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Synthesis, antiproliferative and antibacterial activity of new amides of salinomycin

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ABSTRACT

A series of 11 novel amides of salinomycin were synthesized for the first time. All the obtained compounds were found to show potent antiproliferative activity against human cancer cell lines including the drug-resistant cancer cells. Four new salinomycin derivatives revealed good antibacterial activity against clinical isolates of methicillin-resistant *Staphylococcus epidermidis* (MRSE).

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In last decades natural substances have been in a sense rediscovered as important ingredients in pharmaceutical industry either in their chemically unchanged or synthetically modified forms.[1,2]

Salinomycin (**SAL**) isolated from *Streptomyces albus* is an antibiotic belonging to natural polyether ionophores,[3] exhibiting a large spectrum of antimicrobial activity against Gram-positive bacteria, including *Staphylococcus aureus*, mycobacteria, *Plasmodium falciparum* or *Eimeria* spp, parasites, and protozoa.[4] For this reason, sodium salt of salinomycin (Bio-cox™, Sacox™) found commercial application as a coccidiostatic and non-hormonal growth promoting agent in livestock and poultry breeding.[5] Screening of about 16.000 chemical compounds performed in 2009 showed that **SAL** was the most effective against breast cancer stem cells, nearly 100-fold more active than the commonly used anticancer drug–*Paclitaxel* (*Taxol*).[6] Since this discovery, extensive research has been carried out all over the world to elucidate the unusual properties of **SAL**. Further studies proved that **SAL** shows antiproliferative activity against various types of human tumour cells, for example leukemic stem cells, including lymphocytic leukemia, colon carcinoma stem cells, prostate cancer stem cells, as well as lung cancer cell lines.[7] Additionally, it has been shown that the application of **SAL** enhances the anticancer effect of radio- and chemotherapy.[8] Moreover, the sodium salt of **SAL** is able to selectively deplete the breast cancer stem cells with efficiency comparable to that of **SAL**.[9]

The preliminary clinical study of salinomycin has been performed by Cord Naujokat et al. on a small group of patients with metastatic breast cancer or metastatic head and neck cancers. The patients treated with 200–300 μg/kg of **SAL**, every second day for three weeks, have shown partial tumour regression and only transient acute side effects, including tachycardia and mild tremor, with neither severe nor long-term side effects that can be observed to accompany the use of conventional chemotherapeutic drugs. Thus, the preliminary results have permitted determination of a drug dosage that yields clinically significant benefits without excessive toxicity.[10]

The simplest method for preparing biologically effective compounds is chemical modification of substances with proven high biological activity. The synthesis, structure, as well as biological activity of a series of *O*-acyl derivatives,[11] amides[12,13] and one ester[14] of **SAL** have been already described. The **SAL** derivatives showed antimicrobial activity, among others against methicillin-resistant hospital strains of *Staphylococcus* and anticancer activity

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in various cell phenotypes in the low micromolar range, providing an excellent starting point for further drug discovery optimisation. In addition, tests of **SAL** and its derivatives have clearly proven that some of these compounds have a high antiproliferative effect against normal and drug resistant cancer cells. These results indicate that the biological effects of **SAL** derivatives are diverse on different bacterial or cancer cell lines and are strongly dependent on chemical nature of *O*-acyl, amide or ester substituent.[11–14]

The main aim of this Letter is evaluation of anticancer and antimicrobial activity of new derivatives of **SAL**. Therefore, a series of new **SAL** amides was synthesized, characterized by X-ray and spectroscopic methods and tested against their antiproliferative and antibacterial activity. Moreover, as the biological activity of the new **SAL** derivatives is closely related to their ability to make complexes with monovalent and divalent metals, it was tested using the electrospray ionisation mass spectrometry (ESI MS).

In the present study, the antiproliferative effect of eleven **SAL** amides (**1**–**11**) was tested in vitro using human promyelocytic leukemia (HL-60) and its vincristine-resistant subline (HL-60/vinc), human colon adenocarcinoma cell line (LoVo) and doxorubicin resistant subline (LoVo/DX), and normal murine embryonic fibroblast cell line (Balb 3T3). Multi-drug chemoresistance (MDR) remains one of the most common reasons for failure of chemotherapy. The membrane transporter protein belonging to the ABC transporters family has been shown in vitro to effectively reduce the intracellular concentration of several anticancer chemotherapeutic agents such as doxorubicin. On the other hand, it is known that cancer stem cells may act as master regulators during the process of chemoresistance acquisition and are characterized by MDR phenotype.[15,16]

Taking into account this phenomenon, we decided to study the antiproliferative activity of salinomycin derivatives on drug resistant cells, expressing various transporters (e.g., *p*-glycoprotein) and their parent cell lines to observe not only the antiproliferative activity against cancer cells, but also the possibility to break the barrier of chemoresistance.

Antimicrobial activity of compounds **1**–**11** was also tested in vitro on Gram-positive and Gram-negative bacteria and fungi, as well as against a series of clinical isolates of *Staphylococcus*. To investigate the effect of different substitutions of the carboxylic group of **SAL** on its bioactivity, eight new amide derivatives (**1**–**8**) and three dimers (**9**–**11**) of **SAL** were synthesized using the procedure developed previously by our group.[12] To facilitate the structural activity relationship analysis (SAR) we chose salinomycin amides with different substituents such as: unsaturated alkyl chain (propargylamine, **1**), alkyl chains containing oxygen atoms (2-(2-aminoethoxy)ethanol, **3**) biogenic amines like cysteamine, (**2**), putrescine (**4**), histamine (**7**), dopamine (**8**), containing fluorinated aromatic ring (4-fluorobenzylamine, **6**) and crown ether (2-aminomethyl-15-crown-5, **5**). It is generally believed that dimers of biologically active compounds, such as antibiotics, can show enhanced biological activity relative to that of the single ligand. Thus, the symmetrical dimeric **SAL** ligands, in which two **SAL** molecules are linked by different spacer units (1,4-butanediamine **9**; *p*-phenylenediamine **10**; 4,4′-diaminobiphenyl, **11**) were also prepared to check the effects of linker length and its flexibility on the biological activity of **SAL**.

Salinomycin sodium salt was isolated from veterinary premix–SACOX®. Amide derivatives of salinomycin (**1**–**11**) were obtained in the reaction between salinomycin acid (**SAL)** and amines with addition of DCC (*N,N′*-dicyclohexylcarbodiimide) and HOBt (1-hydroxybenzotriazole) following the procedures described previously.[12]

All **SAL** amides can be easily isolated in pure form following the purification by dry vacuum column chromatography.[17] This method was efficient and gave amides **1**–**8** in high yields of up to 43–88% (Scheme 1). The **SAL** dimers (**9**–**11**) were obtained in the moderate yields of about 30% (Scheme 1). The purity and structures of compounds **1**–**11** were determined on the basis of elemental analysis, FT-IR and NMR methods. The ^{1}H and ^{13}C NMR signals were assigned using one- and two-dimensional (COSY, HETCOR, HMBC and NOESY) spectra. The exemplary NMR spectra are included in the Supplementary material (Figs. S1–S4). The analytical signals in the ^{1}H and ^{13}C NMR spectra and the position of the amide I band in the FT-IR spectra of compounds **1**–**11**, are collected in Table S1.

Detailed structural analysis of the biologically active compounds is very important for better understanding of their anticancer and antimicrobial properties for structure–activity relationship analysis (SAR), and related investigation. Therefore, one exemplary compound of the studied series of derivatives that is *p*-fluorobenzylamide (**6**) was characterized by single crystal X-ray diffraction method. The single crystals of **6** were grown by crystallisation in acetonitrile and their structure was determined using X-ray crystallographic technique (Fig. 1). The crystallographic data and structure refinement of compound **6** are summarized in Table S2 (Supplementary material).

The pseudo-cyclic conformation of **6** is stabilised by four N1–H···O10, O10–H···O6, O9–H···O7 and O8–H···O7 weak intramolecular hydrogen bonds showed in Figure 1, and the parameters of this compound are collected in Table S3. The six-membered rings of **6** exhibit the typical chair conformation. The intermolecular O8–H···O1^{i} hydrogen bond between the terminal hydroxyl group (O8–H) of one molecule and the carbonyl atom of amide group of the neighbouring molecule, together with the van der Waals forces, stabilise the arrangement of **6** molecules in the crystal (Fig. S5, Supplementary data). The bond lengths and angles characterizing the geometry of the molecule are presented as Supplementary material (Table S4). The absolute configuration of **6** is unchanged and is the same as determined previously for salinomycin, its amides and benzotriazole ester.[12–14,18,19]

The presence of the pseudo-cyclic structure of salinomycin amides confirmed by X-ray is facilitates the formation of the lipid-soluble pseudo-cyclic complexes of these compounds with the metal cations. Since the biological activity of the polyether antibiotics and its derivatives strongly depends on their ionophoretic properties, the ability of the new **SAL** derivatives to form complexes with monovalent cations such as Li^{+}, Na^{+}, K^{+}, Rb^{+}, Cs^{+} and divalent cations such as Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+} was studied by us using ESI MS technique. The ESI MS spectra of the mixtures of respective **SAL** amides with the monovalent and divalent metal perchlorates (Fig. S6-S10) demonstrate that the amides (**1**–**4**, **6**–**8**) form exclusively 1:1 complexes with both types of metal cations. Only the amide with the crown moiety (**5**) is able to form different complexes with divalent cations (M) that is the $(\mathbf{5}+M)^{2+}$ and $(\mathbf{5}+MClO_4)^{+}$ (Fig. S9). The last type of complexes has been previously observed for different monensin derivatives.[12,13] Additionally, dimers of **SAL** (**9**–**11**) are able to form complexes with monovalent cations in 1:1 and 1:2 stoichiometry (Fig. S10).

In contrast to unmodified salinomycin (**SAL**), which forms complexes of 1:1 stoichiometry only with monovalent cations, especially Na^{+} and K^{+}, salinomycin amides are able to form complexes with both monovalent and divalent metal cations and of different stoichiometries.

The reason why **SAL** and its derivatives exhibit biological effects is their ability to form lipid-soluble pseudo-cyclic complexes with metal cations and transport them trough cell and mitochondrial membranes. Derivatives of **SAL** with modified carboxylic groups like amides can transport cations via an electrogenic mechanism. The ESI MS measurement show the ability of SAL amides to form complexes with monovalent and divalent cation, therefore the biological activity of these compounds is confirmed also by their ionophoretic properties.

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