutic index of new biometal(II) compounds, further detailed studies on their toxicity are required.

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