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# Cd(II) and Pb(II) complexes of the polyether ionophorous antibiotic salinomycin

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## Abstract

**Background:** The natural polyether ionophorous antibiotics are used for the treatment of coccidiosis in poultry and ruminants. They are effective agents against infections caused by Gram-positive microorganisms. On the other hand, it was found that some of these compounds selectively bind lead(II) ions in *in vivo* experiments, despite so far no Pb(II)-containing compounds of defined composition have been isolated and characterized. To assess the potential of polyether ionophores as possible antidotes in the agriculture, a detailed study on their *in vitro* complexation with toxic metal ions is required. In the present paper we report for the first time the preparation and the structure elucidation of salinomycin complexes with ions of cadmium(II) and lead(II).

**Results:** New metal(II) complexes of the polyether ionophorous antibiotic salinomycin with Cd(II) and Pb(II) ions were prepared and structurally characterized by IR, FAB-MS and NMR techniques. The spectroscopic information and elemental analysis data reveal that sodium salinomycin (SalNa) undergoes a reaction with heavy metal(II) ions to form  $[\text{Cd}(\text{Sal})_2(\text{H}_2\text{O})_2]$  (**1**) and  $[\text{Pb}(\text{Sal})(\text{NO}_3)]$  (**2**), respectively. Abstraction of sodium ions from the cavity of the antibiotic is occurring during the complexation reaction. Salinomycin coordinates with cadmium(II) ions as a bidentate monoanionic ligand through the deprotonated carboxylic moiety and one of the hydroxyl groups to yield **1**. Two salinomycin anions occupy the equatorial plane of the Cd(II) center, while two water molecules take the axial positions of the inner coordination sphere of the metal(II) cation. Complex **2** consists of monoanionic salinomycin acting in polydentate coordination mode in a molar ratio of 1: 1 to the metal ion with one nitrate ion for charge compensation.

**Conclusion:** The formation of the salinomycin heavy metal(II) complexes indicates a possible antidote activity of the ligand in case of chronic/acute intoxications likely to occur in the stock farming.

## Background

The natural polyether ionophorous antibiotics produced by *Streptomyces spp.* are used for the treatment of coccidiosis in poultry (chickens, turkeys and quail) and ruminants (cattle, sheep and goats). They are agents controlling ketosis and bloat in cattle and are applied as growth promoter feed additives in cattle and sheep. The polyether ionophores are mainly effective against Gram-positive bacteria, interfering with ion flux across bacterial membranes thus causing reallocation of bacterial energy resources to maintain cellular pH and ion balance. Effects on feed conversion efficiency arise from the ability of antibiotics to shift rumen fermentation

towards the more energetically efficient propionate pathway, to reduce methane production and to increase nitrogen retention by reducing dietary protein deamination and urinary ammonia excretion [1-9].

From chemical point of view these compounds are polyether derivatives of monocarboxylic acids and represent potential ligands able to bind metal cations due to the presence of oxygen atoms of various types (carboxylic, ether, hydroxyl) in the antibiotic molecule. Monensin, salinomycin, narasin etc. are best known as monovalent polyether ionophores for their affinity to complex with alkali ions,  $\text{Ag}^+$  and  $\text{Tl}^+$  [10-22]. Recently we have shown that they react also with metal(II) ions to form complexes of different composition and of various coordination mode of the ligands [23-28]. Due to their enhanced cytotoxicity against Gram-positive microorganisms as compared to

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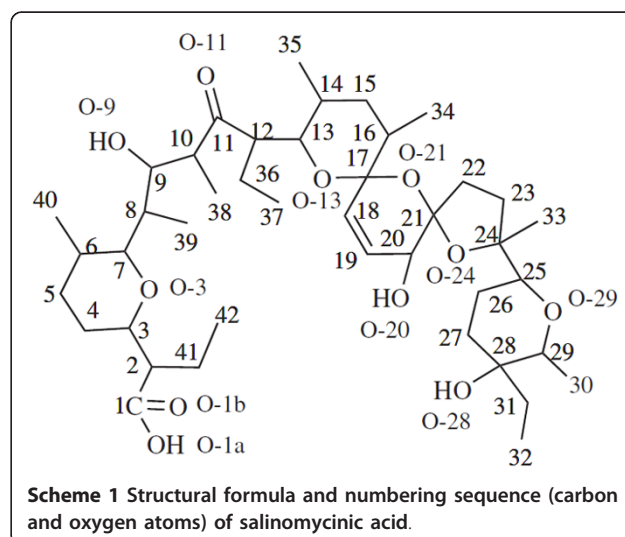
the non-coordinated compounds, the complexes of monensin and salinomycin with biometal(II) ions could be discussed as potential antibacterial agents [23-27]. These new compounds (especially the salinomycin complexes) exert also a pronounced antitumor activity against cell lines established from various malignancies (Ivanova J, Pantcheva IN, Zhorova R, Momekov G, Simova S, Stoyanova R, Zhecheva E, Ivanova S, Mitewa M: Synthesis, spectral properties, antibacterial and antitumor activity of salinomycin complexes with Co(II), Ni(II), Cu(II) and Zn(II) transition metal ions, unpublished), [29,30].

On the other hand, it was found that some polyether ionophores as monensin, ionomycin and nigericin selectively bind lead(II) although the Pb(II)-containing complex species were not isolated and characterized [31-33]. It has been shown that monensin significantly improves the effectiveness of the traditional antidote DMSA (dimercaptosuccinic acid) in reducing Pb(II) concentration in brain and bones of rats, intoxicated with a Pb(II) salt [34]. We recently reported that monensinic acid (MonH) reacts with Cd(II) and Hg(II) ions to form stable complex compounds with different coordination mode of the ligand [28]. Our studies with an animal model (ICR male mice) proved the ability of monensin to act as an efficient antidote in cases of subchronic cadmium intoxication. In contrast to DMSA and EDTA, monensin significantly reduces cadmium(II) concentration in mice organs, subjected to subchronic intoxication, when applied as tetraethyl ammonium salt, without affecting the homeostasis of Cu(II) and Zn(II) ions (Ivanova J, Gluhcheva Y, Kamenova K, Ganeva S, Mitewa M: Tetraethyl ammonium salt of monensinic acid decreases cadmium concentration in organs of mice subjected to subchronic cadmium intoxication, unpublished). These findings prove the necessity of an extensive study on *in vitro* complexation of polyether ionophorous antibiotics with toxic metal ions. A preliminary assessment of their potential as chelating agents will considerably facilitate the treatment of intoxication with toxic metal ions. To the best of our knowledge until now no information on the ability of salinomycin to form complexes with Cd(II), Pb(II) and Hg(II) has been published. In the present paper we report for the first time the preparation and the structure characterization of salinomycin complexes with ions of cadmium(II) and lead(II). As a starting material the commercially available sodium form of salinomycin (SalNa) was used. Extensive NMR data in CDCl<sub>3</sub>, DMSO-d<sub>6</sub> and in isotropic bicelles have been published for this compound [35-37]. The new complexes isolated are analyzed and characterized by IR, FAB-MS, NMR spectroscopies and elemental analysis data.

## Results and Discussion

Complexation of salinomycin (Scheme 1) with ions of Cd(II) and Pb(II) proceeds at mild conditions in the absence of additives and at metal-to-ligand molar ratio = 2: 1. The isolation and characterization of the new compounds confirm the ability of polyether ionophores to react with toxic metal(II) cations and to produce mononuclear complex species. Contrary to sodium monensin (MonNa), which forms heterometallic complexes of composition [M(MonNa)<sub>2</sub>Cl<sub>2</sub>] (M = Co, Mn, Cu) [23,25], sodium salinomycin (SalNa) produces only homometallic complexes [M(Sal)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>] (M = Co, Ni, Cu, Zn) where the sodium ions are abstracted from the ligand cavity (Ivanova J, Pantcheva IN, Zhorova R, Momekov G, Simova S, Stoyanova R, Zhecheva E, Ivanova S, Mitewa M: Synthesis, spectral properties, antibacterial and antitumor activity of salinomycin complexes with Co(II), Ni(II), Cu(II) and Zn(II) transition metal ions, unpublished).

Despite the use of different techniques (slow evaporation of single solvents; slow evaporation of multi solvent systems, slow diffusion method) our efforts to grow crystals from the new compounds were not successful. Only plate-like crystals were obtained which were not appropriate for a refinement of the structures of the new complexes by X-ray diffraction method. This is not surprising since literature data indicate that in contrast to other polyether ionophorous antibiotics, salinomycin and its metal derivatives do not easily crystallize. It should be pointed out that up to now only two crystal structures of salinomycin derivatives are published [11,20]. In order to become acquainted with the structure of the new complexes of this compound with Cd(II) (1) and Pb(II) (2) ions their properties were studied by IR, NMR and FAB-MS spectroscopies.



Due to the similar coordination mode of the ligand in its sodium and in the presently investigated metal forms, the main bands of the coordinated groups in the IR spectra of complexes **1** and **2** do not significantly change as compared to the spectrum of sodium salinomycin. The stretching vibrations of the hydroxyl groups in the spectra of **1** and **2** are both observed at  $3380\text{ cm}^{-1}$  in analogy to the broad band at  $3400\text{-}3300\text{ cm}^{-1}$  in the spectrum of SalNa. The two bands at  $1550\text{ cm}^{-1}/1410\text{ cm}^{-1}$  (**1**) and at  $1520\text{ cm}^{-1}/1410\text{ cm}^{-1}$  (**2**) for the deprotonated carboxylic function are assigned to the asymmetric and symmetric COO-vibrations, respectively. The new intensive band at  $1300\text{ cm}^{-1}$  in the spectrum of the Pb(II) complex (**2**) can be attributed to the presence of nitrate ions originating from the initial lead(II) salt.

The structure of complexes **1** and **2** in solution is corroborated by the unambiguous assignment of their proton and carbon-13 NMR spectra. The data obtained are compared with those observed for acidic (SalH) and sodium (SalNa) forms of salinomycin. The  $^1\text{H}$ -NMR

chemical shifts are listed in the Experimental Section;  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra are placed in Figure 1 and 2, respectively. Chemical shifts for carbon-13 atoms are presented in Table 1, following the numbering of salinomycin shown in Scheme 1.

Comparison of the proton and carbon-13 chemical shifts of the complexes with the data for SalH and SalNa confirm that both **1** and **2** remain intact in acetonitrile solution. Characteristic is the downfield  $^{13}\text{C}$  shift by *approx.* 7 ppm of the deprotonated carboxylic carbon atom. The chemical shifts of most carbon atoms in the four compounds do not change significantly and are in a narrow range of  $\pm 0.5$  ppm. However, several observed down- and upfield shifts of 1 to 3 ppm in the spectra of **1-2** indicate that the new compounds possess slightly different conformations in solution when compared to both salinomycinic acid and its sodium complex.

The FAB-MS spectra confirm the presence of toxic metal(II) ions in the corresponding complexes **1** and **2** (Figure 3). The spectrum of complex **1** contains eight

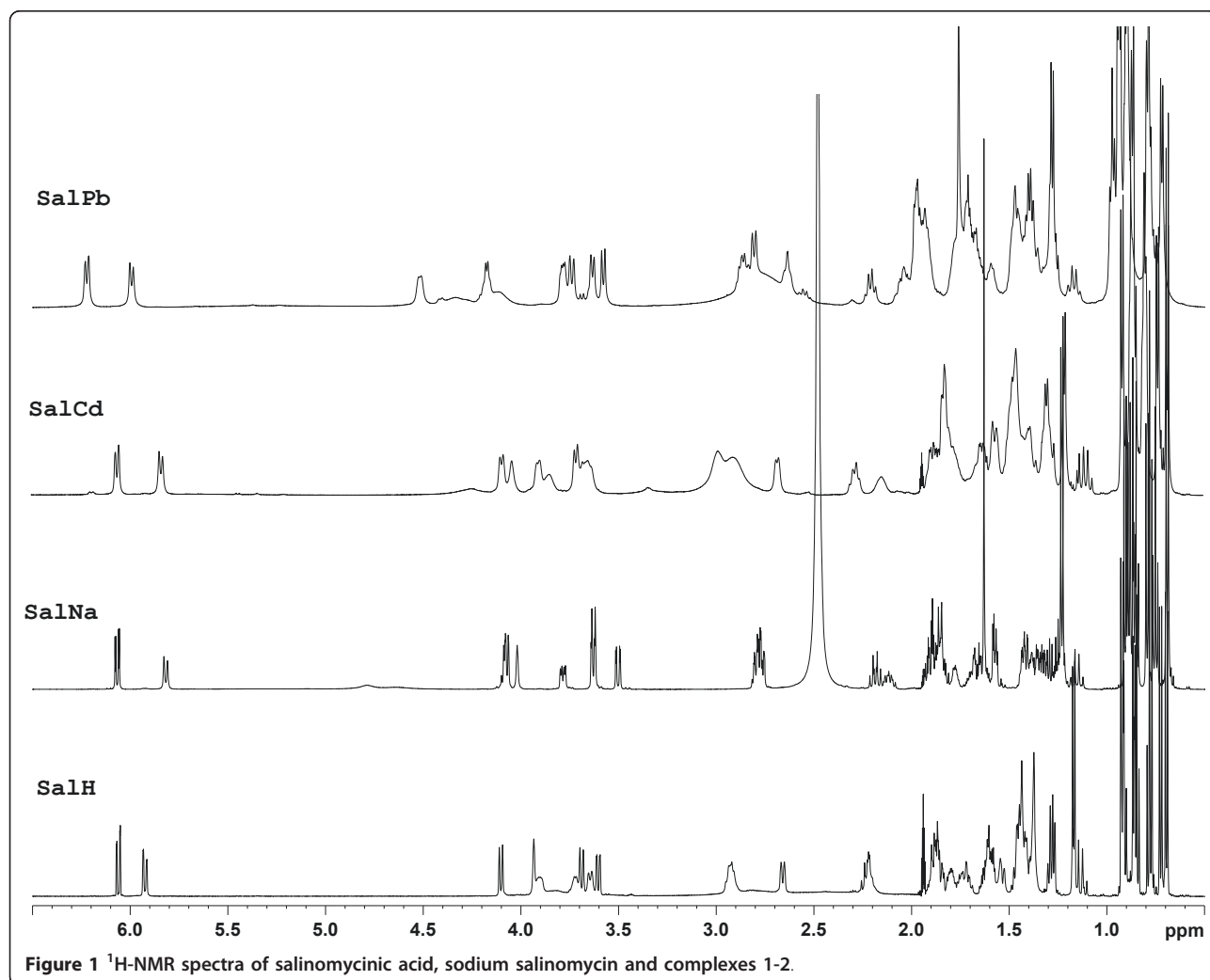
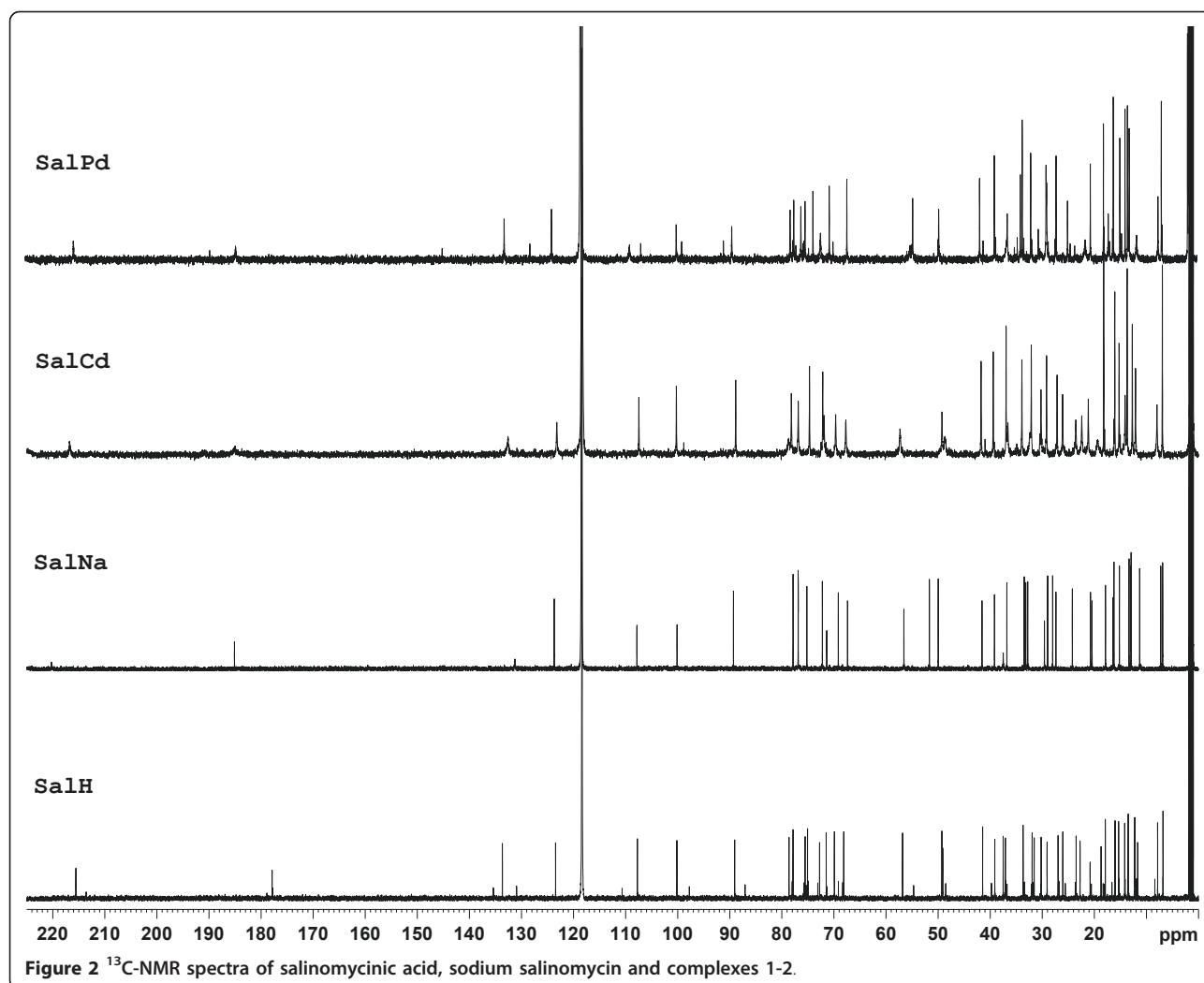


Figure 1  $^1\text{H}$ -NMR spectra of salinomycinic acid, sodium salinomycin and complexes 1-2.

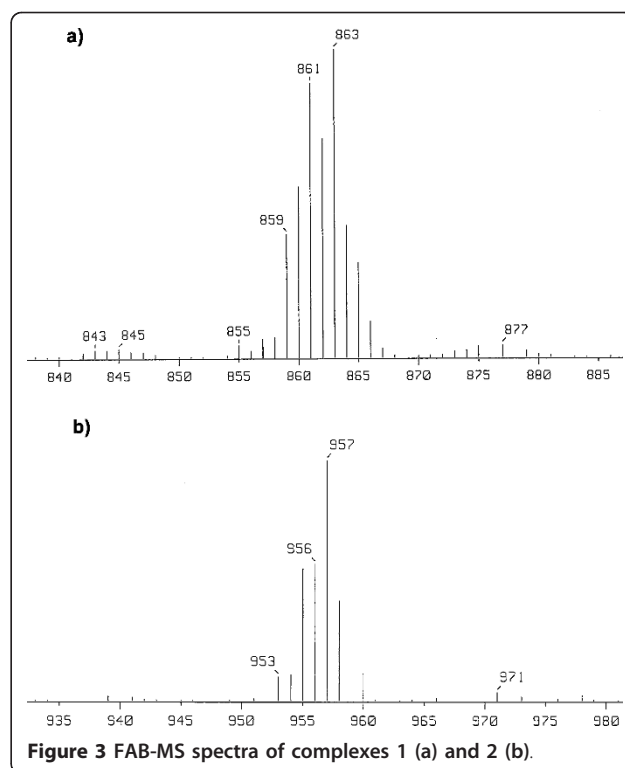


peaks assigned to the formation of  $[\text{C}_{42}\text{H}_{69}\text{O}_{11}\text{Cd}]^+$  (**1a**) (*calc.*  $m/z = 863$ , 100%;  $m/z = 861$ , 83.5%;  $m/z = 862$ , 67.7%;  $m/z = 860$ , 49.2%;  $m/z = 864$ , 42.4%;  $m/z = 859$ , 32.9%;  $m/z = 865$ , 30.9%;  $m/z = 866$ , 11.7%). The FAB-MS spectrum of complex **2** consists of peaks attributed to  $[\text{C}_{42}\text{H}_{69}\text{O}_{11}\text{Pb}]^+$  (**2a**) (*calc.*  $m/z = 957$ , 100%;  $m/z = 956$ , 46.7%;  $m/z = 955$ , 33.6%). The obtained isotopic patterns of **1** and **2** as shown in Figure 3 are consistent with the calculated peaks. It should be noted that the toxic metal(II) species are very stable in the gas phase and no formation of sodium-containing ions  $[\text{C}_{42}\text{H}_{70}\text{O}_{11}\text{Na}]^+$  was observed compared to the behaviour of other salinomycin complexes known up to now (Ivanova J, Pantcheva IN, Zhorova R, Momekov G, Simova S, Stoyanova R, Zhecheva E, Ivanova S, Mitewa M: Synthesis, spectral properties, antibacterial and anti-tumor activity of salinomycin complexes with Co(II), Ni(II), Cu(II) and Zn(II) transition metal ions, unpublished).

On the basis of the spectroscopic studies and elemental analysis evidence, it can be concluded that sodium salinomycin reacts with ions of Cd(II) and Pb(II) to form coordination compounds with different composition and structure. It was found that cadmium(II) ion reacts with two carboxylate anions of salinomycin, extracting sodium ions from the cavity of the starting compound. Salinomycin monoanions are bound to the metal(II) center in a bidentate coordination mode via terminal deprotonated carboxylic group and terminal hydroxyl group achieving head-to-tail cyclization. Most probably oxygen atoms O-1 (carboxylate) and O-28 (secondary hydroxyl) occupy the equatorial places in the inner coordination sphere of the metal(II) ion. Two axially located aqua molecules could stabilize the pseudocyclization of the complex. Cadmium(II) disalinomycinate (**1**) is expected to possess a distorted octahedral geometry similarly to the structures reported for the complexes of salinomycin with ions of Co(II), Ni(II), Cu

**Table 1**  $^{13}\text{C}$ -NMR chemical shifts (150 MHz, acetonitrile- $\text{d}_3$ ) of salinomycin acid (SalH), sodium salinomycin (SalNa) and complexes 1-2

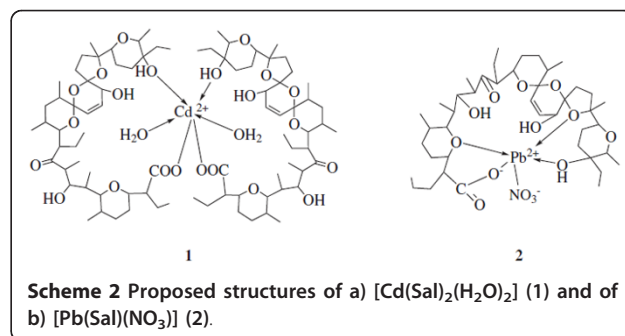
C-atom	SalH	SalNa	1	2
11C = O	215.53	220.23	216.77	215.75
1COOH	177.86	185.06	184.98	184.60
19CH =	133.65	130.58	132.59	133.03
18CH =	123.43	123.57	122.23	123.95
*21C	107.72	107.52	107.44	108.96
*17C	100.14	100.00	100.24	99.99
*24C	89.04	89.20	88.84	89.33
29CH	77.83	77.88	78.14	78.11
13CH	78.60	76.87	78.76	77.41
3CH	75.51	76.99	76.84	75.24
25CH	75.05	75.11	74.67	76.05
7CH	72.73	72.12	72.13	72.30
*28C	71.47	70.8	71.87	73.74
9CH	69.89	69.24	69.63	70.60
20CH	68.10	67.46	67.70	67.22
12CH	56.80	56.55	57.28	54.58
2CH	49.25	51.62	49.24	55.08
10CH	49.09	49.91	48.69	49.59
16CH	41.42	41.48	41.73	41.74
15CH <sub>2</sub>	39.09	39.16	39.39	38.90
22CH <sub>2</sub>	37.49	38.10	36.57	36.64
8CH	37.06	36.76	36.92	36.43
14CH	33.66	33.44	33.89	33.55
23CH <sub>2</sub>	31.53	33.15	32.37	33.91
31CH <sub>2</sub>	31.92	32.86	32.09	31.92
27CH <sub>2</sub>	30.19	29.57	30.24	30.47
6CH	29.06	28.94	29.15	28.97
33CH <sub>3</sub>	26.03	27.76	26.07	28.97
5CH <sub>2</sub>	26.94	27.43	27.13	27.02
41CH <sub>2</sub>	23.47	24.17	23.56	24.85
26CH <sub>2</sub>	22.72	20.42	22.41	20.47
4CH <sub>2</sub>	20.77	20.72	21.16	21.50
35CH <sub>3</sub>	17.86	17.87	18.19	17.95
36CH <sub>2</sub>	18.68	16.20	19.39	17.02
34CH <sub>3</sub>	15.99	16.29	16.07	16.06
30CH <sub>3</sub>	15.31	15.12	15.24	14.8
38CH <sub>3</sub>	13.47	12.94	13.70	13.34
37CH <sub>3</sub>	14.15	13.31	14.09	13.81
42CH <sub>3</sub>	12.20	12.94	12.71	13.01
40CH <sub>3</sub>	11.65	11.27	12.09	11.59
39CH <sub>3</sub>	7.83	7.18	7.97	7.48
32CH <sub>3</sub>	6.79	6.87	6.94	6.83



**Figure 3** FAB-MS spectra of complexes 1 (a) and 2 (b).

(II) and Zn(II) (Ivanova J, Pantcheva IN, Zhorova R, Momekov G, Simova S, Stoyanova R, Zhecheva E, Ivanova S, Mitewa M: Synthesis, spectral properties, antibacterial and antitumor activity of salinomycin complexes with Co(II), Ni(II), Cu(II) and Zn(II) transition metal ions, unpublished).

In contrast to Cd(II), Pb(II) ions react with sodium salinomycin, extracting sodium ions from the ligand cavity, but forming complex 2 of a composition  $[\text{Pb}(\text{Sal})(\text{NO}_3)]$ . The extremely low solubility of this compound in water suggests that the nitrate ion is most probably coordinated in the inner coordination sphere of the metal center. The registered downfield shifts for C1, C2,



**Scheme 2** Proposed structures of a)  $[\text{Cd}(\text{Sal})_2(\text{H}_2\text{O})_2]$  (1) and of b)  $[\text{Pb}(\text{Sal})(\text{NO}_3)]$  (2).

C23, C28 and C33, and upfield shifts for C12, C13 and C26 in the  $^{13}\text{C}$ -NMR spectrum of Pb(II) salinomycin as compared with the spectra of SalH and SalNa suggest coordination of the carboxylate oxygen O-1a, of the hydroxyl oxygen O-28 and the ether oxygens O-3 and O-24 to the Pb(II) ion. The coordination of nitrate anion at axial position in the inner coordination sphere of the metal center secures the overall neutral charge of the complex. It should be noted that the observed enhancements in the NOESY spectra of complexes **1-2** confirm the different metal-to-ligand stoichiometry. The positive NOE's for all signals in **2** indicate formation of 1:1 complex with a lower molecular mass while the negative NOE's observed for **1** corroborate the 1:2 stoichiometry, corresponding to a molecular mass higher than 1500 D.

The different complexation modes of the ligand to ions of Cd(II) and Pb(II) compared to the coordination of salinomycin to sodium ions is corroborated by the chemical shift differences for most carbon atoms in the corresponding  $^{13}\text{C}$ -NMR spectra. The proposed structures of complexes **1** and **2**, derived indirectly on the basis of their spectral properties are shown on Scheme 2a and Scheme 2b, respectively.

## Conclusions

Metal(II) complexes of salinomycin with ions of Cd(II) and Pb(II) were prepared and characterized in gas phase (FAB-MS), in solution (NMR) and in solid state (IR). The data reveal that sodium salinomycin forms homometallic mononuclear complexes, where sodium ions are abstracted from the cavity of the ligand. Two monoanions of salinomycin occupy four places in the inner coordination sphere of the Cd(II) ions in the complex **1** with a composition  $[\text{Cd}(\text{Sal})_2(\text{H}_2\text{O})_2]$  with two water molecules coordinated at axial positions. Contrary to Cd(II) disalinomycin, the complex of salinomycin with Pb(II) ions (**2**) is of a composition  $[\text{Pb}(\text{Sal})(\text{NO}_3)]$  and is in accordance with a square pyramidal geometry. The formation of  $[\text{Cd}(\text{Sal})_2(\text{H}_2\text{O})_2]$  and  $[\text{Pb}(\text{Sal})(\text{NO}_3)]$  complexes proceeds at mild conditions referring to the practical application of salinomycin as potential antidote in case of intoxications with toxic metal ions occurring in poultry and in ruminants.

## Experimental and Methods

### Physical measurements

Elemental analysis data (C, H, O) were obtained with VarioEL V5.18.0 Elemental Analyzer (Germany). The metal content was determined by AAS on a Perkin Elmer 1100 B (USA) apparatus using stock standard solution (1000 mg/mL, Merck). Working reference solutions were prepared after suitable dilution.

IR spectral analysis (in a nujol mull) was performed on a Specord 75-IR (Carl-Zeiss, Germany). FAB-mass analysis data were registered using JEOL JMS-700 (JEOL, Japan).  $^1\text{H}$  (600.13 MHz) and  $^{13}\text{C}$  (150.92 MHz) NMR spectra were acquired on an AVANCE AV600 II+ NMR spectrometer (Bruker, Germany). All spectra were recorded in acetonitrile- $d_3$  at room temperature ( $293.0 \pm 0.1^\circ$ ). The residual methyl solvent signal was used as an internal standard for the  $^1\text{H}$  (1.94 ppm) and  $^{13}\text{C}$  spectra (1.40 ppm). 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: cosygpmfqq, dipsi2etgpsi, hsqc-detgpsisp2.2, hmbcgpplndqf, roesyph.2, 2007). The chemical shift values of the protons with overlapping signals in the complexes have been determined from the HSQC spectra (Figures S1 and S2 [see Additional files 1 and 2]).

### Materials

All chemicals used were of reagent grade. Sodium salinomycin was supplied from Biovet Ltd. (Bulgaria). Cd  $(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$  and  $\text{Pb}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$  were purchased from Fluka (Switzerland). Acetonitrile (MeCN), MeOH and HCl were received from Merck (Germany). In all experiments deionized water was used.

### Synthesis of $[\text{Cd}(\text{C}_{42}\text{H}_{69}\text{O}_{11})_2(\text{H}_2\text{O})_2]$ (**1**)

To a solution of  $\text{C}_{42}\text{H}_{69}\text{O}_{11}\text{Na}$  (0.25 mmol, 193 mg in 2 mL  $\text{H}_2\text{O}$  and 20 mL  $\text{CH}_3\text{CN}$ )  $\text{Cd}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$  (0.5 mmol, 135 mg in 2 mL  $\text{H}_2\text{O}$ ) was slowly added. The colourless reaction mixture was stirred for 15 min. The solution was concentrated at room temperature (r.t.) for 7 days to produce white precipitates of composition  $[\text{Cd}(\text{C}_{42}\text{H}_{69}\text{O}_{11})_2(\text{H}_2\text{O})_2]$  (**1**). The same reaction occurs also in mixed solvent system MeCN: MeOH = 1:10. The solid phase was filtered off, washed with  $\text{H}_2\text{O}$  and dried at r.t. The complex is soluble in MeOH, MeCN,  $\text{CHCl}_3$  and is not soluble in  $\text{H}_2\text{O}$ . Yield 140 mg, 68%. Anal. Calcd. for  $\text{C}_{84}\text{H}_{142}\text{O}_{24}\text{Cd}$  (%): H, 8.68; C, 61.20; Cd, 6.82. Found: H, 8.14; C, 60.51; Cd, 6.95.  $^1\text{H}$ -NMR (600.13 MHz,  $\delta$  (assignment), acetonitrile- $d_3$ ) 6.06 (18CH), 5.84 (19CH), 4.09 (9CH), 4.04 (20CH), 3.90 (3CH), 3.85 (29CH), 3.72 (7CH), 3.65 (13CH), 3.63 (25 CH), 2.99 (10CH), 2.91 (2CH), 2.67 (12CH), 2.28 (22 $\text{CH}_2'$ ), 2.15 (23 $\text{CH}_2'$ ), 1.94 (36 $\text{CH}_2'$ ), 1.88 (22 $\text{CH}_2''$ ), 1.83 (4 $\text{CH}_2'$ , 5 $\text{CH}_2'$ , 6CH), 1.80 (23  $\text{CH}_2'$ ), 1.78 (14CH), 1.68 (26  $\text{CH}_2'$ ), 1.65 (27 $\text{CH}_2'$ ), 1.62 (16CH), 1.58 (27 $\text{CH}_2''$ ), 1.57 (26 $\text{CH}_2'$ ), 1.56 (15 $\text{CH}_2'$ ), 1.49 (8CH), 1.48 (4 $\text{CH}_2''$ ), 1.46 (33 $\text{CH}_3$ ), 1.43 (41 $\text{CH}_2'$ ), 1.40 (36 $\text{CH}_2''$ ), 1.39 (5 $\text{CH}_2''$ ), 1.31 (41 $\text{CH}_2''$ ), 1.30 (31 $\text{CH}_2$ ), 1.21 (30 $\text{CH}_3$ ), 1.09 (15 $\text{CH}_2''$ ), 0.91 (40 $\text{CH}_3$ ), 0.87 (35 $\text{CH}_3$ , 42 $\text{CH}_3$ ), 0.86 (32 $\text{CH}_3$ ), 0.80 (37 $\text{CH}_3$ , 38 $\text{CH}_3$ ), 0.73 (39 $\text{CH}_3$ ), 0.68 (34 $\text{CH}_3$ ).

### Synthesis of [Pb(C<sub>42</sub>H<sub>69</sub>O<sub>11</sub>)(NO<sub>3</sub>)] (2)

Pb(NO<sub>3</sub>)<sub>2</sub>·H<sub>2</sub>O (0.5 mmol, 165 mg) was dissolved in 4 mL H<sub>2</sub>O and was slowly mixed with a solution of sodium salinomycin (0.25 mmol, 193 mg in 22 mL mixed solvent (H<sub>2</sub>O: MeCN = 1: 10)) and the reaction mixture was stirred for 15 min at room temperature. The solvent was slowly evaporated (7 days) to give the complex [Pb(C<sub>42</sub>H<sub>69</sub>O<sub>11</sub>)(NO<sub>3</sub>)] (2) as white precipitates. The compound was washed with H<sub>2</sub>O and dried at room temperature. Yield 190 mg, 75%. Anal. Calcd. for C<sub>42</sub>H<sub>69</sub>O<sub>14</sub>NPb (%): H, 6.82; C, 49.50; N, 1.37; Pb, 20.33. Found: H, 6.20; C, 49.61; N, 1.20; Pb, 19.95. The complex is soluble in MeOH, MeCN, CHCl<sub>3</sub> and is not soluble in H<sub>2</sub>O. <sup>1</sup>H-NMR (600.13 MHz, δ (assignment), acetonitrile-d<sub>3</sub>) 6.19 (18CH), 5.96 (19CH), 4.49 (9CH), 4.34 (20CH), 4.14 (29CH), 3.76 (3CH), 3.71 (25CH), 3.60 (7CH), 3.55 (13CH), 2.84 (10CH), 2.77 (12CH), 2.61 (2CH), 2.17 (22CH<sub>2</sub>'), 2.04 (27CH<sub>2</sub>'), 2.02 (26CH<sub>2</sub>'), 1.95 (23CH<sub>2</sub>, 36CH<sub>2</sub>'), 1.93 (22CH<sub>2</sub>"'), 1.92 (5CH<sub>2</sub>'), 1.90 (4CH<sub>2</sub>'), 1.76 (6CH), 1.73 (33CH<sub>3</sub>), 1.71 (14CH), 1.66 (15CH<sub>2</sub>'), 1.62 (16CH), 1.57 (8CH), 1.47 (27CH<sub>2</sub>"'), 4.1CH<sub>2</sub>'), 1.45 (5CH<sub>2</sub>"'), 1.43 (41CH<sub>2</sub>'), 1.42 (26CH<sub>2</sub>"'), 1.40 (31CH<sub>2</sub>'), 1.37 (36CH<sub>2</sub>'), 1.34 (4CH<sub>2</sub>"'), 1.25 (30CH<sub>3</sub>'), 1.15 (15CH<sub>2</sub>"'), 0.94 (42CH<sub>3</sub>'), 0.91 (35CH<sub>3</sub>, 40CH<sub>3</sub>'), 0.87 (32CH<sub>3</sub>, 38CH<sub>3</sub>'), 0.76 (39CH<sub>3</sub>'), 0.75 (37CH<sub>3</sub>'), 0.69 (34CH<sub>3</sub>').

### NMR data of sodium salinomycin

The <sup>1</sup>H-NMR signals for the starting compound sodium salinomycin in acetonitrile-d<sub>3</sub> are unambiguously assigned as follows: <sup>1</sup>H-NMR (600.13 MHz, δ (assignment), acetonitrile-d<sub>3</sub>): 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<sub>2</sub>"', 26CH<sub>2</sub>"'), 1.94 (22CH<sub>2</sub>'), 1.90 (23CH<sub>2</sub>"'), 1.88 (36CH<sub>2</sub>"'), 1.86 (4CH<sub>2</sub>"', 23CH<sub>2</sub>'), 1.84 (5CH<sub>2</sub>"'), 1.71 (6CH), 1.69 (14CH), 1.67 (15CH<sub>2</sub>"'), 1.62 (16CH, 33CH<sub>3</sub>'), 1.58 (27CH<sub>2</sub>'), 1.43 (5CH<sub>2</sub>'), 1.40 (4CH<sub>2</sub>'), 1.42 (8CH), 1.35 (26CH<sub>2</sub>'), 1.33 (41CH<sub>2</sub>"'), 1.32 (36CH<sub>2</sub>'), 1.30 (31CH<sub>2</sub>"'), 1.26 (31CH<sub>2</sub>'), 1.24 (30CH<sub>3</sub>'), 1.23 (41CH<sub>2</sub>'), 1.16 (15CH<sub>2</sub>'), 0.91 (40CH<sub>3</sub>'), 0.89 (35CH<sub>3</sub>'), 0.87 (32CH<sub>3</sub>'), 0.85 (42CH<sub>3</sub>'), 0.79 (38CH<sub>3</sub>'), 0.76 (37CH<sub>3</sub>'), 0.70 (34CH<sub>3</sub>, 39CH<sub>3</sub>').

### NMR data of salinomycinic acid

Salinomycinic acid was prepared as previously reported [29]. <sup>1</sup>H-NMR (600.13 MHz, δ (assignment), acetonitrile-d<sub>3</sub>): 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<sub>2</sub>"', 23CH<sub>2</sub>"'), 1.88 (22CH<sub>2</sub>'), 4CH<sub>2</sub>"', 36CH<sub>2</sub>"'), 1.85 (5CH<sub>2</sub>"'), 1.80 (6CH), 1.71 (14CH, 23CH<sub>2</sub>'), 1.70 (26CH<sub>2</sub>"'), 1.61 (27CH<sub>2</sub>"'), 1.59 (16CH), 1.58 (15CH<sub>2</sub>"'), 1.54 (27CH<sub>2</sub>'), 1.50 (26CH<sub>2</sub>'), 1.47 (8CH), 1.45 (5CH<sub>2</sub>'), 1.44 (41CH<sub>2</sub>, 4CH<sub>2</sub>'), 1.42 (36CH<sub>2</sub>'), 1.36 (33CH<sub>3</sub>'), 1.29 (31CH<sub>2</sub>'), 1.16 (30CH<sub>3</sub>'), 1.12 (15CH<sub>2</sub>'), 0.94 (40CH<sub>3</sub>'),

0.91 (42CH<sub>3</sub>'), 0.86 (35CH<sub>3</sub>'), 0.85 (32CH<sub>3</sub>'), 0.79 (37CH<sub>3</sub>'), 0.77 (38CH<sub>3</sub>'), 0.73 (39CH<sub>3</sub>'), 0.69 (34CH<sub>3</sub>').

### Additional material

**Additional file 1: Supplementary figure 1.** Edited HSQC spectrum of complex 1.

**Additional file 2: Supplementary figure 2.** Edited HSQC spectrum of complex 2.

### Acknowledgements and funding

The authors are thankful to Assoc. Prof. A. Nakov and MSci P. Dorkov (BIOVET Ltd.) for providing the sodium salinomycin and AQUACHIM JSC, Bulgaria for the deuterated solvent. The technical support of students M. Dimitrova and V. Nansenova (Sofia University) is gratefully acknowledged. The present research was conducted with the financial support from the Bulgarian National Science Fund - Projects DO-02-84/2008 and DRNF-02-13/2009 and Sofia University Fund for Scientific Research (Grant 068/2009).

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### Authors' contributions

Jl performed the synthetic procedures, purification, IR spectroscopic characterization, element analysis interpretation, MT and KO performed the FAB-MS measurements. INP and SS carried out the NMR analyses and drafted the manuscript. All authors read and approved the final manuscript.

### Competing interests

The authors declare that they have no competing interests.

Received: 6 May 2011 Accepted: 9 September 2011

Published: 9 September 2011

### References

1. Chappel LR: The site of action of the anticoccidial salinomycin (Coxistac). *J Parasitol* 1979, **65**:137-143.
2. Taylor RW, Kauffman RF, Pfeiffer DR: Cation complexation and transport by carboxylic ionophores. In *Polyether antibiotics: naturally occurring acid ionophores*. Edited by: Westley. New York: Marcel Dekker; 1982:103-184.
3. Westley JW, Liu CM, Evans RH Jr, Sello LH, Troupe N, Hermann T: Preparation, properties and biological activity of natural and semisynthetic urethanes of monensin. *J Antibiot (Tokyo)* 1983, **36**:1195-1200.
4. Gad SC, Reilly C, Siino K, Gavigan FA, Witz G: Thirteen cationic ionophores: their acute toxicity, neurobehavioral and membrane effects. *Drug Chem Toxicol* 1985, **8**:451-468.
5. Folz SD, Nowakowski LH, Lee BL, Conder GA, Rector DL, Brodasky TF: Anticoccidial activity of alborixin. *J Parasitol* 1987, **73**:29-35.
6. Augustine PC, Smith CK, Danforth HD, Ruff MD: Effect of ionophorous anticoccidials on invasion and development of *Eimeria*: comparison of sensitive and resistant isolates and correlation with drug uptake. *Poult Sci* 1987, **66**:960-965.
7. Logan NB, McKenzie ME, Conway DP, Chappel LR, Hammett NC: Anticoccidial efficacy of semduramicin. 2. Evaluation against field isolates including comparisons with salinomycin, maduramicin, and monensin in battery tests. *Poult Sci* 1993, **72**:2058-2063.
8. Satoh H, Tsuchida K: Pharmacological actions of monovalent ionophores on spontaneously beating rabbit sino-atrial nodal cells. *Gen Pharmacol* 1999, **33**:151-159.

9. Page SV: **The role of enteric antibiotics in livestock production.** Canberra, Australia: Avicare Ltd; 2003.
10. Lutz WK, Winkler FK, Dunitz JD: **Crystal structure of the antibiotic Monensin. Similarities and differences between free acid and metal complex.** *Helv Chim Acta* 1971, **54**:1103-1108.
11. Kinashi H, Otake N, Yonehara H, Sato S, Saito Y: **Studies on the ionophorous antibiotics. I. The crystal and molecular structure of salinomycin p-iodophenacyl ester.** *Acta Cryst* 1975, **B31**: 2411-2415.
12. Pointud Y, Tissier C, Juillard J: **Neutral and charged complexes of alkali metal cations with the ionophore nigericin in methanol. A thermodynamic study.** *J Sol Chem* 1983, **12**:473-485.
13. Garcla-Rosas J, Schneider H, Cox BG: **Silver complexation by the ionophorous antibiotic monensin in nonaqueous solvents.** *J Phys Chem* 1983, **87**:5467-5472.
14. Cox BG, Vantruong N, Rzeszotarska J, Schneider H: **Rates and equilibria of alkali-metal and silver ion complex-formation with monensin in ethanol.** *J Am Chem Soc* 1984, **106**:5965-5969.
15. Walba DM, Hermsmeier M, Haltiwanger RC, Noordik JH: **Crystal structures of monensin B lithium and silver salts.** *J Org Chem* 1986, **51**:245-247.
16. Pangborn W, Duax WL, Langs D: **The hydrated potassium complex of the ionophore monensin A.** *J Am Chem Soc* 1987, **109**:163-2165.
17. Mimouni M, Perrier S, Pointud Y, Juillard J: **Selectivity of bacterial ionophore monensin for monovalent metal cations - solvent effects in methanol and biphasic water-organic systems.** *J Sol Chem* 1993, **22**:769-785.
18. Mimouni M, Hebrant M, Dauphin G, Juillard J: **Monovalent cation salts of the bacterial ionophore monensin in methanol. Structural aspects from NMR experiments.** *J Chem Res* 1996, **S6**: 278-279.
19. Wagner-Czauderna E, Rzeszotarska J, Orłowska E, Kalinowski MK: **Complexing ability of monensin anion for alkali metal cations in binary mixtures of dipolar aprotic solvents.** *Phys Chem Chem Phys* 1997, **101**:1154-1157.
20. Paulus EF, Kurz M, Matter H, Vertesy L: **Solid-state and solution structure of the salinomycin-sodium complex: Stabilization of different conformers for an ionophore in different environments.** *J Am Chem Soc* 1998, **120**:8209-8221.
21. Riddell FG: **Structure, conformation, and mechanism in the membrane transport of alkali metal ions by ionophoric antibiotics.** *Chirality* 2002, **14**:121-125.
22. Paz FAA, Gates PJ, Fowler S, Gallimore A, Harvey B, Lopes NP, Stark CBW, Staunton J, Klinowski J, Spencer JB: **Sodium monensin dihydrate.** *Acta Cryst* 2003, **E59**: m1050-m1052.
23. Dorkov P, Pantcheva IN, Sheldrick WS, Mayer-Figge H, Petrova R, Mitewa M: **Synthesis, structure and antimicrobial activity of manganese(II) and cobalt(II) complexes of the polyether ionophore antibiotic sodium monensin A.** *J Inorg Biochem* 2008, **102**:26-32.
24. Pantcheva IN, Mitewa M, Sheldrick WS, Oppel IM, Zhorova R, Dorkov P: **First divalent metal complexes of the polyether ionophore Monensin A: X-ray structures of [Co(Mon)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>] and [Mn(Mon)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>] and their properties.** *Curr Drug Discov Techn* 2008, **5**:154-161.
25. Pantcheva IN, Dorkov P, Atanasov V, Mitewa M, Shivachev BL, Nikolova RP, Mayer-Figge H, Sheldrick WS: **Crystal structure and properties of the copper(II) complex of sodium monensin A.** *J Inorg Biochem* 2009, **103**:1419-1424.
26. Pantcheva IN, Ivanova J, Zhorova R, Mitewa M, Simova S, Mayer-Figge H, Sheldrick WS: **Nickel(II) and zinc(II) dimonensinates: crystal structure, spectral properties and bactericidal activity.** *Inorg Chim Acta* 2010, **363**:1879-1886.
27. Pantcheva IN, Zhorova R, Mitewa M, Simova S, Mayer-Figge H, Sheldrick WS: **X-ray crystal structure of [M(Mon)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>] (M = Mg, Ca), spectral properties and cytotoxicity against Gram-positive bacteria.** *Biomaterials* 2009, **23**:59-70.
28. Ivanova J, Pantcheva IN, Mitewa M, Simova S, Mayer-Figge H, Sheldrick WS: **Crystal structures and spectral properties of new Cd(II) and Hg(II) complexes of monensic acid with different coordination modes of the ligand.** *Centr Eur J Chem* 2010, **8**:852-860.
29. Mitewa M, Pantcheva I, Alexandrova R: **Antitumor activity of the polyether ionophorous antibiotic monensin and its metal(II) complexes.** In *Recent Researches in Modern Medicine*. Edited by: Braissant O, Wakamatsu H, Kuo-Kang I, Allegaert K, Lenbury Y, Wachholtz A. Cambridge: WSEAS Press; 2011:439-444.
30. Alexandrova R, Zhivkova T, Pantcheva IN, Mitewa M: **Cytotoxic and antiproliferative activities of monensic acid and its metal(II) complexes against drug sensitive and multidrug resistant human tumor cell lines.** *Intern J Biol Biomed Eng* 2011, **5**:93-101.
31. Erdahl WL, Chapman CJ, Taylor RW, Pfeiffer DR: **Ionomycin, a carboxylic acid ionophore, transports Pb<sup>2+</sup> with high selectivity.** *J Biol Chem* 2000, **275**:7071-7079.
32. Hamidinia SA, Shimelis Ol, Tan B, Erdahl WL, Chapman CJ, Renkes GD, Taylor RW, Pfeiffer DR: **Monensin mediates a rapid and selective transport of Pb<sup>2+</sup>- possible application of monensin for the treatment of Pb<sup>2+</sup> intoxication.** *J Biol Chem* 2002, **277**:38111-38120.
33. Hamidinia SA, Tan B, Erdahl WL, Chapman CJ, Taylor RW, Pfeiffer DR: **The ionophore nigericin transports Pb<sup>2+</sup> with high activity and selectivity: a comparison to monensin and ionomycin.** *Biochemistry* 2004, **43**:15956-15965.
34. Hamidinia SA, Erdahl WL, Chapman CJ, Steinbaugh GE, Taylor RW, Pfeiffer DR: **Monensin improves the effectiveness of mesodimercaptosuccinate when used to treat lead intoxication in rats.** *Environm Health Persp* 2006, **114**:484-493.
35. Riddell FG, Tompsett SJ: **The complete assignment of the <sup>1</sup>H and <sup>13</sup>C NMR spectra of the alkali metal salts of salinomycin and narasin.** *Tetrahedron* 1991, **47**:10109-10118.
36. Matsumori N, Morooka A, Murata M: **Conformation and location of membrane-bound salinomycin-sodium complex deduced from NMR in isotropic bicelles.** *J Am Chem Soc* 2007, **129**:14989-14995.
37. Matsumori N, Murata M: **3D structures of membrane-associated small molecules as determined in isotropic bicelles.** *Nat Prod Rep* 2010, **27**:1480-1492.

doi:10.1186/1752-153X-5-52

Cite this article as: Ivanova et al.: Cd(II) and Pb(II) complexes of the polyether ionophorous antibiotic salinomycin. *Chemistry Central Journal* 2011 5:52.

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